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Note

Thin-layer chromatographic separation of diaminodicarboxylic acid stereoisomers and their dansyl derivatives

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Thin-layer and paper chromatographic methods have been widely employed for the separation of the diastereoisomers of amino acids[1–4], peptides[5,6], protected peptide derivatives[7] and depsipeptides[8]. The separation of such mixtures has also been used for the quantitative determination of the diastereoisomer content[9]. The separation of diaminosuccinic[10] and diaminopimelic acids[11] and dinitrophenyl derivatives of the latter[12] has been reported.

In the course of our studies on N-hydroxyamino acids, we obtained a series of unknown di-N-hydroxyaminodicarboxylic acids[13]. As the configuration of these compounds can be readily established after catalytic reduction to diaminodicarboxylic acids, we have developed a method for separating the stereoisomers of diaminodicarboxylic acids. Such amino acids contain two asymmetric carbon atoms and may exist in two optically inactive forms, *viz.*, *meso* and racemic, or as one *meso* and two optically active forms (L,L and D,D).

EXPERIMENTAL

Materials

Meso and racemic diaminosuccinic acids were prepared by standard methods[14]. The methyl esters of diphthalyldiaminoadipic acids were resolved into racemic (m.p. 160–165°) and *meso* forms (m.p. 232–235°) according to the described method[14]. Samples of free acid isomers were obtained from these esters by N-deprotection with hydrazine hydrate and subsequent acid hydrolysis, as described below. The isomers of diaminopimelic acids (*meso*, L,L and D,D) were prepared by the method of Wade *et al.*[15]. The *meso* (m.p. 202–204°) and racemic (m.p. 135–140°) dicarbobenzoxydiaminosuberic acids were obtained by the Mori and Kumagae method[16]. The dicarbobenzoxydiaminosebacic acids ($C_{26}H_{32}N_2O_8$, requires N 5.59%) were separated by fractional crystallization from ethyl acetate giving the *meso* form (m.p. 161–163°; found, N 5.57%) and the racemic form (m.p. 117–119°; found, N 5.71%).

The *meso* and D,D isomers of diaminoazelaic acid derivatives were obtained by the papain digestion procedure, as described in detail for the resolution of diaminosuberic acid isomers[17]. By adapting this procedure, we obtained dicarbobenzoxy-L,L-diaminoazelaic acid dianilide (m.p. 223°; $C_{37}H_{40}N_4O_6$ requires N 8.79%; found, N 8.45%), dicarbobenzoxy-*meso*-diaminoazelaic acid monoanilide (m.p. 95–98°;

$C_{31}H_{35}N_3O_7$ requires N 7.48%; found, N 7.52%) and unreacted dicarbobenzoxy-D,D-diaminoazelaic acid isolated as the dicyclohexylammonium salt (m.p. 172–175°; $C_{49}H_{65}N_4O_8$ requires N 6.69%; found, N 6.71%; $[\alpha]_D^{20} = -13.8$, *c* 5, ethanol). A sample of the *meso* form of the last amino acid was obtained from the monoanilide after hydrolysis with a hydrochloric–acetic acid mixture. Diaminodicarboxylic acid standards were obtained from their dicarbobenzoxy derivatives by treatment with a solution of hydrogen bromide in acetic acid. Dansylamino acid derivatives were prepared in solutions according to Deyl and Rosmus[18].

Preparation of diaminodicarboxylic acids

Amounts of 20 mmoles of dibromoacid diethyl ester and 50 mmoles of potássium phthalimide were heated at 140° in dimethyl sulphoxide for 2 h. The mixture was poured into water and extracted with chloroform. The extracts were dried over sodium sulphate and, after evaporation, a crude oily ester was deposited, which was then refluxed in methanol with 4 ml of hydrazine hydrate. The methanol was evaporated and the crude amino acid ester was hydrolyzed by refluxing with hydrochloric acid. After separation of phthalalazine-1,4-dione, the residue was neutralized and the crystalline amino acids were collected: diaminosuberic acid, yield 65% ($C_8H_{16}N_2O_4$ requires N 13.23%; found, N 13.15%); diaminoazelaic acid, yield 70% ($C_9H_{18}N_2O_4$ requires N 12.84%; found, N 12.47%); and diaminosebacic acid, yield 61% ($C_{10}H_{20}N_2O_4$ requires N 12.07%; found, N 11.57%).

Thin-layer chromatography

The chromatography of free amino acids was performed on thin layers of cellulose powder (Merck, Darmstadt, G.F.R.) with methanol–water–acetic acid (40:10:2) as solvent. The spots of the amino acids were detected with ninhydrin. Thin-layer chromatographic separations of dansyl derivatives were carried out on silica gel G (Merck) thin layer activated at 110° for 1 h. The chromatograms of the dansyl derivatives of amino acids were developed in benzyl alcohol–chloroform–ethyl acetate–acetic acid (8:12:10:1)[19] and benzene–pyridine–acetic acid (40:10:1)[20] systems. The dansyl derivatives were made visible with the aid of a UV lamp.

RESULTS AND DISCUSSION

The results of the separation of diaminodicarboxylic acid stereoisomers are presented in Table I. With diaminosuccinic acid, the *meso* isomer separates from the racemic form. With diaminoadipic acids, on an optically active carrier such as cellulose, the racemate separates additionally into the L,L and D,D forms. As observed by Rhuland *et al.*[11], the *meso* and D,D isomers of diaminopimelic acid did not separate whereas the L,L isomer travelled more rapidly. The diastereoisomers of diaminosuberic, diaminoazelaic and diaminosebacic acids did not separate in the solvent system used. Such separations also failed in other systems and for this reason we tried to use the strongly fluorescent 1-dimethylamino-5-naphthalenesulphonyl (dansyl) derivatives[21]. The dansyl derivatives of amino acids are being used increasingly because of their advantages of requiring no development of chromatograms, high sensitivity and resistance to light and hydrolysis[19–21]. The results of the separation of the diastereoisomers of the didansyl derivatives of diaminodicarboxylic acids in

TABLE I

SEPARATION OF DIAMINODICARBOXYLIC ACID STEREOISOMERS ON CELLULOSE THIN LAYERS

Solvent system: methanol–water–acetic acid (40:10:2).

Acid	*R_F value*	
	Meso	D,D + L,L
Diaminosuccinic	0.05	0.16
Diaminoadipic	0.64	0.23 + 0.28
Diaminopimelic	0.25	0.25 + 0.37
Diaminosuberic	0.41	
Diaminoazelaic	0.47	
Diaminosebacic	0.48	

TABLE II

R_F VALUES OF DIDANSYL DERIVATIVES OF DIAMINODICARBOXYLIC ACIDS ON SILICA GEL THIN LAYERS

Didansyl derivative of amino acid	*Solvent system*			
	*A**		*B***	
	Meso	D,D + L,L	*Meso*	D,D + L,L
Diaminosuccinic	0.0	0.01	0.08	0.12
Diaminoadipic	0.04	0.22	0.13	0.38
Diaminopimelic	0.06	0.21	0.26	0.79
Diaminosuberic	0.08	0.28	0.37	0.71
Diaminoazelaic	0.11	0.32	0.46	0.75
Diaminosebacic	0.13	0.34	0.54	0.81

* Benzene–pyridine–acetic acid (40:10:1).

** Benzyl alcohol–chloroform–ethyl acetate–acetic acid (8:12:10:1).

two systems are summarized in Table II. The R_F values of the *meso* isomers are usually lower than those of the racemic forms, probably owing to the existence of molecules in different conformations. It must be stressed that in the separation of diastereoisomeric dipeptides and didepsipeptides, the L,L or D,D compounds had higher R_F values than the L,D and D,L isomers, which are the equivalent of the *meso* form[5–7].

As diaminodicarboxylic acids are compounds of great biological significance, it is important to develop simple methods for identifying their stereoisomers. The method that we have developed can be used for identifying amino acids of natural origin.

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