HYDROGENOLYTIC OPENING OF 4,4-DIMETHYL-4a,5-EPOXY-A-HOMOCHOLESTANE DERIVATIVES AND MASS SPECTRA OF SOME REACTION PRODUCTS

Helena VELGOVÁ and Antonín TRKA

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

Received August 26th, 1981

The hydrogenolytic opening of the epoxide ring of all four stereoisomeric 3-acetoxy-4,4-dimethyl--4a,5-epoxy-A-homocholestanes was investigated. The reaction proceeds at the more substituted carbon atom $C_{(5)}$, giving rise to 4a-hydroxy-5,6-unsaturated derivatives. The hydrogenation of the 5,6-double bond of these allylic alcohols affords 4,4-dimethyl-A-homo-5β-cholestane derivatives. The mass spectra of 3,4a-diketones *XI* and *XVII* and 4a-ketones *XVI* and *XXVIII* to *XXX* were also studied. In the case of the saturated diketone *XVII* it was observed that the most abundant metastable peak corresponds to a two-step fragmentation process. The decomposition of the molecular ion of the unsaturated diketone *XI* is associated with ring A expansion. The mass spectra of 3-acetoxy ketones *XVI* and *XXVIII* are characterized by an elimination of a C_4H_8 molecule from the contracted ring A of the ion $[M-CH_3COOH]^+$. The unexpected elimination of the species C_6H_{11}, C_6H_{12}, and C_6H_{13} from the ring A of the molecular ions of 3-hydroxy ketones *XXIX* and *XXX* is shown to be preceded by hydroxyl migration and ring A rearrangement.

In our preceding communication[1] we investigated the effect of the oxygen-containing substituent in the position 3 on the stereochemistry of the epoxidation of 4,4-dimethyl-A-homo-4a-cholestene derivatives. In this paper the hydrogenolytic opening of the 4a,5-epoxide ring of all stereoisomeric 3-acetoxy-4,4-dimethyl-4a,5-epoxy--A-homocholestanes is dealt with.

The hydrogenolytic opening of epoxides *I–IV*, the preparation of which has been described in the preceding paper[1], was carried out in acetic acid on Adams catalyst (the yields of the main products of cleavage are given in Table I). The opening of the epoxide ring takes place at the side of the more substituted carbon atom $C_{(5)}$ under formation of 4a-hydroxy-5,6-unsaturated derivatives *V*, *VIII*, *XVIII* and *XXI* which were characterized as diacetates *VI*, *IX*, *XIX* and *XXII*. The structures of the unsaturated alcohols *V*, *VIII*, *XVIII* and *XXI* followed from the ^{1}H NMR data (Table II). In the ^{1}H NMR spectra of compounds *V*, *VIII*, *XVIII* and *XXI* the signal

* Part CCLXXX in the series On Steroids; Part CCLXXIX: This Journal *48*, 173 (1983).

TABLE I

Yield (in % of the total yield) of the cleavage products of epoxides *I*–*IV*

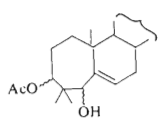
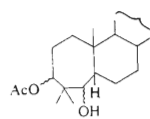
Epoxide	% of the total yield 		Total yield, %
I	91 (*V*)	9 (*XII*)	91
II	55 (*VIII*)	45 (*XIV*)	100
III	97 (*XVIII*)	3 (*XXIV*)	96
IV	67 (*XXI*)	33 (*XXVI*)	100

TABLE II

^{1}H NMR Data of the cleavage products of epoxides *I*–*IV*

^{1}H NMR spectra were measured in deuteriochloroform using tetramethylsilane as internal reference. The chemical shifts are given in δ-scale (ppm), the coupling constants and the half-widths $W_{1/2}$ in Hz. The following abbreviations were used for the characterization of the signals: s singlet, d doublet, bd broad doublet, dd doublet of a doublet, mt multiplet

Compound	$C_{(3)}$—H	$C_{(4a)}$—H	$C_{(6)}$—H
V[a]	4·61 (mt, $W_{1/2} = 9$)	4·31 (s)	5·82 (mt)
VIII[a]	4·79 (mt, $W_{1/2} = 9$)	3·89 (d, $J_{H,OH} = 6$) 3·89 (s)[c,d]	5·38 (mt)
XII[b]	4·83 (dd, $J = 2·9 + 9$)	3·43 (d, $J_{H,OH} = 3·6$) 3·43 (s)[d]	—
XIV[b]	4·46 (mt, $W_{1/2} = 18$)	3·53 (bd, $J_{4a,5} = 8$)	—
XVIII[c]	4·48 (mt, $W_{1/2} = 15$)	4·00 (s)	5·83 (mt)
XXI[b]	4·95 (dd, $J = 9·8 + 4·7$)	3·885 (d, $J_{H,OH} = 3·7$) 3·88 (s)[c,d]	5·43 (mt)
XXIV[c]	[e]	3·50 (s)	—
XXVI[c]	4·70 (mt, $W_{1/2} = 8$)	3·74 (bd, $J_{4a,5} = 8$)	—

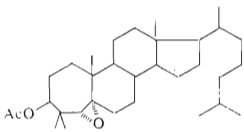
[a] Measured on a Varian HA 100 instrument; [b] measured on a Varian XL 200 instrument; [c] measured on a Tesla B 476 instrument (60 MHz); [d] the values were obtained after exchange in heavy water; [e] owing to great noise the signal of $C_{(3)}$—H could not be read.

of one CH—OH proton is present which appears as a singlet, in agreement with the localization of the hydroxyl group in the position 4a (the doublet observed in the case of the 3aβ-hydroxy derivative *VIII* and *XXI* is converted to a singlet after exchange with heavy water). Further, the signal of one olefinic proton appears in the form of a multiplet in agreement with the localization of the double bond in the position 5,6. The diols *VII*, *X*, *XX* and *XXIII* were oxidized with the chromium trioxide–pyridine complex to the same diketone *XI*.

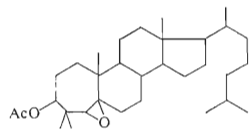
Under the given reaction conditions a hydrogenation of the 5,6-double bond of the primarily formed allylic alcohols *V*, *VIII*, *XVIII* and *XXI* also takes place to some extent, the 5,6-double bond being hydrogenated far more easily in the case of the 4aβ-hydroxy derivatives *VIII* and *XXI* than in the case of the 4aα-hydroxy derivatives *V* and *XVIII* (Table I). Since oxidation of the alcohols *XII* and *XIV* leads to the same ketone *XVI*, oxidation of the alcohols *XXIV* and *XXVI* to the same ketone *XXVIII*, and the oxidation of diols *XV* and *XXVII* to the same diketone *XVII*, it is evident that the configuration at the carbon atom $C_{(5)}$ is identical in compounds *XII*, *XIV*, *XXIV* and *XXVI*. In the case of alcohols *XII* (after exchange with heavy water) and *XXIV* the signal of the CH—OH proton appears as a singlet, while in the case of alcohols *XIV* and *XXVI* it appears as a doublet with a coupling constant about 8 Hz (Table II). According to the Karplus relation[2,3] it would mean that in the case of alcohols *XII* and *XXIV* the dihedral angles between the protons on the carbon atoms $C_{(4a)}$ and $C_{(5)}$ should be about 90. An inspection of Dreiding models has shown that in the case of an α-configuration of the proton on $C_{(5)}$ the dihedral angle between 4aβ- and 5α-protons of 4aα-hydroxy derivatives *XII* and *XXIV* may be about 90° only in such conformations of the seven-membered ring A that seem very improbable from the sterical point of view (for example the conformation $TC_{(5)}$-twist chair, $TB_{(3)}$-twist boat, $B_{(10)}$-boat). On the other hand, for the $C_{(5)}$ proton in β-configuration several sterically favourable conformations of the seven-membered ring A may be found, in which the dihedral angle between 4aβ- and 5β-protons is about 90° (for example the conformations $C_{(10)}$-chair, $TC_{(4)}$-twist chair). Hence, we assume that alcohols *XII*, *XIV*, *XXIV* and *XXVI*, formed on hydrogenation of the 5,6-double bond of 4a-hydroxy-5,6-unsaturated derivatives *V*, *VIII*, *XVIII* and *XXI* in acid medium, are 4,4-dimethyl-A-homo-5β-cholestane derivatives.

The reduction of 4a-ketones *XVI* and *XXVIII* with lithium aluminum hydride gives predominantly corresponding 4aα-hydroxy derivatives, *i.e.* the diols *XIII* and *XXV*. During the alkaline saponification of the 3β-acetoxy group in ketone *XVI* an isomerization of the substituent in the position 3 already takes place under relatively mild conditions (potassium hydrogen carbonate in boiling methanol), leading to 3β- and 3α-hydroxy ketones *XXIX* and *XXX* in an approximately 4 : 1 ratio. The structure of hydroxy ketones *XXIX* and *XXX* was confirmed by their reacetylation to corresponding 3β- and 3α-acetoxy derivatives *XVI* and *XXVIII*.

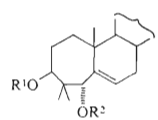
In connection with the study of the mass spectra of the products of the hydrogenolytic cleavage of epoxides *I–IV*, which will be published elsewhere, the mass spectra of diketones *XI* and *XVII* and the ketones *XVI* and *XXVIII–XXX* were also investi-



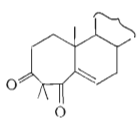
I



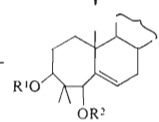
II



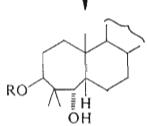
V, R^1 = Ac, R^2 = H
VI, $R^1 = R^2$ = Ac
VII, $R^1 = R^2$ = H



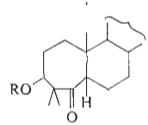
XI



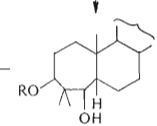
VIII, R^1 = Ac, R^2 = H
IX, $R^1 = R^2$ = Ac
X, $R^1 = R^2$ = H



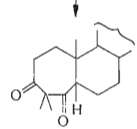
XII, R = Ac
XIII, R = H



XVI, R = Ac
XXIX, R = H

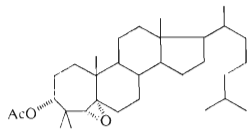


XIV, R = Ac
XV, R = H

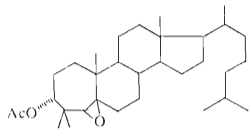


XVII

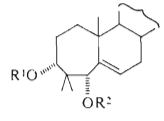
gated. The molecular peak of the saturated diketone *XVII* is very stable (base peak). Its main fragmentation paths starting mostly with a cleavage of the $C_{(3)}$—$C_{(4)}$ and the $C_{(4)}$—$C_{(4a)}$ bonds lead to the best stabilized carbonium ions as intermediates[4]



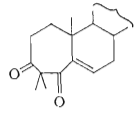
III



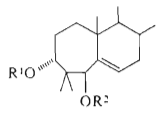
IV



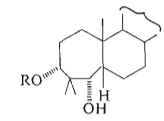
XVIII, R^1 = Ac, R^2 = H
XIX, R^1 = R^2 = Ac
XX, R^1 = R^2 = H



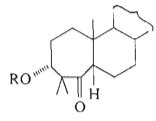
XI



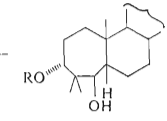
XXI, R^1 = Ac, R^2 = H
XXII, R^1 = R^2 = Ac
XXIII, R^1 = R^2 = H



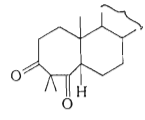
XXIV, R = Ac
XXV, R = H



XXVIII, R = Ac
XXX, R = H

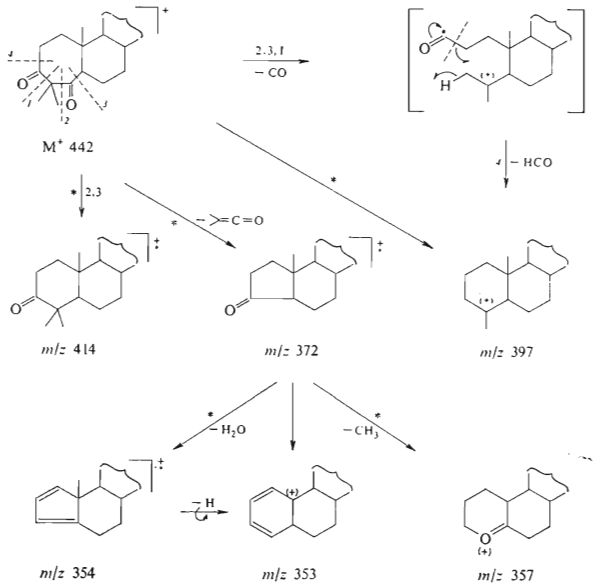


XXVI, R = Ac
XXVII, R = H



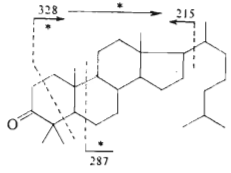
XVII

(Scheme 1, paths *1*, *2*). The ion m/z 414, a product of CO expulsion from the molecular ion, is further decomposed under formation of the most abundant ions m/z 328, 287 and 215 (Scheme 2). The competitive loss of $(CH_3)_2CO$ from M^+ leads to a less



SCHEME 1

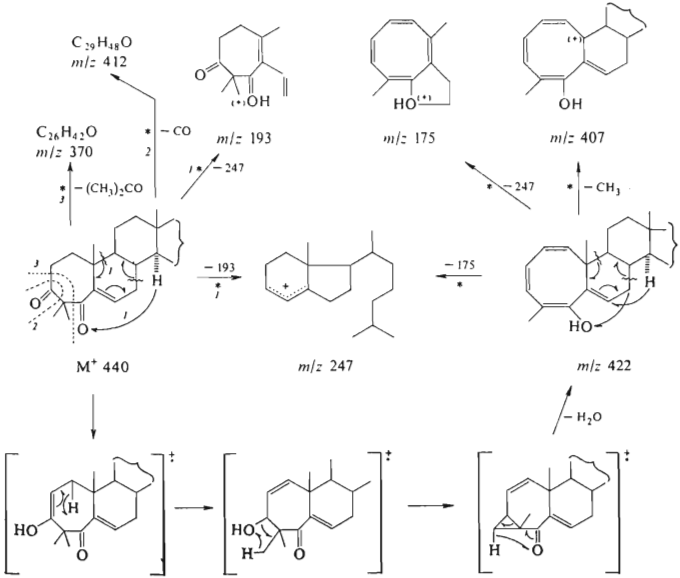
abundant ion m/z 372, which eliminates H_2O under formation of ion m/z 354 (Scheme 1, paths *1*, *3*). The loss of a hydrogen atom from the ion m/z 354 (or the loss of H_2O + H from the ion m/z 372) leads to a carbonium ion m/z 353 (Scheme 1) with the ring A expanded. Similarly, the loss of a methyl group from the ion m/z 372 gives rise to an oxonium ion m/z 357. The elimination of the species CO and HCO from the molecular ion of *XVII* (to give the prominent ion m/z 397, Scheme 1, path *4*) is one of the rare cases when a two-step process is accompanied by a single metastable ion[5] (the corresponding m^* 356·6 being the most abundant metastable ion in the spectrum).



m/z 414

SCHEME 2

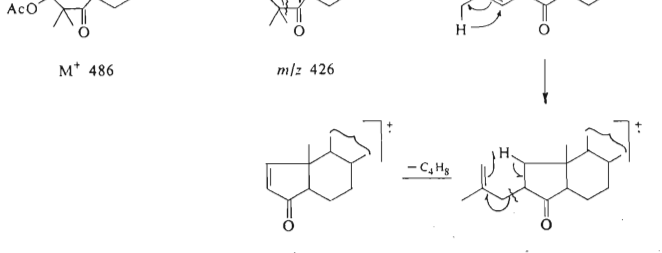
The mass spectrum of the unsaturated diketone *XI* is dominated by the peak M^+ which, in comparison with the molecular ion of the saturated diketone *XVII*, is better stabilized by the presence of the 5,6-double bond. The expulsion of a water molecule from the molecular ion must represent a very complex process connected



SCHEME 3

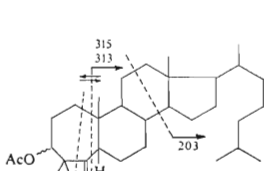
with a skeletal rearrangement of the ring A (Scheme 3). This elimination is accompanied by the most abundant metastable transition (m^* 404·7). The daughter ion m/z 422, for which a cyclooctatetraene structure is assumed, splits off the 19-methyl to give cyclooctatetraene carbonium ion m/z 407 or it undergoes ring B cleavage by a retro-Diels–Alder mechanism with hydrogen rearrangement and charge retention, alternatively, on both fragments (m/z 175 and m/z 247, Scheme 3). The last mentioned process indicates the presence of a 5,6-double bond in the parent ion; hence, in the water elimination from M^+ only the ring A must be involved. An elimination of the neutral fragment $C_{18}H_{31}$ (247 mass units) from the molecular ion leads to ion m/z 193. Abundant metastable peaks corresponding to both the last transitions were observed (m/z 440 → m/z 193, m^* 84; m/z 422 → 175, m^* 72·6). Their presence also supports the cyclic mechanism with hydrogen rearrangement, proposed in Scheme 3. However, it is not quite clear which of the carbonyl groups is expelled from M^+, to give ion m/z 412. The same ambiguity is also associated with the formation of the ion m/z 370 which arises from M^+ by elimination of $(CH_3)_2CO$.

The mass spectra of α- and β-acetoxy ketones *XVI* and *XXVIII* are very similar; they differ merely by the abundance of M^+. The most characteristic feature of the mass spectra of these substances is the elimination of a molecule C_4H_8 from the ion $[M-CH_3COOH]^+$. The process is accompanied by an intensive metastable transition (m/z 426 → m/z 370, m^* 321·4). In the mass spectrum of compound *XXXI* (4,4-[$^2H_6$]-dimethyl analogue of *XVI*), the peak m/z 370 was not shifted. This result indicates that the carbon atom $C_{(4)}$ together with both methyl groups must be included in the species eliminated. Accordingly, the mechanism proposed in Scheme 4



SCHEME 4

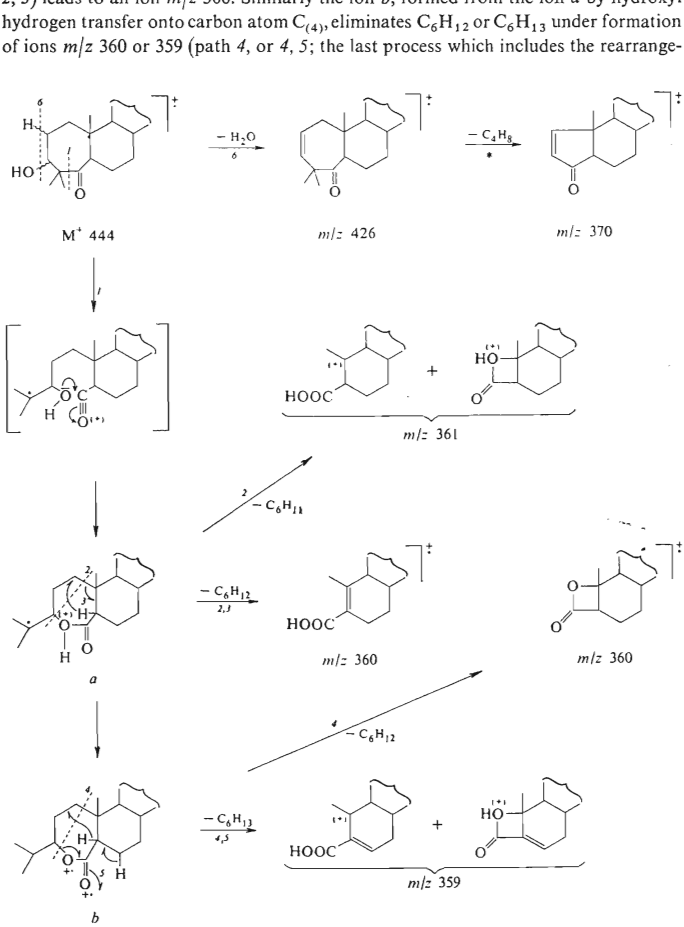
assumes the elimination of CH_3COOH from M^+ by McLafferty rearrangement and a contraction of the 2,3-unsaturated seven-membered ring A to a five-membered one, which easily splits off C_4H_8 by cyclic mechanism after rearrangement of the double bond in the side chain formed. It is interesting that a similar elimination of C_4H_7, preceded by rearrangement of ring A, was shown[6,7] to be a characteristic feature of the mass spectra of normal steroids with a 4,4-dimethyl-Δ^2-grouping. However, in the cited case it was found that the leaving carbon atoms were the $C_{(1)}$—$C_{(3)}$ atoms plus one of the methyls bonded to $C_{(4)}$. The origin of the less important fragments is represented in Scheme 5. Under electron impact the 3-hydroxy



SCHEME 5

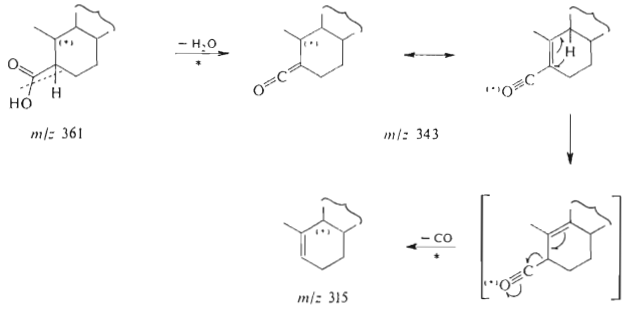
ketones *XXIX* and *XXX* undergo a very unusual and unexpected fragmentation. Their mass spectra (which are almost identical) contain three characteristic abundant ions m/z 359, 360 and 361 with elemental composition corresponding to the loss of C_6H_{13}, C_6H_{12} and C_6H_{11}, respectively, from the molecular ion. For the determination of the origin of these species the 4,4[$^2H_6$]-dimethyl analogue *XXXI* of the 3β-hydroxy ketone *XXIX* was prepared, and its mass spectrum was measured. No shift of the peaks m/z 361, 360 and 359 (as well as of the peaks m/z 370, 343, 315 and 247) was observed. A shift by 6 mass units was observed with ions m/z 331 (loss of the side chain C_8H_{17} from M^+) and m/z 313 (m/z 331 − H_2O). From these results it follows that the C_6 species, split off from M^+, arise from A-ring. This is only possible if the hydroxyl from the A-ring is removed by migration or the entire A-ring is rearranged, because the carbon atom $C_{(3)}$ must be also included in the $C_{(6)}$-chain split off. The probable mechanism of such a rearrangement of the A-ring, permitting to rationalize the observed fragmentation, is shown in Scheme 6 (path *1*; compare a similar unusual fragmentation including the rearrangement of the 3-hydroxyl group, observed[8] in a number of 4-alkylated cholesterols); the cleavage of the $C_{(4)}$—$C_{(4a)}$ bond (which represents the usual initiation of the main fragmentation path of M^+ of the compounds of this series) is followed by the recombination of the seven-membered ring by addition of the free electron pair of the hydroxyl to the electron-defficient carbon atom $C_{(4a)}$. The rearranged molecular ion (Scheme 6, ion *a*) splits off C_6H_{11} (path *2*)

to give ion m/z 361 (base peak). The same cleavage with a hydrogen transfer (paths *2*, *3*) leads to an ion m/z 360. Similarly the ion *b*, formed from the ion *a* by hydroxyl hydrogen transfer onto carbon atom $C_{(4)}$, eliminates C_6H_{12} or C_6H_{13} under formation of ions m/z 360 or 359 (path *4*, or *4*, *5*; the last process which includes the rearrange-



SCHEME 6

ment of two hydrogen atoms is accompanied by an intensive metastable transition ($m^* = 290{\cdot}3$). The ions m/z 361, 360 and 359 are very stable owing to charge delocalization in at least two possible structural forms. In agreement with the proposed structure the ion m/z 361 splits off a molecule of H_2O (Scheme 7) and the ion m/z 343 thus formed loses CO, giving rise to ion m/z 315. The loss of a water molecule from the M^+ of 3-hydroxy ketones *XXIX* and *XXX* proceeds evidently as 1,2-elimination; similarly as with ion $[M-CH_3COOH]^+$ in the spectrum of 3-acetoxy ketones *XVI* and *XXVIII*, the ion $[M-H_2O]^+$ of 3-hydroxy ketones *XXIX* and *XXX* also undergoes the elimination of the species C_4H_8 (Scheme 6, path *6* and Scheme 4) under formation of ion m/z 370.



SCHEME 7

EXPERIMENTAL

The melting points were determined on a Kofler block and they are not corrected. The optical rotation values were measured in chloroform. The infrared spectra were measured on a Zeiss UR 20 instrument in tetrachloromethane, unless stated otherwise. The ^1H NMR spectra were measured, unless otherwise stated, on a Tesla B 476 (60 MHz) apparatus in deuteriochloroform, using tetramethylsilane as internal reference. The chemical shifts are given in ppm. The CD spectra were measured on Dichrographe II (Jouan–Roussel) in dioxane. The mass spectra were measured on a mass spectrometer AEI MS 902 (associated Electric Industries, Manchester, Great Britain) with double focussing. The exact masses found are within ± 3 ppm of the theoretical values. The identity of the samples prepared by various routes was checked by mixture melting points and infrared spectra. The term "conventional work-up" means: The solution was washed with 5% hydrochloric acid, 5% potassium hydrogen carbonate solution and water, dried over anhydrous sodium sulfate and the solvent was evaporated in a vacuum. The crude products were submitted to preparative chromatography on silica gel plates (20 × 20 cm) with light petroleum ether 9 : 1, unless stated otherwise. The required zones were combined, eluted with ether and the solvent was evaporated under reduced pressure.

Hydrogenolytic Opening of the Epoxide Ring in Epoxides *I—IV*

Adams catalyst (50 mg) was added to a solution of epoxide (135 mg) in acetic acid (36 ml) and the mixture was shaken with hydrogen for 90 min. The catalyst was filtered off and the filtrate was poured into water. The product was extracted with ether and the extract washed with 5% aqueous potassium hydrogen carbonate solution and water. After drying of the organic phase over sodium sulfate and filtration the solvent was evaporated in a vacuum. The residue was submitted to preparative thin-layer chromatography on 3 silica gel plates. The yields of the products are given in Table I. The ^{1}H NMR data of the products are presented in Table II and their analytical data and physical constants in Table III.

3β,4aα-Diacetoxy-4,4-dimethyl-A-homo-5-cholestene (*VI*)

Alcohol *V* (120 mg) was acetylated with acetic anhydride (1 ml) in pyridine (4 ml) for three days. The conventional work-up afforded 120 mg of a crude product which was chromarographed preparatively on 3 silica gel plates. The corresponding less polar zones afforded, after working up, 110 ml of diacetoxy derivative *VI* which was crystallized from methanol (68 mg), m.p. 154·5 to

TABLE III

Analytical and physical data of the cleavage products of epoxides *I—IV*

Compound	Formula (m.w.)	Calculated/Found		M.p., °C $[\alpha]_D^{20}$
		% C	% H	
V	$C_{32}H_{54}O_3$	78·96	11·18	141—142
	(486·8)	78·72	11·07	+57°
VIII	$C_{32}H_{54}O_3$	78·96	11·18	146—148
	(486·8)	78·54	11·42	−22°
XII	$C_{32}H_{56}O_3$	78·63	11·55	200·5—201·5
	(488·8)	78·13	11·17	+30°
XIV	$C_{32}H_{56}O_3$	78·63	11·55	208·5—210·5
	(488·8)	78·44	11·85	+27°
XVIII	$C_{32}H_{54}O_3$	78·96	11·18	oil
	(486·8)	78·80	11·10	+22°
XXI	$C_{32}H_{54}O_3$	78·96	11·18	165—167
	(486·8)	78·70	10·95	−32°
XXIV	$C_{32}H_{56}O_3$	78·63	11·55	158—160
	(488·8)	78·41	11·25	− 4°
XXVI	$C_{32}H_{56}O_3$	78·63	11·55	146—148
	(488·8)	78·34	11·08	− 8°

156·5°C, $[\alpha]_D^{20}$ +75° (*c* 0·5). Infrared spectrum: 1 741, 1 248, 1 239, 3 065, 3 025, 1 666 cm^{-1}, For $C_{34}H_{56}O_4$ calculated mol. weight: 528·79; found (mass spectrometry): 528. The more polar fractions were combined and worked up, affording 9 mg of the starting alcohol *V*, m.p. 141–142°C, $[\alpha]_D^{20}$ +57° (*c* 0·5).

4,4-Dimethyl-A-homo-5-cholestene-3β,4aα-diol (*VII*)

To a solution of the acetoxy derivative *V* (40 mg) in ether (5 ml) an excess of lithium aluminum hydride was added and the mixture was allowed to stand at room temperature for 15 min. The excess of the hydride was decomposed with a saturated aqueous sodium sulfate solution and the mixture was filtered through a small column of sodium sulfate. The filtrate was concentrated in a vacuum and the residue (40 mg) crystallized from an ether–heptane mixture affording 30 mg of diol *VII*, m.p. 159–161·5°C, $[\alpha]_D^{20}$ +55° (*c* 0·5). Infrared spectrum (chloroform): 3 625, 1 073; 1 046, 988 cm^{-1}. For $C_{30}H_{52}O_2$ (444·72) calculated: 81·02% C, 11·79% H; found: 81·12% C, 11·52% H.

3β,4aβ-Diacetoxy-4,4-dimethyl-A-homo-5-cholestene (*IX*)

Alcohol *VIII* (150 mg) was acetylated with acetic anhydride (5 ml) in pyridine (5 ml) for 3 days. The conventional work-up afforded 150 mg of a crude product, which was submitted to preparative chromatography on 3 thin-layer silica gel plates. The corresponding less polar zones were worked-up, giving 35 mg of diacetoxy derivative *IX* which was crystallized from methanol (15 mg), m.p. 143–144·5°C, $[\alpha]_D^{20}$ +10° (*c* 0·5). Infrared spectrum: 1 740, 1 733, 1 254, 1 238, 1 656 cm^{-1} For $C_{34}H_{56}O_4$ (528·8) calculated: 77·22% C, 10·68% H; found: 77·44% C, 10·76% H. The corresponding more polar zones were also worked up, giving 110 mg of the starting alcohol *VIII*, m.p. 146–148°C, $[\alpha]_D^{20}$ −22° (*c* 0·5).

4,4-Dimethyl-A-homo-5-cholestene-3β,4aβ-diol (*X*)

An excess of lithium aluminum hydride was added to a solution of acetoxy derivative *VIII* (50 mg) in ether (5 ml) and the mixture was allowed to stand at room temperature for 15 min. The excess of the hydride was decomposed with an aqueous saturated sodium sulfate solution and the mixture was filtered through a small column of sodium sulfate. The filtrate was concentrated in a vacuum and the residue (50 mg) crystallized from methanol, affording 36 mg of diol *X*, m.p. 162–164°C, $[\alpha]_D^{20}$ −11° (*c* 0·5). Infrared spectrum (chloroform): 3 625, 1 046, 1 020, 1 652 cm^{-1}. For $C_{30}H_{52}$, $.O_2$ (444·7) calculated: 81·02% C, 11·79% H; found: 80·94% C, 11·48% H.

4,4-Dimethyl-A-homo-5-cholestene-3,4a-dione (*XI*)

a) Chromium trioxide (45 mg) was added to a solution of diol *VII* (45 mg) in pyridine (2 ml) and the mixture was allowed to stand at room temperature overnight. The conventional work-up afforded 40 mg of a crude product which was chromatographed preparatively on one silica gel thin-layer plate. The required zone was worked up, affording 21 mg of dione *XI* which was crystallized from methanol (6 mg), m.p. 121·5–123·5°C, $[\alpha]_D^{20}$ −73° (*c* 0·5). Infrared spectrum: 1 720, 1 693, 1 660, 1 630 cm^{-1}. For $C_{30}H_{48}O_2$ (440·7) calculated: 81·76% C, 10·98% H; found: 81·48% C, 10·75% H.

b) Chromium trioxide (50 mg) was added to a solution of diol *X* (50 mg) in pyridine (3 ml) and the mixture was allowed to stand at room temperature overnight. The conventional work-up

afforded 50 mg of a crude product which was chromatographed as under *a*), affording 45 mg of dione *XI* which was crystallized from methanol (28 mg), m.p. 121·5–123·5°C, $[\alpha]_D^{20}$ −73° (*c* 0·5).

c) Chromium trioxide (20 mg) was added to a solution of diol *XX* (20 mg) in pyridine (1 ml) and the mixture was allowed to stand at room temperature for 3 days. The conventional work-up afforded 20 mg of a crude product which was chromatographed as under *a*). Yield, 12 mg of dione *XI*, which was crystallized from methanol, m.p. 121·5–123·5°C, $[\alpha]_D^{20}$ −73° (*c* 0·5).

d) Chromium trioxide (20 mg) was added to a solution of diol *XXIII* (20 mg) in pyridine (1 ml) and the mixture was allowed to stand at room temperature for 2 days. The conventional work-up afforded 20 mg of a crude product which was chromatographed as under *a*), affording 18·5 mg of dione *XI* which was crystallized from methanol (10 mg), m.p. 121·5–123·5°C $[\alpha]_D^{20}$ −73° (*c* 0·5).

4,4-Dimethyl-A-homo-5β-cholestane-3β,4aα-diol (*XIII*)

a) An excess of lithium aluminum hydride was added to a solution of acetoxy derivative *XII* (50 mg) in ether (5 ml) and the mixture was allowed to react at room temperature for 15 min, The excess of the hydride was decomposed with a saturated aqueous sodium sulfate solution and the reaction mixture was filtered through a small column of sodium sulfate. The filtrate was concentrated in a vacuum and the residue (50 mg) chromatographed preparatively on a silica gel plate. The required zone was worked up affording 40 mg of diol *XIII* which was crystallized from aqueous methanol (29 mg), m.p. 120–122°C, $[\alpha]_D^{20}$ +25° (*c* 0·5). Infrared spectrum (chloroform): 3 625, 1 049, 1 025 cm^{-1}. For $C_{30}H_{54}O_2$ (446·7) calculated: 80·65% C, 12·18% H; found: 80·16% C, 12·05% H.

b) Lithium aluminum hydride (in excess) was added to a solution of ketone *XVI* (40 mg) in ether (5 ml) and the mixture was allowed to stand at room temperature for 15 min. The same working up procedure as under *a*) gave 40 mg of a crude product which was chromatographed as under *a*). The corresponding more polar zone was eluted and 35 mg of diol *XIII* were obtained, which was crystallized from aqueous methanol (11 mg), m.p. 120–122°C, $[\alpha]_D^{20}$ +25° (*c* 0·5). The corresponding less polar zone afforded, after working up, 3 mg of acetoxy derivative *XII*, m.p. 200–201·5°C, $[\alpha]_D^{20}$ +30° (*c* 0·5).

4,4-Dimethyl-A-homo-5β-cholestane-3β,4aβ-diol (*XV*)

An excess of lithium aluminum hydride was added to a solution of acetoxy derivative *XIV* (40 mg) in ether (4 ml) and the mixture was allowed to react at room temperature for 15 min. The same working up procedure as in the preparation of diol *XIII* gave 40 mg of a crude product which was chromarographed preparatively on one silica gel thin-layer plate in light petroleum–ether 8 : 2. The required zone was worked up, affording 36 mg of diol *XV* which was crystallized from methanol (20 mg), m.p. 190–192°C, $[\alpha]_D^{20}$ +25° (*c* 0·5). Infrared spectrum (chloroform): 3 630, 1 040, 998 cm^{-1}. For $C_{30}H_{54}O_2$ (446·7) calculated: 80·65% C, 12·18% H; found: 80·34% C, 12·03% H.

3β-Acetoxy-4,4-dimethyl-A-homo-5β-cholestan-4a-one (*XVI*)

a) Chromium trioxide (20 mg) was added to a solution of alcohol *XII* (20 mg) in pyridine (2 ml) and the mixture was allowed to stand at room temperature for 2 days. The conventional work-up afforded 20 mg of a crude product which was crystallized from methanol. Yield, 10 mg of ketone

XVI, m.p. 153–155°C, $[\alpha]_D^{20}$ +34° (*c* 0·5). Infrared spectrum: 1 705, 1 740, 1 241, 1 226 cm^{-1}. CD spectrum: $\Delta\varepsilon_{305} = +2{\cdot}09$. For $C_{32}H_{54}O_3$ (486·75) calculated: 78·96% C, 11·18% H; found: 78·67% C, 11·09% H.

b) Chromium trioxide (120 mg) was added to a solution of alcohol *XIV* (120 mg) in pyridine (5 ml) and the mixture was allowed to stand at room temperature for 2 days. The conventional work-up afforded 120 mg of a crude product which was crystallized from methanol, affording 100 mg of ketone *XVI*, m.p. 153–155°C, $[\alpha]_D^{20}$ +34° (*c* 0·5).

c) Alcohol *XXIX* (50 mg) was acetylated with acetic anhydride (0·5 ml) in pyridine (3 ml) overnight. The conventional work-up afforded 48 mg of a crude product which was chromatographed preparatively on a silica gel thin-layer plate. The corresponding zone was worked up, giving 45 mg of ketone *XVI*, which was crystallized from methanol (31 mg), m.p. 153–155°C, $[\alpha]_D^{20}$ +34° (*c* 0·5).

4,4-Dimethyl-A-homo-5β-cholestane-3,4a-dione (*XVII*)

a) Chromium trioxide (20 mg) was added to a solution of diol *XIII* (30 mg) in pyridine (2 ml) and the mixture was allowed to stand at room temperature for 4 days. The conventional work-up led to 30 mg of a crude product which was crystallized from methanol, giving 20 mg of dione *XVII*, m.p. 116–118°C, $[\alpha]_D^{20}$ +53° (*c* 0·5). Infrared spectrum (chloroform): 1 720, 1 691 cm^{-1}, For $C_{30}H_{50}O_2$ (442·7) calculated: 81·39% C, 11·38% H; found: 81·11% C, 11·14% H.

b) Chromium trioxide (25 mg) was added to a solution of diol *XXVII* (25 mg) in pyridine (2 ml) and the mixture was allowed to stand at room temperature overnight. The conventional work-up gave 25 mg of crude product which was purified by preparative thin-layer chromatography on one silica gel plate (10 × 20 cm), using light petroleum–ether 95 : 5 for development. The zone corresponding to the required product was worked up, affording 18 mg of dione *XVII* which was crystallized from methanol, m.p. 116–118°C, $[\alpha]_D^{20}$ +53° (*c* 0·5).

3α,4aα-Diacetoxy-4,4-dimethyl-A-homo-5-cholestene (*XIX*)

Alcohol *XVIII* (100 mg) was acetylated with acetic anhydride (0·5 ml) in pyridine (3 ml) overnight. After the conventional work-up 100 mg of a crude product were obtained which was chromatographed on two preparative silica gel thin-layder plates. The required zones were combined and worked up affording 60 mg of diacetoxy derivative *XIX* which was crystallized from methanol (41 mg), m.p. 147–149°C, $[\alpha]_D^{20}$ +42° (0·5). Infrared spectrum: 1 741, 1 249, 1 030, 1 019, 1 664, 3 050 cm^{-1}. For $C_{34}H_{58}O_4$ calculated mol. weight: 528·79; found (mass spectrometry): 528. The corresponding more polar zones were combined and worked up to afford 30 mg of the starting alcohol *XVIII*, which was resistant to all the attempts at crystallization, $[\alpha]_D^{20}$ +22° (0·5).

4,4-Dimethyl-A-homo-5-cholestene-3α,4aα-diol (*XX*)

An excess of lithium aluminum hydride was added to a solution of acetoxy derivative *XVIII* (30 mg) in ether (5 ml) and the mixture was allowed to react at room temperature for 10 min. The excess of the hydride was decomposed with a saturated aqueous sodium sulfate solution and the mixture was filtered through a small column of sodium sulfate. The filtrate was concentrated under reduced pressure and the residue (30 mg) was crystallized from heptane, giving 18 mg of diol *XX*, m.p. 170–172°C, $[\alpha]_D^{20}$ +33° (0·5). Infrared spectrum (chloroform): 3 615, 1 033, 1 015, 1 005, 3 055, 1 661 cm^{-1}. For $C_{30}H_{52}O_2$ (444·72) calculated: 81·02% C, 11·79% H; found: 81·10% C, 11·64% H.

3α,4aβ-Diacetoxy-4,4-dimethyl-A-homo-5-cholestene (*XXII*)

Alcohol *XXI* (70 mg) was acetylated with acetic anhydride (1 ml) in pyridine (8 ml) for 3 days. The conventional work-up procedure led to 70 mg of a crude product which was purified by preparative chromatography on two silica gel plates. The corresponding less polar zones were worked up affording 40 mg of diacetoxy derivative *XXII*. This was crystallized from methanol (28 mg), m.p. 146–148°C. $[\alpha]_D^{20}$ −23° (*c* 0·5). Infrared spectrum: 1 742, 1 236, 1 023, 1 655 cm^{-1}. For $C_{34}H_{58}O_4$ calculated mol. weight: 528·79; found (mass spectrometry): 528. The working up of the corresponding more polar zones gave 30 mg of the starting alcohol *XXI*, m.p. 165 to 167°C, $[\alpha]_D^{20}$ −32° (*c* 0·5).

4,4-Dimethyl-A-homo-5-cholestene-3α,4aβ-diol (*XXIII*)

An excess of lithium aluminum hydride was added to a solution of acetoxy derivative *XXI* (90 mg) in ether (5 ml) and the mixture was allowed to react at room temperature for 15 min. The excess of the hydride was decomposed with a saturated aqueous sodium sulfate solution and the mixture filtered through a small column of sodium sulfate. The filtrate was concentrated in a vacuum and the residue (90 mg) crystallized from heptane to give 56 mg of diol *XXIII*, m.p. 211–213°C, $[\alpha]_D^{20}$ −13° (*c* 0·5). Infrared spectrum (chloroform): 3 320, 1 037, 1 026, 1 017, 1 651 cm^{-1}. For $C_{30}H_{52}O_2$ (444·7) calculated: 81·02% C, 11·79% H; found: 81·10% C, 11·37% H.

4,4-Dimethyl-A-homo-5β-cholestane-3α,4aα-diol (*XXV*)

a) An excess of lithium aluminum hydride was added to a solution of acetoxy derivative *XXIV* (20 mg) in ether (3 ml) and the mixture was allowed to react at room temperature for 10 min. The same work-up as in the case of diol *XXIII* gave 20 mg of a crude product which was chromatographed preparatively on one silica gel thin-layer plate (10 × 20 cm) using light petroleum–ether 8 : 2 for development. The required zone was worked up, giving 17 mg of diol *XXV* which was crystallized from methanol (9 mg), m.p. 133–135°C, $[\alpha]_D^{20}$ −4° (*c* 0·5). Infrared spectrum (chloroform): 3 630, 1 058, 1 030 cm^{-1}. For $C_{30}H_{54}O_2$ (446·7) calculated: 80·65% C, 12·18% H; found: 80·35% C, 12·03% H.

b) An excess of lithium aluminum hydride was added to a solution of ketone *XXVIII* (20 mg) in ether (3 ml) and the mixture was allowed to react at room temperature for 15 min. Working up as under *a*) gave 20 mg of a crude product which was chromatographed as under *a*), giving 15 mg of diol *XXV*. After crystallization from methanol its m.p. was 133–135°C and $[\alpha]_D^{20}$ −4° (*c* 0·5).

4,4-Dimethyl-A-homo-5β-cholestane-3α,4aβ-diol (*XXVII*)

An excess of lithium aluminum hydride was added to a solution of acetoxy derivative *XXVI* (42 mg) in ether (5 ml) and the mixture was allowed to react at room temperature for 10 min. The excess of the hydride was decomposed with a saturated aqueous sodium sulfate solution and the mixture filtered through a small column of sodium sulfate. The filtrate was concentrated under reduced pressure giving 40 mg of a crude product which was purified by chromatography on a silica gel thin-layer plate in light petroleum–ether 8 : 2. The required zone was worked up, affording 38 mg of diol *XXVII* which was crystallized from heptane (30 mg), m.p. 140–141°C Infrared spectrum (chloroform): 3 630, 1 041, 1 026 cm^{-1}. For $C_{30}H_{54}O_2$ (446·7) calculated: 80·65% C, 12·18% H; found: 80·27% H, 12·09% H.

3α-Acetoxy-4,4-dimethyl-A-homo-5β-cholestan-4a-one (*XXVIII*)

a) Chromium trioxide (20 mg) was added to a solution of alcohol *XXIV* (20 mg) in pyridine (1 ml) and the mixture was allowed to stand at room temperature overnight. The conventional work-up gave 20 mg of a crude product which was chromatographed on one preparative silica gel plate (10 × 20 cm) in light petroleum–ether 95 : 5. The proper zone was worked up, giving 17 mg of ketone *XXVIII* which was crystallized from methanol (11 mg), m.p. 166–167·5°C, $[\alpha]_D^{20}$ +40° (*c* 0·5). Infrared spectrum: 1 738, 1 240, 1 027, 1 709 cm^{-1}. CD spectrum: $\Delta\varepsilon_{302} = +3{\cdot}12$. For $C_{32}H_{54}O_3$ (486·7) calculated: 78·96% C, 11·18% H; found: 78·47% C, 11·25% H.

b) Chromium trioxide (20 mg) was added to a solution of alcohol *XXVI* (20 mg) in pyridine (1 ml) and the mixture was allowed to stand at room temperature overnight. The conventional work-up gave 20 mg of a crude product which was chromatographed as under *a*). Yield, 15 mg of ketone *XXVIII* which was crystallized from methanol, m.p. 166–167·5°C, $[\alpha]_D^{20}$ +40° (*c* 0·5).

c) Alcohol *XXX* (18 mg) was acetylated with acetic anhydride (0·5 ml) in pyridine (3 ml) overnight. The conventional work-up gave 18 mg of a crude product which was crystallized from methanol to give 10 mg of ketone *XXVIII*, m.p. 166–167·5°C, $[\alpha]_D^{20}$ +40° (*c* 0·5).

4,4-Dimethyl-3β-hydroxy-A-homo-5β-cholestan-4a-one (*XXIX*)

An aqueous solution of potassium hydrogen carbonate (110 mg, 1 ml) was added to a solution of acetoxy derivative *XVI* (110 mg) in methanol (10 ml) and the mixture was refluxed for 1 h. After pouring it into water the product was extracted with ether and the ethereal extract washed with water, dried over sodium sulfate and the solvent evaporated under reduced pressure. The residue (110 mg) was chromatographed preparatively on three silica gel thin-layer plates in light petroleum–ether 95 : 5 (double development). The corresponding less polar zones were combined and worked up affording 80 mg of alcohol *XXIX* which was crystallized from methanol (68 mg) m.p. 141–143°C. Infrared spectrum (KBr pellet): 1 075, 1 044, 1 699, 1 689 cm^{-1}. For $C_{30}H_{52}O_2$ (444·7) calculated: 81·02% C, 11·79% H; found: 81·10% C, 11·48% H.

4,4-Dimethyl-3α-hydroxy-A-homo-5β-cholestan-4a-one (*XXX*)

The corresponding more polar zones after the separation of the 3β-alcohol *XXIX* from the preceding experiment afforded 25 mg of alcohol *XXX* which was crystallized from methanol (19 mg), m.p. 158–160°C. Infrared spectrum (chloroform): 1 693, 3 615, 1 043, 1 030 cm^{-1}. For $C_{30}H_{52}O_2$ (444·7) calculated: 81·02% C, 11·79% H; found: 80·89% C, 11·67% H.

Mass Spectra

The mass spectra were measured on a double focussing mass spectrometer AEI MS 902 (Associated Electric Industries, Manchester, Great Britain). The samples were introduced by direct inlet into the ion source heated at 140–170°C. The low resolution mass spectra were recorded using resolving power 1 000 and electron energy of 70 eV. The high resolution measurements were carried out using the resolving power 10 000. The exact masses found are within ±4 ppm of theoretical value.

The partial mass spectra (*i.e.* the most important peaks from the upper part of spectra) of substances *XI*, *XVI*, *XVII*, *XXVIII*–*XXX* and of 4,4-[$^2H_6$]-dimethyl analogues of compounds *XVI* and *XXIX*, *i.e.* of substances *XXXI* and *XXXII* which were prepared from 4,4-[$^2H_6$]-dimethyl analogues of 4aα,5α-epoxide *I* using the same reaction sequence as for compounds *XVI*

and *XXIX*, are presented. The 4,4-[$^1H_6$]-dimethyl analogue of 4aα,5α-epoxide *I* was prepared according to a previously described procedure[1,9] from 4,4-[$^2H_6$]-dimethyl analogue of 4,4-dimethyl-A-homo-4a-cholesten-3-one which was prepared on methylation of A-homo-4a-cholesten-3-one[10] with [$^2H_6$]-methyl iodide[11]. The masses and the corresponding relative abundances (in brackets) in percents of the base peak are given. The elemental composition, corresponding to the found exact mass (if determined) is given in the brackets after the relative abundance.

XI: 175 (18·2, $C_{12}H_{15}O$); 193 (19, $C_{12}H_{17}O_2$); 247 (4); 257 (2); 313 (2·3); 327 (2·1, $C_{22}H_{31}O_2$ + + $C_{24}H_{39}$, 1 : 1); 241 (2·1, $C_{24}H_{37}O$); 353 (3·2, $C_{26}H_{41}$); 355 (2·7); 370 (12·8, $C_{26}H_4aO$); 397 (6·3, $C_{28}H_{45}O$); 407 (9·2, $C_{29}H_{43}O$); 412 (6·5, $C_{29}H_{48}O$); 422 (18·2); M^+ 440 (100; $C_{30}H_{48}O_2$).

XVII: 215 (84, $C_{16}H_{23}$); 247 (9); 259 (8); 283 (6·6); 287 (100, $C_{21}H_{35}$); 301 (24, $C_{21}H_{33}O$); 313 (12, $C_{23}H_{37}$); 315 (24, $C_{23}H_{39}$); 328 (60, $C_{24}H_{40}$); 329 (26); 353 (7, $C_{26}H_{41}$); 354 (8·6, $C_{26}H_{42}$); 357 (9, $C_{25}H_{41}O$); 372 (9, $C_{26}H_{44}O$); 397 (52, $C_{29}H_{49}$); 398 (16·8); 399 (11·6); 414 (32, $C_{29}H_{50}O$); 427 (5·6); M^+ 442 (100; $C_{30}H_{50}O_2$).

XVI: 203 (18); 247 (9); 257 (9·2); 271 (12·5); 313 (36); 315 (42); 343 (10); 355 (6·6); 361 (6); 370 (82); 383 (4); 408 (3·8); 411 (13·3); 426 (100); 443 (1·6); 444 (3); 471 (3·3); M^+ 386 (4·7).

XXVIII: 203 (12·6; $C_{15}H_{23}$ + $C_{14}H_{19}O$, 4 : 1); 247 (6·5;) 257 (8, $C_{18}H_{25}O$); 271 (10·4, C_{19}·$H_{27}O$); 313 (30, $C_{22}H_{33}O$ + $C_{23}H_{37}$, 3 : 1); 315 (35, $C_{23}H_{39}$); 343 (12·8, $C_{24}H_{39}O$); 355 (6·8, $C_{25}H_{39}O$ + $C_{26}H_{43}$, 6 : 5); 356 (4·6); 361 (4·6, $C_{24}H_{41}O_2$); 370 (95, $C_{26}H_{42}O$); 383 (3·8, $C_{27}H_{43}O$ + $C_{28}H_{47}$, 1 : 1); 384 (4·6, $C_{27}H_{44}O$ + $C_{28}H_{48}$. 2 : 1); 408 (3·4); 411 (12); 426 (100, $C_{30}H_{50}O$); 444 (3·6); 471 (2·2); 484 (0·6); 485 (0·8); M^+ 486 (1·4).

XXXI: 203 (11·4); 277 (8·6); 315 (32); 319 (22); 370 (80); 417 (9·7); 432 (100); 450 (3·4); 477 (3·2); M^+ 492 (7).

XXIX: 193 (36); 207 (42); 221 (23); 235 (23); 247 (31·4); 313 (23); 315 (56); 331 (47, $C_{22}H_{35}O_2$) 343 (37, $C_{24}H_{39}O$); 355 (7) 359 (84, $C_{24}H_{39}O_2$); 360 (60, $C_{24}H_{40}O_2$); 361 (100, $C_{24}H_{41}O_2$); 370 (39, $C_{26}H_{42}O$); 375 (5); 383 (4); 397 (4·3); 398 (3·7); 411 (8·3); 426 (34); 429 (23); M^+ 444 (60, $C_{30}H_{52}O_2$).

XXX: 193 (31.4); 207 (40); 221 (23); 235 (20); 247 (29); 313 (20.6); 315 (50); 331 (43); 343 (30); 355 (14·6); 359 (76); 360 (62); 361 (100); 370 (31·4); 373 (12·3); 375 (5·4); 383 (5·7); 397 (12), 401 (60); 402 (5); 411 (8·6); 426 (39); 429 (22); M^+ 444 (77).

XXXII: 193 (27); 207 (35); 221 (19); 235 (19); 247 (26); 313 (7·4); 315 (50); 319 (12·5); 337 (35); 343 (32); 355 (9); 359 (70); 360 (56); 361 (100); 370 (34); 373 (7); 375 (6); 403 (7); 404 (5); 410 (8); 417 (7); 418 (8·6); 432 (36); 435 (19); M^+ 450 (60).

The analyses were carried out in the Analytical Laboratory of this Institute under the direction of Dr J. Horáček. We thank Mrs K. Matoušková for the measurement of the infrared spectra and Dr J. Smolíková for their interpretation. Our thanks also due to Dr S. Vašíčková for the measurement of the CD *spectra, Dr M. Synáčková and Mrs J. Jelínková for the measurement of the* ^{1}H NMR *spectra, and Mrs P. Loudová for the recording of the mass spectra. Technical assistance was provided by Mrs M. Bárová.*

REFERENCES

1. Velgová H., Trka A.: Thts Journal *47*, 315 (1982).
2. Karplus M.: J. Amer. Chem. Soc. *85*, 2870 (1963).
3. Karplus M.: J. Chem. Phys. *30*, 11 (1959).

4. Budzikiewicz H. in the book: *Biochemical Applications of Mass Spectrometry* (G. R. Waller, Ed.), p. 257. Wiley, New York 1980.
5. Bursey J. T., Bursey M.M., Kingston D. G. J.: Chem. Rev. *73*, 191 (1973).
6. Flaskamp E., Kesterke H. J., Budzikiewicz H.: Monatsh. Chem. *107*, 815 (1976).
7. Waller G. R. (Ed.): *Biochemical Applications of Mass Spectrometry*, 1st Supplem. Vol., p. 213. Wiley, New York 1980.
8. Knapp F. F. jr, Schroepfer G. J. jr: J. Chem. Soc. Chem. Commun. *1974*, 537.
9. Velgová H., Černý V.: This Journal *41*, 3131 (1976).
10. Johnson W. S., Neeman N., Birkeland S. P., Fedoruk N. A.: J. Amer. Chem. Soc. *84*, 989 (1962).
11. Cotton F. A., Fassnacht J. H., Horrocks W. D. jr, Nelson N. A.: J. Chem. Soc. *1959*, 4138.

Translated by Ž. Procházka.