Electron Impact Fragmentation of Steroidal Ethylene Hemithioketals and Ethylene Dithioketals[1]

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Received November 13, 1968

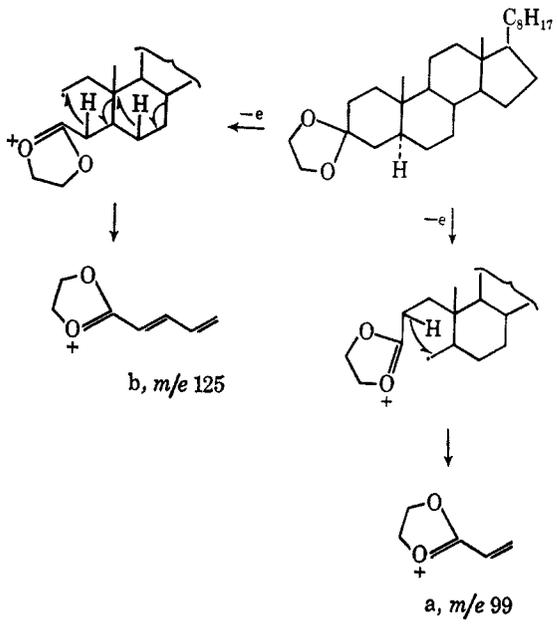
The mass spectra of the 3-ethylene dioxy ketal, hemithioketal, and dithioketal derivatives of cholestane and cholestan-4-one are compared. The hemithioketal is found to be a poor derivative for characterizing steroidal 3-ketones because its fragmentation generates ions resembling the ionized 3-keto compound. The 3-dithioketal is also found to undergo primary fragmentation in the dithiolane ring. Introduction of a carbonyl group at C-4 changes the fragmentation of the dioxy ketal very little, but alters the patterns of the sulfur-containing compounds a great deal. Alteration of configuration at C-5 in the cholestan-4-one derivatives has essentially no effect on the fragmentation pattern.

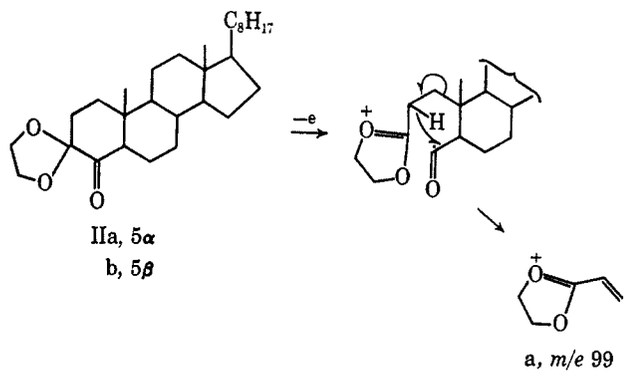
The mass spectra of steroidal ethylene ketals and ethylene thioketals were compared by Djerassi and coworkers[2] several years ago. The fragmentation of ethylene ketals was found to be simpler and more specific than that of the thioketals and this derivative has been recommended[3] for mass spectral characterization of steroidal ketones. In this report the spectra of ethylene dioxy, hemithio-, and dithioketal derivatives of cholestan-3-one are compared with each other and with their α-carbonyl analogs, in an effort to probe the reasons for the simpler fragmentation of the ethylene dioxy ketals. A number of stereoisomers were also investigated.

Results

It has been pointed out[2-4] that the spectra of ethylene ketal derivatives of 3-keto steroids are dominated by two peaks irrespective, to a considerable extent, of other functional groups in the system. Formation of the diagnostic ions[2,3] a and b require α cleavage on either side of the ketal group accompanied by hydrogen transfer, as shown in Scheme I. When no hydrogen is available on C-2 and C-4, the corresponding ion is not formed. This is the case for the epimeric 3-ethylenedioxy derivatives (II)[5] of 5α-cholestan-4-one and 5β-cholestan-4-one. The spectra of this epimeric pair are nearly identical. The largest peak (Table I) is contributed by the diagnostic ion of mass 99. The absence of hydrogen on C-4 accounts for the absence of a peak at *m/e* 125 for the second diagnostic ion, b. No other peak has a relative intensity greater than 20% (Table I) when the spectrum is obtained under the conditions reported. Thus, while the presence of the α-carbonyl group precludes hydrogen transfer from C-4 and genesis of one of the diagnostic ions, it introduces no equally facile new fragmentation.

SCHEME I





In the spectrum of 3-(ethylene-1′-oxy-2′-thio)cholestane (III) the sulfur-containing peaks analogous[4] to diagnostic ions a and b have relative intensities of 37 and 18%, respectively. The key to the fragmentation of this compound appears to be the primary elimination of ethylene sulfide from the oxathiolane ring, generating ions of mass 60 and 386 (Table I). The base peak in the spectrum is at *m/e* 231. Major peaks are found at this mass in the spectra[6] of many ketocholestanes. The ion of mass 231 is generated in the fragmentation of cholestan-3-one, for example, by loss of carbons 15, 16, and 17 and the side chain, with transfer of one

(1) This work was supported in part by U. S. Public Health Service Grant HE 08913 (to C. H. R.), U. S. Public Health Service Grant FR 4378, National Science Foundation Grant GB 7866, and National Institutes of Health Program Grant GM-16492.

(2) G. v. Mutzenbecher, Z. Pelah, D. H. Williams, H. Budzikiewicz, and C. Djerassi, *Steroids*, **2**, 475 (1963).

(3) C. Djerassi, *Pure Appl. Chem.*, **9**, 159 (1964), and references therein.

(4) H. Audier, J. Bottin, A. Diara, M. Fetizon, P. Foy, M. Golfier, and W. Vetter, *Bull. Soc. Chim. Fr.*, 2292 (1964).

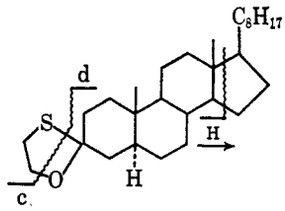
(5) (a) C. H. Robinson and L. Milewich, Abstracts, 153rd National Meeting of the American Chemical Society, Miami Beach, Fla., April 1967, No. O-67; (b) C. H. Robinson, L. Milewich, G. Snatzke, W. Klyne, and S. R. Wallis, *J. Chem. Soc., C*, 1245 (1968); (c) C. H. Robinson and L. Milewich, manuscript in preparation.

(6) H. Budzikiewicz and C. Djerassi, *J. Amer. Chem. Soc.*, **84**, 1430 (1962).

TABLE I
INTENSITIES OF MAJOR PEAKS IN THE MASS SPECTRA OF COMPOUNDS I–VI

Compd no.	M+ RI[b]	M+ TI[c]	M − 28 RI	M − 28 TI	M − 56 RI	M − 56 TI	M − 60 RI	M − 60 TI	M − 88 RI	M − 88 TI	m/e 231 RI	m/e 231 TI	Diagnostic ions: Type a RI	Type a TI	a + 1[a] RI	a + 1[a] TI	Type b RI	Type b TI	Ion k RI	Ion k TI	m/e 60 RI	m/e 60 TI
I	<1	2.78											100	27.85	17	4.74	40	11.14				
II	<1	0.56	2	1.12									100	55.87	15	8.37					32	2.47
III	11	0.85	1	0.08			49	3.78			100	7.72	37	2.86	23	1.78	18	1.40			6	0.92
IV	10	1.54	13	2.00	10	1.54			25	3.85			100	15.38	45	6.92			10	1.54	100	4.35
V	80	3.48	24	1.04			32	1.39					25	1.10	26	1.13	16	0.70			6	0.42
VI	14	0.98	5	0.35	49	3.46			3	0.21			100	7.06	78	5.51			33	2.33		

[a] Corrected for ^{13}C. [b] RI = per cent relative intensity. [c] TI = per cent total ionization, Σ_{29}.

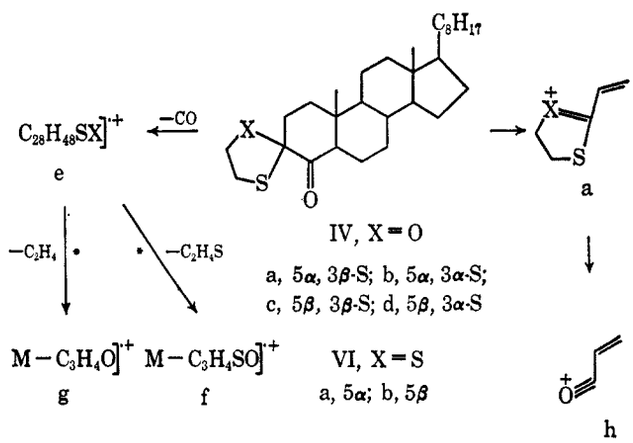


IIIa, 3β-S, 3α-O
b, 3α-S, 3β-O

hydrogen atom.[6,7] In the hemithioketal system III the ion appears to result from loss of ethylene sulfide from one end of the molecule followed by loss of carbons 15, 16, and 17, the side chain, and a transferred hydrogen atom. An intermediate d resembling ionized cholestan-3-one might be postulated because the rest of the spectrum resembles that[6] of cholestan-3-one.

In the spectra of the 4-keto analogs[5] (IVa–d) of this hemithioketal, the base peak is again generated by the diagnostic ion a (mass 115), and the spectra of all four epimers are indistinguishable. An M − 28 ion e is present in this system and is identified in the complete high-resolution spectrum of compound IV as formed by the loss of carbon monoxide from the molecular ion (Table I). This differs from the pattern of the α-keto dioxy ketal (II) and suggests that the carbonyl group exerts more influence on fragmentation in the α-keto hemithioketal compound. The primary elimination of carbon monoxide (*m/e* 432) is followed by fragmentation in the 1,3-oxathiolane ring. (See Scheme II).

SCHEME II



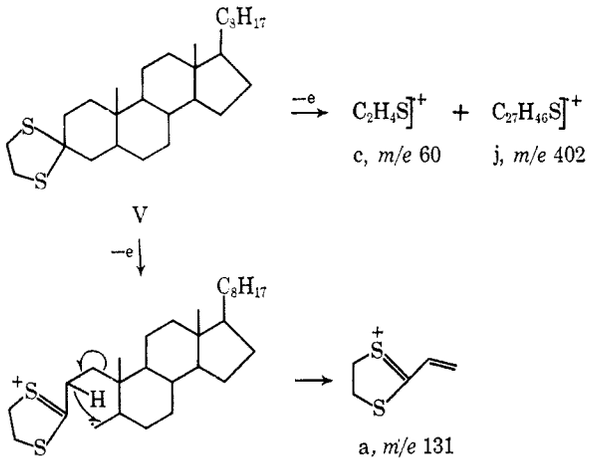
Secondary elimination of C_2H_4S leads to the M − 88 ion f (*m/e* 372) whose composition is $C_{26}H_{44}O$. A flattop metastable peak is observed in the region *m/e* 320–323, supporting this sequence (m − 28 → m − 88) as one route to the mass 372 ions. Elimination of carbon monoxide may also be followed by loss of ethylene, perhaps from the 1,3-oxathiolane ring, generating the only other prominent peak in the high-mass half of the spectrum, M − C_3H_4O, *m/e* 404. In the low-mass range the peak at *m/e* 55 has a relative intensity of 20%. This ion is thought to be formed by secondary decomposition of the mass 115 ion.

Thus the base peak (*m/e* 231) in the spectra of both cholestan-4-one[6] and 3-(ethylene-1′-oxy-2′-thio)cholestane (III) requires fragmentation characteristic of the

(7) L. Tökés, G. Jones, and C. Djerassi, *J. Am. Chem. Soc.*, **90**, 5465 (1968).

hydrocarbon skeleton. When these two functional groups are present together in ring A (IV), this peak occurs to only a few per cent (Table I), and scission of the doubly activated 3,4 bond becomes the major primary process. This leads to generation of the base peak corresponding to diagnostic ion a.

The loss of ethylene sulfide is again an important primary process in the fragmentation of the cholestan-3-one dithioketal V. The base peak (ion c) occurs at *m/e* 60 (Table I). Ethylene is also eliminated from the molecular ion. The molecular ion has a relative intensity of 80%, the peak at *m/e* 131 (corresponding to



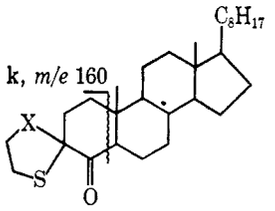
diagnostic ion a) has a relative intensity of 25%, and the second diagnostic peak (*m/e* 157) has a relative intensity of only 16%. The intensity of the peak at *m/e* 132 (26%) is comparable with that of the peak at *m/e* 131 and the mass 132 ion is probably formed with the same C–C bond scissions without hydrogen transfer from C-2, or with a reciprocal transfer. Here, as in the case of the hemithioketals, the diagnostic mass 131 ion has a higher relative intensity in the fragmentation of the α-carbonyl thioketal VI than in the fragmentation of the thioketal itself.

The fragmentation of the C-5 epimers of the 3-ethylene thioketal derivative of cholestan-4-one (VI)[5] leads to nearly identical spectra in which the diagnostic peak has a relative intensity of 100%. A prominent peak (49%) in these spectra occurs at M − 56, *m/e* 420. Accurate mass measurements confirm these mass 420 ions g to have the composition $C_{26}H_{44}S_2$, and a metastable peak at 393.9 suggests that they are formed by sequential loss of carbon monoxide and ethylene. Ethylene is probably lost from the dithiolane ring, such loss having precedent in the fragmentation of tetrahydrothiophene.[8]

Here, as in the α-carbonyl hemithioketal IV, the possibility cannot be eliminated that the diagnostic ion a is formed from the M − CO ion e in addition to, or instead of, the molecular ion.

A moderately intense ion (33%) of mass 160 may be composed of carbons 1–4 and the functional groups. The occurrence of this ion k suggests that not all initial bond cleavage occurs between the thioketal and the carbonyl group. The analogous $C_6H_8O_2S$ ion (mass 144) is present to a small extent (Table I) in the spectrum

(8) A. M. Duffield, H. Budzikiewicz, and C. Djerassi, *J. Am. Chem. Soc.*, **87**, 2920 (1965).



VIa, 5α
b, 5β

of the α-keto hemithioketal IV, and the corresponding mass 128 ion is absent in the spectrum of the α-keto dioxy ketal.

Discussion

One of the most severe limitations of electron impact fragmentation is its inability to distinguish between stereoisomers. The virtual identity of spectra among each of the four sets of epimers discussed here accords with the majority of cases reported.

In the fragmentation of the dioxy ketal I the two most facile α cleavages are those in the A ring which lead to diagnostic ions a and b. When oxygen is replaced by sulfur in the cyclic ketal, scission of C–S bonds (α to the second heteroatom) completes favorably, and primary fragmentation occurs in the oxathiolane or dithiolane ring as well as in the steroid A ring. A larger variety of ions is formed; species arising from scission of C–S bonds contribute the base peaks.

The introduction of a carbonyl group at C-4 facilitates α scission of the 3,4 bond relative to the C–S bond, and in all three of the α-keto ketals the base peak again represents diagnostic ions whose formation involves this A-ring scission. Fission in the oxathiolane and dithiolane rings appears only in secondary processes in these compounds, following elimination of carbon monoxide.

Experimental Section

The ethylene dioxy, hemithio-, and dithioketals described in this paper were prepared by standard procedures, using a benzene solution of the appropriate ketone together with excess ketalizing reagent (ethylene glycol, 2-mercaptoethanol, or ethane-1,2-dithiol) and *p*-toluenesulfonic acid as catalyst. The mixture was refluxed under a Dean–Stark water separator until the reaction was judged complete from tlc of aliquots of the reaction mixture.

All compounds were homogeneous as judged by tlc, and each had infrared and nuclear magnetic resonance spectra consistent with its structure.[9]

The ethylene dioxy ketal I of 5 α-cholestan-3-one had mp 114–115° (lit.[10] mp 115.5–116°), and the corresponding ethylene dithioketal V had mp 143–144° (lit.[11] mp 146.5–147.5°). The known 3-ethylenedithio-5β-cholestan-4-one (VI) had mp 131–132° (lit.[12] mp 128°). The isomeric 3-hemithioketals (IIIa and b) derived from 5α-cholestan-3-one had mp 100–101° (IIIa) and mp 143–145° (IIIb). The high-melting isomer IIIb has been described[13] as existing in two polymorphic modifications of mp 135–136° or 144–145°. We observed only the higher melting modification. In the case of the isomer IIIa, described[13] as showing mp 112–113°, we attribute the difference in melting

(9) All new compounds (IIa, IIb, IVa–d, VIa) gave satisfactory combustion analyses, and a detailed account of the preparation and characterization of these ketals will be given in a forthcoming publication[5] dealing with some of their chemical reactions.

(10) Z. Pelah, D. H. Williams, H. Budzikiewicz, and C. Djerassi, *ibid.*, **86**, 3723 (1964).

(11) L. F. Fieser, *ibid.*, **76**, 1945 (1954).

(12) R. Stevenson and L. F. Fieser, *ibid.*, **78**, 1409 (1956).

(13) E. L. Eliel, L. A. Pilato, and V. G. Badding, *ibid.*, **84**, 2377 (1962).

point of our sample (mp 100–101°) to polymorphism, as our sample was chromatographically homogeneous (in particular, free from starting material and the isomeric IIIb) and had the expected nmr spectrum. All melting points are corrected and determined on a Kofler block.

Low-resolution mass spectra reported in Table I were obtained on an Hitachi RMU 6 mass spectrometer using 80-eV ionization energy with source and direct-inlet temperatures of 180–200°. The complete high-resolution mass spectrum of compound IV was obtained on a CEC-110 mass spectrometer. Individual exact ion masses were determined using the RMU 7 high-resolution instrument.

Registry No.—IIa, 18897-72-8; IIb, 18897-73-9; IIIa, 2760-91-0; IIIb, 2760-93-2; IVa, 18897-78-4; IVb, 17021-85-1; IVc, 18897-79-5; IVd, 18897-77-3; VIa, 18897-74-0; VIb, 18897-75-1.

Acknowledgment.—The assistance of Mr. W. R. Landis of the National Institutes of Health and of the Purdue University Mass Spectrometry Center is most gratefully acknowledged. We also thank Miss M. Pyles for her skillful assistance.

Intramolecular Catalysis in the Acetylation of Methyl Cholate[1]

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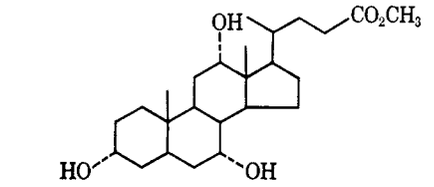
Received November 27, 1968

The hydroxyl groups of methyl cholate decrease in reactivity toward acetic anhydride in the order 3 > 12 > 7 when they are present in compounds free of intramolecular influences. In methyl cholate, however, the 7-hydroxyl is acetylated in preference to the 12-hydroxyl as a result of three interactions: (1) deactivation of the 12-hydroxyl by the side chain, (2) enhancement of 7-hydroxyl reactivity by a 3-acetoxy group, and (3) enhancement of 7-hydroxyl reactivity by the 12-hydroxyl. Preferential acetylation at the 7-hydroxyl occurs also in the absence of a 3-acetoxy group, as in methyl 3α,7α-dihydroxycholanate.

The order of reactivity of the hydroxyl groups of methyl cholate (1) toward acylating agents is established as 3 > 7 > 12, based in part on its acetylation to the 3,7-diacetate by acetic anhydride and pyridine.[2] On conformational grounds, however, the reverse order of reactivity for the 7- and 12-hydroxyls might have been predicted. From inspection of structure 2, it is evident that the 7-hydroxyl is surrounded axially by two hydrogens and a methylene group, while merely three hydrogens surround the 12-hydroxyl, giving rise to less steric inhibition. Some possible explanations for this anomaly include (a) an indirect route for acylation at C-7, (b) inhibition of reactivity of the 12α-hydroxyl, and (c) enhancement of 7α-hydroxyl group reactivity.



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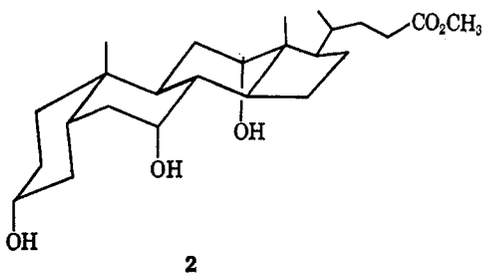

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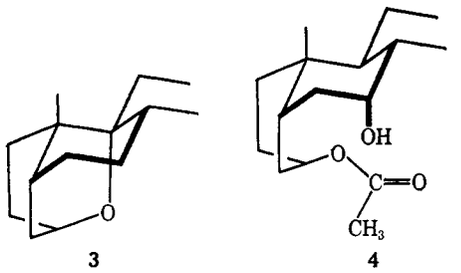

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An indirect route *via* acetyl migration from the 3 position is conceivable. When the A ring assumes a chair conformation (as in 2), the 3- and 7-hydroxyls are relatively remote. In the conformational equilibrium some portion of the molecules could exist in a boat conformation, however (as is required in the 3,9-oxide 3[3]), producing a structure 4 which could permit acetyl migration from the more reactive 3 position. Acetyl migration does *not* occur, however, under the conditions which produce the 3,7-diacetate of methyl cholate.[4]

In the work reported here we confirm the reactivity sequence predicted on conformational grounds, and examine the latter two explanations of the methyl cholate anomaly. Relative reactivities of 3α-, 7α-, and 12α-hydroxyl groups were assessed by comparing yields of acetate produced under identical conditions with methyl lithocholate (5), methyl 7α-hydroxycholanate (6), and 5β-pregnan-12α-ol (7). Inhibition of the reactivity of the 12-hydroxyl by the bile acid side chain was assessed by comparing 5β-pregnan-12α-ol (7) with methyl 12α-hydroxycholanate (8). The acetylation of methyl 7α-12α-dihydroxycholanate (9) was studied in order to determine whether a 3α-acetoxy group influences the course of the reaction, and acetylation yields of other bile acid derivatives were compared for the purpose of detecting enhancement of 7α-hydroxyl group reactivity, should it exist.

In order to test the prediction that the inherent relative reactivity is 3 > 12 > 7 when these three hydroxyl groups are free of influence by any other group in the molecule, we chose compounds in which

(1) Taken in part from the M.S. thesis of B. Orwig, Indiana University, Indianapolis, Ind., 1967.

(2) L. F. Fieser and S. Rajagopalan, *J. Amer. Chem. Soc.*, **72**, 5530 (1950).

(3) V. R. Mattox, *et al.*, *J. Biol. Chem.*, **164**, 569 (1946); R. B. Turner, *et al.*, *ibid.*, **166**, 345 (1946).

(4) R. T. Blickenstaff and B. Orwig, *J. Org. Chem.*, **32**, 815 (1967).