NOTE

Absolute Configurations of Mytiloxanthin and 9-*E*-Mytiloxanthin

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Abstract : Absolute configurations of mytiloxanthin and 9-*E*-mytiloxanthin isolated from the mussel *Mytilus coruscus* were confirmed to be 3*R*, 3'*S*, 5'*R* by using modified Mosher's method.

Key words : Carotenoid, Mytiloxanthin, Absolute configuration, NOE, Mosher's method

1 Introduction

Mytiloxanthin (1), a red carotenoid which was first isolated from the mussel *Mytilus californianus* by Sheer¹⁾, is wide spread in marine shellfish and sea squirt^{2),3)}. 9-*E*-Mytiloxanthin (2) is also occurred with 1 as a minor component in these marine animals. In 1973, Khare *et al.*⁴⁾, proposed structure of 1 to be 3,3',8'-trihydroxy-7,8-didehydro- β,κ -caroten-6'-one by chemical and spectroscopic studies. Subsequently, Chopra *et al.*^{5),6)}, reported the total synthesis of 9-*E*-(3*R*,3'*S*,5'*R*)-mytiloxanthin and proposed the same chirality for natural 1 by comparison of stereomutation product of 1 on thin layer chromatography (TLC). However, the absolute configurations of natural 1 and 2 have not been determined yet since the circular dichroism spectra (CD) of 1 and 2 are extremely weak.

On the other hand, it is well known that the Mosher's method⁷⁾ and modified Mosher's method^{8),9)} using [α -methoxy- α -(trifluoromethyl)phenylacetic acid (MTPA) ester are convenient and important chemical process for determination of the absolute configuration of secondary alcohols and amines. Thus we have applied this method for the determination of the absolute configuration of natural 1 and 2. This paper reports the elucidation of the absolute stereostructures of 1 and 2 isolated from the mussel *M. coruscus*.

2 Experimental

2.1 General experimental procedures

UV and visible light absorption spectra (UV-VIS) and CD were recorded in ether with a Shimadzu UV-240 and Jasco J-500 C, respectively. ¹H-NMR spectra were measured at 300 MHz in CDCl₃ on a Varian XL-300 with TMS as internal standard at 22~24°C. Mass spectra (MS) were measured with JEOL SX 102A. Preparative TLC was carried out on a plate (20×20 cm, 0.5 mm thick) coated with silica gel 60 (Merck) using hexane/acetone (vol/vol, 7 : 3) as solvent. High performance liquid chromatography (HPLC) was performed on a Shimadzu LC-6 AD instrument with a Shimadzu SPD-6 AV spectrophotometer set at 460 nm. The column used was a Shim-pack PREP-ODS with a mobile phase of CH₃CN/CH₂Cl₂ (vol/vol, 8 : 2).

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2.2 Isolation of mytiloxanthin (1) and 9-*E*-mytiloxanthin (2)

According to the method described previously¹⁰⁾, **1** (8 mg) and **2** (2 mg) were isolated from *M. coruscus* (20 kg, 30 specimens) purchased from the fish market in Toba city.

Mytiloxanthin (**1**) showed UV-VIS : 294 and 469 nm. CD : nm ($\Delta\epsilon$) 225 (-0.5), 280 (0), 294 (+0.7), 305 (0) and 360 (-1.0). EI-MS : m/z (relative intensity) 598 $[M]^+$ (23%), 580 $[M-18]^+$ (5), 562 $[M-36]^+$ (1), 506 $[M-92]^+$ (4), 471 $[M-127]^+$ (1), 401 $[M-197]^+$ (20), 197 (17), 179 (25), 109 (90) and 92 (100). $^1\text{H-NMR}$ (Table 1).

9-*E*-Mytiloxanthin (**2**) : UV-VIS 358 and 465 nm. CD : nm ($\Delta\epsilon$) 230 (-0.8), 247 (0), 258 (+1.0), 269 (0), 295 (-1.6), 318 (0), 350 (+1.1) and 368 (0). EI-MS : m/z 598 $[M]^+$ (20%), 580 $[M-18]^+$ (5), 562 $[M-36]^+$ (1), 506 $[M-92]^+$ (3), 471 $[M-127]^+$ (1), 401 $[M-197]^+$ (5), 197 (6), 179 (20), 109 (50) and 92 (100). $^1\text{H-NMR}$ (Table 1).

Table 1 $^1\text{H-NMR}$ (300 MHz) Data for Mytiloxanthin (**1**) and 9-*E*-Mytiloxanthin (**2**) in CDCl_3 .

H	δ Values in ppm: multiplicity (coupling constants in Hz)	
	Mytiloxanthin (1)	9- <i>E</i> -Mytiloxanthin (2)
H-2 ax	1.46 d, d (12, 12)	1.49 d, d (12, 12)
H-2 eq	1.84 d, d, d (12, 3.5, 2)	1.86 d, d, d (12, 3.5, 2)
H-3	3.99 d, d, d, d (12, 9.5, 5.5, 3.5)	4.01 d, d, d, d (12, 9.5, 5.5, 3.5)
H-4 ax	2.07 d, d (17.5, 9.5)	2.09 d, d (17.5, 9.5)
H-4 eq	2.43 d, d, d (17.5, 5.5, 2)	2.46 d, d, d (17.5, 5.5, 2)
H-10	6.46 d (11.5)	6.31 d (11)
H-11	6.54 d (14.5, 11.5)	6.87 d, d (15, 11)
H-12	6.36 d (14.5)	6.35 d (15)
H-14	6.28 d (10.5)	6.27 d (11)
H-15	6.70 d, d (14, 10.5)	6.70 d, d (14, 11)
CH_3 -16 ax	1.146 s	1.191 s
CH_3 -17 eq	1.202 s	1.257 s
CH_3 -18	1.925 s	1.974 s
CH_3 -19	2.014 s	2.006 s
CH_3 -20	1.976 s	1.950 s
H-2' α	2.19 d, d (13.5, 8)	2.19 d, d (13.5, 8)
H-2' β	1.72 d, d (13.5, 4.5)	1.72 d, d (13.5, 4.5)
H-3'	4.53 d, d, d, d (8.5, 8, 4.5, 2.5)	4.53 d, d, d, d (8.5, 8, 4.5, 3)
H-4' α	2.88 d, d (14.5, 8.5)	2.88 d, d (14.5, 8.5)
H-4' β	1.55 d, d (14.5, 2.5)	1.55 d, d (14.5, 3)
H-7'	5.861 s	5.861 s
H-10'	7.23 d (9.5)	7.23 d (10)
H-11'	6.62 d, d (15, 9.5)	6.62 d, d (15, 10)
H-12'	6.65 d (15)	6.65 d (15)
H-14'	6.38 d (10.5)	6.38 d (10.5)
H-15'	6.65 d, d (14, 10.5)	6.65 d, d (14, 10.5)
CH_3 -16' α	0.851 s	0.851 s
CH_3 -17' β	1.189 s	1.189 s
CH_3 -18' β	1.349 s	1.349 s
CH_3 -19'	1.976 s	1.976 s
CH_3 -20'	1.990 s	1.990 s
8' OH	16.299 s	16.299 s

s, singlet; d, doublet,

2.3 Preparation of (*R*) and (*S*)-MTPA esters of **1** and **2**

Commercially available (*R*) and (*S*)-MTPA (Aldrich) were used without further purification. To a solution of **1** (1 mg) in CH₂Cl₂ (5 mL) added (*R*)-MTPA (10mg), 1,3-dicyclohexylcarbodiimide (DCC) (60 mg) and 4-pyrrolidinopyridine (6 mg) and the solution was allowed to stand at room temperature for 12h under the darkness. After filtration of reaction mixture, the red solution was subjected to preparative TLC and preparative HPLC affording the (*R*)-MTPA ester of **1**.

(*R*)-MTPA ester of **1** : UV-VIS : 294 and 469 nm. High resolution-FAB MS : m/z 1030.4905 (M^+ , C₆₀H₆₈O₈F₆, calc. 1030.4821).

Another MTPA esters, *i.e.* (*S*)-MTPA of **1**, (*R*)-MTPA of **2** and (*S*)-MTPA of **2** were also prepared by the similar methods described above.

3 Results and Discussion

In order to obtain unambiguous total ¹H-NMR assignments and confirm the stereostructures of **1** and **2**, ¹H-¹H nuclear Overhauser effect (NOE) experiments¹¹⁾ were performed in CDCl₃ solution. The results of NOE experiments and complete ¹H-NMR assignments for **1** and **2** are compiled in Fig. 1 and Table 1, respectively. The 3',5'-*trans* configuration in κ -end group both **1** and **2** was clearly determined by NOE experiments as shown in Fig. 1. Furthermore, NOEs between H-19 and H-10 and between H-18 and H-11 were observed in **2**. These results clearly proved Δ 9-*E* geometry in **2** and indicated that H-18 in β -end group is spatially close to H-11 in polyene as depicted in Fig. 1.

The absolute configurations at C-3 in β -end group and C-3' in κ -end group in **1** were established by the modified Mosher's method. The (*R*) and (*S*)-MTPA esters of **1** were prepared by treatment of **1** with the MTPA acids and 1,3-dicyclohexylcarbodiimide (DCC) in CH₂Cl₂. The assignments of protons in (*R*) and (*S*)-MTPA esters of **1** were performed by NOE and ¹H-¹H decoupling experiments. The $\Delta\delta$ ($=\delta S-\delta R$) values⁹⁾ in the β -end group and κ -end group in MTPA esters of **1** were shown in Fig. 2. The positive $\Delta\delta$ values for the protons oriented on the right sides of the MTPA plane⁹⁾ are the negative $\Delta\delta$ values for the protons located on the left sides of MTPA in the β -end group disclosed the *R*-configuration at C-3. The *S* configuration at C-3' in κ -end group was also deduced by the $\Delta\delta$ values of the protons as

Fig. 1 NOEs of Mytiloxanthin (**1**) and 9-*E*-Mytiloxanthin (**2**).

$$\Delta\delta = \delta(S) - \delta(R)$$

Fig. 2 $\Delta\delta$ Value (ppm) Obtained from MTPA Ester of Mytiloxanthin (1).

shown in Fig. 2. Based on the results of relative stereochemistry in κ -end group described above, chirality at C-5' was assigned to be *R*. Consequently, the absolute configuration of **1** has been confirmed to be 3*R*,3'*S*,5'*R*. As the similar manner described above, the absolute configuration of **2** was also assigned to be 3*R*,3'*S*,5'*R*.

In conclusion, the absolute configuration of natural **1** and **2** were confirmed by NOE experiments and by using of the modified Mosher's method. This method is useful for determination of the absolute configuration of secondary alcohols in carotenoids.

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References

- 1) B.T. Sheer, *J. Biol. Chem.*, **136**, 275 (1940).
- 2) T.W. Goodwin, "The Biochemistry of Carotenoids," Vol. II. Animals 2nd ed., Chapman and Hall, London, (1984).
- 3) T. Matsuno, S. Hirao, *Marine Biogenetic Lipids, Fats and Oils.*, Vol. I. ed. by R.G. Ackmann, CRC Press, Florida, (1989), p. 251.
- 4) A. Khare, G.P. Moss, B.C.L. Weedon, *Tetrahedron Lett.*, **1973**, 3921.
- 5) A.K. Chopra, G.P. Moss, B.C.L. Weedon, *J. Chem. Soc. Chem. Commun.*, **1977**, 467.
- 6) A.K. Chopra, A. Khare, G.P. Moss, B.C.L. Weedon, *J. Chem. Soc. Perkin Trans. I.*, **1988**, 1383.
- 7) J.A. Dale, H.S. Mosher, *J. Am. Chem. Soc.*, **95**, 512 (1973).
- 8) I. Ohtani, T. Kusumi, M.O. Ishitsuka, H. Kakisawa, *Tetrahedron Lett.*, **30**, 3147 (1989).
- 9) I. Ohtani, T. Kusumi, Y. Kashman, H. Kakisawa, *J. Am. Chem. Soc.*, **113**, 4092 (1991).
- 10) T. Matsuno, T. Maoka, *Bull. Japan. Soc. Sci. Fish.*, **47**, 377 (1981).
- 11) Y. Fujiwara, T. Maoka, M. Ookubo, T. Matsuno, *Tetrahedron Lett.*, **33**, 4941 (1992).

[ノート] **Mytiloxanthinおよび9-E-mytiloxanthin
の絶対構造**

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海産二枚貝のイガイ (*Mytilus coruscus*) から得られたmytiloxanthinおよび9-E-mytiloxanthinの絶対構造をNOEおよび改良Mosher法を用いるNMR実験によりそれぞれ3R, 3'S, 5'Rと決定した。

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[ノート] ***Boreava orientalis*のフェノール化合物の
酸化防止活性**

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*Boreava orientalis*の果実より得られた2種の新規物質を含む11種のフェノール化合物の酸化防止作用を、2, 2'-アゾビス(2, 4-ジメチルバレロニトリル) (AMVN) によるリノール酸メチルの過酸化系を用いて検討した。これらの化合物はいずれも酸化防止活性が認められた。シナビン酸(10)およびその誘導体(9), (11)の活性は、特に顕著であった。

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