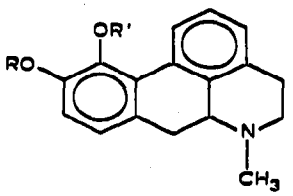
Journal of Chromatography, 77 (1973) 419–421

CHROM. 6536

Note

Gas chromatographic determination of apomorphine O-methyl metabolites in urine

There is renewed interest in the biological activity and metabolism of apomorphine(1).



1. R, R' = H
2. R = CH_3, R' = H
3. R = H, R' = CH_3

In vitro experiments performed in this laboratory[1] and by others[2] have revealed that 1 is a substrate for rat liver catechol O-methyltransferase (COMT). WHITE AND MCKENZIE[2] have suggested that COMT-methylation of 1 leading to apocodeine (2) and/or isoapocodeine (3) may be an important *in vivo* metabolic pathway.

A study of the metabolism of 1 in rats required a method for the determination of 2 and 3 in urine. A gas chromatographic (GC) procedure suitable for this purpose is described in this communication.

Materials and methods

Gas chromatography. A Hewlett-Packard 5750B gas chromatograph equipped with a flame ionization detector was used throughout. The glass column employed (6 ft. × $\frac{1}{8}$ in. I.D.) was packed with 3% OV-17 on 100–120 mesh Gas-Chrom Q and conditioned at 280° for 12 h. Operating conditions were: column and injection port temperature, 260°; detector temperature, 320°; carrier gas (He) flow, 70 ml/min (50 p.s.i.g.); hydrogen flow, 40 ml/min (13 p.s.i.g.); air flow, 440 ml/min (30 p.s.i.g.); range, 10^2; attenuation, 16.

Materials. Compound 2 was prepared according to SMALL *et al.*[3] using *n*-pentane in the final recrystallization step. Preparation of 3 was accomplished by the literature procedure[1] with final purification effected by preparative TLC on 500-μ alumina layers developed in chloroform–benzene (9:1). All reagents and solvents were reagent grade.

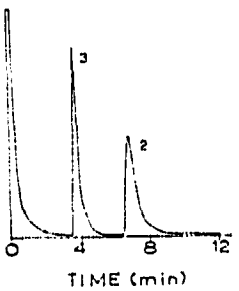
Standard curves. Solutions containing 0.5 to 2 mg/ml of 2 or 3 in ethyl acetate or 1% isoamyl alcohol in *n*-heptane (1% IAH) were prepared. Curves of peak height *vs.* amount chromatographed were constructed following injection of 5-μl aliquots of standard solutions.

Recovery vs. pH. 0.5 to 1.0 ml of 0.1 *N* hydrochloric acid solutions of **2** or **3** (0.5 to 1.0 mg/ml) were added to 15 ml of 0.1 *M* citrate (final pH 6.6), 0.1 *M* TES (final pH 7.3) and 0.2 *M* TRIS (final pH 8.6) buffers. The aqueous mixtures were extracted with three 15-ml portions of 1% IAH. The combined organic layers were reduced to dryness *in vacuo* and the residues dissolved in 0.5 to 1.0 ml of 1% IAH for GC analysis.

Urine analyses. 24-h rat urines (mean volume, 10 ml) were spiked with **2** or **3** as indicated above. The final mixtures were adjusted to pH 8.6 with 0.1 *N* sodium hydroxide and extracted with four equal volumes of 1% IAH. The extracts were taken to dryness *in vacuo* and the residues were treated with 0.5 to 1.0 ml of 1% IAH. 5-μl quantities of the resulting solutions were gas chromatographed.

Results and discussion

Columns prepared with OV-17 or similar internal phases have been utilized in gas chromatographing a number of aporphine alkaloids[4-6]. Among the latter are vicinally substituted hydroxy-methoxy analogs of **2** and **3** (refs. 4, 5). Thus, it was not surprising that **2** and **3** could be successfully developed on an OV-17 column. It was unexpected, however, that these isomeric compounds readily separate as shown in Fig. 1.



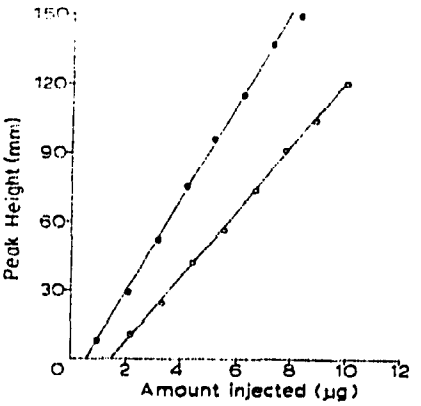


Fig. 1. Separation of **2** and **3** on a 6 ft. × $^1/_8$ in. (glass) 3% OV-17 on Gas-Chrom Q column.

Fig. 2. Standard curves for **2** (○—○) and **3** (●—●).

Examples of standard curves for **2** and **3** are depicted in Fig. 2. Correlation coefficients for these curves were uniformly greater than 0.996. With both compounds, standard curves did not pass through the origin. This is presumably due to loss on the column or irreversible adsorption of a constant amount of compound during GC development. This effect could not be reversed either by primer injections or prior treatment of the column with silylating reagents.

It had been shown earlier that a combination of 1% isoamyl alcohol in *n*-heptane (1% IAH) is an efficient mixture for extraction of aporphines similar to **2** and **3** (ref. 5). Recovery of **2** and **3** using 1% IAH was studied as a function of pH. As shown in Fig. 3, essentially quantitative recoveries are achieved at pH 8.6. Gas

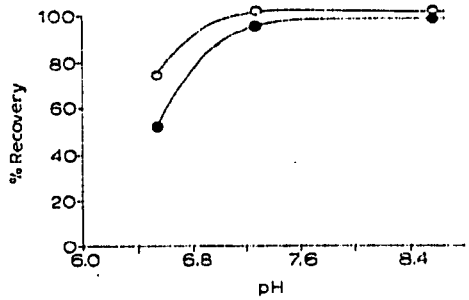


Fig. 3. Extraction of **2** (○—○) and **3** (●—●) from buffers with 1% isoamyl alcohol in *n*-heptane.

TABLE I

ACCURACY AND PRECISION OF GC METHOD

Compound	*Amount added*[a] *(μg)*	*% recovery*		*RSD*
		Range[b]	*Mean*	
2	500–1000	85.8–93.8	89.7	2.8
3	500–1000	80.0–89.2	85.3	3.5

[a] Added to 24-h rat urines (mean vol. ~10 ml).
[b] 12–13 determinations.

chromatogramas of extracts of blank urines revealed no interferences following extraction at this pH.

Urine samples spiked with **2** and **3** were analyzed to determine the accuracy and precision of the GC method. Results of these assays are indicated in Table I. The levels of O-methyl metabolites analyzed would allow measurement of 5 to 50% conversion of **1** to **2** and/or **3** assuming dosages of up to 40 mg/kg and nearly complete excretion of these metabolites in urine. The method permits detection of lower percent conversions but recoveries and precision are poorer.

This work was supported in part by Grant NS-04349, National Institute of Neurological Diseases and Stroke.

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Received November 27th, 1972