

A METHOD FOR DETERMINING ABSOLUTE CONFIGURATION OF  $\beta$ -AMINO ACIDS  
BY CD SPECTRA OF THEIR DNP DERIVATIVES

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Summary: Aromatic  $\beta$ -amino acids were converted to the Dnp derivatives, and aliphatic ones to Dnp-pMA derivatives. The sign of the Cotton effect near 400 nm reflected the configuration at the  $\beta$ -position without exception. Thus, the relation seems to afford a new reliable method for determining the absolute configuration of  $\beta$ -amino acids.

N-2,4-Dinitrophenyl(Dnp) derivatives of aromatic  $\alpha$ -amino acids exhibit characteristic CD spectra above 300 nm. The sign of the band near 400 nm has high degree of correlation to the absolute configuration at the  $\alpha$ -carbon atom (Dnp-aromatic rule).<sup>1)</sup> The characteristic CD pattern is considered to be caused by interaction of the Dnp- and the aromatic chromophores. CD spectra of similar pattern but of opposite sign were observed for *p*-methoxyanilides (pMA) of aliphatic Dnp- $\alpha$ -amino acids with the same configuration.<sup>2)</sup> In this paper, extension of the two rules to  $\beta$ -amino acids is described.

Some antibiotic peptides, such as bottromycin, bleomycin, iturin A, blastocidin S, and so on, contain  $\beta$ -amino acids as their components. Since usual enzymatic methods cannot be applied to  $\beta$ -amino acids, chiroptical methods are precious for determining their configuration. Only a few papers appeared in literature concerning the chiroptical relations of  $\beta$ -amino acid derivatives,<sup>3-6)</sup> and the relations are not always satisfactory for determining the configuration of various  $\beta$ -amino acids because some exceptional cases were pointed out in each case.<sup>7,8)</sup> The method to measure the CD spectra of dicyclohexylammonium(DCHA) salts of N-dithiocarbamoyl derivatives in CHCl<sub>3</sub> or benzene seems to be the most reliable so far reported.<sup>6)</sup>

For the purpose of offering a new method for determining the absolute configuration of  $\beta$ -amino acids and of confirming the scope of the Dnp-aromatic rule in its generalized form,<sup>9)</sup> CD spectra of a number of Dnp- $\beta$ -amino acids and their pMA derivatives were measured.

Figs. 1 and 2 reproduce CD spectra of the Dnp-derivatives of five aromatic  $\beta$ -amino acids and Dnp-pMA derivatives of five aliphatic  $\beta$ -amino acids, respectively. The CD curves indicate that the same rule as for  $\alpha$ -amino acids are applicable also to  $\beta$ -amino acid derivatives: i.e., in the case of L-series compounds the Cotton effect of the band near 400 nm has negative sign for the Dnp-derivatives of aromatic amino acids, and positive sign for the Dnp-pMA derivatives of aliphatic amino acids. The relation will be useful for determining the stereochemistry of newly found  $\beta$ -amino acids.

In fact, the stereochemistry of an iturinic acid was assigned by this method,<sup>10)</sup> and the

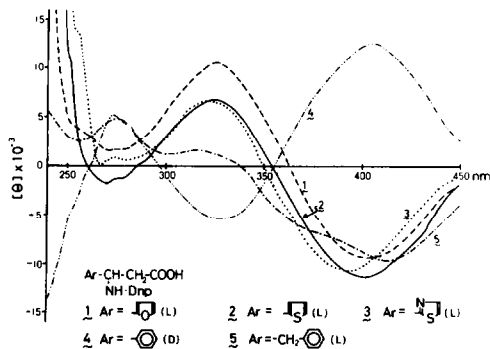


Fig. 1: CD Spectra of Dnp-derivatives of aromatic  $\beta$ -amino acids

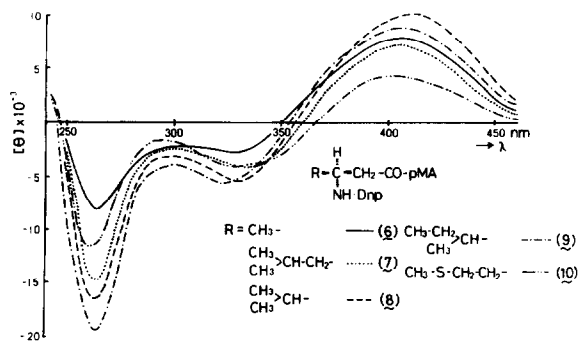
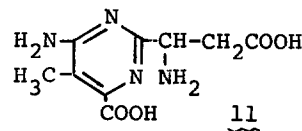


Fig. 2: CD Spectra of Dnp-L- $\beta$ -aminoacyl-pMA

assignment has been further confirmed by the present results. Moreover, the rule was applied to a  $\beta$ -amino acid obtained from the hydrolysate of tallysomicin,<sup>11)</sup>  $\beta$ -amino- $\beta$ -(4-amino-6-carboxy-5-methylpyrimidin-2-yl)propionic acid (11), whose configuration was not determined yet due to the scarcity of the sample. Its Dnp-derivative showed positive Cotton effect near 400 nm ( $[\theta]_{\max} = +910$ ) indicating D-configuration at the  $\beta$ -carbon atom. The same compound isolated from bleomycin has also D-configuration.<sup>7)</sup> The low  $[\theta]_{\max}$ -value would probably be due to partial racemization during hydrolysis.

Dinitrophenylation was carried out according to the Sanger's procedure,<sup>12)</sup> and pMA derivatives were prepared by treating the Dnp- $\beta$ -amino acids dissolved in  $\text{CH}_2\text{Cl}_2$  with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide, 1-hydroxybenzotriazole, and *p*-anisidine.<sup>13)</sup> The purity and structure of each compound were confirmed by TLC,  $^1\text{H-NMR}$ , and elemental analyses except 11, which could not be purified completely.



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