Diterpenoids from the Rhizomes of *Alpinia calcarata*

Ling-Yi Kong,[†,‡] Min-Jian Qin,[‡] and Masatake Niwa*[,†]

Faculty of Pharmacy, Meijo University, Tempaku, Nagoya 4688503, Japan, and China Pharmaceutical University, Nanjing 210009, People's Republic of China

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Four new labdane-type diterpenoids, calcaratarins A−D (**1**−**4**), along with six known labdane-type diterpenoids, a known elemane-type sesquiterpenoid, and a known coumarin, were isolated from the rhizomes of *Alpinia calcarata*. The structures of **1**−**4** were elucidated on the basis of spectroscopic studies.

Plants of the genus *Alpinia* (Zingiberaceae) are used as traditional herbs in some areas of the People's Republic of China and certain countries of southeast Asia for relieving stomachache, treating colds, invigorating the circulatory system, and reducing swellings. As part of our studies on the constituents and chemotaxonomy of the plants of the genus *Alpinia* found in mainland China, we have investigated the constituents of the rhizomes of *A. calcarata* Rosc., a perennial herb that grows in shaded woodland areas in Guangxi Province. Four new labdane-type diterpenoids, calcaratarins A−D (**1**−**4**); six known labdane-type diterpenoids, γ-bicyclohomofarnesal, (*E*)-15,16-bisnorlabda-8(17),11-dien-13-one, labda-8(17),11,13-trien-15(16)-olide, (*E*)-labda-8(17),12-dien-15-ol-16-al, zerumin A, and isocoronarin D; a known elemane-type sesquiterpenoid, shyobunone; and a known 7-methoxycoumarin were isolated from the rhizomes of *A. calcarata*. This paper describes the isolation and structure elucidation of compounds **1**−**4**.

Results and Discussion

Calcaratarin A (**1**), a colorless oil, was indicated by HRFABMS to have the molecular formula $C_{22}H_{36}O_3$. The ^{13}C NMR spectrum exhibited 22 carbon signals, including five methyls (two of which were methoxyl groups), eight methylenes, five methines, and four quaternary carbons. The ^1H NMR signals at δ 0.87 (3H, s), 0.81 (3H, s), and 0.73 (3H, s), as well as those at δ 4.41 (1H, dd, J = 2.4, 1.2 Hz) and 4.82 (1H, dd, J = 2.4, 1.2 Hz), were characteristic of a labdane-type diterpenoid[1] and were assigned to the methyl groups at C-18, C-19, and C-20 and to the methylene group at C-17, respectively. The ^{13}C NMR spectral data, exhibiting signals for three methyl groups at δ 33.61, 21.74, and 14.41; a quaternary carbon at δ 148.27; and a methylene at δ 107.86, also provided evidence for **1** being a labdane-type diterpenoid. The structure of the side-chain (C-11 to C-16) of **1** was deduced from its HMQC and HMBC spectra. In the HMBC spectrum, the olefinic signal at δ 6.53 (1H, t, J = 6.0 Hz, H-12) correlated with the signals of the aldehyde group at δ 194.98 (C-16) and the methylene group at δ 29.06 (C-14). In the HMQC spectrum, the signal at δ 29.06 correlated with the signal at δ 2.56 (2H, dd, J = 8.4, 5.4 Hz, H-14), which, in the HMBC spectrum, correlated with the methine signal at δ 103.91 (C-15), and in turn correlated with two methoxyl signals at δ 3.33 (6H, s, MeO). The stereochemistry of the double bond between C-12 and C-13 of calcaratarin A (**1**) was determined from the NOESY spectrum. A NOE correlation of the signal of the aldehyde group at δ 9.32 (H-16) and the olefinic signal at δ 6.53 (H-12) indicated this double bond to be in the *E*-configuration. Accordingly, the structure of the side-chain was established as shown. The structure of **1**, (*E*)-labda-8(17),12-dien-15,15-dimethoxy-16-al, was further confirmed by reacting **1** with hydrochloric acid to give the parent compound, (*E*)-labda-8(17),12-diene-15,16-dial (**5**), which has been known since 1980.[2] The HPLC analysis of an ethanol extract of the fresh rhizomes of *A. calcarata* showed the presence of **1**, which was then isolated from the ethanol extract. Therefore, calcaratarin A (**1**) appears to be a natural product and not an artifact.

Calcaratarin B (**2**), a colorless oil, was assigned the molecular formula $C_{20}H_{32}O_2$, as determined by HRFABMS. Absorption bands at 3300−2600 and 1708 cm^{-1} in the IR spectrum indicated the presence of a carboxylic acid group. The ^{13}C and ^1H NMR spectra showed clearly the characteristics of a labdane-type diterpenoid. The structure of the side-chain (C-11 to C-16) was deduced from the HMBC spectrum. The olefinic signal at δ 5.31 (1H, t, J = 6.0 Hz, H-12) correlated with the methyl signal at δ 23.87 (C-16) and the methylene signal at δ 37.39 (C-14). Furthermore, the proton at δ 3.08 (2H, br d, J = 1.8 Hz, H-14) correlated with the carboxyl carbonyl at δ 177.21 (C-15). The stereochemistry of the double bond between C-12 and C-13 of calcaratarin B (**2**) was deduced from the NOESY spectrum. The NOE correlation of the methyl signal at δ 1.74 (H-16) and the olefinic resonance at δ 5.31 (H-12) showed a *Z*-configuration of this double bond and supported the side-chain as shown. The structure of calcaratarin B was therefore elucidated as (*Z*)-labda-8(17),12-dien-15-oic acid (**2**). From the point of view of its biogenesis, **2** may to be related to zerumin A,[3] by reduction of the C-16 aldehyde in the latter to a methyl group.

Calcaratarin C (**3**), an amorphous solid, was assigned the molecular formula $C_{20}H_{30}O_3$ from its HRFABMS. The ^{13}C and ^1H NMR spectra showed the presence of a labdane-type skeleton. The structure of the side-chain (C-11 to C-16) was elucidated from the HMQC and HMBC spectra. In the HMQC spectrum, the methylene signal at δ 31.16 (C-12) correlated with the signals of the protons at δ 1.75 and 1.76. In the HMBC spectrum, the signals at δ 1.75 (H-12α) and 1.76 (H-12β) correlated with the methine at δ 67.40 (C-11), which was connected to a hydroxyl group, and with the methine at δ 51.97 (C-9). The olefinic signal at δ 5.96 (H-14) correlated with signals for the quaternary carbon at δ 172.98 (C-13), the carbonyl at δ 173.45 (C-15), and the methylene at δ 70.98 (C-16). The chemical shift value of C-13 (δ 172.98) suggested that the carbonyl was located at C-15 rather than C-16,[4] and that an α,β-unsaturated

* To whom correspondence should be addressed. Fax: +81-52-834-8090. E-mail: masa@meijo-u.ac.jp.
† Meijo University.
‡ China Pharmaceutical University.

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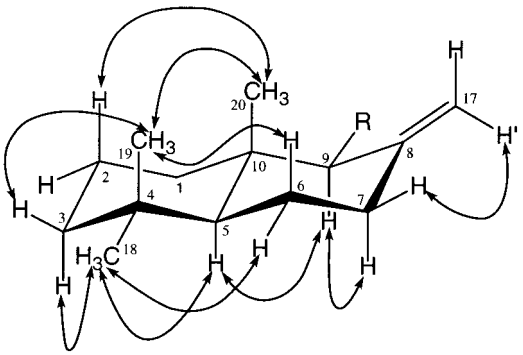
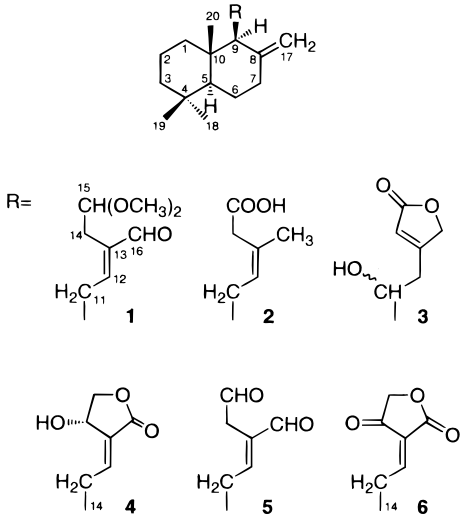


Figure 1. Relative stereochemistry of the skeleton of compounds **1**−**4** determined from their NOESY NMR spectra.

γ-lactone ring was formed between C-15 and C-16. Thus, the structure of calcaratarin C was established as labda-8(17),13-dien-11-ol-15(16)-olide (**3**). The stereochemistry at C-11 remains to be clarified.

Calcaratarin D (**4**) was obtained as an amorphous solid, and its spectral data, including ^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H−^{1}H COSY, NOESY, HMQC, HMBC, and FABMS, indicated this compound to be a stereoisomer of isocoronarin D, a known labdane-type diterpenoid.[5] The only structural difference between **4** and isocoronarin D was the stereochemistry at C-14. Because the relative stereochemistry at C-14 of isocoronarin D has been determined as 14β-hydroxy (*S* relative configuration) by X-ray diffraction,[5] the hydroxyl group at C-14 for **4** must therefore be α-positioned (*R* relative configuration). Calcaratarin D is accordingly 14-*epi*-isocoronarin D or labda-8(17),12-dien-14α-ol-16(15)-olide (**4**). Oxidation of calcaratarin D and isocoronarin D with manganese(IV) oxide both gave labda-8(17),12-dien-14-one-16(15)-olide (**6**).



The relative configurations about the common skeleton of calcaratarins A−D (**1**−**4**) were identical with those of labdane-type diterpenoids isolated from the plants in the family Zingiberaceae, according to their NOESY spectra (Figure 1) and comparison of ^{13}C NMR data with literature values.[1,5,6]

Eight known compounds were identified, in turn, as γ-bicyclohomofarnesal,[7] (*E*)-15,16-bisnorlabda-8(17),11-dien-13-one,[8] labda-8(17),11,13-trien-15(16)-olide,[1] (*E*)-labda-8(17),12-dien-15-ol-16-al,[8] zerumin A,[3] isocoronarin D,[5] shyobunone,[9] and 7-methoxycoumarin,[10] from their spectral data. The isolation of γ-bicyclohomofarnesal from a natural source has not been reported previously.[7] Shyobunone,[9] a known elemane-type sesquiterpenoid, was isolated from a plant in the family the Zingiberaceae for the first time.

Experimental Section

General Experimental Procedures. Optical rotations were taken on a JASCO P-1020 polarimeter (cell length 100 mm). UV spectra were measured on a JASCO V-560 UV/vis spectrophotometer (cell length 10 mm). IR spectra were recorded on a JASCO FT/IR-410 infrared spectrophotometer. 1D and 2D NMR spectra were recorded on JEOL A-400 and A-600 spectrometers. EIMS were measured with a Hitachi M-80 spectrometer. FABMS were measured with a JEOL HX-110 spectrometer using *m*-nitrobenzyl alcohol as matrix. Column chromatography was performed with BW-820MH Si gel (Fuji-Silysia Chemical Co., Ltd., Aichi, Japan). Preparative thin-layer chromatography (TLC) was conducted on Si gel 60 F_{254} 0.5-mm plates (Merck), and analytical TLC on Si gel 60 F_{254} 0.25-mm plates (Merck), with spots visualized under UV light (254 nm) and by spraying with 10% molybdic acid and subsequent heating of the plates. Preparative HPLC was performed with a JASCO PU-980 pump, using a PREP-SIL 20 mm × 25 cm column, equipped with a JASCO UV-970 UV/vis detector.

Plant Material. The rhizomes of *A. calcarata* were collected in the suburbs of Nanning City, Guangxi Province, People's Republic of China, in October 1997, and were identified by M.-J.Q., Department of Medicinal Plants, China Pharmaceutical University, with a voucher specimen (no. 971006) having been deposited there.

Extraction and Isolation. The fresh rhizomes (2.8 kg) were cut into pieces and extracted with MeOH (3 × 15 L) at room temperature. The MeOH extract (129 g) was partitioned between EtOAc (2 L) and water (4 L). The EtOAc solution was concentrated in vacuo to yield 88 g of residue. A part of the residue (44 g) was subjected to column chromatography on Si gel (600 g) and eluted with hexane, hexane−$CHCl_3$ (10:2), hexane−$CHCl_3$ (10:4), and $CHCl_3$ (each 1.5 L), to give fractions 1−4, respectively. Fraction 3 (1.9 g) was subjected to column chromatography on Si gel (100 g) with hexane−$CHCl_3$ (10:3) as an eluent and to repeated preparative TLC, using hexane−benzene (10:4) and hexane−$CHCl_3$ (10:5) as solvents, to give shyobunone (25.7 mg), 7-methoxycoumarin (13.7 mg), and γ-bicyclohomofarnesal (17.8 mg). Fraction 4 (17.8 g) was subjected to column chromatography on Si gel (250 g) with gradient mixtures of hexane−EtOAc (100:5, 100:10, 100:20, and 100:30, successively) (each 500 mL), to give fractions 4-1 (1.8 g), 4-2 (2.7 g), 4-3 (1.5 g), and 4-4 (3.1 g). Fraction 4-1 was subjected to column chromatography on Si gel (30 g) and repeated preparative TLC, eluted with hexane−EtOAc (10:1) and hexane−$CHCl_3$ (10:6), to give (*E*)-15,16-bisnorlabda-8(17),11-dien-13-one (3.8 mg) and **1** (3.2 mg), with final purification by preparative HPLC with hexane−2-propanol (95:5). Fraction 4-2 was purified with column chromatography on Si gel (40 g) and repeated preparative TLC, eluted with hexane−EtOAc (10:1) and benzene−EtOAc (20:1) to give labda-8(17),11,13-trien-15(16)-olide (10.3 mg) and **2** (8.9 mg). Fraction 4-3 was purified by column chromatography and repeated preparative TLC, eluted with hexane−EtOAc (100:15) and hexane−$CHCl_3$ (1:1) to afford (*E*)-labda-8(17),12-dien-15-ol-16-al (59.8 mg) and zerumin A (12.4 mg). Fraction 4-4 was separated by column chromatography and repeated preparative TLC, eluted with $CHCl_3$−EtOAc (4:1), and by preparative HPLC, eluted with hexane−2-propanol (80:20), to give **3** (1.1 mg) and fraction 4-4-2. The latter was subjected to recycled HPLC to afford **4** (4.1 mg) and isocoronarin D (2.8 mg).

An extract (2.5 g) from the fresh rhizomes (60 g) made with EtOH (2 × 500 mL) was subjected to column chromatograhy (hexane−EtOAc, 10:1), followed by preparative HPLC (hexane−2-propanol, 99:1) to give **1** (1.2 mg).

Compound 1: colorless oil, $[\alpha]^{21}_D$ +12.4° (*c* 0.08, $CHCl_3$); UV (CH_3CN) λ_{max} (log ϵ) 235 (4.01) nm; IR (film) ν_{max} 3083,

Table 1. ^{1}H NMR Data of Compounds **1**−**4** at 600 MHz[a]

H	1	2	3	4
1α	1.07 td (13.2, 3.6)	1.03 td (12.9, 3.6)	l.04 td (12.6, 4.2)	1.05 td (12.6, 4.2)
1*β*	1.72 br d (13.2)	1.73 br d (12.9)	1.67 br d (12.6)	l.70 br d (12.9)
2α	1.48 dp(14.4, 3.6)	1.46 m	1.49 m	1.48 dp (13.8, 3.6)
2*β*	1.56 qt (14.4, 3.6)	1.54 qt (13.8, 3.6)	1.56 qt (13.8, 3.6)	1.57 qt (13.8, 3.6)
3α	1.17 td (13.5, 3.6)	1.16 td (13.8, 4.2)	1.18 td (13.5, 4.2)	l.18 td (13.2, 4.8)
3*β*	1.40 br d (13.5)	1.37 br d (13.2)	1.39 br d (13.2)	1.41 br d (13.2)
5	1.12 dd (12.3, 3.0)	1.08 dd (12.6, 2.4)	1.15 dd (12.6, 2.4)	1.12 dd (12.6, 2.4)
6α	1.73 m	1.24 m	1.78 m	1.74 ddt 12.6, 4.8, 3.0)
6*β*	1.33 qd (12.9, 4.8)	1.30 qd (13.2, 4.2)	1.34 qd (13.2, 4.2)	1.31 qd (12.9, 4.2)
7α	2.01 td (12.9, 5.4)	1.99 td (13.2, 5.4)	2.01 td (13.2, 3.6)	1.98 td (13.8, 5.4)
7*β*	2.39 ddd (12.9, 4.2, 2.4)	2.36 ddd (12.9, 4.5, 2.4)	2.42 ddd (12.9, 4.2, 2.4)	2.37 ddd (13.2, 4.5, 2.4)
9	1.88 br d (l0.8)	1.52 br d (9.3)	2.00 br d (9.0)	l.93 br d (10.2)
11	2.44 ddd (17.4, 11.4, 6.6)	2.01 ddd (15.6, 10.5, 5.4)	4.64 m	2.37 ddd (16.4, 10.8, 6.6)
11′	2.62 ddd (17.4, 6.0, 3.0)	2.24 ddd (15.6, 6.6, 1.8)		2.66 ddd (16.4, 7.8, 3.6)
12	6.53 t (6.0)	5.31 t (6.0)	1.75 ddd 12.0, 9.0, 3.6)	6.94 td (6.9, 1.8)
12′			1.76 ddd (12.0, 9.0, 1.2)	
14	2.56 dd (8.4, 5.4)	3.08 br d (1.8)	5.96 q (1.8)	5.04 t (6.0)
15α	4.42 t (5.4)			4.22 dd (10.5, 1.8)
15*β*				4.42 dd (10.5, 5.4)
16	9.32 s	1.74 br d (1.2)	4.87 dd (4.2, 1.8)	
17	4.41 dd (2.4, 1.2)	4.45 dd (3.0, 1.2)	4.39 d (1.2)	4.34 br s
17′	4.82 dd (2.4, 1.2)	4.80 dd (3.3, 1.2)	4.89 d (1.2)	4.81 br s
18	0.87 s	0.85 s	0.87 s	0.87 s
19	0.81 s	0.79 s	0.79 s	0.80 s
20	0.73 s	0.68 s	0.67 s	0.72 s
MeO	3.33 s			

[a] The coupling constants (*J*) in parentheses are given in Hz.

Table 2. ^{13}C NMR Data of Compounds **1**−**4** at 150 MHz[a,b]

C	1	2	3	4
1	39.20 CH_2 (2α, 2*β*, 3α, 3*β*, 20)	39.10 CH_2 (2α, 3*β*, 5, 20)	39.06 CH_2 (2α, 2*β*, 3*β*, 20)	39.24 CH_2 (2α, 2*β*, 20)
2	19.32 CH_2 (1*β*, 3α, 3*β*)	19.38 CH_2 (1*β*, 3α)	19.24 CH_2 (1α, 3α, 3*β*)	19.31 CH_2 (1*β*, 3α)
3	42.06 CH_2 (1*β*, 18, 19)	42.12 CH_2 (1α, 1*β*, 18, 19)	41.94 CH_2 (1*β*, 18, 19)	41.98 CH_2 (1*β*, 18, 19)
4	33.56 C (3α, 5, 18,19)	33.55 C (3α, 3*β*, 5, 18, 19)	33.60 C (5, 18, 19)	33.58 C (5, 18, 19)
5	55.46 CH (3*β*, 6α, 18, 19)	55.39 CH (3α, 3*β*, 6*β*, 18, 19)	55.50 CH (3α, 18, 19)	55.41 CH (18, 19, 20)
6	24.14 CH_2 (5, 7*β*)	24.23 CH_2 (5, 7α)	24.34 CH_2 (5, 7α, 7*β*)	24.13 CH_2 (5, 7α)
7	37.91 CH_2 (6α, 9)	38.10 CH_2 (17, 17′)	38.18 CH_2 (9, 17, 17′)	37.84 CH_2 (17, 17′)
8	148.27 C (9, 11′)	148.56 C (7α, 7*β*, 11)	148.69 C (6α, 11)	148.19 C (9, 11′)
9	56.58 CH (11, 11′, 17, 17′, 20)	57.10 CH (11, 11′, 17, 17′, 20)	51.97 CH (12, 12′, 20)	56.46 CH (11, 17, 17′, 20)
10	39.58 C (1α, 9, 20)	39.51 C (1*β*, 5, 20)	39.43 C (5, 20)	39.75 C (5, 20)
11	24.54 CH_2 (9, 12)	22.76 CH_2 (9, 12)	67.40 CH (12, 12′)	25.15 CH_2 (9, 12)
12	159.93 CH (9, 11, 11′)	130.24 CH (11, 11′, 14, 16)	31.16 CH_2 (9, 11)	149.87 CH (11, 11′)
13	138.16 C (11, 11′, 14, 16)	126.63 C(11, 11′, 14, 16)	172.98 C (12, 12′, 14)	127.88 C (11, 11′, 14*β*)
14	29.06 CH_2 (12, 16)	37.39 CH_2 (12, 16)	114.52 CH (12, 12′, 16)	66.54 CH (12, 15α)
15	103.91 CH (14, OMe)	177.21 C (14)	173.45 C (14, 16)	74.21 CH_2 (14*β*)
16	194.98 CH (12, 14)	23.87 CH_3 (12, 14)	70.98 CH_2 (14)	169.96 C (12, 14*β*, 15α)
17	107.86 CH_2 (9, 7α)	107.37 CH_2 (9)	106.51 CH_2 (7α, 9)	107.49 CH_2
18	33.61 CH_3 (3α, 5, 19)	33.62 CH_3 (3α, 3*β*, 5, 19)	33.55 CH_3 (5, 19)	33.54 CH_3 (5, 19)
19	21.74 CH_3 (3α, 5, 18)	21.75 CH_3 (3α, 5, 18)	21.64 CH_3 (5, 18)	21.69 CH_3 (5, 18)
20	14.41 CH_3 (1α, 5, 9)	14.38 CH_3 (5, 9)	14.63 CH_3 (5, 9)	14.42 CH_3 (5, 9)
MeO	54.30 CH_3 (15)			

[a] Multiplicities of carbons were assigned by DEPT spectra. [b] HMBC corelations are shown in parentheses.

2932, 2868, 2843, 1683, 1642, 1458, 1388, 1366, 1261, 1192, 1162, 1121, 1079, 1015, 974, 889, 757 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 2; FABMS *m*/*z* 371 (100) $[M + Na]^+$, 347, 319, 285, 215, 191, 154, 137; HRFABMS *m*/*z* 371.2563 (calcd for $C_{22}H_{36}O_3Na$, 371.2562).

Compound 2: colorless oil, $[\alpha]^{20}_D$ +14.8° (*c* 0.5, $CHCl_3$); UV (CH_3CN), no absorption over 210 nm; IR (film) ν_{max} 3082, 2929, 2868, 1708, 1644, 1459, 1441, 1412, 1388, 1295, 1216, 891, 758 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 2; FABMS *m*/*z* 305 $[M + H]^+$, 289, 245, 217, 191, 176, 154 (100), 137, 136; HRFABMS *m*/*z* 305.2473 (calcd for $C_{20}H_{33}O_2$, 305.2481).

Compound 3: amorphous solid, $[\alpha]^{24}_D$ +36.6° (*c* 0.05, $CHCl_3$); UV (CH_3CN) λ_{max} (log ϵ) 211 (3.86) nm; IR (film) ν_{max} 3388, 3077, 2963, 2939, 2868, 2855, 1737, 1632, 1442, 1429, 1388, 1272, 1177, 1142, 1082, 1011, 898, 869 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 2; FABMS *m*/*z* 341 (100) $[M + Na]^+$, 321, 309, 289, 176, 154, 137, 136; HRFABMS *m*/*z* 341.2095 (calcd for $C_{20}H_{30}O_3$-Na, 341.2093).

Compound 4: amorphous solid, $[\alpha]^{22}_D$ +21.5° (*c* 0.15, $CHCl_3$); UV (CH_3CN) λ_{max} (log ϵ) 223 (3.94) nm; IR (film) ν_{max} 3397, 2997, 2919, 2845, 1726, 1676, 1647, 1456, 1435, 1386, 1364, 1292, 1219, 1089, 1046, 1011, 979, 907 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 2; FABMS *m*/*z* 319 $[M + H]^+$, 303, 289, 245, 219, 191, 177, 154 (100), 137, 136; HRFABMS *m*/*z* 319.2272 (calcd for $C_{20}H_{31}O_3$, 319.2273).

TLC R_f values using hexane−EtOAc (2:1) were as follows: **1**, 0.84; **2**, 0.26; **3**, 0.52; **4**, 0.49.

Hydrolysis of Compound 1. A solution of compound **1** (3.0 mg), 0.25 N HCl (2.5 mL), and dioxane (1.5 mL) was stirred at 60 °C for 4 h. After dilution with water (5 mL), the reaction mixture was extracted with $CHCl_3$ (3 × 3 mL). The combined extracts were washed with saturated aqueous NaCl solution (2 × 3 mL), dried over $MgSO_4$, and then concentrated in vacuo. The residue was purified by preparative HPLC (hexane−2-

propanol, 98:2) to give **5** (1.4 mg):[2] ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.08 (1H, m, H-1α), 1.74 (1H, m, H-1*β*), 1.51 (1H, m, H-2α), 1.55 (1H, m, H-2*β*), 1.20 (1H, m, H-3α), 1.43 (1H, br d, J = 13.0 Hz, H-3*β*), 1.15 (1H, dd, J = 12.8, 3.2 Hz, H-5), 1.75 (1H, m, H-6α), 1.35 (1H, m, H-6*β*), 2.02 (1H, m, H-7α), 2.42 (1H, br d, J = 12.6 Hz, H-7*β*), 1.91 (1H, br d, J = 9.5 Hz, H-9), 2.35 (1H, m, H-11), 2.50 (1H, m, H-11′), 6.72 (1H, dd, J = 7.0, 6.5 Hz, H-12), 3.42 (1H, d, J = 16.0 Hz, H-14), 3.51 (1H, d, J = 16.0 Hz, H-14′), 9.65 (1H, br s, H-15), 9.44 (1H, s, H-16), 4.36 (1H, br s, H-17), 4.89 (1H, br s, H-17′), 0.90 (3H, s, H-18), 0.82 (3H, s, H-19), 0.75 (3H, s, H-20); EIMS *m*/*z* 302 (M^+), 273, 191, 177, 137 (100), 95, 81.

Oxidation of Compound 4. A mixture of **4** (3.5 mg) and freshly prepared MnO_2 (4 mg) in MeOH (5 mL) was stirred at 35 °C for 10 h. After filtration, the filtrate was concentrated and purified by preparative TLC (hexane−EtOAc 5:1) to give **6** (0.6 mg): IR (KBr) ν_{max} 3020, 2932, 2844, 1735, 1711, 1668, 1639, 1457, 1346, 1082, 1045, 992, 891 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.05 (1H, m, H-1α), 1.71 (1H, m, H-1*β*), 1.44 (1H, m, H-2α), 1.55 (1H, m, H-2*β*), 1.14 (1H, m, H-3α), 1.40 (1H, br d, J = 13.0 Hz, H-3*β*), 1.11 (1H, dd, J = 12.5, 2.5 Hz, H-5), 1.76 (1H, m, H-6α), 1.31 (1H, m, H-6*β*), 2.00 (1H, m, H-7α), 2.41 (1H, m, H-7*β*), 1.91 (1H, br d, J = 9.5 Hz, H-9), 2.62 (1H, m, H-11), 2.71 (1H, m, H-11′), 7.09 (1H, t, J = 6.0 Hz, H-12), 4.64 (2H, br s, H-15), 4.45 (1H, br s, H-17), 4.87 (1H, br s, H-17′), 0.86 (3H, s, H-18), 0.81 (3H, s, H-19), 0.73 (3H, s, H-20); EIMS *m*/*z* 316 (M^+), 259, 191, 167, 137 (100); HRFABMS *m*/*z* 317.2119 (calcd for $C_{20}H_{29}O_3$, 317.2117).

Oxidation of Isocoronarin D. Isocoronarin D (2.5 mg) was treated with freshly prepared MnO_2 (4 mg) in MeOH (5 mL) by the same procedure as described above to give **6** (0.5 mg), and its IR and ^{1}H NMR spectra were identical with those of the ketone from **4**.

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