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Short communication

Fragmentation pathway of dopamine transporter ligands: *N*-substituted-2β-carbomethoxy-3β-phenylnortropane derivatives

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Abstract

Electron impact mass spectra of iodinated tropane derivatives which are of clinical importance are described. The major fragment ions were found to come from the cleavage of the Cα–Cβ bond of the bridgehead nitrogen, affording to hydrogenopyrrolidine derivatives ions. The fragment ions generated from the EI mass spectra were assigned and fragmentation mechanisms were proposed.

Keywords: Tropane derivatives; EI mass fragmentation

1. Introduction

Cocaine **1** is a well known psychoactive drug [1] which has been shown to bind at the dopamine, serotonin and norepinephrine transporters (DAT, SERT, NET, respectively) in the central nervous system (CNS) [2–5]. Moreover, as the DAT inhibition induces an increase in the dopamine concentration in the synapse cleft, affording to the potentiation of the dopaminergic neurotransmission [6], the inhibition of dopamine reuptake sites by cocaine was found to be responsible of its psycho stimulant effects. In addition, as dysfunction of the dopaminergic neurotransmission is implicated in several neurodegenerative disorders such as Parkinson's disease [7], the in vivo quantification of the DAT would be helpful to understand this disease, as well as to diagnose it earlier [8,9]. In this aim, nuclear imaging techniques such as positron emission tomography (PET) or single photon emission computed tomography (SPECT) are suitable to quantify the DAT concentration in vivo. These imaging techniques require the development of radiopharmaceuticals, labeled with β^+ (PET) or γ (SPECT) emitters, with high affinity and selectivity for the biological target (DAT). Our efforts in this field were focused towards the design and the synthesis of iodinated cocaine derivatives **2** (Fig. 1) which exhibited high affinity and specificity for the DAT [8].

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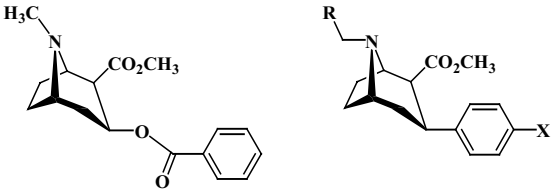


1 Cocaine **2a-l**

Fig. 1. Cocaine **1** and iodinated tropane derivatives **2**.

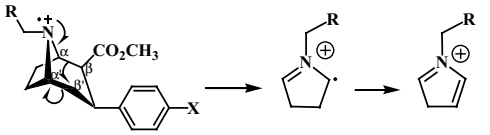
Among these, a compound named PE2I (**2j**), in which the nitrogen substituent is a (*E*)-3-iodoprop-2-enyl group and the phenyl substituent is a methyl moiety, exhibited in vitro the highest affinity and selectivity for the DAT [10]. Moreover, when labeled with iodine-125, PE2I showed a sufficient lipophilicity to cross the blood brain barrier and presented ex vivo, in rats, a high affinity and selectivity for the DAT [10]. In addition to these goods results, in vivo kinetic parameters demonstrated that PE2I labeled by iodine-123 or carbone-11 could be used to explore the DAT in the living human brain either by PET or SPECT [11]. As these cocaine-like compounds are now available as clinical tools to image the DAT in vivo, the general fragmentation pathway of *N*-alkyl tropane derivatives **2a**–**l**, compared to cocaine itself is described here, in electron impact (EI) mass spectral conditions.

2. Experimental

All the tropane derivatives were synthesized in our laboratory as it was published [8]. For general unknown screening in cases of acute poisoning, libraries of EI-mass spectra are extremely helpful for the identification of drugs and organic compounds and their metabolites. Library search with good fit is only possible if the same standard ionization conditions are used for the sample as were used for setting up the library, i.e., 70 eV electron energy and approximately unit resolution for GC/EI-MS. We have used a quadrupole mass spectrometer (Hewlett-Packard 5989A, Palo Alto, California) tuning of the source potentials and mass resolution have been performed, before use, with calibration compound like perfluorotributylamine (PFTBA) to obtain defined ranges of relative ion abundances for good library search results. The following source conditions were maintained: source temperature 200 °C, quadrupole temperature 100 °C, trap current 250 μA. All samples (1 μl) were introduced using a HP 5890 Series II gas chromatograph with a HP-5 (30 m length, 0.32 mm i.d. and 0.25 μm film thickness) capillary column, and helium (99.99%) carrier gas at a flow rate of 1.3 ml min^{-1} were used. The injector and interface temperature were both set at 250 °C. The column temperature was programmed to start at 50 °C and after 2 min to rise at 10 ml min^{-1} to 250 °C. Data was collected in full scan mode (*m/z* 40–600) using ChemSystem software that was running under HP-UX operating system on a workstation HP Apollo 9000 Series 400.

3. Results and discussion

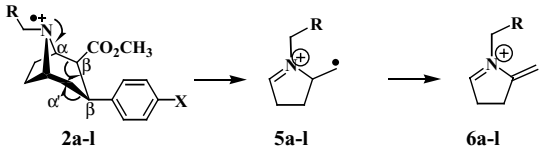
In order to develop new dopamine transporter ligands, we have synthesized several iodinated tropane derivatives. The structures of these compounds are reported in Fig. 1. Comparatively to the cocaine structure, these compounds exhibit an alkynyl, alkenyl, or benzyl substituent at the bridgehead nitrogen and a substituted phenyl ring directly linked at the 3β-position. Usual quantitative methodology for the determination of cocaine in biological matrices includes: high-performance liquid chromatography (HPLC) [12], gas chromatography/mass spectroscopy (GC-MS) [13], eventually coupled to positive ion chemical ionization [14], or liquid chromatography-mass spectrometry (LC-MS) [15,16]. The high sensitivity and selectivity provide by atmospheric pressure ionization mass spectrometry (API/MS) when coupled to liquid chromatography/tandem mass spectrometry (LC/MS/MS) have reduced the time required for method development and sample analysis of drugs [17,18]. We did the mass spectra of the tropane derivatives under EI conditions, without any decomposition by introduction sample through the direct-inlet probe heated at 200 °C. The elemental compositions of the major ions determined by accurate mass measurement are given in Schemes 1–4, and are illustrated by one spectrum in Fig. 2. The presence of a nitrogen and an oxygen could lead to two types of primary ionization. The mass spectrum of cocaine presents a peak in small amount of 2% at *m/z* 272 (M^+ minus OCH_3) suggesting the ionization of the oxygen of the carbomethoxy



2a-l 3a-l 4a-l

	R	X	GCt_R	*m/z* (M^+) (2)	*m/z* (%) (3)	*m/z* (%) (4)
1	Cocaine		23.2	303 (4%)	83 (43%)	82 (100%)
a	CH=CH-CH_3	I	21.5	425 (16%)	123 (65%)	122 (100%)
b	C(Br)=CH_2	I	26.5	489-491 (3-2%)	187-189 (26-23%)	186-188 (22-26%)
c	C#CH	I	21.2	409 (9%)	107 (100%)	106 (46%)
d	C_6H_5	I	28.2	461 (16%)	159 (12%)	158 (89%)
e	CH=CH-C_6H_5	I	47.2	487 (22%)	185 (29%)	184 (44%)
f	C(CH_3)=CH_2	I	22.2	425 (24%)	123 (32%)	122 (100%)
g	C_6H_4-*o*-CH_3	I	25.7	475 (19%)	173 (58%)	172 (100%)
h	CH=CHI	H	25.3	411 (15%)	235 (39%)	234 (33%)
i	CH=CHI	I	31.7	537 (26%)	235 (50%)	234 (41%)
j	CH=CHI	CH_3	27.3	425 (24%)	235 (62%)	234 (40%)
k	CH=CHI	$CH(CH_3)_2$	26.8	453 (38%)	235 (77%)	234 (45%)
l	CH=CHI	$CH_2CH_2CH_3$	27.9	453 (39%)	235 (71%)	234 (45%)

Scheme 1. GC-MS data of the tropane skeleton leading to fragments **3** and **4**.



2a-l 5a-l 6a-l

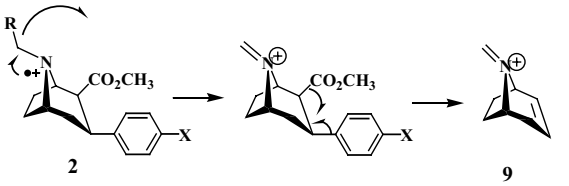
	m/z (%) (5)	*m/z* (%) (6)
1	97 (9%)	96 (19%)
a	137 (9%)	136 (24%)
b	201-203 (2%)	200-202 (8%)
c	121 (0%)	120 (44%)
d	173 (13%)	172 (28%)
e	199 (5 %)	198 (13%)
f	137 (7 %)	136 (24%)
g	187 (4 %)	186 (12%)
h	249 (3%)	248 (14%)
i	249 (3%)	248 (15%)
j	249 (4%)	248 (19%)
k	249 (4%)	248 (21%)
l	249 (2%)	248 (20%)

Scheme 2. Fragmentation and abundance leading to 2-methyl-3,4-dihydropyrrole derivatives **5** and **6**.

with a demethoxylation. This mechanism is observed for all the derivatives **2** in a very few amount (less than 2%). It seemed that the most important ionization is on the nitrogen atom as the two general types of fragmentation have been observed for all these tropane derivatives and are issued from a homolytic Cα–Cβ bond cleavage of the tropanic skeleton. The first fragmentation pathway is presented in Scheme 1. The Cα′–Cβ′ cleavage in a concerted push/pull type mechanism could lead to a radical positive 3,4-dihydro-2*H*-pyrrolinium 3, and, upon subsequent loss of $H^{\bullet}$, a to cationic *N*-substituted-3*H*-pyrrolinium **4** shifted to lower mass by 1 amu. This fragmentation pathway is observed for all the compounds and the abundance of ions **3** and **4** is given in Scheme 1.

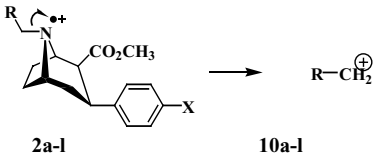
The second pathway is represented in Scheme 2 and could be explained by the cleavage of the Cγ–Cβ′ bond. These fragments, 2-methylene-3,4-dihydropyrrole derivatives **5** and **6**, led to peaks at 14 amu higher comparatively to the peaks obtained for the fragments **3** and **4** described above. The fragment **5** with the methyl radical positive ion is obtained in a very small amount (Scheme 2).

The loss of an iodo radical $\mathbf{I}^{\bullet}$ of the corresponding iodovinylic compounds (**2h–l**) led to the major abundance fragment (**7h–l**) (100%) (Table 1). By contrast, for the compounds where the iodine atom was substituted on the phenyl ring (**2a–g**), the fragment at *m/z* ($M^{+\bullet}$ minus $\mathbf{I}^{\bullet}$), corresponding of the loss of $\mathbf{I}^{\bullet}$, was observed in a very small amount (1–3% for (**7a–g**)) (Table 1). Moreover, compounds (**2h–l**) showed a peak at *m/z* 167 corresponding to the cation



	m/z 122 (%) (9)
1	(5%)
a	(100%)
b	(84%)
c	(14%)
d	(1%)
e	(4%)
f	(100%)
g	(1%)
h	(66%)
i	(50%)
j	(83%)
k	(67%)
l	(57%)

Scheme 3. Abundance in tropidine fragmentation **9**.



	m/z (%) (10)
1	-
a	55 (77%)
b	119-121 (10-7%)
c	39 (0%)
d	91 (100%)
e	117 (100%)
f	55 (32%)
g	105 (84%)
h	167 (57%)
i	167 (55%)
j	167 (56%)
k	167 (49%)
l	167 (44%)

Scheme 4. Abundance of the side chain cleavage **10**.

iodopropenylium **8** (ICH–$CHCH_2^+$) in an abundance around 50% (Table 1).

Interestingly, all derivatives with an allyl group linked at the nitrogen (**2a–b**, **2f**, **2h–l**) exhibited an abundant ion at *m/z* 122. As compound **2b**, which bears a bromine atom at the *N*-substituent, did not presented doublet of peaks around 122 Da, but only a singlet at *m/z* 122, a fragmentation mechanism involving the *N*-substituent as leaving group could be envisaged. This ion could be obtained by a push/pull mechanism involving the cleavage of Cα–Cβ on the extra cyclic chain, and the concomitant departure of the phenyl and the ester groups (Scheme 3). The intermediate ion fragment is always observed, but in a

Table 1
Abundance of the deiodo fragments 7 and abundance of the fragments coming from the departure of the iodovinylic group (ICH=$CHCH_2^+$) **8**

	m/z (%) (**7**)	*m/z* 167 (%) (**8**)
1	–	–
a	298 (1)	–
b	364 (1)	–
c	282 (1)	–
d	334 (1)	–
e	360 (1)	–
f	299 (3)	–
g	349 (1)	–
h	284 (100)	57
I	410 (100)	55
j	298 (100)	56
k	356 (100)	49
l	356 (100)	44

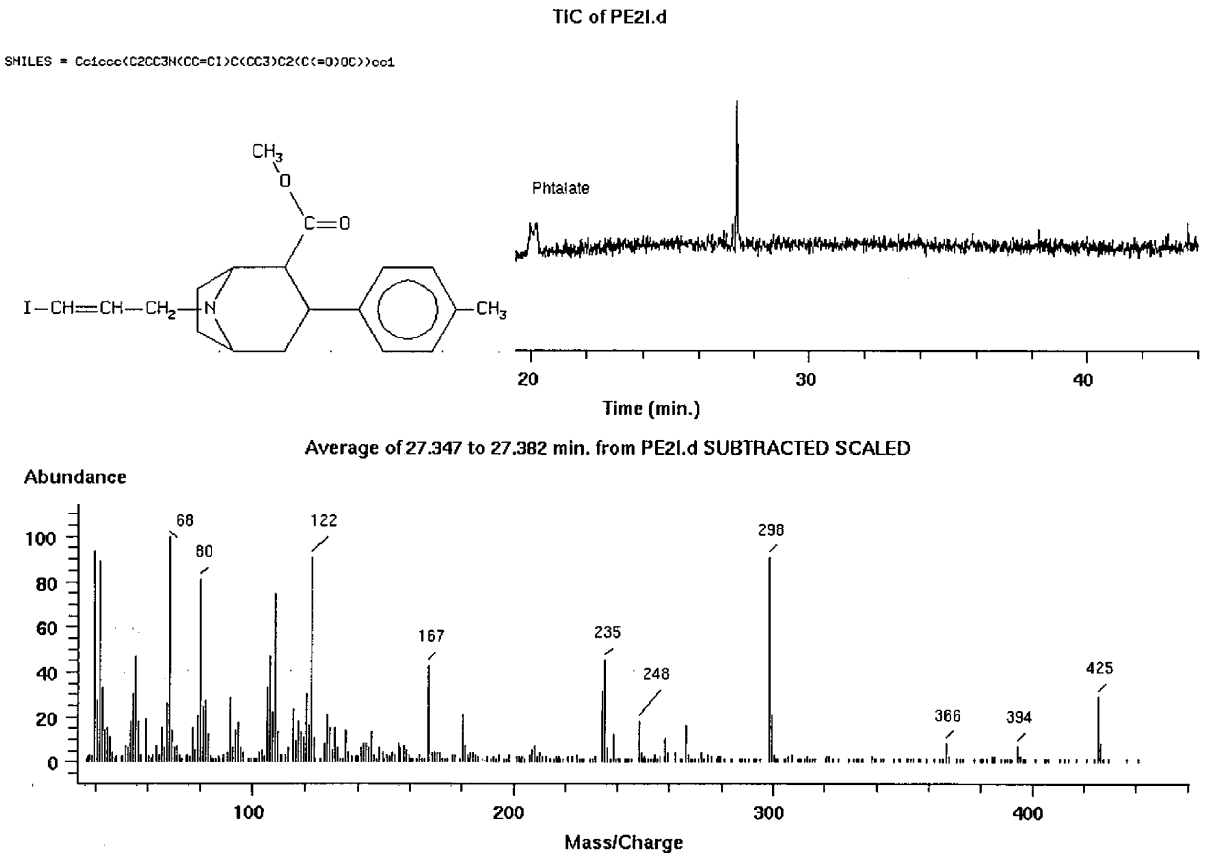


Fig. 2. Mass spectrum of the leader compound PE2I (**2j**).

very small amount (less than 2%). The corresponding tropidine fragment **9** is characterized at *m/z* 122.

Both **2a** and **2f**, which are allylic compounds, showed fragments detected at *m/z* 122 with a maximal abundance. This abundance could be explained with the both mechanisms explained previously (Schemes 1 and 3) which led to different fragments, but with the same mass in these cases. The heterolytic cleavage of the N–C bond of the extra cyclic chain leading to the cation **10** could explained the peaks with a great abundance of 100%, for compounds **2d**, **2e** and **2g** at *m/z* 91, *m/z* 117, and *m/z* 105, respectively (Scheme 4).

4. Conclusion

Mass spectral analysis of tropane derivatives has been carried out, explaining the different major pathway. It would be useful for the routine analysis of tropane alkaloids in cases of suspected tropane alkaloids poisoning.

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References

[1] L.G. Bouknight, R.R. Bouknight, Postgrad. Med. 83 (1988) 115–118, 121–114, 131.
[2] M.C. Ritz, E.J. Cone, M.J. Kuhar, Life Sci. 46 (1990) 635–645.
[3] M.J. Kuhar, Life Sci. 13 (1973) 1623–1634.

[4] M.J. Kuhar, M.C. Ritz, J.W. Boja, Trends Neurosci. 14 (1991) 299–302.
[5] J.A. Javitch, R.O. Blaustein, S.H. Snyder, Mol. Pharmacol. 26 (1984) 35–44.
[6] F.I. Carroll, A.H. Lewin, J.W. Boja, M.J. Kuhar, J. Med. Chem. 35 (1992) 969–981.
[7] H.B. Niznik, E.F. Fogel, F.F. Fassos, P. Seeman, J. Neurochem. 56 (1991) 192–198.
[8] P. Emond, L. Garreau, S. Chalon, M. Boazi, M. Caillet, J. Bricard, Y. Frangin, L. Mauclaire, J.C. Besnard, D. Guilloteau, J. Med. Chem. 40 (1997) 1366–1372.
[9] P.D. Mozley, J.B. Stubbs, H.J. Kim, W. McElgin, M.P. Kung, S. Meegalla, H.F. Kung, J. Nucl. Med. 37 (1996) 151–159.
[10] D. Guilloteau, P. Emond, J.L. Baulieu, L. Garreau, Y. Frangin, L. Pourcelot, L. Mauclaire, J.C. Besnard, S. Chalon, Nucl. Med. Biol. 25 (1998) 331–337.
[11] J.T. Kuikka, J.L. Baulieu, J. Hiltunen, C. Halldin, K.A. Bergstrom, L. Farde, P. Emond, S. Chalon, M. Yu, T. Nikula, T. Laitinen, J. Karhu, E. Tupala, T. Hallikainen, V. Kolehmainen, L. Mauclaire, B. Maziere, J. Tiihonen, D. Guilloteau, Eur. J. Nucl. Med. 25 (1998) 531–534.
[12] L.J. Murphey, G.D. Olsen, R.J. Konkol, J. Chromatogr. Biomed. Appl. 613 (1993) 330–335.
[13] N. De Giovanni, S.S. Rossi, J. Chromatogr. B Biomed. Sci. Appl. 658 (1994) 69–73.
[14] J.A. Bourland, E.F. Hayes, R.C. Kelly, S.A. Sweeney, M.M. Hatab, J. Anal. Toxicol. 24 (2000) 489–495.
[15] P.M. Jeanville, E.S. Estape, S.R. Needham, M.J. Cole, J. Am. Soc. Mass Spectrometry 11 (2000) 257–263.
[16] S. Pichini, R. Pacifici, M. Pellegrini, E. Marchei, E. Perez-Alarcon, C. Puig, O. Vall, O. Garcia-Algar, J. Chromatogr. B 794 (2003) 281–292.
[17] Y. Xia, P. Wang, M.G. Bartlett, H.M. Solomon, K.L. Busch, Anal. Chem. 72 (2000) 764–771.
[18] K. Srinivasan, P.P. Wang, A.T. Eley, C.A. White, M.G. Bartlett, J. Chromatogr. B Biomed Sci. Appl. 745 (2000) 287–303.