Fragmentation studies of tetrahydropyridocarbazole derivatives by EI, ESI-MS/MS and FAB

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Abstract. Four tetrahydropyridocarbazole derivatives were analysed by different mass spectrometry techniques: electrospray ionization, fast atom bombardment and by low and high resolution 70 eV electron ionization. Retro Diels Alder is the main fragmentation pathway, whereas other pathways leading to $[M-1]^+$, $[M-CH_3]^+$ and double charge ions also occur to considerable extents. Semi-empirical calculation provided some evidence on the nature of tropylium ions $[M-1]^+$. Calculation of Δ Hf⁰ indicated that $[M^+-1]$ could be formed preferentially when a hydrogen atom is lost from the methyl substituent of the homoaromatic ring.

1. Introduction

Ellipiticine and olivacine are two important pyridocarbazoles alkaloids that exhibit antitumor activity and their synthetic routes [1] and biological activity [2,3] have been widely studied. Although the pyridocarbazole system is present in several important compounds, few mass spectrometry studies have been performed on their fragmentation pattern.

Continuing our work toward the synthesis of substances with antitumor activity we synthesized new angular derivatives of pyridocarbazoles starting from alkylquinolines. Here we describe the preparation of 1-4 (Fig. 1) and report our results on their fragmentation pattern by EI, ESI-MS/MS and FAB.

2. Experimental

The infrared spectra were recorded in a Perkin Elmer model 1420 instrument. The nuclear magnetic resonance spectra, ¹³C and ¹H, were obtained from a Varian-Unity Plus instrument (300 MHz for ¹H and 75.0 MHz for ¹³C). Elemental analysis were obtained using a Carlo Erba instrument model 1104. Low-resolution EI mass spectra were recorded on a Finnigan MAT 711A instrument. The ionization energy used was 70 eV with the source at 200°C and with an accelerating voltage of 8 kV. Samples were introduced by the standard direct insertion probe. High resolution data were obtained with the same

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Fig. 1. Pyridocarbazoles 1-4.

instrument using 10,000 resolution. FAB positive-ion mass spectra were recorded on a Finnigan MAT-TSQ 70 triple quadrupole mass spectrometer. For FAB analysis, *m*-nitrobenzyl alcohol (NBA) was used as the matrix. Xenon was used to generate the bombarding atoms and the FAB gun was operated at 2 mA emission, providing fast atoms with an energy of 10 kV. Ionspray mass spectra were acquired with a triple quadrupole instrument, Perkin Elmer (API III TAGA 6000E) equipped with a ionspray source (Sciex, Toronto, Canada). Samples were infused into the electronspray source, in methanol solution, using a syringe inlet. The semi-empirical calculation were performed using the AM1 [23] and PM3 [24] methods as implemented in the MOPAC 93 program [25]. All geometries were fully optimized using the EigenVector following optimization routine [26] until the gradient norm dropped bellow 0.05. Geometry optimizations were followed by frequency calculations to certify that the geometries found are true minima.

2.1. Chemicals

Compounds 1,3,5-trimethyl-7,8,9,10-tetrahydro-11H-pyrido[3,2-<u>a</u>]carbazole (1), 1,3,5-trimethyl-8,9, 10,11-tetrahydro-7H-pyrido[2,3-<u>c</u>]carbazole (2), 1,3,5-trimethyl-7,8-dihydro-13H-benzo[1]pyrido[2, 3-<u>c</u>]carbazole (3) and 3-methyl-8,9,10,11-tetrahydro-7H-pyrido[2,3-<u>c</u>]carbazole (4) were synthesized using 2,4,8-trimethyl-5-aminoquinoline, 2,4,5,8-tetramethyl-6-aminoquinoline, 2,4,8-trimethyl-5-aminoquinoline, respectively, as starting material in the Fischer indole synthesis. Compounds 1, 2 and 4 were synthesized using cyclohexanone in the last step, while α -tetralone was used for 3. The structures of the starting aminoquinolines were establish by using ¹H and ¹³C techniques.

2.2. General procedure

To a solution of the appropriated alkylaminoquinoline (2,4,8-trimethyl-5-aminoquinoline, 2,4,5,8-tetramethyl-6-aminoquinoline or 2-methyl-6-aminoquinoline) (10 mmoles) and 2 ml of concentrated hydrochloric acid externally cooled to 0°C, an aqueous solution of sodium nitrite (11 mmoles) was added. The reactional mixture was stirred for 30 min and then the resulting diazonium salt was slowly added to a solution of stannous chloride (26 mmoles) in concentrated hydrochloric acid. The mixture was stirred for 1 h at room temperature and then added 10 ml of glacial acetic. The was raised to 80°C and kept constant for 4 h. The solution was cooled down to room temperature and solid filtered, washed with water (20 ml), sodium hydroxide solution (20 ml, 50% W/V), water (20 ml), air dried and recrystallized in ethanol to yield the compounds:

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1,3,5-Trimethyl-7,8,9,10-tetrahydro-11H-pyrido[*3,2-<u>a</u>]<i>carbazole* (*1*). Obtained in 40% yield; m.p. 180°C; IR (KBr) cm⁻¹-3400 (NH), 2960, 2840, 1600, 1570, 1460, 1360, 1340, 1320, 1230, 1160, 1030, 860, 840; ¹H-NMR (CDCl₃) δ 1.80–2.00 (m, 4H, 2CH₂), 2.62 (s, 3H, CH₃), 2.72–2.76 (2s, 10H, 2CH₃, 2CH₂), 6.90 (s, 1H, Ar), 7.50 (s, 1H, Ar), 8.26 (l, 1H, NH) ppm; ¹³C-NMR (CDCl₃) δ 0.67, 0.87, 1.07, 1.26, 2.24, 19.33, 20.88, 22.37, 22.65, 23.42, 24.41, 25.00, 110.93, 121.08, 122.04, 127.83, 132.71, 139.83 ppm; Anal. Calcd. for C₁₈H₂₀N₂: C-81.81, H-7.57, N-10.6, found C-80.90, H-7.43, N-10.04.

1,3,5-Trimethyl-8,9,10,11-tetrahydro-7H-pyrido[*2,3-<u>c</u>]<i>carbazole* (**2**). Obtained in 25% yield; m.p. 197–199°C; IR (KBr) cm⁻¹ 3140 (NH), 1590, 1580; 1¹H-NMR (MHz, CDCl₃) δ 1.70–2.20 (m, 4H, 2CH₂), 2.47 (s, 3H, CH₃), 2.67–2.74 (2s, 10H, 2CH₃,2CH₂)), 6.83 (s, 1H, Ar), 7.52 (s, 1H, Ar), 8.40 (l, 1H, NH) ppm; ¹³C-NMR (MHz, CDCl₃) δ 0.72, 0.85, 1.17, 1.22, 2.36, 19.37, 20.80, 21.95, 22.45, 24.41, 25.40, 25.65, 112.43, 121.05, 122.17, 127.82, 133.01, 139.80 ppm; Anal. Calcd. for C₁₈H₂₀N₂: C-81.81, H-7.57, N-10.6, found C-80.75, H-7.82, N-10.04.

1,3,5-Trimethyl-7,8-dihydro-13H-benzo[l]pyrido[2,3- \underline{c}]carbazole (3). Obtained in 55% yield; m.p. 205°C; IR (KBr) cm⁻¹ 3550 (NH), 2960, 2850, 1620, 1460, 1420, 1340, 1300, 880, 840, 760; ¹H-NMR (CDCl₃) δ 2.0 (s, 3H, CH₃), 2.72 (s, 4H, 2 CH₂), 2.74 (s, 3H, CH₃), 2.96 (s, 3H, CH₃), 6.94 (s, 1H, Ar), 7.06–7.30 (m, 4H, Ar), 7.58 (s, 1H, Ar), 8.64 (l, 1H, NH) ppm; ¹³C-NMR (CDCl₃) δ 17.37, 17.66, 20.59, 23.14, 27.63, 111.31, 113.76, 117.27, 119.36, 120.40, 120.98, 124.40, 124.69, 126.64, 126.94, 127.13, 128.11, 129.97, 134.05, 137.98, 144.13, 152.23 ppm; Anal. Calcd. for C₂₂H₂₀N₂: C-82.01, H-7.81, N-10.07, found C-81.23, H-7.82, N-9.94.

3-Methyl-8,9,10,11-tetrahydro-7H-pyrido[2,3-<u>c</u>]carbazole (**4**). Obtained in 70% yield; m.p. 208°C; IR (KBr) cm⁻¹ 3200–3100 (NH), 2900, 2825, ¹H-NMR (200.13 MHz, CDCl₃) δ 2.75 (s), 7.30 (1H, d, J = 8.52 Hz), 7.62 (1H, d, J = 9.0 Hz), 7.76 (1H, d, J = 9.0 Hz), 8.43 (1H, sl, NH), 8.56 (1H, d, J = 8.5 Hz) ppm; ¹³C-NMR (MHz, CDCl₃) δ 23.78 (<u>CH</u>₂), 23.39 (<u>CH</u>₂), 23.25 (<u>CH</u>₂), 22.51 (<u>CH</u>₂), 24.47 (<u>CH</u>₃), 111.99, 120.07, 121.68, 131.00, 133.80, 144.36, 154.00 ppm; Anal. Calcd. for C₁₆H₁₆N₂: C-81.35, H-6.77, N-11.86, found C-80.74, H-6.06, N-11.04.

3. Results and discussion

The usual preparation of these substances is based on the well known Fischer indole cyclization between an acyclic or cyclic ketone and appropriated hydrazine derivative of quinoline. Then an appropriated alkylaminoquinoline, 2,4,8-trimethyl-5-aminoquinoline, 2,4,5,8-tetramethyl-6-aminoquinoline or 2methyl-6-aminoquinoline is diazotized and reduced *in situ* to give the hydrazine which was not isolated. When the hydrazine was reacted with cyclohexanone or α -tetralone the only product obtained were the angular tetrahydropyridocarbazoles **1**–**4**. During the preparation of compound **2** the methyl group at position 5 was eliminated. Although this is an unusual result, similar observation had been already reported in the literature.

3.1. Mass spectrometry studies

Compounds 1, 2 and 4 in EI showed high intensities for the molecular ion M^{+} and the retro Diels Alder (RDA) ion, and low intensities for the $[M-1]^+$ ion. Compound 3 produced intense molecular and $[M-1]^+$ ions and no measurable RDA ion (Table 1).

ESI-MS/MS, FAB and EI mass spectrometry [4-11] techniques were used to investigate the competitive RDA fragmentation (**1a**) and a possible pathway leading to the azatropyliumion (**1c**) (Fig. 2). To

Electron ionization (70 eV) spectra data for $1-4$				
Compound	M^{+}	$[M-1]^+$	$[M-28]^+$	Others m/z
1	264 (100)	263 (21)	236 (90)	265 (20), 266 (3), 132 (18), 118 (30)
2	264 (100)	263 (21)	236 (85)	265 (18), 266 (2), 205 (3), 186 (10), 185 (12), 132 (18), 118 (37)
3	312 (100)	311 (92)	None	313 (27), 314 (3), 297 (4), 293 (2), 156 (32), 155 (43)
4	236 (97)	235 (24)	208 (100)	237 (18), 238 (2), 209 (17), 118 (10), 104 (7).

 Table 1

 Electron ionization (70 eV) spectra data for 1–4



Fig. 2. Two possible pathways for the fragmentation of 1.

confirm that an ethylene molecule is lost when the $[M-28]^+$ ions are formed, high resolution mass spectra were obtained (Table 2). Indeed, the fragments $[M-28]^+$ were consistent with $[M-C_2H_4]^+$ and not with $[M-H-HCN]^+$. Another evidence for the RDA fragmentation of **1**, **2** and **4** is the absence of the RDA fragment for **3** suppressed by its condensed benzene ring.

The FAB mass spectra of 1–4 (Table 3) show mainly the molecular ion M^+ , which is not so often observed in FAB spectra [12–15], the protonated molecule $[M+1]^+$, the $[M-1]^+$ and the $[M-28]^+$ RDA fragments. FAB generates the RDA fragment in moderate intensity for 1, 2 and 4.

Table 4 reports the ESI-MS spectra of 1–4, in which the RDA fragment $[M-28]^+$ was also observed for 1, 2, and 4. RDA also occurs from MH⁺ by collision-induced dissociation (CID). When the protonated molecule is subjected to ESI-MS/MS, those for 1, 2 and 4 form the RDA ion as the major fragment (spectra not shown).

The RDA fragment of m/z 236 from both 1 and 2, also subjected to CID, forms m/z 221 by methyl loss, a fragment of m/z 206 by CH₂O loss, and a fragment of m/z 194 by the combined loss of [H+CH₃CN].

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Compound		HR M ^{+.}		HR [M-28] ⁺
1	$C_{18}H_{20}N_2$	264.16265 ($\Delta = 0.00095$)	$C_{16}H_{16}N_2$	236.1306 ($\Delta = 0.00075$)
2	$C_{18}H_{20}N_2$	264.16265 ($\Delta = 0.00047$)	$C_{16}H_{16}N_2$	236.1313 ($\Delta = 0.00005$)
3	$C_{22}H_{20}N_2$	312.1626 ($\Delta = 0.00005$)	_	_
4	$C_{16}H_{16}N_2$	236.1304 ($\Delta = 0.00095$)	$C_{14}H_{12}N_2$	208.1011 ($\Delta = 0.00105$)

 $\label{eq:able 2} Table \ 2 \\ High \ Resolution \ (HR) \ data \ for \ M^{+ \cdot} \ and \ [M-28]^+ \ fragments$

FAB mass spectra of 1-4 in a matrix of NBA				
Compound	M ^{+.}	[M+1] ⁺	[M-1] ⁺	RDA
1	264 (100)	265 (86)	263 (60)	236 (52)
2	264 (100)	265 (87)	263 (50)	236 (40)
3	312 (100)	313 (58)	311 (70)	None
4	236 (62)	237 (100)	235 (23)	208 (24)

Table 3

Table 4 ESI-MS mass spectra of **1–4**

	-	
Compound	$[M+1]^+$	RDA
1	265 (82)	236 (72)
2	265 (92)	236 (48)
3	313 (67)	None
4	237 (100)	208 (45)

The $[M-1]^+$ fragment is observed by EI (Table 1) and FAB (Table 3). Hydrogen loss is common for benzyl derivatives [16–18], methylquinolines and isoquinolines [19–21]. However, when a methyl group is attached to the homoaromatic ring the rearrangement of the benzyl cation to the tropylium ion often occurs, and when the methyl group is attached to the pyridine ring, the analogous azatropylium ion is observed. These alternative dissociations are exemplified for the model compound 2,4,8-trimethylquinoline **5** in Fig. 3, yielding either the tropylium ion **5f** or the azatropylium ion **5b** or **5d**.

Draper and Maclean [22] studied the azatropylium mechanism by deuterium labeling dimethylquinoline. They suggested hydrogen atom loss and ring expansion, followed by loss of CH₃CN or HCN. However, our results showed that the loss of hydrogen atom occurred but without HCN loss [?].

To stablish the more favorable H atom loss from M^+ (Fig. 3), we performed semi-empirical AM1 and PM3 calculations for the model ions **5a**–**f**.

Both the AM1 and PM3 results (Table 5) indicate that the most stable $[M-H]^+$ ion is **5e**, that formed when the hydrogen atom is lost from the methyl group at position 8. Loss of a hydrogen atom from the methyl group in positions 2 (**5a**) or 4 (**5c**) leads to ions that are at least 10 kcal/mol less stable than **5e**. Similarly, the tropylium ion **5f** formed from **5e** is more stable by near 5 kcal/mol than that originated from the rearrangement of the cation of position 2 (**5b**) or 4 (**5d**).

This finding for the model compound **5** suggests that the m/z $[M-1]^+$ in the tetrahydropyridocarbazoles **1**, **2** and **3** correspond to H loss from the methyl group attached to the homoaromatic ring. Compounds **1**, **2** and **4** (Table 1) also show doubly charged M^{2+} and $[M-28]^{2+}$ ions, the latter corresponding to RDA fragmentation of M^{2+} . Compound **3** shows two doubly charged ions, m/z 156 $[M]^{2+}$ and 155 $[M-1]^{2+}$.



Fig. 3. Possible pathways for H loss of the model compound 5.

	Δ Hf ⁰ (kcal/mol) AM1	$\Delta \mathrm{Hf}^0$ (kcal/mol) PM3
5a	243.45	234.94
5b	231.81	230.37
5c	242.65	234.13
5d	231.33	229.06
5e	227.60	223.13
5f	227.33	223.81

 Table 5

 Semi-empirical calculations using AM1 and PM3 methods for the ions formed upon the H loss from 5

4. Conclusions

This study showed that the $[M-28]^+$ fragment ions of tetrahydropyridocarbazolesare formed by RDA fragmentation. Although the formation of azatropylium was observed, it was not possible establish it subsequent loss of HCN. Semi-empirical calculation provided some evidence on the nature of tropy-

lium ions $[M-1]^+$. Calculation of ΔHf^0 indicated that $[M^+-1]$ could be formed preferentially when a hydrogen atom is lost from the methyl substituent of the homoaromatic ring.

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