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Mass spectrometric structure determination of metabolites of 3-[2-(N,N-dimethylaminomethyl)phenylthio]phenol

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ABSTRACT

Metabolites of 3-[2-(N,N-dimethylaminoethyl)phenylthio]phenol (I) were isolated from the urine of rats, mice, rabbit and dog and from the faeces of rats by extraction and thin-layer chromatography. From the mass spectrum of I and using characteristics mass shifts in the spectra of metabolites, the structures of four metabolites were determined, whereas for two other isomeric metabolites the structures were resolved by means of IR spectra. Conclusions regarding the structures derived from the spectra were confirmed by comparing them with synthetic standards. Generally, metabolic changes of I can be characterized by demethylation, hydroxylation combined with methylation, S-oxidation and by a combination of these metabolic reactions.

INTRODUCTION

Usually metabolites isolated from biological material are obtained in small amounts and are not completely pure, which makes mass spectrometry the only physico-chemical method suitable for the determination of their structures. The final confirmation of the structure is provided by a comparison between spectral and chromatographic parameters on the one hand, and synthetic standards on the other. In those instances where this is made possible by the amount and purity of the isolated metabolite, IR and NMR spectrometry can also be applied. Correct determination of the structure of metabolites based on mass spectra plays an important role in the effective further development of drugs, and especially in the effective synthesis of the particular metabolites and in their pharmacological and toxicological investigation.

The objective of this study was to determine the structure of metabolites of 3-[2-(N,N-dimethylaminomethyl)phenylthio]phenol (I) (Fig. 1).

This drug possesses the properties of a selective inhibitor of the re-uptake of 5-hydroxytryptamine in rat brain. In clinical practice a reduced cardiotoxic and anticholinergic action is assumed. The study was carried out as part of a pharmacological investigation of I.

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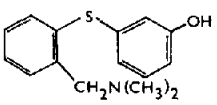


I

Fig. 1. Structure of 3-[2-(N,N-dimethylaminomethyl)phenylthio]phenol (**I**).

EXPERIMENTAL

Materials

Compound **I** was synthesized in the Research Institute of Pharmacy and Biochemistry (Prague, Czechoslovakia) [1]. Methanol and chloroform as solvents for spectroscopy were purchased from Merck (Darmstadt, F.R.G.). β-Glucuronidase, marine mollusc lyophilized, was purchased from Calbiochem–Behring (La Jolla, CA, U.S.A.). Ammonia solution (25%) of analytical-reagent grade was purchased from Lachema (Brno, Czechoslovakia).

Sample preparation

The biological material used in the isolation of metabolites was urine from rats, mice, rabbit and dog. The test animals were male Wistar rats (weight 150–180 g), male NMRI mice (weight *ca.* 30 g), male Rosice dog (weight 13.5 kg) and male Chincilla rabbit (weight 3.6 kg). The procedures used in the single application and sampling of urine and faeces have been reported in previous papers [2,3].

All urine samples prior to the application of **I** and after the application of 50 mg/kg doses (in rats 100 mg/kg) were extracted with benzene and ethyl acetate at pH 7, and subsequently at increased pH (8.75). The solvents were evaporated *in vacuo* and the remaining parts separated on a thin layer of silica gel (Merck DC-Fertigplatten 60-F-254) with the system chloroform–methanol–25% ammonia (80:7:0.7) (S_1). Selected stains which were not present in the extract of blank urine were scraped off, eluted with chloroform–methanol (80:20) and rechromatographed in the system chloroform–methanol–25% ammonia (85:15:0.1) (S_2). After elution of the corresponding stains and evaporation of the solvent, the isolated metabolites were analyzed by mass spectrometry.

The aqueous phases after extraction were enzymatically cleaved with β-glucuronidase and the released metabolites were extracted again as described above. Rat faeces were hydrolysed with 25% potassium hydroxide solution before extraction and the mixture was diluted to double the volume and extracted similarly to urine samples. The limit of detection was 1 μg.

Mass spectrometry

The isolated metabolites were analysed with a Model MAT-44S quadrupole mass spectrometer (Varian MAT, Bremen, F.R.G.) using a direct inlet system. The ion-source temperature was 200°C and the electron energy was 70 eV. The

temperature of the direct inlet was regulated in the range 140–250°C as appropriate. The molecular weights of some metabolites which did not show a molecular peak in their electron impact (EI) mass spectra were determined by chemical ionization (CI) with methane as the reactant gas.

IR spectrometry

With metabolite **IV**, which required a more exact knowledge of the position of substituents on the aromatic ring, the isolation was repeated in order to obtain a sufficient amount for recording IR spectra. The IR spectra were recorded using potassium bromide discs with a Perkin-Elmer Model 298 IR spectrometer.

RESULTS AND DISCUSSION

The mass spectrum of **I** is shown in Fig. 2. The metabolite structure determinations were made using the characteristic peaks A–E, as shown in Fig. 3. Owing to metabolic substitutions of groups R_1, R_2 and R_3, mass shifts occurred at these peaks, which were sufficient for complete or partial structure determination of the respective metabolites. The molecular peak of **I**, $M^{+\cdot}$ corresponds to *m/z* 259. Its intensity (20%) is so pronounced that also for metabolites arising by a simple substitution of groups R_1, R_2 and R_3 a sufficient intensity of their molecular peaks can be assumed. The A-moiety of molecule **I** is reflected in the spectra in intense peaks A − H (*m/z* 165) and in peaks B − 2H (*m/z* 132). From their shifts, metabolic substitutions in the A part can be determined. If the transformations concern only the dimethylaminomethyl group of the A part of the molecule, they can moreover be distinguished from the shifts or the absence of the intense peak of **I** at *m/z* 58 (peak D). A characteristic peak in the mass spectrum of **I** is an abundant peak at *m/z* 213 which corresponds to the hydroxythioxanthenyl cation C. Shifts of this peak in the case of the metabolites indicates metabolic substitution on one or both aromatic rings. The abundant peak at *m/z* 137 (E) is interpreted as the rearrangement product of the dimethylamino group to the

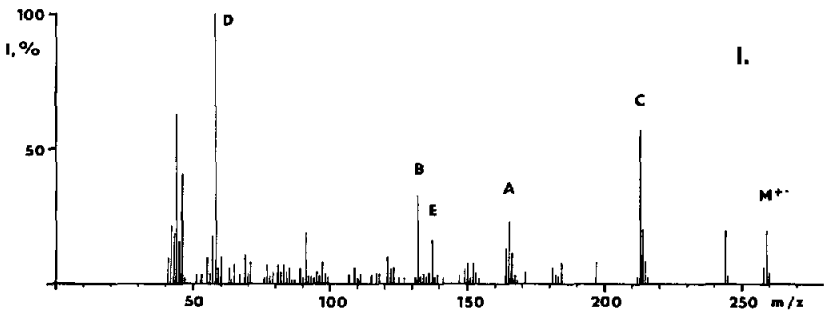


Fig. 2. EI mass spectrum of **I**.

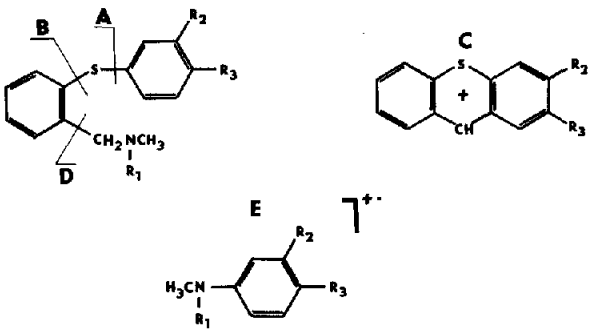


Fig. 3. Specific fragments in the EI mass spectra.

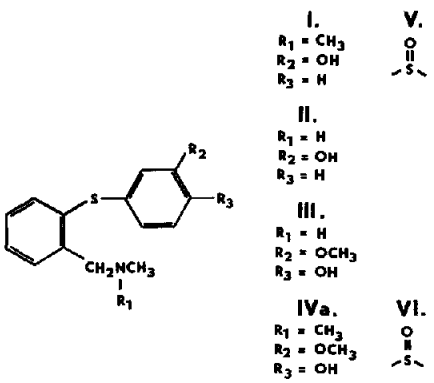


Fig. 4. Structures of **I, II, III, IVa, V** and **VI**.

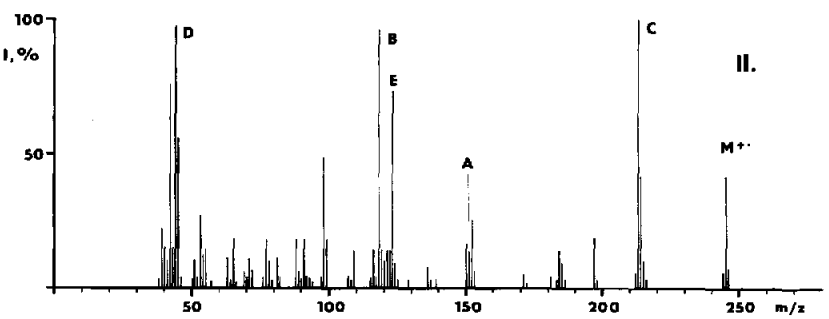


Fig. 5. EI mass spectrum of the metabolite **II**.

hydroxyphenyl ring of **I**. Its shifts allow us to assume simultaneous metabolic changes of both these parts of the molecule of **I**. Mapping of mass shifts of all these characteristic peaks leads to structure determination of the metabolites (Fig. 4). The R_F value of **I** in system S_2 is 0.43.

*Metabolite **II** (R_F = 0.25)*

The characteristic peaks (Fig. 5) $M_{II}^{+\cdot} = M_I - 14$ (*m/z* 245), $A_{II} = A_I - 14$ (*m/z* 151), $B_{II} = B_I - 14$ (*m/z* 118), $C_{II} = C_I$ (*m/z* 213), $D_{II} = D_I - 14$ (*m/z* 44) and $E_{II} = E_I - 14$ (*m/z* 123) determine **II** unambiguously as N-demethylated **I**, *i.e.*, 3-[2-N-(methylaminomethyl)phenylthio]phenol. The confirmation was made by comparing the metabolite **II** with the synthetic standard prepared subsequently [1].

*Metabolite **III** (R_F = 0.33)*

Peaks (Fig. 6) were observed at $M_{III}^{+\cdot} = M_I + 16$ (*m/z* 275), $A_{III} = A_I - 14$ (*m/z* 151), $B_{III} = B_I - 14$ (*m/z* 118), $C_{III} = C_I + 30$ (*m/z* 243), $D_{III} = D_I - 14$ (*m/z* 44) and $E_{III} = E_I + 16$ (*m/z* 153).

Series of characteristic peaks displaced by 16 mass units with respect to the original compound **I** could suggest hydroxylated **I**. However, the absence of a peak at *m/z* 58 or its shift to *m/z* 44 can be explained only by N-demethylation of the dimethylaminomethyl group. Complete agreement with the observed shifts seems to suggest that **III** is analogous to the metabolite **IVa** (see below), *i.e.*, 2-methoxy-4-[2-N-(methylaminomethylphenyl)thio]phenol (R_1 = H, R_2 = OCH_3, R_3 = OH). The synthetic standard confirms this suggestion.

*Metabolite **IV** (Fig. 7)*

This metabolite was detected in two isomeric chromatographically different forms, *i.e.*, **IVa** (R_F = 0.54) and **IVb** (R_F = 0.63). Their mass spectra (Fig. 8) differ only in the intensities of the characteristic peaks. These are $M_{IV}^{+\cdot} = M_I + 30$ (*m/z* 289), $A_{IV} = A_I$ (*m/z* 165), $B_{IV} = B_I$ (*m/z* 132), $C_{IV} = C_I + 30$ (*m/z* 243), $D_{IV} = D_I$ (*m/z* 58) and $E_{IV} = E_I + 30$ (*m/z* 167).

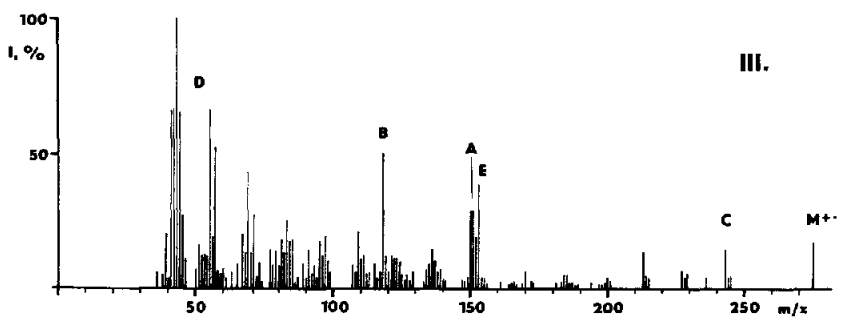
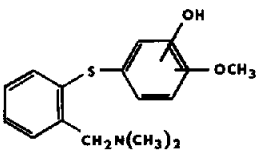


Fig. 6. EI mass spectrum of the metabolite **III**.



IV.

Fig. 7. Structure of **IV**.

Differences in the intensities of characteristic peaks are not so significant for isomers **IVa** and **IVb** as to be reliably distinguishable on the basis of the spectra of metabolites isolated from the biological material. Isomer **IVa** as the product of metabolic hydroxylation and methylation was isolated in a sufficient amount to record its IR spectrum and to compare the latter with that of a synthetic standard (Figs. 9 and 10). The identity can easily be seen. For isomer **IVb**, a structure has been suggested with a mere exchange of substituents R_2 and R_3 of isomer **IVa**. The amount and

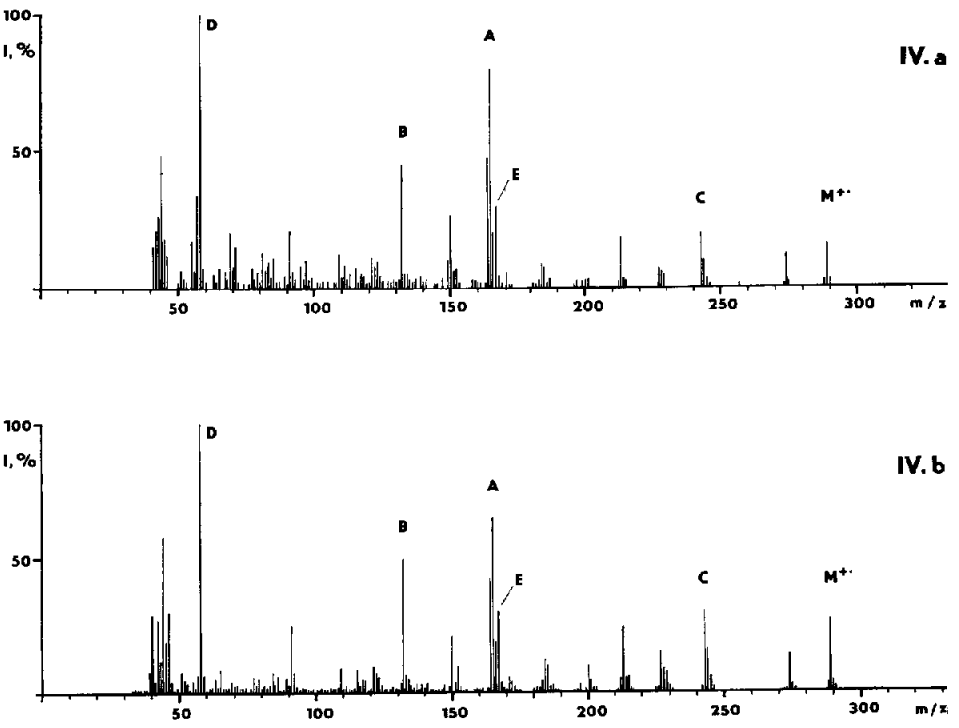


Fig. 8. EI mass spectrum of the metabolites **IVa** and **b**.

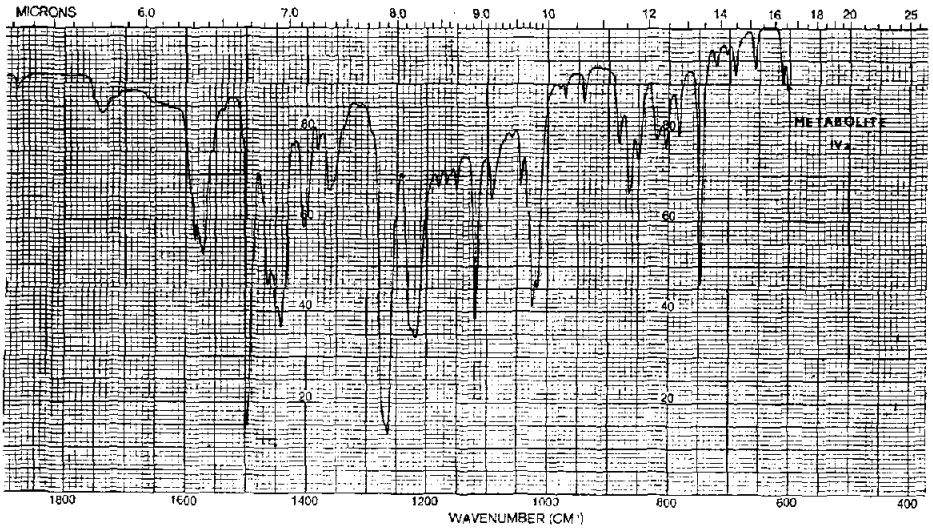


Fig. 9. IR spectrum of the metabolite **IVa**.

purity of the isolated metabolite **IVb** were not sufficient to use the IR spectra to confirm this suggestion, so further isomers differing in the position of the -OH or OCH_3 groups cannot be ruled out. Compund **IVa** correspondes unambiguously to 2-methoxy-4-[2-N,N-(dimethylaminomethyl)phenylthio]phenol.

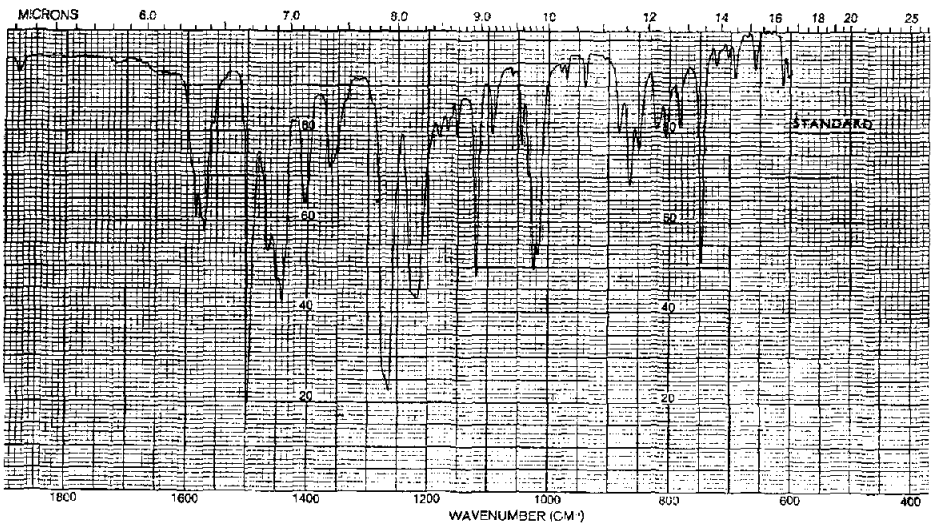


Fig. 10. IR spectrum of the synthetic standard **IVa**.

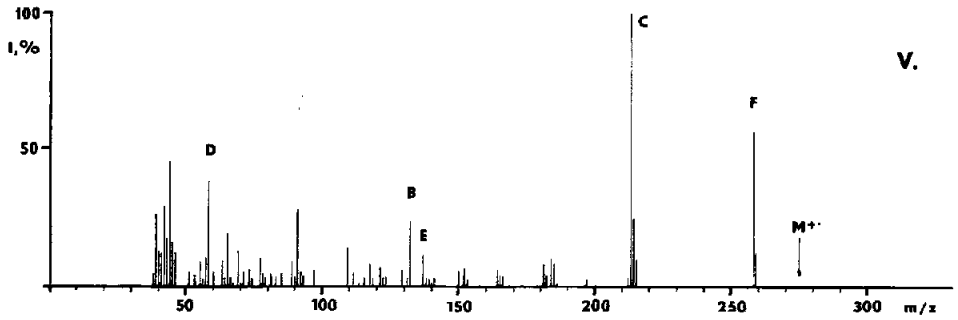


Fig. 11. EI mass spectrum of the metabolite V.

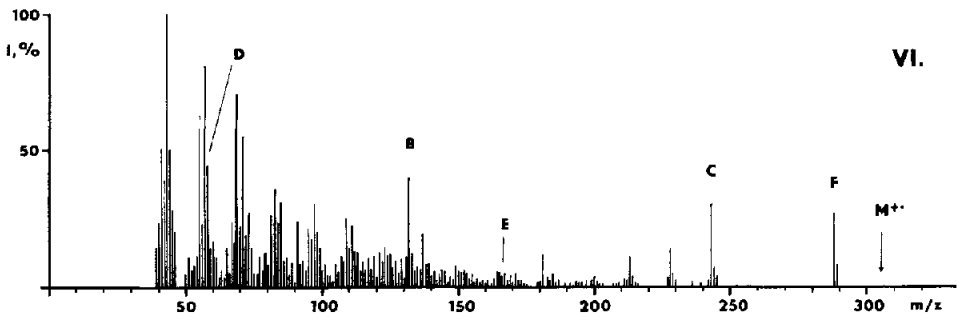


Fig. 12. EI mass spectrum of the metabolite VI.

TABLE I

DETECTION OF I AND ITS METABOLITES IN THE INDIVIDUAL ANIMAL SPECIES

Compound	Rat		Mouse urine	Dog urine	Rabbit urine
	Urine	Faeces			
I	+	+	+	+	+
II	+	+	n.d.[a]	+	n.d.
III	+	n.d.	n.d.	n.d.	n.d.
IVa	+	+	+	+	n.d.
IVb	+	+	n.d.	n.d.	n.d.
V	n.d.	n.d.	n.d.	+	+
VI	+	n.d.	n.d.	n.d.	+

[a] n.d. = Not detected.

Metabolites ***V*** *(R_F = 0.37) and* ***VI*** *(R_F = 0.63)*

These metabolites differ considerably in their mass spectra (Figs. 11 and 12) from those of the preceding metabolites. The molecular peaks are not present in the EI mass spectra. The molecular weights of these metabolites were confirmed by means of CI mass spectra. $[M + H]^+$ at *m/z* 276 in the CI spectrum of metabolite **V** confirms its molecular weight (275). The EI spectrum contains some structurally characteristic peaks, such as $B_V = B_I$ (*m/z* 132), $C_V = C_I$ (*m/z* 213), $D_V = D_I$ (*m/z* 58) and $E_V = E_I$ (*m/z* 137). The fragment F_V (*m/z* 258 = M − OH) is important. On the basis of the mass spectra the structure of metabolite **V** was attributed to the S-oxide of **I**, *i.e.*, 3-[2-N,N-(dimethylaminomethyl)phenylsulphinyl]phenol. This was confirmed by comparing it with the synthetic standard [1]. The structure of metabolite **VI** as the S-oxide of metabolite **IV** or of one of its isomers was determined similarly from its mass spectra.

CONCLUSION

The metabolites detected in the individual animal species are summarized in Table I. Both free metabolites and their conjugates with glucuronic acid (released after enzymatic cleavage) were detected. The largest number and the highest amounts of metabolites were detected in rat urine.

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