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Epoxides Formed by Chlorination of Cholesterol and Cholest-4-en-3β,6β-diol

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A substance formed in low yield by chlorination of cholesterol in aqueous solutions has been identified as 5α,6β-dichloro-3β,4β-epoxy-cholestane (VI). In order to check a possible route for its formation (Scheme 1), the chlorination product of cholest-4-en-3β,6β-diol (VIII) was examined. It was found to consist mainly of 4α-chloro-5β,6β-epoxy-cholestan-3β-ol (XI).

Chlorination of cholesterol in a water/*t*-butanol solution yields a complex mixture of substances (about 3 % of the cholesterol by weight) which move considerably faster on TLC than does cholesterol. From the mixture a compound (*A*) was isolated with the formula $C_{27}H_{44}Cl_2O$, m.p. 133.5 – 136°, and $[\alpha]_{578}$ −69°.[1] The yield was about 1 %. This communication describes the identification of this compound and suggests a route for its formation.

The dominating mass spectrometric fragmentations of *A* involve losses of 2Cl, 2HCl, CH_3, and ($C_8H_{17}+C_3H_6$). The IR spectrum shows the absence of hydroxyl and carbonyl groups, and therefore the oxygen atom in the molecule is evidently present as an ether group. A strong peak at 678 cm^{-1} is very likely due to the carbon-chlorine link.[2]

Reduction of *A* by lithium borohydride gave a complex mixture from which cholesterol (30 % yield) and cholest-5-en-3β,4β-diol (very low yield) were isolated. This indicates that *A* has an epoxy function probably in 3β,4β-position. The formation of a Δ^5-bond during the reduction suggests that *A* contains a 5,6-dichloro group, whose chlorine atoms are probably *trans*-related, since they are relatively easily eliminated. 3β-Acetoxy-5α,6β-dichloro-cholestane was also dehalogenated and hydrolysed to cholesterol (yield 36 %) by a lithium borohydride treatment.

The NMR signals of *A* (Table 1) confirm the presence of the 5,6-dichloro- and 3β,4β-epoxy groups. The signal from the 6-proton (geminal to the chloro

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Table 1. The NMR signals from some steroidal epoxides. They are determined in $CDCl_3$ solutions with the exception of those of *A* marked with*, which are determined in a benzene solution. The magnetic field corresponds to 100 Mc for 3β-acetoxy-4α-chloro-5β,6β-epoxy-cholestane and to 60 Mc for the others. For NMR spectra of other steroidal epoxides, see Tori *et al.*[3]

Compound	Signal [a] for proton			
	in the 19-CH_3 group	geminal to		
		chlorine	epoxy oxygen	hydroxyl acetoxyl trimethyl-siloxyl
A=5α,6β-Dichloro-3β,4β-epoxy-cholestane (VI)	1.70s*	4.47u (*W*6)*	2.88t (*J*4)*	
			3.27d (*J*4)*	
	1.58s[b]	4.58u (*W*7)	3.29u	
			3.37d (*J*4)	
B=4α-Chloro-5β,6β-epoxy-cholestan-3β-ol (XI)	1.01s	4.29d (*J*10)	3.75d (*J*3)	3.6u
3β-Acetoxy-4α-chloro-5β,6β-epoxy-cholestane	1.05s	4.45d (*J*10)	3.79d (*J*3)	5.0u[c]
4α-Chloro-5β,6β-epoxy-3β-trimethylsiloxy-cholestane	1.03s	4.31d (*J*10)	3.78d (*J*3)	3.6u[d]
5β,6β-Epoxy-cholestan-3β-ol	1.00s	—	3.05u (*W*5)	3.7u

[a] The first figure is the chemical shift (δ-value in ppm), the latter shows the signal character (s, singlet: d, doublet: t, triplet: u, unresolved or undetermined). The figure inside brackets is the value of either the coupling constant (*J*) or the half-band width (*W*), both in cps.

[b] Calculated value 1.54 ppm (assuming that the interaction between the 3β,4β-epoxy group and the 19-CH_3 is the same as that between the 9β,11β-epoxy group and the 18-CH_3). For 5β,6β-dichloro-3β,4β-epoxycholestane, the calculated value is lower than 1.30 ppm (assuming that the $\Delta\delta$-value for 5β-Cl is not greater than 0.1 ppm).

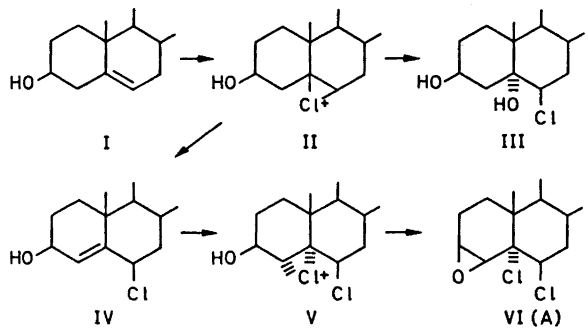
[c] For the acetate protons the signal is at δ 2.13.

[d] For the trimethylsiloxyl protons the signal is at δ 0.15.

group) has a half-band width of only 6-7 cps, which indicates that the proton is equatorial. The 6-chlorine is then β-oriented and the 5-chlorine probably α-oriented.

If the 5-chlorine is assumed to be α-orientad, the δ-value for the 19-methyl group is calculated by the additivity rule [4] to be close to that observed (see Table 1, foot-note *b*). The calculated value for a 5β-chloro compound is considerably lower. This indicates that *A* is 5α,6β-dichloro-3β,4β-epoxy-cholestane (VI).

The reduction of the epoxide *A* gives as the main product the equatorial alcohol (*viz.* cholesterol) instead of the expected axial 4β-alcohol. The explanation for this may be that a Δ^5-bond initially formed by dechlorination may facilitate the cleavage of the allylic C–O bond or that the 6β-chloro and 19β-CH_3 groups may sterically hinder the formation of the 4β-alcohol.



Scheme 1. Suggested route for the formation of compound *A* (VI) from cholesterol (I).

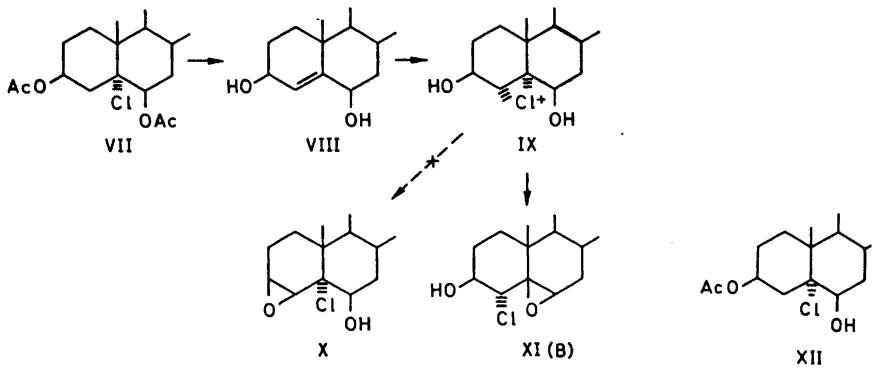
A possible route from cholesterol to *A* is shown in Scheme 1. β-Attack of chlorine on cholesterol (I) gives an intermediate represented by formula (II). The occurrence of such an attack, besides the dominating α-attack, is demonstrated by the formation of the chlorohydrin (III).[1,6] As the intermediate (II) is a chloronium ion from a trisubstituted olefin, it may lose a proton, yielding the allyl chloride (IV).[7] Chlorine may then attack the double bond of (IV) preferentially from the sterically less hindered α-side, to give the intermediate (V) leading to compound *A* (VI).

To test the validity of this scheme a study of the chlorination of the allyl chloride (IV) should be of interest. As this compound is difficult to synthesise, the chlorination of the easily available cholest-4-en-3β,6β-diol (VIII) was instead examined (Scheme 2). This compound is generally prepared by dehydration of 3β,6β-diacetoxy-cholestan-5α-ol followed by saponification.[8] We obtained it by dehydrochlorination of 3β,6β-diacetoxy-5α-chloro-cholestane (VII) and saponification of the di- and monoacetates thus obtained. When 1,5-diazabicyclo[4.3.0]non-5-ene[9] was used as base in the dehydrochlorination only small amounts of epoxides were formed.

Chlorination of (VIII) in a dilute solution of water/*t*-butanol gave a substance *B* (yield 72 %) with the formula $C_{27}H_{45}ClO_2$, m.p. 166–168°, and $[\alpha]_D + 11°$ ($CHCl_3$). The dominating mass spectrometric fragmentations of *B* involved losses of Cl, HCl and $(Cl + C_2H_4O)$.

Compound *B* formed a monoacetate and a monotrimethylsilylether neither of which contained a hydroxy group (IR). Neither the alcohol not the silyl ether have a carbonyl group (IR). Thus the oxygen atoms of *B* have to be located in a hydroxyl and an ether group.

This finding suggests that *B* is (X) or (XI) (see Scheme 2), which are expected to be formed from (VIII) *via* the intermediate (IX). A preference for (XI) was suggested by the observation that 3β-acetoxy-5α-chloro-cholestan-6β-ol (XII), which has an axial, sterically hindered hydroxyl group similar to that of (X), did not react under the mild conditions (hexamethyldisilazane in pyridine) used for silylation of *B*. Neither cathylation [10] (reaction with ethyl chloroformate) nor acetylation discriminated as distinctly as silylation between the compounds *B* and XII.



Scheme 2. The chlorination of cholest-4-en-3β,6β-diol.

The NMR signals of *B* confirm the proposed structure (XI) (see Table 1). The 19-methyl signal of *B* and that of 5β,6β-epoxy-cholestan-3β-ol (see the table) are at similar δ-values which is expected as a 4-chloro group in a 5β-sterol would not cause a considerable shift in the signal.

The chlorination of the unsaturated diol (VIII) thus shows that epoxides can be formed by chlorination of allyl alcohols with suitable configuration. The result supports the assumption that the epoxide (VI) is formed during the chlorination of cholesterol *via* the allyl chloride (IV).

EXPERIMENTAL

5α,6β-Dichloro-3β,4β-epoxy-cholestane (A) (VI). The compound was obtained according to the method of Lindgren.[1] Besides the characteristics already published [1] the compound gave the following mass spectrometric data: distinct peaks at *m/e* 458:456:454 (molecular ions) (4:20:29 % of the base peak), 403 (15 %), 401 (15 %), 385 (30 %), 384 (100 %), 383 (37 %), 382 (42 %), 305 (22 %), 301 (28 %), 300 (29 %), 299 (37 %), 271 (24 %), 265 (22 %), 263 (25 %), 247 (32 %), and 229 (57 %).

Lithium borohydride reduction of 5α,6β-dichloro-3β,4β-epoxy-cholestane (*A*) (VI). The dichloro epoxide (*A*) (78 mg) was added to a clear solution of lithium borohydride (80 mg) in tetrahydrofuran (75 ml). The solution was refluxed for 18 h, acidified to pH 3—4 with dilute sulphuric acid and then stirred for 2 h. The tetrahydrofuran was evaporated and the residue was separated by preparative TLC [adsorbent, silica gel layer with a thickness of 1 mm; eluent, petroleum ether/isopropyl ether (1/1 v/v)].

Two fractions were collected from the complicated reaction mixture. One fraction (20 mg, yield 30 %) consisted of almost pure cholesterol which after recrystallisations from methanol had m.p. 146—148° and $[\alpha]_D^{23}$ −36° (*c*, 0.4; $CHCl_3$). The identification of the isolated compound as cholesterol was confirmed by mixed m.p., mass spectroscopy, GLC, and argentative TLC.

The other fraction (a very small amount) was recrystallised from methanol, m.p. 175°. This compound was indistinguishable from cholest-5-en-3β,4β-diol (m.p. 175°[11]) by mixed m.p., mass spectrum [distinct peaks at *m/e* 402 ($C_{27}H_{46}O_2$, the molecular ion) (50 % of the base peak), 387 (47 %), 384 (100 %) and 358 (92 %)], and NMR spectrum [δ 0.68 s (18-CH_3); δ 1.18 s (19-CH_3); δ 3.6 u (3α-H); δ 4.15 d, $J_{3,4}$=3.5 cps (4α-H): 60 Mc, $CDCl_3$].

Reduction of 3β-acetoxy-5α,6β-dichloro-cholestane with lithium borohydride. The acetoxy dichloride (250 mg) was added to a clear solution of lithium borohydride (250 mg) in tetrahydrofuran (150 ml) and the solution was refluxed for 36 h. The reaction mixture was

worked up as described above for the dichloro epoxide (VI). The reaction product was then silylated and the mixture (trimethyl-silyl ethers, solvent and excess reagent) thus obtained was directly used for an argentative preparative TLC fractionation.[12]

The main fraction was treated with a trace of acid [12] to cleave the ether bond and the alcohol was recrystallised from methanol to yield a product (70 mg, 36 %), m.p. 146–148° $[\alpha]_{578}^{23}$ −34° (*c.* 1.0, $CHCl_3$) which was indistinguishable from cholesterol by mixed m.p. and mass spectrum.

Formation of cholest-4-en-3β,6β-diol (VIII) from 3β,6β-diacetoxy-5α-chloro-cholestane (VII). The diacetoxy-chloro compound (VII) (1.00 g) was stirred at 90° for 7 h with dry 1,5-diazabicyclo[4.3.0]non-5-ene (Aldrich) (10 ml).[9] The reaction product was poured into ice-cold 1 N sulphuric acid (100 ml) and extracted with ether. An oil (0.94 g) was obtained after drying and evaporation. This oil contains unsaturated diacetate and monoacetates together with some 3β-acetoxy-5β,6β-epoxy-cholestane.

The oil was refluxed with 1 N methanolic potassium hydroxide (75 ml) for 45 min. The precipitate of cholest-4-en-3β,6β-diol formed (0.46 g) was collected, washed with water and recrystallised from chloroform-acetone (0.41 g, yield 53 %), m.p. 255–258°, $[\alpha]_D^{23}$ +7° (*c*, 0.6; pyridine) (lit. values;[8] m.p. 258° and [α] +9°) A further amount of the unsaturated diol [0.47 g (6 %), m.p. 255–258°] was obtained from the filtrate by addition of water, extraction with ether and recrystallisations as described above and from ethanol.

Chlorination of cholest-4-en-3β,6β-diol (VIII); formation of 4α-chloro-5β,6β-epoxy-cholestan-3β-ol (XI) (B). The unsaturated diol (VIII) (0.50 g, 1.25 mmole) was dissolved in *t*-butanol (350 ml). An aqueous chlorine solution (78 ml, 1.37 mmole) was added and the solution was stirred for 1/2 h. The products were collected by dichloromethane extraction. The evaporation residue from the extract (0.58 g) was recrystallised from methanol, yielding a product (*B*) (0.39 g, 72 %) with the m.p. 166–168° and $[\alpha]_D^{23}$ +11° (*c*, 0.6; $CHCl_3$) (Found: C 74.1; H 10.4; Cl 8.0; O 7.5. Calc. for $C_{27}H_{45}ClO_2$: C 74.2; H 10.4; Cl 8.1; O 7.3). For NMR signals, see Table 1. Mass spectral data: distinct peaks at *m/e* 436:438 (the molecular ions 721:8 % of the base peak), 401 (100 %), 400 (94 %), and 357 (87 %).

3β-Acetoxy-4α-chloro-5β,6β-epoxy-cholestane. Acetylation of the chloro epoxide (XI) (*B*) with acetic anhydride and pyridine at room temperature overnight gave its acetate, m.p. 125° $[\alpha]_D^{23}$ +18° (*c.* 0.5; $CHCl_3$). For NMR signals, see Table 1. Mass spectral data: distinct peaks at *m/e* 478:480 m.u. ($C_{29}H_{47}ClO_3$, the molecular ions) (12:4 % of the base peak), 418 (100 %), 384 (76 %), 383 (72 %), and 443 (54 %).

Trimethylsilylation of 4α-chloro-3β-hydroxy-5β,6β-epoxy-cholestane (B) (XI). The chloro epoxide (XI) (*B*) (70 mg) was dissolved in pyridine (3 ml) and hexamethyl-disilazane (1 ml) was added. After 11 h at 35° the solution was evaporated to dryness and the residue was recrystallised once from methanol (68 mg, yield 83 %, m.p. 133.5–135°) and then from acetone (m.p. 136°, $[\alpha]_D^{23}$ +18°). (Found: Si 4.7. Calc. for $C_{30}H_{53}ClO_2Si$: Si 5.5). For NMR signals, see Table 1. Mass spectral data: distinct peaks at *m/e* 508:510 (the molecular ions) (11:4 % of the base peak), 473 (100 %), 198 (42 %), 383 (35 %), and 129 (40 %).

Trimethylsilylation of 3β-acetoxy-5α-chloro-cholestan-6β-ol (XII). When applying the conditions used above for silylation of the chloro epoxide (XI) (*B*) no reaction of (XII) occurred as observed on TLC.

To affect silylation the following procedure was used. Compound (XII) (150 mg) was dissolved in pyridine (5 ml) and a 2:1 mixture of hexamethylsilazane and trimethyl-chloro-silane (2 ml) was added. After 1 h at room temperature the solution was evaporated to dryness and the residue was recrystallised from methanol. The product (151 mg, 88 %) melted at 90°, $[\alpha]_D^{23}$ −35° (*c.* 0.5 $CHCl_3$). (Found: Si 4.4. Calc. for $C_{32}H_{57}ClO_3Si$: Si 5.1). NMR signals: δ 0.09 (6β-O-Si$(CH_3)_3$); δ 0.68 s (18-CH_3): δ 1.25 s (19-CH_3); δ 2.06 s (3β-O-$COCH_3$); δ 3.95 (6α-H); δ 5.4 u (3α-H) : 60 Mc, $CDCl_3$. Mass spectral data: distinct peaks at *m/e* 552:554 (the molecular ions) (19:8 % of the base peak), 477 (31 %), 456 (73 %), 441 (46 %), 367 (32 %), 366 (32 %), 321 (100 %), and 129 (35 %).

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