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ARIZONA FLORA: THE STEROLS OF *PENIOCEREUS GREGGII*[1]

JOHN C. KNIGHT and GEORGE R. PETTIT

Department of Chemistry, Arizona State University, Tempe, Arizona 85281

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Abstract—Roots of the cactus *Peniocereus greggii* have been found to contain sucrose, peniocerol, desoxyviperidone, viperidone, viperidinone, and β-sitosterol. Two other sterols, which may be artifacts arising from desoxyviperidone, were also isolated.

Peniocereus greggii is a comparatively rare cactus found in northern Mexico and the southern part of Arizona. The plant possesses a large tuberous root which has been known to weigh up to 50 kg,[2] although interestingly the above-ground part is small and inconspicuous. The large, fragrant, white flower of *P. greggii* blooms nocturnally and is responsible for the common name "Arizona Queen of the Night". Two other members of the same genus (*P. fosterianus*[3] and *P. macdougallii*[4]) have been found to contain the 14α-methylsterol macdougallin (I). Investigation of *P. greggii* constituents was therefore undertaken to determine if 14α-methylsterols were common to other members of the genus (and hence of some taxonomic importance), and to see if 14α-methylsterols other than macdougallin, which is still the only known naturally occurring sterol of this type, could be isolated.

The roots of four plants were dried and ground, and extracted successively with petroleum ether and ethanol. The ethanol extract was concentrated to a small volume and left to stand several days at room temperature. Large colorless prisms of sucrose slowly deposited. Concentration of the ethanolic mother liquors gave a solid, from which organic material was extracted with chloroform. Recrystallizing the chloroform residue from methanol gave a sterol, m.p. 179–181°, which exhibited i.r. and PMR spectra identical to those of peniocerol[3] (II). The diacetate, m.p. 50–51°, did not depress the m.p. of an authentic sample of peniocerol diacetate.[3,5] I.r. and mass spectra confirmed the identification. Additional peniocerol was obtained from the ether-soluble portion of the ethanol extract by chromatography on silica gel together with small quantities of two other sterols (acetates m.p. 148–150° and 186–187°) which proved identical with desoxyviperidone acetate[5,6] (III) and viperidone acetate[5,6] (IV) respectively.

The oily petroleum ether extract showed a strong ester band in the i.r. spectrum at 1735 cm^{-1}. Following saponification and methylation of the acid fraction a GLC examination of

[1] For the previous contribution in this series, see Steroids and Related Natural Products XLV: J. C. KNIGHT and G. R. PETTIT, *J. Org. Chem.* **33**, 1684 (1968). This investigation was supported by Public Health Service Research Grant No. CA-10612-01 from the National Cancer Institute.

[2] *The Fantastic Clan*, p. 26, J. J. THORNBER and F. BONKER, Macmillan, New York (1932).

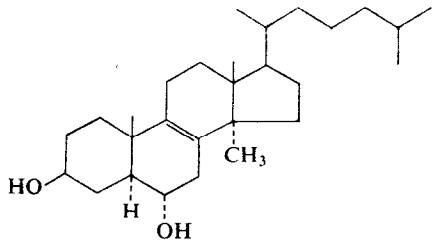
[3] C. DJERASSI, R. D. H. MURRAY and R. VILLOTTI, *J. Chem. Soc.* 1160 (1965).

[4] J. C. KNIGHT, D. I. WILKINSON and C. DJERASSI, *J. Am. Chem. Soc.* **88**, 790 (1966).

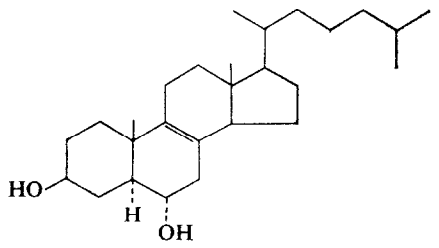
[5] We thank Prof. CARL DJERASSI, Stanford University, for kindly supplying us with authentic samples of compounds II–V.

[6] C. DJERASSI, J. C. KNIGHT and H. BROCKMANN, JR., *Chem. Ber.* **97**, 3118 (1964).

the methyl esters indicated the presence of ten compounds, the principal components being octanoate, laurate, myristate and palmitate. The neutral fraction was chromatographed on silica gel. Elution with benzene-ethyl acetate (1:2) provided more peniocerol and a relatively non-polar yellow oily fraction. The latter material was rechromatographed on silica gel and elution with ligroin–ethyl acetate (9:1) gave a crystalline fraction with approximately the same TLC mobility as cholesterol, plus several yellow semi-solid fractions. The crystalline material showed three peaks when examined by GLC, and as with similar mixtures isolated from *P. macdougallii*[4] and *Wilcoxia viperina*[6] the principal component was β-sitosterol.



(I)



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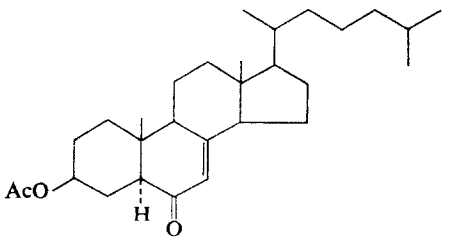

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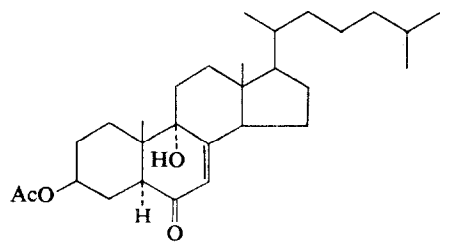

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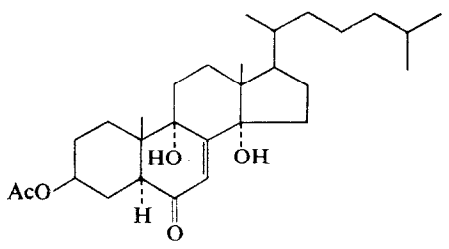

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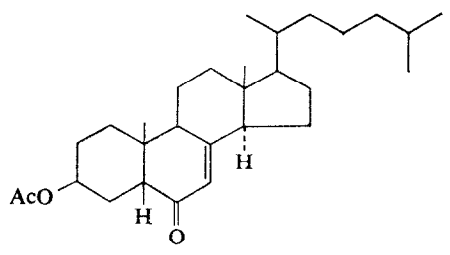
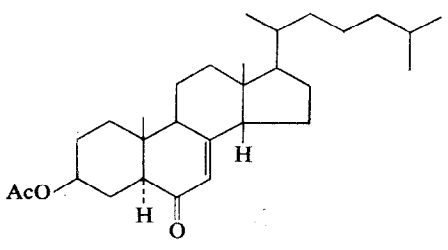
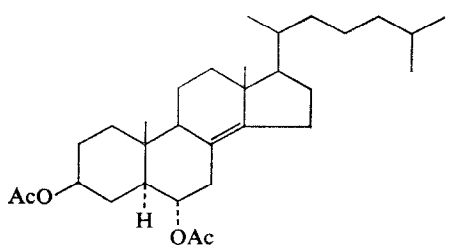


(VI)



(VII)



(VIII)

Of the remaining semi-solid fractions, all except two (A and B) were quite complex. Both A and B were acetylated and rechromatographed on silica gel. Principal components were isolated by preparative TLC. The more polar fraction (from A) was essentially viperidinone acetate (V).[5,6] The less polar fraction (from B) was separated into a major and a minor component. The mass spectrum of the latter indicated an isomer of desoxyviperidone acetate (III), and its i.r. and NMR spectra were also very similar to those of III, the major difference being replacement of the broad PMR signal at 4·70 (axial 3α-H) by a sharper response at 5·10. This indicated either a 5α-3α- or a 5β-3β-structure. Differentiation between these two possibilities was made on the basis of the rotatory dispersion curve. A comparison with the RD curves of an authentic pair of 7-en-6-ones,[7] isomeric at position 5, showed that beyond any doubt the new sterol had the 5β-configuration and it was assigned structure VI. The major component displayed an i.r. spectrum almost superimposable on that of desoxyviperidone acetate (III), but the m.p. was much lower (115–120°). Repeated preparative TLC resolved this fraction into two very similar components. The more polar proved to be desoxyviperidone acetate (III). The second and slightly less polar component gave an i.r. spectrum and ORD curve scarcely distinguishable from that of ketone III. However, in the PMR spectrum the 18-methyl resonance was shifted downfield by about 0·3δ and thereby hidden beneath signals from the secondary methyl groups. The mass spectrum indicated empirical formula $C_{29}H_{46}O_3$, isomeric with desoxyviperidone acetate. Therefore, the new substance and desoxyviperidone may differ at the C/D ring juncture and a structure such as VII may be considered. Due to the paucity of material and considerations noted in the following paragraph, efforts directed at a more definitive assignment were not undertaken.

Considering the difficulty previously encountered[6] in saponification of desoxyviperidone acetate and the ease with which artifacts are formed from the *W. viperina* steroids,[6] it is probable that the two new steroids herein reported are also artifacts formed during base saponification of the ligroin extract. Model experiments showed that treatment of desoxyviperidone acetate with 5% methanolic KOH for longer than a few minutes, followed by reacetylation, resulted in increasingly complex mixtures. Evidently base saponification procedures can be quite unsatisfactory for dealing with fatty acid ester mixtures so frequently encountered in natural products chemistry.

Since no evidence had so far been uncovered for presence of any new 14α-methylsteroid, the various crops of peniocerol were pooled and recrystallized carefully. The mother liquors were examined unsuccessfully for macdougallin. The recrystallized peniocerol was then acetylated, and isomerized to the $\Delta^{8(14)}$-isomer (VIII)[3] by treatment with palladium catalyst (under hydrogenation conditions). The isopeniocerol (VIII) was also fractionally recrystallized, again without revealing macdougallin. Thus, it would seem 14α-methylsterols are not particularly abundant in the roots of *P. greggii*, a fact which sets this cactus apart from the other two members of the genus, *P. fosterianus* and *P. macdougallii*, so far examined. Also of interest is the chemical similarity of the two distinct species, *P. greggii* and *W. viperina*.

EXPERIMENTAL

Solvent extracts of aqueous solutions were dried over anhydrous magnesium sulfate. Chromatographic solvents were redistilled and ligroin refers to a fraction boiling at 60–70°. Column chromatograms were prepared using silica gel (0·2–0·5 mm) supplied by E. Merck (Darmstadt). Preparative thin-layer chromatograms were prepared using silica gel HF 254 (E. Merck) in 2-mm layers on 400 × 200 mm plates. Gas–liquid

[7] A. Furlenmeier, A. Fürst, A. Langemann, G. Waldvogel, U. Kerb, P. Hocks and R. Weichert, *Helv. Chim. Acta* **49**, 1591 (1966).

chromatograms were obtained using a Varian Aerograph Model 1200-2 instrument equipped with a glass column packed with 3% QF-1 on Chromsorb W.

M.p.s were observed using Kofler apparatus and are uncorrected. The u.v. (MeOH solution, Cary Spectrophotometer), i.r. (KBr discs, Beckman IR-12) and NMR spectra (deuterochloroform solution with tetramethylsilane as internal standard, Varian A-60) and optical rotatory dispersion (Jasco ORD/UV-5) measurements were recorded by Miss K. Reimer. We are indebted to Dr. Peter Brown of this department for the mass spectra, which were determined employing an Atlas CH-4B spectrometer equipped with molecular beam inlet system. Element microanalyses were provided by Dr. A. Bernhardt, Max Planck Institut, Mülheim, Germany.

Extraction Procedure

Peniocereus greggii roots[8] (25 lb fresh weight) were cut into ½ in. thick slices and left (1 wk) to dry in a current of air. The dried material (1843 g) was ground (Waring Blendor) in ligroin and the suspension left to stand in the cold with occasional shaking 2 days. The yellow ligroin layer was decanted and replaced with fresh solvent, and the process repeated three more times. The combined extract was evaporated under reduced pressure, to yield a yellow-orange oil (54·5 g).

The plant material was successively spread out to dry in a fume hood several days, suspended in ethanol (95%) at room temperature, and stirred for 2 days, and filtered. The plant residue was extracted in this manner four times, and the combined extract concentrated to about 100 ml under reduced pressure (rotatory evaporator).

Ethanol Extract

A gum which separated during concentration slowly crystallized on standing several days. The dark brown, viscous supernatant liquid was decanted and the solid material dissolved in hot aqueous ethanol. Over a period of several days, three successive crops of large pale yellow prisms (totalling 31·7 g) were collected. Upon recrystallization from aqueous ethanol colorless prisms of sucrose, m.p. 188–192°, were obtained. A mixed m.p. with an authentic sample of sucrose was not depressed, and the PMR and i.r. spectrum completed confirmation.

Peniocerol. Further concentration of the sucrose mother-liquors gave another crystalline crop and this material was triturated with $CHCl_3$ and the solution filtered. The $CHCl_3$ was removed under reduced pressure and the residue crystallized from methanol to yield peniocerol (II) as fine needles (1·33 g), m.p. 173–181°. The m.p. was raised by three more crystallizations from the same solvent to 179–181° (lit.,[6] m.p. 181–183°); mass spec., *m/e* 402 (correct for $C_{27}H_{46}O_2$); ν_{max} strong hydroxyl absorption at 3300 cm^{-1}; PMR 0·61; 0·84; 0·88; 0·92; 0·98; and 3·64 (broad, multiplet) δ. Acetylation with acetic anhydride-pyridine (1:1 at room temperature, overnight) gave a diacetate, m.p. 50–51° (lit.,[6] m.p, 50–51°) which showed an M^+-60 peak at *m/e* 440 in the mass spectrum (loss of acetic acid from $C_{31}H_{50}O_4$). The i.r. spectrum of the diacetate was superimposable on that of an authentic sample.[5]

Viperidone and desoxyviperidone. The combined mother liquors from isolation of peniocerol, plus remainder of the ethanol extract (71·3 g) were dissolved in hot methanol (250 ml). Following cooling to room temperature, diethyl ether (1 l.) was added, and the brown solution decanted from precipitated material. The viscous precipitate was washed with ether (500 ml) and the combined ethereal solutions were evaporated to dryness. The 19·2 g residue was redissolved in ether. The solution was filtered to remove an insoluble sticky solid (3·15 g) and washed successively with 2 N HCl, saturated $NaHCO_3$, and water. Solvent was removed and the resultant brown semi-solid (14·0 g) was chromatographed on silica gel (300 g). Elution with ethyl acetate-benzene (1:2) gave more peniocerol (2·04 g) and smaller amounts of two less polar compounds. The latter fractions were purified by preparative TLC in $CHCl_3$/acetone/methanol (80:20:5) and then acetylated.

The more polar of the two fractions crystallized from ligroin-benzene giving needles of viperidone acetate (0·23 g) m.p. 186–187°; mass spec. M^+ 458 (correct for $C_{29}H_{46}O_4$); ν_{max} 3400 (hydroxyl), 1660 (α,β-unsaturated ketone), 1740 and 1250 (acetate) cm^{-1}; PMR 0·61 (18-methyl), 0·81, 0·92 (sec. methyls), 0·98 (19-methyl) 2·04 (3-acetate), 4·7 (3α-H), and 5·66 (7-H) δ; RD, (MeOH, c, 0·06) $[\alpha]_{550}$ −94°; $[\alpha]_{500}$ −94°; $[\alpha]_{450}$ −78°; $[\alpha]_{392}$ 0°; $[\alpha]_{349}$ +847° (peak); $[\alpha]_{334}$ 0°; $[\alpha]_{320}$ −1884°; $[\alpha]_{312}$ −3015°. The m.p. of an authentic sample[5] (187–192°, lit.[6] 190–191°) was not depressed by admixture with the above material.

The less polar fraction crystallized from ligroin to afford small prisms of desoxyviperidone acetate (60 mg) m.p. 147–149°; mass spec. M^+ 442 (correct for $C_{29}H_{46}O_2$); ν_{max} 1740 (acetate), 1680 (α,β-unsaturated ketone), 1628 (double bond), and 1250 (acetate) cm^{-1}; PMR 0·60 (18-methyl), 0·82, 0·88 (19-methyl), 0·92, 0·96 (sec. methyls), 2·04 (3-acetate), 4·70 (3α-H), and 5·74 (7-H) δ; RD (c, 0·14) $[\alpha]_{550}$ 0°; $[\alpha]_{450}$ 0°; $[\alpha]_{400}$ +169°; $[\alpha]_{345}$ +1489° (peak); $[\alpha]_{327}$ 0°; $[\alpha]_{292}$ −4567°. The m.p. of an authentic sample[5] (152–154°, lit.[6] 151°) was not depressed by admixture with the above material.

[8] Collected on July 14, 1966 and January 8, 1967 at south end of the Rincon Mountains, Pima County, Arizona, by Robert J. Barr, College of Pharmacy, University of Arizona, Tucson.

Ligroin Extract

The crude ligroin extract (54·5 g) was triturated with hot methanol and cooled. The yellow methanol solution was decanted from insoluble oil and the process repeated until nearly all methanol-soluble material had dissolved. The residue was a pale yellow viscous oil (7·2 g). Concentrating the methanol solution under reduced pressure gave an orange-yellow semi-solid (47·3 g), which was redissolved in methanol (475 ml). 25 ml of 50% KOH was added and the mixture heated at reflux 1 hr. After being poured onto crushed ice and extracted with ether, the ethereal solution was washed well with water, dried, and evaporated to a pale yellow solid (29·4 g). The alkaline solution was acidified and extracted with ether. Following removal of solvent the residual red oil was dissolved in methanol (25 ml)-boron trifluoride etherate (1 ml). The solution was heated at reflux 30 min, diluted with water and extracted with ether. The ether solution was washed with saturated $NaHCO_3$, dried and evaporated. A GLC analysis showed that the resulting mixture contained at least ten fatty acid methyl esters, with octanoate, laurate, myristate and palmitate being present in largest amounts (10% DEGS; programmed 100–165°).

Neutral material from the above saponification was dissolved in ethyl acetate and chromatographed in the same solvent on silica gel, providing more peniocerol (8·82 g) (total yield of peniocerol 12·2 g, 0·68% based on dry cactus), and a complex, less polar, yellow oily fraction (20·0 g).

The less polar oily material was rechromatographed on silica gel (250 g) in 9:1 ligroin-ethyl acetate. Three fractions were obtained and shown to be substantially pure by TLC ($CHCl_3$/acetone/methanol 16:4:1 mobile phase). The least polar was a white crystalline solid, m.p. 100–105°, raised by two more crystallizations from methanol/$CHCl_3$ to 130–133° with the same R_f (silica gel) as cholesterol. A GLC analysis showed this fraction to consist of three components with retention times of 6, 7½, and 9 min at 250°. The major component (longest retention time) was β-sitosterol, and the mixture was found similar to phytosterol mixtures isolated from *P. macdougallii*[4] and *W. viperina*.[6]

Viperidinone acetate. The more polar of the other two fractions (see preceding paragraph) was acetylated and purified by chromatography on silica gel using ligroin/ethyl acetate 17:3. The resulting acetate was crystallized from methanol as needles (0·08 g), m.p. 195–198°. Preparative TLC on silica gel HF_{254} with ligroin/ethyl acetate 7:3, followed by recrystallization from methanol gave needles of *Viperidinone acetate* (V), m.p. 199–201°, mass spec. *m/e* 456 (loss of water from M^+ 474, $C_{29}H_{46}O_5$); ν_{max} 3400 (−OH), 1740 (acetate), 1660 (unsaturated ketone), and 1240 (acetate) cm^{-1}; PMR 0·7 (18-methyl), 0·97 (19-methyl), 2·04 (acetate), 3·22 (5α-H), 4·70 (3α-H), 5·88 (7-H) δ; RD (dioxane, c, 0·462) $[\alpha]_{600}$ −22°; $[\alpha]_{500}$ −11°; $[\alpha]_{464}$ 0°; $[\alpha]_{400}$ +195°; $[\alpha]_{376}$ +725° (peak); $[\alpha]_{366}$ +606° (min); $[\alpha]_{361}$ +693° (peak); $[\alpha]_{352}$ 0°; $[\alpha]_{346}$ −292° (inflexion); $[\alpha]_{340}$ −758°. When mixed with an authentic sample,[5] m.p. 194–197° (lit.[6] 197–198°), the m.p. was undepressed.

Desoxyviperidone (III) and related compounds. The fraction of medium polarity was acetylated and chromatographed on silica gel (200 g). Elution with ligroin/ethyl acetate (4:1) provided a solid (1·50 g) which crystallized from methanol as prisms (1·07 g), m.p. 115–120°. This solid could not be purified satisfactorily by fractional crystallization from methanol, and careful TLC using ligroin/ethyl acetate (85:15 five developments) showed that it was a mixture of two closely related components. TLC on four plates (400 × 200 × 2 mm) using the same solvent system (multiple development) gave two fairly pure fractions. The more polar of these (0·3 g) was recrystallized (five times) from methanol to give large prisms of desoxyviperidone acetate (III), m.p. 148–150°, identified by comparison TLC and i.r. spectra. After five crystallizations from methanol, the less polar component was obtained as large hexagonal prisms (0·45 g), m.p. 113–115°. This was a new sterol, tentatively assigned structure VII with the following physical constants: ν_{max} 1730, 1670, 1610, and 1240 cm^{-1}; $[\alpha]_D$ 0°; λ_{max} 247 mμ (ε 15,800); PMR ($CDCl_3$) 0·86, 0·94, 0·98, 2·04, 4·7, 5·88 δ; (C_6H_6) 0·64, 0·86, 0·89, 0·94, 0·98, 1·76 δ; RD (ligroin, c, 0·485) $[\alpha]_{500}$ +21°; $[\alpha]_{450}$ +52°, $[\alpha]_{400}$ +166°; $[\alpha]_{367}$ +825°; $[\alpha]_{358}$ +518°; $[\alpha]_{352}$ +866°; $[\alpha]_{345}$ 0°; $[\alpha]_{340}$ −268°; $[\alpha]_{336}$ −83°; $[\alpha]_{326}$ −1278°; $[\alpha]_{322}$ −1196°; $[\alpha]_{312}$ −1814°; $[\alpha]_{308}$ −1773°; $[\alpha]_{300}$ −1938°; mass spec. M^+ 442 (correct for $C_{29}H_{46}O_3$).

Concentration of the mother liquors from the initial crystallization gave an oil which was chromatographed in ligroin/ethyl acetate (17:3) on silica gel. The initial fractions provided more desoxyviperidone acetate (III) (0·1 mg), m.p. 148–150°, closely followed by another compound [5β-desoxyviperidone acetate (VI)] which formed thin flakes from methanol (250 mg), m.p. 176–177°; $[\alpha]_D$ +60·2° (c, 1·08) mass spec. M^+ 442 (correct for $C_{29}H_{46}O_3$); PMR 0·63 (18-methyl), 0·94, 0·98 (19-methyl), 1·02, 2·06 (O-acetate), 5·10 (3α-H), 5·72 (7-H); RD (ligroin, c, 0·115) $[\alpha]_{550}$ +108°; $[\alpha]_{450}$ +239°; $[\alpha]_{400}$ +412°; $[\alpha]_{375}$ +847°; $[\alpha]_{367}$ +738°; $[\alpha]_{359}$ +1064°; $[\alpha]_{348}$ +347°; $[\alpha]_{342}$ +543°; $[\alpha]_{338}$ 0°; $[\alpha]_{332}$ −304°; $[\alpha]_{327}$ −196°; $[\alpha]_{318}$ −695°; $[\alpha]_{313}$ −608°; $[\alpha]_{306}$ −759°; $[\alpha]_{282}$ −608°; $[\alpha]_{261}$ −1151°; ν_{max} 1743, 1667, 1627, 1278 cm^{-1}.

These two new compounds are therefore isomeric with desoxyviperidone acetate and similar to it in structure. Since they were evidently not the hoped-for 14α-methylsterols, and were probably artifacts obtained during the saponification step (as noted in the discussion), they were not further investigated.

Isomerization of peniocerol diacetate. Peniocerol diacetate (3·34 g) was dissolved in ethyl acetate (200 ml) containing acetic acid (25 ml), and 10% palladium on charcoal added (0·25 g). The solution was stirred under hydrogen for 19 hr, filtered and washed well with water. Evaporation of solvent gave an oil that readily

crystallized from methanol, providing 3β-6α-diacetoxycholest-8(14)-ene (VIII) as large plates (2·72 g) m.p. 140–142° (lit.[3] 141–143°). Fractional recrystallization failed to reveal any macdougallin diacetate.

Diacetate VIII (2·0 g) was dissolved in 5% methanolic KOH (60 ml) and the solution heated at reflux 2 hr, diluted with water and extracted with $CHCl_3$. The $CHCl_3$ was washed with dilute HCl acid and water, dried and evaporated. The residue crystallized from ethyl acetate, providing fine needles of 3β,6α-dihydroxycholest-8(14)-ene (1·35 g), m.p. 197–199° (lit.[3] 195–196°).