Mass Spectra of Steroid Sapogenins

A. M. DAWIDAR and M. B. E. FAYEZ[x]

Abstract □ A study of the mass spectra of 15 natural and synthetic steroid sapogenins confirmed the general modes of fragmentation in the side chain and revealed additional features due to breakdown of ring D and to special structural and stereochemical characteristics.

Keyphrases □ Sapogenins, steroid—mass spectra and fragmentation patterns □ Mass spectrometry—fragmentation patterns of steroid sapogenins □ Steroid sapogenins—mass spectra and fragmentation patterns

This note reports examples of steroid sapogenins which behave under electron impact in accordance with the generalizations put forward by Budzikiewicz *et al.* (1). In addition, other breakdown pathways were identified, and their detection in compounds of unsettled nature may be diagnostically useful.

DISCUSSION

The commonly recognized (1) steroid sapogenin fragmentation products are the spiroketal ring F-containing ion species *a* and *b* (appearing at *m/e* 139 and 115, respectively, for the ring F-unsubstituted compounds, the first being the strongest ion in the spectra) and *c–h*, comprising an intact steroid nucleus. All of these species, conveniently explicable (1) as resulting from transition states involving cleavage of any of the C—C and C—O bonds attached to C-22, are well represented in the mass spectra of the compounds examined (I–XIII, Table I). However, the C-23 brominated derivatives XIV and XV show ion species *a* and *b*, with the appropriate mass shifts, only with very low intensities due to loss of the halogen content (partly as HBr) from the molecular ions as primary reactions. For the same reason, no evidence is available for ion species *f* and *h* in the spectrum of XIV and for *f*, *g*, and *h* in that of XV. The absence of species *h* in the spectrum of 23b-bromotigogenin acetate (XIV)—where the bromine atom is axially bound—is likely due to the unfavorable configuration of the C-23 hydrogen atom which would participate in the breakdown mechanism (1); a different situation may exist in 23ξ-bromodeoxytigogenin which shows ion *h* in a published (2) spectrum.

Another mode of cleavage is observed to have representation in nearly all spectra and may have general utility. This cleavage occurs by fission of the C-13/C-17 and C-14/C-15 bonds, with elimination of the entire spiroketal side chain together with ring D and an additional hydrogen atom, and is analogous to the principal type of fragmentation known (3) in sterols. The primary product of this breakdown is ion species *i*, which shows intact in some spectra but only as an artifact [resulting from loss of the C-3 substituent with or without subsequent expulsion of 54 mass units (butadiene) in a retro-Diels–Alder reaction, leading to ion *j*] in others (Table I).

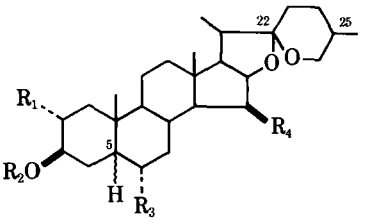
The spectra are complicated by presence of satellite ions resulting from the various species by losses of the nuclear substituents (as water or acetic acid). The loss of the C-3 substituent is frequently followed by collapse of ring A in a retro-Diels–Alder type of reaction, which is inhibited, obviously, where a double bond or additional hydroxy group(s) is present in or close to the ring. The latter type of breakdown of ring A is observed to take place in most ion species comprising the steroid nucleus arising from smilagenin acetate (III) and sarsasapogenin (IV) as opposed to tigogenin acetate (I) and neotigogenin acetate (II), a fact that may be associated with the specific stereochemistry at C-5.

The presence of additional functions remote from ring A appears to induce other special types of reactions (Scheme I). Thus, in addition to the expectable loss of CO from some ion species of hecogenin (X) and 9-dehydrohecogenin acetate (XI), the spectrum of the latter compound contains an appreciable ion at *m/e* 229, which may be the outcome of a γ-hydrogen transfer to the C-12 carbonyl group with loss of ring D to give ion *k*. Another peak (*m/e* 332) in the same spectrum may be due to an additional McLafferty hydrogen transfer with loss of the spiroketal side chain, giving structure *l* for the resulting ion. The spectrum of

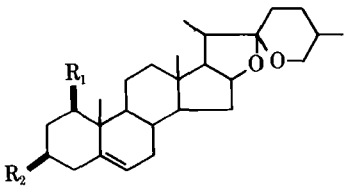
Table I—Mass Spectral Data

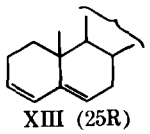
Compounds	Ion Species[a]									
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*
Tigogenin acetate (I)	139[1]	115	315[2]	344[2]	329[2]	386	389	399	—[2]	161
Neotigogenin acetate (II)	139[1]	115	315[2]	344	329[2]	386[2]	389	399	—[2]	161
Smilagenin acetate (III)	139[1]	115	315[2]	344[2,4]	329[2]	386[2,4]	389[2]	399[2,4]	—	161
Sarsasapogenin (IV)	139[1]	115	273[3,4]	302[3,4]	287[3,4]	344[3,4]	347[3,4]	357[3,4]	233[3]	161
Yamogenin acetate (V)	139	115	313	—[1,2]	—[2]	—[2]	—[2]	—[2]	—[2]	—
Gitogenin diacetate (VI)	139[1]	115	373[2]	402[2]	387[2]	444	447	457	—[2]	—
Chlorogenin diacetate (VII)	139[1]	115	373[2]	402[2]	387[2]	444	447	457[2]	—[2]	—
Ruscogenin diacetate (VIII)	139[1]	115	—[2]	—[2]	—[2]	—[2]	445	—[2]	—[2]	—
Digitogenin 2,3-diacetate (IX)	139[1]	115	389[2]	418[2]	403[2,3]	460[2]	463[2,3]	473	—	—
Hecogenin (X)	139[1]	115	287[3]	316[3,5]	301[3,5]	358[3]	361[3]	371[3]	247[3]	175
9-Dehydrohecogenin acetate (XI)	139[1]	115	327[2,4,5]	356[2]	341[2,4,5]	398[2]	401	411[5]	287[2,5]	173
3β-Chloro-25D-spirost-5-ene (XII)	139[1]	115	289[6]	318[6]	303[6]	360[6]	363[6]	373[6]	249	—
25D-Spirosta-3,5-diene (XIII)	139[1]	115	253	282[1]	267	324	327	337	213	—
23b-Bromotigogenin acetate (XIV)	218	—	315[2]	344	329[2,4]	—	389[2,4]	—	—	161
23,23-Dibromosarsasapogenin acetate (XV)	—	273	315[2]	344[2]	329	—	—	—	—	161

[a] Additional information pertaining to observed fragment ions in the various spectra. [1] Base peak (100% intensity ion). Satellite ions resulting from indicated ion species by losses of: [2] acetic acid, [3]water, [4]butadiene (54 mass units) in addition to loss of acetic acid or water, [5]carbon monoxide, and [6]hydrogen chloride.



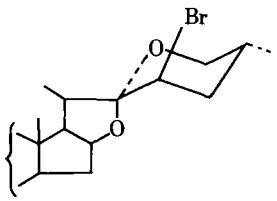
I: $R_1 = R_3 = R_4 = H$, $R_2 = COCH_3$ (5α, 25R)
II: $R_1 = R_3 = R_4 = H$, $R_2 = COCH_3$ (5α, 25S)
III: $R_1 = R_3 = R_4 = H$, $R_2 = COCH_3$ (5β, 25R)
IV: $R_1 = R_2 = R_3 = R_4 = H$ (5β, 25S)
VI: $R_1 = OCOCH_3$, $R_2 = COCH_3$, $R_3 = R_4 = H$ (5α, 25R)
VII: $R_1 = R_4 = H$, $R_2 = COCH_3$, $R_3 = OCOCH_3$ (5α, 25R)
IX: $R_1 = OCOCH_3$, $R_2 = COCH_3$, $R_3 = H$, $R_4 = OH$ (5α, 25R)



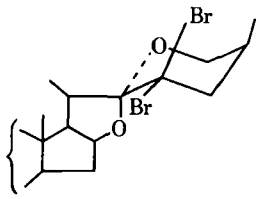


XIII (25R)

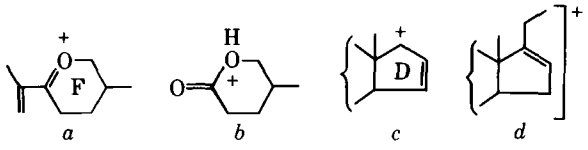
V: $R_1 = H$, $R_2 = OCOCH_3$ (25S)
VIII: $R_1 = R_2 = OCOCH_3$ (25R)
XII: R_1 = H, R_2 = Cl (25R)



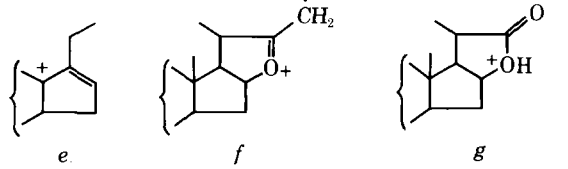
XIV (3β-$OCOCH_3$; 5α-H)



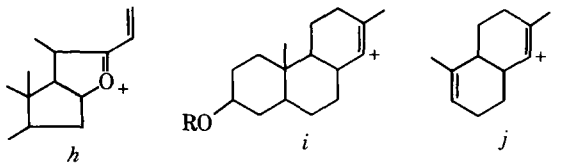
XV (3β-$OCOCH_3$; 5β-H)



a b c d

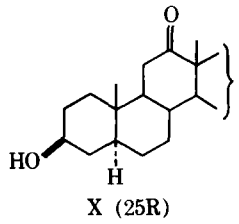


e f g

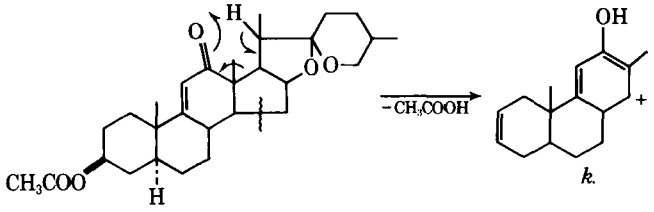


h i j

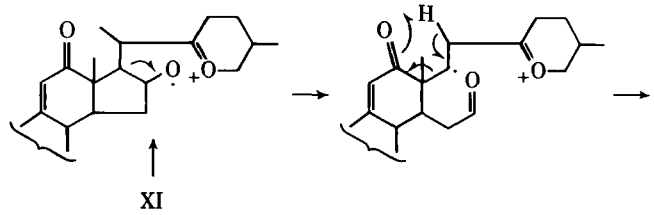
digitogenin 2,3-diacetate (IX) contains two prominent ions at m/e 126 and 168 which, presumably, comprise the spiroketal side chain and result from unusual modes of fragmentation. In the absence of more direct evidence from labeling experiments, it may be assumed that the first ion results by cleavage across ring E, leading to structure *m*, and the second by cleavage across ring D, giving *n*. Although the C-15 hydroxyl group is not directly involved according to the proposed mechanisms, the presence of this function seems to be the sole factor leading to these ion species.



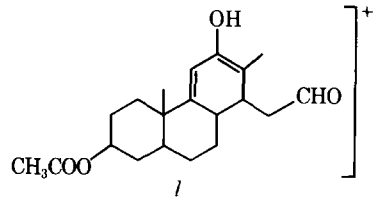
X (25R)



XI



XI



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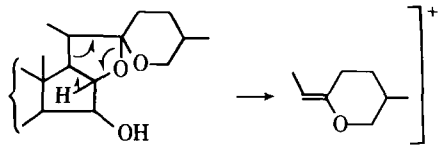
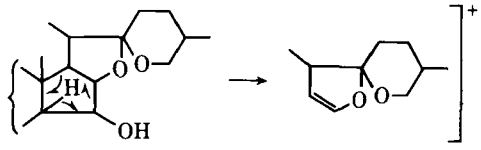


IX *m*



IX *n*

Scheme I

EXPERIMENTAL[1]

All compounds used in this study are known and were obtained by isolation from natural sources, by synthesis using published methods, or as gifts. Melting points are uncorrected. Optical rotations were measured in chloroform solution. Purity of the samples used in the mass spectrometric study was ascertained by TLC using the conditions given.

TLC Conditions—The following four solvent systems were used: A, adsorbent of silver nitrate-impregnated alumina, solvent of cyclohexane–ethyl acetate (10:1); B, adsorbent of alumina G, solvent of *n*-hexane–ethanol (10:1); C, adsorbent of silica gel G, solvent of *n*-hexane–ethyl acetate (20:1); and D, adsorbent of silica gel G, solvent of cyclohexane–ethyl acetate–isopropanol (20:1:2). The spray reagents used were chlorosulfonic acid–acetic acid (1:3) and *p*-anisaldehyde–acetic acid–sulfuric acid (1:100:2).

TLC Data—*Compound I*—mp 204–206° [lit. (4) mp 204°], $[\alpha]_D$

[1] The mass spectra were measured on an MS-9 spectrometer, using an electron energy of 70 ev at a temperature of 140–150°.

−71.6° [lit. (4) $[\alpha]_D$ −73°], R_f 0.62 (A), isolated from *Trigonella foenum-graecum* L. seeds (5).

Compound II—mp 173–175° [lit. (6) mp 172–174°], $[\alpha]_D$ −77.8° [lit. (6) $[\alpha]_D$ −73.4°], R_f 0.60 (A), isolated from *Agave filifera* var. *compacta* L. leaves (7).

Compound III—mp 149–151° [lit. (4) mp 150°], $[\alpha]_D$ −62.6° [lit. (4) $[\alpha]_D$ −60°], R_f 0.55 (A), isolated from *Agave ghiesbrechtii* Koch. leaves (7).

Compound IV—mp 201–204° [lit. (4) mp 199–200°], $[\alpha]_D$ −76° [lit. (4) $[\alpha]_D$ −78°], R_f 0.80 (B), isolated from *Yucca filamentosa* L. leaves (8).

Compound V—mp 184–186° [lit. (9) mp 182°], $[\alpha]_D$ −118° [lit. (9) $[\alpha]_D$ −113°], R_f 0.40 (A).

Compound VI—mp 233–237° [lit. (10) mp 237–242°], $[\alpha]_D$ −91.7° [lit. (10) $[\alpha]_D$ −98°], R_f 0.57 (A), isolated from *Trigonella foenum-graecum* L. seeds (5).

Compound VII—mp 155–157° [lit. (11) mp 155°], $[\alpha]_D$ −38° [lit. (11) $[\alpha]_D$ −38°], R_f 0.40 (A), isolated from *Furcraea gigantea* Vent. leaves (12).

Compound VIII—mp 190–193° [lit. (13) mp 192–194°], $[\alpha]_D$ −80.6° [lit. (13) $[\alpha]_D$ −85°], R_f 0.25 (A), isolated from *Ruscus alexandrinus* cladophylls (14).

Compound IX—mp 240–242° [lit. (15) 241.5–242°], $[\alpha]_D$ −101° [lit. (15) $[\alpha]_D$ −104°], R_f 0.64 (B).

Compound X—mp 257–259° [lit. (4) mp 254–258°], $[\alpha]_D$ +12° [lit. (4) $[\alpha]_D$ +10°], R_f 0.59 (B), isolated from *Yucca aloifolia* L. leaves (8).

Compound XI—mp 226–228° [lit. (4) mp 227°], $[\alpha]_D$ −9.5° [lit. (4) $[\alpha]_D$ −7°], R_f 0.08 (A).

Compound XII—mp 208–211° [lit. (16) mp 213°], $[\alpha]_D$ −109° [lit. (16) $[\alpha]_D$ −101°], R_f 0.61 (C), obtained as an artifact from *Balanites aegyptiaca* L. leaves (17).

Compound XIII—mp 162–163° [lit. (18) mp 164°], $[\alpha]_D$ −179° [lit. (18) $[\alpha]_D$ −175°], R_f 0.72 (C), obtained as an artifact from *Trigonella foenum-graecum* L. seeds (5).

Compound XIV—mp 225–228° [lit. (19) mp 220–230°], $[\alpha]_D$ −78° [lit. (19) $[\alpha]_D$ −81°], R_f 0.78 (D), obtained by synthesis (19).

Compound XV—mp 238–240° [lit. (20) mp 242–244°[, $[\alpha]_D$ −68.3° [lit. (20) $[\alpha]_D$ −73.1°], R_f 0.78 (D), obtained by synthesis (20).

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[x] To whom inquiries should be directed. Present address: College of Pharmacy, Howard University, Washington, DC 20001