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GLC Determination of the Polyvalent Saluretic Uricosuric Agent (2-Cyclopentyl-6,7-dichloro-2-methyl-1-oxo-5-indanyloxy)acetic Acid in Biological Fluids

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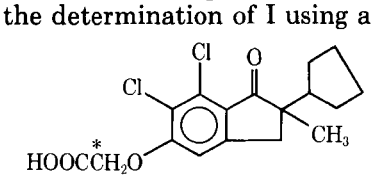
Abstract □ A specific and quantitative GLC method was developed for the determination of (2-cyclopentyl-6,7-dichloro-2-methyl-1-oxo-5-indanyloxy)acetic acid, a novel saluretic uricosuric agent, in biological fluids. The procedure involves the addition of an internal standard to the biological specimens followed by extraction of the acids into benzene at pH 1. The extracted acids, following back-extraction into base and reextraction into methylene chloride at an acidic pH, are converted to the respective methyl esters by reaction with ethereal diazomethane. The sensitivity of the method is such that 2 μg of material can be detected per aliquot of plasma or urine. In the 2.5–50-μg/ml range, recoveries were 98.1 ± 9.6% (plasma, n = 157) and 99.3 ± 6.4% (urine, n = 181). GLC–mass spectrometric techniques established analysis specificity.

Keyphrases □ (2-Cyclopentyl -6,7- dichloro-2-methyl-1-oxo-5-indanyloxy)acetic acid—GLC analysis in biological fluids □ GLC—analysis, (2-cyclopentyl-6,7-dichloro-2-methyl-1-oxo-5-indanyloxy)acetic acid in biological fluids □ Uricosuric agents—(2-cyclopentyl-6,7-dichloro-2-methyl-1-oxo-5-indanyloxy)acetic acid, GLC analysis in biological fluids □ Indanyloxyacetic acid, substituted—GLC analysis in biological fluids

(2-Cyclopentyl-6,7-dichloro -2-methyl-1-oxo-5-indanyloxy)acetic acid (I) is a new nonsulfonamide saluretic agent with uricosuric and antihypertensive activities. The chemical syntheses of this agent and other similar analogs were described recently (1–3). To investigate the physiological disposition of this compound in several species, it was essential that an analytical method for I in biological fluids be developed.

This report describes a specific and sensitive GLC method for the determination of I using a related com-



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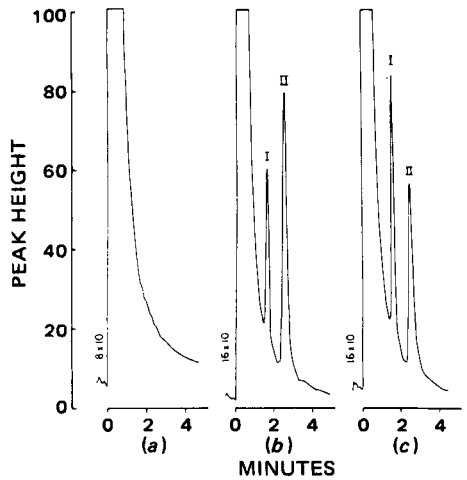


Figure 1—*Gas chromatograms of: control dog plasma* (a), *control dog plasma to which I (5 μg) and II (10 μg) were added* (b), *and dog plasma obtained 30 min after dosing* (c). *All samples were analyzed as described. Aliquots of 5 μl out of a 100-μl final volume were injected on a 1% QF-1 column.*

pound as an internal standard, derivatization with diazomethane, and a flame-ionization detector. Biological specimens from dogs, baboons, and monkeys were used to demonstrate the applicability of the assay following pharmacological I doses. The method also can be used to estimate the levels of the major cyclopentyl ring-hydroxylated metabolites.

EXPERIMENTAL

Reagents and Chemicals—The reagents and chemicals used were I, (2-cyclohexyl-6,7-dichloro-1-oxo-5-indanyloxy)acetic acid (II) as the internal standard, benzene, methylene chloride, ethyl acetate, and freshly prepared 0.5 *M* diazomethane (generated from *N*-nitroso-*N*-methylurea[1]).

Instrumentation—*GLC*—The gas chromatograph[2] was equipped with a flame-ionization detector and a 183-cm × 4-mm (i.d.) glass column packed with either 1% QF-1 or 1% OV-210 on 80–100-mesh Chromosorb W-AW. However, better results with respect to metabolite peaks were obtained when the derivatized extracts were analyzed on a 122-cm × 4-mm (i.d.) glass column packed with 3% OV-210[3]. The column temperature was maintained isothermally between 240 and 250°, depending on the column employed. The detector and injection port temperatures were about 30° higher than the column temperature. Helium was used as the carrier gas with flow rates between 40 and 52 ml/min, depending on the column used.

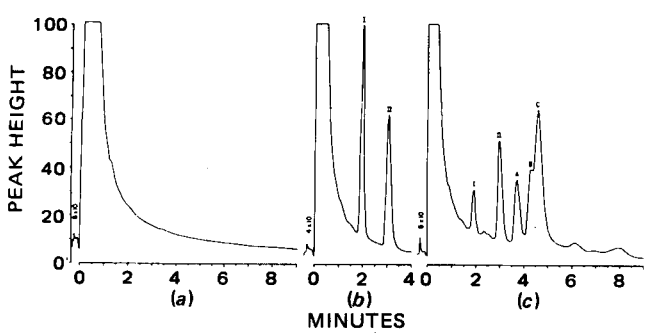


Figure 2—*Gas chromatograms of: control baboon urine* (a), *control baboon urine to which I (10 μg) and II (10 μg) were added* (b), *and baboon urine (0–24 hr)* (c). *Aliquots of 5 μl were analyzed on a 3% OV-210 column.*

[1] K & K Laboratories, Plainview, N.Y.
[2] Hewlett-Packard model 5750.
[3] OV-210 on 80–100-mesh Chromosorb W-HP, Supelco, Inc., and OV-210 on 100–120-mesh Gas Chrom Q, Applied Science Laboratories.

Table I—Recovery of I from Plasma and Urine Using GLC Method

Amount of I Added, μg		Amount Recovered[a] Plasma n	Plasma Mean ± SD	Urine n	Urine Mean ± SD
50.0	A	22	50.49 ± 2.00	19	50.48 ± 2.76
	B		100.97 ± 4.03[b]		100.99 ± 5.52
40.0	A	22	40.30 ± 2.20	28	40.62 ± 1.45
	B		100.71 ± 5.51		101.56 ± 3.63
30.0	A	20	30.61 ± 1.60	18	30.08 ± 0.96
	B		102.02 ± 5.36		100.28 ± 3.22
20.0	A	25	20.26 ± 1.26	30	20.41 ± 1.07
	B		101.29 ± 6.28		102.02 ± 5.29
10.0	A	21	9.90 ± 0.78	29	9.78 ± 0.47
	B		99.00 ± 7.81		97.80 ± 4.66
5.0	A	25	4.62 ± 0.55	31	4.80 ± 0.34
	B		93.23 ± 10.58		95.80 ± 6.85
2.5	A	22	2.28 ± 0.39	26	2.45 ± 0.25
	B		90.74 ± 15.44		97.71 ± 9.97
2.5–50	B	157	98.06 ± 9.60	181	99.31 ± 6.39

[a] Values in A rows represent micrograms recovered; values in B rows represent percent recovery.

GLC–Mass Spectrometry—All mass spectra were obtained on a mass spectrometer[4] with a GLC inlet system. A 122-cm × 3-mm (i.d.) glass column packed with 3% OV-210 (80–100-mesh Chromosorb W-HP) was used. The gas chromatograph was operated isothermally at 240° with a helium flow rate of 30 ml/min. The mass spectrometer ionizing and accelerating potentials were 20 ev and 3.5 kv, respectively. The source, separator, and injection port temperatures were 270, 250 and 245°, respectively.

Measurement of I in Biological Samples—The concentration of I in biological specimens was determined as follows. To an appropriate aliquot (usually 1.0 ml) of plasma or urine in a 50-ml glass-stoppered centrifuge tube were added 10.0 μg of II in 1 ml of pH 8 phosphate buffer, 1 ml of 2 *N* HCl, and 25 ml of benzene. The tube was shaken for 10 min and centrifuged, and at least 20 ml of the organic phase was transferred to a similar tube containing 2 ml of 0.1 *N* NaOH. After the tube was shaken for 10 min, it was centrifuged and the organic phase was removed by aspiration.

The pH of the aqueous phase was adjusted to 1 by the addition of 0.2 ml of 2 *N* HCl, and the free acids were extracted into 5 ml of methylene chloride (vortex, 1 min). After centrifugation, the methylene chloride phase was transferred to a 13-ml centrifuge tube and then reacted with 100 μl of ethereal diazomethane. The content of the tube was evaporated to dryness under nitrogen in a warm water bath (~50°). The residue was subsequently dissolved in 50 μl of ethyl acetate, and appropriate aliquots, usually 5 μl, were injected into the gas chromatograph. The retention times of I and II as the methyl ester derivatives on a 3% OV-210 column were 2.0 and 3.0 min, respectively.

Standard plasma and urine curves were constructed by plotting the peak height ratios (I to II) *versus* weight ratios (I to II). A linear relationship was obtained in the 2.5–50-μg/sample range. The peak height ratio obtained from an unknown sample was then used to determine the amount of I initially present in the sample. The standard recovery sam-

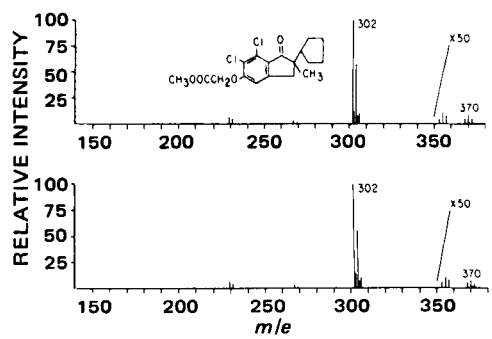


Figure 3—*Comparison of the mass spectrum of I (top) carried through the GLC method with the mass spectrum of material (bottom) obtained from monkey urine. The mass spectra were obtained as the material emerged from the GLC column.*

[4] LKB-9000S.

Table II—Comparison of the Plasma Radioactivity with Plasma I Levels following Administration of ^{14}C-I to Monkeys at 5 mg/kg

	Plasma I Level, μg/ml							
	Monkey 1, Intravenous		Monkey 2, Intravenous		Monkey 3, Oral		Monkey 4, Oral	
Hours	A[a]	B[b]	A	B	A	B	A	B
0.12	77.7	82.3	60.1	59.1	—	—	—	—
0.25	68.9	68.9	49.5	51.1	16.4	16.4	12.1	11.4
0.5	59.7	59.9	40.0	42.3	27.9	30.8	22.5	20.5
1.0	47.3	51.3	31.0	32.3	35.4	37.4	31.8	31.1
1.5	—	—	—	—	37.8	41.9	31.6	30.9
2	38.8	38.9	23.8	23.1	38.0	41.5	31.2	31.0
4	26.9	26.3	15.9	13.6	29.6	27.8	28.1	27.4
6	19.7	16.9	13.0	11.1	23.7	21.1	23.5	21.3
24	12.8	5.4	9.1	3.4	12.7	4.6	18.4	7.6
48	3.0	1.8	1.9	1.0	3.0	2.0	4.0	2.6
72	1.7	1.4	1.2	0.7	1.9	—	2.3	2.1

[a] Determined as I equivalents using radioactivity data. [b] Determined as unchanged I using GLC method.

ples were run concurrently with the unknown specimens as previously described.

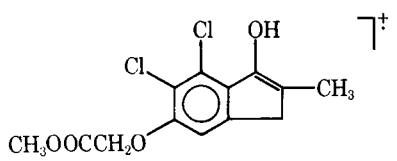
Biological Studies—Four beagle dogs (10.5–11.6 kg), four Rhesus monkeys (3.7–4.7 kg), and four anubis baboons (6.2–7.5 kg) received 5 mg of I/kg po or iv. In each instance, the administered drug was ^{14}C-radiolabeled in the methylene carbon (asterisk in Structure I). The details of the radiochemical synthesis and metabolism studies will be reported elsewhere.

The animals were fasted overnight prior to drug administration. Blood specimens were collected in heparinized tubes, plasma was separated by centrifugation, and aliquots were removed for analysis. Urine specimens were immediately frozen upon collection and kept frozen until analyzed. The samples were analyzed for I using the described procedure.

RESULTS AND DISCUSSION

The developed method is similar to that described previously for a related indanyloxyacetic acid compound (4). GLC analysis of I (as the methyl ester) provided a quantitative analysis of intact drug in urine and plasma. Figure 1 shows gas chromatograms of the following samples using flame-ionization detection: (*a*) control dog plasma, (*b*) I and II added to control dog plasma, and (*c*) dog plasma obtained 30 min after dosing. The retention times of I and II were 1.6 and 2.6 min, respectively. Gas chromatograms of control samples (dog, monkey, or baboon) did not exhibit any extraneous peaks with retention times similar to either I or II as the methyl esters. A typical GLC tracing from a control sample is illustrated in Fig. 1*a*.

A linear relationship was obtained when peak height (or peak area) was plotted *versus* the amount of I injected. Linear curves also were obtained following the analysis of various amounts of I (2.5–50.0 μg) and II (10.0 μg) added to control dog, monkey, or baboon plasma and urine samples (Table I). These data were obtained from analyses performed on the 1% QF-1, 1% OV-210, and 3% OV-210 GLC columns. All analyses were performed over 1 year. As indicated, the mean recoveries of I added to control plasma varied from 90.7 to 102% with a mean recovery of 98.1 ± 9.6% (n = 157) over the entire concentration range. Mean recoveries of I added to control urine in amounts of 2.5–50.0 μg ranged from 95.8 to 102.0% with a mean value of 99.3 ± 6.4% (n = 181). As is evident, the GLC method for determining the levels of I in biological specimens is sensitive and exhibits a high degree of accuracy and precision.



III

Typical levels of I in monkey plasma following a 5-mg/kg dose are presented in Table II. Total radioactivity levels, calculated as I equivalents, are also presented for comparative purposes. As indicated, peak plasma levels of I and radioactivity occurred about 2 hr after oral administration. Furthermore, essentially all radioactivity in the plasma (0–6 hr) following either intravenous or oral administration was intact I. At 48 hr, about half of the plasma radioactivity was attributed to metabolites. Extensive metabolism was observed in the baboon, as illustrated by the results obtained from the GLC analysis of 0–24-hr baboon urine (Fig. 2). The large peaks at 3.5–5 min are attributed to the hydroxylated metabolites. GLC–mass spectral studies showed that hydroxylation occurred on the 2-cyclopentyl moiety, giving rise to several stereochemical isomers (5).

Confirmation of method specificity was obtained when several representative biological specimens (after GLC analysis) were analyzed by combined GLC–mass spectrometry using the aforementioned conditions. A comparison of the mass spectrum of authentic I carried through the GLC procedure with material isolated from monkey urine is presented in Fig. 3. The mass spectra, which were obtained as the material emerged from the GLC column, were identical. The relative intensity of the molecular ion (m/e 370) was less than 0.2% of the intensity of the base peak (m/e 302). The peak at m/e 302 resulted from a McLafferty rearrangement mechanism to form ion III.

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