71. *The Action of Selenium Dioxide on Sterols and Bile Acids. Part IV. Cholestane-2 : 3-dione from Cholestan-3-one.*

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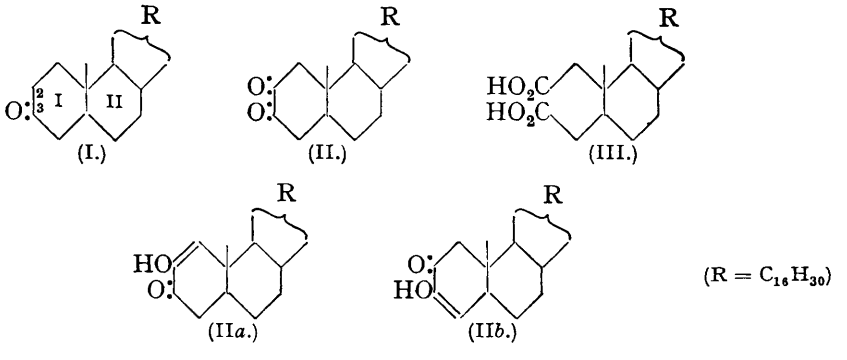
The action of selenium dioxide on unsaturated hydroxylated steroids leads in the first instance to α-glycols. By the oxidation of saturated and of unsaturated steroid ketones with the same reagent, α-diketones (diosphenols) have now been obtained. *Cholestane-2 : 3-dione* has been examined in detail and found to exist in two modifications, probably representing the tautomeric enolic forms of the diketone.

The use of selenium dioxide as an oxidising agent in the steroid series, which has so far been applied only to unsaturated hydroxylated steroids (Callow and Rosenheim, J., 1933, 387; Callow, J., 1936, 462; Rosenheim and Starling, J., 1937, 377; cf. Butenandt and Hausmann, *Ber.*, 1937, **70**, 1154), has now been extended to saturated and unsaturated steroid ketones. Of the ketones examined, only cholestanone reacted readily in alcoholic solution. By analogy with the behaviour on bromination of the ketonic steroids belonging to the *trans*-series (Butenandt and Wolff, *Ber.*, 1935, **68**, 2091) it was to be expected that selenium dioxide would attack cholestanone (I) at the methylene group C_2 in ring I, introducing a carbonyl group. The reaction product, *cholestane-2 : 3-dione* (II), is a diosphenol and was readily separated from unchanged cholestanone by making use of the insolubility of its *potassium* salt in 20% aqueous potassium hydroxide and ether. The yield of the pure diosphenol was found to be dependent on the amount of the oxidising agent used and rose to a maximum of 30% when the proportion of cholestanone to selenium dioxide was 1 : 8 by weight. In spite of the large excess of oxidising agent a variable amount of unchanged cholestanone was recovered.

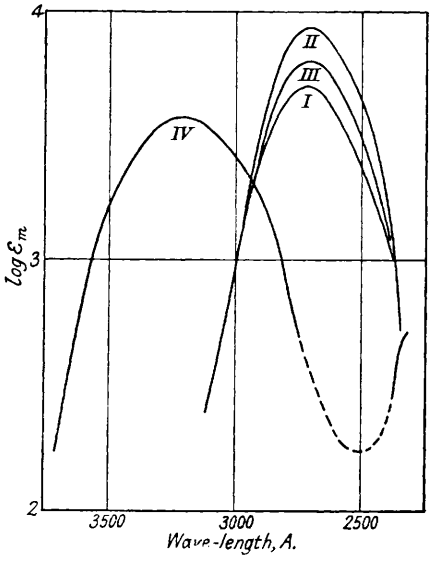


The constitution of the diosphenol was established by the following evidence: The substance gives a purple colour with alcoholic ferric chloride and is further characterised as an α-diketone by its ready reaction in alcoholic solution with *o*-phenylenediamine,

yielding a *quinoxaline* derivative, m. p. 180°. As a diosphenol the diketone can exist in the mono-enol modification (II*a* or II*b*), and yields a *monobenzoate* and a *monoacetate*, from which it is again obtained on hydrolysis. The position of the carbonyl groups at C_2 and C_3 was established by the quantitative oxidation with alkaline hydrogen peroxide to the dicarboxylic acid $C_{27}H_{46}O_4$ (III), previously obtained from cholestanone by oxidation with chromic acid (Windaus and Uibrig, *Ber.*, 1914, **47**, 2384).

The melting point (144—145°) of the diosphenol, regenerated from its potassium salt, remained unchanged after repeated crystallisations from different solvents. Some specimens, however, showed a surprising behaviour, the melting point occasionally falling to 130—132°, or rising to 156° or 168°, on further recrystallisation. An explanation for these perplexing changes was afforded by the observation that the substance occurred in two interconvertible modifications, form (A), m. p. 144—145°, and form (B), m. p. 168—169°. The two forms occur further as an equimolecular mixture (C), m. p. 130—132°.

Absorption spectrum of cholestane-2 : 3-dione.



(I), *Form* (A); (II), *form* (B); (III), [(A) + (B)]/2 = (C); (IV), (A), (B), *and* (C) *in alkaline solution.*

The interconversion of (A) and (B) can be brought about at will by means of mineral acids and alkalis. Thus, when form (A) is heated to 100° with glacial acetic acid containing a trace of hydrochloric acid, and the product recrystallised from ethyl acetate, form (B) is obtained. On the other hand, by converting form (B) into its enolic potassium salt and regenerating the diosphenol, form (A) is obtained. The dione, m. p. 130—132°, occasionally observed on recrystallisation of form (A), is also obtained on recrystallising together equivalent amounts of forms (A) and (B). From the following table it will be seen that the specific rotation, the rotatory dispersion α_{5461}/α_D, and log ϵ_m at 2700 A. (see figure) are those of an equimolecular mixture of forms (A) and (B) of the diosphenol.

Form.	M. p.	$[\alpha]_{5461}$.	$[\alpha]_D$.	α_{5461}/α_D.	Log ϵ_m (2700 A.).
(A)	144—145°	+91·5°	+79·1°	1·16	3·70
(B)	168—169	+67·3	+57·2	1·18	3·93
$\frac{(A) + (B)}{2}$	130—132	+79·4	+68·2	1·17	3·81
(C)	130—132	+79·9	+68·2	1·17	3·80

Forms (A) and (B), as well as the dione of m. p. 130—132°, yield the same quinoxaline derivative with *o*-phenylenediamine and the same dicarboxylic acid, $C_{27}H_{46}O_4$, on oxidation. The two forms give, however, different enolic monoacetates, m. p. 137—138° and 141—143° respectively, their mixture showing a depression of 30° in melting point. Both (A) and (B) give a purple ferric chloride reaction of the same intensity and a yellow colour with tetranitromethane, but differ characteristically in their colour reactions with concentrated sulphuric acid and other reagents.

The two substances are probably best regarded as the two tautomeric enolic forms (II*a* and II*b*) of the diosphenol, but it is not improbable that (A) is one of the enolic forms and (B) the diketone form which, in alcoholic solution, is in equilibrium with the other enolic form. Form (B) is evidently identical with the 2 : 3-diketone recently obtained in small yield by debromination of 2 : 4-dibromocholestanone by Inhoffen (*Ber.*, 1937, **70**, 1695). The variations in melting point and optical activity observed by this author may have been due to slight admixtures of form (A) of the diosphenol in his preparations.

The preparation of *hydroxymethylenecholestan-3-one*, the oxidation of which offers another approach to cholestane-2 : 3-dione, is described.

Experimental.

The reactivities of the various steroid ketones with selenium dioxide were tested as previously described (Callow and Rosenheim, J., 1933, 387). The following results were obtained:

(1) Cholestanone reacted readily and coprostanone slightly in alcoholic solution.

(2) The following ketones did not react in alcohol, but reacted readily in glacial acetic acid at 100°: Δ^4-cholesten-3-one, cholestan-3-ol-6-one, cholestan-6-one, cholestane-3 : 6-dione, Δ^4-cholestene-3 : 6-dione, 3 : 12-diketocholanic acid.

(3) Cholestan-3-ol-7-one acetate, $\Delta^{3:5}$-cholestadien-7-one, and 12-ketocholanic acid did not react in alcohol or glacial acetic acid at 100°.

Experiments on a larger scale with cholestanone, coprostanone, cholestenone, and 3 : 12-diketocholanic acid gave products which formed insoluble enolic potassium salts and gave a purple colour with alcoholic ferric chloride.

Cholestane-2 : 3-dione, Form (*A*).—A solution of cholestanone (10 g.) in boiling alcohol (200 c.c.) was added to a hot solution of selenium dioxide (80 g.) in water (45 c.c.) and alcohol (200 c.c.). On refluxing, the mixture immediately turned yellow and a red precipitate of selenium was formed, which was removed after 15 minutes' boiling and thoroughly washed with hot alcohol (100 c.c.). The filtrate deposited a flocculent brown precipitate (1·6—1·8 g.) on cooling, which was discarded. The resulting clear yellow solution was diluted with ether (400 c.c.) and poured into half-saturated salt solution (700 c.c.). After being washed with water and saturated sodium bicarbonate solution (200 c.c.), the ethereal layer was vigorously shaken with 20% aqueous potassium hydroxide (200 c.c.) in a separating funnel, which was subsequently kept immersed in ice-water until the pale yellow precipitate of the enolic potassium salt had collected at the interface. The aqueous layer was run off, and the ethereal layer decanted from the potassium salt, which was twice washed by decantation with ether. A second extraction with 20% aqueous potassium hydroxide (100 c.c.) of the combined ethereal layer and washings gave a further small quantity of the potassium salt. When a suspension of this salt in ether (200 c.c.) was shaken with an excess of dilute hydrochloric acid, an ethereal solution of the diosphenol was obtained, which was washed with water and dried over anhydrous sodium sulphate. On removal of the solvent in a vacuum at room temperature, the crude diosphenol was obtained as a pale yellow, crystalline solid (5·2 g.).

The product from three such preparations (15·4 g.) was dissolved in cold petrol (450 c.c.) and freed from insoluble flocculent material. On concentration to 200 c.c. at room temperature in a vacuum and after standing at 0°, the petrol solution yielded pale yellow, prismatic needles, m. p. 144—145° (8·5 g.). A further crop (2·0 g.) of the same m. p. was obtained on concentration of the mother-liquor. The product was recrystallised from glacial acetic acid, from which *cholestane-2 : 3-dione* (*A*) separated in prismatic needles, m. p. 144—145°, $[\alpha]^{20°}_{5461}$ + 91·5°, $[\alpha]^{20°}_{D}$ + 79·1° (*c*, 0·954) *; $\alpha_{5461}/\alpha_D = 1{\cdot}16$ (Found †: C, 80·8; H, 10·9. $C_{27}H_{44}O_2$ requires C, 80·9; H, 11·1%). The absorption spectrum of an alcoholic solution (0·005%) is shown in the figure: maximum 2720 A., $\log \varepsilon_m = 3{\cdot}70$. According to the observation made by Dr. R. K. Callow, to whom we are indebted for the spectroscopic measurements, the maxima of both forms of the diosphenol show in alkaline solution (N/200-sodium hydroxide in 95% alcohol) an equal shift in wave-length to 3200 A., $\log \varepsilon_m = 3{\cdot}57$ (figure).

Form (A) of the diosphenol is readily soluble in dioxan, pyridine, and ethyl acetate, sparingly soluble in cold methyl alcohol, ethyl alcohol, or glacial acetic acid, and may be recrystallised from petrol or mixtures of pyridine, dioxan or ethyl acetate with methyl alcohol. The Liebermann–Burchard colour reaction is given in the typical way. In the Salkowski reaction the chloroform layer remains colourless and the lower layer turns from yellow to cherry-red. A solution of form (A) in concentrated sulphuric acid is at first lemon-yellow and changes on standing to a deep cherry-red [cf. behaviour of form (B)].

After removal of the diosphenol, the residue (4 g.) of the ethereal mother-liquor was repeatedly crystallised from alcohol and yielded colourless needles, m. p. 128—129°, of cholestanone, which was characterised as the *o*-tolylsemicarbazone. This derivative is obtained in quantitative yield by dissolving the ketone (0·2 g.) and *o*-tolylsemicarbazide (0·2 g.) in alcohol (10 c.c.) containing a drop of acetic acid. After crystallisation from benzene–alcohol the colourless needles of *cholestanone-o-tolylsemicarbazone*, m. p. 228—229° (decomp.), showed no depression of m. p. in admixture with a specimen prepared from authentic cholestanone (Found: N, 8·0. $C_{35}H_{55}ON_3$ requires N, 7·8%).

* The optical rotations were measured in a 4 dm. tube in chloroform solutions.

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

The *monoacetate* of *form* (*A*), prepared by refluxing the diosphenol (2·7 g.) with acetic anhydride (15 c.c.) for 40 minutes, crystallised in glistening plates on cooling, and was recrystallised from 85% alcohol; m. p. 137—138°, $[\alpha]^{18°}_{5461}$ + 109·8°, $[\alpha]^{18°}_{D}$ + 91·8° (*c*, 1·138), α_{5461}/α_{D} = 1·19 (Found: C, 78·7; H, 10·5. $C_{29}H_{46}O_3$ requires C, 78·7; H, 10·5%). The acetate did not react with ferric chloride, but gave, in contrast to the diosphenol, a purple colour with *m*-dinitrobenzene and alcoholic potassium hydroxide (Zimmermann, *Z. physiol. Chem.*, 1935, **233**, 257). U.V. absorption in alcohol: Maximum, 2380 A., log ε_m = 3·87.

Hydrolysis was effected rapidly when sodium ethoxide (1·2 mols.) in alcohol (10 c.c.) was added to a solution of the acetate (0·5 g.) in ether (4 c.c.). The solution immediately turned yellow and after 5 minutes 50% alcohol (1·4 c.c.) was added drop by drop until crystallisation of the white sodium salt began. After cooling in the ice-box the sodium salt was collected and dissolved in warm glacial acetic acid (3·5 c.c.), from which well-formed prismatic needles of the diosphenol, form (A), m. p. 144—145°, separated on cooling.

The *monobenzoate* was obtained by dissolving the diosphenol (0·5 g.) in pyridine (12 c.c.) and adding benzoyl chloride (1 c.c.). After standing overnight, the product was isolated in the usual way and crystallised from chloroform–methyl alcohol or ethyl acetate. The benzoate was dimorphous, crystallising from ethyl acetate in prismatic needles, which on standing were gradually converted into transparent rhombic crystals; the two forms were readily interconvertible and showed identical clearing and melting points. In polarised light, in Kofler's micro-apparatus, the crystals were seen to melt to an anisotropic liquid at 162—163°, which changed to the isotropic melt at 193—194°, showing a play of colours on cooling (Found: C, 80·4; H, 9·6. $C_{34}H_{48}O_3$ requires C, 80·9; H, 9·6%).

Bromine titration (by the pyridine sulphate–dibromide method). After 15 minutes at room temperature, 0·0989 g. of the diosphenol absorbed 17·5 mg. of bromine (0·89 atom of Br).

Quinoxaline derivative. The diosphenol (50 mg.) was refluxed in alcoholic solution (2 c.c.) with *o*-phenylenediamine (50 mg.) for an hour. The solution on cooling deposited clusters of colourless plates (44 mg.), which were collected and washed with acetone. After recrystallisation from chloroform–acetone, the *quinoxaline* was obtained as clusters of colourless blades, m. p. 179—180° (Found: N, 6·2. $C_{33}H_{48}N_2$ requires N, 5·9%).

Oxidation to the acid $C_{27}H_{46}O_4$ (III). Hydrogen peroxide (100-vol., 3 c.c.) and 10% aqueous potassium hydroxide (6 c.c.) were added in three equal portions, at intervals of 15 minutes, to a solution of the diosphenol (0·5 g.) in alcohol (50 c.c.), which was kept gently boiling on a steam-bath. After the cooled solution had been diluted with water and extracted with ether, the acid was liberated with dilute sulphuric acid and taken up in ether. The white crystalline residue obtained on evaporation was recrystallised from ethyl acetate, from which the acid $C_{27}H_{46}O_4$ (0·5 g.) separated in transparent hemihedral crystals, m. p. 194—195°, showing no depression in admixture with an authentic specimen prepared from cholestanone (0·0998 g. required 4·4 c.c. of N/10-sodium hydroxide for neutralisation. Calc. for $C_{27}H_{46}O_4$, 4·5 c.c.).

Cholestane-2 : 3-dione, Form (*B*).—A solution of form (A) of the diosphenol (0·5 g.) in glacial acetic acid (2·5 c.c.) containing concentrated hydrochloric acid (0·05 c.c.) was kept on a steam-bath for a few minutes. A mixture of the two forms, m. p. 130—132°, clearing at 150°, was deposited, on cooling, in colourless prismatic needles (0·4 g.). Separation was effected by recrystallisation from ethyl acetate, from which the less soluble *form* (*B*) crystallised in clusters of needles, m. p. 168—169°, $[\alpha]^{22°}_{5461}$ + 67·3°, $[\alpha]^{22°}_{D}$ + 57·2° (*c*, 1·010); α_{5461}/α_{D} = 1·18 (Found: C, 81·0; H, 11·1. $C_{27}H_{44}O_2$ requires C, 80·9; H, 11·1%). The absorption spectrum of an alcoholic solution (0·005%) has a maximum at 2700 A., log ε_m = 3·93. In alkaline solution (N/200-sodium hydroxide in 95% alcohol) the same shift to a longer wave-length takes place as in form (A) (see figure).

Form (B) of the diosphenol is much less soluble than form (A) in the same solvents and dissolves in concentrated sulphuric acid with a permanent lemon-yellow colour. The Liebermann–Burchard reaction is negative and in the Salkowski reaction the lower layer turns yellow, whilst the chloroform layer remains colourless.

The *monoacetate* of *form* (*B*) was prepared by the usual technique and crystallised in colourless needles, m. p. 140—144° (Found: C, 78·6; H, 10·2. $C_{27}H_{46}O_3$ requires C, 78·7; H, 10·5%). A mixture with the acetate of form (A), m. p. 137—138°, melted at 110—114°. U.V. absorption in alcohol: Maximum, 2370 A., log ε_m = 3·95.

The ferric chloride reaction was negative; with tetranitromethane a yellow colour was produced in chloroform solution.

The quinoxaline derivative of form (B), m. p. 179—180°, was produced in the same yield and under the same conditions as that of (A), with which it is identical (mixed m. p.).

The oxidation to the acid $C_{27}H_{46}O_4$ with alkaline hydrogen peroxide was effected as described above. The dicarboxylic acid was obtained in quantitative yield, m. p. and mixed m. p. 194—195°.

Conversion of Form (B) into Form (A).—A solution of form (B) (0·2 g.), m. p. 168—169°, in ether (10 c.c.) was shaken with 20% aqueous potassium hydroxide (5 c.c.) at 0°. The colourless potassium salt collected at the interface and was washed twice with ether by decantation, the aqueous layer having been removed previously. The suspension of the salt in ether was shaken with an excess of dilute hydrochloric acid, and the solvent removed from the washed and dried ethereal layer. The crystalline solid, m. p. 140—142°, was recrystallised from petrol (5 c.c.) and obtained in colourless needles, m. p. 143—144°, identical with form (A) of the diosphenol (mixed m. p.). On admixture with form (B), m. p. 168—169°, however, the melting point was depressed to 133—135°, clearing at 155°.

Hydroxymethylenecholestan-3-one.—To a suspension of powdered sodium (0·46 g.) in ether (120 c.c.) and cholestanone (7·7 g.), amyl formate (3·2 c.c.) was slowly added. A yellow solution resulted, the sodium gradually dissolving. After 12 hours, ice-water (100 c.c.) was added, and the alkaline solution was separated and extracted with ether to remove unchanged cholestanone (4 g.). The yellow aqueous layer was freed from ether by aeration and acidified with 30% acetic acid. A white granular precipitate formed immediately, which was removed and thoroughly washed with water. On crystallisation of the dried product from chloroform–methyl alcohol, *hydroxymethylenecholestanone* was obtained as a colourless microcrystalline powder, m. p. 182—184°, clearing at 195°, which gave an intense purple colour with alcoholic ferric chloride (Found : C, 81·0; H, 11·1. $C_{28}H_{46}O_2$ requires C, 81·1; H, 11·2%). Hydroxymethylenecholestanone is sparingly soluble in alcohol, ethyl acetate, and glacial acetic acid, moderately soluble in dioxan, heptane, and benzene, and easily soluble in chloroform. Conditions for its oxidation to the diosphenol are being investigated.

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