STRUCTURAL STUDIES ON FEBRIFUGINE AND ISOFEBRIFUGINE, ALKALOIDS FROM *DICHROA FEBRIFUGA*

STUDIES ON 4-QUINAZOLONES

SYNTHESIS OF SOME 8-AMINOQUINOLINES

Thesis by

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I wish to express my sincere appreciation to the staff members, and to my fellow graduate students in the departments of chemistry and biology for their inspiration and help.

My especial thanks go to Dr. J. B. Koepfli who guided the largest part of the work described in this thesis, and to Dr. E. R. Buchman who was my advisor during the leave of absence of Dr. Koepfli.

To Prof. N. H. Horowitz of the Division of Biology I would like to express my appreciation for his interest in guiding some research undertaken in my minor subject.

Summary

Structural studies on two isomeric, antimalarial alkaloids, febrifugine and isofebrifugine, isolated from Dichroa febrifuga, have been continued. The basic structure of both alkaloids is proposed to be 3-[β-keto-γ-(3-hydroxy-2-piperidyl)propyl]-4-quinazolone, and the relationship of febrifugine and isofebrifugine to this structure is discussed.

Febrifugine is shown to be polymorphic, and evidence is presented that the alkaloid γ-dichroine, from Dichroa febrifuga, is not chemically distinct, but a mixture of two of the crystalline modifications of febrifugine.

The synthesis of some 3-substituted-4-quinazolones is described. These are shown to be unreactive to benzenesulfonyl chloride, while the parent substance, 4-quinazolone, forms 3-benzenesulfonyl-4-quinazolone. The structure of a hydrolysis product of the latter compound is proposed to be N-(N'-formyl-o-aminobenzoyl)benzenesulfonamide, and the synthesis of this and of some related compounds is described.

The synthesis of some 8-aminoquinolines related to isoplasmocid was carried out as part of a cooperative project to study their action on experimental poliomyelitis in monkeys. These compounds were tested elsewhere and found to be without specific activity.

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PART I. STRUCTURAL STUDIES ON FEBRIFUGINE AND ISOFEBRIFUGINE, ALKALOIDS FROM *DICHROA FEBRIFUGA*

Section 1

Introduction

The present investigation on *Dichroa febrifuga* has been a collaborative investigation under the direction of Dr. J. B. Koepfli since 1942. Those taking part have been Dr. James F. Mead, Dr. John A. Brockman Jr., Dr. James Moffat, and the author.

The isolation and characterization work and the preliminary structural studies were carried out principally by Drs. Mead and Brockman and are described in detail in Dr. Brockman's thesis (1) and in publications (2,3). The studies leading to a proposed structure were carried out primarily by Dr. Moffat and the author. A portion of this work and a proposed structure has been published in a communication (4).

For purposes of continuity and clarity of presentation, this thesis takes up where Dr. Brockman's thesis ended and describes work carried out by Drs. Brockman, Moffat, Koepfli, and the author. In the experimental sections, experiments carried out by collaborators other than the author will be designated by suitable initials.

The knowledge of the structures of febrifugine and isofebrifugine at the time of the writing of Dr. Brockman's thesis may be briefly summarized as follows:

1. *The empirical formula.* Both febrifugine and iso-

febrifugine have the same empirical formula, $C_{16}H_{19}O_3N_3$. Furthermore, the alkaloids are easily interconvertible on warming in solution.

2. Presence of a Secondary Aliphatic Amine. Both compounds give various tests characteristic of a secondary aliphatic amine, including the formation of nitroso compounds with sodium nitrite and hydrochloric acid. Both form dibenzenesulfonyl derivatives which are insoluble in dilute hydrochloric acid and dilute sodium hydroxide. Both have a basic group of pK_B 6.3.

3. The Aromatic Portion of the Molecule is a 3-Substituted-4-quinazolone.

a) Both are oxidized by aqueous alkaline permanganate at room temperature to 4-quinazolone. When the alkaloids are heated for a time above the melting point in vacuo, 4-quinazolone sublimes out.

b) The ultraviolet absorption spectra of the alkaloids and of 4-quinazolones are very similar. The absorption spectrum of 3-substituted-4-quinazolone gives the best agreement (fig. 1), the position of the extremes agreeing within 1 mμ.

c) Alkaline hydrolysis of either alkaloid gives a good yield of anthranilic acid, a low yield of ammonia, an unknown yield of formic acid, and oils and resins.

d) The alkaloids and dihydro-alkaloids are not readily soluble in dilute base in contrast to 4-quinazolones unsubstituted in the 3-position.

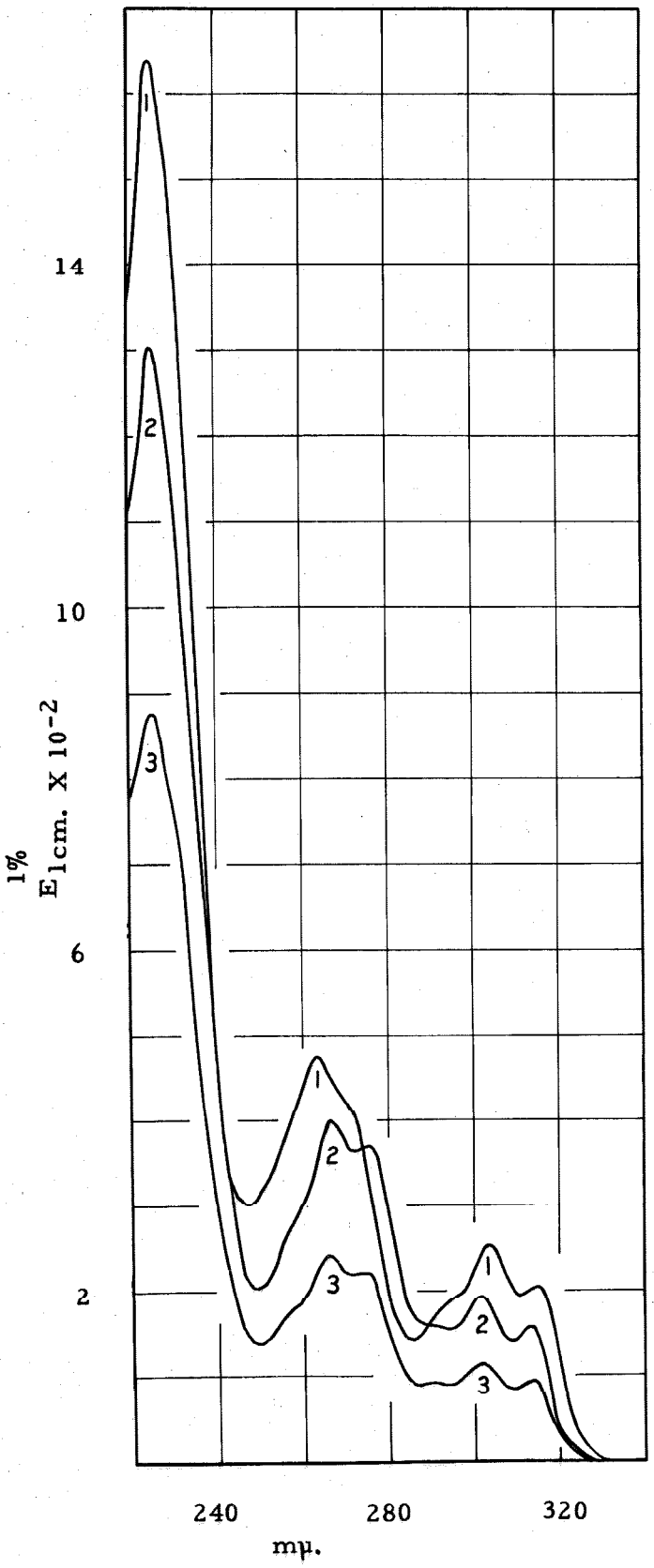


Figure 1. Absorption spectra in absolute ethanol: (1), 2-methyl-4-quinazolone; (2), 3-allyl-4-quinazolone; and (3), febrifugine.

e) The alkaloids and the dihydro-alkaloids are quite easily hydrolyzed at room temperature in 2.5N sodium hydroxide. Hydrolysis of 4-quinazolones unsubstituted in the 3-position is normally much slower.

4. Oxygen Functional Groups. Neither alkaloid gives a positive spot test for aldehyde or methyl ketone. Febrifugine forms an oxime and a semicarbazone; isofebrifugine does not. Both compounds reduce Tollens' reagent.

Both compounds give only dibenzenesulfonyl derivatives. Presumably one benzenesulfonyl group is attached to the secondary amine nitrogen, and the other to one of two atoms, either an oxygen which was originally a hydroxyl group or the 1-nitrogen of the quinazolone ring.

Thus, in addition to the quinazolone oxygen, there is in febrifugine a ketone and a hydroxyl group. In isofebrifugine, a hydroxyl group is indicated.

5. Catalytic (Pt) Hydrogenation. Both alkaloids in ethanol take up one mole of hydrogen, but crystalline dihydro-products are not quantitatively isolated. In glacial acetic acid, febrifugine absorbs one mole of hydrogen but isofebrifugine absorbs 2 or more.

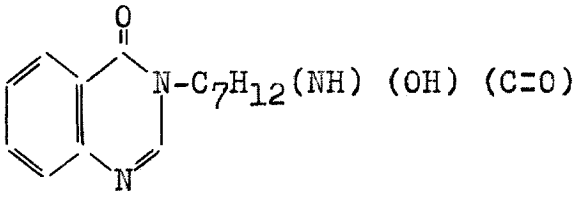
Presumably, the reduction has not affected the quinazolone portion of the molecule as the absorption spectra of the crystalline dihydro-compounds are very similar to the original alkaloids. After reduction, neither compound reduces Tollens' reagent, and febrifugine has apparently lost its keto-function.

6. Hydrolysis of Dihydrofebrifugine. Alkaline hydrolysis

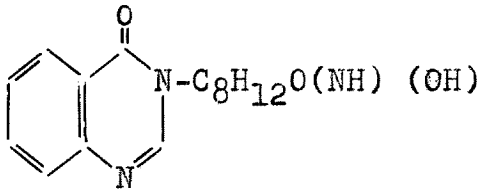
of dihydrofebrifugine gives anthranilic acid, formic acid, no ammonia or other volatile base, and a very water soluble fragment which gives a crystalline benzenesulfonyl derivative of the empirical formula $C_{26}H_{28}O_7S_3N_2$ (tentative).

7. Periodate Oxidation. Oxidation of febrifugine or isofebrifugine with periodate in basic solution uses four equivalents of oxidizing agent, and yields a crystalline product which is tentatively assigned the formula $C_{11}H_{12}O_2N_2$. It appears to be a 3-substituted-4-quinazolone.

8. Best Partial Formulae.



Febrifugine



Isofebrifugine

The research leading to the elucidation of the structures will be presented in sections for the purpose of readability. The material is roughly arranged in chronological order, though

experiments of a similar nature are grouped even though this disturbs the chronology. The deductions to be drawn from the experimental work are presented in each section, but the correlation of the experimental data with a proposed structure is reserved for the end as was the case chronologically.*

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* Subsequent to the elucidation of the structure of febrifugine (4), but prior to the writing of this thesis, a group at Lederle Laboratories (Division of American Cyanamid) published a series of papers on "An Antimalarial Alkaloid from Hydrangea" (5-19). In this series of papers the isolation, degradation, and synthesis of an alkaloid and some analogues was described. The authors of these papers identify this alkaloid as febrifugine. Certain of the degradations given in this thesis are also contained in their series of papers. The degradative work done by them however was carried only to the point where a limited number of probable structures could be written for the alkaloid and these were synthesized as the optically inactive modifications. Their proposed structure, confirming ours (4), was the culmination of some excellent synthetic organic chemistry, though their comparison of the optically inactive synthetic product with the natural material left something to be desired.

During the writing of this thesis a second series of papers by the above group appeared (20-23). In these papers, the preparation of additional analogues and isosters of febrifugine and the preparation of optically active febrifugine and isofebrifugine was described. A structure for isofebrifugine was proposed.

Section 2

Investigations of the Dibenzenesulfonyl Derivatives of Febrifugine and Isofebrifugine

The alkaloids both readily form dibenzenesulfonyl derivatives (1,3). The non-basic nature of these derivatives suggested that one of the benzenesulfonyl groups had reacted with the 1-nitrogen of the quinazolone ring (1).*

Oxidative degradation of dibenzenesulfonylisofebrifugine gave an excellent yield of benzenesulfonamide and a moderate yield of a material tentatively identified as sodium benzenesulfonate. This confirms the attachment of one of the benzenesulfonyl groups to a nitrogen and suggests that the second was on an oxygen.

Later work (part II of this thesis) showed that 3-substituted-4-quinazolones do not react with benzenesulfonyl chloride under the conditions used in the preparation of the dibenzenesulfonyl derivatives of the alkaloids. In addition it was shown that 3-(β-benzenesulfonoxyethyl)-4-quinazolone is insoluble in 1N, 3N, or 6N hydrochloric acid.

The insolubility of the dibenzenesulfonyl derivatives of the alkaloids in acid is thus not good evidence that the 1-nitrogen is substituted. The base strengths of 3-substituted-4-quinazolones have not been determined, but the pK_B of

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* In the case of rutacarpine, a monoacetyl and a monobenzoyl derivative involving the 1-nitrogen of the 4-quinazolone portion are formed (24). In this case, however, the quinazolone is already substituted at the 2-and 3-positions and a double bond is formed elsewhere in the molecule, replacing that at the 1-nitrogen.

4-quinazolone itself is about 11.7, and the alkaloids have a second basic constant of pK_{B_2} 12 (3). The water solubility of the free base thus becomes a factor in the solubility in acid media.

It is believed that the dibenzenesulfonyl derivatives do not involve the 1-nitrogen. The complete inertness of simple 3-substituted-4-quinazolones to benzenesulfonyl chloride under similar conditions is very strong evidence in favor of this conclusion.

Attempts to hydrolyze with hydriodic acid or to make the oximes of the dibenzenesulfonyl derivatives failed to yield crystalline material. In the case of dibenzenesulfonylisofebrifugine, starting material was almost quantitatively recovered after heating with hydroxylamine hydrochloride in pyridine at 70° for two hours.

Experimental*

Oxidative Degradation of Dibenzenesulfonylisofebrifugine (JAB). A solution of 101 mg. of dibenzenesulfonylisofebrifugine, 270 mg. of potassium permanganate (10 moles/mole), 9 ml. of water, and 1 ml. of 10% sodium hydroxide was heated on a steam bath until all permanganate color was gone (1½ hours). On acidification with sulfuric acid, a gas was evolved, presumably carbon dioxide. After the manganese

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* Melting points were in general taken in thin-walled capillary tubes in an electrically heated copper block and are corrected. The rate of heating was one degree per minute at the melting point.

dioxide was dissolved by the addition of sodium bisulfite, a crystalline residue remained. This residue after washing with water and drying weighed 38 mg., and melted at 181-183° with no depression on admixing with starting material (m.p. 182.5-183.5°). The aqueous solution was extracted continuously for 4 hours with chloroform. Evaporation of the chloroform yielded 15 mg. of solid which on crystallization from ethanol-benzene and from aqueous ethanol melted at 149.5-151° and gave no depression on admixing with an authentic sample of benzene-sulfonamide.

The aqueous phase, after the extraction with chloroform, was made strongly alkaline. No significant amount of material could be extracted with chloroform, nor could anything be extracted after neutralization with carbon dioxide. The aqueous phase was then evaporated to dryness on a steam bath and the solid residue extracted with 25 ml. of hot acetonitrile. Evaporation of the acetonitrile gave a white crystalline solid, very soluble in water and soluble in methanol, but not very soluble in other organic solvents. It was recrystallized from hot ethanol to give 5 mg. of platelets which did not melt below 300°. A fusion test indicated the presence of sulfur. Its most probable identity is sodium benzenesulfonate. Thus, on the basis of unrecovered starting material, 88% of benzene-sulfonamide and 26% of sodium benzenesulfonate (tentative) was isolated.

Section 3

Phthalic Anhydride Adducts of Febrifugine and Isofebrifugine

Febrifugine and isofebrifugine each form an adduct in excellent yield on heating with phthalic anhydride in acetonitrile. The melting point and mixed melting point behavior would indicate that the two adducts were possibly the same compound. Acid hydrolysis, however, regenerated the respective alkaloids in good yield, precluding any possibility that the adducts are the same.

The analysis indicated the addition of one phthalic anhydride as was expected. There is some question, however, whether the compounds are phthalamic acids, the expected products. Both adducts give a positive spot test for a primary or secondary amino group.* In addition, the absence of the phthalic acid moiety on an oxidative fragment containing the secondary amine (sect. 4) would indicate that the adducts are half phthalate esters. This formulation is somewhat surprising both for the lack of reaction with the secondary amine and for the fact that isofebrifugine has long been tentatively formulated as a hemiketal because of the lack of a demonstrable carbonyl group. This would mean that the phthalic

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* This test depends on the formation of a non-volatile dithiocarbamate by reaction with carbon disulfide (25a). Apparently some amides interfere, for acetamide and 4-quinazolone were both shown to give the test. However, the test was very strong in the case of the phthalic anhydride adducts.

anhydride reacted with the hydroxyl group of a hemiketal structure in preference to what has been thought to be a non-hindered secondary amine. B. R. Baker and co-workers have recently confirmed our belief that isofebrifugine exists as a hemiketal (23).

Experimental*

Phthalate Adduct of Isofebrifugine (JAB). A solution of 105 mg. of isofebrifugine, 55.8 mg. of phthalic anhydride, and 5 ml. of acetonitrile was boiled for 15 seconds. On cooling, 152 mg. of crystals separated, which after recrystallization from methanol melted at 195-197° (with decomposition). No material would extract into chloroform from an alkaline solution, thus indicating the adduct not to be a phthalate salt of isofebrifugine.

Anal. Calcd. for $C_{24}H_{23}O_6N_3$; C, 64.13; H, 5.16; N, 9.35. Found: C, 65.29; H, 5.58; N, 9.58.

For comparison purposes, the adducts of febrifugine and isofebrifugine were prepared in parallel. The derivative of febrifugine melted at 195-196°. A roughly 1:1 mixture of the two melted at 194-196.5°. Decomposition at the melting point made accurate comparison difficult.

Into a 3.5 mm. (i.d.) capillary tube, 21.3 mg. of the phthalic anhydride adduct of isofebrifugine and a 20 mm.

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* Microanalyses were carried out by Elek Micro Analytical Laboratory, Los Angeles, and G. A. Swinehart and the late G. Oppenheimer, Division of Biology, California Institute of Technology.

column of 6N hydrochloric acid were introduced. The tube was sealed and heated for 2 hours in a boiling bath of 6N hydrochloric acid. On cooling and shaking, 5.9 mg. of crystals separated. These softened at 165° and melted at 177-187° but after allowing to solidify, remelted at 130°, indicating the material to be phthalic acid. The aqueous solution was made basic with 10% sodium hydroxide and extracted five times with equal volume portions of chloroform. After drying with sodium sulfate, the chloroform solution was evaporated to an oil and treated with acetone, yielding 10.8 mg. of crystals melting at 129-131°, and showing no depression on mixing with a sample of isofebrifugine. The yield of isofebrifugine was thus 76% and of the presumed phthalic acid, 75%.

In a similar manner 9.3 mg. (74%) of febrifugine was recovered from 18.8 mg. of its adduct with phthalic anhydride.

Section 4

Oxidative Degradation of the Alkaloids other than with Periodate

Previous oxidation studies on the alkaloids (3) had shown that oxidation with permanganate produced 4-quinazolone. As yet, no workable fragment containing the non-quinazolone portion of the molecule had been obtained. Hydrolytic degradation of the alkaloids seemed infeasible because of the resins produced, and hydrolysis of the dihydroalkaloids had failed to supply an answer (1). Oxidative degradation was returned to in the hope of isolating fragments containing the secondary amine.

Small test experiments established that febrifugine and isofebrifugine are oxidized by selenium dioxide, while dihydrofebrifugine, the periodate oxidation product (sect. 5) and the phthalic anhydride adducts of the alkaloids are not. It was hoped that selenium dioxide would preferentially attack the postulated methylene group attached to the quinazolone ring, but no crystalline material could be isolated from a somewhat larger scale oxidation of isofebrifugine.

Since at the time it was believed that the phthalic anhydride adducts were phthalamic acids, it was felt that the presence of this group might lend some stability to oxidative fragments and perhaps improve the crystalline properties of any fragment. Permanganate oxidation of the adduct of isofebrifugine gave a small yield of the acid phthalate salt of 4-quinazolone, and a small amount of a sublimable amine

hydrochloride.

The amine hydrochloride, though apparently from the non-quinazolone portion of the alkaloid, did not appear to contain the phthalate adduct. Accordingly, isofebrifugine was oxidized in a similar manner, and the same amine hydrochloride was obtained. In addition, from the oxidation of isofebrifugine, was isolated a small yield of 4-quinazolone-3-acetic acid.

The sublimable amine hydrochloride was very water soluble and optically active. Spot tests indicated the presence of an ionizable chlorine, a secondary (or primary) amine, and an ester linkage (25b). Analysis indicated an empirical formula of $C_7H_{11}O_2N.HCl$. The C_7 formulation was substantiated by a saponification equivalent. The ultra-violet absorption spectrum in water was quite non-characteristic, showing very little absorption in the near ultraviolet region. The compound slowly decolorized alkaline (0.2N) potassium permanganate. On warming the compound with dilute alkali, no ammonia or volatile amine was detected. On pyrolysis, the material gave a positive pine splinter test for pyrrole.

Although the alkaloids have no C-methyl groups (Kuhn-Roth) the amine gave a positive iodoform test. That this was no artifact was shown by the actual isolation and identification of iodoform after reaction with iodine in alkali. The iodoform test does not, however, require the original presence of a methyl ketone. If a compound is capable of generating such a group by oxidation and cleavage the compound

will show the reaction (26). Thus certain amines analogous to the known type of alcohols giving the test, yield iodoform (26). Dibenzoylmethane and analogous compounds give the reaction by cleavage (26).

After alkaline hydrolysis, the sublimable amine hydrochloride very slowly consumes a small amount of periodate. The reduction of a small amount of periodate indicates that the molecule probably contains a group intrinsically capable of oxidation by periodate but hindered sterically. It may mean nothing as certain compounds not having an α-glycol or a vicinal amino alcohol structure react slowly with periodic acid, though usually elevated temperatures are required (27).

Thus, a considerable amount is known about the sublimable amine hydrochloride. Since it is non-aromatic it undoubtedly carries the third nitrogen and is thus probably a secondary amine. The empirical formula, in connection with the slow reaction with permanganate, indicates a bicyclic compound, probably a lactone. The compound thus very likely contains a piperidine or pyrrolidine ring fused with a γ - or δ-lactone ring. The pine splinter test would indicate the pyrrolidine ring, but is not very reliable, especially on material which has been pyrolyzed. Either formulation would suggest two centers of asymmetry, thus indicating at least this number in isofebrifugine.

Oxidation of febrifugine with permanganate did not yield any of the lactone obtained from isofebrifugine nor did the mother liquors contain more than a trace of esters. In

addition to 4-quinazolone and 4-quinazolone-3-acetic acid, a small yield of an unidentified fragment was obtained. This fragment was very water soluble and difficult to purify and was not obtained pure in amounts sufficient for analysis. It is apparently an amino acid and sublimable as the hydrochloride. It is optically active and opposite in sign (though not equal) to the lactone from isofebrifugine.

The absence on oxidation of febrifugine of any of the lactone obtained from isofebrifugine is considered to be a significant result. The failure to obtain more than a trace test for esters from solutions where the lactone would have appeared shows that the oxidation proceeds differently with febrifugine and isofebrifugine. It is not likely that the fragment from febrifugine is merely the open form of the lactone from isofebrifugine. The conditions of isolation (sublimation under acid conditions) would be expected to form the lactone ring (especially as the structures of the alkaloids, deduced later from other evidence, indicate that the product from isofebrifugine is 3-hydroxypiperidine-2-acetic acid lactone, thus a γ-lactone).

The different course of the oxidation in febrifugine and isofebrifugine could have its basis in one or both of at least two factors. If the difference between febrifugine and isofebrifugine is merely that febrifugine is a hydroxy ketone and isofebrifugine is the corresponding hemiketal, the oxidation could well proceed quite differently, for at the least there would be in febrifugine an unprotected hydroxyl

group (later shown to be secondary). A second possibility is that febrifugine and isofebrifugine differ in configuration about one of the centers of asymmetry. If this is the case then perhaps the substituents of the saturated heterocyclic ring are trans in the case of febrifugine and cis in the case of isofebrifugine, making the formation of cyclic structure difficult in the former case. The opposite sign of rotation of the two fragments is probably not significant as concerns possible differences in configuration.*

The isolation of 4-quinazolone-3-acetic acid and the lactone accounts for all the carbon, oxygen and nitrogen atoms in isofebrifugine. The carboxyl group of each fragment presumably represents the same carbon atom in isofebrifugine and suggests that this is a masked carbonyl function.

Experimental

Permanganate Oxidation of the Phthalic Anhydride Adduct of Isofebrifugine (JAB). A solution of 0.62 g. of the phthalic anhydride adduct of isofebrifugine in 5 ml. of water was made basic with 1.2 ml. of 2.5N sodium hydroxide. Saturated aqueous potassium permanganate was added dropwise at room temperature until a permanent (2-3 minutes) pink color was obtained (7.2 ml. required). The excess permanganate was removed with hydroxylamine hydrochloride. Manganese dioxide was removed

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* B. R. Baker and co-workers have recently presented evidence that febrifugine and isofebrifugine have the same configuration about their centers of asymmetry and have proposed that febrifugine is a hydroxy ketone and that isofebrifugine is the corresponding hemi-ketal (22, 23).

by filtration, and the solution neutralized with carbon dioxide, causing a precipitate. The solution was extracted with ether. The ether was evaporated and the residue combined with the above precipitate to give 153 mg. (76%) of 4-quinazolone (identified by melting point and mixed melting point). The aqueous solution was acidified to pH 1-2 with 12N hydrochloric acid and extracted five times with 60 ml. portions of ether. Evaporation of the ether yielded 30 mg. of solid which on crystallization from hot ethyl acetate and hot butanone, combined with sublimation (120-140°/0.2 mm.) gave a few milligrams of a compound melting at 178-183° with decomposition.

Anal.: Calcd. for $C_{16}H_{12}O_5N_2$: C, 61.5; H, 3.9; N, 9.0. Found: C, 61.82; H, 4.12; N, 8.94.

The acidic aqueous solution was evaporated to dryness and the dry solids extracted with hot ethanol. Evaporation of the ethanol yielded 263 mg. of solid. This, on dissolving in about 1 ml. of ethanol and adding 1 ml. of acetone, gave 21.5 mg. of crystals. Sublimation of the crystals (150°/0.6 mm.) and crystallization from ethanol yielded 10.6 mg., melting at 236.5-238° (dec.).

Anal. Calcd. for $C_7H_{12}O_2NCl$: C, 47.33; H, 6.81; N, 7.89; Cl, 19.96. Found: C, 47.97; H, 7.02; N, 7.99; Cl, 19.81.

Later a saturated solution of 4-quinazolone in ethanol added to a saturated alcoholic solution of phthalic acid slowly precipitated a light fluffy solid which melted at 182-183°. The 178-183° material was not available for comparison,

but was presumably (by analysis and melting point) the acid phthalate salt of 4-quinazolone.*

Permanganate Oxidation of Isofebrifugine (JAB). To a solution of 1.00 g. of isofebrifugine (3.32 millimoles) in 30 ml. of water containing 3.3 ml. of 1N hydrochloric acid, was added 6.6 ml. of 5% sodium carbonate. A mixture of 66 ml. of 0.10F potassium permanganate and 3.3 ml. of 1N hydrochloric acid was added dropwise with stirring over a period of 40 minutes. A few drops of 10% sodium hydroxide were added occasionally to keep the solution slightly basic. A little hydroxylamine hydrochloride was added to clarify the solution and it was then filtered through sintered glass and the manganese dioxide washed with water. The combined filtrate and washings were then saturated with carbon dioxide and extracted four times with 250 ml. portions of chloroform to remove 4-quinazolone. The aqueous phase was made acid with 12N hydrochloric acid and evaporated with a stream of air on a steam bath. During this evaporation, the mixture became quite dark. It was evaporated down once with ethanol and the portion soluble in 80% ethanol was transferred to a sublimation pistol and sublimed 8 hours (150-170°/0.1 mm.). A second crop was obtained by subliming an additional 5 hours. The

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* Though 4-quinazolone is quite soluble in dilute acid, its basic strength is about pK_B 11.7 and thus would not be difficult to extract from a pH 2 solution. It is probable that the 4-quinazolone and the phthalic acid were extracted independently into the ether. The solubility of 4-quinazolone in water and ether is comparable (about 0.2-0.5%) so that the original extraction of the neutral solution would not have quantitatively removed the 4-quinazolone.

two sublimates were dissolved in hot 1:1 ethanol-water, evaporated to thick oils and treated with about equal volumes of acetone. The first sublimate produced 162 mg. and the second 12.8 mg. of crystals. The crystals were combined and stirred with 0.5 ml. of water and left overnight in the refrigerator. The water insoluble fraction weighed 45 mg., and after sublimation (180-200°/0.3 mm.) and crystallization from aqueous ethanol, melted at 241.5-243.0° with no depression on mixing with an authentic sample of 4-quinazolone-3-acetic acid (28). The aqueous mother liquor was evaporated to dryness and the residue crystallized from 90% ethanol to give 41.9 mg. of crystals melting with decomposition at 225-231°. This material was identical with the $C_7H_{12}O_2NCl$ compound from the oxidation of the phthalic anhydride adduct of isofebrifugine. The specific rotation (2% in water) was -64.8°.

Oxidation of the $C_7H_{12}O_2NCl$ Compound with Sodium Hypoiodite (JAB). A solution of 10.1 mg. of the sublimable amine hydrochloride in 0.58 ml. of 1N sodium hydroxide was allowed to stand overnight. A solution 0.5F in iodine and 1.7F in potassium iodide was then added till a permanent dark iodine color was obtained (0.60 ml.). The mixture was warmed at 60° for 5 minutes and a few drops of sodium hydroxide were added to decolorize the solution. A yellow precipitate formed which was collected after one hour at 0°, yielding 1 mg. of solid. The aqueous solution was extracted with ether, yielding on evaporation of the ether, 6.8 mg. of

yellow solid melting at 118-119.5°. The iodoform isolated represents a 35% yield on a mole for mole basis. Treatment of the ice-cold solution with sodium nitrite and extraction with chloroform yielded on evaporation of the chloroform about 1 mg. of oil which did not give a Lieberman N-nitroso test. Subsequent treatment of the solution with sodium hydroxide and benzenesulfonyl chloride followed by extraction with chloroform yielded about 3 mg. of an oil on evaporation of the chloroform.

Periodate Oxidation of the C_7 Compound (JAB). A solution of 5.7 mg. of the C_7 compound in 4 ml. of water containing 1 ml. of 2.5N sodium hydroxide was heated for one and one-half hours on a steam bath. After saturation with carbon dioxide and addition of 0.5 g. of sodium bicarbonate, the solution was treated with standard periodate solution at room temperature for 3 hours. Titration (27) indicated an uptake of 0.127 equivalents per mole. A similar sample (5.8 mg.) hydrolyzed for 50 hours at room temperature and treated in the same manner for 19 hours with periodate showed an uptake of 0.346 equivalents per mole. L-hydroxyproline treated in sodium bicarbonate solution with periodate consumed 0.376 equivalents per mole in 30 minutes at room temperature, and 0.83 equivalents per mole in 1 hour.

Saponification Equivalent of the C_7 Compound (JAB). A solution of 5.45 mg. of the C_7 compound in 1 ml. of standard (0.1N) sodium hydroxide was heated for 2 hours at 80-90°. Formalin was added and the solution was back titrated with

standard (0.02N) hydrochloric acid to give a saponification equivalent of 95 (theory for $C_7H_{12}O_2NCl$, 89). As a check, 2.17 and 1.62 mg. samples of glycine ethylester hydrochloride were treated in the above manner and gave values of 70.1 and 69.5 (theory, 69.8), while a 3.11 mg. sample without the addition of formalin gave a value of 113.

Permanganate Oxidation of Febrifugine. To 0.90 g. of febrifugine dihydrochloride in 20 ml. of water was added, at 0°, 9.6 ml. of 5% sodium carbonate. At 0°, 48 ml. of 0.1F potassium permanganate containing 2.2 ml. of 1N hydrochloric acid was added dropwise over a period of 45 minutes, and an additional 1.5 ml. of 5% sodium carbonate was added near the end as the solution approached neutrality. After the addition of 4 ml. of 1N sodium bicarbonate, the solution was filtered and the filtrate extracted five times with 100 ml. portions of chloroform. The aqueous solution was acidified with 3 ml. of 6N hydrochloric acid and evaporated with a stream of air on a steam bath. The residue was evaporated twice with 2 ml. portions of ethanol and the portion of the residue soluble in 1:1 ethanol-water was sublimed for 7½ hours (150°/0.02 mm.). The sublimate was dissolved in aqueous ethanol, evaporated to an oil and again evaporated with ethanol. Two volumes (8 drops) of acetone were added and the mixture chilled to give 48.3 mg. of crystals. Treatment with 3 drops of water left 24 mg. of material undissolved (4-quinazolone-3-acetic acid). The mother liquor was evaporated as in the oxidation of isofebrifugine and the residue crystallized from 90%

ethanol, yielding 1.9 mg. of solid which would not melt at 310°. This material gave an immediate white precipitate with 1% silver nitrate in 3F nitric acid and a negative test for the ester grouping (25b). The mother liquor gave only a very faint test for the ester grouping and a very strong test for halide ion. The mother liquor was evaporated to an oil (about 22 mg.). It was very insoluble in organic solvents, readily soluble in ethanol, methanol, or acetonitrile containing small amounts of water, but attempts at crystallization from these aqueous solvents failed to yield crystalline material.

The oil was dissolved in 5 drops of water and extracted with methylene chloride to remove any neutral compounds. The aqueous phase was stirred with 30 mg. of silver oxide until the supernatant liquid gave a negative test for chloride ion. The supernatant was removed and saturated with hydrogen sulfide, yielding 3.7 mg. of silver sulfide. The aqueous phase, after removal of the silver sulfide was evaporated to 11.8 mg. of oil which on treatment with 2 drops of methanol and three drops of ethanol yielded 1.5 mg. of white crystals softening at 175° and melting at 185-192°. The mother liquor, evaporated and treated with 3 drops of ethanol and 1 drop of methanol, produced 4.5 mg. of a gummy solid. From a similar fraction from the oxidation of 0.62 g. of febrifugine dihydrochloride was obtained 6.7 mg. of solid melting at about 190°. Recrystallization of this from 20 drops of 1:1 ethanol-methanol containing 20 microliters of water yielded

1.7 mg. of crystals melting (with decomposition) sharply at 200-203°.

The ultra-violet absorption spectrum was taken of 1.04 mg. of this material in 4 ml. of water containing 1 drop of 1N hydrochloric acid. It showed a non-characteristic absorption very similar to the C_7 compound from isofebrifugine with a very low maximum at 264 and a minimum at 246 millimicrons. The optical density of the maximum was only 0.01. The spectra solution was evaporated with nitrogen, dried and dissolved in 0.44 ml. of water, and the rotation taken in a 1 dm. micro polarimeter tube. The conditions of reading were poor. The author obtained an observed rotation of +0.036° and a fellow student (George K. Helmkamp) obtained an observed rotation of +0.104°. There is no doubt that this compound has a positive rotation.

The compound is quite insoluble in ethanol, somewhat more soluble in methanol, and very soluble in water. It does not precipitate a picrate from ethanol.

Section 5

Oxidation with Periodic Acid

Dr. Brockman, in his thesis (1), describes a $C_{11}H_{12}O_2N_2$ compound produced by the action of periodic acid on the alkaloids in alkaline media. In most experiments about 4 equivalents of periodate were used, but the alkaloids do not consume a definite amount of the periodate. Approximately two equivalents are consumed very rapidly at room temperature in sodium bicarbonate solution (less than ten minutes). After an initial rapid reaction period the rate of consumption falls off roughly exponentially with time. After 53½ hours at room temperature 7.2 equivalents of periodate had been consumed by febrifugine (Table I).

The oxidation of either febrifugine or isofebrifugine leads to the same product (3). The yield of the periodate product varies with the number of equivalents of oxidizing agent, but the product is the same. In addition to the periodate product, in each case it was possible to isolate approximately 10-15% of 4-quinazolone. In a few instances, where acidic products were looked for, approximately 6% of 4-quinazolone-3-acetic acid was found. Though a characteristic amine odor is noticed in the alkaline solution after oxidation, it was shown by aspirating a stream of air through the reaction mixture into a solution of standard acid during the course of the oxidation, that no significant amount of volatile base was produced.

On pyrolysis, the periodate product gave a positive pine splinter test for pyrroles. Since this test is given by the parent alkaloids but not by 4-quinazolone, this is indicative evidence that the compound still contains the third nitrogen. Re-examination of the analytical data (3) showed that the previously assumed formula $C_{11}H_{12}O_2N_2$, fits not much better than $C_{16}H_{17}O_3N_3$, in other words, a loss of 2H from the parent alkaloid. In addition, the data from preparative experiments showed that the highest crude yield of periodate product (83%) was obtained in an experiment where 2.1 equivalents (1.05 moles/mole), instead of 4 equivalents, of oxidizing agent were used.

The ultraviolet absorption spectrum of the oxidation product in ethanol is almost identical with that of the parent compounds. The obvious presumption was therefore that the periodate product is also a 3-substituted-4-quinazolone. Since the molecular extinctions of the various 3-substituted-4-quinazolones are moderately constant (1), the molecular weight of the periodate product was calculated from the 1% extinction of two maxima in ethanol. The estimated molecular weight, 294, is in excellent agreement with the figure of 299 required for the C_{16} formulation.

As do febrifugine and isofebrifugine, the periodate product gives a negative test for reactive methylene and amino groups.

There is no observable rotation of the periodate product

in either ethanol or pyridine. The lack of rotation in two such different solvents would indicate that the product is optically inactive. Since the two parent alkaloids do not have equal and opposite rotation, it is presumed that they have at least two centers of asymmetry. The loss of rotation on removal of two hydrogens indicates that these centers are in some manner closely linked.

The periodate product is attacked by periodate in slightly alkaline media. This result was expected from the continued uptake of periodate by the alkaloids past the two equivalents (one mole) required for the formation of the product.

The periodate product is not attacked by selenium dioxide in boiling methanol.

One of the most characteristic reactions of the periodate product is an irreversible yellowing in dilute hydrochloric acid followed by an amorphous yellow precipitate on making basic. This reaction was not extensively investigated. The yellow material can, with difficulty, be obtained crystalline, but no analysis has been obtained. Even the later proposed structure of the periodate oxidation product (4), sheds little light on the identity of this material. It is possibly a polymer of some sort.

No crystalline benzenesulfonyl derivative of the oxidation product could be obtained on treatment with benzenesulfonyl chloride in pyridine. The solution colored badly and only dark amorphous material appeared on acid-

ification of the pyridine solution.

Since periodate oxidation would be expected to produce carbonyl groups, an attempt was made to prepare the oxime of the periodate product. Analysis of the oxime indicated $C_{12}H_{12}O_3N_4$, suggesting that a cleavage occurred in the preparation. The ultraviolet absorption spectrum of the oxime was typical of a 3-substituted-4-quinazolone and the extinction indicated a molecular weight of 263, which is consistent with the 260 required by the analytical data.

The oxime is readily oxidized by periodate in slightly alkaline or acid media. About 3.4 equivalents of periodate are consumed at room temperature over a considerable range of reaction times. Volatile bases were looked for in one case, and about 2.2% (calculated as ammonia) was found. The presence of ammonia in the volatile bases was confirmed by the preparation of the characteristic octahedra of the ammonium salt of chloroplatinic acid.* No crystalline material could be obtained from the solution after periodate oxidation of the oxime.

A test for the α-dioxime grouping with nickel and ammonium hydroxide (25c) was negative.

The oxime gave a positive test for the presence of a primary or secondary amine (25a), but in an unusual manner.

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* In a similar experiment with diethanolamine, 20% of the theoretical ammonia was liberated. Under comparable conditions, dimethylglyoxime was shown to consume 1.47 and acetone oxime 0.17 equivalents of periodate.

The test, requiring the formation of a dithiocarbamate by reaction with carbon disulfide was positive only in the presence of triethylamine, the addition of which is usually necessary only when the amine is in salt form. The triethylamine did not seem to be contaminated as a negative blank was obtained.

The oxime gave a positive test for the presence of reactive methylene or amino groups.

The oxime does not give a yellow color in acid as does the periodate product. Vigorous acid hydrolysis yielded hydroxylamine and a solid, $C_{12}H_9O_2N_3$, which gave a hydrochloride, $C_{12}H_{10}O_2N_3Cl$. The absorption spectrum of the hydrolysis product is that of a 3-substituted-4-quinazolone with an estimated molecular weight of 221, in excellent agreement with the 227 required by the analytical data.

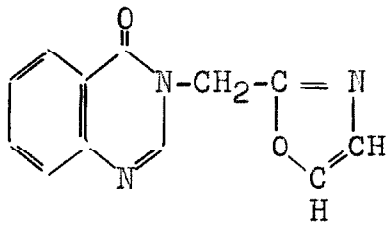
The hydrolysis product gave a negative test for reactive methylene or amino groups, and a negative test for primary or secondary amino groups with or without added triethylamine. The product also gave a negative test with Schiff's reagent.

The hydrolysis product, in contrast to the oxime, consumed only 0.15 equivalents of periodate.

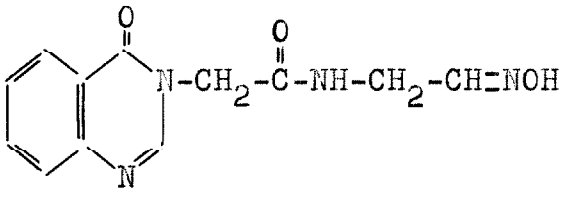
Alkaline hydrolysis of the material yielded a volatile material giving an orange color with Nessler's reagent. This volatile material, presumably a base, was not identified other than to show that it was not ammonia. The residue from the alkaline hydrolysis after neutralization gave a purple color with ninhydrin indicating the presence of an

α-amino acid. Paper strip chromatography indicated this amino acid to be glycine.

The identification of glycine as a hydrolysis product of the hydrolyzed oxime, coupled with the empirical formula and the knowledge that the group next to the 4-quinazolone moiety is a methylene group, led to the proposal of an oxazole, I, as the structure of the product. This then leads to the structure II for the oxime.

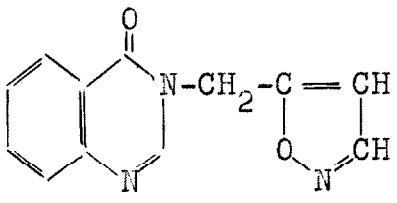


I

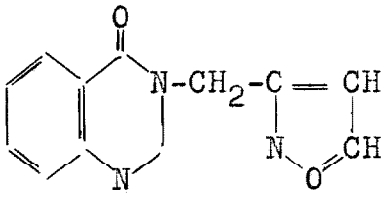


II

Numerous attempts were made to synthesize this oxazole (I) without success until Dr. Moffat's suggestion that an isoxazole (III or IV) would yield glycine on alkaline hydrolysis.



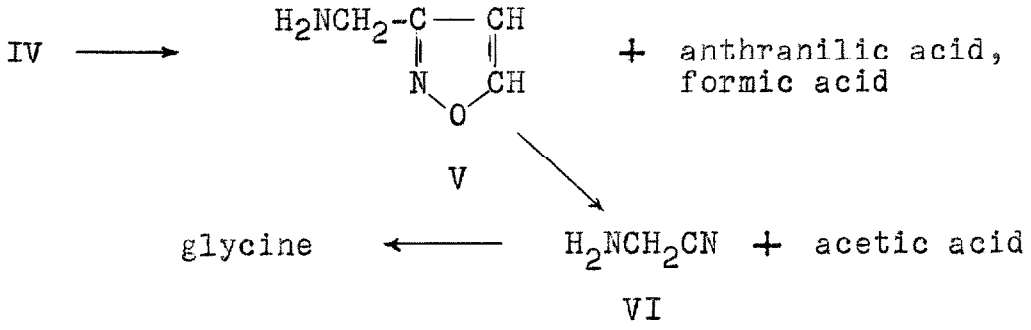
III



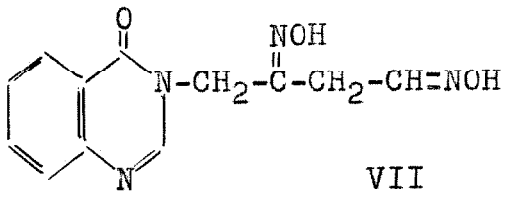
IV

It has been shown that 3-substituted isoxazoles having a free 5-position decompose in warm alcoholic potassium hydroxide to

give nitriles and carboxylic acids, yielding in the case of 3-methylisoxazole, acetonitrile and acetic acid (29). 5-Substituted isoxazoles give β-ketonitriles, while the 3,5-disubstituted isoxazoles are very resistant to alkali (29). Thus, of the proposed isoxazoles, IV might be expected to yield glycine on alkaline hydrolysis, schematically, by hydrolysis to anthranilic acid, formic acid and 3-amino-methylisoxazole (V), decomposition of the isoxazole to acetic acid and aminoacetonitrile (VI), and hydrolysis of the nitrile to glycine.



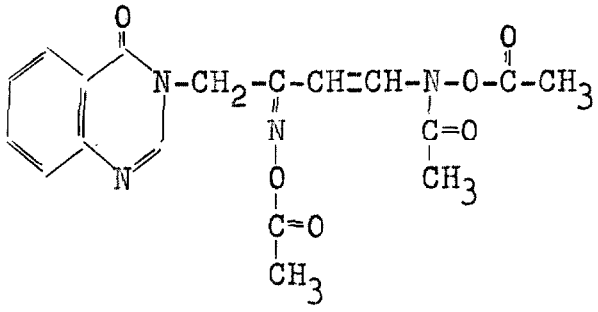
Isoxazoles are readily formed from the monoximes of β-dicarbonyl compounds by elimination of water, and are quite frequently encountered in the laboratory when working with nitroso or isonitroso compounds (30). An isoxazole could thus have arisen from a structure such as VII by hydrolysis to a monoxime, or perhaps directly, by loss of hydroxylamine.



VII

Preliminary hydrolysis of the terminal group would be expected to yield the isoxazole, IV, and prior loss of the ketoxime goup would yield the isomer, III.

For the decision between the structures II and VII for the oxime of the periodate product, a relatively simple test was possible. The oxime was treated with acetic anhydride. A triacetyl derivative was isolated in good yield. Only structure VII is capable of forming a triacetyl derivative, the structure of which would then be VIII.



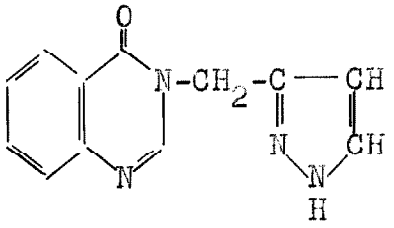
VIII

Because of the possible ambiguity of the structure of the proposed isoxazole and the difficulty of unequivocal synthesis, it was decided to look for an analogous derivative which would be more easily identified.

Since β-dicarbonyl compounds are known to form pyrazoles on vigorous treatment with semicarbazide (31, 32, 33), it was decided to try the decomposition of the periodate product with semicarbazide.

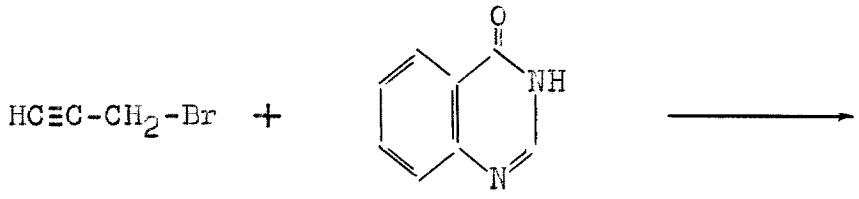
When the periodate oxidation product was treated with semicarbazide, a $C_{12}H_{10}ON_4$ compound was isolated. A rough ultraviolet absorption spectrum showed the compound to contain

the quinazolone ring system. If the reaction with semicarbazide is analogous to the formation of the oxime, this compound should be α-(3-pyrazolyl)-3-methyl-4-quinazolone (IX).

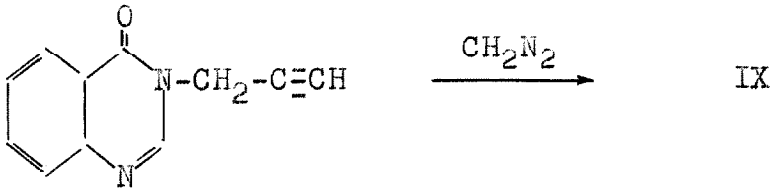


IX

It was decided to attack the proof of structure of the product of reaction with semicarbazide synthetically. The compound was prepared by condensing propargyl bromide (X) with 4-quinazolone (XI) in ethanolic potassium hydroxide to yield 3-propargyl-4-quinazolone (XII). Reaction with diazomethane then yielded the desired pyrazole, IX.



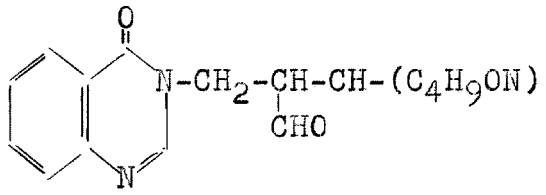
X XI



XII

The synthetic pyrazole proved to be identical with the compound isolated from the reaction of the periodate product with semicarbazide.

The mode of addition of diazomethane to monosubstituted alkynes in general yields the corresponding 3-substituted pyrazole instead of a 4-substituted pyrazole. In addition, that the product was a 4-substituted pyrazole was made unlikely by the fact that this would require the alkaloid to have the grouping XIII. This grouping is unlikely to lead to

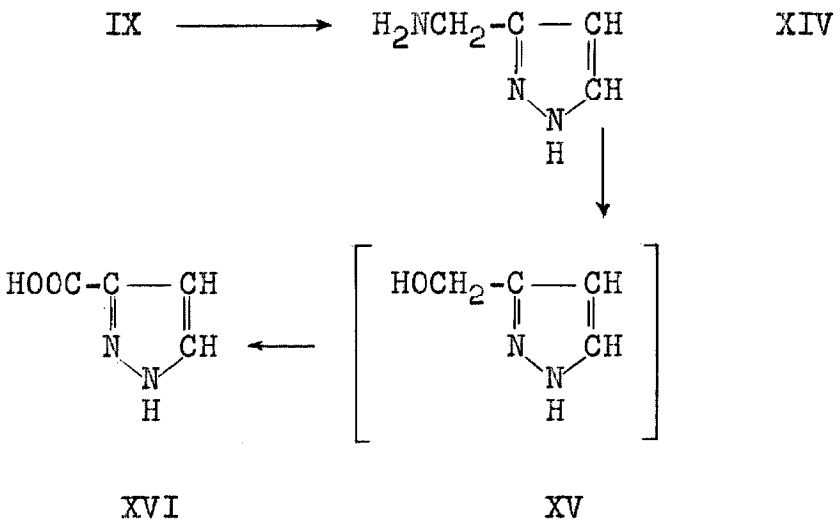


XIII

4-quinazolone-3-acetic acid or a $C_7H_{11}O_2N$ lactone on permanganate oxidation. Nevertheless, because of possible ambiguity, it was decided to degrade the pyrazole to the corresponding pyrazolecarboxylic acid, since both the 3- and 4-pyrazolecarboxylic acids are known (34, 35).

Several attempts at direct oxidation of the pyrazole to the pyrazolecarboxylic acid, including the method of Knorr (34), failed to yield any of the desired product. It is possible that the desired material was actually present and was not detected as the isolation procedure failed to take into account the weakly basic nature of the pyrazolecarboxylic acid.

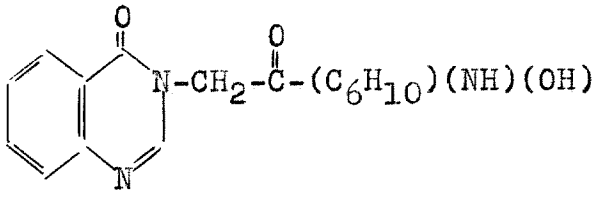
The actual isolation of the pyrazolecarboxylic acid was accomplished by hydrolysis of the quinazolone-pyrazole to 3-aminomethylpyrazole (XIV), deamination with nitrous acid and oxidation of the presumed (not isolated) hydroxymethyl-pyrazole, XV, to the acid, XVI. The resulting product was identified as pyrazole-3-carboxylic acid (XVI).



The structure of the pyrazole was thus confirmed as IX and that of the product from hydroxylamine as an isoxazole, III or IV. There then remains a four carbon fragment to be identified. It is possible, however, to assign a structure at this point.

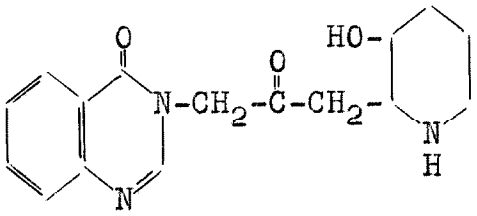
Earlier, all the probable structures for febrifugine were written out simply by devising all possible isomers with the assumption that the molecule was represented by XVII.

The left side of the molecule is derived from the isolation of 4-quinazolone-3-acetic acid on oxidation of



XVII

febrifugine, and a C_7 lactone on oxidation of isofebrifugine. The non-acidic nature of the dibenzenesulfonyl derivative and the formation of a colorless N-nitroso derivative (3) was taken as evidence that the nitrogen function was secondary. The hydroxyl group could be either primary or secondary. A tertiary hydroxyl group was made unlikely by the stability of the alkaloid to strong acid. The lack of a C-methyl group eliminated all those structures having such a feature. The requirement that the compound be oxidizable by periodate (contain oxygen or nitrogen functions on adjacent carbon atoms) eliminated others. Of the remaining structures, all but one were eliminated by the elucidation of the cleavage of the periodate oxidation product. The remaining structure, 3-[β-keto-γ-(3-hydroxy-2-piperidyl)propyl] -4-quinazolone XVIII must then be the structure of the alkaloid.

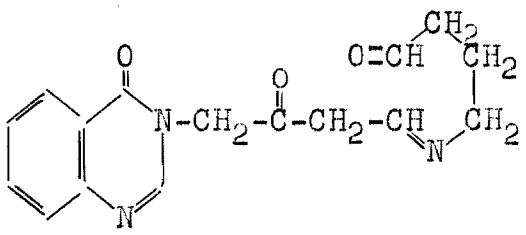


XVIII

It must be emphasized that at this point very little may

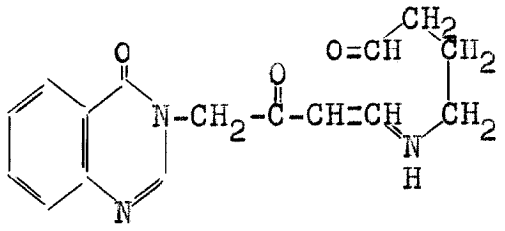
be said about the stereochemistry of the structure or of the difference between febrifugine and isofebrifugine. Since the oxidation of either alkaloid with periodate yields the same (optically inactive) product by loss of two hydrogens, the supposition is that the two isomers are closely related, possibly differing only in configuration about one of the asymmetric carbon atoms. The ease of interconversion of the alkaloids (warming in solution) also suggests that the difference is slight. It will be assumed for the present that the chemical reactions of both febrifugine and isofebrifugine are derived from the basic structure XVIII, and that the difference in their reactions arises from a subtle rearrangement of this structure.

The structure of the periodate oxidation product follows readily from the proposed structure of the alkaloids, XVIII, and from the proposed structure of the cleavage product with hydroxylamine, VII, or with semicarbazide, IX. By analogy with glycols, the oxidation of febrifugine, XVIII, with one equivalent of periodate might be expected to open the piperidine ring to give an aldehyde and an imino group as in XIX.



XIX

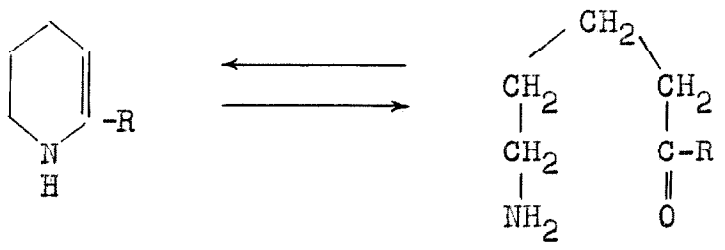
Evidence has been presented that such compounds exist as the corresponding α,β-unsaturated ketones (36). The proposed structure of the periodate oxidation product would be then more correctly represented as XX.



XX

It is noted that XX is structurally a vinylog of an amide. The chemical reactions of analogous compounds have been shown to be like those of amides rather than ketones (36). The cleavage of the periodate product, XX, to a derivative of a β-keto aldehyde, VII or IX, is thus not surprising. In additional support of this, it might be mentioned that Δ^1-tetrahydropyridines do not seem to exist if it is possible for the double bond to be in the Δ^2-position (37). Further, the 2-alkyl-Δ^2-tetrahydropyridines (XXI) apparently exist in aqueous solution in equilibrium with the corresponding alkyl δ-aminobutyl ketones (XXII) (37). On isolation, they readily lose water to yield the original tetrahydropyridine.

It is evident from the proposed structure of the periodate oxidation product, XX, and from the discussion of the cleavage, that the previously mentioned four carbon fragment is probably present as a derivative of γ-aminobutyraldehyde. Thus, in the case of the cleavage with semicarbazide, γ-aminobutyralde-



XXI XXII

hyde semicarbazone should be in the mother liquors after removal of the pyrazole, IX.

It was noted that when the odorless residue from one of the preparations of the pyrazole, IX, by cleavage of the periodate product, was heated with acetone, a strong amine odor was observed. On taking to dryness, this odor disappeared but could be regenerated by again boiling with acetone. This was taken to mean that possibly the acetone was releasing γ-aminobutyraldehyde by exchange of the semicarbazone moiety and that the aminoaldehyde was then cyclizing to give a pyrroline. When the residue was decomposed with cyclohexanone and the effluent vapors collected in a solution of ethanolic picrolonic acid, crystals were obtained. These crystals melted with decomposition at the reported temperature for the picrolonate of Δ^3-pyrroline (38). A sample of this compound was prepared and compared with the isolated material. The melting points were the same but the mixed melting point behavior was inconclusive. The rather high decomposition point made accurate measurement difficult, and the observed

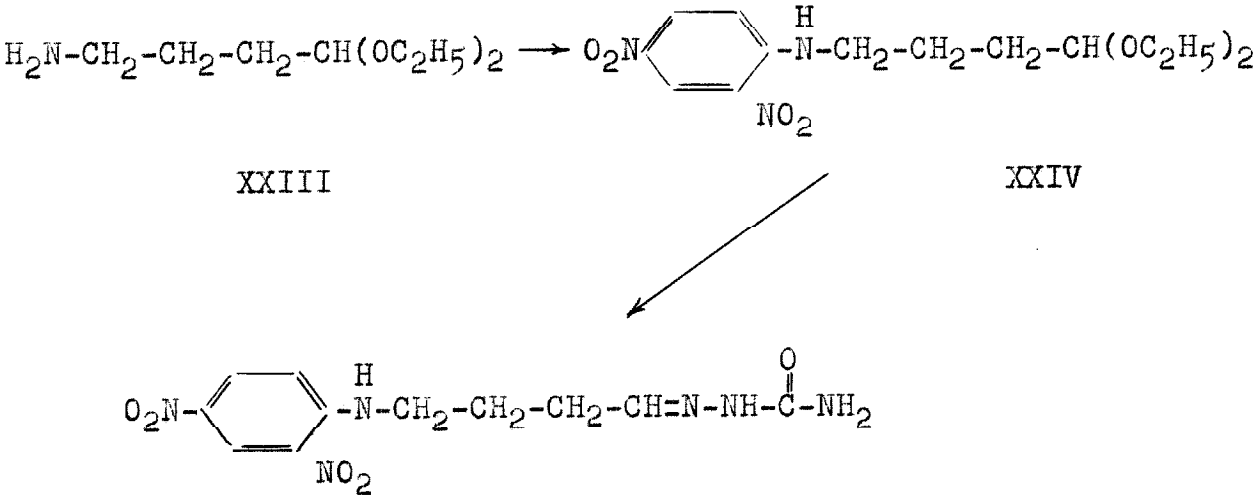
depression of 3 degrees was not necessarily significant. In addition, the mixed melting point was quite sharp. The analysis on the isolated picrolonate was almost meaningless. It is suspected that the material was a mixture, containing in addition to any pyrroline picrolonate, possibly ammonium picrolonate and the monopicrolonate of hydrazine. The latter is especially probable as the reported melting point and solubility characteristics (Beilstein) are those of the isolated material.

Later consideration showed that ring closure of γ-aminobutyraldehyde would probably yield the unknown Δ^2-pyrroline by analogy with γ-and δ-aminoketones (37). Although many 2-alkyl derivatives are known, neither of the parent compounds, Δ^2-pyrroline or Δ^2-tetrahydropyridine, have been described. It is possible that these compounds polymerize so readily as to be incapable of existence.

It was thus decided to attempt the isolation of the aminoaldehydesemicarbazone as such or as a derivative. The use of 2,4-dinitrofluorobenzene in the preparation of the colored N-(2,4-dinitrophenyl)-α-aminoacids (39) suggested its possible use here. A colored derivative would make practical a chromatographic isolation of the desired compound from the rather complex mixture. A test tube experiment showed that the reagent gives an almost instantaneous yellow-orange color with simple amines.

γ-Aminobutyraldehyde diethylacetal (XXIII) (40, 41) was prepared and reacted with dinitrofluorobenzene in an alkaline

medium to give the N-(2,4-dinitrophenyl) derivative (XXIV). This in turn was decomposed without isolation, in acid, in the presence of semicarbazide to give N-(2,4-dinitrophenyl)-γ-aminobutyraldehyde semicarbazone (XXV).



XXIII XXIV XXV

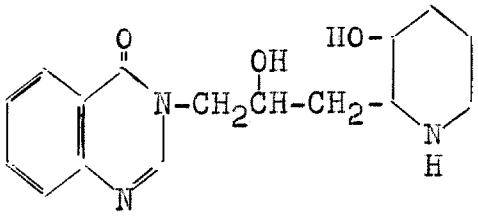
An investigation of the chromatographic behavior of this derivative led to the selection of alumina as adsorbent and methylene chloride containing ½% acetic acid and 20% acetone as a developer as a trial system for isolation purposes. The aqueous mother liquor from one of the decompositions of the periodate product with semicarbazide was evaporated to a thick paste and treated with dinitrofluorobenzene in aqueous-alcoholic sodium bicarbonate. The material which was soluble in methylene chloride was then chromatographed on alumina using ½% acetic acid and 20% acetone in methylene chloride as developer. A yellow zone appeared at the expected location and was easily cut away from several other zones. The material

thus isolated proved to be identical with the synthesized derivative of γ-aminobutyraldehyde by the criteria of melting point, mixed melting point, and chromatographic behavior on two different adsorbent systems.

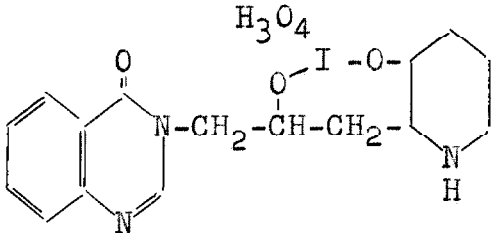
The structure of the periodate oxidation product is thus confirmed as XX. There is little doubt that febrifugine and isofebrifugine have structures based on XVIII.

It must be pointed out that there is an observation which is not easily correlated with the proposed scheme for the periodate oxidation of the alkaloids. The dihydro alkaloids are only very slowly attacked by periodate. The only apparent difference between febrifugine and dihydrofebrifugine is the presence in dihydrofebrifugine of a hydroxyl group in place of the carbonyl of febrifugine. The scheme of periodate oxidation, as developed, does not involve the carbonyl group of febrifugine, yet this function seems necessary for rapid oxidation. It is possible that it is not the presence of the ketone but the absence of the corresponding hydroxyl group that is required. The usual mechanism given for the oxidation of vicinal hydroxyl groups by periodate involves the formation of a cyclic diester with paraperiodic acid (27). A possible situation in the case of dihydrofebrifugine (XXVI) is that a cyclic ester (XXVII) is formed and is stable enough to effectively restrict the bridging of the paraperiodic acid between the required hydroxyl and somewhat hindered amine.

After hydrolysis with alkali, the dihydro alkaloids are

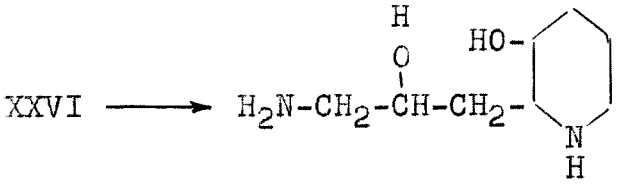


XXVI

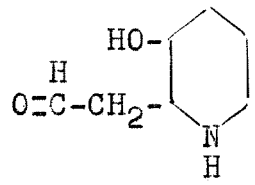


XXVII

oxidized quite rapidly, consuming four equivalents of periodate in less than an hour. This is expected, for hydrolysis of dihydrofebrifugine should yield XXVIII, which on cleavage with



XXVI



XXVIII XXIX

periodate would give XXIX. This compound, XXIX, has the same grouping as the alkaloids and would then oxidize in a manner similar to febrifugine. The cyclic ester proposed in the case

of dihydrofebrifugine, could of course operate on XXVIII to slow its reaction with periodate. It is felt, however, that the effect would not be as serious in this case. In dihydrofebrifugine (or febrifugine) the first attack by periodate involves a nitrogen atom which by its presence in a ring is prevented from swinging into the most favorable position for the attack by periodate. In XXVIII, the first grouping to be split has no such restrictions.

Experimental

Quantitative Oxidations with Periodate (JAB). For quantitative estimation of the amount of periodate consumed, a small sample (usually about 5 mg.) was treated at the desired pH with an excess of standard 0.01N sodium paraperiodate. After standing for the desired length of time at room temperature, the excess of periodate was estimated by the addition of sodium arsenite and potassium iodide and titration with standard iodine solution (27). In those cases where the oxidation was not run in sodium bicarbonate solution, the pH was adjusted to about 8 before analysis. The results are tabulated in Table I.

Preparative Oxidations of Febrifugine with Periodate and Search for Side Products (JAB). The preparation of the periodate oxidation product of the alkaloids has already been reported (3). Since then, the preparation has been tried under a variety of conditions, varying the length of the reaction time, the amount of periodate added, and the pH of

Table I

Oxidations with Excess Periodate
In Sodium Bicarbonate Solution

Compound	Time (Hours)	Eq. IO_4^- Consumed per Mole
Febrifugine	1/6	2.27, 2.42
Febrifugine	5/12	3.05
Febrifugine	1	3.65
Febrifugine	2	3.91
Febrifugine	4	4.27
Febrifugine	8	4.82
Febrifugine	24	6.02
Febrifugine	53½	7.17
Isofebrifugine	1	2.78
Periodate Oxid. Prod.	3/4	0.636
Periodate Oxid. Prod.	2 3/4	1.28
Periodate Oxid. Prod.	24	5.51
Oxime of Periodate Prod.	1¼	3.42
Oxime of Periodate Prod.	2½	3.47
Oxime of Periodate Prod.[a]	2½	1.43
Acetone Oxime	1 3/4	0.166
Dimethylglyoxime	1	1.47
Isoxazole (IV)	1½	0.15
Dihydrofebrifugine	17	0.74
Alkali Hydrolyzed Dihydrofebrifugine	1	4.11

(a) In 0.1N hydrochloric acid.

the solution. The results were quite irregular and no correlation is possible. Consistent yields of 30-37% of pure product were obtained by running the oxidation for from 1 to 1 3/4 hours in either sodium bicarbonate or sodium carbonate solution with 3 to 5 equivalents of periodate per mole of alkaloid. The highest crude yield (83%) was obtained on oxidation with 2.1 equivalents of periodate in sodium bicarbonate solution for 1½ hours. Recrystallization gave only 17% of pure product. It is not known whether some error was made in the purification.

After one typical oxidation of febrifugine (155 mg.), the ethanolic mother liquor from the crystallization of the crude product was evaporated to dryness. The residue on crystallization from ethanol followed by recrystallization from acetonitrile yielded 12.5 mg. of needles melting with sublimation at 216-217° and showing no depression on mixing with an authentic sample of 4-quinazolone.

In another oxidation of febrifugine (800 mg.), the slightly alkaline aqueous phase (after removal of the oxidation product) was acidified and evaporated to dryness. The residue was treated with a large excess of diazomethane in methanol. It was then evaporated to dryness again and the residue extracted with chloroform until the extracts gave an essentially negative test for the presence of esters (25b). Evaporation of the chloroform gave an oil which on standing with ethyl acetate yielded 5 mg. of crystals melting at 149-152°. This material was not compared with a sample of

methyl 4-quinazolone-3-acetate (m.p. 151.5-152° (6)) but was very probably this compound.

In an oxidation of 200 mg. (about 0.5 millimole) of febrifugine dihydrochloride in sodium bicarbonate solution with 4.8 equivalents of periodate, a stream of air was aspirated through the reaction mixture for 1 3/4 hours into a solution of standard 0.1 N hydrochloric acid. Back titration with standard 0.1N sodium hydroxide using a bromphenol blue endpoint indicated about 0.007 millimoles of volatile bases had been liberated.

Qualitative Tests on the Periodate Oxidation Product (JAB). A small sample of the periodate product was heated with a free flame in the bottom of a 10 x 70 test tube. A pine splinter moistened with hydrochloric acid, held in the vapors, turned red. This test was positive when applied to isofebrifugine, and negative with 4-quinazolone.

A test for reactive methylene and amino groups using sodium 1,2-naphthoquinone-4-sulfonate (25d), was negative for the periodate oxidation product. Febrifugine and isofebrifugine also gave a negative test.

A small sample treated with selenium dioxide in boiling methanol gave no precipitate of selenium.

Ultraviolet Absorption Spectrum of the Periodate Product (JAB). The ultraviolet absorption spectrum of the periodate oxidation product in ethanol (0.00561 mg./ml.) was that of a typical 3-substituted-4-quinazolone (3). The molecular weight was calculated from the 1% extinction of the major peaks using

the average molal extinctions of the alkaloids (1). The estimated value was 294. $C_{16}H_{17}O_3N_3$ requires 299.

Optical Rotation of the Periodate Product (JAB). Rotations were taken in a 1 dm. semimicro polarimeter tube holding approximately 1.5 ml. of solution. The polarimeter used was manufactured by Winkel-Zeiss and was equipped with a sodium lamp. The accuracy of the observed rotations is approximately 0.01°.

A solution of 10.5 mg. of the periodate product in 2.0 ml. of ethanol had an observed rotation of -0.005°, and 20.3 mg. in 2.0 ml. of pyridine had an observed rotation of 0.00°.

Oxime of the Periodate Oxidation Product (JAB). A mixture of 53.1 mg. of the periodate product, 93.3 mg. of hydroxylamine hydrochloride, 20 drops of ethanol and 20 drops of pyridine was warmed until complete solution occurred and allowed to stand overnight at room temperature. The mixture was then refluxed for 8 hours and evaporated to a thick oil with a stream of air. The solution was made strongly alkaline with 2.5N sodium hydroxide and, after scratching, was allowed to crystallize at 0°. Recrystallization of the product from absolute ethanol gave 32.3 mg. of colorless needles melting at 174.5-175.5° with evolution of gas. The ultraviolet absorption spectrum in ethanol was that of a typical 3-substituted-4-quinazolone.

Anal. Calcd. for $C_{12}H_{12}O_3N_4$; C, 55.38; H, 4.65; N, 21.52; Mol. Wt., 260. Found: C, 54.87, 55.89, 55.07; H, 4.64, 4.67, 5.10; N, 20.56, 20.64, 21.34, 21.06; Mol. Wt. (UV spectrum),

263.

Quantitative studies on the periodate oxidation of the oxime are recorded in Table I. As in the oxidation of febrifugine, volatile bases were looked for, and 2.2% (calculated as ammonia) were found. The presence of ammonia in the volatile bases was qualitatively demonstrated by the preparation of the characteristic octahedra of the ammonium salt of chloroplatinic acid (42).

Treatment of a small sample of the oxime with nickel acetate solution and ammonium hydroxide gave no color (25c). The dithiocarbamate test for primary or secondary amines (25a) was negative. Addition of triethylamine caused the test to be positive, while a blank with triethylamine was negative.

A test for reactive methylene or amino groups using sodium 1,2-naphthoquinone-4-sulfonate (25d) was positive.

Acid Hydrolysis of the Oxime of the Periodate Product (JAB). A solution of 50 mg. of the oxime in 1 ml. of 12N hydrochloric acid was heated to 100° for 3 hours. A qualitative test for hydroxylamine (25e), was positive. After evaporation to dryness, the residue was dissolved in water and made alkaline with sodium hydroxide, causing the precipitation of 41 mg. of solid. The solid, on crystallization from 1:2 methanol-water, weighed 33 mg. and melted at 102-104°. In contrast to the periodate oxidation product, no yellowing was observed during the acid treatment. The ultraviolet absorption spectrum in ethanol was that of a typical 3-substituted-4-quinazolone.

Anal. Calcd. for $C_{12}H_9O_2N_3$: C, 63.5; H, 3.97; N, 18.52; Mol. Wt., 227. Found: C, 62.54; H, 4.06; N, 18.17; Mol. Wt. (UV spectrum), 221.

A hydrochloride was obtained by dissolving the free base in the minimum of hot ethanol and adding the required amount of 12N hydrochloric acid. The hydrochloride melted at 188-190° (dec.) after crystallization from 90% ethanol.

Anal. Calcd. for $C_{12}H_{10}O_2N_3Cl$: C, 54.66; H, 3.82; N, 15.94; Cl, 13.45. Found: C, 54.72; H, 3.86; N, 15.99; Cl, 13.45.

Preliminary tests on the free base indicated the absence of reactive methylene or amino groups (25d), and the absence of a primary or secondary amine (25a). The compound gave no color with Schiff's reagent.

Alkaline Hydrolysis of the $C_{12}H_9O_2N_3$ Compound (JAB). About 0.5 mg. of the product from acid hydrolysis of the oxime was heated to 100° with a 10 ul. column of 10% sodium hydroxide in the bottom of a cane tube (prepared from a capillary tube of ca. 1 mm. diameter). In about 10-15 minutes, a red-brown precipitate was observed in some Nessler's reagent in the upper arm of the cane tube. On repeating this, replacing the Nessler's reagent with 1N hydrochloric acid and testing the solution qualitatively for ammonia (42), none was found. When the alkaline residue from the hydrolysis was neutralized with carbon dioxide and heated to 100° with 1% aqueous ninhydrin, a purple color developed.

When a similar hydrolyzate was acidified and spotted on

strips of Whatman #1 filter paper and developed (descending) with 77% ethanol (43), ninhydrin showed a spot in the same location as glycine chromatographed in the presence of a comparable amount of sodium chloride and hydrochloric acid. The spot did not separate from added glycine, but separated into two spots when alanine was added. Previous experiments with a mixture of anthranilic acid and glycine showed that these two have widely different Rf values (0.40 for glycine, and 0.81 for anthranilic acid). In the case of the hydrolyzed material, there appeared 3 fluorescent spots leading the location of the ninhydrin spot.

Reaction of the Oxime of the Periodate Product with Acetic Anhydride (JM). A solution of 50 mg. of the oxime in 5 ml. of acetic anhydride was heated at 140 degrees for 15 minutes and then evaporated *in vacuo*. The residue was taken up in chloroform and extracted with sodium bicarbonate, followed by water. The chloroform was dried with sodium sulfate and evaporated, leaving a colored residue which on several crystallizations from hot ethanol gave colorless crystals melting at 173-174.5°. The ultraviolet absorption spectrum in ethanol was that of a typical 3-substituted-4-quinazolone.

Anal. Calcd. for $C_{18}H_{18}O_6N_4$: C, 55.95; H, 4.70; N, 14.50. Found: C, 56.13; H, 4.91; N, 14.30.

Reaction of the Periodate Oxidation Product with Semicarbazide (JBK). A mixture of 74 mg. of the periodate oxidation product, 0.3 g. of semicarbazide hydrochloride, 25 drops of 96% ethanol, and 30 drops of pyridine were heated at

35° for 24 hours, 70-75° for 6 hours, and 90° for ½ hour. Evaporation of the solution and crystallization of the residue from water yielded a mixture of needles and prisms. The needles were soluble in 0.1N hydrochloric acid but the prisms were not, and a separation was made in this manner. The prisms proved to be the amide of hydrazinedicarboxylic acid, a degradation product of semicarbazide (44). Recrystallization of the needles from ethanol yielded 11 mg. of colorless crystals melting at 187-188°. On treatment of the needles with silver nitrate in nitric acid, a copious white precipitate formed which redissolved on addition of ammonium hydroxide. A rough ultraviolet absorption spectrum in ethanol indicated the needles to contain the quinazolone ring.

Anal. Calcd. for $C_{12}H_{10}ON_4$; C, 63.70; H, 4.46; N, 24.77. Found: C, 63.38; H, 4.44; N, 24.45.

On treatment of the material in ethanol with ethanolic picric acid, crystals separated which on recrystallization from ethanol melted at 199-199.5°.

Anal. Calcd. for $C_{18}H_{13}O_8N_7$; N, 21.53. Found: N, 21.48.

3-Propargyl-4-quinazolone (JM). Twelve grams of propargyl bromide was added dropwise to 11 g. of 4-quinazolone and 4.2 g. of potassium hydroxide in 96% ethanol. A rapid reaction ensued. The precipitated potassium bromide was filtered off and the ethanol removed *in vacuo* at 35°. The residue was dissolved in chloroform, and the solution washed with alkali, dried, and concentrated to crystallize the product. Recrystallization of the product from ethanol gave 9 g. of colorless

crystals melting at 116-118°.

Anal. Calcd. for $C_{11}H_8ON_2$; C, 71.72; H, 4.38; N, 15.21. Found: C, 71.66; H, 4.42; N, 15.25.

α-(3-Pyrazolyl)-3-methyl-4-quinazolone (JM). To 0.50 g. of 3-propargyl-4-quinazolone dissolved in absolute ether was added a slight excess of a 0.5M solution of diazomethane in ether. The next day needles began to separate from the ether and on the fifth day the solvent was decanted and the residue crystallized from 96% ethanol to yield colorless needles melting at 187.5-188°. A picrate melting at 199-199.5° was prepared with ethanolic picric acid. Neither the picrate nor the free base showed any depression in melting point on mixing with similar material from the decomposition of the periodate oxidation product with semicarbazide.

Hydrolysis of α-(3-pyrazolyl)-3-methyl-4-quinazolone to 3-aminomethylpyrazole (JBK). A solution of 0.7 g. of the pyrazole in 2.5N potassium hydroxide was heated on a steam bath for 5 hours. The smell of ammonia was evident throughout the hydrolysis. The solution was adjusted to pH 4 to 5 with hydrochloric acid and continuously extracted with ether to remove anthranilic acid. The aqueous phase was then adjusted to pH 10 to 12 with potassium hydroxide and extracted with benzene and then ether. Practically nothing was extracted. The aqueous solution was again made acid, evaporated to dryness and extracted with ethanol. From some of the ethanol solution, a hydrochloride was obtained. As first obtained, it was somewhat hygroscopic but on drying at 100° became non-hygroscopic

and melted at 199-201°. A nitrate was obtained which melted at 115-120°, crude. The free base appeared to be very water soluble, only very slightly soluble in benzene and slightly soluble in ether. The main amount of the ethanol solution was precipitated with picric acid, yielding a large amount of potassium picrate and a few mg. of a picrate melting at 215°. Recrystallization from ethanol yielded yellow, chunky microprisms melting with decomposition at 221-222°.

Anal. Calcd. for $C_4H_7N_3 \cdot 1\frac{1}{2}(C_6H_3O_7N_3)$: N, 24.0. Found: N, 24.26.

Degradation of α-(3-Pyrazolyl)-3-methyl-4-quinazolone to Pyrazole-3-carboxylic acid (JBK). A suspension of 0.2 g. of the pyrazole in 2.5N potassium hydroxide was allowed to stand for 4 days at room temperature. The material went slowly into solution over this period of time. The alkaline solution was acidified to pH 4 to 5 with hydrochloric acid and continuously extracted with ether to remove anthranilic acid. It was then acidified to pH 2, cooled to 0°, treated with sodium nitrite (0.15 g.), allowed to come to room temperature and then warmed on a steam bath. The acid solution was adjusted to pH 10 to 12 with potassium hydroxide and 0.15 g. of potassium permanganate was added. At first a bright green precipitate formed which slowly turned brown. The solution was heated on a steam bath for 3 hours, filtered, and the residue extracted twice with boiling water. The combined aqueous solutions were made acid with hydrochloric acid, taken to dryness, and the

residue extracted with hot ethanol. The ethanol was evaporated and the residue crystallized successively from water, ether-benzene, and ether, to give crystals melting at 210-212° with a crystal transition at about 165-170°. This material did not depress the melting point (212-214°) of an authentic sample of pyrazole-3-carboxylic acid prepared by saponification of ethyl pyrazole-3-carboxylate prepared by the method of v. Auwers and Cauer (45).

Examination of the Mother Liquor after Isolation of α-(3-Pyrazolyl)-3-methyl-4-quinazolone (JBK). The aqueous mother liquor, after decomposition of the periodate oxidation product (74 mg.) with semicarbazide and removal of the pyrazole, was evaporated to a thick odorless, semi-crystalline gum. The final drying was done in a vacuum desiccator over sulfuric acid. It was observed that a strong amine odor appeared when the gum was boiled with acetone, which disappeared on evaporation to dryness. This could then be repeated.

The major portion of the material was heated at 90-100° with cyclohexanone for 2 hours while bubbling a stream of nitrogen through the solution. The nitrogen was run into a solution of 0.15 g. of picrolonic acid in 5 ml. of 96% ethanol cooled in an ice bath. Crystals appeared in the alcohol, and on concentration to about 3 ml., a few mg. of bronze colored prisms melting at 258-260° (decomposition) were collected. Recrystallization from a large volume of boiling 96% ethanol did not change the

melting point.

Anal. Found: C, 43.59; H, 4.14; N, 24.84.

Fractionation of the isolated material from ethanol showed no difference in melting behavior between the first and second crops, but both crops melted 2 degrees higher than the original sample.

Preparation of γ-Aminobutyraldehyde diethylacetal. γ-Aminobutyraldehyde diethylacetal was prepared by the method of Wohl (40, 41). The sequence of compounds and the observed constants are as follows: β-chloropropionaldehyde diethylacetal (46) (b.p. 59-62°/8 mm., n_D^{25} 1.4197); β-cyanopropionaldehyde diethylacetal (b.p. 95-97°/5 mm., n_D^{25} 1.4172; rep. b.p. (41) 106°/45 mm.); γ-aminobutyraldehyde diethylacetal (b.p. 92.2-93°/14 mm., n_D^{25} 1.4266; rep. b.p. (40) 96°/21 mm.). The large discrepancy with the reported boiling point of β-cyanopropionaldehyde diethylacetal deserves mention. The reported boiling point of Wohl (41) must be in error, as this is approximately the boiling point of β-chloropropionaldehyde diethylacetal, and is further than would be predicted from the boiling point of the amine. The boiling points obtained by the author are in good agreement with the usual differences between the boiling points of a nitrile, an amine, and the chloride of one less carbon atom.

N-(2,4-dinitrophenyl)- γ-aminobutyraldehyde Semicarbazone. A solution of 0.5 g. of γ-aminobutyraldehyde diethylacetal and 1.15 g. of 2, 4-dinitrofluorobenzene in

15 ml. of methylene chloride was shaken 15 minutes with a second phase of 7 ml. of 1N sodium bicarbonate. Evaporation of the yellow methylene chloride solution yielded an oil. This oil was dissolved in 8 ml. of 50% ethanol and 2 drops of 12N hydrochloric acid and 1.0 g. of semicarbazide dihydrochloride were added. The mixture was shaken for 40 minutes. A pasty yellow solid slowly separated, which on gentle warming and scratching became particulate. The solid was washed with 50% ethanol and then with water until free of acid. Crystallization from 450 ml. of ethanol yielded 0.56 g. of an orange-yellow solid melting at 175-178°. Solution in glacial acetic acid and precipitation with an equal volume of water removed the orange cast and subsequent recrystallization from acetonitrile yielded yellow crystals melting at 180-182°, with decomposition.

Anal. Calcd. for $C_{11}H_{14}O_5N_6$; C, 42.58; H, 4.55; N, 27.09. Found: C, 42.72; H, 4.59; N, 26.89.

Isolation of N-(2,4-dinitrophenyl)-γ-aminobutyraldehyde Semicarbazone from the Semicarbazide Cleavage of the Periodate Product. Investigation of the chromatographic behavior of the synthetic material had led to the selection of 3:1 Alumina(Alorco, 200 mesh)-Celite 545 as adsorbent, and ½% acetic acid and 20% acetone in methylene chloride as developer as a combination to try in the isolation of the expected derivative.

Accordingly, 0.16 g. of the gummy residue from the

evaporated aqueous phase of one of the decompositions of the periodate oxidation product with semicarbazide was shaken for 15 minutes with 0.45 g. of 2,4-dinitrofluorobenzene, 0.45 g. of sodium bicarbonate, 3 ml. of water and 5 ml. of ethanol. After the solution was evaporated to dryness, the residue was extracted with portions of methylene chloride until the methylene chloride came away colorless. The orange methylene chloride solution was chromatographed in five portions on #2 columns. A yellow zone appeared at the expected location on each column (about 6 cm. from the top with 4½ volumes of developer). The desired zone was easily cut away from other colored zones. Elution of the combined desired zones with acetic acid and precipitation with water, followed by recrystallization from acetonitrile yielded 18 mg. of yellow needles melting at 179-181°. There was no depression of melting point on mixing with a sample of the synthetic N-(2,4-dinitrophenyl)-γ-amino-butyraldehyde semicarbazone.

Section 6

Structure of the $C_7H_{11}O_2N$ Lactone from Permanganate Oxidation of Isofebrifugine

In section 4 there was described a seven carbon fragment obtained by the permanganate oxidation of isofebrifugine. This fragment had the empirical formula $C_7H_{11}O_2N$, and was tentatively assigned a bicyclic structure with one ring being a γ- or δ-lactone and the other a piperidine or pyrrolidine ring. Though it was not possible at the time to assign a more definite structure, it nevertheless proved useful in assigning a structure to the alkaloids. Only after the assignment of XVIII as the gross structure of febrifugine and isofebrifugine was it possible to propose a definite structure for this fragment.

Examination of XVIII reveals that oxidative cleavage at "Y" would yield the previously mentioned 4-quinazolone-3-acetic acid (XXX). Cleavage at "X" would lead to 4-quinazolone (XI), presumably by way of an easily hydrolyzed derivative such as 3-formyl-4-quinazolone (XXXI) or 3-carboxy-4-quinazolone (XXXII). Cleavage at "X" might also yield 3-hydroxypiperidine-2-acetic acid (XXXIII) which would cyclize under acid conditions to the lactone, XXXIV.* The most logical structure for the $C_7H_{11}O_2N$ compound is then

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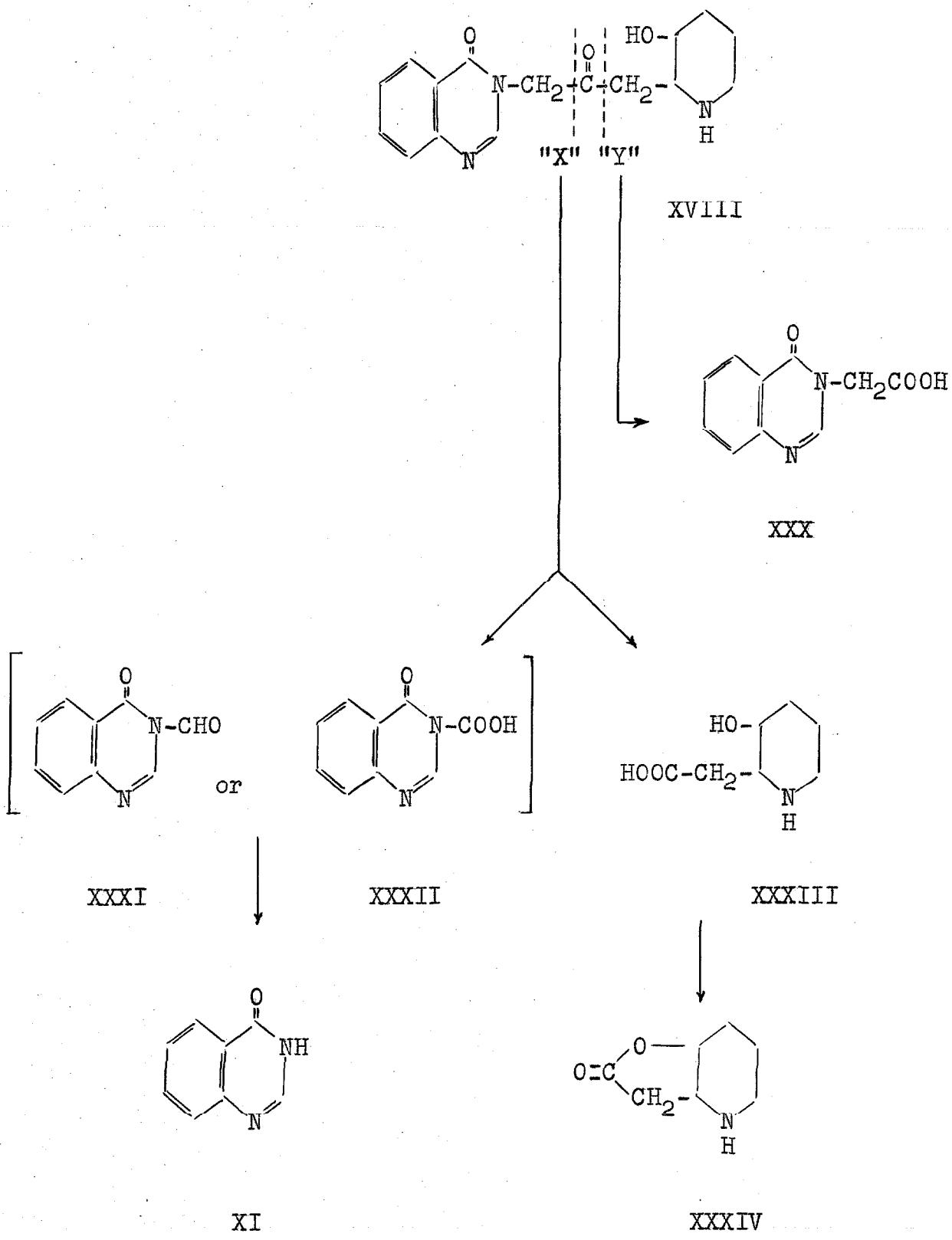
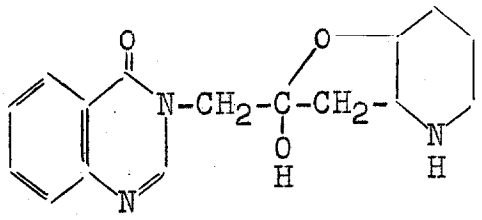
* It has been recently shown that the groupings on the piperidine ring must be cis in order for lactonization to occur (22).

2-hydroxypiperidine-3-acetic acid lactone (XXXIV).



The large amounts of 4-quinazolone obtained in the oxidation of either febrifugine or isofebrifugine indicate that the oxidative cleavage takes place to a large extent at "X". The hydroxypiperidine, XXXIII, should be quite labile to further oxidation. The isolated ester was attacked by permanganate, but relatively slowly. The low yield of the lactone on oxidation of isofebrifugine (less than 10%) is thus to be expected. On the other hand, none of this material could be detected on oxidation of febrifugine. This could result from any of at least three conditions: (a) Febrifugine and isofebrifugine may exist as hemiketals (XXXV) and have different configurations about the asymmetric carbon thus introduced. This could have a profound effect on the course of the oxidation. (b) Febrifugine and isofebrifugine may differ in configuration about one of the asymmetric carbon atoms in the piperidine ring. Thus, febrifugine would have

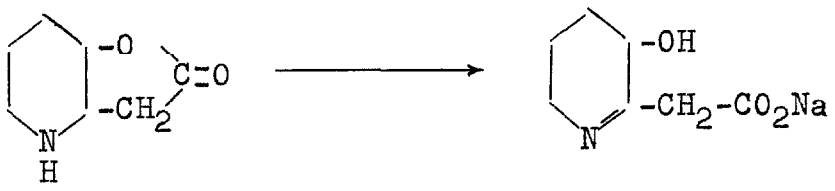


XXXV

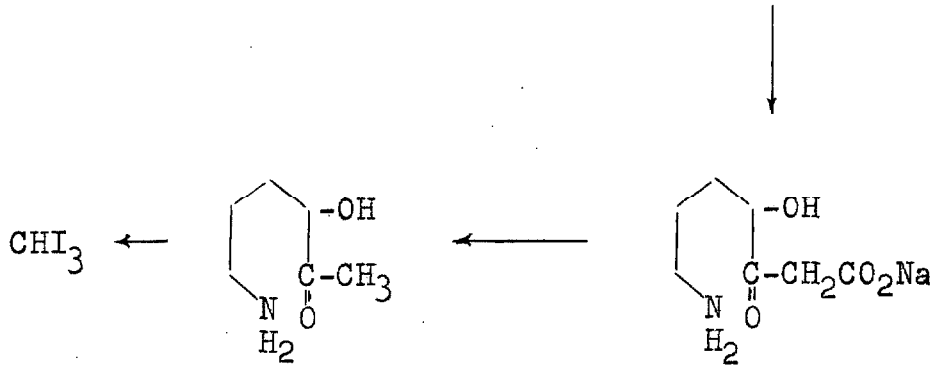
a <u>trans</u> grouping of substituents on the piperidine ring and could not yield the lactone. (c) As has been recently proposed by other workers (23), febrifugine and isofebrifugine may differ only in that the former is the hydroxy ketone,

XVIII, and the latter one of the two forms of XXXV. The lactone could be formed by direct oxidation of the hemiketal of isofebrifugine, and in the case of febrifugine would have to form, on isolation, from a more labile structure such as XXXIII.

For some time the most disturbing feature of the isolated ester was the fact that it reacted with sodium hypoiodite to give iodoform. The alkaloids have no C-methyl group and it did not seem possible that one was produced in the oxidative degradation. A possible explanation of the iodoform reaction on the basis of structure XXXIV is as follows:



XXXIV XXXVI



XXXVIII XXXVII

Amines having the usually required carbon skeleton undergo the haloform reaction (47), thus the oxidation shown in the first step is not unlikely under the conditions of the haloform reaction. Compound XXXVI very probably does not exist more than transiently. As was mentioned before in the discussion of the tetrahydropyridines, such compounds probably exist as the Δ^2 structure and in aqueous solution are in equilibrium with the open aminoketone, XXXVII (37). The effect of the hydroxyl group in the 3-position is not known. If the double bond shifts into the 2,3-position, the effect might then merely be an oxidation of the hydroxyl to a ketone. A second oxidation of the amino group would then be necessary to yield a β-ketoacid.

The actual process is probably not as simple as the schematic outline. Substitution of the methylene groups with iodine could occur before and after the decarboxylation. Indeed it is felt that the pictured decarboxylation would not proceed well at the low temperature (60°) of the iodoform test, but would most certainly proceed as a result of the increased activation introduced by α-halo atoms.

Section 7

Summary of Evidence for the Structures of Febrifugine and Isofebrifugine

For the moment, isofebrifugine will be assumed to have a structure based on that of febrifugine. Possible differences between the two isomeric alkaloids will be discussed in the next section.

At the conclusion of Brockman's thesis (1), febrifugine had been shown to be a 4-quinazolone substituted only at the 3-position. The remainder of the molecule had been shown to contain a keto-group, a secondary amine, and a hydroxyl group.

The isolation of 4-quinazolone-3-acetic acid and a lactone containing the secondary amine after oxidation of isofebrifugine indicated that the carbonyl group was beta to the quinazolone moiety. In addition it showed that the hydroxyl group was either γ or δ to the carbonyl.

Since the alkaloids are oxidizable by periodate, and the oxidation does not affect the quinazolone ring, the secondary amine must be vicinal to either the carbonyl or to the hydroxyl group. The cleavage product of the periodate oxidation product with hydroxylamine was shown to be the dioxime of a β-ketoaldehyde by the formation of the triacetyl derivative. The cyclization of the dioxime on heating with acid further indicated the carbonyl functions to be β. The cleavage of the periodate product with semicarbazide to yield the 3-substituted pyrazole, IX, confirmed that the basic structure of the cleavage product is 3-(β, δ-dioxobutyl)-

4-quinazolone. This means that the secondary amine in the alkaloid is beta to the ketonic group and alpha to the hydroxyl group. The only structure satisfying this requirement and having no C-methyl group is XVIII. The identification of γ -aminobutyraldehyde semicarbazone as the other cleavage product of the periodate oxidation product confirmed this structure.

Section 8

The Relationship of Febrifugine to Isofebrifugine

The nature of the difference between febrifugine and isofebrifugine makes an unequivocal assignment of exact structures difficult. Febrifugine has a demonstrable carbonyl group and isofebrifugine does not. However, the ease of interconversion, the similarity of febrifugine and isofebrifugine in reduction to dihydro compounds (3), the identity of the products of oxidation with periodate (3), and the isolation of the lactone, XXXIV, on permanganate oxidation of isofebrifugine, all point to the presence in isofebrifugine of a masked carbonyl group in the same location as that in febrifugine. The failure of isofebrifugine to react with carbonyl reagents indicates that the ketonic group is either sterically hindered or does not exist except as a potential carbonyl group.

Since the carbonyl function is apparently associated with the difference, a few of the properties of the dihydro compounds will be reviewed. The dihydro derivatives of the alkaloids are not identical (3). The point of reduction seems to be the carbonyl group. Though the possible interconversion of dihydrofebrifugine and dihydroisofebrifugine has not been studied extensively, the dihydro compounds appear to be stable to heat and can be crystallized from hot solvents without isomerization (1). The dihydrocompounds are hydrolyzed in alkali without the formation of resins (1). The lack of resin formation on hydrolysis, and ultraviolet

absorption spectra practically identical to the parent compounds (1) were results to be predicted (6) on the basis of the original alkaloids having a carbonyl group beta to the quinazolone ring. The dihydrocompounds are only slowly attacked by periodate, requiring 17 hours to consume 0.74 equivalents per mole. After alkaline hydrolysis, however, 4.11 equivalents are consumed in one hour. The cyclic structure proposed in section 5 to explain this anomaly may indicate that dihydrofebrifugine has a cis configuration of the groups on the piperidine ring. However, a seven membered ring might as easily form from the trans configuration.

In the preparation of the dihydrocompounds, in spite of the uptake of almost exactly one mole of hydrogen, the yield of the reduction products is quite low (1). If the two asymmetric centers of the piperidine ring are not involved in the difference between the alkaloids, then it would be reasonable to predict that the reduction should not be completely stereospecific. In other words, some dihydroisofebrifugine might be expected to result from the reduction of febrifugine and vice versa.

Crystallization of the mother liquors from the preparations of the dihydrocompounds proved unprofitable. A few unsuccessful attempts at the chromatographic separation of the dihydro derivatives soon showed that the development of conditions for the chromatography of colorless, nonfluorescent compounds is a time consuming process. Accordingly,

the N-dinitrophenyl derivatives of the dihydrocompounds were prepared, and their chromatography studied. Conditions were not found which would separate the derivatives.

A second avenue of attack on the difference between the alkaloids was that of permanganate oxidation. The oxidation of febrifugine, as previously mentioned, did not yield the lactone obtained from isofebrifugine. It would be desirable to further identify the fragment obtained from febrifugine and to compare its configuration with that of the lactone from isofebrifugine. The low yields of both fragments, especially that from febrifugine would have made this procedure too costly of the limited supply of the alkaloids. It was reasoned that the large amount of 4-quinazolone produced in the oxidation of isofebrifugine indicated that the oxidation was proceeding in such a manner as to give large yields of the desired fragment. The small yield of the lactone was thought to be due to the lability of the product to further oxidation. The substitution of the secondary amine with a negative substituent might stabilize the fragment to oxidation and, if the substituent was also colored, would aid in the isolation of the fragment by chromatography.

The dinitrophenyl derivatives of febrifugine and isofebrifugine were prepared. The reaction of the alkaloids with the reagent was very slow and the yields were not very satisfactory, though there did not seem to be appreciable side reactions and the unreacted alkaloid could be recovered. Crystallization of the derivatives was difficult. They tended

to precipitate from solvents as gums rather than as crystalline material. With the hope of improving the properties of the derivatives, it was decided to use a chromatographic isolation. Brockman had previously shown (1) that the alkaloids were very tightly held by silicic acid. A developer solution of 20% acetone in methylene chloride gave a very satisfactory separation of the derivatives and left any unreacted alkaloids at the top of the column. Unreacted dinitrofluorobenzene moved practically with the front with methylene chloride alone, and its hydrolysis product, the phenol, moved very rapidly with the developers used. The chromatographically purified derivatives still crystallized only with difficulty.

The dinitrophenyl derivative of the lactone from isofebrifugine was prepared, using about 1 mg. of the lactone. At least four yellow zones were obtained when the reaction mixture was chromatographed. Thus there exists some uncertainty in the reaction of dinitrofluorobenzene with polyfunctional compounds.

The attempt to compare the oxidative fragments of febrifugine and isofebrifugine was set aside because of the uncertainty of the lactone derivative and the difficulty of crystallization of the derivatives of the alkaloids. The supply of alkaloids was quite small, making it necessary to work with relatively small quantities. Thus, small yields on crystallization could not be tolerated.

Another possibility of getting some information on the problem of the difference between the alkaloids lay in pre-

paring the same derivative involving the carbonyl group. The difficulty involved is that isofebrifugine does not form such derivatives as an oxime or semicarbazone at room temperature, and warming under alkaline conditions causes conversion to febrifugine. It was reasoned, that since the alkaloids are stable (do not interconvert) to acid, no ambiguity would result from forcing isofebrifugine to give a derivative under acid conditions. If isofebrifugine exists as a hemiketal, then the displacement of this with a divalent derivative such as 2,4-dinitrophenylhydrazine would be ideal. Rotations on the dinitrophenylhydrazones of the two alkaloids would then determine whether the configurations about the asymmetric carbon atoms of the piperidine ring were the same for the two alkaloids. A monovalent derivative such as a mercaptol would be of some use, as febrifugine might form a di- and isofebrifugine a mono-derivative with the mercaptan.

As it developed, it was not possible to prepare a dinitrophenylhydrazone of even febrifugine. Starting material was recovered after heating febrifugine dihydrochloride at 100° for 40 minutes with dinitrophenylhydrazine in glacial acetic acid containing about 2% of concentrated hydrochloric acid. This would indicate that the ketonic group is sterically hindered.

An attempt was made to prepare the dimethylmercaptol of febrifugine, using methylmercaptan, freshly fused zinc chloride, and anhydrous sodium sulfate. Starting material was recovered after 21 hours at room temperature.

Isofebrifugine could not be methylated with methanolic hydrogen chloride at 100°.

Though it was known that the interchange between febrifugine and isofebrifugine was reciprocal (3), it seemed advisable to obtain some information as to the position of the equilibrium (if an actual equilibrium existed), the effect of different types of solvents, and at least a rough determination of the rate. The large difference of specific rotation of febrifugine and isofebrifugine in chloroform (3) suggested the use of the polarimeter for analysis of mixtures of the two.

For the purposes of the equilibrium studies, it was decided to study the interconversion in benzene and in methanol. After heating for the desired length of time at the arbitrary temperature of 75°, the rotation of the sample was taken in chloroform (fig. 2).

In methanol, both febrifugine and isofebrifugine approach the same mixture of approximately 45% isofebrifugine and 55% febrifugine. The interconversion in methanol at 75° is quite rapid. In ten minutes a sample of pure febrifugine developed approximately half the equilibrium concentration of isofebrifugine. Prolonged heating (5½ hours) at 75° did not materially affect the rotation of the equilibrium mixture, indicating that little or no decomposition was occurring. With benzene as the medium, the interconversion was less rapid. The position of the equilibrium was poorly defined, but appeared to be about 82% isofebrifugine. A solution initially febrifugine contained about 41% isofebrifugine after 1½ hours heating.

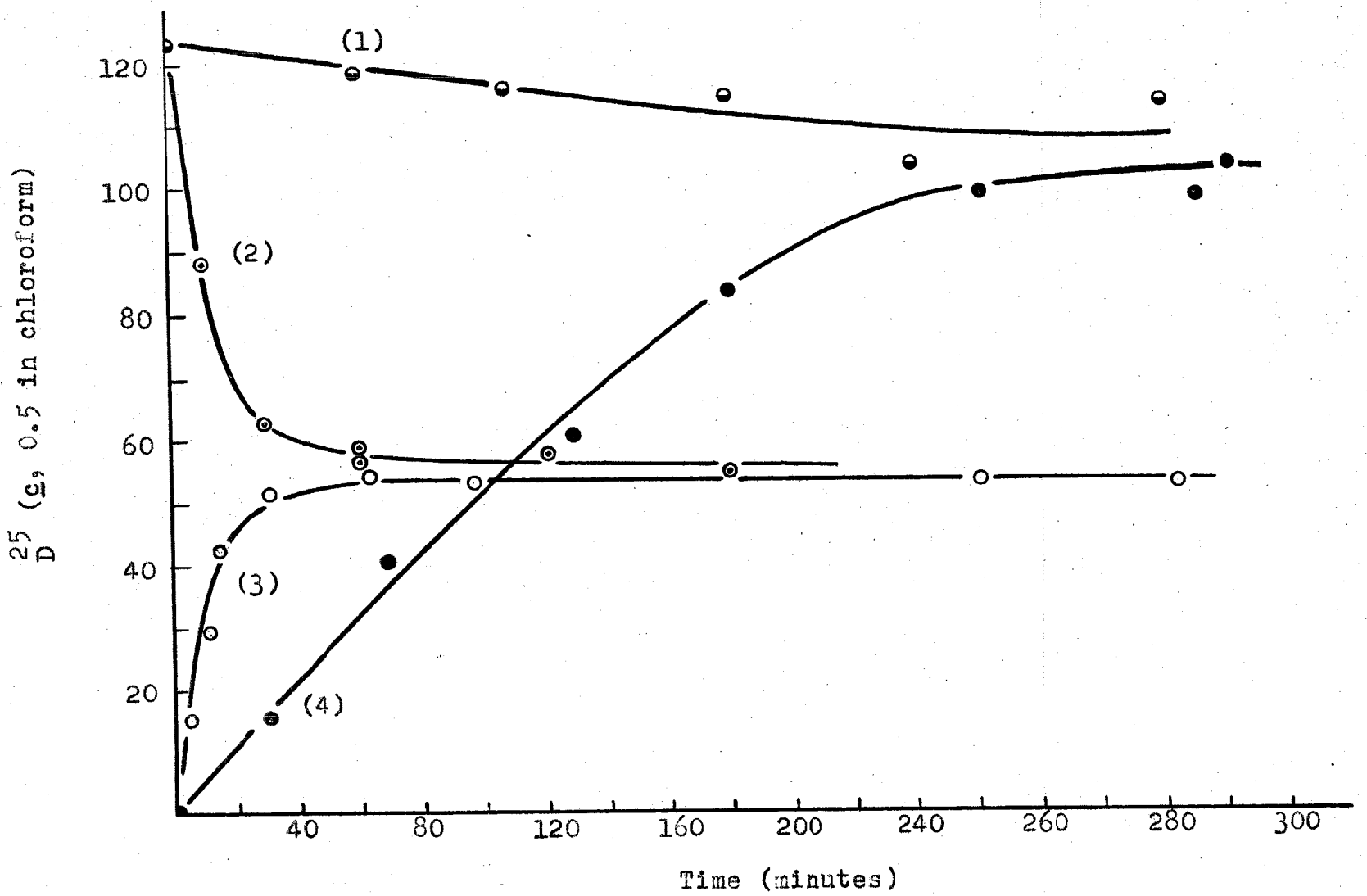


Figure 2. Studies on febrifugine-isofebrifugine interchange at 75°C.: (1), isofebrifugine in benzene; (2), isofebrifugine in methanol; (3), febrifugine in methanol; and (4), febrifugine in benzene.

The increased rate of interconversion in methanol as opposed to benzene is not explained. The complete stability of the alkaloids to acid conditions indicates that the interconversion is base catalyzed. It seems unlikely that the only difference between febrifugine and isofebrifugine is the formation of a hemiketal. If such were the case the interchange would be expected to occur under acid conditions and not under basic conditions. Rather, it seems more likely that febrifugine and isofebrifugine differ in configuration about one of the asymmetric carbon atoms in the piperidine ring. The chemistry of the molecule would indicate that the hydroxyl group and the carbonyl side chain have a trans configuration in febrifugine and a cis configuration in isofebrifugine. The geometry of the molecule makes it improbable that an interaction between the hydroxyl group and the carbonyl (in the case of the lactone, lactonization) could take place from the trans configuration, whereas an easy interaction would be expected from a cis configuration. A cis configuration would also be expected to block access to the carbonyl group more than would the trans structure, thus offering an explanation for the non-reactivity of isofebrifugine as a ketone.

Thus the available data, though inconclusive, suggests that febrifugine has the structure XVIII, and is one of the two antipodes having a trans configuration on the piperidine ring. Isofebrifugine would then be one of the two antipodes having a cis configuration, and quite possibly exists as a hemiketal (XXXV) rather than the hydroxy ketone.

It had been originally planned to include two possible mechanisms for the interconversion of febrifugine and isofebrifugine, based on the above interpretation of the structures. Since these were quite speculative and no actual support of the structures, their discussion was deleted after the appearance of papers by Baker, et. al., proposing the cis configuration for both febrifugine and isofebrifugine (22, 23). A comment on this interpretation however, might be in order.

The facile interconversion of the two alkaloids as the free bases, and the stability to acid (3) would appear to be in contradiction to the proposal (23), that febrifugine is a *hydroxy ketone and isofebrifugine the corresponding hemiketal.* Such isomerism is generally considered to be acid catalyzed. Under this interpretation isofebrifugine would be expected also to form carbonyl derivatives (identical with those of febrifugine) since it is well known from the carbohydrates that hemiketal formation does not prevent such reactions. Baker, et. al., to explain the non-reactivity, propose stabilization of the hemiketal structure by intramolecular hydrogen bonding. The chelate so formed involves a seven membered ring containing one double bond. Significant contribution to stability by such a chelate, is open to question.

It must be pointed out that the weight of the total evidence lies with the explanation of Baker, et. al. Their demonstration (22) that in febrifugine the two groups attached to the piperidine ring are cis was quite straightforward.

Experimental

Preparation of the N-dinitrophenyl Derivatives of Dihydrofebrifugine and Dihydroisofebrifugine. A mixture of 11 mg. of dihydrofebrifugine and 22 mg. of dinitrofluorobenzene was shaken with aqueous-ethanolic sodium bicarbonate solution for 1 hour. The solution was evaporated and the residue dissolved in 6N hydrochloric acid and extracted with ether to remove the excess reagent. The aqueous solution was then made basic and extracted three times with equal volume portions of methylene chloride. This transferred most of the yellow color from the aqueous to the organic phase. Evaporation of the methylene chloride and recrystallization of the residue from aqueous methanol yielded 5.8 mg. of yellow solid melting at 145-152°, setting to a sort of glass on melting.

Dihydroisofebrifugine (14.5 mg.) was treated in a similar manner, except that the yellow methylene chloride solution was placed on a #2 chromatographic column containing 3:1 alumina-cellite. Development with 100 ml. (about 3½ volumes) of 0.25% acetic acid in methylene chloride produced several bands, but one of these (1.7 cm. from the top) was clearly the main band. Development with 100 ml. of 0.5% acetic acid in methylene chloride caused this to move free of others to a position 6 cm. from the top. After elution of the zone with methanol and removal of water soluble components (arising from the adsorbent) 9.9 mg. of a yellow residue was obtained. The only solvent found which would crystallize the material at all, and that

not well, was isopropyl ether to give a material with a very poor melting point of about 120° with gross decomposition.

The use of a two phase reaction mixture of methylene chloride and 1N sodium bicarbonate, was found to be very effective in the preparation of these derivatives. After about 1 hour of shaking, the organic phase was dried and placed directly on the column.

The behavior of the isomeric derivatives was investigated briefly on #1 columns of alumina-cellite. Approximately 0.2 mg. quantities of the derivatives were used by taking aliquots of methylene chloride solutions containing ca. 1 mg./ml. It was found that a small amount of acid was needed in the developer solution in order to obtain zones narrow enough for their color to be seen. The results are given in Table II. Briefly, no difference in behavior was demonstrated.

Preparation of the N-dinitrophenyl Derivatives of Febrifugine and Isofebrifugine. The derivatives were prepared by the two phase procedure described for the dihydro compounds. The reaction of dinitrofluorobenzene with the alkaloids was much slower than with the dihydro alkaloids. In a sample preparation 36 mg. of febrifugine, 80 mg. of dinitrofluorobenzene, 20 ml. of methylene chloride, 5 ml. of water and 0.5 g. of sodium bicarbonate were shaken for 16 hours at room temperature. Chromatographic isolation of the derivative yielded 7.6 mg. of a yellow-orange gum which would not crystallize. The corresponding derivative of isofebrifugine was gotten to crystallize from benzene by the addition of a small

amount of acetone and ligroin, but the yield on recrystallization was very poor. This derivative melted at 175-177°.

The chromatographic procedure was not the same as for the dihydro alkaloids. Because of its cleaner nature, silicic acid (Mallinckrodt Reagent, sold as a specially prepared silicic acid for chromatographic use) mixed with 1/3 part of celite was used as adsorbent and 20% acetone in methylene chloride was used as the developer solution. A standard activating prewash consisting of 0.2V of ether, 1V of 1:1 ether-acetone, 0.8V ether, and IV of methylene chloride was used.* In the first trials, the retention of the unreacted alkaloids at the top of the column was confirmed by streaking the column with an aqueous solution containing 0.1% potassium permanganate and 1% sodium hydroxide (3). For small columns it was found that the use of a camel's hair brush for streaking was not satisfactory. It was more convenient to apply the reagent from a bulbless dropper which had been drawn out to a capillary tip. The dropper was inclined to a quite low angle with the extruded column and drawn fairly rapidly along the length of the column with the tip in light contact. Capillarity of the column drew the reagent from the dropper. A quite uniform line of most any desired width could be obtained by regulating the speed of traverse.

The use of 20% acetone in methylene chloride caused the dinitrophenyl derivatives of febrifugine and isofebrifugine

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* Recommended by W. A. Schroeder.

Table II

Attempted Chromatographic Separation of the N-dinitrophenyl Derivatives of Dihydrofebrifugine and Dihydroisofebrifugine on Alumina-Celite

Compound[a]	Developer[b]	Distance of zone from top of column (cm.)
I, F	2V 0.2% AcOH 0.2% EtOAc	1
I	3V 0.4% AcOH 0.5% EtOAc	2.1
F	3V 0.4% AcOH 0.5% EtOAc	2.1
I, F	2V 0.4% AcOH 0.5% EtOAc	1.5
(same column)	3V 0.4% AcOH 4.0% EtOAc	3.5
(same column)	1% MeOH added to above developer and run to end	--
I, F	10V 0.5% AcOH 4.0% EtOAc 2.0% Acetone	--

(a) The derivative of dihydroisofebrifugine is denoted by I, and of dihydrofebrifugine by F.

(b) The base of all the developers was methylene chloride.

to move down the column, leaving the unreacted alkaloids in the top few millimeters. The derivative of isofebrifugine was the faster moving of the two and could easily be separated from the febrifugine derivative.

Reaction of Dinitrofluorobenzene with the $C_7H_{12}O_2NCl$ Compound. A small amount, 1.1 mg., of the C_7 lactone hydrochloride from permanganate oxidation of isofebrifugine was stirred 3 hours at room temperature with 2 mg. of sodium bicarbonate, 2 to 3 mg. of dinitrofluorobenzene, 2 drops of water and 3 drops of ethanol. The reaction mixture was evaporated to dryness and the yellow residue leached with methylene chloride till no more yellow color was removed (solution A). The yellow solid which remained was treated with excess 1N hydrochloric acid and extracted with methylene chloride (solution B). Solutions A and B were chromatographed separately on #1 columns of silicic acid-celite using 10 ml. (1½V) of 4% acetone in methylene chloride as developer. Solution A produced three yellow zones, and solution B two yellow zones the faster moving of which was probably dinitrophenol. The fastest moving of the zones from solution A appeared to contain more material than any of the other zones but was judged to contain less than half of the total material (excluding dinitrophenol).

Reaction of Febrifugine with Dinitrophenylhydrazine. A mixture of 10 mg. of febrifugine dihydrochloride, 5 mg. of 2,4-dinitrophenylhydrazine, 10 drops of glacial acetic acid, and 10 µl. of 12N hydrochloric acid was heated in a sealed

capillary tube at 100° for 40 minutes. Colorless crystals (7.6 mg.) separated on cooling. These were identified by melting point and mixed melting point as febrifugine dihydrochloride.

Reaction of Febrifugine with Methylmercaptan. About 25 to 50 mg. of zinc chloride was fused in vacuo in the bottom of an ampoule (prepared from 10 mm. pyrex tubing), and after cooling, 25 mg. of anhydrous sodium sulfate, 21 mg. of febrifugine, and 1 ml. of methylmercaptan were introduced. The ampoule was sealed and allowed to stand at room temperature for 24 hours. After opening the ampoule and allowing the methylmercaptan to evaporate, the residue was treated with 2 ml. of 5% sodium carbonate and extracted with chloroform. Evaporation of the chloroform yielded 17.5 mg. of colorless crystals, which after crystallization from ethanol were identified by melting point and mixed melting point as febrifugine.

Reaction of Isofebrifugine with Methanolic Hydrogen Chloride. A solution of 24.0 mg. of isofebrifugine in 2 ml. of methanol was saturated with dry hydrogen chloride at 0°. The reaction tube was sealed and heated at 100° for 7¼ hours. The mixture was evaporated with a stream of nitrogen (after the initial spontaneous evaporation) to a small volume, 1 ml. of water added, and made basic with 5N sodium hydroxide. It was then extracted seven times with 2 ml. portions of chloroform, and the chloroform evaporated, yielding 22.7 mg. of material identified by melting point and mixed melting point

as isofebrifugine.

Interconversion of Febrifugine and Isofebrifugine. For the study of the interchange, an approximately 10 mg. sample of the desired alkaloid was weighed into an ampoule prepared from a 10 x 70 mm. pyrex test tube. The desired solvent (3 ml. of methanol, or 5 ml. of benzene) was introduced. The ampoule was sealed and heated in an oil bath at 75° for the desired length of time. The ampoule was then cooled, opened, and the contents evaporated at a temperature of approximately 10° with a stream of nitrogen. The evaporation was then completed in a vacuum desiccator. The residue was dissolved in 2 ml. of chloroform and the rotation taken in a 1 dm. semimicro polarimeter tube (Figure 2). It was shown that putting febrifugine or isofebrifugine through either procedure but without heating, gave the rotation of the pure starting material. The specific rotation of the solutions from heating in benzene did not become constant till approximately 4-5 hours of heating. The specific rotation (pure febrifugine, 0°; pure isofebrifugine, +125°) of the equilibrium mixture starting with febrifugine was about 100° and starting with isofebrifugine, about 104°, indicating an equilibrium concentration of isofebrifugine of about 82%. A number of points were taken from ½ hour to 5½ hours of heating. Though the curve obtained was not smooth, it could be estimated that a solution initially febrifugine contained half the equilibrium concentration of isofebrifugine after about 1½ hours heating. The curves obtained on heating in

methanol were quite smooth and agreed very well at the point of equilibrium. The specific rotation at equilibrium (reached in less than 1 hour) was about 55°, indicating an equilibrium concentration of about 45% isofebrifuge. The half-way point was reached in 10 minutes. Prolonged heating in methanol (5½ hours) did not detectably affect the rotation of the equilibrium mixture, indicating that decomposition was probably not occurring.

Section 9

The Polymorphism of Febrifugine and the Identity of γ-Dichroine

Febrifugine has been occasionally encountered in higher melting crystalline modifications. It has been the practice of this laboratory to distinguish between the forms by means of greek letters. The material (m.p. 140°) referred to throughout the previous sections of this thesis as febrifugine was the form most commonly obtained on crystallization and would have been more properly designated as α-febrifugine. Polymorphism has been noted only of the free base of febrifugine and not of isofebrifugine or of any of the derivatives of either alkaloid.

Other workers (48, 49, 50) have assigned different names to the alkaloids of D. febrifuga, causing some confusion (3). As an aid to the reader it seemed advisable to preface the discussion with a table (Table III) of the compounds to be considered and the conclusions reached concerning them.

In 1948, Chinese investigators, Chou, et al. (48), reported the isolation from Dichroa febrifuga of three "isomeric" alkaloids, convertible to one another under proper conditions. These alkaloids were named α-, β-, and γ-dichroine, and were reported to have antimalarial activity increasing in that order. In 1949, Koepfli, et al. (3) reported that in spite of the disagreement in melting points it was evident from the data of Chou, et al., that α-, and β-dichroine were isofebrifugine and febrifugine (α-febri-

Table III

Cryst. Mod. of Febrifugine	Melting Point °C	Probable Identity or Mode of Preparation
α-Febrifugine[a]	139-140	Crystallization of any form from most solvents
β-Febrifugine	154-156	Heat isomerization of isofebrifugine (no solvent)
γ-Febrifugine	151-153	Spont. cryst. from chloroform
δ-Febrifugine	153-156	Spont. cryst. from chloroform
β-Dichroine	145[b]	α-febrifugine
γ-Dichroine	160[b]	Mixture of β-, and γ-febrifugine; heat isomerization of α-dichroine
Isomers of Febrifugine		
Isofebrifugine	129-130	
α-Dichroine	136[b]	Isofebrifugine

(a) Referred to as febrifugine in publications (2, 3) and in the previous sections of this thesis.

(b) Taken from the literature (49), and is probably four to six degrees lower than this, as the melting point of γ-dichroine was determined in this laboratory to be 154-156°.

fugine), respectively, the isolation and properties of which had been reported in 1947 (2). It was further suggested that γ-dichroine was identical with a material (β-febrifugine) which had been shown to be a high melting crystalline modification of febrifugine, and thus not an isomer of β-dichroine.* This would require the physiologic data on the dichroines to be in error. The evidence for this was very strong as the reported preparation of γ-dichroine was substantially identical with that of β-febrifugine, namely the heating of the lowest melting isomeride (α-dichroine, isofebrifugine). The only reported difference in the preparation was the claim of the Chinese investigators that γ-dichroine was crystallized from acetone. Any attempt in this laboratory to actually crystallize β-febrifugine from acetone yielded the α-form. The Chinese investigators further claimed that all derivatives (including the hydrochlorides) of γ-dichroine were identical with those of β-dichroine, but occasionally referred to crude hydrochlorides as a mixture of β-, and γ-dichroine hydrochlorides. It might be remarked that the

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* It should be noted here that this paper of Koepfli, et al. (3), contained a statement which is probably misleading. They reported that the higher melting base was occasionally encountered during early isolation studies, and subsequently obtained by neutralization of febrifugine dihydrochloride and by spontaneous crystallization of melted isofebrifugine (isomerization). The crystalline form of febrifugine obtained from spontaneous crystallization of melted isofebrifugine has since been designated as β-febrifugine. The other preparations were probably not the same crystalline form. They may have been γ-, or δ-febrifugine or forms not encountered by the author.

physiologic testing is done in 0.01N hydrochloric acid.

In June, 1948, K. K. Chen, Eli Lilly and Company, Indianapolis, received a sample of γ-dichroine from Chou. A portion of this was sent to L. H. Schmidt, Institute of Medical Research, Christ Hospital, Cincinnati. In May, 1949, Dr. Chen supplied us with a few milligrams of this γ-dichroine, and in 1950 Dr. Schmidt sent us what remained (a few mg.) of the sample he had received. The results of pharmacologic tests by Chen (51) indicated γ-dichroine to have a higher activity than febrifugine (α-febrifugine) against Plasmodium lophurae in ducklings. The tests of Dr. Schmidt (52) indicated febrifugine and γ-dichroine to have the same acute toxicities for the white mouse, but indicated γ-dichroine to have a lower subacute toxicity for the rhesus monkey, and a lower suppressive activity against P. cynomolgi than did febrifugine.

It should be pointed out that the pharmacologic tests of γ-dichroine were conducted some two years after those of febrifugine and that the tests on monkeys were made with a small number of animals (four).

Parallel melting and mixed melting point determinations of γ-dichroine and β-febrifugine were identical. Microscopic examination showed the two to have a different appearance.

At this point, a communication (53) from Chen stated that X-ray powder photographs of γ-dichroine and β-febrifugine showed them to be different.

Brockman, in this laboratory, took X-ray powder photographs of the two forms of febrifugine and of γ-dichroine. The photographs of all three were quite distinct from one another, though as was later shown by the author, certain of the reflections of γ-dichroine are probably identical with strong reflections of β-febrifugine.

By evaporation of a saturated chloroform solution of α-febrifugine and seeding with γ-dichroine Brockman obtained crystals (#4, P. 259) whose powder photograph bore some resemblance to that of γ-dichroine. There were, however, serious discrepancies between the two photographs.

By heating two mixed samples of isofebrifugine and α-febrifugine and seeding one with β-febrifugine and the other with γ-dichroine he obtained, in both cases, material with an X-ray powder photograph like that of β-febrifugine. Other attempts were made by Brockman to prepare γ-dichroine, but without success.

Further (unsuccessful) attempts were made by Dr. Koepfli and the author to prepare γ-dichroine by seeding experiments.

In one preparation of the free base of febrifugine from its dihydrochloride the crystals first separating on evaporation of a chloroform extract bore a strong physical resemblance to γ-dichroine. As the melting point indicated the material to be one of the high melting modifications, an X-ray powder photograph was promptly taken. The photograph had a striking resemblance to that of γ-dichroine, and the material was named γ-febrifugine. The physiological activity

of γ-febrifugine was tested in parallel with that of α-febrifugine, and no difference could be detected.*

Microscopic examination of all the forms at hand were made. The observations are recorded in Table IV.

In yet another isolation of the free base of febrifugine from its dihydrochloride, a fourth crystal modification of febrifugine was obtained by spontaneous crystallization. In this case, crystals suddenly appeared suspended in the organic phase while extracting an alkaline solution of febrifugine dihydrochloride with chloroform. The X-ray powder photograph of these crystals was again different from the other forms. This modification was named δ-febrifugine.

As a check, the rotations of the various forms were taken in diverse solvents (Table V). γ-Febrifugine had the same specific rotation as α-febrifugine in chloroform, ethanol, and in 1N hydrochloric acid. δ-Febrifugine was compared only in chloroform and in ethanol, but the rotations were identical with α-febrifugine. Brockman (3) had previously shown that in rotational and chromatographic studies β-febrifugine was identical with α-febrifugine, and that they were interconvertible by cross seeding of saturated solutions in the cold.

No attempt was made to interconvert α-, γ-, and δ-febrifugine by seeding experiments in the cold. However,

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* The author is indebted to L. H. Schmidt and the Institute of Medical Research, Christ Hospital, Cincinnati for the testing of these compounds.

Table IV

Microscopic Examination of Crystal Forms

Compound	Appearance	Extinction	Sign of Birefringence as Determined by 1st Order Red Compensator
α-febrifugine	Platelets	Parallel	High index is parallel to long axis
β-febrifugine	Rod-like needles- contains some platelets	Largely parallel with some fragments with 45° extinction	Opposite from α-febrifugine
γ-febrifugine	Fibrous needles	Parallel	Crystals of each sign (?)
2nd crop of γ-febrifugine	Fibrous needles	Parallel	Opposite (?) from α-febrifugine
No. 4 p. 257 (Abt. 1 yr. after powd. photo.)	Some platelets and Some rods	Parallel	Same as α-febrifugine Opposite from α-febrifugine
γ-dichroine	Fibrous needles, perhaps somewhat more bunched than γ-febrifugine	Parallel	Crystals of each sign (?)
isofebrifugine	Chunky crystals with apparently no edges or faces parallel (triclinic?)	Parallel	--

Table V

Rotations of the Alkaloids in Various Solvents

Compound	$[\alpha]_D^{25}$ (c, 0.5)					
	$CHCl_3$	EtOH	0.1N HCl	0.5N HCl	1.0N HCl	2.0N HCl
α-Febrifugine[a]	0	+22	+18	+28	+32	+35
β-Febrifugine		+28[b]				
γ-Febrifugine	+2	+21			+32	
δ-Febrifugine	0	+19				
Isofebrifugine[c]	+125	+30		+18	+18	+16

(a) Reported (3) $[\alpha]_D^{25}$ + 6° (c, 0.5 in chloroform) and +28° (c, 0.5 in ethanol); reported (54) $[\alpha]_D^{25}$ + 21° (c, 1.4 in ethanol).

(b) Taken from the literature (3).

(c) Reported (3) $[\alpha]_D^{25}$ + 131° (c, 0.35 in chloroform); reported (54) $[\alpha]_D^{25}$ + 31° (alcohol) and + 120° (c, 0.8 in chloroform).

since the new forms arose from treatment of febrifugine dihydrochloride in solutions not above room temperature and have the rotation of α-febrifugine, there seems little doubt that these are polymorphic forms and not isomers. Each of the forms, on crystallization from hot ethanol, yields α-febrifugine.

The overall resemblance of the X-ray powder photograph of γ-febrifugine to that of γ-dichroine suggested that a detailed examination of the photographs might be profitable.

Visual estimation of relative line intensities was made of the photographs taken in this laboratory. In addition, data from the powder photographs of γ-dichroine and β-febrifugine taken at Eli Lilly and Co. was made available to us.* The data from our photographs (reproduced in Fig. 3) was recorded directly in graphic form (Figure 4). It may be readily seen from an examination of Figure 4 that with some minor discrepancies, the X-ray powder photograph of γ-dichroine can be reproduced by assuming this material to be a mixture of β-, and γ-febrifugine, with γ-febrifugine predominating. An estimate of the composition would be perhaps 75% γ-febrifugine and 25% β-febrifugine.*

An analysis of a second set of X-ray powder photographs at Eli Lilly and Co., was made by Dr. S. F. Kern of that laboratory (53).* Dr. Kern had available only β-febrifugine

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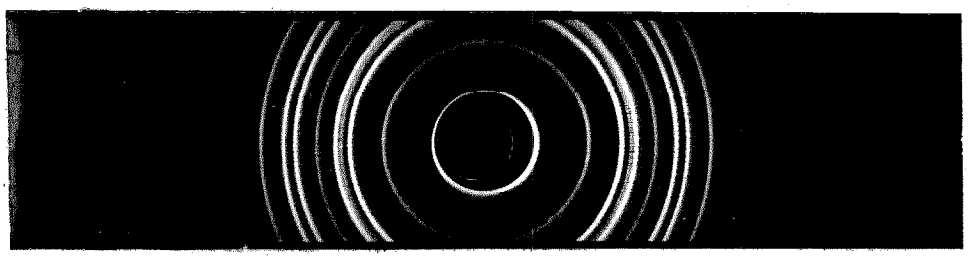
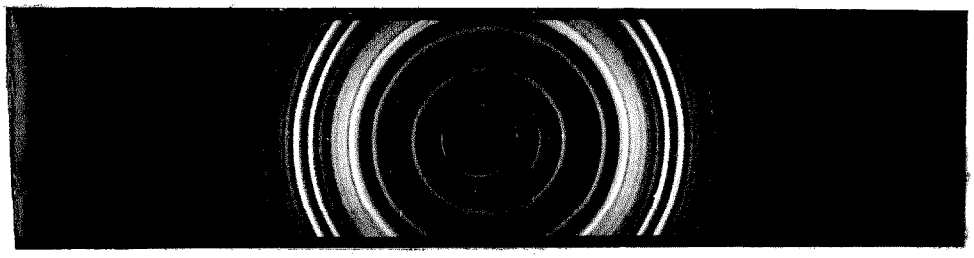
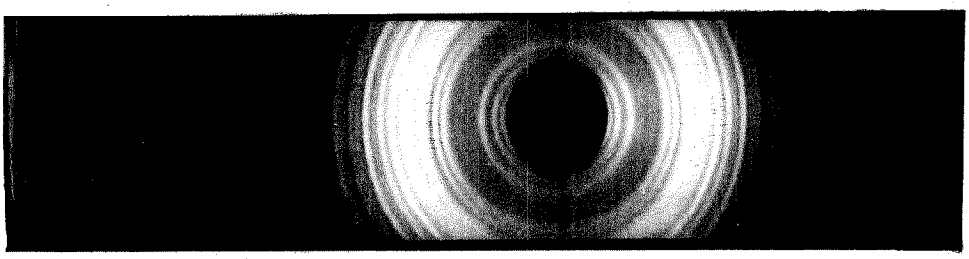
* The author is indebted to Dr. R. B. Corey, Calif. Inst. of Technology, for his generous help in interpreting the photographs, and to Drs. K. K. Chen and S. F. Kern, and Eli Lilly and Company for making their data available to us.



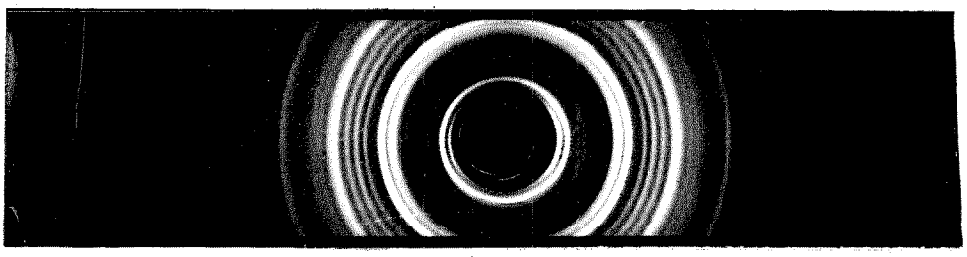
α-Febrifugine



β-Febrifugine



γ-Dichroine

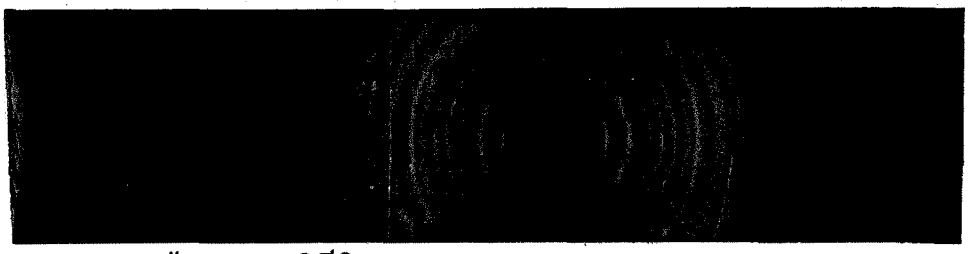


γ-Febrifugine

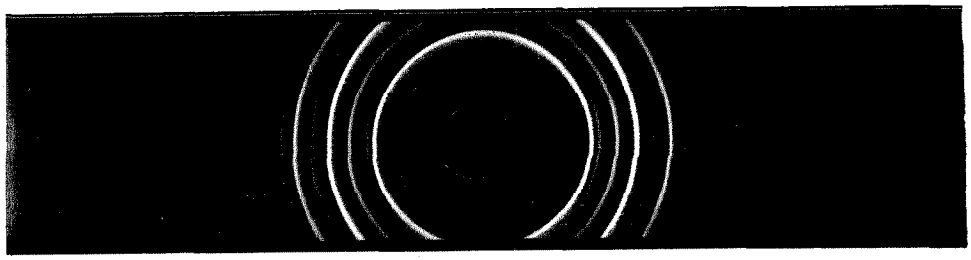
Figure 3. X-Ray Powder Photographs of the Alkaloids.

Figure 3 (cont'd).

δ-Febrifugine



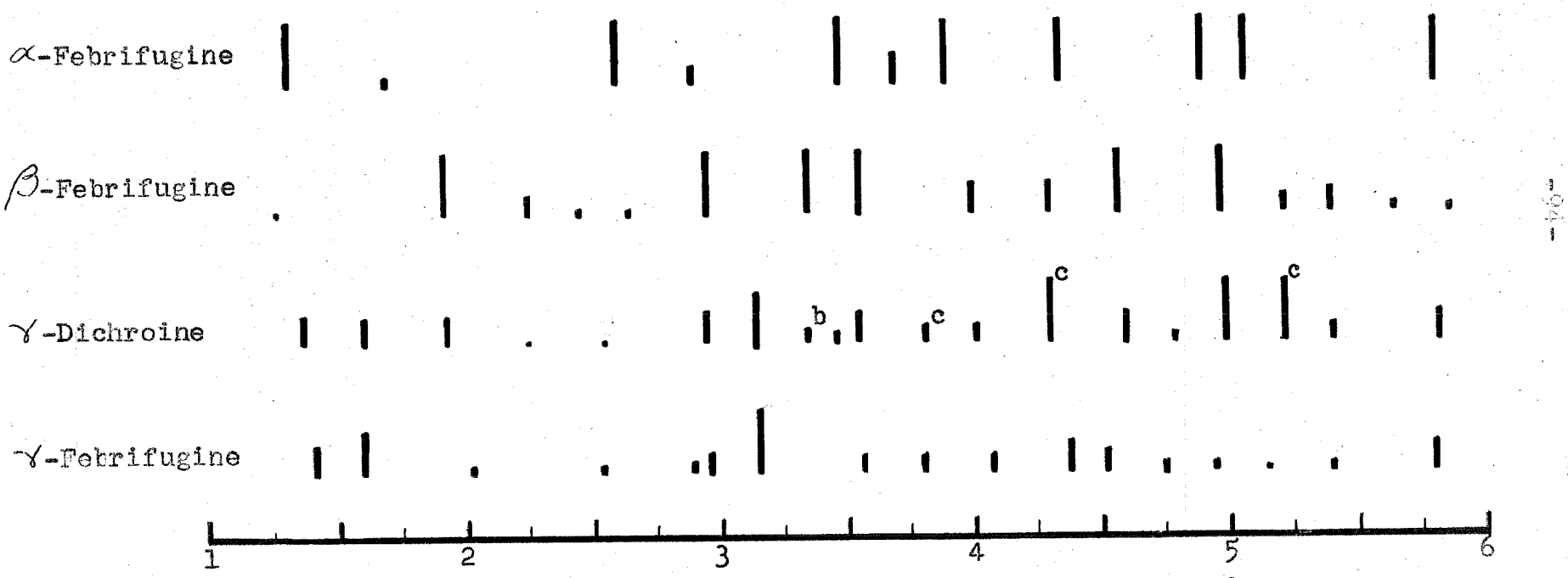
#4, p. 259



Isofebrifugine

Figure 4

Visual Intensities from X-Ray Powder Photographs[a]



[a] The height of a line represents a visual estimate of the relative intensity of a reflection (str., med., wk., v. wk.) on an individual photograph, and the intensities on different photographs are not necessarily directly comparable. [b] Taken from the Lilly photographs; not present in ours. [c] Shown as a weak reflection on the photographs from Lilly.

and γ-dichroine. It was his conclusion that they contain a common morphological constituent. He also recrystallized both samples from cold acetone by evaporation, and obtained identical powder photographs. The data on the recrystallized material agrees with our photographs of α-febrifugine. Dr. Kern's photographs of β-febrifugine and γ-dichroine showed little change over those taken at Lilly some two years before. During this time their samples had sat on a laboratory shelf in closed tubes.

The author retook X-ray powder photographs of the material studied by Dr. Brockman to see if some of the minor discrepancies in intensity could be resolved. Those pictures taken by Dr. Brockman were taken with the powdered sample mounted on vaselined glass fibers, while those taken by the author were with the material in special thin-walled capillary tubes. Thus, there was a possibility that orientation could account for the inability to exactly match relative intensities in the analysis.

Both samples of γ-dichroine gave X-ray powder photographs identical to that of α-febrifugine.

It is of course impossible to say at what point the conversion to α-febrifugine occurred. The later seeding experiments with γ-dichroine may well have been fruitless by reason of not having any γ-dichroine. The γ-dichroine, when not in use had been stored in the refrigerator. It is reasonable that the small amount of moisture picked up by possible opening of the samples before they had warmed to room temperature

could have caused the samples to slowly recrystallize to α-febrifugine. In view of the stability of the samples stored at Lilly at room temperature, it is not likely that the material converted as the dry solids.

Brockman's sample of β-febrifugine was unchanged, but his sample #4, p. 259 had reverted to α-febrifugine. The disposition of these two samples is somewhat indefinite. It is believed that these were in the refrigerator also, but neither would have been opened as frequently as the γ-dichroine.

An old sample of very pure isofebrifugine, stored in the refrigerator for several years, gave an excellent powder photograph identical with later samples.

In summary then, four crystalline modifications of febrifugine (α-, β-, γ-, and δ-febrifugine) have been prepared in this laboratory. The form of febrifugine depends on the conditions of crystallization, but one form (α-febrifugine) is the most easily obtained. The solvents which are most suitable for recrystallization of febrifugine, convert all others to the α-form.

Because of the facile isomerization of febrifugine to isofebrifugine it was necessary to take considerable pains to be certain that the various "crystalline modifications" were not other isomers. With the exception of β-febrifugine, the mode of preparation would normally exclude isomerization from consideration. Koepfli, et al., had already shown by the criteria of rotation, chromatographic behavior, and

reciprocal interchange by cross seeding in cold solvents that α-, and β-febrifugine were identical in solution (3). The remaining forms were shown by their rotations in various solvents, to be identical with α-febrifugine and each on recrystallization, yielded the α-form. In addition, α-, and γ-febrifugine were identical in parallel physiologic experiments.

There remained the rather confusing problem presented by γ-dichroine. The chemical data of its proponents indicated it to be β-dichroine (except for melting point), yet their physiologic data indicated it to be different. Since there was no question but what β-dichroine was febrifugine, the physiologic data on the dichroines was confirmed when investigators in this country claimed that febrifugine and γ-dichroine were different. These latter physiologic experiments lack weight, however, because the tests on the two compounds were run some two years apart. Unfortunately, there is no γ-dichroine available at the present time, and it is unlikely that it will ever be possible to perform parallel pharmacologic tests on febrifugine and γ-dichroine.

γ-Dichroine has been shown to convert very easily to α-febrifugine, even converting during storage in the refrigerator. This was independently confirmed by Dr. Kern when he attempted recrystallization of γ-dichroine and β-febrifugine, and obtained α-febrifugine in both cases. In short, the behavior is just that which would be expected of a polymorphic system. In fact, if one assumes γ-dichroine

is chemically different from febrifugine, the conversion to febrifugine is so facile that it is still difficult to explain how the compounds could behave differently in pharmacologic experiments. It was further shown that the X-ray powder photograph of γ-dichroine indicated it to be a mixture of β-, and γ-febrifugines, two crystalline modifications of febrifugine.

It is concluded that γ-dichroine is not a third isomeric alkaloid from D. febrifuga, but is a mixture of β-, and γ-febrifugines.

Experimental

X-Ray Powder Photographs. All X-ray powder photographs were taken with a Phillips powder camera (57.3 mm.). All photographs taken here and the first photographs at Lilly (1949) were with $CuK\alpha$ radiation. The later photos at Lilly were with $CrK\alpha$ radiation. Dr. Kern's results were submitted with the interplanar spacing given (d/n). This data was recalculated here as 4 θ using the wavelength of $CuK\alpha$ radiation so that the spacing could be compared with the 4 θ measured directly from our photographs.

Dr. Brockman was assisted by R. A. McAllister in the taking of the photographs. The mounting of the author's samples and the photographing of them was done by Dr. John E. Leonard of these laboratories.

Contact prints of the photographs used in the preparation of figure 4 are given in figure 3.

Melting Points. All melting points in these experiments were taken in thin walled capillary tubes in an electrically heated copper block, using a 10X wide field magnifier for observation. In all cases, the rate of temperature rise at the melting point was approximately one degree per minute. All melting points are corrected.

The melting point of isofebrifugine is 129-130°, of α-febrifugine, 139-140°, and of β-febrifugine, 154-156° (3).

In the comparison of γ-dichroine with β-febrifugine, the samples were melted in parallel, introducing them into the apparatus at a temperature of 130°. In each, a softening was noted at 153°, and each melted at 154.0-156.0°. An approximately 1:1 mixture treated in this manner, softened at 153°, and melted at 153.5-156°. It is to be noted that the melting of any of the forms of the alkaloid is accompanied by decomposition and that differences in particle size and sample size may cause a variation of a degree or more in the observed melting point. The identical melting point of γ-dichroine and γ-febrifugine was probably fortuitous.

Preparation of #4, p. 259 (JAB). A sample of α-febrifugine was dissolved in chloroform containing a little benzene to give a saturated solution at room temperature. The solution was centrifuged to clarify it and seeded with γ-dichroine. The solution was then evaporated to about $\frac{1}{2}$ volume with an air stream at room temperature, stirring continuously. After chilling in ice for $\frac{1}{2}$ hour, the crystals were collected and washed with ether, to give a material

melting at 152-154°.

Preparation of γ-Febrifugine. To 3.00 g. of febrifugine dihydrochloride in 25 ml. of water was added 4.5 ml. of 10% sodium hydroxide. The solution was extracted five times with 90 ml. portions of chloroform. After drying with anhydrous sodium sulfate, the chloroform was evaporated with nitrogen, at below room temperature (about 10°), to 125 ml., at which point it was mushy with solid. An equal volume of ether was added and the suspension chilled to 0°. After collecting and washing with 1:1 chloroform-ether, the product (γ-febrifugine) weighed 1.5 g. and melted at 151-153°. A small amount rapidly recrystallized from ethanol (hot about 10 seconds) melted at 137.0-139.0°. By evaporation and addition of more ether, second and third crops of γ-febrifugine were obtained having a combined weight of 0.40 g. and melting at 144.5-148°. Crystallization of the combined second and third crops from 4 ml. of ethanol yielded 0.26 g. of α-febrifugine melting at 136-138°.

Preparation of δ-Febrifugine. To 0.07 g. of febrifugine dihydrochloride dissolved in ½ ml. of water was added 3 drops of 10% sodium hydroxide. The aqueous solution was shaken with 3 ml. of chloroform. The separatory funnel containing the two phases was inadvertently left for 45 minutes. On returning, there were feathery needles suspended in the chloroform. The chloroform suspension was drawn off and filtered and the crystals washed twice with ½ ml. portions of chloroform and once with 1 ml. of ether. The material (δ-febrifugine

sample A) melted at 153-156°.

The above experiment was repeated. In this case the crystals formed within seconds in the chloroform. In a few minutes, the chloroform layer was quite solid with the feathery needles. The aqueous phase was extracted four times with 3 ml. portions of chloroform and all extracts combined. It was necessary to dilute to 20 ml. with chloroform in order to dissolve all the material at room temperature. After drying with potassium carbonate the chloroform was evaporated at reduced pressure and the residue recrystallized from ethanol, yielding α-febrifugine.

The preparation of δ-febrifugine was repeated. In this case, the chloroform suspension of crystals was washed three times with $\frac{1}{2}$ volume portions of water. This treatment largely dissolved the suspended matter, but that remaining (B) had a melting point of 150-152°. Samples A and B gave identical X-ray powder photographs, and the crystal form was designated as δ-febrifugine.

Later, the remainder of sample B was rapidly recrystallized (hot about 5 seconds) from ethanol to give α-febrifugine of melting point 136-137°.

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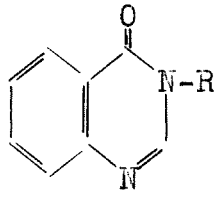
PART II. STUDIES ON 4-QUINAZOLONES

Section 1

Introduction

In part I of this thesis, the structures of the dibenzenesulfonyl derivatives of febrifugine and isofebrifugine were discussed. The insolubility of the derivatives in acid had suggested the possibility that the 1-nitrogen of the quinazolone ring was involved in the reaction with benzenesulfonyl chloride. Though this appeared unlikely, it seemed desirable to experimentally determine if benzenesulfonyl chloride would react with 3-substituted 4-quinazolones, i.e. compounds of the general structure I.



I

The original problem was then the relatively simple task of reacting benzenesulfonyl chloride with a few representative 3-substituted-4-quinazolones. Since the parent substance, 4-quinazolone, was available it was decided to round out the investigation by testing its reactivity to the reagent. Though the substituted quinazolones did not react, 4-quinazolone did.

The determination of the structure of the initial product and its degradation products constitutes the largest section of this part of the thesis.

Section 2 of this part describes the preparation of model 3-substituted-4-quinazolones and the demonstration that the 1-nitrogen is unreactive to benzenesulfonyl chloride. Section 3 describes the reaction of 4-quinazolone with benzenesulfonyl chloride and the synthesis of certain model compounds used in the determination of the structure of the reaction product. In the course of the investigation, there was encountered an interesting reaction of one of the degradation products. This, and certain miscellaneous experiments are described in section 4. Ultraviolet absorption curves of the various compounds discussed are presented in the appendix.

Section 2

The Preparation of Model 3-Substituted-4-quinazolones and Their Reaction With Benzenesulfonyl Chloride

Three model compounds were chosen for the attempted reaction with benzenesulfonyl chloride. The first compound, the known 3-methyl-4-quinazolone, was chosen as an example of a 4-quinazolone with an inert substituent in the 3-position. The second and third compounds, 3-(β -ketopropyl)-4-quinazolone and 3-(β -hydroxyethyl)-4-quinazolone were chosen because of the presence of an oxygen function β- to the 4-quinazolone moiety in febrifugine and isofebrifugine.

3-(β -Ketopropyl)-4-quinazolone had been previously prepared in these laboratories by Rafat Mirza by the condensation of 4-quinazolone and chloroacetone in alcoholic potassium hydroxide, but no analysis had been obtained. The unknown 3-(β-hydroxyethyl)-4-quinazolone was prepared by the interesting method of Leonard and Ruyle (1) by heating a mixture of 4-quinazolone and ethanolamine.

3-Methyl-4-quinazolone and 3-(β -ketopropyl)-4-quinazolone did not react with benzenesulfonyl chloride in pyridine at room temperature. 3-(β -Hydroxyethyl)-4-quinazolone reacted only to form the ester, 3-(β -benzenesulfonoxyethyl)-4-quinazolone, and as secondary by-products on longer reaction times, 3-(β-chloroethyl)-4-quinazolone, and anthranilic acid. The presence of the anthranilic acid as a secondary reaction product was somewhat of a mystery, as

significant hydrolysis of the various 3-substituted-4-quinazolones would not be expected under the conditions of the reaction and isolation. 3-Substituted-4-quinazolones are in general quite stable to acid conditions but are typically hydrolyzed to anthranilic acid by heating with alkali for a short time, or on treatment with dilute alkali for several days at room temperature (2). To see if perhaps one of the quinazolones involved was unusually susceptible to hydrolysis, the chloride, the alcohol, and the ester were each subjected to conditions comparable to the experiment producing the anthranilic acid. No anthranilic acid could be detected. However, on treatment of pure 3-(β-benzenesulfonoxyethyl)-4-quinazolone with pyridine at room temperature for about one day, a compound was formed which appears to be a quaternary salt formed between the ester and pyridine.

To gain some knowledge concerning the point at which the anthranilic acid arose, the reaction yielding it was repeated. Immediately after decomposition with ice and acidification to pH 3 to 4 (keeping cold), the solution showed a strong blue fluorescence under ultraviolet light indicating the presence of anthranilic acid. Thus, it is indicated that the anthranilic acid was either formed in the pyridine solution during the reaction with the benzenesulfonyl chloride or in the cold aqueous solution by decomposition of some unknown labile side product. The anhydrous conditions existing during the reaction with the benzenesulfonyl chloride makes the latter explanation seem the more likely.

The structure of the 3-(β-benzenesulfonoxyethyl)-4-quinazolone was confirmed by hydrolysis with boiling water to 3-(β-hydroxyethyl)-4-quinazolone. The ester is apparently only very slightly more soluble in dilute acid than in water, in which it is extremely insoluble.

The structure of the chloride was proved by synthesis from the alcohol on treatment with thionyl chloride. The chloride is very slightly soluble in water and readily soluble in 1N hydrochloric acid.

Experimental

3-Methyl-4-quinazolone. 3-Methyl-4-quinazolone was prepared by the method of Knape (3). It had been recently prepared by the same method in this laboratory by Rafat Mirza, who obtained a material melting at about 102° instead of at 71° reported in Beilstein (4) and in Richter (5). Späth (6), in a paper on peganine, reported a melting point of 105° but without comment. After several attempts by Mirza and the author to obtain the lower melting form it was discovered that this had been investigated by Bogert and Geiger and reported in a paper dealing with some other problems (7). They found that the lower melting material is a monohydrate and that drying at 80° in vacuo over sulfuric acid gives the anhydrous compound melting at 105°.* In 1946, Leonard and Curtin (9)

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* A recheck of the literature showed that this paper had been missed because it is not cross indexed in the decennial indices of Chemical Abstracts under the compound in question, though it is listed in Meyer and Jacobson (8).

failed to recognize a material melting at 103.5-105.5°, isolated from methylation of 4-quinazolone with diazomethane, as being 3-methyl-4-quinazolone.

A sample of the higher melting material crystallized from water, and air dried, melted at 68-69.5°. Drying at room temperature *in vacuo* over anhydrous calcium chloride for 2 hours yielded the higher melting material.

Preparation of 3-(β-Ketopropyl)-4-quinazolone*. A solution of 10.0 g. of 4-quinazolone and 4.5 g. of potassium hydroxide in 100 ml. of absolute ethanol was heated to boiling. Six milliliters of chloroacetone in 20 ml. of ethanol were run in over a period of 15 minutes and the mixture was refluxed for a total of 1 hour. The solvent was removed under reduced pressure, the residue ground with 40 ml. of 0.5N sodium hydroxide and allowed to stand overnight at 4°. The sodium hydroxide solution was filtered off and the precipitate washed with cold water, yielding 8.2 g. (58.5%) of 3-(β-ketopropyl)-4-quinazolone. After successive recrystallization from water and acetone it melted at 164-166°.

Anal. Calcd. for $C_{11}H_{10}O_2N_2$: C, 65.33; H, 4.99. Found: C, 65.35; H, 5.12.

Preparation of 3-(β-Hydroxyethyl)-4-quinazolone*. A mixture of 4.0 g. of 4-quinazolone and 10 ml. of redistilled

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* The preparation of these compounds was subsequently reported by Baker, et al. (10). The preparations are reported here because of the use of different conditions in the case of 3-(β-ketopropyl)-4-quinazolone, and a different method in the case of 3-(β-hydroxyethyl)-4-quinazolone.

ethanolamine was heated at 150-155° for 2 hours, at which time the initial vigorous evolution of ammonia had ceased. On cooling, the reaction mixture crystallized to a thick paste. The total material was dissolved in 100 ml. of 1N hydrochloric acid and made basic with 5N sodium hydroxide, which caused the separation of 1.6 g. of a white precipitate. The alkaline solution was extracted five times with 25 ml. portions of chloroform yielding, on evaporation of the chloroform, 1.6 g. of material for a total crude yield of 3.2 g. (61.5%). (It later developed that the distribution coefficient between chloroform and dilute alkali is only 1.2, thus probably about 0.5 g. of product was left in the aqueous phase.) The material was crystallized from absolute ethanol to a constant melting point of 154-156°. A sample held at 157° for ½ hour and allowed to resolidify, remelted at the same temperature.

Anal. Calcd. for $C_{10}H_{10}O_2N_2$: C, 63.15; H, 5.30; N, 14.73. Found: C, 63.21; H, 5.28; N, 14.76.

The ultraviolet absorption spectrum in ethanol showed maxima at 314, 302, 291, 267, and 225 mμ, and a plateau at 270-275 mμ.

Attempted Reaction of 3-Methyl-4-quinazolone with Benzenesulfonyl Chloride. To 1.0 g. of 3-methyl-4-quinazolone (m.p. 103-104°) in 5 ml. of pyridine*, was slowly added 3.8 ml. of benzenesulfonyl chloride. The solution became warm. It was

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* This and all pyridine later mentioned was either Baker & Adamson or Merck, reagent grades, and was allowed to stand over potassium hydroxide pellets for at least one week before use.

stoppered tightly and allowed to stand at room temperature overnight. Long clear needles had separated from the pyridine. These were collected, washed with pyridine and then with ether and dried, yielding 0.7 g. of colorless crystals. The material was very water soluble to give a solution of pH ca. 4, very insoluble in boiling benzene, very soluble in ethanol, and soluble (ca. 3%) in boiling dioxane. Crystallization from 4:1 benzene-ethanol or from dioxane gave feathery plates melting at 131-132° and giving positive qualitative tests for the presence of nitrogen and sulfur, and a negative test for halide (by the Zn-CaO fusion method (11)). The material proved to be identical with that obtained when pyridine, benzenesulfonyl chloride and a trace of water were allowed to stand at room temperature. This material was not investigated further.*

The original pyridine phase was evaporated to dryness and treated with 40 ml. of 1N sodium hydroxide. The sodium hydroxide solution was extracted seven times with 40 ml. portions of chloroform. Evaporation of the chloroform yielded 0.9 g. of material which after crystallization from benzene

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* Before the origin of the 131-132° melting material was discovered, it was thought that it might be the benzenesulfonic acid salt of 3-methyl-4-quinazolone. Accordingly 3-methyl-4-quinazolone and an excess of benzenesulfonic acid were boiled in a mixture of benzene and ethanol. On cooling, jagged needles appeared which were recrystallized from benzene-ethanol to a constant melting point of 205-207°. No analysis was obtained.

was identified by melting point and mixed melting point as 3-methyl-4-quinazolone. The alkaline solution was then made acid with hydrochloric acid and extracted three times with 40 ml. portions of chloroform. Evaporation of the chloroform yielded 0.06 g. of a red paste.

Attempted Reaction of 3-(β-Ketopropyl)-4-quinazolone with Benzenesulfonyl Chloride. To 0.80 g. of 3-(β-ketopropyl)-4-quinazolone (m.p., 165°) in 4.42 ml. of pyridine, was added 1.42 ml. of benzenesulfonyl chloride and the mixture was tightly stoppered. After one day at room temperature, the mixture was poured over about 30 g. of ice and water. Filtration yielded 0.25 g. of solid melting at 164-165°. The solution was made quite basic with 20% sodium hydroxide and extracted three times with 40 ml. portions of chloroform. Evaporation of the chloroform yielded 0.43 g. of material melting at 161-163°. After crystallization from water the combined material melted at 163.7-165.2° and gave no depression on admixing with starting material. The starting material was thus recovered to the extent of 96%. Reaction periods up to 5 days gave the same result.

Reaction of 3-(β-Hydroxyethyl)-4-quinazolone with Benzenesulfonyl Chloride. (A) One gram of 3-(β-hydroxyethyl)-4-quinazolone and 2.02 ml. (2 fold excess) of benzenesulfonyl chloride were dissolved in 4.42 ml. of pyridine at room temperature. The temperature rose spontaneously to 30-35°. In about 20 minutes, crystals began to appear. After a total of 1 hour and 5 minutes the mixture was shaken which caused it to

become solid with crystals. The mass was then scraped into 30 g. of ice and made acid with 12N hydrochloric acid. The mixture was filtered and the almost white solid washed thoroughly with cold water to yield 1.45 g. (83.5%) of the ester, 3-(β-benzenesulfonoxyethyl)-4-quinazolone. The material was purified by crystallization from hot methanol, which did not significantly alter its melting behavior. When the point was taken slowly, the ester softened at 125-130°, setting to a glass-like material which then melted quite indefinitely over a wide range, and did not in general become completely liquid till above 200° where darkening became pronounced. When the material was placed in the melting point apparatus at 150°, it melted immediately to clear liquid which was rapidly converted to the glass-like substance mentioned above.

Anal. Calcd. for $C_{16}H_{14}O_4N_2S$: C, 58.17; H, 4.27; N, 8.49. Found: C, 57.71; H, 4.41; N, 8.42.

The ultraviolet absorption spectrum in ethanol showed maxima at 314, 302, 292, 272, 266, and 223 mμ.

On treatment of the ester with water or 1N or 3N hydrochloric acid, no material was observed to dissolve. In the case of the acid treatment, the supernatant liquid was made basic and a trace of the ester (identified by melting point) precipitated. Treatment of the ester with 6N hydrochloric acid caused a change in appearance of the solid, but no solution was apparent.

A suspension of 0.15 g. of the ester in 5 ml. of water was

heated to reflux. The original suspension was neutral, but became acid almost immediately on heating, and at the end of 5 minutes all the material had dissolved. On cooling, the solution remained clear, but making the solution basic with 10% sodium hydroxide caused a white precipitate. The alkaline mixture was extracted five times with 2 ml. portions of chloroform, the first portion completely dissolving the solid. Evaporation of the chloroform yielded 72 mg. of material melting at 152°. On recrystallization from ethanol it melted at 153-154.5° and showed no depression on mixing with a sample of 3-(β-hydroxyethyl)-4-quinazolone. From the distribution coefficient of the material (1.2) between chloroform and dilute alkali, it can be seen that at least 1/7 of the total product must have remained in the aqueous phase. The actual amount of alcohol formed must have then been 84 mg. (97%).

(B) The reaction mixture as in (A) was allowed to stand for 48 hours and then scraped into 30 g. of ice and water. Filtration yielded 0.5 g. of solid. The solid was leached twice with 6 ml. portions of 1N hydrochloric acid. The residue melted in the characteristic manner of the ester, and amounted to 0.19 g. The acid solution, on being made basic precipitated 0.30 g. of white solid melting without decomposition at 123-125°. The aqueous pyridine mother liquor from the first precipitate was brought to pH of ca. 2 or less with hydrochloric acid and extracted four times with 15 ml. portions of chloroform. Evaporation of the chloroform yielded 0.205 g. additional of the new material. Recrystallization from

methanol brought it to a constant melting point of 122-125°.

Anal. Calcd. for $C_{10}H_9ON_2Cl$: C, 57.56; H, 4.35; N, 13.43; Cl, 16.99. Found: C, 56.79; H, 4.34; N, 14.22; Cl, 16.99.

The compound was very slightly soluble in water, and readily soluble in 1N hydrochloric acid. It was recovered unchanged from its acid solution by precipitation with alkali. The ultraviolet absorption spectrum in ethanol showed maxima at 314, 302, 266, and 226 mμ, and plateaus at 270-275, and 291-295 mμ. The material proved to be identical with that obtained on treatment of 3-(β-hydroxyethyl)-4-quinazolone with thionyl chloride in the presence of a trace of pyridine.

The acid solution, after the extraction of the chloro compound, was brought to pH ca. 4-5 with sodium hydroxide (brown reaction on congo red indicator paper) and extracted five times with 25 ml. portions of chloroform. Evaporation of the chloroform yielded 0.201 g. of solid. Continuous extraction of the aqueous phase (6 hours) with chloroform yielded an additional 0.040 g. After recrystallization from aqueous methanol, the combined material melted at 143.9-145.4°. The material was soluble in 1N hydrochloric acid and sodium bicarbonate solution and showed no depression in melting point when mixed with an authentic sample of anthranilic acid.

Thus, on the basis of the 3-(β-hydroxyethyl)-4-quinazolone, about 33% of anthranilic acid, 46% of 3-(β-chloroethyl)-4-quinazolone, and 11% of 3-(β-benzenesulfonoxyethyl)-4-quinazolone were isolated.

In one instance where the reaction was allowed to proceed for 6 days, none of the ester could be detected. Only the chloro compound was isolated (anthranilic acid was not looked for).

Reaction (B) was repeated. Immediately after decomposition of the reaction mixture with ice, the solution was brought to a pH of 4-5 with hydrochloric acid (keeping cold). A trace of solid remained. On examination of the mixture with an ultraviolet lamp a strong blue fluorescence was observed.

Synthesis of 3-(β-Chloroethyl)-4-quinazolone. One-half gram of 3-(β-hydroxyethyl)-4-quinazolone was added slowly to 1.25 ml. of cold thionyl chloride in 10 ml. Kjeldahl flask. All went into solution. After the addition of 5 ml. of chloroform and 1 drop of pyridine, the mixture was boiled down to about half its volume, and twice more boiled down to a small volume with 10 ml. portions of chloroform (total heating time, ca. 40 minutes). Addition of 5 ml. of water gave a clear solution, and on making this basic with 10% sodium hydroxide, a precipitate appeared. The slurry was extracted twice with 5 ml. portions and twice with 3 ml. portions of chloroform. Evaporation of the chloroform yielded 0.557 g. of solid melting at 119-123°. Recrystallization from aqueous methanol yielded 0.466 g. (85%) of white needles melting at 123-125°. This material gave no depression in melting point when mixed with the chloro compound isolated from the reaction of benzenesulfonyl chloride with 3-(β-hydroxyethyl)-4-quinazolone.

Attempted Hydrolysis of 3-(β-Hydroxyethyl)- and 3-(β-

Chloroethyl)-4-quinazolone with Base and Acid. Ninety-nine milligrams of 3-(β-hydroxyethyl)-4-quinazolone was dissolved in 30 ml. of water and 10 drops of 10% sodium hydroxide were added to bring the pH to greater than 10. After 1 hour and 10 minutes at room temperature, the aqueous solution was extracted four times with 5 ml. portions of chloroform. Evaporation of the extracts yielded 66.7 mg. of solid. The aqueous phase was acidified to pH ca. 4 with hydrochloric acid and continuously extracted for 8 hours with chloroform. Evaporation of the chloroform yielded 39 mg. of material. Both extracts were identified by melting point and mixed melting point as the starting material. From the amount of the starting material extracted from the dilute base with chloroform it may be calculated that about 1/5 of the remaining material was extracted each time. Since the aqueous phase was about 6 times the organic, it is seen that the distribution coefficient of 3-(β-hydroxyethyl)-4-quinazolone between chloroform and water is about 1.2.

In a similar manner it was shown that solution in pyridine for 2 days at room temperature and re-isolation after dilution with water, did not alter the alcohol or the chloride. Each was recovered quantitatively. In addition, examination of the diluted pyridine solutions with an ultraviolet lamp showed no fluorescence. Under these conditions, 5 mg. of anthranilic acid showed a very strong fluorescence. The chloride was quantitatively recovered after 3 days at room temperature in 1N hydrochloric acid.

Reaction of 3-(β-Benzenesulfonoxyethyl)-4-quinazolone with Pyridine. A solution of 113 mg. of 3-(β-benzenesulfonoxyethyl)-4-quinazolone in 1 ml. of pyridine was allowed to stand at room temperature. After 20 hours the solution was still clear, but on being picked up for examination, rapidly became solid with crystals. After a total of 48 hours, the solid was collected by centrifugation and washed twice with 5 drops of pyridine and 6 times with ½ ml. portions of ether to yield 0.10 g. of white needles, melting at 185-187°. The material was extremely water soluble, giving a neutral solution (universal indicator paper). After crystallization from benzene-ethanol, the melting point became constant at 187-188°. A sample left in air for 4 days showed no visible change, and there was no change in the melting point.

Anal. Calcd. for $C_{21}H_{19}O_4N_3S \cdot \frac{1}{2}H_2O$: C, 60.27; H, 4.82; N, 10.04; S, 7.66. Found: C, 60.61; H, 4.60; N, 9.12; S, 7.43.

The ultraviolet absorption spectrum in ethanol showed maxima at 314, 302, 290.5, 265, and 218 mμ, and inflections at 271-279 and 228-233 mμ.

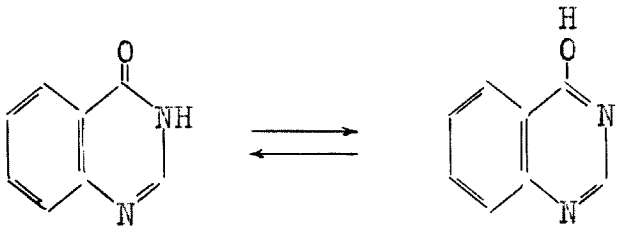
The mother liquor and washings from the isolation of the crude material were diluted with 5 ml. of water and examined with an ultraviolet lamp. No fluorescence could be observed. Addition of 1 ml. of saturated sodium bicarbonate solution and extraction with chloroform yielded 23 mg. of solid identified by melting behavior as starting material. The yield of the presumed quaternary compound was then 71%, or on the basis of unrecovered starting material, 89%.

Section 3

The Reaction of 4-Quinazolone with Benzenesulfonyl Chloride

It was mentioned in the introduction that the original purpose of the series of experiments described in Part II of this thesis was to determine if benzenesulfonyl chloride would react with 3-substituted-4-quinazolones. The parent compound, 4-quinazolone (II), was available as it had been used as an intermediate in the preparation of the 3-substituted-4-quinazolones. It was not expected that this compound would react with benzenesulfonyl chloride. Under Schotten-Baumann conditions (aqueous alkali), no reaction occurred, and when the mixture was acidified, the benzenesulfonic acid salt of 4-quinazolone precipitated. When the reaction was tried in pyridine, a derivative was formed involving the 3(N)-position of the 4-quinazolone.

Before describing this reaction and the ensuing products, it might be instructive to discuss briefly some of the properties of 4-quinazolone. Examination shows it to have



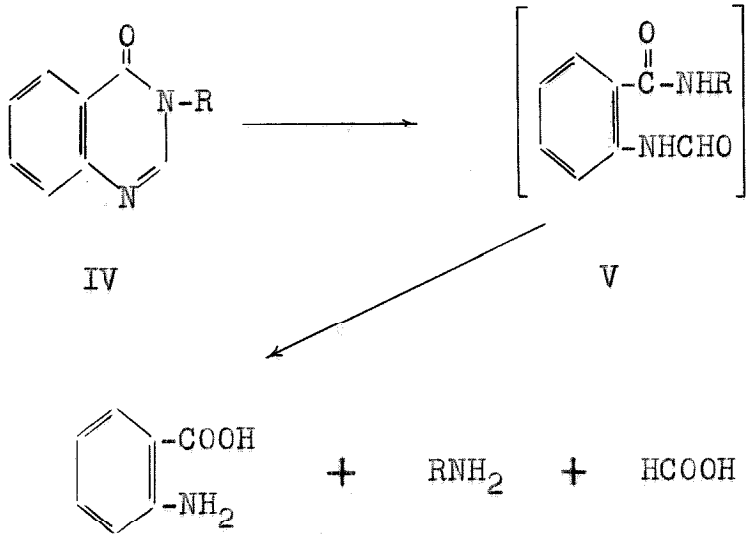
II III

a structure which can be represented by the two tautomeric forms II and III. There is some evidence for this as 4-quinazolone is more soluble in alkali than in water alone, and

potentiometric titration of its benzenesulfonic acid salt (section 4) indicated two acidic groups of pK_{A1} 2.3, and pK_{A2} 8.9. The figure of 2.3 quite obviously belongs to the salt of the tertiary nitrogen, and the 8.9 to the phenolic group of structure III. Elderfield, et al., have reported on the base strengths of various quinazolines (12), including quinazoline, pK_A 3.3, 3,4-dihydroquinazoline, pK_A 8.8, and 3-acetyl-3,4-dihydroquinazoline, pK_A 2.8. It is interesting to note that 4-quinazolone is a weaker base than 3-acetyl-3,4-dihydroquinazoline in which the 3-nitrogen is in the form of an amide as it is in structure II for 4-quinazolone. The difference could be due to the carbonyl group being able, in the case of 4-quinazolone, to exert more influence on the 1-nitrogen. The influence of structure III on the base strength of the 1-nitrogen is not known. Resonance, in such a structure, would be expected to decrease the basicity by lowering the availability of electrons at the nitrogen. This is the usual explanation of the weakly basic nature of pyridine, yet quinoline is a stronger base than aniline by a factor of nearly five. The acid strength of the tautomeric group in 4-quinazolone is seen to be more than ten times that of phenol. This is a rather surprising acidity and no ready explanation is available.

As is usual with such systems, reaction with alkylating agents in an alkaline medium produces largely the 3(N)-substituted derivative, yet reaction with diazomethane yields both the O-, and N-methyl compounds (9). Treatment of the silver

salt with alkylating agents might be expected to yield largely the O-alkyl derivatives. Replacement of the hydroxyl group of structure III with a chlorine or with an alkoxyl group yields a compound with the characteristic reactivity of an acid chloride or an ester (13). As was previously mentioned, 4-quinazolone is quite stable to hydrolysis by acid or alkali, but substitution in the 3(N)-position renders it labile to alkaline hydrolysis. The alkaline hydrolysis of a 3-substituted-4-quinazolone (IV) yields anthranilic acid (VI), formic acid, and a primary amine (VII), presumably by way of a substituted formylanthranilamide such as V.



IV V

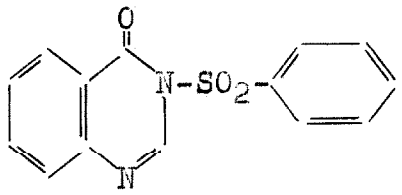
VI VII

When a pyridine solution of benzenesulfonyl chloride and 4-quinazolone was allowed to stand at room temperature for several days, decomposition of the reaction mixture with ice yielded a colorless solid, insoluble in either acid or base. At first, considerable difficulty was had with the purification

of the reaction product. Though the product could be crystallized from most organic solvents of a polar nature, the melting points of successive crystallizations were quite erratic, generally rising gradually to the region of 200° and then unaccountably dropping. Occasionally in the crystallization of the product a much less soluble fraction would suddenly appear. This latter fraction melted at approximately 160-170°, but with a peculiar decomposition. It melted, resolidified, and then did not remelt till about 230-250°. Sometimes this material did not melt, but merely softened, at the lower temperature. This lower melting compound was soluble in sodium bicarbonate solution and insoluble in acid, yet extraction of the initial crude solid with bicarbonate solution did not seem to help in the purification of the higher melting compound. The reason for the erratic behavior of the neutral compound became obvious when it was discovered that a few minutes boiling in a wet organic solvent converted it quantitatively to the acidic compound. The use of carefully dried solvents finally yielded an analytical sample of the neutral compound (A), melting at 195-199°. Analysis indicated $C_{14}H_{10}O_3N_2S$ (mono-benzenesulfonyl derivative of 4-quinazolone). It was confirmed that warm, aqueous organic solvents converted it to the acidic compound (B), $C_{14}H_{12}O_4N_2S$ (addition of the elements of water to the neutral compound). As might be expected, considerable amounts of the acid were found in the original reaction mixture whenever the temperature was allowed to rise during the decomposition with ice (or water), or the

mixture allowed to stand at room temperature for several days after the decomposition with water. Both compounds are very insoluble in water and only slightly soluble in cold organic solvents, the acid being the least soluble in most solvents.

The ultraviolet absorption spectrum of the neutral compound, A, is very similar to that of a 3-substituted-4-quinazolone, but with the positions of the maxima shifted about 4 mμ. toward the visible (Figure 1). This in combination with the analysis, was a strong indication that A is 3-benzene-sulfonyl-4-quinazolone (VIII).



VIII

The base soluble compound, B, has an ultraviolet absorption spectrum which lacks much of the fine structure typical of a 3-substituted-4-quinazolone (Figure 1). Solution of B in alkali and back-titration with acid, using phenolphthalein as indicator, gave a neutral equivalent of 274, considerably lower than the 304 required by the empirical formula, $C_{14}H_{12}O_4N_2S$. Since phenolphthalein has its color change around pH 9 or 10, a low neutral equivalent indicated the possibility of two acid groups with the second ionization constant quite small. The extreme insolubility of the free acid prevented the use of a potentiometric titration to determine the ionization constant

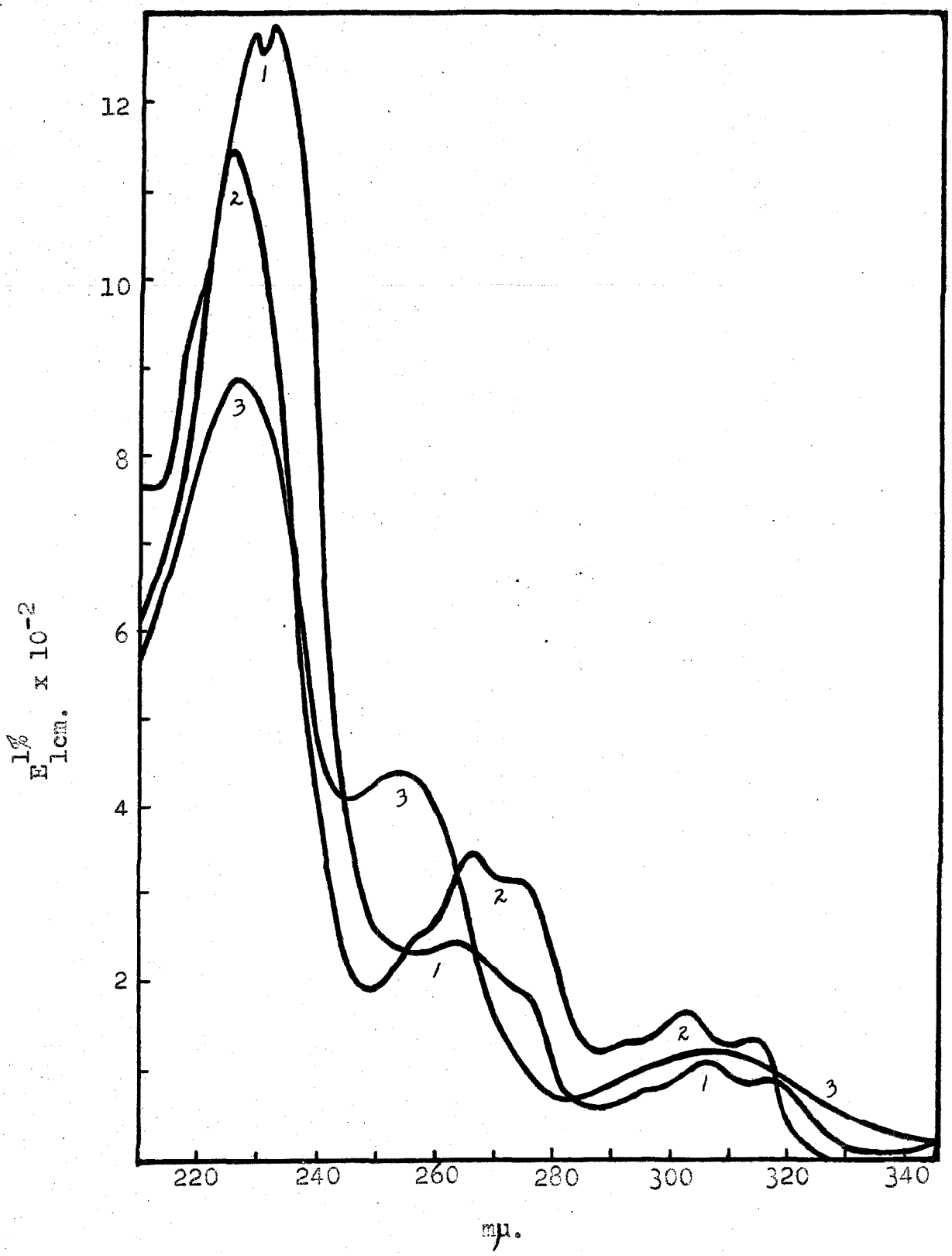


Figure 1. Absorption spectra in absolute ethanol: (1), compound A; (2), 3-methyl-4-quinazolone; and (3), compound B.

(or constants) of B. As it is, the solubility in sodium bicarbonate solution, places the pK_A at about 6 or less.

The melting behavior of compound B was briefly investigated, as the resolidification on heating above the melting point could have been indicative of anhydride or lactone formation. The crude pyrolysis product of B was shown to contain several substances, none of which were isolated pure or identified. Treatment of the crude material with water partially dissolved the solids to give a solution having a pH of ca. 2 or less, indicating at least one of the products to be a strong acid. The crude pyrolysis product gave a negative test for a primary aromatic amine. It is probable, as will be discussed in section 4, that the pyrolysis yields the benzenesulfonic acid salt of 4-quinazolone.

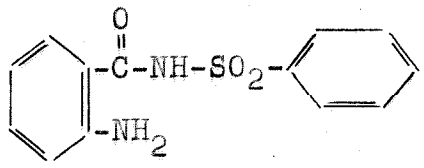
As was discussed earlier for 3-substituted-4-quinazolones, VIII would be expected to be stable to acid and to yield formic acid, anthranilic acid, and benzenesulfonamide on alkaline hydrolysis. Small test experiments showed that both A and B are resistant to boiling 12N hydrochloric acid. Hydrolysis of A with hot 20% sodium hydroxide yielded formic acid and benzenesulfonamide, but the presence of anthranilic acid was not demonstrated. There was isolated what is presumed to have been a mixture of anthranilic acid and a compound having similar properties, probably that which was later isolated from hydrolysis of B.

Vigorous alkaline hydrolysis of B yielded anthranilic acid, formic acid, benzenesulfonamide, and a pale yellow com-

pound of empirical formula $C_{13}H_{12}O_3N_2S$. The yellow compound was very insoluble in water but soluble in sodium bicarbonate solution. It would not dissolve directly in dilute acid, but when excess acid was added rapidly to a dilute solution of the material in alkali, it did not precipitate. If the acid solution was then neutralized carefully with alkali to a pH of ca. 2 or 3, the compound separated from solution. As first precipitated by neutralization of either its acid or alkaline solution, the compound was white, but within a minute or so turned a pale yellow. Crystallization from ethanol did not remove this yellow color. If the crystals were suspended in hydrochloric acid of greater strength than 2N, the yellow material gradually disappeared and within about fifteen minutes the suspension became solid with white, fibrous needles. The more concentrated the acid, the more rapid was the transformation. When a few of the fibrous needles were dried *in vacuo* their appearance did not change. On treatment with a drop of water, the needles did not appear to dissolve, but were converted to the yellow material, yielding an aqueous supernatant which was strongly acid and which gave a white precipitate with silver nitrate in nitric acid. The original yellow material gave a weak test for the presence of a primary aromatic amine.

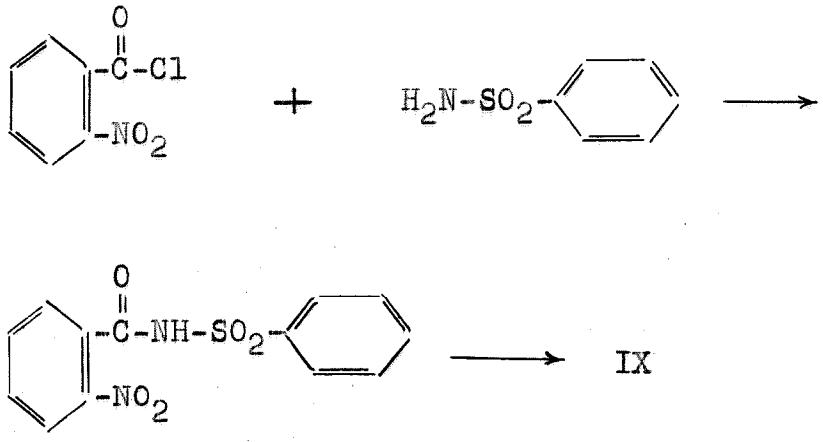
The above solubility behavior of the hydrolysis product of B indicated an acid function having a pK_A of 6 or less. The data also indicated the presence of a very weak primary aromatic amine forming a sparingly soluble hydrochloride. On this basis, the most probable structure consistent with the empirical

formula of $C_{13}H_{12}O_3N_2S$ and the probable structure of A (VIII), is N-(o-aminobenzoyl)-benzenesulfonamide (IX). A few compounds



IX

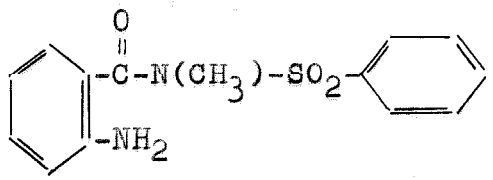
having the N-benzoylbenzenesulfonamide structure have been prepared (14-20) and are known to be soluble in bicarbonate solution. The structure of the hydrolysis product as IX was proved synthetically. The simplest and most unequivocal synthesis appeared to be the direct fusion of o-nitrobenzoyl chloride and benzenesulfonamide by the method of Gerhardt (14) to yield N-(o-nitrobenzoyl)-benzenesulfonamide (X), and subsequent reduction to the desired amine. The synthetic



X

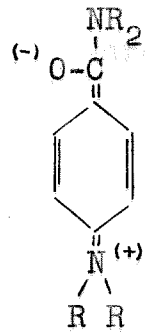
material was identical in every respect with that isolated from hydrolysis of B.

The picture is marred slightly by the unexplained observation of the change in color, from white to yellow, immediately after precipitation from solution. The absorption spectrum, in ethanol, was not determined at wavelengths greater than 350 mμ., but at this point the absorption was increasing and was apparently near the peak of a very broad absorption band. The yellow color most probably arises from the tailing of this band into the violet end of the visible. A similar compound, N-methyl-N-(o-aminobenzoyl)-benzenesulfonamide (XI), was also yellow and showed a similar broad absorption band

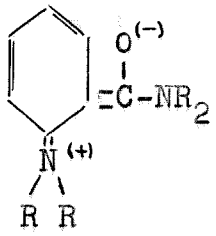


XI

centered at 358 mμ. with a corresponding molecular extinction of 3,800. The molecular extinction of IX at 350 mμ. was 5,200. The formyl derivative of XI was colorless, as was the later described tri-N-methyl derivative of IX. Other compounds of the series, but which lack the amino group, are colorless. The standard compilations list m-aminobenzamide and p-aminobenzamide as yellow solids. No reference could be found concerning the color of the ortho isomer. The most obvious explanation of a yellow color in ortho and para isomers of such compounds would be the assumption that quinoid forms such as XII or XIII contribute significantly to the structures of the molecules. This explanation is in agreement with observation



XII



XIII

that the hydrochloride salt of IX is colorless. It is also in accord with the tri-N-methyl derivative being colorless and a slightly stronger base, for an o-dimethylamino group would have difficulty approaching co-planarity with the benzene ring.* It does not, however, explain why the initial precipitate of IX is colorless, and why m-aminobenzamide is yellow.

In spite of the difficulties with color, the structure of the hydrolysis product of B seems established as N-(o-aminobenzoyl)-benzenesulfonamide (IX). In compound B, then, there is a benzenesulfonyl group attached to a nitrogen atom which was originally the 3-nitrogen of a 4-quinazolone, and the structure of A is confirmed as 3-benzenesulfonyl-4-quinazolone (VIII).

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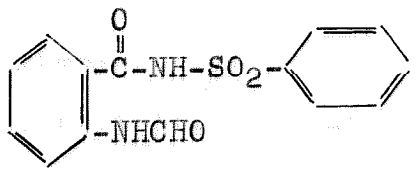
* The base strength of o-aminobenzoic acid and its esters is increased by about 3.5 $p\overline{K}$ units by di-methylation of the amine (21). The relative basicities noted above were based on somewhat qualitative observations of the solubilities in dilute acid and are not at all certain.

It is noted that N-(o-aminobenzoyl)-benzenesulfonamide arose from B by loss of one carbon and one oxygen. This could represent the hydrolytic removal of a formyl group, and the presence of formic acid in the hydrolyzate has been qualitatively demonstrated. A tentative structure for B would then be N-(N'-formyl-o-aminobenzoyl)-benzenesulfonamide (XIV). Since the free amine, IX, was available it was heated with formic acid



XIV

in the hopes of preparing the N-formyl derivative XIV, and comparing it with B. The reaction product was not identical with B. This caused a certain amount of confusion until it was discovered that the synthetic material, though it had the correct empirical formula, was not XIV, but the benzenesulfonic acid salt of 4-quinazolone.* Formylation of IX at room temperature with a mixture of formic acid and acetic anhydride gave an excellent yield of B, leaving little doubt that the structure of B is XIV. This structure has only one acidic group capable of detection by titration. Evidence was accumulated, however, that significant hydrolysis of B, to IX and formic acid, occurs at room temperature in aqueous alkali.

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* This interesting reaction will be discussed in Section 4.

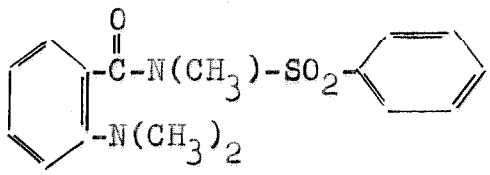
The low neutral equivalent of B was not, therefore, due to a second acidic function, but resulted from partial hydrolysis.

In the course of the investigation of the structure of B, its reaction with various methylating agents was studied. The results are presented here, and interpreted on the basis of structure XIV.

Reaction with methyl iodide in aqueous alkali gave inconclusive results. It was clear that a reaction had taken place, but the individual reaction products appeared to be difficult to isolate in the pure state. It was clear that at least two compounds were formed. One was yellow, insoluble in dilute acid, and soluble in alkali. The other was amphoteric and white, but rapidly became yellow on standing in organic solvents or on exposure to air. This reaction was investigated prior to the discovery of the amine, IX, as a hydrolysis product of B. It is likely that the yellow, acid-insoluble compound was this amine, formed by hydrolysis under the alkaline conditions of the reaction, and was not a methylation product. The other compound could well have been a methylation product. Being amphoteric, it was possibly a methyl derivative of the aromatic amine of IX. Its rapid conversion from a white to a yellow compound could have been the same phenomenon as observed with IX, and is again not explained.

Methylation of B with dimethyl sulfate in aqueous alkali gave a small yield of a $C_{16}H_{18}O_3N_2S$ compound. The same compound was obtained in about the same yield on similar treatment of IX. Analysis indicated the presence of three N-methyl

groups. The derivative was insoluble in water and alkali, and though sparingly soluble in acid was qualitatively more soluble than IX or XI, possibly indicating a slightly stronger basic group. The empirical formula, the presence of three N-methyl groups, the lack of solubility in alkali, and the preparation from N-(o-aminobenzoyl)-benzenesulfonamide lead to the structure N-methyl-N-(o-dimethylaminobenzoyl)-benzene-sulfonamide (XV) for this compound.



XV

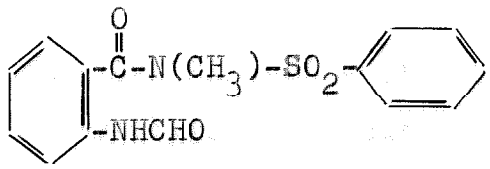
The fact that this trimethyl derivative was obtained in about the same yield from the amine, IX, as from B, indicates that it arose from B via hydrolysis to the amine. This combined with the tentative evidence that some hydrolysis occurred (without methylation) in the attempted methylation with methyl iodide, leads to the conclusion that B is slowly hydrolyzed by dilute alkali at room temperature.

Reaction with methyl iodide and dimethyl sulfate thus yielded very little additional information in support of the proposed structure of B.

Attention was focused on the group in B causing the solubility in sodium bicarbonate solution. When a solution of diazomethane was added slowly (at 0^{o}) to a solution of B, the yellow color of the diazomethane was discharged very rapidly

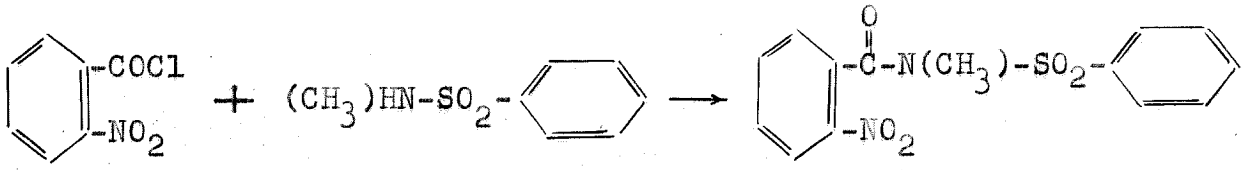
until about an equimolar amount had been added. The color of an additional portion of diazomethane solution remained indefinitely. From the resulting solution, it was possible to isolate three compounds, melting respectively at 195-200°, 154-155°, and 118-119°. The empirical formulae indicated these to be isomeric, mono-methyl derivatives of B. The separation of these derivatives was a tedious process. The compound to be considered first, the one melting at 118-119°, was not detected for some time, but once conditions were found for its crystallization from the residual oils, it could be consistently isolated in yields of 20 to 30 percent.

This derivative was found to be identical with a previously prepared model compound, N-methyl-N-(N'-formyl-o-aminobenzoyl)-benzenesulfonamide (XVI), the structure of which was

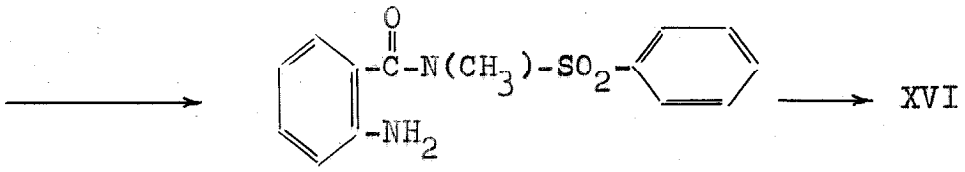


XVI

quite certain; it had been synthesized by fusion of o-nitrobenzoyl chloride with N-methyl-benzenesulfonamide to give N-methyl-N-(o-nitrobenzoyl)-benzenesulfonamide (XVII), followed by reduction to N-methyl-N-(o-aminobenzoyl)-benzenesulfonamide (XVIII), and formylation with formic acid to XVI. Saccharin has been shown to yield a mixture of the O- and N-methyl derivatives on treatment with



XVII



XVIII

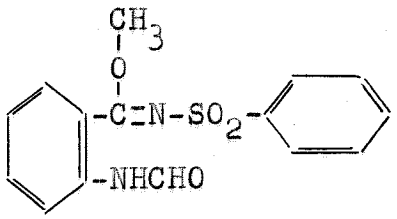
diazomethane (22). By analogy then, N-(N'-formyl-o-amino-benzoyl)-benzenesulfonamide (XIV) would yield the N-methyl compound, XVI, as one of its methylation products. This is then independent confirmation of XIV as the structure of B.

The structures of the two remaining methylation products of B, melting at 155° and 200°, are not clearly established. These compounds were both insoluble in dilute acid and dilute alkali (as was XVI). Both gave faint, atypical tests for the presence of esters.

Two alkali insoluble products would have presented no problem. One of these would be the already described N-methyl compound, and the other an O-methyl derivative, XIX. It is not clear where a methyl group could be placed on XIV to yield a third, alkali-insoluble derivative.

The derivative melting at 155° accounted for 20 to 30 percent of the starting material. A quantitative methoxyl

determination indicated the presence of one methyl group attached to oxygen. Its ultraviolet absorption spectrum in



XIX

ethanol showed only one well defined maximum (224 mμ.). The general appearance of the absorption curve was almost identical with that of the N-methyl derivative (XVI), but with the whole curve shifted 9 or 10 mμ. toward longer wavelengths. It was not found possible to correlate the ultraviolet absorption spectra of the various model compounds in this series with their structures, as the effect of the substituents is apparently quite complex. A shift of 10 mμ., however, is not unreasonable in going from the N-methyl compound (XVI) to the O-methyl compound (XIX). The methyl derivative of B melting at 155° has been, therefore, tentatively assigned the structure XIX.

The product melting at 200° was isolated in inconsistent yields. In some cases, none was detected. In no case was there more than about 8 percent isolated. A quantitative methoxyl determination showed the material to have no methyl groups attached to oxygen, and probably none attached to

nitrogen.* The ultraviolet absorption spectrum was identical in every respect with that of the first product of reaction of benzenesulfonyl chloride with 4-quinazolone, compound A (m.p., 195-199°). Unfortunately, the similarity of the supposed methylation product of B to A was not noticed at first. Without re-isolation of an additional supply of the derived product, it was not possible to check the original analysis, and time did not permit this when the possibility of error in the empirical formula was discovered. A trace of the supposed methylation product was available, however, and a mixed melting point determination was made with a sample of A. No depression of the melting point was observed in parallel melting with the individual samples, but neither sample was of high purity, and the result must be considered as inconclusive. It is felt that the samples of B used in the methylation experiments were not contaminated with A; a stage in the purification of B involved solution in alkali, followed by filtration and precipitation with acid. However, the rather erratic appearance of the high melting compound in various repetitions of the methylation of B, is perhaps significant in this respect, and contamination cannot be completely ruled out. It is concluded that the highest melting material isolated from the reaction of diazomethane with B was possibly not a methyl derivative of B, but was compound A.

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* The determination showed 0.00% methoxyl, and it was the opinion of the analyst, Dr. Elek, that this indicated the absence of N-methyl groups as well.

In summary then, 4-quinazolone is unreactive to benzenesulfonyl chloride in aqueous alkali, but reacts in pyridine solution to yield 3-benzenesulfonyl-4-quinazolone (VIII). This compound is readily hydrolyzed by warm aqueous solvents to N-(N'-formyl-o-aminobenzoyl)-benzenesulfonamide (XIV), an acidic substance. Hydrolysis of this material with alkali yielded the free amine, N-(o-aminobenzoyl)-benzenesulfonamide (IX). Reaction of the formyl derivative (XIV) with diazomethane yielded three compounds. The structure of one of these was proved by synthesis to be N-methyl-N-(N'-formyl-o-aminobenzoyl)-benzenesulfonamide (XV), and another was tentatively assigned the isomeric structure with the methyl group on oxygen instead of nitrogen. The third compound was a minor product, and is suspected to have been 3-benzenesulfonyl-4-quinazolone, the presence of which is unexplained.

Experimental

Attempted Reaction of 4-Quinazolone with Benzenesulfonyl Chloride in Aqueous Alkali. A solution of 0.5 g. of 4-quinazolone in 5 ml. of 10% sodium hydroxide was shaken vigorously with 0.4 ml. of benzenesulfonyl chloride until all of the acid chloride had gone into solution. The resulting clear solution was acidified with 6N hydrochloric acid and chilled to 0°. Filtration yielded 0.7 g. of colorless needles which when recrystallized from water and from dilute hydrochloric acid (less soluble in this) melted at 259-260°. The material was slightly soluble in water at room temperature to give a solution acid to litmus.

To prepare the benzenesulfonic acid salt of 4-quinazolone, a slight excess of benzenesulfonic acid was added to 0.25 g. of 4-quinazolone dissolved in 2 ml. of 0.5N hydrochloric acid. On cooling to 0°, there were obtained colorless needles which were then crystallized from 90% ethanol to a constant melting point of 258-260°. A mixture of this and the material from reaction with benzenesulfonyl chloride in alkali showed no depression in melting point. No analysis was obtained, but the material did not depress the melting point of a sample prepared by a different route (section 4) having the correct empirical formula, $C_{14}H_{12}O_4N_2S$.

Reaction of 4-Quinazolone with Benzenesulfonyl Chloride in Pyridine. This reaction was repeated many times and under various conditions. A typical reaction will be presented, and afterward certain variations will be indicated.

A solution of 1.00 g. of 4-quinazolone and 2 ml. of benzenesulfonyl chloride in 4 ml. of pyridine was allowed to stand at room temperature for 4 days. The reaction mixture was carefully decomposed with 2 ml. of water, and filtered. The solid material was washed four times with 2 ml. portions of water, three times with 5 ml. portions of half-saturated sodium bicarbonate solution, and again thoroughly washed with water. After drying in vacuo at room temperature, the almost white solid weighed 0.78 g. It was recrystallized seven times from

1:1.5 benzene-dioxane* which caused the melting point to slowly rise from about 180-190° to 204-209°. It was then crystallized three times from dioxane alone. The melting point immediately became 195-199° and remained constant. This material (A) was moderately soluble in polar, organic solvents, but insoluble in water and in dilute acid and dilute alkali. The ultraviolet absorption spectrum, in ethanol (figure 1), showed maxima at 229, 232, 264, 305, and 318 mμ., minima at 230, 260, 287, and 313 mμ., and plateaus at 272-275 and 295-300 mμ.

Anal. Calcd. for $C_{14}H_{10}O_3N_2S$: C, 58.73; H, 3.52; N, 9.79; S, 11.20. Found: C, 58.80; H, 3.63; N, 9.82; S, 11.10.

The aqueous pyridine mother liquors and the washings from A were acidified with 6N hydrochloric acid which yielded, after filtration and drying, 0.30 g. of powdery solid. This was dissolved in a few ml. of water containing 3 drops of 6N ammonium hydroxide, precipitated by the addition of 0.5N hydrochloric acid, and the operation repeated yielding an almost white solid (B) melting at 165-166° (dec.). One crystallization from dioxane yielded white crystals melting at 166-167° (dec.), and a second crystallization from benzene-dioxane caused no change. Melting points as high as 175° have been frequently noted for this material, but these usually dropped to the lower value on further crystallization. The product was soluble at room temperature to the extent of about 32 mg., 90 mg., and

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* The dioxane was purified by refluxing for 7 hours over solid sodium hydroxide, followed by refluxing over sodium until no more was consumed, and distillation from the excess sodium. The benzene was prepared by distillation of reagent grade benzene, discarding a liberal fore-run in order to remove water.

12 mg. in 100 ml. of benzene, chloroform, and ether, respectively. It was very soluble in pyridine, moderately soluble in dioxane, insoluble in water, and dilute acid, but soluble in sodium bicarbonate solution. Nothing could be extracted into chloroform from the solution in 1N sodium bicarbonate. The ultraviolet absorption spectrum, in ethanol (figure 1), showed maxima at 226, 254, and 304 mμ., and minima at 245, and 282 mμ.

Anal. Calcd. for $C_{14}H_{12}O_4N_2S$: C, 55.25; H, 3.98; N, 9.21; S, 10.53; neut. equiv., 304. Found: C, 55.38; H, 4.02; N, 9.30; S, 10.43; neut. equiv., 274.

If the reaction mixture is kept cold during the decomposition with water and filtered shortly thereafter, the yield of the base insoluble compound (A) is increased at the expense of the base soluble compound (B). If the temperature is allowed to rise during the decomposition with water or the aqueous suspension is allowed to stand at room temperature, the yield of B is increased at the expense of A. Four to six days at room temperature or a few minutes at 80-100° are sufficient to destroy all of the base insoluble compound. The use of either a small or large excess of benzenesulfonyl chloride (over 1 mole) had no apparent effect on the yield. The best yield of A (89%) was obtained with a reaction time of 12 days.

When 256 mg. of A was heated on a hot water bath with 1 ml. of water and 2 ml. of dioxane it went rapidly into solution, and in a few minutes solid began to appear. After a

total of 15 minutes, the mixture was evaporated to dryness with a stream of air yielding a residue which was largely soluble in alkali. Acidification of its alkaline solution yielded 230 mg. of white solid which melted at 165-166°, and which did not depress the melting point of a sample of B. The use of aqueous pyridine, or aqueous ethanol gave substantially the same result.

Pyrolytic Decomposition of B. If a sample of B is held at a temperature above the melting point for a few minutes it resolidifies and requires a temperature of 230-250° to re-melt. When a small sample was decomposed in this manner in an evacuated melting point tube and the tube opened under water, the tube was filled completely with water. A portion of the solid dissolved and the pH of the aqueous phase became about 2 or 3.

A somewhat larger sample of B was decomposed by heating at 170-180° for 15 minutes. A small sample of the crude product gave no color when treated with nitrous acid and then β-naphthol in alkali (23). Another sample was successively leached with 1N sodium bicarbonate, 1N sodium hydroxide, and 2N hydrochloric acid. Acidification of the sodium bicarbonate solution yielded a precipitate with the characteristics of B. The sodium hydroxide and hydrochloric acid solutions each yielded a precipitate on neutralization.

Alkaline Hydrolysis of A. Approximately 1 mg. of A was was heated to 100° in a sealed capillary tube containing 10 microliters of 20% sodium hydroxide. The solid dissolved

within about 2 minutes. After 15 minutes, the solution was tested for the presence of formaldehyde (24a) and the test was negative. When similar hydrolyzates were tested for the presence of formic acid (24b), and a diazotizable amine (23), the tests were positive. A small sample treated as above, but in a bent capillary tube having some Nessler's reagent in the unheated upper arm, gave no coloration of the reagent. Heating with 12N hydrochloric acid for 2 hours at 100° yielded only a very faint test for formic acid.

A suspension of 200 mg. of A in 1 ml. of 20% sodium hydroxide was heated at 100° until a clear solution was obtained. The solution was then neutralized with carbon dioxide and diluted to 5 ml. A precipitate formed, and the whole was extracted three times with 5 ml. portions of ether, the first of which dissolved the precipitate. Evaporation of the ether yielded a white solid which after crystallization from aqueous ethanol melted at 153-155°, and did not depress the melting point of a sample of benzenesulfonamide.

After the above extraction with ether, the aqueous phase was acidified with hydrochloric acid to pH ca. 4, causing a trace of solid to precipitate. The mixture was extracted with ether, evaporation of which yielded a reddish paste. Sublimation of the paste (110°/1 mm.) gave a white solid which was very slightly more soluble in dilute acid than in water. Recrystallization from water brought the melting point to 130-145°. The crystals were small and chunky, and not the characteristic needles of anthranilic acid.

Alkaline Hydrolysis of B. The small test hydrolyses described for A were repeated with B, and the same results were obtained.

A solution of 1.00 g. of B in 8 ml. of 20% sodium hydroxide was refluxed for 5½ hours, diluted to 40 ml., neutralized with carbon dioxide, and extracted four times with 40 ml. portions of chloroform. Evaporation of the chloroform yielded 0.21 g. of material identified, as in the case of A, as benzenesulfonamide. The aqueous solution was then made strongly acid (blue reaction with congo red indicator paper) and 0.25 g. of yellow precipitate was collected by filtration. Partial neutralization of the solution (to pH ca. 4) caused the precipitation of an additional 0.12 g. of the yellow material.

The aqueous phase was exhaustively extracted with chloroform in a continuous extraction apparatus. Evaporation of the chloroform yielded 0.21 g. of solid, which after sublimation (110°/1 mm.), and crystallization from aqueous ethanol, melted at 145-146°, and did not depress the melting point of an authentic sample of anthranilic acid.

The yellow precipitates (0.25 g., and 0.12 g.) were combined and crystallized from 50% ethanol, giving tiny, pale yellow crystals melting at 177-178°.

Anal. Calcd. for $C_{13}H_{12}O_3N_2S$: C, 56.50; H, 4.34; N, 10.15; S, 11.62. Found: C, 56.67; H, 4.58; N, 10.15; S, 10.78.

The ultraviolet absorption spectrum, in ethanol, showed maxima at 228, 252, and 272 mμ., and minima at 244, 271, and 283 mμ. The molecular extinction at 350 mμ. was 5,260, and was

approaching a broad maximum in the direction of longer wavelengths. A small sample gave an orange-red-precipitate when treated with nitrous acid followed by β-naphthol in alkali (23). The material did not dissolve in dilute acid, but dissolved in 1N sodium bicarbonate with evolution of carbon dioxide. When an alkaline solution of the material was slowly acidified, a white precipitate was obtained. The precipitate turned yellow within about one minute. When a second alkaline solution was rapidly made strongly acid, no precipitate appeared. Partial neutralization (to pH ca. 4) yielded a white precipitate which rapidly turned yellow. Treatment of the yellow material with 3N hydrochloric acid caused a slow metamorphosis (6N, rapid) to white, fibrous needles, the color of which was stable on drying in air or *in vacuo*. Treatment of the white needles with water caused a metamorphosis to the small yellow crystals. The supernatant liquid had a pH of ca. 2, or less, and gave a copious white precipitate when treated with 5% silver nitrate in 3N nitric acid.

N-(o-nitrobenzoyl)benzenesulfonamide (X). A mixture of 10 g. of o-nitrobenzoic acid and 23.8 g. of thionyl chloride was refluxed until reaction was complete (40 minutes). The excess thionyl chloride was distilled off and the residue was boiled down twice with benzene to remove the last of the thionyl chloride. The crude o-nitrobenzoyl chloride was then mixed with an equivalent quantity (9.26 g.) of benzenesulfonamide and heated in an oil bath at 150-155°, at which temperature a steady stream of hydrogen chloride was evolved. After 1¼ hours, the

flow of gas had diminished, and the mixture was allowed to cool, yielding a dark, semi-crystalline mass. Crystallization from 200 ml. of 50% ethanol yielded 14.7 g. (80%) of colorless, chunky crystals melting at 167-168°.* The ultraviolet absorption spectrum, in ethanol, showed maxima at 218, 247, 259, and 265 mμ., minima at 242, and 264 mμ., and a plateau at 271-274 mμ.

Anal. Calcd. for $C_{13}H_{10}O_5N_2S$: N, 9.14. Found: N, 9.22.

N-(o-aminobenzoyl)benzenesulfonamide (IX). A solution of 20.0 g. of the nitro compound (X) in 100 ml. of 6N ammonium hydroxide was poured in a thin stream into 300 ml. of boiling water containing 126.8 g. of ferrous sulfate heptahydrate (7 molecular equivalents) (25). The resulting black solution was treated with concentrated ammonium hydroxide in small portions, shaking vigorously between additions, until the reaction mixture was definitely alkaline. It was then boiled for five minutes and immediately filtered with suction. The clear, yellow filtrate was then chilled rapidly in an ice bath (the yield is lowered if the solution is allowed to remain hot). Colorless crystals (identified by mixed melting point as benzenesulfonamide) separated, and were removed. With vigorous stirring, 6N hydrochloric acid was slowly added until the solution turned congo red indicator paper a definite blue. The resulting

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* There is evidence that this material is dimorphic. In the first preparation, the first three crystallizations yielded colorless needles, and the melting point leveled off at 136-137°. The fourth crystallization yielded the higher melting material, and the fifth required more than twice the amount of solvent. In subsequent preparations, the lower melting material was not observed.

yellow precipitate, when collected and dried, weighed 9.29 g. and melted at 172-176°. Crystallization by solution in 200 ml. of hot ethanol, addition of an equal volume of hot water, and chilling, yielded tiny, pale yellow crystals, melting at 176-178°. A mixed melting point with similar material from the alkaline hydrolysis of B, showed no depression.

N-(N'-formyl-o-aminobenzoyl)benzenesulfonamide (XIV). To a mixture of 0.40 ml. of acetic anhydride and 0.16 ml. of 99% formic acid, was added 0.80 g. of N-(o-aminobenzoyl)benzenesulfonamide. The yellow color of the amine rapidly disappeared, and the temperature rose spontaneously to ca. 40°. The mixture was allowed to stand for 4 hours, dissolved in 50 ml. of 1N sodium bicarbonate, and filtered to remove a trace of insoluble material. Acidification of the solution with 1N hydrochloric acid gave a voluminous precipitate, which was collected, washed thoroughly with water, and dried. The yield was 0.72 g., melting at 165-167°. A mixed melting point with B showed no depression.

Reaction of B with Methyl Iodide. Roughly equimolar quantities of B and methyl iodide were added to an excess of 10% sodium hydroxide. Vigorous shaking yielded a clear, yellow solution. Gradual acidification gave at first a tacky, yellow precipitate, which partially dissolved on addition of more acid. The supernatant was removed and adjusted to pH 8 by the addition of 1N sodium hydroxide. A flocculent white precipitate separated from the slightly alkaline solution. The yellow material melted at 170-175° after crystallization from

aqueous ethanol to give long, colorless needles, which turned yellow on standing in the solvent or on exposure to air. Both compounds were insoluble in dilute acid, but when an alkaline solution of either material was rapidly acidified, no precipitate appeared until the pH was raised to ca. 3 or 4. When excess alkali was then added, the precipitate redissolved.

Reaction of B with Dimethyl Sulfate. Dimethyl sulfate (3.6 ml.) was added dropwise over a period of 2½ hours to a rapidly stirred solution consisting of 2.00 g. of B in 20 ml. of 1N sodium hydroxide. Additional sodium hydroxide was added, as necessary, to maintain the reaction mixture strongly alkaline. A precipitate appeared after about 1 ml. of the dimethyl sulfate had been added and gradually increased in amount during the course of the reaction. After the addition was completed, the mixture was stirred for ½ hour. The residual dimethyl sulfate was then destroyed by the addition of 1 ml. of concentrated ammonium hydroxide. Extraction with 20 ml. of chloroform gave, on evaporation of the chloroform, 0.37 g. of colorless needles. An additional 0.2 g. of this material was obtained by acidification of the aqueous phase, extraction with chloroform, evaporation of the chloroform, and treatment of the resulting oil with dimethyl sulfate as described above. Crystallization from hot methanol yielded colorless, elongated prisms melting at 133-135°. The ultraviolet absorption spectrum, in ethanol, showed maxima at 223.5 and 320 mμ., a minimun at 292 mμ., and plateaus at 264-267 and 270-273 mμ.

Anal. Calcd. for $C_{16}H_{18}O_3N_2S$: C, 60.35; H, 5.70; N,

8.80; S, 10.07; N-CH_3, 9.12 (per group); O-CH_3, 9.75 (per group). Found: C, 60.86; H, 5.74; N, 8.56; S, 9.97; N-CH_3, 23.5; O-CH_3, 2.18.*

When 0.55 g. of N-(o-aminobenzoyl)benzenesulfonamide was treated in a similar manner, but without recycling, there was obtained 78 mg. of product melting at 133-135°. A mixture of this and the material obtained from B showed no depression in melting point.

Reaction of B with Diazomethane. A distilled ethereal solution of diazomethane was prepared by the method of Arndt (26), and 14 ml. (0.0105 moles) was added, with swirling in an ice-bath, to a suspension of 3.04 g. (0.010 moles) of B in 150 ml. of methylene chloride. All but the last portion of diazomethane decolorized rapidly on addition. The resulting clear, yellow solution was poured into an evaporating dish and allowed to evaporate in the hood, yielding a yellow oil. Treatment of the oil with 12 ml. of chloroform gave a yellow solution and 200 mg. of crystals (fraction (a)).** Addition of 6 ml. of ligroin, to the chloroform solution caused the precipitation of 0.84 g. of a somewhat gummy solid (fraction (b)). The mother liquor was evaporated with a stream of air, and the

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* The analyst, Dr. Elek, stated that the determination of N-methyl groups frequently gives low values, and that the result from an O-methyl determination is high if N-methyl groups are present.

** In several repetitions of this experiment, considerable variation was observed in the amount of fraction (a). The maximum was about 8% of the weight of the starting material; in some cases none of this fraction was detected.

residue was twice dissolved in 4 ml. portions of ethanol, evaporating to a thick oil each time. The residue was again dissolved in 4 ml. of ethanol. Scratching gave 0.88 g. of crystalline material (fraction (c)). The mother liquor was alternately evaporated to an oil and the oil redissolved in ethanol, for several repetitions of the cycle. Each time, the ethanolic solution was induced to give a solid fraction of from 10 to 100 mg. These fractions corresponded successively to (a), (b), (a), and (c).

Fraction (a) was crystallized twice from absolute ethanol and five times from a 50-50 mixture of benzene and absolute ethanol to give 50 mg. of tiny, colorless platelets melting at 195-200°. The ultraviolet absorption spectrum, in absolute ethanol, was identical with that of compound A.

Anal. Calcd. for $C_{15}H_{14}O_4N_2S$: C, 56.59; H, 4.43; N, 8.80; S, 10.07. Found: C, 56.45; H, 4.40; N, 8.80; S, 10.08; O-CH_3, 0.00.

The pure material was soluble in hot ethanol, benzene, dioxane, and methanol to the extent of about 0.3%, 0.7%, 3-5%, and 0.5%, respectively, and to the extent of about 0.9% in cold chloroform or dioxane. When tested for the presence of a carboxylic ester with hydroxylamine and ferric chloride, it gave a red-brown color (24c).

Fraction (b) was crystallized several times from ethanol to a constant melting point, giving 0.63 g. of colorless crystals melting at 153-156°. The ultraviolet absorption spectrum, in ethanol, showed a maximum at 224 mμ., and a

plateau at 280-285 mμ.

Anal. Calcd. for $C_{15}H_{14}O_4N_2S$: C, 56.59; H, 4.43; N, 8.80; S, 10.07; O-CH_3, 9.75. Found; C, 56.55; H, 4.57; N, 8.70; S, 9.95; O-CH_3, 9.30.

The approximate solubilities in hot ethanol, benzene, dioxane, and methanol were determined to be 5%, 0.9%, 8.5%, and 3%, respectively, and in cold chloroform, dioxane, and methanol, to be 4%, 2.5%, and 0.8%, respectively. A test for the presence of a carboxylic ester gave a brown color with a faint violet cast.

Fraction (c) was crystallized once from 1 ml. of ethanol, and twice from 3 ml. of a 50-50 mixture of benzene and acetonitrile, giving 0.6 g. of colorless, chunky crystals melting at 118-119°. The ultraviolet absorption spectrum, in ethanol, showed a maximum at 215 mμ., and plateaus at 272 - 274, and 283-286 mμ.

Anal. Calcd. for $C_{15}H_{14}O_4N_2S$: C, 56.59; H, 4.43; N, 8.80; S, 10.07. Found: C, 56.50; H, 4.48; N, 8.69; S, 10.15.

N-methyl-N-(o-nitrobenzoyl)benzenesulfonamide XVII). This material was prepared by fusing a mixture of o-nitrobenzoyl chloride and N-methylbenzenesulfonamide in the manner described for N-(o-nitrobenzoyl)benzenesulfonamide. The crude product was crystallized from 95% ethanol, giving colorless rods melting at 96-98°. The yield was 57%. The ultraviolet absorption spectrum, in ethanol, showed maxima at 217, 261, and 266 mμ., minima at 216, 247, and 263.5 mμ., and a plateau at 271-274 mμ.

Anal. Calcd. for $C_{14}H_{12}O_5N_2S$: C, 52.50; H, 3.78; N,

8.75; S, 10.01. Found; C, 52.46; H, 3.87; N, 8.66; S, 10.02.

N-methyl-N-(o-aminobenzoyl)benzenesulfonamide (XVIII). Several methods of reduction of the corresponding nitro compound were tried. Ferrous sulfate and ammonium hydroxide yielded oils and unreacted starting material. Starting material was recovered after refluxing for a half-hour with zinc dust and glacial acetic acid. Treatment with a mixture of mossy zinc, glacial acetic acid, and 6N sulfuric acid gave an oil. The use of tin and hydrochloric acid gave a small yield of the desired product.

A mixture of 1.00 g. of N-methyl-N-(o-nitrobenzoyl)-benzenesulfonamide, 0.74 g. of mossy tin, and 5 ml. of 12N hydrochloric acid was heated on a steam bath until all of the tin had dissolved (45 minutes), and then for an additional ½ hour. The reaction mixture was cooled, made strongly alkaline with 2N sodium hydroxide, and extracted with chloroform. Evaporation of the chloroform gave 0.47 g. of oil. The oil was treated with 1 ml. of ethanol, giving 0.20 g. of yellow needles. Three crystallizations from methanol gave 0.145 g. (16%) of pure product, pale yellow needles melting at 119-120°. The ultraviolet absorption spectrum, in ethanol, showed maxima at 225.5, and 357.5 mμ., a minimum at 282 mμ., and a shelf at 250-260 mμ.

Anal. Calcd. for $C_{14}H_{14}O_3N_2S$: C, 57.90; H, 4.86; N, 9.65; S, 11.04. Found: C, 58.04; H, 4.93; N, 9.61; S, 11.06.

N-methyl-N-(N'-formyl-o-aminobenzoyl)benzenesulfonamide (XVI). A mixture of 1.00 g. of N-methyl-N-(o-aminobenzoyl)-

benzenesulfonamide and 5 ml. of 90% formic acid was refluxed for ½ hour. The mixture was cooled and treated with 10 ml. of water, giving 0.84 g. of crystalline material. One crystallization from ethanol and three from methanol gave 0.58 g. of colorless, chunky crystals melting at 118-119°. A mixture of this and similar material from the treatment of B with diazomethane showed no depression in melting point.

Section 4

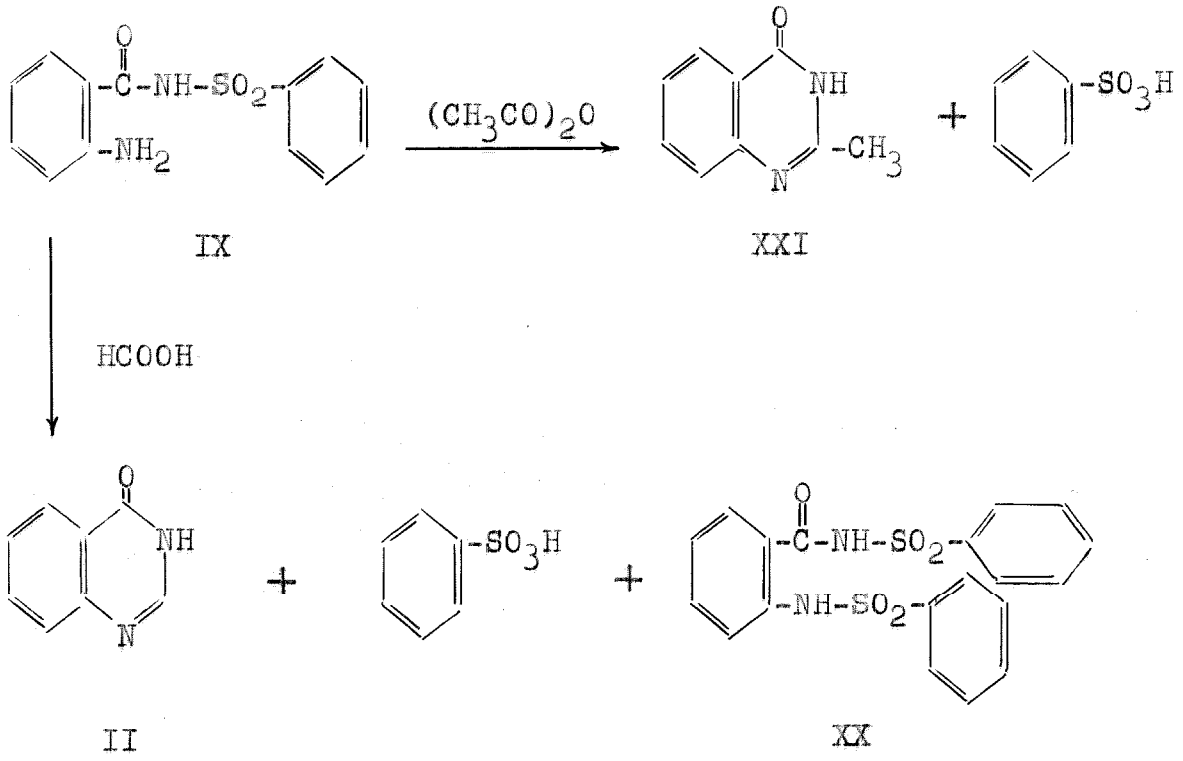
Miscellaneous Experiments

In the course of the investigations described in section 3, there arose the question of what dissociation constant (as an acid) would be expected for a formylated aromatic amine. A search of the literature disclosed that in 1896, Ewan (27) had measured the electrical conductivity of aqueous solutions of formanilide and reported a dissociation constant of 5×10^{-10}. He further reported (from conductivity) that in aqueous solution the sodium salt was almost completely hydrolyzed to formanilide and sodium hydroxide.

Calculation showed that at the concentration employed, 0.01F, the sodium salt of an acid of the reported strength should have been largely unhydrolyzed. Potentiometric titration of a 0.1F solution of formanilide with 0.15F sodium hydroxide gave a curve indistinguishable from that of pure water. Material with a dissociation constant as small as 2×10^{-12} would have been readily detected. A similar titration of N-formylanthranilic acid yielded no inflection past one equivalent of the alkali. The insolubility of the free acid prevented accurate determination of the dissociation constant of the carboxyl group. The dissociation constant reported by Ewan is thus in error. The conductivities reported probably arose largely from contamination of the formanilide with traces of electrolytes; a most logical contaminant is aniline formate.

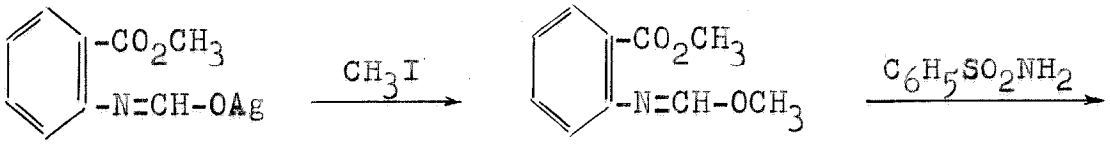
An incidental consequence of the investigation of the

structure of compound B (section 3), was the discovery of an interesting reaction of N-(o-aminobenzoyl)benzenesulfonamide (IX). When heated with formic acid for a short time it gave a fifty-five percent yield of 4-quinazolone (II) and benzenesulfonic acid. There was also obtained fifteen percent of N-(o-benzenesulfonamidobenzoyl)benzenesulfonamide (XX), which was identified by synthesis from the amine, IX, and benzenesulfonyl chloride. Prolonged heating with the formic acid, or the use of less concentrated acid, did not significantly alter the yield of either compound. The reaction was extended to include acetic anhydride, which gave an eighty percent yield of 2-methyl-4-quinazolone (XXI), and benzenesulfonic acid. Glacial acetic acid, however, had no effect on IX, which was recovered unchanged after an hour's heating. The presence of

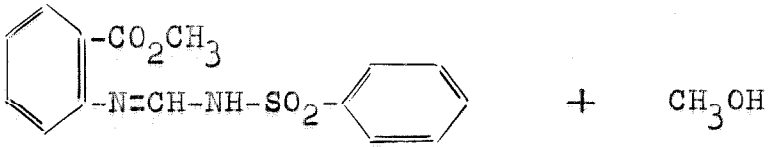


the hydrogen on the amide group of IX would appear to be necessary for the reaction, as the compound having a methyl group in its place (XVIII) gave the normal formyl derivative (XVI). It also seems reasonable to assume that acylation of the amine is the first step in the reaction. Thus, the behavior of compound B, the N-formyl derivative of IX, on pyrolysis (section 3), can be explained on the basis of the major product being the high melting benzenesulfonic acid salt of 4-quinazolone.

A carboxylic acid isomeric with compound B was prepared as the methyl ester. This compound, N-benzenesulfonyl-N'-(o-carbomethoxyphenyl)formamidine (XXII), was prepared by the scheme shown. The general method has been described by Knott (31). The methyl ester of N-formylanthranilic acid was converted to its silver salt, (XXIII), and reacted with methyl iodide to give methyl N-(o-carbomethoxyphenyl)formimidate (XXIV). This was then treated with benzenesulfonamide to give the desired compound.



XXIII XXIV



XXII

The structures of sulfonyl derivatives of amidines have been discussed with respect to the position of the double bond and the existence of stable and unstable isomers has been postulated (32, 33). The position of the double bond in XXII was not rigorously established, but was so placed because of the solubility of the compound in alkali and the general resemblance of its ultraviolet absorption spectrum to that of the 3-substituted-4-quinazolones.

Potentiometric Titration. The substances to be examined were dissolved in distilled water which had been boiled to remove carbon dioxide, and titrated with carbonate-free sodium hydroxide solution (28). The solutions were stirred with a magnetic "flea". The pH was followed with a Beckman glass electrode pH meter, model G, standardized at pH 10 with the furnished buffer solution. Dissociation constants were calculated from the pH at the equivalence point using the simple mass action expression for the neutralization of a weak acid with a strong base. The effect of ionic strength was neglected and K_W was taken as 10^{-14}.

Formanilide (29), 20.2 mg. (0.167 millimole), in 1.50 ml. of water was titrated with 0.14N base in 0.05 ml. portions until 1.4 ml. (0.198 millimoles) had been added. The pH at each step was the same as the corresponding value when the titration was repeated omitting the formanilide.

N-formylanthranilic acid (30), 17.0 mg. (0.103 millimoles), was suspended in 2.00 ml. of water and titrated with 0.14N base. The free acid was only very slightly soluble, and the rate of

solution was such that solution was not complete until somewhat more than one equivalent of the base had been added. The addition was continued until 2.00 ml. (0.282 millimoles) of the base had been added. No inflection was observed past one equivalent of base.

A solution of 4-quinazolone, 20.3 mg. (0.139 millimoles), in 3.00 ml. of water, was titrated with 0.10N base. The point of inflection was estimated to be at pH 10.65 ± 0.1 and 1.50 ml. of base, giving a calculated K_A for 4-quinazolone of 1.6×10^{-9}.

A solution of 4-quinazolone, 2.29 mg. (0.0157 millimoles), in 1.00 ml. of water, was titrated with 0.00989N base. The inflection was discernible, but was too flat to permit accurate location of the flex point. From the pH (10.27) at the theoretical equivalence point, the dissociation constant was calculated to be 1.6×10^{-9}.

The benzenesulfonic acid salt of 4-quinazolone, 5.20 mg. (0.0171 millimoles), in 2.00 ml. of water was titrated with 0.01075N base. There was a very sharp inflection at 1 equivalent of base and a discernible inflection in the region of 2 equivalents. From the initial pH of the solution (2.45), K_{A1} was calculated to be 8.5×10^{-3}. The pH (10.17) at the theoretical second equivalence point gave a K_{A2} of 1.5×10^{-9}. The constants were re-determined from a titration of 30.2 mg. (0.0993 millimoles) in 2.00 ml. of water with 0.141N base. The initial pH was 1.81 giving a K_{A1} of 4.8×10^{-3}. The second point of inflection was estimated to be at pH 10.72 for a K_{A2} of 1.0×10^{-9}.

Reaction of Formic Acid with N-(o-aminobenzoyl)benzenesulfonamide (IX). A mixture of 1.00 g. of the amine and 5 ml. of 99% formic acid was refluxed for 15 minutes and then evaporated to dryness at reduced pressure. The residue was dissolved in 110 ml. of boiling absolute ethanol. On cooling, there was obtained 0.60 g. of colorless needles, melting at 250-254°. The analytical sample, prepared by crystallization from ethanol, melted at 254-255°.

Anal. Calcd. for $C_{14}H_{12}O_4N_2S$: C, 55.25; H, 3.98; N, 9.21; S, 10.53. Found: C, 55.28; H, 4.00; N, 9.15; S, 10.40.

A mixture of this and material prepared by addition of benzenesulfonic acid to a solution of 4-quinazolone, showed no depression in melting point. A 63 mg. sample was dissolved in 0.5N sodium bicarbonate and extracted with chloroform. Evaporation of the chloroform gave 25 mg. of colorless needles melting at 216-218° and showing no depression in melting point when mixed with an authentic sample of 4-quinazolone.

The ethanolic mother liquor, from the first crystallization of the crude product, was evaporated to a small volume. Treatment with 35 ml. of water yielded a white precipitate, and crystallization of this from 15 ml. of ethanol gave 220 mg. of fibrous needles melting at 222-223°. The analytical sample, prepared by crystallization from ethanol, melted at 224-225°. The ultraviolet absorption spectrum, in ethanol, showed maxima at 215, 272, and 305 mμ., minima at 271 and 281 mμ., and a plateau at 240-250 mμ.

Anal. Calcd. for $C_{19}H_{16}O_5N_2S$: C, 54.79; H, 3.87; N, 6.73;

S, 15.40. Found: C, 55.34; H, 3.63; N, 6.95; S, 14.66.

The use of 87% instead of 99% formic acid, or increase of the reaction time to 4½ hours, did not appreciably alter the yield of either product.

A sample of N-(o-benzenesulfonamidobenzoyl)benzenesulfonamide (XX) was prepared by reacting 46 mg. of the amine with 0.05 ml. of benzenesulfonyl chloride in ½ ml. of pyridine for 11 hours at room temperature. The product was isolated by dilution of the reaction mixture with water and crystallization of the resulting precipitate from methanol, yielding 41 mg. (58%) of colorless, fibrous needles. A mixture of these and the material from reaction with formic acid, showed no depression in melting point.

Reaction of Acetic Anhydride with N-(o-aminobenzoyl)-benzenesulfonamide (IX). A mixture of 0.50 g. of N-(o-aminobenzoyl)benzenesulfonamide and 11 ml. of acetic anhydride was refluxed for 30 minutes. A bulky precipitate appeared during the course of the heating. The mixture was cooled and filtered, yielding 0.46 g. of thin, colorless platelets melting at 242-244°. Recrystallization from acetic anhydride did not alter the melting point. A sample dissolved in water gave a solution of pH ca. 2, or less. When the solution was made alkaline with sodium hydroxide and then neutralized with carbon dioxide, there were obtained colorless needles. These, after crystallization from ethanol, melted at 237-239°, and showed no depression in melting point when mixed with an authentic sample of 2-methyl-4-quinazolone (reported m.p. (34), 238-239°). A

picrate was prepared of each. The melting point of each was 205-208° (reported (34), 207.5-208.5°), and a mixed melting point showed no depression. The benzenesulfonic acid salt of the authentic sample was prepared in ethanol, and after crystallization from ethanol, melted at 242-244°, and showed no depression on mixing with the first material obtained.

Silver N-(o-carbomethoxyphenyl)formimidate (XXIII). The method of preparation was that of Comstock and Kleeberg (35). To a rapidly stirred solution of 16.8 g. (0.094 moles) of methyl N-formylanthranilate (36) and 15.8 g. (0.094 moles) of silver nitrate in 80 ml. of 50% ethanol, was added a solution of sodium hydroxide (0.094 moles) in 20 ml. of 50% ethanol. An immediate brown precipitate appeared. The mixture was diluted with 200 ml. of water, filtered with suction, and the precipitate washed twice with 100 ml. portions of water. The solid, when dry, was grey in color and weighed 24.0 g.

Methyl N-(o-carbomethoxyphenyl)formimidate (XXIV). The method of Lander (37) was used. The silver salt, 23.9 g. (0.0835 moles), and methyl iodide, 11.9 g. (0.0835 moles), were mixed with 15 ml. of sodium-dried ether in a flask equipped with a small reflux condenser and protected from atmospheric moisture with a drying tube. The mixture spontaneously refluxed for several hours. After standing overnight at room temperature, the resulting paste was diluted with 50 ml. of ether and filtered. The ether was stripped, and the resulting oil distilled, yielding 10.6 g. (66%) of product boiling at 100-104° (1.5 mm.). The analytical sample, prepared by redistillation,

was a yellow oil boiling at 99.5-99.6° (1.50 mm.). The ultraviolet absorption spectrum, in ethanol, showed maxima at 213 and 294 mμ., and a minimum at 273 mμ.

Anal. Calcd. for $C_{10}H_{11}NO_3$; C, 62.16; H, 5.74; N, 7.25. Found: C, 62.76; H, 6.00; N, 7.18.

N-benzenesulfonyl-N'-(o-carbomethoxyphenyl)formamidine (XXII). The general method was that of Knott (31). A solution of 1.93 g. (0.010 moles) of methyl N-(o-carbomethoxyphenyl) formimidate and 1.57 g. (0.010 moles) of benzenesulfonamide in 15 ml. of ethanol was refluxed for 15 minutes. Chilling gave 3.24 g. of crystals, which after crystallization from ethanol melted at 145-147°. The crystals were soluble in 5% sodium hydroxide, but not in sodium bicarbonate or hydrochloric acid. The ultraviolet absorption spectrum, in ethanol, showed maxima at 218, 270 and 310 mμ., minima at 250, and 292 mμ., and a plateau at 274-276 mμ.

Anal. Calcd. for $C_{15}H_{14}O_4N_2S$: C, 56.59; H, 4.43; N, 8.80; S, 10.07. Found: C, 57.11; H, 4.62; N, 8.65; S, 9.49.

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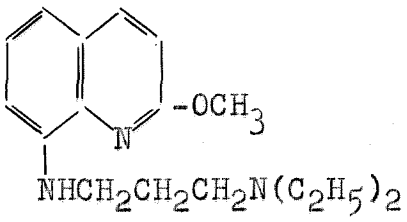
PART III. SYNTHESIS OF SOME 8-AMINOQUINOLINES. *

As part of the general program during the last war to develop a drug with the desirable antimalarial properties of pamaquine but without its high toxicity, workers in this laboratory, under Dr. J. B. Koepfli, prepared some seventy compounds for screening. Included were a number of 2-substituted 8-(3-diethylaminopropylamino)quinolines (1). These latter compounds were tested on monkeys by Dr. L. H. Schmidt, The Christ Hospital Institute of Medical Research, Cincinnati, Ohio, and found to be extremely toxic and were abandoned as potential antimalarials. Dr. Ida G. Schmidt, a cytologist, made the observation that one of this series, the 2-methoxy compound, I, seemed to have a particular effect on the neurons.



I

Isoplasmocid

As a result it was suggested to Dr. A. B. Sabin, University of Cincinnati College of Medicine, that he test the drug

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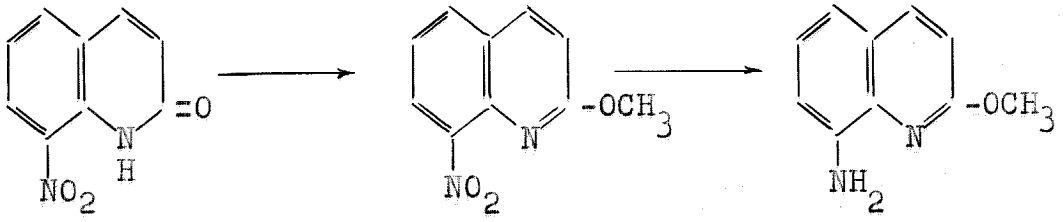
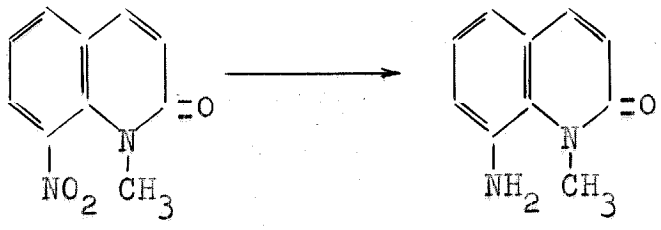
* This work was financed by the National Foundation for Infantile Paralysis through a sub-grant by the Children's Hospital Research Foundation, Cincinnati, Ohio.

on polio. This was done in 1948 on experimental poliomyelitis in monkeys. The tests indicated that the drug, isoplasmocid, was capable in some instances of retarding the process and in others of completely preventing the paralytic disease, while the isomeric 6-methoxy compound, plasmocid, had an adverse effect. Under the auspices of the National Foundation for Infantile Paralysis, a collaborating group consisting of Drs. N. L. Drake, R. C. Elderfield, J. B. Koepfli, L. H. Schmidt, and A. B. Sabin, was formed to explore this further.

At a conference of the group, a question arose concerning the structure of isoplasmocid.* The synthesis by Mislow and Koepfli (1), had involved reaction of dimethyl sulfate with 8-nitro-2-quinolone (II) to give 2-methoxy-8-nitroquinoline (III), followed by reduction to 2-methoxy-8-aminoquinoline (IV)



II III IV



V VI

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* Dr. Elderfield suggested that it could well have the isomeric 1-methyl-2-quinolone structure.

and condensation with 2-diethylaminopropyl chloride to give I. The reaction under suspicion was the O-methylation of II with dimethyl sulfate; for in all similar cases (2), reaction with methyl iodide or dimethyl sulfate invariably gives the isomeric N-methyl compound, which in this case would be 1-methyl-8-nitro-2-quinolone (V). Decker (3), on the basis of a quantitative methoxyl determination, had proposed the O-methyl structure, III, for the methylation product of II. An unequivocal synthesis of the N-methyl compound, V, melting at 128° and its reduction to the corresponding amine, VI, melting at 180°, has been described (4,5). The reported melting point of V seemed suspiciously close to that of the supposed O-methyl isomer, III, 124-125°. Though the analytical sample of the amine obtained by Mislow and Koepfli melted at 75-76°, it seemed desirable to confirm that this was the major product. It was barely possible that the analytical sample had not adequately represented the material used in the preparation of isoplasmocid.

The preparation of isoplasmocid was repeated, both to obtain a new sample (one-hundred fifty grams) for further virologic tests, and to check the intermediate material. Distillation of the crude reduction product of the nitro compound in question gave a 70% yield of the amine described by Koepfli and Mislow as IV. Crystallization of the still-pot residue gave 12% of the isomeric compound, VI. The simultaneous isolation of both isomers lends weight in confirmation of their structures. There is little possibility of more than

the two isomers described, of which the O-methyl isomer would be expected to be the more volatile and the lower melting. The methylation is thus seen to yield only a small amount of the undesired isomer, and this is removed in the purification procedure. There is little doubt that the major fraction is the desired product, and that isoplasmocid has the structure I.*

The tests, on monkeys, of the new sample of isoplasmocid, largely substantiated the earlier results. Treatment with the drug increased the average incubation period for paralysis from 6.6 days to 11.75 days, and lowered the fatality rate among paralyzed monkeys from 75% to 50%. The drug, however, displayed such high neurotoxicity as to preclude its clinical use. It was decided to evaluate the effect of blocking groups other than methoxy in the 2-position, and to investigate modifications of the side chain on the amine in the 8-position. This was for the twofold purpose of diminishing the toxicity and throwing light on the configuration or composition that is of importance in antagonizing the propagation of poliomyelitis virus. The compounds to be tested were chosen carefully, as the size of the test animal made testing expensive and required that each drug be furnished in the rather large quantity of one-hundred grams of the dihydroiodide salt.

Two previously described compounds, 2,6-dimethoxy-8-

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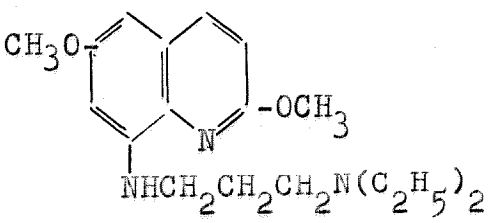
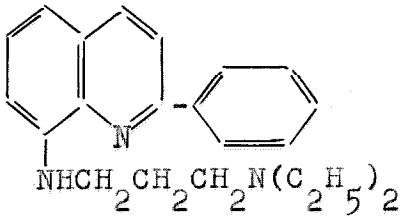
* It was subsequently reported by Elderfield (6) that he had prepared the 2-methoxy drug by an unequivocal route; its infrared and ultraviolet absorption spectra were identical with those of the isoplasmocid. He further reported that he was unable to attach a side chain to 8-amino-1-methyl-2-quinolone.

(3-diethylaminopropylamino)quinoline (VII) (1), and 2-phenyl-8-(3-diethylaminopropylamino)quinoline (VIII) (7) were prepared for testing. Though a confirmatory analysis was obtained, and



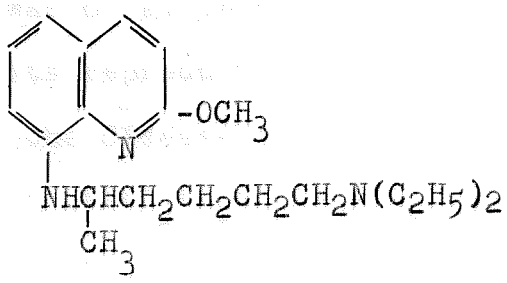
VII



VIII

the melting point agreed with the reported value (7), the dihydroiodide of the 2-phenyl compound was scarlet, an observation not previously reported. Since the free base was pale yellow and the dihydroiodides of the other members of the series are colorless when pure, this phenomenon was briefly investigated. The color did not seem to be due to an impurity (or at least to one which could be easily removed), nor did it appear to be due to oxidation. By treatment with a large excess of hydriodic acid it was occasionally possible to obtain colorless crystals, the composition of which was not established, and which were unstable with respect to the colored material. Since the scarlet material seemed to be the reasonably pure dihydrioiodide of VIII, it was submitted for testing.

The final drug prepared was the previously unknown 2-methoxy-8-(4-diethylamino-1-methylbutylamino)quinoline (IX). This compound was made by the condensation, by a standard procedure (7), of 2-methoxy-8-aminoquinoline (IV) with



IX

1-diethylamino-4-bromopentane (noval bromide). No difficulty was encountered except in the preparation of the required noval bromide hydrobromide. The results of Elderfield, et. al. (8), who prepared this material, as the hydrobromide salt, by the addition of thionyl bromide to a solution of the corresponding alcohol in benzene, could not be duplicated. To obtain other than dark, tarry products, it was found necessary to reverse the order of addition of the reactants. The resulting noval bromide hydrobromide, when purified, melted some six degrees higher than reported. Though there could be little doubt, its identity was confirmed by condensation with 6-methoxy-8-aminoquinoline to give plasmochin.

The other collaborators submitted a number of compounds embracing a variety of groups in the 2-position and several variations of the side chain.

The results of virological assay were not encouraging. A few of the compounds (including IX) had antipoliomyelitic activity, but none approached the activity of the original compound, isoplasmocid. In many of the cases, the toxic effects

were greater. After a study of the results, the collaborators felt that there was no evidence to indicate a specific effect on the virus or its reproduction, and that the observed effects on the course of the disease were due to a generalized toxemia. It was therefore decided that the preparation of additional compounds would not be justified, and the project was abandoned.

2-Methoxy-8-(3-diethylaminopropylamino)quinoline (Isoplasmocid, I). 2-Methoxy-8-nitroquinoline was prepared* and hydrogenated according to the directions of Mislow and Koepfli (1). Distillation of the crude product from 133 g. of the nitro compound gave 80 g. of distillate in the temperature range 103-105° (0.20 mm.), and 31 g. of a non-distillable residue. The residue was crystallized from 300 ml. of ethanol, giving 13.5 g. of light orange crystals melting at 178-184°. Several recrystallizations from ethanol gave pale yellow crystals melting at 182-184°. These, treated with acetic anhydride gave, after crystallization from ethanol, colorless platelets melting at 176-178° (reported for 8-amino-1-methyl-2-quinolone (7): free base, 180°; acetyl derivative, 174°).

The distillate solidified spontaneously to almost colorless crystals melting at 70-74°. A small sample recrystallized from ethanol melted at 75-76° (reported (1), 75-76°). The main portion of the distillate was used without further purification to prepare the desired 2-methoxy-8-(3-diethylaminopropylamino) quinoline as the dihydroiodide. The batch submitted for the

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* The nitro compound was prepared by R. Mirza, California Institute of Technology.

pharmacologic tests melted at 141.2-141.7° (reported (1), 140-142°).

2-Phenyl-8-(3-diethylaminopropylamino)quinoline (VIII). The free base was prepared by the published method (7), and was a pale yellow, viscous oil having the reported boiling point. The base was dissolved in ethanol, and hydriodic acid (spec. gr., 1.7) added in order to prepare the dihydroiodide. The first portions of acid caused temporary local coloring (red) of the solution. When slightly more than one equivalent had been added, the solution became permanently red and was quite dark by the time the required two equivalents had been added. The resulting red solid was crystallized several times to give scarlet needles melting at 179-182° (reported, 184-185°). Further crystallization did not change the melting point or the color.

Anal. Calcd. for $C_{22}H_{29}N_3I_2$: C, 44.83; H, 4.96; N, 7.13; I, 43.07. Found: C, 44.89; H, 5.00; N, 7.20; I, 42.95.

A small sample of the colored salt was boiled with water, giving a yellow solution which on long standing deposited tiny yellow crystals. A sample of the free base was dissolved in ethanol and a 200% excess of hydriodic acid added. The solution was red, but on long standing deposited colorless needles. In each case, when the melting point was taken of the product, the crystals turned red at 110-120°, and melted at 179-182°. A solution of the colorless product in ethanol, either alone or with added hydroquinone or sodium bisulfite, was red, and on chilling deposited the original scarlet needles.

A sample of the free base was divided into five fractions by molecular distillation at 170° (0.02 mm.). No difference could be detected in their behavior with hydriodic acid.

1-Diethylamino-4-bromopentane Hydrobromide (Noval Bromide Hydrobromide). Elderfield et al. (8,9) report the preparation of this compound by the addition of thionyl bromide to a solution of 1-diethylamino-4-hydroxypentane (noval alcohol). On four separate occasions, the published directions were carefully followed.* In each case, instead of the reported crystalline product, there was obtained a dark, tarry mass which could not be induced to crystallize. When the mode of addition of the reactants was reversed, the desired crystalline product was obtained.

Thionyl bromide (382 g.) was dissolved in 1.8 liters of sodium-dried benzene and chilled to 5° with an ice-salt bath, carefully excluding atmospheric moisture by means of drying tubes on all outlets. Then, with vigorous stirring, noval alcohol (1.84 moles) was added at such a rate that the temperature of the reaction mixture remained at 4.5-5.5°. The addition required 40 minutes, after which the mixture was allowed to stir for 5 hours, gradually warming to room temperature. Two liquid phases were obtained. The solvent was

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* Noval ketone, from which the alcohol was prepared by catalytic hydrogenation (8), was kindly supplied by Dr. R. C. Elderfield, Columbia University, along with more detailed directions than those published for the preparation of the alcohol and the bromide.

removed with a water-pump, leaving a pale-brown residue which was dried at 80° in a vacuum oven. A small sample, crystallized from alcohol-ether, melted at 100-101° (reported (8), 94-94.5°).

A sample of the crude product was condensed with 6-methoxy-8-aminoquinoline (Winthrop Chemical Co.) to give plasmochin (8), identified as the citrate, m.p. 123-124° (reported (8), 125-127°).

2-Methoxy-8-(4-diethylamino-1-methylbutylamino)quinoline (IX). Procedure D of Elderfield, et. al. (7), was followed in this condensation. A solution of 10.5 g. of 2-methoxy-8-aminoquinoline, and 36.6 g. of 1-diethylamino-4-bromopentane hydrobromide, in 20 ml. of water and 20 ml. of McIlvaine's standard buffer (pH 4.8) was heated at 49° for 8½ hours, 70° for 1½ hours, and 95-100° for 4½ hours. Sodium hydroxide solution (10%) was added from time to time, as necessary, to maintain a pH of ca. 4.5-5.0. The reaction mixture was cooled and made acid to congo red by the addition of hydrochloric acid. The solution was filtered, made neutral to congo red by the addition of sodium acetate, and extracted three times with 50 ml. portions of ether. The aqueous phase was made strongly alkaline with sodium hydroxide and heated to 80° for 5 hours. After cooling, the mixture was extracted with ether. The ether extract was dried, the solvent removed, and the residue distilled in vacuo under nitrogen, giving 7.9 g. (42%) of the desired base, b.p. 157-160° (0.30 mm.).

The dihydroiodide was obtained by dissolving the base in

15 ml. of ethanol and adding a solution of 7.6 ml. of hydriodic acid (spec. gr., 1.7) in 15 ml. of ethanol. The mixture was chilled to 0°, giving 8.2 g. (57%) of almost white crystals melting at 153-154° (dec.). A second crop of 5.3 g. was obtained by addition of 75 ml. of ether to the mother liquor. The analytical sample, prepared by crystallization from ethanol, melted at 154-155° (dec.). The salt is sensitive to both air and light, the colorless needles turning yellow within two weeks on exposure to either.

Anal. Calcd. for $C_{19}H_{31}ON_3I_2$: C, 39.94; H, 5.47; N, 7.36. Found: C, 39.98; H, 5.36; N, 7.61.

A citrate, prepared from the free base in ether-alcohol and crystallized from acetone, melted at 100-101°.

References

1. K. Mislow, and J. B. Koepfli, J. Am. Chem. Soc. 68, 1553 (1946).

2. M. T. Bogert and H. A. Seil, J. Am. Chem. Soc. 29, 517 (1907).

3. H. Decker, Ber. 38, 1151 (1905).

4. H. Decker and A. Stavrolopoulos, J. prakt. Chem. 68, 100 (1903).

5. H. Decker and H. Engler, Ber. 42, 1736 (1909).

6. Private communication from R. C. Elderfield, Columbia University, New York.

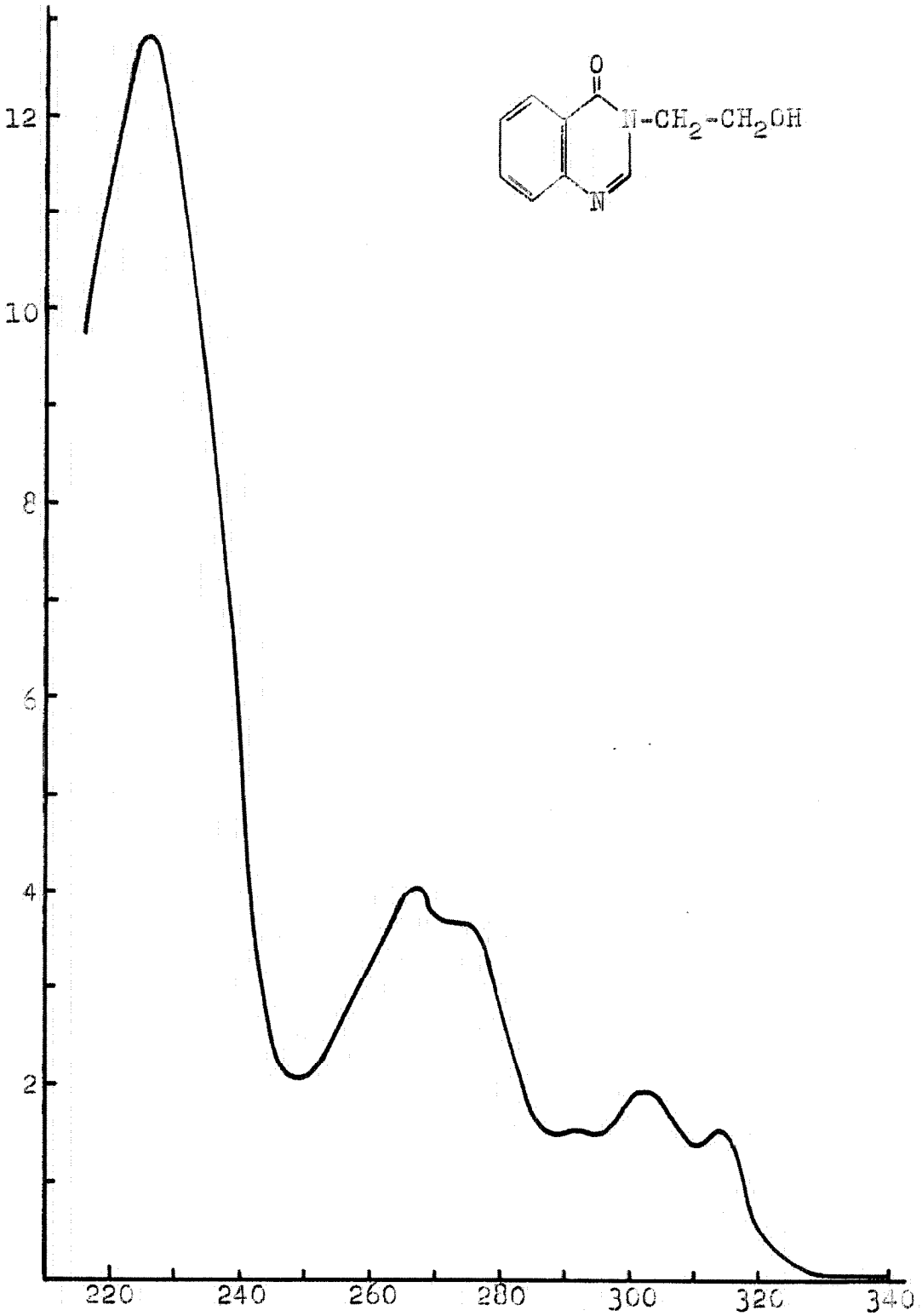
7. R. C. Elderfield, W. J. Gensler, J. D. Head, H. A. Hageman, C. B. Kremer, J. B. Wright, A. D. Holley, B. Williamson, J. Galbreath, L. Wiederhold III, R. Frohardt, S. M. Kupchan, T. A. Williamson and O. Birstein, J. Am. Chem. Soc. 68, 1524 (1946).

8. R. C. Elderfield, L. C. Craig, W. M. Lauer, R. T. Arnold, W. J. Gensler, J. D. Head, T. H. Bembry, H. R. Mighton, J. Tinker, J. Galbreath, A. D. Holley, L. Goldman, J. T. Maynard and N. Picus, J. Am. Chem. Soc. 68, 1516 (1946).

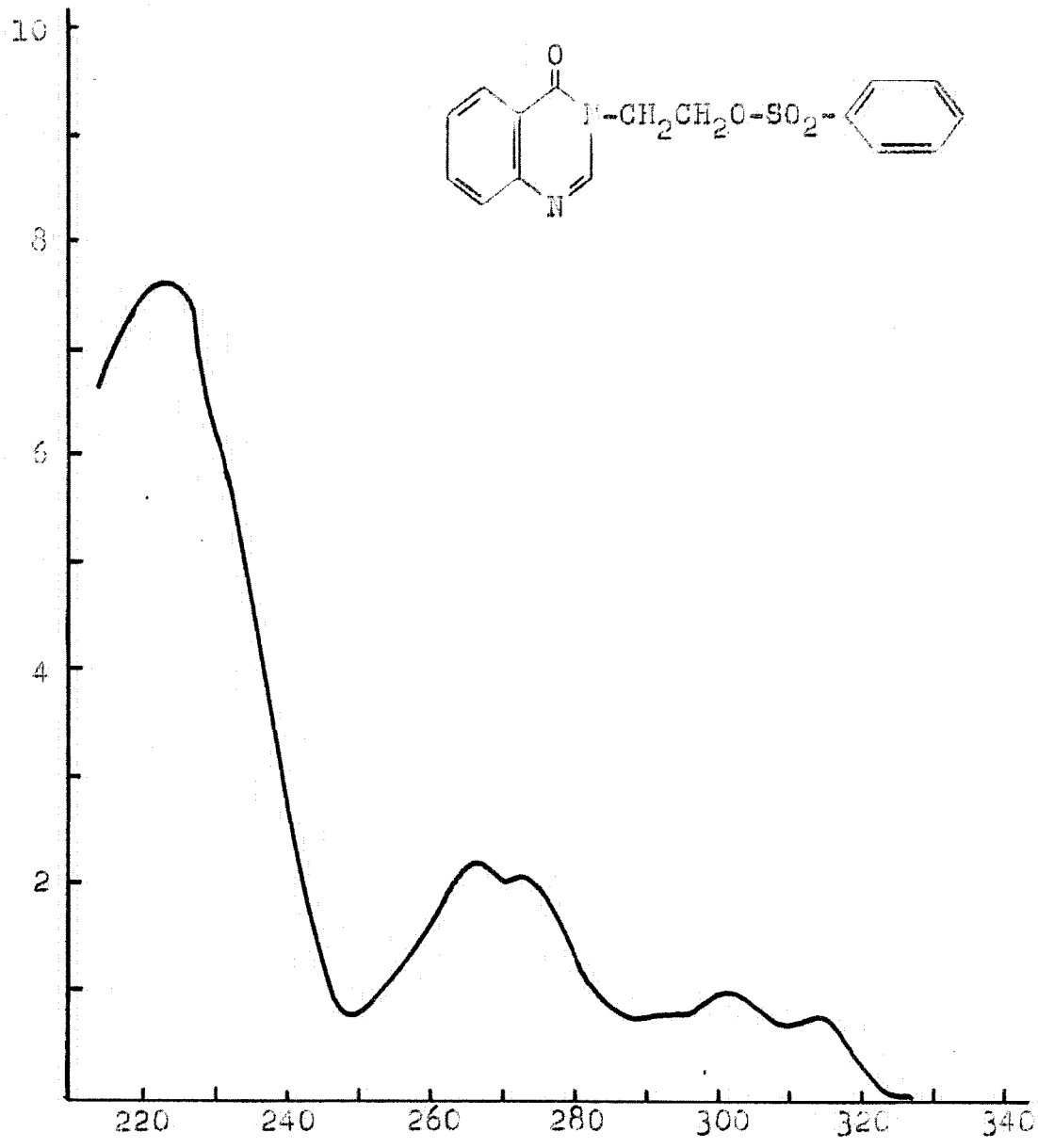
9. R. C. Elderfield, W. J. Gensler, F. Brody, J. D. Head, S. C. Dickerman, L. Wiederhold III, C. B. Kremer, H. A. Hageman, F. J. Kreysa, J. M. Griffing, S. M. Kupchan, B. Newman and J. T. Maynard, J. Am. Chem. Soc. 68, 1579 (1946).

APPENDIX

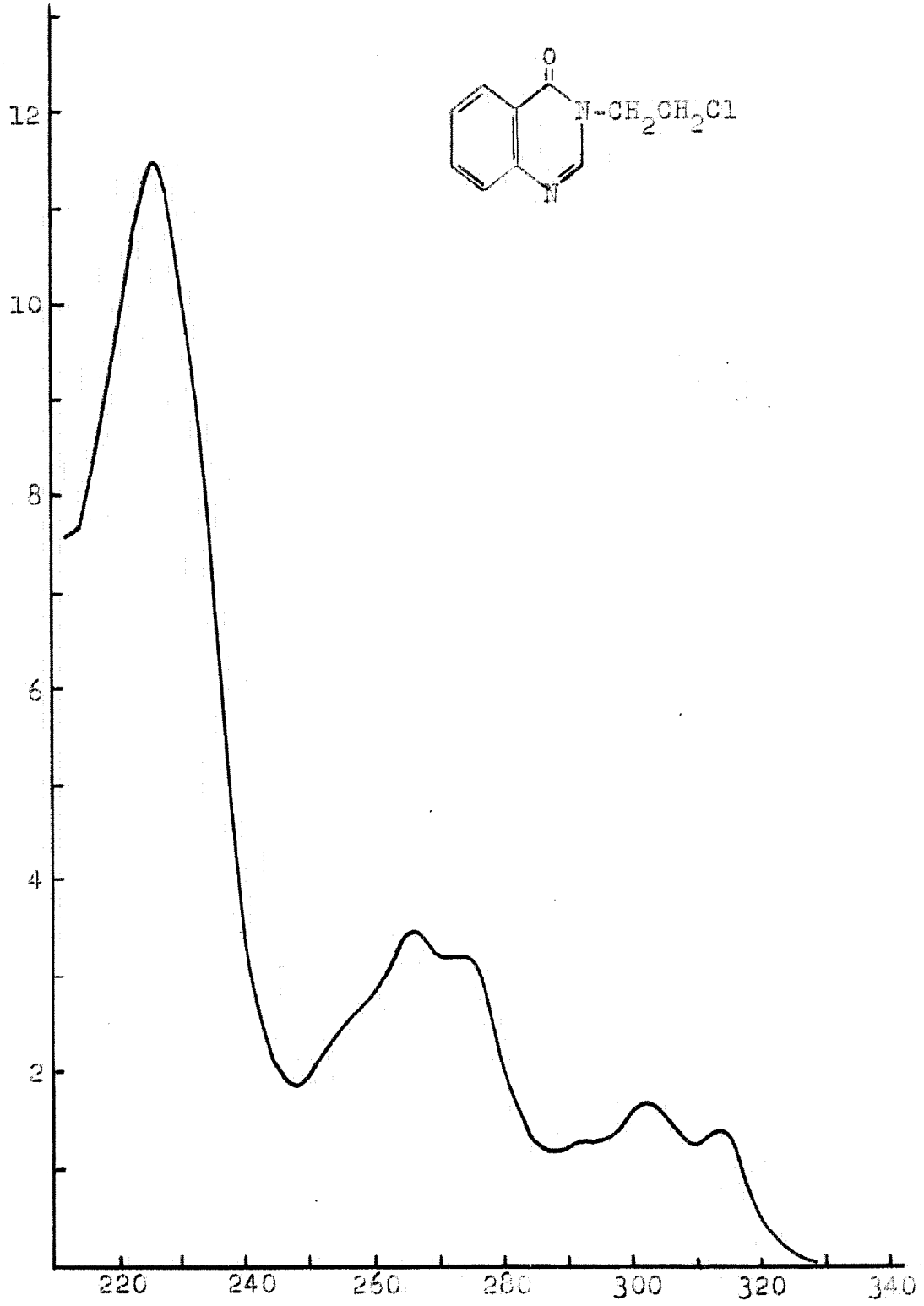
Herein are presented ultraviolet absorption curves of the various compounds discussed in part II. All curves were taken in absolute ethanol in 1 cm. quartz cells with a Beckman, Model DU, Quartz Photoelectric Spectrophotometer. The ordinate of all curves is $E^{1\%}_{1cm.}$, and the abscissa is millimicrons. The structure (or tentative structure) of each compound is indicated. The page numbers which are given refer to the page in the text where the compound is first discussed, and the compounds are not in the order of their appearance in the text. Two compounds are included which are not discussed in the text; in one case a reference is given to the preparation, and in the other, the mode of preparation is indicated.



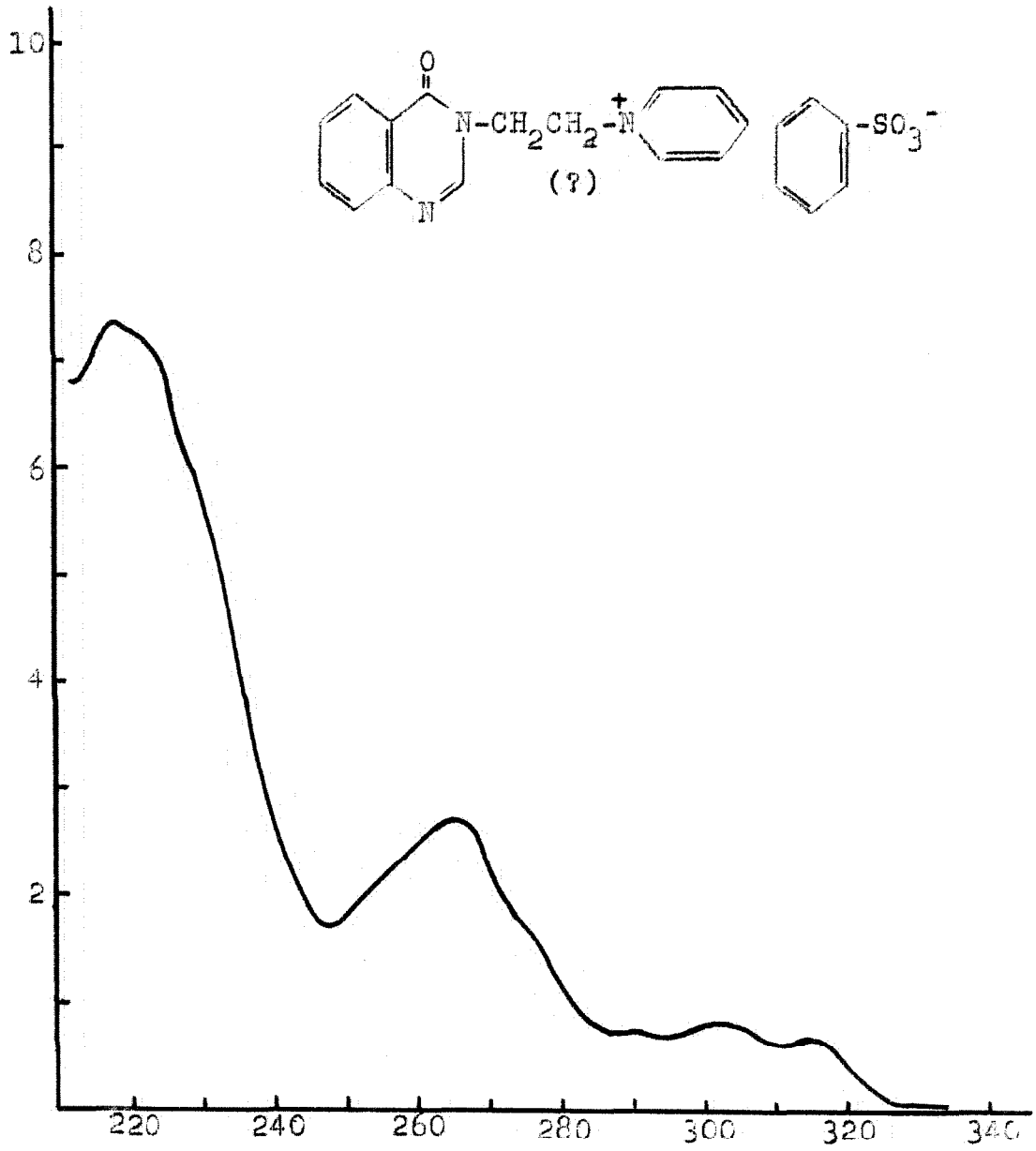
3-(β-Hydroxyethyl)-4-quinazolone, p. 107.



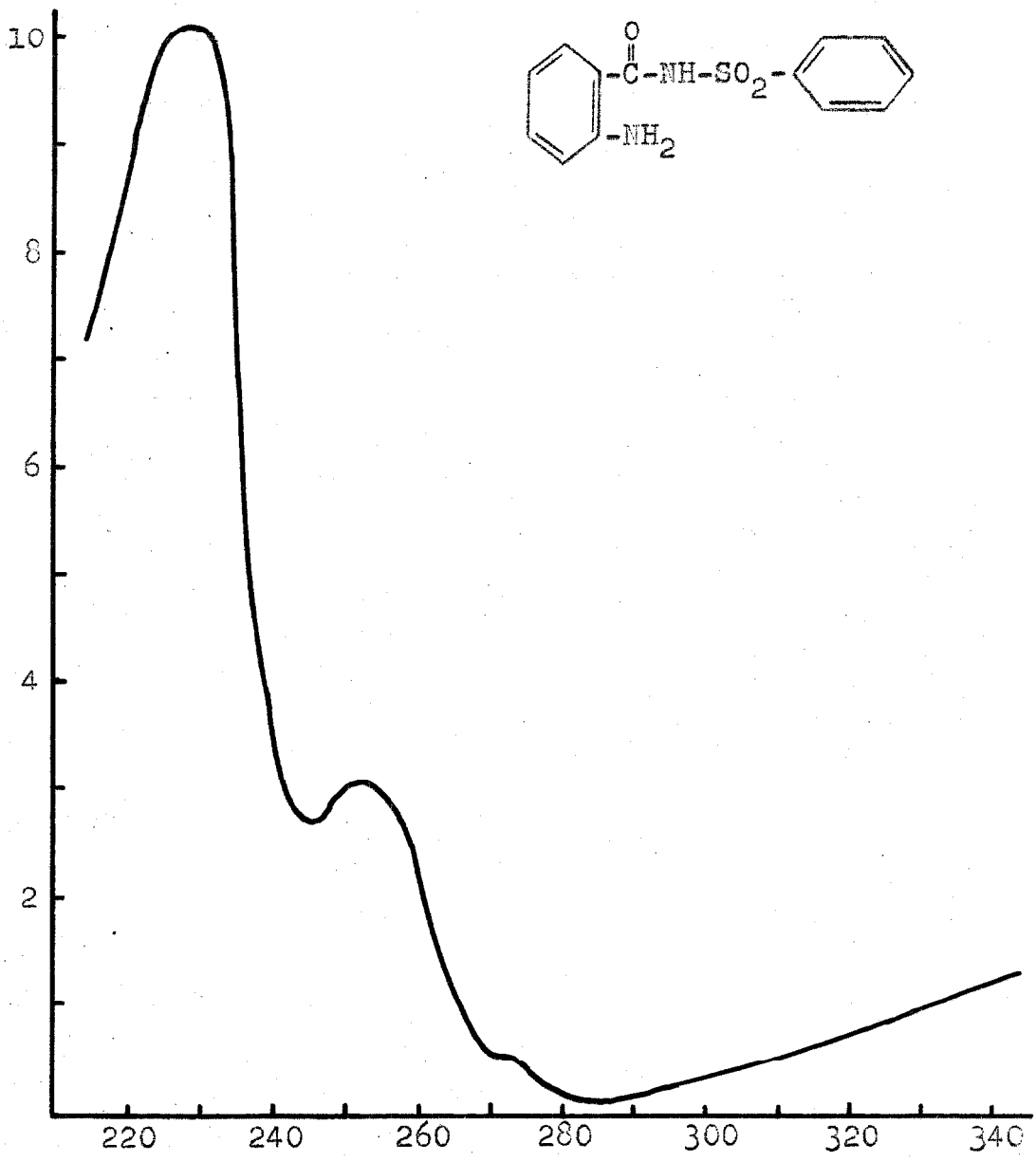
3-(β-Benzenesulfonoxyethyl)-4-quinazolone, p. 107.



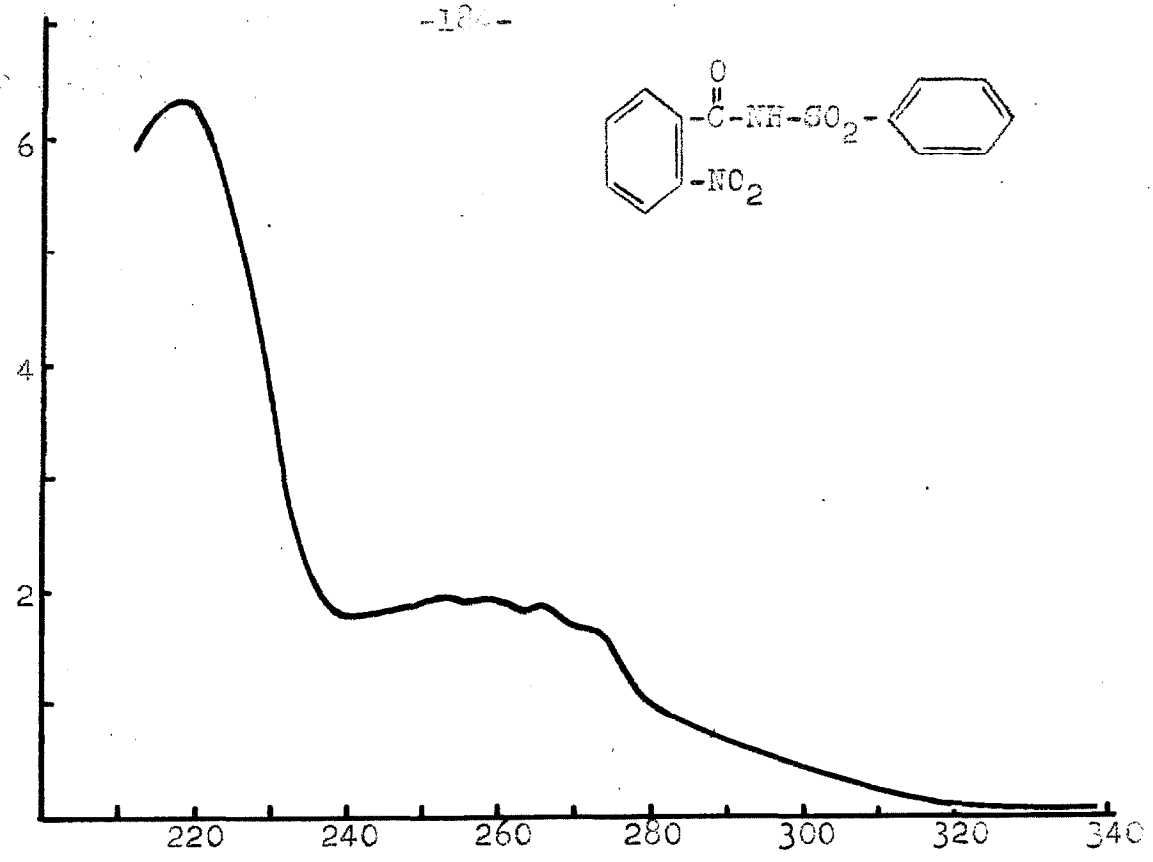
3-(β-Chloroethyl)-4-quinazolone, p. 107.



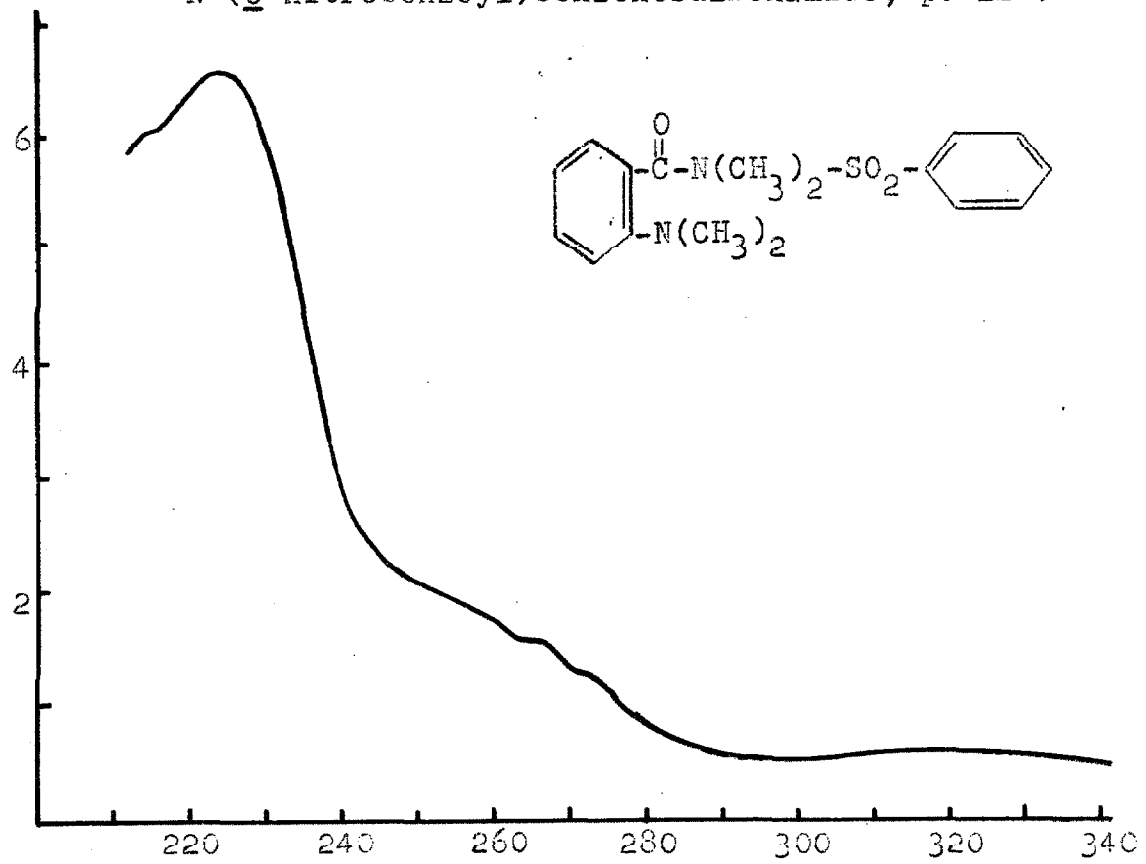
Possible structure, see p. 100.



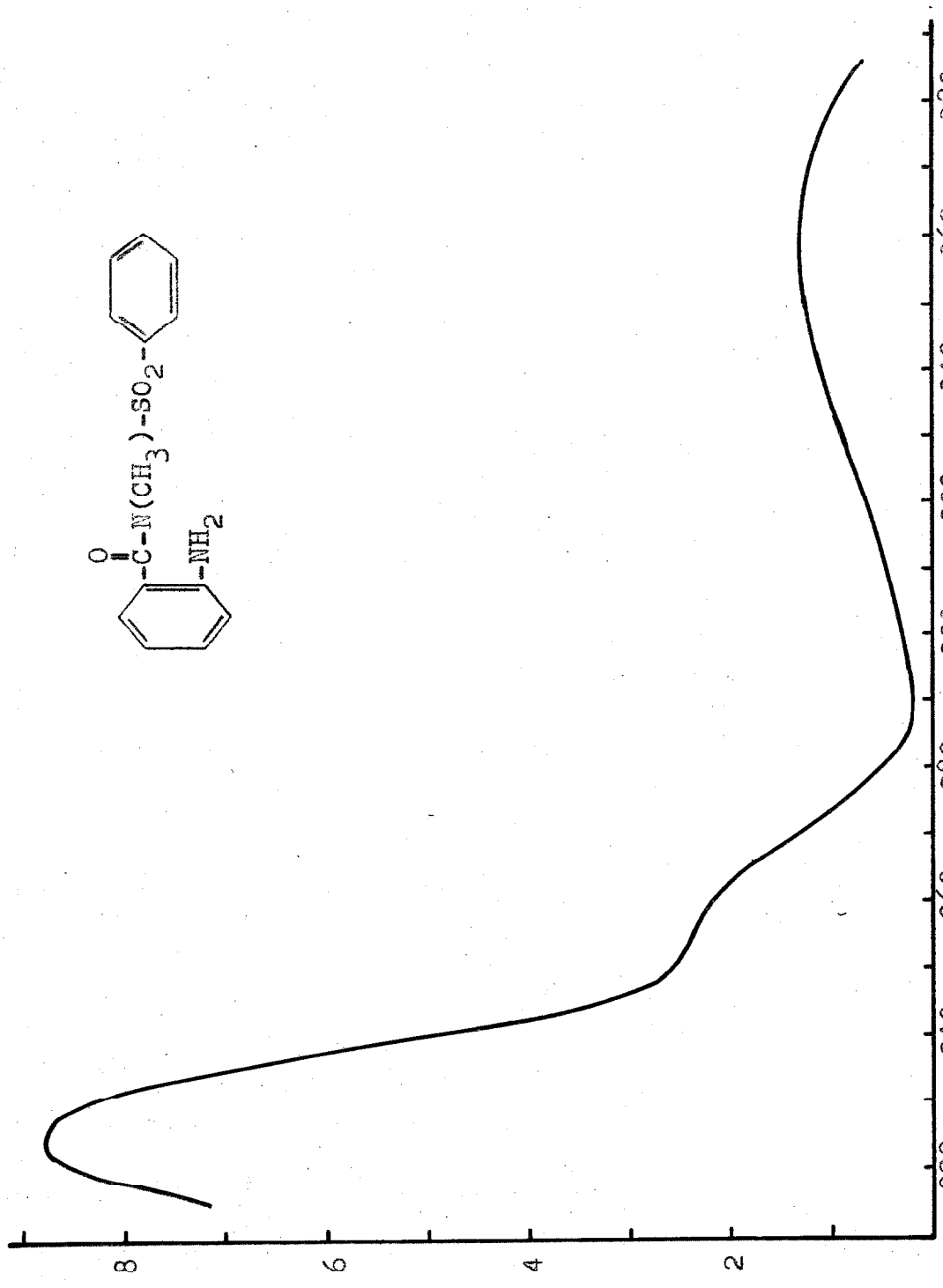
N-(o-aminobenzoyl)benzenesulfonamide, p. 128.



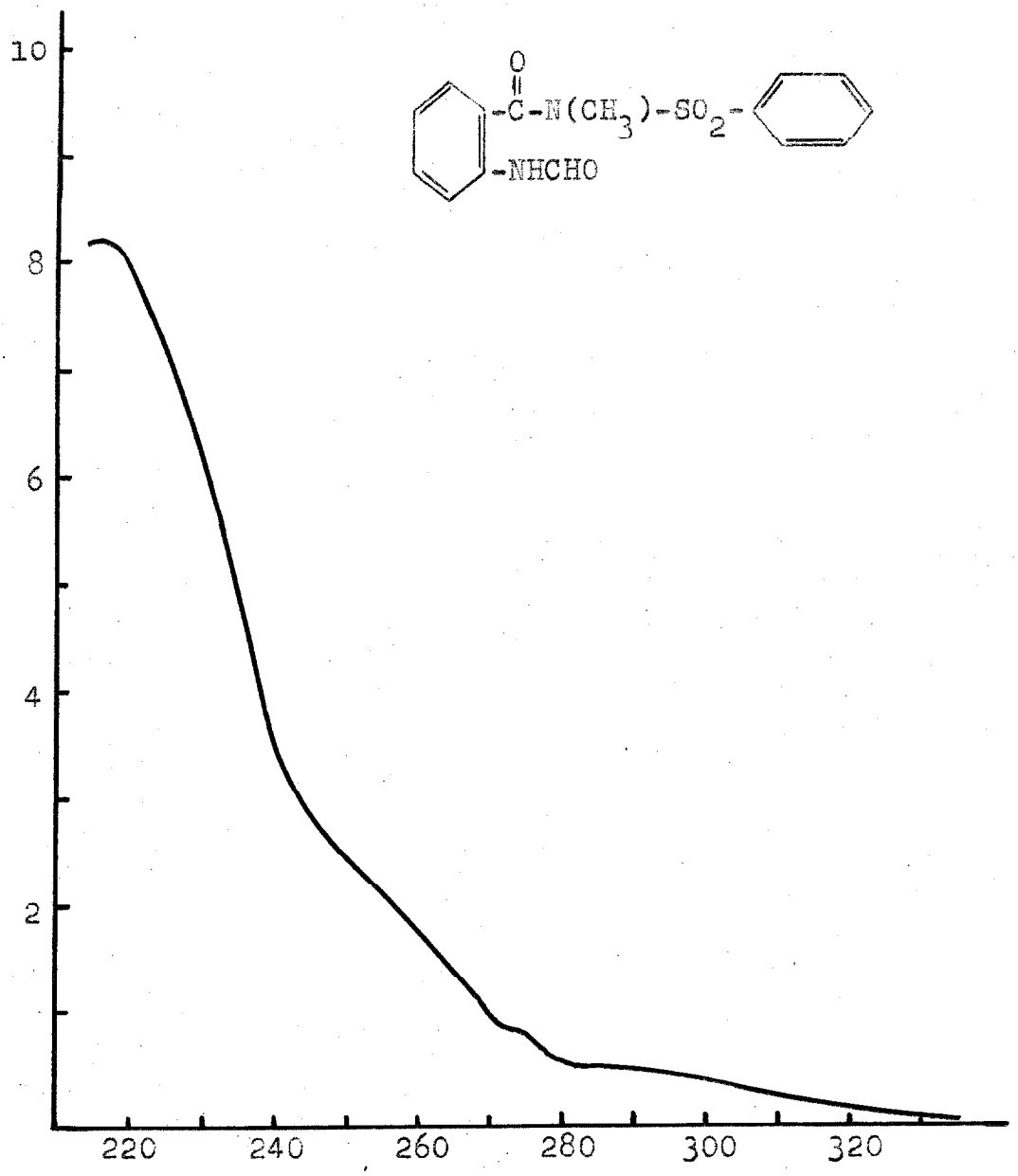
N-(o-nitrobenzoyl)benzenesulfonamide, p. 128.



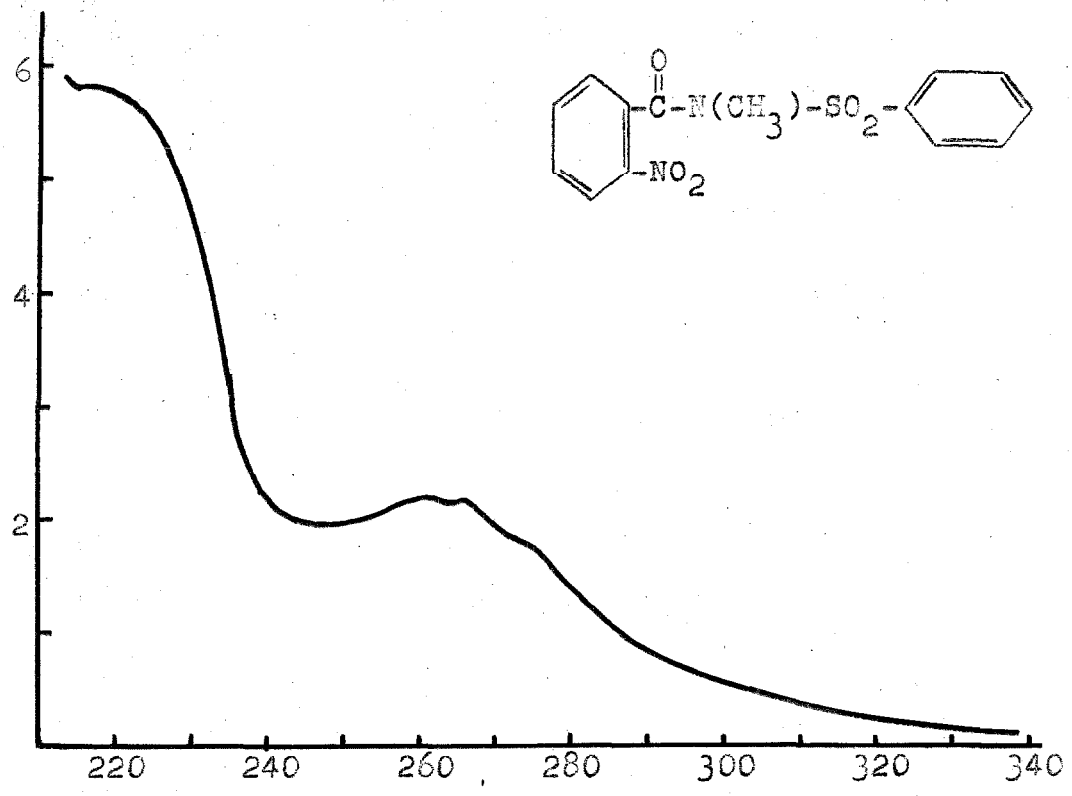
N-methyl-N-(o-dimethylaminobenzoyl)-benzenesulfonamide, p. 133.



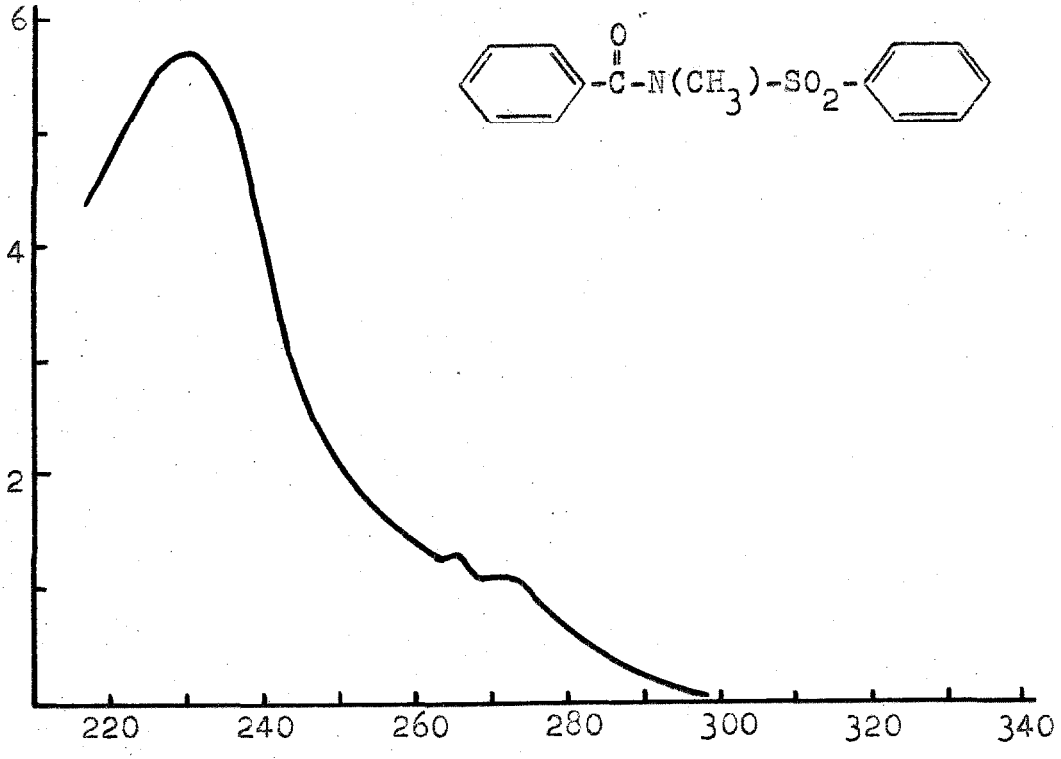
N-methyl-N-(o-aminobenzoyl)benzenesulfonamide, p. 129.



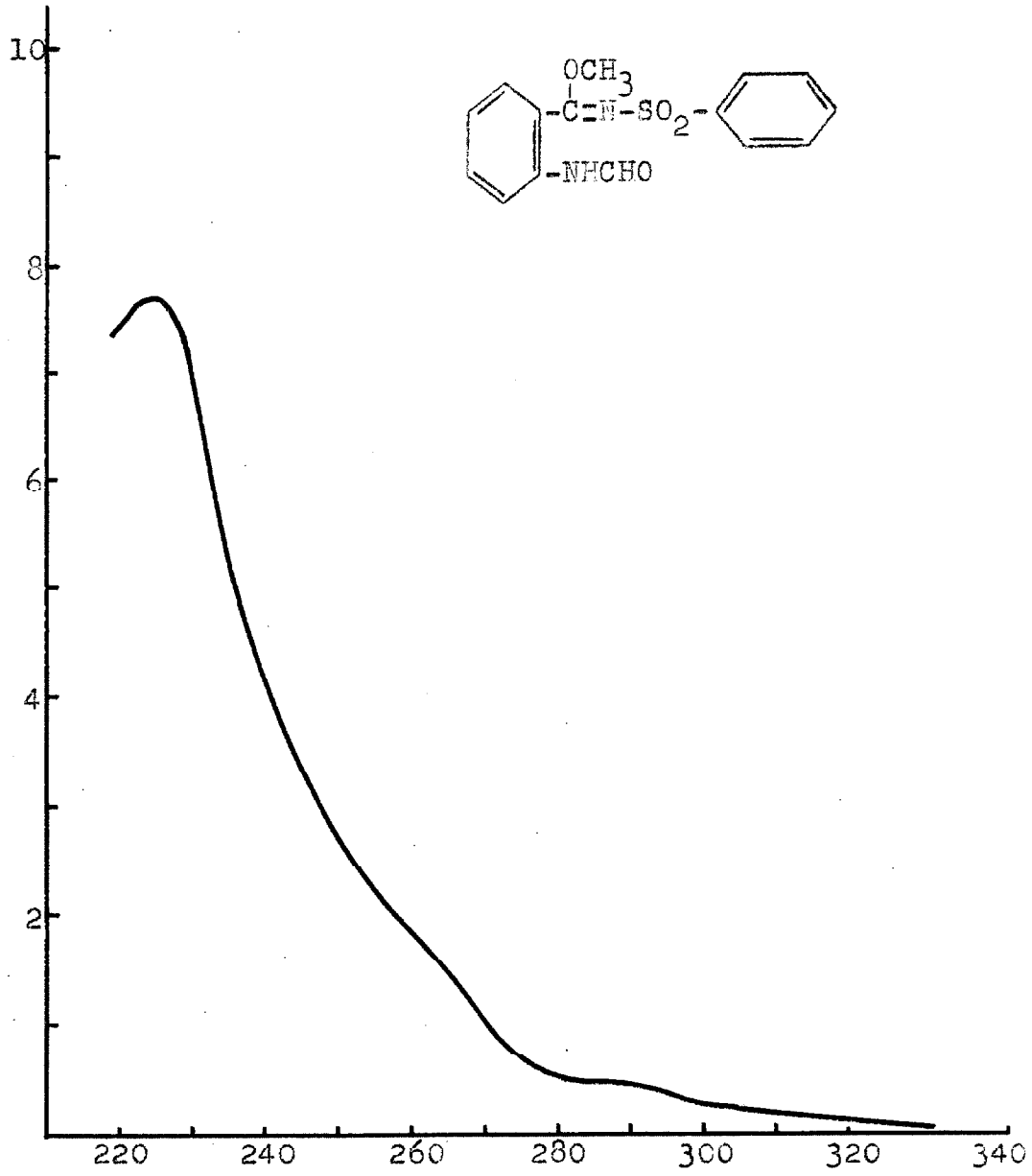
N-methyl-N-(N'-formyl-o-aminobenzoyl)-benzenesulfonamide, p. 134.



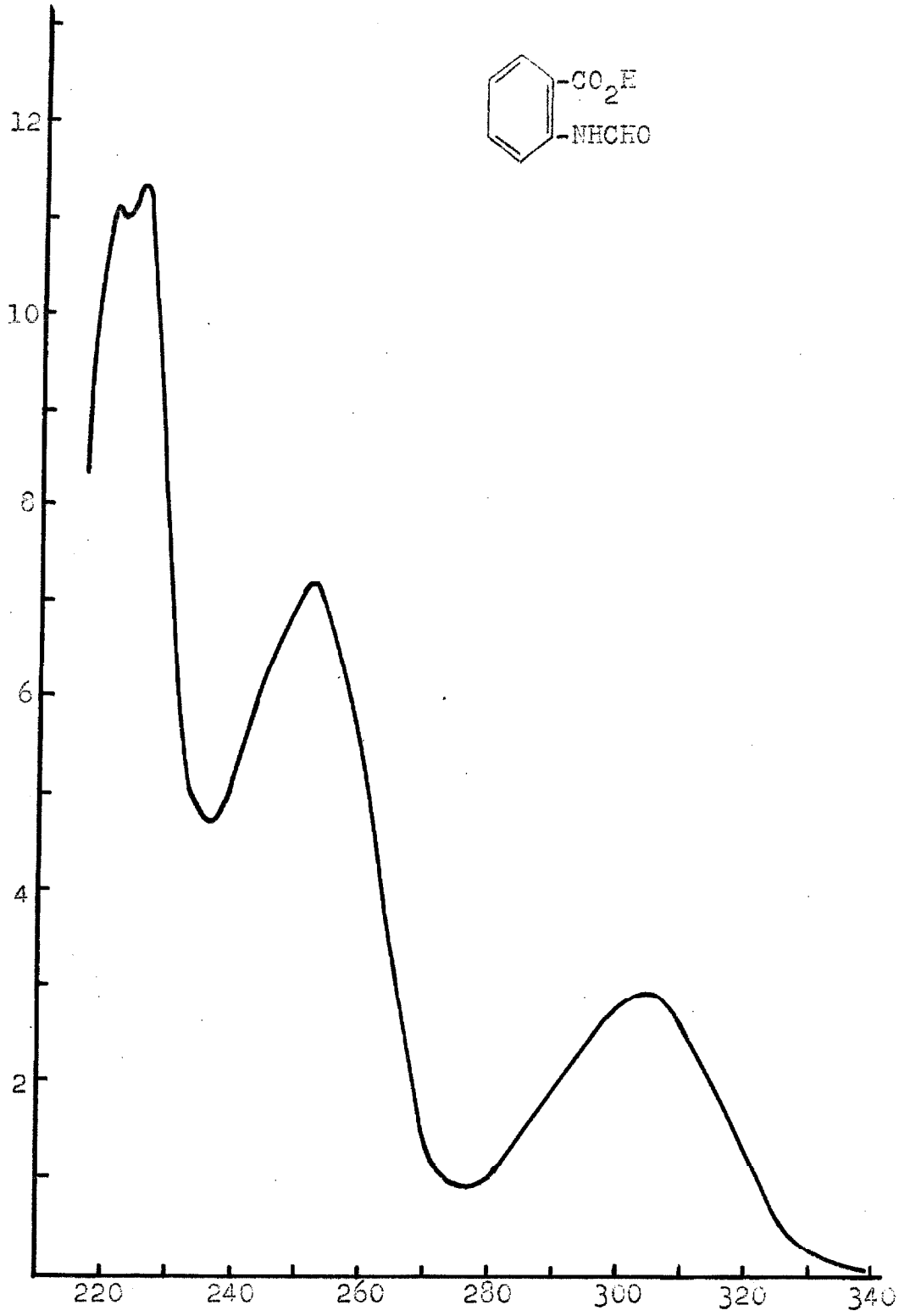
N-methyl-N-(o-nitrobenzoyl)benzenesulfonamide, p. 135.



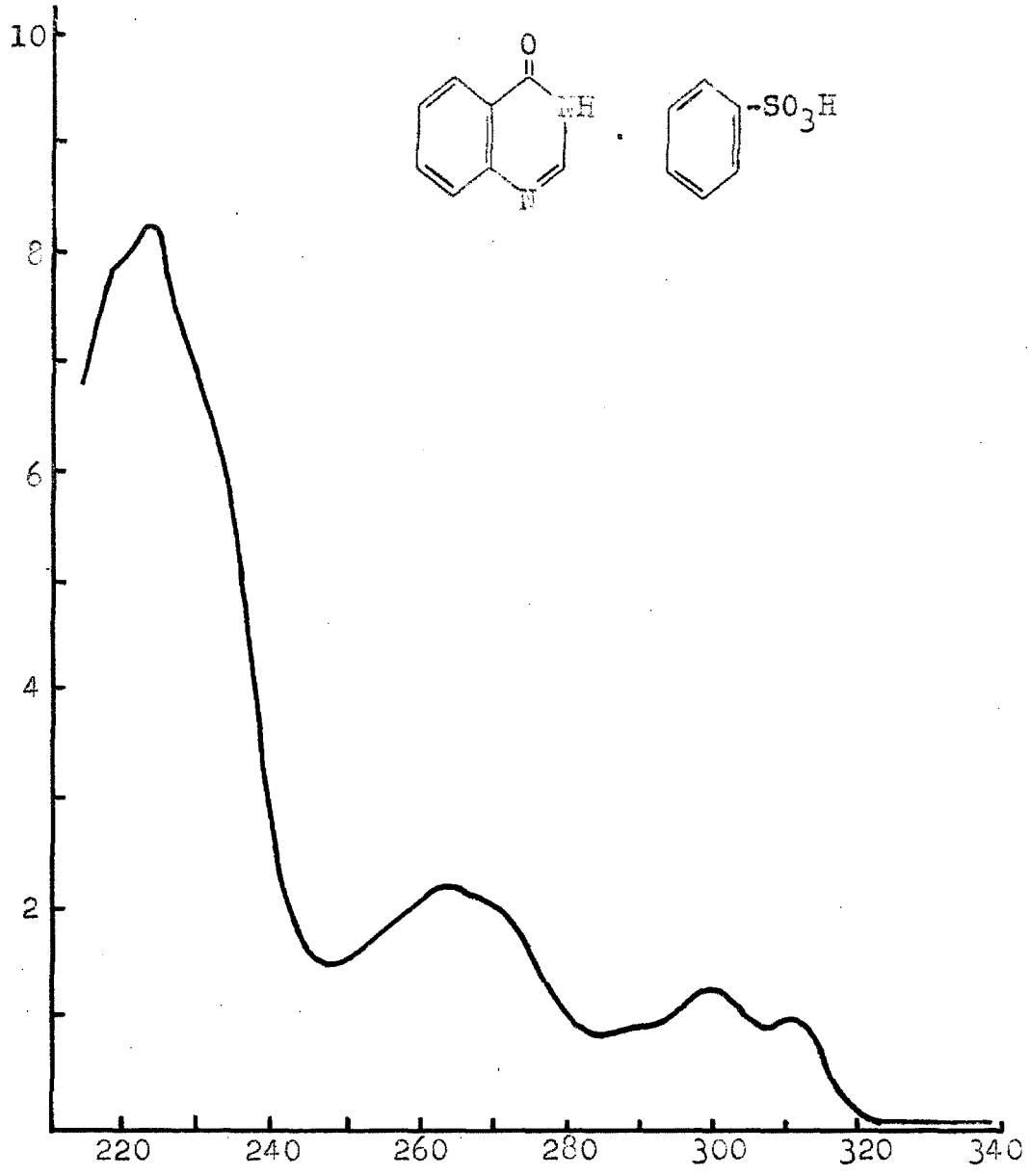
N-methyl-N-benzoylbenzenesulfonamide, prepared by fusion of benzoyl chloride and N-methylbenzenesulfonamide; m.p. 88-89°.



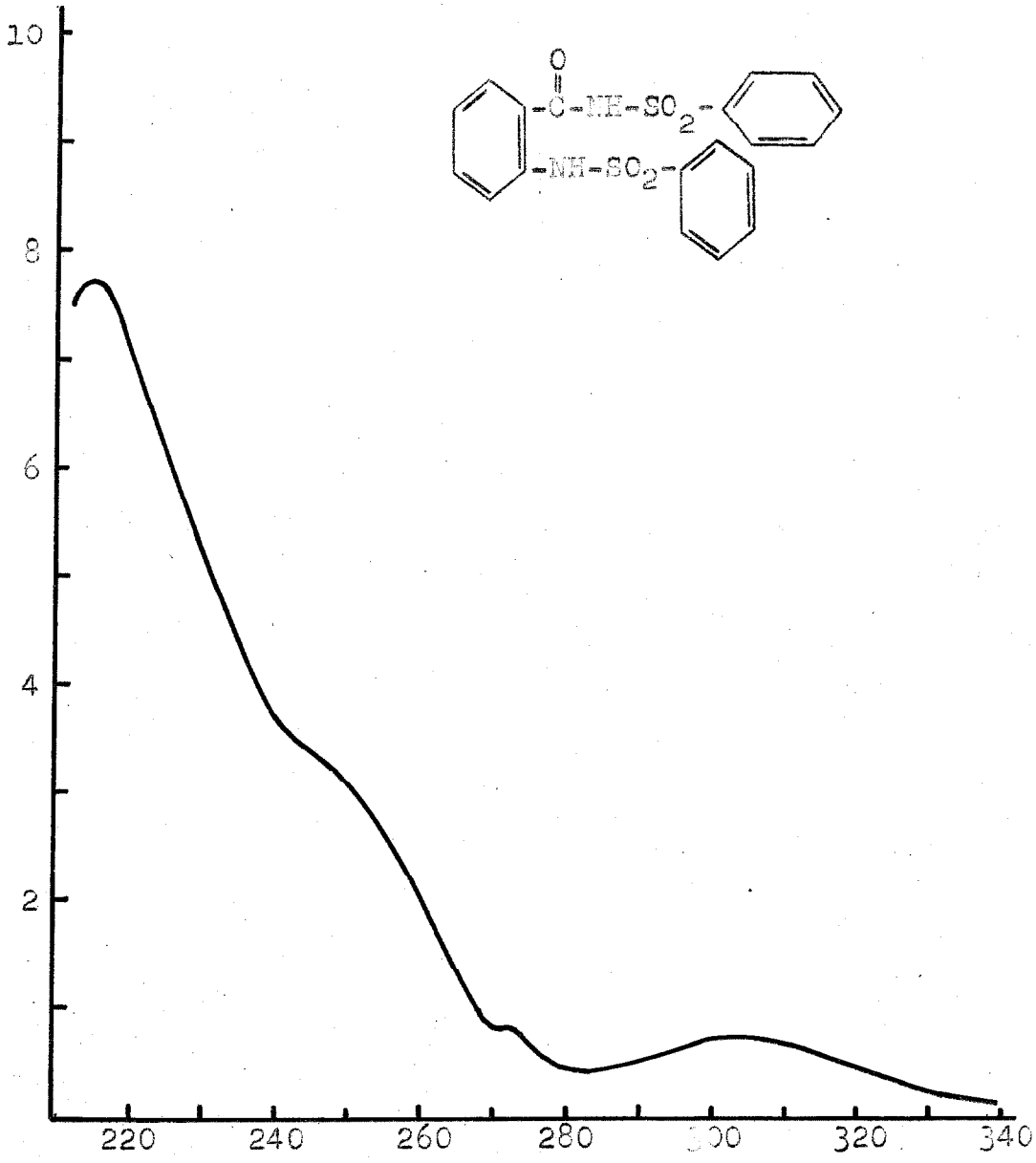
Methyl N-benzenesulfonyl-o-formamidobenzimidate, p. 136.



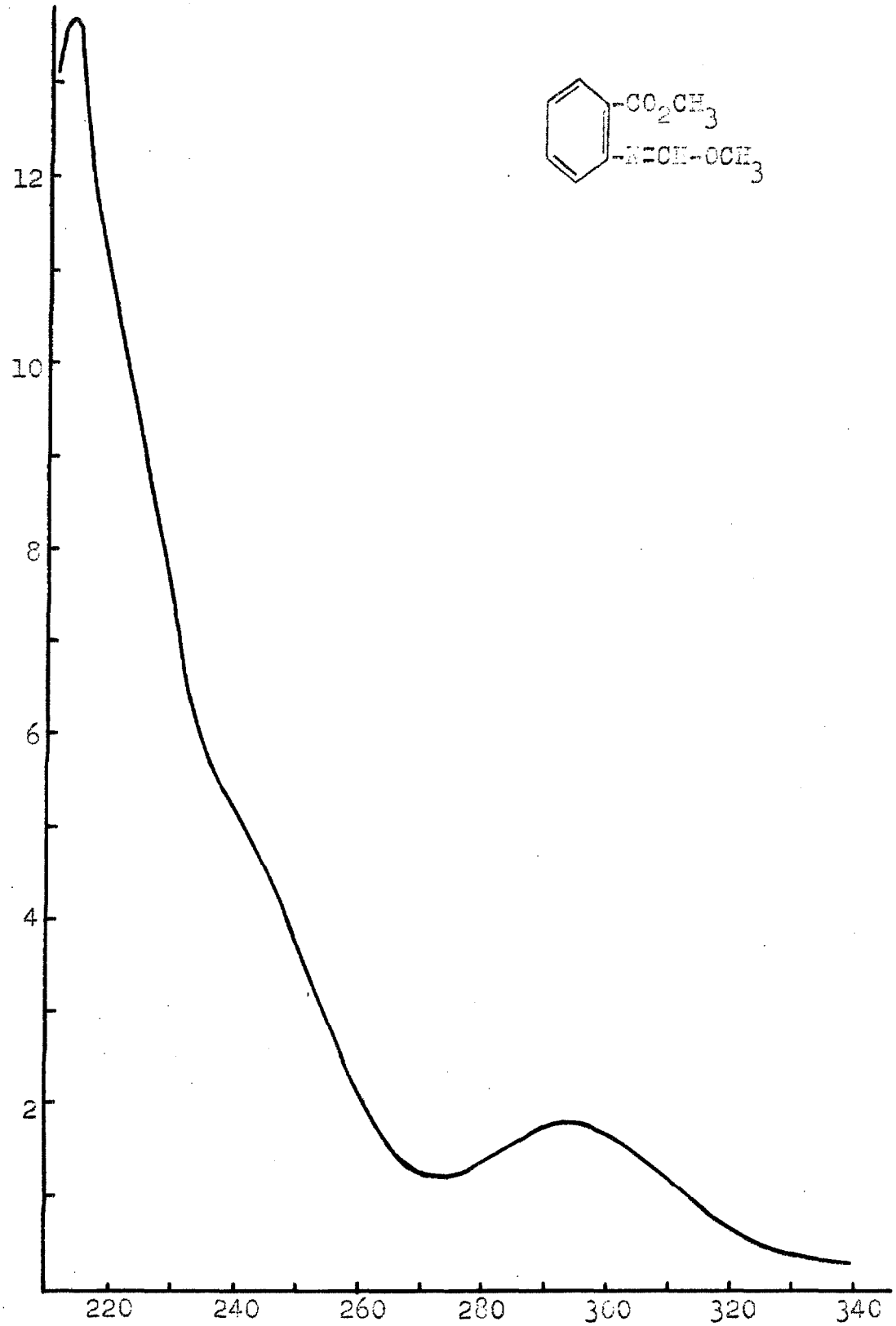
N-formylanthranilic acid, p. 154.



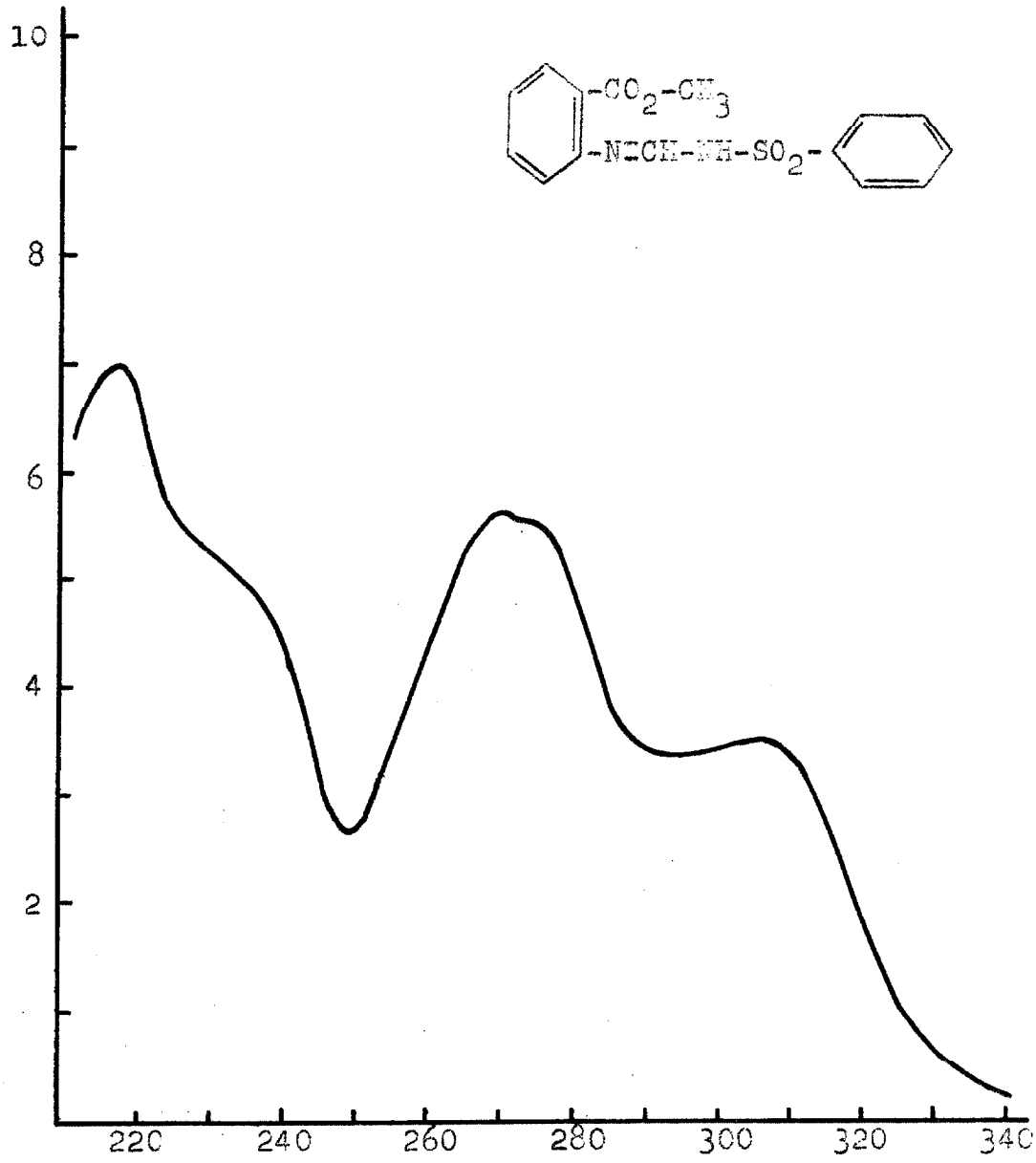
Benzenesulfonic acid salt of
4-quinazolone, p. 155.



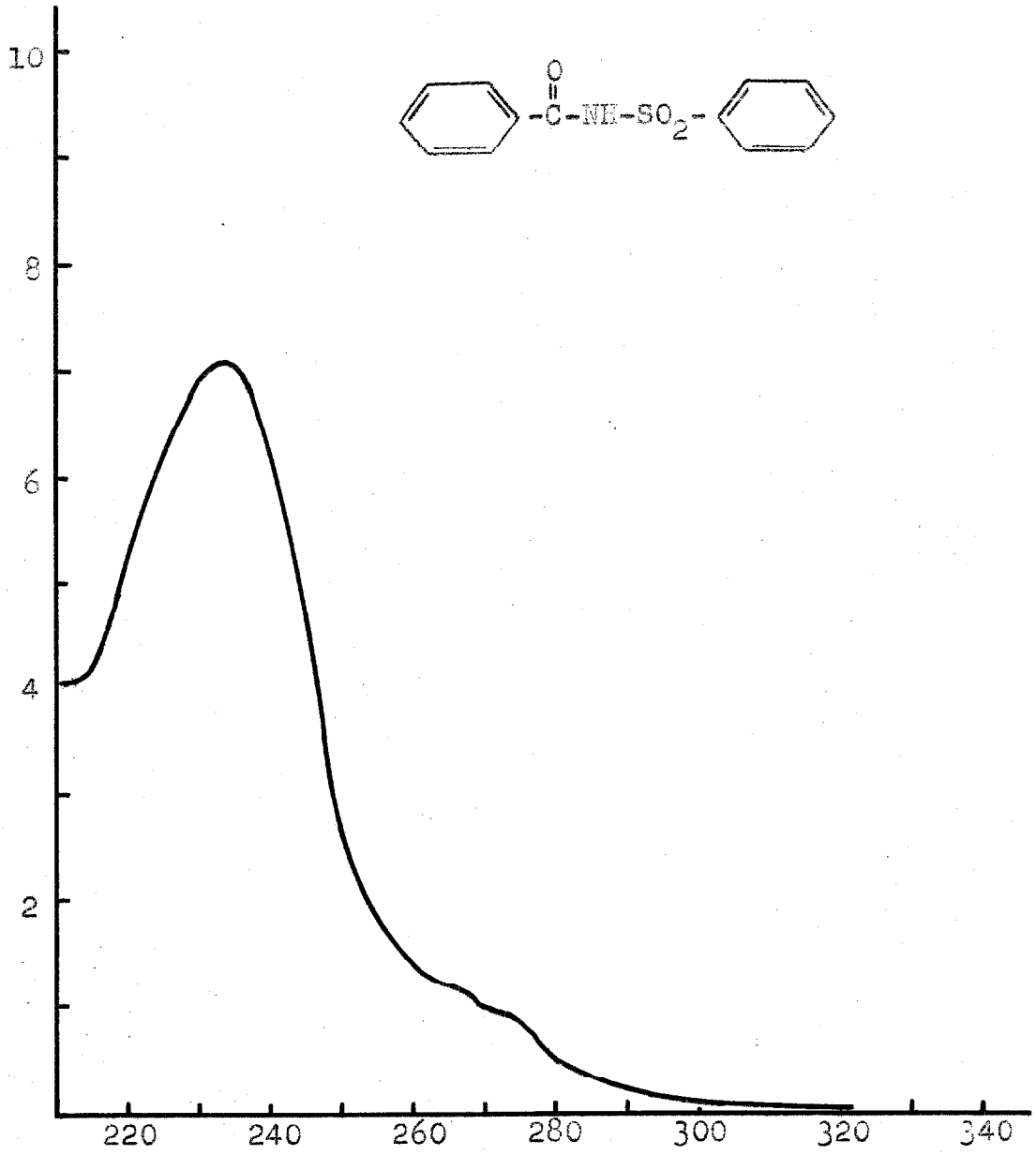
N-(o-benzenesulfonamidobenzoyl)-benzenesulfonamide, p. 155.



Methyl N-(o-carbomethoxyphenyl)formimidate, p. 156.



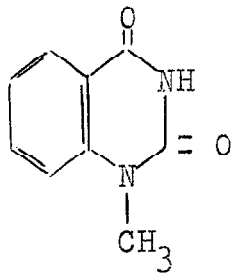
N-benzenesulfonyl-N'-(o-carbomethoxy-phenyl)formamidine, p. 156.



N-benzoylbenzenesulfonamide, for preparation see Ch. Gerhardt and L. Chiozza, Ann. chim. et phys. (3) 46, 145 (1856).

Propositions

1. Wang and Christensen have concluded that 1-(N)-methyl-2,4-diketo-1,2,3,4-tetrahydroquinazoline (I) and its 3(N)-isomer each exist in two stereoisomeric forms. The evidence presented does not lead inevitably to this conclusion.



I

C. H. Wang and B. E. Christensen, J. Am. Chem. Soc. 71, 1440 (1949).

2. A scheme is proposed whereby 3-hydroxypiperidine-2-acetic acid (or its lactone) will give iodoform on treatment with sodium hydroxide and iodine. See this thesis, pages 62 and 63.

3. Reissert and Schaaf have prepared 4-hydroxy-2-keto-3-phenyl-1,2,3,4-tetrahydroquinazoline-4-carboxylic acid by action of phenylisocyanate on o-aminophenylglyoxylic acid. The unknown 4-alkoxy-2-quinazolones might be prepared by reacting o-aminophenylglyoxylic acid with cyanic acid, converting the hydroxy acid to the chloro acid, degrading the silver salt with bromine, dehydrobrominating, and treating the resulting 4-chloro-2-quinazolone with a sodium alkoxide.

A. Reissert and H. Schaaf, Ber. 59, 2494 (1926).

4. The ortho and meta, isomers of N,N-dimethylnitroaniline are reported to be red. These compounds would not be expected to be highly colored.

5. The failure of dihydrofebrifugine to react rapidly with paraperiodic acid can be explained on the basis of the formation of a cyclic ester (see pp 42-44, this thesis).

6. The mechanisms of ammonolysis and saponification of esters can be extended to include the opening of anhydrides and imides with aqueous ammonia and alkali, respectively. The direction of opening unsymmetrical molecules can thus be predicted.

E. R. Alexander, Principles of Ionic Organic Reactions, John Wiley and Sons, Inc., New York, N. Y., 1950, p. 231 ff.

7. The alkylation under alkaline conditions of such compounds as 2-pyridone in general leads largely to the N-alkyl product. The usual explanation is inadequate and is not in accord with the available information concerning the structure of these compounds.

R. C. Elderfield, Heterocyclic Compounds, Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1950, p. 534 ff.

8. In part II of this thesis, there is described the reaction of N-(N'-formyl-o-aminobenzoyl)benzenesulfonamide with diazomethane (p. 133 ff). It is proposed that the product melting at 200° is 3-benzenesulfonyl-4-quinazolone, which arose as a consequence of the reaction and was not orginally present as an impurity.

9. Phage-host and phage-host-nuclear relationships could

be best studied in a haploid organism whose genetics are well known and whose chromosomes are orthodox. A systematic search should be made for a phage in Neurospora.

10. Cytologic data on the blue-green algae indicate in some cases that the nuclear material is diffuse. Information on this might be obtained by determination of the killing curve and the sensitive volume with X-rays.

L. W. Sharp, Fundamentals of Cytology, McGraw-Hill, New York, N. Y., 1943, p. 162.