Polyamide layer chromatography
Further studies on dinitrophenyl amino acids

In the previous paper[1], we have described an excellent separation of seventeen dinitrophenyl (DNP) amino acids together with 2,4-dinitrophenol and 2,4-dinitroaniline by polyamide layer chromatography. Here we want to report on the separation of fourteen additional DNP amino acids* by the same method. Two-dimensional chromatograms of all 31 amino acids are also shown.

Experimental

Preparation of polyamide layer and chromatographic techniques. Details are to be found in the previous paper[1]. The polyamide resin was Amilan CM 1007s (poly-ε-caprolactam) of Toyo Rayon Co., Tokyo, Japan. Ascending methods were used in all developments as before. All solvents were purified to meet chromatographic requirements. U.V. contact photography was used to record the results (light source: a germicide lamp).

Results and discussion

Table I shows the R_F values for one-dimensional developments of the 31 DNP amino acids. The DNP amino acids are numbered according to their R_F values in solvent system II. Figs. 1-5 show the two-dimensional developments of all 31 DNP amino acids on 15 × 15 cm polyamide layers. The solvent systems are the same as those used in the previous paper[1] except solvent I and II. We changed solvent I and II to continuous development in order to separate slower moving spots. 2,4-Dinitrophenol was used as standard and the development was stopped after the 2,4-dinitrophenol had run 10.0 cm from the origin. Overall times required (from the application of sample to recording the chromatograms by U.V. contact photography) are as shown in Table II.

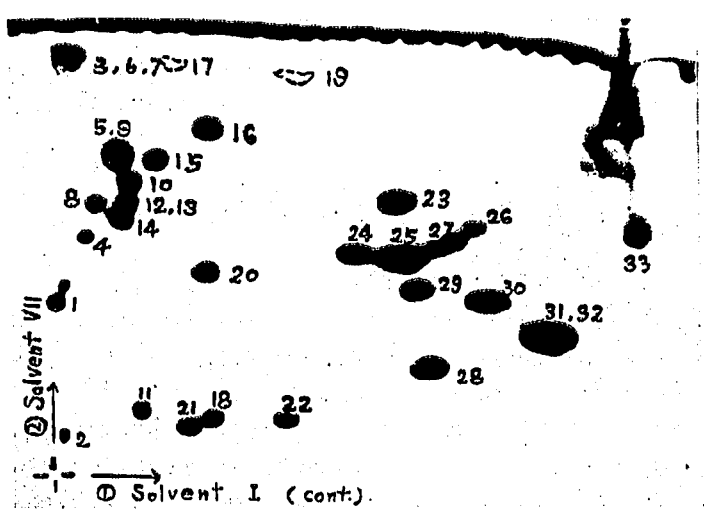

Fig. 1. Two-dimensional chromatogram. Solvent: 1st dimension: I (cont.), 2 h, 10.5 cm; 2nd dimension: VII, 1 h, 10 cm. Layer: ε-polycaprolactam resin CM 1007s. Loading: *ca.* 0.2 μg of each DNP derivative. Numbers: *cf.* Table I.

* We wish to thank Dr. A. TSUGITA, Osaka University, Osaka, Japan, for the gift of these samples.

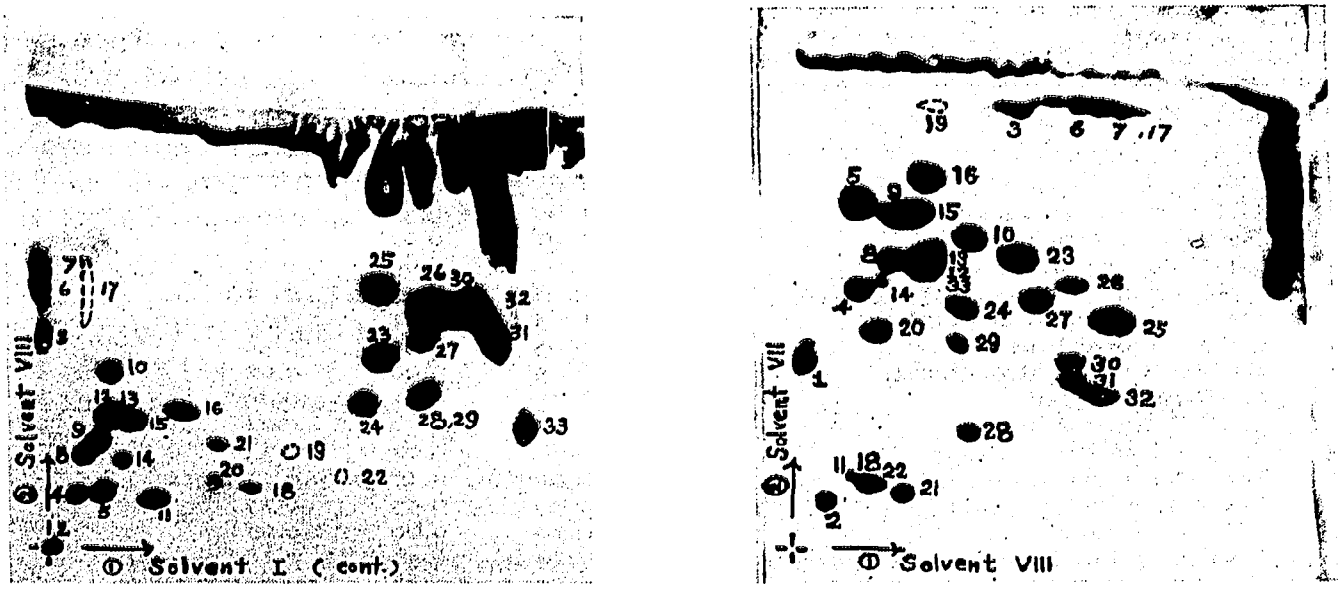

Fig. 2. Two-dimensional chromatogram. Solvent: 1st dimension: I (cont.), 2 h, 9 cm; 2nd dimension: VIII, 3.5 h, 10 cm. Layer: ε-polycaprolactam resin CM 1007s. Loading: *ca.* 0.2 μg of each DNP derivative. Numbers: *cf.* Table I.

Fig. 3. Two-dimensional chromatogram. Solvent: 1st dimension: VIII, 3.5 h, 9 cm; 2nd dimension: VII, 1 h, 11 cm. Layer: ε-polycaprolactam resin CM 1007s. Loading: *ca.* 0.2 μg of each DNP derivative. Numbers: *cf.* Table I.

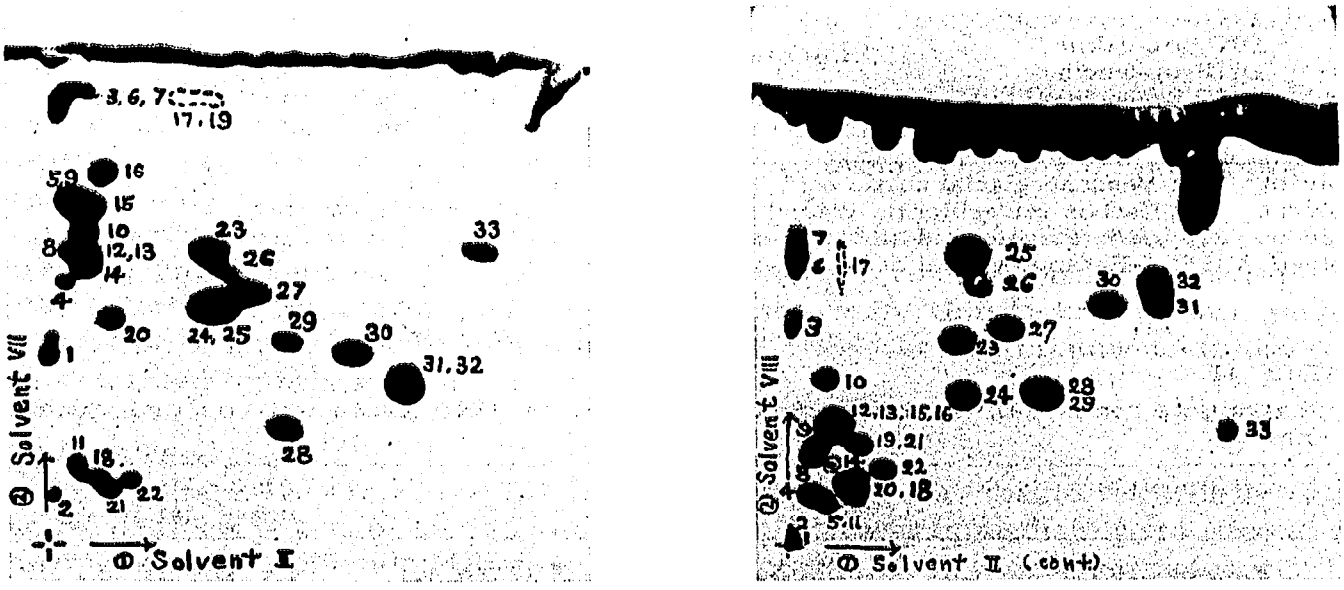

Fig. 4. Two-dimensional chromatogram. Solvent: 1st dimension: II (cont.), 3 h, 8.5 cm; 2nd dimension: VII, 1 h, 11 cm. Layer: ε-polycaprolactam resin CM 1007s. Loading: *ca.* 0.2 μg of each DNP derivative. Numbers: *cf.* Table I.

Fig. 5. Two-dimensional chromatogram. Solvent: 1st dimension: II (cont.), 3 h, 8.5 cm; 2nd dimension: VIII, 3.5 h, 10 cm. Layer: ε-polycaprolactam resin CM 1007s. Loading: *ca.* 0.2 μg of each DNP derivative. Numbers: *cf.* Table I.

TABLE I

R_F VALUES OF DNP AMINO ACIDS ON A POLYAMIDE LAYER

No.	*DNP derivative*	*Solvent system*			
		I	*II*	*VII*	*VIII*
1	DNP-cysteic acid sodium salt	0.00	0.00	0.38	0.01
2	Bis-DNP-L-cystine	0.01	0.01	0.11	0.01
3	α-DNP-L-arginine	0.03	0.01	0.90	0.47
4	DNP-aspartic acid	0.06	0.04	0.55	0.12
5	DNP-DL-methionine sulphone	0.09	0.05	0.72	0.10
6	δ-DNP-L-ornithine	0.09	0.05	0.90	0.56
7	ε-DNP-lysine hydrochloride	0.12	0.05	0.90	0.62
8	DNP-DL-serine	0.08	0.05	0.61	0.20
9	DNP-L-asparagine	0.10	0.05	0.70	0.21
10	DNP-L-hydroxyproline	0.12	0.08	0.64	0.33
11	Bis-DNP-L-ornithine	0.17	0.09	0.17	0.11
12	DNP-DL-allo-threonine	0.13	0.09	0.59	0.27
13	DNP-threonine	0.16	0.10	0.60	0.27
14	DNP-L-glutamic acid	0.15	0.10	0.56	0.18
15	DNP-L-glutamine	0.17	0.10	0.70	0.24
16	DNP-DL-methionine sulphoxide	0.22	0.10	0.75	0.26
17	O-DNP-L-tyrosine	0.20	0.10	0.88	tailing
18	Bis-DNP-lysine	0.35	0.14	0.14	0.15
19	Bis-DNP-L-histidine	0.48	0.15	0.88	0.23
20	DNP-glycine	0.32	0.16	0.48	0.21
21	DNP-tryptophane	0.32	0.17	0.12	0.23
22	Bis-DNP-DL-tyrosine	0.58	0.24	0.12	0.16
23	DNP-sarcosine	0.63	0.41	0.61	0.46
24	DNP-alanine	0.62	0.42	0.49	0.33
25	2,4-Dinitroaniline	0.64	0.42	0.49	0.64
26	DNP-DL-β-alanine	0.75	0.44	0.56	0.60
27	DNP-L-proline	0.75	0.51	0.50	0.46
28	DNP-phenylalanine	0.85	0.62	0.23	0.33
29	DNP-DL-methionine	0.77	0.62	0.41	0.32
30	DNP-DL-valine	0.88	0.76	0.38	0.53
31	DNP-DL-leucine	0.94	0.90	0.32	0.53
32	DNP-isoleucine	0.94	0.90	0.31	0.56
33	2,4-Dinitrophenol	1.00	1.00	0.60	0.26

Solvent I: Benzene–glacial acetic acid (80:20) (continuous flow).
Solvent II: Carbon tetrachloride–glacial acetic acid (80:20) (continuous flow).
Solvent VII: 90% Formic acid–water (50:50).
Solvent VIII: *n*-Butanol–glacial acetic acid (90:10).

TABLE II

TIME REQUIRED FOR TWO-DIMENSIONAL CHROMATOGRAPHY OF DNP AMINO ACIDS ON A POLYAMIDE LAYER

Fig.	*1st dimension*	*2nd dimension*	*Time required (h)*
1	Benzene–HOAc (80:20) (cont.) (Solvent I)	90% HCOOH–H_2O (50:50) (Solvent VII)	3.5
2	Benzene–HOAc (80:20) (cont.) (Solvent I)	*n*-BuOH–HOAc (90:10) (Solvent VIII)	6
3	*n*-BuOH–HOAc (90:10) (Solvent VIII)	90% HCOOH–H_2O (50:50) (Solvent VII)	5
4	CCl_4–HOAc (80:20) (cont.) (Solvent II)	90% HCOOH–H_2O (50:50) (Solvent VII)	4.5
5	CCl_4–HOAc (80:20) (cont.) (Solvent II)	*n*-BuOH–HOAc (90:10) (Solvent VIII)	7

As little as 0.1 μg of DNP amino acids are easily recognizable by U.V. contact photography after two-dimensional development. DNP-proline, DNP-hydroxyproline, DNP-tryptophan and DNP-sarcosine give an orange colour when the chromatograms are dried, while the rest are yellow. Furthermore, DNP-glycine always gives a characteristic shape on development in a *n*-butanol–glacial acetic acid system. These observations greatly help the assignment of spots on the chromatograms of 31 DNP amino acids.

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A solvent system for the thin layer chromatographic separation of hippuric and mandelic acids

During the course of studies on the metabolism of drugs our attention has been focused on thin-layer chromatography of organic acids.

In recent years many paper chromatographic separations of organic acids have been reported. Techniques for the efficient separation of hippuric acid and mandelic acid by paper chromatography[1,2] and thin-layer chromatography do not appear to have been developed.

In the practice of thin-layer chromatography the developing solvent systems are frequently complex. The purpose of this report is to describe a water saturated ether–methanol–87% formic acid system to facilitate the thin-layer chromatography separation of hippuric acid and mandelic acid. Other aromatic organic acids also can be separated by this system.

Experimental

Silica gel HF (E. Merck A.G., Darmstadt), 25 g, was shaken vigorously with 50 ml distilled water for 60 sec, and this suspension was immediately transferred to an open spreader (Shandon Unoplan Spreader) on 23 glass plates (50 × 200 mm). The glass plates were coated to a thickness of 250 μ. The plates were then allowed to stand for 30 min at room temperature and were activated by drying in a oven at 100–110° for 3–4 h. Hippuric acid, mandelic acid, and other organic acids were applied and the plates developed in the solvent systems described. The system having the best solvent combination for the general separation of organic acids was water saturated ether–