Journal of Chromatography, 90 (1974) 178–180

CHROM. 7162

Note

A new solvent system for the thin-layer chromatographic separation of the Dansyl derivatives of some biogenic amines

GRAHAM C. BOFFEY and GLENN M. MARTIN
Clinical Pathology Service, Royal Inland Hospital, Kamloops, British Columbia (Canada)
(Received October 9th, 1973)

During our investigations of pharmacologically active compounds present in tick tissues, a methodology of high sensitivity and resolution was required for the separation of biogenic amines. The procedure was to accomplish these criteria without significant interference from amino acids.

Procedures are available for the separation of biogenic amines utilizing various chromatographic systems including paper, thin-layer and gas–liquid (GLC) chromatography. Seiler and Wiechmann[1–3], Seiler[4] and Creveling *et al.*[5] have described the derivative formation of primary and secondary amines with 5-dimethylamino-1-naphthalenesulfonyl chloride (Dansyl-Cl) to produce intensely fluorescent compounds which may subsequently be separated by chromatography on silica gel thin layers using semi-polar solvent systems. The utilization of the reaction of Dansyl-Cl with the amino groups of amino acids and peptides in procedures for the separation of these derivatives was described by Gray and Hartley[6] and subsequently by Boulton and Bush[7] and others[2,8]. The chromatographic resolution of the Dansyl derivatives of amine-containing biological materials is impaired by the diversity of derivatives produced during the reaction of Dansyl-Cl with constituents of biological extracts. Reaction of Dansyl-Cl with hydroxyl groups of phenolic compounds[1] and some alcohols[4] has been reported. Similar difficulties may also be anticipated using the more recently reported reaction[9] of 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) with amino groups to produce fluorescent derivatives of amines since reaction also occurs with other electron donating groups but produces derivatives of substantially relatively weaker fluorescence.

The use of GLC to separate and identify biogenic amines involves certain fundamental difficulties such as the low volatility of the free amines and their basicity which produce tailing, long retention times and peak asymmetry. Reactions to produce trimethylsilyl[10,11], dinitrophenyl[12], trifluoracetyl[13], perfluoropropionyl[14] and isothiocyanate[15] derivatives of amines derived from biological samples have been proposed in order to reduce some of the problems of GLC separation. These approaches have also been of limited value even in conjunction with various stationary and liquid phases. As a result of the inherent problems of the above procedures it was decided to reinvestigate the feasability of developing a procedure for biogenic amine separation utilizing the proven Dansyl-Cl reaction as the basis of the method.

MATERIALS AND METHODS

Dansyl-Cl was recrystallized twice from hexane and stored desiccated at room temperature until required. Analytical grade reagents were used throughout the study except when otherwise specified. Reference amines were used as obtained from the supplier without further purification. The Dansyl derivatives of the reactive amino compound (0.01 to 1.00 μM) were prepared from the biological sample by a modification of the method of Creveling *et al.*[5] and after lipid removal with benzene as described the Dansyl derivatives of the amine were extracted into 3 ml of ethyl acetate–cyclohexane (1:9, v/v) by mechanical shaking for 2 min. The mixture was centrifuged at $1500 \times g$ for 10 min, the ethyl acetate–cyclohexane extract was removed and the solvent was evaporated at room temperature under a stream of dry nitrogen. The residue contained predominantly the Dansyl derivatives of the amines which were redissolved in 50 μl of ethyl acetate. The solution of the amine derivative was applied to a 20×20 cm silica gel coated thin-layer plate (E. Merck, Darmstadt, G.F.R. Cat. No. 5763 distributed by Brinkmann Instruments (Canada) Ltd.) of layer thickness 0.25 mm. Dansyl derivatives of standard biogenic amines (0.01 to 1.00 μM) were prepared as described and also applied to the chromatoplate.

The chromatogram was developed in the carbon tetrachloride–ethylene glycol monomethyl ether (85:15, v/v) solvent system until the solvent front had ascended

TABLE I

R_F VALUES FOR DANSYL DERIVATIVES OF SOME BIOGENIC AMINES

Solvent system: carbon tetrachloride–ethylene glycol monomethyl ether (85:15).

Substance	$R_F \times 100$	*S.D.* $\times 100$ ($n=10$)	*Coefficient of variation*
Agmatine	36	4.5	12.7
Ammonia	22	1.5	6.7
D-Amphetamine	47	1.9	4.0
Cadaverine	36	4.2	11.5
Diethylamine	56	1.2	2.1
Histamine	7	2.1	30.0
5-Hydroxytryptamine	14	3.1	22.0
Metanephrine	27	1.7	6.3
Methylamine	34	2.2	6.3
Normetanephrine	20	2.1	10.6
Octopamine	15	1.5	9.8
Phenylethylamine	46	1.6	3.5
Phenylpropanolamine	33	5.1	15.4
Piperidine	60	2.4	4.1
Putrescine	35	3.5	10.1
Spermidine	33	5.2	15.8
Spermine	35	6.1	7.4
Synephrine	22	1.4	6.3
Triethylamine	55	1.5	2.7
Tryptamine	31	2.6	8.5
Tyramine	24	1.8	7.5
Dansyl-Cl	65	2.5	3.8

15 cm from the origin during approximately 100 min at 20°. The chromatogram was blown completely free of solvent to remove any residual carbon tetrachloride which had a marked quenching effect on the fluorescence of the Dansyl derivatives. The location of the separated derivatives was determined by inspection under long wavelength (366 nm) ultraviolet light.

RESULTS

Table I shows the mean R_F values for a number of amine derivatives using this solvent system and the reproducibility for a series of ten separate runs.

DISCUSSION

The procedure described resulted in an effective preliminary separation of amino acids and amines as their Dansyl derivatives as was demonstrated by tracer studies using the Dansyl derivatives of ^{14}C-labelled glycine and putrescine. These studies indicated that the derivative of putrescine was preferentially extracted into the ethyl acetate–cyclohexane solvent with an efficiency of at least 94% in comparison with the glycine derivative over a concentration range of 1.00 to 0.01 μM. The chromatographic system described produced reproducible, high-resolution separations of several biologically important primary and secondary amines. The sensitivity of the procedure was greater than 0.01 μM.

ACKNOWLEDGEMENTS

This work was supported in part by grants from the Medical Research Council of Canada (MA-375) and the Kamloops Medical Foundation.

REFERENCES

1 N. Seiler and M. Wiechmann, *Z. Anal. Chem.*, 220 (1966) 108.
2 N. Seiler and M. Wiechmann, *J. Chromatogr.*, 28 (1967) 351.
3 N. Seiler and M. Wiechmann, *Hoppe-Seyler's Z. Physiol. Chem.*, 348 (1967) 1285.
4 N. Seiler, *Hoppe-Seyler's Z. Physiol. Chem.*, 348 (1967) 601.
5 C. R. Creveling, K. Kondo and J. W. Daly, *Clin. Chem.*, 14 (1968) 303.
6 W. R. Gray and B. S. Hartley, *Biochem. J.*, 89 (1963) 59P.
7 A. A. Boulton and I. E. Bush, *Biochem. J.*, 92 (1964) 11P.
8 N. Seiler and J. Wiechmann, *Experientia*, 20 (1964) 559.
9 P. B. Ghosh and N. W. Whitehouse, *Biochem. J.*, 108 (1968) 155.
10 W. J. A. VandenHeuvel, *J. Chromatogr.*, 36 (1968) 354.
11 J. L. Cashaw, N. J. Wash, Y. Yamanaka and V. E. Davis, *J. Chromatogr. Sci.*, 9 (1971) 98.
12 S. Baba and S. Ogiya, *Yakugeku Zasshi*, 89 (1969) 1704.
13 W. J. Irvine and N. J. Saxby, *Phytochemistry*, 8 (1969) 473.
14 F. Cattabeni, S. H. Koslaw, F. Karoum and E. Costa, *Trans. Amer. Soc. Neurochem.*, 2 (1971) 60.
15 N. Narasimhachari and P. Vouros, *Anal. Biochem.*, 45 (1972) 154.