Globosumones A−C, Cytotoxic Orsellinic Acid Esters from the Sonoran Desert Endophytic Fungus *Chaetomium globosum*[1]

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Three new esters of orsellinic acid, globosumones A−C (**1**−**3**), and three known compounds, orsellinic acid (**4**), orcinol, and trichodion (**5**), were isolated from *Chaetomium globosum* endophytic on *Ephedra fasciculata* (Mormon tea). The structures of the new compounds **1**−**3** were established spectroscopically, which included 2D NMR experiments and ^{1}H NMR studies on Mosher's ester derivatives. All compounds were evaluated for inhibition of cell proliferation in a panel of four cancer cell lines, NCI-H460 (non-small cell lung cancer), MCF-7 (breast cancer), SF-268 (CNS glioma), and MIA Pa Ca-2 (pancreatic carcinoma), and normal human fibroblast cells (WI-38). Only globosumones A (**1**) and B (**2**) were found to be moderately active.

Endophytic fungi consisting mostly of Ascomycetes and Fungi Imperfectii inhabiting the intercellular spaces of higher plants represent one of the largest—conservatively 1.5×10^6 species—and relatively untapped resource of secondary metabolites.[2,3] In their symbiotic association, the higher plant (macrophyte) protects and feeds the endophyte, which "in return" produces bioactive metabolites to enhance the growth and competitiveness of the host and to protect it from herbivores and plant pathogens.[4] Accordingly, some endophytes could be valuable sources of bioactive compounds. Until now most studies of endophytic fungi of higher plants for bioactive agents have focused on those that occur in northern temperate and tropical rain forests. Adaptation of endophytic communities in plants that grow in extreme habitats other than arctic or alpine is generally unknown, and a recent review has suggested the importance of host plant as well as the ecosystem in influencing the general metabolism of endophytic microbes.[3] Desert plants have largely been ignored as a source of endophytes probably due to the assumption that endophyte infection requires humidity.[5,6] Contrary to this contention Schulthess and Faeth have recently isolated and partially identified more than 400 endophytic fungal taxa from Arizona fescue.[7] It is also possible that strains of endophytes found in plants having highly modified anatomy and physiological adaptations, such as those found in desert environment, may produce secondary metabolites different from the same species inhabiting plants growing in less extreme environments.

The possibility that endophytic fungal diversity is influenced by the diversity of plant species and environmental factors suggests a hitherto unutilized opportunity to harvest unique natural products from endophytic fungi of floristically diverse plant communities such as those in the U.S. Southwestern desert. We have recently initiated a research program to discover and develop anticancer agents from endophytic fungi of the U.S. Southwest plants and thus far collected and cultured over 400 fungal strains inhabiting about 20 Sonoran desert plant species. The EtOAc extracts obtained from these cultures have been tested for inhibition of cell proliferation using a panel of four human cancer cell lines [NCI-H460 (non-small cell lung), MCF-7 (breast), SF-268 (CNS glioma), MIA Pa Ca-2 (pancreatic carcinoma)] and normal human fibroblast (WI-38) cells. Several of these extracts exhibiting ≥90% inhibition of at least one of the four cancer cell lines at a dose of 10 μg/mL were selected for fractionation, and one such extract was derived from *Chaetomium globosum* Ames (Ascomycete), occurring in the stem tissue of Mormon tea (*Ephedra fasiculata* A.; family Ephedraceae). Bioactivity-guided fractionation of this extract resulted in the isolation of three novel orsellinic acid esters, globosumones A (**1**), B (**2**), and C (**3**), and the known compounds orsellinic acid [orsellic acid (**4**)], orcinol, and trichodion (**5**). Herein we report the isolation of **1**−**5** and orcinol, structure elucidation of **1**−**3**, and cytotoxicities of **1** and **2**. Previous investigations of soil-derived *C. globosum* strains have resulted in the isolation of the toxic indole derivative chetomin,[8] cytotoxic indole-3-yl-[13]cytochalasans chaetoglobosins A−B,[9] C−F,[10] G and J,[11] Q, R, and T,[12] 19-*O*-acetylchaetoglobosins B and D,[13] immunomodulating and antitumor compound TAN-1142,[14] the sesquiterpene antibiotic heptelidic acid,[15] antimicrobial indole derivative dethitetra(methylthio)chetomin,[16] the azaphilones chaetoviridins A−D,[17] the indole-2,3-dione prenisatin,[18] unidentified epoxysuccinate-type cysteine protease inhibitors PF1138A and B,[19] and anti-TB compounds chaetomanone and echinulin.[20] The occurrence of orsellic acid (**4**) in *C. cochliodes* has also been reported.[21]

Results and Discussion

The cytotoxic EtOAc extract of *C. globosum* on solvent−solvent partitioning[22] yielded a bioactive $CHCl_3$ fraction, which on gel permeation, silica gel, and reversed-phase chromatography afforded compounds **1**−**5** and orcinol. Globosumone A (**1**) was obtained as a colorless solid that analyzed for $C_{13}H_{14}O_5$ by a combination of HRFABMS and ^{13}C NMR spectroscopy. Its IR spectrum had absorption bands at 3344, 1724, 1685, and 1620 cm^{-1}, suggesting the presence of hydroxyl, ester carbonyl, and α,β-unsaturated ketone carbonyl functions. The ^{1}H NMR spectrum of **1** indicated the presence of two *meta*-coupled aromatic protons at δ 6.29 and 6.31 (d, J = 2.0 Hz), a methyl group on an aromatic system at δ 2.51 (s), a 2H singlet at δ 5.28,

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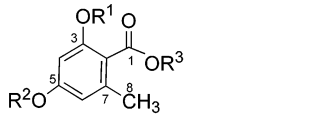

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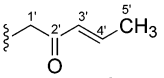

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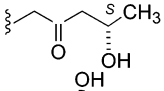

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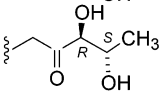
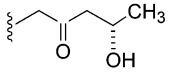
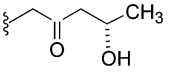


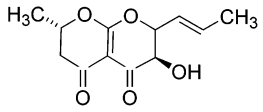
4 $R^1 = R^2 = R^3 = H$

6 $R^1 = H$, $R^2 = CH_3$, R^3 =



7 $R^1 = R^2 = CH_3$, R^3 =





5

and a spin system consisting of two 1H double quartets at δ 7.08 (J = 16.0 and 6.5 Hz) and 6.30 (J = 16.0 and 1.5 Hz) and a 3H double doublet at δ 1.96 (J = 6.5 and 1.5 Hz). On the basis of the chemical shifts and coupling constants of the protons, this spin system was suspected to be due to a $-CH{=}CH-CH_3$ moiety with *E* configuration and attached to a carbonyl group. The ^{13}C NMR spectrum of **1** when analyzed with the help of HSQC showed the presence of a ketone carbonyl (δ 192.0), an ester carbonyl (δ 170.5), six aromatic carbons of which two were oxygenated (δ 165.4 and 163.0) and two were protonated (δ 111.9 and 101.1), two protonated olefinic carbons (δ 144.9 and 128.1), a methylene carbon (δ 67.5), and two methyl carbons (δ 23.6 and 18.1). The ^1H and ^{13}C chemical shifts of the methylene group (δ_H 5.28 and δ_C 67.5) suggested it to be sandwiched by a carbonyl group and an oxygen atom. On the basis of the HMBC correlations (Figure 1) the aromatic moiety of globosumone A was determined to be that of orsellinic acid (**4**). The foregoing spectral data, together with ^2J and ^3J correlations in its HMBC spectrum, were useful in elucidating the structure of globosumone A as 2′-oxo-pent-3′-enyl orsellinate (**1**).

Globosumone B (**2**), obtained as a colorless solid, analyzed for $C_{13}H_{16}O_6$ by a combination of HRFABMS and ^{13}C NMR spectroscopy and indicated six degrees of unsaturation. The IR spectrum of **2** had absorption bands at 3355, 1732, 1651, and 1589 cm^{-1}, suggesting the presence of hydroxyl, ester carbonyl, and ketone carbonyl groups. The ^1H NMR spectrum was similar to that of **1** except that the signals due to the two olefinic protons were replaced by a 1H multiplet at δ 4.05 and two 1H double doublets (J = 15.0 and 7.5 Hz) at δ 2.59 and 2.56 and that one of the methyl signals has shifted upfield from δ 1.96 to 1.10 and has changed from a double doublet (J = 6.5 and 1.5 Hz) to a doublet (J = 6.0 Hz). The ^{13}C NMR spectrum of **2** also showed a very close resemblance to that of **1**, the significant differences being the downfield shift of the signal due to the carbonyl group from δ 192.0 to 203.0 and the upfield shift of signals due to C-3′ and C-4′ from δ 128.1 and 144.9 to δ 48.1 and 62.8, respectively. The chemical shift of C-4′ indicated that it is oxygenated, and the 2′-oxo-4′-hydroxypentyl side chain in **2** was confirmed by the presence of HMBC correlations between the methyl protons at δ 1.10 and the carbons at δ 62.8 and 48.1. Methylation of globosumone B (**2**) afforded its monomethyl and dimethyl derivatives **6** and **7**, respectively. The 4′ *S*-configuration was deduced from a modified Mosher's ester method[23] using the (*S*)- and (*R*)-MTPA esters (**8a** and **8b**, respectively) of monomethylglobosumone B (**6**) (Figure 2), identifying globosumone B as 2′-oxo-4′*S*-hydroxypentyl orsellinate (**2**).

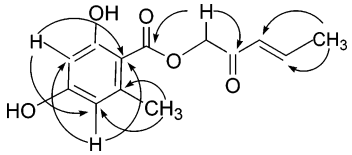
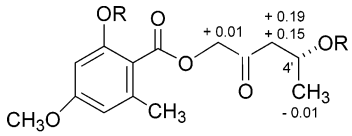
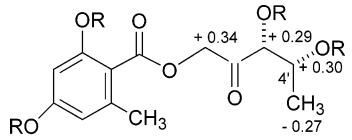


Figure 1. Selected HMBC correlations for **1**.



8a : R = (*S*)-MTPA
8b : R = (*R*)-MTPA

Figure 2. $\Delta\delta$ value [$\Delta\delta$ (in ppm) = $\delta_S - \delta_R$] obtained for (*S*)- and (*R*)-MTPA esters (**8a** and **8b**, respectively) of monomethylglobosumone B (**6**).



9a : R = (*S*)-MTPA
9b : R = (*R*)-MTPA

Figure 3. $\Delta\delta$ value [$\Delta\delta$ (in ppm) = $\delta_S - \delta_R$] obtained for (*S*)- and (*R*)-MTPA esters (**9a** and **9b**, respectively) of globosumone C (**3**).

Globosumone C (**3**) was obtained as a colorless solid that analyzed for $C_{13}H_{16}O_7$ by a combination of HRFABMS and ^{13}C NMR spectroscopy and indicated six degrees of unsaturation. Its IR spectrum had absorption bands at 3398, 1732, 1651, and 1620 cm^{-1}, suggesting the presence of hydroxyl, ester carbonyl, and ketone carbonyl groups. The ^1H and ^{13}C NMR spectra of **3** showed very close resemblance to those of **1** and **2**. In its ^1H NMR spectrum, in addition to the signals due to two aromatic protons, aromatic methyl, and a methylene at δ 4.51, it had a 1H doublet (J = 4.0 Hz) at δ 4.48, a 1H double quartet (J = 6.5 and 4.0 Hz) at δ 5.49, and a methyl doublet (J = 6.5 Hz) at δ 1.34. Compared with **1**, the ^{13}C NMR spectrum of **3** had its signal due to a carbonyl group shifted downfield to δ 210.9, while the signals due to C-3′ and C-4′ were shifted upfield to δ 76.7 and 72.7 from 128.1 and 144.9, respectively. The molecular formula of **3** together with the above NMR data suggested it to be a 3′,4′-dihydroxy derivative of globosumone A (see above). The configuration at C-4′ of globosumone C (**3**) was determined to be *S* by application of a modifed Mosher's ester method[23] using the (*S*)- and (*R*)-MTPA esters (**9a** and **9b**, respectively) of **3** (Figure 3). On the basis of the 3′-H/4′-H coupling constant (4.0 Hz) observed in the ^1H NMR[24] of **3**, the relative configurations of the OH groups at these positions were deduced to be *erythro*, suggesting the *R* configuration for C-3′. The foregoing identified globosumone C as 2′-oxo-3′*R*,4′*S*-dihydoxypentyl orsellinate (**3**). Comparison of physical and/or spectral data with those reported in the literature allowed the remaining compounds encountered in *C.*

Table 1. Cytotoxicities of Compounds **1**−**5** and Orcinol against a Panel of Four Tumor Cell Lines and Normal Human Primary Fibroblast Cells[a]

	cell line[b]				
compound	NCI-H460	MCF-7	SF-268	MIA Pa Ca-2	WI-38
1	6.50	21.30	8.80	10.60	13.00
2	24.80	21.90	29.10	30.20	14.20
doxorubicin	0.01	0.07	0.04	0.05	0.30

[a] Results are expressed as IC_{50} values in μM; compounds **3**−**5** and orcinol were found to be inactive in all cell lines at 10.0 μg/mL. [b] Key: NCI-H460 = human non-small cell lung cancer; MCF-7 = human breast cancer; SF-268 = human CNS cancer (glioma); MIA Pa Ca-2 = human pancreatic cancer; WI-38 = normal human primary fibroblast cells.

globosum to be identified as orsellinic acid (**4**),[25] orcinol,[26] and trichodion (**5**).[27]

Compounds **1**−**5** and orcinol were evaluated for in vitro inhibition of cell proliferation using a panel of four cancer cell lines [NCI-H460 (non-small cell lung), MCF-7 (breast), SF-268 (CNS glioma), and MIA Pa Ca-2 (pancreatic cancer)] and normal human fibroblast (WI-38) cells. Cells were treated with test compounds for 72 h in RPMI-1640 media supplemented with 10% fetal bovine serum, and cell viability was evaluated by the MTT assay.[28] As shown in Table 1, only globosumones A (**1**) and B (**2**) were found to be cytotoxic.

Experimental Section

General Experimental Procedures. Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO Dip-370 polarimeter using $CHCl_3$ as solvent. IR spectra for KBr disks were recorded on a Shimadzu FTIR-8300 spectrometer. 1D and 2D NMR spectra were recorded in $CDCl_3$, acetone-d_6, and DMSO-d_6 and using residual solvents as internal standards with a Bruker DRX-500 instrument at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR. The chemical shift values (δ) are given in parts per million (ppm), and the coupling constants are in Hz. Low-resolution and high-resolution MS were recorded on Shimadzu LCMS QP8000α and JEOL HX110A spectrometers, respectively.

Cytotoxicity Bioassays. The tetrazolium-based colorimetric assay (MTT assay)[28] was used for the in vitro assay to measure inhibition of proliferation of human non-small cell lung carcinoma (NCI-H460), human breast carcinoma (MCF-7), human glioma (SF-268), and human pancreatic carcinoma (MIA Pa Ca-2) cell lines and normal human primary fibroblast (WI-38) cells as previously reported.[22] All samples for assays were dissolved in DMSO. During bioassay-guided fractionation, cytotoxicity of fractions was monitored using the NCI-H460 cell line.

Fungal Isolation, Identification, and Cultivation. The endophytic fungus, *Chaetomium globosum*, was isolated from the stem tissues of *Ephedra fasciculata* collected from South Mountain Park in Phoenix, Arizona, on April 19, 2002. Stem tissues were collected from naturally growing plants and returned on ice to the laboratory. Stems were cut into 2−5 cm long fragments and washed in 95% ethanol to begin surface sterilization to remove epiphytic fungi. Stem fragments were then placed in 70% ethanol for 1 min, the liquid was decanted, and a solution of 33% bleach (sodium hypochlorite) was added for 5 min followed by washing in sterile water (× 3). All fragments were then cut aseptically into smaller fragments (~0.5 cm) for plating, and these fragments were then plated aseptically on three different media: corn meal extract agar, potato dextrose agar, and malt extract agar (all from Difco, Plymouth, MN) to ensure culturing a wide variety of endophytic fungi that vary in nutrient requirements and growth rates. Each plant part was plated three times on each media type. Distinct cultures on each plate were isolated on new plates, and preliminary identifications were made based upon hyphae, mycelial, and spore morphology. Final identification of the endophyte was based upon morphological characteristics and partial LSU rRNA sequences compared to MicroSeq library (Microbial ID, Newark, DE) and GenBank sequence database (99.7% match with *Chaetomium globosum*). All isolates were stored on multiple plates at 5 °C. In addition, slant tubes of each culture were maintained at 5 °C as a library of endophytic fungi at Arizona State University Biology Department and the Southwest Center for Natural Products Research and Commercialization of the University of Arizona microbial culture collection under the code name EPH1Sb. The strain was subcultured using Petri dishes with PDA. To produce culture medium for isolation of secondary metabolites, the fungus was cultured in potato dextrose broth (PDB, Difco, Plymouth, MN) in five 4 L shaker flasks at 120 rpm, each containing 2 L of the medium at 26 °C for 15 days.

Extraction and Isolation. The resulting fermentation broth (10 L) was filtered through Whatman No. 1 filter paper and a layer of Celite 545. The filtrate was extracted with EtOAc (3 × 3 L). The EtOAc extracts were evaporated under reduced pressure to afford a yellow semisolid (3.56 g), which was partitioned between hexane and 80% aqueous MeOH. The 80% aqueous MeOH fraction was diluted to 50% aqueous MeOH by the addition of H_2O and extracted with $CHCl_3$. Evaporation of $CHCl_3$ under reduced pressure yielded a dark semisolid (593 mg). A portion (590 mg) of this was subjected to gel permeation chromatography on a column of Sephadex LH-20 (20.0 g) in hexane/CH_2Cl_2 (1:4) and eluted with hexane/CH_2Cl_2 (1:4) (100 mL), CH_2Cl_2/acetone (3:2) (100 mL), CH_2Cl_2/acetone (1:4) (100 mL), CH_2Cl_2/MeOH (1:4) (100 mL), and MeOH (100 mL). Three cytotoxic fractions [A (150 mg), B (118 mg), and C (35 mg)] and one noncytotoxic fraction [D (209 mg)] were collected. Column chromatography of fraction A (150 mg) on reversed-phase silica gel and elution with increasing amounts of MeCN in H_2O afforded a cytotoxic subfraction, A1 (26.7 mg). This subfraction was further purified by preparative TLC on silica gel (CH_2Cl_2/2-propanol, 95:5) followed by preparative TLC on reversed-phase silica gel (40% MeCN in H_2O) to obtain trichodion (**5**) (6.8 mg). Column chromatography of fraction B (117 mg) on reversed-phase silica gel and elution with increasing amounts of MeCN in H_2O afforded subfraction B1 (22.5 mg), which was further purified by preparative TLC on silica gel (CH_2Cl_2/2-propanol, 97:3) to afford globosumone A (**1**) (5.0 mg). Preparative TLC of fraction C (35.0 mg) on reversed-phase silica gel (50% MeCN in H_2O) afforded orsellinic acid (**4**) (9.1 mg) and globosumone C (**3**) (5.1 mg). Column chromatography of fraction D (208.0 mg) on reversed-phase silica gel and elution with increasing amounts of MeCN in H_2O afforded two major subfractions, D1 (34.0 mg) and D2 (91.1 mg). Subfraction D1 (34.0 mg) was further purified by preparative TLC on reversed-phase silica gel (45% MeCN in H_2O) to yield an additional quantity (8.8 mg) of **4** and orcinol (15.2 mg). Subfraction D2 (44.0 mg) was further purified by preparative TLC on reversed-phase silica gel (45% MeCN in H_2O) to afford additional quantities of **4** (17.2 mg) and **3** (15.2 mg) and globosumone B (**2**) (5.4 mg).

Globosumone A (1): white solid; mp 148 − 149 °C; UV (MeOH) λ_{max} (log ϵ) 299.5 (4.58), 265.5 (5.01), 219.0 (5.32) nm; IR (KBr) ν_{max} 3344, 1724, 1685, 1620, 1585, 1461, 1431, 1245, 1195, 1153, 1103 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) δ 7.08 (1H, dq, J = 16.0, 6.5 Hz, H-4′), 6.31 (1H, d, J = 2.0 Hz, H-6), 6.30 (1H, dq, J = 16.0 and 1.5 Hz, H-3′), 6.29 (1H, d, J = 2.0 Hz, H-4), 5.28 (2H, s, H-1′), 2.51 (3H, s, H-8), 1.96 (3H, dd, J = 6.5, 1.5 Hz, H-5′); ^{13}C NMR (125 MHz, acetone-d_6) δ 192.0 (C, C-2′), 170.5 (C, C-1), 165.4 (C, C-3), 163.0 (C, C-5), 144.9 (CH, C-4′), 144.2 (C, C-7), 128.1 (CH, C-3′), 111.9 (CH, C-6), 105.0 (C, C-2), 101.1 (CH, C-4), 67.5 (CH_2, C-1′), 23.6 (CH_3, C-8), 18.1 (CH_3, C-5′); HRFABMS *m/z* 251.2596 [M + H]$^+$ (calcd for $C_{13}H_{15}O_5$, 251.2591).

Globosumone B (2): white solid; mp 168−170 °C; $[\alpha]_D^{25}$ +4.95 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 301.0 (4.08), 265.5 (4.52), 217.5(4.72) nm; IR (KBr) ν_{max} 3352, 2927, 1732, 1651, 1589, 1450, 1380, 1319, 1261, 1203, 1164, 1118, 1095, 462 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.49 (1H, s, OH),

10.19 (1H, s, OH), 6.20 (1H, d, J = 2.0 Hz, H-4), 6.17 (1H, d, J = 2.0 Hz, H-6), 5.03 (1H, d, J = 18.0 Hz, H-1′a), 4.98 (1H, d, J = 18 Hz, H-1′b), 4.05 (1H, m, H-4′), 2.58 (1H, dd, J = 15.0, 7.0 Hz, H-3′b), 2.49 (1H, dd, J = 15.0, 7.0 Hz, H-3′a), 2.35 (3H, s, H-8), 1.10 (3H, d, J = 6.0 Hz, H-5′); ^{13}C NMR (125 MHz, DMSO-d_6) δ 203.0 (C, C-2′), 169.0 (C, C-1), 161.4 (C, C-3), 161.3 (C, C-5), 141.0 (C, C-7), 110.4 (CH, C-6), 106.8 (C, C-2), 100.5 (CH, C-4), 69.0 (CH_2, C-1′), 62.8 (CH, C-4′), 48.1 (CH_2, C-3′), 23.7 (CH_3, C-8), 22.2 (CH_3, C-5′); HRFABMS *m/z* 269.2749 [M + H]$^+$ (calcd for $C_{13}H_{17}O_6$, 269.2744).

Globosumone C (3): white solid; mp 162−165 °C; $[\alpha]_D^{25}$ −18.97° (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ϵ) 301.5 (3.76), 265.5 (4.18), 218.0 (4.41) nm; IR (KBr) ν_{max} 3398, 2920, 1732, 1620, 1651, 1458, 1392, 1315, 1257, 1203, 1161, 1107, 1072 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) δ 6.19 (1H, d, J = 1.5 Hz, H-6), 6.15 (1H, d, J = 1.5 Hz, H-4), 5.49 (1H, dq, J = 6.5, 4.0 Hz, H-4′), 4.51 (2H, s, H-1′), 4.48 (1H, d, J = 4.0 Hz, H-3′), 2.46 (3H, s, H-8), 1.34 (3H, d, J = 6.5 Hz, H-5′); ^{13}C NMR (125 MHz, acetone-d_6) δ 210.9 (C, C-2′), 171.1 (C, C-1), 165.1 (C, C-3), 162.9 (C, C-5), 143.8 (C, C-7), 111.5 (CH, C-6), 104.9 (C, C-2), 100.7 (CH, C-4), 76.7 (CH, C-3′), 72.7 (CH, C-4′), 66.5 (CH_2, C-1′), 23.4 (CH_3, C-8), 13.9 (CH_3, C-5′); HRFABMS *m/z* 285.2742 [M + H]$^+$ (calcd for $C_{13}H_{17}O_7$, 285.2738).

Orsellinic acid (4): colorless amorphous solid; physical data were consistent with those reported in the literature.[25]

Orcinol: colorless amorphous solid; ^{1}H and ^{13}C NMR and MS data were consistent with those reported in the literature.[26]

Trichodion (5): colorless solid; mp 162−163 °C; APCIMS (+)-ve mode *m/z* 239 [M + 1]$^+$, APCIMS (−)-ve mode *m/z* 237 [M − 1]$^+$; ^{1}H and ^{13}C NMR data were consistent with those reported in the literature.[27]

Methylation of Globosumone B. Globosumone B (**2**) (2.5 mg) in dry acetone was stirred with excess K_2CO_3 and CH_3I (2 drops) at 26 °C for 1.5 h (TLC control). The reaction mixture was filtered through Celite 545, and solvent and excess CH_3I were removed under reduced pressure to give the crude product, which was purified by preparative TLC (silica gel) using 2% methanol in CH_2Cl_2 as eluant to give **6** (1.4 mg) and **7** (0.7 mg). Monomethyl globosumone B (**6**): colorless amorphous solid; ^{1}H NMR ($CDCl_3$) δ 11.45 (1H, s, OH), 6.36 (1H, d, J = 2 Hz, H-4), 6.34 (1H, d, J = 2.0 Hz, H-6), 4.97 (1H, d, J = 17.5 Hz, H-1′a), 4.94 (1H, d, J = 17.5 Hz, H-1′b), 4.34 (1H, m, H-4′), 3.83 (3H, s, OCH_3) 2.66 (1H, s, H-3′a), 2.65 (1H, d, J = 2.5 Hz, H-3′b), 2.58 (3H, s, H-8), 1.28 (3H, d, J = 6.5 Hz, H-5′). Dimethyl globosumone B (**7**): colorless amorphous solid; ^{1}H ($CDCl_3$) δ 6.37 (1H, d, J = 2 Hz, H-4), 6.34 (1H, d, J = 2.0 Hz, H-6), 4.87 (1H, d, J = 17.0 Hz, H-1′a), 4.82 (1H, d, J = 17.0 Hz, H-1′b), 4.33 (1H, m, H-4′), 3.84 (6H, s, 2 × OCH_3), 2.74 (1H, dd, J = 17.5, 3.5 Hz, H-3′a), 2.66 (1H, dd, J = 17.5, 8.5 Hz, H-3′b), 2.41 (3H, s, CH_3-8), 1.27 (3H, d, J = 6.5 Hz, CH_3-5′).

Preparation of the (*R*)- and (*S*)-MTPA Ester Derivatives of 6 by a Convenient Mosher Ester Procedure.[23a] Compound **6** (0.7 mg) was transferred into a clean NMR tube and was dried completely under vacuum of an oil pump. Pyridine-d_5 (0.5 mL) and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (5 μL) were added into the NMR tube immediately under a N_2 gas stream, and then the NMR tube was shaken carefully to mix the sample and MTPA chloride evenly. The reaction NMR tube was allowed to stand at 0 °C and monitored every 24 h by ^{1}H NMR. The reaction was found to be complete after 7 days. ^{1}H NMR data of the (*S*)-MTPA ester derivative (**8a**) of **6** (500 MHz, pyridine-d_5): δ 6.659 (1H, d, J = 2.5 Hz, H-6), 6.482 (1H, d, J = 2.0 Hz, H-4), 5.396 (2H, s, H-1′), 4.949 (1H, m, H-4′), 3.688 (3H, s, OCH_3), 3.124 (1H, dd, J = 14.5, 8.0 Hz, H-3′a), 2.904 (1H, dd, J = 14.5, 4.5 Hz, H-3′b), 2.637 (3H, s, H-8), 1.694 (3H, d, J = 7.0 Hz, CH-5′). In the manner described for **8a**, another portion of **6** (0.7 mg) was reacted in a second NMR tube with (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (5 μL) at 0 °C for 7 days using pyridine-d_5 (0.5 mL) as solvent, to afford the (*R*)-MTPA ester derivative (**8b**) of **6**. ^{1}H NMR data of **8b** (500 MHz, pyridine-d_5): δ 6.641 (1H, d, J = 2.5 Hz, H-6), 6.466 (1H, d, J = 2.0 Hz, H-4), 5.390 (2H, s, H-1′), 4.607 (1H, m, H-4′), 3.688 (3H, s, OCH_3), 2.937 (1H, dd, J = 14.5, 8.0 Hz, H-3′a), 2.754 (1H, dd, J = 14.5, 4.5 Hz, H-3′b), 2.640 (3H, s, H-8), 1.700 (3H, dd, J = 7.0, 1.5 Hz, H-5′).

Preparation of the (*R*)- and (*S*)-MTPA Ester Derivatives of 3 by a Convenient Mosher Ester Procedure.[23a] Compound **3** (2.1 mg) was transferred into a clean NMR tube and was dried completely under vacuum of an oil pump. Pyridine-d_5 (0.5 mL) and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (10 μL) were added into the NMR tube immediately under a N_2 gas stream, and then the NMR tube was shaken carefully to mix the sample and MTPA chloride evenly. The reaction NMR tube was allowed to stand at 0 °C and monitored every 24 h by ^{1}H NMR. The reaction was found to be complete after 7 days. Solvent was removed completely in a vacuum, and the dry residue was dissolved in $CDCl_3$. ^{1}H NMR data of the (*S*)-MTPA ester derivative (**9a**) of **3** (500 MHz, $CDCl_3$; data were assigned on the basis of the correlation of the ^{1}H−^{1}H COSY spectrum): δ 6.332 (1H, brd, J = 2 Hz, H-6), 6.321 (1H, brd, J = 2 Hz, H-4), 5.295 (2H, s, H-1′a, H-1′b), 5.504 (1H, dq, J = 6.0, 3.0 Hz, H-4′), 4.699 (1H, d, J = 3.5 Hz, H-3′), 2.389 (3H, s, H-8), 1.349 (3H, d, J = 6.5 Hz, H-5′). In the manner described for **9a**, another portion of compound **3** (2.1 mg) was reacted in a second NMR tube with (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (10 μL) at 0 °C for 7 days using pyridine-d_5 (0.5 mL) as solvent, to afford the (*R*)-MTPA ester derivative (**9b**) of **3**. ^{1}H NMR data of **9b** (500 MHz, $CDCl_3$): δ 6.770 (1H, brs, H-6), 6.687 (1H, brs, H-4), 5.204 (1H, dq, J = 6.0, 3.0 Hz, H-4′), 4.955 (2H, s, H-1′a, H-1′b), 4.406 (1H, d, J = 3.0 Hz, H-3′), 2.369 (3H, s, H-8), 1.617 (3H, d, J = 6.5 Hz, H-5′).

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