Upon DNP-aromatic Rule. Effect of Introducing a Methyl Group to the β-Position upon the CD Spectra of DNP Derivatives of Aromatic α-Amino Acids

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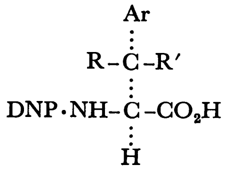
CD spectra of *N*-DNP-*O*-benzoyl derivatives of L-serine, L-threonine, and L-allothreonine and DNP derivatives of L-phenylalanine and (β*R*)- and (β*S*)-β-methyl-L-phenylalanines were studied. The effect of methyl substitution at the β-position upon their CD spectra was explained by the change in the population of the rotamer g^- ($\chi^1=-60°$) which was shown to be responsible for the characteristic negative Cotton effect at ≈400 nm. The negative sign of the $[\theta]^{\max}_{\approx 400}$ values of the DNP derivatives of aromatic L-α-amino acids described by DNP-aromatic rule was proved to be due to the contribution of the rotamer g^-.

The 2,4-dinitrophenyl (DNP) derivatives of L-α-amino acids having aromatic group on the side chain were found to show a negative Cotton effect around 400 nm. Extensive study upon the chiroptical properties of DNP-α-amino acids and related compounds[1–5] lead us to the proposal of DNP-aromatic rule[3] which predicts a negative $[\theta]_{\approx 400}$ for the compounds $DNP\cdot NHCHR_1R_2$ with the absolute configuration corresponding to the aromatic L-α-amino acids, where R_1 and R_2 are an aromatic and a nonaromatic groups, respectively. The R_1 group, which carries an aromatic chromophore, must not contain an additional chiral center, since *N*-DNP-*O*-benzoyl-L-threonine (**1a**) was found to exhibit the positive Cotton effect at 410 nm. The corresponding L-serine derivative (**1b**) showed normal CD spectrum expected as an analog of aromatic L-α-amino acid.[3)]



$$\begin{array}{c} Ar \\ \vdots \\ R\text{-}C\text{-}R' \\ \vdots \\ DNP\cdot NH\text{-}C\text{-}CO_2H \\ \vdots \\ H \end{array}$$

	Ar=OCOPh	Ar=Ph
$R=H, R'=CH_3$	**1a**	**2a**
$R=H, R'=H$	**1b**	**2b**
$R=CH_3, R'=H$	**1c**	**2c**

For the purpose of investigating the reason why the L-threonine derivative (**1a**), which has one extra methyl group at the β-position of the L-serine derivative (**1b**), exhibits an exceptional CD spectrum, *N*-DNP-*O*-benzoyl-L-allothreonine (**1c**) was prepared. The corresponding derivatives (**2a** and **2c**) of DNP-L-phenylalanine (**2b**) were also synthesized. As summarized in Table 1, **1c** exhibited a negative Cotton effect at 405 nm larger than that of **1b**; *i.e.*, the effect of methyl group in **1c** on $[\theta]^{\max}_{\approx 400}$ was opposite to that in **1a**. The similar tendency was observed in **2** series, though $[\theta]_{405}$ of **2a** remained negative; *i.e.*, the magnitude of the negative Cotton effects was in the order **2a**<**2b**<**2c**.

Relative orientation of the two chromophores, which is assumed to be the determining factor of the CD spectra, is regulated by the molecular conformation described by the three torsional angles ϕ, χ^1, and χ^2.[6)] Restricted rotation around $DNP\cdot NH\text{-}C^\alpha$ bond confines the ϕ value to very narrow regions near −160° and near −70°.[7)] Assuming that the Cotton effects are due to the exciton coupling between the transition moments of the two chromophores, the rotational strength (RS_1) at the longest wavelength band (400 nm) was calculated using the following equation:[8,9)]

$$RS_1=\frac{2\pi}{ch}\cdot\frac{\lambda_1\lambda_2}{\lambda_1^2-\lambda_2^2}\cdot\left(\frac{(\boldsymbol{\mu}_1\cdot\boldsymbol{\mu}_2)}{R^3}-\frac{3(\boldsymbol{\mu}_1\cdot\boldsymbol{R}_{21})(\boldsymbol{\mu}_2\cdot\boldsymbol{R}_{21})}{R^5}\right)\cdot[\boldsymbol{R}_{21}\cdot(\boldsymbol{\mu}_2\times\boldsymbol{\mu}_1)].$$

The results of the calculation for **2b** with varying χ^1 and χ^2 values were summarized in Fig. 1. As to the side chain conformation, the molecules can be considered to be populated around the three staggered conformations, g^- ($\chi^1=-60°$), g^+ ($\chi^2=+60°$), and t ($\chi^1=180°$), which were expected to exhibit negative, positive, and negligible or slightly negative Cotton effects, respectively. Essentially similar conclusion was obtained by the calculation for **1b**; *i.e.*, in both of **1** and **2** series, the conformer g^- was considered to contribute negative to the observed Cotton effect at ≈400 nm.

Conformational changes induced by the introduction of a methyl group at the β-position of **1b** and **2b** were studied by ^{1}H-NMR spectra as summarized in Table 2.[10)] As shown in Fig. 2 the *ortho*-proton of the DNP group of **2** was expected to experience an anisotropic shielding effect of the phenyl ring in the conformer g^-, which was considered to be responsible for the negative Cotton

Table 1. CD spectral data recorded in methanol

Compound	Molecular ellipticity($[\theta]\times 10^{-2}$) and wavelength(λ/nm) in parentheses[a)]
1a	+34(410),0(360),−19(337),0(290),−135(227),0(213)
1b	−34(408),0(374),+77(340),+20(290),+117(238),0(219)
1c	−80(407),0(361),+73(330),+25(290),+67(270sh),+190(236),+147(220)
2a	−42(405),0(347),+16(320),+13(300),+30(272),0(256),−99(233),0(224)
2b	−91(410),0(363),+74(334),+21(296),+46(270),+23(254),+590(212)
2c	−102(410),−44(350sh),0(316),+70(268),+52(253)

a) sh: Shoulder.

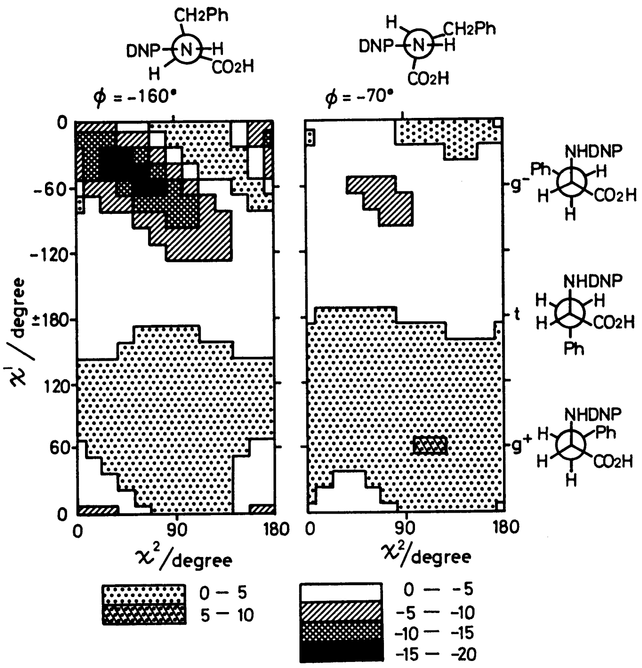


Fig. 1. Calculated rotatory strength (in 10^{-40} c.g.s.) at 400 nm of DNP-L-phenylalanine (**2b**).

Table 2. ^{1}H-NMR spectral data

Compound	Chemical shift of aromatic protons[a] (δ)				Coupling constant between $C^\alpha H$ and $C^\beta H$ (J/Hz)
	o	*m*	*m′*	Ph	
1a[b]	7.50	8.40	9.07	7.57,7.70,8.09	2.8
1b[b]	7.45	8.35	8.94	7.52,7.65,7.97	9.2,7.9
1c[b]	7.42	8.30	9.04	7.49,7.65,7.95	2.9
2a[c]	7.12	8.27	9.01	7.37	6.62
2b[c]	7.05	8.24	9.03	7.27	6.63,4.96
2c[c]	6.69	8.06	9.02	7.31	4.47

a) For notations *o*, *m*, *m′*, see Fig. 2. b) Recorded in CD_3COCD_3. c) Recorded in CD_3OD.

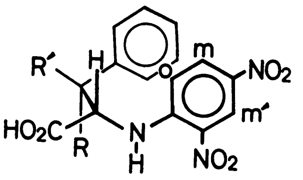


Fig. 2. Conformer g^- of **2a**—**c** in which anisotropic shielding effect of phenyl ring upon the *o*-proton of the DNP group is expected.

effect around 400 nm. The significant difference observed in the chemical shifts of the *ortho*-protons in **2b** and its methyl substituted analogs seem to indicate decrease and increase in the population of the g^- conformer caused by the presence of a methyl group in **2a** and **2c**, respectively, which is consistent with the CD spectral data. In the case of **1b** and its methylated derivatives (**1a** and **1c**), the difference in the chemical shifts of the *ortho*-protons is not prominent, though the same tendency as found in **2** series was observed. The coupling constant between $C^\alpha H$ and $C^\beta H$, however, clearly indicated that the population of g^- conformer is very small for **1a**; *i.e.*, the small coupling constant (2.8 Hz) indicated the two protons to take gauche instead of *trans* positions, to which the g^- conformer corresponds. Thus, the positive $[\theta]_{410}$ value observed for **1a** is explained by significant decrease of the g^- conformer caused by introduction of the methyl group.

In conclusion, the g^- conformer was shown to be responsible for the negative Cotton effect around 400 nm and the effect of methyl substitution at the β-position upon the CD spectra was explained by the change in population of the g^- conformer. Combination of conformational analysis and rotational strength calculation seem to be a useful method for interpreting or predicting chiroptical properties of compounds which show CD spectra due to exciton coupling.

Experimental

Melting points were uncorrected. Compounds **1b** and **2b** were already described in Ref. 3.

N-*DNP-O-benzoyl-*L-*threonine (1a).* DNP-L-threonine (713 mg, 2.5 mmol) was benzoylated with Schotten-Baumann method using benzoyl chloride (0.87 cm^3, 7.5 mmol) and NaOH (10 mmol). Crystallization from acetone–benzene afforded **1a** (76 mg) as yellow needles: mp 184—187 °C. Found: C, 52.15; H, 3.85; N, 10.75%. Calcd for $C_{17}H_{15}N_3O_8$: C, 52.45; H, 3.88; N, 10.79%.

N-*DNP-O-benzoyl-*L-allo*threonine (1c).* This compound was prepared in the same manner as **1a**, starting from the corresponding L-*allo*threonine derivative: yellow powder; mp 176—177 °C.

As for **2a** and **2c**, the compounds actually synthesized were their enatiomers as described below, starting from the samples supplied by Prof. S. Kuwata of Kōnan University.

*(2*R*, 3*R*)-*N*-DNP-2-amino-3-phenylbutanoic Acid (Enantiomer of 2a).* 2,4-Dinitrophenylation of 38 mg (0.2 mmol) of (2*R*, 3*R*)-2-amino-3-phenylbutanoic acid ($[\alpha]_D^{20}$ +24.2°; *c* 1, H_2O)[11] with the procedure of Sanger[12] afforded the enantiomer of **2a** (45 mg): yellow prisms from ethyl acetate–benzene; mp 169—170 °C. Found: C, 55.59; H, 4.41; N, 12.18%. Calcd for $C_{16}H_{15}N_3O_6$: C, 55.65; H, 4.38; N, 12.17%.

*(2*R*, 3*S*)-*N*-DNP-2-amino-3-phenylbutanoic Acid(Enantiomer of 2c).* This compound was synthesized from (2*R*, 3*S*)-2-amino-3-phenylbutanoic acid ($[\alpha]_D^{22}$ +6.90°; *c* 1, H_2O)[11] in the same manner as described above. Yellow prisms from ethyl acetate–benzene; mp 159—160 °C. Found: C, 55.58; H, 4.32; N, 12.08%. Calcd for $C_{16}H_{15}N_3O_6$: C, 55.65; H, 4.38, N, 12.17%.

Measurements. CD spectra were recorded on a JASCO spectropolarimeter model J-20 or J-40 at room temperature. PMR spectral data given in Table 2 were measured at 270 MHz on a Bruker WH-270 spectrometer at room temperature.

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References

1) M. Kawai, U. Nagai, and M. Katsumi, *Tetrahedron*

Lett., **1975**, 2845.

2) M. Kawai and U. Nagai, *Tetrahedron Lett.*, **1977**, 3889.

3) M. Kawai, U. Nagai, M. Katsumi, and A. Tanaka, *Tetrahedron*, **34**, 3435 (1978).

4) U. Nagai, M. Katsumi, T. Yamada, S. Kuwata, and H. Watanabe, *Tetrahedron Lett.*, **1981**, 653.

5) U. Nagai, N. Taki, and M. Kawai, *Chem. Pharm. Bull.*, **29**, 1750 (1981).

6) IUPAC-IUB Comission on Biochemical Nomenclature, *Biochemistry*, **9**, 3471 (1970).

7) M. Kawai and U. Nagai, *Bull. Chem. Soc. Jpn.*, **55**, 1327 (1982).

8) J. A. Schellman, *Acc. Chem. Res.*, **1**, 144 (1968).

9) For the calculation of the rotatory strengths (RS_1), each chromophore was assumed to have the following electric transition moments (wavelength (λ) in nm, dipole strength ($|\mu|^2$) in 10^{-36} c.g.s. and the direction of the moment in degree with respect to the long axis of the chromophore): DNP·NH 400, 8.6, 44;[13,14] $PhCO_2$ 230, 13.3, 6.5;[15] 185, 27.3, 4;[14] Ph 208.5, 7.5, 0; 182.8, 20.9, 0; 182.8, 20.9, 90.[16]

10) ^{1}H-NMR spectra of **1a**—**c** were recorded in acetone-d_6 instead of methanol-d_4. Similar CD spectra were observed in acetone, too, as follows: **1a**, $[\theta]_{403}$ +3100; **1b**, $[\theta]_{407}$ −4200; **1c**, $[\theta]_{402}$ −6900.

11) Y. Kataoka, Y. Seto, M. Yamamoto, T. Yamada, S. Kuwata, and H. Watanabe, *Bull. Chem. Soc. Jpn.*, **49**, 1081 (1976).

12) F. Sanger, *Biochem. J.*, **39**, 507 (1945).

13) M. Kawai, U. Nagai, and T. Kobayashi, *Tetrahedron Lett.*, **1974**, 1881.

14) The direction of the electric transition moment was given by a modified SCF-MO-CI calculaion; Z. Yoshida and T. Kobayashi, *J. Chem. Phys.*, **54**, 4538 (1971).

15) J. Tanaka, *Bull. Chem. Soc. Jpn.*, **36**, 833 (1963).

16) S. Nagakura and K. Kimura, *Nippon Kagaku Zasshi*, **86**, 1 (1965).