NMR study of acutanol, a new cembrene alcohol, and sarcophytol A isolated from the soft coral *Sarcophyton acutangulum*

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Abstract. A new cembrene alcohol, named acutanol, has been isolated from the soft coral, *Sarcophyton acutangulum*. Its structure has been determined on the basis of extensive NMR study. Absolute configuration of the major component, sarcophytol A, has been confirmed by use of a chiral anisotropic reagent, 1-naphthylmethoxyacetic acid (1NMA).

1. Introduction

An increasing number of unique natural products has been found from marine organisms, and they have been attracting the interests of organic chemists and biochemists because of their novel chemical structures and remarkable biological activities.

We have been studying the chemical constituents of marine organisms inhabiting along Japanese coasts, and this paper deals with the spectroscopic analysis of a new compound, acutanol, produced by a soft coral as well as the absolute configuration study of another chemical ingredient, sarcophytol A, of the same marine creature.

2. Structure of a new cembrene alcohol

A soft coral *Sarcophyton acutangulum* was collected off the Ishigaki Island, Okinawa Prefecture, Japan, in 1997 and the whole body was freeze-dried. The freeze-dried soft coral was extracted with hexane at room temperature for one week. Chromatographic separation of the extract afforded sarcophytol A (**1**) [1] as a major component and other minor cembranoids together with (+)-alloaromadendrene [7]. During the purification procedure of **1**, we noticed that the major component was accompanied by a small amount of a compound, which was indicated by the ¹H NMR spectra of the chromatographic fractions.

This minor compound was difficult to remove by several separation methods such as HPLC, and, therefore, the fraction consisting mainly of **1** was treated with acetic anhydride and pyridine. The product showed two spots on TLC (Merck; Kieselgel 60 F₂₅₄; hexane : ethyl acetate = 9 : 1), the major spot (R_f = 0.7) due to the acetate (**3**) and the minor one (R_f = 0.3) due to a new compound, named acutanol (**2**). As will be described later, this compound turned out to have a tertiary hydroxy group that resisted acetylation.

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Molecular formula of acutanol (**2**), $[\alpha]_D + 26.0^\circ$ (CHCl_3), was established as $\text{C}_{20}\text{H}_{32}\text{O}$ by the molecular ion of HR-MS at m/z 288.2433. The ^1H NMR spectrum of **2** exhibited the signals of five olefin protons, δ 5.04 (1H, brt, H-11), 5.16 (1H, brt, H-7), 5.57 (1H, t, $J = 8$ Hz, H-14), 5.94 (1H, d, $J = 15.8$ Hz, H-3) and 6.37 (1H, d, $J = 15.8$ Hz, H-2), two olefinic methyls, δ 1.46 (s, H-19) and 1.59 (s, H-20), isopropyl methyls, δ 1.16 (d, $J = 7$ Hz, H-16) and 1.17 (d, $J = 7$ Hz, H-17), and another singlet methyl, δ 1.25 (s, H-18).

The ^{13}C NMR spectrum indicated the signals assignable to four olefins at δ 121.3 (d), 122.6 (d), 124.8 (d), 126.7 (d), 133.7 (s), 134.0 (s), 137.3 (d), 145.7 (s). The signal at δ 73.5 (s) indicated the presence of a tertiary alcohol.

Assignment of all the ^1H and ^{13}C NMR signals (Table 1) was accomplished by the H,H-COSY, HSQC, and HMBC spectra, which concomitantly led to the structure of acutanol (**2**). The correlations found in the H,H-COSY [a], HMBC [b] and NOESY [c] spectra are indicated by arrows in Fig. 1. The *E*-configuration of the double bond at 2-position is obvious from the large coupling constant ($J = 15.8$ Hz) between H-2 and 3, and the configurations of the double bonds at 7- and 11-positions were deduced to be *E* from the carbon chemical shifts of 19- (δ 14.9) and 20-methyls (δ 17.0) as well as the NOE cross peaks between H-6/H19 and H-10/H-20. The *Z*-configuration of $\text{C}_1=\text{C}_{14}$ was confirmed by the NOE correlations between H-15/H-14 and H-16,17/H-14.

Investigation of the absolute configuration of **2** has been unsuccessful so far, because ozonization of **2** carried out in an attempt to obtain 2-hydroxy-2-methylpentanedioic acid, whose absolute configuration would be determinable by the PGME (phenylglycine methyl ester) method [6], yielded only an intractable mixture.

Fig. 1. Correlations found in the (a) H,H-COSY, (b) HMBC and (c) NOESY spectra of acutanol (**2**) are shown by arrows.

Table 1

The ^1H and ^{13}C NMR assignment of acutanol (C_6D_6 ; 400 MHz for ^1H and 100 MHz for ^{13}C). The chemical shifts of ^1H and ^{13}C are relative to $\text{C}_6\text{D}_5\text{H}$ (δ 7.20) and C_6D_6 (δ 128.0), respectively

Position	^{13}C (δ)	^1H (δ) (J)
1	145.7	—
2	122.6	6.37 (d, 15.8 Hz)
3	137.3	5.94 (d, 15.8 Hz)
4	73.5	—
5	42.2	1.73 (m)
6	24.4	2.15 (m)
7	126.7	5.16 (br.t, 6.4 Hz)
8	133.7	—
9	39.6	2.10 (m)
10	25.1	2.10 (m), 2.25 (m)
11	124.8	5.04 (m)
12	134.0	—
13	37.6	2.71, 2.86 (each dd, 8.1, 15.5 Hz)
14	121.3	5.57 (t, 8.1 Hz)
15	32.3	2.59 (sept, 7.0 Hz)
16	22.7	1.16 (d, 7.0 Hz)
17	22.8	1.17 (d, 7.0 Hz)
18	29.7	1.25 (s)
19	14.9	1.46 (s)
20	17.0	1.59 (s)

3. Absolute configuration of sarcophytol A

We next turned our attention toward sarcophytol A (**1**). We have developed several chiral anisotropic reagents, such as 1-naphthylmethoxyacetic acid (1NMA), which are applicable to determination of the absolute configuration of secondary alcohols [3,5]. We were interested in the effectiveness of 1NMA for elucidating the absolute configuration of such a medium-sized-ring compound.

The stable conformation of the 1NMA ester is depicted in Fig. 2a. Due to the strong diamagnetic anisotropic effect of the naphthalene ring, ^1H NMR signals for the protons on the same side ($\text{H}_{\text{X-Z}}$) of the 1-naphthyl group shift upfield greater than those of the opposite side ($\text{H}_{\text{A-C}}$) in case of an (*R*)-1NMA ester. The reverse should hold true for an (*S*)-1NMA ester. $\Delta\delta$ values are obtained for the respective protons by subtracting the chemical shifts of the (*S*)-ester from those of the (*R*)-ester (Fig. 2b). The absolute configuration is obtained by positioning the protons with positive and negative $\Delta\delta$ values on the right and left side of the model B (as for model A, see [4]), respectively. An advantage of this method is that the $\Delta\delta$ values are usually two or three times greater than those obtained by the conventional modified Mosher's method using MTPA [4].

Sarcophytol A (**1**) was esterified with (*R*)- and (*S*)-1NMA. The proton chemical shifts were determined by the H,H-COSY and HSQC experiments and the $\Delta\delta$ values ($= \delta_{R\text{-ester}} - \delta_{S\text{-ester}}$) were calculated for the protons. The results are shown in Fig. 3. These values are about three times greater than those usually obtained when the MTPA esters are used. All the assigned protons with positive and negative $\Delta\delta$ values are located on the right and left sides of the 1NMA plane, respectively, when the *S*-configuration of the hydroxyl group is assigned for sarcophytol A. This result is the same as reported for sarcophytol A, the absolute configuration of which has been established by X-ray crystallography worked on its

(a) The stable conformation of the 1NMA ester of a secondary alcohol.

(b) Model B to determine the absolute configurations of secondary alcohols.

Fig. 2. (a) The structure of 1-naphthylmethoxyacetic acid (1NMA) and the stable conformation of the 1NMA ester of a secondary alcohol. (b) Model B to show the correct absolute configuration of secondary alcohols possessing the protons with positive and negative $\Delta\delta$ values on the right and left sides of the 1NMA plane, respectively.

Fig. 3. The $\Delta\delta$ ($= \delta_{R\text{-ester}} - \delta_{S\text{-ester}}$) values (in ppm) obtained for the protons of sarcophytol A (**1**). The ^1H NMR spectra were recorded at 400 MHz for CDCl_3 solutions.

methoxytrifluoromethylphenylacetic acid (MTPA) ester [2]. Thus, use of 1NMA for determination of the absolute configuration of **1** has been verified.

4. Experimental

4.1. General

^1H and ^{13}C NMR spectra were recorded on JEOL AL-300 and Bruker ARX-400 spectrometers. High-resolution mass spectra were taken under electron impact (EI) conditions using a JEOL JMS-SX102A spectrometer with a direct inlet system. Optical rotations were determined for solutions in CHCl_3 on a JASCO DIP-370 polarimeter.

4.2. Separation of sarcophytol A and acutanol

The soft coral *Sarcophyton acutangulum*¹ collected at the Ishigaki Island, Okinawa Prefecture, Japan, in 1997 was freeze-dried. The resulting material (900 g) was soaked in hexane at room temperature for one week. Evaporation of the hexane gave an oil (20.6 g). Repeated chromatographic separation (Merck; Kieselgel 60) afforded a fraction containing sarcophytol A (**1**) (4.2 g). The ¹H and ¹³C NMR signals of this product were mostly identical with the reported ones [1]. Purity of this material seemed to be more than 90% (¹H NMR), its TLC showing only one spot under several solvent systems. A small portion (46 mg) of this material was successively separated by preparative TLC (Merck; Kieselgel F₂₅₄; hexane: ethyl acetate = 7 : 3) yielding pure sarcophytol A (32 mg) : [α]_D + 148.5° (c 0.11, CHCl₃) (lit. +141°) [1]. The crude sarcophytol A (3.85 g) was treated with acetic anhydride (8 ml) and pyridine (10 ml) at room temperature for 18 h. The mixture was worked up as usual, giving an oily product (3.56 g). Chromatographic separation afforded sarcophytol A acetate (**3**) (3.3 g) [1] and acutanol (**2**) (330 mg). Acutanol (**2**) HR-MS *m/z* 288.2433 (Calcd for C₂₀H₃₂O: 288.2453); [α]_D + 26.0° (c = 0.31, CHCl₃); IR (film) 3400 cm⁻¹.

4.3. Esterification of sarcophytol A with (R)- and (S)-INMA

(R)-INMA ester of sarcophytol A. To a solution of sarcophytol A (17.0 mg; 0.06 mmol), (*R*)-1NMA (1-naphthylmethoxyacetic acid) (19.3 mg; 0.09 mmol) and 4-dimethylaminopyridine (15.7 mg; 0.13 mmol) in dry dichloromethane (1.5 ml) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (23.2 mg; 0.12 mmol), and the mixture was stirred for 19 h under N₂ atmosphere. The mixture was treated with water and CH₂Cl₂, and the organic layer was washed with 10% aqueous citric acid, water, 5% aqueous NaHCO₃, water, and brine. The organic layer was dried over Na₂SO₄ and concentrated. Purification of the crude ester by preparative TLC gave 14.6 mg of (*R*)-1NMA ester of sarcophytol A. (*S*)-1NMA ester of sarcophytol A was obtained in the same manner.

(R)-INMA ester of sarcophytol A. ¹H NMR (CDCl₃, 400 MHz) δ 0.947 (3H, d, *J* = 6.8 Hz, H-17), 0.977 (3H, d, *J* = 6.8 Hz, H-16), 1.310 (3H, s, H-20), 1.434 (3H, s, H-19), 1.702 (3H, s, H-18), 2.273 (1H, sept, *J* = 6.8 Hz, H-15), 3.459 (3H), 4.926 (1H, brs, H-7), 4.940 (1H, brs, H-11), 5.321 (1H, s), 6.042. (1H, d, *J* = 10.4 Hz, H-3), 6.137 (1H, d, *J* = 10.4 Hz, H-2), 6.015 (1H, dd, *J* = 8.8, 3.6 Hz, H-14), 7.43–8.29 (7H, naphthyl). ¹³C NMR (CDCl₃, 100 MHz) δ 141.6 (C-1), 122.3 (C-2), 121.3 (C-3), 136.9 (C-4), 39.6 (C-5), 25.4 (C-6), 124.3 (C-7), 134.3 (C-8), 38.5 (C-9), 24.4 (C-10), 126.2 (C-11), 129.9 (C-12), 41.5 (C-13), 73.8 (C-14), 27.7 (C-15), 23.9 (C-16), 24.5 (C-17), 16.2 (C-18), 15.5 (C-19), 17.7 (C-20), 169.7, 133.8, 132.4, 131.0, 129.1, 128.5, 126.4, 126.2, 125.7, 125.1, 124.1, 81.2, 57.5 (1NMA).

(S)-INMA ester of sarcophytol A. ¹H NMR (CDCl₃, 400 MHz) δ 0.312 (3H, d *J* = 6.8 Hz, H-16), 0.810 (3H, d, *J* = 6.8 Hz, H-17), 1.447 (3H, s, H-19), 1.542 (3H, s, H-20), 1.648 (3H, s, H-18), 1.975 (1H, sept, *J* = 6.8 Hz, H-15), 3.451 (3H), 4.942 (1H, brt, *J* = 5.6 Hz, H-7), 5.021 (1H, brt, *J* = 5.6 Hz, H-11), 5.375 (1H, s), 5.997 (1H, d, *J* = 11.6 Hz, H-2), 6.043 (1H, d, *J* = 11.6 Hz, H-3), 6.087 (1H, dd, *J* = 9.8, 4 Hz, H-14), 7.409–8.30 (7H, naphthyl). ¹³C NMR (CDCl₃, 100 MHz) δ 141.5 (C-1), 122.3 (C-2), 121.3 (C-3), 136.7 (C-4), 39.6 (C-5), 25.4 (C-6), 124.4 (C-7), 134.2 (C-8), 38.5 (C-9), 24.4 (C-10), 126.0 (C-11), 129.8 (C-12), 41.9 (C-13), 73.5 (C-14), 27.2 (C-15), 23.4 (C-16), 24.1 (C-17), 16.1

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(C-18), 15.4 (C-19), 17.9 (C-20), 169.6, 133.8, 132.2, 131.1, 129.1, 128.4, 126.4, 126.2, 125.6, 125.1, 124.1, 81.0, 57.4 (1NMA).

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