Chem. Pharm. Bull. 29(6)1750—1752(1981)

Chiroptical Properties of the N-2,4-dinitrophenyl(Dnp)-Derivatives of Unsaturated α-Amino Acids: Extension of the Dnp-Aromatic Rule

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(Received December 19, 1980)

The circular dichroism spectra of the N-2,4-dinitrophenyl(Dnp)-derivatives of some aliphatic olefinic and acetylenic α-amino acids were examined in order to compare them with those of N-Dnp-α-amino acids with alkyl and aryl side chains. All of the L-series compounds showed negative Cotton effects of moderate strength near 400 nm. The results were explained by application of exciton chirality theory to the probable conformers. Such an explanation can also be extended to S-containing and aromatic amino acids. Thus, the results should be useful for assigning the absolute configurations of new amino acids and related amines.

Keywords——unsaturated amino acids; absolute configuration; Dnp-derivatives; CD spectra; Dnp-aromatic rule

The N-2,4-dinitrophenyl(=Dnp) derivatives of aromatic L-α-amino acids show strong negative Cotton effects ($[\theta]_{max} \approx -10000$) near 400 nm (Dnp-aromatic rule), but those of aliphatic ones commonly show only very weak Cotton effects of variable sign ($-2000 < [\theta]_{max} < +2000$) at this wavelength.[1] Aliphatic unsaturated α-amino acids are intermediate between the aromatic and saturated aliphatic ones from the viewpoint of side-chain unsaturation. Thus, the circular dichroism(CD) spectral behaviour of their Dnp-derivatives has attracted our interest. Hatanaka *et al.* have isolated a number of unsaturated L-α-amino acids from several species of mushrooms and elucidated the structures.[2] The present study was begun with samples kindly provided by Professor Hatanaka.

Experimental

The unsaturated amino acids were converted to the Dnp-derivatives according to the procedure described by Sanger,[3] and the derivatives were purified by silica gel chromatography and through formation of the dicyclohexylammonium salt. Purities of the Dnp-derivatives were checked by TLC (silica gel; $CHCl_3$: MeOH: AcOH=95: 5: 1 or 90: 10: 1) and ^{1}H-NMR. They all showed reasonable ^{1}H-NMR spectra, although traces of impurities were detected in addition to the single major spot on TLC for some of the samples. It was judged that the derivatives were sufficiently pure for the present purpose.

CD spectra were all measured in MeOH with a Jasco J-40A automatic recording spectropolarimeter. Concentrations of the solutions were estimated from their optical densities at the absorption maxima (348 nm) using a mean molecular absorption coefficient of $\varepsilon = 17500$.

Results and Discussion

All of the Dnp-derivatives exhibited negative Cotton effects near 400 nm, as shown in Figs. 1 and 2. The $[\theta]_{max}$-values range from −1800 to −7100, which are intermediate between those of aromatic and aliphatic saturated compounds.

These results can be explained on the basis of exciton chirality theory.[4] By application of the theory to the present case negative Cotton effects were calculated for the interaction of the Dnp-band near 400 nm with the chromophore in the R_2 group (see Formula 1) for most of the probable conformers. Hence, statistical averaging of the contributions from various probable conformers is considered to give a negative value as a whole. The magnitude of the Cotton effect depends largely on the strength (ε_{max}) and position (λ_{max}) of the absorption of the R_2 chromophore, that is, larger ε_{max} and longer λ_{max} yield a larger Cotton effect. Similarly,

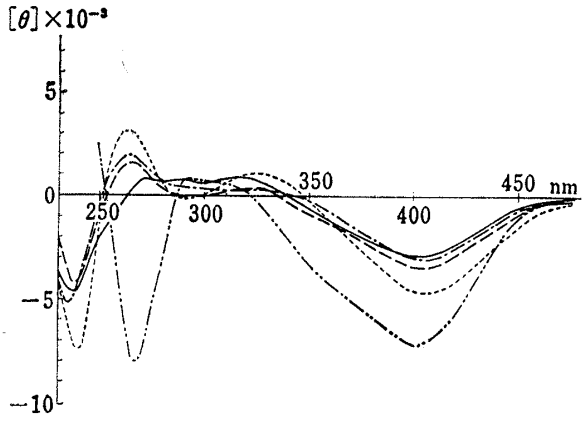


Fig. 1. CD Spectra of Dnp-L-α-amino Acids (DnpNH-CHX-COOH) bearing an Olefinic Bond in the Side-chain

X=
——— $CH_2=C(Cl)-CH_3$
- - - - $CH_2=C(COOH)-CH_2-$
-------- $CH_3-CH_2-CH=C(COOH)-CH_2-$
——— $CH_2=CH-CH_2-$
-------- $CH_3-CH_2-C(=CH_2)-$

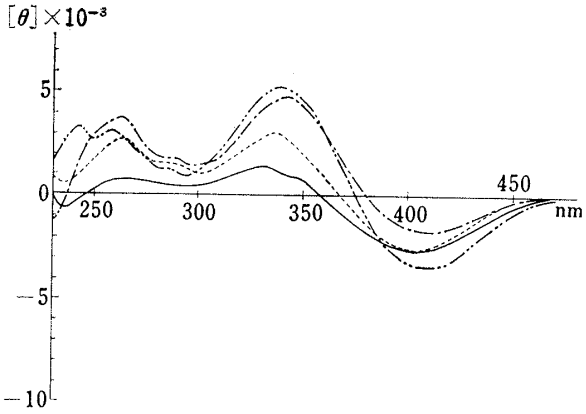
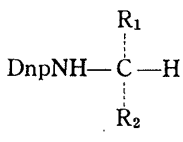


Fig. 2. CD Spectra of Dnp-L-α-amino Acids (DnpNH-CHX-COOH) beaing an Acetylenic or an Allenic Bond in the Side-chain

X=
——— $CH_2=C=CH-CH_2-$
-------- $HC\equiv C-CH_2-$
——— $CH_3-C\equiv C-CH_2-$
-------- $HO-CH_2-C\equiv C-CH_2-$

a positive Cotton effect is expected for the interaction of the Dnp-band with R_1 chromophore. In the present case, both interactions, Dnp-olefin and Dnp-carboxyl, must be considered. Since ethylene has λ_{max} at 193 nm (ε_{max}=10000) and acetic acid has λ_{max} at 160 nm (ε_{max}=2500 ~4200),[5] the contribution of the former apparently exceeds that of the latter to result in the observed negative Cotton effect near 400 nm.

It is interesting to note that Dnp-L-methionine and Dnp-S-methyl-L-cysteine exhibit moderately strong Cotton effects near 400 nm ($[\theta]_{max}\approx$ −4000), as a dialkyl sulfide has moderately strong UV absorption near 200 nm (ε_{max}=4000).[5] Since an aromatic group is known to have strong UV absorption (*e.g.* toluene, λ_{max}=183 nm, $\varepsilon_{max}\approx$70000), the Dnp-aromatic rule[2] is consistent with the above explanation. Thus, we should usually be able to predict the sign of the Cotton effect near 400 nm of any compound represented by Formula 1 on the basis of the considerations discussed above if no additional asymmetric center exists.



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Formula 1

These results should be useful for assigning the absolute configurations of new amino acids and related amines. For example, it is applicable to S-farnesyl-cysteine, which was recently found as a component of the mating-inducing lipopeptide factor of a species of yeast.[6]

Acknowledgement The authors are grateful to Professor Hatanaka for his generous gift of amino acid samples.

References and Notes

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[Chem. Pharm. Bull. 29(6)1752—1754(1981)]

Studies on Peptides. CI.[1,2] Synthesis of a Wasp Venom, Mastoparan M[1,2]

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(Received December 25, 1980)

A tetradecapeptide amide, H–Ile–Asn–Leu–Lys–Ala–Ile–Ala–Ala–Leu–Ala–Lys–Lys–Leu–Leu–NH_2, corresponding to the entire amino acid sequence of a wasp venom, mastoparan M, was synthesized using the thioanisole-mediated trifluoroacetic acid deprotecting procedure.

Keywords——wasp venom; mastoparan; mastoparan X; mast cell degranulating activity; histamine release from mast cell; TFA-thioanisole deprotection

Mastoparan M is a tetradecapeptide amide isolated from *Vespa mandarinia*[3] and its amino acid sequence was shown to have a much closer structural homology to those of previously isolated wasp venoms, mastoparan X[4] and mastoparan,[5] than to that of polistes mastoparan[6] (Fig. 1).

Mastoparan M	H–Ile–Asn–Leu–Lys–Ala–Ile–Ala–Ala–Leu–Ala–Lys–Lys–Leu–Leu–NH_2
Mastoparan X	H–Ile–Asn–Trp–Lys–Gly–Ile–Ala–Ala–Met–Ala–Lys–Lys–Leu–Leu–NH_2
Mastparan	H–Ile–Asn–Leu–Lys–Ala–Leu–Ala–Ala–Leu–Ala–Lys–Lys–Ile–Leu–NH_2
P. Mastoparan	H–Val–Asp–Trp–Lys–Lys–Ile–Gly–Gln–His–Ile–Leu–Ser–Val–Leu–NH_2

Fig. 1. Amino Acid Sequences of Wasp Venoms

This new mast cell degranulating peptide has now been synthesized using available peptide fragments from our previous syntheses of mastoparan X[7] and mastoparan,[8] according to the scheme illustrated in Fig. 2.

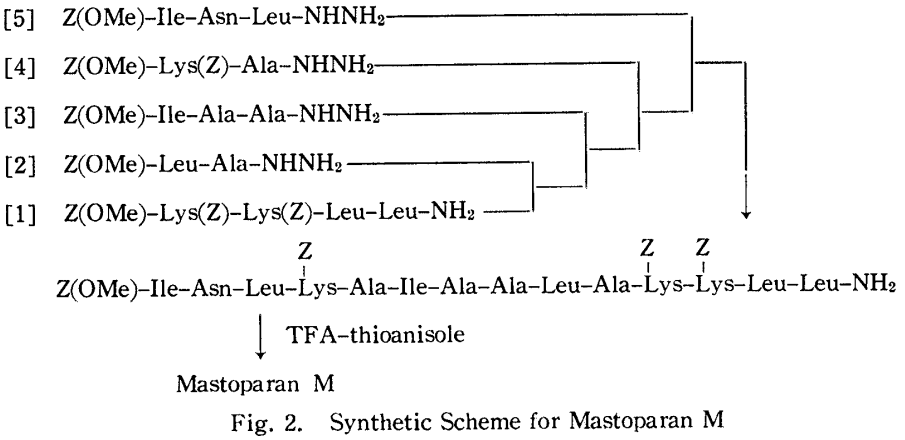

Fig. 2. Synthetic Scheme for Mastoparan M