Brartemicin, an Inhibitor of Tumor Cell Invasion from the Actinomycete *Nonomuraea* sp.[1]

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Brartemicin (**1**), a new trehalose-derived metabolite, was isolated from the culture broth of the actinomycete of the genus *Nonomuraea*. Its structure and absolute configuration were determined by spectroscopic analyses. The new compound inhibited the invasion of murine colon carcinoma 26-L5 cells with an IC_{50} value of 0.39 μM in a concentration-dependent manner without showing cytotoxic effects.

Tumor metastasis is the process by which a tumor cell leaves the primary tumor, disseminates to a distant site via the circulatory system, and establishes a secondary tumor.[2] In the metastatic process, translocation of tumor cells across extracellular matrix barriers, namely invasion, is a critical step to accomplish the metastasis. The process of invasion is understood to include tumor cell adhesion, enzymatic degradation of extracellular matrix proteins, and migration. Inhibition of these steps is thus expected as an effective approach to control metastasis and invasion.[3] In our continuing screening for antiinvasive compounds from natural products,[4] a new trehalose-containing metabolite was isolated from the culture broth of the actinomycete *Nonomuraea* sp. The producing strain *N.* sp. TP-A0870 was isolated from a leaf of the Brazilian medicinal plant *Artemisia vulgaris*. The strain was cultured in our standard medium for actinomycetes,[5] and the whole culture broth was extracted with 1-butanol. The extract showed inhibitory activity toward tumor cell invasion into Matrigel, the reconstituted extracellular matrix proteins.[6] Bioassay-guided fractionation of the extract led to the purification of a new compound, brartemicin (**1**).

Compound **1** was obtained as a colorless powder, which had a molecular formula of $C_{28}H_{34}O_{17}$ by interpretation of high-resolution FABMS ($[M + H]^+$ *m/z* 643.1884) and NMR data. The IR spectrum of **1** indicated the presence of hydroxyl (3320 cm^{-1}) and ester (1620 cm^{-1}) functionalities. The UV spectrum, with absorption bands at λ_{max} 216, 264, and 301 nm, was indicative of a benzoyl chromophore.[7] ^1H and ^{13}C NMR analysis of **1** in combination with the HMQC spectrum revealed the presence of 14 carbons including three carbonyl or oxygenated sp^2 carbons, four olefinic or aromatic carbons, an oxygen-bearing methylene, five oxygen-bearing methines, and a quaternary methyl. Further analysis of $^1H-^1H$ COSY and HMBC spectra provided two distinctive units. The first part was clearly a hexopyranose sugar moiety. The continuous COSY correlations from H-1 to H-6 and an HMBC correlation from H-1 to C-5 established a hexopyranose ring. The large coupling constants $^3J_{H,H}$ for H-2/H-3, H-3/H-4, and H-4/H-5 revealed the axial−axial relationship for these protons. The small coupling constant between H-1 and H-2 (3.6 Hz) established the α-configuration at the anomeric position, thereby establishing the relative configuration of the sugar as an α-glucopyranoside. The second part was the substituted benzene moiety. The downfield shifts of C-9 and C-11 indicated that these carbons possessed oxygen substitution, which was further supported by the upfield shifts of the *ortho*-carbons C-8, C-10, and C-12. HMBC correlations from the H-14 methyl to C-8, C-12, and C-13 allowed its location at C-13. The location of the carbonyl group at C-7 was confirmed by the four-bond HMBC correlations from H-10, H-12, and H-14, thus establishing the 2,4-dihydroxy-6-methylbenzoyl moiety. The sugar and the aromatic parts were connected between C-6 and C-7 through an ester bond on the basis of an HMBC correlation from H-6 to C-7. Overall analysis of the NMR data indicated that **1** possessed 14 carbons, corresponding to one-half the number of carbons determined by HRFABMS. Thus, it became clear that **1** was a symmetrical dimer composed of two identical C_{14} units. Finally, a three-bond correlation between H-1 (H-1′) and C-1′ (C-1) linked the two acylated α-glucopyranosyl units to complete the structure of **1**. Since **1** was optically active, the two glucopyranosyl moieties should have the same absolute configuration. α,α-D-Trehalose derivatives possessing the same substituents at the 6- and 6′-positions are known to show positive $[\alpha]_D$ values. For example, 6,6′-ditosyl-D-trehalose[8] and 6,6′-dihexadecanoyl-D-trehalose[9] show $[\alpha]_D$ values of +90 and +78, respectively. Thereby, the optical rotation of **1**, $[\alpha]_D$ +73, led to the conclusion that the sugar moiety of **1** is α-D-glucopyranosyl-α-D-glucopyranoside (α,α-D-trehalose). This was also supported by the $[\alpha]_D$ values of analogues (**2**: $[\alpha]_D$ +94; **3**: $[\alpha]_D$ +105; **4**: $[\alpha]_D$ +63. Scheme 1) that were synthesized from α,α-D-trehalose for a structure−activity relationship study.

Invasion and cytotoxicity assays were carried out according to the procedure previously described.[4a] Compound **1** inhibited the invasion of murine colon carcinoma 26-L5 cells with an IC_{50} value of 0.25 μg/mL (=0.39 μM). Cytotoxicity against the same cell line was not detected even at 10 μg/mL. **1** possesses the hydroxylated benzoyl moiety that is also contained in myxochelins and lupinacidins, invasion inhibitors found by us.[4] In particular, the 2,3-dihydroxybenzoyl substructure is common in **1** and the myxochelins, leading to a speculation that these compounds have the same mode of action. The process of invasion mainly consists of three steps: cell adhesion, degradation of extracellular matrix, and cell migration. Myxochelins inhibit the second step by inhibiting the protease activity of MMP-2 and MMP-9, but **1** showed no effects on these enzymes. In addition, lupinacidins inhibit the third step, cell migration, but **1** did not. These findings indicate that the mode of action of **1** is different from that of inhibitors previously reported. To gain insights into the pharmacophore, a preliminary SAR analysis was conducted for three analogues (**2**−**4**). These compounds were synthesized by the coupling of a benzyl-protected α,α-D-trehalose and an appropriate benzoate derivative, followed by the removal of protecting groups by hydrogenolysis (Scheme 1). Analogues **2**, **3**, and **4** at 1 μg/mL exhibited 51%, 35%, and 40%

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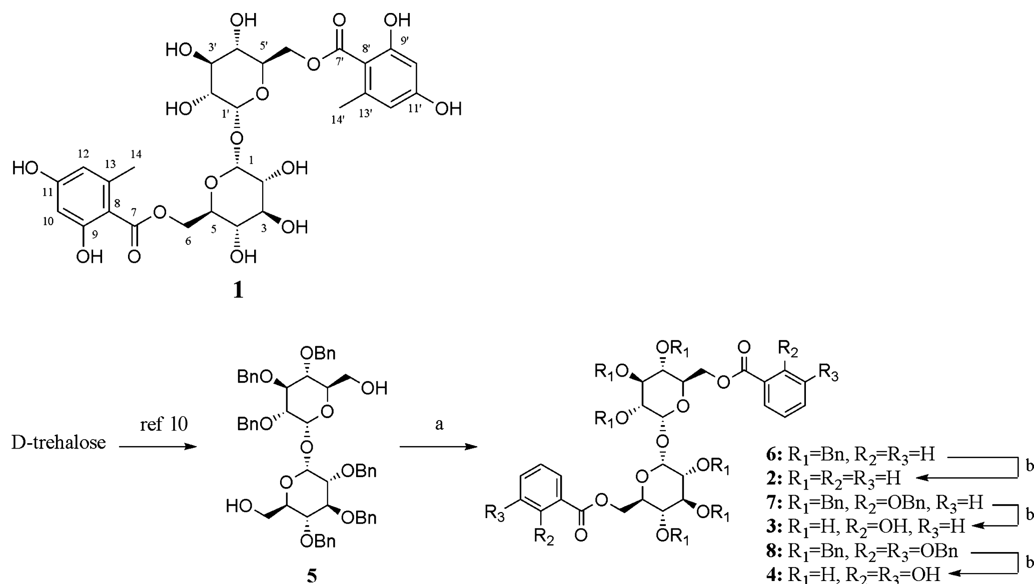
Scheme 1[a]



[a] Reagents: (a) ArCOOH, DEAD, Ph_3P or ArCOOH, EDAC, DMAP; (b) H_2, Pd−C, MeOH.

inhibition, respectively, without showing cytotoxic effects, while **1** displayed 59% inhibition at the same concentration. These findings indicated that the 6,6′-dibenzoyltrehalose-based structure is a novel scaffold for the development of new antiinvasive agents.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-3000 polarimeter. The UV spectrum was recorded on a Hitachi U-3210 spectrophotometer. The IR spectrum was measured on a Perkin-Elmer Spectrum 100. NMR spectra were obtained on a Bruker AVANCE 400 or a Bruker AVANCE 500 spectrometer, using the signals of the residual solvent protons and carbons as internal standard. HRFABMS was measured on a JEOL JMS-HX110 spectrometer. HRESITOFMS were recorded on a Bruker microTOF focus. Silica gel 60-C18 (Nakalai Tesque 250−350 mesh) was used for ODS column chromatography. HPLC separation was performed using a Cosmosil 5C18-AR-II (Nacalai Tesque Inc., 20 × 250 mm) with a photodiode array detector.

Microorganism. Strain TP-A0870 was isolated from a dried leaf of *Artemisia vulgaris* L. purchased in São Paulo, Brazil. A voucher specimen (No. B-042) with identification has been deposited at the Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University (Chiyoda-ku, Tokyo). The strain was identified as a member of the genus *Nonomuraea* on the basis of 99.3% 16S rRNA gene sequence (1483 nucleotides; DDBJ accession number AB449973) identity with the *N. bangladeshensis* type strain (accession number AB274966).

Fermentation. Strain TP-A0870 cultured on a slant agar medium was inoculated into 500 mL K-1 flasks each containing 100 mL of the seed medium consisting of soluble starch 1%, glucose 0.5%, NZ-case 0.3%, yeast extract 0.2%, tryptone (Difco Laboratories) 0.5%, K_2HPO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, and $CaCO_3$ 0.3% (pH 7.0). The flasks were cultivated on a rotary shaker (200 rpm) at 30 °C for 4 days. The seed culture (3 mL) was transferred into 500 mL K-1 flasks each containing 100 mL of the production medium consisting of glucose 0.5%, glycerol 2%, soluble starch 2%, Pharmamedia (Traders Protein) 1.5%, yeast extract 0.3%, and Diaion HP-20 (Mitsubishi Chemical Co.) 1%. The pH of the medium was adjusted to 7.0 before sterilization. The inoculated flasks were cultured on a rotary shaker (200 rpm) at 30 °C for 6 days.

Extraction and Isolation. At the end of the fermentation period, 50 mL of 1-butanol was added to each flask, and they were allowed to shake for 1 h. The mixture was centrifuged at 6000 rpm for 10 min, and the organic layer was separated from the aqueous layer containing the mycelium. Evaporation of the solvent gave approximately 2.0 g of extract from 1 L of culture. The crude extract (20 g) was subjected to silica gel column chromatography with a step gradient of $CHCl_3$/MeOH (1:0, 20:1, 10:1, 4:1, 2:1, 1:1, and 0:1 v/v). Fraction 6 was concentrated to provide 2.0 g of brown powders, which were further purified by reversed-phase ODS column chromatography with a gradient of MeCN/0.15% KH_2PO_4 buffer (pH 3.5) (2:8, 3:7, 4:6, 5:5, 6:4, 7:3, and 8:2 v/v). Fractions 3 and 4 were combined and evaporated. The remaining aqueous solution was extracted with 1-butanol, and the organic layer was concentrated to give semipure **1** as a major component. Final purification of **1** was achieved by repeated C-18 RP HPLC with MeCN/H_2O (30:70), followed by evaporation and lyophilization. Typical recovery of **1** from a 1 L culture was 3.1 mg.

Table 1. ^{1}H and ^{13}C NMR Data for Brartemicin (**1**) in CD_3OD

position	δ_C[a]	δ_H mult (*J* in Hz)[b]	HMBC[b,c]
1, 1′	95.6, CH	5.13, d (3.6)	1′ (1), 2 (2′), 5 (5′)
2, 2′	73.2, CH	3.49, dd (9.5, 3.6)	3 (3′), 4 (4′)
3, 3′	74.5, CH	3.85, dd (9.5, 9.0)	1 (1′), 2 (2′), 4 (4′)
4, 4′	72.2, CH	3.44, dd (10.0, 9.0)	3 (3′), 5 (5′), 6 (6′)
5, 5′	71.4, CH	4.19, ddd (10.0, 4.9, 2.2)	3 (3′), 4 (4′)
6, 6′	65.4, CH_2	4.58, dd (12.0, 2.2)	4 (4′), 5 (5′), 7 (7′)
		4.46, dd (12.0, 4.9)	5 (5′), 7 (7′)
7, 7′	172.8, qC		
8, 8′	105.6, qC		
9, 9′	166.3, qC		
10, 10′	101.7, CH	6.15, d (2.4)	7 (7′), 8 (8′), 9 (9′), 11 (11′), 12 (12′)
11, 11′	163.9, qC		
12, 12′	112.6, CH	6.21, d (2.4)	7 (7′), 8 (8′), 10 (10′), 11 (11′), 14 (14′)
13, 13′	144.9, qC		
14, 14′	24.9, CH_3	2.51, s	7 (7′), 8 (8′), 9 (9′), 12 (12′), 13 (13′)

[a] Recorded at 100 MHz. [b] Recorded at 500 MHz. [c] HMBC correlations are from proton(s) stated to the indicated carbon.

Brartemicin (1): colorless powder; $[\alpha]^{24}_D$ +73 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 216 (4.77), 264 (4.53), 301 (4.14) nm; IR (film) ν_{max} 3320, 1620 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRFABMS $[M + H]^+$ 643.1884 (calcd for $C_{28}H_{35}O_{17}$, 643.1875).

6,6′-Dibenzoyl-α,α-D-trehalose (2). Triphenylphosphine (45 mg, 0.17 mmol), benzoic acid (20 mg, 0.17 mmol), and diethyl azodicarboxylate (40% solution in toluene, 77 μL, 0.17 mmol) were added to a stirred solution of 2,2′,3,3′,4,4′-hexabenzyl-α,α-D-trehalose[10] (**5**, 50 mg, 0.057 mmol) in dry THF (1 mL) at 0−5 °C. After stirring for 2 h at the same temperature, the reaction mixture was diluted with ice−water and extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc, 10:1−1:1) to give

6 (60 mg) in 96% yield: $[\alpha]^{24}_{D}$ +83 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (2H, dd, *J* = 8.5, 1.2 Hz), 7.95 (2H, dd, *J* = 8.5, 1.2 Hz), 7.62 (1H, tt, *J* = 7.2, 1.2 Hz), 7.54 (1H, tt, *J* = 7.2, 1.2 Hz), 7.48 (2H, t, *J* = 7.4 Hz), 7.23−7.42 (12H, m), 5.23 (1H, d, *J* = 3.6 Hz), 5.04 (1H, d, *J* = 10.7 Hz), 4.91 (1H, d, *J* = 10.7 Hz), 4.90 (1H, d, *J* = 10.7 Hz), 4.74 (1H, d, *J* = 11.9 Hz), 4.70 (1H, d, *J* = 11.9 Hz), 4.59 (1H, d, *J* = 10.7 Hz), 4.35 (1H, m), 4.34 (1H, d, *J* = 12.7 Hz), 4.27 (1H, dd, *J* = 12.7, 3.8 Hz), 4.12 (1H, t, *J* = 9.3 Hz), 3.69 (1H, t, *J* = 9.3 Hz), 3.63 (1H, dd, *J* = 9.6, 3.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 138.5, 137.84, 137.81, 133.1, 129.9, 129.65, 128.54, 128.52, 128.50, 128.4, 128.2, 128.1, 128.0, 127.82, 127.78, 127.4, 94.0, 81.8, 79.6, 77.7, 75.9, 75.3, 73.1, 69.3, 63.1; HRESITOFMS *m/z* $[M + Na]^+$ 1113.4387 (calcd for $C_{68}H_{66}O_{13}Na$, 1113.4395).

To a solution of **6** (20 mg, 0.044 mmol) in MeOH/THF (1:1, 5 mL) was added 10% Pd/C (10 mg), and the mixutre was stirred at room temperature under an atmosphere of H_2 for 2 h. The reaction mixture was filtered through Celite and concentrated under reduced pressure. The residue was purified by preparative HPLC in the same manner as described for **1** to yield **2** (14 mg) in 46% yield: $[\alpha]^{24}_{D}$ +94 (*c* 1.0, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 8.03 (2H, d, *J* = 7.5 Hz), 7.60 (1H, t, *J* = 7.5 Hz), 7.47 (2H, t, *J* = 7.5 Hz), 5.13 (1H, d, *J* = 3.7 Hz), 4.59 (1H, dd, *J* = 11.9, 1.8 Hz), 4.47 (1H, dd, *J* = 11.9, 5.0 Hz), 4.21 (1H, ddd, *J* = 10.0, 5.0, 1.8 Hz), 3.85 (1H, t, *J* = 9.3 Hz), 3.54 (1H, dd, *J* = 9.7, 3.7 Hz), 3.49 (1H, t, *J* = 9.5 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 168.0, 134.3, 131.4, 130.6, 129.6, 95.5, 74.7, 73.2, 72.0, 71.7, 65.1; HRESITOFMS *m/z* $[M + Na]^+$ 573.1594 (calcd for $C_{26}H_{30}O_{13}Na$, 573.1579).

6,6′-Bis(2-hydroxybenzoyl)-α,α-D-trehalose (3). To a stirred solution of **5** (50 mg, 57 μmol) in dry DMF (2 mL) were added *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (55 mg, 0.29 mmol), 2-benzyloxybenzoic acid (39 mg, 0.17 mmol), and 4-(dimethylamino)pyridine (35 mg, 0.29 mmol) at room temperature. After stirring for 3 h, the reaction mixture was diluted with ice−water and extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/EtOAc, 10:1−1:1) to give **7** (61 mg) in 75% yield: $[\alpha]^{24}_{D}$ +63 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73 (1H, dd, *J* = 8.0, 1.8 Hz), 7.42 (2H, d, *J* = 7.3 Hz), 7.20−7.39 (19H, m), 6.93 (2H, m), 5.17 (1H, d, *J* = 3.5 Hz), 5.16 (2H, s), 5.00 (1H, d, *J* = 10.8 Hz), 4.86 (1H, d, *J* = 10.9 Hz), 4.82 (1H, d, *J* = 10.5 Hz), 4.61 (2H, s), 4.56 (1H, d, *J* = 10.5 Hz), 4.41 (1H, dd, *J* = 12.3, 3.5 Hz), 4.32 (1H, m), 4.31 (1H, d, *J* = 12.3 Hz), 4.06 (1H, t, *J* = 9.5 Hz), 3.70 (1H, t, *J* = 9.5 Hz), 3.50 (1H, dd, *J* = 9.7, 3.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.8, 158.1, 138.7, 138.0, 137.8, 136.7, 133.4, 131.7, 128.6, 128.5, 128.4, 128.2, 127.9, 127.75, 127.72, 127.6, 127.5, 126.7, 120.6, 120.5, 113.8, 93.9, 81.6, 79.3, 77.7, 75.6, 75.2, 72.8, 70.3, 69.4, 63.1; HRESITOFMS *m/z* $[M + Na]^+$ 1325.5218 (calcd for $C_{82}H_{78}O_{15}Na$, 1325.5238).

In the same manner as described for **2**, **7** gave **3** in 90% yield: $[\alpha]^{24}_{D}$ +110 (*c* 0.6, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 7.90 (1H, dd, *J* = 8.0, 1.7 Hz), 7.48 (1H, ddd, *J* = 8.4, 7.3, 1.3 Hz), 6.94 (1H, dd, *J* = 8.4, 1.3 Hz), 6.91 (1H, ddd, *J* = 8.0, 7.3, 1.3 Hz), 5.12 (1H, d, *J* = 3.7 Hz), 4.62 (1H, dd, *J* = 11.8, 2.2 Hz), 4.50 (1H, dd, *J* = 11.8, 5.1 Hz), 4.22 (1H, ddd, *J* = 10.1, 5.1, 2.2 Hz), 3.84 (1H, t, *J* = 9.4 Hz), 3.53 (1H, dd, *J* = 9.7, 3.7 Hz), 3.46 (1H, dd, *J* = 10.1, 9.0 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 170.3, 162.0, 136.1, 130.4, 119.6, 117.6, 112.9, 94.9, 73.9, 72.4, 71.1, 70.7, 64.5; HRESITOFMS *m/z* $[M + Na]^+$ 605.1465 (calcd for $C_{26}H_{30}O_{15}Na$, 605.1476).

6,6′-Bis(2,3-dihydroxybenzoyl)-α,α-D-trehalose (4). In the same manner as described for **6**, **5** and 2,3-dibenzyloxybenzoic acid[11] gave **8** in 56% yield: $[\alpha]^{24}_{D}$ +45 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16−7.48 (23H, m), 5.10 (1H, d, *J* = 3.5 Hz), 5.09 (4H, m), 4.98 (1H, d, *J* = 10.8 Hz), 4.84 (1H, d, *J* = 10.8 Hz), 4.83 (1H, d, *J* = 10.6 Hz), 4.59 (2H, s), 4.53 (1H, d, *J* = 10.6 Hz), 4.38 (1H, dd, *J* = 12.0, 3.2 Hz), 4.27 (2H, m), 4.04 (1H, t, *J* = 9.3 Hz), 3.62 (1H, t, *J* = 9.6 Hz), 3.44 (1H, dd, *J* = 9.6, 3.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.6, 152.9, 157.8, 138.7, 138.0, 137.9, 137.6, 128.8, 128.63, 128.55, 128.44, 128.40, 128.3, 128.2, 128.0, 127.6, 127.5, 127.4, 123.9, 122.8, 119.4, 118.0, 93.9, 81.6, 79.4, 77.7, 75.8, 75.3, 72.9, 71.4, 71.2, 69.3, 63.2; HRESITOFMS *m/z* $[M + Na]^+$ 1537.6068 (calcd for $C_{96}H_{90}O_{17}Na$, 1537.6070).

In the same manner as described for **2**, **8** gave **4** in 90% yield: $[\alpha]^{24}_{D}$ +63 (*c* 0.57, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 7.40 (1H, dd, *J* = 8.0, 1.2 Hz), 7.02 (1H, dd, *J* = 8.0, 1.2 Hz), 6.76 (1H, t, *J* = 8.0 Hz), 5.12 (1H, d, *J* = 3.7 Hz), 4.63 (1H, dd, *J* = 11.9, 1.8 Hz), 4.50 (1H, dd, *J* = 11.9, 4.8 Hz), 4.21 (1H, ddd, *J* = 10.1, 4.8, 1.8 Hz), 3.84 (1H, dd, *J* = 9.3 Hz), 3.53 (1H, dd, *J* = 9.7, 3.7 Hz), 3.47 (1H, dd, *J* = 9.5 Hz); ^{13}C NMR (100 Hz, CD_3OD) δ 171.5, 151.3, 147.2, 121.8, 121.4, 120.1, 114.0, 95.6, 74.7, 73.2, 71.9, 71.4, 65.3; HRESITOFMS *m/z* $[M + Na]^+$ 637.1369 (calcd for $C_{26}H_{30}O_{17}Na$, 637.1375).

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of brartemicin. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Bioactive microbial metabolites. Part 34. Part 33: Azumi, M.; Ogawa, K.; Fujita, T.; Takeshita, M.; Yoshida, R.; Furumai, T.; Igarashi, Y. *Tetrahedron* **2008**, *64*, 6420–6425.

(2) Kohn, E. C.; Liotta, L. A. *Cancer Res.* **1995**, *51*, 1856–1862.

(3) Geho, D. H.; Bandle, R. W.; Clair, T.; Liotta, L. A. *Physiology* **2005**, *20*, 194–200.

(4) (a) Miyanaga, S.; Obata, T.; Onaka, H.; Fujita, T.; Saito, N.; Sakurai, H.; Saiki, I.; Furumai, T.; Igarashi, Y. *J. Antibiot.* **2006**, *59*, 698–703. (b) Igarashi, Y.; Trujillo, M. E.; Martinez-Molina, E.; Yanase, S.; Miyanaga, S.; Obata, T.; Sakurai, H.; Saiki, I.; Fujita, T.; Furumai, T. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3702–3705. (c) Miyanaga, S.; Sakurai, H.; Saiki, I.; Onaka, H.; Igarashi, Y. *Bioorg. Med. Chem.* **2009**, in press. DOI: 10.1016/j.bmc.2009.02.040.

(5) Igarashi, Y.; Iida, T.; Yoshida, R.; Furumai, T. *J. Antibiot.* **2002**, *55*, 764–767.

(6) Saito, K. I.; Oku, T.; Ata, N.; Miyashiro, H.; Hattori, M.; Saiki, I. *Biol. Pharm.* **1997**, *20*, 345–348.

(7) Agatsuma, T.; Takahashi, A.; Kabuto, C.; Nozoe, S. *Chem. Pharm. Bull.* **1993**, *41*, 373–375.

(8) Birch, G.; Richardson, A. C. *Carbohydr. Res.* **1968**, *8*, 411–415.

(9) Bottle, S.; Jenkins, D. *J. Chem. Soc., Chem. Commun.* **1984**, 385.

(10) Umemura, K.; Matsuyama, H.; Kobayashi, M.; Kamigata, N. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 3026–3028.

(11) Kanai, F.; Isshiki, K.; Umezawa, Y.; Morishima, H.; Naganawa, H.; Takita, T.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1985**, *38*, 31–38.

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