New Cembranes from the Soft Coral *Sarcophyton* Species

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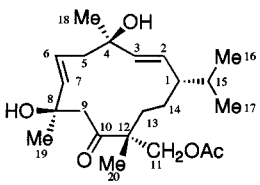
Received January 6, 1999

Three new cembranes, including one with a 13-membered carbocyclic ring, have been isolated from the soft coral *Sarcophyton* sp.

Soft corals belonging to genus *Sarcophyton* have provided many common 14-membered carbocyclic cembrane diterpenes exhibiting ichthyotoxic activity.[1] It has been suggested that the diterpenes were used as chemical defense compounds to avoid predators.[2] Five new rare 13-membered carbocyclic and eight new 14-membered carbocyclic cembranes, some of which showed ichthyotoxicity, have been isolated from *Sarcophyton* sp., collected in the area of Bonotsu, Kagoshima Prefecture.[3,4] We have examined the soft coral *Sarcophyton glaucum* (Quoy & Gaimard, 1883)(Alcyoniidae), collected at Cape Sata, Kagoshima Prefecture. The concentrated methanol extract of the animals was partitioned between dichloromethane and water. Bioassay-guided fractionation of the dichloromethane extract, which showed ichthyotoxicity to killifish, *Oryzia latipes*,[4] using a combination of silica and reversed-phase HPLC, yielded three new cembranes, sartol acetate B (**1**), having a 13-membered carbocyclic ring; epoxysartone B (**2**); and sartone E (**3**), and three known cembranes, sartones A (**4**) and B (**5**)[4] and sarcotol acetate (**6**).[3] In this paper, we describe their isolation and structure elucidation of **1**−**3**.



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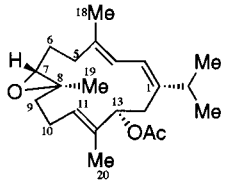

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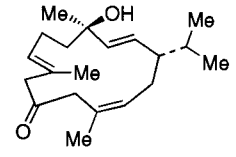

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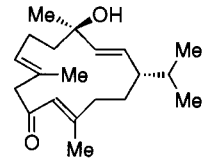

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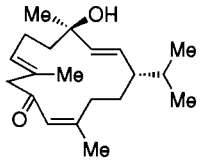

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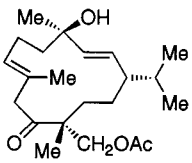

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Sartol acetate B (**1**) showed IR absorption bands of hydroxyl groups (3491 and 3270 cm^{-1}), an acyl group (1738 and 1244 cm^{-1}), and a chelated or an α,β-unsaturated carbonyl group (1680 cm^{-1}). The molecular formula $C_{22}H_{36}O_5$, determined by the HREIMS and ^{13}C NMR (Table 1), indicated five degrees of unsaturation. Resonances due to two olefinic carbons (δ 123.8, d; δ 131.8, d; δ 137.9, d; δ 140.1, d), an acetoxyl carbon (δ 20.7, q; δ 170.8, s), and a carbonyl carbon (δ 213.2, s) in the ^{13}C NMR spectrum accounted for four double-bond equivalents, indicating that **1** was a monocyclic compound. The ^1H NMR spectrum (Table 1) indicated resonances corresponding to six methyl groups: three tertiary methyl groups, two of which were attached to carbons bearing a hydroxyl group (δ 1.11, 1.32, and 1.36, 3H each, s), isopropyl methyls (δ 0.82 and 0.85, 3H each, d, J = 7.2 Hz, δ ca. 1.50, 1H, overlapped), and an oxymethylene group (δ 4.05, 4.20, 1H each, J = 11.4 Hz). This suggested that **1** was a rearranged cembrane.[3] Signal patterns corresponding to H-1 to H-3, H-15 to H-17, and methyl protons (δ 1.32, 3H, s, H-18) on a carbon bearing a hydroxyl group were observed, the assignment of which was established by comparing the resonances with those of the cembranes isolated from *Sarcophyton* sp. from the area of Bonotsu and by the aid of $^1H-^1H$ COSY spectrum of **1** (Figure 1). H-2 (δ 5.15, 1H, dd, J = 9.3 and 15.8 Hz) was coupled to H-3 (δ 5.35, 1H, d, J = 15.8 Hz) and H-1(δ ca. 1.65, 1H, H-1), the latter of which was coupled to H-15 (δ 1.50, 1H, overlapped). H-15 was also coupled to H-16 and H-17 (δ 0.82 and 0.85, 3H each, d, J = 7.2 Hz). The presence of three isolated spin systems was observed. The olefinic proton at δ 5.64 (1H, d, J = 15.9 Hz, H-7) was coupled to an olefinic proton (δ 5.45, 1H, ddd, J = 6.6, 8.3, and 15.9 Hz, H-6), which was coupled to neighboring methylene protons (δ 2.24, 1H, br dd, J = 8.3 and 13.4 Hz, δ 2.32, 1H, ddd, J = 1.3, 6.6, and 13.4 Hz, H-5). Two AB systems appeared at δ 2.62 (2H, d, J = 7.1 Hz, H-9) and 4.13 (2H d, J = 11.4 Hz, H-11). There remained resonances due to two overlapped methylene protons to be assigned. On the basis of the results, compound **1** possessed a 13-membered carbocyclic ring, as in the case of sarcotol acetate (**6**). The geometries of the double bonds at C-3 and C-6 were assigned to be trans from the large coupling constants between H-2 and H-3 (J = 15.8 Hz) and between H-6 and H-7 (J = 15.9 Hz). The gross skeleton was completed by the HMQC and HMBC spectra (Figure 1). The connectivity from C-3 to C-5 resulted from cross-peaks from H-18 to C-3 (δ 137.9, d), C-4 (δ 73.4, s), and C-5 (δ 45.7, t). The linkage from C-7 to C-13 was inferred from H-19 (δ 1.36) to C-7 (δ 140.1, d), C-8 (δ 72.0, s), and C-9 (δ 48.8, t); from H-9 (δ

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10.1021/np9900066 CCC: $18.00
Published on Web 06/19/1999

Table 1. NMR Spectral Data of Compounds **1**−**3**[a]

no.	**1**		**2**		**3**	
1	1.65 (m)	49.4		143.6	ca 1.78 (overlapped)	50.4
2	5.15 (dd, 9.3, 15.8)	131.8	6.15 (d, 11.2)	120.4	5.40 (dd, 7.7, 15.8)	129.9
3	5.35 (d, 15.8)	137.9	5.87 (br d, 11.3)	121.1	5.49 (d, 15.8)	136.2
4		73.4		135.2[b]		73.2
5	2.24 (br dd, 8.3, 13.4)	45.7	2.20 (m)	37.0	ca. 176 (overlapped)	42.6
	2.32 (ddd, 1.3, 6.6, 13.4)		2.26 (overlapped)		ca. 1.84 (overlapped)	
6	5.45 (ddd, 6.6, 8.3, 15.9)	123.8	1.73 (m)	22.1	2.02 (m)	23.5
					2.32 (m)	
7	5.64 (d, 15.9)	140.1	2.75−2.81 (overlapped)	60.9	5.28 (br t, 6.4)	131.9[b]
8		72.0		59.8		128.3
9	2.48 (d, 7.1)	48.8	ca 1.63 (overlapped)	35.9[c]	2.96 (br d, 12.3)	53.6
	2.84 (d, 7.1)				3.01 (br d, 12.3)	
10		213.2	ca 1.99 (overlapped)	25.3		207.2
11	4.05 (d, 11.4)	68.2	5.39 (br t, 6.3)	128.9	3.12 (d, 16.7)	44.8
	4.20 (d, 11.4)				3.25 (d, 16.7)	
12		51.1		134.7[b]		130.5[b]
13	ca 1.50 (overlapped)	31.4	5.05 (dd, 4.0, 7.3)	79.5	5.53 (br t, 7.7)	126.8
14	ca 1.28 (m)	19.7	2.31 (dd, 4.0, 13.7)	35.1[d]	ca 1.84 (overlapped)	31.0
			2.75−2.81 (overlapped)			
15	ca 1.50 (overlapped)	30.7	2.40 (hept, 6.8)	35.1	ca 1.70 (overlapped)	
16, 17	0.82 (d, 7.2)	20.4, 20.9	1.05 (d, 6.8)	21.7, 22.6	0.84 (d, 6.8)	18.3, 21.4
	0.85 (d, 7.2)		1.09 (d, 6.8)		0.87 (d, 6.8)	
18	1.32 (s)	26.4	1.79 (br s)	14.9[d]	1.32 (s)	27.4
19	1.36 (s)	28.4	1.29 (s)	17.1[d]	1.64 (br s)	16.7
20	1.11 (s)	17.8	1.60 (br s)	17.8[d]	1.77 (br s)	24.7
AcO	2.04 (s)	20.7, 170.8	2.04 (s)	21.4, 170.0		

[a] Chemical shift values are in ppm from TMS, and *J* values (in Hz) are presented in parentheses. [b−d] These values may be interchangeable in any vertical column.

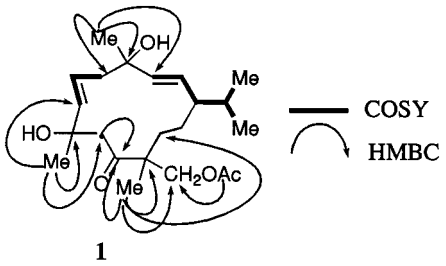

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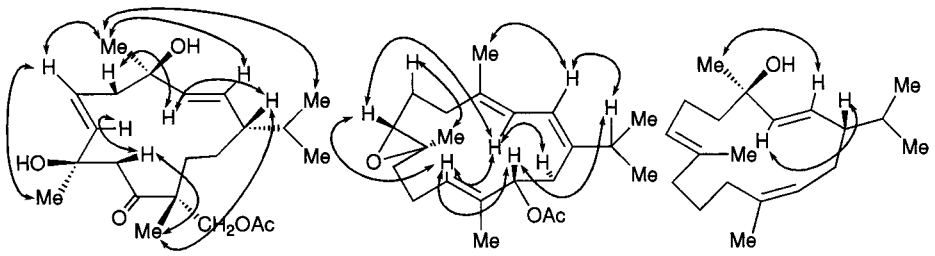
Figure 1. ^{1}H−^{1}H COSY and HMBC correlations of **1**.

2.48 and 2.84, 2H) to C-10 (*δ* 213.2, s); and from H-20 (*δ* 1.11) to C-10, C-11 (*δ* 68.2, s), C-12 (*δ* 51.1, t), and C-13 (*δ* 31.4, t). The acetoxyl group was concluded to be attached to the methylene group at C-12 (*δ* 4.05 and 4.20, 1H each) on the basis of the low chemical shifts and a cross-peak between H-11 and the ester carbonyl (*δ* 170.8, s). The relative stereochemisty of all chiral centers was deduced from observed NOE cross-peaks (Figure 2). NOEs from H-3 to H-1, H-5 (*δ* 2.24), and H-7 and from H-20 (*δ* 1.11) to H-1 and H-9 (*δ* 2.48) showed that H-1 and H-20 occur on the same face of the ring system (*β*); H-18 and H-19 were on the face (α) opposite H-1, because NOEs from H-18 to H-2 and H-6, and H-17 and from H-6 and H-19 were observed.

The IR spectrum of epoxysartone B (**2**), $C_{22}H_{34}O_3$, indicated presence of an ester (1738 cm^{-1}) and an olefinic group (1659 cm^{-1}). The UV spectrum showed absorption at λ_{max} 250 nm (log ϵ 4.33) indicative of conjugation. In the ^{13}C NMR spectra, resonances due to five methyl carbons (*δ* 14.1, 17.1, 17.8, 21.7, 22.6, each s), five methylene carbons (*δ* 22.1, 25.3, 35.1, 35.9, 37.0), two methine carbons (*δ* 35.1, 79.5), epoxide carbons (*δ* 59.8, d; *δ* 60.9, s), acetoxyl carbons (*δ* 21.4, s; *δ* 170.0), and six olefinic carbons (*δ* 120.4, d; *δ* 121.1, d; *δ* 128.9, d; *δ* 134.7, s; *δ* 135.2, s; *δ* 143.6, s) suggested that **2** was probably a bicyclic common cembrane. The downfield chemical shift of H-15 (*δ* 2.40, 1H, hept, *J* = 6.8 Hz) suggested the presence of a double bond at C-1. The presence of four isolated spin systems were elucidated by the ^{1}H−^{1}H COSY spectrum (Figure 1). H-2 (*δ* 6.15, 1H, d, *J* = 11.3 Hz) was coupled to H-3 (*δ* 5.87, 1H, br d, *J* = 11.3 Hz), which in turn was weakly coupled to H-18 (3H, br s). Their positions were assigned by analysis of NOE experiments (Figure 1), as NOEs from H-2 to H-15 and H-18 were observed. The epoxide proton [*δ* 2.80−2.95, 1H (overlapped) H-7] was coupled to methylene protons at *δ* 1.73 (2H, m, H-6), which, in turn, was coupled to methylene protons at *δ* 2.20 (1H, m, H-5) and 2.26 (1H, overlapped, H-5). The downfield chemical shifts of the latter protons suggested that the methylene group was attached to a double bond. An olefinic proton at *δ* 5.39 (1H, br t, *J* = 6.3 Hz, H-11) was coupled allylically to methyl protons (*δ* 1.60, 3H, br s, H-20) and to methylene protons at *δ* ca. 1.99 (2H, overlapped, H-10), the latter of which was furthermore coupled to methylene protons at *δ* ca. 1.63 (2H, overlapped, H-9). An olefinic proton at *δ* 5.05 (1H, dd, *J* = 4.0 and 7.3 Hz, H-13) on a carbon bearing an acetoxyl group (*δ* 2.04, 3H, s) was coupled to methylene protons [*δ* 2.31, 1H, *J* = 4.0 and 13.7 Hz, *δ* 2.75−2.80, 1H (overlapped) H-14] linked to an olefinic bond. At this stage, the epoxide and the olefinic bond were concluded to be located between C-7 and C-8 and between C-11 and C-12, respectively, inasmuch as the higher field chemical shift of the methylene protons (*δ* ca. 1.63) indicated that the position was adjacent to the epoxide, but not to the olefinic bond. Therefore, the location of the acetoxyl group could be placed at C-13. The *E*-configurations of the olefinic bonds at C-3 and C-11 were confirmed by the chemical shifts of C-18 and C-20 (δ_C 14.9, 17.1, or 17.8). On the basis of these results, the gross structure of **2** was determined as depicted in Figure 1. The relative stereochemistry of all chiral centers was elucidated from the NOE experiments on **2** (Figure 2). Irradiation of H-3 resulted in enhancement of H-6, H-7, H-11, H-13, and H-14, while irradiation of H-11 induced enhancement of H-3, H-7, and H-13. A NOE between H-7 and H-19 was not observed. This suggested that H-7 and H-13 occurred



1 **2** **3**

Figure 2. NOE correlations of **1**−**3**.

on the same face (β) of the ring and the methyl group at C-8 on the opposite face (α) of the ring.

The IR data of sartone E (**3**) was compatible with the presence of a hydroxyl (3412 cm^{-1}), a carbonyl (1711 cm^{-1}), and an olefinic (1651 cm^{-1}) function. The presence of five methyl carbons (δ 16.7, 18.3, 21.4, 24.7, 27.4), five methylene carbons (δ 23.5, 31.0, 42.6, 44.8, 53.6), two methine carbons (δ 30.1, 50.4), a tertiary carbon bearing a hydroxyl group (δ 73.2), six olefinic carbons [δ 126.8, d; 128.3, s; 129.9, d; 130.5, s; 131.9, d; 136.2, d], and a carbonyl carbon (δ 207.2) in the ^{13}C NMR spectrum (Table 1) and the molecular formula $C_{20}H_{32}O_2$ suggested that **3** was monocyclic. The partial structures were assigned by analysis of $^1H-^1H$ COSY spectrum (Figure 1). The first olefinic proton (δ 5.49, 1H, d, J = 15.8 Hz, H-3) of four was coupled to the second olefinic proton at δ 5.40 (1H, dd, J = 7.7 and 15.8 Hz, H-2), which, in turn, was coupled to a proton at δ ca. 1.78 (1H, overlapped, H-1). This last proton was furthermore coupled to an isopropyl methine (δ ca. 1.76, 1H, overlapped, H-15, δ 0.84, 0.87, 3H each, d, J = 6.8 Hz, H-16 and H-17, isopropyl Me). The protons seemed to be corresponding to H-1 to H-3 and to H-15 to H-18 by comparing the coupling patterns with those of cembranes isolated in the area of Bonotsu.[3] The third olefinic proton at δ 5.28 (1H, br t, J = 6.4 Hz, H-7) was coupled weakly to olefinic methyl protons (δ 1.64, 3H, br s, H-19) and to methylene protons (δ 2.02, 1H, m, δ 2.32, 1H, m, H-6), which were also coupled to other methylene protons [δ ca. 1.76, 1H, overlapped, δ ca. 1.84, 1H (overlapped) H-5]. Two AB systems appeared at δ 2.96 and 3.01 (1H each, br d, J = 12.3 Hz, H-9) and δ 3.12 and 3.25 (1H each, d, J = 16.7 Hz), the chemical shifts of which suggested that the two isolated methylenes were located between the olefinic bond and the carbonyl function. The fourth olefinic proton (δ 5.53, 1H, br t, J = 7.7 Hz, H-13) was coupled weakly to methyl protons (δ 1.77, 3H, br s, H-20) and to methylene protons (δ ca. 1.84, 2H, overlapped, H-14). The gross structure, except for the geometry of the olefinic bonds at C-7 and C-12, was determined on the basis of the above results. The *Z* configuration of the olefinic bond at C-12 was confirmed by the observation of an NOE between H-13 and H-20 in the NOE experiment. Therefore, sartone E (**3**) was deduced to be isomeric with sartones A (**4**) and B (**5**). Comparison of the ^{13}C NMR data of **3** with those of sartones A and B was used to make the assignment of all peaks in the ^{13}C NMR spectrum of **3**. The chemical shift of C-19 (δ 16.7) was thus consistent with *E*-configuration of the olefinic bond at C-7. The relative stereochemistry of H-1 and H-18 was elucidated by the NOE correlations (Figure 2). NOEs from H-3 to H-1 and from H-2 to H-18 suggested that the configurations of H-1 and H-18 were β and α, respectively. Therefore, the structure of sartone E was assigned as **3**.

Compounds **1** and **3** did not show ichthyotoxicity against killifish, *Oryzia latipe*s, at a concentration of 20 μg/mL. The ichthyotoxicity test was not performed for **2**, because it had decomposed.

Experimental Section

General Experimental Procedures. UV and IR spectra were recorded on a UV-210 and a JASCO FT/IE 5300 spectrometers. NMR spectra were recorded with a JEOL JNM-GX 400 or a VARIAN UNITY-500 NMR spectrometer using TMS as internal standard and $CDCl_3$ as a solvent. MS were obtained with JEOL JMS DX-300 instrument.

Animal Material. The soft coral *Sarcophyton* sp., identified by Mr. K. Takemura (Sankei Kagaku, Co., Ltd.), was collected by using scuba at −15 m at Cape Sata, Kagoshima Prefecture. The reference sample (collection no. 204) was deposited at Faculty of Science, Kagoshima University.

Extraction and Isolation. The fresh organism (dry wt 273 g) was chopped into small pieces and extracted with MeOH (30 L). The MeOH extract was suspended in H_2O and extracted with CH_2Cl_2. A portion (5.0 g) of the CH_2Cl_2 extract (6.9 g) was absorbed on Si gel and subjected to chromatography on Si gel (40 g) packed in hexane, and fractions (100 mL) were collected as follows: 1−2 (CH_2Cl_2−hexane, 1:1), 3−4 (CH_2Cl_2−hexane, 4:1), 5−6 (CH_2Cl_2), 7−8 (MeOH−CH_2Cl_2, 1:49), 9−11 (MeOH−CH_2Cl_2, 1:19), 12−13 (MeOH−CH_2Cl_2, 1:9), 14−15 (MeOH−CH_2Cl_2, 1:4), 16−17 (MeOH−CH_2Cl_2, 1:1), and 18−20 (MeOH). Fractions 1−5, which showed ichthyotoxicity against killifish, *O. latipes*, were chromatographed on Si gel using MeOH and CH_2Cl_2, increasing the proportion of MeOH to elute the fractions from the column. The fractions eluted with MeOH−CH_2Cl_2 (1:99) were applied to HPLC (ODS) with MeOH−H_2O (3:2), giving epoxysartone B (**2**) (3.7 mg), sartone B (**5**) (5.4 mg), sartone A (**4**) (15.2 mg), sartone E (**3**) (1.6 mg), and sarcotol acetate (**6**) (10.4 mg). Sartol acetate B (**1**) (3.3 mg) was isolated from fractions 8−11, which exhibited strong ichthyotoxicity, by Si gel chromatography with MeOH−CH_2Cl_2, (1:49) and then HPLC with MeOH−H_2O (7:3).

Sartol acetate B (1): oil; $[\alpha]_D$ +11.1° (*c* 0.19, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 nm (3.40); IR (film) ν_{max} 3491, 3270, 1738, 1680, 1244 cm^{-1}; ^1H NMR (500 MHz) and ^{13}C NMR (150 MHz) (see Table 1); LREIMS *m*/*z* 362 $[M]^+$, 347 $[M - 15]^+$, 302 $[M - AcOH]^+$; HREIMS *m*/*z* 362.24778 $[(M - H_2O)^+$, calcd for $C_{22}H_{34}O_4$, 362.24757].

Epoxysartone B (2): oil; $[\alpha]_D$ +50.7° (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ϵ) 250 nm (4.33); IR (film) ν_{max} 1738, 1659, 1238 cm^{-1}; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) (see Table 1); HREIMS *m*/*z* 346.2500 (M^+, calcd for $C_{22}H_{34}O_3$, 346.2505).

Sartone E (3): oil; $[\alpha]_D$ +6.2° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 nm (3.58); IR (film) ν_{max} 3412, 1711, 1651 cm^{-1}; ^1H NMR (500 MHz) and ^{13}C NMR (150 MHz) (Table 1); HREIMS *m*/*z* 286.2258 $[(M - H_2O)^+$, calcd for $C_{20}H_{30}O$, 286.2295)].

Acknowledgment. The authors are grateful to Mr. Kaoru Takemura (Sankei Kagaku Co., Ltd.) for identifying the coral.

References and Notes

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(4) Iwagawa, T.; Nakamura, S.; Okamura, H.; Nakatani, M. *Bull. Chem. Soc. Jpn*. **1996**, *69*, 3543−3549.
(5) The ^{13}C NMR spectral data of **4** and **5**; **4**: C-1 (*δ* 46.3, d), C-2 (*δ* 130.0, d), C-3 (*δ* 137.4, d), C-4 (*δ* 72.4, s), C-5 (*δ* 42.4, t), C-6 (*δ* 22.3, t), C-7 (*δ* 133.4, d), C-8 (*δ* 128.8, s), C-9 (*δ* 57.1, t), C-10 (*δ* 199.9, s), C-11 (*δ* 122.1, d), C-12 (*δ* 157.8, s), C-13 (*δ* 38.6, t), C-14 (*δ* 27.6, t), C-15 (*δ* 32.8, d), C-16, C-17 (*δ* 19.1, q, *δ* 20.6, q), C-18 (*δ* 28.4, q), C-19 (*δ* 16.5, q), C-20 (*δ* 17.7, q); **5**: C-1 (*δ* 47.6, d), C-2 (*δ* 129.7, d), C-3 (*δ* 137.8, d), C-4 (*δ* 72.8, s), C-5 (*δ* 42.2, t), C-6 (*δ* 22.9, t), C-7 (*δ* 128.9, d), C-8 (*δ* 129.7, s), C-9 (*δ* 52.1, t), C-10 (*δ* 200.9, s), C-11 (*δ* 128.3, d), C-12 (*δ* 155.4, s), C-13 (*δ* 31.1, t), C-14 (*δ* 29.0, t), C-15 (*δ* 33.1, d), C-16, C-17 (*δ* 18.9, q, *δ* 20.3, q), C-18 (*δ* 28.1, q), C-19 (*δ* 18.0, q), C-20 (*δ* 23.9, q).

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