I. THE BIOGENESIS OF PHENAZINE PIGMENTS

II. β-FERROCENYL CARBONIUM IONS

III. CHEMICAL SHIFTS AND π-ELECTRON DENSITIES

Thesis by

Robert Everett Carter

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TO MY PARENTS

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I am indebted to my parents for their encouragement and support and for impressing upon me the importance of taking advantage of every opportunity for education.

ABSTRACT

PART I

The biogenesis of phenazine pigments in bacteria has been studied by the use of radioactive substrates. The results indicate that the pigment of Pseudomonas chlororaphis originates in the dimerisation of precursors derived from anthranilic acid. The structures of all the other known phenazine pigments are compatible with such a derivation.

PART II

β-Ferrocenyl carbonium ions have been studied in solvolytic experiments with β-ferrocenylethyl and β-ferrocenylisopropyl tosylates. A deuterium-labelling experiment with the ethyl compound indicates the absence of cyclopentadienyl ring participation. The isotope effect in the solvolysis (in 80% acetone) of β-ferrocenylethyl-α,α-d_2 tosylate was determined. The racemization of optically active β-ferrocenylisopropyl tosylate in 60% acetone at pH 6.9 is discussed; it is suggested that the racemization may come about through a special iron-carbon interaction which leads to a symmetrical carbonium ion. A simple diagram of a β-ferrocenyl carbonium ion stabilized by iron participation is presented and discussed to scrutinize the plausibility of such participation.

PART III

The use of the equation $\delta = aq$ which relates the chemical shift (δ) of an aromatic proton to the excess π-electron density q on the carbon to which the proton is attached is discussed and illustrated by examples from the current literature.

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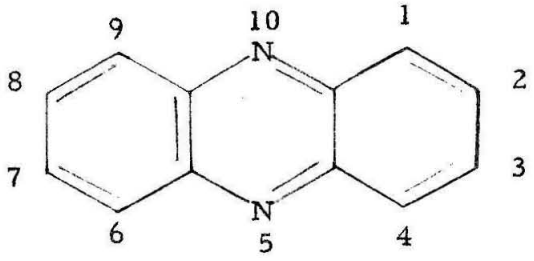
PART I

THE BIOGENESIS OF PHENAZINE PIGMENTS

THE BIOGENESIS OF PHENAZINE PIGMENTS

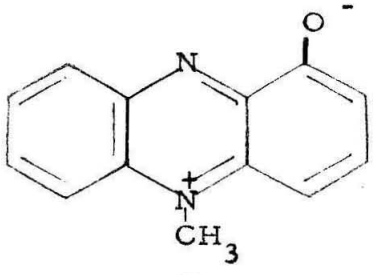
Introduction

The aromatic phenazine nucleus (I) occurs in nature in a



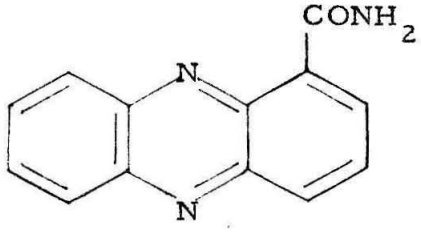
I

number of brightly colored compounds produced by bacteria of the genus Pseudomonas. The deep blue pigment pyocyanine (II) is found



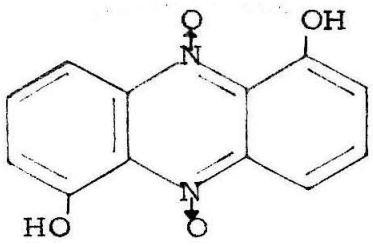
II

in cultures of Ps. aeruginosa and represents the first reported occurrence of the phenazine nucleus in a natural product (1). Chlororaphin (a 3:1 molecular compound of phenazine-1-carboxamide (III)



III

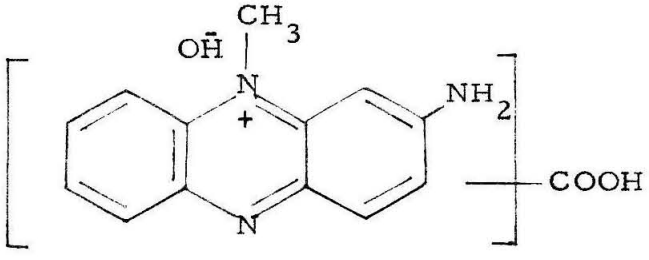
and its 5, 10-dihydro derivative (2)), iodinin (IV) (the purple pigment



IV

of Ps. iodinum (3)), phenazine-1-carboxylic acid (4) (a bright yellow compound produced by Ps. aureofaciens), and 1-hydroxyphenazine (a derivative of pyocyanine produced by a strain of Ps. aeruginosa (5)) complete the list of bacterial phenazine pigments which have been described completely in the literature. Iodinin and its reduced form, 1,6-dihydroxyphenazine, were isolated from Brevibacterium crystalloiodinum by Irie, Kurosawa and Nagaoka (5a).

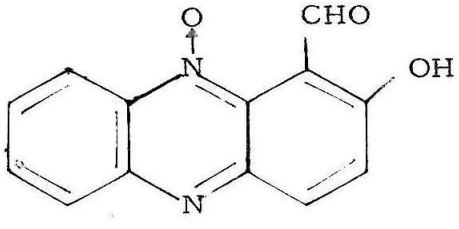
The isolation of other phenazines from bacteria has been reported but complete structure determinations for these compounds have not yet been described. Holliman (6) has isolated two pigments produced by a red strain of Ps. aeruginosa, one of which was assigned the partial structure (V). A similar structure is proposed for the other compound,



V

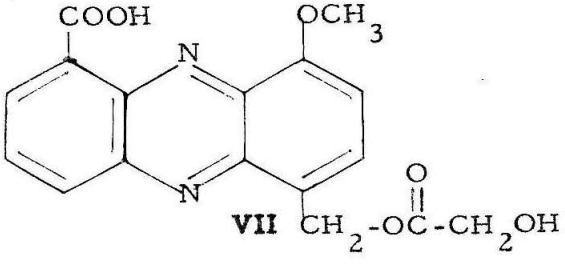
but with the addition of a methyl group and a sulfur-containing substituent. Studies by E. Olson (7) in these laboratories concerning the red-orange phenazine pigment which accompanies phenazine-1-carboxylic acid in Ps. aureofaciens cultures (4) have led

to the proposal of the tentative structure (VI).



VI

The production of phenazine derivatives is not monopolized by bacteria: certain species of the mold Streptomyces have been shown to synthesize phenazines. Phenazine-1-carboxylic acid and a similar monobasic acid ($C_{17}H_{16}N_2O_2$, tubermycin A) were isolated from a Streptomyces misakiensis culture by Isono and his co-workers (8). A compound related to iodinin, viz, 1,6-dihydroxyphenazine, was obtained by Akabori and Nakamura from S. thioluteus (9) and shown to be active as an anti-fungal agent. The most fertile source of phenazine pigments is the mold Streptomyces griseoluteus, an organism isolated from the soil of a potato field in Tokyo. No less than five interrelated compounds have been obtained from this species by S. Nakamura and his co-workers. The first two compounds were named griseoluteins A(10) and B(11) and are reported to have structures (VII) and (VIII), respectively. Further investigation has now produced three



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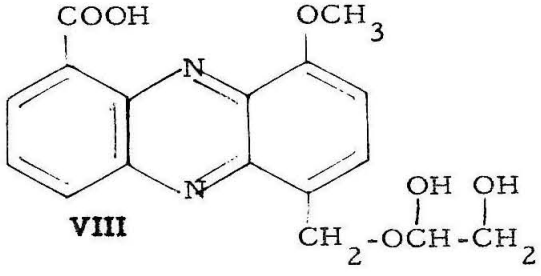
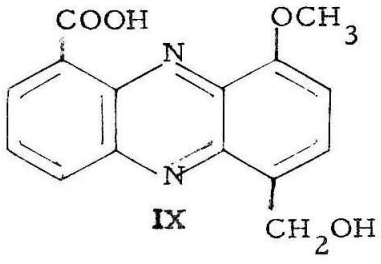
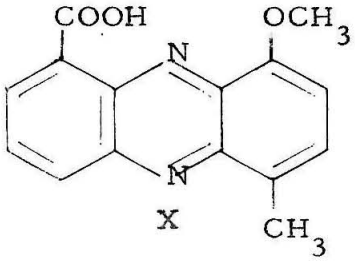


VIII

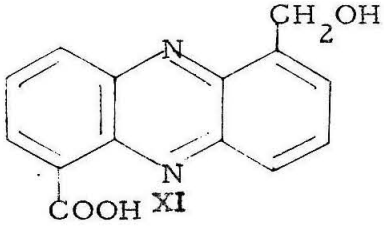
additional compounds: griseoluteic acid (12) (IX), 1-methoxy-4-methyl-9-carboxy phenazine (13) (X), and 1-hydroxymethyl-6-carboxy phenazine (13) (XI).



IX



X



XI

Antibiotic and Anti-tumor Properties

The anti-tumor and bacteriostatic effects of griseoluteins A and B and related compounds were investigated by Ogata (14). Diacetyl griseolutein B and griseoluteic acid showed strong inhibitory effects upon HeLa cancer cells and weaker effects upon Ehrlich carcinoma in mice. The bacteriostatic effects of these compounds were tested against seven species of bacteria; they were all found to be at least partially inhibitory to all the bacteria tested.

Akabori and M. Nakamura found 1,6-dihydroxyphenazine from S. thioluteus (9) to be effective against several species of pathogenic plant fungi and a yeast, Zygosaccharomyces salsus.

Schoental (15) studied the antibacterial properties of pyocyanine and 1-hydroxyphenazine and concluded that although the former had strong bactericidal activity, it was too toxic and unstable for therapeutic purposes. The hydroxy compound proved to be less toxic and non-irritating and it exhibited strong antibacterial action against more than twenty bacterial strains.

The potential value of phenazine pigments as antibiotics gives the study of their biosynthesis more than academic interest.

Historical Perspective

Pyocyanine, the phenazine pigment which has been given the greatest amount of biogenetic attention, has some importance as an antibiotic (15), a contaminant causing pigmentation of wool (16), and a respiratory catalyst in bacteria (17).

Friedheim (17b) has shown that pyocyanine added to cells of Ps. aeruginosa suspended in a phosphate buffer actually increases oxygen consumption by as much as 24-fold. The effect of pyocyanine probably depends upon the presence of other respiratory catalysts such as the cytochrome shown spectroscopically by Yaoi and Tamiya (18) to be present in Ps. aeruginosa. Indirect evidence for this dependence is given by Friedheim (17b): pyocyanine increased (in varying degree) the respiration of other microorganisms containing cytochrome, viz.

Staphylococcus aureus and Pneumococcus Type III, whereas with obligatory anaerobes such as Bacillus tetani, pyocyanine had no effect.

Ramakrishnan and Campbell (19) studied the gluconic dehydrogenase system of Ps. aeruginosa which specifically catalyzes the oxidation of gluconic acid to 2-keto gluconic acid, a reaction which occurs on the pentose phosphate pathway of glucose metabolism. (See p. 12.) They found pyocyanine to be a very active hydrogen acceptor for the enzyme.

Thus it is quite clear that pyocyanine is not simply a metabolic by-product; it plays a very useful role in the physiology of Ps. aeruginosa.

Although a significant amount of work on the biosynthesis of pyocyanine has been entered in the literature (e.g., refs. (20), (21), (22)), no definitive proposals concerning possible biogenetic paths for the phenazines in general had appeared until the recent note by Carter and Richards (23), which is a brief summary of the work to be reported in this thesis (Part I) and in which a shikimate pathway is indicated for the biosynthesis of phenazine-1-carboxamide by Ps. chlororaphis.

The studies of Young (24) Hellinger (25) and others (26) will not be discussed here since these workers were primarily interested in the determination of appropriate culture conditions and/or the study of the antibiotic properties of the pigments of Ps. aeruginosa. They

did not speculate about the biosynthetic paths involved in the production of pyocyanine . Their work is adequately reviewed elsewhere (27).

Grossowicz et al. (21) studied the formation of pyocyanine by resting cells of Ps. aeruginosa. The bacteria were incubated for 24 hours at 37° C. and then taken up in saline solution, washed by centrifugation and resuspended in a buffered solution. The concentration of organisms was determined by turbidity measurements; however, as pointed out by Frank and DeMoss (22), data are not given with regard to this point, nor is it made clear whether several measurements were made or merely "initial" and "final" measurements. Substrates were added to the bacterial suspensions and after static incubation at 37° the pyocyanine was determined spectrophotometrically.

The results obtained by Grossowicz et al. are not in general in agreement with those obtained by either Blackwood and Neish (20) or Frank and DeMoss. It should be pointed out that: (a) Grossowicz et al. used a strain of Ps. aeruginosa from the stock collection of the Department of Bacteriology at the Hebrew University in Jerusalem, while the other authors cited above used Ps. aeruginosa ATCC #9027; and (b) Grossowicz et al. claimed to obtain pyocyanine synthesis with nonproliferating cell suspensions while both Blackwood and Neish and Frank and DeMoss worked with growing cultures. Neither of these points, however, affords a reasonable explanation for the completely

contradictory results gathered in Table I. The results of Blackwood and Neish cannot be compared directly with those of the other authors since they are given in terms of relative specific activity (based on that of cell carbon which was set equal to 1.00) of pyocyanine carbon derived from various radioactive substrates, and they are thus not included in the table. In the preparation of this table, pyocyanine formation from γ-aminobutyric acid was arbitrarily chosen as 100% and all the numbers in the table are thus directly comparable since they are all calculated on the same basis. In the work of Grossowicz et al., pyocyanine formation from L-glutamic acid was chosen as 100%, while in the work of Frank and DeMoss, DL-alanine was the chosen standard substrate. It can readily be seen from a glance at the table that while L-glutamic acid proved to be a very good substrate for Grossowicz et al. (91% pyocyanine formation), it was quite a poor substrate for Frank and DeMoss, who observed only 11.8% pyocyanine formation in the presence of this compound. Indeed, Frank and DeMoss found D- rather than L-glutamic acid to be an extremely effective substrate from which almost three times as much pyocyanine was formed as from γ-aminobutyric acid. Unfortunately, Grossowicz et al. do not report any results with this compound. The greatest discrepancy in the results of these two groups of workers is in the use of DL-isoleucine as a substrate. Grossowicz et al. got essentially no pyocyanine formation from this substrate, while Frank and DeMoss found it to be twice as good as γ-aminobutyric

Table I

Pyocyanine Formation from Various Substrates

Substrate	Pyocyanine Formation (%)*	
	Grossowicz et al.(21)	Frank and DeMoss(22)
γ-Aminobutyric Acid	100 (110)**	100 (113)**
L-Glutamic Acid	91	11.8
D-Glutamic Acid	--	292
DL-Isoleucine	0	206
α-Ketoglutaric Acid + NH_4^+	50	0
β-Alanine	63.6	0

*Pyocyanine formation from γ-aminobutyric acid arbitrarily chosen as 100%.

**The number in parentheses is the actually reported figure based in the work of Grossowicz et al. on pyocyanine formation from L-glutamic acid as 100% and in the work of Frank and DeMoss on that from DL-alanine as 100%.

acid. This table demonstrates the futility of attempting to draw any definitive conclusions about phenazine biosynthesis from a study of the work of previous authors.

In view of the objections which have been raised (22) to the experimental methods (and therefore to the results) of Grossowicz et al., their suggestion that "glutamic acid holds a key position in pyocyanine synthesis" loses a good deal of its validity.

Blackwood and Neish (20) found glycerol and dihydroxyacetone to be the only substrates tested which formed pyocyanine with a specific activity higher than the average level of cell constituents, and they conclude that non-phosphorylated trioses may be converted to six-carbon compounds which would condense to form the phenazine ring. However, Frank and DeMoss studied the effect of glycerol on the incorporation of labeled alanine into pyocyanine in the presence of phosphate and obtained results which imply that at the relatively high phosphate concentration used by Blackwood and Neish (3×10^{-3} M) glycerol would indeed be a good substrate. A table from the paper of Frank and DeMoss will clarify this statement:

Substrate	Pyocyanine (cpm/μ mole)	
	0.5×10^{-3} M PO_4	1.3×10^{-3} M PO_4
L-Alanine-U-C^{14}	13087	---
L-Alanine-U-C^{14} + glycerol	8635	524

The table shows that the use of glycerol in the presence of labeled alanine results in a dilution of the labeled pyocyanine produced; if the phosphate concentration is raised from 0.5 to 1.3 x 10^{-3}M, the label dilution is greatly increased.

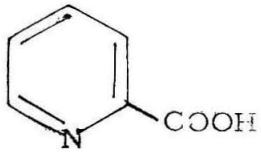
Frank and DeMoss found a close relation between cell growth and pyocyanine synthesis: only those amino acids which supported growth would also support pyocyanine synthesis. They conclude that this close relation "effectively obscures any attempt to decide which of the substrates tested may be a specific precursor of pyocyanine. Thus, even if a given amino acid were incorporated intact into the ring system of the pigment, that same amino acid might also be a good substrate for growth and its efficiency as a substrate for pyocyanine might not be observable." This conclusion is especially valid in the absence of specific methods of degradation by which the distribution of label among the pyocyanine carbon atoms might be determined.

Plausibility of Shikimate Path for Phenazine Biosynthesis

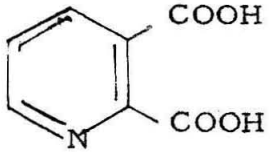
A. Biosynthesis of Shikimic Acid and Anthranilic Acid

The biogenetic origin of aromatic compounds in nature, especially the common aromatic amino acids (phenylalanine, tyrosine and tryptophan), is usually discussed in terms of the shikimic acid

cycle. It has been shown (27) that this important metabolic cycle begins with glucose and ultimately may lead to the amino acids cited above, to compounds other than tryptophan containing the indole nucleus, to six membered heterocycles such as picolinic (XII) and quinolinic (XIII) acids and to anthranilic acid (XIV). We are here concerned with the



XII



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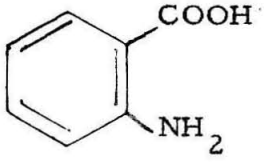

XIV

possibility that anthranilic acid may function as a precursor for the naturally occurring phenazines.

The Embden-Meyerhof or glycolytic pathway for the metabolism of glucose involves the isomerization of glucose-6-phosphate to fructose-6-phosphate, the formation of fructose-1, 6-diphosphate and the cleavage of this diphosphate to two interconvertible three-carbon compounds, viz, dihydroxyacetone phosphate and glyceraldehyde-3-phosphate (28). These reactions and those which convert the three-carbon fragment to phosphoenolpyruvic acid are outlined in Chart I.

In microorganisms an alternative pathway for glucose metabolism is available: the pentose-phosphate or oxidative pathway. Glucose-6-phosphate is oxidized to 6-phosphogluconic acid, which is further oxidized to 6-phospho-3-keto gluconic acid. The decarboxylation of the latter compound produces a pentose (ribulose-5-phosphate) which

Embden-Meyerhof (Glycolytic) Path for Glucose Metabolism

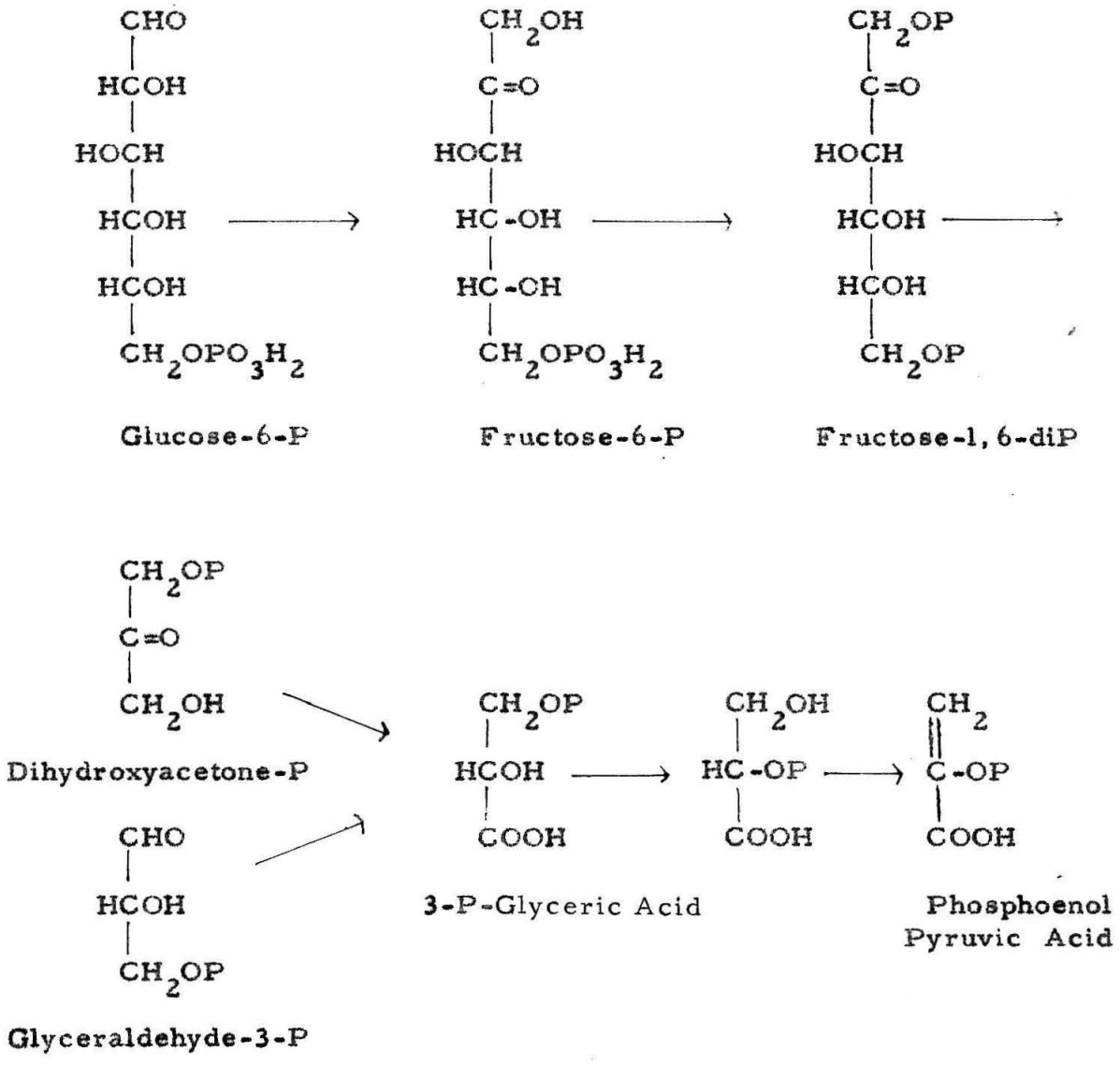

Chart I

is isomerized to xylulose-5-phosphate as well as to ribose-5-phosphate. A reaction mediated by the enzyme transketolase transfers carbon atoms 1 and 2 of the xylulose to the ribose to produce sedoheptulose-7-phosphate and a triose, glyceraldehyde-3-phosphate. The enzyme transaldolase cleaves the heptulose in such a manner that the dihydroxyacetone portion is transferred to the triose phosphate. Thus the 4-carbon compound necessary for the biosynthesis of shikimic acid, erythrose-4-phosphate, is produced along with fructose-6-phosphate (29). (See Chart II.) Another source of the 4-carbon fragment is the breakdown of sedoheptulose-7-phosphate to erythrose-4-phosphate and dihydroxyacetone phosphate by the enzyme aldolase. This source is not usually important since the equilibrium favors the formation of the heptulose rather than its breakdown (30).

Erythrose-4-phosphate and phosphoenol pyruvic acid condense to form the 7-carbon sugar acid 2-keto-3-deoxy-7-phosphoglucoheptonic acid, which cyclizes to form 5-dehydroquinic acid. This compound loses the elements of water to produce the immediate precursor of shikimic acid, 5-dehydroshikimic acid (31). Srinivasan (32) has recently demonstrated the enzymatic conversion of shikimic acid-5-phosphate and L-glutamine to anthranilic acid by a cell-free extract of a mutant of Escherichia coli. The reactions involved in the synthesis of anthranilic acid from erythrose-4-phosphate and phosphoenolpyruvic acid are outlined in Chart III.

Pentose-Phosphate (Oxidative) Path for Glucose Metabolism

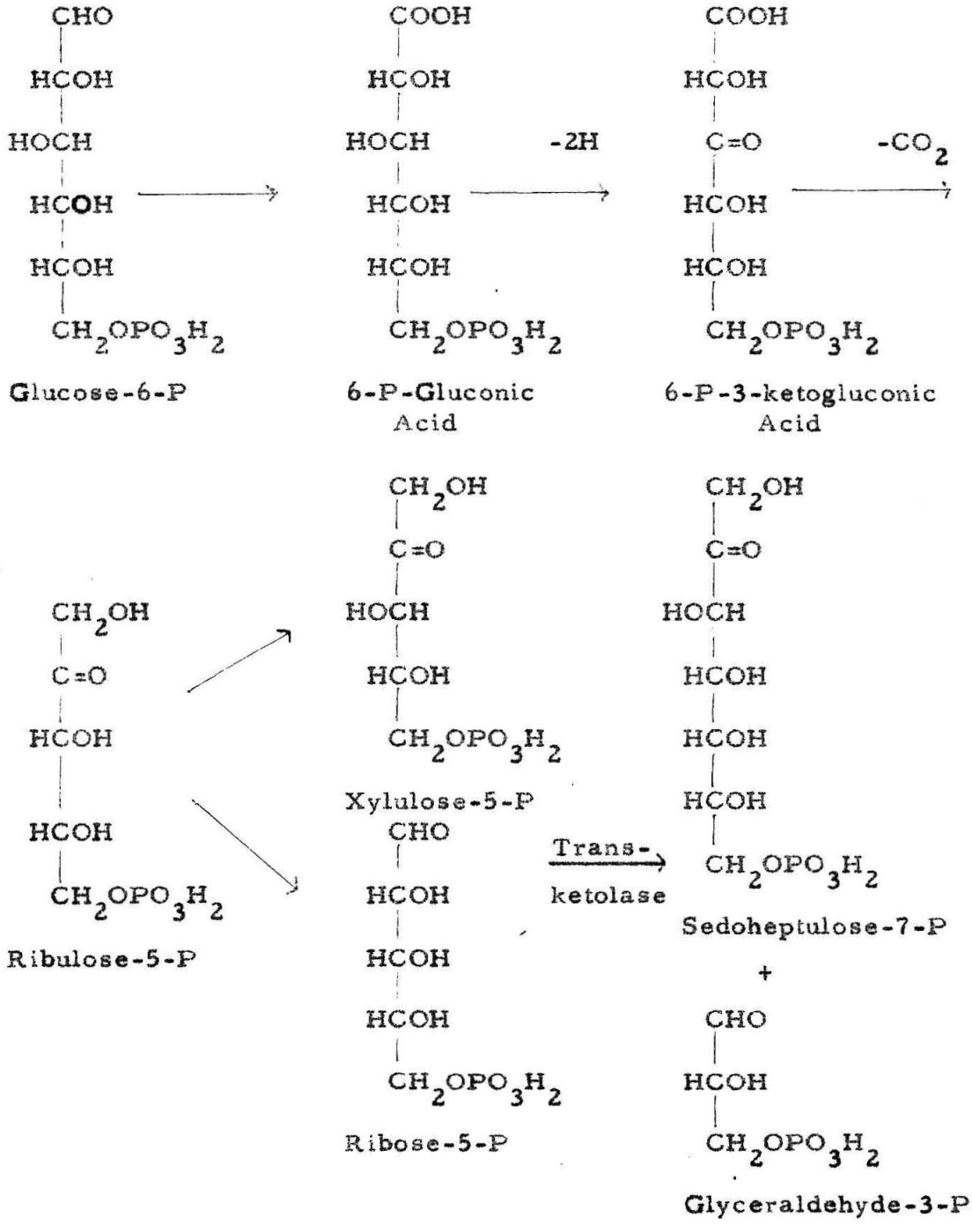

Chart II

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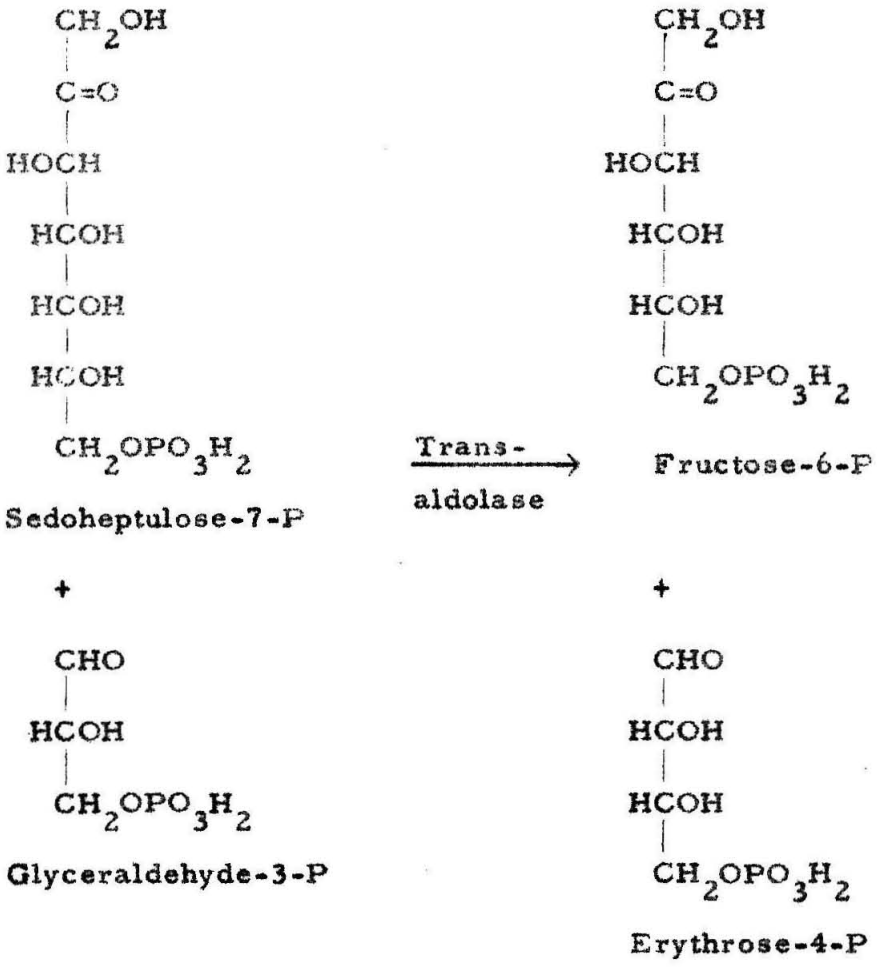
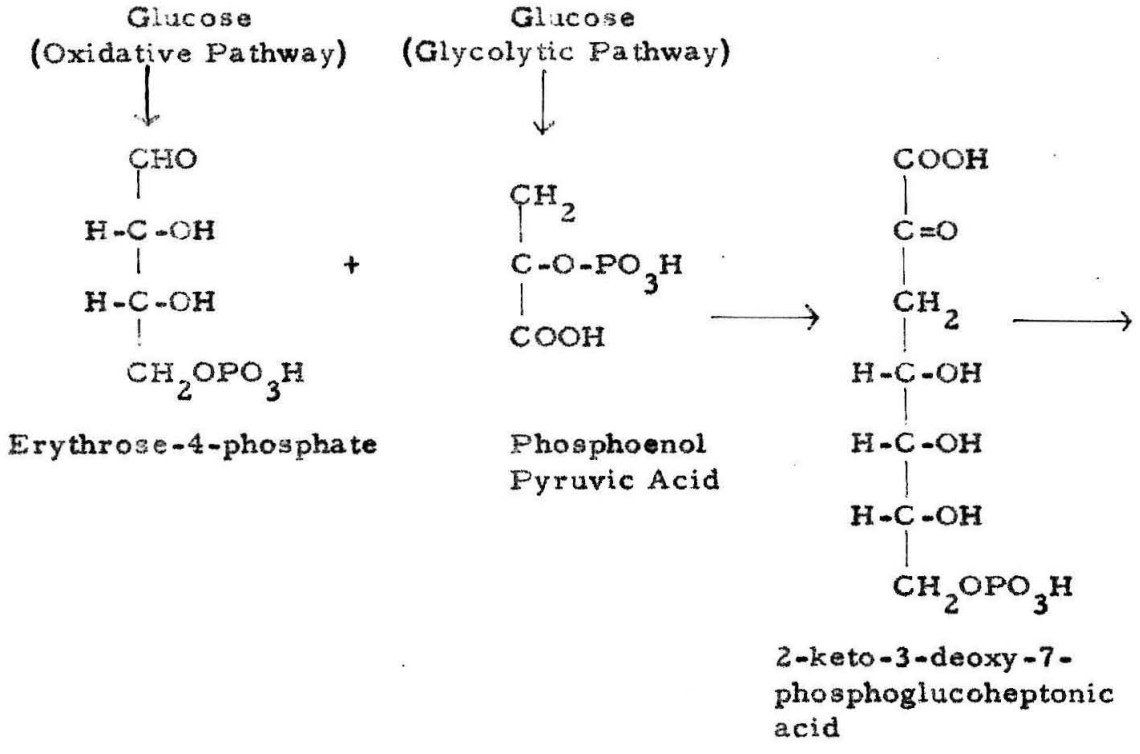


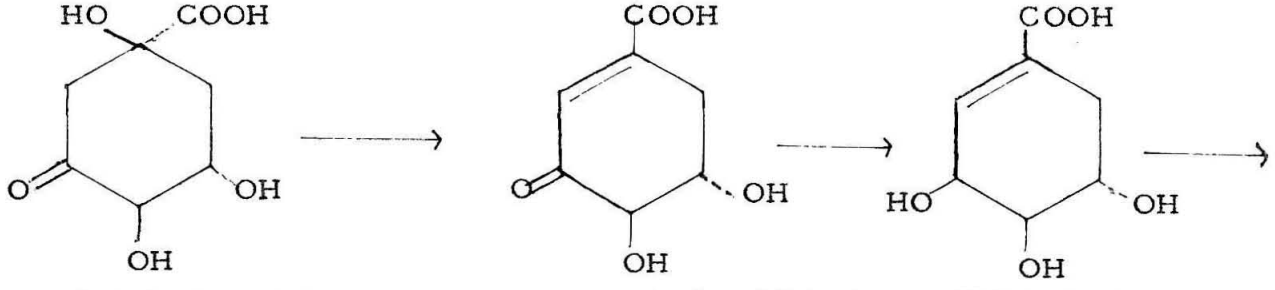
Formation of Anthranilic Acid



Erythrose-4-phosphate

Phosphoenol Pyruvic Acid

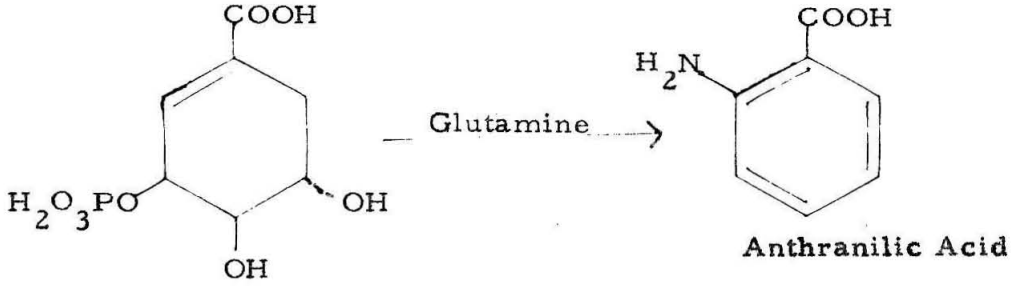
2-keto-3-deoxy-7-phosphoglucoheptonic acid



5-Dehydroquinic Acid

5-Dehydroshikimic Acid

Shikimic Acid



Anthranilic Acid

Chart III

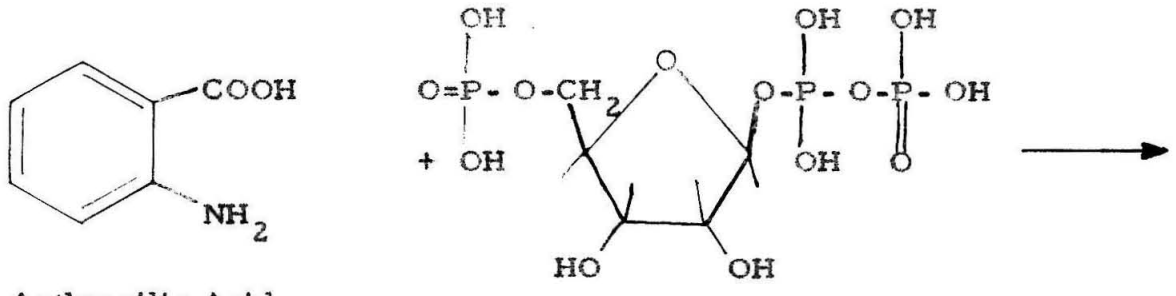
B. Anthranilic Acid Metabolism

Anthranilic acid metabolism is almost invariably closely interrelated with that of tryptophan. The metabolism of tryptophan leads to anthranilic acid; conversely, anthranilic acid is used in the manufactore of the indole nucleus of tryptophan. In certain bacteria the two compounds are apparently interchangeable: anthranilic acid was found capable of replacing tryptophan as a growth factor for two Lactobacillus species (33).

White et al. (27) discuss the metabolism of anthranilic acid in E. coli (Chart IV). It condenses with 5-phosphoribosyl-1-pyrophosphate to form an indole derivative. That this reaction occurs with loss of the carboxyl carbon has been demonstrated by carbon-14 labelling experiments (34). The indole derivative is then degraded to indole itself which is converted to tryptophan by the action of tryptophan synthetase and serine.

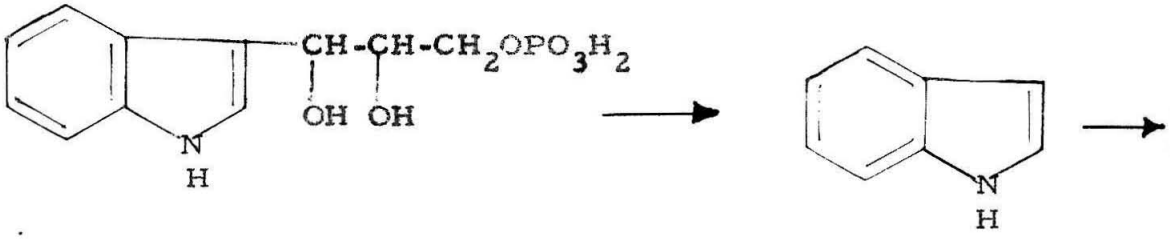
Many Pseudomonads use tryptophan as a major source of carbon, nitrogen and energy (35). In these organisms, tryptophan is oxidized to kynurenine which is hydrolyzed to anthranilic acid and alanine by kynureninase (Chart V). In the study by Stanier (35), 19 out of 24 Pseudomonas strains oxidized tryptophan, kynurenine and anthranilic acid at a rapid and steady rate to the point of substrate exhaustion.

The oxidation of anthranilic acid (Chart VI) proceeds with loss



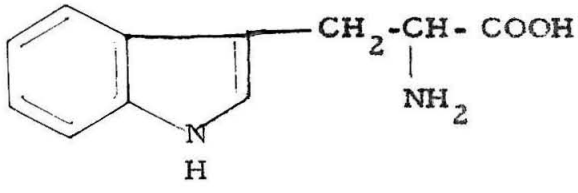
Anthranilic Acid

5-Phosphoribosyl-1-pyrophosphate



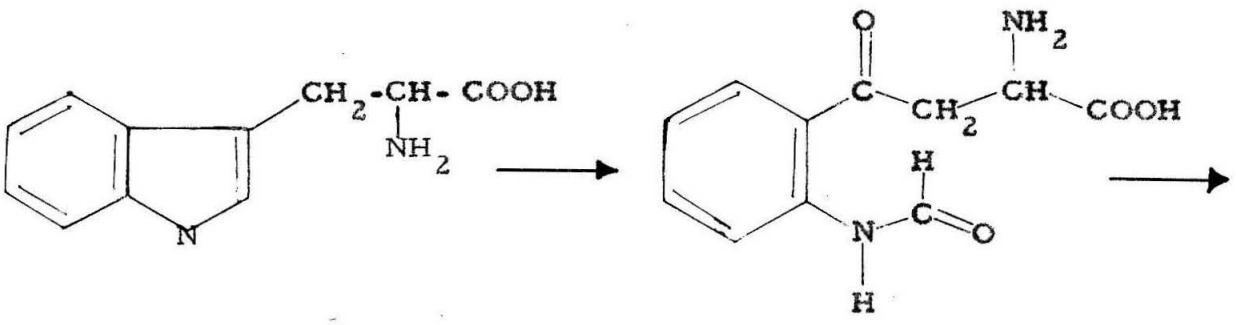
Indolyl-3-glycerol phosphate

Indole

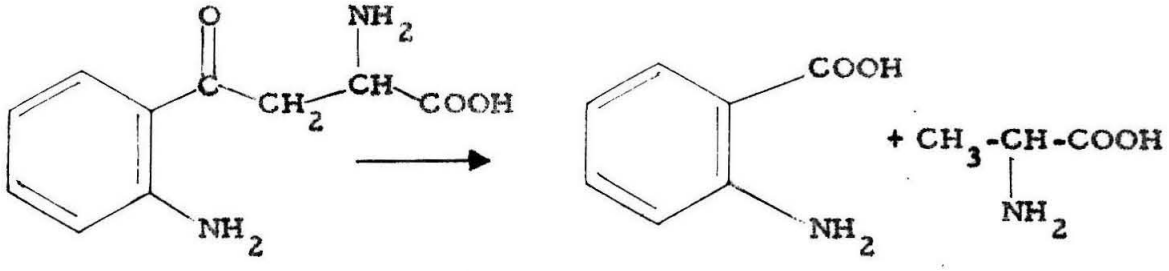


Tryptophan

Chart IV

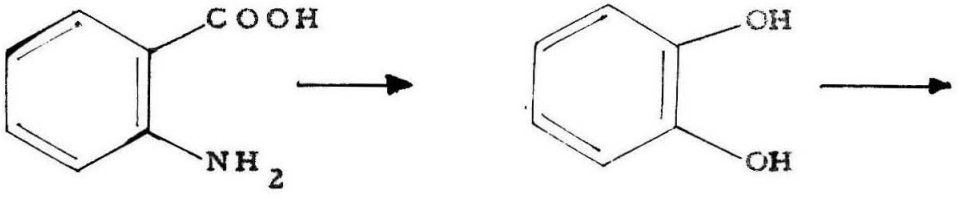


Tryptophan — Formylkynurenine

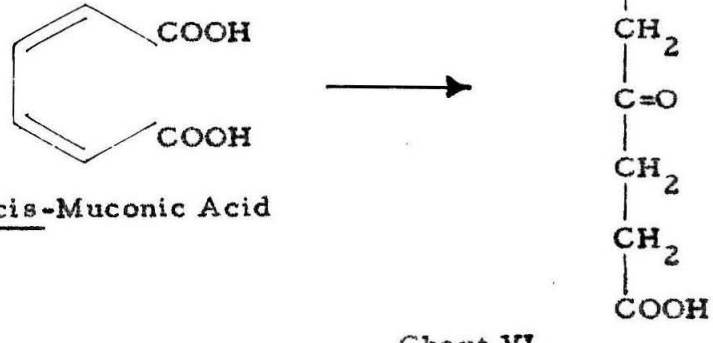


Kynurenine — Anthranilic Acid — Alanine

Chart V



Anthranilic Acid — Catechol



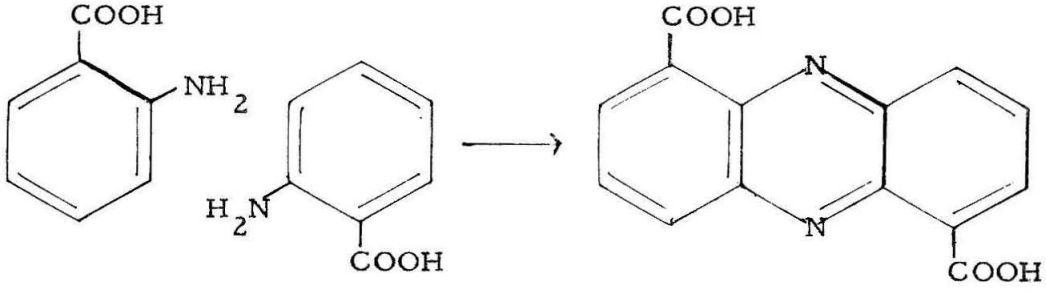
cis, cis-Muconic Acid — β-Keto adipic Acid

Chart VI

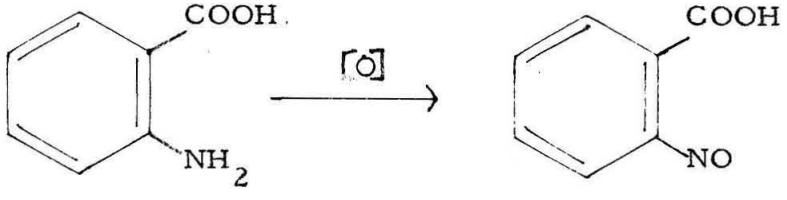
of the carboxyl carbon and formation of catechol (36). The latter is then oxidatively cleaved to cis,cis-muconic acid, which is converted to β-keto adipic acid.

C. Mechanism of Phenazine Biosynthesis

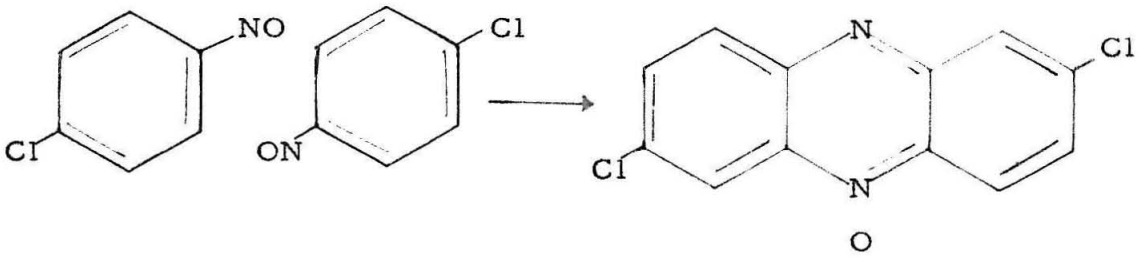
A possible mechanism of phenazine biosynthesis which involves the condensation of two anthranilic acid units may be written:



This dimerization may be rationalized on purely chemical grounds: for example, a mechanism which involves preliminary oxidation at nitrogen is plausible. Bamberger and Ham (37) describe the formation



of phenazine-N-oxides by the action of concentrated sulfuric acid on derivatives of nitrosobenzene in acetic acid at 20°. Under these conditions the mono-N-oxide of 2,7-dichlorophenazine is obtained from p-chloronitrosobenzene. It is interesting to note that certainly one



and possibly two of the naturally occurring phenazines are N-oxides. (See page 2 .) A plausible mechanism for the formation of a phenazine from o-carboxynitrosobenzene is given in Chart VII. Unfortunately, the precise structures of the species that couple are not defined by the experiments reported in this thesis and the selection of one mechanism above all others is thus premature. Furthermore, it should be emphasized that the possibilities are not limited to the dimerization of two identical units.

These experiments do indicate, however, that the ring in the phenazine pigment bearing the carboxyl carbon has not been produced via a preliminary oxidation of the anthranilic acid ring to 3-hydroxy anthranilic acid. The complex series of reactions required for this conversion, which has been given detailed study in the mold Neurospora, results in the loss of the carboxyl carbon of the anthranilic acid substrate. Studies with mutants (34) have shown that tryptophan and nicotinic acid produced in the presence of anthranilic-acid-carboxyl-C^{14} are devoid of detectable radioactivity. Further, it is known that the series of reactions which takes place in the mold is the following (34a):

Possible Mechanism of Phenazine Formation

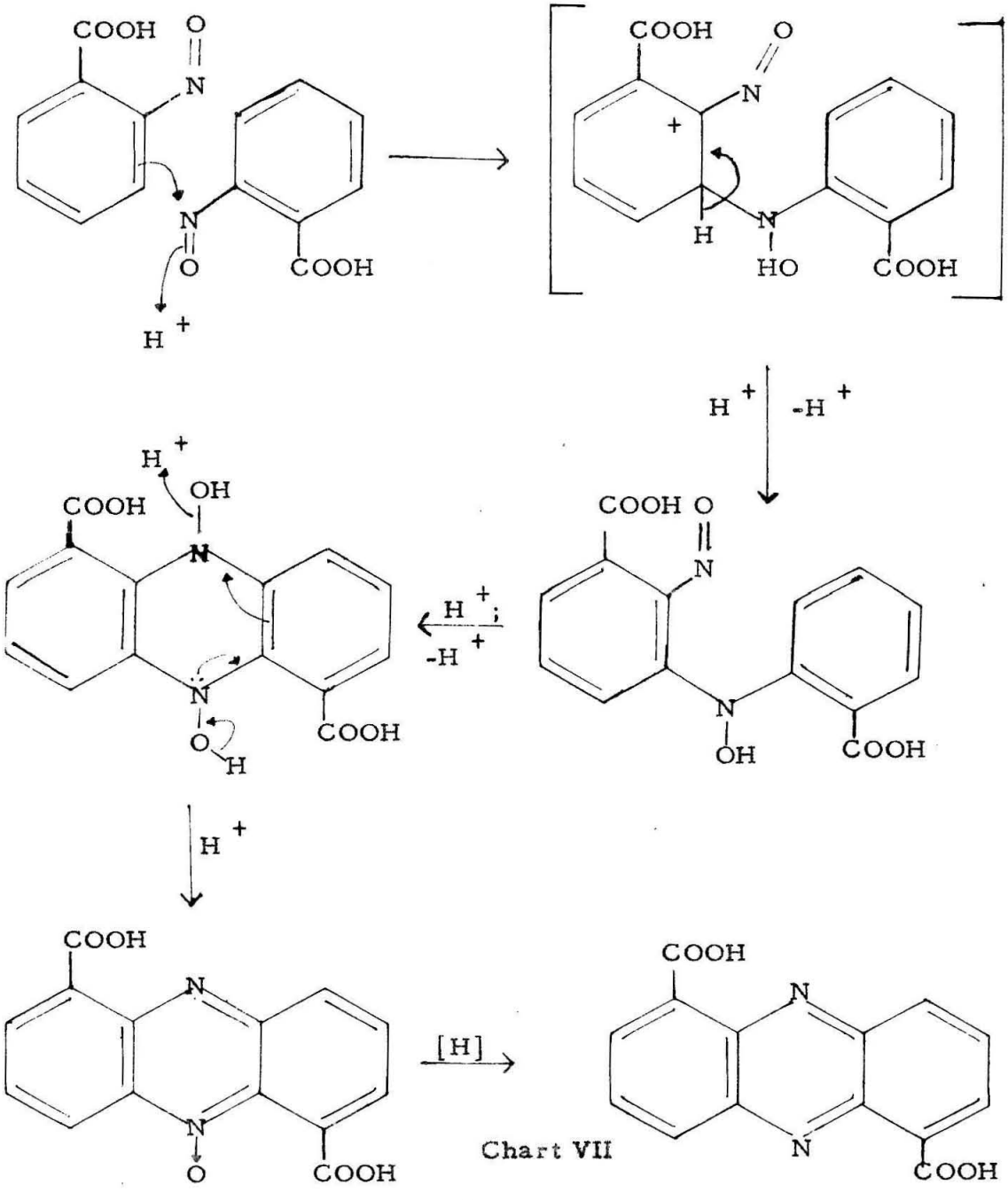
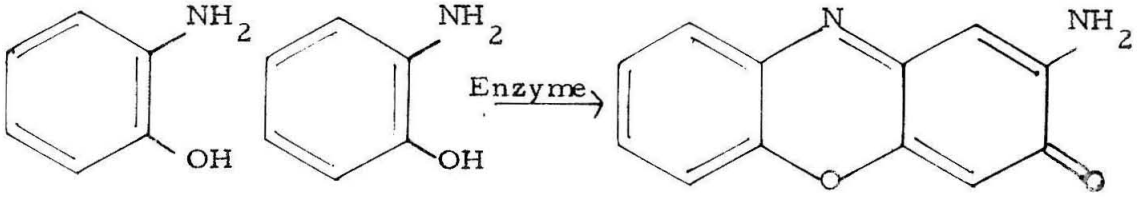


Chart VII

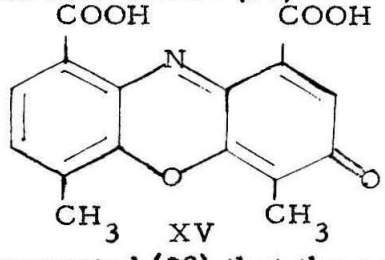
anthranilic acid ⟶ indole⟶ tryptophan ⟶ kynurenine ⟶ 3-hydroxykynurenine ⟶ 3-hydroxyanthranilic acid ⟶ nicotinic acid.

This is simply a branch of the anthranilic acid metabolism scheme shown in Chart V. The enzyme which mediates the key reaction in this sequence, i.e. the hydrolysis of 3-hydroxykynurenine to 3-hydroxy anthranilic acid, is present in mammalian liver, Neurospora, and Pseudomonas (34b).

The formation of the isophenoxazone ring system by some Actinomyces species is somewhat analogous to the proposed anthranilic acid dimerization discussed above and can be given brief mention here. Nagasawa et al. (38) have demonstrated that the enzymatic oxidation of o-aminophenol leads to an isophenoxazone:



Actinomycin D, one of the useful antibiotics isolable from Actinomyces antibioticus, has been established (39) as a bis-peptide derivative of XV.



XV

It has been suggested (39) that the actinomycins arise via an oxidative dimerization of a peptide derivative of 3-hydroxy-4-methyl

anthranilic acid. The dimerization of 3-hydroxy-4-methyl anthranilic acid has been carried out oxidatively in vitro by Brockmann and Muxfeldt (40). Birch and coworkers have shown (40a) by C^{14} labelling experiments that the methyl groups on the isophenoxazone ring in actinomycin are derived from methionine.

The structures of all the known naturally occurring phenazines (see Introduction) are compatible with a scheme of biogenesis which involves the condensation of two appropriately substituted six-membered rings which are on (or close to) the shikimate pathway. None of the natural phenazines has carbon substituents at any position other than the one and/or six positions except those which are N-methylated. However, N-methylation by a source of one-carbon fragments (methionine, for example) very commonly occurs in nature (41).

A study of the incorporation of anthranilic acid-carboxyl-C^{14}- into 1-hydroxymethyl-6-carboxy phenazine (XI), a metabolite of Streptomyces griseoluteus, would certainly prove extremely interesting. It could be easily shown whether or not this compound arises from the dimerization of two anthranilic acid units by removing the two carbon substituents consecutively in a sample of the pigment isolated from a culture to which labeled anthranilic acid had been added. The carboxyl group of the pigment could be removed first by the decarboxylation procedure used in this work (see p. 47); after oxidation of the hydroxymethyl group, it could subsequently be removed by a repetition of the same procedure.

Approach to the Problem

The organism chosen for study was a strain of Pseudomonas chlororaphis which produces an abundance of easily isolable pigment. This bacterium, a member of the class Schizomycetes, the order Pseudomonadales and the family Pseudomonadaceae, was originally isolated from the dead larvae of the cockchafer (42). It has been found to be pathogenic for mice, guinea pigs, frogs, fresh-water fishes and crayfish; its usual habitat, so far as is known (42), is decomposing organic matter and fresh water.

Pseudomonas chlororaphis NRRL B-977 was used in this study. The problem was attacked by the use of substrates specifically labeled with carbon-14. Anthranilic acid-carboxyl-C^{14} was an obvious choice and it was prepared by the method of Murray and Ronzio (43).

Since the pathways leading from alanine to anthranilic acid via shikimate are known (44), it was of interest to investigate the incorporation of alanine-C^{14} and alanine-2-C^{14} into the pigment of Ps. chlororaphis. The pathway discussed below is not unique; it represents just one of the possible paths from alanine to anthranilic acid.

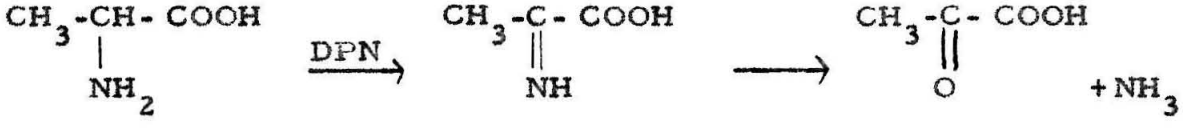
The fourth labeled substrate, sodium carbonate-C^{14}, was used to confirm our suspicion that it is reasonable to observe some of the label from anthranilic acid-carboxyl-C^{14} in the phenazine ring system. The metabolism of anthranilic acid (see p. 18) by Pseudomonas

species usually involves its decarboxylation; thus, if the incorporation of sodium carbonate-C^{14} into the ring system took place, we would surely be justified in assuming that carbonate derived from anthranilic acid would find its way into the ring in similar fashion. This is discussed in more detail in the Results and Discussion section (p. 33).

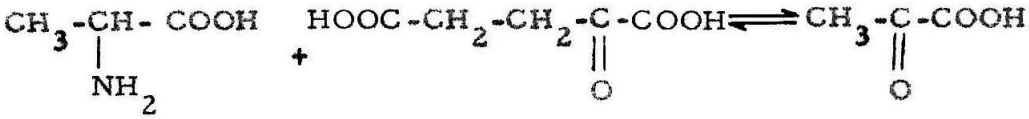
Pathway from Alanine to Anthranilic Acid

The conversion of alanine to pyruvic acid is a well-known biochemical reaction which may be carried out either by oxidative deamination, as, for example, in Bacillus subtilis (45) or by a transamination reaction known to occur in a variety of animal tissues, higher plants, and in many microorganisms (46).

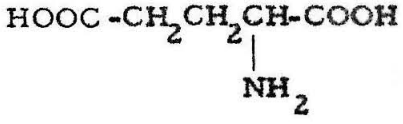
The oxidative deamination reaction in B. subtilis requires diphosphopyridinenucleotide (DPN) and results in a hypothetical intermediate imino propionic acid which is very rapidly hydrolyzed to ammonia and pyruvic acid:



The transamination reaction in which we are interested may be illustrated by a system studied in detail by Cohen (47) in swine heart muscle:



L-Alanine 2-Ketoglutaric Acid Pyruvic Acid +



L-Glutamic Acid

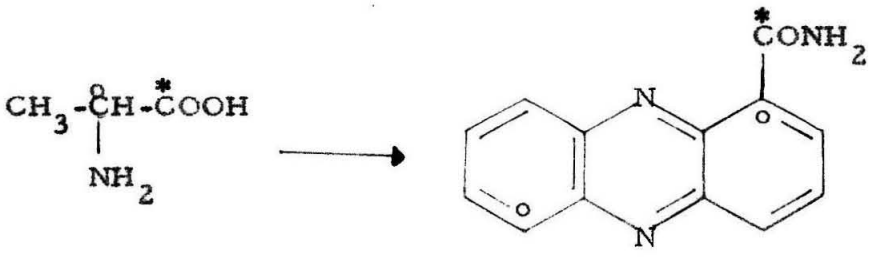
The same transamination occurs in microorganisms, such as Streptococcus faecalis (48).

The transformation of pyruvic acid into phosphoenolpyruvate is usually catalyzed by an enzyme ("pyruvate kinase") which requires magnesium ions and is a reaction of widespread occurrence in nature which needs no further comment here.

Phosphoenolpyruvic acid, as pointed out in the section on the biosynthesis of shikimic and anthranilic acids (see above, p. 14), is the three-carbon fragment which combines with erythrose-4-phosphate to form the seven-carbon sugar acid precursor of shikimic acid. Thus the most straightforward path for the incorporation of radioactive alanine into anthranilic acid is via the direct combination of phosphoenolpyruvate derived from the hot alanine with erythrose-4-phosphate synthesized from a non-radioactive source. The

anthranilic acid formed in this way will be labeled in the carboxyl group if alanine-1-C^{14} was added to the culture medium and in the 1-position if the added substrate was alanine-2-C^{14}*.

The implication here is that, to a first approximation, phenazine-1-carboxamide isolated from a culture to which alanine-1-C^{14} had been added will be labeled solely in the carboxyl group. Similarly, if alanine-2-C^{14} was the substrate, all of the activity will be in the phenazine portion of the molecule. Thus:

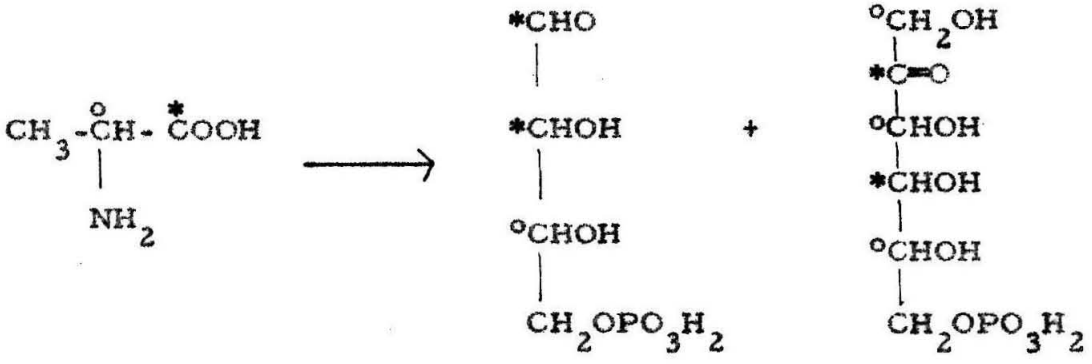


But, of course, this first approximation leaves completely out of consideration the many other metabolic paths open to alanine once it is converted to phosphoenolpyruvic acid. The path to be singled out for discussion here leads to erythrose-4-phosphate and is nothing more nor less than the reverse of the Embden-Meyerhof pathway for glucose metabolism followed by the metabolism of the glucose thus synthesized *via* the pentose phosphate path. Since both these pathways have been outlined above in Charts I and II, it will suffice to point out at this point

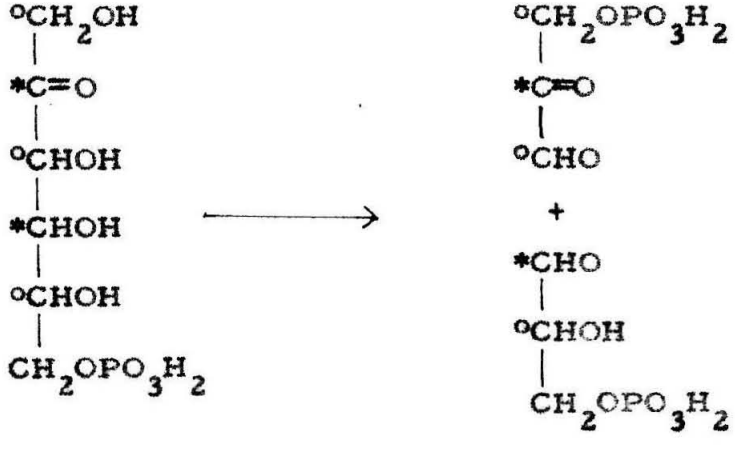
---

*In the drawings of the molecules depicted in the remainder of this section, the following convention will be observed: carbon atoms theoretically derived from alanine-1-C^{14} will be denoted by an asterisk (*) and those from alanine-2-C^{14} by a circle (o).

the distribution of radioactivity in the erythrose-4-phosphate and the fructose-6-phosphate obtained by this circuitous route:



The anthranilic acid ultimately obtained after the condensation of a labeled erythrose with a labeled phosphoenolpyruvate will have carbon-14 in both the ring and the carboxyl group whether the added substrate was alanine-1- or alanine-2-C^{14}. The way in which the label in alanine-2-C^{14} gets into the carboxyl group of anthranilic acid is simply the glycolytic cleavage of the circuitously formed fructose-6-phosphate to two three carbon fragments:



Therefore the label from alanine-2-C^{14} will appear in a molecule of phosphoenolpyruvate in both the one and three positions and will thus appear in the carboxyl group of anthranilic acid.

It should perhaps be briefly mentioned that the label from alanine-1-C^{14} has a path of incorporation which is not available to that from alanine-2-C^{14}. Namely, alanine may be oxidatively decarboxylated to acetic acid (40) and, as indicated in the experiment with sodium carbonate-C^{14} to be described below (p. 34), the carbonate thus formed may be incorporated into phenazine precursors. This path will certainly not be of any great importance and may be disregarded completely for our purposes.

All of these considerations lead to the following qualitative predictions: (1) alanine-1-C^{14} will produce phenazine-1-carboxamide in which the activity is primarily in the carboxyl group; and (2) alanine-2-C^{14} will produce phenazine-1-carboxamide in which the activity is primarily in the ring system.

Results and Discussion

The growth of the bacteria, the isolation and purification of the pigment, and the hydrolysis and decarboxylation of the phenazine-1-carboxamide thus obtained are described in detail in the Experimental section. In brief, the procedure was as follows: the labeled substrate was added to a culture of Pseudomonas chlororaphis NRRL B-977 (50) just before autoclaving.* During the isolation procedure (ether extraction of the culture medium), the original chlororaphin was entirely oxidized to phenazine-1-carboxamide. The amide was purified by chromatography on alumina and several recrystallizations from methanol. It was then hydrolyzed in base and the corresponding acid was purified by recrystallization from ethanol until its activity was the same within experimental error as the constant specific activity of the starting amide, thus rendering improbable the possibility that the observed activity was due to a highly active minor impurity. The amide derived from the incorporation of anthranilic acid-carboxyl-C^{14} was purified in the usual way, but its specific activity was not determined due to the small amount of this material available. This amide was converted directly to the acid and the latter was then purified to constant specific activity.

In order to determine the distribution of radioactivity between

*In the case of anthranilic acid-carboxyl-C^{14}, the sterilized substrate was added to a growing culture over a period of five days.

the phenazine and carboxyl moieties of the various pigment samples, the phenazine-1-carboxylic acid samples were decarboxylated in diphenyl ether at 260° C. in the presence of copper powder. The carbon dioxide was collected and converted to barium carbonate. The phenazine was purified by chromatography on alumina and recrystallization from ethanol, which yielded bright yellow needles with a sharp melting point (171° --- see Experimental section, p. 50 , and Table VII).

The specific activities were determined either by combustion to carbon dioxide, which was counted in an ion chamber by means of a vibrating reed electrometer, or by counting the samples directly on aluminum planchets in a Nuclear Chicago gas-flow counter model D47. The determination of the carbonate activity was used primarily as a check, since the activity in the carboxyl group was in all cases calculated by difference from that in the phenazine.

The results of these experiments are briefly summarized in Table II.

Anthranilic acid-carboxyl-C^{14} was incorporated to the extent of 0.002% and 21 $\pm$ 3% of the activity was found in the phenazine. It is known that anthranilic acid is very rapidly metabolized (35) by Pseudomonas species with loss of the carboxyl carbon as carbon dioxide and the formation of catechol (36) (vide supra). It is likely that some of the carbon dioxide resulting from this metabolic decarboxylation is incorporated into precursors of the phenazine pigments. Thus an

Table II

The Incorporation of Radioactive Substrates into Phenazine-1-Carboxamide

Substrates	% Incorporation	% Activity in Phenazine
Anthranilic acid-$C^{14}OOH$	0.002	21 ± 3
Sodium carbonate-C^{14}	0.0001	60 ± 10
Alanine-1-C^{14}	0.01	25 ± 4
Alanine-2-C^{14}	0.01	72 ± 2

*The fraction of incorporation was calculated by dividing the total added activity by the total activity recovered in the form of phenazine-1-carboxamide.

explanation is offered for both the relatively low percentage of incorporation of labeled anthranilic acid and the fact that all of the activity so incorporated does not reside in the carboxyl carbon of the phenazine pigment: the observed distribution of activity represents the sum of intact anthranilic incorporation and a more circuttous incorporation of the carboxyl carbon of anthranilic acid via a pathway that includes carbonate. The results obtained with added sodium carbonate-C^{14} complement the above data by showing that carbonate can indeed be used to make precursors of the phenazine ring system: 60% of the observed activity from carbonate incorporation was found in the phenazine. These results lend support to the suggested rationalization of the data from anthranilic acid incorporation. The poor incorporation

of the sodium carbonate-C^{14} (0.0001%) is at least partially due to the fact that the pH of the medium decreases as growth proceeds, thus releasing some of the carbonate into the atmosphere as carbon dioxide. It is no doubt also due to the circuitous route by which the carbonate is incorporated. That is, it must first be converted to a biologically active form and then enter the pool of one-carbon fragments from which it may be eventually removed as part of a phenazine precursor. The original radioactive material is greatly diluted during such a process.

The distributions of activity observed in pigment samples obtained after the addition of alanine-1- or 2-C^{14} to Ps. chlororaphis cultures are compatible with their incorporation via shikimate and anthranilic acid. The activity in the pigment from alanine-1-C^{14} was concentrated (~75%) in the carboxyl group, whereas that in the pigment from alanine-2-C^{14} was concentrated in the ring system (72%).

Such a distribution of activity was expected on the basis of the metabolic paths available for the conversion of alanine into possible phenazine precursors, and was discussed in detail above (p. 27). Thus the results of the alanine incorporations lend some support to the proposal of a shikimate path for phenazine biogenesis.

Conclusion

The experiments described herein have led to the first definitive

proposal of a possible biogenetic path for the natural phenazines. As pointed out in the section on the mechanism of biosynthesis, the exact nature of the species which form the phenazine ring are unfortunately not defined by these experiments, but they do at least indicate the appropriate path to be followed by future investigations.

EXPERIMENTAL

I. Materials and Methods

A. Bacteriological: Pseudomonas chlororaphis NRRL B-977 (50) was cultured by Dr. R. Pressman on a peptone-glycerol broth medium (4). A sample of the lyophilized culture from Dr. Haynes was grown on an "inoculum" medium for 48 hours at room temperature (22-24°C). The components of this medium are Difco peptone (2%), glucose (1%), salt solutions "A" and "B" (51) (0.5% each), and distilled water. Salt solution "A" contains dipotassium hydrogen phosphate (10 g) and potassium dihydrogen phosphate (10 g) in water (100 ml); salt solution "B" contains magnesium sulfate heptahydrate (10 g), sodium chloride (0.5 g), manganese sulfate tetrahydrate (0.5 g), and ferrous sulfate heptahydrate (0.5 g) in water (250 ml). The pH of the "inoculum" medium was adjusted to 7.7.

A loopful of a 48-hour growth on this medium was streaked on yeast-glucose agar of the following composition: Difco yeast extract (1%); glucose (1%); agar (2%); and distilled water. The pH of this medium also was adjusted to 7.7. After incubation at room temperature (22-24° C) for three days, colonies were selected and transferred to slants of yeast-glucose agar which were then used as stock cultures. These were kept refrigerated and fresh cultures were prepared at monthly intervals.

Transplants from the stock cultures were made to 500 ml Erlenmeyer flasks containing inoculum medium (100 ml); incubation at room temperature (22-24° C) for 24 hours on a horizontal shaker operating at 80-90 cycles per minute produced cultures which were used to inoculate the "production" medium; Difco peptone (1%), potassium nitrate (0.1%), sodium chloride (0.5%), glycerol (4%), and tap water. The pH of the medium was 7.2. Fernbach flasks (2800 ml) containing "production" medium (800 ml) were inoculated and put on the shaker at room temperature for five days.

All sterilizing was done by autoclaving at 120° C and 15 psi for 15 minutes. When radioactive substrates were used, the cultures were prepared as described above, except that the labeled compound was added to the medium just before autoclaving.

Table III

Addition of Labeled Substrates to Ps. chlororaphis Cultures

Substrate	No. flasks 800 ml. medium	Activity added (total mc)	Pigment yield (mg) (Crop 1; 1st recryst.)
Alanine-1-C^{14}	3	0.15	431.5
Alanine-2-C^{14}	3	0.15	205.7
Anthranilic Acid-$C^{14}OOH$	1	0.04	150 (a)
Sodium Carbonate-C^{14}	3	0.5	337.9

(a) Estimated from yield of phenazine-1-carboxylic acid after hydrolysis of amide.

The data in Table I show that the yield of pigment per flask does not vary greatly with the nature of the labeled substrate except in the case of alanine-2-C^{14}. The reason for this difference is unknown; it may simply reflect a variation in culture conditions which in turn affected the organism's pigment-producing capacity. It is interesting to note that the pigment which incorporated alanine-2-C^{14} had three to five times greater specific activity than that which incorporated alanine-1-C^{14} (vide infra).

B. Substrates:

Alanine-1- and -2-C^{14} were obtained from Merck & Co., Ltd., Canada.

Sodium carbonate-C^{14} was obtained from Nuclear-Chicago Corp., Des Plaines, Illinois.

Anthranilic acid-carboxyl-C^{14} was prepared by the method of Murray and Ronzio (43). However, it was difficult to obtain a good yield by this procedure. The procedure is therefore recorded here in full detail so that the identity of the material added to the cultures in the experiments with labeled anthranilic acid cannot be questioned.

Butyl lithium (14.6 m mole), freshly prepared in ether, was injected into the reaction flask by means of a hypodermic syringe. The syringe needle was equipped with a stopcock to prevent losses. The flask had been previously flushed with purified nitrogen and was now cooled to -30° with Dry Ice-acetone and kept filled with nitrogen.

To the butyl lithium solution was added o-bromoacetanilide (1.72 g, 8.0 m moles) in ether by means of an addition funnel. The mixture was stirred for 15 minutes at -30° and then cooled to -200° with liquid nitrogen. The reaction flask was evacuated to 0.1 mm and carbon dioxide, prepared from barium carbonate (780 mg, 3.9 m mole cold barium carbonate plus 20 mg, 0.1 m mole, 3.4 mc barium carbonate-C^{14}) by the action of concentrated sulfuric acid. Quantitative evolution of carbon dioxide was aided by stirring and warming. The carbon dioxide was stored in a trap at -200°.

The reaction mixture was warmed to -30° and stirred for several minutes; it was then re-cooled to -200° and carbon dioxide was re-admitted. This procedure was repeated until the pressure in the system (read on an open end manometer) became constant. Nitrogen was then admitted and the cold reaction mixture was treated with water (20 ml) containing concentrated hydrochloric acid (2 ml) and allowed to warm up. The ether layer was separated and washed with potassium hydroxide solution (1 N); the washings were combined with the aqueous layer, which was made basic and extracted with ether for several hours.

Dissolved ether was removed by warming and stirring, after which the basic solution was refluxed for 40 hours. It was then extracted with ether for three hours before being adjusted to approximately pH 3 and exhaustively extracted with ether. The ether extract was dried over anhydrous sodium sulfate; the removal of the solvent left a small

amount of a slightly yellow solid. The solid was dissolved in water and the solution was filtered; a very pale yellow solution resulted which will be referred to as the "stock solution" in what follows.

The stock solution was shown to contain primarily anthranilic acid by comparing its ultraviolet spectrum with that of authentic anthranilic acid: λ_{max} 324 mμ, $\epsilon = 1.9 \times 10^3$. The spectra were identical in the entire 230-400 mμ region. The stock solution was calculated to have a concentration of 7.2×10^{-4} M, or about 1 mg/ml. It was further shown to contain primarily anthranilic acid by paper chromatographic comparison with an authentic sample in two solvent systems: aqueous ammonium chloride (3%) and n-butanol saturated with water. The exploration of the paper strips with a Geiger counter showed that the anthranilic acid was separated from a very small amount of non-radioactive impurity.

Stock solution (0.2 ml) was diluted to 100 ml in a volumetric flask. A sample (0.2 ml) of this solution was pipetted onto each of three planchets for counting in a Nuclear-Chicago Gas Flow Counter, Model D47. (See p. 43 for counting technique.) The activity was calculated to be 8.25×10^6 cpm/ml stock solution.

Effect of Autoclaving on Anthranilic Acid: Anthranilic acid (52.6 mg, 0.38 m mole, m.p. 142.5-143°) was dissolved in water (10 ml) by warming on a hot plate. The solution was autoclaved at 120° C

and 15 lbs for 15 minutes. Adjustment of the pH to ~3 and continuous extraction with ether until the aqueous layer had no fluorescence in ultraviolet light led to the recovery of 49.7 mg, m.p. 142-5-143.5°. This represents a 94.5% recovery, so we may conclude that the sterilization procedure does not destroy a significant quantity of anthranilic acid.

C. Isolation and Purification of Pigment: The pigment was isolated by ether extraction of the culture medium. A typical example of the purification procedure follows:

Phenazine-1-carboxamide (103.6 mg, 0.46 m mole) was dissolved in chloroform (~ 10 ml) and chromatographed on a column of aluminum oxide (12 x 2 cm) which had been prepared in hexane. The elution of the column was begun with hexane; the eluent was then changed to ether and ether-chloroform mixtures containing consecutively 20, 40, 60, 70 and 100% chloroform. The amide was eluted with 100% chloroform and was thus separated from a small amount of red material which remained on the column and was not further investigated. After chromatography, the amide was recrystallized from methanol, yielding bright yellow needles (65.4 mg), m.p. 241.5-243° (lit. (52) 241°). A second crop (23.3 mg) of m.p. 239-241° was obtained by concentration of the mother liquor. Further recrystallization of the first crop from methanol yielded 41.6 mg, m.p. 241-242.5° .

D. Determination of Specific Activity by Gas Flow Counter:

A sample (6.968 mg) of phenazine-1-carboxamide which had been chromatographed as described above and recrystallized once from methanol was weighed on a Cahn Electrobalance and dissolved in spectral grade chloroform (10 ml from a volumetric pipet). Three samples (1 ml each) were pipetted onto sandblasted aluminum planchets (3 cm diameter), which had been previously washed in acetone. The solvent was removed by a heat lamp supported 29 cm above the planchets. The samples were counted on a Nuclear-Chicago Gas Flow Counter, Model D47. Each sample was counted at least three times and was sufficiently thin that a self-absorption correction was unnecessary. A typical example of data obtained in this way is given in Table IV.

Table IV

Typical Data Obtained by Gas Flow Counter

Sample: 6.968 mg phenazine-1-carboxamide from alanine-2-C^{14} incorporation.

Planchet No.	Time (Min. for 800 Cts.)
I	6.06
	6.50
	6.20
II	6.24
	6.23
	6.09
III	6.06
	6.21
	6.33

The figures in the table are the minutes required for the machine to register 800 counts from a given sample. The average number of counts per minute was calculated and the standard deviation determined. The number so obtained (123.6 $\pm$ 1.5 cpm in this case) was corrected for background (15.0 cpm) and the corrected value used to calculate the number of counts per minute per millimole (cpm/m mole), which was 3.48×10^4 for this sample. The amide was further recrystallized and its specific activity redetermined until the latter was constant. The first crop of the second recrystallization in this example had 3.54×10^4 cpm/m mole; the average of these two values, $3.51 \pm 0.03 \times 10^4$ cpm/m mole, is the constant specific activity of the amide in this example. A constant activity was reached in all cases by chromatography and 2-3 recrystallizations from methanol. The amide obtained from the incorporation of anthranilic acid-carboxyl-C^{14} was recrystallized twice and converted directly to the acid which was then recrystallized to constant specific activity (see Results and Discussion, p. 32).

E. Determination of Specific Activity by Wet Combustion:

A sample (19.244 mg) of phenazine-1-carboxamide obtained from sodium carbonate-C^{14} incorporation, which had been chromatographed as described above (part C) and recrystallized twice from methanol, was weighed on a Cahn Electrobalance in an aluminum weighing pan; the

sample and pan were transferred to a combustion tube which was then attached to the combustion system. (The details of the oxidation procedure and the complete structure of the combustion system are described in a set of mimeographed notes by W. A. Sheppard entitled "C^{14}-Measurement by Ionization Chamber Technique.") Oxidation fluid (53) (2-3 ml) was added to the sample and the mixture heated with a micro-burner until no more dense white fumes were given off. After the reaction mixture had cooled slightly, another portion (~3 ml) of oxidation fluid was added, and the heating procedure repeated. After slight cooling, cold carbon dioxide was bubbled through the system for 3-5 minutes; care was taken to ensure the complete filling of the ionization chamber, which was then placed on a vibrating reed electrometer for counting. The specific activity was determined by converting the slope of a line representing the build-up of voltage with time to counts per minute (cpm). Equation 1 was used to calculate the current:

$$I = \Delta v/\Delta t \times 10^{-3} \times C \qquad (1)$$

I is the current in amperes, Δv is the voltage change in millivolts, Δt is the time interval in seconds, and C is 10^{-11} farads. Equation 2

$$I \times 10^{16} \times 60 = cpm \qquad (2)$$

relates the current to the number of counts per minute.

At least five determinations of the slope were made for each sample. A typical example of data obtained in this way is given in

Table V for the sample described at the beginning of this section.

Table V

Typical Data Obtained by Wet Combustion-Electrometer Technique

Sample: 19.244 mg phenazine-1-carboxamide from $NaC^{14}O_3$ incorporation.

I ($Amps \times 10^{16}$)	Bkgnd. I (a) ($Amps \times 10^{16}$)	Sample I ($Amps \times 10^{16}$)
3.40		
3.54		
3.47		
3.30	2.41 ± 0.05	0.99 ± 0.06
3.36		
3.34		
Avg. 3.40 ± 0.04		

(a) Average of seven determinations

The figure for the sample in Table V ($0.99 \pm 0.06 \times 10^{-16}$ amp) was converted to 59.4 ± 3.6 counts per minute. Thus the specific activity is $0.69 \pm 0.04 \times 10^{3}$ counts per minute/millimole.

F. Hydrolysis of Phenazine-1-carboxamide: Phenazine-1-carboxamide (57.0 mg, 0.25 m mole, $3.51 \pm 0.03 \times 10^{4}$ cpm/m mole) from alanine-2-C^{14} incorporation and aqueous sodium hydroxide (33%, 70 ml) were placed in a 100 ml flask attached to a condenser by means of a rubber stopper and heated at reflux for 20 hours. After cooling, the reaction mixture was filtered twice through a pad of asbestos in a Gooch filtration apparatus. The greenish yellow residue was dissolved

in concentrated hydrochloric acid, the solution adjusted to $3 < pH < 4$, and then continuously extracted with ether until the aqueous layer was colorless. The extract was dried over anhydrous sodium sulfate and the solvent was evaporated on the steam bath. The residue was recrystallized from absolute ethanol, yielding bright yellow crystals (42.9 mg, 75%), m.p. 238-239° (lit. (52) 237°). Concentration of the mother liquor afforded a small second crop (5.8 mg) with melting point 236-238° . These two crops were combined and recrystallized to yield 39.8 mg, m.p. 238-239° . A sample (4.232 mg) was weighed and counted twice; the average of the two counts was $3.61 \pm 0.08 \times 10^4$ cpm/m mole. This is to be compared with the constant specific activity of the starting amide, which was $3.51 \pm 0.03 \times 10^4$ cpm/m mole. The average of these two values ($3.56 \pm 0.06 \times 10^4$ cpm/m mole) was used to calculate the percentage of radioactivity incorporated into the phenazine portion of the molecule after the latter was obtained from the decarboxylation of the acid. Amide samples obtained from the incorporation of the various radioactive substrates were treated similarly; the pertinent data are collected in Table VI.

G. Decarboxylation of Phenazine-1-carboxylic Acid:

Phenazine-1-carboxylic acid (32.8 mg, 0.146 m mole, $3.56 \pm 0.06 \times 10^4$ cpm/m mole) from alanine-2-C^{14} incorporation, copper powder (15 mg) and diphenyl ether (5 ml) were mixed in a 25 ml Erlenmeyer flask to

Table VI

Hydrolysis of phenazine-1-carboxamide samples obtained from incorporation of radioactive substrates

Origin	Phenazine-1-carboxamide Const. Sp. Act. (cpm/mmole x 10^{-4})	Amt. Hydrolyzed (mg)	Phenazine-1-COOH Yield (mg)	Const. Sp. Act. (cpm/mmole x 10^{-4})	Avg. Const. Spec. Act. (cpm/mmole x 10^{-4})	Counting Method
Alanine-1-C^{14}	1.23 ± 0.02	65.1	---	1.27 ± 0.03	1.25 ± 0.03	Gas flow
Alanine-1-C^{14}	--- (a)	87	38.2	4.10 ± 0.03	4.10 ± 0.03	" "
Alanine-2-C^{14}	3.51 ± 0.03	57.0	42.9	3.61 ± 0.08	3.56 ± 0.06	" "
Anthranilic Acid-$C^{14}OOH$	--- (a)	150(b)	108.8	$0.15_7 \pm 0.01_4$	$0.15_7 \pm 0.01_4$	" "
Anthranilic Acid-$C^{14}OOH$	--- (a)	150(b)	108.8	$0.42_8 \pm 0.01_6$	$0.42_8 \pm 0.01_6$	Wet combustion
$NaC^{14}O_3$	0.066 ± 0.005	199	105.7	0.059 ± 0.005	0.064 ± 0.005	" "

(a) Amide sample had been chromatographed and crystallized at least once and converted directly to acid.

(b) Estimated from yield of acid.

which a 14/35 standard taper joint was attached. A magnetic stirring bar was added and the flask attached to a Mini-Lab condenser through the top of which was passed a stream of purified nitrogen. The nitrogen passed through a U-tube containing mercury just before the reaction flask and thence to a trap containing aqueous sodium hydroxide (1 M, 25 ml), to which boiled water (25 ml) had been added. After the trap was a second U-tube containing mercury connected to the water aspirator. A slight positive pressure was maintained at the beginning of the system and a slight negative pressure at the end.

The reaction mixture was heated and stirred at about 260° (temperature ranged from 234-284° in this run) for five hours. At the end of this time, boiled aqueous ammonium chloride (1 M, 25 ml) was added to the solution in the trap. This solution was then transferred to an iodine flask containing boiled aqueous barium chloride (1 M, 25 ml). The barium carbonate precipitate was filtered through a medium porosity sintered glass filter, which was covered with a watch glass as often as possible during the operation. The precipitate was washed consecutively with boiling water, acetone and ether, and then dried in an oven at 119° . The expected weight of barium carbonate was 28.8 (theoretical) plus 8.3 (blank run) mg, or 37.1 mg; that obtained was 38.3 mg. (A blank run was made before each use of the decarboxylation system.)

The decarboxylation reaction mixture was put on a column of aluminum oxide (13 x 2 cm) made up in hexane. The flask was rinsed once with ether. Elution of the column was begun with hexane (100 ml) and then changed to a 1:3 ether-hexane mixture to elute the phenazine. The crude phenazine (23.4 mg, 89.4%) was recrystallized from absolute ethanol, yielding bright yellow needles (12.4 mg), m.p. 170-171° (lit. (52) 171°). A sample (6.990 mg) was weighed and prepared for counting as described above; its specific activity was calculated to be $2.56 \pm 0.02 \times 10^4$ cpm/m mole.

A sample of the barium carbonate (9.002 mg) was prepared for counting as follows: it was placed in a 25 ml Erlenmeyer flask to which was added ethanol (95%, 10 ml) from a volumetric pipet followed by a magnetic stirring bar. The flask was tightly corked and the suspension stirred for 5.5 hours. The stirrer was then slowed down to a speed of "10" (out of 100) and three samples (1 ml each) were pipetted onto aluminum planchets (3 cm diameter). The solvent was evaporated by a heat lamp supported 25 cm above the samples.

The weight of the barium carbonate sample was roughly corrected on the basis of the crude phenazine yield (89.4%) as follows: the maximum amount of barium carbonate obtained from the decarboxylation was calculated by multiplying the theoretical barium carbonate yield (28.8 mg) by the crude phenazine yield (89.4%), or (0.895) (28.8) = 25.7 mg. This number divided by the amount of barium carbonate

actually isolated in this run (38.2 mg) gives the fraction of the isolated material which is assumed to be radioactive: 25.7/38.3 = 0.671. The corrected weight of the barium carbonate sample was then obtained by taking this fraction of its total weight: (0.671) (9.002) = 6.047 mg. Thus the specific activity of the barium carbonate can be calculated to be $0.84 \pm 0.02 \times 10^4$ cpm/m mole.

The sum of the specific activities of the phenazine and barium carbonate samples is 3.40×10^4 cpm/m mole. Thus, 3.40/3.56 x 100 or 95.5% of the original activity was recovered. This implies that $72 \pm 2\%$ of the activity is in the phenazine (on the basis of the original activity), or $75 \pm 2\%$ on the basis of the recovered activity.

Table VII summarizes the data obtained in the decarboxylation of phenazine-1-carboxylic acid samples derived from the incorporation of the various substrates. The standard deviation of the specific activity as determined by gas flow counter or electrometer is given the greatest weight in calculating the approximate limits of error listed in the table. The percentage of activity in the carboxyl group was calculated by difference: the actual determination of the specific activities of the barium carbonate samples was used primarily as a check. The carbonate activities tended to be higher than would be predicted on the basis of the phenazine activities, and it was later found that this was due to contamination of the apparatus used for collecting hot carbon dioxide.

Table VII

Decarboxylation of Phenazine-1-COOH Samples

Substrate	Phenazine-1-COOH Const. Sp. Activity[a] cpm/mmole x 10^4	Amt. Decarboxylated (mg)	m.p.	Phenazine Sp. Activity gm/mmole x 10^4	% Orig. Activity
Alanine-1-C^{14}	4.10 ± 0.03	34.4	---	0.10 ± 0.006	25 ± 4
Alanine-2-C^{14}	3.56 ± 0.06	32.8	170-171°	2.56 ± 0.02	72 ± 2
Anthranilic Acid-C^{14}-OOH	0.15_7 ± 0.01_4	33.3	171-172°	0.04_1 ± 0.004	26 ± 2
Anthranilic Acid $C^{14}OOH$	0.42_8 ± 0.01_6	33.3	171-172°	0.09_4 ± 0.008	21 ± 3
$NaC^{14}O_3$	0.064 ± 0.005	56.8	171-171.5°	0.03_6 ± 0.003	60 ± 10

(a) See Table VI.

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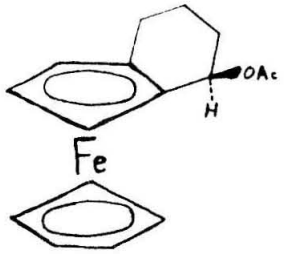
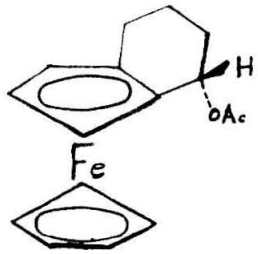
II. β-FERROCENYL CARBONIUM IONS

INTRODUCTION

The electron-releasing effectiveness of the ferrocene nucleus is well-attested by experimental observations. The evidence is discussed in detail by Hill (1) and will therefore be only briefly summarized here. Ferrocene is highly reactive in electrophilic acetylation reactions (2, 3, 4, 5) and in mercuration (6); Hill suggests (7) that the reactivity of ferrocene toward electrophilic substitution reagents places it in a class with such reactive aromatics as thiophene and furan, and that this reactivity implies that ferrocene should easily stabilize an adjacent carbonium ion by conjugative electron release. Hill and Richards (8), in several recent papers, present abundant support for the unusual stability of α-ferrocenyl carbonium ions and propose an iron-carbon interaction at the reaction center in the transition state as an important factor in this stability. Some of the most compelling evidence for metal participation arises from results obtained in the solvolysis of exo- and endo-α-acetoxy -1, 2-tetramethylene ferrocene (8b, 9). The exo isomer (I) solvolyzes in 80% acetone faster than the endo isomer (II) by a factor of



I



II

2570; the product in either case consists solely of the exo alcohol. Thus it is more favorable for the leaving acetate group to depart trans to the iron atom, and backside participation of the metal electrons in the stabilization of the forming carbonium ion is indicated. As Hill and Richards point out (8b), other factors such as steric hindrance by the lower ring to departure of the acetate group and repulsion of the acetate group by the filled iron orbitals may operate to decrease the rate of the endo isomer, but metal participation is probably the dominant effect in the exo isomer. A molecular orbital description (8a) of the iron-carbon interaction shows that it may involve the overlap of $3d_{\pm 1}$ iron orbitals* with a p-orbital of the α-carbon atom.

Thus the case for metal participation in α-ferrocenyl carbonium ions is reasonably well established. β-Ferrocenyl carbonium ions have not been extensively studied. Trifan and Bacskai (9) report a 537-fold rate increase in the solvolysis of β-ferrocenylethyl tosylate in 80% acetone compared with the corresponding phenyl compound. Hill (10) showed that the only product formed in the solvolysis of the ferrocene compound is β-ferrocenylethanol. The remainder of this part of this thesis will be a presentation and discussion of some results obtained in the solvolysis of β-ferrocenylethyl and β-ferrocenylisopropyl tosylates in 80% acetone at 30°C.

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*i.e., d_{xz} and d_{yz}; see footnote 21 in reference 8a.

CHAPTER I

Stabilization of β-Ferrocenyl Carbonium Ions

The fact that β-ferrocenylethyl tosylate solvolyzes 537 times faster than β-phenylethyl tosylate (9) is an indication that the ferrocene compound is an unusual primary tosylate. Trifan and Bacskai point out (9) that only a factor of ca. 3 of this rate difference is reflected in the $\Delta H^{\ddagger}$ terms. This implies, according to transition-state theory (11), that the remaining factor of ca. 179 is to be accounted for by a difference in $\Delta S^{\ddagger}$ for the two reactions. A simple calculation using the equation

$$537 = \frac{k_{Fc}}{k_{Ph}} = e^{(\Delta S^{\ddagger}_{Fc} - \Delta S^{\ddagger}_{Ph})/R} \; e^{-(\Delta H^{\ddagger}_{Fc} - \Delta H^{\ddagger}_{Ph})/RT}$$

where the subscripts Fc and Ph refer to the ferrocenyl and phenyl compounds respectively and the remaining symbols have their usual meanings shows that $\Delta S^{\ddagger}_{Fc}$ is more positive than $\Delta S^{\ddagger}_{Ph}$ by about 10 e.u.* Four theoretically possible driving forces which might account for this enhanced reactivity will now be considered. The first is anchimeric assistance due to hydride migration (10), which will produce an α-carbonium

*Some of the possible implications of this entropy difference are discussed below (p. 89).

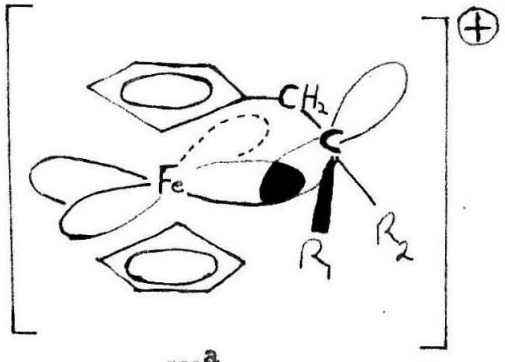
ion (if the hydride shift is complete) or a related species, either of which may be stabilized by the ferrocene group. A result of this could be the formation of a secondary as well as a primary alcohol in the solvolysis in aqueous acetone. Hill performed such a solvolysis (10) and was able to find only the primary alcohol by column chromatography and infra-red spectroscopy. The limit of detection of the secondary alcohol by these methods is about 2% (10).

The second possible driving force is an interaction with the π-electrons of the cyclopentadienyl ring to which the side-chain is attached. This is analogous to the phenyl participation postulated by Winstein et al. (13) to account for the solvolytic behavior of various 2-arylethyl tosylates. Evidence has been obtained concerning this possibility by a combination of deuterium labelling and nuclear magnetic resonance (NMR) spectroscopy, and it will be discussed in detail below (p. 67).

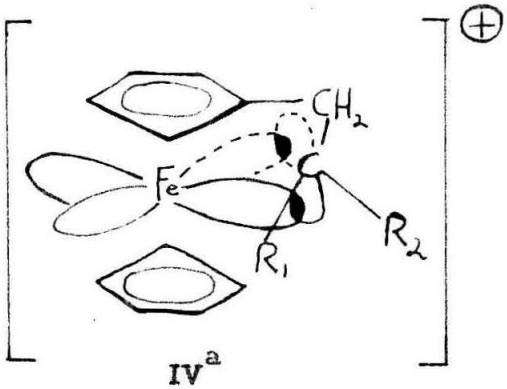
The third possibility is that the ferrocene nucleus may alter the rate by means of an inductive effect. However, as discussed below (p. 86), this effect is expected to be somewhat similar to that of a phenyl ring and to therefore decrease rather than increase the rate of S_N1 solvolysis.

The fourth possibility is the direct participation of the iron electrons in a filled d orbital, in analogy with the interaction proposed by Hill and Richards (8) to exist in α-ferrocenyl carbonium ions. Models for iron participation in β-ferrocenyl carbonium ions were proposed by

Hill (14) and involve a σ-bond formed between one lobe of a $d_{\pm 2}$* iron orbital and one lobe of the carbonium ion p-orbital (III) or a π-bond utilizing both lobes of the $d_{\pm 2}$ orbital and both lobes of the p-orbital (IV).



III[a]



IV[a]

[a] Taken from reference 1.

The former model is essentially backside participation and implies retention of configuration as the stereochemical result of solvolysis; the latter amounts to frontside participation and racemization is the predicted result.

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*$d_{\pm 2}$ refers to d_{xy} and $d_{x^2-y^2}$; see footnote 21 of reference 8a.

CHAPTER II

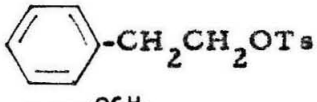
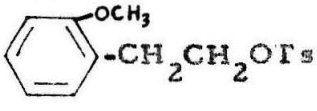
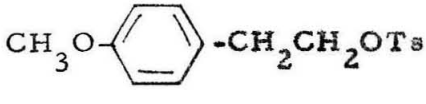
An Investigation of the Solvolysis of β-Ferrocenylethyl Tosylate by Deuterium Labelling and NMR

Introduction

The evidence for the existence of phenyl participation in the solvolyses of appropriate phenyl tosylates may be placed in three categories: (1) kinetic, (2) stereochemical, and (3) isotopic rearrangement. Winstein et al. (13) determined the solvolysis rates of several 2-arylethyl tosylates in ethanol, glacial acetic acid and formic acid at 75° and compared the observed rate constants with that for ethyl tosylate. A table of their data is given below (Table I). These relative

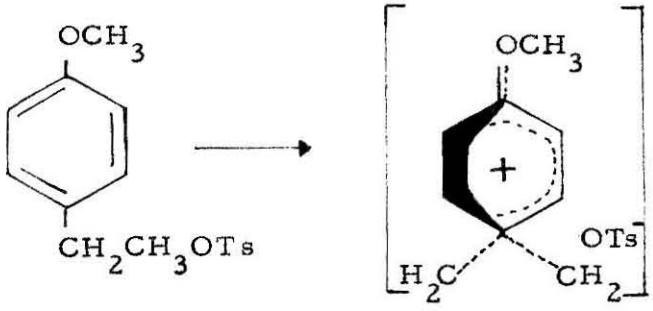
Table I[a]

Relative Rates of 2-Arylethyl Tosylates at 75°

Compound	Rel. k_{EtOH}	Rel. k_{HOAc}	Rel. k_{HCOOH}
CH_3CH_2OTs	1	1	1
	0.24	0.37	2.0
	0.28	8.9	93
	0.45	10.0	94

[a]Taken from reference 13.

rates are most easily explained if it is assumed that phenyl participation assists the ionization of the 2-anisylethyl tosylates in formic acid. In



the more nucleophilic but less ionizing solvent ethanol no rate enhancement is found for any of these compounds since phenyl participation cannot compete effectively with participation of the solvent. In acetic acid there are no clear indications that phenyl participation is very important, but, as Winstein et al. (13) point out, the data would be consistent with some successful competition of phenyl with solvent participation. For 2-p-anisylethyl tosylate Winstein et al. (13) estimated (including the inductive effect of the p-anisyl group, which decreases the rate by about one power of 10*) that the solvolysis of this compound in formic acid is at least 10^3 times as fast as solvolysis would be if it were of the limiting variety (15) (unassisted by backside solvent participation) and anchimerically unassisted.

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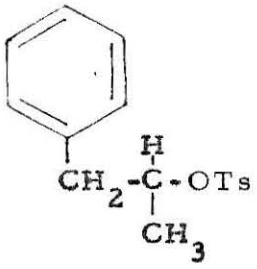
*Winstein et al. (28) estimated a factor of 10 rate decrease for the inductive effect of a phenyl group and assumed (13) that since p-methoxyphenylacetic acid is nearly as strong as phenylacetic acid the inductive effect of the p-anisyl group is about the same as that of the phenyl group.

Winstein and his co-workers (16) studied the stereochemical results of the solvolysis of β-phenylisopropyl tosylate (V) in ethanol,



V

acetic acid and formic acid. Table II (16) summarizes these results, which may be rationalized in the same terms as those used above in the discussion of the kinetic data, i.e., in formic acid participation of phenyl competes sufficiently well with solvent participation to make retention of configuration predominant, whereas in ethanol exactly the reverse is true.

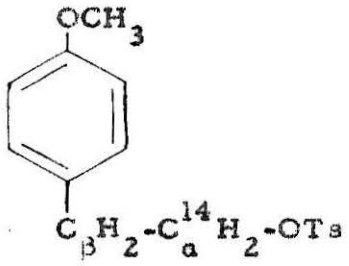
Table II[a]

Steric Results of Solvolysis of β-Phenylisopropyl Tosylate

Solvent	Inversion (%)	Retention (%)
EtOH	93	7
HOAc	65	35
HCOOH	15	85

[a]Taken from reference 16.

Essentially irrefutable evidence for a symmetrical intermediate in the solvolysis of 2-p-anisylethyl tosylate was obtained by Jenny and Winstein (17) by C^{14}-tracer methods. 2-p-anisylethyl tosylate specifically labelled with C^{14} at C_α (VI) was prepared and solvolyzed in ethanol,



VI

acetic acid and formic acid. The solvolysis products were degraded to determine the extent of C^{14}-scrambling between C_α and C_β. The results are presented in Table III, from which it is clear that phenyl participation is present exclusively (i.e., complete scrambling of label observed) in acetic and formic acids, but only partially in the more nucleophilic ethanol.

Table III[a]

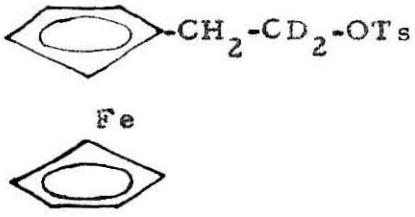
C^{14}-Scrambling in Solvolyses of p-Anisylethyl Tosylate-C^{14}

Solvent	Temp., °C	C^{14} in C_β of Product (%)
EtOH	75	23.9
HOAc	75	51.9
HCOOH	25	50.9

[a]Data from reference 17.

Results and Discussion

The experiments now to be discussed were suggested by analogy with the work of Jenny and Winstein (17)*. β-Ferrocenylethyl tosylate-α,α-d_2 was prepared from the corresponding alcohol by means of the usual tosyl chloride-in-pyridine reaction. The alcohol was obtained from ferrocenyl acetic acid (18) by reduction with lithium aluminum deuteride. The tosylate was solvolyzed in 50% aqueous acetone and in



acetic acid 0.01 M in acetic anhydride. The product in each case was isolated (in 53 and 67.6% yields, respectively**) and nuclear magnetic resonance (NMR) spectra were taken. Within the limits of detectability (see below, p. 71) no rearranged material was obtained from either solvolysis.

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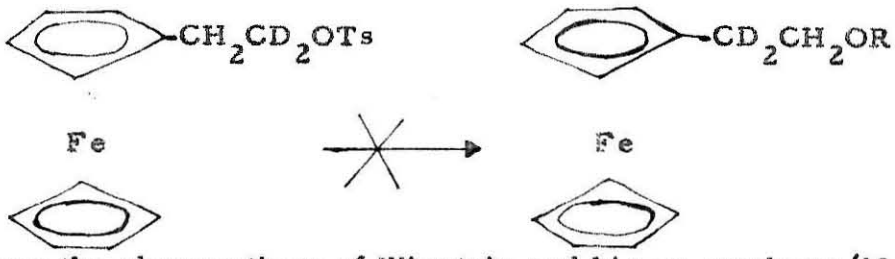
*The use of deuterium and NMR was suggested by Dr. E. A. Hill.

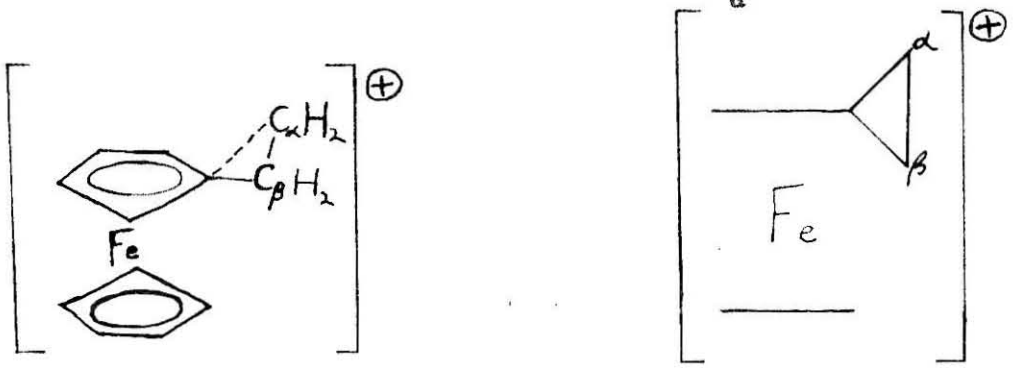
**The 53% yield of $FcCH_2CD_2OH$ was primarily due to losses during two recrystallizations from ligroin (30-60); the 67.6% yield of $FcCH_2CD_2OAc$ was due to the oxidation of some of the product to ferricinium ion which was not recovered from the aqueous layer and to loss during crystallization. (See Experimental Section for details.)



From the observations of Winstein and his co-workers (13,16,17), it is apparent that of the two solvent systems used in the present work, the acetic acid would provide the greater opportunity for the production of rearranged solvolysis product. Fifty percent acetone is certainly a more nucleophilic solvent than acetic acid, which implies a greater tendency for backside intervention in the former solvent. Winstein et al. (16) suggest the following sequence, in order of decreasing tendency for solvent participation: EtOH>MeOH>H_2O>HOAc>HCOOH. Fifty percent acetone would occur between H_2O and HOAc in this sequence. It hardly needs to be said that 50% aqueous acetone is a more ionizing solvent than acetic acid: the dielectric constant of acetic acid is only 6.2 whereas that of 50% acetone is probably near 50.* Therefore a carbonium ion will form much more readily in the aqueous solvent than in the acetic acid. The failure to detect rearrangement in either solvent may be taken as an indication that carbonium ion stabilization by interaction with the π-electrons of the cyclopentadienyl ring does not occur. However, note must be taken of the existence of an alternative

*The dielectric constants of acetone and of water are 20.7 and 78.54 respectively at 25°C ; that of 80% acetone-water is 29.4 (19). Even though the dielectric constant is often an inadequate measure of solvent ionizing power (20), the difference is so large in this case that in spite of inadequacies the conclusion is unaltered.

explanation: the interaction may occur, but C_α and C_β never become equivalent and there is thus solvent collapse at C_α only.



VII

In fact, even if C_α and C_β are allowed to become equivalent (VII), the "lower" ring may sterically hinder solvent collapse at C_β. Unfortunately, the present data do not permit us to rule out this explanation completely, but we may at least cast some doubt upon it by the following considerations. The hydroxyl frequency shifts in the infra-red for OH···ring bonding are essentially the same in β-phenylethanol and in β-ferrocenylethanol: Trifan, Weinmann and Kuhn (21) report a shift (relative to free hydroxyl) of 29 cm^{-1} for the phenyl compound, and Trifan and Bacskai (22) and Hill (23) observed a shift of 27 cm^{-1} for the ferrocenyl compound. This indicates that the "availability" of π-electrons for interaction is similar in these two compounds. An extension of this reasoning* leads to the conclusion that the corresponding tosylates should have similar reactivities in solvolysis if the carbonium ions are stabilized by interaction with the π-electrons of the respective rings.

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*The problem involved in such an extension is that the OH shifts represent ground state interactions whereas the application is to an excited state.

The factor of 537 that lies between the solvolysis rates (9) demonstrates the falseness of this conclusion.

If, however, in spite of all evidence to the contrary, the factor of 537 is attributed to some sort of special interaction between the ferrocenyl ring and the carbonium ion, one is led to predict a rate enhancement (compared to the corresponding phenyl derivative) in the solvolysis of 4-ferrocenyl-1-butyl aryl sulfonates and even in that of the 5-ferrocenyl-1-pentyl compounds. The 3-ferrocenyl-1-propyl compounds would be expected to solvolyze somewhat slower than the other ω-ferrocenyl-1-alkyl aryl sulfonates since a 4-membered ring would be formed in this case. Garwood (42a) measured the rates of acetolysis and hydrolysis (in 80% acetone) of brosylates in the ω-ferrocenyl-1-alkyl series (including 5-ferrocenyl-1-pentyl) and found no rate enhancement whatsoever and no significant differences between members of the series. In fact, the rate of hydrolysis of the 4-phenyl-1-butyl compound is somewhat faster than that of the 4-ferrocenyl-1-butyl compound. An analogy may be drawn (42a) between neighboring aromatic ring participation and electrophilic aromatic substitution: the high reactivity of ferrocene in certain electrophilic substitutions (see above, p. 58) suggests that the ferrocene ring should be an effective neighboring group. The absence of rate enhancement in the ω-ferrocenyl-1-alkyl brosylates, then, is good evidence for the absence of cyclopentadienyl ring participation.

Garwood (42a) investigated the mechanism of electrophilic substitution in metallocenes by means of competitive acetylations of ferrocene and ruthenocene and of ferrocene and 1,1'-diethylferrocene, and interpreted his results in terms of direct interaction between the metal and the entering electrophile. The report (23a) that ferrocene is protonated on the iron atom in BF_3-H_2O is in line with this interpretation. The analogy between neighboring aryl participation and electrophilic aromatic substitution may be extended (42a) to the metallocene case: i.e., since metal participation may occur when the electrophile is an acylonium ion, it may also occur when the electrophile is a β-carbonium ion. When, however, the electrophile is a γ-, δ-, or ε-carbonium ion metal participation cannot occur due to the appearance of unfavorable steric interactions when a ring of the necessary size is closed between the iron atom and the carbonium ion (42a).

The limit of detectability (LD) of rearranged solvolysis product by NMR was roughly determined by the addition of varying amounts of undeuterated β-ferrocenyl ethanol to solutions of the α,α-dideutero compound in carbon tetrachloride. The 60 Mc NMR spectrum of β-ferrocenyl ethanol has peaks at 5.80 τ (ferrocenyl protons), 6.22 τ (triplet for α-protons), 7.34 τ (triplet for β-protons) and 6.83 τ (OH) (see Fig. I). The spectrum of the α,α-dideutero compound has peaks at 5.78 τ (ferrocenyl protons), 7.35 τ (singlet for β-protons) and 6.55 τ (OH) (see Fig. II). (The position of the OH proton peak is concentration-

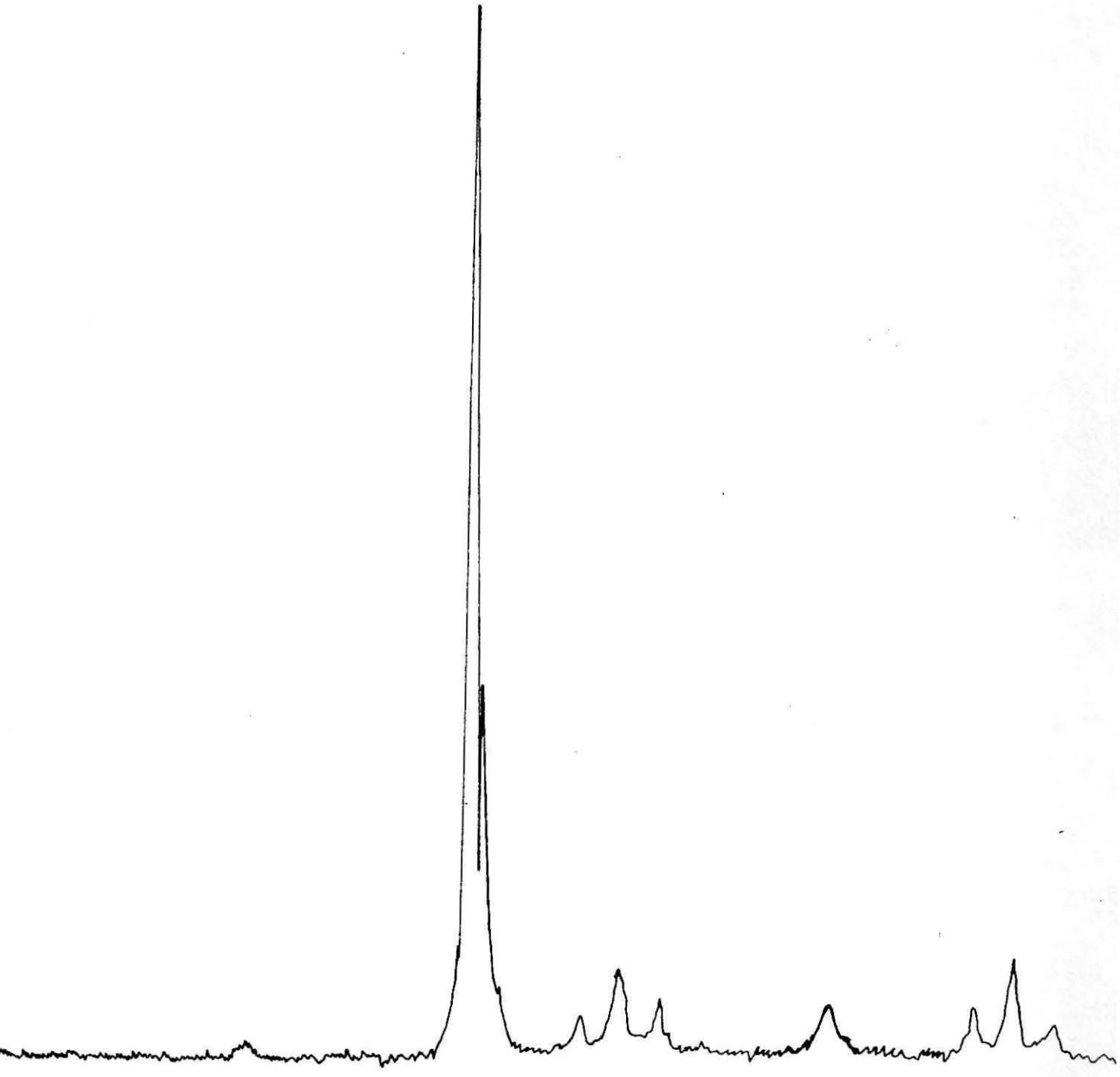


Figure I

60 Mc NMR Spectrum of β-Ferrocenylethanol[a, b]

[a] 14.8% solution in CCl_4

[b] Taken by R. C. Neuman, Jr.

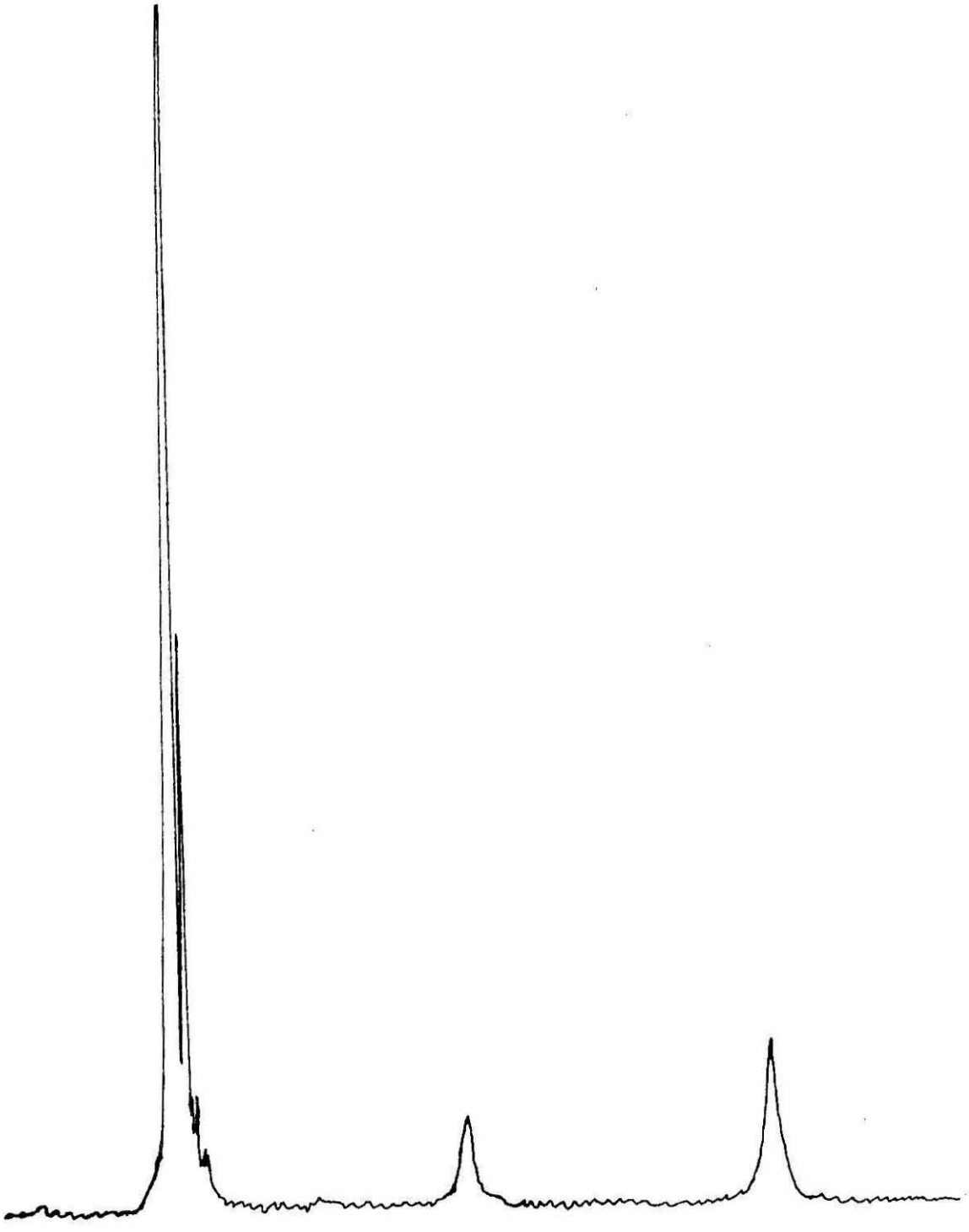


Figure II

60 Mc NMR Spectrum of Authentic β-Ferrocenylethanol-α,α-d_2 [a, b]

[a] 24% solution in CCl_4

[b] Taken by W. F. Beach

dependent.*) The data in Table IV show that the limit of detectability is $4.3\% < LD < 12.5\%$.

Table IV

Approximate Limit of Detectability by NMR of β-Ferrocenylethanol-β,β-d_2 in the Presence of β-Ferrocenylethanol-α,α-d_2

$Fc-\overset{A}{CH_2}CD_2OH$ (mg)	$Fc-\overset{B}{CH_2}CH_2OH$ (mg)	B/A	Remarks
120	30	0.25	Easily detectable
120	15	0.125	Easily detectable
205	8.8	0.043	Not distinctly observed

(Each sample was dissolved in 1 ml. of CCl_4.)

This, of course, is for undeuterated alcohol in the presence of α-deuterated material: the peak for the α-protons of the undeuterated compound is a triplet whereas it is expected to appear as a singlet in β-ferrocenylethanol-β,β-d_2, the rearranged alcohol. Due to this difference, it is most likely that a somewhat smaller amount of β-ferrocenylethanol-β,β-d_2 than of β-ferrocenylethanol itself might be detected since a single peak should be easier to distinguish from baseline noise than a triplet of the same area.** Thus we may assume with some confidence that

*Cf. effect of dilution of EtOH with CCl_4 on the NMR spectrum (24).

**The single α-proton peak in the deuterated compound will be somewhat broadened due to the small magnetic effect of the deuterium atoms, however (25) and thus difficult to see at very low concentrations.

at least 5-10% of rearranged alcohol would have been detected. Similarly, in the solvolysis of β-ferrocenylethyl tosylate-α,α-d_2 in acetic acid, at least 5-10% of the rearranged acetate would have been observed. The spectra were taken on fairly concentrated solutions in both cases for obvious reasons. (See Table V.)

Table V

Concentrations of Samples of β-Ferrocenylethyl tosylate-α,α-d_2 Solvolysis Products Used to Determine NMR Spectra

Solvolysis Medium	Product (mg)	CCl_4 (mg)	Conc'n. (Wgt.%)
50-50 Acetone-H_2O	183.9	480	27.7
Acetic Acid (0.01M Ac_2O)	383.0	656.9	36.8

The NMR spectra of unrearranged synthetic β-ferrocenylethanol-α,α-d_2 and the product from the solvolysis of β-ferrocenylethyl tosylate-α,α-d_2 are presented in Figures II and III, respectively. It is clear that the two spectra are identical except for the position of the OH peak, which, as noted above, is due to the concentration dependence of the OH resonance. A similar comparison of spectra in the acetolysis shows the two corresponding spectra to be completely identical since there is no concentration dependent peak in this case (see Experimental section).

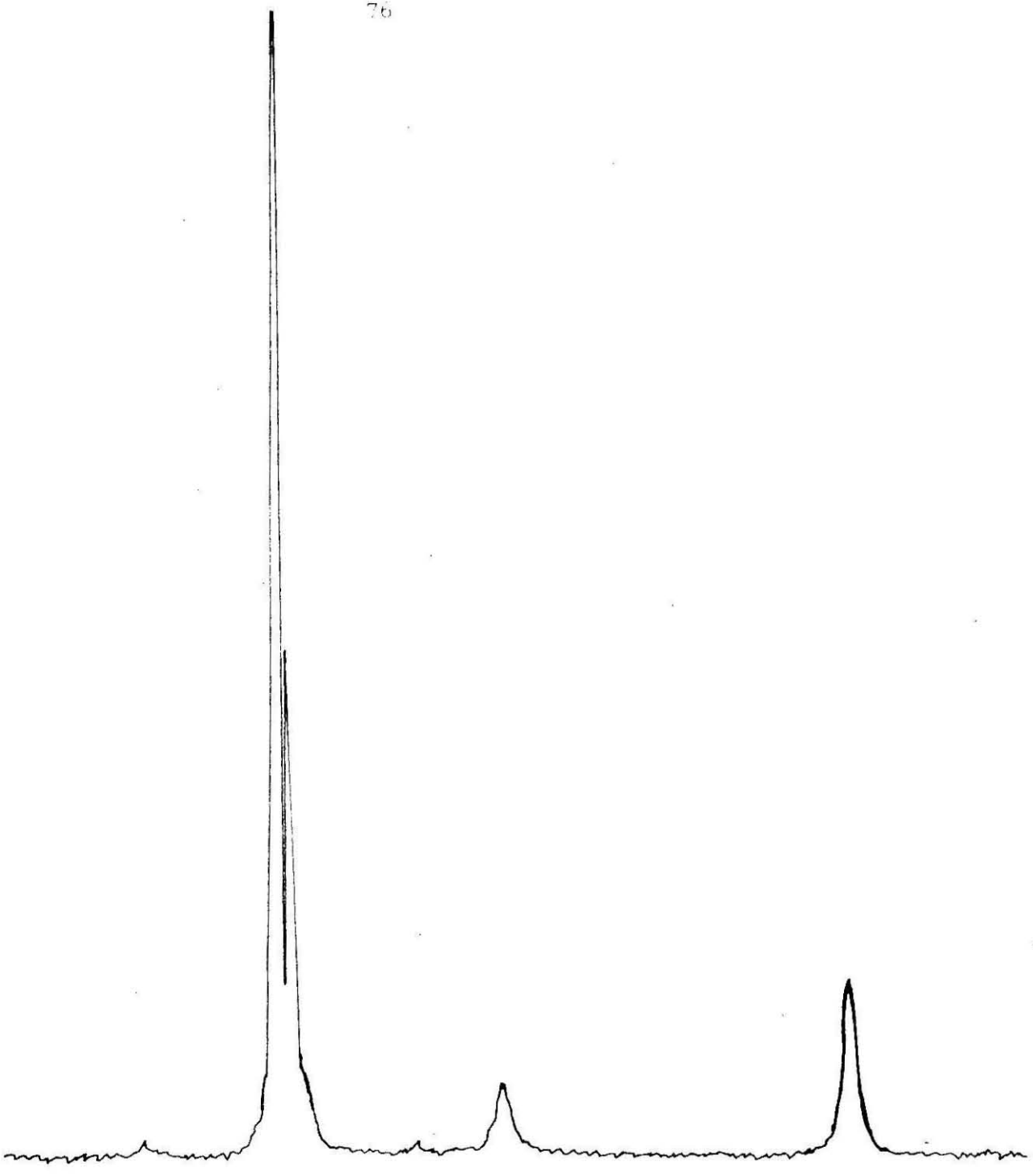


Figure III

60 Mc NMR Spectrum of β-Ferrocenylethanol-α,α-d_2 [a, b]

Recovered from Solvolysis in 80% Acetone

[a] 28% solution in CCl_4

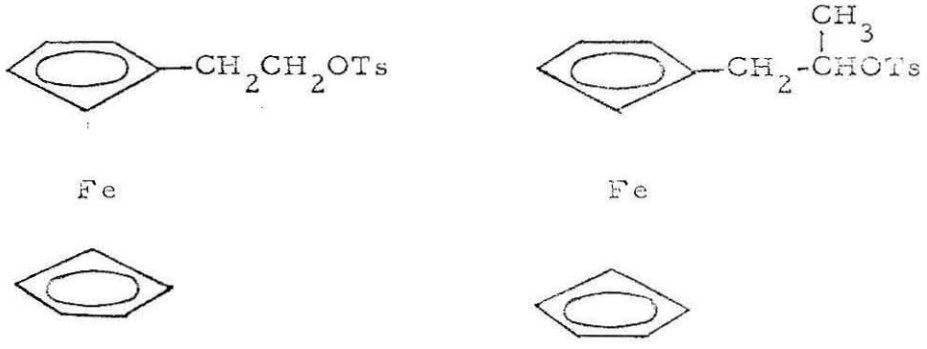
[b] Taken by W. F. Beach.

CHAPTER III

The Solvolytic Behavior of β-Ferrocenyl Tosylates

Introduction

The solvolyses of β-ferrocenylethyl and β-ferrocenylisopropyl tosylates in 80% acetone-water followed first-order kinetics to at least



80% reaction, beyond which there appeared to be a slight slowing down of the rate (in the case of β-ferrocenylethyl tosylate, at least, this amounted to about a 10% decrease in rate). This was not further investigated, but it is certainly not serious for our purposes and may simply be due to the phenomenon of external ion return, an effect proposed by Winstein et al. (26) in which the toluenesulfonic acid produced during the reaction causes a "common ion" rate depression.

In what follows it will be assumed that these solvolyses are unimolecular (S_N1) in nature by analogy with numerous other primary and secondary tosylate solvolyses which have been studied in greater detail and for which an S_N1 mechanism is indicated (27).

Table VI

Solvolyses of β-Ferrocenyl Compounds

Compound	Temp. °C	Initial Concn. (M)	Solvent	$10^5 k(sec^{-1})$
β-Ferrocenyl ethyl tosylate	30.00	0.00483 0.00486	80% acetone	1.10 ± 0.01[a]
b-Ferrocenyl ethyl tosylate-α,α-d_2	30.00	0.00488 0.00495	80% acetone	$0.86_2 \pm 0.00_4$[a]
dl-β-Ferrocenyliso-propyl tosylate	30.00	0.00440	80% acetone	1.53
(-)-β-Ferrocenyliso-propyl tosylate	26[b]	0.00479	60% acetone pH 6.9	5.76[c]

[a] Average of two runs.

[b] Ambient temperature.

[c] The rate levelled off at this value after an initial rapid rise (see Experimental Section for details).

The Solvent

In order that any comparisons at all may be made between the results obtained in the ferrocenyl solvolyses and those in many other systems in a variety of solvents, it will first be necessary to determine the place of 80% acetone in the established solvent sequence (see below) with regard to nucleophilicity and ionizing power. Nucleophilicity refers to the ability of a solvent molecule to act as a displacing group on carbon

in solvolytic displacements (27a). Other expressions which describe this property are "tendency toward backside attack of solvent" and "tendency toward solvent participation" in the transition state. Ionizing power is a collective term which includes all of those properties of a solvent which enable it to solvate ions. The dielectric constant, hydrogen-bonding ability (for solvating anions) and availability of electron pairs (for solvating cations) of a given solvent are important in the assessment of ionizing power (20).

The solvent sequence, in order of decreasing nucleophilicity, which was established by Winstein and his co-workers (16) (see above) is EtOH > MeOH > H_2O > HOAc > HCOOH. The first three members of this series may be considered to have similar nucleophilic powers which are much greater than those of the last two; these last two may be regarded as approximately equal in nucleophilicity (29). Since acetone is probably inert as far as nucleophilicity is concerned, it seems reasonable to place 80% acetone-water in this sequence somewhere between H_2O and HOAc, and probably closer to the latter than to the former. Swain, Mosely and Bown (29a) have determined the following "nucleophilic constants" (relative to 80% ethanol = 0.00) for the indicated solvents: MeOH, -0.05; H_2O, -0.44; 80% acetone, -0.45; HOAc, -4.82; and HCOOH, -4.44.

Grunwald and Winstein (30) have defined the ionizing power (Y) of a solvent as the logarithm of the rate of solvolysis of t-butyl chloride therein relative to its rate of solvolysis in 80% aqueous ethanol:

$$\log (k/k_o) = Y .$$

Fainberg and Winstein (31) give the value -0.673 for 80% aqueous acetone. Thus 80% acetone is found to be a poorly ionizing solvent (e.g., relative to formic acid) and we may write the following solvent sequence in order of decreasing ionizing power: H_2O (3.493) > HCOOH (2.054) > 80% acetone (-0.673) > HOAc (-1.675) > EtOH (-2.033) (31).

The Effect of an α-Methyl Group

β-Ferrocenylisopropyl tosylate solvolyzes 1.4 times faster than the β-ferrocenylethyl compound in 80% acetone at 30° C. It is reasonable to suppose that in S_N1 reactions there is some relation between the effect of an α-methyl group and the amount of positive charge which develops at the nascent carbonium ion in the rate-determining transition state. The effect of an α-methyl group in tosylate solvolyses has been considered in detail by Winstein and his co-workers (13, 28, 33), and in the light of their conclusions the effect observed in the β-ferrocenyl case will now be discussed.

Winstein, Grunwald and Jones (15) proposed the term "limiting" to describe solvolyses in which backside solvent participation is absent. They derived equation 1 to express the effects of solvent ionizing power (Y) and nucleophilic character (N) on rates of solvolysis.

$$d \log k = (\partial \log k/\partial Y)_N dY + (\partial \log k/\partial N)_Y dN \qquad (1)$$

This allows a mathematical definition of "limiting" systems as those for which $(\partial \log k/\partial N)_Y = 0$. Since carbonium ions are stable in the order tertiary > secondary > primary, it is to be expected that sensitivity to N should increase in the order primary > secondary > tertiary. This is strikingly shown for a tertiary relative to a secondary compound in the solvolyses of t-butyl and isopropyl bromides in formic acid, 50% ethanol and absolute ethanol (32): the relative rates ($k_{t\text{-Bu}}/k_{i\text{-Pr}}$) in these solvents are $\sim 10^6$, ~17000 and 1140, respectively. The solvolysis of the tertiary compound in the poorly nucleophilic formic acid should be close to limiting (15); therefore the effect of an α-methyl group in a limiting solvolysis is to increase the rate by at least a factor of 10^6. Winstein and Marshall (33) have pointed out that comparable rate increases are expected for isopropyl relative to ethyl compounds in limiting solvolyses. * The rate factor for the compounds under discussion here (β-ferrocenylisopropyl/β-ferrocenylethyl) is only 1.4 and thus it appears that these solvolyses are very far from limiting. However, as some of the relative rates listed in Table VII will demonstrate, neighboring group participation very effectively decreases the α-methyl effect. As Winstein and Marshall (33) point out, the magnitude of the effect is somewhat dependent on temperature, solvent and structure even for limiting solvolyses and therefore only large differences in rate ratios are meaningful. The

*Winstein and Marshall (33) estimate a factor of at least 10^4 in tosylate solvolyses.

Table VII

Rate Differences in Tosylate Solvolyses Due to α-Methyl Substitution

Compound Pair	Solvent	Temp., °C	Ratio of solvolysis rates	Ref.
(1) $\frac{CH_3CH_2OTs}{CH_3OTs}$	EtOH	75	0.43	33
	HOAc	75	0.87	33
	HCOOH	75	1.8	33
(2) $\frac{(CH_3)_2CHOTs}{CH_3CH_2OTs}$	EtOH	70	3.3	37
	HCOOH	75	200	33
(3) $\frac{(CH_3)_2CHCH(OTs)CH_3}{(CH_3)_2CHCH_2OTs}$	HCOOH	75	2240	33
	HCOOH	25	5200	33
(4) $\frac{(CH_3)_3CCH(OTs)CH_3}{(CH_3)_3CCH_2OTs}$	HCOOH	25	12500	33
(5) $\frac{(C_6H_5)CH_2CH(OTs)CH_3}{(C_6H_5)CH_2CH_2OTs}$	EtOH	30	1.6	16[a]
	HOAc	50	37	13
	HOAc	30	31	a
	HCOOH	30	65	a
	80 Acetone	30	~10	b
(6) $\frac{(p\text{-}CH_3OC_6H_4)CH_2CH(OTs)CH_3}{(p\text{-}CH_3OC_6H_5)CH_2CH_2OTs}$	HCOOH	30	57	13, 16
	HOAc	50	27.7	c
	EtOH	50	~10	c
(7) $\frac{Fc^{d}\text{-}CH(OAc)CH_3}{Fc\text{-}CH_2OAc}$	80% Acetone	30	10.5	36
(8) $\frac{Fc\text{-}CH_2CH(OTs)CH_3}{Fc\text{-}CH_2CH_2OTs}$	80% Acetone	30	1.4	e

[a] Rates at 30°C extrapolated from data at other temperatures in references 13 and 16.

[b] Crudely estimated as described on p. 84

[c] The rate for the primary compound was extrapolated from data in reference 13.

[d] The symbol Fc is for the ferrocenyl group.

[e] Present work.

factor for an α-methyl in compound pairs 3 and 4 in Table VII (10^3-10^4) is of the order of magnitude expected for a limiting tosylate solvolysis and this is not surprising since steric hindrance to backside solvent participation in these compounds (34) is probably important. The methyl, ethyl and isopropyl tosylates (compound pairs 1 and 2) undergo solvolyses which are far from limiting even in formic acid. This is reasonable on two accounts: first, there is little to hinder solvent participation sterically; and second, the carbonium ions produced in these systems have little means for internal stabilization and therefore interact readily with solvent molecules which help disperse the positive charge. Compound pairs 5 and 6, in which participation by neighboring phenyl (in formic acid) has been demonstrated by carbon-14 labelling techniques (17, 35), show quite low α-methyl effects which change in a qualitatively predictable way with the nucleophilic character of the solvent. Thus the rate ratio for the β-phenylisopropyl vs. the β-phenylethyl compound decreases by a factor of ~40 on changing from formic acid to ethanol (at 30°). This is an indication of the fact that solvent participation is not completely absent in systems with neighboring groups.

Ferrocenylcarbinyl acetate and methylferrocenylcarbinyl acetate (compound pair 7) were studied in detail by Hill (36) who concluded that the solvolyses of these compounds in 80% acetone were close to limiting on the grounds that iron participation is indicated to be present by other evidence and that backside solvent attack is sterically hindered. We may

therefore conclude, in the case of the small α-methyl effect in compound pair 8 (β-ferrocenylisopropyl and β-ferrocenylethyl tosylates) that the explanation in terms of a non-limiting solvolysis is not unique. The data presently available are unfortunately insufficient to resolve this question, but it is obvious that what is now needed is the determination of the α-methyl effect in a variety of solvents such as acetic acid, ethanol and ethanol-water mixtures. Formic acid is unfortunately a strongly oxidizing medium and cannot be used for the solvolysis of ferrocene derivatives.

A note about the estimation of the rate factor for β-phenylisopropyl vs. β-phenylethyl tosylate in 80% acetone (compound pair 5) is appropriate here. The rate for the β-phenylethyl compound was calculated from the data of Trifan and Bacskai (9) and the rate of β-ferrocenylethyl tosylate as determined in the present work. The rate of the β-phenylisopropyl compound in 80% acetone was extrapolated from those in formic and acetic acids by use of the Grunwald-Winstein Y-correlation (30) in the following way: Grunwald and Winstein (30) found that the logarithms of the solvolysis rates of many compounds give linear correlations in Y which can be expressed by the equation

$$\log k = mY + \log k_o$$

where k_o is the rate of solvolysis in 80% aqueous ethanol and m measures the sensitivity of the compound, relative to t-butyl chloride, to the ionizing power of the solvent. If the rather poor assumption is made that

80% acetone has a nucleophilicity comparable to those of acetic and formic acids, the second term in equation 1,

$$(\partial \log k/\partial N)_Y \, dN$$

may be dropped ($dN \sim 0$). Then $(\partial \log k/\partial Y)_N = m$, and an "apparent" m value may be calculated for a compound from rates of solvolysis in formic and acetic acids:

$$\frac{\log k_{HCOOH} - \log k_{HOAc}}{Y_{HCOOH} - Y_{HOAc}} = m_{app.}$$

This was done for β-phenylisopropyl tosylate after extrapolating the solvolysis rates in formic and acetic acids to 30° C from the data given by Winstein et al. (13), and an apparent m of 0.750 was obtained. To estimate the rate in 80% acetone at 30° C, this value of m was used in the equation

$$\log(k_{HCOOH}/k_{Me_2CO\text{-}H_2O}) = m_{app.}(Y_{HCOOH} - Y_{Me_2CO\text{-}H_2O}),$$

which led to a value of 2.3×10^{-7} sec^{-1}. As a sort of control calculation, a similar operation was performed for β-phenylethyl tosylate: the extrapolated rate was 2.5×10^{-8} sec^{-1} which agrees fairly well with the experimental value of 2.05×10^{-8} sec^{-1} calculated on the basis of the work of Trifan and Bacskai (9). The crude extrapolation for the β-phenylisopropyl compound allows an estimation of the rate enhancement due to replacement of the phenyl group by a ferrocenyl group in this case

(i.e., $k_{FcPrOTs}/k_{PhPrOTs}$): the rate ratio is ~66. This is smaller by about a factor of 10 than the rate increase for the corresponding ethyl compounds (537) and is simply a reflection of the larger effect of the α-methyl (~10) in the phenyl compound than in the ferrocenyl compound (1.4). It is tempting to speculate that the reason for this difference is to be sought in a larger amount of participation of neighboring iron than of neighboring phenyl, but of course the difference is too small to permit such speculation to have much validity.

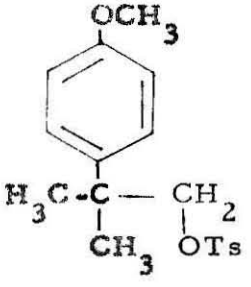
It is important to have some idea of the rate of solvolysis of β-ferrocenylethyl tosylate which would be expected in 80% acetone in the absence of neighboring group participation in order to assess the magnitude of the rate enhancement. The difficulties involved in making such an estimation are great, and are primarily due to the facts that the rate of solvolysis of ethyl tosylate, the "standard" compound, is not known in 80% acetone and that the inductive effect of a ferrocene group on the rate has not been estimated. It will be shown below that the inductive effect of a ferrocene group is probably quite similar to that of a phenyl group (28) and therefore will decrease a solvolysis rate by a factor of about 10. A crude approximation of the rate of ethyl tosylate in 80% acetone at 30° may be made by an extrapolation based on the rates in acetic and formic acids, as described above for the estimation of the rate of β-phenylisopropyl tosylate in 80% acetone. This yields a value of $\sim 10^{-8}$ sec^{-1}. Semenow and Roberts (38) calculated the dipole moment

of 1,1'-di(p-chlorophenyl)-ferrocene to be 3.12 D for the configuration in which the cyclopentadienyl rings are eclipsed. (The corresponding value when the rings are antiprismatic is 2.97 D.) The experimental dipole moment is 3.12 D, in good agreement with the calculated figures. One of the assumptions underlying the calculation is that only the C-Cl moments make important contributions to the dipole moment. This implies that phenylferrocene is expected to have zero dipole moment, or in other words that the inductive effects of the two groups are similar. As Semenow and Roberts point out, this expectation is supported by the close agreement between the dipole moment of acetophenone (2.97 D), measured by Hassel and Uhl (39), and that of acetyl ferrocene (3.02 D), measured by Richmond and Frieser (40). Further, the fact that the pKa values of ferrocene carboxylic acid (4.4) (41) and benzoic acid (4.20) (42) in water are quite close also suggests the similarity of the inductive effects of the phenyl and ferrocenyl groups.* Therefore, if the factor of 10 rate decrease estimated for the phenyl group (28) is applied to the present case, a rate enhancement in the solvolysis of β-ferrocenylethyl tosylate in 80% acetone at 30° by a factor of $\sim 10^3$ may be calculated. This rate enhancement is of the same order of magnitude

*Garwood (42a) determined the following pKa's in ethanol-water (density 0.8916 g/cm^3) at 25°: ferrocene carboxylic acid, 6.61; benzoic acid, 6.13; ferrocenyl acetic acid, 6.4; and phenyl acetic acid, 6.08. This implies that the electron-withdrawing inductive effect of the phenyl group is somewhat stronger than that of the ferrocenyl group and therefore that a factor of 10 for a rate decrease due to β-ferrocenyl may be an overestimate.

as that which may be estimated from the data of Winstein et al. (13) in the solvolysis of p-anisylethyl tosylate in formic acid at 75° compared to ethyl tosylate (at least 10^3), an enhancement which would no doubt be decreased in the more nucleophilic solvent 80% acetone.

Recent work by Smith, Fainberg and Winstein (43a) allows an interesting direct comparison to be made between the rate of solvolysis of p-methoxyneophyl tosylate (VIII) and that of p-ferrocenylethyl tosylate in 80% acetone. The observed first-order rate constant for the p-



VIII

methoxyneophyl compound is considered to be the rate constant for purely anchimerically assisted ionization (43a). (In other words, it is assumed that unassisted solvolysis does not proceed.) The rate for this compound in 80% acetone at 75° is 53.0×10^{-5} sec.$^{-1}$; unfortunately, $\Delta H^{\ddagger}$ was not measured for the reaction in this solvent, but if we estimate a value of ~22 kcal/mole* the extrapolated rate at 30° is ~0.5×10^{-5} sec.$^{-1}$. This is to be compared with the value of

*Smith, Fainberg and Winstein (43a) report the following $\Delta H^{\ddagger}$ values (kcal/mole) for this reaction in the indicated solvents: absolute ethanol, 23.56; 80% ethanol, 21.75; methanol, 23.12; 80% methanol, 21.31; and acetic acid, 23.09.

1.10×10^{-5} sec.$^{-1}$ obtained for β-ferrocenylethyl tosylate in the present work. Thus the rate of the ferrocenyl compound is quite comparable to that of a compound which owes its reactivity solely to neighboring group participation.

Entropy of Activation as an Indication of Participation

Winstein and Heck (43b), on the basis of rate comparisons of a number of primary tosylates in ethanol, acetic acid and formic acid, concluded that anchimerically unassisted solvolyses tend to have a $\Delta S^{\ddagger}$ of ca. -18 ± 2 e.u. while the assisted solvolyses have $\Delta S^{\ddagger}$ of ca. -7 ± 2 e.u. Thus for β-phenylethyl tosylate the values are: ethanol, -20.2 e.u.; acetic acid, -17.3 e.u.; and formic acid, -9.5 e.u. This indicates the predominance of neighboring phenyl participation in formic acid which was later proven by C^{14}-labelling experiments (35) (see above, p. 66) As pointed out in Chapter I (p. 60), $\Delta S^{\ddagger}$ for β-ferrocenylethyl tosylate is ~10 e.u. more positive than that for β-phenylethyl tosylate in 80% acetone (9). It is reasonable to expect the solvolysis of the phenyl compound to be unassisted in 80% acetone and therefore to have a $\Delta S^{\ddagger}$ in the -18 ±2 e.u. class. This indicates that the solvolysis of the ferrocenyl compound is anchimerically assisted. In view of the above discussion of the results of the deuterium experiment (pp. 68ff), the entropy difference is yet another indirect indication of neighboring iron participation.

CHAPTER IV

The Solvolysis of Optically Active β-Ferrocenylisopropyl Tosylate

Introduction

Doering and Zeiss (43) proposed a useful general scheme to account for the stereochemical results of solvolytic displacement reactions which occur in the absence of special effects such as neighboring group participation. Their scheme is summarized in Chart I for the specific case in which the leaving group is the tosylate group and the displacing group is a water molecule.

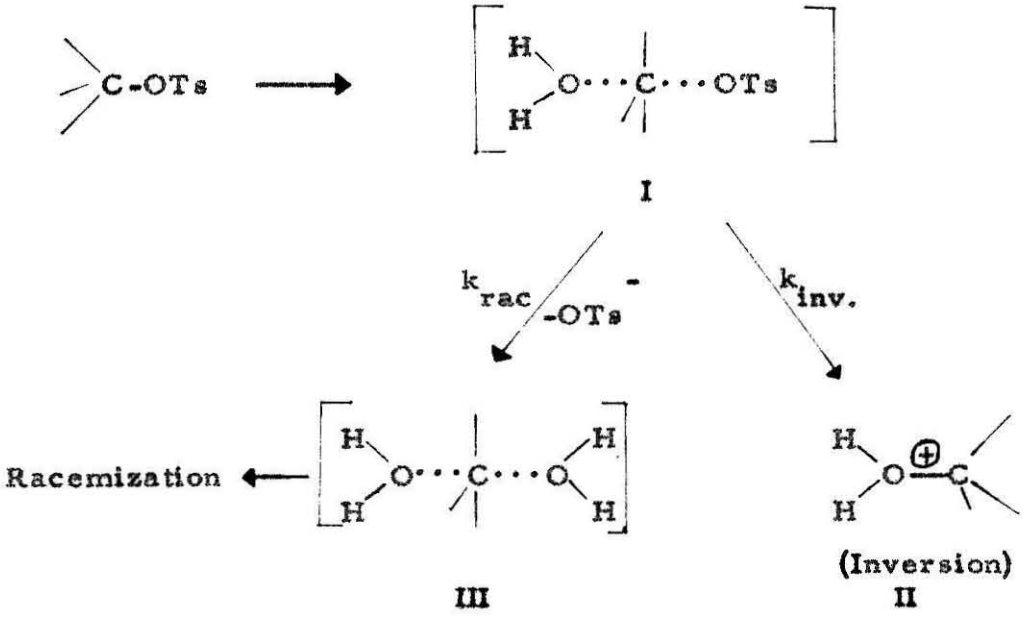


Chart I[a]

[a]Adapted from Streitwieser (44).

The stereochemical outcome of a particular solvolysis reaction is dependent upon the relative magnitudes of k_{inv}, the rate constant for inversion, and k_{rac}, that for racemization. The Doering-Zeiss scheme, as Streitwieser (44) points out, avoids the rather vague concepts of carbonium ion "longevity" and "shielding" which are involved in the Hughes-Ingold theory of the stereochemistry of S_N1 reactions (45). It is obvious that the nature of the solvent (e.g., its capability for backside participation), the stability and structure of the carbonium ion, and the nature of the leaving group will all have some effect on the ratio k_{inv}/k_{rac}. It may be argued, for example, that in the absence of special effects (see below) a relatively more stable carbonium ion will yield more racemized product than a less stable one in the same solvent system simply due to the greater ease of displacement of the leaving group (I ⟶ III) from a more stable carbonium ion. In other words, a less stable carbonium ion requires a stronger solvent-carbon interaction for stabilization than does a more stable one; therefore by the time intermediate I is reached covalent bond formation has proceeded further in the former case than in the latter and an inverted product (II), which contains a full solvent-carbon covalent bond, is more readily formed. The fact that the ratio k_{inv}/k_{rac} is usually greater in the solvolysis of secondary carbinyl systems than in those of tertiary systems may be easily rationalized on the basis of the above considerations. The basic

Doering-Zeiss scheme must sometimes be modified and/or extended to accommodate special effects which are observed in certain systems, as the following examples will demonstrate.

The solvolysis of optically active 2-octyl tosylate in absolute ethanol results in 2-ethoxy octane which is 100% inverted (46). This is perhaps a reasonable result in view of the high nucleophilicity of ethanol and its tendency toward backside participation. But even in the poorly nucleophilic solvent acetic acid at 75°, the solvolysis of 2-octyl tosylate is accompanied by about 90% inversion (46). The explanation suggested for this phenomenon is the formation of a tight ion pair, from which the tosylate group does not very easily exchange with a solvent molecule, as in the conversion I ⟶ III in Chart I, but from which the tosylate group may be eventually displaced by backside attack of a solvent molecule with consequent inversion of configuration. The tight ion pair is thought (46) to be stabilized by the interaction of the empty p orbital of the carbonium ion with the oxygen orbitals of the leaving group.

Recent work by Winstein et al. (19, 47) and by Pocker (48) on the racemization of optically active p-chlorobenzhydryl chloride in 80% aqueous acetone at 25° has shown that the rate of racemization is 2-3 times greater than the rate of solvolysis. The simple Doering-Zeiss scheme predicts that k_{rac} should at most be equal to the solvolysis rate. This implies, therefore, the presence of an additional mechanism for racemization which either accompanies the one suggested in the scheme

of Doering and Zeiss or operates alone. The mechanism proposed by Winstein and his co-workers (19, 47) and by Pocker (48) is the following: ion pairs are present even in 80% acetone (which one would think should be, relative to acetic acid at least, capable of solvating ions rather efficiently) and, in the words of Winstein et al. (47) these ion pairs "lose configuration and return to the covalent condition" more rapidly than they dissociate or react with solvent to yield solvolysis products. Pocker (48) suggests that the members of the ion pair rotate relative to one another and so lose configuration.

The effect of a neighboring group is another "special effect" which requires an extension of the Doering-Zeiss scheme. In the presence of a neighboring group which can participate at the backside of a carbonium ion, in effect replacing the solvent molecule pictured in I, predominant retention of configuration is observed, provided that the solvent is of a suitably non-nucleophilic character and does not compete effectively with the neighboring group. An example of this, the solvolysis of β-phenyl-isopropyl tosylate in formic acid, was taken from the work of Winstein et al. (16) and discussed above (p. 65) in another connection.

Results and Discussion

Ferrocenyl acetic acid was prepared by the method of Lednicer, Lindsay and Hauser (18) and was converted to ferrocenyl acetone by reaction with methyl lithium in anhydrous ether.* The ketone was not isolated,

*The methyl lithium reaction was suggested by Dr. K. Kopecky.

but was converted directly to dl-β-ferrocenylisopropanol by lithium aluminum hydride reduction. Optically active β-ferrocenylisopropanol ($[\alpha]_D^{25°}$ + 17.24° $CHCl_3$, c 2.09) was obtained via the acid phthalate ester and the strychnine salt. The alcohol was converted to the tosylate ($[\alpha]_D^{25°}$ -66.34° $CHCl_3$, c 2.05) with tosyl chloride in pyridine. The rotation of the tosylate did not change significantly upon recrystallization (see Experimental Section, p. 136) and was thus assumed to be essentially optically pure.

The solvolysis of optically active β-ferrocenylisopropyl tosylate in 60% aqueous acetone at 26° in a "pH stat" set at pH 6.9 (see Experimental Section for details) produced β-ferrocenylisopropanol, the rotation of which could not be accurately determined. This may have been due to the presence of minor impurities which blocked the path of the light through the polarimeter tube. In any case, the alcohol was converted to the tosylate which has about a 4-fold greater rotation, and is much easier to work with due to its better crystallizing properties. Furthermore, a fairly accurate value of the rotation of optically pure tosylate was available whereas this was not true for the alcohol. The tosylate was completely racemic; i.e., its rotation was 0.00±0.01°.

The solvolysis was run in 60% acetone rather than the usual 80% acetone due to the very sluggish response of the pH meter electrodes in the latter solvent.* Sixty percent acetone may reasonably be expected to be more nucleophilic than 80% acetone; that is, the former is closer

*See Experimental Section for further discussion of this point.

to absolute ethanol in the solvent sequence discussed above than is the latter (p. 79): EtOH >MeOH>H_2O>60% Acetone > 80% Acetone > HOAc> HCOOH. One would predict, on the basis of this greater nucleophilicity, that k_{inv}/k_{rac} for an ordinary secondary tosylate would be greater in 60% acetone than in 80% acetone. However, the ionizing power of a solvent also has an effect on k_{inv}/k_{rac}: an increase in ionizing power would tend to lower the amount of net inversion. The Y value for 60% acetone (31) is +0.796, compared to a value of -0.673 for 80% acetone. Unfortunately, since appropriate data are not available, it is impossible to estimate the net result of these opposing effects, and we therefore cannot predict how different k_{inv}/k_{rac} is expected to be in 60% acetone compared to 80% acetone. It might be expected, however, on the basis of the place of 60% acetone in the solvent sequence and by analogy with results obtained in other solvents with secondary tosylates, that the stereochemical result of the solvolysis of such a compound in 60% acetone would be predominant net inversion. Obviously more experiments are necessary to justify this expectation: for example, the stereochemistry of the solvolysis of 2-octyl tosylate in 80% and in 60% acetone would be interesting.

The rate of solvolysis of β-ferrocenylisopropyl tosylate in 60% acetone at 26° is greater by a factor of about 3.8 than the rate at 30° in 80% acetone.* This is of course due to the combined effects of the

*See Experimental Section.

increased nucleophilicity and ionizing power of the 60% solvent.

A constant pH was desirable for this solvolysis since it was shown in separate preliminary experiments that the product is racemized in the presence of p-toluene sulfonic acid (in 80% acetone) and that the tosylate yields alcohol with complete inversion of configuration in 80% acetone in the presence of a 10% excess of hydroxide ion. The latter observation is due, no doubt, to the predominance of an S_N2 mechanism for the solvolysis under these conditions. The racemization of β-ferrocenylisopropanol by p-toluenesulfonic acid probably proceeds via the same carbonium ion which is involved in the solvolysis. In the absence of special effects one would predict predominant inversion in the

$$ROH + H^+ \rightleftharpoons ROH_2^+ \rightleftharpoons R^+ + H_2O$$

solvolysis in 60% acetone (as pointed out above) and racemization of the alcohol by acid in 60%* acetone. Since on the contrary we observe complete racemization in the solvolysis, it may be that the reason for this observation is to be sought in the nature of the carbonium ion rather than in the nature of the solvent. If some "special effect," such as neighboring group participation, is present in the formation of the carbonium ion, it must obviously operate to produce racemized product.

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*The fact that the alcohol is racemized by TsOH was actually determined in 80% acetone, but it is certainly reasonable to expect the same thing to occur in the 60% solvent. It is also reasonable to expect the alcohol to be stable to racemization in 60% acetone at pH 6.9; a control experiment was not done to test this point, but it does not appear possible to visualize a rational path for racemization under neutral conditions.

Iron participation is an attractive rationalization for these observations, as will be indicated in the sequel. A simple diagram representing a carbonium ion stabilized by interaction with the iron atom is discussed below (p. 114): an interesting result of this discussion is that while the iron-carbon interaction may be quite weak, the predicted stereochemical result is racemization. In the absence of more data such as the rate of the racemization compared to that of the solvolysis, iron participation must remain conjectural.

The argument that the observed racemization is simply a fortuitous combination of equal amounts of retention and inversion or equal amounts of retention, inversion and racemization, cannot be ruled out on the basis of the present data. It is considered unlikely, however, that such is the case since there are only two possible reasonable ways in which retention of configuration could occur in this system: the first is backside participation by the cyclopentadienyl ring to which the side chain is attached, and this has been shown to be unlikely by experiments reported in this thesis (p. 67), by the similar H-bonding abilities of phenyl and ferrocenyl rings, and most conclusively by the work of Garwood (42a) which was discussed above (p. 70). The second is backside participation by iron electrons which, as mentioned above, is indicated to be less favorable than the "frontside" participation that yields racemized product. (See below, p. 123-124)

Another argument which must be considered is that the racemization

is due to the formation of an ion pair which loses configuration upon internal return as discussed above in the case of p-chlorobenzhydryl chloride (19, 47, 48). In 80% acetone, the rate of racemization reported by Winstein, Hojo and Smith (19) is only twice that of the solvolysis. In acetic acid the rate ratio is 32 (in the presence of 0.012M LiOAc)(19). It seems very reasonable to assume that in 60% acetone, which must be even more dissociating than 80% acetone, this ratio would be very close to 1.0; i.e., ion pair formation is not expected to be very important in 60% acetone. Furthermore the fact that the p-chlorobenzhydryl carbonium ion is somewhat more stable than the β-ferrocenyl isopropyl carbonium ion implies that the racemization of the former should occur more readily than that of the latter. A crude indication of the greater stability of the p-chlorobenzhydryl cation is that even though the chloride ion is a poorer leaving group than the tosylate group by at least a factor of 100 (49), the solvolysis of p-chlorobenzhydryl chloride in 80% acetone at 25° is faster by a factor of 1.5 than the solvolysis of β-ferrocenylisopropyl tosylate in the same solvent at 30°.

CHAPTER V

The Secondary α-Deuterium Isotope Effect in the Solvolysis of β-Ferrocenylethyl Tosylate-α,α-d_2

Introduction

The term "secondary deuterium isotope effect" (k_H/k_D) refers to an alteration in the rate of a reaction which is brought about by the substitution of a deuterium for a hydrogen in a bond which remains unbroken in the rate-determining step. The first such effect was reported by Lewis and Boozer in 1952 for the decomposition of an alkyl chlorosulfite in dioxane (50): k_H/k_D was found to be 1.37 at 61.5° and 1.58 at 77.5°. In this case the deuterium was in the β-position relative to the reaction center; we are here concerned with the type of secondary isotope effect due to deuterium in the α-position. Streitwieser et al. (51) carefully studied the α-effect in the acetolysis of cyclopentyl-1-d p-toluenesulfonate, for which k_H/k_D = 1.15 at 50° C. As will be seen later (p. 106), this figure is representative of the ordinary size of this effect.

The Origin of the α-Deuterium Effect in Solvolytic Reactions

The major source of the α-deuterium effect in S_N1 solvolyses is considered by Streitwieser (51, 52) to be the rehybridization (sp^3 to sp^2) of the carbon at the reaction center in proceeding from the tetrahedral ground state to the trigonal, carbonium ion-like transition state (52). Such a change in hybridization is usually associated with changes in the stretching and bending frequencies of the C-H bonds at the reaction center. Since the zero-point energy (energy of the first vibrational level) of a C-D bond is lower than that for the corresponding C-H bond and since the zero-point energy (E_o) is related to the frequency by the equation

$$E_o = \frac{1}{2} h\nu_o \qquad (2)$$

it is obvious that if the frequency of vibration of a bond is lowered in a transition state, the zero-point energy of that bond is also decreased. It has been shown by Streitwieser et al. (51) and by Melander (53) that the Bigeleisen equation for the calculation of isotope effects on the rates of reactions (54) may be reduced by suitable approximations and assumptions to a form in which zero-point energy is the dominant effect to be considered:

$$k_H/k_D \cong \prod_i \exp\left[\left(1 - \frac{1}{1.35}\right)\left(\frac{h}{kT}\left(\nu_{H_i} - \nu^{\ddagger}_{H_i}\right)\right)\right] \Big/ 2 \qquad (3)$$

No term in ν_o appears in this equation since the approximation

$$\nu_o = \nu_H/1.35 \qquad (4)$$

has been made (55). When the values of the constants h and k are inserted into equation 3 and a small amount of algebraic juggling is performed, equation 5 is the result.

$$k_H/k_D \cong \exp\left[\frac{0.187}{T}\sum_i\left(\nu_{H_i} - \nu^{\ddagger}_{H_i}\right)\right] \qquad (5)$$

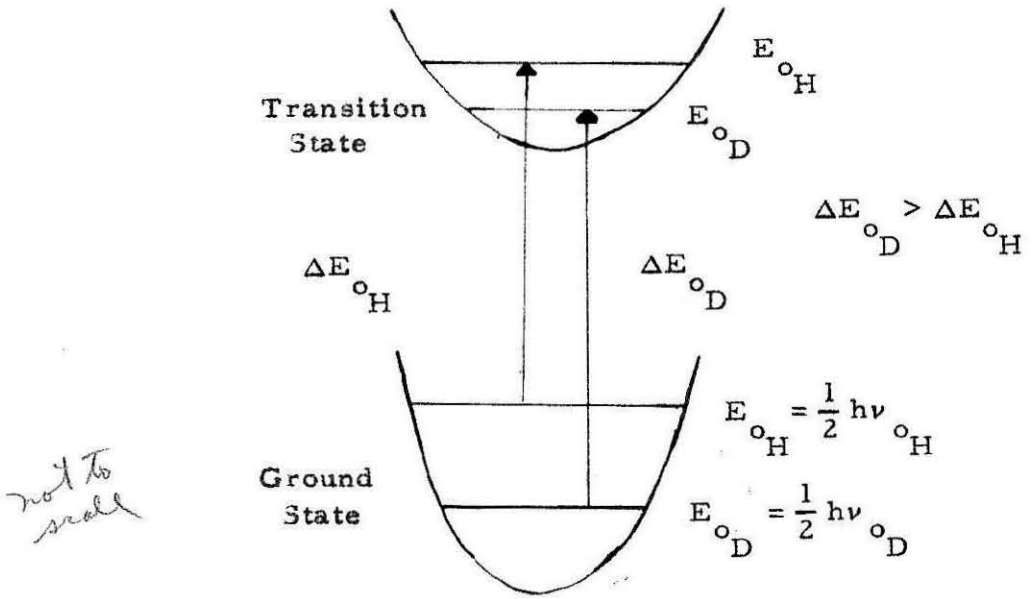
The sum is taken over all frequencies of isotopic C-H bonds in the ground state and in the transition state, and ν is expressed in wavenumbers.

The frequencies which have been assigned to the in-phase and out-of-phase C-H stretching vibrations of a secondary C-H bond ($-CH_2-$) in the ground state are 2926 $\pm$ 10 and 2853 $\pm$ 10 cm^{-1} respectively (56). The

deformation or bending modes of $-CH_2-$ hydrogens give rise to absorption close to 1465 $\pm$ 20 cm^{-1} (57). (In β-ferrocenylethyl tosylate these three bands occur at 2920, 2856 and ~1465 cm^{-1}, respectively.) Streitwieser et al. (51) used the aldehyde C-H ($-\overset{O}{\overset{\|}{C}}-H$), where the hybridization is sp^2 and there is a δ^+ charge on the carbon, as a model for the transition state. However, we are here concerned with finding a model for a secondary rather than a tertiary C-H in an sp^2 transition state. The best approximation is probably a vinyl group ($-CH{=}CH_2$), which has absorption for C-H stretching in the region near 3000 cm^{-1} (58) and for $=CH_2$ in-plane and out-of-plane bending at ~1415 and ~910 cm^{-1}, respectively (59). As pointed out by Streitwieser et al. (51) "the presence of a net positive charge on a trigonal carbon apparently does little to the vibration frequencies of attached bonds. For example, the vibration frequencies of the tropylium cation* are very similar to those of benzene (60). The greatest change in frequency in this model occurs in one of the C-H bending vibrations: 1465 → ~910 cm^{-1}, a difference of ~550 cm^{-1}. By using equation 4 to calculate the corresponding frequencies for the C-D bond it can easily be estimated that the corresponding change in frequency will be ~450 cm^{-1}. Since the frequency change of one of the bending modes of a C-H bond in going from the ground state to the transition state is greater than that of the corresponding C-D

*Of course, the carbons in the tropylium cation have only 1/7 positive charge each, but due to the admittedly approximate nature of the calculation of the frequency change this is not expected to affect the validity of the result.

vibration in S_N1 solvolyses, the decrease in the zero-point energy of the C-H bond will be greater. This leads to a larger activation energy for the deuterium compound and a slower rate of reaction.* A schematic energy level diagram based on a figure in a review by Streitwieser (61) will serve to clarify this statement:



If the values 2985 (62) and 3085 cm^{-1} (58) are assigned to the symmetrical and asymmetrical C-H modes, respectively, in our model transition state, equation 4 may be used to calculate the isotope effect (corrected for the presence of two α-deuterium atoms) in an S_N1 solvolysis at 30° C : $k_H/k_D = 1.46$.

In the above discussion it has been tacitly assumed that C-D and C-H bonds are harmonic oscillators; i.e., a plot of the potential energy

*A more complete discussion of this theory of the α-deuterium isotope effect is given in references (51) and (52).

as a function of bond length, for example, will be a parabola. In fact, however, due to anharmonicity such plots are slightly skewed; the effect of this anharmonicity is manifested in the fact that isotopic bonds have slightly different bond lengths, for example (52, 63). The magnitude of the anharmonicity effect may be indicated by the following data: Bartell has recently found (64) by electron diffraction that the C-D bond of deuteromethane is 0.004_7 Å shorter than the corresponding C-H bond. Bell and Coop (65) report that the dipole moment of DCl is 0.003-0.007 D greater than that of HCl; and deBruyne and Smyth (66) have observed a difference of 0.012-0.015 D in dipole moment between ND_3 and NH_3. Thus the effect appears to be on the order of 0-2 per cent.

Halevi (67) has interpreted the decrease in acidity (10-13%) between $C_6H_5CD_2COOH$, CD_3COOH, $C_6H_5CD_2NH_3^+$ and their protium analogs in terms of an inductive effect of α-deuterium which arises as a consequence of the slightly greater electron density on carbon attached to deuterium which in turn arises from the shorter C-D bond length. Weston (63) and Streitwieser et al. (51) have pointed out, however, that this decrease in acidity may better be explained by recourse to the argument involving vibrational zero-point energy differences outlined above. In the case of phenylacetic acid, for example, the reaction under consideration may be expressed as an equilibrium (63):

$$C_6H_5CD_2COOH + C_6H_5CH_2COO^- \rightleftharpoons C_6H_5CD_2COO^- + C_6H_5CH_2COOH$$

The inductive explanation ignores differences in C-H vibrational frequencies between the acid and the anion when, between acetic acid and acetate anion at least, there is enough of a difference to account for the observed isotope effect (51, 67a).* If inductive differences between H and D were an important source of the α-deuterium isotope effect, a faster rate for the deutero compound would be predicted due to inductive stabilization of the nascent carbonium ion; however, the experimental facts do not bear this out.

Recently, Bartell (68) has considered the role of non-bonded repulsions in secondary isotope effects and in the case of the α-effect has related his model to that of Streitwieser et al. (51). Bartell's theory rests on the fact that non-bonded repulsions involving deuterium atoms, when averaged over stretching and bending vibrations, are smaller than those for hydrogen which has a characteristically greater amplitude of vibration. Thus, in proceeding from the ground to the transition state, more relief of non-bonded repulsions is associated with the transformation from a tetrahedral ground state to a trigonal transition state in protium compounds than in deuterium compounds and the former therefore react faster. It can be seen (qualitatively, at least) that the isotope

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*Halevi has contradicted this statement (69) on the basis of more recent determinations of the IR spectra of CH_3COOH and CH_3COO^- (70). But he fails to mention that the spectra were not determined on the same phase: that of CH_3COOH was taken on the vapor while that of CH_3COO^- was determined in solution.

effect will be somewhat reduced by anharmonicity: since carbon-hydrogen bonds are slightly longer than carbon-deuterium bonds, hydrogen non-bonded distances are greater than those for deuterium. Thus the effect of the larger hydrogen amplitude is somewhat reduced and the isotope effect is correspondingly decreased. Bartell (68a) demonstrates that in the case of the solvolysis of cyclopentyl-1-d tosylate the isotope effect is calculated to be 1.31 (Streitwieser's maximum estimate (51) was 1.44). He then takes into account a difference of 0.004 Å between hydrogen and deuterium non-bonded distances and the calculated isotope effect decreases to 1.17, in good agreement with the observed value of 1.15. Bartell states (68a) that "the result of this simple treatment based on intramolecular non-bonded interactions is pleasing but serves as a sharp reminder of the sensitivity of the effect to commonly neglected factors. It is apparent that the quantitative deficiences of these and other qualitatively understood interactions preclude the definitive deduction of the nature of the transition state from the magnitude of the α-effect."

Leffek, Llewellyn and Robertson (71) have also postulated an important role for intramolecular van der Waals interactions in the α-effect and have suggested that while the hybridization change ($sp^3 \rightarrow sp^2$) may well be a necessary part of the reaction, it is not necessarily responsible for the decrease in zero point energy (and therefore in frequency) which seems to account for the effect. They point out that theoretical calculations based on the hybridization change will be sensitive to the model

chosen for the transition state, and this is to some extent illustrated below (p. 109) where it is shown that if an olefinic =C-H rather than an aldehydic -C(=O)-H is used as a model for the transition state in Streitwieser's example (51) of the solvolysis of cyclopentyl-1-d tosylate, the calculated k_H/k_D decreases from 1.44 to 1.18.

It is obvious that the separation of a given α-isotope effect into its zero-point energy, inductive, and non-bonded repulsive components (if, indeed, such separate components exist) is an impossible task in the present state of our knowledge of the subject. However, it is nonetheless useful to speculate a bit about what an isotope effect may mean in terms of transition state geometry as long as such speculation is seen in the proper perspective.

Magnitude of the α-Deuterium Effect

The magnitude of the α-deuterium isotope effect in S_N1 solvolyses is ordinarily between 10-15% per deuterium, as shown by the large number of examples collected in Table VIII. The data have been corrected for the number of deuterium atoms per molecule and for the effect of temperature by equation 6 which relates the isotope effect to the difference

$$(RT/n)(\ln k_H/k_D) = \Delta(\Delta F_a) \qquad (6)$$

in free energy of activation between deuterated and undeuterated molecules (72), and in which n is the number of deuteriums per molecule. The average value of the isotope effect is 1.14 at 30° C for fifteen S_N1

Table VIII[a]

Secondary α-Deuterium Isotope Effects in S_N1 Solvolyses

Reaction	Temp., °C	Atoms D	(k_H/k_D) obs.	$(k_H/k_D)^b$ corr.	Ref.
1. Acetolysis of cyclopentyl-1-d tosylate	50	1.07	1.15	1.16	51
2. Solvolysis of 1-p-tolylethyl-1-d chloride	-	1.00	1.10	1.11	72
3. Solvolysis of 1-methylheptyl-1-d brosylate	-	1.00	1.10	1.11	c
4. Acetolysis of isopropyl-2-d brosylate	70.0	1.01	1.12	1.14	73
5. Acetolysis of cyclohexyl-1-d tosylate	75.0	1.03	1.19	1.22	73
6. Acetolysis of cyclodecyl-1-d tosylate	25.0	1.05	1.17	1.17	73
7. Acetolysis of benzyl-α-d tosylate	50.0	1.00	1.12	1.13	73
8. Acetolysis of benzyl-α,α-d_2 tosylate	50.0	2.06	1.25	1.14	73
9. Formolysis of β-phenylethyl-α,α-d_2 tosylate	75.3	1.83	1.17	1.11	74
10. Formolysis of p-anisylethyl-α,α-d_2 tosylate	50	2.0	1.20	1.11	75
11. Formolysis of β,β-diphenylethyl-α,α-d_2 tosylate	75	2.0	1.21	1.12	75
12. Acetolysis of p-anisylethyl-α,α-d_2 tosylate	75	2.0	1.18	1.11	75
13. Acetolysis of β,β-diphenylethyl-α,α-d_2 tosylate	75	2.0	1.21	1.12	75
14. Solvolysis of benzhydryl-α-d chloride	25	-	1.16	1.16	76
15. Solvolysis of β-ferrocenylethyl-α,α-d_2 tosylate	30.00	2	1.27	1.14	d

Table VIII (continued)

[a] Adapted and expanded from Table IV in S. Seltzer, J. Amer. Chem. Soc. 83, 2625-2629 (1961).

[b] The $k_H k_D$ ratio is corrected to 30° and for only one α-D. In cases where more than the theoretical number of atoms of deuterium was present, the theoretical value for n in the equation $(RT/n)(\ln k_H/k_D) = \Delta(\Delta F_a)$ (72) was used.

[c] Ref. 2 in ref. (72).

[d] Present study.

solvolyses. That the effect should be fairly constant from reaction to reaction is no doubt a reflection of the facts that: (1) these reactions probably all proceed by mechanisms which have in common the presence of an electron-deficient carbon atom in the transition state and (2) C-H bending frequencies are remarkably independent of the débris attached to the carbon atom in question, and the changes in frequency on going to grossly similar transition states are therefore comparable.

The Isotope Effect in the Solvolysis of β-Ferrocenylethyl Tosylate-α,α-d_2

When the rate of solvolysis of β-ferrocenylethyl tosylate-α,α-d_2 in 80% acetone at 30° is compared with that of the undeuterated compound, k_H/k_D is found to be 1.27, or about 14% per deuterium atom.* (See Table VIII.) The experimental value is reasonably close, considering the approximations involved, to the figure of 1.46 calculated above (p. 102) on the basis of frequency changes between ground and transition states, and is completely in line with the other α-D effects gathered in Table VII. Perhaps the vinyl $=CH_2$ model for the transition state in this solvolysis is a rather better approximation than the aldehyde C-H model chosen by Streitwieser et al. (51) for the corresponding transition state in the cyclopentyl tosylate solvolysis; their calculated k_H/k_D was a maximum of

*An integrated NMR spectrum of the deuterated alcohol from which the tosylate was prepared indicates the presence of 2 deuterium atoms per mole since the ratio ring protons/side-chain protons found by NMR is 3.04 and it should theoretically be 3.00; and, of course, there is no detectable peak for the α-protons, indicating their complete replacement by D.

Table IX

Isotope Effect in the Solvolysis of β-Ferrocenylethyl Tosylate

Compound	Temp., °C	$10^5 k$ (Avg.)[a]	k_H/k_D
β-Ferrocenylethyl Tosylate	30.00	1.10±0.01	1.27
β-Ferrocenylethyl Tosylate-α,α-d_2	30.00	0.86_2±0.00$_4$	

[a] Average of two determinations; k in sec^{-1}.

about 1.44 while the observed value was 1.15. This is probably due to the fact that although both models involve sp^2 carbon atoms, the one is a carbon-carbon sp^2 bond while the other is a carbon-oxygen sp^2 bond. As a matter of fact, if an olefinic C-H (-CH=CH-) is used as a model in the cyclopentyl tosylate solvolysis, and the frequency changes (76) 2890-3025 cm^{-1} (C-H stretching), 1340-965 cm^{-1} (C-H out-of-plane bending) and 1340-1300 cm^{-1} (C-H in-plane bending) are considered, the calculated isotope effect becomes 1.18, in excellent agreement with the observed value. This is an indication of the sensitivity of these calculations to the transition state model chosen. It should be pointed out that the olefinic frequencies given above are those for a trans -CH=CH- since the correlations for the bending modes of the cis structure are uncertain: the out-of-plane C-H bending mode is thought to occur somewhere near 690 cm^{-1} (77), but the absorption for the in-plane

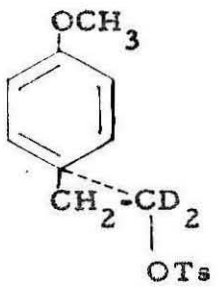
mode has not been surely assigned (78).

Whether the zero point decrease is actually due to a hybridization change, to a change in intramolecular van der Waals forces, or to a combination of both, the reasons which Streitwieser et al. (51) give for the difference between their calculated and observed isotope effects remain of interest since their reasoning is based upon the effect which is common to all of these causes, and may be applied to the present situation. Namely, if the change in out-of-plane bending frequency ($1465 \rightarrow 910\ cm^{-1}$) is the greatest contributor to the isotope effect, it is apparent that if the leaving tosylate group is fairly close to the nascent carbonium ion, it can damp the C-H bending motion by simple steric interference. This will decrease the frequency change ($\nu_H - \nu_H^{\ddagger}$) and thereby lower the isotope effect. The argument that the proximity of leaving and/or entering groups in the transition state lowers the isotope effect is supported by the following observations: Shiner (79) found $k_H/k_D = 1.00$ for the α-D compound in the reaction of isopropyl bromide with sodium ethoxide, an S_N2 displacement. In this case the transition state contains the entering ethoxide ion and the leaving bromide ion on either side of a planar carbonium ion, and the C-H bending motion is greatly hindered; in fact, the bending frequency could reasonably be quite close to that of a tetrahedral C-H bond. Johnson and Lewis (72) compiled results which showed that increasing necessity for nucleophilic

attack from the rear is accompanied by a weakening of the α-deuterium isotope effect. Robertson and his co-workers (71, 80) have greatly extended the work of Johnson and Lewis (72) and have presented evidence which supports the general conclusion that the α-isotope effect may be interpreted as a measure of the crowding about the carbon atom which forms the reaction center in the transition state.

Solvolytic reactions have been classified by Winstein et al. (15) according to the degree of carbon-solvent covalent character in the transition state: "pure" S_N1 reactions, in which there is little or no solvent participation in the transition state, are said to be limiting whereas at the other end of the scale are nucleophilic reactions in which the solvent plays a major role in the transition state. (See above, p. 80.) With this classification and the work of Johnson and Lewis (72) and of Robertson et al. (71, 80) on the use of the isotope effect as a measure of transition state hindrance in mind it may be easily seen that if the solvolysis of β-ferrocenylethyl tosylate in 80% acetone is an S_N1 reaction (which is most likely), the isotope effect indicates the presence of few (if any) solvent molecules at the backside of the carbonium ion in the transition state: i.e., k_H/k_D in this reaction is quite comparable to effects observed in reactions known to be somewhat limiting. For example, the acetolysis of cyclopentyl-1-d tosylate will have little or no backside solvent participation in the transition state, according to Streitwieser et al. (51), and the isotope effect per deuterium is 1.16 at

30° compared to the value of 1.14 per D observed in the present case. The observed isotope effect is also compatible with the presence of some neighboring group participation, as shown by comparison with a reaction in which such participation is known to exist from independent experiments (16,17) and in which an α-isotope effect of similar magnitude is observed. In the acetolysis and formolysis of p-anisylethyl tosylate-α,α-d_2, k_H/k_D was found to be 1.18 and 1.20, respectively (75). An unsymmetrical transition state such as (IX) was suggested when it was observed that the β,β-dideutero compound does not show an isotope effect (75), which indicates that the β-C-H frequencies do not change between ground and transition states. The observed isotope effects correspond to a value of 1.11 per deuterium at 30°. The simple diagram of a β-



IX

ferrocenyl carbonium ion stabilized by neighboring iron participation presented below (p. 114) indicates that an iron-carbon interaction, while it may be weak, is at least permissible on steric grounds. This, of course, is a diagram for an intermediate rather than a transition state, but in any case the isotope effect is compatible with some

neighboring iron participation. However, this is by no means diagnostic evidence.

CHAPTER VI

The Plausibility of an Iron-Stabilized β-Ferrocenyl Carbonium Ion

A speculative diagram of a β-ferrocenyl carbonium ion stabilized by iron participation will now be discussed. (See Fig. IVa.) Although the diagram may have more geometrical than chemical validity, it will nevertheless enable us to form an approximate idea of some important interatomic distances. Since no structural data on representative carbonium ions are available certain assumptions about bond distances and angles must be made: namely, it is assumed that the carbonium ion is planar and in a state of sp^2 hybridization; and it is assumed that the angle between C_α and the attached atoms is ~120°. If normal C-O and C-H single bond distances (81) and tetrahedral angles are assumed where appropriate, and if the ring to ring distance of 3.32 Å found by Dunitz, Orgel and Rich in ferrocene (82) is accepted, Figure IVa may be constructed. To maintain clarity of presentation, the cyclopentadienyl rings themselves have not been depicted, but the two planes in which they lie are indicated by two straight lines. C_1, C_β, C_α and the two C_α hydrogens (the carbonium ion is that derived from β-ferrocenylethyl tosylate, for the sake of concreteness) are represented as lying in the plane of the paper. It is highly likely that the two cyclopentadienyl rings are staggered in this derivative as they are in the ferrocene crystal (82) since this minimizes steric interactions. Therefore C_1 of the upper ring will

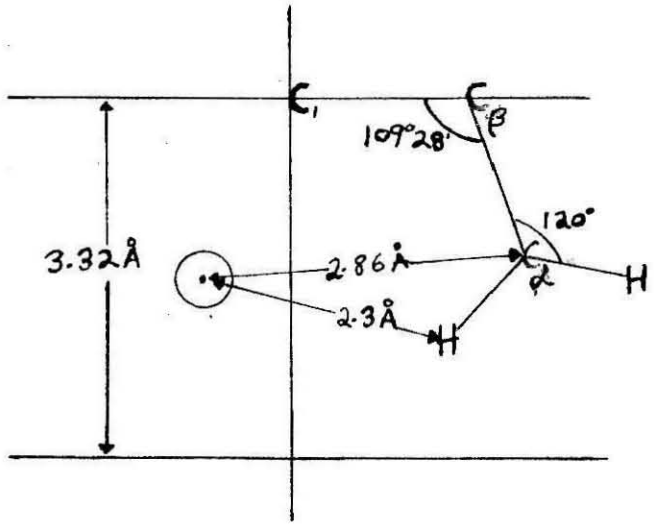


Figure IVa

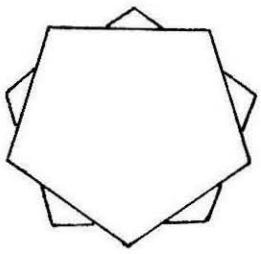


Figure IVb

lie 3.32 Å above the middle of one of the C-C bonds in the lower ring. (Fig. IVb shows this staggered configuration for two regular pentagons.) The following estimations of interatomic and other distances may be made by direct measurement (the scale of both Figs. IVa and b is 1.5 cm/Å), geometric calculation or a combination of both: (1) the distance from the nearest C-C bond of the lower ring to the nearest H on C_a is about 1.8 Å; (2) the distance between the nearest H on C_a and the iron atom is about 2.3 Å ; (3) the distance between the nearest H on C_a and either of the two nearest hydrogens on the lower ring is calculated to be about 1.7 Å ; and (4) the distance from C_a to a line passing through the center of both rings (approximate iron-carbon distance) is about 2.86 Å. In the ensuing discussion of these distances and their implications for iron participation, the following van der Waals radii (83) will be used: hydrogen, 1.2 Å ; C, ca. 1.5 Å *; and iron, ca. 2 Å.**

By virtue of the adjacent positive charge the hydrogens on C_a are in an electronic environment quite different from that surrounding hydrogen atoms attached to neutral carbon. In effect, the C_a-hydrogens are attached to an electronegative atom and might be expected to form

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*Calculated by assuming a covalent radius of 0.67 Å (appropriate for sp^2 carbon (81) and following the rule proposed by Pauling (84) according to which a van der Waals radius equals the covalent radius plus 0.80 Å.

**If iron is assigned the single-bond covalent radius 1.165 Å (85) the calculated van der Waals radius is 1.96 Å ; if the radius of 1.135 Å derived from Pauling's resonating bond treatment of ferrocene (86) is used, the van der Waals radius becomes 1.94 Å .

hydrogen bonds with suitable electron donors.* The first two distances listed above may therefore be inspected for hydrogen-bonding possibilities. The 1.8 Å distance from an H on C_a to the lower ring is probably rather far for effective H $\cdots \pi$ interaction. The existence of H$\cdots\pi$ bonds has been inferred from O-H frequency shifts in the infra-red by Baker and Shulgin (87) and others (21, 22, 88, 89, 90). Specifically, shifts ($\nu_{free}-\nu_{bonded}$) of 70-127 cm^{-1} were observed for various o-allyl phenols (87). An estimation of the H$\cdots\pi$ distance in these compounds may be made by drawing a simple geometric picture of the atomic positions: it is readily seen that o-allylphenol is a particularly favorable case since the H$\cdots\pi$ approach may be made less than 1 Å with no bond or angle deformation. The O-H frequency shift (29 cm^{-1}) in β-phenylethanol has been attributed to H$\cdots\pi$ bonding (21, 90). In this molecule the estimated H$\cdots\pi$ distance is ~1 Å, which seems quite

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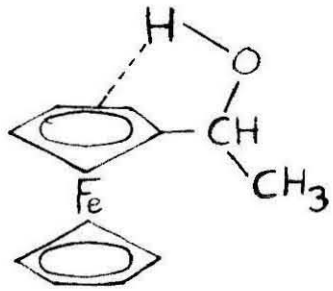
*This statement requires some comment. Good evidence for the formation of H bonds between hydrogen on carbon and suitable electron donors is available only for the hydrogens in $CHCl_3$, HCN and R-C≡CH (86a). Unfortunately, none of these examples is germane to the case at hand since we are dealing with a carbon atom which has been deprived of the usual octet of electrons and is left with only a sextet. The importance of the resonance form (A) will determine whether the aldehyde hydrogen

(A)

may be a suggestive first approximation to our situation. However, as Pimentel and McLellan (86a) point out, the question of hydrogen bonding by the aldehydic C-H is not completely resolved. Schneider and Bernstein found no evidence for H bonding in the IR spectra of solid formaldehyde and acetaldehyde (86b), but on the other hand various authors (86a) have presented evidence for the presence of such a bond from kinetic

reasonable: the sum of the covalent radii of hydrogen (0.30 Å (91)) and sp^2 carbon (0.667 Å) is ~0.97 Å . Trifan and Bacskai (22) interpreted the O-H stretching band at 3617 cm^{-1} in the infra-red spectrum of methyl ferrocenyl carbinol as due to a hydroxyl group hydrogen-bonded to the



upper ring. This is a questionable assignment since this frequency is rather close to that normally accepted for a free O-H**: Baker and Shulgin (87), for example, found free O-H absorption between 3611 and 3623 cm^{-1} for 12 variously substituted o-allylphenols. Further, the estimated H···π distance for methyl ferrocenyl carbinol is ~1.7 Å , which seems rather long in the light of the above data. This brings us back to the statement which began this discussion: viz, the distance of 1.8 Å between an H on C_α and the lower ring seems a bit long for effective H···π bonding.

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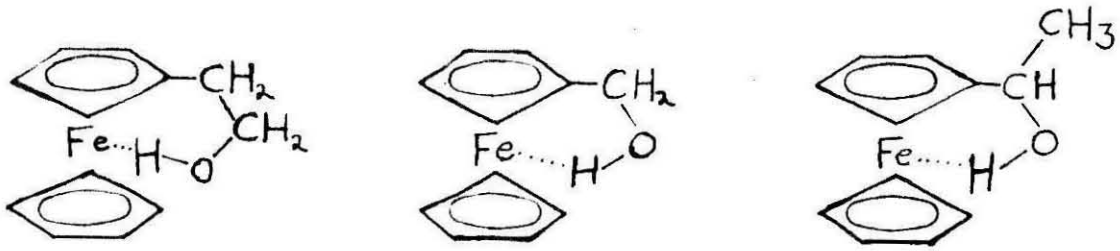
studies, IR spectra, and the fluorescence of benzaldehyde adsorbed on metals. In spite of the absence of a clear analogy, it will still be profitable to inquire into the possibility for hydrogen bond formation in our system if we keep in mind the fact that only a rather fuzzy line separates weak hydrogen bonds from attractive van der Waals interactions (86c).

**Of course, the relatively high frequency may just be indicative of the weakness of the interaction.

Hydrogen bonds to iron in ferrocenyl alkanols have been observed by Trifan and Bacskai (22) and by Hill (92). The latter presents an admirably detailed discussion of the spectral and other evidence for the existence of such bonding and it will therefore not be given here. Hill reports the values 3537, 3578 and 3581 cm^{-1} for the bands due to H···Fe bonding in β-ferrocenylethanol, ferrocenyl carbinol and methylferrocenylcarbinol, respectively. The strongest of these bonds is apparently



that in β-ferrocenylethanol since the change in frequency from the higher frequency band (~3600 cm^{-1}) is 85 cm^{-1} for this compound whereas the change is 32 cm^{-1} for ferrocenyl carbinol and 24 cm^{-1} for methylferrocenyl carbinol. A geometric estimation of the H···Fe distance in β-ferrocenylethanol shows it to be about 1.46 Å; this distance in the other two compounds is ~2.15 Å (92). From the covalent single-bond radii of iron and hydrogen (1.165 and 0.30 Å, respectively (85, 91)) an approximate H···Fe bond length of ~1.46 Å may be calculated. Thus the H···Fe distance of 2.3 Å estimated from the diagram of a β-ferrocenylethyl carbonium ion under discussion appears to be too long for any but fairly weak bond formation.

Although the model predicts little or no hydrogen bond formation, it must be borne in mind that van der Waals interactions will be present: the estimated H···Fe distance is about 1 Å shorter than the combined van der Waals radii of iron and hydrogen (2 + 1.2 = 3.2 Å) and the H···lower ring distance (~1.8 Å) is about 1 Å shorter than the corresponding combination for H and sp^2 carbon (~ 2.7 Å). These van der Waals forces are likely to be more attractive than repulsive for the same reasons given above (p. 116) for the possibility of hydrogen-bond formation.

The distance between the nearest H on C_a and either of the two nearest hydrogens on the lower ring (distance #3 above) is about 1.7 Å . This is 0.7 Å shorter than the minimum van der Waals interaction distance of 2.4 Å, calculated on the basis of a 1.2 Å van der Waals radius for hydrogen. Mulliken (93) has estimated the energy of non-bonded H···H interactions for various H···H distances in the ground states of several ordinary hydrocarbon molecules (CH_4, C_2H_6, C_2H_4, C_6H_6) by means of molecular orbital calculations of H···H overlap integrals. A plot of Mulliken's data is given in Figure V, from which it may be determined that the energy of an H···H non-bonded repulsion at 1.7 Å is about 0.406 e.v. This represents a contribution of 9.3_6 kcal/mole to the energy of the compound. An important point must be borne in mind in considering this result: Mulliken's calculations were made for hydrogens attached to either the same carbon or to adjacent carbons in

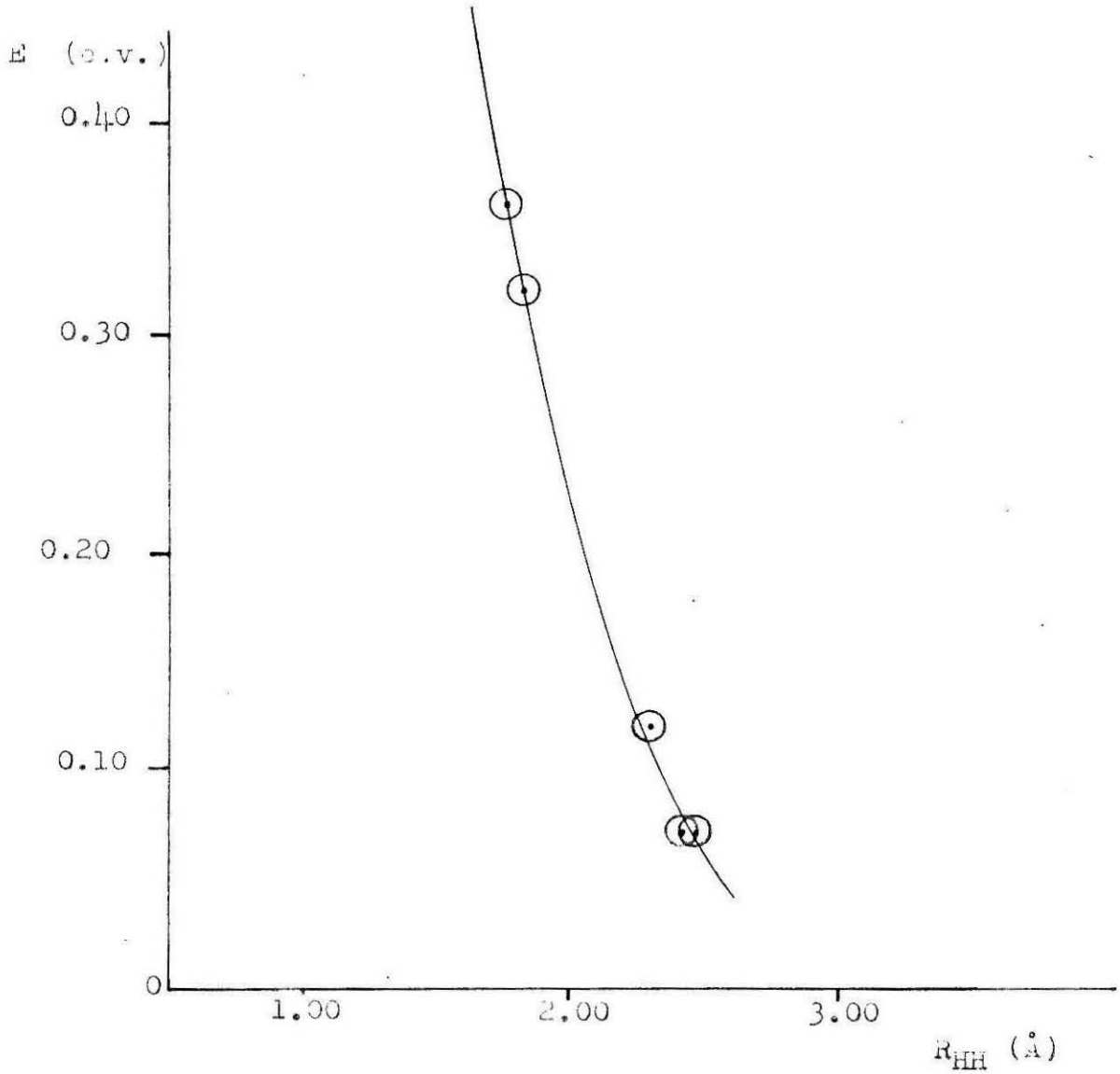


Figure V

the same state of hybridization. In the molecule under consideration here, both of the carbons in question are assumed to be sp^2 hybridized, but one of them bears a positive charge. As discussed above, this charge will surely have an effect on the electron distribution at the attached hydrogen and will probably somewhat decrease its van der Waals radius. It is likely, therefore, that the energy of the H...H interaction in this case will be lower than what might be expected if the two hydrogens concerned were exactly alike.

The Fe-C_α distance obtained from our simple diagram is 2.86 Å. The covalent radii of iron and sp^2 carbon (81, 85) are 1.165 and 0.677 Å, respectively. The sum of these, 1.832 Å, should be the length of a full iron-carbon single bond.* The equation (94)

$$D(n) = D(1) - 0.60 \log n$$

where D(n) is the bond length for bond number n and D(1) is the bond length for a single bond of similar type, may be used to estimate that the Fe-C bond is slightly less than 2/100 of an iron-carbon single bond. The distance between the iron atom and the carbinyl carbon in α-ferrocenyl carbonium ions corresponds to less than 1/100 of a single bond, as pointed out by Hill(95). It is suggested (95) that if the iron exerts a driving force on ionization in α-ferrocenyl carbonium ions, it will shift to a position where it can bond more favorably with the electron

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* This figure changes to 1.802 Å if the single bond radius of iron calculated by Pauling (87) in ferrocene is used.

deficient carbon. In a molecular orbital description of metal participation in α-ferrocenyl carbonium ions (8a, 96) it is calculated that the overlap integral of the iron $d_{\pm 1}$ orbital with the empty p-orbital on the carbinyl carbon may be increased from 0.020 to 0.032 if the "upper" ring is allowed to shift 0.2 Å in the direction which shortens the distance between the iron and the carbinyl carbon. An analogous shift in the case of the β-ferrocenyl carbonium ion, accompanied by a deformation of the C_1-C_β-C_α angle from 109°28' to ~100°, will decrease the Fe-C_α distance to about 2.46 Å which corresponds to 8.9 per cent of an iron-carbon single bond. Of course, the results of the MO calculations for α-carbonium ions are not strictly applicable to the β-series since the positive center in the latter is not conjugated with the ring: thus the "ring shift" may be more reasonable in the α-carbonium ions since there it is a means of maximizing the overlap of the iron orbitals with p-orbitals on all six of the carbons involved. One consequence of the fact that ferrocenyl carbonium ions are not conjugated with the ring is that the positive charge cannot be delocalized through the rings, and some other means of stabilization must be found.

If one of the hydrogens on C_α is replaced by a methyl group to form the carbonium ion derived from β-ferrocenylisopropyl tosylate, the means for eliminating any unfavorable steric interactions between an H on C_α and the "lower" ring which is available to the β-ferrocenylethyl carbonium ion (viz. a rotation of the C_α-C_β bond by 90°) is no longer

feasible due to important non-bonded repulsions between the methyl group hydrogens and the "lower" ring. That this is the case may be appreciated as follows: the distance between C_α and the plane which is tangent and perpendicular to the plane of the "lower" ring is only about 2.1 Å; it is assumed that the CH_3-C_α-H angle is near 120° and therefore that the carbon of the CH_3 group is in the same plane as C_α (i.e., perpendicular to the plane of the paper); then since the C-H bond distance in the methyl group is 1.09 Å, it stands to reason that the distance between the methyl hydrogens and the cyclopentadienyl hydrogens will be considerably less than the H···H non-bonded repulsion distance of 2.4 Å. The implication of this reasoning for the stereochemical outcome of the solvolysis of optically active β-ferrocenylisopropyl tosylate is racemization: backside (d-p → σ) iron participation, which would lead to retention, requires the presence of more steric repulsions than "frontside" (d-p → π) iron participation. The experimental result, as discussed above (p. 94), is complete racemization.

Two further points are of interest: (1) According to Pauling's resonating bond treatment of ferrocene (86) the iron atom bears a small negative charge (-0.22). It is reasonable to expect this charge to be present in the ferrocene derivatives under discussion; it is further reasonable to expect such a charge to exert an attractive influence on an electron-deficient carbon in its immediate neighborhood. This would no

doubt be a factor in increasing the strength of the Fe···C interaction beyond that estimated above on the basis of the covalent radii of neutral molecules.*

(2) Since the combined van der Waals radii of iron and C_a equal 3.5 Å, there will most likely be some attractive forces which must be taken into account when the two atoms are about 0.5 Å closer than the calculated van der Waals interaction distance.

The models for iron participation in β-ferrocenyl carbonium ions derived by Hill (14) and mentioned above (p. 62) cannot be accepted or rejected simply on the basis of the above discussion; however, metal participation remains an attractive rationalization for the effects observed in the solvolyses of β-ferrocenyl derivatives.

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*Simple electrostatic theory (97) tells us that the change in free energy involved in bringing two charges Z_A and Z_B from infinity to a distance r from each other in a medium of dielectric constant D is $\Delta F = (Z_A Z_B e^2)/Dr$, where e is the charge on the electron. If this may be applied to the present situation, in which $Z_A = -0.22$, $Z_B = +1$, $D = 20.7$ and $r = 3.06 \times 10^{-8}$ cm, then ΔF is -1.2 kcal/mole. This might be taken as an indication of the size of the Fe-C_a interaction at this distance; however, it is well to remember that due to the approximations and simplifications involved in this calculation and to the fact that the result is relatively small, its significance is doubtful. For example, the solvent is assumed to be a continuous dielectric medium, and the effects of other nearby ions (OTs^-, e.g.) are ignored. Furthermore, the equation is rigorously applicable only in infinitely dilute solutions.

Summary and Conclusions

Four possible explanations for the enhanced reactivity of β-ferrocenyl derivatives are hydride migration to form an α-carbonium ion, interaction with the π-electron system of the cyclopentadienyl ring, the inductive effect of the ferrocene nucleus, and direct participation by iron electrons. The first of these was shown to be unlikely by Hill (10); the second has been investigated and discounted as reported in Chapter II of this thesis; the third was indicated to decrease the rate; and no incontrovertible evidence was obtained concerning the fourth explanation. The rate increase due to the methyl group in β-ferrocenylisopropyl tosylate was shown to be interpretable either in terms of a solvolysis which is far from limiting (15) or in terms of neighboring group participation. The α-deuterium isotope effect in the solvolysis of β-ferrocenylethyl tosylate is equally compatible with a close-to-limiting solvolysis or with neighboring group participation, but it is not compatible with a far-from-limiting solvolysis, since one would expect a considerably decreased isotope effect in such a case. For example, Leffek, Llewellyn and Robertson (71) found k_H/k_D to be only 1.03_8 in the solvolysis of ethyl tosylate-α,α-d_2 in water at 54°, a reaction which is almost certainly very far from limiting. Thus, the α-methyl effect and the α-isotope effect taken together constitute circumstantial evidence for the presence of neighboring group (i.e., iron) participation in β-ferrocenyl tosylate solvolyses. The racemization of (-)-β-ferrocenylisopropyl

tosylate in 60% acetone was discussed from the point of view that one would expect predominant inversion in this solvent in the absence of special effects. It is pointed out that the racemization is compatible with "frontside" iron participation and that in this case this is indicated to be more favorable than the backside participation which would lead to retention by a simple diagram of an iron-stabilized β-ferrocenyl carbonium ion. Finally, the $\Delta S^{\neq}$ in the solvolysis of the ferrocene compound is another piece of circumstantial evidence for iron participation. The analogy between electrophilic substitution and neighboring metal participation, as discussed by Garwood (42a), adds to the plausibility of metal-carbonium ion interaction. It is fairly obvious, from the somewhat preliminary nature of some of the experiments described herein, that a great deal of effort must be expended before any really definitive conclusions about carbonium ion stabilization in β-ferrocenyl derivatives may be drawn. Some of the more necessary effort is indicated in the suggested experiments described in the following Appendix.

APPENDIX

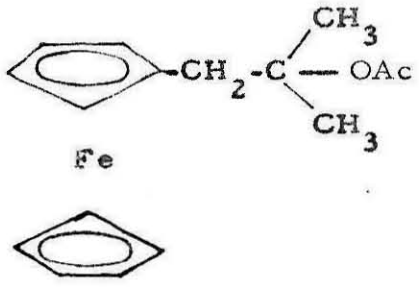
Suggested Future Experiments:

(1) Extensive kinetic experiments must be run to determine the rates of β-ferrocenyl derivatives in a wide range of solvents besides 80% acetone, such as acetic acid, ethanol and ethanol-water mixtures, and of course $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ should be obtained for these reactions. It would also be desirable to obtain kinetic data for the corresponding β-phenyl derivatives under the same conditions.

(2) The polarimetric rate in the solvolysis of β-ferrocenylisopropyl tosylate should be determined, as pointed out above (p. 97).

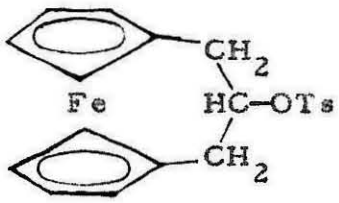
(3) The kinetic isotope effect in the solvolysis of β-ferrocenylisopropyl tosylate would be of interest, since the model predicts that the vibration of the α-hydrogen will be interfered with by the ferrocenyl system, thus lowering the k_H/k_D ratio.

(4) The rate of solvolysis of (I), which presumably cannot be assisted by iron participation due to steric hindrance by the methyl groups, should be determined. Complications may arise, however, since in this case the α-methyl effect is likely to be more pronounced than it was in β-ferrocenylisopropyl tosylate.



(I)

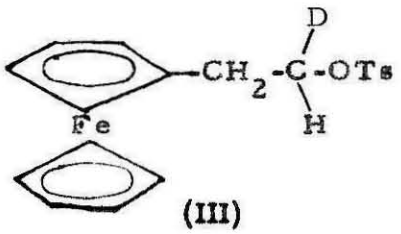
(5) The solvolytic behavior of (II) should be investigated. Hill (105) reports that a crude sample of this compound contained a fast com-



(II)

ponent which solvolysed at a rate ~75 times faster than that of cyclopentyl tosylate (extrapolated). This compound is of interest since there is opportunity neither for backside solvent participation nor for interaction with the cyclopentadienyl rings.

(6) If a sample of (III) could be obtained with sufficient optical



(III)

activity, the stereochemical result of its solvolysis would certainly be of great interest. However, great experimental difficulties would no doubt be encountered simply due to the yellow color of ferrocene derivatives: the rotations of compounds which are asymmetric due to the presence of a deuterium and a hydrogen on the same carbon tend to be very small* and thus a large concentration or a long polarimeter tube is required to determine the rotation with any accuracy. The yellow color of (III) either in high concentration or in a long tube would effectively block the light path, rendering the determination of a rotation completely impossible.

*For example, Streitwieser (52) reports that the rotation of optically pure ethylbenzene-α-d is $[\alpha]_D$ -0.7°.

CHAPTER VII

Experimental

All melting points are uncorrected. Analyses were done by Schwarzkopf Microanalytical Laboratories, Woodside, N. Y. (S) and the Spang Microanalytical Laboratory, Ann Arbor, Mich. (Sp).

NMR spectra were determined on either the Varian 4300 B nmr spectrometer or the Varian A 60 by W. F. Beach and R. C. Neuman, Jr., to both of whom many thanks are due.

I.R. spectra were taken on the Beckmann IR-7 spectrometer.

Ferrocene was obtained from K. & K. Laboratories, Inc., Jamaica 33, N. Y. Its melting point was 171-173° and it was used without further purification.

N,N-Dimethylaminomethylferrocene was prepared by the reaction of paraformaldehyde and N,N,N',N'-tetramethyldiaminoethane with ferrocene in acetic acid, as described by Lindsay and Hauser (98).

Ferrocenylacetonitrile was prepared by reacting potassium cyanide with the methiodide of N,N-dimethylaminomethylferrocene in water, as described by Lednicer, Lindsay and Hauser (18). The hydrolysis of the nitrile to the corresponding acid and the reduction of the latter to the corresponding alcohol with lithium aluminum hydride were carried out as directed by Lednicer et al. (18).

β-Ferrocenylethanol-α,α-d_2 was prepared from ferrocenyl acetic acid by reduction with lithium aluminum deuteride (95.6%) obtained from Metal Hydrides Co., Beverly, Mass. Two recrystallizations from ligroin (30-60°) gave alcohol with m.p. 40-42°. This material was shown to be fully deuterated in the α-position by NMR analysis. (See footnote on p. 108.)

β-Ferrocenylethyl tosylate was prepared from ferrocenyl ethanol and p-toluene sulfonyl chloride in pyridine, as described by E. A. Hill (99). The p-toluenesulfonyl chloride had been purified by washing a benzene solution thereof with aqueous sodium hydroxide (5%), followed by drying over anhydrous sodium sulfate. After the addition of petroleum ether (30-60°) to the benzene solution and refrigeration at -10° overnight colorless crystals formed (m.p. 67-69°). The pyridine was reagent grade which was kept over barium oxide.

β-Ferrocenylethyl tosylate-α,α-d_2 was similarly prepared from β-ferrocenylethanol-α,α-d_2. M.P. 76-78°.

β-Ferrocenylethyl acetate (100):- β-Ferrocenylethanol (250 mg., 1.09 m mole) and acetic anhydride (0.5 ml) were allowed to react in pyridine (1.3 ml) for 24 hours at room temperature. All volatile material was evaporated at the mechanical pump, leaving a dark oil which was crystallized from ethanol-water to yield 235.6 mg (79.6%) of bright yellow crystals, m.p. 40-42°. An I.R. in CCl_4 showed strong absorption at 1745 (carbonyl) and 1250 (acetate) cm^{-1}.

Analysis (S): Calcd. for $C_{14}H_{16}O_2Fe$: C, 61.79%; H, 5.93%; Fe, 20.52%. Found: C, 61.78%; H, 6.19%; Fe, 20.56%.

dl-Ferrocenylisopropanol*:- Methyl lithium (0.07 mole), freshly prepared in anhydrous ether, was added to ferrocenyl acetic acid (5 g, 0.02 mole) in anhydrous ether (150 ml) stirred with a high-speed stirrer in a Morton flask. The reaction mixture was refluxed for 6 hours and, after cooling, was treated with dilute sulfuric acid. The ether layer was separated, washed with aqueous sodium hydroxide (5%) and water, and then dried on a column of anhydrous sodium sulfate containing a small amount of ascorbic acid. The dry solution was added to a suspension of lithium aluminum hydride in anhydrous ether and then stirred and refluxed overnight. The cooled reaction mixture was treated with hydrochloric acid (0.1 N) and water and extracted with ether. The extract was washed with aqueous sodium hydroxide (5%) and water; it was not dried, but was evaporated to a small volume to which benzene was added to form an azeotrope with the water. The residue was dissolved in dichloromethane and chromatographed on deactivated aluminum oxide (57 x 2.5 cm). The racemic alcohol was eluted with dichloromethane-hexane (50-50) with small quantities of methanol (~1%) added after the elution of a small bright yellow band which moved rapidly down the column preceding the alcohol. The removal of the solvent from the alcohol fraction left a reddish oil (2.12 g, 43% crude yield based on ferrocenyl acetic

*Procedure suggested by Dr. Karl Kopecky.

acid). An analytical sample, prepared by two recrystallizations from ethanol-water, had m.p. 40-41.5°. Analysis (S): Calcd. for $C_{13}H_{16}OFe$: C, 63.97; H, 6.61; Fe, 22.88. Found: C, 63.70; H, 6.93; Fe, 23.13. A large-scale preparation of this compound was later carried out in 88.6% yield: 26.6 grams of unrecrystallized alcohol was obtained from 30 grams of ferrocenyl acetic acid.

dl-Ferrocenylisopropyl Acid Phthalate (101):- dl-Ferrocenylisopropanol (2 g unrecrystallized oil) was dissolved in dry pyridine (3 ml). Phthalic anhydride (1.4 g, 9.5 m mole) was added and the mixture gently warmed to assist solution. After sitting at room temperature overnight the solution was heated on the steam bath for half an hour and then poured into a mixture of ice and excess hydrochloric acid. The phthalate ester was extracted with chloroform and the extract was washed with water and dried on a column of anhydrous sodium sulfate. After evaporation of the solvent the residue was treated with excess aqueous sodium carbonate (10%). The yellow solid thus produced was collected and washed on the filter with ether to remove unreacted alcohol; it was then transferred to a separatory funnel to which a mixture of ortho phosphoric acid (85%), ice and ether had been added. The ether layer was separated, washed with water and dried on a column of anhydrous sodium sulfate containing a small amount of ascorbic acid. The oil which remained after the evaporation of the solvent was recrystallized from benzene-petroleum ether (30-60°) to yield a yellow powder (2.19 g, 70%, m.p.

121-125°. A second recrystallization produced material of m.p. 125-127.°)

Resolution of dl-Ferrocenylisopropyl Acid Phthalate and Conversion of Phthalate to Corresponding Alcohol: - dl-Ferrocenylisopropyl acid phthalate (5.3 g, 14 m mole) was dissolved in acetone and a solution of strychnine (4.7 g, 14 m mole) in warm chloroform was added. The mixture was allowed to sit overnight at room temperature. The volume was reduced to remove the chloroform, which forms an azeotope with acetone. Acetone was then added and the solution decanted from unreacted strychnine. Two recrystallizations from acetone produced bright golden yellow crystals (1.36 g), m.p. 180-181°, $[\alpha]_D^{25}$ + 15.71° (chloroform, c 2.8). Analysis (S): Calcd. for $C_{41}H_{42}O_6N_2Fe$: C, 68.91; H, 5.92; N, 3.92; Fe, 7.82. Found: C, 68.65; H, 6.15; N, 4.09; Fe, 8.03. The resolved strychnine salt was easily reconverted to the acid phthalate by the addition of an ethanolic solution of the salt to a large excess of dilute aqueous ammonia. After the precipitation of strychnine was complete the solution was filtered and the filtrate was acidified with aqueous hydrochloric acid and extracted with ether. The extract was washed with water, dried over anhydrous sodium sulfate and evaporated to dryness. The phthalate was thus obtained as a viscous dark oil which was converted directly to the alcohol by reduction with lithium aluminum hydride in anhydrous ether. The reaction was worked up in the usual way: aqueous hydrochloric acid (20%) and water were added to the cooled reaction mixture; the ether layer was separated and washed with aqueous sodium hydroxide (5%) and

water, and evaporated almost to dryness. Benzene was added to remove the water by azeotropic distillation and the residue was dissolved in chloroform and chromatographed on deactivated aluminum oxide. β-Ferrocenylisopropanol was eluted with hexane-chloroform (50-50) and was obtained as an oil which slowly crystallized (m.p. 47-49°), $[\alpha]_D^{25}$ +17.24° (chloroform, c 2.09).

Optically Active Ferrocenylisopropyl Tosylate:- The tosylate was prepared in the usual manner in pyridine (see above, p. 132) in 34% yield. Crystallization from hexane after chromatography on deactivated aluminum oxide (the tosylate was eluted with chloroform) produced orange crystals, m.p. 78-79°, $[\alpha]_D^{25}$ -66.34° (chloroform, c 2.05). Analysis (Sp): Calcd. for $C_{20}H_{22}O_3FeS$: C, 60.16; H, 5.55; Fe, 13.99. Found: C, 60.27; H, 5.56; Fe, 13.83. A second recrystallization produced material of m.p. 78-79.5°, $[\alpha]_D^{28}$ -65.73° (chloroform, c 1.78).

dl-Ferrocenylisopropyl tosylate was prepared from dl-ferrocenylisopropanol in pyridine as described above for the optically active compound. Orange crystals, m.p. 68-69.5°, were produced. Analysis (Sp): Calcd. for $C_{20}H_{22}O_3FeS$: C, 60.16; H, 5.55; Fe, 13.99. Found: C, 60.18; H, 5.64; Fe, 14.06.

Solvolysis of β-Ferrocenylethyl Tosylate-α,α-d_2:-

A. Acetone-water (50-50): β-Ferrocenylethyl tosylate-α,α-d_2 (600 mg, 1.56 m mole) and acetone-water (50-50, 250 ml) were placed in a loosely stoppered erlenmeyer flask set in an oil bath at 50° C and

stirred for 12 hours. The reaction mixture was cooled and diluted with water (300 ml) and extracted with dichloromethane. The extract was washed with water, aqueous sodium hydroxide (5%) and water and dried on a column of anhydrous sodium sulfate. After evaporation of the dichloromethane the residue was twice crystallized from ligroin (30-60°) to yield 183.9 mg of β-ferrocenylethanol-α,α-d_2 (53%), m.p. 43-45°. NMR spectrum: the recovered product (183.9 mg) was dissolved in carbon tetrachloride (0.3 ml); nitrogen was slowly bubbled through the solution for fifteen minutes. This represents a 28% solution (by weight) of the alcohol. Tetramethylsilane was used as an external standard. The spectrum was essentially identical to that of synthetic β-ferrocenylethanol-α,α-d_2, with peaks at 5.77 τ (ferrocenyl protons), 7.33 τ (β-protons) and 6.38 τ (OH). The OH peak was slightly shifted from its position in the authentic sample (6.55 τ), but this is simply due to the different concentration of this sample: it was a 24% solution rather than a 28% solution. (See above, p. 71 .) Integration of the areas under the peaks in the sample of alcohol recovered from the solvolysis showed the ratio of ferrocenyl to side-chain protons to be 8.5/2.8, or 3.04. The theoretical ratio is 3.00.

B. Glacial acetic acid: Baker acetic acid (99.9%) was refluxed with acetic anhydride for 20 hours and then distilled. A portion of the distillate (100 ml) was made 0.01 M in acetic anhydride and this solution was used for the solvolysis. β-Ferrocenylethyl tosylate-α,α-d_2 (800 mg,

2.08 m mole) and acetic acid-acetic anhydride (40 ml) were placed in a 50 ml RBSN flask to which a stopcock was attached. The flask was cooled in dry-ice-acetone and evacuated to ~0.1 mm. It was warmed to room temperature, re-frozen and re-evacuated, and then placed in a constant temperature bath at 70° C for five hours.

The reaction mixture was cooled in running tap water and then poured into an excess of distilled water. The product was extracted with dichloromethane (the remaining aqueous layer was slightly blue due to oxidation to ferricinium ion) and the extract washed twice with aqueous sodium hydroxide (5%) and once with water. The oxidized material was not recovered. After drying (anhydrous sodium sulfate) and evaporation of the solvent, the residue was recrystallized from ethanol-water. Yield: 383 mg, 67.6%, m.p. 40-41.5°.

NMR spectrum: The above 383 mg of product was dissolved in 656.9 mg (0.4 ml) of a solution made by putting tetramethylsilane (one drop) in carbon tetrachloride (1 ml). This represents a 36.8% solution of the acetate. The spectrum showed no evidence of rearranged product: peaks at 6.86 τ (ferrocenyl protons), 7.31 τ (β-protons), and 7.90 τ ($OCOCH_3$), identical with an authentic sample.

Kinetic procedure:- All of the solvolytic rates (except the run on the "pH stat"---see below, p. 148) were determined in 80% acetone which was prepared as described by Hill (102). An automatic microburet (1 ml) with a Teflon stopcock was used to perform all titrations, which

TABLE X

NMR Spectra of β-Ferrocenylethyl Compounds

Compound		Peaks		
		τ	δ	cps below $SiMe_4$
β-Ferrocenylethanol	Fc	5.80	4.20	252.0
	α-CH_2	6.22	3.78	226.8
	β-CH_2	7.34	2.66	159.6
	OH	6.83	3.17	190.2
β-Ferrocenylethanol-α,α-d_2 (Synthetic)	Fc	5.78	4.22	253.2
	β-CH_2	7.35	2.65	159.0
	OH	6.55	3.45	207.0
β-Ferrocenylethanol-α,α-d_2 (Recovered from solvolysis)	Fc	5.77	4.23	253.8
	β-CH_2	7.33	2.67	160.2
	OH	6.38	3.62	217.2
β-Ferrocenylethylacetate-α,α-d_2	Fc	5.86	4.14	248.4
	β-CH_2	7.31	2.69	161.4
	$OCOCH_3$	7.90	2.10	126.0

were done under nitrogen in a flask of the following design: two standard taper female joints (19/38) were set approximately two centimeters apart on a flat-bottomed flask of about 50 milliliters capacity. A nitrogen inlet tube was passed through a standard taper male joint (19/38) and into the flask. The nitrogen was purified by being passed through a drying tower containing Drierite and through two gas bubblers containing Ascarite. A quenching solution (10-20 ml of 85% acetone-water) was added to the flask along with three drops of cresol red indicator and a magnetic stirring bar. The solution was stirred and cooled in an ice-salt bath and nitrogen was bubbled in for at least five minutes before a titration was begun. Aliquots of the solvolysis mixture (5 ml) were removed and titrated at appropriate time intervals. During the titration the nitrogen inlet tube was raised above the surface of the solution and the tip of the buret was placed just below the surface. A fresh flask was used for each titration and a clean volumetric pipet (5 ml) was used for each aliquot. The samples were solvolyzed in volumetric flasks (100 ml) in a constant temperature bath set at 30.00±0.02° C. In this manner, duplicate titrations (infinity titers, for example) could be reproduced within 0.003 ml or better out of a total volume of 0.700 ml.

Calculation of rate constants:- The solvolysis rate constants were calculated using the standard integrated first-order rate equation (103):

$$\ln(a-x) = kt + \ln a$$

where a is the infinity titer, x is the titer at time t, and k is the

first-order rate constant. Values of the rate constant were estimated from plots of $-\ln(a-x)$ vs t: what appeared to be the best straight line was drawn, the half-life was estimated from this plot, and the rate constant was calculated from the equation (105):

$$k = \ln 2/t_{\frac{1}{2}} = 0.693/t_{\frac{1}{2}}$$

Typical kinetic data are presented in Tables XI, XII, XIII and XIV.

TABLE XI

Run 2. Solvolysis of β-Ferrocenylethyl Tosylate, 80% Acetone, 30.00° C.

Sample: 185.8 mg. Initial concentration: 0.00483 m/l.

Titration	Titer (x ml) 0.0352 M NaOH	Titer of unchanged tosylate	t (hr)
1	0.043	0.649	1.54
2	0.082	0.610	3.56
3	0.283	0.409	13.02
4	0.363	0.329	18.51
5	0.460	0.232	28.32
6	0.495	0.197	33.64
7	0.556	0.136	44.16

Infinity titers: 0.696, 0.697 ml

Theoretical infinity titer: 0.692 ml

% Followed to completion: 91.2----Points 8-10 are not included above due to downward drift in rate at end of reaction (see Table X).

Rate constant: 1.09×10^{-5} sec^{-1} (estimated from plot)

TABLE XII

Run 2. Solvolysis of β-Ferrocenylethyl Tosylate, 80% Acetone, 30.00° C.

Sample: 185.8 mg. Initial concentration: 0.00483 m/l.

Calculation of Instantaneous Rate Constants to Demonstrate Downward Drift in Rate at End of Reaction

Time (min.)	ROTs (m mole)	$10^5 k$ (sec^{-1})
0	0.483	----
92.5	0.456	1.152
213.5	0.430	0.982
781	0.288	1.122
1110.5	0.232	1.116
1699.5	0.163	1.072
2018.5	0.139	1.038
2649.5	0.096	1.024
3210	0.068	1.026
3598.5	0.058	0.988
4174	0.044	0.963

TABLE XIII

Run 3. Solvolysis of β-Ferrocenylethyl Tosylate-α,α-d_2,

80% Acetone, 30.00° C.

Sample: 186.8 mg. Initial concentration: 0.00488 m/l.

Titration	Titer (× ml 0.0352 M NaOH	Titer of unchanged tosylate	t (hr)
1	0.030	0.668	1.72
2	0.084	0.614	3.73
3	0.238	0.460	13.11
4	0.323	0.375	19.76
5	0.407	0.291	28.40
6	0.448	0.250	33.98
7	0.517	0.181	44.27

Infinity titers: 0.699, 0.700 ml

Theoretical infinity titer: 0.698 ml

% Followed to completion: 74.1

Rate constant: $0.86_7 \times 10^{-5}$ sec^{-1} (estimated from plot)

TABLE XIV

Run 6. Solvolysis of dl-Ferrocenylisopropyl Tosylate, 80% Acetone, 30.00° C.

Sample: 175.3 mg

Initial concentration: 0.00440 m/l.

Titration	Titer (x ml 0.0352 M NaOH	Titer of unchanged tosylate	t (min)
1	0.037	0.585	61
2	0.067	0.555	123
3	0.153	0.469	300
4	0.270	0.352	603
5	0.354	0.268	905
6	0.378	0.244	1016
7	0.430	0.192	1262
8	0.452	0.170	1442
9	0.471	0.151	1559

Infinity titer: 0.622 ml

Theoretical infinity titer: 0.631 ml

% Followed to completion: 75.8

Rate constant: 1.53×10^{-5} sec^{-1} (estimated from plot)

Kinetic Isotope Effect in Solvolysis of β-Ferrocenylethyl Tosylate:- Separate samples of β-ferrocenylethyl tosylate and its α,α-dideutero analog were solvolyzed simultaneously on two separate occasions at 30.00° C. The pertinent data are gathered below in Table XV.

TABLE XV

Kinetic Isotope Effect in Solvolysis of β-Ferrocenylethyl Tosylate

Compound	Run #	Concn[a] (10^3 m/l)	% Followed	$10^5 k$ (sec^{-1})	$10^5 k$ (Avg.)	k_H/k_D
β-Ferrocenylethyl Tosylate	2	4.83	91.2	1.09	1.10±0.01	
	4	4.86	78.4	1.11		
β-Ferrocenylethyl Tosylate-α,α-d_2	3	4.88	74.1	0.86_7	$0.86_2 \pm 0.00_4$	1.27
	5	4.95	70.0	0.85_6		

[a]Initial concentration

Solvolysis of Optically Active Ferrocenylisopropyl Tosylate in Presence of Hydroxide Ion:- Optically active β-ferrocenylisopropyl tosylate (298.6 mg, 0.748 m mole, $[\alpha]_D^{25}$ -66.34°, chloroform c 2.05) was dissolved in reagent grade acetone through which nitrogen had been bubbled and added to aqueous sodium hydroxide (23.4 ml, 0.0352 M) in a volumetric flask (100 ml). The flask was filled to the mark with reagent grade acetone and nitrogen was bubbled in; a slightly cloudy solution resulted. The flask was then placed in a constant temperature bath maintained at 44° C where it remained for 19 hours. The reaction mixture was diluted with water (300 ml) and extracted with dichloromethane. The extract was washed with aqueous sodium hydroxide (5%) and water. After drying on a column of anhydrous sodium sulfate and evaporation to dryness, the product was chromatographed on deactivated aluminum oxide. One single band was observed which was eluted with chloroform. An IR spectrum of this band was identical to that of authentic β-ferrocenylisopropanol. The entire amount of this band (170.8 mg, 94%) was dissolved in spectral grade chloroform (5 ml). This proved too opaque to obtain a rotation; thus a portion of this solution (1 ml) was diluted to 5 ml and the rotation taken: $[\alpha]_D^{25}$ -23.42° (chloroform, c 0.683).

Racemization of Optically Active β-Ferrocenylisopropanol by p-Toluenesulfonic Acid:- The rotation of β-ferrocenylisopropanol in 80% acetone (solvent used for kinetic runs) was measured: $[\alpha]_D^{25}$ -18.47°

(80% acetone, c 0.812). (This material was a sample of that isolated from the solvolysis in the presence of hydroxide ion reported above.) β-Ferrocenylisopropanol (86.0 mg, 0.352 m mole $[\alpha]_D^{25}$ -18.47° was dissolved in 80% acetone (45 ml) to which p-toluenesulfonic acid monohydrate (66.5 mg, 0.35 m mole) was added. The solution was kept at 45° C for 8 hours; it was then poured into a mixture of ice and water and extracted with dichloromethane. (Sodium hydrosulfite was added to reduce ferricinium ion.) The extract was washed with aqueous sodium hydroxide (5%) and water, dried on a column of anhydrous sodium sulfate, and evaporated to dryness. The residue was dissolved in spectral grade chloroform and was found to possess zero rotation.

The Solvolysis of Optically Active β-Ferrocenylisopropyl Tosylate at Constant pH:-

The Apparatus

The pH-stat used in this experiment was built by M. D. Cannon, International Instruments Co., Canyon, California. The instrument and its use are described by Applewhite, Martin and Niemann (104). The essential features for use in constant pH titrations are: an "Agla" micrometer syringe driven by a variable speed, reversible motor controlled by a moving coil galvanometer (Constant Meter Relay) with manually adjustable fixed contacts that permit the choice of the desired pH; a Leeds and Northrup Model 7664-41 A.C.-operated pH meter; and a Leeds and Northrup Speedomax Type G Recorder.

In this experiment the reaction was run at ambient temperature (26°) in a beaker (250 ml) which was covered with a Lucite plate containing holes for the pH meter electrodes, a nitrogen inlet tube, a small glass propelier driven by a stirring motor and a hypodermic needle from the micrometer syringe. The nitrogen was bubbled through an acetone-water mixture in an attempt to minimize volume change due to evaporation. At appropriate times during the course of the reaction small amounts of acetone (3-6 ml) had to be added to maintain the starting volume. The pH stat was set to maintain the pH at 6.95 at the beginning of the reaction; after about 50 minutes of reaction the setting was changed to pH 6.9 since this was necessary to take into account the slowness of the electrode response and avoid overshoot in the addition of base (see below).

The reaction had to be run in 60-40 acetone-water rather than the usual 80% acetone since the response of the electrodes is very sluggish in the latter solvent, causing considerable excess base to be added to the solution before the pH meter caught up with the actual pH of the system and closed the Contact Meter Relay to stop the micrometer syringe. In 60% acetone the electrodes were much less sluggish and the rate of the reaction was about 5 times as fast (see below): the combined effect of these two factors allowed the rate of the addition of standard base to be adjusted to essentially that required by the reaction. The syringe was refilled with base 16 times during the course of the reaction (~17.5 hours); this usually led to a fall in the pH to as low as 4 before the syringe

could be replaced. However, the amount of time which the reaction spent at pH < 6.9 is quite trivial compared to the whole reaction time, since it takes less than 5 minutes to refill and replace the syringe and, in any case, after the first 3 half-lives or so (~ 9 hours) the production of acid had considerablyslowed and the pH then fell very little during syringe changes. Toward the end of the reaction, the pH sometimes reached about 8.0 due to overshoot and the addition of base was for the most part controlled by manipulation of the controls for the micrometer syringe motor.

The chart paper moved at the constant speed of one inch per minute in one dimension and since from previous calibration it was known that each division in the other dimension corresponds to 4.888 μl of standard base, both time and volume data could be taken directly from the chart.

The Reaction

(-)-β-Ferrocenylisopropyl tosylate (287.1 mg, 0.719 m mole $[\alpha]_D^{25}$ -66.34° chloroform, c 2.05) was dissolved in purified acetone (90 ml). Time zero was taken as that moment when carbon dioxide-free water (60 ml) was added to make a total volume of 150 ml 60-40 acetone-water. The standard base was 0.0803 M; it was found necessary to add a small amount of more concentrated (~0.2 M) base along with 993.2 μl of 0.0803 M base at the very beginning of the reaction to adjust the pH to 6.95. The amount of concentrated base added was neglected in the

calculation of the instantaneous rate constants (see below). The reaction was allowed to run for 17.5 hours. It was then diluted with ice-water and extracted with dichloromethane. The extract was washed with water, dried on a column of anhydrous sodium sulfate and evaporated to dryness. The residue weighed 164.8 mg; the theoretical yield of β-ferrocenylisopropanol is 175.5 mg. An attempt to determine the rotation of this material met with difficulties (see p. 94) which were overcome by converting it to the tosylate. The identity of the alcohol was shown by an IR spectrum, and it was not further purified before conversion to the tosylate which was carried out in the usual manner with tosyl chloride in pyridine. The tosylate was purified by chromatography on deactivated aluminum oxide (eluted with chloroform) and the identity of the tosylate was proven by IR spectral comparison with an authentic sample. The rotation of this material was within experimental error (±0.01°) the same as that of the blank.

Approximate Rate Constant

Instantaneous rate constants were calculated from the equation (106)

$$k = \frac{2.303 \log(a/a-x)}{t}$$

where x, the amount of base consumed at time t, was calculated from the chart. A correction for the amount of base added to adjust the pH at the beginning of the reaction (993.2 μℓ) which neglects the small amount

of concentrated (~0.2 M) base also added at this time. The rate data are listed in Table XVI, where it is seen that the initial rate increased rapidly during the first half-life of the reaction. The reason for this initial rapid rise in rate was not determined, but one possibility is that it was a function of the machine rather than the reaction itself (106).

TABLE XVI

Rate of Solvolysis of β-Ferrocenylisopropyl Tosylate at Constant pH Ambient Temperature 26°

Solvent: 60-40 acetone-water

Sample: 287.1 mg

Initial concentration: 0.00479 m/l

t (min)	ROTs (m mole)	$10^5 k$ (sec^{-1})
0	0.719	----
62.8	0.609	4.407
78.8	0.574	4.769
98.8	0.536	4.952
107.2	0.505	5.490
127.8	0.467	5.632
145.6	0.436	5.735
168.2	0.401	5.793
194.4	0.364	5.838
229.8	0.324	5.786

Average k: $5.378 \pm 0.177 \times 10^{-5}$ sec^{-1} for all points.

$5.757 \pm 0.073 \times 10^{-5}$ sec^{-1} for last five points.

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III. CHEMICAL SHIFTS AND π-ELECTRON DENSITIES

Introduction

The NMR chemical shifts (relative to benzene) of the isoelectronic molecules cyclopentadienyl anion ($C_5H_5^-$), benzene (C_6H_6) and tropylium cation ($C_7H_7^+$) were recently reported by Fraenkel, Carter, McLachlan and Richards (1)* in a paper in which careful consideration was given to the effect of π-electron density on the chemical shift and an electrostatic model was derived which suggests that chemical shifts for aromatic protons are primarily due to interaction between charge localized in the π-orbital of a carbon atom and the electrons in the C-H bond. The chemical shifts for this isoelectronic series had previously been measured by Leto, Cotton and Waugh (2) who did not use a common solvent for all measurements and who drew no conclusions concerning the origin of these shifts from their data. In the work under discussion here (1) the shifts were measured in two common solvents (dimethyl sulfoxide and acetonitrile) for several salts of $C_5H_5^-$ and $C_7H_7^+$ which were prepared by literature methods (see Experimental Section of reference 1 for details). The results are listed in Table I from which the average values (relative to benzene) +1.85 ppm for $C_5H_5^-$ and -1.90 ppm for $C_7H_7^+$ may be obtained.

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*The credits for this paper should be allocated as follows: experimental, Carter and Fraenkel; discussion, Fraenkel and McLachlan; and theory, McLachlan.

TABLE I[a]

Chemical Shifts of Salts of $C_5H_5^-$ and $C_7H_7^+$

Compound[b]	Solvent	δ ± 0.004 ppm
$C_7H_7^+ ClO_4^-$	Dimethyl sulfoxide	-1.938
$C_7H_7^+ ClO_4^-$	Acetonitrile	-1.873
$C_7H_7^+ BF_4^-$	Dimethyl sulfoxide	-1.934
$C_7H_7^+ BF_4^-$	Acetonitrile	-1.873
$C_5H_5^- Na^+$[c]	Dimethyl sulfoxide	1.970
$C_5H_5^- Na^+$[c]	Acetonitrile	1.785

[a] Taken from reference (1).

[b] Concentration ~2% in all cases.

[c] The purple color of these solutions[(1)] was due to impurities; $C_5H_5^-Na^+$ made under very pure N_2 forms colorless solutions.

Equation 1 was suggested (1) to relate the chemical shift (δ) of an aromatic hydrogen (relative to benzene) to the excess π-electron density q on the carbon atom; a is a constant for which the value

$$\delta = aq \qquad (1)$$

+ 10 ppm/electron was adopted and from a theoretical consideration of the polarization of the aromatic C-H bond due to q, it was estimated that this polarization contributes 4-7 ppm to the value of a. The remainder of this part of this Thesis will demonstrate the usefulness of equation 1 in the estimation of charge distributions in aromatic molecules.

I. Aromatic π-Electron Densities

In Table III of reference 1 the charge distributions of nitrobenzene, aniline and pyridine, estimated from chemical shifts (relative to benzene) and equation 1, were compared with charge distributions calculated by molecular orbital methods. It was pointed out (1) that "since the theoretical calculations are based on highly simplified assumptions and are not intended to provide accurate electron densities, it is not surprising that there are, at times, fairly large discrepancies between the calculated and experimental values. In particular, they involved assigning an 'electronegativity parameter' to nitrogen and oxygen. A recent theoretical discussion (3) suggests that some of the [theoretical calculations] are based on too large a value for this parameter and may exaggerate the non-uniformity of the charge distribution." In this connection, it is interesting to note the work of Brown and Heffernan (4) on the calculation of π-electron densities in pyridine: an "electronegativity parameter" of 0.5 is assigned to the nitrogen* and the resulting charge densities are overall in better agreement with those from the NMR data. This is shown in Table II where the theoretical and experimental π-electron densities for several aromatic and heterocyclic molecules are compared. Included are the comparisons made in reference 1 as well as those for pyrimidine, pyrrole, furan and thiophene. Unfortunately, all of the NMR data was not obtained with reference to the same standard and none of the values was with reference to

*Longuet-Higgins and Coulson (5), whose work was quoted in reference 1, used a value of 2.0.)

TABLE II

Charge Distributions Estimated from Chemical Shifts

Compound	Excess electron density, q			Origin
	o	m	p	
Nitrobenzene	-0.097	-0.030	-0.042	NMR[a]
	-0.15	0.00	-0.17	b
Aniline	0.077	0.013	0.040	NMR[a]
	0.02	0.00	0.02	b
Pyridine	-0.13	0.00	-0.037	NMR[c,d]
	-0.151	-0.053	-0.178	e
	-0.077	0.00	-0.050	f
	-0.048	0.00	-0.019	g
	2		5	
Pyrimidine	-0.21		0.02	NMR[h]
	-0.16		0.01	f
	α		β	
Pyrrole	0.02		0.06	NMR[i]
	0.15		0.03	j
	0.1		0.06	e
Furan	-0.01		0.10	NMR[k]
	0.01		0.11	m
Thiophene	-0.03		-0.02	NMR[n]
	0.00		0.06	p

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benzene. However, all of the data in the table have been converted to relate to an internal benzene reference (see notes to table). Despite the presence of some discrepancies (see above), the results in Table II are on the whole encouraging.

The recent literature provides a great deal of support for the simple proportionality expressed by equation 1 and for the value +10 ppm/electron for the constant a. Smith and Schneider (6) compared the NMR spectra of pyridine and pyridinium ion and suggested that the change in local charge densities (Δq) between the two molecules is simply proportional to the changes in chemical shifts for the several protons, i.e., $\Delta\delta = a\,\Delta q$.* The constant a was found to have the value 9.5 ppm. This work will be discussed below (p. 173) in connection with some considerations of the changes in chemical shifts associated with the protonation of metallocenes.

Spiesecke and Schneider (7) determined π-electron densities in azulene from C^{13} and hydrogen chemical shifts. The chemical shifts of $C_5H_5^-$, C_6H_6 and $C_7H_7^+$, taken from reference 1, were plotted against the known local π-electron densities of these molecules, for which the symbol ρ was used: i.e., $\rho_{C_6H_6} = 1.0$, $\rho_{C_5H_5^-} = 1.2$ and $\rho_{C_7H_7^+} = 0.86$. The straight line thus obtained had a slope of 10.6, which is the constant a in equation 1. The C^{13} chemical shifts of

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*These symbols are used for the sake of uniformity. Smith and Schneider (6) used $\Delta\delta = k\Delta\rho$ instead.

$C_5H_5^-$, C_6H_6, $C_7H_7^+$ and $C_8H_8^=$ were measured (7) and similarly plotted.* The equation $\Delta\delta_{C^{13}} = 160q$ was obtained. In both cases a lack of strict proportionality was noted: the deviations of the individual points from the lines are greater than the experimental error in the chemical shift measurements.** This was also noted in reference 1 where it was pointed out that the correlation between chemical shifts and theoretical electron densities in azulene (7) suggests that the lack of strict proportionality should not be regarded as evidence against equation 1 but rather is due to special effects as yet unexplained. The work of Spiesecke and Schneider (7) confirms this view: Table III presents some of their data compared with theoretical excess π-electron densities of azulene calculated by three different methods, and it is apparent that the values from equation 1 are in reasonable agreement with the theoretical values. The chemical shifts of the various azulene protons were corrected (7, 8) for the shift due to the ring current of the neighboring ring.

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* $\rho_{C_8H_8^=} = 1.25$.

** The proton chemical shift of $C_8H_8^=$ has been measured by Katz (7a) and by Fritz (7b). A value of +1.15 ppm relative to benzene was reported (7a). Equation 1 predicts a shift of 2.5 ppm and thus there is a rather sizeable discrepancy in this case. The shift for cyclooctatetraene itself is quite close to that of $C_8H_8^=$ (7a, b); Fritz (7b) suggests that this is due to the operation of two opposing effects. One is the ring current due to the 10 π-electrons which should cause a shift to lower field (-1.95 ppm relative to cyclooctatetraene) and the other effect is a "charge effect" which should cause a shift to higher field.

TABLE III[a]

Chemical Shifts and π-Electron Densities of Azulene

Position	δ[b]		q from	q calculated		Brown and
	Meas.	Corr.[c]	Eq. (1)[d]	Pariser[e]	Julg[f]	Heffernan[g]
2	-0.583	-0.23	-0.022	-0.021	-0.003	-0.012
3,1	-0.067	+0.58	+0.055	+0.096	+0.049	+0.059
4,8	-0.945	-0.34	-0.032	-0.121	-0.092	-0.045
5,7	+0.280	+0.48	+0.045	+0.049	+0.034	+0.011
6	-0.170	-0.03	-0.003	-0.052	-0.062	-0.031

[a] Data from reference 7.

[b] Ppm relative to benzene.

[c] Corrected for ring current of neighboring ring.

[d] a = 10.6 ppm/electron.

[e] R. Pariser, J. Chem. Phys. 25, 1112-1116 (1956).

[f] A. Julg, J. Chim. Phys. 52, 377-381 (1955).

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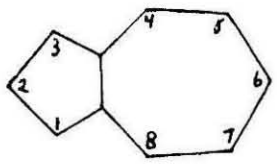


MacLean and Mackor (9) obtained the NMR spectra of a number of aromatic carbonium ions such as those derived from pentamethylbenzene, 9,10-dimethylanthracene, and anthrone. They calculated excess charge densities from chemical shifts by a consideration of the linear term in equation 2. The value for a_1 obtained by these authors is

$$\delta = a_1 q + a_2 q^2 + \cdots \qquad (2)$$

13.4 ppm/electron which, they note, corresponds satisfactorily with the value 10 ppm suggested in reference 1. The second term in equation 2 may be in part accounted for by the "distortion of the hydrogen 1s orbital by the electrostatic field of the excess charge"(9). MacLean and Mackor (9) suggest that the lack of strict proportionality between δ and q (see above) may be due to this second term.

This discussion provides ample demonstration of the usefulness of equation 1 in connection with the determination of charge densities in aromatic compounds. In Table IV the values of the proportionality constant a which have been suggested by the various authors are summarized.

TABLE IV

Values of a in $\delta = aq$

a (ppm/electron)	Ref.
10	1
9.5	6
10.6	7
13.4	9

II. Changes in Chemical Shift Following Protonation

The chemical shifts (relative to benzene) of the metallocenes ferrocene, ruthenocene and osmocene in tetrahydrofuran are given in reference 1. Curphey, Santer, Rosenblum and Richards (10) report shifts for these same compounds (relative to $SiMe_4$) in carbon tetrachloride or deuterochloroform as well as the shifts after protonation by BF_3-H_2O. These data are presented in Table V along with the calculated change in chemical shift ($\Delta\delta$) due to protonation.

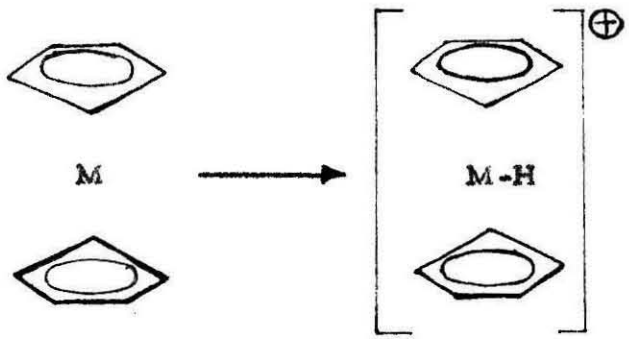


TABLE V

Changes in Chemical Shift Following Protonation

Compound	Solvent	(ppm rel. to φH)	Δδ	Ref.
Ferrocene	THF	3.200		1
Ferrocene	CCl_4	3.19	-0.97	a
Ferrocene	BF_3-H_2O	2.23		10
Ruthenocene	THF	2.805		1
Ruthenocene	CCl_4	2.81	-0.90	10
Ruthenocene	BF_3-H_2O	1.90		10
Osmocene	THF	2.696		1
Osmocene	$CDCl_3$	2.52	-0.59;-0.41	10
Osmocene	BF_3-H_2O	2.11		10

[a] From value reported by G. V. D. Tiers, abstracts of the 137th Meeting of the American Chemical Society, April 1960, p. 4-0.

In each case, the transformation from metallocene to (metallocene-H)$^+$ must involve a decrease in the available electron density by one unit charge for the whole metallocenyl nucleus. According to Pauling's resonating bond treatment of ferrocene (11), the iron atom in the unprotonated material bears a charge of -0.22. We may say, then,

that protonation will raise a demand for 0.88 unit of charge from the two cyclopentadienyl rings, or 0.088 unit per C-H bond.* Therefore, according to equation 1, the chemical shift for protonated ferrocene (relative to ferrocene itself) should be moved to lower field by about 0.9 ppm, i.e., $\Delta\delta = -0.9$ ppm. The data in Table V demonstrate that the observed shift for ferrocene due to protonation (as well as that for ruthenocene) is in line with this calculation ($\Delta\delta = -0.97$ ppm).** The rather low value of $\Delta\delta$ for osmocene (-0.59 ppm) may be attributed at least in part to a smaller degree of protonation in this case, as suggested by Curphey, Santer, Rosenblum and Richards on the basis of other considerations (10).

The work of Smith and Schneider (6) on the protonation of pyridine in trifluoroacetic acid was mentioned above (p. 167) and may be briefly outlined here. The chemical shifts of pyridine and pyridinium ion were determined relative to CH_2Cl_2; the $\Delta\delta$ values for the various hydrogens are given in ppm in Table VI.

The formation of pyridinium ion from pyridine cannot be directly compared with the protonation of metallocenes since the charge distributions (on carbon) are not uniform in both cases. However, if it is assumed that the change in chemical shift for each proton is proportional to the amount of charge removed from each carbon in the formation of

*Other treatments of ferrocene (Shustorovich and Dyatkina(13), for example) place a + charge on the iron. If this is the case, we must take one whole unit of charge from the rings upon protonation, which leads to a predicted shift of 1 ppm to lower field.

**No correction for bulk diamagnetic susceptibility effects was made since all of the data from which Table V was compiled were obtained with reference to interrelatable internal standards.

TABLE VI[a]

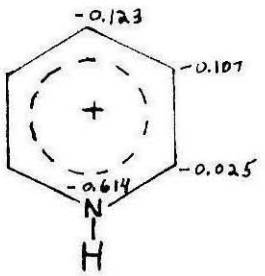
Chemical Shifts of Pyridine and Pyridinium Ion

(ppm relative to internal CH_2Cl_2)

	Pyridine	Pyridinium Ion	Δδ, ppm
H_α	-3.33	-3.58	-0.25
H_β	-1.87	-2.94	-1.07
H_γ	-2.28	-3.50	-1.22

[a]Data from reference 6.

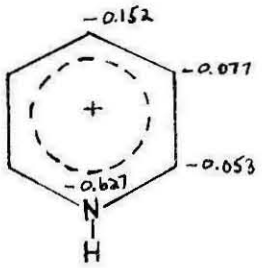
the ion, the difference in charge distribution between the ion and the free base may be calculated. If equation 1 is used in the form $\Delta\delta = a\Delta q$ with $a = 10$, the following differential charge distribution is obtained:



I

Smith and Schneider (6) used the value 9.5 for the constant a, which they calculate from the chemical shifts coupled with the fact that the equation $\Sigma\Delta q = 1$ must be satisfied. It is of interest to compare this "experimental" difference in charge distribution with that obtained theoretically. Brown and Heffernan (12) have calculated the π-electron

densities for the various atoms in pyridine and in pyridinium cation. From their data the differences in π-electron densities shown in **(II)** may be calculated:



II

The agreement is quite acceptable; the data are summarized in Table **VII**.

TABLE VII

Changes in π-Electron Densities on Protonation

(Pyridine ⟶ Pyridinium cation)

Position	Δq		
	NMR(a = 10)	NMR (a = 9.5)[a]	Theory[b]
N	-0.614	-0.594	-0.627
C_α	-0.025	-0.026	-0.053
C_β	-0.107	-0.113	-0.077
C_γ	-0.123	-0.128	-0.152

[a] Ref. (6)

[b] Ref. (12)

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PROPOSITIONS

PROPOSITION I

The Hammett equation has been very widely applied to many

$$\log (k/k_o) = \sigma\rho$$

reactions involving meta- and para-substituted phenyl compounds (1); it is the most general relationship known for the effect of substituents on rates or equilibria (1c), but it generally fails when applied to reactions in which steric factors at the reaction center are not constant. It is for this reason that the Hammett equation has been very little used to correlate rates or equilibria in olefin addition reactions. This Proposition suggests a way in which a modified form of the Hammett equation may be employed for such a correlation.

An attractive mechanistic possibility in many olefin addition reactions is the intermediacy of a π-complex which would presumably also be present to some extent in the rate-determining transition state. For example, Skell (2) has suggested such a possibility in the addition of carbenes to olefins; Simmons and Smith (3) proposed an intermediate similar to a π-complex in the reaction between methylene iodide and olefins in the presence of a zinc-copper couple; and recent proposals by Taft and his coworkers (4, 5) have implicated a π-complex in the hydration of olefins.

Equilibria involving cyclic olefins and complexing agents (Ag^+, I_2) have been studied by Traynham and Olechowski (6), who determined equilibrium constants for π-complex formation in these systems. If such equilibria could be correlated with rate constants for olefin addition reactions by means of a Hammett-type equation, the information obtained from the correlation would be a great aid in elucidating the detailed mechanism of such reactions. If the rate-determining transition state involved a species analogous to that found in a molecular complex equilibrium, a good correlation might be expected. However, steric effects in olefin π-complex equilibria and in olefin addition reactions are likely to be of sufficient importance to prevent Hammett-equation correlations. It is to avoid this difficulty that the following proposal is suggested.

If a compromise is made, the steric factor may be kept constant to all intents and purposes while at the same time substituent effects on π-complex equilibria involving an olefinic bond may be studied: the compromise is the use of styrene as the donor molecule. It is therefore proposed that "σ^{π}" constants be obtained for π-complex equilibria involving p-substituted styrenes for use in the equation

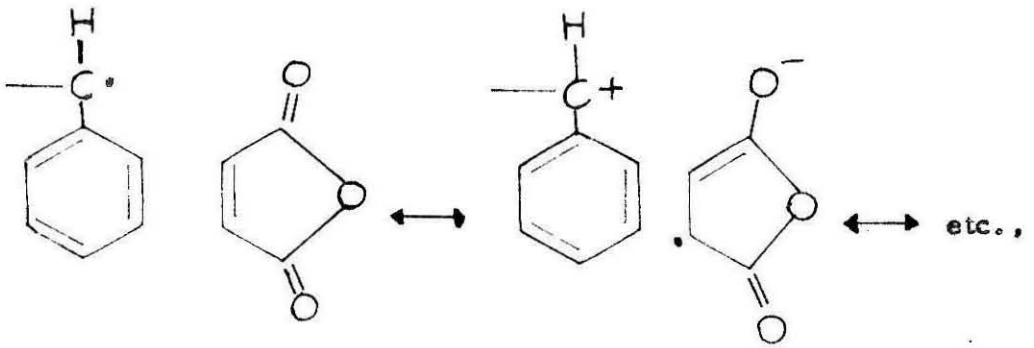
$$\log (K/K_o) = \sigma^{\pi} \rho$$

where the symbol σ^{π} is used to differentiate between these constants and those of the original Hammett equation. σ^{π} is simply $\log K'/K'_o$, where K' and K'_o are equilibrium constants for substituted and unsubstituted compounds, respectively, in an equilibrium chosen as standard.

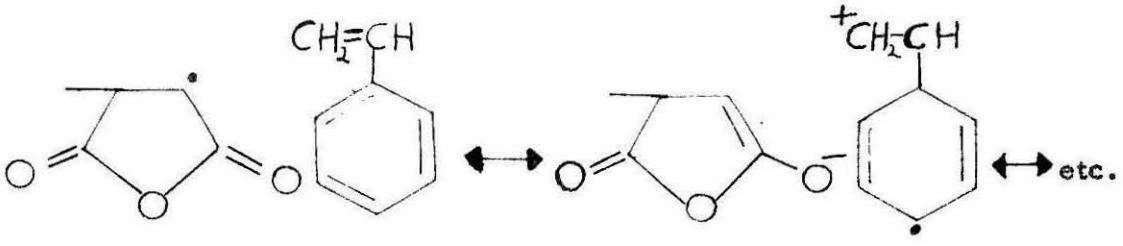
This standard equilibrium might be that between p-substituted styrenes and Ag^+: there is evidence that the silver ion is complexed preferentially with the olefinic side-chain in styrene itself, at least. Andrews and Keefer (7) found that the equilibrium constant for the interaction of styrene with silver ion is exceptionally high in comparison with the constants for the other aromatic donor molecules investigated. Since the equilibrium constants for olefin-Ag^+ complexes are much higher than those for aromatic-Ag^+ complexes (7,8), a specific coordination of the silver ion with the vinyl linkage in styrene is indicated. It is pertinent that Holden and Baenziger (9) have shown by X-ray diffraction studies that in the crystalline $PdCl_2$-styrene complex the palladium is associated preferentially with the vinyl side-chain. Complications may arise from this choice of a standard equilibrium due to the existence of 1:2 as well as 1:1 donor:acceptor complexes in this case and also due to the fact that with iodine as the substituent, anomalous results may be obtained because of preferential complexing of the silver cation with the iodine (10). Nevertheless, once σ^π values are obtained they may be used in attempts to correlate rate data for addition reactions involving the olefinic side-chain. The appropriate equilibrium constants for calculating σ^π values could be determined spectrophotometrically using the methods of Andrews and Keefer (10,11).

Addendum

It is interesting to note that in a study of the "alternating effect" that occurs in many free radical copolymerizations and leads to the alternation of the two monomer units along the polymer chain, Walling and his coworkers (12) suggested that in strongly alternating systems such as styrene plus maleic anhydride the energy of the transition state for the radical-olefin addition may be lowered by the participation of resonance structures in which electron transfer has occurred between radical and olefin. For the attack of a styrene radical on maleic anhydride such structures might be (12)



and, for the conjugate reaction, in which a maleic anhydride radical adds to styrene (12),



The basis for this suggestion is a study of the effects of meta- and para-substituents on the reactivity of styrene (and methyl-styrene) towards a series of reference radicals of differing polarity: the styrene radical itself, the methyl methacrylate radical and the maleic anhydride radical (12). Hammett plots of the logarithms of the relative reactivities (i.e., relative to unsubstituted styrene) versus the σ values for the various substituents produced an excellent linear relationship only for the data involving the styrene radical. The other two systems are strongly alternating, and styrenes with electron-supplying groups showed abnormally increased reactivities. This led Walling and his coworkers (12) to draw a parallel between the transition state for polymerization and the formation of molecular complexes between donors such as substituted styrenes and acceptors such as methyl methacrylate and maleic anhydride. This is reasonable; however, a good linear correlation between log (relative reactivity) versus σ^{π} values obtained from equilibrium constants for styrene-maleic anhydride π-complex formation would have been a valuable substantiation of these conclusions in line with the tenor of this Proposition.

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PROPOSITION II

A large number of biochemical reactions involve the replacement of a C-H bond by a C-O bond. Metabolic hydroxylations and epoxidations and the formation of dihydrodiols from aromatic compounds are examples of this process (1).

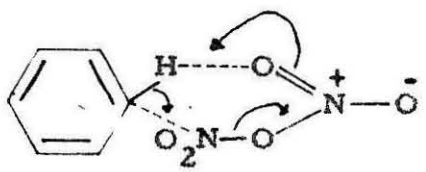
The proposals outlined below are concerned with the electrophilic hydroxylation of aromatic compounds which occurs, for instance, as a detoxification mechanism in many organisms (2). The salient characteristics of the enzyme systems that catalyze these reactions are: (a) dependence upon the presence of both a reducing agent and oxygen; (b) origin of the introduced hydroxyl group in O_2 and not in H_2O; and (c) hydroxylation at positions on the aromatic ring which are normally attacked by electrophilic agents (3). Thus the overall reaction has many of the characteristics of a classical electrophilic aromatic substitution.

A model system which hydroxylates aromatic and heterocyclic compounds was discovered and carefully studied by Udenfriend and his coworkers (4). Nonspecific, nonenzymatic hydroxylation occurs in the presence of O_2, ascorbic acid, ferrous ion, and ethylenediaminetetraacetic acid. No firm conclusions about the detailed mechanism of the reaction have been published, but the appearance of the hydroxyl group at positions normally attacked by electrophiles suggested the replacement of ring hydrogen by OH^+ (5); an alternative theory proposes

that the effective reagent is a product of the reaction of hydrogen peroxide with ascorbic acid (4).

The purpose of this proposition is to obtain information about the intimate mechanism of the hydroxylation of aromatic substrates catalyzed both by model systems and by enzymes. The work of Melander and others (6) concerning the use of the isotope effect to study the mechanism of electrophilic aromatic substitution is extensive and affords a great body of data for comparison with the results obtained in this study.

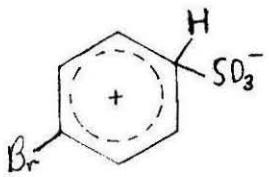
There have been a number of studies in which isotope effect data in an aromatic substitution have provided evidence of great utility in the establishment of reaction mechanisms. For example, Halvarson and Melander (7) obtained $k_H/k_T = 1.0 \pm 0.1$ in the nitration of anisole with benzoyl chloride plus silver nitrate in acetonitrile. This result ruled out a possible concerted mechanism,



which had been proposed earlier (8).

A more common situation is that in which an observed isotope effect is used as a criterion for timing the formation of a new bond or the breaking of an old bond at the reaction center. The isotope effect in the sulfonation of bromobenzene with sulfuric acid in nitrobenzene was determined in competitive experiments with bromobenzene-4-t by

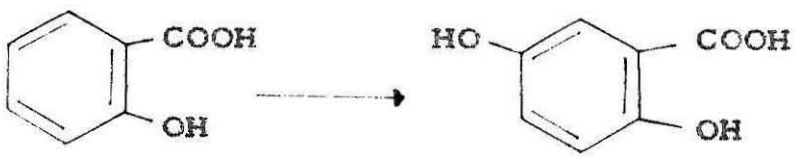
Berglund-Larsson and Melander (9). k_H/k_T was of the order of 2. The absence of an isotope effect in nitration (see above) was interpreted in terms of a rate-determining bond formation; the presence of an effect in sulfonation must therefore imply the participation of C-H bond-breaking in the rate-controlling step. This type of reasoning, coupled with the fact that ordinary sulfonic acids are quite strongly acidic, led to the proposal that, in the intermediate (I), the negative charge not far from the hydrogen to be removed in the final step is likely to slow down



I

the proton expulsion, which might then become partly rate-controlling (9a). That the removal of the proton is not altogether rate-controlling is indicated by the size of the isotope effect: a k_H/k_T of about 5 or greater would be expected if proton removal were rate-determining.

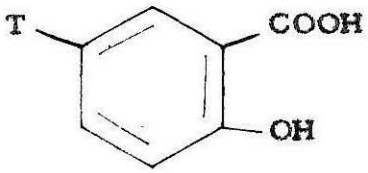
An example of a reaction which can profitably be studied is the hydroxylation of salicylic acid to gentisic acid. This conversion is



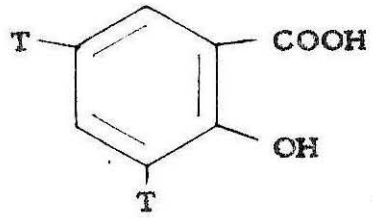
brought about by Udenfriend's model system in 30% yield (10); horse-radish peroxidase catalyzes the same reaction in the presence of O_2

and dihydroxyfumaric acid (11).

The desired labelled substrate (II) cannot be be easily made since the ortho and para hydrogens as well as the carboxyl hydrogen and the phenolic hydrogen will exchange rapidly in the presence of acid or base. However, the compound (III) can easily be prepared by the treatment of salicylic acid with T_2SO_4-T_2O followed by exchange of the carboxylic



II



III

and phenolic tritium atoms with scrupulously neutral H_2O. The presence of the tritium in the ortho position will at most have a small secondary effect (if any at all) on the substitution of the para tritium in the hydroxylation and its presence can be corrected for in the calculation of the isotope effect.

All of the substances to be used as substrates will be simple, readily available aromatic or heterocyclic compounds which yield predominantly or entirely one product on hydroxylation. Of course, substrates must be chosen from which the same product arises whether the conversion is carried out enzymatically or in the presence of a model system. The great variety of aromatic substances which can be hydroxylated by natural systems (Mason (1) lists about 100) makes the choice of appropriate substrates an easy task.

Thus, two proposals are suggested:

I. Determine the deuterium and/or tritium isotope effects on the rates of hydroxylation of various substrates in Udenfriend's model system.

II. Determine the isotope effect on the rates of enzymatic hydroxylation of the same substrates.

In both cases the effect can be measured by competitive reactions. A mixture of labelled and unlabelled compound may be allowed to react in the presence of the appropriate hydroxylating system and the disappearance of the label can be followed conveniently by nuclear magnetic resonance or infrared spectroscopy if the isotope used is deuterium or by radioactivity measurements if it is tritium. The isotope of choice is tritium since it gives, in general, larger kinetic isotope effects than deuterium and its radioactivity makes it detectable in very small amounts.

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PROPOSITION III

A promising approach to the study of hydrogen bonding is the correlation of results obtained by IR and NMR spectroscopic methods (1). This approach has been applied to a limited extent in the investigation of the relatively weak hydrogen bonds between chloroform and suitable donor solvents. Korinek and Schneider (2) determined the chemical shifts(relative to unassociated $CHCl_3$) of the chloroform proton in solutions of various concentration in triethylamine, ether, propionitrile and propyl fluoride.* Huggins, Pimentel and Shoolery (3) had previously measured the shift in triethylamine as well as that in acetone. These data are summarized in Table I along with IR data obtained by Huggins and Pimentel (4) and by Lord, Nolin and Stidham (5) for $CDCl_3$ in triethylamine, acetone and ether. There is apparently a rough correspondence between the chemical shift and the IR band half-width.

Person and his coworkers (6) studied the IR spectra of charge-transfer complexes of iodine monochloride, iodine cyanide, bromine and chlorine with various donor solvents. The equation (7)

$$(\epsilon_o + \epsilon_a) = 1.537 \times 10^{-2}\sqrt{B/\mu}$$

was used to calculate ϵ_a, the additional charge on the Y atom due to resonance structure b:

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*The data were extrapolated to infinite dilution to correct for the presence of self-associated $CHCl_3$.

TABLE I

NMR Chemical Shifts of $CHCl_3$ and IR Data for $CDCl_3$

Solvent	NMR $\Delta\delta$, ppm[a]	IR $\Delta\nu$[b], cm^{-1}	IR $\nu_{1/2}$, cm^{-1}
Triethylamine	1.20	84(4)	42(4)
Acetone	0.81	0(4,5)	16(4);11(5)
Ether	0.76	10(5)	16(5,c)
Propionitrile	0.55	----	----
Propyl fluoride	0.32	----	----

[a] $\Delta\delta = \delta_o - \delta$ where δ_o is the shift for unassociated $CHCl_3$ determined by extrapolation to infinite dilution of shifts for $CHCl_3$ in inert hydrocarbon solvents. δ_o = 0.30 ppm (2).

[b] Relative to unassociated $CDCl_3$ (4,5).

[c] Incorrectly quoted as 15 in ref. 2.

$$\underset{a}{D\cdots X\text{-}Y} \longleftrightarrow \underset{b}{(D\text{-}X)^{+}\cdots Y^{-}}$$

In the above equation ϵ_o is the effective charge of the uncomplexed molecule (in carbon tetrachloride solution), μ is the reduced mass of the atoms involved in the X-Y bond, and B is the integrated intensity. Values of ϵ_a for the various complexes were plotted versus the relative change in force constant $(k_o - k/k_o)$, where k_o is the force constant for the uncomplexed X-Y bond, and a reasonable correlation was obtained (6b).

Included in this correlation were values of ϵ_a and k/k_o for some of the hydrogen-bonded complexes studied by Huggins and Pimentel (8) (pyrrole and various donor solvents, methanol and the same solvents, and acetic acid dimer). If we consider the fact that all of these data were not determined in exactly the same way (i.e., different temperatures for the IR spectra and different integration limits for B were used (6b, 8)), the correlation obtained is a striking experimental confirmation of the theoretical relation between charge-transfer complexes and hydrogen bonds (6).

The purpose of this proposition is to correlate $\Delta\delta$ and ϵ_a values for a large number of hydrogen-bond complexes of $CHCl_3$. Such a correlation may then be related to the equation $\delta = aq$ which was proposed by A. McLachlan in a paper by Fraenkel, Carter, McLachlan and Richards (9) to relate the excess π-electron density (q) on carbon in an aromatic C-H bond to the chemical shift. (See Part III of this Thesis.) This proposition may be divided into three parts:

1. Determine ϵ_a for $CHCl_3$ hydrogen-bond complexes with non-associating solvent donors such as triethylamine, acetone, various ethers, ethyl acetate, acetic anhydride, etc. at infinite dilution. Experimental difficulties which will arise due to overlapping bands of $CHCl_3$ and the solvent may be overcome by the use of deuterated solvents or $CDCl_3$.

2. Determine $\Delta\delta$ for the same series of complexes at the same temperature and at infinite dilution.

3. Correlate ϵ_a with $\Delta\delta$. A positive correlation is certainly expected on the basis of the above discussion of data already in the literature. The slope of the plot of $\Delta\delta$ vs. ϵ_a is presumably the constant a in the equation $\Delta\delta = a\epsilon$. The chemical shifts observed in these $CHCl_3$-donor systems are probably caused primarily by the polarization of the C-H bond: i.e., the effects due to the presence of an aromatic system which complicated the full estimation of a in reference 9 will be absent from the $CHCl_3$-donor systems. If the C-H bond in these systems may be described by a wave function

$$\Psi = \Psi_o + \lambda\Psi_1$$

where Ψ_o and Ψ_1 represent wave functions for the ground and excited (i.e., polarized) states of the C-H bond, respectively, the experimental value of a may be used to estimate λ (9). In any case, the experiments suggested in this proposition will aid in the formulation of theories of hydrogen bonding.

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PROPOSITION IV

Methylene (CH_2) may be produced from diazomethane in various ways (1), three of which are: (a) a direct photolysis reaction; (b) a decomposition catalyzed by Fe(DPM)$_3$* ; and (c) the use of benzophenone as a photosensitizer. The first of these methods produces singlet methylene which is a highly reactive (and therefore unselective) intermediate. For example, the reaction between methylene from diazomethane photolysis and various hydrocarbons appears to proceed by random insertion (2, 3). The Fe(DPM)$_3$-catalyzed decomposition of diazomethane has been studied by Kopecky, Hammond and Leermakers (1) and was shown to lead to a selective methylene which apparently only adds to the double bond; no insertion product was detected. A similarly selective methylene was produced in the photosensitized reaction which leads to the triplet state of methylene:

$$CH_2N_2 \xrightarrow{h\nu} CH_2 \uparrow\downarrow + N_2$$

$$(C_6H_5)_2CO \xrightarrow{313\ m\mu} (C_6H_5)_2CO^{*1} \longrightarrow (C_6H_5)_2CO^{*3}$$

$$(C_6H_5)_2CO^{*3} + CH_2N_2 \longrightarrow CH_2N_2^{*3}$$

$$CH_2N_2^{*3} \longrightarrow CH_2 \uparrow\uparrow + N_2$$

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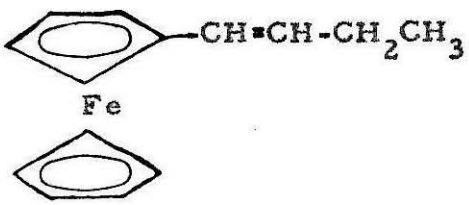
*DPM = dipivaloylmethide.

It is proposed that the possible reactions of the two species of methylene with ferrocene and its derivatives be investigated. These reactions would be intrinsically interesting and they represent an opening wedge into the field of metallocene photochemistry.

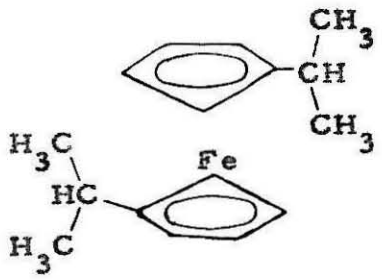
One way to study these reactions would be to compare both products and product ratios from the reactions of the two kinds of methylene with a suitable olefin, e.g., cyclohexene, in the presence of ferrocene. Another suitable experiment would be the use of a ferrocene derivative such as 2-ethyl vinylferrocene (I) in similar experiments. (A relative of (I), methyl(2-ferrocenylvinyl) ketone, has been prepared by Hill (4).)



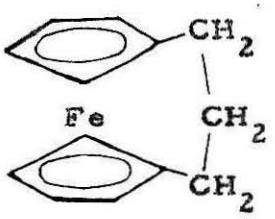
I

One kind of product which may be expected from these reactions arises from the insertion of methylene on the cyclopentadienyl rings of the ferrocenyl group, and is of interest as a new example of electrophilic substitution in the ferrocene series. The relatively high reactivity of ferrocene in electrophilic substitution reactions such as mercuration and Friedel-Crafts acylation has been discussed in terms of a preliminary coordination of the electrophile with the iron atom (5), but the evidence for such interaction is mostly indirect. If the results of the

above investigations were to indicate a somewhat exclusive preference for ring C-H over side-chain C-H insertion, this might be interpreted as more support (indirect, however) for the notion of iron-electrophile interaction. Such a preference might be considered somewhat analogous to the report that the reaction of pyridine with methylene from the photolysis of CH_2N_2 produces a good yield of α-picoline (6); it is suggested that this compound results from the rearrangement of an adduct formed by preliminary interaction of the methylene with the nitrogen atom (6). The mechanism of the substitution in ferrocenes may be investigated in more detail as follows: it is proposed that alkyl-substituted ferrocenes be allowed to react with methylene obtained from the photosensitized decomposition of diazomethane and the product compositions be determined. A high ratio of ring C-H insertion product to side chain C-H insertion product in such compounds as methyl and ethyl ferrocene would not of itself demand an interpretation in terms of preferential initial coordination of the electrophile with the metal. If, however, the amount of side chain insertion (statistically corrected for the number of aliphatic C-H bonds present) increases when the approach to the metal is sterically hindered, as in (II) or (III), a less ambiguous interpretation could be offered.



II[a]



III[b]

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These experiments may of course be extended to include other metal-organic compounds. Dibenzenechromium would be of particular interest: the reactivity of this compound in electrophilic substitution reactions cannot be compared with that of ferrocene (with which it is isoelectronic) due to the ease with which the chromium compound undergoes a transformation to the corresponding cation (7); i.e., in acylation, alkylation, sulfonation and mercuration, the cation forms rapidly and does not react further (7). It would be of interest to attempt the reaction of dibenzenechromium with methylene and to compare the result with that obtained in the ferrocene reaction. The only electrophilic substitution so far known which is common to both of these compounds is metalation by butyl sodium (7).

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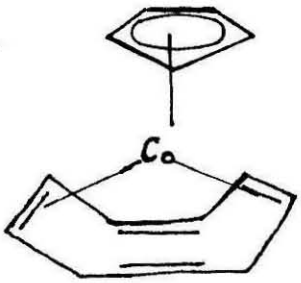
PROPOSITION V

A wide variety of organometallic compounds containing the aromatic cyclopentadienyl ring has been prepared in the past ten years. The type of compound to be considered in this Proposition is of the very general formula $(C_5H_5)M(R)$, where M is the metal atom and R is of extremely variable nature: C_5H_5, C_5H_4R', C_5H_6, C_5H_5R', diolefins, monoölefins, C_8H_8, $(CO)_x$, etc., etc. The NMR spectra of many of these compounds have been reported, but no systematic study has been made of the effect of a variety of R groups or of different metals in a given class of compounds on the position of the NMR peak for the C_5H_5 protons. The peak for these protons is very sharp and unsplit in all of the compounds to be considered here; its position may be determined to a rather high degree of accuracy, and small effects will thus not go unnoticed. It is the purpose of this Proposition to point out some of the interesting qualitative correlations which may be made from the existing data and to suggest at least one of the paths which a systematic study of these kinds of compounds might take. All of the NMR chemical shifts referred to in this Proposition will be for protons in the C_5H_5 portion of $(C_5H_5)M(R)$ in ppm relative to benzene; where necessary (i. e. in most of the cases) appropriate conversions from other reference standards have been made.

I. Stable complexes of the general formula $(C_5H_5)Co(diene)$ have been made by King, Treichel and Stone (1) and by Green, Pratt and

Wilkinson (2); the structures of some of these complexes along with the chemical shifts of the respective C_5H_5 protons are presented below:



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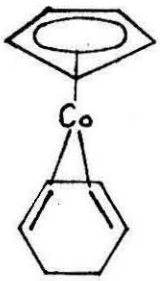

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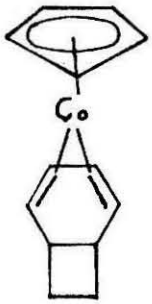
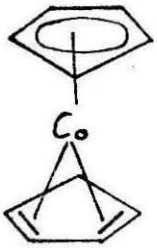
II



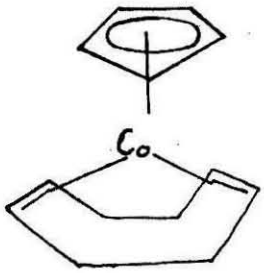
2.61

III



2.63

IV



2.78

V

The interesting thing to note in this series is that there seems to be a rough inverse correlation between the extent of conjugation in the olefinic part of the molecule and the shift of the C_5H_5 protons. All of these compounds have in common the coordination of a C_5H_5Co moiety with two double bonds; thus the process of bond formation should be at least comparable in all of these cases. A double bond conjugated with a second double bond will surely have a lower π-electron density than an unconjugated one and in the process of bond formation with the cobalt the conjugated system will effectively have "fewer electrons to offer"; this is then reflected in the fact that the chemical shift of the C_5H_5 protons in the 1,5-cyclooctadiene complex (V) is at somewhat higher field than those in the conjugated diene complexes*. These simple considerations do not take into account differences in geometry in these compounds (complex I, especially) which may perhaps affect electron distributions and

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* In Part III of this Thesis the existence of approximately a direct proportionality between chemical shifts and excess π-electron densities (3) was discussed.

chemical shifts, but obviously no definitive conclusions can be drawn from such limited data and chemical shift measurements of this kind must be extended to complexes of this type with other olefins (cycloheptadienes and -trienes, e.g.) and with metals other than cobalt (rhodium, e.g.)

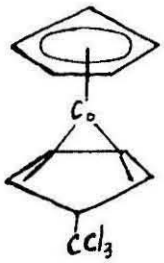
II. The chemical shifts of ferrocene, ruthenocene and osmocene are 3.2, 2.8 and 2.7, respectively (3). The shifts of the C_5H_5 protons in the compound pair $C_5H_5Cr(CO)_2C_5H_6$ (4) and $C_5H_5Re(CO)_2C_5H_6$ (2) are 1.9 and 1.6, respectively; in $C_5H_5CoC_5H_6$ and $C_5H_5RhC_5H_6$ the corresponding values are 2.6 and 2.1 (2). In Table I these chemical shifts are listed along with the Pauling electronegativities of the various metal atoms (5); a correlation (within each series) is apparent.

TABLE I

Chemical Shifts of C_5H_5 Protons and Electronegativities of Metal Atoms

Compound	δ, ppm	Electronegativity of Metal (5)
Ferrocene	3.2	1.8
Ruthenocene	2.8	2.2
Osmocene	2.7	2.2
$C_5H_5Cr(CO)_2C_5H_6$	1.9	1.6
$C_5H_5Re(CO)_2C_5H_6$	1.6	1.9
$C_5H_5CoC_5H_6$	2.6	1.8
$C_5H_5RhC_5H_6$	2.1	2.2

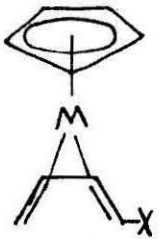
That such a correlation should exist is certainly reasonable if we simply consider an effect of the electronegativity of the metal on the effective π-electron density of the C_5H_5 ring. But of course the inherent magnetic properties of the metal as well as the effect of an external magnetic field on the motion of electrons in the metal and in the C_5H_5 ring may also have some effect. The above correlation should be extended to all of the possible compounds within a given series: a very large number of cyclopentadienyl metallocenes have been prepared, for example, many of which are diamagnetic and thus amenable to NMR measurements. It is interesting that the C_5H_5 chemical shift in such compounds as $C_5H_5Co(CO)(CF_3CF_2CF_2)I$, which have been prepared by King, Treichel and Stone (1), is downfield from the corresponding resonance in the cobalt complexes discussed above. (The peak for $C_5H_5Co(CO)(CF_3CF_2CF_2)I$ is at 1.59 ppm, for example.) It is suggested (1) that the electronegativity of the fluorocarbon group is responsible for the downfield shift. Finally, a comparison of the C_5H_5 shift for compound (IV) above with that of (VI) (2) in which a trichloromethyl group replaces one of the hydrogens on the C_5H_6 ring shows that a shift to lower field (2.63→2.48 ppm)



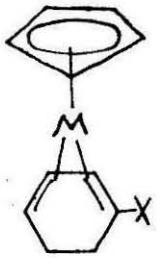
VI

occurs in this case also.

III. One of the paths which may be followed in a systematic study of $(C_5H_5)M(R)$ compounds has the possibility of leading to information about ground state electronic interactions of groups of interest to the organic chemist and probably also to a clearer picture of the nature of the bonding in metal-organic compounds. It is proposed that compounds of the type (VII) and (VIII) be prepared and the shifts of the C_5H_5 protons be determined in each case*. The X groups may be,



VII



VIII

e.g. halogen, alkyl, aryl, ether or halomethyl functions. The results of this study could perhaps be used to set up a relative scale of "functional group electronegativities" which would be useful in comparing the effects of these groups quantitatively in other systems. Of course this scale would be related to the Hammett/Taft scale of sigma values with the difference that the "electronegativity" scale would be based on the effect of X on the undisturbed, non-reacting state of the molecule; i.e., no reaction is involved in the measurement of a NMR chemical

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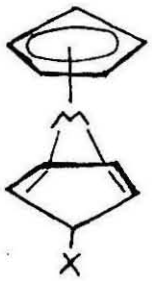
* The reason for the suggestion of the substituted butadiene complexes (VII) is that the necessary olefins may be more readily accessible than the corresponding cyclohexadienes.

shift. As pointed out above, the shifts in these systems may be determined quite accurately due to the unsplit and sharp nature of the C_5H_5 proton peak. It would be of interest to compare differences between the effects of X groups in systems such as VII or VIII with effects in systems such as IX, which have been prepared by Green, Pratt and Wilkinson (2) with M = Co and X = H, D, CH_3, CCl_3 and



IX

$CHCl_2$. This would amount to a comparison of vinylic and allylic systems (non-reacting).

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