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Note

Separation of the Dns derivatives of polyamines and related compounds by thin-layer and high-pressure liquid chromatography

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Since the initial report by Russell[1], describing the elevated levels of polyamines in the urine of one cancer patient, which was later confirmed in a number of independent studies involving large numbers of patients, there has been an increasing interest in the possible use of urinary polyamine levels as a clinical test in the diagnosis, management and follow-up of patients with cancer. The successful use of this clinical test would necessitate the availability of an analytical procedure for the determination of polyamines and their conjugates in biological fluids, and a number of these methods have been described in the literature[2–9].

The most sensitive method for the determination of polyamines is probably the method which was originally developed by Seiler and Wiechmann[9] and has been since modified by a number of investigators[10–12]. This method involves the formation of the Dns (5-dimethylaminonaphthalene-1-sulfonyl) derivatives of polyamines and their separation on thin-layer chromatography (TLC) plates followed by the measurement of the fluorescence of the spot corresponding to each amine after elution. Studies in our laboratories have indicated that high-pressure liquid chromatography (HPLC) is a potentially useful alternative to TLC in the aforementioned analytical procedure and the present communication describes the separation of the Dns derivatives of the diamines, polyamines and monoacetyldiamines and polyamines using HPLC.

EXPERIMENTAL

Thin-layer chromatography

Silica gel GF and alumina G plates (250 μ) were purchased from Analtech (Newark, Del., U.S.A.). All reagents and solvents used were analytical grade. Chloroform was stored over anhydrous calcium chloride over night and distilled. Isopropanol was distilled from magnesium and a catalytic amount of iodine.

Solutions of the Dns derivatives in chloroform were applied to the plates. Silica gel plates were developed in chloroform–isopropanol (50:2 or 10:1) and alumina plates in chloroform–dioxane–isopropanol (45:2:5 or 45:2:1). The compounds were visualized under long-wavelength (366 nm) UV light.

High-pressure liquid chromatography

A Varian L.C. 4100 liquid chromatograph was used throughout this investigation. The detector used was a Laboratory Data Control UV detector (280 nm). All solvents were de-gased before use. The columns used were: Micropak Si-10 (50 cm × 2.2 mm I.D., Varian Aerograph), Micropak A1-5 (25 cm × 2.2 mm I.D., Varian Aerograph), and Corasil II (100 cm × 2.0 mm I.D.). The Corasil II column was prepared by dry-packing with Corasil II de-activated by the addition of 40 mg of water to 7.0 g of Corasil II.

RESULTS AND DISCUSSION

The Dns derivatives of fourteen diamines, polyamines and monoacetyldiamine and polyamines were synthesized and unequivocally characterized for use in this study. The detailed synthetic methods used are beyond the scope of this report and will be published in a future communication. The separation of these Dns derivatives was first investigated using TLC and the chromatographic systems which seemed promising were then adopted to HPLC.

There are a number of chromatographic systems reported for the separation of polyamines. None of these systems, however, was satisfactory for the separation of the large number of derivatives we examined. Therefore, we developed more satisfactory chromatographic systems for the separation of the Dns derivatives of diamines, polyamines and monoacetyldiamines and polyamines using TLC.

The R_F values of the Dns derivatives of the diamines, polyamines and acetyldiamines and polyamines on silica gel plates using a solvent system composed of chloroform–isopropanol (25:1) are listed in Table I under A. The Dns derivatives of

TABLE I

R_F VALUES FOR THE Dns DERIVATIVES OF DIAMINES, POLYAMINES AND THE ACETYLDIAMINES AND POLYAMINES

(A) Silica gel GF plates and chloroform–isopropanol (25:1) as a solvent; (B) silica gel GF plates and chloroform–isopropanol (10:1) as a solvent; (C) alumina G plates and chloroform–dioxane–isopropanol (45:2:1) as a solvent.

Dns derivative of	*A*	*B*	*C*
Ammonia	0.55	0.69	0.24
1,2-Diaminoethane (I)	0.70		0.42
1,3-Diaminopropane (II)	0.69		0.43
1,4-Diaminobutane (III)	0.68	0.81	0.45
1,5-Diaminopentane (IV)	0.72		0.54
Spermidine (V)	0.78	0.89	0.64
Spermine (VI)	0.84		0.82
Monoacetyl 1,2-diaminoethane (VII)		0.14	
Monoacetyl 1,3-diamonopropane (VIII)		0.13	
Monoacetyl 1,4-diaminobutane (IX)	0.07	0.10	0.20
Monoacetyl 1,5-diaminopentane (X)		0.16	0.31
N^1-Acetylspermidine (XI)	0.18	0.25	0.40
N^8-Acetylspermidine (XII)		0.20	0.41
N^1-Acetylspermine (XIII)	0.28	0.37	0.62
N^4-Acetylspermine (XIV)		0.36	0.39
$N^{1,12}$-Diacetylspermine (XV)		0.03	

spermine, spermidine, cadaverine and putrescine appeared as well-defined and well separated spots. The Dns derivatives of 1,2-diaminoethane and 1,3-diaminopropane were not well separated from each other or from the di-Dns putrescine. The Dns derivatives of all the diamines and polyamines were well separated from those of ammonia and monoacetyldiamines and polyamines.

The R_F values of the Dns derivatives of monoacetyldiamines and polyamines on silica gel plates using chloroform–isopropanol (10:1) as a solvent are listed in Table I under B. The separation of the Dns derivatives of N^1-acetylspermine, N^1-acetylspermidine, N^8-acetylspermidine, acetylcadaverine and acetylputrescine was satisfactory. The impressive separation of the isomers N^1-acetyl di-Dns spermidine and N^8-acetyl di-Dns spermidine is worthy of attention. The Dns derivatives of monoacetyl 1,2-diaminoethane and monoacetyl 1,3-diaminopropane were not separated from each other and from the mono-Dns monoacetyl putrescine.

The R_F values of the Dns derivatives of the diamines, polyamines and monoacetyldiamines and polyamines on alumina G TLC plates using chloroform–dioxane–isopropanol (45:2:1) as a solvent are listed in Table I under C. The separation of the Dns derivatives of the diamines and polyamines is superior to that achieved using silica gel TLC plates. Furthermore, the Dns derivatives of the diamines and polyamines were separated well from each other. However, the Dns derivatives of the monoacetyldiamines and polyamines as a group were not separated from the Dns derivatives of the diamines and polyamines. Furthermore, the N^1-monoacetyl di-Dns spermidine was not separated from the N^8-monoacetyl di-Dns spermidine. Therefore, this chromatographic system is not recommended for the analysis of samples which contain the acetyl derivatives of polyamines.

The separation of the Dns derivatives of the diamines and polyamines using HPLC was next studied. Fig. 1 represents the separation of these compounds on a 25-cm Micropak A1-5 column using chloroform–isopropanol (100:1) as solvent. The seven compounds tested were well separated in a total separation time of 18 min.

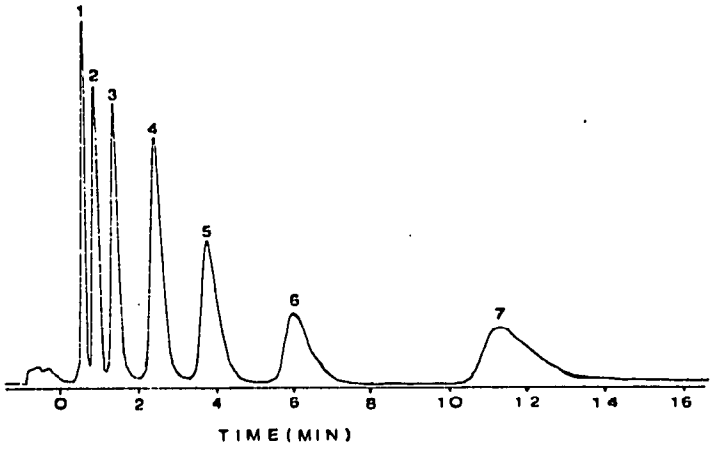

Fig. 1. HPLC separation of Dns derivatives of diamines and polyamines. Column, Micropak Al-5 (25 cm × 2.2 mm I.D.); solvent, chloroform–isopropanol (100:1); flow-rate, 60 ml/h. Compounds tested were the Dns derivatives of: 1, spermine; 2, spermidine; 3, cadaverine; 4, putrescine; 5, 1,3-diaminopropane; 6, 1,2-diaminoethane; 7, ammonia.

The separation of the Dns derivatives of the monoacetyldiamines and polyamines was carried out on a 50-cm Micropak Si-10 column using a mobile phase composed of chloroform–isopropanol (250:15), at a flow-rate of 20 ml/h. The Dns derivatives of the monoacetyldiamines and polyamines were well separated from the Dns diamines and polyamines. Furthermore, the N^1-acetyl di-Dns spermidine and N^8-acetyl di-Dns spermidine were well separated from each other. The separation of the Dns derivatives of the monoacetyl cadaverine and 1,3-diaminopropane was not very satisfactory (Fig. 2).

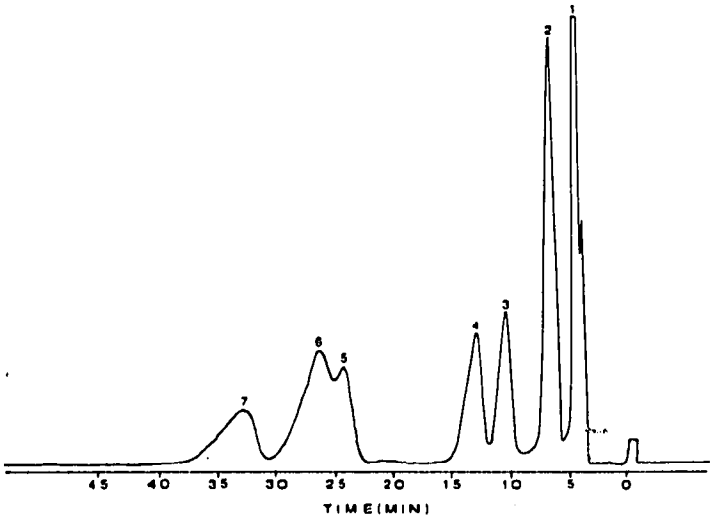

Fig. 2. HPLC separation of Dns derivatives of monoacetyldiamines and polyamines. Column, Micropak Si-10 (50 cm × 2.2 mm I.D.); solvent, chloroform–isopropanol (250:15); flow-rate, 20 ml/h. Compounds tested were the Dns derivatives of: 1, diamines, polyamines and ammonia; 2, N^1-acetylspermine; 3, N^1-acetylspermidine; 4, N^8-acetylspermidine; 5, acetylcadaverine; 6, acetyl-1,3-diaminopropane; 7, acetylputrescine.

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