I. THE STRUCTURES OF PHYTOTOXIC COMPOUNDS FROM *THAMNOSMA MONTANA*.

II. THE ACTION OF PHYTOTOXIC COMPOUNDS ON PLANTS.

III. PREPARATION AND REACTIONS OF S-PHENYLTHIOLCARBAMATES.

IV. STUDIES ON THE SYNTHESIS OF ACTIDIONE.

Thesis by

Donald Gibson Crosby

In Partial Fulfillment of the Requirements

For the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

1954

Acknowledgements.

I would like to express my appreciation to Prof. Carl Niemann for his interest and helpful advice during my work at the California Institute of Technology, and for his aid in obtaining several of the most important compounds discussed in Part I of this thesis. I also wish to thank Prof. James Bonner for suggesting the interesting problem described in Part I, and Dr. E.R. Buchman for his aid and advice during that investigation.

To Prof. Howard Lucas I must express my gratitude for his friendship and generous good counsel, both in our professional and personal relationships. I also appreciate the cooperation of R.A. Bernhard and P.E. Peterson during the administration of my teaching duties.

I am indebted to Prof. R.N. Smith of the Department of Chemistry of Pomona College, Claremont, for allowing me to use the facilities of his laboratories during the latter phase of the work described in Part IV, and during the writing of this thesis.

Prof. F.W. Went freely offered the facilities of the Earhart Plant Research Laboratory for the work described in Part II, and I can never forget the friendly associations with him, both in the laboratory and on the desert. Likewise, the friendship of Dr. Don Yost, Dr. A.J. Haagen-Smit, Dr. H.R. Rinderknecht, and many of the Caltech students will be long remembered.

Abstract

The chemistry of the three phytotoxic compounds isolated from Thamnosma montana by E.L. Bennett in 1949 has been studied, and two of the substances have been identified as the known furocoumarins isopimpinellin and byakangelicin. Degradation and partial synthesis of the third compound have shown that it is also a furocoumarin, and have indicated its probable structure.

The effects of several naturally-occurring phytotoxic substances on plants have been observed under controlled conditions, and have been compared with the effects noted in nature. The mechanism of the toxic action is still ambiguous.

Convenient methods for the synthesis of S-substituted thiolcarbamates have been found, and the scope and limitations of their reactions with primary and secondary amines, amides, and hydrazines to produce ureas, acylureas, and semicarbazides, respectively, have been determined. The mechanism of the general reaction and its application to the identification of organic compounds also have been considered.

Attempts have been made to synthesize the antibiotic substance "Actidione" by four different routes, and although this end has not been achieved, much groundwork has been laid for the eventual preparation of the compound.

Contents

PART I. THE STRUCTURES OF PHYTOTOXIC COMPOUNDS FROM *THAMNOSMA MONTANA*.

A. Introduction

"What influence does any plant exert over its vegetable neighbors, and to what extent is this influence felt?" These questions have intrigued both the practical grower and the plant scientist for years, the former because of the direct bearing which the phenomena have on the growth of his crops, and the latter for the great value and wide application which are held in the answers, as well as for numerous more subtle reasons. For a long time, it was suspected that some kind of "chemical warfare" was being carried on between at least certain species of plants, in addition to the well known effects created by competition for light, water, and mineral nutrients, but surprisingly little success was achieved in confirming this suspicion conclusively. This early history has been reviewed in an excellent article by Bonner (1) and it is unnecessary to reiterate here.

Partly at the suggestion of F. W. Went (2), an investigation was undertaken to determine the role and importance of naturally-occurring phytotoxic substances in the ecology of plants in the

California deserts. Bonner and Galston (3) independently had shown that a phytotoxic compound was produced by guayule (Parthenium argentatum) which inhibited the growth of surrounding plants of the same species, and which was subsequently identified as trans-cinnamic acid.

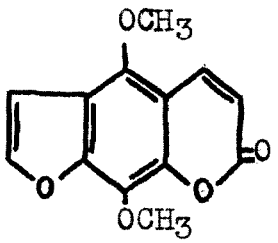
Gray and Bonner (4) investigated the toxic principle extracted from brittlebush (Encelia farinosa), a very common plant on the Sonoran Desert. Degradation and synthesis showed that the compound was 3-acetyl-6-methoxybenzaldehyde. A substance was extracted from Encelia native to Southern Arizona, which was shown to be entirely different chemically from that found in the same species native to California, although exhibiting similar toxic properties. This latter compound may be closely related to α-santonin.

Continuing the study of these natural herbicides, a survey was made of eleven desert plant species to determine the extent to which each showed plant-toxic activity (5). One of the most effective antagonists was Thamnosma montana, and this species yielded an aqueous extract from which three toxic compounds were isolated by chromatography (5).

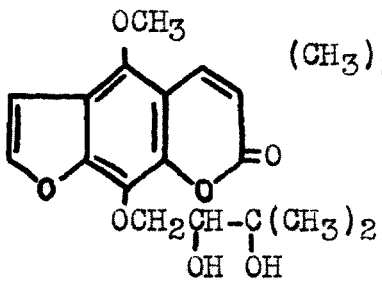
The purpose of the present investigation was to elucidate the chemical structure of each of these substances from Thamnosma, and to determine their effect on other plant species.

It will be shown in the succeeding sections of this part that the compounds belong to the furocoumarin series, and that two of them have been described previously. Evidence of the chemical nature of the third compound has been accumulated, and

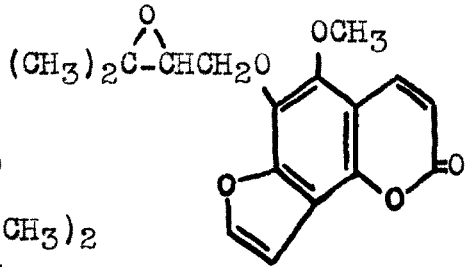
the following structures are now proposed for the toxic substances:



(I)



(II)



(III)

Other members of this series have been isolated from various plant sources and their structures have been determined. In each case, the substance has been given a trivial name, usually derived from that of the parent plant, and these names will be used here when discussing such compounds rather than reference to the more cumbersome systematic nomenclature. An exception to the use of classical names has been made in the case of the parent furocoumarin nucleus known as "angelicin." For the purpose of the present argument, the name "isopsoralen" has been found to be more descriptive, and hence will be employed.

No attempt has been made to indicate the validity of structures for the reference compounds described later. The reader is referred to a review article by Späth (6) from which the location of the original sources may be obtained.

Several different numbering systems have been used in previous descriptions of the furocoumarin series. In this thesis the nomenclature will follow the system outlined in "The Ring Index" by Patterson and Capell which is based on current Chemical Abstracts usage.

Table 1

Phytotoxic Compounds from Desert Plants

Compound	Parent Plant	Location	Reference
trans-Cinnamic Acid	*Parthenium argentatum*	S. California	3
3-Acetyl-6-methoxy-benzaldehyde	*Encelia farinosa*	S. California	4
Isopimpinellin	*Thamnosma montana*	S. California	This thesis
Byakangelicin	*Thamnosma montana*	S. California	This thesis
Thamnosmin	*Thamnosma montana*	S. California	This thesis
Unknown lactone	*Encelia farinosa*	S. Arizona	7
Unknown lactone	*Artemesia absinthium*	Western Europe	8

B. Physical Properties

Purification of the three toxic compounds yielded crystalline solids exhibiting the properties listed in Table 2. In general, the physical properties of (I) are considered to be representative for all three compounds.

The best solvents from which to effect crystallization were found to be acetone and acetic acid. Although aqueous ethanol was used almost exclusively by investigators who studied closely related compounds, this solvent proved unsatisfactory in the present investigation, particularly where (I) was involved. In these cases, crystallization revealed two series of crystals; both fine needles and clumps of microscopic plates were produced simultaneously. Both forms melted at the same temperature, possessed identical absorption spectra in the ultraviolet, and were interconvertible upon recrystallization. In general, solvent systems containing water were found to be less useful in this investigation than were purely organic solvents.

(I) crystallizes from organic solvents in bright yellow needles, while (II) and (III) usually separate as small cream-colored plates, although (III) was found to form in tiny hair-like needles when crystallized from a small amount of acetone by addition of ether.

When heated, (I) gives off a heavy, vanilla-like odor which disappears on cooling. The substance may be sublimed either <u>in vacuo</u> or at ordinary pressure to form tangled needles closely resembling yellow cotton, and melting at the same temperature as the original compound.

All three toxic compounds fluoresce brightly under ultraviolet light, and this feature greatly aids in their separation by chromatographic adsorption. After exposure to an argon lamp for several hours, (I) was recovered apparently unchanged.

Qualitative analyses showed that the compounds did not contain N, P, S, or halogen, but that alkoxyl groups were present in each case. Quantitative analyses gave the results tabulated in Table 3, and from these data it was concluded that the most probable formulas for (I), (II), and (III) were $C_{11}H_4O_3(OCH_3)_2$, $C_{16}H_{15}O_6(OCH_3)$, and either $C_{16}H_{13}O_5(OCH_3)$ or $C_{16}H_{15}O_5(OCH_3)$, respectively. Although values found for C and H were high in each case, a partial survey of the literature dealing with the structure determination of other natural products now known to be closely related to those from Thamnosma shows that this same difficulty invariably has occurred in the past.

The absorption spectra of (I), (II), and (III) in the ultraviolet region, and of (III) in the infrared are presented in Figures 1 and 2. Their significance in the structure proof of the compounds will be shown in a later section.

Table 2.

Physical Properties of Thamnosma Toxic Compounds.

Compound	Formula	M.p. °C	$[\alpha]_D^{25}$	Solubility[a]	
				Water	Organic Solvents
I	$C_{13}H_{10}O_5$	147.5-148.5°	inactive	15 mg/l.	Sol. acetone, benzene, ethanol, methanol, ethyl acetate; sl. sol. ether, chloroform; insol. carbon tetrachloride.
II	$C_{17}H_{18}O_7$	122.0-123.0°	23.0°[b]	150 mg/l.	
III	$C_{17}H_{16}O_6$	175.5-176.5°	-11.9°[c]	30 mg/l.	

a. The solubility characteristics of the three compounds are similar.

b. c = 3.25% in pyridine.

c. c=2.10% in 1:1 ethanol-acetone.

Table 3

Quantitative Analyses of Thamnosma Toxic Compounds.[a]

		% C		% H		% OCH_3		M.W.	
Compound	Calc'd for:	Found	Calc'd	Found	Calc'd	Found	Calc'd	Found	Calc'd
I	$C_{13}H_{10}O_5$	63.73	63.41	4.52	4.09	25.50	25.20	248	246.2
		63.54	63.41	4.17	4.09	24.53	25.20		
II	$C_{17}H_{18}O_7$	61.38	61.07	5.46	5.43	9.15	9.28	357	334.3
III	$C_{17}H_{16}O_6$	64.84	64.54	5.80	5.10	9.98	9.81	313	318.3
		64.80		5.99					
		64.58		5.79					
		64.78		5.78		9.87			
	Av.	64.75	64.54	5.84	5.10	9.92	9.81		
	$C_{17}H_{15}O_6$	64.75	64.74	5.84	4.80	9.92	9.84		317.3
	$C_{17}H_{17}O_6$	64.75	64.34	5.84	5.40	9.92	9.78		319.3
	$C_{17}H_{18}O_6$	64.75	64.14	5.84	5.70	9.92	9.75		320.3
	$C_{17}H_{19}O_6$	64.75	63.94	5.84	6.00	9.92	9.72		321.4

a. Analyses by G. Oppenheimer, G. Swinehart, and A. Elek.

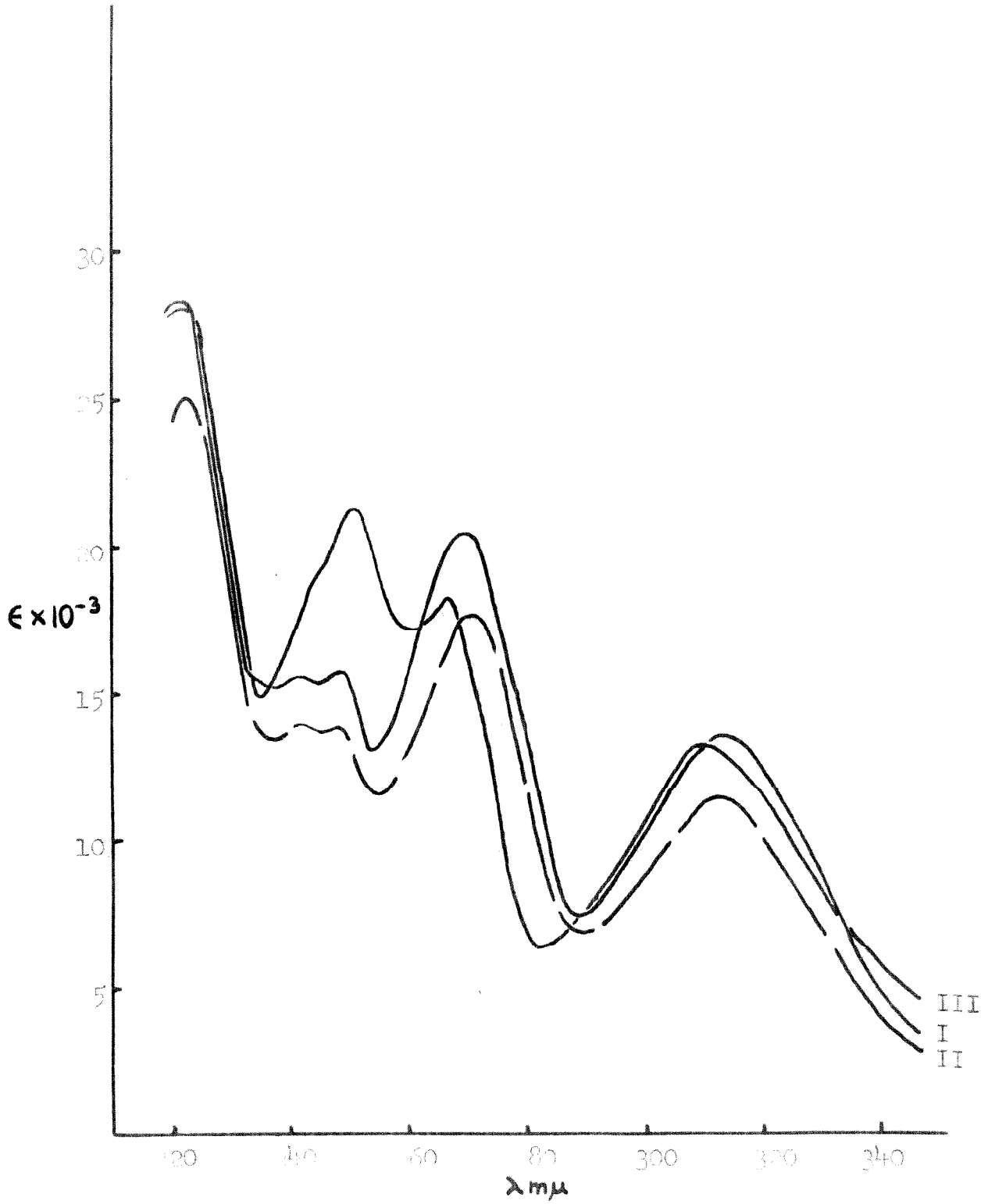


Fig. 1. U.v. Spectra of Thevnosma Toxic Compounds.

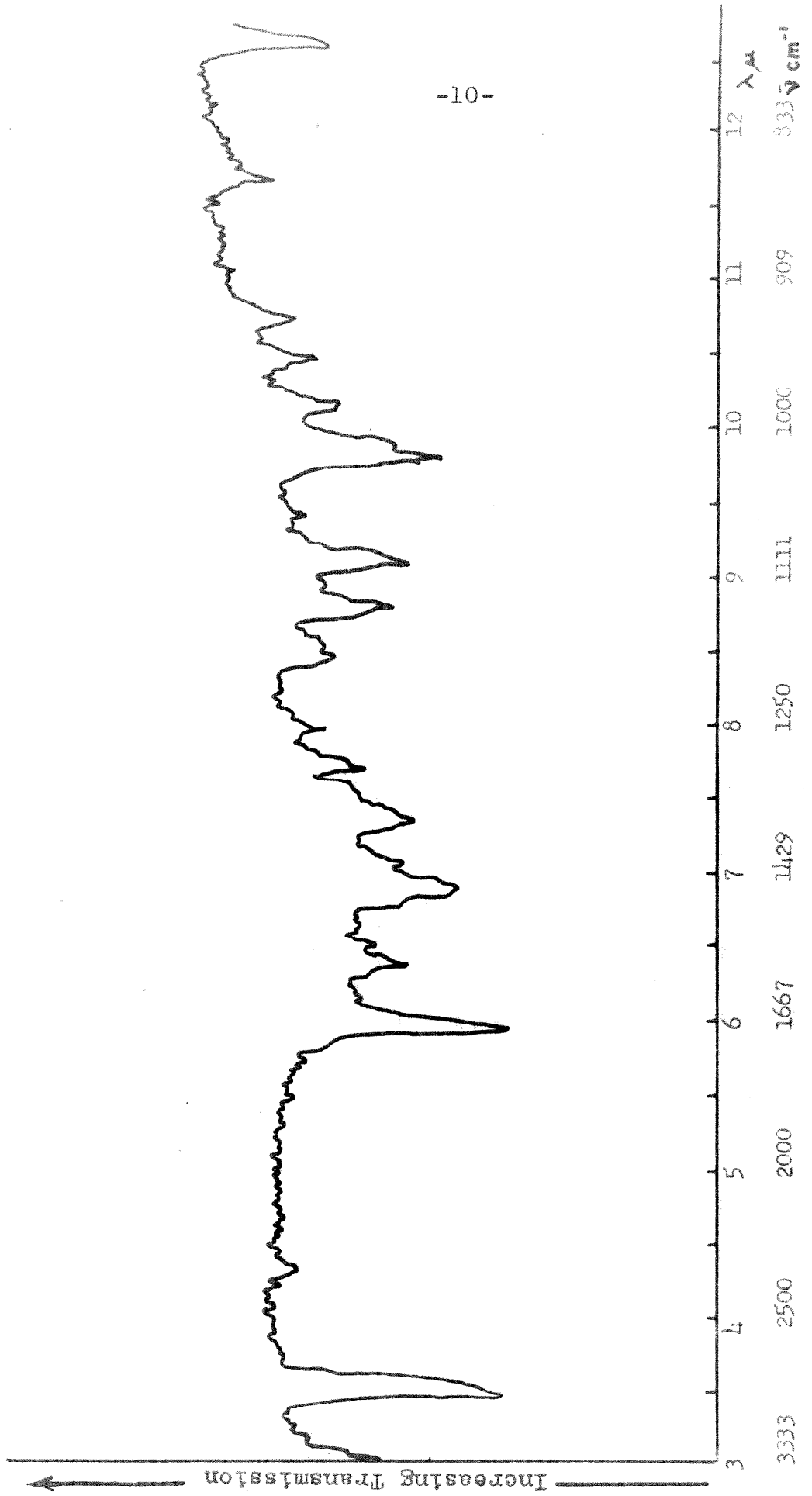


Fig. 2. Infrared Absorption Spectrum of Compound (III).

C. Chemical Properties

1. General Properties

The Thamnosma compounds exhibited several common chemical properties, and these will be grouped together. All of them were found to be practically insoluble in water (cf., Table 2), and only slightly soluble in ether, insoluble in 5% aqueous sodium bicarbonate and 5% aqueous sodium hydroxide solutions when cold, but soluble in the latter when hot. They also were soluble in cold concentrated sulfuric acid with formation of a red color, but insoluble in dilute aqueous sulfuric and hydrochloric acids.

When dissolved in warm aqueous sodium hydroxide or potassium hydroxide solutions, the substances imparted a yellow color, and upon acidification, the original compound was recovered apparently unchanged. This reversible transformation was followed in very dilute solution by means of the Beckmann spectrophotometer, and from the graphs shown in Figure 3 it is seen that while the solution process is quite slow, the reversal is almost instantaneous, at least for a part of the molecule. Further, it appears that the reaction with hydroxide affects strongly the chromophoric group present in the original substances. Boiling with dilute hydrochloric acid and with aqueous alkali for several hours did not cause appreciable alteration in the original absorption spectra upon return to pH 7.

Upon dilution of the red solutions of the three substances in concentrated sulfuric acid with water, a solid was precipi-

tated in each case, and these were found to exhibit the properties of the original solutes.

Ignition of the compounds caused them to burn with a luminous flame, and no residue remained after combustion. This indicated unsaturation, probably the presence of an aromatic system.

2. Specific Properties

The effects of various chemical reagents on the toxic compounds were observed on a micro scale, either in a small-diameter pyrex capillary tube or in a depression on a porcelain spot plate. In each case, control samples were tested simultaneously, with both a blank and at least one pure substance of known constitution. An estimated 0.05-0.1 mg. of unknown was used for each of these tests. The results of this examination are shown in Table 4.

A further series of classification reactions was carried out in order to determine the chemistry of the chromophoric group of compound (I). These tests were conducted in a 1 cm. Corex absorption cell, and reaction was detected by changes in the ultraviolet absorption spectrum. The unknown was used at a dilution of $1:10^5$, and the reagents were diluted to a concentration of about 0.01% in aqueous solution. Typical examples of both positive and negative tests are shown in Figure 4, and the combined results are summarized in Table 5. It will be noted in Figure 4 that no attempt was made to measure the extinction of the spectral curves; only shifts in the wavelengths of maxima and minima were recorded.

From these data, it appears that the following conclusions may be drawn:

1. All three compounds contain a chromophoric group which is disrupted upon reaction with alkali under conditions of saponification, but which is reinstated upon acidification. These phenomena are typical of phenols, enols, and lactones.

2. The compounds all contain a readily oxidizable position, but no definite statements can be made about its nature except that it is not a carbonyl function.

3. Compound (II) contains hydroxyl groups, probably as a 1,2-glycol.

4. Compound (I) does not contain any functional groups with the exception of the chromophore mentioned above, and the negative results of the various phenol tests and tests for the carbonyl group virtually limit this to a lactone ring. It is probable that the chromophoric groups in (II) and (III) are similar to that in (I).

Table 4

Classification Tests.

Test	Blank	Control [a]	Thamnosma Compound (I)	(II)	(III)
Tollens'	- [b]	A(+)	+	+	+
Schiff's	-	A(+), B(-)	-	-	-
Dinitrophenyl-hydrazine	-	A(+)	-	-	-
Ceric Nitrate	-	A(+), C(-), D(-)	-	+	-
Ferric Chloride	-	A(+), E(+)	-	-	-
Millon's phenol	-	A(+), F(+)	-	-	-
HIO_4	-	A(-), G(+)	-	+	-
Ferric hydroxamate	-	A(-), H(+)	+	+	+

a. A. Vanillin
   B. Benzyl alcohol
   C. Acetone
   D. Anisaldehyde
   E. p-Hydroxybenzaldehyde
   F. Hydroquinone
   G. Propylene glycol
   H. Ethyl Benzoate

b. (-) and (+) indicate negative and positive tests respectively.

Table 5

Effects of Reagents on the Absorption Spectrum of Compound (I)[a].

Reagent	Spectral Change	
	Cold	After Heating
Hydroxylamine hydrochloride	-[b]	-
Hydrogen peroxide (neutral)	-	-
Periodic acid	-	-
Sodium bisulfite	-	-
Sodium hydroxide	+	+
Hydrochloric acid	-	-
Bromine water	+	c
Chromium nitrate	-	-

a. Spectra were measured in 1 cm. Corex cells with a Beckmann Model DU Quartz Spectrophotometer.

b. (+) Indicates that the spectrum was altered; (-) indicates that no change was observed.

c. Not measured.

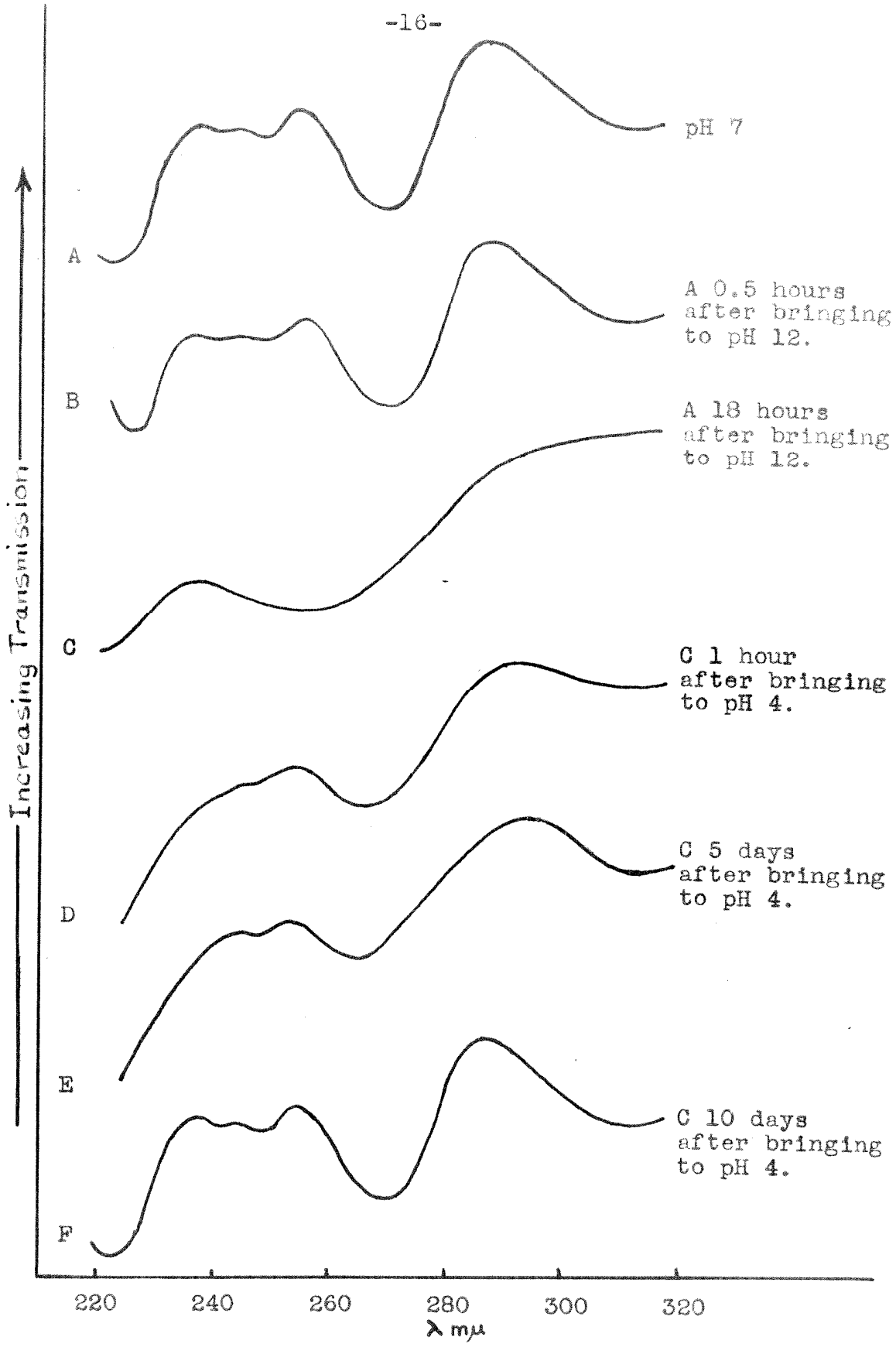


Fig. 3. Effect of Alkali on the Spectrum of Isopimpinellin (Compound I).

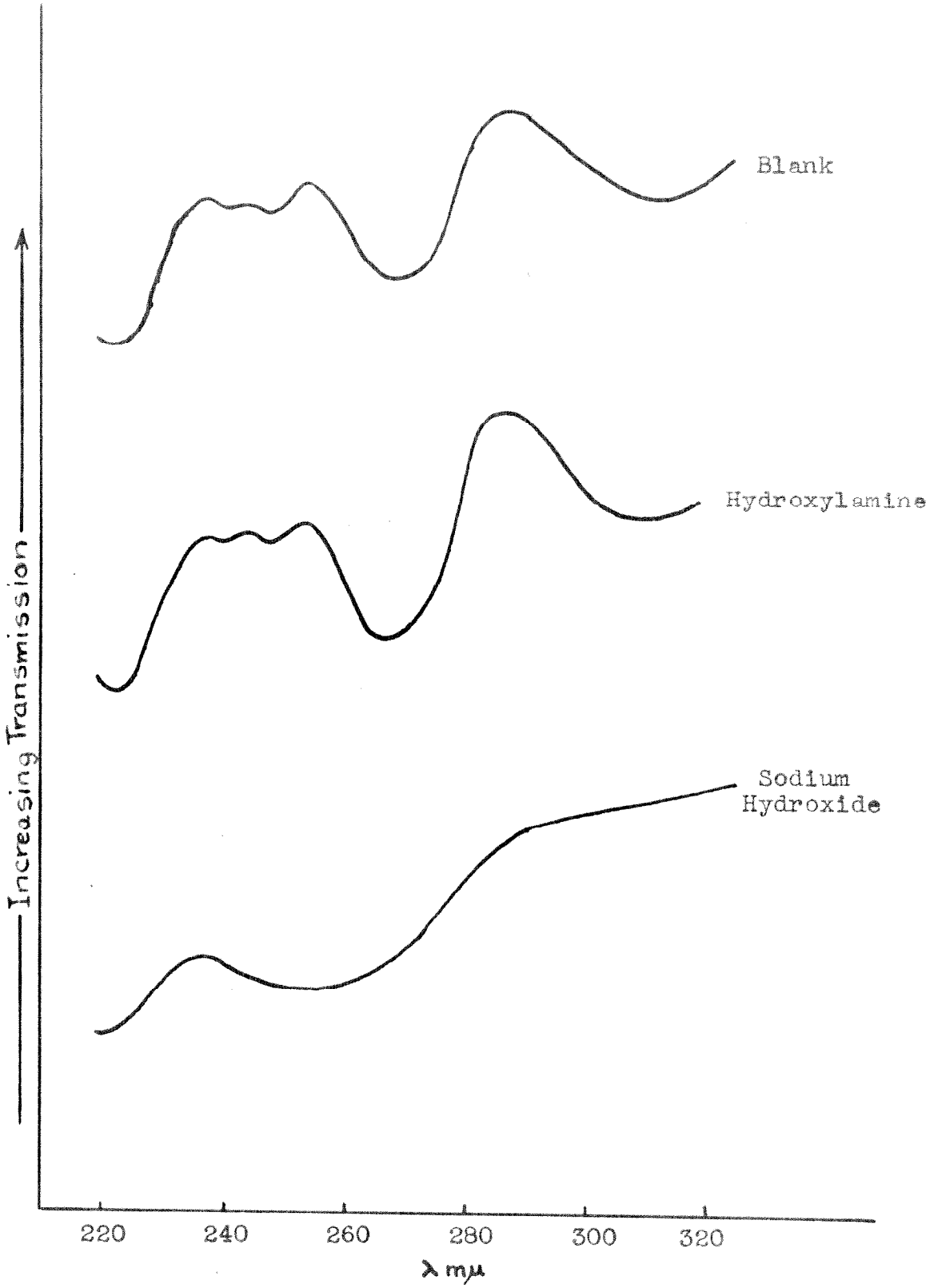


Fig. 4. Effects of Reagents on the Spectrum of Isopimpinellin (I).

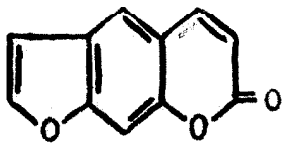
D. Chemical Structure:
Evidence from Absorption Spectra

The ultraviolet absorption spectra of the toxic compounds from Thamnosma have been presented in Figure 1, and the evidence of chemical reactivity as shown by absorption spectra has been presented in subsequent figures. It is the purpose of this section to combine and compare spectral data from several sources, and to indicate the deductions drawn from such comparison regarding the overall structure of the unknown substances.

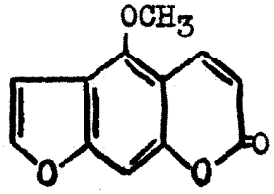
Figure 5 gives the spectra of several compounds which were thought to possess structures similar to those of (I), (II), and (III). Although minor similarities may be seen in retrospect, these curves in general afforded no help during the investigation, and are included here only as a matter of record.

The spectra of several substituted coumarins are presented in Figure 6 (9), showing the first appearance of the curves characteristic of the toxic compounds.

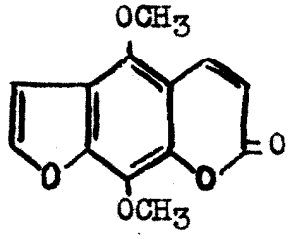
In Figure 7, the spectra of selected members of the psoralen system of compounds are shown. Psoralen (IV) is the parent substance, and it is apparent that its spectrum is altered considerably by the introduction of a methoxyl group into the benzene ring, as in bergapten (V), and again by introduction of still another methoxyl, as in isopimpinellin (VI).



(IV)



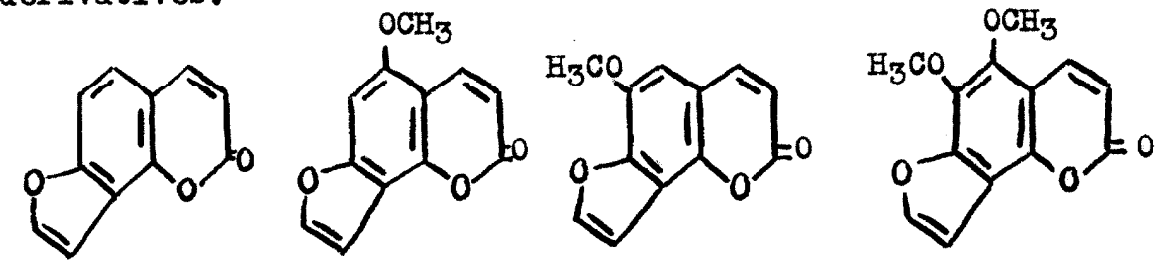
(V)



(VI)

Although the shift in the extinction and wavelength of the maxima are not regular enough to permit accurate prediction of the difference between curves at a given wavelength (Table 6), the comparison is important because it shows the great dependence of spectrum on the position of substituent methoxyl groups, and allows a distinction to be made among members of the series.

Similarly, corresponding members of the isopsoralen group are differentiated to some degree by changes in spectra caused by methoxyl substitution, as shown in Figure 8. However, in this case the curves are sufficiently similar (Table 7) to preclude the prediction of positions of substitution from the spectrum with certainty. The value of these curves lies in their display of the characteristic spectrum of isopsoralen derivatives.



(VII) (VIII) (IX) (X)

Figures 9, 10, and 11 compare the spectra of the toxic compounds from Thamnosma with curves of known compounds (cf., Table 8). Discussion of these comparisons will be postponed until the last section of this part of the thesis.

The next figure shows the radicle change in spectrum caused by saturation of the isolated double bond of psoralen with hydrogen. Although the absorption curve for a dihydrofurocoumarin saturated in the pyrone ring unfortunately is not

available, Table 9 indicates the change in absorption maxima upon hydrogenating isopimpinellin (VI) to 5,6-dihydroisopimpinellin. It is also apparent in this case that the alteration of the spectrum upon saturation at these points is considerable.

The absorption spectrum of (III) in the infrared region between 3μ and 13μ has been presented in Figure 2 (p. 17). Surprisingly little information could be obtained from this graph other than the already apparent facts that the substance was aromatic, probably highly substituted, and perhaps contained some sort of acid-carbonyl group. The spectrum was measured in Nujol mull in a recording Perkin-Elmer infrared spectrophotometer with the aid of Dr. M. Ikawa.

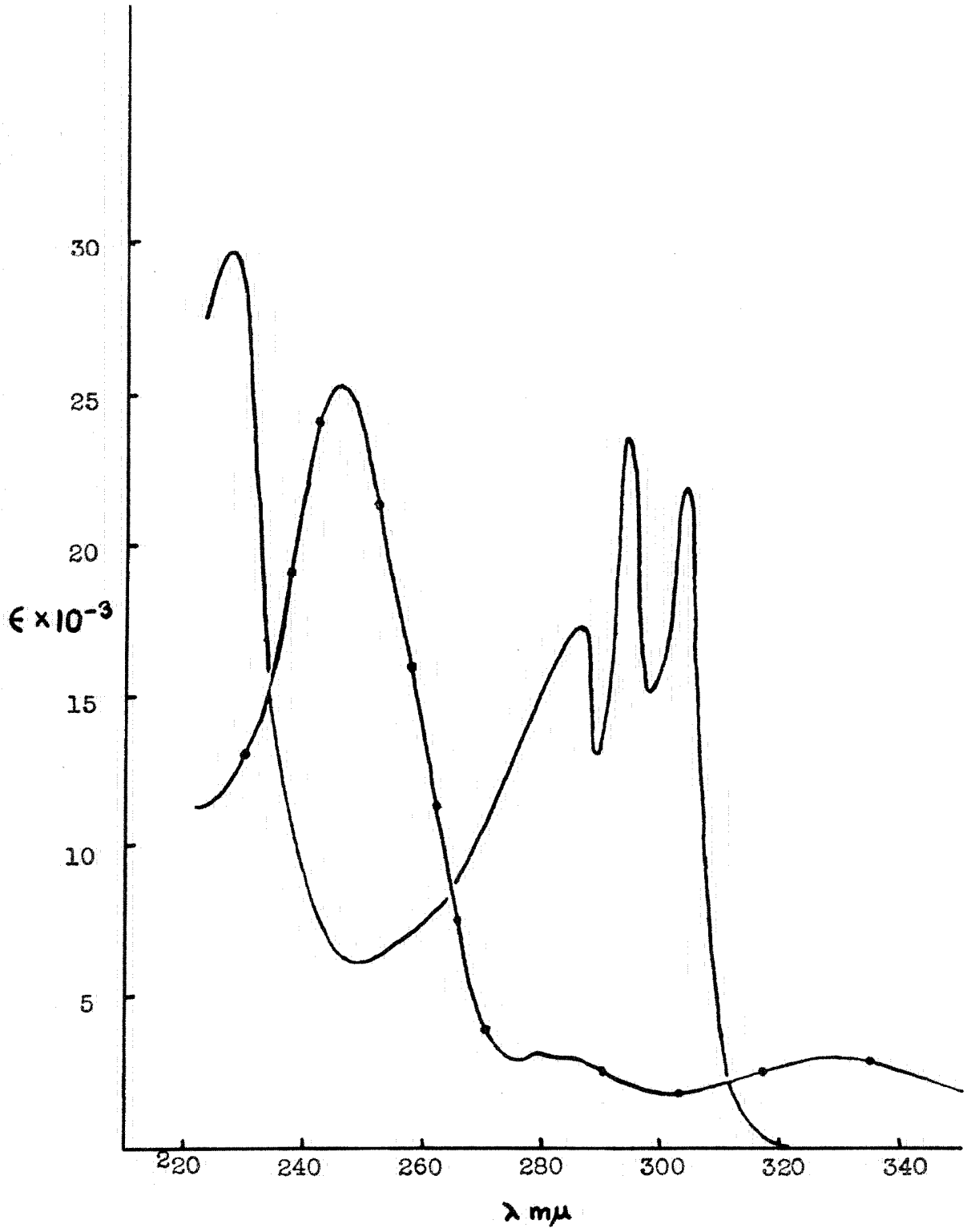


Fig. 5. U.v. Absorption Spectra.

Khellin(⟷); 3,7-Dicarbethoxy-2,6-dimethyl-p-benzdifuran(——).

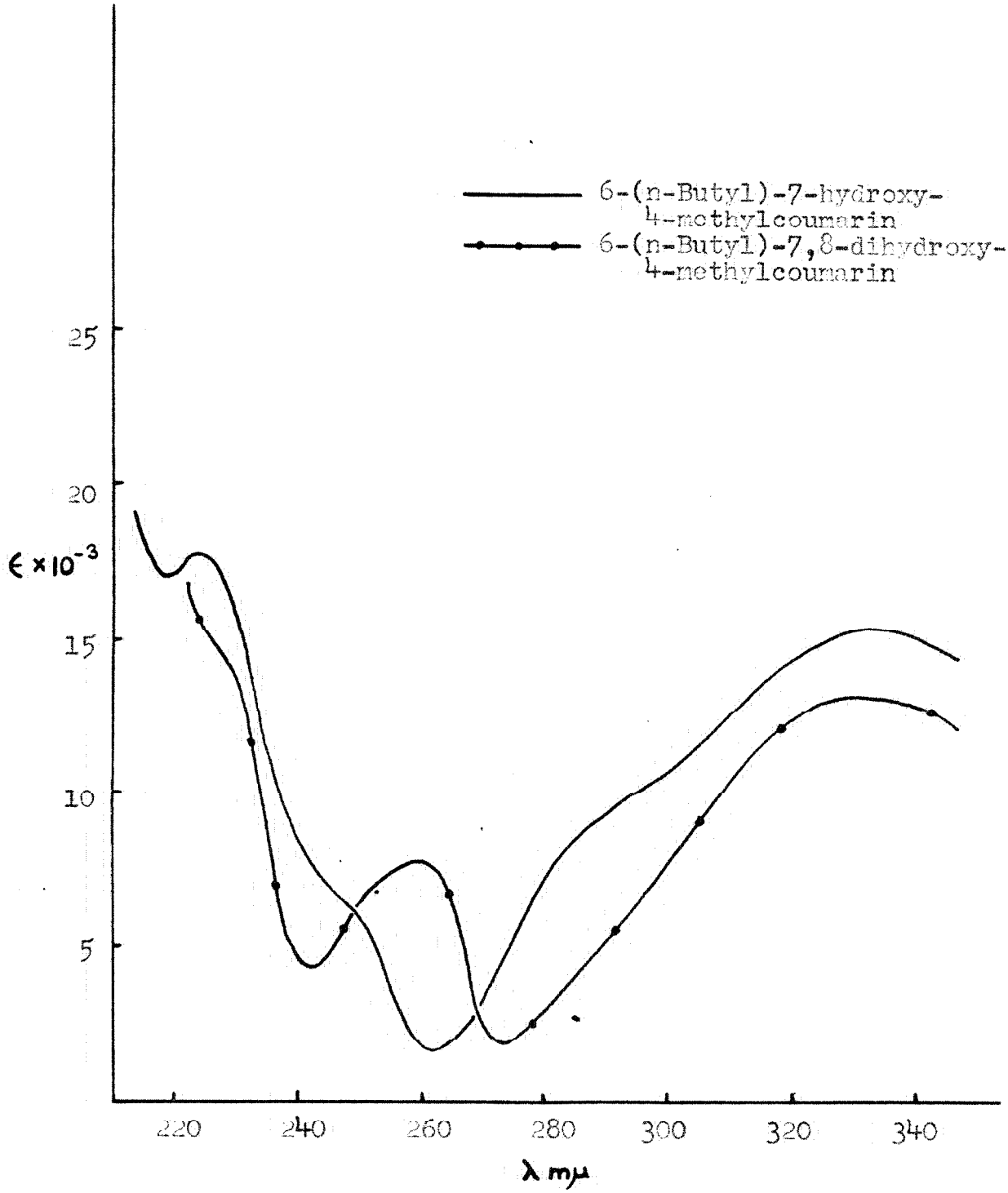


Fig. 6. U.v. Spectra of Selected Coumarins.

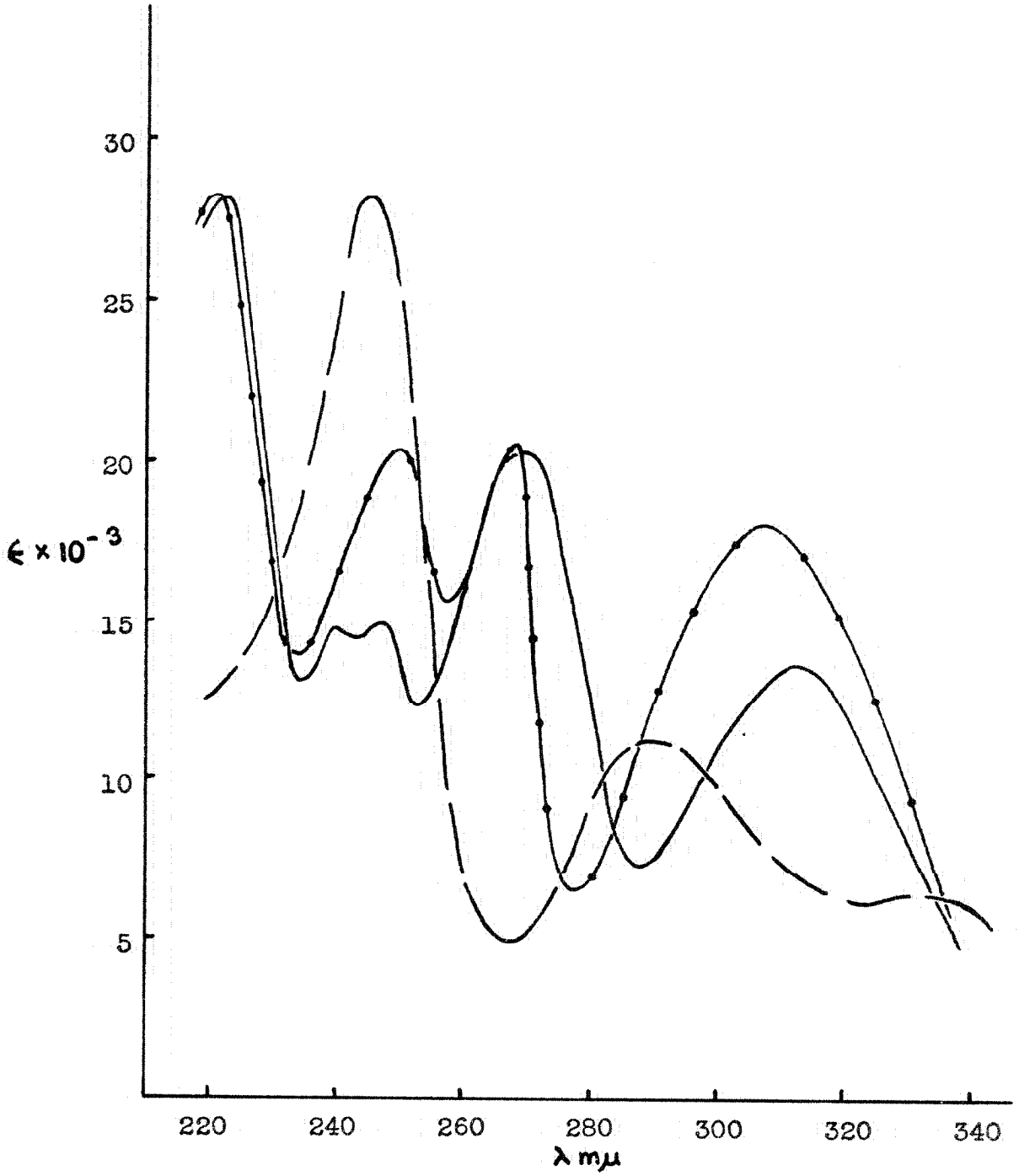
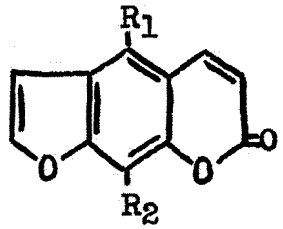


Fig. 7. U.v. Absorption Spectra of Psoralens.

Psoralen(– –); Bergapten(–•–); Isopimpinellin(——).

Table 6

Effects of Substituents on Absorption Spectra of Furocoumarins.



			Maximum Extinction									
Compound	R_1	R_2	λ_1[a]	ϵ_1[b]	λ_2	ϵ_2	λ_3	ϵ_3	λ_4	ϵ_4	λ_5	ϵ_5
Psoralen[c]	H	H	---	---	244	28.20	---	---	290	11.21	332	6.60
Bergapten[d,e]	OCH_3	H	220	24.55	---	---	250	17.00	268	17.00	307	14.48
Isopimpinellin	OCH_3	OCH_3	222	28.30	240	14.85	247	24.92	269	20.35	313	13.53

a. All values of λ are in mμ.

b. $\epsilon_n = \epsilon \times 10^{-3}$.

c. Data of Horning and Reisner (10).

d. Spectral data on xanthotoxol, (R_1 = H, R_2 = OCH_3) are unavailable.

e. Data of Caldwell and Jones (11).

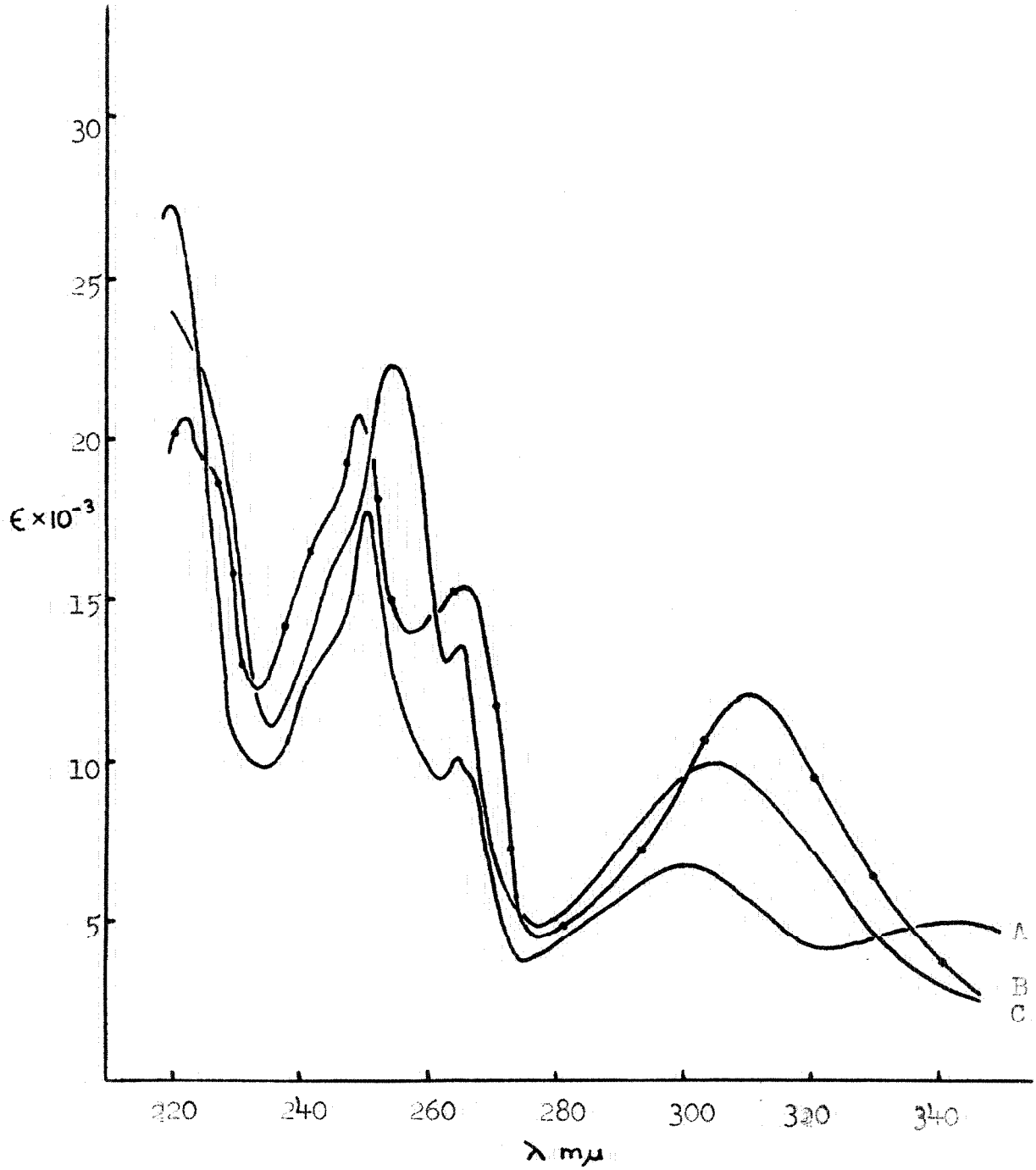
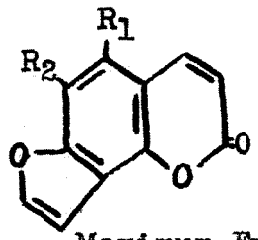


Fig. 8. U.v. Spectra of Selected Isopsoralens.
A. Sphondin; B. Isobergapten; C. Pimpinellin.

Table 7

Effects of Substituents on Absorption Spectra of Furocoumarins.



Compound	R_1	R_2	Maximum Extinction λ_1[a]	ϵ_1[b]	λ_2	ϵ_2	λ_3	ϵ_3	λ_4	ϵ_4
Isopsoralen[c]	H	H	---	---	234	20.	268	4.5	---	---
Isobergapten	OCH_3	H	222	20.70	249	20.80	266	15.59	310	11.90
Sphondin	H	OCH_3	220	27.2	251	17.8	265	10.1	301	6.8
Pimpinellin	OCH_3	OCH_3	220	24.0	254	22.3	265	13.6	305	9.9

a. All values of λ are in mμ.

b. $\epsilon n = \epsilon \times 10^{-3}$.

c. Data of Jois and Manjunath (12).

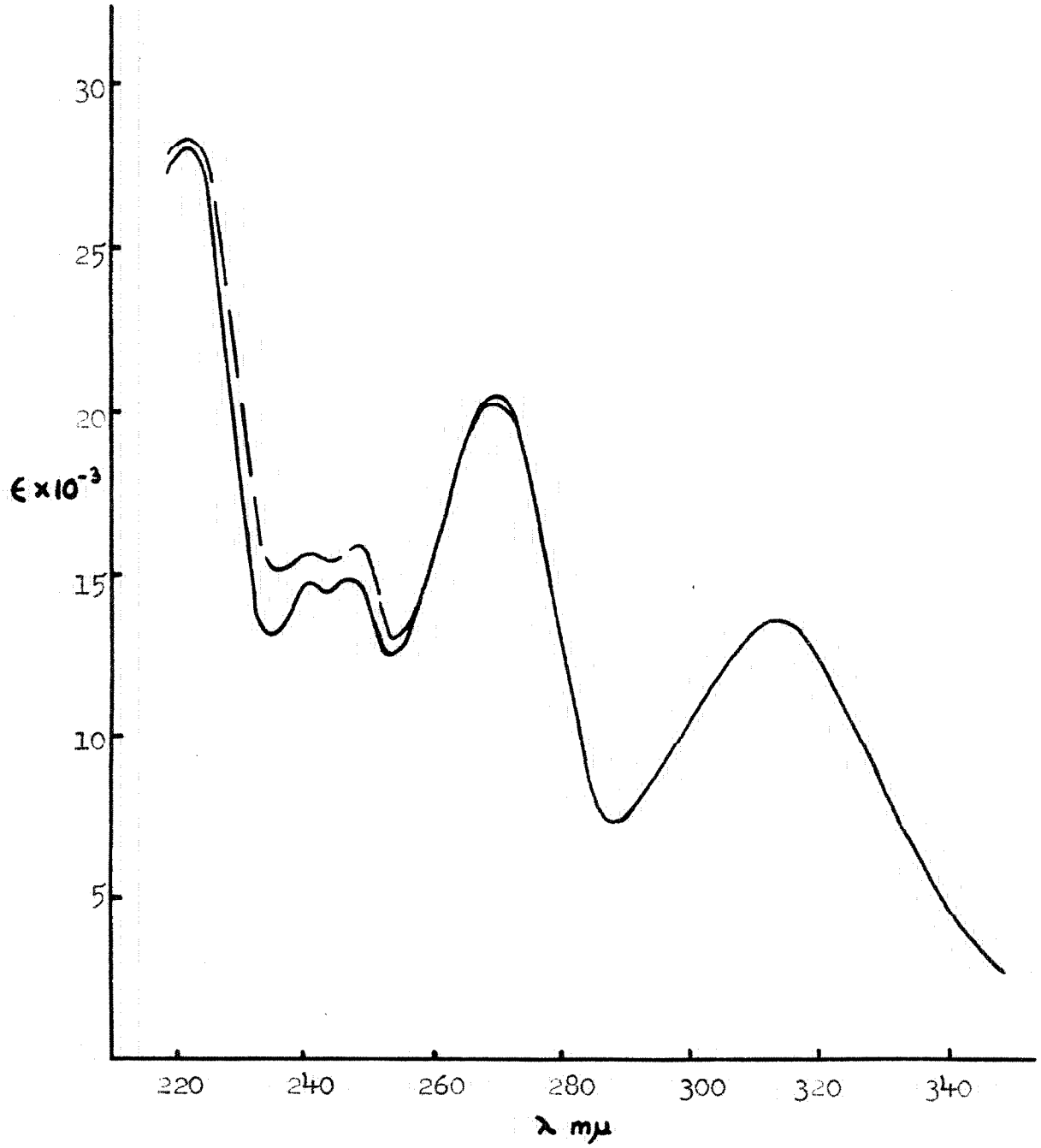


Fig. 9. U.v. Spectra of (I)(---) and Isopimpinellin.

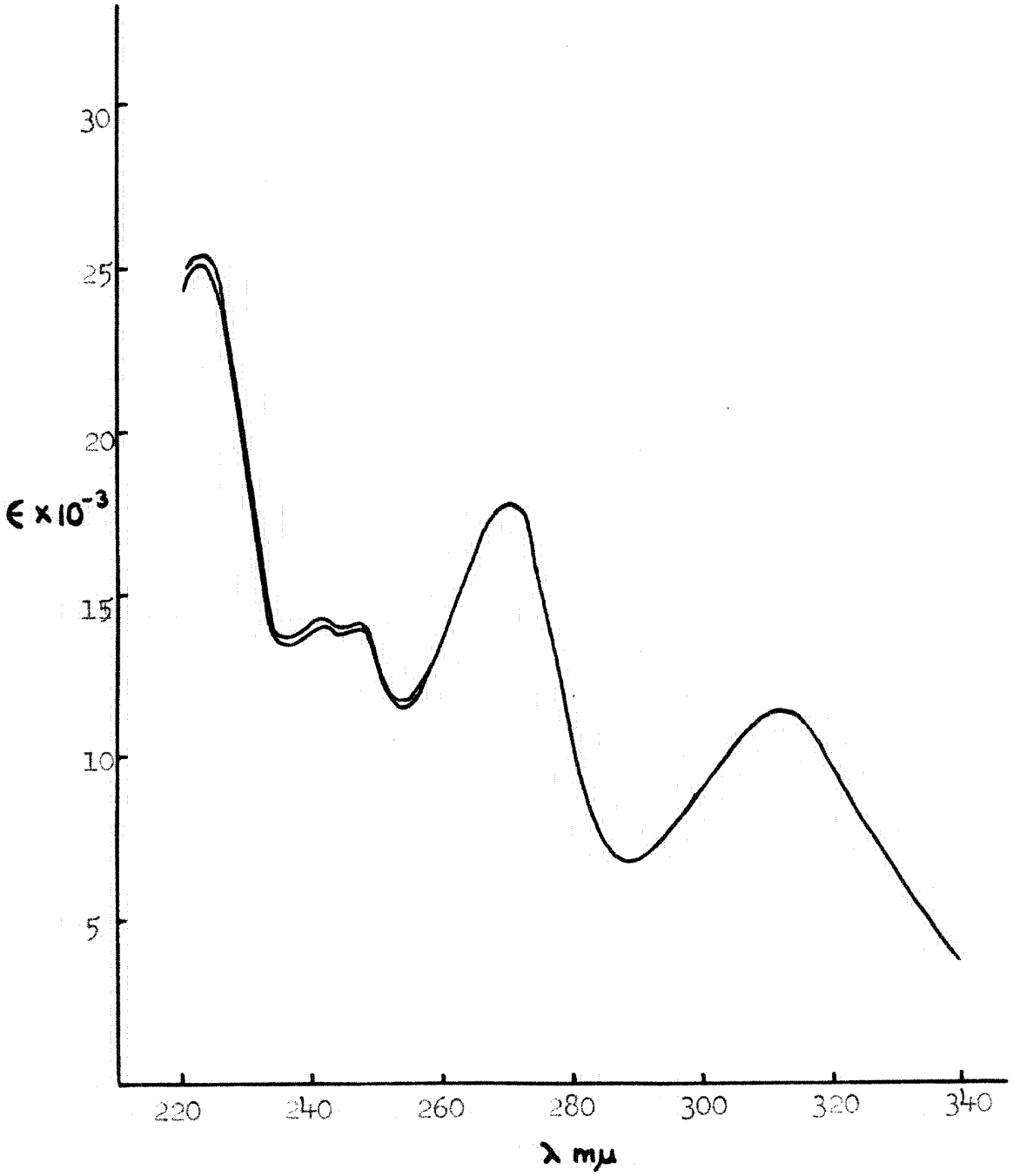


Fig. 10. U.v. Spectra of (II) and Byakangelicin.

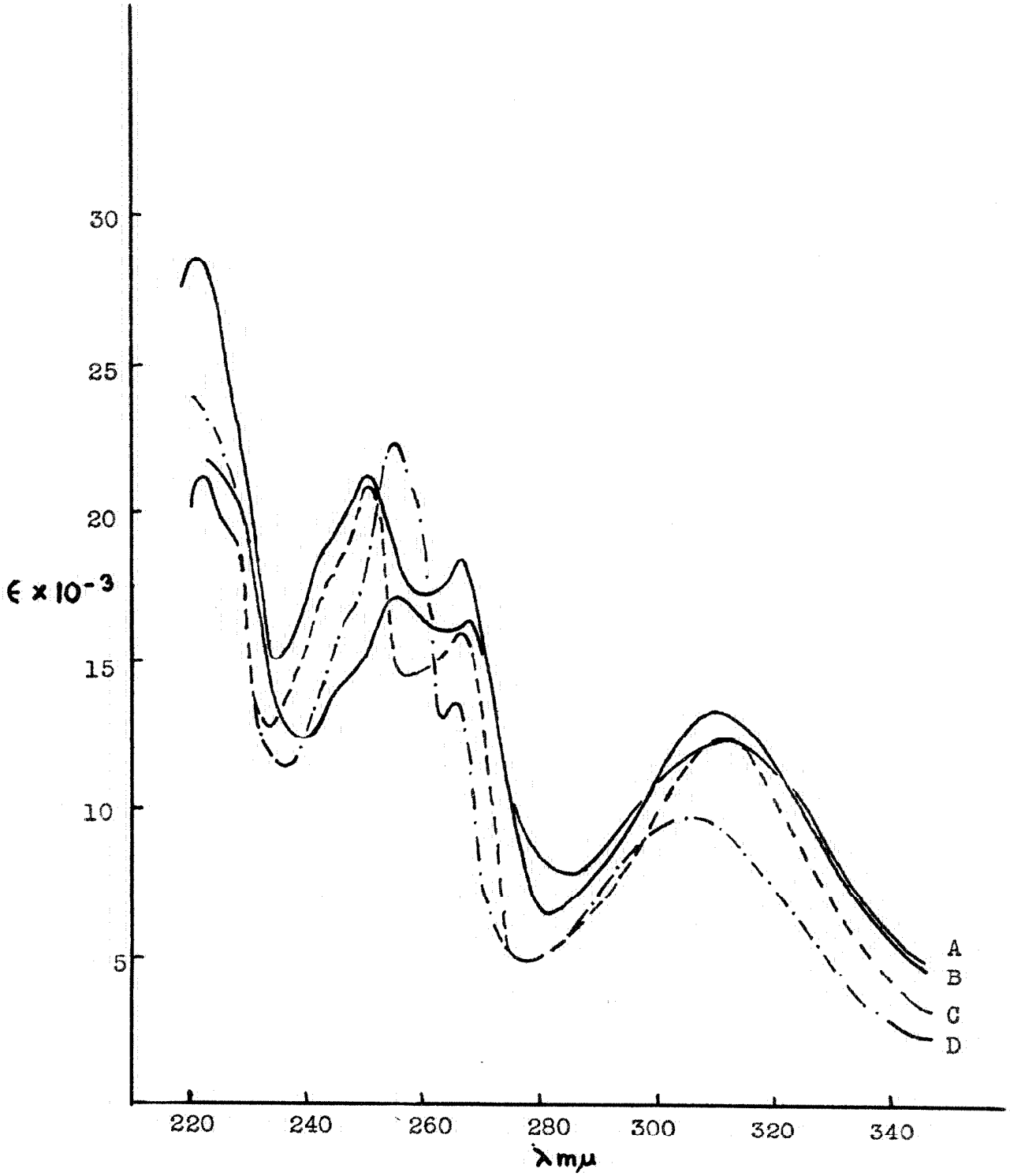
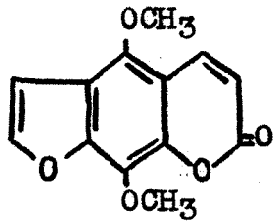


Fig. 11. Comparison of U.v. Absorption Spectra.

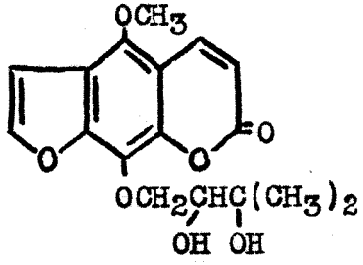
A. Thamnosmol; B. (III); C. Isobergapten; D. Pimpinellin.

Table 8

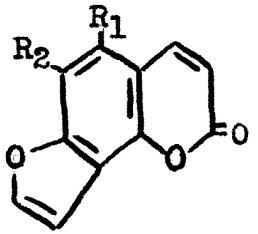
Comparison of Spectra of Thamnosma Compounds with Known Spectra.



(VI)



(XI)



(VIII - X)

Compound	Maximum Extinction									
	λ_1	ϵ_1	λ_2	ϵ_2	λ_3	ϵ_3	λ_4	ϵ_4	λ_5	ϵ_5
Thamnosma Compound I	222	28.30	241	15.60	248	15.70	270	20.40	312	13.40
Isopimpinellin (VI)	222	28.30	240	14.85	247	14.92	269	20.35	313	13.53
Thamnosma Compound II	223	25.00	242	14.00	248	13.90	270	17.70	312	11.40
Byakangelicin (XI)	223	24.90	242	13.80	247	13.70	270	17.70	312	11.40
Thamnosma Compound III	221	28.60	251	21.20	266	18.40	308	13.30	---	---
Isobergapten (VIII)	222	20.70	249	20.80	266	15.59	310	11.90	---	---
Sphondin (IX)	220	27.2	251	17.8	265	10.1	301	6.8	---	---
Pimpinellin (X)	220	24.0	254	22.3	265	13.6	305	9.9	---	---

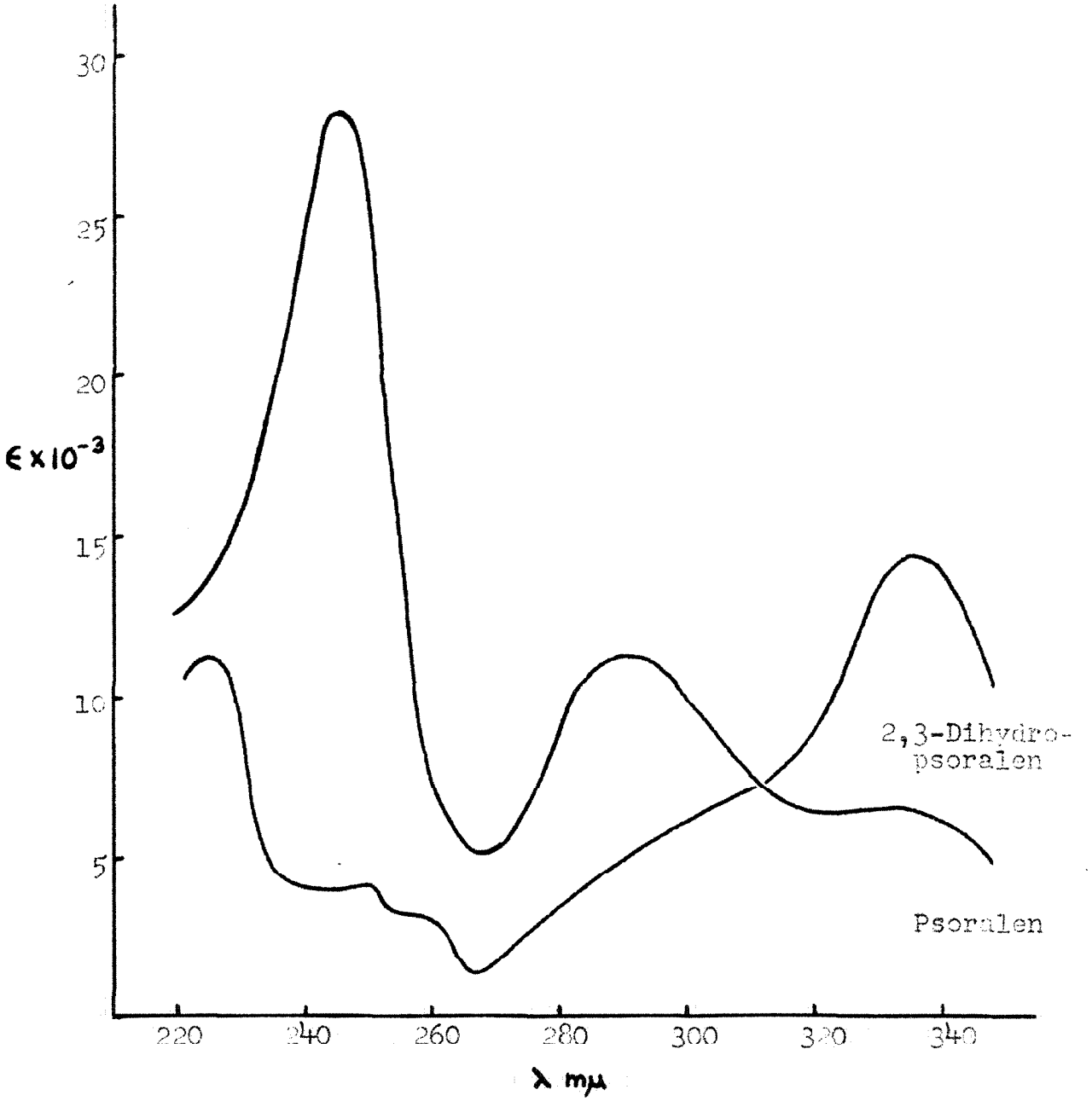
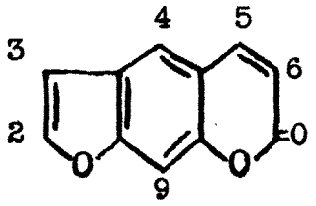


Fig. 1. Effect of saturation on the Psoralen Spectrum.

Table 9

Effects of Hydrogenation on Absorption Spectra of Furocoumarins.



Compound	Maximum Extinction									
	λ_1[a]	ϵ_1[b]	λ_2	ϵ_2	λ_3	ϵ_3	λ_4	ϵ_4	λ_5	ϵ_5
Psoralen[c]	244	28.20	290	11.21	332	6.60	---	---	---	---
2,3-Dihydropsoralen[c]	225	11.23	250	4.20	335	14.13	---	---	---	---
Isopimpinellin	222	28.30	240	14.85	247	14.92	269	20.35	313	13.53
5,6-Dihydroiso-pimpinellin[d]	220	27.55	255	4.16	---	---	---	---	---	---

a. All values of λ are in mμ.

b. $\epsilon n = \epsilon \times 10^{-3}$.

c. Data of Horning and Reisner (10).

d. Data of Caldwell and Jones (11).

E. Chemical Structure:

Evidence from Chemical Degradation.

1. Compound (I)

This compound was subjected to but few degradation studies, partly because of its unreactivity and partly because the peculiar structural requirements soon resolved the possibilities into a few definite categories.

The stability of (I) to hot and cold acid and alkali was mentioned in an earlier section.

Oxidation of (I) with chromium trioxide in glacial acetic acid produced an orange quinone (XII) which proved the presence of the psoralen nucleus, and which permitted tentative identification of the unknown prior to comparison with an authentic sample (cf., p. 40).

2. Compound (II).

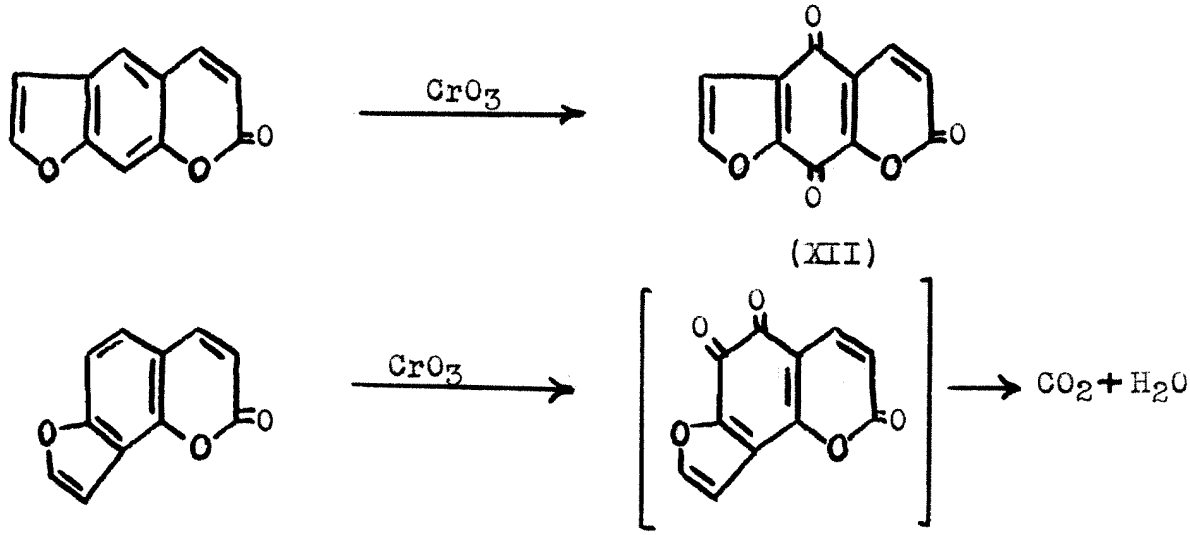
Oxidation of compound (II) with alkaline hydrogen peroxide yielded a crystalline acid which melted at the correct point for furan-2,3-dicarboxylic acid, and oxidation with chromium trioxide produced psoralen quinone identical with that obtained from (I). Acetylation of (II) with acetic anhydride permitted isolation of an acetyl derivative which was subsequently shown to be identical with that of a known furocoumarin (cf., p. 40).

After identification, (II) was employed as a model for degradation of (III) in several instances, and these reactions will be discussed later.

3. Compound (III).

The degradative reactions imposed upon (III) are summarized in Figure 12. The compound proved to be a rather difficult material to handle, because unless reactions were conducted rapidly and the products worked up immediately, there was a tendency for a black tar to form which was very difficult to manipulate on a micro scale. In addition, supersaturated solutions of (III) and related compounds were found to be quite stable and derivatives were slow to crystallize from solution.

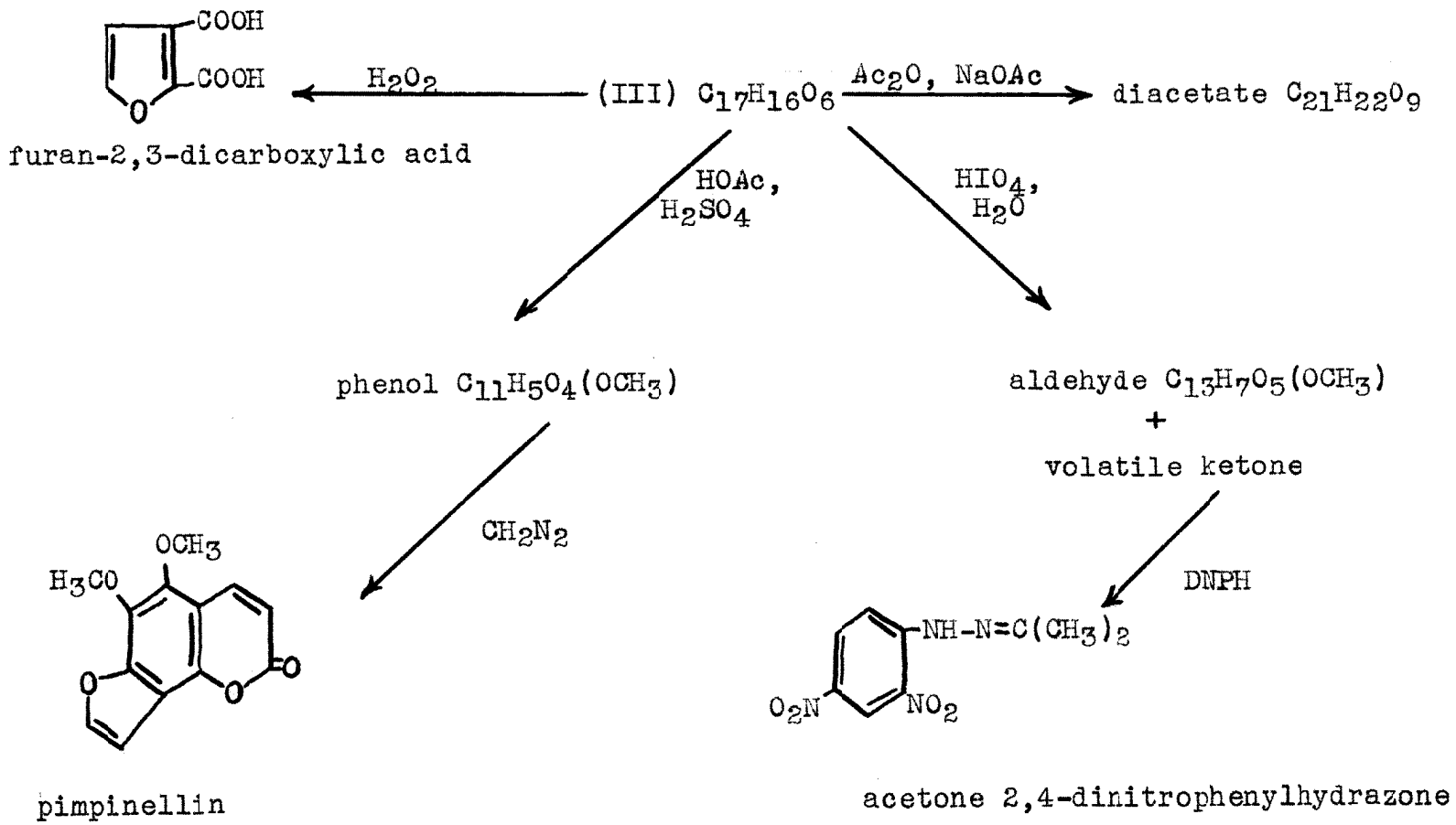
The substance was oxidized by dilute alkaline hydrogen peroxide to yield furan-2,3-dicarboxylic acid, confirming the belief that it belonged to the furocoumarin series. However, when oxidized by chromium trioxide, no organic products were obtained. Under these conditions, derivatives of psoralen such as isopimpinellin were oxidized to psoralen quinone while isobergapten was completely oxidized, leaving no organic residue. These facts indicated that (III) had an isopsoralen nucleus.



(XII)

Figure 12

Chemical Degradation of Thamnosma Compound (III).



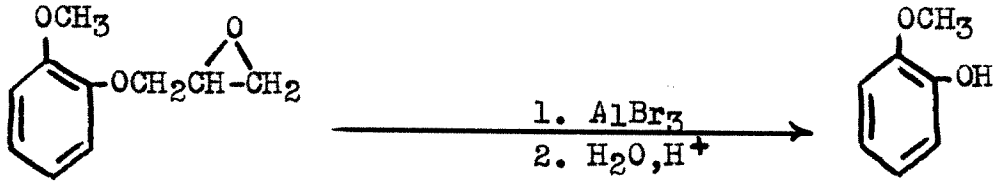
Because (III) did not react with aqueous periodic acid at once, but gave a strong positive reaction after warming in acidic solution, a side-chain epoxyl group was indicated. When acylated with acetic anhydride and sodium acetate, a diacetyl compound was obtained which confirmed the presence of such a group.

In order to take advantage of the presence of the 1,2-epoxide ring, it was proposed to open it with dilute acid, and cleave the resulting glycol with periodic acid. The model compounds, 3-(o-methoxyphenoxy)propylene glycol, 3-(p-methoxyphenoxy)propylene glycol, and 3-(o-methoxyphenyl)glycidic ether were prepared by reaction of the appropriate phenol and chlorohydrin in the presence of aqueous sodium hydroxide. To prove the formation of an aldehyde by cleavage of the models with periodic acid, (o-methoxyphenoxy)acetaldehyde was prepared from guaiacol and dimethyl chloroacetal in aqueous alkali followed by acid hydrolysis of the resulting acetal to the free aldehyde.

After successfully splitting the models to yield formaldehyde and the phenoxyacetaldehyde, (III) was treated in an identical manner. By heating the neutralized reaction mixture, a distillate was obtained from which acetone was recovered as its 2,4-dinitrophenylhydrazone, and upon cooling and centrifuging the residue, a solid aldehyde was obtained whose empirical formula corresponded to that of furocoumarin substituted with a methoxyl and an oxyacetaldehyde group.

To prove the presence of the epoxyisopentenyloxy side chain, cleavage to the corresponding phenol was contemplated. Reaction

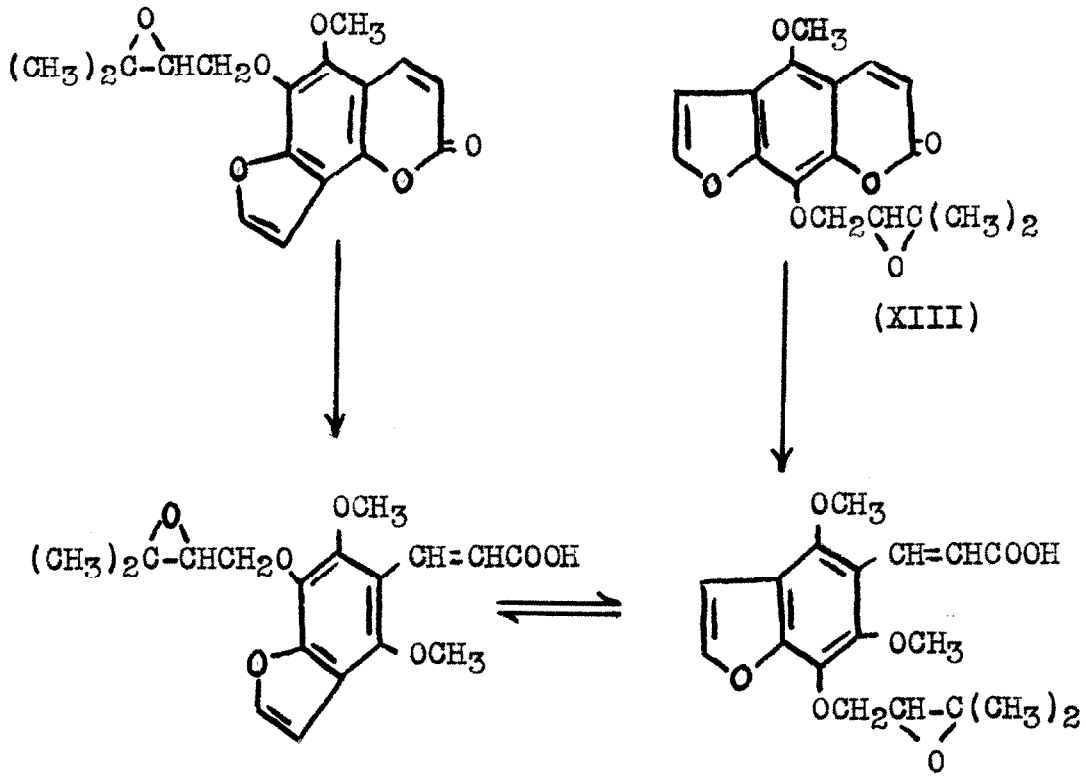
of 3-(o-methoxyphenyl)glycidic ether with anhydrous aluminum bromide in purified benzene produced a solid addition compound which was subsequently hydrolyzed to yield guaiacol.



The tendency of the reagent to react with the β,γ-double bond of coumarin (13) raised doubt as to the advisability of its use. At the time of this part of the investigation, the Thamnosma compound (II) had been identified as a furocoumarin containing the $-O-CH_2\underset{OH}{CH}-\underset{OH}{C}(CH_3)_2$ side chain. Consequently, this substance was split with aluminum bromide in order to test the use of the reagent on a compound closely related to (III). It was found that although (II) was cleaved to yield the expected phenol, the yield was so low that the method was abandoned.

The reagent employed to bring about such a cleavage in previous studies was sulfuric acid in solvent acetic acid. When warmed with this mixture, (III) reacted in the expected manner to produce a phenol (XIV). This substance was methylated with diazomethane in ether to form the known principle of Pimpinella saxifraga, pimpinellin (X).

As a final proof of structure, an attempt was made to methylate (III) in the presence of alkali to a substituted cinnamic acid as shown below with the tentative structure of the Thamnosma compound. Identical methylation of the known furocoumarin byakangelicol (XIII) should produce an identical acid.

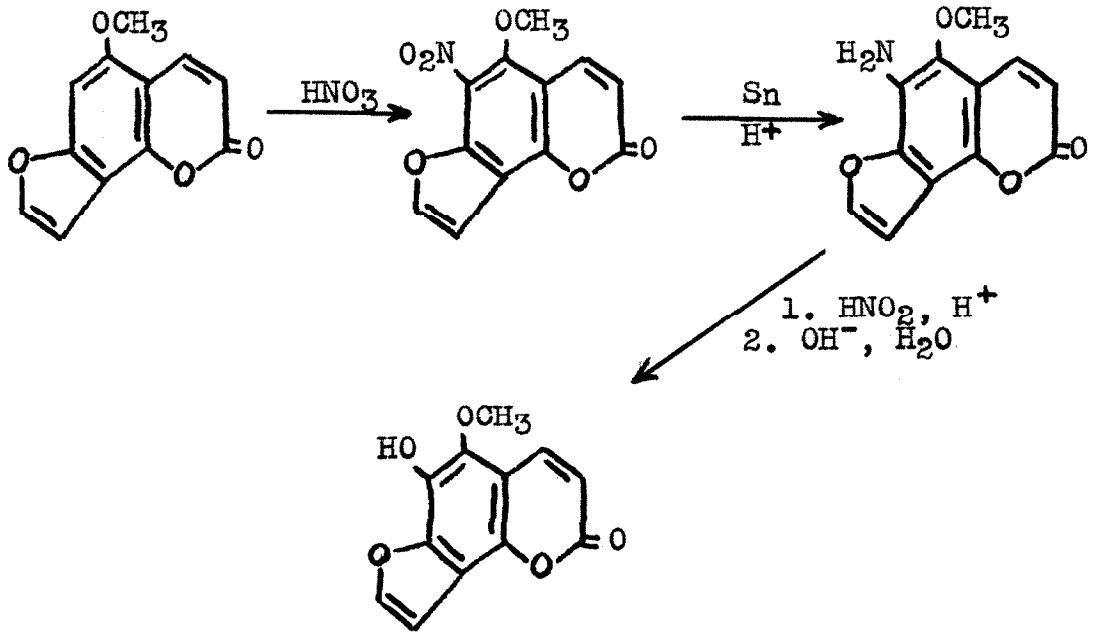


Although several attempts were made to carry out such a transformation, no success was achieved. In each case, a brown tar was formed which could only be partly crystallized, and isolation by the use of chromatography was unsuccessful.

F. Chemical Structure: Evidence from Synthesis

In order to prove further the structure of (III), an attempt was made to synthesize it, or one of its closely related derivatives. Isobergapten (100 mg.) was supplied by the Smith, Kline, and French Laboratories, this quantity being a major part of the known supply as far as could be estimated. The furocoumarin was nitrated to yield the previously undescribed 6-nitroisobergapten, and this was reduced to the corresponding amine, although the compound was not isolated. The

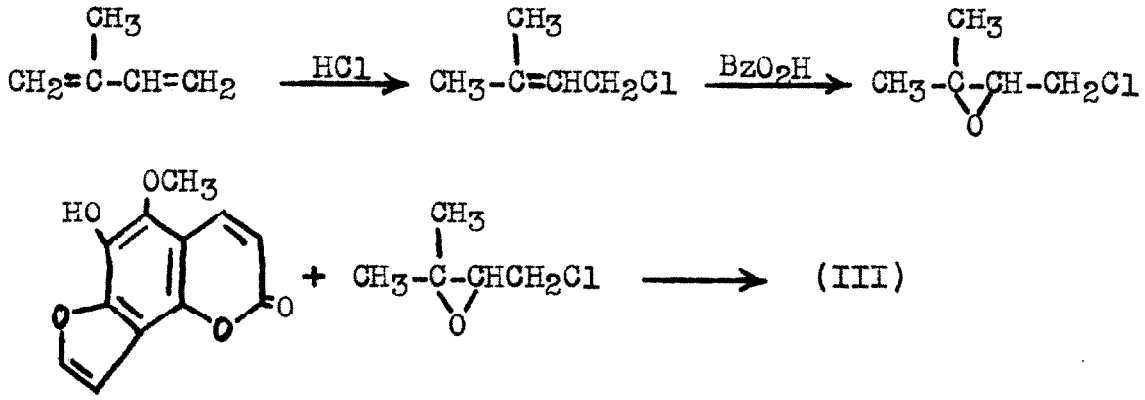
amine was subsequently diazotized, and the diazonium sulfate hydrolyzed to yield 5-methoxy-6-hydroxyisopsoralen. The reactions are summarized below.



(XIV)

The phenol (XIV) was found to be identical with that obtained by cleavage of (III) (cf., p. 35) thus confirming the presence of the isopsoralen nucleus and designating clearly the position of the epoxyisopentenyloxy substituent.

It was originally proposed to synthesize the entire molecule of the Thamnosma substance by reaction of the phenol (XIV) with 1-chloro-2,3-epoxy-3-methylbutane, prepared by addition of hydrogen chloride to isoprene followed by reaction of the resulting substituted allyl halide with perbenzoic acid, as shown in the following diagram.



However, preparation of the phenol itself proved conclusively the structure of (III) when considered in the light of the other physical and chemical data accumulated, and the quantity of starting material for this final synthesis was considered insufficient to warrant an attempt at the present time.

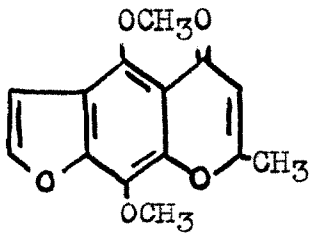
G. Conclusions

Compound I.

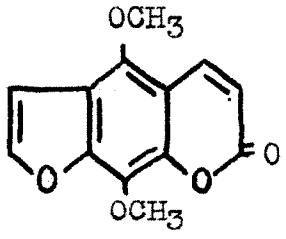
Due to its peculiar empirical formula, $C_{11}H_4O_3(OCH_3)_2$, compound (I) was studied first in hope that evidence for its structure might also give insight into the structures of the other two compounds, apparently related to it according to their spectra.

The extremely high ratio of C to H made necessary the presence of several rings and a high degree of unsaturation, and according to the Calandra equation (14), three rings might be expected. The results of classification tests showed that while it was possible that the compound might be a phenol, a lactone was more strongly suggested, and would also supply one of the necessary rings.

The sudden medical interest in the drug "Khellin" (XV) at the time of this investigation was extremely fortunate, for it was apparent that the empirical formula of this compound differed from (I) only by addition of one methylene group, and that the structural formula could be transformed into another which possessed all of the requirements for (I) simply by removing the C-methyl group and placing the carbonyl group in the α- instead of the γ-position.



(XV)



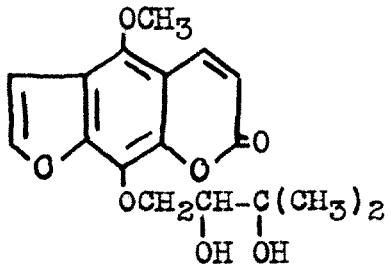
(VI)

A search of the literature revealed that a considerable number of such furocoumarins were known to occur naturally, and that a compound having the structure (VI) was indeed known, and exhibited physical and chemical properties identical with (I) (15). The substance was known as "isopimpinellin", and subsequently, an authentic sample was obtained from the Smith, Kline, and French Laboratories which confirmed the identity of the toxic substance from Thamnosma.

Although isopimpinellin has been isolated from various genera of Umbelliferae, it has only once been reported to occur in a plant belonging to the Rutacae, as does Thamnosma (11).

Compound II.

The close relation of the u.v. absorption spectrum of this substance to that of (I) suggested immediately that (II) was also a furocoumarin having a structure closely related to (I, VI). A search of the literature on naturally-occurring coumarins again revealed that a substance called "byakangelicin" (XI) was known which appeared to be identical with (II). A sample of byakangelicin was kindly supplied by Dr. T. Noguchi of Tokyo, the first investigator to describe the substance (16), and this corresponded exactly to the second Thamnosma compound.



(XI)

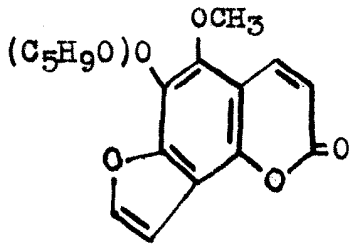
Byakangelicin is the active principle of the Japanese drug byakusi, and previously has been isolated solely from species of Angelica (Umbelliferae).

Compound III.

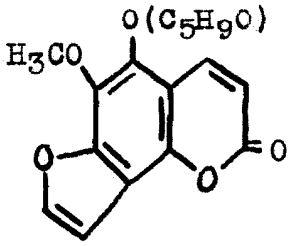
A thorough search of the literature did not reveal any compound corresponding to (III). The u.v. absorption spectrum of the furocoumarin isobergapten (VIII), supplied by the Smith, Kline, and French Laboratories, was found to be very similar to that of the Thamnosma compound, and samples of the related sphondin (IX) and pimpinellin (X), obtained from Dr. Richard

Goodwin of the Connecticut State College, showed this similarity also (cf., p. 28).

If (III) was assumed to contain the furocoumarin nucleus, subtraction of the empirical formula left the residue $C_5H_9O_2(OCH_3)$ undefined, and the transformations indicated in the previous section on chemical degradation showed the C_5H_9O portion to be an epoxyisopentenyloxy group, and separate from the methoxyl group. This type of structure was found in the previously described furocoumarins byakangelicol (XIII), oxypeucedanin, and auropten, and was supported in this case by the Calandra equation which predicted that (III) would contain four rings. Consequently, (III) might possess either (XVI) or (XVII), depending on the relative positions of the substituents.



(XVI)



(XVII)

The empirical formula of (III) as calculated from analytical data did not distinguish clearly whether 16 or 18 hydrogens were present in the molecule, and since the side chain could not accommodate the extra atoms, it was presumed that if present, they must reside in either the pyrone or furan ring. However, spectrographic evidence and the formation of furan-2,3-dicarboxylic acid by oxidation indicated that the

unsaturation in these rings was intact, and subsequent synthesis confirmed this fact and the formula $C_{17}H_{16}O_6$.

Synthesis also proved the exact location of the two substituents in the isopsoralen nucleus, and showed the most probable structure of (III) to be (XVI).

A comparison of the properties of Thamnosma compounds with substances of known structure is presented in Table 10. Following the customary method of naming naturally-occurring furocoumarins, the name "thamnosmin" is proposed for the new compound.

The author is indebted to Dr. T. A. Geissman of Los Angeles, Dr. Richard Goodwin of New Haven, Dr. T. Noguchi of Tokyo, and the Smith, Kline, and French Laboratories for their generous assistance and for authentic samples of the furocoumarins employed in this study.

H. Experimental

All melting points in this section are corrected unless marked otherwise, and all microanalyses were performed by Dr. A. Elek.

Oxidation of Furocoumarins with Chromium Trioxide.--The furocoumarin (10-30 mg.) was dissolved in 1 ml. of glacial acetic acid, and a solution of one half its weight of pure chromium trioxide in the same solvent was added. After standing at room temperature for 2 months, 1 ml. of water was added, the dark solution centrifuged, and the solid recrystallized from 0.1 ml. of 80% aqueous ethanol. The melting point of the

orange substance was determined on a Kohfler hot stage, m.p. ca. 250° (d.).

Isopimpinellin (I) and byakangelicin (II) yielded the expected quinone, although less than 1 mg. was obtained in each case. Compound (III) and isobergapten (VIII) failed to give any product.

Oxidation of (II) with Hydrogen Peroxide.--Compound (II) (24 mg.) was dissolved in 3 ml. of 5% aqueous potassium hydroxide by warming, and 3 ml. of 30% aqueous hydrogen peroxide slowly was added dropwise with stirring. After standing overnight, the yellow solution was acidified with dilute aqueous sulfuric acid, extracted with three 20 ml. portions of ethyl acetate, and the solvent was distilled off. The residue was dissolved in a few drops of ethanol, and a portion of the solution was evaporated to dryness on a microscope slide and the m.p. determined on a Kohfler stage. Beautiful rhombic crystals, m.p. 210-212° (subl.).

Although the highest value reported for the m.p. of furan-2,3-dicarboxylic acid is 221° (16), most investigators (e.g., 11) have found the value to lie in approximately the above range.

Oxidation of (III) with Hydrogen Peroxide.--Compound (III) (100 mg.) was dissolved in a mixture of 4 ml. water and 0.5 ml. 50% aqueous potassium hydroxide solution in a centrifuge cone by warming. Hydrogen peroxide (30%) (1 ml.) slowly was added dropwise to the yellow solution with constant stir-

ring, and a vigorous reaction took place. After standing for 4 hours at room temperature, the mixture was almost colorless, and after addition of another 0.5 ml. of 30% hydrogen peroxide, and 18 hours standing at room temperature, the color had entirely disappeared. The solution was then heated on a water bath to 80°, and maintained at this temperature while an additional 2 ml. 30% hydrogen peroxide were added slowly over a period of 4 hours. Upon cooling, it was saturated with sodium sulfate, acidified with 2 M aqueous sulfuric acid, and extracted repeatedly with a total of 85 ml. of ether. After being dried over anhydrous magnesium sulfate, the solvent was removed *in vacuo*, the residue dissolved in 1 ml. of ethyl acetate and transferred to a centrifuge cone, and the solvent again removed. The dark brown residue was warmed with 0.1 ml. of glacial acetic acid, and upon cooling, white crystals separated. Recrystallization from the same solvent yielded thin white plates, m.p. 215-216°.

Anal. Calc'd. for $C_6H_4O_5$(156.1): C,46.2; H,2.6.
Found: C,46.2; H,2.6.

Attempts to recrystallize the furan-2,3-dicarboxylic acid from ethyl acetate, ether, acetone, and ethanol were unsuccessful. A previous oxidation of 30 mg. of (III) in the same manner apparently was incomplete, because a sticky gum was obtained which when chromatographed from ether on a 20 x 2 cm. column of 1:1 alumina-Celite produced a vividly fluorescent band which yielded a fraction of a mg. of an acid, m.p. >200°, upon elution of the band with ether.

Acetylation of (II).--Compound (II) (14 mg.) was heated at 100° for 2 hours with 0.8 ml. of redistilled acetic anhydride, 1 ml. of water was added, the mixture allowed to stand until homogeneous, made alkaline with dilute aqueous sodium carbonate solution, and extracted with ether. After removal of moisture with magnesium sulfate, the ether was allowed to evaporate spontaneously to yield pale yellow needles, and the substance was recrystallized from ethanol, m.p. 117-118°. When mixed with a sample of compound (II), m.p. < 98°. The acetate of byakangelicin melts at 118-119° (16).

Acetylation of (III).--Compound (III) (20 mg.) was refluxed with 1 ml. of redistilled acetic anhydride and 100 mg. anhydrous sodium acetate for two hours, and the resulting solution was poured into 20 ml. of cold water. After standing in the cold for several days, the precipitated needles were centrifuged down, the supernatant liquid decanted, and the residue recrystallized from 10% ethanol. The yield was 4 mg., m.p. 81° (Kohfler block).

Anal. Calc'd for $C_{21}H_{22}O_9$ (418.4): C,60.3; H,5.3.
Found: C,59.9; H,6.1.

Preparation of 3-(o-Methoxyphenyl)glycidic Ether.--Guaiacol (21.8 ml., 0.20 mole) was dissolved in 65 ml. of 15% aqueous sodium hydroxide, and 50 ml. of water were added. Epichlorohydrin (15.7 ml., 0.20 mole) was added, the mixture was allowed to stand at 20° for 4 hours with frequent vigorous shaking, extracted with two 100 ml. portions of ether, the

extract dried over magnesium sulfate, and the solvent removed by distillation.

Fractional distillation in vacuo through an efficient, jacketed column yielded a fraction boiling at 137-138°/6 mm. which solidified on cooling to white crystals, m.p. 35-36°. Marle (17) reported m.p. 36°.

Preparation of 3-(o-Methoxyphenoxy)propylene Glycol.--Guaiacol (21.8 ml., 0.20 mole) was dissolved in 65 ml. 15% aqueous sodium hydroxide and 50 ml. of water, and a few grams of ice were added. Glycerine α-monochlorohydrin (15.7 ml., 0.17 mole) was added, the mixture was allowed to stand for an hour, and 1.0 ml. (0.03 mole) additional chlorohydrin was introduced. After standing for 5 hours with occasional shaking, the dark solution was extracted with two 100 ml. portions of benzene, the extract was dried over potassium carbonate, and solvent was stripped off. The resulting dark oil was distilled in vacuo to yield a colorless product, b.p. 200-205°/6 mm., which solidified almost immediately to white crystals, m.p. 78-79° (Marle reports 78.5-79°). The yield was 24 g. (71%).

Preparation of 3-(p-Methoxyphenoxy)propylene Glycol.--Hydroquinone monomethyl ether (24.8 g., 0.20 mole) was mixed with a solution of 9 g. (0.23 mole) sodium hydroxide in 100 ml. of absolute ethanol, 50 ml. of additional ethanol were added, the solution was heated to boiling, and 16.7 ml. (0.20 mole) of glycerine α-monochlorohydrin were added dropwise over

a period of 45 minutes. After boiling under reflux for 1 hour, the mixture was chilled in ice, filtered, and the solvent was removed by distillation in vacuo. The residue crystallized on cooling, and was dissolved in 200 ml. of hot benzene, boiled briefly with Norite, filtered hot, and 50 ml. of ligroin were added.

After standing overnight at 4°, the solid was filtered, washed with cold ligroin, and sucked dry, m.p. 80-81°. The reported value (18) is 80.5-81.5°. The yield was 80%.

Preparation of o-Methoxyphenoxyacetaldehyde.-- Guaiacol (12.4 g., 0.10 mole) was dissolved in 12.5 g. (0.10 mole) of dimethylchloroacetal, and a solution of 5.6 g. of potassium hydroxide in 20 ml. of water was added. After boiling under reflux for 4 hours, 50 ml. of ethanol were added, boiling was resumed for 6 hours, the mixture was cooled, 100 ml. of 2 N aqueous sulfuric acid were added, the solution warmed for 2 hours, chilled, and extracted with two 100 ml. portions of ether. The combined extracts were dried over magnesium sulfate, the solvent removed by distillation, and the residue fractionally distilled in vacuo to provide 6 g. of an oil, b.p. 118-121°/6 mm., which partially solidified on standing. Sabetay (19) reports the same boiling point.

Cleavage of a Glycol with Periodic Acid: Preparation of p-Methoxyphenoxyacetaldehyde.--In order to prepare this compound 500 mg. of 3-(p-methoxyphenoxy)propylene glycol and 575 mg. of paraperiodic acid were dissolved in 15 ml. of water, boiled for 10 minutes, and finally chilled in ice. The pre-

cipitated orange solid was isolated by filtration, washed with cold water, boiled briefly in 15 ml. of water with Norite, and the filtered solution was allowed to stand. After several days, the solid was removed by filtration, washed with water, and dried in air. The yield was 200 mg. of white crystals, m.p. 75-76°. Stoermer (20) indicates that the compound with this m.p. is a hydrate.

Cleavage of (II) with Periodic Acid.--Compound (II) (20 mg.) was dissolved in 1.0 ml. of dry dioxane, 2 ml. of water were added followed by 1 drop of 25% aqueous sulfuric acid, the mixture was warmed to 60°, allowed to stand for 10 minutes, and 15 mg. of paraperiodic acid were added, followed by 1 ml. of water.

The solution was boiled in a centrifuge tube fitted with a micro distillation head, the brown residue was cooled, made slightly alkaline with sodium carbonate, and 17mg. of dimedone were added with no result. Even after prolonged standing, no solid separated, and evaporation to dryness left only a black tar which was not manipulated further.

In a second experiment, 15 mg. of (II) were dissolved in 0.5 ml. of dioxane, 1 ml. of 0.5 N aqueous sulfuric acid was added, the solution was warmed at 60° for 10 minutes, chilled well in ice, and 12 mg. of paraperiodic acid were added. Water (1 ml.) was added, the mixture allowed to stand at 20° for 15 minutes, chilled again in ice, and pure solid barium carbonate was added slowly with stirring until no more gas evolution was evident. After standing for 2 hours, the solid was centri-

fuged down, the supernatant was boiled in the same apparatus used in the first experiment, the distillate collected in an ice-cooled receiver, and 2,4-dinitrophenylhydrazine reagent was added with immediate formation of a yellow precipitate. Isolation of this solid showed it to be acetone dinitrophenylhydrazone, m.p. 120-121°.

The residue remaining after distillation of the reaction mixture was mixed with 14 mg. of dimedone and 0.2 ml. of ethanol, heated to 90° for 10 minutes, and allowed to stand overnight. The precipitated crystals were centrifuged, washed with 10% aqueous ethanol, and recrystallized from aqueous acetone, m.p. 178-179°. The compound was not analyzed, but was presumed to be the methone of the expected aldehyde.

Cleavage of (III) with Periodic Acid.--Compound (III) (36 mg.) was dissolved in 1 ml. of purified dioxane by heating, and 0.5 ml. of water and 2 drops of 2 N aqueous sulfuric acid were added. The yellow solution was warmed to 80° for 20 minutes, cooled in ice, and a mixture of 1 ml. dioxane, 1 ml. water, and 40 mg. of periodic acid were added at about 0°. After the mixture had come to room temperature, it was allowed to stand for 20 minutes, and 0.5 g. of solid barium carbonate was added with stirring. When neutral to pH paper, the mixture was spun in a centrifuge, and the supernatant was transferred to a small distillation apparatus and carefully distilled until the volume of the residue had diminished by one-half. Dioxane (0.5 ml.) was added to the boiler and distillation was continued until only a small volume of liquid remained.

When cooled, the residue deposited a yellowish solid which was recrystallized twice from ethanol to yield finally 2 mg. of helical crystals, m.p. 215-215.5°.

Anal. Calc'd. for $C_{14}H_{10}O_6$(274.2): C,61.4; H,3.7.

Found: C,63.0; H,3.4.

The distillate, which had been collected in an ice-cooled receiver, was mixed with 1 ml. of a solution of 2,4-dinitrophenylhydrazine in absolute ethanol saturated with hydrogen chloride, and the solution was warmed, water was added dropwise until a precipitate commenced to form, and crystallization was allowed to take place. The resulting orange needles were crystallized three times from aqueous ethanol, m.p. 123-124°. When mixed with a sample of authentic acetone dinitrophenylhydrazone, the m.p. remained at 123-124°.

Cleavage of 3-(o-Methoxyphenoxy)glycidic Ether with Aluminum Bromide.--Thiophene-free benzene was purified by drying over anhydrous calcium chloride, distilling, boiling with anhydrous aluminum chloride for 5 hours, distilling again, washing with water, then with 6M aqueous sodium hydroxide, and again with water until the aqueous layer was neutral. The solvent was finally distilled from metallic sodium (21).

The glycidic ether (20 mg.) was dissolved in 2 ml. of the above solvent, 30 mg. of anhydrous aluminum bromide were added, and the mixture was boiled under reflux for 5 minutes. The gelatinous precipitate was centrifuged, washed with several 1 ml. portions of absolute ether, and warmed on a water bath under an inert atmosphere until a fine, dry powder remained.

This was heated on a water bath for 1 hour with 1 ml. of 3 M aqueous sulfuric acid, the cloudy mixture extracted with two 2 ml. portions of ether, the extract dried over magnesium sulfate, and the ether stripped off.

The residual oil which smelled strongly of guaiacol was converted to the tribromo derivative by the method of Shriner and Fuson (22), m.p. 115-116° (lit. 116°).

Attempts to employ purified carbon tetrachloride as solvent, and to add the aluminum bromide as its benzene solution proved unsatisfactory.

Cleavage of (II) with Aluminum Bromide.--Compound (II) (16 mg.) was dissolved in 2 ml. of specially purified benzene by warming, and about 100 mg. of anhydrous aluminum bromide were added. An immediate yellow precipitate formed, and the solution became yellow in color. The mixture was boiled under reflux for 1.5 hours, cooled, centrifuged, and the precipitate was washed with 0.5 ml. of benzene, 2 ml. of fresh benzene were added followed by 3 ml. of 2 N aqueous sulfuric acid, and the mixture was warmed to 50° for 5 minutes, the upper layer decanted, the lower aqueous layer washed with 2 ml. of benzene, and the combined extracts dried over magnesium sulfate.

A solution of diazomethane in ether was prepared from 2.5 g. of N-methyl-N-nitroso-N'-nitroguanidine by the method of Fieser (23), and the dry solution was added to the benzene solution of the phenol. After standing overnight, the almost colorless mixture was evaporated to dryness, the yellow residue dissolved in 0.2 ml. of acetone, one drop of water and 2 drops

of ethanol were added, and the mixture was allowed to stand overnight again. The pale yellow powder was isolated by centrifugation and dried in air, m.p. 144-145°. The m.p. of a mixture with isopimpinellin was not depressed. The yield was less than 1 mg..

A second experiment gave identical results, except that the final product appeared to be very impure, m.p. ca. 120-140°, indicating that cleavage probably was incomplete.

Cleavage of (III) with Sulfuric Acid in Acetic Acid.-- Compound (III) (50 mg.) was dissolved in 2 ml. of pure glacial acetic acid in a centrifuge cone, 2 drops of concentrated sulfuric acid were added, and the mixture was allowed to stand at room temperature for 20 hours, chilled in ice, and 5 g. of ice were added slowly in small pieces with stirring. The pale yellow-green solid was centrifuged down, the supernatant decanted, and the precipitate washed with 0.1-0.2 ml. of cold acetone. Recrystallization from 0.5 ml. of acetone yielded fine needles after allowing the solution to stand for several days at 4°. The yield was ca. 5 mg. of yellow-green needles, m.p. 134-135°.

Anal. Calc'd. for $C_{12}H_8O_5$ (232.2): C,62.1; H,3.5.

Found: C, 61.7; H, 4.0.

In a previous attempt to cleave (III), the substance was dissolved in a mixture of 2 ml. of glacial acetic acid and 2 drops of concentrated sulfuric acid, and heated at 110° for 2 hours in a sealed tube. The black mixture was poured over ice, the dark precipitate centrifuged, the resulting solid

dissolved in 2 ml. of ethanol, 2 ml. of water added, and the mixture allowed to stand. The solid which separated was dark and gummy, and became continually more difficult to handle as attempts were made to crystallize it from benzene, acetic acid, or acetone. At last, the tar was dissolved in benzene and chromatographed on a 20 x 2 cm. column of 1:1 alumina-Celite, using acetone as developer and following the development of the chromatogram by means of the phenol's yellow fluorescence in ultraviolet light. Elution with boiling ethanol and subsequent removal of solvent in an inert atmosphere yielded only about 1 mg. of product, m.p. ca. 135°.

Formation of Pimpinellin (X).--About 2 mg. of the phenol from the previous reaction were dissolved in 2 ml. of ether and 2 ml. of a dry ethereal solution of diazomethane were added. After standing at room temperature for 1 hour, an additional 2 ml. of ethereal diazomethane were added, and the ether was boiled off on a warm water bath. The solid residue melted at 121-122°, and when mixed with an authentic sample of pimpinellin no depression of the m.p. was observed. It is interesting to note that the supply of natural pimpinellin in the United States consisted of about 1 mg. owned by Richard Goodwin of the Connecticut State College, and of which he generously sent one half. The absorption spectrum of the compound shown in Table 7 also was determined with this sample.

Preparation of 6-Nitroisobergapten (XIV).--The nitration was conducted according to Thoms and Baetcke (24). SKF iso-

bergapten (50 mg.) was dissolved in 1.0 ml. of pure glacial acetic acid, and a solution of 10 drops of concentrated nitric acid in 1.0 ml. of acetic acid was added. The mixture was heated to 100° for 2 hours and allowed to stand overnight. The precipitated orange crystals were centrifuged, washed with 0.1 ml. of glacial acetic acid and then with 0.2 ml. of acetone, and finally were allowed to dry in air. The yield was 40 mg. (66%), m.p. 238-239°.

Preparation of 6-Hydroxyisobergapten (VIII).--The method employed was that of Thoms and Baetcke (24). Nitrobergapten (30 mg.) was suspended in 5 ml. of 4 M aqueous hydrochloric acid in a 15 ml. centrifuge tube fitted with a mechanical stirrer and an inlet tube, and 100 mg. of granulated tin (ca. 30 mesh) was introduced slowly in small portions with agitation. The mixture was then heated to 100° for 0.5 hour, cooled in ice, and neutralized with 2 M aqueous sodium hydroxide until the precipitate commenced to redissolve.

After extraction with three 15 ml. portions of chloroform, the amine was reextracted into 5 ml. of 4 M aqueous hydrochloric acid, chilled to 0°, and ca. 1 M sodium nitrite solution was added dropwise until the starch-iodide test showed the presence of excess nitrous acid. The mixture was allowed to stand for several days at room temperature, made slightly alkaline with 2 M aqueous sodium hydroxide, warmed to 60° for 1 hour, cooled, and shaken with ether. After the extract had been dried over magnesium sulfate, the solvent was removed in vacuo, the

yellowish solid dissolved in 5 ml. of ethyl acetate, transferred to a centrifuge tube, and the solvent again removed. An attempt to crystallize the product from aqueous ethanol gave only a brown resin (cf., ref. 25), but when this was dissolved in 2 drops of acetone and allowed to stand, the product crystallized, m.p. 134-135°. Only about 2 mg. were finally obtained. A mixed m.p. with a sample of the phenol obtained from the Thamnosma compound (III) was not depressed.

Attempted Methylation of (III).--Compound (III) (70 mg.) was dissolved in 5 ml. of 2 M aqueous potassium hydroxide and 4 drops of pure methyl sulfate were added with stirring and warming. After 5 minutes, an additional 4 drops of methyl sulfate were added, causing the slow formation of a precipitate. Potassium hydroxide solution (1 ml.) was added to dissolve the solid, 4 drops more of methyl sulfate were added, and the mixture was allowed to stand at 50° for one hour, made alkaline with aqueous potassium hydroxide, extracted with ether, the aqueous layer made acid with 2 M sulfuric acid, and the precipitate centrifuged. The brown solid was dissolved in 1 ml. of 1 M aqueous sodium carbonate solution with evolution of carbon dioxide, 1 ml. of water was added, and the solution was boiled briefly with Norite, filtered, and allowed to cool. The color was not removed. Acidification reprecipitated the product, which next was mixed with several drops of acetone, the crystals isolated by centrifugation, washed with 1 drop of acetone, and dried in air. When dry, only a minute amount of

dark solid remained, and chromatographic adsorption of the acetone supernatant on alumina did not permit resolution of the colored solute, which could not be induced to crystallize.

Table 10.

Comparison of Toxic Compounds with Known Substances.[a]

Compound	Formula	M.p. °C.	$[\alpha]_D^{25}$
I	$C_{13}H_{10}O_5$	147.5-148.5°	inactive
Isopimpinellin	$C_{13}H_{10}O_5$	149-151°	inactive
II	$C_{17}H_{18}O_7$	122.0-123.0°	23.0°
Byakangelicin	$C_{17}H_{18}O_7$	125-126°	24.6°
XIV[b]	$C_{12}H_8O_5$	134-135°	inactive
Pimpinellin	$C_{13}H_{10}O_5$	118-119°	inactive
Isobergapten	$C_{12}H_8O_4$	224°	inactive

a. For comparison of absorption spectra see Figures 9,10, and 11.

b. Phenol from Thamnosmin.

References

1. J.F. Bonner, Bot. Rev., 16, 51(1950).
2. F.W. Went, Bull. Torrey Bot. Club, 69, 100(1942).
3. J.F. Bonner and A.W. Galston, Bot. Gaz., 106, 185(1944).
4. R. Gray and J.F. Bonner, J.A.C.S., 70, 1249(1948).
5. E.L. Bennett and J.F. Bonner, Am. J. Bot., 40, 29(1953).
6. E. Späth, Ber., 70A, 83(1937).
7. E.L. Bennett, Thesis, California Institute of Technology, 1949.
8. H.R. Bode, Planta, 30, 567(1940).
9. C.R. Jacobson, K.R. Brower, and E.D. Amstutz, J. Org. Chem., 18, 1117(1953).
10. E.C. Horning and D.B. Reisner, J.A.C.S., 70, 3619(1948).
11. A.G. Caldwell and E.R.H. Jones, J. Chem. Soc., 1945, 540.
12. H.S. Jois and B.L. Manjunath, Ber., 70B, 434(1937).
13. E.J. King, J.A.C.S., 49, 562(1927).
14. A. Calandra, Chem. News, 144, 327(1932).
15. F. Wessely and F. Kallab, Monatsh., 59, 163(1933).
16. T. Noguchi and M. Kawanami, Ber., 71B, 344(1938).
17. E.R. Marle, J. Chem. Soc., 101, 308(1912).
18. H.L. Yale, E.J. Pribyl, W. Baker, F.H. Bergeim, and W.A. Lott, J.A.C.S., 72, 3710(1950).
19. S. Sabetay, Bull. soc. chim., 45, 1166(1929).
20. R. Stoermer, Ann., 312, 335(1900).
21. P. Pfeiffer and E. Haack, ibid, 460, 156(1928).

22. R.L. Schriner and R.C. Fuson, "The Systematic Identification of Organic Compounds", John Wiley and Sons, Inc., New York, N.Y., 1948.

23. L.F. Fieser, "Experiments in Organic Chemistry", D.C. Heath and Co., New York, N.Y., 1941.

24. H. Thoms and E. Baetcke, Ber., 45, 3705(1912).

25. T. Noguchi and M. Kawanami, ibid., 71B, 1428(1938).

PART II. THE ACTION OF PHYTOTOXIC COMPOUNDS ON PLANTS.

A. Introduction

Unsaturated lactones are well known for their pronounced physiological activity (1). In fact, furocoumarins were first isolated and studied because of their effect on fish, as low a concentration as $1:10^5$ being lethal in some cases. Primitive tribes in the Americas used this property as a means of obtaining food, and extraction of various Rutaceous and Umbelliferous plants used for such a purpose showed the invariable presence of furocoumarins or closely related compounds (2). These substances were shown by Späth (3) to be the active fish poisons in the extracts.

The effect of furocoumarins on plants has not been reported, but a large number of less complicated coumarins ~~has~~ have been tested and found to be powerful inhibitors of germination and growth. The experiments involved in this section were concerned with the germination and growth of plants in the presence of various naturally-occurring toxic substances, particularly under conditions which might elucidate the means by which such substances are effective in nature. The three Thamnosma compounds, khellin and khellol (from Ammi visnaga), naringin (a flavanone from Citrus), and the widely-distributed coumarin were employed in the tests. The inhibition of germination by the active principle of California Encelia farinosa, 3-acetyl-6-methoxybenzaldehyde (AMB), has been studied previously (4), and was measured again here for comparison.

The experiments in this section of the thesis were conducted under controlled conditions in the Earhart Plant Research Laboratory under the direction of Dr. F. W. Went.

B. Germination

The results of experiments conducted to determine the effect of various toxic substances on seed germination are summarized in Tables 1 and 2. Seeds of Marglobe tomato, mung bean, and Kanata oat were chosen as representative of several important plant groups, and were allowed to germinate in 250 ml. wide-necked Erlenmeyer flasks or Petri dishes on filter paper soaked with 4 ml. samples of the test solutions. Tests were conducted at 27° in the dark. It is apparent that the compounds tested exhibited only low activity at the concentrations employed, although isopimpinellin and thamnosmin were used in their saturated aqueous solutions.

Previous tests with AMB (4) showed that much higher concentrations of the toxic substances might be necessary for inhibition of germination, and for this reason the most soluble of the test compounds, byakangelicin and khellin were employed in an experiment at their maximum concentration in aqueous solution. In addition, isopimpinellin was converted to the sodium salt of the corresponding cinnamic acid, and was tested in this more soluble form. No inhibition of the germination of bean was observed in any of these cases.

Due to the reported sensitivity of carrot to the presence of coumarin (5), this species (Danvers variety) was tested also (Table 3). Although definite inhibition was demonstrated in several cases, the high activity ascribed to coumarin in the literature could not be confirmed.

An attempt was made to study the effects of the toxic compounds on the germination of several of the desert annuals known to be affected in their natural environment, but the experiment was abandoned when no success could be achieved in germination of the control plants. The species tested were Thamnosma montana, Layia platyglossa typica, Cucurbita palmata, Datura meteloides, Dalea spinosa, and Malocothrix californica glabrata.

C. Root Elongation

Root elongation was measured in seedlings grown on moist filter paper in wide-mouthed Erlenmeyer flasks. Table 4 indicates the effects produced by concentrations of toxic compounds too low to inhibit germination appreciably. Audus and Quastel (5) have found that root elongation is much more sensitive to the presence of coumarins than is germination, and the present investigation confirms this observation.

Although the root elongation of carrot was not measured, it was observed that while elongation of the stem was inhibited (Table 3), elongation of the root appeared to be affected to a lesser degree.

D. Stem Elongation

Stem elongation experiments also were performed on Marglobe tomato, mung bean, and Kanata oat. The test plants were germinated in plastic trays at 25-27° in the dark, then grown in normal light at 25° for several days until a convenient height was attained. Uniform plants were transferred to 15 ml. shell vials and allowed to grow in half-strength Hoagland's solution for an additional 48 hours, this solution was replaced by 12 ml. of a solution of the toxic compound in half-strength Hoagland's solution, and subsequent growth was measured at a day temperature of 23° and night temperature of 17°. The distance measured was that from the base of the first leaf to the point at which the plant was secured to the container. The volume was maintained at 12 ml. by addition of deionized water.

The results of such experiments are summarized in the following Figures 1 - 9. Stem elongation of tomato is plotted both as % elongation and as difference in elongation (Δ), but because of the close similarity of the two graphs, only the latter values are employed in the remaining figures. A graph comparing elongation in terms of % of the control appeared meaningless under the present circumstances.

Although all of the experiments apparently were conducted in an identical manner, two different types of growth were

observed (Figs. 1-3). This phenomenon should be investigated further.

Figure 10 indicates the effect of two toxic compounds on excised tomato tops. The controls for each compound were not excised, but a separate excised and chemically untreated control was included also.

Table 5 lists the stem elongation of seedlings germinated and grown in contact with the toxic compounds. Although the concentrations of these substances were too low to inhibit germination to any great extent, it is apparent that subsequent growth of the shoot is affected considerably.

E. Miscellaneous

In order to determine the effect of the toxic substances when applied to the leaves of growing tomato plants, selected plants (20-30) were grown in vermiculite contained in plastic cups, supplied with standard nutrient, and sprayed daily with 25 ml. of a 10^{-4} M aqueous solution of the test compound. After 4 days, no effect was observable, so spraying was discontinued. The plants still were identical after 1 week.

The extent of reversibility of the action on plants was demonstrated by the following experiment. Uniform tomato seedlings were transferred to pyrex vials containing openings in the bases which allowed the roots to be washed. The plants were permitted to grow in half-strength Hoagland's solution which contained either 9 mg./l. of khellin or 9 mg/l. of isopinellin, and after seven days all were found to be shrivelled

and stunted. The roots then were washed thoroughly with nutrient solution and allowed to grow for 9 days in half-strength Hoagland's solution.

At the end of this test, the unwashed plants treated with isopimpinellin were only one-half the size of the controls, while those which had been washed were almost identical with the untreated plants. Washing of the seedlings treated with khellin had not reversed the action, however, and all of the plants were dead.

F. Discussion

Although the sensitivity of germination to the presence of phytotoxic substances has been seen to vary considerably among different species, it is doubtful that inhibition of this phase of development is responsible for the activity of the toxic compounds under natural conditions. A previous investigation showed that AMB caused 90% inhibition of germination at a concentration of 500 mg./l., but it is improbable that concentrations of this order of magnitude could exist or be effective under desert conditions. The Thamnosma compounds appear almost ineffective even in saturated solution, and the inactivity of the sodium derivative of isopimpinellin shows that conversion to a more soluble form does not increase toxicity.

Observation of the roots of plants grown in test solutions showed a remarkable lack of rootlet growth. While the control

developed a bushy mass of root hairs, only a single main root could be seen in the case of the test plants. This fact, and the inhibition of root elongation presented a possible mechanism for toxic action, but the constant uptake of water by treated plants and the discovery that the effect of test substances upon stem elongation was the same both in the presence and absence of roots again made the mechanism less attractive.

The inhibition of root elongation also demonstrated the adverse effect on this part of the plant. The first indication of the imminent death of a test plant was a drying and withering of the leaf tips and a drastic diminution of the stem diameter, and shortly after this, the shoot and leaves wilted. While this might be due to failure of the plant to absorb water, the facts that water uptake was apparent almost until death, and that the inhibition of stem elongation proceeded whether a root was present or not led one to doubt that lack of water was responsible for the action of the toxic compounds.

The stem elongation of both tomato and bean is considerably affected even at concentrations of toxic compounds on the order of 10^{-5} molar, and the growth curves under such conditions take two forms. Not only does isopimpinellin differ from khellin in its effect upon growth, but also in the degree to which this effect is reversible. Oat does not appear to be appreciably affected by these toxic compounds, and in fact,

there is evidence that they operate to promote growth to some extent.

The present investigation has shown the general nature of the action of naturally-occurring phytotoxic substances under laboratory conditions, but there is too little detailed evidence to permit speculation on the exact mechanism of the effect, if indeed a discrete mechanism exists. Direct measurement of respiration and water uptake in the presence of the toxins might offer such evidence.

Table 1

Effects of Toxic Compounds on Germination at 27°.

Compound	Conc. moles/l.	Beans (12 plants) Days 1	Tomatoes (15 plants) Days 1	2	3	4	5	6	Oats (15 plants) Days 1	2	3	4	5
Control	---	12[a]	0[a]	0	2	7	12	13	0[a]	1	1	1	1
AMB	1×10^{-4}	12	0	0	3	7	14	15	0	2	3	3	3
Coumarin	1×10^{-4}	12	0	0	0	7	12	13	0	3	4	4	5
Khellin	1×10^{-4}	12	0	0	0	4	8	12	0	2	3	3	4
Isopimpinellin	7.5×10^{-5}	12	0	0	0	5	11	11	0	2	2	2	2
Thamnosmin	1×10^{-4}	12	0	0	2	8	12	12	0	2	2	2	2
Byakangelicin	1×10^{-4}	12	0	0	0	4	11	13	0	1	2	4	4

a. Number of seeds germinated.

Table 2

Effects of Toxic Compounds on Germination at 27°.

Compound	Conc. moles/l.	Bean Plants	Bean Days 1	Bean Days 6	Tomato Plants	Tomato Days 1	Tomato Days 6	Oat Plants	Oat Days 1	Oat Days 6
Khellin	3×10^{-4}	22	6	22	20	0	15	22	0	20
Thamnosmin	2×10^{-4}	23	8	23	30	0	28	20	0	17
Khellol	2×10^{-4}	22	3	20	22	0	20	20	0	18
Naringin	2×10^{-4}	31	5	31	35	0	30	20	0	18
Control	---	23	-	23	35	-	25	20	0	19

Table 3

Effect of Toxic Compounds on Germination and Growth of Carrot at 27°.[a]

Compound	Concentration moles/l.	Seeds Germinated after 100 hrs.	Height[b] in mm. after 170 hrs.
Control	---	14	30
AMB	1×10^{-4}	15	20
Coumarin	1×10^{-4}	11	0[c]
Khellin	1×10^{-4}	15	8
Isopimpinellin	7.5×10^{-5}	7	5
Thamnosmin	1×10^{-4}	12	12
Byakangelicin	1×10^{-4}	13	15

a. Twenty plants were tested.

b. Approximate.

c. Germination complete, but with no further development.

Table 4

Effects of Toxic Compounds on Root Elongation at 27°.

Compound	Conc. moles/l.	Mung Bean[a,b] Av. length mm.	Mung Bean[a,b] Av. length % of control	Tomato[b,c] Av. length mm.	Tomato[b,c] Av. length % of control
Control	---	29	100	77	100
AMB	1×10^{-4}	28	97	78	101
Coumarin	1×10^{-4}	20	69	51	66
Khellin	1×10^{-4}	24	83	38	49
Isopimpinellin	7.5×10^{-5}	34	117	47	61
Thamnosmin	1×10^{-4}	23	77	81	105
Byakangelicin	1×10^{-4}	24	83	76	99

a. Measured 2 days after germination.

b. Twelve plants.

c. Measured 5 days after germination.

Table 5

Effects of Toxic Compounds on Stem Elongation at 27°.[a]

Compound	Conc. moles/l.	Mung Bean[b,c] Av. Length mm.	Mung Bean[b,c] Av. Length % of Control	Tomato[c,d] Av. Length mm.	Tomato[c,d] Av. Length % of control
Control	---	30	100	47	100
AMB	1×10^{-4}	23	77	49	104
Coumarin	1×10^{-4}	19	63	30	64
Khellin	1×10^{-4}	25	83	22	47
Isopimpinellin	7.5×10	33	110	31	66
Thamnosmin	1×10^{-4}	24	80	44	94
Byakangelicin	1×10^{-4}	25	83	39	83

a. Seedlings germinated in test solutions.

b. Measured 2 days after germination.

c. Twelve plants.

d. Measured 5 days after germination.

Table 6.

Growth of Excised Tomato Tops.

Compound	Excised	5 Days % Increase	13 Days % Increase	21 Days %Increase
Control	+	90	305	460
Isopimpinellin[a]	+	43	115	190
Isopimpinellin[a]		53	123	170
Khellin[a]	+	35	100	150
Khellin[a]		53	85	93

a. Concentration 9 mg./l.

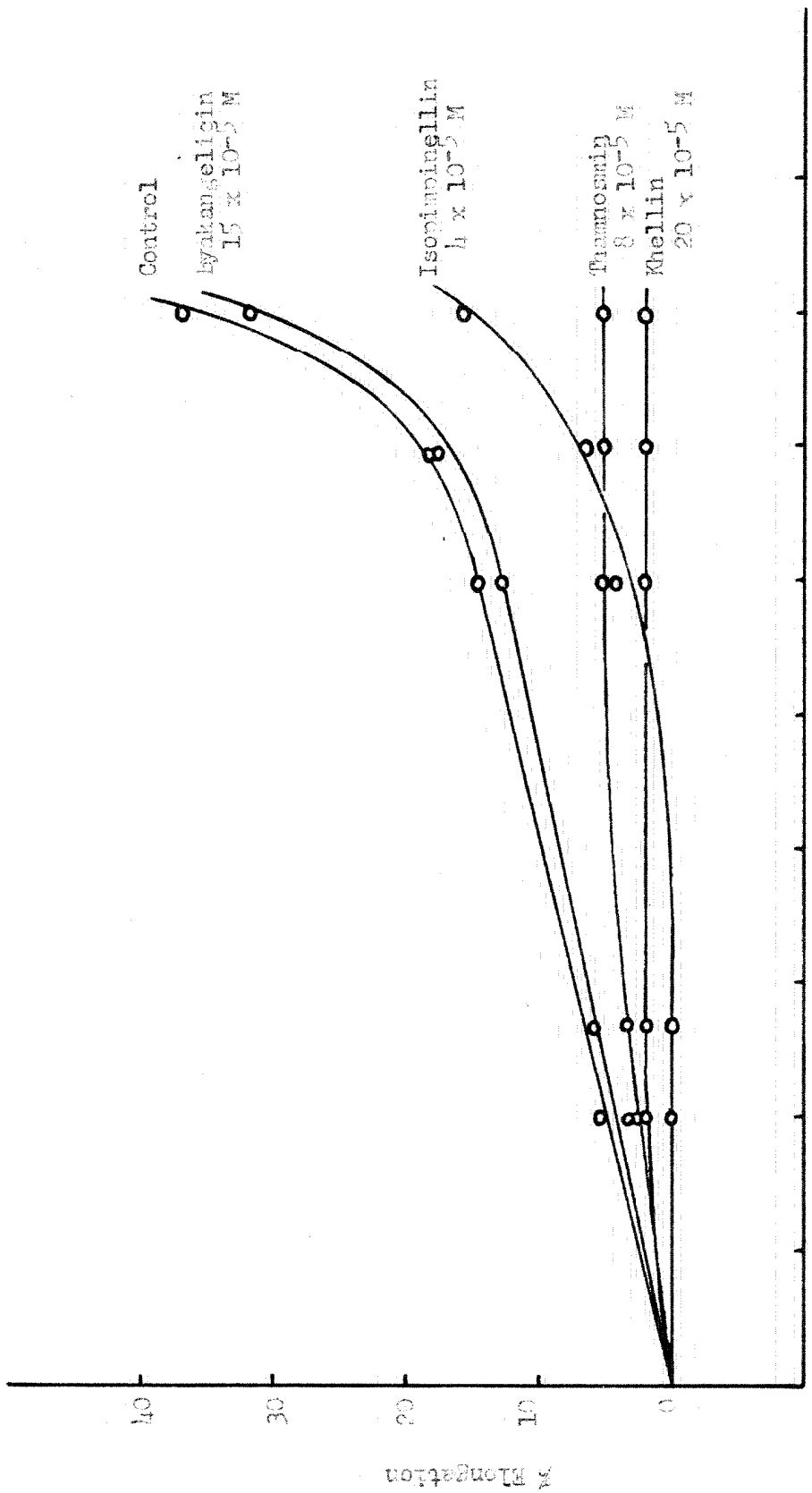


Fig. 1. Effects of Toxic Compounds on Stem Elongation of Tomato.

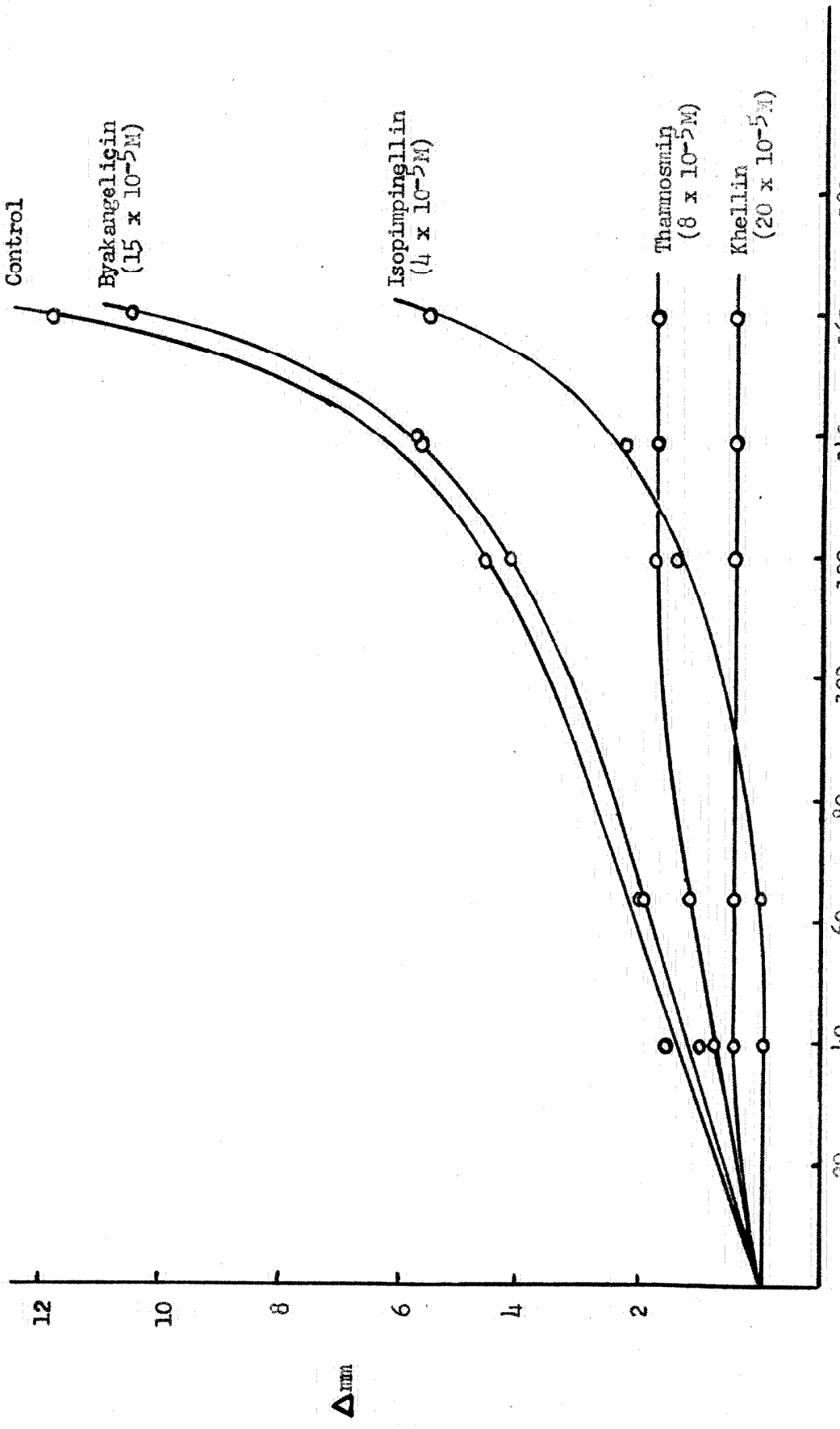


Fig. 2 Effects of Toxic Compounds on Stem Elongation of Tomato.

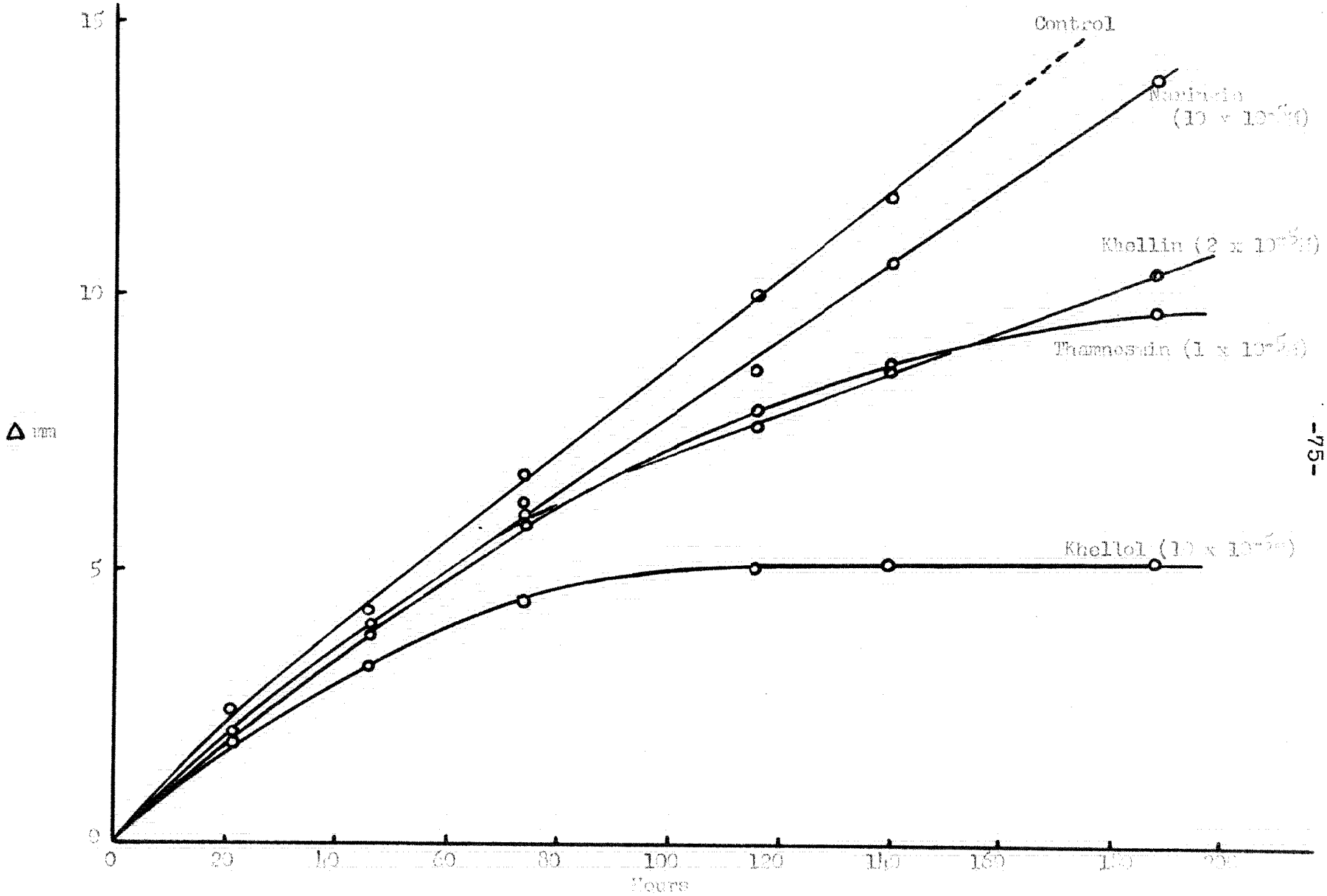


Fig. 2. Effects of Toxic Compounds on Shoe Elongation of Tender

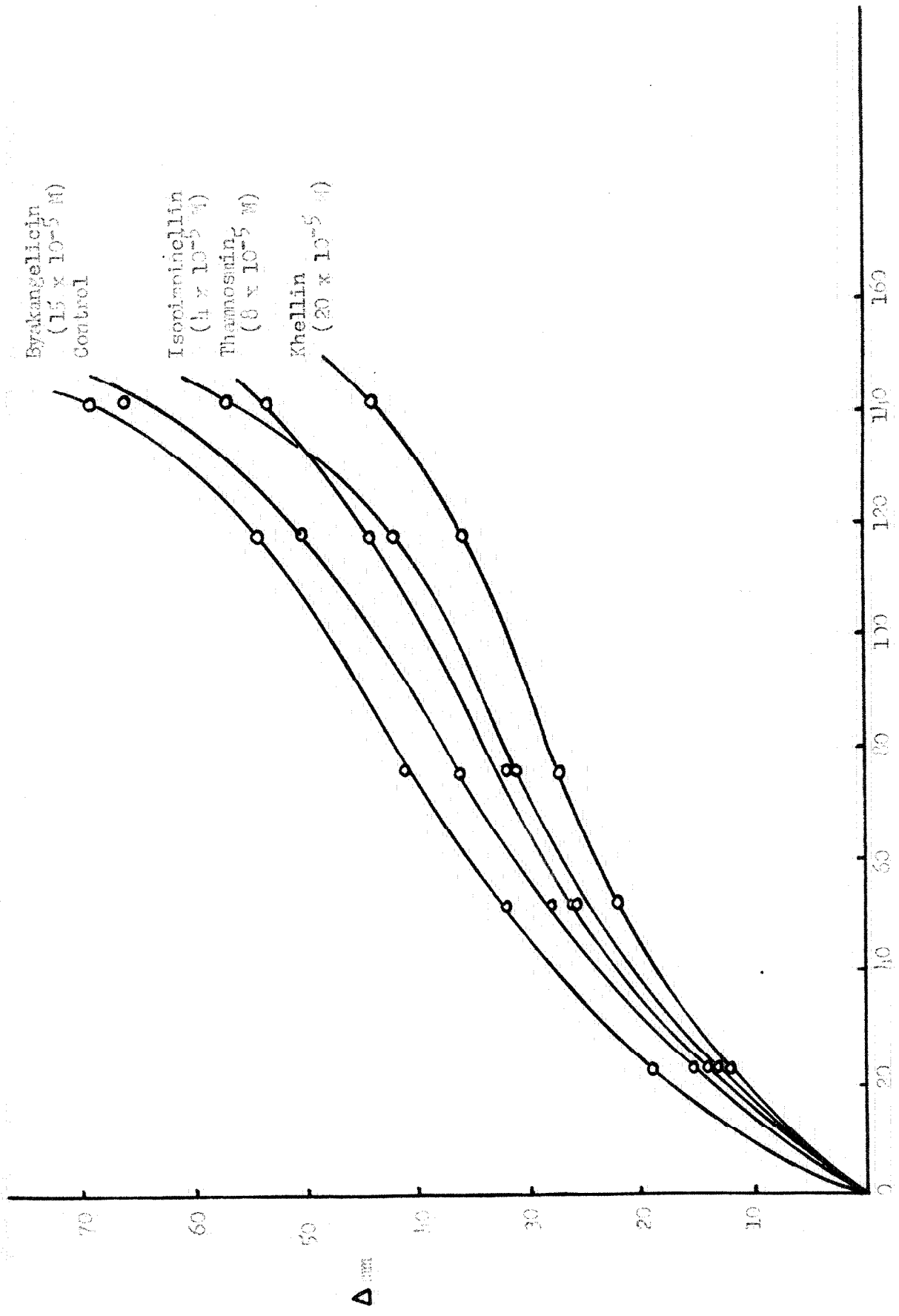


Fig. 4. Effects of Toxic Compounds on Stem Elongation of Bean.

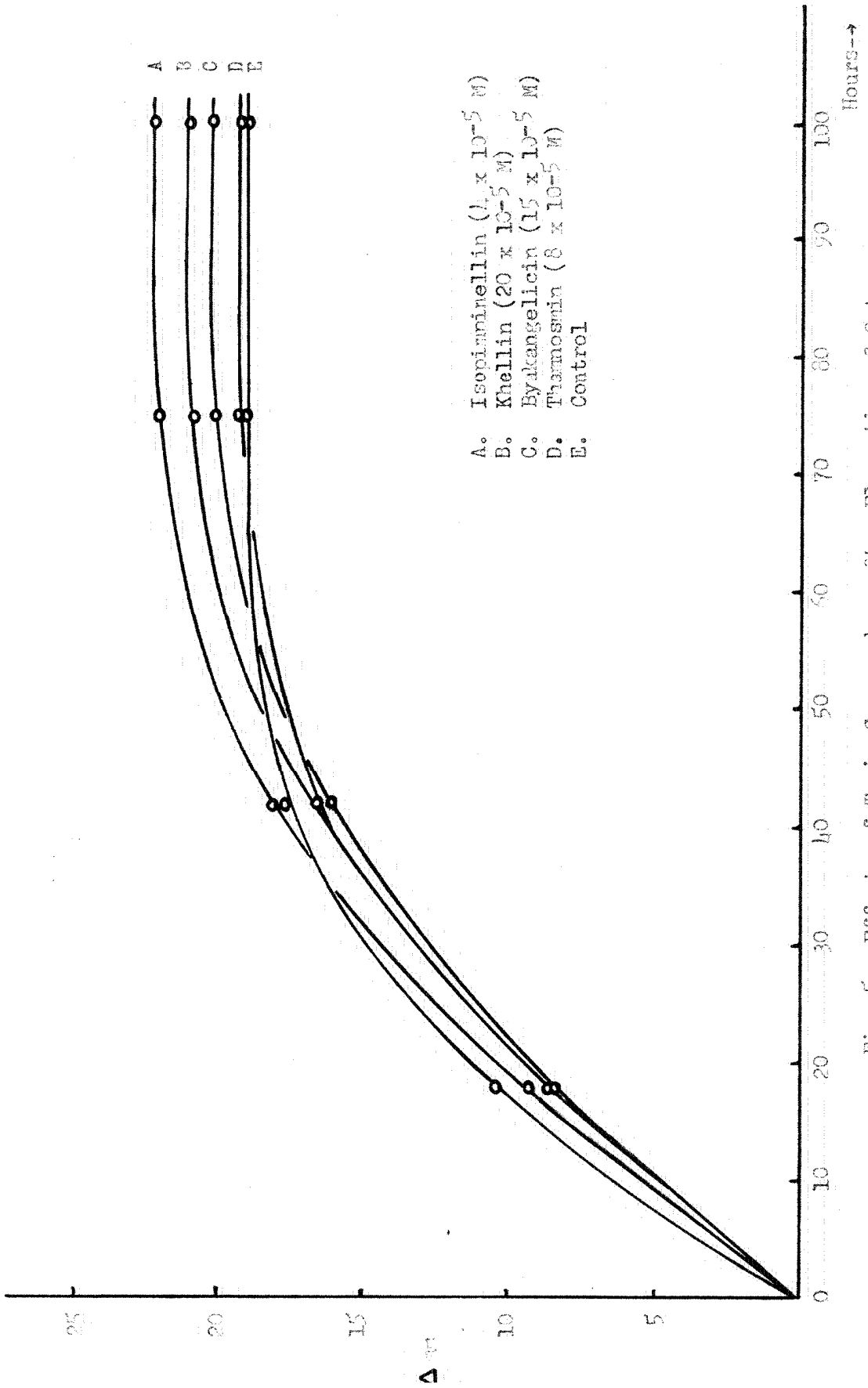


Fig. 5. Effects of Toxic Compounds on Stem Elongation of Oat.

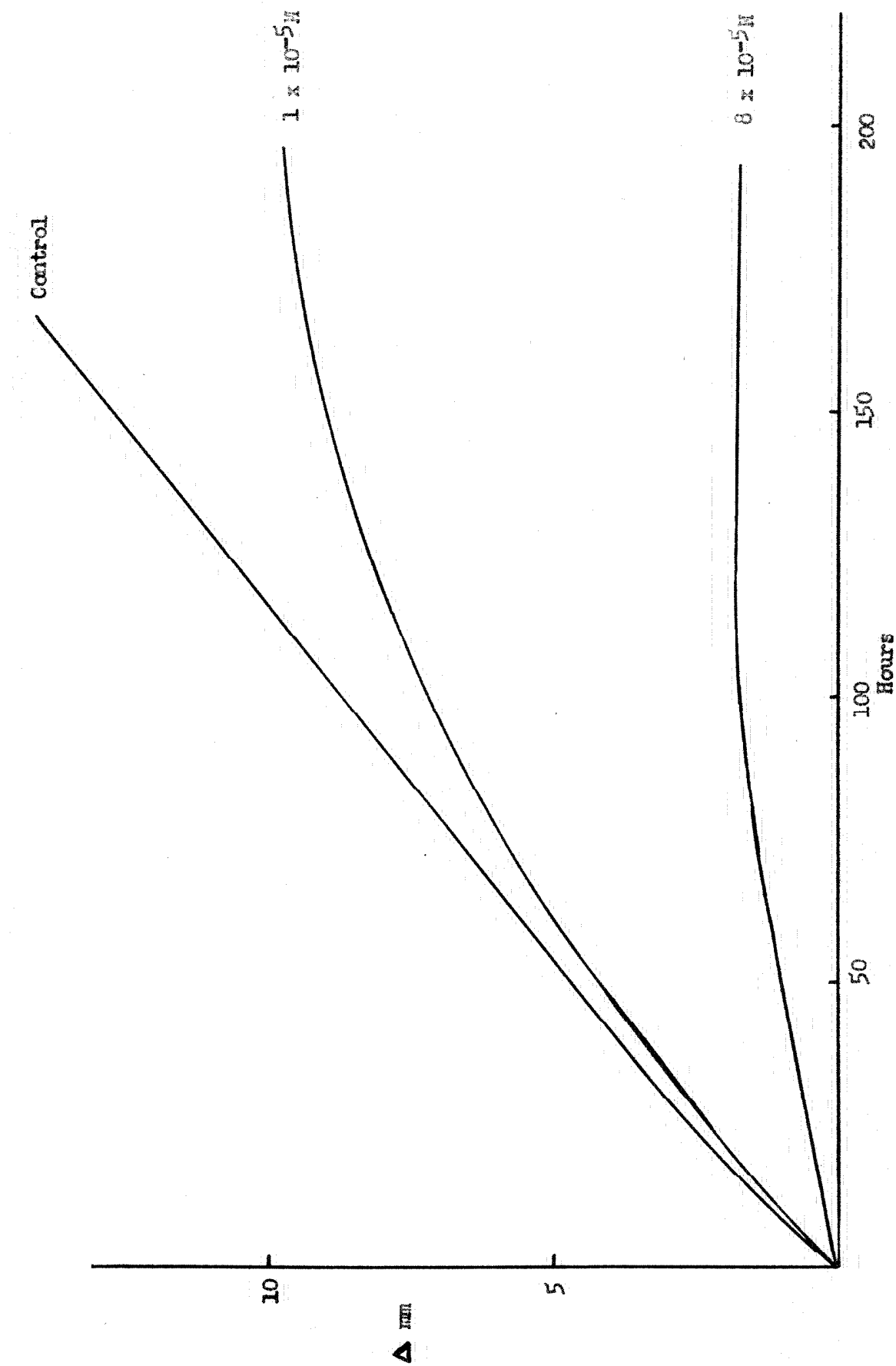


Fig. 5. Effect of Thammosmin on Stem Elongation of Tomato.

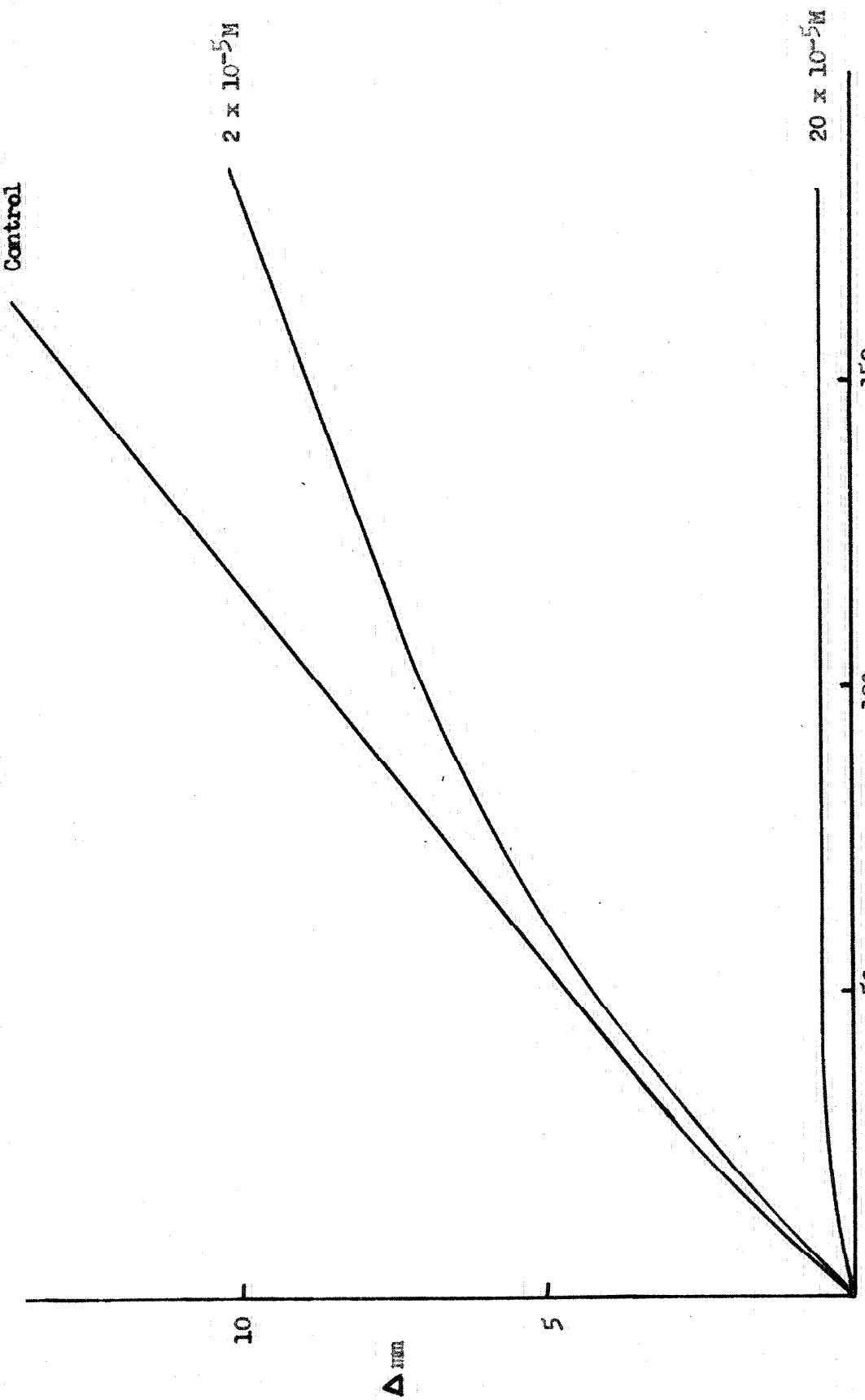


Fig. 7. Effect of Khellin on Stem Elongation of Tomato.

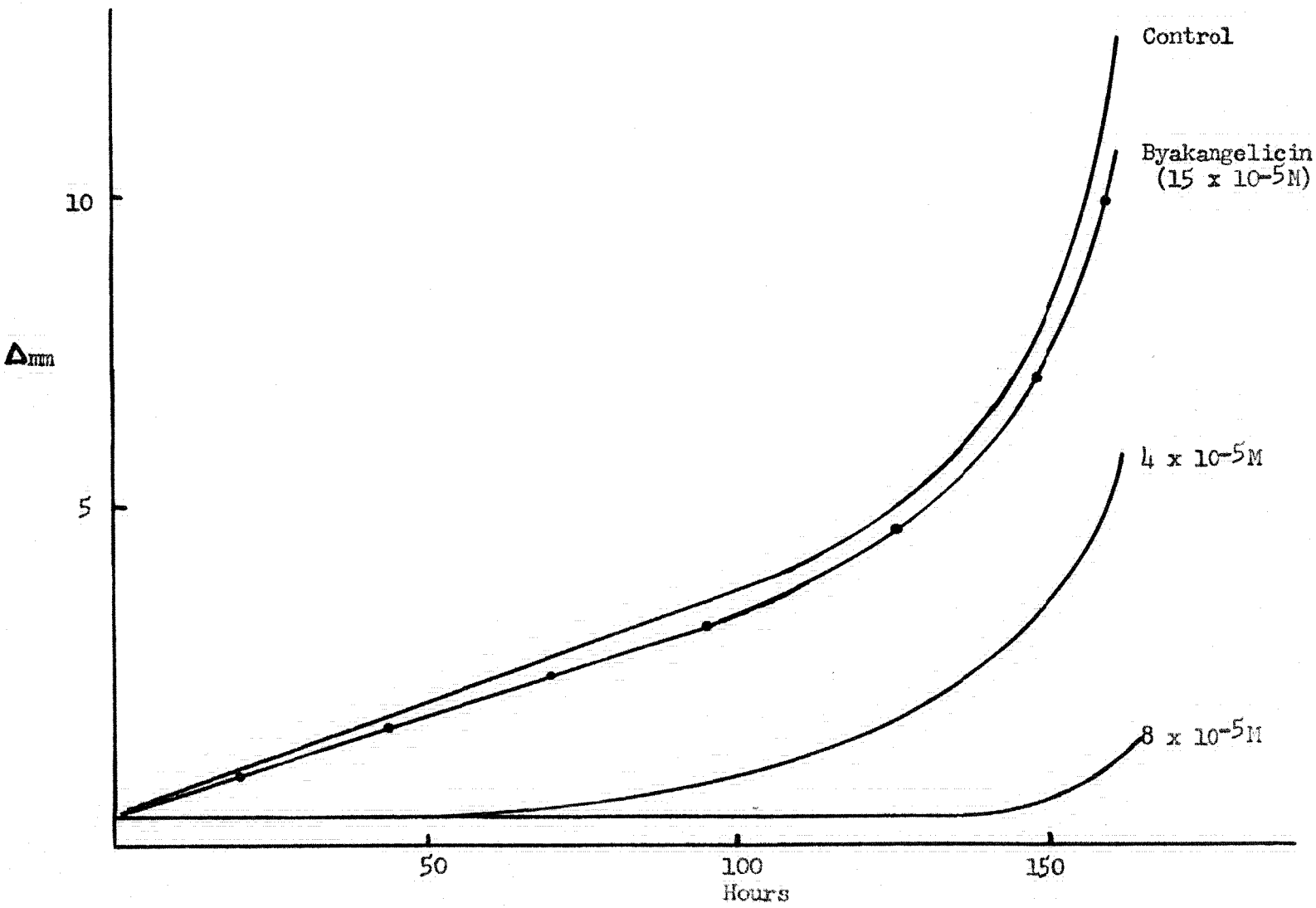


Fig. 8. Effect of Isopimpinellin on Stem Elongation of Tomato.

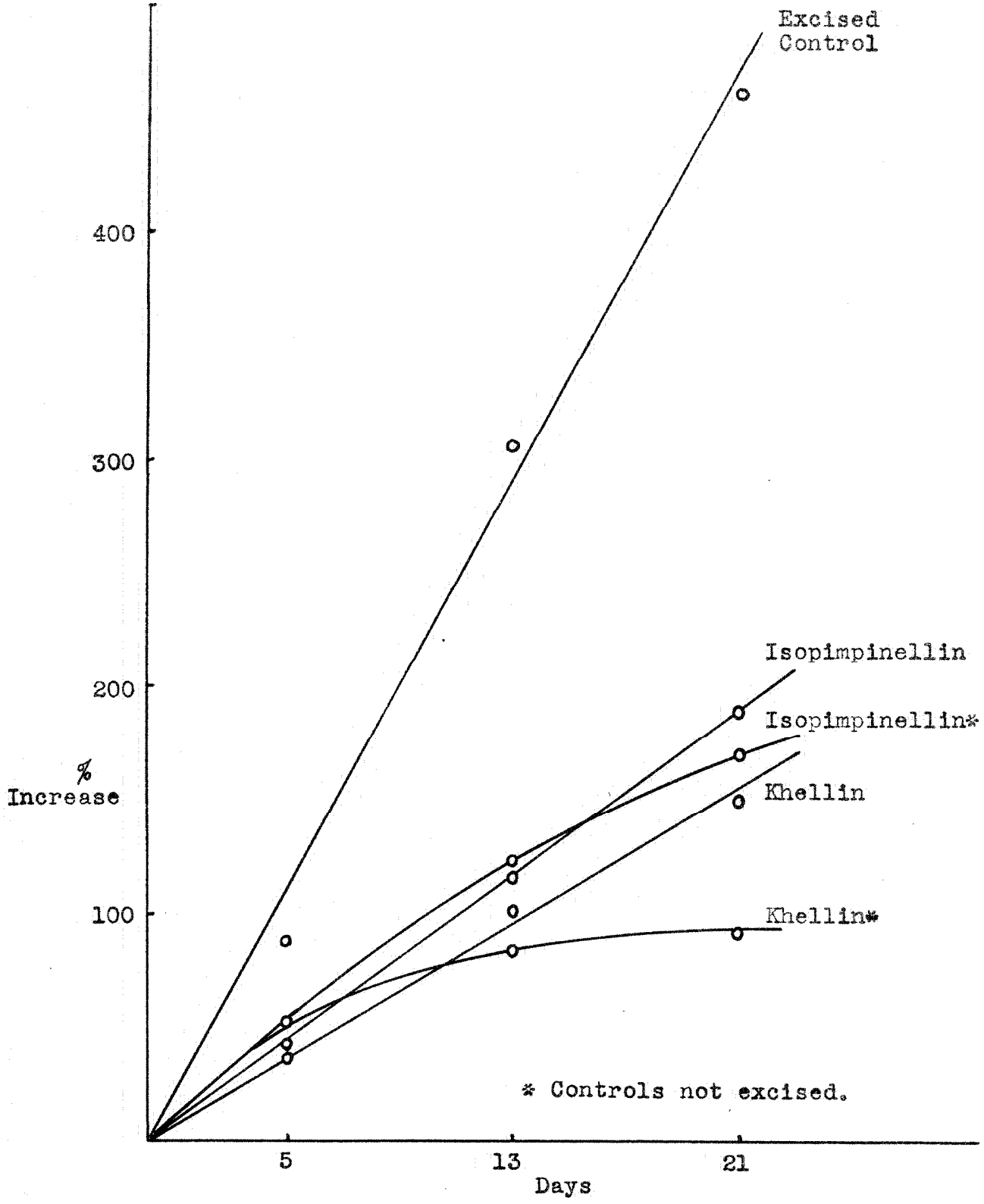


Fig. 9. Effects of Toxic Compounds on Stem Elongation of Excised Tomato Tops.

References

1. L.J. Haynes, Quart. Revs., 2, 46(1948).
2. H. Priess, Ber. deut. Pharm. Ges., 21, 267(1911).
3. E. Späth, Ber., 70A, 83(1937).
4. H.K. Garner, Thesis, California Institute of Technology, 1948.
5. L.J. Audus and J.H. Quastel, Nature, 159, 320(1947).

PART III. PREPARATION AND REACTIONS OF S-PHENYLTHIOLCARBAMATES.

A. Introduction

It has been shown by Schuller and Niemann (1) that certain amides of S-phenylthiocarbonic acid (usually called S-phenylthiolcarbamates) react with primary and secondary amines to produce the corresponding ureas, as shown below.

$$R_1\text{-S-CONHR}_2 + HNR_3R_4 \longrightarrow R_1\text{-SH} + R_2NH\text{-CONR}_3R_4$$

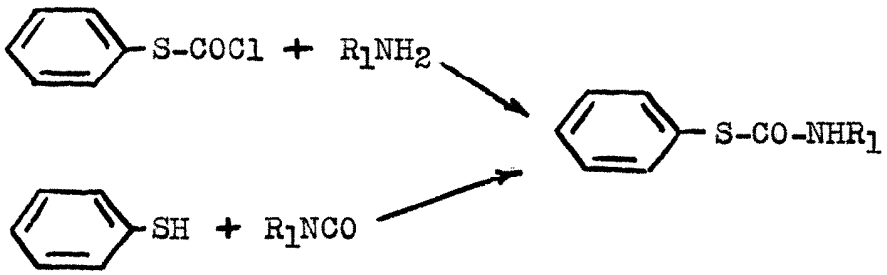
Because of the ease and rapidity with which the reaction goes to completion, it was considered that the method might be useful for the preparation of a variety of ureas and urea-like compounds, and the present investigation was conducted to ascertain the scope, limitations, and optimum conditions of this type of reaction.

Thiolcarbamates not only react with primary and secondary amines to yield ureas, but also with hydrazines and amides to give semicarbazides and acylureas respectively. The use of dithiocarbamates as starting materials permits the synthesis of thioureas by this method, but substitution of either oxygen or nitrogen for the thiolcarbamate sulfur in the preparation of ureas generally gave unsatisfactory results.

B. Preparation of S-Substituted Thiolcarbamates.

Thiolcarbamates (R_1-S-CO-NR_2R_3) were prepared in which R_1 was aryl or aralkyl, and R_2 and R_3 were hydrogen, alkyl, aryl, or heterocyclic. Although it was found possible to synthesize these amides from an isocyanate and the corresponding mercaptan, most of the preparations were made by reaction of the more

versatile S-phenylthiocarbonyl chloride (2,3) with an appropriate amine, or with ammonia.



Recrystallization of the amides from aqueous ethanol produced excellent crystals which melted over short ranges, but analysis of these solids gave results which deviated from the calculated values by large factors, even after careful removal of moisture. However, a recrystallization from toluene yielded crystals for which the analytical values were satisfactory. The melting points of samples crystallized from the different solvents were identical.

The properties of a series of representative N-monosubstituted-S-phenylthiolcarbamates are shown in Table 1. While the amides obtained from secondary amines are relatively stable in the presence of both acids and bases (1,3) those formed from ammonia or primary amines are readily decomposed in alkaline medium (1,3). Therefore, in the preparation of amides of the latter types it is desirable to add a solution of two moles of the amine to one of the acid chloride except in those cases where the amine has a pK_B greater than ca. 8 where the reverse order of addition is equally satisfactory. Although the above procedure requires a second mole of the amine

for neutralization of the hydrogen chloride formed during the reaction it should be noted that the yields obtained by this method are generally greater than 90% of the theoretical.

The addition of two moles of the amine to one of the acid chloride was found to proceed rapidly and smoothly in dioxane or absolute ethanol solutions. The amides so formed were recovered either by pouring the reaction mixtures into cold water, or by their evaporation to dryness, extraction of the neutral residue with either benzene or toluene and subsequent precipitation from these solutions by the addition of ligroin.

The stoichiometry of the reaction of phenylthiocarbonyl chloride with a simple primary amine in the presence of an excess of base may be represented by the following equations:

$$\phi SCCl + RNH_2 + B^- \longrightarrow \phi SCNHR + BH + Cl^-$$

$$\phi SCNHR + RNH_2 + B^- \longrightarrow RNHCNHR + BH + \phi S^-$$

$$\phi SCCl + \phi S^- \longrightarrow \phi SCS\phi + Cl^-$$

Thus, it is evident that under the above conditions two moles of the acid chloride will react with two moles of the primary amine to give one mole of the corresponding urea and one mole of diphenyldithiocarbonate. If more than two moles of the acid chloride are added to the original reaction mixture the amount in excess of two moles does not appear to react as is

apparent from the persistence of an oily emulsion of the acid chloride even after prolonged agitation. However, if the simple primary amine is replaced by an equivalent amount of an α-amino acid it appears that an unlimited amount of the acid chloride can be consumed, if sufficient base is present, *except in those cases where the urea is periodically removed* from the reaction mixture. In a particular experiment in which six moles of the acid chloride was allowed to react with two moles of DL-phenylalanine, in the presence of an excess of aqueous sodium hydroxide, there were obtained three moles of diphenyl dithiocarbonate and, after acidification of the reaction mixture, one mole of the urea and two moles of carbon dioxide. These facts may be explained, at least in part, in terms of the following equations.

$$\phi SCCl + NH_2CHRCO_2^- + OH^- \longrightarrow \phi SCNHCHRCO_2^- + Cl^- + H_2O$$

$$\phi SCNHCHRCO_2^- + NH_2CHRCO_2^- + OH^- \longrightarrow CO(NHCHRCO_2^-)_2 + \phi S^- + H_2O$$

$$\phi SCCl + \phi S^- \longrightarrow \phi SCS\phi + Cl^-$$

$$CO(NHCHRCO_2^-)_2 + 2\phi SCCl \longrightarrow CO(NHCHRCO_2COS\phi)_2 + 2Cl^-$$

$$CO(NHCHRCO_2COS\phi)_2 + 8OH^- \longrightarrow CO(NHCHRCO_2^-)_2 + 2\phi S^- + 2CO_3^= + 4H_2O$$

$$2\text{ØS}\overset{\overset{O}{\|}}{C}\text{Cl} + 2\text{ØS}^- \longrightarrow 2\text{ØS}\overset{\overset{O}{\|}}{C}\text{SØ} + 2\text{Cl}^-$$

The above phenomenon is not only of practical interest in connection with the synthesis of ureas derived from the α-amino acids by the technic outlined above and described in detail earlier (1) but it also suggests two interesting possibilities, i.e., one, that of stopping the reaction at the mixed anhydride stage by limiting the amount of base present in the reaction system and ~~to~~ thereby gaining an intermediate that would be useful for the subsequent synthesis of derivatives of the carboxyl function present in these ureas, and two, that the role of the urea in catalyzing the hydrolytic degradation of phenylthiocarbonyl chloride may be of sufficient general interest to justify a detailed study of the mechanism of this reaction. It is not known at present whether the particular urea used in this study has any special function in the hydrolytic degradation of phenylthiocarbonyl chloride or whether the same reaction can be observed with any carboxylic acid.

C. Reactions of S-Substituted Thiolcarbamates.

For the synthesis of the various substituted ureas the requisite S-phenylthiolcarbamate, derived from either ammonia or a primary amine, was dissolved in dioxane, or absolute ethanol, and to this solution was added the appropriate primary or secondary amine and a base such as triethylamine. After the reaction mixture had been allowed to stand for 1

hour at ca. 25° the urea was isolated by one of the several methods described below. Triethylamine was found to be the most satisfactory catalyst for general use because it is a sufficiently strong base to function as an effective proton acceptor in the reaction under discussion, it is freely soluble in the usual reaction media, and it can be readily removed from the reaction mixture by distillation. Where the added amine has a pK_B of less than ca. 6 it may of course be added in excess so as to assume the role of both reactant and catalyst. Sodium hydroxide, while convenient in certain cases, e.g., with α-amino acids, does not appear to be as generally useful as triethylamine.

Water-insoluble ureas are conveniently isolated in yields greater than 90% by a procedure in which the reaction mixture is poured into water containing an excess of 1 N hydrochloric acid, the suspension so formed steam distilled until the residual suspension is practically odorless, the precipitated urea collected, washed with aqueous sodium carbonate and with water and finally recrystallized from toluene or absolute ethanol. If the steam distillation is omitted several recrystallizations from toluene are necessary to obtain an odorless product. A more general method of isolation is based upon the removal of the solvent, the tertiary amine catalyst and thiophenol by evaporation of the reaction mixture to dryness on a steam bath, and under an inert atmosphere, followed by recrystallization of the residue from toluene or aqueous

ethanol. In those cases where the added amine is a gas and can also serve as the catalyst, the reaction is conveniently conducted by passing the gas into a toluene solution of the appropriate amide followed by subsequent precipitation of the urea by the addition of ligroin.

In many instances the ureas may be prepared without the actual isolation of the intermediate S-phenylthiolcarbamate. In addition to the procedure described earlier (1) the following method may be applied. S-Phenylthiocarbonyl chloride, and a one fold excess of ammonia or a primary amine are allowed to react in a water-insoluble solvent, the amine hydrochloride removed by extraction of the reaction mixture with dilute aqueous hydrochloric acid, the non-aqueous phase dried, the second amine, and triethylamine if necessary, added to this solution and the urea isolated as described above. If a symmetrical disubstituted urea is to be prepared, the above procedure may be modified by allowing the acid chloride to react with two moles of the requisite primary amine in the presence of an excess of triethylamine.

Some of the ureas prepared from S-phenylthiolcarbamate, or N-monosubstituted derivatives thereof, are listed in Table 2. In general the yields were 90% or greater. In addition to a comparison of the melting points of the various ureas with those given in the literature (4-23) all of the ureas listed in Table 2 which contained a phenyl group were also prepared from phenyl isocyanate in order that a direct comparison of the melting points could be made in these cases.

The extension of the base-catalyzed reaction of S-phenylthiolcarbamate, or N-monosubstituted derivatives thereof, with ammonia, or with primary or secondary amines to the synthesis of analogous compounds was demonstrated by the synthesis of N-phenyl-N'-benzoylurea (21) from N,S-diphenylthiolcarbamate and benzamide, by the preparation of 1,4-diphenylsemicarbazide (22) from the same carbamate and phenylhydrazine, and by the synthesis of N,N'-diphenylthiourea (23) in 82% yield from N,S-diphenyldithiolcarbamate and aniline. In all three cases triethylamine was used as the base, and in the latter instance the dithiolcarbamate was prepared by the reaction of phenyl isothiocyanate with thiophenol rather than by the reaction of aniline with the known S-phenyldithiocarbonyl chloride (3).

A further modification was made by the replacement of N,S-diphenylthiolcarbamate with O,N-diphenylcarbamate in the synthesis of N-phenyl-N'-n-butylurea (8) from n-butylamine, and with N-phenyl-S-(β-phenethyl)thiocarbamate in a synthesis of N,N'-diphenylurea (10) from aniline. However, with these latter carbamates, and in contrast to the S-phenylthiolcarbamates, it was found necessary to conduct the reactions at 80°, rather than at 25°, in order to obtain good yields of the ureas in the course of one hour.

It has been pointed out (1) that the base catalyzed reaction of S-phenylthiolcarbamate, or its N-monosubstituted derivatives, with primary or secondary amines may be formula-

ted in terms of a 1:2 elimination reaction (24-26) to give an intermediate isocyanate which then participates in a 1:2 addition reaction with the amine to give the urea. However, all attempts to isolate this supposed intermediate, or to detect it by means other than its usual addition reactions, were totally unsuccessful.

While it is possible to formulate the reaction under discussion in a manner analogous to that employed for the aminolysis of esters (27,28) and of thioesters (29), there are several facts that are difficult to explain on the basis of such a mechanism. The principal difficulty resides in the fact that N-disubstituted-S-phenylthiolcarbamates are extremely stable in the presence of both acids and bases, and although a number of attempts were made to cause a reaction between such thiolcarbamates and primary or secondary amines to form tri- and tetra-substituted ureas no such reaction could be demonstrated even though the reaction conditions included temperatures up to 180° and prolonged reaction times. Although it is known that electron releasing groups in the immediate neighborhood of the carboxyl function will decrease the rate of ammonolysis of esters (27) and that esters of α-substituted carboxylic acids undergo ammonolysis with difficulty (30) there appears to be no valid explanation, in terms of the above reaction mechanism, of why for example N-ethyl-S-phenylthiolcarbamate should react with a primary or secondary amine under the conditions specified and the N-diethyl

compound should not, particularly in view of the fact that a number of esters of thioacetic acid are rapidly and completely aminolyzed by aniline even under slightly acidic conditions (29).

The clear requirement for the presence of an amide hydrogen atom in the thiolcarbamate in order that subsequent reaction to form a urea be observed led to the suggestion (1) that the first step in the over-all reaction is the formation of a resonance stabilized intermediate ion, i.e.,

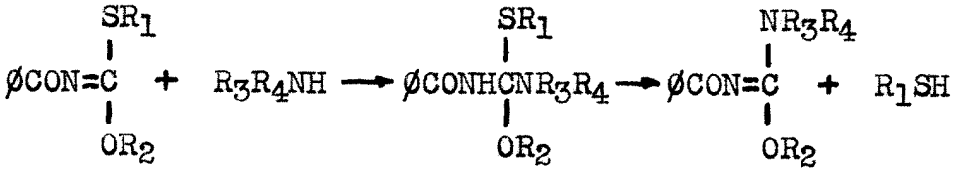
$$\phi SCNHR_1 + B^- \rightleftharpoons \left[\phi SCNR_1 ;\ \phi SC{=}NR_1\right]^- + BH,$$

which is then attacked by the primary or secondary amine with the concomitant elimination of thiophenate ion resulting in the formation of the urea, i.e.,

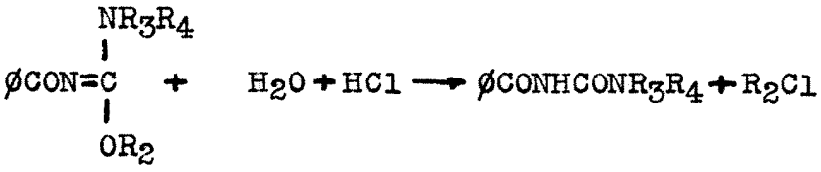
$$\left[\phi SCNR_1 ;\ \phi SC{=}NR_1\right]^- + R_2R_3NH \rightleftharpoons \left[\phi S{-}C(=O){-}NHR_1 \atop NR_2R_3\right]^- \longrightarrow R_1NHCONR_2R_3 + \phi S^-.$$

This formulation is compatible with all of the known experimental facts relative to the behavior of the S-phenylthiolcarbamates, provides an adequate explanation of the lack of reactivity of the O-alkylcarbamates under conditions where the corresponding S-aryl- and S-alkylthiolcarbamates and the O-arylcarbamates are readily converted into ureas, is compatible with the fact that S-arylthiolcarbamates are more reactive than the corresponding S-alkylthiolcarbamates or O-arylcarbamates, and is capable of extension to explain the behavior of compounds which are analogs of the thiolcarbamates.

The reaction mechanism given immediately above also derives support from the investigations of Wheeler and coworkers (31,32) who found that the reaction



was both general and facile and that the O-alkylisourea so formed reacts with cold aqueous hydrochloric acid to give an alkyl halide and a practically quantitative yield of the substituted urea, i.e.,

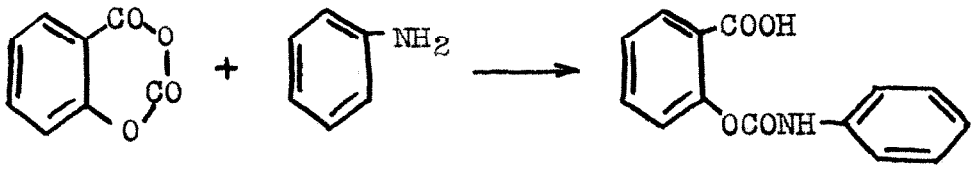


D. Preparation and Reactions of Compounds Related to S-Substituted Thiolcarbamates.

The reactivity of the thiolcarbamates suggested that the oxygen analogs (carbamates) might also possess interesting properties. Although O,N-diphenylcarbamate prepared both from phenyl isocyanate and phenol, and from phenoxycarbonyl chloride and aniline, was converted to substituted ureas by aniline and by n-butylamine at 100°, O-ethyl-N-phenylcarbamate was completely inert under these conditions.

Anhydrocarboxysalicylic acid (benzo-1,3-dioxandione-2,4) (33) was prepared from disodium salicylate and phosgene, and when treated with aniline, O-(o-carboxyphenyl)-N-phenyl-

carbamate was formed.



When the product of this reaction was allowed to react with n-butylamine at room temperature, N-phenyl-N'-(n-butyl)-urea was formed in 81% yield. No substituted urea could be isolated from the mixture resulting after the attempted reaction of O-(o-carboxyphenyl)-N-phenylcarbamate with N-methylaniline and triethylamine.

The solubility of such a carbamate in dilute aqueous alkali suggested possible application for the preparation of ureas in aqueous solution. Thiosalicylic acid reacted with phenyl isocyanate to yield a yellowish solid, m.p. ca. 200°, which was assumed to be S-(o-carboxyphenyl)-N-phenylthiolcarbamate. Both this and the corresponding oxygen analog mentioned above were soluble in 1 M aqueous sodium hydroxide solution, and produced precipitates of N-phenyl-N'-(n-butyl)urea upon addition of n-butylamine. Further details of this procedure were not sought, and it might be of interest to investigate such reactions in greater detail.

Since substitution of the O-phenyl group with an electron-attracting group in the ortho position had promoted the formation of a substituted area upon reaction with an amine, a similar compound was synthesized which contained a para nitro group in the O-phenyl moiety. Reaction of this

O-(p-nitrophenyl)-N-phenylcarbamate with n-butylamine gave a 73% yield of the appropriate urea. It was further proposed to synthesize the sulfur analog of this carbamate, and for this purpose, p-nitrothiophenol was prepared from p-nitrochlorobenzene, and allowed to react with phenyl isocyanate. Although the crystalline product appeared to be quite pure, results of a microanalysis did not correspond to the composition of any predicted compound, and the substance was not investigated further.

E. The Use of S-Phenylthiolcarbamates in Qualitative Organic Analysis.

Because of the frequent use of ureas as derivatives for the identification of organic compounds, the reaction of S-phenylthiolcarbamates with amines immediately suggested that the reagents might prove of use in qualitative organic analysis. These thiolcarbamates are apparently indifferent to moisture, easily stored, handled, and weighed as crystalline solids of moderate melting points, and are stable over a period of years. Their reaction with the amine group is quantitative under appropriate conditions, and is specific so far as their general chemical properties have been studied.

This last point is of particular value, because it permits derivatization of primary and secondary amines in the presence of alcohols and phenols under conditions which

would not permit the use of the more reactive isocyanates generally employed for the purpose. Primary amines have now been derivatized quantitatively by reaction with S-phenylthiolcarbamates in the presence of tertiary amines, amides, hydrocarbons, alcohols, phenols, ethers, carbonyl compounds, and carboxylic acids and esters.

The preparation of the derivative may be conducted on any convenient scale in dioxane, ethanol, or toluene at temperatures preferably below 60°, and the product obtained by methods indicated previously. The resulting ureas may be crystallized satisfactorily from aqueous ethanol.

The principal advantages gained by the use of S-phenylthiolcarbamates instead of the corresponding isocyanates in this application lie in the stability to moisture and resultant ease of storage and use of the reagents. Testing of six previously unopened bottles of p-nitrophenyl isocyanate showed that no appreciable quantity of the compound remained, all of it having been converted spontaneously to p,p'-dinitrodiphenylurea. Under similar conditions, S-phenyl-N-(p-nitrophenyl)thiolcarbamate remained unchanged for a period of two years, and even in the event of some unforseen reaction or decomposition, the reagent may be purified easily by recrystallization from toluene.

Primary and secondary amines have been derivatized previously by the formation of substituted ureas containing the phenyl, p-chlorophenyl (34), p-ethoxyphenyl (35), p-nitro-

phenyl (19), and cyclohexyl groups (36), and examples of each of these cases now have been prepared successfully from the corresponding S-phenylthiolcarbamates.

F. Experimental.

Many of the processes described in this section are included as examples of general procedures developed and used with many different compounds, most of which are listed in the tables at the end of the section. Microanalyses were performed by Dr. A. Elek. All m.p.'s are corrected unless otherwise indicated.

S-Phenylthiocarbonyl Chloride.--Cylinder phosgene was washed with cottonseed oil, dried by bubbling through concentrated sulfuric acid, and passed into 700 ml. of dry toluene in a 5-l. three-necked flask with constant mechanical agitation until a total of 154 g. (1.56 moles) had dissolved. Thiophenol (170 g., 1.54 moles) was then run in over a period of 10 minutes, followed by a total of 1500 ml. of 1 M aqueous sodium carbonate solution (equivalent to 1.50 moles). Addition of the alkaline solution required 1 hour, during which the mixture was constantly stirred and chilled in ice. After stirring for an additional 20 minutes, the layers were separated and the upper organic phase was shaken with 300 ml. additional 1 M aqueous sodium carbonate solution, the layers separated, and the organic phase dried over anhydrous magnesium sulfate.

After removal of the toluene *in vacuo*, the product was fractionally distilled under diminished pressure. Following a fore-run of thiophenol, S-phenylthiocarbonyl chloride was collected, b.p. 88.0-91.0°/5mm., n_D^{26} 1.5805-1.5811. The yield was 131.5 g. (61.2%) with recovery of 32.5 g. of thiophenol.

Pure S-phenylthiocarbonyl chloride boils at 89.5-90.0°/-5mm., n_D^{26} 1.5801, D_4^{23} 1.256.

Phenoxycarbonyl Chloride.--To an ice cold and well stirred solution of 61 g. (0.62 mole) of phosgene, previously washed by successive passage through cottonseed oil and concentrated sulfuric acid, in 250 ml. of toluene was added, at the rate of *ca.* 5 ml. per minute, a solution of 47 g. (0.5 mole) of phenol in 500 ml. of 1 N aqueous sodium hydroxide, the reaction mixture stirred for an additional hour at 0°, the layers separated, and the non-aqueous layer dried over magnesium sulfate and freed of excess phosgene by aeration of the dried solution. The solvent was removed *in vacuo* and the residue fractionally distilled to give 26.0 g. (33.3%) of phenoxycarbonyl chloride b.p. 63-63.5°/7 mm. The distillate crystallized to give the solid acid chloride, m.p. 38.0-38.5°. Reaction of the solid acid chloride with aniline gave a practically quantitative yield of O,N-diphenylcarbamate, *vide post*.

N,S-Diphenylthiolcarbamate.--To an ice cold and well stirred solution of 13.8 ml. (0.10 mole) of phenylthiocarbonyl

chloride in 100 ml. of dry dioxane was added dropwise 18.6 ml. (0.20 mole) of aniline, the reaction mixture allowed to stand at 25° for two hours, the slurry slowly poured with vigorous stirring into a mixture of 300 ml. of water and 100 g. of ice, the precipitated solid collected, washed with 300 ml. of 2 N hydrochloric acid and then with 500 ml. of water and finally air dried. This product was recrystallized from aqueous ethanol to give 20.0 g. (87.5%) of N,S-diphenylthiolcarbamate, m.p. 123.3-124°. The mother liquor was concentrated to give an additional 1.6 g. of product which brought the total yield to 94%.

The other thiolcarbamates listed in Table 1, with the exception of N-methyl-S-phenylthiolcarbamate, were prepared essentially as described above except that it was found preferable to recrystallize the crude products from toluene rather than from aqueous ethanol.

N-Methyl-S-phenylthiolcarbamate.--Dry gaseous methylamine, 0.06 mole, was slowly passed into a cold solution of 4.14 ml. (0.03 mole) of phenylthiocarbonyl chloride in 15 ml. of dry dioxane, the reaction mixture allowed to stand at 25° for 15 minutes, the precipitated methylammonium chloride collected, the colorless filtrate evaporated to dryness at 60° and the solid residue recrystallized from toluene to give 3.5 g. (70%) of N-methyl-S-phenylthiolcarbamate, m.p. 103-104°.

N-Phenyl-S-(β-phenethyl)thiolcarbamate.--A mixture of 1.19 g. (0.01 mole) of phenyl isocyanate, 1.38 g. (0.01 mole)

of β-phenethyl mercaptan, and 5 drops of anhydrous pyridine was allowed to stand at 25° for 16 hours. The solid reaction product was then recrystallized from a mixture of 60-80° ligroin and toluene to give 2.50 g. (97%) of N-phenyl-S-(β-phenethyl)thiolcarbamate, m.p. 109.5-110.5°.

Anal. Calc'd for $C_{15}H_{15}NOS$(257.4): C, 70.0; H, 5.9; N, 5.4.

Found: C, 70.2; H, 5.9; N, 5.1.

O,N-Diphenylcarbamate.--Redistilled aniline, 3.4 g. (0.038 mole) was added dropwise and with vigorous agitation to a solution of 3 g. (0.019 mole) of phenoxycarbonyl chloride in 20 ml. of dioxane, the reaction mixture allowed to stand for ten minutes and then poured into ice water. To this suspension was added 15 ml. of 2 N hydrochloric acid , the mixture stirred, the solid collected, washed with 2 N hydrochloric acid and with water, and air dried. The crude product was recrystallized from aqueous ethanol to give 3.4 g. (95%) of O,N-diphenylcarbamate, m.p. 126.3-126.8° with no depression of the m.p. by admixture of the same urethane prepared from phenol and phenyl isocyanate.

Preparation of Substituted Ureas.--The preparations which follow are representative of those which were used for the synthesis of the various ureas which are listed in Table 2. In general all of these ureas were prepared in yields which were greater than 90% of the theoretical.

N-Phenyl-N'-α-pyridylurea.--To a solution of 1.14 g. (0.005 mole) of N,S-diphenylthiolcarbamate in 15 ml. of dry dioxane was added 0.47 g. (0.005 mole) of α-aminopyridine and

1.0 ml. (0.007 mole) of triethylamine, the reaction mixture allowed to stand at 25° for several hours, the solvent removed by evaporation at ca. 60° in a stream of illuminating gas, the solid residue triturated with 20 ml. of 60-80° ligroin, the solid collected, repeatedly washed with ligroin and air dried. The crude product was recrystallized from aqueous ethanol to give 1.03 g. (97%) of N-phenyl-N'-α-pyridylurea, m.p. 179-180° with no depression of the m.p. upon admixture with the same urea prepared by the reaction of α-aminopyridine with phenyl isocyanate.

Piperidine N-Carboxylic Anilide.--Piperidine, 1.0 ml. (0.01 mole) was added to a solution of 0.57 g. (0.0025 mole) of N,S-diphenylthiolcarbamate in 10 ml. of toluene, the exothermic reaction allowed to proceed to completion, the crystalline reaction product triturated with 2 ml. of 60-80° ligroin, the suspension cooled in an ice-bath, the solid collected, washed with a warm 1:1 mixture of ligroin and toluene, the filtrate and washings combined, evaporated to dryness, the residue taken up in ligroin, the solid collected, washed with ligroin and air dried. Recrystallization of the crude product from aqueous ethanol gave 0.48 g. (95%) of product m.p. 168-169° which upon an additional recrystallization from the same solvent gave piperidine N-carboxylic anilide, m.p. 171-172°, with no depression of the m.p. when mixed with an authentic sample prepared from piperidine and phenyl isocyanate.

N-Phenyl-N',N'-dimethylurea.--An excess of dry dimethylamine was passed into a solution of 0.57 g. (0.0025 mole) of N,S-diphenylthiolcarbamate in 15 ml. of toluene until the formation of a crystalline precipitate had ceased. An equal volume of 60-80° ligroin was added to the suspension, the latter cooled in an ice-bath, the solid collected and washed with a 1:1 mixture of ligroin and toluene to give 0.33 g. (81%) of N-phenyl-N',N'-dimethylurea, m.p. 127.5-128°. Admixture of this product with an authentic sample prepared from dimethylamine and phenyl isocyanate failed to depress the m.p.

N-Phenyl-N'-n-butylurea.--To 0.54 g. (0.0025 mole) of O,N-diphenylcarbamate in 10 ml. of dry dioxane was added 0.5 ml. (0.005 mole) of n-butylamine, the mixture heated under refluxing conditions for 1 hour, the cooled reaction mixture poured into 75 ml. of water, the solid collected, washed with 2 N hydrochloric acid and with water and dried *in vacuo* to give 0.45 g. (94%) of product, m.p. 126-127°. Recrystallization of this product from a mixture of benzene and ligroin gave N-phenyl-N'-n-butylurea, m.p. 127.8-128.2° which was not depressed when admixed with an authentic sample prepared from phenyl isocyanate and n-butylamine.

1,4-Diphenylsemicarbazide.-- To a solution of 0.57 g. (0.0025 mole) of N,S-diphenylthiolcarbamate and 0.25 ml. (0.0025 mole) of phenylhydrazine in 10 ml. of dry dioxane was added 0.7 ml. (0.005 mole) of triethylamine, the reaction mixture allowed to stand at 25° for 1 hour, the clear solution poured into 50 ml. of water and 30 g. of crushed ice,

the precipitated solid collected, washed with 70 ml. of 2 N hydrochloric acid and then with 50 ml. of water and then air dried. Recrystallization of this product from 50% aqueous-ethanol gave 0.52 g. (93%) of 1,4-diphenylsemicarbazide, m.p. 177.8-178.5° with no depression of the m.p. when mixed with an authentic sample prepared from phenylhydrazine and phenyl isocyanate.

N,N'-Diphenylthiourea.--To 0.61 g. (0.0025 mole) of N,S-diphenyldithiocarbamate, prepared from thiophenol and phenyl isothiocyanate, in 10 ml. of dry dioxane was added 0.93 ml. (0.01 mole) of aniline and 1.4 ml. (0.01 mole) of triethylamine, the reaction mixture allowed to stand for 1 hour, the clear solution diluted with 30 ml. of 60-80° ligroin, the solid collected and dried to give 0.47 g. (82%) of thiocarbanilide, m.p. 153-154° with no depression of the m.p. when mixed with an authentic sample.

N-Phenyl-N',N'-diethylurea.--Aniline, 2 ml. (0.022 mole) was added to a solution of 1.4 ml. (0.01 mole) of phenylthiocarbonyl chloride in 15 ml. of benzene, the reaction mixture stirred for 10 minutes, 25 ml. of 2 N hydrochloric acid added, the mixture again stirred, and the non-aqueous phase separated, washed with water and dried over sodium sulfate. To this solution was added 3.0 ml. (0.03 mole) of diethylamine, the reaction mixture allowed to stand for 30 minutes, the solvent removed on a steam bath, the crystalline residue

triturated with 10 ml. of 60-80° ligroin, and the solid collected and washed with 30 ml. of ligroin to give 1.76 g. (92%) of N-phenyl-N',N'-diethylurea, m.p. 80-82° with no depression of the m.p. when mixed with an authentic sample prepared from phenyl isocyanate and diethylamine.

Reaction of N-Phenyl-S-(β-phenethyl)thiolcarbamate with Aniline.--The thiolcarbamate (0.52 g., 0.002 mole), aniline (0.2 g., 0.002 mole) and 0.2 ml. of triethylamine in 5 ml. of dry dioxane were heated at 80° for 1 hour. The cooled reaction mixture was poured into a mixture of 20 g. of ice and 50 ml. of 2 N hydrochloric acid, the precipitate collected, washed with water, and dried to give 0.43 g. (100%) of sym-diphenylurea, m.p. 230-232°. A similar experiment conducted at 25° gave only 0.2 g. (45%) of sym-diphenylurea after extraction of the unreacted thiolcarbamate with hot carbon tetrachloride.

Identification of Products Formed in Reaction of N,S-Diphenylthiolcarbamate with Aniline.--To a solution of 0.57 g. (0.0025 mole) of N,S-diphenylthiolcarbamate and 0.9 ml. (0.01 mole) of aniline in 10 ml. of dry dioxane was added 1.4 ml. (0.01 mole) of triethylamine, the mixture allowed to stand for 45 minutes at 25°, the clear solution washed into a distilling flask with 250 ml. of water containing 15 ml. of 2 N sulfuric acid, the contents of the flask steam distilled, the distillate (50 ml.) extracted with ether, the ethereal extract dried over magnesium sulfate, the solvent removed and the oily residue allowed to react with dinitrochlorobenzene

in the presence of alkali (37) to give 0.36 g. (68%) of 2,4-dinitrodiphenylsulfide, m.p. 118-119°. The residue remaining after the steam distillation was filtered, the solid washed with water, dried, and recrystallized from ethanol to give 0.52 g. (98%) of sym-diphenylurea, m.p. 233-234°.

Reaction of Phenylthiocarbonyl Chloride with DL-Phenylalanine.--S-Phenylthiocarbonyl chloride, 12.95 g. (0.075 mole) was weighed into a small separatory funnel and the acid chloride was added in small portions to a vigorously stirred solution of 4.13 g. (0.025 mole of DL-phenylalanine in 15 ml. of 3 N aqueous sodium hydroxide and 10 ml. of water. When ca. 1/5 of the acid chloride had been added an additional 10 ml. of the aqueous alkali was introduced into the reaction mixture followed by more of the acid chloride until ca. 4 g. of the latter remained in the funnel. Since no reaction was apparent at this stage, stirring was discontinued and the reaction mixture allowed to stand overnight. After an interval of 9 hours, during which time a precipitate had separated, an additional 15 ml. of 3 N alkali were added followed by the remainder of the acid chloride which was added dropwise and with stirring. After all of the acid chloride had been added a final portion of 10 ml. of 3 N alkali was added and the reaction mixture stirred for an additional 8 hours. The solid was then collected, washed with water and dried to give 9.20 g. (99.5%) of diphenyldithiocarbonate, m.p. 43.5-44°. The alkaline filtrate was quantitatively transferred to a flask

equipped with a dropping funnel and an absorption train for the collection of carbon dioxide. The system was swept free of air by a stream of nitrogen, the carbon dioxide absorption tube weighed, the solution carefully acidified with 2 N sulfuric acid to pH 5 and the evolved carbon dioxide swept into the absorption tube by a stream of nitrogen. The amount of carbon dioxide collected, corrected for the amount of carbonate present in the added alkali and that remaining in solution at 20° was 1.11 g. or 0.025 mole. The solid which had precipitated from the alkaline solution upon acidification was collected, washed with water and dried to give 4.27 g. (96%) of N,N'-carbonyl-bis-DL-phenylalanine. Thus from the reaction of 2 moles of the α-amino acid with 6 moles of the acid chloride in the presence of an excess of aqueous sodium hydroxide there was obtained 1 mole of the urea, 3 moles of diphenyldithiocarbonate and 2 moles of carbon dioxide.

Preparation of Anhydrocarboxysalicylic Acid.--Salicylic acid (55 g., ~~0.04~~ 0.4 mole) was dissolved in a warm solution of 32 g. of sodium hydroxide in 50 ml. of water, the water was removed by evaporation, and the product finally was dried at 110°.

Cylinder phosgene was washed by bubbling through cottonseed oil and then concentrated sulfuric acid, and then was dissolved in 100 ml. of toluene until the solution contained 50 g. (0.51 mole) of the compound. Disodium salicylate (45 g., 0.25 mole) was added slowly in portions with ice

cooling and stirring, followed by 60 ml. of toluene, and the mixture was stirred at room temperature for 7 hours. After filtration through a sintered-glass funnel, excess phosgene was removed by drawing a rapid stream of washed air through the solution, solvent was removed at 50° *in vacuo* until the volume was reduced to 50 ml., and the solution was allowed to stand overnight. The crystalline precipitate was filtered off, washed with cold toluene, and sucked dry. The yield was 5 g. (21%), m.p. 139-140° (d.).

The yield in a second preparation was 26%.

Preparation of O-(o-Carboxyphenyl)-N-phenylcarbamate.--This compound was prepared by the method of Davies (33) from anhydrocarboxysalicylic acid and aniline in 98% yield. Its reactions with amines were conducted in a manner identical with those of S-substituted thiolcarbamates.

Preparation and Reaction of S-(o-Carboxyphenyl)-N-phenylthiolcarbamate.--Thiosalicylic acid (5 g., 0.033 mole) and phenyl isocyanate (4 g., 0.033 mole) were mixed, 20 ml. of toluene were added, and finally 2 drops of pyridine were introduced. The warm mixture was heated at 70° for 1 hour, cooled, and the solid was recrystallized from aqueous ethanol, m.p. 200°. The yield was 9 g. (*ca.* 100%).

The thiolcarbamate (0.43 g., 0.002 mole) was dissolved in 10 ml. of 1 M aqueous sodium hydroxide solution, 1 ml. (0.01 mole) n-butylamine was added, and the mixture was allowed to stand overnight. The precipitate was filtered off, washed

with water, and recrystallized from aqueous ethanol, yield 0.4 g. (ca. 100%), m.p. 128-129°.

Preparation of p-Nitrothiophenol.--This compound was prepared from p-nitrochlorobenzene and sodium disulfide by the procedure described by Shirley (38).

Attempted Preparation of S-(p-Nitrophenyl)-N-phenylthiolcarbamate.--p-Nitrothiophenol (10 g., 0.065 mole) was dissolved in 10 ml. of dry dioxane and 7.0 ml. (0.065 mole) of phenyl isocyanate were added. The mixture was warmed until a vigorous reaction commenced, allowed to stand for 40 minutes, cooled, and the precipitated crystals were filtered off and recrystallized from toluene. The yield was almost quantitative, m.p. 181.0-182.0°.

Anal. Calc'd. for $C_{13}H_{10}N_2O_3S$ (278.3): C,56.0; H,3.6; N,10.1.

Found: C,47.0; H,2.7; N,11.0.

Preparation of O-(p-Nitrophenyl)-N-Phenylcarbamate.--p-Nitrophenol (13.9 g., 0.10 mole) was dissolved in 25 ml. of tetrahydrofuran, and 10.9 ml. (0.10 mole) of phenyl isocyanate were added. The mixture was heated under reflux for several hours, cooled, and the crystalline solid filtered off and recrystallized from toluene. The yield was 12 g. (46.5%), m.p. 147-148°.

N-(p-Nitrophenyl)-S-phenylthiolcarbamate.--Phenylthiocarbonyl chloride (1.72 g., 0.01 mole) and 2.76 g. (0.02 mole) p-nitroaniline were dissolved in 10 ml. dry dioxane, and the solution was allowed to stand for 1 1/2 hours with frequent

shaking. The mixture was poured into 100 ml. of ice water, and the precipitated solid was filtered off, the product being rubbed to induce crystallization if necessary. The yellow crystals were washed with water, 1 M aqueous hydrochloric acid, and then again with water, and after crystallization from aqueous ethanol, melted at 142-143°. The yield was 2.52 g. (92%).

The results of microanalysis of this preparation were unsatisfactory, but recrystallization from toluene instead of aqueous ethanol yielded yellow needles, m.p. 142.2-142.8°, for which the analysis was acceptable.

Anal. Calcd. for $C_{13}H_{10}N_2O_3S$ (274.3): C,56.9; H,3.7; N,10.2.

Found: C,56.9; H,3.7; N,10.1.

Reaction of p-Nitrophenyl Isocyanate with N-methylaniline. --para-Nitrophenyl isocyanate (Eastman practical grade)(0.8 g.) was heated with 3 ml. dry carbon tetrachloride on an oil bath until the solvent boiled. The resulting mixture was filtered while hot through a sintered glass plate, and 0.5 g. N-methylaniline was added to the yellow filtrate. The solution was again refluxed for 1 hour, cooled, the pale yellow precipitate centrifuged down, and the supernatant decanted. The residue was heated with 50% aqueous ethanol, filtered hot, and allowed to crystallize, m.p. 122-123°.

Reaction of N-(p-Nitrophenyl)-S-phenylthiolcarbamate with N-Methylaniline.--N-(p-Nitrophenyl)-S-phenylthiolcar-

bamate (0.27g., 0.001 mole) was dissolved in 3 ml. of dry dioxane and 0.5 g. N-methylaniline and 5 drops of triethylamine were added. After standing at room temperature for 30 minutes, the mixture was poured into 50 ml. ice-cold 1 N aqueous hydrochloric acid, the solid precipitate filtered off, washed with water, and recrystallized from aqueous ethanol. The compound formed yellow-green needles, m.p. 122-123°. The melting point of a mixture of this urea and that from the previous preparation remained undepressed.

Identical results were obtained when the thiolcarbamate and amine were heated in dioxane to 100° for 30 minutes in the presence of a few drops of pyridine, but heating in the presence of triethylamine gave a yellowish crystalline solid, m.p. 218°, which was not further investigated.

Table 1

N-Monosubstituted S-Phenylthiolcarbamates[a,b].

($R_1HNCOSC_6H_5$)

R_1	M.P. °C	% C		% H		% N	
		Calcd.	Found	Calcd.	Found	Calcd.	Found
Methyl	103-104	57.5	58.2	5.4	5.6	8.4	7.9
Ethyl	81-82	59.6	59.8	6.1	6.2	7.7	7.7
Cyclohexyl	113-113.5	66.4	66.4	7.3	7.3	6.0	5.9
Phenyl	125.5-126.	68.2	68.2	4.9	4.8	6.1	6.1
p-Chlorophenyl	141-141.5	59.4	59.4	3.8	3.8	5.3	5.3
p-Ethoxyphenyl	144.5-145	65.9	66.0	5.5	5.5	5.1	5.0
p-Nitrophenyl	142.2-142.8	56.9	56.9	3.7	3.7	10.2	10.1
o-Carbomethoxyphenyl	117.5-118.3	62.7	62.8	4.6	4.6	4.9	4.9
α-Pyridyl	151.2-152	62.6	62.7	4.4	4.4	12.2	12.1

a. Batty and coworkers (British Patents 599178 and 599179; C. A., 42, 7331, 7332 (1948)) appear to have prepared a number of N-substituted S-phenyl- and S-(p-tolyl)thiolcarbamates, but detailed information concerning their synthesis and properties is not available.

b. Several N-disubstituted S-phenylthiolcarbamates were also prepared, i.e., N-methyl-N-phenyl-S-phenylthiolcarbamate (1,3) and N,N-pentamethylene-S-phenylthiolcarbamate, m.p. 60-60.5°, anal. calcd. for $C_{12}H_{15}ONS$: C, 65.2; H, 6.8; N, 6.3: found: C, 65.2; H, 6.7; N, 6.2.

Table 2

Ureas Prepared from N-Substituted S-Phenylthiolcarbamates

Urea	M. P. °C Found	Lit.	Ref.
N-n-Butyl	94-95	96	4
N-Phenyl	144.6-145.6	147	5
N-(p-Anisyl)	166-167	164-165	6
N-(p-Phenetyl)	160-161	160-161	6
N-(p-Nitrophenyl)	237-238	238	7
N-Phenyl-N'-methyl	145-146	151	8
N-Phenyl-N'-allyl	115.5-116	115.5	9
N-Phenyl-N'-n-butyl	130-131	130	8
N,N'-Diphenyl	238-239	237-238	10
N-Phenyl-N'-(p-chlorophenyl)	237-238	237-238	11
N-Phenyl-N'-(p-nitrophenyl)	211-212	212	12
N-Phenyl-N'-(α-pyridyl)	185-186	187	13
N-Phenyl-N',N'-dimethyl	127.5-128	134	14
N-Phenyl-N',N'-diethyl	84-85	85	15
N-Phenyl-N',N'-tetramethylene	133-134	133-134	16
N-Phenyl-N',N'-pentamethylene	171-172	171-172	16
N-Phenyl-N',N'-dicyclohexyl	167-168	169	17
N-(p-Phenetyl)-N'-n-butyl	145-146	146	8
N-(p-Chlorophenyl)-N'-n-butyl	171-172	173	8
N-(α-Pyridyl)-N'-n-butyl	87-88	88	8
N-Methyl-N,N'-diphenyl	104-105	104	18
N-Methyl-N-phenyl-N'-(p-nitrophenyl)	122-123	123	19
Imidazole-N-carboxylic acid anilide [a]	114-115	114.5-115.5	16

a. In these cases a more convenient alternative nomenclature has been used to describe the urea.

Table 2 Cont'd.

Urea	M. P. °C Found	Lit.	Ref.
Indole-N-carboxylic acid anilide[a]	135-136	136	16
Tetrahydroquinoline-N-carboxylic acid anilide[a]	96-97	96	16
Morpholine-N-carboxylic acid anilide[a]	161-162	161.5-162	16
2-Hydroxybenzimidazole[a]	310	310-312	20
N-Phenyl-N'-benzoyl	210-211	210	21
1,4-Diphenylsemicarbazide[a]	177.8-178.5	177	22
N,N'-Diphenylthio	153-154	154	23

a. In these cases a more convenient alternative nomenclature has been used to describe the urea.

References

1. W.H. Schuller and C.G. Niemann, J.A.C.S., 75, 3425(1953).
2. R.Riemschneider and O. Lorenz, Monatsh., 84, 518(1953).
3. H. Rivier, Bull.Soc.chim., [4] 1, 733(1907).
4. T.L. Davis and K.C. Blanchard, J.A.C.S., 51, 1790(1929).
5. idem., ibid., 45, 1816(1923).
6. P.P.T. Sah and K. Chang, Ber., 69B, 2762(1936).
7. R. Stolle and F. Henke-Stark, J. prakt. Chem., [2], 124, 261(1930).
8. J.W. Boehmer, Rec. trav. chim., 55, 379(1936).
9. A.E. Dixon, J. Chem. Soc., 67, 556(1895).
10. J.R. Scott and J.B. Cohen, ibid., 121, 2034(1922).
11. H. Goldschmidt and B. Bardach, Ber., 25, 1347(1892).
12. R. Leuckart, J. prackt. Chem., [2], 41, 301(1890).
13. K. Feist, W. Awe, J. Schultz and F. Klatt, Arch. Pharm., 272, 100(1934).
14. R. Stolle, J. prakt. Chem., [2], 117, 185(1927).
15. W. Gebhardt, Ber., 17, 3033(1884).
16. R.A. Henry and W.M. Dehn, J.A.C.S., 71, 2297(1949).
17. P. Sabatier and J.B. Sendersens, Ann. chim., [8], 4, 319(1905).
18. W. Gebhardt, Ber., 17, 2088(1884).
19. P.P.T. Sah, Rec. trav. chim., 59, 231(1940).
20. S.V. Niementowaki, Ber., 43, 3012(1910).

References Cont'd.

21. H. Lakra and F.B. Dains, J.A.C.S., 51, 2220(1929).

22. M. Busch and O. Limpach, Ber., 44, 560(1911).

23. W. Autenrieth and N. Hefner, ibid., 58, 2151(1925).

24. E.D. Hughes and C.K. Ingold, Trans. Faraday Soc., 37, 657(1941).

25. M.L. Dhar, E.D. Hughes, C.K. Ingold, A.M.M. Mandour, G.A. Maw and L.I. Woolf, J. Chem. Soc., 2093(1948).

26. N. Kornblum and H.E. De La Mare, J.A.C.S., 73, 880(1951).

27. M. Gordon, J.G. Miller, and A.R. Day, J.A.C.S., 70, 1946(1948).

28. R. Baltzly, I.M. Berger, and A.A. Rothstein, ibid., 72, 4149(1950).

29. R. Schwyzer, Helv. Chim. Acta, 36, 414(1953).

30. E. Fischer, and A. Dilthey, Ber., 35, 844(1902).

31. H.L. Wheeler and B. Barnes, Am. Chem. J., 24, 60(1900).

32. H.L. Wheeler and T.B. Johnson, ibid., 24, 185(1900); 26, 408(1901).

33. W.H. Davies, J. Chem. Soc., 1951, 1357.

34. P.P.T. Sah, et. al., J. Chinese Chem. Soc., 3, 137(1935).

35. T. Curtius and W. Ulmer, J. prakt. Chem., 125, 23(1930).

36. W. Siefken, Ann., 562, 75(1949); S. Olsen and E. Enkemeyer, Chem. Ber., 81, 359(1948).

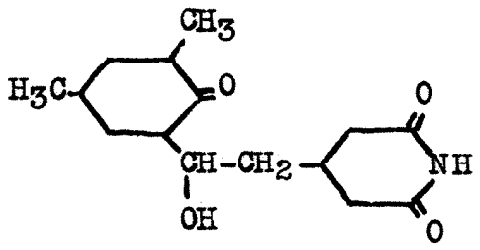
37. N.D. Cheronis and J.B. Entrikin, "Semimicro Qualitative Organic Analysis," T.Y. Crowell Co., New York, N.Y., 1947.

38. D.A. Shirley, "Preparation of Organic Intermediates," John Wiley and Sons, Inc., New York, N.Y., 1951.

PART IV. STUDIES ON THE SYNTHESIS OF ACTIDIONE.

A. Introduction.

In 1947, Ford and Leach of the Upjohn Research Laboratories reported the isolation from Streptomyces griseus of a new antibiotic substance which did not appear to belong to the streptomycin group (1), and following a rather exhaustive study of the chemistry of this compound, Kornfeld and a group at the Eli Lilly Laboratories postulated its structure to be (I) (2).



(I)

The name arose from an erroneous suggestion that the substance contained two keto groups, and the alternative name of "Cycloheximide" has been proposed as being less ambiguous.

Actidione has several interesting and valuable properties. It is very active against yeasts and many fungi, but almost without effect on other microorganisms, and this peculiar characteristic makes the compound suitable for soil fumigation where destruction of beneficial bacteria is undesirable. The lethal effect upon a number of wheat smuts has suggested an enormous value as a seed fumigant (3).

Actidione is a powerful rodent repellant, and has been used effectively to protect sacks and boxes from entry and destruction by rodents. Since plants and seeds appear

unaffected by low concentrations of the antibiotic, it is possible that it might find use in seed protection, particularly in applications such as reforestation where destruction of seeds by rodents is a major problem.

Whiffen (4) has obtained evidence that the biosyntheses of both actidione and streptomycin by S. griseus are based upon the same substrate, and that formation of one is accomplished at the expense of the other. Thus, by blocking the synthesis of actidione at some intermediate stage, it might be possible to increase the yield of the valuable streptomycin considerably.

Unfortunately, the utility of actidione is diminished by its high mammalian toxicity, and by its vesicant action. Although its closely related derivatives have no biological activity (2), structural analogs might conceivably be found which would retain some useful features but be less dangerous to handle. Before such analogs could reasonably be considered, it was felt necessary to confirm the proposed actidione structure by synthesis, and such confirmation was the chief goal of the present study.

Although the synthesis of actidione has not been achieved, much ground work has been laid for an eventual successful preparation of the antibiotic.

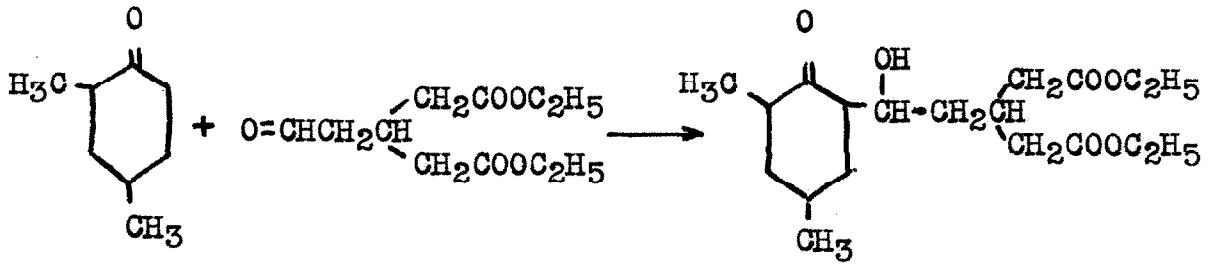
Studies on the synthesis of actidione were divided into three separate stages: (1) The formation of the actidione skeleton, consisting of a methylated cyclanone ring to which

an appropriate side-chain was attached; (2) necessary refinements on this skeleton to yield some derivative of actidionic acid, whose ester is (III), and (3) closure of the sensitive glutarimide ring. Each stage will be treated separately in an appropriate section of this thesis. Because of the presumption that the starting materials involved in any actual synthesis of actidione would be difficult to obtain, most of the determination of synthetic route and reaction conditions has been conducted with model compounds designed to combine the important reactive features of the original substance with comparative ease of preparation. Wherever possible, known and well-characterized compounds have been employed as models, and in such cases, a minimum of analysis and structure proof has been performed; the reader is referred to the original sources where such data may be found.

B. Synthesis of the Actidione Skeleton.

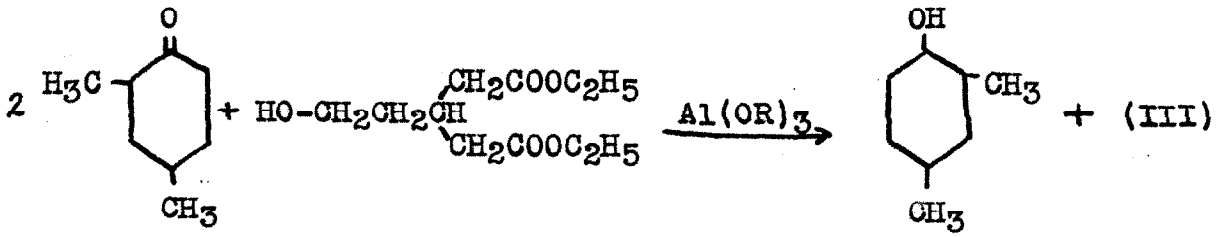
1. An Approach Through the Oppenauer Reaction.

A method of synthesis was first proposed which would permit the direct formation of actidionic ester (III), from which actidione could readily be formed. Since the hydroxyl group in formula (I) is in β-position with respect to the carbonyl group, it seemed reasonable to assume that a β-substituted glutaric acid derivative and the dimethylcyclohexanone ring might be joined by means of an aldol type condensation, as shown below.



(II) (III)

Although the evident instability of the free aldehyde (II) (2) precluded its use, considerable evidence is available (5) to indicate that such a compound might be prepared by use of the Oppenauer reaction with coincident condensation of the product with the ketone employed as oxidizing agent:



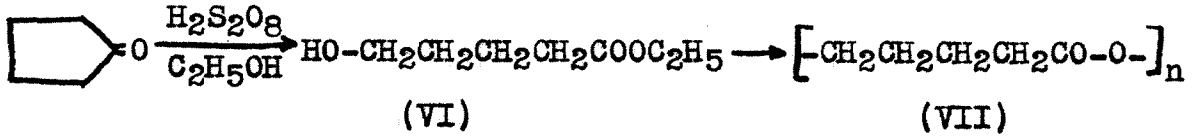
(IV) (V)

A satisfactory synthesis of 2,4-dimethylcyclohexanone starting with 2,4-xylenol has recently been described (2), and it is possible that the carbinol (V) might be prepared from β-ethoxypropionaldehyde by the series of reactions indicated on p. 130 of this thesis.

It was decided that the combination Oppenauer-condensation reaction should be tested with model compounds, due to the difficulty involved in obtaining the intermediates (IV)

and (V). For this purpose, 2-methylcyclohexanone (X) was prepared in good yield by oxidation of the commercially-available 2-methylcyclohexanol with sulfuric acid and sodium dichromate, and the product was purified by conversion to its sodium bisulfite compound, recrystallization, and regeneration of the ketone by steam distillation.

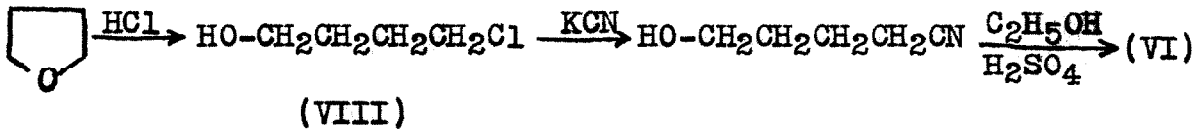
Attempts to prepare ethyl γ-hydroxybutyrate by alcoholysis of γ-butyrolactone were unsuccessful, so the next higher homolog was prepared as a model of the hydroxy ester (V). Oxidation of cyclopentanone with persulfuric acid in ethanol according to the method of Büchi and Jeger (6) gave the desired ethyl δ-hydroxyvalerate, but upon distillation *in vacuo*, the ester decomposed to give a white solid, m.p. 58.8-59.2°, apparently a polyester (VII).



Fichter and Beisswenger (7) have described the thermal polymerization of δ-valerolactone to a solid polyester, m.p. 47-48°, which has the same appearance and chemical properties as does our compound, and which gives the same results upon analysis. Van Natta, *et al.*, (8) have studied the polymers of ε-caprolactone, and have shown that both a cyclic dimer (m.p. 111-113°) and a true polymer (m.p. 53-56°) are formed, which may account for the discrepancy between the melting point of our polymer and that reported in the literature.

Upon warming (VII) with absolute ethanol and concentrated sulfuric acid, and carefully freeing the product from traces of acid, a sample of ethyl δ-hydroxyvalerate was obtained which did not decompose upon distillation or storage.

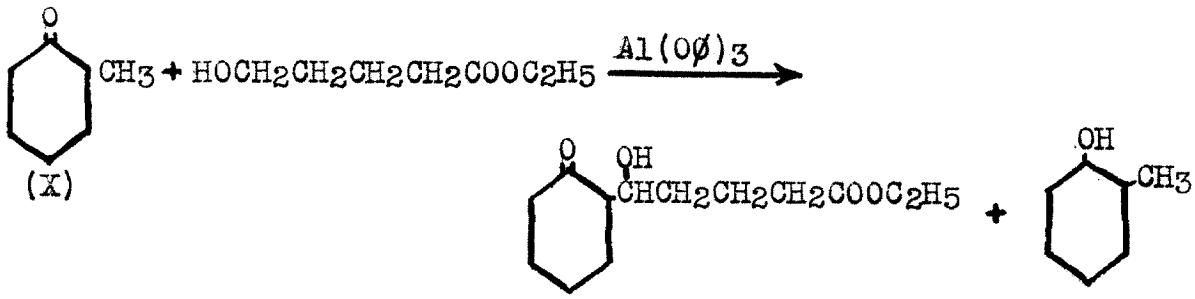
The ester was also prepared by the following sequence of reactions, starting with tetrahydrofuran:



(VIII)

The final product was again stable when entirely free from acid, and was identical with that obtained from cyclopentanone.

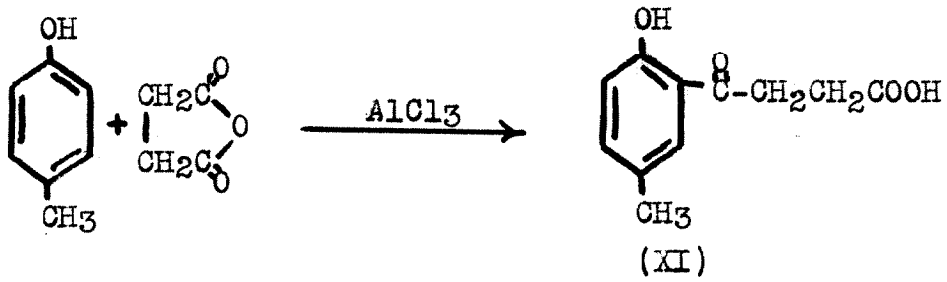
An examination of the possible catalysts for the Oppenauer reaction showed that best results might be expected from the use of aluminum phenoxide (IX), subsequently prepared from metallic aluminum and phenol by the method of Fuchs and Reichstein (9). Upon reaction of ethyl δ-hydroxyvalerate, 2-methylcyclohexanone, and aluminum phenoxide in hot toluene, a dark solution was obtained from which 2-methylcyclohexanol and unreacted 2-methylcyclohexanone were isolated by distillation. The residual tar could not be resolved; apparently, the aldehyde ~~(III)~~ was formed to some extent but perhaps was too unstable to be useful. When the reactants were allowed to stand at room temperature, no 2-methylcyclohexanol was isolated, the original ketone being recovered intact.



Failure of the model compounds to undergo the desired reaction discouraged more complicated attempts along the same line, and the method was abandoned.

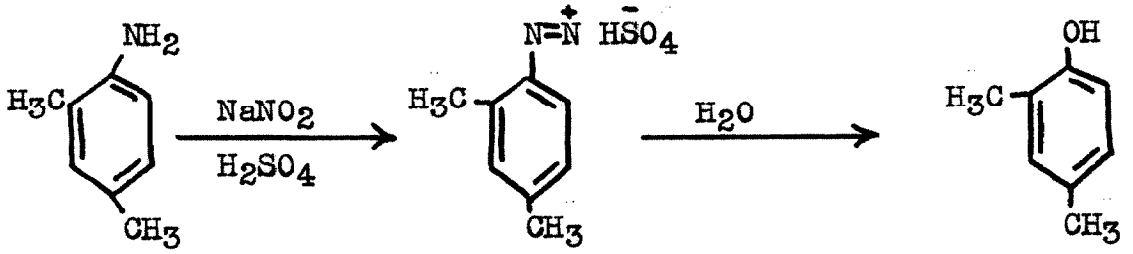
2. An Approach Through the Friedel-Crafts Reaction.

A possible route to the actidione skeleton lay in a Friedel-Crafts reaction of 2,4-xylenol with some derivative of methanetriacetic acid (XVI) to give a dibasic aromatic keto acid. As a model for such a synthesis, p-cresol was acylated by succinic anhydride in the presence of anhydrous aluminum chloride to give β-(2-hydroxy-5-methylbenzoyl)propionic acid:

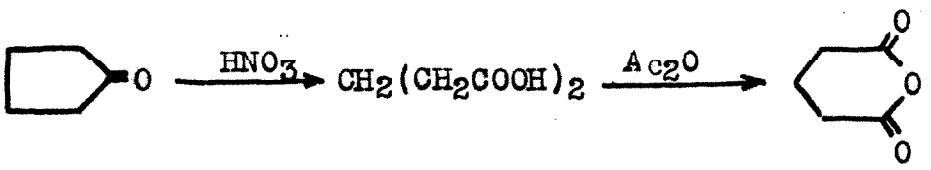


As a further model, and in order to prepare a closer analog of the actidione skeleton, it was proposed to prepare γ-(2-hydroxy-3,5-dimethylbenzoyl)butyric acid (XV) from glutaric anhydride and 2,4-xylenol. Cyclopentanone was

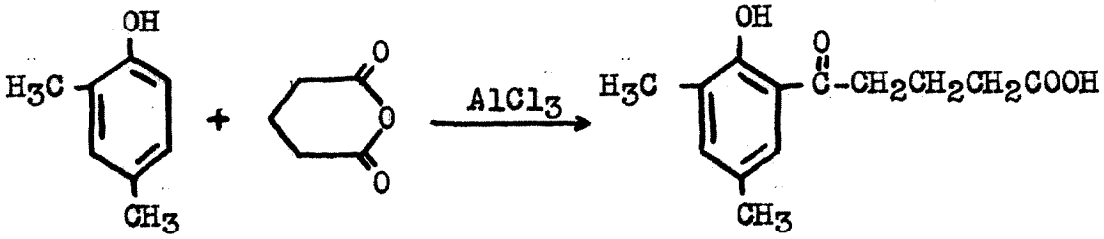
oxidized by nitric acid to yield glutaric acid, and this was converted to the anhydride by boiling with acetic anhydride. The xylenol was prepared from 2,4-dimethylaniline by the diazo reaction, and from it the keto acid was obtained in the same way as was the analogous compound (XI). The reactions leading to the formation of (XV) are summarized below:



(XII)

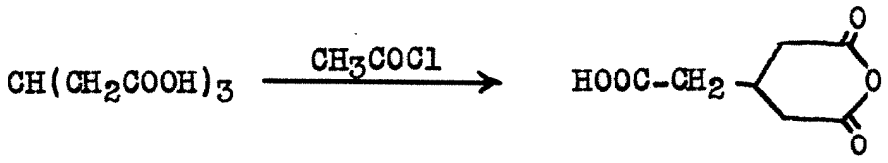


(XIII) (XIV)

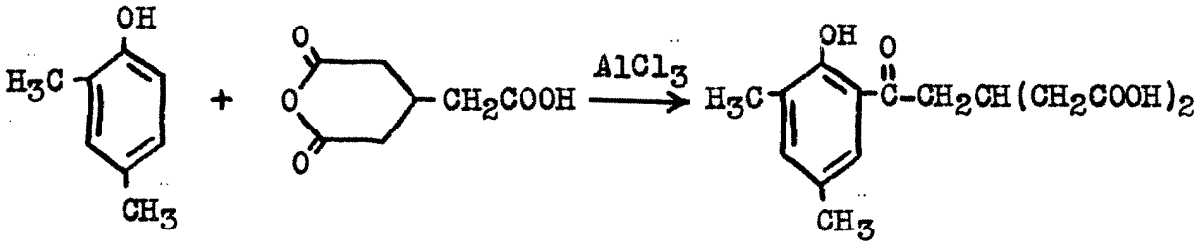


(XV)

In order to continue along this line of research, it was next necessary to prepare methanetriacetic acid. A small quantity of the substance was obtained after considerable difficulty (<u>vide post</u>), converted to its anhydride by prolonged boiling with acetyl chloride, and the gummy product was allowed to react with 2,4-xylenol in the presence of anhydrous aluminum chloride, as shown below:



(XVI) (XVII)



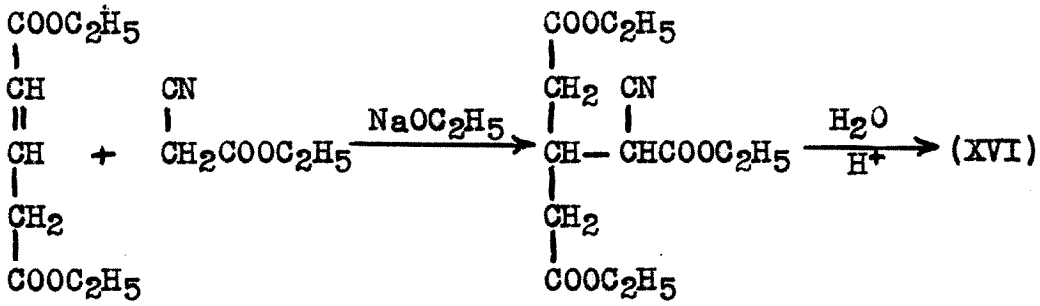
A dark resinous substance was obtained which was soluble in alkali, but which could not be decolorized in alkaline solution by Norite or by sulfur dioxide. Upon heating with a large volume of water, it dissolved and the solution was successfully decolorized by Norite, but neither continuous ether extraction nor final evaporation to dryness yielded any organic residue.

Although insufficient methanetriacetic acid was available for another attempt at the synthesis, it is possible that further experiments of this type might produce satisfactory results.

3. The Methanetriacetic Acid Problem.

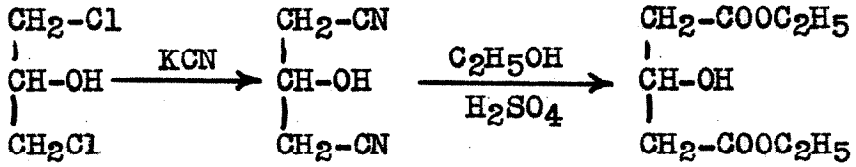
Of the several methods reported in the literature for the synthesis of methanetriacetic acid (XVI), that of Kohler and Reid (10) was chosen as the most satisfactory. According to these investigators, ethyl glutaconate undergoes a Michael condensation with a cyanoacetic ester in the presence of

sodium ethylate to form the cyano ester (XVIII), which may be hydrolyzed to yield the desired acid.



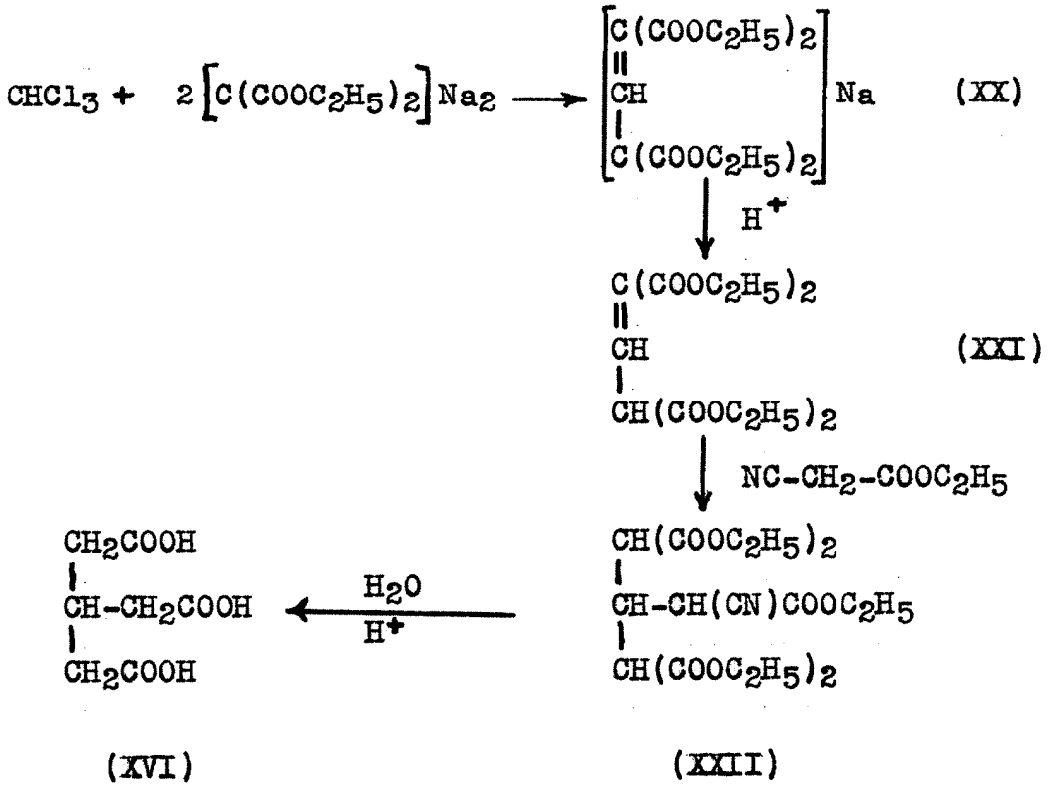
(XVIII)

The glutaconic ester may be obtained by dehydration of the corresponding β-hydroxyglutaric ester, and this in turn may be prepared from glycerol α,γ-dichlorohydrin by the following reactions (12):



(XIX)

Several attempts to obtain the ester in this way were unsuccessful, and it was decided to abandon the method in favor of that suggested by Ingold and Perrin (11). Reaction of sodiomalonic ester with chloroform produced the sodium derivative of α,α′-dicarbethoxyglutaconic ester, from which the free ester was liberated by acidification. Although glutaconic acid could be obtained by hydrolysis at this point, prolonged reaction of the tetraester with ethyl cyanoacetate gave a pentaester (XXII) related to (XVIII), from which methanetriacetic acid was obtained by hydrolysis.



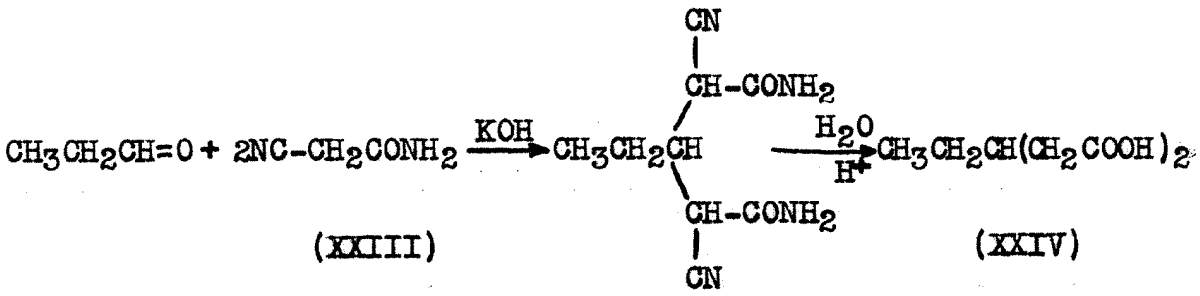
Addition of the sodiomalonic ester to the chloroform, rather than the standard reversed procedure did not lead to $\alpha, \alpha', \alpha''$-tricarbethoxymethanetriacetic ester, as might be expected from a simple malonic ester synthesis (cf., p. 158).

Although time-consuming, the method of Ingold and Perrin afforded the desired acid in 18% overall yield.

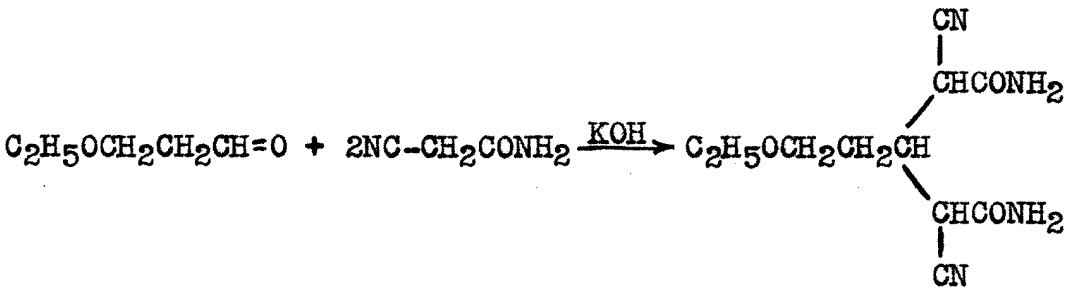
The remaining synthetic method recorded in the literature (12) was examined (cf., p. 177) and was found to be inaccurately reported. According to Ingold and Nickolls (13) ethyl β-bromoglutarate, prepared from the corresponding hydroxy ester, was allowed to react with sodioacetoacetic ester, followed by hydrolysis to give methanetriacetic acid. Inaccurate description of the properties of intermediate substances led to such low yields that the synthesis was terminated

with the isolation of the bromo ester, the use of which is described later in this thesis.

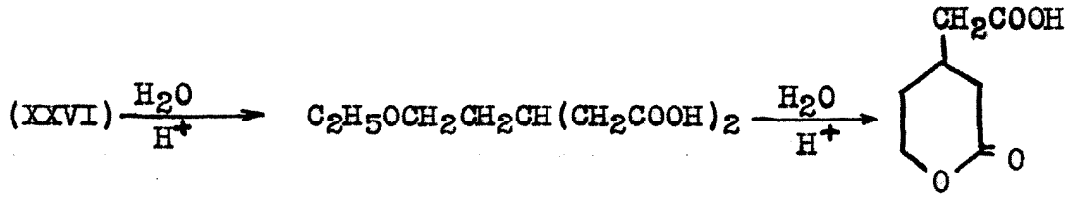
Due to the interest in methanetriacetic acid for this investigation, a study was conducted to determine the possibility of devising a novel method for its synthesis. Attention was primarily directed toward the use of condensation reactions, particularly the Knoevenagel method and its modifications. Jeffry and Vogel (14) found that a wide variety of aldehydes and ketones could be condensed with cyanoacetamide in alkaline medium to produce solid intermediates which yielded β-substituted glutaric acids upon hydrolysis. The reaction was carried out starting with propionaldehyde, and was found to proceed smoothly.



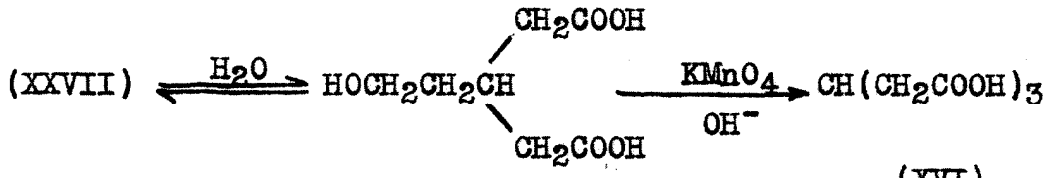
The proposed route to methanetriacetic acid involved such a condensation of β-ethoxypropionaldehyde with cyanoacetamide or a similar compound containing reactive hydrogens, followed by hydrolysis of the resulting intermediate to 2-ketotetrahydropyran-4-acetic acid and oxidation of this to the desired product with alkaline potassium permanganate solution. The last step would utilize the stability of methanetriacetic acid toward oxidation (11).



(XXV) (XXVI)

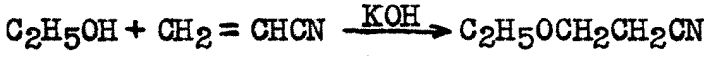


(XXVII)

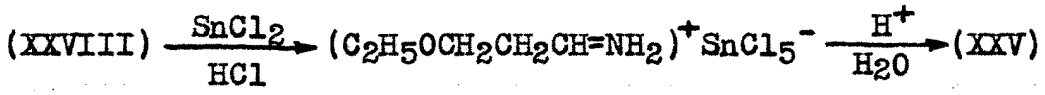


(XVI)

β-Ethoxypropionaldehyde was prepared by the Stephen reaction, starting with β-ethoxypropionitrile obtained from ethanol and acrylonitrile. The corresponding acetal was prepared from ethanol and acrolein in the presence of hydrogen chloride.



(XXVIII)



$$C_2H_5OH + CH_2{=}CHCH{=}O \xrightarrow{H^+} C_2H_5OCH_2CH_2CH(OC_2H_5)_2$$

(XXIX)

Cyanoacetamide was obtained by ammonolysis of ethyl cyanoacetate with aqueous ammonia.

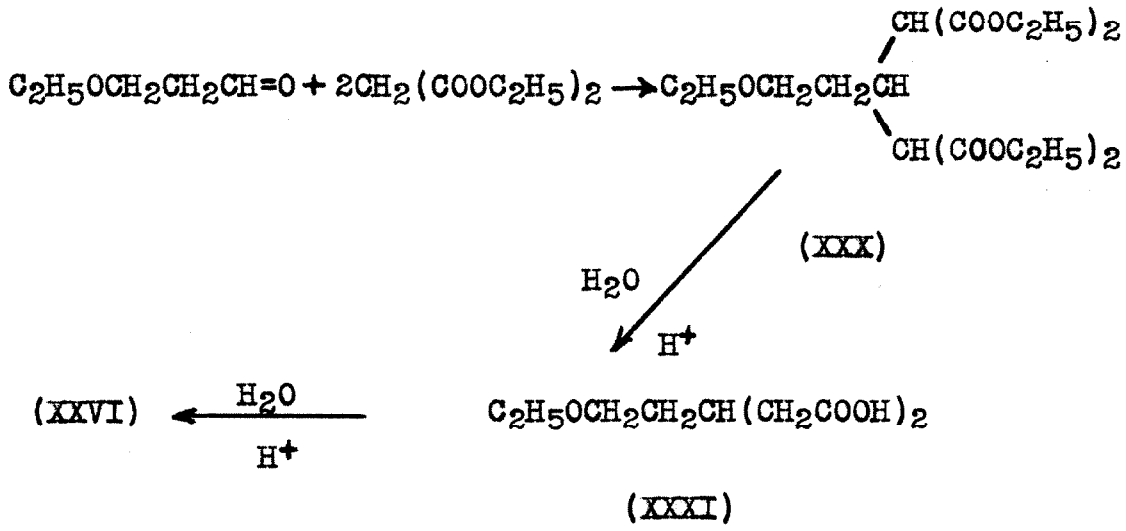
$$NC\text{-}CH_2COOC_2H_5 + NH_3 \longrightarrow NC\text{-}CH_2CONH_2 + C_2H_5OH$$

Many attempts to carry out the condensation of the aldehyde and amide invariably resulted in low yields, as indicated in Table 1. Similarly, the Guareschi reaction (16), usually a satisfactory procedure, gave only a small amount of the intermediate (XXVI) in this case.

$$C_2H_5OCH_2CH_2CH{=}O + 2NC\text{-}CH_2COOC_2H_5 + NH_3 \longrightarrow (XXVI)$$

The condensation of β-ethoxypropionaldehyde with ethyl malonate in the presence of piperidine gave rather low yields of the appropriate tetra ester (XXX), and the isolation of the product was made difficult by its high boiling point, 200° at 1.5 mm. pressure, and by its high viscosity. Although apparently satisfactory yields of the crude ester were obtained on each attempt at its preparation, the contents of the boiler invariably set to a black carbonaceous solid before much distillate could be collected, no matter how carefully heat was applied.

Alkaline hydrolysis of both the Guareschi base (XXVI) and the above ester (XXX) led to β-(2-ethoxyethyl)glutaric acid (XXXI), as did hydrolysis with sulfuric acid.

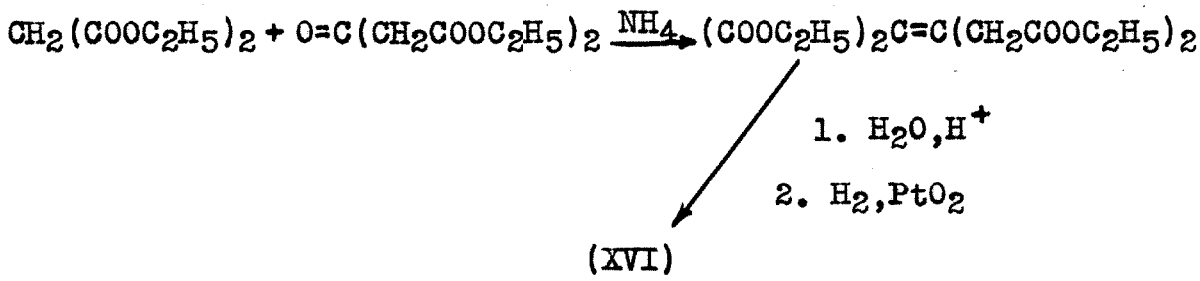


Hydrolysis by boiling with either aqueous hydriodic or hydrobromic acids produced only a trace of an acidic substance which was not identified. However, when (XXX) was heated to 150-190° in a sealed tube with concentrated hydrochloric acid, the desired 2-ketotetrahydropyran-4-acetic acid (XXVII) was obtained (15).

The final quantity of the lactone (XXVII) was so small that oxidation to methanetriacetic acid was not attempted. It was apparent that the condensation reactions involving β-ethoxypropionaldehyde which were investigated were impractical for large-scale use, and actually offered no advantage over the methods already discussed for the preparation of methanetriacetic acid.

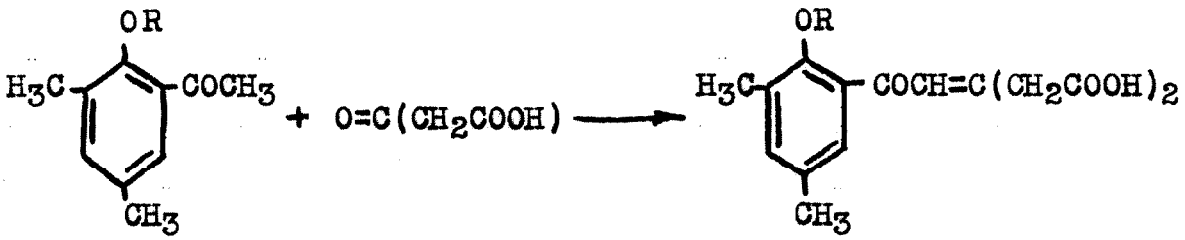
Still another reaction was attempted in this study. The conditions for the condensation of ketones with malonic acid and its derivatives have been thoroughly studied and standardized, and in a recent investigation (17) acetone was employed

in a variety of such reactions with uniformly high yields. However, utilization of ethyl acetonedicarboxylate in an identical manner did not produce any sign of a reaction. The reason for this is entirely obscure.



4. Approaches Through 2-Hydroxy-3,5-dimethylacetophenone.

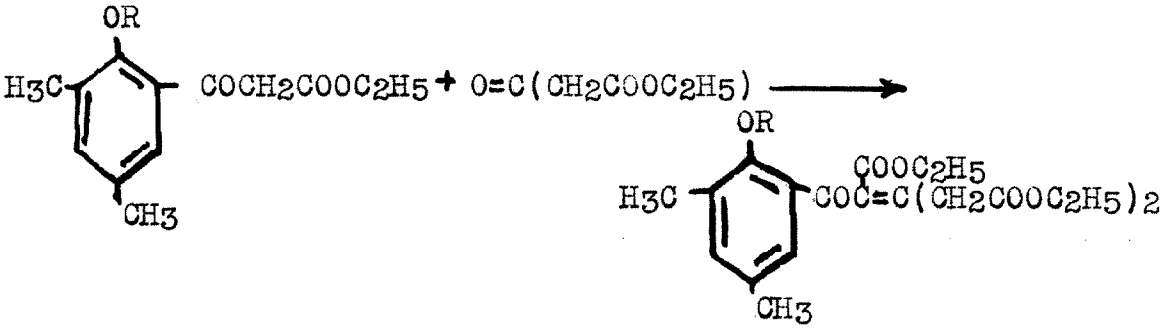
The ease with which many aldehydes and ketones condense with acetophenone suggested that a substituted acetophenone might react with acetonedicarboxylic acid to produce a compound having the actidione skeleton:



Although acetonedicarboxylic acid has been found to condense in the presence of sulfuric acid with many compounds containing active hydrogens (18), no reaction with acetophenone was observed to take place. Similarly, use of alkaline conditions under which many carbonyl compounds have been shown to react with acetophenone gave negative results. However, the usual

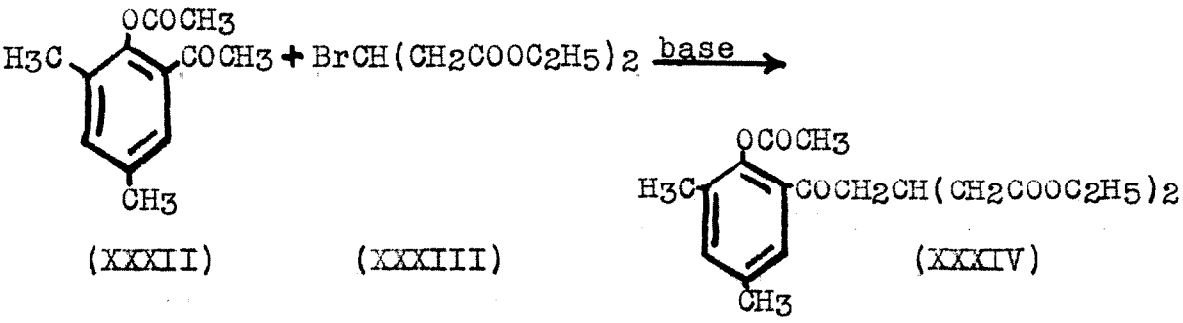
reactivity of the α-hydrogens of such a ketone led to further speculation about its application to the actidione problem.

An approach was considered which involved synthesis of a benzoylacetic ester followed by a Knoevenagel Condensation with ethyl acetonedicarboxylate.



The complete unreactivity of the acetonedicarboxylic ester with ethyl malonate under standardized conditions discouraged further work along this line.

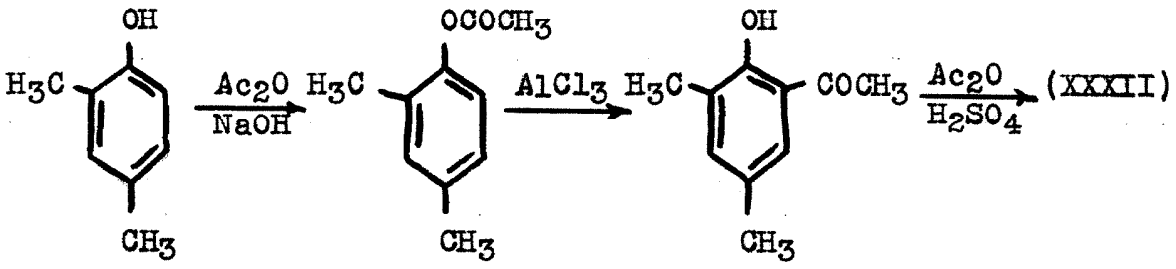
Another attempt to obtain the actidione skeleton was based on observations of Claisen (19), Freer and Lachman (20), and Haller and Ramart-Lucas (21) that acetophenone may be C-alkylated by means of organic halides under conditions similar to those of the Darzens reaction. It was proposed that 2-acetoxy-3,5-dimethyl acetophenone should be alkylated by ethyl β-bromoglutarate in the presence of a base according to the equation below.



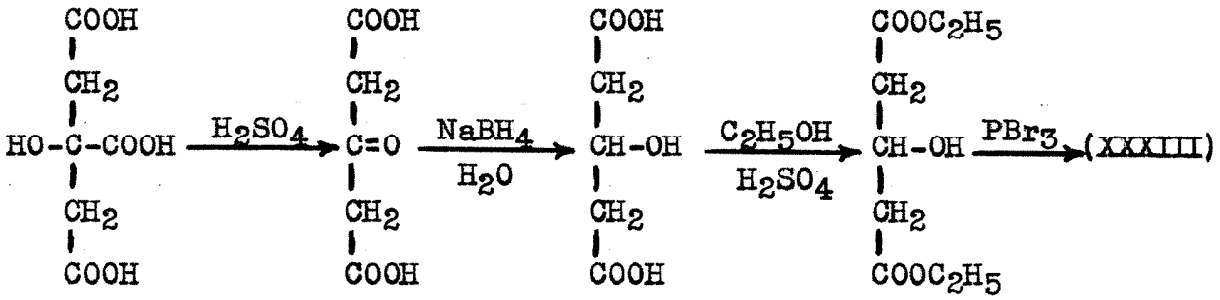
(XXXII) (XXXIII) (XXXIV)

Sodium methoxide was chosen as the base upon recommendation of Newman and Magerlein (22).

The necessary bromoester was prepared from citric acid by conversion into acetonedicarboxylic acid, reduction of this with sodium borohydride to β-hydroxyglutaric acid, esterification, and reaction of the hydroxyester with phosphorus tribromide. The acetophenone derivative used in the reaction was prepared by acetylating 2,4-dimethylphenol, rearranging the ester to a methyl ketone, and again acetylating. The series of reactions used in preparation of the starting materials are summarized by the following schemes:



(XXXV)



(XXXVI) (XIX)

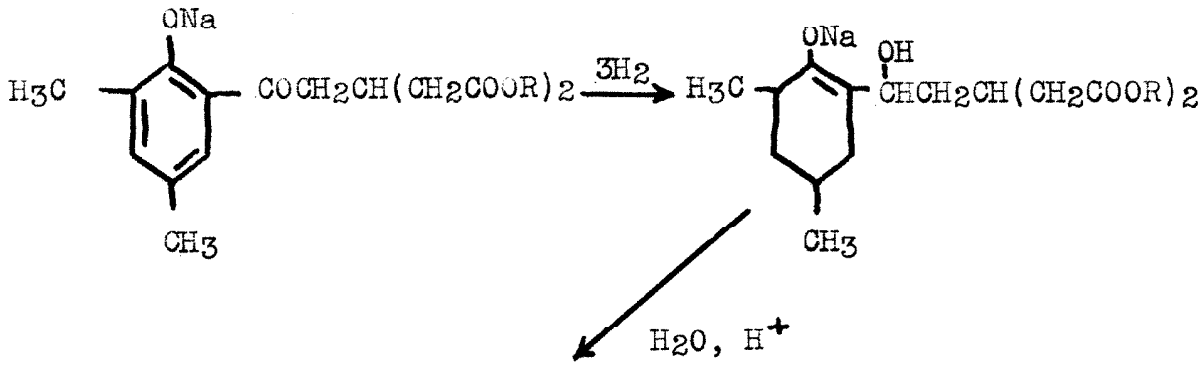
The reaction of the acetoxyacetophenone with the bromoester was conducted in purified toluene in the presence of freshly prepared sodium methylate, and produced a black tar

from which a considerable quantity of oily fore-run was distilled *in vacuo*. The residue was heated to 150° at 2 mm. pressure, the dark non-volatile substance taken up in ethanol, and the solvent removed *in vacuo*. Upon addition of ligroin, a crystalline product melting at 153-154° separated. This substance gave a precipitate with dinitrophenylhydrazine reagent, but analysis of a sample recrystallized twice from ether showed that it was not the expected product, and due to the small quantity available, no further manipulation was attempted.

C. Modification of the Actidione Skeleton.

1. Hydrogenation.

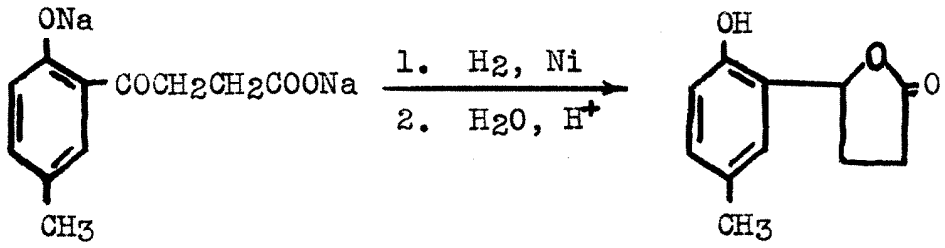
Formation of the actidione skeleton by either the Friedel-Crafts method or through 2-hydroxy-3,5-dimethylacetophenone as shown in the preceding section of this thesis would result in the formation of an aromatic keto acid or its derivative, and in order to obtain an acetidionic acid derivative (XXXVII) it would be necessary to saturate the intermediate with hydrogen at several points. Ideally, the aromatic ring could be partially saturated by the method of Thompson (23) to yield a cyclohexanone directly, while the side-chain keto group could be reduced simultaneously to an alcohol.



(XXXVII)

Complete saturation of the aromatic ring would lead to a derivative of dihydroactidionic acid, which might be converted to actidionic acid as shown later in this section.

Attempts to convert the model keto acid (XI) into a substituted cyclohexanone were unsuccessful, and in each case an aromatic lactone was obtained as the only product.



(XI) (XXXVIII)

(XXXVIII) proved to be identical with the lactone formed by reduction of (XI) with sodium borohydride, a reagent which appears to be incapable of saturating an aromatic nucleus.

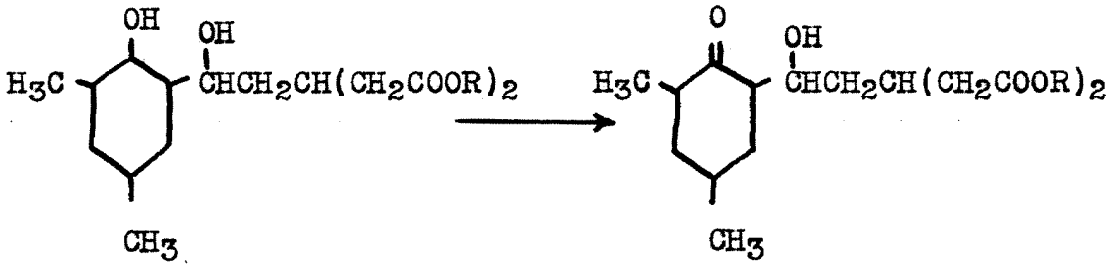
Similarly, repeated attempts to saturate the aromatic ring of (XI) under conditions which would not reduce the

lactone to an alcohol, or the intermediate hydroxyl group to a methylene group were entirely unsuccessful, although conditions were employed under which phenols previously had been converted to cyclohexanols with ease.

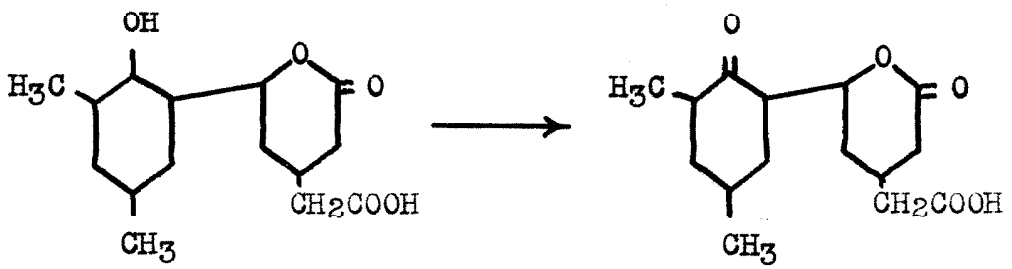
The entire study was hampered by lack of suitable equipment for catalytic hydrogenation at either high or low pressure, and it is certain that once this difficulty is overcome, a satisfactory method for conversion of the aromatic actidione skeleton into a suitable relative of actiononic acid will present itself.

2. Oxidation.

In the event that only dihydroactidionic acid could be obtained, it would be necessary to devise a satisfactory method for its conversion into actidionic acid without oxidation of the side chain hydroxyl group.



Although the two hydroxyl groups of the dihydro compound should be almost identical chemically, there is good evidence (2) that when R=H, the side chain hydroxyl will lactonize preferentially, allowing oxidation of the ring hydroxyl as long as the medium is kept acidic.



(XXXIX)

It was hoped that the keto acid (XI) could be completely hydrogenated to furnish a model for use in the determination of suitable conditions for the oxidative step, but this end was not realized. However, from the experience gained in the oxidation of 2-methylcyclohexanol and cyclopentanone, it was concluded that the use of sodium dichromate and acid might be entirely satisfactory for this purpose.

D. Formation of the Glutarimide Ring.

Regardless of the route by which the actidione skeleton was formed, it appeared that final formation of the glutarimide ring would involve manipulation of either a lactone such as (XXXIX) or a β-substituted glutaric ester.

Direct opening of γ-butyrolactone with either ammonium hydroxide or anhydrous ammonia proved unsuccessful, and as a consequence, a method was developed which employed the readily obtainable corresponding sodium salt as starting material. Using glutaric acid derivatives, it was shown that following the opening of the lactone ring with aqueous sodium hydroxide solution to form a disodium salt, addition of silver nitrate

solution caused precipitation of the insoluble silver salt, which upon shaking with aqueous ammonium iodide was converted to the diammonium salt with separation of silver iodide. Distillation of a solution of the ammonium salt to dryness yielded the desired glutarimide. The following is a summary of the steps involved in the above transformation.

$$\text{lactone} \xrightarrow[\text{NaOH}]{H_2O} R\text{-}CH(CH_2COONa) \xrightarrow{Ag^+} R\text{-}CH(CH_2COOAg) \xrightarrow{NH_4I}$$

$$R\text{-}CH(CH_2COONH_4)_2 \longrightarrow R\text{-}\langle\text{glutarimide ring}\rangle$$

The use of metal salts other than those of silver proved unsatisfactory (cf., p. 161).

Glutarimide was also prepared by a method which involved heating of glutaric acid or a substituted glutaric acid to 200° with urea. However, it appears that this procedure will not be applicable to the present study of actidione.

An attempt was made to ammonolyze ethyl glutarate to either glutarimide or glutaramide, but the low yield and prolonged reaction time caused the method to be unsatisfactory.

E. Experiments.

All melting points are corrected unless marked otherwise. Microanalyses were performed by Dr. A. Elek.

Attempted Preparation of Ethyl γ-Hydroxybutyrate.--γ-Butyrolactone (15.2 ml., 0.20 moles) was dissolved in 50

ml. of absolute ethanol saturated with dry hydrogen chloride. After standing at room temperature for 2 days, the mixture was heated under reflux for 4.5 hours, shaken with dilute aqueous sodium carbonate solution, and dried over anhydrous magnesium sulfate. Distillation gave 16 g. (61%) of a colorless liquid, b.p. 129.0-130.6°/105 mm., 94.8-95.2°/22 mm., n_D^{25} 1.4337.

Anal. Calc'd for $C_6H_{12}O_3$: C,54.5; H,9.1.
Calc'd for $C_4H_6O_2$: C,55.8; H,7.0.
Found: C,52.6; H,6.7.

The substance smells like butyrolactone, and the literature value for the b.p. of this compound is 94°/20 mm., n_D^{25} 1.4343.

In a second experiment, the above procedure was repeated with substitution of 1 ml. of concentrated sulfuric acid for the hydrogen chloride. Distillation at 5 mm. pressure gave 2.2 ml. of a liquid boiling at 67-70°, and 8.0 ml. boiling at 77-84° and having a pronounced pineapple odor. However, neither fraction gave a positive test with ceric nitrate reagent, and neither reacted with phenyl isocyanate. Pure γ-butyrolactone boils at 89°/12 mm. or 84°/ 5 mm.; the b.p. of ethyl γ-hydroxybutyrate has not definitely been reported (24).

Ethyl δ-Hydroxyvalerate (VI). (Method I).--Ammonium persulfate (230 g., 1.0 mole) was stirred with a mixture of 130 ml. of water and 390 ml. concentrated sulfuric acid at room temperature, and the mixture was cooled in a bath of

ice and concentrated hydrochloric acid. Ordinary ethanol (500 ml.) was added slowly dropwise, the temperature lowered to -5°, and a solution of 50 g. (0.6 mole) of cyclopentanone in 130 ml. of ethanol was added dropwise over a period of 2 hours, while the temperature of the mixture was kept below 0°. After addition of all the ketone, stirring at -5° was continued for 20 hours to give a homogeneous solution, 3 l. of half-saturated ammonium sulfate solution was run in at such a rate that the temperature remained below 15°, and the mixture was allowed to stand at 4° overnight.

After continuous extraction with ether for 30 hours and removal of solvent, the product was fractionally distilled in vacuo to yield 46 g. (77%) of colorless oil, b.p. 90°/5 mm., which immediately solidified to a crystalline wax, m.p. 58.8-59.2°.

The solid did not give a ferric hydroxamate test for the ester group, did not react with phenyl isocyanate, and did not dissolve in aqueous-alcoholic potassium hydroxide solution. It was insoluble in water, but readily soluble in organic solvents.

Anal. Calc'd for $C_7H_{14}O_3$: C,57.5; H,9.6.
Calc'd for $C_5H_8O_2$: C,59.8; H,8.1.
Found : C,59.2; H,8.1.

The solid polyester (30 g.) was dissolved in 50 ml. of absolute ethanol, 2 ml. concentrated sulfuric acid were added, and the mixture was heated under reflux for 4 hours, poured

over ice, the resulting solution was extracted with ether, and the extract was washed with dilute aqueous sodium bicarbonate solution, dried over anhydrous magnesium sulfate, and the solvent was removed on a steam bath. Distillation gave a colorless liquid, b.p. 110°/5 mm., yield 12 g.. The residue solidified upon cooling.

A small quantity of liquid ester was heated on a steam bath for 1 hour with 1 ml. 100% hydrazine hydrate and sufficient ethanol to give a homogeneous solution. Excess hydrazine hydrate and alcohol were removed in vacuo by warming on a steam bath, the residual oil cooled in dry ice and scratched until crystals appeared, and finally the solid was recrystallized from ethanol by addition of ether, m.p. 105-106°. The hydrazide is very soluble in water, and care must be taken to insure complete removal of all volatile matter before crystallization is attempted. A preliminary experiment in which water was not entirely removed was unsuccessful. Robinson and Smith (25) report identical values for both the b.p. of the ester and m.p. of the hydrazide.

Tetramethylene Chlorohydrin (VIII).-- The method employed is that of Starr and Hixon (26). Sodium-dried tetrahydrofuran (163 ml., 2.0 moles) was heated under refluxing conditions while dry hydrogen chloride was introduced until the temperature of the mixture was 100° (about 6 hours). After standing for several days, excess tetrahydrofuran was distilled off at 100 mm. pressure, and the residue was

fractionally distilled *in vacuo* through an 8" Vigreux column and distillation head. Almost all of the crude compound distilled at 73.5-74.0°/8 mm., yield 105 g. (70%). About 45 g. of unreacted tetrahydrofuran was recovered.

Ethyl δ-Hydroxyvalerate (Method II).-- Potassium cyanide (39.0 g., 0.60 mole) was dissolved in 50 ml. of water, and 175 ml. of 95% ethanol was added. The mixture was heated under refluxing conditions, mechanically stirred, and 54.3 g. (0.50 mole) tetramethylene chlorohydrin were added dropwise over a period of 2 hours. The dark solution was then heated to reflux temperature for an additional 8 hours and poured into 1 l. of water. The product was extracted with two 100 ml. portions of chloroform, and the extract was decolorized with Norite, dried over anhydrous magnesium sulfate, and freed of solvent on a steam bath.

The residual oil was dissolved in 115 ml. of absolute ethanol, 50 ml. concentrated sulfuric acid were added, and the mixture was heated under refluxing conditions for 6 hours, allowed to cool, and poured over ice. After extraction with three 100 ml. portions of ether, washing with dilute aqueous sodium carbonate, drying over anhydrous magnesium sulfate, and removal of solvent on a steam bath, the remaining oil was distilled *in vacuo*, b.p. 107-110°/5 mm., yield, 21 g. (29%).

The hydrazide of the corresponding acid was prepared in the manner indicated above, m.p. 105-106°, and when mixed

with a sample of the hydrazide prepared previously, the m.p. was not depressed.

Aluminum Phenoxide (IX).--Granulated aluminum (3 g., 0.11 atom), 50 g. (0.53 mole) of colorless crystalline phenol, a crystal of iodine, and 50 mg. of mercuric chloride were heated on a steam bath with exclusion of moisture until a vigorous reaction commenced. External heat was removed, and after about 10 minutes, the liquid began to solidify. Dry, redistilled benzene (100 ml.) was added, and the mixture was boiled under refluxing conditions for 1 hour, 300 ml. additional benzene were added, and boiling was continued for 2.5 hours.

The resulting mixture was filtered while hot, the volume reduced rapidly to 200 ml. in vacuo, and the hot, green solution was diluted with 200 ml. of 60-70° ligroin, allowed to cool somewhat, and an additional 150 ml. of ligroin were added. After standing at 4° for several days, the white, crystalline product was carefully filtered off as quickly as possible, washed with 50 ml. of ligroin, and freed of solvent by placing in a vacuum desiccator and exhausting through a dry ice trap for several hours. The final, slightly gray product was stored in a glass stoppered bottle in a desiccator, yield 30 g. (89%).

2-Methylcyclohexanone (X).--Sodium dichromate (160 g., 0.52 mole) was placed in a 2 l. flask fitted with a stirrer, thermometer well, and dropping funnel, and 750 ml. of water

were added. Stirring was commenced and 72 ml. (1.35 moles) of concentrated sulfuric acid were poured in. Eastman practical grade 2-methylcyclohexanol (100 ml., 0.80 mole) was added dropwise over a period of 20 minutes, while the temperature of the mixture was held at 55-65°, the temperature was lowered to 35° over a period of 1.5 hours, and the dark solution allowed to stand for 20 hours. Ether (250 ml.) was added, and the mixture was shaken in a separatory funnel. The organic layer was then washed with four 125 ml. portions of 5% aqueous sodium hydroxide solution followed by 200 ml. of water, 100 ml. additional ether were added to break the resulting strong emulsion, and the combined washings were again extracted with 200 ml. of ether.

The combined ethereal extracts were placed in a 1-l. wide-mouthed bottle, and the solvent was removed on a steam bath. A saturated solution of sodium bisulfite was freshly prepared by heating a mixture of 220 g. of sodium bisulfite and 400 ml. of water, cooling to room temperature, and allowing attainment of equilibrium by standing overnight. This solution was filtered into the bottle containing the ketone, mechanically shaken for 7 hours, and allowed to stand for 3 days. The pearly white flakes were filtered off, washed with 30 ml. of saturated sodium bisulfite solution, pressed free of moisture, washed well with 100 ml. of ether, and finally sucked dry and pressed on filter paper. The yield in this preparation amounted to 135 g. (71%); a second afforded a yield of 91%.

Upon adding 100 g. of the bisulfite addition compound to a mixture of 110 ml. of 15% aqueous sodium hydroxide solution and 70 ml. of water, followed by distillation with steam, a biphasic distillate was obtained. This was half-saturated with sodium chloride, extracted with three 50 ml. portions of ether, and the combined extracts were dried over anhydrous magnesium sulfate. Distillation gave 28 g. of pure 2-methylcyclohexanone, b.p. 163-166°, corresponding to a yield of 53% based on the amount of addition compound used.

The dinitrophenyl hydrazone of the ketone was prepared by the method of Shriner and Fuson (27), m.p. 136-137° (lit. 137°).

Reaction of 2-Methylcyclohexanone with Ethyl δ-Hydroxyvalerate.--2-Methylcyclohexanone (5.6 g., 0.05 mole), ethyl δ-hydroxyvalerate (7.3 g., 0.05 mole), and 15 g. of aluminum phenoxide were mixed with 100 ml. of dry toluene and heated to boiling for 1 hour, cooled, and transferred to an apparatus for steam distillation. Steam was passed into the mixture until about 300 ml. of distillate had been collected, the dark residue was cooled in ice and extracted with 200 ml. of ether, the ethereal solution washed with water, dried over magnesium sulfate, and the solvent was removed *in vacuo* at room temperature. An attempt to distill the dark tarry residue did not result in any distillate, although the temperature of the boiler was raised to 160°/1 mm., and no solid

could be induced to form, even on prolonged storage at 4° with frequent vigorous rubbing.

The organic layer from the steam distillation was separated and dried over magnesium sulfate. Slow fractional distillation through a 14" heated column packed with glass helices and fitted with a good still head allowed recovery of the toluene (b.p. 110-111°); the residue was transferred to a small flask fitted with a 5" semimicro Vigreux column and still head, and careful distillation resulted in isolation of 5.2 g. of liquid boiling at 162-165°, amounting to 93% recovery. The product gave an immediate precipitate with dinitrophenylhydrazine reagent.

The colorless liquid was dissolved in 10 ml. of ether and shaken with 10 ml. of a saturated aqueous solution of sodium bisulfite for 2 hours, the solid filtered off and washed with ether, and the filtrate again shaken after addition of 2 g. of pure sodium bisulfite. The small amount of solid was again separated by filtration, and the resulting filtrate was extracted with 50 ml. of ether, the extract dried over magnesium sulfate, and the solvent was removed on a steam bath after addition of 1 g. of α-naphthyl isocyanate. The solid residue was recrystallized from carbon tetrachloride to give 1.3 g. (9.2%) of 2-methylcyclohexanol α-naphthylurethane, m.p. 153-155°.

An authentic sample prepared from 2-methylcyclohexanol did not depress the m.p..

The experiment was conducted again in an identical manner, with the exception that the suspension in toluene was allowed to stand at room temperature for 5 days with frequent shaking. The residue after steam distillation was almost colorless, and the dried ethereal extract of this yielded only a minute quantity of oily residue after removal of solvent on a steam bath.

Distillation of the dried organic layer from the steam distillate gave 5.5 g. (98%) of a liquid boiling at 57-60°/20 mm. which gave a precipitate with dinitrophenylhydrazine reagent, followed by 4 g. (55%) of ethyl δ-hydroxyvalerate, b.p. 105-110°/5 mm.. The residue in the boiler solidified upon cooling to give ca. 3 g. (41%) of the polyester for a total recovery of ca. 96%. No attempt was made to resolve the low-boiling ketone fraction.

β-(2-Hydroxy-5-methyl)benzoylpropionic Acid (XI).--Succinic anhydride (21.5 g., 0.215 mole), p-cresol (25 g., 0.23 mole), and 100 ml. of dry tetrachloroethane were stirred together in a three-necked flask fitted with a reflux condenser, sealed stirrer, and powder dispenser, while 60 g. (0.45 mole) of finely powdered anhydrous aluminum chloride were added in small portions over a period of 30 minutes. The temperature did not rise above 40°. After stirring for 1 hour, the temperature was raised to 130° for 2.5 hours, the mixture allowed to cool, and 50 ml. of water were added slowly, followed by 140 ml. of concentrated hydrochloric acid.

The yellow mixture was steam-distilled until no more volatile substances distilled (ca. 500 ml. of distillate), the residue was cooled to 10°, and the precipitated solid was isolated by filtration.

In order to purify the crude acid, it was dissolved in hot, dilute aqueous sodium carbonate solution, filtered free from insoluble particles, and finally acidified and the precipitated acid collected. The yield was 25 g. (56% based on the succinic anhydride used).

The keto acid was too soluble in ethanol to permit recrystallization from this solvent, and difficulty was experienced in recovering the compound from aqueous ethanol, either due to excessive solubility or supersaturation. Water was found to be a satisfactory solvent although a large volume was required. The brown color of the crude acid was removed by boiling the acid aqueous solution with Norite; the color was not removed from alkaline or neutral solution even on repeated treatment. Recrystallization of 25 g. of the crude acid from 4 l. of boiling water, after treatment with Norite, gave 15 g. of pure, white plates, m.p. 133.5-134°.

A second preparation of β-(2-hydroxy-5-methyl)benzoylpropionic acid was carried out in the same manner as was the first, except that the crude product was crushed, mixed well with Norite, and the mixture was placed in an extraction thimble. Extraction with 1-l. of water in a Soxhlet apparatus gave a very efficient recrystallization, and after allowing

extraction to proceed for 30 hours, the white, crystalline solid was removed from the boiler and sucked dry. The yield was 15.6 g. (40%) of pure acid, m.p. 133.5-134°. Rosenmund and Shapiro (28) report a m.p. of 136-137° for this compound.

2,4-Xylenol (XII).--The method used here is an adaptation of that employed by Koelsch (29). Eastman Kodak Co. pure 4-amino-1,3-dimethylbenzene (85 g., 0.70 mole) was dissolved in a hot solution of 85 ml. (1.6 moles) of concentrated sulfuric acid in 600 ml. of water. After chilling in ice, a cold solution of 50 g. (0.725 mole) of sodium nitrite in 75 ml. of water was added in small portions with stirring followed by 3 g. of urea, and the mixture was allowed to stand for 10 minutes.

Portions of 100 ml. of the diazonium sulfate solution were added slowly to a boiling solution of 100 ml. concentrated sulfuric acid in 300 ml. of water, through which a rapid stream of steam was passing. After addition was completed (about 4 hours), about 2 l. of additional distillate were collected to make a total of about 4 l., this was half-saturated with sodium chloride, extracted with a total of 1-l. of ether in 150 ml. portions, the organic layer dried over anhydrous magnesium sulfate, and solvent removed on a steam bath.

Fractional distillation through a 5" Vigreux column fitted with a distillation head gave 65.3 g. (76.5%) of a liquid, b.p. 109-114°/27 mm., having a very strong phenolic odor. Reaction with phenyl isocyanate according to the

method of Shriner and Fuson (27) gave a phenylurethane, m.p. 111-112° (lit. 112°).

Glutaric Acid (XIII).--Dilute nitric acid (d. 1.20, 720 g.) was heated almost to boiling in a three-necked flask equipped with a condenser, thermometer well, and a dropping funnel, and containing several porous chips. A few drops of cyclopentanone were added, and as soon as the vigorous reaction became established, 100 g. (1.19 moles) of cyclopentanone were added dropwise at a rate of 2 drops/sec., the last 30 ml. being added while external heat was applied to the oxidizing mixture. The nitric acid and water were then distilled off until the volume was only about 100 ml., the red solution was transferred to an evaporating dish, and more solvent was removed by prolonged heating on a steam bath. Although the mixture partly crystallized, attempts to remove the last traces of nitric acid by extraction of the organic material with ethyl acetate, benzene, or ether were unsuccessful. When removal of the nitric acid by washing with ice water was tried, the glutaric acid also dissolved almost entirely.

Vacuum distillation proved successful for the isolation of the glutaric acid; following a fore-run of water and nitric acid, the pure substance distilled at 185-195°/10 mm., yield 85 g. (64%), m.p. 92-95°.

Glutaric Anhydride (XIV).--This substance was prepared by the method of Cason and Rappoport (30). Glutaric acid (11 g., 0.083 mole) was mixed with 50 ml. of pure acetic

anhydride and the solution was heated under reflux for 10 hours, transferred to a distillation apparatus, and distilled *in vacuo*. Following a fore-run of acetic acid and acetic anhydride, glutaric anhydride distilled at 160-165°/20 mm., yield 9 g. (95%).

The product was recrystallized by dissolving in warm benzene and adding a four-fold amount of absolute ether, chilling, and washing the crystals with cold ether, m.p. 55-56°.

γ-(2-Hydroxy-3,5-dimethyl)benzoylbutyric Acid (XV).--Glutaric anhydride (10.0 g., 0.088 mole) was dissolved in 100 ml. of tetrachloroethane, 10.8 ml. (0.092 mole) of 2,4-xylenol were added, and the mixture was stirred vigorously while 26 g. (0.2 mole) fresh, anhydrous aluminum chloride were introduced slowly over a period of 45 minutes at a temperature below 40°. After stirring for 1.5 hours, the mixture was heated to 130-140° for 3 hours with vigorous stirring, allowed to cool, and 50 ml. of water were added dropwise, followed by 50 ml. concentrated hydrochloric acid.

After standing overnight, the yellow mixture was steam distilled until no more volatile matter could be detected in the distillate. Cooling the residue in ice caused the precipitated yellow oil to solidify. The solid product was dissolved in 100 ml. of 10% aqueous sodium carbonate solution by heating, the solution was filtered hot, cooled, and concentrated hydrochloric acid was added with stirring until the

supernatant solution was strongly acid. The yellowish precipitate was filtered, washed with water, and sucked dry to yield 6.0 g. (29% based on the glutaric anhydride used).

A small sample was dissolved in boiling water, the solution decolorized with Norite, filtered hot, and the acid allowed to crystallize. Like β-(2-hydroxy-5-methyl)benzoylpropionic acid, Norite is ineffective for decolorization of alkaline solutions of this compound.

The product was obtained as white leaflets, m.p. 110-111°.

Anal. Calc'd for $C_{13}H_{16}O_4$: N.E.,236.

Found: N.E.,236.

Methanetriacetic Anhydride (XVII).--Semicrystalline methanetriacetic acid (ca. 5 g.) was heated to reflux for 8 hours with 20 ml. of freshly-distilled acetyl chloride, the dark mixture was allowed to stand overnight, acetic acid and unreacted acetyl chloride were distilled off, 15 ml. of fresh acetyl chloride were added and distilled off, and the process was repeated. The residue was then heated to 100° under vacuum until the odor of acetic acid no longer was detectable (12 hours), and the gummy residue (4.6 g.) was dissolved in 50 ml. of purified tetrachloroethane and used at once for the next step.

The anhydride was described in Ingold (31) as a viscous gum, which was not obtained in a crystalline form despite repeated attempts. However, the substance was obtained in a sufficiently pure state for analysis, the results of which were in agreement with the expected values.

Reaction of Methanetriacetic Anhydride with 2,4-Xylenol.--

Pure 2,4-xylenol (4.0 g., 0.033 mole) was added to the solution of 4.6 g. (0.027 mole) methanetriacetic anhydride in 50 ml. of tetrachloroethane, and finely-powdered, anhydrous aluminum chloride (15 g., 0.11 mole) was added slowly with vigorous stirring over a period of 1 hour. The warm mixture was heated to 60° for 2 hours, then to 140° for 1 hour, when it became so thick that it could not be stirred.

While still hot, the dark sludge was poured into a mixture of 50 ml. of concentrated hydrochloric acid and 300 g. of ice, steam-distilled until no more volatile material could be detected in the distillate, and the residue allowed to cool. The dark oil which had precipitated during the distillation with steam solidified upon cooling, and after standing overnight at 4°, the solid was filtered off and dried in a vacuum desiccator. The yield was 3.0 g. of a dark, brittle solid.

The product was found to be insoluble in water, but soluble in dilute aqueous sodium carbonate solution, and in organic solvents. Although the solutions in organic solvents such as acetic acid, toluene, and acetone could not be decolorized with Norite, repeated boiling of an alkaline aqueous solution with the agent finally removed most of the color.

The colorless alkaline solution was acidified to pH 2 with dilute aqueous sulfuric acid, and continuously extracted for 60 hours with ether. Upon drying the ethereal solution with magnesium sulfate and removal of the solvent under

reduced pressure, no residue remained. The extracted aqueous solution was carefully evaporated to dryness, the dry solid residue was boiled with 100 ml. of ethanol, and the solvent was removed on a warm water bath under an inert atmosphere. Again, there was no residue.

A reaction appears to have taken place between the anhydride and the xylenol, and it is possible that the successive treatments with decolorizing charcoal eventually removed all of the product from solution; the Norite was discarded after use without any attempt at elution being made.

Reaction of Glycerol α,γ-Dichlorhydrin with Potassium Cyanide.--Glycerol α,γ-dichlorhydrin (69 g., 0.54 mole) was dissolved in 100 ml. of methanol in a three-necked flask, and the solution was heated to boiling under refluxing conditions. A solution of 70 g. of crystalline potassium cyanide in 80 ml. of water was added dropwise over a period of 30 minutes with vigorous mechanical stirring, the mixture was stirred and boiled for 1 hour, cooled in ice, and filtered. The dark filtrate was evaporated *in vacuo* on a Glas-col heater, and subsequently was overheated and decomposed. The potassium chloride remaining on the filter weighed 61 g., corresponding to 76% yield.

A second experiment was conducted in an identical manner, with removal of solvent *in vacuo* on a steam bath. The dark brown residue was dissolved in 100 ml. of ethanol, the solution was filtered, and 40 g. of concentrated sulfuric acid were added. After boiling under refluxing conditions

for 4 hours, the mixture was cooled, poured over ice, and the resulting solution was extracted with 200 ml. of ether, the extract dried over magnesium sulfate, and ether was removed by distillation, leaving only a small residue which was not manipulated further.

Much later experience showed that the directions of Dreifuss and Ingold, from which these preparations were made, were in error concerning the properties and solubility of the resulting ethyl β-hydroxyglutarate (cf., p. 177).

Sodium Ethyl α,α′-Dicarbethoxyglutaconate (XX).--Freshly-distilled ethyl malonate (40 g., 0.25 mole) was added dropwise to the hot solution resulting from the reaction of 11.5 g. (0.5 atom) of clean metallic sodium with 250 ml. of absolute ethanol which had been distilled from sodium directly into the reaction flask. Redistilled, dry chloroform (15 g., 0.12 mole) was then added dropwise over a period of 40 minutes with vigorous stirring, the resulting bright yellow, opaque mixture was boiled under refluxing conditions for 1 hour, filtered hot, and the residue was washed with 100 ml. of hot ethanol. Ligroin (50 ml.) was added to the hot, red solution, and it was allowed to cool.

After storage at 4° overnight, the bright lemon-yellow crystals were filtered off, washed with 25 ml. of cold ethanol, and sucked dry to yield 25 g. (59% based on chloroform) of the desired compound. Ingold and Perrin (11) report a 47% yield, with recovery of an additional 14% by concentration of the mother liquor; concentration of the mother liquor

in the above preparation yielded only a trace of additional product.

A second preparation in which twice the above quantities were employed gave a 49% yield, although the amount of sodium chloride which was recovered after filtration of the hot reaction mixture corresponded exactly to the stoichiometric weight.

Addition of Sodiomalonic Ester to Chloroform.--A major source of difficulty in the preparation of methanetriacetic acid from chloroform and sodiomalonic ester (cf., preceding experiment) lies in the dehydrohalogenation of some intermediate substance by the base during the course of the reaction. To circumvent this problem, it was proposed to maintain a constant excess of chloro-compound throughout the addition of the sodioester.

$$CHCl_3 + 3\ CH(COOC_2H_5)_2Na \rightarrow CH\left[CH(COOC_2H_5)_2\right]_3 + 3NaCl$$

For this purpose, 15 g. (0.12 mole) of chloroform were dissolved in 50 ml. of absolute ethanol in a flask fitted with a reflux condenser, sealed stirrer, and dropping funnel. Freshly distilled ethyl malonate (60 g., 0.37 mole) was dissolved in a solution of sodium ethylate prepared by addition of 8.5 g. (0.37 atom) of c.p. sodium metal to 200 ml. of absolute ethanol. Stirring was started, and the solution of the sodioester was run in over a period of 1 hour, the white slurry was boiled for 3 hours, and excess alcohol finally was distilled off.

The residue was shaken with dilute aqueous hydrochloric acid until the aqueous layer was acid, then washed with water, dried over anhydrous potassium carbonate, and distilled *in vacuo*. Only unchanged chloroform and malonic ester were recovered.

Ethyl α,α'-Dicarbethoxyglutaconate (XXI).--The sodium salt obtained from malonic ester and chloroform (53 g., 0.15 mole) was shaken in a Squibb funnel with 300 ml. of water and 50 ml. of concentrated hydrochloric acid, and 100 ml. of ether were added. After shaking for 15 minutes, the layers were separated, the organic phase was dried over magnesium sulfate, and the solvent was removed by distillation. The residual oil was then heated to 130° at 25 mm. pressure for 2 hours, cooled, and used directly in the next experiment.

Methanetriacetic Acid (XVI).--The above ester was mixed with 15 g. of freshly distilled ethyl cyanoacetate and 1.5 g. of piperidine, and the mixture was allowed to stand in a tightly-stoppered flask. After 10 days, an additional 2 g. of piperidine were added.

After a period of 6 weeks at 25°, the red mixture was shaken with dilute aqueous hydrochloric acid and ether, the ethereal solution was washed with water, dried over magnesium sulfate, and low-boiling compoments of the solution were distilled off at 135°/20 mm.. The residue was mixed with an equal volume (50 ml.) of concentrated sulfuric acid, allowed to stand at room temperature overnight, and after addition

of 25 ml. of water, the solution was boiled under refluxing conditions for 8 hours. An additional 50 ml. of water were added and the mixture was distilled, with frequent addition of water, until 300 ml. of distillate had been collected.

After dilution with 200 ml. of water, continuous extraction with ether for 3 days, and evaporation of the solvent, the residue was made alkaline with 2 M aqueous sodium carbonate solution, again extracted with ether, the residual aqueous solution boiled to free it of ether, and a solution of 6 g. of potassium permanganate in 100 ml. of water was added slowly with stirring. After 15 minutes, the mixture was chilled in ice, filtered, excess permanganate removed by careful addition of aqueous sodium bisulfite solution, the colorless solution acidified and heated to boiling, cooled, and continuously extracted with ether for 3 days.

Upon drying the ethereal extract over magnesium sulfate and removing the solvent by evaporation, a colorless crystalline slush was obtained, which yielded pure methanetriacetic acid, m.p. 119-121°, upon recrystallization from chloroform. The yield was 5 g. (18%).

Because of the high yields reported for the hydrolytic steps involved in this preparation, it was suspected that considerable loss of product might have occurred in the several solvent extractions required. In order to circumvent the use of extraction for isolation of methanetriacetic acid from its aqueous solutions, a method was developed by

which the substance could be precipitated specifically as its lead salt, with subsequent regeneration. Table 1 indicates the solubility of certain metallic salts of organic acids in water, from which it is apparent that methanetriacetic acid is easily precipitated as the lead salt while the barium salt remains in solution. Thus, the sulfuric acid solution resulting from hydrolysis of a substituted methanetriacetic acid could be freed of sulfate ion by precipitation with barium nitrate, followed by treatment of the sulfate-free solution with lead nitrate. After removal of the lead methanetriacetate, the free acid might be obtained by metathesis with hydrogen sulfide.

α,α'-Dicyano-β-ethylglutaramide.--Cyanoacetamide (16.8 g., 0.20 mole) was dissolved in 120 ml. of water by warming, the solution was cooled in ice, and 7.4 ml. (0.10 mole) of freshly redistilled propionaldehyde were added, followed by 10 ml. of ethanol and 5 drops of 50% aqueous sodium hydroxide solution.

After standing at 25° for 2 days, very little solid had separated but upon shaking, a white precipitate appeared, and within 3 hours the contents of the flask were solid. The product was filtered off, washed with cold water, and sucked dry. The yield was 14 g. (74%), m.p. 139-140°.

β-Ethylglutaric Acid (XXIV).-- α,α'-Dicyano-β-ethylglutaramide (3.0 g., 0.044 mole) was dissolved in a mixture of 4 g. of sodium hydroxide, 20 ml. of water, and 20 ml. of ethanol, and the solution was boiled under

Table 1.

Solubility of Metallic Salts of Carboxylic Acids in Water at 20°.

	Ion			
Acid	Ba^{++}	Zn^{++}	Pb^{++}	Ag^{+}
Succinic	- [a]	-	-	±
Glutaric	-	-	-	+
Adipic	-	-	±	-
Methanetriacetic	-	-	+	±

a. (-) indicates no formation of a precipitate upon addition of 1 ml. of 0.1 N aqueous solution of the ion to 15 ml. of 0.005 N acid solution, (±) indicates noticeable turbidity, (+) indicates immediate precipitation.

refluxing conditions for 5 hours until evolution of ammonia had ceased entirely. Concentrated sulfuric acid (8 ml.) was added slowly, accompanied by vigorous evolution of carbon dioxide, and the acid solution was boiled briefly, allowed to cool, and extracted with ether. After drying the ethereal extract over magnesium sulfate, the solvent was removed to give 2.3 g. (91%) of β-ethylglutaric acid, m.p. 71-72°. The literature value (14) is 73°.

Hydrolysis with 10% aqueous sulfuric acid followed by isolation in the manner described above gave the same product in 90% yield.

β-Ethoxypropionitrile (XXVIII).--Ethanol (95%) (19 ml., 0.3 mole) was dissolved in 15 ml. of 1 M aqueous sodium hydroxide solution, 19.6 ml. (0.3 mole) of freshly redistilled acrylonitrile were added, and the mixture was mechanically shaken for 1.5 hours. The phases were then separated, the aqueous layer washed with 40 ml. of ether, and the combined organic phases were dried over anhydrous magnesium sulfate.

Fractional distillation through an 8" Vigreux column gave 22 g. (73%) of pure nitrile, b.p. 170-172°. A second attempt gave only 60% yield, although the original description of the method (32) claimed a yield of 90%.

β-Ethoxypropionaldehyde (XXV).--Anhydrous stannous chloride was prepared by heating the pure dihydrate in a nickel crucible until the crystalline solid melted, then resolidified to a yellow powder. Upon continued heating, this powder melted also, whereupon it was allowed to cool and solidify, and was ground in a mortar.

The anhydrous salt was covered with 150 ml. of absolute ether, and the mixture was shaken vigorously while hydrogen chloride was bubbled in over a period of 40 minutes until two layers separated. Then 18 g. (0.18 mole) of β-ethoxypropionitrile were added, the mixture again vigorously shaken, and allowed to stand at 4° for 2 days. The colorless, crystalline solid was separated by filtration, washed with 50 ml. of cold ether, and heated to boiling under reflux with 100 ml. of water. After cooling spontaneously, the solution was extracted with two 100 ml. portions of ether, the ethereal extract dried, the solvent removed and the product distilled through a 12" helix-packed column with an ice-water cooled still head. The yield was 12 g. (65%), b.p. 135-140° (uncorr.).

β-Ethoxypropionaldehyde Acetal (XXIX).--This compound was prepared by the method of Pingert (33) from acrolein and ethanol in the presence of hydrogen chloride. The preparation was performed exactly as indicated with the exception that only half of the stated quantities was used. The final product was distilled through a 15" ring-packed column to yield 50 g. (20%) of colorless, fragrant acetal, b.p. 80-85°/21 mm..

Cyanoacetamide (XXIII).--The method employed in this synthesis was that of Corson, Scott, and Vose (34). Ethyl cyanoacetate (200 g., 1.77 moles) was added to 150 ml. of concentrated ammonium hydroxide in a wide-mouthed flask, and the mixture was shaken and allowed to stand for 10 minutes.

The warm, homogeneous solution was then cooled in an ice-salt freezing mixture for 1 hour, the solid was filtered, pressed, washed with two 25 ml. portions of ice-cold ethanol, and finally sucked dry.

The yield of snow-white powder, m.p. 119-120°, was 68%. Repetition of the synthesis gave a 62% yield; recrystallization from ethanol did not materially decrease the yield, but also did not improve the m.p..

Reaction of Cyanoacetamide with β-Ethoxypropionaldehyde.--Cyanoacetamide (8.4 g., 0.10 mole) was dissolved in 25 ml. of warm water, and the solution was cooled to room temperature. Freshly-distilled β-ethoxypropionaldehyde (4.0 ml., 0.036 mole) was added, followed by 0.5 ml. of 50% aqueous potassium hydroxide solution, and the mixture shaken. Heat was evolved.

After standing overnight, the flask contained a white solid and a yellow supernatent liquid. At the end of 8 days, the solid was separated by filtration, washed with 50 ml. of water, and sucked dry. The yield was 3.2 g (35%) of white crystalline powder, m.p. 218°.

This experiment produced the highest yield observed in the series of ten runs summarized in Table 2. The method is based on a known and previously satisfactory procedure (cf., p. 129 of this thesis), and consequently, efforts were extended to determine if possible the cause of the unsatisfactory yields.

Measurement of the solubility of the "Guareschi base" in the reaction medium was made by attempting to dissolve

Table 2

The Condensation of Cyanoacetamide with β-Ethoxypropionaldehyde

Run	Moles Aldehyde	Moles Amide	Solution Volume(ml.)	Catalyst	T°C	Time(hrs.)	Yield (%)	Remarks
1.	0.05	0.10	30	NaOH	25°	120	0	a
2.	0.10	0.20	95	KOH	25°	500	trace[b]	
3.	0.036	0.10	30	KOH	25°	200	35	
4.	0.045	0.11	55	KOH	50°	2	30	
5.	0.036	0.10	55	$C_5H_{11}N$	50°	20	trace	
6.	0.045	0.10	55	KOH	25°	5	18	Continuously shaken
7.	0.045	0.10	300	KOH	4°	150	16	
8.	0.031	0.062	45	$C_5H_{11}N$	25°	120	trace	
9.	0.01	0.10	30	Triton "B"	25°	150	trace	
10.	0.135	0.33	140	KOH	25°	50	18	Soln.kept alkaline

a. β-Ethoxypropionaldehyde acetal was hydrolyzed to the aldehyde immediately before use.

b. The product is assumed to be the usual "Guareschi base": $CH_3CH_2OCH_2CH_2CH(CHCONH_2)_2$ (CN).

50 mg. of the white solid in both the filtrate from an experiment which produced only a trace of product, and in a 10% aqueous solution of pure β-ethoxypropionaldehyde which contained 1% of potassium hydroxide. After shaking for 1 hour at 25°, the white insoluble residue was isolated by filtration, dried, and weighed. There was no loss, showing that the yield discrepancy was not due to incomplete precipitation. Evaporation of the filtrate from Run 7 *in vacuo* yielded a red oil, which partially crystallized upon standing at 4° for 1 week. The solid portion was separated by filtration, washed with cold ethanol, and sucked dry to yield 5 g. (60%) of unchanged cyanoacetamide, m.p. 116-118°.

Preparation of the Guareschi Base from β-Ethoxypropionaldehyde and Ethyl Cyanoacetate.--The method is that outlined by Vogel (16). A mixture of 55 g. (0.49 mole) of redistilled ethyl cyanoacetate and 25 g. (0.24 mole) of redistilled β-ethoxypropionaldehyde was cooled below 0°, and a saturated solution of dry ammonia in 100 ml. of absolute ethanol, also below 0°, was added all at once. A stopper was wired onto the bottle, and it was allowed to stand at 4° for 3 days. The yellowish solid was removed by filtration, washed with 50 ml. of cold absolute ethanol followed by 50 ml. of absolute ether, and the product was sucked dry. The yield was 16 g., m.p. 218° (d), corresponding to 28% of the theoretical quantity.

Ethyl α,α'-Dicarbethoxy-β-(2-ethoxyethyl)glutarate (XXVI).--Redistilled ethyl malonate (82 ml., 0.50 mole) and

redistilled β-ethoxypropionaldehyde (28 ml., 0.25 mole) were mixed, cooled in ice, and 6.0 ml. of diethylamine were added in four portions over a period of 1 hour at temperatures below 10°. After standing overnight, the mixture was boiled under refluxing conditions for 10 hours, cooled, washed with dilute aqueous hydrochloric acid, and 50 ml. of ether were added.

The organic phase was dried over anhydrous magnesium sulfate, and after removal of ether on a steam bath, the residue was fractionally distilled in vacuo through a 3" insulated Vigreux column fitted with a standard distillation adapter whose vapor path must be at least 15 mm. in diameter. Following a fore-run of malonic ester, a substance distilled which was assumed to be ethyl α-carbethoxy-β-(2-ethoxyethyl) crotonate, b.p. 174-180°/1.3 mm., followed by the desired product. During the final stage of distillation, the temperature of the boiler was regulated very carefully to avoid overheating, which caused extensive charring and polymerization.

The ester was obtained as a yellow liquid, b.p. 194-197°/1.8 mm., n_D^{23} 1.4580, D_4^{23} 1.1143, M.R. calc'd. 98.54, M.R. found 99.05. The yield in this case was 24 g. (24%).

In a second experiment, 150 g. (0.94 mole) of ethyl malonate, 34 g. (0.33 mole) of β-ethoxypropionaldehyde, and 10 ml. of piperidine were mixed, and the hot solution was allowed to stand for 4 hours, boiled under refluxing conditions for 6 hours, allowed to stand overnight, and again boiled for

5 hours. The resulting red oil was poured into a mixture of 300 ml. of ice-water and 15 ml. concentrated hydrochloric acid, extracted into 200 ml. of ether, the ethereal extract washed with dilute aqueous sodium carbonate solution and with water, and dried over magnesium sulfate.

After removal of the ether on a steam bath, the residue was fractionally distilled in vacuo in a Claisen flask to which a side-arm 10 mm. in diameter had been attached. The product distilled at 188.5-190° (uncorr.)/1.5 mm. to give a 29% yield (39 g.) of yellow oil, after which the contents of the boiler suddenly decomposed with gas evolution to form a black solid polymer.

A third experiment employed a Claisen flask as modified above for the final distillation, but including a 3" column of glass rings in the distillation neck. The temperature of the boiler was carefully controlled by means of an electrically-heated metal bath in hope that the loss of the major portion of the product by decomposition could be moderated. Ethyl malonate and an intermediate fraction were collected as a fore-run, but before any of the desired product had distilled, the contents of the boiler again decomposed vigorously, as did liquid trapped in the fractionating column.

These experiments indicate that although the crude reaction mixture need not be purified before distillation, it is absolutely necessary that the distillation be conducted as rapidly as is possible, and because of the viscous nature of the product, a wide-diameter system is essential for this purpose.

β-(2-Ethoxyethyl)glutaric Acid (XXXI).--Ethyl α,α'-dicarbethoxy-β-(2-ethoxyethyl)glutarate (8 g., 0.02 mole) was mixed with 30 ml. of 4 M aqueous sodium hydroxide and 30 ml. of water, and the mixture was boiled under refluxing conditions for 10 hours. The homogeneous solution was extracted with ether, the aqueous layer filtered, acidified with evolution of carbon dioxide, and the acid solution boiled for 5 hours. After extraction with ether, the extract was dried over magnesium sulfate, and solvent was removed on a water bath to yield 3 g. of red oil, n_D^{21} 1.4718.

Distillation of the oil at 5 mm. pressure produced a colorless liquid which distilled at ca. 200° (bath temperature), but very little product was collected because of decomposition in the boiler. Attempts to form the S-benzylthiouronium salt of the acidic substance were unsuccessful, and reaction with phenylhydrazine produced a solid phenylhydrazide of doubtful purity (35). However, the acid showed the expected neutralization equivalent, and since this approach to the methanetriacetic acid problem appeared unpromising the β-(2-ethoxyethyl)glutaric acid was not studied further.

Anal. Calc'd for $C_9H_{16}O_5$: N.E., 102

Found: N.E., 105

Ethyl α,α'-dicarbethoxy-β-(2-ethoxyethyl)glutarate (8.0 g., 0.02 mole) was mixed with 10 ml. of concentrated sulfuric acid and allowed to stand overnight. The resulting solution, and the slightly alkaline mixture was extracted

with 100 ml. of ether. The aqueous residue was acidified with dilute hydrochloric acid, extracted with three 75 ml. portions of ether, the extract dried over magnesium sulfate, and the solvent removed in an inert atmosphere to yield 2.3 g. (57%) of an orange oil, n_D^{21} 1.4791. The product could not be induced to crystallize even when chilled in dry ice and rubbed, but again the neutralization equivalent was satisfactory.

Anal. Calc'd for $C_9H_{16}O_5$: N.E., 102

Found: N.E., 107

When the Guareschi base (XXVI) was subjected to hydrolysis under each of the above conditions, identical results were obtained.

2-Ketotetrahydropyran-4-acetic Acid (XXVII).--The ester (5.0 g., 0.012 mole) was placed in a pyrex bomb tube, 20 ml. of concentrated hydrochloric acid were added, and the tube was sealed and allowed to stand overnight. The brown, homogeneous solution was heated to 140-190° for 5 hours in a steel hydrogenation bomb containing a little water and mounted on a mechanical rocker. After cooling and standing for 1 day, the bomb tube containing the brown biphasic mixture was cooled in ice and opened carefully by allowing internal pressure to blow out a softened spot. The upper mobile layer evaporated rapidly with effervescence, and after removal of the remaining hydrochloric acid in vacuo, a brown residue was obtained from the lower phase.

The residue was dissolved in a 50 ml. water, boiled briefly with Norite, filtered, and the colorless solution was

shaken with two 75 ml. portions of ether, the combined extracts dried over magnesium sulfate, and the solvent removed *in vacuo*. Although the resulting oil did not solidify upon chilling in dry ice or upon standing for several months, addition and subsequent spontaneous evaporation of 10 ml. of ether caused the formation of a crystalline slush which deposited fine needles when recrystallized from dry chloroform, m.p. 49-50°, yield 0.4 g. (23%).

Anal. Calc'd. for $C_7H_{10}O_4$ (158.2): C,53.1; H,6.4.

Found: C, 52.8; H, 6.6.

Attempted Reaction of Ethyl Acetonedicarboxylate with Ethyl Malonate.--This procedure was carried out according to the method of Prout (17). Ethyl acetonedicarboxylate (20 ml., 0.10 mole), freshly distilled ethyl malonate (40 ml., 0.25 mole), 150 ml. of redistilled benzene, and 0.5 g. of α-aminophenylacetic acid were boiled under refluxing conditions in an apparatus fitted with a water trap and solvent return system. After 48 hours, no water had collected in the trap, and the experiment was not continued further. Distillation allowed recovery of the unchanged starting material.

Acetonedicarboxylic Acid (XXXVI).--The method employed was that of Adams, Chiles, and Rassweiler (37). Fuming sulfuric acid (20% SO_3)(350 ml.) was cooled to 0° and 230 g. (1.1 moles) of powdered U.S.P. citric acid was slowly added with efficient cooling in an ice-salt bath, and with vigorous mechanical stirring. After addition was completed, the mix-

ture was stirred for 7 hours at temperatures below 30°, with vigorous evolution of carbon monoxide.

The almost clear, amber solution was cooled to -12°, and a mixture of 600 g. of ice and 100 g. of powdered dry ice was added in 6 portions with stirring. The slurry was filtered as rapidly as possible through glass cloth stretched on a Büchner funnel, excess sulfuric acid was pressed out, and the solid was transferred to a beaker where it was stirred into a smooth paste with 70 ml. of ethyl acetate. The paste was filtered on a sintered glass plate, and sucked free of solvent. The yield was 56 g. (38%) of a white, free-flowing powder, m.p. 130°(d).

A similar preparation of the acid in which the solid was filtered initially on a fritted glass plate yielded only 36 g. (25%) of the desired product, although much more was present before filtration. The acetonedicarboxylic acid quickly clogged the filter, and due to its considerable solubility in sulfuric acid, and the length of time required for filtration, a very significant part of the product was lost. It is absolutely necessary that filtration of the viscous mixture be as rapid as possible.

Although it is generally accepted that acetonedicarboxylic acid decomposes very rapidly, a sample of the dry acid obtained above was perfectly stable for more than one week at room temperature, even without storage in a desiccator. A slightly damp and acid sample decomposed after several days' storage in an evacuated, dark desiccator.

Attempted Reaction of Acetophenone with Acetonedicarboxylic Acid.--Acetophenone (8 g.) was added to a cold solution of acetonedicarboxylic acid prepared by warming 16 g. of citric acid with 20 ml. of concentrated sulfuric acid to 80° for 20 minutes (18). After standing for 2 days, the yellowish viscous liquid was poured into cold water, and the resulting mixture was distilled with steam. The acetophenone was recovered unchanged.

In a second experiment, 12 g. (0.1 mole) of acetophenone was poured into a cold solution of 35 g. of pure sodium hydroxide in a mixture of 130 ml. of ethanol and 220 ml. of water. After chilling the acetophenone solution to 10°, 18 g. of acetonedicarboxylic acid in 50 ml. of water were added with vigorous stirring, the mixture was stirred at 10° for 2 hours, and then at 25° for 10 hours.

After standing overnight, the colorless, homogeneous solution was steam-distilled to remove alcohol and unreacted acetophenone, cooled to -10°, and acidified with 2 M aqueous sulfuric acid. Acidification was accompanied by evolution of a gas. The cold acid solution was continuously extracted with ether for 36 hours, the ethereal solution dried over anhydrous magnesium sulfate, and the solvent was removed *in vacuo* below 20°. There was no residue.

When carried out under identical conditions, reaction of acetophenone with benzaldehyde gave benzalacetophenone (38), and reaction with acetone gave isopropylidene acetophenone (39).

2-Hydroxy-3,5-dimethylacetophenone (XXXV).--A solution of 24 ml. (0.2 mole) of 2,4-xylenol in 100 ml. of 3 M aqueous sodium hydroxide was cooled in ice, 50 g. of ice were added to the mixture, and 25 ml. (0.26 mole) of pure acetic anhydride were poured in rapidly. After being shaken vigorously for 5 minutes, the biphasic mixture was made alkaline by addition of 2 M aqueous sodium hydroxide solution, the phases were separated, and the aqueous layer was extracted with 100 ml. of ether, the combined organic layers dried over anhydrous magnesium sulfate, and the ether removed on a steam bath. The yield of pale yellow, fragrant oil was 31 g. (95%).

Powdered aluminum chloride (27 g.) was added slowly, and after the vigorous reaction had subsided somewhat, the mixture was heated to 120-130° for 3 hours, allowed to cool to about 60°, and poured into a mixture of 200 g. of ice and 100 ml. of 3 M aqueous hydrochloric acid. The crystalline slurry was stirred vigorously, filtered, washed with 250 ml. of 3 M aqueous hydrochloric acid, then with water, and was finally pressed and sucked dry. The yield was 30 g. (97%) of yellow solid. A small sample was recrystallized from aqueous ethanol, m.p. 53-54°, corresponding exactly to the value presented in the literature (40).

2-Acetoxy-3,5-dimethylacetophenone (XXXII).--A portion of the product of the preceding synthesis (27 g.) was heated to the boiling point with 100 ml. of 3 M aqueous sodium hydroxide solution, but it would not dissolve to an apprecia-

ble extent. The solid was filtered off, washed with 300 ml. of water, and sucked dry.

This residue was then dissolved in 50 ml. of pure acetic anhydride, 5 drops of concentrated sulfuric acid were added, and the dark mixture was boiled under refluxing conditions for 5 hours, cooled, poured over ice, and the purple solid was filtered off, washed with water, and finally pressed dry on a porous plate. The produce was recrystallized from ethanol, but boiling the solution with Norite did not decolorize it completely. The yield was 12 g. (35%), m.p. 72-73°.

Anal. Calc'd dor $C_{12}H_{14}O_3$: C,69.9; H,6.8

Found: C,69.8; H,6.8.

β-Hydroxyglutaric Acid.--Dry acetonedicarboxylic acid (56 g., 0.38 mole) was dissolved in a solution of 43 g. (0.41 mole) of pure sodium carbonate in 200 ml. of water. Powdered sodium borohydride (4.0 g., 0.11 mole) was then added in four 1 g. portions over a period of 30 minutes with frequent shaking.

After standing at room temperature for 24 hours, a solution of 10 g. (0.25 mole) of sodium hydroxide in 100 ml. of water was added, the mixture allowed to stand for 0.5 hour, and the pH brought to ca. 4 with concentrated hydrochloric acid (85 ml.). After filtration, water was stripped off under vacuum, the white crystalline residue heated to reflux with 100 ml. of 95% ethanol, and the extract was dried over

anhydrous magnesium sulfate and used for the preparation of the ester without isolation of the acid itself.

Ethyl β-Hydroxyglutarate (XIX).--The alcoholic solution of β-hydroxyglutaric acid from the preceding preparation was mixed with 10 ml. of concentrated sulfuric acid, and heated under refluxing conditions for 8 hours. After cooling, the mixture was poured over several hundred grams of ice, and the homogeneous solution was extracted with three 100 ml. portions of ether, the ethereal solution dried, and solvent removed in vacuo. Fractional distillation through a 10" Vigreux column with an efficient semimicro still head gave 22.4 g. (29%) of a colorless, heavy oil, b.p. 130-132°/ 10 mm.. There was neither fore-run nor residue.

The product was slowly soluble in water, and did not react with aqueous potassium permanganate solution, bromine in carbon tetrachloride, or dinitrophenylhydrazine reagent. After boiling with 2 N aqueous sulfuric acid for several minutes, the compound rapidly decolorized permanganate solution and bromine in carbon tetrachloride. These tests were conducted for the reason that the three possible products, i.e., ethyl β-hydroxyglutarate, ethyl acetonedicarboxylate, and ethyl glutaconate, all boil at the same temperature at the pressure used.

Dreifuss and Ingold (12), who described the preparation of the ester, indicated that it was not appreciably soluble in water, while our findings are to the contrary. It is

probable that if the original acid solution of the ester had been continuously extracted, a high yield of ester would have been obtained.

Ethyl β-Bromoglutarate (XXXIII).--Ethyl β-hydroxyglutarate (22.4 g., 0.110 mole) was dissolved in 60 ml. of anhydrous ether, the solution was chilled in ice, and 7 ml. (0.074 mole) of redistilled phosphorus tribromide were added slowly over a period of 1 hour with vigorous stirring under anhydrous conditions. The mixture was allowed to warm to room temperature and to stand for 3 days, after which it was poured over ice, and the precipitated oil was separated, the aqueous supernatent extracted with 100 ml. of ether, the combined organic phases washed with 10% aqueous sodium bicarbonate until the washing were alkaline, and the ethereal solution dried over anhydrous potassium carbonate.

The ether was distilled *in vacuo* to leave 13.4 g. (46%) of a heavy, colorless oil which did not appear to react with cold alcoholic silver nitrate solution, but which gave a pale yellow, nitric acid-insoluble precipitate upon warming with the reagent. The product was not distilled upon recommendation of Ingold and Nickolls (13).

Reaction of Ethyl β-Bromoglutarate with 2-Acetoxy-3,5-dimethylacetophenone.--Commercial toluene was purified by shaking repeatedly with cold, concentrated sulfuric acid until the acid layer remained colorless, which required about 3 volumes of acid per volume of toluene. The organic layer was then shaken with successive portions of distilled water

until the washings were neutral, the suspended water was removed by storage over anhydrous magnesium sulfate, and the final product was distilled to yield a middle fraction which was considered to be pure.

Pure sodium methylate was prepared by dissolving clean c.p. sodium metal in freshly prepared absolute methanol, and stripping off unreacted solvent in vacuo at 70° to produce a free-flowing, white powder.

Purified 2-acetoxy-3,5-dimethylacetophenone (10.3 g., 0.050 mole) was dissolved in 50 ml. of the dry toluene prepared above, 2.0 g. of fresh sodium methylate were added, the mixture was chilled in ice, and 13.3 g. (0.050 mole) of dried ethyl β-bromoglutarate were added dropwise over a period of 1 hour, during which an additional 1.5 g. of sodium methylate were added. The reaction was conducted in an all-glass system consisting of a flask equipped with a sealed stirrer, a reflux condenser, a dropping funnel, and an inlet tube, and the apparatus was well dried before use. During the reaction, a constant slow stream of dried nitrogen was passed in, and moisture was excluded by means of drying tubes filled with magnesium perchlorate.

After addition of the ester, the mixture was allowed to warm to room temperature for 14 hours, and then was heated to 100° for 3 hours, cooled, and poured over ice. The aqueous layer was separated and extracted with 100 ml. of toluene, the combined organic layers were washed with water until

neutral, dried over anhydrous sodium sulfate, most of the toluene stripped off *in vacuo* at 80°, and the residue was distilled from a Claisen flask fitted with a 10 mm. diameter side-arm.

The residue remaining in the boiler after removal of substances volatile below 150°/2 mm. was dissolved in ethanol, the solvent removed *in vacuo*, and 50 ml. of 30-60° ligroin were added. After standing overnight in a refrigerator, the precipitated solid was filtered and recrystallized from ether, m.p. 153-154°. Only about 200 mg. of the material was obtained in a pure state, but evaporation of the ligroin supernatent produced 1 g. of additional solid.

Anal. Calc'd for $C_{21}H_{28}O_7$ (392.4): C,64.3; H,7.2.
Found: C,70.1; H,6.1.

No attempt was made to resolve the small quantity of black oil remaining uncrystallized.

Hydrogenation of β-(2-Hydroxy-5-methylbenzoyl)propionic Acid.--In the first experiment, an attempt was made to partially saturate the aromatic ring by the method of Thompson (23) to obtain a substituted cyclohexanone. The keto acid (10.4 g., 0.05 mole) was dissolved in a solution of 4.5 g. (0.113 mole) of sodium hydroxide in 50 ml. of water, and 3 g. of freshly prepared Raney nickel (Robert Ghirardelli) were added. The mixture was then shaken in a glass liner at 35° under 1500 lbs./sq. in. of hydrogen for 0.5 hour, and then at 50° for 6.5 hours. There was a continuous drop in pressure, and

at 500 lbs./sq. in. the bomb was again charged to full pressure. Again the pressure dropped to 700 lbs./sq. in. and remained at this point.

The catalyst was filtered off and the homogeneous alkaline solution was cooled in ice and acidified with 10 ml. of concentrated hydrochloric acid, heated to 50° for 10 minutes, chilled at 4° overnight, and the heavy, precipitated oil was extracted into 100 ml. of ether. After washing the extract with 2% aqueous sodium carbonate, it was dried over magnesium sulfate, the ether was evaporated, and the colorless product was allowed to stand at 4°.

After about 1 month, the oil suddenly crystallized, and the solid product melted at 113-115°. The yield was almost quantitative.

Further experiments employed Adams' platinum oxide catalyst. The β-(2-hydroxy-5-methylbenzoyl)propionic acid (2 g., 0.01 mole) was dissolved in 10 ml. of 1 M aqueous potassium hydroxide, 40 ml. of water and 0.1 g. of catalyst were added, and the mixture was shaken under 40 lbs/sq. in. hydrogen pressure at 25° for 2 hours with a pressure drop of 1 lb./sq. in.. The catalyst was filtered off, 20 ml, of 1 M aqueous sulfuric acid was added to the alkaline filtrate, and the solution was warmed. Upon cooling, the precipitated oil was extracted into ether, the extract dried over magnesium sulfate, and the solvent was distilled off. The oily residue crystallized when rubbed, and recrystallization from absolute

ethanol gave beautiful rods, m.p. 116-117°. The yield was 2.0 g. (100%).

An identical experiment conducted with the free keto acid in ethanol instead of aqueous alkali gave identical results.

Several further experiments were conducted under varied conditions, but by this time the equipment was all so leaky that it was finally abandoned as useless.

γ-(2-Hydroxy-5-methyl)-γ-butyrolactone (XXXVIII).--The above keto acid (2.0 g., 0.01 mole) was dissolved in a solution of 0.54 g. (0.005 mole) of pure sodium carbonate in 25 ml. of water and 0.5 g. (0.015 mole) of sodium borohydride was added in small portions with vigorous swirling over a period of 10 minutes. The colorless solution was heated to 100° for 10 minutes, acidified, cooled, and extracted with ether, the ethereal extract was dried over magnesium sulfate, the solvent was distilled off, and the residual oil was rubbed until solid. Recrystallization from a mixture of absolute ethanol and ligroin gave 1.9 g. (95%) of colorless rods, m.p. 116-116.8°. Mixture with the products from any of the hydrogenation experiments failed to depress this m.p.. The value in the literature (28) is 116-117°.

Preparation of Glutarimide from Sodium Glutarate.--Glutaric acid (1.32 g., 0.010 mole) was dissolved in 20 ml. of water and neutralized with 1.06 g. (0.010 mole) of dry sodium carbonate. After the evolution of carbon dioxide ceased, a solution of 3.40 g. (0.020 mole) of silver nitrate

in 60 ml. of water was added, the precipitate filtered off, washed with 50 ml. of water, a dried at 110° for 2 hours to give 3.30 g. (95.5%) of very pale yellow silver glutarate.

The silver salt was shaken with a solution of 2.90 g. of ammonium iodide in 50 ml. of water, at which time the solid became bright yellow and flocculent. After standing overnight the precipitate was filtered off, washed with water, and the filtrate was distilled to dryness on an oil bath, the residue heated to 150° for 3 hours, cooled, and scraped out. Recrystallization from benzene yielded 0.3 g. (27%) of glutarimide, m.p. 152-153°.

Upon boiling the product with dilute aqueous sodium hydroxide solution, ammonia was expelled, and after acidification, extraction with ether, drying the extract, and removal of solvent, a small quantity of glutaric acid, m.p. 96-97°, was obtained.

Preparation of Glutarimide from Ethyl Glutarate.--Ethyl glutarate (9 ml., 0.05 mole) and 40 ml. of 28% aqueous ammonia were mixed together and allowed to stand for three weeks at room temperature. The mixture became homogeneous and a small quantity of solid precipitated. Ammonia, water, and unreacted ester were removed by distillation *in vacuo*, the solid residue was extracted with several portions of boiling absolute ethanol, and the alcohol was again removed *in vacuo* to leave a white solid residue of glutarimide, m.p. 151-152°, yield 1.4 g. (25%).

Preparation of Glutarimide from Glutaric Acid and Urea.--
Glutaric acid (1.3 g., 0.01 mole) was mixed with 5 g. of urea and the solid was heated to 200° for 2 hours, during which ammonia was evolved vigorously. After cooling, the solid was scraped out, boiled for several minutes with water to remove unreacted urea, etc., filtered, and the residue was dissolved in hot benzene, the solution boiled briefly with Norite, filtered, and allowed to stand. After several days, the precipitated solid was isolated by filtration, m.p. 151-152°, yield 0.5 g. (44%).

References

1. J.H. Ford and B.E. Leach, J.A.C.S., 70, 1223(1948).
2. E.C. Kornfeld, R.G. Jones, and T.V. Parke, ibid., 71, 150(1949).
3. A.J. Whiffen, Mycologia, 42, 253(1950).
4. A.J. Whiffen, J. Bact., 56, 283(1948).
5. C. Djerassi, in Adams, "Organic Reactions", Vol. VI, John Wiley and Sons, Inc., New York, N.Y., 1950, p. 207.
6. G. Büchi and O. Jeger, Helv.Chim.Acta, 32, 538(1949).
7. F. Fichter and A. Beisswenger, Ber., 36, 1200(1903).
8. F.J. Van Natta, J. Hill, and W.H. Carothers, J.A.C.S., 56, 455(1934).
9. H.G. Fuchs and T. Reichstein, Helv.Chim.Acta, 26, 523(1943).
10. E.P. Kohler and G.H. Reid, J.A.C.S., 47, 2803(1925).
11. C.K. Ingold and E.A. Perrin, J.Chem.Soc., 121, ~~1638~~ 1924 (1922).
12. M.H. Dreifuss and C.K. Ingold, ibid., 1923, 2964.
13. C.K. Ingold and L.C. Nickolls, ibid., 121, 1638(1922).
14. G.H. Jeffry and A.I. Vogel, ibid., 1939, 446.
15. R. Ghosh, J.Ind.Chem.Soc., 13, 323(1936).
16. A.I. Vogel, "Practical Organic Chemistry", Longmans, Green and Co., Inc., New York, N.Y., 1948.
17. F.S. Prout, J.Org.Chem., 18, 928(1953).
18. B.B. Dey, J.Chem.Soc., 107, 1606(1915); A.M. Gakhokidze and A.P. Guntsadze, J.Gen.Chem.(U.S.S.R.), 17, 1642(1947).

References Cont'd.

19. L. Claisen, Ber., 38, 693(1905).

20. P.C. Freer and A. Lachman, Am.Chem.J., 19, 878(1897).

21. A. Haller and P. Ramart-Lucas, Compt.rend., 159, 143 (1914).

22. M.S. Newman and B.J. Magerlein, in Adams, "Organic Reactions," Vol. V, John Wiley and Sons, Inc., New York, N.Y., 1949, p. 413.

23. R.B. Thompson, Org. Synth., 27, 21(1947).

24. T. Curtius and E. Müller, Ber., 37, 1261(1904).

25. R. Robinson and L.H. Smith, J.Chem.Soc., 1937, 371.

26. D. Starr and R.M. Hixon, "Organic Syntheses," John Wiley and Sons, Inc., New York, N.Y., Coll. Vol. II, p. 571.

27. R.L. Shriner and R.C. Fuson, "The Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N.Y., 1948.

28. K.W. Rosenmund and D. Shapiro, Arch.Pharm., 272, 313 (1934).

29. C.F. Koelsch, J.A.C.S., 61, 969(1939).

30. J. Cason and H. Rapoport, "Laboratory Text in Organic Chemistry," Prentice-Hall Inc., New York, N.Y., 1950.

31. C.K. Ingold, J.Chem.Soc., 119, 353(1921).

32. J.H. Mac Gregor and C. Pugh, ibid., 1945, 535.

33. F.P. Pingert, Org. Synth., 25, 1(1945).

34. B.B. Corson, R.W. Scott, and C.E. Vose, "Organic Syntheses," John Wiley and Sons, Inc., New York, N.Y., Coll. Vol. I, p. 179.

References Cont'd.

35. G.H. Stemple and G.S. Schaffel, J.A.C.S., 64, 470(1942).

36. R. Adams, H.M. Chiles, and C.F. Rassweiler, "Organic Syntheses," John Wiley and Sons, Inc., New York, N.Y., Coll. Vol. I, p. 10.

37. E.P. Kohler and H.M. Chadwell, ibid., p. 79.

38. I.G. Farbenindustrie, Ger.Pat., 495, 878.

39. Kv. Auwers, M. Lechner, and H. Bundesmann, Ber., 58, 36(1925).

Propositions

1. I propose that the extremely active phytotoxic compound isolated from Arizona Encelia farinosa (Gray, Thesis, 1948) is closely related to santonin, and that Gray's empirical formula is incorrect.

2. I propose that hydrazine formate may be used to prepare substituted hydrazines by means of the Leuckart reaction.

$$R_2C{=}O + 2\ HCOONH_3NH_2 \longrightarrow R_2CHNH\overset{CHO}{\underset{|}{N}}H + CO_2 + N_2H_4 + 2\ H_2O$$

3. I propose that the furocoumarins isolated from Thamnosma montana (This Thesis, Part I) are not the agents responsible for plant growth inhibition in desert plant communities.

4. Efficient small-scale filing of chemical information is becoming increasingly difficult. A modification of the Coordinate Index System is proposed which would greatly increase the effectiveness and value of a personal filing system.

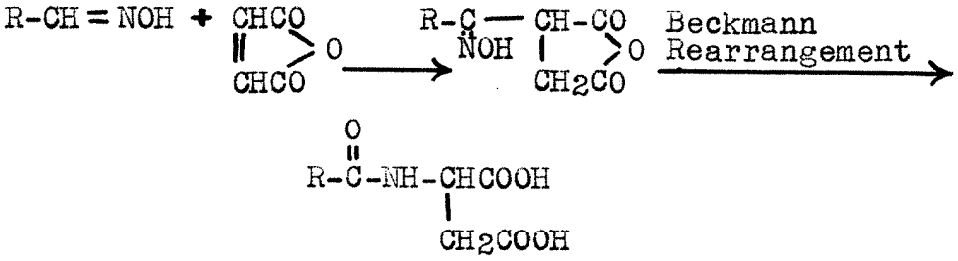
5. I propose that optically active synthetic polymers might be employed for the chromatographic resolution of optical isomers.

6. I propose that a mechanism of action of at least some of the antibiotic substances produced by Streptomyces griseus involves inhibition of protein synthesis in the susceptible organism. (1).

7. I propose that picric acid may be employed to determine with accuracy the molecular weights of members of most classes of organic compounds on a micro scale.

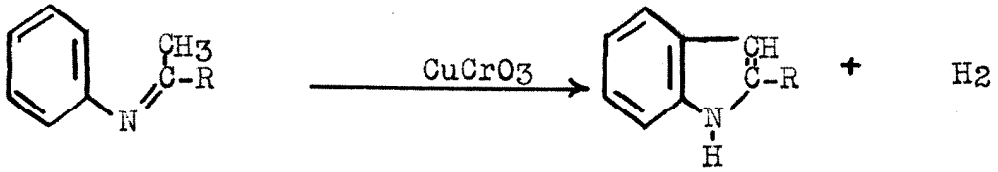
8. I propose that phloroglucinol and phloroglucinol derivatives constitute one of the important bridges between simple aliphatic compounds and aromatic compounds in plants, and that they are formed from acetate.

9. The reaction of aldoximes with maleic anhydride to form N-acylated aspartic acids has been studied by La Parola(2,3) and the mechanism proposed by him has been generally accepted.



I propose that other mechanisms can be formulated which are more consistent with the facts observed.

10. I propose that the cyclodehydrogenation reaction (4) may be conducted under conditions which are much milder than those used at the present time, and thus become of major importance for the synthesis of many heterocyclic compounds.



11. Separation of compounds of the rare earth elements from one another still presents difficulty. I propose that fractional distillation or sublimation of their chelate compounds might prove effective for such a purpose.

References

1. S. Waksman, "Streptomycin," The Williams and Wilkins Co., Baltimore, Md., 1949.
2. G. La Parola, Gazz. chim. ital., 67, 481(1937); C.A., 32, 1675(1938).
3. G. La Parola, Gazz. chim. ital., 73, 94(1943); C.A., 38, 5211(1944).
4. C. Hansch, D.G. Crosby, et al., J.A.C.S., 73, 704(1951).