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Separation of thebaine, some of its metabolites and congeners on glass fibre sheets

Thebaine, a close congener of morphine, is primarily a central stimulant and in contrast to morphine does not produce physical dependence[1,2]. During the course of our investigation on the pharmacokinetics and metabolism of [^{3}H]thebaine in the rat, need arose for the development of suitable methods for the separation and identification of thebaine from some of its known metabolites and congeners (Fig. 1). Microbiological metabolism of thebaine has been shown[3,4] to produce 14-hydroxy codeine, 14-hydroxy codeinone and 14-hydroxy codeinone N-oxide as major metabolites. Extensive studies[5–9] on biogenesis of opium alkaloids have shown that the most probable route for the biosynthetic conversion of thebaine to morphine involves initial demethylation of thebaine to neopinone, rearrangement to codeinone, reduction to codeine followed by demethylation to morphine. *In vivo* O- and N-demethylation of codeine and morphine to norcompounds and glucuronide conjugation have also been demonstrated[10–13] in experimental animals. The voluminous literature on separation of opium alkaloids and other drugs of abuse has been covered in recent reviews[14,15].

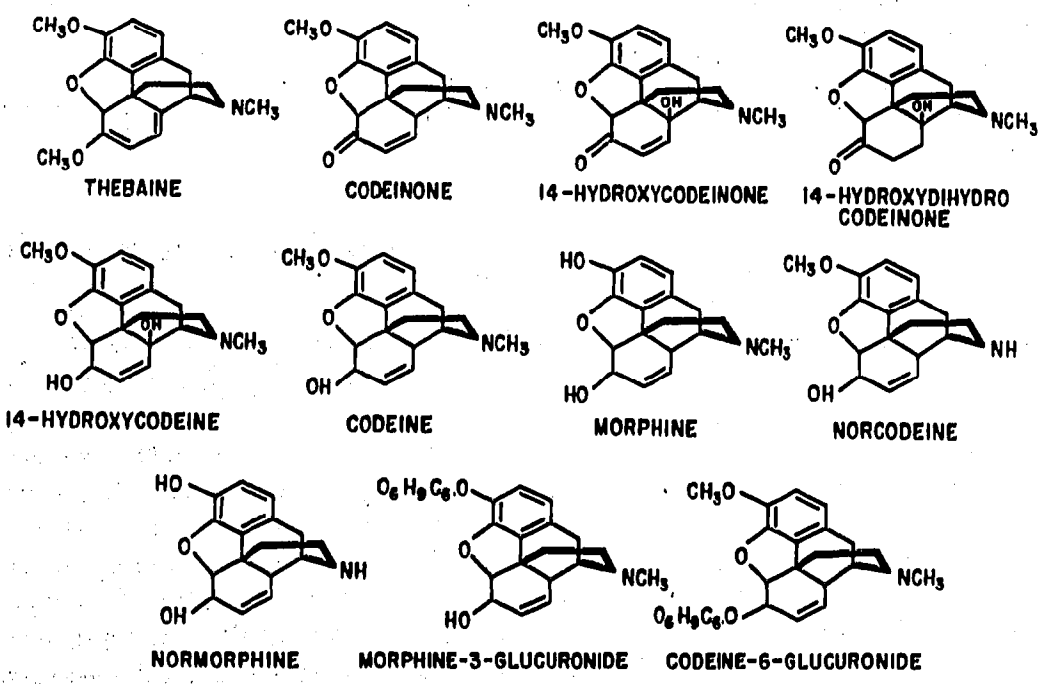

Fig. 1. Structure of thebaine and related compounds.

The present study, however, required specific methods that could be adapted for the separation and assay of metabolites of [^{3}H]thebaine in rat brain and urine. This communication describes the application of instant thin-layer chromatography (ITLC) for the separation of these compounds (Fig. 1), which possesses the advantages of speed, convenience and ease of radioscanning by direct transfer of sectioned planimetric strips of glass fibre silica gel-impregnated sheets to counting vials, addition of eluant and toluene–phosphor and assay of radioactivity in a liquid scintillation counter.

Materials and methods

14-Hydroxycodeine[16], codeinone[17], 14-hydroxycodeinone[18], morphine-3-glucuronide[12] were prepared by methods previously described. Codeine-6-glucuronide was a kind gift from Dr. S. Y. YEH, Addiction Research Centre, Lexington, Ky. Other samples were obtained commercially. Gelman instant TLC media Silica Gel ITLC (obtained from Gelman Instrument Company, Ann Arbor, Mich.), was used for TLC with application of standard techniques. The compounds were localized after development by spraying with iodoplatinate spray reagent.

TABLE I

CHROMATOGRAPHIC MOBILITIES ON GELMAN ITLC (SILICA GEL) OF THEBAINE, SOME OF ITS KNOWN METABOLITES AND CONGENERS WITH DIFFERENT SOLVENT SYSTEMS

S_1 = benzene–ethyl acetate–methanol–conc. ammonia (80:20:6.5:0.1); S_2 = *n*-hexane–ethyl acetate–conc. ammonia (60:40:0.1); S_3 = *n*-butanol–*n*-butyl ether–conc. ammonia (25:70:2); S_4 = *n*-butanol–acetic acid–water (35:3:10).

Compound	$R_F \times 100$			
	S_1	S_2	S_3	S_4
Thebaine	86	33	87	92
14-Hydroxycodeinone	98	79	90	89
14-Hydroxydihydrocodeinone	100	75	86	84
14-Hydroxycodeine	87	57	83	85
Codeinone	76	18	79	87
Codeine	52	15	67	85
Morphine	27	6	40	85
Norcodeine	17	2	32	95
Normorphine	5	0	11	94
Codeine-6-glucuronide	0	0	0	47
Morphine-3-glucuronide	0	0	0	29

Results

The results of TLC mobilities of different compounds are given in Table I. A good separation of thebaine from other compounds with the exception of 14-hydroxycodeine was obtained in solvent system S_1. The compounds with higher mobilities *e.g.* thebaine, codeinone, 14-hydroxycodeinone and 14-hydroxycodeine separated very well in system S_2, those with lower mobilities as normorphine, norcodeine, morphine, codeine and codeinone in system S_3. The polar glucuronides which remained at the origin in systems S_1, S_2, S_3 could be separated from other compounds in system S_4. Thus using combinations of systems S_1, S_2, S_3, S_4, thebaine, some of its known metabolites and congeners could be adequately separated from each other in a very short time.

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