Eujavanicols A–C, Decalin Derivatives from *Eupenicillium javanicum*

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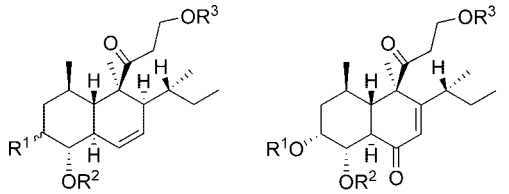
Three new decalin derivatives, eujavanicols A–C (**1**–**3**), were isolated from an extract of *Eupenicillium javanicum* IFM 54704. Their structures were determined by chemical and spectroscopic methods.

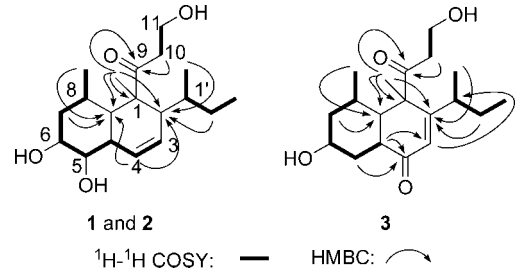
Our group has been searching for fungal metabolites that show antifungal activity against the pathogenic filamentous fungi *Aspergillus fumigatus* and *Aspergillus niger* and/or the pathogenic yeasts *Candida albicans* and *Cryptococcus neoformans*. During previous research for antifungal substances from fungal source,[1] it was found that an organic extract of *Eupenicillium javanicum* IFM 54704 showed characteristic and potent antifungal activity against *A. fumigatus*. Fractionation of this extract has led to the isolation of three new decalin derivatives, designated eujavanicols A (**1**), B (**2**), and C (**3**), along with mevalonolactone.[2] We report herein the isolation and structure determination of eujavanicols A (**1**), B (**2**), and C (**3**).

The molecular formulas of both **1** and **2** were confirmed as $C_{19}H_{32}O_4$ by HRFABMS. The ^{13}C NMR spectra of **1** and **2** revealed the presence of three sp^2 carbons containing one ketone carbon. Hence, **1** and **2** could be assigned as bicyclic. Acetylation of **1** provided a triacetyl derivative (**4**), indicating the presence of three hydroxyl groups.



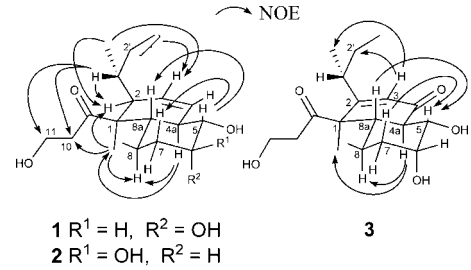
1 R^1 = α-OH, R^2 = R^3 = H
2 R^1 = β-OH, R^2 = R^3 = H
3 R^1 = R^2 = R^3 = H
7 R^1 = R^2 = R^3 = COPhBr
4 R^1 = α-OAc, R^2 = R^3 = Ac
5 R^1 = α-OCOPhBr, R^2 = R^3 = COPhBr
6 R^1 = β-OCOPhBr, R^2 = R^3 = COPhBr
9 R^1 = α-OCOCH=C(Me)CH_2CO_2H, R^2 = R^3 = H

The structural fragments of **1** and **2** shown by bold lines in Figure 1 were established from their ^1H–^1H COSY and HMQCNMR spectra. The HMBC correlations of the olefinic proton (δ_H 6.00 in **1**; δ_H 6.03 in **2**) with C-2 (δ_C 52.4 in **1** and **2**) and C-8a (δ_C 43.1 in **1**; δ_C 42.7 in **2**), of the singlet methyl protons (δ_H 1.25 in **1**; δ_H 1.22 in **2**) with C-1 (δ_C 52.6 in **1**; δ_C 52.5 in **2**), C-2 and C-8a, and of the C-8 methyl protons (δ_H 0.60 in **1**; δ_H 0.65 in **2**) with C-8a suggested that **1** and **2** are decalin derivatives. Further correlations of the methyl protons at C-1′ (δ_H 0.93 in **1** and **2**) with C-2, and of the singlet methyl protons (δ_H 1.25 in **1**; δ_H 1.22 in **2**) and two protons at C-10 (δ_H 2.66 and 2.86 in **1**; δ_H 2.67 and 2.85 in **2**)



1 and **2** **3**
^1H-^1H COSY: HMBC:

Figure 1. H–H COSY and HABCNMR correlation of eujavanicols A (**1**), B (**2**), and C (**3**).



1 R^1 = H, R^2 = OH **3**
2 R^1 = OH, R^2 = H

Figure 2. Difference NOE interactions for eujavanicols A (**1**), B (**2**), and C (**3**).

with the aliphatic ketone (δ_C 215.7 in **1**; δ_H 215.4 in **2**), indicated that **1** and **2** are decalin derivatives having a 3-hydroxypropionyl group at C-1 and a 1-methylpropyl group at C-2, as shown in Figure 1. This conclusion was supported by other HMBC correlation peaks also indicated in this figure.

The relative configuration of eujavanicols A (**1**) and B (**2**) was established by detailed analysis of their respective ^1H NMR spectrum and by difference NOE experiments (Figure 2). In both cases, when the signal for H-5 (δ_H 3.44 in **1**; δ_H 3.22 in **2**) was irradiated, enhancements of the upfield proton at C-7 (δ_H 1.52 in **1**; δ_H 1.40 in **2**) and H-8a (δ_H 1.93 in **1**; δ_H 2.05 in **2**) were observed. Irradiation of the H-4a proton signal (δ_H 2.14 in **1**; δ_H 1.74 in **2**) caused enhancements of the C-1 methyl signal (δ_H 1.25 in **1**; δ_H 1.22 in **2**) and H-8 (δ_H 1.73 in **1**; δ_H 1.43 in **2**). Furthermore, irradiation of the C-1 methyl signal resulted in the enhancement of H-2 (δ_H 1.94 in **1** and **2**), along with enhancements of H-8 and H-10 (δ_H 2.86 in **1** and δ_H 2.85 in **2**). These results indicated that the methyl at C-8, the 1-methylpropyl at C-2, and the 3-hydroxypropionyl at C-1 in both **1** and **2** are on the same side as H-5. A difference between **1** and **2** in the ^1H NMR spectrum was observed in terms of the coupling patterns at H-5 (δ_H 3.44, br d, J = 10.7 Hz in **1**; δ_H 3.22, dd, J = 10.7 and 8.5 Hz in **2**). Therefore, the relationship of the diol in the cyclohexane ring of **1** is cis, and **2** is

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trans. The only uncertainty remaining to be solved was the configuration of C-1′ in the 1-methylpropyl substituent at C-2. Irradiation of the signal for H-1′ (δ_H 1.13 in **1**; δ_H 1.12 in **2**) caused enhancement of H-2, along with enhancements of H-10 and H-11 (δ_H 3.84 and 3.90 in **1**, and δ_H 3.84 and 3.91 in **2**). Irradiation of the signal for Me-1′ (δ_H 0.93 in **1** and **2**) caused enhancements of H-2 and H-3 (δ_H 5.71 in **1** and **2**). In turn, irradiation of the methyl signal for C-3′ (δ_H 0.76 in **1** and **2**) resulted in the enhancement of H-3. Consequently, the relative configuration of **1** and **2** was assigned as depicted in structures **1** and **2**.

In order to confirm the absolute configuration by the exiton chirality method,[3] eujavanicols A (**1**) and B (**2**) were derivatized as their respective tri-*p*-bromobenzoate, **5** and **6**, respectively. The CD spectrum of **5**, derived from **1**, exhibited strong negative exiton chirality [$\Delta\varepsilon$ −49.0 (253 nm); $\Delta\varepsilon$ +15.6 (234 nm)], whereas that of **6** exhibited a positive exiton chirality [$\Delta\varepsilon$ +20.5 (254 nm); $\Delta\varepsilon$ −15.4 (238 nm)]. From the result, the chiralities of C-5 and C-6 in **5** and **6** were found to be 5*S* and 6*R,* and 5*S* and 6*S*, respectively. The absolute structures of eujavanicols A and B were thus determined as depicted in **1** and **2**, respectively.

The molecular formula of eujavanicol C (**3**) was confirmed as $C_{19}H_{30}O_5$ by HRFABMS. The IR absorption regions at 1703 and 1646 cm^{-1} and ^{13}C NMR signals at δ_C 203.2 and 213.4 indicated the presence of both an aliphatic ketone and a conjugated ketone in **3**. The ^{13}C NMR spectrum revealed the presence of four sp^2 carbons containing two ketone carbons. Hence, **3** was also assigned as a bicyclic compound.

The structural fragments of **3** shown by bold lines in Figure 1 were established from the ^{1}H–^{1}H COSY and HMQC spectra. All signals in the ^{1}H NMR spectrum of **3** displayed broad peaks. The HMBC correlations of H-5 (δ_H 3.88) with the carbon of the conjugated ketone (δ_C 203.2), the C-8 methyl protons (δ_H 0.77) with C-8a (δ_C 45.9), and the C-1 methyl protons (δ_H 1.37) with the quaternary carbon at C-1 (δ_C 59.4), C-2 (δ_C 173.6), and C-8a suggested that **3** is also a decalin derivative. Further correlations of the C-1′ methyl protons (δ_H 1.08) with C-2 and the C-1 methyl protons (δ_H 1.37) and two protons at C-10 (δ_H 2.57 and 2.88) with the aliphatic ketone (δ_C 213.4) indicated that **3** has a 3-hydroxypropionyl group at C-1 and a 1-methylpropyl group at C-2, as shown in Figure 1.

The relative stereochemistry of **3** was established by difference NOE experiments in acetone-d_6 as solvent (Figure 2) and detailed analysis of the ^{1}H NMR spectrum in $CDCl_3$. When the signal for H-8a (δ_H 2.29) or the upfield proton at C-7 (δ_H 1.30) was irradiated, enhancement of H-5 (δ_H 3.68) was observed. Irradiation of H-4a (δ_H 2.45) caused enhancements of the C-1 methyl group (δ_H 1.22) and H-8 (δ_H 1.99). From the above results, and the coupling pattern at H-5 (δ_H 3.88, dd, J = 9.4 and 2.0 Hz) in $CDCl_3$, the relative configuration of **3** was assigned as being the same as **1**, except for the stereochemistry of C-1′.

In order to confirm the absolute configuration by the exiton chirality method, **3** was derivatized to the tri-*p*-bromobenzoate (**7**) followed by the reduction with $NaBH_4$, to give one main product, **8**. The ^{1}H NMR spectrum revealed that **8** is a dihydro compound reduced only at C-4. The CD spectrum of **8**, derived from **7**, exhibited negative exiton chirality [$\Delta\varepsilon$ −30.0 (256 nm); $\Delta\varepsilon$ +14.2 (239 nm)]. From this result, the chiralities of C-5 and C-6 in **3** were found to be 5*S* and 6*R*, respectively. The stereochemistry of C-1′ in **3** was not determined, but it may be assumed to be as shown in the structure **3** because of its co-occurrence with **1**, for biogenetic reasons.

Eujavanicol A (**1**) is a hydrolysis product of trichoharzin (**9**),[4] isolated from *Trichoderma harzianum*. Previously, we found that an organic extract of *E. javanicum* IFM 52670 showed characteristic and strong antifungal activity against *A. fumigatus* and this active substance was the acyclic form of the lactone in compactin.[5] It was found that an organic extract of *E. javanicum* of the other strain (IFM 54704) showed potent antifungal activity against *A. fumigatus*. Compactin and the acyclic form of the lactone in compactin were not found in the organic extract of *E. javanicum* (IFM 54704). Eujavanicols A (**1**), B (**2**), and C (**3**) were isolated from the antifungal-active fraction, instead of compactin derivatives, but **1**–**3** showed no antifungal activity. Further investigation of the active fraction led to the isolation of a new cyclic depsipeptide having antifungal activity. The structure of this active principle is now under investigation.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 photopolarimeter. The UV and IR spectra were recorded on a JASCO V-560 and a JASCO FT/IR-4100 spectrophotometer, respectively. CD curves were recorded on a JASCO J-820 spectropolarimeter.^{1}H and ^{13}C NMR spectra were recorded on a JNM ECA-500 (^{1}H, 500.16 MHz; ^{13}C, 125.77 MHz) and JNM AL-300 (^{1}H, 300.40 MHz; ^{13}C, 75.45 MHz) spectrometer, using $CDCl_3$ solutions containing tetramethylsilane as an internal standard. EIMS and FABMS were taken with a JEOL-MS600W spectrometer.

Fungal Material. The examined strain was isolated from a cultivated soil in Chiba, Japan, identified as *Eupenicillium javanicum* based on morphology (by T. Yaguchi), and deposited at the Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, under the accession number IFM 54704. The strain IFM 54704 was cultured at 25 °C for 21 days in 20 Roux flasks containing 160 g of moist rice in each flask.

Extraction and Isolation. The fermented rice was extracted with CH_2Cl_2–MeOH (3:2), and the organic layer was evaporated in vacuo. The resultant extract was suspended in H_2O and extracted with EtOAc, and then, the organic layer was evaporated in vacuo. The EtOAc extract (46 g), which showed strong antifungal activity against *A. fumigatus*, was separated by column chromatography on silica gel (800 g) into six fractions eluted with CH_2Cl_2 (19.3 g), CH_2Cl_2–EtOH (20:1) (5.9 g), CH_2Cl_2–EtOH (10:1) (13.3 g), CH_2Cl_2–EtOH (5:1) (1.9 g), and EtOH (3.8 g). The third fraction [CH_2Cl_2–EtOH (10:1)] was purified by low-pressure liquid chromatography (LPLC) on a silica gel column using cyclohexane–acetone (3:1) followed by further purification by high-pressure liquid chromatography (HPLC) on a silica gel column eluting with cyclohexane–acetone (2:1) to give eujavanicol A (**1**) (340 mg), with benzene–acetone (5:2) to give eujavanicol B (**2**) (8.6 mg), and with $CHCl_3$–acetone (3:1) to give eujavanicol C (**3**) (9.1 mg) and mevalonolactone[2] (6 mg), in order.

Eujavanicol A (1): colorless amorphous solid; $[\alpha]_D^{22}$ +49.9 (*c* 1.33, MeOH); UV (MeOH) λ_{max} (log ε), end absorption; IR ν_{max} 3355 (OH), 1697 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.00 (1H, dt, J = 10.7, 2.0 Hz, H-4), 5.71 (1H, ddd, J = 10.7, 4.6, 2.7 Hz, H-3), 4.04 (1H, q, J = 3.1 Hz, H-6), 3.90 (1H, m, H-11), 3.84 (1H, m, H-11), 3.44 (1H, br d, J = 10.7 Hz, H-5), 2.86 (1H, ddd, J = 18.9, 7.3, 3.9 Hz, H-10), 2.66 (1H, ddd, J = 18.9, 6.1, 3.7 Hz, H-10), 2.14 (1H, tq, J = 10.4, 2.4 Hz, H-4a), 1.94 (1H, m, H-2), 1.93 (1H, t, J = 10.4 Hz, H-8a), 1.85 (1H, dt, J = 14.6, 2.9 Hz, H-7eq), 1.73 (1H, m, H-8), 1.52 (1H, ddd, J = 14.6, 12.1, 2.6 Hz, H-7ax), 1.47 (1H, m, H-2′), 1.25 (3H, s, Me-1), 1.13 (1H, m, H-1′), 0.93 (3H, d, J = 6.7 Hz, Me-1′), 0.76 (3H, m, H-3′), 0.76 (1H, m, H-2′), 0.60 (3H, d, J = 7.0 Hz, Me-8); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 215.7 (C-9), 126.1 (C-4), 124.0 (C-3), 75.3 (C-5), 69.7 (C-6), 58.1 (C-11), 52.6 (C-1), 52.4 (C-2), 43.1 (C-8a), 41.3 (C-7), 41.2 (C-10), 39.1 (C-4a), 37.2 (C-1′), 30.6 (C-8), 24.5 (C-2′), 22.4 (Me-8), 19.4 (Me-1), 19.3 (Me-1′), 12.6 (C-3′); HRFABMS *m/z* 325.2375 [M + H]$^+$ (calcd for $C_{19}H_{33}O_4$, 325.2379).

Eujavanicol B (2): colorless amorphous solid; $[\alpha]_D^{23}$ +28.0 (*c* 0.33, MeOH); UV (MeOH) λ_{max} (log ε), end absorption; IR ν_{max} 3356 (OH), 1698 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.03 (1H, dt, J = 10.5, 1.9 Hz, H-4), 5.71 (1H, ddd, J = 10.7, 4.8, 2.7 Hz, H-3), 3.91 (1H, m, H-11), 3.84 (1H, m, H-11), 3.55 (1H, m, H-6), 3.22 (1H, dd, J = 10.7, 8.5 Hz, H-5), 2.85 (1H, ddd, J = 18.9, 7.4, 3.9 Hz, H-10), 2.67 (1H, ddd, J = 18.9, 6.1, 3.5 Hz, H-10), 2.05 (1H, t, J = 9.9 Hz, H-8a), 1.94 (1H, m, H-2), 1.94 (1H, m, H-7eq), 1.74 (1H, m, H-4a), 1.46 (1H, m, H-2′), 1.43 (1H, m, H-8), 1.40 (1H, m, H-7ax), 1.22 (3H, s, Me-1), 1.12 (1H, m, H-1′), 0.93 (3H, d, J = 7.0 Hz, Me-1′), 0.76 (3H, m, H-3′),0.76 (1H, m, H-2′), 0.65 (3H, d, J = 6.4 Hz, Me-8); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 215.4 (C-9), 125.2 (C-4), 124.0 (C-3), 78.7 (C-5), 75.7 (C-6), 58.0 (C-11), 52.5 (C-1), 52.4 (C-2), 42.7 (C-8a), 42.7 (C-4a), 42.3 (C-7), 41.2 (C-10), 37.2 (C-1′), 35.1 (C-8), 24.4 (C-

2′), 22.5 (Me-8), 19.3 (Me-1), 19.2 (Me-1′), 12.5 (C-3′); HRFABMS *m/z* 325.2345 $[M + H]^+$ (calcd for $C_{19}H_{33}O_4$, 325.2379).

Eujavanicol C (3): colorless amorphous solid; $[\alpha]_D^{23}$ +52.3 (*c* 0.37, MeOH); UV (MeOH) λ_{max} (log ε), 244 (4.01) nm; IR ν_{max} 3427 (OH), 1703 (CO), 1646 (CO) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.08 (1H, br s, H-3), 4.00 (1H, m, H-6), 3.88 (1H, dd, J = 9.4, 2.0 Hz, H-5), 3.83 (2H, br s, H-11), 2.88 (1H, m, H-10), 2.57 (1H, m, H-10), 2.57 (1H, m, H-4a), 2.17 (1H, m, H-8), 2.16 (1H, m, H-8a), 1.91 (1H, br d, H-7eq), 1.74 (1H, m, H-1′), 1.37 (3H, br s, Me-1), 1.37 (2H, m, 2′-H), 1.30 (1H, br t, H-7ax), 1.08 (3H, d, J = 6.3 Hz, Me-1′), 0.89 (3H, m, H-3′), 0.77 (3H, br s, Me-8); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 213.4 (C-9), 203.2 (C-4), 173.6 (C-2), 126.0 (C-3), 72.3 (C-5), 67.8 (C-6), 59.4 (C-1), 57.9 (C-11), 45.9 (C-8a), 44.7 (C-4a), 41.7 (C-10), 38.8 (C-7), 37.0 (C-1′), 30.3 (C-2′), 27.6 (C-8), 22.8 (Me-1′), 18.9 (Me-8), 14.6 (Me-1), 12.2 (C-3′); ^1H NMR (acetone-d_6, 500 MHz) δ 5.94 (1H, br s, H-3), 3.73 (1H, m, H-6), 3.68 (1H, m, H-5), 3.64 (2H, m, H-11), 2.85 (1H, m, H-10), 2.56 (1H, m, H-10), 2.45 (1H, dd, J = 12.9. 9.5 Hz, H-4a), 2.29 (1H, m, H-8a), 1.99 (1H, m, H-8), 1.62 (1H, dt, J = 13.8, 3.4 Hz, H-7eq), 1.57 (1H, m, H-1′), 1.36 (2H, m, H-2′), 1.30 (1H, m, H-7ax), 1.22 (3H, br t, Me-1), 0.97 (3H, d, J = 6.3 Hz, Me-1′), 0.75 (3H, m, H-3′), 0.63 (3H, br d, Me-8); ^{13}C NMR (acetone-d_6, 125 MHz) δ 212.0 (C-9), 204.1 (C-4), 174.1 (C-2), 126.3 (C-3), 73.2 (C-6), 68.5 (C-5), 60.1 (C-1), 57.7 (C-11), 45.7 (C-8a), 45.6 (C-4a) [the preceding two assignments may be reversed], 43.2 (C-10), 40.1 (C-7), 37.6 (C-1′), 30.7 (C-2′), 28.5 (C-8), 22.9 (Me-1′), 19.1 (Me-8), 14.8 (Me-1), 12.5 (C-3′); HRFABMS *m/z* 339.2176 $[M + H]^+$ (calcd for $C_{19}H_{31}O_5$, 339.2171).

Acetylation of 1. Eujavano1 A (**1**, 88 mg) was dissolved in pyridine (1 mL) containing acetic anhydride (1 mL) and kept overnight at rt. The mixture was poured into ice water and extracted with ether. The crude product was purified by LPLC on a silica gel column, eluting with benzene–acetone (20:1) to give triacetyleujavanicol (**4**, 40 mg).

Triacetate (4) of 1: ^1H NMR ($CDCl_3$, 500 MHz) δ 5.70 (1H, ddd, J = 10.6, 4.0, 2.9 Hz, H-3), 5.65 (1H, br d, H-4), 5.39 (1H, q, J = 2.9 Hz, H-6), 4.70 (1H, dd, J = 11.7, 2.9 Hz, H-5), 4.37 (2H, m, H-11), 2.91 (1H, dt, J = 18.9, 5.7 Hz, H-10), 2.82 (1H, dt, J = 18.9, 6.3 Hz, H-10), 2.36 (1H, br t, H-4a), 2.10 (3H, s, Me), 2.09 (1H, m, H-8a), 2.04 (3H, s, Me), 2.02 (3H, s, Me), 1.96 (1H, m, H-2), 1.75 (1H, dt, J = 13.7, 2.9 Hz, H-7eq), 1.70 (1H, m, H-8), 1.60 (1H, ddd, J = 13.7, 11.5, 2.9 Hz, H-7ax), 1.46 (1H, m, H-2′), 1.28 (3H, br s, Me-1), 1.15 (1H, m, H-1′), 0.92 (3H, d, J = 6.9 Hz, Me-1′), 0.75 (3H, m, H-3′), 0.72 (1H, m, H-2′), 0.59 (3H, d, J = 6.9 Hz, Me-8); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 209.9 (C-9), 170.8, 170.3, 170.3, 124.6 (C-3), 124.4 (C-4) [the preceding two assignments may be reversed], 75.3 (C-5), 69.0 (C-6), 59.0 (C-11), 52.1 (C-1), 52.0 (C-2), 43.3 (C-8a), 38.9 (C-7), 38.0 (C-10), 37.0 (C-4a), 36.7 (C-1′), 31.3 (C-8), 24.3 (C-2′), 21.9 (Me-8), 21.1, 20.8, 20.7, 19.1 (Me-1′), 19.0 (Me-1) [the preceding two assignments may be reversed], 12.3 (C-3′); HRFABMS *m/z* 451.2688 $[M + H]^+$ (calcd for $C_{25}H_{39}O_7$, 451.2696).

***p*-Bromobenzoylation of Eujavanicols A–C (1–3).** General procedure: Eujavanico1s A, B, and C (**1–3**; 3 mg each) and *p*-bromobenzoyl chloride (50 mg) were individually dissolved in pyridine (0.3 mL), and the mixture was kept at rt for 12 h. Each reaction mixture was poured into ice water and extracted with EtOAc. The organic layer was 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) (cyclohexane–acetone, 5:1) to afford the analogue tri-*p*-bromobenzoate (2.5 mg from A, 3.4 mg from B, and 3.1 mg from C, respectively).

Tri-*p*-bromobenzoate of Eujavanico1 A (5): CD (MeOH) $\Delta\varepsilon$ (nm) −49.0 (253), +15.6 (234); ^1H NMR ($CDCl_3$, 500 MHz; other than phenyl signals) δ 5.74–5.68 (3H, m, H-4,5,6), 5.07 (1H, dd, J = 11.5, 3.5 Hz, H-3), 4.74–4.59 (2H, m, H_2-11), 3.14–2.92 (2H, m, H_2-10), 2.63 (1H, t, J = 10.9 Hz, H-4a), 2.27 (1H, t, J = 9.8 Hz, H-8a), 1.99 (2H, m, H-2, H-7), 1.81 (2H, m, H-7, H-8), 1.52 (1H, m, H-2′), 1.38 (3H, s, Me-1), 1.19 (1H, m, H-1′), 0.86 (3H, d, J = 6.8 Hz, Me-1′), 0.72 (4H, m, H-2′, H-3′), 0.68 (3H, d, J = 6.3 Hz, Me-8).

Tri-*p*-bromobenzoate of Eujavanico1 B (6): CD (CH_2Cl_2) $\Delta\varepsilon$ (nm) +20.5 (254 nm), −15.4 (238 nm); ^1H NMR ($CDCl_3$, 500 MHz; other than phenyl signals) δ 5.69 (1H, ddd, J =10.3, 4.6, 2.9 Hz, H-3), 5.59 (1H, br d, H-4), 5.32–5.23 (2H, m, H-5, H-6), 4.71–4.59 (2H, m, H_2-11), 3.08–2.95 (2H, m, H_2-10), 2.36–1.63 (7H, m), 1.30 (3H, s, Me-1), 1.22 (1H, m, H-1′), 0.86 (3H, d, J = 6.3 Hz, Me-1′), 0.73 (3H, d, J = 6.3 Hz, H-3′), 0.72 (1H, m, H-2′), 0.71 (3H, br d, Me-8).

Tri-*p*-bromobenzoate of Eujavanico1 C (7): ^1H NMR ($CDCl_3$, 500 MHz; other than phenyl signals) δ 6.08 (1H, br s, H-3), 5.68 (1H, br s, H-6), 5.54 (1H, dd, J = 10.4, 3.5 Hz, H-5), 4.65 (1H, dt, J = 11.5, 7.2 Hz, H-11), 4.46 (1H, m, H-11), 3.17 (1H, dt, J = 18.3, 7.2 Hz, H-10), 3.05 (1H, m, H-10), 2.91 (1H, br s, H-4a), 2.42 (1H, m, H-8), 2.23 (1H, m, H-8a) [the preceding two assignments may be reversed], 2.08 (1H, dt, J = 14.7, 3.5 Hz, H-7eq), 1.78 (1H, m, H-1′), 1.63 (1H, br d, H-7ax), 1.43 (3H, s, Me-1), 1.38 (1H, m, H-2′), 1.12 (3H, d, J = 6.9 Hz, Me-1′), 0.87 (7H, m, Me-8, H-3′, H-2′).

Reduction of Eujavanico1 C Tri-*p*-bromobenzoate (7) with $NaBH_4$. Compound **7** (2 mg) and $NaBH_4$ (10 mg) were dissolved in EtOH (3 mL), and the mixture was kept at rt for 5 min. After the addition of acetone, the reaction mixture was poured into ice water and extracted with CH_2Cl_2, and the extract dried over Na_2SO_4. On evaporation of the solvent, the residue was purified by HPLC (silica gel) (cyclohexane–acetone 4:1) to afford **8** (0.8 mg), one of the main products from **7**.

Compound 8: CD (CH_2Cl_2) $\Delta\varepsilon$ (nm) −30.0 (256 nm), +14.2 (239 nm); ^1H NMR ($CDCl_3$, 500 MHz) δ 5.93 (1H, d, J = 9.9 Hz, 3-H), 5.66 (1H, m, H-6), 5.43 (1H, dd, J = 10.9, 2.9 Hz, H-5), 4.65 (1H, m, H-11), 4.44 (1H, m, H-11), 3.98 (1H, m, H-4), 3.25 (1H, m, H-10), 3.08 (1H, m, H-10), 2.45 (1H, br t, J = 11.5 Hz, H-8a), 2.18 (1H, m, H-7), 2.04–2.16 (3H, m, H-4a, H-7eq, H-8), 1.64–1.59 (2H, m, H-7ax, H-1′), 1.43 (3H, s, Me-1), 1.32 (1H, m, H-2′), 1.00 (3H, d, J = 6.3 Hz Me-1′), 0.86 (5H, m, H-2′, H-3′, Me-8).

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