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Positive and Negative Ion Mass Spectrometry of Benzophenones, the Acid-Hydrolysis Products of Benzodiazepines

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Summary. Positive electron impact (EI), positive chemical ionization (CI), and negative CI mass spectra of 14 benzophenones are presented. In the positive EI mode, intense molecular peaks appeared for most compounds; some other peaks due to splitting at both sides of the carbonyl group also appeared. In the positive CI mode, $[M+1]^+$ quasi-molecular ions together with $[M+C_2H_5]^+$ peaks were observed for all compounds; some fragment peaks were common to those in the positive EI mode. In the negative CI mode, the spectra were much simpler than those in the positive EI or CI mode. In the 1 Torr negative CI mode, some spectra showed only single molecular anions; in the 0.01 Torr negative CI mode, halogen or nitro peaks appeared in addition to the molecular anions. An extraction procedure for benzophenones from human urine and plasma after heating in strong acid, and their separation by gas chromatography (GC) are also presented to serve for their actual identification by GC/mass spectrometry.

Key words: Benzophenones, mass spectrometry – Mass spectrometry, negative ion chemical ionization – Benzodiazepines, mass spectrometry

Zusammenfassung: Es werden positive Elektronenstoß-(EI)-, positive chemische Ionisierungs-(CI)- und negative CI-Massenspektren von Benzophenonderivaten beschrieben. Beim positiven EI-Modus wurden bei fast allen Verbindungen intensive Molekülpeaks beobachtet; andere Peaks entstehen durch Spaltungsreaktionen beiderseits der Carbonylgruppe. Beim positiven CI-Modus erschienen quasi-molekulare Ionen $[M+1]^+$ zusammen mit $[M+C_2H_5]$-Peaks bei fast allen Verbindungen; einige Fragmentierungs-

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ionen entsprachen denen des positiven EI-Modus. Beim negativen CI-Modus waren die Spektren viel einfacher als beim positiven EI- oder CI-Modus. Im Falle des 1 Torr negativen CI-Modus zeigten einige Spektren lediglich einzelne Molekül-Anionen; beim 0,01 Torr negativen CI-Modus erschienen Halogen- oder Nitro-Peaks zusätzlich zu den Molekül-Anionen. Weiterhin wird ein Extraktionsverfahren zur Isolierung der Benzophenone aus Urin und Plasma nach stark saurer Hydrolyse beschrieben sowie die Trennung mittels Gas-Chromatographie (GC) als Vorbereitung zur Identifizierung durch GC/Massenspektrometrie.

Schlüsselwörter: Benzophenone, Massenspektrometrie – Massenspektrometrie, Benzophenone – Benzodiazepine, Massenspektrometrie

Introduction

Benzodiazepines are now the bestsellers in pharmaceutical markets in the world and used as antianxietics, hypnotics and, antiepileptics. These drugs are frequently encountered in forensic chemistry and clinical toxicology. They undergo dealkylation (R_2), reduction of a nitro group into an amino group (R_1), or hydroxylation followed by its conjugation (R_4) in the human body [1–3]. To identify benzodiazepines, detection of benzophenones, the acid-hydrolysis products, is widely used [4–6], because benzodiazepines per se and their metabolic products, such as conjugates, equally give benzophenones after acid hydrolysis, which are stable and easily extractable into organic solvents. In this paper, we present positive electron impact (EI), positive chemical ionization (CI), and negative CI mass spectra of 14 benzophenones; and also methods for their extraction from human samples and their separation by gas chromatography (GC) to serve for their actual identification by GC/mass spectrometry (MS).

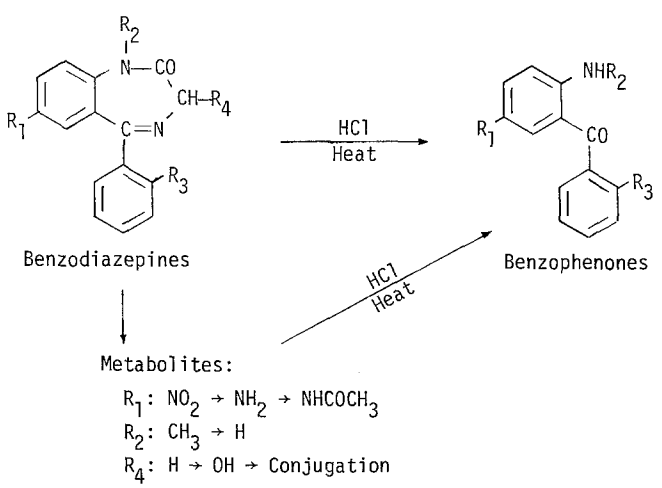


Fig. 1. Conversion of benzodiazepines and their metabolites to benzophenones by heating in acid

Materials and Methods

The 14 benzophenones investigated are listed in Table 1. The precursor benzodiazepines and their derivatives were obtained from Hoffmann-La Roche & Co., AG, Basel, Switzerland, except haloxazolam obtained from Sankyo Co., Ltd., Tokyo. The benzophenones were synthesized according to de Silva et al. [4]. Each benzodiazepine was heated in 6 N HCl at 100°C for 1 h. The pH of the solution was brought to 8–9 with 6 N KOH solution. It was extracted with ethyl ether and evaporated to dryness under the stream of nitrogen. The resulting yellow crystals of benzophenones were almost pure when checked by GC and GC/MS. Extrelut was purchased from Merck, Darmstadt (FRG); and 5% SP-2100 on Chromosorb W AW DMCS (60/80 mesh) from Gasukuro Kogyo Inc., Tokyo. Other common chemicals used were of the highest purity commercially available.

The urine and plasma obtained from healthy subjects were also used for the extraction experiments.

MS Conditions

Mass spectra in the positive EI, positive CI, and medium pressure (1 Torr) negative CI modes were recorded on a JMS-D300 (GC) MS instrument with a JMA-2000E computer-controlled data analysis system by the direct inlet method in Hamamatsu. Less than 1 μg of each benzophenone dissolved in hexane was applied to the instrument. MS conditions were: accelerating voltage 3.0 kV, ionization current 300 μA, separator temperature 280°C, and ion source temperature 220°C; in the positive EI mode, electron energy 70 eV; in the positive and negative CI modes, electron energy 200 eV, reagent gas methane and chamber pressure 1 Torr.

Mass spectra in the low pressure negative CI mode were recorded in Zürich on a LKB 2091 MS instrument, modified for CI MS as described by Ryhage [7]. Its MS conditions were: accelerating voltage 3.5 kV, ionization current 250 μA, electron energy 100 eV, separator temperature 270°C, ion source temperature 300°C and chamber pressure 0.01 Torr.

Extraction and GC Separation

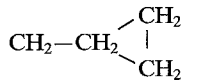
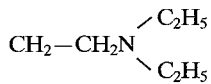
One milliliter of a test sample (urine or serum) was mixed with 1 ml 12 N HCl and heated at 100°C for 1 h. After cooling the pH of the solution was brought to 8–9 with 6 N KOH solution. It was applied to 3 g Extrelut packed in a 10-ml glass syringe. After standing for 30 min, the benzophenones were eluted with 10 ml hexane and evaporated to dryness in vacuo. The residue was dissolved in 40 μl hexane and subjected to GC analysis. GC was carried out on a Shimadzu GC-4CM instrument with a 1.0 m × 3 mm (i.d.) glass column packed with 5% SP-2100 on Chromosorb W AW DMCS (60/80 mesh). The GC conditions were: injection temperature 250°C, column temperature 220°C, and nitrogen flow rate 45 ml/min. The peaks appearing in the gas chromatogram were identified with the above JMS-D300 GC/MS instrument.

Results

Positive EI Mass Spectra. Major ions in positive EI mass spectra of 14 benzophenones are listed in Table 2. Intense molecular ions were observed for most compounds. The peaks at m/z 105 or 77, m/z 139 or 111, and m/z 123 or 95 were commonly observed for compounds with $R_3 = H$, $R_3 = Cl$, and $R_3 = F$, respectively.

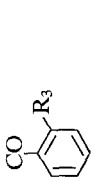
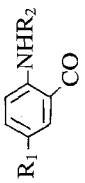
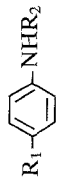
Positive CI Mass Spectra. In the positive CI mode, intense $[M+1]^+$ quasi-molecular ions together with $[M + C_2H_5]^+$ peaks appeared in all compounds (Table 3). The peaks at m/z 105, 139, and 123 appeared according to the group of R_3; these ions were common to those in the positive EI mode (Table 2). For the

Table 1. Chemical structure, retention time in GC, and corresponding precursor benzodiazepine(s) for 14 benzophenones

	Benzophenone	R_1	R_2	R_3	Retention time[a] (min)	Precursor benzodiazepine
I.	2-Amino-5-chlorobenzophenone	Cl	H	H	2.84	Chlordiazepoxide, oxazepam, chlorazepate, oxazolam
II.	2-Methylamino-5-chlorobenzophenone	Cl	CH_3	H	3.42	Diazepam, temazepam, medazepam
III.	2-Cyclopropylmethylamino-5-chlorobenzophenone	Cl		H	8.31	Prazepam
IV.	2-Amino-2′,5-dichlorobenzophenone	Cl	H	Cl	4.20	Lorazepam
V.	2-Methylamino-2′,5-dichlorobenzophenone	Cl	CH_3	Cl	4.88	N-Methyl-lorazepam
VI.	5-Chloro-2-(2-diethylaminoethylamino)-2′-fluorobenzophenone	Cl		F	12.4	Flurazepam
VII.	2-Amino-5-nitrobenzophenone	NO_2	H	H	7.82	Nitrazepam
VIII.	2-Amino-5-nitro-2′-chlorobenzophenone	NO_2	H	Cl	10.6	Clonazepam
IX.	2-Amino-5-nitro-2′-fluorobenzophenone	NO_2	H	F	6.80	Nor-flunitrazepam
X.	2-Methylamino-5-nitro-2′-fluorobenzophenone	NO_2	CH_3	F	8.22	Flunitrazepam
XI.	2,5-Diamino-2′-chlorobenzophenone	NH_2	H	Cl	6.20	7-Amino-clonazepam
XII.	2,5-Diamino-2′-fluorobenzophenone	NH_2	H	F	4.00	7-Amino-nor-flunitrazepam
XIII.	2-Amino-5-bromo-2′-fluorobenzophenone	Br	H	F	3.46	Haloxazolam
XIV.	2-Amino-5-bromobenzoylpyridine	Br	H	–	4.84	Bromazepam

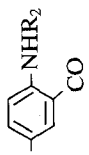
[a] The retention time was measured with a 1.0 m × 3 mm (i.d.) glass column packed with 5% SP-2100 on Chromosorb W AW DMCS (60/80 mesh) at 220°C with nitrogen flow at 45 ml/min

Table 2. Major ions in positive EI mass spectra of benzophenones

Compound number	m/z (% I)								
	M	M–1					$M-R_1-H$	$M-R_3$	Other
I.	231 (86)	230 (100)	105 (28)	154 (24)	77 (34)	126 (12)	195 (8)	nd[a]	nd
II.	245 (100)	244 (77)	105 (24)	168 (19)	77 (38)	140 (6)	nd	nd	228 (39)
III.	285 (100)	284 (11)	105 (38)	nd	77 (43)	180 (17)	nd	nd	270 (66)
IV.	265 (78)	264 (29)	139 (28)	154 (35)	111 (21)	126 (16)	229 (10)	230 (100)	nd
V.	279 (100)	278 (15)	139 (26)	168 (36)	111 (29)	140 (8)	nd	244 (87)	229 (52)
VI.	348 (2)	nd	nd	nd	nd	nd	nd	nd	86 (100)
VII.	242 (100)	241 (91)	105 (37)	165 (19)	77 (37)	nd	195 (24)	nd	nd
VIII.	276 (66)	275 (16)	139 (32)	165 (25)	111 (20)	nd	229 (6)	241 (100)	195 (24)
IX.	260 (100)	259 (50)	123 (39)	165 (20)	95 (19)	nd	213 (14)	241 (9)	nd
X.	274 (100)	273 (28)	123 (22)	179 (13)	95 (14)	nd	257 (24)	255 (6)	nd
XI.	246 (100)	245 (31)	139 (7)	135 (10)	111 (6)	107 (24)	nd	211 (54)	nd
XII.	230 (100)	229 (45)	123 (10)	135 (8)	95 (9)	107 (23)	nd	211 (19)	nd
XIII.	293 (100)	292 (51)	123 (54)	198 (27)	95 (34)	170 (17)	213 (20)	274 (12)	nd
XIV.	276 (31)	nd	nd	198 (20)	78 (19)	170 (21)	nd	nd	249 (100)

[a] nd: not detectable (intensity less than 5%)

Table 3. Major ions in positive CI mass spectra of benzophenones with methane as reagent gas

Combound number	m/z (% I)						
	M	M + 1	M + C_2H_5			M–R_1	Other
I.	231 (19)	232 (100)	260 (17)	105 (30)	154 (7)	196 (5)	nd[a]
II.	245 (35)	246 (100)	274 (16)	105 (14)	168 (6)	210 (24)	nd
III.	285 (44)	286 (100)	314 (10)	105 (21)	208 (6)	250 (33)	nd
IV.	265 (13)	266 (100)	294 (20)	139 (74)	154 (13)	230 (10)	nd
V.	279 (35)	280 (100)	308 (15)	139 (46)	168 (7)	244 (17)	nd
VI.	348 (26)	349 (100)	377 (13)	123 (7)	nd	313 (38)	86 (97)
VII.	242 (7)	243 (60)	271 (5)	105 (18)	nd	nd	213 (100)
VIII.	nd	277 (36)	305 (8)	139 (22)	nd	nd	247 (100)
IX.	nd	261 (46)	289 (5)	123 (13)	nd	nd	231 (100)
X.	nd	275 (69)	303 (10)	123 (6)	nd	nd	245 (100)
XI.	246 (38)	247 (100)	275 (15)	nd	nd	nd	nd
XII.	230 (36)	231 (100)	259 (14)	123 (22)	nd	nd	nd
XIII.	293 (18)	294 (100)	322 (11)	123 (61)	198 (5)	nd	216 (39)
XIV.	276 (31)	277 (100)	305 (15)	106 (48)	198 (14)	nd	263 (37)

[a] nd: not detectable (intensity less than 5%)

Table 4. Major ions in negative CI mass spectra of benzophenones measured at 1 and 0.01 Torr chamber pressures with methane as reagent gas

Compound number	m/z (% I)					
	Chamber pressure 1.0 Torr			Chamber pressure 0.01 Torr		
	M	Halogen or nitro	Other(s)	M	Halogen or nitro	Other(s)
I.	231 (100)	nd[a]	nd	231 (29)	35 (100)	nd
II.	245 (100)	nd	nd	245 (37)	35 (100)	nd
III.	285 (100)	nd	nd	285 (26)	35 (100)	nd
IV.	nd	35 (55)	229 (100)	–	–	–[b]
V.	279 (5)	35 (96)	229, 243 (63) (100)	nd	35 (100)	229, 243 (47) (50)
VI.	348 (100)	nd	241, 261 (45) (26)	348 (19)	35 (100)	241, 261 (27) (13)
VII.	242 (100)	nd	241, 226 (36) (8)	242 (100)	46 (5)	226 (5)
VIII.	nd	nd	210, 240 (22) (100)	nd	35, 46 (97) (11)	210, 240 (28) (100)
IX.	260 (100)	nd	259, 244 (26) (6)	260 (100)	nd	nd
X.	274 (100)	nd	nd	–	–	–
XI.	nd	35 (8)	210 (100)	210 (46)	35 (100)	nd
XII.	230 (100)	nd	209 (10)	–	–	–
XIII.	293 (4)	79 (100)	nd	–	–	–
XIV.	276 (29)	79 (100)	nd	nd	79 (100)	nd

[a] nd: not detectable (intensity less than 5%)
[b] Not determined

nitro-benzophenones (Compounds VII–X), the peaks at m/z M-29, which probably correspond to $[MH-NO_2+NH_2]^+$ were the base peaks.

Negative CI Mass Spectra. The results in both medium (1 Torr) and low (0.01 Torr) pressure negative CI modes are presented in Table 4. The negative spectra were much simpler than those in the positive EI or CI mode. In many compounds the molecular anions constituted base peaks at 1 Torr of the chamber pressure; there were almost no peaks except the molecular anions in Compounds I–III and X.

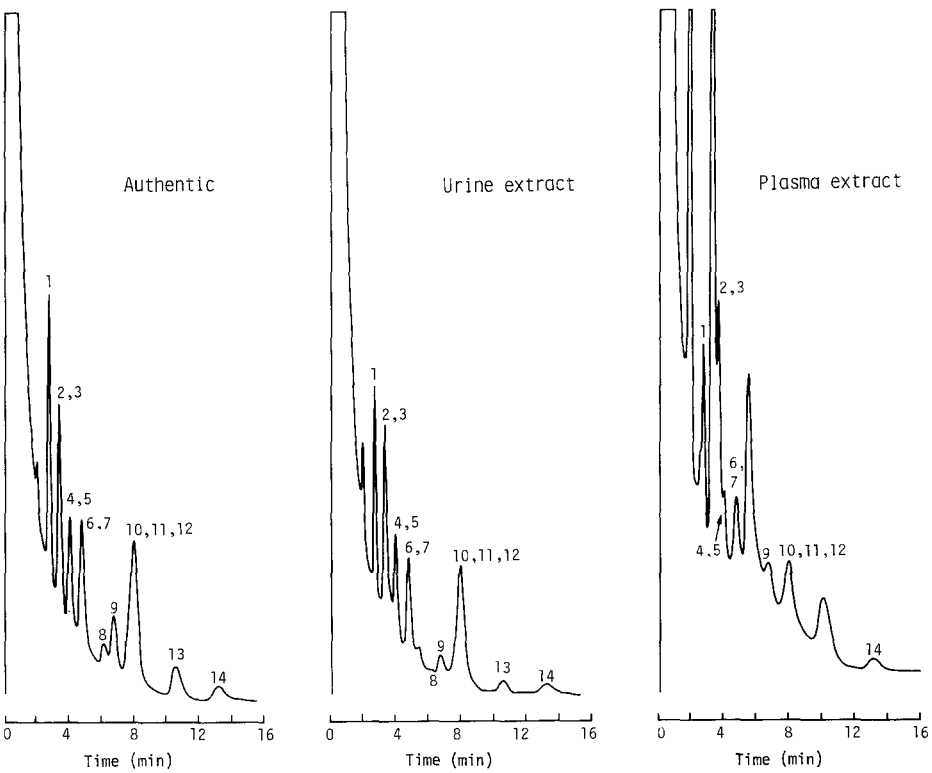


Fig. 2. GC for 14 benzophenones extracted from human urine or plasma. *Keys:* 2-amino-5-chlorobenzophenone (Compound I), 1; 2-methylamino-5-chlorobenzophenone (Compound II), 2; 2-amino-5-bromo-2′-fluorobenzophenone (Compound XIII), 3; 2,5-diamino-2′-fluorobenzophenone (Compound XII), 4; 2-amino-2′,5-dichlorobenzophenone (Compound IV), 5; 2-amino-5-bromobenzoylpyridine (Compound XIV), 6; 2-methylamino-2′,5-dichlorobenzophenone (Compound V), 7; 2,5-diamino-2′-chlorobenzophenone (Compound XI), 8; 2-amino-5-nitro-2′-fluorobenzophenone (Compound IX), 9; 2-amino-5-nitrobenzophenone (Compound VII), 10; 2-methylamino-5-nitro-2′-fluorobenzophenone (Compound X), 11; 2-cyclopropylmethylamino-5-chlorobenzophenone (Compound III), 12; 2-amino-5-nitro-2′-chlorobenzophenone (Compound VIII), 13; 5-chloro-2-(2-diethylaminoethylamino)-2′-fluorobenzophenone (Compound VI), 14. GC was isothermally carried out with a 1.0 m × 3 mm (i.d.) glass column packed with 5% SP-2100 on Chromosorb W AW DMCS (60/80 mesh). Its conditions were: column temperature 220°C and nitrogen flow rate 45 ml/min. The mixture of 14 benzophenones, 10 μg of each, was added to 1 ml of urine or plasma prior to heating and extraction (procedure see text)

In the low pressure (0.01 Torr) negative CI mode, the spectra were generally similar to those at 1 Torr of chamber pressure. However, halogen or nitro (m/z 46) peaks appeared much more easily, and constituted base peaks in Compounds I–III, V, VI, and XI.

Separation by GC. To actually identify benzodiazepines in human samples by GC/MS, the mixture of benzophenones, 10 μg of each, was added to 1 ml of urine or plasma, heated at 100°C for 1 h, extracted with the Extrelut column, and then applied to a GC column (1 m × 3 mm) of 5% SP-2100 on Chromosorb W AW DMCS. An isothermal gas chromatogram at 220°C is shown in Fig. 2. The respective retention times are also listed in Table 1. Separation of the compounds from biologic impurities was satisfactory for urine. However, some interfering peaks appeared in the chromatogram for plasma.

Discussion

In this paper, we have presented positive EI, positive CI, and negative CI mass spectra of 14 benzophenones, the acid-hydrolysis products of benzodiazepines and their metabolites. Although the positive EI mass spectra of some benzophenones were reported in 1981 [6], the data on their positive CI and negative CI spectra have never been reported before to our knowledge.

There are some advantages of detecting benzophenones over detecting benzodiazepines per se. Not only benzodiazepines, but also their conjugates and other metabolites equally give benzophenones after acid-hydrolysis (Fig. 1). Hydrolysis of the conjugates can also be achieved by glucuronidases, but typically takes more than 5 h [8]. Thus, the acid-hydrolysis method is especially useful for urine samples, because the drugs are excreted to urine largely in a hydroxylated or conjugated form [2]. In addition, the benzophenones are very stable, easily extractable into organic solvents and thus very suitable for GC/MS assays; while the direct detection of benzodiazepines by GC often suffers from their decomposition during the passage through the column [9].

We have presented negative CI mass spectra at both low (0.01 Torr) and medium (1 Torr) pressures (Table 4). The low pressure mode was first introduced by Ryhage and Brandenberger [10] in 1978, and is successfully being used in their laboratory [11] in actual forensic science practice. The negative spectra at both pressures were somewhat similar to each other, but halogen or nitro peaks appeared much more easily at the low pressure (Table 4). The negative modes gave much simpler spectra (Table 4) than did the positive EI (Table 2) and positive CI (Table 3) modes. At 1 Torr of the chamber pressure in the negative mode, many compounds showed molecular anions constituting base peaks with no other or minor fragment peaks (Table 4), showing a possibility that much higher sensitivity can be obtained in selected ion monitoring in the negative CI mode than in the positive modes. This line of experiments on sensitivity for some benzophenones is now under way in our laboratory.

Our interest is focused on identification of a drug in samples obtained from victims in actual forensic science practice. The peaks at m/z 105, 139, and 123 in

both positive EI and CI modes (Tables 2, 3) are useful for screening; they also give information on the group in the 2′-position. The same is true for the peaks at m/z 77, 111, and 95 in the positive EI mode (Table 2). To test the presence of a halogen or nitro group in the structure, the negative CI spectra at the low pressure are most useful (Table 4).

We have added a method for extraction of benzophenones from human urine and plasma, and also their GC conditions (Fig. 2). These studies together with the mass spectra in different modes seem very useful especially for screening in forensic chemistry and clinical toxicology.

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