Tetrahedron, 1966, Vol. 22, pp. 1103 to 1111. Pergamon Press Ltd. Printed in Northern Ireland

CONSTITUENTS OF *WITHANIA SOMNIFERA* DUN—V

STUDIES ON SOME MODEL STEROIDAL EPOXIDES[1]

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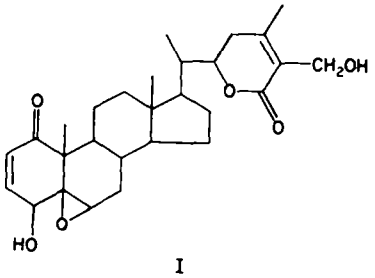
(*Received* 30 *September* 1965)

Abstract—Several steroidal 5,6-epoxy-derivatives bearing an oxygen substituent at C_4 have been synthesized and the stereochemistry of rings A and B studied.

IN CONNECTION with our studies[1] on the stereochemistry of the naturally occurring withaferin A (I), a compound with a steroid like structure in which rings A and B have a rather unusual substitution pattern, an investigation was undertaken on steroidal 5,6-epoxy derivatives bearing an oxygen substituent at C_4. Steroidal epoxides have been extensively studied, however it is interesting to note that no reports have been recorded in the literature for such epoxidic compounds.



I

For the preparation of the proposed epoxides the required 4β-acetoxy-Δ^5-cholestene (IV)[3] was prepared by the hydrolytic opening in the presence of perchloric acid[3b] of the oxide ring in 4α,5α-epoxy-cholestane (II)[3a] affording in excellent yields the known 4β,5α-diol (IIIa); Darzens' dehydration[3c] performed at 0° on the corresponding acetate (IIIb) resulted in the desired compound IV. When this product (IV) was treated with perbenzoic acid in benzene solution a mixture consisting of the α-oriented epoxide (Vb) and its β isomer (VIb) was obtained in a ratio of about 9:1 respectively and the two compounds were separated in a pure form by careful chromatography on acid washed alumina.

The α-orientation of the epoxide in V was derived by the treatment of Vb with hydrobromic acid in acetic acid solution yielding, following the diaxial opening of the epoxide,[4] the 4β-acetoxy-5α-hydroxy-6β-bromo-derivative (VII); debromination using

[1] D. Lavie, E. Glotter and Y. Shvo, *J. Chem. Soc.* in press (1965).
[2] Recipient of a scholarship from "Plantex Ltd", Nathania, Israel.
[3] [a] R. E. Ireland, T. I. Wrigley and W. G. Young, *J. Amer. Chem. Soc.* **80**, 4604 (1958); [b] A. Bowers, E. Denot, R. Urquiza and L. M. Sanchez-Hidalgo, *Tetrahedron* **8**, 116 (1960); [c] D. N. Jones, J. R. Lewis, C. W. Shoppee and G. H. R. Summers *J. Chem. Soc.* 2876 (1955).
[4] A. Fürst and P. A. Plattner, *Helv. Chim. Acta* **32**, 275 (1949).

Raney nickel formed the known 4β-acetoxy-5α-hydroxy-cholestane (IIIb) which was previously used in our preparation sequence.

The β-orientation of the epoxide in the second isomer which was obtained from the above mixture, was demonstrated by a similar treatment of the compound with hydrobromic acid, yielding now the bromohydrin (VIII) 4β-acetoxy-5β-hydroxy-6α-bromo-cholestane. This assignment was confirmed by debromination with Raney nickel to the 4β-acetoxy-5β-hydroxy-cholestane (IX), the structure of which was proven unequivocally by comparison with a synthetic sample prepared for this purpose.[5] According to the reported data[6] concerning the process of the opening of 5β,6β-steroidal epoxides bearing a substituent at C_3, the diaxial 5α-bromo-6β-hydroxy derivative should be obtained. Unexpectedly the diequatorial 5β-hydroxy-6α-bromo compound (VIII) was formed; its formation could be rationalized by a critical examination of the Dreiding models of both VIb and VIII.[7]

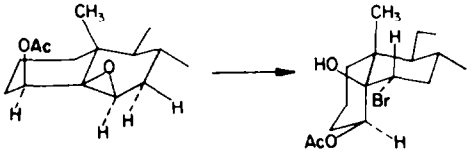


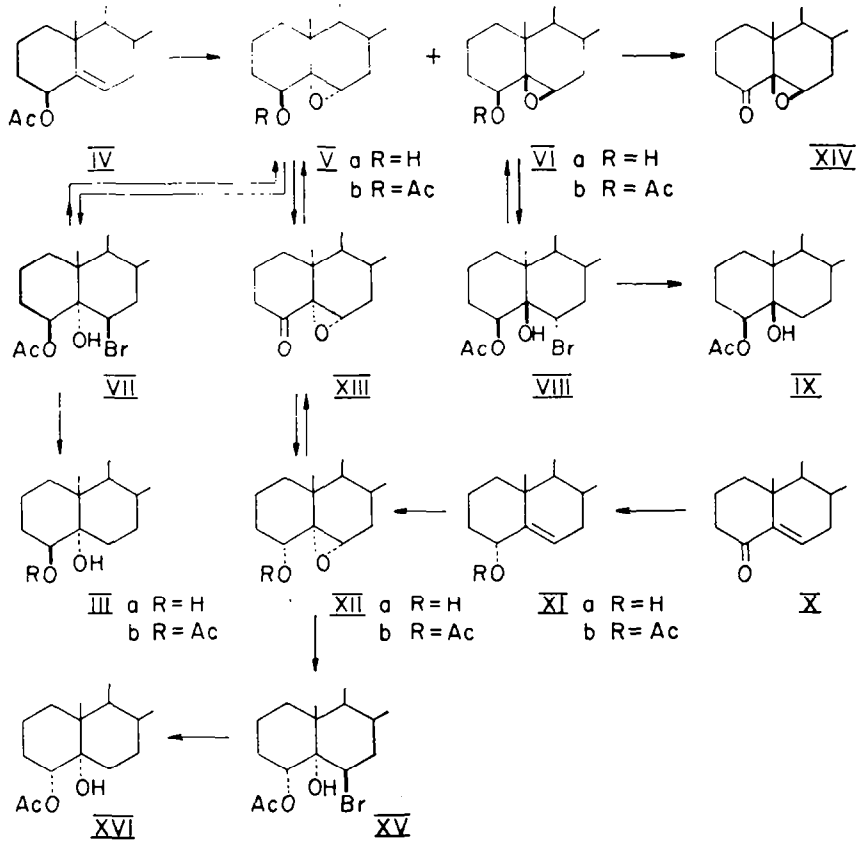
FIG. 1

In the β-epoxide (VIb) ring B can be seen to have a half chair form and ring A which is in a normal chair conformation is fused to B in a way approaching a cholestane type structure as drawn in Fig. 1. The β-oriented acetoxyl substituent at C_4 is thereby axial and indeed the equatorial C_4 proton exhibits in the NMR spectrum a triplet at δ 4·57. Following the protonation of the oxidic oxygen during the process of the epoxide opening, ring B develops a boat conformation as a consequence of which the C_4β-acetoxyl becomes equatorial and thereby obstructs the approach of the bulky bromine anion at C_5 on the rear side of the molecule. In such a situation a quasi *trans* diaxial 5β-hydroxy-6α-bromo intermediate is assumed to form, and only subsequently conversion to the more stable all chair coprostane type conformation occurs in which the above substituents are *trans* diequatorially oriented. Indeed examination of the NMR spectrum of the bromohydrin (VIII) reveals that the C_4 proton signal exhibits a double doublet pattern centered at δ 5·66 indicative for its axial orientation, while the C_6 proton which is adjacent to the bromine atom gives also rise to a double doublet at δ 4·58, confirming thereby the equatorial orientation of the bromine atom itself. Following the debromination step, the C_4–H in IX kept the same double doublet pattern as in VIII (δ 5·40) characteristic for the β-equatorial orientation of the C_4 acetoxyl, while the β-orientation of the hydroxyl group at C_5 was obvious by comparison with the synthetic 4β-acetoxy-5β-hydroxy-cholestane, in which the orientation of the substituents is well established.[5] However in those cases in which an acetoxyl group at C_4 is absent, the rear access to C_5 is not obstructed any more and therefore a normal 5α-bromo-6β-hydroxy derivative is formed during this

[5] J. F. Eastham, G. B. Miles and C. A. Krauth, *J. Amer. Chem. Soc.* **81**, 3114 (1959).

[6] D. R. James and C. W. Shoppee, *J. Chem. Soc.* 4224 (1954); C. W. Shoppee and R. E. Lack, *Ibid.* 4864 (1960); T. Komeno, *Chem. Pharm. Bull. Tokyo* **8**, 672 (1960).

[7] For reviews see: [a] J. Levisalles, *Bull. Soc. Chim. Fr.* 551 (1960); [b] R. E. Parker and N. S. Isaacs, *Chem. Revs.* **59**, 737 (1959); also D. H. R. Barton, D. A. Lewis and J. F. McGhie, *J. Chem. Soc.* 2907 (1957); D. H. R. Barton and R. C. Cookson, *Quart. Rev.* **10**, 44 (1956).



reaction. A well chosen example[8] is the reduction of 5β-6β-epoxy-cholestane: treatment with lithium in ethylamine will lead to 80% of the 6β-hydroxy derivative and ~6% of the 5β-hydroxy isomer, while during the treatment with LAH, the approach of the bulky AlH_4^- anion to the position 5 is inhibited, resulting in the obtention of ~60% of the equatorial 5β-hydroxy derivative. It seems that in our experiments we are facing an extreme case of such hindrance which leads to the unique formation of the diequatorial bromohydrin (VIII).

The reverse reactions—namely the formation of the epoxide rings from the two bromohydrins (VII and VIII) described above were interesting as well. In the case of the 5α-hydroxy-6β-bromo-derivative (VII) in which the groups involved are *trans* diaxial, ring closure to the epoxide takes place already during filtration of a benzene solution of the compound through acid washed alumina, leading to the 4β-acetoxy-5α,6α-epoxy-cholestane (Vb) used in our reaction sequence; however, in order to convert the *trans* diequatorial bromohydrin (VIII) to the corresponding 5β,6β-epoxide stronger conditions were required, i.e. boiling for 1 hr in a methanolic solution of sodium methoxide; as expected, at the same time the C_4 acetoxyl group underwent hydrolysis thus leading to VIa. The conditions required in order to induce the reaction in the latter case, can well be explained[7a] assuming that ring B in its chair

[8] A. S. Halsworth and H. B. Henbest, *J. Chem. Soc.* 4604 (1957).

conformation (VIII) has to be forced into a boat form in which the groups which are involved become *trans* diaxially oriented, thus enabling the normal displacement of the $C_6\alpha$-bromine by the anion of the $C_5\beta$-hydroxyl group.

The preparation of the second pair of isomeric epoxides, namely 4α-hydroxy (acetoxy)-5α,6α-and 5β,6β-epoxy-cholestane was next attempted. While the β-epoxide has not yet been obtained, the α-isomer was prepared by a sequence involving the sodium borohydride reduction of Δ^5-cholestene-4-one[9] to a mixture of the two epimeric hydroxyl derivatives, in which the 4α-hydroxy-Δ^5-cholestene was preponderant; subsequent epoxidation by means of perbenzoic acid yielded the 4α-hydroxy-5α,6α-epoxy derivative (XIIa). In contradistinction to the epoxidation reaction of IV, no isolatable

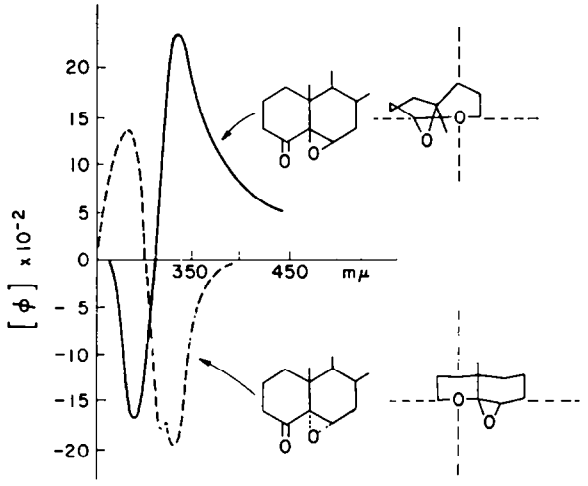


FIG. 2. ORD curves of 5α,6α-epoxy-cholestane-4-one (XIII) and 5β,6β-epoxy-cholestane-4-one (XIV).

quantities of the β-epoxide could now be detected. Acetylation of XIIa yielded the α-acetoxy derivative (XIIb) which was also obtained by acetylation of the hydroxy derivative (XIa) followed by epoxidation. The α-orientation of the epoxide in XII was unequivocally demonstrated by the chromic acid oxidation of XIIa to 5α,6α-epoxy-cholestane-4-one (XIII), which is identical with the epoxy-ketone obtained by a similar oxidation step of the hydroxyl group in Va. Inversely, the reduction with sodium borohydride of the epoxy-ketone (XIII) yielded a mixture of the C_4 epimeric alcohols (Va and XIIa). Ultimately when the 4β-hydroxy-5β,6β-epoxy-derivative (VIa) was subjected to chromic acid oxidation, the isomeric 5β,6β-cholestane-4-one (XIV) was obtained.

In view of recent observations[10] concerning the optical rotatory dispersion of α-epoxy or α-cyclopropyl ketones, it is of interest to present the ORD curves of the two epoxy-ketones (XIII and XIV; Fig. 2). It was found[10] that the contributions to the Cotton effect around 290 mμ of cyclopropane and epoxide rings adjacent to the carbonyl group are opposite in sign to those made by alkyl groups. In the terms of the proposed "reversed" octant rule,[10] the Cotton effect of the α-epoxide (XIII) has

[9] [a] W. Reusch and R. LeMahieu, *J. Amer. Chem. Soc.* **86**, 3068 (1964); [b] C. W. Shoppee and R. E. Lack, *J. Chem. Soc.* 3271 (1961).

[10] C. Djerassi, W. Klyne, T. Norin, G. Ohloff and E. Klein, *Tetrahedron* **21**, 163 (1965).

to be negative and indeed this compound displays such a negative curve. The β-epoxide (XIV) exhibits a positive Cotton effect. The octant projection of this molecule as visualized from the examination of a Dreiding model in which ring A is in a *chair* conformation is not in accordance with the observed Cotton effect. However, this effect can well be explained when ring A of the molecule has a *boat* conformation. In this connection it is noteworthy that in such a case the plane of the epoxide ring is almost perpendicular to the plane of the carbonyl group which is in accordance with the requirements for maximum interaction between these two groups;[10] if ring A however has a chair conformation the angle between these two planes is wide. In the α-epoxide XIII, the two planes are perpendicular.

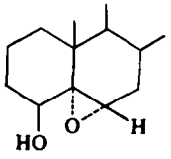
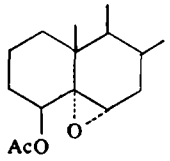
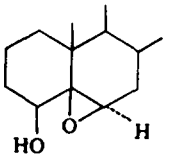
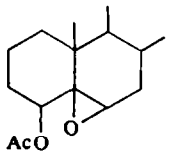
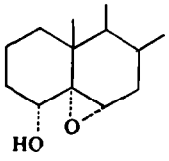
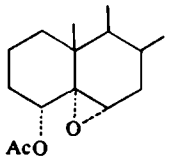
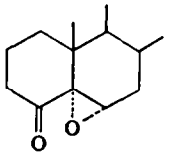
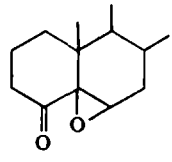
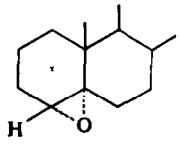
The analysis of the NMR spectrum of the epoxide (XIV) led to interesting observations. When ring A is in a chair form, the dihedral angle between the proton at C_6 and the $C_7\alpha$–H has a value of $80° \pm 5°$ and consequently a doublet should be expected with a coupling constant of $\sim$3 c/s, due to the sole interaction with the $C_7\beta$ proton (the dihedral angle measured is of $40° \pm 5°$; such a coupling is in accordance with the Karplus equation[11] as applied to epoxy derivatives,[12] *vide infra*). In the case of a boat, in which the plane of the carbonyl group is perpendicular to the plane of the epoxide ring, the angles observed between C_6–H and the two $C_7\alpha$–H and $C_7\beta$–H are $75° \pm 5°$ and $45° \pm 5°$ respectively, resulting in a doublet with a calculated coupling constant of 2·5 c/s, a value which actually fits well to the measured coupling constant. These data are indeed not unequivocal in order to enable a differentiation between the two conformations of ring A, however, taking into consideration the positive Cotton effect and the requirement of perpendicularity of the planes between the epoxide and the carbonyl, it can be concluded that ring A probably assumes a boat shape in the epoxy-ketone (XIV).

The orientation of the epoxide ring in all the compounds described in the present paper has been derived through various methods not involving NMR techniques. The first order approximation analyses of the NMR signal of the C_6 proton in the epoxy-derivatives described above, are in good agreement with the sterochemical assignments. Through the analysis[13] of a large number of 5,6-steroidal epoxides substituted at C_3, it has been pointed out that, notwithstanding the fact that the observed coupling constants do not fit those derived from the Karplus equation, the angles between the C_6–H and the two neighbouring C_7 protons are sufficiently different in order to permit a differentiation between the α and β isomeric epoxides. Recently, in order to fit the experimental coupling constants measured on systems containing epoxy and epithio-rings, a revised variant of the Karplus equation has been proposed[12] on empirical grounds, in which the value of 5·1 has been assigned to the constant k_1 of the original equation ($J = 5{\cdot}1 \cos^2\theta$; $0 < \theta < 90°$). The J values which have been calculated using this equation agree well to the coupling constants measured for the epoxides described herewith.[5] In Table 1 we have collected the data of the chemical shifts and the coupling constants of the epoxidic protons in the various cholestane derivatives described in the present work.

An observation which is worth reporting, refers to the chemical shift of the proton adjacent to the hydroxyl or the acetoxyl group in the epoxides Va,b and XIIa,b. In

[11] M. Karplus, *J. Chem. Phys.* **30**, 11 (1959).
[12] K. Tori, T. Komeno and T. Nakagawa, *J. Org. Chem.* **29**, 1136 (1964).
[13] A. D. Cross, *J. Amer. Chem. Soc.* **84**, 3206 (1962).

TABLE 1. SIGNALS OF EPOXIDIC PROTONS

Compd	Cholestane derivatives	δ	J c/s	Compd	Cholestane derivatives	δ	J c/s
Va		3·00	d, 3·5	Vb		3·17	d, 3·8
VIa		3·16	d, 2·0	VIb		3·12	d, 2·2
XIIa		3·43	d, 4·0	XIIb		3·11	d, 3·8
XIII		3·60	d, 4·0	XIV		3·17	d, 2·5
II		2·90	t, 2·0				

d = doublet, t = triplet.

a cyclohexane ring an axial proton is known[14] to be more shielded than the corresponding equatorial one. However, exceptions to this rule have already been reported in several instances: for example in α-haloketones,[15] the reverse to the above rule is observed and in every pair of epimeric compounds, the proton adjacent to the halogen atom is shielded when equatorial and deshielded when axial. Similar effects have been also recorded for α-acetoxy-ketones[16] in which the usual axial-equatorial relationship is reversed. This effect has been attributed to the influence of the vicinal carbonyl function. A similar differential shielding effect has been also disclosed for

[14] L. M. Jackman, *Applications of NMR Spectroscopy in Organic Chemistry* p. 115. Pergamon Press, London (1959).

[15] A. Nickon, M. A. Castle, R. Harada, C. E. Berkoff and R. O. Williams, *J. Amer. Chem. Soc.* **85**, 2185 (1963).

[16] K. L. Williamson and W. S. Johnson, *J. Amer. Chem. Soc.* **83**, 4623 (1961).

a 3,4,5 cyclopropyl ring in the epimeric 3α,5α-cyclosteroidal-C_6-alcohols and acetates,[17] the C_6 equatorial proton resonating at higher field than the axial counterpart.

In our series a similar observation has been done by comparing in the α-oriented epoxides the epimeric pairs of 4α-and 4β-hydroxyl and acetoxyl derivatives respectively (Va, XIIa, and Vb, XIIb). In the 4β substituted derivatives the equatorial C_4α–H exhibits a triplet at δ 3·20 for Va and δ 4·30 for Vb, while in the 4α derivatives the signal of the axial C_4β–H is a double doublet centered at δ 3·90 for XIIa and δ 5·23 for XIIb. It can therefore be inferred that factors of a similar nature to those responsible for the different shielding effect of the equatorial and axial protons adjacent to a cyclopropane ring are also effective for oxirane rings.

Added December 1965

While this paper was in print, the fourth isomer, namely 4α-hydroxy, 5β,6β-epoxy-cholestane, m.p. 127–129°, $[\alpha]_D$ +12° (c 0·8) has been prepared by sodium borohydride reduction of the epoxy-ketone (XIV). (Found: C, 80·62; H, 11·45. $C_{27}H_{46}O_2$ requires: C, 80·54; H, 11·52%.) In the NMR spectrum of this compound, the C_6 epoxidic proton appears as expected as a doublet, J = 2·5 c/s, at δ 3·63.

Completing the observation concerning the differential shielding of the C_4 axial and equatorial protons in Va and XIIa respectively, in the epimeric pair of the 4-hydroxy-5β,6β-epoxy-cholestanes, the same relationship could now be disclosed: in VIa the C_4 equatorial proton resonates at δ 3·35 (triplet), while in the above new isomer the signal of the C_4 axial proton is at lower field, δ 3·98 (double doublet).

EXPERIMENTAL

M.ps were taken on a Fisher–Johns m.p. apparatus. All optical rotation measurements were carried out in $CHCl_3$ solution. UV absorption spectra were done on a Cary 14 spectrophotometer in EtOH solution. IR spectra were recorded on a Perkin–Elmer Infracord model 137 spectrometer equipped with a NaCl prism and, unless otherwise stated, were determined in $CHCl_3$ solution of 5–10% concentration. NMR spectra were recorded on a Varian A-60 spectrometer. The line positions given are δ values. The spectra were determined in $CDCl_3$ solutions of about 5–10% concentration and containing tetramethylsilane as internal standard. The optical rotatory dispersion measurements were done on a Jasco model ORD/UV-5 instrument. TLC was done on chromatoplates of silica gel G (Merck) and spots were developed with I_2 vapors. In the chromatographies, alumina refers to acid washed alumina Merck. Microanalytical determinations were carried out in the microanalytical laboratory of our Institute, under the direction of Mr. R. Heller.

Preparation of 4β-acetoxy-Δ⁵-cholestene (IV). A solution in acetone (150 ml) of 4α,5α-epoxy-cholestane (4 g; prepared by the epoxidation with perbenzoic acid of Δ⁴-cholestene[24c]) was left over night in the presence of 7% aqueous perchloric acid (4 ml). The reaction mixture was then poured onto ice, the solid filtered, washed with water and dried.[24b] The crude diol was chromatographed on alumina; elution with hexane–ether (7:3) yielded IIIa (3·5 g) which crystallized from MeOH, m.p. 171–172°. The diol was acetylated overnight at room temp with acetic anhydride and dry pyridine; following the usual work-up, IIIb was obtained and crystallized from acetone, m.p. 175–176°. This product was converted to IV by dehydration with $SOCl_2$ in the presence of pyridine at 0°,[24c] m.p. 108° overall yield from II, 60%.

Epoxidation of 4β-acetoxy-Δ⁵-cholestene to 4β-acetoxy-5α,6α-epoxy-cholestane (Vb) *and 4β-acetoxy-5β,6β-epoxy-cholestane* (VIb). To a solution of IV (1 g) in dry benzene (25 ml), a solution of perbenzoic acid in the same solvent (1·5 equivs peracid) was added at room temp. After 2·5 hr the solution was washed with cold aqueous 5% Na_2CO_3aq and water, then dried over Na_2SO_4 and the solvent removed *in vacuo*. The residue (1 g) was carefully chromatographed through alumina (150 g). Elution with hexane yielded unreacted starting material (80 mg) while with hexane–ether (98:2) Vb (800 mg) emerged and was crystallized twice from MeOH, m.p. 100–102°, $[\alpha]_D$ −40° (*c*, 1·0), ν_{max}^{KBr}

[17] J. Tadanier and W. Cole, *J. Org. Chem.* **27**, 4610 (1962).

1736 cm^{-1}; no maximum absorption in the UV region. (Found: C, 78·60; H, 10·73. $C_{29}H_{48}O_3$ requires: C, 78·32; H, 10·88%.)

Further elution with hexane–ether (96:4) yielded VIb (85 mg) which crystallized from MeOH, m.p. 89–91°, ν_{max} 1736 cm^{-1}; no maximum absorption in the UV region (Found: C, 78·50; H, 10·90; $C_{29}H_{48}O_3$ requires: C, 78·32; H, 10·88%.)

4β-Hydroxy-5α,6α-epoxy-cholestane (Va). Compound Vb (200 mg) in MeOH (20 ml) was left overnight at room temp in the presence of 2% methanolic KOH (20 ml). Following acidification to pH ~ 3, most of the solvent was evaporated *in vacuo*, water was then added and the solid which precipitated was collected, washed with water and dried. Crystallized from MeOH (150 mg), m.p. 136–138°, $[\alpha]_D$ −36° (*c*, 1·0). (Found: C, 80·25; H, 11·41. $C_{27}H_{46}O_2$ requires: C, 80·54; H, 11·52%.) By reacetylation Vb was obtained.

4β-Hydroxy-5β,6β-epoxy-cholestane (VIa). Compound VIb (50 mg) was subjected to the above hydrolytic conditions; the product crystallized from MeOH, m.p. 106—108°, $[\alpha]_D$ +10° (*c*, 1·0). (Found C, 80·84; H, 11·62. $C_{27}H_{46}O_2$ requires: C, 80·54; H, 11·52%.) Reacetylation yielded VIb.

Preparation of 4β-acetoxy-5α-hydroxy-6β-bromo-cholestane (VII). To a solution of Vb (80 mg) in glacial acetic acid (5 ml), an acetic acid solution of HBr (45% w/v; 0·2 ml) was added at 15°. The mixture was stirred for 2 hr at the same temp, then poured onto ice-water, the product was extracted with ether, washed with water and $NaHCO_3$aq. Evaporation of the solvent left an oily residue (85 mg) which could not be induced to crystallize, but exhibited one spot on a chromatoplate, ν_{max} 1730 cm^{-1}. On attempted purification by chromatography on neutral or acid washed alumina the epoxide ring reclosed, thus resulting in a quantitative recovery of the pure starting compound Vb.

Debromination of the bromohydrin VII *to 4β-acetoxy-5α-hydroxy-cholestane.* The above bromohydrin (80 mg) in abs. alcohol (25 ml) was heated to reflux overnight while stirring with Raney Ni (1 teaspoonful). The crude product was filtered through alumina and crystallized from acetone (50 mg); it was identical in all respects with IIIb, described above.

4β-Acetoxy-5β-hydroxy-6α-bromo-cholestane (VIII). Compound VIb (110 mg) was treated with HBr in acetic acid, as described above. The crude reaction product exhibited one spot on a chromatoplate, however it could not be crystallized. Following chromatography on alumina, the bromohydrin was recovered unchanged.

Formation of the epoxide VIa *from the bromohydrin* VIII. Compound VIII (100 mg) in MeOH (20 ml) was heated during 1 hr at reflux with a 2% methanolic MeONa (10 ml). Following acidification at pH ~ 3, the solvent was evaporated, water was added and the solid collected by filtration; it crystallized from MeOH, and was found identical in all respects with VIa. Acetylation with acetic anhydride–pyridine yielded the corresponding VIb, having the same characteristics as the original sample.

4β-Acetoxy-5β-hydroxy-cholestane (IX). Compound VIII (95 mg) was treated with Raney Ni as described above and the product was chromatographed through alumina; it crystallized from MeOH, m.p. 115–117°, $[\alpha]_D$ +31° (*c*, 1·0), and was identified by direct comparison with an authentic sample prepared according to literature indications.[5]

Preparation of 4α-hydroxy-Δ⁵-cholestene (XIa). 5α-Cholestane-4-one[9a] was brominated according to literature data[9b] resulting in a mixture of 5α- and 5β-bromo-derivatives (1 g) which was then subjected to dehydrobromination in dimethylformamide (20 ml) in the presence of LiCl (1 g) and Li_2CO_3 (1 g). After heating for 2 hr in a stream of N_2, the reaction mixture was poured into water and extracted with ether. The residue was chromatographed yielding pure Δ⁵-cholestene-4-one, m.p. 109–111°. $NaBH_4$ reduction of this ketone in MeOH afforded a mixture of the C_4 epimeric alcohols, containing preponderantly the $C_4\alpha$ isomer (XIa), which was purified by chromatography and recrystallization from acetone–hexane, m.p. 143–144°. Acetylation of XIa in the usual conditions, gave XIb, m.p. 123°.

Epoxidation of 4α-acetoxy-Δ⁵-cholestene (XIb) *to 4α-acetoxy-5α,6α epoxy cholestane* (XIIb). The reaction was performed as described above for IV. Following careful chromatography on alumina, only XIIb could be isolated. Crystallized from MeOH, m.p. 99–101° $[\alpha]_D$ −16° (*c*, 1·0) ν_{max}^{KBr} 1739 cm^{-1}, no maximum absorption in the UV. (Found: C, 78·59; H, 10·77. $C_{29}H_{48}O_3$ requires: C, 78·32; H, 10·88%.)

Epoxidation of 4α-hydroxy-Δ⁵-cholestene (XIa), *to 4α-hydroxy-5α,6α-epoxy-cholestane* (XIIa). The same conditions were used as above, the product crystallized from MeOH, m.p. 128–131°, $[\alpha]_D$

−29° (*c*, 1·0); (Found: C, 80·73; H, 11·40. $C_{37}H_{46}O_3$ requires: C, 80·54; H, 11·52%.) Compound XIIa was also obtained by the hydrolysis of the acetate group in XIIb, as described for Va.

Formation of the bromohydrin XV *and subsequent debromination to* XVI. The corresponding bromohydrin (XV) was obtained in the same manner as previously described. The crude product displayed a single spot on a chromatoplate. Raney Ni debromination afforded the corresponding 4α-acetoxy-4α-hydroxy-cholestane m.p. 149°, identified by direct comparison with an authentic sample.[5]

Oxidation of Va *and of* XIIa *to 5α,6α-epoxy-cholestane-4-one* (XIII). Compound Va (110 mg) in pure acetone solution (20 ml) was oxidized at 0–5° with a solution of CrO_3 in dil H_2SO_4 (Jones reagent). The excess oxidant was destroyed with MeOH, water was added and the product extracted with $CHCl_3$. The solution was washed and dried; evaporation of the solvent left a residue which was chromatographed through alumina. Elution with hexane–ether (95:5) yielded fractions which were combined (80 mg) and crystallized from MeOH, m.p. 86–87°; RD (*c*, 0·071; dioxan), 24°; $[\Phi]_{400}$ −310°; $[\Phi]_{331}$ −1,915°; $[\Phi]_{314}$ −1,690°; $[\Phi]_{310}$ −1,800°; $[\Phi]_{282}$ +1,350°. ν_{max}^{KBr} 1718 cm^{-1}. (Found C, 80·81; H, 10·91. $C_{37}H_{44}O_3$ requires: C, 80·94; H, 11·07%.) Oxidation of XIIa in conditions similar to those described above yielded the same epoxy-ketone XIII.

Sodium borohydride reduction of the epoxy-ketone XIII. A solution of XIII (170 mg) in MeOH (20 ml) containing NaOH (17 mg) was treated with $NaBH_4$ (40 mg) in water (0·4 ml), during 1 hr at 20°. The solution was then acidified and the product was extracted with $CHCl_3$. The residue was chromatographed through alumina; elution with hexane–ether (85:15) yielded XIIa (20 mg) while with hexane ether (75:25) the epimer Va emerged (120 mg). Both compounds were found identical in all respects with those described above.

Oxidation of VIa *to 5β,6β-epoxy-cholestane-4-one* (XIV). The oxidation of VI (100 mg) with CrO_3 was performed as already described. Chromatography of the crude reaction product on alumina (elution with hexane–ether 9:1), yielded the pure XIV which crystallized from MeOH, m.p. 102–104°; RD (*c*, 0·066; dioxan), 24°; $[\Phi]_{400}$ +788°; $[\Phi]_{326}$ +2360°; $[\Phi]_{294}$ −1697°. ν_{max}^{KBr} 1721 cm^{-1}. (Found C, 80·93; H, 10·90. $C_{37}H_{44}O_3$ requires: C, 80·94; H, 11·07%.)

Acknowledgement—We would like to express our thanks to Dr. P. Pfeffer of "Plantex Ltd." Nathania, Israel for his interest and helpful discussions.