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Note

Biotransformation of epomediol

III. Gas chromatographic analysis of rat plasma, bile, urine and faeces

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Epomediol [Clesidren®, Camillo Corvi; 1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane-6,7-*endo*,*endo*-diol] is a terpenoid drug acting on hepatic ATPase pumps and on cellular hepatic membranes (osmotic, compartmental and concentration effects). The biotransformation of epomediol has been studied in rat urine, bile and faeces[1,2] and in dog and human urine[2]. The mono-β-glucuronide (M3) of the intact drug represented the major metabolite in rat urine and the sole metabolite found in rat bile and faeces[1].

This note describes the development of a selective gas chromatographic (GC) assay for the unchanged drug in rat plasma, bile, urine and faeces, and, consequently, after enzymatic hydrolysis, its β-glucuronide.

EXPERIMENTAL

Reagents and materials

β-Glucuronidase from *Helix pomatia* was obtained from Boehringer (Mannheim, G.F.R.). Epomediol and *trans*-sobrerol (used as internal marker) were in-house reference standards. All other reagents and solvents were of analytical grade.

Extraction procedure

Plasma (1 or 2 ml) was diluted to 3 ml in 0.2 *M* acetate buffer (pH 4.6) containing the internal standard (40 or 4 μg), washed with *n*-hexane (3 ml), saturated with sodium chloride and extracted with chloroform (3 × 5 ml). The organic layer was evaporated to dryness *in vacuo* and, for GC, the residue was dissolved in methylene chloride (0.5 or 0.1 ml).

Urine (0.5 ml) was diluted in 0.8 ml of the acetate buffer containing the internal standard (200, 20 or 2 μg) and with 0.2 ml of 20% (w/v) ascorbic acid solution and handled as described above for plasma. For GC the organic residue was dissolved in methylene chloride (5, 0.5 or 0.2 ml).

Alternatively, urine (0.5 ml) was added to 0.8 ml of the acetate buffer, 0.5 g of sodium sulphate decahydrate and 0.2 ml of β-glucuronidase, incubated at 37°C for 72 h and then 0.25 ml of the acetate buffer containing sobrerol (200 or 20 μg) were added. The extraction and GC stages were as described for non-incubated urine.

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Bile (0.5 ml) was diluted in 1.0 ml of the acetate buffer containing sobrerol (20 μg) and handled as for plasma. The organic residue was rinsed with 0.5 ml of methylene chloride.

Alternatively, bile (0.5 ml) was added to 1.3 ml of the acetate buffer, 0.8 g of sodium sulphate decahydrate and 0.2 ml of β-glucuronidase, incubated at 37°C for 72 h and then 0.25 ml of the acetate buffer containing the internal standard (200 μg) were added. The mixture was then handled as for plasma. The organic residue was dissolved in methylene chloride (5 ml).

Faeces (1 g) were homogenized in water (10 ml) and methanol (15 ml) and centrifuged (2200 *g*, 5 min). The precipitate was re-suspended in methanol, shaken for 5 min and centrifuged again. The methanol was evaporated *in vacuo* and the aqueous residue was diluted to 10 ml with the acetate buffer and centrifuged (solution F). One aliquot of F (2 ml) was added to 0.25 ml of the acetate buffer containing sobrerol (20 or 2.5 μg), washed with *n*-hexane (2 × 4 ml), saturated with sodium chloride and extracted with chloroform (3 × 5 ml). After evaporation of the organic solvent *in vacuo*, the residue was dissolved in methylene chloride (0.5 ml). Another aliquot of F (2 ml) was added to 1 g of sodium sulphate decahydrate and 0.2 ml of β-glucuronidase, incubated at 37°C for 72 h and then 0.25 ml of the acetate buffer containing sobrerol (20 μg) were added. The extraction and GC stages were as described for non-incubated faeces.

Gas chromatography

Two gas chromatographs were used. A Carlo Erba Model 2400 T was equipped with a flame ionization detector (FID) and a Pyrex glass column (2 m × 3 mm I.D.) packed with 3% Carbowax 20M on Gas-Chrom Q (80–100 mesh). For bile and faeces analysis, the oven temperature was 160°C and the injector and detector temperatures were 265°C and 285°C, respectively. For plasma and urine the oven temperature was programmed from 110 to 180°C at 2°C/min with an initial hold of 5 min; the injector/detector temperature was 280°C. Nitrogen was the carrier gas and the flow-rate was 42 ml/min at 160°C. For plasma analysis, a Perkin-Elmer Model Sigma 2 gas chromatograph was equipped with a FID and a 25-m fused-silica capillary column coated with OV-101. The operating conditions were as follows: oven temperature, 110 to 155°C, programmed at 5°C/min (initial hold of 1 min); injector temperature, 200°C; detector temperature, 225°C; carrier gas (helium) precolumn flow-rate, 40 ml/min with a splitting ratio of 52:1. The peak areas were integrated by a Perkin-Elmer Sigma 10 Data Station.

Calibration curves and reproducibility

Calibration curves were constructed by spiking drug-free biological samples with 0.2 *M* acetate buffer solutions containing the drug and its internal standard. The concentration ranges of epomediol (μg/ml or μg/g) were: for plasma, 0.3–25 and 30–200; for urine, 2–20, 20–200, 200–3000 and (incubation) 10–200 and 200–3000; for bile, 10–150 and (incubation) 200–3000; for faeces, 15–75, 100–1000 and (incubation) 100–1000. The ratios of the peak area (height) of epomediol to that of the internal standard were plotted against concentration. Linear equations were obtained upon regression analysis[3].

The reproducibility of the drug assay was evaluated by performing replicate

TABLE I

REPRODUCIBILITY OF ANALYSIS OF EPOMEDIOL IN BIOLOGICAL SAMPLES

Biological sample	*Drug concentration (μg/ml or μg/g)*	*Coefficient of variation (CV; areas, n = 6) (%)*
Plasma	5	5.86*
	10	3.26*
Urine	10	4.62
	100	2.45
Bile	50	4.68
Faeces	75	5.77

* Capillary column.

analyses of the spiked biological samples and calculating the coefficient of variation (Table I).

RESULTS AND DISCUSSION

Gas chromatograms typical of extracted blank and spiked samples of bile and faeces are shown in Figs. 1 and 2, respectively. Good reproducibility of results (Table

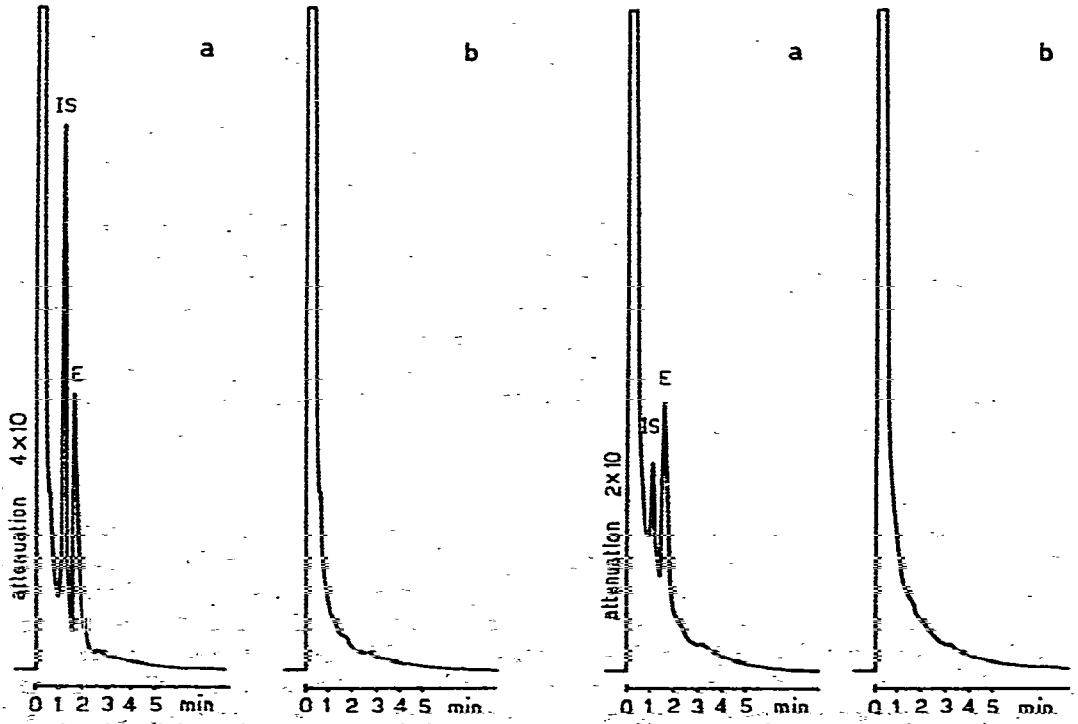

Fig. 1. Gas chromatograms of extracts from bile: a, spiked sample; b, blank sample. Peaks: IS = sobrerol (20 μg/ml); E = epomediol (25 μg/ml). Injection volume: *ca.* 2 μl.

Fig. 2. Gas chromatograms of extracts from faeces: a, spiked sample; b, blank sample. Peaks: IS = sobrerol (12.5 μg/g); E = epomediol (50 μg/g). Injection volume: *ca.* 1 μl.

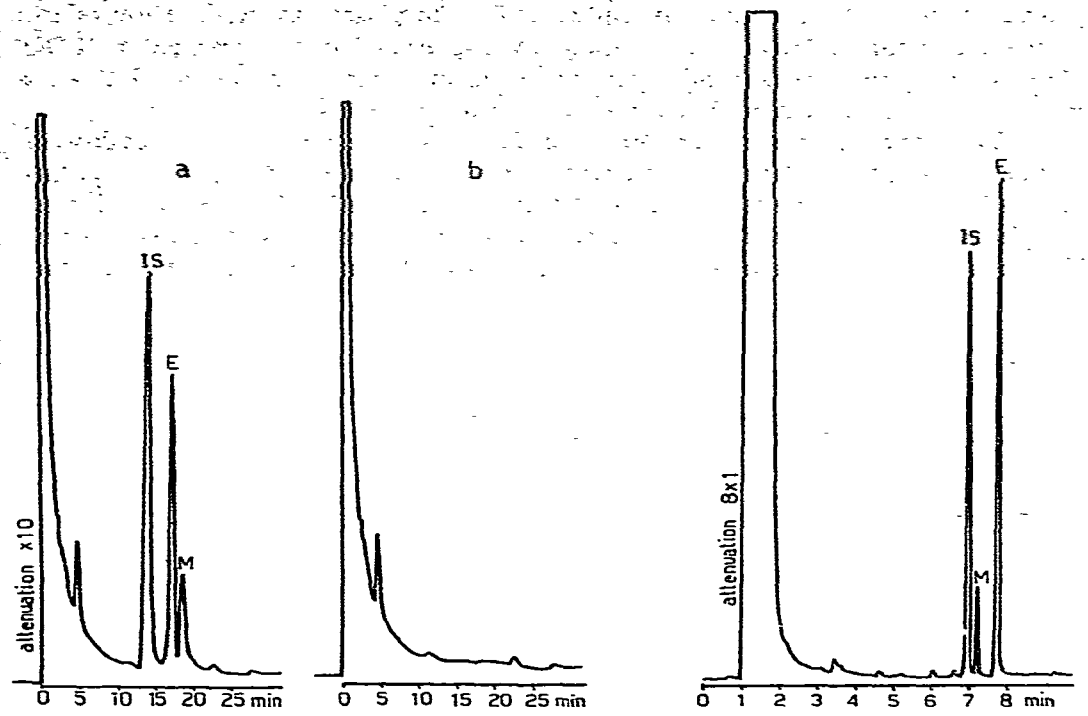

Fig. 3. Gas chromatograms from urine: a, treated rat urine; b, blank sample. Peaks: IS = sobrerol (40 μg/ml); E = epomediol (*ca.* 60 μg/ml); M = metabolite M1. Injection volume: *ca.* 2 μl.

Fig. 4. Gas chromatogram from treated rat plasma (capillary column). Peaks: IS = sobrerol (4 μg/ml); M = metabolite M1; E = epomediol (*ca.* 6 μg/ml). Injection volume: *ca.* 2 μl.

I) and good linearity of the calibration curves were found. Moreover, the incubated samples showed no peaks that could interfere with the peaks of the drug and of its internal standard. The limit of quantitation (LOQ)[4] of epomediol in bile was about 2 μg/ml, whereas in faeces it was about 15 μg/g.

The temperature programming of the packed column was required in urine and plasma analysis in order to separate the drug from one of its metabolites, namely 6-hydroxy-1,3,3-trimethyl-2-oxabicyclo[2.2.2]octan-7-one (M1)[1], which can also be quantified (Fig. 3). Under these conditions the LOQ of epomediol, in urine and

TABLE II

GC ESTIMATION OF EPOMEDIOL AND M3 EXCRETION IN BILE, FAECES AND URINE

No. of female rats	*Biological sample*	*Collection time (h)*	*Unchanged drug (% of administered dose ± S.D.)*	*Unchanged drug + M3 (% of administered dose ± S.D.)*
6	Bile	0–3	0.63 ± 0.12	13.7 ± 1.83
9	Faeces	0–72	1.27 ± 0.32	1.93 ± 0.39
9	Urine	0–72	23.41 ± 4.13	83.85 ± 3.36

plasma, was about 1.5 μg/ml, not sufficient for the plasma assay. The use of the capillary column enabled us to quantify lower plasma levels of epomediol (LOQ about 0.25 μg/ml) with a good separation from interfering compounds (Fig. 4), a good linearity of the calibration curves and a shorter analysis time.

The presence of an enterohepatic circulation of the drug due to hydrolysis and/or reabsorption of its β-glucuronide (from bile) has been previously hypothesized[1]. The method presented here has been applied to the analysis of biological samples of female albino Wistar rats dosed by oral gavage with 100 mg/kg epomediol. The results (Table II) are in agreement with those obtained for enterohepatic recycling[5].

REFERENCES

1 P. Ventura and A. Selva, *Biomed. Mass Spectrom.*, 9 (1982) 18.
2 P. Ventura and R. Pria, *Il Farmaco, Ed. Sci.*, in press.
3 J. S. Hunter, *J. Ass. Offic. Anal. Chem.*, 64 (1981) 574.
4 ACS Committee on Environmental Improvement, *Anal. Chem.*, 52 (1980) 2242.
5 R. J. Parker, P. C. Hirom and P. Millburn, *Xenobiotica*, 10 (1980) 689.