Colopsinols D and E, New Polyhydroxyl Linear Carbon Chain Compounds from Marine Dinoflagellate *Amphidinium* sp.

Takaaki KUBOTA,^a Masashi TSUDA,^a Miho TAKAHASHI,^a Masami ISHIBASHI,^a Seiko OKA,^b and Jun'ichi KOBAYASHI^{*,a}

Graduate School of Pharmaceutical Sciences^a and Center for Instrumental Analysis,^b Hokkaido University, Sapporo 060-0812, Japan. Received May 31, 2000; accepted July 6, 2000

Colopsinols D (1) and E (2), two new polyhydroxyl linear carbon chain compounds, have been isolated from the cultured marine dinoflagellate *Amphidinium* sp. The structures of 1 and 2 were elucidated on the basis of two-dimensional NMR and FAB-MS/MS data.

Key words dinoflagellate; *Amphidinium* sp.; polyhydroxyl compound; FAB-MS/MS

Marine dinoflagellates are an important source of bioactive natural products. During our search for bioactive metabolites from marine dinoflagellates,^{1–3} we found three new polyhydroxyl linear carbon chain compounds with a glucoside moiety, colopsinols A, B, and C, from laboratory cultured marine dinoflagellate *Amphidinium* sp.^{4,5} Further investigation of the extract resulted in the isolation of two minor polyhydroxyl linear carbon chain compounds, colopsinols D (1) and E (2). This paper describes the isolation and structure elucidation of 1 and 2.

The harvested cells of the dinoflagellate were extracted with MeOH/toluene (3:1). The toluene-soluble materials of the extract were subjected to a silica gel column (CHCl₃/MeOH, 95:5→MeOH). The fraction eluted with MeOH was separated by gel filtration on Sephadex LH-20 (CHCl₃/MeOH, 1:1) and centrifugal partition chromatography (CPC, ascended mode, CHCl₃/MeOH/H₂O, 5:6:4) followed by C₁₈ column chromatography (iso-PrOH/H₂O, 45:55) to yield colopsinols D (1, 0.0001%, wet weight) and E (2, 0.0003%) together with colopsinols A⁴ (3), B, and C.⁵

The physico-chemical data of colopsinol D (1) were similar to those of colopsinol A⁴ (3). The IR spectrum was indicative of the presence of hydroxyl group(s) ($\nu_{\max}$ 3430 cm⁻¹), ketone carbonyl(s) ($\nu_{\max}$ 1710 cm⁻¹), and sulfate ester ($\nu_{\max}$ 1250 cm⁻¹).⁶ The presence of sulfate ester was also suggested by characteristic fragment ions observed in the negative ion fast atom bombardment (FAB) MS [m/z 80 (SO₃⁻), 97 (HSO₄⁻)]. Positive ion high resolution electron spray ionization (HR-ESI) MS data [m/z 1431.7490, (M+Na)⁺, Δ +3.9 mmu] of colopsinol D (1) indicated the molecular formula, C₇₁H₁₁₇NaO₂₄S, corresponding to that of a dehydrated form of 3. The ¹³C-NMR data including distortionless enhancement by polarization transfer (DEPT) experiments revealed that 1 contained two ketones, one *sp*² quaternary carbon, seven *sp*² methines, two *sp*² methylenes, twenty-three oxymethines, two oxymethylenes, two *sp*³ methines, twenty-nine *sp*³ methylenes, and three methyl groups, thus accounting for total 71 carbons. The number of hydroxyl groups was deduced from the following ¹³C-NMR deuterium-induced shift experiment using CD₃OH and CD₃OD as solvents. Of 25 signals observed for oxygenated carbons (δ_C 59–106), 15 oxygenated carbons (14 oxymethines and one oxymethylene) did not show deuterium-induced upfield shifts, while colopsinol A (3) included 13 un-

changed oxygenated carbons in the ¹³C-NMR deuterium-induced shift experiment.⁴ Of the unchanged oxygenated carbons for 1, two low-field methine carbons at δ_C 104.86 (C-1') and 105.81 (C-1'') in CD₃OH were attributed to anomeric carbons of sugar units, while four high-field ones at δ_C 61.14 (C-45), 60.58 (C-46), 59.84 (C-51), and 60.26 (C-52) were involved in two epoxide rings. The remaining unchanged oxygenated carbons were assigned as those bearing ether-oxygen (C-4, δ_C 73.85; C-8, δ_C 74.64, C-9, δ_C 82.86, C-12, δ_C 82.07), glycoside linkages (C-18, δ_C 78.19; C-6'; δ_C 71.08), and a sulfate ester⁷ (C-5, δ_C 80.10) from the NMR and MS data described below. Thus, colopsinol D (1) was indicated to have an ether ring more than colopsinol A (3).

Interpretation of ¹H-¹H correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), and ¹H-detected heteronuclear single quantum coherence (HSQC) spectra led to the following six partial structures: a) from C-1 to C-13, b) from C-15 to C-21, c) from C-28 to C-48, d) from C-50 to C-56, e) from C-1' to C-6', and f) from C-1'' to C-6'' (Fig. 1). ¹H-Detected heteronuclear multiplebond correlation (HMBC) cross-peaks for H₂-13/C-14 (δ_C 212.20), H₂-15/C-14, H₂-21/C-22 (δ_C 215.37), and H₂-23/C-22 suggested that the partial structures a, b, and c were connected through two ketone carbonyls (C-14, C-22). The presence of an *exo*-methylene group at C-49 was deduced from HMBC correlations for H₂-59/C-48 (δ_C 34.57), H₂-59/C-49 (δ_C 147.37), and H₂-59/C-50 (δ_C 40.42). The relative stereochemistry of the two epoxide rings at C-45–C-46 and C-51–C-52 in the partial structure c was assigned as both *trans* by ¹H-¹H coupling constants ($J_{45/46}$ =2.5 Hz, $J_{51/52}$ =2.0 Hz).⁸ The *E*-geometries of three disubstituted double bonds at C-31–C-32, C-35–C-36, and C-39–C-40 in the partial structure c were deduced from the carbon chemical shifts of allylic carbons (C-30, δ_C 41.97⁹); C-33, δ_C 38.41¹⁰); C-34, δ_C 42.26⁹); C-37, δ_C 34.65; C-38, δ_C 34.65; C-41, δ_C 34.26). The linear aliphatic chain at C-23–C-28 was revealed by the substantial product ion peaks at m/z 931, 917, 903, 889, 875, and 861 observed in the FAB-MS/MS spectrum of 1 [precursor ion m/z 1385 (M–Na)⁻] (Fig. 2). Two sugar units (e, f) were both assigned as β -glucose on the basis of the ¹H-¹H coupling constants ($J_{1'/2'}$ =7.5 Hz, $J_{1''/2''}$ =8.1 Hz). Both sugars were elucidated to be D-form by chiral HPLC analyses of 1-*O*-methyl-2,3,4,6-tetra-*O*-benzoyl derivatives of the methanolysis products of 1.¹¹ Nuclear Overhauser effects

* To whom correspondence should be addressed. e-mail: jkobay@pharm.hokudai.ac.jp
Dedicated to the memory of Dr. Kyosuke Tsuda.

Stereochemistry of the tetrahydropyran and tetrahydrofuran rings are relative, and two epoxide rings are *trans*.

Stereochemistry of the tetrahydropyran ring is relative, and two epoxide rings are *trans*.

Chart 1. Structures of 1—3

Fig. 1. 2D-NMR Correlations of Colopsinol D (1)

(NOE) were observed from H-1' to H-18 and from H-1" to H-6', indicating that a β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside was connected to C-18. The presence of a tetrahydropyran ring in the partial structure **a** was implied by HMBC cross-peaks from H-4 to C-8 (δ_C 74.64). The relative stereochemistry of the tetrahydropyran ring at C-4—C-8 was deduced as a chair form from ^1H - ^1H coupling constants ($J_{4/5}$ = 3.5 Hz; $J_{5/6}$ = 3.0 Hz; $J_{6/7\alpha}$ = 10.0 Hz; $J_{6/7\beta}$ = 4.3 Hz; $J_{7\alpha/8}$ = 9.0 Hz; $J_{7\beta/8}$ = 3.3 Hz). The carbon signal (δ_C 80.10) of

C-5 in **1** was located at a lower-field than those (*ca.* δ_C 70) of the axially-oriented hydroxy-bearing carbons on two tetrahydropyran rings of luteophanol A,^[12] suggesting that the sulfate ester was attached to C-5. The existence of a tetrahydrofuran ring at C-9—C-12 was deduced from HMBC correlation from H-9 to C-12 (δ_C 82.07). The oxymethine carbon signals at C-9 (δ_C 82.86) and C-12 (δ_C 82.07) of **1** were located at a relatively lower-field than those of **3** (C-9, δ_C 74.75; C-12, δ_C 69.54), the chemical shift differences of geminal methylene protons of C-10 (δ_H 2.05, 1.73) and C-11 (δ_H 2.19, 1.60) were relatively large,^[13] and the FAB-MS/MS spectrum (Fig. 2) of **1** showed product ion peaks at m/z 283 and 281, supporting the presence of a tetrahydrofuran ring at C-9—C-12. The relative stereochemistry of the tetrahydrofuran ring was elucidated to be *trans* by NOE to H₂-13 obtained from the irradiation of H-9, while the relative stereochemistry between C-8 and C-9 was not defined. Thus, the structure of colopsinol D was elucidated to be **1**.

Colopsinol E (**2**) had the molecular formula of $\text{C}_{65}\text{H}_{109}\text{NaO}_{20}\text{S}$ as revealed by the HR-ESI-MS [m/z 1287.7058, ($\text{M}+\text{Na}$)⁺, Δ +3.0 mmu]. The ^1H - and ^{13}C -NMR data (Table 1) of **2** were similar to those of colopsinol A (**3**), except for lack of resonances for one glucose in **2**. Two-dimensional (2D)-NMR data including the ^1H - ^1H COSY, TOCSY, and HSQC spectra as well as negative ion FAB-

Table 1. ^1H - and ^{13}C -NMR Data of Colopsinols D (1) and E (2)

Position	1				2			
	$\delta_{\text{H}}^{(a)}$	(m, Hz)	$\delta_{\text{C}}^{(b)}$	m	$\delta_{\text{H}}^{(a)}$	(m, Hz)	$\delta_{\text{C}}^{(b)}$	m
1	1.25 ^(c)	(d, 6.2)	24.72	q	1.25 ^(c)	(d, 6.3)	24.86	q
2	3.97	(m)	65.81	d	3.97	(m)	65.83	d
3	1.86	(m)	39.98	t	1.89	(m)	39.98	t
	1.61	(m)			1.59	(m)		
4	4.50	(dt, 10.4, 3.5)	73.85	d	4.52	(dt, 10.6, 3.5)	73.76	d
5	4.36	(br t, 3.1)	80.10	d	4.36	(br t, 3.1)	80.33	d
6	4.12	(ddd, 3.0, 4.3, 10.0)	67.34	d	4.10	(ddd, 3.0, 4.4, 10.0)	67.47	d
7	1.86	(m)	33.23	t	1.88	(m)	39.98	t
	1.77	(m)			1.78	(m)		
8	3.60	(ddd, 9.0, 6.0, 3.3)	74.64	d	3.55	(ddd, 9.3, 5.8, 3.5)	74.94	d
9	4.23	(br dt, 1.3, 6.0)	82.86	d	3.68	(m)	74.75	d
10	2.05	(m)	41.97	t	1.66	(m)	35.29	t
	1.73	(m)			1.60	(m)		
11	2.19	(m)	40.28	t	1.65	(m)	30.57	t
	1.60	(m)			1.60	(m)		
12	4.40	(m)	82.07	d	4.12	(m)	69.54	d
13	2.81 ^(d)	(m)	53.17 ^(a)	t	2.67 ^(d)	(m)	53.34 ^(a)	t
14			212.20	s			212.18	s
15	2.71 ^(d)	(m)	52.56 ^(a)	t	2.66 ^(d)	(m)	52.69 ^(a)	t
16	4.44	(m)	66.11	d	4.44	(m)	66.17	d
17	1.71	(m)	44.51	t	1.71	(m)	44.37	t
	1.61	(m)			1.58	(m)		
18	3.99	(m)	78.19	d	3.98	(m)	78.45	d
19	1.86 ^(d)	(m)	31.78	t	1.85 ^(d)	(m)	31.79	t
20	1.86 ^(d)	(m)	31.78	t	1.87 ^(d)	(m)	31.49	t
21	2.69 ^(d)	(m)	40.08	t	2.68 ^(d)	(m)	40.12	t
22			215.37	s			215.47	s
23	2.53 ^(d)	(m)	44.51	t	2.53 ^(d)	(t, 7.4)	44.42	t
24	1.59 ^(d)	(m)	25.82	t	1.58 ^(d)	(m)	25.73	t
25	1.33 ^(d)	(m)	28.98	t	1.33 ^(d)	(m)	28.97	t
26	1.35 ^(d)	(m)	31.23	t	1.35 ^(d)	(m)	31.21	t
27	1.33 ^(d)	(m)	31.51	t	1.33 ^(d)	(m)	31.49	t
28	1.37	(m)	35.21	t	1.37	(m)	38.39	t
	1.13	(m)			1.13	(m)		
29	1.48	(m)	39.13	d	1.48	(m)	35.23	d
30	2.02	(m)	41.97	t	2.00	(m)	42.01	t
	1.87	(m)			1.88	(m)		
31	5.37	(m)	128.94	d	5.36	(m)	128.98	d
32	5.35	(m)	139.21	d	5.34	(m)	139.25	d
33	2.16	(m)	38.41	d	2.16	(m)	39.20	d
34	2.01 ^(d)	(m)	42.26	t	2.00 ^(d)	(m)	42.30	t
35	5.42	(m)	132.92	d	5.42	(m)	132.96	d
36	5.42	(m)	130.91	d	5.42	(m)	130.96	d
37	2.08 ^(d)	(br s)	34.65	t	2.07 ^(d)	(br s)	34.69	t
38	2.08 ^(d)	(br s)	34.65	t	2.07 ^(d)	(br s)	34.67	t
39	5.45	(m)	132.22	d	5.45	(m)	132.26	d
40	5.45	(m)	132.05	d	5.45	(m)	132.08	d
41	2.04 ^(d)	(m)	34.26	t	2.04 ^(d)	(m)	34.31	t
42	1.46 ^(d)	(m)	27.25	t	1.46 ^(d)	(m)	27.30	t
43	1.46 ^(d)	(m)	31.23	t	1.45 ^(d)	(m)	31.27	t
44	1.55 ^(d)	(m)	33.73	t	1.54 ^(d)	(m)	33.78	t
45	2.77	(ddd, 9.3, 5.0, 2.5)	61.14	d	2.77	(ddd, 9.3, 5.0, 2.5)	61.18	d
46	2.77	(ddd, 9.3, 5.0, 2.5)	60.58	d	2.77	(ddd, 9.3, 5.0, 2.5)	60.62	d
47	1.74	(m)	32.06	t	1.74	(m)	32.10	t
	1.69	(m)			1.69	(m)		
48	2.28 ^(d)	(m)	34.57	t	2.27 ^(d)	(m)	34.61	t
49			147.37	s			147.41	s
50	2.28 ^(d)	(m)	40.42	t	2.29 ^(d)	(m)	40.45	t
51	2.88	(ddd, 6.2, 5.0, 2.0)	59.84	d	2.88	(ddd, 6.2, 5.0, 2.0)	59.87	d
52	2.81	(ddd, 6.2, 5.0, 2.0)	60.26	d	2.80	(ddd, 6.2, 5.0, 2.0)	60.29	d
53	1.65 ^(d)	(m)	33.23	t	1.68 ^(d)	(m)	33.27	t
54	2.25 ^(d)	(m)	32.06	t	2.24 ^(d)	(m)	32.07	t
55	5.90	(ddt, 17.1, 10.3, 6.9)	139.73	d	5.90	(ddt, 17.0, 10.3, 6.7)	139.77	d
56	5.10	(m)	116.35	t	5.10	(m)	116.40	t
	5.02	(m)			5.03	(m)		
57	0.90 ^(c)	(d, 6.9)	20.77	q	0.90 ^(c)	(d, 6.7)	20.81	q
58	1.00 ^(c)	(d, 6.2)	21.56	q	0.99 ^(c)	(d, 6.7)	21.62	q

droxyl groups, a glucoside moiety, and a sulfate ester. Colopsinol D (**1**) has a tetrahydrofuran ring at C-9—C-12, while colopsinol E (**2**) is the mono-deglucosyl form of colopsinol A (**3**). Colopsinol E (**2**) exhibited cytotoxicity against L1210 murine leukemia cells (IC_{50} value: $7 \mu\text{g/ml}$), while colopsinol D (**1**) did not show such cytotoxicity ($IC_{50} > 20 \mu\text{g/ml}$).

Experimental

IR and UV spectra were recorded on JASCO FT/IR-5300 and JASCO Ubest-35 spectrophotometers, respectively. ESI mass spectra were obtained on a JEOL JMS-700TZ, JEOL SX-102A, or Shimadzu LCMS-QP8000 spectrometer using a sample dissolved in MeOH with a flow rate of $200 \mu\text{l/min}$. The MS/MS spectra were obtained on a JEOL JMS-700TZ tandem mass spectrometer equipped with a charge coupled device (CCD) array detector. The mass spectrometer was operated at an accelerating voltage of 3 kV and in the negative mode. Argon collision gas was used with the pressure to reduce the selected precursor ion intensity by 30%. A 1 : 1 mixture of glycerol and nitrobenzyl alcohol was used as a matrix of FAB-MS/MS.

Cultivation and Isolation The dinoflagellate *Amphidinium* sp. (strain number Y-5) was uniaxially cultured at 25°C for two weeks in seawater medium enriched with 1% Erd-Schreiber (ES) supplement. The harvested cells of the cultured dinoflagellate (1205 g wet weight, from 4960 l of culture) were extracted with MeOH/toluene (3 : 1, 31×3). After addition of 1 M NaCl (1 l), the mixture was extracted with toluene (41×3). The toluene soluble fraction was evaporated under reduced pressure to give a residue (44.4 g), which was partially (26.7 g) subjected to a silica gel column eluted with $\text{CHCl}_3/\text{MeOH}$ and then MeOH. Part (2.33 g) of the fraction (3.3 g) eluted with MeOH was purified by gel filtration on Sephadex LH-20 ($\text{CHCl}_3/\text{MeOH}$, 1 : 1), CPC (ascended mode, $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$, 5 : 6 : 4), and C_{18} column (iso- $\text{PrOH}/\text{H}_2\text{O}$, 45 : 55) to afford colopsinols D (**1**, 0.8 mg, 0.0001%, wet weight) and E (**2**, 1.9 mg, 0.0003%) together with colopsinols A (**3**, 0.0008%), B (0.001%), and C (0.0002%).

Colopsinol D (**1**): IR (KBr) cm^{-1} : 3430, 1710, 1250. ^1H - and ^{13}C -NMR (Table 1); ESI-MS (negative mode) m/z : 1385 $[\text{M}-\text{Na}]^-$. HR-ESI-MS (positive mode) m/z ($\text{M}+\text{Na}$) $^+$: Calcd for $\text{C}_{71}\text{H}_{117}\text{O}_{24}\text{SNa}_2$: 1431.7451. Found 1431.7490.

Colopsinol E (**2**): IR (KBr) cm^{-1} : 3430, 1705, 1250. ^1H - and ^{13}C -NMR (Table 1); ESI-MS (negative mode) m/z : 1241 $[\text{M}-\text{Na}]^-$; HR-ESI-MS (positive mode) m/z ($\text{M}+\text{Na}$) $^+$: Calcd for $\text{C}_{65}\text{H}_{109}\text{O}_{20}\text{SNa}_2$: 1287.7028. Found 1287.7058.

Determination of Stereochemistry of the Sugar Units of Colopsinols D (1**) and E (**2**) by Chiral HPLC** Colopsinol D (**1**, 0.1 mg) or E (**2**, 0.1 mg) was treated with 3% HCl/MeOH ($300 \mu\text{l}$) at 110°C for 1 h. After the solvent was removed by nitrogen stream, to the residue was added CHCl_3 ($100 \mu\text{l}$) and the CHCl_3 solution was extracted with H_2O ($100 \mu\text{l}\times 3$). The aqueous fraction evaporated *in vacuo* was treated with pyridine ($100 \mu\text{l}$), triethylamine ($15 \mu\text{l}$), and benzoyl chloride ($15 \mu\text{l}$) at room temperature for 21 h. After addition of MeOH ($100 \mu\text{l}$), the reaction mixture was extracted with hexane ($100 \mu\text{l}\times 3$). The hexane-soluble fraction was evaporated *in vacuo* to afford the 1-*O*-methyl-2,3,4,6-tetra-*O*-benzoyl derivative of each sugar unit. Authentic D- and L-glucose were treated with benzoyl chloride as described

above to give the 1-*O*-methyl-2,3,4,6-tetra-*O*-benzoyl derivatives of D- and L-glucose, respectively. The 1-*O*-methyl-2,3,4,6-tetra-*O*-benzoyl derivatives were subjected to chiral HPLC analyses using Chiralpak OP(+) (Daicel Chemical Industry, Ltd., $4.6\times 250 \text{ mm}$; MeOH; flow rate, 0.5 ml/min ; UV detection at 254 nm). The retention time of the 1-*O*-methyl-2,3,4,6-tetra-*O*-benzoyl derivative of the methanolysis product of **1** or **2** was found to be both 23.8 min, while the retention times of the 1-*O*-methyl-2,3,4,6-tetra-*O*-benzoyl derivatives of authentic D- and L-glucose were found to be 23.8 and 25.8 min, respectively.

Acknowledgments We thank Mr. K. Watanabe, GC-MS & NMR Laboratory, Faculty of Agriculture, Hokkaido University, for measurements of ESI-MS of colopsinol E. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan.

References and Notes

- 1) Tsuda M., Endo T., Kobayashi J., *Tetrahedron*, **55**, 14565—14570 (1999).
- 2) Tsuda M., Endo T., Kobayashi J., *J. Org. Chem.*, **65**, 1349—1352 (2000).
- 3) Kubota T., Tsuda M., Kobayashi J., *Tetrahedron Lett.*, **41**, 713—716 (2000).
- 4) Kobayashi J., Kubota T., Takahashi M., Ishibashi M., Tsuda M., Naoki H., *J. Org. Chem.*, **64**, 1478—1482 (1999).
- 5) Kubota T., Tsuda M., Takahashi M., Ishibashi M., Naoki N., Kobayashi J., *J. Chem. Soc., Perkin Trans. 1*, **1999**, 3483—3488.
- 6) Kobayashi J., Ishibashi M., Nakamura H., Ohizumi Y., Hirata Y., *J. Chem. Soc., Perkin Trans. 1*, **1989**, 101—103.
- 7) The sulfate-bearing position (C-34; δ_{C} 82.1) of theonezolid A: Kobayashi J., Kondo K., Ishibashi M., Wälchli M. R., Nakamura T., *J. Am. Chem. Soc.*, **115**, 6661—6665 (1993).
- 8) Kobayashi J., Ishibashi M., Nakamura H., Ohizumi Y., Yamasu T., Sasaki T., Hirata Y., *Tetrahedron Lett.*, **27**, 5755—5758 (1986).
- 9) These chemical shifts were closer to that of the methine-bearing allylic methylene carbon (C-24; δ_{C} 41.9) on the *E*-double bond of (22*E*)-cholesta-5,22-diene-3 β ,7 β ,19-triol [Aiello A., Fattorusso E., Mienna M., *J. Nat. Prod.*, **55**, 321—325 (1992)] than that (C-13; δ_{C} 32.8) on the *Z*-double bond of patellazole B (Corley D. G., Moore R. E., Paul V. J., *J. Am. Chem. Soc.*, **110**, 7920—7922 (1988)).
- 10) The chemical shift corresponded to that of the allylic methine carbon (C-19; δ_{C} 36.2) on the *E*-double bond of theonezolid A⁷⁾ rather than that (C-10; δ_{C} 48.5) on the *Z*-double bond of patellazole B.⁹⁾
- 11) Kobayashi J., Doi Y., Ishibashi M., *J. Org. Chem.*, **59**, 255—257 (1994).
- 12) The axially-oriented hydroxy-bearing positions (C-27; δ_{C} 69.7, C-40; 69.5) on two tetrahydropyran rings of luteophanol A [Doi Y., Ishibashi M., Nakamichi H., Kosaka T., Ishikawa T., Kobayashi J., *J. Org. Chem.*, **62**, 3820—3823 (1997)].
- 13) Geminal proton signals of C-21 (δ_{H} 2.10, 1.50) and C-22 (δ_{H} 1.95, 1.65) on the tetrahydrofuran ring in amphidinolide C; Kobayashi J., Ishibashi M., Wälchli M. R., Nakamura H., Hirata Y., Sasaki T., Ohizumi Y., *J. Am. Chem. Soc.*, **110**, 490—494 (1988).