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Note

Detection of spermine and thermospermine by thin-layer chromatography

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In recent years several unusual polyamines, *e.g.* *sym*-norspermidine[1], *sym*-homospermidine[2], *sym*-norspermine[1], thermospermine[3], canavalmine[4], and caldopentamine[5], have been detected in a variety of living cells. Most of these compounds, including the widely distributed spermidine and spermine, can be separated by thin-layer chromatography (TLC), or liquid chromatography, but so far no report has been published on the separation of spermine and thermospermine, an isomer of spermine. A simple method for the separation of these compounds is also important for the identification of the ubiquitous spermine. During the course of our synthetic studies on polyamines, we have found that TLC with a solvent system containing formaldehyde is suitable for this purpose; the system also shows a unique separation profile for a series of di- and polyamines.

EXPERIMENTAL

Chemicals

The diamines, 1,3-propanediamine, 1,4-butanediamine (putrescine), 1,5-pentanediamine (cadaverine), 1,6-hexanediamine, 1,7-heptanediamine and 1,8-octanediamine, were of the highest available purity. The triamines 1,7-diamino-4-azaheptane (*sym*-norspermidine), 1,8-diamino-4-azaoctane (spermidine), 1,7-diamino-4-aza-4-methylheptane were obtained from Tokyo Kasei (Tokyo, Japan); 1,9-diamino-4-azanonane (aminopropylcadaverine), 1,10-diamino-4-azadecane, 1,11-diamino-4-azaundecane, 1,12-diamino-4-azadodecane, and 1,9-diamino-5-azanonane (*sym*-homospermidine) were synthesized by the published methods[6,7]. The tetraamines 1,8-diamino-3,6-diazaoctane, 1,11-diamino-4,8-diazaundecane (*sym*-norspermine) and 1,12-diamino-4,9-diazadodecane (spermine) were purchased from Sigma (St. Louis, MO, U.S.A.) and 1,13-diamono-4,10-diazatridecane and 1,14-diamino-4,11-diazatetradecane were synthesized by the published method[6]; 1,12-diamino-4,8-diazadodecane (thermospermine), 1,13-diamino-5,9-diazatridecane (canavalmine), 1,13-diamino-4,9-diazatridecane and 1,14-diamino-5,10-diazatetradecane were synthesized by a selective method which will be reported elsewhere. The structures of these compounds were confirmed by ^{13}C nuclear magnetic resonance and elemental analysis. Formaldehyde (35%) was obtained from Junsei (Tokyo, Japan);

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all other solvents and chemicals used were commercially available highly purified reagents.

Thin-layer chromatography

The TLC systems used were as follows: system A, pre-coated silica gel plates (5 × 5 cm, Silica gel 60F-254, E. Merck, Darmstadt, G.F.R.) with a developing solvent system of *n*-butanol–acetic acid–pyridine–water (3:3:2:1); system B, silica gel plates as for system A with a developing solvent system of *n*-butanol–acetic acid–pyridine–formaldehyde (3:3:2:1); system C[8], pre-coated cellulose plates (10 × 10 cm, Avicel SF, Funakoshi, Tokyo, Japan) with a developing solvent system of isopropanol-concentrated hydrochloric acid–water (8:3:2). Di- and polyamines on the plates were detected with ninhydrin.

RESULTS AND DISCUSSION

The ready formation of a hexahydropyrimidine ring by the reaction of spermidine with formalin[9] led us to use a formaldehyde-containing solvent system for the TLC separation of polyamines. The R_F values for a series of di- and triamines in TLC systems A or B are summarized in Table I. The chromatographic profiles of the diamines in the two systems are markedly different, showing that R_F values increase in accordance with the elongation of the methylene chain in system A, while in the formaldehyde-containing system B, the R_F values for 1,3-propanediamine, putrescine and cadaverine decrease in this order, with cadaverine having the lowest value. Similarly, the R_F values of the triamines in system A increase along with elongation of the methylene chain, while in system B those of *sym*-norspermidine, spermidine and aminopropylcadaverine decrease in this order and a further chain elongation contributes to increase in the R_F value (as in system A). These results

TABLE I

R_F VALUES OF DIAMINES AND TRIAMINES

Structure	R_F	
	System A	*System B*
$H_2N(CH_2)_3NH_2$	0.40	0.72
$H_2N(CH_2)_4NH_2$ (putrescine)	0.43	0.62
$H_2N(CH_2)_5NH_2$ (cadaverine)	0.49	0.58
$H_2N(CH_2)_6NH_2$	0.57	0.66
$H_2N(CH_2)_7NH_2$	0.66	0.73
$H_2N(CH_2)_8NH_2$	0.76	0.82
$H_2N(CH_2)_3NH(CH_2)_3NH_2$ (*sym*-norspermidine)	0.15	0.67
$H_2N(CH_2)_3N(CH_3)(CH_2)_3NH_2$	0.14	0.33
$H_2N(CH_2)_3NH(CH_2)_4NH_2$ (spermidine)	0.18	0.50
$H_2N(CH_2)_3NH(CH_2)_5NH_2$ (aminopropylcadaverine)	0.22	0.43
$H_2N(CH_2)_3NH(CH_2)_6NH_2$	0.26	0.51
$H_2N(CH_2)_3NH(CH_2)_7NH_2$	0.35	0.63
$H_2N(CH_2)_3NH(CH_2)_8NH_2$	0.42	0.72
$H_2N(CH_2)_4NH(CH_2)_4NH_2$ (*sym*-homospermidine)	0.20	0.52

TABLE II

R_F VALUES OF TETRAAMINES

Structure	R_F		
	System A	*System B*	*System C*
$H_2N(CH_2)_2NH(CH_2)_2NH(CH_2)_2NH_2$	0.05	0.36	0.10
$H_2N(CH_2)_3NH(CH_2)_5NH(CH_2)_3NH_2$	0.06	0.27	0.19
$H_2N(CH_2)_3NH(CH_2)_6NH(CH_2)_3NH_2$	0.08	0.36	0.25
$H_2N(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH_2$ (*sym*-norspermine)	0.04	0.27	0.13
$H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$ (spermine)	0.06	0.26	0.17
$H_2N(CH_2)_3NH(CH_2)_3NH(CH_2)_4NH_2$ (thermospermine)	0.07	0.40	0.18
$H_2N(CH_2)_4NH(CH_2)_3NH(CH_2)_4NH_2$ (canavalmine)	0.07	0.34	0.22
$H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_4NH_2$	0.08	0.24	0.22
$H_2N(CH_2)_4NH(CH_2)_4NH(CH_2)_4NH_2$	0.08	0.23	0.26

clearly show that the mobility of di- and triamines in system B does not simply depend on chain elongation and that there should be additional factors influencing their mobility. One possibility is hexahydropyrimidine ring formation. This appears to be true on account of the high R_F value in 1,3-propanediamine or *sym*-norspermidine and the low value in 1,7-diamino-4-aza-4-methylheptane which lacks a hydrogen on the 4-aza group necessary for ring formation. However this ring formation

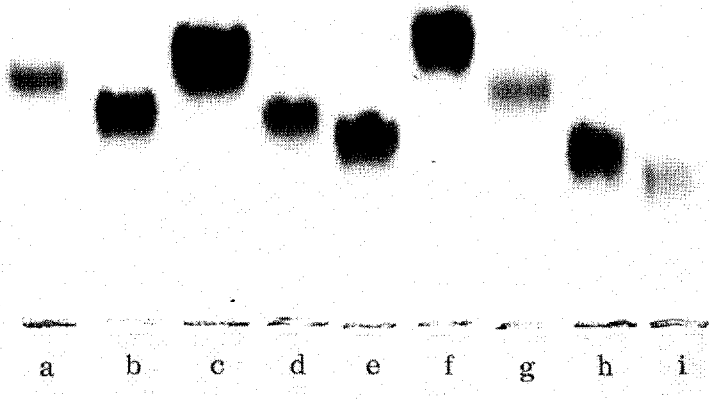

Fig. 1. Thin-layer chromatogram of tetraamines using system B. Approximately 10 nmol of each compound was placed on the plate. A ninhydrin spray was used for detection. Spots: a = 1,8-diamino-3,6-diazaoctane; b = 1,13-diamino-4,10-diazatridecane; c = 1,14-diamino-4,11-diazatetradecane; d = *sym*-norspermine; e = spermine; f = thermospermine; g = canavalmine; h = 1,13-diamino-4,9-diazatridecane; i = 1,14-diamino-5,10-diazatetradecane.

alone is insufficient to explain the higher R_F values of putrescine or spermidine over those of cadaverine or aminopropylcadaverine, respectively. In any event, it is possible that di- and triamines suffer a reduction in their hydrophilicity after interaction with the formaldehyde, and that this reduction is more conspicuous in those amines with a shorter methylene chain, *e.g.*, 1,3-propanediamine, putrescine, *sym*-norspermidine and spermidine.

The R_F values for a series of tetraamines in three systems are summarized in Table II. In systems A and C, the mobility of the tetraamines depends principally on the numbers of methylene groups, *i.e.*, the lowest R_F value is for 1,8-diamino-3,6-diazaoctane and the highest for 1,14-diamino-5,10-diazatetradecane. As can be seen from Table II, one methylene group is sufficient to produce a distinguishable difference in system C, the system which is thought to be the best one so far known for the TLC separation of tetraamines. However, it is difficult, even in this system, to separate isomers such as spermine and thermospermine, or canavalmine and 1,13-diamino-4,9-diazatridecane. Separation of the isomers can be achieved with use of system B as shown in Table II and Fig. 1. Although the reasons remain to be elucidated, system B is highly useful for the separation and detection of naturally occurring spermine and thermospermine. This also applies to canavalmine and 1,13-diamino-4,9-diazatridecane of which the latter compound has not been found in natural sources.

From these results, it can be seen that a combination of the three TLC systems described above will help to identify all naturally occurring di- and polyamines.

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