Thin-layer chromatographic separation and detection of methysergide and methergine

While the separation of certain ergot alkaloids by both paper[1,2] and thin-layer chromatographic (TLC) techniques[3–6] have been described, these reports generally have dealt with naturally occurring alkaloids. A rapid and sensitive method for the separation and identification of two clinically important ergot derivatives, 1-methyl-D-lysergic acid (+) butanolamide (methysergide) and D-lysergic acid (+) butanolamide (methergine) was needed. Therefore we have defined the conditions for the TLC separation and identification of methysergide and methergine both in pure chemical form and after extraction from biologic tissues and fluids. D-Lysergic acid (+) 2-propanolamide (ergonovine), previously studied with TLC[3], was included as a reference compound.

Materials and methods

Standard TLC plates (20 × 20 cm) were coated with Silica Gel (E. Merck A.G., Darmstadt) or aluminum oxide (Camag) to a thickness of 250 μ using Desaga-Brinkmann equipment. Plates were dried at room temperature, activated in an oven at 100° for 30 min and allowed to cool before using. Preformed Eastman thin-layer chromagram sheets Type K301R2 (silica gel without fluorescent indicator) were also used. Several solvent systems were tested on each of these plates (Table I).

A 1–5 μl aliquot was applied at a point 2 cm from the bottom of the plate and run for a standard length of 15 cm in a saturation tank in which the walls were lined with solvent soaked filter paper. After completion of run, plates were air dried and compounds localized with a U.V. lamp (300 mμ) or by spraying the plate with VAN URK reagent[7].

In other experiments, methysergide and methergine were extracted from sodium chloride saturated biologic fluid into *n*-heptane at an alkaline pH according to the method of DOEPFNER[8]. Solvent was then evaporated at 45° and pressure of 0.01 mm Hg. The dry residue was redissolved in one of the solvents tested and spotted on TLC plates.

After methysergide and methergine spots are localized with U.V. light, they may be eluted and quantitated spectrophotofluorometrically according to the method of DOEPFNER[8]. The biologic activity of the methysergide spot was confirmed by the oestrus rat uterus technique[9,10]; methysergide blocks uterine contraction induced by serotonin more effectively than methergine.

Results

Data obtained using pure compounds on silica gel, preformed Eastman plates and Aluminum Oxide G are summarized in Table I.

Chloroform–methanol (4:1) (solvent system X), on Eastman preformed silica plates yielded excellent migration and uniform round spots which allow easy discrimination between methysergide and methergine. In this system, ergonovine has an hR_F* value of 34 ± 1, which compares well with the value found for ergonovine by AGURELL[3] (27 ± 1) in a similar system. Standard Silica Gel G plates had similar round

* $hR_F = R_F \times 100$.

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TABLE I

hR_F VALUES OF SELECTED ERGOT DERIVATIVES*

Key to solvent systems: I = chloroform–acetone (5:4); II = chloroform–benzene–glacial acetic acid (34:45:10); III = chloroform–benzene–heptane (3:6:5); IV = chloroform–cyclohexane–diethylamine (4:5:1); V = chloroform–cyclohexane–diethylamine (70:30:0.5); VI = chloroform–diethylamine (9:1); VII = chloroform–ethanol (4:1); VIII = chloroform–ethanol (9:1); IX = chloroform–ethanol (24:1); X = chloroform–methanol (4:1); XI = chloroform–methanol (9:1).

Solvent system	*Eastman Chromagram sheet*			*Silica Gel G*			*Aluminum Oxide G*		
	Methysergide $X \pm S.D.$	*Methergine* $X \pm S.D.$	*Ergonovine* $X \pm S.D.$	*Methysergide* $X \pm S.D.$	*Methergine* $X \pm S.D.$	*Ergonovine* $X \pm S.D.$	*Methysergide* $X \pm S.D.$	*Methergine* $X \pm S.D.$	*Ergonovine* $X \pm S.D.$
I	5 ± 0.2	3 ± 0.4	0	2 ± 0.4	2 ± 0.4	0	5 ± 1	3 ± 0.1	3 ± 1
II	0	0	0	0	0	0	68 ± 4	46 ± 2	40 ± 1
III	0	0	0	0	0	0	0	0	0
IV	6 ± 0.4	0	0	0	0	0	5 ± 1	0	0
V	0	0	0	0	0	0	3 ± 0.5	0	0
VI	33 ± 1	14 ± 1	12 ± 1	10 ± 0.4	4 ± 0.4	4 ± 0.4	16 ± 1	4 ± 2	4 ± 2
VII	49 ± 3	34 ± 1	22 ± 1	27 ± 8	17 ± 5	8 ± 5	90 ± 1	80 ± 2	76 ± 1
VIII	29 ± 2	15 ± 2	6 ± 1	12 ± 2	6 ± 1	0	12 ± 2	6 ± 1	0
IX	8 ± 0.4	3 ± 0	0	5 ± 0.1	2 ± 0	0	39 ± 0.5	20 ± 1	18 ± 1
X	63 ± 6	52 ± 6	34 ± 4	33 ± 0.2	23 ± 2	12 ± 1	89 ± 1	84 ± 1	83 ± 0.8
XI	59 ± 14	32 ± 10	16 ± 6	22 ± 5	12 ± 4	1 ± 1	88 ± 3	80 ± 2	78 ± 2

* The number of determinations with each system varied between 3 and 11.

spots, but the migration rate was slower. Chloroform–ethanol (4:1) (VII) yielded good hR_F values, but spots were moderately ovoid in shape.

Chloroform–benzene–glacial acetic acid (45:45:10) (II) provided separation on Aluminum Oxide G plates but resulted in marked tailing. Chloroform–ethanol (4:1) (VII) provided slightly ovoid spots and reasonably good separation on Aluminum Oxide G plates.

The chloroform–methanol (4:1) system with either Eastman preformed silica plates or the usual silica gel plates was also found effective in separating methysergide from methergine in extracts of biologic tissues and fluids. Methysergide, 0.1 μg, was the minimum amount detectable. Absorption and emission spectra of eluted spots[1] of methysergide and methergine extracted from biologic fluids were identical to known reference spots. Methysergide extracted from biologic tissue, separated by TLC, eluted, and quantitated spectrophotofluorometrically was capable of blocking the effect of serotonin on the rat uterus *in vitro*. For example, 10 mμg of methysergide blocked the effect of 80 mμg of serotonin. Preliminary studies indicate that methergine may be one of the metabolites of methysergide in both man and the rabbit.

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Department of Medicine (Division of Clinical Pharmacology), Johns Hopkins University School of Medicine, Baltimore, Md. (U.S.A.)

JOSEPH R. BIANCHINE
ADELE NIEC
PLACIDO V. J. MACARAEG, Jr.

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