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# The Structure of Steroid D-Ring Lactones[1]

BY N. L. WENDLER, D. TAUB AND H. L. SLATES

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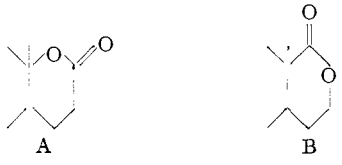
D-Ring lactones resulting from peracid oxidation of 17-keto steroids and 17a-keto D-homo steroids have been shown by unambiguous means to be the products of oxygen interpolation between carbon atoms 13,17 and 13,17a, respectively. The directional course of the Demianov rearrangement of 17-hydroxy-20-amino-norpregnanes was found to be independent of the configuration at $C_{17}$.

In 1942, Westerfeld[2] observed that estrone on treatment with alkaline hydrogen peroxide produced a lactone. Part structure A was assigned to this lactone in virtue of the fact that the corresponding hydroxy acid could be esterified by alcohol and acid, a fact considered to be less compatible with a tertiary acid derived from the alternative isomeric lactone part structure B.

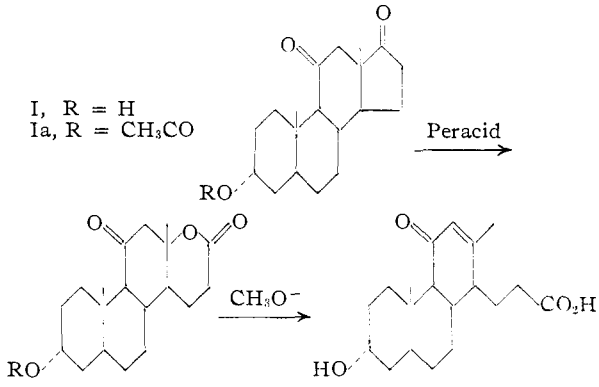


A B

Since the time of these original observations numerous reports[3] have appeared bearing on the structure of the lactones produced from peracid oxidation of 17-keto steroids. These reports unfortunately do not provide a completely unambiguous solution to the problem.[4] It is the purpose of the present paper, therefore, to present unequivocal proof that these steroid D-ring lactones are indeed to be formulated by the structural type A.

Etiocholane-3$\alpha$-ol-11,17-dione[5] (I) was converted by perbenzoic acid in good yield to a crystalline lactone, m.p. 216–220°. The latter, on treatment in refluxing methanol with less than one equivalent of sodium methoxide, gave a 90% yield of the crystalline $\alpha,\beta$-unsaturated ketonic acid III, m.p. 178–179°, $\lambda_{max}$ 237 m$\mu$ ($\epsilon$ 12,100). The production of an $\alpha,\beta$-unsaturated ketonic acid establishes unequivocally that the original lactone possesses structure II with oxygen interpolated between $C_{13}$ and $C_{17}$ in a position for ready $\beta$-elimination with base.[5a]

In 1943, Goldberg and Wydler[6] observed that hydrogenation of dehydroepiandrosterone acetate cyanohydrin followed by treatment with nitrous acid produced two ketones formulated as 17-




II, R = H
IIa, R = $CH_3CO$ III

and 17a-keto D-homo systems. The structural assignments were based on conversion of the 17a-D-homo ketone into 1-methylchrysene. Recently Heusser and his associates[7] have intimated that the appearance of the two ketones was probably to be ascribed to the presence of two $C_{17}$-stereoisomeric hydroxyamines which in turn arose from an original mixture of 17$\alpha$- and 17$\beta$-cyanohydrins. As if to confirm this conclusion dehydroepiandrosterone acetate was converted to its cyanohydrin and the latter acetylated to give pure $\Delta^5$-3$\beta$,17$\beta$-diacetoxy-17-isoetianic acid nitrile.[7] The latter compound on successive reduction with lithium aluminum hydride and treatment with nitrous acid was reported[7] to give the 17a-D-homo ketone exclusively.[8]

In the course of the present work we were able to establish that the Demianov rearrangement of 17-hydroxy-20-amino-nor-pregnanes is independent of the configuration at $C_{17}$ and gives in each instance a mixture of 17- and 17a-ketones in a ratio of *ca.* 1:6. Thus etiocholane-3$\alpha$-ol-11,17-dione (I) was converted to 3$\alpha$,17$\beta$-diacetoxy-11-keto-17-isoetianic acid nitrile (IVa). Reduction of IVa with lithium aluminum hydride and subsequent rearrangement of the intermediate amine IVb with nitrous acid followed by oxidation at $C_{11}$ produced 10–15% of D-homoetiocholane-3$\alpha$-ol-11,17-dione acetate (VI), m.p. 203–205° and 85–90% of D-homoetiocholane-3$\alpha$-ol-11,17a-dione acetate (VII), m.p. 164–165°. By an alternative approach pregnane-3$\alpha$,17$\alpha$,21-triol-11,20-dione (V) was reduced with lithium aluminum hydride and the reduction product cleaved with periodic acid to the corresponding $C_{20}$-aldehyde, the latter being isolated as its alkali-

(1) Presented in part at the American Chemical Society Meeting-in-miniature, January 24, 1955, Newark, N.J.

(2) W. W. Westerfeld, *J. Biol. Chem.*, **143**, 177 (1942).

(3) (a) R. P. Jacobsen, *ibid.*, **171**, 61 (1947); (b) H. Levy and R. P. Jacobsen, *ibid.*, **171**, 71 (1947); (c) R. P. Jacobsen, G. M. Picha and H. Levy, *ibid.*, **171**, 81 (1947); (d) M. Keller and J. Weiss, *J. Chem. Soc.*, 1247 (1951); (e) R. N. Jones, P. Humphries and K. Dobriner, THIS JOURNAL, **72**, 956 (1950); (f) C. von Seemann and G. A. Grant, *ibid.*, **72**, 4073 (1950); (g) E. B. Hershberg, E. Schwenk and E. Stahl, *Arch. Biochem.*, **19**, 300 (1948); (h) G. Picha, THIS JOURNAL, **74**, 703 (1952); (i) J. Fried, R. W. Thoma and A. Klingsberg, *ibid.*, **75**, 5764 (1953).

(4) For a recent reference on this topic see: N. S. Leeds, D. K. Fukushima and T. F. Gallagher, *ibid.*, **76**, 2265 (1954).

(5) L. H. Sarett, *J. Biol. Chem.*, **162**, 601 (1946).

(5a) The configuration at $C_{13}$ would not be expected to be altered in the peracid reaction; *c.f.* T. F. Gallagher and T. H. Kritchevsky, THIS JOURNAL, **72**, 882 (1950); R. B. Turner, *ibid.*, **72**, 878 (1950).

(6) For a leading reference see M. W. Goldberg and R. Wydler, *Helv. Chim. Acta*, **26**, 1142 (1943).

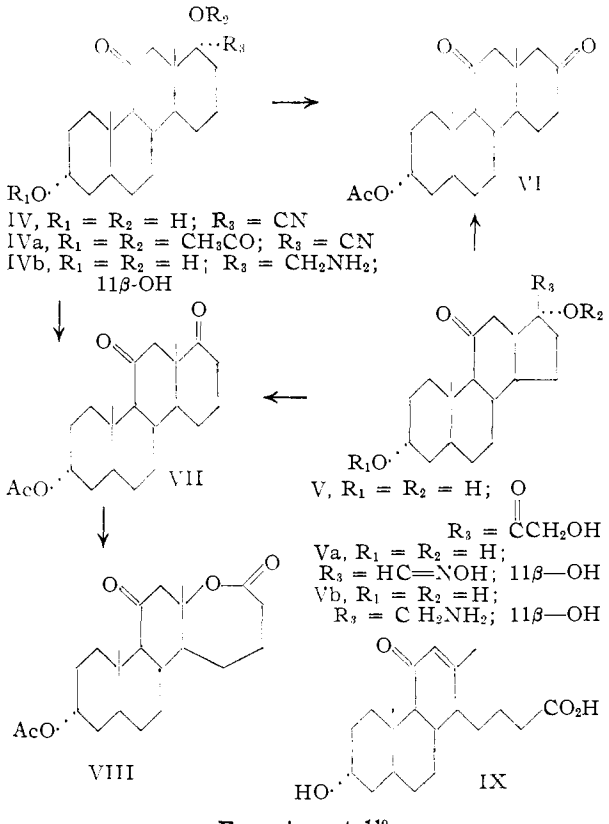
(7) H. Heusser, P. Th. Herzig, A. Fürst and Pl. A. Plattner, *ibid.*, **33**, 1093 (1950).

(8) F. Ramirez and S. Stafiej, THIS JOURNAL, **77**, 134 (1955), recently have advanced a mechanism which would accommodate dependence of the course of rearrangement on the configuration at $C_{17}$.

soluble oxime Va. Hydrogenation of Va and nitrous acid rearrangement of the intermediate amine Vb followed by chromic acid oxidation at $C_{11}$ afforded the two ketones VI and VII in the identical ratio as had been experienced from IVb.

The structure of the 17a-ketone VII, and consequently the 17-ketone VI as well, was established by converting VII to the 7-membered lactone VIII with perbenzoic acid. Treatment of this lactone with refluxing base in the manner described for its 6-membered counterpart II (see above) produced the $\alpha,\beta$-unsaturated ketonic acid IX, m.p. 164–166°, $\lambda_{max}$ 237.5 m$\mu$ ($\epsilon$ 12,500).

Thus the preponderant 16,17-bond migration involved in the Demianov rearrangements stands in marked contrast to the essentially exclusive 13,17-bond transposition of the peracid reaction. The mechanistic distinction between these two reactions is, therefore, not without an element of subtlety inasmuch as both are presumed to share the common feature of an electron-deficient center in the rearranging species.[9]




## Experimental[10]

**Etiocholane-3α-ol-11,17-dione Acetate** (Ia).[5,11]—To a solution of pregnane-3α,17α-diol-11,20-dione 3α-acetate, m.p. 202–205° (60 g.) in acetic acid (600 ml.) was added chromic oxide (11.3 g., equivalent ratio 1.1:1) in water (5 ml.) and acetic acid (100 ml.). After three days at 25° the solvent was removed *in vacuo*, water added and the product extracted with ethyl acetate. The organic solution was washed with 5% aqueous sodium carbonate and water and dried over anhydrous magnesium sulfate. The residue remaining after removal of the solvent was dissolved in 2:1 petroleum ether–benzene (1200 ml.) and chromatographed on acid-washed alumina (500 g.). The column was eluted with petroleum ether–benzene, benzene and benzene–chloroform mixtures. From the petroleum ether–benzene fractions there was obtained etiocholane-3α-ol-11,17-dione acetate (Ia), 21.5 g., m.p. 159–161°, and 6.5 g., m.p. 156–159°. From the benzene and benzene–chloroform eluates starting material was recovered; 15 g., m.p. 194–200°. The conversion yield of 17-ketone Ia was 70%.[12]

**Etiocholane-3α-ol-11,17-dione** (I).[5]—Etiocholane-3α-ol-11,17-dione acetate (Ia) (14.0 g.) was dissolved in a solution of sodium hydroxide (12.0 g.) in 50% methanol (240 ml.). After 30 minutes at 25° acetic acid (18 ml.) was added, the mixture was concentrated to 150 ml. and water (300 ml.) was added. The product was filtered, washed several times with water and dried *in vacuo;* 12.2 g. (99%), m.p. 186–188°.

**Etiochololactone-3α-ol-11-one** (II).—To a solution of etiocholane-3α-ol-11,17-dione (I) (15.0 g.) in benzene (225 ml.) was added perbenzoic acid in benzene (100 ml., 0.32 *M*). After 48 hours at room temperature the mixture was concentrated *in vacuo* to a thick slurry, triturated with ether (75 ml.), filtered and washed with ether. Crystallization from acetone–ether gave the lactone II as massive prisms, 7.3 g., m.p. 212–218°; 3.4 g., m.p. 202–206° (75%). Recrystallization from acetone–petroleum ether raised the melting point to 216–220°, $[\alpha]_D$ −12° (*c* 1.08); $\lambda_{max}^{CHCl_3}$ 2.92, 5.80, 5.85 $\mu$.

*Anal.* Calcd. for $C_{19}H_{28}O_4$: C, 71.23; H, 8.81. Found: C, 71.65; H, 9.05.

**Etiochololactone-3α-ol-11-one Acetate** (IIa).—To a solution of etiocholane-3α-ol-11,17-dione acetate (Ia) (1.73 g.) in acetic acid (5.0 ml.) was added peracetic acid in acetic acid (4.8 ml., 1.25 *M*) and the colorless mixture was kept at 25° for 24 hours. Slow addition of cold water (100 ml.) gave the crystalline product, 1.41 g. (77%), m.p. 170–180°. Crystallization from acetone–ether gave pure lactone acetate IIa as elongated prisms, m.p. 181–184°, $[\alpha]_D$ +14° (*c* 0.77); $\lambda_{max}^{CHCl_3}$ 5.78, 5.86 $\mu$.

*Anal.* Calcd. for $C_{21}H_{30}O_5$: C, 69.58; H, 8.34. Found: C, 69.24; H, 8.10.

**Unsaturated Acid** III.—To etiochololactone-3α-ol-11-one (II) (0.500 g.) in refluxing methanol (10 ml.) was added over the course of one hour 5.2 ml. of 0.27 *N* methanolic sodium methoxide (equivalent ratio 0.9:1). Removal of the methanol *in vacuo* and addition of ether afforded the sodium salt of the acid III which was filtered, washed with ether and dissolved in a few ml. of water. Addition of dilute hydrochloric acid followed by ether extraction in the usual way gave crystalline unsaturated acid III which was recrystallized from acetone–Skellysolve B; glistening plates 350 mg., m.p. 178–179°; 50 mg., m.p. 174–176°; corrected yield, 89%.[13] Further recrystallization did not raise the m.p. above 178–179°, $[\alpha]_D$ −32.4° (*c* 0.79); $\lambda_{max}^{CH_3OH}$ 237 m$\mu$, $\epsilon$ 12,100; $\lambda_{max}^{nujol}$ 3–4, 5.87, 6.00, 6.12 $\mu$.

*Anal.* Calcd. for $C_{19}H_{28}O_4$: C, 71.23; H, 8.81. Found: C, 70.57; H, 8.79.

The unsaturated acid III also was obtained from the acetoxy lactone IIa by treatment with sodium hydroxide in refluxing methanol (equivalent ratio 1.9:1).

**A. Transformations in the 17β-Hydroxy-21-norpregnane Series.**—Etiocholane-3α-ol-11,17-dione acetate (5 g.) was

(9) Arguments of a kind have been advanced to rationalize differences observed between solvolytically induced Wagner–Meerwein rearrangements and those evoked by the nitrous acid–amine reaction. See, for example, J. G. Burr, Jr., and L. S. Ciereszko, THIS JOURNAL, **74**, 4526 (1952); **74**, 5431 (1952); P. S. Bailey and J. G. Burr, Jr., *ibid.*, **75**, 2951 (1953); J. D. Roberts, C. C. Lee and W. H. Saunders, Jr., *ibid.*, **76**, 4501 (1954).

(10) Melting points are corrected. The rotations were taken in chloroform at 25°.

(11) T. H. Kritchevsky, D. L. Garmaise and T. F. Gallagher, THIS JOURNAL, **74**, 483 (1952). Reduction of the relative quantity of chromic oxide used by these authors resulted in an improved yield.

(12) When 3.3 equivalents of $CrO_3$ per equivalent of starting material were used (18 hours, 25°) there was obtained along with the 17-ketone Ia (50%) and recovered starting material, about 10% of etiochololactone-3α-ol-11-one acetate (IIa), m.p. 182–184°, identical with the lactone produced by reaction of peracetic acid with the ketone Ia (see below). The analogous direct formation of isoandrololactone acetate by perbenzoic acid treatment of Reichstein's Substance "L" acetate was reported recently by Leeds, Fukushima and Gallagher.[4]

(13) Sufficient base was present for reaction with only 450 mg. of lactone II.

dissolved in 150 cc. of ethanol and treated at 0° with 30 g. of potassium cyanide followed by the dropwise addition of 34 cc. of acetic acid with stirring. The temperature was maintained at 0–5° during the addition and for 1 hour thereafter. The reaction mixture was stirred additionally at room temperature for 2 hours, then poured into ice-water and extracted with ethyl acetate. The ethyl acetate extracts were dried, concentrated to dryness and the residue acetylated in 25 cc. of pyridine with 10 cc. of acetic anhydride to afford 4 g. (first crop) of IVa from ethyl acetate–Skellysolve B, m.p. 183–185° $[\alpha]_D$ +23°.

*Anal.* Calcd. for $C_{24}H_{33}O_5N$: C, 69.40; H, 7.95; N, 3.37. Found: C, 68.92; H, 7.64; N, 3.63.

A solution of 2 g. of the cyanohydrin acetate (IVa) in 50 cc. of benzene was reduced by refluxing for 1 hour with 3 g. of lithium aluminum hydride in 250 cc. of ether. The reaction mixture was worked up in the described manner[7] and extracted with acetone to give the **oxazolidine derivative of 21-norpregnane-3α,11β,17β-triol-20-amine** as rod-like prisms from ethyl acetate–Skellysolve B, m.p. 190–192°.

*Anal.* Calcd. for $C_{24}H_{39}O_3N$: C, 73.20; H, 10.32; N, 3.72. Found: C, 73.45; H, 10.29; N, 3.73.

A solution of 1 g. of the above amine in 95 ml. of 5% aqueous acetic acid was chilled to 0° and treated with a solution of 0.35 g. of sodium nitrite in 8 cc. of water. The reaction mixture was allowed to stand 3 hours at 0° and overnight at room temperature. During this period 0.6–0.7 g. of solid product separated. The latter was acetylated with 4 ml. of pyridine and 2 ml. of acetic anhydride at room temperature overnight followed by oxidation in 5 ml. of acetic acid containing 130 mg. of chromic acid. Chromatography of the product on acid-washed alumina yielded from eluates of 1–5% ether in benzene, 340 mg. of D-homoetiocholane-3α-ol-11,17a-dione acetate (VII)[14] as needles from acetone–Skellysolve B, m.p. 167–168°, $[\alpha]_D$ +28.7° (*c* 1.02).

*Anal.* Calcd. for $C_{22}H_{32}O_4$: C, 73.33; H, 8.88. Found: C, 73.50; H, 8.77.

**2,4-Dinitrophenylhydrazone** crystallized as yellow needles from methanol–ethyl acetate, m.p. 220–225°.

*Anal.* Calcd. for $C_{28}H_{36}O_7N_4$: N, 10.37. Found: N, 10.48.

The eluates from the chromatography consisting of 10–20% ether in benzene yielded 52 mg. of **D-homoetiocholane-3α-ol-11,17-dione acetate (VI)** as plates from acetone–ether, m.p. 205–207°, $[\alpha]_D$ +23.0° (*c* 1.09).

*Anal.* Calcd. for $C_{22}H_{32}O_4$: C, 73.33; H, 8.88. Found: C, 73.29; H, 8.91.

**2,4-Dinitrophenylhydrazone** crystallized as needles from methanol–ethyl acetate, m.p. 281–283°.

*Anal.* Calcd. for $C_{28}H_{36}O_7N_4$: N, 10.37. Found: N, 10.54.

B. **Transformations in the 17α-Hydroxy-21-norpregnane Series.**—A solution of 6 g. of pregnane-3α,17α-21-triol-11,-20-dione 21-acetate in 600 ml. of tetrahydrofuran was reduced with 6 g. of lithium aluminum hydride. Addition was effected at room temperature after which the reaction mixture was refluxed for 1.5 hours. The excess lithium aluminum hydride was decomposed with ethyl acetate followed by 200 ml. of a saturated solution of sodium sulfate and 200 g. of magnesium sulfate. The reaction mixture was filtered and the filtrate evaporated to give 6 g. of crude pentol.

The above crude pentol (6 g.) in 250 ml. of dioxane was treated with 3.72 g. of periodic acid ($H_5IO_6$) in 70 ml. of water and the reaction solution allowed to stand for 4 hours. At the end of this period the reaction mixture was evaporated to near-dryness, the residue extracted with ether and the ether extract washed with water and evaporated to an oil. This oil was dissolved in 100 ml. of methanol and treated with 2.5 g. of hydroxylamine hydrochloride and 3 g. of sodium acetate in 10 ml. of water, heated to boiling on a water-bath and allowed to stand overnight at room temperature. The reaction mixture from the oximation was evaporated nearly to dryness, the residue dissolved in ether and the oxime Va extracted with 10% aqueous sodium hydroxide. Acidification of the alkaline extracts with hydrochloric acid deposited the crude oxime Va as a gum. The latter was taken up in ether–ethyl acetate, dried over magnesium sulfate and evaporated to give 3.8 g. of crude Va as an amorphous residue.

A solution of 2.8 g. of the above crude oxime Va in 50 cc. of acetic acid was hydrogenated using 280 mg. of platinum oxide. The uptake of hydrogen was rapid and conformed to essentially 1 mole.[15] The catalyst was filtered and the filtrate made *ca.* 50% with water, then treated dropwise at 0° with a solution of 1 g. of sodium nitrite in 30 ml. of water. The reaction product, which had separated largely (1.6 g.) during the overnight reaction period, was acetylated, back oxidized at $C_{11}$ with chromic acid and chromatographed. From the 1–2% ether in benzene eluates was obtained 340 mg. of the 17a-ketone VII, m.p. 164.5–165.5°. The 10% ether–benzene eluates yielded 50 mg. of the 17-ketone VI, m.p. 203–206°.

**D-Homoetiochololactone-3α-ol-11-one Acetate (VIII).**—D-Homoetiocholane-3α-ol-11,17a-dione acetate (VII) (1.08 g.) in benzene (14 ml.) was mixed with perbenzoic acid in benzene (19.2 ml., 0.391 *M*). After 3 days at 25° the remaining peracid was destroyed and the reaction mixture worked up by addition of water and ether extraction. The product was crystallized from ether; 760 mg., m.p. 170–172°; 102 mg., m.p. 163–166°; yield 85%. Recrystallization from ether–petroleum ether gave plates m.p. 172–174°, $[\alpha]_D$ −24° (*c* 0.77); $\lambda_{max}^{CHCl_3}$ 5.80, 5.85 μ.

*Anal.* Calcd. for $C_{22}H_{32}O_5$: C, 70.18; H, 8.57. Found: C, 69.78; H, 8.31.

**Unsaturated Acid IX.**—A solution of D-homoetiochololactone-3α-ol-11-one acetate (188 mg.) in methanol (20 ml.) and aqueous 1.00 *N* sodium hydroxide (1.50 ml.) was refluxed one hour under nitrogen. Acetic acid (0.5 ml.) was added, the solvent removed *in vacuo* and the acidic product extracted into ether and worked up in the usual way. It crystallized spontaneously on removal of solvent and was recrystallized several times from acetone–ether as matted needles, m.p. 164–166°, $[\alpha]_D$ −27° (*c* 0.78); $\lambda_{max}^{CH_3OH}$ 237.5 mμ, ε 12,500; $\lambda_{max}^{CHCl_3}$ 3–4; 5.85, 6.00, 6.18 μ.

*Anal.* Calcd. for $C_{20}H_{30}O_4$: C, 71.81; H, 9.04. Found: C, 71.70; H, 8.79.

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(14) The best preparative procedure for this compound consists in the direct hydrogenation of the crude cyanohydrin (IV) followed, without isolation, by reaction with nitrous acid and chromatography. In this way an over-all yield of 67–77% of VII based on I can be realized (*cf.* also Ref. 6).

(15) Attempts to isolate this amine were unsuccessful. Unlike its 17β-hydroxy counterpart IVb, which gave a crystalline oxazolidine and was soluble in dilute mineral acid, the 17α-hydroxyamine (Va) did not give an isolable oxazolidine derivative and with dilute hydrochloric acid, precipitated an insoluble hydrochloride. The latter fact constitutes a facile method of separating the two $C_{17}$-stereoisomeric amines.