CHROM. 6282

Cactus alkaloids

XVII. Thin-layer chromatography of Dansylated non-phenolic β-phenethylamines

Thin-layer chromatographic (TLC) separation of the free amines of the cactus alkaloids, mescaline (3,4,5-trimethoxy-β-phenethylamine) and 3,4-dimethoxy-β-phenethylamine, is difficult to achieve. Similarly it is difficult to distinguish between N-methylmescaline and N-methyl-3,4-dimethoxy-β-phenethylamine. In a recent study[1] we identified traces of these four compounds in the non-phenolic alkaloid extracts of the cactus, *Pelecyphora aselliformis* Ehrenberg. In the course of this investigation we found that these simple alkaloids, as well as β-phenethylamine, N-methyl-β-phenethylamine, 4-methoxy-β-phenethylamine, and N-methyl-4-methoxy-β-phenethylamine, could be more easily separated by TLC as their Dansyl conjugates.

Under alkaline conditions Dansyl chloride (1-dimethylamino-naphthalene-5-sulfonyl chloride) reacts almost quantitatively with such primary and secondary amines to produce fluorescent conjugates[2]. Often this fluorescence can be useful to visualize these compounds on previously developed TLC plates after spraying the plates with a solution of Dansyl chloride[3]. Alternately the Dansyl conjugates can be formed prior to chromatography and the conjugates themselves separated chromatographically[4].

This note reports slight modification of preparation methods and solvent systems previously reported for the separation of such Dansyl conjugates[2,4] and extends the method to include the non-phenolic N-methyl-β-phenethylamines. The two-dimensional procedure allowed us to resolve, on a single plate, sufficient quantities for mass spectral analyses of the Dansyl conjugates of the above-mentioned eight non-phenolic β-phenethylamines. This separation method should be useful and adaptable for the rapid identification and differentiation of these simple amines in additional complex biological extracts, such as urine[5].

Experimental

To prepare the Dansyl conjugates[6,7], we added 5 mg of Dansyl chloride in 0.2 ml of acetone to 0.5–2 mg of amine or amine salt in 0.2 ml of distilled water. Sufficient sodium carbonate was added to saturate the solution, and the mixture was placed on a rotary shaker for 4 h. The mixture was then extracted by adding 1 ml each of water and ethyl acetate, and shaking. The ethyl acetate was drawn off and the extraction repeated with two more portions of ethyl acetate.

The combined ethyl acetate extracts were condensed under reduced pressure, applied in a single spot near the corner of a 1-mm thick, 20 × 20-cm plate of Silica Gel G, and developed two-dimensionally[2]. The first dimension employed chloroform–butyl acetate (10:1) and the second dimension utilized benzene–triethylamine (10:1). The positions of the compounds on the developed plates were determined by means of their brilliant yellow fluorescence under long wavelength ultraviolet light. The spots were scraped from the plate and eluted by rinsing three times with ethyl acetate; the

filtered rinses were combined and concentrated under reduced pressure to a volume suitable for application to the probe of the mass spectrometer[5–7]. Table I summarizes the average R_F values calculated and their standard deviations.

TABLE I

R_F VALUES AND THEIR STANDARD DEVIATIONS OF DANSYLATED REFERENCE β-PHENETHYLAMINES

Two dimensional TLC on Silica Gel G, first dimension: chloroform–butyl acetate (10:1), second dimension: benzene–triethylamine (10:1).

Dansylated conjugate	R_F *value*	
	First dimension	*Second dimension*
β-Phenethylamine	0.53 ± 0.06	0.38 ± 0.03
N-Methyl-β-phenethylamine	0.77 ± 0.06	0.55 ± 0.03
4-Methoxy-β-phenethylamine	0.41 ± 0.06	0.34 ± 0.02
N-Methyl-4-methoxy-β-phenethylamine	0.67 ± 0.07	0.52 ± 0.03
3,4-Dimethoxy-β-phenethylamine	0.25 ± 0.05	0.26 ± 0.02
N-Methyl-3,4-dimethoxy-β-phenethylamine	0.46 ± 0.07	0.45 ± 0.03
Mescaline	0.14 ± 0.07	0.45 ± 0.03
N-Methylmescaline	0.29 ± 0.06	0.44 ± 0.03

The authors are grateful to Dr. A. BROSSI, Hoffmann-La Roche, Inc., for samples of certain reference compounds. This work was supported by U. S. Public Health Service Grants MH-17128-03 and MH-21448-01 from the National Institute of Mental Health.

Drug Plant Laboratory, School of Pharmacy, University of Washington, Seattle, Wash. 98105 (U.S.A.) J. M. NEAL

School of Pharmacy and Pharmacal Sciences, Purdue University, Lafayette, Ind. 47907 (U.S.A.) J. L. MCLAUGHLIN*

1 J. M. NEAL, P. T. SATO, W. N. HOWALD AND J. L. MCLAUGHLIN, *Science*, 176 (1972) 1131.
2 N. SEILER AND M. WIECHMANN, in A. NIEDERWIESER AND G. PATAKI (Editors), *Progress in Thin-Layer Chromatography and Related Methods*, Vol. 1, Ann Arbor-Humphrey Science Publishers, Ann Arbor, 1970, pp. 95–144.
3 J. L. MCLAUGHLIN AND A. G. PAUL, *Lloydia*, 29 (1966) 315.
4 N. SEILER AND M. WIECHMANN, *Experientia*, 21 (1965) 203.
5 C. R. CREVELING AND J. W. DALY, *Nature*, 216 (1967) 190.
6 C. R. CREVELING, K. KONDO AND J. W. DALY, *Clin. Chem.*, 14 (1968) 302.
7 J. REISCH, H. ALFES, N. JANTOS AND H. MOLLMANN, *Acta Pharm. Suecica*, 5 (1968) 393.

Received June 12th, 1972

* To whom inquiries should be directed.