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Note

Quantitation of (*d,l*)-3-ethyl-2,6-dimethyl-4,4*a*,5,6,7,8,8*a*,9-octahydro-4*a*,8*a*-*trans*-1H-pyrrolo[2,3-*g*] isoquinolin-4-one in human plasma by gas chromatography—chemical ionization mass spectrometry

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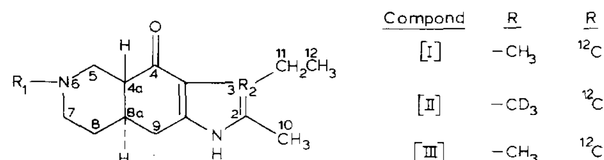
The compound (*d,l*)-3-ethyl-2,6-dimethyl-4,4*a*,5,6,7,8,8*a*,9-octahydro-4*a*,8*a*-*trans*-1H-pyrrolo[2,3-*g*]isoquinolin-4-one [I] is one of a number of pyrrolo-[2,3-*g*]isoquinolines which exhibit neuroleptic activity and are being investigated as possible antipsychotic agents [1]. A high-performance liquid chromatographic (HPLC) method [2] for determination of [I] lacked the sensitivity (25 ng ml^{-1}) necessary for monitoring [I] in plasma following a single therapeutic dose of the drug. This paper describes a gas chromatography—chemical ionization mass spectrometry (GC—CIMS) assay which can quantitate between 2 and 50 ng ml^{-1} of [I] in plasma. The sensitivity of the assay is sufficient to measure the concentration of the drug in human plasma for 4—8 h after the oral administration of a single 5-mg dose of the drug.



EXPERIMENTAL

Materials

Compounds [I] and [II] were synthesized by Dr. G.L. Olson, Chemical Research Department and compound [III] was synthesized by the isotope synthesis group, Hoffmann-La Roche Inc. (Nutley, NJ, U.S.A.). The specific

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activity of [III] was 0.046 μCi mg^{-1}. Methanol, chloroform and ethyl acetate were purchased from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). Phosphate buffer (1.0 *M*, pH 11) was prepared by mixing 530 ml of 1 *M* $K_2HPO_4 \cdot 3H_2O$ and 470 ml of a saturated solution of sodium phosphate, and adjusting the solution to pH 11 with 1 *M* $KH_2PO_4 \cdot 3H_2O$.

Analytical procedure

Stock solutions (1 mg ml^{-1}) of compounds [I] and [II] were prepared in methanol. Standard solutions were prepared by dissolving the stock solution in methanol to give final concentrations of 4—100 ng of [I] per 100 μl. For preparation of standard curves, 100 μl of the standard solutions were added to 2 ml of the control plasma containing 40 ng of compound [II] and either 4, 10, 20, 40 or 100 ng of compound [I]. The spiked plasma samples were then taken through the complete analytical procedure described below. The experimental plasma samples were thawed, and 2-ml aliquots of each were pipetted into 16-ml screw-capped tubes (Pyrex 9825) with Teflon®-lined screwcaps. A 40-ng amount of the internal standard [II] was added to each plasma sample. A 2-ml volume of 1 *M* phosphate buffer pH 11 and 6 ml of chloroform were added and the tubes were shaken on a variable-speed reciprocating shaker (Eberbach, Ann Arbor, MI, U.S.A.) for 15 min. The sample tubes were centrifuged, the aqueous layer was discarded, the organic phase was transferred to a 5-ml centrifuge tube (Pyrex No. 8061), and the organic solvent was evaporated at 50°C under a stream of nitrogen. The residue was reconstituted in 50 μl of ethyl acetate and a (5-μl) aliquot of this solution was injected into the GC—MS system.

Stability study

Sufficient compound [I] was added to several control plasma samples to give a drug concentration of 3 ng ml^{-1}. The fortified plasma samples were stored at —20°C in 16-ml tubes with Teflon-lined screw-caps.

Efficiency of extraction

The recovery of compound [I] was determined by adding known amounts of ^{14}C-labelled compound [III] to three individual 2-ml plasma control samples and extracting the samples using the procedure described. The radioactivity in the organic layer was counted on a Mark III liquid scintillation spectrometer (Searle Analytic, Des Plaines, IL, U.S.A.) using 10 ml of Aquasol (New England Nuclear, Boston, MA, U.S.A.) as counting solution.

Effect of the blood collection container on extractability of [I]

Control human blood (100 ml) was spiked with approximately 700 ng of compound [III] (7 ng ml^{-1}). The stoppers of six Vacutainers (B & D., Vacutainer No. 6527) were removed. Aliquots (5 ml) of spiked blood were transferred into the Vacutainers which were then restoppered. Additional 5-ml aliquots of blood were transferred to six of the same 16-ml culture tubes (Pyrex No. 9825) which were used for plasma extractions. The culture tubes were capped and all twelve tubes were placed on a horizontal shaker and gently shaken for 30 min. Three of the Vacutainer blood samples and three

of the culture tube blood samples were centrifuged and plasma collected. A 1-ml aliquot each of the six plasma samples and 1 ml each of the remaining whole blood samples were radioassayed according to the procedure described previously.

Instrumentation

GC—MS analysis was performed using a Finnigan Model 1015 mass spectrometer operated in the CI mode. Data were acquired using a Finnigan 6000 data system with revision I software. GC separations were performed on a glass column (1 m × 1 mm I.D.) packed with 3% OV-17 on 120—140 mesh Gas-Chrom Q (Applied Science Labs., State College, PA, U.S.A.). Prior to use, the column was conditioned with no flow for 2 h at 300°C and with a 60 ml min^{-1} helium flow overnight at 280°C. The column was mounted in a Finnigan 9500 gas chromatograph and was connected to the mass spectrometer via a direct transfer line. Methane was used as carrier gas at a pressure of 1.2 kg m^{-2}. The ion source pressure of methane was approximately 53 Pa. The reagent gas (ammonia) was added using the direct insertion probe gas inlet to give a total ion source pressure of 80 Pa. The temperatures of the injection port, GC oven and transfer line were 275°C, 280°C and 250°C, respectively. Under these conditions, compound [I] had a retention time of 1.3 min. The filament was operated at an ionization energy of 70 eV and an emission current of 0.92 MA. The voltage to the conversion dynode was supplied by a Hewlett-Packard Model 6516A, 0—3000 V, power supply. Typically, the conversion dynode electron multiplier was operated at 1.9 kV. The electron multiplier was operated at 1.8 kV and the preamplifier was set to 10^{-8} A/V.

The slope (m) and intercept (b) values from a least-squares analysis of the m/z 247 to m/z 250 ion ratio versus amount added data from the calibration curve samples were used to calculate the amount (x) of [I] in an experimental sample from the measured ion ratio (R) using the equation $x = (R - b)/m$.

RESULTS AND DISCUSSION

Ammonia—methane CIMS spectra of [I] and [II] are shown in Fig. 1. As expected, the mass spectra consist principally of MH^+ ions. Fig. 2 shows typical selected ion current profiles from the analysis of plasma taken from a subject both before and after a dose of [I]. The analysis of the pre-dose plasma gave no response in the selected ion current profile corresponding to [I].

Linear calibration curves (correlation coefficients >0.99) were observed for the determination of between 2 and 50 ng/ml of [I] using 20 ng ml^{-1} of [II] as the internal standard. The precision and accuracy of the method were determined by analyzing samples of human control plasma which were fortified to give concentrations of [I] of 2, 5, 10, 20 and 50 ng ml^{-1}. The samples were prepared and analyzed in duplicate on three different days. The inter-assay precision of calibration curves is shown in Table I. The mean relative standard deviation for all the concentrations used to prepare the calibration curve was 4.7%. Mean intra-assay precision was 4.7% (Table II). The lower limit of quantitation of the assay is 2 ng ml^{-1} using 2.0 ml of human plasma. The extractability of [I] from human plasma was 88—89%.

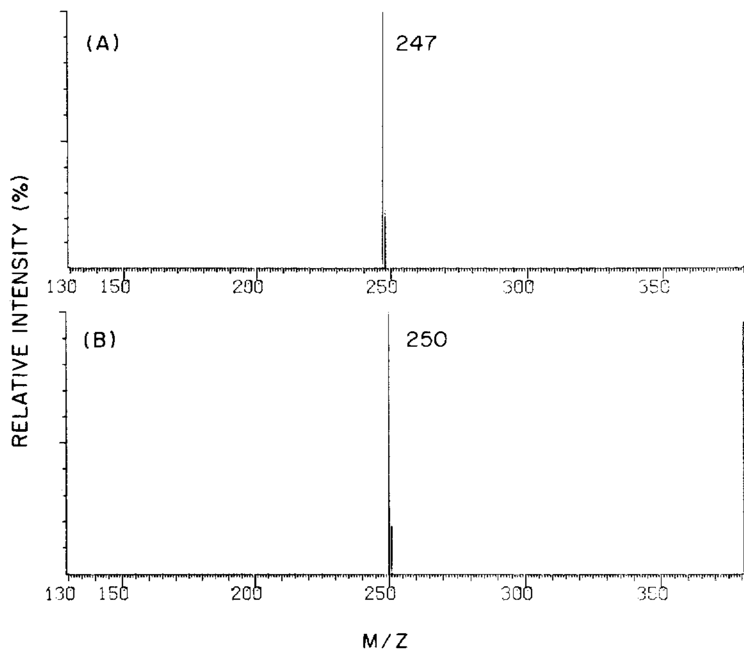

Fig. 1. Ammonia—methane CI mass spectra of [I] (A) and its trideuterated analogue [II] (B).

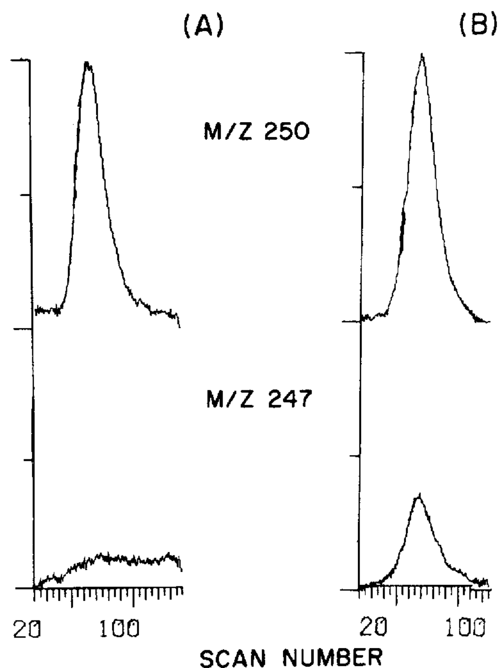

Fig. 2. Ion chromatograms from the analysis of 1 ml of plasma from a subject who had received a single 5-mg oral dose of [I]. (A) Plasma taken just prior to dosing; (B) plasma taken 30 min post dosing. The concentration of [I] in this last sample was 8.2 ng ml^{-1}. The ions at *m/z* 247 and 250 represent [I] and [II], respectively. For these ion chromatograms, 1 μl out of 50 μl was inejcted.

TABLE I

INTER-ASSAY VALIDATION DATA FOR [I] IN PLASMA

Calibration curve	Concentration (ng ml^{-1})				
	2	5	10	20	50
1	2.0	5.1	9.4	19.0	51.6
	2.0	5.8	9.6	19.3	50.5
2	1.9	4.9	9.3	20.4	50.2
	2.3	5.0	9.9	19.9	50.2
3	2.1	5.0	9.8	20.4	49.6
	1.8	5.0	10.3	20.4	49.6
Mean	2.0	5.1	9.7	19.9	50.3
± S.D.	0.17	0.33	0.37	0.62	0.74
R.S.D.* (%)	8.5	6.5	3.8	3.1	1.5
			Mean R.S.D. =		4.7

*R.S.D. = relative standard deviation.

TABLE II

INTRA-ASSAY VALIDATION DATA FOR [I] IN PLASMA (n=3)

Concentration added (ng/ml^{-1})	Calculated concentration (ng/ml^{-1} ± S.D.)	R.S.D. (%)
2	2.3 ± 0.12	5.2
5	4.7 ± 0.51	10.9
10	9.7 ± 0.31	3.2
20	19.0 ± 0.43	2.3
50	51.3 ± 1.06	2.1
	Mean R.S.D. =	4.7

The effect of the collection container (B & D, Vacutainer No. 6527) on assay accuracy was evaluated. For blood samples fortified with 7 ng ml^{-1} of [I], the mean concentration of [I] (± S.D.) determined was 6.6 ± 0.1 ng ml^{-1} for samples exposed to the culture tubes and 6.6 ± 0.2 ng ml^{-1} for samples exposed to the Vacutainers. In plasma, the mean [I] concentration (± S.D.) determined was 5.2 ± 0.2 ng ml^{-1} for the blood samples exposed to the culture tubes and 5.2 ± 0.2 ng ml^{-1} for the blood samples exposed to the Vacutainers. Thus, the exposure of the blood samples to the Vacutainers caused no artifactual increase or decrease in the concentration of [I]. Assuming a hematocrit (Ht) of 0.45, the red blood cell/plasma partition ratio for [I] was calculated to be approximately 1.6.

The stability of [I] in plasma on storage at —20°C over a 2-week storage period was demonstrated by reanalysis of the plasma samples fortified with [I]. Table II contains the results of ten repeat determinations of the plasma concentration of [I] assayed on three separate days. The mean (± S.D.) concentration of [I] determined was 3.3 ± 0.2 (R.S.D. = 6.1%). Compound [I] was stable in plasma for at least 2 weeks.

TABLE III

PLASMA CONCENTRATION (ng ml^{-1}) OF [I] FROM THE REPEAT ANALYSES OF A FORTIFIED HUMAN PLASMA SAMPLE

Control human plasma was pooled (100 ml) and spiked with 300 ng of [I] (i.e. 3 ng ml^{-1}).

Date	No.	[I] found (ng ml^{-1})	$\frac{\text{Found} - \text{mean}}{\text{mean}} \times 100$
11/9/82	1	3.5	+6.1
	2	3.5	+6.1
	3	3.4	+3.0
	4	3.6	+9.1
11/10/82	1	3.3	0
	2	3.1	−6.1
	3	3.1	−6.1
	4	3.3	0
11/23/82	1	3.3	0
	2	3.2	−3.0
		Mean ± S.D. = 3.3 ± 0.2	(R.S.D. = ±6.1)

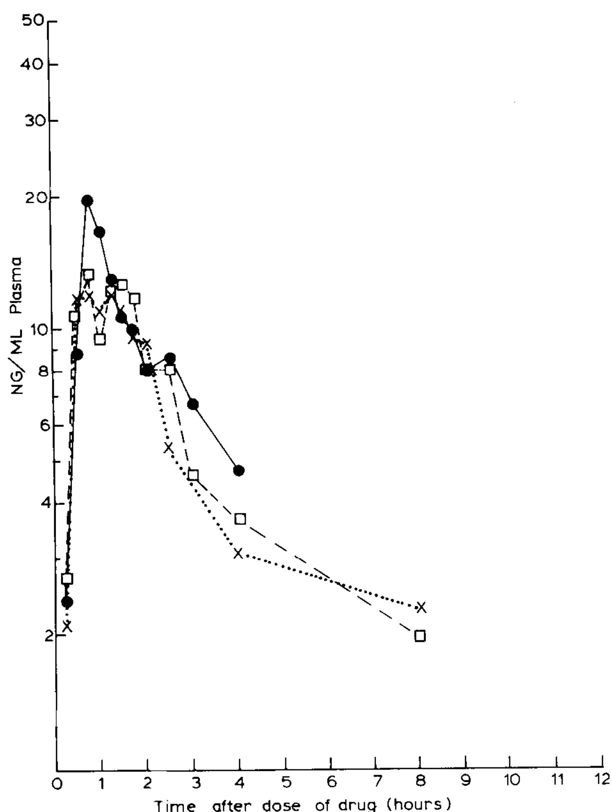

Fig. 3. Plasma concentration—time curves of [I] after oral administration of a 5-mg oral dose of [I] to three healthy volunteers.

The concentrations of [I] in the plasma of three human subjects given single 5-mg oral doses of drug are shown in Fig. 3. The mean (± S.D.) peak concentration of [I] was 17.5 ± 2.3 ng ml^{-1}. The peak plasma concentrations were observed at 1—2 h after drug administration and fell to below the limit of quantitation of the assay (<2 ng ml^{-1}) from 4—8 h post-dose. The half-life in these three subjects was approximately 2 h.

To summarize, a GC—MS method for [I] was developed which is sensitive to 2 ng ml^{-1} of plasma. The assay was used to determine [I] in plasma for 4—8 h following a single oral dose of the drug.

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