CONFIGURATIONAL STUDIES ON THIOMARINOL

Sir:

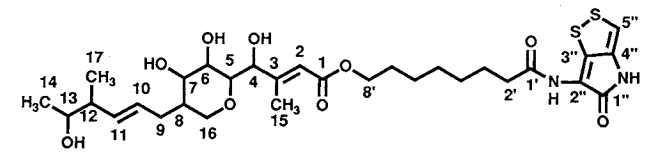
A new antibiotic, thiomarinol, was isolated from the culture broth of a marine bacterium, *Alteromonas rava* sp. nov. SANK 73390[1]. The structure of thiomarinol was deduced to be a hybrid of a pseudomonic acid analogue and holothin[2]. In a previous paper[2], we suggested that the stereochemistry of the six-membered ether ring in the monic acid moiety of thiomarinol was very similar to that of pseudomonic acid C by the coupling constants and acetonide formation. In this paper, we report the absolute configuration of the 4-hydroxymonic acid C moiety of thiomarinol, except at C-12. Hydrolysis of thiomarinol (**1**) with 0.1 N NaOH in 60% MeOH solution afforded a 4-hydroxymonic acid C (**2**) and its methyl ester (**3**), as described previously[2], and a new bicyclic compound (**4**, $C_{17}H_{28}O_7$; FAB-MS: *m/z* 345 $(M+H)^+$), as shown in Fig. 1.

The formation of the bicyclic compound (**4**) proceeded from intramolecular Michael attack of the C-6 hydroxy group on the acrylate ester function in the monic acid[3]. This compound (**4**), having a rigid structure, is a suggestive one, because the relative configuration at C-4 can be solved by NMR analysis. In the ^1H NMR spectrum of **4**, the allyl methyl at C-15 disappeared, and a methyl and an isolated methylene signals newly appeared at δ 1.25 and 2.62, both as singlets. Also, comparison of the ^{13}C NMR spectra between **2** and **4** suggested the disappearance of 2,3-double bond. Acetylation of **4** with acetic anhydride in pyridine gave a triacetate (**5**, $C_{23}H_{34}O_{10}$; FAB-MS: *m/z* 471 $(M+H)^+$). In the ^1H NMR spectrum of **5**, protons at 4-H, 7-H and 13-H were shifted to low field at δ 5.53, 5.26 and 4.82, and all carbinol protons at 4-H, 5-H, 6-H, 7-H, 13-H and 16-H were well separated. The relative configuration of the bicyclic ring of **5** was deduced from the coupling constants of $J_{4,5}=8.7$ Hz, $J_{5,6}=10.7$ Hz, $J_{6,7}=2.4$ Hz, $J_{7,8}=2.4$ Hz, $J_{8,16a}=0$ Hz and $J_{8,16b}=2.9$ Hz, as shown in Fig. 2. The veracity of this conformation was strengthened with the aid of additional information on the NOEs between 2-H and 4-H, 4-H and 6-H, 6-H and 7-H, 6-H and 9-H, and 5-H and 15-H_3.

The absolute configuration of **4** was elucidated by the modified MOSHER's method of 2-methoxy-2-(trifluoromethyl)-2-phenylacetic acid (MTPA) derivatives[4]. The *S* or *R* MTPA ester derivatives of thiomarinol were prepared by the reaction with *S* or *R* MTPA and dicyclohexylcarbodiimide (DCC) in THF solvent[5]. We isolated mono *O*- (*S* and *R*) MTPA esters of C-4 (**6**), C-7 (**7**), and C-13 (**8**), respectively, by preparative HPLC (Senshu-pak, ODS, H-4251, 10×250 mm, 60% CH_3CN, 5 ml/minute). ^1H NMR spectra of all compounds were measured in $CDCl_3$ solution with 400 MHz. The $\Delta\delta$ (ppm) values are shown in Fig. 3.

From these results, the absolute configurations of secondary alcohols at C-4, C-6 and C-13 were *R*, *R* and *S*, respectively. In addition, the absolute configurations at C-5, C-7 and C-8 were also deduced as *S*, *R* and *S*, respectively, from the relative configuration of **4**. Finally, the geometry of double bonds at C-2～C-3 and C-10～C-11 were assigned as both *E*, by NMR spectra. ALEXANDER *et al.* reported the geometry of the C-2～C-3 double bond of

Fig. 1. Structures of thiomarinol (**1**) and its hydrolysis products.



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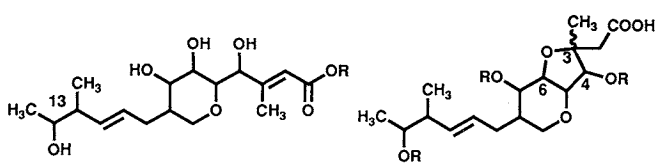
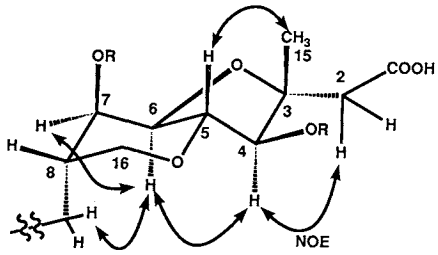


2 R=H
3 R=CH_3

4 R=H
5 R=$COCH_3$

Fig. 2. Relative configuration of **5** (R = $COCH_3$).



Chemical shifts (δ) and J values (Hz)
2-H_2 (2.67, 2.81, ABq, J=15.1)
4-H (5.53, d, J=8.7)
5-H (3.91, dd, J=8.7, 10.7)
6-H (4.02, dd, J=10.7, 2.4)
7-H (5.26, t, J=2.4)
9-H (2.24, m)
15-H_3 (1.19, s)
16-H_2 (3.74, 3.78, ABX, J=11.7, 2.9)

Fig. 3. $\Delta\delta_{ppm}$ values obtained for the MTPA esters of **6**, **7**, and **8**.

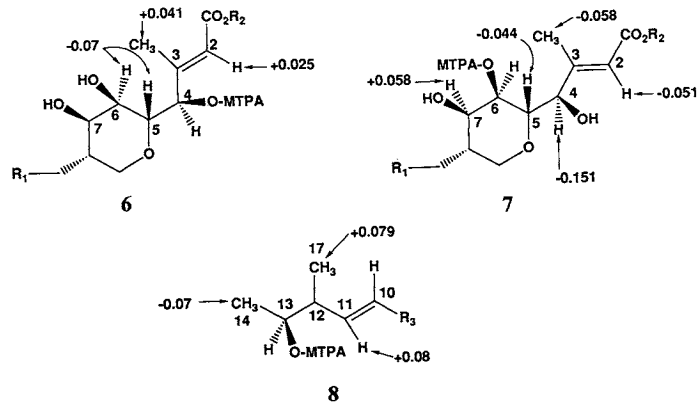
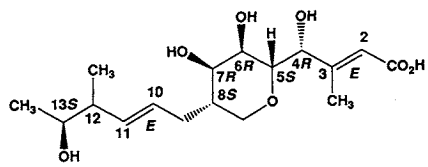


Fig. 4. Stereochemical structure of 4-hydroxymonic acid C (**2**).



pseudomonic acid A and its isomer by ^1H and ^{13}C NMR spectra[6]. In its ^{13}C NMR spectra, chemical shifts of the C-15 signal in the *E* and *Z* configurations of the C-2 ~ C-3 double bond appeared at δ 19.1 and 27.4, respectively. On the other hand, obvious NOE between 2-H and 15-H was only observed in the *Z*-isomer. The C-15 signal of thiomarinol in the ^{13}C NMR spectrum appeared at δ 15.7, near the *E*-configuration of pseudomonic acid A, and the NOE between 2-H and 15-H was not observed. As additional evidence, the NOEs in **2** were not observed between 2-H and 15-H, but between 2-H and 4-H. The coupling constant between 10-H and 11-H in thiomarinol was observed at 15.5 Hz. This value was assigned as signifying a *trans* double bond. Therefore, the five asymmetric centers except at C-12 of 4-hydroxymonic acid derivative of thiomarinol were established as 4*R*, 5*S*, 6*R*, 7*R*, 8*S* and 13*S*, and the geometry of double bonds at C-2 ~ C-3 and C-10 ~ C-11 were both *E*, as shown in Fig. 4. These configurations and geometries, except at C-4 and C-12, of thiomarinol are the same as those of pseudomonic acid C[3].

Recently, two new pseudomonic acid derivatives were isolated from a marine bacterium, *Alteromonas* sp.[7]. The structure of one of then contained the 4-hydroxymonic acid derivative, which was identical with that of thiomarinol. However, the absolute configuration of this compound was not reported.

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