Table I—Antitumor Activity of Ketocaine Analogues Against Ehrlich Ascites Carcinoma in Mice

Compound	Dose, mg/kg i.p.	Number per Group	MST[a]	%,T/C[b]
Control[c]	—	10	16.0	—
II	1	5	15.0	93.7
	5	5	23.8	148.7
	20	5	—[d]	—
V	10	5	19.6	122
	50	5	27.4	171.2
	200	5	—[d]	—

[a] Mean Survival Time (days). [b] % T/C ≥ 125 denotes significant activity. [c] Saline. [d] Death due to drug-related toxicity.

EXPERIMENTAL[1]

Synthesis of Compounds II and V—*o*-Hydroxybutyrophenone (III: 16.4 g, 0.1 mole) and tris(2-chloroethyl)amine hydrochloride (IV: 24.1 g, 0.1 mole) were added to a solution of sodium ethoxide (0.2 mole) in absolute ethanol (150 ml). The mixture was refluxed for 3 hr, and the solvent was evaporated under reduced pressure. The residue was treated with water and a few drops of 34% ammonium hydroxide until alkaline, then extracted with chloroform, dried with anhydrous sodium sulfate, and evaporated under reduced pressure. Column chromatography of the residue gave 6 g of starting material (III), then 12 g of II, and finally 3 g of V. Compounds II and V, oily at room temperature, were then converted into the corresponding hydrochlorides: II HCl: mp 113–115° (absolute ethanol).

Anal.—Calc. for $C_{16}H_{23}Cl_2NO_2\cdot HCl$: C, 52.11; H, 6.56; N, 3.80. Found: C, 52.19; H, 6.44; N, 3.72. IR (cm^{-1}): 2400–2300, 1690, 1600, 1580, 975, 760. ^1H-NMR (δ): 0.96 (t, 3, CH_3); 1.68 (m, 2, CH_2—CH_3); 2.84 (t, 2, $COCH_2$); 3.86 (m, 6, 3 $CH_2N^{(+)}\lessgtr$), 4.10 (m, 4, 2 CH_2Cl); 4.61 (t, 2, O—CH_2); 6.9–7.7 (m, 4 aromatic).

V·HCl: mp 83–85° (toluene). *Anal.*—Calc. for $C_{26}H_{34}ClNO_4\cdot HCl$: C, 62.90; H, 7.11; N, 2.82. Found: C, 63.18; H, 7.32; N, 2.81. IR (cm^{-1}): 2400–2300, 1670, 1600, 1580, 1125, 750. ^1H-NMR (δ): 0.95 (t, 6, 2 CH_3); 1.66 (m, 4, 2 CH_2—CH_3); 2.82 (t, 4, 2 $COCH_2$); 3.88 (m, 6, 3 $CH_2N^{(+)}\lessgtr$); 4.12 (t, 2, CH_2Cl); 4.67 (t, 4, 2 O—CH_2); 6.9–7.7 (m, 8, aromatic).

[1] Melting points are uncorrected. Bakerflex plates (Silica-gel IB2-F) were used for TLC. For column chromatography, Kieselgel 60 (Merck) was used, activated at 120° for 2 hr: the eluent was a mixture of petroleum ether (bp 60–80°)-acetone 80:20. The IR spectra were recorded in Nujol with a Perkin-Elmer 298 spectrometer. ^1H-NMR spectra were recorded in $CDCl_3$ (10% w/v) with a 90-MHz spectrometer (Varian EM 390) using TMS as an internal standard.

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ACKNOWLEDGMENTS

Presented in part at the 21st Congress of the Italian Pharmacological Society in Naples, June 2–5, 1982.

Dedicated to Prof. M. Amorosa on the occasion of his 70th birthday.

Section VIII: A. Andreani, M. Rambaldi, D. Bonazzi, G. Fabbri, L. Greci, I. Galatulas, and R. Bossa, *Arch. Pharm.*, **316**, 141 (1983).

GLC Determination of (−)-1-Cyclopropylmethyl-4-(3-trifluoromethylthio-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)piperidine in Human Plasma and Urine

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Received July 30, 1981, from the *Merck Institute for Therapeutic Research, West Point, PA 19486.* Accepted for publication June 18, 1982.

Abstract □ (−)-1-Cyclopropylmethyl-4-(3-trifluoromethylthio-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)piperidine (MK-160) was extracted from human plasma and urine with petroleum ether and quantitated by GLC using a nitrogen-sensitive detector. A homologue of the drug served as the internal standard. The method is specific for the drug in the presence of potential metabolites and is capable of measuring concentrations in plasma as low as 6 ng/ml.

Keyphrases □ GLC determination—(−)-1-Cyclopropylmethyl-4-(3-trifluoromethylthio -5*H*- dibenzo[*a*,*d*]cyclohept-5-ylidene)piperidine (MK-160) in plasma and urine

(−)-1-Cyclopropylmethyl-4-(3-trifluoromethylthio-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)piperidine (I)[1] shows stereospecific antipsychotic, antidopaminergic, and anticholinergic activities in the mouse, rat, and squirrel monkey (1, 2). Clinical trials in human subjects necessitated an analytical method capable of measuring low nanogram concentrations of I in plasma without interference from potential metabolites of the drug. This report describes a GLC method using a nitrogen–phosphorus detector which meets these requirements with acceptable precision and accuracy.

EXPERIMENTAL

Chemicals and Reagents—Heptane, petroleum ether (35–60°), and methanol were reagent grade; isoamyl alcohol was spectroscopic quality. Stock solutions of I HCl and II were prepared in methanol and serially diluted with methanol to the desired concentrations. All concentrations are expressed as the free base of I.

Apparatus—GLC analysis was performed with a gas chromatograph[2] equipped with a nitrogen–phosphorus detector and a 91-cm × 2-mm column packed with 3% OV-17 on Gas Chrom Q (80–100 mesh)[3]. The

[1] This compound is designated as MK-160 by Merck & Co., Inc.

[2] Hewlett-Packard Model 5840A.
[3] Supelco.

0022-3549/ 83/ 0700-0815$01.00/ 0

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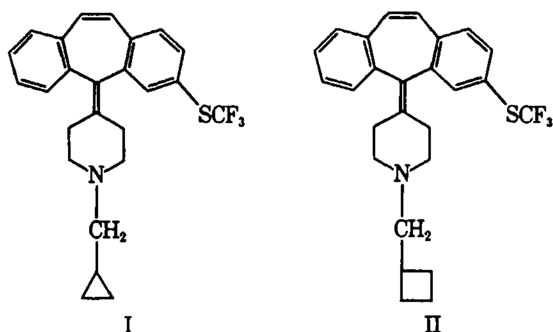

instrument was operated isothermally with column oven, detector, and injection port temperatures of 250, 275, and 300°, respectively. The carrier gas (helium), hydrogen, and air flow rates were 30, 3, and 50 ml/min, respectively. With daily use, the usable lifespan of the rubidium bead detector was ~2 months.

Procedure—To 1 ml of plasma or urine in a 13-ml glass-stoppered centrifuge tube were added 100 ng of II in 50 µl of methanol and, for plasma analysis only, 1 ml of 0.1 *N* HCl. Eight milliliters of petroleum ether–isoamyl alcohol (100:1) was added, and the tube was shaken for 10 min and centrifuged (600×*g*). Approximately 6.5 ml of the organic layer was transferred to a 13-ml centrifuge tube. The bulk of the solvent was evaporated in a stream of nitrogen in a water bath at 45°, leaving a small volume of isoamyl alcohol. The contents were dissolved in 50 µl of heptane, and 3 µl was injected into the GLC column. A standard curve was prepared by analysis of control human plasma or urine samples containing 6.25, 12.5, 25, 50, and 100 ng of I/ml and 100 ng of II/ml in a total volume of 50 µl of methanol. The ratios of the peak areas of I–II were plotted *versus* nanograms of I added.

Table I—Precision and Accuracy of the GLC Assay for I in Plasma

I Added, ng/ml	I Found[a], ng/ml	RSD, %	Relative Error, %
6.25	6.0 ± 1.1	18.3	−4.0
12.5	12.2 ± 1.7	13.9	−2.0
25	25.0 ± 2.4	9.6	0.0
50	50.3 ± 3.3	6.6	+0.6
100	99.1 ± 2.9	2.9	−0.9

[a] *N* = 40–48 for each concentration except at 100 ng/ml where *N* = 9.

Table II—Plasma Levels of I in a Human Subject after a Single Oral 120-mg Dose

Hours Postdose	Plasma I, ng/ml
1	8
2	100
4	61
6	31
8	29
24	8
32	4[a]

[a] Estimated value since 6.25 ng/ml was the lowest standard concentration analyzed.

RESULTS AND DISCUSSION

As shown in Fig. 1, I and II were adequately separated by the method, with retention times of 2.4 and 3.2 min, respectively. Control plasma or urine samples assayed concurrently gave no significant interfering peaks. Several potential metabolites of I (its sulfoxide, sulfone, and descyclopropylmethyl analogues) had retention times of 5.7, 4.9, and 1.3 min, respectively, and thus would not interfere in the determination of I.

The precision and accuracy of the method were demonstrated by analysis of replicate plasma samples containing known concentrations of I. The results are shown in Table I. The reproducibility of the method was indicated by the relative standard deviations of 18.3, 13.9, 9.6, 6.6, and 2.9%, respectively, for 6.25, 12.5, 25, 50, and 100 ng of I/ml. The accuracy of the procedure was demonstrated by the relative errors of −4, 2, 0, +0.6, and −0.9%, respectively, for 6.25, 12.5, 25, 50, and 100 ng of I/ml. The precision and accuracy of urine analyses were similar to that observed for plasma. The relative standard deviations were 15.9, 2, and 2.9%, respectively, for 6.25, 12.5, and 25 ng of I/ml of urine. The relative errors were +0.8, −4, and 3.2%, respectively, for the same concentrations. The overall recoveries for plasma and urine were ~92 and 78%, respectively.

Although I is a basic compound, its recovery from plasma was considerably higher at slightly acidic, rather than alkaline, pH values. A similar phenomenon has been reported for cinnarizine, a basic substituted piperazine with antihistaminic properties (3).

Plasma levels of I in a human subject are listed in Table II. Peak levels of drug were present at 2 hr postdose; only very low concentrations were observed after 24 or 32 hr. The urine contained no detectable amounts of I at any time after drug administration, nor was any detected in the urine of volunteers given multiple oral doses of I[4]. These results suggest that I is extensively metabolized in humans; studies with [^{3}H]I in animals indicated that I was well absorbed after oral administration. Approximately 10–30% of the dose was excreted in the urine but little or no unchanged I was present in the urine or feces[5].

This report describes a method which has adequate sensitivity to determine I in human plasma after therapeutic dosing. The use of a structurally similar internal standard obviated any need to correct the results for extraction volumes or for incomplete recovery of I from plasma or urine. Lack of interference in the assay by several potential metabolites of the drug was demonstrated.

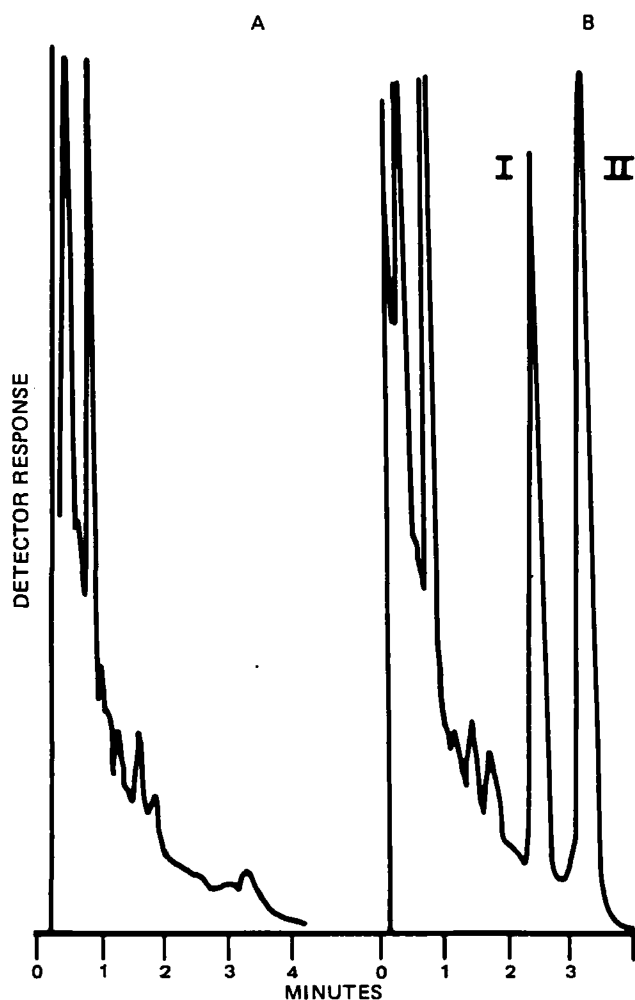

Figure 1—*Gas-liquid chromatograms (nitrogen detector) of human plasma extracts. Key: A, 0-hr human plasma; B, human plasma, containing 100 ng of I/ml to which was added 100 ng of II.*

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ACKNOWLEDGMENTS

The authors thank Dr. D. C. Remy for a sample of the internal standard and Drs. J. D. Arnold and J. D. Irwin for supplying the human plasma samples.

Oral Bioavailability and Intravenous Pharmacokinetics of Amrinone in Humans

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Received April 19, 1982, from the **Department of Drug Metabolism, Sterling-Winthrop Research Institute, Rensselaer, NY 12144* and the *‡Drug Studies Unit, University of California, San Francisco, CA 94143.* Accepted for publication June 24, 1982.

Abstract □ Fourteen healthy males received two 75-mg doses of amrinone as a single capsule and as an intravenous solution in a single-dose crossover study. The mean (±*SD*) bioavailability, based on the area under the plasma concentration *versus* time curves, was 0.93 ± 0.12. The plasma data for these subjects during the intravenous phase was described by an open two-compartment body model with a mean (±*SD*) apparent first-order terminal elimination rate constant, β, of 0.19 ± 0.06 hr^{-1}, which corresponds to a half-life of 3.6 hr.

Keyphrases □ Amrinone—plasma levels in humans after oral and intravenous doses, pharmacokinetics, bioavailability □ Pharmacokinetics—amrinone after an intravenous dose, described by an open two-compartment body model □ Bioavailability—amrinone after oral and intravenous doses, plasma levels, pharmacokinetics

Amrinone[1], 5-amino[3,4′-bipyridin]-6(1*H*)-one, is a novel cardiotonic agent (1–4) which has demonstrated inotropic activity after both oral and parenteral administration to patients suffering from congestive heart failure (5–8). Previous investigations into the relationship between intravenous and peroral amrinone doses have suggested that it required twice as much amrinone by the oral route to achieve the same inotropic response as with an intravenous bolus (9). This report describes the results of our investigations into the bioavailability of amrinone, and includes a nonlinear least-squares estimate of the pharmacokinetic parameters of amrinone following intravenous administration.

EXPERIMENTAL

Study in Human Volunteers—In this single-dose crossover study, 14 healthy male volunteers each received two 75-mg doses of amrinone, as a single oral capsule and as an intravenous solution, with a 1-week washout interval between medications. The sequence of drug administration for each subject was determined by random assignment. Appropriate institutional review and approval were obtained. No subject had a history suggestive of renal, hepatic, or cardiac dysfunction. The mean (±*SEM*) age of these volunteers was 28.9 ± 1.2 years; the mean weight was 77.4 ± 3.4 kg and the mean height was 180 ± 1.7 cm. Blood samples were collected (potassium oxalate) before medication and at 0.17, 0.33, 0.50, 0.75, 1.0, 1.25, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 15.0, and 24.0 hr postdose. The blood was centrifuged; plasma was separated and frozen until it was assayed.

Assay Procedure—The analysis of plasma amrinone followed a published procedure (10) with the following minor changes: a 40° water bath was used to evaporate the residual ethyl acetate and ~2.3% (by volume) of tetrahydrofuran was added to the mobile phase. Plasma standards, which were prepared in normal human plasma, were extracted and analyzed with each set of plasma samples from the subjects in the study. The concentration of amrinone in plasma was determined by comparison with the regression line of the peak height ratios of the standards. The minimum quantifiable level of amrinone was estimated as the concentration whose lower 80% confidence limit just encompassed zero[2] and was ~0.02 μg/ml.

Five separate high-performance liquid chromatographic (HPLC)

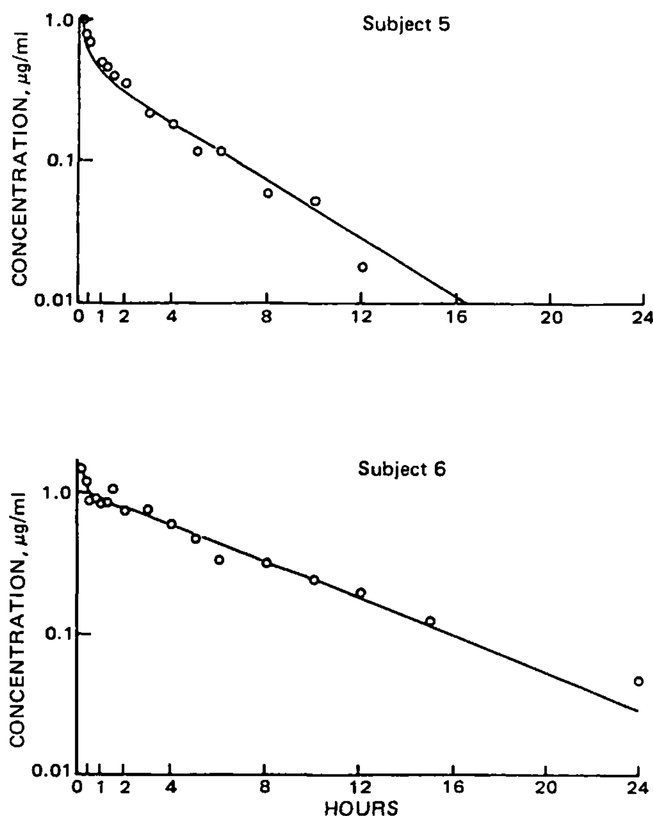

Figure 1—*Plasma concentration of amrinone in human volunteers after intravenous administration of a solution containing 75 mg of amrinone. Plasma concentrations observed (O) in two subjects with widely divergent clearance rates, and concentrations predicted by the open two-compartment body model (—).*

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0022-3549/ 83/ 0700-0817$01.00/ 0
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