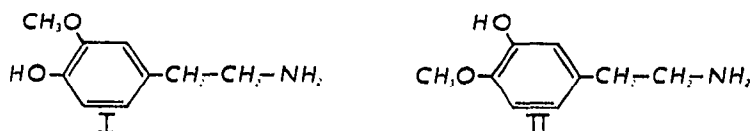


CHROM. 6102

3- and 4-O-methyl ethers of dopamine: gas-liquid chromatography and mass spectrometric studies of their isothiocyanate derivatives

Both 3-methoxytyramine (3-MT), the 3-O-methyl ether of dopamine (I), and its isomer, 4-O-methyldopamine (4-methoxy-*m*-tyramine, 4-MmT) (II) have been reported to be metabolites of L-Dopa. These substances could be formed as normal



metabolites in the process of detoxification of L-Dopa by methylation through catechol-O-methyltransferase (COMT) activity and by decarboxylation. Thus elevated levels of 3-MT could indicate an increase in COMT activity. According to the literature O-methylation in the *meta* position is the preferred one for COMT, and the ratios of the *m* and *p* isomers for various substrates have been reported^{1,2}. Interest in the mode of action of L-Dopa has risen with its increasing use in the treatment of Parkinsonism and studies on the various metabolites of L-Dopa may make an important contribution towards understanding the mechanism by which it ameliorates the symptoms of the disease. These metabolites of L-Dopa may be of considerable physiological importance, for 3-MT has also been reported to be present in certain types of carcinoma³ and 4-MmT has been implicated as a possible intermediate in the formation of 3:4-dimethoxyphenylethylamine (DMPEA) as an abnormal metabolite in schizophrenia^{4,5}.

Prerequisite to an evaluation of the physiological significance of the above compounds are separation by a selective method, positive identification and quantitation. In studies on 3-MT, thin-layer chromatography (TLC) and paper chromatography, followed by diazotized sulfanilic acid¹ or ninhydrin spray⁴, and fluorometric analysis have been used⁶. Gas-liquid chromatography (GLC) of the trifluoroacetyl (TFA) derivative of 3-MT and its mass spectrum have also been reported³. However, this method does not lend itself to quantitation. BRANDENBERGER AND HELLBACH⁷ have reported the use of an isothiocyanate (NCS) derivative of the primary amino group in the analytical determination of amphetamine (phenylisopropylamine) by GLC and mass spectrometry (MS). We have recently extended the use of NCS derivatives for the separation of a series of biogenic amines and amphetamines and have also described the GC conditions and mass spectral characteristics⁸. In this communication we report a further application of these derivatives in the GC-MS analysis of the two monomethyl ethers of dopamine, 3-MT and 4-MmT.

Materials and methods

3-Methoxy-4-hydroxyphenylethylamine (3-O-methyldopamine) and 4-methoxy-3-hydroxyphenylethylamine (4-O-methyldopamine) as hydrochlorides and Regisil-TMCS, were obtained from Regis Chemical Company.

Solutions of 3-MT and of 4-MmT were prepared by dissolving 12 mg of the hydrochloride of the respective compound in 10 ml of distilled water to give a free

base concentration of 1 mg/ml. One milliliter of the solution was taken, and after the pH had been adjusted to 8 with sodium bicarbonate solution, was extracted into 10 ml of ethyl acetate. The ethyl acetate extract was dried over sodium sulfate and evaporated to dryness under vacuum. The residue was redissolved in 1 ml of ethyl acetate and shaken with 0.1 ml of carbon disulfide for 30 min. The solution was again evaporated to dryness and reconstituted into 1 ml of ethyl acetate. This corresponded to 1 mg/ml (or 1 $\mu\text{g}/\mu\text{l}$) of the original base. To prepare the trimethylsilyl (TMS) derivatives, a 5- μl aliquot of the NCS derivative in pyridine was mixed with 5 μl of Regisil reagent and allowed to stand for 5 min. Perdeuterio-trimethylsilyl (TMS- d_9) derivatives were prepared by reacting the NCS derivatives with a 5:1 mixture of bis-perdeuterio-trimethylsilyl acetamide (BSA- d_{18}) and perdeuterio-trimethylchlorosilane (TMC- d_9) in pyridine⁹. TFA derivatives were prepared by treating the ethyl acetate solution of the NCS compounds with a few drops of trifluoroacetic anhydride and allowing it to stand at room temperature for 30 min.

A 1% OV-101 on GCQ 6-ft. column and a 2.5% OV-225 on GCQ 4-ft. column were used for GLC at 170° and 190° isothermal, respectively, for the two columns. Methylene unit (M.U.) values were obtained by running the samples with hydrocarbon mixture under programmed temperatures, 130° \rightarrow 6°/min and 150° \rightarrow 5°/min, respectively. Varian MAT CH-7 and LKB 9000 were used for GC-MS recording.

Results and discussion

The NCS derivatives of the isomeric monomethyl ethers separated on both the OV-101 and the OV-225 columns. The retention times of the 3- and 4-O-methyl compounds were compared with those of DMPEA-NCS. On OV-225 the compounds were well separated, while on OV-101 the 4-O-methyl ether was separated only as a shoulder from DMPEA (see Fig. 1). The TMS derivatives of the two compounds were not separated on either of the two columns. Attempts to separate the TFA derivatives were not successful on either column. The GC data on the two monomethyl ethers are presented in Table I.

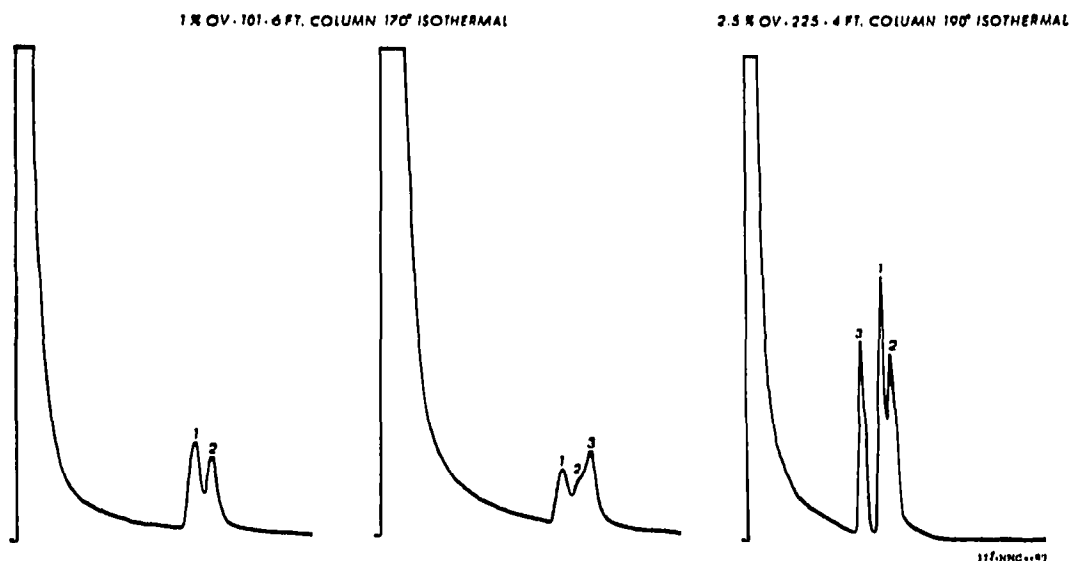


Fig. 1. Gas chromatograms of NCS derivatives 3-MT (1), 4-MmT (2) and DMPEA (3).

TABLE I

GAS CHROMATOGRAPHIC DATA

Compound	1% OV-101		2.5% OV-225	
	RT ^a	M.U.	RT ^b	M.U.
3-MT-NCS	2.50	18.27	4.50	26.87
4-MmT-NCS	2.75	18.44	4.88	27.11
DMPEA-NCS	2.80	18.44	3.86	26.05
3-MT-NCS-TMS	3.4	19.21	3.5 ^c	25.11
4-MmT-NCS-TMS	3.4	19.21	3.5 ^c	25.11
3-MT-TFA	1.8	17.73	3.0 ^c	24.19
4-MmT-TFA	1.8	17.73	3.0 ^c	24.19

^a Retention time in minutes; 170° isothermal. Carrier gas, helium, 30 ml/min. Varian, Model 2710.

^b Retention time in minutes; 190° isothermal. Carrier gas, nitrogen, 30 ml/min. Barber-Colman, Model 5000.

^c 180° isothermal.

The mass spectra of the NCS derivatives of both the 3- and 4-O-methyl dopamines (IIIa and IVa) were virtually identical. As an example the mass spectrum of IVa (4-MmT-NCS) is reproduced in Fig. 2. The molecular ion (m/e 209) is of moderate

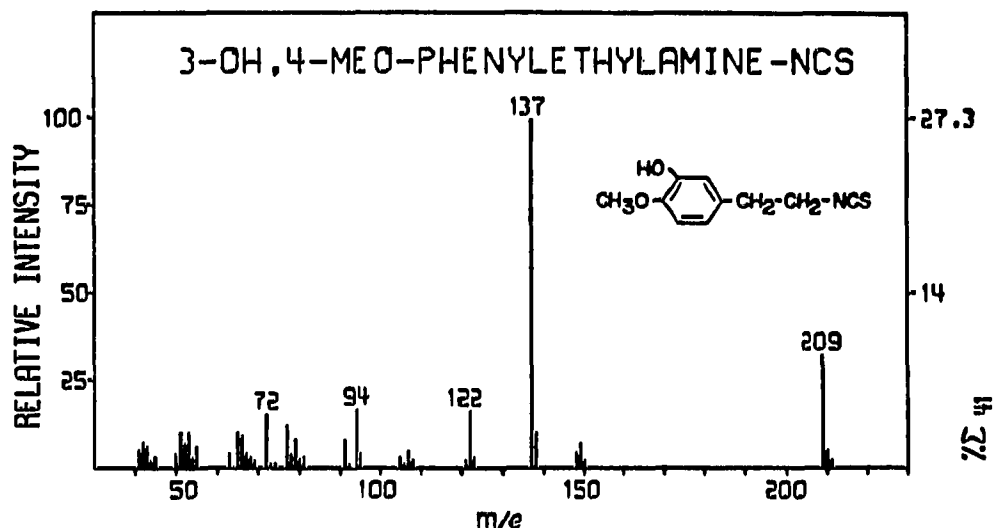
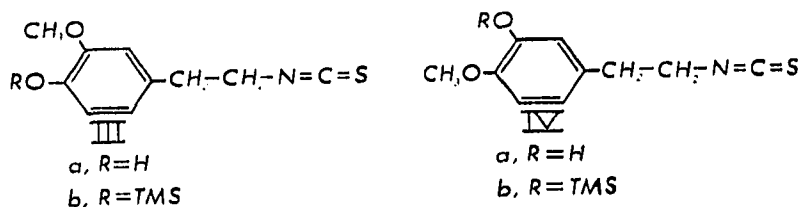
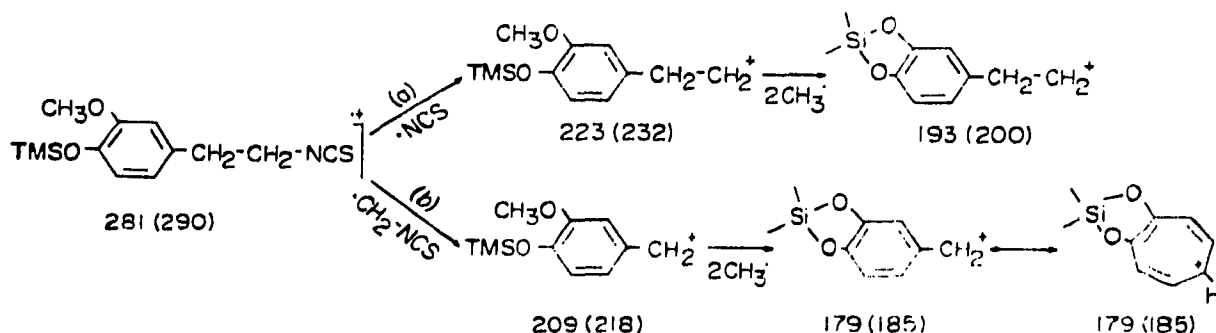


Fig. 2. Mass spectrum of 4-MmT-NCS.

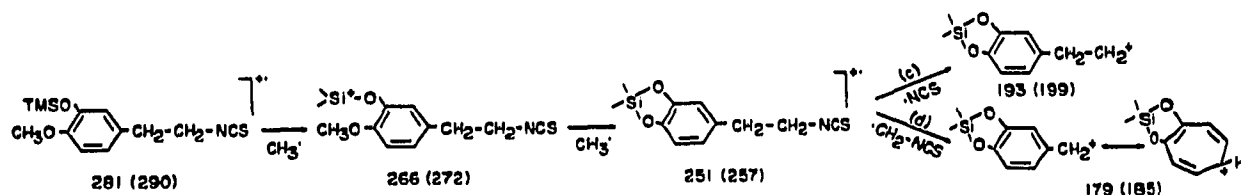
abundance (9% Σ_{40}) and the predominant fragmentation process involves the loss of $\cdot\text{CH}_2\text{-NCS}$ (72 amu) to give m/e 137. On the other hand the mass spectra (Fig. 3) of the NCS-TMS derivatives (IIIb and IVb, 3-MT-NCS-TMS and 4-MmT-NCS-TMS, respectively) of the two isomers are, in spite of many similarities, sufficiently unique to allow their differentiation. The leading fragmentation processes in IIIb and IVb



are summarized in Schemes 1 and 2, respectively. Pathways (a) and (b) (Scheme 1) are equally favorable in the fragmentation of the NCS-TMS derivative of 3-MT (IIIb) and as a result the ions at m/e 179 and m/e 193 are of virtually equal relative



Scheme 1. Fragmentation mechanism of IIIb (3-MT-NCS-TMS).



Scheme 2. Fragmentation mechanism of IVb (4-MmT-NCS-TMS).

abundance. The ion at m/e 223 is assumed to be an intermediate to the formation of m/e 193, but no metastable evidence was available to confirm this. The significantly higher abundance of the m/e 179 over the m/e 193 ion in the spectrum of the NCS-TMS derivative of 4-MmT (IVb) may be rationalized by the mechanism suggested in Scheme 2. This fragmentation mode is supported by the relatively greater abundance of the (M-15) (m/e 266) and (M-30)⁺ (m/e 251) ions in IVb than in IIIb. The apparent more favorable elimination of $\cdot\text{CH}_2\text{-NCS}$ (pathway d) rather than $\cdot\text{NCS}$ (pathway c) from m/e 251 results in the higher relative abundance of the m/e 179 ion in the spectrum of IVb than in that of IIIb. The numerals given in parentheses in Schemes 1 and 2 refer to the ion mass values in the corresponding perdeuterotrimethylsilyl derivatives and are in agreement with the proposed ion structures.

The TFA derivatives gave very similar spectra, the molecular ion (m/e 305) being fairly abundant (30 % of base peak, m/e 233). The 3-methoxy compound showed a fragment ion at m/e 205 which was absent in the other isomer.

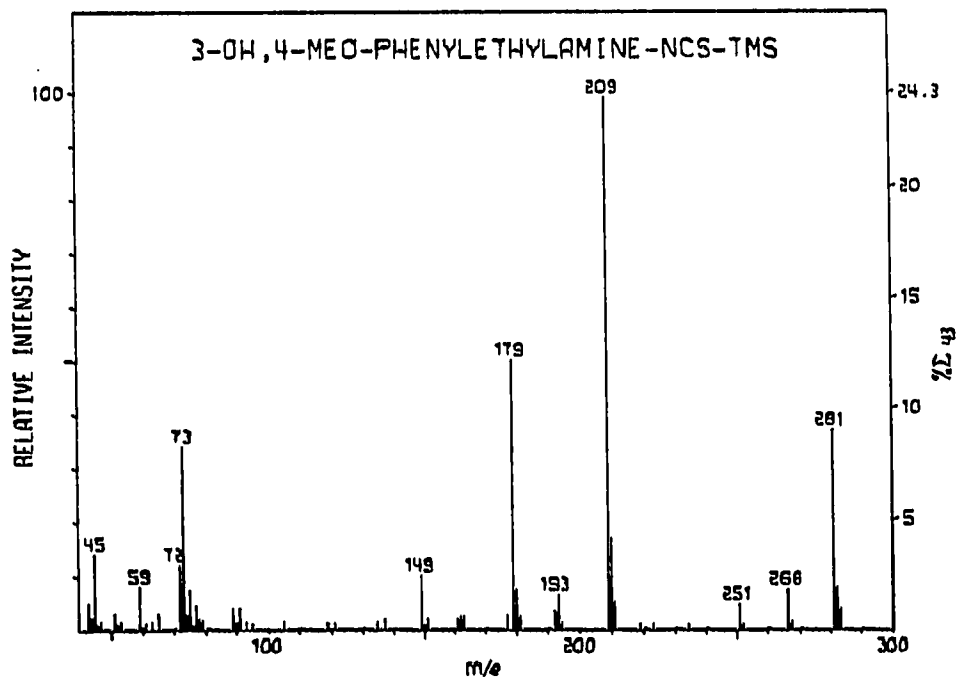
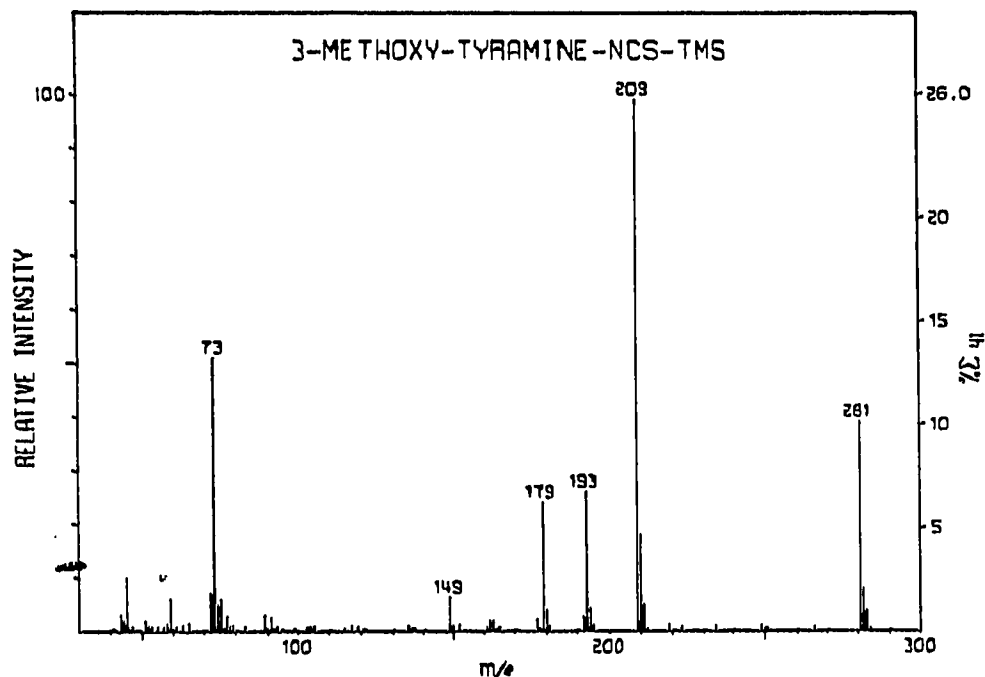


Fig. 3. Mass spectrum of 3-MT-NCS-TMS (upper) and of 4-MmT-NCS-TMS (lower).

The methods reported so far for the identification and quantitation of the two monomethyl ethers of dopamine are not very specific either in qualitative identification or quantitative determination. The method reported in this communication provides both qualitative identification by the GC-MS method and, after such

identification, quantitative measurement by GLC alone. This method should prove a convenient tool for studies on the metabolism of L-Dopa and the study of methylation using COMT with dopamine as substrate.

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