Thus, the enzyme would not be expected to act as a general base catalyst if, as the model studies show, such catalysis would probably be ineffective and unnecessary.

Possible mechanisms of lysozyme catalysis involving three distinct intermediates have been considered: (a) protonated glycosyl bond fission leading to pyranosyl carbonium ion,[2b] (b) nucleophilic displacement by aspartate to give a covalently bound α-glycosyl–enzyme, and (c) intramolecular nucleophilic displacement by the 2-acetamido group to yield a protonated oxazolene. If general acid catalysis by glutamic acid-35 is involved in the enzyme mechanism as has been suggested,[2b] the following possible mechanisms may be written.

Rupley and Gates,[4] in examining transfer reactions catalyzed by lysozyme, have shown that retention of configuration of the β anomeric carbon occurs. The reaction of a possible glycosyl–enzyme intermediate (from eq 6b) or an oxazolene intermediate (from eq 6c) with water or other acceptor would lead of necessity to β product. Thus, the stereochemistry of the product for either eq 6b or 6c would be predetermined to be the β configuration by the double-displacement nature of the over-all reaction.

Acknowledgments. This work was supported by a grant from the National Institutes of Health. We wish to thank Professor Bernard Weissmann for his generous gift of compounds II and IV. We also wish to thank Professor John A. Rupley for allowing us to examine his space-filling model of the lysozyme–substrate complex, and for his helpful discussions.

The Reaction of Indolenine Salts with Nucleophiles

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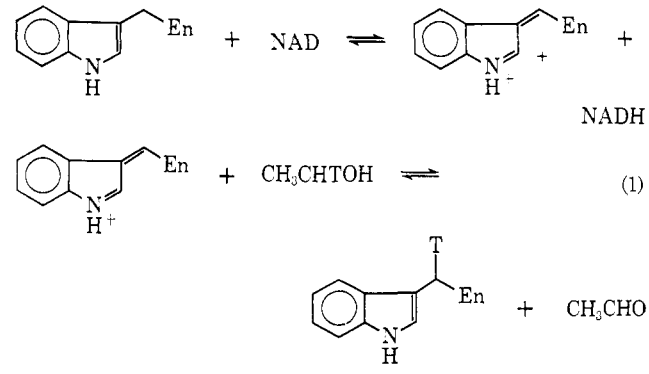
Abstract: The suggestion that an indolenine moiety may be an intermediate in the mechanism of action of the dehydrogenase enzymes has led to a study of the reduction of substituted phenylindolenine hydrosulfates by diethyl 2,6-dimethyl-1,4-dihydropyridyl-3,5-dicarboxylate (Hantzch ester). These reactions affording 3,5-dicarbethoxyl-2,6-dimethylpyridinium hydrosulfate and the corresponding 3-benzylindole as products were found to be first order with respect to each reactant with rate constants 500 times greater in acetonitrile than in ethanol. The extinction coefficient of the visible band of the indolenine salts undergoes a 100-fold parallel change in these solvents. These effects were interpreted as being due to tighter solvation of the indolenine salt by ethanol as compared to acetonitrile. The presence of intermediate charge-transfer complexes could not be determined because of the magnitude of the rate constants. The inclusion of radical inhibitors in the reaction solutions had no effect on the rate of the reaction. The reaction of the phenylindolenine salts with secondary amines was found to yield the corresponding adducts II (R = H), although the nmr spectrum of the imidazole adduct does not seem to be consistent with this structure. Kinetic studies of the reaction of the indolenine salts with secondary amines showed the reactions to be complex (*i.e.*, see Figures 3 and 4). In contrast the reaction of secondary amines with the phenyl-N-methylindolenine salt was simple first order with respect to each reactant with rate constants comparable to reduction by the Hantzch ester. The complexity of the reaction with the phenylindolenine salt is attributed to acid–base equilibria between the protonated indolenine (protonated imine) and the amine. By comparison of the rate constants for the reaction of the unprotonated phenylindolenine salt with aziridine and morpholine (*i.e.*, Scheme I (h)) with the constants for the reaction of the phenyl-N-methyl analog with these amines it is concluded that protonation increases the reaction rate 12000-fold. These phenomena are discussed with references to the dehydrogenase enzymes.

In 1965 Schellenberg[3a] reported that tritium was transferred to a tryptophan residue of yeast alcohol dehydrogenase during the enzymatic conversion of ethanol-1-^{3}H to acetaldehyde. Establishment of the position of the tritium label led to the postulation of (1) for the mechanism of action of yeast alcohol dehydrogenase. Analogous experiments with L-lactate and L-malate dehydrogenases gave similar results.[3b] Recently, Palm has shown that tritium labeling of yeast alcohol dehydrogenase takes place more slowly than does NADH formation and that A-NADT transfers tritium to the enzyme in the absence of acetaldehyde.[4] It



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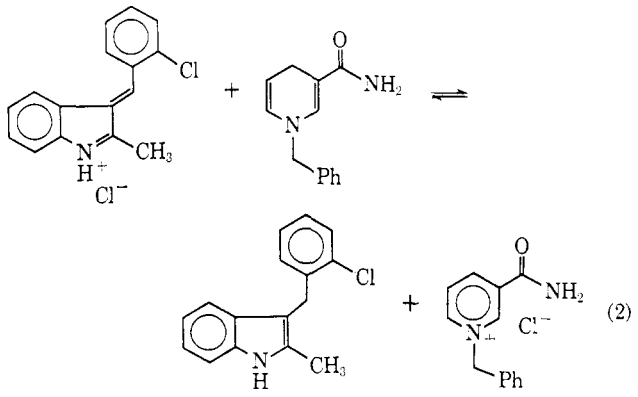
(3) (a) K. A. Schellenberg, *J. Biol. Chem.*, **240**, 1165 (1965); (b) T.-L. Chan and K. A. Schellenberg, *Federation Proc.*, **26**, 1709 (1967); K. A. Schellenberg, *J. Biol. Chem.*, **242**, 1815 (1967).

(4) D. Palm, *Biochem. Biophys. Res. Commun.*, **22**, 151 (1966); A referee has stated that under the experimental conditions used by Palm, a moiety other than tryptophan is labeled.

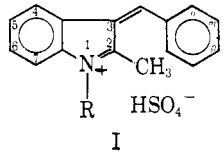
appears that enzyme labeling may be independent of the hydrogen transfer process. The availability of indolenine salts[5] made it possible for Schellenberg[6] to investigate (2) as a possible model for (1). This facile reaction



(2)

was reported to be first order with respect to each component with a rate constant of 1200 M^{-1} min^{-1} and through deuterium labeling it was shown, in accord with (1), that hydrogen was transferred from the dihydropyridine to the methine carbon of the indolenine salt. Recently Schellenberg and co-workers have shown that thiols add to *o*-chlorophenyl-2-methylindolidenemethane hydrochloride to form the corresponding *o*-chlorophenyl-3-(2-methylindolyl)alkylthiomethane in a facile reaction and that this product affords the corresponding 3-benzylindole and mercaptan upon reduction with acidic dithionite.[6b] These findings led to speculation that the protein indolenine moiety may be stabilized as a cysteine adduct which can be reductively cleaved when the indolenine moiety is needed. Indolenine and indolenine-like compounds have also been reported as reactive intermediates in other reactions.[7]

In this paper we report studies of the reaction of variously substituted phenyl-2-methylindolidenemethane hydrosulfates (I) with amines and the hydride transfer reagent diethyl 1,4-dihydro-2,6-dimethylpyridyl-3,5-dicarboxylate (Hantzsch ester).



I

Experimental Section

Materials. 2-Methylindole, 5-chloro-2-methylindole, 1,2-, 2,5-, and 2,7-dimethylindoles, benzaldehyde, *p*-hydroxybenzaldehyde, and *o*- and *p*-chlorobenzaldehydes were purchased from Eastman or Aldrich Chemicals Companies and used without further treatment. Diethyl 2,6-dimethyl-1,4-dihydropyridyl-3,5-dicarboxylate was prepared according to a modification[8] of the method of Singer and McElvain. The bright yellow needles melted at 192–193° (lit.[8] mp 189–190°). Imidazole (Eastman White Label) was recrystallized from acetone–petroleum ether (bp 60–90°). Piperidine (bp 103–104°), morpholine (bp 127°), and aziridine (bp 55–56°) were freshly distilled from BaO through a 1-ft Vigreux column. The amines were obained from the Eastman Chemical Company in red- and white-labeled containers. The acetonitrile (bp 82°) was obtained from Matheson Coleman and Bell as their number Ax149 and twice fractioned from P_2O_5 through a 6-ft column packed with glass helices.

Procedures for the preparation of the substituted phenyl-2-methylindolinenemethane hydrosulfates are modifications of the method provided by Burr and Gortner.[5] Hydrosulfates rather than hydrochlorides were prepared because phenyl-2-methylindolindenemethane hydrochloride could not be prepared by Burr and Gortner or ourselves and a common anion was desired for the kinetic studies.

Procedure A. *p*-Hydroxyphenyl-2-methylindolidenemethane Hydrosulfate. To a solution of 0.5 g (0.0038 mole) of 2-methylindole and 1 ml (0.019 mole) of concentrated sulfuric acid in 5 ml of absolute ethanol at 0° was added 0.46 g (0.0038 mole) of *p*-hydroxybenzaldehyde with vigorous stirring. The bright red precipitate which formed after about 10 min was collected on a sintered disk and thoroughly washed with absolute ethanol (some of the salts were washed with acetonitrile) followed by ether. The red crystals were dried over P_2O_5 *in vacuo* in a dark container; infrared λ_{max}^{KBr} 3000 (broad), 1580, and 1550 cm^{-1}.

Anal. Calcd for $C_{16}H_{15}NO_5S$: C, 57.70; H, 4.54; N, 4.21; S, 9.63. Found: C, 57.05; H, 4.51; N, 4.05; S, 9.72.

Heating this material results in its slow decomposition. Thus, recrystallization three times from absolute ethanol followed by drying at 80° in a dark container over P_2O_5 *in vacuo* resulted in a product exhibiting the following elemental analysis: C, 55.48; H, 5.48; N, 4.29; S, 10.04.

***p*-Chlorophenyl-2-methylindolidenemethane hydrosulfate** resulted as a yellow powder; infrared λ_{max}^{KBr} 2550 (broad), 1602, and 1580 cm^{-1}.

Anal. Calcd for $C_{16}H_{14}ClNO_4S$: C, 54.62; H, 4.01; Cl, 10.08; N, 3.98; S, 9.12. Found: C, 54.33; H. 4.16; Cl, 9.88; N, 3.84; S, 9.09.

***o*-Chlorophenyl-2-methylindolidenemethane hydrosulfate** was produced as a yellow powder; infrared λ_{max}^{KBr} 2600 (broad), 1820 (weak), 1620, and 1570 cm^{-1}.

Anal. Calcd for $C_{16}H_{14}ClNO_4S$: C, 54.62; H, 4.01; Cl, 10.08; N, 3.98; S, 9.11. Found: C, 54.61; H, 4.19; Cl, 10.03; N, 3.79; S, 9.25.

Procedure B. Phenyl-2-methylindolidenemethane Hydrosulfate. One-half gram of α-methylindole was dissolved in 3 ml of benzaldehyde and concentrated sulfuric acid was added a little at a time on a spatula until the mass of material solidified. For some of the compounds prepared by this method it was necessary to add a little ether in order to solidify the material. The yellow solid was collected by suction on a sintered disk and treated as in procedure A above to produce a bright yellow powder; infrared λ_{max}^{KBr} 2600 (broad), 1600, and 1550 cm^{-1}.

Anal. Calcd for $C_{16}H_{15}NO_4S$: C, 60.60; H, 4.77; N, 4.42; S, 10.11. Found: C, 60.36; H, 4.71; N, 4.09; S, 9.88.

Phenyl-1,2-dimethylindolidenemethane hydrosulfate resulted as a yellow powder; infrared λ_{max}^{KBr} 2900 (broad), 1600, and 1560 cm^{-1}.

Anal. Calcd for $C_{17}H_{17}NO_4S \cdot 0.33H_2SO_4$: C, 56.59; H, 4.92; N, 3.88; S, 11.55. Found: C, 56.35; H, 5.19; N, 3.43; S, 11.05.

Phenyl-2,5-dimethylindolidenemethane hydrosulfate resulted as a yellow powder; infrared λ_{max}^{KBr} 2700 (broad), 1600, and 1560 cm^{-1}.

Anal. Calcd for $C_{17}H_{17}NO_4S$: C, 61.61; H, 5.17; N, 4.42; S, 9.67. Found: C, 61.83; H, 5.19; N, 4.44; S, 9.75.

Phenyl-2,7-dimethylindolidenemethane hydrosulfate was a brown powder; infrared λ_{max}^{KBr} 2800 (broad), 1600, and 1560 cm^{-1}.

Anal. Calcd for $C_{17}H_{17}NO_4S$: C, 61.61; H, 5.17; N, 4.42; S, 9.67. Found: 61.61; H, 5.40; N, 4.35; S, 9.77.

Phenyl-2-methyl-5-chloroindolidenemethane hydrosulfate was produced as a yellow powder; infrared λ_{max}^{KBr} 2600 (broad), 1600, and 1550 cm^{-1}.

Anal. Calcd for $C_{16}H_{14}ClNO_4S$: C, 54.62; H, 4.01; Cl, 10.08; N, 3.98; S, 9.11. Found: C, 54.64; H, 4.27; Cl, 9.81; N, 4.03; S, 9.15.

3-(*p*-Hydroxybenzyl)-2-methylindole. To 400 mg (7.42 mmoles) of potassium borohydride dissolved in 5 ml of water was added 5 ml of ether followed by 100 mg (0.3 mmole) of the *p*-hydroxyphenylindolenine salt. This resulting mixture was stirred until all the solid disappeared. After 2 hr the bubbling subsided, the ether phase was separated, and the aqueous phase was washed with ether. All ether solutions were combined and dried 0.5 hr over sodium sulfate. Removal of sodium sulfate and ether left 65 mg (90% yield) of reddish solid residue. Three crystallizations from

(5) G. O. Burr and R. A. Gortner, *J. Am. Chem. Soc.*, **46**, 1224 (1924).
(6) (a) K. A. Schellenberg and G. W. McLean, *ibid.*, **88**, 1070 (1966); (b) K. A. Schellenberg, G. W. McLean, H. L. Lipton, and P. S. Lietman, *ibid.*, **89**, 1948 (1967).
(7) (a) T. C. Bruice and T. H. Fife, *ibid.*, **83**, 1124 (1961); (b) J. H. Thesing and J. Meyer, *Ber.*, **87**, 1084 (1954).
(8) E. A. Braude, J. Hannah, and R. Linstead, *J. Chem. Soc.*, 3249 (1960).

an ethanol–water mixture gave small white needles, mp 146–147°; infrared λ_{max}^{KBr} 3550 (sharp), 3500 (sharp), 3050, 2950, and 1610 (doublet) cm^{-1}; nmr ($CDCl_3$, τ, in ppm from tetramethylsilane) 2.60 (multiplet, 8 H), 5.78 (singlet, 2 H), and 7.53 (singlet, 3 H).

Anal. Calcd for $C_{16}H_{15}NO$: C, 80.98; H, 6.37; N, 5.90. Found: C, 81.05; H, 6.51; N, 6.03.

3-Benzyl-2-methylindole. Phenyl-2-methylindolidenemethane hydrosulfate (100 mg, 0.315 mmole) was treated as above affording 45 mg (70% yield) of a yellow solid residue which was recrystallized three times from ethanol–water to colorless prisms, mp 118–120°; infrared λ_{max}^{KBr} 3380 (sharp), 3000, 2900, 1600 (weak), and 1450 (triplet) cm^{-1}; nmr 2.77 (multiplet, 10 H), 5.92 (singlet, 2 H), and 7.67 (singlet, 3 H).

Anal. Calcd for $C_{16}H_{15}N$: C, 86.84; H, 6.83; N, 6.33. Found: C, 86.73; H, 6.66; N, 6.37.

3-Benzyl-1,2-dimethylindole. Phenyl-1,2-dimethylindolidenemethane hydrosulfate (116 mg, 0.35 mmole) was treated as above affording 63 mg (76% yield) of a yellow solid residue which was recrystallized four times from ethanol–water mixture to colorless needles, mp 56.6–57°; infrared λ_{max}^{KBr} 3100, 2970, 1610, 1560, and 1470 (strong and sharp pentet) cm^{-1}; nmr 2.77 (multiplet, 9 H), 5.88 (singlet, 2 H), 6.33 (singlet, 3 H), and 7.65 (singlet, 3 H).

Anal. Calcd for $C_{17}H_{17}N$: C, 86.77; H, 7.28; N, 5.95. Found: C, 86.93; H, 7.41; N, 6.17.

Diethyl 2,6-dimethylpyridyl-3,5-dicarboxylate was prepared according to the method of Braud, Hannah, and Linstead,[8] and obtained as white crystals, nmr 1.40 (singlet, 1 H), 5.54 (quartet, J = 7 cps, 4 H), 7.12 (singlet, 7 H), and 8.57 (triplet, J = 7 cps, 6 H).

Apparatus. Spectrophotometric measurements were performed on a Gilford Model 2000 spectrophotometer or a Durrum-Gibson Model 13001 stopped-flow spectrophotometer at 30 ± 0.1°.

Kinetics. Acetonitrile solutions which were about 10^{-4} M in indolenine salt were prepared by stirring a suspension of the salt in acetonitrile for 15 min. This was done in a tightly stoppered volumetric flask which was covered with aluminum foil. The indolenine salt solutions were mixed with equal volumes of acetonitrile solutions of amine or Hantzch ester (10^{-4} to 1.0 M) in the mixing chamber of the stopped-flow spectrophotometer. The reactions were followed by observing the time dependence for the disappearance of absorbance due to the indolenine salt. The wavelengths employed are provided in Table I.

Table I. Wavelengths Employed to Follow Disappearance of Indolenine Salt

Phenyl-2-methyl-indolidene-methane hydrosulfate substituent	Added reactant, mμ				
	Imid-azole	Hantzch ester	Piper-idine	Morpho-line	Aziri-dine
Phenyl	360	430	360	360	360
p-Hydroxyphenyl		430			
p-Chlorophenyl		430			
o-Chlorophenyl		430			
Phenyl-5-chloro		420			
Phenyl-N-methyl	390	420	390	390	
Phenyl-5-methyl		420			
Phenyl-7-methyl		420			

For those kinetic experiments carried out in the Gilford spectrophotometer, 1 ml of the amine solution was pipetted into a 2-ml cuvette, and the cuvette was allowed to equilibrate in the thermostated block of the instrument. The indolenine salt solution was thermostated in a water bath. When both solutions were thermally equilibrated the cuvette was withdrawn from the thermostated block, 1 ml of the indolenine salt solution was added, and after shaking the cuvette was returned to the spectrophotometer to initiate the recording of the absorbance change. For the reactions carried out in absolute alcohol, solutions with concentrations of 10^{-3} M were used. The second-order rate constants were obtained from the slope of plots of $\log (a - x)/(b - x)$ *vs.* time using eq 3 where

$$k = \frac{\text{slope } 2.303}{a - b} \tag{3}$$

a and b are the initial concentrations of absorber and nonabsorber, respectively. The values of $\log (a - x)/(b - x)$ were calculated from the experimental data using eq 4. The pseudo-first-order

$$\log \left(\frac{a - x}{b - x}\right) = \log \left[\frac{1}{\frac{b}{a} - 1^{(OD_0/OD_t)} + 1}\right] \tag{4}$$

rate constants were obtained from the slopes of $\log (OD_0/OD_t)$ *vs.* time.

Reaction of Phenyl-2-methylindolidenemethane with Diethyl 2,6-Dimethyl-1,4-dihydropyridyl-3,5-dicarboxylate. The dihydropyridine and indolenine salts (100 and 124 mg, respectively; 0.393 mmole each) were allowed to stand together 10 min at room temperature in 7 ml of acetonitrile. The solvent was removed *in vacuo* at 50° leaving 210 mg of yellow glass which crystallized on the sides of the flask. The yellow solid was recrystallized from benzene–hexane to yellow prisms, mp 83–98°; infrared λ_{max}^{KBr} 3230, 2650 (broad), and 1720 (sharp) cm^{-1}.

The yellow crystals (107 mg, 0.188 mmole) were recrystallized from an ethanol–water mixture to yield 44 mg of white crystals. Three recrystallizations from ethanol–water afforded colorless plates, mp 119.5–120°, infrared λ_{max}^{KBr} 3400 (sharp), 3050, 2930, 1580 (broad and weak), 1480 (sharp), and 1421 cm^{-1}. A mixture melting point of this material and 3-benzyl-2-methylindole (*loc. cit.*) was determined to be 118–120°, undepressed over that of the components. The original aqueous–alcoholic mother liquor was diluted with water and made basic with aqueous potassium hydroxide. The white precipitate was collected by centrifugation, washed twice with water, and dried *in vacuo* over P_2O_5. The white crystals weighted 27 mg, infrared λ_{max}^{KBr} 3400 (small), 3000 (sharp), 1720 (sharp), and 1590 cm^{-1}, identical with the infrared spectrum of diethyl 2,6-dimethylpyridyl-3,5-dicarboxylate (*loc. cit.*).

3-Benzyl-2-methylindole (33 mg) and 38 mg of diethyl 2,6-dimethylpyridyl-3,5-dicarboxylate were dissolved in about 2 ml of absolute ethyl alcohol which was previously saturated with hydrogen chloride. Removal of the solvent produced a solid yellow residue. The material was twice recrystallized from benzene–hexane to yellow needles, mp 83–98°, infrared λ_{max}^{KBr} 3250, 2650 (broad), and 1725 cm^{-1}, This spectrum was identical with the spectrum of the original product complex except for the absence of strong bands at 1040 and 870 cm^{-1}.

Reaction of *p*-Hydroxyphenyl-2-methylindolidenemethane Hydrosulfate with Diethyl 2,6-Dimethyl-1,4-dihydropyridyl-3,5-dicarboxylate. The dihydropyridine and indolenine salts (100 and 131 mg, respectively; 0.395 mmole each) were allowed to stand together at room temperature about 10 min in about 7 ml of acetonitrile. The solvent was removed leaving 222 mg of yellow glass which became crystalline on the side of the flask. The solid (53 mg) was recrystallized from ethanol–water mixture affording 29 mg of brown solid which was recrystallized three more times from aqueous–ethanol to yield white needles, mp 143.5–144°, infrared λ_{max}^{KBr} 3550 (sharp), 3400 (sharp), 3030, 2920, and 1610 (small doublet) cm^{-1}. A mixture melting point of this material and 3-(*p*-hydroxybenzyl)-2-methylindole (*loc. cit.*) was determined to be 142.5–144°, undepressed over that of the components. The original aqueous–ethanolic mother liquor was diluted with water and made basic with aqueous potassium hydroxide. The white precipitate resulting was collected by centrifugation, washed twice with water, and dried *in vacuo* over P_2O_5. The white solid weighed 21 mg; infrared λ_{max}^{KBr} 3400 (broad), 3000, 1720 (strong and sharp), and 1590 (sharp) cm^{-1}, identical with the infrared spectrum of diethyl 2,6-dimethylpyridyl-3,5-dicarboxylate (*loc. cit.*).

Reaction of Phenyl-1,2-dimethylindolidenemethane Hydrosulfate with Diethyl 2,6-Dimethyl-1,4-dihydropyridyl-3,5-dicarboxylate. The dihydropyridine and indolenine salts (50 and 65 mg, respectively; 0.196 mmole each) were mixed together in 6 ml of acetonitrile and allowed to stand about 10 min at room temperature. The solvent was removed *in vacuo* at 50° and the deep red solution left a dark gum which did not become crystalline. The gum was taken up in alcohol and the solution heated to boiling. Water was added to the hot solution until it became slightly turbid. Upon cooling and standing at room temperature, 53 mg of gray solid separated. The solid was recrystallized three times from aqueous ethanol to yield colorless prisms, mp 56.5–57°, infrared λ_{max}^{KBr} 3080, 2950, 1610, 1570, and 1470 cm^{-1}. A mixture melting point of this material and 3-benzyl-1,2-dimethylindole (*loc. cit.*) was determined to be 55.5–57°, undepressed over that of the components. The original aqueous ethanolic mother liquor was diluted with water

and made basic with aqueous potassium hydroxide. The resulting white precipitate was collected by centrifugation, washed twice with water, and dried *in vacuo* over P_2O_5. The white solid weighed 35 mg, infrared λ_{max}^{KBr} 3400 (small), 3000 (sharp), 1720 (sharp and strong), and 1590 cm^{-1}, identical with the infrared spectrum of diethyl 2,6-dimethylpyridyl-3,5-dicarboxylate (*loc. cit.*).

Reaction of *o*-Chlorophenyl-2-methylindolidenemethane Hydrosulfate with Diethyl 2,6-Dimethyl-1,4-dihydropyridyl-3,5-dicarboxylate. Since nmr analysis can account for all of the protons of the products of this reaction nmr analyses were conducted on the reaction products of the remaining indolenine salts with diethyl 2,6-dimethyl-1,4-dihydropyridyl-3,5-dicarboxylate. Their preparation is identical with the one described below.

The dihydropyridine and indolenine salts (50 and 60 mg, respectively; 0.196 mmole each) were allowed to react 10 min at room temperature in about 5 ml of acetonitrile. The solvent was removed *in vacuo* at 50° and the residue shaken thoroughly with ether and 1 *M* aqueous potassium hydroxide. The layers were separated, and the organic layer was washed with water and dried over sodium sulfate. The ether solution was decanted from the salt and the ether was removed leaving an 80% yield of a yellow crystalline residue, nmr 1.27 (singlet, 1 H), 2.97 (multiplet, 9 H), 5.60 (quartet, J = 7 cps, 4 H), 5.85 (singlet, 1 H), 7.13 (singlet, 6 H), 7.70 (singlet, 3 H), and 8.60 (triplet, J = 7 cps, 6 H).

***p*-Chlorophenyl-2-methylindolidenemethane hydrosulfate** gave the following nmr spectrum: 1.28 (singlet, 1 H), 2.83 (multiplet, 9 H), 5.60 (quartet, J = 7 cps, 4 H), 5.98 (singlet, 2 H), 7.13 (singlet, 6 H), 7.68 (singlet, 3 H), 8.60 (triplet, J = 7 cps, 6 H).

Phenyl-5-chloro-2-methylindolidenemethane hydrosulfate gave the following nmr spectrum: 1.28 (singlet, 1 H), 2.78 (multiplet 9 H), 5.58 (quartet, J = 7 cps, 4 H), 5.98 (singlet, 2 H), 7.13 (singlet, 6 H), 7.68 (singlet, 3 H), and 8.60 (triplet, J = 7 cps, 6 H).

Phenyl-2,5-dimethylindolidenemethane hydrosulfate gave the following nmr spectrum: 1.28 (singlet, 1 H), 2.78 (multiplet, 9 H), 5.60 (quartet, J = 7 cps, 4 H), 5.95 (singlet, 2 H), 7.13 (singlet, 6 H), 7.62 (singlet, 3 H), 7.70 (singlet, 3 H), and 8.60 (triplet, J = 7 cps, 6 H).

Phenyl-2,7-dimethylindolidenemethane hydrosulfate gave the following nmr spectrum: 1.30 (singlet, 1 H), 2.78 (multiplet, 9 H), 5.60 (quartet, J = cps, 4 H), 5.93 (singlet, 2 H), 7.13 (singlet, 6 H), 7.57 (singlet, 3 H), 7.63 (singlet, 3 H), and 8.60 (triplet, J = 7 cps, 6 H).

Reaction of Phenyl-2-methylindolidenemethane Hydrosulfate with Amines. Morpholine. The indolenine salt (172 mg, 0.541 mmole) and 281 mg (3.22 mmoles) of morpholine were mixed in about 10 ml of acetonitrile. When the yellow color disappeared, the white precipitate was collected by centrifugation, washed with ether, and dried to 44 mg of morpholinium sulfate, infrared λ_{max}^{KBr} 3500, 3330, 3000 (broad), and 1560 cm^{-1}. The solvent was removed *in vacuo* at 50° from the centrifugate leaving a colorless gum which solidified upon being washed with water to a light yellow crystalline solid, 165 mg (100% yield) upon drying. The solid could be recrystallized from ethanol to white prisms of phenyl-3-(2-methylindolyl)morpholinomethane, mp 176–179°; infrared λ_{max}^{KBr} 3400, 2860 (broad), and 1580 (small doublet) cm^{-1}; nmr 2–3 (multiplet, 10 H), 5.55 (singlet, 1 H), 6.30 (triplet, J = 5 cps, 4 H), 7.57 (triplet, J = 5 cps, 4 H), and 7.65 (singlet, 3 H).

Anal. Calcd for $C_{20}H_{22}N_2O$: C, 78.39; H, 7.24; N, 9.14. Found: C, 77.98; H, 7.44; N, 9.25.

About 100 mg of these crystals was dissolved in 7 ml of ethyl alcohol and a small amount of concentrated sulfuric acid was introduced. The solution darkened and a yellow precipitate formed. The material was collected by centrifugation and washed with ethanol and ether, infrared λ_{max}^{KBr} 2600 (broad), 1580, and 1550 cm^{-1}, identical with the spectrum of the starting indolenine salt.

Imidazole. This procedure is the same as for morpholine. The indolenine salt (100 mg) and 288 mg of imidazole gave 73 mg of imidazolium sulfate, infrared λ_{max}^{KBr} 3200 (broad), 1480, and 1570 cm^{-1}, and 90 mg (100% yield) of white crystalline material from the solution which was recrystallized from aqueous ethyl alcohol to white crystals of imidazole adduct, mp 117–119°; infrared λ_{max}^{KBr} 3200 (broad) and 1550 (small doublet); nmr (CD_3COCD_3) 3.33 (multiplet, 12 H), 7.67 (singlet, 2 H), and 8.15 (singlet, 3 H).

Anal. Calcd for $C_{19}H_{17}N_3$: C, 79.50; H, 5.96; N, 14.62; mol wt, 287. Found: C, 79.70; H, 6.01; N, 14.13; mol wt (Rast), 245.

Piperidine. The procedure here is the same as for morpholine. The indolenine salt (105 mg) was treated with 690 mg of piperidine. Piperidinium sulfate (70 mg) was obtained. Eighty milligrams (78%) of white precipitate was obtained from the reaction solution. The material was recrystallized from methanol and dried *in vacuo* over P_2O_5 to white crystals of phenyl-3-(2-methylindolyl)piperidinomethane, mp 130–132°; infrared λ_{max}^{KBr} 3450 (sharp), 2900 (broad), and 1580 (small triplet) cm^{-1}; nmr ($CDCl_3$) 2.90 (multiplet, 11 H), 5.48 (singlet, 1 H), 7.61 (singlet on top of broad band, 7 H), and 8.5 (multiplet, 6 H).

Anal. Calcd for $C_{21}H_{24}N_2$: C, 82.85; H, 7.94; N, 9.20. Found: C, 82.46; H, 8.34; N, 9.44.

Aziridine. The procedure here was the same as for morpholine. The indolenine salt (110 mg) was treated with 500 mg of azirdine. Aziridinium sulfate (40 mg) was obtained as a precipitate, and 81 mg (89%) of white solid was obtained from the reaction solution. The solid was recrystallized from methanol–water twice and dried *in vacuo* over P_2O_5 to white crystals of phenyl-3-(2-methylindolyl)aziridinomethane, mp 144–146°; infrared λ_{max}^{KBr} 3100 (broad), 1620–1500 (six small bands), and 1460 cm^{-1}; nmr 2.74 (multiplet, 10 H), 6.18 (singlet, 1 H), 7.62 (singlet, 3 H), and 8.83–7.83 (multiplet, 4 H).

Anal. Calcd for $C_{18}H_{18}N_2$: C, 82.40; H, 6.91; N, 10.67. Found: C, 82.79; H, 6.95; N, 10.24.

Reaction of Phenyl-1,2-dimethylindolidenemethane Hydrosulfate with Imidazole. The procedure here was the same as for morpholine. The indolenine salt (135 mg) was treated with 246 mg of imidazole. Imidazolium sulfate, 114 mg, infrared λ_{max}^{KBr} 3150 (broad) and 1580 (sharp) cm^{-1}, was precipitated and 15 mg (42%) of reddish solid was obtained from the reaction solution. Recrystallization three times from aqueous ethanol produced small prisms of imidazole adduct, mp 172–175°; infrared λ_{max}^{KBr} 3400 (broad), 3100, 2900, and 1600 (triplet) cm^{-1}; nmr 2.77 (multiplet, 14 H), 6.35 (singlet, 3 H), and 7.73 (singlet, 3 H).

Anal. Calcd for $C_{20}H_{19}N_3$: C, 79.70; H, 6.35; N, 13.94. Found: C, 79.88; H, 6.62; N, 13.40.

Results

Hydrogen Transfer. The rate of disappearance of substituted phenyl-2-methylindolidenemethane hydrosulfates (I) in the presence of approximately equal concentrations of diethyl 2,6-dimethyl-1,4-dihydropyridyl-3,5-dicarboxylate was followed in acetonitrile. The latter was chosen because of its solvent properties and the instability of the indolenines in protic solvents like absolute alcohol. Plots of log $(a - x)/(b - x)$ *vs.* time were linear to three or four half-lives indicating the reaction to be first order in each component (Figure 1). The values obtained for the second-order rate constants are shown in Table II. Some experiments were conducted in absolute alcohol in order to study solvent effects on the reaction. In these cases the disappearance of the Hantzch ester was followed. It was found that whenever the ratio (phenylindolenine salt)/(Hantzch ester) was less than about 1.3, residual absorption was observed and the ultraviolet spectra of systems at equilibrium in which the ratio (indolenine salt)/(Hantzch ester) was between one and 1.3 was identical with the spectrum of the Hantzch ester. This seemed to indicate that these reactions were proceeding to equilibrium. However, no retrograde reaction could be detected under conditions in which it should have been clearly visible if the residual absorption observed in the forward step had been due to an approach to equilibrium. Table III lists the second-order rate constants determined in absolute ethanol.

Hammett plots (*i.e.*, log k *vs.* σ) for the reaction of Hantzch ester (acetonitrile) with the three indolenine salts of Table II which are substituted in the *para* position of the phenyl ring and the three salts substituted in the 5 position of the indolenine nucleus gave ρ values of +1.5 and +0.77, respectively. A plot of log k *vs.* σ^+ for substituents in the *para* position of the phenyl ring showed slightly more scatter than did the σ plot. The plot for substituents on the

Table II. Rate Data for the Reaction of Substituted Phenyl-2-methylindolidenemethane Hydrosulfates with Hantzch Ester (solvent acetonitrile, 30°)

Phenyl-2-methylindolidene-methane hydrosulfate Substituent	$M \times 10^5$	Hantzch ester, $M \times 10^5$	$k_{rate} \times 10^{-6}$, M^{-1} min^{-1}
Phenyl	7.04	11.46	1.60
Phenyl	4.82	4.90	2.28
Phenyl	4.82	2.45	2.33
p-Hydroxyphenyl	5.12	11.46	0.363
p-Hydroxyphenyl	5.75	5.54	0.240
p-Hydroxyphenyl	5.75	2.77	0.467
p-Chlorophenyl	5.02	11.46	2.76
Phenyl-5-chloro	5.25	11.62	3.17
Phenyl-5-methyl	5.75	11.62	1.37
Phenyl-N-methyl	6.27	11.62	0.79
Phenyl-7-methyl	6.88	11.62	2.65

Table III. Rate Constants for the Reaction of Substituted Phenyl-2-methylindolidenemethane Hydrosulfates with Hantzch Ester (solvent absolute ethanol, 30°)

Phenyl-2-methylindolidenemethane hydrosulfate Substituent	$M \times 10^4$	Hantzch ester, $M \times 10^4$	k_{rate}, M^{-1} min^{-1}
Phenyl	4.47	2.29	3900
Phenyl-N-methyl	3.78	2.29	1330
o-Chlorophenyl	3.78	2.29	625

5 position of the indole nucleus was best with σ constants for *meta* substituents. Table IV shows the effect of radical inhibitors[9] on the reaction of the *p*-hydroxyphenylindolenine salt with the Hantzch ester. Table V shows the marked solvent effects on the ultraviolet maxima and extinction coefficients for the N-methyl- and phenylindolenine salt, but shows the Hantzch ester to be almost unaffected.

Table IV. Rate Constants for the Reaction of *p*-Hydroxy-2-methylindolidenemethane Hydrosulfate with the Hantzch Ester in the Presence of Radical Inhibitors at 30 ± 0.1° in Acetonitrile

Added inhibitor	Indolenine salt, $M \times 10^5$	Hantzch ester, $M \times 10^5$	k_{rate}, M^{-1} min^{-1}
None	4.01	4.98	0.177
Picric acid 5×10^{-4} *M*	4.01	4.98	0.244
Benzoic acid 1.3×10^{-3} *M*	4.01	4.98	0.188

The reaction of 0.4 mmole of phenyl-2-methylindolidenemethane hydrosulfate with 1 equiv of Hantzch ester proceeds rapidly in 7 ml of acetonitrile or ethanol with concurrent loss of yellow color and solution of the undissolved indolenine salt. The only isolated product was a yellow crystalline solid which melted over a 15° range. The two components of this material were separated by recrystallizing the product from aqueous ethanol. The indole component precipitates while the pyridinium salt remains in the solution. Each component was identified by comparison to independently synthesized material. Diethyl 2,6-dimethylpyridine-3,5-dicarboxylate was prepared by oxidation of the Hantzch ester with chloranil,[8] and 2-methyl-3-benzylindole was prepared by reduction of the phenylindolenine salt by potassium borohydride.[6] The initial yellow solid product could be reconstituted as the hydrochloride salt by treatment of equivalent amounts of diethyl 2,6-dimethylpyridyl-3,5-dicarboxylate and 2-methyl-3-benzylindole with gaseous hydrogen chloride. Analogous results were obtained from the products of the reaction of the Hantzch ester with the *p*-hydroxyphenyl and N-methylindolenine salts. Nmr analysis indicated that analogous products are obtained from the reaction of the other indolenine salts with the Hantzch ester.

(9) F. A. Bovey and I. M. Kolthoff, *Chem. Rev.*, **42**, 491 (1948).

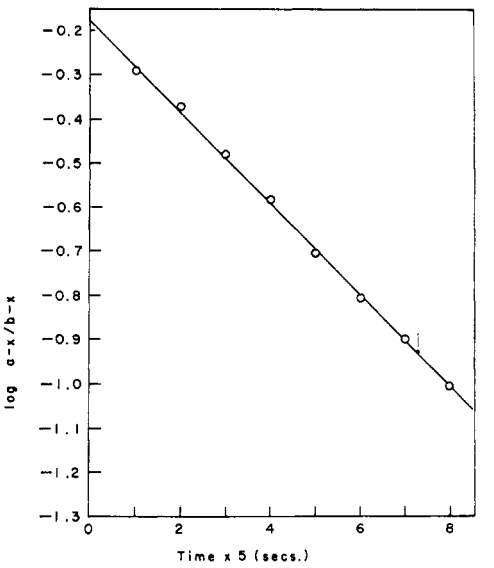


Figure 1. Plot of log $(a - x)/(b - x)$ *vs.* time for the reaction of 7×10^{-5} *M* phenylindolenine hydrosulfate with 11.5×10^{-5} *M* Hantzch ester in acetonitrile at 30°.

Aminolysis. In acetonitrile the rate of disappearance of phenyl-2-methylindolidenemethane hydrosulfate in the presence of imidazole, morpholine, piperidine, and aziridine was followed under the pseudo-first-order conditions of [amine] ≫ [indolenine]. No attempt was made to control pH.[10] Plots of log $(OD_0)/(OD_t)$ *vs.* time were linear up to four half-lives (Figure 2). Plots of k_{obsd} *vs.* amine concentration are shown for each amine in Figures 3 and 4. The points are experimental and the curves are derived from eq 5a–d.[11]

Imidazole

$$k_{obsd} = \frac{12.5A + 2000A^2}{5 \times 10^{-5} + 800A^2} \quad (5a)$$

Aziridine

$$k_{obsd} = \frac{4.7A + 8.3A^2 + 145A^3}{5 \times 10^{-5} + 0.2A + 3.5A^2} \quad (5b)$$

Morpholine

$$k_{obsd} = \frac{8A + 142A^2 + 2880A^3}{5 \times 10^{-5} + 1.6A + 110A^2} \quad (5c)$$

(10) Methods for the determination of hydrogen ion activity by the glass electrode in acetonitrile have recently been discussed. See J. F. Coetzee, G. R. Padmanabhan, and G. P. Cunningham, *Talanta*, **11**, 93 (1964); W. S. Muney and J. F. Coetzee, *J. Am. Chem. Soc.*, **66**, 89 (1962); J. F. Coetzee and G. P. Cunningham, *ibid.*, **87**, 2534 (1965); J. F. Coetzee and G. R. Padmanabhan, *ibid.*, **87**, 5005 (1965).

(11) Abbreviations used: IH^+ = indolenine salt, H = Hantzch ester, IH_0^+ = initial indolenine salt concentration, H_0 = initial Hantzch ester concentration, I_T = IH + I...$HNHR_2$ + I + $INHR_2$, A = R_2NH = imidazole, piperidine, aziridine, or morpholine.

Table V. Absorption Maxima and Extinction Coefficients of Indolenine Salts and the Hantzch Ester

Phenyl-2-methylindolidenemethane hydrosulfate substituent	Acetonitrile			Ethanol		
	Concn, M	λ_{max}	ϵ	Concn, M	λ_{max}	ϵ
Phenyl	2.27×10^{-4}	394	15900	2.86×10^{-3}	382	101
Phenyl-N-methyl	1.09×10^{-4}	380	10640	3.24×10^{-3}	380	93
Hantzch ester	8.77×10^{-5}	363	7900	1.74×10^{-4}	372	9800

Piperidine

$$k_{obsd} = 80A + 2613A^2 \quad (5d)$$

The reaction of 0.5 mmole of phenyl-2-methylindolidenemethane hydrosulfate with excess morpholine, piperidine, imidazole, and aziridine occurs rapidly in 10 ml of acetonitrile with loss of yellow color, solution

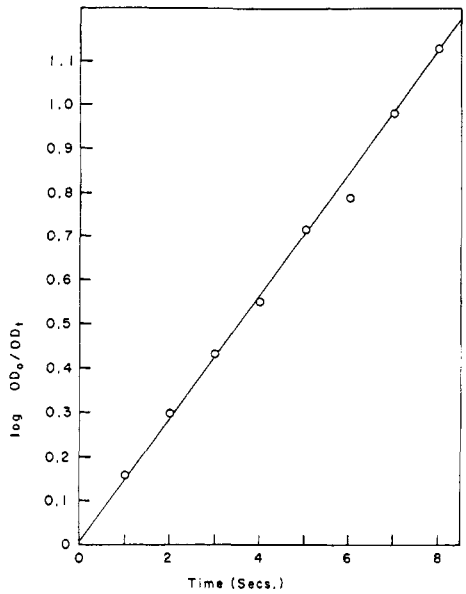
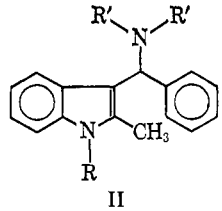


Figure 2. Plot of log OD_0/OD_t *vs.* time for the reaction of 7×10^{-5} M phenylindolenine hydrosulfate with 0.395 M aziridine in acetonitrile at 30°.

of the undissolved indolenine salt, and deposition of a white precipitate of the amine sulfate. The infrared, nmr, and analytical data are consistent with the assignment of structure II (R = H) to the other products from the reactions involving morpholine, piperidine, and aziridine.



II

The nmr spectrum of the imidazole product however does not seem to be consistent with the proposed structure II (R = H). The methine proton resonance seen at about τ 5.5 in the spectra of the other amine adducts is absent in that of imidazole while a resonance peak integrating to two protons is observed in the imidazole spectrum at τ 7.67 that is absent in the spectra of the other amines. Treatment of an ethanol solution of the morpholine adduct with sulfuric acid afforded the starting indolenine salt thus demonstrating the reversibility of the reaction. The rate of disappearance of phenyl-1,2-dimethylindolidenemethane hydrosulfate in acetonitrile solutions of imidazole, piperidine, and morpholine was followed under second-order conditions as described in the experiments with the Hantzch ester. The rate constants obtained are shown in Table VI. Thus substitution of a methyl group for the proton on the nitrogen atom of the indolenine nucleus has changed the kinetic behavior of the reaction to simple second order with rate constants greater in magnitude than in the hydride-transfer reaction.

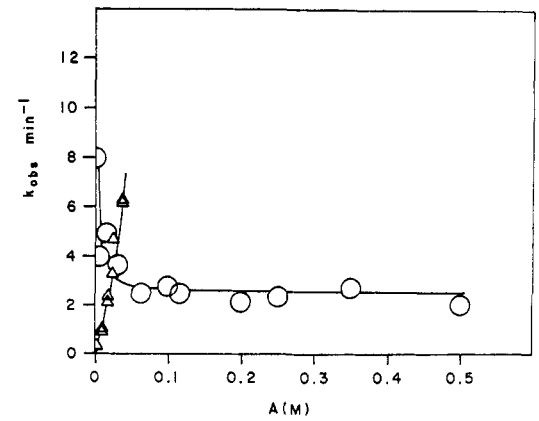


Figure 3. Plot of k_{obsd} *vs.* concentration of amine for the reaction of the phenylindolenine hydrosulfate with (O) imidazole and (Δ) piperidine in acetonitrile at 30°.

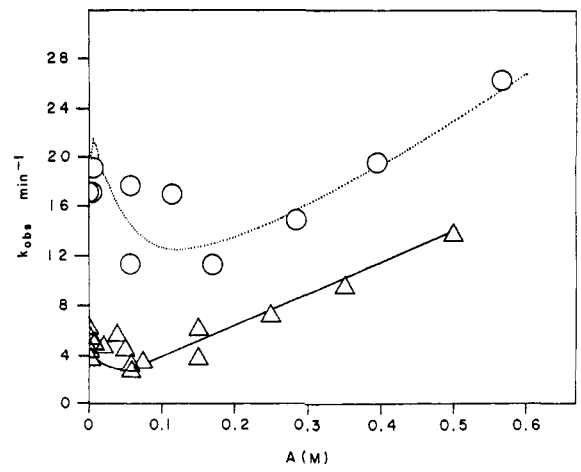


Figure 4. Plot of k_{obsd} *vs.* concentration of amine for the reaction of the phenylindolenine hydrosulfate with (O) aziridine and (Δ) morpholine in acetonitrile at 30°.

Table VI. Rate Constants for the Reaction of Phenyl-1,2-dimethylindolidenemethane Hydrosulfate with Amines in Acetonitrile at 30 ± 0.1°

Amine	Concn, M	M^{-1} min^{-1} × 10^{-6} [a]
Imidazole	5×10^{-4}	18.7
	1×10^{-4}	32.4
Morpholine	1.39×10^{-3}	6.9
Piperidine	7.8×10^{-5}	13.8

[a] The numbers represent only one rate determination each (two for imidazole).

The reaction of phenyl-1,2-dimethylindolidenemethane hydrosulfate with imidazole proceeded rapidly in acetonitrile with the deposition of imidazolium sulfate. Removal of this salt followed by the solvent produced a white solid residue whose microanalysis is consistent with the structure II (R = CH_3). The nmr spectrum of this product, like the spectrum of the product of the reaction of the phenylindolenine salt with imidazole, contained no methine resonance but unlike the spectrum of the latter substance did not contain a two-proton resonance peak.

Discussion

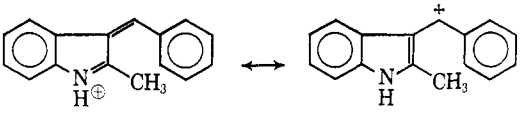
The most striking observation in the hydrogen transfer reactions to indolenine salts is the 500-fold rate enhancement observed when acetonitrile rather than ethanol is used as the solvent (Tables II and III). The parallel 100-fold increase in extinction coefficient (Table V) observed for the visible absorption band of the salt and the fact that there is almost no effect on the extinction coefficient of the Hantzch ester indicate that ethanol and acetonitrile have very different abilities to solvate the indolenine salt. Acetonitrile is known to be less capable of solvating polar molecules than protic solvents and acids and bases exist in the solvent as hydrogen-bonded conglomerates.[10] The fact that the N-methylindolenine salt also shows a rate and extinction coefficient enhancement on change of solvent established that the proton on the indole nitrogen atom is not the only atom involved in solvation. Thus, this hydrogen-transfer reaction may be a simple bimolecular process whose rate is inhibited in ethanol due to the formation of a stable tight solvation shell. Whether or not a charge-transfer complex between the reactant salt and Hantzch ester molecules is involved cannot be discerned because the usual methods of detection of such complexes are precluded due to the rate of the reaction.

The ρ value of 1.5 obtained from the slope of the Hammett plot for the reduction by Hantzch ester of indolenines with *para* substituents on the phenyl ring compares favorably to the value of 1.5 found for the reduction by lithium tetrakis(N-dihydropyridyl)aluminate of substituted benzophenones[12] but is somewhat less than the value of 2.63 found by plotting the data of Abeles, Hutton, and Westheimer for the reaction of substituted thiobenzophenones with N-benzylnicotinamide.[13] It is also less than values found for the reduction of aromatic ketones by sodium borohydride.[12]

(12) P. T. Lansbury and R. E. MacLeay, *J. Am. Chem. Soc.*, **87**, 831 (1965).

(13) R. H. Abeles, R. F. Hutton, and F. H. Westheimer, *ibid.*, **79**, 712 (1957).

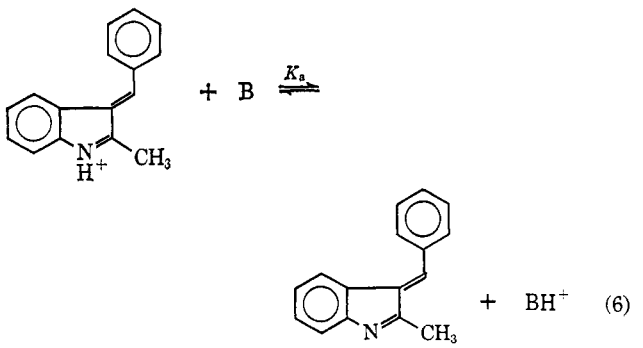
Since only three *p*-phenyl-substituted indolenine salts were studied the significance of the slightly better Hammett correlation with σ compared to σ^+ is questionable. However, the substantially positive value obtained for ρ probably means that the reaction site has little "carbonium ion" character. This in turn would suggest that the resonance contribution from the ground-state structure IIIa is greater than for IIIb.



IIIa IIIb

That the plot for substituents in the 5 position of the indolenine nucleus correlates best with σ for *meta* substituents indicates that the greatest effect for these substituents on the reaction site is through the carbon–carbon single bond bridge of the indole nucleus rather than around the heterocyclic ring (*i.e.*, through the indole nitrogen atom). The lesser value of $\rho = 0.77$ for this plot is consistent with the greater distance of these substituents from the reaction site. From Table IV it is seen that benzoic and picric acids have little effect on the reaction. This supports the conclusion of Schellenberg and McLean[6] that free radicals are not involved in the reaction of the *o*-chlorophenylindolenine hydrochloride with N-benzylnicotinamide. They found little rate reduction in the presence of ferric nitrate or thioglycol. The analysis of the products is consistent with the report of Schellenberg and McLean[6] that the transfer of hydrogen is to the methine carbon of the indolenine salt from the dihydropyridine. It is curious that the component products crystallize together upon removal of solvent. Perhaps some sort of complex is responsible.

The reaction of phenyl-2-methyindolidenemethane hydrosulfate with amines is shown to be quite complex. However, the reaction of amines with the phenyl-N-methylindolenine salt is a simple second-order reaction very comparable in rate to hydride transfer; thus the proton on the indolenine nitrogen atom seems to be the cause of the abnormal second-order plots in Figures 3 and 4. The indolenine salts are protonated imines and would be expected to undergo proton transfer with a suitable base as shown in eq 6. The pK_a' of the conjugate acid

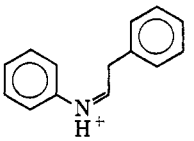


(6)

of N-benzylideneaniline (IV) is 2.80[14] and if the indolenine salt may be compared to this imine, it would possess sufficient acidity to interact with the amine bases in these experiments provided unexpected solvent

(14) E. H. Cordes and W. P. Jencks, *ibid.*, **84**, 832 (1962).

effects are not operative in acetonitrile. This would appear not to be the case from acid–base equilibrium studies conducted in acetonitrile. Coetzee and coworkers[10] have shown that the glass electrode can be used to measure the hydrogen ion concentration of acid solutions in anhydrous acetonitrile. Furthermore, a plot of pK_a' of aliphatic amines determined in acetonitrile *vs.* pK_a' values determined in water is approximately linear. It also turns out that plots of conductance or glass electrode response *vs.* $(B)/(BH^+)$ are curved especially with secondary amines in acetonitrile.[10] This is explained as being due to the equilibria of eq 7. The latter equilibrium is referred to as homoconjugation. This situation apparently results from the lower dielectric constant and weaker base properties of acetonitrile compared to water. Thus polar species are forced to rely on themselves for stabilization rather than to solvation by acetonitrile solvent. It would seem then that a complex situation might have been anticipated for the reactions in question. Taking this and the nature of eq 5a–d into account, Scheme I is proposed to explain the data of Figures 3 and 4.



IV

$$HA + B \rightleftharpoons BH^+A$$

$$BH^+A \rightleftharpoons BH^+ + A \qquad (7)$$

$$BH^+A + HA \rightleftharpoons AHA + BH^+$$

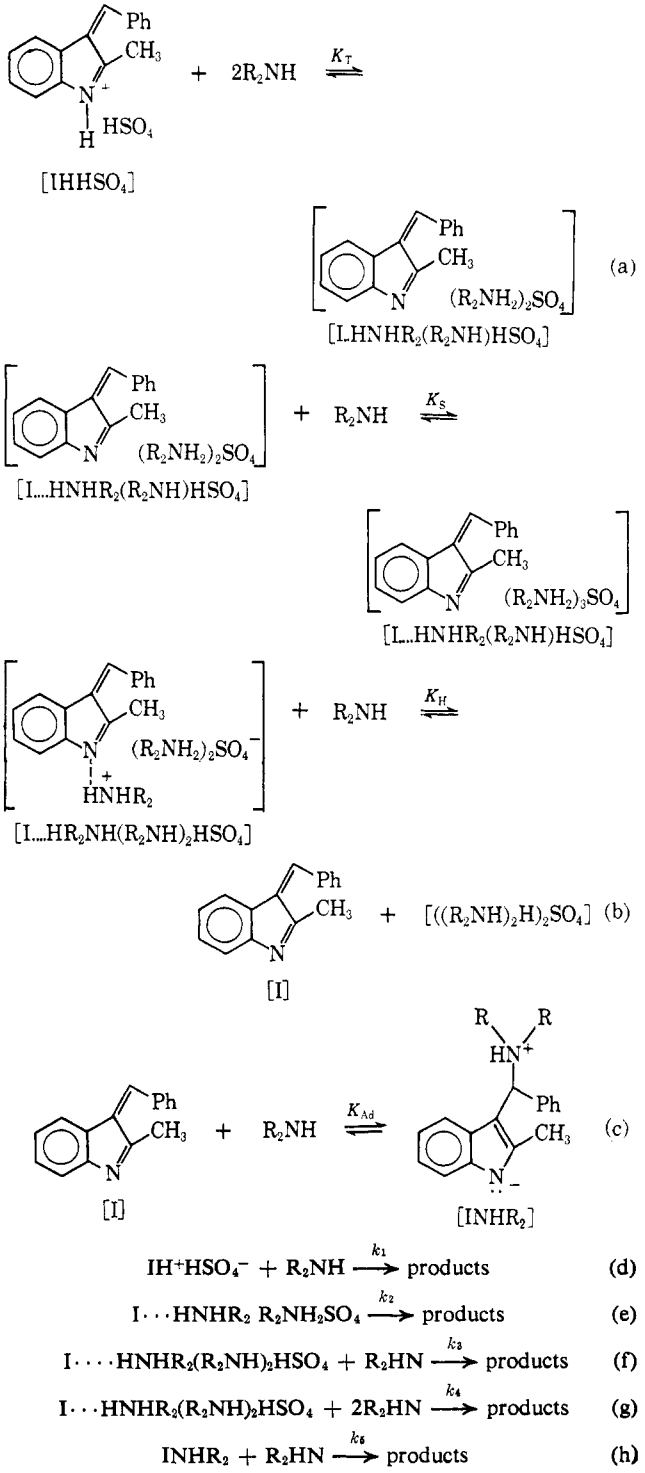
$$\text{rate} = k_{obsd}[I_T] = \frac{k_1[(R_2NH_2)_2SO_4](A) + k_2K_T(A)^2[(R_2NH_2)_2SO_4]}{K_T(A)^2 + (R_2NH_2)_2SO_4}[I_T] \qquad (8)$$

$$\text{rate} = k_{obsd}[I_T] = \frac{k_3[((R_2NH_2)_2H)_2SO_4](A) + k_4[((R_2NH)_2H)_2SO_4](A)^2 + k_5K_HK_{Ad}(A)^3}{[((R_2NH)_2H)_2SO_4] + K_H(A) + K_HK_{Ad}(A)^2}[I_T] \qquad (9)$$

$$k_{obsd} = \frac{k_4[(R_2NH_2)_2SO_4](A)}{K_H} + k_5K_{Ad}(A)^2 \qquad (10)$$

The rate law for the reaction with imidazole, eq 5a, has the form of eq 8, derived from parts a, d, and e of Scheme I, while the reaction with aziridine and morpholine, eq 5b and c, respectively, has the form of eq 9, derived from parts b, c, f, g, and h of Scheme I. For piperidine eq 5d, eq 9 may be rewritten as eq 10 considering the k_3 term to be unimportant as well as K_{Ad} and $[((R_2NH)_2H)_2SO_4]$ to be smaller than K_H. The constants thus obtained are listed in Table VII. These constants were obtained assuming that the concentrations of $(R_2NH_2)_2SO_4$ and $((R_2NH)_2H)_2SO_4$ were approximately equal to the initial indolenine salt concentration because of the much greater initial concentration of amine compared to indolenine salt. The uniqueness of the rate law for imidazole arises from its weak base strength because it is the only amine for which a kinetic pathway to products from the protonated indolenine is observed. Its weak base strength also apparently prevents it from attacking the unprotonated indolenine (*i.e.*, species I in Scheme I). The very strong base properties of piperidine, in an analogous manner, make its rate law unique. In piperidine solutions the predominate species is anticipated to be the free indolenine (*i.e.*, I in Scheme I); thus pathway k_5 is the major contributor to products. The values of K_H and K_{Ad} cannot be determined kinetically for imidazole and piperidine but they were estimated from two-point plots of log K *vs.* pK_a' using the data of the other two amines (see Table VII). For piperidine both K_H and K_{Ad} equal 10^4. On this basis it would seem that the assumption that $K_{Ad} \ll K_H$ for piperidine

Scheme I



$IHHSO_4$ + $2R_2NH$ $\overset{K_T}{\rightleftharpoons}$ $[IHHSO_4]$

$[IHHSO_4]$ + R_2NH $\overset{K_S}{\rightleftharpoons}$ $[I\cdots HNHR_2(R_2NH)HSO_4]$ (a)

$[I\cdots HNHR_2(R_2NH)HSO_4]$ + R_2NH $\overset{K_H}{\rightleftharpoons}$ $[I\cdots HR_2NH(R_2NH)_2HSO_4]$

$[I]$ + $[((R_2NH)_2H)_2SO_4]$ (b)

$[I]$ + R_2NH $\overset{K_{Ad}}{\rightleftharpoons}$ $[INHR_2]$ (c)

$$IH^+HSO_4^- + R_2NH \xrightarrow{k_1} \text{products} \qquad (d)$$

$$I\cdots HNHR_2\ R_2NH_2SO_4 \xrightarrow{k_2} \text{products} \qquad (e)$$

$$I\cdots HNHR_2(R_2NH)_2HSO_4 + R_2HN \xrightarrow{k_3} \text{products} \qquad (f)$$

$$I\cdots HNHR_2(R_2NH)_2HSO_4 + 2R_2HN \xrightarrow{k_4} \text{products} \qquad (g)$$

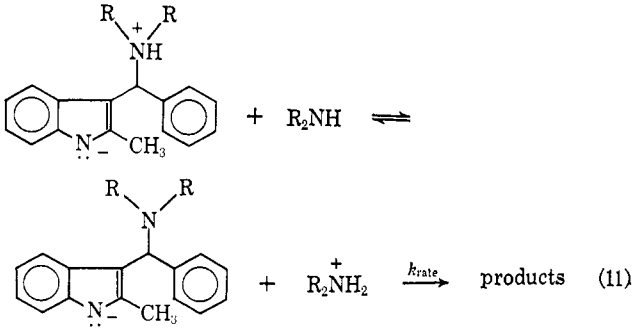
$$INHR_2 + R_2HN \xrightarrow{k_5} \text{products} \qquad (h)$$

Table VII. Rate and Equilibrium Constants Obtained from a Fit of Eq 8 to Eq 5a and Eq 9 to Eq 5b, c, and d[a]

Amine	K_T	K_H[b]	k_{Ad}[b]	k_1	k_2	k_3	k_4	k_5
Imidazole	800	(0.005)	(0.08)	2.5×10^5	0.5×10^5	...	...	...
Aziridine		0.20	17.5	...	...	0.94×10^5	1.55×10^5	41.5
Morpholine		1.6	69.0	...	...	1.6×10^5	2.84×10^6	26.2
Piperidine		(10^4)	(1.41×10^4)	...	...	...	...[c]	...[c]

[a] The data enclosed in parentheses is estimated (see footnotes *b* and *c*). [b] The values of K_H and K_{Ad} for imidazole and piperidine were estimated from two-point plots of log K_H and K_{Ad} *vs.* pK_a' of the amine. [c] These numbers cannot be evaluated from eq 10 because K_H and K_{Ad} are not known.

is unjustified. But since the proposed equilibria is correct only to the extent that it is reasonable and satisfies the observed kinetics, it does not appear useful to pursue this possible discrepancy. One could probably write other equilibria which would be just as satisfactory but it would seem of little consequence in view of the complexity of the data. The equilibrium constants K_H and K_{Ad} in Table VII appear to increase with the basicity of the amine. The rate constants k_1, k_2, k_3, and k_4 are all nearly identical while k_5 seems to be inversely proportional to amine basicity. This latter inverse effect may be due to a two-step mechanism such as is shown in eq 11. The rate-determining step is donation of a proton to the indole nitrogen by the protonated amine.



The indolenine salts are seen to be very unstable compounds which will resort to many different ways to reduce their energy content. Reactions of these salts are much slower in ethanol than in acetonitrile because of increased stability due to solvation. This solvation may involve hydrogen bonding between the solvent molecules and the acidic proton of the salt but also involves some charge contribution from the solvent to the positively charged chromophore of the salt. When these salts are in solutions containing amines a third method of stabilization is open to them. The amines, being more basic than ethanol, can accept their acidic proton. That this stabilizes the indolenine is seen by the fact that after proton donation the free phenylindolenine reacts about 12,000 times slower than its salt with morpholine and aziridine (see Table VI). The amine reactions with these salts are reversible. The retrograde reaction has been established to be acid catalyzed. By the principle of microscopic reversibility the forward reaction must then involve a base. This helps to explain the function of the extra molecules of amine in the rate steps of Scheme I. Schellenberg[6b] has suggested that an indolenine moiety might be stabilized in a protein by formation of a thioether from which it could then be reductively regenerated. A similar proposal may be made for stabilization by addition of amine from which product indolenine could be regenerated by general acid catalysis.

The nucleophilicity and/or basicity of the nucleophile appear to have little bearing on the reaction rate. Thus, amines varying in basicity over three pK_a units appear to react at about the same rate with this salt (see Table VI). The rate constant for hydride reduction by the Hantzch ester is 18 times slower with the phenyl-N-methylindolenine salt than nucleophilic attack by amines.

No explanation can be offered at present for the differences encountered in nmr spectra of the imidazole products compared to products from the reaction with the other amines. The structure of these products may be different.

If enzymes are, as has been suggested, capable of excluding solvent molecules from their reaction sites by burying them within their lyophobic regions the much greater rate constants obtained in acetonitrile as compared to ethanol for reduction by the Hantzch ester suggests that exclusion of water would increase the energy of the ground state of the reactants, thus allowing more facile conversion to products. The dehydrogenase enzyme may also regulate the hydride transfer to indolenine salts (if they are intermediates) by allowing solvation and/or proton removal by its own bases (for example, imidazole from histidine residues or the ε-amino group of lysyl residues).

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