3α-Acetoxy-16α,17α-oxido-16β-acetyl-5β-androstane-11-one (XVIIIa). Acetylation of 600 mg. of XVIII in 2 ml. of acetic anhydride and 2 ml. of pyridine at 25° for 18 hr., and crystallization of the product from acetone-ether gave 555 mg. of the 3-acetate XVIIIa, m.p. 166–167°; $\lambda_{max}^{CHCl_3}$, 5.80, 5.90, 8.04 μ.

Anal. Calcd. for $C_{23}H_{32}O_6$: C, 71.10; H, 8.30. Found: C, 70.72; H, 8.18.

3α-Acetoxy-16β-acetyl-5β-androstane-11-one (XIa) *and 3α-hydroxy-16β-acetyl-5β-androstane-11-one* (*XI*) *from the 16α,17α-oxide* (XVIIIa). To a solution of 350 mg. of the 16α,17α-oxide (XVIIIa) in 14 ml. of acetic acid kept at 12° was added 1.5 ml. of 24% hydrogen bromide in acetic acid. After 30 min. at 12° the solution was concentrated to dryness under vacuum and flushed with benzene several times to give the bromohydrin *3α-acetoxy-16α-hydroxy-17β-bromo-16α-acetyl-5β-androstane-11-one* (XIX) as an amorphous solid; $\lambda_{max}^{CHCl_3}$ 2.90–3.00, 5.80, 5.85, 8.0 μ; negligible ultraviolet absorption in the 220–400 mμ region.

A solution of 370 mg. of the bromohydrin (XIX) in 20 ml. of methanol and 2 ml. of water was treated with hydrogen at atmospheric pressure over 400 mg. of 25% palladium on calcium carbonate catalyst. When hydrogen uptake ceased, the catalyst was removed by filtration and the filtrate concentrated to a small volume. Water was added and the mixture was extracted with ether. The ether extract (260 mg.), which gave a negative Beilstein test, was chromatographed on 8.0 g. of acid washed alumina. From the 50% petroleum ether–benzene to 100% benzene fractions (130 mg.) was obtained 3α-acetoxy-16β-acetyl-5β-androstane-11-one (XIa), m.p. 118–120° identical with an authentic sample by mixed melting and infrared spectra comparisons. The 100% chloroform eluates (78 mg.) on crystallization from acetone-hexane gave the corresponding 3α-ol (XI), m.p. 203–207° undepressed on admixture with an authentic sample. The respective infrared spectra were identical.

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[CONTRIBUTION FROM THE CHEMICAL RESEARCH DIVISION, G. D. SEARLE & Co.]

The Configuration of 7-Hydroxy-Δ⁴-3-oxosteroids[1a]

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Received October 17, 1960

The configurations of 7-hydroxy-Δ⁴-3-oxosteroids have been correlated by comparison of the molecular rotations of the corresponding acetates with 7β-acetoxycholestenone and by the analysis of NMR spectra.

In recent years a number of 7-hydroxy-Δ⁴-3-oxosteroids have been reported in the literature.[2] The configurations of these compounds were assigned by various methods, the most popular of which was comparison of molecular rotatory differences. The values obtained were compared to those in the literature for saturated or Δ⁵ unsaturated 7-hydroxysteroids,[3] for which the molecular rotatory contribution [$\Delta M_D = M_D$ (7-OR)-M_D (7-H)] for a 7β-hydroxy group is positive while that for the 7α-group is negative. The data reported by McAleer *et al.*[2e] show, by contrast, that with a Δ⁴-3-ketone grouping in the molecule, both 7α- and 7β-hydroxyls cause negative shifts of about the same magnitude. Thus it appears to be impossible to assign configuration to a 7-hydroxy-Δ⁴-3-oxosteroid on the basis of its molecular rotation.

Some of the acetates of these hydroxy compounds have been reported and here the differences in rotation seemed to offer more chance for differentiating the 7α and 7β isomers. Consequently, when we isolated the isomeric 7-hydroxy-4-androstene-3,17-diones from fermentations, we took the opportunity to prepare their acetates and compare their properties with those reported for the acetate of 7β-hydroxycholestenone.[2a] This is the one 7β-hydroxy-Δ⁴-3-oxosteroid whose structure is unequivocal, as it was prepared from the well-characterized[4] compound, 7β-hydroxycholesterol.[5] This comparison, as seen in Table I, indicated that the

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(2) (a) C. W. Greenhalgh, H. B. Henbest, and E. R. H. Jones, *J. Chem. Soc.*, 2375 (1952). (b) C. Meystre, E. Vischer, and A. Wettstein, *Helv. Chim. Acta*, **38**, 381 (1955). (c) W. J. McAleer, M. A. Kozlowski, T. H. Stoudt, and J. M. Chemerda, *J. Org. Chem.*, **23**, 958 (1958). (d) K. Tsuda, T. Asai, E. Ohki, A. Tanaka, and M. Hattori, *Chem. and Pharm. Bull. (Tokyo)*, **6**, 387 (1958). (e) S. Bernstein, W. S. Allen, M. Heller, R. H. Lenhard, L. I. Feldman, and R. H. Blank, *J. Org. Chem.*, **24**, 286 (1959) and S. Bernstein, L. Feldman, W. S. Allen, and R. H. Blank, U. S. Pat. 2,962,512 (Nov. 29, 1960). (f) J. Fried, R. W. Thoma, D. Perlman, and J. R. Gerke, U. S. Pat. 2,836,608 (May 27, 1958). (g) A. Schubert, K. Heller, R. Siebert, K. Zetsche, and G. Langbein, *Naturwissenschaften*, **45**, 264 (1958). (h) E. L. Dulaney and W. J. McAleer, U. S. Pat. 2,888,469 (May 26, 1959). (i) K. Tanabe, R. Hayashi, R. Takasaki, and M. Shirasaka, *Chem. and Pharm. Bull. (Tokyo)*, **7**, 811 (1959). (j) K. Tsuda, T. Asai, E. Ohki, A. Tanaka, and T. Matsuhisa, *Chem. and Pharm. Bull. (Tokyo)*, **7**, 369 (1959). (k) A. L. Nussbaum, G. Brabazon, T. L. Popper, and E. P. Oliveto, *J. Am. Chem. Soc.*, **80**, 2722 (1958). (l) K. Tsuda, T. Asai, Y. Sato, T. Tanaka, T. Matsuhisa, and H. Hasegawa, *Chem. and Pharm. Bull. (Tokyo)*, **8**, 626 (1960). (m) K. Tsuda, T. Asai, Y. Sato, T. Tanaka, and M. Kato, *J. Gen. App. Microbiol. (Tokyo)*, **5**, 1 (1959); *Chem. Abstr.*, **53**, 22250 (1959). (n) R. W. Thoma and J. Fried, U. S. Pat. 2,960,513 (Nov. 15, 1960).

(3) L. F. Fieser and M. Fieser, *Steroids*, Reinhold, New York, 1959, p. 179.

(4) H. Heymann and L. F. Fieser, *Helv. Chim. Acta*, **35**, 631 (1952).

(5) The structures of the 7α-hydroxy-steroids reported by Nussbaum *et al.*,[2k] as deduced from the method of synthesis, are also very probably correct. However, as the authors did not report rotations for their acetates, the compounds have been omitted from our table.

TABLE I

Parent Compound	7β-OH			7β-OAc			7α-OH			7αOAc		
	M.P.	$[\alpha]_D$[k]	ΔM_D[l]	M.P.	$[\alpha]_D$[k]	ΔM_D[l]	M.P.	$[\alpha]_D$[k]	ΔM_D[l]	M.P.	$[\alpha]_D$[k]	ΔM_D[l]
Cholestenone	183.5–184	+63	−86[a]	101–102	+77	+3[a]						
4-Androstene-3,17-dione	220–222.5	+178	−29[e]				255–256.5	+164	−71	177–179	+78	−298
	220.5–222.5	+186	−4	182–183	+180	+53	242.5–245[m]					
							248–250	+155	−98[n]			
Progesterone	188–191	+141	−169[c]				229–230	+167	−83[f]	237–238	+71	−368[f]
		+158 (di)	−44[c]				227–231	+154 (di)	−57[c]			
	190.5–192.5	+144[m] (di)										
21-Hydroxyprogesterone	178–181.5	+151	−111[c]				216–226	+158	−87[b]			
							216–225	+144	−136[c]			
21-Acetoxyprogesterone							217–219	+162 (et)	−56[b]	189–192	+83	−348[b]
14α-Hydroxyprogesterone	208–214	+154	−144[f]	191.5–192.5	+161	−52[f]	252–255	+175	−71[g]	222–225	+63	−433[g]
							280	+177[h] (me)		227–235[h]		
							233–234	+173[j] (di)		223–225 and 245–250	+75	−386[j]
15β-Hydroxyprogesterone	231–233	+136	−28[d]	194–195	+123	−21[d]						
	226–228	+122	−76	194–195	+132	+14						
17α,21-Dihydroxyprogesterone	209–211	+94 (me)	−109[e]				228–230	+146 (me)	+79[m]			
							248–250	+97 (et)	−98[n]			
21-Acetoxy-17α-progesterone	210–211.5	+111	−79[e]	246–248	+107	−50[e]	240.5–241[m]			180–182[m]		
				243–244	+110	−36				180–182	+39	−375[n]

[a] Ref. 2a. [b] Ref. 2b. [c] Ref. 2c. [d] Ref. 2l. [e] Ref. 2e. [f] Ref. 2f. [g] Ref. 2g. [h] Ref. 2h. [i] Ref. 2i. [j] Unpublished work by R. D. Muir, R. Ray, and R. M. Dodson of these laboratories. [k] Rotations in chloroform, unless indicated to be in dioxane (di), ethanol (et), or methanol (me). [l] Rotations of parent compounds taken from J. P. Mathieu and A. Petit, *Tables de Constantes et Donees Numeriques 6. Constantes Selectionees Pouvoir Rotatoire Naturel, I. Steroides*, Masson and Cie., Editeurs, Paris, 1956; except 15β-hydroxyprogesterone, ref. 2f; 17α,21-dihydroxyprogesterone, ref. 2e; 14α-hydroxyprogesterone, $[\alpha]_D$ +205, ($CHCl_3$). ref. 2i; 21-hydroxyprogesterone $[\alpha]_D$ +192° ($CHCl_3$); 21-acetoxyprogesterone, $[\alpha]_D$ +189° ($CHCl_3$); and 21-acetoxy-17α-hydroxyprogesterone, $[\alpha]_D$ +136° ($CHCl_3$). [m] Ref. 2m. [n] Ref. 2n.

7β-acetoxy-Δ⁴-3-oxosteroids have small ΔM_D values, while the 7α-acetates have negative values of −300° or greater.

In an effort to place these assignments on an even more solid footing, we examined the optical rotatory dispersion spectra[7a] of the two 7-acetoxy-4-androstene-3,17-diones. Djerassi, Halpern, Halpern, and Riniker[7b] have shown that 7β-methyltestosterone has the same spectrum as testosterone, while the axial 7α-methyl derivative has a distinctly different spectrum. Unfortunately, the spectra of both of the acetoxy compounds were quite similar to that of 4-androstene-3,17-dione; and so they provided no clue to the configurations.

Fortunately, examination of the NMR spectra[8] proved more fruitful. The equatorial 7β-hydrogen produced a peak in the spectrum of 7α-acetoxy-4-androstene-3,17-dione at 4.883 p.p.m., while the axial 7α-hydrogen of the 7β-acetoxy compound gave a broader multiplet signal at 5.233 p.p.m. as would be predicted from the increased shielding of the axial hydrogen and the increased spin coupling with the axial 8β and 6β-hydrogens.[10]

McAleer *et al.*[2c] have reported that 7-hydroxy-Δ⁴-3-oxosteroids can be distinguished by the use of the "blue tetrazolium" test. We found this to be the case, although, in our hands the results varied widely from one day to another and required the use of adequate standards. Of course, the presence of an easily oxidizable group in the molecule will invalidate the test.

As a result, of the above analysis of the configurations of the 7-hydroxy-Δ⁴-3-oxosteroids, some reassignments of configurations of previously described compounds must be made.

The 7-hydroxy-4-androstene-3,17-dione characterized here as β was recently assigned the α-configuration by Bernstein *et al.*[2e] As their only argument for the assignment was the molecular rotatory contribution of the hydroxyl group, we feel that the evidence fits the 7β-configuration better for this compound as well as for the 7,17α,21-trihydroxy-4-pregnene-3,20-dione from which it was derived.[2e]

Another 7,17α,21-trihydroxy-4-pregnene-3,20-dione has recently been isolated by Tsuda and his co-workers.[2m] They did not assign a configuration or report a rotation for their 7-acetoxy compound, but the assignment of the 7α-configuration seems reasonable on the basis of the non-identity of Tsuda's compound with Bernstein's[2e] material. Tsuda also degraded his product to a 7-hydroxy-4-androstene-3,17-dione, whose melting point matches our 7α-hydroxy compound best.[6] More recently still, Thoma and Fried[2n] have prepared the same trihydroxyprogesterone and also assigned to it the 7α-configuration.

In the progesterone series, Tanabe *et al.*,[2i] isolated two compounds from a progesterone fermentation using *Absidia regnieri*. One was a compound to which Tsuda *et al.*[2l] have now assigned the structure 7β,14α,15β-trihydroxyprogesterone. The properties of this compound have been omitted from Table I, since the parent compound 14α,15β-dihydroxyprogesterone is unknown. However, Tsuda's proof of structure is unambigous. The second compound was a 7,14α-dihydroxyprogesterone, to which Tanabe had assigned the 7α configuration because his compound formed an acetal derivative with benzaldehyde.

Schubert and co-workers[2g] had isolated a different 7,14α-dihydroxyprogesterone and had assigned to it the 7α-configuration on the basis of an analysis of the infrared spectrum of the 7-monoacetate. As this analysis is convincing and as the molecular rotations of the 7-monoacetates of both 7,14α-dihydroxy compounds fit well into our general analysis on the basis of Schubert's assignment, we have assigned the structure, 7β,14α-dihydroxyprogesterone, to Tanabe's compound. Under the conditions used, it seems possible that the acetal formation, observed by Tanabe, was accomplished by inversion of one of the hydroxyl groups.

EXPERIMENTAL[11]

7β-Acetoxy-4-androstene-3,17-dione. 7β-Hydroxy-4-androstene-3,17-dione, m.p. 218.5–221°, 0.30 g., was dissolved in 1.5 ml. of acetic anhydride and 3 ml. of pyridine and allowed to stand overnight at room temperature. The solution was poured into a dilute solution of sodium carbonate. After the evolution of carbon dioxide was complete, the solution was extracted two times with methylene chloride. The extracts were washed three times with water, dried, and concentrated. The residue was dissolved in acetone, filtered, and evaporated under nitrogen until all the pyridine was gone. The residue was crystallized from ether-petroleum ether (b.p. 35–40°) to yield 0.26 g. of 7β-acetoxy-4-androstene-3,17-dione, m.p. 182–183°, $\lambda_{max}^{CH_3OH}$ 237.3 mμ, ε 16,500.

Anal. Calcd. for: $C_{21}H_{28}O_4$: C, 73.23; H, 8.19. Found: C, 73.27; H, 8.38.

7α-Hydroxy-4-androstene-3,17-dione. 4-Androstene-3,17-dione, 10.0 g., was fermented by the methods previously

(6) This result indicates that the organism used by Tsuda,[2m] *Diplodia Tubericola* (Ell & Ev. Gan), gives different configurations depending on the substrate, since they isolated 7β-hydroxyprogesterone as the product from progesterone.

(7a) Very kindly determined for us by Professor Carl Djerassi, Stanford Univ.

(7b) C. Djerassi, O. Halpern, V. Halpern, and B. Riniker, *J. Am. Chem. Soc.*, 80, 4001 (1958).

(8) Very kindly determined and interpreted for us by Dr. N. L. McNiven of the Worcester Foundation for Experimental Biology. They were run at 60 mc. using deuterochloroform as the solvent. The shifts are reported as τ values relative to tetramethylsilane as an internal standard.[9]

(9) G. V. D. Tiers, *J. Phys. Chem.*, 62, 1151 (1958).

(10) R. U. Lemieux, R. K. Kulling, H. J. Bernstein, and W. G. Schneider, *J. Am. Chem. Soc.*, 79, 1005 (1957).

(11) The rotations were taken in chloroform at 24 ± 2°. All melting points were taken on a Fisher-Johns melting point apparatus. We wish to thank Dr. R. D. Muir and his associates for the fermentations reported.

described[12] using a *Neurospora sp.* (M 714). The methylene chloride extracts were concentrated to dryness, dissolved in 10% ethyl acetate in benzene and chromatographed on 1400 g. of silica gel. The chromatographic column was eluted successively with 10%, 12%, 15%, 25%, 35%, 40%, and 50% ethyl acetate in benzene. The 50% eluates were concentrated to dryness and the residue was crystallized from acetone-cyclohexane, then from methanol, and finally from acetone-cyclohexane again to yield 394 mg. of 7α-hydroxy-4-androstene-3,17-dione, m.p. 255–256.5°, $\lambda_{max}^{CH_3OH}$ 241 mμ, ε 16,000. The maximum in the ultraviolet spectrum in 0.10*N* methanolic potassium hydroxide shifted on standing from 241 mμ to 283 mμ.

Anal. Calcd. for: $C_{19}H_{26}O_3$: C, 75.46; H, 8.67. Found: C, 75.36; H, 8.63.

7α-Acetoxy-4-androstene-3,17-dione. 7α-Hydroxy-4-androstene-3,17-dione, 0.10 g., was dissolved in 1.5 ml. of acetic anhydride and 3 ml. of pyridine and allowed to stand overnight at room temperature. Then the solution was concentrated under vacuum at 40–50°. Toluene was added and distilled twice followed by ether–petroleum ether (b.p. 35–40°). The residue solidified and was crystallized from acetone–petroleum ether (b.p. 65–70°) to yield 0.07 g. of 7α-acetoxy-4-androstene-3,17-dione, m.p. 177–179°, $\lambda_{max}^{CH_3OH}$ 238 mμ, ε 15,700.

Anal. Calcd. for: $C_{21}H_{28}O_4$: C, 73.23; H, 8.19. Found: C, 73.21; H, 8.18.

Sources of previously reported compounds. 7β-Hydroxy-4-androstene-3,17-dione was one of the products from a fermentation of 4-androstene-3,17-dione using *Rhizopus stolonifer*, ATCC No. 6227-B. The 7-position was proved by the shift of the ultraviolet maximum in base from 241 to 283 mμ. 7β,21-Diacetoxy-17α-hydroxy-4-pregnene-3,20-dione was isolated after acetylation of a portion of the product obtained by the action of a *Penicillium sp.*, ATCC No. 12556 on 17β,21-dihydroxy-4-pregnene-3,20-dione. We were unable to separate the free trihydroxy compound from a mixture with other products. The action of the same organism on progesterone produced 7β,15β-dihydroxy-4-pregene-3,20-dione which was selectively acetylated to give the 7β-monoacetate.[2d]

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(12) D. H. Peterson, H. C. Murray, S. H. Eppstein, L. M. Reineke, A. Weintraub, P. D. Meister, and H. M. Leigh, *J. Am. Chem. Soc.*, 74, 5933 (1952).

[CONTRIBUTION FROM THE CHEMICAL PROCESS IMPROVEMENT DEPARTMENT, LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID CO.]

16α-Hydroxysteroids. X.[1] 2β-Hydroxylation of 9α-Fluorohydrocortisone by *Streptomyces roseochromogenus*

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Received November 7, 1960

9α-Fluorohydrocortisone is hydroxylated by *Streptomyces roseochromogenus* in the 16α- position, the 2β- position, and in both the 2β- and 16α- positions.

The fermentative 16α-hydroxylation of 9α-fluorohydrocortisone I by *Streptomyces roseochromogenus*[3] is accompanied by a complex spate of steroidal by-products. Isomerization of the major product 9α-fluoro-16α-hydroxyhydrocortisone II, has already been described.[1,4] The present paper deals with other reducing steroids formed in the fermentation. A later communication will deal with nonreducing steroids elaborated.

Paper chromatographic examination of broth extracts revealed the presence of several reducing steroids including 9α-fluoro-16α-hydroxyhydrocortisone, contaminated with a reducing steroid III of slightly less mobility, the D-homoannulation product 9α-fluoro-11β,16α,17aα-trihydroxy-17aβ-hydroxymethyl-4-D-homoandrostene-3,17-dione IV, and a still more polar component V.

Isolation of the steroid III was accomplished from enriched mother liquors from which remaining 16α,17α-diol II and other 16α,17α-diols were removed by extraction with aqueous sodium borate solution. The new monohydroxylated 9α-fluorohydrocortisone III was distinguished from other known monohydroxylated (1ξ-,[5] 6β-,[6] 16α-[7]) 9α-fluorohydrocortisones. The diacetate of III is further distinguished from the known diacetate of 9α-fluoro-16β-hydroxyhydrocortisone.[8]

The more polar steroid V was extracted into aqueous borate and was thus recognized as being 16α-hydroxylated. Satisfactory isolation from the borate extract was not possible as boron was present in the preparation after acidification and chromatography. A cyclic 16α,17α-acetonide VI was formed both by conventional means[7,9] using crystalline mixtures containing V and by micro reaction on

(1) Paper IX. J. J. Goodman and L. L. Smith, *Applied Microbiology*, 8, 363 (1960).

(2) Present address: Wyeth Laboratories, Philadelphia, Pa.

(3) R. W. Thoma, J. Fried, S. Bonanno, and P. Grabowich, *J. Am. Chem. Soc.*, 79, 4818 (1957).

(4) L. L. Smith, M. Marx, J. J. Garbarini, T. Foell, V. E. Origoni, and J. J. Goodman, *J. Am. Chem. Soc.*, 82, 4616 (1960).

(5) W. J. McAleer, M. A. Kozlowski, T. H. Stoudt, and J. M. Chemerda, *J. Org. Chem.*, 23, 508 (1958).

(6) L. L. Smith, H. Mendelsohn, J. J. Goodman, J. P. Dusza, and S. Bernstein, *J. Org. Chem.*, 26, 974 (1961).

(7) S. Bernstein, R. H. Lenhard, W. S. Allen, M. Heller, R. Littell, S. M. Stolar, L. I. Feldman, and R. H. Blank, *J. Am. Chem. Soc.*, 78, 5693 (1956); 81, 1689 (1959).

(8) S. Bernstein, M. Heller, and S. M. Stolar, *J. Am. Chem. Soc.*, 81, 1256 (1959).

(9) J. Fried, A. Borman, W. B. Kessler, P. Grabowich, and E. F. Sabo, *J. Am. Chem. Soc.*, 80, 2338 (1958).