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Note

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

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During the course of our study on the physiological disposition and metabolism of tritium ring labeled cocaine in experimental animals, need arose for the development of sensitive methods for the separation of labeled cocaine, some of its metabolites and congeners (Fig. 1). Paper¹⁻³, thin-layer^{4,5} and gas chromatographic⁶ methods have earlier been reported for the separation of cocaine from its metabolites, benzoylecgonine and ecgonine⁷⁻¹¹. Pulmonary excretion of labeled carbon dioxide in experimental animals following administration of cocaine-N-¹⁴CH₃ has provided suggestive evidence¹² for the formation of norecgonine.

This communication describes the application of glass fibre silica gel impregnated sheets (ITLC) to the separation of cocaine, some of its metabolites and congeners. This technique possesses the advantages of speed, convenience and ease

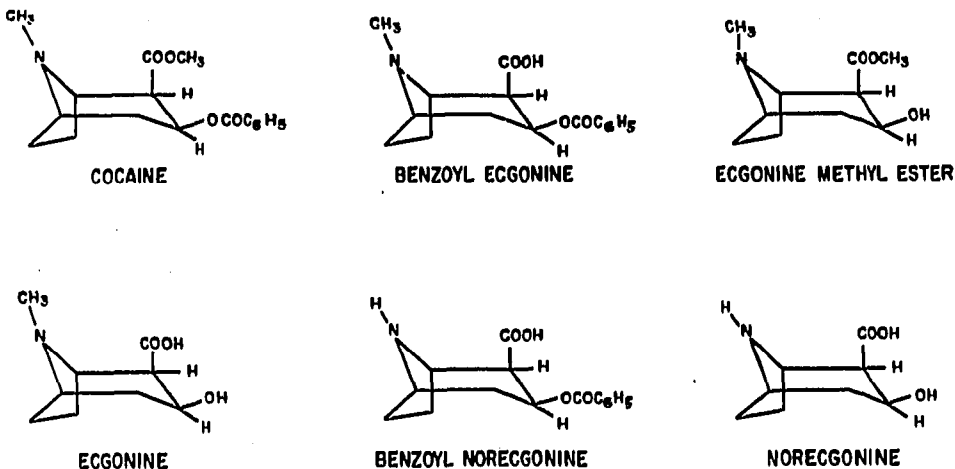


Fig. 1. Cocaine and related compounds.

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of radioscanning by direct transfer of sectioned planimetric strips of silica gel-glass fibre sheets to counting vials, addition of eluant and toluene-phosphor and assay of radioactivity in a liquid scintillation spectrometer.

MATERIALS AND METHODS

Cocaine was obtained commercially (Merck & Co., Inc., N.J.), ecgonine¹³, ecgonine methyl ester¹⁴ were prepared by methods previously described. Benzoyl ecgonine and benzoyl norecgonine were a kind gift from Dr. E. L. May, N.I.H., Bethesda, Md., and norecgonine was prepared from benzoyl norecgonine by modified acid hydrolysis procedure¹³. Gelman instant thin-layer chromatography 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 (a) acidified iodoplatinate reagent and (b) Ludy Tenger's reagent¹⁵ prepared fresh by dissolving 500 mg of bismuth carbonate in 1.5 ml of conc. HCl, adding 3 g of KI and making the volume to 50 ml with distilled water.

RESULTS

The results obtained with the mobilities of cocaine and related compounds on ITLC are given in Table I. Cocaine and ecgonine methyl ester could be separated from other compounds with solvent system S_1 , and could be separated from each other with system S_2 . Benzoyl norecgonine and norecgonine separated well from benzoyl ecgonine and ecgonine in systems S_3 , S_4 and S_5 . The compounds with higher mobility in system S_6 , e.g. cocaine, benzoyl ecgonine and norcompounds, could be separated from slower moving polar conjugated *in vivo* metabolites in this system. Combinations of systems S_1 , S_2 , S_4 , S_5 , S_6 , therefore could adequately separate cocaine, some of its metabolites and congeners.

TABLE I

CHROMATOGRAPHIC MOBILITIES ON GELMAN ITLC (SILICA GEL) OF COCAINE, SOME OF ITS METABOLITES AND CONGENERS IN DIFFERENT SOLVENT SYSTEMS

S_1 = Chloroform-acetone-conc. ammonia (5:94:1); S_2 = benzene-ethyl acetate-methanol-conc. ammonia (80:20:1.2:0.1); S_3 = chloroform-acetone-diethylamine (5:4:1); S_4 = ethyl acetate-methanol-conc. ammonia (17:2:1); S_5 = ethyl acetate-methanol-conc. ammonia (15:4:1); S_6 = *n*-butanol-acetic acid-water (35:3:10).

Compound	$R_F \times 100$					
	S_1	S_2	S_3	S_4	S_5	S_6
Cocaine	98	98	98	98	98	83
Benzoyl ecgonine	0	0	29	50	73	88
Ecgonine methyl ester	98	64	98	98	98	66
Ecgonine	0	0	33	11	28	48
Benzoyl norecgonine	0	0	12	30	46	95
Norecgonine	0	0	12	4	15	75

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