

69.13; H, 5.80; N, 14.23; N-acetyl, 14.57. Found: C, 69.48; H, 6.10; N, 14.23; N-acetyl, 14.37). Intramolecular cyclization of VI and subsequent decarboxylation, as well as further transformations of VII and related substances, will be reported shortly.

DEPARTMENT OF PHARMACOLOGY
HARVARD MEDICAL SCHOOL
BOSTON 15, MASS.

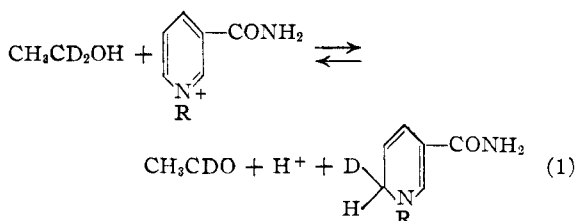
FREDERICK C. UHLE

RECEIVED FEBRUARY 15, 1951

THE ENZYMIC TRANSFER OF HYDROGEN FROM ALCOHOL TO DPN

Sir:

The oxidation of ethyl alcohol to acetaldehyde by DPN (diphosphopyridine nucleotide) in the presence of alcohol dehydrogenase has been investigated with deuterium as a tracer. It has been found that a hydrogen atom is transferred directly from the alpha carbon atom of the alcohol to the DPN molecule (see Equation 1) and therefore that the hydrogen atoms of the solvent (water) do not enter the reduction product.



where R represents the ribose-pyrophosphate-ribose-adenine groups of DPN. (Equation 1 is illustrated above with Karrer's structure¹ for reduced DPN).

$\text{CH}_3\text{CD}_2\text{OH}$ was prepared by the reduction of phenyl acetate with lithium aluminum deuteride; the procedure was analogous to that used for making $\text{CH}_3\text{CDOHCH}_3$.² (*Anal.*³ Deuterium atoms/molecule: calcd. 2.0, found 2.1). The deuterioalcohol (0.2 cc.) was equilibrated for 30 minutes with 4 mg. of crystalline alcohol dehydrogenase⁴ and 0.05 g. of DPN of 93% purity⁵ in 5 cc. of 0.5 M aqueous $(\text{HOCH}_2)_3\text{CNH}_2$ buffer⁶ at pH 9.0. The enzyme was heat inactivated and removed by precipitation with 10 cc. of alcohol. Then 45 cc. of alcohol and 0.4 cc. of 6 N HCl were added to precipitate the reduced DPN, which was redissolved in 1 cc. of the buffer at pH 7.4, and reprecipitated with 15 cc. of alcohol. The purpose of the reprecipitation was to remove any exchangeable deuterium. *Anal.* Found: C, 38.45; H, 6.36; deuterium atoms per molecule, 1.1. Calculation of the last figure was based on the hydrogen analysis of the isolated amine salt, which contained approximately 65 atoms of hydrogen present per molecule of enzymatically active pyridine nucleotide.

(1) P. Karrer, B. H. Ringier, J. Büchi, H. Fritzsche and U. Solmssen, *Helv. Chim. Acta*, **20**, 55 (1937); P. Karrer and O. Warburg, *Biochem. Z.*, **285**, 297 (1935).

(2) A. Leo, unpublished results.

(3) R. B. Alfin-Slater, S. M. Rock and M. Swislocki, *Anal. Chem.*, **22**, 421 (1950).

(4) E. Racker, *J. Biol. Chem.*, **184**, 313 (1950).

(5) A. Kornberg and B. L. Horecker, private communication.

(6) G. Gomori, *Proc. Soc. Exptl. Biol. Med.*, **62**, 33 (1946).

Identical results were obtained when the procedure above was repeated with twice the concentration of enzyme and twice the equilibration period. The results were also confirmed by the following control experiments. The procedure was repeated with unlabeled ethyl alcohol in a medium of D_2O , and the reduced DPN was found to contain no excess deuterium. Reduced DPN was also prepared by chemical reduction⁷ with $\text{Na}_2\text{S}_2\text{O}_4$ in D_2O , and precipitation with ethyl alcohol. The product was dissolved in H_2O and reprecipitated. *Anal.* Deuterium atoms per molecule; found, 1.0. Repetition of the solution and precipitation procedure did not change the deuterium content of the reduced DPN; the deuterium atom in the molecule therefore does not exchange with hydrogen atoms of the solvent at neutral pH.

The rate of the enzymatic reduction of DPN by deuterioalcohol, the stereochemistry of the reduction, and other aspects of this problem are currently under investigation.

We wish to thank Dr. P. Ofner, who carried out the purification of the DPN and Dr. H. S. Anker, who assisted with some of the mass spectrometer analyses.

(7) P. Ohlmeyer, *Biochem. Z.*, **297**, 66 (1938).

DEPARTMENT OF CHEMISTRY
DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF CHICAGO
CHICAGO, ILLINOIS

F. H. WESTHEIMER
HARVEY F. FISHER
ERIC E. CONN
BIRGIT VENNESLAND

RECEIVED MARCH 19, 1951

THE TOTAL SYNTHESIS OF A STEROID¹

Sir:

We wish to record the total synthesis of methyl *dl*-3-keto- $\Delta^{4,9(11),16}$ -etiocholatrienate (IV). This represents the first synthesis of a compound possessing the full hydroaromatic steroid nucleus of the correct stereochemical configuration.

Condensation of 5-methoxytoluquinone with butadiene in benzene gave *cis*-1,4-diketo-2-methoxy-4a-methyl-1,4,4a,5,8,8a-hexahydronaphthalene (m.p. 94.5–95.5°).² The latter was transformed, by acidification of its solution in basic aqueous dioxane under carefully controlled conditions, to the *trans*-isomer (m.p. 130–131°, found, C, 69.38; H, 6.85). Reduction with lithium aluminum hydride gave the corresponding glycol (m.p. 139–140°, found, C, 68.78; H, 8.77), transformed by dilute mineral acid in aqueous dioxane to 1-hydroxy-2-keto-4a-methyl-1,2,4a,5,8,8a-hexahydronaphthalene (m.p. 71.5–72.5°). Acetylation, followed by treatment with zinc in boiling acetic anhydride or xylene, gave the *trans*-bicyclic ketone (I) (b.p. 75° at 0.2 mm., m.p. 34–35°, max. 224 μ (log $E = 4.01$), found, C, 81.02; H, 8.98). This was converted to the hydroxymethylene ketone (b.p. 88–90° at 0.015 mm., max. 229 μ (4.00) and 361 μ (3.88)), which with ethyl vinyl ketone in the presence of potassium *t*-butoxide in *t*-butanol yielded 1-formyl-1- γ -ketopentyl-2-keto-4a-methyl-1,2,4a,5,8,8a-hexahydronaphthalene (m.p. 97.5–98.5°, found, C, 74.43; H, 8.15).

(1) First announced at the Centenary Lecture of the Chemical Society presented at Burlington House, London, on April 26, 1951.

(2) Orchin and Butz, *J. Org. Chem.*, **8**, 509 (1943).