Notes

Chapecoderins A−C, New Labdane-Derived Diterpenoids from *Echinodorus macrophyllus*

Jun'ichi Kobayashi,*,† Mitsuhiro Sekiguchi,† Hideyuki Shigemori,† and Ayumi Ohsaki*,‡

Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan, and Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Tokyo 101-0062, Japan

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A new *seco*-labdane-type diterpenoid, chapecoderin A (**1**), and two new rearranged labdane-type diterpenoids, chapecoderins B (**2**) and C (**3**), were isolated from the leaves of the Brazilian medicinal plant *Echinodorus macrophyllus* ("Chapéu-de-couro"), and their structures and relative stereochemistry were elucidated by spectroscopic data. Chapecoderins A−C (**1**−**3**) possess in common an α,β-unsaturated γ-lactone ring in the side chain.

Brazilian medicinal plants have proven to be a rich source of compounds that might be useful for the development of new pharmaceutical agents.[1] In our search for bioactive compounds from Brazilian medicinal plants, a new *seco*-labdane-type diterpenoid, chapecoderin A (**1**), and two new rearranged labdane-type diterpenoids, chapecoderins B (**2**) and C (**3**), with an α,β-unsaturated γ-lactone ring in the side chain, were isolated from the leaves of *Echinodorus macrophyllus* (Kunth) Micheli (Alismataceae). This plant is known in Brazil as "Chapéu-de-couro" and is used to treat difficulties in urination, hepatitis, and rheumatism. In this paper we describe the isolation and structure elucidation of compounds **1**−**3**.

The leaves of the Brazilian medicinal plant *E. macrophyllus* were extracted with MeOH, and the extracts were partitioned between EtOAc and H_2O. The EtOAc-soluble portions were subjected to passage over a Si gel column ($CHCl_3$−MeOH, 98:2) and then over a reversed-phase column (MeOH−H_2O, 80:20), to afford chapecoderins A (**1**), B (**2**), and C (**3**).

The molecular formula, $C_{20}H_{30}O_4$, of chapecoderin A (**1**) was established by HREIMS [*m*/*z* 334.2130 (M^+), Δ −1.4 mmu]. The IR spectrum suggested the presence of unsaturated lactone carbonyl (1752 and 1655 cm^{-1}) and ketone carbonyl (1701 cm^{-1}) groups. The gross structure of **1** was deduced from detailed analysis of the ^1H and ^{13}C NMR data (Table 1) aided with 2D NMR experiments ($^1H-^1H$ COSY, HMQC, and HMBC). The ^{13}C NMR data indicated that the molecule possessed two ketone carbonyls, one unsaturated ester carbonyl, one trisubstituted olefin, two sp^3 quaternary carbons, eight methylenes (one of them bearing an oxygen atom), one methine, and four methyl groups. Because four out of six unsaturations were thus accounted for, it was concluded that **1** contains two rings. The $^1H-^1H$ COSY spectrum revealed connectivities of C-1 to C-3, C-5 to C-7, C-11 to C-12, and C-14 to C-15. HMBC correlations (Figure 1) of H_3-18 and H_3-19 to C-3, C-4, and C-5 and of H_3-20 to C-1, C-5, and C-10 revealed the presence of a cyclohexane ring, which possessed Me-18 and Me-19 attached to C-4 and Me-20 at C-10. HMBC correlations of H_3-17 and H_2-7 to C-8 (δ_C 208.0) indicated the connectivity of Me-17 to C-7 through a ketone carbonyl (C-8). Cross-peaks of H_3-20 and H-11a to C-9 (δ_C 215.0) in the HMBC spectrum implied

Table 1. ^1H NMR Data of Chapecoderins A−C (**1**−**3**)

position	**1**[a] (δ, ppm)	**2**[b] (δ, ppm)	**3**[b] (δ, ppm)
1a	1.56 (m)	2.08 (dd, 5.0, 12.7)	1.70 (td, 3.5, 14.0)
b	1.36 (m)	1.40 (m)	1.24 (td, 3.5, 14.0)
2a	1.70 (m)	1.59 (m)	1.59 (m)
b	1.60 (m)		1.48 (m)
3a	1.48 (m)	1.40 (m)	1.37 (m)
b	1.42 (m)	1.17 (m)	1.06 (m)
5	1.72 (m)	2.32 (dd, 8.1, 12.9)	1.34 (dd, 6.2, 12.0)
6a	1.32 (m)	1.55 (m)	2.11 (dd, 12.0, 14.0)
b	1.25 (m)	1.45 (m)	2.09 (dd, 6.2, 14.0)
7	2.42 (dd, 8.2, 8.2)[c]	2.60 (dd, 5.0, 12.2)	
11a	2.86 (td, 7.2, 18.0)	1.82 (m)	2.94 (dt, 5.1, 16.7)
b	2.74 (td, 7.2, 18.0)	1.80 (m)	2.36 (dt, 4.5, 16.7)
12a	2.56 (t, 7.2)[c]	2.52 (td, 5.0, 7.0)	2.69 (m)
b		2.34 (m)	2.50 (m)
14	7.16 (s)	5.94 (s)	6.36 (s)
15	4.75 (s)[c]	3.80 (s)[c]	3.85 (s)[c]
17	2.02 (s)[d]	1.84 (s)[d]	1.94 (s)[d]
18	0.91 (s)[d]	0.88 (s)[d]	0.92 (s)[d]
19	0.92 (s)[d]	0.87 (s)[d]	0.88 (s)[d]
20	1.21 (s)[d]	0.76 (s)[d]	0.78 (s)[d]

[a] In $CDCl_3$. [b] In C_6D_6. [c] 2H. [d] 3H.

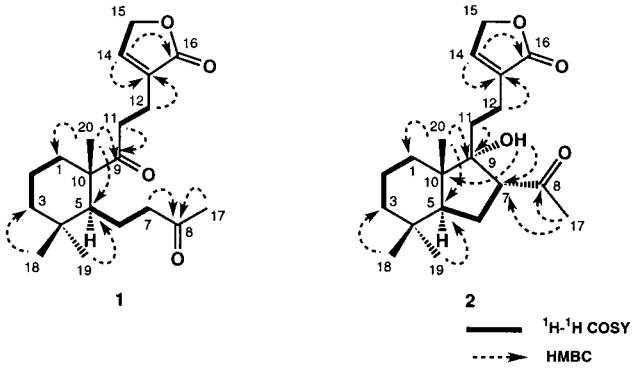

Figure 1. Selected 2D NMR correlations of chapecoderins A (**1**) and B (**2**).

* To whom correspondence should be addressed. Tel.: +81-11-706-4985. Fax: +81-11-706-4989. E-mail: jkobay@pharm.hokudai.ac.jp (J. Kobayashi). Tel.: +81-3-5280-8153. Fax: +81-3-5280-8005. E-mail: a-ohsaki@i-mde.tmd.ac.jp (A. Ohsaki).
† Hokkaido University.
‡ Tokyo Medical and Dental University.

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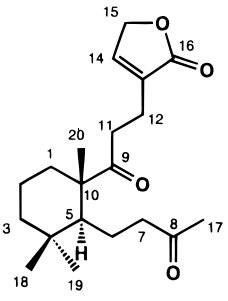

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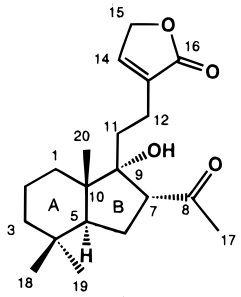

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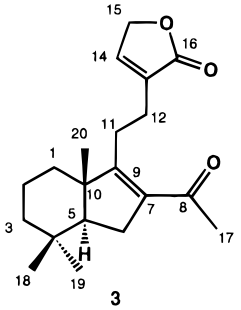

3

the presence of a ketone carbonyl (C-9) between C-10 and C-11. HMBC correlations of H_2-15 to C-13, H-14 to C-13 and C-16, and H_2-12 to C-13 and the unsaturations from the elemental formula revealed the presence of a γ-lactone ring (C-13 through C-16 and O-15) connected to C-12. Thus, the gross structure of chapecoderin A was elucidated as **1**. NOESY correlations of H_3-20 to H-2a and H_3-18, and H-5 to H-1b, H-3b, and H_3-19 indicated a β-orientation of Me-20, an α-orientation of H-5, and a trans relationship between Me-20 and H-5 (Figure 2). A chair conformation of the cyclohexane ring was elucidated from NOESY correlations of H-3b to H-1b and H-5 and of H_3-18 to H2a. Thus, the relative stereochemistry of chapecoderin A was assigned as **1**.

Chapecoderin B (**2**) showed the molecular ion peak at *m/z* 334 (M^+) in the EIMS. HREIMS analysis revealed the molecular formula to be $C_{20}H_{30}O_4$ [*m/z* 334.2119 (M^+), Δ −2.5 mmu]. IR absorptions implied that **2** possessed hydroxyl (3426 cm^{-1}), unsaturated lactone (1745 and 1647 cm^{-1}), and ketone (1730 cm^{-1}) groups. The ^1H NMR spectrum showed a hydroxyl proton (δ_H 5.52 s) exchangeable with D_2O. Analysis of the ^1H and ^{13}C NMR data and the HMQC spectrum provided one ketone carbonyl, one ester carbonyl, one trisubstituted olefin, one oxygenated quaternary carbon, two sp^3 quaternary carbons, two methines, seven methylenes (one of them bearing an oxygen atom), and four methyl groups. Detailed analysis of ^1H−^1H COSY spectrum implied connectivities of C-1 to C-3, C-5 to C-7, C-11 to C-12, and C-14 to C-15. In the HMBC spectrum H_3-18 and H_3-19 showed long-range ^1H−^{13}C correlations with C-3, C-4, and C-5, and H_3-20 showed cross-peaks with C-1, C-5, and C-10, indicating the presence of a cyclohexane ring with Me-18 and Me-19 affixed to C-4 and Me-20 at C-10 (Figure 1). HMBC correlations of H_3-20 to C-9 (δ_C 84.7); OH-9 to C-7, C-9, and C-10; and H_3-17 to C-7 and C-8 (δ_C 215.0) revealed that the presence of a cyclopentane ring with a hydroxyl group at C-9 and an acetyl group at C-7. HMBC correlations of H_2-15 to C-13, H-14 to C-13 and C-16, H_2-12 to C-13, and H_2-11 to C-9 revealed the presence of a γ-lactone ring (C-13 through C-16 and O-15) connected to C-12 and the connection between C-9 and C-11. Thus, the gross structure of chapecoderin B was elucidated as **2**. NOESY correlations of H_3-20 to H-2a and H_3-18, and H-5 to H-1b and H-3b indicated a chair conformation of ring A, a β-orientation of Me-20 and α-orientation of H-5, and a trans relationship between rings A and B, while the correlations of H-7 to H_3-20, OH-9 to H-5, and H-11a to H_3-20 indicated a β-orientation of H-7 and an α-orientation of OH-9 (Figure 2). Thus, the relative stereochemistry of chapecoderin B was elucidated as **2**.

The molecular formula, $C_{20}H_{28}O_3$, of chapecoderin C (**3**) was established by HREIMS [*m/z* 316.2053 (M^+), Δ +1.5 mmu], indicating that **3** was a dehydrated form of **2**. Comparison of the ^1H and ^{13}C NMR spectra of **3** with those of **2** revealed that a carbinol (δ_H 5.52, OH-9; δ_C 84.7, C-9) and a methine (δ_H 2.60, H-7; δ_C 53.7, C-7) in **2** were

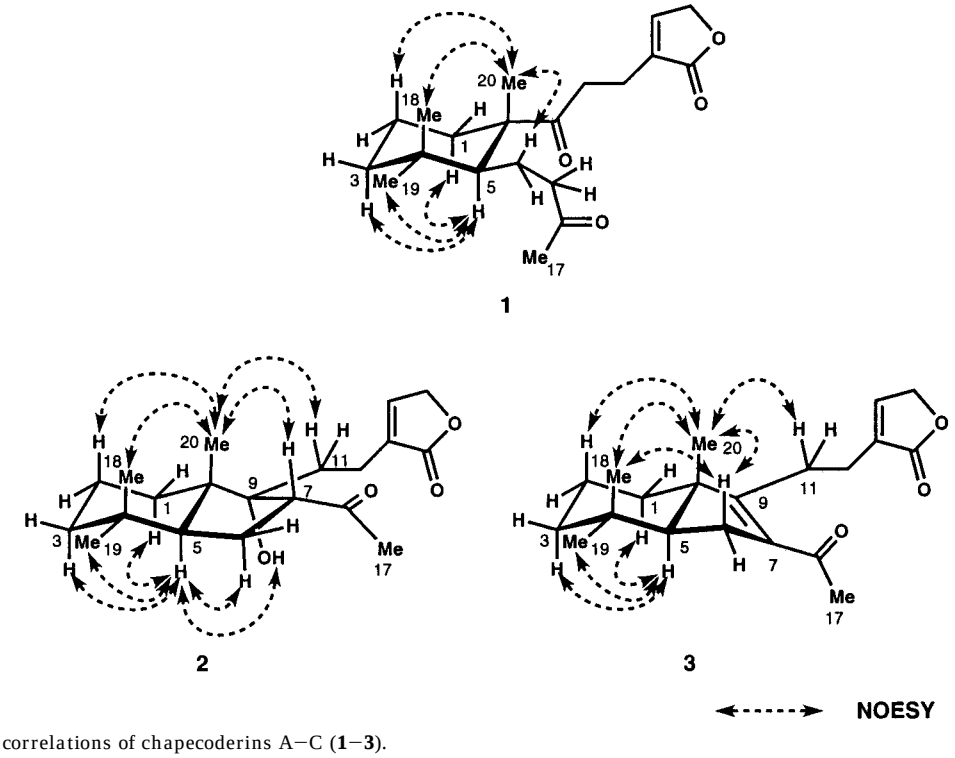

Figure 2. NOESY correlations of chapecoderins A−C (**1**−**3**).

Table 2. ^{13}C NMR Data of Chapecoderins A−C (**1**−**3**)

position	**1**[a] (δ, ppm)	**2**[b] (δ, ppm)	**3**[b] (δ, ppm)
1	37.5	31.0	33.5
2	18.5	20.0	19.0
3	41.5	41.2	41.5
4	34.5	33.0	33.0
5	48.0	51.3	57.0
6	22.8	27.0	30.5
7	30.0	53.7	123.0
8	208.0	215.0	197.0
9	215.0	84.7	136.0
10	53.5	49.3	50.0
11	35.0	36.0	25.5
12	20.7	22.0	24.5
13	133.0	134.0	134.0
14	146.5	142.0	143.5
15	70.3	69.1	69.0
16	175.0	173.0	173.0
17	30.5	31.0	29.5
18	22.8	21.0	20.5
19	33.8	33.0	32.5
20	19.5	16.2	16.3

[a] In $CDCl_3$. [b] In C_6D_6.

replaced by a tetrasubstituted olefin (δ_C 123.0 and 136.0, C-7 and C-9, respectively) in **3**. The presence of the tetrasubstituted olefin (C-7 and C-9) was also supported by HMBC correlations of H-11a to C-7 and H-6a to C-9. The UV absorption at 255 nm indicated the presence of an unsaturated ketone (C-9, C-7, C-8, and O-8). NOESY correlations of H_3-20 to H-2a and H_3-18 and of H-5 to H-1b and H-3b indicated the β-orientation of Me-20 and the α-orientation of H-5 (Figure 2). Thus, the relative stereochemistry of chapecoderin C was assigned as **3**.

Chapecoderins A−C (**1**−**3**) are new labdane-derived diterpenoids isolated in the present investigation from the Brazilian medicinal plant *E. macrophyllus*, although some 8,9-*seco*-labdane-type and rearranged labdane-type diterpenoids have been obtained from the liverwort *Jungermannia infusca*,[2] and the higher plants *Gypothamnium pinifolium*[3] and *Galeopsis angustifolia*.[4] Biogenetically, chapecoderin C (**3**) may be derived from chapecoderin A (**1**) through intramolecular aldol condensation between C-7 and C-9 to give chapecoderin B (**2**) followed by dehydration at C-7 and C-9. Chapecoderins B (**2**) and C (**3**) exhibited cytotoxicity against murine lymphoma L1210 cells with IC_{50} values of 7.2 and 6.0 μg/mL, respectively, while chapecoderin A (**1**) showed no significant inhibitory activity (IC_{50} >10 μg/mL).

Experimental Section

General Experimental Procedures. The 7.26 and 7.20 ppm resonances of residual $CDCl_3$ and C_6D_6, respectively, and 77.0 and 128.0 ppm of $CDCl_3$ and C_6D_6, respectively, were used as internal references for ^1H and ^{13}C NMR spectra, respectively. EIMS were obtained operating at 70 eV.

Plant Material. The leaves of *E. macrophyllus* ("Chapéu-de-couro") was purchased in São Paulo, Brazil. The plant was identified by Dr. T. Nakasumi (Instituto de Pesouisas de Plantas Medicinais do Brasil), and a voucher specimen has been deposited at Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University.

Extraction and Separation. The leaves were extracted with MeOH (500 mL × 2), and the extracts were partitioned between EtOAc (50 mL × 2) and H_2O (50 mL). The combined EtOAc-soluble portion (558 mg) was subjected to Si gel column chromatography ($CHCl_3$−MeOH, 98:2) to afford a fraction (67 mg), which was partitioned between hexane (10 mL × 3) and 90% MeOH. The MeOH-soluble materials were purified by passage over C_{18} HPLC (Develosil ODS-HG-5, Nomura Co. Ltd., Seto, Japan; 10 × 250 mm, flow rate 2.5 mL/min, eluent MeOH−H_2O, 80:20, UV detection at 254 nm) to give chapecoderins A (**1**, 1.7 mg), B (**2**, 2.2 mg), and C (**3**, 0.8 mg).

Chapecoderin A (1): a colorless amorphous solid; $[\alpha]^{23}_D$ +5.5° (*c* 0.86, $CHCl_3$); UV (MeOH) λ_{max} 210 (ϵ 6200) nm; IR (KBr) ν_{max} 1752, 1701, 1655, 1636 cm^{-1}; ^1H and ^{13}C NMR (Tables 1 and 2); EIMS *m*/*z* 334 [M^+]; HREIMS *m*/*z* 334.2130 [M^+] (calcd for $C_{20}H_{30}O_4$, 334.2144).

Chapecoderin B (2): a colorless amorphous solid; $[\alpha]^{23}_D$ −4.6° (*c* 1.1, $CHCl_3$); UV (MeOH) λ_{max} 210 (ϵ 6400) nm; IR (KBr) ν_{max} 3426, 1745, 1730 (sh), 1647 cm^{-1}; ^1H and ^{13}C NMR (Tables 1 and 2); EIMS *m*/*z* 334 [M^+]; HREIMS *m*/*z* 334.2119 [M^+] (calcd for $C_{20}H_{30}O_4$, 334.2144).

Chapecoderin C (3): a colorless amorphous solid; $[\alpha]^{23}_D$ +5.7° (*c* 0.31, $CHCl_3$); UV (MeOH) λ_{max} 208 (ϵ 5200) and 255 (ϵ 2900) nm; IR (KBr) ν_{max} 1746, 1645 cm^{-1}; ^1H and ^{13}C NMR (Tables 1 and 2); EIMS *m*/*z* 316 [M^+]; HREIMS *m*/*z* 316.2053 [M^+] (calcd for $C_{20}H_{28}O_3$, 316.2038).

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