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## Note

# Resolution of six polar DL-amino acids by chromatography on native cellulose\*

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The resolution of amino acid facemates by paper chromatography was first reported more than three decades ago<sup>1-4</sup>. We have developed the method for separating D- and L-amino acids on cellulose columns and by thin-layer chromatography during biochemical investigations, and successfully resolved many DL-amino acids (proteinic and non-proteinic)<sup>5-10</sup>. However, six polar racemates (aspartic acid, glutamic acid, lysine, arginine, asparagine and glutamine) have remained unresolved. Recently, we have found novel elution mixtures that resolve not only the charged DL-amino acids, which was close to an isoelectric point for the respective amino acid, but also the uncharged ones (asparagine and glutamine).

The purposes of this note are to describe the resolution of the polar DL-amino acids, and to summarize all the resolutions of the proteinic DL-amino acids on cellulose columns by adding the present data to the previous results<sup>8</sup>.

### **EXPERIMENTAL**

The packed cellulose column (Merck, lot No. 2331; unmodified microcrystalline; column size:  $250 \times 0.85$  cm I.D.; bed height: 240 cm) was first washed with 10 ml of 1 N hydrochloric acid, then neutralized with 30 ml of 0.01 N sodium hydroxide and equilibrated with the elution mixture as described below. The chromatograms, which were poorly resolved, were analysed by a computer to display a reasonable resolution profile as shown in Fig. 1. On the basis of the computer-analysed patterns, enantiomers with high optical purity were obtained on repeated rechromatography.

All the DL-amino acids (non-derivatized) were resolved with the elution mixture close to their isoelectric point. DL-Asp and DL-Glu (300  $\mu$ g each) were thus resolved with an elution mixture containing acetonitrile, pyridine and  $5 \cdot 10^{-3}$  M hydrochloric acid (5:5:2, v/v/v). The eluents were monitored after mixing with ninhydrin reagent. For DL-Lys (1 mg) and DL-Arg (500  $\mu$ g), the mixture was acetonitrile-pyridine-1 · 10<sup>-3</sup> M sodium hydroxide (2:2:1 for DL-Lys and 1:1:1 for DL-Arg), and for DL-Asn (200  $\mu$ g) and DL-Gln (300  $\mu$ g) it was acetonitrile-pyridine-water (2:2:1).

<sup>\*</sup> LEB/OU Contribution No. 84.

### RESULTS AND DISCUSSION

Fig. 1 shows the resolved chromatograms for each pair of D- and L-amino acids. Resolution of charged amino acids (Asp, Glu, Lys and Arg) was achieved with the elution mixture close to their isoelectric point, without which no resolution was obtained. For instance, D- and L-Asp were eluted at 7.5 h (acetonitrile-pyridine- $2 \cdot 10^{-3} M$  hydrochloric acid, 2:2:1) and at 10 h (acetonitrile-pyridine- $1 \cdot 10^{-3} M$  hydrochloric acid, 5:5:2), respectively, but no resolved chromatogram for D- and L-Asp was obtained. Under conditions close to their isoelectric points, therefore, it seems that the enantiomers of amino acids interact with cellulose slightly differently and might consequently be resolved as shown in Fig. 1. On the other hand, DL-Asn and DL-Gln were eluted with acetonitrile-pyridine-water, because they are polar but uncharged. It was already known that acetonitrile was very effective for resolving these DL-amino acids.

Since not all the racemates were completely resolved, the chromatograms thus obtained were analysed by a computer to show the elution pattern of the enantiomers (Fig. 1 inset). Applying the method employed here, we could establish the optical purity of the resolved enantiomers and give a strategy for rechromatography to enhance the optical purity of the peak.

We have previously resolved DL-amino-acids on cellulose <sup>5-10</sup>, and it is interesting to collect here all the data on cellulose column chromatography (Table I). Some of the data are comparable with those from other liquid chromatography methods involving a chiral stationary phase <sup>11-16</sup>. Since our method of chromatographic resolution has marked characteristics in comparison with other leading resolution techniques, as mentioned earlier<sup>8</sup>, it should be improved further to give high resolution capability and hence make a contribution to a variety of different investigations.

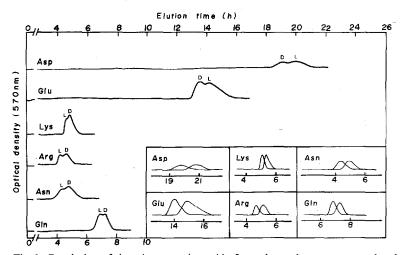


Fig. 1. Resolution of six polar DL-amino acids. Inset shows the computer-analysed patterns. Amino acid abbreviations are listed in Table I.

SUMMARY OF RESOLUTION OF ALL PROTEIN DL-AMINO ACIDS

TABLE I

Containing  Containing  D.049) A-P-5 · 10 <sup>-3</sup> N HCI (5:5:2) C  D.060) A-P-5 · 10 <sup>-3</sup> N HCI (5:5:2) C  D.051) A-P-1 · 10 <sup>-3</sup> N NaOH (2:2:1) C  D.103) A-P-W (2:2:1) B,C  D.066) P-E-W (4:1:1) A  D.065) P-E-W (4:1:1) A  P-E-W (4:1:1) A  D.050) P-E-W (5:1:1) B	DL-Amino acids		Retention time (min,	ime (min)		Resolution	Resolution	Identifi-	Ref.
acid Asp 1184 (1183) 1242 (1243) 1213 (1213) 0.048 (0.049) A-P-5 · 10 <sup>-3</sup> N HCI (5:5:2) C is acid Giu 844 (840) 882 (892) 863 (866) 0.044 (0.060) A-P-5 · 10 <sup>-3</sup> N HCI (5:5:2) C is acid Giu 844 (840) 882 (892) 863 (866) 0.044 (0.060) A-P-5 · 10 <sup>-3</sup> N HCI (5:5:2) C is acid Giu 844 (840) 82 (892) 310 (312) 0.048 (0.061) A-P-1 · 10 <sup>-3</sup> N NaOH (2:2:1) C is acid Giu 434 (436) 409 (408) 277 (276) 291 (291) 0.093 (0.103) A-P-1 · 10 <sup>-3</sup> N NaOH (2:1:1) C is acid Giu 434 (436) 409 (408) 422 (422) 0.057 (0.066) A-P-W (2:2:1) C is acid Giu 434 (436) 927 (927) 966 (966) 0.082 (0.082) P-E-W (4:1:1) A is acid Giu 674 (1043) 1015 (1016) 0.048 (0.053) P-E-W (4:1:1) A is acid Giu 674 (644) 0.059 (0.059) P-E-W (4:1:1) A is acid Giu 674 (644) 0.059 (0.059) P-E-W (5:1:1) B is acid Giu 634 (644) 0.059 (0.059) P-E-W (5:1:1) B is acid Giu 644 (644) 0.059 (0.056) P-E-W (5:1:1) B is acid Giu 644 (644) 0.059 (0.059) P-E-W (5:1:1) B is acid Giu 644 (644) 0.059 (0.056) P-E-W (5:1:1) B is acid Giu 644 (644) 0.092 (0.066) P-E-W (5:1:1) B is acid Giu 644 (644) 0.092 (0.066) P-E-W (5:1:1) B is acid Giu 644 (644) 0.092 (0.066) P-E-W (5:1:1) B is acid Giu 644 (644) 0.092 (0.066) P-E-W (5:1:1) B is acid Giu 644 (644) 0.092 (0.066) P-E-W (5:1:1) B is acid Giu 644 (644) 0.092 (0.066) P-E-W (5:1:1) B is acid Giu 644 (644) 0.092 (0.066) P-E-W (5:1:1) B is acid Giu 644 (644) 0.092 (0.066) P-E-W (5:1:1) B is acid Giu 644 (644) 0.092 (0.066) P-E-W (5:1:1) B is acid Giu 644 (644) 0.092 (0.066) P-E-W (5:1:1) B is acid Giu 644 (644) 0.093 (0.066) P-E-W (5:1:1) B is acid Giu 644 (644) 0.093 (0.066) P-E-W (5:1:1) B is acid Giu 644 (644) 0.093 (0.066) P-E-W (5:1:1) B is acid Giu 644 (644) 0.093 (0.066) P-E-W (5:1:1) B is acid Giu 644 (644) 0.093 (0.066) P-E-W (5:1:1) B is acid Giu 644 (644) 0.093 (0.066) P-E-W (5:1:1) B is acid Giu 644 (644) 0.093 (0.066) P-E-W (5:1:1) B is acid Giu 644 (644) 0.093 (0.066) P-E-W (5:1:1) B is acid Giu 644 (644) 0.093 (0.066) P-E-W (5:1:1) B is acid Giu 644 (644) 0.093 (0.066) P-E-W (5:1:1) B is acid Giu 644 (644) 0.093 (0.066)	Name	Abbrev- iation	D (peak)	L (peak)	Trough	Jacon	conmittons	method***	
te acid Glu 844 (840) 882 (892) 863 (866) 0.044 (0.060) A-P-5 . 10 <sup>-3</sup> N HCl (5:5:2) C  Lys 317 (321) 302 (302) 310 (312) 0.048 (0.061) A-P-1 . 10 <sup>-3</sup> N NaOH (2:2:1) C  sinc As 304 (306) 277 (276) 291 (291) 0.093 (0.103) A-P-1 . 10 <sup>-3</sup> N NaOH (1:1:1) C  sinc As 344 (436) 409 (408) 422 (422) 0.057 (0.066) A-P-W (2:2:1) C  sinc Glin 434 (436) 409 (408) 422 (422) 0.057 (0.066) P-E-W (4:1:1) A  sinc Cys 990 (889) 1040 (1043) 1015 (1016) 0.048 (0.053) P-E-W (4:1:1) A  sinc Cys 990 (889) 1040 (1043) 1015 (1016) 0.048 (0.053) P-E-W (4:1:1) A  sinc Ma 892 (892) 938 (938) 915 (915) 0.050 (0.050) P-E-W (4:1:1) B  sinc Met 623 (623) 663 (663) 664 (644) 0.059 (0.059) P-E-W (5:1:1) B  nine Met 634 (995) 664 (644) 0.096 (0.066) P-E-W (5:1:1) B  lanine Phe 641 711 679 0.066 (0.066) P-E-W (5:1:1) A  hlan Trp 380 435 413 413 0.133 P-E-W (1:1:1) A  hlan Trp 380 435 915 915 913 0.066 (0.066) P-E-W (5:1:1) B  hlan Trp 380 435 915 913 0.033 P-E-W (3:1:1) A  hlan Trp 380 435 913 913 0.033 P-E-W (3:1:1) A  hlan Trp 380 435 913 913 0.033 P-E-W (3:1:1) A  hlan Trp 380 435 913 913 0.033 P-E-W (3:1:1) A  hlan Trp 380 435 913 913 0.033 P-E-W (3:1:1) A  hlan Trp 380 435 913 913 0.033 P-E-W (3:1:1) A  hlan Trp 380 435 913 913 0.033 P-E-W (3:1:1) A  hlan Trp 380 435 913 913 0.033 P-E-W (3:1:1) A  hlan Trp 380 435 913 913 0.033 P-E-W (3:1:1) A  hlan Trp 380 435 913 913 0.033 P-E-W (3:1:1) A  hlan Trp 380 435 913 913 0.033 P-E-W (3:1:1) A  hlan Trp 380 435 913 913 0.033 P-E-W (3:1:1) A  hlan Trp 380 435 913 913 0.033 P-E-W (3:1:1) A  hlan Trp 380 435 913 913 0.033 P-E-W (3:1:1) A  hlan Trp 380 435 913 913 0.033 P-E-W (3:1:1) A	Aspartic acid	Asp	1184 (1183		1213 (1213)	0.048 (0.049)	A-P-5 · 10 <sup>-3</sup> N HCl (5:5:2)	S	
Lys 317 (321) 302 (302) 310 (312) 0.048 (0.061) A-P-1 · 10 <sup>-3</sup> N NaOH (2.2.1) C gine 3 dd (306) 277 (276) 291 (291) 0.093 (0.103) A-P-1 · 10 <sup>-3</sup> N NaOH (1.2.1) C c line 3 dd (306) 277 (276) 291 (291) 0.093 (0.103) A-P-1 · 10 <sup>-3</sup> N NaOH (1.2.1) C c line 434 (436) 409 (408) 422 (422) 0.057 (0.066) A-P-W (2.2.1) C c line 434 (436) 409 (408) 422 (422) 0.057 (0.066) P-E-W (2.2.1) C c line 5 c line 1263 (1263) 1337 (1348) 1300 (1300) 0.057 (0.066) P-E-W (4.1.1) A line	Glutamic acid	੶ਜ਼ੑ	844 (840	887	863 (866)	0.044 (0.060)	$A-P-5 \cdot 10^{-3}N \text{ HCl } (5:5:2)$	C	
Fig. 4rg 304 (306) 277 (276) 291 (291) 0.093 (0.103) A-P-1 · 10 <sup>-3</sup> N NaOH (1:1:1) C included Asin 286 (293) 252 (258) 269 (276) 0.126 (0.127) A-P-W (2:2:1) B,C C C C C C C C C C C C C C C C C C C	Lysine§	Lys		302	310 (312)	0.048 (0.061)	A-P-1 10 <sup>-3</sup> N NaOH (2:2:1)	၁	
şine\$         Asn         286 (293)         252 (258)         269 (276)         0.126 (0.127)         A-P-W (2.2.1)         B.C           ine\$         Glin         434 (436)         409 (408)         422 (422)         0.057 (0.066)         A-P-W (2.2.1)         C           Ser         1263 (1263)         1337 (1348)         1300 (1300)         0.057 (0.066)         P-E-W (4:1.1)         A           c Cys         990 (989)         1040 (1043)         1015 (1016)         0.048 (0.053)         E-W (4:1.1)         A           e         Tyr         596         674         635         0.120         P-E-W (4:1.1)         A           e*         Tyr         596         674         635         0.120         P-E-W (4:1.1)         A           e*         Tyr         596         674         635         0.120         P-E-W (4:1.1)         A           e*         Tyr         596         674         635         0.120         P-E-W (4:1.1)         A           e*         Tyr         596         674         635         0.120         P-E-W (4:1.1)         A           e*         Tyr         820         872         0.120         P-E-W (5:1.1)         B           e*<	Arginine§	Arg		277	291 (291)	0.093 (0.103)	A-P-1 · 10 <sup>-3</sup> N NaOH (1:1:1)	C	
Ser   1263 (1263)   1337 (1348)   1300 (1300)   0.057 (0.066)   P-E-W (4:1:1)   A     Ser   1263 (1263)   1337 (1348)   1300 (1300)   0.057 (0.066)   P-E-W (4:1:1)   A	Asparagine§	Asn		_	269 (276)	0.126 (0.127)	A-P-W (2:2:1)	B,C	
Ser         1263 (1263)         1337 (1348)         1300 (1300)         0.057 (0.066)         P-E-W (4:1:1)         A           r         Th         1006 (1006)         927 (927)         966 (966)         0.082 (0.082)         P-E-W (4:1:1)         A           e         Tyr         596         674         635         0.123         P-E-W (6:1)         B           e*         Tyr         596         674         635         0.123         P-E-W (4:1:1)         A           e*         Tyr         596         674         635         0.120         P-E-W (4:1:1)         A           e*         Tyr         596         674         635         0.120         P-E-W (4:1:1)         A           e*         Tyr         595         679         915 (915)         0.050 (0.050)         P-E-W (5:1:1)         B           val         625 (625)         663 (663)         644 (644)         0.059 (0.059)         P-E-W (5:1:1)         B           Leu         523 (515)         540 (542)         531 (529)         0.066 (0.066)         P-E-W (5:1:1)         B           inine         Met         634         695         664         0.092         P-E-W (5:1:1)         B <th< td=""><td>Glutamines</td><td>Gln</td><td></td><td></td><td>422 (422)</td><td>0.057 (0.066)</td><td>A-P-W (2:2:1)</td><td>င</td><td></td></th<>	Glutamines	Gln			422 (422)	0.057 (0.066)	A-P-W (2:2:1)	င	
Fig. 1. (1006 (1006) 927 (927) 966 (966) 0.082 (0.082) P-E-W (4:1:1) A  E. Cys 990 (989) 1040 (1043) 1015 (1016) 0.048 (0.053) E-W (6:1) B  E. Tyr 596 674 635 0.123 P-E-W (6:1) A  Ala 892 (892) 820 872 0.120 P-E-W (1:1:1) A  Ala 892 (892) 938 (938) 915 (915) 0.050 (0.050) P-E-W (5:1:1) B  Leu 523 (515) 540 (542) 531 (529) 0.032 (0.053) P-E-W (5:1:1) B  Inine Met 634 695 664 (644) 0.092 P-E-W (5:1:1) B  Inine Met 634 695 664 (644) 0.066 (0.066) P-E-W (5:1:1) B  Inine Met 634 695 664 0.092 P-E-W (5:1:1) B  Inine Met 641 711 679 0.081 P-E-W (5:1:1) A  Inine Met 641 770 740 0.081 P-E-W (5:1:1) A  Inine Met 641 770 740 740 0.081 P-E-W (5:1:1) A  Inine Met 641 770 740 740 0.081 P-E-W (5:1:1) A  Inine Met 641 770 740 740 0.081 P-E-W (5:1:1) A  Inine Met 641 770 740 0.081 P-E-W (5:1:1) A  Inine Met 641 770 740 0.081 P-E-W (5:1:1) A	Serine	Ser			1300 (1300)	0.057 (0.066)	P-E-W (4:1:1)	A	∞
Cys         990 (989)         1040 (1043)         1015 (1016)         0.048 (0.053)         E-W (6:1)         B           Tyr         596         674         635         0.123         P-E-W (4:1:1)         A           His         925         820         872         0.120         P-E-W (4:1:1)         A           Ala         892 (892)         938 (938)         915 (915)         0.050 (0.050)         P-E-W (5:1:1)         B           Val         625 (625)         663 (663)         644 (644)         0.050 (0.059)         P-E-W (5:1:1)         B           Le         522 (515)         540 (542)         531 (529)         0.056 (0.065)         P-E-W (5:1:1)         B           le         512 (512)         547 (547)         529 (529)         0.066 (0.066)         P-E-W (5:1:1)         B           ine         Met         634         664         0.092         P-E-W (5:1:1)         B           ine         Phe         641         770         740         0.081         P-E-W (5:1:1)         B           ine         Fr         641         0.103         P-E-W (5:1:1)         B         B           ine         Fr         642         0.092         P-E-W (5:1:1)         B	Threonine§	Thr	1006 (1006)	_	(996) 996	0.082 (0.082)	P-E-W (4:1:1)	A	<b>∞</b>
Tyr         596         674         635         0.123         P-E-W (4:1:1)         A           His         925         820         872         0.120         P-E-W (4:1:1)         A           Ala         892 (892)         938 (938)         915 (915)         0.050 (0.050)         P-E-W (5:1:1)         B           Val         625 (625)         663 (663)         644 (644)         0.059 (0.059)         P-E-W (5:1:1)         B           Le         523 (515)         540 (542)         531 (529)         0.066 (0.066)         P-E-W (5:1:1)         B           le         512 (512)         547 (547)         529 (529)         0.066 (0.066)         P-E-W (5:1:1)         B           nine         Phe         644         0.092         P-E-W (5:1:1)         B           pro         710         770         740         0.081         P-E-W (5:1:1)         B           n         770         73         613         9.133         P-E-W (5:1:1)         A	Cysteine	Ç			1015 (1016)	0.048 (0.053)	E-W (6:1)	В	<b>~</b>
His 925 820 872 0.120 P-E-W (1:1:1) A  Ala 892 (892) 938 (938) 915 (915) 0.050 (0.050) P-E-W (5:1:1) B  Val 625 (625) 663 (663) 644 (644) 0.059 (0.059) P-E-W (5:1:1) B  Leu 523 (515) 540 (542) 531 (529) 0.052 (0.053) P-E-W (5:1:1) B  Le 512 (512) 547 (547) 529 (529) 0.066 (0.066) P-E-W (5:1:1) B  Le 64 0.092 P-E-W (5:1:1) B  Ind Phe 641 711 679 0.103 P-E-W (5:1:1) A  Ind 770 740 0.081 P-E-W (3:1:1) A  Ind 770 740 0.081 P-E-W (3:1:1) A  Ind 770 740 0.081 P-E-W (3:1:1) A	Tyrosine	Tyr	296	674	635	0.123	P-E-W (4:1:1)	¥	5,6,8,9
Ala         892 (892)         938 (938)         915 (915)         0.050 (0.050)         P-E-W (5.51:1)         B           Val         625 (625)         663 (663)         644 (644)         0.059 (0.059)         P-E-W (5:1:1)         B           Lou         523 (515)         540 (542)         531 (529)         0.032 (0.053)         P-E-W (5:1:1)         B           e         Ile         512 (512)         547 (547)         529 (529)         0.066 (0.066)         P-E-W (5:1:1)         B           anine         Met         693         664         0.092         P-E-W (5:1:1)         B           anine         Phe         641         711         679         0.103         P-E-W (4:1:1)         A           pro         710         770         740         0.081         P-E-W (3:1:1)         B           nan         Trp         380         435         0.133         P-E-W (1:1)         A	Histidine§	His	925	820	872	0.120	P-E-W (1:1:1)	¥	8,9,5
Val         625 (625)         663 (663)         644 (644)         0.059 (0.059)         P-E-W (5:1:1)         B           Leu         523 (515)         540 (542)         531 (529)         0.032 (0.053)         P-E-W (5:1:1)         B           e         Ile         512 (512)         547 (547)         529 (529)         0.066 (0.066)         P-E-W (5:1:1)         B           ine         Met         634         695         664         0.092         P-E-W (5:1:1)         B           anine         Phe         641         711         679         0.103         P-E-W (4:1:1)         A           pro         710         770         740         0.081         P-E-W (3:1:1)         B           nan         Trp         380         435         413         0.133         P-E-W (1:1)         A	Alanine	Ala		938		0.050 (0.050)	P-E-W (5.5:1:1)	æ	<b>∞</b>
the State St	Valine	Val		999		0.059 (0.059)	P-E-W (5:1:1)	<b>8</b>	∞
ine Re 512 (512) 547 (547) 529 (529) 0.066 (0.066) P-E-W (5:1:1) B B B B B B B B B B B B B B B B B B B	Leucine	Leu		<del>2</del>		0.032 (0.053)	P-E-W (5:1:1)	æ	<b>∞</b>
mine         Met         634         695         664         0.092         P-E-W (5:1:1)         B           alanine         Phe         641         711         679         0.103         P-E-W (4:1:1)         A           Pro         710         770         740         0.081         P-E-W (3:1:1)         B           phan         Trp         380         435         413         0.133         P-E-W (1:1:1)         A	Isoleucine	el Ile		547		0.066 (0.066)	P-E-W (5:1:1)	B	<b>∞</b>
alanine         Phe         641         711         679         0.103         P-E-W (4:1:1)         A           Pro         710         770         740         0.081         P-E-W (3:1:1)         B           phan         Trp         380         435         413         0.133         P-E-W (1:1:1)         A	Methionine	Met		695		0.092	P-E-W (5:1:1)	В	6,8
Pro 710 770 740 0.081 P-E-W (3:1:1) B phan Trp 380 435 413 0.133 P-E-W (1:1:1) A	Phenylalanine Phenylanine Phenylalanine Phenylalanine Phenylalanine Phenylalanine Phen	Phe	<u>\$</u>	711	629	0.103	P-E-W (4:1:1)	A	5,6,8,9
Trp 380 435 413 0.133 P-E-W	Proline	Pro	710	<i>170</i>	740	0.081	P-E-W (3:1:1)	8	∞
	Tryptophan	Trp	380	435	413	0.133	P-E-W (1:1:1)	<b>V</b>	5,6,7,8,9

<sup>\*</sup> Resolution factor = [D(peak) - I(peak)]/trough; values in parentheses were obtained by computer.

<sup>\*\*</sup> P = Pyridine; E = ethanol; W = water; A = acetonitrile. All ratios are v/v.

<sup>\*\*\*</sup> A = C.D. spectra; B = co-chromatography, by which sample mixtures containing enantiomers in different ratios (e.g. D.L = 1:3, or vice versa) were eluted; and C = thin-layer chromatography on cellulose plates (Avicel SF, Funakoshi Yakuhin, Osaka, Japan), which was carried out by ascending development of D- and L-enantiomers alternately spotted at the origin.  ${}^{\S}$  L-Enantiomers were eluted faster.

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