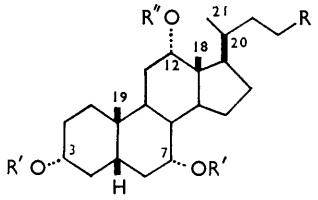
549. *Scymnol Sulphate and Anhydroscymnol.*

By A. D. Cross.

Anhydroscymnol, the product of alkaline hydrolysis of scymnol sulphate,[1] has been shown to contain a trimethylene oxide ring (IIIa). The nomenclature, structures, and biogenesis of some bile alcohol sulphates and the derived alcohols are commented upon.

Hammarsten [2] isolated α-scymnolsulphuric acid from the bile of the shark *Scymnus borealis*. The homogeneity of his "β-scymnolsulphuric acid" remains unsubstantiated. Isolation of the α-form, more commonly termed scymnol sulphate, from rays and other sharks has been reported,[3] and Cook [3] considered it a typical component of the bile of Elasmobranch fishes. Vigorous alkali hydrolysis furnished a free bile alcohol, scymnol.[2-7] In view of the structure established here for this compound, and from the known properties of other bile alcohol sulphate esters, it is proposed that this hydrolysis product be renamed anhydroscymnol, which name is employed throughout this communication.*



(I); R = CO_2H; R′ = R″ = H

(II); R = $-CH(O)-C(Me)(CH_2\cdot OH)$; R′ = R″ = H

(III); R = $-CH(O-CH_2)-CH-CH_2\cdot OR'$ (a); R′ = R″ = H; (b); R′ = Ac; R″ = H; (c); R′ = R″ = Ac

(IV) R = $-CH(OH)-CH(Me)-CH_2\cdot OH$; R′ = R″ = H

(V) R = $-CH(Cl)-CH(CH_2\cdot OH)-CH_2\cdot OH$; R′ = R″ = H

(VI) R = $-CH(O-CH_2)-CH-CH_2-O-H$:M; R′ = R″ = H

(VII) R = $-CH=CH\cdot CH_2\cdot OH$; R′ = R″ = H

(VIII) R = $-CH(OH)-CH(CH_2\cdot OH)-CH_2\cdot OH$; R′ = R″ = H

(IX) R = $-CH(CH_2\cdot OH)-CH_2\cdot OH$

(X) R = $-CH(CH_2)(CH_2)O$

Early work established the presence in anhydroscymnol of one primary and three secondary hydroxyl groups, and an oxide ring opened by hydrogen chloride to a chlorohydrin from which the oxide ring was regenerated by the action of alkali.[5] Oxidation of anhydroscymnol tetra-acetate, followed by hydrolysis, gave cholic acid (I).[6,7] Structure

* The proposed change standardises the nomenclature for hydrolysis products of bile alcohol sulphates.

[1] In a preliminary account of this work (Cross, *Proc. Chem. Soc.*, 1960, **344**), the expression "scymnol," the hydrolysis product of a constituent of shark bile was incorrectly abbreviated.

[2] Hammarsten, *Z. physiol. Chem.*, 1898, **24**, 322.

[3] Cook, *Nature*, 1941, **147**, 388; Igarashi and Kakubata, *Mem. Fac. Agric., Hokkaido Univ.*, 1952, **1**, 181; Oikawa, *J. Biochem.* (*Japan*), 1925, **5**, 63.

[4] Fieser and Fieser, "Natural Products Related to Phenanthrene," 3rd Edn., Reinhold Publ. Corp., New York, 1949, pp. 112—113.

[5] Windaus, Bergmann, and König, *Z. physiol. Chem.*, 1930, **189**, 148.

[6] Asikari, *J. Biochem.* (*Japan*), 1939, **29**, 319.

[7] Bergmann and Pace, *J. Amer. Chem. Soc.*, 1943, **65**, 477.

(II) for anhydroscymnol[6] was accepted until Fieser and Fieser[4] remarked on the improbability of the epoxide (II) remaining inert during the vigorous oxidation of anhydroscymnol to a trioxo-carboxylic acid.[5] A trimethylene oxide structure (IIIa) appeared more acceptable and the applicability of nuclear magnetic resonance spectroscopy to the structural problem was noted.[8]

Acetylation of anhydroscymnol afforded the triacetate (IIIb) [showing infrared absorption at 3632 cm.$^{-1}$ (OH)] and the tetra-acetate (IIIc).* Assignment of the unacetylated hydroxyl group to the 12α-position (IIIb) follows from molecular-rotation changes coincident upon further acetylation,[1] and from the known hindrance to attack at this position by the side-chain. In the nuclear magnetic resonance spectrum neither anhydroscymnol triacetate (in chloroform) nor scymnol sulphate (in pyridine) showed absorption assignable to epoxide protons or to a methyl group attached to a carbon atom also bearing oxygen,[9] except acetates, thus rendering untenable structure (II) for anhydroscymnol. Reduction of anhydroscymnol with lithium aluminium hydride furnished dihydroanhydroscymnol (deoxyscymnol) (IV). Slomp and Mackellar[10] recently recorded nuclear magnetic resonance spectra of hydroxylated steroids in pyridine solutions and observed that, compared with spectra of solutions in halogenated hydrocarbons, the *C*-methyl proton absorp-

Nuclear magnetic resonance: methyl-proton absorption data.[a]

Protons (no.)	18-Methyl (3)	19-Methyl (3)	21-Methyl[d] (3)	26-Methyl[d] (3)	Acetyl (various)
Methyl cholate in C_5H_5N	9·20 (2·6)	9·00 (3·1)	8·84, 8·78 (3·3)	—	—
Methyl 3α,12α-diacetyl-7-deoxycholate in $CHCl_3$	9·28	9·09	9·17[g]	—	7·97, 7·90
Methyl 3α,7α,12α-triacetylcholate in CCl_4	9·27	9·08	9·16, 9·11	—	8·05, 7·96, 7·93
Anhydroscymnol in C_5H_5N	9·20 (2·6)	9·00 (2·8)	8·83, 8·76 (3·6)	—	—
Anhydroscymnol triacetate in $CHCl_3$	9·31	9·07	8·98[g]	—	7·96 (3), 7·92 (6)
Anhydroscymnol triacetate in C_6H_5N	9·28	9·11	8·86, 8·81	—	8·14 (3), 8·00 (6)
Anhydroscymnol tetra-acetate in CCl_4	9·27	9·07	9·17, 9·12	—	8·03, 7·99, 7·94, 7·91
Dihydroanhydroscymnol in C_5H_5N	9·18 (2·7)	9·00 (2·7)	8·83, 8·77, 8·71 (total 6·6)		—
Anhydroscymnol chlorohydrin in C_5H_5N	9·19	8·98	8·79, 8·74	—	—

[a] Absorptions are expressed as τ[b] values. Spectra were obtained with a Varian Associates V-4300 spectrometer equipped with a 56·445 megacycle oscillator and using *ca.* 10% solutions in chloroform, carbon tetrachloride, or pyridine.[c] Tetramethylsilane was used as an internal reference for calibration by the conventional sideband technique. [b] Tiers, *J. Phys. Chem.*, 1958, **62**, 1151. [c] Pyridine was purified by gas-liquid chromatography. [d] Secondary *C*-methyl absorption was never well-resolved [e] and consisted of a main peak with a shoulder at a higher τ value (splitting 3—4 c.p.s.). [e] A simple well-resolved doublet is not expected for a methyl group in the situation CHMe if the chemical shift between the two types of proton is of the same order as the coupling constant between them.[f] [f] Abraham, Pople, and Bernstein, *Canad. J. Chem.*, 1958, **36**, 1302. [g] The marked shift of this proton absorption on complete acetylation illustrates the steric proximity of the $C_{(21)}$-methyl to the 12α-hydroxyl.

tions are often shifted, with clarification of the spectrum. Our spectral results (see Table) confirm their observations and, moreover, establish that reduction of the oxide ring of

* " Scymnol " (anhydroscymnol) and some derivatives from the late Professor Bergmann's collection were made available through the kindness of Dr. W. M. Stokes, Providence College, Rhode Island, U.S.A.

[8] Cross, *Quart. Rev.*, 1960, in the press. L. F. Fieser, *Nucleus*, 1959, Dec., p. 80 (the Author thanks the Editor for drawing attention to this published lecture).

[9] For characteristic absorptions of methyl protons in various environments see Jackman, " Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London, 1959, Ch. 4.

[10] Slomp and MacKellar, *J. Amer. Chem. Soc.*, 1960, **82**, 999.

anhydroscymnol generates a new methyl group attached to a carbon atom bearing one hydrogen but no oxygen atom. Approximate area counts support this conclusion (see Table). Methyl cholate shows the same *C*-methyl proton absorption τ values and areas as anhydroscymnol and the derived chlorohydrin (V), all in pyridine solution: similarly, for carbon tetrachloride solution, *C*-methyl proton absorption of methyl triacetylcholate strongly resembles that of anhydroscymnol tetra-acetate.

In the infrared spectrum (potassium chloride disc) anhydroscymnol shows absorptions at 961 (s) and 878 cm.$^{-1}$ (w) attributed to the trimethylene oxide [11] since in the chlorohydrin (V), dihydroanhydroscymnol (IV), and scymnol sulphate, both bands are absent, removal of the former revealing the weak absorption (*ca.* 950 cm.$^{-1}$) of the cholic acid nucleus.

Support for structure (IIIa) for anhydroscymnol came from the failure of periodic acid to attack either dihydroanhydroscymnol or anhydroscymnol chlorohydrin. In each case structure (II) for anhydroscymnol requires oxidisable molecules. An early sample † labelled " periodic acid oxidation product of scymnol chlorohydrin " showed no carbonyl absorption in the infrared spectrum and, on recrystallisation, proved to be anhydroscymnol chlorohydrin hydrate. When heated with copper–bronze both anhydroscymnol and dihydroanhydroscymnol yielded formaldehyde. This reaction is characteristic of the 1,3-glycol system $>CH(OH)\cdot C\cdot CH_2\cdot OH$,[12] but with anhydroscymnol constitutes a new example of " fragmentation " [13] in which relief of the oxide ring strain promotes the reaction (VI ⟶ VII + H·CHO).[3] Barton and Brooks,[14] and Tarbell and his co-workers,[15] have described formally analogous C–C bond cleavages in αβ-epoxy-δ-hydroxy-systems of morolic acid and fumagillin derivatives, respectively, with aluminium hydride as the proton acceptor.

These observations are compatible solely with structures for anhydroscymnol (IIIa), scymnol (revised nomenclature) (VIII), and scymnol sulphate (VIII, sulphated on a side-chain hydroxyl group *). Scymnol, the hexol, still awaits preparation, and anhydroscymnol is an artefact of the hydrolytic method, a possibility envisaged by Haslewood a decade ago,[16] and now independently proved.[17] Reassessment of Hammarsten's analytical figures [2] for sodium scymnol sulphate revealed their better agreement with structure (VIII, monosulphated), $C_{27}H_{47}O_5\cdot SO_4Na$, than his suggested formula $C_{27}H_{45}O_4\cdot SO_4Na$.

Sodium ranol sulphate is found in the bile of the frog *Rana temporia*.[18] Acidic and alkaline hydrolyses lead respectively to ranol and anhydroranol, and evidence for a trimethylene oxide ring in the latter is being sought. Scymnol sulphate represents an interesting departure from the hitherto-assumed β-oxidative degradation of the cholesterol side-chain since both terminal methyl groups are now oxidised. It is considered very probable that other C_{27} bile alcohols incorporate this structural unit (IX), and the recent isolation of the first disulphate bile ester from the hagfish [19] is in accord with this notion. These C_{27} bile alcohols are found only in fishes and amphibia, and the side-chain structure of the bile alcohol sulphate of the coelacanth [20] is of obvious interest. Extension of this work will cover other bile alcohols and a search for the terminal structural unit (X).

* Though attachment of the sulphate ester at the $C_{(26)}$ or $C_{(27)}$ primary hydroxyl group is probable, published evidence does not yet exclude esterification at the $C_{(24)}$ hydroxyl group, which would give a mechanistically more favourable structural unit for formation of the trimethylene oxide ring (III).

[11] Barrow and Searles, *J. Amer. Chem. Soc.*, 1953, **75**, 1175, quote strong absorption at 970—980 cm.$^{-1}$ for simple trimethylene oxides. Gates and Roe, *Tetrahedron*, 1960, **11**, 148, have summarised data for similar absorptions, and all bands appear in the range 940—985 cm.$^{-1}$.
[12] Tsuda and Kitigawa, *Ber.*, 1938, **71**, 1604.
[13] Grob, *Experientia*, 1957, **13**, 126.
[14] Barton and Brooks, *J.*, 1951, 257.
[15] Tarbell, Carman, Chapman, Huffman, and McCorkindale, *J. Amer. Chem. Soc.*, 1960, **82**, 1005.
[16] Haslewood, *Biochem. Soc. Symp.*, 1951, **6**, 83.
[17] Briggs and Haslewood, *Biochem. J.*, in the press.
[18] Haslewood, *Biochem. J.*, 1952, **51**, 139.
[19] Haslewood, *Biochem. J.*, in the press.
[20] Haslewood, *Biochem. J.*, 1957, **66**, 22.

Experimental

Melting points were taken on a Kofler block. Analyses were determined by Miss Cuckney and her staff, infrared spectra by Mrs. Boston on a Grubb Parsons S4/DB1 spectrophotometer with calcium fluoride and sodium chloride prisms as appropriate, and nuclear magnetic resonance spectra by Dr. J. W. Lown. Light petroleum refers to the fraction, b. p. 60—80°.

Acetylation of Anhydroscymnol.—Anhydroscymnol (50 mg.) was treated with acetic anhydride (0·5 ml.) and pyridine (3 ml.) at room temperature during 24 hr. *Anhydroscymnol triacetate* (51 mg.) crystallised from chloroform–light petroleum as needles, m. p. 169—171°, $[\alpha]_D$ 34·4° (*c* 1·03 in $CHCl_3$) and 44·2° (*c* 1·96 in pyridine); infrared absorption peaks were at 3632 (OH) and 1734 cm.$^{-1}$ with shoulders at 1748 and 1743 cm.$^{-1}$ (3 acetates) (Found: C, 68·5; H, 9·2; Ac, 21·9. $C_{33}H_{52}O_8$ requires C, 68·7; H, 9·1; 3 Ac, 22·4%).

Chromatography on Grade III alumina of " acetylated scymnol," * prepared by keeping anhydroscymnol in pyridine with acetic anhydride at 100° during 1 hr., furnished anhydroscymnol triacetate and anhydroscymnol tetra-acetate. The latter crystallised from light petroleum as needles, m. p. 147—150° (lit.[7] 145—147°), $[\alpha]_D$ 75·8° (*c* 1·51 in $CHCl_3$) and 84·9° (*c* 1·70 in pyridine); no infrared hydroxyl absorption.

Dihydroanhydroscymnol.—Anhydroscymnol (100 mg.) in dry dioxan (10 ml.) was added to an excess of lithium aluminium hydride (100 mg.) in boiling dioxan (10 ml.), and the mixture kept boiling for 3 days. The excess of hydride was destroyed (ethyl acetate) and, after dilution (H_2O) of the mixture, dioxan was removed by azeotropic distillation. Saturated sodium potassium tartrate solution was then added and the suspension extracted continuously with hot chloroform for 2 days to yield *dihydroanhydroscymnol* (93 mg.), which crystallised as the monohydrate from ethanol–light petroleum or " wet " ethyl acetate as prisms, m. p. 118—124°, and 182—183° after resolidification or after being dried for 48 hr. at 105°. The m. p. of a mixture with anhydroscymnol (m. p. 189—190°) was 165—173°. Anhydroscymnol had $[\alpha]_D$ 39·1° (*c* 0·93 in pyridine) and dihydroanhydroscymnol $[\alpha]_D$ 36·2° (*c* 0·72 in pyridine) (Found, in sample dried at 105°: C, 68·8; H, 10·7. $C_{27}H_{48}O_5,H_2O$ requires C, 68·9; H, 10·7%). The solvent of crystallisation was removed during 48 hr. at 125° (Found: C, 71·4; H, 10·9. $C_{27}H_{48}O_5$ requires C, 71·6; H, 10·7%).

In an alternative procedure, after destruction of the excess of hydride, the hot aqueous dioxan solution was filtered and evaporated and dihydroanhydroscymnol extracted from the residue in 94% yield by 1 : 1 acetone–chloroform.

Glycol Reactions.—(a) *Periodic acid.* Dihydroanhydroscymnol in methanol was treated with aqueous periodic acid (0·02N; 10 ml.). A blank was similarly prepared but the sterol was omitted, and consumption of periodic acid was followed by iodometric titration. No periodic acid was consumed after 24 hr. at room temperature. The product was a gum showing strong hydroxyl but no carbonyl absorption in the infrared spectrum.

Anhydrous anhydroscymnol chlorohydrin, m. p. 196°, after treatment with periodate,* gave as the sole isolable compound anhydroscymnol chlorohydrin monohydrate, m. p. 132—135° (lit.,[5] m. p. 126°; Kofler block m. p. 130—135° for an authentic sample), showing no carbonyl absorption in the infrared spectrum.

(b) *Pyrolyses with copper–bronze.* An intimate mixture of powdered dihydroanhydroscymnol (80 mg.) and copper–bronze powder (200 mg.) was heated to 270—300° (metal bath) for 2 hr. in a stream of oxygen-free, dry nitrogen. Effluent gases were bubbled through freshly-prepared, ice-cold, aqueous dimedone solution. The precipitate was filtered off and, after recrystallisation from ethanol, identified as formaldehyde dimethone by m. p., mixed m. p., and comparative infrared spectra. A " blank " reaction, run simultaneously, gave no formaldehyde.

Anhydroscymnol similarly afforded formaldehyde dimethone under identical reaction conditions.

Acetylation of Dihydroanhydroscymnol.—Dihydroanhydroscymnol (55 mg.) was acetylated at room temperature as described for anhydroscymnol. Chromatography of the crude product on Grade III alumina yielded only a gum (30 mg.), chromatographically homogeneous, which appeared, from analysis, to be *dihydroanhydroscymnol tetra-acetate*, showing infrared absorption at 3545 cm.$^{-1}$ (hydroxyl) (Found: Ac, 26·3. $C_{35}H_{56}O_9$ requires 4 Ac, 27·7%). The nuclear

* See footnote on p. 2818.

magnetic resonance spectrum showed proton absorption equivalent to two secondary *C*-methyl groups (cf. dihydroanhydroscymnol, Table).

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DEPARTMENT OF CHEMISTRY, IMPERIAL COLLEGE, SOUTH KENSINGTON,
LONDON, S.W.7.
[Present address: RESEARCH LABORATORIES, SYNTEX S.A., APARTADO 2679,
MEXICO, D.F.] [*Received, January 27th*, 1961.]