Identification of Nonpolar Methotrimeprazine Metabolites in Plasma and Urine by GLC–Mass Spectrometry

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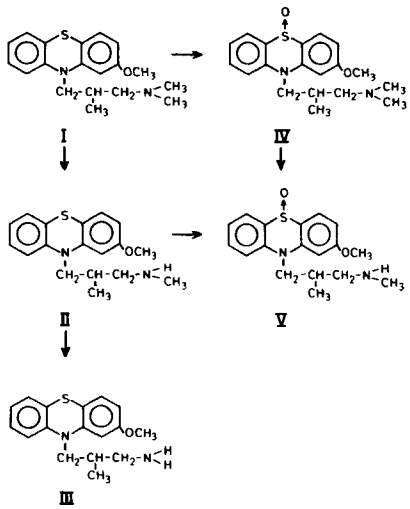
Abstract □ Two metabolites of methotrimeprazine, the sulfoxide and the demethylated analog, were identified in extracts from patient plasma by combined GLC–mass spectrometry. Methotrimeprazine and its sulfoxide had similar mass spectra but different GLC retention times. In addition to the metabolites found in plasma, two other metabolites, the didesmethyl analog and the monodesmethyl sulfoxide, were identified in a urine extract.

Keyphrases □ Methotrimeprazine and metabolites—GLC–mass spectral analysis, human plasma and urine □ GLC–mass spectrometry—analysis, methotrimeprazine and four metabolites, human plasma and urine □ Phenothiazines—methotrimeprazine and four metabolites, GLC–mass spectral analysis, human plasma and urine □ Analgesics—methotrimeprazine and four metabolites, GLC–mass spectral analysis, human plasma and urine

Information about plasma levels of the phenothiazine drug methotrimeprazine[1] (I) and a metabolite (IV) recently was published (1). The biological half-life of I appeared similar to that of its congener chlorpromazine (2), and the apparent volume of distribution (V_β or $V_{d,area}$) appeared somewhat larger. A striking observation was that the plasma levels of the sulfoxide (IV) were about twice as high as the levels of I after oral drug administration. *In vitro* studies later demonstrated that IV must be considered pharmacologically active with regard to cardiac effects, differing in this respect from the corresponding metabolite of chlorpromazine (3).

The plasma levels of I and IV were measured by a GLC method based on flame-ionization detection (4), and the probable identity of the extracted compounds with I and IV was shown by their GLC retention times. The present study was carried out to ascertain the identity of the compounds extracted from plasma by combined GLC–mass spectrometry. A urine extract was analyzed by the same procedure to verify that the metabolites excreted were identical to the corresponding reference compounds.

The molecular formulas of I and its monodesmethyl (II), didesmethyl (III), sulfoxide (IV), and monodesmethyl sulfoxide (V) metabolites are shown in Scheme I.



Scheme I

EXPERIMENTAL

Standard Solutions—Reference samples were supplied by various companies[2,3]. Compounds I, III, and IV were dissolved in heptane containing 1.5% isopentyl alcohol; the bases of II and V were extracted from 0.5 ml of 5% NaOH into 10 ml of the same heptane–isopentyl alcohol mixture.

Blood and Urine Sampling—Blood and urine were collected from four somatically healthy psychiatric patients treated with I tablets for at least 4 weeks, without any additional medication. Subjects 1 and 2 were females, 49 and 43 years old, respectively; Subjects 3 and 4 were males, 46 and 38 years old, respectively. Blood samples were collected 3 and 5 hr after the morning dose and immediately centrifuged, and the plasma was frozen and kept at −24°. Urine was collected before and 5 hr after the morning dose and kept at −24° until analyzed.

Extraction and GLC—The plasma concentrations of I and IV were determined by a GLC method (4) prior to the GLC–mass spectral analysis. The urine concentrations of I, II, and IV were determined by the same procedure but without the internal standard, using peak heights for quantitation.

The plasma and urine extracts analyzed by GLC–mass spectrometry were obtained by the same extraction procedure. For each patient, two 4.5–6-ml aliquots of the plasma sample containing the highest sulfoxide concentrations (Table I) were extracted simultaneously, and the organic phases from the two aliquots of plasma were combined before evaporation to dryness. A 6-ml aliquot of urine from Subject 1, collected 5 hr after the morning dose, was extracted by the same procedure.

GLC–Mass Spectrometry—The evaporated extracts from plasma or urine were dissolved in 10 μl of heptane containing 20% isopentyl alcohol, and 4 μl of this solution was analyzed on a computerized GLC–mass spectrometer[4] (5). The gas chromatograph was equipped with a 120-cm silanized glass column, 2 mm i.d., packed with 3% OV-17 on 80–100-mesh Gas Chrom Q. The injector and column temperatures were 255 and 242°, respectively; the flow rate of helium carrier gas was 30 ml/min. The ionizing potential, trap current, and accelerating voltage were 20 ev, 60 μamp, and 3.5 kv, respectively; the temperatures of the separator and ion source were 270 and 290°, respectively.

Mass spectra were obtained by repetitive scanning over the mass range of *m/e* 10–500 every 2.9 sec and stored in the external memory of the computer. The scan speed was 0.8 sec/spectrum. Gas chromatograms showing total ion current as a function of scan number and mass chromatograms showing the intensity of particular ions as a function of scan number were later generated by the computer.

RESULTS

Reference Samples—The relative retention times of I, II, III, IV, and V were 1, 1.25, 1.40, 3.95, and 4.70, respectively, in the GLC system connected to the mass spectrometer.

Mass spectra of reference samples of I, II, and IV are shown in Fig. 1. The mass spectrum of IV, which was similar to that of I, contained only traces (relative intensity 0.2%) of the molecular ion (*m/e* 344). The base

[1] Known as levomepromazine in Europe.
[2] Société des Usines Chimiques Rhône-Poulenc, 94, Vitry-sur-Seine, France.
[3] AB Mekos, Helsingborg, Sweden.
[4] LKB 9000, LKB, Stockholm, Sweden.

Table I—Dosages and Concentrations of Methotrimeprazine and Metabolites in Plasma 3 and 5 hr after, and in Urine before (0 hr) and 5 hr after, Administration of the Morning Dose

Subject	I Dose, mg		Plasma Concentration, ng/ml				Urine Concentration, ng/ml					
			I		IV		I		II		IV	
	Morning	Evening	3 hr	5 hr	3 hr	5 hr	0 hr	5 hr	0 hr	5 hr	0 hr	5 hr
1	100	200	59	36	257	163	135	432	220	850	4,510	4081
2	200	200	141	135	231	336	500	—	555	—	10,594	—
3	175	175	76	66	389	237	175	371	345	857	5,359	7714
4	100	200	51	61	208	353	84	125	128	150	4,414	2120

peak in both mass spectra had mass number 58, corresponding to the dimethylaminomethylene group at the end of the side chain. The most abundant ion in both mass spectra was the molecular ion of I (*m/e* 328).

The molecular ion of II (*m/e* 314) was the most abundant peak in its mass spectrum. The mass spectra of II and its sulfoxide (V) were similar, like the mass spectra of I and IV. The predominant peaks in the mass spectrum of V had the following mass numbers (and relative intensities): 44 (14%), 58 (20%), 229 (96%), 242 (61%), 269 (36%), 312 (27%), and 314 (100%).

The predominant peaks in the mass spectrum of the reference sample of III were at 228 (41%), 242 (70%), 269 (18%), 300 (100%), and 314 (13%). The ion of mass 314 probably arises from contamination with II in the reference sample, since the molecular weight of III is only 300.

Plasma and Urine Extracts—The concentrations of I and IV in plasma and of I, II, and IV in urine are given in Table I.

Figure 2 shows computer-generated mass chromatograms of ions with mass numbers 314 and 328, obtained after injection of a plasma extract. The chromatogram of mass number 328 contained two peaks with retention times similar to those of I and IV, while the chromatogram of mass number 314 contained one peak with a retention time similar to that of II. The mass spectra corresponding to the peak intensities in the mass chromatograms shown in Fig. 2 are shown in Fig. 3.

The total ion current gas chromatograms of plasma extracts from all subjects showed three peaks with retention times similar to I, II, and IV. The mass numbers and relative intensities of the predominant peaks in the corresponding mass spectra are summarized in Table II.

Figure 4 shows mass chromatograms of ions with mass 314, 300, and 328, obtained from a urine extract. The *m/e* 314 mass chromatogram contained two peaks with maximum intensities obtained in scan numbers 230 and 300, corresponding to the retention times of II and V. The mass spectrum recorded in scan number 230 was similar to the mass spectrum of II (Fig. 1). The predominant peaks in the mass spectrum recorded in scan number 300 had the following mass numbers (and relative intensities): 58 (100%), 229 (72%), 242 (52%), 269 (28%), and 314 (77%).

The *m/e* 300 mass chromatogram showed one small peak with peak intensity obtained in scan number 232, corresponding to the retention time of III. The predominant peaks in the mass spectrum recorded in scan number 232 had the following mass numbers (and relative intensities): 44 (25%), 86 (12%), 229 (74%), 242 (45%), 269 (17%), 300 (10%), and 314 (100%).

The *m/e* 328 mass chromatogram (Fig. 4) contained two peaks with retention times similar to I and IV. The mass spectra recorded in scan numbers 225 and 300 were similar to the mass spectra of I and IV shown in Fig. 1.

It was previously reported that peaks from two unidentified substances appeared in the flame-ionization gas chromatograms of samples extracted

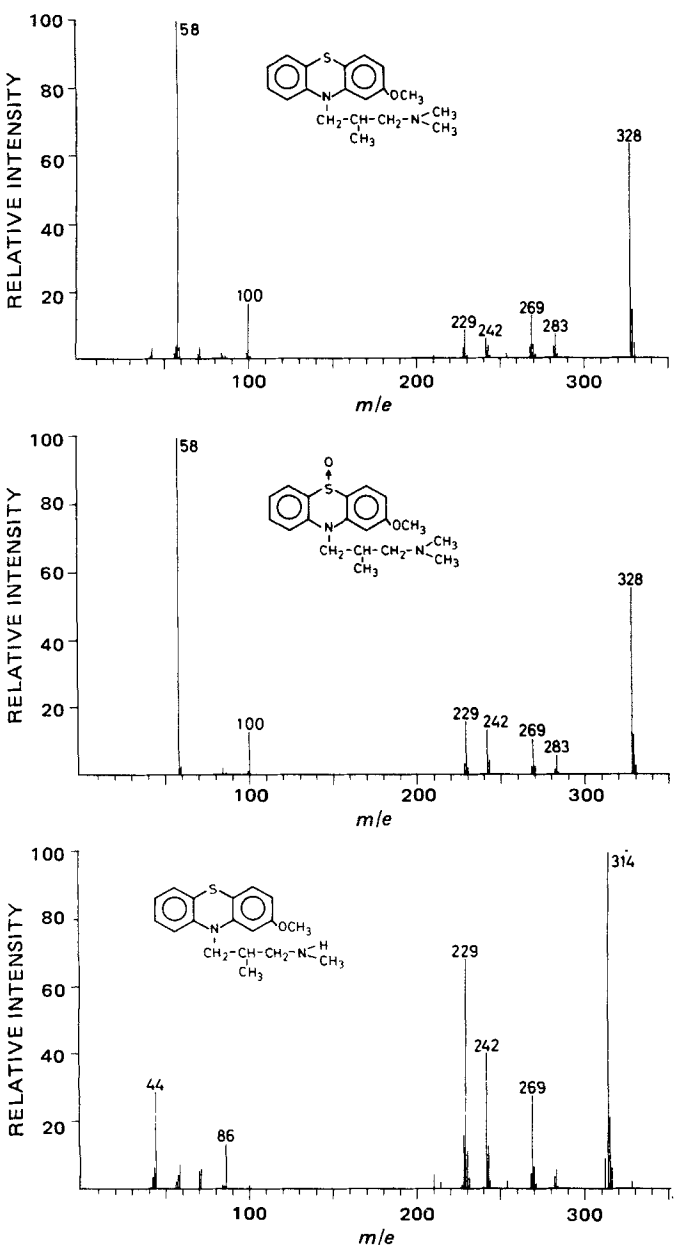


Figure 1—*Mass spectra of I, IV, and II obtained after injection of 1 μg of each compound from organic standard solutions.*

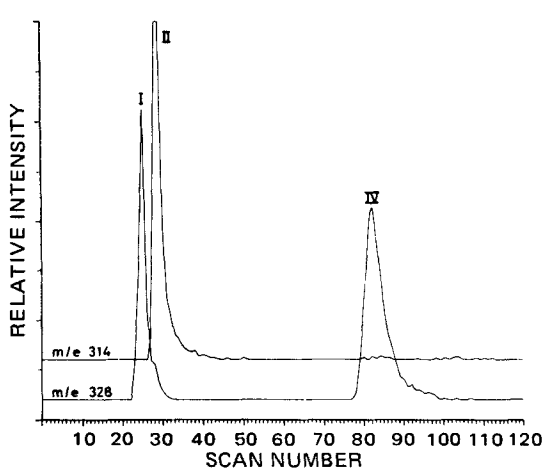


Figure 2—*Mass chromatograms obtained from an extract of 11.3 ml of plasma from Subject 1, collected 3 hr after administration of the morning dose.*

Table II—Predominant Peaks in Mass Spectra from Plasma Extracts

Subject	Mass Number (Relative Intensity, %) RRT[a] 1.0	RRT 1.25	RRT 3.95
1	58 (100), 328 (50), 43 (18), 100 (18)	314 (100), 229 (61), 242 (36), 44 (29)	58 (100), 328 (63), 100 (15), 229 (13)
2	58 (100), 328 (54), 100 (15), 229 (10)	314 (100), 229 (64), 242 (37), 269 (27)	58 (100), 328 (73), 229 (34), 242 (15)
3	58 (100), 328 (53), 100 (16), 229 (9)	314 (100), 229 (65), 242 (40), 44 (25)	58 (100), 328 (55), 229 (17), 100 (14)
4	58 (100), 42 (64), 328 (58), 71 (49), 100 (24)	314 (100), 44 (88), 72 (65), 229 (53)	58 (100), 328 (54), 229 (16), 207 (15)
	I[b]	II[b]	IV[b]
	58 (100), 328 (64), 100 (17), 269 (13)	314 (100), 229 (68), 242 (40), 44 (29), 269 (27)	58 (100), 328 (56), 229 (15), 242 (13), 100 (12)

[a] RRT = relative retention time. [b] Reference sample.

from plasma by this procedure (4). The mass spectrum of the one with the shortest retention time on the OV-17 column showed relatively high intensities of ions with mass numbers 149, 167, and 279, indicating that this compound was the commonly used plasticizer bis(2-ethylhexyl) phthalate (6).

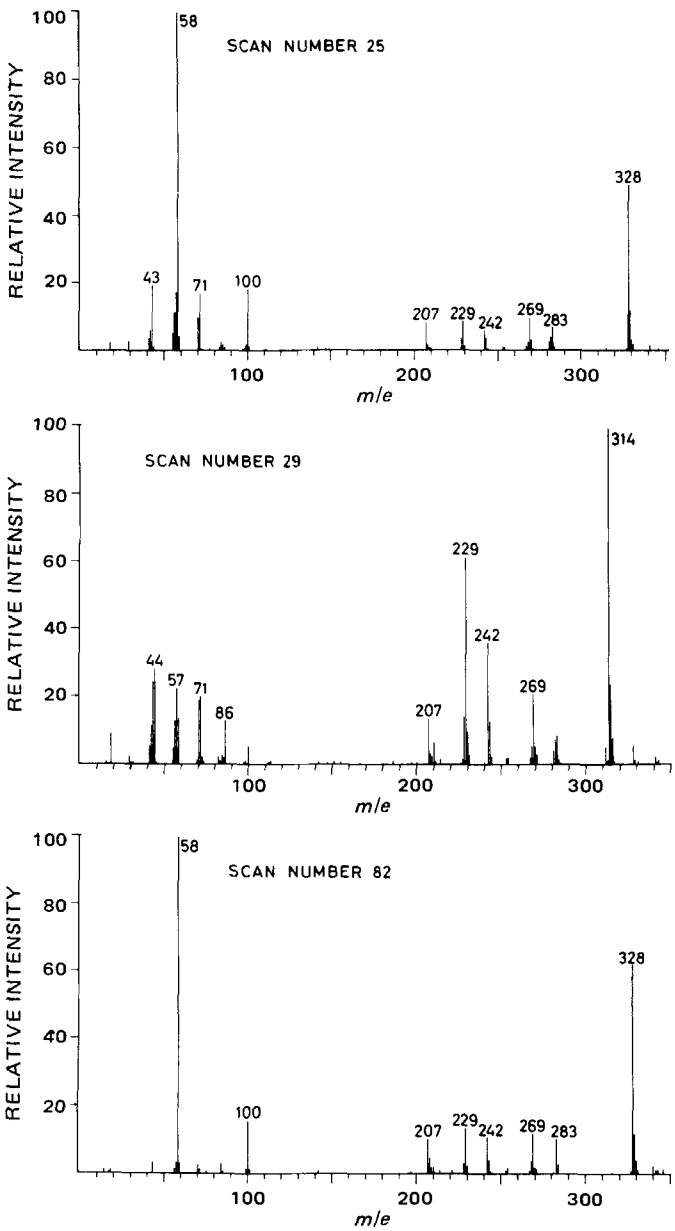


Figure 3—*Mass spectra obtained from the same sample as the mass chromatograms shown in Fig. 2.*

DISCUSSION

The 70-ev mass spectra of chlorpromazine sulfoxide and promazine sulfoxide were reported to contain an ion of mass corresponding to the dimethylamino side chain less two hydrogen atoms, which was absent from the mass spectra of the parent drugs (7). It was later reported that the two hydrogen atoms were eliminated from the methylene group in the middle of the side chain (8). In methotrimeprazine, one of these hydrogen atoms is substituted by a methyl group; no ion of mass corresponding to the side chain less one or two hydrogen atoms was seen in the mass spectra of I and IV, which were similar (Fig. 1).

It was reported previously that the 70-ev mass spectrum of IV, obtained by using a direct inlet system at 150°, showed a relatively high intensity of the molecular ion (*m/e* 344) and was distinguishable from the mass spectrum of I by the absence of ions with mass numbers 100 and 149 (9). The absence of the molecular ion of IV in the mass spectrum shown in Fig. 1 might have been due to reduction of the sulfoxide in the molecule separator, which was kept at 270°. A 70-ev mass spectrum of IV, recorded under otherwise the same conditions, was similar to the 20-ev mass spectrum and showed a relative intensity of the molecular ion of 0.9%.

The blood and urine samples were collected from patients receiving relatively large doses of I (Table I) and at the times of the day when the highest concentrations of I, II, and IV were expected. The method used for assay of I, II, and IV in urine was less accurate than the original procedure for I and IV in plasma, since no internal standard was used. Nevertheless, the results were sufficiently accurate for the scaling of the mass spectrometric analysis.

Several different components supposed to be conjugated metabolites of I were found previously in urine from psychiatric patients (10). Relatively polar components like glucuronides or hydroxylated metabolites of I are not, however, likely to be extracted by the present procedure. Compounds I, II, and IV previously were identified and quantitated in human urine (9), and the results given in Table I are in good agreement with the previously reported concentrations.

The peak in the *m/e* 300 mass chromatogram shown in Fig. 4 was not resolved from the peak of II (*m/e* 314), which had a much higher intensity;

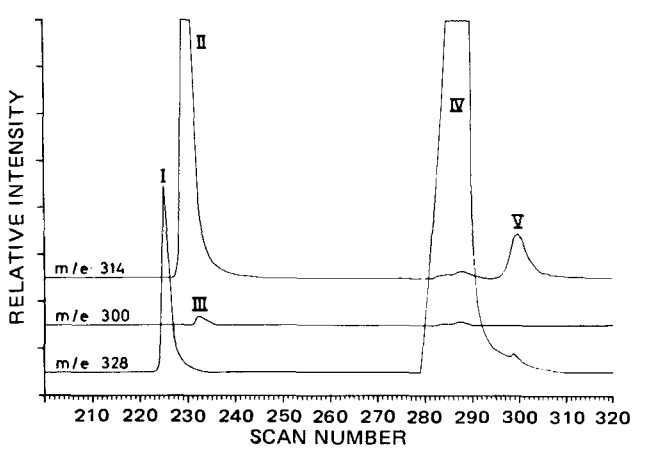


Figure 4—*Mass chromatograms obtained from an extract of 6 ml of urine from Subject 1, collected 5 hr after administration of the morning dose.*

therefore, the mass spectrum recorded in scan number 232 was similar mainly to that of II. Since the mass spectra of reference samples of I and II contained no ion with mass number 300, which was the base peak in the mass spectrum of III, the peak in the *m/e* 300 mass chromatogram still indicates that the urine contained III, although in apparently low concentration compared to I, II, and IV. The total recoveries of III and V by the extraction procedure were, however, not determined and may have been lower than the recoveries of I, II, and IV.

The mass spectrum recorded in scan number 300 was similar to the mass spectrum of V, confirming that the urine also contained this metabolite.

It may be concluded from the data given in Table II that all plasma extracts analyzed by combined GLC–mass spectrometry contained I, II, and IV. Some additional peaks of relatively high intensities, originating from the background, appeared in the mass spectra of plasma extracts from Subject 4.

In vitro studies indicated that IV must be considered pharmacologically active with regard to cardiac effects, while chlorpromazine sulfoxide possesses much less activity (3). The chlorpromazine (11–13) and promazine (14) metabolites analogous to II have proved pharmacologically active in different biological systems, and it seems reasonable that II should also possess significant pharmacological activity. Therefore, it would be desirable to have a sufficiently sensitive and specific method for quantitation of I and both of its nonpolar metabolites, II and IV, in plasma.

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Potential Long Acting Opiate Antagonists: Preparation, Pharmacological Activity, and Opiate–Receptor Binding of *N*-Substituted 2′-Hydroxy-5-methyl-9α-propyl-6,7-benzomorphans

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Abstract □ A homologous series of *N*-substituted 2′-hydroxy-5-methyl-9α-propyl-6,7-benzomorphans (hydrogen to octyl inclusive, allyl, and cyclopropylmethyl) was prepared. In contradistinction to the normetazocine, normorphine, and (−)-3-hydroxymorphinan series, the *N*-pentyl and *N*-hexyl derivatives do not have the analgesic potency of the parent *N*-methyl compound; instead, they are narcotic antagonists with a long duration of action. All of the *N*-substituted 9α-propylbenzomorphans, except for methyl, heptyl, and octyl, have antagonist activity. The receptor binding constants of the *N*-alkyl compounds are uniformly two- to threefold lower than those of the *N*-substituted normetazocines.

Keyphrases □ Opiate antagonists, potential—various *N*-substituted benzomorphans synthesized, pharmacological activity and opiate–receptor binding evaluated □ Benzomorphans, *N*-substituted—synthesized, pharmacological activity and opiate–receptor binding evaluated □ Structure–activity relationships—various *N*-substituted benzomorphans synthesized, pharmacological activity and opiate–receptor binding evaluated □ Binding, opiate–receptor—various *N*-substituted benzomorphans evaluated

Narcotic analgesics based on fused ring systems, such as the 6,7-benzomorphans and the more complex morphinans and morphines, have commonly been converted to antagonists by modification of the *N*-substituent (1, 2) [classically by replacing the *N*-methyl with allyl (3), cyclopropylmethyl (1), or propyl (1)]. Many antagonists prepared in this manner are highly potent and some are used clinically (2). Most antagonists studied have only a short duration of action, for reasons that are not exactly clear. Long duration of action is a useful property in treating opiate overdosage and in some addiction treatment programs.