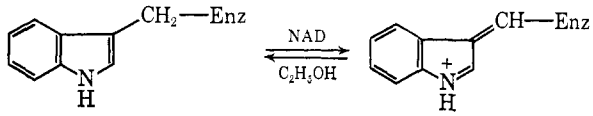
Oxidation–Reduction of 2-Substituted 3-Benzylindoles and 3-Benzylidene-3H-indoles. Model Reactions for Alcohol Dehydrogenase

Sir:

Schellenberg[1] has recently proposed a mechanism of action of yeast alcohol dehydrogenase in which the 3-alkylidene-3H-indole (**2**) of a tryptophan residue of the enzyme **1** participates in the oxidation of ethanol. He also suggested a possible stabilization of the postulated indolenine **2** by reaction with a nearby cysteine residue.

Scheme I

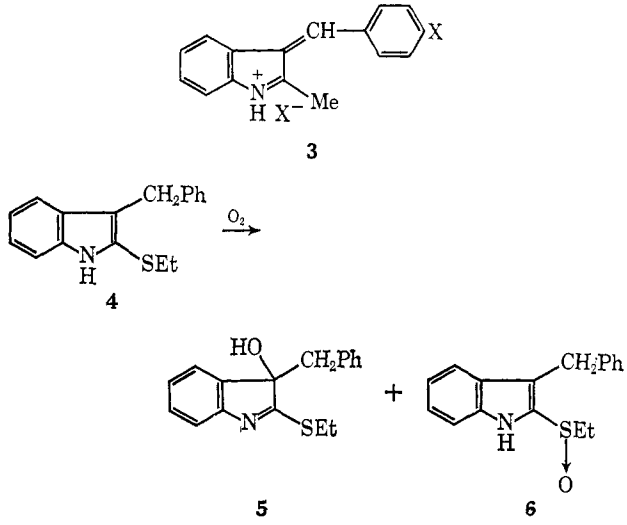


1 **2**

Schellenberg,[2] Bruice,[3] and coworkers demonstrated model reactions for reductions of **2** with dihydronicotinamide derivatives and found additions of thiols or amines to 3-benzylidene-2-methylindoles (**3**), chosen as model compounds of **2**. However, the two important model reactions for the hypothesis, (i) oxidation of **1** to **2** and (ii) oxidation of ethanol with **2**, have remained unsuccessful.

We now wish to report these model reactions as an extention of autoxidation of indole derivatives[4] having a substituent at position 2. 3-Benzyl-2-ethylthioindole (**4**), mp 48–49°, prepared by the action of ethanesulfenyl chloride on 3-benzylindole, was autoxidized to a 1:1 mixture of **5** and **6** when a chloroform solution of **4** was stirred at room temperature, providing results corresponding to those for 3-methyl derivatives.[4] Structures of **5** and **6** were proved by their spectral

Scheme II

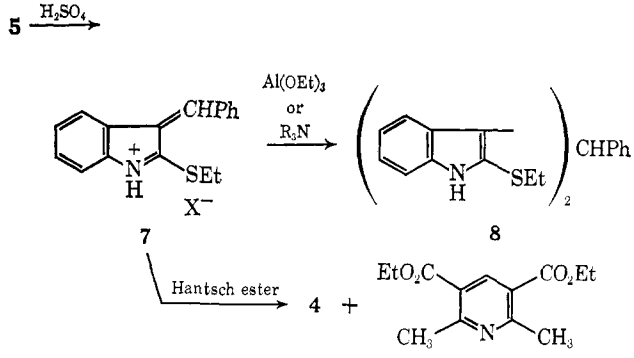


data as well as elemental analyses: 3-hydroxy derivative **5**: mp 174–175°; λ_{max}^{EtOH} 308, 296, 286, 229 mμ (ε 7800, 7600, 6900, 16,400); ν_{max}^{KBr} 3300 cm^{-1} (OH); $\delta_{TMS}^{CDCl_3}$ 3.09 (q, 2 H, benzylic methylene); mass spectrum, *m/e* 283 (M$^+$); S-oxide **6**: mp 163–164°; λ_{max}^{EtOH} 294, 228 mμ (ε 15,300, 29,900); ν_{max}^{KBr} 3100 (NH), 1005 cm^{-1} (S–O); $\delta_{TMS}^{CDCl_3}$ 4.25 (q, 2 H, benzylic methylene), 10.57 (broad s, 1 H, NH); mass spectrum, *m/e* 283 (M$^+$).

The dehydration of **5** to **7** was accomplished with concentrated sulfuric acid at room temperature, but was unsuccessful with other acids such as trifluoroacetic acid and fluoroboric acid.[5] Salts **7** failed to crystallize; however, the assignment of structure was based on spectral data ($\lambda_{max}^{CH_3CN}$ 400, 275, 245 mμ; $\delta_{TMS}^{F_3CCOOH}$ 1.64 (t, 3 H, CH_2CH_3) 3.55 (q, 2 H, CH_2CH_3), 8.45 (s, 1 H, benzylidene CH)) which were identical with that of a specimen prepared by the condensation of 2-ethylthioindole[4] and an excess of benzaldehyde in the presence of sulfuric[6] or fluoroboric acids. Furthermore the reduction of **7** with sodium borohydride gave **4** in 90% yield.

When **7** was treated with an equimolar amount of the Hantzsch ester in acetonitrile at room temperature, an excellent yield of **4** and pyridine derivative was obtained in accordance with previous findings.[3] That 2-ethylthio not only serves as a protective group of the 2 position of the indole toward oxidation but also facilitates the oxidation at the 3 position of the indole led to the speculation that the participation of a thiol group in the enzyme not only stabilizes **2** but also facilitates the oxidation of a tryptophan residue to the indolenine.[7]

Scheme III



The oxidation of ethanol to acetaldehyde with **7** met some difficulties, as 1,4 addition of ethanol[8] to **7** occurred as reported on **3**.[9] Treatment of **7** with aluminum ethoxide gave the biindolylmethane **8**: mp 158–159°; λ_{max}^{EtOH} 302, 293, 285 mμ; mass spectrum, *m/e* 442 (M$^+$). The same compound was obtained from **7** when treated with triethylamine in acetonitrile. This indicates the free base of **7** is unstable, and forms **8** before hydrogen transfer occurs.

However, the 2-methyl derivative **3** (R = H, sulfate) was reduced by aluminum ethoxide and isopropoxide. 3-Benzyl-2-methylindole (**9a**), mp 118–120°, was

(1) K. A. Schellenberg, *J. Biol. Chem.*, **242**, 1815 (1967); **241**, 2446 (1966); **240**, 1165 (1965).

(2) K. A. Schellenberg, G. W. McLean, H. Lipton, and P. S. Lietman, *J. Am. Chem. Soc.*, **89**, 1948 (1967); K. A. Schellenberg and G. W. McLean, *ibid.*, **88**, 1077 (1966).

(3) R. W. Hoffman and T. C. Bruice, *ibid.*, **89**, 6243 (1967).

(4) T. Hino, M. Nakagawa, and S. Akaboshi, *Chem. Commun.*, 656 (1967); T. Hino, M. Nakagawa, T. Wakatsuki, K. Ogawa, and S. Yamada, *Tetrahedron*, **23**, 1441 (1967).

(5) Attempted direct oxidation of 4 to 7 with lead dioxide or manganese dioxide under a stream of nitrogen failed, but did give **5**. Further investigation along this line is now under progress.

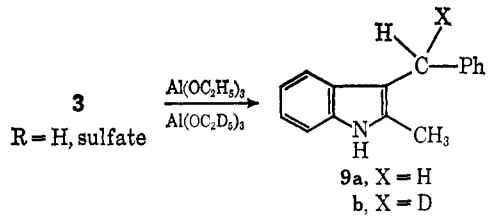
(6) G. O. Burr and R. A. Gortner, *J. Am. Chem. Soc.*, 46, 1224 (1924).

(7) An amino group in the enzyme also might be responsible for the acceleration of oxidation of the tryptophan residue, since 2-amino-3-methylindoles were more sensitive toward autoxidation than 2-ethylthioindole derivatives. On the other hand, it is well known that 2,3-dialkylindoles give the corresponding 3-hydroperoxide and their rearranged products by air oxidation. See ref 4.

(8) The ultraviolet spectrum of 7 in acetonitrile gives an indolic absorption by the addition of a small amount of ethanol, but the ethoxy compound has not yet been isolated.

(9) J. D. Albright and H. R. Snyder, *J. Am. Chem. Soc.*, **81**, 2239 (1959).

Scheme IV



obtained in 10–15% yield[10] when the free base of **3**, prepared in methylene chloride with triethylamine under a stream of nitrogen, was heated with freshly prepared aluminum alkoxide in methylene chloride for 30 min. The solvent was then replaced by toluene and refluxed for several hours. The formation of acetaldehyde in the case of aluminum ethoxide was proved using the 2,4-dinitrophenylhydrazone. Further evidence for the hydrogen transfer from the ethanol was demonstrated by the reduction of **3** with deuterated aluminum ethoxide[11] under similar reaction condition. 3-Benzyl-2-methylindole (**9b**) obtained by using deuterated aluminum ethoxide showed the presence of a deuterium at the benzylic position by nmr ($\delta_{TMS}^{CDCl_3}$ 2.30 (s, 3 H), 4.00 (s, 1 H)) and mass spectra (*m/e* 222 (M^+)). The deuterated acetaldehyde was obtained as its 2,4-dinitrophenylhydrazone, mp 150–152°, *m/e* 228 (M^+), 227, 226, 225. Although the yield of the reaction was low, the results support the dehydrogenase mechanism proposed by Schellenberg. It is interesting to note that the conjugated double bond has been reduced by aluminum alkoxides.

(10) Crystalline by-products were obtained from the reaction mixture; none of them was found to be a biindolylmethane derivative. The details will be reported in a full paper.

(11) The reagent was prepared from ethanol-d_6 and aluminum thin foil following the procedure in A. H. Blatt, "Organic Syntheses," Coll. Vol. 2, John Wiley & Sons, Inc., New York, N. Y., 1943, p 598.

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Miserotoxin, a New Naturally Occurring Nitro Compound

Sir:

Livestock poisoning due to the ingestion of various *Astragalus* (Leguminosae) species (locoweeds, poison vetches) has been known for many years. These plants can be divided[1] into three groups based upon fundamentally different properties: (1) selenium toxicity caused by selenium accumulation, (2) the chronic, true "loco" symptoms particularly observed with horses, and (3) an acute poisoning caused by several botanically related species and typified by that due to timber milkvetch (*Astragalus miser*).

We report here the isolation and characterization of miserotoxin, the first isolated poison from the third group of plants.

Above-ground parts of *A. miser* Dougl. var. *oblongifolius* (Rydb.) Cronq., collected near Logan, Utah, were dried and ground, and the powder was extracted with hot ethanol. The ethanol solution was cooled and decanted from a solid residue. The ethanol was evaporated to dryness and the residue was dissolved in water and washed several times with chloroform and then with *n*-butyl alcohol. The resulting aqueous solution was purified by automatic countercurrent distribution between water and *n*-butyl alcohol. All fractions were tested by chick bioassay[2] and the only poisonous material was found to be concentrated in several consecutive fractions. Only the poisonous fractions gave a positive test[2] for a nitro group and gave identical nmr spectra. These fractions were combined to give a homogeneous oil (dubbed miserotoxin), $[\alpha]^{25}D$ −22° (*c* 2.0, H_2O), corresponding to about 3% by weight of the dried plant material. All attempts at crystallization of miserotoxin failed.

The 60-MHz nmr spectrum (in D_2O with added DCl to displace the HDO peak) showed a quintet (2 H, J = 6 cps, $-CH_2CH_2CH_2NO_2$) centered at 2.34 ppm (from external TMS), a triplet (2 H, J = 6 cps, $-CH_2NO_2$) at 4.68 ppm, and a doublet (1 H, J = 7.5 cps, anomeric α proton of a pyranose sugar) at 4.51 ppm. Double irradiation[3] at 100 MHz of the quintet reduced the triplet to a singlet, and irradiation of the triplet reduced the quintet to a triplet. The only other absorptions were in the 3.0–4.2-ppm range, and the complex multiplet was not successfully analyzed even at 100 MHz. The mass spectrum of miserotoxin did not show an identifiable molecular ion, but mainly showed fragments explicable as arising from a glucopyranose derivative. However, a high-resolution mass spectrum[4] identified a large *m/e* 88 peak as $C_3H_6NO_2$, which, along with the above nmr analysis, the chemical tests, and an infrared spectrum showing typical NO_2 group absorptions at 1380 and 1520 cm^{-1}, indicated the presence of a $-CH_2CH_2CH_2NO_2$ group in miserotoxin. Miserotoxin thus appeared to be a β-D-glucoside of 3-nitro-1-propanol. However, treatment of miserotoxin with acetic anhydride and sodium acetate produced in high yield a hexaacetyl derivative (mol wt 519, $C_{21}H_{29}NO_{14}$, by mass spectrum[4]) rather than the expected tetraacetate. Studies on model primary nitro compounds showed, however, that oxidative diacetylation of such compounds is in general a high yield reaction,[5] and hence all data now indeed point to I as the structure of miserotoxin. The correctness of this conclusion was proven by hydrolysis of miserotoxin to D-glucose (identified by nmr, tlc, and an osazone derivative) and 3-nitro-1-propanol (identified by comparison with an authentic sample synthesized from 3-bromo-1-propanol). Oral treatment of a 140-kg calf with 8 g of

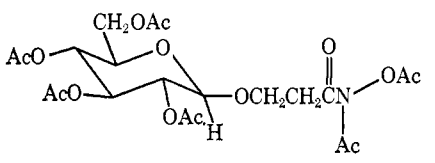
(1) J. M. Kingsbury, "Poisonous Plants of the United States and Canada," Prentice-Hall, Inc., Englewood, Cliffs, N. J., 1964, p 306.

(2) M. C. Williams and F. A. Norris, *Weed Sci.* **17**, 236 (1969).

(3) We are indebted to Dr. D. A. Nelson of the University of Wyoming for performing this experiment.

(4) High-resolution spectra were obtained from the Purdue Mass Spectrometry Center which is supported under U. S. Public Health Service Grant FR-00354.

(5) Thus, 1-nitropropane is converted readily to $CH_3CH_2CON(OAc)(Ac)$ and miserotoxin to a. This reaction will be discussed more fully elsewhere.



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