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Mass spectrometry as an aid to the identification of ergots and dihydroergots: comparison of hard and soft ionization techniques

ALAN F. CASY*

School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, UK

Abstract: An analysis of the 70 eV electron impact (EI) and fast atom bombardment (FAB) mass spectral features of a variety of ergoline and dihydroergoline derivatives of therapeutic importance is presented with emphasis upon analytical utility. Derivatives which carry non-peptide based C-8 substituents are fully characterized by EI-MS through provision of molecular weight evidence and fragment ions diagnostic of both the ergoline skeleton and the C-8 substituent. Peptidic ergolines and dihydroergolines are poorly characterized by EI-MS, but their FAB-MS clearly reveal $[M + 1]^+$ (high intensity) and $[M - 1]^-$ (high to low intensity) ions in positive and negative ion spectra, respectively. Negative FAB spectra of salts also display diagnostic anion–base conjugate ions.

Keywords: *Mass spectrometry (MS); electron impact (EI); fast atom bombardment (FAB); ergots; dihydroergots.*

Introduction

Mass spectrometry, like NMR spectroscopy, is a technique well-suited to the identification of members of closely related groups of compounds, and has the advantage of economy in analyte size. Ergoline (ergot) derivatives represent such a group, and the value of ^{1}H-NMR spectroscopy as an aid to characterizing individual members has been presented [1]. The availability of electron impact (EI) [13] and fast atom bombardment (FAB) (this report) MS data has prompted the present evaluation of mass spectrometry in the same regard. Several reports of the MS features of ergots have been made over the past 20 or so years (chiefly in reference to EI spectra) [2–13], but no survey of the overall potential of MS in the analysis of ergots of pharmaceutical importance has been presented.

Materials and Methods

Samples of bromocriptine mesylate, dihydroergotamine mesylate, ergotamine tartrate, methysergide maleate and ergometrine maleate were supplied by Sandoz Pharmaceuticals, and pergolide mesylate by Lilly Research Laboratories. The Home Office Forensic Science Service provided samples of dihydroergocornine, α- and β-dihydroergocryptine and dihydroergocristine mesylates. Ergocryptine and ergocristine bases were purchased from Sigma.

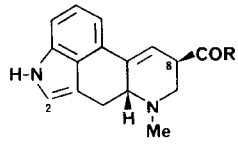
Positive and negative FAB mass spectra were obtained using a 7070E VG Analytical instrument. Mixtures of analyte and glycerol were examined by standard procedures and original spectra were corrected by subtraction of ions due to matrix ions [14]. The chemical ionization (CI) spectrum was obtained with isobutane as the reactant gas.

Results and Discussion

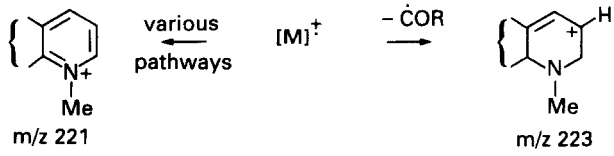
70 eV EI data

The 1985 publication *Pharmaceutical Mass Spectra* [13] contains a large number of mass spectrograms of ergot derivatives and this compilation has provided most of the EI data for this analysis. For identification purposes, an ideal mass spectrogram is one which provides both molecular weight and characteristic fragmental ion evidence. Such is the case only for those of the ergolines **1a–f** with simple (non-peptidic) substituents at C-8. In addition to prominent $[M]^+$ ions (base peaks except for ergometrine, see legend for **1**), mass spectra of these derivatives display well-defined (intensities mostly above 20%) *m/z* lines character-

*Correspondence to 5 Piplar Ground, Bradford-on-Avon BA15 1XF, UK.



			$[M]^+$
(a)	lysergic acid	R = OH	268 (100)
(b)	lysergamide	R = NH_2	267 (100)
(c)	lysergide (LSD)	R = NEt_2	323 (100)
(d)	ergometrine	R = $NHCH(Me)CH_2OH$	325 (88)
(e)	methylergometrine	R = $NHCH(Et)CH_2OH$	339 (100)
(f)	methysergide	R = $NHCH(Et)CH_2OH$ 1-Me(NMe)	353 (100)



Scheme 1

Table 1
Percentage abundance of fragment ions generated by 70 eV electron impact common to non-peptidic ergots **1a–f***

Ion (*m/z*)†	**1a** 8-CO_2H	**1b** 8-$CONH_2$	**1c** 8-$CONEt_2$	**1d** Ergometrine	**1e** Me-ergometrine	**1f** Methysergide‡
223	30 (43)	28	33	41	44	43
221	26 (41)	47	73	100	77	94
207	34 (41)§	40	38	41	46	48
196‖	6 (5)	25	22	53	46	97
192	30 (49)	15	12	24	21	27
191	9 (10)	5	5	6	8	17
181	19 (20)	20	48	41	46	51
180	43 (51)	37	19	29	24	26
167	19 (26)	15	13	18	19	23
154	49 (55)	27	12	24	23	27
127	17 (20)	12	4	12	9	—

* Data from spectra compilation of ref. 13, data for **1a** in parentheses from ref. 9.
† See Scheme 1 and ref. 9 for structures of most of these ions.
‡ Corresponding ion + 14 (**1f** carries Me at N-1).
§ Two contributors.
‖ *m/z* 223 − 27 (HCN)?

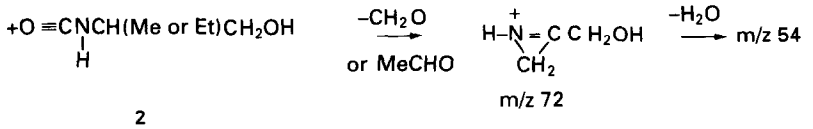
istic of the ergoline skeleton and common to all, which arise as a result of loss of the C-8 substituent from the molecular ion and subsequent fragmentations (Scheme 1).

Full analysis of the 70 eV EI spectrum of one of these derivatives, namely lysergic acid (**1a**), has been reported [9] (proposed fragment ion structures supported by metastable ion evidence) and the results of this work may be applied to data on **1a–f** reported in *Pharmaceutical Mass Spectra*. Mass spectral lines diagnostic of the ergoline skeleton for these ergots are listed in Table 1.

Ions of *m/z* value 196, prominent in all spectra except that of lysergic acid reported in ref. 9, may arise through loss of HCN (27) from the ion *m/z* 223. Apart from the prominent molecular ions, those diagnostic of the C-8 substituent in spectra of **1a–f** are sparse or absent. Spectra of ergometrine (**1d**), methylergometrine (**1e**) and methysergide (**1f**) all display lines due to $[M]^+$-H_2O ions of intensities 24, 40 and 11%, respectively. The low value ions *m/z* 72 and 54, prominent in all these spectra, distinguish but do not differentiate this trio; such ions probably arise through loss of an aldehyde from the ion **2** followed by elimination of water.

Peptidic examples

70 eV EI data [13] on the ergolines **3a–f** which carry peptide-based C-8 substituents are relatively uninformative as aids to identification.



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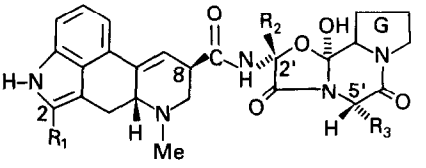
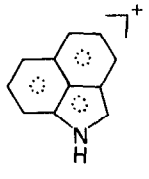


		R_1	R_2	R_3	MWt
(a)	ergotamine	H	Me	CH_2Ph	581
(b)	ergocristine	H	$CHMe_2$	CH_2Ph	609
(c)	ergocryptine (α)*	H	$CHMe_2$	CH_2CHMe_2	575
(d)	bromocriptine	Br	$CHMe_2$	CH_2CHMe_2	653, 655
(e)	ergocornine	H	$CHMe_2$	$CHMe_2$	561
(f)	ergosine	H	Me	CH_2CHMe_2	547

β-isomer: R_3 = $CH(Me)CH_2Me$

Molecular ions are absent while ions diagnostic of the molecular framework as described above for non-peptidic examples (Table 1), are generally of low intensity (<20%), a result, most likely, of fragmentations being dominated by features of the peptidic substituent. The most prominent ion of this series is *m/z* 154 (**4**) [9]; intensities are: **3a** (3) (*m/z* 153 14), **3b** (41), **3c** (51), **3d** (68) (2-Br replaced by H), **3e** (41) and **3f** (48).



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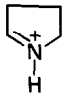

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The base peak and other high intensity ions provide evidence of the 2′- and 5′-substituents. Spectra of ergolines with 5′-benzyl substituents display prominent *m/z* 91 ions: **3a** (49), **3b** (40). Those of derivatives with isopropyl features ($CHMe_2$ and CH_2CHMe_2) display strong lines due to *m/z* 43 (often the base peak) and *m/z* 41 ions, while *m/z* 55/57 lines are seen in addition in spectra of certain 5′-CH_2CHMe_2 derivatives; **3c** (19, 20), **3d** (9, 2), **3f** (41, 70). All spectra of this group show a prominent *m/z* 70 line (often the base peak) which may be attributed to an ion formed from the pyrrolidino ring G (**5**) portion of the C-8 substituent [6]. The prominent *m/z* ion 125 ions in spectra of ergotamine **3a** (100) and ergocristine **3b** (58) are unassigned (present but of low intensity, ~10, in spectra **3c–f**).

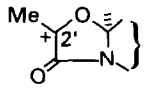
Although EI mass spectral data on the peptidic ergots **3** provide clues to structure, definitive identification of a member of the group requires MWt evidence. Information of this kind is fortunately provided from spectra run under conditions of soft ionization using the fast atom bombardment (FAB) technique. In FAB spectra of **3a–d** lines due to $[M + 1]^+$ and $[M - 1]^-$ ions were prominent (often the base peak) in positive and negative ion spectra respectively: **3a** 582 (100), 580 (~30); **3b** 610 (100), 608 (100); **3c** 576 (100), 574 (20); **3d** 654/656 (~30), 652/654 (~20). In the case of **3d**'s spectrum, the approximately 1:1 doublet nature of these lines revealed the presence of bromine isotopes.

Ions characteristic of the ergoline nucleus, as observed in EI spectra, were present in positive ion FAB spectra (although not especially prominent in that of **3c**), together with those produced by loss of water from $[M + 1]^+$ ions (Table 2). Negative ion FAB spectra displayed far fewer lines than positive spectra; those of salts registered the appropriate anion (**3a** tartrate *m/z* 149, **3d** mesylate 95, **1d** maleate 115, base peak for **3a** and **3d**, 35% for **1d**) together with diagnostically valuable ergoline–anion conjugate ions: **1d** *m/z* 468 (4), **3d** 748, 750 (~10, 10). A chemical ionization MS of ergotamine tartrate **3a** showed the ion *m/z* 315 as base peak, assigned as shown (**6**). This

Table 2
Percentage abundance* of positive fragment ions generated by FAB common to peptidic ergots **3a–d**

Ion (*m/z*)†	**3a** Ergotamine	**3b** Ergocristine	**3c** Ergocryptine	**3d** Bromocriptine
$[M + 1]^+ - H_2O$	15 (564)‡	30 (592)	20 (228)	10 (636) 10 (638)§
268	25	60	35	<5
223	45	80	abs:224 (30)	85
221	35	70	abs:222 (40)	70
208	55	85	35:221 (95)	100
207	35	60	20	100
196‖	15	25	25	20
192	25	30	25	60
180	25	30	35	55
168	25	35	abs	40
167	15	abs	abs	55
154	35	50	abs	50
127	5	abs	10	15
120¶	35	45	abs	5
98¶	15	30	20	40

*To nearest multiple of 5.
†As footnote † of Table 1.
‡*m/z* value of $[M + 1]^+ - H_2O$ ion.
§*m/z* 576 (40%) $[M + 1]^+ - Br + H$ ion prominent.
‖*m/z* 223 − 27 (HCN)?
¶Unassigned structure.



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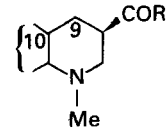

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ion and corresponding ions of other ergot derivatives were not prominent in FAB spectra.

FAB spectra of the non-peptidic ergot methysergide **1f** likewise displayed lines due to $[M + 1]^+$ (*m/z* 354, 100%) and $[M - 1]^-$ (*m/z* 352, 6%) ions but were otherwise less informative than the EI spectra.

Dihydroergolines

Fragment ions of the EI-MS spectra of 9,10-dihydrolysergic acid have decreased abundances compared with the spectrum of lysergic acid, and the structures of several of these (which lack the C-8 substituent) have been proposed on the basis of metastable ion evidence [9]. The non-peptidic dihydroergoline pergolide **7** behaved similarly under electron impact. Its EI spectrum displayed a $[M]^+$ base peak and several characteristic lines common to that of dihydrolysergic acid: *m/z* 223 (~5), 167 (~10), 154 (~20), 127 (~5). The most

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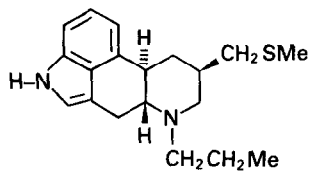


prominent fragment ion was *m/z* 285 (40) (loss of Et· from Pr^n of N-6) while ions formed by loss of Me· (*m/z* 299, 5%) and ·SMe (*m/z* 267, 10%) were also seen. EI Spectra of the peptidic dihydroergolines **8a**, **b**, **c** and **e**, like those of corresponding ergots, lacked molecular ions. (The structure shown for **8** is a partial structure, legend and details of R as for **3**.) Certain fragment ions of prominent intensities were common to all, but these (with some exceptions), did not aid differentiation of individual members (Table 3).

Some ions gave evidence of C-8 substituent structure, e.g. *m/z* 91: **8a** (65), **8b** (57) − 25% however for **8e** (a non-5′-benzyl derivative). Spectra of 5′-benzyl derivatives also displayed prominent ions at *m/z* 125: **8a** (96), **8b** (100) of unknown structure, seen likewise in spectra of the ergoline parents. In contrast, members of the same peptidic group were defined by prominent $[M + 1]^+$ ions in their FAB spectra: **8a** (100), **8b** (65), **8c** (α, 75; β, 70), **8e** (90); these ions were accompanied by corresponding $[M + 1]^+ - H_2O$ ions of intensities 5–10%. Ions characteristic of the dihydroergoline nucleus and certain features of the C-8 sub-

Table 3
Percentage abundance of fragment ions of FAB and 70 eV EI-MS of the peptide dihydroergolines **8a**, **b**, **c** and **e***

	8a Dihydroergotamine		**8b** Dihydroergocristine		**8c** Dihydroergocryptine¶		**8e** Dihydroergocornine	
Ion (*m/z*)†	FAB	EI	FAB	EI	FAB	EI	FAB	EI
$[M + 1]^+ - H_2O$	5 (566)‡	—	10 (594)	—	10 (560)	—	10 (546)	—
270	90	5	100	abs	95	2	100	3
269	20	18	10	1	10	4	10	64
253§	80	—	70	2	65	3	50	1
225	60	6	60	9	50	20	40	21
223	30	9	30	9	30	21	20	18
209	20	3	15	1	20	4	15	10
182‖	35	4	30	1	25	6	20	8
168	50	5	50	2	45	11	35	11
167	45	11	50	6	45	25	40	21
154	75	22	80	14	60	100	50	57
153	10	55	15	48	10	17	10	14
144	20	11	5	2	15	7	10	19
125§	10	96	5	100	5	10	5	11
120§	60	7	40	7	5	abs	5	abs
91**	40	65	25	57	5	abs	5	25
72§	††	abs	25	abs	25	abs	50	12
70‡‡	††	100	95	88	100	34	85	100

*EI Data from spectral compilation of ref. 13, FAB data from spectra of this report. Percentage abundance for FAB data to nearest multiple of 5.

†Several are +2 analogues of ions seen in MS of corresponding ergot derivatives (Table 2), e.g. ions *m/z* 223, 225 and 209; others correspond with fragment ions of dihydrolyservic acid [9].

‡*m/z* value of $[M + 1]^+ - H_2O$ ion.

§Unassigned structure.

‖*m/z* 223 − 41 (MeCN)?

¶FAB spectra of **8c** (α) and that of its β-isomer (5′-$CHMeCH_2Me$) were indistinguishable. However, their ^{1}H-NMR features in DMSO-d_6 allow ready identification (270 MHz spectra; ppm from TMS; approx. ^3J in parentheses; d, doublet; t, triplet) α: 5′-H t 4.32 (6.6), Me signals (2′-$CHMe_2$, 5′-CH_2CHMe_2) d, 1.08 (6.6), 0.95 (6.6), 0.90 (6.6), 0.85 (6.6); β: 5′-H d 4.35 (2.2), Me signals (2′-$CHMe_2$, 5′-$CH(Me)CH_2Me$) d, 1.09 (6.6), 0.94 (6.6), 0.93 (6.6), t, 0.86 (7.3).

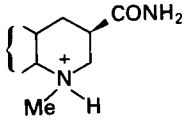
Prominent line in MS of 5′-benzyl derivatives **8a and **8b**.

††No data below *m/z* 80.

‡‡Ring D characteristic, see **5**.

stituent were also clearly seen (Table 3). The intensities of ions present in both FAB and EI spectra often varied considerably. Thus the ion *m/z* 125 of high intensity in EI spectra of **8a** and **8b** (see above) had intensities 10 and 5%, respectively, in corresponding FAB spectra (true also for spectra of the parent ergots **3a** and **3b**). In contrast, FAB ionization conditions favour generation and stability of the ion *m/z* 270 (base peak or close to 100% in all dihydroergot spectra), an ion of low intensity (5% or less) in corresponding EI spectra. A possible structure of this ion is **9**, formed by protonation of amide nitrogen followed by separation of the peptidic fragment (as cation) and protonation of the ergot residue at N-6.

In related negative ion FAB spectra,



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$[M - 1]^-$ ions were of low intensity (1–2.5%) but well defined nevertheless. Such spectra, all of mesylate salts, showed a base peak at *m/z* 95 ($MeSO_3^-$ anion) and distinct low intensity (1–2.5%) ions due to dihydroergoline–mesylate conjugates.

Summary

70 eV EI MS of the ergoline derivatives **1a–f** and the dihydroergoline **7**, which carry non-peptide-based C-8 substituents, fully characterize individual members through provision of molecular weight evidence and fragment ions diagnostic of both the ergoline skeleton and the C-8 substituent. In contrast, molecular ions are absent in EI-MS of the peptidic ergolines **3a–f** and dihydro analogues **8a**, **b**, **c** and **e**, and individual members are difficult to differentiate (apart from evidence of the 2′- and 5′-substituents in certain cases). However, FAB-MS of peptidic ergolines and dihydroergolines clearly reveal the molecular size of an analyte

through the presence of $[M + 1]^+$ (high intensity) and $[M - 1]^-$ (high to low intensity but well-defined in all cases) ions in positive and negative ion spectra, respectively. Negative FAB spectra of salts also display diagnostic anion–base conjugate ions.

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