79. *The Action of Selenium Dioxide on Sterols and Bile Acids. Part III. Cholesterol.*

By O. Rosenheim and W. W. Starling.

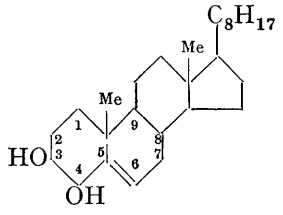
Although selenium dioxide reacts with ergosterol and *apo*cholic acid in alcoholic solution at room temperature (Callow and Rosenheim, J., 1933, 387; Callow, J., 1936, 462), the main reaction consisting in partial dehydrogenation, yet cholesterol is remarkably resistant to this reagent in alcoholic solution, even on boiling. Reaction, indicated by a precipitate of red selenium, takes place readily, however, when its solution in glacial acetic acid, nitrobenzene, dioxan, or acetonitrile is heated with selenium dioxide. In the first two solvents the main crystallised reaction product (yield, 35—40%) is a compound $C_{27}H_{46}O_2$, m. p. 176—177°, which was characterised as an unsaturated diol. Its constitution as a cholestene-α-glycol, *viz.*, cis-$\Delta^{5:6}$-*cholestene*-3 : 4-*diol* (I), was established by the following evidence :

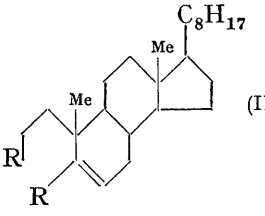
(1) The presence of a single ethenoid linkage was demonstrated by (*a*) quantitative perbenzoic acid titration and preparation of a saturated *monoxide*, $C_{27}H_{46}O_3$, m. p. 173—174°; (*b*) bromine titration; and (*c*) catalytic reduction with Adams's catalyst to cis-*cholestane*-3 : 4-*diol*, $C_{27}H_{48}O_2$, m. p. 202—203° (reduction with a palladium catalyst yields a mixture of cholestane, coprostane, and cholestan-3-ol). (2) Evidence accounting for the functions of the two oxygen atoms is afforded by the preparation of various mono-, di-, and mixed esters, proving that the compound is a disecondary alcohol. (3) The relative position of the hydroxyl groups and their function as a disecondary α-glycol follow from the results of quantitative titration with lead tetra-acetate by Criegee's method (*Ber.*, 1931, **64**, 260). The unsaturated dialdehyde, $C_{27}H_{44}O_2$ (II, R = CHO), resulting on oxidation of the diol with lead tetra-acetate, was characterised by its *di*-o-*tolylsemicarbazone* (and other derivatives), and furnished on further oxidation with hydrogen peroxide an unsaturated dicarboxylic acid, $C_{27}H_{44}O_4$, m. p. 292—293° (II, R = CO_2H). The same acid was also obtained on oxidation of the diol with potassium hypobromite, and was found to be identical with Diels's acid (Diels and Abderhalden, *Ber.*, 1903, **36**, 3179; 1904, **37**, 3092). Further,

the action of lead tetra-acetate on the saturated diol $C_{27}H_{48}O_2$ and oxidation of the product with hydrogen peroxide yielded the saturated acid $C_{27}H_{46}O_4$, characterised by its dimethyl ester, and identical with the saturated Diels's acid (Windaus, *Ber.*, 1919, **52**, 170). This fact incidentally furnishes evidence that, in the saturated diol, addition of hydrogen at C_5 had occurred in the *trans*-position relative to the 10-methyl group.

These observations prove that selenium dioxide attacks the cholesterol molecule mainly at the CH_2 group situated between the 5 : 6 ethenoid linkage and the secondary alcohol group at C_3. No evidence for hydroxylation at C_7 or for ketone formation was obtained.

(I.)





(II.)

The diol is quantitatively precipitated by digitonin in alcoholic solution. The digitonide, m. p. 240—242°, contains its constituents in equimolecular proportion, like cholesterol digitonide. The mono-esters, formed by the 3-hydroxyl group, are not precipitable by digitonin, thus proving that the 4-hydroxyl group does not combine with digitonin (cf. Tschesche and Hagedorn, *Ber.*, 1935, **68**, 2247). Characteristic blue colour reactions are given by the substance with arsenic trichloride, trichloroacetic acid, and by sulphuric acid or zinc chloride in glacial acetic acid. In the Salkowski reaction the chloroform layer is blue, and the sulphuric acid layer red with a green fluorescence. The glycol distils without decomposition in a high vacuum.

As was to be expected, the presence of the α-glycol group confers great reactivity upon this derivative of cholesterol, the first of its type. For instance, it rapidly loses one molecule of water on warming with dilute mineral acids, yielding a ketone and a compound $C_{27}H_{46}O_2$ (?), m. p. 139—140°, which is being investigated. The ketone was identified as coprostenone (cholestenone) by its characteristic o-*tolylsemicarbazone*. With Brady's reagent no reaction takes place at room temperature, but on warming, the 2 : 4-dinitrophenylhydrazone of coprostenone rapidly crystallises.

It is somewhat surprising that the action of selenium dioxide on the esters of cholesterol (the acetate and benzoate have so far been examined) proceeds in a different direction from that on cholesterol itself. In the case of the acetate, a small percentage only of the monoacetate of the *cis*-diol can be isolated, the main crystallised reaction product (yield 25—30%) being the *diacetate*, m. p. 135—136°, of the isomeric trans-$\Delta^{5:6}$-*cholestene*-3 : 4-*diol*, $C_{27}H_{46}O_2$, m. p. 257—258°. Cholesteryl benzoate in glacial acetic acid solution yields on oxidation with selenium dioxide a mixture in about equal proportions of the monobenzoate of the *cis*-diol with a compound, m. p. 128—129°. The latter, a *cholestene*-3 : 4-*diol* 3-*benzoate*-4-*acetate*, $C_{36}H_{32}O_4$, gives on hydrolysis the *trans*-diol, m. p. 257—258°. The preferential acetylation of the 4-hydroxyl group in the *trans*-diol is analogous to the behaviour of the monobenzoate of *trans-cyclo*hexane-1 : 2-diol, which reacts more readily with nitrobenzoyl chlorides than the benzoate of the corresponding *cis*-diol (Wilson and Read, J., 1935, 1269).

The constitution of the *trans*-diol was established by the same methods as in the case of its isomeride. That it contains a single ethenoid linkage follows from the results of (*a*) titration with bromine and preparation of the *dibromide* $C_{27}H_{46}O_2Br_2$, and (*b*) titration with perbenzoic acid and isolation of the saturated trans-*cholestanediol oxide*, $C_{27}H_{46}O_3$, m. p. 164—165°. Catalytic reduction (platinum oxide) yields trans-*cholestane*-3 : 4-*diol*, $C_{27}H_{48}O_2$, m. p. 194—195°. On heating with dilute mineral acids, the *trans*-diol rapidly loses one molecule of water and yields coprostenone (cholestenone), like its isomeride. It gives intense blue colours with various sterol reagents and is precipitable by digitonin like the *cis*-diol, but it differs from the latter by its sparing solubility in most organic solvents. This relative insolubility enabled us (*Chem. and Ind.*, 1933, **52**, 1056) to recognise the *trans*-diol

as a constituent of the resinous product called " Oxycholesterol " by Lifschütz (*Z. physiol. Chem.*, 1919, **106**, 279), which is obtained from cholesterol dibromide by debromination with sodium acetate.

The assignation of the respective configurations to the two isomeric α-glycols is based on the difference in the speed of their reaction with lead tetra-acetate (Criegee, Kraft, and Rank, *Annalen*, 1933, **507**, 159). The action of this reagent on the diol, m. p. 177°, is extremely rapid (reaction velocity $k_{20°} = 51{\cdot}1$), but the diol, m. p. 258°, reacts so slowly that accurate determinations of its reaction velocity were not attempted. These results, in agreement with the melting point rule, justify the alternative designation of the two α-glycols as *cis-cis-* and *cis-trans-*$\Delta^{5:6}$-cholestene-3 : 4-diol respectively—the nomenclature refers to the 3- and 4-hydroxyl group as *cis* or *trans* relative to the 10-methyl group (cf. Schoenheimer and Evans, *J. Biol. Chem.*, 1936, **114**, 571; Callow and Young, *Proc. Roy. Soc.*, 1936, *A*, **157**, 195). Measurements of the surface films of the diols and their diacetates were kindly carried out by Dr. Askew, the results agreeing with the above conclusions (Adam, Askew, and Danielli, *Biochem. J.*, 1935, **29**, 1786).

The ease with which these primary oxidation products of cholesterol are converted by loss of water into coprostenone, readily reducible to coprostanone and coprostanol *in vitro*, makes them of considerable biochemical interest in connection with the conversion by the animal organism of cholesterol into coprostanol. This conversion involves a reduction of the 5 : 6 ethenoid linkage. Although the animal organism is known to effect the reduction of cholesterol to cholestanol (the *trans*-decalin derivative), the mechanism of the conversion into coprostanol, the *cis*-isomeride, has remained hitherto unexplained (for a recent discussion of the problem, see Gardner, Gainsborough, and Murray, *Biochem. J.*, 1935, **29**, 1139). The unusual reactivity of the cholestene-α-glycols and their easy conversion into coprostenone suggested that they, and not cholesterol itself, are the immediate precursors of coprostanol, produced subsequently by intestinal bacterial reduction. On the assumption that primary oxidation to one of the α-glycols may play a rôle in cholesterol metabolism, feeding experiments with the above-described substances were undertaken (Rosenheim and Webster, *Nature*, 1935, **136**, 474). A large increase in the excretion by rats of fæcal coprostanol was observed after the administration of coprostenone, and a distinct effect was obtained with the *cis*-diol, whereas the *trans*-diol was without action, being excreted unchanged. Similar results with coprostenone were obtained simultaneously by Schoenheimer, Rittenberg, and Graf (*J. Biol. Chem.*, 1935, **111**, 183), who made use also of deuterium as an indicator.

Experimental.

cis-$\Delta^{5:6}$-*Cholestene*-3 : 4-*diol and its Derivatives.*

A solution of selenium dioxide (25 g.) in water (10 c.c.), to which glacial acetic acid (500 c.c.), warmed to 80°, has been added, is rapidly mixed with a solution of cholesterol (50 g.) in benzene (250 c.c.), also warmed to 80°. The mixture, which rapidly turns yellow and then red, is refluxed on a steam-bath for 1 hour. After the addition of sodium acetate (crystals, 100 g.), and heating for a few minutes, the black modification of selenium is deposited and removed. The filtrate is poured into half-saturated salt solution (1 l.), the benzene layer separated, washed with water, and dried over sodium sulphate, and the solvent removed under reduced pressure. The residue (60 g.) is suspended in light petroleum (b. p. 40—50°; 1500 c.c.), allowed to settle in a tall stoppered cylinder, and washed twice with the same solvent by decantation. The crude, creamy-white product (26 g.), m. p. 174—175°, is crystallised once from acetone (norit) and from 85% alcohol, from which the cis-*diol* (20 g.) separates in inch-long monoclinic needles, m. p. 176—177°, b. p. 255—260°/0·2 mm., $[\alpha]_D^{20°}$ — 60·0°, $[\alpha]_{5461}^{20°}$ — 71·0° (*c*, 1·566*); $\alpha_{5461}/\alpha_D = 1{\cdot}19$ (Found : † C, 80·4; H, 11·3. $C_{27}H_{46}O_2$ requires C, 80·5; H, 11·5%). The diol gives an intense blue coloration with the trichloroacetic acid reagent (Rosenheim, *Biochem. J.*, 1929, **23**, 47).

The *diacetate*, prepared by refluxing the *cis*-diol (1 g.) with acetic anhydride (10 c.c.) for 1 hour, crystallised in needles on cooling, and was recrystallised from spirit; m. p. 169—170°, $[\alpha]_D^{23°}$ — 96·1°, $[\alpha]_{5461}^{23°}$ — 117·1° (*c*, 1·576); $\alpha_{5461}/\alpha_D = 1{\cdot}20$ (Found : C, 76·3; H, 10·4. $C_{31}H_{50}O_4$

* The optical rotations were measured in a 4-dm. tube; all solutions were in chloroform unless otherwise stated.

† Micro-analyses by Dr. G. Weiler, Oxford.

requires C, 76·5; H, 10·4%). Hydrolysis of the diacetate with sodium ethoxide yielded the *cis*-diol. The diacetate gives blue colour reactions with trichloroacetic acid, as well as with antimony trichloride in chloroform. In acetic acid solution the free diol also gives a blue colour on warming with the antimony trichloride reagent, whereas it does not react in chloroform solution, remaining colourless for some time and turning reddish-purple only after some hours.

cis-*Cholestene*-3 : 4-*diol* 3-*benzoate* is conveniently prepared by boiling for 5 minutes a solution of cholesteryl benzoate (10 g.) in glacial acetic acid (100 c.c.) with a solution of selenium dioxide (2 g.) in water (1 c.c.), to which glacial acetic acid (100 c.c.) has been added. Sodium acetate (crystals, 7·5 g.) is added, and the black deposit of selenium removed. On cooling, the crude monobenzoate (3·8 g.) crystallises, and is recrystallised from methyl alcohol–ethyl acetate; needles, m. p. 209—210°, not melting to an anisotropic liquid; $[\alpha]_D^{20°}$ — 30·7°, $[\alpha]_{5461}^{23°}$ — 36·9° (*c*, 0·758); α_{5461}/α_D = 1·20 (Found: C, 80·7; H, 10·0. $C_{34}H_{50}O_3$ requires C, 80·6; H, 9·9%). Hydrolysis furnished the *cis*-diol. The 3-monobenzoate is also obtained from the *cis*-diol in pyridine by treatment with benzoyl chloride (1 equiv.) at room temperature.

cis-*Cholestenediol* 3-*benzoate*-4-*acetate*, prepared from the 3-benzoate by refluxing with acetic anhydride, crystallises in plates, m. p. 166—167°, $[\alpha]_D^{20°}$ — 55·9°, $[\alpha]_{5461}^{20°}$ — 66·6° (*c*, 1·667); α_{5461}/α_D = 1·19 (Found: C, 78·8; H, 9·5. $C_{36}H_{52}O_4$ requires C, 78·7; H, 9·6%). The *dibenzoate*, obtained by heating the diol in dry pyridine with slightly more than the calculated amount of benzoyl chloride for 5 hours on the water-bath, crystallises from alcohol in prismatic needles, m. p. 150—151°, $[\alpha]_D^{23°}$ — 53·9°, $[\alpha]_{5461}^{23°}$ — 63·5° (*c*, 0·835), α_{5461}/α_D = 1·18 (Found: C, 80·4; H, 8·8. $C_{41}H_{54}O_4$ requires C, 80·6; H, 8·9%). The *bis*-3 : 5-*dinitrobenzoate*, prepared by the pyridine method, crystallises in yellowish plates, m. p. 220—221°, $[\alpha]_D^{22°}$ — 39·6°, $[\alpha]_{5461}^{22°}$ — 45·5° (*c*, 1·054), α_{5461}/α_D = 1·15 (Found: C, 62·3; H, 6·5. $C_{41}H_{50}O_{12}N_4$ requires C, 62·3; H, 6·4%).

Perbenzoic acid titration. After 48 hours at 0°, 0·1030 g. of *cis*-diol consumed the equivalent of 3·86 mg. of oxygen, corresponding to 0·94 ethylenic linkage, and after 100 hours 0·1000 g. absorbed 3·93 mg. (0·98 ethylenic linkage).

cis-*Cholestane*-3 : 4-*diol Oxide*.—The *cis*-diol (4 g.) in chloroform (200 c.c.) was kept at 0° with a solution of perbenzoic acid (1·1 atoms of O) in chloroform (20 c.c.). The reaction was complete after 70 hours. The solution, after washing with aqueous sodium carbonate and water, was dried over sodium sulphate, and the solvent removed. The *oxide* was crystallised from spirit and from acetone, separating in slender needles, m. p. 173—174°, $[\alpha]_D^{19°}$ + 3·9°, $[\alpha]_{5461}^{19°}$ +4·5° (*c*, 1·142); α_{5461}/α_D = 1·15 (Found: 77·7; H, 11·1. $C_{27}H_{46}O_3$ requires C, 77·4; H, 11·1%). The oxide gave no coloration either with tetranitromethane in chloroform or with trichloroacetic acid. In the Salkowski test it behaves like cholesteryl oxide (colourless chloroform layer, red acid layer; cf. Westphalen, *Ber.*, 1915, **48**, 1064). The *diacetate* of the *cis*-diol oxide, prepared by acetylation with acetic anhydride, separated from methyl alcohol in clusters of needles, m. p. 178—179°; $[\alpha]_D^{19°}$ — 22·1°, $[\alpha]_{5461}^{19°}$ — 27·5° (*c*, 0·610); α_{5461}/α_D = 1·25 (Found: C, 74·1; H, 9·8. $C_{31}H_{50}O_5$ requires C, 74·0; H, 10·0%). On admixture with the oxide (m. p. 174°), a m. p. depression of 35° was observed.

Bromine titration (by the pyridine sulphate dibromide method). After 5 minutes at room temperature, 0·1054 g. of the *cis*-diol absorbed 40·3 mg. bromine (0·96 ethylenic linkage), and after 30 minutes, 0·0510 g. absorbed 20·8 mg. (1·02 ethylenic linkages).

cis-*Cholestene*-3 : 4-*diol dibromide*. Solutions of the *cis*-diol (5 g.) in chloroform (15 c.c.) and of bromine (0·66 c.c.) in acetic acid (120 c.c.) are rapidly mixed. Crystallisation of the *dibromide* begins after about one minute, and is completed by cooling in ice. The product (4·2 g.), m. p. 95—97°, rapidly decomposes on recrystallisation from hot solvents, but may be recrystallised from chloroform–acetic acid at room temperature, giving prismatic needles, m. p. 110—112° (decomp.) (Found: C, 57·5; H, 8·3. $C_{27}H_{46}O_2Br_2$ requires C, 57·6; H, 8·2%).

The dibromide is rapidly debrominated on mixing its solution in benzene with a solution of sodium iodide in alcohol at room temperature (cf. Schoenheimer, *J. Biol. Chem.*, 1935, **110**, 461), yielding the *cis*-diol. A solution of the dibromide in acetone rapidly turns yellow on warming, and gives off hydrogen bromide. On cooling, a neutral bromine-free substance, m. p. 133—134°, crystallises out; this is iso*propylidene cholestene*-3 : 4-*diol*, $[\alpha]_D$ — 38·2°, $[\alpha]_{5461}$ — 46·9° (*c*, 1·406); α_{5461}/α_D = 1·23 (Found: C, 81·4; H, 11·4. $C_{30}H_{50}O_2$ requires C, 81·4; H, 11·7%), also obtained from the *cis*-diol and acetone containing 1% of hydrogen chloride. The *trans*-diol does not yield an acetone compound, a fact supplying additional evidence for the assignation of the *cis*- and the *trans*-configuration to the respective isomeric α-glycols.

Hydrogenation. A suspension of platinum oxide (0·45 g.) in acetic acid (40 c.c.) was reduced with hydrogen, a solution of the *cis*-diol (10 g.) in acetic acid (300 c.c.) then added, and the whole shaken with hydrogen. Absorption took place rapidly and was complete in 2½ hours. Nearly

2 mols. of hydrogen were absorbed, no break in the absorption curve being noticeable after the addition of 2 atoms. A crystalline precipitate was dissolved by warming, the catalyst removed, and the solution concentrated under reduced pressure until crystallisation began. The crude product (6·5 g.) was dissolved in ether, in which it is sparingly soluble, by extraction (Soxhlet) and crystallised in the extraction flask, m. p. 193—194°. Crystallisation from heptane and from acetone yielded shiny leaflets of cis-*cholestane*-3 : 4-*diol*, m. p. 202—203°, $[\alpha]_D^{24°}$ + 18·8°, $[\alpha]_{5461}^{24°}$ + 22·2° (*c*, 1·206); $\alpha_{5461}/\alpha_D = 1{\cdot}18$ (Found : C, 80·4; H, 12·0. $C_{27}H_{48}O_2$ requires C, 80·1; H, 12·0%). The *diacetate*, obtained by refluxing the saturated diol with acetic anhydride, crystallised from methyl alcohol in long needles, m. p. 136—137°, $[\alpha]_D^{22°}$ − 7·1° (*c*, 1·0) (Found : C, 76·3; H, 10·8. $C_{31}H_{52}O_4$ requires C, 76·2; H, 10·7%).

After removal of the saturated diol, the mother-liquors were precipitated with digitonin in alcoholic solution. The digitonide was dissolved in pyridine, the digitonin precipitated with ether and removed. The residue of the ethereal filtrate yielded cholestan-3-ol, crystallising in triclinic plates, m. p. 141—142°, unchanged after admixture with an authentic specimen (Found : C, 79·6; H, 12·4. Calc. for $C_{27}H_{48}O,H_2O$: C, 79·7; H, 12·4%). The mixture of hydrocarbons, not precipitated by the above digitonin treatment, was recovered and separated by fractional crystallisation. The least soluble fraction furnished cholestane, crystallising in monoclinic plates, m. p. 80—81°, not depressed in admixture with authentic cholestane (Found : C, 87·0; H, 13·0. Calc. for $C_{27}H_{48}$: C, 87·0, H, 13·0%). From the more soluble fractions, small amounts of coprostane were obtained, crystallising in orthorhombic needles, m. p. and mixed m. p. 70—71° (Found : C, 87·0; H, 13·0. Calc. for $C_{27}H_{48}$: C, 87·0; H, 13·0%).

When the diacetate of the *cis*-diol was catalytically reduced over palladised charcoal in neutral or acetic acid solution, rapid absorption of nearly 3 mols. of hydrogen took place, yielding a mixture of cholestane and coprostane. The same catalyst acting on the *cis*-diol in alcoholic solution furnished cholestan-3-ol in addition to the hydrocarbons. The constituents of the mixtures were separated and identified as described above. In no instance was the saturated *cis*-diol obtained by the use of a palladium catalyst.

Lead tetra-acetate titration. After 24 hours at room temperature, 0·1046 g. of *cis*-diol consumed 5·08 c.c. of *N*/10-lead tetra-acetate, corresponding to 0·97 atom of oxygen, and after 48 hours, 0·1030 g. consumed 5·22 c.c. (1·02 atoms of O).

Di-o-tolylsemicarbazone of dialdehyde, $C_{27}H_{44}O_2$. A solution of lead tetra-acetate (2·5 g.) in acetic acid (100 c.c.) is added to a suspension of the *cis*-diol (1 g.) in acetic acid (50 c.c.). The clear solution resulting after 2 minutes' shaking is kept for 24 hours at room temperature. The crude dialdehyde (0·9 g.), obtained on extraction of the reaction mixture with light petroleum as a colourless oil, is dissolved in alcohol (10 c.c.). A solution of *o*-tolylsemicarbazide (1 g.) (cf. Lei, Sak, and Shih, *J. Chinese Chem. Soc.*, **1935**, **3**, 247) in hot spirit (20 c.c.) and 5 drops of acetic acid are added. Crystallisation begins within 10 minutes. After two recrystallisations from alcohol, the *di-o-tolylsemicarbazone* is obtained in clusters of needles, m. p. 192—193° (Found : C, 74·7; H, 9·1; N, 12·4. $C_{43}H_{62}O_2N_6$ requires C, 74·4; H, 8·9; N, 12·1%). The *bis*-2 : 4-*dinitrophenylhydrazone*, prepared from the crude dialdehyde by means of Brady's reagent, crystallised from benzene as a microcrystalline crimson powder (Found : N, 14·7. $C_{39}H_{52}O_8N_8$ requires N, 14·7%). The *disemicarbazone*, obtained in the usual way, was purified by digestion with boiling alcohol; m. p. 218—219° (Found : N, 16·5. $C_{29}H_{50}O_2N_6$ requires N, 16·4%).

Oxidation of Dialdehyde, $C_{27}H_{44}O_2$, *to Diels's Acid.*—Hydrogen peroxide (100-vol., 0·5 c.c.) is added to a solution of the aldehyde (1·0 g.) in acetic acid (5 c.c.). An addition product, m. p. 121—122°, crystallises out, which dissolves on warming. The solution is kept on a steam-bath for 2 hours, crystallisation of the acid starting after ½ hour's heating. After recrystallisation from acetic acid and methyl ethyl ketone, Diels's acid is obtained in typical tetragonal crystals, m. p. 292—293°, not depressed in admixture with authentic acid prepared from cholesterol (Found : C, 74·9; H, 10·1. Calc. for $C_{27}H_{44}O_4$: C, 75·0; H, 10·2%. 0·1130 G. required 5·4 c.c. of *N*/10-KOH for neutralisation. Calc. : 5·5 c.c.). The monomethyl ester crystallises in plates, m. p. 125—126°, alone or in admixture with an authentic specimen (0·1034 g. required 2·3 c.c. *N*/10-KOH. Calc. : 2·3 c.c.).

Diels's acid was also obtained by shaking the finely powdered *cis*-diol (5 g.) for 20 hours with a solution of potassium hypobromite, prepared by adding bromine (3 c.c.) to a mixture of ice (40 g.) and potassium hydroxide (25%, 50 c.c.). The acid and its mono-methyl and -ethyl esters were identified by mixed m. p.'s with authentic specimens and by elementary analyses.

These observations are consistent with the view that both *cis*-cholestenediol and the dialdehyde $C_{27}H_{44}O_2$ are intermediate products in the oxidation of cholesterol by potassium hypobromite to Diels's acid (Diels and Abderhalden, *loc. cit.*).

Dihydro-Diels's Acid from cis-*Cholestane*-3 : 4-*diol*.—After 40 hours, 0·2078 g. of the saturated *cis*-diol consumed 10·27 c.c. of *N*/10-lead tetra-acetate, corresponding to 1·0 atom of oxygen. The saturated dialdehyde was extracted from the reaction mixture with ether, and oxidised with hydrogen peroxide in acetic acid solution. The resulting acid crystallised from acetic acid in leaflets, m. p. 248—250° (Found: C, 74·6; H, 11·0. Calc. for $C_{27}H_{46}O_4$: C, 74·6; H, 10·7%). The dimethyl ester, prepared with diazomethane, crystallised from methyl alcohol in prisms, m. p. 125—126° (Found: C, 75·6; H, 10·9. Calc. for $C_{29}H_{50}O_4$: C, 75·3; H, 10·9%). The m. p.'s of the acid and of its dimethyl ester are given by Windaus (*Ber.*, 1919, **52**, 175) as 252° and 123—124° respectively, whilst the isomeric acid, m. p. 249°, from coprosterol yields a dimethyl ester, m. p. 61° (*idem*, *Ber.*, 1916, **49**, 1732).

Digitonin Precipitation.—A solution of the *cis*-diol (542 mg.) in boiling spirit (8 c.c.) was mixed with a 1% solution of digitonin in hot 90% alcohol (30 c.c.). The digitonide separated on cooling in starry aggregates of needles. After 24 hours at 0°, the precipitate was collected, washed with 90% alcohol and with ether, dried at 100°, and weighed. Yield of digitonide, 0·2108 g., m. p. 240—242° (decomp.). Like cholesterol digitonide, it contains one-quarter of its weight of sterol (Windaus, *Z. physiol. Chem.*, 1910, **65**, 112), which is recovered nearly quantitatively by the pyridine–ether method (Schoenheimer and Dam, *ibid.*, 1933, **215**, 59).

Coprostenone (*Cholestenone*) *from* cis-*Cholestenediol*.—(a) A solution of the *cis*-diol (2·5 g.) in acid alcohol (180 c.c., containing 7 c.c. of concentrated hydrochloric acid) is heated for 10 minutes on a water-bath. After the addition of potassium acetate (8 g.), the solution is cooled and the precipitated potassium chloride removed. A solution of *o*-tolylsemicarbazide (2 g.; see p. 381). in hot alcohol (20 c.c.) is added. *Coprostenone-o-tolylsemicarbazone* crystallises from butyl alcohol in feathery needles, m. p. 243—244°, alone or in admixture with an authentic specimen (Found: N, 8·0. $C_{35}H_{53}ON_3$ requires N, 8·0%). A solution of the *cis*-diol (0·2 g.) in acid alcohol (15 c.c., containing 0·6 c.c. of concentrated hydrochloric acid) is added to a suspension of 2 : 4-dinitrophenylhydrazine (0·1 g.) in alcohol (10 c.c.). After warming on a water-bath a voluminous brick-red precipitate is formed within a few minutes. Coprostenone-2 : 4-dinitrophenylhydrazone (cf. Ralls, *J. Amer. Chem. Soc.*, 1933, **55**, 2092) separates from benzene–alcohol as a woolly mass of hair-like crystals, which change into stout transparent prisms on standing, m. p. 233—234°, not depressed in admixture with an authentic specimen (Found: C, 70·0; H, 8·9; N, 10·2. Calc. for $C_{33}H_{48}O_4N_4$: C, 70·2; H, 8·6; N, 10·0%). The same reaction takes place on warming a solution of the *cis*-diol with Brady's reagent (J., 1931, 756) and may be used to demonstrate the formation of coprostenone from the *cis*-diol at body temperature in an incubator.

(b) Coprostenone, m. p. 80—81°, alone or admixed with an authentic specimen, was obtained by heating the semicarbazone, m. p. 233—234° (0·9 g., prepared from the *cis*-diol as above by means of semicarbazide acetate), with a mixture (50 c.c.) of acetic and hydrochloric acids (1 : 1) for 30 minutes on a water-bath (Found: C, 84·4; H, 11·3. Calc. for $C_{27}H_{44}O$: C, 84·3; H, 11·5%). Coprostenone was also isolated and identified by the usual methods from the reaction products obtained by heating the finely-powdered *cis*-diol (1 g.) with water (10 c.c.) in a sealed tube to 200° for 8 hours.

trans-$\Delta^{5:6}$-*Cholestene*-3 : 4-*diol and its Derivatives*.

Hot solutions of cholesteryl acetate (20 g.) and selenium dioxide (10 g., 5 c.c. water), each in 200 c.c. of acetic acid, are mixed and boiled for 5 minutes. After the addition of sodium acetate (crystals, 60 g.), the mixture is reheated and the black deposit of selenium removed. The cooled filtrate, to which ether (200 c.c.) has been added, is poured into half-saturated salt solution (1 l.). The ethereal layer is separated, washed with water, dried over sodium sulphate, and the solvent removed. The resinous reddish residue contains a mixture (which may be separated by appropriate selective solvents) of the mono- and the di-acetates of the *cis*- and the *trans*-diol. Acetylation is completed by refluxing the resin with acetic anhydride for ½ hour. The crystals, which separate on cooling, are removed, dried over soda–lime in a desiccator, and washed with spirit (60 c.c.) by grinding in a mortar. The crude diacetates (10·8 g.) are hydrolysed by boiling their solution in methyl alcohol (300 c.c.) with 5% methyl-alcoholic sodium methoxide (120 c.c.). Crystallisation of the *trans*-diol begins after a few minutes and is complete in ½ hour. The diol is sparingly soluble in light petroleum, ether, alcohol, ethyl acetate, etc., but may be recrystallised from nitrobenzene, dioxan, or pyridine. Purification of the crude product (5 g., m. p. 256—257°) is preferably effected by acetylation, recrystallisation of the diacetate, and subsequent hydrolysis. trans-*Cholestene*-3 : 4-*diol* crystallises in orthorhombic needles, m. p. 257—258°, b. p. 255—260°/0·2 mm., $[\alpha]_D^{20°}$ + 6·0°, $[\alpha]_{5461}^{20°}$ + 7·5° (*c*, 0·50 in pyridine); $\alpha_{5461}/\alpha_D = 1{\cdot}25$ (Found: C, 80·6; H, 11·6. $C_{27}H_{46}O_2$ requires C, 80·5; H, 11·5%).

trans-*Cholestene*-3 : 4-*diol diacetate*, prepared by refluxing the *trans*-diol with acetic anhydride, crystallised from methyl alcohol in long needles, m. p. 135—136°, $[\alpha]_D^{18°}$ — 13·3°, $[\alpha]_{5461}^{18°}$ — 16·4° (*c*, 1·11); α_{5461}/α_D = 1·23 (Found : C, 76·3; H, 10·2. $C_{31}H_{50}O_4$ requires C, 76·5; H, 10·4%). The *dibenzoate*, prepared by the pyridine method, crystallises in prismatic needles, m. p. 181—182°, $[\alpha]_D^{20°}$ — 74·4°, $[\alpha]_{5461}^{20°}$ — 91·4° (*c*, 1·35); α_{5461}/α_D = 1·23 (Found : C, 80·7; H, 9·0. $C_{41}H_{54}O_4$ requires C, 80·6; H, 8·9%). The 3-*benzoate*-4-*acetate* is obtained, together with the monobenzoate of the *cis*-diol, when cholesteryl benzoate is treated with selenium dioxide in acetic acid solution. The filtrate from *cis*-cholestene-3 : 4-diol 3-benzoate (see above) is poured into water, and the granular precipitate taken up in light petroleum. After removal of the solvent and repeated crystallisation of the residue from chloroform–methyl alcohol and from spirit, the mixed ester crystallises in prisms, m. p. 128—129°, $[\alpha]_D^{20°}$ — 21·2°, $[\alpha]_{5461}^{20°}$ — 26·2° (*c*, 1·448); α_{5461}/α_D = 1·23 (Found : C, 78·8; H, 9·5. $C_{36}H_{52}O_4$ requires C, 78·7; H, 9·6%). On hydrolysis in ethereal solution with sodium ethoxide, the ester yielded the *trans*-diol.

Perbenzoic acid titration. Suspensions of the *trans*-diol (50 mg.) in chloroform (5 c.c.), kept at 0° and titrated after 48, 96, and 120 hours, absorbed amounts of oxygen corresponding to 0·88, 0·94, and 0·98 ethylenic linkage respectively.

trans-*Cholestane*-3 : 4-*diol Oxide*.—A suspension of the *trans*-diol (2 g.) in chloroform (200 c.c.), mixed with a solution of perbenzoic acid (1·1 atoms of O) in chloroform, was kept at 0° for 9 days, the reaction then being complete. The *oxide* was isolated as described for that of the *cis*-diol, and crystallised from acetone in long needles, m. p. 164—165°, $[\alpha]_D^{20°}$ — 7·5°, $[\alpha]_{5461}^{20°}$ — 9·2° (*c*, 0·648); α_{5461}/α_D = 1·23 (Found : C, 77·5; H, 10·7. $C_{27}H_{46}O_3$ requires C, 77·4; H, 11·1%). Its *diacetate* crystallises from methyl alcohol in rhombic plates, m. p. 154—155°, $[\alpha]_D^{19°}$ — 58·5°, $[\alpha]_{5461}^{19°}$ — 69·6° (*c*, 1·028); α_{5461}/α_D = 1·19 (Found : C, 74·0; H, 9·9. $C_{31}H_{50}O_5$ requires C, 74·0; H, 10·0%).

Bromine titration (by the pyridine sulphate dibromide method). After 30 minutes at room temperature, 0·1000 g. of the *trans*-diol absorbed 44·2 mg. of bromine, corresponding to 1·1 ethylenic linkages.

trans-*Cholestene*-3 : 4-*diol dibromide*. A suspension of the *trans*-diol (1 g.) in chloroform (100 c.c.) was shaken for 1 hour with pyridine sulphate dibromide solution (200 c.c.). The excess of bromine was removed with *N*/10-sodium thiosulphate, the chloroform layer separated, washed with water and with sodium carbonate solution. After drying over sodium sulphate, the solvent was removed under reduced pressure, and the residue crystallised repeatedly from alcohol or acetone. The *dibromide* crystallises in fine needles from benzene, m. p. 196—197° (decomp.) (Found : C, 57·7; H, 7·9; Br, 28·8. $C_{27}H_{46}O_2Br_2$ requires C, 57·7; H, 8·2; Br, 28·5%).

Unlike the dibromide of the *cis*-diol, that of the *trans*-diol is remarkably stable. Whereas the former is instantly debrominated by sodium iodide at room temperature, the latter is recovered unchanged after 5 hours' boiling of its benzene solution with an alcoholic solution of sodium iodide. On boiling in alcoholic solution for 2 hours with zinc dust, however, bromine is removed and the *trans*-diol is recovered.

Hydrogenation. On account of the insolubility of the *trans*-diol, the more soluble diacetate (10 g.) in ether–acetic acid solution was catalytically reduced with platinum oxide (0·47 g.) under the same conditions as for the *cis*-diol (see above). Again, nearly 2 mols. of hydrogen were rapidly absorbed (1 hour) without any break in the absorption curve. After removal of the catalyst and evaporation of the ether, a semicrystalline waxy deposit (1·9 g.), consisting mainly of cholestane, was removed. The solution was diluted with water and extracted with ether. After removal of the solvent, the residue (6·5 g.) was fractionally crystallised from methyl alcohol and separated into two main fractions. The least soluble fraction, m. p. 192—193°, was crystallised from benzene–petrol and finally from acetone, yielding stout hexagonal plates of trans-*cholestane*-3 : 4-*diol*, m. p. 194—195°, $[\alpha]_D^{20°}$ + 10·2°, $[\alpha]_{5461}^{20°}$ + 12·1° (*c*, 1·184); α_{5461}/α_D = 1·16 (Found : C, 80·0; H, 12·1. $C_{27}H_{48}O_2$ requires C, 80·1; H, 12·0%). The *diacetate*, prepared by refluxing with acetic anhydride, crystallised in thin plates, m. p. 140—141° (Found : C, 76·4; H, 10·8. $C_{31}H_{52}O_4$ requires C, 76·2; H, 10·7%).

The diol gave no coloration either with trichloroacetic acid or with tetranitromethane in chloroform. After 48 hours' treatment with lead tetra-acetate, no measurable consumption of oxygen took place, and the *trans*-diol was recovered unchanged.

Cholestane and cholestan-3-ol were isolated, and identified by the usual methods, from the more soluble fractions of the reduction products. Catalytic reductions of the *trans*-diol diacetate over palladium catalysts yielded in every instance a mixture of cholestane, coprostane, and cholestan-3-ol, in the same proportions as in the case of the *cis*-diol described above.

Coprostenone (*Cholestenone*) *from* trans-*Cholestene*-3 : 4-*diol*.—A suspension of the *trans*-

diol (0·5 g.) in alcohol (30 c.c., containing 1·5 c.c. of concentrated hydrochloric acid) is refluxed for 10 minutes on a water-bath and treated with *o*-tolylsemicarbazide (see above), yielding *coprostenone-o-tolylsemicarbazone*, m. p. 243—244° (Found: N, 8·1. $C_{35}H_{53}ON_3$ requires N, 8·0%). After removal of the tolylsemicarbazone, the filtrate yielded a compound $C_{27}H_{46}O_2$(?), m. p. 139—140°, which is being investigated. A mixture with the substance of the same m. p., obtained under similar conditions from the *cis*-diol, showed no depression of m. p.

Coprostenone-2 : 4-dinitrophenylhydrazone, m. p. 233—234° (Found : N, 10·1. $C_{33}H_{48}O_4N_4$ requires N, 10·0%), was obtained by adding Brady's reagent (5 c.c.) to a suspension of the *trans*-diol (0·5 g.) in alcohol (25 c.c.) and heating for a short time on a water-bath. The *semicarbazone*, m. p. 233—234° (Found : N, 9·5. $C_{28}H_{47}ON_3$ requires N, 9·5%), prepared from the *trans*-diol as above by means of semicarbazide acetate, was heated with a mixture of acetic and hydrochloric acids (1 : 1) on a water-bath and yielded coprostenone, m. p. and mixed m. p. 80—81° (Found : C, 84·0; H, 11·2. Calc. for $C_{27}H_{44}O$: C, 84·3; H, 11·5%). Coprostenone was also isolated, and identified by the usual methods, from the reaction products obtained by heating the *trans*-diol with anhydrous copper sulphate to 180° under reduced pressure, or with water in a sealed tube to 200°.

trans-*Cholestene-3 : 4-diol from "Oxycholesterol."*—The reaction product obtained according to Lifschütz (*loc. cit.*) from cholesterol (50 g.), brominated in ethereal solution, was poured into water and extracted with light petroleum (1500 c.c.). The extract was filtered through a folded filter, and the extraction repeated until a drop of 90% trichloroacetic acid no longer produced a blue spot on the filter-paper used for filtration. Well-formed orthorhombic crystals were deposited from the extracts on standing. They were collected, and washed with light petroleum; yield 1·5 g., m. p. 235—236°. On recrystallisation from ethyl acetate (1 : 600), the m. p. was raised to 242—244°, but complete purification was only effected by acetylation and subsequent hydrolysis of the diacetate, yielding *trans*-cholestene-3 : 4-diol, m. p. 256—258° (Found : C, 80·5; H, 11·5%). The diacetate, m. p. 135—136°, not depressed in admixture with the diacetate obtained by the selenium dioxide process, crystallised in long needles from methyl alcohol (Found : C, 76·5; H, 10·2%). The results of titrations with perbenzoic acid and with bromine were identical with those given above for the *trans*-diol prepared from cholesteryl acetate and selenium dioxide. On treatment with Brady's reagent, coprostenone-2 : 4-dinitrophenylhydrazone, m. p. 233—234°, was obtained (Found : N, 10·1%).

The diol gives with the Lifschütz reagent (acetic–sulphuric acid, 1 : 10), and also with trichloroacetic acid, the intense blue colour reaction which is considered typical for "oxycholesterol" by Lifschütz. On addition of a drop of dilute ferric chloride, the blue colour changes to green, the second phase of the reaction, showing a well-marked absorption band at 6500 A. characteristic of "oxycholesterol."

National Institute for Medical Research,
London, N.W. 3.

[*Received, December 29th,* 1936.]