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OPTICAL ROTATORY DISPERSION OF NITROBENZENE DERIVATIVES—III

N-2,4-DINITROPHENYL-α-AMINO ACIDS

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Abstract—Optical rotatory dispersion (ORD) of N-2,4-dinitrophenyl-α-amino acids (DNP-amino acids) were measured over the wavelength range 200–600 nm. All the DNP-amino acids measured showed a strong peak near 220 nm, which can be utilized for microdetermination of the configuration of component amino acids of some peptide antibiotics. A pair of marked Cotton effects centered at 370 nm were observed for compounds containing two DNP-groups. The Cotton effects in the visible region proved to be applicable to conformational analysis of such peptides as gramicidin S that can be doubly dinitrophenylated.

ORD-CURVES of *o*-nitrobenzoyl derivatives of optically active secondary alcohols and of α-amino acids show a Cotton effect centered near 330 nm, the sign of which is controlled by the chirality of the alcohols and amino acids.[1,2] DNP-amino acids can be considered similar to *o*-nitrobenzoyl derivatives in the sense that they both have an asymmetric center in the vicinity of the aromatic nitro group. Hence, we are interested in the ORD-behaviour of DNP-amino acids.

Some typical ORD-curves are presented in Fig 1. Extrema are observed near 450, 360, 330, 300, 250 and 220 nms. Molecular rotation values at each extremum

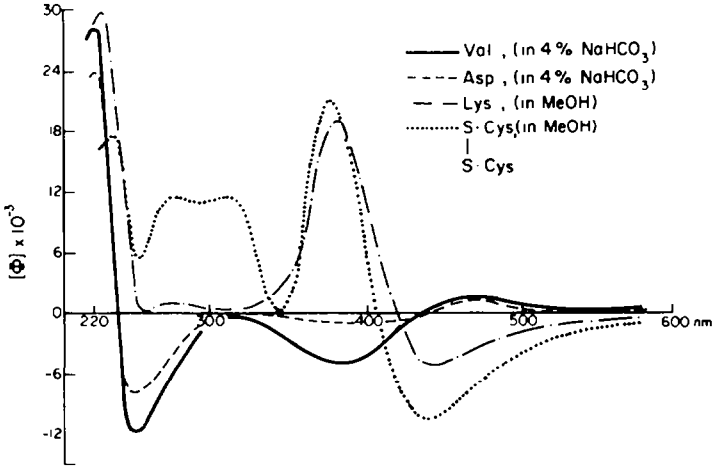

FIG 1. Some typical ORD curves of DNP amino acids

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in methanolic and in aqueous $NaHCO_3$ aq are listed in Tables 1 and 2. Two characteristic features are observed in these ORD-data: the first is the very big rotation ($[\phi] = +2 \sim 5 \times 10^4$) near 220 nm, especially in aqueous solution. This feature can be used to obtain the configuration (D or L) and further the optical purity (D/L-ratio) of the constituent amino acids of peptides containing D-α-amino acids such as some antibiotics.

TABLE 1. ORD-DATA OF DNP-AMINO ACIDS MEASURED IN MeOH

Parent Amino Acid	Molecular Rotation Value at Extrema near				
	220 nm	250 nm	300 nm	360 nm	450 nm
Alanine	+12200(220)	+2900(250,tr)	+ 5000(300,pk)		+ 130(450)
Valine	+14900(220,sh)	−3200(250,tr)	+ 2900(300)		− 250(455,tr)
Leucine	+15200(220,sh)	−330(250,tr)			− 470(450,tr)
Phenylalanine	+22400(224,pk)		+ 2800(300,tr)	+13000(360,pk)	− 4500(440,tr)
Tyrosine(di)	+ 8200(230,pk)		− 6500(308,tr)	+17000(360,pk)	− 5200(440,tr)
Aspartic Acid	+12000(220)	+1600(255,tr)	+ 5300(295,pk)	0(360)	+ 25(450)
Glutamic Acid	+12800(220)	+3200(250,tr)	+ 4800(320,pk)	+ 2800(350)	− 1650(440,tr)
Cystine(di)	+17400(234,pk)	+5200(252,tr)	+10400(280,pl)	+21000(374,pk)	−10400(440,tr)
Lysine(di)	+30000(225,pk)	0(250,tr)	0(300)	+20000(380,pk)	− 5000(440,tr)
Arginine	+14900(220)	+4700(250,pk)	+ 3000(300,tr)	+ 5100(350,pk)	− 730(450,tr)
Serine	+12300(220)	0(250,tr)	+ 3400(300,pk)		+ 50(450)
Proline	+15700(220,pk)	0(250,tr)	+25000(298,pk)		−13300(425,tr)

The numbers and letters in parentheses mean the wavelength where extrema are found and the types of extrema, respectively. −pk: peak, tr: trough, sh: shoulder, pl: plateau. When no extremum was found near the wavelength, no letters are in parentheses. Blanks mean that rotations could not be determined because of excess absorption.

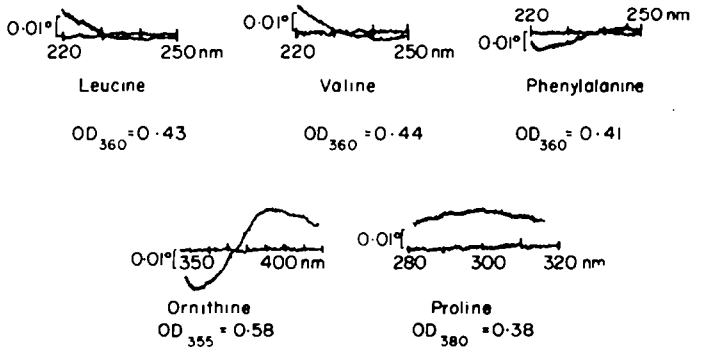
In order to confirm the usefulness, some synthetic mixtures of D- and L-aspartic acid were prepared and converted to the DNP-derivatives. Their rotations at 220 nm measured in aqueous solution are presented in Table 3 together with the optical density at 360 nm. The D/L-ratio calculated from the observed rotation shows good agreement with the prepared ratio. Thus, it should be possible to obtain the optical purity of amino acids in general if the R_1-values (Table 3) which are specific for each DNP-amino acid are pre-determined. Next, gramicidin S, a peptide antibiotic, was hydrolyzed, DNP-ylated and separated by means of two-dimensional paperchromatography. Each spot was cut out, extracted with $NaHCO_3$ aq, and the rotation and optical density of the extracts measured (Fig 2). The results show that only phenylalanine is in the D-form, and are consistent with the known configurations. It is convenient to use the rotation maxima of comparable strength at 300 nm for proline and 380 nm for ornithine, which are two of the five constituent amino acids of gramicidin S, since solvent absorption is negligibly small in the longer wavelength region but is significant near 220 nm to affect the accuracy of rotational measurements.

The second characteristic feature of the ORD-curves of the DNP-amino acids is the double Cotton effect centered near 360 nm observed only for those having two-DNP-residues per molecule, exemplified by di-DNP-cystine (Fig 1). To confirm

TABLE 2. ORD-DATA OF DNP-AMINO ACIDS MEASURED IN 4%-$NaHCO_3$

Parent Amino Acid	Molecular Rotation Extrema	
	Near 220 nm	Near 450 nm
Alanine	+26700	+ 2300
Valine	+28300	+ 2200
Leucine	+27400	+ 1800
Isoleucine	+33000	—
Phenylalanine	+37800	− 1700
Tyrosine	+12000	− 1700
Threonine	+32300	—
Serine	+37100	+ 4200
Tryptophan	+37300	—
Methionine	+29700	—
Cystine	+53100	− 7900
Aspartic Acid	+23900	+ 1500
Glutamic Acid	+25000	—
Proline	+11600	−11600
Hydroxypyproline	+ 7700	−19300
Histidine	+29200	− 2400
Arginine	+24300	—

the generality of this finding, some additional di-DNP-compounds were prepared and subjected to ORD-measurements. Fig 3 shows the ORD-curves of the di-DNP-derivatives and of δ-mono-DNP-ornithine for comparison. Similar double Cotton effects due to the interaction of two chromophores in a molecule are found for dibenzoates of steroidal glykols[3] and dinucleoside monophosphates[4] and can be explained by a dipole coupling mechanism.[5]



Extracted from PPC with 1% $NaHCO_3$ (l=1cm for OD; l=2cm for RD)

FIG 2. ORD curves of DNP-amino acids obtained from the hydrolysate of gramicidin S

Although the relation between the Cotton effects (sign and magnitude) and the spatial arrangement of the two DNP-groups is not elucidated, it can be said at least that the two DNP-groups must be close together to allow mutual coupling of the transition moments of each chromophore. In flexible molecules conformational equilibria should also play an important role in determining the magnitude of the Cotton effects. Hence, the observed difference in magnitude of the Cotton effects among various di-DNP-amino acids should be interpreted on the basis of two factors: possible conformational equilibria and the geometry of the two DNP-groups in each conformer.

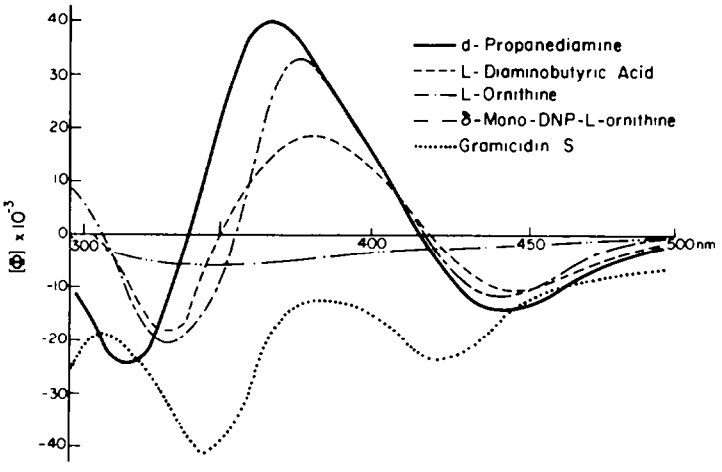


FIG 3. ORD curves of di-DNP-derivs of diamino-compounds in MeOH

Since gramicidin S has two free amino groups on the δ-carbon atoms of the two ornithine residues, it gives a di-DNP-derivative, which shows similar double Cotton effects in the same wavelength region (Fig. 3). This suggests that the two DNP-groups are close together in the favoured conformation, and supports the conformation proposed by Craig *et al.*[6] and Schwyzer *et al.*[7] in which the delta-amino groups of the ornithine residues lie on the same side of the decapeptide ring and in close proximity.

TABLE 3. MEASUREMENT OF OPTICAL PURITY OF DNP-ASPARTIC ACID

Mix. Ratio (D:L)	Opt. Purity Calc.	A_{360}	$100\alpha_{220}$	R	Opt. Purity Obs.
4:2	−0·33	0·65	−0·70	−2·15	−0·34
5:1	−0·67	0·55	−1·10	−4·00	−0·64
pure D	−1·00	0·52	−1·60	−6·15	−0·99

Optical Purity(calc.) = (L − D)/(L + D), $R = (100\alpha_{220}/A_{360}) \times (1/1')$.
Optical Purity(obs.) = R/R_1, $1 = 1{\cdot}0$ cm, $1' = 0{\cdot}5$ cm, $R_1 = +6{\cdot}21$.
R_1 is the R-value for the pure L-isomer, i.e. $R_1 = [\phi]_{220}/\varepsilon_{360}$.

It is also interesting to note that the magnitude of the Cotton effects of di-DNP-amino acids is sensitive to solvent MeOH to $NaHCO_3$ aq as well as to the difference in the chain length which separates the two DNP-groups, because it implies that the spatial arrangement of the two DNP-groups is a very important factor in determining the magnitude of the Cotton effect.

EXPERIMENTAL

Most DNP-amino acids were purchased from WAKO Pure Chemicals Industries Ltd. and used without further purification. Their mps. and molecular rotations are described together with the data reported by Rao *et al.*[8] (in parentheses) for purity criteria. The other DNP-derivatives were prepared by the procedure of Sanger.[9] Their mps. and elemental analyses are described. All rotations were measured at room temp. (10–30°) with a Jasco Model ORD/UV-5 Optical Rotatory Dispersion Recorder.

DNP-alanine. M.p. 176–178°, $[M]_D$ + 414° (c = 0·08 in N-NaOH). (M.p. 177°, $[M]_D$ + 367°).

DNP-valine. M.p. 132–134°, $[M]_D$ − 313° (c = 0·13 in AcOH). (M.p. 132°, $[M]_D$ − 79°).

DNP-glutamic acid. M.p. 143–145°, $[M]_D$ − 313° (c = 0·16 in AcOH). (M.p.: no description, $[M]_D$ −253°).

DNP-leucine. M.p. 98–100°, $[M]_D$ + 172° (c = 0·20 in 4%-$NaHCO_3$). (M.p. 94–95°, $[M]_D$ + 176°).

Di-DNP-cystine. M.p. 117–122°, $[M]_D$ − 1540° (c = 0·23 in 4%-$NaHCO_3$). (M.p. 118–121°, $[M]_D$ −1487°).

DNP-phenylalanine. M.p. 186–189°, $[M]_D$ − 232° (c = 0·23 in 4%-$NaHCO_3$). (M.p. 189°, $[M]_D$ − 261°).

DNP-serine. M.p. 176–180°, $[M]_D$ + 274° (c = 0·31 in 4%-$NaHCO_3$). (M.p. 173–174°, $[M]_D$ + 341°).

DNP-aspartic acid. M.p. 184–187°, $[M]_D$ + 301° (c = 0·35 in N-NaOH). (M.p. 186–187°, $[M]_D$ + 275°).

Di-DNP-lysine. M.p. 172–173°, $[M]_D$ − 166° (c = 0·09 in AcOH). (M.p. 170–172°, $[M]_D$ − 127°).

DNP-arginine. M.p. 222–226°, $[M]_D$ − 160° (c = 0·13 in AcOH). (M.p. 260°, $[M]_D$ − 121°).

DNP-proline. M.p. 137–139°, $[M]_D$ − 2325° (c = 0·015 in 4%-$NaHCO_3$). (M.p. 138°, $[M]_D$ − 2172°).

Di-DNP-tyrosine M.p. 175–180° (dec.), $[M]_D$ − 124° (c = 0·20 in AcOH). (M.p. 178–182°dec., $[M]_D$ − 60°); (Found: C, 47·23, H, 3·18, N, 13·24. $C_{21}H_{15}O_{11}H_2O$ requires: C, 47·46, H, 3·22, N, 13·18%).

Di-DNP-ornithine. M.p. 153–156°, ORD (c = 0·0020 in 4%-$NaHCO_3$): $[\phi]_{500}$ −16240°, $[\phi]_{460}$ −32480°(tr), $[\phi]_{440}$ 0°, $[\phi]_{390}$ +153120°(pk), $[\phi]_{370}$ 0°, $[\phi]_{355}$ −139200°(tr), $[\phi]_{325}$ 0°, $[\phi]_{300}$ +53360° (pk), $[\phi]_{255}$ 0°, $[\phi]_{250}$ −32480°(tr), $[\phi]_{240}$ 0°, $[\phi]_{224}$ +92000°; (c = 0·0184 in MeOH): $[\phi]_{450}$ −6300°, $[\phi]_{435}$ −8830°(tr), $[\phi]_{420}$ 0°, $[\phi]_{370}$ +37900°(pk), $[\phi]_{350}$ 0°, $[\phi]_{330}$ −27200°(tr), $[\phi]_{310}$ 0°, $[\phi]_{300}$ +7570°. (Found: C, 42·06, H, 3·62, N, 17·00. $C_{17}H_{16}O_{10}N_6$ H_2O requires: C, 42·33, H, 3·74, N, 17·42%).

Di-DNP-diaminobutyric acid. M.p. 120–122°, ORD(c = 0·0020 in 4%-$NaHCO_3$): $[\phi]_{490}$ −4500°, $[\phi]_{470}$ −9000°(tr), $[\phi]_{445}$ 0°, $[\phi]_{390}$ +29250°(pk), $[\phi]_{370}$ 0°, $[\phi]_{350}$ −36000°(tr), $[\phi]_{300}$ 0°; (c = 0·0040 in MeOH): $[\phi]_{490}$ −2250°, $[\phi]_{450}$ −9000°(tr), $[\phi]_{420}$ 0°, $[\phi]_{380}$ +20250°(pk), $[\phi]_{350}$ 0°, $[\phi]_{335}$ −18000° (tr), $[\phi]_{310}$ 0°, $[\phi]_{270}$ +9000°(pk), $[\phi]_{250}$ +4500°. (Found: C, 42·29, H, 3·41, N, 18·41, $C_{16}H_{14}O_{10}N_6$ requires: C, 42·67, H, 3·13, N, 18·66%).

Di-DNP-d-propanediamine. M.p. 273°, ORD(c = 0·0040 in dioxane): $[\phi]_{500}$ −2030°, $[\phi]_{450}$ −14210° (tr), $[\phi]_{415}$ 0°, $[\phi]_{365}$ +406008(pk), $[\phi]_{340}$ 0°, $[\phi]_{320}$ −24360°(tr), $[\phi]_{300}$ −1050°. (Found: C, 43·97, H, 3·38, N, 20·18. $C_{15}H_{14}O_8N_6$ requires: C, 44·34, H, 3·47, N, 20·69%).

Di-DNP-gramicidin S. M.p. 181°. ORD(c = 0·0050 in MeOH); $[\phi]_{450}$ −11784°. $[\phi]_{420}$ −23568°(tr), $[\phi]_{375}$ −11784°(pk), $[\phi]_{345}$ −41244°(tr), $[\phi]_{300}$ −29460°, $[\phi]_{250}$ −145000°, $[\phi]_{234}$ −276000°(tr). (Found: C, 57·12, H, 6·54, N, 14·79. $C_{72}H_{96}O_{18}N_{16}$ $2H_2O$ requires: C, 57·28, H, 6·68, N, 14·86%). Amino acid analysis showed the ratio δ-DNP-ornithine: ornithine = 99:1. It showed a single spot at R_f = 0·42 when developed with CCl_4:AcOH = 100:4·5 on a polyamide TLC-plate.[10]

δ-DNP-ornithine. The commercial product of Schwarz/Mann was used without further purification. ORD(c = 0·0040, MeOH): no significant Cotton effect was observed in the visible spectral range, but a faint trough was found near 340 nm. ($[\phi]_{340}$ −5960°), the maximal rotation so far measured (λ > 280 nm.).

Analysis of the configuration of the component amino acids in gramicidin S. ca. 1 mg. of gramicidin S was hydrolyzed with 6N-HCl at 110° in an evacuated sealed tube for 24 hr. The hydrolysate was evaporated, and the residue dinitrophenylated. A ½ portion of the mixture of DNP-amino acids obtained were separated by two-dimensional paperchromatography.[11] Each spot was removed and extracted with 3·0 ml 1%-$NaHCO_3$ aq. The optical densities and the rotations of each aqueous extract was measured at the appropriate wavelength region. Gramicidin J supplied from Nikken Kagaku Ltd. Co. was used as gramicidin S. The identity of Gramicidin J with gramicidin S is already proved.[12]

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