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Identification of a urinary metabolite of perazine as a piperazine-2,5-dione derivative

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A non-basic perazine metabolite has been isolated from the urine of schizophrenic patients ingesting perazine. Identification of this compound as 10-[3′-(2″,5″-dioxo-4″-methyl-piperazinyl)-propyl]-phenothiazine sulphoxide was achieved using ultraviolet, infrared, nuclear magnetic resonance and mass spectroscopy.

In a previous paper, Breyer (1969) has described the isolation and identification of those perazine (Taxilan) metabolites that result from demethylation, sulphoxidation, *N*-oxidation and aromatic hydroxylation followed by glucuronide conjugation of the molecule. A further metabolic product, referred to as substance VIII, which seemed to possess an altered piperazine ring, was also regularly detected and isolated (Breyer, 1969; Kanig & Breyer, 1969). This paper gives details of the isolation of substance VIII on a milligram scale and of the elucidation of its structure by chemical and physico-chemical methods.

EXPERIMENTAL

Urine was collected from patients receiving orally 300–600 mg of perazine daily.

Chemical reactions were as described by Breyer (1969). Alkaline hydrolysis was achieved by heating with 0·1 N NaOH/tetrahydrofuran (1 : 1, v/v) to 100° for 30 min.

Isolation of substance VIII. Urine (1 litre) was adjusted to pH 2 by addition of 5 N HCl and extracted with successive portions of 200 and 100 ml of 1,2-dichloroethane. The combined organic layers were washed with 30 ml of N ammonia and evaporated under reduced pressure at 40°. The dark brown residue was subjected to thin-layer chromatography (TLC) on a 400 × 200 mm silica gel H (Merck) plate with dichloroethane–ethyl acetate–ethanol–acetic acid–water (30 : 28 : 8·5 : 8·5 : 5 by volume) as developing solvent. After the solvent had been allowed to rise twice to a height of 23 cm above the application line, the position of substance VIII was determined using a reference strip which was sprayed with concentrated HCl. The band containing compound VIII was removed and treated as described for the other metabolites (Breyer, 1969). The isolated organic material was further purified by TLC in isopropanol–chloroform–water–25% ammonia (35 : 30 : 4 : 1 by volume) on 200 × 200 mm silica gel G (Merck) plates. Extraction residues from several chromatograms were combined, dissolved in ethanol and centrifuged at 3000 rev/min for 15 min, in order to remove traces of silica gel. The solution was evaporated under a stream of nitrogen and the oily residue obtained was warmed with benzene, whereupon it crystallized. Recrystallization from methanol resulted in nearly colourless crystals

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which melted at 255°. The yield was about 1 mg from 1 litre of urine. The material proved to be homogeneous upon TLC in 5 different solvent systems (Breyer, 1969). Elementary analysis*: C, 62·5; H, 5·5; N, 11·0%

Ultraviolet spectroscopy. Small amounts of substance VIII were dissolved in 0·1 N HCl, the solution extracted with dichloroethane–methanol (1 : 1 v/v) and the material obtained by evaporation of the solvent. The spectrum was obtained using a Zeiss PMQ II spectrophotometer from 220 to 380 nm.

Infrared spectroscopy. Spectra were recorded with a Perkin-Elmer model 621 grating spectrophotometer. The sample of substance VIII was prepared with KI (1 mg in 200 mg KI). The spectrum was compared with the spectra of perazine and perazine sulphoxide which were recorded similarly.

Nuclear magnetic resonance spectroscopy. A Varian HA-100 spectrometer was used. Substances were dissolved in $CDCl_3$–CD_3OD (1 : 1 v/v), and tetramethylsilane served as internal reference compound.

Mass spectroscopy. The spectrum was obtained with a Varian MAT SM 1 mass spectrometer at 70 eV, using the direct oven inlet system H heated to 220°; the source temperature was 250°. Exact mass measurements were made using a resolution of 15000.

RESULTS AND CONCLUSIONS

Chemical properties. Substance VIII is extracted into dichloroethane from acidic (pH 2) as well as from alkaline (pH 11) aqueous solutions. In pure form it is slightly soluble in dichloroethane, chloroform and alcohols and easily soluble in a chloroform–methanol mixture (1 : 1 v/v). It fails to react with sulphur dioxide (no *N*-oxide), methyl iodide, acetic anhydride–pyridine (no N–H or O–H), diazotized sulphanilic acid (no aromatic hydroxyl) or hydrogen peroxide (no sulphide or tertiary amine), but zinc–HCl produces a substance with higher Rf values in TLC with basic solvent systems. With NaOH in tetrahydrofuran compound VIII is decomposed to a more polar product, judging from the behaviour in TLC.

Ultraviolet spectrum. This closely resembles that of perazine sulphoxide and the other sulphoxides described by Breyer (1969), but absorption maxima and minima are slightly shifted. (For compound VIII, λ_{max} 228, 272, 301, 344, λ_{min} 256, 285, 317; for perazine sulphoxide λ_{max} 233, 271, 298, 341, λ_{min} 256, 283, 314 nm, for 5×10^{-5} M solutions in 0·1 N HCl). The fluorescence properties of compound VIII and the colour reaction given upon spraying TLC plates with concentrated HCl are also consistent with the sulphoxidic character, while the reaction product formed with zinc–HCl behaves like a sulphide in fluorescence and HCL staining.

Infrared spectrum. That the aromatic system of the parent compound perazine was preserved is shown in the spectrum of VIII by the presence of ν(CH) bands at 3115, 3070 and 3015 cm^{-1}, strong γ(CH) bands at 772 and 756 cm^{-1}, as well as bands at 1610, 1586 and 1572 cm^{-1}. The latter are typical of aromatic rings. In contrast to perazine, the spectra of perazine sulphoxide and VIII show strong bands at 1015 and 1030 $cm,^{-1}$ respectively. These bands and the absence of a ν(OH) vibration at short wave lengths indicate the presence in VIII of a S=O group. A conspicuous difference between the spectrum of VIII and those of perazine and perazine sulphoxide is the presence of two strong bands at 1681 and 1668 cm^{-1} in VIII. These bands stem from ν(C=O) vibrations. The long wavelength is characteristic of the amide group. The

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absence of short wave ν(NH) and amide II absorptions near 1550 cm^{-1} indicate that C_2NCO groups are probably present. The intensity of the aliphatic ν(CH) bands is much less with VIII than with perazine or perazine sulphoxide. Further, the strong long wave ν(CH) bands which are present in the comparison spectra are missing in the spectrum of VIII. These bands are characteristic of CH_2 groups in amines. Their absence can be seen as an indication of C=O groups in the piperazine ring.

Nuclear magnetic resonance spectrum. This is indicative of the presence of two chemically equivalent *ortho*-disubstituted phenyl rings: there is a quartet at 2·06 τ (2 H) coupled to a multiplet at 2·72 τ (2 H) with about 7·5 Hz and to a multiplet at 2·4 τ (4 H) with about 1·5 Hz. The multiplet at 2·72 τ is coupled with about 6·4 Hz and with ~1·9 Hz to the multiplet at ~2·4 τ. The trimethylene fragment gives rise to two triplets at 5·56 τ (2 H) and 6·46 τ (2 H) coupled with 7·0 and 6·8 Hz, respectively, to a multiplet at 7·75 τ (2 H). Further there are a singlet at 6·56 τ indicative of isolated CH_2 groups and another singlet at 6·98 τ which probably belongs to a N-CH_3 group. The chemical shift of this latter signal is more consistent with an amide N-CH_3 than with one belonging to a trialkylamine. The N-CH_3 of sarcosine anhydride (2,5-dioxo-1,4-dimethylpiperazine) which was measured for a comparison forms a singlet at 7·04 τ.

Mass spectrum. This confirmed that the metabolite must be an oxidation product. The molecular ion was found at m/e 383 with an accurate mass of 383·1281 corresponding to an elemental composition of $C_{20}H_{21}N_3O_3S$ (calculated: 383·1300). Loss of oxygen, OH and H_2O from the molecular ion is in agreement with the assumption of a sulphoxide (Bowie & others, 1966). The base peak at m/e 238 with an elemental composition of $C_{15}H_{12}NS$ may be best rationalized as the stable △1-propenylen-(3)-phenothiazinium ion depicted in Fig. 1 showing that further oxidation took place in the piperazine ring. Accurate mass measurements of the most prominent ions (Table 1) allow discussion of the fragmentation scheme presented in Fig. 1 where the oxidized piperazine ring is symbolized by R.

Table 1. *Accurate mass data of the molecular ion and the main fragments of substance VIII obtained in mass spectroscopy*

m/e	Composition	Calculated	Found
383	$C_{20}H_{21}N_3O_3S$	383·130356	383·128056
367	$C_{20}H_{21}N_3O_2S$	367·135441	367·134787
366	$C_{20}H_{20}N_3O_2S$	366·127616	366·125301
365	$C_{20}H_{19}N_3O_2S$	365·119791	365·119658
238	$C_{15}H_{12}NS$	238·069044	238·069509
226	$C_{14}H_{12}NS$	226·069044	226·068430
212	$C_{13}H_{10}NS$	212·053394	212·053632
198	$C_{12}H_8NS$	198·037744	198·037188
180	$C_{13}H_{10}N$	180·081320	180·081404
169	$C_8H_{13}N_2O_2$	169·097696	169·097157
167	$C_8H_{11}N_2O_2$	167·082047	167·081928

Conclusions. The formula $C_{20}H_{21}N_3O_3S$ derived from the molecular peak in the mass spectrum is in accordance with the elementary analysis (calculated C, 62·7; H, 5·5; N, 11·0; found C, 62·5; H, 5·5; N, 11·0%). The excellent agreement indicates that the relatively strong peak at m/e 367 is due rather to thermal release of the sulphoxide oxygen during mass spectroscopy than to admixture of the sulphide with the purified substance VIII. This possibility can further be excluded by consideration of the ultraviolet spectrum and the homogeneity on TLC.

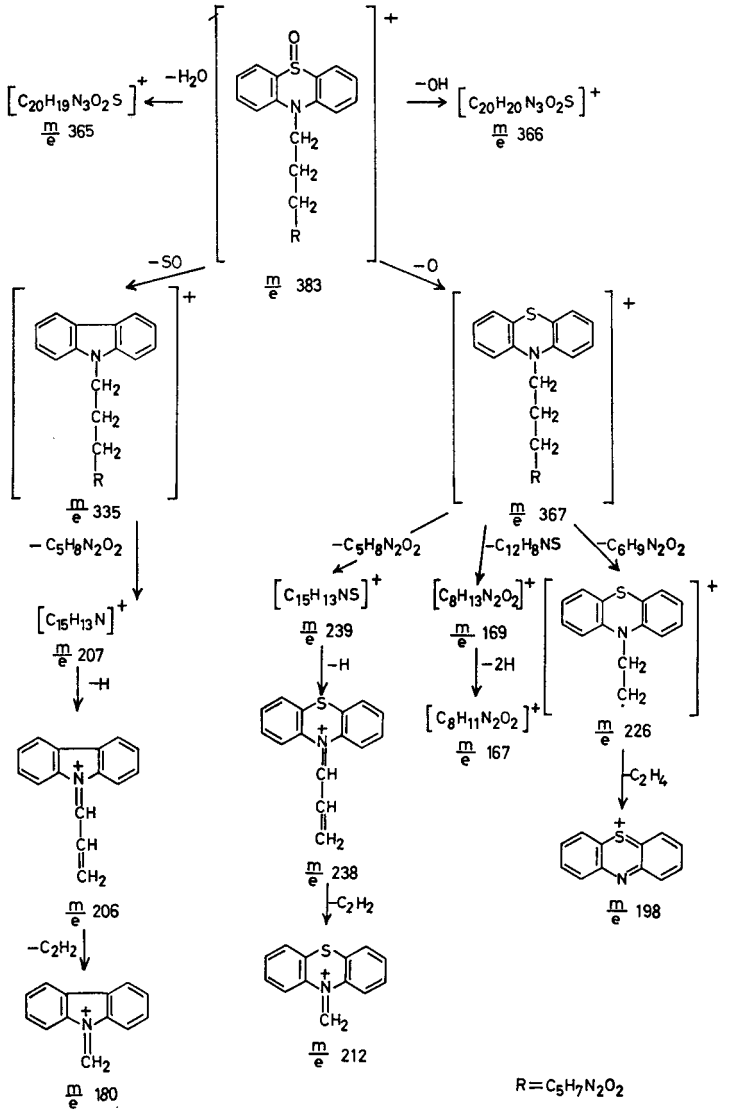
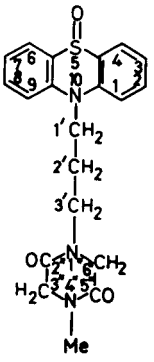


FIG. 1. Tentative fragmentation scheme of substances VIII in mass spectrometry.

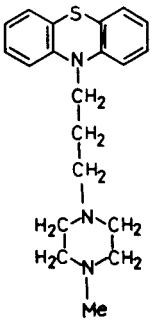
From the formula, $C_{20}H_{21}N_3O_3S$, it follows that three oxygen atoms have been added to the parent molecule, $C_{20}H_{25}N_3S$, and four hydrogen atoms have been removed. Since one oxygen is bound to the sulphur, the two others have each replaced two hydrogens. The occurrence of ether functions is ruled out by the infrared spectrum, so that the formation of CO from CH_2 groups is the most probable oxidative reaction. This is confirmed by the amide bands at 1681 and 1668 cm^{-1} in the infrared spectrum and by the loss of basic character. The elemental composition of the ions m/e 169 $C_8H_{13}N_2O_2$ and m/e 167 $C_8H_{11}N_2O_2$ is further support for the assumption that two oxygen functions occur in the side-chain. That both CO groups are situated in the piperazine ring is proved by the nmr spectrum showing a CH_2–CH_2–CH_2 sequence which must be due to the propyl chain on N–10 and a peak at 6·98 τ indicative of a methyl group.

There are four possibilities left for the position of the two keto-groups in the piperazine ring: 2″, 3″-, 3″,5″-, 2″,6″-, and 2″,5″-. Vicinal CO groups (2″,3″-) would mean vicinal CH_2 groups, too, but this is not compatible with the nmr spectrum suggesting isolated CH_2 groups. The possibility of two CO groups adjacent to one nitrogen (3″,5″- or 2″,6″-) is ruled out by the absence of basic character, since the other N atom would be surrounded by methylene (and possibly methyl) groups and should be basic. The absence of ν(CH) bands at high wavelengths in the infrared spectrum which would indicate CH_2 groups in amines also points to the same conclusion.

Therefore the only constitution which agrees with all findings is that of 10-[3′-(2″,5″-dioxo-4″-methylpiperazinyl)-propyl]phenothiazine sulphoxide (VIII).



VIII



Perazine

DISCUSSION

Piperazine-2,5-diones have been described as urinary metabolites resulting from amino-acids (Kibrick, Hashiro & others, 1965; Perry, Richardson & others, 1965), as metabolites from microbial cultures (Birkinshaw & Mohammed, 1962) and as products of protein hydrolysis simulating fermentation metabolites (Mitscher, Kunstmann & others, 1967). Apparently they have not yet been detected as oxidative metabolites of piperazine or piperazine derivatives despite the widespread use of these compounds as anthelmintics (see, for instance, Oelkers, 1959) and for other purposes.

The easy hydrolysis of diketopiperazines to dipeptides (Sykes, Robertson & others, 1966) would suggest the possibility that hydrolysis products of substance VIII might occur in the urine of patients ingesting perazine. Since as amino-acids these would not be extracted into organic solvents they are not easily detected. Radioactive labelling would indicate the presence of further metabolites in addition to those isolated. Until now, however, labelled perazine has not been available so that no conclusions about the occurrence of such polar degradation products can be made.

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