Pergamon

Journal of Pharmaceutical & Biomedical Analysis, Vol. 12, No. 1, pp. 27–40, 1994

0731-7085/94 $6.00 + 0.00

Rapid identification of ergot derivatives by ^{1}H-NMR spectroscopy

ALAN F. CASY*

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

Abstract: The 400 MHz ^{1}H-NMR spectra of some therapeutically important ergot derivatives (three bases, four protonated bases and four dihydroergoline salts) are analysed in terms of the low field chemical shift region (above 5 ppm), common resonances of rings C and D (below 5 ppm) and C-8 substituent features. Attention is drawn to data of specific analytical value, and a scheme for the rapid identification of members of this group of ergots proposed. Features which provide evidence of the solute conformation of ring D, and isomerization to less active C-8 epimers are also emphasized.

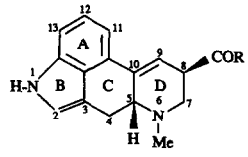
Keywords: *Ergots (ergolines); dihydroergolines; ^{1}H-NMR.*

Introduction

^{1}H-NMR Spectroscopy is a valuable technique for the identification of individual members of a closely related group of compounds with similar electronic, vibrational and other properties. Examples of the application of NMR in this regard are differentiation of the penicillin, cephalosporin and tetracycline families of antibiotics [1–3]. Ergot (ergoline) derivatives, both natural and synthetic variants, likewise form a group which presents problems of specific identification. This paper presents a review of reports on the NMR features of the group and extends it to include most derivatives in therapeutic use [4]. Data of specific analytical value are highlighted; in addition features that provide evidence of stereochemistry (notably the conformation of ring D) and isomerization to less active C-8 epimers are emphasized.

The described analyses concern first common features of the ergoline nucleus **1** (low field: aromatic, vinylic and acidic protons above 5 ppm: alicyclic ring protons), and secondly those of the C-8 substituent (peptide-based moiety or simple function as in ergometrine **1** and methysergide **2**) which primarily carry specificity (Structure **1** and **2**).

Literature reports on ergolines refer exclusively to free bases [5–8] whose ^{1}H-NMR spectra are generally well resolved, as confirmed in the further examples of this paper.



1 R = ^{20}NHCH(Me)CH$_2$OH
2 R = NHCH(Et)CH$_2$OH with H-N^1 replaced by Me-N^1

Structures 1 and 2

Data on protonated bases, the usual therapeutic form of ergots, make up the bulk of the present results. With the exception of certain C-8 function and aromatic signals, broad resonances often of poor resolution were encountered. These features of salt spectra limited signal assignment in many cases but, as will be shown, did not preclude the designation of diagnostic points. The broad nature of resonances due to alicyclic ring protons of ergoline salts may be a consequence of conformational equilibria set up as a result of proton exchange at 6-N and/or changes in conformation about the C-8 amido bond (less probable: single COR features were observed, mostly sharply resolved).

Spectral assignments shown in Tables 1–3 were aided by previous analyses of lysergic acid diethylamide (220 MHz) [6] and ergotamine (270 MHz) [7] made by consideration of chemical shifts and coupling constants

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

Table 1
^{1}H-NMR Spectral characteristics of some ergoline bases[a]

Compound and solvent	Low field region (above 5 ppm)					
	1-NH	20-NH	12,13,14-H	2-H	9-H	15′-OH
Ergotamine (**3a**) in $CDCl_3$ 270 MHz [7]	8.14 d (<1.5)	9.04 s	7–7.5 m[b]	6.91 dd (1.8,0.5)	6.34 dd (5.5,2.0)	6.97 s
Present work tartrate in $CDCl_3$-NaOD	not seen	not seen	7.1–7.5 m[b]	6.94 d (~2)	6.37 dd (~6,2)	not seen
Ergocristine (**3b**) in $CDCl_3$	8.03 brs[c]	9.7 s	7.13–7.57 m[b]	6.94 brs[c]	6.4 brd (5.8)	within 7.46–7.57
in DMSO-d_6	(9.18)[d]	(10.74)[d]	7.02–7.35 m[b]	within 7–7.4	6.3 brs 6.5 brd[c]	within 7–7.4
Ergocryptine (**3c**) in $CDCl_3$	8.05 brs[c]	9.82 s	7.24 dd (7.5,<1) 7.19 t (7–8) 7.15 dd (~7,1)	6.94 brs[c]	6.39 dd (6,~1)	7.38 d (1.5)
in DMSO-d_6	(9.21)[d]	(10.74)[d]	6.95–7.21 m	within 6.9–7.3	6.22 brs 6.45 m[a]	7.45 brs

Table 1
Continued

Compound and solvent	Common resonances of rings C and D (below 5 ppm)						
	4α-H	4β-H	5β-H	6-NMe	7α-H	7β-H	8α-H
Ergotamine (**3a**) in $CDCl_3$ 270 MHz [7]	2.79 ddd (14.2,11.9,1.8)	3.32 ddd (14.2,5.0,<0.5)	3.73 m (11.9,5.0,2.0,1.5)	2.61 s	2.96 dd (11.9,3.9)	2.78 dd (11.9,3.4)	3.18 m (5.5,3.9,3.4,1.5)
Present work tartrate in $CDCl_3$-NaOD	2.8 m[f]	3.35 dd (~14,4)	3.77 brd (12–13)[g]	2.63 s	2.97 dd (~11,4)	2.8 m[f]	3.18 brs[g]
Ergocristine (**3b**) in $CDCl_3$	2.86 m[f]	3.34 dd (14,4.8)	3.85 brd (~12)[g]	2.66 s	3.00 dd (~10,2.5)	2.86 m[f]	3.18 brs[g]
in DMSO-d_6	2.5 m[f]	3.48 dd (14.5,5.5)	3.68 brm[g]	2.5 s[k]	[h]	[h]	3.18 brs[g]
Ergocryptine (**3c**) in $CDCl_3$	2.86 dt (12.5,12.5,<1)	3.33 dd (14,5)	3.89 dd[g] (11.3,4)	2.67 s	2.96 m[i]	2.96 m[i]	3.16 brs[g]
in DMSO-d_6	[h]	3.45 dd (~13,5)	3.7 brd[g,j]	2.5 s[k]	[h]	[h]	3.18 brs[g]

Table 1
Continued

Compound and solvent	C-8 Substituent features					
	2′-Me	5′-H	8′ and 11′-H	9′ and 10′-H	13′-C<u>H</u>$_2$Ph	Other signals
Ergotamine (**3a**) in $CDCl_3$ 270 MHz [7]	1.51 s	4.69 dd (6.6,5.5)	3.6 m	2.1 m	3.46 dd (14,6.6) 3.26 dd (14,5.5)	—
Present work in $CDCl_3$-NaOD	1.52 s	4.69 t (6)	3.5–3.7 m	1.5–2.2 4 m	3.47 dd (13,4) 3.26 dd (13,5)	—
Ergocristine (**3b**) in $CDCl_3$	—	4.68 t (~6)	3.5–3.7 m	1.8,2.2 m	3.44 dd (14,7) 3.27 dd (14,5)	2′-CH<u>Me</u>$_2$ 0.89 d(6.7), 1.0 d(7) 2′-C<u>H</u>Me$_2$ within m near 2.07
in DMSO-d_6	—	4.57 t (~5)	3.4 m 3.75 brt (~7)	1.7,1.8 m	3.22 dd (14,7) 3.11 dd (14,6.5)	2′-CH<u>Me</u>$_2$ 0.9 d(6.7), 1.04 d(6.7) 2′-C<u>H</u>Me$_2$ m near 2.1
Ergocryptine (**3c**) in $CDCl_3$	—	4.52 t (~7)	3.5–3.7 m	1.78 m	—	2′-CH<u>Me</u>$_2$ + 5′CHCH$_2$CH<u>Me</u>$_2$ 0.91 d(6.7), 1.01 apparent t[l], 1.05 d(6.4) 5′-CHC<u>H</u>$_2$ 1.86, 2.0 m; 2 × C<u>H</u>Me$_2$ 2.15 m
in DMSO-d_6	—	4.35 t (~5) 4.25 t[e]	3.7 m	1.7–2.1 m	—	2′-CH<u>Me</u>$_2$ + 5′-CHCH$_2$CH<u>Me</u>$_2$ 0.90 d(6.4)[m], 0.98 d(6.7), 1.05 d(6.7) 5′-CHC<u>H</u>$_2$ 1.7–2.1 m; 2 × C<u>H</u>Me$_2$ 2.15 m

[a] Chemical shifts (ppm) from TMS, coupling constants or line separations (Hz) follow signal descriptions. Abbreviations: br, broad (indicative of small unresolved coupling(s)); s, singlet; d, doublet; t, triplet; m, multiplet plus combinations, e.g. dd, doublet of doublets. Spectra recorded at 400 MHz unless otherwise stated.
[b] Includes 5′-CH$_2$<u>Ph</u> protons.
[c] Linked to geminal and/or vicinal neighbour(s) by COSY cross-peak(s).
[d] Interchangeable assignments.
[e] Minor resonance due to C-8 epimer.
[f] 4α- and 7β-signals overlap.
[g] Composed of several unresolved couplings.
[h] Within band centred on 2.5 ppm which includes the solvent resonance.
[i] 7α- and 7β-signals overlap (both dd).
[j] Includes 11′-H signal.
[k] Near the solvent signal.
[l] Formed by a pair of overlapping Me doublets.
[m] Two coincident Me doublet (integral 6).

supported by spin-decoupling experiments. In this paper spectra were run at 400 MHz and analyses assisted by 2D ^1H–^1H correlation (COSY) plots. Salts were examined as solutions in DMSO-d_6 and, when solubility allowed, D_2O. The former solvent allowed the detection of N—H and O—H resonances, both D_2O-exchangeable.

Materials and Methods

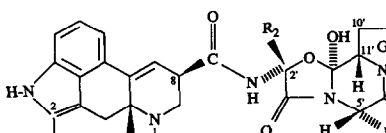
^1H-NMR Spectra were obtained on a JEOL GX 400 spectrometer. Samples of approximately 10 mg were dissolved in the appropriate solvent (0.5 ml) and examined without degassing at the ambient probe temperature (20°C) and employing the standard conditions of 32K data points with digital resolution of 0.18 Hz per point [9]. The homonuclear ^1H–^1H chemical shift correlated 2D plots were obtained using the standard COSY-45 pulse sequence [10]. Solvents employed were $CDCl_3$ and CD_3OD for bases, and DMSO-d_6, CD_3OD and D_2O for salts. TMS served as reference in all cases except that of D_2O when DSS was used.

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 and dihydroergocristine mesylates. Ergocryptine and ergocristine bases were purchased from Sigma.

Results and Discussion

Free base ergolines

The ^1H-NMR features of three C-8 peptidic ergots, ergotamine **3a**, ergocryptine **3c** and ergocristine **3b** (Structure **3**) are given in Table 1. It was possible to identify 1-H(NH) and 2-H by a COSY cross-peak in spectra of ergocryptine (**3c**) and ergocristine (**3b**). The vinylic 9-H signal (broad resonance 6.34–6.4 ppm in $CDCl_3$, 6.22–6.3 ppm in DMSO-d_6) was accompanied by a minor signal to low field (near 6.5 ppm) in DMSO-d_6 spectra, evidence that this solvent induces epimerization at C-8. Most aliphatic ring proton signals were resolved in all three base spectra and their multiplicities and ^3J coupling magnitudes were consistent with a pseudo-axial C-8 substituent half-chair conformation (**4**) (Structure **4**) for ring D stabilized by an intramolecular hydrogen bond [7]. The coupling interactions of 5β-H and 8α-H include both allylic and homoallylic contributions [6, 7], and in consequence both resonances are characteristically broad (Fig. 1).



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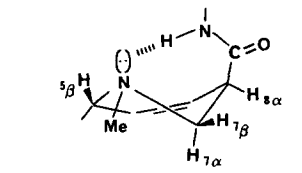
	R_1	R_2	R_3
(a) ergotamine	H	Me	CH_2Ph
(b) ergocristine	H	$CHMe_2$	CH_2Ph
(c) ergocryptine	H	$CHMe_2$	CH_2CHMe_2
(d) bromocriptine	Br	$CHMe_2$	CH_2CHMe_2
(e) erogcornine	H	$CHMe_2$	$CHMe_2$

Structure 3



Structure 4
Base conformation.

C-8 Substituent features

The 5′-H resonance was well defined in all spectra as a narrow triplet near 4.6 ppm, as were those of the benzyl methylene protons (AB of ABX system) of ergotamine (**3a**) and ergocristine (**3b**) (dd near 3.4 and 3.25 ppm). Methyl features distinguished ergotamine **3a** (2′-Me, s near 1.5 ppm), ergocristine **3b** (2′-$CHMe_2$, 2d near 1 ppm) and ergocryptine **3c** (2′-$CHMe_2$ and 5′-CH_2CHMe_2, 4d near 1 ppm). The presence of the C-8 epimer of ergocryptine was apparent from the minor 5′-H resonance upfield of the major signal in the spectrum run in DMSO-d_6.

Protonated ergolines

Ergometrine **1** and methysergide **2** maleates were the simplest cases examined since they do not carry peptidic units at C-8 (Table 2). Only a single NH resonance (20-NH) was present in the spectrum of methysergide run in DMSO-d_6 — the 1-NH signal was replaced by an NMe singlet near 3.8 ppm additional to that of 6-NMe. Aryl signal integrals distinguished ergotamine **3a** tartrate (9, includes 5′-CH_2Ph) and bromocriptine **3d** mesylate (3, 2-H re-

Table 2
^{1}H-NMR Spectral characteristics of some ergoline salts (protonated bases)[n]

	Low field region (above 5 ppm)					
Compound and solvent	1-NH	20-NH	12,13,14-H	2-H	9-H	Counter ion[q]
Ergometrine (**1**) maleate in DMSO-d_6	10.97 brs[c]	8.26 d(7)[c]	7.11–7.18 m 7.29 d(7)	within 7.11–7.2[c]	6.52 brs	6.05 s maleate
in D_2O	—	—	7.13 brs 7.23 m 7.39 dd(6, 2.5)	within 7.13–7.23	6.48 brs	6.21 s maleate
Methysergide (**2**) maleate in DMSO-d_6	—	8.14 d(7.5)[c]	7.16–7.21 m 7.34 dd(6, 2)	within 7.16–7.21	6.55 brs	6.05 s maleate
in CD_3OD	—	—	7.01 d(4.5) 7.17–7.3 m	within 7.01–7.3	6.62 brs	6.24 s maleate
Ergotamine (**3a**) tartrate in DMSO-d_6	10.75 brs[c]	9.4 s	7.05–7.35 m[b]	near 7[c]	6.57 brs 6.72 brs[c]	4.24 s tartrate
Bromocriptine (**3d**) mesylate in DMSO-d_6	11.8 s	9.5 s	7.06–7.27 m	—	6.42 brs	3.37 s mesylate
in CD_3OD	—	—	7.15–7.26 m[p]	—	6.56 brs	3.24 s mesylate

Table 2
Continued

Compound and solvent	Common resonances of rings C and D (below 5 ppm)						
	4α-H	4β-H	5β-H	6-NMe	7α-H	7β-H	8α-H
Ergometrine (**1**) maleate in DMSO-d_6	3.5 m[r]	within 3.6–3.8 m	within 3.6–3.8 m	3.06 brs	within 3.6–3.8 m	2.85 t(~10)[c]	4.25 brs[c,g]
in D_2O	3.47 brt (~10)	within 3.68–3.9 m	within 3.68–3.9 m	3.11 brs	within 3.68–3.9 m	2.88 brt (~12)	4.01 brs[g,u]
Methysergide (**2**) maleate in DMSO-d_6	3.55 t(12)	within 3.75 m	within 3.75 m	3.08 s 3.8 s (1-NMe)	within 3.75 m[c]	2.83 t(12)[c]	4.25 brs[g]
in CD_3OD	within 3.75 m	within 3.84 m	within 3.84 m	3.12 s 3.8 s (1-NMe)	within 3.75 m[c]	2.94 t(~12)[c]	4.19 brs[g]
Ergotamine (**3a**) tartrate in DMSO-d_6	[s]	[s]	[s]	3.1 m[t]	3.5 dd(12,~5)[c]	2.58 t(~11)[c,k]	3.6 (?) m[c,g]
Bromocriptine (**3d**) mesylate in DMSO-d_6	[s]	[s]	4.08 brm	3.17 brs	3.6 brdd (~12,3)[c]	2.8 t(~12)[c]	4.4 brs[g]
in CD_3OD	3.75 t(11–12)	[s]	4.1 brm	3.24 brs	[s]	2.87 t(12.5)	4.37 brs[g]

Table 2
Continued

Compound and solvent	C-8 Substituent features								
	Me signals	CH_2 signals		5′-H	8′-H	9′-H	10′-H	11′-H	Other signals
Ergometrine (**1**) maleate in DMSO-d_6	20-NHCHMe 1.09 d(6.7)[c]	20-NHCHMeCH$_2$OH 3.4 m(8-line)[c]		—	—	—	—	—	20-NHCHMe 3.84 m[c] CH$_2$OH 4.83 m[c]
in D_2O	1.16 d(6.7)	3.55 dd(11.3,6.4) 3.64 dd(11.5,4.8)		—	—	—	—	—	4.01 m (overlaps 8α-H) —
Methysergide (**2**) maleate in DMSO-d_6	20-NHCHCH$_2$Me 0.87 t(7.3)	20-NHCHCH$_2$Me 1.38,1.62 m(8-line)	20-NHCHEtCH$_2$OH 3.5 m	—	—	—	—	—	20-NHCHEt 3.69 m
in CD_3OD	0.97,0.99 t(overlap)	1.51,1.68 m(8-line)	3.6 m	—	—	—	—	—	3.84 m
Ergotamine (**3a**) tartrate in DMSO-d_6	2′-Me 1.5 s 1.49 s[e]	13′-CH$_2$Ph 3.1 m 3.2 dd(12.5,6)		4.51 t(5–6)	3.1 m	1.75 m	1.9 m[e]	3.75 t(7.5)[e]	—
Bromocriptine (**3d**) mesylate in DMSOpd$_6$	2′-CHMe$_2$ + 5′-CH$_2$CHMe[2] 0.79 d(6.4) 0.92 d(6.8) 0.95 d(7.3) 1.08 d(6.8)	13′-CH$_2$CHMe$_2$ 1.6–1.8 m[e]		4.36 t(~7)[e]	[s]	[s]	1.9 m[e]	3.55–3.75 m[e]	2′-CHMe$_2$ + 5′-CH$_2$CHMe$_2$ 1.85–2.3 m[e]
in CD_3OD	1.0 t[l] 0.82 d(6.7)[e] 1.17 d(6.7)[e]	1.8 m		4.55 t(6–7)	3.5 m[e]	1.95 m[e]	2.33 m[e]	3.85 t(6–7)[e]	1.95 m(2′)[e] 2.24 m(5′)[e]

[n] Footnotes of Table 1 apply.
[o] Absent after D_2O exchange; linked to CH_2 of C-8 substituent by COSY cross-peak.
[p] The integral of 3 is relative to that of the 9-H resonance (1).
[q] Mesylate and tartrate resonances fall below 5 ppm.
[r] 3.55 t(~10) after D_2O.
[s] Unresolved.
[t] Overlaps with the 13′-CH_2 signal.
[u] 8α-H and CHMe signals overlap.

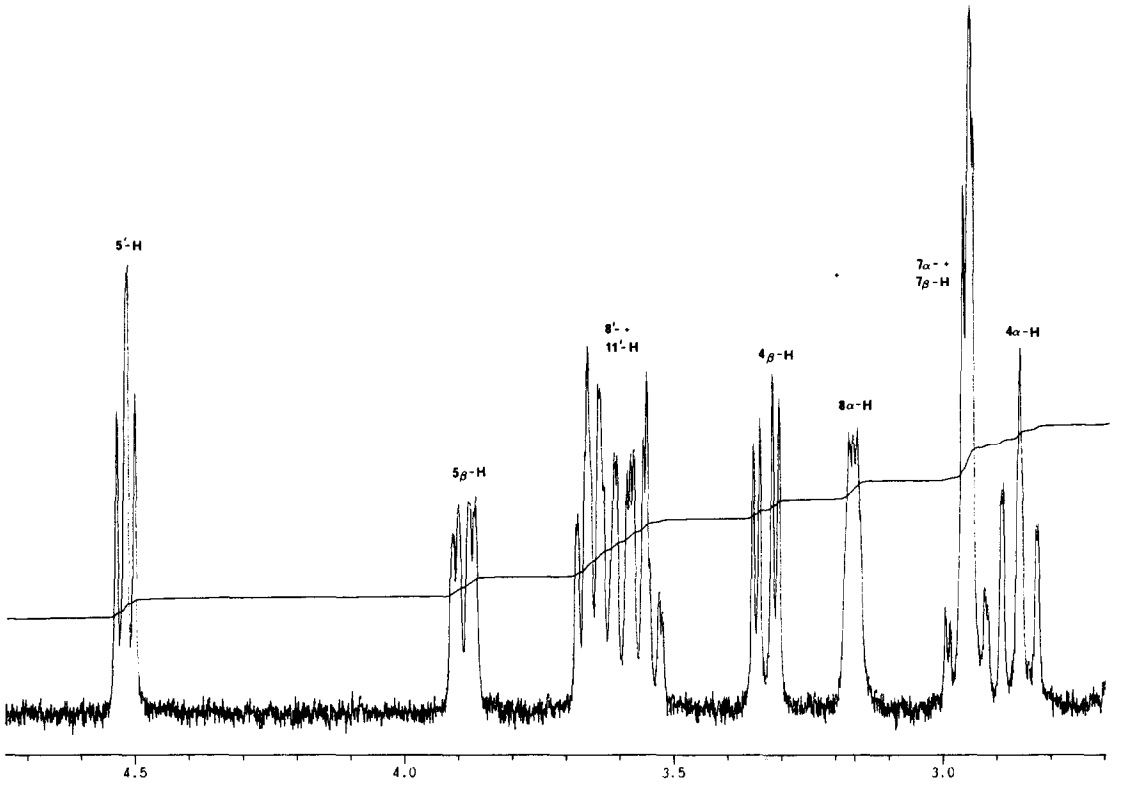


Figure 1
Part of the 400 MHz ^{1}H-NMR spectrum of ergocryptine base **3c** in $CDCl_3$. The chemical shift scale is ppm from TMS.

placed by Br) from other members of this group.

Although resolution of ring C–D proton signals of salts was inferior to that of the Table 1 bases, it proved possible to assign many individual resonances by the aid of COSY plots and other means. The 7β-H signals (mostly 2.8–2.9 ppm) were generally well resolved; their appearance revealed a strong coupling (~12 Hz) to 8α-H indicative of preference for a pseudo-equatorial 8-R half chair conformation of ring D (**5**) (Structure **5**). The 8α-H resonances near 4 ppm were characteristically broad (the 4β-H signal was obscured by overlap with that of 5β-H).

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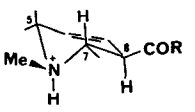


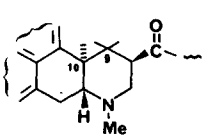
Structure 5
Ring D conformation for salts.

C-8 Substituent features

COSY plots clearly delineated the C-8 features of ergometrine **1** maleate (the 8-line $CHC\underline{H}_2OH$ signal was specially well resolved in the D_2O spectrum), and methysergide maleate. In the peptidic examples **3a** and **3d**, characterized by 5′-H triplet signals near 4.5 ppm, 2′ (Me for **3a**, $CHMe_2$ for **3d**) and 5′ (CH_2Ph for **3a**, CH_2CHMe_2 for **3d**) spectral features were all resolved. Signals due to ring G (pyrrolidino protons) of **3a** and **3d**, absent from spectra of the non-peptidic examples, were assignable to various multiplet resonances (8′, 11′ near 3 and 3.8, respectively, 9′, 10′ 1.9–2.33 ppm).

Dihydroergoline protonated bases

The absence of a vinylic proton resonance near 6.5 ppm in spectra of the examples given in Table 3 immediately revealed them as 9,10-dihydroergoline derivatives **6** (Structure **6**). Integrals of the aryl proton band differentiated pergolide **7** (Structure **7**) and dihydrocornine



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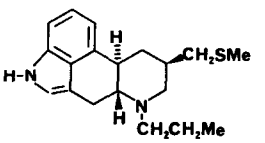


7

Structures 6 and 7
For structure **6**: (a)–(e) as for **3**.

Table 3
^{1}H-NMR Spectral characteristics of some dihydroergoline salts[a]

Compound and solvent	Low field region (above 5 ppm)				
	1-NH	20-NH	12,13,14-H	2-H	Other acidic-H
Pergolide (**7**) mesylate in DMSO-d_6	11.04 brs	—	H-12,14: 7.0 d(7), 7.36 d(7.9) H-13: 7.23 t(7.9)	7.25 brs	9.9 brs
Dihydroergotamine (**6a**) mesylate in DMSO-d_6	11.94 brs	9.6 brs	H-12 or 14: 6.9 d(7) 7.13–7.3[b]	within 7.13–7.3	9.95 brs 6.42 s
in D_2O	—	—	6.54 d(7.2) 1pr 6.96 brs 1pr 7.04 t(~7) 2pr 7.09 t(~7) 2pr 7.17 d(7.9) 1 pr 7.23 d(7.9) 2 pr	within 6.96–7.23	—
dihydroergocristine (**6b**) mesylate in DMSO-d_6	10.9 s	9.3 s	6.86 d(7) 1pr 7.06–7.28 m 8pr[b]	within 7.06–7.28	10.0 brs
Dihydroergocornine (**6e**) mesylate in DMSO-d_6	10.9 s	9.25 s	H-12,14: 6.83 d(7), 7.22 d(7.9) H-13: 7.09 t(7–8)	7.16 brs	7.16

Table 3
Continued

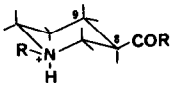
	Common resonances of rings C and D (below 5 ppm)										
Compound and solvent	4α-H	4β-H	5β-H	6-Me	7α-H	7β-H	8α-H	9α-H	9β-H	10α-H	Me of mesylate
Pergolide (**7**) mesylate in DMSO-d_6	within 2pr m 2.9–3.1	(3.69)[d] brd(11)	within 4pr m 3.3–3.5	(6-Pr[n])[w]	(3.58)[d] brd(14)	within 2pr m 2.9–3.1	base of 2.4 Me signal	2.85 brd(14)	1.4 brq(~14)	within 4pr m 3.3–3.5	2.41 s
Dihydroergotamine (**6a**) mesylate in DMSO-d_6	[s]	(3.7)[d] brm	[s]	3.03 s	(3.58)[d] brd(~11)	2.8–3.5[s]	[s]	[s]	1.62 brq(~11)	[s]	2.33 s
in D_2O	[s]	(3.72)[d] dd (10,5.5)	[s]	(2.74)[d] s	(3.3–3.6)[d] m[s]	[s]	[s]	[s]	within m 1.75–2.2	[s]	(2.73)[d] s
Dihydroergocristine (**6b**) mesylate in DMSO-d_6	[s]	(3.7)[d] brm	[s]	3.0 brs	(3.5)[d] m[s]	[s]	[s]	[s]	1.61 brq(~12.5)	[s]	2.34 s
Dihydroergocornine (**6e**) in DMSO-d_6	[s]	[s]	[s]	3.0 brs	[s]	[s]	[s]	[s]	~1.6 brq(~12)	[s]	2.43 s

Table 3
Continued

Compound and solvent	C-8 Substituent features							
	Me signals	CH_2 signals	5′-H	8′-H	9′-H	10′-H	11′-H	Other signals
Pergolide (**7**) mesylate in DMSO-d_6	S-Me 2.4 s	8-CHCH$_2$S 2.58 dd(13,7) 2.69 dd(13,5)	—	—	—	—	—	—
Dihydroergotamine (**6a**) in DMSO-d_6	2′-Me 1.53 s	13′-CH$_2$Ph 3.07 dd(~14,5)[x] 3.18 dd(~14,7)	4.52 t(5.5)	3.2–3.5 m	within 1.7–1.8, 1.8–2.0 m	within 9′-H signals	3.75 t(~7)	—
in D_2O	1.52 s	3.05 dd(14.6) 3.26 dd(14,7)	4.56 t(6)	3.35–3.48 m	within 1.7–1.8, 1.8–2.0 m	within 9′-H signals	3.72 dd(10,5.5)	
Dihydroergocristine (**6b**) in DMSO-d_6	2′-CHMe$_2$ 0.92,1.05 d(6.7)	13′-CH$_2$Ph 3.05 dd(14,7) 3.15 dd(14,4)	4.56 t(4.5)	[x]	within 1.7–2.1 m	within 1.7–2.1 m	3.8 t(~7)	2′-CHMe$_2$ 2.1 m(6.7)
Dihydroergocornine (**6e**) in DMSO-d_6	2′-CHMe$_2$ + 5′-CHMe$_2$ 1.00 d(6.7) 1.05 d(7.0) 1.10 d(7.0) 1.16 d(6.7)	—	4.36 d(3)	[x]	within 1.8–2.2 m	within 1.8–2.2 m	3.82 dd(9.6)	2′-CHMe$_2$ 2.22 m(6.7) 5′-CHMe$_2$ 2.48 m

[y] Footnotes of Tables 1 and 2 apply.
[w] 6-NPr^n: Me1.03 t(7); MeCH$_2$ 1.7–1.85 m; NCH$_2$ within a 4pr m 3.3–3.5.
[x] The higher field doublet is masked by other resonances.

6e (both 4) from dihydroergotamine **6a** and dihydrocristine **6b** (both 9). The spectrum of the non-peptidic example pergolide **7** was best resolved in regard to ring C–D proton signals. Entry to its analysis was gained by assignment of C_8–$C\underline{H}_2S$ (a typical 8-line signal) which led via COSY cross-peaks to 8α-H and other resonances. The 9β-H signal near 1.4 ppm was unique in displaying three large couplings (producing an apparent quartet) consistent with a pseudo-eq-8-CH_2SMe ring D chair conformation **8** (Structure **8**) for this dihydroergoline derivative (in dihydroergolines with bulky 6-N substituents a twist-boat conformation of ring D is favoured [8]). The low field position of the signal due to its geminal partner (9α-H) may be attributed to aromatic deshielding. Ring C–D assignments are in accord with those made for festuclavine base (6-NMe, 8-Me analogue of **7**) once allowance for N-protonation effects are made [5]. Proton signals below 4 ppm were poorly resolved in all peptidic dihydroergoline spectra due to extensive overlap of ring C–D and pyrrolidino (ring G) resonances. However, all spectra revealed an apparent quartet near 1.5 ppm of separations ~12 Hz assigned to 9β-H.



8

Structure 8

C-8 Features

The presence of a peptidic C-8 substituent in derivatives **6a**, **b** and **e** was revealed by observation of a 5′-H resonance near 4.5 ppm in their spectra; signal multiplicities (t for **6a** and **6b**, d for **6e**) served to pin-point dihydroergocornine (5′-$C\underline{H}Me_2$). The 8-line signal diagnostic of 5′-$C\underline{H}_2Ph$ was clearly resolved in spectra of dihydroergotamine **6a** and dihydroergocristine **6b**, as were 2′ and 5′ methyl features (2′-Me for **6a**, 2′-$CHMe_2$ for **6b**, 2′- and 5′-$CHMe_2$ for **6e**). COSY cross-peaks allowed assignment of other features of the C-8 substituent.

Proposed diagnostic scheme

Once there is reason to suspect an analyte to be an ergot, e.g. by its positive response in the coloration reaction with *p*-dimethylaminobenzaldehyde [11, 12], examination of the pattern of the low field region of its ^{1}H-NMR spectrum run in DMSO-d_6 will confirm the presence of an ergoline skeleton (moving from low to high field: two 1 pr singlets, multiplet of integral 3 or more, 1 pr singlet). Absence of the highest field 1 pr singlet of this group near 6.5 ppm (due to the 9-H vinylic proton) reveals a dihydroergoline derivative. Only three low field features of this kind are seen in the spectrum of methysergide **2** (1-NH → 1-NMe).

C-8 Substituent evidence. The next point to check is the presence or absence of a 1 pr resonance near 4.5 ppm (usually a narrow triplet). Such signals are due to 5′-H of a peptide-based C-8 substituent and take the form of triplet in spectra of **3/6 a–d** and doublets in those of the ergocornines **3e** and **6e**. The count of high field Me doublet signals near 1 ppm differentiates ergocryptine **3c** and bromocriptine **3d** (both 4) from ergocristine **3b** (2) and ergotamine **3a** (nil). Identities of the last two examples are confirmed by additional C-8 substituent signals (8-line 5′-$C\underline{H}_2Ph$ resonance within 3.0–3.5 ppm for **3a** and **3b**, 2′-Me singlet near 1.5 ppm for **3a**). Dihydroanalogues **6a–d** may be differentiated similarly from their spectra. Careful integration of the aromatic signal near 7.0 ppm serves to distinguish ergocryptine (integral 4) from its 2-bromo congener **3d** (integral 3).

C-8 Spectral features of the non-peptidic derivatives readily identify ergometrine **1** (Me doublet near 1.1 ppm, 8-line $C\underline{H}_2OH$ signal near 3.5 ppm) and methysergide **2** (Me triplet near 0.9 ppm, $C\underline{H}_2Me$ resolved 1 pr multiplets near 1.4 and 1.6 ppm). The dihydro-derivative pergolide **7** is best characterized by its 6-$NCH_2C\underline{H}_2Me$ (m and t near 1.8 and 1.0, respectively) and S-Me (s 2.4 ppm) resonances.

Acknowledgements — Thanks are due to Sandoz Pharmaceuticals (Dr W.S. Phillips), Lilly Research Laboratories (T.L. Jeatran) and the Home Office Forensic Science Service (Dr D. Osselton) for generous provision of the ergots detailed under "Materials and Methods", and Messrs R. Hartell and D. Wood for recording the spectra.

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[Received for review 19 November 1992; revised manuscript received 18 January 1993]