Biochemical Pharmacology, Vol. 23, pp. 313–322. Pergamon Press, 1974. Printed in Great Britain.

FORMATION OF IDENTICAL METABOLITES FROM PIPERAZINE- AND DIMETHYLAMINO-SUBSTITUTED PHENOTHIAZINE DRUGS IN MAN, RAT AND DOG

URSULA BREYER, HANS JÖRG GAERTNER and AXEL PROX

Institut fur Toxikologie der Universität Tübingen, 74 Tübingen, and Dr. Karl Thomae GmbH, 795 Biberach/Riss, Germany

(*Received* 13 *June* 1973; *accepted* 19 *July* 1973)

Abstract—Degradation of the piperazine ring in the phenothiazine drugs perazine, trifluoperazine, fluphenazine, prochlorperazine and perphenazine *in vivo* leads to the formation of γ-(phenothiazinyl-10)-propylamine (PPA) and of its ring-substituted analogues CF_3-PPA and Cl-PPA. The sulfoxides of these metabolites have been identified as urinary biotransformation products in patients ingesting perazine or fluphenazine, in rats treated chronically with perazine, trifluoperazine, prochlorperazine or perphenazine, and in a dog given fluphenazine. The structures of the compounds have been confirmed by mass spectrometry. The primary amines are also known metabolites of dimethylamino-substituted phenothiazines, since PPA results from didemethylation of promazine, and CF_3-PPA and Cl-PPA (= nor_2-chlorpromazine) are formed from triflupromazine and chlorpromazine, respectively.

WHEN neuroleptic drugs of the phenothiazine series that carry a piperazine ring in the side chain are incubated with rat liver microsomes, they are oxidatively attacked in a manner analogous to other phenothiazine drugs. Besides the introduction of an oxygen at the sulfur, at an aliphatic nitrogen and at the aromatic ring system to form the sulfoxides, the *N*-oxides and the phenols, respectively, a major biotransformation reaction is the removal of the methyl or the β-hydroxyethyl group from the terminal nitrogen atom.[1,2] Studies *in vivo* revealed that *N*-dealkylation in this class of drugs is not confined to these groups, but that the piperazine ring is degraded to a small extent, probably by an oxidative attack at the carbon atoms. Ethylenediamine derivatives have been detected as intermediates in this biodegradation.[3,4] Complete removal of the alkyl bridges connecting the two nitrogen atoms of the ring will result in the formation of γ-(phenothiazinyl-10)-propylamine (PPA) and its 2-substituted analogues, respectively. These compounds have been described as *in vivo* metabolites of dimethylamino-substituted phenothiazine drugs (for instance l.c.[5–7]), from which they are produced by two consecutive *N*-demethylation reactions.

The occurrence of PPA in the organs of rats treated chronically with perazine and the excretion of its sulfoxide in the urine of rats and patients following administration of perazine have already been mentioned.[3,8,9] The present paper will describe the isolation and characterization of the phenothiazinyl-propylamine sulfoxides excreted in urine by various species during ingestion of perazine, trifluoperazine, fluphenazine, prochlorperazine or perphenazine. For a comparison, the same metabolites were obtained from the urine of rats treated with promazine, triflupromazine or chlorpromazine. In the case of prochlorperazine and chlorpromazine, the non-sulfoxidic compound was demonstrated as a common tissue metabolite in rats.

MATERIALS AND METHODS

Drugs. These were received from the companies mentioned previously[2] and from the following: perazine dimalonate (Taxilan®, Chemische Fabrik Promonta, Hamburg, Germany), prochlorperazine bis-methanesulfonate (Témentil®, Rhône-Poulenc, Paris, France), promazine hydrochloride (Protactyl®, Wyeth-Pharma, Münster, Germany), triflupromazine hydrochloride (Psyquil®, Chemische Fabrik von Heyden, Regensburg, Germany) and chlorpromazine hydrochloride (Megaphen® Bayer, Leverkusen, Germany).

Drug metabolites. γ-(Phenothiazinyl-10)-propylamine (PPA) as hydrochloride and *N*[γ-(phenothiazinyl-10)-propyl]piperazine (desmethyl perazine) as dimalonate were kindly supplied by Chemische Fabrik Promonta (Hamburg, Germany). γ-(2-Trifluoromethyl-phenothiazinyl-10)-propylamine (CF_3-PPA) and the 2-chloro analogue Cl-PPA were prepared by cyanoethylation of the corresponding phenothiazines[10] followed by $LiAlH_4$ reduction of the nitriles.[11] A sample of Cl-PPA (nor$_2$-chlorpromazine) was also supplied by Dr. A. A. Manian (National Institute of Mental Health, Bethesda, U.S.A.). The sulfoxides of the compounds were prepared by H_2O_2 oxidation.[12]

N[γ-(Phenothiazinyl-10)-propyl]ethylenediamine (PPED, IV: R_1 = H, Fig. 1), its 2-chloro analogue Cl-PPED (IV: R_1 = Cl) and the *N'*-methyl derivative Cl-PPMED (III: R_1 = Cl, R_2 = CH_3) were synthesized by reacting γ-(phenothiazinyl-10)-propylchloride and γ-(2-chloro-phenothiazinyl-10)-propylchloride,[13] respectively, with ethylenediamine or *N*-methyl-ethylenediamine.*

Drug regimen and urine collection in patients. Psychiatric in-patients studied were two males receiving fluphenazine (6 mg t.i.d. and 12 mg t.i.d., respectively) and 10 males and two females under treatment with perazine (200–600 mg daily in three divided doses). Three out of these patients were also studied for 18–31 days following termination of a treatment with 600 mg perazine. In a further patient, several investigations were carried out 3 months after withdrawal of perazine (final dose 900 mg daily). Urine was either collected during 24 hr, or a morning specimen was obtained. The samples were stored at −18° until analyzed.

Animal treatment. Male Wistar rats weighting 280–350 g were treated chronically with piperazine-substituted phenothiazine drugs. The compounds were given daily by oesophageal tube as aqueous solutions of their salts in a volume of 5 ml/kg body wt. In the case of Cl- and CF_3-substituted drugs, the doses had to be increased slowly over several weeks. The quantities administered (expressed as free base) were: perazine 50 mg/kg twice daily, trifluoperazine 5–40 mg/kg, prochlorperazine 10–50 mg/kg, perphenazine 2–20 mg/kg. When the final doses had been reached and also during application of 25 mg/kg prochlorperazine and 10 mg/kg perphenazine, urine was collected for a period of 12 hr.[2]

Twelve-hour urine collections were also carried out following administration of single doses of triflupromazine (10 mg/kg), and promazine, chlorpromazine, prochlorperazine, desmethyl perazine, PPED, Cl-PPED or Cl-PPMED (25 mg/kg).

For studying tissue metabolites, chlorpromazine was given to rats during 2 weeks, the dose being increased from 2 × 10 to 50 mg/kg.

A female Beagle dog weighing 11 kg was fed fluphenazine in gelatine capsules. The

* U. BREYER *et al.*, to be published.

dose was 10 mg/kg for 6 days and 20 mg/kg for 10 days. Twelve hr after drug ingestion, the dog was placed into a metabolic cage, and 6 hr later urine was obtained by catheterization.

Isolation and quantitation of metabolites. Urine from patients (400 ml) was adjusted to pH 9 with 5 N NaOH and extracted three times with 200 ml redistilled chloroform. The organic phase was evaporated and the residue transferred to a 20 × 20 cm plate coated manually with 0·4 mm silica gel GF_{254} (Merck, Darmstadt, Germany). The extract was applied on a band 8 cm wide along with reference compounds, and the first separation was carried out in solvent C_1 (Table 1). The u.v. absorbing band corresponding to PPA-SO or CF_3-PPA-SO was removed and the substances isolated by partitioning[14] using two 2-ml portions of chloroform as extractant. The material obtained was rechromatographed in solvent A and subsequently in solvent B (Table 1).

TABLE 1. R_f VALUES OF PPA, CF_3-PPA, Cl-PPA AND OF THEIR SULFOXIDES IN THIN LAYER CHROMATOGRAPHY ON SILICA GEL

Compound	R_f value in solvent*			
	A	B	C_1	C_2
PPA	0·60	0·72	0·38	0·52
CF_3-PPA	0·73	0·85	0·46	0·60
Cl-PPA	0·67	0·80	0·42	0·58
PPA-SO	0·26	0·39	0·15	0·30
CF_3-PPA-SO	0·47	0·65	0·23	0·43
Cl-PPA-SO	0·38	0·57	0·21	0·37

* Solvents—A: isopropanol–chloroform–25% ammonia–water, 32:16:2:1. B: acetone–isopropanol–1 N ammonia, 27:21:6. C_1: 1,2-dichloroethane–ethyl acetate–ethanol–acetic acid water, 25:28:8·5:8·5:5. C_2: 1,2-dichloroethane–ethyl acetate–ethanol–acetic acid–water, 15:26:12:8:7·5.

Dog urine (100–180 ml) was processed analogously. Rat urine was processed as described by Gaertner *et al.*,[2] in most cases specimens from two rats being pooled. Following chromatography in solvent A, bands containing PPA-SO, CF_3-PPA-SO or Cl-PPA-SO were rechromatographed in solvent B. When rats had received promazine, triflupromazine or chlorpromazine, urinary metabolites were subject to a first separation in solvent B followed by a purification of the primary amine sulfoxides in solvent C_2.

Rats treated chronically with 50 mg/kg prochlorperazine or chlorpromazine were sacrificed 24 hr after the last dose, and 5 g of liver tissue was extracted.[8] Chromatography of the extracts was performed in chloroform–isopropanol, 10:1, followed by solvent A. The band containing Cl-PPA was removed and the material purified in solvent B.

The purified metabolites were dissolved in 3 ml 0·1 N HCl and the u.v. absorption measured at two characteristic wavelengths,[2,14] if necessary following dilution.

For mass spectrometric investigation, the compounds were re-extracted from the aqueous solutions after addition of ammonia, and pooled samples were further purified by chromatography in methanol–25% ammonia, 20:1, on silica gel plates prewashed with this solvent and with hexane.

Mass spectrometry. High and low resolution mass spectra were recorded using a MS 902 S-DS 30 instrument (A.E.I., Manchester, England). All samples were directly introduced into the ion source. The ion source temperature varied between 120 and 150°. A trap current of 500 and 100 μA was used for recording of the high and low resolution mass spectra, respectively. High-resolution mass spectrometry was performed on-line with a 16 sec scan and a resolving power better than 10,000. All mass measurements were within 10 ppm.

Dansyl derivatives. Sulfoxides were reduced to the sulfides with Zn–HCl before derivatization. The reaction with 5-dimethylamino-1-naphthalene sulfonyl chloride was carried out at 45°.[15] The derivatives were extracted with benzene and chromatographed on silica gel foils (Polygram Sil G/UV$_{254}$, Macherey-Nagel, Düren, Germany) in solvents D and E (Table 2).

RESULTS

In all five piperazine-substituted phenothiazine drugs tested (I, Fig. 1), the piperazine ring was degraded *in vivo* leaving an amino group on the propyl side chain. The resulting compounds, γ-(phenothiazinyl-10)-propylamine (PPA), γ-(2-trifluoromethyl-phenothiazinyl-10)-propylamine (CF_3-PPA) and the 2-chloro analogue (Cl-PPA) were excreted in urine as their sulfoxides PPA-SO, CF_3-PPA-SO and Cl-PPA-SO (Fig. 1). Also following administration of intermediates in the piperazine ring degradation, PPED (IV: R_1 = H, Fig. 1), Cl-PPED (IV: R_1 = Cl) and Cl-PPMED (III: R_1 = Cl, R_2 = CH_3), the primary amine sulfoxides PPA-SO and Cl-PPA-SO, respectively, were obtained as urinary metabolites.

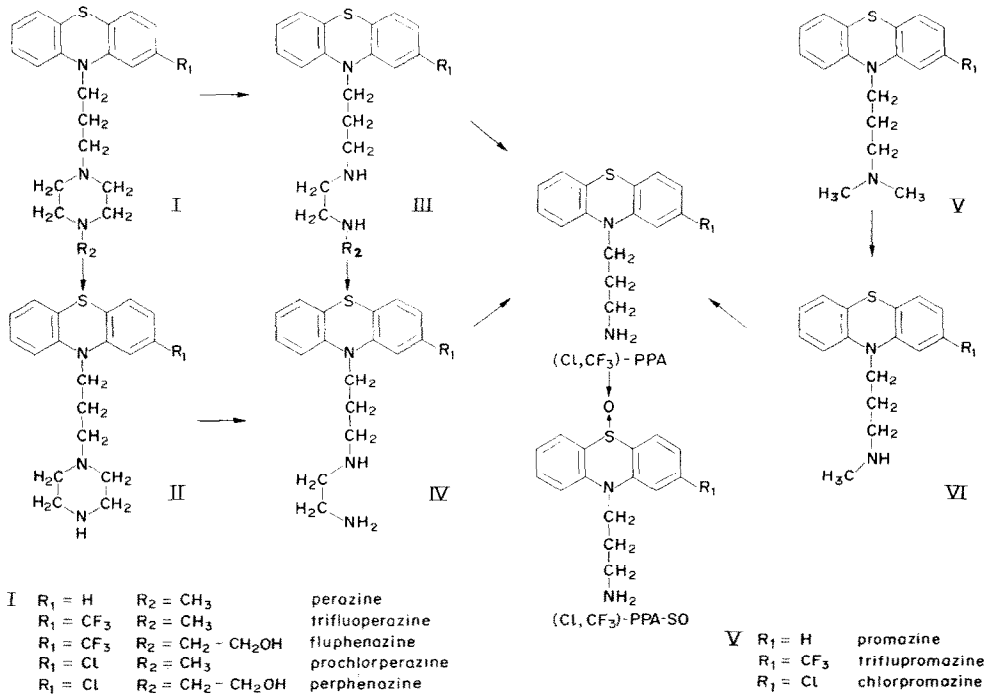

FIG. 1. Metabolic pathways leading to the formation of phenothiazinyl-propylamines from piperazine- and dimethylamino-substituted phenothiazine drugs.

Identical products resulted from demethylation and sulfoxidation of the corresponding dimethylamino-substituted phenothiazines (V, Fig. 1). The biotransformation of promazine to PPA-SO[6] and of chlorpromazine to Cl-PPA-SO[5] was confirmed, and the analogous degradation of triflupromazine to CF_3-PPA-SO could be demonstrated.

In addition, Cl-PPA was detected in rat liver not only following chlorpromazine, but also after treatment with prochlorperazine.

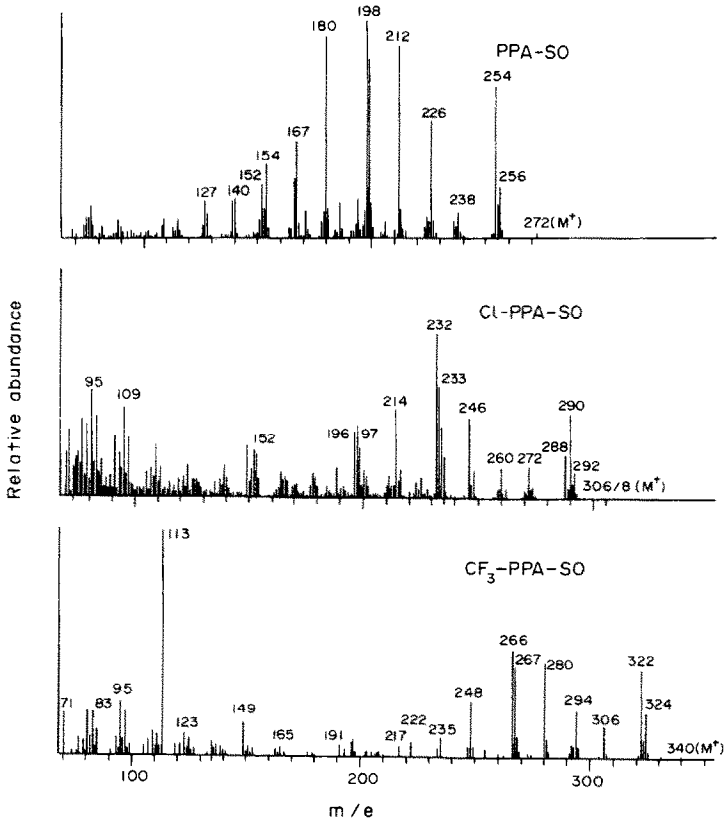

FIG. 2. Mass spectra of PPA-SO from the urine of patients medicated with perazine, of Cl-PPA-SO from the urine of rats treated with prochlorperazine, and of CF_3-PPA-SO from the urine of a patient receiving fluphenazine.

The identity of the metabolites was established by criteria being in some cases the mass spectra, in the other cases the u.v. spectra and the thin layer chromatographic characteristics of the substances in the form of the sulfides and of the sulfoxides and the chromatographic properties of the dansyl derivatives.

Mass spectra (Figs. 2 and 3). The spectra of PPA-SO and Cl-PPA-SO obtained as urinary metabolites of perazine and prochlorperazine, respectively, were compared to those of the synthetic substances and found to be identical. In the case of CF_3-PPA-SO, the fluphenazine metabolite only was investigated.

The metabolites showed a very characteristic fragmentation pattern. Mass spectrometry of these compounds, however, was complicated by their thermal lability. It

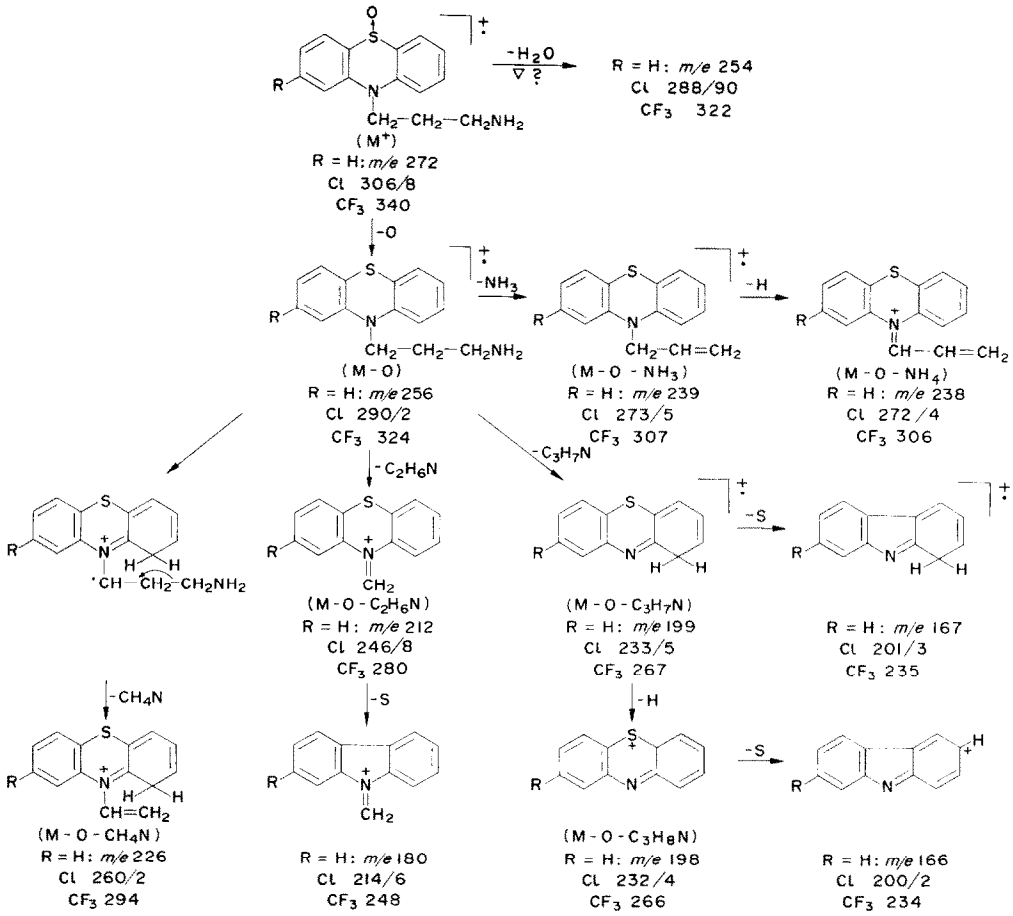

FIG. 3. Fragmentation pattern of PPA-SO, Cl-PPA-SO and CF_3-PPA-SO in mass spectrometry.

has been observed that the elimination of a water molecule from the molecular ion was at least partially due to a thermal process. The intensity of the corresponding ions increased with increasing probe temperature. Thus, changes in the intensity ratios will be observed using different evaporation temperatures. Since this may affect structure elucidation it should be emphasized, however, that there were no changes in the fragmentation pattern.

The initial process in all of these sulfoxides under electron impact was the loss of an oxygen atom. High resolution mass measurements revealed no fragments retaining the sulfoxide oxygen. All of them were derived from the (M–O) ions. Both processes, the elimination of water and the loss of oxygen, may cause the low abundance of the molecular ions. Nevertheless, the elemental composition of these ions could be fully confirmed by high resolution mass spectrometry.

α-Cleavage in the side chain gave rise to one of the most prominent ions at *m/e* 30 (CH_4N). Thus, the presence of a —CH_2—NH_2 residue became evident. It was further established by the complement loss of 30 amu (CH_4N) from the M—O) ion. As seen in Fig. 3, this may be promoted by a hydrogen rearrangement. The presence

of a primary amine was also indicated by the elimination of NH_3 and $NH_3 + H$, respectively, from the (M—O) ion. This finding is in full agreement with those made on other phenothiazine derivatives.[16]

Loss of a C_2H_6N radical was due to a C—C bond cleavage α to the phenothiazine nitrogen. Thus, a $—CH_2—CH_2—CH_2—NH_2$ substituent at this position may be suggested. It was confirmed by the occurrence of a McLafferty rearrangement which eliminated the entire side chain under hydrogen rearrangement ($M—O—C_3H_7N$). The resulting ion lost a further hydrogen atom. As illustrated in Fig. 3, several of the ion fragments underwent elimination of a sulfur atom from the phenothiazine nucleus.

TABLE 2. R_f VALUES OF 5-DIMETHYLAMINO-1-NAPHTHALENE-SULFONYL (DANSYL) DERIVATIVES OF PHENOTHIAZINYL-PROPYLAMINES ON SILICA GEL

	R_f value in solvent*	
Compound	D	E
Dansyl-PPA	0·42	0·49
Dansyl-CF_3-PPA	0·43	0·41
Dansyl-Cl-PPA	0·43	0·38

* Solvents—D: chloroform–ethyl acetate, 15:1. E: ethanol–water, 2:1.

Thin layer chromatography. PPA-SO, CF_3-PPA-SO and Cl-PPA-SO isolated from urine after administration of the various drugs were compared with the corresponding sulfoxides obtained by H_2O_2 oxidation of the synthetic reference compounds and found to be indistinguishable in four solvent systems (Table 1). They also exhibited chromatographic properties identical to those of authentic material following reduction to the sulfides. Cl-PPA extracted from livers of rats treated with prochlorperazine cochromatographed with that produced from chlorpromazine and with the reference compound in all solvents. In addition, the ninhydrin reaction was carried out routinely and found to be consistently positive.

Dansyl derivatives of PPA, CF_3-PPA and Cl-PPA from the various sources behaved identically in two solvents (Table 2) upon comparison with each other and with the derivatives of the synthetic compounds.

By these and previous experiments, metabolic degradation to phenothiazinyl-propylamines could be shown to occur in the following situations:

PPA was found in rat tissues following chronic treatment with perazine.[3]

PPA-SO was excreted.

(a) by patients under medication with perazine (200–600 mg daily); in addition for up to more than 4 weeks following treatment with 600 mg daily (3 patients) and at least for 3 months after termination of a treatment with increasing perazine doses (300–900 mg daily) for 4 weeks (1 patient).
(b) by rats fed perazine chronically (twice 50 mg/kg daily).
(c) in small quantities by rats given a single dose of desmethyl perazine (II: R_1 = H, Fig. 1; 25 mg/kg).

(d) by rats that had received an acute dose of PPED (25 mg/kg).
(e) by rats treated with promazine (25 mg/kg).

CF_3-PPA-SO was excreted
(a) by patients during treatment with fluphenazine (18 or 36 mg daily).
(b) by a dog receiving chronically 10 or 20 mg/kg fluphenazine per day.
(c) by rats given chronically trifluoperazine (40 mg/kg).
(d) by rats following acute administration of triflupromazine (10 mg/kg).

Cl-PPA was detected in livers of rats chronically treated
(a) with prochlorperazine (50 mg/kg).
(b) with chlorpromazine (50 mg/kg).

Cl-PPA-SO was excreted
(a) by rats during acute or chronic treatment with prochlorperazine (25 or 50 mg/kg).
(b) by rats having received once the prochlorperazine metabolites Cl-PPED or Cl-PPMED (25 mg/kg).
(c) by rats given chronically perphenazine (10 or 20 mg/kg).
(d) by rats following a single dose of chlorpromazine (25 mg/kg).

Following the administration of dimethylamino-substituted drugs, the primary amine sulfoxides were excreted in greater quantities than after application of the piperazine derivatives. For instance, 10 rats given acutely 25 mg/kg prochlorperazine excreted an average 0·2 per cent of the dose as Cl-PPA-SO within 12 hr, whereas an acute dose of 25 mg/kg chlorpromazine gave rise to Cl-PPA-SO in urine in a quantity of 1·4 per cent of the dose. Prolonged treatment with prochlorperazine produced a doubling of the Cl-PPA-SO excretion. In patients receiving perazine or fluphenazine, the quantities of PPA-SO or CF_3-PPA-SO, respectively, in urine accounted for approximately 0·2–0·4 per cent of the drug dose. A relation to the duration of treatment could not yet be established, but in the dog given fluphenazine this was clearly present, since the quantity of CF_3-PPA-SO increased sixfold relative to fluphenazine sulfoxide in the course of drug administration.

DISCUSSION

The results obtained show that *in vivo* a metabolic convergence takes place leading to common metabolites from the two major classes of phenothiazine drugs, namely those carrying a dimethylamino group and those containing a piperazine ring in the side chain. The reaction sequence by which the piperazine ring is degraded occurs irrespective of whether the substituent on the ring is methyl (perazine, prochlorperazine, trifluoperazine) or β-hydroxyethyl (fluphenazine, perphenazine), and it is observed in all three series with different substituents in the 2-position of the phenothiazine nucleus (H, CF_3, Cl).

Whether the metabolic relationships bear relevance for the pharmacodynamic properties of the drugs remains open to question. Due to a lack of pharmacological test systems that are unequivocally accepted to measure effects of drugs that correlate with their antipsychotic activity, a possible contribution of the primary amines to the therapeutic action of the phenothiazine drugs will not be easily established.

Reports from the literature on pharmacological effects are available for Cl-PPA only, and the results are conflicting. The compound had little influence on the behaviour of rats[17,18] and proved unable to suppress amphetamine-induced stereotypies

in rats.[19] In contrast, it was comparable to chlorpromazine in its potency to stimulate the dopamine synthesis and the accumulation of homovanillic acid in mouse brain.[20,21]

Also concerning the quantities of the primary amine found in chlorpromazine treatment contradictory reports have been published. Forrest *et al.*[7] detected low concentrations of Cl-PPA only in the organs of patients chronically medicated up to the time of death, and Curry and Marshall[22] measured small quantities in the plasma. However, Turano *et al.*[23] described Cl-PPA as the most abundant plasma metabolite in patients receiving chlorpromazine.

A remarkable feature is the continuing urinary excretion of the primary amines as sulfoxides after termination of drug administration. Following treatment with piperazine-substituted phenothiazines, the compounds obviously result from further degradation of the ethylenediamine derivatives (III and IV, Fig. 1) which accumulate in tissues during drug administration.[4,8] In rats having received perazine, the excretion of PPA-SO could be observed as long as PPED levels in excess of 20 μg/g were present in the liver. In psychiatric patients withdrawn from perazine, the sustained excretion of PPA-SO was paralleled by an excretion of the sulfoxides of the ethylenediamine derivatives themselves.[9] That the ethylenediamines can serve as precursors of the propylamines has been confirmed by the experiments presented in this paper.

When in chronic treatment the doses of the piperazine-substituted drugs are high enough to allow for an accumulation of the ethylenediamines, there will be an increase in the relative amount of the propylamines with time. This has been shown in the dog receiving fluphenazine and in the rats treated with prochlorperazine. Another reason why the piperazine-substituted drugs were given chronically is that they are poorly tolerated by rats. Since total urinary metabolites represent only a small fraction of the dose,[2,24] the primary amine sulfoxides are below the level of detectability in acute experiments with low doses. Upon repeated administration, tolerance develops to the drugs, and the doses can be increased to a level where the metabolites described are easily demonstrable.

Following cessation of chlorpromazine medication, the primary amine seems to be eliminated slowly. In connection with investigations on the "pink spot" a chlorpromazine metabolite was shown to be excreted by patients for up to 25 days[25] or even 65 days[26] after drug withdrawal, especially following a high-dose regimen.[26] This metabolite could be identified as nor_2-chlorpromazine sulfoxide (Cl-PPA-SO).[27,28] Unfortunately detailed studies on individual urinary metabolites during the period of elimination following chlorpromazine therapy are lacking, so that it is not known whether other metabolites show a prolonged excretion similar to that of Cl-PPA-SO. Careful evaluations would be particularly interesting in view of a report that "reappearance of clinical symptoms coincided with the disappearance of detectable amounts of chlorpromazine metabolites from the urine".[29]

Acknowledgements—The authors wish to thank the pharmaceutical companies mentioned above for supplying the drugs. Thanks are due to Dr. R. Bertele and Mr. U. Künzel for synthesizing some of the metabolites, to Mr. H. Wachsmuth for recording the mass spectra and to Mr. J. Strecker for the contribution of some measurements. The skilful technical assistance of Miss G. Liomin and Mr. G. Joachim is gratefully acknowledged. The work has been supported by the Deutsche Forschungsgemeinschaft.

REFERENCES

1. U. BREYER, *Biochem. Pharmac.* **20,** 3341 (1971).
2. H. J. GAERTNER, U. BREYER and G. LIOMIN, *Biochem. Pharmac.* **23,** 303 (1974).
3. U. BREYER, D. KRAUSS and J. C. JOCHIMS, *Experientia* **28,** 312 (1972).
4. H. J. GAERTNER and U. BREYER, *Arzneimittel-Forsch.* **22,** 1084 (1972).
5. H. GOLDENBERG and V. FISHMAN, *Proc. Soc. exp. Biol. Med.* **108,** 178 (1961).
6. H. GOLDENBERG, V. FISHMAN, A. HEATON and R. BURNETT, *Proc. Soc. exp. Biol. Med.* **115,** 1044 (1964).
7. I. S. FORREST, A. G. BOLT and M. T. SERRA, *Biochem. Pharmac.* **17,** 2061 (1968).
8. U. BREYER, *Biochem. Pharmac.* **21,** 1419 (1972).
9. U. BREYER and H. J. GAERTNER, *Proc. Eur. Soc. for the Study of Drug Toxicity*, Excerpta Medica, Int. Congr. Series **288,** 59 (1973).
10. K. FUJII, *J. pharm. Soc. Japan* **76,** 637 (1956).
11. E. F. GODEFROI and E. L. WITTLE, *J. org. Chem.* **21,** 1163 (1956).
12. M. BERGER WECHSLER and I. S. FORREST, *J. Neurochem.* **4,** 366 (1959).
13. M. H. SHERLOCK and N. SPERBER, U.S. Pat. No. 2,860,138; *Chem. Abstracts* **53,** 7212 (1959).
14. U. BREYER, *Biochem. Pharmac.* **18,** 777 (1969).
15. R. W. FREI and J. F. LAWRENCE, *J. Chromatogr.* **61,** 174 (1971).
16. J. N. T. GILBERT and B. J. MILLARD, *Org. Mass Spectrom.* **2,** 17 (1969).
17. G. G. BRUNE, H. H. KOHL, W. G. STEINER and H. E. HIMWICH, *Biochem. Pharmac.* **12,** 679 (1963).
18. H. H. KOHL, G. G. BRUNE and H. E. HIMWICH, *Biochem. Pharmac.* **13,** 539 (1964).
19. S. LAL and T. L. SOURKES, *Eur. J. Pharmac.* **17,** 283 (1972).
20. H. NYBÄCK and G. SEDVALL, *Psychopharmacologia* **26,** 155 (1972).
21. J. DAILEY, G. SEDVALL and B. SJOQUIST, *J. Pharm. Pharmac.* **24,** 580 (1972).
22. S. H. CURRY and J. H. MARSHALL, *Life Sci.* **7,** 9 (1968).
23. P. TURANO, J. E. MARCH, W. J. TURNER and S. MERLIS, *J. Med.* **3,** 109 (1972).
24. A. P. MELIKIAN and I. S. FORREST, *Proc. West. Pharmac. Soc.* **15,** 78 (1972).
25. K. PIND and A. FAURBYE, *Acta psychiat. scand.* **42,** 246 (1966).
26. M. SIEGEL and H. TEFFT, *J. nerv. ment. Dis.* **152,** 412 (1971).
27. H. R. STEINBERG and J. ROBINSON, *Nature, Lond.* **217,** 1054 (1968).
28. N. WAD and K. CLOSS, *J. Pharm. Pharmac.* **23,** 131 (1971).
29. S. SVED, A. PERALIS and D. PALAIC, *Br. J. Psychiat.* **119,** 589 (1971).