Indolenines. Reduction by a Dihydropyridine and Addition to Thiols[1,2]

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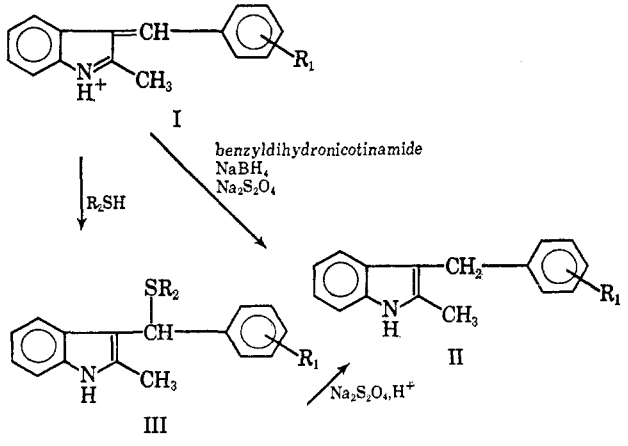
Abstract: Indolenines were studied as models for the postulated indolenine intermediate derived from tryptophanyl residues in dehydrogenase enzymes. An indolenine salt (*o*-chlorophenyl(2-methyl-3H-indolylidene)methane hydrochloride) was rapidly reduced by 1-benzyl-1,4-dihydronicotinamide. The products of the reaction were the corresponding indole and pyridinium salt. Similar reduction by 1-benzyl-1,4-dideuterionicotinamide produced the indole with one deuterium atom in the methylene group, indicating direct transfer of a hydride ion in the reaction. The effect of phenyl substituents on the rate of reduction was examined. Reduction of *p*-methoxy-, *o*-chloro-, and *p*-nitrophenyl(2-methyl-3H-indolylidene)methane hydrochloride by 1-benzyl-1,4-dihydronicotinamide in ethanol at 25° followed the rate law $v = k_2$(indolenine)(dihydropyridine), with k_2 = 7, 25, and 32 l. mole^{-1} sec^{-1}, respectively. The *o*-chloroindolenine salt readily added to mercaptobenzene, benzyl mercaptan, and methyl thioglycollate to give the corresponding thioether–indole, *o*-chlorophenyl(2-methyl-3-indolyl)alkylthio- (or arylthio-) methane. Acidic dithionite reductively cleaved such a thioether–indole adduct to the free indole and mercaptan.

Mechanistic studies of the enzymes yeast alcohol dehydrogenase[4,5] and rabbit muscle lactate dehydrogenase[6] had led to the finding of tritium-labeled transferred hydrogen in the methylene group of tryptophan, following interruption of the enzymatic reaction and hydrolysis of the protein. Such labeling could be reasonably explained by the hypothesis that a specific tryptophanyl residue participates in the enzymatic reaction by means of a reversible dehydrogenation to a cationic indolenine (3H-indolylidenemethane). We wish to report here studies of indolenine reactions that might serve as models for reactions of the postulated enzyme indolenine.

Several indolenine salts were synthesized and shown to undergo reduction by a dihydropyridine and to form adducts with thiols. The reactions are summarized in Scheme I.

Scheme I



Reduction of Indolenines by 1-Benzyl-1,4-dihydronicotinamide. The indolenine I (R_1 = *o*-Cl) and benzyldihydronicotinamide, each 0.05 *M* in ethanol, were mixed and allowed to react 5 min at 25°. After ether–aqueous acid extraction and recrystallization, the indole II and 1-benzylnicotinamide chloride were obtained in 88 and 86% yields, respectively. Both products were identified by infrared spectra and melting point. Similar reduction of the indolenines I (R_1 = *p*-methoxy- and *p*-nitro-) also gave the corresponding indoles II identified by infrared spectra. Reduction of the *o*-chloroindolenine I by 1-benzyl-1,4-dideuterionicotinamide has been shown to give the indole II in which one H of the methylene group was replaced by deuterium.[2] Rate studies (Table I) indicate a second-order reaction

Table I. Indolenine Reduction by 1-Benzyl-1,4-dihydronicotinamide[a]

I, *M*	R_1	Benzyldihydronicotinamide, *M*	k_2, l./mole sec
0.002	*o*-Cl	0.0002	26
0.0001	*o*-Cl	0.0002	23
0.001	*p*-CH_3O	0.001	7.1
0.0005	*p*-CH_3O	0.001	7.2
0.0001	*p*-CH_3O	0.0001	8.2
0.0002	*p*-NO_2	0.0001	32.9
0.0001	*p*-NO_2	0.0001	32.3

[a] Reactions were carried out in absolute ethanol in thermostated cuvettes at 25.0°. The disappearance of the dihydronicotinamide was measured by automatically recording the change in absorbance at 354 mμ with time. Measurements over two to four half-lives followed the rate law $v = k_2$(I)(dihydropyridine). The previously reported rate data for the *o*-chloroindolenine[2] is included for comparison.

with moderate enhancement of rate by electron-withdrawing substituents on the phenyl group of the indolenine. This substituent effect suggests a hydride transfer mechanism.

Addition of Indolenine to Thiols. The thioether–indoles III (R_1 = *o*-Cl; R_2 = Ph, benzyl, carbomethoxy-

(1) Supported by Research Grant GM-11799 from the National Institutes of Health. A preliminary account of part of this work has been published.[2]
(2) K. A. Schellenberg and G. W. McLean, *J. Am. Chem. Soc.*, **88**, 1077 (1966).
(3) John and Mary R. Markle Scholar in Academic Medicine.
(4) K. A. Schellenberg, *J. Biol. Chem.*, **240**, 1165 (1965).
(5) K. A. Schellenberg, *ibid.*, **241**, 2446 (1966).
(6) K. A. Schellenberg, *ibid.*, in press.

methyl) were formed rapidly and in good yield following addition of indolenine I to a solution of the appropriate mercaptan in ethanol (see Experimental Section). The position of the thio linkage is almost certainly that shown in Scheme I, rather than the previously reported addition to the 2 position of the indole ring.[2,7] The revised structure is based on the following. (1) The adduct was insoluble in aqueous HCl, whereas an alkylaniline would be expected to form a salt. (2) The nmr spectra of the adducts indicated a marked influence of the group attached to sulfur on the singlet CH chemical shift. Thus, for phenylthio, benzylthio, and carbomethoxymethylthio adducts, the δ was 6.06, 5.45, and 5.96. An ethylenic CH (as previously suggested) would probably be less influenced by such variations in structure of a thioether at the 2 position of the indole. Also, the indole 2-methyl chemical shift was relatively constant (δ was 2.20, 2.05, and 2.24 in the series), again consistent with the indole structure. (3) The ultraviolet spectra of the adducts were consistent with those of indoles; for the phenylthio adduct ultraviolet λ_{max}^{EtOH} 259 mμ (ϵ 10,400), 280 mμ shoulder (ϵ 9700), and 289 mμ (ϵ 8000). The spectrum was that of an indole superimposed on a phenyl thioether, and the spectrum of the benzylthio adduct was also that of a typical indole (see Experimental Section).

Reduction of Indolenine and Thioether–Indole by Dithionite. Gas chromatographic assay indicated that indolenine and thioether–indole were reduced to the indole. Reduction was optimal when water or aqueous HOAc was added to a mixture of sodium dithionite and reagent I or III in absolute ethanol. The results are summarized in Table II. The reduction of indolenine required the addition of water last, and the indolenine apparently decomposed in the presence of water, since addition of water to ethanolic indolenine I (R_1 = *o*-Cl) resulted in immediate precipitation of a material that was apparently a polymeric indole derivative as indicated by infrared and ultraviolet spectra and nonvolatility on gas chromatography.

The thioether–indole was reductively cleaved in good yield to indole and mercaptobenzene in similar experiments. Mercaptobenzene was identified by its odor. Unlike the indolenines, the thioether–indole was stable in the presence of water, and reduction occurred when dithionite was added last.

It is seen that borohydride, as may be expected, reduced only the cationic indolenine and not the thioether–indole (Table II).

Table II. Reductions by Dithionite[a]

Reagent	Changes in expt	Indole, % yield[b]
I	None	15
I	Omit water	0
I	Omit dithionite	0
I	Add water before dithionite	0
I	NH_4OH, 1 *M*, in place of water	9
I	HOAc, 1 *M*, in place of water	16
I	$NaBH_4$, 5 mg, in place of dithionite	75
III	None	61
III	Omit water	8
III	Omit dithionite	0
III	Add water before dithionite	20
III	NH_4OH, 1 *M*, in place of water	8
III	HOAc, 8 *M*, in place of water	73
III	HOAc, glacial, in place of water	53
III	Imidazole Cl, pH 6.5, 1 *M*, in place of water	17
III	$NaBH_4$, 5 mg, in place of dithionite	0

[a] Sodium dithionite, 5 mg, was suspended in 0.1 ml of an absolute ethanolic solution of indolenine I (R_1 = *o*-Cl), 0.02 *M*, or thioether–indole III (R_1 = *o*-Cl; R_2 = Ph), 0.02 *M*. Water, 0.1 ml, was then added, and 10 μl of the liquid phase was assayed for indole by vpc. Other additions or changes are noted in the table. [b] Yields were calculated from the area of the peak with retention time characteristic of the indole II (R_1 = *o*-Cl), and were calibrated with a known solution of the indole.

(7) D. C. DeLuca suggested the revised structure.

Discussion of Results

Some of these reactions are possibly analogous to certain steps in enzyme-catalyzed dehydrogenations. In particular, the rapid reduction of indolenine by the dihydropyridine, with direct transfer of hydrogen, might be analogous to enzymatic tritium transfer.[4–6] The correlation of rate with electron-withdrawing substituents on the phenyl group (Table I) is consistent with a hydride transfer,[8] and a crude calculation, based only on the two *para*-substituted indolenines, gives a Hammett ρ value of +0.6. The rapid rate of this reduction ($k_2 \sim 20$ l./mole sec) is also consistent with enzyme rates if certain assumptions are made. For example, dehydrogenases, such as alcohol dehydrogenase and lactate dehydrogenase, have maximum catalytic activities of about 400 μmoles/mg enzyme/min[9,10] and with enzyme molecular weights of about 150,000,[10,11] give a substrate turnover of about 10^3 sec^{-1}. Assuming the "active site" of the enzyme is equivalent to a 10 *M* solution of the reacting functional group,[12,13] then the indolenine reduction by dihydropyridine would give a turnover of 200 sec^{-1}. Such agreement in rates has no significance except that it does not exclude indolenine reduction as a step in the enzymatic reaction. Such a comparison omits cooperative effects of other groups at the active site,[14] differences in effective dielectric constant in the two systems, and the multistep nature of the over-all enzyme-catalyzed reaction.

The indolenine cation I (R_1 = *o*-Cl) was stabilized by 1,4 addition of thiols. Possibly an enzyme–indolenine is similarly stabilized by addition of a cysteine residue of the enzyme. If so, one could postulate the cleavage of such an adduct with concomitant activation of the indolenine, caused by a change in enzyme conformation on binding to coenzyme or substrate.[15]

The model system suffers several deficiencies, and may, therefore, be invalid. Most importantly, the reverse reaction, the oxidation of indole to indolenine, has not been shown. Also, a potential model substrate, ethanol, does not reduce the indolenines. Other as yet undefined groups in the enzyme must be postu-

(8) R. H. Abeles, R. F. Hutton, and F. H. Westheimer, *J. Am. Chem. Soc.*, **79**, 712 (1957).
(9) J. H. R. Kägi and B. Vallee, *J. Biol. Chem.*, **235**, 3188 (1960).
(10) H. J. Fromm, *ibid.*, **238**, 2938 (1963).
(11) J. E. Hayes, Jr., and S. F. Velick, *ibid.*, **207**, 225 (1954).
(12) F. H. Westheimer, *Advan. Enzymol.*, **24**, 441 (1962).
(13) M. L. Bender, F. J. Kezdy, and C. R. Gunter, *J. Am. Chem. Soc.*, **86**, 3714 (1964).
(14) M. L. Bender and F. J. Kezdy, *ibid.*, **86**, 3704 (1964).
(15) J. A. Yankeelov, Jr., and D. E. Koshland, Jr., *J. Biol. Chem.*, **240**, 1593 (1965).

lated to function in the activation of substrate and in the activation of indole to permit the reverse reaction.

Experimental Section

Melting points were usually taken with sealed evacuated capillaries and are corrected. Analyses were performed by Galbraith Laboratories, Inc. Ultraviolet spectra were recorded with Bausch and Lomb 505. Rates were measured from the ultraviolet absorption with the aid of a time–rate accessory connected to a Honeywell 10-mv recorder. Temperature regulation was maintained with the constant-temperature cell holder connected with a circulating thermostat. Infrared and nmr spectra were obtained with the Perkin-Elmer 21 and Varian A-60 instruments, respectively. Gas chromatography was performed with an F & M 700 instrument with thermal conductivity detector. Columns were $^1/_8$ in. × 6 ft Hi-Pak, 10% silicone rubber. Chromatographs were run at a temperature of 220°, with helium pressure of 30 psi. Under these conditions the indole I (R_1 = *o*-Cl) retention time was 8.5 min, and the thioether–indole III (R_1 = *o*-Cl; R_2 = Ph) retention time was 6.3 min. The indolenine I, indole II, and phenyl thioether adduct III (R_1 = *o*-Cl) have been described.[2] The ultraviolet spectrum of the latter is reported here in comparison with the benzyl thioether–indole (see text, Results).

***p*-Methoxyphenyl(2-methyl-3H-indolylidene)methane Hydrochloride.** Compound I (R_1 = *p*-CH_3O), was prepared according to Burr and Gortner,[16] and was twice recrystallized from methanol–ether. The yield of elongated golden crystals was 35%, mp 188–189° (lit.[16] 181°); infrared ν_{max}^{KBr} 3450, 2630, 2410, 1580, 1555, 1480, and 1435 cm^{-1}. *Anal.* Calcd for $C_{17}H_{16}ClNO$: C, 71.44; H, 5.64. Found: C, 71.43; H, 5.80.

***p*-Methoxyphenyl(2-methyl-3-indolyl)methane.** Indolenine I (R_1 = *p*-CH_3O), 0.5 g, was added to a solution of 0.17 g of $NaBH_4$ in 50 ml of dimethylformamide. After ether extraction, washing, and recrystallization from ethyl acetate–heptane, fine colorless crystals were obtained, mp 119–120°; infrared ν_{max}^{KBr} 3330 (sharp), 1622 (weak), 1605, 1580, 1485, and 1460 cm^{-1}. *Anal.* Calcd for $C_{17}H_{17}NO$: C, 81.24; H, 6.82; N, 5.57. Found: C, 81.22; H, 6.94; N, 5.56. Gas chromatography showed a single peak with retention time of 14.4 min.

***p*-Nitrophenyl(2-methyl-3H-indolylidene)methane Hydrochloride.** This was similarly prepared from *p*-nitrobenzaldehyde and 2-methylindole.[16] Yellow-brown crystals separated from the reaction mixture and were washed with ethyl acetate. The yield was 66–69%, mp 205–210° dec. The material apparently decomposed during attempts at recrystallization from ethyl acetate. Spectra were: infrared ν_{max}^{KBr} 3400, 2420, 1625, 1600, 1570, and 1515 cm^{-1}; ultraviolet λ_{max}^{EtOH} 278 mμ (ε 17,200), 290 mμ shoulder (ε 13,600), and 340 mμ shoulder (ε 1700). *Anal.* Calcd for $C_{16}H_{13}ClN_2O_2$: C, 63.90; H, 4.36; Cl, 11.79; N, 9.32. Found: C, 63.75; H, 4.53; Cl, 11.68; N, 9.11.

***p*-Nitrophenyl(2-methyl-3-indolyl)methane.** Indolenine I (R_1 = *p*-NO_2), 0.12 g, was added to a solution of 0.1 g $NaBH_4$ in 5 ml of dimethylformamide. After ether extraction and alumina chromatography, yellow crystals were obtained, mp 117–118°; infrared ν_{max}^{KBr} 3420 (sharp), 1625 (weak), 1606, 1520, and 1470 cm^{-1}; ultraviolet λ_{max}^{EtOH} 279 mμ (ε 16,600), 290 mμ shoulder (ε 13,200), and 340 mμ shoulder (ε 1200). *Anal.* Calcd for $C_{16}H_{14}N_2O_2$: C, 72.16; H, 5.30; N, 10.52. Found: C, 72.00; H, 5.52; N, 10.34.

***o*-Chlorophenyl(2-methyl-3-indolyl)benzylthiomethane.** Indolenine I (R_1 = *o*-Cl), 0.155 g, was added to benzyl mercaptan, 0.21 g, in 3 ml of absolute ethanol, and allowed to stand 5 min at 25°. After ether extraction, washing, and crystallization from ethyl acetate–heptane, colorless crystals were obtained in 88% yield, mp 112–115°; infrared ν_{max}^{KBr} 3420, 1625, 1605, 1585, 1560, 1500, and 1460 cm^{-1}; ultraviolet λ_{max}^{EtOH} 274 mμ (ε 9140), 279 mμ (ε 9250), and 289 mμ (ε 7750); nmr ($CDCl_3$) δ 2.05 (singlet, 3 H), 3.48 (singlet, 2 H), 5.45 (singlet, 1 H), 7–8 (multiplet, 14 H). *Anal.* Calcd for $C_{23}H_{20}ClNS$: C, 73.09; H, 5.33. Found: C, 73.07; H, 5.45.

***o*-Chlorophenyl(2-methyl-3-indolyl)carbomethoxymethylthiomethane.** This was prepared similarly to the preceding compound, with substitution of methyl thioglycollate for benzyl mercaptan. After extraction and vain attempts at crystallization, a pale tan oil was obtained in 90% yield: infrared ν_{max}^{neat} 3390, 1735, 1625, 1560, 1460, and 1430 cm^{-1}; nmr ($CDCl_3$) δ 2.24 (singlet, 3 H), 3.00 (singlet, 2 H), 3.50 (singlet, 3 H), 5.96 (singlet, 1 H), 7–8 (multiplet, 9 H). *Anal.* Calcd for $C_{19}H_{18}ClNO_2S$: C, 63.41; H, 5.04. Found: C, 63.15; H, 4.91.

Attempted Condensations with Trifluoroacetaldehyde and Pivalaldehyde. Condensation of trifluoroacetaldehyde ethyl hemiacetal with 2-methylindole in HCl–ethanol or HCl–ethanol–dimethylformamide resulted in a red tar with infrared spectrum suggestive of an indole. Similar attempts with pivalaldehyde gave a mixture of products including a violet solid that did not melt at 350°, and with analysis and molecular weight suggesting a trimer containing three residues each of indole and aldehyde.

Acknowledgment. We wish to thank Mrs. Evelyn Connor for technical assistance, and Dr. D. P. Hollis for assistance with the nmr spectra.

(16) G. O. Burr and R. A. Gortner, *J. Am. Chem. Soc.*, **46**, 1224 (1924).

Communications to the Editor

Copper-Catalyzed Decomposition of Benzenesulfonyl Azide in Hydroxylic Media

Sir:

Copper is well known to be an effective catalyst for the decomposition of diazoalkanes and diazocarbonyl compounds. Its role in these reactions has been described[1] as stabilization of a carbene intermediate in a copper–carbene complex. We have now found that copper is an extremely effective catalyst for the decomposition of benzenesulfonyl azide (**1**). In refluxing methanol containing 1 g of freshly reduced copper powder per 0.1 mole of azide, **1** decomposes smoothly while evolving a stoichiometric quantity of nitrogen. Benzenesulfonamide (**2**) was isolated in *ca.* 80% yield, together with methylenebis(benzenesulfonamide) (**3**) and 1,3,5-tris(benzenesulfonyl)hexahydro-*s*-triazine (**4**), the minor products apparently resulting from condensation of benzenesulfonamide with formaldehyde formed in major amounts through dehydrogenation of methanol.

In 2-propanol, **2** was again obtained in nearly quantitative yield, and acetone was detected to the extent of approximately 15% of the nitrogen evolved.[2] Azide decomposition did not occur with copper in dry *t*-butyl alcohol, but on addition of a trace of water to this medium reaction took place at nearly the same rate as in pure methanol, producing complete conversion to the sulfonamide **2**. In this case acetone and smaller

(1) J. Hine, "Divalent Carbon," Ronald Press, New York, N. Y., 1964, pp 118, 125. One may regard the Simmons–Smith reagent as a copper–carbene complex in the sense intended; see E. P. Blanchard and H. E. Simmons, *J. Am. Chem. Soc.*, **86**, 1337 (1964), and earlier references cited therein.

(2) The total amount of dehydrogenation product dissolved in a very large amount of the precursor alcohol was estimated by a vpc procedure. It must be assumed that the quantitative result is subject to some gross inaccuracies and therefore is only tentative.