Structures and Absolute Stereochemistry of Five New Secospatanes and a Spatane Isolated from the Brown Alga *Dilophus okamurai* Dawson

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Five new secospatane diterpenoids, secospatacetal A, B, C, D, and E have been isolated from the brown alga *Dilophus okamurai* Dawson, and their structures as well as absolute configurations have been elucidated by means of NMR spectroscopy and chemical transformations. Also, the structure of a known spatane diterpene has been revised.

**Key words** *Dilophus okamurai*; secospatacetal; absolute configuration; marine natural product; chiral anisotropic reagent

An increasing number of unique natural products is being found from marine organisms, and the marine products have been attracting the interest of organic chemists and biochemists because of their novel chemical structures and remarkable biological activities. Studies of marine natural products has also prompted the development of new methodology for spectroscopic elucidation of the complex chemical structures and absolute configurations of the intriguing secondary metabolites.

The brown algae of Dictyotaceae are known to produce a variety of diterpenoids which proved to have pharmaceutical activities and to be antifeedants against mollusks and ichthyotoxins. This report deals with the structures and absolute configurations of five new secospatane diterpenoids, secospatacetal A (1), B (2), C (3), D (4), and E (5), which were isolated from the brown alga *Dilophus okamurai* Dawson.

**Results and Discussion**

*D. okamurai* Dawson (Japanese name: Fukurin-amiji) was collected at Kikizu beach of Ehime Prefecture and immediately soaked in methanol. The methanol extract was condensed and the residue was partitioned between hexane and water. The hexane soluble material was repeatedly chromatographed to give five new diterpenoids.

Secospatacetal A (1) gave a molecular ion at m/z 436.2797 confirming the molecular formula to be C$_{25}$H$_{40}$O$_6$. The $^1$H-NMR spectrum in C$_6$D$_6$ revealed the signals for three singlet methyls at $\delta$ 1.55 (H-20), 1.62 (H-14) and 1.66 (H-19), one doublet methyl at $\delta$ 1.06 (H-11) and two olefinic protons at $\delta$ 5.18 (H-17) and 5.23 (H-15). In addition, the $^1$H–$^1$H COSY spectrum of secospatacetal B (2) was used for the assignment of the protons and carbons. The proton networks of the compound were easily deduced by the $^1$H–$^1$H COSY spectrum, because the connectivity of the protons is only interrupted by the quaternary carbons at C-10, 13 and 18. The heteronuclear multiple bond correlation (HMBC) spectrum (see 1a) allowed the complete connectivity of the carbons, which revealed the secospatane skeleton and the planar structure of 1. The nuclear Overhauser enhancement and exchange spectroscopy (NOESY) cross-peaks depicted in 1b allowed assignment of the relative stereochemistry of secospatacetal A (1).

The absolute configuration of 1 was determined using the OMe-mandelate method. Alkaline hydrolysis of 1 afforded the deacetyl product, and the hydroxy group of the product was esterified with (R)- and (S)-methoxypyphenolic acids (MPA). The $\Delta\delta$ values ($\Delta\delta$ = $\delta_S$ - $\delta_R$) obtained for all the protons are indicated in 1c. The systematic arrangement of the $\Delta\delta$ values enabled elucidation of the absolute configuration of 1 as shown in the structure.

The structures including the relative stereochemistry of secospatacetals B—E, 2—5, were determined by the spectral analyses essentially similar to those described for 1. The spectral data along with the assignment of protons and carbons are summarized in Experimental. We then focused on the absolute configuration of these four compounds.

The two acetyl moieties of secospatacetal B (2) were removed by hydrolysis, and the resultant hydroxy groups were esterified with (R)- and (S)-MPA to give the diester. Usually, introduction of more than one anisotropic moiety in a small-sized molecule is unfavorable if the anisotropic effects from the multiple aromatic rings interfere with each other. In this case, however, based on the ideal conformation of the mandelates proposed by Trost and Curran, the two phenyl rings of MPA in the diester may be oriented so that the anisotropic effects (upfield shift in this case) from the phenyl rings are in harmony with each other. Therefore, the signs of the $\Delta\delta$ values obtained for 2 must be systematically distributed and the values would be larger than those of 1. As can be seen in 2a, the speculation turned out to be correct: Arrangement of the positive and negative $\Delta\delta$ values is quite orderly and most of the values exceed those observed for 1. From these data, the absolute configuration of 2 was determined to be 1S, 2S, 5R, 7R, 8R, 9R, 10S, 12R.

The yields of secospatacetals C (3) and D (4) were extremely poor, 0.8 mg and 0.6 mg, respectively. The problem of consuming the sample by derivatization into two diastere-

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omers, (R)- and (S)-mandelates, can be greatly relieved if only one of the enantiomers of a certain chiral anisotropic reagent is effectively applied. Thus, methoxy(2-naphthyl)acetic acid (2NMA),\(^7,8\) having a stronger anisotropic effect than the mandelic acid, was adopted for determining the absolute configuration of these compounds (The ideal conformation\(^7\) of an (R)-2NMA ester is shown in Fig. 1).

The hydrolytic products of compounds 3 and 4, each 0.3 mg, were esterified with excess (R)-2NMA, and the chemical shifts of all the protons were assigned by means of \(^1\)H–\(^1\)H COSY. We defined \(\Delta\delta'\) value as \(\Delta\delta' = \delta_{\text{acetate}} - \delta_{(R)-2NMA\ \text{ester}}\) and \(\Delta\delta = 0.1\) (ppm) as a threshold value. In structures 3a and 4a, the \(\Delta\delta'\) values are assigned to the respective protons and the values above the threshold are underlined. It should be noted that, in each compound, introduction of the highly diamagnetic naphthalene moiety causes the upfield shift of the protons on both sides of the 2NMA plane. The protons with the \(\Delta\delta'\) values larger than 0.1 ppm are located only on the left side of the 2NMA plane, thus leading to the R-configuration at C-5 of 3 and 4.

We next turned our attention to methoxy(1-naphthyl)acetic acid (1NMA). Molecular models show that the anisotropy of the naphthalene ring is more conveniently used for a cyclic compound in a 1NMA ester (A) than in a 2NMA ester (B) (Fig. 2): The anisotropy of the two benzene rings of naphthalene is effectively given on the protons of the cyclic alcohol (L-menthol as an example) in 1NMA ester (A), whereas anisotropy of only one benzene ring is effective in 2NMA ester (B).

To confirm this assumption and compare the anisotropic effects between 1NMA and 2NMA, secospatacetal E (5), which was obtained in a very small quantity and whose relative stereochemistry was determined by spectroscopic analyses, was converted to 1NMA ester. The \(\Delta\delta'\) values are as-
signed in structure 5a. They are clearly larger than those of 2NMA esters 3a and 4a. The distribution of the $\Delta \delta'$ values (>0.1 ppm) led to the R-configuration at C-5 of 5.

This methodology, in which only one of the enantiomers of 1NMA or 2NMA (the former preferable for cyclic compounds) is used to determine the absolute configuration, seems to be valid in the series of the compounds described above. The authors, however, feel that it is somewhat risky to make it a general method at this stage. More examples to verify the method may be necessary, and the proper threshold of the $\Delta \delta'$ value, which is rather arbitrary in the present study, must be established. We recently reported a similar method using one enantiomer of (R)- and (S)-2NMA, which was applied to acyclic compounds.9)

The spectral properties of compound 6 were identical with those reported by an Australian group.10) In their report, structure 7 was proposed for this compound, and the relative and absolute stereochemistry was presumed by comparison of its spectral data with those of spatol (10), the absolute stereostructure of which had been determined by X-ray crystallography.11)

Fig. 1. An Ideal Conformation of a Methoxy-2-naphthylacetate (2NMA Ester)

(i) In the phase-sensitive NOESY experiments (400 MHz, CDCl₃) carried out for 6 and its hydrolytic product 8, H-5 gives an intense cross peak to H-6ₐ. This intensity was as large as the one between H-7 and H-6₉. (ii) The coupling pattern of H-5 ($J_{5,6ₗ}=0$ Hz; $J_{5,6₉}=4.5$ Hz) was similar to that reported for spatol. This coupling constant as well as other $J$ values suggested that the conformation of the cyclopentane ring was as shown in 6a, in which H-5 was oriented anti to H-7.

To obtain other evidence of the $\beta$-orientation of H-5,
chemical reactions were performed. Compound 6 was subjected to Swern oxidation, and the resulting ketone was reduced with NaBH₄ to give only one product. Consideration of the stereochemical pathway suggested that the hydride attacked from the convex side of the molecule, and, therefore, the H-5 of the product should have \( \alpha \)-configuration. The optical rotation was determined for solutions in methanol on a JASCO DIP-370 polarimeter. For column chromatography, Kieselgel 60 (Merek) was used. Preparative HPLC was performed on a JAI LC-908 instrument with LiChrosorb Si 60 columns (5 mm, 250×25 mm i.d. and 250×10 mm i.d., Merck).

**Extraction and Isolation** Diplophus okamurai Dawson (40 kg) was collected on Kikizuka Beach, Ehime Prefecture, Japan in May 1995. The whole plant was extracted with MeOH at room temperature for 1 month. The MeOH extract was concentrated under reduced pressure to give a residue, which was partitioned between hexane and H₂O. A part of the hexane-soluble portion (30 g) was subjected to silica gel chromatography eluting with hexane–AcOEt (4:1) to afford secospatacetal D (1 mg) to give five fractions (frs. 1—5). Fraction 3 was chromatographed on silica gel eluting with hexane–AcOEt (4:1) to give seven fractions (frs. 3-1—6). Fraction 3-3 was subjected to silica gel chromatography eluting with hexane–AcOEt (8:1) to yield seven fractions (frs. 3-1—7). Fraction 3-3-2 was purified by preparative HPLC [hexane–AcOEt (2:1)] to afford secospatacetal A (5 mg).

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1 Hydrolytic Product of Secospatacetal D 1H-NMR (CDCl3): δ 0.96 (3H, d, J = 7.3 Hz, H-11), 1.59 (3H, H-Me-6z), 1.69 (3H, H-Me-6x), 1.70 (3H, H-Me-19), 1.71 (3H, H-Me-19), 1.91 (3H, H-Me-3f), 1.93 (3H, H-Me-6f), 2.13 (1H, dd, J = 13.2, 5.2 Hz, H-3a), 2.21 (1H, H-Me-1), 2.36 (1H, dd, J = 14.4, 11.1 Hz, H-8), 2.51 (1H, dd, J = 13.0, 8.3, 4.7 Hz, H-4), 2.62 (1H, dd, J = 11.1, 9.6 Hz, H-9), 2.78 (1H, H-Me-16), 2.91 (1H, H-Me-16), 3.30 (3H, H-10-Me), 3.45 (3H, 12-O-Me), 3.53 (1H, dd, J = 5.3, 9.1 Hz, H-17), 3.78 (1H, q, J = 5.9, 12.5 Hz, H-12), 4.29 (1H, H-Me-1), 5.10 (1H, H-Me-8), 5.24 (1H, t, J = 7.1 Hz, H-17), 5.28 (1H, t, J = 7.3 Hz, H-15).

13C-NMR (CDCl3): δ 14.1 (C-11), 17.8 (C-20), 22.0 (C-14), 25.7 (C-19), 27.3 (C-16), 37.7 (C-8), 37.8 (C-7), 40.8 (C-6), 42.7 (C-3), 46.3 (C-16), 48.1 (C-9), 48.5 (C-4), 50.3 (10-OMe), 55.4 (12-Ome), 71.5 (C-5), 77.4 (C-2), 102.1 (C-12), 110.6 (C-10), 124.0 (C-17), 127.4 (C-13), 131.3 (C-18), 155.8 (C-13).

(2) R-MPA Ester of Secospatacetal D 1H-NMR (CDCl3): δ 0.96 (3H, d, J = 7.3 Hz, H-11), 1.59 (3H, H-Me-6z), 1.69 (3H, H-Me-6x), 1.70 (3H, H-Me-19), 1.71 (3H, H-Me-19), 1.91 (3H, H-Me-3f), 1.93 (3H, H-Me-6f), 2.13 (1H, dd, J = 13.2, 5.2 Hz, H-3a), 2.21 (1H, H-Me-1), 2.36 (1H, dd, J = 14.4, 11.1 Hz, H-8), 2.51 (1H, dd, J = 13.0, 8.3, 4.7 Hz, H-4), 2.62 (1H, dd, J = 11.1, 9.6 Hz, H-9), 2.78 (1H, H-Me-16), 2.91 (1H, H-Me-16), 3.30 (3H, H-10-Me), 3.45 (3H, 12-O-Me), 3.53 (1H, dd, J = 5.3, 9.1 Hz, H-17), 3.78 (1H, q, J = 5.9, 12.5 Hz, H-12), 4.29 (1H, H-Me-1), 5.10 (1H, H-Me-8), 5.24 (1H, t, J = 7.1 Hz, H-17), 5.28 (1H, t, J = 7.3 Hz, H-15).

13C-NMR (CDCl3): δ 14.1 (C-11), 17.8 (C-20), 22.0 (C-14), 25.7 (C-19), 27.4 (C-16), 37.7 (C-8), 37.8 (C-7), 40.8 (C-6), 42.7 (C-3), 46.3 (C-16), 48.1 (C-9), 48.5 (C-4), 50.3 (10-Ome), 55.4 (12-Ome), 71.5 (C-5), 77.4 (C-2), 102.1 (C-12), 110.6 (C-10), 124.0 (C-17), 127.4 (C-13), 131.3 (C-18), 155.8 (C-13).

(8) 1H-NMR of Hydrolytic Product of Secospatacetal D 1H-NMR (C6D6): δ 2.66 (1H, t, J = 7.1 Hz, H-17), 5.41 (1H, d, J = 7.3 Hz, H-15), 5.21 (1H, t, J = 7.0 Hz, H-17), 5.27 (1H, t, J = 6.7 Hz, H-15), 5.83 (1H, dd, J = 5.9, 2.6 Hz, H-2), 6.07 (1H, dd, J = 6.0, 1.6 Hz, H-3). 13C-NMR (C6D6): δ 17.6 (C-11), 17.7 (C-20), 22.0 (C-14), 25.7 (C-19), 27.3 (C-16), 37.6 (C-8), 37.9 (C-7), 40.3 (C-6), 40.7 (C-15), 47.5 (C-9), 48.5 (C-4), 50.6 (10-Ome), 55.4 (12-Ome), 71.7 (C-5), 102.2 (C-12), 114.0 (C-10), 124.0 (C-17), 127.3 (C-13), 127.4 (C-15), 129.0 (C-18), 135.9 (C-13), 141.4 (C-2).

(2) R-2NMA Ester of Secospatacetal D 1H-NMR (CDCl3): δ 0.88 (8H, -1H-Me-8), 1.65 (H-14 and H-20), 1.72 (H-15, 2.00 (H-6), 2.03 (H-8), 2.13 (H-9), 2.21 (H-6), 2.38 (H-4), 2.67 (H-3), 2.78 (H-2), 2.80 (10-Ome), 3.19 (10-Ome), 3.47 (H-7), 3.49 (2NMA), 4.01 (H-12), 5.06 (H-17), 5.11 (2NMA), 5.25 (H-15), 5.36 (H-5), 5.86 (H-3), 5.97 (2), 7.53—8.01 (2NMA).

(1) R-2NMA Ester of Secospatacetal D 1H-NMR (CDCl3): δ 0.88 (8H, -1H-Me-8), 1.65 (H-14 and H-20), 1.72 (H-15, 2.00 (H-6), 2.03 (H-8), 2.13 (H-9), 2.21 (H-6), 2.38 (H-4), 2.67 (H-3), 2.78 (H-2), 2.80 (10-Ome), 3.19 (10-Ome), 3.47 (H-7), 3.49 (2NMA), 4.01 (H-12), 5.06 (H-17), 5.11 (2NMA), 5.25 (H-15), 5.36 (H-5), 5.86 (H-3), 5.97 (2), 7.53—8.01 (2NMA).

17), 5.15 (H-15), 5.51 (H-5), 7.29—7.43 (MP A).

References