Journal of Chromatography, 283 (1984) 211–221
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,287

IDENTIFICATION AND QUANTITATION OF 1-ARYLPIPERAZINES, METABOLITES RESULTING FROM SIDE-CHAIN CLEAVAGE OF (4-SUBSTITUTED ARYL-1-PIPERAZINYL)ALKYL HETEROCYCLIC DERIVATIVES IN RAT PLASMA AND BRAIN

S. CACCIA*, A. NOTARNICOLA, M. H. FONG and E. BENFENATI
Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea 62, 20157 Milan (Italy)
(First received July 20th, 1983; revised manuscript received September 9th, 1983)

SUMMARY

Many drugs contain the arylpiperazine moiety in the side-chain of their molecules. A common metabolic pathway of such drugs is cleavage of the side-chain with the formation of 1-arylpiperazines. This has been verified by combined gas chromatography–mass spectrometry of biological samples from rats given orally a series of heterocyclic derivatives bearing a 4-aryl(phenyl, pyrimidinyl, pyridyl or thiazolyl)-1-piperazinylalkyl moiety (oxypertine, zolertine, millipertine, empiprazole, dapiprazole, antrafenine, piribedil, azaperone). A sensitive and selective electron-capture gas–liquid chromatographic procedure for 1-arylpiperazines in rat plasma and brain is described. The overall recovery from plasma and brain was 70–90%. The limit of detection for substituted (halogenated) phenylpiperazines was 10–25 ng/ml or ng/g and 25–100 ng/ml or ng/g for other derivatives. Preliminary data are reported on the time course of the production and elimination of 1-arylpiperazines after oral administration of representative compounds with the arylpiperazine moiety (oxypertine, azaperone and S-3608).

INTRODUCTION

Cleavage of the arylpiperazine side-chain, leading to the formation of piperazine derivatives, is important in the metabolism of trazodone[1,2], an antidepressant agent recently introduced into clinical practice (see ref. 3 for a review), as well as its structurally and pharmacologically related compounds containing the *m*-chlorophenylpiperazine (mClPP) moiety in their chemical structure[4] and buspirone[5], a newly developed antianxiety agent[6,7] with a pyrimidinylpiperazine (PmP) side-chain. It has been found that these metabolites, like many other piperazine derivatives, are biochemically and pharmacologically active[8–14], and thus may contribute to their parent drugs' pharmacological effects. These findings suggested that 1-arylpiperazine formation could be a pharmacologically important pathway for other structurally related compounds, hence our interest in the metabolism of other drugs containing the arylpiperazine moiety in the side-chain. As a basis for these investigations we

0021-9673/84/$03.00

developed the present gas–liquid chromatographic procedure with electron-capture detection (GLC–ECD) for the quantitation of 1-arylpiperazines in biological samples. Preliminary data are given on the plasma and brain concentrations of 1-arylpiperazines formed during the biotransformation of representative compounds of this category.

EXPERIMENTAL

Drugs and their metabolites were kindly supplied by the following companies: dapiprazole hydrochloride by Angelini (Rome, Italy); 1-*m*-trifluoromethylphenylpiperazine (mCF_3PP) hydrochloride and 1-phenylpiperazine (PP) hydrochloride by Clin-Midy (Milan, Italy), 1-*p*-fluorophenylpiperazine (pFPP) by Centre de Recherche Delalande (Rueil-Malmaison, France), piribedil methane sulphonate, S-3608 [coumaranyl-5-methyl)-4-(thiazolyl-2)-1-piperazine hydrochloride] and 1-(2-thiazolyl)piperazine (TZP) by Les Laboratoires Servier (Orléans, France), 1-*o*-chlorophenylpiperazine (oClPP), 1-*m*-chloropenylpiperazine (mClPP), 1-*p*-chlorophenylpiperazine (pClPP) hydrochlorides and empiprazole hydrochloride by Merck (Darmstadt, F.R.G.), azaperone, 1-*o*-methoxyphenylpiperazine ($oOCH_3PP$) dihydrochloride and 1-(2-pyridyl)piperazine (PdP) dihydrochloride by Janssen Pharmaceutica (Beerse, Belgium), oxypertine and milipertine hemitartrate by Sterling Winthrop (Milan, Italy) and antrafenine by L.E.R.S.-Synthelabo (Paris, France).

Heptafluorobutyric anhydride (HFBA) was obtained from Pierce (Rockford, IL, U.S.A.). Formic acid, acetone, *n*-heptane, chloroform, ethyl acetate and benzene (Pestenal grade) were obtained from Farmitalia-Carlo Erba (Milan, Italy).

Apparatus

1-Arylpiperazine heptafluorobutyrates were analysed on a Carlo Erba Fractovap 2150 gas chromatograph equipped with a ^{63}Ni electron-capture detector. The chromatographic column was a glass tube (2 m × 3 mm I.D.) packed with 80–100-mesh Supelcoport with 3% OV-17 as the stationary phase (Supelco, Bellefonte, PA, U.S.A.). The oven, injection port and detector temperatures were 185, 250 and 250°C, respectively. The carrier gas was nitrogen at a flow-rate of 40 ml/min.

Gas chromatography–mass spectrometry (GC–MS) was performed on an LKB 2091 instrument equipped with an LKB 2130 computer system for data acquisition and calculation and used in the electron-impact mode. The GC–MS operating conditions were as follows: chromatographic column and operating conditions as previously indicated; ion source temperature, 250°C; electron energy, 70 eV; trap current, 50 μA; and accelerating voltage, 3.5 kV.

Animals

Male CD-COBS rats (Charles River, Como, Italy) weighing about 200 g were used. They were treated orally with substituted N-piperazine derivatives and killed at various times after administration. Blood was collected in heparinized test-tubes, centrifuged and the plasma stored at −20°C. Brains were immediately removed and stored at −20°C.

Procedure

Plasma and brain homogenate, after addition of an appropriate internal standard, were processed as previously described[15]. Drug-free plasma and brain samples with known amounts of a given arylpiperazine were analysed concurrently with each set of unknown samples. Concentrations of 1-arylpiperazines in the unknown samples were obtained from the ratio of their peak areas to that of the internal standard. Percentage recoveries were calculated by comparing the peak-area ratios of arylpiperazine heptafluorobutyrates after plasma and brain extraction with the peak-area ratios obtained by direct injection of standard solutions of 1-arylpiperazine heptafluorobutyrates.

RESULTS AND DISCUSSION

The heptafluorobutyryl derivatives of 1-arylpiperazines were easily prepared and appeared to be stable in benzene solution for several days. As expected, they

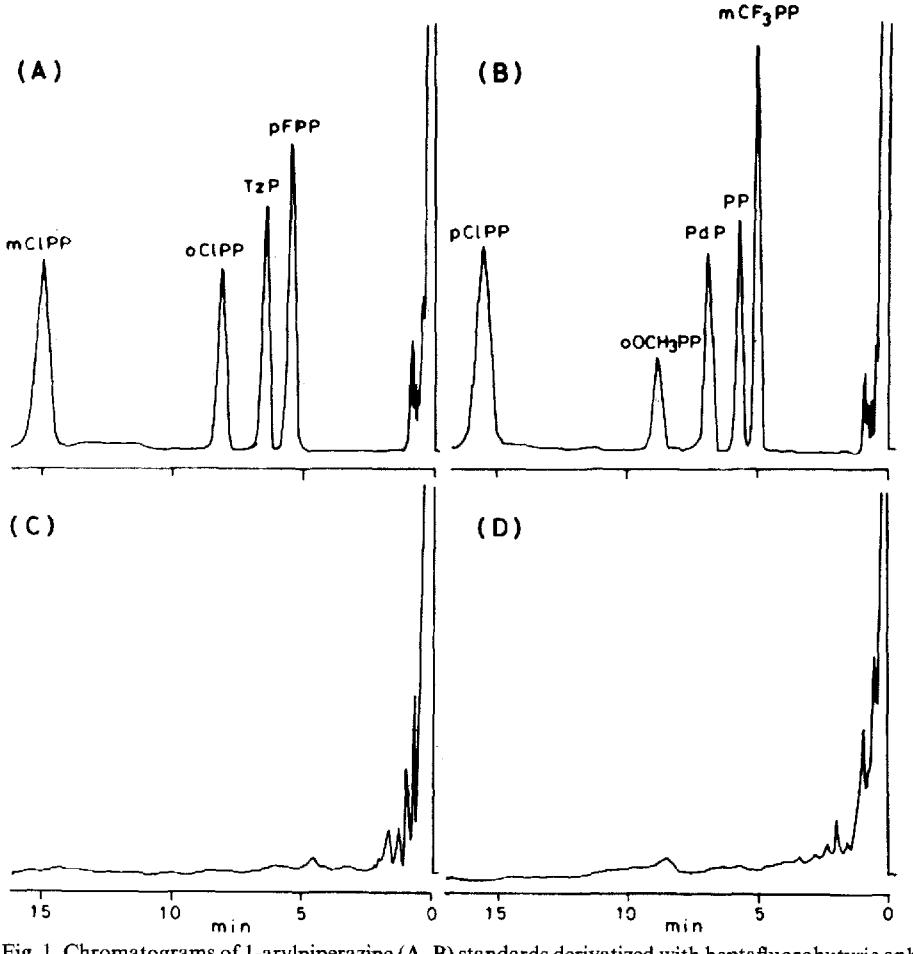

Fig. 1. Chromatograms of 1-arylpiperazine (A, B) standards derivatized with heptafluorobutyric anhydride and of plasma (C) and brain (D) blank extracts.

showed good GLC properties and high electron affinities. Representative GLC traces obtained from mixtures of these derivatives are shown in Fig. 1A and B. The GLC conditions described permitted the rapid and sensitive determination of most of the compounds of interest without interference from endogenous constituents (see Fig. 1C and D for GLC traces of plasma and brain blank extracts). Under these experimental conditions, approximate retention times ranged from 5.1 min (mCF_3PP) to 15.3 min (pClPP). Obviously these GLC conditions could be slightly modified[1] to improve the analysis of the derivatives with relatively long retention times (mClPP, pClPP).

Under the appropriate conditions, the detection limits for the halogen-substituted phenylpiperazines was 10–25 ng per ml of plasma or ng per g of brain tissue. Unsubstituted derivatives (and $oOCH_3PP$) had higher detection limits (25–100 ng/ml or ng/g) because of the lower electron affinities. The calibration graphs were linear from the detection limits up to about 500–1000 ng/ml or ng/g.

Of the solvent systems we studied, benzene represented the best compromise with respect to extraction efficiency and selectivity. Other solvents increased the back-

TABLE I

RECOVERIES OF 1-ARYLPIPERAZINES FROM BRAIN HOMOGENATES

Compound	*Amount added (ng/g)*	*Recovery (%) (mean* ± S.D.)*	*Coefficient of variation (%)*
pFPP	10	85.5 ± 8.5	9.9
	25	89.0 ± 5.0	5.6
	100	88.2 ± 5.6	6.3
	250	90.1 ± 4.3	4.8
mCF_3PP	10	81.0 ± 9.0	11.1
	25	81.0 ± 7.0	8.6
	100	85.0 ± 6.0	7.0
	250	88.0 ± 4.0	4.5
oClPP	25	75.5 ± 7.5	9.9
	50	79.0 ± 6.0	7.6
	100	79.0 ± 6.0	7.6
	250	80.0 ± 5.0	6.2
PP	50	79.5 ± 9.5	12.9
	100	76.0 ± 6.5	8.5
	250	80.0 ± 6.0	7.5
	500	80.0 ± 6.0	7.5
PdP	50	75.0 ± 5.0	6.7
	100	75.0 ± 5.0	6.7
	250	84.0 ± 10.0	11.9
	500	80.0 ± 4.0	5.0
TzP	25	70.0 ± 8.0	11.4
	50	72.7 ± 7.4	10.2
	100	75.0 ± 5.0	6.7
	250	75.0 ± 5.0	6.7

* Mean of 3–4 determinations.

ground interference in the blank or resulted in significant losses of some 1-arylpiperazines.

Table I gives the results of a recovery experiment in which, depending on the calibration graph of the particular 1-arylpiperazine, 10–500 ng were added to brain homogenates. Recoveries were excellent for halogenated phenylpiperazines and acceptable for the other derivatives investigated. Generally the procedure was more precise at higher concentrations because the recoveries were greater. The coefficient of variation at the detection limits, however, was still within generally accepted limits for drug assays for all the 1-arylpiperazines investigated. The recovery from plasma and the precision were similar to or better than those from brain.

Biological samples from rats treated orally with compounds possessing a phenyl- (oxypertine, zolertine), *o*-methoxyphenyl- (millipertine), *o*-chlorophenyl- (empiprazole), *m*-trifluoromethylphenyl- (antrafenine), pyrimidinyl- (piribedil), pyridyl- (azaperone) and thiazolyl- (S-3608) piperazine side-chain were analysed as described. The chromatograms of each extract showed a peak with a retention time consistent with that of the corresponding 1-arylpiperazine. The identities of the peaks were checked by combined GC–MS. The mass spectra were identical with those obtained after injection of authentic 1-arylpiperazine heptafluorobutyrates, confirming that the metabolic pathway of all these drugs includes cleavage of the arylpiperazine side-chain with the formation of piperazine derivatives.

Figs. 2–4 show the mass spectra of these piperazine derivatives. The molecular ion was generally important; it was the base peak in mClPP (Fig. 3B) and in $oOCH_3PP$ (Fig. 2C). There was often a small ion corresponding to the expulsion of $F^{\cdot}$ ($H^+ - 19$); this loss was more noticeable in mCF_3PP (Fig. 3C). All the 1-arylpiperazines containing a nitrogen in the aromatic ring (heteroaromatic compounds) showed a small ion at *m/z* 281, corresponding to elimination of the aromatic ring. These derivatives also showed a loss of 169 a.m.u. ($C_3F_7^{\cdot}$) which could not be found in the other spectra. The ejection of 197 a.m.u. ($COC_3F_7^{\cdot}$) was characteristic of all the spectra. Less common was the expulsion of 212 a.m.u., which gave ions higher than 5% only in the heteroaromatic compounds and in $oOCH_3PP$. The expelled fragment could be $C_3F_7CONH^{\cdot}$ (or $C_3F_3CO^{\cdot}$ plus $CH_3^{\cdot}$ in the case of $o\text{-}OCH_3PP$). The elimination of a neutral fragment, $F_7C_3CON{=}CH_2$, and of $H^{\cdot}$ (total 226 a.m.u.) gave an important ion and followed a trend typical of the piperazines[16]. The ejection of 239 a.m.u. probably gave an ion $ArNC_2H_4^{+\cdot}$ (Ar = phenyl, *m*-CF_3-phenyl, *o*-Cl-phenyl, *m*-Cl-phenyl, *o*-OCH_3-phenyl, thiazolyl). In the spectra of PdP and PmP (Fig. 4A and B) and $oOCH_3PP$ (Fig. 2C), the elimination of 240 a.m.u. was more prominent than the loss of 239 a.m.u. Following opening of the piperazine ring, the subsequent losses were those of 253 and 254 a.m.u., to give ions analogous to those indicated for piperidine[16].

Surprisingly, all the spectra of the derivatives presented in Fig. 4 show elimination of 252 a.m.u., giving the base peak. This loss may be due to the transfer of one $H^{\cdot}$ from the aromatic ring to the nitrogen, as exemplified in Fig. 5 for PdP. Another exception regarding the expulsion of 253 and 254 a.m.u. is that generally the loss of 253 a.m.u. was more important but in the oCH_3PP (identified in brains of dapiprazole-treated rats) spectrum elimination of 254 a.m.u. was the base peak. This could be explained by fragmentation involving the *ortho* group also, as indicated in Fig. 6. Analogous fragmentation is suggested for $oOCH_3PP$, leading to the ex-

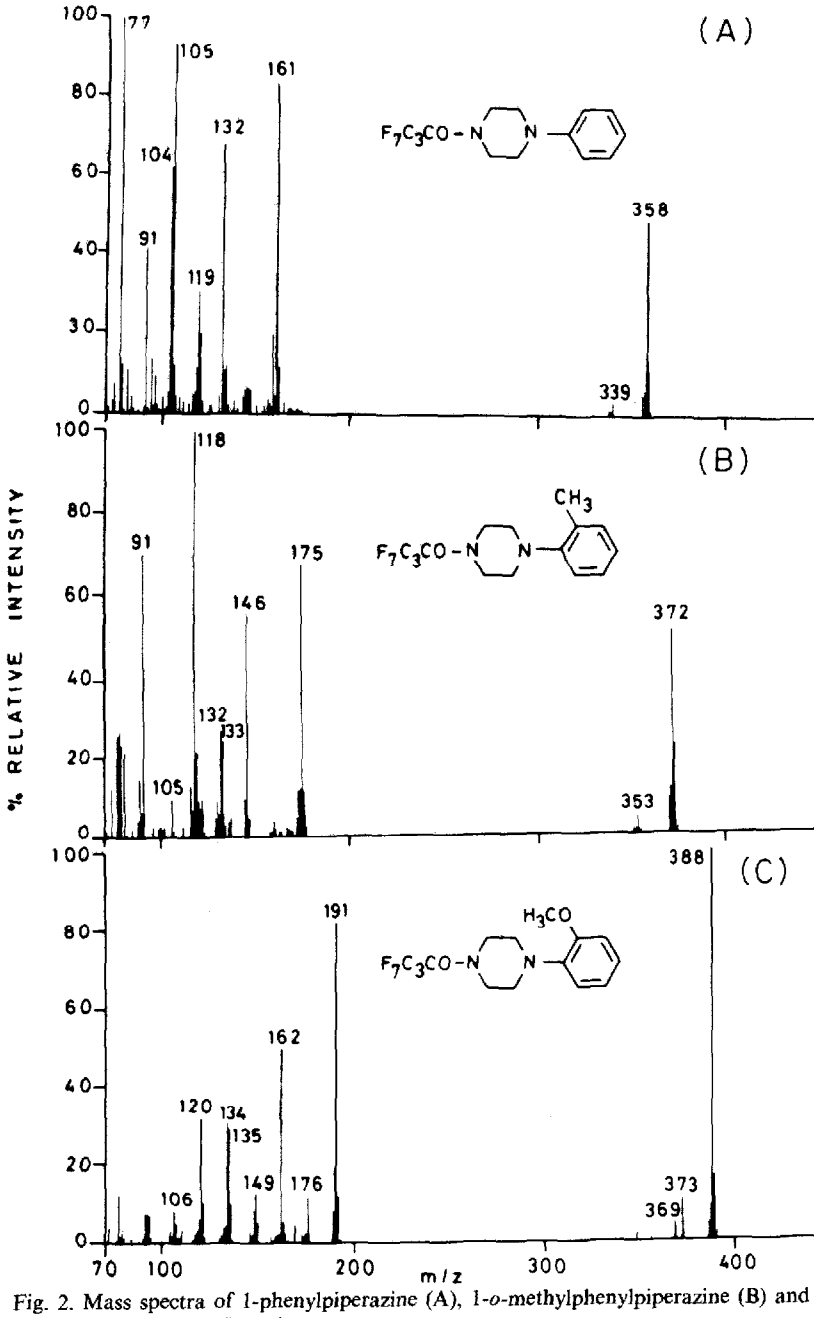

Fig. 2. Mass spectra of 1-phenylpiperazine (A), 1-*o*-methylphenylpiperazine (B) and 1-*o*-methoxyphenylpiperazine (C) heptafluorobutyrates.

pulsion of 268 a.m.u. The elimination of 267 a.m.u., however, is typical in all instances and probably involves the cleavage of both the aliphatic chains of the nitrogen linked to the aromatic ring.

Plasma and brain concentration-time profiles of piperazine derivatives follow-

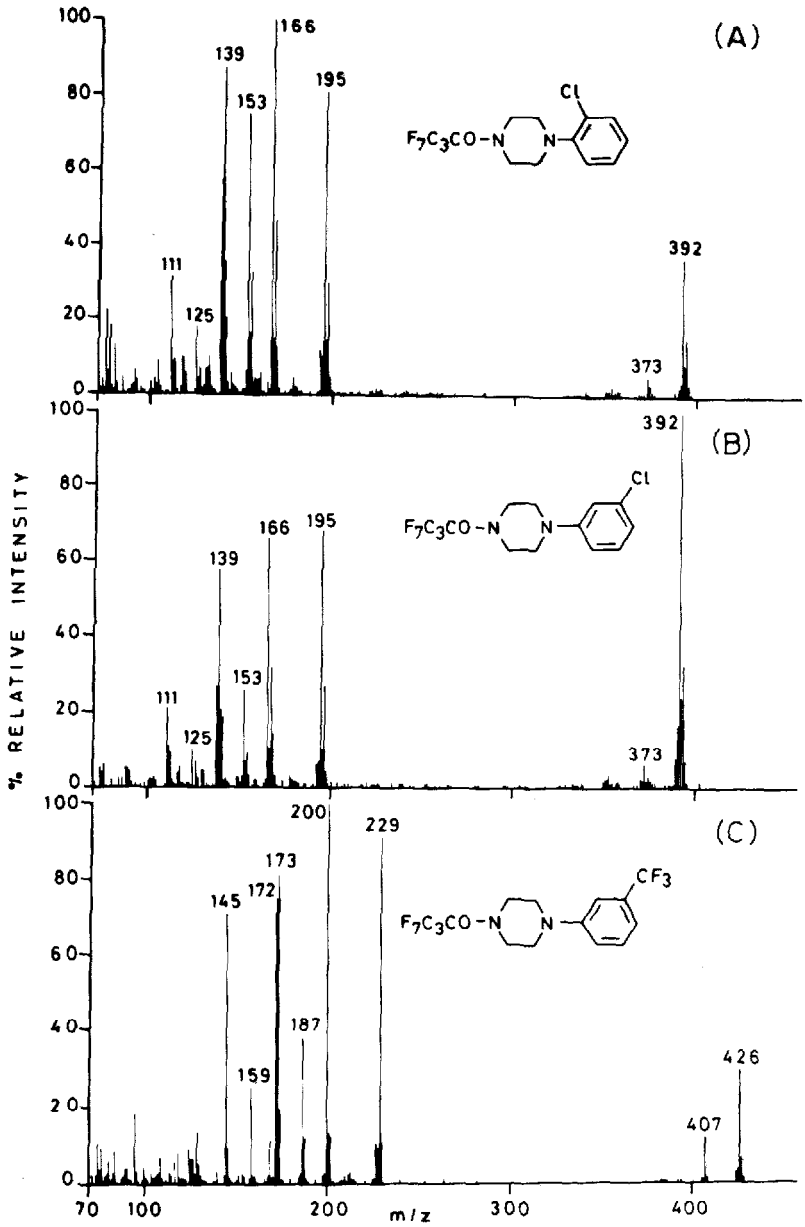

Fig. 3. Mass spectra of 1-*o*-chlorophenylpiperazine (A), 1-*m*-chlorophenylpiperazine (B) and 1-*m*-trifluoromethylphenylpiperazine (C) heptafluorobutyrates.

ing oral administration to rats of representative compounds with the arylpiperazine moiety are shown in Fig. 7. 1-Arylpiperazine was formed to differing extents by different compounds. It was apparently rapid and extensive with oxypertine (25 mg/kg, p.o.), an antipsychotic agent belonging to the 1-(indolylalkyl)-4-arylpipera-

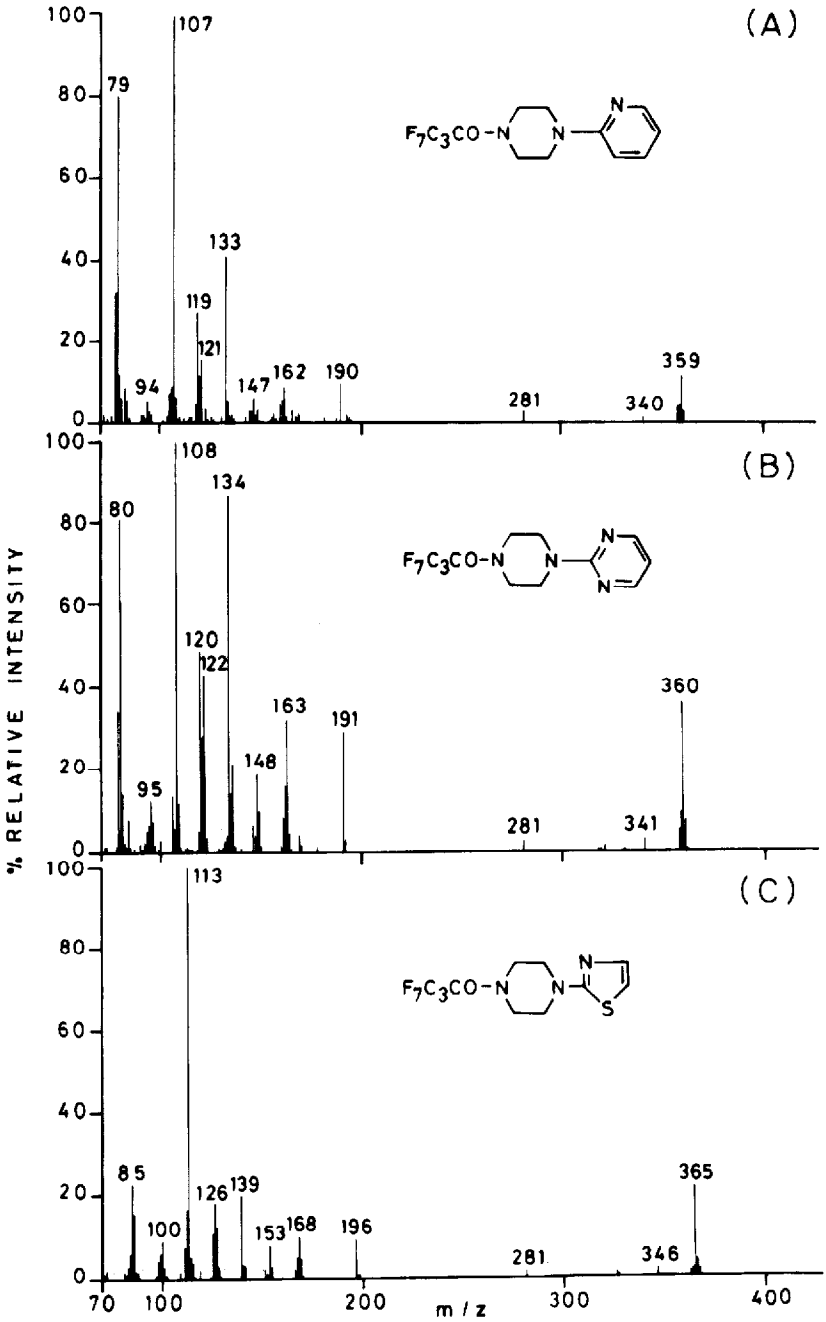

Fig. 4. Mass spectra of 1-(2-pyridyl)piperazine (A), 1-(2-pyrimidinyl)piperazine (B) and 1-(2-thiazolyl) piperazine (C) heptafluorobutyrates.

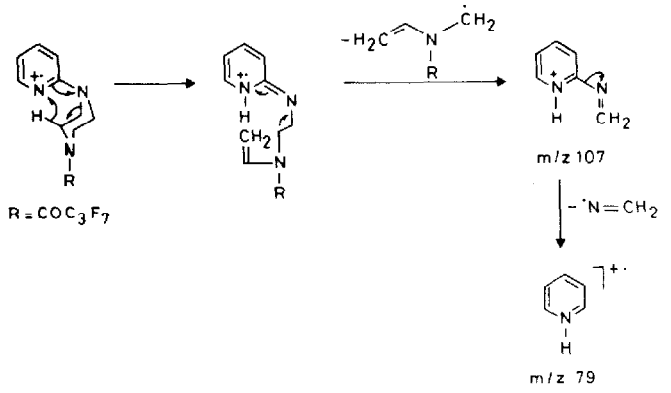

Fig. 5. Possible route of fragmentation of 1-(2-pyridyl)piperazine, leading to ions at *m/z* 107 and 99.

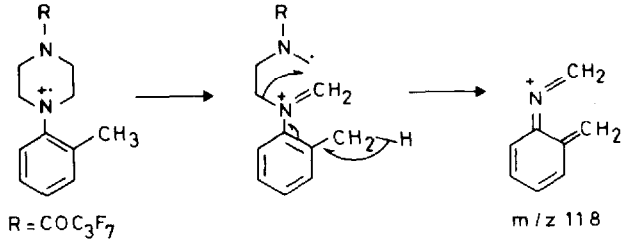

Fig. 6. Possible route of fragmentation of 1-*o*-methylphenylpiperazine, leading to an ion at *m/z* 118.

zine class[17], and azaperone (25 mg/kg, p.o.), a butyrophenone of the 4-arylpiperazine type[18]. Concentrations of the metabolites, PP (Fig. 7A) and PdP (Fig. 7B), of oxypertine and azaperone, respectively, rose rapidly and significant concentrations were observed in both plasma and brain within hours of administration of the parent drugs. With S-3608, a derivative of the dopamine agonist piribedil[19], this reaction apparently occurred to a limited extent and even after large oral doses (100 mg/kg, p.o.) the TzP concentrations of only a few ng/g were observed in rat brain; these concentrations were very short-lasting (Fig. 7C). No TzP could be detected in rat plasma (< 25 ng/ml), but this is not surprising as this and previous studies[2,5] have demonstrated that 1-arylpiperazines accumulate markedly in brain tissue, reaching concentrations 4–26 times those in plasma, expressed as area under the curve (see Table II). Like S-3608, piribedil (100 mg/kg, p.o.) yielded only trace amounts of the corresponding metabolite (PmP) (data not presented), whereas its structurally related compound buspirone (10 mg/kg, p.o.) had been previously found to form significant amount of PmP (ref. 15 and Table II).

Studies with other drugs are still in progress and a more detailed account of the time course of the production and elimination of their metabolites will be presented later. The findings with the compounds mentioned in this paper represent the extremes of the data observed to date.

In conclusion, drugs belonging to different chemical and pharmacological

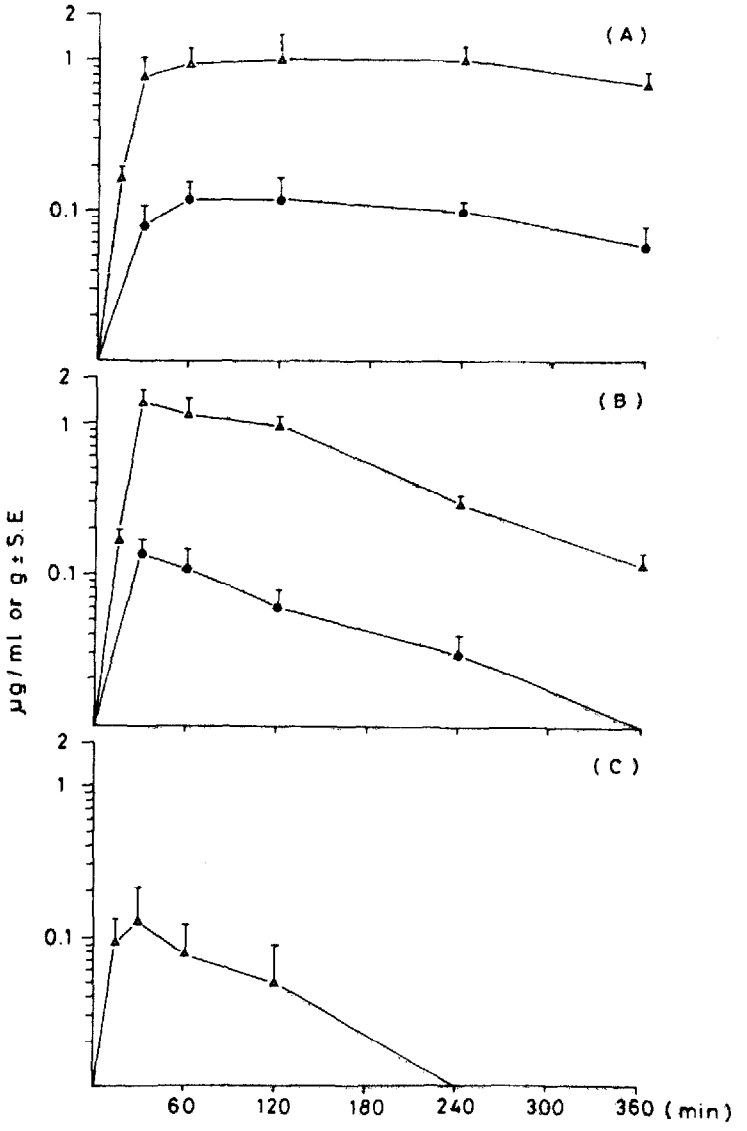

Fig. 7. Plasma (●) and brain (△) concentration–time curves of 1-phenylpiperazine (A), 1-(2-pyridyl)piperazine (B) and 1-(2-thiazolyl)piperazine (C) after oral administration of oxypertine (25 mg/kg), azaperone (25 mg/kg) and S-3608 (100 mg/kg).

classes but all possessing the arylpiperazine side-chain have been found to form 1-arylpiperazines during biotransformation in the rat. These metabolites, which are biologically active[8–14], reach brain concentrations several times those in plasma. The formation of 1-arylpiperazine, therefore, may be a pharmacologically significant pathway, at least in the rat, for these derivatives undergoing extensive cleavage of the arylpiperazine side-chain.

TABLE II

PLASMA AND BRAIN AREAS UNDER THE CURVES (AUC) OF REPRESENTATIVE 1-ARYLPIPERAZINES AFTER ORAL ADMINISTRATION OF THE CORRESPONDING PARENT DRUG

AUC values were calculated by the trapezoidal rule and extrapolated to infinity.

Parent drug	*Dose (mg/kg, p.o.)*	*Metabolite*	*AUC (μg/ml or g min)*	
			Plasma	*Brain*
Trazodone*	25	mClPP	10	262
Oxypertine	25	PP	34***	311***
Azaperone	25	PdP	20	217
Buspirone**	10	PmP	57	250
S-3608	100	TzP	N.D.§	14

* From Caccia *et al.*[1].
** From Caccia *et al.*[15].
*** Calculated only up to 6 h.
§ N.D. = not determinable.

ACKNOWLEDGEMENTS

This study was supported by CNR, Rome, Italy, Gruppo Nazionale Farmacologia Contract No. 81.00258.04.

REFERENCES

1 S. Caccia, M. Ballabio, R. Fanelli, G.Guiso and M. G. Zanini, *J. Chromatogr.*, 210 (1981) 311.
2 S. Caccia, M. H. Fong, S. Garattini and M. G. Zanini, *J. Pharm. Pharmacol.*, 34 (1982) 605.
3 R. N. Brogden, R. C. Heel, T. H. Speight and G. S. Avery, *Drugs*, 21 (1981) 401.
4 M. H. Fong, S. Garattini and S. Caccia, *J. Pharm. Pharmacol.*, 34 (1982) 674.
5 S. Caccia, M. Muglia, A. Mancinelli and S. Garattini, *Xenobiotica*, 13 (1983) 147.
6 Yao-Hua, Wu., J. W. Rayburn, L. E. Allen, H. C. Ferguson and J. W. Kissel, *J. Med. Chem.*, 15 (1972) 477.
7 H. Goldberg and R. Finnerty, *Amer. J. Psychiatry*, 136 (1979) 1184.
8 R. W. Fuller, N. R. Mason and B. B. Molloy, *Biochem. Pharmacol.*, 29 (1980) 833.
9 R. W. Fuller and N. R. Mason, *Advan. Exp. Med. Biol.*, 133 (1981) 359.
10 J. Maj, A. Rawlòw, W. Palider and A. Lewandowska, *Abstracts of 7th International Congress of Pharmacology, Paris, 1978*, p. 637, abstr. no. 2040.
11 R. Invernizzi, S. Cotecchia, A. De Blasi, T. Mennini, R. Pataccini and R. Samanin, *Neurochem. Int.*, 3 (1981) 239.
12 L. Cervo, M. Ballabio, S. Caccia and R. Samanin, *J. Pharm. Pharmacol.*, 33 (1981) 813.
13 P. E. Keane, M. Sarelin Benedetti and J. Dow, *Neuropharmacology*, 21 (1982) 163.
14 S. Garattini, S. Caccia and T. Mennini, *J. Clin. Psychiatry*, 43 (1982) 19.
15 S. Caccia, S. Garattini, A. Mancinelli and M. Muglia, *J. Chromatogr.*, 252 (1982) 310.
16 H. Budzikiewicz, C. Djerassi and D. H. Williams, *Mass Spectrometry of Organic Compounds*, Holden-Day, San Francisco, 1967.
17 S. Archer, D. W. Wylie, L. S. Harris, T. R. Lewis, J. W. Schulenberg, M. R. Bell, R. K. Kullnig and A. Arnold, *J. Amer. Chem. Soc.*, 84 (1962) 1306.
18 P. A. J. Janssen and W. F. M. Van Bever, in E. Usdin and I. S. Forrest (Editors), *Psychotherapeutic Drugs, Part II, Applications*, Marcel Dekker, New York, 1977, p. 869.
19 J.-C. Poignant, H. Gressier, M. Petitjean, G. Regnier and R. Canevari, *Experientia*, 31 (1975) 1204.