**Tetraacetate of *exo*-Oxabicyclo[2.2.1]hept-5-ene-2,3-diol (8).**—Oxabicyclo[2.2.1]-hept-5-ene-2,3-diol carbonate (**1b**) (600 mg, 3.8 mmol) was dissolved in 15 ml of the ethyl acetate and 0.77 ml of dry pyridine; 1 g of osmium tetroxide dissolved in 2 ml of ethyl acetate was added. This mixture was sealed and left for 24 hr at room temperature. The solution was filtered, and a dry, black precipitate weighing 1.4 g was collected; 60 g of sodium sulfite ($Na_2SO_3$), previously dissolved in 300 ml of water, and 300 ml of ethyl alcohol were added to the black precipitate. The mixture was boiled for 7 hr, producing a new, black precipitate, $Na_4[OS(SO_3)_3]\cdot 6H_2O$. This solution was concentrated, and its volume was reduced to 50 ml. Sodium hydroxide solution (5 ml), 5% (w/v) was added. This mixture was maintained at room temperature for 1 hr. Once again it was neutralized with acid, then concentrated to dryness, and dried at 60–80° for 4 hr.

**Acetylation of the Anhydro *cis*-Inositol 8.**—Acetic anhydride (50 ml) was added to the dry residue previously obtained. This mixture was warmed for 24 hr in an electric bath. Then the aceetic anhydride was removed *in vacuo*, and the residue resembled needles, mp 188–190°, yield 40 mg (6.6%), ir max 1750, 1250, 840, 820 $cm^{-1}$.

***epi*-Inositol (9,11).**—A solution (5 ml) consisting of 80% (v/v) acetic acid, 20% (v/v) water, and 1% (v/v) sulfuric acid was added to 10 mg (0.061 mmol) of hydroxylated material **8**. The mixture was warmed over a water bath for 14 hr, then tested by paper chromatography.[19,20] One spot was revealed which corresponded to *epi*-inositol, $R_f$ 0.20.[20] Also the Scherer reaction[18] was positive. The dry residue was acetylated by adding 5 ml of acetic anhydride and a few drops of concentrated sulfuric acid. This mixture was left at 40° for 24 hr and poured into cold water, and then yielded crystals of **11**. Further purification from toluene yielded crystals, mp 186–190°.[7]

**Registry No.**—**1a**, 32384-16-0; **1b**, 32384-17-1; **2** tetraacetate, 36912-06-8; **4**, 36912-07-9; **5** (R = Ac), 36912-08-0; **6**, 643-10-7; **7**, 87-89-8; **8** tetraacetate, 36912-10-4; **10**, 36912-11-5; **11**, 20108-71-8; monochloroethylene carbonate, 3967-54-2; vinylene carbonate, 872-36-6.

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# Sterol Metabolism. XX. Cholesterol 7β-Hydroperoxide[1]

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3β-Hydroxycholest-5-ene 7β-hydroperoxide was isolated along with 6β-hydroperoxycholest-4-en-3-one from autoxidation of crystalline cholesterol. Epimerization of 3β-hydroxycholest-5-ene 7α-hydroperoxide also provided the 7β-hydroperoxide in low conversion yield. The structure of 3β-hydroxycholest-5-ene 7β-hydroperoxide was established by sodium borohydride reduction to cholest-5-ene-3β,7β-diol and by spectral means. The 7β-hydroperoxide decomposed thermally to cholest-5-ene-3β,7β-diol and 3β-hydroxycholest-5-en-7-one, thereby accounting for the ubiquitous presence of cholest-5-ene-3β,7β-diol in cholesterol autoxidation products. An alternate pathway of derivation of cholest-5-ene-3β,7β-diol *via* epimerization of cholest-5-ene-3β,7α-diol was also demonstrated. Autoxidation of cholesterol 3β-acetate afforded the acetate derivatives of the cholesterol 7β-, 20α-, and 25-hydroperoxides.

The autoxidation of cholesterol (**1a**) under a variety of conditions leads to formation of the well-known epimeric cholest-5-ene-3β,7-diols (**3b**, **4b**), 3β-hydroxycholest-5-en-7-one (**5a**), cholesta-3,5-dien-7-one, cholest-5-ene-3β,25-diol, and 5α-cholestane-3β,5,6β-triol. Chromatographic evidence[2] and isolation work[3] have established that autoxidation proceeds *via* initial hydroperoxide fomation followed by thermal decomposition to give the better known stable autoxidation products mentioned. The numerous stable autoxidation products of cholesterol oxidized in the side-chain are satisfactorily accounted in this manner, arising *via* initial formation of the cholesterol 20α-, 24-, 25-, and 26-hydroperoxides.[3] The well-known B-ring autoxidation products **3b**, **5a**, and cholesta-3,5-dien-7-one are likewise properly accounted for *via* reduction and dehydration processes acting on the Δ⁵-7α-hydroperoxide **3a**, formed by stereospecific rearrangement[4] of the Δ⁶-5α-hydroperoxide **2a** formed by initial attack of oxygen on cholesterol.[5]

Such direct pathways do not account for the ubiquitous presence in autoxidized cholesterol of the 3β,7β-diol **4b** in substantial amounts along with the 3β,7α-diol **3b**. As established in the present study, the 7β-alcohol **4b** may be derived by two pathways, one proceeding *via* the previously unrecognized epimerization of the 7α-alcohol **3b**, the other *via* similar epimerization of the 7α-hydroperoxide **3a** to give the previously undescribed 7β-hydroperoxide **4a** whose thermal decomposition provides the 7β-alcohol **4b** and the 7-ketone **5a**.

In continued examination of cholesterol autoxidation products[3] we isolated for the first time from crystalline cholesterol samples heated in air 6β-hydroperoxy-

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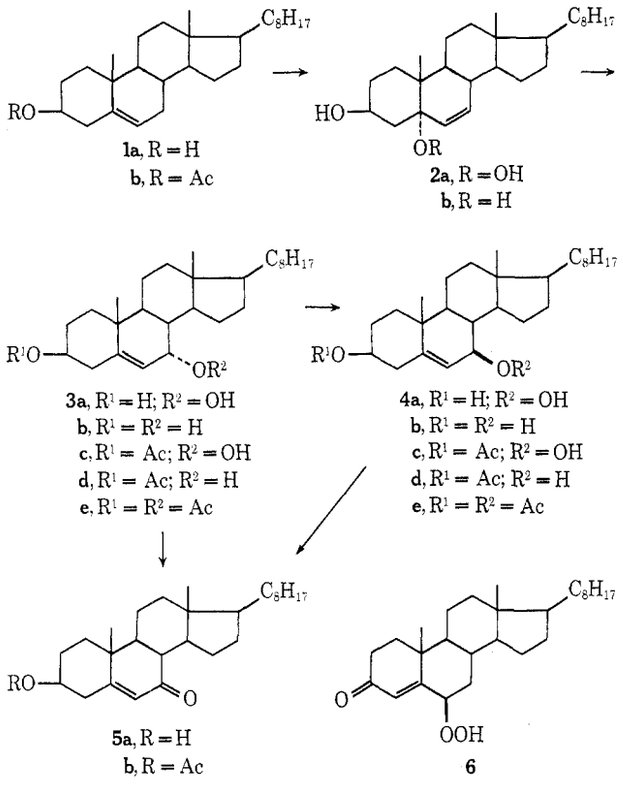
(2) (a) L. L. Smith and F. L. Hill, *J. Chromatogr.*, **66**, 101 (1972). Chromatographic evidence for cholesterol hydroperoxide derivatives in various autoxidation conditions has previously been provided: (b) F. Neuwald and K. E. Fetting, *Pharm. Ztg.*, **108**, 1490 (1963); (c) C. Horvath, *J. Chromatogr.*, **22**, 52 (1966); (d) L. L. Smith, W. S. Matthews, J. C. Price, R. C. Bachmann, and B. Reynolds, *ibid.*, **27**, 187 (1967).

(3) (a) J. E. van Lier and L. L. Smith, *J. Org. Chem.*, **35**, 2627 (1970); (b) J. E. van Lier and L. L. Smith, *Steroids*, **15**, 485 (1970); (c) J. E. van Lier and L. L. Smith, *J. Org. Chem.*, **36**, 1007 (1971); (d) J. E. van Lier and G. Kan, *ibid.*, **37**, 145 (1972).

(4) (a) G. O. Schenck, O.-A. Neumüller, and W. Eisfeld, *Angew. Chem.*, **70**, 595 (1958); *Justus Liebigs Ann. Chem.*, **618**, 202 (1958); (b) B. Lythgoe and S. Trippett, *J. Chem. Soc.*, 471 (1959); (c) A. Nickon and J. F. Bagli, *J. Amer. Chem. Soc.*, **83**, 1498 (1961). A specific research for cholesterol 7β-hydroperoxide in reactions leading to **3a** failed to detect **4a**.[4c]

(5) (a) G. O. Schenck, K. Gollnick, and O.-A. Neumüller, *Justus Liebigs Ann. Chem.*, **603**, 46 (1957); (b) G. O. Schenck and O.-A. Neumüller, *ibid.*, **618**, 194 (1958).




cholest-4-en-3-one (**6**)[5a,6,7] and the new cholesterol 7β-hydroperoxide **4a**. The 7β-hydroperoxide was also isolated from cholesterol autoxidation products enriched in **2a** and **3a** which had been stored for several months. Notably autoxidation of crystalline cholesterol 3β-acetate (**1b**) gave a major isolable peroxidic product 3β-acetoxy 7β-hydroperoxide (**4c**) together with smaller amounts of the 3β-acetates of cholesterol 20α- and 25-hydroperoxides and the secondary products **3d**, **4d**, and **5b**.

The structure of the 7β-hydroperoxide **4a** was established by its sodium borohydride reduction to the 3β,7β-diol **4b** obtained as the sole product. Similar borohydride reduction of the epimeric 7α-hydroperoxide **3a** yielded the corresponding 3β,7α-diol **3b** as the sole product. Molecular rotation of the dextrorotatory 7β-hydroperoxide **4a**[8] and proton spectra further support the assigned 7β-hydroperoxide structure of **4a**. The 7α-proton signal of **4a** appears as a doublet of doublets, coupled with the C-6 vinyl proton ($J_{6,7}$ = 1.5 Hz) and the axial 8β proton ($J_{7,8}$ = 8 Hz), deshielded by 0.3 ppm from its chemical shift in spectra of the 7β-alcohol **4b**.[10] Identical B-ring conformations for **4a** and **4b** and quasiequatorial character for the 7β-hydroperoxide and 7β-hydroxyl groups of **4a** and **4b**, respectively, are suggested by these coupling patterns and by the C-19 angular methyl proton signals at 1.04 ppm for **4a** and **4b** in distinction to a shielded (0.97 ppm) position in the spectrum of the 7α-alcohol **3b**.

Attempted acetylation of **4a** with acetic anhydride and pyridine afforded the 3β-acetoxy 7-ketone **5b**, which reaction finds precedence in the dehydration of cholesterol 24-hydroperoxide by acetic anhydride and pyridine to give 3β-acetoxycholest-5-en-24-one.[3c] Thermal decomposition of **4a** gave the 7-ketone **5a** as a major product together with the 7β-alcohol **4b**.

It is evident that the 7β-hydroperoxide **4a** in some autoxidized cholesterol preparations is formed from the 7α-hydroperoxide **3a**, which is formed in turn from the 5α-hydroperoxide **2a** initially formed from cholesterol. The indicated derivation of **4a** from **2a** *via* **3a** was established by direct chromatographic observation of solutions of **2a** and **3a**. In these experiments the facile stereospecific rearrangement of **2a** to the 7α-hydroperoxide **3a** previously reported[4] was confirmed, and the epimerization of **3a** to the 7β-hydroperoxide **4a** was established.

The 7β-hydroperoxide **4a** was not epimerized under the conditions which epimerized **3a**. Epimerization of the quasiaxial **3a** to the quasiequatorial **4a** accordingly appears to be under thermodynamic control, the 1,3-diaxial interactions associated with a B-ring chair conformation for **3a** being dominant over 1,3 interactions with the syn-parallel C-15 methylene group of the product quasiequatorial 7β-hydroperoxide bond of **4a**. Under conditions epimerizing **3a**, the 7β-hydroperoxide **4a** decomposed to the 3β,7β-diol **4b** and to the 7-ketone **5a**. In general the 7β-hydroperoxide **4a** appears to be more labile toward thermal decomposition than its 7α epimer **3a**, and isolation of pure samples of **3a** free of its decomposition products **3b** and **5a** is considerably easier than is isolation of **4a** free from **4b** and **5a**.

A rearrangement and epimerization sequence was similarly observed which linked the 5α-alcohol **2b** through the 7α-alcohol **3b** to the 3β,7β-diol **4b**. Although epimerization of the allylic alcohol **3b** is unexceptional,[11] the reaction has not been previously recorded. However, the cholest-5-ene-3β,7-diol diacetates **3e** and **4e** are interconverted in hot acetic acid[12a] but not in refluxing benzene solutions of lead diacetate.[12b]

To our knowledge the conversion of **3a** to **4a** constitutes the first recorded instance of secondary hydroperoxide epimerization. However, cases of allylic hydroperoxide epimerization may have been involved in other prior studies without being recognized as such. Whereas photosensitized oxidation of lanost-8-en-3β-ol acetate afforded the 7α-hydroperoxide,[13] extended autoxidation in ethyl acetate at 50° gave the epimeric 7β-hydroperoxide.[14] Although no evidence for B-ring conformation in these derivatives is available, a B-ring modified chair conformation is reasonable, giving quasi-

(6) (a) L. F. Fieser, T. W. Greene, F. Bischoff, G. Lopez, and J. J. Rupp, *J. Amer. Chem. Soc.*, **77**, 3928 (1955); (b) A. J. Cox, *J. Org. Chem.*, **30**, 2052 (1965); (c) A. Nickon and W. L. Mendelson, *ibid.*, **30**, 2087 (1965); (d) R. Gardi and A. Lusignani, *ibid.*, **32**, 2647 (1967).

(7) Identity of **6** rests on its physical and chemical properties, including sodium borohydride reduction to the known cholest-5-ene-3β,6β-diol.

(8) Molecular rotational increments (Δ[M]D) for the 7α-hydroperoxide **3a** and the 7β-hydroperoxide **4a** compared with cholesterol are −427 and +322, respectively; for the 7α-alcohol **3b** and the 7β-alcohol **4b**, −204 and +168, respectively, calculated using [M]D values for **1a**, −154;[9] **3a**, −581;[4a] **4a**, +168; **3b**, −358;[9] **4b**, +14.[9]

(9) *Physical data from* J. Jacques, H. Kagan, and G. Ourisson, "Tables of Constants and Numerical Data," Vol. 14, S. Allard, Ed., Pergamon Press, Oxford, 1965, pp 472, 475.

(10) Comparison proton spectra of the 7β-alcohol **4b** revealed the 7α proton as a doublet of doublets, $J_{6,7}$ = 1.5, $J_{7,8}$ = 7 Hz; of the 7α-alcohol **3b**, as a doublet of doublets, $J_{6,7}$ = 5.5, $J_{7,8}$ = 1.5 Hz.

(11) Ready epimerization of $C_{19}$-$\Delta^5$-7 alcohols in acidic systems has been reported; *cf.* (a) R. Hampl and L. Stárka, *J. Steroid Biochem.*, **1**, 47 (1969); (b) L. Stárka and R. Hampl, "Hormonal Steroids," V. H. T. James and L. Martini, Ed., Excerpta Medica, Amsterdam, 1971, pp 150–157; (c) P. Morand and A. Van Tongerloo, *Chem. Commun.*, 7 (1972).

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(13) J. E. Fox, A. I. Scott, and D. W. Young, *Chem. Commun.*, 1105 (1967); *J. Chem. Soc., Perkin Trans. 1*, 799 (1972).

(14) (a) D. H. S. Horn and D. Ilse, *J. Chem. Soc.*, 2280 (1957); (b) J. Scotney and E. V. Truter, *J. Chem. Soc. C*, 1911 (1968).

axial 7α and quasiequatorial 7β substituents, thereby implying potential epimerization of the 7α- to the 7β-hydroperoxide in analogy to our own findings with **3a** and **4a**. Furthermore, our isolation of the 6β-hydroperoxide **6** but not its 6α epimer and the variable recovery under other conditions by other investigators of either the 6β-hydroperoxide **6** alone[5a,7a] or of both the 6β-hydroperoxide **6** and its 6α epimer,[7b-d] together with the recognized ease with which 6β-hydroxy-Δ⁴ 3-ketones are epimerized, suggests that epimerization of the 6β-hydroperoxide **6** might account in part for the presence of its 6α epimer in some studies.[7b-d] In that the 6β-hydroperoxyl group of **6** appears to be axial,[15] its epimerization in parallel with that of **3a** to **4a** might be expected by analogy. Previous evidence indicating that epimerization of **6** does not occur[7b] cannot be considered as conclusive, and, in matters dealing with the mechanisms of allylic hydroperoxide formation,[7c,17] it may be necessary to consider allylic hydroperoxide epimerization as well as allylic alcohol epimerization as possible complicating features.

## Experimental Section[18]

**Cholesterol Autoxidation.**—Cholesterol (1 kg), recrystallized several times from methanol to remove autoxidation products, was held at 70° in air but in the dark. After 4 weeks the material was recrystallized from ethanol and the crystalline cholesterol therefrom recovered was heated again at 70° for 4 weeks, after which time the sample was recrystallized from ethanol, etc. The ethanol mother liquors were evaporated directly, and a 3-month collection of mother liquor residues (stored in a deep freezer until processed) was chromatographed on silica gel to give five major fractions, fractions A–E, as previously described.[3]

**6β-Hydroperoxycholest-4-en-3-one (6).**—Fraction A contained compounds more mobile than **1a** on thin layer chromatography, including the 6β-hydroperoxide **6**. Rechromatography of fraction A on Sephadex LH-20 developed with methylene chloride gave retarded fractions enriched in **6**, which was recovered by evaporation and crystallization from methanol, thus giving 64 mg of **6** as colorless needles: mp 177–180° (lit. mp 180°,[6a] 177 and 181°,[5a] 180–181°[6b]); $\lambda_{max}^{CH_3OH}$ 237 nm [lit. $\lambda_{max}^{C_2H_5OH}$ 236 nm (ε 16,850),[6a] 235 nm[6b]]; $\tilde{\nu}_{max}^{KBr}$ 3300 (broad), 1670 cm⁻¹; $\tilde{\nu}_{max}^{CHCl_3}$ 3515 cm⁻¹; $R_c$ 1.25 (yellow color with sulfuric acid); positive Wurster red color with *N,N*-dimethyl-*p*-phenylenediamine; $R_v$ 1.45; nmr δ 0.72 (s, 3 H, C-18 protons), 0.82 (d, 6 H, $J$ = 5 Hz, C-26, C-27 protons), 0.92 (d, 3 H, $J$ = 5 Hz, C-21 protons), 1.35 (s, 3 H, C-19 protons), 4.50 (d, $J$ = 2 Hz, 1 H, 6α proton), 6.00 ppm (s, 1 H, C-4 vinyl proton); mass spectrum *m/e* (rel intensity) 416 (2, M), 400 (100, M − O), 398 (24, M − $H_2O$), 382 (10, M − $H_2O_2$), 385 (17, M − $CH_3O$), etc.

**Cholest-4-ene-3β,6β-diol.**—Excess sodium borohydride was added to a solution of 10 mg of **6** in 5 ml of methanol. After 15 min a few drops of acetic acid was added, followed by 20 ml of water. The product was extracted with diethyl ether, evaporated, and crystallized from methanol. Thus was obtained 6 mg of cholest-4-ene-3β,6β-diol: mp 257–258° (lit. mp 257–258°,[20a,b] 254°,[20c] 256–257°,[20d]); $R_c$ 0.24; $t_R$ (as the trimethylsilyl ether) 0.70 (3% QF-1), 1.50 (3% SE-30);[21] mass spectrum *m/e* (rel intensity) (of the trimethylsilyl ether) 546 (20), 531 (24), 519 (9), 457 (32), 404 (100), etc.; identical in all respects with an authentic sample of cholest-4-ene-3β,6β-diol.

**3β-Hydroxycholest-5-ene 7β-Hydroproxide (4a). A. From Cholesterol.**—Fraction D from the initial chromatography on silica gel of autoxidized cholesterol which contained **4a**, cholest-5-ene-3β,25-diol, and other sterols of similar polarity was chromatographed on 60-cm-long, 2.5-cm-diameter columns of Sephadex LH-20 irrigated with methylene chloride containing 1% (v/v) ethanol. The retarded fractions containing **4a**, well separated from other sterols in fraction D, were evaporated and the hydroperoxide **4a** was recrystallized from methanol–diethyl ether. Thin layer chromatographic analysis of the purification showed contaminant 3β,7β-diol **4b**, formed apparently during processing. Rechromatography on Sephadex LH-20 and recrystallization gave the same contamination of the 7β-hydroperoxide with 3β,7β-diol **4b**. The analytical sample of 7β-hydroperoxide **4a** was prepared by rechromatography a third time on Sephadex LH-20, with the eluates most concentrated in **4a** taken to dryness and subjected to immediate analysis. Thus was obtained pure **4a**: mp 148–150°; $[\alpha]_{578}$ +40.2°; $R_c$ 0.60 (blue color with sulfuric acid; positive Wurster red color with *N,N*-dimethyl-*p*-phenylenediamine); $\tilde{\nu}_{max}^{KBr}$ 3350 (OH), 1625 (C=C), 1430, 1340, 1040, 945, 590 cm⁻¹ (distinguished from spectra of the 7α-hydroperoxide **3a**, $\tilde{\nu}_{max}^{KBr}$ 3325, 1640, 1425, 1350, 1045, 945, 635 cm⁻¹, by small frequency differences); nmr ($CDCl_3$) δ 0.69 (s, 3 H, C-18 protons), 0.85 (d, 6 H, $J$ = 5 Hz, C-26, C-27 protons), 0.92 (d, 3 H, $J$ = 5 Hz, C-21 protons), 1.04 (s, 3 H, C-19 protons), 3.58 (broad, 1 H, $W_{1/2}$ = 12 Hz, 3α proton), 4.15 (q, 1 H, $J_{6,7}$ = 1.5 Hz, $J_{7,8}$ = 8 Hz, 7α proton), 5.61 ppm (d, 1 H, $J_{6,7}$ = 1.5 Hz, C-6 vinyl proton).

*Anal.* Calcd for $C_{27}H_{46}O_3$: C, 77.46; H, 11.07. Found: C, 77.29; H, 11.07.

A separate isolation of **4a** from a preparation enriched in **2a**, **3a**, and other autoxidation products of cholesterol but free from cholesterol and not initially containing **4a** was accomplished after inadvertent storage of the mixture for several months at room temperature. Chromatography on silica gel gave a fraction eluted with 10% ethyl acetate in benzene enriched in **4a**, which was rechromatographed on Sephadex LH-20 developed with benzene. The **4a** fraction, 12 mg, was chromatographed on a 0.25 mm thick silica gel $HF_{254}$ plate irrigated twice with acetone–chloroform (1:4) thereby affording 7 mg of **4a**, identified by thin layer and gas chromatographic properties, infrared absorption spectra, and sodium borohydride reduction to **4b**.

(15) Axial character of the 6β-hydroperoxyl group in **6** is suggested by the hypsochromic shift (4–6 nm) in the absorption spectrum maximum of **6** and by strong 1,3-diaxial effects (a paramagnetic shift of 0.17 ppm) on the C-19 methyl protons chemical shift, both in comparison with the parent cholest-4-en-3-one. Furthermore, the singlet character of the C-4 proton resonance signal[15b] and the absence of 1,2-trans-diaxial coupling between the 6α and 7β protons of **6** exclude a B-ring boat conformation and an equatorial 6β-hydroperoxyl group. Rather, weak coupling ($J$ = 2 Hz) exhibited in the doublet signal of the 6α proton support a B-ring chair conformation for **6** in which an equatorial 6α proton is coupled equally with both vicinal 7-protons.

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(17) A. Nickon, V. T. Chuang, P. J. L. Daniels, R. W. Denny, J. B. DiGiorgio, J. Tsunetsugu, H. G. Vilhuber, and E. Werstiuk, *J. Amer. Chem. Soc.*, **94**, 5517 (1972).

(18) Solvents used in this study were redistilled prior to use. All evaporations were conducted under vacuum in all glass rotary evaporator units. Melting points were taken on a Fisher-Johns melting point apparatus. Optical rotations at 578 nm were taken on chloroform solutions of steroids using a Zeiss digital readout polarimeter. Infrared absorption spectra were recorded on 1.5-mm pressed KBr disks incorporating the samples and on solutions in $CCl_4$ (1.0 mm path), using Perkin-Elmer Model 337 and Model 357 spectrophotometers. Proton nmr spectra were recorded in deuteriochloroform solutions using a Varian T-60 spectrometer, with tetramethylsilane as an internal standard. Mass spectra were obtained using an AEI MS-30 double beam instrument, using heptacosafluorobutylamine in the reference beam. The sample beam was connected *via* a membrane separator operated at 215° to a Pye Unicam Model 104 gas chromatograph equipped with a 5-ft-long 3-mm-i.d. coiled glass column packed with 3% QF-1 on 80–100 mesh Gas-Chrom Q (Applied Science Laboratories, State College, Pa.). Oven temperature was 244°; helium at 30 ml/min was used as a carrier gas. Spectra of resolved sterol components were obtained at 24 eV with a resolution of 1000 and a scanning speed of 10 sec per decade.

Thin layer chromatography was conducted using previously described procedures.[2a,d,3] Mobility data are given for triple ascending irrigation of silica gel $HF_{254}$ 0.25-mm-thick chromatoplates using toluene–ethyl acetate (3:2), except where other solvent systems are designated. *N,N*-Dimethyl-*p*-phenylenediamine[2a] and 50% aqueous sulfuric acid[2d] were used for visualization. Gas chromatography was conducted on 3% SE-30 and 3% QF-1 phases as previously described.[19a] Preparative and analytical liquid chromatography on Sephadex LH-20 columns was carried out as previously described.[19b] Thin layer chromatographic mobilities ($R_c$), gas chromatographic retention times ($t_R$), and liquid chromatographic void volumes on Sephadex LH 20 ($R_v$) were all measured *vs.* cholesterol as unity.

(19) (a) J. E. van Lier and L. L. Smith, *Anal. Biochem.*, **24**, 419 (1968); (b) J. E. van Lier and L. L. Smith, *J. Chromatogr.*, **41**, 37 (1969).

(20) (a) O. Rosenheim and W. W. Starling, *J. Chem. Soc.*, 377 (1937); (b) V. A. Petrow, O. Rosenheim, and W. W. Starling, *ibid.*, 679 (1938); (c) V. Prelog, L. Ruzicka, and P. Stein, *Helv. Chim. Acta*, **26**, 2222 (1943); (d) L. F. Fieser, J. E. Herz, M. W. Klohs, M. A. Romero, and T. Utne, *J. Amer. Chem. Soc.*, **74**, 3309 (1952).

(21) The epimeric cholest-4-ene-3β,6-diols are readily distinguishable by both thin layer and gas chromatography.[19a]

**B. From the 7α-Hydroperoxide 3a.**—A sample of **3a** (15.4 mg) meticulously freed from **4a** and other detectable sterols was dissolved in 7 ml of ethyl acetate and warmed at 40° in a water bath. The extent of epimerization of **3a** to **4a** was followed directly by thin layer chromatography and by sodium borohydride reduction and thin layer chromatography of the better resolved alcohols **3b** and **4b**. After 48 hr epimerization had proceeded to about 25–30%. The solution was concentrated to 0.5 ml and applied to a 0.25 mm thick silica gel $HF_{254}$ chromatoplate. The applied sample was converged into a fine line with acetone, and the prepared chromatoplate was irrigated with benzene–ethyl acetate (18:7) three times. The more mobile **4a** zone was eluted with acetone, and dilution of the concentrated solution with petroleum ether (bp 30–60°) gave 3.2 mg of **4a**, mp 147–149°, with thin layer and gas chromatographic properties and infrared absorption spectra identical with those of authentic **4a**. Sodium borohydride reduction of the sample gave **4b**, mp 176–178°, with thin layer and gas chromatographic properties and infrared absorption spectra identical with those of authentic **4b**.

Epimerization of **3a** to **4a** was also achieved using acetone, benzene, carbon tetrachloride, and methanol as solvents, the product **4a** being recovered and identified by the means described for the epimerization in ethyl acetate. At slightly higher temperatures (50°) or after 72–120 hr at 40° thermal decomposition of both **3a** and **4a** occurred, giving thin layer chromatograms bearing **3b**, **4b**, and **5a** as well as **3a** and **4a**.

Separation of small amounts of **4a** in the presence of larger amounts of the more polar **3a** required careful attention. Resolution was not achieved when chromatoplates thicker than 0.25 mm were employed. Multiple irrigations were routinely used with the solvent system benzene–ethyl acetate (17:8), in which system the hydroperoxides had the relative mobilities: **4a**, 1.00; **3a**, 0.96 (**2a**, 0.96; **2b**, 0.71; **3b**, 0.48; **4b**, 0.54). Repeated thin layer chromatography was necessary for complete purification of **4a** free from **3a** and thermal decomposition products.

**Autoxidation of Cholesterol Acetate.**—Crystalline cholesterol acetate (**1b**) (35.5 g) was stirred and exposed to a stream of air in a flask heated in an oil bath at 90–100° in the dark. After 3 days the material became sticky and stirring was difficult. After 2 weeks the light yellow syrup obtained was cooled to room temperature and dissolved in 50 ml of diethyl ether, and 100 ml of methanol was then added to the ether solution. Crystals of **1b** (18 g) were removed by filtration, and the mother liquor was concentrated, yielding a second crop of **1b** (4.3 g). The mother liquor was evaporated, and the solids (9.2 g) were chromatographed on silica gel using toluene containing 5% (v/v) diethyl ether. Using thin layer chromatographic analyses of individual column fractions, five major fractions (fractions A–E) were collected.

Fraction A on evaporation yielded 3.8 g of **1b**, $R_c$ 1.63 (magenta color with sulfuric acid), identified by melting point and nmr with an authentic sample. Fraction B yielded 0.1020 g of **5b**, $R_c$ 1.48 (yellow-green color with sulfuric acid), identified by melting point, nmr, and sodium borohydride reduction to the characteristic mixture of epimeric 3β,7-diols **3b** and **4b**.

Fraction C containing peroxidic components of thin layer chromatographic mobility $R_c$ 1.40–1.48 was rechromatographed on Sephadex LH-20 using methylene chloride containing 1% methanol. Four subfractions with thin layer chromatographic mobilities of 1.40, 1.41, 1.44, and 1.48 were taken.

**3β-Acetoxycholest-5-en-7-one (5b).**—The $R_c$ 1.48 subfraction from fraction C from autoxidation of **1b** was evaporated to give 0.058 g of **5b**: $R_c$ 1.48; $t_R$ 7.1 (3% QF-1), 2.7 (3% SE-30); nmr δ 0.68 (s, 3 H, C-18 protons), 0.85 (d, $J$ = 5 Hz, 6 H, C-26, C-27 protons), 0.92 (d, $J$ = 5 Hz, 3 H, C-21 protons), 1.21 (s, 3 H, C-19 protons), 2.05 (s, 3 H, 3β-acetyl protons), 4.70 (broad, 1 H, 3α proton), 5.70 ppm (s, 1 H, C-6 vinyl proton), identical in every respect with an authentic sample of **5b**.

Samples of **5b** isolated on attempted acetylation of **4a** and of **4c** were crystallized from methanol and identified by the same physical methods in comparison with an authentic sample.

**3β-Acetoxycholest-5-ene 20α-Hydroperoxide.**—The $R_c$ 1.44 subfraction from fraction C from autoxidation of **1b** was evaporated and crystallized from methanol to give 0.032 g of 3β-acetoxycholest-5-ene 20α-hydroperoxide: mp 92–95°; $R_c$ 1.44 (brown color with sulfuric acid, positive Wurster red color with *N*,*N*-dimethyl-*p*-phenylenediamine); nmr δ 0.82 (s, 3 H, C-18 protons), 0.92 (s, 3 H, C-19 protons), 0.95 (d, $J$ = 5 Hz, 6 H, C-26, C-27 protons), 0.96 (s, 3 H, C-21 protons), 2.05 (s, 3 H, 3β-acetoxyl protons), 4.50 (broad, 1 H, 3α proton), 5.35 ppm (d, $J$ = 5 Hz, 1 H, C-6 vinyl proton).

Reduction with sodium borohydride and hydrolysis with 5% sodium methoxide in methanol gave cholest-5-ene-3β,20α-diol: $R_c$ 0.88; $t_R$ 2.13 (3% QF-1), 2.09 (3% SE-30); mass spectrum $m/e$ (rel intensity) 384 (100), 369 (20), 366 (18), 351 (43), 317 (8), 299 (52), 281 (20), 271 (44), 258 (12), 253 (22), etc., identical with similar physical properties of an authentic sample of cholest-5-ene-3β,20α-diol.

**3β-Acetoxycholest-5-ene 25-Hydroperoxide.**—The $R_c$ 1.41 subfraction from fraction C from autoxidation of **1b** was evaporated to give 0.029 g of 3β-acetoxycholest-5-ene 25-hydroperoxide as a syrup: $R_c$ 1.41 (brown color with sulfuric acid, positive Wurster red color with *N*,*N*-dimethyl-*p*-phenylenediamine); nmr δ 0.66 (s, 3 H, C-18 protons), 0.90 (d, $J$ = 5 Hz, 3 H, C-21 protons), 1.00 (s, 3 H, C-19 protons), 1.20 (s, 6 H, C-26, C-27 protons), 2.05 (s, 3 H, 3β-acetoxyl protons), 4.50 (broad, 1 H, 3α proton), 5.35 ppm (d, $J$ = 5 Hz, 1 H, C-6 vinyl proton).

Sodium borohydride reduction and hydrolysis with 5% sodium methoxide in methanol gave cholest-5-ene-3β,25-diol: $R_c$ 0.60; $t_R$ 2.40 (3% QF-1), 1.60 (3% SE-30); mass spectrum $m/e$ (rel intensity) 402 (7), 384 (75), 382 (16), 370 (35), 367 (60), 351 (52), 299 (32), 273 (47), 271 (100), 255 (30), 253 (25), etc., identical in these properties with an authentic sample of cholest-5-ene-3β,25-diol.

**3β-Acetoxycholest-5-ene 7β-Hydroperoxide (4c).**—The $R_c$ 1.40 subfraction from fraction C from autoxidation of **1b** was evaporated to give 0.249 g of **4c**: mp 80–82°; $[\alpha]_{578}$ +91.1°; $R_c$ 1.40 (blue color with sulfuric acid, positive Wurster red color with *N*,*N*-dimethyl-*p*-phenylenediamine); nmr δ 0.68 (s, 3 H, C-18 protons), 0.84 (d, 6 H, $J$ = 5 Hz, C-26, C-27 protons), 0.92 (d, 3 H, $J$ = 5 Hz, C-21 protons), 1.05 (s, 3 H, C-19 protons), 2.10 (s, 3 H, 3β-acetate protons), 4.15 (m, 1 H, $W_{1/2}$ = 12 Hz, 7α proton), 4.65 (m, 1 H, $W_{1/2}$ = 16 Hz, 3α proton), 5.82 ppm (d, 1 H, $J$ = 5 Hz, C-6 vinyl proton).

Sodium borohydride reduction of **4c** followed by hydrolysis with 5% sodium methoxide in methanol gave **4b**, identified by thin layer and gas chromatographic properties and proton nmr spectra.

**Cholest-5-ene-3β,7β-diol (4b). A. From the 7β-Hydroperoxide 4a.**—A solution of 50 mg of **4a** in methanol was reduced with an excess of sodium borohydride. Thin layer chromatographic analysis of the reduction mixture established that no **3b** was present and that **4b** only had been formed. The crude product was recrystallized from diethyl ether–hexane, yielding 23 mg of **4b**: mp 176–179° (lit.[9] mp 172–179°); $[\alpha]_D$ +3.3° (lit.[9] $[\alpha]_D$ +3.5°); $R_c$ 0.33 (blue color with sulfuric acid); $t_R$ 2.3 (3% QF-1), 1.6 (3% SE-30); $R_v$ 1.6; nmr δ 0.70 (s, 3 H, C-18 protons), 0.86 (d, $J$ = 5 Hz, 6 H, C-26, C-27 protons), 0.92 (d, $J$ = 5 Hz, 3 H, C-21 protons), 1.04 (s, 3 H, C-19 protons), 3.53 (broad, 1 H, 3α proton), 3.86 (q, $J$ = 1.5, 7 Hz, 7α proton), 5.30 ppm (d, $J$ = 1.5 Hz, 1 H, C-6 vinyl proton); mass spectrum $m/e$ (rel intensity) 402 (1), 384 (48), 382 (14), 366 (100), etc.; identical in these respects with an authentic sample of **4b**.

**B. From Cholest-5-ene-3β,7α-diol.**—Pure **3b**, mp 185–186°, free from **4b** and other detectable sterols, was dissolved in acetone (10 mg/5 ml) and warmed at 50° in a water bath. Aliquots (80 μg) were withdrawn at intervals for thin layer chromatographic analysis using benzene–ethyl acetate (3:7). After 72 hr sufficient **4b** was present to warrant isolation. The sample was chromatographed on 0.25 mm thick silica gel $HF_{254}$ chromatoplates using benzene–ethyl acetate (3:7) with triple ascending irrigation. The **4b** was eluted from the chromatoplate and crystallized from diethyl ether–hexane to yield **4b**: mp 176–177° (lit.[9] mp 172–179°); $\tilde{\nu}_{max}^{KBr}$ 3320, 1664 $cm^{-1}$, identical with spectra obtained from an authentic sample of **4b**. Full identity of the sample with an authentic sample of **4b** was also demonstrated using thin layer and gas chromatographic properties.

**C. From 5α-Cholest-6-ene-3β,5-diol-4-$^{14}C$.**—A sample of cholesterol-4-$^{14}C$ was converted by photosensitized oxidation in pyridine[4c,5] to **2a**-4-$^{14}C$ of specific activity 9700 dpm/mg. Sodium borohydride reduction of 20 mg of **2a**-4-$^{14}C$ in methanol gave 16 mg of **2b**-4-$^{14}C$ (9200 dpm/mg) purified by repeated thin layer chromatography. The pure **2b**-4-$^{14}C$, 4 mg, was dissolved in 2 ml of acetone and warmed at 50° for 72 hr, after which time the **2b**, **3b**, and **4b** zones were excised from the chromatoplate and the associated radioactivity was measured by scintillation counting methods. The amount of radioactivity recovered in each fraction was as follows: **2b**, 80%; **3b**, 14.2%; 5.8%.

**Cholest-5-ene-3β,7α-diol (3b). A. From the 7α-Hydroperoxide 3a.**—A solution of 1 mg of **3a** in methanol was reduced with an excess of sodium borohydride. Thin layer chromatographic analysis of the reduction mixture established that only **3b** was present and that no **4b** had been formed. Pure **3b** was recovered by preparative thin layer chromatography and identified by thin layer and gas chromatographic means.

**B. From 5α-Cholest-6-ene-3β,5-diol.**—Pure **2b**, mp 148–149° (lit. mp 147–150°,[4c] 170–175, 166–171, and 134–135°,[5] 181°[22]), prepared by sodium borohydride reduction of **2a**, free from **3a** and all other detectable sterols, was dissolved in acetone (10 mg/5 ml) and warmed at 50° on a water bath. Aliquots (80 μg) were removed at intervals for thin layer chromatographic analysis. The intensity of the **3b** spot on chromatograms increased over the period 24–72 hr. After 72 hr the sample was chromatographed using benzene–ethyl acetate (3:7) with triple ascending irrigation. The **3b** zone was eluted and the pure product was crystallized from diethyl ether–hexane, thus yielding pure **3b**: mp 185–186° (lit.[9] mp 158–161 and 176–187°); $\nu_{max}^{KBr}$ 3350, 1630 $cm^{-1}$, identical with spectra of an authentic sample. The **3b** preparation was also identical in thin layer and gas chromatographic properties with an authentic sample of **3b**.

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**C. From Cholesterol Acetate (1b).**—Fraction D, 2.994 g, obtained from autoxidation of **1b**, characterized by thin-layer chromatographic mobility $R_c$ 1.11 with an intense blue color with sulfuric acid spray, was composed of **3c** and **4c** in the proportion 3:2. Hydrolysis of the material with 5% sodium methoxide in methanol followed by chromatography on Sephadex LH-20 and crystallization several times from methanol gave pure **3b**: mp 182–184° (lit.[9] mp 158–161 and 176–187°); $[\alpha]_D$ −75.8° (lit.[9] $[\alpha]_D$ −89°); $R_c$ 0.28 (blue color with sulfuric acid); $t_R$ 2.2 (3% QF-1), 1.6 (3% SE-30); $R_v$ 1.5; nmr δ 0.68 (s, 3 H, C-18 protons), 0.86 (d, $J$ = 5 Hz, 6 H, C-26, C-27 protons), 0.92 (d, $J$ = 5 Hz, C-21 protons), 0.99 (s, 3 H, C-19 protons), 3.50 (m, $W_{1/2}$ = 12 Hz, 1 H, 3α proton), 3.85 (q, $J_{6,7}$ = 5.5, $J_{7,8}$ = 1.5 Hz, 1 H, 7β proton), 5.60 ppm (d, $J$ = 5.5 Hz, 1 H, C-6 vinyl proton); mass spectrum identical with that of the 3β,7β-diol **4b**. In addition to **3b** thus recovered there was obtained from the Sephadex LH-20 column a pure sample of **4b**, identified by melting point, chromatographic, and spectral properties with an authentic sample.

**Registry No.**—**4a**, 36871-91-7; **4c**, 36871-92-8; 3β-acetoxycholest-5-ene 20α-hydroperoxide, 36871-93-9; 3β-acetoxycholest-5-ene 25-hydroperoxide, 36871-94-0.

# Syntheses of 2,5-Dimethyl-4-hydroxy-2,3-dihydrofuran-3-one (Furaneol), a Flavor Principle of Pineapple and Strawberry

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Three syntheses of furaneol, a flavor component of strawberry and pineapple, are described. Oxidation of the known 2,5-dimethyl-2,5-dimethoxy-2,5-dihydrofuran with potassium chlorate in the presence of catalytic amounts of osmium tetroxide in aqueous solution gave *erythro*-3,4-dihydroxyhexane-2,5-dione, while hydrodimerization of methylglyoxal with zinc yielded the threo isomer. Both dihydroxy diketones on exposure to mildly basic reagents were converted to furaneol. Acidic reagents did not lead to furaneol but its aliphatic isomer 3-hydroxy-3-hexene-2,5-dione and 3-acetyl-2,5-dimethyl-4,5-dihydrofuran-4-one, the latter originating from cleavage to pyruvic acid followed by condensation with starting material. In a third synthesis hexane-3,4-dione was transformed to the symmetrical dibromide and then to furaneol by hydrolysis.

Among the many hundreds of compounds isolated from the volatile portions of fruit aromas,[2] furaneol [2,5-dimethyl-4-hydroxy-2,3-dihydrofuran-3-one (**5**)] occupies a central position. It was isolated at the same time from the organoleptic principle of pineapple[3] and from strawberry flavor.[4] Since this flavor principle with a powerful caramel-like odor has found many applications in the food and beverage industry, its chemical synthesis has become of some interest. Furaneol was first prepared accidentally, in unspecified yield, from rhamnose and piperidine acetate in hot ethanol solution.[5] Two rational syntheses[6,7] of furaneol have been described, but both seem unpractical for production purposes. In this paper we describe syntheses of furaneol from three different, readily available starting materials. Oxidation of 2,5-dimethyl-2,5-dimethoxy-2,5-dihydrofuran (**2**) prepared by bromination of 2,5-dimethylfuran (**1**) in methanol solution,[8] with potassium chlorate and a catalytic amount of osmium tetroxide[9] in aqueous tetrahydrofuran containing sodium bicarbonate, gave the diol **3** in 10% yield. Since we suspected that most of the diol **3** was lost by hydrolysis the oxidation was performed in a more aqueous reaction medium and in the absence of bicarbonate. The dihydroxy diketone **4** was thus obtained in nearly quantitative yield. The diol **3** is formed also upon oxidation of the olefin with potassium permanganate and we concluded that it has cis stereochemistry and the resulting dihydroxy diketone **4** the erythro configuration. Parenthetically, infrared measurements indicate the presence of only one intramolecular hydrogen bond in the erythro isomer, suggesting the preferred conformation **4**. Efforts to convert the cis diol **3** to furaneol by elimina-

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