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URINARY METABOLITES OF 10-[3'-(4''-METHYL-PIPERAZINYL)-PROPYL]-PHENOTHIAZINE (PERAZINE) IN PSYCHIATRIC PATIENTS—I

ISOLATION, IDENTIFICATION AND DETERMINATION OF METABOLITES

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Abstract—From the urine of patients receiving daily 300–600 mg of perazine drug metabolites have been extracted and separated by thin-layer chromatography. Metabolic conversions taking place include demethylation, sulfoxidation, *N*-oxidation and aromatic hydroxylation followed by conjugation with glucuronic acid. The total amount of extractable urinary metabolites accounted for 15–30 per cent of the dose in a group of 15 patients. Besides the hydroxy perazines the *N*-oxides constitute a major part of the metabolic products excreted in urine.

INVESTIGATIONS on the metabolism of phenothiazine drugs have until now centered on chlorpromazine. Since a comparison with the metabolic reactions of further phenothiazine compounds seemed interesting, we undertook to study the metabolites of perazine (Taxilan®) which in Germany was established as an effective antipsychotic drug and proved to exert only little side effects.[1]

This compound is likely to undergo similar metabolic conversions as chlorpromazine, with the exception that there is only one demethylation step possible with a concomitant reduction in the number of demethylated products. On the other hand, the piperazine ring in the side chain would offer special problems if it were split, but until now metabolites of this kind have not been identified.

EXPERIMENTAL

Materials

Perazine base was kindly supplied by Chemische Fabrik Promonta, Hamburg, Germany.

Silica gel G and H were obtained from E. Merck, Darmstadt, Germany. 1,2-Dichloroethane (Riedel-de Haen, Seelze, Germany) and other solvents were redistilled before use.

An aqueous solution of β-glucuronidase (5·2 U/ml) and aryl sulfatase (2·6 U/ml) was obtained from Boehringer und Soehne, Mannheim, Germany, solid β-glucuronidase "reinst" (400 Fishman units per mg) from Serva Entwicklungslabor, Heidelberg, Germany.

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24 hr urine specimens from 15 patients receiving 300–600 mg of perazine daily were collected with addition of 2 ml acetic acid. When the collection was finished, the samples were checked for nitrite according to Grieß-Ilosvay[2] or with "Nitur Test" sticks (Boehringer und Soehne, Mannheim, Germany). Samples reacting positive were discarded, negative ones were at once frozen and stored at − 25° until work-up. As a qualitative test for the presence of phenothiazine metabolites the FPN test of Forrest and Forrest[3] was used. An approximate estimate of the completion of urine collection was obtained by creatinine determinations according to Jaffé.[4]

Methods

Extraction procedures. (1) Unpolar unconjugated metabolites: Urine (150 ml) was adjusted to pH 11 by addition of 5 N NaOH under control of a glass electrode. Extraction was done by shaking with successive portions of 60, 60, 40 and 40 ml dichloroethane and centrifuging to get clean separation of the two layers. The combined organic extracts were washed with 20 ml of 0·2 N NH_3 and evaporated under reduced pressure. The residue was dissolved in 1·5 ml ethanol. When the urine was very dilute, 250 ml were extracted with correspondingly larger volumes of dichloroethane. The residue volume was again adjusted to 1 per cent of the original urine volume. The extracted urine was acidified to pH 4·5 with 5 N HCl and used for isolation of further metabolites.

(2) Polar unconjugated metabolites: (a) For quantitative analysis. 30–50 ml (depending upon concentration) of previously extracted urine (pH 4·5) were saturated with SO_2 gas and left at room temperature for 30 min. SO_2 was partly removed by bubbling nitrogen through the solution, and the pH was adjusted to 11. Extractable metabolites formed were isolated by shaking with three 30–50 ml portions of dichloroethane. Further work-up according to (1).

(b) For qualitative analysis. 20 ml urine samples pre-extracted according to (1) and (3) were used. They were extracted at pH 11 with three 20 ml portions of *n*-butanol, the residue of the extract was dissolved in 5 ml 0·1 N acetic acid and applied to a column containing Amberlite IR-120 (0·5 g). After washing with acetic acid and water, basic substances were eluted with 0·2 N NH_3. The first 24 ml of alkaline eluate were evaporated under reduced pressure and the residue extracted with ethanol. The ethanol solution was subjected to TLC in solvent system S_{IV} (Table 1). The chromatograms were either sprayed with conc. HCl or the areas expected to contain the substances in question were scraped off, eluted with methanol + 3% conc. ammonia and the evaporated eluate rechromatographed in system S_{II}.

(3) Polar conjugated metabolites:[5] To 50 ml pre-extracted urine (pH 4·5) was added 0·2 ml β-glucuronidase + aryl sulfatase solution or 20 mg β-glucuronidase powder. After incubating at 37° for 18–20 hr the sample was brought to pH 9, and free phenols were extracted with 50, 50, 40 and 30 ml of dichloroethane. The extract was evaporated and dissolved in a fixed volume of ethanol from which aliquots were taken for TLC in solvent system S_V.

The residual urine was in some instances checked for more polar substances. Quantitative analysis was done by SO_2 reduction according to (2a) with the exception that dichloroethane extraction was carried out at pH 9. For qualitative analysis a 10 ml aliquot was extracted with *n*-butanol at pH 9, the residue of the extract was dissolved in 1 ml ethanol and impurities were partly removed by precipitation with

3 ml ether. The supernatant was evaporated and extracted with 0·2 ml acetone for thin layer chromatography in solvent S_{IV}.

Thin layer chromatography (TLC). Glass plates were coated with silica gel G or H to 0·4 mm thickness. Solvent systems used are contained in Table 1. Chromatography in system S_I was carried out on 400 × 200 mm silica gel H plates allowing the solvent to run twice to a height of 23 cm above the application line, or on 200 × 200 mm silica gel G plates with two runs to the upper edge. Between the two runs the plates were left to dry at the air for 8 min. For chromatography in other solvent systems, 200 × 200 mm plates with silica gel G were run to a height of 12–14 cm.

TABLE 1. R_f VALUES OF PERAZINE METABOLITES ON THIN LAYER CHROMATOGRAMS. SILICA GEL H (FOR S_I) OR G (FOR THE OTHER SYSTEMS), THICKNESS 0·4 mm; RUNNING HEIGHT 12–14 cm

Compound	Solvent system				
	S_I*	S_{II}	S_{III}	S_{IV}	S_V
Ia	0·33	0·73	0·52	0·90	0·60
Ib	0·33	0·45	0·28	0·58	0·28
IIa	0·08	0·60	0·31	0·65	0·28
IIb	0·08	0·32	0·13	0·43	0·08
IIIa	0·20	0·75	0·45	0·79	0·63
IIIb	0·20	0·48	0·18	0·45	0·29
IV	0·42	0·45	0·20	0·43	0·28
V	0·08	0·40	0·10	0·36	0·12
VI				0·50	
VII				0·52	
VIII	0·42	0·52	0·37	0·52	0·67

S_I = dichloroethane/ethyl acetate/acetic acid/ethanol/water (30:28:8·5:8·5:5) (acetic ester equilibrium mixture according to Green *et al.*[12] saturated with dichloroethane)

S_{II} = methanol/25% ammonia (95:5)

S_{III} = isopropanol/chloroform/water/25% ammonia (35:30:4:1)

S_{IV} = isopropanol/chloroform/water/25% ammonia (40:20:5:5)

S_V = acetone/isopropanol/1 N ammonia (27:21:12) (according to Goldenberg, Fishman, Heaton and Burnett[13] who used 1% NH_3)

* Run twice to a height of 23 cm above the application line.

For the first separation of unpolar metabolites carried out in solvent S_I, extracts corresponding to 1 mg (approximately 3 μmoles) of metabolites were spotted on a line of 12 cm length. For further purification extracts of one band were applied on a 6 cm line. The substances can be localized on the plates by their fluorescence under u.v. light. With irradiation at 254 mμ the intensity of fluorescence decreases in the order hydroxy perazine sulfoxides > perazines > hydroxy perazines > perazine sulfoxides. Under 366 mμ light the hydroxy perazine sulfoxides still fluoresce brightly and perazine sulfoxides are easier detected than under 254 mμ light, but the other types of substances exhibit a weak fluorescence only. As spraying reagent concentrated hydrochloric acid was used (the addition of nitrite[6] proved to be unnecessary) which gives immediately appearing light red spots with those phenothiazine compounds substituted in 10 position only and purple spots with phenolic derivatives, whereas sulfoxides slowly appear as dark red spots.

Before scratching the gel off the plate, it was slightly wetted by spraying with water. Areas marked under u.v. light or found by the aid of a reference strip sprayed with

HCl were taken out with a spatula, the gel was suspended in a centrifuge tube in 1 ml 1 N NH_3, and the substances were isolated by shaking with 2 ml dichloroethane, centrifuging and removing the organic layer with a Pasteur pipette. The procedure was repeated three times with 1 ml portions of dichloroethane, and the combined extracts were taken to dryness under a stream of nitrogen at 35°. Highly polar derivatives were extracted from the gel with methanol/25% ammonia (97:3).

Spectrophotometric measurements. For reading the u.v. spectrum substances were dissolved in 0·1 N HCl. Solutions obtained from unchromatographed extracts were equilibrated with half the volume of dichloroethane which removed coloured impurities. Spectra were read from 220 to 380 mμ on a Zeiss PMQ II spectrophotometer.

Quantitative determinations from spectrophotometric data were made by calculating the extinction difference E_{255}–E_{280} for sulfides and E_{270}–E_{285} for sulfoxides and correlating them to the extinction differences of solutions with known concentrations of reference compounds (Table 2).

TABLE 2. ABSORPTION DIFFERENCES OF PERAZINE DERIVATIVES USED FOR QUANTITATIVE DETERMINATIONS (0·1 N HCl, 1 cm LIGHT PATH)

	E_{255}–E_{280}	E_{270}–E_{285}
Perazine, 5×10^{-5}M	1·29	0·003
Perazine sulfoxide, 5×10^{-5}M	− 0·035	0·320

Phenolic metabolites were measured in HCl solution as the other compounds and under the form of their azo dyes: The phenol solution in 0·1 N HCl (3 ml) was mixed with 0·2 ml 0·33% sulfanilic acid solution in 30% (v/v) acetic acid followed by dropwise addition of 0·2 ml 0·05% $NaNO_2$ solution under vigorous shaking. After 5 min the extinction of the purple solution was measured at 555 mμ.

Chemical interconversions. Oxidation of sulfides to sulfoxides: This was done with perazine on a preparative scale by a procedure analogous to that used by Berger, Wechsler and Forrest[7] for analytical purposes. 500 mg perazine were dissolved in 50 ml 2 N CH_3COOH and heated after addition of 0·5 ml 33% hydrogen peroxide in a boiling water bath for 2 min. The cooled solution was alkalinized to pH 9 with sodium hydroxide and extracted with dichloroethane. Evaporation of the organic phase resulted in a crystalline residue which was recrystallized from benzene/petroleum ether. 400 mg perazine sulfoxide, melting point 146–147·5°.

On a smaller scale formation of sulfoxides can be carried out by leaving the sulfide (0·1–1 μmole) for 1 hr at 37° in an ethanol solution containing 3% H_2O_2 and 2% CH_3COOH.

Oxidation of tertiary amines to *N*-oxides: A solution of 0·5–3 μmole of the amine in 1 ml ethanol + 0·1 ml 33% H_2O_2 + 0·1 ml 25% ammonia was left at 37° for 4 hr. After evaporation under nitrogen the residue was spotted on a silica gel G plate for chromatographic separation of the reaction products in solvent S_{IV}. The sulfoxide and *N*-oxide sulfoxides were contained besides the *N*-oxide if the starting material was a sulfide.

Reduction of sulfoxides to sulfides: Zinc powder was added to a solution of the sulfoxide in 0·1 N HCl and the mixture left for 10 min with occasional shaking. The

sulfide was extracted from the alkalinized solution with dichloroethane. This procedure also reduces *N*-oxides to amines.[8, 9]

Selective reduction of *N*-oxides to amines: This is possible with sulfur dioxide according to Auerbach and Wolffenstein.[10] SO_2 gas is bubbled into a solution of the *N*-oxide in water or methanol for 2 min. The reaction is complete after leaving the mixture for 30 min at room temperature. Thermical splitting[11] was carried out in benzene solution, 1 hr at 60°.

N-Methylation was achieved by methyl iodide.[5] The phenolic secondary amine was reacted for 30 min at room temperature, other secondary amines for 2 hr.

O-Methylation: Overnight with diazomethane in methanol/ether solution.[5]

Acetylation was carried out with acetic anhydride + pyridine in benzene solution.

RESULTS

Unpolar unconjugated metabolites

Recovery of this fraction from urine can be considered quantitative, since re-extraction of the urine resulted in an amount of u.v. absorbing material corresponding to 1–2 per cent of the first fraction only. Besides, experiments with perazine and perazine sulfoxide added to urine free from drug metabolites gave recoveries of 97 and 95%, respectively. The u.v. spectrum of the crude extract of unpolar metabolites can be interpreted as being composed of the spectra of the perazine type and the perazine sulfoxide type (Fig. 1). The fact that these two compounds have identical

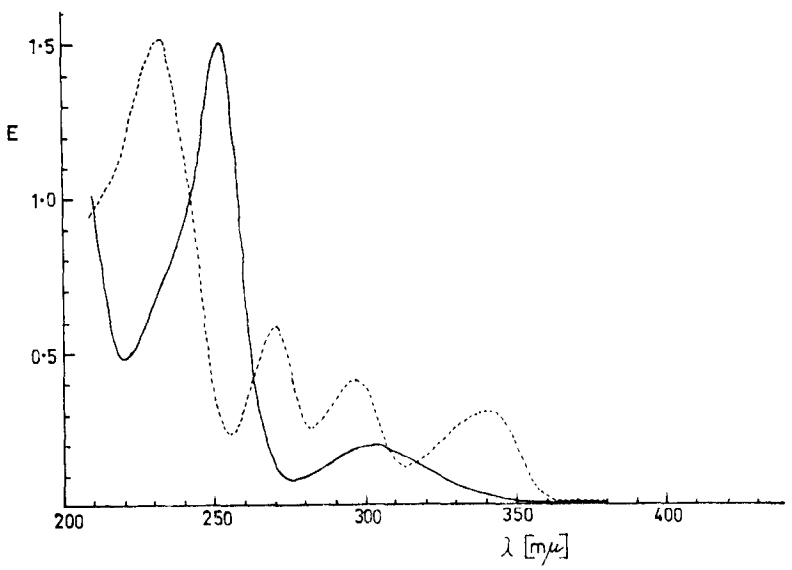

FIG. 1. Ultraviolet spectra measured in 0·1 N HCl (1 cm light path) ————, perazine, 5×10^{-5} M; — — — — — perazine sulfoxide, 5×10^{-5} M.

extinction coefficient at 263 and 308 mμ can be used to gain an estimate of the total amount of metabolites in the extract. The extinctions of an aliquot of the extract at 263 and 308 mμ were correlated to those of the reference compounds in a known concentration and from these data the amount of the total fraction was calculated. It usually represented 6–12 per cent of the dose administered. On the basis of this estimate the extract volumes subjected to thin layer chromatography were determined since

the separations became optimal with approximately 3 μmoles of material. This amount was contained in 15–180 ml of urine.

For a preliminary separation of the metabolites on silica gel layers the acidic solvent system of Green, Forrest, Forrest and Serra[12] modified by saturation with benzene, chloroform or dichloroethane proved to be the most favourable one. The chromatographic pattern obtained is shown in Fig. 2. All of the bands contained mixtures of two or more metabolites which were separated by further chromatography in basic solvent systems. Thus, from band A perazine sulfoxide (IIa) and desmethyl perazine sulfoxide (IIb) were purified in solvent S_{II}; band E was resolved in S_{III} into perazine (Ia) and desmethyl perazine (Ib) and band F into perazine *N*-oxide (IV) and a com-

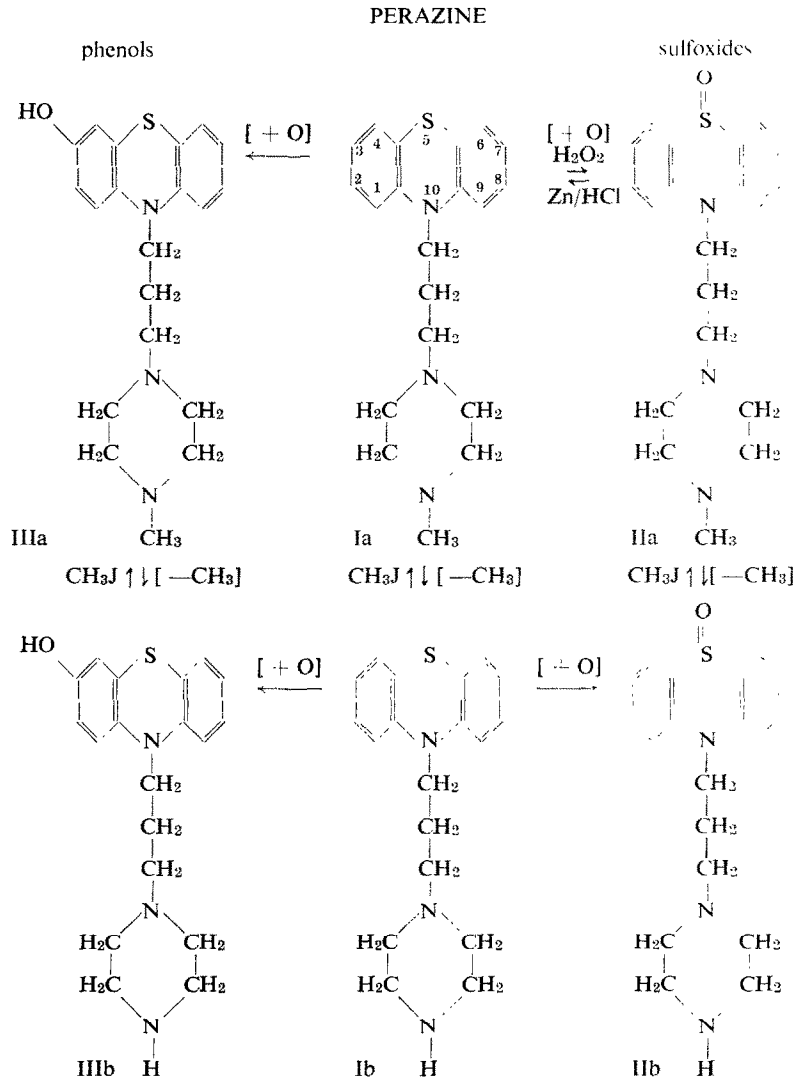

FIG. 3. Demethylated, hydroxylated and sulfoxidic metabolites of perazine and their chemical interconversions. Metabolic steps are indicated in brackets, alternative pathways of metabolism are represented by broken arrows.

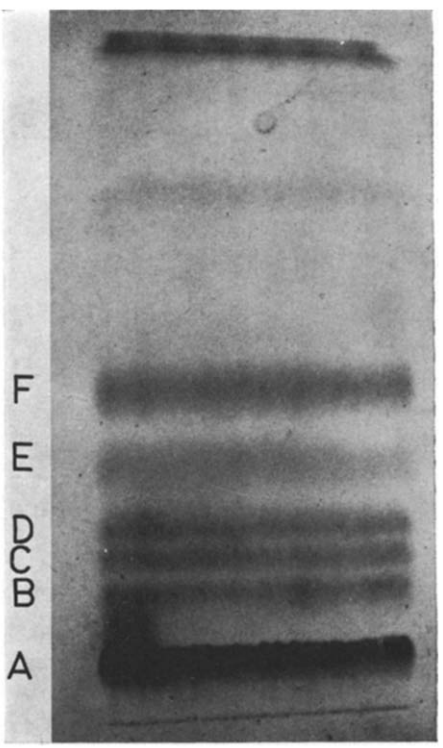

FIG. 2. Thin layer chromatogram of the unpolar metabolite fraction run in solvent system S_1. Colours developed with conc. HCl: band A, B, D and F dark red; band C purple; band E light red; Composition: band A IIa + IIb; band B mixture of minor sulfoxides; band C IIIa + IIIb; band D 2 or 3 minor sulfoxides; band E Ia + Ib; band F IV + VIII.

pound VIII with yet unknown structure. The *Rf* values of the single metabolites in the solvent systems used are summarized in Table 1.

In order to prove the identitities of the compounds, Ia was compared to the pure perazine and IIa to the sulfoxide obtained on a preparative scale by oxidation of perazine. R_f values were identical for synthetic materials and metabolites isolated from urine in all solvent systems. Zinc powder in 0·1 N HCl reconverted the sulfoxide to perazine. The demethylated compounds Ib and IIb could be remethylated to Ia and IIa, respectively, by methyl iodide, and IIb was reduced to Ib by zinc/HCl. The secondary amines also reacted with acetic anhydride. Chemical interconversions and possible metabolic pathways are depicted in Fig. 3.

Compound IV is reduced to perazine by zinc/HCl or by sulfur dioxide, and thermical release of the oxygen proceeds at 60°. IV can therefore be assumed to represent a

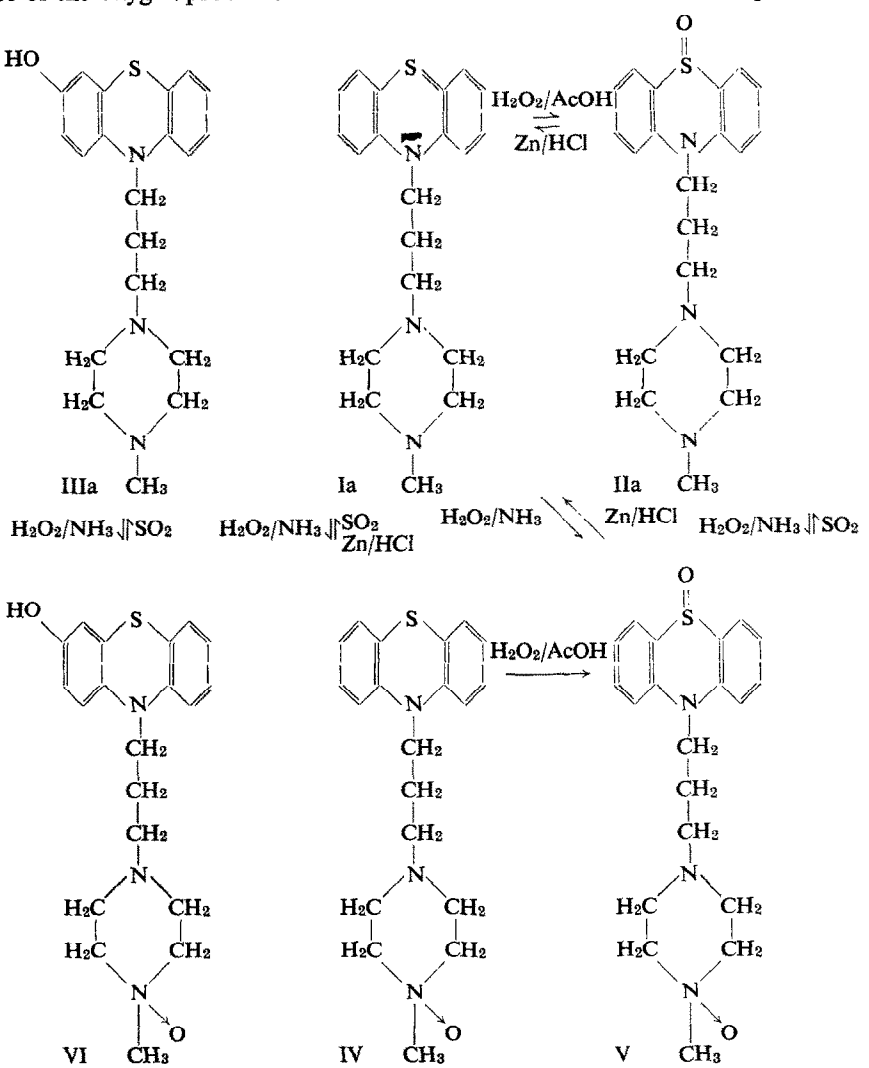

FIG. 4. Chemical interconversions of the *N*-oxide metabolites of perazine and its hydroxy and sulfoxide derivatives.

N-oxide of perazine. From the investigations of Beckett[14] on preferred sites of *N*-oxide formation the metabolite must be assumed to carry the *N*-oxygen on the same nitrogen as the methyl group, so that formula IV (Fig. 4) should be correct. The *N*-oxide can be obtained together with other oxidation products by reacting perazine with an ethanolic solution of hydrogen peroxide containing ammonia. The possibility of a formation of the *N*-oxide *in vitro* during the work-up procedure was ruled out by a control experiment: Perazine extracted from a urine sample was oxidized in trace amounts to the sulfoxide, but no *N*-oxide was detectable in accordance with observations in the chlorpromazine series.[15]

Substance VIII is a non-basic sulfoxide still containing three nitrogen atoms. Work is going on to elucidate the structure of this compound.

Band C in the chromatograms in S_I consists of the hydroxy perazines IIIa and IIIb; their separation and quantitative determination was not routinely done from band C, since their amount in the unconjugated metabolite fraction constituted a small and fairly constant part (1–3 per cent) of the total phenolic metabolites. Band B is composed of a large number of minor sulfoxidic metabolites out of which none was isolated in pure form. From band D two sulfoxides were purified in small amounts. None of them reacted with methyl iodide or SO_2 but the one with the lower R_f values in basic solvent systems is acetylated with acetic anhydride so that it may be assumed to contain an aliphatic hydroxy group.

Quantitative determination of purified substances was achieved by u.v. spectroscopy. The major metabolites of this fraction exhibit a spectrum of one of the two types: the perazine spectrum with maxima at 252 and 302 mμ and the perazine sulfoxide spectrum with maxima at 233, 271, 298 and 342 mμ (Fig. 1) in close agreement with the u.v. spectra of other phenothiazines.[16, 17] Desmethyl perazine and perazine *N*-oxide coincide in spectrum with perazine, desmethyl perazine sulfoxide and roughly also substance VIII with perazine sulfoxide. It was assumed that the molar extinction coefficients, too, are the same for all substances of one spectral type. The absorption differences used for quantitation, E_{255}–E_{280} for sulfides and E_{270}–E_{285} for sulfoxides, were chosen in a manner that values for the one type of spectrum are great, but for the other type negligible (Table 2).

The extracted substances from the second chromatography were measured and from the u.v. data the amounts of the individual compounds per litre of urine calculated. Over-all recoveries in the two consecutive chromatographic separations were checked for each metabolite and found to vary between 76% (Ib) and 85% (IIa). The average deviation between duplicate determinations from one extract was $\pm$ 5 per cent.

The relative amount of the substances in this fraction usually decreases in the order perazine *N*-oxide > perazine sulfoxide > desmethyl perazine sulfoxide > substance VIII > desmethyl perazine > perazine.

Polar unconjugated metabolites

Upon sulfur dioxide reduction this fraction yields mainly perazine sulfoxide (IIa). The amount of this product usually is around that of perazine *N*-oxide in the unpolar fraction. Chemical identification of the compound from which IIa is formed was achieved with a certain degree of reliability by synthesising model compounds through hydrogen peroxide oxidation of perazine or perazine sulfoxide. Because of the larger

number of products obtained with the former, oxidation of the sulfoxide is preferable. This results in three different products separable by TLC in solvent S_{IV}. The one with the highest R_f value runs together with the substance isolated from a butanol extract of pre-extracted urine by ion exchange chromatography. R_f values also coincide in solvent S_{II}. This substance is also formed by H_2O_2 oxidation of the *N*-oxide IV in acidic solution, so that we ascribe to it the constitution of perazine *N*-oxide sulfoxide (V, Fig. 4). The second substance from the H_2O_2 oxidation of perazine sulfoxide follows the first one very closely on the chromatogram and probably is the isomeric *N*-oxide sulfoxide with N^1 of the piperazine ring converted to an *N*-oxide. The third product with a distinctly lower R_f value is supposed to be the di-*N*-oxide sulfoxide. These two latter substances could not be detected in urine extracts. All three reaction products exhibit u.v. absorption spectra and fluorescence properties identical to those of perazine sulfoxide and are reconverted to the starting material by SO_2, while zinc HCl produces perazine.

Besides perazine sulfoxide the extracts after sulfur dioxide reduction of pre-extracted urine contain minor amounts of the secondary amines desmethyl perazine and desmethyl perazine sulfoxide. Acidification alone to pH 1 (which is reached upon saturation of urine samples with SO_2) will not liberate comparable amounts of material but rather gives the same traces of substances contained in re-extracts of urine freed from the unpolar fraction. Whether the source of the secondary amines are *N*-oxides on N^1 of the piperazine ring or molecules with some oxygen function on N^4 cannot yet be decided. The possibility of a demethylation as a side reaction of the *N*-oxide reduction with SO_2[18] was checked with perazine *N*-oxide. Only traces of desmethyl perazine corresponding to less than 1 per cent of the starting material could be detected.

Purification and quantitation of the compounds of this fraction were done on dichloroethane extracts of the reduced products according to the methods described above.

Polar conjugated metabolites

Treatment of urine samples, from which the unpolar fraction had been extracted, with pure β-glucuronidase or with an enzyme preparation containing aryl sulfatase in addition yielded equal amounts of phenolic metabolites judging from the coupling reaction with diazotized sulfanilic acid. Therefore it can be concluded that practically the whole conjugated fraction consists of glucuronides. Complete recovery of this fraction was checked by re-extraction which did not yield any appreciable further amount of phenols. The two main products accounting for 80–95 per cent of the dichloroethane-extractable phenolic fraction were separated by chromatography in solvent S_V and identified as 3-hydroxy perazine (IIIa) and desmethyl 3-hydroxy perazine (IIIb) (Fig. 3). Besides these only minor amounts of further phenolic metabolites with higher R_f values in solvent S_V occurred in this fraction.

The 3 position of the hydroxy group was derived from a comparison of the colour reactions with those given by the isomeric hydroxy promazines.[19] The properties of hydroxy perazine matched those of 3-hydroxy promazine exactly, since it gave a purple persulfate stain and the solution in 25% sulfuric acid showed maximal extinction at 567 mμ as compared to 564 mμ with 3-hydroxy promazine. The desmethyl derivative IIIb was converted to the tertiary amine by methyl iodide.

3-Hydroxy perazine sulfoxide (VII) and 3-hydroxy perazine *N*-oxide (VI) could be detected in butanol extracts after removal of the hydroxy perazines IIIa and IIIb. Whether VII is a naturally occurring metabolite of perazine cannot be decided, since small amounts of this substance are also formed during the work-up procedure. This was shown in a model experiment, where a solution of hydroxy perazine in an urine sample free from drug metabolites was subjected to the extraction procedures described above. In contrast, the *N*-oxide VI was not formed *in vitro*. The occurrence of its glucuronide as a metabolic product can be derived from the following criteria: urine samples from which the phenols IIIa and IIIb had been removed by glucuronidase incubation and extraction into dichloroethane yielded upon sulfur dioxide treatment and subsequent extraction with dichloroethane further amounts of hydroxy perazine corresponding to about 10 per cent of the original phenolic fraction. Extraction with *n*-butanol at pH 9 instead of SO_2 treatment resulted in a substance running closely behind hydroxy perazine sulfoxide in solvent S_{IV}, exhibiting a relatively weak fluorescence and giving a purple HCl stain. Upon removal from the plate and reduction with SO_2 hydroxy perazine was formed. Identical chromatographic and chemical properties were shown by a synthetic product obtained by oxidating hydroxy perazine with hydrogen peroxide in ethanol containing ammonia.

3-Methoxy perazine was synthetically prepared from IIIa using diazomethane and stated not to occur as an urinary metabolite which is in accordance with observations on chlorpromazine.[4]

For quantitative determination of the phenols the visible absorption of the coupling products with diazotized sulfanilic acid was measured. In order to gain a basis for the calculation of the absolute amounts from spectroscopic data we made use of the finding of Beckett *et al.*[17] that related phenothiazine derivatives exhibit closely similar extinction coefficients at the u.v. absorption maxima. In the case of perazine this was found to be true for perazine and its sulfoxide (Fig. 1). Since in addition the u.v. spectrum of hydroxy perazine closely resembles that of 7-hydroxy chlorpromazine derivatives[17] showing a maximum at 250 mμ, we assume that this rule applies to the hydroxy perazines, too. Therefore we suppose an absorption of 1·5 at 250 mμ to indicate a concentration of 5×10^{-5} M. Starting from this basis, we checked for a large number of samples the relation between u.v. absorption of the original compound and visible absorption of the coupling product. From the maximal value for the ratio $E_{80}/(E_{250}-E_{555})$ which was found to be 1·0, a factor for direct calculations from visible absorption data was derived.

The amount of hydroxy perazine usually exceeded that of the desmethyl derivative, while the sum of these two substances used to be constant for each single patient and in most of the cases roughly equal to the total unpolar fraction.

Extremely low single values for the phenol fraction occasionally observed in some patients resulted from the presence of nitrite in urine, most probably caused by contaminations with Coli bacteria. Model experiments with nitrite added to aliquots of urine confirmed this finding by considerably lowering the amount of phenols detectable after enzyme incubation.

DISCUSSION

The methods used for separation and determination of single metabolites in this study, i.e. TLC followed by u.v. spectrophotometry, are relatively laborious, but they

are the only ones which allow for a complete recovery of unchanged substances in not too small amounts after determination. This is advantageous when a new drug is under investigation and the metabolites are to be subjected to concomitant qualitative and quantitative analysis. The extraction from silica gel by distribution between aqueous ammonia and dichloroethane was chosen because attempts to extract the metabolites directly from the gel by 0·1 N hydrochloric acid resulted in partial decomposition of the non-sulfoxides yielding a pink colour with perazine and a lavender colour with hydroxy derivatives. In extracts with ammoniacal methanol, inorganic material from the silica gel was consistently contained.

The metabolic reactions found to occur in perazine are the same as those observed in chlorpromazine and related drugs.[4, 5, 8, 13, 15, 19–21] The relative amounts of metabolites detectable in urine, however, differ characteristically, since the *N*-oxides which constitute relatively minor urinary metabolites only in chlorpromazine[4, 8, 15, 22] are the main representatives of the non-hydroxylated metabolite fraction of perazine. An explanation for this peculiarity may be found in the relatively polar character of perazine caused by the presence of three nitrogen atoms. Introduction of a *N*-oxide into this molecule is likely to make it so hydrophilic that it is no longer reabsorbed in the kidney tubules and therefore appears in urine in relatively large amounts. The same thing is even more true for the *N*-oxide sulfoxide. In contrast, chlorpromazine *N*-oxide which is a main metabolite of chlorpromazine *in vitro*[9] can be supposed to be reabsorbed and then may be reconverted to the amine. That the reduction of chlorpromazine *N*-oxide is easily catalysed by liver enzymes has been shown by *in vitro* experiments.[23]

Whether more perazine metabolites are contained in urine than those extracted by our procedures cannot yet be decided, since no experiments with radioactively labelled material have been done. Butanol extracts prepared after removal of the unpolar and the phenolic fractions did not show major metabolites besides those described. But more polar substances, for instance with a split piperazine ring, cannot be excluded.

Details on the pattern of metabolites in individual psychiatric patients and on the change of pattern during treatment will be the subject of a separate paper.[24]

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