COMMUNICATIONS TO THE EDITOR

OXIDATION OF STEROIDS BY MICROÖRGANISMS. III. SIDE CHAIN DEGRADATION, RING D-CLEAVAGE AND DEHYDROGENATION IN RING A

Sir:

In recent publications from our own[1] as well as from other laboratories[2] concerned with transformations of steroids by microörganisms, the main emphasis has been on the introduction of one or more hydroxyl groups into the steroid nucleus. We now wish to report some transformations of progesterone and related steroids involving scission of carbon–carbon linkages in the side chain and in ring D, as well as the introduction of a new double bond in ring A.

Fermentation of progesterone with *Streptomyces lavendulae* (Rutgers University No. 3440-14) in a medium containing soybean meal, glucose and soybean oil followed by extraction of the culture filtrate[3] with chloroform afforded after chromatography on alumina $\Delta^{1,4}$-androstadien-3,17-dione (I),[4] m.p. 138–139.5°; $[\alpha]^{23}D$ +115° (*c*, 0.56 in $CHCl_3$); $\lambda_{max.}^{Nujol}$ 5.74 μ (17-ketone), 6.04, 6.16 and 6.24 μ ($\Delta^{1,4}$-3-ketone) (7% yield), identified by comparison with an authentic sample, and $\Delta^{1,4}$-androstadien-17β-ol-3-one (II)[4], m.p. 167–168°; $[\alpha]^{23}D$ +21° (*c*, 1.28 in $CHCl_3$); $\lambda_{max.}^{alc.}$ 243 mμ (ϵ = 16,100); $\lambda_{max.}^{Nujol}$ 2.96 μ (OH), 6.02, 6.18 and 6.24 μ ($\Delta^{1,4}$-3-ketone); (*Anal.* Found: C, 79.65; H, 9.10. Calcd. for $C_{19}H_{26}O_2$: C, 79.66; H, 9.15) (12% yield), which on oxidation with chromic acid was converted to I. The utility of I and II as intermediates in the synthesis of estradiol and estrone is well known.[4] In addition to providing a two step synthesis of estrone and estradiol from progesterone the above bioconversion suggests progesterone, I and II, as possible intermediates in the synthesis of these hormones in the mammalian organism.[5]

Oxidative degradation involving not only elimination of the side chain but cleavage between carbon atoms 13 and 17 as well has been observed with a variety of organisms. Thus, fermentation of progesterone with *Penicillium chrysogenum* (University of Wisconsin No. 49–133)[6] in a medium containing corn steep liquor, $NH_4H_2PO_4$, $CaCO_3$ and soybean oil afforded in 70% yield the known testololactone (III),[7] m.p. 207–209°; $[\alpha]^{23}D$ +43° (*c*, 1.0 in $CHCl_3$); $\lambda_{max.}^{alc.}$ 237 mμ (ϵ = 17,900); $\lambda_{max.}^{Nujol}$ 5.82 μ (lactone carbonyl), 5.99 μ and 6.18 μ (Δ^4-3-ketone); (*Anal.* Found: C, 75.90; H, 8.83. Calcd. for $C_{19}H_{26}O_3$: C, 75.46; H, 8.67) identical with an authentic sample of the substance.[8] Reduction of III with PtO_2 in glacial acetic acid followed by oxidation with chromic acid yielded after chromatography 5α-dihydrotestololactone (IV),[7] m.p. 171–172°; $[\alpha]^{23}D$ −18° (*c*, 0.76 in $CHCl_3$); $\lambda_{max.}^{Nujol}$ 5.82 μ (lactone carbonyl and 3-ketone); (*Anal.* Found:. C, 75.14; H, 8.98. Calcd. for $C_{19}H_{28}O_3$: C, 74.95; H, 9.27) identical with an authentic sample,[8] and the hitherto undescribed 5β-dihydrotestololactone (V), m.p. 202–203°; $[\alpha]^{23}D$ −25° (*c*, 1.0 in $CHCl_3$); $\lambda_{max.}^{Nujol}$ 5.83 μ; (*Anal.* Found: C, 74.94; H, 9.50).

Bioöxidation involving both lactone formation in ring D and dehydrogenation in ring A is less widespread and has been observed with but a small number of organisms. Thus, when progesterone, Reichstein's compound S or testosterone are fermented with *Cylindrocarpon radicola* (A.T.C.C. No. 11011) there results in about 50% yield Δ^1-dehydrotestololactone (VI), m.p. 218–219°; $[\alpha]^{23}D$ −44° (*c*, 1.29 in $CHCl_3$)[9]; $\lambda_{max.}^{alc.}$ 242 mμ (ϵ = 15,800); $\lambda_{max.}^{Nujol}$ 5.83 μ (lactone carbonyl)[10] 6.01, 6.15 and 6.22 μ($\Delta^{1,4}$-3-ketone); (*Anal.* Found: C, 76.29; H, 7.87. Calcd. for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05). The structure of VI is based on the following evidence: Reduction with PtO_2 in glacial acetic acid followed by chromic acid oxidation yielded IV and V. VI forms a semicarbazone, m.p. 250–260° dec.; (*Anal.* Found: N, 11.19. Calcd. for $C_{20}H_{27}N_3O_3$: N, 11.76) showing the ultraviolet spectrum, $\lambda_{max.}^{alc.}$ 244 mμ(ϵ = 12,200), 297 mμ (ϵ = 22,600) char-

(1) D. Perlman, E. Titus and J. Fried, THIS JOURNAL, **74**, 2126 (1952); J. Fried, R. W. Thoma, M. N. Donin, J. Herz, J. R. Gerke and D. Perlman, *ibid.*, **74**, 3962 (1952).

(2) D. H. Peterson, *et al.*, *ibid.*, **75**, 421 (1953), and earlier papers; D. R. Colingsworth, J. N. Karnemaat, F. R. Hanson, M. P. Brunner, M. Mann and W. J. Haines, *J. Biol. Chem.*, **203**, 807 (1953); F. W. Kahnt, Ch. Meystre, R. Neher, E. Vischer and A. Wettstein, *Experientia*, **8**, 432 (1952); O. Mancera, A. Zaffaroni, B. A. Rubin, F. Sondheimer, G. Rosenkranz and C. Djerassi, THIS JOURNAL, **74**, 3711 (1952).

(3) Extraction of the dried mycelium with acetone yielded a reduction product of progesterone, m.p. 170–172°; $[\alpha]^{20}D$ +81° (*c*, 0.93 in $CHCl_3$); $\lambda_{max.}^{alc.}$ 240 mμ (ϵ = 15,900); $\lambda_{max.}^{Nujol}$ 2.91 μ (OH), 5.98 and 6.20 μ (Δ^4-3-ketone), which was identified as Δ^4-pregnen-20β-ol-3-one by reoxidation to progesterone and comparison with an authentic sample of the hydroxy ketone kindly furnished by Dr. R. B. Turner (*cf.* P. Wieland and K. Miescher, *Helv. Chim. Acta*, **32**, 922 (1949), and R. B. Turner and D. M. Voitle, THIS JOURNAL, **73**, 2283 (1951)).

(4) H. H. Inhoffen, G. Zuehlsdorff and Huang-Minlon, *Ber.*, **73**, 451 (1940); H. H. Inhoffen, *Angew. Chem.*, **59**, 207 (1947).

(5) Evidence for or against such a hypothesis could be adduced by extending the perfusion studies using radioactive acetate recently reported by N. T. Werthessen, E. Schwenk and C. Baker, *Science*, **117**, 380 (1953), to labelled progesterone, I and II.

(6) This reaction has also been accomplished using organisms of the genera *Aspergillus* and *Mucor*, *e.g.*, *A. flavipes* (A.T.C.C. No. 11013) and *M. mucedo* (A.T.C.C. No. 7941).

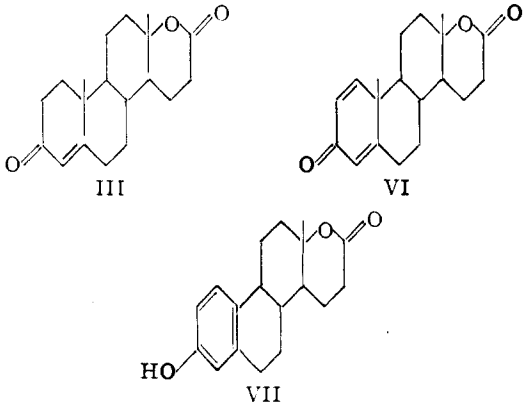
(7) H. Levy and R. P. Jacobsen, *J. Biol. Chem.*, **171**, 71 (1947). These authors left open the question, whether the lactones described by them possess structures exemplified by III, VI and VII or the alternate structures, in which ring scission has occurred between carbon atoms 16 and 17. Evidence favoring the formulation given in this paper has since been accumulated by various authors and is well summarized in reference 8 of a recent publication by G. M. Picha, THIS JOURNAL, **74**, 703 (1952). To this may be added the observation made in our own laboratories that the hydroxy acid derived from VI remained unchanged during treatment with chromic acid indicating that it possesses a tertiary hydroxyl group rather than a primary one as required by the alternate formulation of the lactone.

(8) We are indebted to Dr. H. Levy for kindly furnishing the samples of testololactone, 5α-dihydrotestololactone and estrololactone.

(9) The difference in molecular rotation between Δ^1-dehydrotestololactone and testololactone $\Delta[M]_D^{VI-III}$ −257°. The average contribution for the 1,2-double bond in $4\Delta^{1,4}$-3-ketones is −231°.

(10) This band shifts to 5.76μ when the spectrum is taken in carbon disulfide solution; *cf.* R. N. Jones, P. Humphries and K. Dobriner, THIS JOURNAL, **72**, 956 (1950).

acteristic of $\Delta^{1,4}$-3-semicarbazones.[4] Pyrolysis of VI at 550–600° in mineral oil[11] furnished Westerfeld's lactone (estrololactone)[12,13] (VII), identified after conversion into the acetate, m.p. 149–151°; $\lambda_{max.}^{alc.}$ 267 mμ(ϵ = 1080) and 275 mμ(ϵ = 950); $\lambda_{max.}^{Nujol}$ 5.70 μ (phenolic acetyl), 5.80 μ (lactone carbonyl), by comparison with an authentic sample of the latter.[8]



III VI

VII

The biochemical conversion of the β-oriented acetyl side chain in progesterone to a 17β-hydroxyl group parallels the degradation of 20-ketosteroids by peracids, which likewise proceeds with retention of configuration at C_{17}.[14] Similarly, the formation of ring D lactones has its chemical parallel in the reaction sequence: progesterone $\xrightarrow{RCO_3H}$ testosterone $\xrightarrow{-H_2}$ androstenedione $\xrightarrow{RCO_3H}$ III. A bioöxidation mechanism involving these same intermediates is not out of the question since testosterone is readily converted into VI by *C. radicola*.

(11) E. B. Hershberg, M. Rubin and E. Schwenk, *J. Org. Chem.*, **15**, 232 (1950).

(12) W. W. Westerfeld, *J. Biol. Chem.*, **143**, 177 (1943).

(13) R. P. Jacobsen, *ibid.*, **171**, 61 (1947).

(14) T. F. Gallagher and T. Kritchevsky, THIS JOURNAL, **72**, 882 (1950); R. B. Turner, *ibid.*, **72**, 878 (1950).

THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH
NEW BRUNSWICK, NEW JERSEY

JOSEF FRIED
RICHARD W. THOMA
ANNA KLINGSBERG

RECEIVED OCTOBER 19, 1953

THE SYNTHESIS OF ERYTHRINANE[1]

Sir:

The purpose of this communication is to report on a simple method of probable general applicability for the elaboration of the entire ring system of the *Erythrina abyssinica* Lam. alkaloids, the constitution of which has been elucidated recently by Prelog and his co-workers.[2] The conversion of 2-carbethoxycyclohexanone to erythrinane in five steps and about 12% over-all yield is described. It is anticipated that a number of substituted erythrinanes will now be easily accessible for chemical and pharmacological studies.

(1) The term "erythrinane" is proposed to designate the basic ring system of the Erythrina alkaloids.

(2) M. Carmack, B. C. McKusick and V. Prelog, *Helv. Chim. Acta*, **34**, 1601 (1951); H. G. Khorana, G. W. Kenner and V. Prelog, *ibid.*, **34**, 1969 (1951). Concerning the structure of β-erythroidine, the exhaustive investigations of Boekelheide and his collaborators (ref. 7 and accompanying papers) should be consulted.

Through the condensation of the potassio derivative of 2-carbethoxycyclohexanone with 2-bromoethylphthalimide in boiling toluene, there was obtained as a viscous oil, 2-carbethoxy-2-(β-phthalimidoethyl)-cyclohexanone which, without purification, was hydrolyzed with boiling concentrated hydrochloric acid to 2,3,4,5,6,7-hexahydroindole (35% over-all yield), [b.p. 80° (19 mm.)]; *Anal.* Calcd. for $C_8H_{13}N$: N, 11.37. Found: N, 11.28. *Picrate*,[3] m.p. 132–133° [*Anal.* Calcd. for $C_{14}H_{16}O_7N$: C, 47.72; H, 4.57. Found: C, 47.85; H, 4.45]. Over Adams catalyst in ethanol, the latter base absorbed 0.98 molar proportion of hydrogen to yield octahydroindole isolated as the picrate, m.p. 135–137°, which proved identical with a sample prepared according to the literature.[4] Treatment of hexahydroindole with phenylacetyl chloride under the Schotten-Baumann conditions afforded in 80% yield, *2-(β-phenylacetamidoethyl)-cyclohexanone* (I), m.p. 53–54°, (*Anal.* Calcd. for $C_{16}H_{21}O_2N$: C, 74.09; H, 8.16. Found: C, 73.96; H, 8.18.); maxima at 2.92 μ (NH—), 5.85 μ (C=O), 6.02 and 6.60 μ (—CONH—) and 6.70 μ (phenyl) in the infrared region. When heated for twenty-four hours at 100° in excess polyphosphoric acid, (I) was converted in 60% yield to *8-oxoerythrinane*[5] (II), m.p. 132–133° (*Anal.* Calcd. for $C_{16}H_{19}ON$: C, 79.62; H, 7.93; N, 5.80. Found: C, 79.71; H, 8.02; N, 5.62.), whose structure was deduced from the following evidence: (a) its infrared spectrum shows a single peak at 6.14 μ (besides the phenyl bands) which is characteristic of a disubstituted amide carbonyl group; (b) it is unaffected by prolonged heating in concentrated hydrochloric acid, a behavior inconsistent with an acyclic amide structure but consistent with a lactam structure[6]; (c) when treated with lithium aluminium hydride in boiling ether it is converted in 70% yield to the corresponding base *erythrinane*, a colorless oil, b.p. 195° (bath temp.) (0.1 mm.) (*Anal.* Calcd. for $C_{16}H_{21}N$: C, 84.52; H, 9.31. Found: C, 84.38; H, 9.17.); *picrate:* m.p. 184–185° (*Anal.* Calcd. for $C_{22}H_{24}O_7N_4$: N, 12.27. Found: N, 12.29); *methiodide:* m.p. 201–203° (*Anal.* Calcd. for $C_{17}H_{24}NI$: I, 34.3. Found: I, 34.1.). The infrared spectrum of the free base lacks the band of (II) at 6.14 μ; (d) on vigorous oxidation with nitric acid it yields 4-nitrophthalic acid isolated as its anhydride and further characterized as its anil derivative; both proved identical with authentic specimens.

Therefore, (II) is the only reasonable structure accommodating the evidence and further work in the methoxylated series is contemplated.

The biogenetic implications of the facile conversion of (I) to (II) are obvious and in keeping with Boekelheide's recent suggestion.[7]

Several attempts to ring close N-(β-phenylethyl)-

(3) Boiling points and melting points are uncorrected.

(4) R. Willstätter and D. Jaquet, *Ber.*, **51**, 767 (1918), report 137–138° as the m.p. of octahydroindole picrate.

(5) For the numbering of the ring system, see ref. 2.

(6) The opened form of the lactam undoubtedly exists in the hot acid mixture but cannot be isolated presumably because of spontaneous ring closure during the process of isolation. A similar apparent refractoriness of a lactam to boiling hydriodic acid can be found in the morphinane series (M. Gates, R. B. Woodward, W. F. Newhall and R. Kunzli, THIS JOURNAL, **72**, 1141 (1950)).

(7) V. Boekelheide, *et al.*, *ibid.*, **75**, 2550 (1953).