Improved Method for Determining Dibenzepine and Its *N*-Demethylated Metabolites in Human Urine

A. De LEENHEER▲ and A. HEYNDRICKX

Abstract □ Urine samples taken from two psychiatric patients who ingested known amounts of the drug dibenzepine hydrochloride were extracted by a two-phase procedure with ether. A first screening by UV spectrophotometry showed the presence of compound(s) with a dibenzepine structure. The TLC separation of aliquots of extracts revealed four spots: two upper spots, each containing two substances, and two lower spots corresponding to single *N*-demethylated metabolites. GLC on the acetylated extracts by use of a polar column enabled the qualitative and quantitative (with prochlorperazine as internal standard) determinations of free dibenzepine and its five *N*-demethylated metabolites. IR and mass spectroscopic analyses of these compounds, isolated by micropreparative GLC of an aliquot of the acetylated extract, confirmed the specificity of results and gave detailed structural information.

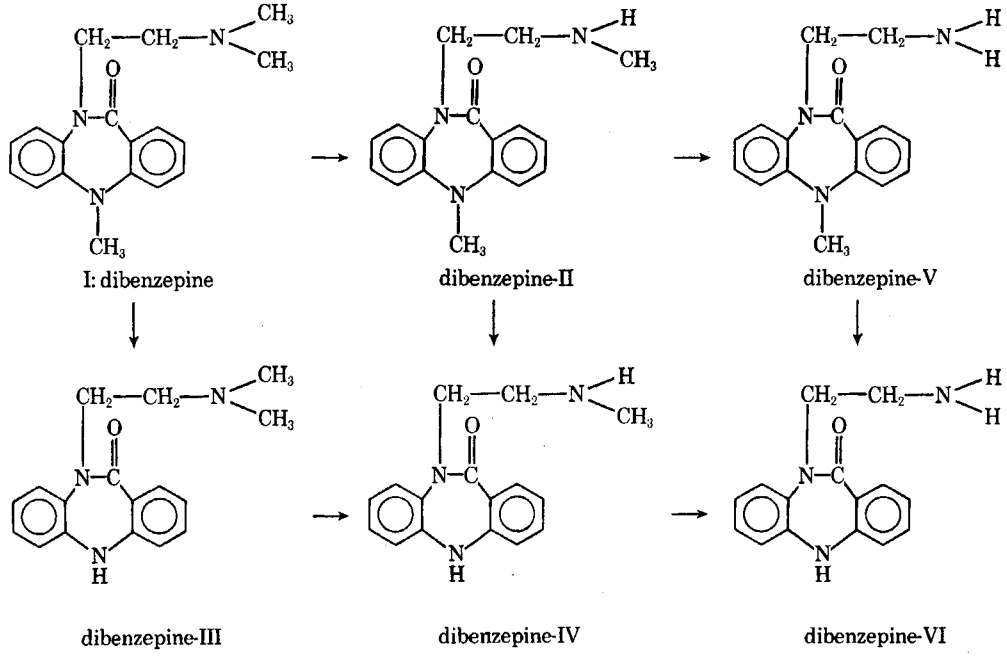
Keyphrases □ Dibenzepine and *N*-demethylated metabolites—characterization, analysis, human urine □ GLC—analysis, dibenzepine and *N*-demethylated metabolites, human urine

Dibenzepine (I) is a dibenzodiazepine derivative used as a drug with antidepressive properties. From the structural formula it may be deduced that the compound has a basic character due to the presence of an aliphatic tertiary amine function. Dibenzepine has been described to undergo *N*-demethylation and phenolic hydroxylation, followed by glucuronide formation in man, mouse, rat, rabbit, and dog (1–3). These studies were performed using UV spectrophotometry, paper chromatography, TLC, GLC, and radiochromatography and yielded insight into dibenzepine metabolism. However, a valuable method for the quantitative determination of dibenzepine and its *N*-demethylated metabolites was not developed.

Current interest in these laboratories in the metabolism of I in man led to the identification of the previously described (1–3) five *N*-demethylated products (II–VI) as important metabolites (Scheme I). By a different experimental approach, a procedure of improved specificity was developed for qualitative and quantitative determinations of free dibenzepine and its five *N*-demethylated metabolites (II–VI) in urine of man. Special attention was paid to the complete characterization of free basic compounds and their assay by GLC using an internal standard.

EXPERIMENTAL

Over periods of 4 successive days, the total urine outputs of two male patients who ingested, for the indicated period, a known amount of I (orally administered as chlorhydrate) together with a



I: 5-methyl-10-β-dimethylaminoethyl-10,11-dihydro-11-oxo-5*H*-dibenzo[*b*,*e*][1,4]diazepine
II: 5-methyl-10-β-methylaminoethyl-10,11-dihydro-11-oxo-5*H*-dibenzo[*b*,*e*][1,4]diazepine
III: 10-β-dimethylaminoethyl-10,11-dihydro-11-oxo-5*H*-dibenzo[*b*,*e*][1,4]diazepine
IV: 10-β-methylaminoethyl-10,11-dihydro-11-oxo-5*H*-dibenzo[*b*,*e*][1,4]diazepine
V: 5-methyl-10-β-aminoethyl-10,11-dihydro-11-oxo-5*H*-dibenzo[*b*,*e*][1,4]diazepine
VI: 10-β-aminoethyl-10,11-dihydro-11-oxo-5*H*-dibenzo[*b*,*e*][1,4]diazepine

Scheme I—N-*Demethylation metabolism of dibenzepine occurring in man*

Table I—Case Histories of Psychiatric Patients under Major Dibenzepine Treatment

Patient	Age, years	Daily Dose (in mg.) of Orally Administered: Dibenzepine (I) (Chlorhydrate)	Methotrimeprazine (Maleate)	Diazepam	Time Medication Began before First Urine Collection
1	62	6 × 80	25	—	6.5 months
2	36	5 × 80	25	3 × 10	3 weeks

much lower dose of methotrimeprazine[1] and diazepam (only for the second patient) were collected separately (Table I). Each sample was refrigerated immediately. Each 24-hr. urine collection was homogenized, and a 200-ml. aliquot was extracted prior to analysis without delay.

Screening Test—As a first indication of the presence of compounds, a ferrichloride, perchloric acid, and nitric acid (FPN) test (4, 5) and a chromium^{+6} test (6) were performed on each urine sample. The FPN reagent (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. The chromium^{+6} reagent (6) consisted of 200 mg. of $K_2Cr_2O_7$, 75 ml. of concentrated H_2SO_4, 125 ml. of concentrated HNO_3, 50 ml. of concentrated $HClO_4$, and distilled water to 1000 ml.

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); 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 its five *N*-demethylated metabolites (II–VI) were washed with 5-ml. portions of 0.001 *N* NaOH until the aqueous layer remained colorless. The washed ether was evaporated in a rotary evaporator to a final volume of about 100 ml.; it was then 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 combined ether phases were dried over anhydrous 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] using 1-cm. silica cells in the 450–190-nm. wavelength range.

TLC—Aliquots (0.1 ml.) of the alcoholic extracts were 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 dibenzepine hydrochloride and its demethylated products II[3], III, IV[3], V[3], and VI[4] and 10 mcg. of methotrimeprazine maleate and methotrimeprazine sulfoxide, were spotted in a 2-cm. line at 3 cm. from the lower edge of 250-μ thin-layer plates, 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 consecutively sprayed with ferric reagent (7), vanadium^{+5} reagent, and chromium^{+6} reagent (6) for visualization.

For the ferric reagent (7), 500 mg. of $Fe(NO_3)_3$ is dissolved in 80 ml. of concentrated H_2SO_4 and the solution is diluted to 1000 ml. For the vanadium^{+5} reagent, 500 mg. of V_2O_5 is dissolved in 80 ml. of concentrated H_2SO_4 and the solution is diluted to 1000 ml.

GLC—GLC analysis was performed with a research gas chromatograph[5] equipped with dual flame-ionization detectors. The column was 1% FFAP[6] coated on Diatoport S[7], 80–100 mesh, and was used for single-column operation.

The operating conditions were: glass spiral column, 1.80-m. length, 4-mm. i.d.; carrier gas, nitrogen, 70 ml. min.$^{-1}$ (bubble flowmeter); oven temperature, 245°; temperature of injector block, 250°; temperature of detector block (flame-ionization detector), 248°; and air and hydrogen, calibrated for optimum sensitivity.

Qualitative Determination—Qualitative assays of urinary dibenzepine and its *N*-demethylated metabolites were performed with the acetylated derivatives. For this purpose, 0.5-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 acid 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 in 40 μl. of ethyl acetate with the aid of a whirlmixer.

From the standards of I, its five *N*-demethylated compounds (II–VI), methotrimeprazine maleate, and methotrimeprazine sulfoxide, 10-mg. amounts were treated in the same way, but the residues obtained were taken up in 2 ml. of ethyl acetate (0.5% solutions). Volumes varying from 1 to 2 μl. were separately injected on top of the column with a syringe (Hamilton) of 10-μl. capacity.

Quantitative Determination—The same treatment was applied as for the qualitative determination, except that a known amount (200 mcg., but 160 mcg. for recovery experiments) of prochlorperazine was added as an internal standard before the first evaporation.

Calibration was made with a standard mixture. This was prepared by combining 50-μl. volumes of the 0.5% ethyl acetate solutions of the acetylated active compounds and a 1% ethyl acetate solution of prochlorperazine-(āc.)[8]. After evaporation in a water bath at 40° with the aid of a nitrogen stream, the residue was dissolved in 50 μl. of ethyl acetate. The composition of the standard solution was: 250 mcg. of I-(āc.), 250 mcg. of II-ac.[9], 250 mcg. of III-(āc.), 250 mcg. of IV-ac., 250 mcg. of V-ac., 250 mcg. of VI-ac., and 500 mcg. of prochlorperazine-(āc.) in 50 μl. of ethyl acetate.

For each active compound, a calibration factor k_i was calculated:

$$k_i = 2 \times \frac{S_x}{S_s} \qquad \text{(Eq. 1)}$$

where S_x/S_s is the quotient of peak areas[10] on chromatograms of the compound to be determined and the internal standard prochlorperazine-(āc.), and 2 is the reciprocal of the weight proportion of the compound to be quantitated (250 mcg.) w.r.t. internal standard prochlorperazine-(āc.) (500 mcg.).

The arithmetic mean $\bar{k}$ of all calibration factors k_i for a given compound was determined from three chromatograms according to:

$$\bar{k} = \left(\sum_1^3 k_i\right) \Big/ 3 \qquad \text{(Eq. 2)}$$

Results of C_u (mcg./100 ml. urine) were obtained with the equation:

$$C_u = \frac{10}{\bar{k}} \times \frac{S_x'}{S_s'} \times m_s \qquad \text{(Eq. 3)}$$

where S_x'/S_s' is the quotient of peak areas of the compound to be determined as present in the extract and the internal standard prochlorperazine-(āc.), m_s is total amount in micrograms of the internally added standard prochlorperazine-(āc.), and 10 is the conversion factor because 0.5 ml. alcoholic extract corresponds to a 10-ml. urine sample.

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

Trapping of GLC Effluents—A splitting system[11] was installed at the exit of each column, yielding a 10:1 ratio. As a collection device,

[1] Known in Europe as levomepromazine.
[2] Unicam SP800.
[3] As the chlorhydrate.
[4] As the oxalate.
[5] Hewlett-Packard 5 750.
[6] Reaction product of Carbowax 20M and *m*-dinitroterephthalic acid.
[7] Acid and silane-treated, Hewlett-Packard.

[8] (āc.) indicates that the compound underwent the acetylation treatment without being changed (no acetylable function present).
[9] ac. indicates that the compound was transformed to the acetamide.
[10] Measured using the method of peak height times peak width at half height ($h_{max} \times W_{0.5h}$).
[11] Hewlett-Packard.

Table II—Volume, pH, FPN, and Chromium^{+6} Test of Urine Samples Collected from Psychiatric Patients

Patient	Sample	Volume of Urine, ml./24 hr.)	pH	FPN Test	Chromium^{+6} Test
1	1 (1st day)	1000	6.0	—	—
	2 (2nd day)	800	5.5	—	—
	3 (3rd day)	1050	6.0	—	—
	4 (4th day)	750	5.5	—	—
2	1 (1st day)	800	5.0	± (blue)	± (blue)
	2 (2nd day)	900	5.5	± (blue)	± (blue)
	3 (3rd day)	600	6.0	± (blue)	± (blue)
	4 (4th day)	1200	5.0	—	± (blue)

a Pyrex capillary (12.5-cm. length and 1.5-mm. i.d.) fixed with two Teflon O-rings in a brass tube (10.5-cm. length, 7-mm. o.d., and 4-mm. i.d.) provided with an 1/8 swagelok nut, was used. A 1.0-ml. aliquot of the alcoholic extract was acetylated, and the residue was dissolved in 25 μl. of ethyl acetate. This solution was at once preparatively chromatographed on the 1% FFAP column at 245° for the trapping of separated compounds.

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 or 7 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 or 1 × 4-mm. hole), and the IR spectrum was recorded from 625 to 4000 cm.$^{-1}$ with a spectrophotometer[12] equipped with a microbeam condenser[13]. The mass spectrum was recorded with an instrument[14], 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, FPN test (4, 5), and chromium^{+6} test (6) of the urine samples were determined just after each 24-hr. collection. These preliminary data are summarized in Table II.

Only for Patient 2 were slightly positive FPN and chromium^{+6} tests noticed. In fact, the administered doses of methotrimeprazine were too low for good color reactions. Dibenzepine and diazepam did not develop any color under these experimental conditions.

The UV spectra of all extracts showed a striking correspondence with the reference spectrum of I (λ_{max}: 207 and 221 nm. with ϵ 28,652 and 28,419, 283 Nm.[15]; λ_{min}: 215 nm.). UV spectra of the five *N*-demethylated metabolites were qualitatively almost identical. UV spectrophotometry, being essentially an additive method, gave an overall pattern of all UV absorbing compounds as present in the acidic extract and no differentiation could be made at this stage of the analysis.

TLC of reference compounds in the described system gave: V, R_f 0.59 yellow; I, R_f 0.54 yellow; VI, R_f 0.50 blue; III, R_f 0.47 blue; II, R_f 0.24 yellow; and IV, R_f 0.19 blue. Notwithstanding the presence of six substances, four spots were obtained for all extracts: V and I yielded one yellow spot, VI and III appeared as one blue spot, and only the two lower spots (II yellow and IV blue) were single. *N*-Demethylation of the ring nitrogen resulted in formation of a blue color with the spray reagents used. This may be explained on the basis that essentially the entire ring system is responsible for the colors given, with the aminoalkyl side chain having no influence.

GLC analysis of a 0.5-ml. acetylated fraction of the alcoholic extracts on the 1% FFAP column (245°) showed the presence of free I-(āc.) (R_t 3 min. 40 sec.), III-(āc.) (R_t 7 min. 46 sec.), II-ac. (R_t 25 min. 5 sec.), V-ac. (R_t 33 min. 11 sec.), IV-ac. (R_t 58 min. 4 sec.), and VI-ac. (R_t 76 min. 54 sec.) in all extracts. Inspection of qualitative chromatograms permitted the choice of prochlorperazine as a suitable standard (R_t 11 min. 5 sec.) which did not overlap with any peak from the extracts. Calibration factors $\bar{k}$, obtained as previously described, are: I-(āc.), 2.14; II-ac., 4.74; III-(āc.), 1.28; IV-ac., 4.34; V-ac., 4.43; and VI-ac., 2.42.

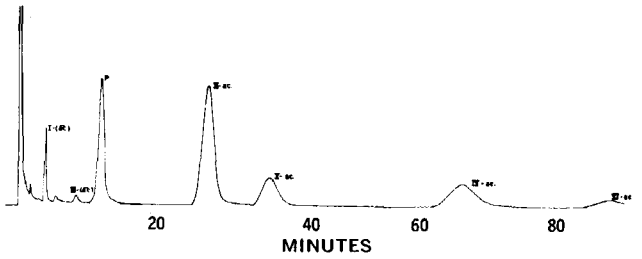


Figure 1—*Quantitative GLC on the 1% FFAP column from urinary extract 4 of Patient 2. Sample size was: 200 ml. urine, 0.5 ml. alcoholic extraction liquid evaporated and residue redissolved in 30 μl. of ethyl acetate, and 1.1 μl. injected on top of the column (corresponding to 0.37 ml. urine). Attenuation settings were: range, × 10; and attenuation, × 16. Key (from left to right): I-(āc.); III-(āc.); P, prochlorperazine-(āc.) internal standard; II-ac.; V-ac.; IV-ac.; and VI-ac.*

As seen from Table III, monodemethylation of the aminoalkyl side chain is predominant. *N*-Demethylation of the ring nitrogen is only a minor pathway. Patients 1 and 2 show differences as to their preferences for described *N*-demethylation processes. An example of the GLC quantitation of free I and its five *N*-demethylated metabolites in a single run on the 1% FFAP column is shown in Fig. 1.

Recovery experiments for I yielded 78% for the first urine sample examined and 86% for the second. These results indicate a reasonable extraction recovery, which may be extrapolated for the *N*-demethylated compounds as well. Previous results obtained by UV, TLC, and GLC did not reveal any methotrimeprazine or some of its metabolites. This result is certainly due to the major intake of I *versus* a minor intake of methotrimeprazine (about 20 times lower doses) and the small sample aliquots taken for analysis.

For confirmation of the specificity of the GLC results, a 1-ml. alcoholic extract (Sample 2 of Patient 1 and Sample 1 of Patient 2, Tables II and III) was separately evaporated under a nitrogen stream; the residue was acetylated and chromatographed on the 1% FFAP column installed for trapping. Under identical conditions, 20-μl. aliquots of 0.5% standard solutions of acetylated reference substances were chromatographed and separately collected. IR and mass spectra obtained on the purified I, II-ac., IV-ac., V-ac., and VI-ac., as isolated from mentioned urine samples (Figs. 2, 3, and 5–7), agreed completely with those obtained from reference compounds. III-(āc.) could not be isolated in a sufficient amount, but its IR and mass spectra (Fig. 4) were obtained from a reference substance[16].

IR spectroscopy enabled easy differentiation of each compound on the basis of its fingerprint characteristics. An N—H absorption around 3300 cm.$^{-1}$ showed up in all spectra except for I-(āc.) and II-ac. Structures containing the aliphatic tertiary amine function N—$(CH_3)_2$, such as I and III-(āc.), gave a 2770-cm.$^{-1}$ band which was of weak intensity but easily distinguishable. The C=O amide frequency around 1600 cm.$^{-1}$ permitted a classification in three groups, each including two compounds: I-(āc.) and III-(āc.) had the sharpest band, II-ac. and IV-ac. showed a medium broad band (tertiary amide functions), and II-ac. and VI-ac. gave the broadest absorption (secondary amide function). The 1458-cm.$^{-1}$ absorption had a definite lower intensity for III-(āc.), IV-ac., and VI-ac. (CH_3 at the seven ring substituted by H). This feature was opposite to the sharp band identified for I, II-ac., and V-ac. (*N*-ring CH_3). The 1250-cm.$^{-1}$ strong band, which was present only in I, II-ac., and V-ac., could be assigned to the *N*-ring CH_3 function. As a general phenomenon, substitution of N—CH_3 by N—H in the diazepine ring resulted in spectra with broader bands and less fine structure.

Mass spectroscopic results gave the right molecular ion for each compound: I-(āc.) at *m/e* 295 ($C_{18}H_{21}N_3O$), II-ac. at *m/e* 323($C_{19}H_{21}N_3O_2$), III-(āc.) at *m/e* 281 ($C_{17}H_{19}N_3O$), IV-ac. at *m/e* 309

[12] Perkin-Elmer 257.
[13] RIIC C-41.
[14] LKB 9000.
[15] Shoulder.
[16] With identical treatment by acetylation and GLC.

Table III—Quantitative Results of GLC Determination of Dibenzepine and Its Five *N*-Demethylated Metabolites

Patient	Sample	Concentrations, C_u, mcg./100 ml. Urine						Percent of Dose Recovered as Metabolites
		I	II	III	IV	V	VI	
1	1	42.0 (0.09)[a]	901 (1.97)	Traces	289 (0.66)	626 (1.43)	442 (1.08)	5.14
	2	75.9 (0.13)	1356 (2.37)	63.6 (0.11)	410 (0.75)	779 (1.43)	480 (0.94)	5.60
	3	12.3 (0.03)	462 (1.06)	Traces	205 (0.49)	394 (0.95)	347 (0.89)	3.39
	4	32.4 (0.05)	1356 (2.22)	Traces	403 (0.69)	763 (1.31)	525 (0.96)	5.18
2	1	224 (0.45)	1201 (2.52)	98.4 (0.21)	507 (1.11)	412 (0.91)	384 (0.90)	5.65
	2	601 (1.35)	1039 (2.45)	106.0 (0.25)	323 (0.80)	272 (0.67)	289 (0.76)	4.93
	3	258 (0.39)	799 (1.26)	67.2 (0.11)	307 (0.51)	162 (0.27)	172 (0.30)	2.45
	4	173 (0.52)	683 (2.15)	63.1 (0.20)	326 (1.08)	226 (0.75)	189 (0.66)	4.84

[a] Percent of administered dose recovered as unchanged drug.

($C_{18}H_{19}N_3O_2$), V-ac. at *m/e* 309 ($C_{18}H_{19}N_3O_2$), and VI-ac. at *m/e* 295 ($C_{17}H_{17}N_3O_2$). A low relative intensity was obtained for the molecular ion of the aliphatic tertiary amine structures I-(ac.) and III-(ac.) *versus* the high relative intensities for the aliphatic secondary and tertiary amides. Side chains were identified by three typical mass spectroscopic fragmentation patterns. Indeed, the six structures examined could be subdivided in three groups of two compounds each, just differing by one methylene group (14 a.m.u.) located at the ring nitrogen (not interfering with the side-chain fragmentation). The tertiary aliphatic amines I-(ac.) and III-(ac.) showed peaks due to α-cleavage at *m/e* 251 and 237 (M − 44 or M − C_2H_6N), α-cleavage and hydrogen-ion transfer to the eliminated fragment at *m/e* 250 and 236 (M − 45 or M − C_2H_7N), β-cleavage at *m/e* 237 and 223 (M − 58 or M − C_3H_8N), and a major γ-cleavage with hydrogen-ion transfer to the radical ion at *m/e* 224 and 210 (M − 71 or M − C_4H_9N) (base peak). In the low mass range, an intense ion was observed at *m/e* 58 [$H_2C{=}N^+(CH_3)_2$] which was due to simple β-cleavage.

The aliphatic secondary amide structures V-ac. and VI-ac. gave peaks due to fission β to the carbonyl at *m/e* 251 and 237 (M − 58 or M − C_2H_4NO), fission γ to the carbonyl with hydrogen-ion transfer (resulting in elimination of acetamide CH_3CONH_2) at *m/e* 250 and 236 (M − 59 or M − C_2H_5NO) (base peak), fission δ to the carbonyl at *m/e* 237 and 223 (M − 72 or M − C_3H_6NO), and γ-cleavage with hydrogen-ion transfer to the radical ion at *m/e* 224 and 210 (M − 85 or M − C_4H_7NO). The major fragmentation M − C_2H_5NO was further proven by occurrence of metastable ions at 202.3 for V-ac. and 188.8 for VI-ac. The aliphatic tertiary amide structures II-ac. and IV-ac. produced peaks due to fission β to the carbonyl at *m/e* 251 and 237 (M − 72 or M − C_3H_6NO), fission β to the carbonyl with hydrogen-ion transfer (resulting in elimination of methylacetamide $CH_3CONHCH_3$) at *m/e* 250 and 236 (M − 73 or M − C_3H_7NO), fission γ to the carbonyl at *m/e* 237 and 223 (M − 86 or M − C_4H_8NO), and fission δ to the carbonyl with hydrogen-ion transfer to the radical ion at *m/e* 224 and 210 (M − 99 or M − C_5H_9NO). The predominant fragmentation M − C_3H_7NO was also indicated by the metastable ions at 193.5 for II-ac. and at 180.2 for IV-ac.

I-(ac.)—ν^{KBr}: 3065 (phenyl), 2960 (CH_3), 2820 (N—CH_3), 2775 [$N(CH_3)_2$], 1640 (C=O amide), 1593 and 1505 (phenyl), 1478 (CH_3 and CH_2), 1458 and 1392 (CH_3), 1325 (Ar—N), 1252 (N—CH_3), 1167 and 1152 (C—N), and 768 (*o*-substituted phenyl) cm.$^{-1}$. *m/e* (relative intensity): 295(16) $C_{18}H_{21}N_3O$, 251(4) $C_{16}H_{15}N_2O$, 250(11) $C_{16}H_{14}N_2O$, 237(10) $C_{15}H_{13}N_2O$, 236(14) $C_{15}H_{12}N_2O$, 224 (1000) $C_{14}H_{12}N_2O$, 210(16) $C_{13}H_{10}N_2O$, 209(10) $C_{13}H_9N_2O$, 195(9) $C_{13}H_9NO$, 177(16) $C_{13}H_7N$, 149(48) $C_{12}H_5$, 59(18) C_3H_9N, and 58 (524) C_3H_8N.

II-ac.—ν^{KBr}: 3065 (phenyl), 2955 (CH_3), 2815 (N—CH_3), 1640 (C=O amide), 1590 and 1503 (phenyl), 1477 (CH_3 and CH_2), 1452 and 1392 (CH_3), 1322 (Ar—N), 1248 (N—CH_3), 1167 and 1140

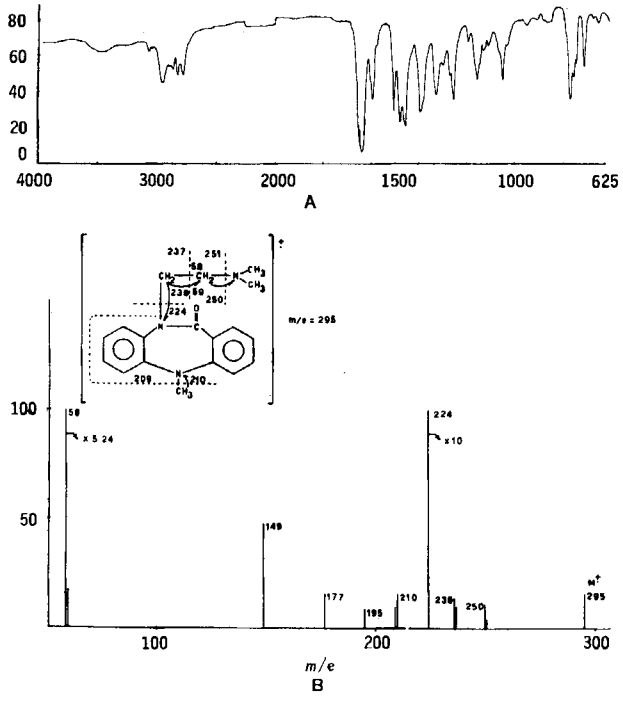


Figure 2—*IR spectrum (A) and mass spectrum (B) of trapped I-(ac.) isolated from urinary extract 2 of Patient 1.*

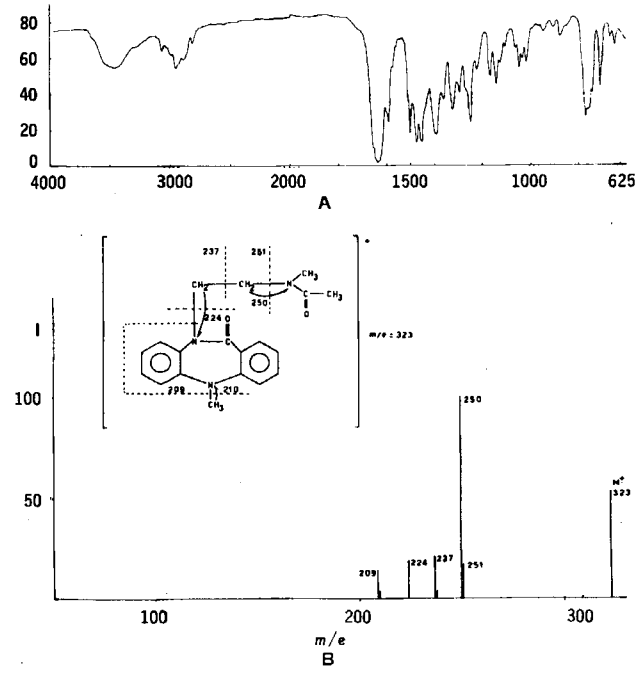


Figure 3—*IR spectrum (A) and mass spectrum (B) of trapped II-ac. isolated from urinary extract 2 of Patient 1.*

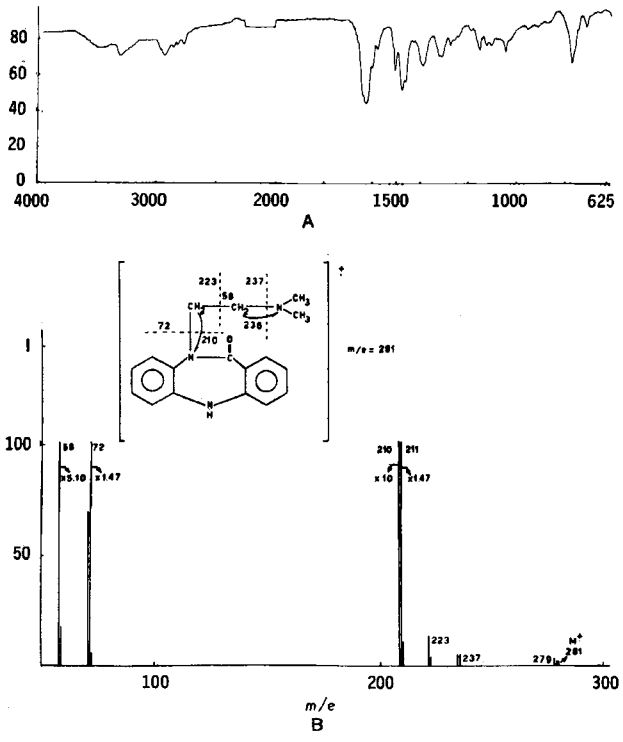


Figure 4—*IR spectrum (A) and mass spectrum (B) of trapped III-(ac.) reference substance.*

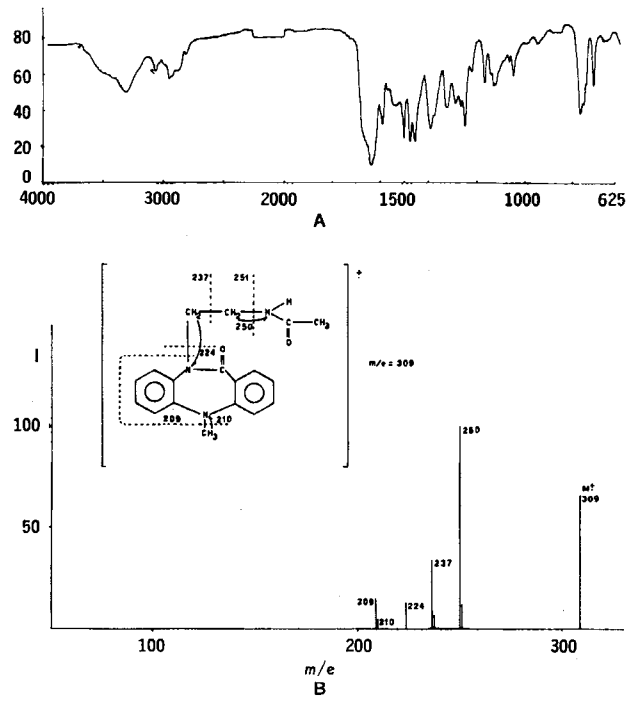


Figure 6—*IR spectrum (A) and mass spectrum (B) of trapped V-ac. from urinary extract 2 of Patient 1.*

(C—N), and 770 (*o*-substituted phenyl) cm.$^{-1}$. *m/e* (relative intensity): 323(53) $C_{19}H_{21}N_3O_2$, 309(1) $C_{18}H_{19}N_3O_2$, 251(17) $C_{16}H_{15}N_2O$, 250(100) $C_{16}H_{14}N_2O$, 238(4) $C_{15}H_{14}N_2O$, 237(21) $C_{15}H_{13}N_2O$, 224 (19) $C_{14}H_{12}N_2O$, 210(4) $C_{13}H_{10}N_2O$, and 209(14) $C_{13}H_9N_2O$; m^* = 193.5 [323(M^+) → 250].

III-(ac.)—ν^{KBr}: 3300 (N—H), 2930 (CH_3), 2820 (N—CH_3), 2775 [N(CH_3)$_2$], 1625 (C=O amide), 1598 and 1505 (phenyl), 1476 (CH_3 and CH_2), 1460 and 1388 (CH_3), 1318 (Ar—N), 1308 (*N*-ring H), 1150(C—N), and 767 (*o*-substituted phenyl) cm.$^{-1}$. *m/e* (relative intensity): 281(2) $C_{17}H_{19}N_3O$, 280(2) $C_{17}H_{18}N_3O$, 279(3) $C_{17}H_{17}N_3O$, 237(5) $C_{15}H_{13}N_2O$, 236(5) $C_{15}H_{12}N_2O$, 224(4) $C_{14}H_{12}N_2O$, 223(13) $C_{14}H_{11}N_2O$, 212(11) $C_{13}H_{12}N_2O$, 211(147) $C_{13}H_{11}N_2O$, 210(1000) $C_{13}H_{10}N_2O$, 73(6) $C_4H_{11}N$, 72(147) $C_4H_{10}N$, 71(69) C_4H_9N, 59(18) C_3H_9N, and 58(510) C_3H_8N.

IV-ac.—ν^{KBr}: 3295 (N—H), 3060 (phenyl), 2930 (CH_3), 1630 (C=O amide), 1598 and 1502 (phenyl), 1476 (CH_3 and CH_2), 1454 and 1392 (CH_3), 1300 (*N*-ring H), 1249 (N—CH_3), 1162 and 1142 (C—N), and 762 (*o*-substituted phenyl) cm.$^{-1}$. *m/e* (relative intensity): 309(63)

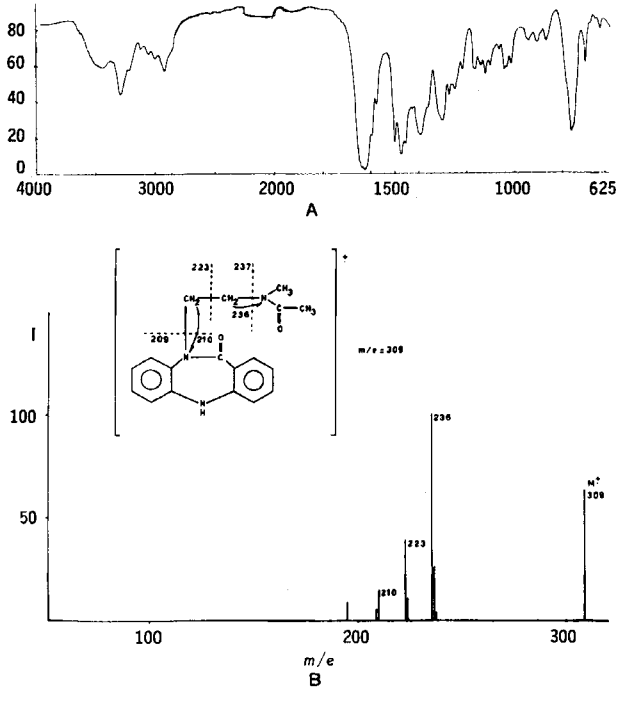


Figure 5—*IR spectrum (A) and mass spectrum (B) of trapped IV-ac. from urinary extract 2 of Patient 1.*

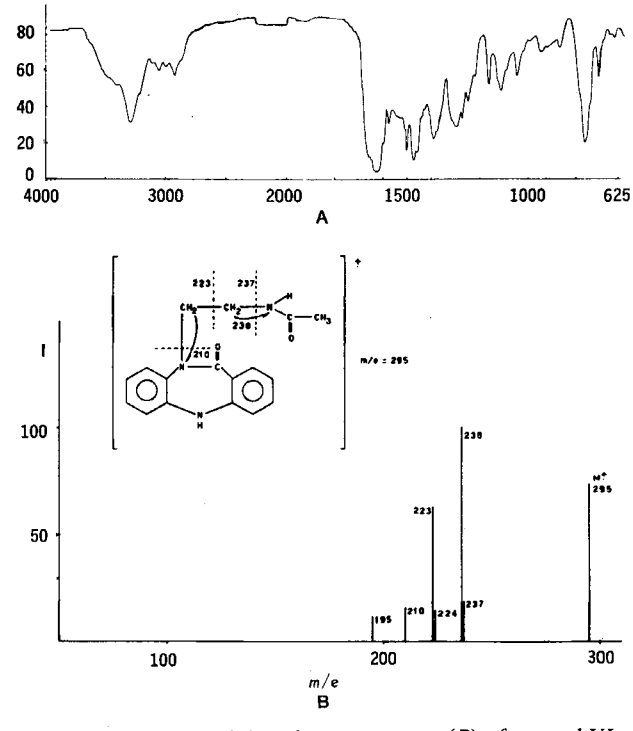


Figure 7—*IR spectrum (A) and mass spectrum (B) of trapped VI-ac. from urinary extract 2 of Patent 1.*

$C_{18}H_{19}N_3O_2$, 238(4) $C_{15}H_{14}N_2O$, 237(26) $C_{15}H_{13}N_2O$, 236(100) $C_{15}H_{12}N_2O$, 224(11) $C_{14}H_{12}N_2O$, 223(39) $C_{14}H_{11}N_2O$, 210(15) $C_{13}H_{10}N_2O$, 209(6) $C_{13}H_9N_2O$, and 195(9) $C_{13}H_9NO$; m^* = 180.2 [309(M^+) → 236].

V-ac.—ν^{KBr}: 3315 (N—H), 3070 (phenyl), 2960 (CH_3), 2820 (N—CH_3), 1640 (C=O amide), 1592 and 1506 (phenyl), 1478 (CH_3 and CH_2), 1457 and 1392 (CH_3), 1327 (Ar—N), 1250 (N—CH_3), 1168 and 1142 (C—N), and 770 (*o*-substituted phenyl) $cm.^{-1}$. *m/e* (relative intensity): 309(66) $C_{18}H_{19}N_3O_2$, 251(12) $C_{16}H_{15}N_2O$, 250(100) $C_{16}H_{14}N_2O$, 238(7) $C_{15}H_{14}N_2O$, 237(34) $C_{15}H_{13}N_2O$, 224(13) $C_{14}H_{12}N_2O$, 210(5) $C_{13}H_{10}N_2O$, and 209(15) $C_{13}H_9N_2O$; m^* = 202.3 [309(M^+) → 250].

VI-ac.—ν^{KBr}: 3295 (N—H), 3060 (phenyl), 2930 (CH_3), 1630 (C=O amide), 1597 and 1502 (phenyl), 1475 (CH_3 and CH_2), 1458 and 1390 (CH_3), 1298 (*N*-ring H), 1249 (N—CH_2), 1160 (C—N), and 762 (*o*-substituted phenyl) $cm.^{-1}$. *m/e* (relative intensity): 295(74) $C_{17}H_{17}N_3O_2$, 237(19) $C_{15}H_{13}N_2O$, 236(100) $C_{15}H_{12}N_2O$, 224(15) $C_{14}H_{12}N_2O$, 223(63) $C_{14}H_{11}N_2O$, 210(16) $C_{13}H_{10}N_2O$, and 195(12) $C_{13}H_9NO$; m^* = 188.8 [295(M^+) → 236].

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Inhibition of MAO by β-Carbolinium Halides

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Abstract □ In the search for a compound capable of selective inhibition of peripheral MAO, a number of 2,9-disubstituted β-carbolinium compounds were synthesized. With tryptamine as the substrate, these compounds generally exerted greater inhibition of the enzyme from human liver mitochondria than that from bovine liver. An exception to this was 2-propargyl-9-methyl-β-carbolinium bromide (VII), which inhibited the enzyme from both sources to a nearly equal extent. The inhibitory activity of 2,9-dimethyl-β-carbolinium iodide (I) was further studied with mitochondrial MAO of human liver, heart, and brain; rat liver, heart, and brain; mouse liver; and bovine liver; tryptamine and tyramine were used as substrates. Compound I was more effective than pargyline in inhibiting tryptamine oxidation by human MAO from peripheral tissues but was less active than pargyline toward tyramine oxidation. Kinetic studies on the inhibition of tryptamine oxidation indicated I to be a mixed-type inhibitor with MAO from human tissues and rat liver and competitive with MAO from rat heart and brain and bovine liver. The inactivation of tryptamine oxidation by I was reversible in nature, as was VII which bears a propargyl ($CH_2C{\equiv}CH$) group, the same group that endows pargyline with its irreversible binding to MAO. Since the rate of irreversible inactivation of MAO by pargyline was decreased by the presence of I, there exists the possibility of a common binding site for the two compounds.

Keyphrases □ MAO inhibitors, potential—synthesis of 2,9-disubstituted β-carbolinium halides □ β-Carbolinium halides, 2,9-disubstituted—synthesis, inhibition of MAO

Accumulated evidence for multiple forms of MAO in various tissues suggests differences in the metabolism of biogenic amines in the central and peripheral nervous systems (1–8). Many compounds, including those of the potent irreversible type such as iproniazid, pargyline, and tranylcypromine, do not exert selective inhibition of MAO of either the central or peripheral nervous system. An inhibitor whose action is specific for MAO of peripheral tissues may be useful in determining the origin of hypertension and the mechanism of reserpine action and would prove valuable in the therapy of angina pectoris because of the absence of central behavioral effects. In addition, selective inhibition of centrally localized MAO could be achieved with the combination of a nonspecific irreversible inhibitor and a specific peripheral reversible inhibitor; this would involve pretreatment with the latter to saturate the peripheral enzyme binding site, thus allowing exposure of only central MAO to the irreversible inactivation by the former. The effects of peripheral inhibition would be short lived, while the central effects from the irreversible inhibitor would be manifested until the enzyme could be regenerated (9).

One approach to designing an inhibitor with these characteristics involves exploiting the impermeability of cerebral tissue to highly polar compounds. Quater-