alcohol while $(sec\text{-OH})_t$ is the concentration of α-methylallyl alcohol at time t. Similarly, the conversion of *cis*-crotyl alcohol to *trans*-crotyl alcohol *via* α-methylallyl alcohol can be calculated by using the relationship

$$\frac{k_2\bar{C}_{sec\text{-OH}}}{k_4\bar{C}_{sec\text{-OH}}}(sec\text{-OH})_t = (trans\text{-OH})_t$$

Vapor Phase Chromatography. The instrument employed in this work was a Perkin-Elmer Model 800 gas chromatograph epuipped with a differential flame ionization detector. Analyses of the reaction mixtures were carried out on two separate vpc columns: a 20-ft copper column (1/8 in. o.d.) packed with 10% (w/w) β,β'-thiodipropionitrile (TDPN) on 80–100 mesh Chromosorb W; and a 10-ft copper column (1/8 in. o.d.) packed with 10% (w/w) Ucon 550HB on 80–100 mesh Chromosorb W. A suggested antitailing agent, ATPET-80 (0.1% by weight), was added to the above column packings.

α-Methylallyl alcohol and crotyl α-methylallyl ethers were analyzed on the (TDPN) vpc column operated at 70° column temperature/160° preheator, 23 cc/min nitrogen carrier gas flow, and 15 psi of hydrogen gas for detector response. While the (Ucon) column operated at 70° column temperature/160° preheator, 23 cc/min nitrogen carrier gas flow provided data for the per cent composition of the reaction mixture with respect to *cis*- and *trans*-crotyl alcohol, di-α-methylallyl ethers, and the dicrotyl ethers. Under these operating conditions, the geometric isomers (*cis* and *trans*) of the butenyl ethers could not be satisfactorily resolved for quantitative measurements. Consequently, all ether measurements are reported without regard for isomeric composition.

Calibration factors were determined for each butenyl compound using *n*-butyl alcohol as an internal standard. Peak areas were integrated with a Disc Chart integrator Model 203 connected to the recorder of the Perkin-Elmer 800 gas chromatograph. Individual peak areas were corrected for base-line drift by employing a drift corrector part no. 1295 with each individual area measurement.

Control experiments performed with mixtures of known compositions indicated that the analyses are accurate to +0.6%. In some cases the accuracy is considerably greater.

H_0 Measurement for the Reaction Solvent. The determination of H_0 for the reaction solvent (70% aqueous dioxane, 2.90 M in sulfuric acid) was carried out as described by Braude.[22] The indicator base used in the determination of H_0 was *o*-nitroaniline ($pk_\beta = -0.29$; λ_{max} 415 μ (ϵ 3050), $A = 0.22$).

Instruments. All infrared spectra were taken on a Beckman I.R. 5-A. Nmr spectra were determined on a Varian Model A-60 nmr spectrometer.

(22) E. H. Braude, *J. Chem. Soc.*, 1971 (1948).

The Structure of the Cactus Sterol Macdougallin (14α-Methyl-Δ^8-cholestene-3β,6α-diol). A Novel Link in Sterol Biogenesis[1,2]

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Contribution from the Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois, and the Department of Chemistry, Stanford University, Stanford, California. *Received October 16, 1965*

Abstract: The nonsaponifiable fraction of the ethanolic extract of *Peniocereus macdougallii* has been found to contain β-amyrin, lophenol, peniocerol, a mixture of sterols presumed to be cholesterol, campesterol, and β-sitosterol, and a new sterol named macdougallin. Degradative experiments are described which establish the structure of macdougallin as 14α-methyl-Δ^8-cholestene-3β,6α-diol. Its biogenetic significance as the first naturally occurring 14α-methyl sterol is discussed.

During the systematic investigation of a number of cactus species for the presence of alkaloids and triterpenoids,[4] it was observed that certain species contained appreciable neutral fractions. Investigation of the neutral constituents of *Lophocereus schottii* resulted in the identification of lophenol[5] (4α-methyl-Δ^7-cholesten-3β-ol) (I) which was of particular interest since it appeared to be an intermediate in the biological conversion of lanosterol to cholesterol.[6] This was confirmed by its independent isolation from rat skin and feces,[7] and the reports of its *in vitro* conversion to cholesterol by tissue homogenates.[8,9] In the hope of finding further intermediates that might shed some light on the biosynthetic processes involved in the demethylation of lanosterol, several other species of cactus were examined. Particular attention was given to the genera *Peniocereus* and *Wilcoxia*, which both possess relatively large, tuberous roots, an unusual feature among the *Cactaceae*. This study has resulted in the isolation and identification of several unusual sterols, among them peniocerol[10] (II, $R_1 = R_2 = R_3 = H$) and viperidinone[11] (III) from *Peniocereus fosteria-*

(1) Supported by the U. S. Atomic Energy Commission and by Grant GM-06840 from the National Institutes of Health.

(2) For a preliminary report of the structure of macdougallin see C. Djerassi, J. C. Knight, and D. I. Wilkinson, *J. Am. Chem. Soc.*, 85, 835 (1963).

(3) (a) Postdoctoral Research Fellow at Stanford University, 1962–1963, and Resident Research Associate, Argonne National Laboratory, 1963–1965; present address: Department of Chemistry, Arizona State University, Tempe, Ariz.; (b) Postdoctoral Research Fellow at Stanford University, 1961–1962.

(4) For a review see C. Djerassi in "Festschrift Arthur Stoll," Birkhäuser Verlag, Basel, Switzerland, 1957, pp 330–352.

(5) C. Djerassi, G. W. Krakower, A. J. Lemin, L. H. Liu, J. S. Mills, and R. Villotti, *J. Am. Chem. Soc.*, 80, 6284 (1958).

(6) R. B. Clayton, *Quart. Rev.* (London), **19**, 168 (1965).

(7) D. H. Neiderhiser and W. W. Wells, *Arch. Biochem. Biophys.*, 81, 300 (1959).

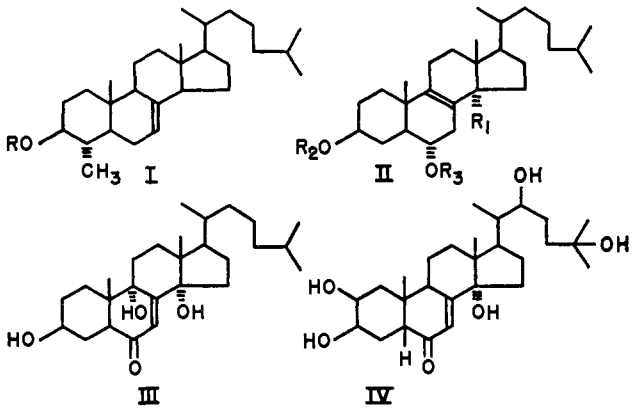
(8) M. Lindbergh, F. Gautschi, and K. Bloch, *J. Biol. Chem.*, 238, 1661 (1963).

(9) J. Pudles and K. Bloch, *ibid.*, 235, 3417 (1960).

(10) C. Djerassi, R. D. H. Murray, and R. Villotti, *Proc. Chem. Soc.*, 450 (1961); *J. Chem. Soc.*, 1160 (1965).

(11) C. Djerassi, J. C. Knight, and H. Brockmann, Jr., *Chem. Ber.*, 97, 3118 (1964).

nus and *Wilcoxia viperina*, respectively. The isolation of the latter is particularly interesting because it is the first plant sterol possessing some of the structural features of the insect hormone ecdysone[12] (IV). During the course of this work it was observed by Dr. R. D. H. Murray that the mother liquors from which peniocerol had been crystallized deposited, on standing for a long period, well-formed needles of a compound melting at 173–175°, $[\alpha]_D$ +71.8°. The mass spectrum revealed a molecular weight of 416, corresponding to a molecular formula $C_{28}H_{48}O_2$ which was confirmed by analysis, and indicating that the new compound was a homolog of peniocerol. The low yield of this new sterol greatly hindered the determination of its structure, and it was not until a better source was discovered in the cactus *P. macdougallii* that progress could be made with the structural work. The ether-soluble part of the crude ethanolic extract of the cactus roots was saponified, and the neutral portion was extracted with ether and acetylated. Chromatography on alumina gave, in addition to the diacetate of the new sterol (now named macdougallin), an equivalent amount of peniocerol diacetate (II, R_1 = H; R_2 = R_3 = Ac) and smaller amounts of lophenol acetate (I), β-amyrin acetate, and a mixture of the acetates of β-sitosterol, campesterol, and cholesterol similar to that isolated from *W. viperina*.[11]



Structure of Macdougallin. The mixture of macdougallin and peniocerol diacetates obtained by chromatography as described above could not be satisfactorily separated by further column chromatography or preparative thin layer chromatography, but macdougallin diacetate was readily obtained in a pure form by repeated crystallization from methanol, in which it was much less soluble than peniocerol diacetate. The diacetate, mp 124–126°, $[\alpha]_D$ 55.4° (*c* 1.17), gave correct analytical figures for $C_{32}H_{52}O_4$ and showed a molecular ion at 500 mass units, together with prominent peaks at *m/e* 440 and 380 corresponding to the loss of one and two molecules of acetic acid, respectively. Given a normal tetracyclic skeleton, this indicated the presence of one double bond, which was confirmed by the high terminal absorption in the ultraviolet (ϵ_{210} 4800; ϵ_{220} 1600), and which was evidently tetrasubstituted because of the absence of olefinic proton signals in the nmr spectrum. The hindered nature of the double bond was emphasized by its lack of reactivity toward osmium tetroxide. Ozonolysis, followed by oxidative decomposition of the ozonide with hydrogen peroxide, resulted in the formation of an epoxide (V), mp 146–148°, $[\alpha]_D$ 28.3° (*c* 0.92). Similar epoxidation of an unreactive double bond has previously been reported in the case of the α- and β-amyrins.[13]

Partial saponification of macdougallin diacetate (II, R_1 = CH_3; R_2 = R_3 = Ac) was carried out with potassium carbonate in methanol at 45°, the course of the reaction being followed by thin layer chromatography. Column chromatography of the resulting mixture gave the monoacetate (II, R_1 = CH_3; R_2 = H; R_3 = Ac) as an amorphous, glass-like solid. This was oxidized by Jones reagent[14] to a crystalline keto acetate (VI, R = Ac), mp 113–116°, $[\alpha]_D$ 77.9° (*c* 1.04), with infrared absorption bands at 1720 (ketone), 1745 and 1245 cm^{-1} (acetate), the rotatory dispersion curve of which showed the typical positive Cotton effect of a 3-ketone.[15] The same compound could be obtained by acetylation of the Oppenauer oxidation product of macdougallin, which gave almost exclusively the 6-hydroxy 3-ketone (VI, R = H) as the primary product, mp 120–130°, $[\alpha]_D$ +80.2° (*c* 0.91), with infrared maxima at 3420 (hydroxyl) and 1700 cm^{-1} (ketone), and a molecular ion peak at *m/e* 414 (correct for $C_{28}H_{46}O_2$).

Evidence for the location of the "extra" methyl group could be adduced from molecular rotation data. Subtraction of the molecular rotation $[\phi]_D$ of peniocerol and its diacetate from those of macdougallin and its diacetate gives +62° and +58° as the increments due to the additional methyl group (Table I). From biogenetic considerations, the most probable locations for the methyl were C-4, C-24, and C-14. The ϕ_D increment for the insertion of a methyl at C-24 in Δ^8-cholestenol is −37° for the free sterol and −27° for the acetate, values which are of opposite sign to those found for macdougallin and its diacetate and which render a C-24 location unlikely. Introduction of a methyl group at C-4 in Δ^8-cholestenol gives increments which are of the correct sign but which differ widely (+27° and +148°), since the proximity of the methyl to the 3β-hydroxyl causes an increase in rotation on acetylation instead of the usual decrease. In the case of dihydrolanosterol and 4,4-dimethyl-Δ^8-cholestenol and their acetates, the increment due to the 14α-methyl group is positive and about the same in each case (100 and 110°), indicating that of the three positions under consideration the methyl group was most likely to be located at C-14. The mass spectral evidence was also against the side chain location. The mass spectrum of macdougallin shows major peaks in the mass range corresponding to increasing fragmentation of the nucleus after loss of the side chain (at *m/e* 285, 261, 243, and 225) which are 14 mass units higher than the corresponding peaks in the spectrum of peniocerol (*m/e* 271, 247, 229, and 211), revealing that the methyl group is almost certainly attached to the nucleus.

Positive evidence ruling out C-4 was provided by the rotatory dispersion curve of VI (R = Ac), which did not show a Cotton effect in methanolic HCl because of

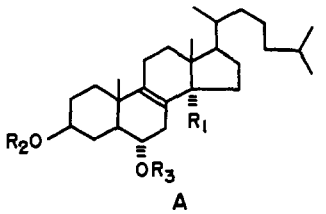
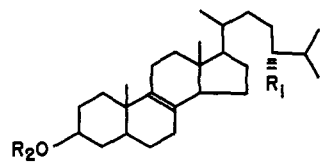
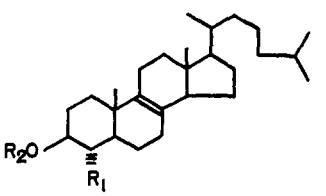
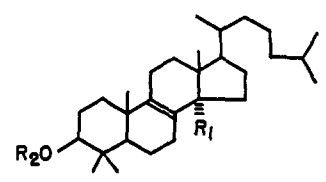
(12) W. Hoppe and R. Huber, *Chem. Ber.*, 98, 2403 (1965).

(13) O. Jeger, *Progr. Chem. Org. Nat. Prod.*, 7, 1 (1950).

(14) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).

(15) C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill Book Co., Inc., New York, N. Y., 1960, p 42.

Table I. Effect of Methyl Groups on Molecular Rotations

	[α]D, degrees	[φ]D, degrees	
 A			
$R_1 = CH_3$; $R_2 = R_3 = H$	71.8	299	$\Delta\phi_D$ +62°
$R_1 = R_2 = R_3 = H$	59	237	
$R_1 = CH_3$; $R_2 = R_3 = Ac$	55.4	277	$\Delta\phi_D$ +58°
$R_1 = H$; $R_2 = R_3 = Ac$	45	219	
 B			
$R_1 = CH_3$; $R_2 = H$	39[a]	156	$\Delta\phi_D$ −37°
$R_1 = R_2 = H$	50[b]	193	
$R_1 = CH_3$; $R_2 = Ac$	24.5[a]	108	$\Delta\phi_D$ −27°
$R_1 = H$; $R_2 = Ac$	31.5[a]	135	
 C			
$R_1 = CH_3$; $R_2 = H$	55[c]	220	$\Delta\phi_D$ +27°
$R_1 = R_2 = H$	50[b]	193	
$R_1 = CH_3$; $R_2 = Ac$	64[c]	283	$\Delta\phi_D$ +148°
$R_1 = H$; $R_2 = Ac$	31.5[a]	135	
 D			
$R_1 = CH_3$; $R_2 = H$	63[d]	270	$\Delta\phi_D$ +100°
$R_1 = R_2 = H$	41[e]	170	
$R_1 = CH_3$; $R_2 = Ac$	60[f]	280	$\Delta\phi_D$ +112°
$R_1 = H$; $R_2 = Ac$	37[e]	168	

[a] D. H. R. Barton and J. D. Cox, *J. Chem. Soc.*, 214 (1949). [b] P. Bladon, J. M. Fabian, H. B. Henbest, H. P. Koch, and G. W. Wood, *ibid.*, 2402 (1951). [c] A. A. Kandutsch and A. E. Russell, *J. Biol. Chem.*, **235**, 2253 (1960). [d] R. G. Curtis, *J. Chem. Soc.*, 1017 (1950). [e] F. Gautschi and K. Bloch, *J. Biol. Chem.*, **233**, 1343 (1958). [f] L. Ruzicka, E. Rey, and A. C. Muhr, *Helv. Chim. Acta*, **27**, 472 (1944).

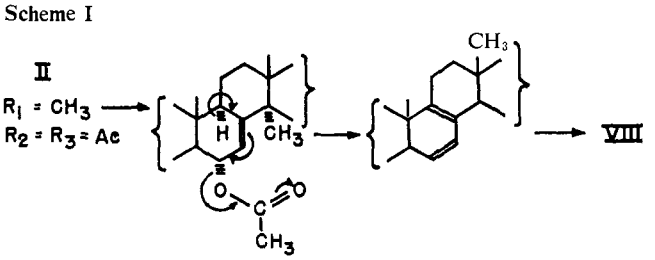
complete ketal formation,[16] and also by a deuterium exchange experiment on the ketone VI (R = H), which showed the presence of four exchangeable hydrogens.

(16) C. Djerassi, L. A. Mitscher, and B. J. Mitscher, *J. Am. Chem. Soc.*, 81, 947 (1959).

It therefore seemed likely that macdougallin possessed an 8(9)-double bond and a 14α-methyl group, which explained the resistance of the double bond toward reduction or isomerization, even in the presence of acids at elevated temperatures and pressures.[17] In contrast to peniocerol diacetate which was isomerized on treatment with a platinum catalyst in the presence of hydrogen, and fully reduced by hydrogenation under acid conditions, macdougallin diacetate was invariably recovered unchanged because the 14α-methyl group blocked migration of the double bond.

The combination of a 14α-methyl substituent and an 8(9)-double bond is a characteristic of many tetracyclic triterpenes such as lanosterol and eburicoic acid[18] (VII), which can readily be isomerized to an equilibrium mixture of 7(8)- and 8(9)-enes on treatment with hydrogen chloride in acetic acid.[18] Under these conditions, macdougallin diacetate was recovered largely unchanged, but a very small amount of a less polar compound was detected by thin layer chromatography. This material was isolated by column chromatography on alumina, and the recovered starting material then was retreated with hydrogen chloride in acetic acid. After several such treatments the pooled reaction product was purified to yield a substance mp 77–79°, $[\alpha]_D$ +66.3° (*c* 1.00). The analysis indicated a formula $C_{30}H_{48}O_2$, corresponding to the loss of the elements of acetic acid, and this was supported by the mass spectrum, which gave a molecular ion of mass 440 and an M − 60 peak at *m/e* 380. Infrared maxima at 1745 and 1245 cm^{-1} and a three-proton signal at 2.04 ppm in the nmr spectrum showed that one acetate still remained, and in addition the nmr spectrum revealed the presence of two olefinic protons giving rise to a triplet centered at 5.45 ppm. The ultraviolet spectrum showed the characteristic three-banded spectrum of a steroid 7,9(11)-diene with maxima at 234, 243, and 252 mμ, all of which evidence pointed to the structure 14α-methyl-$\Delta^{7,9(11)}$-cholestadien-3β-ol acetate (VIII), presumably formed by a mechanism such as that given in Scheme I. Although this compound had been previously reported in the literature,[19] no constants were given. However, it proved possible to synthesize a sample of its immediate precursor 14α-methyl-Δ^7-cholesten-3β-ol (IX),[20] which was oxidized by selenium dioxide to a compound shown to be identical with the diene VIII by mixture melting point determination and infrared spectral comparison. The location of the methyl group was therefore definitely established at

Scheme I



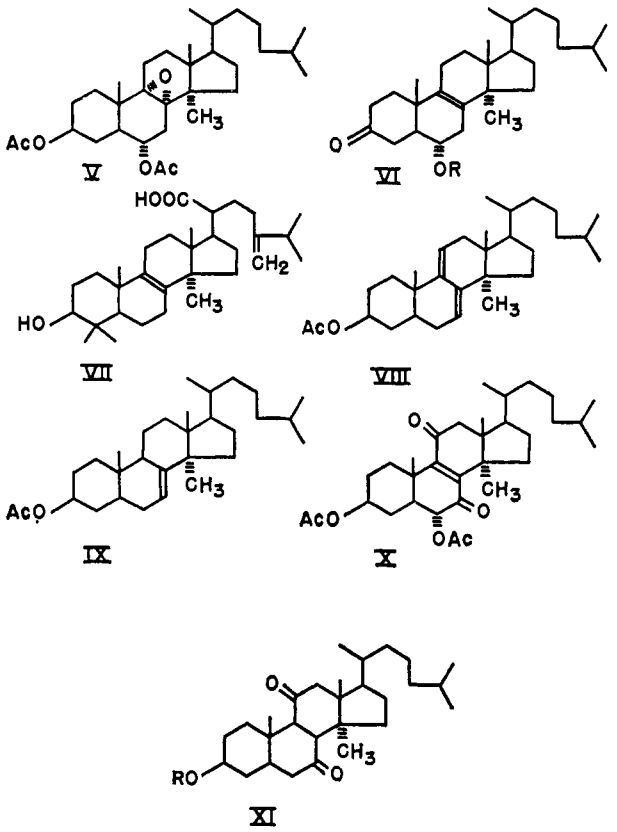
(17) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, pp 273, 368.

(18) J. S. E. Holker, A. D. G. Powell, A. Robertson, J. J. H. Simes, R. S. Wright, and R. M. Gascoigne, *J. Chem. Soc.*, 2422 (1953).

(19) R. B. Woodward, A. A. Patchett, D. H. R. Barton, D. A. J. Ives, and R. B. Kelly, *ibid.*, 1131 (1957).

(20) We are grateful to Professor D. H. R. Barton for providing an authentic sample of this intermediate.

C-14, and macdougallin could be defined as 14α-methyl-Δ⁸-cholestene-3β,6α-diol (II, $R_1 = CH_3$; $R_2 = R_3 = H$).



A compound of this structure would be expected to undergo oxidation with chromium trioxide at the allylic 7 and 11 positions, to give a yellow, enolic enedione analogous to those obtained from lanosterol[21] and similar triterpenes.[22] This proved to be the case, and a compound (X) was isolated from the reaction mixture with characteristic ultraviolet absorption at 270 mμ (ε 6378)[23] and infrared maxima at 1739, 1709, and 1681 cm^{-1} (CCl_4). The analytical data were in accordance with the formula $C_{32}H_{48}O_6$, and the mass spectrum showed a molecular ion of mass 528, in addition to major peaks at *m/e* 468 and 408 corresponding to the loss of one and two acetates. This compound was readily reduced with zinc dust and acetic acid with concomitant loss of the 6α-acetoxy group, to give 14α-methyl-7,11-dioxocholestan-3β-ol acetate (XI, R = Ac). This compound exhibited no ultraviolet maxima between 220 and 350 mμ, and absorbed in the infrared at 1724 (acetate) and 1701 cm^{-1} (ketone). The nmr spectrum showed no olefinic protons and a three-proton signal at 2.04 ppm corresponding to a single acetate group. The analysis and mass spectrum gave a molecular formula $C_{30}H_{48}O_4$ (M^+ 472). Saponification and benzoylation gave the benzoate (XI, R = COC_6H_5), mp 168–170°, $[\alpha]D$ +42°, the physical constants of which were very similar to those given in the literature[19] (mp 168°, $[\alpha]D$ +52°). For purposes of comparison, synthetic samples of the acetate and benzoate of the 7,11-dione (XI, R = Ac and R = COC_6H_5) were prepared by the method of Woodward, *et al.*[19] (Scheme II), and each proved to be identical with the material obtained by degradation of macdougallin diacetate, as determined by mixture melting points, thin layer chromatography, and infrared spectral comparisons.

Finally, Wolff–Kishner reduction of the 7,11-dione (XI, R = Ac) under the rigorous conditions of Barton, Ives, and Thomas[24] gave 14α-methylcholestanol (XVII, R = H) identified by its mass spectrum (M^+ 402) and analysis, and also by its physical constants and spectra which were almost identical with those given in the literature[19] (Table II), as were those of the acetate and benzoate. Mixture melting points of all three compounds with synthetic samples were undepressed.

Table II

	Found		Reported[19]	
XVII	Mp, °C	$[\alpha]D$, degrees	Mp, °C	$[\alpha]D$, degrees
R = H	145–147	+43.8	144–145	+39
R = Ac	98–99	+24.5	98–99	+27
R = COC_6H_5	169–171	+28	167–168	+32

When reduction of the 8(9)-ene-7,11-dione X was attempted by catalytic hydrogenation, a complex mixture of products resulted from which a small amount of a relatively nonpolar compound, mp 76–77°, was isolated by chromatography. The mass spectrum gave a molecular ion peak at *m/e* 442 and a strong peak at 382 (M^+ − 60) indicating an acetate of formula $C_{30}H_{50}O_2$, which was confirmed by the analysis and the infrared peaks at 1710 and 1245 cm^{-1}. Saponification gave the free alcohol, mp 123–124°, with molecular ion peak at *m/e* 400, and a peak due to loss of water at *m/e* 382. Oxidation with Jones reagent[14] gave the ketone, mp 131–135°, molecular ion *m/e* 398, for which the rotatory dispersion curve showed the positive Cotton effect of a 3-ketone.[15] The nmr spectrum of the original acetate showed all the peaks due to 14α-methyl-Δ⁷-cholestenol acetate (IX) plus several additional peaks, and the integral of the small olefinic proton signal at 5.15 ppm showed that it corresponded to about half of one proton.

It was thus apparent that the compound was a mixture of 14α-methyl-Δ⁷-cholestenol acetate and a double-bond isomer, probably the Δ⁸ derivative. This was confirmed by treatment of the Δ⁷ compound (IX) with hydrogen chloride gas in chloroform solution, and isolation of the Δ⁸ isomer by fractional crystallization from methanol. The progress of the crystallization was conveniently followed by gas chromatography, the retention times of the two isomers differing by 1 min at 260° on a column of 3% SE30. In this way a 95% pure sample of 14α-methyl-Δ⁸-cholesten-3β-ol acetate (XVIII) was obtained, the nmr spectrum of which

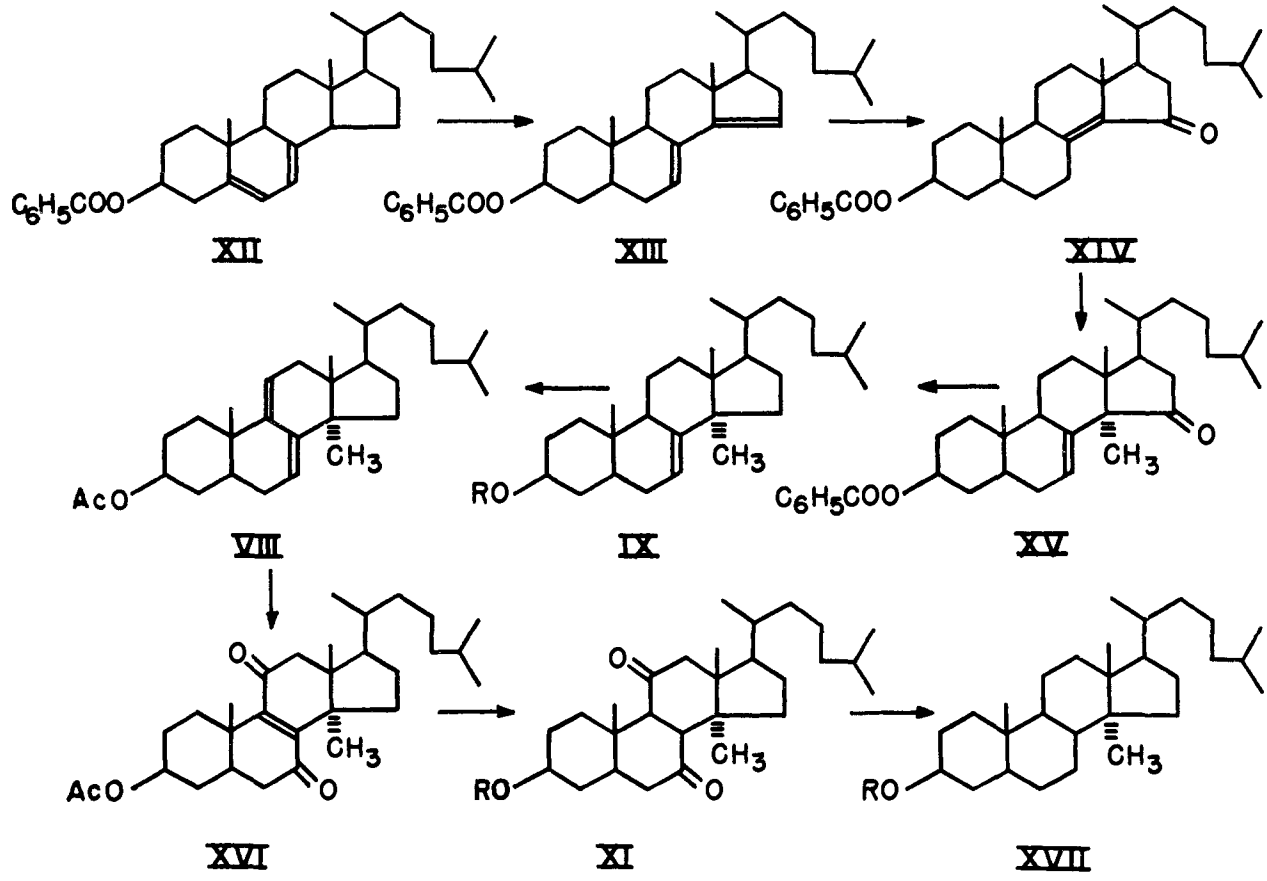
(21) L. Ruzicka, E. Rey, and A. C. Muhr, *Helv. Chim. Acta*, 27, 472 (1944).

(22) J. Fried, P. Grabowich, E. F. Sabo, and A. I. Cohen, *Tetrahedron*, 20, 2297 (1964).

(23) The absorption maximum for the -8(9)-ene-7,11-dione system is variously given as (a) 269 mμ (ε 8500) (L. F. Fieser and J. E. Herz, *J. Am. Chem. Soc.*, 75, 5356 (1953)); (b) 268 mμ (ε 6310) (L. F. Fieser, J. E. Herz, and W. Y. Huang, *ibid.*, 73, 2397 (1951)); (c) 271 mμ (ε 7900) (L. F. Fieser, W. P. Schneider, and W. Y. Huang, *ibid.*, 75, 124 (1953)); (d) 270 mμ (ε 8700) (E. M. Chamberlin, W. V. Ruyle, A. E. Erickson, J. M. Chermeda, L. M. Aliminosa, R. L. Erickson, G. E. Sita, and M. Tishler, *ibid.*, 73, 2396 (1951)).

(24) D. H. R. Barton, D. A. J. Ives, and B. R. Thomas, *J. Chem. Soc.*, 2056 (1955).

Scheme II



exhibited all of the "extra" peaks seen in that of the macdougallin degradation product. Saponification of the acetate (XVIII), mp 71–72°, $[\alpha]_D$ +35°, gave the alcohol (XVIII, OH for OAc), mp 114–116°, $[\alpha]_D$ +39°, and oxidation with Jones reagent[14] provided the ketone (XIX) mp 134-135°, $[\alpha]_D$ +60°.

Initial attempts to interpret the nmr spectra of the macdougallin degradation products were complicated by the fact that the effect of a 14α-methyl group on the 18- and 19-methyl resonances was not known. The theoretical values for the corresponding 14α-H compounds were therefore calculated from a published table of increments[25] and on comparison with the 14α-methyl compounds it was found that there was a reasonably constant difference between the theoretical and observed values (Table III). In the case of the 19-methyl group the effect was small and of the order of ± 0.045, while in the case of the 18-methyl substituent it was approximately +0.125 ppm.

Biogenetic Implications. It is now generally accepted that squalene is the common precursor of sterols and triterpenoids in both plant and animal metabolism, the differing modes of cyclization being due to the action of enzymes which impose differing conformations on the squalene chain while catalyzing the cyclization.[6] In this respect it is of interest to note the occurrence in the same plant of a pentacyclic triterpene (β-amyrin), a 4α-methyl sterol (lophenol), a 14α-methyl sterol (macdougallin) and its corresponding demethyl derivative (peniocerol), and also the phytosterol mixture. Similar mixtures have been isolated from *Lophocereus schottii*[5] which contained lupeol (XX), lophenol (I), and Δ^7-stigmastenol (XXI), and also from orange peel oil[26,27] in which citrostadienol (XXII) was accompanied by friedelin (XXIII) and β-sitosterol (XXIV).

The conversion of lanosterol to cholesterol involves three steps: the saturation of the side-chain double bond, the removal of three methyl groups, and the transfer of a double bond from 8(9) to 5(6). The first of these can take place at any stage[6] in the conversion. The removal of the methyl groups, which has been shown to require oxygen,[28] is thought to proceed by stepwise oxidation of each methyl to carboxyl, followed by its elimination as CO_2. In the case of the 4,4-dimethyl grouping, this would be assisted by temporary conversion of the 3β-hydroxy to a carbonyl group. The isolation of 14α-desmethyllanosterol (XXV) from rat liver by Bloch, *et al.*,[29] has long been considered to mean that the 14α-methyl group is the first to be removed, followed by the 4β- and 4α-methyls, though the order in which the last two are removed is not clear in view of the probable participation of the enolic form of a 3-keto group, which would presumably cause the remaining 4-methyl to assume the most stable α configuration on reketonization.

The isolation of macdougallin showed that, in plants at least, it is possible that attack on the 4,4-dimethyl

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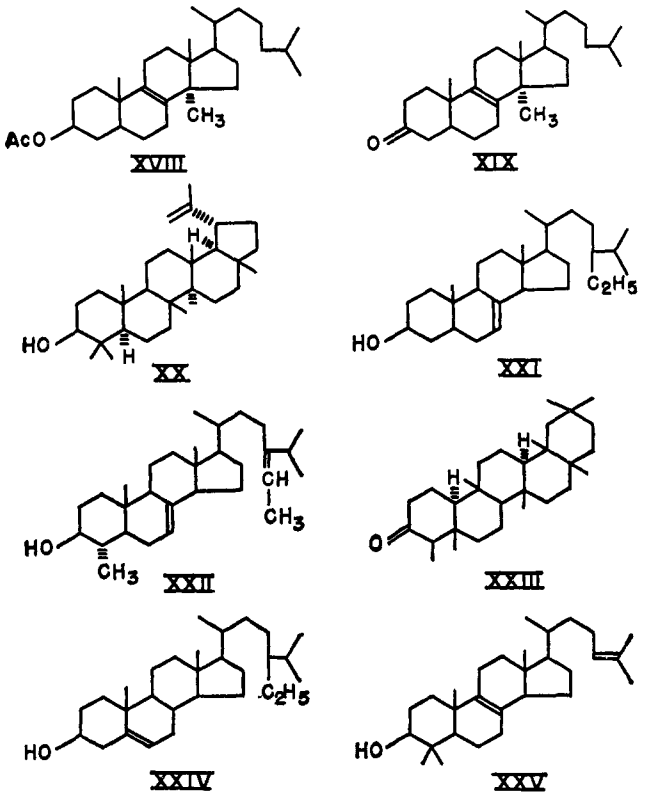
Table III. Effect of 14α-Methyl Group on Chemical Shifts of 18- and 19-Methyls

Compound	19-Methyl, ppm			18-Methyl, ppm		
	Calcd[a]	Obsd[b]	Δ^{14-Me}	Calcd[a]	Obsd[b]	Δ^{14-Me}
14α-Methylcholestan-3β-ol (XVII, R = H)	0.808	0.840	+0.032	0.650	0.800	+0.150
14α-Methyl-Δ^7-cholesten-3β-ol acetate (IX)	0.775	0.820	+0.045	0.533	0.660	+0.127
14α-Methyl-Δ^8-cholesten-3β-ol acetate (XVIII, R = Ac)	0.950	0.950	−0.000	0.567	0.700	+0.133
14α-Methyl-$\Delta^{7,9(11)}$-cholestadien-3β-ol acetate (VIII)	0.917	0.920	+0.003	0.500	0.580	+0.080
14α-Methyl-7,11-dioxocholestan-3β-ol acetate (XI, R = Ac)	1.317	1.260	−0.057	0.625	0.750	+0.125
Macdougallin diacetate (II, $R_1 = CH_3$; $R_2 = R_3 = Ac$)	0.992	1.040	+0.048	0.575	0.700	+0.125

[a] Values calculated from table published in ref 25. [b] Observed values.

group can occur prior to the removal of the 14α-methyl, and it raised the question of whether this alternate pathway might not also operate in mammalian sterol biosynthesis. Although macdougallin itself has been shown to be inert in animal tissue,[30] recent work has demonstrated that homogenates of rat liver can convert 14α-methyl-Δ^7-cholesten-3β-ol (IX) to cholesterol,[31] the rate of conversion being approximately one-fourth of that of the corresponding demethyl compound, Δ^7-cholesten-3β-ol. The fact that a reaction can be shown to occur *in vitro* is not proof that it necessarily occurs in the intact animal, but nevertheless it is evident that some aspects of the currently accepted scheme for the conversion of lanosterol to cholesterol must be reevaluated.



Experimental Section

Melting points were taken on a Kofler Block or a Fisher-Johns apparatus and are uncorrected. Optical rotations (all in chloroform) and infrared and ultraviolet spectra were measured at Stanford by Mrs. D. Aguilar with a Zeiss Model 50-370 Polarimeter, a Perkin-Elmer 421 spectrophotometer, and a Cary Applied Physics Model 14 spectrophotometer, respectively, and at Argonne by Dr. J. C. Knight using a Lippisch manual polarimeter or a Bendix photoelectric polarimeter, a Perkin-Elmer 21 spectrophotometer, and a Cary Model 11 MS spectrophotometer. Rotatory dispersion curves were recorded by Mrs. R. Records with a Japan Spectroscopic Manufacturing Co., Model ORD-5 spectropolarimeter. Drs. M. Ohashi and J. M. Wilson ran the mass spectra with a CEC mass spectrometer Model 21-103 C, and the nuclear magnetic resonance spectra were measured at Stanford by Dr. L. Durham and at Argonne by Dr. J. C. Knight using a Varian A-60 nmr spectrometer. Microanalyses were performed at Stanford Microanalytical Laboratory by Messrs. E. Meier and J. Consul, and also in the laboratory of Dr. A Bernhardt in the Max Planck Institute at Mülheim, Germany. Silica Gel HF_{254}, supplied by Brinkmann Instruments, Inc., was used for all thin layer chromatograms.

The phrase "in the usual manner" may be taken to mean the following procedure. The reaction mixture was diluted with water and extracted several times with ether. The ether extract was washed with dilute hydrochloric acid (2 *N*) and water, then with saturated sodium bicarbonate solution and water again, after which it was dried over anhydrous sodium sulfate.

Extraction of *Peniocereus macdougallii*. The dried and ground roots of *Peniocereus macdougallii* (16 kg) were extracted with hot ethanol,[32] yielding a dark brown oil (822 g). This was heated with ethanol (500 ml) and the viscous solution was poured into ether (4000 ml), stirred vigorously, and left to settle. The brown ether solution was decanted, and the process was repeated until the ether was no longer colored. The ether-insoluble brown powder thus obtained (300 g) was discarded, and the ether solution was evaporated to yield a brown oil (500 g). This was dissolved in methanol (2000 ml) containing potassium hydroxide (200 g) and water (100 ml) and the mixture was refluxed for 2 hr. The cooled solution was diluted with water and shaken with ether, then filtered through glass wool to remove insoluble black tars (which were discarded). The ether layer was washed well with dilute hydrochloric acid (2 *N*) and water, then dried over sodium sulfate and evaporated to yield a granular brown solid (168 g). This was acetylated by warming with a mixture of acetic anhydride (250 ml) and pyridine (750 ml) until the mixture was homogeneous, and then leaving to stand at room temperature overnight. Work-up in the usual manner gave an oily, red-brown acetate (180 g). The alkaline solution from the saponification was filtered and acidified with hydrochloric acid (2 *N*), then ether extracted. Evaporation of the washed and dried extract gave a crude red semisolid acid (41.8 g) which was redissolved in ether and methylated with diazomethane. Thin layer chromatography and infrared spectroscopic examination of

(30) K. Bloch, *Science*, 150, 19 (1965); M. Slaytor and K. Bloch, *J. Biol. Chem.*, 240, 4598 (1965).

(31) J. C. Knight, P. D. Klein, and P. A. Szczepanik, *J. Biol. Chem.*, in press.

(32) Collected by Dr. D. K. Cox near La Escondida, Oaxaca, Mexico. We are indebted to Dr. Cox for numerous collections and to Dr. R. Villotti, Syntex, S. A., Mexico City, for large-scale extractions performed in Mexico.

the resulting red, oily mixture of methyl esters (35.0 g) indicated that it consisted mainly of a mixture of straight-chain esters, so further work in this direction was discontinued.

Chromatography of the Crude Acetates. The crude acetates were subjected to preliminary chromatography on alumina (Merck basic, 2600 g) and the various fractions were pooled on the basis of thin layer chromatography to give seven main fractions: (1) 4:1 hexane–benzene, colorless oil, 1.33 g (discarded); (2) 4:1 hexane–benzene, colorless oil, 29.6 g; (3) 4:1 hexane–benzene, crystalline, 18.25 g; (4) 4:1 hexane–benzene, semisolid, 13. 74g; (5) 4:1 hexane–benzene, pale yellow oil, 15.00 g; (6) ether, pale yellow oil, 10.23 g; (7) 1:1 ether–methanol, black tar, 55.87 g (discarded). Fractions 2 to 5 were each rechromatographed on alumina (Woelm neutral, grade 2) in hexane–benzene 4:1, and similar fractions again were pooled to yield three distinct fractions.

A. Monoacetate Fraction. This was a mixture of steroid and triterpenoid monoacetates (see below), weight 6.8 g, 0.043% based on dry cactus.

B. Macdougallin Diacetate Fraction. Repeated crystallization of this fraction (40.2 g), mp 105–115°, separated it from the residual peniocerol diacetate that could not be removed by chromatography, and gave a pure product (30.7 g), mp 124–127°, $[\alpha]^{24}D$ +55.4° (*c* 1.165) (0.19% based on dry cactus). *Anal.* Calcd for $C_{32}H_{52}O_4$: C, 76.75; H, 10.47. Found: C, 76.82; H, 10.41. The ultraviolet spectrum showed no maxima (ϵ_{210} 4800), (ϵ_{200} 1600); ν_{max} 1735, 1250 cm^{-1} ($CHCl_3$). The nmr spectrum showed (A) at 60 Mc ($CDCl_3$) 0.7, 0.8, 0.9, 1.04, 2.0, 2.04, 4.3–5.2 ppm; (pyridine) 0.7, 0.85, 0.92, 0.98, 2.00, 4.5–5.2 ppm; (B) at 100 Mc ($CDCl_3$) 0.7, 0.84, 1.04, 2.02, 2.04 ppm. The mass spectrum showed an $M^+ - 60$ peak at *m/e* 440.

Saponification with 5% methanolic potassium hydroxide or reduction with lithium aluminum hydride gave the parent sterol macdougallin (II, $R_1 = R_2 = R_3 = H$), mp 173–174°, $[\alpha]^{25}D$ +71.8° (*c* 0.75). *Anal.* Calcd for $C_{28}H_{48}O_2 \cdot H_2O$: C, 77.36; H, 11.59. Found: C, 77.41; H, 11.45. The mass spectrum showed M^+ 416. The ultraviolet spectrum showed no maxima (ϵ_{210} 4009), (ϵ_{220} 1445). The nmr spectrum showed 0.81, 0.88, 0.90, 0.95 ppm ($CDCl_3$).

C. Peniocerol Diacetate. This was obtained as fine needles (30.5 g) (0.19% based on dry cactus) from methanol, mp 48–50°, raised by drying *in vacuo* for 2 days at room temperature to 90–93°, and further raised to 95–103° by drying for an additional period *in vacuo* at 50°, $[\alpha]D$ +45° (*c* 0.79). *Anal.* Calcd for $C_{31}H_{50}O_4$: C, 76.50; H, 10.36. Found: C, 76.51; H, 10.57. The mass spectrum showed $M^+ - 60$ at *m/e* 426. The ultraviolet spectrum showed no maxima (ϵ_{210} 10,350), (ϵ_{220} 4800); ν_{max} 1735, 1243 cm^{-1} (KBr). The nmr spectrum showed (A) at 60 Mc ($CDCl_3$) 0.8, 0.83, 0.90, 2.03 ppm; (B) 100 Mc ($CDCl_3$) 0.6, 0.85, 0.90, 1.05, 2.06, 2.10 ppm. Saponification with 5% methanolic potassium hydroxide or reduction with lithium aluminum hydride gave peniocerol, crystallizing as needles from methanol, mp 168–171°, $[\alpha]^{25}D$ +59° (*c* 0.75). *Anal.* Calcd for $C_{27}H_{46}O_2 \cdot CH_3OH$: C, 77.36; H, 11.59. Found: C, 77.41; H, 11.45. The mass spectrum showed M^+ 402. The ultraviolet spectrum showed λ_{max} 204 mμ (ϵ 4650), (ϵ_{210} 3900), (ϵ_{220} 1340). The nmr spectrum (60 Mc) showed 0.70, 0.81, 0.90, 0.95 ppm.

Purification of Monoacetate Fraction. 1. β-Amyrin Acetate. The monoacetate fraction was recrystallized from methanol–chloroform to give plates (2.8 g), mp 110–115°, raised by several crystallizations from methanol–ether to 126–128°, $[\alpha]^{24}D$ −23.7° (*c* 0.93). A small amount of material not readily soluble in the methanol–chloroform mixture was filtered off and recrystallized from methanol–ether as needles (135 mg, 0.00084% based on dry cactus), mp 237–239°, $[\alpha]^{27}D$ +79.13° (*c* 1.11). A mixture melting point with a sample of β-amyrin acetate was not depressed and their infrared spectra could be superimposed (lit.[33] mp 241°, $[\alpha]D$ +82°). Saponification with 10% methanolic potassium hydroxide provided β-amyrin, mp 198–199°, mass spectrum M^+ 426 ($C_{30}H_{50}O$) (lit.[33] mp 197°). A mixture melting point with authentic β-amyrin was not depressed, and their infrared spectra were superimposable.

2. Lophenol Acetate. The mother liquors from the crystallization of the monoacetate fraction gave on evaporation *in vacuo* a pale yellow oil (3.03 g) which was reduced with lithium aluminum hydride to give the parent sterols. The solid sterol mixture (2.00 g) was chromatographed on alumina (Woelm neutral, grade 2, 80 g) in benzene. Two crystalline fractions, each showing only one spot on a chromatoplate, were eluted. The first of these (0.25 g) (0.0016% based on dry cactus) melted at 148–150°, raised to 151–153° by four crystallizations from methanol, $[\alpha]^{29}D$ +1.01° (*c* 0.99). Mass spectrometry gave the molecular weight as 400, correct for $C_{28}H_{48}O$: A mixture melting point with a specimen of lophenol (lit.[5] mp 149–151°, $[\alpha]D$ +5°) was not depressed, and after intensive drying *in vacuo* both samples gave identical infrared spectra. On examination by gas chromatographic analysis both samples had the same retention time, 12.5 min. A stainless steel column, 6 ft × 1/8 in., packed with 5% SE30 on Chromsorb W, at 290°, and a Hi-Fi instrument (Wilkens Instrument and Research Co.) were used.

3. Cholesterol, Campesterol, and β-Sitosterol Acetates. The second crystalline fraction (eluted after lophenol) was reacetylated and shown by gas chromatographic analysis to consist of three sterol acetates corresponding to the mixture (mp 110–115, $[\alpha]D$ −23.7°), obtained by direct crystallization of the monoacetate fraction (see above). Examination of this mixture and the corresponding dihydrosterol acetates by mass spectrometry and gas chromatography showed that it was a mixture of cholesterol, campesterol, and β-sitosterol acetates, similar to that isolated from *P. fosterianus* and *W. viperina*,[11] the characterization of which was already described in detail elsewhere.[11]

Reactions of Macdougallin (II, $R_1 = CH_3$; $R_2 = R_3 = H$). Ozonolysis of Macdougallin Diacetate (II, $R_1 = CH_3$; $R_2 = R_3$ = Ac). Macdougallin diacetate (240 mg) was ozonized in methylene chloride (10 ml) at −70°. Hydrogen peroxide (7 ml of 30%) was added and the mixture was stirred for 16 hr. The organic phase was washed with ferrous sulfate solution, dried, and evaporated. The residue solidified in contact with methanol, and **14α-methyl-8α,9α-oxidocholestane-3β,6α-diol diacetate** (V) could be crystallized for the same solvent as needles (90 mg), mp 146–148°, $[\alpha]D$ 28.3° (*c* 0.92). *Anal.* Calcd for $C_{32}H_{52}O_5$: C, 74.37; H, 10.14. Found: C, 74.49; H, 10.37. The mass spectrum showed $M^+ - 60$ at *m/e* 456; ν_{max} 1730, 1240–1260 cm^{-1} ($CHCl_3$).

Partial Saponification of Macdougallin Diacetate (II, R = CH_3; $R_2 = R_3$ = Ac). Macdougallin diacetate (1.25 g) was dissolved in a mixture of methanol (44 ml) and dioxane (14 ml) and a solution of potassium carbonate (0.25 g) in water (4.8 ml) was added. The mixture was stirred under nitrogen for 2.5 hr while the temperature was maintained at 45°, and then poured into water (200 ml) and extracted with ether. The extract was washed with water, dried over magnesium sulfate, and evaporated to yield a colorless oil which was chromatographed on alumina (Woelm grade 2, neutral, 80 g). Elution with ether–benzene 1:19 gave unchanged starting material (15 mg) and continued elution with increasing concentrations of ether in benzene gave first what is presumed to be **14α-methylcholest-8-ene-3β,6α-diol 3β-acetate** (II, $R_1 = CH_3$; R_2 = Ac; $R_3 = H$) (12 mg) and then **14α-methylcholest-8-ene-3β,6α-diol 6α-acetate** (II, $R_1 = CH_3$; $R_2 = H$; R_3 = Ac) (0.51 g) as an amorphous, glass-like solid; ν_{max} 3590, 1725, 1260 cm^{-1}. *Anal.* Calcd for $C_{30}H_{50}O_3$: C, 78.55; H, 10.99. Found: C, 77.88; H, 10.87.

Finally, elution with ether–methanol 1:4 gave macdougallin (II, R = CH_3; $R_2 = R_3 = H$) (0.53 g) which was directly reacetylated and crystallized from methanol as needles, mp 122–126°.

14α-Methyl-Δ⁸-cholesten-3-on-6α-ol Acetate (VI, R = Ac). A solution of 14α-methyl-Δ⁸-cholestene-3β-6α-diol 6α-acetate (II, $R_1 = CH_3$; $R_2 = H$; R_3 = Ac) (300 mg) in acetone (12 ml) was stirred and cooled to 0° under nitrogen, and Jones reagent[14] added until the solution had a faint orange tinge. After an additional 3 min, methanol was added and the solution was diluted and ether extracted. The washed and dried extract was evaporated to yield a crystalline solid (170 mg) which crystallized from methanol, giving needles of **14α-methyl-Δ⁸-cholesten-3-on-6α-ol acetate** (VI, R = Ac) (140 mg), mp 117–118°, $[\alpha]^{26}D$ +77.9° (*c* 1.04). *Anal.* Calcd for $C_{30}H_{48}O_3$: C, 78.89; H, 10.59. Found: C, 78.63; H, 10.75. The mass spectrum showed $M^+ - 60$ at *m/e* 396; ν_{max} 1720, 1705, 1250 cm^{-1} ($CHCl_3$). Rotatory dispersion showed α_{600} +68°, α_{359} +71°, α_{310} +716° (peak), α_{280} +517° (trough), α_{270} +557° (*c* 1.01 MeOH). The rotatory dispersion curve in methanolic HCl showed no Cotton effect.

Deuterium Exchange of 14α-Methyl-Δ⁸-cholesten-3-on-6α-ol (VI, R = H). 14α-Methyl-Δ⁸-cholesten-3-on-6α-ol acetate (VI, R = Ac) (30 mg) was dissolved in a solution of sodium methoxide prepared from sodium (20 mg) and deuteriomethanol (2.5 ml) and containing deuterium oxide (1 drop). The mixture was refluxed for 16 hr under nitrogen, then cooled and diluted with deuterium oxide (1 ml), and extracted with dry ether (15 ml). The ether layer was separated, dried, and evaporated, and the residue of

(33) L. C. King, C. D. Ball, B. Riegel, C. E. Schweitzer, P. G. Smith, and E. W. Meyer, *J. Am. Chem. Soc.*, **65**, 1168 (1943).

14α-methyl-Δ⁸-cholesten-3-on-6α-ol (VI, R = H) was purified by percolation through alumina (Woelm grade 2, neutral, 3 g) deactivated by addition of 3% deuterium oxide. Examination by mass spectrometry established the presence of molecules containing up to four atoms of deuterium, with molecular ions at 414 to 418. The calculated percentages are: d_0, 22.2%; d_1, 21.5%; d_2, 25.9%; d_3, 22.2%; d_4 8.2%.

14α-Methyl-Δ⁸-cholesten-3-on-6α-ol (VI, R = H). 14α-Methyl-Δ⁸-cholesten-3-on-6α-ol acetate (VI, R = Ac) (50 mg) was dissolved in methanol (3.5 ml) and added to a solution of sodium (100 mg) in methanol (2.4 ml) and water (1.2 ml). The mixture was refluxed for 1 hr under nitrogen, then evaporated to dryness *in vacuo*. Water was added, and the hydroxy ketone was extracted into ether and purified by chromatography on alumina (Woelm, neutral, grade 2, 5 g). Elution with ether–benzene 1:4 produced 22 mg of **14α-methyl-Δ⁸-cholesten-3-on-6α-ol** (VI, R = H), mp 120–130°. The mass spectrum showed M⁺ 414 ($C_{28}H_{46}O_2$). Rotatory dispersion showed α_{600} +80°, α_{589} +85°, α_{370} +270°, α_{300} + 575° (peak), α_{280} + 350°, α_{260} 0° (*c* 0.11, MeOH).

Oppenauer Oxidation of Macdougallin. (II, R_1 = CH_3; R_2 = R_3 = H) Macdougallin (1.0 g) was dissolved in a mixture of toluene (100 ml) and cyclohexanone (50 ml) and 50 ml of the solvent was removed by distillation. The solution then was cooled slightly, and aluminum isopropoxide (0.5 g) was added. Slow distillation was continued for 15 min, then the solution was allowed to reflux for an additional 15 min, cooled, and diluted with aqueous sodium potassium tartrate. The solvents were removed from the mixture by steam distillation, and the residue was extracted from the cooled solution with ether. The ether solution was dried over $MgSO_4$ and evaporated, and the residual oil was chromatographed on alumina (Woelm neutral, grade 2, 30 g). Benzene eluted a small amount of crystalline material, which was crystallized from methanol twice to yield needles (3 mg) of what is presumably **14α-methyl-Δ⁸-cholestene-3,6-dione**, mp 169–171°. The mass spectrum showed M⁺ 412 ($C_{22}H_{44}O_2$); ν_{max} 1700 cm⁻¹. Further elution with benzene–ether 9:1 gave **14α-methyl-Δ⁸-cholesten-3-on-6α-ol** (VI, R = H) (0.29 g) as a white solid, mp 120–130°, $[\alpha]^{31}D$ 80.2° (*c* 0.91), the melting point of which was unchanged after three crystallizations from hexane–benzene. The mass spectrum showed M⁺ 414 ($C_{28}H_{46}O_2$); ν_{max} 3420, 1700 cm⁻¹. Acetylation of VI (R = H) with acetic anhydride and pyridine at room temperature overnight gave, on working up in the usual manner, needles of 14α-methyl-Δ⁸-cholesten-3-on-6α-ol acetate (VI, R = Ac), mp 113–115° after crystallization from methanol. A mixture melting point with a sample prepared by partial hydrolysis and oxidation of Macdougallin diacetate (see above) was not depressed. Finally, ether–methanol eluted unchanged macdougallin, giving needles of the diacetate (0.5 g), mp 122–124°, on reacetylation and crystallization from methanol.

Attempted Hydrogenation of Macdougallin Diacetate. Macdougallin diacetate was recovered unchanged after attempted hydrogenation under the following conditions: (1) 10% Pd–C catalyst in ethyl acetate overnight; (2) PtO_2 catalyst in ethyl acetate overnight; (3) PtO_2 catalyst in acetic acid overnight; (4) PtO_2 catalyst in acetic acid containing a little HCl at 100° and 45 psi overnight.

Attempted Isomerization of Macdougallin Diacetate. Dry hydrogen chloride was passed into a solution of Macdougallin diacetate (122 mg) in chloroform (25 ml) at −30° for 3 hr. Dry nitrogen was then passed through the solution for 1 additional hr, and the solvent was removed under reduced pressure. The residue, after recrystallization from methanol, proved to be identical with the starting matrial, as shown by a mixture melting point determination and infrared spectrum comparison. Similar treatment of the parent sterol also resulted in recovery of the starting material.

14α-Methyl-Δ⁷,⁹⁽¹¹⁾-cholestadien-3β-ol Acetate (VIII). A. From Macdougallin Diacetate. Macdougallin diacetate (400 mg) was dissolved in acetic acid (60 ml) and the solution was saturated with hydrogen chloride at room temperature and left to stand for 18 hr. Dry nitrogen then was blown through the solution to remove the hydrogen chloride, and the solvent was removed under reduced pressure. The residue, a pale yellow oil which showed three spots on a chromatoplate (developed in 5% ethyl acetate–hexane), was chromatographed on alumina (Woelm grade 2, neutral, 20 g) in hexane. The first few fractions, containing all the material that could be eluted with hexane, were pooled, and the column then was stripped with ether to give a fraction that consisted almost exclusively of unchanged starting material. This was recycled through the above procedure several times, and the combined hexane eluates from five successive treatments were pooled and evaporated to give a crystalline solid (100 mg). Purification by preparative thin layer chromatography on silica gel HF_{254}, followed by crystallization from methanol, gave **14α-methyl-Δ⁷,⁹⁽¹¹⁾-cholestadien-3β-ol acetate** (VIII) as large flat plates (60 mg), mp 77–78°, $[\alpha]^{29}D$ +66.3° (*c* 1.0). *Anal.* Calcd for $C_{30}H_{48}O_2$: C, 81.76; H, 10.98. Found: C, 82.21; H, 10.83. The mass spectrum showed M⁺ 440; ν_{max} 1740, 1250 cm⁻¹ (KBr); λ_{max} 234 mμ (ε 17,300), 243 (19,900), 251 (ε 13,400). The nmr spectrum showed 0.58, 0.84, 0.92, 2.0, 4.5–5.00, 5.30–5.50 ppm. After collection of the final hexane eluate, the column was stripped with ether to yield an oil from which macdougallin diacetate (150 mg) was recovered by crystallization from methanol.

B. From Synthetic 14α-Methyl-Δ⁷-cholesten-3β-ol Acetate (IX). A sample of 14α-methyl-Δ⁷-cholesten-3β-ol acetate (IX) was prepared by the method of Woodward, *et al.*,[19,31] and had the following constants: mp 98–99°, $[\alpha]^{28}D$ +4.7° (*c* 1.04) (lit.[19] mp 95–96°, $[\alpha]D$ +4°). *Anal.* Calcd for $C_{30}H_{50}O_2$: C, 81.39; H, 11.38. Found: C, 81.34; H, 11.36. The nmr spectrum showed 0.66, 0.82, 0.90, 0.96, 2.02, 4.5–5.0, 5.15 ppm ($CDCl_3$); ν_{max} 1715, 1250 cm⁻¹ ($CHCl_3$). A sample of this material (800 mg) was dissolved in acetic acid (50 ml) and refluxed for 3 hr with selenium dioxide (800 mg). The solution then was filtered through Celite, diluted with water, and extracted with ether. The ether solution was washed well with water and sodium bicarbonate solution, then dried over sodium sulfate and evaporated. The resulting orange oil was chromatographed on alumina in 9:1 hexane–benzene, and the **14α-methyl-Δ⁷,⁹⁽¹¹⁾-cholestadien-3β-ol acetate** (VIII) was obtained as a colorless oil that crystallized from methanol as large, thin plates (570 mg), mp 78–79°, $[\alpha]^{26}D$ +68.9° (*c* 1.12). *Anal.* Calcd for $C_{30}H_{48}O_2$: C, 81.76; H, 10.98. Found: C, 81.43; H, 10.95.

A mixture melting point with the sample obtained from macdougallin was undepressed, and their infrared, ultraviolet, and nmr spectra were identical in every way.

Chromium Trioxide Oxidation of Macdougallin Diacetate. Macdougallin diacetate (5.0 g) was dissolved in acetic acid (200 ml) and treated with chromium trioxide (7.2 g) in 90% acetic acid (200 ml) at 17–20°, and left at room temperature for 44 hr. The solution then was diluted with water and ether extracted, and the extract was washed with water, sodium bicarbonate solution, and water again, then dried over magnesium sulfate and evaporated. The resulting yellow oil was dissolved in a small amount of methanol and left to crystallize, and in this way two crops of yellow prisms, totalling 0.85 g, were obtained. The mother liquors were concentrated *in vacuo* and chromatographed on three large chromatoplates (20 cm × 100 cm × 2 mm) spread with silica gel HF_{254}, and developed with hexane containing 20% ethyl acetate. The yellow band due to the enedione diacetate was visible without the need for a spray reagent, and after elution with ether from the excised absorbent gave a yellow oil that crystallized from methanol, yielding an additional 0.95 g of **14α-methyl-7,11-dioxo-Δ⁸-cholestene-3β,6α-diol diacetate** (X) (total yield 1.8 g). The analytical specimen was further purified by chromatography on a small (20 × 20 cm) chromatoplate, and had the following constants: mp 125–127°, $[\alpha]^{26}D$ +122.4° (*c* 1.3). *Anal.* Calcd for $C_{32}H_{48}O_6$: C, 72.69; H, 9.15. Found: C, 72.87; H, 9.27. The mass spectrum showed M⁺ 528; λ_{max} 270 mμ (ε 6378); ν_{max} 1739, 1709, 1681, 1245 cm⁻¹ (CCl_4). The nmr spectrum showed (A) ($CDCl_3$) 0.82, 0.91, 1.23, 1.40, 2.02, 2.18, 2.46, 2.70, 4.5–4.9, 5.13, 5.36 ppm; (B) (C_6H_6) 0.64, 0.88, 0.98, 1.15, 1.25, 1.75, 1.85.

14α-Methyl-7,11-dioxocholestan-3β-ol Acetate (XI, R = Ac). A. From Zinc–Acetic Acid Reduction of the Enedione Diacetate X. 14α-Methyl-Δ⁸-cholestene-7,11-dioxo-3β,6α-diol diacetate (X, 1.00 g) was dissolved in acetic acid (60 ml) and stirred under reflux with zinc dust (1.00 g) for 1 hr. The solution then was filtered, diluted, and ether extracted, and the extract was worked up in the usual way to give a colorless oil that slowly crystallized. Recrystallization from methanol gave **14α-methyl-7,11-dioxocholestan-3β-ol acetate** (XI, R = Ac) as long, white needles (340 mg), mp 157–159°, $[\alpha]^{26}D$ 40.2° (*c* 1.10). *Anal.* Calcd for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24. Found: C, 76.05; H, 10.28. The mass spectrum showed M⁺ 472; ν_{max} 1724, 1701, 1250 cm⁻¹ ($CHCl_3$). The nmr spectrum showed 0.75, 0.85, 0.95, 1.26, 2.02, 2.36, 2.55, 2.62, 4.4–4.9 ppm ($CDCl_3$). The melting point was not depressed by admixture with a synthetic sample prepared as described below, and their infrared and nmr spectra were superimposable. Saponification with 5% methanolic potassium hydroxide gave the corresponding alcohol (XI, R = H), which was treated with benzoyl chloride in pyridine to give the benzoate (XI, R = COC_6H_5), mp 168–170°, $[\alpha]^{26}D$ +42° (*c* 1.0) (lit.[19] mp 168°, $[\alpha]D$ +52°). *Anal.* Calcd for $C_{35}H_{50}O_4$: C, 78.61; H, 9.42. Found: C, 78.55; H, 9.38.

B. By Synthesis from 14α-Methyl-$\Delta^{7,9(11)}$-cholestadien-3β-ol Acetate (VIII). A synthetic sample of 14α-methyl-$\Delta^{7,9(11)}$-cholestadien-3β-ol acetate (VIII, 350 mg) in acetic acid (150 ml) was added to a solution of chromium trioxide (325 mg) in acetic acid (10 ml). The mixture was kept at 90° for 2 hr, then diluted and worked up in the usual way to give a yellow oil which was chromatographed on a 2-mm thick chromatoplate (20 × 20 cm) spread with silica gel HF_{254} and developed in hexane containing 6% ethyl acetate. The yellow band corresponding to the enedione monoacetate (XVI) was cut out and eluted with ether, yielding a yellow oil which gave needles (134 mg) of **14α-methyl-7,11-dioxo-Δ^8-cholesten-3β-ol acetate** (XVI) on crystallization from methanol, mp 116–118°, $[\alpha]^{26}D$ +94.2° (*c* 1.28); λ_{max} 271 mμ (ε 8404). *Anal.* Calcd for $C_{30}H_{46}O_4$: C, 76.55; H, 9.85. Found: 76.33; H, 9.78. The nmr spectrum showed 0.82, 0.93, 1.20, 1.30, 2.04, 2.7, 3.0 ppm ($CDCl_3$).

The enedione monoacetate XVI (80 mg) was dissolved in acetic acid (10 ml) and stirred under reflux for 1 hr with zinc dust (200 mg). The cooled and filtered solution was diluted with water and worked up in the usual manner to give a white solid which gave, on crystallization from methanol, needles (56 mg) of 14α-methyl-7,11-dioxocholestan-3β-ol acetate (XI, R = Ac), mp 157–158°, $[\alpha]^{26}D$ +36° (*c* 1.04). This compound was identical with that obtained by reduction of the enedione diacetate X derived from macdougallin, as determined by a mixture melting point and infrared spectral comparison.

14α-Methyl-Δ^8-cholesten-3β-ol Acetate (XVIII, R = Ac). A. By Catalytic Reduction of 14α-Methyl-7,11-dioxo-Δ^8-cholestene-3β-6α-diol Diacetate (X). The enedione diacetate X (300 mg) was dissolved in acetic acid (9 ml) and stirred in an atmosphere of hydrogen in the presence of platinum dioxide catalyst (155 mg) for 14 hr at a temperature of about 50°. The solution then was filtered, diluted, and worked up in the usual way to give a colorless oil that showed no maxima in the ultraviolet. Chromatography on alumina (Woelm neutral, grade 2, 20 g) using gradient elution (ether into 500 ml of hexane) gave a small amount of a crystalline solid, further purified by preparative tlin layer chromatography, after which it crystallized from methanol as needles (40 mg), mp 76–77°. *Anal.* Calcd for $C_{30}H_{50}O_2$: C, 81.39; H, 11.38. Found: C, 81.10; H, 11.50. The mass spectrum showed M^+ 442; ν_{max} 1710, 1250 cm^{-1} ($CHCl_3$). The nmr spectrum showed 0.70, 0.80, 0.95, 2.0, 4.4–5.0, 5.15 ppm ($CDCl_3$), clearly showing that this compound was a mixture of double bond isomers. Saponification of the acetate (20 mg) with 10% methanolic potassium hydroxide gave the parent alcohols as needles from methanol (15 mg), mp 123–124°. The mass spectrum showed M^+ 400 ($C_{28}H_{48}O$). Oxidation with Jones reagent[14] under the usual conditions provided the corresponding ketones as a pale yellow oil that spontaneously crystallized (9 mg). After two sublimations at 130° (10^{-5} mm) this was obtained as a colorless crystalline solid, mp 131–135°, mass spectrum M^+ 398 ($C_{28}H_{46}O$); ν_{max} 1700 cm^{-1} ($CHCl_3$). The rotatory dispersion curve showed α_{600} +31.8°, α_{589} +31.8°, α_{308} +695° (peak), α_{282} 0°, α_{256} −410° (trough), α_{250} −368° (*c* 0.11, MeOH).

B. By Isomerization of 14α-Methyl-Δ^7-cholesten-3β-ol Acetate (IX). 14α-Methyl-Δ^7-cholestene-3β-ol acetate (IX, 410 mg) was dissolved in chloroform (25 ml) and the solution was saturated with hydrogen chloride at room temperature. After standing for 2 hr, the solution was evaporated to dryness, and the resulting colorless oil crystallized from methanol as needles (270 mg), mp 86–89°. A second crop (90 mg), mp 78–79°, was obtained on concentration of the mother liquors, and the residual mother liquors were set aside. The two crystalline crops then were pooled and again were treated with hydrogen chloride as described above. Crystallization of the product gave fine needles (170 mg) of starting material, mp 90–91°. The mother liquors of this crop were pooled with those obtained from the first HCl treatment, and concentrated to give four successive crops of crystalline material. The last two crops (crop D, 40 mg; and crop E, 15 mg) were shown by gas chromatographic analysis to consist of 90 and 95% **14α-methyl-Δ^8-cholesten-3β-ol acetate** (XVIII, R = Ac), respectively, the remainder being unchanged Δ^7 isomer. An A-660 instrument (Wilkens Instrument and Research Co.) was used, with a column containing 3% SE-30 on 60–80 mesh Chromsorb W previously treated with hexamethyldisilazane, at 265°; the retention times were 8.9 (Δ^8) and 9.8 min (Δ^7). Crop D had $[\alpha]^{26}D$ +28° (*c* 0.89); crop E had $[\alpha]^{26}D$ +35.9° (*c* 0.75). The nmr spectrum showed 0.70, 0.82, 0.90, 0.96, 2.02, 4.4–4.9 ppm. Crops D and E were each saponified by refluxing with 5% methanolic potassium hydroxide, and the free sterols were extracted from the cooled solution with ether and recrystallized from methanol to give **14α-methyl-Δ^8-cholesten-3β-ol** (XVIII, R = H), mp 115–116°, $[\alpha]^{26}D$ +42° (*c* 1.12), from crop D, and mp 114–116°, $[\alpha]^{26}D$ +39° (*c* 0.47), from crop E. Oxidation of crop D with Jones reagent[14] in the usual way gave **14α-methyl-Δ^8-cholesten-3-one** (XIX) as a crystalline solid, mp 133–135°, raised to 134–135° by one crystallization from methanol, $[\alpha]D$ 59.8° (*c* 0.99); ν_{max} 1715 cm^{-1} (CCl_4).

Wolff–Kishner Reduction of 14α-Methyl-7,11-dioxocholestan-3β-ol Acetate (XI, R = Ac). Sodium (200 mg) was dissolved in redistilled diethylene glycol (20 ml), and hydrazine (previously dried by refluxing with potassium hydroxide pellets) was distilled into the stirred solution (under nitrogen) until it refluxed at 180°. The mixture was then cooled to 80°, and 14α-methyl-7,11-dioxocholestan-3β-ol acetate (XI, R = Ac; obtained from macdougallin *via* the enedione diacetate X, 300 mg) was added, after which the mixture was allowed to reflux at 180° for 21 hr. Hydrazine then was allowed to distil from the mixture until the temperature had risen to 210°, where it was maintained for an additional 24 hr. After cooling, the solution was diluted with water and the product was isolated by ether extraction in the usual manner, as a white crystalline solid, which gave long thin needles of **14α-methylcholestan-3β-ol** (XVII, R = H, 170 mg), mp 145–147°, $[\alpha]^{26}D$ 43.8° (*c* 1.25) (lit.[19] mp 144–145°, $[\alpha]D$ +39°). *Anal.* Calcd for $C_{28}H_{50}O$: C, 83.51; H, 12.52. Found: C, 83.44; H, 12.44. The mass spectrum showed M^+ 402. The nmr spectrum showed 0.80, 0.84, 0.90, 1.22. Treatment with acetic anhydride and pyridine at room temperature overnight gave the **acetate** XVII (R = Ac), mp 98–99°, $[\alpha]^{26}D$ +24.5° (*c* 1.14) (lit.[19] mp 98–99°, $[\alpha]D$ +27°). *Anal.* Calcd for $C_{30}H_{52}O_2$: C, 81.02; H, 11.79. Found: C, 80.97; H, 11.70. Benzoylation with benzoyl chloride and pyridine overnight gave the **benzoate** XVII (R = COC_6H_5), mp 169–171°, $[\alpha]^{26}D$ +28° (*c* 1.04) (lit.[19] mp 167–168°, $[\alpha]D$ +32°). *Anal.* Calcd for $C_{35}H_{54}O_2$: C, 82.95; H, 10.74. Found: C, 83.03; H, 10.77.

Samples of 14α-methylcholestan-3β-ol and its acetate were also prepared by Wolff–Kishner reduction of synthetic 14α-methyl-7,11-dioxocholestan-3β-ol acetate (XI, R = Ac). Mixture melting points with the samples described above were not depressed, and their respective infrared spectra were identical.