product[6] is provided by the action of lithium aluminum hydride on this intermediate V.[7]

Acknowledgment.—The authors are grateful to Dr. H. T. Openshaw for a sample of isoemetine hydrobromide. Financial support was provided by the research committee of the Graduate School.

(7) It is pertinent that in the case of the Δ^3-dehydroyohimbane (D-E *trans*) and similar (*cf.* ref. 4) systems, catalytic reduction also affords the more stable 3,15,20-*cis-trans* product (E. E. van Tamelen, M. Shamma and P. E. Aldrich, THIS JOURNAL, **78**, 4628 (1956).

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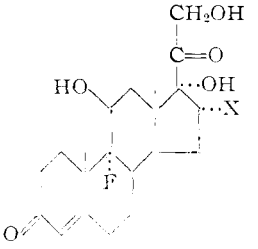
OXIDATION OF STEROIDS BY MICROORGANISMS. IV. 16α-HYDROXYLATION OF 9α-FLUOROHYDROCORTISONE AND 9α-FLUOROPREDNISOLONE BY *Streptomyces roseochromogenus*

Sir:

The introduction of a 16α-hydroxyl group into 9α-fluorohydrocortisone (I) and 9α-fluoroprednisolone (II) has been shown by Bernstein, *et al.*,[1] to result in complete suppression of the salt-retaining properties of these steroids without appreciably impairing their glucocorticoid activity. Moreover, preliminary studies in man[2] have demonstrated the anti-arthritic activity of 9α-fluoro-16α-hydroxyprednisolone (triamcinolone) and have confirmed its lack of salt-retaining activity. An efficient synthesis of this complex steroid is therefore of considerable practical interest.



I, X = H
II, X = H; $\Delta^{1,2}$
III, X = OH
IV, X = OH; $\Delta^{1,2}$

Applying the microbiological 16α-hydroxylation reaction first reported from this laboratory[3,4] to I and II we have succeeded in preparing 16α-hydroxy-9α-fluorohydrocortisone (III) and 16α-hydroxy-9α-fluoroprednisolone (IV) in yields of 50% and 20%, respectively.

A fermentation medium containing soybean meal (30 g.), glucose (20 g.), soybean oil (4.4 g.) and calcium carbonate (0.050 g.) in distilled water (2 l.) was distributed over 40 250-ml. erlenmeyer flasks, steam-sterilized for 30 minutes at 120° and after addition of the steroid (1 g. dissolved or suspended in 40 ml. of methanol) inoculated with vegetative growth of *Streptomyces roseochromogenus*.[5] The flasks were incubated at 25° for 4 to 7 days with rotatory mechanical shaking at 280 r.p.m. in a 2 in. radius, the mycelium filtered off and washed with water. The filtrate was extracted with methyl isobutyl ketone (three 800-ml. portions), the extract concentrated to small volume, cooled and the resulting crystals recrystallized from ethyl alcohol. The properties of III (m.p. 250–252°, $[\alpha]D$ +97° (*c* 0.99 in pyridine); λ_{max}^{alc} 238 mμ (15,000); λ_{max}^{Nujol} 2.79, 2.98, 5.82, 6.00, 6.17μ[6]; *Anal.* Found: C, 63.77; H, 7.32) and IV (m.p. 248–250°; $[\alpha]^{23}D$ +71° (c 0.35 in acetone); λ_{max}^{Nujol} 2.95, 5.85, 6.02, 6.16, 6.24, 11.26 μ; diacetate: m.p. 170–180° (with gas evolution); $[\alpha]^{23}D$ +28° (*c* 0.38 in $CHCl_3$); *Anal.* Found: C, 62.34; H, 6.74) are in harmony with those reported by Bernstein, *et al.*[1]

Oxidation of the diacetates of III and IV with chromic and sulfuric acids in acetone gave, respectively, the diacetates of 16α-hydroxy-9α-fluorocortisone, m.p. 215–217°; $[\alpha]^{23}D$ +94° (*c* 0.35 in $CHCl_3$); λ_{max}^{alc} 235 mμ (15,100); λ_{max}^{Nujol} 2.94, 5.75, 6.00, 6.19 μ; *Anal.* Found: C, 63.03; H, 6.53, and of 16α-hydroxy-9α-fluoroprednisone, m.p. 204–206°; $[\alpha]^{23}D$ +90° (c 0.41 in $CHCl_3$); λ_{max}^{alc} 235 mμ (16,200); λ_{max}^{Nujol} 2.97, 5.75, 5.99, 6.15, 6.22, 11.28 μ; *Anal.* Found: 63.13; H, 6.25.

The biosynthetic conversion of III into IV by means of *Corynebacterium simplex* has been reported.[1] We have performed this reaction in 65% yield with a strain of *Mycobacterium rhodochrous*[7] adding the steroid (0.5 g./l.) to a 24-hour old culture in a medium containing yeast extract, tryptone, pentane, glucose calcium, and incubating for five hours.

(1) S. Bernstein, R. H. Lenhard, W. S. Allen, M. Heller, R. Littell, S. M. Stolar, L. I. Feldman, and R. H. Blank, THIS JOURNAL, **78**, 5693 (1956).

(2) L. Hellman, B. Zumoff, M. K. Schwartz, T. F. Gallagher, C. A. Berntsen and R. H. Freyberg, Abstract of Papers presented at the 3rd Interim Meeting of the American Rheumatism Association, Bethesda, Md., Nov. 30, 1956.

(3) D. Perlman, E. O. Titus and J. Fried, THIS JOURNAL, **74**, 2126 (1952).

(4) J. Fried, R. W. Thoma, D. Perlman, J. E. Herz, and A. Borman, *Recent Progr. Hormone Research*, **9**, 149 (1955).

(5) Waksman Collection Number 3689, Rutgers University, New Brunswick, N. J.

(6) Occasionally a polymorphic form of III was obtained, which lacked the 2.79 μ band and showed an entirely different picture in the fingerprint region.

(7) This culture, isolated by Dr. J. O. Lampen of our laboratories, is identified as SC 2318 in our collection. With regard to its classification *cf.* R. Gordon and J. M. Mihm, *J. Bact.*, **73**, 15 (1957). Its dehydrogenating properties were discovered in these laboratories by Dr. H. Kroll.

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TOTAL SYNTHESIS OF 17-DESMETHOXYDESERPIDINE

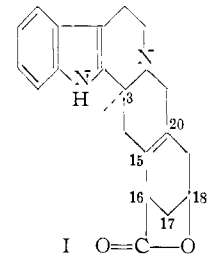
Sir:

In a previous communication[1] we described the synthesis of the unsaturated lactone (I). We now wish to report the conversion of this intermediate to 17-desmethoxydeserpidine.

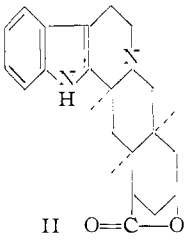
Reduction of the unsaturated lactone (I) with hydrogen and a highly active platinum catalyst for sixteen hours gave predominantly the saturated lactone (II), m.p. 267–269° (found: C, 74.81; H, 6.98; λ_{max}^{Nujol} 5.71 μ) as well as a small amount of an isomeric lactone (III), m.p. 307–309° (found: C, 74.25; H, 6.86; λ_{max}^{Nujol} 5.65 μ). When the product was allowed to remain in contact with the

(1) F. L. Weisenborn and H. E. Applegate, THIS JOURNAL, **78**, 2021 (1956).

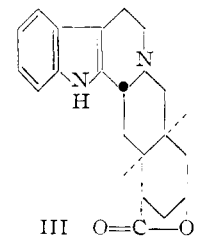
platinum catalyst for five days after reduction was complete, an equilibrium was reached in which the initially formed lactone (II) containing a C_3 α-hydrogen had been transformed almost completely into its more stable C_3 β-hydrogen isomer (III).[2] The lactones (II and III) were shown to be C_3 epimers possessing the same configurations at C_{15}, C_{16} and C_{20} as α-yohimbine[3] by transformation of both compounds to *d,l*-17-desoxy-α-yohimbine (IVc). Thus, hydrogen bromide in acetic acid at 100° converted the two isomers to the same bromo acid (IVa), isolated as the hydrobromide, m.p. 278–279° (found: C, 49.90; H, 4.97; Br, 32.78). Compound



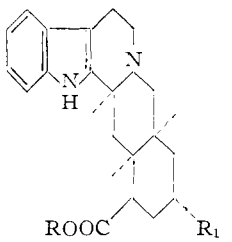
I



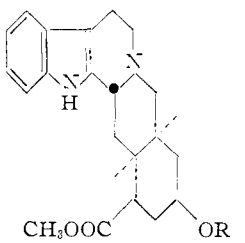
II



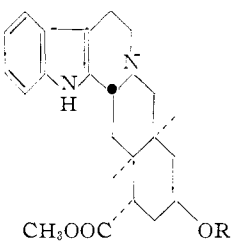
III



IVa, R = H, R_1 = Br
IVb, R = CH_3, R_1 = Br
IVc, R = CH_3, R_1 = H



Va, R = H
Vb, R = $OC(C_6H_2)(OCH_3)_3$



VIa, R = H
VIb, R = $OC(C_6H_2)(OCH_3)_3$

III gives the same bromo acid as lactone II in this reaction since the hydrogen bromide catalyzes an inversion at C_3 to the more stable α-hydrogen[4] once the lactone ring is opened. Esterification of the bromo acid (IVa) with diazomethane yielded the bromoester (IVb), m.p. 194–196° (found: C, 60.39; H, 6.08), which was reduced with hydrogen and palladium on calcium carbonate in the presence of ammonium acetate to the ester (IVc), m.p. 205–206° (found: C, 74.51; H, 7.60). This compound was shown to be identical with authentic 17-desoxy-α-yohimbine[5] by comparison of their infrared spectra. Thus, compound III must have the same stereochemical configuration at the five asymmetric centers, C_3, C_{15}, C_{16}, C_{18} and C_{20} as deserpidic acid lactone.[4] *d,l*-17-Desmethoxydeserpidic acid lactone (III) on treatment with cold sodium methoxide-methanol solution produced the hydroxy ester (Va), m.p. 220–222° (found: C, 71.16; H, 6.87) as well as a small amount of an isomeric compound (VIa), m.p. 288–289° (found: C, 71.30; H, 7.32). The infrared spectra of both esters (Va and VIa) in chloroform showed the type of absorption in the 3.5 μ region characteristic of β-oriented C_3 hydrogens.[6] Treatment of the lower melting hydroxy ester (Va) with boiling sodium methoxide-methanol solution converted it to the higher melting isomer (VIa). That the base catalyzed isomerization involved inversion of the C_{16} carbomethoxy group was proved by the observations: (1) the amino acid derived from the hydroxy ester (VIa) by basic hydrolysis did not relactonize with pyridine and acetic anhydride; (2) the amino acid obtained by the action of cold aqueous sodium hydroxide on lactone III readily reverted to the parent lactone and (3) the latter amino acid gave the hydroxy ester (Va) on esterification with diazomethane. The hydroxy ester (Va), therefore is d,l-methyl 17-desmethoxydeserpidate.[7] *d,l*-methyl 16-iso-17-desmethoxydeserpidate (VIa) and *d,l*-methyl 17-desmethoxydeserpidate (Va) were esterified with trimethoxybenzoyl chloride in pyridine to give *d,l*-16-iso-17-desmethoxydeserpidine (VIb), m.p. 150–153° (found: C, 67.64; H, 6.84) and *d,l*-17-desmethoxydeserpidine (Vb), m.p. 247–248° (found: C, 67.79; H, 6.45), respectively.

d,l-17-Desmethoxydeserpidine at a dose of 2.5 mg./kg. intravenous in the unanesthetized dog produced the typical reserpine-like response (delayed hypotension, miosis and somnolence). *d,l*-16-Iso-17-desmethoxydeserpidine (VIb) was inactive.

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(2) The stability of the C_3 β-hydrogen configuration in this ring system has been observed previously in the acid isomerization of isoreserpic acid lactone to reserpic acid lactone (*cf.* Woodward, *et al.*, *ibid.*, **78**, 2023 (1956)) and may be explained rationally by conformational considerations.

(3) For a recent discussion of the relationship of deserpidine to α-yohimbine *cf.* E. Schlittler, "Rauwolfia," Little, Brown and Co., Boston, Mass., 1957, p. 81.

(4) H. B. MacPhillamy, C. F. Huebner, E. Schlittler, A. F. St. Andre and P. R. Ulshafer, THIS JOURNAL, **77**, 4335 (1955).

(5) This compound was prepared by Dr. P. A. Diassi of this laboratory and will be described in a forthcoming publication.

(6) E. Wenkert and D. K. Roychaudhuri, THIS JOURNAL, **78**, 6417 (1956).

(7) Methyl reserpate on treatment with methanolic sodium methoxide does not suffer inversion of the carbomethoxy group since this would force both the C_{17} methoxyl and the C_{18} hydroxyl groups to be axially oriented in order to gain an equatorial C_2–C_3 bond. In the present case, however, inversion of the carbomethoxy group is favored since an equatorial C_2–C_3 bond is thereby obtained while being opposed only by one axial substituent (the C_{18} hydroxyl). In testing the generality of the inversion shown by the ester (Va), we found that the carboxyl group of 3-epi-α-yohimbine (Bader, *et al.*, THIS JOURNAL, **77**, 3547 (1955)) also inverts in order to gain an equatorial C_2–C_3 bond. Thus, 3-epi-α-yohimbine (m.p. 126–128°, $[\alpha]_D$ −68° (methanol), kindly supplied by Dr. E. Wenkert) on treatment with methanolic sodium methoxide was converted to 16-iso-3-epi-α-yohimbine (m.p. 220–222°, $[\alpha]_D$ +64° (methanol)).