each of the steps leading to the formation of aminobutyric acid from acetaldehyde and animal livers appears to contain enzymes which can catalyze these same reactions. The enzyme reported by Vilenkina[2] should catalyze reactions (1) and Lien and Greenberg[5] have reported conversion of threonine to aminobutyric acid, apparently by reactions (2) and (3) in rat livers. Though this synthetic pathway may not be used by animals it may be of importance in some organisms.

(5) O. G. Lien, Jr., and D. M. Greenberg, *J. Biol. Chem.*, **200**, 367 (1953).

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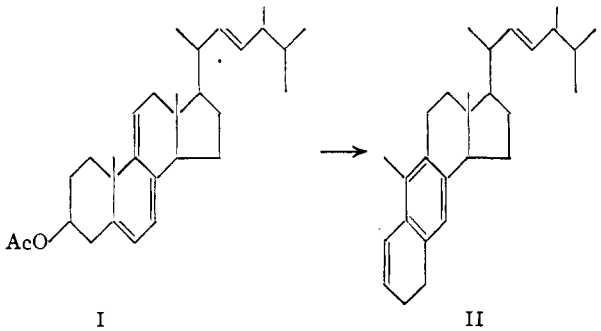
RECEIVED MAY 8, 1953

THE REARRANGEMENT OF DEHYDROERGOSTERYL ACETATE TO A *s*-OCTAHYDROANTHRACENE DERIVATIVE

Sir:

On treatment of a chloroform solution of dehydroergosteryl acetate (I) with catalytic amounts of hydrogen chloride at room temperature a skeletal rearrangement of the steroid takes place. The pure product (II) obtained in a yield of about 30% lacks an oxygen function and shows an ultraviolet absorption spectrum characteristic of an aromatic ring with one conjugated double bond, $\lambda_{max.}$ (isooctane) 222, 227, 266, 296, and 308 mμ. (ε 26,100, 27,100, 18,600, 2,760, 2,220, respectively); $\lambda_{max.}$ (CS_2) 968 cm.$^{-1}$; m.p. 105–107°; $[\alpha]^{20}D$ −70° ($CHCl_3$); *Anal.* Calcd. for $C_{28}H_{40}$: C, 89.29; H, 10.70. Found: C, 88.96; H, 10.74. It is proposed that, by the rupture of the C_1–C_{10} bond and reattachment of C_1 to C_6, 1,2,3,4,7,8-hexahydro-3′-(5,6-dimethyl-3-heptenyl-2)-2,10-dimethyl-1,2-cyclopentanthracene (II) is formed. (Positions 7,8 and 3,4 for the conjugated double bond have not been ruled out experimentally.) Kinetic measurements by ultraviolet spectrophotometry show that this rearrangement is first order in steroid and approximately second order (1.85) in hydrogen chloride. The reaction rate constant is equal to 0.146 ± 0.003 liter^2 moles^{-2} sec.$^{-1}$ at 20°.



I II

By catalytic hydrogenation (PtO_2, ethyl acetate–acetic acid) the double bond in the side chain and the conjugated olefinic double bond are saturated to give the corresponding *s*-octahydroanthracene derivative (III), m.p. 106–107°; $[\alpha]^{20}D$ +21° ($CHCl_3$); $\lambda_{max.}$ (isoöctane) 273, 278 and 282 mμ (ε 670, 550 and 695 respectively), $\lambda_{min.}$ 247 mμ (ε 95); *Anal.* Calcd. for $C_{28}H_{44}$: C, 88.34; H, 11.65. Found: C, 88.42; H, 11.47. Oxidation of II with 70% nitric acid and subsequent esterification of the resulting compound with diazomethane leads to 1-methyl-2,3,5,6-tetracarbomethoxybenzene (IV), m.p. 121–123°; *Anal.* Calcd. for $C_{15}H_{16}O_8$: C, 55.55; H, 4.97. Found: C, 55.43; H, 5.06. The structure of IV was confirmed by its comparison with a sample obtained by an analogous oxidation of 9-methyl-*s*-octahydroanthracene. Compound IV, incidentally, was found to be identical with the methyl tetracarbomethoxybenzene obtainable by the nitric acid oxidation of various steroids.[1] From the analogous oxidation of 9-methyl-*s*-octahydrophenanthrene we obtained pentacarbomethoxybenzene instead of the expected, unknown 1-methyl-2,3,4,5-tetracarbomethoxybenzene (V).

We are considering the possibility that this type of facile rearrangement, *i.e.*, the transformation of steroids into anthracene derivatives, is involved in spontaneous carcinogenesis.

(1) (a) H. H. Inhoffen, *Ann.*, **494**, 122 (1932); (b) A. Windaus and G. Zühlsdorff, *ibid.*, **536**, 204 (1938); (c) M. Müller, *Z. physiol. Chem.*, **233**, 223 (1935).

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RECEIVED APRIL 30, 1953

ENZYMES OF THE FATTY ACID CYCLE. II. ETHYLENE REDUCTASE[1]

Sirs:

We have recently reported on the identification and isolation of β-keto thiolase and β-keto reductase.[2] Similar results have been obtained in other laboratories.[3,4,5] Through the combined action of these two enzymes the cell elongates the chain of the CoA thioester derivatives of fatty acids by the addition of a C_2 carbon chain from acetyl-S-CoA forming the corresponding β-hydroxy-CoA-thioester derivatives. In this way β-hydroxy-butyryl-S-CoA is formed from acetyl-S-CoA (Reactions 1 and 2).

$$2CH_3\text{—}CO\text{—}S\text{—}CoA \rightleftharpoons CH_3\text{—}CO\text{—}CH_2\text{—}CO\text{—}S\text{—}CoA + HS\text{—}CoA \quad (1)$$

$$CH_3\text{—}CO\text{—}CH_2\text{—}CO\text{—}S\text{—}CoA + DPNH + H^+ \rightleftharpoons CH_3\text{—}CHOH\text{—}CH_2\text{—}CO\text{—}S\text{—}CoA + DPN^+ \quad (2)$$

$$CH_3\text{—}CHOH\text{—}CH_2\text{—}CO\text{—}S\text{—}CoA \rightleftharpoons CH_3\text{—}CH{=}CH\text{—}CO\text{—}S\text{—}CoA + H_2O \quad (3)$$

$$\text{Leuco-safranine} + CH_3\text{—}CH{=}CH\text{—}CO\text{—}S\text{—}CoA \longrightarrow \text{Safranine} + CH_3\text{—}CH_2\text{—}CH_2\text{—}CO\text{—}S\text{—}CoA \quad (4)$$

(1) This work was supported in part by a grant from the Research Foundation of Germany. The following abbreviations are used: Coenzyme A, CoA-SH; acyl coenzyme A derivatives, acyl-S-CoA; oxidized and reduced diphosphopyridine nucleotide, DPN$^+$ and DPNH; reduced triphosphopyridine nucleotide, TPNH; flavinadenine dinucleotide, FAD; micromoles, μM.

(2) F. Lynen, L. Wessely, O. Wieland and L. Rueff, *Angew. Chem.*, **64**, 687 (1952).

(3) J. R. Stern, M. J. Coon and A. del Campillo, THIS JOURNAL, **75**, 1517 (1953).

(4) A. L. Lehninger and G. D. Greville, *ibid.*, **75**, 1515 (1953).

(5) D. E. Green and S. Mii, *Federation Proc.*, **12**, 211 (1953).

The remaining two enzymes of the cycle have been recently characterized. One of them, which may be referred to as crotonase (Reaction 3), is the subject of a preceding note.[6] The other enzyme, ethylene reductase, catalyzes Reaction 4. Our method of replacing the naturally occurring CoA compounds by the readily synthesized analogs of N-acetylthioethanolamine again proved useful in this case. We found that in place of crotonyl-S-CoA the simpler compound S-crotonyl-N-acetyl thioethanolamine is reduced through the action of ethylene reductase.

S-Crotonyl-N-acetylthioethanolamine was obtained through reaction of crotonyl chloride with the lead salt of N-acetyl thioethanolamine, m.p. 61.5–62°. In aqueous solution it shows two characteristic absorption bands with peaks at 224 mμ (ϵ = 11500) and 262 mμ (ϵ = 6750). The method used by Fischer and Eysenbach[7] to study fumarate reductase, namely, the oxidation of a leuco dye, such as leucosafranin, was used to assay ethylene reductase as shown in Reaction 4.

In this reaction crotonic acid cannot replace the thioester derivative. The enzyme assay, in which the appearance of color from the leuco dye is followed, is illustrated in Fig. 1. By the use of this assay ethylene reductase was purified about 50-fold from sheep liver extracts through steps involving acetone fractionation, adsorption and elution from calcium phosphate gel and ammonium sulfate fractionation. The solution of the purified enzyme is yellow. A colorless, almost inactive protein can be precipitated from the above solution with ammonium sulfate at *p*H 3.6. Activity of this protein can be partially restored by addition of yeast Kochsaft or crude preparations of FAD. This suggests that, like fumarate reductase, ethylene reductase may be a flavoprotein. The two enzymes, however, are not identical. DPNH or TPNH cannot substitute for the leuco dye.

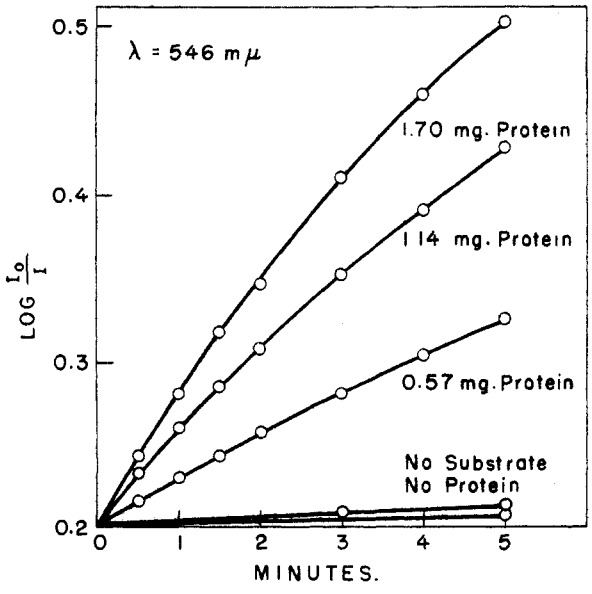

Fig. 1.—2.6 μM. S-crotonyl-N-acetylthioethanolamine and 0.5 μM. leucosafranin T in 2.1 ml. of 0.066 *M* phosphate buffer, *p*H 7.1; enzyme as indicated; temp. 17° (*d*, 0.5 cm.).

With cruder preparations of the enzyme the crotonyl-thioethanolamine derivative can be replaced by β-hydroxybutyryl-S-CoA (prepared either enzymatically[2] or synthetically[8]) indicating that the preparations also contain crotonase,[6] the enzyme catalyzing Reaction 3. These observations prove that ethylene reductase reacts with crotonyl CoA.

(6) J. R. Stern and A. del Campillo, THIS JOURNAL, **75**, 2277 (1953). Joint work on this enzyme is being carried out in the New York University and Munich laboratories.

(7) F. G. Fischer and H. Eysenbach, *Ann. Chem.*, **530**, 99 (1953).

(8) T. Wieland and L. Rueff, *Angew. Chem.*, in press.

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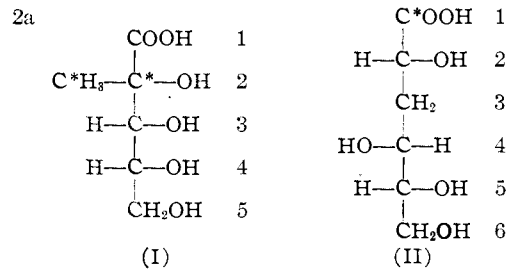
RECEIVED APRIL 3, 1953

CONCERNING THE MECHANISM OF FORMATION OF SACCHARINIC ACIDS

Sir:

Since the discovery of the saccharinic acids some seventy years ago, the mechanism by which they arise through the action of alkali on reducing sugars has remained obscure. Nef[1] first suggested as the crucial step in their formation an intramolecular isomerization and hydration similar to the benzilic acid rearrangement. This proposal was later modified and modernized by Isbell,[2] who interpreted the reaction sequence in terms of consecutive electron displacements. An alternative mechanism, involving the intermolecular recombination of fragments of the original sugar has been largely disregarded on account of the failure to observe formation of higher-carbon saccharinic acids from the action of alkali on lower-carbon sugars.

We now have examined, by means of C^{14}-labeling experiments, the formation of two saccharinic acids, "D-glucosaccharinic" acid (I) and "D-galacto-α-metasaccharinic" acid (II). Our results indicate that the branched-chain and the straight-chain acid studied are formed by *different* general mechanisms.



1-C^{14}-D-Mannose[3] was converted by the action of saturated lime-water at room temperature[4] to C^{14}-"D-glucosaccharinic acid." The latter was degraded, by oxidation with sodium metaperiodate, to carbon dioxide (C-1), acetic acid (C-2a, C-2), formic acid (C-3, C-4) and formaldehyde (C-5). Over 95% of the original radioactivity was found in the acetic acid fragment and degradation of the latter showed that the labeling was distributed approximately in the ratio C-2a:C-2, 2:3.

(1) J. U. Nef, *Ann.*, **357**, 294 (1907); **376**, 1 (1910).

(2) H. S. Isbell, *J. Research Natl. Bur. Standards*, **32**, 45 (1944).

(3) J. C. Sowden, *J. Biol. Chem.*, **180**, 55 (1949).

(4) H. Kiliani, *Ber.*, **15**, 701, 2953 (1882).