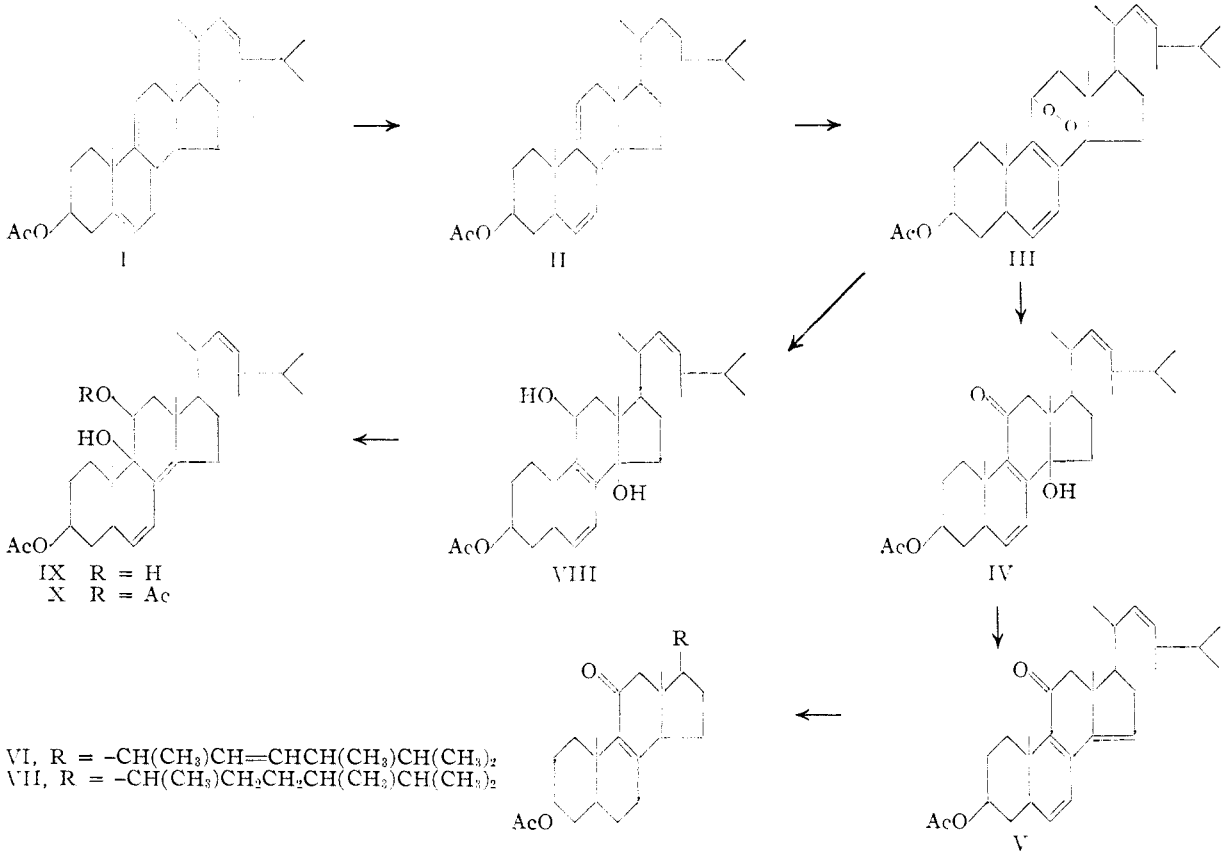
CORTICOSTEROID INTERMEDIATES. II. A NEW ROUTE TO 11-OXYGENATED STEROIDS

Sir:

A new synthetic route has been devised for the conversion of C-ring unsubstituted steroids to cortisone. In contrast to recently published methods, which involve epoxidation of steroid 7,9(11)-dienes,[1-6] our synthesis employs photochemical peroxidation of homoannular C-ring dienes to introduce 11-oxygen as an 11,14-peroxide bridge. The C-ring endoperoxide system undergoes facile rearrangement to form directly 11-keto steroids suitable for conversion to cortisone. The preparation of the C-ring dienes required in this synthesis is accomplished by a heretofore unreported isomerization of nuclear trienes of the dehydroergosterol type. This communication reports the application of the new synthesis to ergosterol.



$\Delta^{5,7,9(11),22}$-Ergostatetraen-3β-ol acetate (dehydroergosterol acetate) (I),[7] prepared by mercuric acetate dehydrogenation of ergosterol acetate, was catalytically isomerized with liquid sulfur dioxide in over 80% yield to the C-ring diene $\Delta^{6,8(14),9(11),22}$-ergostatetraen-3$\beta$-ol acetate (II), m.p. 149.0–151.0°; $[\alpha]_D$ −94° ($CHCl_3$); $\lambda_{max.}$ 287.5 mu (log ϵ = 3.82), $\lambda_{max.}$ 232.5 mu (log ϵ = 4.25) (ether); found: C, 82.39; H, 10.26.[8] Photoperoxidation[9] of II afforded $\Delta^{6,8,22}$-ergostatrien-3β-ol acetate 11,14-peroxide (III), m.p. 164.6–166.4°; $[\alpha]_D$ −19° ($CHCl_3$); $\lambda_{max.}$ 272 mu (log ϵ = 3.61) (ether); found: C, 77.03; H, 9.50. Experimental evidence in support of the structure III was obtained by selective hydrogenation over a lead–palladium catalyst to form a glycol, $\Delta^{6,8,22}$-ergostatrien-3β,11,14-triol 3-acetate (VIII), m.p. 160.8–163.4°; $[\alpha]_D$ −34° ($CHCl_3$); $\lambda_{max.}$ 274 mu (log ϵ = 3.63) (ether); found: C, 76.61; H, 9.75, which underwent acid-catalyzed anionotropic rearrangement to a readily acylated isomer, $\Delta^{6,8(14),22}$-ergostatrien-3β,9,11-triol 3-acetate (IX), m.p. 203.0–206.0°; $\lambda_{max.}$ 248 mu (log ϵ = 4.42) (ether); found: C, 76.68; H, 9.80; active hydrogen, 1.87 moles per mole. 11-Acetate (X), m.p. 169.2–170.6°; $[\alpha]_D$ −47° ($CHCl_3$); $\lambda_{max.}$ 247 mu (log ϵ = 4.43) (ether); found: C, 75.05; H, 9.60. The ultraviolet spectra of III, VIII and IX and the acetylation of IX to X are consistent with the structures shown.

Mild base-catalyzed rearrangement of the per-

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oxide[10,11] III yielded $\Delta^{6,8,22}$-ergostatrien-3β,14-diol-11-one 3-acetate (IV), m.p. 188.8–192.4°; $[\alpha]_D$ +34° ($CHCl_3$); $\lambda_{max.}$ 308 mu (log ϵ = 3.84) (ether); found: C, 76.72; H, 9.65. Acid-catalyzed dehydration, followed by reacetylation led to $\Delta^{6,8,14,22}$-ergostatetraen-3β-ol-11-one acetate (V), m.p. 145.0–146.8°; $[\alpha]_D$ −82° ($CHCl_3$); $\lambda_{max.}$ 326 mu (log ϵ = 3.95), $\lambda_{max.}$ 233 mu (log ϵ = 4.18) (ether); found: C, 79.72; H, 9.54. The position of the carbonyl group at C-11 in V was established by hydrogenation over palladium-charcoal or W-7 Raney nickel[12] to form Δ^{8}-ergosten-3β-ol-11-one acetate (VII), m.p. 137.8–138.6°; $[\alpha]_D$ +125° ($CHCl_3$); $\lambda_{max.}$ 248 mu (log ϵ = 3.90) (ether); found: C, 78.91; H, 10.74; melting point undepressed on admixture with an authentic sample prepared by hydrogenation of VI obtained by an independent route.[2b,4]

Intermediates retaining the unsaturated side chain and suitable for conversion to cortisone were prepared by selective hydrogenation of V over W-2 nickel[13] to form $\Delta^{8,14,22}$-ergostatrien-3β-ol-11-one acetate, m.p. 127.0–128.2°; $[\alpha]_D$ +20° ($CHCl_3$); $\lambda_{max.}$ 291 mu (log ϵ = 4.06) (ether); found: C, 79.51; H, 9.92; or over W-7 nickel to form the known cortisone intermediate, $\Delta^{8,22}$-ergostadien-3β-ol-11-one acetate (VI), m.p. 131.4–131.8°; $[\alpha]_D$ +110° ($CHCl_3$); $\lambda_{max.}$ 248 mu (log ϵ = 3.95) (ether); melting point undepressed on admixture with an authentic sample.[2b,4]

Details of this work and alternate conversions of III and IV will be the subject of later communications from this Laboratory.

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THE ENZYMATIC OXIDATION OF *d*- AND *l*-β-HYDROXYBUTYRATE[1]

Sirs:

Although the *l*-isomer of β-hydroxybutyric acid is often regarded as the "naturally occurring" isomer[2] the *d*-isomer is also known to undergo biological oxidation.[3,4,5]

We have found that the mechanisms of enzymatic oxidation of the two pure isomers[6] in clear, dialyzed extracts of acetone-dried rat liver mitochondria are quite different. Such extracts contain the already known[2] diphosphopyridine nucleotide (DPN)-linked, *l*-specific β-hydroxybutyric dehydrogenase catalyzing the following reaction

$$\text{1-}\beta\text{-Hydroxybutyrate} + \text{DPN} \rightleftarrows \text{acetoacetate} + \text{DPNH} \quad (1)$$

This reaction requires no components beyond extract, DPN and *l*-β-hydroxybutyrate. Under these circumstances *d*-β-hydroxybutyrate is not oxidized.

However such extracts will cause the reduction of DPN by the *d*-isomer if they are supplemented with adenosine triphosphate (ATP), Coenzyme A, and Mg^{++}. Such additions have no stimulatory effect on the reduction of DPN by *l*-β-hydroxybutyrate. Furthermore, the *l*-specific β-hydroxybutyric dehydrogenase is not involved in the oxidation of the d-isomer in the presence of these additional cofactors, since fractionation of the extracts yielded preparations with high activity toward *d*-β-hydroxybutyrate and little or none toward the *l*-isomer (Table I). These findings therefore suggested

TABLE I

ENZYMATIC OXIDATION OF *d*-β-HYDROXYBUTYRATE

The test system contained 0.10 ml. of dialyzed fraction of acetone-dried rat liver mitochondria, 10 μM. cysteine, 2.5 μM. ATP, 5 μM. $MgCl_2$, 50 μM. tris-(hydroxymethyl)-aminomethane buffer *p*H 8.0, 0.5 μM. CoA, 100 μM. KCl, 1.0 μM. DPN, 25 μM. *d*- or *l*-β-hydroxybutyrate and H_2O to make 1.00 ml.; temp. 20°; time, 20 min. Appearance of DPNH measured spectrophotometrically at 340 mμ.

System	Substrate	Δ log I_0/I
Complete	None	0.015
Complete	*d*-Isomer	.670
ATP omitted	*d*-Isomer	.005
CoA omitted	*d*-Isomer	.062
Mg^{++} omitted	*d*-Isomer	.149
Complete	*l*-Isomer	.040
ATP + CoA + Mg^{++} omitted	*l*-Isomer	.052

that the reduction of DPN by the *d*-isomer proceeds as follows

$$d\text{-}\beta\text{-Hydroxybutyrate} + \text{CoA} \xrightarrow{\text{ATP}} d\text{-}\beta\text{-hydroxybutyryl-CoA} \quad (2)$$

$$d\text{-}\beta\text{-Hydroxybutyryl-CoA} + \text{DPN} \rightleftarrows \text{acetoacetyl-CoA} + \text{DPNH} \quad (3)$$

Further evidence for this formulation follows: In the absence of DPN but with hydroxylamine present as a "trapping" agent the *d*-isomer forms a hydroxamic acid derivative, detected by colorimetry and paper chromatography.[7] This reaction requires the presence of ATP, Mg^{++} and CoA. The formation of acetoacetyl-CoA as the end-product of the over-all reaction was established by the finding that citrate was formed as product of oxidation of *d*-β-hydroxybutyrate when oxalacetate, excess CoA, and "condensing enzyme" were present, *via* the following known reactions[8]

$$\text{Acetoacetyl-CoA} + \text{CoA} \rightleftarrows 2\ \text{acetyl-CoA} \quad (4)$$

$$\text{Acetyl-CoA} + \text{oxalacetate} \rightleftarrows \text{citrate} + \text{CoA} \quad (5)$$

Free acetoacetate formed no citrate under these conditions.

(1) This work was supported in part by grants from the Nutrition Foundation, Inc., the U. S. Public Health Service, and the John Simon Guggenheim Foundation.

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