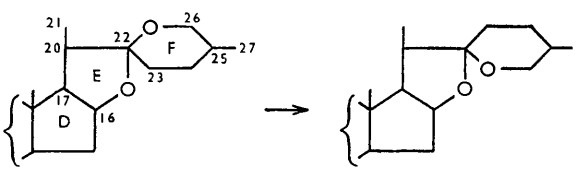
## 543. *Steroidal Sapogenins: The Isomerisation of Normal to* iso-*Sapogenins.*

By R. K. CALLOW and (MISS) P. N. MASSY-BERESFORD.

Support for a hypothesis of the mechanism of the isomerisation by acid of " normal " sapogenins (25*L*) into " *iso* "-sapogenins (25*D*) has been obtained by effecting the change with deuterium ions, followed by location of the deuterium in the isomerisation product by degradation. The reversibility of the isomerisation has been confirmed, and it has been shown that, under the conditions for inversion at position 25, deuterium–hydrogen exchange also occurs at position 20.

MARKER and ROHRMANN [1] reported the conversion, by prolonged treatment with 2N-ethanolic hydrochloric acid of the " normal " sapogenin sarsasapogenin into the " *iso* "-sapogenin smilagenin, and suggested that the reaction involves the opening and reclosure of the terminal oxide ring:



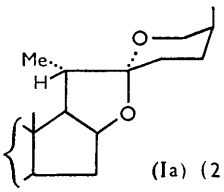

" Normal " sapogenin " *iso* "-Sapogenin

The isomerisation is a general reaction of the " normal " sapogenins.

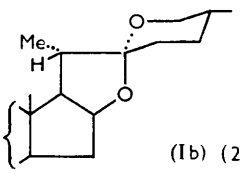
[1] Marker and Rohrmann, *J. Amer. Chem. Soc.*, 1939, **61**, 846.

Further investigation [2] of the isomerisation of sarsasapogenin showed that the reaction yields a mixture containing sarsasapogenin, smilagenin, and the corresponding $\Delta^2$- or $\Delta^3$-3-deoxysapogenins, and also that the isomerisation is an equilibration in which the formation of the more stable " *iso* "-sapogenin is favoured.

Callow and James [3] investigated alternative conditions of isomerisation and found that the reaction takes place in hot dioxan containing hydrochloric acid, and also occurs in the presence of acetic anhydride under similar, anhydrous conditions, so that the change must take place without the opening of ring F. Scheer, Kostic, and Mosettig [4] showed that the " normal " and the " *iso* "-sapogenins differ in configuration at position 25; James [5] related the configuration at this asymmetric centre to glyceraldehyde, and it has been proved [6] that *neo*tigogenin (25*L*) and tigogenin (25*D*) have the same configuration at all asymmetric centres except $C_{(25)}$. The absolute configuration at $C_{(22)}$ has been discussed [6] and structures (Ia) and (Ib) have been proposed for the 25*L*- and the 25*D*-sapogenins. It must follow that the isomerisation (Ia) ⟶ (Ib) consists of an inversion of configuration at $C_{(25)}$, in which the methyl group attached to $C_{(25)}$ changes from the axial to the equatorial conformation.



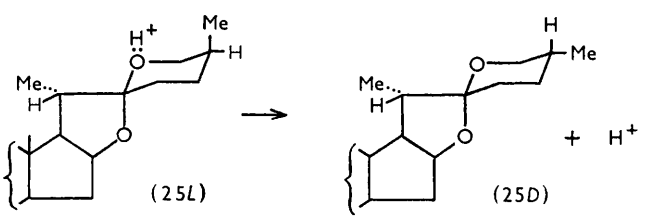
(Ia) (25*L*)



(Ib) (25*D*)

Cornforth [7] suggested that the inversion occurs by the addition of a proton to the oxygen atom of ring F of the 25*L*-sapogenin, the proton then being well placed to initiate a displacement reaction with inversion of configuration at $C_{(25)}$.

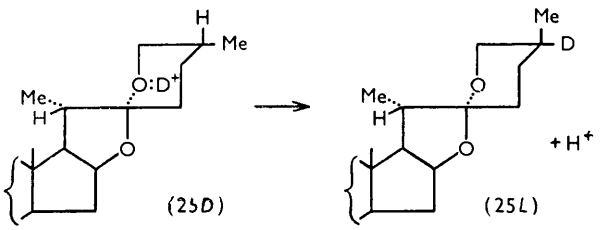
If the isomerisation takes place by this process, treatment of a 25*L*-sapogenin with deuterium ions should give a 25*D*-sapogenin containing, at position 25, one deuterium atom stable towards brief treatment with acids.[8] Proof of the position of the deuterium should be obtainable by degradation. If the isomerisation is reversible,[2] similar treatment of a




25*D*-sapogenin with deuterium ions should also introduce deuterium at position 25. This reverse process could occur by a mechanism similar to that suggested by Cornforth if the sapogenins can exist with ring F in the less stable [9] " upright " conformation under the conditions of isomerisation.

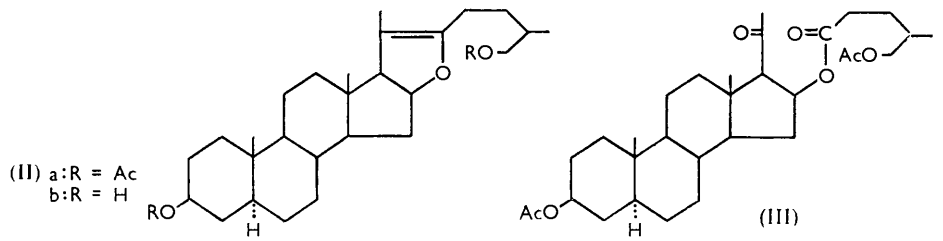
[2] Wall, Serota, and Witnauer, *J. Amer. Chem. Soc.*, 1955, **77**, 3086.
[3] Callow and James, *J.*, 1955, 1671. [It should have been made clear in this paper that the reaction in the presence of acetic anhydride was carried out under anhydrous conditions, with dioxan (10 ml.) saturated with hydrogen chloride in place of the concentrated hydrochloric acid (15 ml.) used in the experiment described immediately previously.]
[4] Scheer, Kostic, and Mosettig, *J. Amer. Chem. Soc.*, 1953, **75**, 4871.
[5] James, *J.*, 1955, 637.
[6] Callow and Massy-Beresford, *J.*, 1957, 4482.
[7] Cornforth, *Ann. Reports*, 1953, **50**, 219.
[8] Callow, James, and Massy-Beresford, *Chem. and Ind.*, 1956, R26.
[9] Hirschmann, Hirschmann, and Corcoran, *J. Org. Chem.*, 1955, **20**, 572.

It was found that *neo*tigogenin acetate (25*L*) was isomerised when heated in a sealed tube on the steam-bath for 55 hr. with 6·5 molecular proportions of 1·4N-deuterium chloride in dioxan. After treatment of the product with boiling acetic acid [8] or 0·5N-methanolic hydrochloric acid to remove labile deuterium, and alkali-treatment to complete




saponification of the acetate group, deuterated tigogenin containing 1·0 D (where the symbol D signifies gram-atoms of deuterium per mole of steroid) was obtained in 50% yield. The same product was obtained by similar treatment of tigogenin acetate in full accordance with the assumed reversibility of the isomerisation. The deuterated tigogenin showed weak carbon–deuterium stretching absorption bands in the infrared spectrum at 2175 and 2130 cm.$^{-1}$, and the spectrum differed from that of tigogenin in the " fingerprint " region, especially in that the 915 cm.$^{-1}$ band was more intense than the band at 895 cm.$^{-1}$.

It was originally intended to determine the position of the deuterium by oxidative degradation of the corresponding *pseudo*sapogenin, followed by isolation and degradation of the side-chain. A method of isolation of the side-chain without oxidation at position 26 was devised using unlabelled starting material. Oxidation of *pseudo*tigogenin diacetate (IIa) by chromic acid at room temperature gave 3β-acetoxy-16β-(δ-acetoxy-γ-methylvaleroyloxy)-5α-pregnan-20-one (III), which, on methanolysis with N-hydrogen chloride in dry methanol, yielded a mixture of methyl δ-hydroxy-γ-methylvalerate (IV), a γ-lactone,



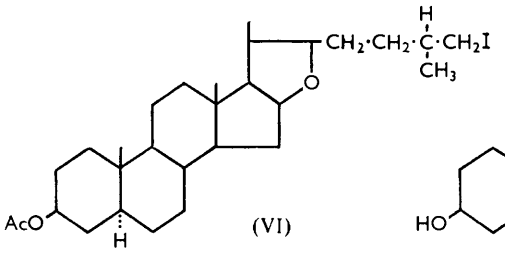

(IV) $HO{\cdot}CH_2{\cdot}CHMe{\cdot}CH_2{\cdot}CH_2{\cdot}CO_2Me$ $HO{\cdot}CH_2{\cdot}CHMe{\cdot}CH_2{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CHPh_2$ (V)

and a δ-lactone. Treatment of the mixture with diphenylmethylamine yielded the crystalline amide (V) of δ-hydroxy-γ-methylvaleric acid. When the process was repeated with deuterated tigogenin (1·0 D), the mixture containing the methyl ester was found to contain only 0·13 D and did not yield a crystalline diphenylmethylamide. This method of degradation was abandoned when another approach proved successful.

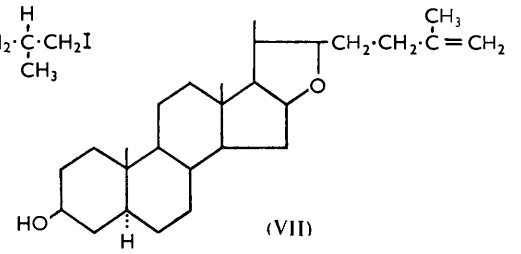
Sato and Latham [10] reported the conversion of tigogenin into 3β-acetoxy-16 : 22-epoxy-26-iodocholestane (VI); this was unstable, being extremely sensitive to treatment with alkali,[11] which caused simultaneous deacetylation and dehydrohalogenation to give 16 : 22-epoxycholest-25-en-3β-ol (VII). This reaction suggested a method of determining the position of the deuterium in deuterated tigogenin, since a deuterium atom at position 25 would be completely removed by dehydroiodination of a 26-iodo-derivative.

[10] Sato and Latham, *J. Amer. Chem. Soc.*, 1956, **78**, 3150.
[11] Sato, Latham, and Scheer, *J. Org. Chem.*, 1956, **21**, 689.

Tigogenin was converted into dihydrotigogenin (VIIIa) in high yield by catalytic hydrogenation with a trace of perchloric acid as a promoter.[12] Dihydrotigogenin was converted into the ditoluene-*p*-sulphonate (VIIIb) which on treatment with sodium iodide in ethyl methyl ketone gave 16 : 22-epoxy-3ξ : 26-di-iodocholestane (IX), a stable, highly

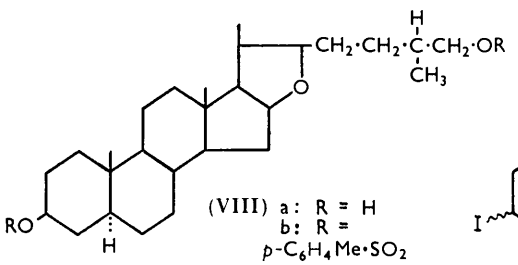



(VI)

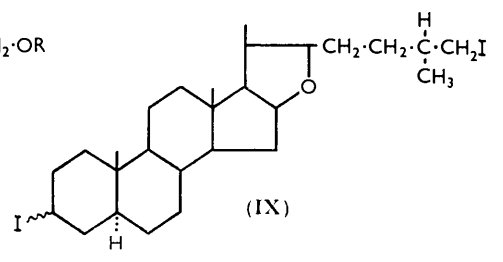



(VII)

crystalline solid, in 52% yield. Fractional crystallisation indicated that two isomers were present which differed in solubility, crystalline form, melting point, and infrared absorption spectrum both in the solid state and in $CS_2$ solution. Analysis of the infrared absorption




(VIII) a: R = H
b: R = *p*-$C_6H_4Me\cdot SO_2$




(IX)

spectra in the low-frequency range suggested that the compounds might be isomeric at $C_{(3)}$, although the frequency shift for the carbon–iodine linkage (about 2 cm.$^{-1}$) is smaller than would be expected for such isomerism. The less soluble isomer, thought to be 16 : 22-epoxy-3β : 26-di-iodocholestane, absorbs at 680 and 604 cm.$^{-1}$ (in $CS_2$ solution), and the other isomer at 678 and 604 cm.$^{-1}$. The absorption at 604 cm.$^{-1}$ is tentatively assigned to the carbon–iodine linkage at position 26, since it is absent from the spectra of compounds (X) and (XI) described below.

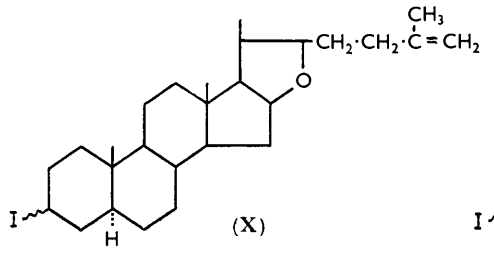
Treatment of the di-iodide with 10% potassium hydroxide in boiling ether–methanol yielded a 4 : 1 mixture, separable by chromatography, of 16 : 22-epoxy-3ξ-iodocholest-25-ene (X) and 16 : 22-epoxy-3ξ-iodo-26-methoxycholestane (XI). Both compounds were stable crystalline solids.

This series of reactions was repeated with deuterated tigogenin (0·9 D) (prepared from *neo*tigogenin acetate by isomerisation with deuterium chloride), which gave deuterated dihydrotigogenin (0·8 D), the ditoluene-*p*-sulphonate (0·8 D), and deuterated 16 : 22-epoxy-3ξ : 26-di-iodocholestane containing 0·95 D. Dehydroiodination gave 16 : 22-epoxy-3ξ-iodocholest-25-ene which unexpectedly contained 0·46 D. Evidently only half the deuterium content of the deuterated tigogenin had been situated at position 25. That the remainder was situated at position 20 was proved by the following series of experiments in which specific measures designed to remove deuterium from position 20, by introduction of the 20 : 22-double bond, were taken.

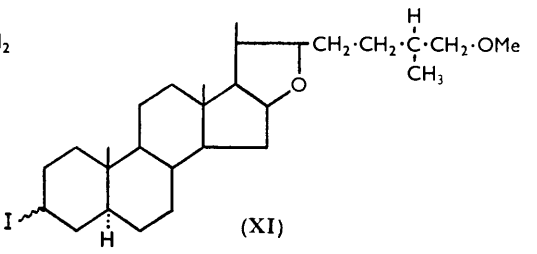
Since further supplies of *neo*tigogenin were not available, advantage was taken of the reversibility of the isomerisation and tigogenin acetate was used for the preparation of

[12] Hershberg, Oliveto, Rubin, Staeudle, and Kuhlen, *J. Amer. Chem. Soc.*, 1951, **73**, 1144.

deuterated tigogenin. The amount of 1·4N-deuterium chloride used in the reaction (12·5 molecular proportions) was twice that employed in previous experiments. After removal of labile deuterium and purification, the deuterated tigogenin containing 1·56 D. This was converted into deuterated *pseudo*tigogenin (IIb) contained 0·6 D, which was cyclised by acid to give deuterated tigogenin containing 0·48 D. The infrared absorption spectrum of this compound showed a single C–D stretching band at 2120 cm.$^{-1}$, and differed from that of tigogenin in the " fingerprint " region, having three bands of equal intensity



(X)

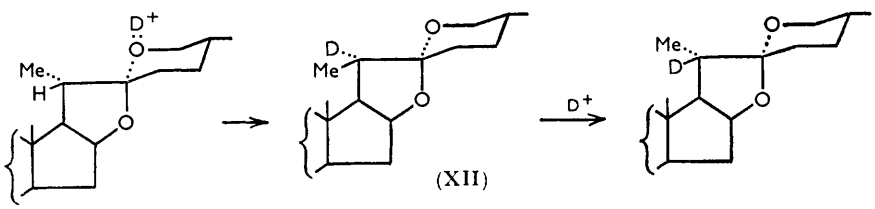


(XI)

at 920, 896, and 885 cm.$^{-1}$, and no absorption at 865 cm.$^{-1}$. The deuterated tigogenin was converted into deuterated 16 : 22-epoxy-3ξ : 26-di-iodocholestane (0·47 D), which was dehydroiodinated to give 16 : 22-epoxy-3ξ-iodocholest-25-ene that contained no detectable deuterium.

These results support the hypothesis that the isomerisation of the sapogenins is a reversible reaction which proceeds by the mechanism suggested by Cornforth.[7] In addition it has been shown that deuterium–hydrogen exchange occurs at position 20 under the conditions for inversion at $C_{(25)}$, since deuterated tigogenin (1·56 D) loses one deuterium atom when converted, by introduction of the 20 : 22-double bond, into deuterated *pseudo*-tigogenin (0·6 D).

This exchange at position 20 could occur by two alternative processes. When a deuteron adds to the oxygen atom of ring F, it is equally well placed to cause displacement with inversion at either $C_{(25)}$ or $C_{(20)}$. Inversion at $C_{(20)}$ would give the 20-*iso*sapogenin (XII) with the 20-methyl group in the hindered β-position. These isomers are



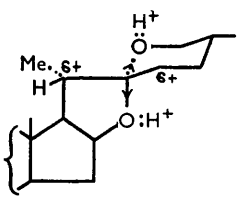
(XII)

obtained [13-15] by very mild acid treatment of *pseudo*sapogenins, and are readily converted into 20α-sapogenins by further acid treatment. If a 20β-sapogenin were formed during the isomerisation, it would be converted immediately into the 20α-isomer in the presence of 1·4N-deuterium chloride.

An alternative and more probable explanation is that the spiroketal group causes activation of the hydrogen atom at $C_{(20)}$ towards deuterodeprotonation. The well-known reactivity of position 23 of the sapogenins under acid conditions is thought to result from the addition of protons to the oxygen atoms of rings E and F, which causes an electron deficiency at $C_{(23)}$ by electromeric changes *via* the spiroatom as indicated in the diagram.

[13] Callow, Dickson, Elks, Evans, James, Long, Oughton, and Page, *J.*, 1955, 1966.
[14] Wall, Serota, and Eddy, *J. Amer. Chem. Soc.*, 1955, **77**, 1230.
[15] Ziegler, Rosen, and Shabica, *ibid.*, 1954, **76**, 3865.

Similar activation of the hydrogen atom at $C_{(20)}$ must also occur, but is probably somewhat reduced by the inductive effect of the 20-methyl group. The exchange which occurs [8] when the sapogenins are boiled with deuterium acetate is thought to occur at position 23. Deuterium at position 23 is readily removed by brief treatment with acids, but the deuterium introduced at position 20 by deuterium chloride in hot dioxan is evidently stable to brief acid treatment. This must be due partly to steric effects, since the deuterium atom at $C_{(20)}$ is shielded by the 20α- and the 13β-methyl group, partly to the reduced activation at $C_{(20)}$ compared with that at $C_{(23)}$ as a result of the inductive effect of the 20-methyl group, and partly to the greater stability of a C–D bond compared with the corresponding C–H bond which is a result of the deuterium isotope effect.




## Experimental

M. p.s were determined in a Kofler apparatus with polarised light, and are corrected. Optical rotations were determined for $CHCl_3$ solutions of concentration 1·0% unless otherwise stated. Samples for analysis were recrystallised to constant m. p., and in the case of dihydrotigogenin and tigogenin were dried for 8 hr. in a slow stream of air at 100° and 140° respectively. For deuterated compounds, the percentages of hydrogen found are " apparent " since they were computed as though the water formed was pure $H_2O$. The required values given for comparison have been calculated in the same way, and all " apparent " values for hydrogen are designated by " H ". Infrared absorption was measured with a double-beam instrument with a rock-salt prism (Perkin-Elmer Model 21) in KCl discs unless otherwise stated.

*Deuterium Analyses.*—The percentage of deuterium oxide in the water formed by combustion of a sample (30—60 mg.) of deuterated steroid was determined by infrared analysis, by a method derived from those described by Trenner and Walker,[16] and Berglund-Larsson.[17] The HOD absorption at 2500 cm.$^{-1}$ of a film of the water sample was determined in a cell made of two crystalline quartz plates 0·5 mm. in thickness, separated by aluminium foil strips 0·017 mm. in thickness. The absorption of the empty cell was balanced by that of two similar quartz plates in the compensating beam. The slit width was 30 μ. For each analysis, a calibration curve was recorded by using solutions of $D_2O$ in $H_2O$ of known concentration, and the concentration of the unknown was determined by interpolation of the plot of the logarithm of the percentage of $D_2O$ against the percentage absorption at 2500 cm.$^{-1}$, which is a straight line over the concentration range 1—8% w/w of $D_2O$ in $H_2O$. The method gave reproducible results with an accuracy of ±0·1 D for compounds which yielded water containing 1—8% w/w of $D_2O$ in $H_2O$.

pseudo*Tigogenin Diacetate.*—Tigogenin (10 g.) was boiled with *n*-octanoic acid (20 ml.) for 2 hr. under nitrogen. The water formed in the reaction was collected in a trap. After cooling, ether (200 ml.) was added and the solution extracted twice with 0·5N-sodium hydroxide solution (300 ml.). The aqueous layers were extracted with ether (100 ml.), and the combined ether extracts washed once with water, dried, and evaporated. The residual oil was boiled with 5% potassium hydroxide in methanol for 30 min. Hot water (~800 ml.) was added slowly to the hydrolysis solution. After cooling, the solid was collected, washed with hot, slightly alkaline water, and dried *in vacuo* over sodium hydroxide.

The crude *pseudo*tigogenin (7·6 g.) was acetylated in pyridine–acetic anhydride (1 : 1) on the steam-bath for 30 min. The solvents were evaporated under reduced pressure and the residue was dissolved in carbon tetrachloride and evaporated to dryness six times. The gummy diacetate was used for the next stage without further purification.

3β-*Acetoxy*-16β-(δ-*acetoxy*-γ-*methylvaleroyloxy*)-5α-*pregnan*-20-*one.*—A solution of chromic oxide (7·5 g.) in 80% acetic acid (75 ml.) was added to a solution of *pseudo*tigogenin diacetate in glacial acetic acid (75 ml.) at 30°. The temperature rose to 49°, and the mixture was stirred at room temperature for 1½ hr. Ethanol (5 ml.) was added, and the mixture was poured into water, saturated with sodium chloride, and extracted with ether. The extract was washed

[16] Trenner and Walker, *Perkin-Elmer Instrument News*, 1952, **4**, No. 1.
[17] Berglund-Larsson, *Acta Chem. Scand.*, 1956, **10**, 701.

with sodium hydrogen carbonate solution until free from acid, then with water, dried and evaporated.

*N-Diphenylmethyl-δ-hydroxy-γ-methylvaleramide.*—The oxidation product (7·85 g.) was boiled with N-hydrogen chloride in dry methanol (70 ml.) for 3 hr. and left overnight at room temperature. The solution was adjusted to pH 6 with 1·88N-potassium hydroxide in methanol (25·5 ml.), and the solvent was evaporated under reduced pressure at room temperature. The gum was dissolved in ether, which was then dried ($MgSO_4$) and filtered to remove inorganic salts. The filtrate was evaporated under reduced pressure at the lowest possible temperature. The residual gum was heated slowly under reduced pressure to 130°. A distillate was obtained, infrared examination of which indicated that it was a mixture of a methyl ester, a γ-lactone, and a δ-lactone, which were not separated by fractional distillation. The combined fractions (600 mg.) were dissolved in dry benzene (25 ml.), diphenylmethylamine (2·0 g.) was added, and the solution boiled for 18 hr. with the exclusion of atmospheric carbon dioxide. The benzene was evaporated under reduced pressure and the residue treated with aqueous orthophosphoric acid. The precipitate was collected, washed with aqueous orthophosphoric acid, and dried *in vacuo* over $P_2O_5$. The solid (500 mg.) was crystallised twice from benzene, to give needles of *N-diphenylmethyl-δ-hydroxy-γ-methylvaleramide*, m. p. 118—119°, $[\alpha]_D$ +8° (Found: C, 77·0; H, 7·8; N, 4·8. $C_{19}H_{23}O_2N$ requires C, 76·7; H, 7·8; N, 4·7%), $\nu_{max.}$ 3275, 3020, 2920, 2840, 1647, 1534, 1500, 1245, 1227, 1040, 980, 753, 738, 695 cm.$^{-1}$, $\nu_{max.}$ (in $CCl_4$) 3270, 3010 (w), 2900, 2840, 1645, 1500, 1040, 690 cm.$^{-1}$.

*Dihydrotigogenin.*—Tigogenin acetate (2·0 g.) in glacial acetic acid (200 ml.) containing 3 drops of 72% perchloric acid was hydrogenated over platinum oxide (300 mg.) at room temperature and 32 lb./sq. in. for 18 hr. The catalyst was removed and the filtrate diluted with water, neutralised with 50% sodium hydroxide solution, and extracted with ether. The ether extract was washed with sodium hydrogen carbonate solution and water, dried, and evaporated. The residue was boiled with 3% potassium hydroxide in methanol for 1 hr. The solution was poured into water and neutralised, and the solid collected, washed, and dried (1·64 g., 90%). Dihydrotigogenin had m. p. 167—171° (from acetone), $[\alpha]_D$ −4° (Found: C, 77·0; H, 11·0. Calc. for $C_{27}H_{46}O_3$: C, 77·5; H, 11·1%) (lit.,[18] m. p. 163—166°, $[\alpha]_D$ −4°).

*Dihydrotigogenin 3β : 26-Ditoluene-p-sulphonate.*—Dihydrotigogenin (1·18 g., 2·8 mmoles) was dissolved in pyridine (15 ml.) (which had been dried, distilled, and kept over barium oxide) and cooled to −10°. Toluene-*p*-sulphonyl chloride (3·6 g., 19 mmoles) was added and the mixture swirled, with cooling, until the acid chloride had dissolved. The solution was kept at 0° for 2 hr. and at room temperature overnight. Ice was added, and after 30 min. the mixture was extracted with ether. The extract was washed well with cold N-hydrochloric acid, with water, with 2% sodium hydrogen carbonate solution, and with water, dried, and evaporated under reduced pressure. The gummy product (1·97 g., 96%) had no infrared absorption due to hydroxyl, but bands at 1710 (m), 1595 (aromatic C=C); 1365, 1185, 1170 (S=O stretching); 1090, 925, 860, 835, 805, 660 cm.$^{-1}$, and was used for the next stage without further purification.

*16 : 22-Epoxy-3ξ : 26-di-iodocholestane.*—Dihydrotigogenin 3β : 26-di-*p*-toluenesulphonate (1·97 g.) was dissolved in ethyl methyl ketone (50 ml.), sodium iodide (4·0 g.) was added, and the mixture boiled for 17 hr. After cooling, the insoluble salts were filtered off, the solvent was evaporated under reduced pressure at room temperature, and the residue partitioned between chloroform and water. The chloroform layer was washed with sodium thiosulphate solution and water, dried, and evaporated under reduced pressure at room temperature. On treatment of the residual gum with ether, white crystals were obtained (739 mg., 52% from dihydrotigogenin). The product was crystallised twice from ether–methanol, to give plates of *16 : 22-epoxy-3(β?) : 26-di-iodocholestane*, m. p. 140—146°, $[\alpha]_D$ +6° (Found: C, 50·9; H, 6·8; I, 39·6. $C_{27}H_{44}OI_2$ requires C, 50·8; H, 7·0; I, 39·8%), $\nu_{max.}$ 1145, 1105, 1050, 990, 670, 640 cm.$^{-1}$, $\nu_{max.}$ (in $CS_2$) 680, 650, 604 cm.$^{-1}$. A more soluble *isomer* was obtained from the mother-liquors as needles, m. p. 146—151°, $[\alpha]_D$ +6° (Found: C, 51·1; H, 7·2; I, 39·0%), $\nu_{max.}$ 1173, 1047, 672, 640 cm.$^{-1}$, $\nu_{max.}$ (in $CS_2$) 678, 604, 475 cm.$^{-1}$.

*16 : 22-Epoxy-3ξ-iodocholest-25-ene.*—16 : 22-Epoxy-3ξ : 26-di-iodocholestane (500 mg.) was dissolved in ether (50 ml.). A solution of potassium hydroxide (15 g.) in methanol (150 ml.) was added, some of the ether was evaporated, and the solution boiled for 6 hr., poured into water, cooled, and extracted with ether. The extract was washed with water, dried, and evaporated

[18] Doukas and Fontaine, *J. Amer. Chem. Soc.*, 1951, **73**, 5917.

under reduced pressure at room temperature. The residue (397 mg.) was chromatographed on alumina (Spence Type H). Benzene–light petroleum (b. p. 60—80°) (1 : 4) eluted material (324 mg., 81% from the di-iodide) which was crystallised from ethanol to give needles of 16 : 22-*epoxy*-3ξ-*iodocholest*-25-*ene*, m. p. 92—95°, $[\alpha]_D$ +7° (*c* 0·62%) (Found: C, 63·9; H, 8·7; I, 24·5. $C_{27}H_{43}OI$ requires C, 63·5; H, 8·5; I, 24·9%), $\nu_{max.}$ 3020 (w), 1650, 1110, 882, 675, 640 cm.$^{-1}$, $\nu_{max.}$ (in $CS_2$) 1650 and 885 (>C=$CH_2$), 681 cm.$^{-1}$.

Benzene–light petroleum (b. p. 60—80°) (2 : 3) eluted 16 : 22-*epoxy*-3ξ-*iodo*-26-*methoxycholestane* (57 mg., 13·4% from the di-iodide), m. p. 124—131° (plates from acetone), $[\alpha]_D$ +6° (*c* 0·7%) (Found: C, 62·1; H, 8·7; I, 23·1; MeO, 6·5. $C_{28}H_{47}O_2I$ requires C, 62·0; H, 8·7; I, 23·4; MeO, 5·7%), $\nu_{max.}$ 1110 (s), 676, 640 cm.$^{-1}$, $\nu_{max.}$ (in $CS_2$) 1108 (C·OMe), 680 cm.$^{-1}$.

*Preparation of a Solution of Deuterium Chloride in Dioxan.*—A mixture of phosphorus pentachloride (62·5 g., 0·3 mole) and phosphorus oxychloride (70 ml.) was cooled in an ice-bath in a flask equipped with a dropping funnel, stirrer, inlet tube leading into the solution, and an outlet lead. The outlet lead was connected to a cold-trap cooled in alcohol–carbon dioxide, and this was connected to a flask containing pure dioxan (75 ml.) equipped with inlet lead, condenser, and calcium chloride guard tube. A slow stream of nitrogen was passed through the apparatus, and deuterium oxide (6·02 g., 0·3 mole) was added slowly from the dropping funnel. After the addition, stirring was continued for 30 min. and nitrogen was passed through the apparatus. The resulting solution of deuterium chloride in dioxan was found by titration to be 4·79N (yield 60% from deuterium oxide).

*Isomerisation of* neo*Tigogenin Acetate with Deuterium Chloride.*—*neo*Tigogenin acetate (2·0 g., 4·36 mmoles) was dissolved in dioxan (10 ml.) in a Carius tube. The solution was frozen, the tube was flushed with nitrogen, and 2·85N-deuterium chloride in dioxan (10 ml., 28·5 mmoles) was added. The tube was sealed and heated on the steam-bath for 60 hr. After cooling, the solution was poured into sodium carbonate solution, and the solid collected, washed, and dried. The crude product (1·98 g.) was dissolved in methanol (100 ml.), concentrated hydrochloric acid (5 ml.) was added, and the solution boiled for 30 min., then poured into water. The solid was collected, washed, and dried. The partially acetylated product was boiled in methanol containing 2% of potassium hydroxide for 1 hr., the solution was poured into water, and the solid collected, washed, and dried. The product (1·93 g.) was allowed to percolate through neutral alumina in benzene solution to remove coloured impurities and crystallised from methanol. The *deuterated tigogenin* had m. p. 195—199°, $[\alpha]_D$ −71° (Found: C, 77·7; "H", 10·8; 0·98 D. $C_{27}H_{43}DO_3$ requires C, 77·7; "H", 10·6%), $\nu_{max.}$ 2175 (w) and 2130 (w) (C–D stretching), 915 > 895, 870 (m) cm.$^{-1}$.

*Deuterated Tigogenin from Tigogenin Acetate.*—Treatment of tigogenin acetate (13 g., 28·3 mmoles) with 1·4N-deuterium chloride (350 mmoles) as described above yielded crude material containing 3·1 D. After treatment with 0·5N-methanolic hydrochloric acid, chromatography, and crystallisation from ether the deuterated tigogenin had m. p. 189—195°, $[\alpha]_D$ −64° (*c* 0·69%) (Found: C, 77·6; "H", 10·5; 1·56 D), $\nu_{max.}$ 2170 (w), 2120 (w) cm.$^{-1}$.

*Conversion of Deuterated Tigogenin into Deuterated* 16 : 22-*Epoxy*-3ξ-*iodocholest*-25-*ene*.—Deuterated tigogenin (0·9 D) prepared from *neo*tigogenin acetate was converted, by the methods described previously, into *deuterated dihydrotigogenin*, m. p. 165—170° (from acetone), $[\alpha]_D$ −5° (*c* 1·2%) (Found: C, 77·2; "H", 11·3; 0·8 D. $C_{27}H_{45}DO_3$ requires C, 77·3; "H", 11·1%). This was converted into the ditoluene-*p*-sulphonate (0·8 D) which was then converted into *deuterated* 16 : 22-*epoxy*-3ξ : 26-*di-iodocholestane*, m. p. 142—146° (from ether–methanol), $[\alpha]_D$ +6° (Found: C, 51·0; "H", 6·7; I, 39·1; 0·95 D. $C_{27}H_{43}DOI_2$ requires C, 50·7; "H", 7·0; I, 39·7%). Dehydroiodination gave *deuterated* 16 : 22-*epoxy*-3ξ-*iodocholest*-25-*ene*, m. p. 93—96° (from ethanol), $[\alpha]_D$ +7° (*c* 0·74%) (Found: C, 63·3; "H", 8·5; I, 24·7; 0·46 D), and *deuterated* 16 : 22-*epoxy*-3ξ-*iodo*-26-*methoxycholestane*, m. p. 125—130° (from acetone), $[\alpha]_D$ +6° (*c* 0·39%) (Found: C, 61·7; "H", 9·1; I, 23·4; MeO, 6·4; 0·98 D. $C_{28}H_{46}DO_2I$ requires C, 61·9; "H", 8·7; I, 23·3; MeO, 5·7%).

*Conversion of Deuterated Tigogenin into* 16 : 22-*Epoxy*-3ξ-*iodocholest*-25-*ene* via pseudo-*Tigogenin*.—Deuterated tigogenin (1·56 D), prepared from tigogenin acetate as described above, was converted by the method described previously into deuterated *pseudo*tigogenin (0·6 D). The crude product was dissolved in methanol (100 ml.), concentrated hydrochloric acid (10 ml.) was added, and the solution boiled for 45 min., then poured into water. The solid was collected, washed, dried, chromatographed on neutral alumina, and crystallised from methanol to give deuterated tigogenin, m. p. 200—203°, $[\alpha]_D$ −63° (*c* 0·75%) (Found: C, 77·9; "H", 10·6;

0·48 D), $\nu_{max.}$ 2120 (w), 920, 896, 885 cm.$^{-1}$. This was converted into deuterated dihydrotigogenin, m. p. 169—171°, $[\alpha]_D$ −4° (*c* 0·76%) (Found: C, 77·8; "H", 11·0; 0·49 D). The ditoluene-*p*-sulphonate (0·48 D) was converted into deuterated 16 : 22-epoxy-3ξ : 26-di-iodocholestane, m. p. 141—146°, $[\alpha]_D$ +4·5° (*c* 0·73%) (Found: C, 51·2; "H", 6·6; I, 39·5; 0·47 D). Dehydroiodination gave 16 : 22-epoxy-3ξ-iodocholest-25-ene, m. p. 93—95°, $[\alpha]_D$ +7° (*c* 0·83%) (Found: C, 63·6; H, 8·2; I, 24·8; no deuterium), and deuterated 16 : 22-epoxy-3ξ-iodo-26-methoxycholestane, m. p. 124—129°, $[\alpha]_D$ +7° (*c* 0·54%) (Found: C, 62·5; "H", 8·7; I, 22·4; MeO, 7·0; 0·55 D).

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