GLC Determination of *S*-2-(3-*tert*-Butylamino-2-hydroxypropoxy)-3-cyanopyridine in Human Plasma

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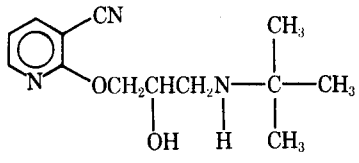
Abstract □ *S*-2-(3-*tert*-Butylamino-2-hydroxypropoxy)-3-cyanopyridine was recovered (~90%) from human plasma and detected by conversion to a diheptafluorobutyryl derivative for electron-capture GLC. A homolog served as an internal standard. The method measures plasma drug concentrations at the 5-ng/ml level and is suitable for plasma analysis from humans who receive a therapeutic oral dose.

Keyphrases □ *S*-2-(3-*tert*-Butylamino-2-hydroxypropoxy)-3-cyanopyridine—analysis, electron-capture GLC, diheptafluorobutyryl derivative, human plasma, antihypertensives □ Antihypertensives—*S*-2-(3-*tert*-butylamino-2-hydroxypropoxy)-3-cyanopyridine, analysis, electron-capture GLC, human plasma □ GLC, electron capture—*S*-2-(3-*tert*-butylamino-2-hydroxypropoxy)-3-cyanopyridine, human plasma

S-2-(3-*tert*-Butylamino-2-hydroxypropoxy)-3-cyanopyridine (I) hydrochloride is a potent antihypertensive β-adrenoceptor antagonist with peripheral vasodilating activity[1]. It lowers arterial pressure in spontaneously hypertensive rats and in hypertensive humans.

A sensitive and specific analytical method was required to measure I in human plasma. The use of electron-capture techniques to quantitate low drug levels in biological fluids was validated by many workers. A suitable derivatizing reagent sometimes must be used to impart electron-capturing properties to the assayed compound. Initial attempts at derivatization of I with either trifluoroacetic anhydride or pentafluoropropionic anhydride were unsuccessful because two products were formed.

A trifluoroacetic anhydride–trimethylamine mixture was used successfully for propranolol analysis (1); however, when applied to the analysis of I, the resultant derivative was not endowed with sufficient electron-capturing properties. Heptafluorobutyrylimidazole (III) is a reagent used in the analysis of indolealkylamines (2–4) and timolol maleate, another β-blocker (5). In the procedure reported here, I was extracted from plasma, derivatized to form the diheptafluorobutyryl analog, and determined by electron-capture GLC at concentrations as low as 5 ng/ml.



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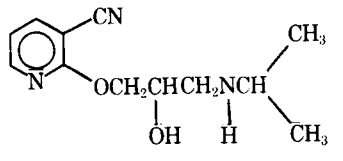

II

Table I—Reproducibility of the Electron-Capture GLC Assay for I

I, ng/ml	Peak Height Ratio ± *SD* (I/II) (*n* = 3)
100	1.08 ± 0.11
50	0.52 ± 0.01
25	0.31 ± 0.01
10	0.10 ± 0.01
5	0.055 ± 0.005

Table II—Major Ions in the Mass Spectrum of I Diheptafluorobutyryl Derivative

m/e	Ion	Fragment(s) Lost
626	M − 15⁺	CH_3
506	M − 135⁺	$C_6H_4N_2O + CH_3$
371	M − 270⁺	$C_4F_7O_2 + C_4H_9$

EXPERIMENTAL

Reagents—Hexane and toluene were nanograde quality. Ether (absolute) was ACS reagent grade. Compound III was obtained in 1-ml ampuls[2]. Compound I was used as the hydrochloride salt, and *S*-2-(3-isopropylamino-2-hydroxypropoxy)-3-cyanopyridine (II) served as the internal standard. All concentrations are expressed in terms of the free base.

Apparatus—GLC analysis was performed using a gas chromatograph[3] equipped with a ^{63}Ni-electron-capture detector (15 mCi) and a 122 × 0.64-cm (4 ft × 0.25 in.) column packed with 3% OV-1 on Gas Chrom Q[4] (60–80 mesh). The instrument was operated isothermally with the oven, detector, and injection port temperatures at 180, 300, and 250°, respectively. The carrier gas (5% methane in argon) flow rate was 78 ml/min. The pulse frequency was variable. Gas lines were fitted with filters containing molecular sieves (15 Å).

Procedure—Plasma, 1 ml, was mixed with II (50 μl of a 1-μg/ml solution of II in water) and 1 *N* NaOH (0.2 ml) and was then shaken with toluene–ether (4 ml of a 4:1 mixture) in a 13-ml glass-stoppered centrifuge tube for 10 min. After centrifugation, the organic layer was transferred as completely as possible to a 5-ml centrifuge tube containing 0.1 *N* HCl (0.3 ml). After being stoppered and shaken for 10 min, the tube contents were centrifuged, the organic layer was removed by aspiration, and the aqueous phase was mixed with 2 *N* NaOH (0.1 ml). Toluene–ether (1.5 ml of a 4:1 mixture) was added, and the tube was shaken for 10 min.

The organic phase was separated (after centrifugation), transferred to a 5-ml centrifuge tube, and evaporated under a nitrogen stream. Compound III, 25 μl, and hexane, 0.1 ml, were added to the residue. After heating at 90–100° for 1 hr, the tubes were allowed to cool, hexane (50 μl) and 0.1 *N* HCl (0.1 ml) were added, and the contents were agitated for 30 sec. The top hexane layer was analyzed by GLC on a 3% OV-1 column with electron-capture detection, using 1-μl aliquots/injection. The retention times of the derivatized internal standard and I were 4.8 and 6.8 min, respectively. GLC–mass spectrometry showed that the acylation products of these compounds are the diheptafluorobutyryl de-

[1] Dr. C. S. Sweet, Merck Institute for Therapeutic Research, West Point, Pa., unpublished results.

[2] Pierce Chemical Co.

[3] Hewlett-Packard model 5730A.

[4] Supelco.

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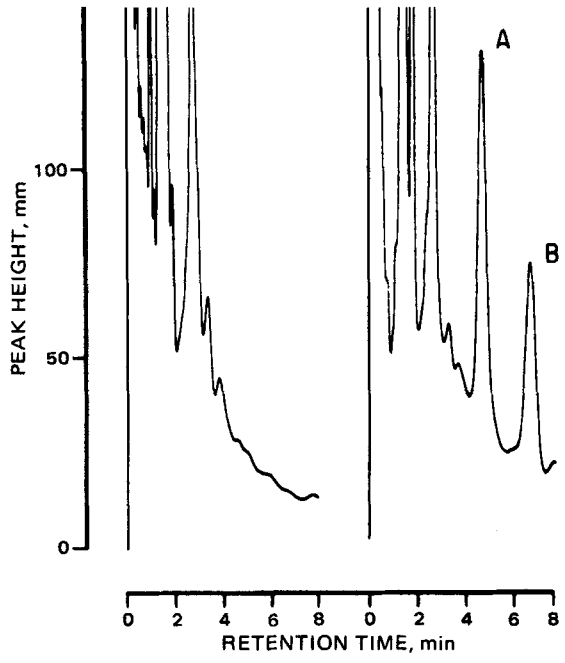

Figure 1—*Gas-liquid chromatograms (electron-capture detector) of human plasma extracts. Left: 0-hr human plasma. Right: 50 ng of II (as the diheptafluorobutyryl derivative A) and 50 ng of I (as the diheptafluorobutyryl derivative B) added to 1.0 ml of plasma.*

rivatives. A standard curve was constructed by analysis of control plasma samples containing known quantities of I and II.

Compound I in Human Plasma—Four normotensive human subjects each received a single 6-mg oral dose of ^{14}CN-I. Blood was collected in heparinized containers at intervals during the first 24 hr following the dose, and the plasma was harvested and frozen until assayed.

In a single-blind study, I was administered to five patients in a dosage range from 5 mg *sid* to 40 mg *bid* over a period ranging from several days to several weeks. On days when the daily dosage was increased, blood was drawn at 0, 1, 2, 4, 8, and 12 hr; the plasma was harvested for subsequent analysis.

RESULTS AND DISCUSSION

There was a linear relationship between the GLC peak height ratio, *i.e.*, I to II, and the plasma I concentration over a range of 5 ng to several micrograms. The method reproducibility was determined by analyzing plasma samples (1 ml) containing I, *e.g.*, 100, 50, 25, 10, and 5 ng/ml (Table I). To determine the recovery of I, the same procedure was used to extract ^{14}CN-I from human plasma. At 50 and 100 ng/ml, the radioactivity recoveries were 89.6 ± 4.1% (n = 3) and 88.4 ± 7.2% (n = 3), respectively. There was no radioactivity loss when the samples were evaporated to dryness prior to derivatization.

The reagent III was stored at −20° and subsequently was thawed and mixed with hexane for the derivatization reaction, which yielded the diheptafluorobutyryl derivative (Table II and Fig. 1). Substitution of hexane with the more polar solvent ethyl acetate did not give satisfactory results, contrary to published reports (5, 6). The derivatization had to be performed at 90–100°, although lower temperatures had been used (6) for a number of amines.

No interference was produced by plasma constituents. Occasionally, there was some day-to-day variation in the peak height ratio of I and its analog II. However, the assay of control samples at different times during a day indicated no fluctuation of this ratio over 8 hr.

Plasma I levels were measured in four humans who received an oral dose of ^{14}CN-I (see *Experimental*). Initially, I levels were similar to plasma radioactivity levels when the latter were expressed as I nanogram equivalents. A maximum I concentration (16 ± 2 ng/ml) was reached at 3 hr. At later times, there was a pronounced decrease in the plasma I levels, and the majority of the plasma radioactivity was apparently represented by ^{14}CN-I metabolites (Table III).

Table III—Mean Plasma I Levels in Four Normotensive Subjects after a Single Oral 6-mg Dose of ^{14}CN-I [a]

Hours after Dose	Plasma I ± *SD*, ng/ml: Radioactive Equivalents	Plasma I ± *SD*, ng/ml: I
0.25	<5	<5
0.5	11 ± 3 (n = 3)	7 ± 3 (n = 3)
1.0	16 ± 5 (n = 4)	12 ± 4 (n = 3)
1.5	20 ± 2 (n = 4)	10 ± 5 (n = 3)
2.0	21 ± 3 (n = 4)	15 ± 4 (n = 4)
3.0	22 ± 3 (n = 4)	16 ± 2 (n = 4)
4.0	22 ± 4 (n = 4)	8 ± 2 (n = 4)
5.0	19 ± 3 (n = 4)	7 ± 2 (n = 4)
6.0	18 ± 3 (n = 4)	7 ± 1 (n = 4)
7.0	16 ± 2 (n = 4)	6 ± 1 (n = 3)
8.0	14 ± 2 (n = 4)	6 ± 2 (n = 3)
12.0	9 ± 2 (n = 4)	<5
24.0	<5	<5

[a] Compound I had a specific activity of 8323 dpm/μg.

Table IV—Dependence of Plasma I Concentration on Dose in Five Subjects [a]

Hours	Dose, mg: 5[b]	10[b]	20	30	40
1	25 ± 11	17 ± 6	85 ± 26	131 ± 20	180 ± 72
2	12 ± 5	30 ± 8	75 ± 18	108 ± 35	169 ± 29
4	<5	19 ± 5	52 ± 18	97 ± 20	144 ± 47
8	<5	8 ± 3	23 ± 5	28 ± 24	74 ± 43
12	<5	<5	12 ± 2	24 ± 4	36 ± 19

[a] Values are mean and *SD* for five subjects. [b] n = 3.

The plasma I levels were dependent on the dose over a range of 5–40 mg (Table IV); *i.e.*, I did not display nonlinear pharmacokinetics with a therapeutic dosage regimen. This finding suggests that the I elimination routes are not easily saturated and that the drug metabolism enzymes are neither induced nor inhibited by I.

The GLC method reported here provides a sensitive assay for I in the plasma of humans following therapeutic doses. The use of an internal standard compensates for quantitation error during sample manipulation. The ^{14}C-tracer study indicated that the I metabolites present in human plasma are extremely water soluble (unlike the identified urinary metabolites of I[5]) and are not detectable under the electron-capture GLC assay conditions used. The assay is selective for I.

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[5] Dr. S. Vickers, unpublished results.