64 ml. of hydrogen were absorbed. Calcd. for 3 equivalents: 66 ml. After filtering from the catalyst, removal of the solvent left the free base which could not be crystallized. The picrate, m.p. 184–186°, and the hydrobromide, m.p. 154–155°, did not depress the melting points of the salts prepared above. The infrared spectra of the salts prepared by the two routes were identical.

3-Carbomethoxy-1-[β-(3-indolyl)ethyl]pyridinium bromide. To a solution of 10.6 g. of 3-(β-bromoethyl)indole in 100 ml. of absolute methanol and 75 ml. of anhydrous ether was added 7.1 g. of methyl nicotinate. After standing at room temperature for 3 days, the mixture was concentrated. On cooling it crystallized. The crystalline material was recrystallized from absolute methanol yielding 2.3 g. (21%) of stout yellow needles which darkened at 207° and decomposed at 218–220° (m.p. block) (uncorr.).

Anal. Calcd. for $C_{17}H_{17}BrN_2O_2$: C, 56.52; H, 4.74; N, 7.76. Found: C, 56.48; H, 4.81; N, 7.84.

3-Carboxy-1-[β-(3-indolyl)ethyl]pyridinium bromide. To a solution of 4.85 g. of 3-(β-bromoethyl)indole in 125 ml. of absolute ethanol was added 2.60 g. of nicotinic acid. After warming to effect solution, the mixture was allowed to stand 4 days at room temperature. The yellow solid which deposited after concentration at reduced pressure was recrystallized from 95% ethanol giving 3.16 g. (42%) of yellow needles, m.p. 269–270° (dec.). The ultraviolet spectrum of an ethanolic solution showed absorption maxima at 289 (log ε 3.82) and 270 (log ε 4.00) mμ and a shoulder at 280 mμ (log ε 3.92). Minima occurred at 241 (log ε 3.54) and 287 (log ε 3.80) mμ.

Anal. Calcd. for $C_{16}H_{15}BrN_2O_2$: C, 55.34; H, 4.35; N, 8.07. Found: C, 55.55; H, 4.30; N, 8.09.

5-Carboxy-1-[β-(3-indolyl)ethyl]-2-methylpyridinium bromide. This was prepared from 5.25 g. of 3-(β-bromoethyl)-indole and 3 g. of 6-methylnicotinic acid by the same procedure as was used for the preceding compound. The yield of pale yellow feathery needles from 95% ethanol was 4.84 g. (61%). The substance darkens at about 263° and decomposes at 274–275°. The ultraviolet spectrum of an ethanolic solution showed a maximum at 272 mμ (log ε 3.94), a shoulder at 289 mμ (log ε 3.70), and a minimum at 240–242 mμ (log ε 3.33).

Anal. Calcd. for $C_{17}H_{17}BrN_2O_2$: C, 56.52; H, 4.74; N, 7.76. Found: C, 56.56; H, 4.94; N, 7.69.

Dihydrostilbazole [4-(β-phenethyl)pyridine] (XVI). A solution of 1.83 g. of 4-stilbazole (XV) in 100 ml. of ethanol was shaken under hydrogen at room temperature and atmospheric pressure with 100 mg. of 5% palladium on charcoal catalyst. During 12 hr. 273 ml. of hydrogen was absorbed. Calcd. for 1 equivalent: 255 ml. The resultant dihydrostilbazole crystallized in quantitative yield from benzene-ligroin. It melted at 69–70°. Reported m.p. 69–71°[16] and 65°.[17] The picrate melted at 162–163°. Reported m.p., 162–163°.[16]

The same base was obtained when the reduction was done in ethanol over platinum oxide.

Octahydrostilbazole (4-phenethylpiperidine) (XVII). A solution of 1.83 g. of 4-stilbazole in 100 ml. of glacial acetic acid was shaken under hydrogen at room temperature and atmospheric pressure with 200 mg of 5% palladium on charcoal. After 6 hr., 4 equivalents of hydrogen (1,121 ml.) had been absorbed. Removal of the solvent from the filtered solution left an oil which was dissolved in water. After making the solution basic with sodium hydroxide solution, it was extracted with ether. The oil remaining after concentration of the dried ether solution was converted directly to the benzenesulfonamide, m.p. 130° (uncorr.), from ethanol. Reported m.p. 130°.[18]

4-(β-Cyclohexylethyl)piperidine (XVIII). A solution of 1.83 g. of 4-stilbazole in 100 ml. of ethanol containing an excess of anhydrous hydrogen chloride was shaken with 350 mg. of Adams' platinum oxide catalyst under hydrogen at room temperature and atmospheric pressure. During the course of 9 hr., 7 equivalents of hydrogen were absorbed. The free base, m.p. 33–35° showed no absorption in the ultraviolet.

For characterization, the base was converted to the 3,5-dinitrobenzamide by treatment with 3,5-dinitrobenzoyl chloride in pyridine for 1 hr. On dilution the amide crystallized. After recrystallization from ethanol, it melted at 154°.

Anal. Calcd. for $C_{20}H_{27}N_3O_5$: C, 61.68; H, 6.99; N, 10.79. Found: C, 61.63; H, 6.97; N, 10.95.

The benzenesulfonamide, prepared as in the preceeding case, melted at 120° after recrystallization from ethanol.

Anal. Calcd. for $C_{19}H_{29}NO_2S$: C, 68.03; H, 8.71; N, 4.18. Found: C, 67.76; H, 8.49; N, 4.14.

ANN ARBOR, MICH.

(16) B. Fels, *Ber.*, **37**, 2147 (1904).
(17) K. Friedländer, *Ber.*, **38**, 2837 (1905).
(18) S. M. McElvain, *J. Am. Chem. Soc.*, **52**, 1637 (1930).

[CONTRIBUTION FROM THE CHEMICAL LABORATORY, HARVARD UNIVERSITY]

Cholesterol and Companions. X.[1] The Diol Fraction

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A technique of inverse chromatography facilitated isolation from various cholesterol samples of 25-hydroxycholesterol, cerebrostenediol (24-OH isomer), 7-ketocholesterol, and an alkane-1,2-diol mixture. 25-Hydroxycholesterol appears not to be a product of animal origin but to result from air-oxidation of crystalline cholesterol.

Oxidation of cholesterol with a variety of reagents has afforded no less than twenty oxidation products in which the original 27 carbon atoms are retained. The structure, and indeed the origin, of one of these products still awaits elucidation. This substance, designated ketone 104,[3] is a somewhat hindered ketone of the formula $C_{27}H_{44}O_3$[3,4] which is formed in 0.5–0.9% yield on oxidation of either commercial cholesterol or

(1) Paper IX: L. F. Fieser and R. Stevenson, *J. Am. Chem. Soc.*, **76**, 1728 (1954).

(2) A friendship with the late Lyndon F. Small which began when we were fellow graduate students was later enlivened by his outstanding work on morphine and on general phenanthrene chemistry. Reviewing his work in books has been a pleasure, both because the experimentation is always meticulously done, and because I have known and admired this keen experimentalist. L.F.F.

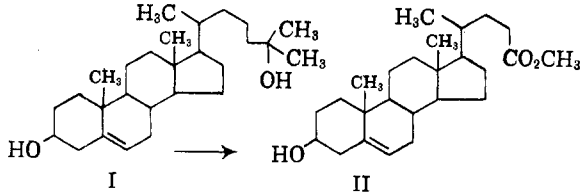
(3) L. F. Fieser, *J. Am. Chem. Soc.*, **75**, 4395 (1953).

(4) L. F. Fieser and B. K. Bhattacharyya, *J. Am. Chem. Soc.*, **75**, 4418 (1953).

cholesterol purified through the dibromide. The previous observations suggested that the substance contains two oxidic bridges and that it probably is a product of oxidation of an oxygen-rich companion substance rather than of cholesterol itself. The present investigation was undertaken in the hope that isolation of a precursor might cast light on the nature of the anomalous oxidation product.

Since an oxygen-rich precursor would be expected to be more hydrophilic than cholesterol and more strongly adsorbed on alumina than this sterol, we designed a technique for isolation based upon these predicted properties. An initial crystallization from ethanol eliminates, in the crystallizate, the bulk of the cholesterol (and the accompanying cholestanol and lathosterol[3]). The material recovered from the mother liquor is then crystallized from acetic acid (quickly, in order to repress acetylation), when an additional crop of cholesterol separates as the acetic acid complex. The residual glassy material recovered from the mother liquor is then processed by a scheme of inverse chromatography: a solution of the material in ether is stirred with two or three successive, relatively small, batches of alumina (Merck acid-washed), and these are collected, washed extensively with benzene, dried, and made into a chromatograph column. Elution with suitable benzene-ether mixtures (or with convenient ether–petroleum ether equivalents) removes residual cholesterol and readies the column for delivery of more strongly adsorbed companions, or for stripping with ether-methanol for recovery of the total diol fraction for chromatography after acetylation. Selective adsorption of this fraction onto alumina seems to effect separation as efficiently as the conventional, far lengthier process of chromatography and greatly facilitated the processing of batches of several hundred grams.

The more strongly adsorbed material is called the diol fraction because diols are indeed the most characteristic components. Thus the first substance isolated was identified as 25-hydroxycholesterol (I) by comparison with synthetic material[5,6]



I II

kindly supplied by Dr. A. I. Ryer. The substance was further characterized by hydrogenation to the stanediol, Oppenauer oxidation to the Δ^4-ene-3-one, and oxidation as the acetate dibromide and conversion to methyl 3β-acetoxy-Δ^5-cholenate,[7] II. Hydroxylation at the tertiary position C_{25} may well be the initial step in the chromic acid oxidation by which the sterol side chain is transformed into the bile acid side chain and acetone.[8]

On checking the history of the cholesterol from which the 25-hydroxy compound was first isolated, we were informed by Dr. David Klein of the Wilson Co. that the material was from a lot produced just four years prior to the isolation experiment. We therefore decided to investigate samples stored in ordinary containers for longer and for shorter periods. Dr. Konrad Bloch, then at Chicago, kindly supplied us with a batch of Wilson Co. cholesterol remaining from a research of F. C. Koch[9] and estimated to be 24 years old. The melting point had dropped off about 30°, and the material afforded a very large diol fraction. The presence of black gums and noncrystalline chromatograph fractions probably obscured the actual content of companions, but even so 25-hydroxycholesterol was isolated in 0.34% yield, as compared to a yield by the same technique of 0.14% from four-year old cholesterol. We then processed 450 g. of a batch of cholesterol two months after it has been produced in the Wilson Laboratories. The glassy residue prior to adsorption on alumina in this case amounted to only 0.9% of the starting material, as compared to 2.4% and 18% for the 4- and 24-year old samples. No 25-hydroxycholesterol was encountered and the only crystalline product found was 7-ketocholesterol, isolated in 0.005% yield. The ketone probably was present in the older samples and obscured isolation of the 3,25-diol.

The results obtained with cholesterol samples manufactured in a standard process and differing only in age indicate that cholesterol undergoes oxidation to the 3,25-diol on storage in the crystalline state in the presence of air.[10] A few exploratory autoxidation experiments were made with the idea that appreciable hydroxylation at any of the tertiary positions 17, 20, or 25 might open a route to production of hormones. After bubbling air through a refluxing solution of cholesterol in benzene for five days the melting point had dropped about 50°. The diol fraction in this case was segregated by extraction from petroleum ether with aqueous methanol. Early extracts afforded cholestane-3β,5α,6β-triol, and later extracts afforded, after acetylation, 7-ketocholesteryl acetate

(5) A. I. Ryer, W. H. Gebert, and N. M. Murrill, *J. Am. Chem. Soc.*, **72**, 4247 (1950).

(6) W. G. Dauben and H. L. Bradlow, *J. Am. Chem. Soc.*, **72**, 4248 (1950).

(7) E. S. Wallis and E. Fernholz, *J. Am. Chem. Soc.*, **57**, 1504 (1935).

(8) A. Windaus and K. Neukirchen, *Ber.*, **52**, 1915 (1919). See comments by J. R. Billeter and K. Miescher, *Helv. Chim. Acta*, **30**, 1409 (1947); L. F. Fieser, *J. Am. Chem. Soc.*, **70**, 3239 (1948).

(9) F. C. Koch, E. M. Koch, and I. K. Ragins, *J. Biol. Chem.*, **85**, 141 (1929).

(10) Compare W. G. Dauben and P. H. Payot, *J. Am. Chem. Soc.*, **78**, 5657 (1956).

in 1.6% yield; 25-hydroxycholesterol was not encountered. Treated in the same way, cholestanol yielded cholestanone as the only identified oxidation product.

Dr. R. T. Rapala of the Armour Laboratories kindly supplied us with material from the mother liquor from a final crystallization of cholesterol from beef spinal cord. The processing of 600 g. of this material by inverse chromatography afforded 60 mg. of material identical with cerebrostenediol, a 24-hydroxycholesterol isolated by Ercoli and de Ruggieri[11] from horse brain cholesterol. Later fractions, when acetylated and rechromatographed, afforded 24 mg. of 7-ketocholesteryl acetate. In confirmation of the Italian work, we isolated cerebrostenediol from a batch of horse brain cholesterol provided by Dr. Ercoli.

Cerebrostenediol was also isolated from a concentrate from wool fat (100 g.) kindly supplied by Dr. Lester I. Conrad of the American Cholesterol Co. The cholesterol derivative was a minor component (10 mg.) of the diol fraction in comparison to another diol (2.2 g.) eluted in later fractions and characterized by being readily soluble in both petroleum ether and in methanol. In a preliminary characterization we had established that the substance is an alkane-1,2-diol by glycol cleavage to formaldehyde and a higher aldehyde, when a paper by Horn and Hougen[12] appeared reporting an extensive investigation of the nonsaponifiable fraction of wool wax. By using amounts of material large enough for fractional distillation of the acetates, these authors isolated five alkane-1,2-diols. Our material undoubtedly is a mixture, but comparison of the constants suggests that a major component is 20-methylheneicosane-1,2-diol, $(CH_3)_2CH(CH_2)_{17}CHOHCH_2OH$.

It is evident that, contrary to our original expectations, this investigation failed to disclose an oxygen-rich precursor of ketone 104. The results indeed confirm the conclusion reached in a subsequent analytical study (to be reported) that ketone 104 is a product of oxidation of cholesterol.

EXPERIMENTAL

Isolation of 25-hydroxycholesterol (L.F.F.). (a) From 4-yr. old cholesterol. Wilson Co. cholesterol (from spinal cord and brain of cattle) lot No. 74708, produced in March, 1949, was processed just four years later. The dibromide from 150 g. of sterol was debrominated in ether-acetic acid[13] and a first crop of nearly pure cholesterol was let crystallize and combined with successive crops (melting in the range 146–150°) obtained by concentration and recrystallization as required. The tail fraction from the mother liquor was a a brown glass (2.4 g.), the next-to-tail fraction a low-melting solid (2.3 g.). Chromatography of the tail fraction afforded 50 mg. of cholesteryl acetate, 200 mg. of crude cholesterol then bromine-containing material (colorless glass; crystals from methanol, m.p. 125–140°; stable to zinc dust in ether–acetic acid[13]), and then, on elution with 1:4 benzene-ether and with ether, a total of 225 mg. of 25-hydroxycholesterol. An eluate fraction containing this diol uncontaminated with companions is recognizable from the fact that on evaporation the diol is left as a white scum on the walls of the flask. Crystallization from acetone gave a microcrystalline powder, m.p. 172–173°, α_D −41.0° Chf (c 0.85),λ^{Chf} 2.8, 6.08 μ.

Anal. Calcd. for $C_{27}H_{46}O_2$: C, 80.54: H, 11.52. Found: C, 80.25; H, 11.29.

Earlier, glassy fractions containing brominated material on digestion with petroleum ether afforded more solid diol, which was readily purified by dissolving it in ether, adding 2–3 volumes of petroleum ether, and concentrating to the point of crystallization. The next-to-tail fraction afforded only 5 mg. of the 3,25-diol, and the total cholesterol removed in the fractional crystallization when reprocessed yielded 58 mg. more of the diol; total yield 288 mg. (0.19%).

Once the diol was identified, purification of the starting material through the dibromide was recognized as not only unnecessary but disadvantageous, because of the formation of troublesome bromine-containing substances. Thus a 600-g. batch of the same cholesterol when processed as described in (b) (crystallization from acetic acid; adsorption of the mother liquor from ether onto two 75-g. portions of alumina) easily afforded 840 mg. (0.14%) of the diol.

(b) *From 24-yr. old cholesterol.* The Wilson Co. cholesterol remaining from a research of F. C. Koch was yellowish, had a strong urine odor, and melted at 116–128°. Crystallization of 199 g. of material from 1 l. of 95% ethanol gave 107 g. of crystals, and the material recovered from the mother liquor on crystallization from 300 ml. of acetic acid gave 9.6 g. of sterol, m.p. 138–140°. An ethereal extract of the diluted acetic acid mother liquor was almost black, but when shaken with alkali the upper layer cleared to a light tan and an oily brown sodium salt separated at the interface above a dirty, milky suspension. The aqueous layer and the oil were drawn off and back extracted with ether, and the dried ethereal solution gave 37 g. of a light yellow, glassy residue. A solution of this material in 200 ml. of ether was stirred for 10 min. with 75 g. of alumina and the solid collected, washed on the Büchner with 400 ml. of benzene, and dried at 80° for 10 min. (A, 78.3 g.). The ethereal mother liquor was stirred with two more 75-g. portions of alumina and the solids (B, C) washed and dried as before. A column was then prepared with a lower layer of 75 g. of fresh alumina overlaid with A, B, and C. Elution with 1:1 ether-methanol removed a series of early fractions to give a diol concentrate amounting to 9.7 g. By chromatography on 300 g. of alumina with solvents in the usual range from benzene to methanol, the material was spread over 100 fractions, but only two homogeneous crystalline products were encountered. The first was cholesterol (from methanol: 0.57 g., m.p. 146–148°). Later fractions eluted by petroleum ether–ether (2:3 and 3:7) afforded 25-hydroxycholesterol contaminated with a petroleum ether–soluble companion. Crystallization from ether–petroleum ether gave a total of 680 mg. (0.34%) of 25-hydroxycholesterol, m.p. 170–171°.

(c) *Examination of freshly extracted cholesterol.* Wilson Co. cholesterol lot No. 86529 was processed two months after production as follows: 450 g. was crystallized from 2.4 l. of 95% ethanol (384 g. of crystals) and the residue left on evaporation of the mother liquor (21 g.) crystallized from acetic acid (170 ml.; 16.3 g. of crystals). The fraction recovered from the mother liquor on crystallization from methanol afforded a few crops of crystals melting in the cholesterol range and afforded 4.0 g. (0.9%) of a residual glass (compare 2.4 and 18.5%, from the 4- and 24-yr. old samples). Selective adsorption from ether onto two 75-g. portions of alumina and elution with methanol gave 1.7

(11) A. Ercoli and P. de Ruggieri, *J. Am. Chem. Soc.*, **75**, 3284 (1953); A. Ercoli, S. di Frisco, and P. de Ruggieri, *Gazz. chim. ital.*, **83**, 78 (1953). For configuration, see W. Klyne and W. M. Stokes, *J. Chem. Soc.*, 1987 (1954).

(12) D. H. S. Horn and F. W. Hougen, *J. Chem. Soc.*, 3533 (1953).

(13) L. F. Fieser, *J. Am. Chem. Soc.*, **75**, 5421 (1953).

g. of a "diol" fraction, which was first chromatographed as acetate. The only identifiable material separated from early petroleum ether eluates as an oil (0.57 g.), which on saponification and crystallization from acetic acid gave crude cholesterol, m.p. 140–142° (0.21 g.). On chromatography of the saponified "diol" fraction, benzene eluted a little cholesterol, and 1:1 benzene-ether afforded, in late fractions, 25 mg. of a solid that crystallized from ether–petroleum ether in clusters of white needles, m.p. 169–170°; very faint color with tetranitromethane. The substance depressed the m.p. of both 25-hydroxycholesterol and of cerebrostenediol but gave no depression in m.p. when mixed with an authentic sample of *7-ketocholesterol*, m.p. 171–172°, α_D −107° Chf (c 24.8), −97° Di (c 23.3).[14]

Characterization of 25-hydroxycholesterol (*W.-Y. H.*). Further crystallization of the diol isolated from commercial cholesterol raised the melting point only to 175–177°, slightly lower than reported values: 181.5–182.5°, α_D −39.3° Chf;[5] 177–179°, α_D −38.6° Chf.[6] Our material may have contained a little cerebrostenediol (see below); it did not depress the melting point of a synthetic sample kindly supplied by Dr. Ryer and the infrared spectra were identical except that our material gave a slightly more intense band at 6.08 μ. The *monoacetate* of the isolated diol when crystallized from aqueous acetone melted at 141–142°, α_D −40.2° Chf (c 1.54), λ^{Chf} 2.8, 2.9, 5.82, 8.0 μ; undepressed on admixture with synthetic material from Dr. Ryer (m.p. 142–142.8°, α_D −40.4° Chf).

Anal. Calcd. for $C_{29}H_{48}O_3$: C, 78.32; H, 10.88. Found: C, 77.92; H, 10.96.

Cholestane-3β,25-diol (*W.-Y. H.*). Hydrogenation of 25-hydroxycholesterol (isolated) in ethanol in the presence of Adams catalyst and a trace of perchloric acid, and crystallization from methanol, gave a product saturated to tetranitromethane, m.p. 194–195°, α_D +24° Chf (c 0.83).

Anal. Calcd. for $C_{27}H_{48}O_2$: C, 80.14; H, 11.96. Found: C, 80.16; H, 11.89.

The *monoacetate*, crystallized from aqueous acetone, melted at 124–125°, α_D +8° Chf (c 0.90).

Anal. Calcd. for $C_{29}H_{50}O_3$: C, 77.97; H, 11.25. Found: C, 77.48; H, 11.20.

Oxidation of 25-hydroxycholesteryl acetate dibromide (*W.-Y. H.*). A solution of 778 mg. of 25-hydroxycholesteryl acetate in 5 ml. of ether was treated with 295 mg. of bromine in 2 ml. of acetic acid and the resulting semisolid mixture let stand overnight in a dry ice bath. The ether was then removed at reduced pressure (25°) and the residue diluted with 25 ml. of acetic acid, treated with 2 g. of chromic anhydride, and the suspension was stirred mechanically for 1.5 hr. and then let stand for 30 min. The oxidized material was then heated with zinc dust and acetic acid for 30 min. on the steam bath to effect debromination, and the product was separated into neutral and acidic fractions by extraction from ether with 4*N* aqueous alkali. The neutral fraction was crystallized from methanol (m.p. 125–135°) and then chromatographed. Early fractions afforded 150 mg. of crystals, m.p. 120–130°, and late fractions gave 257 mg. of starting material, m.p. 140–142°. The acidic fraction, esterified with diazomethane and chromatographed, gave as the main product *methyl 3β-acetoxy-Δ⁵-cholenate*, m.p. (from methanol) 154–156°, α_D −50° Chf (c 0.70). The substance did not depress the melting point of a comparison sample, m.p. 155–157°, −43° Chf (c 1.81), prepared from a sample of 3β-hydroxy-Δ⁵-cholenic acid, m.p. 240–241°, α_D −32.4° Di, supplied by the Research Division of Schering Corp. Both samples showed distinctly stronger levorotation than previously reported[7]: m.p. 156°, α_D −18.7° Chf.

25-Hydroxy-Δ⁴-cholestene-3-one (*B. K. B.*). A mixture of 200 mg. of 25-hydroxycholesterol, 1 ml. of cyclohexanone, 600 mg. of redistilled aluminum isopropoxide, and 7 ml. of toluene was refluxed for 3 hr. and the reaction product isolated in the usual way and chromatographed (m.p. 147–149°). Two crystallizations from ether–petroleum ether and one from petroleum ether–acetone raised the m.p. to 149–150°, α_D +96° Chf (c 0.6), λ^{Chf} 241.5 mμ (16,440); yield 80 mg.

Anal. Calcd. for $C_{27}H_{44}O_2$: C, 80.94; H, 11.07. Found: C, 81.29; H, 11.21.

Cerebrostenediol (a). From beef spinal cord (*L. F. F.*). The starting material from the Armour Laboratories was material from the ethanol mother liquor of a first crystallization that produced cholesterol of high purity. On digestion of 600 g. of the material with 2.2 l. of 95% ethanol, a little dark solid remained undissolved and the crystals that separated on cooling were dirty, m.p. 141–147°. Evaporation of the mother liquor left a glassy residue that only partially dissolved on digestion with ether. Suction filtration (slow) left a horn-like residue of ether-insoluble material (25.5 g.), and evaporation of the filtrate gave a glassy residue (32 g., contained solvent). When this was rubbed with acetic acid at room temperature it partly dissolved and gave a paste of cholesterol–acetic acid, which was removed (dry weight 9.8 g.). Extraction of the diluted mother liquor gave 12 g. of residue; this was dissolved in ether and the solution stirred with 250 g. of alumina, which was then collected, washed with benzene, dried, and made into a column. Petroleum ether–ether (1:1) eluted mobile yellow oils (fractions 1–4), then solid sterol (5–7), and then a series of fractions (11–17) of material which initially was a glass but which on standing afforded crystalline granules embedded in the glass. On digestion with petroleum ether the granules remained undissolved and could be collected. The total solid (60 mg., m.p. 168–170°) dissolved slowly in acetone, and after the solution had been concentrated to a small volume the product crystallized in tufts of needles, m.p. 173–174°. The substance depressed the melting point of 25-hydroxycholesterol and of 7-ketocholesterol, but a mixture with a sample of cerebrostenediol[11] supplied by Dr. A. Ercoli melted at 173.5–175°.

Anal. Calcd. for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52. Found: C, 80.83; H, 11.57.

Fractions 21–30 (1:4 petroleum ether–ether) afforded glassy material very soluble in petroleum ether; when rechromatographed as the acetate this afforded in terminal fractions (4:1 petroleum ether–ether) a solid that on crystallization from methanol gave 24 mg. of rosettes of blades, m.p. 156–158°, identified as *7-ketocholesteryl acetate* by mixed melting point determination (158–159°).

Further elution with ether removed no more material, and then 1:1 ether-methanol (36–37) eluted a total of 0.8 g. of a substance similar in properties to the lipid diol described below.

(b) *From horse brain.* A 17.4-g. sample of total sterol (m.p. 135–140°) from horse brain kindly supplied by Dr. A. Ercoli was crystallized from methanol (12.6 g., m.p. 147–148°) and the material recovered from the mother liquor was crystallized from acetic acid (1 g., m.p. 145–147°). The glassy residue (1.9 g.) recovered on ether extraction of the acetic acid mother liquor on chromatography afforded, after removal of early sterol fractions (m.p. 141–145°), a series of solid fractions eluted by 1:1 benzene-ether that on crystallization from acetone slowly gave a round cluster of well-formed needles of cerebrostenediol (8 mg.), m.p. 173.5–175°, mixed m.p. 174–175° (also identified by comparison of the infrared spectra).

Cerebrostenediol dibenzoate (*W.-Y. H.*), prepared by benzoylation in pyridine at 25° overnight, was crystallized from ether-acetone and had the constants: m.p. 182–183°, α_D −19° Chf (c 1.20), λ^{Chf} 5.85, 6.2, 6.86, 7.8 μ.

Anal. Calcd. for $C_{41}H_{54}O_4$: C, 80.61; H, 8.91. Found: C, 81.06; H, 8.95.

Cerebrostenediol and a lipid diol from wool fat (*L. F. F.*). The starting material (American Cholesterol Co.) was a commercial concentrate designated Amerchol concentrate

(14) S. Bergstrom and O. Wintersteiner, *J. Biol. Chem.*, **141**, 597 (1941), report m.p. 170–172, α_D −104 Chf.

C-53, a fraction intermediate between cholesterol and lanolin and including material from the ethanolic mother liquor from crystallization of cholesterol. One hundred g. of the light tan, soft resin was dissolved in 500 ml. of methanol and the solution was cooled and scratched until the oil that initially separated had solidified. The solid was removed by filtration, the mother liquor evaporated, and a solution of the residue (24 g.) in ether stirred with two successive 100-g. portions of alumina, each of which was washed liberally with benzene and briefly dried (108, 103.5 g.). A column was then constructed with a lower layer of fresh alumina (50 g.), followed by the second and then the first adsorbate. Elution with 1:1 petroleum ether–ether removed a series of solid fractions (6–8) melting in the range 120–135°. A 1:4 mixture first eluted only mixtures (9–11) and then (12–17), material identified as cerebrostenediol; this appeared as dots of solid that could be triturated with petroleum ether and collected (10 mg.). The substance crystallized from acetone in small blades, m.p. 170–171.5°, mixed m.p. 170–172°.

Fractions 22–32 (1:4 petroleum ether–ether) and 33–34 (ether) afforded small amounts of glassy material which solidified on standing and which when dissolved in a small amount of petroleum ether separated on cooling as a white powder (0.8 g.). More of the same material, designated *lipid diol*, was obtained by terminal elution with ether–methanol (4:1); a solution of the brown eluate in hot petroleum ether was filtered from small amounts of tar and a white solid, evaporated (2.9 g.) and crystallized from acetone, to give 1.45 g. of slightly yellowish solid, m.p. 68–69°. The colorless solid (0.8 g.) from fractions 22–34 on successive crystallizations from acetone melted at 68–70°, 72–74°, 76–77°, 78–79°, 78–79°, α_D^{24} +1.5° Chf (c 2.02), λ^{Chf} 2.8, 2.9, 8.9, 9.4 μ (see below for characterization).

Preliminary characterization of the lipid diol (W.-Y. H.). The material from wool fat, m.p. 78–79° (after several crystallizations; a larger sample, rechromatographed and recrystallized, m.p. 79–80°) appeared to be a mixture of average composition corresponding to the formula $C_{22}H_{46}O_2$. Horn and Horgen[12] report for 20-methylheneicosane-1,2-diol the m.p. 84–84.4°.

Anal. Calcd. for $C_{22}H_{46}O_2$: C, 77.12; H, 13.53. Found: C, 77.21, 77.38; H, 13.44; 13.43.

The derivatives briefly investigated are seen from the analyses to differ somewhat from the starting material in homolog composition; the theoretical values cited are those corresponding to the C_{22}-formula for the diol. A mono-*p*-nitrobenzoate (excess reagent in pyridine at 25°) separated from methanol as a granular solid, m.p. 75–76°.

Anal. Calcd. for $C_{29}H_{49}O_5N$: C, 70.84; H, 10.05; N, 2.85. Found: C, 69.92; H, 9.57; N, 3.12.

Periodic acid titrations indicated the substance to be a *vic*-diol of molecular weight 380, 374. Oxidation of 100 mg. of material in ethanol (25 ml.)-water (5 ml.) gave, after chromatography, material which appeared to be a mixture of an aldehyde (medium band at 5.78 μ) and its diethyl acetal (strong band at 8.9 μ). Conversion to the aldehyde 2,5-dinitrophenylhydrazone and chromatography gave a light yellow microcrystalline powder, m.p. 81–82°, λ^{EtOH} (1% Chf) 358 mμ (20,100).

Anal. Calcd. for $C_{27}H_{46}O_4N_4$: C, 66.09; H, 9.45; N, 11.47. Found: C, 65.45; H, 9.17; N, 11.50.

In another experiment the product, after crystallization from ether-ethanol, appeared to consist exclusively of the acetal: m.p. 63–64°, λ^{Chf} 8.9 μ (no carbonyl band). Drying for analysis effected conversion to the free aldehyde.

Anal. Calcd. for $C_{21}H_{42}O$: C, 81.21; H, 13.63. Found: C, 80.95; H, 13.69.

Oxidation of the diol (100 mg.) with lead tetraacetate gave a volatile component isolated and identified as formaldehyde 2,4-dinitrophenylhydrazone. Chromatographed and crystallized from aqueous methanol, this formed orange needles, m.p. 164–165°, λ^{EtOH} (1% Chf) 348 mμ (19,400), identical with an authentic sample in UV and IR spectra and in mixed melting point. The nonvolatile component was extracted with ether and the solution washed with potassium iodide–sodium acetate and then with sodium thiosulfate solution. An acid was obtained as a granular solid, m.p. 60° (characteristic carboxyl IR absorption; no ether band).

Anal. Calcd. for $C_{21}H_{42}O_2$: C, 77.23; H, 12.96. Found: C, 77.73; H, 13.11.

Treatment of the above aldehyde-acetal mixture with sulfuric acid in aqueous dioxane followed by oxidation with dichromate in acetic acid gave a similar acidic product, m.p. 58–59°; direct dichromate oxidation of the diol gave an acid, m.p. 54–56°. The *p*-bromophenacyl ester melted at 80–82°. The amide, obtained from acetone as a granular solid, melted at 97° (IR spectrum almost identical with that of stearamide).

Anal. Calcd. for $C_{21}H_{43}ON$: C, 77.48; H, 13.32. Found: C, 77.12; H, 13.17.

The sample did not depress the melting point of either of the amides:[15] $(CH_3)_2CH(CH_2)_{19}CONH_2$, m.p. 100°; acid, m.p. 62°, $(CH_2)_2CH(CH_2)_{17}CONH_2$ (m.p. 94°; acid, m.p. 55.6°).

Autoxidation experiments (B. K. B.). A solution of 200 g. of cholesterol (purified through the dibromide) in 400 ml. of benzene was oxidized by bubbling air through the boiling solution for 120 hr. Melting points observed during this period were as follows: 24 hr., 133–141°; 48 hr., 125–133°; 72 hr., 110–125°; 96 hr., 100–123°. After evaporation of the benzene, 70 g. of the residue was dissolved in 1 l. of petroleum ether and the solution was equilibrated with two 350-ml. portions of 50% methanol-water (by volume). The material recovered from the two extracts (5 g.) on chromatography afforded cholesterol and cholestane-3β,5α,6β-triol, m.p. 235°; no depression in mixed melting point. Further extraction of the petroleum ether solution with two 350-ml. portions of 75% aqueous methanol removed 2 g. of material of which the only component identified was cholesterol. Three extractions with 90% methanol gave 10.8 g. of material which was chromatographed. After removal of cholesterol (2 g.), the column was stripped with methanol and the material eluted was acetylated. Direct crystallization afforded 500 mg. of 7-ketocholesteryl acetate, m.p. 158–160°, α_D +97.5° Chf (c 1.0); no m.p. depression. Chromatography afforded a further 600 mg. of the same product, m.p. 158–160°.

Cholestanol (48 g.) was air-oxidized in the same way and a solution of the residue remaining on evaporation of the solvent dissolved in petroleum ether (500 ml.) and sufficient ether to produce a homogeneous solution. This was extracted three times with 95% methanol and the combined extracts evaporated and diluted with water. Extraction with ether gave 7 g. of yellow solid, which was chromatographed. The only product identified, other than cholestanol, was eluted by 10% ether in benzene and melted at 126–128°. Two crystallizations from methanol gave 400 mg. of cholestanone, m.p. 129.2–129.8°, α_D +43.8° Chf (c 1.03); no depression in melting point.

Anal. Calcd. for $C_{27}H_{46}O$: C, 83.87; H, 11.99. Found: C, 84.22; H, 11.99.

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