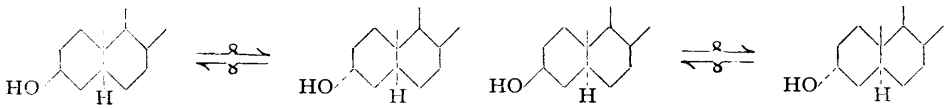
49. *Steroids and Walden Inversion. Part IX.* Epimerisation at* $C_{(17)}$.

By J. Elks and C. W. Shoppee.

Androstan-17α-ol, prepared from androstan-17β-yl toluene-*p*-sulphonate by acetolysis and hydrolysis, and androstan-17β-ol failed to undergo interconversion on treatment with sodium ethoxide at 180° in the presence of a ketone to act as hydrogen-acceptor.

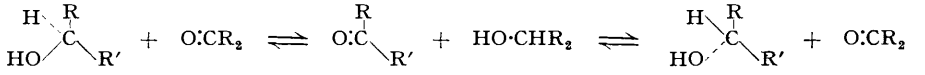
Epimerisation of a 3-hydroxy-steroid was first observed by Dorée and Gardner (*J.*, 1908, 1630) who found that coprostanol (I) was converted into *epi*coprostanol (II) when heated with sodium–amyl alcohol. Interconversion of epimeric 3-hydroxy-steroids was first demonstrated by Windaus and Uibrig (*Ber.*, 1914, **47**, 2384; 1915, **48**, 857; 1916, **49**, 1724) who showed that cholestanol (III) and *epi*cholestanol (IV) by treatment with sodium ethoxide at 180° furnished an equilibrium mixture containing ~90% of the former, whilst coprostanol (I) and *epi*coprostanol (II) gave an equilibrium mixture containing ~90% of the latter.



(I) (II) (III) (IV)

Subsequently, Marker, Kamm, Wittle, Oakwood, Lawson and Laucius (*J. Amer. Chem. Soc.*, 1937, **59**, 2291) converted pregnane-3β : 20β-diol (as I) into pregnane-3α : 20β-diol (as II) and prepared *allo*pregnane-3β : 20α-diol (as III) from *allo*pregnane-3α : 20α-diol (as IV) by treatment with sodium–xylene. It appears that here the steroid alcohol furnishes steroid alkoxide for its own interconversion, since Barnett, Heilbron, Jones, and Verrill (*J.*, 1940, 1390) were able to epimerise cholesterol, cholestanol, lumisterol, and *neo*ergosterol by use of aluminium *iso*propoxide–xylene. Beynon, Heilbron, and Spring (*J.*, 1937, 406) similarly observed almost complete epimerisation of *epi*cholestanol (IV) to cholestanol (III) in the presence of potassium in boiling benzene.

The mechanism of epimerisation has been shown to involve an oxidation–reduction process, analogous to that operating in Oppenauer oxidation and Meerwein–Ponndorf reduction, by the elegant experiments of Aschner and Doering (*J. Amer. Chem. Soc.*, 1949, **71**, 838; cf. Hückel and Naab, *Ber.*, 1931, **64**, 2137); these authors showed that optically active secondary alcohols are not racemised by alkoxides unless a trace of a ketone can arise, or is added, to permit establishment of the equilibrium :



The position of equilibrium in the epimerisations (I ⇌ II) and (III ⇌ IV) is now recognised to result from the greater steric compression to which atoms or groups possessing the polar conformation are subjected compared with the same atoms or groups possessing the corresponding equatorial conformation † (Shoppee, *Chem. and Ind.*, 1952, 86; cf. Barton, *Experientia*, 1950, **6**, 316). Such steric compressions have been observed to control the thermodynamic stabilities of steroid hydroxy-compounds epimeric at $C_{(4)}$ (Barton and Rosenfelder, *J.*, 1951, 1048), $C_{(6)}$ (Shoppee and Summers, *J.*, 1952, 3361), $C_{(7)}$ (Barton, *loc. cit.*; cf. Heilbron, Shaw, and Spring, *Rec. Trav. chim.*, 1938, **57**, 529; Cremlyn and Shoppee, unpublished observation), $C_{(11)}$ (Gallagher *et al.*, *J. Biol. Chem.*, 1946, **162**, 511, 521, 533;

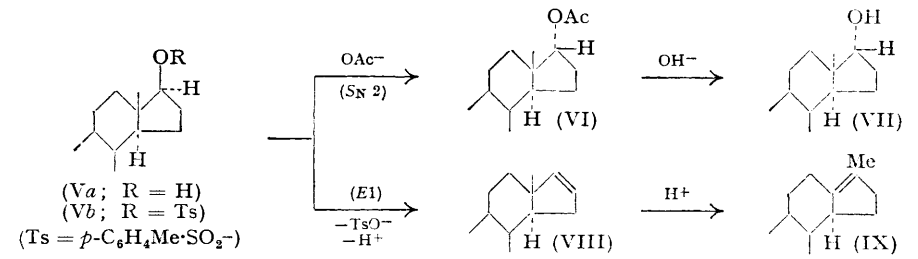
* Part VIII, *J.*, 3374.

† Because ring A in cholesterol is a chair form, it is consistent that the equilibrium cholesterol (90%) ⇌ *epi*cholesterol (10%) (Heilbron *et al.*, *loc. cit.*) favours the former compound (3β-OH : equatorial). In the case of the equilibrium lumisterol (60%) ⇌ *epi*lumisterol (40%) it is to be noted that, despite *cis*-union of rings A and B, ring A is a chair form but the 3β-hydroxyl group here possesses the polar conformation, whilst in the case of lumistanol (3β-OH : polar) sodium methoxide at 200° yields *epi*lumistanol (3α-OH : equatorial) (Ahrens, Fernholz, and Stoll, *Annalen*, 1933, **500**, 109).

Fieser, *Experientia*, 1950, **6**, 312), and $C_{(12)}$ (Gallagher *et al.*, *J. Biol. Chem.*, 1946, **162**, 539; Gallagher and Borgstrom, *ibid.*, 1946, **164**, 791). It seemed therefore of interest to examine the situation at $C_{(17)}$.

Both reaction rates and equilibria at $C_{(17)}$ are strongly influenced by steric compression. The situation has been discussed by Gallagher and Kritchevsky (*J. Amer. Chem. Soc.*, 1950, **72**, 882), Fieser (*loc. cit.*), and by Shoppee (*Nature*, 1950, **166**, 107) who suggested that asymmetric induction may be operative in addition to steric compression. The products of reactions at $C_{(17)}$ in C/D-*trans*-steroids consist largely of the epimerides in which the original 17-substituent takes up the β-configuration which, as Barton (*loc. cit.*) has pointed out, may be regarded as possessing the equatorial conformation. Thus epimerisation of (*a*) 17-acyl or (*b*) 17-carbalkoxy-steroids by acid or alkaline reagents furnishes mixtures in which the 17β-form predominates [(*a*) Butenandt *et al.*, *Ber.*, 1935, **68**, 1847; 1937, **70**, 96; 1939, **72**, 1112; Shoppee, *J.*, 1949, 1671; (*b*) Shoppee, *Helv. Chim. Acta*, 1940, **23**, 925; von Euw and Reichstein, *ibid.*, 1944, **27**, 1851; Sorkin and Reichstein, *ibid.*, 1946, **29**, 1218; Heusser, Meier, and Ruzicka, *ibid.*, p. 1250]. It might therefore be expected that a 17α-hydroxy-steroid would undergo almost complete transformation into the 17β-hydroxy-epimeride.

The compounds chosen for examination were the androstan-17-ols. A quantity of androstan-17β-ol (V*a*) was most kindly made available by Dr. G. Rosenkranz of Syntex Ltd., Mexico City, and a small quantity of androstan-17α-ol (VII) was prepared from it.



After treatment of androstan-17β-ol (V*a*) with toluene-*p*-sulphonyl chloride in pyridine at 20°, the alcohol was recovered unchanged, but prolonged reaction at 45—50° gave (V*b*) in satisfactory yield. Plattner and Fürst (*Helv. Chim. Acta*, 1943, **26**, 2266), and Prelog and Szpilfogel (*ibid.*, 1944, **27**, 390), have shown that acetolysis of 3-toluene-*p*-sulphonyloxy-steroids (saturated at $C_{(5)}$) occurs with inversion of configuration at $C_{(3)}$; the replacement described by Plattner and Fürst is accompanied by elimination, a considerable quantity of the Δ^2- or Δ^3-olefin being formed. Treatment of (V*b*) with potassium acetate in glacial acetic acid gave rise to much unsaturated material and no pure compound with the properties of the acetate (VI) could be isolated. In subsequent experiments, the crude acetolysis product was hydrolysed with alcoholic potassium hydroxide and the resulting mixture subjected to chromatography. From the more strongly absorbed fractions a small amount (~5% yield) of androstan-17α-ol (VII) was isolated together with a little of the starting material (V*b*). That the chief reaction was elimination rather than replacement of the toluene-*p*-sulphonyloxy-group was indicated by the fact that the greater part of the reaction product was rapidly eluted from the column by pentane and gave a yellow colour with tetranitromethane–chloroform. This material could not be caused to crystallise and, although androst-16-ene (VIII), which exists in two forms, m. p. 44°, $[\alpha]_D$ +18° (Kägi and Miescher, *ibid.*, 1939, **22**, 683), and m. p. 74·5—75·5°, $[\alpha]_D$ +17° (Prelog, Ruzicka, and Wieland, *ibid.*, 1944, **27**, 66), may have been present, it seems probable that the main constituent was ψ-androstene (IX), an oil which is produced by treatment of (VIII) with acidic reagents (Miescher and Kägi, *ibid.*, 1949, **32**, 761); the colour test described by Miescher and Kägi for ψ-androstene was positive.

Reduction of androstan-17-one with lithium aluminium hydride gives almost exclusively androstan-17β-ol, but it has been found possible chromatographically to isolate small quantities (*ca.* 0·25%) of androstan-17α-ol. A gift of androstan-17α-ol (200 mg.) by

Dr. K. Miescher of CIBA, Basle, supplemented the small quantities available by the above method, and enabled the investigation to be completed.

It was found that a synthetic mixture of androstan-17α-ol and androstan-17β-ol could be separated quite satisfactorily by chromatography (see above), the 17α-ol being eluted with a mixture of equal volumes of pentane and benzene and the 17β-ol with pure benzene. The intermediate mixed fraction amounted to less than 10%.

The epimerisation reactions were carried out with alcoholic sodium methoxide at 180° (Windaus, *Ber.*, 1916, **49**, 1724). After such treatment of androstan-17β-ol for 16 hours, some 90% of it was recovered by chromatography. Moreover, there was no evidence of the presence in the more readily eluted fractions of the 17α-ol as judged by the Kägi–Miescher colour reaction (*Helv. Chim. Acta*, 1939, **22**, 683). Since this result might indicate that under the conditions of reaction there was an equilibrium which was very much in favour of the 17β-epimeride (as indeed analogy suggests), androstan-17α-ol was subjected to similar treatment. Here again some 90% was recovered and no trace could be found of the 17β-epimeride. These experiments were repeated with the addition of a small quantity of androstan-17-one with precisely similar results. It seems that under the conditions which cause interconversion of the epimeric forms of 3-hydroxy-steroids, 17-hydroxy-groups (like 20-hydroxy-groups in the side-chain) are unaffected.

Experimental

M. p.s were determined thermo-electrically on a Kofler block; limit of error ±2°. Solvents for chromatographic operations were rigorously purified and dried and, unless stated otherwise, aluminium oxide (Spence type H, activity II) was used. For drying of ethereal extracts, brief treatment with anhydrous sodium sulphate was used. Microanalyses are by Drs. Weiler and Strauss, Oxford.

Androstan-17β-yl Toluene-p-sulphonate (V*b*).—Androstan-17β-ol (1·0 g.) was dried by repeated evaporation with benzene at 10 mm. and by heating at 100°/0·01 mm. for 30 minutes. It was dissolved in specially dried pyridine (20 c.c.), purified toluene-*p*-sulphonyl chloride (3·0 g.) added, and the mixture kept at 45—50° for 4 days. The cold solution was treated with water (5 c.c.) whereupon an oil separated which rapidly crystallised in needles. After some hours the crystals were washed with N-hydrochloric acid and with water and dried (m. p. 133—138°). Recrystallisation from acetone gave needles, m. p. 138—139° (1·03 g.); a second crop obtained from the mother-liquors weighed 0·17 g. Again recrystallised from acetone *androstan-17β-yl toluene-p-sulphonate* had m. p. 139—140°, $[\alpha]_D$ 0° ± 2° (*c* = 1·00 in $CHCl_3$) (Found, after drying at 20°/0·01 mm.: C, 72·7; H, 8·8. $C_{26}H_{38}O_3S$ requires C, 72·5; H, 8·9%).

Acetolysis. The toluene-*p*-sulphonate (V*b*) (200 mg.), anhydrous potassium acetate (200 mg.), and acetic acid (4 c.c.) were refluxed for 3 hours. The cold solution was diluted with water and the resultant oil extracted with ether. The extract was washed with 2N-sodium carbonate solution, then with water, dried, and evaporated. The residue was refluxed for 2 hours with a solution of potassium hydroxide (0·3 g.) in water (0·3 c.c.) and ethanol (3 c.c.); ethanol was removed in a vacuum, the residue diluted with water, and the product repeatedly extracted with ether. The extract was washed successively with 2N-hydrochloric acid, sodium carbonate solution, and water, dried, and evaporated. The residual yellow oil (129 mg.) was dried by repeated azeotropic distillation with benzene and chromatographed in pentane on aluminium oxide (4 g.) prepared in pentane; 15-c.c. batches of each eluant were used. The column was eluted with pentane (Fr. 1—5), pentane–benzene (1 : 1) (Fr. 6—15), benzene (Fr. 16—20), and ether (Fr. 21—25). Fractions 1—3 (90·4 mg.) were combined but the oil did not crystallise. A small portion was dissolved in acetic acid, and one drop of concentrated sulphuric acid added; addition of a dilute solution of bromine in acetic acid gave an intense blue colour characteristic of ψ-androstene (Miescher and Kägi, *loc. cit.*). Fractions 6—11 crystallised partly when kept; they were combined and crystallised from aqueous acetone, to give androstan-17β-yl toluene-*p*-sulphonate, m. p. 133—137°, mixed m. p. 134—139°. Fractions 16—20 contained insignificant traces of oil. Fractions 21—24 were crystalline and were combined and recrystallised from methanol, to give androstan-17α-ol, double m. p. 139° with transformation to needles, m. p. 147° (5 mg.); a second crystallisation from methanol gave small needles, m. p. 146·5—147·5°, not depressed by admixture with a genuine specimen, m. p. 148—151°. The colour test for androstan-17α-ol described by Miescher and Kägi was positive, whilst by admixture with androstan-17β-ol the m. p. was depressed to *ca.* 120°.

Chromatographic Separation of Androstan-17α-ol and Androstan-17β-ol.—A synthetic mixture of androstan-17α-ol (28 mg.) and androstan-17β-ol (25 mg.) was chromatographed in pentane–benzene (1 : 5; 6 c.c.) on aluminium oxide (2 g.) prepared in pentane (5-c.c. eluant batches). Fractions 1—20 obtained by elution with pentane–benzene (1 : 1) gave androstan-17α-ol (18·8 mg.), m. p. 152—153° after crystallisation from methanol. Further elution (Fr. 21—24) and with benzene (Fr. 25) gave mixtures, m. p. 130—155°, of the epimerides (4·7 mg.). Fractions 26—29 obtained with benzene, fractions 30—32 with benzene–ether (1 : 1), and fractions 22—35 with ether gave androstan-17β-ol (21·2 mg.), m. p. 168—169° after recrystallisation from benzene–pentane.

Reduction of Androstan-17-one with Lithium Aluminium Hydride [By M. FINKELSTEIN].—Androstan-17-one (m. p. 118—119°; 791 mg.) was treated with lithium aluminium hydride (500 mg.) in ether. The reaction mixture was worked up in the usual way and the product (780 mg.) chromatographed on a column of aluminium oxide (20 g.) prepared in pentane; 100 c.c. of solvent were used for each elution. The column was eluted successively with pentane, pentane–benzene (1 : 1), benzene, and benzene–ether mixtures (1 : 19, 1 : 7 twice), and finally twice with ether, giving fractions 1—8. Fraction 2 was an oil [11 mg.; (—)] (+, − refer to response to the Kägi–Miescher test); fraction 3 was semicrystalline [2 mg.; weak (+)]. Fraction 4 had m. p. 129—157° [8 mg.; (+)]; fractions 5 and 6 had m. p. 132—147° [20 mg.; (+)] and m. p. 169° [284 mg.; (−)] respectively. Fractions 7 and 8 had m. p. 169° [449 mg.; (−)]. Fractions 2—5 were united (22 mg.) and rechromatographed on aluminium oxide (1 g.) in pentane (3-c.c. eluant batches). Elution with pentane–benzene (1 : 1) gave oil [3 mg.; (−)]; use of benzene gave material, m. p. 99—103° [1 mg.; (−)]. Further elution with benzene and with benzene–ether (1 : 19) gave fractions 4′ and 5′, each showing a double m. p. 137—139° and 150—152° [1 mg.; 1 mg.; (+), (+)]; further elution with benzene–ether (1 : 19) gave fractions 6′ and 7′, m. p. 157—160° and 165—168° [5 mg.; 4 mg.; (−); (−)]. Elution with benzene–ether (1 : 9) (Fr. 8′) gave material, m. p. 166—168° (4·5 mg.; (−)]. Fractions 3′ and 4′ consisted of androstan-17α-ol, which showed the characteristic double m. p. 137—139° with transformation to needles, m. p. 152°, unchanged by admixture with a genuine specimen. Fractions 6′, 7′, and 8′ consisted of androstan-17β-ol.

Equilibration Experiments.—(*a*) Androstan-17β-ol (m. p. 168°; 100 mg.) was heated with a solution of sodium (0·4 g.) in dry ethanol (10 c.c.) in a sealed tube at 180° for 15 hr. The product was chromatographed in pentane–benzene (1 : 1), on aluminium oxide (4 g.) prepared in pentane (10-c.c. eluant batches). Elution with benzene–pentane (1 : 1) (Fr. 1—7) and with benzene (Fr. 8, 9) furnished no crystalline material. Further elution with benzene gave crystals, m. p. 101—115°, and a series of products (Fr. 11—22) having m. p. 132—140° rising to 159—162°. Use of benzene–ether (1 : 4) (Fr. 23—34) gave material, m. p. 162—167°. A single elution with ether gave a trace of material, m. p. 152—158°. Fractions 18—35 (86·1 mg.) were combined and crystallised from benzene–pentane to give androstan-17β-ol (65·8 mg.), m. p. 166—167°, undepressed by admixture with a genuine specimen. Fractions 9—17 were united (8 mg.) and melted between 132° and 156°; the absence of androstan-17α-ol was shown by a negative Kägi–Miescher test, and crystallisation from pentane gave androstan-17β-ol (3·7 mg.), m. p. 166—167°. No further crystalline material could be obtained from the mother-liquor.

(*b*) Androstan-17α-ol (m. p. 148—151°; 25 mg.) was heated with a solution of sodium (0·1 g.) in dry ethanol (2·5 c.c.) in a sealed tube at 180° for 15 hr. The product (25 mg.) melted at 140—146° after softening from 130° and gave a strongly positive Kägi–Miescher test; it was chromatographed on aluminium oxide (1 g.) prepared in pentane (5-c.c. eluant batches). Elution with pentane and with benzene–pentane (1 : 1) (Fr. 6) furnished no material; further elution with benzene–pentane (1 : 1) (Fr. 7—19) gave crystalline material, m. p. 146—148°. Use of benzene (Fr. 20—24) gave crystals, m. p. 146—147°, 147—148°, 146—148°, 144—147°, and 144—146°. Further elution with benzene and benzene–ether (1 : 1) furnished only traces of material, whilst elution with ether furnished no material. Fractions 7—25 were united (22·6 mg.) and crystallised from methanol, to give androstan-17α-ol (13·2 mg.), m. p. 148—149°; a second crop (5·8 mg.) obtained by concentration of the mother-liquors had m. p. 144—148°. These products mixed with androstan-17α-ol gave no depression, but by admixture with androstan-17β-ol melted from 124° upwards.

(*c*) [By G. H. R. SUMMERS.] Androstan-17α-ol (m. p. 148—151°; 73 mg.) was heated with a solution of sodium in *tert.*-butanol (5 c.c., prepared from 0·25 g. of sodium and 45 c.c. of *tert.*-butanol) in the presence of androstan-17-one (m. p. 118—119°; 18 mg.) in a sealed tube at 180° for 48 hr. The product, a white crystalline solid, was chromatographed on aluminium oxide (3 g.) in pentane (20-c.c. eluant batches). Elution with pentane gave androstan-17-one (16·8

mg.), m. p. and mixed m. p. 118°; elution with pentane–benzene (2 : 1, 1 : 1, and 1 : 3) gave androstan-17α-ol (57 mg.), m. p. 148—152° undepressed by admixture with a genuine specimen. Elution with benzene and benzene–ether (1 : 1) gave crystalline material (20·8 mg.) which was rechromatographed on aluminium oxide (1 g.) in benzene–pentane (1 : 1) (eluant batches of 20 c.c.). Fractions 1—7 obtained with benzene–pentane (1 : 1) had the following m. p.s: 149—153°, 150—152°, 149—151°, 150—151°, 151°, 150°, and 146—153°, in each case after transition to needles at *ca.* 138°, and all gave a positive Kägi–Miescher test; fraction 8 obtained with benzene contained only a trace of material, but fraction 9 obtained with benzene–ether (3 : 2) gave material of m. p. 138° with transition to needles, m. p. 150°, giving a positive Kägi–Miescher test; elution with ether gave no material. The total solid recovered amounted to 17 mg. and consisted of androstan-17α-ol, no trace of androstan-17β-ol being detected.

One of us (J. E.) thanks Glaxo Laboratories Ltd. for the opportunity to participate in this work. We gratefully acknowledge gifts of androstan-17-one and androstan-17β-ol from Dr. G. Rosenkranz of Syntex Ltd., Mexico City, androstan-17α-ol from Dr. K. Miescher of CIBA, Basle, and a grant from the Royal Society which has partly defrayed the cost of this work.

UNIVERSITY COLLEGE, SWANSEA, UNIVERSITY OF WALES. [*Received September 9th*, 1952.