Methotrimeprazine and Its Sulfoxide and Desmethyl Metabolites in Urine of Psychiatric Patients

A. De LEENHEER▲ and A. HEYNDRICKX

Abstract □ Urine samples taken from three psychiatric patients who ingested known amounts of the drug methotrimeprazine maleate were extracted by a two-phase procedure with ether. A first screening by UV spectrophotometry showed the preponderant presence of the sulfoxide metabolite. The TLC separation of aliquots of extracts revealed free methotrimeprazine and its sulfoxide, desmethyl, and desmethyl sulfoxide metabolites. GLC on the acetylated extracts, using two different columns, permitted the qualitative and quantitative (with trifluoperazine as internal standard) determination of free methotrimeprazine and the sulfoxide and desmethyl metabolites. IR and mass spectral analyses of these compounds, isolated by micropreparative GLC of an aliquot of the acetylated extract, gave detailed structural information and confirmed the specificity of results.

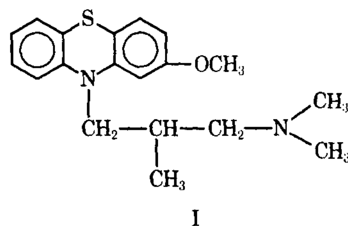
Keyphrases □ Methotrimeprazine and metabolites—isolation, analysis, in urine of psychiatric patients □ GLC—isolation of methotrimeprazine and its metabolites from urine □ IR spectrophotometry—identification, analysis, methotrimeprazine and its metabolites from urine □ Mass spectrometry—identification, analysis, methotrimeprazine and its metabolites from urine

Methotrimeprazine[1] (I) is a dialkylaminopropylphenothiazine derivative used as a drug with tranquilizing properties. From the general formula, it may be deduced that the compound has a basic character due to the presence of a tertiary amine function on an alkyl chain. Methotrimeprazine has been reported to undergo sulfoxidation in man (1) and hydroxylation, *O*-demethylation, sulfoxidation, and *N*-demethylation in rats (2, 3). Previous studies on methotrimeprazine assay were performed using colorimetric, UV spectrophotometric, paper chromatographic, and TLC methods.

Current interest in these laboratories into the metabolism of methotrimeprazine (I) in man led to the identification of its sulfoxide (II), desmethyl (III), and desmethyl promazinesulfoxide (IV) as three important, less polar metabolites (Scheme I). A method of improved specificity was developed for qualitative and quantitative determination of I, II, and III in urine of man.

EXPERIMENTAL

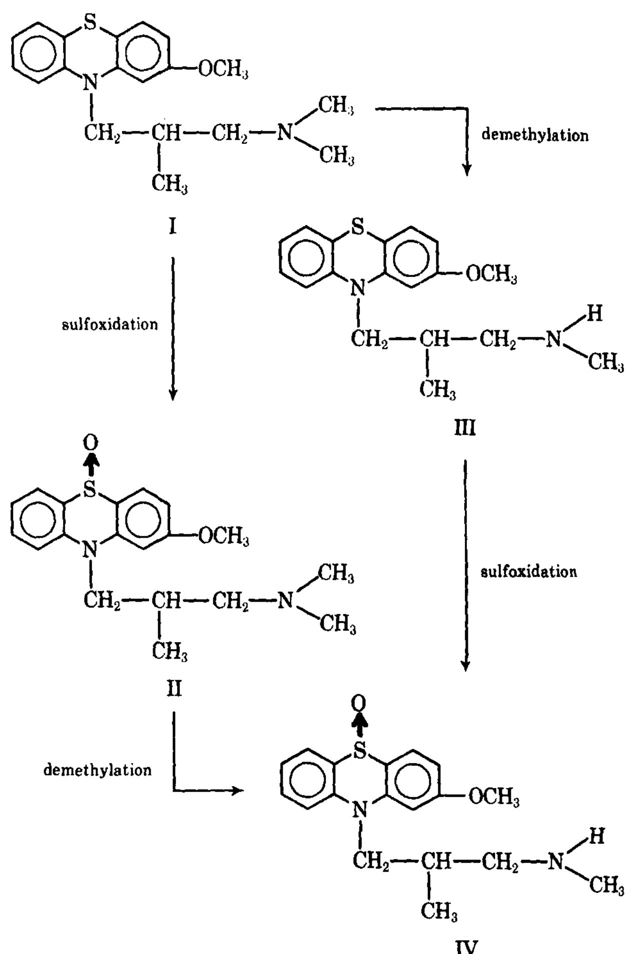
Over 4 successive days, the total urine output of three patients who ingested a known amount of I (orally administered as maleate)



was collected separately (Table I). Each sample was refrigerated immediately. Each 24-hr. urine collection was homogenized, and a 200-ml. aliquot was extracted without delay prior to analysis.

Screening Test—As a first indication, an FPN test was performed on each urine sample. The FPN reagent (ferrichloride, perchloric acid, and nitric acid) (4, 5) was prepared by combining 5 ml. of 5% (w/v) $FeCl_3$, 45 ml. of 20% (concentration 1:5) $HClO_4$, and 50 ml. of 50% (concentration 1:2) HNO_3.

Extraction Procedure—A 200-ml. aliquot of each urine sample was acidified with 2 *N* HCl and extracted twice under nitrogen atmosphere with 400 and 200 ml. of peroxide-free ether (freshly distilled over hydroquinone, reagent grade); then the ether layers were discarded. The aqueous phase was alkalized with 10 *N* NaOH and again extracted twice under nitrogen atmosphere with 200 ml. of ether. The ether layers, which contained I and the less polar metabolite fraction (II, III, and IV) were washed with 5-ml. portions of 0.001 *N* NaOH until the aqueous layer remained colorless. The washed ether was evaporated in a rotatory evaporator to a final volume of about 100 ml., saturated with nitrogen, and extracted twice with 10 ml. of 0.1 *N* HCl. The total acid extracts were made basic with 1 ml. of 10 *N* NaOH, saturated with nitrogen, and extracted twice with 40 ml. of peroxide-free ether. The com-



Scheme I—Partial metabolism of methotrimeprazine (I) occurring in man (less polar metabolite fraction)

[1] Known in Europe as levomepromazine.

Table I—Case Histories of Psychiatric Patients under Methotrimeprazine (I) Treatment

Patient			Daily Dose of I Administered Orally as Maleate, mg.	Duration of Medication before First Urine Collection
Number	Sex	Age, years		
1	Male	61	25, 25, 25	7 weeks
2	Male	23	25, 25, 50	14 days
3	Male	63	25, 25, 25	2 years

bined ether phases were dried over sodium sulfate, filtered, and evaporated on a water bath at 40° under a slow stream of nitrogen. The residue was dissolved in 10 ml. of ethanol and kept in a well-closed tube at −15° for further analysis (alcoholic extraction liquid).

UV Spectrophotometry—The UV spectra of the acidic extracts (20 ml. 0.1 *N* HCl) were automatically recorded with a double-beam spectrophotometer[2] in the wavelength range of 450–190 nm.

TLC—A 1-ml. aliquot of each alcoholic extract was evaporated to a small volume on a water bath at 40°, with the aid of a nitrogen stream. These, together with 20 mcg. of the reference compounds I-maleate, II, III-maleate, and IV-oxalate were spotted in a 2-cm. line 3 cm. from the lower edge of a 250-μ thin-layer plate. The plate was prepared with equal parts of silica gel H_{254} and cellulose MN 300. Chloroform, acetone, and ammonia (25%) (50:50:1) were used as the solvent system. After development the plates were sprayed with ferric reagent (6) for visualization. This reagent was prepared by dissolving 500 mg. of ferric nitrate in 80 ml. of concentrated sulfuric acid and diluting with distilled water to 1000 ml.

GLC—GLC analysis was performed with a research gas chromatograph[3] equipped with dual flame-ionization detectors (FID). Two packed columns of different polarity were installed for simultaneous single-column operation. One was prepared with 2% FFAP (a polyester, a reaction product of Carbowax 20M and *m*-dinitroterephthalic acid) and the other with 5% OV-1 (a methylsilicone polymer) (7) by coating the liquid phases on Diatoport S[4] (acid and silane treated), 80-100 mesh, as a support.

The operating conditions were: glass spiral columns, 1.80-m. length, 4-mm. i.d.; carrier gas, nitrogen, 70 ml. $min.^{-1}$ (bubble flowmeter); oven temperature, 230°; temperature of injector block, 235°; temperature of detector block (FID), 235°; and air and hydrogen, calibrated for optimum sensitivity.

Qualitative Determination—Qualitative assays of urinary I and its metabolites were performed with the acetylated derivatives. For this purpose, 1-ml. aliquots of the individual alcoholic extracts were transferred to conical siliconized tubes of 15-ml. capacity and evaporated on a water bath at 40° under a slow stream of nitrogen. To the final residues, 0.2 ml. of pyridine (refluxed and distilled over potassium hydroxide) and 0.2 ml. acetic anhydride (refluxed and distilled over calcium carbide) were added. After a 1-hr. reaction time in a phosphorus pentoxide desiccator, the reaction mixtures were evaporated again under the above-mentioned conditions. The residues obtained were dissolved[5] in 25 μl. of ethyl acetate. The standards (I-maleate, II, III-maleate, and IV-oxalate, 10-mg. amounts) were treated in the same way, but the residues obtained were taken up in 10 ml. of ethyl acetate (0.1% solutions). Volumes varying from 1 to 2 μl. were separately injected[6] on both columns.

Quantitation—The same treatment was applied as for the qualitative determination, except that a known amount (20 mcg., but 40 mcg. for recovery experiments) of trifluoperazine was added as an internal standard before the first evaporation.

Calibrations were made with three series of mixtures, prepared from 0.2% ethanolic solutions of the active compounds (μl.): (*a*) I-($\overline{ac}$.)[7]-trifluoperazine-(ac.) (20:80, 30:70, 40:60, and 50:50); (*b*) II-($\overline{ac}$.)-trifluoperazine-(ac.) (60:40, 70:30, and 80:20); and (*c*) III-acetamide trifluoperazine-($\overline{ac}$.) (60:40, 70:30, and 80:20). For each mixture of a given series, calibration factors k_i were calculated using the equation:

$$k_i = \frac{S_x}{S_s} \times \frac{v_s}{v_x} \quad \text{(Eq. 1)}$$

where S_x/S_s is the quotient of peak surfaces[8] on chromatograms—for a given mixture *i*— of the compound to be determined and internal standard trifluoperazine-($\overline{ac}$.), and v_s/v_x is the reciprocal of volume proportions— for a given mixture *i*—of the compound to be quantitated with respect to internal standard trifluoperazine-($\overline{ac}$.). The arithmetic mean $\bar{k}$ of all calibration factors k_i of the same series was calculated self-evidently according to:

$$\bar{k} = \sum_{i=n}^{i=n} k_i/n \quad \text{(Eq. 2)}$$

where $n = 4$ for (*a*) and 3 for (*b*) and (*c*). Results, expressed in mcg. compound/100 ml. urine or mcg. %, were obtained with the equation:

$$\text{mcg. } \% = \frac{5}{\bar{k}} \times \frac{S_x'}{S_s'} \times m_s \quad \text{(Eq. 3)}$$

where S_x'/S_s' is the quotient of peak surfaces of the compound to be determined as present in the extract and internal standard trifluoperazine-($\overline{ac}$.), m_s is total amount in mcg. of internally added standard trifluoperazine-($\overline{ac}$.), and 5 is the conversion factor because 1 ml.

Table II—Volume, pH, and FPN Test of Urine Samples Collected from Psychiatric Patients

Patient	Sample	Volume of Urine, ml./24 hr.	pH	FPN Test
1	1 (1st day)	890	6.0	± (blue)
	2 (2nd day)	880	6.0	+ (blue)
	3 (3rd day)	2000	5.5	± (blue)
	4 (4th day)	550	5.5	+ (blue)
2	1 (1st day)	2350	6.0	+ (blue)
	2 (2nd day)	750	6.0	+ (blue)
	3 (3rd day)	980	5.5	+ (blue)
	4 (4th day)	1780	5.5	+ (blue)
3	1 (1st day)	750	9.0	+ (blue)
	2 (2nd day)	500	8.0	+ (blue)
	3 (3rd day)	600	8.0	+ (blue)
	4 (4th day)	500	5.0	+ (blue)

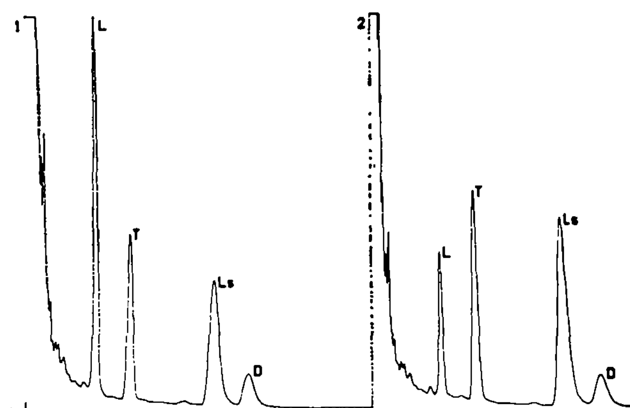


Figure 1—*Gas chromatograms of urine extracts 1 and 2 (Patient 1). The column was 5% OV-1, quantitative determination (acetylation), and the internal standard was trifluoperazine-($\overline{ac}$.) (T). Key (from left to right): urine extract 1—I-($\overline{ac}$.) (L), trifluoperazine-($\overline{ac}$.) (T), II-($\overline{ac}$.) (Ls), and III-acetamide (D); urine extract 2—same sequence as for 1.*

[2] Unicam SP 800.
[3] Hewlett-Packard 5750.
[4] Hewlett-Packard.
[5] With the aid of a Whirlmixer.
[6] With an Hamilton syringe of 10-μl. capacity.
[7] The (ac.) compound has undergone the acetylation treatment without having been changed (no acetylatable function present).

[8] Measured using the method of peak height times peak width at half-height ($h_{max.} \times w_{0.5h}$).

Table III—Calibration Factors $\bar{k}$ Used for Subsequent Determinations of Urinary Methotrimeprazine (I) and Its Metabolites

Compound Quantitated	Internal Standard	Column	$\bar{k}$
I-(ac.)	Trifluoperazine-(ac.)	2% FFAP	1.04
I-(ac.)	Trifluoperazine-(ac.)	5% OV-1	0.97
II-(ac.)	Trifluoperazine-(ac.)	5% OV-1	0.80
III-acetamide	Trifluoperazine-(ac.)	5% OV-1	0.46

Table IV—Quantitative Results of GLC Determinations of Methotrimeprazine (I) and Two of Its Metabolites

Patient	Sample	I, mcg./100 ml. Urine		II, mcg./100 ml. Urine	III, mcg./100 ml. Urine
		2% FFAP	5% OV-1	5% OV-1	5% OV-1
1	1	122	124	185	55.7
	2	57.2	54.8	306	129
	3	71.3	71.0	109	61.9
	4	39.4	39.5	131	40.7
2	1	49.4	50.3	335	14.7
	2	52.9	45.8	473	45.4
	3	27.8	26.7	193	Traces
	4	34.1	35.4	374	23.5
3	1	121	120	675	83.6
	2	76.3	76.5	553	135
	3	78.6	75.1	600	76.5
	4	141	135	995	109

alcoholic extract corresponds to 20 ml. urine sample (expressed per 100 ml. urine, 1/20 × 100 = 5).

Recovery Experiments—To 200 ml. urine, collected as a pool of samples from subjects under no drug medication, 400 mcg. I-maleate was added and the sample was taken through the whole experimental procedure as described.

Trapping of GLC Effluents—A splitting system[9] was installed at the exit of each column, yielding a 10:1 ratio. As a collection device, a pyrex capillary of 12.5-cm. length and 1.5-mm. i.d., fixed with two Teflon O-rings in a brass tube of 10.5-cm. length, 7-mm. o.d., and 4-mm. i.d., provided with a 1/8 swagelok nut, was used. A suitable aliquot of the alcoholic extract was acetylated, and the residue was dissolved in 50 µl. of ethyl acetate. This solution was at once preparatively chromatographed on the 5% OV-1 column at 230° for trapping of compounds that separated.

IR and Mass Spectrometry—After collection, each compound was rinsed out from its capillary with 2 × 10 µl. of ethyl acetate. This solution was poured on 25 mg. of KBr in an agate mortar, and the solvent was allowed to evaporate. A micropellet was prepared by means of a punched disk (2 × 10-mm. hole), and the IR spectrum was recorded[10] from 625 to 4000 cm.$^{-1}$. The mass spectrum was recorded[11] using the direct insertion probe system loaded with a small pulverized piece of the KBr pellet and running the spectra in standard conditions (70 ev., 150°).

RESULTS AND DISCUSSION

The volume, pH, and FPN test (4, 5) of the urine samples were determined just after each 24-hr. collection. These preliminary data are summarized in Table II.

The UV spectra of all extracts showed a striking correspondence with the reference spectrum of II ($\lambda_{max.}$: 217 p[12], 249, 274 s[13], 294, and 332 nm. with ϵ: 18,740, 28,636, 8853, 4237, and 4237; $\lambda_{min.}$: 227, 219.5, and 308). UV spectrophotometry, being essentially an additive method, gave an overall pattern of all phenothiazine compounds as present in the acidic extract. A sulfoxide pattern (four-peak maxima) occurred because, as confirmed by quantitative GLC (*vide infra*), this compound was excreted in the highest amount. Besides a small amount of free I (R_f 0.77 blue), II (R_f 0.53 blue), III (R_f 0.43 blue), and IV (R_f 0.09 blue) were detected by TLC in all extracts. Oxidized and/or demethylated compounds were separated according to the sequence given, which is always obtained for the corresponding metabolites of other phenothiazine drugs.

GLC analysis of a 1-ml. fraction of the alcoholic extracts on the 2% FFAP column (230°) showed the presence of free I-(ac.) (R_t 14 min. 48 sec., theoretical plates 2700) in all urine samples. On the 5% OV-1 column (230°), free I-(ac.) (R_t 4 min. 39 sec., theoretical plates 3200), II-(ac.) (R_t 12 min. 58 sec.), and III-acetamide (R_t 15 min. 30 sec.) were revealed in all extracts. Results indicated that the 2% FFAP column permitted only the determination of free I, while the 5% OV-1 column separated well free I, its sulfoxide

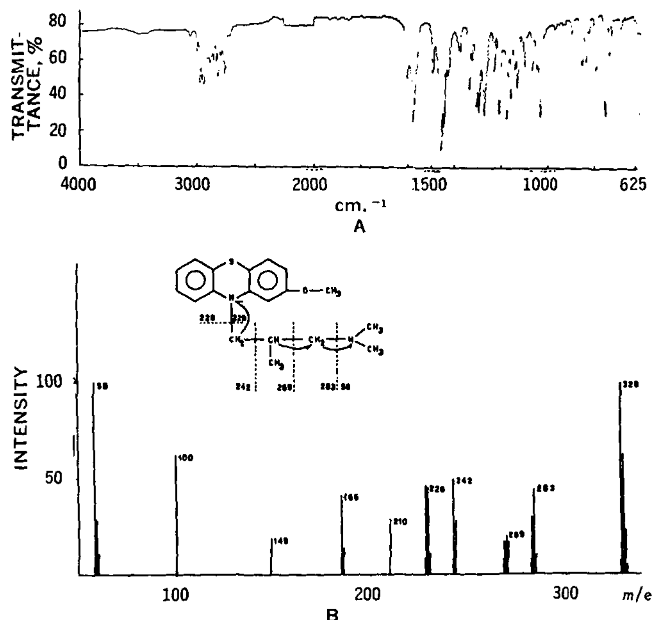


Figure 2—*IR spectrum (A) and mass spectrum (B) of trapped I isolated from urinary extract 4 of Patient 3.*

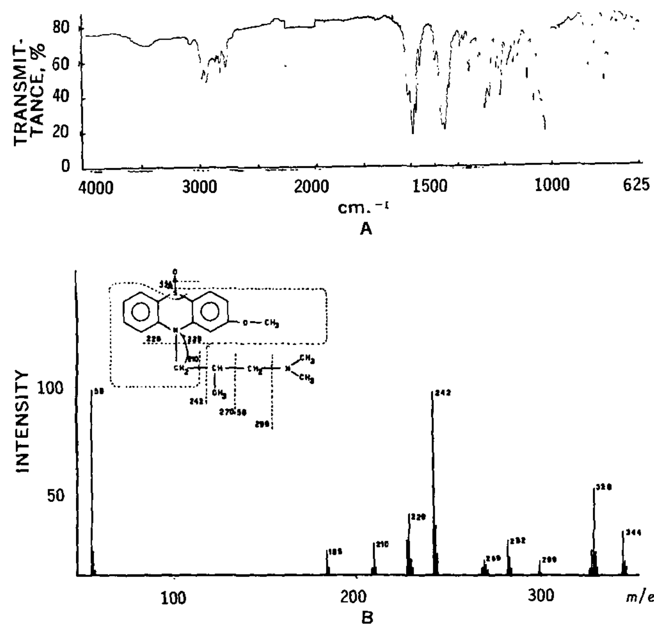


Figure 3—*IR spectrum (A) and mass spectrum (B) of trapped II isolated from urinary extract 4 of Patient 3.*

[9] Hewlett-Packard.
[10] With a Perkin Elmer 257 spectrophotometer equipped with microbeam condensor RIIC C-41.
[11] With an A.E.I. MS9 instrument.
[12] p: plateau.
[13] s: shoulder.

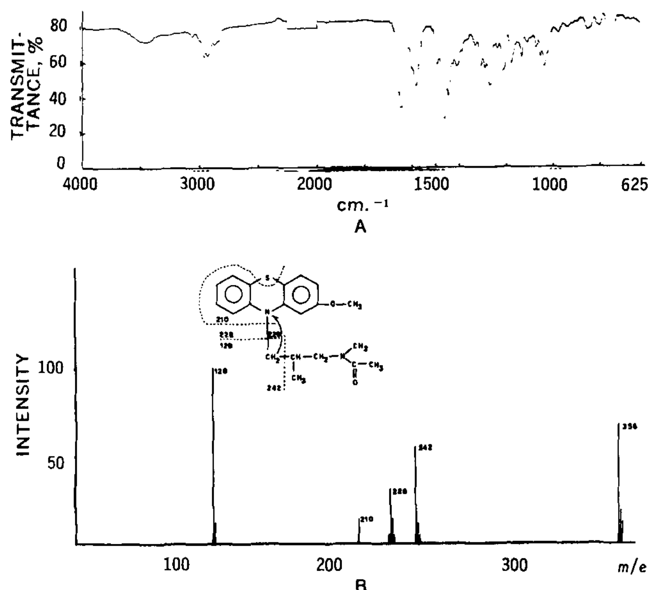


Figure 4—*IR spectrum (A) and mass spectrum (B) of trapped III acetamide isolated from urinary extract 4 of Patient 3.*

(II), and the monodemethylation compound III. A third metabolite, IV, occurred in the extracts as shown by TLC analysis. The last compound could not have been chromatographed under the described conditions. However, because of complete degradation, it did not interfere with the determinations of the other products.

Inspection of qualitative chromatograms permitted the choice of trifluoperazine as a suitable internal standard (R_t 18 min. 5 sec. on 2% FFAP and 6 min. 58 sec. on 5% OV-1), which did not overlap with any peak from the extracts. Calibration factors $\bar{k}$, obtained as described, are given in Table III.

As shown in Table IV, the values obtained for free urinary I determined in parallel on the 2% FFAP and 5% OV-1 columns agreed well. Furthermore, the excretion of II is dominant and its concentrations were always higher than those of I and the monodemethylation product III. An example of the GLC quantitation of three products in a single run on the 5% OV-1 column is shown in Fig. 1. For IV, an individual, perhaps indirect, method has yet to be worked out; *e.g.*, the reduction of the sulfoxide (IV) to the sulfide form (III) prior to acetylation should enable one to chromatograph this compound as III-acetamide (sum of III and IV). Subtraction of the original III concentration should then give the IV concentration.

Recovery experiments for I yielded 91 (on 2% FFAP) and 90% (on 5% OV-1) for a first urine sample and 85 (on 2% FFAP) and 85% (on 5% OV-1) for a second urine sample. These results indicated a good extraction recovery and a high reproducibility of the entire procedure.

For confirmation of the specificity of the GLC results, the remaining alcoholic extract, 4, of Patient 3 (Table IV) was evaporated under a nitrogen stream; the residue was acetylated and chromatographed on the 5% OV-1 column installed for trapping.

The IR and mass spectral results described here and presented in Figs. 2–4 refer to physical data obtained for the compounds isolated from the mentioned urine sample. IR spectroscopy enables easy differentiation between I, II, and III-acetamide. Indeed, II is readily recognized by the presence of strong 1028- and 769-cm.$^{-1}$ bands due to the sulfoxide group ($>S \rightarrow O$), while III-acetamide shows a specific 1650-cm.$^{-1}$ (C=O amide I *tert*) absorption. Fingerprint regions of the three compounds isolated are also highly characteristic and correspond completely with those of IR spectra of the reference compounds. This in itself is strong evidence for the powerful purification offered by the micropreparative GLC system. Mass spectral results gave the right molecular ion for each compound: I at *m/e* 328 ($C_{19}H_{24}N_2OS$), II at *m/e* 344 ($C_{19}H_{24}N_2O_2S$), and III-acetamide at *m/e* 356 ($C_{20}H_{24}N_2O_2S$). A relative high intensity for all M + 2 peaks indicates the presence of a sulfur atom (^{32}S and ^{34}S) in all structures examined. Side chains are identified by mass spectral fragmentation patterns. I shows peaks at *m/e* 283 (M-45 or M-C_2H_7N), 269 (M-59 or M-C_3H_9N), 242 (M-86 or M-$C_5H_{12}N$), 229 (M-99 or M-$C_6H_{13}N$), 228 (M-100 or M-$C_6H_{14}N$), and 58 [$H_2C = N^+ (CH_3)_2$] base peak. II loses oxygen as seen from its mass spectral peak at *m/e* 328 (M-16 or M-O) and gives identical degradation for its side chain, with peaks at *m/e* 299 (M-45 or M-C_2H_7N), comparable to the *m/e* 283 I peak, 242, 229, 228, and 58 (base peak). III-acetamide differs from the former compounds by the acetylated nature of its side chain. Nevertheless, peaks at *m/e* 242, 229, and 228 are still representative of a preferential cleavage starting from the higher branch. Lack of the typical base peak at *m/e* 58 and occurrence of the latter at *m/e* 128 are good evidence of a stabilized $C_7H_{14}NO^+$ ion.

Spectral Data for I—ν^{KBr}: 3060 and 3005 (phenyl), 2960 (CH_3), 2945 (CH_2), 2890 (CH), 2865 (CH_3), 2840 (CH_2), 2825 (N—CH_3), 2800 and 2780 (N—CH_2), 2765 and 2730 (N—CH_3), 1603, 1582, and 1494 (phenyl), 1462 (CH_3 and CH_2), 1448 (phenyl), 1382 (CH_3), 1337 (C—N aromatic), 1275 (C—N aliphatic), 1232 and 1159 (CH aromatic), 1143 (1:2:4 substituted phenyl), 1133 and 1102 (C–N aliphatic), 1033 (CH aromatic), 838 (1:2:4 substituted phenyl), and 753 (*o*-substituted phenyl) cm.$^{-1}$; *m/e* (relative intensity): 330 (23) $C_{19}H_{24}N_2O\text{-}^{34}S$, 328 (99) $C_{19}H_{24}N_2OS$, 283 (44) $C_{17}H_{17}NOS$, 269 (20) $C_{16}H_{15}NOS$, 242 (49) $C_{14}H_{12}NOS$, 229 (45) $C_{13}H_{11}NOS$, 228 (46) $C_{13}H_{10}NOS$, and 58 (100) C_3H_8N.

Spectral Data for II—ν^{KBr}: 3070 and 3000 (phenyl), 2945 (CH_2), 2862 (CH_3), 2848 (CH_2), 2825 (N—CH_3), 2778 (N—CH_2), 2725 (N—CH_3), 1608, 1588, 1576, 1557, and 1492 (phenyl), 1467 (CH_3 and CH_2), 1453 (phenyl), 1348 (C—N aromatic), 1260 (C—N aliphatic), 1230 and 1158 (CH aromatic), 1140 (1:2:4 substituted phenyl), 1035 (CH aromatic), 1028 (SO), 838 (1:2:4 substituted phenyl), 769 (SO), and 752 (*o*-substituted phenyl) cm.$^{-1}$; *m/e* (relative intensity): 346 (5) $C_{19}H_{24}N_2O_2\text{-}^{34}S$, 344 (24) $C_{19}H_{24}N_2O_2S$, 328 (47) $C_{19}H_{24}N_2OS$, 299 (8) $C_{17}H_{17}NO_2S$, 242 (99) $C_{14}H_{12}NOS$, 229 (33) $C_{13}H_{11}NOS$, 228 (19) $C_{13}H_{10}NOS$, 210 (17) $C_{12}H_{12}NO$, and 58 (100) C_3H_8N.

Spectral Data for III-Acetamide—ν^{KBr}: 3060 and 3005 (phenyl), 2965 (CH_3) 2938 (CH_2), 2878 (CH_3), 2840 (CH_2), 1650 (C=O amide I *tert*), 1603, 1585, and 1493 (phenyl), 1463 (CH_3 and CH_2), 1448 (phenyl), 1268 (C—N aliphatic), 1235 (CH aromatic), 1130 (C—N aliphatic), 1105 (C—N aliphatic), 1033 (CH aromatic), and 753 (*o*-substituted phenyl) cm.$^{-1}$; *m/e* (relative intensity): 358 (12) $C_{20}H_{24}N_2O_2\text{-}^{34}S$, 356 (67) $C_{20}H_{24}N_2O_2S$, 242 (55) $C_{14}H_{12}NOS$, 229 (14) $C_{13}H_{11}NOS$, 228 (31) $C_{13}H_{10}NOS$, 210 (19) $C_{13}H_{11}NO$, and 128 (100) $C_7H_{14}NO$.

REFERENCES

(1) L. G. Allgén, L. Hellstrom, and C. G. Sant-Orp, *Acta Psychiat. Scand., Suppl. 169*, **39,** 366(1963).

(2) A. H. M. Afifi and E. L. Way, *J. Pharmacol. Exp. Ther.*, **160,** 397(1968).

(3) A. H. M. Afifi and E. L. Way, *Agressologie*, **IX,** 353(1968).

(4) I. S. Forrest and F. M. Forrest, *Clin. Chem.*, **6,** 11(1960).

(5) *Ibid.*, **6,** 362(1960).

(6) H. Leach and W. R. C. Crimmin, *J. Clin. Pathol.*, **9,** 164 (1956).

(7) A. De Bruyn, *Belg. Chem. Ind.*, **33,** 155(1968).

ACKNOWLEDGMENTS AND ADDRESSES

Received November 26, 1971, from the *Department of Toxicology, State University of Gent, Gent 9000, Belgium.*

Accepted for publication February 16, 1972.

The authors gratefully acknowledge the help of Prof. Dr. A. Claeys from the Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, with programming the calculations of results on a high-speed electronic calculator (Diehl), and Dr. K. Van Cauwenberghe from the Department of Organic Chemistry for running the mass spectra. They also thank the Rhône-Poulenc (Paris) Pharmaceutical Co. for providing reference samples of I (levomepromazine maleate; 7044 R.P.), II (levomepromazine-sulfoxide; 19609 R.P.), III (desmethyllevomepromazine maleate; 16584 R.P.), and IV (desmethyllevomepromazinesulfoxide oxalate; 21682 R.P.).

▲ To whom inquiries should be directed. Present address: Laboratorium voor Medische Biochemie en Klinische Analyse, Akademisch Ziekenhuis, De Pintelaan 135, Gent 9000, Belgium.