204. *The Structure of the Cactus Sterol, Peniocerol (Cholest-8-ene-3β,6α-diol).*

By Carl Djerassi, R. D. H. Murray, and R. Villotti.

The constitution and stereochemistry of a new cactus sterol, peniocerol, has been shown to be that of cholest-8-ene-3β,6α-diol by its conversion into cholest-8(14)-ene, cholestan-6α-ol, cholestan-3β-yl acetate, and cholestane-3β,6α-diol.

Our systematic investigations [1] of the aerial portions of giant cacti have led to the isolation of numerous new triterpenoids as well as a new sterol, lophenol (4α-methylcholest-7-en-3β-ol) [2] which may well be an intermediate in the lanosterol-to-cholesterol biogenetic pathway.[3] Our attention has now been focused on the relatively rare genus *Peniocereus* [4] which together with *Wilcoxia* is unique in possessing large tuberous roots. We reported [5] the isolation of the known cactus triterpene, chichipegenin,[6] from the aerial parts of *Peniocereus fosterianus* Cut. Herein we report the isolation, from the roots of the same cactus, of a new sterol, peniocerol, to which we assign [5] the structure cholest-8-ene-3β,6α-diol (I).—Apart from the isolation of 22α-hydroxycholesterol from *Narthecium ossifragum*,[7] this represents the first isolation of a cholestane derivative from a higher plant. Recently, cholesterol has been isolated from *Solanum tuberosum* [8] and *Dioscorea spiculiflora*,[8] and

[1] For summary see Djerassi, " Festschrift Arthur Stoll," Birkhauser, Basel, 1957, pp. 330—352.
[2] Djerassi, Krakower, Lemin, Liu, Mills, and Villotti, *J. Amer. Chem. Soc.*, 1958, **80**, 6284.
[3] Wells and Lorsch, *J. Biol. Chem.*, 1960, **235**, 978.
[4] Britton and Rose, " The Cactaceae," Carnegie Institution, Washington, 1920, vol. II, p. 112; Bravo, " Las Cactaceae de Mexico," Imprenta Universitaria, Mexico, D.F., 1937, pp. 282—284.
[5] Sandoval, Manjarrez, Leeming, Thomas, and Djerassi, *J. Amer. Chem. Soc.*, 1957, **79**, 4468.
[6] Preliminary communication, Djerassi, Murray, and Villotti, *Proc. Chem. Soc.*, 1961, 450.
[7] Stabursvik, *Acta Chem. Scand.*, 1953, **7**, 1220.
[8] Johnson, Bennett, and Heftmann, *Science*, 1963, **140**, 198.

observed, by gas chromatography, in *Avena sativa*.[9] It is likely that cholestane derivatives occur widely in plants but that the methods of detection used previously were not adequate.

Chromatography of the ether-soluble neutral fraction of an ethanolic extract of the roots of *P. fosterianus* provided, in *ca.* 1% yield (based on dry root), a new sterol, peniocerol (I). Peniocerol (I) [$\nu_{max.}$ (in chloroform) 3330 cm.$^{-1}$ (OH)] had the molecular formula $C_{27}H_{46}O_2$ and had terminal ultraviolet absorption characteristic of a tetrasubstituted ethylenic linkage,[10] confirmed by the absence of olefinic protons in the nuclear magnetic resonance (n.m.r.) spectrum. The ready formation of a diacetate (II) and a dibenzoate (III) indicated the presence of two reactive hydroxyl groups. Peniocerol was thus tetracyclic and probably a sterol containing an 8,9- or 8,14-double bond.

More precise information about the location of the double bond could be adduced from hydrogenation experiments. Peniocerol diacetate, on treatment with palladised charcoal in ethyl acetate containing acetic acid, was not reduced but converted into the isomeric diacetate (V). That the double bond had moved to the more stable tetrasubstituted 8,14-position was apparent from the increased terminal ultraviolet absorption [ε (210 mμ) 10350; ε (220 mμ) 4800] together with the absence of vinyl protons in the n.m.r. spectrum. The use of ultraviolet spectroscopy in distinguishing between a double bond in the 8,9-position and the doubly exocyclic 8,14-isomer is well established.[10] Compelling evidence for a sterol skeleton having neither the double bond nor either hydroxyl in the side-chain was found in the mass spectrum.[11] Although no molecular ion was found, substantial peaks at *m/e* 426 ($M - 60$) and 366 ($M - 2 \times 60$) were observed, corresponding to the loss of one and two molecules of acetic acid, respectively. Significantly, two major fragments were present at *m/e* 313 ($M - 60 - 113$) and 254 ($M - 2 \times 60 - 113$), from which it was deduced that the molecule contained a C_8H_{17} moiety, thereby strengthening support for a dihydroxycholest-8(14)-ene structure for the double-bond isomer (VI) of peniocerol.

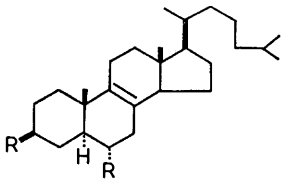
Slow crystallisation of the mother-liquors of crystallisation of peniocerol afforded a new diol, macdougallin (IV), $C_{28}H_{48}O_2$, since isolated in greater quantity from *Peniocereus macdougalli* Cut.[12] The ratio of peniocerol to macdougallin in our extract was calculated approximately from the mass spectrum of the crude diacetate mixture, obtained by acetylation of the crude sterol fraction followed by hydrogen chloride catalysed isomerisation. The ratio of the peaks *m/e* 426 ($C_{31}H_{50}O_4 - 60$) to *m/e* 440 ($C_{32}H_{50}O_4 - 60$) was 20 : 9, corresponding roughly to two parts of the double-bond isomer of peniocerol diacetate to one of macdougallin diacetate.

The presence of a cholest-8-ene carbon framework in peniocerol (I) was shown unambiguously by the following transformations. The ditoluene-*p*-sulphonate (VIII), derived from the double-bond isomer (VI) of peniocerol, on treatment with alkaline alumina,[13] followed by catalytic hydrogenation of the unstable cholesta-2,6,8(14)-triene (IX), provided cholest-8(14)-ene (X) identical with an authentic specimen [14] synthesised from 7-dehydrocholesterol.

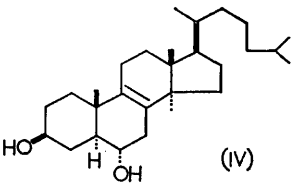
The location and stereochemical disposition of the hydroxyl functions was determined as follows. The triene (IX) had $\lambda_{max.}$ 247 mμ (ε 15,600), indicative of a double bond between carbon atoms 6 and 7 conjugated to the 8,14-olefin,[15] thus showing that one of the hydroxyls could be assigned to position 6 or 7 of the cholest-8(14)-ene nucleus. Partial saponification of the isomeric diacetate (V) with methanolic potassium carbonate afforded both monoacetates in unequal amounts, the major component (XI) containing a 6α-acetate, as shown by the following reactions. Oxidation with chromium trioxide in pyridine gave

[9] Knights, University of Glasgow, personal communication.
[10] Bladon, Henbest, and Wood, *J.*, 1952, 2737.
[11] de Mayo and Reed, *Chem. and Ind.*, 1956, 1481.
[12] Djerassi, Knight, and Wilkinson, *J. Amer. Chem. Soc.*, 1963, **85**, 835.
[13] Douglas, Ellington, Meakins, and Swindells, *J.*, 1959, 1720.
[14] Cremlyn and Shoppee, *J.*, 1954, 3515; Turner, Meador, and Winkler, *J. Amer. Chem. Soc.*, 1957, **79**, 4122.
[15] Windaus, Linsert, and Eckhardt, *Annalen*, 1938, **543**, 22.

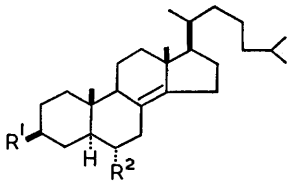
a keto-acetate (XIII), which had $\nu_{max.}$ (in chloroform) 1708 cm.$^{-1}$ (6-membered ketone), the ketone probably being in position 3 from the nature of the positive Cotton effect curve.[16] Treatment of the keto-acetate with ethanedithiol and perchloric acid gave a thioketal



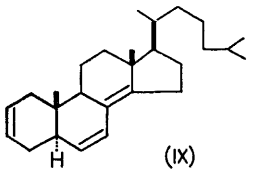
(I : R = OH)
(II: R = OAc)
(III: R = OBz)



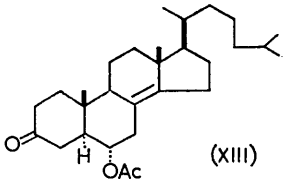
(IV)



(V : $R^1 = R^2 = OAc$)
(VI : $R^1 = R^2 = OH$)
(VII: $R^1 = R^2 = OBz$)
(VIII: $R^1 = R^2 = OTs$)



(IX)

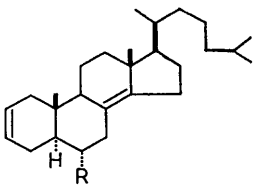


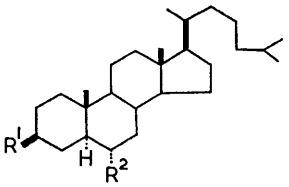
(XIII)

(X : $R^1 = R^2 = H$)
(XI : $R^1 = OH$, $R^2 = OAc$)
(XII: $R^1 = OAc$, $R^2 = OH$)

(XIV: $R^1 = H$, $R^2 = OAc$)
(XV: $R^1 = H$, $R^2 = OH$)
(XVI: $R^1 = OTs$, $R^2 = OAc$)

(XVII: R = OAc)
(XVIII: R = OH)





(XIX: $R^1 = H$, $R^2 = OH$)
(XX : $R^1 = R^2 = OAc$)
(XXI: $R^1 = R^2 = OH$)
(XXII: $R^1 = OAc$, $R^2 = H$)

which, without isolation, was desulphurised with Raney nickel in ethanol. Saponification of the resulting acetate (XIV) yielded cholest-8(14)-en-6α-ol (XV) which was more efficiently prepared by the following route.

The toluene-*p*-sulphonate (XVI) derived from the more abundant monoacetate, when shaken with alkaline alumina,[13] readily afforded a non-conjugated diene-acetate (XVII). The n.m.r. spectrum shows two olefinic protons as a doublet, τ 4·33, 4·37, virtually identical with the corresponding region in cholest-2-ene (τ 4·38, 4·42), thus supporting the proposed formulation for the triene (IX). The direction of elimination was in agreement with the findings of Chang and Blickenstaff [17] who isolated mainly cholest-2-ene as the hydrocarbon fraction from alkaline alumina treatment of cholestan-3β-yl toluene-*p*-sulphonate. The non-acetylated hydroxyl in the monoacetate (XI) derived from peniocerol was therefore probably in the 3β-position. Saponification to the diene-alcohol (XVIII), followed by catalytic hydrogenation over palladised charcoal, gave the same enol (XV) as above. Although the 8,14-double bond could not be reduced directly, saturation was effected as follows. Treatment with hydrogen chloride isomerised the double bond to the 14,15 position, now reducible with platinum oxide in ethyl acetate. Barton, Cox, and Holness [18] showed that a 1 : 1 equilibrium mixture of 8,14- and 14,15-double-bond isomers results from acid-catalysed isomerisation of ergosta-8(14),22-dien-3β-yl acetate. Repeated isomerisation-hydrogenation eventually led to almost complete conversion into cholestan-6α-ol (XIX), identical with an authentic sample [19] supplied by Dr. Ruth Lack (Sydney).

The assignment of the 3β-configuration to the remaining hydroxyl was shown by

[16] Djerassi, Closson, and Lippman, *J. Amer. Chem. Soc.*, 1956, **78**, 3163.
[17] Chang and Blickenstaff, *Chem. and Ind.*, 1958, 590.
[18] Barton, Cox, and Holness, *J.*, 1949, 1771.
[19] Shoppee and Summers, *J.*, 1952, 1786.

exposure of the diacetate (V) at −30° to hydrogen chloride in chloroform, whereby the double bond migrated to the 14,15-position. Hydrogenation with platinum oxide in acetic acid gave cholestane-3β,6α-diol diacetate (XX) and also a trace of cholestan-3β-yl acetate (XXII), which again indicated the close proximity of one of the acetate groups to the double bond. Hydrolysis afforded cholestane-3β,6α-diol (XXI), identical with an authentic sample [20] provided by Dr. Ruth Lack (Sydney).

Cholest-8(14)-ene-3β,6α-diol (VI) was synthesised by Barton and Rosenfelder,[21] and the derived diacetate (V) was identical with that of the peniocerol diacetate isomerisation product by direct comparison (mixture m. p. and mass spectra) with an authentic sample kindly supplied by Professor D. H. R. Barton (London). This result confirms our assignment of cholest-8-ene-3β,6α-diol (I) to peniocerol. We obtained values of melting point for the double-bond isomer (VI) of peniocerol and its dibenzoate higher than those previously recorded.[21]

Peniocerol is the first naturally occurring plant sterol oxygenated at C-6, although steroidal 6-ketones have recently been isolated from a related cactus.[22] Whether it represents an intermediate in the biosynthesis of cholesterol in plants remains an open question. However, Bloch has shown [23] that rat-liver homogenates efficiently convert peniocerol under anaerobic conditions into cholest-7-enol.

Experimental

Ultraviolet spectra were determined with a Cary model 14 spectrophotometer and are for solutions in ethanol, and infrared spectra with a Perkin-Elmer Infracord or model 21 spectrometer. Optical rotations were measured in chloroform solutions. The light petroleum used was of b. p. 40—60°. Chromatographic alumina was Merck Standardised, deactivated, if required, according to Brockmann.[24] Merck Silica Gel G was used for thin-layer chromatography on glass (chromatoplate technique). Microanalyses were carried out by Mr. E. Meier. N.m.r. spectra were determined, with a Varian AR-60 instrument, by Dr. L. J. Durham, mass spectra were measured by Dr. H. Budzikiewicz using a Consolidated Electrodynamics Corporation model 21-103 C mass spectrometer with an all-glass heated inlet system, and optical rotatory dispersion measurements were made by Mrs. R. Records, with a Japan Spectroscopic Co. automatically recording spectrophotometer.

Extraction of Peniocereus fosterianus *Cut.*—The ethanolic extract (from 7 kg. of dried cactus roots collected by Dr. D. K. Cox in the State of Colima, Mexico) was suspended in ether (20 l.) and stirred for 4 hr. The ether-soluble brown syrup (390 g.) was redissolved in ether, washed with dilute acid, water, and dilute alkali (3 × 1 l. in each case), and water (6 × 1 l.), and dried. Evaporation gave a semi-solid residue (376 g.) of which a portion (200 g.) was adsorbed on alumina (Grade IV; 6 kg.) in benzene. Extended elution with ether–benzene (3 : 1) afforded peniocerol (I) (52·4 g.), prisms, m. p. 160—165° (from methanol). After nine crystallisations from methanol the m. p. remained constant at 168—171°, $[\alpha]_D$ +59° (*c* 0·76) (Found: C, 80·65; H, 11·45. $C_{27}H_{46}O_2$ requires C, 80·55; H, 11·5%), ε (210 mμ) 3900, ε (220 mμ) 1340. The derived *diacetate* (II) formed needles, m. p. 48—50° (from acetone), $[\alpha]_D$ +45° (*c* 0·79), which after prolonged drying in a high vacuum at 50° had m. p. 95—103°, $\nu_{max.}$ (KBr) 1737, 1240 cm.$^{-1}$ (OAc), ε (210 mμ) 3645, ε (220 mμ) 1360 (Found: C, 76·5; H, 10·55. $C_{31}H_{50}O_4$ requires C, 76·5; H, 10·35%). Saponification of the diacetate with 5% potassium hydroxide in refluxing methanol regenerated peniocerol, m. p. 167—170°. The corresponding *dibenzoate* (III) formed plates, m. p. 202—204° (from methylene chloride–methanol), $[\alpha]_D$ +101° (*c* 0·76) (Found: C, 80·5; H, 8·85. $C_{41}H_{54}O_4$ requires C, 80·6; H, 8·9%). The fractions (25 g.) preceding peniocerol were refluxed with 5% potassium hydroxide in methanol for 2 hr. Dilution with water and extraction with ether afforded a dark gum (20 g.) which by rechromatography as above and crystallisation from methanol furnished peniocerol (5·9 g.), m. p. 163—166°. Slow crystallisation of a dilute methanolic solution of the peniocerol crystallisation mother-liquors gave a new diol, macdougallin (IV),[12] m. p. 173—174·5°, $[\alpha]_D$ +72° (*c* 0·75).

[20] Plattner and Lang, *Helv. Chim. Acta*, 1944, **27**, 1872.
[21] Barton and Rosenfelder, *J.*, 1951, 2381.
[22] Djerassi, Knight, and Brockmann, *Ber.*, 1964, **97**, 3118.
[23] Bloch, Harvard University, personal communication.
[24] Brockmann, *Ber.*, 1941, **74**, 73.

Isomerisation. Peniocerol diacetate (II) (25 mg.) in ethyl acetate (5 ml.), after shaking with 10% palladium–charcoal in a hydrogen atmosphere, and freeing from catalyst and solvent, afforded the isomeric diacetate (V), plates, m. p. 141—143° (from methanol), $[\alpha]_D$ +30° (*c* 1·42) (lit., m. p. 141—142°, $[\alpha]_D$ +28°) (Found: C, 76·5; H, 10·35. $C_{31}H_{50}O_4$ requires C, 76·5; H, 10·35%), ε (210 mμ) 10,350, ε (220 mμ) 4800. A sample of cholest-8(14)-ene-3β,6α-diol diacetate, m. p. 138—141° (supplied by Professor D. H. R. Barton), showed a mixed m. p. 137·5—141° with the isomeric diacetate (V), and the mass spectra were identical. The diacetate (V) (1 g.) was refluxed with 5% potassium hydroxide in methanol for 3 hr. and diluted with water, to give the isomeric diol (VI) (0·8 g.), m. p. 195—196° (from acetone, $[\alpha]_D$ +26° (*c* 1·08) (lit., m. p. 179—180°, $[\alpha]_D$ +24°) (Found: C, 80·2; H, 11·6. $C_{27}H_{46}O_2$ requires C, 80·55; H, 11·5%), ε (210 mμ) 9700, ε (220 mμ) 3900. The derived *dibenzoate* formed needles, m. p. 191—192° (from methylene chloride–methanol), $[\alpha]_D$ +55° (*c* 1·66) (lit., m. p. 181—182°, $[\alpha]_D$ +51°) (Found; C, 80·35; H, 8·9. $C_{41}H_{54}O_4$ requires C, 80·6; H, 8·9%).

*Conversion into cholest-*8(14)*-ene.* The diol (VI) (2·63 g.) and toluene-*p*-sulphonyl chloride (6·94 g.) in pyridine (20 ml.) were kept in the dark for 2 days; the solution was poured on to crushed ice and after 2 hr. extracted with ether. The ether extract was washed with dilute acid, water, dried, and evaporated under reduced pressure. The residue was chromatographed over alumina (Grade IV; 40 g.), elution with ether–light petroleum (1 : 1) furnishing the ditoluene *p*-sulphonate (VIII) (4·85 g.), rods, m. p. 115—119° (from methanol–ether), $[\alpha]_D$ + 14° (*c* 1·65) (Found: C, 69·05; H, 8·0. $C_{41}H_{58}O_6S_2$ requires C, 69·24; H, 8·2%), $\lambda_{max.}$ (in MeOH) 223 mμ (ε 26,800). A mixture of the ditoluene-*p*-sulphonate (VIII) (3 g.), alumina (Woelm basic, Grade I; 50 g.), and dry benzene (60 ml.) was stirred for 4 days, then filtered through a short column of alumina (Grade I). Chromatography of the resultant oil (0·95 g.) over alumina (Grade I; 66 g.) and elution with hexane afforded cholesta-2,6,8(14)-triene (IX) (0·85 g.), needles, m. p. 28—44°, $\lambda_{max.}$ 247 mμ (ε 15,600) [Found: *M*, 366 (mass spectrometry). $C_{27}H_{42}$ requires *M*, 366]. The triene (0·53 g.) in ethyl acetate was hydrogenated over 10% palladium–charcoal (100 mg.). After 10 min. hydrogen (2·04 mol.) had been absorbed, and the product, after freeing from catalyst and solvent, was adsorbed on alumina (Grade I; 24 g.). Elution with hexane gave cholest-8(14)-ene (0·52 g.), needles, m. p. 53—54·5° (from acetone–methanol), $[\alpha]_D$ +23° (*c* 1·42 in CCl_4) (lit., m. p. 53·5—55°, $[\alpha]_D$ +20°), identical (mixed m. p., rotation, infrared and mass spectra, chromatoplate and gas chromatography, SE 30 at 250°) with a sample prepared from 7-dehydrocholesterol.

Partial saponification. Potassium carbonate (916 mg.) in water (10 ml.) was added to a solution of the diacetate (V) (6·3 g.) in methanol (150 ml.) and dioxan (150 ml.), kept for 3 days, and evaporated under reduced pressure at room temperature. The residue was extracted into ether, washed with water, dried, evaporated, and the product (6·1 g.) adsorbed on alumina (Grade II; 385 g.). The column was eluted by a gradient technique (ether flowing into light petroleum), affording successively unchanged diacetate (2·31 g.), cholest-8(14)-ene-3β,6α-diol 3-acetate (XII) (0·36 g.), rods, m. p. 133—135° (from hexane), $[\alpha]_D$ +15° (*c* 1·20), $\nu_{max.}$ (in $CHCl_3$) 3605, 3445 (OH), and 1724 cm.$^{-1}$ (OAc) (Found: C, 77·95; H, 10·5. $C_{29}H_{48}O_3$ requires C, 78·3; H, 10·9%), and cholest-8(14)-ene-3β,6α-diol 6-acetate (XI) (3·3 g.), needles (from acetonitrile), m. p. 125·5—127°, $[\alpha]_D$ +52° (*c* 0·92), $\nu_{max.}$ (in $CHCl_3$) 3590, 3415 (OH), and 1721 cm.$^{-1}$ (OAc) (Found: C, 78·1; H, 10·75%). Acetylation of each monoacetate with acetic anhydride in pyridine overnight afforded the diacetate (V) (mixed m. p., infrared spectrum, and chromatoplate mobility).

*Cholest-*8(14)*-en-*6α*-ol.*—(a) *Through ketone* (XIII). The monoacetate (XI) (200 mg.) in pyridine (15 ml.) was treated with chromium trioxide (2·5 g.) in pyridine (100 ml.) overnight. The mixture was poured into water, extracted into ether, and the extract washed with dilute hydrochloric acid and water, dried, and evaporated. 3-Oxocholest-8(14)-en-6α-yl acetate (XIII) (100 mg.) formed needles, m. p. 99—100° (from light petroleum), $\nu_{max.}$ (in $CHCl_3$) 1718 (OAc) and 1708 cm.$^{-1}$ (6-membered ketone) (Found: C, 78·45; H, 10·55. $C_{29}H_{46}O_3$ requires C, 78·7; H, 10·45%), optical rotatory dispersion (*c* 0·14 in dioxan), 25°, $[\alpha]_{650}$ +19°, $[\alpha]_{589}$ +27°, $[\alpha]_{360}$ +423°, $[\alpha]_{280}$ −154°, $[\alpha]_{265}$ −202°. The derived semicarbazone formed needles, m. p. 195—199° (from methanol) (Found: C, 72·2; H, 10·1; N, 8·65. $C_{30}H_{49}N_3O_3$ requires C, 72·1; H, 9·9; N, 8·4%). The keto-acetate (605 mg.) in ethanedithiol (0·9 ml.) and benzene (4 ml.) containing 2 drops of 70% perchloric acid was kept for 1 hr. The solution was diluted with ether, washed with water, dried, ethanol (10 ml.) added, and the ether evaporated. The ethanolic solution was added to W2 Raney nickel in ethanol (150 ml.), and the mixture refluxed

with stirring for 12 hr. Removal of the inorganic material and solvent yielded an oil (500 mg.) which was chromatographed over alumina (Grade I; 50 g.). Elution with ether–light petroleum (1 : 19) gave cholest-8(14)-en-6α-yl acetate (XIV) (305 mg.) as a colourless oil, $\nu_{max.}$ (in $CHCl_3$) 1718 cm.$^{-1}$ (OAc). The acetate (300 mg.), in ether, was added to lithium aluminium hydride in ether and the mixture stirred for 15 min. The excess of reagent was decomposed with saturated sodium sulphate solution, and the ethereal solution decanted and evaporated, to give cholest-8(14)-en-6α-ol (XV) (260 mg.), needles, m. p. 93—96° (from acetonitrile), $[\alpha]_D$ +25° (*c* 0·78) (Found: C, 83·65; H, 12·05. $C_{27}H_{46}O$ requires C, 83·85; H, 12·0%).

(b) *Through toluene-*p*-sulphonate* (XVI). The monoacetate (XI) (2·145 g.) and toluene-*p*-sulphonyl chloride (2·85 g.) in pyridine (25 ml.) were kept for 2 days. Work up in the usual way furnished the toluene-*p*-sulphonate (XVI) (2·62 g.), needles, m. p. 136—138° (from ether–methanol), $[\alpha]_D$ +24° (*c* 1·07) (Found: C, 72·1; H, 8·9. $C_{36}H_{54}O_5S$ requires C, 72·2; H, 9·1%). The toluene-*p*-sulphonate (2·6 g.) in benzene (100 ml.) was stirred vigorously with alumina (Woelm basic, Grade I; 50 g.) for 4 days and the mixture added to a column of alumina (Grade I; 200 g.). Elution with ether–hexane (2 : 3) afforded cholesta-2,8(14)-dien-6α-yl acetate (XVII) as a thick oil (986 mg.), $[\alpha]_D$ +82° (*c* 1·24). The diene-acetate (90 mg.), in ether, was stirred with lithium aluminium hydride in ether for 15 min. Work up in the normal fashion gave the diene-alcohol (XVIII) (75 mg.), needles, m. p. 128—132° (from acetonitrile), $[\alpha]_D$ +63·5° (*c* 0·80) (Found: C, 84·6; H, 11·55. $C_{27}H_{44}O$ requires C, 84·3; H, 11·55%). The diene-alcohol in ethyl acetate smoothly absorbed hydrogen (1·0 mol.) over 10% palladium–charcoal, giving, after removal of catalyst and solvent, cholest-8(14)-en-6α-ol, identical (mixed m. p., infrared absorption, and chromatoplate behaviour) with material prepared as in (a).

Cholestan-6α-ol.—Dry hydrogen chloride was bubbled through cholest-8(14)-en-6α-ol (XV) (295 mg.) in chloroform (20 ml.) at −30° for 2 hr. Excess of hydrogen chloride was removed under reduced pressure at −30°, and the solution washed with sodium hydrogen carbonate solution, water, dried, and evaporated. The residual oil, in ethyl acetate, was hydrogenated over platinum oxide (300 mg.) for 12 hr., and freed from catalyst and solvent to give an oil (290 mg.), ε (220 mμ) 1100. This process of acid treatment followed by hydrogenation was repeated twice until the product (283 mg.) crystallised spontaneously. Repeated crystallisation gave cholestan-6α-ol (XIX), plates, m. p. 125·5—127° (from acetonitrile), $[\alpha]_D$ +36° (*c* 1·04) (lit., m. p. 126—129°, $[\alpha]_D$ +35°), identical (mixed m. p., infrared absorption, mass spectrum, and chromatoplate mobility) with an authentic sample.

Isomerisation-hydrogenation. Dry hydrogen chloride was bubbled through the diacetate (V) (2·19 g.) in chloroform (25 ml.) at −30° for 2 hr. Working up in the usual manner gave a thick oil which was hydrogenated over platinum oxide in acetic acid overnight. This procedure of acid treatment followed by hydrogenation was repeated twice, when the product showed ε (220 mμ) 550 (corresponding to 11·5% starting material). The oil in methylene chloride at −70° was treated with ozone, then stirred with water (20 ml.) and 30% hydrogen peroxide (6 ml.) for 2 hr. The methylene chloride extract was washed with water, dried, and evaporated, and the residue (2·26 g.) chromatographed on alumina (Grade I; 150 g.). The column was eluted by a gradient technique (ether flowing into light petroleum), affording cholestan-3β-yl acetate (XXII) (14 mg.), plates, m. p. 108—111° (from methanol), $[\alpha]_D$ +13° (*c* 1·05), identical (mixed m. p., rotation, infrared absorption, and chromatoplate behaviour) with an authentic sample, and cholestane-3β,6α-diol diacetate (XX) (1·5 g.), plates, m. p. 52° (from ethanol) (lit., 54—55°). Recrystallisation furnished needles (from methanol), m. p. 106—108°, $[\alpha]_D$ +39° (*c* 1·10) (lit., m. p. 107—108°, $[\alpha]_D$ +39·5°). Saponification with 5% potassium hydroxide in refluxing methanol gave cholestane-3β,6α-diol (XXI), needles, m. p. 211—216° (from methanol), $[\alpha]_D$ +37° (*c* 1·01), identical (mixed m. p. and infrared absorption) with an authentic sample.

We are indebted to Dr. D. K. Cox (Syntex Botanical Department) and Syntex, S.A., Mexico City, for the plant collections and preliminary extractions. Financial support was provided by the National Institutes of Health, U.S. Public Health Service, and a Fulbright travel award is gratefully acknowledged by one of us (R. D. H. M.).

Department of Chemistry, Stanford University, Stanford, California, U.S.A.

[Present addresses: (R. D. H. M.) Chemistry Department, The University, Glasgow, W.2. (R. V.) Syntex Laboratories, Palo Alto, California, U.S.A.]

[*Received, June 11th*, 1964.]