An Unusual Mass Spectral Fragmentation of C-4 Alkylated Cholesterols

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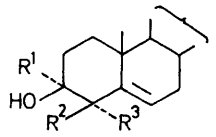
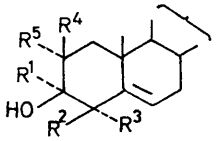
Summary The mass spectra of C-4-alkylated cholest-5-en-3β-hydroxysterols are characterized by an ion (*m/e* 331) resulting from the unusual intramolecular transfer of the 3β-hydroxy-group to the charge-retaining fragment with concomitant loss of ring A.

THE mass spectral fragmentation properties of steroids have received considerable attention during the last decade.[1,2] Although cholesterol (I) and related monohydroxy-sterols generally undergo rather complex fragmentation, the high mass region of their mass spectra

show ions which correspond formally to rather simple fragmentations involving the loss of H_2O, CH_3, and the iso-octyl sidechain.[3] The mass spectra of the isomeric C-4-methylated sterols (II) and (III)† contain an additional moderately intense ion at *m/e* 331 [(II) Σ_{67} 1·8%, rel. int. 12%, (III) Σ_{65} 2·6%, rel. int. 36%]‡. This ion is not found in the spectrum of (I) and appears to represent a fragmentation specific to C-4 alkylated cholesterols, arising from a process involving fragmentation of the nucleus. Compounds (IV) and (IX) also give the *m/e* 331 ion [(IV) Σ_{67} 3·6%; (IX) Σ_{67} 2·5%], as do the analogues deuteriated at C-2 [(X), (XII), (XIV), and (XVI)], C-3 [(V), (VI), (XI), (XII), (XV), and (XVI)], C-4 [(V) and (VI)], and C-30 and C-31 [(VII), and (XIII)—(XVI)]. High resolution measurements show that the *m/e* 331 ion had the composition $C_{23}H_{39}O$. In the mass spectrum of 4α-methylcholest-5-en-3β-[^{18}O]ol the ion shifted to *m/e* 333 (Σ_{67} 1·1%) while it shifted to *m/e* 332 in that of 4β-methylcholest-5-en-3β-[^{2}H]ol (Σ_{67} 2·4%).

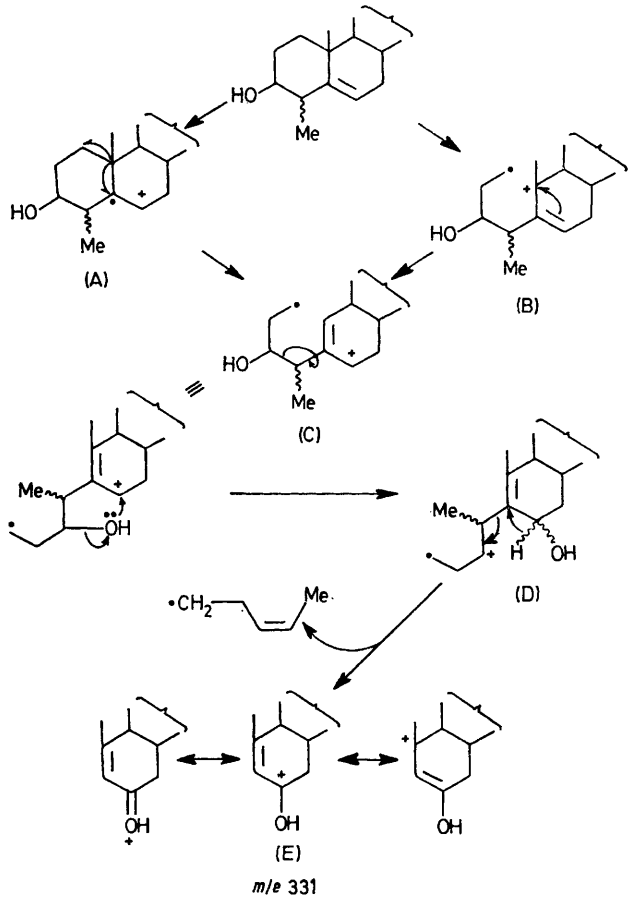


	R^1	R^2	R^3
(I)	H	H	H
(II)	H	H	Me
(III)	H	Me	H
(IV)	H	Et	H
(V)	D	D	Me
(VI)	D	Me	D
(VII)	H	H	CD_3
(VIII)	H	CD_3	H



	R^1	R^2	R^3	R^4	R^5
(IX)	H	Me	Me	H	H
(X)	H	Me	Me	D	D
(XI)	D	Me	Me	H	H
(XII)	D	Me	Me	D	D
(XIII)	H	CD_3	CD_3	H	H
(XIV)	H	CD_3	CD_3	D	D
(XV)	D	CD_3	CD_3	H	H
(XVI)	D	CD_3	CD_3	D	D

These results indicate that the *m/e* 331 ion is formed by a process involving loss of elements of ring A with transfer of the hydroxy O and H atoms to the charge-retaining species. One possible mechanism for its formation is in the Scheme. Ionization of either 4α-methyl or 4β-methyl-cholest-5-en-3β-ol could form either ion (A) or (B). Rearrangement to (C) and rotation about the C-4,5 bond would then position the C-3 OH group favourably for transfer to C-6 with concomitant scission of the C–O bond. Species (D) could then be cleaved in a number of ways one of which would involve transfer of the 6-H to C-5 with loss of the mass 69 fragment. The *m/e* 331 ion (E) is very stable since many resonance forms can be written. The mechanism of the effect of the 4-alkyl substituents which leads to the formation of this ion in the spectra of 4-alkylated-Δ^5-sterols but not in that of cholesterol is not yet clear.



SCHEME

The *m/e* 331 ion is not found in the spectra of C-4 alkylated cholestanols, cholesta-5,7-dienols, cholest-7-enols, the acetates of compounds (I)—(XVI), or the 3β-trimethylsilyl ether of 4β-methyl-cholest-5-en-3β-ol.[5,6]

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† All compounds were prepared by well established routes except (V) and (VI). Reduction of 6β-bromo-4-methylcholest-4-en-3-one with a large excess of $LiAlD_4$ gave (V). Reduction with a small excess of $LiAlD_4$ gives (II) and the C-4-isomer (III) (*ca.* 31% yield). The substances were fully characterized by i.r., mass spectral, and n.m.r. methods. Purity was established by t.l.c. and g.l.c.

‡ The mass spectra were obtained using a CEC Model 21-110-B double-focussing instrument at 70 eV; Inlet temperature 200—220°. We thank Dr. J. Hudson for assistance and also helpful discussions. In addition, some samples were analysed using an LKB Model 9000 single focussing instrument.

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