ABSOLUTE CONFIGURATIONS OF CYTOTOXIC MARINE CEMBRANOLIDES; CONSIDERATION OF MOSHER'S METHOD

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Abstract. Stereochemistry of the cembranolides 1 and 5, isolated from the soft coral <u>Sinula-ria mayi</u>, has been elucidated by means of 2D NMR spectroscopy and X-ray crystallography. The absolute configuration of denticulatolide **6** has been determined by X-ray analysis, and the validity of Mosher's method is discussed as to the MTPA esters **3**, **4**, **7**, and **8**.

An abundance of cembranolides have been found from marine organisms such as soft corals and sponges,¹ and they have been attracting the attention of many scientists because some of them have been found to exhibit remarkable biological activities such as ichtyotoxicity.² Herein, we describe the determination of the stereochemistry of cembranolides 1^3 and 5^4 together with the absolute configuration of denticulatolide 6,² which were obtained from the Okinawan soft coral Sinularia mayi.



Chromatographic separation of the MeOH extract of \underline{S} . <u>mayi</u> by monitoring cytotoxicity gave three cytotoxic compounds A, B, and C.

Cembranolide A was obtained as colorless crystals, mp 180-181 °C, $[\alpha]_D$ +50.5 (c 0.30, CHCl₃). By comparison of its physical and spectroscopic properties, this substance was identified with 1, which had been obtained from Lobophytum pauciflorum.³ Because the stereo-chemistry of this compound was not known, we wanted to elucidate the relative configurations of the substituents and the geometry of the double bonds by ¹H-NMR (500 MHz) spectroscopy. There have been very few reports on the stereochemical elucidation of cembranoids by NMR studies.⁵ Fortunately, 1 showed well-defined signals in the ¹H-NMR spectrum, and the coupling patterns of all the protons as well as the carbon signals could be assigned by ¹H-¹H and ¹H-¹³C COSY and decoupling difference spectra (Table 1). Also, the phase-sensitive NOESY afforded a number of NOE cross peaks, by which we could propose the stereochemical features for 1 (as to conformation, see 1a). The stereochemistry was verified by X-ray crystal-



lography, ⁶ which showed that, in a crystalline state, **1** exists in the same conformation (1a)

Fig. 1. Stereoview of 1 and 9.

Cembranolide B, a pale yellow oil, $[\alpha]_D$ -5.8° (c 0.11, CHCl₃), was identified to be 5 (isolated from the genus <u>Lobophytum</u>), the stereochemistry of which has not yet been revealed.⁴ By means of 2D NMR spectroscopy, the stereochemistry of 5 was deduced to be as described in structure 5 (see also 5a), and this was confirmed by the following chemical conversions. 1 was saponified (LiOH/dioxane/H₂O), and the product, after acidification and isolation, was heated in benzene with continuous removal of water. The resulting hydroxylactone (2) was oxidized (PCC/CH₂Cl₂) to give a ketone, $[\alpha]_D$ -4.8° (c 0.07, CHCl₃), which was identified with 5 by spectroscopic comparison. The chiloptical properties of both compounds are the same, showing that their absolute configurations are identical.

Cembranolide C, mp 118-119 °C , $[\alpha]_D$ -18.8° (c 0.40, CHCl₃), was identified with denticulatolide (6) by comparison of its physical properties with those of the authentic sample.² The relative stereochemistry of this compound has been firmly established by X-ray crystallography.² Our 2D NMR studies led to the conformation 6a (in a solution) identical with that in a crystalline state.² The absolute configuration of 6, however, has not been determined yet, and therefore we focused our attention on elucidation of the absolute configurations of 6 and 1. Initially, Mosher's method using 19F-NMR spectroscopy⁷ was applied. The acetates 1and 6 were converted to the MTPA esters 3, 4 and 7, 8, respectively, $[(i) LiOH/dioxane/H_2O,$ (ii) H^+ , (iii) heat in benzene, (iv) (+) and (+/-)-MTPA chlorides/CH₂Cl₂/DMAP/Et₃N], and their ¹⁹F-NMR spectra were compared. In these pairs of MTPA esters, the trifluoromethyl groups of (+)-esters 3 and 7 exhibit lower chemical shifts than those of the corresponding (-)-esters 4 and 8, thus suggesting 13R-configuration of 1 and 7- \underline{R} of 6, while detailed analyses of the 1 H-NMR (500 MHz) spectra revealed that there were significant and systematic differences between the proton chemical shifts of MTPA esters 7 and 8 (Fig. 2). According to Mosher's method using ¹H-chemical shifts,⁸ we could assume the 7<u>S</u>-configuration for 6. The same analysis was carried out on 1 and we obtained the 13S-configuration.

Eventually the 7<u>S</u>-configuration of **6** was unambiguously established by X-ray crystallography⁶ performed for the <u>p</u>-bromobenzoate **9** (Fig.1).

Considering that the 1 H-NMR behaviors of the MTPA ester pairs 7, 8 and 3, 4, are in parallel, and that 1 was isolated from the same organism as 6 was obtained, the 13S-configuration was assigned for 1. Since 1 had been transformed to 5, the present work also led to 1S, 2R-configuration for 5.

The incorrect prediction on the absolute configuration of **6** by ¹⁹F-NMR spectroscopy requires comment. In Mosher's (¹⁹F) rule the major factor causing the ¹⁹F-chemical shift difference between the (+) and (-)-MTPA esters is assumed to be the steric repulsion from the bulkier β (compared with the smaller β ')-substituent to the phenyl group, which results in the rotation of the methoxytrifluoromethylphenylmethyl group in the direction shown in Fig. 3 [(+)-CF₃ shows lower chemical shift than (-)-CF₃.].⁷

the present cembranolides, the substituents on the carbons other than β -carbon may cause a greater steric compression. Actually, molecular models of the (-)-MTPA ester (8) reveal that the distance from the phenyl group to 3-H (ϵ -H) is much shorter than that to 19-Me (β -substituent). Also, in the (+)-MTPA ester (7) the phenyl group is closer to 9-H (γ -H) and 10-H (δ -H) than 19-Me (on β -carbon). Thus, consideration of the steric effect from β -substituents alone may lead to an erroneous prediction of the absolute configuration.

Tab	le l. ^l H	and 1 <u>1</u>	³ C-NMR (CDCl ₃)	Constan	ts of <u>5</u>	<u>1, 5</u> , and <u>6</u> ^a		<u>6</u>	
No.	δ ¹³ c	δ ¹ H	(J in Hz)	δ ¹³ C	δ ¹ H	(J in Hz)	δ ¹³ c	δ ¹ H	(J in Hz)
1	38.5	2.93	(12,8,3 ^b)	37.0	3.58	(11,9,3 ^b)	44.6	2.77	(12,7,2,1.5 ^e)
2	77.4	5.40	(10,8)	76.9	5.54	(10,9)	79.4	5.13	(7 ^e)
3	119.8,	4.90		118.7	4.76	(brd,10)	123.3	5.44	(brd,7)
4	138.2 ^a			138.0 ⁿ			138.5		
5	40.1	2.1	(m)	40.0	1,95	(m)	35.6	2.07	(m)
		2.27	(m)		2.26	(m)		1.94	(m)
6	24.7	2.1	(m)	24.4	2.15	(2H,m)	26.1	1.55	(m)
		2.27	(m)			b		1.94	(m)
7	125.7	4.92 [°]		125.8	4.90	(8,2 ^D)	70.3	5,72	(10)
8	134.2			133.4			80.1		
9	36.9	2.1	(2H,m)	36.4	2.06	(14,10,4)	31.1	1.71	(14,4,5,2)
			-		2,15	(14,9,4)		1.46	(5,14 ^e)
10	23.8	1.93	(14,3,5 [°])	23.9	1.96	(14,10,4)	24.8	1.55	(14,9,2.5,2)
		1.47	(14,10,8,6)		1.42	(14,4,9")		2.07	(12,9,4.5,5)
11	60.2	2.74	(10,3)	60.9	2.95	(9,4)	84.4	4,27	(12,2.5)
12	61.2			63.6			135.4		1-
13	72.1	4.94	(11,2)	207.8			125.6	5.31	(11,4,2)
14	27.3	1.39	(13,12,2)	35.2	2.57	(20,11)	28.2	3.02	(14,11,2)
	đ	1.83	(13,11,3)	h	2.70	(20,3)		1.78	(14,12,4,2 ^D)
15	143.9 [°]			145.5"			140.2		
16	170.2 ¹			169.9			170.5		
17	121.0	5.46	(brd,3)	120.8	5.42	(brd,3)	121.4	5.56	(1.5)
		6.22	(brd,3)		6.20	(brd,3)		6.16	(1.5)
18	15.7	1.82	(2)	14.8	1.62	(brs)	16.5	1.61	(brs)
19	15.69	1.56	(brs)	15.1	1.56	(2)	19.6	1.03	(s)
20	15.5 ⁹	1.11	(s)	12.3	1.37	(s)	24.5	1.70	(2)
21	171.2						170_6		
22	20,9	2.14	(s)				21.1	2.04	(s)

a 1 H and ¹³C-NMR spectra were recorded at 500 and 125 MHz, respectively. ^bquartet. ^CThese signals were obscured by other signals. ^{d,f-h}These assignments can be interchanged. ^etriplet.







5<u>a</u>

Cembranolides 1, 5, and 6 exhibit cytotoxic activity against B16 (mouse melanoma) cells at 8.4, 2.1, and 3.6 ppm, respectively.



 $\begin{array}{c} & \beta \\ \hline MeO \\ \hline OCF_3 \\ \hline OCF_3 \\ \hline OCF_3 \\ \hline OMe \\ \hline OMe \\ \hline OCF_3 \\ \hline OCF_3 \\ \hline OMe \\ \hline OCF_3 \\ \hline OC$

(-)-esters

Fig. 2. ¹H-Chemical shift differences [δ (-MTPA) - δ (+MTPA)] of the MTPA esters.

Fig. 3. (+) and (-)-MTPA esters of an R-alcohol.

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- 6. Crystal data of 1: $C_{22}H_{30}O_5$, mp 180-181 °C (MeOH), monoclinic, space group P2₁, a = 10.590(1), b = 10.399(2), c = 9.497(2) Å, $\beta = 92.37(1)$, and Z = 2. The structure was solved by direct methods and refined by full-matrix least squares calculation to R = 0.050. Crystal data of 9: $C_{27}H_{31}O_6$ Br, mp 187-189 °C (AcOEt), orthorhombic, space group P2₁2₁2₁, a = 13.863(2), b = 19.661(3), c = 9.433(1) Å, and Z = 4. The absolute configuration was established by measuring 30 Bijovoet pairs. The final R was 0.085 (Rw = 0.078).
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