New Decalin Derivatives, Eujavanoic Acids A and B, from *Eupenicillium javanicum*

Shigeru Okamoto,[†] Tomoo Hosoe,[†] Takeshi Itabashi,[†] Koohei Nozawa,*[,†] Kaoru Okada,[‡] Galba Maria de Campos Takaki,[‡] Minoru Chikamori,[§] Takashi Yaguchi,[§] Kazutaka Fukushima,[§] Makoto Miyaji,[§] and Ken-ichi Kawai[†]

Faculty of Pharmaceutical Sciences, Hoshi University, Ebara 2-4-41, Shinagawa-ku, Tokyo 142-8501, Japan, Nucleo de Pesquisas em Ciencias Ambientais, Universidade Catolica de Pernambuco, Rua Nunes Machado, 42 Boa Vista, Bloco J, Recife, 50050-590, Pernambuco, Brazil, and Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Inohana 1-8-1, Chuo-ku, Chiba 260-8673, Japan

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Two new decalin derivatives, eujavanoic acids A (**1**) and B (**2**), were isolated from *Eupenicillium javanicum*, along with several compactin derivatives. The structures of **1** and **2** were determined by spectroscopic methods and modified Mosher's method. The side chain (2-methylbutanoyloxy) and acid functionalities of compactin derivatives were necessary to show the antifungal activity.

We have been searching for fungal metabolites with antifungal activity against pathogenic filamentous fungi, *Aspergillus fumigatus* and *A. niger*, and/or pathogenic yeasts, *Candida albicans* and *Cryptococcus neoformans*. During our research, we found that an organic extract of *Eupenicillium javanicum* IFM 52670 showed characteristic and strong antifungal activity against *A. fumigatus*.[1] Fractionation of the extract led to the isolation of new decalin derivatives, designated eujavanoic acids A (**1**) and B (**2**), along with compactin (**3**)[2] and its derivatives: dihydrocompactin (**4**),[3] ML-236A (**5**),[4] 3,5-dihydro-3α-hydroxy-ML-236C (**6**),[5] 3,5-dihydro-3-oxo-ML-236C (**7**), the acid form (**8**), and the ethyl ester (**9**) of compactin (**3**). In this paper, we report the isolation and structure determination of eujavanoic acids A (**1**) and B (**2**) and the isolation of a compound having anti-*A. fumigatus* activity from *E. javanicum*. Furthermore, we describe the relationship between the antifungal activity and structures of compactin derivatives.

The molecular formula of **1** was established as $C_{14}H_{20}O_4$ by HREIMS. The IR absorption regions at 3200, 3600−2400, 1720, and 1650 cm^{-1} and ^{13}C NMR signals at δ_C 177.7 and 203.1 indicated the presence of a hydroxyl group, a carboxyl group, and a conjugated ketone in **1**. The ^{13}C NMR spectrum revealed the presence of four sp^2 carbons including two carbonyl carbons. Hence **1** is bicyclic.

The structural fragment of **1** shown by the bold line in Figure 1 was established by the $^1H-^1H$ COSY and HMQC spectra of **1**. The cross-peaks between the carboxyl carbon (δ_C 177.7) and four protons at C-9 and C-10 (δ_H 1.66, 1.91, 2.31, and 2.40) in the HMBC spectrum proved that the carboxyl group is connected at C-10. Furthermore, the HMBC correlations from 1-H (δ_H 1.78) and 2-methyl protons (δ_H 1.03) to the conjugated ketone (δ_C 203.1) suggested that the conjugated ketone was attached to C-2. Considering the above result and the presence of an olefinic singlet (δ 5.80) in **1**, we concluded that **1** was the decalin derivative as shown in Figure 1. This conclusion was supported by other HMBC correlation peaks, which are indicated in Figure 1.

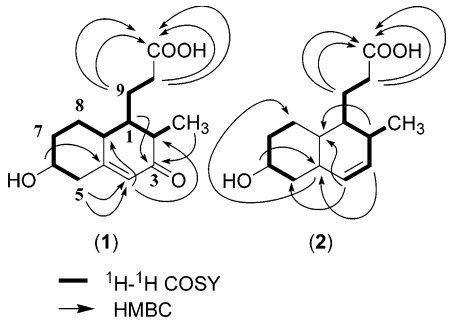

Figure 1. $^1H-^1H$ COSY and HMBC correlation of eujavanoic acids A (**1**) and B (**2**).

The molecular formula of **2** was established as $C_{14}H_{22}O_3$ by HRCI-MS. The IR spectrum of **2** showed absorptions at 3400, 3300−2500, and 1710 cm^{-1}, indicating the presence of a hydroxyl and one carboxyl group. Since the ^{13}C NMR spectrum of **2** displayed three sp^2 carbon peaks that included a carboxyl group, it was clear that **2** was also bicyclic.

The structural fragment of **2** shown by the bold line in Figure 1 was established by the $^1H-^1H$ COSY and HMQC spectra. The cross-peaks between the carboxylic carbon (δ_C 175.0) and the four protons at C-9 and C-10 (δ_H 1.38, 1.85−1.95, 2.18, and 2.37) in the HMBC spectrum of **2** proved that the carboxyl group is connected with C-10. We concluded that **2** was the decalin derivative as shown in Figure 1, because pairwise correlations in the HMBC spectrum of **2** existed between 2-H at δ_H 2.28 and C-8a at δ_C 39.6, 3-H at δ_H 5.60 and C-4a at δ_C 42.9, 4-H at δ_H 5.33 and C-5 at δ_C 43.4, and even 6-H at δ_H 3.56 and C-4a. The conclusion was supported by other HMBC correlation peaks, which are indicated in Figure 1.

The relative configuration of eujavanoic acids A (**1**) and B (**2**) is proposed on the basis of NOESY data (Figure 2). NOESY correlations of 2-Me to 9-H and 8a-H and of 8ax-H to 1-H and 6-H observed in both **1** and **2** suggested that 2-Me, 9-H, 8a-H, and 6-OH are all on the same face of the ring system, with 2-Me and 8a-H in axial orientations and 6-OH in equatorial orientation.

To confirm the absolute configuration, the advanced Mosher's method[6] was applied to eujavanoic acids A (**1**) and B (**2**). The (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)-

* To whom correspondence should be addressed. Tel: +81-3-5498-5789. Fax: +81-3-5498-5789. E-mail: nozawa@hoshi.ac.jp.
[†] Hoshi University.
[‡] Universidade Catolica de Pernambuco.
[§] Chiba University.

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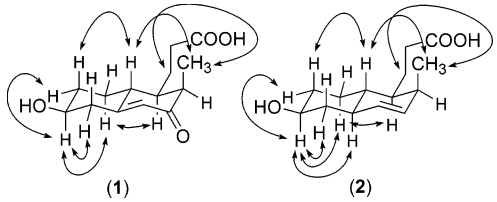
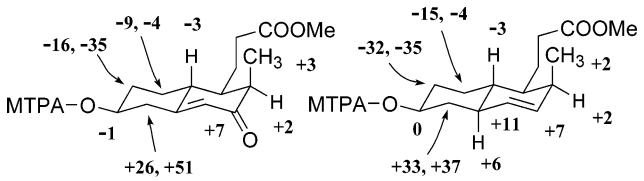


Figure 2. NOESY correlation of eujavanoic acids A (**1**) and B (**2**).



(**10**) (**11**)

Figure 3. Differences of chemical shifts ($\Delta\delta$ in hertz) between the (*R*)- and (*S*)-MTPA esters of **10** and **11**.

phenylacetic acid (MTPA) esters of methyl esters (**10** and **11**) of **1** and **2** were synthesized, and the values of the chemical shift differences between the (*R*)- and (*S*)-MTPA esters [$\Delta\delta = \delta_S - \delta_R$ in hertz (500 MHz)] were calculated. From the results (Figure 3), both eujavanoic acids A and B were assigned the *S*-configuration at C-6, as depicted in **1** and **2**.

The analyses of ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY proved that **6** was the lactone form of sodium 3α-hydroxy-3,5-dihydro-ML-236C, originally isolated from *Paecilomyces viridis* L-68.[5] The ketone derived from **6** by treatment with active MnO_2 was identical to **7** on the basis of spectroscopic data. Therefore, the structure of **7** was confirmed. Compound **9** was identical to the ethyl ester derived from **3** by treatment with KOH in EtOH.

The antifungal activity was determined by the paper disk method against *A. fumigatus*. Bioassay-directed separation of the extract led to the isolation of the active compound (**8**). The molecular formula of **8** was established as $C_{23}H_{36}O_6$ by electron-impact mass spectrometry (EIMS) (M^+, 408) and the analysis of ^{1}H and ^{13}C NMR spectra. The ^{1}H NMR spectrum of **8** was similar to that of **3**, except for the chemical shifts of the protons in the lactone ring. Compound **8** was readily converted to **3** by heating in $CHCl_3$. Hence, active compound **8** was determined as the acyclic form of the lactone in compactin (**3**). **8** showed strong growth inhibition against *A. fumigatus* (18 mm inhibition zone at 1.0 μg/disk) and *C. albicans* (17 mm inhibition zone at 5.0 μg/disk), whereas other metabolites (**1**−**7** and **9**) showed no antifungal activity at 100 μg/disk.

Compounds **4**−**7** and 4a-α-tetrahydrocompactin (**12**) derived from **4** by hydrogenation with 10% Pd−C showed no antifungal activity. When treated with 5% sodium hydroxide in DMF, the acyclic analogues **13**−**17** were formed. After purification, the antifungal assay of **13**−**17** against *A. fumigatus* was performed (Table 1). Compound **13** derived from **4** and **17** from **12** showed strong activity as well as that of **8** against *A. fumigatus*, but the other acids **14**−**16** showed no antifungal activity up to 100 μg/disk. Therefore, it was clear that a 2-methylbutanoyloxy residue and carboxyl group in the side chains of compactin derivatives are necessary in order to show the anti-*A. fumigatus* activity.

Experimental Section

General Experimental Procedures. General experimental procedures were described in the previous paper.[1] IR spectra were recorded on a JASCO FT/IR-5300 spectrophotometer. HPLC was performed with a Senshu SSC-3160 pump (flow rate, 7 mL/min), equipped with a Shimamura YRD-883 RI-detector and HPLC column, YMC Pack SIL 06 (10ϕ × 300 mm) or Senshu Pack Peagasil ORD (20ϕ × 250 mm). TLC was conducted on precoated Kieselgel 60 F254 plates (5714; Merck). Spots on TLC were detected by UV light of 254 nm and/or by spraying with phosphomolybdic acid (5%)−ceric acid (trace) in 5% H_2SO_4 and subsequent heating of the plates.

Table 1. Antifungal Activity of Acid Forms of Compactin Derivatives against *A. fumigatus*[a]

compound (mg/disk)	AMPB[b]	**8**	**9**	**13**	**17**
10	13	30	−	32	22
5	14	27	−	21	18
1	11	18	−	16	+
0.5	+	(16)	−	−	−

[a] The diameter of inhibition circle is indicated in mm. The parentheses mean slightly growing in the inhibition circle. The plus (+) means slight inhibition, and the minus (−) means no inhibition. [b] Amphotericin B.

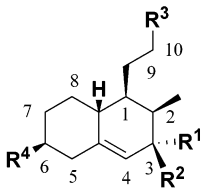
Culture, Extraction, and Isolation. *E. javanicum* IFM52670 was cultured at 25 °C for 21 days in 10 Roux flasks containing 150 g of moist rice in each flask. The fermented rice was extracted with CH_2Cl_2−MeOH (1:1), and the organic layer was evaporated in vacuo. The resultant extract (25 g) was suspended in H_2O and extracted with EtOAc, and then the H_2O and the organic layers were evaporated in vacuo, respectively. The EtOAc extract (12 g) showed strong antifungal activity against *A. fumigatus*.

The EtOAc extract was separated by column chromatography over silica gel (240 g) into six fractions: CH_2Cl_2, CH_2Cl_2−EtOH (20:1) (2 g), CH_2Cl_2−EtOH (10:1) (650 mg), CH_2Cl_2−EtOH (5:1) (580 mg), CH_2Cl_2−EtOH (1:1) (230 mg), and EtOH. The fifth fraction [CH_2Cl_2−EtOH (1:1)] showed antifungal activity against *A. fumigatus* and was further separated by low-pressure liquid chromatography (LPLC) on silica gel with benzene−EtOH (5:1) and then using CH_2Cl_2−EtOH (5:1) to give eujavanoic acid A (**1**) (9 mg) and eujavanoic acid B (**2**) (12 mg), with benzene−EtOH (2:1), followed by repeated purification by HPLC on an ODS column (85% CH_3CN) to obtain the active compound (**8**) (7 mg). The second fraction [CH_2Cl_2−EtOH (20:1)] was separated into three fractions by LPLC on a silica gel column using CH_2Cl_2−EtOH (20:1). The first fraction was purified with HPLC on an ODS column (90% CH_3OH) to give **9** (6 mg). The next fraction including a main metabolite was purified with HPLC on an ODS column (85% MeOH) to give compactin (**3**)[2] (300 mg) and dihydrocompactin (**4**)[3] (10 mg). The third fraction [CH_2Cl_2−EtOH (10:1)] was also purified with LPLC on a silica gel column using benzene−EtOAc−EtOH (5:1:0.3) to give ML-236A (**5**)[4] (6 mg), 3,5-dihydro-3α-hydroxy-ML-236C (**6**) (20 mg), and 3,5-dihydro-3-oxo-ML-236C (**7**) (6 mg). Compounds **3**, **4**, and **5** were identified by comparison with published data.[2−4]

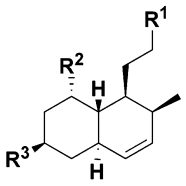
Eujavanoic acid A (1): colorless needles (EtOAc); mp 130−130.5 °C; $[\alpha]_D^{24}$ +84° (*c* 0.14, MeOH); IR (KBr) ν_{max} 3200 (OH), 3600−2400 (CO*OH*), 1720 (*CO*OH), 1650 (CO) cm^{-1}; UV (MEOH) λ_{max} (log ϵ) 239 (4.09) nm; EIMS *m*/*z* 252 (M^+), 234 ($M^+ - H_2O$), 161 (base peak); HREIMS *m*/*z* 252.1372 (252.1361 for $C_{14}H_{20}O_4$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.80 (1H, brs, H-4), 3.72 (1H, m, H-6), 2.74 (1H, ddd, J = 2.1, 4.6, 13.1 Hz, H-5), 2.48 (1H, m, H-2), 2.40 (1H, m, H-10), 2.31 (1H, ddd, J = 6.8, 8.0, 15.5 Hz, H-10), 2.21 (1H, m, H-5), 2.19 (1H, m, H-8), 2.16 (1H, m, H-7), 2.05 (1H, m, H-8a), 1.91 (1H, m, H-9), 1.78 (1H, m, H-1), 1.66 (1H, m, H-9), 1.50 (1H, m, H-7), 1.23 (1H, m, H-8), 1.03 (3H, d, J = 7.3 Hz, 2-C*H*$_3$); ^{13}C NMR ($CDCl_3$, 125.43 MHz) δ 203.1 (C, C-3), 177.7 (C, C-11), 161.3 (C, C-4a), 123.9 (CH, C-4), 70.5 (CH, C-6), 44.9 (CH_2, C-5), 41.9 (CH, C-1), 41.7 (CH, C-2), 39.5 (CH, C-8a), 34.7 (CH_2, C-7), 31.2 (CH_2, C-10), 28.9 (CH_2, C-8), 23.5 (CH_2, C-9), 11.2 (2-*C*H$_3$).

Eujavanoic acid B (2): colorless microcrystal (benzene−MeOH); mp 167−168 °C; $[\alpha]_D^{24}$ +37.3° (*c* 0.20, MeOH); IR (KBr) ν_{max} 3400 (OH), 3300−2500 (CO*OH*), 1710 (*CO*OH) cm^{-1};

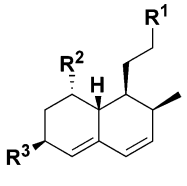
Chart 1



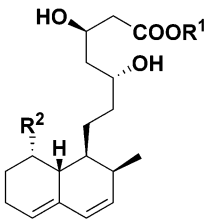
	R^1	R^2	R^3	R^4
1	=O		COOH	OH
6	OH	H	A	H
7	=O		A	H
10	=O		COOMe	OH
15	OH	H	B	H
16	=O		B	H



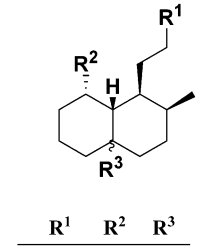
	R^1	R^2	R^3
2	COOH	H	OH
4	A	C	H
11	COOMe	H	OH
13	B	C	H



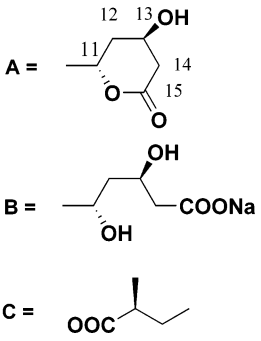
	R^1	R^2	R^3
3	A	C	H
5	A	OH	H
14	B	OH	H



8 R^1 = H R^2 = C
9 R^1 = Et R^2 = C



	R^1	R^2	R^3
12	A	C	αH
17	B	C	αH



UV (MeOH) λ_{max} (log ϵ) end absorption; CI-MS *m/z* 239 (M^+ + H), 221 (base peak, $M^+ + 1 - H_2O$), 203 ($M^+ + 1 - 2H_2O$); HRCIMS *m/z* 239.1642 (239.1647 for $C_{14}H_{23}O_3$); ^{1}H NMR (acetone-d_6, 500 MHz) δ 5.60 (1H, ddd, J = 2.9, 4.7, 9.7 Hz, H-3), 5.33 (1H, d, J = 9.7 Hz, H-4), 3.56 (1H, tt, J = 10.8, 4.4, H-6), 2.37 (1H, ddd, J = 5.3, 9.9, 15.7 Hz, H-10), 2.28 (1H, m, H-2), 2.18 (1H, ddd, J = 6.7, 9.5, 15.7 Hz, H-10), 2.02 (1H, brd, H-7), 1.92 (1H, m, H-5), 1.89 (1H, m, H-9), 1.86 (1H, m, H-8), 1.72 (1H, brt, H-4a), 1.49 (1H, m, H-1), 1.38 (1H, m, H-9), 1.22 (1H, m, H-7), 1.02 (1H, m, H-8), 1.00 (1H, m, H-8a), 0.99 (1H, m, H-5), 0.85 (3H, d, J = 7.0 Hz, 2-CH_3). ^{13}C NMR (acetone-d_6, 125.43 MHz) δ 175.0 (C, C-11), 133.7 (CH, C-3), 131.5 (CH, C-4), 70.7 (CH, C-6), 43.4 (CH_2, C-5), 42.9 (CH, C-4a), 41.8 (CH, C-1), 39.6 (CH, C-8a), 37,1 (CH_2, C-7), 32.9 (CH, C-2), 32.2 (CH_2, C-10), 28.3 (CH_2, C-8), 25.1 (CH_2, C-9), 15.2 (2-CH_3).

Methylation of 1 and 2 with Diazomethane. An excess ethereal solution of diazomethane was added to a solution of eujavanoic acid A (7 mg) or B (8 mg) in CH_2Cl_2 (1 mL), respectively, and the solution allowed to stand for 5 min followed by evaporation to give a colorless solid.

Eujavanoic acid A methyl ester (10): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.79 (1H, brs, H-4), 3.71 (1H, m, H-6), 3.68 (3H, s, COOCH_3), 2.74 (1H, ddd, J = 2.4, 4.6, 13.1 Hz, H-5), 2.45 (1H, m, H-2), 2.37 (1H, m, H-10), 2.26 (1H, m, H-10), 2.17 (3H, m, H-5, H-7, H-8), 2.04 (1H, m, H-8a), 1.91 (1H, m, H-9), 1.75 (1H, m, H-1), 1.64 (1H, m, H-9), 1.50 (1H, m, H-7), 1.21 (1H, m, H-8), 1.02 (3H, d, J = 7.3 Hz, 2-CH_3); ^{13}C NMR ($CDCl_3$, 125.43 MHz) δ 203.1 (C, C-3), 173.6 (C, C-11), 161.3 (C, C-4a), 123.9 (CH, C-4), 70.6 (CH, C-6), 51.7 (CH_3, COOCH_3), 44.9 (CH_2, C-5), 42.0 (CH, C-1), 41.7 (CH, C-2), 39.5 (CH, C-8a), 34.8 (CH_2, C-7), 31.5 (CH_2, C-10), 29.0 (CH_2, C-8), 23.8 (CH_2, C-9), 11.2 (2-CH_3).

Eujavanoic acid B methyl ester (11): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.62 (1H, m, H-3), 5.36 (1H, d, J = 9.8 Hz, H-4), 3.67 (3H, s, COOCH_3), 3.66 (1H, m, H-6), 2.40 (1H, ddd, J = 5.0, 10.1, 15.1 Hz, H-10), 2.25 (1H, m, H-2), 2.20 (1H, m, H-10), 2.09 (1H, brd, H-7), 1.99 (1H, brd, H-5), 1.92 (2H, m, H-8, H-9), 1.75 (1H, brt, H-4a), 1.45 (1H, m, H-1), 1.42 (1H, m, H-9), 1.27 (1H, m, H-7), 1.05 (3H, m, H-5, H-8, H-8a), 0.85 (3H, d, J = 7.0 Hz, 2-CH_3); ^{13}C NMR ($CDCl_3$, 125.43 MHz) δ 174.3 (C, C-11), 132.9 (CH, C-3), 130.1 (CH, C-4), 70.7 (CH, C-6), 51.6 (CH_3, COOCH_3), 42.0 (CH_2, C-5), 41.7 (CH, C-4a), 40.8 (CH, C-1), 38.3 (CH, C-8a), 36.0 (CH_2, C-7), 31.9 (CH, C-2), 31.9 (CH_2, C-10), 27.3 (CH_2, C-8), 24.2 (CH_2, C-9), 14.8 (2-CH_3).

Synthesis of (*S*)-and (*R*)-MTPA Esters of 10. Dicyclohexylcarbodiimide (16 mg), 4-(dimethylamino)pyridine (6 mg), and (*S*)- or (*R*)-MTPA (16 mg) were added to a solution of eujavanoic acid A methyl ester (**10**) (3.5 mg) in CH_2Cl_2 (3 mL). The reaction mixture was kept at 40 °C for 1.5 h and then washed with 0.5 M HCl, saturated $NaHCO_3$, and water, successively, and dried over Na_2SO_4. After removal of the solvent by evaporation, the residue was purified by HPLC (silica gel) (CH_2Cl_2−EtOAc 20:1) to afford the (*R*)- or (*S*)-MTPA ester of **10** [4.5 mg for (*S*), 4.6 mg for (*R*)].

(*R*)-MTPA ester of 10: ^{1}H NMR ($CDCl_3$, 500 MHz; other than phenyl signals) δ 5.83 (1H, brs, H-4), 5.01 (1H, tt, J = 4.6, 11.3 Hz, H-6), 3.68 (3H, s, COOCH_3), 2.80 (1H, ddd, J = 2.1, 4.7, 13.3 Hz, H-5), 2.47 (1H, dq, J = 4.3, 7.2 Hz, H-2), 2.37 (1H, ddd, J = 6.2, 9.5, 15.7 Hz, H-10), 2.28 (1H, m, H-5), 2.26 (2H, m, H-7, H-10), 2.25 (1H, m, H-8), 2.07 (1H, m, H-8a), 1.90 (1H, m, H-9), 1.77 (1H, m, H-1), 1.69 (1H, m, H-7), 1.62 (1H, m, H-9), 1.30 (1H, m, H-8), 1.01 (3H, d, J = 7.2 Hz, 2-CH_3).

(*S*)-MTPA ester of 10: ^{1}H NMR ($CDCl_3$, 500 MHz; other than phenyl signals) δ 5.84 (1H, brs, H-4), 5.01 (1H, tt, J = 4.6, 11.3 Hz, H-6), 3.67 (3H, s, COOCH_3), 2.85 (1H, ddd, J = 2.3, 4.9, 13.1 Hz, H-5), 2.47 (1H, dq, J = 4.4, 7.1 Hz, H-2), 2.39 (1H, m, H-5), 2.37 (1H, m, H-10), 2.28 (1H, m, H-10), 2.24 (2H, m, H-7, H-8), 2.06 (1H, m, H-8a), 1.90 (1H, m, H-9), 1.78 (1H, dq, J = 4.4, 13.1 Hz, H-1), 1.62 (1H, m, H-9), 1.61 (1H, m, H-7), 1.29 (1H, m, H-8), 1.01 (3H, d, J = 7.1 Hz, 2-CH_3).

Synthesis of (*S*)- and (*R*)-MTPA Ester of 11. Dicyclohexylcarbodiimide (12 mg), 4-(dimethylamino)pyridine (4 mg), and (*S*)- or (*R*)-MTPA (12 mg) were added to a solution of eujavanoic acid B methyl ester (**11**) (3.0 mg) in CH_2Cl_2 (2 mL). The reaction mixture was kept at 40 °C for 1.5 h and then washed with 0.5 M HCl, saturated $NaHCO_3$, and water, successively, and dried over Na_2SO_4. After removal of the solvent by evaporation, the residue was purified by normal-phase HPLC (benzene) to afford the (*R*)- or (*S*)-MTPA ester of **11** [2.0 mg for (*S*), 2.1 mg for (*R*)].

(*R*)-MTPA ester of 11: ^{1}H NMR ($CDCl_3$, 500 MHz; other than phenyl signals) δ 5.62 (1H, ddd, J = 2.7, 4.9, 9.9 Hz, H-3), 5.32 (1H, d, J = 9.9 Hz, H-4), 5.03 (1H, tt, J = 4.6, 11.3 Hz,

H-6), 3.68 (3H, s, COOCH_3), 2.40 (1H, ddd, J = 5.2, 10.4, 15.6 Hz, H-10), 2.26 (1H, m, H-2), 2.20 (1H, m, H-10), 2.18 (1H, m, H-7), 2.05 (1H, m, H-5), 1.96 (1H, m, H-8), 1.92 (1H, m, H-9), 1.85 (1H, m, H-4a), 1.50 (1H, m, H-1), 1.46 (1H, m, H-7), 1.40 (1H, m, H-9), 1.22 (1H, m, H-5), 1.10 (1H, m, H-8), 1.09 (1H, m, H-8a), 0.84 (3H, d, J = 7.0 Hz, 2-CH_3).

(*S*)-MTPA ester of 11: ^{1}H NMR ($CDCl_3$, 500 MHz; other than phenyl signals) δ 5.64 (1H, ddd, J = 2.7, 4.9, 9.9 Hz, H-3), 5.34 (1H, d, J = 9.9 Hz, H-4), 5.03 (1H, tt, J = 4.6, 11.3 Hz, H-6), 3.67 (3H, s, COOCH_3), 2.40 (1H, ddd, J = 6.4, 10.2, 15.7 Hz, H-10), 2.26 (1H, m, 2-H), 2.20 (1H, ddd, J = 6.1, 9.9, 15.7 Hz, H-10), 2.12 (2H, m, H-5, H-7), 1.93 (1H, m, H-8), 1.91 (1H, m, H-9), 1.86 (1H, m, H-4a), 1.48 (1H, m, H-1), 1.39 (2H, m, H-7, H-9), 1.29 (1H, m, H-5), 1.09 (1H, m, H-8), 1.08 (1H, m, H-8a), 0.84 (3H, d, J = 7.0 Hz, 2-CH_3).

3,5-Dihydro-3-oxo-ML-236C(7): colorless amorphous powder; UV (MeOH) λ_{max} (log ϵ) 239 nm (4.08); HREIMS m/z 306.1809 (306.1831 for $C_{18}H_{26}O_4$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.74 (1H, brs, H-4), 4.39 (1H, m, H-13), 2.72 (1H, dd, J = 4.9, 17.7 Hz, H-14), 2.63 (1H, ddd, J = 1.7, 3.5, 17.7 Hz, H-14), 2.48 (1H, dd, J = 4.8, 7.3 Hz, H-2), 2.44 (1H, m, H-5), 2.16 (2H, m, H-5, H-8), 2.09 (1H, m, H-8a), 1.94 (2H, m, H-6, H-12), 1.89 (1H, m, H-7), 1.77 (1H, m, H-1), 1.74 (1H, m, H-12), 1.71 (1H, m, H-10), 1.62 (1H, m, H-9), 1.53 (2H, m, H-9, H-10), 1.49 (1H, tq, J = 3.4, 13.1 Hz, H-7), 1.38 (1H,tq, 3.5, J = 13.0 Hz, H-6), 1.21 (1H, dq, J = 3.4, 12.5 Hz, H-8), 1.02 (3H, d, J = 7.3 Hz, 2-CH_3); ^{13}C NMR ($CDCl_3$, 125.43 MHz) δ 203.9 (C, C-3), 170.4 (C, C-15), 166.0 (C, C-4a), 122.2 (CH, C-4), 77.5 (CH, C-11), 62.7 (CH, C-13), 42.8 (CH, C-1), 41.9 (CH, C-2), 41.0 (CH, C-8a), 38.6 (CH_2, C-14), 36.1 (CH_2, C-5), 36.0 (CH_2, C-12), 33.0 (CH_2, C-8), 32.7 (CH_2, C-10), 27.5 (CH_2, C-6), 25.9 (CH_2, C-7), 23.7 (CH_2, C-9), 11.4 (2-CH_3).

Oxidation of 3,5-Dihydro-3α-hydroxy-ML-236C(6) with MnO_2. MnO_2 (100 mg) was added to a stirred solution of 3,5-dihydro-3α-hydroxy-ML-236C (**6**)[5] (20 mg) in CH_2Cl_2 (3 mL). After 2 h, MnO_2 was filtered off and the solvent was evaporated. The residue was subjected to LPLC, eluting with benzene−EtOAc−EtOH (5:1:0.3), to give a ketone (5 mg) and starting material (12 mg). This ketone derivative was identical to 3,5-dihydro-3-oxo-ML-236C (**7**) on the basis of spectroscopic data.

Acid form (8) of compactin (3): colorless amorphous powder; EIMS m/z 408 (M^+); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 5.92 (1H, d, J = 9.7 Hz, H-4), 5.73 (1H, dd, J = 9.7, 6.1 Hz, H-3), 5.51 (1H, brs, H-5), 5.16 (1H, brs, H-8), 3.95 (1H, m, H-13), 3.47 (1H, m, H-11), 2.31 (4H, m, H-2, H-8a, H-14, H-2′), 2.20 (1H, dd, J = 15.0, 8.2 Hz, H-14), 2.07 (2H, m, H-6), 2.0 (1H, m, H-7), 1.66 (1H, m, H-7), 1.55 (2H, m, H-1, H-3′), 1.47 (1H, m, H-12), 1.40 (1H, m, H-12), 1.36 (3H, m, H-3′, H-9, H-10), 1.27 (1H, m, H-9), 1.09 (1H, m, H-10), 1.04 (1H, d, J = 7.0 Hz, 2′-CH_3), 0.84 (3H, d, J = 7.0 Hz, 2-CH_3), 0.82 (1H, t, J = 7.6 Hz, H-4′); ^{13}C NMR (DMSO-d_6, 125.43 MHz) δ 175.3 (C, C-1′), 172.8 (C, C-15), 133.6 (C, C-4a), 132.8 (CH, C-3), 127.9 (CH, C-4), 123.0 (CH, C-5), 68.5 (CH, C-11), 67.0 (CH, C-8), 65.8 (CH, C-13), 44.4 (CH_2, C-12), 42.4 (CH_2, C-14), 40.7 ((CH, C-2′), 36.7 (CH, C-8a), 36.4 (CH, C-1), 34.3 (CH_2, C-10), 30.3 (CH, C-2), 26.1 (CH_2, C-3′), 25.4 (CH_2, C-7), 23.8 (CH_2, C-9), 20.3 (CH_2, C-6), 16.6 (2′-CH_3), 13.5 (2-CH_3), 11.3 (CH_3, C-4′).

Hydrogenation of 4. 10% Pd−C (20 mg) was suspended in a solution of dihydrocompactin (**4**) (20 mg) in MeOH (4 mL) and the mixture stirred at room temperature in a hydrogen atmosphere for 2 h. The catalyst was filtered off and the solvent evaporated in vacuo. The residue was purified by HPLC on silica gel with CH_2Cl_2−acetone (10:1) to give 4a-α-tetrahydrocompactin (**12**) (7.5 mg).

4a-α-Tetrahydrocompactin (12): colorless amorphous powder; $[\alpha]_D^{24}$ +113° (c 0.34); EIMS m/z 394 (M, $C_{23}H_{38}O_5$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.17 (1H, brs, H-8), 4.58 (1H, m, H-11), 4.35 (1H, m, H-13), 2.72 (1H, dd, J = 5.2, 17.7 Hz, H-14), 2.61 (1H, ddd, J = 1.4, 3.9, 17.7 Hz, H-14), 2.38 (1H, m, H-2′), 1.95 (2H, m, H-2, H-12), 1.93 (1H, m, H-7), 1.84 (1H, m, H-10), 1.67 (2H, m, H-12, H-3′), 1.55 (3H, m, H-3, H-5, H-5), 1.49 (1H, m, H-6), 1.44 (2H, m, H-9, H-3′), 1.43 (1H, m, H-1), 1.38 (1H, m, H-4), 1.36 (1H, m, H-6), 1.34 (1H, m, H-7), 1.32 (1H, m, H-4a), 1.19 (1H, m, H-9), 1.18 (1H, m, H-10), 1.17 (1H, m, H-4), 1.15 (3H, d, J = 6.7 Hz, 2′-CH_3), 1.12 (1H, dt, J = 2.1, 11.0 Hz, H-8a), 1.02 (1H, m, H-3), 0.91 (3H, t, J = 7.5 Hz, H-4′), 0.82 (3H, d, J = 7.0 Hz, 2-CH_3); ^{13}C NMR (DMSO-d_6, 125.43 MHz) δ 176.4 (C, C-1′), 170.5 (C, C-15), 76.5 (CH, C-11), 68.9 (CH, C-8), 62.7 (CH, C-13), 43.9 (CH, C-8a), 41.9 (CH, C-2′), 40.0 (CH, C-4a), 38.6 (CH_2, C-14), 37.0 (CH, C-1), 36.2 (CH_2, C-12), 33.7 (CH_2, C-3), 33.1 (CH_2, C-10), 32.7 (CH_2, C-5), 30.9 (CH_2, C-7), 29.0 (CH, C-2), 28.3 (CH_2, C-4), 26.8 (CH_2, C-3′), 24.5 (CH_2, C-9), 20.4 (CH_2, C-6), 17.0 (2′-CH_3), 11.9 (2-CH_3), 11.8 (CH_3, C-4′).

Hydrolysis of Compactin-Related Compounds. A solution of compounds **4**, **5**, **6**, **7**, and **12** in DMF (1 mL) was added separately to 5% NaOH (3 mL) and kept at 60 °C for 2 h. After concentration, the residue was separated by chromatography on a column of HP20 resin (DIAION) (resin volume, 10 mL in H_2O) into four fractions (each 20 mL): H_2O, 30% MeOH, 50% MeOH, 70% MeOH, and 100% MeOH. Each hydrolysate was eluted with 70% MeOH.

Sodium salt (13) of 4: ^{1}H NMR (CD_3OD, 270 MHz) δ 0.76 (3H, d, J = 7.1 Hz, 2-CH_3), 0.83 (3H,t, J = 7.4 Hz, H-4′), 1.03 (3H, d, J = 6.9 Hz, 2′-CH_3), 0.8−1.7 (15H, m), 1.9 (1H, m), 2.1−2.4 (4H, m), 3.6 (1H, m), 4.0 (1H, m), 5.06 (1H, m, 8-H), 5.29 (1H, d, J = 9.8 Hz, H-4), 5.5 (1H, m, H-3).

Sodium salt (14) of 5: ^{1}H NMR (CD_3OD, 270 MHz) δ 0.80 (3H, d, J = 7.1 Hz, 2-CH_3), 1.0−2.4 (15H, m), 3.7 (1H, m), 4.01 (1H, m), 4.13 (1H, m, H-8), 5.37 (1H, m, H-5), 5.60 (1H, dd, J = 5.9, 9.7 Hz, H-3), 5.81 (1H, d, J = 9.6 Hz, H-4).

Sodium salt (15) of 6: ^{1}H NMR (CD_3OD, 270 MHz) δ 0.70 (3H, d, J = 6.9 Hz, 2-CH_3), 0.8−2.4 (19H, m), 3.67 (2H, m), 4.00 (1H, m), 5.28 (1H, m, 4-H).

Sodium salt (16) of 7: ^{1}H NMR (CD_3OD, 270 MHz) δ 0.91 (3H, d, J = 7.25 Hz, 2-CH_3), 1.1−2.6 (19H, m), 3.65 (1H, m), 3.90 (1H, m), 5.61 (1H, m, 4-H).

Sodium salt (17) of 12: ^{1}H NMR (CD_3OD, 270 MHz) δ 0.75 (3H, d, J = 7.1 Hz, 2-CH_3), 0.83 (3H,t, J = 7.4 Hz, H-4′), 1.06 (3H, d, J = 6.9 Hz, 2′-CH_3), 0.8−1.7 (17H, m), 1.8−2.0 (2H, m), 2.1−2.4 (3H, m), 3.59 (1H, m), 3.98 (1H, m), 5.04 (1H, m, H-8).

Antifungal Assay. The antifungal assay was performed by the same method as in the previous paper, except for the test culture medium (potato dextrose agar) and the recorded time (after 48 h incubation).[7]

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