

222° (reported<sup>13</sup> m.p. 219–221°, reported<sup>15</sup> m.p. 223–225°), was obtained by crystallization from chloroform-

(15) J. von Euw, A. Lardon and T. Reichstein, *ibid.*, **27**, 1287 (1944).

petroleum ether.

*Anal.* Calcd. for  $C_{22}H_{32}O_6$ : C, 70.18; H, 8.57. Found: C, 70.34; H, 8.52.

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[CONTRIBUTION FROM SECTION OF BIOCHEMISTRY, MAYO CLINIC]

## Steroids Derived from Bile Acids. XX. Degradation of $3\alpha,9\alpha$ -epoxy-11-Ketonorcholanic Acid to $3\alpha,9\alpha$ -Epoxy-11-ketoetiocholanolic Acid<sup>1</sup>

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Methyl  $3\alpha,9\alpha$ -epoxy-11-ketonorcholanate has been degraded to  $3\alpha,9\alpha$ -epoxy-11-ketoetiocholanolic acid by a combination of the Barbier-Wieland and Hoehn-Mason procedures in a yield of 36%.

Cleavage of the oxide ring in methyl  $3\alpha,9\alpha$ -epoxy-11-ketocholanate with hydrogen bromide and degradation of the  $3\alpha$ -hydroxy steroid thus obtained to give  $3\alpha$ -acetoxypregnane-11,20-dione and  $3\alpha$ -acetoxy-11-ketoetiocholanolic acid have been reported.<sup>2</sup> Degradation of the side chain was achieved by a modification of the Barbier-Wieland method, and the pregnane was converted into the etio acid by the Hoehn-Mason scheme. This paper describes the use of these procedures for conversion of methyl  $3\alpha,9\alpha$ -epoxy-11-ketonorcholanate into  $3\alpha,9\alpha$ -epoxy-11-ketoetiocholanolic acid.

ether was inert toward oxidation it was advantageous to degrade the side chain to the etio acid before restoration of the reactive hydroxyl group at C-3. However, in a study of the cleavage of the oxide ring at the nor, bisnor and etio ester stages it was found that there was a progressive decrease in the reactivity of the oxide ring toward hydrogen bromide.<sup>3</sup> The conditions which were satisfactory for the ester of  $3\alpha,9\alpha$ -epoxy-11-ketonorcholanolic acid failed to open the oxide completely when the ester of the etiocholanolic acid was used; more severe conditions<sup>3</sup> led to formation of the  $3,12\alpha$ -dibromo-11-keto derivative of etiocholanolic acid.

### Experimental<sup>4</sup>

**$3\alpha,9\alpha$ -Epoxy-11-keto-23,23-diphenylnorcholan-23-ol (II).**—A solution of 19.49 g. of methyl  $3\alpha,9\alpha$ -epoxy-11-ketonorcholanate in 100 ml. of benzene was added slowly with mechanical stirring to 200 ml. of an ethereal solution of 1.5 M phenylmagnesium bromide in a flask immersed in a bath at  $-20^\circ$ . The solution of steroid was added at such a rate that the temperature in the flask was  $0$  to  $3^\circ$ . The reaction mixture was stirred for 3 hours at  $0^\circ$ , the flask was sealed and stored in an ice-bath. After 24 hours the solution was poured over ice and ammonium chloride, the organic phase was washed and concentrated to dryness and the residue was crystallized from acetone-methanol. The volatile products were removed from the filtrate by steam distillation and more material was separated from acetone-methanol; total yield 81%. The analytical sample melted at  $152$ – $153^\circ$ ,  $[\alpha]_D^{25} +61 \pm 1^\circ$ .

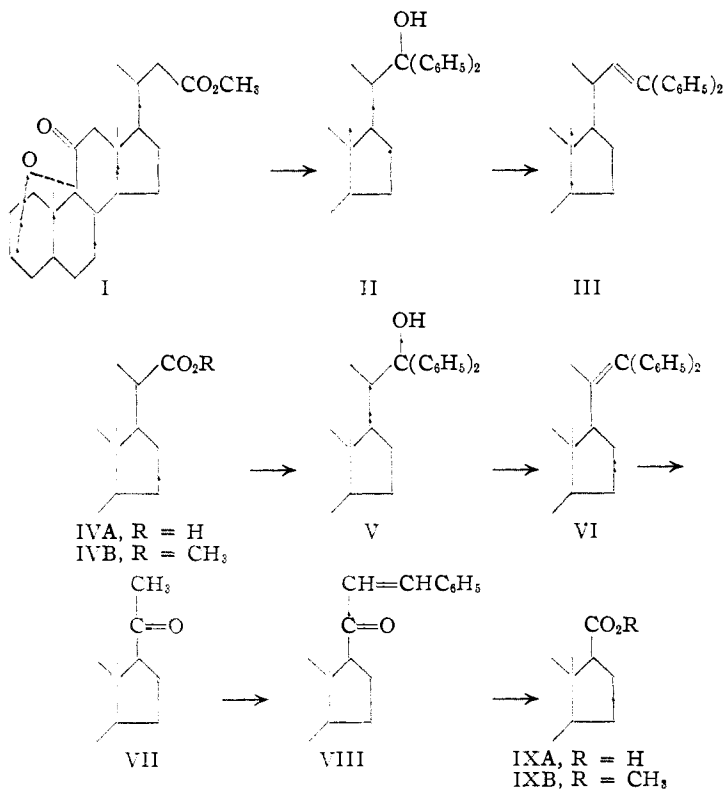
*Anal.* Calcd. for  $C_{35}H_{44}O_3$ : C, 81.99; H, 8.65. Found: C, 82.22; H, 8.55.

**$3\alpha,9\alpha$ -Epoxy-23,23-diphenyl- $\Delta^{22}$ -norcholen-11-one (III).**—A solution of 5.39 g. of  $3\alpha,9\alpha$ -epoxy-11-keto-23,23-diphenylnorcholan-23-ol in 10 ml. of acetic acid was refluxed for 1 hour and poured into water. The precipitate was collected, dried and recrystallized from acetone-methanol; wt. 5.02 g. (97%), m.p.  $136$ – $139^\circ$ . The analytical sample melted at  $136.5$ – $137.5^\circ$ ,  $[\alpha]_D^{25} +172 \pm 2^\circ$ .

*Anal.* Calcd. for  $C_{35}H_{42}O_2$ : C, 84.97; H, 8.56. Found: C, 85.12; H, 8.61.

(3) R. B. Turner, V. R. Mattox, L. L. Engel, B. F. McKenzie and E. C. Kendall, *J. Biol. Chem.*, **166**, 345 (1946).

(4) All melting points were determined on the Fisher-Johns apparatus. We are indebted to Merck & Co., Inc., Rahway, New Jersey, and to Mr. William Saschek, Department of Biochemistry, Columbia University, for microanalyses. Optical rotations were taken in chloroform ( $c \sim 1$ ) at  $27 \pm 2^\circ$ .



Presence of the  $3,9$ -epoxide instead of the  $3\alpha$ -hydroxy group facilitated formation of the diphenylcarbinol derivatives, and since the cyclic

(1) The work reported in this paper was carried out in 1943–1946.

(2) R. B. Turner, V. R. Mattox, W. F. McGuckin and E. C. Kendall, *THIS JOURNAL*, **74**, 5814 (1952).

**3 $\alpha$ ,9 $\alpha$ -Epoxy-11-ketobisnorcholanolic Acid (IVA).**—3 $\alpha$ ,9 $\alpha$ -Epoxy-23,23-diphenyl- $\Delta^{22}$ -norcholen-11-one (33.1 g.) was oxidized as described in the preparation of 3 $\alpha$ ,9 $\alpha$ -epoxy-11-ketonorcholanolic acid.<sup>3</sup> The product, crystallized from acetone, separated in three crops and was dried to constant weight at 105°; I, 17.89 g., m.p. 181–182°; II, 1.91 g., m.p. 179–181°; III, 0.28 g., m.p. 178–180°; total crude yield, 79%. A sample of the acid that had been crystallized from acetone and dried at about 40° melted at 78–80° with effervescence, recrystallized on the slide and remelted at 182–184°. The sample lost 13.3% in weight when dried at 105°. Calculated for 1 mole of acetone, 13.9%. The dried sample melted at 182.5–183.5°,  $[\alpha]_D^{25} +69 \pm 2^\circ$ .

*Anal.* Calcd. for C<sub>22</sub>H<sub>32</sub>O<sub>4</sub>: C, 73.30; H, 8.95. Found: C, 73.37; H, 8.88.

**Methyl 3 $\alpha$ ,9 $\alpha$ -Epoxy-11-ketobisnorcholanate (IVB).**—3 $\alpha$ ,9 $\alpha$ -Epoxy-11-ketobisnorcholanolic acid (18.9 g.) was esterified in 300 ml. of methanol, which contained 3 ml. of concentrated sulfuric acid. The solution was refluxed for 8 hours, and cooled in an ice-bath. Crystals (16.38 g., m.p. 138–139°) which separated were filtered and washed with water. Concentration of the filtrate gave 2.56 g. of ester (m.p. 137–138°), and a third crop (0.68 g., m.p. 137–138°) was obtained after dilution with water, extraction with benzene, concentration and crystallization from cold methanol. The total yield was 99.5%,  $[\alpha]_D^{25} +77 \pm 2^\circ$ .

*Anal.* Calcd. for C<sub>23</sub>H<sub>34</sub>O<sub>4</sub>: C, 73.76; H, 9.15. Found: C, 74.05; H, 9.16.

**3 $\alpha$ ,9 $\alpha$ -Epoxy-11-keto-22,22-diphenylbisnorcholan-22-ol (V).**—Methyl 3 $\alpha$ ,9 $\alpha$ -epoxy-11-ketobisnorcholanate (3.75 g.) as a dry powder was added in portions with mechanical stirring to 29 ml. of an ethereal solution of 2.1 molar phenylmagnesium bromide and 29 ml. of dry ethyl morpholine at 0°. The solution, protected from moisture with a calcium chloride tube, was kept at room temperature for 24 hours and at 45–50° for 48 hours and was then poured over ice, in dilute acetic acid. The aqueous phase was extracted thrice with benzene, the extracts were combined, washed with dilute acetic acid, dilute sodium hydroxide solution and with water. The solvent was removed and 4.19 g. (84%) of crystals, m.p. 201°, was obtained from methanol which contained a small amount of acetone. A purified sample melted at 204–205°,  $[\alpha]_D^{25} +9.5 \pm 1^\circ$ .

*Anal.* Calcd. for C<sub>34</sub>H<sub>42</sub>O<sub>3</sub>·1/2H<sub>2</sub>O: C, 80.43; H, 8.54. Found: C, 80.48, 80.57; H, 8.65, 8.51.

**3 $\alpha$ ,9 $\alpha$ -Epoxy-22,22-diphenyl- $\Delta^{20(22)}$ -bisorcholen-11-one (VI).**—A solution of 2.23 g. of 3 $\alpha$ ,9 $\alpha$ -epoxy-11-keto-22,22-diphenylbisnorcholan-22-ol in 10 ml. of acetic acid was refluxed for 2 hours and cooled. The ethylene, after recrystallization from acetic acid, weighed 1.98 g. (92%) and melted at 197–198°,  $[\alpha]_D^{25} +275 \pm 2^\circ$ .

*Anal.* Calcd. for C<sub>34</sub>H<sub>40</sub>O<sub>2</sub>: C, 84.95; H, 8.93. Found: C, 84.86; H, 8.64.

**3 $\alpha$ ,9 $\alpha$ -Epoxy-11-ketopregnane-11,20-dione (VII).**—A solution of 10.0 g. (20.8 millimoles) of 3 $\alpha$ ,9 $\alpha$ -epoxy-22,22-diphenyl- $\Delta^{20(22)}$ -bisorcholen-11-one in a mixture of 30 ml. of chloro-

form and 30 ml. of methanol was cooled in an ice-salt-bath, and 22.5 millimoles of ozone was passed into the solution. Twenty grams of zinc dust and 40 ml. of 95% acetic acid were added and the solution was stirred until it gave a negative starch-iodide test. The zinc was removed by filtration, the solution was concentrated and the residue was crystallized (4.48 g., m.p. 123°) from methanol. Volatile products were removed from the filtrate with steam and more of the product (1.76 g., m.p. 123°; 0.18 g., m.p. 120°) was crystallized from methanol. The total crude yield was 93%. Purified material melted at 126–127°,  $[\alpha]_D^{25} +167 \pm 1^\circ$ .

*Anal.* Calcd. for C<sub>21</sub>H<sub>30</sub>O<sub>3</sub>: C, 76.32; H, 9.15. Found: C, 76.22; H, 8.96.

**3 $\alpha$ ,9 $\alpha$ -Epoxy-21-benzylidenepregnane-11,20-dione (VIII).**—3 $\alpha$ ,9 $\alpha$ -Epoxy-11-ketopregnane-11,20-dione (3.30 g.) was dissolved in 25 ml. of hot methanol, the solution was cooled to room temperature and 3.0 ml. of benzaldehyde (freshly distilled) was added. After addition of a solution of sodium methylate (1.0 g. of sodium in 10 ml. of methanol) the mixture was allowed to stand at room temperature for 48 hours. The product which separated was filtered and washed with cold methanol. After recrystallization from chloroform-methanol (1:10) the material (3.74 g.) melted at 194–195°,  $[\alpha]_D^{25} +91 \pm 2^\circ$ .

*Anal.* Calcd. for C<sub>23</sub>H<sub>34</sub>O<sub>3</sub>: C, 80.34; H, 8.19. Found: C, 80.43; H, 8.31.

**3 $\alpha$ ,9 $\alpha$ -Epoxy-11-ketoetiocholanolic Acid (IXA).**—A solution of 4.18 g. of 3 $\alpha$ ,9 $\alpha$ -epoxy-21-benzylidenepregnane-11,20-dione in 30 ml. of chloroform, 15 ml. of ethyl acetate and 15 ml. of methanol was cooled to 0° and 11 millimoles of ozone was passed into the solution. Four grams of zinc dust and 20 ml. of 85% acetic acid were added and the solution was stirred for 20 minutes at 0°. The zinc was filtered off, washed with ether, the combined filtrates were washed with water and concentrated under reduced pressure. The residue was dissolved in 30 ml. of ethyl acetate and 20 ml. of acetic acid, and to the solution 15 ml. of 7.7 N periodic acid and 1.5 ml. of concentrated sulfuric acid were added. After 3 hours at room temperature the solution was diluted with water, the product was extracted with ether, and the ethereal solution was washed with water and with 0.5 N potassium hydroxide. Acidification of the alkaline solution liberated the etiocholanolic acid which was filtered off, washed and dried, wt. 2.92 g. (88%), m.p. 232–233°. Repeated recrystallization of the acid from ether-petroleum ether did not raise the melting point,  $[\alpha]_D^{25} +116 \pm 6^\circ$ .

*Anal.* Calcd. for C<sub>29</sub>H<sub>38</sub>O<sub>4</sub>: C, 72.26; H, 8.49. Found: C, 72.33; H, 8.49.

**Methyl 3 $\alpha$ ,9 $\alpha$ -Epoxy-11-ketoetiocholanate (IXB).**—The ester prepared from 3 $\alpha$ ,9 $\alpha$ -epoxy-11-ketoetiocholanolic acid with diazomethane in ether and crystallized from cold methanol, melted at 132–133°,  $[\alpha]_D^{25} +111 \pm 2^\circ$ .

*Anal.* Calcd. for C<sub>21</sub>H<sub>30</sub>O<sub>4</sub>: C, 72.80; H, 8.73. Found: C, 72.86; H, 8.69.

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