[CONTRIBUTION NO. 142 FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF TENNESSEE]

Ring-D Reactions of 3β-Acetoxy-5α-pregn-16-ene-12,20-dione

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The alkaline addition of methanol to the 16-dehydro-12,20-diketone II takes place if air is excluded. When this precaution is not taken the double bond is oxidized with the formation of the epoxide III. The latter was converted to 16α,17α-epoxy-5α-pregnane-3,12,20-trione (VIII), whose melting point is the same as Marker's "*allo*-pregnanetrione-3,12,20 hydrate." Proof of the epoxide structure was obtained by conversion to the bromohydrin V, reduction of which with zinc and acetic acid led to the pregnane VII, instead of the expected 17α-hydroxypregnane.

Early in our work with hecogenin we noted that treatment of 3β-acetoxy-5α-pregn-16-ene-12,20-dione (II) with methanolic potassium hydroxide yielded a substance identical with a by-product of the preparation of II itself from hecone diacetate I. Hydrolysis of I with potassium carbonate or potassium hydroxide in moist methanol, *t*-butyl alcohol or dioxane gave rise in part to this new product. The method of formation suggested that it belonged to the "hydrate" or "17-hydroxy" series of which "*allo*-pregnan-3,12,20-trione hydrate" was the first example.[2,3]

Like Marker's triketone this product could not be acetylated, showed no conjugated unsaturation and gave analytical values corresponding to the presence of an extra oxygen atom over that required for II. The compound did not belong to the 16α-methoxy series of Fukushima and Gallagher since it could be formed in the absence of methanol and failed repeatedly to display methoxyl on analysis.[4,5] An earlier experiment had suggested that this substance was in equilibrium with 3β-acetoxy-5α-pregn-16-ene-12,20-dione (II)[6] in the presence of alkali.

Lately we have been unable to duplicate the result of this experiment and find the new product does not acquire absorptivity in the 225–240 mμ range upon standing in methanolic alkali. The previously noted similarity between this compound and 3β-acetoxy-16α,17α-epoxy-5α-pregnane-12,20-dione,[6] which shows similar stability in alkali, was reconsidered and we find they are identical so that III is the structure of our "hydrate." This epoxide was originally made by oxidation of II with alkaline hydrogen peroxide. Its formation in moist alkaline alcohols or dioxane is probably due to air oxidation of the highly reactive double bond. Further proof of the epoxide structure was adduced from the conversion of the new product to the known bromohydrin V,[6] also obtained from the hydroxybromohydrin VI. Either III or V with alkali yielded the same hydroxyepoxide IV.

In an attempt to prepare further quantities of 3β-acetoxy-17α-hydroxy-5α-pregnane-12,20-dione with zinc and acetic acid, as reported previously, we found V to be converted mainly to VII. Although the reasons for departure from our previous results have not been determined,[7] it may be assumed that elimination of the elements of hypobromous acid followed by reduction of the C-16 double bond led to the product VII. Eliminations of hypobromous acid with zinc from positions 3,4[8] and 11,12[9] in steroids have been observed and the zinc reduction of conjugated double bonds in even less active systems than the one in question also has been reported.[10]

Moore and Wittle recorded the first addition of alcohol to a 16-dehydropregnane-12,20-dione, *viz.*, 2α,3β-diacetoxy-5,16-pregnadiene-12,20-dione which was obtained from kammogenin.[11] Working under ordinary conditions, they found none of the material of the "hydrate" series which Marker had subsequently considered to be 17α-hydroxy steroids.[3] Conversely, in many experiments we have never encountered any product which gave a true methoxyl analysis. Consequently, we were skeptical that the results with 3β-acetoxy-5,16-pregnadien-20-one can be extended to explain Marker's results with the 12,20-diketones.[4] We now have prepared 3β-acetoxy-16α-methoxy-5α-pregnane-12,20-dione (IX) by treating II with methanolic potassium hydroxide under nitrogen. This product is clearly different from III and the presence of the methoxyl group has been shown quantitatively. The MR_D in dioxane for the sequence IX (M_D +421) − II (M_D +461) is −40. The MR_D in chloroform for the analogous derivatives from kammogenin is −42.[11] With respect to the parent pregnanedione VII, the contribution of the 16α-methoxyl is −102 as compared with an average of about −200 for steroids not oxygenated at C-12.[4]

For purposes of comparison with Marker's "*allo*-pregnantrione-3,12,20 hydrate" ("*allo*-pregnanol-17-trione-3,12,20"),[2,3] we oxidized IV with sodium dichromate and obtained 16α,17α-epoxy-5α-pregnane-3,12,20-trione (VIII), melting at 265–268°.[12] The melting point given for Marker's compound is 262–264°; this is the only constant available.

We believe that compound VIII is identical with Marker's triketone and that one gets oxidation at

(1) G. D. Searle and Co., Skokie, Illinois.

(2) R. E. Marker, R. B. Wagner, P. R. Ulshafer, E. L. Wittbecker, D. P. J. Goldsmith and C. H. Ruof, THIS JOURNAL, **69**, 2167 (1947).

(3) R. E. Marker, *ibid.*, **71**, 2656, 4149 (1949).

(4) D. K. Fukushima and T. F. Gallagher, *ibid.*, **73**, 196 (1951).

(5) All quantitative analyses are by Galbraith Microanalytical Laboratories, Knoxville, Tenn.

(6) G. P. Mueller, R. E. Stobaugh and R. S. Winniford, THIS JOURNAL, **76**, 4888 (1953).

(7) Dr. E. S. Rothman has kindly informed us that the Raney nickel–ethanol reduction of V is satisfactory for preparing the 17α-hydroxysteroid as we described it but that VII was also formed as a contaminating substance. In his hands, however, the zinc–acetic acid reduction of V gave about 50% of the epoxide III.

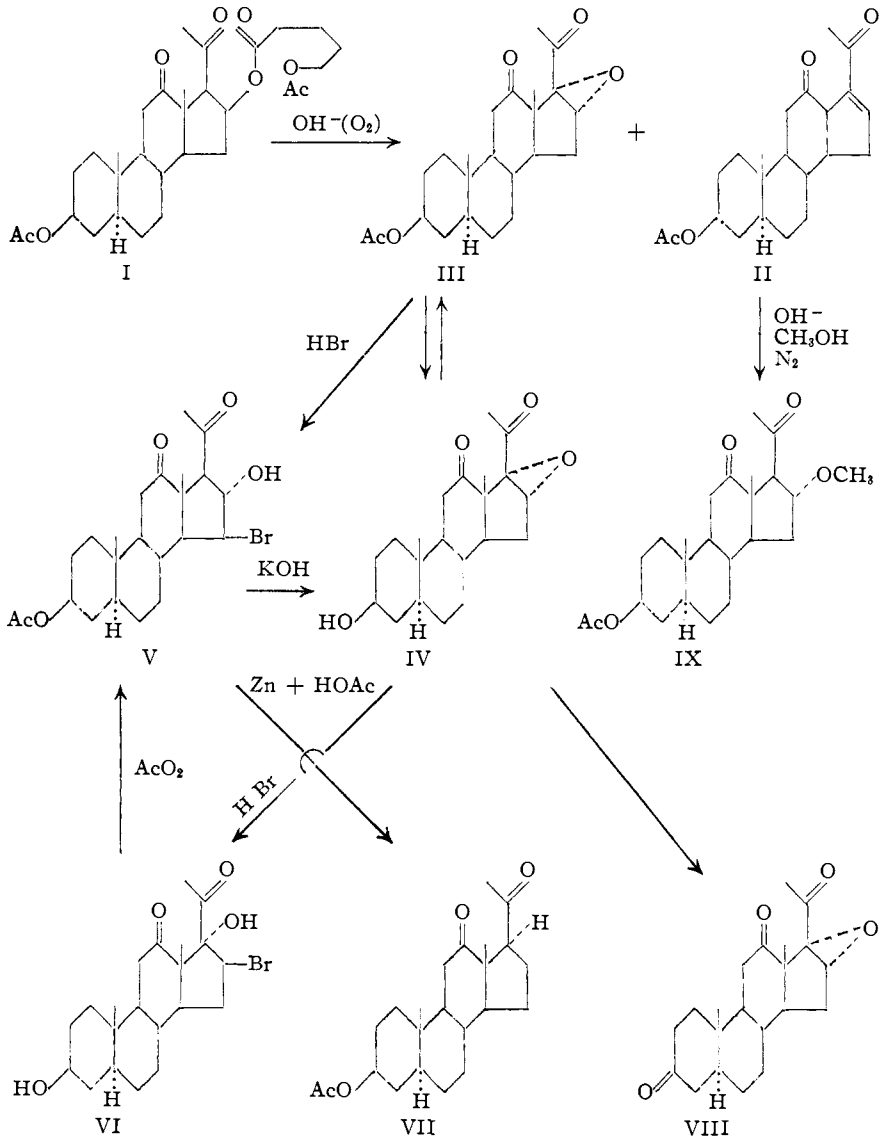
(8) L. F. Fieser and R. Ettorre, THIS JOURNAL, **75**, 1700 (1953).

(9) J. W. Cornforth, J. M. Osbond and G. H. Phillips, *J. Chem. Soc.*, 907 (1954).

(10) R. B. Woodward, F. Sondheimer, D. Taub, K. Heusler and W. M. McLamore, THIS JOURNAL, **74**, 4223 (1952), footnote 18.

(11) J. A. Moore and E. L. Wittle, *ibid.*, **74**, 6287 (1952).

(12) All melting points were taken on the Kofler hot-stage and are corrected.



the C-16 double bond on reaction of the 16-dehydropregnane-12,20-diones in old solvents under sufficiently alkaline conditions, as with potassium hydroxide or to some extent with potassium carbonate. It may be that the alcohol adduct is formed in freshly purified methanol or ethanol without the need of a nitrogen atmosphere to prevent oxidation. When comparing our product with what data appear in the literature on Marker's compound, one should note that VIII is a triketone and its infrared spectrum in potassium bromide shows no hydroxyl absorption. But in discussing different portions of an infrared spectrum, presumably of "*allo*-pregnan-3,12,20-trione hydrate," Wagner, Moore and Forker[13] considered a band at 3510 cm.$^{-1}$ possibly due to hydroxyl absorption, and Jones, Ramsay, Keir and Dobriner[14] by integration of carbonyl absorption intensities showed this compound to be a diketone. Direct comparison of infrared spectra showed that our compound VIII is indeed different from that represented in the foregoing work,[13,14] but there may be some question whether these spectra really represent Marker's original product.[15] Since no direct comparison with an authentic sample has been possible, our conclusion as to the nature of Marker's product is to this extent limited. Nevertheless, the other facts that have been deduced support the interpretation that has been made.

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Experimental

3β-Acetoxy-16α,17α-epoxy-5α-pregnane-12,20-dione (III) and 3β-Acetoxy-5α-pregn-16-ene-12,20-dione (II).—A solution of 3.5 g. of crude hecone diacetate I,[6] in 175 ml. of *t*-butyl alcohol, was treated at room temperature with 7 ml. of 12.7 *N* aqueous potassium hydroxide. The two-phase[16] mixture was stirred for 3 hours, poured into brine and worked up with ether after neutralizing the alkali. Distillation of the dried ether-butanol extracts *in vacuo*, acetylation of the residue in boiling acetic anhydride and removal of the latter *in vacuo* left 2.36 g. of yellow residue showing absorption, λ_{max} 229 mμ (ε 4669), corresponding to about 55 mole per cent. of II. This was chromatographed on 70 g. of acid-washed alumina; 1:1 petroleum ether–benzene eluted 698 mg. from which some hecogenin acetate was crystallized; next eluted with the same eluant and finally benzene was 1131 mg. of product from which III was crystallized using ether–hexane, ether–acetone and pure acetone. The epoxide crystallized as rectangular prisms, m.p. 237.0–238.5° (sweating at 233°), $[\alpha]_D$ +100°.[17] *Anal.* Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.32. Found: C, 71.05; H, 8.32. All physical properties and infrared spectra of this product and corresponding fractions obtained in other experiments as well as the epoxide prepared with alkaline hydrogen peroxide[6] were nearly identical. The third fraction, eluted from the column with 10% ether in benzene weighed 509 mg. and yielded 187 mg. of II, m.p. 174–175°, $[\alpha]_D$ +123°, λ_{max}^{alc} 227 mμ (log ε 3.82).

In another experiment aqueous methanolic potassium carbonate was used and the ultraviolet absorption again

(13) R. B. Wagner, J. A. Moore and R. F. Forker, THIS JOURNAL, **71**, 4159 (1949).

(14) R. N. Jones, D. A. Ramsay, D. S. Keir and K. Dobriner, *ibid.*, **74**, 80 (1952).

(15) Correspondence with the investigators principally interested in this problem during the last few years revealed that all the original samples had been used. Dr. Gallagher, who kindly made the infrared comparisons for us, observed that our spectrum was not identical with any of those made at the Sloan-Kettering Institute a few years ago. He felt that any conclusion beyond the non-identity of our products with any of the others available was unwarranted.

(16) The use of a two-phase system instead of our original procedure was suggested by Dr. Rothman.

(17) Rotations are in dioxane unless otherwise specified.

showed about 50 mole per cent. of 16-dehydro steroid. Both II and III were isolated as just described.

3β-Hydroxy-16α,17α-epoxy-5α-pregnane-12,20-dione (IV).—A 250-mg. sample of III was hydrolyzed for one hour in 50 ml. of boiling 0.5 *N* methanolic potassium hydroxide. The solution was neutralized, concentrated to 25 ml. and diluted with 15 ml. of water. Shiny leaflets, 221 mg., separated on cooling. These were recrystallized twice from aqueous methanol, m.p. 202–203° (sweating at 178°, partially melting at 190° and recrystallizing as spherulites), $[\alpha]_D$ +112°. The sample was air-dried. *Anal.* Calcd. for $C_{21}H_{30}O_4 \cdot H_2O$: C, 69.18; H, 8.85. Found: C, 69.45; H, 8.99. A sample recrystallized from acetone–hexane was extremely hygroscopic. Rigorous drying over phosphorus pentoxide at 110° did not remove all of the water of crystallization. *Anal.* Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 71.02; H, 8.94.

Acetates obtained earlier from II in alkaline media, as referred to in the previous experiment, yielded the same product on hydrolysis. Reacetylation in boiling acetic anhydride yielded III.

16β-Bromo-3β-acetoxy-17α-hydroxy-5α-pregnane-12,20-dione (V).[6]—A solution of 350 mg. of III in 5.4 ml. of glacial acetic acid was treated in the cold with 3.5 ml. of 48% hydrobromic acid in 26 ml. of acetic acid. After 30 minutes at 40° the mixture was precipitated in water. The product was fractionally crystallized giving some starting material and 218 mg. of V from which the analytical sample was obtained as transparent cubes from ether, m.p. 178.0–179.5°, $[\alpha]_D$ +30°; +45.7° (ethanol). *Anal.* Calcd. for $C_{23}H_{33}O_5Br$: C, 58.83; H, 7.08; Br, 17.02. Found: C, 58.95; H, 7.20; Br, 16.91.

Treatment of V, 103 mg., for 24 hours in 25 ml. of 0.5 *N* methanolic potassium hydroxide gave 94% of IV which was recrystallized from aqueous methanol yielding 41 mg. of flat, transparent needles, m.p. 202–203° (recrystallizing as spherulites at 190°), $[\alpha]_D$ +113°. This did not depress the melting point of the sample of IV described above.

3β,17α-Dihydroxy-16β-bromo-5α-pregnane-12,20-dione (VI).—3β-Hydroxy-16α,17α-epoxy-5α-pregnane-12,20-dione, 175 mg., was treated with hydrobromic acid in acetic acid as described above yielding 91% of VI. This was recrystallized from acetone–hexane and appeared as granular crystals, m.p. 168.5–170.0°, $[\alpha]_D$ +31°; +45.3° (ethanol). *Anal.* Calcd. for $C_{21}H_{31}O_4Br$: C, 59.00; H, 7.31. Found: C, 58.59; H, 7.09.

Acetylation of VI with boiling acetic anhydride gave V.

Reduction of 3β-Acetoxy-17α-hydroxy-16β-bromo-5α-pregnane-12,20-dione (V) to 3β-Acetoxy-5α-pregnane-12,20-dione (VII).—A solution of V, 279 mg. in 35 ml. of glacial acetic acid, was treated with 3 g. of zinc dust, portions being added at 30-minute intervals for 2 hours while stirring at 95° in a nitrogen atmosphere. The hot solution was filtered into cold water and the product isolated with ether. Evaporation of the washed and dried ether solution left a residue which was triturated with ether. The insoluble residue was then crystallized four times from acetone yielding irregular transparent prisms of VII, m.p. and m.m.p. with authentic sample[6] 195.5–197.0° (with sweating at 184°), $[\alpha]_D$ +140°. The analysis was correct and the total yield after reprocessing the mother liquors was 40%.

Hydrolysis of the acetate overnight in methanolic sodium hydroxide and recrystallization from acetone afforded 3β-hydroxy-5α-pregnane-12,20-dione crystallizing as elongated transparent prisms, m.p. 195.0–197.5°, $[\alpha]_D$ +164°. *Anal.* Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.69; H, 9.59.

Reacetylation of the latter gave VII.

16α,17α-Epoxy-5α-pregnane-3,12,20-trione (VIII).—A solution of 650 mg. of sodium dichromate dihydrate in 15 ml. of glacial acetic acid was added over a 25-minute period to 225 mg. of IV in 60 ml. of 1:1 glacial acetic acid–benzene at 10°. After an hour an additional 325 mg. of oxidant in 5 ml. of solvent was added and the mixture allowed to stand 24 hours at 0°. The mixture was poured into cold, aqueous sodium bisulfite solution and extracted with ether. The washed and dried ether solution was evaporated and the residue triturated with more ether. The insoluble residue remaining was recrystallized four times from acetone; the elongated transparent prisms melted at 265.0–268.0° (sweating at 234° and becoming translucent at 239°, followed by slow growth of needles radiating from the crystalline mass at 247°) $[\alpha]_D$ +143°. *Anal.* Calcd. for $C_{21}H_{28}O_4$: C, 73.22; H, 8.19. Found: C, 73.17; H, 8.30. Reworking the mother liquors brought the over-all yield to 40%.

3β-Acetoxy-16α-methoxy-5α-pregnane-12,20-dione (IX).—A solution was prepared under nitrogen containing 1 g. of potassium hydroxide in 50 ml. of methanol which had been freshly distilled in a nitrogen atmosphere. To this was added 0.50 g. of 3β-acetoxy-5α-pregn-16-ene-12,20-dione. After standing at 40° for 15 minutes, the whole was poured into 100 ml. of cold water containing 2 ml. of concentrated hydrochloric acid. The dry product, isolated by ether extraction, was reacetylated overnight in 10 ml. of pyridine and 10 ml. of acetic anhydride. The mixture of acetates remaining after ether extraction washing and removal of the ether weighed 0.44 g. and was an oil. This was chromatographed on 20 g. of silica gel, the desired product, 0.31 g. being eluted with 10% ether in benzene and crystallized from cyclohexane. The heavy rectangular plates melted at 144.0–145.2°, $[\alpha]_D$ +104° ($CHCl_3$). There was no appreciable absorption in the 225–245 mμ region. *Anal.* Calcd. for $C_{24}H_{36}O_5$: C, 71.25; H, 8.97; OCH_3, 7.67. Found: C, 71.31; H, 9.35; OCH_3, 7.22.

KNOXVILLE, TENNESSEE

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SYNTEX, S. A.]

Steroids. LX.[1] Synthesis of C-2 Oxygenated Derivatives of Reichstein's Substance S and of Cortisone

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Syntheses of 2α-hydroxy-substance S (IVa) and 2α-hydroxy-cortisone (IVb), as well as of their 2,21-diacetates (IIIa and IIIb) and 2,17,21-triacetates (VIa and VIb), are described.

A number of hormone analogs bearing an α-hydroxy group at C-2 have previously been prepared by two different methods. The one involves direct acetoxylation of a Δ^4-3-ketone with lead tetraacetate,[2] followed by saponification of the resulting 2α- or 2β-acetoxy-Δ^4-3-ketone; the other involves acetolysis of a 6-bromo-Δ^4-3-ketone with potassium acetate,[2d,3] again followed by saponification. We now describe the employment of both these methods for the synthesis of 2α-hydroxy-substance S (IVa) and 2α-hydroxy-cortisone (IVb), as well as of their 2,21-diacetates (IIIa and IIIb) and 2,17,21-triacetates (VIa and VIb).

(1) Steroids. LIX, E. Batres, G. Rosenkranz and F. Sondheimer, THIS JOURNAL, **76**, 5171 (1954).

(2) (a) G. Ehrhart, H. Ruschig and W. Aumüller, *Angew. Chem.*, **52**, 363 (1939); *Ber.*, **72**, 2035 (1939); (b) T. Reichstein and C. Montigel, *Helv. Chim. Acta*, **22**, 1212 (1939); (c) E. Seebeck and T. Reichstein, *ibid.*, **27**, 948 (1944); (d) F. Sondheimer, S. Kaufmann, J. Romo, H. Martinez and G. Rosenkranz, THIS JOURNAL, **75**, 4712 (1953).

(3) (a) D. E. A. Rivett and E. S. Wallis, *J. Org. Chem.*, **15**, 35 (1950); (b) L. F. Fieser and M. A. Romero, THIS JOURNAL, **75**, 4716 (1953); (c) J. Herran, G. Rosenkranz and F. Sondheimer, *ibid.*, **76**, 5531 (1954).