Aromatic Substitution. XXVI.[1] Propionylation and Benzoylation of Benzene and Alkylbenzenes with Ethyloxocarbonium (Propionylium) and Phenyloxocarbonium (Benzoylium) Hexafluoroantimonate in Nitromethane and Tetramethylene Sulfone Solution

George A. Olah, Joachim Lukas,[2] and Erika Lukas

Contribution from the Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106. *Received March 20, 1969*

Abstract: Competitive propionylation and benzoylation of benzene and alkylbenzenes with ethyloxocarbonium (propionylium) and phenyloxocarbonium (benzoylium) hexafluoroantimonate, respectively, in nitromethane and tetramethylene sulfone solution at 25° were investigated. The high selectivity acylation reactions proceed according to a mechanism involving σ complex type of transition states. Investigation of the acylation of ring-deuterated toluene and benzene showed primary kinetic isotope effects. Secondary isotope effects in the acylation of side-chain deuterated toluene and *m*-xylene were also observed.

The isolation of stable alkyloxocarbonium (acylium) ion salts[3] enables the study of Friedel–Crafts type of acylation reaction with isolated oxocarbonium complexes in aprotic solvents without the use of acid catalysts. In previous work[4] we reported on the acetylation of benzene and its derivatives with methyloxocarbonium (acetylium) hexahaloantimonates. We have now studied propionylation and benzoylation.

$$ArH + RCO^+SbF_6^- \longrightarrow ArCOR + HSbF_6$$
$$R = CH_3, C_2H_5, C_6H_5$$

Results

Nature of the Acylating Agents. Ethyloxocarbonium hexafluoroantimonate (I) and phenyloxocarbonium hexafluoroantimonate (II) were prepared as described previously from the corresponding acyl fluorides and antimony pentafluoride in 1,1,2-trifluorodichloroethane (Freon 113) solution.

$$CH_3CH_2COF + SbF_5 \longrightarrow CH_3CH_2\overset{+}{C}O\ SbF_6^-$$

$$C_6H_5COF + SbF_5 \longrightarrow C_6H_5\overset{+}{C}O\ SbF_6^-$$

In order to prepare the oxocarbonium ions under strictly anhydrous conditions without the necessity of drybox or vacuum line techniques, an all-glass reaction vessel was used[5] (see Experimental Section). Through the fritted glass bottom a continuous stream of dry nitrogen was passed, keeping the reaction mixture under an inert atmosphere. Equimolar amounts of the acyl fluoride and antimony pentafluoride dissolved in Freon 113 were allowed to react at 0°. The nitrogen stream kept the solution well stirred, although when preparing larger quantities (10 g or more) a stirrer was also fitted into the reaction vessel. After the reaction was completed, the nitrogen stream was reversed and the solvent forced through the fritted glass bottom. The reaction vessel was then warmed to room temperature and the white crystalline oxocarbonium ion salt washed several times with dry Freon 113 reversing the direction of the nitrogen stream being used alternatively. After removing residual solvent under vacuum, the oxocarbonium salts were dried under vacuum.

The ir and nmr spectra of the ethyl- and phenyloxocarbonium ions, when obtained under strictly anhydrous conditions, indicate the presence of only pure oxocarbonium ions (Figures 1 and 2) with only trace amounts of the corresponding protonated acids present (due to hydrolysis). If solutions are exposed to moisture, however, partial hydrolysis to the protonated acids takes place (O acylation of water).

$$RCO^+ \xrightarrow{H_2O} RCO_2H_2^+$$

Propionylation of Benzene and Alkylbenzenes. In order to investigate the relative reactivity of benzene and alkylbenzenes with ethyloxocarbonium (propionylium) hexafluoroantimonate, the competitive method of relative rate determination was used. Competitive propionylations of alkylbenzenes *vs.* benzene were carried out, with the exception of *o*- and *m*-xylene and mesitylene which were run against toluene (the substantially higher rates in these cases otherwise would cause significant error and the rates relative to benzene were calculated by employing the toluene–benzene relative rate constant). Equimolar quantities of the competing aromatics used in tenfold excess were propionylated in nitromethane solution by adding to the well-stirred reaction mixture a solution of $CH_3CH_2CO^+SbF_6^-$ in nitromethane at 25°. The relative amount of propiophenone and alkylpropiophenones and the isomer distribution of the alkylpropiophenone isomers were determined by gas–liquid chromatography. Table I summarizes the propionylation data of benzene and alkylbenzenes in nitromethane solution.

All the competitive propionylations were found to be first order in aromatics by investigating the variation of concentration of benzene and toluene, respectively, in

(1) Part XXV: G. A. Olah and N. A. Overchuk, *J. Amer. Chem. Soc.*, 87, 5786 (1965).
(2) Postdoctoral Research Investigator, 1966–1967.
(3) G. A. Olah, S. J. Kuhn, W. S. Tolgyesi, and E. B. Baker, *J. Amer. Chem. Soc.*, 84, 2733 (1962); G. A. Olah, W. S. Tolgyesi, S. J. Kuhn, M. E. Moffatt, I. J. Bastien, and E. B. Baker, *ibid.*, 85, 1328 (1963).
(4) G. A. Olah, S. J. Kuhn, S. H. Flood, and B. A. Bardie, *ibid.*, 86, 2203 (1964).
(5) J. Lukas, Ph.D. dissertation, University of Karlsruhe, Karlsruhe, Germany, 1965.

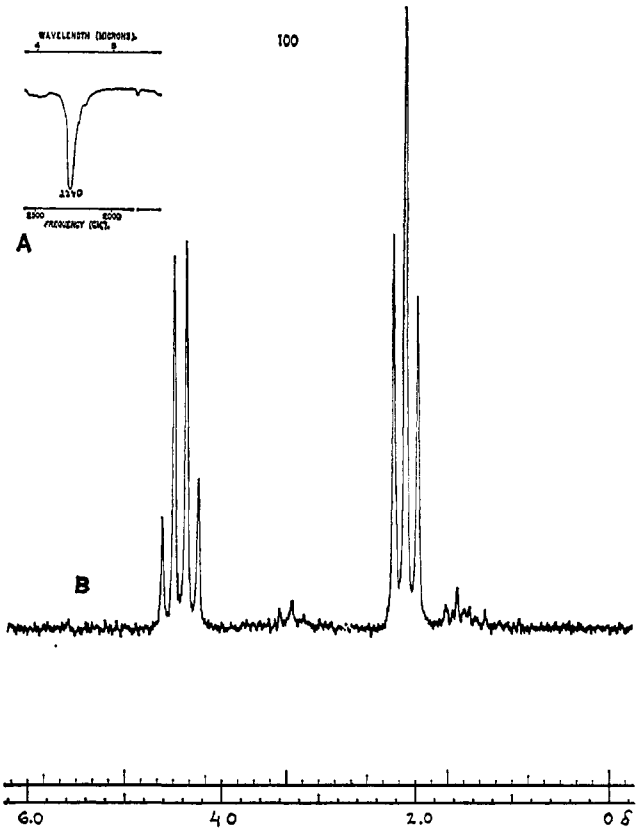

Figure 1 (A) Infrared carbonyl stretching frequency region of $CH_3CH_2CO^+SbF_6^-$ (fluorolube mull); (B) pmr spectrum of $CH_3CH_2CO^+SbF_6^-$ in SO_2 solution at $-60°$.

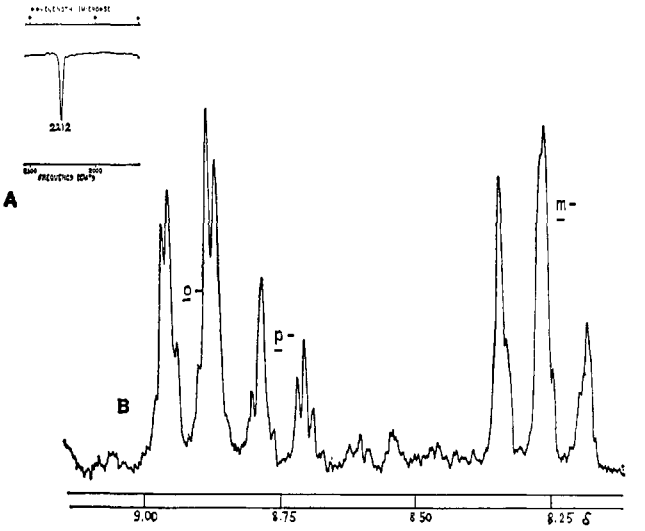

Figure 2. (A) Infrared carbonyl stretching frequency region of $C_6H_5CO^+SbF_6^-$ (fluorolube mull); (B) pmr spectrum of $C_6H_5CO^+SbF_6^-$ in SO_2 solution at $-10°$.

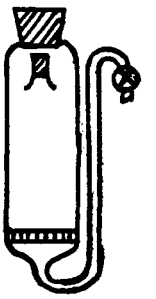

Figure 3. Reaction vessel for preparation of oxocarbonium ion salts.

competitive exponents using toluene–benzene ratios from 1:10 to 10:1.

Table I. Competitive Propionylation of Benzene and Alkylbenzenes with $CH_3CH_2CO^+SbF_6^-$ in Nitromethane Solution at 25°

Aromatic	$k_{AR}:k_{benzene}$	Isomer distribution, % ortho	meta	para	
Benzene	1.0				
Toluene	92.2	2.6	2.2	95.2	
p-Xylene	13.7	100			2,5-dimethylpropiophenone
m-Xylene	203.5	>99			2,4-dimethylpropiophenone
o-Xylene	652.0	>99			3,4-dimethylpropiophenone
Mesitylene	663.0	100			2,4,6-trimethylpropiophenone

For studying the possible effect of the anion we also studied propionylation with ethyloxonium hexachloroantimonate in the same solvent (Table II). The gegenion seems to have little effect, besides a slight steric effect (as shown in the decreased amount of *o*-methylpropiophenone).

Table II. Competitive Propionylation of Benzene and Alkylbenzenes with $CH_3CH_2CO^+SbCl_6^-$ in Nitromethane Solution at 25°

Aromatic	$k_{AR}:k_{benzene}$	Isomer distribution, % ortho	meta	para
Benzene	1.0			
Toluene	98.8	0.7	2.2	96.1
p-Xylene	15.8			
m-Xylene	272.7			
o-Xylene	743.9			
Mesitylene	550.0			

The propionylation of benzene and alkylbenzenes with ethyloxocarbonium hexafluoroantimonate and hexachloroantimonate also shows close resemblance to the aluminum chloride catalyzed propionylations with propionyl chloride and propionic anhydride. (Data are summarized in Tables III and IV.)

Table III. Competitive Aluminum Chloride Catalyzed Propionylation of Benzene and Alkylbenzenes with Propionyl Chloride in Nitromethane Solution at 25°

Aromatic	$k_{AR}:k_{benzene}$	Alkylpropiophenone, % ortho	meta	para	
Benzene	1.0				
Toluene	80.0	0.8	2.2	97.0	
p-Xylene	15.4	100			2,5-dimethylpropiophenone
m-Xylene	237.0	>99			2,4-dimethylpropiophenone
o-Xylene	786.0	>99			3,4-dimethylpropiophenone
Mesitylene	1125.0	100			2,4,6-trimethylpropiophenone

Benzoylation. Benzoylation of benzene and alkylbenzenes was carried out with phenyloxocarbonium hexafluoroantimonate in tetramethylene sulfone or nitromethane solution. Data showed no significant differences, and since results were well reproducible in tetramethylene sulfone solution and no change of isomer distributions with reaction time was observed even in

Table IV. Competitive $AlCl_3$–CH_3NO_2 Catalyzed Propionylation of Benzene and Methylbenzenes with Propionic Anhydride in Nitromethane Solution at 25°

Aromatic	$k_{AR}:k_{benzene}$	Methylpropiophenone, %		
		ortho	*meta*	*para*
Benzene	1.0			
Toluene	106.0	3.2	0.90	95.90
p-Xylene	17.6	100 2,5-dimethylpropiophenone		
m-Xylene	300.2	>99 2,5 dimethylpropiophenone		
o-Xylene	1180.0	>99 3,4-dimethylpropiophenone		
Mesitylene	2194.0	100 2,4,6-trimethylpropiophenone		

though the gradually decreasing amount of *ortho* benzoylation going from toluene to ethylbenzene to isopropylbenzene to *t*-butylbenzene (see Table V) reflects steric hindrance. The isomer distribution in the benzoylation of toluene-α,α,α-d_3 with $C_6H_5CO^+SbF_6^-$ was found to be 10.54% *ortho*, 0.95% *meta*, and 88.5% *para* isomer, indicating very little change from the isomer distribution obtained in the benzoylation of toluene. The isomer distribution of benzoylation of *m*-xylene-α-d_6 is 2.5% of 2,5- and 97.5% of 2,4-dimethylbenzophenone, again indicating little change from the

Table V. Competitive Benzoylation of Benzene and Alkylbenzenes with Phenyloxocarbonium Hexafluoroantimonate ($C_6H_5CO^+SbF_6^-$) in Tetramethylene Sulfone Solution at 25°

Aromatic	$k_{AR}:k_{benzene}$	Isomer distribution, %		
		ortho	*meta*	*para*
Benzene	1.0			
Toluene	190.5	10.3	0.9	88.8
Ethylbenzene	157.6	6.0	1.3	92.7
Isopropylbenzene	93.0	3.1	1.7	95.2
t-Butylbenzene	67.9	0.6	2.7	96.7
o-Xylene	64.6	4.2 2,3-dimethylbenzophenone	95.8 3,4-dimethylbenzophenone	
m-Xylene	564.0	1.8 2,6-dimethylbenzophenone	98.2 2,4-dimethylbenzophenone	
p-Xylene	227.8	100.0 2,5-dimethylbenzophenone		
Mesitylene	262.0	100.0 2,4,6-trimethylbenzophenone		

the case of *t*-butylbenzophenones (in nitromethane solution the amount of *o*-*t*-butylbenzophenone decreased over a period of time on account of increasing amount of *meta* isomer), this solvent was used predominantly in our studies. Table V summarizes the data of the benzoylation of benzene and alkylbenzenes with phenyloxocarbonium hexafluoroantimonate. Brown and Jensen's[6–8] previous data on the aluminum chloride catalyzed benzoylation of benzene and alkylbenzenes are in good agreement with present data both relating to positional and substrate selectivity.

Comparison of Propionylation and Benzoylation. The propionylation and benzoylation of benzene and alkylbenzenes show high selectivity relating to both substrate and positional selectivity. Data indicate a substantially σ-complex type of mechanism and good correlation with Brown's selectivity principle.

Comparison of the isomer distributions of the acylation or toluene with methyl-, ethyl-, and phenyloxocarbonium hexafluoroantimonate warrants comment. Data summarized in Table VI show that benzoylation

Table VI. Isomer Distribution in the Acylation of Toluene with Oxocarbonium Hexafluoroantimonates, $RCO^+SbF_6^-$

R	Solvent	Isomer distribution, %		
		ortho	*meta*	*para*
CH_3	CH_3NO_2	1.2	1.3	97.5
C_2H_5	CH_2NO_2	2.6	2.2	95.2
C_6H_5	TMS	10.3	0.9	88.8

gives significantly more *ortho* isomer then either acetylation or propionylation. Steric considerations alone can not, in our opinion, account for this observation, although the substantially higher substrate selectivity found in the benzoylations as compared with propionylations and the previously studied acetylations, using stable oxocarbonium hexafluorantimonate complexes, relate the greater ability of the phenyloxocarbonium ion salt to substitute in the *ortho* position relative to an alkyl group. This is particularly evident in the relative reactivities of *m*- and *p*-xylene and mesitylene as compared to benzene.

Hydrogen Kinetic Isotope Effect. In order to determine whether there is a kinetic hydrogen isotope effect in the propionylation and benzoylation of deuterated aromatics, as compared with the protium compounds, the previously described competitive methods of kinetic isotope effect determination were used.[4]

Competitive propionylation and benzoylation of benzene and benzene-d_6 was carried out with $CH_3CH_2CO^+SbF_6^-$ and $C_6H_5CO^+SbF_6^-$, respectively, in nitromethane solution at 25°. The reaction mixtures were analyzed by mass spectroscopy to determine the propiophenone–propiophenone-d_5 and benzophenone–benzophenone-d_5 ratios. The comparison of the relative amounts of products gave the kinetic isotope effect: the $k_H:k_D$ was 2.75 for $CH_3CH_2CO^+SbF_6^-$, and 1.58 for $C_6H_5CO^+SbF_6^-$. The same kinetic isotope effect was also obtained (with good agreement of results) from the competitive propionylation and benzoylation of toluene–benzene as compared with toluene–benzene-d_6 (Table VII). In this case the relative amounts

isomer distribution obtained in the benzoylation of *m*-xylene. We suggest that the weaker electrophilic nature of the phenyloxocarbonium ion, due to charge delocalization into the phenyl ring as compared to the methyl- or ethyloxocarbonium ions, contributes to the observed substitution pattern in accordance with Klopman's[9] conception of "soft" and "hard" reactivity in electrophilic aromatic substitutions. We are further testing this conception on suitable model systems.

(6) H. C. Brown and F. R. Jensen, *J. Amer. Chem. Soc.*, **80**, 2291, 2296 (1958).
(7) F. R. Jensen, G. Marino, and H. C. Brown, *ibid.*, **81**, 3303 (1959).
(8) H. C. Brown and G. Marino, *ibid.*, **81**, 3308 (1959), and previous references given in these papers.
(9) G. Klopman, *ibid.*, **90**, 223 (1968).

Table VII. Kinetic Hydrogen Isotope Effects in Propionylation and Benzoylation

	$k_T:k_B$		
$CH_3CH_2CO^+SbF_6^-$			
Toluene–benzene-d_6	261	$k_H:k_D$ = Benzene-d_6	$^{261}/_{92}$ = 2.84
Toluene–benzene	92		
Toluene-d_5–benzene	30	$k_H:k_D$ = Toluene-d_5	$^{92}/_{30}$ = 3.06
$C_6H_5CO^+SbF_6^-$			
p-Xylene–benzene	227.8	$k_H:k_D$ = Benzene-d_6	1.80
p-Xylene–benzene-d_6	410		
Toluene–benzene	190.5		
Toluene-d_5–benzene	115.5	$k_H:k_D$ = Toluene-d_5	1.65

of propiophenone and methylpropiophenone could be determined by gas–liquid partition chromatography.

The observed kinetic hydrogen isotope effect is greater for propionylation than benzoylation.

The primary kinetic isotope effects indicate that the proton elimination step in the propionylation and benzoylation of benzene, toluene, and *p*-xylene is at least partially rate determining, similar to previous observations relating to acetylation. Friedel–Crafts acylations show significant differences from the previously investigated alkylations, halogenations, and nitrations, all involving only small secondary isotope effects. The proton elimination being kinetically significant in Friedel–Crafts acylation could at least partially account for the observed high selectivities and therefore merits further consideration.

The high $k_T:k_B$ rates in Friedel–Crafts acylations seem to indicate that in this case both the substrate and positional selectivity is determined in the same step, namely, formation of a σ-complex type of transition state, as suggested by Pfeiffer and Wizinger,[10] Wheland,[11] and particularly by the work of Brown and his coworkers.[12]

If the transition state in the rate-determining step is indeed of σ-complex nature, then it could be expected that the proton elimination step could be at least partially rate determining and subsequently a primary kinetic isotope effect could be observed. The data have shown that this is indeed the case when comparing the rates of acylation of benzene and benzene-d_6.

If we continue this argument, we should also conclude that isotopic substitution of the methyl group in toluene should affect the conjugative stabilization of the σ complexes (the conjugative effect of CD_3 being smaller than that of CH_3) and thus a secondary kinetic isotope effect should also be observable. To prove this suggestion, we extended our investigations to the benzoylation of $CD_3C_6H_5$ and *m*-$(CD_3)_2C_6H_4$ and compared their reactivities in competitive acylations with benzene. The data are summarized in Table VIII.

As may be seen from the data of Table VIII, deuteration of the methyl group indeed causes a secondary isotope effect. Thus it was possible to demonstrate that

(10) P. Pfeiffer and R. Wizinger, *Ann. Chem.*, **461**, 136 (1928).
(11) G. W. Wheland, *J. Amer. Chem. Soc.*, **64**, 9001 (1942).
(12) For a summary see, for example, H. C. Brown and K. L. Nelson in "The Chemistry of Petroleum Hydrocarbons," Vol. III, B. T. Brooks, S. S. Kurtz, C. B. Boord, and L. Schmerling, Ed., Reinhold Publishing Corp., New York, N. Y., 1955, pp 465–578, and references therein.

Table VIII. Secondary Kinetic Isotope Effect in Benzoylation of Toluene and *m*-Xylene with $C_6H_5CO^+SbF_6^-$ in Tetramethylene Sulfone Solution at 25° as a Consequence of Deuteration of the Methyl Groups

	$k_{AH}:k_{benzene}$	k_Hk_D
Toluene–benzene	190.5	
Toluene-α,α,α-d_3–benzene	183.4	1.04
m-Xylene–benzene	564.0	
m-Xylene-α-d_6–benzene	456.0	1.24

electrophilic aromatic substitutions involving a σ-complex type of rate-determining transition state show both primary and secondary kinetic isotope effect. The observation of secondary isotope effects in Friedel–Crafts acylation using stable oxocarbonium ion salts as acylating reagents is one of the clear-cut cases, where the secondary isotope effects *cannot* be caused by exclusive steric reasons, as suggested by Brown.[13] The acylation reactions have a great preference for the *para* positions in alkylbenzenes, as reflected by the isomer distributions; thus substitution of the methyl group by deuterium cannot be expected to affect the steric requirements, as indicated by the observed isomer distributions, but reflects the changes of the electronic effects of the CD_3 group as compared to the CH_3 group.

Experimental Section

Benzene, alkylbenzenes, and halobenzenes were used in purities comparable to those reported in previous papers of this series. Nitromethane was purified as described. Propionyl chloride and propionic anhydride were commercially available chemicals of highest purity, redistilled before used. Propionyl and benzoyl fluorides were prepared as reported.[3]

Preparation of Ethyl- and Phenyloxocarbonium Hexafluoroantimonates. Equimolar amounts of propionyl or benzoyl fluoride and SbF_5 were allowed to react by dissolving both components in Freon 113 and adding the SbF_5 solution slowly to the acyl fluoride solution at 0°. The reaction was carried out in a glass reaction vessel which has a fritted glass plate as a bottom (Figure 1). Through the fritted glass bottom, a continuous stream of nitrogen was kept flowing keeping the reaction mixture under an inert atmosphere. When the reaction is carried out in larger quantities it should also be stirred. After the reaction was completed, the nitrogen stream was reversed and the solvent pressed through the fritted glass bottom. The reaction vessel was then warmed to room temperature and the white precipitate washed several times with Freon 113. After that the N_2 stream was again reversed and the salt was dried this way. Residual amounts of solvent were removed under vacuum.

Substituted propionophenone and benzophenone isomers were all known from the literature and were available either from commercial sources or prepared by standard methods. Their purity was checked by glpc analysis and infrared and nmr spectroscopy. When necessary, they were purified by preparative scale gas chromatography using an Aerograph Autoprep instrument with polypropylene glycol column.

Competitive Acylation with Oxocarbonium Ion Salts. Equimolar amounts of two arenes were dissolved in nitromethane or sulfolane and a freshly prepared solution of the oxocarbonium ion salt in the same solvent was added to the vigorously stirred solution. The mole ratio of reagents was 10:10:1. The reaction mixture is then worked up by quenching with water, neutralizing with sodium carbonate, and extracting the benzophenones in ether. In a typical run, 3.3 g (0.0096 mol) of phenyloxocarbonium hexafluoroantimonate in 50 ml of sulfolane was added to 7.49 g (0.0959 mol) of benzene and 10.19 g (0.0959 mol) of ethylbenzene dissolved in 50 ml of sulfolane. It was then kept with stirring in a constant-temperature bath at 25°.

Competitive Propionylation of Benzene and Alkylbenzenes in CH_3NO_2 Solution. To a solution of 0.05 mol of $AlCl_3$ dissolved in 50 g of CH_3NO_2 was added 0.25 mol each of benzene and alkyl-

(13) H. C. Brown, M. E. Azzaro, J. G. Koehling, and G. J. McDonald, *J. Amer. Chem. Soc.*, **88**, 2520 (1966).

benzene in a three-necked flask equipped with a thermometer, dropping funnel, and reflux condenser connected through a drying tube to a hydrogen halide absorber. The reaction flask was placed in a constant-temperature bath at 25 ± 0.5°. With vigorous stirring (magnetic stirrer), 0.05 mol of propionyl chloride or propionic anhydride dissolved in 30 g of CH_3NO_2 was added dropwise over a period of 10 min. The reaction was allowed to proceed an additional 10 min. The solution was then washed with water three times with approximately 200 ml of a 5% solution of NaOH (to remove CH_3NO_2) and again with water. The organic layer was separated, dried over $CaCl_2$, and analyzed by gas–liquid partition chromatography.

Determination of Isotope Effect. (a) Benzene-d_6 (0.025 mol) and toluene (0.025 mol) were dissolved in 5 g of tetramethylene sulfone and acetylated with 0.01 mol of $RCO^+SbX_6^-$ as previously described. The products were analyzed by gas–liquid partition chromatography. (b) Benzene-d_6 (0.025 mol) and benzene (0.025 mol) were dissolved in 5 g of tetramethylene sulfone and acetylated with 0.01 mol of $RCO^+SbX_6^-$ as previously described. The products were analyzed by mass spectroscopy. (c) Benzene (0.025 mol) and toluene-d_8 (0.025 mol) were dissolved in 5 g of tetramethylene sulfone and acetylated with 0.01 mol of $RCO^+SbF_6^-$ as previously. The products were analyzed by gas–liquid partition chromatography. (d) Benzene-d_6 (0.025 mol) and toluene-d_8 (0.025 mol) were dissolved in 5 g of tetramethylene sulfone and acetylated with 0.01 mol of $RCO^+SbF_6^-$ as previously described. The products were analyzed by gas–liquid partition chromatography. (e) Benzene (0.025 mol) and toluene-α,α,α-d_3 (0.025 mol) were dissolved in 5 g of tetramethylene sulfone and acetylated with 0.01 mol of $R^+SbF_6^-$ as described in a. The products were analyzed by gas–liquid partition chromatography. (f) Benzene (0.025 mol) and toluene-d_5 (0.025 mol) were acylated as in e. (g) Benzene (0.025 mol) and *m*-xylene-α-d_6 (0.025 mol) were acetylated as in e.

Gas–Liquid Partition Chromatography Analyses. The analyses were carried out on a Perkin-Elmer Model F 11 gas chromatography, using a Disc integrator or on a Perkin-Elmer 226 chromatograph with electronic integrator. On both instruments, capillary polypropylene glycol columns of 150 and 100 ft length, respectively, were used. The products were identified by comparison with authentic samples. When these were not available, they were prepared by standard methods. Molar response factors were determined by standard methods.

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A Conformationally Defined Imine Derivative of Pyridoxal: 7,8-Dihydro-3-methyl-2,6-naphthyridin-4-ol[1]

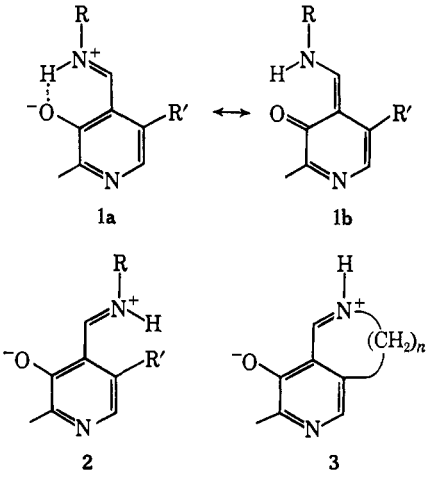
Tom L. Fisher and David E. Metzler

Contribution from the Department of Biochemistry and Biophysics, Iowa State University, Ames, Iowa 50010. *Received April 28, 1969*

Abstract: 7,8-Dihydro-3-methyl-2,6-naphthyridin-4-ol (**9**), a cyclic imine derivative of pyridoxal, has been synthesized. The C=N group of this compound lies in a fixed position corresponding to one of the possible conformations of the imine group in pyridoxal phosphate dependent enzymes and opposite to the conformation of hydrogen-bonded imines of pyridoxal previously studied. Although the cyclic imine is catalytically inactive, due to the stability of the six-membered ring which includes the imine group, its electronic absorption spectrum is strikingly similar to those of some pyridoxal phosphate containing enzymes. Derivatives of the cyclic imine have been prepared by oxidation, reduction, and reaction with hydroxylamine.

Studies on several B_6-dependent enzymes have revealed that an imine bond links the aldehyde function of the coenzyme to the ε-amino group of a lysine residue on the apoenzyme.[2] The imine group is thought to have a central function in the catalytic mechanism of these enzymes. Thus, a thorough understanding of the chemistry involved is of fundamental importance.

Imines of pyridoxal and related substances with amino acids and amines have been studied.[3–5] At low pH most of these substances exist as internally hydrogen-bonded structures, **1**. Resonance form **1a** depicts the compound as a protonated imine, emphasizing its direct relationship to the parent aldehyde and amine, while resonance form **1b** shows more clearly the keto–enamine nature of structure **1**, which Heinert and Martell have shown to predominate in aqueous solution.[4] The hydrogen bonding is assumed to hold the molecule in conformation **1**. However, a second conformation, **2**, in which the imine double bond and the pyridine ring are again coplanar, is also possible, and doubtless exists at high pH where the chelated hydrogen has dissociated.



1a **1b** **2** **3**

(1) This investigation was supported by a grant from the U. S. Public Health Service (AM-01549). Use of the Varian HA-100 nmr spectrometer was made possible by an equipment grant to Iowa State University from the National Science Foundation.

(2) B. M. Guirard and E. E. Snell in "Comprehensive Biochemistry," Vol. 15, M. Florkin and E. H. Stotz, Ed., American Elsevier Publishing Co., New York, N. Y., 1964, pp 138–174.

(3) D. E. Metzler, *J. Am. Chem. Soc.*, 79, 485 (1957).

(4) D. Heinert and A. E. Martell, *ibid.*, 85, 183 (1963).

(5) T. C. French, D. S. Auld, and T. C. Bruice, *Biochemistry*, 4, 77 (1965).