533. *Steroids and Related Compounds. Part VI. The Stereochemical Configuration and Dehydration of the Isomeric Androstane-3 : 5 : 6-triol-ones.*

By M. Davis and V. Petrow.

Oxidation of 3β : 5α : 6β-triacetoxycholestane * (I; R′ = R″ = Ac) gives 3β : 5α : 6β-triacetoxyandrostan-17-one (I*a*; R′ = R″ = Ac) identical with the compound obtained from androstane-*trans*-3 : 5 : 6-triol-17-one, the stereochemical configuration of which is thus rigidly proved.

Dehydration of 3β : 6β-diacetoxyandrostan-5α-ol-17-one (I*a*; R′ = Ac, R″ = H) with thionyl chloride in pyridine gives 3β : 6β-*diacetoxyandrost*-4-*en*-17-*one* (II*a*; R′ = Ac), hydrolysed to the *diol* which gives (i) androstane-3 : 6 : 17-trione (III) on oxidation by the Oppenauer method and (ii) *androst*-4-*en*-3β-*ol*-6 : 17-*dione* by oxidation with chromic acid.

Androst-4-*ene*-3β : 6α-*diol*-17-*one* (IV*a*; R′ = H) is obtained through a Darzens dehydration of 3β : 6β-diacetoxyandrostan-5α-ol-17-one (V*a*; R′ = Ac) and passes into androstane-3 : 6 : 17-trione on oxidation with aluminium *tert.*-butoxide in the presence of acetone.

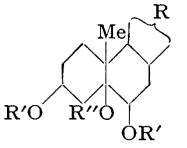
Oxidation of cholesterol with hydrogen peroxide leads to the formation of a *trans*-triol to which the stereochemical configuration of a cholestane-3β : 5α : 6β-triol (I; R′ = R″ = H) has been assigned (Part IV; Ellis and Petrow, *J.*, 1939, 1078). The $C_{(5)}$-hydroxyl group of this compound possesses unexpected mobility and may be eliminated in three different ways. By dehydration of the triol diacetate with thionyl chloride in pyridine, 3β : 6β-diacetoxycholest-4-ene (II; R′ = Ac) is obtained (Petrow, Rosenheim, and Starling, *J.*, 1938, 677). Interaction of the triol with methanolic hydrogen chloride leads to the formation of the 5-chloro-derivative (Windaus, *Z. physiol. Chem.*, 1921, **117**, 155), the dibenzoate of which passes smoothly on pyrolysis into the enol-benzoate, 3β : 6-dibenzoyloxycholest-5-ene (Petrow, Rosenheim, and Starling, *loc. cit.*; Lettre and Muller, *Ber.*, 1937, **70**, 1947). This result is best interpreted by postulating inversion of configuration at $C_{(5)}$ during replacement of the tertiary hydroxyl group by halogen, leading to the formation of a 5(β)-*chlorocoprostane*-3β : 6β-*diol*, followed by " *trans* "-elimination of hydrogen chloride with the grouping at $C_{(6)}$. Finally, dehydration of cholestane-*trans*-triol diacetate with sulphuric acid in acetic anhydride (Westphalen, *Ber.*, 1915, **48**, 1064) or with potassium hydrogen sulphate in acetic anhydride (Petrow, *J.*, 1939, 998) leads to the formation of the diacetate of the dextrorotatory " Westphalen's diol " to

* The nomenclature proposed by Fieser and Fieser (*Experientia*, 1948, **4**, 285; cf. this vol., p. 1672) is adopted.

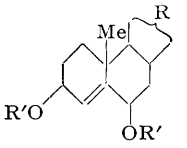
which the constitution of a 5-methylnorcholest-8(9)-ene-3 : 6-diol has been assigned (Petrow, Rosenheim, and Starling, *loc. cit.*). Oxidation of cholesterol with potassium permanganate or osmic acid, on the other hand, gives rise to a cholestane-*cis*-triol, identified as cholestane-3β : 5α : 6α-triol (V; R′ = H) by Prelog and Tagmann (*Helv. Chim. Acta*, 1944, **27**, 1867), who dehydrated it by the Darzens method to cholest-4-ene-3β : 6α-diol.

Two similar triols have been obtained in the androstane series. Direct oxidation of dehydro-*iso*androsterone with hydrogen peroxide (Ehrenstein, *J. Org. Chem.*, 1939, **4**, 506), or hydrolysis of 3β-acetoxy-5α : 6α-epoxy- and 3β-acetoxy-5β : 6β-epoxy-ætiocholan-17-one (Ushakov and Lutenberg, *J. Gen. Chem. Russia*, 1937, **7**, 1821; Ehrenstein, *J. Org. Chem.*, 1940, **5**, 544; Ruzicka, Grob, and Raschka, *Helv. Chim. Acta.*, 1940, **23**, 1518), affords the *trans*-triol. Oxidation of dehydro*iso*androsterone with osmic acid gives the *cis*-isomer (Ushakov and Lutenberg, *Nature*, 1937, **140**, 466). These two triolones yield the same androstan-5α-ol-3 : 6 : 17-trione on oxidation (Ehrenstein, 1939, *loc. cit.*) and thus differ solely in the configuration of the hydroxyl group at $C_{(6)}$. On the basis of this observation and from analogy with the corresponding cholestanetriols, Ehrenstein (*J. Org. Chem.*, 1948, **13**, 214) provisionally assigned the constitution of an androstane-3β : 5α : 6β-triol-17-one (I*a*; R′ = R″ = H) to the *trans*-isomer.

We now find that oxidation of 3β : 5α : 6β-triacetoxycholestane (I; R′ = R″ = Ac) with chromic acid gives a small neutral ketonic fraction from which 3β : 5α : 6β-*triacetoxyandrostan*-17-*one semicarbazone* may be isolated. This compound melts indefinitely and is thus unsuitable for purposes of characterisation. On hydrolysis with pyruvic acid with the technique of Hershberg (*J. Org. Chem.*, 1948, **13**, 542), however, it readily yields 3β : 5α : 6β-triacetoxy-androstan-17-one (I*a*; R′ = R″ = Ac); this and its *oxime* are identical with the corresponding derivatives prepared from the "*trans*"-triol. The stereochemical configuration of the "*trans*"-triol, and hence of the *cis*-isomer, is thus rigidly proved for the first time. We observed *inter alia* that alkaline hydrolysis of (I*a*; R′ = R″ = Ac) gives 5β : 6β-*epoxyætiocholan*-3β-*ol*-17-*one*, a rather surprising result as 3β : 5α : 6β-triacetoxycholestane (I; R′ = R″ = Ac) furnishes only the 5-monoacetate (I; R′ = H, R″ = Ac) under the same experimental conditions. (I*a*; R′ = R″ = Ac) thus resembles 3β-acetoxy-6β-methanesulphonyloxycholestan-5α-ol and the corresponding androstan-17-one which pass into the respective α-oxides on treatment with alcoholic potash (Fürst and Koller, *Helv. Chim. Acta*, 1947, **30**, 1454).



(I; R = $C_{16}H_{30}$.)
(I*a*; R = $C_{10}H_{14}O$.)



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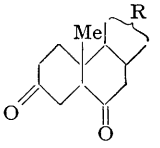

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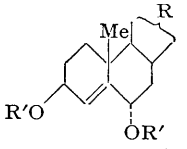
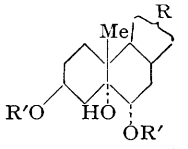


(IV; R = $C_{16}H_{30}$.)
(IV*a*; R = $C_{10}H_{14}O$.)



(V; R = $C_{16}H_{30}$.)
(V*a*; R = $C_{10}H_{14}O$.)

Earlier work has shown that (I; R′ = Ac, R″ = H) is readily converted into 3β : 6β-diacetoxycholest-4-ene (II; R′ = Ac) by treatment with thionyl chloride in pyridine at 0° (Petrow, Rosenheim, and Starling, *loc. cit.*). 3β : 6β-Diacetoxyandrostan-5α-ol-17-one (I*a*; R′ = Ac, R″ = H), in contrast, is recovered unchanged after this treatment. However, if a large excess of thionyl chloride is used and the mixture is heated under reflux for at least 30 minutes, a new, lævorotatory *diacetate*, $C_{23}H_{32}O_5$, is obtained in yields never exceeding 50%. Hydrolysis gives a *diol*, reconverted into the original diacetate by acetic anhydride. The diacetate (but not the diol) gives a blue colour with trichloroacetic acid, but neither compound gives a colour with tetranitromethane or the Tortelli–Jaffé reagent. These observations rule out its formulation as the enol-acetate of an androstan-3β-ol-6 : 17-dione (cf. Petrow, Rosenheim, and Starling, *loc. cit.*), the 3-monoacetate of which is known (Ruzicka and Muller, *Helv. Chim. Acta*, 1944, **27**, 503), and lead us to assign to it the constitution of a 3β : 6β-*diacetoxyandrost*-4-*en*-17-*one* (II*a*; R′ = Ac).

Oppenauer oxidation of androst-4-ene-3β : 6β-diol-17-one (II*a*; R′ = H) gives androstane-3 : 16 : 17-trione (III), identified by comparison with an authentic specimen prepared by the method of Ushakov and Lutenberg (*J. Gen. Chem. Russia*, 1939, **9**, 69). A similar result has previously been recorded in the cholestane series by Prelog and Tagmann (*loc. cit.*). Oxidation of (II*a*; R′ = H) with chromic acid, however, leads to the formation of a new, αβ-unsaturated *diketo-alcohol*, $C_{19}H_{28}O_3$, also formed in lower yield in the presence of excess of the oxidising agent. This compound must clearly be the hitherto unknown *androst-4-en-3β-ol-6 : 17-dione* as it yields an acetate which differs from the 6(β)-acetoxyandrost-4-ene-3 : 17-dione of Ehrenstein (*J. Org. Chem.*, 1941, **6**, 626).

Experiments on the Darzens dehydration of 3β : 6α-diacetoxyandrostan-5α-ol-17-one (V*a*; R′ = Ac) did not prove very satisfactory. *Androst-4-ene-3β : 6α-diol-17-one* (IV*a*; R′ = H) was ultimately obtained with great difficulty and in low yield. Its oxidation by the Oppenauer method gave androstane-3 : 6 : 17-trione (III).

Experimental.

M.p.s are corrected. Microanalyses are by Drs. Weiler and Strauss, Oxford. Optical rotations were measured in a 2-dm. tube. Activated alumina was used for the chromatographic purifications.

3β : 5α : 6β-Triacetoxyandrostan-17-one (I*a*; R′ = R″ = Ac).—(*a*) Treatment of 3β-acetoxy-5β : 6β-epoxyætiocholan-17-one with acetic acid gave 3(β) : 5(α)-diacetoxyandrostan-6β-ol-17-one, m. p. 231° (Ehrenstein, *loc. cit.*, gives m. p. 204°), converted into the triacetate (I*a*; R′ = R″ = Ac), m. p. 185°, by further acetylation. (*b*) 3β : 6β-Diacetoxyandrostan-5α-ol-17-one (50 mg.) and toluene-*p*-sulphonic acid (15 mg.) in an excess of acetic anhydride were heated at 100° for 30 minutes, yielding the triacetate, m. p. 182—183°, alone or in admixture with an authentic specimen.

The *semicarbazone* of (I*a*; R′ = R″ = Ac), obtained by heating the above triacetate (100 mg.) with semicarbazide hydrochloride (500 mg.) and sodium acetate (500 mg.) in ethanol (10 ml.) for 3½ hours, separated from pyridine– or acetone–light petroleum in plates which slowly decomposed between 180° and 220° according to the rate of heating (Found : N, 8·6. $C_{26}H_{39}O_7N_3$ requires N, 8·3%). The corresponding *oxime*, prepared by heating the keto-triacetate (45 mg.) with hydroxylamine hydrochloride (45 mg.) and sodium acetate (70 mg.) in ethanol (3 ml.) for 5½ hours, crystallised from ether–light petroleum in long needles, m. p. 145—152° (decomp.) (sealed tube) (Found : N, 3·4. $C_{25}H_{37}O_7N$ requires N, 3·0%).

5β : 6β-Epoxyætiocholan-3β-ol-17-one.—(*a*) 3β : 5α : 6β-Triacetoxyandrostan-17-one (20 mg.) and potassium hydroxide (30 mg.) were heated in ethanol (1 ml.) for 15 minutes. Concentration in a vacuum, followed by dilution with water, gave *5β : 6β-epoxyætiocholan-3β-ol-17-one*, needles, m. p. 166—167°, from ether–light petroleum (Found : C, 74·4; H, 9·6. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%).

(*b*) 3β : 5α-Diacetoxyandrostan-6β-ol-17-one, on hydrolysis as above, gave the same β-oxide, m. p. 165·5—166°, not depressed in admixture with a sample prepared as in (*a*) and converted by acetylation into the known 3β-acetoxy-5β : 6β-epoxyætiocholan-17-one.

3β : 5α : 6β-Triacetoxycholestane.—The following improved method was used. A solution of 3β : 6β-diacetoxycholestan-5α-ol (200 mg.) in acetic anhydride containing toluene-*p*-sulphonic acid (60 mg.) was heated at 100° for 15 minutes, and the mixture diluted with water. Crystallisation of the product from aqueous acetone gave 3β : 5α : 6β-triacetoxycholestane (150 mg.), m. p. 148—149°, not depressed in admixture with an authentic specimen prepared by the method of Hattori (*J. Pharm. Soc. Japan*, 1939, **59**, 411, 129).

Oxidation of 3β : 5α : 6β-Triacetoxycholestane.—To a well-stirred solution of the triacetate (100 g.) in glacial acetic acid (3 l.) was added, during 6¼ hours, a solution of chromic acid (155 g.) and concentrated sulphuric acid (72 ml.) in acetic acid (723 ml.) and water (175 ml.). The temperature of the mixture rose to 39°. After a further 3¼ hours the solids which had separated were filtered off and washed with ether. The filtrate was diluted with 2—3 volumes of water and extracted with ether (total, 4—5 l.), and the washed ethereal extract extracted with sodium hydroxide solution. The oil remaining after the removal of the ether, in methanol (100 ml.), was kept overnight at 0° whereupon unchanged triacetoxycholestane (7·5 g.) separated and was removed. The filtrates were mixed with semicarbazide hydrochloride (10 g.) and sodium acetate (10 g.) in methanol (50 ml.), heated under reflux for 3 hours, and evaporated to dryness under reduced pressure, and the residues treated with ether and water and filtered from insoluble matter. The ethereal layer was removed and repeatedly treated with water, whereupon the semicarbazone of (I*a*; R′ = R″ = Ac) (3 g.) separated at the ether–water interface and was collected. The semicarbazone (2·45 g.) was heated with pyruvic acid (2 g.) and sodium acetate (1 g.) in water (2 ml.) and acetic acid (10 ml.) for 1 hour. The regenerated ketone separated on pouring of the mixture into water. Purification by chromatography in benzene–light petroleum (yield, 335 mg.), followed by crystallisation from ether–light petroleum, gave 3β : 5α : 6β-triacetoxyandrostan-17-one, m. p. 184—186°, $[\alpha]_D^{21}$ −8·55° (*c*, 0·486 in acetone) (Found : C, 66·5; H, 7·9. Calc. for $C_{25}H_{36}O_7$: C, 67·0; H, 8·1%), not depressed in admixture with an authentic specimen. The oxime had m. p. 145—148° (decomp.) (sealed tube), not depressed in admixture with an authentic specimen. Alkaline hydrolysis gave 5β : 6β-epoxyætiocholan-17-one, m.p. 166—166·5°, alone or in admixture with an authentic specimen.

3β : 6β-Diacetoxyandrost-4-en-17-one (II*a*; R′ = Ac).—A solution of 3β : 6β-diacetoxyandrostan-5α-ol-17-one (680 mg.) in dry pyridine (6 ml.) was treated with redistilled thionyl chloride (670 mg.), and the mixture heated under reflux (oil-bath) for 40 minutes. The solution was poured on ice, and the product extracted with ether and purified by chromatography in benzene–light petroleum, giving 3β : 6β-*diacetoxyandrost-4-en-17-one* (50%; m. p. 157—160°) which, after repeated crystallisation from

ether–light petroleum or aqueous acetone, formed shiny plates, m. p. 163—164°, $[\alpha]_D^{14}$ 55·7° ± 7° (*c*, 0·3425 in chloroform) (Found : C, 70·9; H, 8·2. $C_{23}H_{32}O_5$ requires C, 71·1; H, 8·3%).

Androst-4-en-3β : 6β-diol-17-one (II*a*; R′ = H), obtained by hydrolysis of the foregoing diacetate formed prismatic needles, m. p. 269—270°, from acetone–benzene (Found : C, 74·4; H, 9·3. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%). Treatment of the diolone (10 mg.) with benzoyl chloride in pyridine for 1 hour at 100°, followed by purification of the product by chromatography, gave a dibenzoate, needles (from ether–light petroleum), m. p. 174°, giving a greenish-blue colour with trichloroacetic acid. There was insufficient material for analysis. When this diolone (50 mg.) in ethanol (2·5 ml.) was heated with 30% sulphuric acid (0·125 ml.) for 20 minutes under reflux, and the product purified by chromatography (1 : 1-benzene–light petroleum) and crystallised from light petroleum, androst-4-ene-3 : 17-dione was obtained in very small amount, m. p. 163—165°, not depressed in admixture with an authentic specimen (cf. cholest-4-ene-3β : 6β-diol ⟶ cholest-4-en-3-one; Rosenheim and Starling, *J.*, 1937, 377).

Oxidation of Androst-4-ene-3β : 6β-diol-17-one.—(*a*) The diolone (50 mg.) and aluminium *tert.*-butoxide (200 mg.) in acetone (2 ml.) and benzene (6 ml.) were heated under reflux for 17 hours. After being washed with dilute sulphuric acid and water, the solvent was removed. The residue, in benzene–light petroleum (1 : 1), was purified by chromatography. Elution of the column with benzene gave androstane-3 : 6 : 17-trione (III) (10 mg.), needles (after crystallisation from ether), m. p. 191—192°, not depressed in admixture with an authentic specimen but lowered to 180° in admixture with androst-4-ene-3 : 6 : 17-trione.

(*b*) The diolone (45 mg.) in acetic acid (3 ml.) and chloroform (few drops) was treated with chromic acid (15 mg.) in 95% acetic acid (0·75 ml.) and kept for 3¾ hours at room temperature. The solution was diluted with ethanol and then evaporated under reduced pressure, and the residue treated with water. Extraction with chloroform and ether, followed by chromatography, in benzene–light petroleum, of the neutral fraction of the product, gave *androst-4-en-3β-ol-6 : 17-dione* (16 mg.), colourless needles (from ether–light petroleum), m. p. 192—193° (Found : 75·5; H, 8·7. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·7%). Some unchanged diolone was also recovered. The ultra-violet absorption spectrum (in *iso*propyl alcoholic solution), for which we are indebted to Dr. R. E. Stuckey and Mr. P. S. Stross, B.Sc. (Analytical Dept., The British Drug Houses Ltd.), showed a maximum at 237 mμ., $E_{1\,cm.}^{1\%}$ = 432, characteristic of an αβ-unsaturated ketone.

Treatment of the diketo-alcohol with acetic anhydride in pyridine afforded *3β-acetoxyandrost-4-ene-6 : 17-dione*, needles (from ether–light petroleum), m. p. 200° (Found : C, 73·7; H, 8·5. $C_{21}H_{28}O_4$ requires C, 73·2; H, 8·2%).

Androstane-3β : 5α : 6α-triol-17-one (V*a*; R′ = H).—The following improved method was used (cf. Prelog and Tagmann, *loc. cit.*). To a solution of androst-5-en-3β-ol-17-one (570 mg.) in dry ether (50 ml.) was added a solution of osmic acid (500 mg.) in dry ether (100 ml.) and pyridine (0·5 ml.). After three days at room temperature the mixture was evaporated *in vacuo* and the residue shaken with 0·1N-potassium hydroxide (50 ml.) containing mannitol (2 g.) for 24 hours. The *cis*-triolone was collected and crystallised from ethyl acetate, m. p. 239—242°. Yield, 440 mg.

Androst-4-ene-3β : 6α-diol-17-one (IV*a*; R′ = H).—A solution of 3β : 6α-diacetoxyandrostan-5α-ol-17-one (80 mg.) in dry pyridine (5 ml.) was treated with redistilled thionyl chloride (0·2 ml.), and the mixture heated under reflux (oil-bath) for 45 minutes. The oily product, which gave a blue colour with trichloroacetic acid, failed to crystallise and was hydrolysed with potassium hydroxide (100 ml.) in boiling methanol (2 ml.) for 25 minutes. Extraction of these alkaline liquors with chloroform (5 times) gave *androst-4-en-3β : 6α-diol-17-one*, needles (from benzene), m. p. 205—206° (Found : C, 74·5; H, 9·3. $C_{19}H_{28}O_3$ requires C, 75·0; H, 9·3%), not showing a colour with the trichloroacetic acid reagent. When the diolone (12 mg.) was heated with 3 : 5-dinitrobenzoyl chloride (25 mg.) in benzene–pyridine for 15 minutes, and the product purified by chromatography in light petroleum, the bis-3 : 5-dinitrobenzoate was obtained, having m. p. 192—195°, but there was insufficient material for analysis.

Oxidation of Androst-4-ene-3β : 6α-diol-17-one.—The diolone (35 mg.) and aluminium *tert.*-butoxide (200 mg.) in acetone–benzene were heated under reflux for 35 hours. The product, after purification by chromatography and crystallisation from ether–light petroleum, yielded androstane-3 : 6 : 17-trione, m. p. 191—192°, not depressed in admixture with an authentic specimen.

3β : 4β-Diacetoxyandrost-5-en-17-one, obtained by acetylation of the 4-monoacetate (Petrow, Rosenheim, and Starling, *J.*, 1943, 135), formed needles, m. p. 156—158°, from aqueous ethanol (Found : C, 71·0; H, 8·1. $C_{23}H_{32}O_5$ requires C, 71·1; H, 8·3%). The compound gave a transient blue colour with trichloroacetic acid.

3β-Acetoxyandrost-5-en-4β-ol-17-one.—The corresponding *cis*-3 : 4-diol (445 mg.) (Petrow, Rosenheim, and Starling, *loc. cit.*) in dry pyridine containing 1·1 mols. of acetic anhydride (*i.e.*, 170 mg.) was kept at room temperature for 20 hours and finally heated for 10 minutes on the steam-bath. The product was purified by chromatography in light petroleum, the first crystalline fractions of the eluates yielding *3β-acetoxyandrost-5-en-4β-ol-17-one* (25 mg.), prisms (from ether–light petroleum), m. p. 163—164° (Found : C, 72·6; H, 8·9. $C_{21}H_{30}O_4$ requires C, 72·8; H, 8·7%), giving a blue colour with trichloroacetic acid. Later fractions, eluted by ether–benzene, gave successively an unidentified mixture (35 mg.), the 4-monoacetate (144 mg.), unchanged diolone (60 mg.), and an oil (80 mg.). As the 3-monoacetate was formed in such low yield experiments on its transformations were abandoned.

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