ACTA CHEMICA SCANDINAVICA 23 (1969) 3110–3119

Derivatives of Sympathomimetic Amines for Gas Chromatography with Electron Capture Detection and Mass Spectrometry

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A variety of derivatives of amphetamine and phenmetrazine were prepared for their analysis by gas chromatography with electron capture (EC) detection. *N*-Trichloroacetyl derivatives were found to have favourable properties in terms of high EC-response, narrow and symmetric peak shape, and facile formation. Mass spectra of derivatives of amphetamine, methamphetamine, *p*-hydroxyamphetamine, phenmetrazine, methyl phenidate, chlorphentermine, and diethylpropion were recorded with a combined gas chromatograph mass spectrometer (GC-MS). The preparation of derivatives was of advantage since the free amines show tailing peaks on the phases which can be used with GC-MS. Also the mass spectrum of the derivative frequently assumed a more complex character giving more structural information and facilitating positive identification.

Current procedures for the gas chromatography of sympathomimetic amines of the amphetamine group involve either the use of alkali treated supports or conversion of the amines into suitable derivatives.[1] These methods work well with ionization detectors which permit analysis down to about 0.1 μg. Recently more sensitive and specific detectors have become available for gas phase analysis to be extended below the nanogram for substances having affinity for free electrons. Compounds with low electron affinity can also be analyzed by conversion into derivatives which give stronger electron capturing properties.

Gas chromatography combined with mass spectrometry (GC-MS) has become a powerful technique for the identification and structure determination at the ultra micro scale.[2] The preparation of derivatives for this analysis can be of advantage for increased sensitivity, better chromatographic properties, and more characteristic and easily identifiable mass spectrum.

The object of the present investigation has been to develop derivatives for primary and secondary amines suitable for gas chromatography with electron capture detectors and to study derivatives of several sympathomimetic amines with GC-MS.

EXPERIMENTAL

Derivatives were prepared from 1 mg portions of the amines in 0.1 ml of ethyl acetate. For the acyl derivatives 0.1 ml of the acid anhydrides or chlorides was added. Generally 30 min at room temperature was allowed for reaction. With perhaloacyl derivatives no free amine remained after 5 min, indicating rapid reaction.

For the *p*-trifluoromethyl benzoyl derivatives 0.1 ml of the acid chloride was used. The reaction was complete in 5 min at room temperature.

The maleamide derivatives were prepared using maleic anhydride in ethyl acetate (50 mg/ml). Reaction was instantaneous and quantitative. The resulting monobasic acid was esterified using diazomethane.

Excess perhaloacid anhydrides was eliminated under a stream of nitrogen. The less volatile anhydrides were hydrolyzed after the reaction was completed and the amide extracted into 0.1 ml of hexane. To form the Schiff bases and carbon disulphide derivative, 50°C for an hour was needed to complete the reaction. The structures of all derivatives were confirmed by mass spectrometry.

The formation of the derivatives was studied with gas chromatography using a flame ionization detector (FID). All derivatives gave single peaks. These solutions were then diluted with hexane or redistilled ethyl acetate to fit the sensitivity studies with EC-detector. The EC-response of the derivatives were compared with that of lindane using area measurements with a ball and disc integrator. The values were corrected for differences in FID-response relative to lindane (hexachlorocyclohexane).

Trifluoroacetic anhydride, pentafluoropropionic anhydride, *p*-trifluoromethylbenzoyl chloride, 1,3-dichlorotetrafluoroacetone, hexachloroacetone, pentafluorobenzoyl chloride, perfluorooctanoic anhydride were from Pierce Chemical Company, Rockford, Ill. Mono- and trichloroacetic anhydrides and trichloroacetyl chloride were from Fulka, Switzerland.

The gas chromatographic analyses were carried out on an F & M Model 402 equipped with two identical columns: one connected to flame ionization and the other to a ^{63}Ni electron capture detector. Columns were 6 feet glass packed with 100—200 mesh Gaschrom Q coated with 1 % SE-30 or 1 % OV-1; conditioning was 300°C for 48 h. N_2 carrier gas pressure was 3 kg/cm², flow rate 60 ml/min. Carrier gas through the column connected to the EC-detector was 10 % methane in argon at a pressure of 3 kg/cm².

The mass spectra were recorded on an LKB 9000 combined gas chromatograph-mass spectrometer. The column was a six feet 1 % SE-30. The flow of the carrier gas (helium) was 30 ml/min. Temperatures were: flash heater 250°, column 130—190°, molecule separator 260°, and ion source 290°. The ionizing current was 60 μA and the energy of the electrons 22.5 eV. Background spectra were recorded at each temperature and the abundance of the back ground peaks subtracted from those at the same *m/e* values in the analyzed derivative.

RESULTS

The prerequisite for a good derivative for EC-detection should be good chromatographic properties at high sensitivity, high EC-response, quantitative formation at the nanogram level and excess reagent should be removed easily and quantitatively. In the following the results are reported for several different approaches to the problem. In each case derivatives were prepared of the primary amine amphetamine [(±)-2-amino-1-phenylpropane], and of the secondary amine phenmetrazine (2-phenyl-3-methyltetrahydro-1,4-

Table 1.

Compound	Derivative	EC-Response (Lindane = 100)
Amphetamine	Trifluoroacetyl	< 0.1
	Pentafluoropropionyl	4
	Heptafluorobutyryl	9
	Perfluorooctanoyl	23
	Monochloroacetyl	0.1
	Trichloroacetyl	54
	Isothiocyanate	0.2
	Maleamide	13
	p-Trifluoromethyl benzoyl	12
	Pentafluorobenzoyl	77
Phenmetrazine	Trifluoroacetyl	< 0.1
	Pentafluoropropionyl	6
	Heptafluorobutyryl	8
	Perfluorooctanoyl	16
	Monochloroacetyl	1
	Trichloroacetyl	47
	Maleamide	7
	p-Trifluoromethylbenzoyl	16
	Pentafluorobenzoyl	52

oxazine). The EC-responses relative to the pesticide lindane (hexachlorocyclohexane) are reported in Table 1.

Attempts to condense amphetamine with halogenated ketones. It was thought that the formation of a Schiff base from amphetamine and halogenated acetone should give a derivative with a favourable EC-response since a halogen-carrying carbon would be next to the carbon nitrogen double bond. Accordingly amphetamine was warmed with an excess of either trichloroacetone, trifluoroacetone, 1,3-dichlorotetrafluoroacetone or hexachloroacetone. In each case a single peak was formed. When analyzed by mass spectrometry these compounds proved to be the acylated amines. This results from preferential elimination of haloform after the initial attack of the amine nitrogen on the carbonyl carbon.

Halogenated benzamides. The amines were reacted with *p*-trifluoromethylbenzoyl chloride and pentafluorobenzoyl chloride as described under Experimental. These derivatives could be expected to give good EC-sensitivity due to the inductive effect of the fluorine on the aromatic electrons. As seen in Table 1 the pentafluorobenzamide of amphetamine as well as phenmetrazine gave an EC-response almost comparable to that of lindane. The peak shape was, however, fairly broad and in nanogram amounts pronounced tailing occurred.

Halogenated acyl derivatives. Perfluoroacylated derivatives of amphetamine and phenmetrazine were prepared using trifluoroacetic, pentafluoropropionic, heptafluorobutyric and pentadecafluorooctanoic anhydrides. In addition monochloro and trichloroacetyl derivatives were prepared of the same compounds. In Table 1, the EC-responses are compared to that of lindane.

The trifluoroacetyl derivatives were not detectable in the largest amount (100 ng) injected. The pentafluoropropionyl derivative gave an EC-response which was 4 % of that of lindane. The heptafluorobutyryl and perfluorooctanoyl derivatives gave EC-signals which were about 9 and 23 %, respectively, relative to lindane. The perfluorooctanoyl derivative's peaks were, however, fairly broad.

Monochloroacetyl amine derivatives injected up to 100 ng produced no response from the EC-detector. The trichloroacetamides on the other hand gave a response which was about 50 % of that of lindane. The peak was sharp and symmetrical even in the lowest sensitivity range. The chromatogram of 0.1 ng of amphetamine-TCA and phenmetrazine-TCA is shown in Fig. 1.

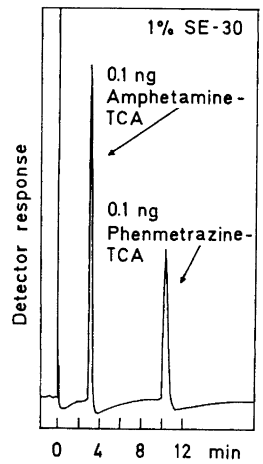

Fig. 1. Separation of 0.1 ng of the trichloroacetyl (TCA) derivatives of amphetamine and phenmetrazine. Conditions: 1 % SE-30, 160°, ^{63}Ni detector operated with a pulse interval of 50 μsec.

The trichloroacetamides of amphetamine and phenmetrazine formed rapidly also in dilute solution (1 μg/ml) of the amines in hexane or dry ethyl acetate using trichloroacetyl chloride. A 50—100 fold excess of the reagent was sufficient to give a quantitative reaction within 37 min at 30°. The reaction mixture could then be injected directly on the column or the excess reagent removed by brief extraction with 0.1 N NaOH.

Other derivatives. Lovelock [3] reported a good electron affinity for diethyl maleate. This is ascribed to the presence of the conjugated enedione

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C—C=C—C

system in the molecule. The maleamides of amphetamine and phenmetrazine were prepared using maleic anhydride. The resulting monobasic acid was converted to the methyl ester before analysis. These compounds possessed relatively good properties for analysis with the EC-detector. Responses were about 15 % relative to lindane (Table 1). Slight tailing was, however, observed, especially in the nanogram range.

Table 2.

Compound	Derivative	Mol. wt.	Molecule ion, %	Base peak	Abundant fragments (%)
Amphetamine	—	135	—	44	91 (20)
»	*N*-acetyl	177	3	44	86(68), 118(41)
»	*N*-propionyl	191	2	44	100(60), 118(37)
»	*N*-TFA	231	—	118	140(90), 91(18) 188(34), 190(34)
»	*N*-TCA	279	—	118	192(12), 91(20)
»	Acetone-eneamine	175	—	84	91(16)
p-Hydroxy amphetamine	TFA	343	—	140	230(45), 203(8)
»	TMSi	295	—	116	179(8)
Methamphet-amine	—	149	—	58	91(3)
»	*N*-acetyl	191	2	58	100(45), 91(9) 119(30) 111(32)
»	*N*-TFA	245	—	154	91(18)
»	*N*-TMSi	221	—	130	—
Phen-metrazine	—	177	13	71	56(23), 42(28)
»	*N*-propio-nyl	233	17	127	70(80), 57(53), 100(32)
»	*N*-TFA	273	8	167	70(53), 180(92)
»	*N*-TCA	322(3)	2	70	216(23), 115(84), 143(66)
»	*N*-TMSi	249	16	100	234(19)
Chlor-phentermine	—	183	—	58	168(3)
»	*N*-acetyl	225	1	58	100(69), 127(16) 166(16)
»	*N*-TFA	279	—	154	166(8)
»	*N*-TMSi	255	0.5	130	—
Methyl-phenidate	—	233	0.5	84	91(69), 150(35)
»	*N*-acetyl	309	—	126	84(96)
»	*N*-TFA		—	180	150(10)
»	*N*-TMSi	295	—	154	—
Diethyl-propion	—	205	1	100	44(68), 56(19), 77(17)

The isothiocyanate of amphetamine gave a relatively weak EC-response.

Mass spectrometry. Amphetamine [(±)-2-amino-1-phenylpropane], methamphetamine [(±)-*N*-methyl-2-amino-1-phenylpropane], *p*-hydroxyamphetamine [(±)-2-amino-1-(4-hydroxy phenyl)propane], phenmetrazine (2-phenyl-3-methyl-tetrahydro-1,4-oxazine), chlorphentermine [2-amino-2-methyl-1-(4-chlorophenyl)propane], and methylphenidate [methyl α-phenyl-α-(2-piperidyl)-acetate] were analyzed with the combined gas chromatograph mass spectrometer (GC-MS). Various derivatives of these amines were prepared to see which would have the best properties for mass spectrometric analysis. The results are summarized in Table 2.

The spectra of the acetyl, trifluoroacetyl, trimethylsilyl derivatives of amphetamine showed that two main cleavages occur, namely between the carbons of the side chain and of the carbon-nitrogen bond. This is exemplified by the mass spectrum of acetylamphetamine shown in Fig. 2.

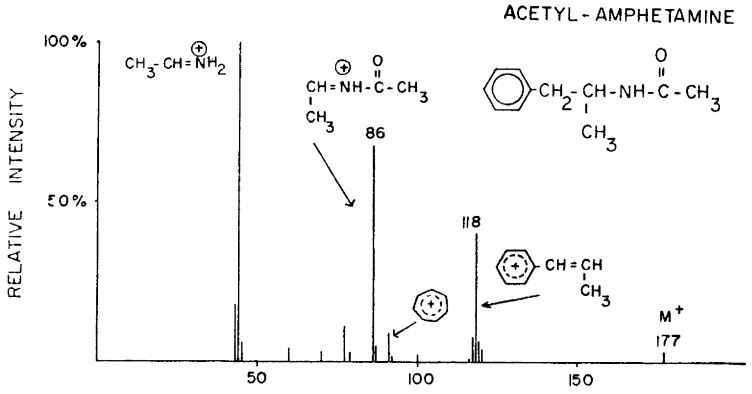

Fig. 2. Mass spectrum of *N*-acetyl amphetamine.

Characteristic peaks for all derivatives were seen at 118 due to the phenylpropenyl residue and at 91 which presumably is the tropylium ion formed from the benzyl cation. Other abundant fragments are the substituted alkyl amines, which have different *m/e* values depending on the nature of the derivative used. With acetyl amphetamine this is 86 (Fig. 2). At the low mass end a prominent peak is usually observed at 44. This is most likely due to $CH_3—CH=N^+H_2$ ion formed after α- and β-cleavage in relation to the nitrogen.

Amphetamine as the free base or after condensation with acetone gave spectra very poor in peaks. The base peak is the alkyl amine fragment. In addition a smaller fragment is seen at mass number 91 due to the benzyl residue.

The derivatives of methamphetamine gave mass spectra which showed basically similar fragmentation patterns as amphetamine (Table 2). With this compound β-fission predominated to give fragments of alkylamine ions with

and without the substituent. The free amine gave practically only one peak (Table 2).

Hydroxyamphetamine does not chromatograph well as the free base due to the presence of the free phenolic group. The trifluoroacetyl and trimethylsilyl derivatives had, however, satisfactory properties both for gas chromatography and mass spectrometry (Table 2).

The mass spectrum of phenmetrazine (Table 2) showed a fairly prominent molecular ion ($m/e=177$). The base peak had a mass number of 71 and is formed from β-cleavage of the morpholine ring with the elimination of the residue containing the benzene ring ($m/e=106$). A smaller fragment due to removal of a methyl radical from the fragment giving the base peak was also observed.

Substitution of the secondary nitrogen in phenmetrazine with acyl or trimethyl silyl groups results in spectra richer in abundant fragments. Cleavage β to the benzene ring and the nitrogen is again typical. The major peaks arise from the substituted alkylamine ion and its further degradation. This is exemplified by the mass spectrum of TMSi-phenmetrazine shown in Fig. 3. The proposed fragmentation pattern is indicated in the figure.

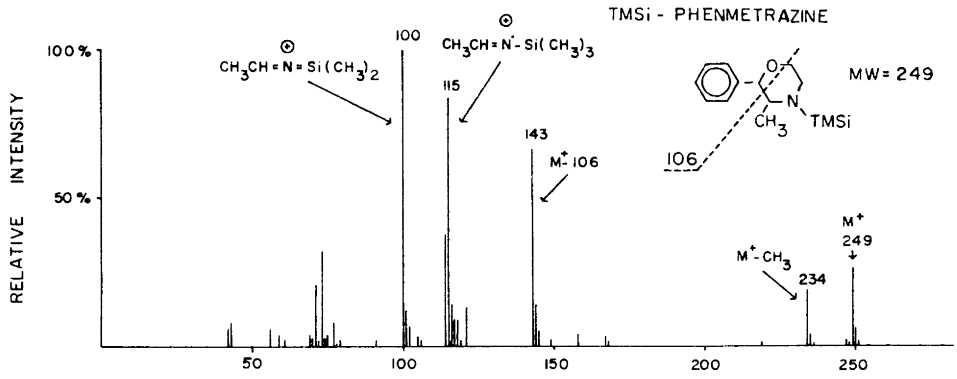

Fig. 3. Mass spectrum of trimethylsilyl (TMSi)-phenmetrazine.

The mass spectra of chlorphentermine as the free amine consisted practically only of the base peak (Table 2). This can be explained by β-fission which in chlorphentermine is favoured due to the tertiary carbon. Small peaks were also recorded at mass numbers 168 (3 %) and 125 (4 %). A single peak type of spectrum was also produced by the TMSi derivative of chlorphentermine as a result of the same mechanism.

Acetyl chlorphentermine, however, produced a spectrum more suitable for identification. A small (3 %) molecular ion was observed. A peak at 166 (18 %) with chlorine isotope at 168 (10 %) was ascribed to elimination of the *N*-acetyl as a radical. The *N*-acetyl cation produced the base peak. Another abundant (80 %) fragment with an m/e value of 100 was formed from the acylated alkylamine.

The spectrum of methyl phenidate (Table 2) is characterized by a base peak ($m/e=84$) formed from the piperidine ring and peaks at mass number 91 and 150. The former fragment is presumably the benzyl ion formed from elimination of the carbomethoxy group ($m/e=59$) of the latter fragment, which is the methyl phenylacetate ion. The hydrogen added during the formation of this fragment probably originates from the amine hydrogen since the mass spectra of acyl derivatives of methyl phenidate have no peaks at 150.

In contrast to the phenylalkylamines the derivatives of methylphenidate gave mass spectra which were poorer in terms of major fragments compared to the unsubstituted amine. Perfluoroacylation or trimethylsilylation gave as a rule only one major peak, respectively, corresponding to the substituted piperidyl. In addition smaller peaks were recorded at m/e 84.

Attempts to prepare derivatives of diethylpropion by condensation of the α-keto group with methoxyamine or dimethylhydrazine were unsuccessful. The free amine can, however, be chromatographed on nonpolar silicone phases. The mass spectrum consisted mainly of peaks from the nitrogen-carrying part of the molecule after the usual β-cleavage (Table 2).

DISCUSSION

The use of derivative formation in the gas chromatographic analysis of amines is desirable for several reasons. Firstly, the free amines are subject to adsorption phenomena in the chromatographic system due to the hydrogen bonding characteristic of the nitrogen. This becomes especially noticeable when the analysis is performed in the submicrogram range. Secondly, the use of sensitive detectors such as electron capture (EC) detectors makes it necessary to convert the amines into derivatives which possess both good chromatographic properties and strong affinity for free electrons. Thirdly, the use of mass spectrometry in combination with gas chromatography requires derivatives which will give mass spectra suitable for identification and for structural analysis. The present investigation was undertaken with these purposes in mind.

A variety of derivatives of amphetamine and phenmetrazine were prepared and tested for electron capture response. The trifluoroacetylation gave a weak response whereas the pentafluoropropionamide, heptafluorobutyramide and perfluorooctanamide gave electron capture responses which increased with the number of fluorine atoms incorporated. This confirms and extends the work of Clarke *et al.*,[4] who showed that a number of *N*-perfluoroacyl substituted alkylamines had fairly weak EC-responses. The perfluorooctanamide gave response which was about 20 % of that of lindane. The peak shape was, however, rather broad. Also the perfluorooctanoic anhydride proved fairly difficult to remove.

The maleamides and pentafluorobenzamides of both amphetamine and phenmetrazine had good EC-responses but at the nanogram level distorsion of peaks were observed.

The isothiocyanate is formed when the primary amine amphetamine is warmed in carbon disulphide.[5,6] When analyzed by GC the peaks are sharp and symmetric but the EC-response did not appear to be significantly better than the flame ionization detector response.

The derivative with best over all properties proved to be the trichloroacetamide. This combines excellent chromatographic properties with a high EC-response. The derivative forms quantitatively and quickly with a small excess of trichloroacetyl chloride. A gas chromatographic method for the analysis of amphetamine and phenmetrazine in blood based on the conversion of the amines into trichloroacetamides is currently is use.

Walle [7] recently prepared dinitrophenyl derivatives for the analysis of primary and secondary amines with EC-detectors and demonstrated their use in the analysis of amphetamine in urine. Although generally a most useful approach the limitation of these derivatives seemed to be the large increase in retention time. This might preclude its use for the analysis of amines with a molecular weight above 170 such as phenmetrazine and methylphenidate, where the temperature needed for elution would be over the temperature limit of the tritium electron capture detector and where column bleed would increase the background signal.

Mass spectra for amphetamine, methamphetamine, phentermine, chlorphentermine, methylphenidate, and other phenylalkamines of medical interest have recently been reported.[8,7] As shown in these investigations and in the present work these compounds undergo a common β-cleavage process and the resulting alkyl amine normally appears as the base peak. The benzyl residue rearranges to the tropylium ion and appears as a small peak at an m/e value of 91. The spectra of the unsubstituted amines are usually poor in abundant heavier fragments. These are the most useful for identification purposes, which involves comparing relative intensities of a number of fragments.

Preparation of derivatives of these amines as performed in the present study proved to be useful for several reasons. Firstly, they can be more readily introduced into the mass spectrometer in small amounts by means of a gas chromatographic inlet system. Secondly, the mass spectrum assumed a more complex character with more abundant peaks at high mass numbers thus giving more structural information and facilitating positive identification. Thirdly it seems that the use of appropriate derivatives would facilitate the detection of these compounds by the technique of mass fragmentography [2] where the mass spectrometer is used as gas chromatographic detector focused on characteristic fragments in the spectrum.

Acknowledgements. This study was supported by grants from the *Swedish Delegation of Medical Defense Research* No. A70—68, the *C. B. Nathhorst Foundation* and the *H. and G. Jeansson Foundation.*

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Received April 3, 1969.