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.

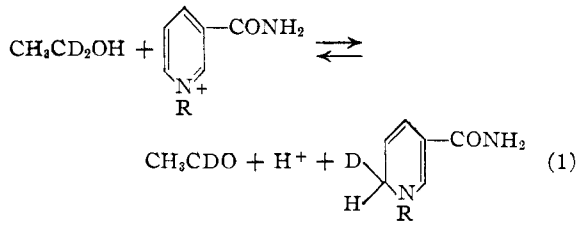
DEPARTMENT OF PHARMACOLOGY
HARVARD MEDICAL SCHOOL FREDERICK C. UHLE
BOSTON 15, MASS.

RECEIVED FEBRUARY 15, 1951

THE ENZYMATIC 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.



(1)

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

CH_3CD_2OH was prepared by the reduction of phenyl acetate with lithium aluminum deuteride; the procedure was analogous to that used for making $CH_3CDOHCH_3$.[2] (*Anal.*[3] Deuterium atoms/molecule: calcd. 2.0, found 2.1). The deuteroalcohol (0.2 cc.) was equilibrated for 30 minutes with 4 mg. of crystalline alcohol dehydrogenase[4] and 0.05 g. of DPN of 93% purity[5] in 5 cc. of 0.5 *M* aqueous $(HOCH_2)_3CNH_2$ buffer[6] at *p*H 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 *p*H 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 reduction[7] with $Na_2S_2O_4$ 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 *p*H.

The rate of the enzymatic reduction of DPN by deuteroalcohol, 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 F. H. WESTHEIMER
DEPARTMENT OF BIOCHEMISTRY HARVEY F. FISHER
UNIVERSITY OF CHICAGO ERIC E. CONN
CHICAGO, ILLINOIS BIRGIT VENNESLAND

RECEIVED MARCH 19, 1951

THE TOTAL SYNTHESIS OF A STEROID[1]

Sir:

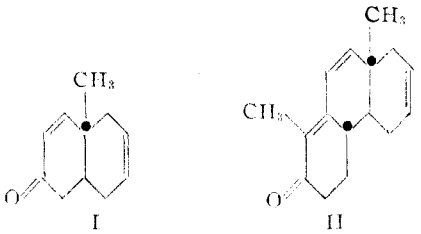
We wish to record the total synthesis of methyl *dl*-3-keto-$\Delta^{4,9(11),16}$-etiocholatrienate (IV). This represents the first synthesis of a compound possessing the full hydroaromatic steroid nucleus of the correct stereochemical configuration.

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 mμ (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 mμ (4.00) and 361 mμ (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).

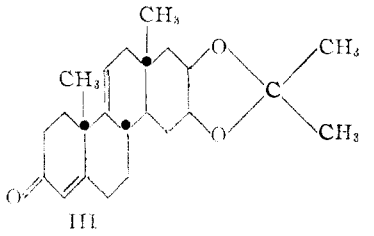
(1) First announced at the Centenary Lecture of the Chemical Society presented at Burlington House, London, on April 26, 1951.

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

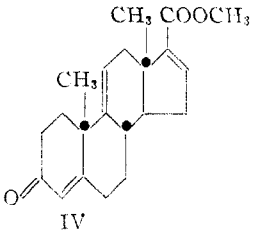
Cyclization[3] in aqueous alkaline dioxane afforded the tricyclic ketone (II) (m.p. 71–72°, max. 289 mμ (4.42), found, C, 83.48; H, 8.82). II with osmium tetroxide in ether gave 1,8a-dimethyl-2-keto-6,7-dihydroxy-$\Delta^{9,10a}$-decahydrophenanthrene as a mixture of stereoisomers (m.p. 156.5–157.5°, found, C, 72.87; H, 8.61, and m.p. 181–182°, found, C, 73.22; H, 8.44). The lower melting form predominated and was converted to the acetonide (m.p. 98–99°, found, C, 75.32; H, 8.73). Partial hydrogenation in dry benzene with a Pd–$SrCO_3$ catalyst led to the corresponding $\alpha\beta$-unsaturated ketone (m.p. 157.5–158.5°, max. 250 mμ (4.18), found, C, 74.85; H, 9.37).



I II



III



IV

The 3-position of the latter was blocked by conversion to the hydroxymethylene compound (m.p. 127–129°, max. 246 mμ (4.08) and 361 mμ (4.00), found C, 72.23; H, 8.58), which with methylaniline in methanol gave the methylanilinomethylene derivative[4] (m.p. 220–224°, found, C, 76.59; H, 8.30; N, 3.51). The protected ketone was condensed with acrylonitrile in the presence of aqueous Triton Bi n *t*-butanol–benzene, and the product on basic hydrolysis yielded 1-β-carboxyethyl-1,8a-dimethyl-2-keto-6,7-dihydroxy-Δ^{10}-dodecahydrophenanthrene acetonide as a mixture of two isomers (α, m.p. 148–150° (labile form) and m.p. 171–173° (stable form), found C, 69.78; H, 8.55; impure β, oil). The β-isomer with hot acetic anhydride and a trace of sodium acetate gave the β-enol lactone[5] (m.p. *ca.* 240°), which on treatment with methylmagnesium bromide, followed by base cyclization,[6] gave *dl*-3-keto-16,17-dihydroxy-$\Delta^{4,9(11)}$-D-homoandrostadiene acetonide (III) (m.p. 199–202°, max. 239 mμ (4.15), found, C, 77.31; H, 9.27) in good yield (comparable to the yield of cholestenone obtained by Fujimoto[6]). In contradistinction, the α-enol lactone (m.p. 177–178°, found, C, 73.53; H, 8.40), treated analogously, gave a poor yield of the C 10 epimer of III (m.p. 168–169°, max. 237 mμ (4.15), found, C, 77.11; H, 9.18). The action of periodic acid on (III) furnished a dialdehyde (m.p. 129–132°), which on cyclization with hot aqueous dioxane gave mainly *dl*-$\Delta^{9,(11),16}$-bisdehydro-20-norprogesterone (m.p. 171–173°). Dichromate oxidation, followed by esterification with diazomethane, led to the *dl*-keto-ester (IV) (m.p. 159–162°).[7]

Dehydration of methyl 3-keto-11β,17β-dihydroxy-Δ^4-etiocholenate[8] (prepared from Kendall's Compound F) with phosphorus oxychloride–pyridine gave the *d*-keto-ester (IV) (m.p. 187–191°).[7] The infrared spectra of the synthetic and natural esters were identical in every respect. The respective powder X-ray diffraction patterns were also identical.[9]

The infrared spectra of all the compounds described were in accord with the assigned structures. The resolution of synthetic IV, and its conversion to naturally occurring steroids, are now under investigation.

We would like to thank the Research Corporation, Merck and Co., and the Monsanto Chemical Co. for support, Dr. Ajay K. Bose for valuable improvements of certain steps, and Mrs. Dorothy Voitle and Mr. Irving Osvar for technical assistance.

CONVERSE MEMORIAL LABORATORY
HARVARD UNIVERSITY
CAMBRIDGE 38, MASSACHUSETTS

R. B. WOODWARD
FRANZ SONDHEIMER
DAVID TAUB
KARL HEUSLER
W. M. MCLAMORE

RECEIVED APRIL 27, 1951

(3) *Cf.* Shunk and Wilds, THIS JOURNAL, **71**, 3946 (1949).
(4) *Cf.* Birch and Robinson, *J. Chem. Soc.*, 501 (1944).
(5) *Cf.* Turner, THIS JOURNAL, **72**, 579 (1950).
(6) *Cf.* Fujimoto, *ibid.*, **73**, 1856 (1951).
(7) These m.ps. were taken on a Kofler micro hot-stage. All others were taken in a capillary.
(8) von Euw and Reichstein, *Helv. Chim. Acta*, **25**, 1019 (1942).
(9) We wish to thank Dr. C. Frondel of the Department of Mineralogy, Harvard University, for these measurements and their interpretation.