Determination of the absolute configuration of marine oxylipin topsentolide A₁ by the synthesis of the enantiomer of the natural product

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A B S T R A C T

Two possible stereoisomers of topsentolide A₁, a cytotoxic oxylipin against human solid tumor cell lines, were prepared in order to determine the stereochemistry of natural product. That is, the enantiomer of topsentolide A₁, (8S,11S,12R)-isomer, and its diastereomer was efficiently synthesized in a stereoselective manner. The stereochemistry of topsentolide A₁ was determined to be 8R,11R,12S by comparing NMR spectra and specific rotations of the synthetic isomers and the natural product.

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A number of oxylipins have been isolated as secondary metabolites of marine organisms such as cyanobacteria, alga, hydrozoa, and sponges. These oxylipins have interesting activities and synthetic studies on them are meaningful, as well as biological studies, because their bioactivities are frequently concerned with structure. Halicholactone (**2**, isolated from *Halichondria okadai*)[1] is an inhibitor of lipoxygenase; mueggelone (**3**, isolated from *Aphanizomenon flos-aquae*)[2] is an inhibitor of fish development; and solandelactone D (**4**, isolated from *Solanderia secunda*)[3] is an inhibitor of farnesyl protein transferase. Previously in our group, the syntheses of these marine oxylipins had been achieved in stereoselective manner and the results had been already reported (Fig. 1).[4–8]

In 2006, Jung's group isolated topsentolide A₁ (**1**)[9] from the extract of a marine sponge *Topsentia* sp. as a cytotoxic oxylipin against human solid tumor cell lines. Topsentolide A₁ has a nine-membered lactone with an epoxide side chain. Stereochemistry of the epoxide was proposed to be *cis* by spectroscopic analysis, however, the relative as well as the absolute configuration of the three stereocenters remained unknown. Therefore, we undertook the synthesis of possible stereoisomers of topsentolide A₁ in order to determine the absolute configuration of the natural product. In this Letter, we report the synthesis of (8S,11R,12S)-isomer (**5**) and (8S,11S,12R)-isomer (**6**), and the determination of the absolute configuration of topsentolide A₁.

Our synthetic strategy of (8S)-isomers (**5** and **6**) is illustrated in Scheme 1. We decided to utilize a key intermediate **7**, which could be transformed into both of (8S,11R,12S)-isomer (**5**) and

(8S,11S,12R)-isomer (**6**) stereoselectively via epoxide formation, where the stereochemistry of the epoxide could be controlled by changing elimination direction.[4,5] Compound **7** would be constructed by Horner–Wadsworth–Emmons (HWE) reaction of a side chain moiety **8** with a lactone moiety **9**. Both the phosphonate **8** and the lactone **9** would be derived from the known aldehyde **10**,[10,11] which could be easily prepared from L-malic acid.

Synthesis of the phosphonate **8** is shown in Scheme 2. The known aldehyde **10**[10,11] was subjected to Wittig reaction with the known phosphonium salt **11**[12] to afford *Z*-olefin **12** selectively (>98%, checked by GC analysis). The *Z*-olefin **12** was simply transformed into the corresponding primary alcohol **14** in four steps, which was then oxidized to give aldehyde **15**. Oxidation of the aldehyde **15** with NaClO₂ and subsequent esterification using CH₂N₂ afforded the desired methyl ester **16** only in poor yield (∼20%), and undesired migration of TBS group was observed. On the other hand, oxidation of **15** with NIS[13] in methanol proceeded smoothly to give ester **16** in good yield without any side reactions.
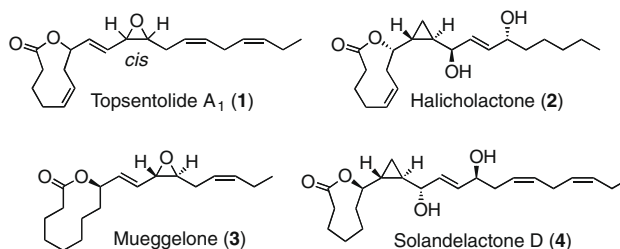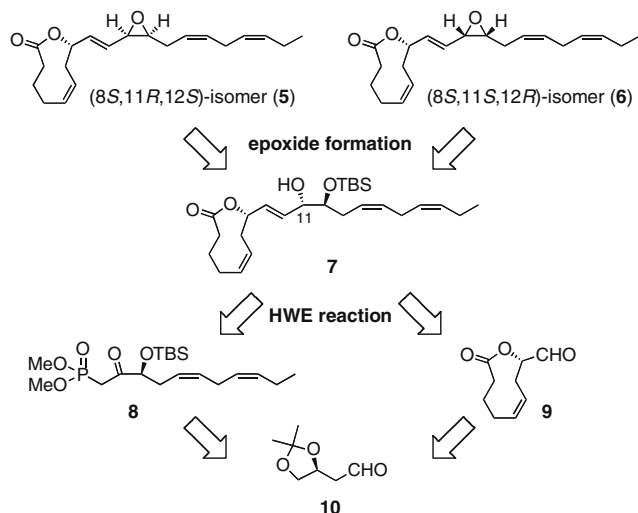

Figure 1. Bioactive marine oxylipins.

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Scheme 1. Retrosynthetic analysis of two possible (8*S*)-stereoisomers of topsentolide A₁.

Finally, ester **16** was reacted with anion of dimethyl methylphosphonate to afford the desired β-ketophosphonate **8**, one of the substrates for HWE reaction.

Synthesis of the lactone **9** is shown in Scheme 3. Wittig reaction of the known aldehyde **10**[10,11] and a commercially available phosphonium salt afforded *Z*-olefin **17** selectively (*E*-isomer was not observed in ¹H NMR spectrum), which was transformed into the corresponding hydroxycarboxylic acid **19** in four steps. Lactonization of **19** was successfully achieved by Yamaguchi's method[14] to provide nine-membered lactone **20** in satisfactory yield, together with a small amount of a dimeric lactone (3%). After the removal of PMB group, **20** was oxidized into the desired aldehyde **9**, another substrate for HWE reaction.

Now that both the side chain moiety **8** and the lactone moiety **9** were obtained enantioselectively, HWE reaction was examined (Scheme 4). Under Masamune's conditions employing DBU-LiCl,[15] HWE reaction of the aldehyde **9** and the β-ketophosphonate **8** proceeded smoothly to give unsaturated ketone **21**, with excellent *E* selectivity without any epimerization (other isomers were not observed in ¹H NMR spectrum). Stereoselective reduction of the ketone **21** using Luche's conditions[16] successfully afforded the key intermediate **7**, the precursor for epoxide formation. Reduction of **21** proceeded under Felkin–Anh control, and the stereochemistry at C-11 was confirmed to be *S* by modified Mosher's method[17] after conversion to the corresponding MTPA esters (Table 1). In order to
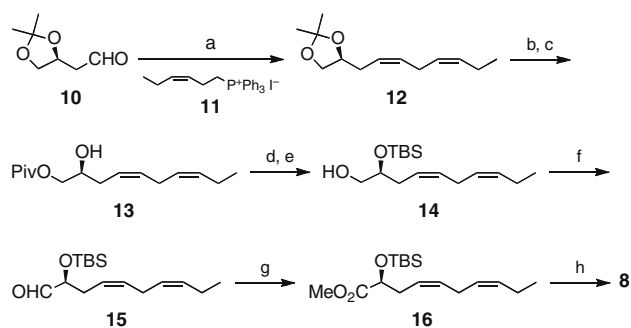
Scheme 2. Synthesis of the phosphonate **8**. Reagents and conditions: (a) **11**, KHMDS, THF, −78 °C to rt, 84%; (b) 1 N HCl, THF, rt, 90%; (c) PivCl, Pyr., rt, 89%; (d) TBSOTf, 2,6-lutidine, CH₂Cl₂, rt, 96%; (e) DIBAL, CH₂Cl₂, −50 °C, 97%; (f) (COCl)₂, DMSO, Et₃N, CH₂Cl₂, −78 °C to rt, 97%; (g) NIS, K₂CO₃, MeOH, rt, 78%; (h) (MeO)₂P(O)Me, *n*-BuLi, THF, −78 °C, 52%.
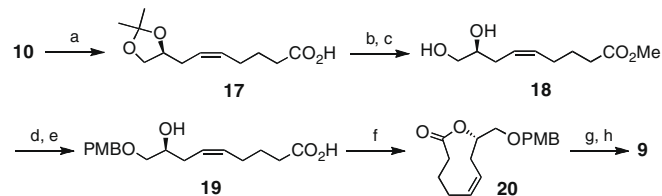

Scheme 3. Synthesis of the lactone **9**. Reagents and conditions: (a) (4-carboxybutyl)triphenylphosphonium bromide, NaHMDS, THF, −78 °C to rt; (b) CH₂N₂, Et₂O, MeOH, 0 °C, 69% in two steps; (c) 1 N HCl, THF, rt, 97%; (d) PMBOC(NH)CCl₃, TsOH·H₂O, CH₂Cl₂, rt, 63%; (e) LiOH·H₂O, H₂O, MeOH, rt, 98%; (f) 2,4,6-trichlorobenzoyl chloride, Et₃N, THF, rt, then DMAP, toluene, reflux, 81%; (g) DDQ, H₂O, CH₂Cl₂, 87%; (h) (COCl)₂, DMSO, Et₃N, CH₂Cl₂, −78 °C, 69%.
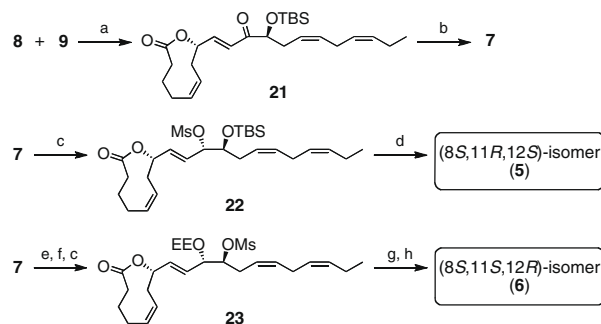
Scheme 4. Synthesis of possible (8*S*)-stereoisomers of topsentolide A₁. Reagents and conditions: (a) DBU, LiCl, MeCN, rt, 67%; (b) NaBH₄, CeCl₃·7H₂O, MeOH, rt, 96%; (c) MsCl, Et₃N, DMAP, CH₂Cl₂, rt; (d) TBAF, THF, rt, 85% in two steps; (e) EVE, PPTS, CH₂Cl₂, rt, 83%; (f) TBAF, THF, rt, 91%; (g) 0.5 N HCl, THF, rt, (h) TBAF, THF, rt, 29% in three steps.

Table 1
¹H NMR (300 MHz, CDCl₃) data for (*S*)- and (*R*)-MTPA ester of **7**

MTPA ester of **7**
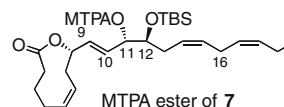
Position	¹H chemical shift (δ)		δ_S–δ_R
	(*S*)-MTPA	(*R*)-MTPA	
9	5.79	5.87	−0.08
10	5.68	5.81	−0.13
12	3.79	3.77	+0.02
16	2.70	2.67	+0.03

construct both α-epoxide and β-epoxide from **7**, the stereochemistry of the epoxide was controlled as described below by changing elimination direction.[4,5] At first, alcohol **7** was converted to the corresponding mesylate **22**, which was treated with TBAF to provide β-epoxide, (8*S*,11*R*,12*S*)-isomer (**5**). On the other hand, alcohol **7** was converted to another mesylate **23** by changing the position of the leaving group. Finally, the removal of EE group was followed by treatment under basic conditions to afford α-epoxide, (8*S*,11*S*,12*R*)-isomer (**6**), successfully.

Having succeeded in the construction of two possible stereoisomers of topsentolide A₁, we analyzed the NMR spectra and specific rotations carefully.[18,19] As for the ¹³C NMR, there was no difference between both the diastereomers (**5** and **6**) to distinguish them. In the ¹H NMR, the signals of both diastereomers (**5** and **6**) appeared at almost similar chemical shifts to those of natural topsentolide A₁, however, slight differences of the chemical shifts and the shapes of peaks were observed between **5** and **6**. As shown in
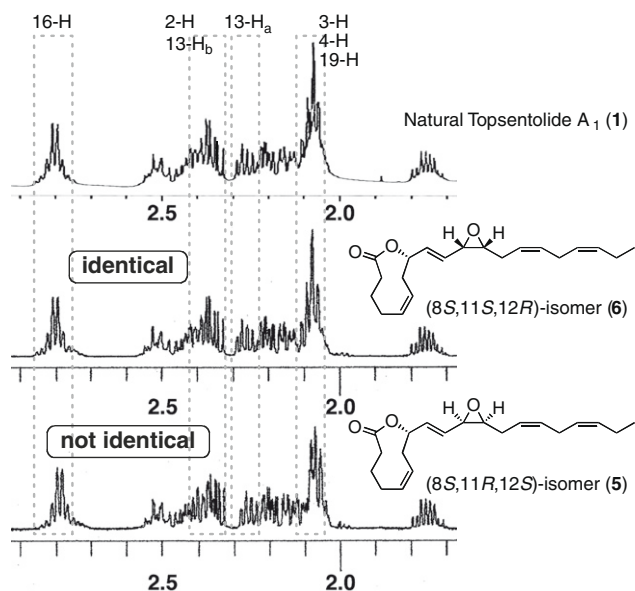
Figure 2. ¹H NMR signals of natural topsentolide A₁ and synthesized stereoisomers (500 MHz, in CD₃OD).
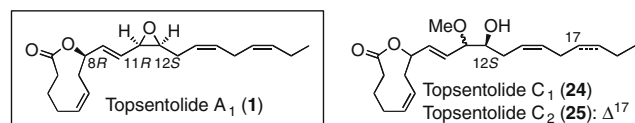
Figure 3. Absolute configuration of topsentolide A₁.

Figure 2, some signals of (8S,11R,12S)-isomer (**5**) were not identical with those of natural topsentolide A₁ [especially at 2.74–2.86 ppm (16-H), 2.32–2.42 ppm (2-H and 13-H$_b$), 2.23–2.30 (13-H$_a$) and 2.03–2.12 ppm (3-H, 4-H and 19-H)], while ¹H NMR spectrum of (8S,11S,12R)-isomer (**6**) was completely identical with that of natural topsentolide A₁. Furthermore, specific rotations of the synthetic diastereomers, $[\alpha]_D^{24}$ −104 (c 0.11, MeOH) for **5** and $[\alpha]_D^{24}$ −88 (c 0.11, MeOH) for **6**, showed opposite sign to $[\alpha]_D^{24}$ +59.4 (c 0.11, MeOH)[9] for the natural material. On the basis of these results, we propose the (8R,11R,12S)-stereochemistry, the enantiomer of **6**, for natural topsentolide A₁ (**1**) as shown in Figure 3. Actually, Jung's group also isolated topsentolide C₁ (**23**) and C₂ (**24**) together with **1**.[9] They suspected these methyl ether analogues to be artifacts formed during the process of extraction with MeOH, and determined the absolute configuration at C-12 as S by Mosher's method. Their determination of stereochemistry at C-12 also supports our results.

In summary, we succeeded in the synthesis of *ent*-topsentolide A₁ (**6**) and its stereoisomer **5**, and determined the stereochemistry

of topsentolide A₁ to be 8R,11R,12S. Our work is under way to prepare (8R)-isomers including the natural form of topsentolide A₁ (**1**) and to submit these isomers to further biological assay. Results will be reported in a full account.

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18. *Analytical and spectral data of synthesized* **5**: $[\alpha]_D^{24}$ −104 (c 0.11, CH₃OH). ¹H NMR (500 MHz, CD₃OD) δ (ppm) 0.96 (3H, t, J = 7.5 Hz, 20-H), 1.76 (1H, m, 3-H$_a$), 2.03–2.29 (7H, m, 2-H$_a$, 3-H$_b$, 4-H$_a$, 7-H$_a$, 13-H$_a$, 19-H), 2.32–2.55 (4H, m, 2-H$_b$, 4-H$_b$, 7-H$_b$, 13-H$_b$), 2.73–2.85 (2H, m, 16-H), 3.13 (1H, dt, J = 4.5, 6.5 Hz, 12-H), 3.47 (1H, br dd, J = 6.5, 4.5 Hz, 11-H), 5.24–5.31 (2H, m, 8-H, 17-H), 5.35–5.52 (5H, m, 5-H, 6-H, 14-H, 15-H, 18-H), 5.76 (1H, ddd, J = 15.5, 7.0, 1.5 Hz, 10-H), 6.03 (1H, ddd, J = 15.5, 5.5, 1.0 Hz, 9-H). ¹³C NMR (125 MHz, CD₃OD) δ (ppm) 14.7, 21.5, 26.3, 26.6, 27.1, 27.5, 34.4, 35.4, 57.2, 59.4, 73.9, 124.9, 125.5, 127.2, 127.9, 132.0, 133.0, 135.2, 136.3, 175.6. IR (film) ν 2962, 1742, 1218, 1136, 969 cm⁻¹. ESI-HRMS *m/z* calcd for C₂₀H₂₈NaO₃ [M+Na]⁺ 339.1931, found 339.1942.
19. *Analytical and spectral data of synthesized* **6**: $[\alpha]_D^{24}$ −88 (c 0.11, CH₃OH). ¹H NMR (500 MHz, CD₃OD) δ (ppm) 0.97 (3H, t, J = 7.5 Hz, 20-H), 1.76 (1H, m, 3-H$_a$), 2.03–2.30 (7H, m, 2-H$_a$, 3-H$_b$, 4-H$_a$, 7-H$_a$, 13-H$_a$, 19-H), 2.32–2.55 (4H, m, 2-H$_b$, 4-H$_b$, 7-H$_b$, 13-H$_b$), 2.74–2.86 (2H, m, 16-H), 3.12 (1H, dt, J = 4.5, 6.5 Hz, 12-H), 3.48 (1H, br dd, J = 7.0, 4.5 Hz, 11-H), 5.25–5.32 (2H, m, 8-H, 17-H), 5.35–5.52 (5H, m, 5-H, 6-H, 14-H, 15-H, 18-H), 5.76 (1H, ddd, J = 15.5, 7.0, 1.5 Hz, 10-H), 6.04 (1H, ddd, J = 15.5, 5.5, 1.0 Hz, 9-H). ¹³C NMR (125 MHz, CD₃OD) δ (ppm) 14.7, 21.5, 26.3, 26.7, 27.1, 27.5, 34.4, 35.3, 57.3, 59.4, 73.8, 124.9, 125.5, 127.3, 127.9, 132.0, 133.0, 135.3, 136.3, 175.6. IR (film) ν 2961, 1743, 1218, 1137, 969 cm⁻¹. ESI-HRMS *m/z* calcd for C₂₀H₂₈NaO₃ [M+Na]⁺ 339.1931, found 339.1933.