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C-15 Substituted Steroids. 15α- and 15β-Hydroxy-Reichstein's Substance S and Transformation Products

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The preparation of 15α- and 15β-hydroxy-Reichstein's substance S by microbiological oxidation is described. Certain transformations of these compounds and stereochemical aspects are discussed.

Some time ago we reported[1] on the microbiological hydroxylation of Reichstein's substance S to form C15-hydroxylated derivatives.[2] This paper presents the experimental details of the previous report.

Fermentation of Reichstein's substance S with the *Spicaria simplicissima* (ATCC13595) provided a monohydroxylated Reichstein's substance S. This compound could be treated with calculated amounts of acetic anhydride in pyridine to form either a monoacetate or a diacetate. Oxidation of the monoacetate with chromium trioxide-pyridine gave a triketone which had an additional carbonyl absorption in the infrared spectrum that was very close to the acetate "C–O" stretching absorption. Treatment of this ketone acetate with sodium methoxide or sodium carbonate in methanol furnished a triketone diol whose saturated carbonyl absorption was at 1756 and 1722 cm.$^{-1}$ in the infrared spectrum. This clearly demonstrated that the new carbonyl function was contained in the D ring and, further, that the microbiologically introduced hydroxyl group from which the new carbonyl group was derived was in the D ring.

Since the above diacetate was not identical to either of the known diacetates of 16α- and 16β-hydroxy substance S[3] it must be presumed that the new hydroxyl group was introduced at the C-15-position. Consequently, the monohydroxylated Reichstein's substance S was assigned the structure 15β,17α,21 - trihydroxy - 4 - pregnene - 3,20 - dione (Ia) (evidence for the configurational assignment is discussed below). The monoacetate was then 21-acetoxy - 15β,17α - dihydroxy - 4 - pregnene - 3,20 - dione (Ib) and the diacetate, 15β,21-diacetoxy-17α-hydroxy-4-pregnene-3,20-dione (Ic). That the monoacetate Ib had its acetoxy function at C-21 was presumed from the expected preferred acylation of a primary hydroxyl group, from the shift in carbonyl absorption typical of a 20-carbonyl-21-acetoxy moiety in the infrared spectrum[4] and from the product of the oxidation of the monoacetate Ib which still contained the Blue Tetrazolium reactive side chain.

The product of oxidation of the monoacetate Ib then was presumed tentatively to be 21-acetoxy-17α-hydroxy-4-pregnene-3,15,20-trione (II). The free steroid, formed upon saponification of the acetate grouping of II, on reacetylation produced a new acetate isomeric with II. Since the new acetate and II are both 15-carbonyl compounds, it was reasonable to assume that they were epimeric at the C-14-position. Because the saponification of II was carried out with relatively strong bases (sodium methoxide or sodium carbonate) it was also assumed that the epimerization occurred at this step, and that the free diol and new acetate have the same configuration at C-14. In view of the difficulty in arbitrarily assigning the more stable configuration at C-14 (*cis*-hydrindanone *versus* *trans*-hydrindanone) when a different attachment on ring D at C-17 is investigated,[5] consideration for the actual configurations must rest first on the assumption that II is a *trans*-hydrindanone (14α-hydrogen) with the postulation that the mild chromium trioxide–pyridine oxidation of the monoacetate Ib would not cause epimerization at C-14. Secondly, it has been suggested by Barton and Laws[6] and further confirmed by others[5,7] that substitution of the C-15-position of a normal (14α-hydrogen) steroid with a keto group afforded a positive molecular rotation contribution to the molecule. On the other hand, if the addition of the keto group was accompanied by epimerization to the 14-iso-(14β-hydrogen)-steroid, a negative molecular rotation contribution was given to the molecule. The accompanying Table I illustrates that in structurally confirmed examples in an etiocholanic acid series[7] and a spirostane series[5] the 14β-hydrogen-15-ketone has a large negative rotation when compared to the molecular rotation of the 14α-hydrogen-15-ketone. In this work the 15-ketone-

(1) S. Bernstein, L. I. Feldman, W. S. Allen, R. H. Blank and C. E. Linden, *Chemistry & Industry*, 111 (1956).

(2) Other microbiological oxidations at C-15 of the steroid molecule are described in several other publications: (a) Ch. Meystre, E. Vischer and A. Wettstein, *Helv. Chim. Acta*, **38**, 381 (1955); (b) J. Fried, R. W. Thoma, D. Perlman, J. E. Herz and A. Borman, *Recent Progress in Hormone Research*, **11**, 149 (1955); (c) A. Wettstein, *Experientia*, **11**, 465 (1955); (d) C. Djerassi, L. B. High, J. Fried and E. F. Sabo, THIS JOURNAL, **77**, 3673 (1955); (e) E. L. Dulaney, W. J. McAleer, M. Koslowski, E. O. Stapley and J. Jaglom, *Appl. Microbiol.*, **3**, 336 (1955); (f) S. H. Eppstein, P. D. Meister, H. C. Murray and D. H. Peterson, *Vitamins and Hormones*, **14**, 359 (1956); (g) B. Klüger, R. Siebert and A. Schubert, *Naturwiss.*, **44**, 40 (1957); (h) A. Schubert and R. Siebert, *Chem. Ber.*, **90**, 2576 (1957); (i) A. Schubert and R. Siebert, *ibid.*, **91**, 1856 (1958); (j) A. Schubert, R. Siebert and L. Koppe, *Angew. Chem.*, **70**, 742 (1958); (k) A. Gubler and Ch. Tamm, *Helv. Chim. Acta*, **41**, 301 (1958); (l) W. J. McAleer, T. A. Jacob, L. B. Turnbull, E. F. Schoenwaldt and T. H. Stoudt, *Arch. Biochem. Biophys.*, **73**, 127 (1958); (m) H. L. Herzog, M. J. Gentles, W. Charney, D. Sutter, E. Townley, M. Yudes, P. Kabasakalian and E. B. Hershberg, *J. Org. Chem.*, **24**, 691 (1959); (n) K. Tsuda, T. Asai, Y. Sato and T. Tanaka, *Chem. Pharm. Bull. (Tokyo)*, **7**, 534 (1959).

(3) (a) W. S. Allen and S. Bernstein, THIS JOURNAL, **78**, 1909 (1956); (b) K. Heusler and A. Wettstein, *Chem. Ber.*, **87**, 1301 (1954). We wish to thank Dr. Wettstein for supplying us with a sample of 16β-21-diacetoxy-17α-hydroxy-4-pregnene-3,20-dione.

(4) R. N. Jones, P. Humphries and K. Dobriner, THIS JOURNAL, **72**, 956 (1950).

(5) See C. Djerassi, T. T. Grossnickle and L. B. High, *ibid.*, **78**, 3166 (1956), for a full discussion of this aspect.

(6) D. H. R. Barton and G. F. Laws, *J. Chem. Soc.*, 52 (1954).

(7) H. Linde and K. Meyer, *Helv. Chim. Acta*, **42**, 807 (1959).

21-acetate II has a more positive molecular rotation of 5 when compared to that of Reichstein's substance S acetate, while the new acetate has a more negative molecular rotation of −478 when compared to that of Reichstein's substance S acetate. This evidence strongly suggested that the new acetate was 21-acetoxy-17α-hydroxy-4,14-isopregnene-3,15-20-trione (IIIb). Again in consideration of the saponification step where epimerization would most likely occur, the free diol would most probably be 17α,21-dihydroxy-4,14-isopregnene-3,20-dione (IIIa).[8]

It was interesting to notice that the mother liquors of the saponification reaction on reacetylation gave a mixture of products. Partition chromatography of the mixture gave both isomeric 15-ketones mentioned above, II and IIIb, and an as yet unknown compound (more polar than II and IIIb) whose infrared spectrum suggested a steroid monoacetate triol. The 15-keto-substance S acetate (II) may have been derived from some 15-keto S in the original mother liquor. The precursor of the unknown acetate is not known at present. A further interesting observation was that 15-keto-14-iso-substance S acetate (IIIb) was less polar than 15-keto-substance S acetate (II) in the chromato graphic system used.

TABLE I

Compound	M_D	ΔM_D(15-C=O-15-CH_2)
17β-Carbomethoxy-etiocholane-3β-ol acetate[a]	+186	
3β-Acetoxy-17β-carbomethoxy-etiocholan-15-one[7]	+262	+ 76
3β-Acetoxy-17β-carbomethoxy-14-isoetiocholan-15-one[7]	− 82	−268
22a,25a,5α-Spirostane-2α,3β-diol diacetate[b]	−486	
2α,3β-Diacetoxy-22a,25a,5α-spirostane-15-one[5]	−451	+ 35
2α,3β-Diacetoxy-22a,25a,5α,14-isospirostane-15-one[5]	−701	−215
21-Acetoxy-17α-hydroxy-4-pregnene-3,20-dione[c]	+571	
21-Acetoxy-17α-hydroxy-4-pregnene-3,15,20-trione (II)[d]	+576	+ 5
21-Acetoxy-17α-hydroxy-4,14-isopregnene-3,15,20-trione (IIIb)[d]	+ 93	−478

"Tables de Constantes et Données Numeriques 6. Constantes Selectionnées Pouvoir Rotatoire Naturel 1. Steroides," Masson & Cie ,Editeurs, Paris, 1956; [a] p. 96; [b] p. 278, [c] p. 79. [d] This work.

Treatment of 21-acetoxy-15β,21-dihydroxy-4-pregnene-3,20-dione (Ib) with methanesulfonyl chloride formed the 15β-methanesulfonate Id which upon reaction with sodium acetate in glacial acetic acid[9] afforded 21-acetoxy-17α-hydroxy-4,14-pregnadiene-3,20-dione (IVa).[10] This facile elimination suggested that the mesylate grouping in Id would be *trans* to the C-14 hydrogen and pseudo-axial. However, it must be remembered that an 11α-tosyloxy grouping *cis* to the C-9α-hydrogen and equatorial has been shown to be easily eliminated under a variety of conditions[9,11] so that no conclusion as to stereochemistry can be drawn from this particular experiment. Saponification of the diene IVa gave 17α,21-dihydroxy-4,14-pregnadiene-3,20-dione (IVb).[12]

When 15β-hydroxy substance S was treated with sodium bismuthate in acetic acid,[13] the product formed was 15β-hydroxy-4-androstene-3,17-dione (V). Chromium trioxide oxidation of the latter compound afforded 4-androstene-3,15,17-trione (VI).[14] The ultraviolet absorption spectrum of VI exhibited the expected peak at 275 mμ[15] for a β-diketone.

Fermentation of Reichstein's substance S with *Hormodendrum olivaceum*[16] (ATCC 13596) produced a different monohydroxylated Reichstein's substance S which was isomeric with the above described product Ia. The new compound in our hands, however, proved difficult to monoacylate preferentially.[17] Treatment of the new triol isomer with an excess of acetic anhydride in pyridine gave a new unknown diacetate. When the quantity of acetic anhydride used was reduced to one mole equivalent, only the free triol and the diacetate were isolated. Oxidation of this triol with sodium bismuthate in acetic acid yielded a monohydroxylated dione isomeric with the 15β-hydroxy-4-androstene-3,17-dione (V) mentioned previously. Oxidation of this new isomer gave 4-androstene-3,15,17-trione (VI) identical with the sample described above. Thus the new series of compounds also has a new hydroxyl group at C-15 and must be epimeric at this position to the first described series.

(8) The possibility of this epimerization occurring was considered at the time our previous note[1] was written, but the above argument was not fully developed. Consequently, this compound was referred to as 15-keto-Reichstein's substance S in that publication.

(9) J. Fried and E. F. Sabo, THIS JOURNAL, **79**, 1130 (1957). Also, see C. Djerassi, *et al.*,[5] for a similar reaction that failed to eliminate the 15β-mesylate grouping.

(10) B. M. Bloom, E. J. Agnello and G. D. Laubach, *Experientia*, **12**, 27 (1956), have also prepared IVa by a different pathway. Dr. Bloom kindly sent us a sample which had an identical infrared spectrum to the sample reported here.

(11) (a) R. Casanova, C. W. Shoppee and G. H. R. Summers, *J. Chem. Soc.*, 2983 (1953); (b) S. Bernstein, R. H. Lenhard and J. H. Williams, *J. Org. Chem.*, **19**, 41 (1954); (c) G. Rosenkranz, O. Mancera and F. Sondheimer, THIS JOURNAL, **76**, 2227 (1954).

(12) B. M. Bloom and G. M. Shull, *ibid.*, **77**, 5767 (1955), also have reported the preparation of IVb.

(13) C. J. W. Brooks and J. K. Norymberski, *Biochem. J.*, **55**, 371 (1953).

(14) The M_D contribution of the 15-keto group of VI to the molecular rotation of 4-androstene-3,17-dione is −192°. This would suggest according to the previous comments[5-7] and Table I that the C,D ring fusion in VI was *cis*. It should be pointed out, however, that the M_D of 3β-acetoxy-15-androsten-17-one [J. Fajkos, *Coll. Czech. Chem. Comm.*, **23**, 1559 (1958)] was 407° more negative than that of 3β-acetoxyandrostan-17-one. Since the ultraviolet absorption spectrum of VI shows that it undoubtedly existed to a large extent in the form of an enol, it was possible that this form was contributing to the large negative rotation. Furthermore, in a different series of compounds it has been noticed (personal communication from R. Littell) that a 14-iso-Δ^{15}-17-one steroid has a markedly more positive rotation than the normal Δ^{15}-17-one and also the saturated normal 17-one. This fact suggests that compound VI has a *trans* C/D ring fusion.

(15) It should be noticed that this peak remains substantially in the same region (275–277 mμ) in neutral or basic solution, a fact more characteristic of open chained β-diketones rather than cyclic β-diketones. The observation does not fit the theory of "*trans*-fixed" cyclic β-diketones offered by B. Eistert and W. Reiss, *Chem. Ber.*, **87**, 108 (1954). It is possible that the large ring system fused to the 5-membered ring, however, has some effect that is not understood at present.

(16) This organism has been reclassified since our original publication[1] in which it was described as *Hormodendrum viride*.

(17) K. Tsuda, T. Asai, Y. Sato and T. Tanaka (ref. 2n) recently have succeeded in isolating a 21-monoacetate and a 15α-monoacetate in low yield by repetitive chromatography.

Thus the new triol was 15α,17α,21-trihydroxy-4-pregnene-3,20-dione (VIIa), the diacetate was the 15α,21-diacetate VIIb and the sodium bismuthate oxidation product was 15α-hydroxy-4-androstene-3,17-dione (VIII).[18]

Comparison of the rates of acetylation of the newly introduced hydroxyl function in the triols Ia and VIIa indicates the stereochemistry at the C-15 position. Since the hydroxyl function that is β at C-15 would be expected on steric grounds to be esterified more slowly due to its pseudo-axial conformation and also to its non-bonded 1,3-interaction with the C-13-methyl group, the ease of formation of a C-21-monoacetate characteristic of Ia showed that this compound contained the 15β-hydroxyl group, and that the isomeric VIIa which was difficult to monoacylate must be the 15α-hydroxy compound. Furthermore, the general rules of molecular rotation of hydroxyl groups in alicyclic systems postulated by Klyne and Stokes[19] suggested that the 15α-hydroxy system should be more dextrorotatory than the 15β-hydroxy compounds, an indication which also confirms our assigned structures. Other steroid compounds hydroxylated at C-15 also conform to this rule.[2b,c] Finally, paper chromatographic analysis of the epimeric hydroxy-androstenes (V and VIII) showed the 15β-hydroxy compound V to be less polar than the 15α-hydroxy VIII. This was to be expected considering the suggestion by Savard[20] that the axial isomer has greater mobility than its corresponding equatorial epimer.

Acknowledgment.—We wish to thank Louis M. Brancone and associates for the analytical data, William Fulmor and associates for the spectral and optical rotational data, and Charles Pidacks and associates for the partition chromatographic separation.

Experimental[21]

15β,17α,21-Trihydroxy-4-pregnene-3,20-dione (Ia).[22]—A 5-gal. fermentation bottle containing a paddle stirrer and aerating assembly was charged with 12 l. of the following medium: N-Z amine (type A), 0.3%; dried yeast (Fleischmanns' 35-DB), 0.03%; papain digest of liver extract, 0.03%; malic acid, 0.01%; corn starch, 1.0%; dibasic ammonium phosphate, 0.2%; potassium dihydrogen phosphate, 0.15%; potassium hydrogen phosphate, 0.05%; magnesium sulfate, 0.025%; sodium chloride, 0.2%; Hoagland's A-Z trace elements salt solution, 0.1%; *p*H adjusted to 7.0. The bottle was sterilized by autoclaving at 120° for 1 hr. The fermentation vessel was inoculated with 400 ml. of a 48-hour shaker flask culture of *Spicaria simplicissima* (ATCC 13595) incubated at 28° with a stirring rate of 200 r.p.m. and an aeration rate of 0.3 vol. of air/vol. of medium/minute. Seventy-two hours after inoculation, 6 g. of Reichstein's substance S dissolved in 120 ml. of methanol was added. After 28 hours of fermentation, paper chromatography of aliquots showed an almost complete conversion of substance S to a more polar material. The mash was filtered and the mycelial cake was washed with 3 l. of acetone. The acetone wash and the filtrate were combined and the acetone was removed by distillation. The resulting aqueous solution was extracted with four successive 2-l. portions of methylene chloride which were combined, washed with saturated saline, dried over anhydrous sodium sulfate, and evaporated. The resultant gummy solid (5 g.) was crystallized from acetone–petroleum ether to give 0.93 g., m.p. 208–211.5°. The analytically pure product had a m.p. 240–242°, $[\alpha]^{24}D$ + 96° (methanol), λ_{max}^{EtOH} 241 mμ (ε 16,600); ν_{max}^{KBr} 3497, 1721, 1664, 1623 and 1130 cm.$^{-1}$.

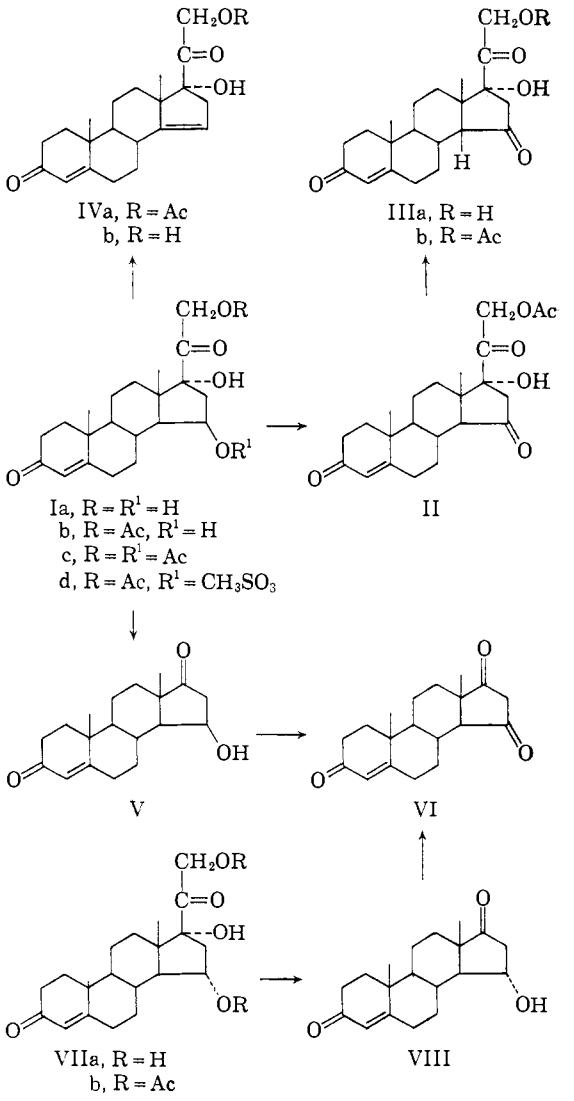
Anal. Calcd. for $C_{21}H_{30}O_5$ (362.45): C, 69.58; H, 8.34. Found: C, 69.87; H, 8.53.

21-Acetoxy-15β,17α-dihydroxy-4-pregnene-3,20-dione (Ib).[22]—A solution of 200 mg. of Ia in 5 ml. of pyridine was treated with 1.1 ml. of a 19:1 mixture of pyridine–acetic anhydride, and was allowed to stand overnight at room temperature. The solution then was poured into water and the product was extracted with ethyl acetate. Evaporation of the ethyl acetate afforded 0.12 g. of solid, m.p. 240.5–244°. Recrystallization from acetone–petroleum ether gave pure Ib, m.p. 245.5–247°, λ_{max}^{EtOH} 240–241 mμ (ε 15,500); ν_{max}^{KBr} 3690, 3454, 1734(shoulder), 1730, 1660, 1618, 1246 and 1128 cm.$^{-1}$.

Anal. Calcd. for $C_{23}H_{32}O_6$ (404.49): C, 68.29; H, 7.97. Found: C, 68.11; H, 8.29.

In another run the product Ib melted at 250–253°, $[\alpha]^{24}D$ +98° (chloroform).

15β,21-Diacetoxy-17α-hydroxy-4-pregnene-3,20-dione (Ic).[22]—The combined mother liquors from the crystallization of Ia were evaporated, and the residue was acetylated in the usual manner. The reaction mixture was poured into water, and the crystalline product was collected by filtra-



(18) Since our original paper,[1] A. Gubler and Ch. Tamm[2k] have reported the preparation of compound VIII by means of direct microbiological hydroxylation of 4-androstene-3,17-dione.

(19) W. Klyne and W. M. Stokes, *J. Chem. Soc.*, 1979 (1954).

(20) K. Savard, *J. Biol. Chem.*, **202**, 457 (1953).

(21) All melting points are uncorrected. The petroleum ether used had a b.p. 60–70° (Skellysolve B).

(22) Since our original publication,[1] this compound has also been reported in ref. 2m.

tion and air-dried. Recrystallization from acetone–petroleum ether gave needles which melted at 252–254°, $[\alpha]^{25}D$ + 109° (chloroform), λ_{max}^{EtOH} 238 mμ (ε 14,500); ν_{max}^{KBr} 3542, 1756, 1730, 1658, 1634, 1240, 1038 cm.$^{-1}$.

Anal. Calcd. for $C_{25}H_{34}O_7$ (446.52): C, 67.24; H, 7.68; OAc, 19.3. Found: C, 67.46; H, 7.91; OAc, 19.0.

21-Acetoxy-17α-hydroxy-15β-mesyloxy-4-pregnene-3,20-dione (Id).—A mixture of Ia (0.6 g.), methanesulfonyl chloride (1.0 ml.) and pyridine (20 ml.) was allowed to stand overnight at 5° and then poured into ice-water. The crystalline precipitate was collected by filtration, and was washed with water and air-dried. This gave 0.48 g. of Id, m.p. 128° dec., ν_{max}^{KBr} 3544, 1760, 1740, 1688, 1622, 1332, 1230, 1172, 1110 cm.$^{-1}$. This compound was not further characterized.

21-Acetoxy-17α-hydroxy-4-pregnene-3,15,20-trione (II).[22]—A solution of Ib (100 mg.) in 2 ml. of pyridine was treated with 100 mg. of chromium trioxide in 1.5 ml. of pyridine. After standing overnight, the mixture was poured into cold water and the product was extracted with ethyl acetate. The extract was washed once with saturated sodium bicarbonate solution, twice with saturated saline, and was dried over anhydrous sodium sulfate. Evaporation gave a solid which was recrystallized from acetone–petroleum ether to give 41 mg. of II, m.p. 240.5–244°. Recrystallization from acetone–petroleum ether raised the melting point to 254–255.5°, λ_{max}^{EtOH} 240 mμ (ε 15,850); ν_{max}^{Nujol} 3332, 1758(shoulder), 1748, 1728, 1652, 1622, 1232, 1133 cm.$^{-1}$.

Anal. Calcd. for $C_{23}H_{30}O_6$ (402.47): C, 68.63; H, 7.51. Found: C, 68.21; H, 7.78.

In another run the trione acetate II melted at 252–254°, $[\alpha]^{24}D$ +143° (chloroform).

17α,21-Dihydroxy-4,14-isopregnene-3,15,20-trione (IIIa). A mixture of II (98 mg.) in methanol (20 ml.) was treated with a solution of sodium (6.7 mg.) in methanol (10 ml.), and was allowed to stand for 10 minutes at room temperature (nitrogen atmosphere). The solution then was neutralized with a few drops of glacial acetic acid, water was added, and the methanol was removed by distillation. The residual water mixture was extracted with chloroform, the extract was washed with saturated saline, dried and evaporated. The residue was crystallized from acetone–petroleum ether to give 32 mg. of IIIa, m.p. 231–233°. Recrystallization from acetone–petroleum ether afforded the analytical sample, m.p. 235–236°, $[\alpha]^{24}D$ +100° (chloroform), λ_{max}^{EtOH} 240 mμ (ε 16,000); ν_{max}^{KBr} 3538, 3356, 1756, 1722, 1668, 1624 and 1098 cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{28}O_5$ (360.44): C, 69.97; H, 7.83. Found: C, 70.04; H, 8.02.

21-Acetoxy-17α-hydroxy-4,14-isopregnene-3,15,20-trione (IIIb). A.—Acetylation of 40 mg. of IIIa with 0.5 ml. of acetic anhydride in 1.5 ml. of pyridine in the usual manner afforded 11 mg. of IIIb, m.p. 202.5–203° after crystallization repeatedly from acetone–petroleum ether.

Anal. Calcd. for $C_{23}H_{30}O_6$ (402.47): C, 68.63; H, 7.51. Calcd. for $C_{23}H_{30}O_6 \cdot 1/2\ H_2O$ (411.47): C, 67.13; H, 7.59. Found: C, 67.37; H, 7.32.

B.—A repeat of the procedure in A on 43 mg. of crude IIIa gave an oil which was combined with the mother liquor of the reaction described above to give 72 mg. of an oil. This was submitted to partition chromatography on Celite[23] with the solvent system: heptane (3 parts), ethyl acetate (3 parts), methanol (3 parts) and water (2 parts) to give three peaks as distinguished by ultraviolet absorption spectrum. The two less polar peaks which overlapped to some extent were separated as well as possible and each individually submitted to partition chromatography on Celite[23] with the solvent system heptane–ethyl acetate–methanol–water (5:3:3:2). The first peak which came off the column in the first half of the second hold-back volume was evaporated and crystallized from acetone–petroleum ether to give 15 mg. of IIIb, m.p. 203.5–204°, λ_{max}^{MeOH} 240 mμ (ε 16,400), $[\alpha]^{25}D$ +23° (chloroform), ν_{max}^{KBr} 3450, 1750, 1730(shoulder), 1680, 1625 and 1235 cm.$^{-1}$.

Anal. Found: C, 66.75; H, 7.54.

Evaporation of the second half of the second hold-back volume gave 13.3 mg. of II, m.p. 248.5–249°.

Evaporation of the third hold-back volume of the first partition column gave 12.7 mg. of a crude solid, m.p. 153–157°, λ_{max}^{MeOH} 240 and 280 mμ (ε 16,700 and 67, based on a molecular weight of 404.5); ν_{max}^{KBr} 3300, 3200, 1751, 1735, 1642, 1607 and 1237 cm.$^{-1}$.

21-Acetoxy-17α-hydroxy-4,14-pregnadiene-3,20-dione (IVa).—A mixture of Id (300 mg.), sodium acetate (1.0 g.) and glacial acetic acid (20 ml.) was refluxed for 1 hour and then was poured into cold water, neutralized with sodium bicarbonate, and extracted with chloroform. The extract was washed with saturated saline, dried and evaporated. The residual oil was chromatographed on 30 g. of silica gel. The product was eluted with 20% ether in benzene. After evaporation, the residue was crystallized from acetone–petroleum ether to give 58 mg., m.p. 182–184°. Recrystallization from acetone–petroleum ether raised the melting point to 187–188°,[24] $[\alpha]^{25}D$ + 104° (chloroform), λ_{max}^{EtOH} 238 mμ (ε 14,600); ν_{max}^{KBr} 3390, 1760, 1740, 1668, 1618, 1234, 1098 cm.$^{-1}$.

Anal. Calcd. for $C_{23}H_{30}O_5$ (386.47): C, 71.48; H, 7.82; OAc, 11.2. Found: C, 71.65; H, 8.14; OAc, 10.7.

In another run 0.48 g. of Id gave 0.27 g. (70%) of IVa, m.p. 185–187°.

17α,21-Dihydroxy-4,14-pregnadiene-3,20-dione (IVb).—A mixture of IVa (250 mg.) in methanol (50 ml.), was treated with a solution of sodium (15 mg.) in methanol (10 ml.), and was allowed to stand for 10 minutes at room temperature (nitrogen atmosphere). The solution then was neutralized with a few drops of glacial acetic acid, water was added and the methanol was removed by distillation. The residual water mixture was extracted with chloroform, the extract washed with saturated saline, dried and evaporated. The residue was crystallized from acetone–petroleum ether giving 140 mg. of IVb, m.p. 182–184°. Recrystallization from acetone–petroleum ether raised the melting point to 186–188°,[25] $[\alpha]^{24}D$ + 84° (chloroform), λ_{max}^{EtOH} 238 mμ (ε 16,000); ν_{max}^{KBr} 3422, 1728, 1674, 1622, 1103 cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{28}O_4$ (344.44): C, 73.22; H, 8.19. Found: C, 73.22; H, 8.52.

15β-Hydroxy-4-androstene-3,17-dione (V).—A mixture of Ia (320 mg.), sodium bismuthate (5.0 g.) and 50% (v./v.) acetic acid (25 ml.) was stirred for 0.5 hour. After neutralization with 10% sodium hydroxide solution, the mixture was shaken with 200 ml. of chloroform and filtered. The residue was washed thoroughly with chloroform, and the extracts combined and washed with saturated saline. The dried chloroform solution was concentrated under vacuum to dryness, and the residue was crystallized from acetone–petroleum ether. This gave 210 mg. (83%) of V, m.p. 196–198°. Recrystallization from acetone–petroleum ether raised the melting point to 199.5–201°, $[\alpha]^{24}D$ +148° (chloroform), λ_{max}^{EtOH} 240 mμ (ε 16,800); ν_{max}^{KBr} 3450, 1744, 1656, 1622, 1060 cm.$^{-1}$.

Anal. Calcd. for $C_{19}H_{26}O_3$ (302.40): C, 75.46; H, 8.67. Found: C, 75.22; H, 8.86.

4-Androstene-3,15,17-trione (VI). A.[22]—A mixture of V (1.90 g.), chromium trioxide (1.60 g.) and pyridine (100 ml.) was allowed to stand overnight at room temperature. After the addition of methanol (10 ml.) and water, the solution was extracted with chloroform. The extract was washed with saturated sodium bicarbonate solution, saturated saline and dried and evaporated. The dark brown residue was extracted with warm ether and the ether solution evaporated to give a solid glass. This was dissolved in benzene and chromatographed on silica gel. Benzene–ether (1:1) eluted the solid material which was crystallized from acetone–petroleum ether to give 140 mg., m.p. 164–166°. Recrystallization from acetone–petroleum ether raised the melting point to 172–173°, $[\alpha]^{25}D$ +124° (chloroform), λ_{max}^{MeOH} 242 mμ (ε 17,400), 275 mμ (ε 5,250) (shoulder); $\lambda_{max}^{1\%\ alc.\ KOH}$ 242 mμ (ε 15,600), 277 mμ (ε 19,700); ν_{max} 1767, 1736, 1675, 1616 cm.$^{-1}$.

Anal. Calcd. for $C_{19}H_{24}O_3$ (300.38): C, 75.97; H, 8.05. Found: C, 75.65; H, 8.23.

(23) Celite is the trademark of Johns-Manville Co. for diatomaceous silica products. For a complete description of this technique see S. Bernstein, M. Heller, R. Littell, S. M. Stolar, R. H. Lenhard, W. S. Allen and I. Ringler, THIS JOURNAL, **81**, 1696 (1959).

(24) Reference 10 reported m.p. 201.4–202.8°, λ_{max}^{EtOH} 240 mμ (ε 16,200), $[\alpha]D$ +75° (dioxane) for IVa.

(25) Reference 12 reported m.p. 196.8–198.8°, λ_{max}^{EtOH} 240 mμ (log ε 4.21), $[\alpha]D$ +52° (dioxane) for IVb.

Further elution of the silica gel column with ether gave after crystallization from acetone–petroleum ether 155 mg. of starting material, m.p. 201–203°.

B.—The 15α-ol VIII (710 mg.) was treated with 750 mg. of chromium trioxide in 30 ml. of pyridine as in the above experiment. The solid resulting from removal of the extracting solvent was chromatographed on silica gel. Elution by ether–benzene (1:1) followed by crystallization from acetone–petroleum ether gave 41 mg. of VI, m.p. 173–174°, ultraviolet and infrared absorption spectra identical to that of the above experiment.

Further elution of the silica gel column with ether gave after recrystallization from acetone–petroleum ether 101 mg. of VIII, m.p. 194–196°.

15α,17α,21-Trihydroxy-4-pregnene-3,20-dione (VIIa).—Six grams of Reichstein's substance S was fermented in the same type of apparatus described for the preparation of Ia using a medium consisting of corn steep liquor, 2%; soy bean meal (Staley extracted soy grits), 1%; dextrin, 1%; potassium hydrogen phosphate, 1%; sodium chloride, 1%; calcium carbonate, 0.01%; *p*H 5.0. An inoculum (400 ml.) of 72-hour old mycelial of *Hormodendrum olivaceum* (ATCC 13596) was used and the mixture was incubated at 21° and aerated with 0.75 l. of sterile air per liter of medium per minute for 72 hours when the substance S in 100 ml. of ethanol was added. Fermentation was continued for 53 hours when paper chromatographic analysis indicated almost complete disappearance of substance S. The work-up was done as in the preparation of Ia. The residue from the methylene chloride extraction was crystallized from acetone–petroleum ether to afford 1.82 g. of VIIa, m.p. 182–188°. Recrystallization from acetone gave a m.p. 227–230°, $[\alpha]^{25}D$ +146° (methanol); λ_{max}^{EtOH} 241 mμ (ε 16,400); ν_{max}^{KBr} 3472, 1715, 1664, 1626, 1109, 1044 cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{30}O_5$ (362.45): C, 69.58; H, 8.34. Found: C, 69.70; H, 8.34.

15α,21-Diacetoxy-17α-hydroxy-4-pregnene-3,20-dione (VIIb).—Acetylation of 100 mg. of VIIa in 6 ml. of pyridine with 2 ml. of acetic anhydride gave after recrystallization from acetone–petroleum ether 70 mg. of VIIb, m.p. 199.5–200.5°, $[\alpha]^{25}D$ +132° (chloroform), λ_{max}^{EtOH} 240 mμ (ε 16,800); ν_{max}^{KBr} 3320, 1745, 1655, 1631 (shoulder) and 1240 cm.$^{-1}$.

Anal. Calcd. for $C_{25}H_{34}O_7$ (446.52): C, 67.24; H, 7.68; OAc, 19.3. Found: C, 67.50; H, 7.84; OAc, 19.0.

15α-Hydroxy-4-androstene-3,17-dione (VIII).—A mixture of 250 mg. of VIIa was stirred for 20 minutes with 4.0 g. of sodium bismuthate and 25 ml. of glacial acetic acid. The mixture then was worked up as in the preparation of V. The final residue was slurried with cold ether to give 28 mg. of VIII, m.p. 179–181°. Recrystallization from acetone–petroleum ether gave a m.p. of 194–196°, $[\alpha]^{24}D$ +206° (methanol), λ_{max}^{EtOH} 240 mμ (ε 16,600); ν_{max}^{KBr} 3545, 1735, 1675 and 1623 cm.$^{-1}$.

Anal. Calcd. for $C_{19}H_{26}O_3$ (302.40): C, 75.46; H, 8.67. Found: C, 75.63; H, 8.69.

PEARL RIVER, N. Y.

[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY[1]]

Steroidal Sapogenins LVII. 11α-Hydroxylation of Tigogenin Derivatives[2]

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Tigogenin (I) was converted to the corresponding 16-dehydro-20-ketopregnene (II) which in turn gave the 16α,17α-epoxide III and thence the 3-ketone IV. Microbiological hydroxylation of IV gave the 11α-hydroxy steroid V which in three steps gave 17α-hydroxy-5α-pregnane-3,11,20-trione (VIII).

This Laboratory has been engaged in the investigation of sources for a variety of steroidal sapogenins[3a,b,c,d] and in studies of the utilization of these compounds and their derivatives. In contrast to diosgenin which is the source for a variety of important steroidal hormones,[4] little work has appeared since Marker's classical researches[5] on the use of the saturated 5β- or 5α-sapogenins for hormonal intermediates. In a previous paper we have discussed the preparation of 11-oxygenated-5β-pregnanes derived from sarsasapogenin or smilagenin.[6] The present report deals with the preparation of 11-oxygenated 5α-derivatives derived from tigogenin. This sapogenin is available in particularly high quantity and purity from *Yucca peninsularis*.

The starting point for the various preparations was 3β-hydroxy-5α-16-pregnen-20-one (II) available from tigogenin (I) *via* the standard side chain degradation procedure used at this Laboratory.[7]

Epoxidation with alkaline hydrogen peroxide[8] gave 16α,17α-epoxy-3β-hydroxy-5α-pregnan-20-one (IIIa), a small portion of which was characterized as the known 3β-acetate IIIb.[9] The major portion of III without purification was oxidized with chromium trioxide–pyridine reagent[10] to give 16α,17α-epoxy-5α-pregnane-3,20-dione (IV), a compound for which surprisingly we could not find a literature reference. The structure of IV is established from its method of preparation, infrared spectrum, analysis and microbial hydroxylation to the known 16α,17α-epoxy-11α-hydroxy-5α-pregnane-3,20-dione (V).[11] The reaction sequence II → IV proceeded in high yield giving better than 80% IV under non-isolation conditions.

Microbiological hydroxylation of IV to the 11α-hydroxy compound Va was carried out with *Rhizopus nigricans* or *Aspergillus ochraceus*. Both of these organisms have been widely used for the 11α-hydroxylation of a number of steroids.[12]

(1) Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture. Article not copyrighted.

(2) Previous paper in this series, Steroidal Sapogenins, LVI, S. G. Levine and M. E. Wall, THIS JOURNAL, **82**, 1444 (1960).

(3) (a) M. E. Wall, *et al.*, *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 1 (1954); (b) **43**, 503 (1954); (c) **44**, 438 (1955); (d) **46**, 653 (1957).

(4) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publ. Corp., New York, N. Y., 1959, p. 661.

(5) R. E. Marker, *et al.*, THIS JOURNAL, **69**, 2167 (1947).

(6) H. E. Kenney, E. A. Weaver and M. E. Wall, *ibid.*, **80**, 5568 (1958).

(7) M. E. Wall, H. E. Kenney and E. S. Rothman, *ibid.*, **77**, 5665 (1955).

(8) P. L. Julian, *et al.*, *ibid.*, **71**, 756, 3574 (1949); **72**, 5145 (1950).

(9) R. E. Marker, E. M. Jones and E. L. Wittbecker, *ibid.*, **64**, 468 (1942).

(10) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, *ibid.*, **75**, 422 (1953).

(11) J. Romo, G. Rosenkranz, C. Djerassi and F. Sondheimer, *ibid.*, **75**, 1277 (1953).

(12) For a comprehensive recent review of this subject *cf.* S. H. Eppstein, P. D. Meister, H. C. Murray and D. H. Peterson, *Vitamins and Hormones*, **14**, 359 (1956).