Journal of Chromatography, 87 (1973) 294–297

CHROM. 6997

Note

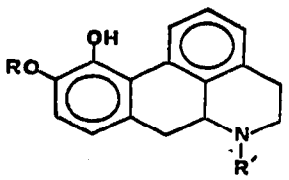
Analysis of apomorphine and norapomorphine in urine by thin-layer chromatographic fluorescence quenching

ROBERT V. SMITH, MELVIN R. COOK and ANDREW W. STOCKLINSKI

Division of Medicinal Chemistry and Natural Products, College of Pharmacy, The University of Iowa, Iowa City, Iowa 52242 (U.S.A.)

(First received May 7th, 1973; revised manuscript received August 14th, 1973)

The metabolic fate of apocodeine (I) is being investigated as part of a systematic study of the mammalian metabolism of aporphine alkaloids[1–3].



(I) R = CH_3, R' = CH_3
(II) R = H, R' = CH_3
(III) R = H, R' = H

Recent experiments revealed that apomorphine (II) and norapomorphine (III) are urinary metabolites of I in rats[4]. Quantitative evaluation of these results was required. A TLC fluorescence quenching procedure has been devised for this purpose and is described in this communication.

MATERIALS AND METHODS

TLC fluorescence quenching

A Nester-Faust Uniscan 900 TLC scanner connected to a Varian 1-mV recorder was used throughout. Plates developed (as indicated below) with compound II and/or III were allowed to stand in the air for 24 h. These were scanned in the fluorescence mode under the following conditions: $\lambda_{exc.} = 254$ nm; span 500 to 1000; neutral density filters, 0.5 to 1.0; gain, $\times 100$; slit width, 1 mm; length, 0.65 cm; scanning rate, 3.2 cm/min; recorder speed, 1.0 cm/min.

Materials

Apomorphine hydrochloride was purchased from Penick Chemical Co. Compound III was prepared by the procedure used by Koch *et al.*[5]. All solvents and reagents were reagent grade.

Standard solutions

Compound II was recovered from its hydrochloride by treating an aqueous solution containing an equivalent of 1 to 5 mg of compound II with 0.1 *M* citrate

buffer (pH 7.0) and extracting with three 5-ml portions of ethyl acetate. The total ethyl acetate extract was reduced to dryness *in vacuo* and the residue dissolved in 1 to 2 ml of acetone. Acetone solutions of compound III (1 to 5 mg/ml) served as standards.

Thin-layer chromatography

Silica gel GF_{254} plates, 250 μm thick (Analtech), were scored into 1-cm channels and spotted with 1 to 4 μl of standard and sample solutions (acetone) containing 0.5 to 6 μg of compound II or III. The spotted plates were developed 10 cm in acetone–methanol (1:1).

Standard curves

Using a triangulation method ($A = {}^1/_2\ b \cdot h$, where b = base, h = height), area measurements were derived from scans of plates developed with three quantities of compound II in the range of 0.5 to 5.0 μg and compound III in the range of 1.0 to 6.0 μg. Plots of A *vs.* amount chromatographed were prepared and utilized in determining levels of compounds II and III in sample solutions which were developed on the same plate as the standards.

Recovery experiments

1.8-mg quantities of compound II or III, dissolved in a minimum of 0.1 M citrate solution (pH 3.0), were added to 10 ml of 0.1 M citrate buffer (pH 7.0) or one-day Sprague Dawley rat urines (mean volume, *ca.* 10 ml). The final mixtures were adjusted to pH 7.0 with 0.1 N hydrochloric acid or sodium hydroxide and extracted with three 10-ml portions of ethyl acetate. The separation funnels employed were rinsed with 10 ml of ethyl acetate; the combined extracts were reduced to dryness *in vacuo* and the residues dissolved in 1 to 2 ml of acetone for TLC analysis.

RESULTS AND DISCUSSION

TLC systems were required for identification of compounds II and III as metabolites of compound I in rats. One of these, acetone–methanol (1:1) (250-μm silica gel GF_{254} plates) provided good resolution of compounds II and III (II, R_F 0.68; III, R_F 0.43) and development of these compounds as well consolidated spots. The previous success in measuring aporphines by TLC fluorescence quenching[1,2] prompted investigation of the possible usefulness of this technique for the analysis

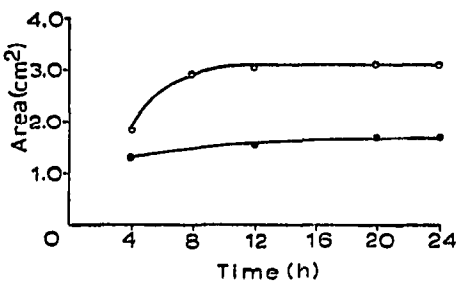


Fig. 1. Fluorescence quenching as a function of time. ●—●, compound II (1.9 μg); ○—○, compound III (4.6 μg).

of compounds II and III. Since the degree of fluorescence quenching will be a function of the chromophoric composition of the developed spots, attention was paid to the fact that compounds II and III slowly air-oxidize to form blue-green products following development. Presumably these pigments resemble the *o*-quinone-type compounds reported as resulting from the autoxidation of compound II (ref. 6). Fortunately, the degradation process is self-limiting with respect to its effect on fluorescence quenching (see Fig. 1). Thus, by allowing developed plates to stand in the air for 24 h prior to scanning, this variable is eliminated.

The response to compound II was linear over the range of 0.5 to 5.0 μg while linearity from 1.0 to 6.0 μg was achieved with compound III. Correlation coefficients consistenly averaged 0.991 for compound II and 0.984 for compound III. Earlier experiments had shown that compound II could be quantitatively recovered from pH 7 buffers by extraction with ethyl acetate[7]. This result was confirmed in the present investigation while recovery of compound III from pH 7 buffer (but not from urine!) (see Materials and methods) was 88% (RSD, ±1%; four trials). Urine samples spiked with compounds II and III were analyzed to determine the accuracy and precision of the TLC fluorescence quenching method. Results of these assays are indicated in Table I. Recovery and reproducibility for compound II are good;

TABLE I

ACCURACY AND PRECISION OF THE REVERSE TLC FLUORIMETRY METHOD

Compound	*Amount added** (*mg*)	*% Recovery*		
		*Range***	*Mean*	*RSD*
II	1.85	84.4–93.3	88.5	3.5
III	1.87	36.8–43.3	41.4	2.1

* Added to 24-h rat urines (mean vol., *ca.* 10 ml).
** Six to seven determinations.

recovery of compound III from urine is poorer but acceptable since precision comparable to compound II was achieved. Attempts were made to improve the recovery of compound III from urine by utilizing mixtures of ethyl acetate and isoamyl alcohol (1 to 10%) as extraction media. These efforts were thwarted by co-extraction of native urinary components which seriously interfered in the measurement step. The levels of compounds II and III analyzed would allow measurement of 5 to 50% conversion of compound I to II and/or III assuming dosages of up to 80 mg/kg and nearly complete excretion of these metabolites in urine.

ACKNOWLEDGEMENTS

This work was supported in part by Grant NS-04349, National Institute of Neurological Diseases and Stroke. The authors are grateful to Professor Joseph G. Cannon for a generous sample of norapomorphine.

REFERENCES

1 R. V. Smith and S. P. Sood, *J. Pharm. Sci.*, 60 (1970) 1654.
2 J. G. Cannon, R. V. Smith, A. Modiri, S. P. Sood, R. J. Borgman, M. A. Aleem and J. P. Long, *J. Med. Chem.*, 15 (1972) 273.
3 R. V. Smith and A. W. Stocklinski, *J. Chromatogr.*, 77 (1973) 419.
4 R. V. Smith and M. R. Cook, *J. Pharm. Sci.*, (1973) in press.
5 M. V. Koch, J. G. Cannon and A. M. Burkman, *J. Med. Chem.*, 11 (1968) 977.
6 H. H. A. Linde and M. S. Ragab, *Helv. Chim. Acta*, 51 (1968) 683.
7 R. V. Smith and S. P. Sood, *Anal. Lett.*, 5 (1972) 273.