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ABSOLUTE CONFIGURATION OF AN ITURINIC ACID AS DETERMINED BY CD SPECTRUM OF ITS DNP-p-METHOXYANILIDE

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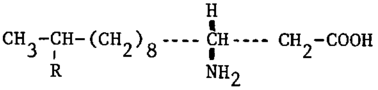
Summary: Absolute configuration of the long chain β-amino acid obtained from the hydrolysate of iturin A has been determined to be R by application of Dnp-aromatic rule. This is the first assignment for the chirality of an iturinic acid.

Recently, Kawai and Nagai reported that Dnp-aromatic rule1) can be used for determining the absolute configuration of aliphatic α-amino acids by deriving them to the N-2,4-dinitrophenyl-*p*-methoxyanilides (=Dnp-pMA)2). The method is applicable also to amino acids having the amino group at other positions than α3). Because the CD spectra of N-Dnp-aminoacyl-pMA have their origin in interaction of the Dnp and the pMA groups, and insertion of a few methylene groups between them does not alter the nature of the interaction significantly, as exemplified in the case of Dnp-Phe homologs1).

In 1965, Delcambe reported the isolation of a lipophilic antifungal peptide, iturin, produced by *Bacillus subtilis*4). Peypoux *et al.* purified the antibiotic, found new β-amino acids as its components, and elucidated the structure5). They were isolated as a mixture of two compounds having the structures, I and II. But, the stereochemistry remained undetermined.



$$\underset{\displaystyle R}{CH_3\text{-}CH}\text{-}(CH_2)_8\text{----}\overset{\displaystyle H}{\underset{\displaystyle NH_2}{CH}}\text{----}CH_2\text{-}COOH$$

I:R=CH_3, II:R=C_2H_5

In 1977, Besson *et al.* have found a series of homologous long chain β-amino acids in bacillomycin L.6). Delcambe *et al.* proposed to call such β-amino acids iturinic acids7). Iturinic acids are also found in some other antibiotics produced by *Bacillus subtilis*.

So, we have planned to determine the absolute configuration of iturinic acids by application of the extended Dnp-aromatic rule2,3). A mixture of I and II was prepared by hydrolysis of iturin A5), converted to the corresponding Dnp-pMA derivative, and subjected to CD measurement. Dinitrophenylation was carried out according to a modified Sanger's procedure8) starting from 10 mg of the β-amino acid mixture. The Dnp derivative obtained was treated with *p*-methoxyaniline, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, and 1-hydroxybenzotriazole in methylene chloride9). After usual work-up, the neutral fraction was purified by preparative TLC to yield the desired product (7 mg) in crystalline form: m.p. 107-108°, UV(MeOH): λ_{max} 347 nm(ε 17600);

Elem. Anal., Found: C,63.51; H,7.89; N,10.53. Calcd. for $C_{27.5}H_{39}O_6N_4$: C,63.28; H,7.53; N,10.73 %. The nmr spectrum measured in $CDCl_3$ also confirmed the structure. The above molecular formula is the averaged one of two formulas $C_{27}H_{38}O_6N_4$ and $C_{28}H_{40}O_6N_4$ because the starting material is known to be a mixture of about equal proportion of C_{14} and C_{15} iturinic acids from analysis of the MS spectrum.

Fig. 1 reproduces the CD spectrum of the Dnp-iturinyl-pMA together with that of Dnp-L-Ala-pMA for comparison. The results indicate apparently that both I and II have D-configuration at C-3. We should, however, be careful to draw a conclusion since we have measured the CD spectrum of a mixture. There are four possible combinations for the chirality of I and II; R-R, R-S, S-R, and S-S. Among them only the first possibility is consistent with the observed CD curve. In cases of the second and the third possibility, the mixture (C_{14}/C_{15}=0.93/1) can be regarded as quasi-racemic and very weak Cotton effect must be observed by mutual cancellation in contradiction to the fairly strong Cotton effect shown in Fig. 1. The lower intensity of the Cotton effect than that of L-Ala derivative would be mainly due to the different distance between the two chromophores and to some factors caused by different steric hindrances by the methyl and the long alkyl side-chains. Therefore, we can now conclude that both I and II have D-configuration at C-3. Biogenetic consideration and the difficulty experienced in resolving the parent peptides containing I and II also suggest that I and II have the same chirality at C-3. This paper is the first report concerning the stereochemistry of iturinic acids, and usefulness of the extended Dnp-aromatic rule for determining absolute configuration of newly found amino acids has been demonstrated.

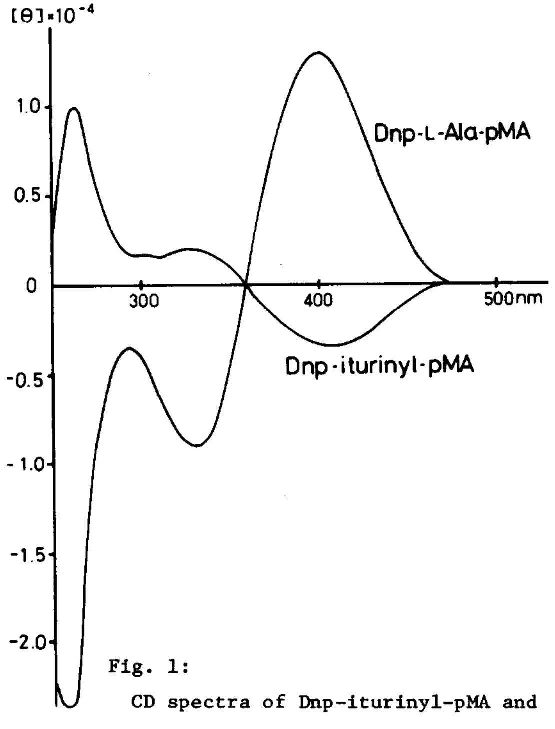


Fig. 1:
CD spectra of Dnp-iturinyl-pMA and Dnp-L-Ala-pMA measured in MeOH.

REFERENCES

1) M.Kawai, U.Nagai and M.Katsumi, *Tetrahedron Letters*, 2845-2848 (1975)
2) M.Kawai and U.Nagai, *ibid.*, 3889-3890 (1977)
3) M.Kawai, U.Nagai, M.Katsumi, and A.Tanaka *Tetrahedron*, 34, 3435-3444 (1978)
4) L.Delcambe, *Bull. Soc. Chim. Belge*, 74, 315-340 (1965)
5) F.Peypoux, M.Guinand, G.Michel, L.Delcambe, B.C.Das, P.Vareune, and E.Lederer, *Tetrahedron*, 29, 3455-3459 (1973)
6) F.Besson, F.Peypoux, G.Michel, and L. Delcambe, *Eur. J. Biochem.*, 77, 61-67 (1977)
7) L.Delcambe, F.Peypoux, M.Guinand, and G. Michel, 10th International Symposium on the Chemistry of Natural Products, Dunedin, N.Z.
8) F.Sanger, *Biochem. J. London*, 39, 507 (1945)
9) J.C.Sheehan, J.Preston, and P.A.Cruickshank, *J. Am. Chem. Soc.*, 87, 2492-2493 (1965); S.Nozaki, A.Kimura, and I.Muramatsu, *Chem. Lett.*, 1057-1058 (1977)

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