Mass Spectral Fragmentation of Bile Acid Ester Derivatives

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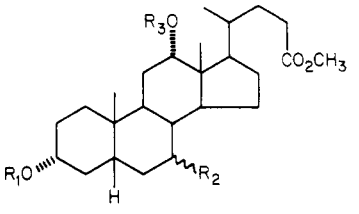
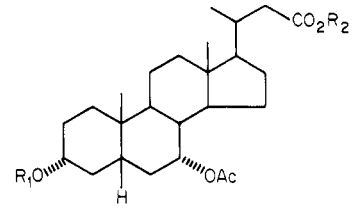
The current mechanistic understanding and evidence for the decreasing preferential order (12α > 7α > 3α) of consecutive loss of three carboxylic acid or three water molecules from the molecular ion of 3α,7α,12α-tricarboxy-5β-cholanoates or 3α,7α,12α-trihydroxy-5β-cholanoates, respectively, are summarized. Additional mechanistic details for water loss have been determined with the aid of deuterium labeling. The fragmentation pathways of 3α,7α,12α-triacetoxy-5β-pregnan-20-one, a bile acid derivative, have been defined by comparison with four deuterated analogues. Evidence for the nominal loss of acetic anhydride or its equivalent from triacetate esters of cholic acid is presented.

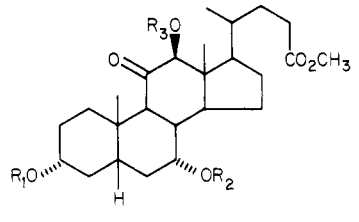
Although the mass spectra of bile acids have been extensively studied, many mechanistic details of the fragmentations after electron impact remain undetermined.[1] Our prior research established that the order of consecutive loss of three acetic acid molecules from the triacetate of methyl cholate occurs such that the 12α-OAc group is extruded first, the 7α-OAc group is lost second, and the 3α-OAc group is extruded last;[2] within the framework of charge localization and participation,[3] this work permitted us to trace the probable movement of charge from the region of the D ring after initial ionization to the region of the A ring. Herein, we present the mass spectra of other new cholic acid ester derivatives and, with additional deuterium labeling on 3α,7α,12α-triacetoxy-5β-pregnan-20-one, further define the genesis of pertinent mass spectral ions.



	R_1	R_2	R_3
1a	EtCO	α-OH	H
1b	EtOCO	α-OH	H
1c	EtOCO	α-$IC_6H_4CO_2$	IC_6H_4CO
1d	H	α-CF_3CO_2	CF_3CO
1e	CF_3CO	α-CF_3CO_2	CF_3CO
1f	H	α-OH	H
1g	HCO	α-HCO_2	HCO
1h	Ac	α-AcO	Ac
1i	EtCO	α-$EtCO_2$	H
1j	Ac-d_3	H	OAc
1k	Ac	β-Cl	OAc



2a, R_1 = Ac; R_2 = H
b, R_1 = Ac-d_3; R_2 = CH_3

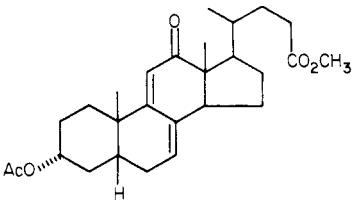


	R_1	R_2	R_3
3a	H	Ac	H
3b	Ac	Ac	H
3c	CH_3	Ac	H
3d	CH_3	H	CH_3

Results and Discussion

Carboxylic Acid Loss. The loss of acetic acid in the mass spectra (Table I) of diacetates **1j** and **2b** conforms with our earlier observations with the triacetate of methyl cholate;[2] i.e., the 12α-OAc group in the spectrum of - or the 7α-OAc group in the spectrum of **2b** are regiospecifically lost as acetic acid before the 3α-OAc group (no $[M - HOAc\text{-}d_3]^{+\cdot}$ ion peaks are observed in the spectra of **1j** and **2b**). Consecutive carboxylic acid loss in the mass spectrum of **1c** is also consistent with these results; the *m/z* 458 ion peak ($[M - 2IC_6H_4CO_2H]^{+\cdot}$) is considerably more intense than the *m/z* 616 ion peak ($[M - IC_6H_4CO_2H - EtOCO_2H]^{+\cdot}$). Similarly, in the spectrum of **1i** the *m/z* 442 peak is more dominant than the *m/z* 386 peak. It is suggested that the observation of significant $[M - EtCO_2H]^{+\cdot}$ and $[M - EtOCO_2H]^{+\cdot}$ ion peaks in the spectra of **1a** and **1b**, respectively, arises because of increased initial ionization at the 3α-ester groups and/or because H_2O loss from hydroxy groups is less facile than carboxylic acid loss from ester groups.

A retro-Diels–Alder cleavage (loss of C_4H_6) from the A ring occurs only after loss of one HOAc in the spectra of **4** and **5b**, loss of two HOAc molecules in the spectra of **1j**, **2a**, **5c**, and **5d**, and loss of three carboxylic acid molecules in the mass spectra of **1c**, **1g**, **1h**, and **5e** (Table I). The



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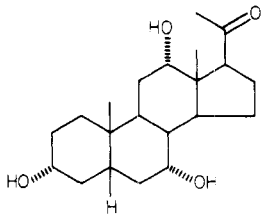
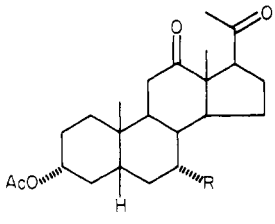
(1) (a) P. Szczepanik, D. Hachey, and P. Klein, *J. Lipid Res.*, **17**, 314 (1976); (b) G. Waller, Ed., "Biochemical Applications of Mass Spectrometry", Wiley-Interscience, New York, 1972.
(2) J. R. Dias and B. Nassim, *Org. Mass Spectrom.*, **13**, 402 (1978).
(3) W. Vetter, W. Meister, and W. J. Richter, *Org. Mass Spectrom.*, 3, 777 (1970).

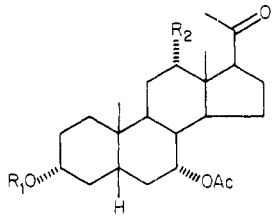
0022-3263/80/1945-0337$01.00/0



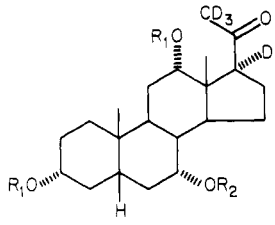
5a



5b, R = H
c, R = OAc



5d, R_1 = Ac; R_2 = H
e, R_1 = Ac; R_2 = OAc
f, R_1 = Ac-d_3; R_2 = OAc
g, R_1 = Ac; R_2 = AcO-d_3



6a, R_1 = R_2 = Ac
b, R_1 = Ac; R_2 = Ac-d_3
c, R_1 = R_2 = H

absence of significant loss of C_4H_6 before *all* the carboxylic ester groups are eliminated provides additional evidence that the 3α-ester group is extruded last in the successive elimination of two and three carboxylic acids from the molecular ion of cholic acid ester derivatives.

Expulsion of the second acetic acid molecule in the spectrum of **2b** (*m/z* 356) is enhanced relative to the same loss (*m/z* 370) in the spectrum of **1j**; presumably, the double bond formed by elimination of the 7α-OAc group in **2b** shifts to the $\Delta^{5,6}$ position, wherein subsequent elimination of the 3α-acetoxy group leads to a more stable conjugated diene that is not as readily produced from the molecular ion of **1j**.

17-Side-Chain Ejection. Placing electronegative formate or trifluoroacetate ester groups on the A, B, and C rings of the steroid skeleton of methyl cholate raises the ionization energy in these rings and thereby increases the localization of charge in the region of the D ring, enhancing fragmentations associated with that ring. Enhanced 17-side-chain loss is observed in the spectra of **1d** (*m/z* 385 and 271), **1e**,[4] and **1g** (*m/z* 299); this electronegative substituent effect needs to be studied further. The presence of a 12α-OAc group enhances mass spectral 17-side-chain loss (*m/z* 318 and 255) in **1j** relative to **2a** or **2b**; this loss is activated in **1j** by the formation of the Δ^{11} double bond which forms a homoallylic carbocation upon ejection of the 17-side-chain. A similar argument can be evoked to explain enhanced 17-side-chain loss (*m/z* 313 and 253) in the spectrum of **5e** compared to the spectrum of **5d**.

Loss of Water. Unlike electron impact induced carboxylic acid loss in steroid esters which occurs predominantly by cis 1,2-elimination, mass spectral water loss in axial hydroxy steroids proceeds mainly by cis 1,3 hydrogen transfer preferentially from tertiary sites to the hydroxy group, followed by extrusion of H_2O; equatorial hydroxy groups or axial hydroxy groups remote to tertiary sites usually undergo nonspecific water loss which is suggested to occur after ring fission.[5] It has been shown that a 3α-hydroxy-5β-steroid system loses its 9α-deuterium together with the hydroxyl group when HDO is lost directly from the molecular ion and must involve an A-ring boat conformation for acquisition of the appropriate proximity between these leaving groups; because the corresponding isomeric 3β-hydroxy-5β-steroid system exhibited a smaller H_2O loss (presumably involving the 5β-hydrogen), it was concluded that the spatial distance between the hydroxy group and an activated hydrogen governed the extent of H_2O elimination.[6]

Consecutive elimination of three HOAc molecules from the molecular ion of methyl cholate triacetate (**1h**) led to ion peaks that progressively increased in relative abundance since the corresponding ions became progressively more stable (Table II). This requires the existence of similar kinetic rate constants for cis 1,2-elimination of HOAc from the 12α-OAc, 7α-OAc, and 3α-OAc groups and subsequent fragmentations from these daughter ions; in this case, loss of the 17-side-chain from the corresponding successive daughter ions also progressively increases (Table II). Consecutive loss of three H_2O molecules from ionized methyl cholate (**1f** in Table II) and 3α,7α,12α-trihydroxycoprostane[4] led to peaks that first increased and then decreased. This can be rationalized by arguing that the 12α- and 7α-OH groups undergo cis 1,3-abstraction of the 9α- and 14α-hydrogens with simultaneous elimination to form progressively more stable ions, leaving no remaining proximate tertiary axial hydrogens (i.e., no 9α-H) for the 3α-OH group to abstract upon flipping the A ring to a boat conformation; thus, the loss of the third consecutive H_2O molecule probably involves prior ring fission and consequently a smaller kinetic rate constant. In fact, the minor loss of 29 mass units after loss of two H_2O molecules in the spectra of 3α,7α,12α-trihydroxycoprostane,[4] methyl cholate (**1f**), **5a**, and **6c** gave ions at *m/z* 355, 357, 285, and 289, respectively, which can best be explained as A-ring fission with 3-hydroxy hydrogen transfer and formyl radical loss. Extrusion of three successive water molecules from the molecular ion of **5a** progressively decreases in extent (Table II), but when the relative abundance for the associated D-ring-cleavage ion plus the corresponding relative abundance of the ion for just water loss are added, then it is seen that the sum of peak intensities for loss of one, two, and three water molecules first increases and then decreases as it does in the spectrum of **1f**. Therefore, consecutive water loss in the mass spectrum of **5a** probably follows a mechanistic pathway similar to the one responsible for consecutive water loss in the spectrum of **1f**. However charge localization on the 20-oxo group in **5a** results in another fragmentation pathway that leads to even more stable ions that are not similarly accessible from molecular ions of **1f** (or **1h** and **5e**). Note that the absence of significant loss of C_4H_6 before all the hydroxy groups are eliminated as H_2O in the spectrum of **1f** provides additional evidence that the A-ring 3α-OH group is lost last.

Comparison of the mass spectra of **5a** and **6c** completely defines the genesis of all the ions above *m/z* 200 (Table I). Consecutive water loss, D-ring cleavage, and 17-side-chain scission are the major processes and, no doubt, proceed via similar mechanisms in methyl cholate (**1f**). In going from the 70-eV ionization voltage spectrum in **6c** to the 12-eV spectrum, one notes that the amount of HDO loss increases (e.g., *m/z* 336 at 70 eV goes to *m/z* 336 and 335 at 12 eV) and that more deuterium retention occurs on the remaining steroid fragment after D-ring cleavage (*m/z* 229 at 70 eV goes to *m/z* 229 and 230 at 12 eV). A mechanism consistent with these data involves exchange of the 12α-hydroxyl proton with the 17α-deuterium (a to c).

(4) R. Ryhage and E. Stenhagen, *J. Lipid Res.*, **1**, 361 (1960).
(5) D. Kingston, B. Hobrock, M. Bursey, and J. Bursey, *Chem. Rev.*, **75**, 693 (1975).
(6) H. Klein and C. Djerassi, *Chem. Ber.*, **106**, 1897 (1973).

Table I. Partial Monoisotopic Mass Spectra of Cholic Acid Ester Derivatives[a]

compd	ionization voltage, eV	m/z (% relative abundance, probable genesis)
1a	12	478 (4, $[M]^{+\cdot}$), 460 (6, $[M - H_2O]^{+\cdot}$), 442 (56, $[M - 2H_2O]^{+\cdot}$), 404 (19, $[M - EtCO_2H]^{+\cdot}$), 386 (21, $[M - EtCO_2H - H_2O]^{+\cdot}$), 368 (100, $[386 - H_2O]^{+\cdot}$ and $[442 - EtCO_2H]^{+\cdot}$), * 355 (12, $[M - H_2O - EtCO_2H - CH_3O]^+$), 353 (10, $[368 - CH_3]^+$),* 345 (14, $[M - H_2O - C_6H_{11}O_2]^+$), 327 (26, $[442 - C_6H_{11}O_2]^+$),* 314 (10, $[M - 2H_2O - EtCO_2H - C_4H_6]^{+\cdot}$), 295 (6, $[M - 2H_2O - EtCO_2H - CH_2CO_2CH_3]^+$), 281 (4, $[368 - C_4H_7O_2]^+$),* 271 (25, $[386 - C_6H_{11}O_2]^+$),* 261 (10, $[C_{17}H_{25}O_2]^+$), 253 (55, $[368 - C_6H_{11}O_2]^+$, $[327 - EtCO_2H]^+$, and $[271 - H_2O]^+$),* 227 (10)
1b	12	494 (1, $[M]^{+\cdot}$), 476 (3, $[M - H_2O]^{+\cdot}$), 458 (10, $[M - 2H_2O]^{+\cdot}$), 404 (10, $[M - EtOCO_2H]^{+\cdot}$), 386 (43, $[M - H_2O - EtOCO_2H]^{+\cdot}$), 368 (93, $[386 - H_2O]^{+\cdot}$),* 361 (6, $[M - H_2O - C_6H_{11}O_2]^+$), 355 (13, $[M - H_2O - EtOCO_2H - CH_3O]^+$), 353 (10, $[M - 2H_2O - EtOCO_2H - CH_3]^+$), 343 (19, $[M - 2H_2O - C_6H_{11}O_2]^+$), 314 (12, $[M - 2H_2O - EtOCO_2H - C_4H_6]^{+\cdot}$), 295 (7, $[M - 2H_2O - EtOCO_2H - CH_2CO_2CH_3]^+$), 281 (7, $[M - 2H_2O - EtOCO_2H - C_4H_7O_2]^+$), 271 (39, $[M - H_2O - EtOCO_2H - C_6H_{11}O_2]^+$), 261 (14, $[C_{17}H_{25}O_2]^+$), 253 (100, $[368 - C_6H_{11}O_2]^+$, $[343 - EtOCO_2H]^+$, and $[271 - H_2O]^+$),* 227 (8)
1c	70	954 (0, MW), 706 (1, $[M - IC_6H_4CO_2H]^{+\cdot}$), 616 (3, $[M - IC_6H_4CO_2H - EtOCO_2H]^{+\cdot}$), 591 (2, $[M - IC_6H_4CO_2H - C_6H_{11}O_2]^+$), 501 (2, $[M - IC_6H_4CO_2H - EtOCO_2H - C_6H_{11}O_2]^+$), 458 (55, $[M - 2IC_6H_4CO_2H]^{+\cdot}$), 443 (2, $[M - 2IC_6H_4CO_2H - CH_3]^+$), 368 (59, $[458 - EtOCO_2H]^{+\cdot}$),* 353 (8, $[M - 2IC_6H_4CO_2H - EtOCO_2H - CH_3]^+$), 343 (12, $[M - 2IC_6H_4CO_2H - C_6H_{11}O_2]^+$), 337 (6, $[M - 2IC_6H_4CO_2H - EtOCO_2H - CH_3O]^+$), 314 (19, $[M - 2IC_6H_4CO_2H - EtOCO_2H - C_4H_6]^{+\cdot}$), 295 (7, $[M - 2IC_6H_4CO_2H - EtOCO_2H - C_2H_5O_2]^+$), 281 (10, $[M - 2IC_6H_4CO_2H - EtOCO_2H - C_4H_7O_2]^+$), 261 (7, $[C_{17}H_{25}O_2]^+$), 253 (100, $[368 - C_6H_{11}O_2]^+$ and $[343 - EtOCO_2H]^+$),* 248 (19, $[M - IC_6H_4CO_2H]^{+\cdot}$), 231 (87, $[M - IC_6H_4CO]^+$)
1d	70	614 (1, $[M]^{+\cdot}$), 500 (2, $[M - CF_3CO_2H]^{+\cdot}$), 482 (3, $[M - CF_3CO_2H - H_2O]^{+\cdot}$), 469 (3, $[M - CF_3CO_2H - CH_3O]^+$), 451 (3, $[M - CF_3CO_2H - H_2O - CH_3O]^+$), 385 (19, $[M - CF_3CO_2H - C_6H_{11}O_2]^+$), 386 (41, $[M - 2CF_3CO_2H]^{+\cdot}$), 369 (25), 368 (18, $[386 - H_2O]^{+\cdot}$),* 353 (8, $[M - 2CF_3CO_2H - H_2O - CH_3]^+$), 337 (3, $[M - 2CF_3CO_2H - H_2O - CH_3O]^+$), 295 (41, $[M - 2CF_3CO_2H - H_2O - C_3H_5O_2]^+$), 281 (10, $[M - 2CF_3CO_2H - H_2O - C_4H_7O_2]^+$), 271 (70, $[386 - C_6H_{11}O_2]^+$), 261 (14, $[C_{17}H_{25}O_2]^+$), 253 (100, $[368 - C_6H_{11}O_2]^+$ and $[271 - H_2O]^+$),* 227 (30), 226 (27)
1e	70	710 (1, $[M]^{+\cdot}$), 596 (2, $[M - CF_3CO_2H]^{+\cdot}$), 565 (3, $[M - CF_3CO_2H - CH_3O]^+$), 482 (19, $[M - 2CF_3CO_2H]^{+\cdot}$), 337 (4, $[M - 3CF_3CO_2H - CH_3O]^+$), 368 (47, $[M - 3CF_3CO_2H]^{+\cdot}$), 367 (67, $[M - 2CF_3CO_2H - C_6H_{11}O_2]^+$), 261 (12, $[C_{17}H_{25}O_2]^+$), 253 (100, $[M - 3CF_3CO_2H - C_6H_{11}O_2]^+$), 227 (25), 226 (20)
1g	12	506 (2, $[M]^{+\cdot}$), 460 (8, $[M - HCO_2H]^{+\cdot}$), 414 (100, $[460 - HCO_2H]^{+\cdot}$),* 399 (10, $[M - 2HCO_2H - CH_3]^+$), 369 (50), 368 (33, $[414 - HCO_2H]^{+\cdot}$),* 353 (19, $[M - 3HCO_2H - CH_3]^+$), 337 (4, $[M - 3HCO_2H - CH_3O]^+$), 327 (14, $[M - 2HCO_2H - C_4H_7O_2]^+$), 314 (11, $[M - 3HCO_2H - C_4H_6]^{+\cdot}$), 299 (100, $[M - 2HCO_2H - C_6H_{11}O_2]^+$), 281 (14, $[M - 3HCO_2H - C_4H_7O_2]^+$), 272 (26, $[M - 2HCO_2H - C_8H_{14}O_2]^{+\cdot}$), 261 (47, [414 to $C_{17}H_{25}O_2]^+$),* 253 (60, $[299 - HCO_2H]^+$ and $[368 - C_6H_{11}O_2]^+$),* 227 (19), 226 (12), 213 (20), 154 (59)
1i	12	534 (1, $[M]^{+\cdot}$), 516 (1, $[M - H_2O]^{+\cdot}$), 460 (4, $[M - EtCO_2H]^{+\cdot}$), 442 (30, $[M - H_2O - EtCO_2H]^{+\cdot}$), 427 (6, $[442 - CH_3]^+$),* 386 (19, $[M - 2EtCO_2H]^{+\cdot}$), 368 (87, $[442 - EtCO_2H]^{+\cdot}$ and $[386 - H_2O]^{+\cdot}$),* 355 (12, $[M - 2EtCO_2H - CH_3O]^+$), 353 (23, $[427 - EtCO_2H]^+$ and $[368 - CH_3]^+$),* 327 (58, $[M - H_2O - EtCO_2H - C_6H_{11}O_2]^+$), 314 (21, $[M - H_2O - 2EtCO_2H - C_4H_6]^{+\cdot}$), 300 (12, $[M - H_2O - EtCO_2H - C_8H_{14}O_2]^{+\cdot}$), 281 (7, $[M - H_2O - 2EtCO_2H - C_4H_7O_2]^+$), 271 (19, $[M - 2EtCO_2H - C_6H_{11}O_2]^+$), 261 (18, $[C_{17}H_{25}O_2]^+$), 253 (100, $[368 - C_6H_{11}O_2]^+$ and $[327 - EtCO_2H]^+$),* 227 (17), 226 (40)
1j	12	493 (0, MW), 433 (8, $[M - HOAc]^{+\cdot}$), 388 (2, $[M - AcOAc\text{-}d_3]^{+\cdot}$), 370 (45, $[M - HOAc - HOAc\text{-}d_3]^{+\cdot}$), 355 (10, $[M - HOAc - HOAc\text{-}d_3 - CH_3]^+$), 318 (16, $[M - HOAc - C_6H_{11}O_2]^+$), 316 (9, $[M - HOAc - HOAc\text{-}d_3 - C_4H_6]^{+\cdot}$), 255 (100, $[370 - C_6H_{11}O_2]^+$ and $[318 - HOAc\text{-}d_3]^+$),* 228 (18, $[M - HOAc - HOAc\text{-}d_3 - C_8H_{14}O_2]^{+\cdot}$), 213 (19, $[M - HOAc - HOAc\text{-}d_3 - C_9H_{17}O_2]^{+\cdot}$), 154 (39, $[C_9H_{14}O_2]^{+\cdot}$)
1k	70	526, 524 (0, 0, MW), 466, 464 (3, 9, $[M - HOAc]^{+\cdot}$), 428 (31, $[M - HOAc - HCl]^{+\cdot}$), 406, 404 (10, 31, $[M - 2HOAc]^{+\cdot}$), 369 (66, [406, 404 − Cl]$^+$),* 368 (34, $[M - 2HOAc - HCl]^{+\cdot}$), 351, 349 (21, 62, $[M - HOAc - C_6H_{11}O_2]^+$), 313 (48, $[M - HOAc - HCl - C_6H_{11}O_2]^+$), 291, 289 (31, 93, $[351, 349 - HOAc]^+$),* 253 (100, $[313 - HOAc]^+$ and $[291, 289 - HCl]^+$),* 154 (100, $[C_9H_{15}O_2]^{+\cdot}$)
	12	526, 524 (0, 0), 466, 464 (3, 10), 428 (23), 406, 404 (7, 21), 369 (36), 368 (32), 351, 349 (33, 100), 313 (36), 291, 289 (12,36), 253 (20), 154 (100)
2a	12	462 (0, MW), 402 (7, $[M - HOAc]^{+\cdot}$), 387 (2, $[M - HOAc - CH_3]^+$), 360 (4, $[M - AcOAc]^{+\cdot}$), 342 (100, $[M - 2HOAc]^{+\cdot}$), 327 (25, $[M - 2HOAc - CH_3]^+$), 288 (12, $[M - 2HOAc - C_4H_6]^{+\cdot}$), 255 (16, $[M - 2HOAc - C_4H_7O_2]^+$), 228 (28, $[M - 2HOAc - C_6H_{10}O_2]^{+\cdot}$), 213 (15, $[M - 2HOAc - C_7H_{13}O_2]^+$)
2b	12	479 (0, MW), 419 (6, $[M - HOAc]^{+\cdot}$), 404 (2, $[M - HOAc - CH_3]^+$), 374 (4, $[M - AcOAc\text{-}d_3]^{+\cdot}$), 356 (100, $[M - HOAc - HOAc\text{-}d_3]^{+\cdot}$), 341 (35, $[356 - CH_3]^+$),* 318 (12, $[M - HOAc - C_5H_9O_2]^+$), 302 (7, $[M - HOAc - HOAc\text{-}d_3 - C_4H_6]^{+\cdot}$), 283 (4, $[M - HOAc - HOAc\text{-}d_3 - CH_2CO_2CH_3]^+$), 255 (11, $[356 - C_5H_9O_2]^+$),* 228 (8, $[M - HOAc - HOAc\text{-}d_3 - C_7H_{12}O_2]^+$), 213 (14, $[356 - C_8H_{15}O_2]^+$ and $[228 - CH_3]^+$)*
3a	12	478 (25, $[M]^{+\cdot}$), 463 (10, $[M - CH_3]^+$), 460 (14, $[M - H_2O]^{+\cdot}$), 445 (14, $[M - CH_3 - H_2O]^+$), 442 (9, $[M - 2H_2O]^{+\cdot}$), 418 (25, $[M - HOAc]^{+\cdot}$), 400 (69, $[M - HOAc - H_2O]^{+\cdot}$), 387 (34, $[M - HOAc - CH_3O]^+$), 386 (15, $[M - HOAc - CH_3OH]^{+\cdot}$), 385 (46, $[M - HOAc - H_2O - CH_3]^+$), 382 (49, $[400 - H_2O]^{+\cdot}$), 371 (34, $[M - HOAc - CH_3OH - CH_3]^+$), 369 (22, $[M - HOAc - H_2O - CH_3O]^+$), 367 (25, $[M - HOAc - 2H_2O - CH_3]^+$),

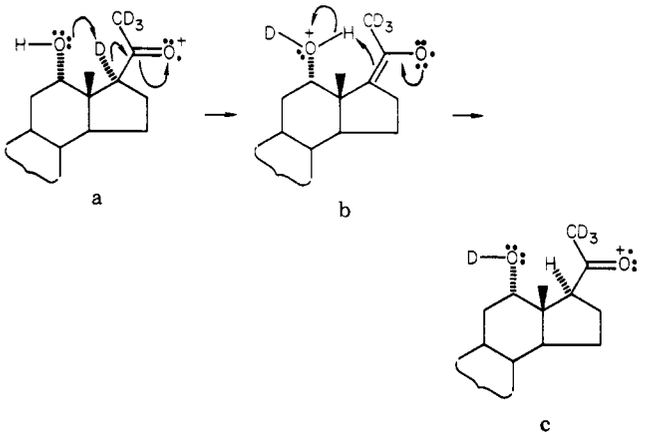
Table I (*Continued*)

compd	ionization voltage, eV	*m/z* (% relative abundance, probable genesis)
		345 (19, $[M - H_2O - C_6H_{11}O_2]^+$), 327 (100, $[M - H_2O - HOAc - C_3H_5O_2]^+$), 309 (29, $[M - 2H_2O - HOAc - C_3H_5O_2]^+$), 285 (51, $[M - HOAc - H_2O - C_6H_{11}O_2]^+$), 267 (67, $[M - HOAc - 2H_2O - C_6H_{11}O_2]^+$), 259 (30), 257 (19), 245 (24), 241 (25), 229 (39)
3b	12	520 (6, $[M]^{+\cdot}$), 505 (1, $[M - CH_3]^+$), 502 (1, $[M - H_2O]^{+\cdot}$), 460 (23, $[M - HOAc]^{+\cdot}$),* 445 (5, $[M - HOAc - CH_3]^+$), 442 (8, $[M - HOAc - H_2O]^{+\cdot}$), 429 (5, $[M - HOAc - CH_3O]^+$), 400 (5, $[M - 2HOAc]^{+\cdot}$), 387 (24, $[M - H_2O - C_6H_{11}O_2]^+$), 382 (37, $[M - 2HOAc - H_2O]^{+\cdot}$), 371 (29), 369 (23, $[M - 2HOAc - CH_3O]^+$), 327 (100, $[M - 2HOAc - C_3H_5O_2]^+$), 309 (27, $[M - 2HOAc - C_3H_5O_2 - H_2O]^+$), 285 (19, $[M - 2HOAc - C_6H_{11}O_2]^+$), 267 (31, $[M - 2HOAc - C_6H_{11}O_2 - H_2O]^+$), 257 (6), 243 (12), 241 (16), 229 (24)
3c	12	464 (100, $[M]^{+\cdot}$), 449 (18, $[M - CH_3]^+$), 446 (36, $[M - H_2O]^{+\cdot}$), 432 (43, $[M - CH_3OH]^{+\cdot}$), 385 (64, $[M - 2CH_3OH - CH_3]^+$), 382 (49, $[M - 2CH_3OH - H_2O]^{+\cdot}$), 369 (44, $[M - 2CH_3OH - CH_3O]^+$), 367 (29, $[M - 2CH_3OH - CH_3 - H_2O]^+$), 317 (27, $[M - CH_3OH - C_6H_{11}O_2]^+$), 299 (29, $[M - CH_3OH - H_2O - C_6H_{11}O_2]^+$), 285 (39, $[M - 2CH_3OH - C_6H_{11}O_2]^+$), 271 (49, $[M - CH_3OH - H_2O - C_6H_{11}O_2 - CO]^+$), 267 (46, $[M - 2CH_3OH - H_2O - C_6H_{11}O_2]^+$), 239 (46, $[M - 2CH_3OH - H_2O - C_6H_{11}O_2 - CO]^+$)
3d	12	506 (59, $[M]^{+\cdot}$), 491 (9, $[M - CH_3]^+$), 474 (14, $[M - CH_3OH]^{+\cdot}$), 459 (16, $[M - CH_3 - CH_3OH]^+$), 446 (15, $[M - HOAc]^{+\cdot}$), 442 (5, $[M - 2CH_3OH]^{+\cdot}$), 414 (100, $[M - HOAc - CH_3OH]^{+\cdot}$), 399 (24, $[M - HOAc - CH_3OH - CH_3]^+$), 382 (100, $[414 - CH_3OH]^{+\cdot}$),* 369 (49), 367 (24, $[M - HOAc - 2CH_3OH - CH_3]^+$), 341 (19), 328 (23), 299 (49), 273 (33), 271 (64), 267 (61), 239 (42)
4	70	442 (9, $[M]^{+\cdot}$), 427 (3, $[M - CH_3]^+$), 424 (1, $[M - H_2O]^{+\cdot}$), 411 (7, $[M - CH_3O]^+$), 382 (31, $[M - HOAc]^{+\cdot}$),* 367 (7, $[382 - CH_3]^+$),* 364 (3, $[M - HOAc - H_2O]^{+\cdot}$), 340 (4, $[M - HOAc - CH_2CO]^{+\cdot}$), 335 (3, $[367 - CH_3OH]^+$),* 328 (4, $[382 - C_4H_6]^{+\cdot}$),* 309 (3, $[M - HOAc - CH_2CO_2CH_3]^+$), 295 (4, $[M - HOAc - C_4H_7O_2]^+$), 287 (10, $[M - C_9H_{15}O_2]^+$), 267 (16, $[M - HOAc - C_6H_{11}O_2]^+$), 249 (4, $[M - HOAc - H_2O - C_6H_{11}O_2]^{+\cdot}$), 241 (21, $[M - HOAc - C_4H_7O_2 - C_4H_6]^+$), 227 (49, $[287 - HOAc]^+$),* 185 (100, $[227 - CH_2CO]^+$)*
	12	442 (50), 427 (3), 424 (1), 411 (6), 382 (100), 367 (8), 364 (2), 340 (5), 335 (3), 328 (7), 309 (4), 295 (5), 287 (30), 267 (17), 249 (2), 241 (20), 227 (58), 185 (51)
5a	12	350 (5, $[M]^{+\cdot}$), 332 (64, $[M - H_2O]^{+\cdot}$),* 314 (21, $[332 - H_2O]^{+\cdot}$),* 299 (6, $[M - 2H_2O - CH_3]^+$), 296 (6, $[M - 3H_2O]^{+\cdot}$), 285 (3, $[M - 2H_2O - CHO]^+$), 265 (51, $[M - C_5H_9O]^+$), 253 (5, $[M - 3H_2O - Ac]^+$), 247 (24, $[332 - C_5H_9O]^+$),* 211 (6, $[M - 3H_2O - C_5H_9O]^+$), 147 (12), 121 (4)
5b	70	374 (94, $[M]^{+\cdot}$), 359 (27, $[M - CH_3]^+$),* 356 (16, $[M - H_2O]^{+\cdot}$),* 314 (100, $[M - HOAc]^{+\cdot}$), 304 (17, $[M - C_4H_6O]^{+\cdot}$), 299 (52, $[359 - HOAc]^+$),* 296 (20, $[314 - H_2O]^{+\cdot}$),* 286 (9, $[M - HOAc - CO]^+$), 281 (10, $[M - HOAc - H_2O - CH_3]^+$), 271 (46, $[M - HOAc - Ac]^+$), 260 (16, $[M - HOAc - C_4H_6]^{+\cdot}$), 253 (39, $[M - HOAc - Ac - H_2O]^+$), 244 (21, $[M - C_4H_6O - HOAc]^{+\cdot}$), 229 (19, $[M - HOAc - C_5H_9O]^+$), 217 (12, $[M - HOAc - C_4H_6 - Ac]^+$)
5c	70	432 (25, $[M]^{+\cdot}$), 417 (26, $[M - CH_3]^+$),* 372 (97, $[M - HOAc]^{+\cdot}$),* 357 (19, $[417 - HOAc]^+$),* 354 (4, $[M - HOAc - H_2O]^{+\cdot}$), 329 (18, $[372 - Ac]^+$),* 312 (100, $[372 - HOAc]^{+\cdot}$),* 297 (90, $[312 - CH_3]^+$ and $[357 - HOAc]^+$),* 294 (16, $[312 - H_2O]^{+\cdot}$),* 284 (9, $[M - 2HOAc - CO]^{+\cdot}$), 279 (16, $[M - 2HOAc - H_2O - CH_3]^+$), 269 (83, $[312 - Ac]^+$),* 258 (12, $[M - 2HOAc - C_4H_6]^{+\cdot}$), 251 (39, $[M - 2HOAc - H_2O - Ac]^+$), 241 (29, $[M - 2HOAc - Ac - CO]^+$), 227 (39, $[M - 2HOAc - CH_3 - C_4H_6O]^+$ or $[M - 2HOAc - C_5H_9O]^+$)
5d	12	418 (0, MW), 403 (2, $[M - CH_3]^+$), 400 (1, $[M - H_2O]^{+\cdot}$), 358 (15, $[M - HOAc]^{+\cdot}$), 343 (2, $[M - HOAc - CH_3]^+$), 340 (2, $[M - HOAc - H_2O]^{+\cdot}$), 316 (6, $[M - AcOAc]^{+\cdot}$), 298 (100, $[358 - HOAc]^{+\cdot}$),* 283 (29, $[298 - CH_3]^+$),* 280 (5, $[298 - H_2O]^{+\cdot}$),* 255 (27, $[298 - Ac]^+$),* 244 (14, $[M - 2HOAc - C_4H_6]^{+\cdot}$), 228 (15, $[M - 2HOAc - C_4H_6O]^{+\cdot}$), 213 (17, $[M - 2HOAc - C_4H_6O - CH_3]^+$)
5e	70	476 (1, $[M]^{+\cdot}$), 461 (3, $[M - CH_3]^+$), 433 (12, $[M - Ac]^+$), 416 (5, $[M - HOAc]^{+\cdot}$), 401 (1, $[M - HOAc - CH_3]^+$), 374 (5, $[M - AcOAc]^{+\cdot}$), 373 (10, $[433 - HOAc]^+$),* 356 (80, $[M - 2HOAc]^{+\cdot}$), 341 (8, $[356 - CH_3]^+$),* 338 (1, $[M - 2HOAc - H_2O]^{+\cdot}$), 314 (14, $[M - AcOAc - HOAc]^{+\cdot}$), 313 (28, $[M - 2HOAc - Ac]^+$), 296 (86, $[356 - HOAc]^{+\cdot}$),* 281 $[296 - CH_3]^+$),* 278 (4, $[M - 3HOAc - H_2O]^{+\cdot}$), 271 (10), 253 (100, $[313 - HOAc]$ and $[296 - Ac]^+$),* 242 (14, $[M - 3HOAc - C_4H_6]^{+\cdot}$), 240 (14, $[296 - C_4H_8]^{+\cdot}$),* 229 (13, $[M - AcOAc - HOAc - C_5H_9O]^+$), 226 (15, $[M - 3HOAc - C_4H_6O]^{+\cdot}$), 211 (15, $[M - 3HOAc - C_4H_6O - CH_3]^+$), 200 (23, $[296 - C_7H_{12}]^{+\cdot}$)*
5f	70	479 (1, $[M]^{+\cdot}$), 464 (4, $[M - CH_3]^+$), 436 (10, $[M - Ac]^+$), 419 (4, $[M - HOAc]^{+\cdot}$), 404 (1, $[M - HOAc - CH_3]^+$), 377 (5, $[M - AcOAc]^{+\cdot}$), 376 (1, $[436 - HOAc]^+$),* 373 (3, $[436 - HOAc\text{-}d_3]^+$),* 359 (60, $[M - 2HOAc]^{+\cdot}$), 344 (9, $[359 - CH_3]^+$),* 341 (2, $[M - 2HOAc - H_2O]^{+\cdot}$), 316 (18, $[M - 2HOAc - Ac]^+$), 314 (13, $[M - AcOAc - HOAc\text{-}d_3]^{+\cdot}$), 313 (14, $[M - Ac - HOAc - HOAc\text{-}d_3]^+$), 296 (75, $[359 - HOAc\text{-}d_3]^{+\cdot}$),* 281 (85, $[296 - CH_3]^+$),* 278 (6, $[M - 2HOAc - HOAc\text{-}d_3 - H_2O]^{+\cdot}$), 271 (9), 253 (100, $[316 - HOAc\text{-}d_3]^+$ and $[296 - Ac]^+$),* 242 (14, $[M - 2HOAc - HOAc\text{-}d_3 - C_4H_6]^{+\cdot}$), 240 (15, $[296 - C_4H_8]^{+\cdot}$), 229 (17, $[M - AcOAc - HOAc\text{-}d_3 - C_5H_9O]^+$), 226 (16, $[M - 2HOAc - C_4H_6O]^{+\cdot}$), 211 (20, $[M - 2HOAc - HOAc\text{-}d_3 - C_4H_6O - CH_3]^+$), 200 (27, $[296 - C_7H_{12}]^{+\cdot}$)*
5g	70	479 (1, $[M]^{+\cdot}$), 464 (3, $[M - CH_3]^+$), 433 (11, $[M - Ac\text{-}d_3]^+$), 416 (4, $[M - HOAc\text{-}d_3]^{+\cdot}$), 401 (1, $[M - HOAc\text{-}d_3 - CH_3]^+$), 374 (9, $[M - AcOAc\text{-}d_3]^{+\cdot}$), 373 (10, $[433 - HOAc]^+$),* 356 (65, $[M - HOAc\text{-}d_3 - HOAc]^{+\cdot}$), 341 (11, $[356 - CH_3]^+$), 338 (1, $[M - HOAc\text{-}d_3 - HOAc - H_2O]^{+\cdot}$), 314 (7, $[M - AcOAc\text{-}d_3 - HOAc]^{+\cdot}$), 313 (26, $[M - HOAc\text{-}d_3 - HOAc$

Table I (*Continued*)

compd	ionization voltage, eV	*m/z* (% relative abundance, probable genesis)
		– Ac]⁺), 296 (80, [356 – HOAc]⁺·),* 281 (85, [296 – CH_3]⁺),* 278 (5, [M – 2HOAc – HOAc-d_3 – H_2O]⁺·), 271 (7), 253 (100, [296 – Ac]* and [313 – HOAc]⁺),* 242 (13, [M – HOAc-d_3 – 2HOAc – C_4H_6]⁺·), 240 (14, [296 – C_4H_8]⁺·),* 229 (26, [M – AcOAc-d_3 – HOAc – C_5H_9O]⁺), 226 (15, [M – HOAc-d_3 – 2HOAc – C_4H_6O]⁺·), 211 (17, [M – HOAc-d_3 – 2HOAc – C_4H_6O – CH_3]⁺), 200 (26, [296 – C_7H_{12}]⁺·)*
6a	70	480 (1, [M]⁺·), 462 (3, [M – CD_3]⁺), 437 (10, [M – Ac]⁺), 420 (4, [M – HOAc]⁺·), 405 (2, [M – HOAc – CH_3]⁺), 378 (7, [M – AcOAc]⁺·), 377 (6, [437 – HOAc]⁺·), 360 (60, [M – 2HOAc]⁺·), 345 (6, [360 – CH_3]⁺),* 341 (2, [M – 2HOAc – DHO]⁺·), 318 (14, [M – AcOAc – HOAc]⁺·), 317 (15, [M – Ac – 2HOAc]⁺), 314 (16, [M – 2HOAc – Ac-d_3]⁺), 300 (61, [360 – HOAc]⁺·),* 285 (87, [300 – CH_3]⁺·),* 281 (7, [M – 3HOAc – DHO]⁺·), 273 (4), 272 (5), 271 (4), 254 (100, [300 – Ac-d_3]⁺ and [314 – HOAc]⁺),* 246 (15, [M – 3HOAc – C_4H_8]⁺·), 244 (16, [300 – C_4H_6]⁺·),* 229 (24, [M – AcOAc – HOAc – $C_5H_5D_4O$]⁺·), 226 (22, [M – 3HOAc – $C_4H_2D_4O$]⁺·), 211 (23, [M – 3HOAc – $C_4H_2D_4O$ – CH_3]⁺), 204 (38, [300 – C_7H_{12}]⁺·)*
6b	70	483 (1, [M]⁺·), 465 (2, [M – CD_3]⁺), 440 (9, [M – Ac]⁺), 423 (3, [M – HOAc]⁺·), 408 (1, [M – HOAc – CH_3]⁺), 380 (6, [440 – HOAc]⁺),* 378 (7, [M – AcOAc-d_3]⁺·), 360 (51, [M – HOAc – HOAc-d_3]⁺·), 345 (5, [360 – CH_3]⁺),* 341 (2, [M – HOAc – HOAc-d_3 – DHO]⁺·), 318 (12, [M – AcOAc-d_3 – HOAc]⁺·), 317 (13, [M – Ac – HOAc – HOAc-d_3]⁺), 314 (14, [M – HOAc – HOAc-d_3 – Ac-d_3]⁺), 300 (83, [360 – HOAc]⁺·),* 285 (88, [300 – CH_3]⁺),* 281 (8, [M – 2HOAc – HOAc-d_3 – DHO]⁺·), 273 (4), 272 (4), 254 (100, [300 – Ac-d_3]⁺ and [314 – HOAc]⁺),* 246 (13, [M – 2HOAc – HOAc-d_3 – C_4H_6]⁺·), 244 (16, [300 – C_4H_8]⁺·), 229 (21, [M – AcOAc-d_3 – HOAc – $C_5H_5D_4O$]⁺), 226 (19, [M – 2HOAc – HOAc-d_3 – $C_4H_2D_4O$]⁺·), 211 (20, [M – 2HOAc – HOAc-d_3 – $C_4H_2D_4O$ – CH_3]⁺), 204 (33, [300 – C_7H_{12}]⁺·)*
6c	70	354 (2, [M]⁺·), 336 (19, [M – H_2O]⁺·),* 318 (8, [336 – H_2O]⁺·),* 303 (4, [M – $2H_2O$ – CH_3]⁺), 300 (5, [M – $3H_2O$]⁺·), 289 (4, [M – $2H_2O$ – CHO]⁺), 285 (7, [M – $3H_2O$ – CH_3]⁺), 265 (14, [M – $C_5H_5D_4O$]⁺), 254 (18, [M – $3H_2O$ – Ac-d_3]⁺), 247 (25, [336 – $C_5H_5D_4O$]⁺),* 229 (100, [318 – C_5H_4O]⁺),* 211 (12, [M – $3H_2O$ – $C_5H_5D_4O$]⁺), 147 (34), 121 (65)
	12	354 (3), 336 (30), 335 (21), 318 (12), 317 (14), 303 (6), 302 (4), 300 (6), 299 (7), 289 (2), 288 (2), 285 (4), 284 (3), 266 (22), 265 (21), 254 (17), 253 (4), 248 (12), 247 (20), 230 (33), 229 (100), 211 (10), 147 (7), 121 (17)

[a] Metastable peaks were observed for processes marked with an asterisk.



Subsequent exchange of the deuterium on the 12α-OD in c with hydrogens on the steroidal system leads to retention of deuterium in the steroid skeleton containing fragment ions. This enhanced deuterium exchange at low ionization energy is an alternative reaction that is less competitive at higher ionization energies where charge is more extensively localized on the 12α-hydroxyl oxygen and where the shorter ion lifetime leads to a predilection for concerted elimination over stepwise elimination. Furthermore, C13–C17 bond scission followed by hydrogen transfer from C18 to C17 may be more competitive with exchange between the 12α-hydroxy proton and the 17α-deuterium at higher ionization energies than at lower ionization energies.

A partial plausible mechanistic sequence for loss of three successive H_2O molecules from the molecular ion of **1f** is presented in Scheme I. The ionized cyclopropyl ring in d results from 9α,12α-diaxial cis 1,3-elimination and in e from 12α,14α-diaxial cis 1,3-elimination. An orbital sym-

Scheme I

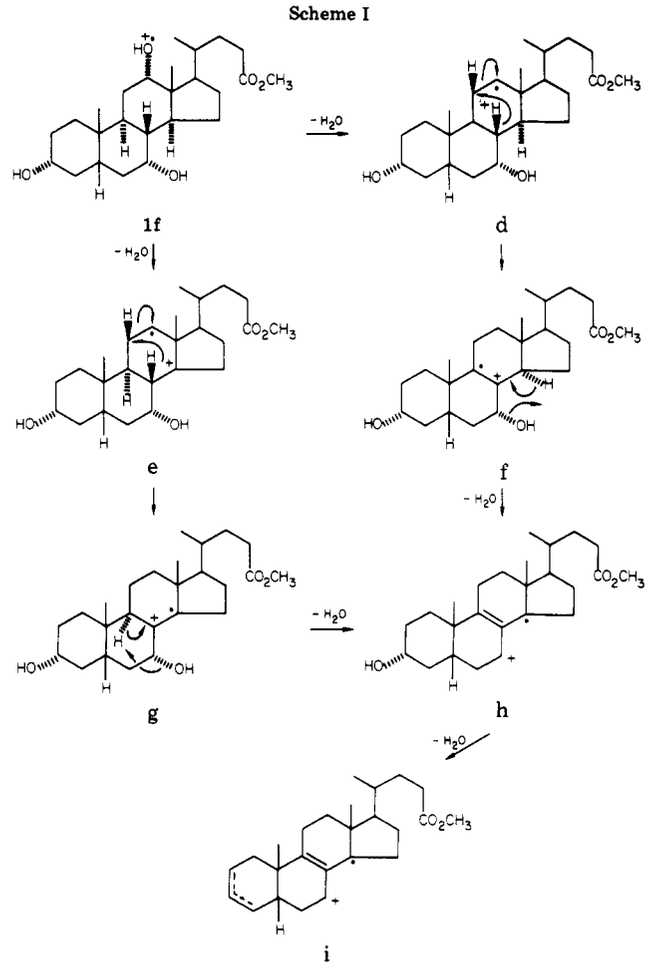


Table II. Mass Spectral (12 eV)[a] Comparison of Consecutive Loss of Acetic Acid and Water

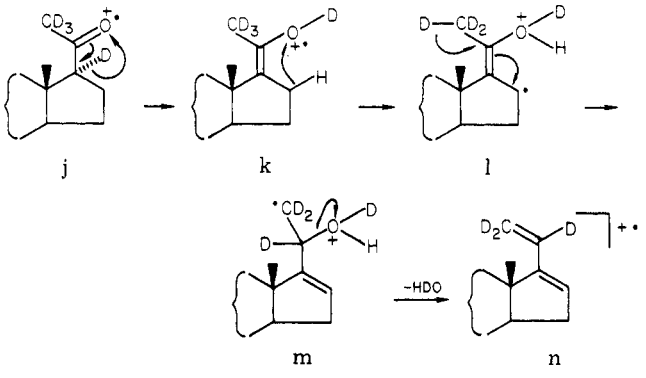
compd	$[M]^{+\cdot}$	$[M - ROH]^{+\cdot}$	$[M - ROH - C_6H_{11}O_2]^+$	sum[b]	$[M - 2ROH]^{+\cdot}$	$[M - 2ROH - C_6H_{11}O_2]^+$	sum[b]	$[M - 3ROH]^{+\cdot}$	$[M - 3ROH - C_6H_{11}O_2]^+$	sum[b]
1h	548 (1)	488 (3)	373 (17)	20	428 (47)	313 (69)	116	368 (72)	253 (100)	172
1f	422 (1)	404 (4)	299 (5)	9	386 (43)	271 (100)	143	368 (33)	253 (59)	92
5a	350 (2)	332 (67)	247 (21)[c]	88	314 (18)	229 (100)[c]	118	296 (7)	211 (2)[c]	9

[a] The mass spectra of **1h** and **1f** were obtained under identical conditions. [b] Sum of relative intensities of prior two columns. [c] Replace $C_6H_{11}O_2$ loss by C_5H_9O loss.

metry allowed 1,2-hydrogen shift followed by a 1,3-hydrogen shift leads to isomerization of d and e to f and g, respectively. Subsequent H_2O ejection leads to the same ion h, which is more stable than any of the prior ions. Although this mechanism incorporates the results delineated above, many details remain undefined in the absence of deuterium labeling studies (e.g., at positions 1α, 9α, and 14α).

Loss of methanol as in the spectra of the methyl ethers **3c** to **3d** is also believed to be similar to H_2O loss from alcohols.[5] Likewise, electron impact induced loss of HCl should parallel the loss of water and also occur via cis 1,3-diaxial elimination. Thus, the presence of chlorine radical loss, $[M - 2HOAc - Cl]^+$, in the spectrum of **1k** is consistent with the equatorial orientation of the 7β-Cl group, which is unfavorable for HCl loss.

Water loss from the carbonyl group of various isomers of diketo and triketo methyl cholanoates is well documented.[1b] Like the 12-oxo methyl cholate acetate derivatives,[2] the spectrum of **4** exhibits a nominal loss of H_2O. Also, the spectra of 20-oxo acetates **5d** and **5e** exhibit H_2O loss. Combining the 12-oxo and 20-oxo groups in compounds **5b** and **5c** results in even more substantial H_2O elimination after ionization (and in the case of **5c**, only after fragmentation) in the mass spectrometer. The observation of exclusive HDO loss in the spectra of deuterated analogues **6a** and **6b** establishes that ketonic H_2O loss in the mass spectra of 20-oxo steroids involves one α-hydrogen. A plausible mechanism (j to n) is shown.

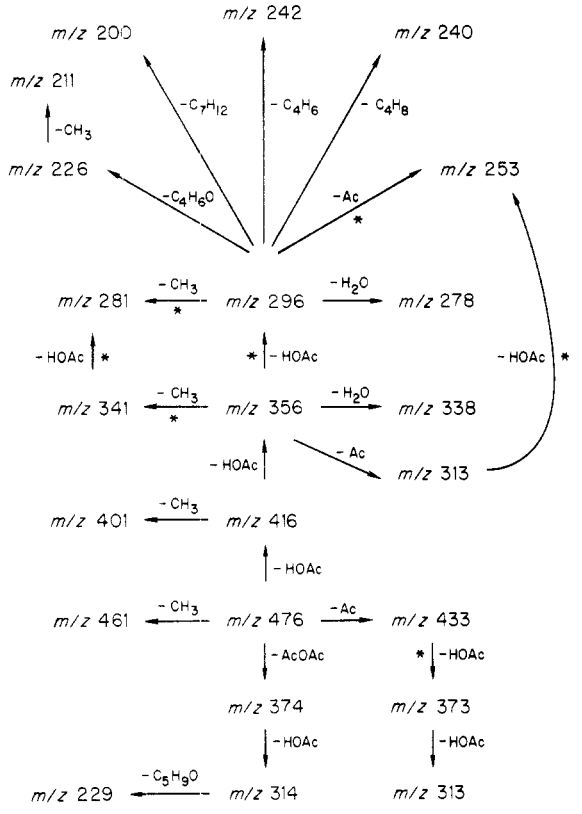


j k l m n

Fragmentation Pathways of $3\alpha,7\alpha,12\alpha$-Triacetoxy-5β-pregnan-20-one. It was convenient to synthesize the additional deuterated analogues **6a** and **6b**, and comparison of their mass spectra with the spectra of **5d** to **5g** has permitted construction of Scheme II. The consecutive loss of acetic acid molecules from the molecular ion of **5e** (Table I) occurs such that the 12α-OAc group is lost first (m/z 479 goes to m/z 416 in the spectrum of **5g**), the 7α-OAc group is lost second, and finally the 3α-OAc group is lost last (m/z 359 goes to m/z 296 in **5f**).[2] Ejection of acetyl radical from the molecular ion of **5e** was determined to derive from the 12α-OAc group ($[M]^{+\cdot}$ goes to m/z 433 in both **5e** and **5g**).[2] Surprisingly, the elimination of HOAc after Ac radical loss comes primarily from the 3α-OAc group (m/z 436 ($[M - Ac]^+$) goes mainly to m/z 373 ($[436 - HOAc\text{-}d_3]^+$) in the spectrum of **5f**). The loss of H_2O has already been discussed (vide supra j to n).

Acetic anhydride loss or successive acetic acid and ketene loss (not necessarily in that order) from the molecular ion of **5e** was established to occur by spectral comparison of **5f**, **5g**, and **6b**. Distinguishing between these two possibilities was not possible because of the low intensity of the corresponding precursor ions expected in the latter case for these low abundance $[M - AcOAc]^{+\cdot}$ ions (approximately 2% in relative abundance). However, on

Scheme II



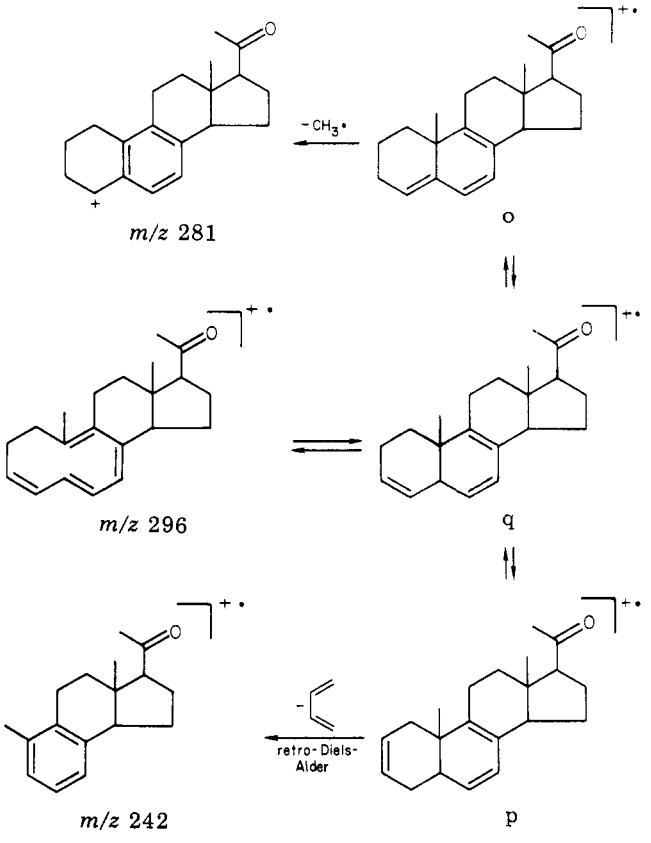
the basis of precedence,[7] the authors favor the latter where a 7α-acetoxy α-methyl hydrogen is transferred to the ionized 12α-acetoxy oxygen, resulting in ketene and acetic acid ejection, respectively, but for convenience have indicated these relevant ions by AcOAc loss; this preference is reinforced by the absence of anhydride loss in the spectra of the formate (**1g**) and trifluoroacetate (**1e**) esters. Regardless, the formation of these ions indicate that some interaction occurs between the acetate groups in ionized diacetate and triacetate derivatives of cholic acid. Note that the deuterium labeling results (Table III) are in harmony with charge localization on the 12α-OAc group after initial ionization and subsequent interaction between the more proximate 12α-OAc and 7α-OAc groups.

The loss of a methyl group becomes progressively more important as the fragment ion becomes more unsaturated and conjugated because of loss of acetic acid molecules. This and deuterium labeling at C-21 in **6a** and **6b** established that it is the C-18 or C-19 methyl groups that are being ejected from fragment ions of **5e** to form ions of m/z 401, 341, and 281. However, the minor loss of a methyl group from the molecular ion originates from the C-21 methyl, since loss of 18 mass units ($[M - CD_3]^+$) is observed in the spectra of **6a** and **6b**; this loss occurs by incipient charge localization directly on the 20-oxo group after ionization and ceases when charge subsequently becomes localized elsewhere on the molecule. An intense metastable peak for the loss of a methyl group from the m/z 296 ion to form the m/z 281 ion was observed; a feeble metastable peak was also observed for m/z 341 ions losing HOAc to form m/z 281 ions. Since the fragmentations of **5e** appear to exhibit a pattern suggesting the migration of localized charge from the region of the D ring to the region of the A ring, it is proposed that the C-19 methyl group is being ejected to form ions m/z 341 and 281. Scheme III presents

Table III. Electron Impact (70 eV) Induced Ions (~2% Relative Abundance) Corresponding to Acetic Anhydride Loss in Methyl Cholate Triacetate

m/z	ion	3α	7α	12α
446	M − AcOAc	OAc	OAc	OAc
446	M − AcOAc-d_3	OAc	OAc	OAc-d_3
446	M − AcOAc-d_6	OAc	OAc-d_3	OAc-d_3
449	M − AcOAc-d_3	OAc-d_3	OAc	OAc-d_3
449	M − AcOAc	OAc-d_3	OAc	OAc

Scheme III



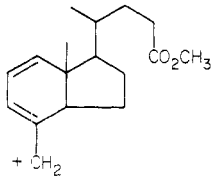
a plausible uniform mechanism for C-19 methyl group loss and A-ring retro-Diels–Alder reaction. The ion (m/z 296) formed from consecutive loss of three HOAc molecules can undergo an electrocyclization reaction to ion q which may isomerize to either o or p. Methyl ejection from ion o produces the stabilized benzyl carbonium ion m/z 281, and retro-Diels–Alder reaction of ion **p** gives the benzenoid ion m/z 242.

Comparing the mass spectra of **1h** vs. **5e**, and **1f** vs. **5a**, provides additional insight concerning the mass spectral fragmentation of bile acids. The peaks at 433, 373, and 313 (Ac· loss from the 12α-OAc group), 461 (C-21 CH_3 loss), and 338 and 278 (ketonic H_2O loss) mass units have no analogous peaks in the spectrum of **1h**. Furthermore, the peaks at m/z 240 and 200 mass units are unique to the spectrum of **5e**, and the mechanisms for their formation are unresolved except that they most likely involve carbon groups from the A-ring region. All other ion peaks in the spectrum of **5e** have corresponding peaks in the spectrum of **1h**. The m/z 211 ion can be formed by the path shown in Scheme II or by an alternate D-ring cleavage where a C_5H_9O fragment is lost from the m/z 296 ion as suggested by other workers;[8] however, deuterium labeling of the C-18

(7) J. R. Dias, R. Ramachandra, and B. Nassim, *Org. Mass Spectrom.*, 13, 307 (1978).

(8) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry", Vol. 2, Holden-Day, San Francisco, 1964, pp 101-103.

and C-19 methyl groups will be necessary to distinguish between these two alternatives. Low intensity peaks due to CH_3O loss or cleavage of the 17-side-chain beyond C-20 (e.g., m/z 281) in the spectrum **1h** are obviously absent in the spectrum of **5e**. The m/z 261 ion formed in the spectrum of **1h** is believed to have the following identity[1b] and has an analogue ion at m/z 189 in the spectrum of **5e**.



m/z 261

The primary difference between the mass spectra of **1f** and **5a** is that in the latter D-ring cleavage prevails.[9] Charge is principally localized on the more easily ionized 20-oxo group in the molecular ion of **5a** leading to D-ring scission ion peaks at m/z 265, 247, 229, and 211, whereas these same ion peaks are barely perceptible in the spectrum of **1f**. Otherwise, the spectra of **1f** and **5a** are similar in their consecutive loss of H_2O and loss of the 177-side-chain, except that the minor loss of CH_3O and fragments of the 17-side-chain beyond C-20 are absent in the spectrum of **5a**.

Conclusion

This paper summarizes the current state of the art in the understanding of how bile acids mechanistically undergo fragmentation to ions above m/z 200 after electron impact. Specifically, the preferential order of consecutive loss of three carboxylic acid or water molecules (12α > 7α > 3α) has been delineated. Electronegative substituents on the B and C rings (as formoxy or trifluoroacetoxy, 12α-OH or 12α-OAc leaving groups, and 20-oxo groups all enhance 17-side-chain loss and/or D-ring cleavage. Elimination of an equatorial 3α-acetoxy group from a 3α,7α,12α-triacetoxy 5β-steroid is more facile than elimination of an equatorial 3α-hydroxy group from a 3α,7α,12α-trihydroxy 5β-steroid.

Observation of exchange between the 12α-OH proton and the 17α-deuterium in the 12-eV spectrum of **6c** provides additional mechanistic evidence for cis 1,3-diaxial elimination of H_2O from cyclic alcohols. Ketonic water loss involves only one α-proton and most likely results in the formation of a butadiene system. Minor loss of the equivalent of acetic anhydride provides proof that interaction occurs between the acetate groups in ionized diacetate and triacetate derivatives of cholic acid.

Experimental Section

All mass spectra were obtained with a Nuclide 12-90-G single focusing instrument having a resolution capability of 10 000. Spectra were obtained at ionization voltages of 12 and 70 eV, a resolution of 600 to 800, and an accelerating voltage of 4 kV. The inlet source temperature ranged from 150 to 180 °C, and the sample probe temperature ranged from 80 to 120 °C. NMR spectra were acquired with a Varian T-60 or Perkin-Elmer R-24B instrument.

The syntheses of **1a**, **1b**, and **1e–i** have been reported in the literature many times, and the syntheses of **3a** to **3d**, **4**, **1k**, and **5a–g** have been recently reported.[2,10] Compound **1d** was synthesized by dissolving **1e** in ether and washing with a $NaHCO_3$ solution several times until TLC (hexane-EtOAc) showed that the 3α-CF_3CO_2 group was totally removed. The triformate **1g** was synthesized by heating (60 °C) methyl cholate (**1f**) in formic acid (87%) for 6 h followed by treatment of the isolated product with diazomethane.[11]

Methyl 3α-(Carbethoxyoxy)-7α,12α-bis(m-iodobenzoxy)-5β-cholanoate (1c). Diol 1b[12] (2.0 g) was dissolved in THF (5 mL), m-iodobenzoyl chloride (1.5 mL) was added, and the solution was heated at reflux for 24 h. TLC indicated one major product, which upon crystallization from ether–hexane gave 1.5 g of **1c**: mp 113–14 °C; ν_{max} 2955 (C-H), 1715 and 1270 (ester), and 1560 cm^{-1}; ^{1}H NMR δ 7.8 (m, 8 H, aromatic), 5.31 (peak, 1 H, 12β-H), 5.23 (peak, 1 H, 7β-H), 4.2 (hump, 1 H, 3β-H), 4.0 (q, 2 H, CH_3CH_2O), 3.56 (s, 3 H, CH_3O), 1.19 (t, 3 H, CH_3CH_2O), 1.00 (s, 3 H, C-19 CH_3), and 0.82 (s, 3 H, C-18 CH_3).

Methyl 3α-(Acetoxy-d_3)-12α-acetoxy-5β-cholanoate (1j). Methyl 3α,12α-diacetoxy-5β-cholate was selectively deacetylated at position-3 with AcCl-CH_3OH[13] and reacted with pyridine and Ac_2O-d_6. The product was purified by TLC: ^{1}H NMR δ 5.06 (peak, 1 H, 12β-H), 4.7 (hump, 1 H, 3β-H), 3.66 (s, 3 H, OCH_3), 2.10 (s, 3 H, 12α-OAc), 0.93 (s, 3 H, C-19 CH_3), and 0.76 (s, 3 H, C-18 CH_3).

Methyl 3α-(Acetoxy-d_3)-7α-acetoxy-24-nor-5β-cholanoate (2b). Acid **2a** (220 mg) was dissolved in CH_3OH (3 mL) to which AcCl (0.15 mL) had been added. After standing at room temperature for 1.5 h, the solvent was removed with a rotary evaporator, and the residue was treated with pyridine and Ac_2O-d_6 at room temperature for 6 h. Removal of the pyridine and TLC purification of the resulting residue gave a near-quantitave yield of **2b**: ^{1}H NMR δ 4.87 (peak, 1 H, 7β-H), 4.6 (hump, 1 H, 3β-H), 3.66 (s, 3 H, OCH_3), 2.04 (s, 3 H, 7α-OAc), 0.96 (s, 3 H, C-19 CH_3), 0.75 (s, 3 H, C-18 CH_3).

17,21,21,21-Tetradeuterio-3α,7α,12α-trihydroxy-5β-pregnan-20-one (6c) and 17,21,21,21-Tetradeuterio-3α,7α,12α-triacetoxy-5β-pregnan-20-one (6a). Trihydroxy ketone **5a** (105 mg) was dissolved in THF (1 mL), and D_2O (3 mL) plus Na_2CO_3 (80 mg) was added. The solution was heated at reflux for 48 h followed by stirring at room temperature for 72 h. The cooled solution was extracted with ether and then $CHCl_3$, the organic layer was dried by percolating through $MgSO_4$, and the solvent was evaporated to yield triol **6c** (100 mg): ^{1}H NMR ($CDCl_3$, Me_4Si internal reference) δ 3.8 (peaks, 2 H, 7β-H and 12β-H), 3.5 (hump, 1 H, 3β-H), 0.92 (s, 3 H, C-19 CH_3), and 0.65 (s, 3 H, C-18 CH_3). Triol **6c** (100 mg) was dissolved in pyridine (1 mL) and Ac_2O (0.5 mL). After standing at room temperature for 24 h, it was heated at reflux for 1 h. The pyridine was removed by a rotary evaporator, and the residue thus obtained was purified by TLC on silica gel (HF_{254}) to give triacetate **6a** (105 mg): mp 151–3 °C; ν_{max} (solid film) 2970 (C—H), 2270 (C—D), 1740 and 1250 (3-AcO), and 1710 (20-C=O) cm^{-1}; ^{1}H NMR δ 5.13 (peak, 1 H, 12β-H), 4.92 (peak, 1 H, 7β-H), 4.5 (hump, 1 H, 3β-H), 2.20 (s, 3 H, 12α-OAc), 2.10 (s, 3 H, 7α-OAc), 2.04 (s, 3 H, 3α-OAc), 0.94 (s, 3 H, C-19 CH_3), and 0.72 (s, 3 H, C-18 CH_3).

17,21,21,21-Tetradeuterio-3α,12α-diacetoxy-7α-(acetoxy-d_3)-5β-pregnan-20-one (6b). Triacetate **5e** (70 mg) was dissolved in THF (1.5 mL), and D_2O (2.5 mL) containing reacted Na (18 mg) was added. After heating at reflux for 144 h, the cooled reaction mixture was extracted with ether and then $CHCl_3$. The solvent from extraction was evaporated, and the resulting residue was treated with pyridine (1 mL) and Ac_2O (0.5 mL) for 9 days at room temperature. The pyridine was removed on a rotary evaporator, and the residue was purified by TLC and recrystallized (ether-hexane); mp 153-5 °C. The ^{1}H NMR spectrum was the same as for **6a** except that the 3 proton singlet at 2.10 was reduced to one-third intensity. The mass spectrum of **6b** was obtained

(9) S. Popov, G. Eadon, and C. Djerassi, *J. Org. Chem.*, **37**, 155 (1972).

(10) (a) J. R. Dias, *J. Chem. Eng. Data*, **22**, 445 (1977); (b) B. Nassim and J. R. Dias, *Org. Prep. Proced. Int.*, 11(6) (1979).

(11) F. Cortese and L. Bauman, *J. Am. Chem. Soc.*, **57**, 1393 (1935).

(12) L. Fieser and S. Rajagopalan, *J. Am. Chem. Soc.*, **71**, 3935 (1949).

(13) J. R. Dias and R. Ramachandra, *Synth. Commun.*, **7**, 293 (1977).

by subtracting the spectrum of **6a** from the spectrum of this mixture.

Acknowledgment. The technical assistance of Barbara Jean Dias and Ashoke Sinha was appreciated.

Registry No. **1a**, 54047-80-1; **1b**, 21059-36-9; **1c**, 72049-90-2; **1d**, 72049-91-3; **1e**, 2342-71-4; **1f**, 1448-36-8; **1g**, 33744-75-1; **1h**, 6818-44-6; **1i**, 55106-11-1; **1j**, 72049-92-4; **1k**, 64219-21-2; **2a**, 72059-84-8; **2b**, 72049-93-5; **3a**, 72049-94-6; **3b**, 72049-95-7; **3c**, 72049-96-8; **3d**, 72049-97-9; **4**, 72049-98-0; **5a**, 601-95-6; **5b**, 72049-99-1; **5c**, 72050-00-1; **5d**, 72050-01-2; **5e**, 61543-88-2; **5f**, 69889-99-2; **5g**, 69890-00-2; **6a**, 72050-02-3; **6b**, 72050-03-4; **6c**, 72050-04-5.

Notes

Electron-Transfer Processes: Oxidation of 4-Phenylbutanoic Acid by Cobaltic Acetate

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The oxidative decarboxylation of arylacetic acids by cobalt(III) acetate in the presence of copper(II) acetate in acetic acid has been ascribed to the formation of an aromatic radical cation intermediate.[1]

The high value of ρ (−2.9) for the decarboxylation of substituted arylacetic acids and the exclusive formation of 4-phenylbutyrolactone in the cobaltic oxidation of 4-phenylbutanoic acid has been reported as evidence for the proposed mechanism.

In this note, we describe our results on oxidation of 4-phenylbutanoic acid by cobalt(III) acetate under various experimental conditions, which include also those previously reported,[1] and by cobalt(III) acetate in the presence of copper(II) acetate, respectively. In all cases, the oxidative decarboxylation of **1** (path A) competes with the formation of **2** (path B) (Scheme I). The 3-phenylpropyl radical reacts by hydrogen abstraction, giving 1-phenylpropane (**3**) in the absence of Cu(II) salts, while in the presence of Cu(II) salts the olefins **4** ($PhCH_2CH{=}CH_2$) and **5** ($PhCH{=}CHCH_2OAc$) are formed.

$$PhCH_2CH_2CH_2\cdot + Cu^{2+} \rightarrow PhCH_2CH{=}CH_2 \underset{\mathbf{4}}{} + Cu^{+} + H^{+}$$

Compound **5** comes from further oxidation of **4**.

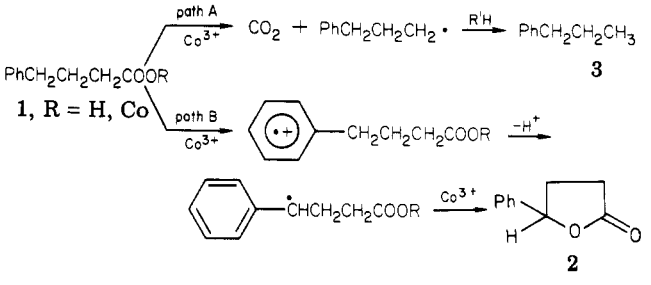
$$PhCH_2CH{=}CH_2 \xrightarrow[-Co^{2+},\,-H^{+}]{Co^{3+}} PhCHCH{=}CH_2 \rightarrow PhCH{=}CHCH_2\cdot$$

$$PhCH{=}CHCH_2\cdot + Co(OAc)_3 \rightarrow PhCH{=}CHCH_2OAc + Co(OAc)_2$$
$$\mathbf{5}$$

Competitive oxidation of acetic acid by Co(III) is also responsible for the observed low conversion of **1**.

Our results are not in favor of a single mechanism[1] and suggest that a dualism of mechanism (electron transfer from the aromatic ring and from the anion carboxylate) is operating in the oxidation of arylacetic acids and of 4-phenylbutanoic acid by Co(III) salts.[2]

Scheme I



A similar mechanism has already been suggested for the oxidation of phenylacetic acid by Ce(IV) salts;[3] this conclusion was criticized on the basis of the results obtained by Co(III) salts.[1]

Very similar results were obtained also in the oxidation of 4-phenylbutanoic acid with peroxydisulfate, indicating that an analogous dualism of mechanism is operating also in that case.

Experimental Section

Potassium peroxydisulfate and copper(II) acetate monohydrate were obtained from Fluka and Carlo Erba, respectively. 4-Phenylbutanoic acid, 3-phenylpropane, and 3-phenylpropene were commercial products (Fluka). The olefin **5** was prepared by $NaBH_4$ reduction of cinnamaldehyde and esterification with acetic anhydride and pyridine of the resulting alcohol. The lactone **2** was identified by comparison with the authentic sample.[4] The 4-phenyl-4-acetoxybutanoic acid was prepared starting with 4-phenyl-4-hydroxybutanoic acid and esterification with acetic anhydride and pyridine. Cobalt(III) acetate was prepared (>97% purity) according to the reported procedure.[5]

All solvents and reagents were checked for purity and found to be free from the products **1**, **2**, **3**, **4**, and **5**.

All experiments were carried out under N_2.

Cobaltic Oxidation of 4-Phenylbutanoic Acid. (a) In the Absence of $Cu(OAc)_2$. A solution of 5.7 g (0.035 mol) of 4-phenylbutanoic acid in a 0.66 M $Co(OAc)_3$ acetic acid solution (50 mL) was heated at reflux for 2 h. The solution was poured into water, acidified with HCl, and extracted by ether (2 × 50 mL). On the combined extracts an acid–base separation was carried out with a saturated solution of Na_2CO_3 and 10% HCl. The neutral fraction, after addition of methyl benzoate as the internal standard, was analyzed by GLC on a glass column (2 m) of 10% UCC W 982 on Chromosorb P (80–100 mesh) with a GLC "Hewlett Packard" Model 575. The yields of the lactone **2** and

(1) R. M. Dessau and E. I. Heiba, *J. Org. Chem.*, **40**, 3647 (1975).

(2) It was suggested by a referee that the detection of oxidative cleavage via the free-radical route from **1** path A indicated that this would be the exclusive pathway for phenylacetic acid oxidation.

(3) W. S. Trahanovsky, J. Cramer, and D. W. Brixin, *J. Am. Chem. Soc.*, **96**, 1077 (1974).

(4) A. Clerici, F. Minisci, and O. Porta, *Tetrahedron Lett.*, 4183 (1974).

(5) S. S. Lande, J. D. Salk, and J. K. Kochi, *J. Inorg. Nucl. Chem.*, **33**, 4101 (1971).

0022-3263/80/1945-0345$01.00/0