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Letter to the Meeting

Dear Sir

Chromophoric Derivatives of Amphetamine Analogues: Structural Characterization by Electron Impact Mass Spectrometry

The widespread abuse of amphetamines has markedly increased, to become a serious social problem. Amphetamine (AMP), methamphetamine (MEAMP), methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) represent the most frequently abused compounds owing to their powerful central stimulant effects.[1]

Clinical and forensic toxicology laboratories are therefore strongly interested in developing reliable analytical procedures to detect such substances in biological fluids. Many analytical approaches have been proposed in this field, mainly based on gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) of suitable derivatives.[2,3] High-performance liquid chromatography (HPLC) represents an interesting alternative technique to GC/MS.[4] Ultraviolet (UV) detection is the most popular approach in HPLC and the development of an HPLC–UV method for the detection of the above amines in urine was therefore undertaken. Unfortunately, amphetamines show relatively weak radiation absorption in the UV range. Highly chromophoric derivatives were therefore prepared by condensing the four amines with sodium naphthoquinone-4-sulphonate (NQS) to obtain compounds **1–4** (Fig. 1). Their structural characterization, required in the development of the complete HPLC method, was studied by electron impact (EI) mass spectrometry.

Compounds **1–4** were synthesized as follows: 1.5 cm^3 of an 8% aqueous solution of $NaHCO_3$ and 1 cm^3 of a 0.5% aqueous solution of NQS (Merck, Darmstadt, Germany) were added to 1 mg of each standard amphetamine (Division of Narcotic Drugs, United Nations, Vienna, Austria). The resulting mixtures were heated at 70 °C for 60 min. After

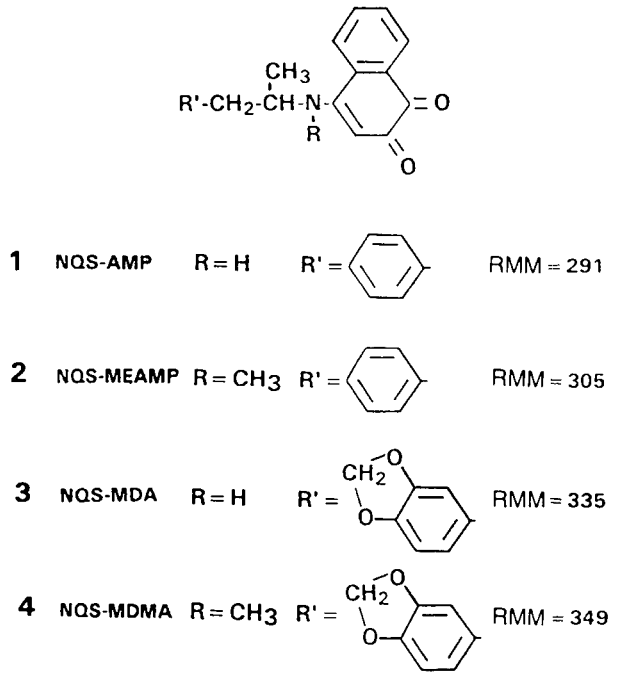

Figure 1. Structures of compounds **1–4**

Table 1. EI mass spectra and MIKE spectra of $M^{+\cdot}$ of compounds 1–4

	1		2		3		4	
Ionic species	EI *m/z* (Relative intensity, %)	MIKE *m/z* (Absolute intensity, %)	EI *m/z* (Relative intensity, %)	MIKE *m/z* (Absolute intensity, %)	EI *m/z* (Relative intensity, %)	MIKE *m/z* (Absolute intensity, %)	EI *m/z* (Relative intensity, %)	MIKE *m/z* (Absolute intensity, %)
$M^{+\cdot}$	291		305		335		349	
	(1)		(2)		(6)		(10)	
$[M - CH_3]^+$	276		290	290				
	(2)		(1)	(9)				
$[M - CO]^{+\cdot}$	263	263	277	277	307		321	
	(0.8)	(19)	(2)	(14)	(0.9)		(1.1)	
b	200	200	214	214	200	200	214	214
	(100)	(81)	(25)	(11)	(91)	(85)	(100)	(73)
d	173		187	187	173		187	187
	(25)		(17)	(15)	(9)		(38)	(3)
c	172		186	186	172		186	186
	(23)		(100)	(48)	(41)		(79)	(9)
$[R' - CH_2]^+$	91		91		135	135	135	135
	(23)		(33)		(100)	(15)	(69)	(15)
$[C_9H_5NRO]^+$	144		158	158	144		158	
	(3)		(6)	(3)	(2)		(14)	
$[C_8H_5NR]^+$	116		130		116		130	
	(4)		(5)		(3)		(15)	
$[C_8H_5N]^{+\cdot}$	115		115		115		115	
	(9)		(15)		(9)		(15)	
$[C_7H_4N]^+$	102		102		102		102	
	(10)		(11)		(30)		(24)	

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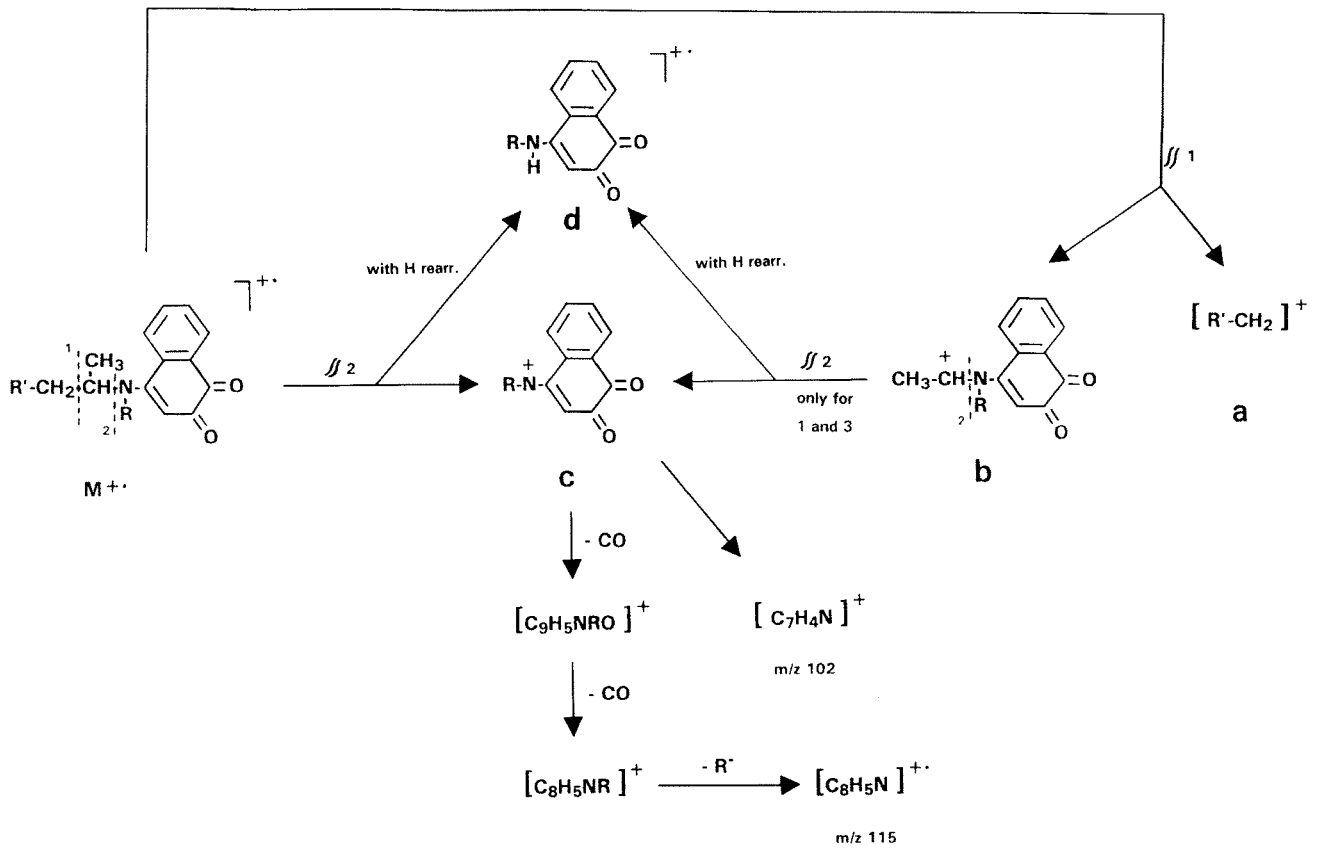

Scheme 1. Common EI-induced fragmentation pattern of compounds **1–4**.

cooling, they were extracted with 5 cm^3 of CCl_4 and the organic phases obtained were evaporated to dryness under a stream of nitrogen at 40 °C. The residues were analysed by mass spectrometry.

All mass spectrometric measurements were performed on a VG-ZAB-2F instrument[5] operating under EI conditions (70 eV, 200 μA, 200 °C source temperature). Samples were introduced by the direct inlet system with a probe temperature of 280 °C. Metastable transitions were detected by mass-analysed ion kinetic energy (MIKE) spectroscopy.[6]

Electron impact mass spectra and the MIKE spectra of $M^{+\cdot}$ species of compounds **1–4** are reported in Table 1. The common fragmentation pattern, as obtained by metastable ion studies, is shown in Scheme 1.

EI mass spectrometry, with the aid of MIKE experiments, allowed confirmation of the expected structures and the study of the characteristic mass spectrometric behaviour of the above compounds.

Although they were not very abundant, molecular ions were detected for all the compounds, and their EI-induced decompositions turned out to be highly diagnostic from the structural point of view. In fact, cleavage 1 in Scheme 1 leads to the two complementary ions (*a* and *b*), specific for each compound (*m/z* 91 and 200 for **1** and 91 and 214 for **2**, 135 and 200 for **3** and 135 and 214 for **4**). However, the formation of both ion species, *a* and *b*, is observed in the MIKE spectra of $M^{+\cdot}$ of **3** and **4** only (see Table 1). The lack of fragment ions *a* at *m/z* 91 in the MIKE spectra of **1** and **2** can be justified in terms of the Stevenson–Audier rule,[7] owing to the lower ionization energy of the nitrogen-containing fragment (structure *b*) with respect to the ionization energy of the tropylium fragment ($a = m/z$ 91).

Cleavage 2 in Scheme 1 leads to fragment ions indicative of the presence of the *N*-methylated moiety. It should be noted that the formation of fragment ions *c* is a favoured process for compounds **2** and **4**, under both EI and MIKE conditions, owing to the inductive effect of the methyl group. For compounds **1** and **3**, fragment ions *c* and *d* do not arise directly from molecular ions but from ions *b*, as proved by MIKE experiments.

The MIKE spectra of ion species at *m/z* 172 and 200 originating from **1** and **3** almost overlap, thus proving the identical structure of ions with the same *m/z* value arising from different compounds. The same results are obtained when analysing the ion species at *m/z* 186 and 214 originating from **2** and **4**.

In conclusion, the present mass spectrometric data allowed the structural characterization of the synthesized chromophoric derivatives. In particular, MIKE spectroscopy provided its unique utility in the structure elucidation of the chemical species of interest. In this context it can be successfully applied also to reveal the possible presence of side products of reactions and to obtain information about their structures. Further, it shows other advantages over the more traditional GC/MS approach in this field: time-consuming multi-step sample pretreatments are avoided, the analysis time is reduced and higher sensitivity and specificity are obtainable.

Yours

G. FRISON, F. CASTAGNA, G. BRUSINI and L. TEDESCHI
Centre of Behavioural and Forensic Toxicology,
Istituto di Medicina Legale,
University of Padova,
Via Falloppio 50,
I-35121 Padova,
Italy

R. SERAGLIA and P. TRALDI
CNR, Servizio di Spettrometria di Massa,
Area della Ricerca di Padova,
Corso Stati Uniti 4,
I-35100 Padova,
Italy

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