(III) Methyl 3β,7α,12α-trihydroxycholanate (14) crystallized as needles from ethyl acetate: mp 184.5–187.5 °C; identical according to TLC, high-pressure LC, and NMR with authentic 14 (below).

(IV) Methyl 3α,7α,12α-trihydroxycholanate (13), dense prisms from MeOH, was identical with authentic methyl cholate.

(13) 3β,7α,12β-Trihydroxycholanic acid (2a) obtained from ester 1 by the general hydrolysis procedure (above) crystallized from aqueous ethanol as colorless prisms: mp, 225–226 °C;[34] $[\alpha]_D$ +23.4° (EtOH); NMR (CD_3COCD_3–D_2O) δ 0.73 (3 H, s, C-18 Me), 0.95 (3 H, s, C-19 Me), 3.43 (1 H, br m, CHOH), 3.82 (1 H, m, CHOH), 3.97 (1 H, m, CHOH).

Anal. Calcd for $C_{24}H_{40}O_5 \cdot 0.5H_2O$: C, 69.51; H, 9.96. Found: C, 69.16; H, 10.04.

(14) Methyl 3β,7α,12α-trihydroxycholanate 3-formate (15), prepared by inversion of methyl cholate 3-tosylate[33] by reaction with DMF as in the preparations of the tosylates **10** and **11**, crystallized out of aqueous methanol: mp 157.0–158.5 °C (lit.[11] mp 110–112 °C); IR 1721 cm^{-1} (formate and COOMe); NMR δ 0.68 (3 H, s, C-18 Me), 0.93 (3 H, s, C-19 Me), 3.85 (1 H, m, CHOH), 3.96 (1 H, m, CHOH), 5.14 (1 H, m, CHOCHO), 8.03 (1 H, s, OCHO).

(15) Methyl 3β,7α,12α-trihydroxycholanate (14) was obtained by hydrolysis of the formate group of **15** either by treatment with MeOH–HCl or by contact with alumina as in the analogous preparations of **12** (section 7). The product was crystallized from ethyl acetate: mp 185.0–187.0 °C (lit.[10] mp 176 °C, from acetone); NMR δ 0.68 (3 H, s, C-18 Me), 0.91 (3 H, s, C-19 Me), 3.64 (3 H, s, OMe), 3.86 (1 H, m, CHOH), 4.01 (2 H, m, merging of 2 CHOH signals).

(16) 3β,7α,12α-Trihydroxycholanic acid (14a) was obtained from any of its derivatives **11**, **14**, or **15** by the general method of hydrolysis to the free acids described at the beginning of the Experimental Section and was crystallized out of aqueous EtOH as needles: mp 196.5–197.0 °C[34] (lit.[6] mp 200–202 °C from EtOAc).

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Registry No. 1, 71883-63-1; **1a**, 71883-64-2; **2**, 71883-65-3; **2a**, 71883-66-4; **3**, 10538-64-4; **4**, 71837-86-0; **4a**, 18069-63-1; **5**, 28535-81-1; **6**, 71837-87-1; **7**, 7432-44-2; **8**, 71837-88-2; **9**, 71837-89-3; **10**, 71837-90-6; **11**, 71837-91-7; **12**, 71837-92-8; **13**, 1448-36-8; **14**, 28050-54-6; **14a**, 3338-16-7; **15**, 42921-40-4; methyl 3α-hydroxycholanate, 1249-75-8; methyl 3β-hydroxycholanate, 5405-42-5; methyl 7α-hydroxycholanate, 28050-19-3; methyl 12α-hydroxycholanate, 1249-70-3; methyl 12β-hydroxycholanate, 28050-18-2; methyl 3α-tosyloxycholanate, 1261-92-3; methyl 3β-formyloxycholanate, 71837-93-9; methyl 7α-acetoxycholanate, 19684-60-7; methyl 12-oxocholanate, 1173-30-4.

Mass Spectra of Nitrate Esters of Cholic Acid Derivatives

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Steroidal nitrate esters preferentially eject HNO_3, HNO_2, and NO_2· over NO_3· and NO·. It is proposed that oxidative elimination of HNO_2 results in the formation of an oxo group. No direct ionization of the nitroxy group itself occurs in the mass spectrometer, but the observed ejections are triggered by movement of charge to the vicinity of the nitroxy group. The electron impact induced loss of HNO_3 and NO_2· from steroidal nitrates has a strong mechanistic similarity to the loss of HOAc and Ac·, respectively, from steroidal acetates, but the former losses usually produce less intense mass spectral peaks. An example of the facile ejection of NO_2· from a carbonium ion to regenerate an ion radical is provided.

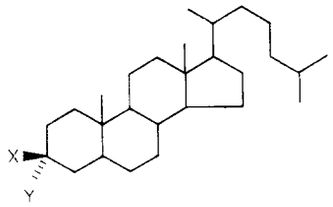
Virtually no definitive mass spectral summary of steroidal nitrates has yet been published. Observations, generalizations, and complexities associated with the mass spectra of steroid nitrate esters are therefore described in this paper. Since the highly electronegative nitrate functional group is expected to possess an ionization energy higher than that of any other functional group, except fluorine, one anticipates that the possible ejection of HNO_3, HNO_2, NO_3·, NO_2·, or NO· from a nitrate-containing molecule will result from ionization and subsequent alteration of the molecular region remote or adjacent to the nitrate group. Thus, the study of mass spectral breakdown of nitrate esters should uniquely provide further insight on fundamental principles which govern mass spectral fragmentation processes, e.g., charge localization, migration, and induction of fragmentations. In addition, intramolecular oxidation and the formation of radical ions from carbonium ions due to ejection of reasonably stable NO_2· or NO· radicals from even-electron ions are processes that need to be fully defined.

Results and Discussion

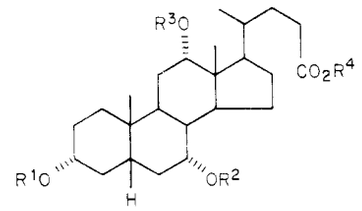
Except for the 12-eV spectra, the partial, monoisotopic mass spectra in Table I are cited in terms of relative percent of the most intense mass peak above m/z 200; the true base peak was usually a peak below m/z 200 with a relative intensity of approximately 2–7 times the cited 100% peak. Two problems complicate the interpretion of the mass spectra of steroid nitrates: (1) thermal decomposition of the sample on the direct-inlet probe, and (2) the low relative intensity of some of the characteristic peaks which could be indiscernible with prominent peaks that have their origin from impurities. Nevertheless, multiple mass spectral scans with increasing probe temperature and successive scans at ionization voltages of 70 and 12 eV gave fairly reproducible spectra except for the trinitrates; the propensity for decomposition of a steroidal nitrate increased with the degree of nitration of the molecule. Trinitrates **5c** and **6b** decomposed slowly enough that most of the characteristic peaks could be identified in the initial spectra taken in the first few scans at the lowest probe temperature giving significant ion current above background. However, trinitrates **6a** and **6c** decomposed more rapidly and only a few significant mass peaks persisted sufficiently in several of the initial successive spectral scans to be reliably reported in Table I. The mass spectra of nitrates **7a**, **7b**, **8a**, and **8c** have been reported[1] and are summarized again in Table I but

0022-3263/79/1944-4572$01.00/0

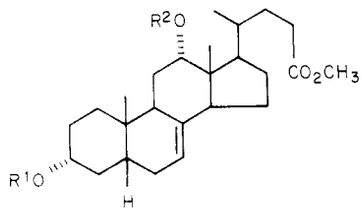
Chart I



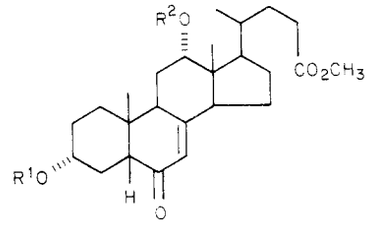
1a, X = ONO_2; Y = H
b, X = ONO_2; Y = D
c, X = D; Y = ONO_2
d, X = OAc; Y = H



2a, R^1 = NO_2; R^2 = H; R^3 = NO_2; R^4 = H
b, R^1 = NO_2; R^2 = H; R^3 = NO_2; R^4 = CH_3
c, R^1 = NO_2; R^2 = H; R^3 = H; R^4 = CH_3
d, R^1 = NO_2; R^2 = Ac; R^3 = NO_2; R^4 = CH_3
e, R^1 = Ac; R^2 = H; R^3 = Ac; R^4 = CH_3

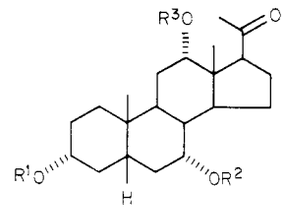


3a, R^1 = R^2 = NO_2
b, R^1 = R^2 = Ac

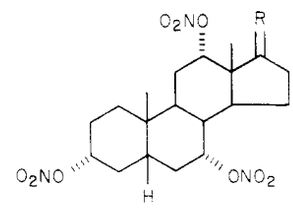


4a, R^1 = R^2 = NO_2
b, R^1 = R^2 = Ac

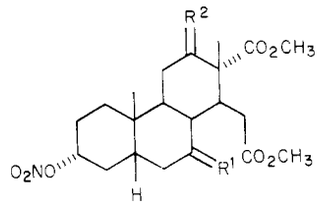
Chart II



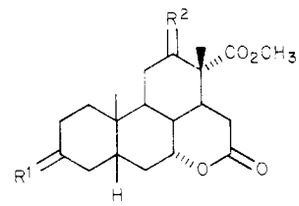
5a, R^1 = NO_2; R^2 = Ac; R^3 = Ac
b, R^1 = Ac; R^2 = Ac; R^3 = NO_2
c, R^1 = NO_2; R^2 = NO_2; R^3 = NO_2
d, R^1 = Ac; R^2 = Ac; R^3 = Ac



6a, R = α-H, β-OAc
b, R = O
c, R = α-H, β-OH



7a, R^1 = R^2 = β-H, α-OAc
b, R^1 = R^2 = O



8a, R^1 = β-H, α-ONO_2; R^2 = β-H, α-OAc
b, R^1 = β-H, α-ONO_2; R^2 = O
c, R^1 = O; R^2 = β-H, α-ONO_2
d, R^1 = β-H, α-OAc; R^2 = O
e, R^1 = O; R^2 = β-H, α-OAc

with more complete peak genesis assignment.

Loss of HNO_3 vs. HOAc. Comparison (Charts I and II) of mononitrates **1a**, **8b**, and **8c**, dinitrates **2b**, **3a**, and **4a**, and trinitrates **5c** and **6b** with monoacetates **1d**, **8d**, and **8e**, diacetates **2e**, **3b**, and **4b**, and triacetates **5d** and 3α,7α,12α-triacetoxy-5β-androstan-17-one,[2] respectively, demonstrates that fragmentation peaks emanating from the loss of HNO_3 are less pronounced than the fragmentation peaks emanating from the loss of HOAc. A particularly marked contrast is provided by the spectra of **1a** and **1d**.[3] In the spectrum of **1a** the base peak is that due to D-ring cleavage[4] (m/z 278 in Table I) and the $[M - HNO_3]^{+\cdot}$ peak is not perceptible, whereas in the spectrum of **1d**, the base peak is the $[M - HOAc]^{+\cdot}$ peak and the peak due to D-ring scission (m/z 215) is the next most intense peak. Since major loss of the nitroxy group, in one form or another, occurs only after D-ring cleavage, within the framework of charge participation,[5] one could argue that in the mass spectrometer incipient ionization of **1a** occurs at the D ring and after scission charge moves down to the region of the A ring where it triggers various fragmentations of the nitroxy group, such as m/z 232 in Table I. However, incipient ionization of **1d** occurs principally at the more easily ionized acetoxy group, and after ejection

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Table I. Partial Mass Spectra of Nitrate Esters of Cholic Acid Analogues[a]

compd	ionization voltage, eV	m/z (percent relative abundance, probable genesis)
1a	12	433 (49, $[M]^{+\cdot}$), 418 (16, $[M - CH_3]^+$)*, 386 (7, $[M - HNO_2]^{+\cdot}$), 355 (4, $[M - CH_3 - HNO_3]^+$), 330 (19, $[M - HNO_2 - C_3H_4O]^{+\cdot}$), 315 (7, $[M - HNO_2 - C_3H_4O - CH_3]^+$), 293 (18, $[M - C_{10}H_{20}]^{+\cdot}$)*, 279 (60, $[M - C_{11}H_{22}]^{+\cdot}$), 278 (100, $[M - C_{11}H_{23}]^{+\cdot}$)*, 264 (20, $[279 - CH_3]^+$)*, 232 (49, $[278 - NO_2]^{+\cdot}$)*, 217 (60, $[M - HNO_2 - C_3H_4O - C_8H_{17}]^+$), 210 (24)
1b	12	434 (51, $[M]^{+\cdot}$), 419 (17, $[M - CH_3]^+$)*, 386 (6, $[M - DNO_2]^{+\cdot}$), 356 (4, $[M - CH_3 - HNO_3]^+$), 330 (13, $[M - DNO_2 - C_3H_4O]^{+\cdot}$), 315 (7, $[M - DNO_2 - C_3H_4O - CH_3]^+$), 294 (20, $[M - C_{10}H_{20}]^{+\cdot}$)*, 280 (60, $[M - C_{11}H_{22}]^{+\cdot}$), 279 (100, $[M - C_{11}H_{23}]^+$)*, 265 (20, $[280 - CH_3]^+$)*, 233 (39, $[279 - NO_2]^{+\cdot}$)*, 217 (46, $[M - DNO_2 - C_3H_4O - C_8H_{17}]^+$), 211 (22)
2a	70	498 (0, MW), 435 (30, $[M - HNO_3]^{+\cdot}$), 417 (76, $[435 - H_2O]^{+\cdot}$)*, 388 (34, $[M - HNO_3 - HNO_2]^{+\cdot}$), 372 (40, $[M - 2HNO_3]^{+\cdot}$), 371 (73, $[M - HNO_3 - H_2O - NO_2]^+$), 370 (27, $[M - HNO_3 - H_2O - HNO_2]^{+\cdot}$), 355 (23, $[M - HNO_3 - H_2O - HNO_2 - CH_3]^+$), 354 (51, $[372 - H_2O]^{+\cdot}$)*, 353 (82, $[371 - H_2O]^+$)*, 339 (25, $[M - 2HNO_3 - H_2O - CH_3]^+$), 335 (20, $[M - HNO_3 - 3H_2O - NO_2]^+$), 316 (35, $[M - H_2O - HNO_3 - C_5H_9O_2]^+$), 271 (56, $[M - 2HNO_3 - C_5H_9O_2]^+$), 270 (25, $[M - HNO_3 - H_2O - NO_2 - C_5H_9O_2]^{+\cdot}$), 269 (33, $[M - HNO_3 - H_2O - HNO_2 - C_5H_9O_2]^+$), 253 (100, $[M - 2HNO_3 - C_5H_9O_2 - H_2O]^+$)
2b	70	512 (0, MW), 449 (2, $[M - HNO_3]^+$), 431 (63, $[M - HNO_3 - H_2O]^{+\cdot}$), 402 (22, $[C_{25}H_{38}O_4]^{+\cdot}$),[b] 386 (40, $[M - 2HNO_3]^{+\cdot}$), 385 (90, $[M - HNO_3 - H_2O - NO_2]^+$), 384 (39, $[M - HNO_3 - H_2O) - HNO_2]^{+\cdot}$), 369 (30, $[C_{25}H_{37}O_2]^+$ and $[C_{24}H_{33}O_3]^+$),[b] 368 (22, $[C_{25}H_{36}O_2]^{+\cdot}$),[b] 367 (85, $[C_{25}H_{35}O_2]^+$),[b] 353 (45, $[M - HNO_3 - H_2O - NO_2 - CH_3OH]^+$), 335 (32, $[M - HNO_3 - 2H_2O - NO_2 - CH_3OH]^+$), 316 (31, $[M - HNO_3 - H_2O - C_6H_{11}O_2]^+$), 270 (53, $[M - HNO_3 - H_2O - C_6H_{11}O_2 - NO_2]^{+\cdot}$), 269 (45, $[M - HNO_3 - H_2O - HNO_2 - C_6H_{11}O_2]^+$), 253 (59, $[M - 2HNO_3 - H_2O - C_6H_{11}O_2]^+$), 216 (100, $[M - HNO_3 - H_2O - C_6H_{11}O_2 - NO_2 - C_4H_6]^{+\cdot}$), 201 (75, $[M - HNO_3 - H_2O - C_6H_{11}O_2 - NO_2 - C_4H_6 - CH_3]^+$)
2c	70	467 (4, $[M]^{+\cdot}$), 449 (4, $[M - H_2O]^{+\cdot}$), 431 (7, $[M - 2H_2O]^{+\cdot}$), 402 (3, $[M - H_2O - HNO_2]^{+\cdot}$), 386 (17, $[M - H_2O - HNO_3]^{+\cdot}$), 385 (26, $[M - 2H_2O - NO_2]^+$), 368 (21, $[M - 2H_2O - HNO_3]^{+\cdot}$), 367 (11, $[385 - H_2O]^+$)*, 355 (11, $[M - H_2O - HNO_3 - CH_3O]^+$), 353 (15, $[M - 2H_2O - NO_2 - CH_3OH]^+$), 334 (11, $[M - H_2O - C_6H_{11}O_2]^+$), 316 (22, $[M - 2H_2O - C_6H_{11}O_2]^+$), 271 (22, $[M - H_2O - HNO_3 - C_6H_{11}O_2]^+$), 270 (13, $[M - 2H_2O - C_6H_{11}O_2 - NO_2]^{+\cdot}$), 269 (15, $[M - 2H_2O - C_6H_{11}O_2 - HNO_2]^+$), 253 (100, $[M - 2H_2O - HNO_3 - C_6H_{11}O]^+$)
	12	467 (2), 449 (2), 431 (12), 402 (8), 386 (45), 385 (40), 368 (47), 355 (6), 353 (12), 334 (12), 316 (12), 271 (30), 253 (100)
2d	70	554 (0, MW), 507 (6, $[M - HNO_2]^{+\cdot}$), 492 (13, $[M - HNO_2 - CH_3]^+$), 447 (19, $[M - HNO_2 - HOAc]^{+\cdot}$), 432 (25, $[M - HNO_2 - HOAc - CH_3]^+$), 431 (30, $[M - HOAc - HNO_3]^{+\cdot}$), 430 (30, $[M - HOAc - H_2O - NO_2]^+$), 385 (95, $[431 - NO_2]^+$)*, 384 (60, $[M - HNO_2 - HOAc - HNO_3]^{+\cdot}$), 369 (59, $[M - HOAc - HNO_3 - HNO_2 - CH_3]^+$), 367 (100, $[385 - H_2O]^+$)*, 335 (35, $[M - HNO_3 - HOAc - NO_2 - H_2O - CH_3OH]^+$), 316 (31, $[M - HOAc - HNO_3 - C_6H_{11}O_2]^+$), 269 (75, $[M - HOAc - HNO_3 - C_6H_{11}O_2 - HNO_2]^+$)
	12	554 (0), 507 (8), 492 (17), 447 (33), 432 (35), 431 (40), 430 (58), 385 (92), 367 (100), 335 (24), 316 (20), 269 (50)
3a	70	494 (1, $[M]^{+\cdot}$), 463 (1, $[M - CH_3O]^+$), 447 (2, $[M - HNO_2]^{+\cdot}$), 431 (3, $[M - HNO_3]^{+\cdot}$), 416 (2, $[M - CH_3O - HNO_2]^{+\cdot}$), 429 (3, $[M - HNO_2 - H_2O]^{+\cdot}$), 401 (9, $[M - HNO_2 - NO_2]^+$), 384 (100, $[M - HNO_3 - HNO_2]^{+\cdot}$), 368 (24, $[M - 2HNO_3]^{+\cdot}$), 366 (55, $[384 - H_2O]^{+\cdot}$)*, 335 (13, $[366 - CH_3O]^+$)*, 325 (18, $[M - HNO_3 - HNO_2 - CO_2CH_3]^+$), 269 (48, $[M - HNO_3 - HNO_2 - C_6H_{11}O_2]^+$), 259 (24, $[M - CH_3O - HNO_2 - C_7H_{11}NO_3]^+$), 253 (20, $[M - 2HNO_3 - C_6H_{11}O]^+$), 251 (32, $[269 - H_2O]^+$)*
4a	70	508 (1, $[M = C_{25}H_{36}N_2O_9]^{+\cdot}$),[b] 480 (2, $[M - CO]^{+\cdot}$), 462 (13, $[M - NO_2]^+$), 461 (3, $[M - HNO_2]^{+\cdot}$), 446 (10, $[C_{25}H_{36}NO_6]^+$),[b] 444 (15, $[462 - H_2O]^+$)*, 430 (4, $[M - NO_2 - CH_3OH]^+$), 416 (20, $[M - NO_2 - H_2O - CO]^+$), 399 (89, $[M - NO_2 - HNO_3]^+$), 398 (48, $[C_{25}H_{34}O_4]^{+\cdot}$),[b] 381 (100, $[399 - H_2O]^+$)*, 367 (21, $[M - NO_2 - HNO_3 - CH_3OH]^+$), 349 (22, $[M - NO_2 - H_2O - HNO_3 - CH_3OH]^+$), 283 (70, $[M - HNO_2 - HNO_3 - C_6H_{11}O_2]^+$), 267 (19, $[M - 2HNO_3 - C_6H_{11}O_2]^+$)
4b	70	502 (9, $[M]^{+\cdot}$), 471 (1, $[M - CH_3O]^+$), 460 (2, $[M - CH_2CO]^{+\cdot}$), 442 (62, $[M - HOAc]^{+\cdot}$), 429 (10, $[M - CH_2CO - CH_3O]^+$), 411 (7, $[M - HOAc - CH_3O]^+$), 400 (9, $[M - CH_2CO - HOAc]^{+\cdot}$), 382 (100, $[442 - HOAc]^{+\cdot}$)*, 369 (8, $[M - CH_2CO - CH_3O - HOAc]^+$), 367 (20, $[M - 2HOAc - CH_3]^+$), 354 (25, $[M - 2HOAc - CO]^{+\cdot}$), 327 (36, $[M - HOAc - C_6H_{11}O_2]^+$), 295 (9, $[M - 2HOAc - CO - CO_2CH_3]^+$), 287 (18, $[M - HOAc - C_9H_{15}O_2]^+$), 267 (100, $[327 - HOAc]^+$)*, 239 (27, $[M - 2HOAc - C_6H_{11}O_2 - CO]^+$)
	12	502 (6), 442 (100), 382 (81)*, 367 (3), 354 (5), 327 (8), 295 (1), 287 (3), 267 (6)
5a	70	479 (3, $[M]^{+\cdot}$), 436 (26, $[M - Ac]^+$), 419 (7, $[M - HOAc]^{+\cdot}$), 404 (2, $[M - HOAc - CH_3]^+$), 376 (11, $[M - Ac - HOAc]^+$), 359 (56, $[M - 2HOAc]^{+\cdot}$), 344 (4, $[M - 2HOAc - CH_3]^+$), 316 (22, $[359 - Ac]^+$)*, 313 (67, $[376 - HNO_3]^+$)*, 297 (26, $[M - 2HOAc - CH_3 - HNO_2]^+$), 295 (100, $[313 - H_2O]^+$)*, 281 (7, $[M - 2HOAc - CH_3 - HNO_3]^+$), 271 (15, $[M - Ac - HOAc - HNO_3 - CH_2CO]^+$), 253 (49, $[271 - H_2O]^+$)*
5b	70	479 (12, $[M]^{+\cdot}$), 433 (17, $[M - NO_2]^+$), 419 (9, $[M - HOAc]^{+\cdot}$), 404 (2, $[M - HOAc - CH_3]^+$), 373 (15, $[M - NO_2 - HOAc]^+$), 359 (87, $[M - 2HOAc]^{+\cdot}$), 344 (4, $[M - 2HOAc - CH_3]^+$), 356 (29, $[M - HOAc - HNO_3]^{+\cdot}$), 313 (40, $[M - NO_2 - 2HOAc]^+$), 297 (20, $[M - 2HOAc - CH_3 - HNO_2]^+$), 295 (71, $[M - NO_2 - 2HOAc - H_2O]^+$), 281 (42, $[M - 2HOAc - CH_3 - HNO_3]^+$), 271 (46, $[M - NO_2 - 2HOAc - CH_2CO]^+$), 253 (100, $[M - NO_2 - 2HOAc - CH_2CO - H_2O]^+$)
5c	70	485 (0, MW), 470 (7, $[M - CH_3]^+$), 439 (27, $[M - NO_2]^+$), 422 (20, $[M - HNO_3]^{+\cdot}$), 407 (4, $[M - CH_3 - HNO_3]^+$), 376 (12, $[M - NO_2 - HNO_3]^+$), 359 (24, $[M - 2HNO_3]^{+\cdot}$), 347 (48), 331 (89), 313 (90, $[M - 2HNO_3 - NO_2]^+$), 312 (59, $[M - 2HNO_3 - HNO_2]^{+\cdot}$), 297 (44, $[M - 2HNO_3 - HNO_2 - CH_3]^+$), 295 (100, $[M - 2HNO_3 - NO_2 - H_2O]^+$), 294 (90, $[M - 2HNO_3 - HNO_2 - H_2O]^{+\cdot}$), 279 (35, $[M - 2HNO_3 - HNO_2 - H_2O - CH_3]^+$), 269 (70, $[M - 2HNO_3 - HNO_2 - Ac]^+$), 253 (49, $[M - 3HNO_3 - Ac]$), 251 (89, $[M - 2HNO_3 - HNO_2 - Ac - H_2O]^+$)
6a	70	501 (1, $[M]^{+\cdot}$), 455 (16, $[M - NO_2]^+$), 227 (100, $[C_{16}H_{19}O]^+$)

Table I (*Continued*)

compd	ionization voltage, eV	*m/z* (percent relative abundance, probable genesis)
6b	70	457 (0, MW), 442 (40, $[M - CH_3]^+$), 429 (11, $[M - CO]^{+\cdot}$), 414 (11, $[M - CH_3 - CO]^+$), 366 (12, $[M - CO - HNO_3]^{+\cdot}$), 331 (24, $[M - 2HNO_3]^{+\cdot}$), 303 (50, $[M - CO - 2HNO_3]^{+\cdot}$), 285 (100, $[M - CO - 2HNO_3 - H_2O]^{+\cdot}$), 268 (60, $[M - 3HNO_3]^{+\cdot}$)
6c	70	459 (1, $[M]^{+\cdot}$), 413 (1, $[M - NO_2]^+$), 396 (1, $[M - HNO_3]^{+\cdot}$), 350 (6, $[M - HNO_3 - NO_2]^+$), 333 (36, $[M - 2HNO_3]^{+\cdot}$), 287 (60, $[M - 2HNO_3 - NO_2]^+$), 286 (48, $[M - 2HNO_3 - HNO_2]^{+\cdot}$), 271 (29, $[M - 2HNO_3 - HNO_2 - CH_3]^+$), 269 (100, $[M - 2HNO_3 - NO_2 - H_2O]^+$), 268 (63, $[M - 2HNO_3 - HNO_2 - H_2O]^{+\cdot}$)
7a	70	527 (4, $[M]^{+\cdot}$), 496 (3, $[M - CH_3O]^+$), 484 (7, $[M - Ac]^+$), 467 (9, $[M - HOAc]^{+\cdot}$), 452 (35, $[484 - CH_3OH]^+$)*, 425 (9, $[M - HOAc - CH_2CO]^{+\cdot}$), 407 (30, $[M - 2HOAc]^{+\cdot}$), 392 (21, $[M - Ac - CH_3OH - HOAc]^+$), 375 (22, $[M - 2HOAc - CH_3OH]^{+\cdot}$), 362 (14, $[M - HOAc - CH_2CO - HNO_3]^{+\cdot}$), 347 (28, $[M - 2HOAc - CH_3OH + CO]^{+\cdot}$), 344 (14, $[M - 2HOAc - HNO_3]^{+\cdot}$), 330 (35, $[M - HOAc - CH_3OH - CH_2CO - HNO_3]^{+\cdot}$), 329 (32, $[M - HOAc - CH_3OH - HNO_3 - Ac]^+$), 313 (32, $[C_{20}H_{25}O_3]^+$ and $[C_{19}H_{21}O_4]^+$),[b] 312 (28, $[M - 2HOAc - CH_3OH - HNO_3]^{+\cdot}$), 301 (43, $[M - HOAc - CH_3OH - HNO_3 - Ac - CO]^+$), 285 (100, $[M - 2HOAc - HNO_3 - CO_2CH_3]^+$), 284 (67, $[M - 2HOAc - HNO_3 - CH_3OH - CO]^{+\cdot}$), 241 (79, $[M - 2HOAc - CH_3OH - HNO_3 - Ac - CO]^+$), 225 (75, $[M - 2HOAc - HNO_3 - CH_3OH - CO - CO_2CH_3]^+$), 223 (77, $[M - 2HOAc - CH_3OH - HNO_2 - CO_2CH_3 - CO - H_2O]^+$)
7b	70	439 (19, $[M]^{+\cdot}$), 421 (9, $[M - H_2O]^{+\cdot}$)*, 408 (55, $[M - CH_3O]^+$), 407 (11, $[M - CH_3OH]^{+\cdot}$), 393 (15, $[M - NO_2]^+$), 392 (4, $[M - HNO_2]^{+\cdot}$), 380 (55, $[M - CO_2CH_3]^+$), 377 (23, $[M - HNO_2 - CH_3]^+$), 362 (65, $[M - H_2O - CO_2CH_3]^+$), 361 (26, $[M - NO_2 - CH_3OH]^+$), 348 (22, $[M - CO_2CH_3 - CH_3OH]^+$), 345 (37, $[M - HNO_2 - CH_3 - CH_3OH]^+$), 343 (40, $[M - NO_2 - CH_3OH - H_2O]^+$), 330 (100, $[M - H_2O - CH_3OH - CO_2CH_3]^+$), 315 (30, $[M - NO_2 - CH_3OH - H_2O - CO]^+$), 301 (40, $[C_{18}H_{21}O_4]^+$),[b] 283 (85, $[M - CO_2CH_3 - CH_3OH - HNO_2 - H_2O]^+$), 273 (40, $[M - CO_2CH_3 - CH_3OH - HNO_2 - CO]^+$), 255 (55, $[M - CO_2CH_3 - CH_3OH - HNO_2 - CO - H_2O]^+$)
8a	70	453 (4, $[M]^{+\cdot}$), 422 (6, $[M - CH_3O]^+$), 411 (100, $[M - CH_2CO]^{+\cdot}$), 393 (4, $[M - HOAc]^{+\cdot}$), 383 (6, $[M - CH_2CO - CO]^{+\cdot}$), 380 (11, $[M - CH_2CO - CH_3O]^+$), 352 (9, $[M - CH_2CO - CO_2CH_3]^+$), 351 (6, $[M - CH_2CO - CH_3OH - CO]^{+\cdot}$), 348 (12, $[M - CH_2CO - HNO_3]^{+\cdot}$), 347 (13, $[M - HOAc - NO_2]^+$), 330 (10, $[M - HOAc - HNO_3]^+$), 329 (15, $[M - HOAc - NO_2 - H_2O]^+$), 315 (19, $[M - HOAc - NO_2 - CH_3OH]^+$), 301 (22, $[M - HOAc - NO_2 - H_2O - CO]^+$), 287 (44, $[M - HOAc - NO_2 - CH_3OH - CO]^+$), 271 (84, $[M - HOAc - HNO_3 - CO_2CH_3]^+$), 270 (44, $[M - HOAc - HNO_3 - CH_3OH + CO]^{+\cdot}$), 269 (56, $[M - HOAc - NO_2 - CH_3OH - CO - H_2O]^+$)
8b	70	409 (2, $[M]^{+\cdot}$), 391 (6, $[M - H_2O]^{+\cdot}$), 378 (12, $[M - CH_3O]^+$), 363 (53, $[M - NO_2]^+$), 362 (16, $[M - HNO_2]^{+\cdot}$), 350 (33, $[M - CO_2CH_3]^+$), 346 (82, $[M - HNO_3]^{+\cdot}$), 345 (23, $[M - NO_2 - H_2O]^+$), 344 (19, $[M - HNO_2 - H_2O]^{+\cdot}$), 331 (21, $[M - HNO_3 - CH_3]^+$), 328 (12, $[M - HNO_3 - H_2O]^{+\cdot}$), 314(26, $[M - HNO_3 - CH_3OH]^{+\cdot}$), 287 (100, $[M - HNO_3 - CO_2CH_3]^+$), 286 (65, $[M - HNO_3 - CH_3OH - CO]^{+\cdot}$), 285 (76, $[M - NO_2 - H_2O - CH_3OH - CO]^+$)
8c	70	409 (16, $[M]^{+\cdot}$), 394 (11, $[M - CH_3]^+$), 376 (16, $[M - CH_3 - H_2O]^+$), 362 (9, $[M - HNO_2]^{+\cdot}$), 334 (13, $[M - HNO_2 - CO]^{+\cdot}$), 302 (100, $[M - HNO_2 - CO - CH_3OH]^{+\cdot}$), 287 (83, $[M - HNO_2 - CO - CH_3OH - CH_3]^+$)

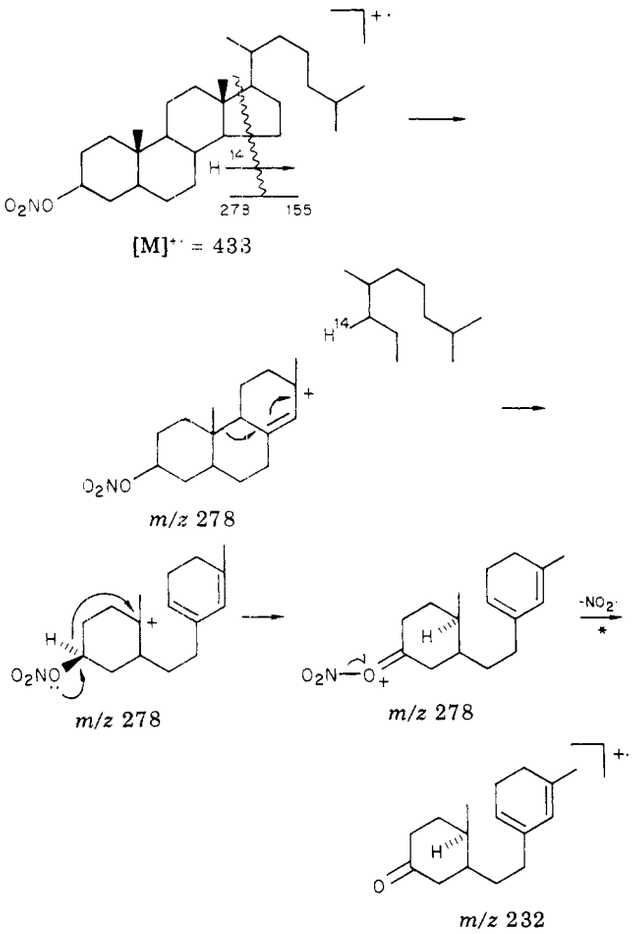
[a] An associated metastable peak was observed for each process marked with an asterisk. [b] The composition of this ion was determined by high-resolution measurement.

of HOAc, charge moves up from the A ring to the vicinity of the D ring. The mass spectra of **1b** and **1c** were indistinguishable and, thus, were without stereochemically induced spectral differences that would be expected from fragmentations generated by a directly ionized nitroxy functional group. Loss of HNO_3 from the molecular ion of mononitrates **8b** and **8c** coincides nicely with the observed loss of HOAc from the molecular ions of monoacetates **8d** and **8e**, respectively. The particularly facile elimination of HOAc from **8d** after electron impact compared to the same elimination in **8e** was attributed to ion–dipole interaction and/or product stability control,[1] and these rationalizations may be equally invoked here to explain the intense loss of HNO_3 from the molecular ion of **8b** but its imperceptible loss from the molecular ion of **8c**. Consecutive loss of two HNO_3 molecules from the molecular ion of dinitrates **2b**, **3a**, and **4a** is observed (Table I). Successive elimination of three HNO_3 molecules after electron impact is observed in the spectra of trinitrates **5c** and **6b**. Although consecutive loss of two and three HOAc molecules also occurs from the corresponding molecular ions of diacetates **2e**, **3b**, and **4b** and the triacetates (e.g., **5d**), significant differences between these nitrate and acetate mass spectra exist.

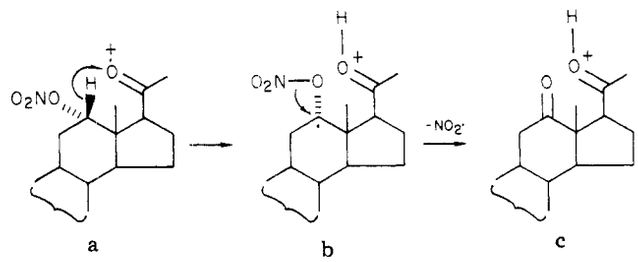
The mass spectra of steroid systems possessing both acetoxy and nitroxy functional groups (**2d**, **5a**, **5b**, **6a**, **7a**, and **8a**) are particularly illuminating in regard to HNO_3 loss. Previous work has established that in ionized cholic acid acetate derivatives the 12α-OAc group is lost as HOAc first from the molecular ion, the 7α-OAc group is lost second, and finally the 3α-OAc group is lost third.[2] In the mass spectrum of **2d** no $[M - HOAc]^{+\cdot}$ is observed but $[M - HNO_3 - HOAc]^{+\cdot}$ is observed suggesting that only after the imperceptible loss of the 12α-ONO_2 as HNO_3 from the molecular ion is the 7α-OAc group lost as HOAc. In the spectrum of **5a** the combined effects of less facile elimination of HNO_3 compared to HOAc and less facile elimination at the 3α-position vs. the 7α- and 12α-positions results in no perceptible loss of HNO_3 from $[M - 2HOAc]^{+\cdot}$, though, HNO_3 is lost from other fragment ions. No $[M - HNO_3]^{+\cdot}$ ion peak is observed in the spectrum of **5b** both because the 12α-ONO_2 group is lost as $NO_2\cdot$ (vide infra) and because the preference for incipient ionization in the vicinity of the D ring may be reduced compared to ionization of **5d**. The $[M - 2HOAc - HNO_3]^{+\cdot}$ and $[M - HOAc - HNO_3]^{+\cdot}$ ion peaks in the spectra of **7a** and **8a**, respectively, correlate well with what is expected in regard to the order of consecutive elimination. The order of consecutive loss of HNO_3 and H_2O in the spectra of **2b** and **2c** also conforms well to our earlier work.[2]

Loss of $NO_2\cdot$ vs. Ac·. Since nitrate esters can eject several different possible fragments (HNO_3, $NO_3\cdot$, HNO_2, $NO_2\cdot$, NO·), a goal in this work is to identify what structural features of a molecule predisposed it to preferentially

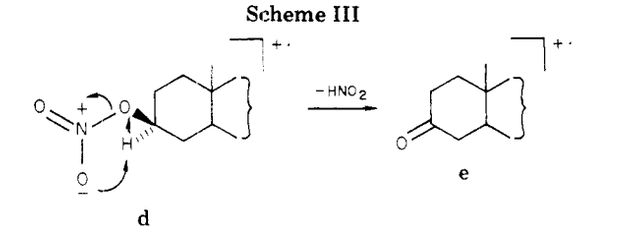
Scheme I



Scheme II



Scheme III



eject one kind of fragment over another. Direct scission of the O–NO_2 bond in nitrate esters or the O–Ac bond in acetate esters to form a two-electron-deficient, positively charged oxygen and a NO_2· or Ac· radical, respectively, is a high-energy process. However, the former involves cleavage of the weaker O–N bond to form the more stable cofragment, NO_2·. Thus, the facile loss of NO_2· from the even-electron carbonium ion system from the D-ring-cleavage product[4] in the spectra of **1a** and **1b** ($[278 - NO_2]^+$ and $[279 - NO_2]^+$)* to form an ion radical may well represent a general process uniquely associated with nitrate esters; an intense metastable peak was observed for this process, and Scheme I provides a plausible mechanism by which positive charge in the D ring moves to the vicinity of the A ring where it can trigger extrusion of NO_2· radical. Loss of NO_2· after D-ring cleavage is also observed in the spectra of **2a**, **2b**, and **2c** (m/z 316 to m/z 270). Ejection of NO_2· from the 12α-NO_3 group in the molecular ions of **5b**, **5c**, and **6a** presumably follows the same mechanistic pathway (a to c) established for the loss of Ac· from the corresponding acetate analogues (e.g., **5d**),[2] since no $[M - NO_2]^+$· peak was present in the spectrum of **5a** (Scheme II).

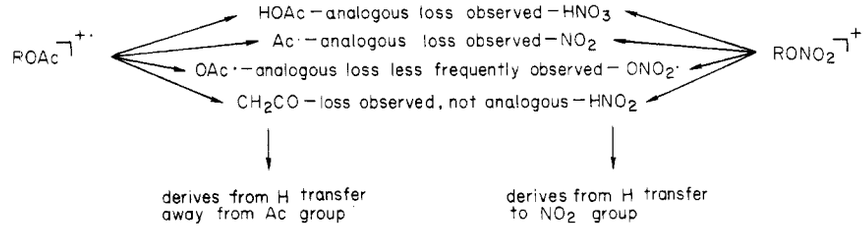
Loss of NO· and NO_3·. In all the mass spectra provided in Table I, it was not necessary to invoke loss of NO· or NO_3· to explain the origin of any fragment ion. Extrusion of NO· would require the transfer of oxygen from the nitrogen and would evidently involve such extensive rearrangement that it is not a prominent process. Although, some fragment ions explained by successive loss of HNO_2 and CH_3· could be equally explained by NO_3· loss, consideration of all the fragmentations presented in Table I suggests that the former combination is more prevalent. This agrees with the fact that NO_3· is the least stable oxide of nitrogen, and fracture of the C–O bond is more endothermic than fracture of the O–N bond in alkyl nitrates. In all our work,[1,2] we have never observed any significant fragmentation of the C–O alkoxy bond in acetate esters to give the AcO· radical. Both these simple scissions should be mechanistically similar, and, therefore, it must be concluded that these particular fragmentations are less frequent processes in the mass spectrometer.

Loss of HNO_2. Oxidative elimination of HNO_2 (the hydrocarbon moiety becomes oxidized) from nitrate esters has no analogue in the fragmentation of ionized aliphatic esters. The minor $[M - HNO_2]^+$· ion peak in the spectrum of **1a** becomes a $[M - DNO_2]^+$· ion peak (m/z 386) in the spectrum of **1b** and is consistent with the mechanism of Scheme III involving a five-membered ring.

Conclusion

Introduction of a functional group of higher ionization energy (e.g., NO_3 or F) into a molecule should raise the average energy ($E + I$, internal energy of the precursor molecule plus the ionization energy).[6] This will increase the probability of decomposition pathways associated with the hydrocarbon moiety (or associated with other functional groups present) for which the threshold value ($E_a + I$, energy of activation plus ionization energy) is not reduced by the presence of this electronegative group and

Scheme IV. Summary Comparison of Fragments Ejected from Acetate Esters vs. Nitrate Esters after Electron Impact



make the hydrocarbon moiety appear more reactive by comparison. Since D-ring cleavage was enhanced in the mass spectrum of **1a** relative to **1d**, it may well be more useful to study this cleavage process in fluoro- or nitroxy-substituted cholestanes rather than in unfunctionalized cholestane; this might permit the determination of the identity of the enhanced product ion which currently cannot be adduced by deuterium labeling[4] by enhancing subsequent daughter ions.

Since excitation energy is not localized in a carbonium ion, fragmentations are more likely to occur in the region where the positive charge is *localized* since it is this region where the critical energy for bond cleavage is lowest. Thus, if one is able to determine the movement of positive charge in a gas-phase carbonium ion, the succession of fragmentations can be predicted from the principles one has learned about carbonium ion solution chemistry. Vicinal charge triggered losses of HNO_3 and HOAc have parallel mechanisms but differ in ionization energies so that more HOAc elimination occurs via direct initial ionization of the corresponding acetate group.

Extrusion of $NO_2\cdot$ radical from nitrate esters is similar to extrusion of Ac· radical from acetate esters and is preceded by transfer of the hydrogen atom geminal with the alkoxy-containing C–O bond system to a remote electron-deficient site. The former is more facile since it also can occur from an even-electron carbonium ion system to regenerate an odd-electron ion radical system.

Oxidative elimination of HNO_2 involves transfer of the hydrogen atom geminal with the alkoxy-containing C–O bond system to the departing N–O system. Ejection of HNO_3, HNO_2, and $NO_2\cdot$ from the nitroxy function group is preferred to loss of $NO_3\cdot$ and NO· radicals. Scheme IV summarizes the fragments ejected from acetate esters vs. nitrate esters. Finally, these processes appear to be electron impact induced; however, thermal reactions on the sample probe cannot unequivocally be excluded.

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 and accelerating voltages of 4–6 kV. The inlet source temperature was 180 °C and the probe temperature ranged between 50 and 100 °C. Successive scans were taken as the temperature increased to verify spectral alterations resulting from decomposition. The dinitrates exhibited some darkening and the trinitrates exhibited significant darkening of the unvolatilized residue remaining in the quartz crucible after acquisition of the spectra. The deuterated nitrate esters **1b** and **1c** were synthesized by reducing 5α-cholestan-3-one with $NaBD_4$ and reacting the isolated deuterated analogues of 3α- and 3β-hydroxy-5α-cholestane with fuming HNO_3 in Ac_2O and $CHCl_3$. The syntheses of all the compounds have been described.[7,8,9]

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Registry No. **1a**, 71883-62-0; **1b**, 71837-72-4; **2a**, 64219-17-6; **2b**, 14942-96-2; **2c**, 71837-73-5; **2d**, 14942-97-3; **3a**, 64219-18-7; **4a**, 64219-19-8; **4b**, 64219-22-3; **5a**, 63533-75-5; **5b**, 63533-73-3; **5c**, 64934-52-7; **6a**, 71837-74-6; **6b**, 64934-54-9; **6c**, 65254-33-3; **7a**, 63533-79-9; **7b**, 63533-84-6; **8a**, 63533-91-5; **8b**, 63533-92-6; **8c**, 63533-97-1.

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Hexafluoro-2-propyl Esters in Peptide Synthesis[1a]

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The use of hexafluoro-2-propanol and its esters for peptide coupling reactions was investigated. Hexafluoro-2-propyl esters of N-protected amino acids and peptides may be prepared without racemization by carbodiimide-mediated coupling of the carboxyl component with hexafluoro-2-propanol (HFP). In peptide coupling reactions HFP esters are about 10^3 times less reactive than the corresponding *p*-nitrophenyl esters. In HFP, which is a powerful peptide solvent, more reactive acyl components are converted to HFP esters under base catalysis. Coupling in HFP is much slower than in dimethoxyethane or dimethylformamide. HFP esters are stable to conditions for removal of benzyloxycarbonyl protecting groups.

In the fragment condensation steps of a large peptide synthesis or in polymerization of peptides to make sequence polymers, solubility is often a problem. The dipolar aprotic solvents in common use, dimethylformamide (DMF) and dimethyl sulfoxide (Me_2SO), are difficult to purify and difficult to remove, and they do not always have sufficient solvent power. A different class of powerful peptide solvents are the fluorinated alcohols, particularly trifluoroethanol (TFE) and hexafluoro-2-propanol (HFP). In contrast to DMF and Me_2SO, they are low boiling (TFE, 74 °C; HFP, 59 °C) and weakly nucleophilic,[2] but like DMF and Me_2SO they are miscible with water and most organic solvents. They have been used in optical and magnetic resonance spectroscopic studies of peptides, but their reported use in synthesis has been limited. TFE has been employed as a solvent in acidolytic deblocking procedures[3,4] and as a cosolvent in the coupling step of sol-

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