Two New Analogues of the Cytotoxic Substance BE-52211 from *Streptomyces* sp.

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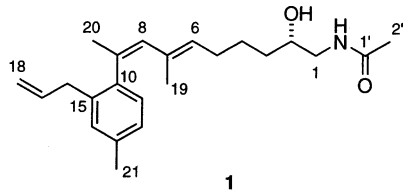
Two new β-hydroxy acetamides, BE-52211 B and BE-52211 C, which were structural analogues of BE-52211, were obtained as an inseparable mixture from an actinomycete, *Streptomyces* sp. Their structures were elucidated on the basis of spectroscopic data. They inhibited cell division of starfish embryos at a concentration of 2.5 μg/mL or greater.

In the course of our search for inhibitors of starfish (*Asterina pectinifera*) embryonic development,[1−14] we found that the *i*-PrOH extract of an actinomycete, *Streptomyces* sp., showed inhibitory activity against cell division of starfish embryos. Bioassay-directed fractionation of the crude extract afforded two new compounds, designated BE-52211 B (**1**) and BE-52211 C (**2**), as an inseparable mixture. Compounds **1** and **2** are structural analogues of the cytotoxic compound BE-52211 (**3**),[15] having a 3-hydroxy-1-butenyl substituent at C-15. In this paper, we report the isolation and structure elucidation of compounds **1** and **2**.



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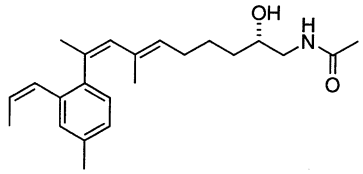

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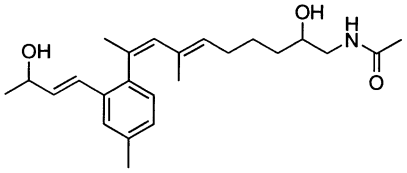

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The *i*-PrOH extract of the fermentation broth was partitioned between EtOAc and H_2O, and the EtOAc layer was dried over anhydrous Na_2SO_4. The EtOAc extract was concentrated and then subjected to chromatography on silica gel using 20−80% EtOAc in hexane as eluent. The biologically active fraction was subsequently chromatographed on ODS (MeOH−H_2O, 9:1) to afford **1** and **2** (5 mg) as an inseparable mixture. They gave only one peak by ODS-HPLC (10−50% CH_3CN in H_2O).

Table 1. ^{1}H NMR Data for the Mixture **1** + **2** in C_6D_6[a]

position	**1**	**2**
1a	2.89 m	2.89 m
1b	3.15 m	3.15 m
2	3.41 m	3.41 m
3a	1.15 m	1.15 m
3b	1.30 m	1.30 m
4a	1.30 m	1.30 m
4b	1.50 m	1.50 m
5	2.00 m (2H)	2.00 m (2H)
6	5.47 br t (7.3)	5.47 br t (7.3)
8	6.23 br s	6.23 br s
11	7.06 d (7.9)	7.11 d (7.9)
12	6.93 br d (7.9)	6.96 br d (7.9)
14	7.09 br s	7.25 br s
16a	3.41 br	6.68 dq (11.6, 1.8)
16b	3.49 br	
17	6.02 dddd (17.1, 10.4, 6.7, 6.7)	5.73 dq (11.6, 7.3)
18a	5.08 dq (10.4, 1.8q)	1.81 dd (7.3, 1.8) (3H)
18b	5.14 dq (17.1, 1.8q)	
19	1.49 br s (3H)	1.53 br s (3H)
20	2.05 br s (3H)	2.08 br s (3H)
21	2.19 s (3H)	2.22 s (3H)
2′	1.51 s (3H)	1.51 s (3H)
NH	4.92 br	4.92 br

[a] ^{1}H chemical shift values (δ ppm) followed by multiplicity and then the coupling constant (*J*/Hz).

BE-52211 B (**1**) and BE-52211 C (**2**) were obtained as an inseparable mixture in the ratio of ca. 4:3, respectively (as calculated from the ^{1}H NMR integral). The FABMS of **1** and **2** showed an $[M + H]^+$ ion at *m*/*z* 356 in the positive mode and an $[M - H]^-$ ion at *m*/*z* 354 in negative mode, compatible with the molecular formula of $C_{23}H_{33}NO_2$. The IR spectrum contained absorption bands at 3330 (OH and NH), 1653 (amide), 1626 (C=C), and 1607 (aromatic ring) cm^{-1}. The ^{1}H NMR spectrum measured in $CDCl_3$ displayed ill-separated aromatic proton signals in the narrow region between δ_H 6.9 and 7.0. Therefore, extensive NMR measurements were performed in C_6D_6 (Table 1). Most of the ^{13}C NMR signals attributable to the aromatic carbons were split into pairs of narrowly separated signals (Table 2), indicating that they arose from a mixture of two closely related compounds, **1** and **2**. Interpretation of the ^{1}H NMR, TOCSY, ^{1}H−^{1}H COSY, and HMQC spectra led to the

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Table 2. ^{13}C NMR Data for the Mixture **1** + **2** in C_6D_6[a]

position	1	2
1	46.3 t	46.3 t
2	71.5 d	71.5 d
3	34.8 t	34.8 t
4	25.8 t	25.8 t
5	28.2 t	28.2 t
6	132.1 d	131.8 d
7	134.2 s	134.3 s
8	132.5 d	132.4 d
9	134.4 s	134.6 s
10	140.2 s	140.7 s
11	128.9 d	129.0 d
12	127.1 d	127.6 d
13	136.4 s	135.7 s
14	130.1 d	130.1 d
15	137.0 s	135.2 s
16	37.6 t	129.6 d
17	138.0 d	126.3 d
18	115.8 t	14.6 q
19	15.5 q	15.4 q
20	28.4 q	27.8 q
21	21.1 q	21.2 q
1′	170.3 s	170.3 s
2′	22.6 q	22.6 q

[a] Multiplicities were determined by DEPT experiments.

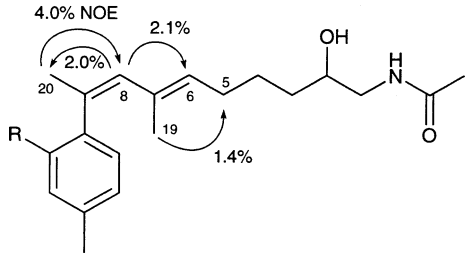

Figure 1. Key NOEs in **1** + **2**.

following structural units: C-1 to C-6, C-11 to C-12, and C-16 to C-18. Connectivities of the partial structure of C-6 to C-10 were established on the HMBC cross-peaks: H_2-5/C-6 and C-7; H-6/C-8 and C-19; H_3-19/C-6, C-7, and C-8; H-8/C-6, C-10, C-19, and C-20; H_3-20/C-8, C-9, and C-10. A $^1H-^1H$ COSY correlation between the NH proton and H_2-1, together with HMBC correlations of H_3-2′/C-1′ and H_2-1/C-1′, provided evidence of the presence of the acetamide group at C-1. The presence of the 1,2,4-trisubstituted benzene ring was established on the HMBC cross-peaks: H-14/C-10, C-12, and C-16; H-12/C-10, C-14, and C-21; H-11/C-9, C-13, and C-15; H_3-21/C-12, C-13, and C-14. The location of the acetamide side chain was established on the basis of HMBC correlations from H-8 and H_3-20 to C-10, and the location of the remaining C_3H_5 unit, 2-propene in **1** and 1-propene in **2**, was determined by observation of an HMBC correlation from H-14 to C-16. Irradiation of the H_3-20 resulted in significant NOE enhancement of H-8 (4.0%), as shown in Figure 1. Irradiation of the H-8 signal resulted in NOE enhancements of H_3-20 (2.0%) and H-6 (2.1%). Irradiation of the H_3-19 signal resulted in 1.4% NOE enhancements of H_2-5. These findings indicated that the geometry of the trisubstituted olefins was 6*E*,8*Z*.

The geometry of the disubstituted olefine Δ^{16} in **2** was determined to be *Z* on the basis of the vicinal ^1H coupling constant ($J_{16,17}$ = 11.6 Hz). Thus, the structures of **1** and **2** were determined to be (6*E*,8*Z*)-*N*-[9-(2-allyl-4-methylphenyl)-2-hydroxy-7-methyl-6,8-decadienyl]acetamide and (6*E*,8*Z*)-*N*-{9-{4-methyl-2-[(*Z*)-1-propenyl]phenyl}-2-hydroxy-7-methyl-6,8-decadienyl}acetamide, respectively.

The absolute stereochemistry of **1** and **2** was elucidated by using the modified MTPA method.[16] The mixture of **1** and **2** was treated with (−)- and (+)-MTPA chlorides in pyridine to afford a mixture of (*S*)-MTPA esters (**1a** and **2a**) and a mixture of (*R*)-MTPA esters (**1b** and **2b**), respectively. The signs of $\Delta\delta$ ($\delta_S - \delta_R$) values for protons of H_2-1 and H_3-2′ were positive, while those for protons of H_2-3, H_2-4, and H_2-5 were negative, as shown in Figure 2. In accordance with the modified Mosher model, the absolute configuration at C-2 was assigned as *S*.

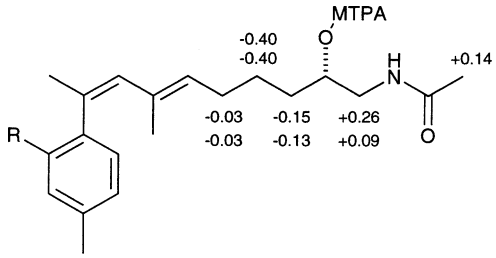

Figure 2. $\Delta\delta$ values for the MTPA esters; $\Delta\delta$ (ppm) = $\delta_S - \delta_R$.

BE-52211 B (**1**) and BE-52211 C (**2**) prevented the first mitotic cell division of fertilized starfish eggs: their minimum effective concentration was 2.5 μg/mL.

Experimental Section

General Experimental Procedures. Optical rotation was measured on a JASCO DIP-370 digital polarimeter at the sodium D line (589 nm). UV and IR spectra were recorded on a Shimadzu UV-160A spectrophotometer and a JASCO FT/IR-5300 spectrometer, respectively. ^1H and ^{13}C NMR spectra were recorded on a JEOL GSX500 spectrometer (500 MHz for ^1H, 125 MHz for ^{13}C) at 25 °C. ^1H and ^{13}C NMR chemical shifts were referenced to residual solvent signals: δ_H 7.26 and δ_C 77.0 for $CDCl_3$ and δ_H 7.20 and δ_C 128.0 for C_6D_6. FABMS and ESIMS were measured on a JEOL SX102A spectrometer.

Biological Material. The microorganism was isolated from soil of province of east Kalimantan, Indonesia, and stored at Kyowa Hakko Kogyo Co. It was identified by one of the authors (M.S.) as *Streptomyces* sp. as follows. The vegetative hyphae of the isolate were well developed and branched. Aerial mycelia were formed on agar media, and slightly curved spore chains were developed at the tip of aerial mycelia. The diaminopimelic acid in whole-cell hydrolysates of the isolate was in the LL configuration. These characteristics indicated that the isolate belongs to the genus *Streptomyces*.

Fermentation. A loopful of cells from a mature slant culture of the above producing strain was inoculated into a test tube (21 mm i.d. × 200 mm) containing 10 mL of a seed medium [glucose 1.0%, soluble starch 1.0%, beef extract 0.3%, yeast extract 0.5%, bactotryptone 0.5%, KH_2PO_4 0.1%, and $Mg_3(PO_4)_2 \cdot 8H_2O$ 0.05% adjusted to pH 7.0]. The inoculated tube was first cultured for 4 days at 28 °C with vigorous shaking. The culture (3.0%) was then inoculated into 50 mL of a seed medium in a 300 mL Erlenmeyer flask and cultured for 3 days at 28 °C on a rotary shaker. The seed culture (2.5 mL) was transferred into a 300 mL Erlenmeyer flask containing 50 mL of a production medium [soluble starch 4.0%, soybean meal 1.0%, corn steep liquor 0.5%, dry yeast 0.5%, KH_2PO_4 0.5%, $ZnSO_4 \cdot 7H_2O$ 0.001%, $CoCl_2 \cdot 6H_2O$ 0.0001%, $NiSO_4$ 0.0001%, and $Mg_3(PO_4)_2 \cdot 8H_2O$ 0.05% adjusted to pH 7.0]. The fermentation was carried out at 28 °C for 5 days under agitation at 220 rpm.

Bioassay. Specimens of *A. pectinifera* were collected from the coastal waters off Japan during their breeding season and kept in seawater at 15 °C in laboratory aquaria. Experiments were performed at 20 °C, and filtered seawater diluted to 90% (v/v) with distilled water was used throughout. Oocytes and sperm were removed from ovarian and testicular fragments, respectively. Oocytes were induced to mature by treatment with 1 μM 1-methyladenine (Sigma, St. Louis, MO).[17] Maturing oocytes were fertilized by the addition of a diluted sperm suspension at 40 min after the start of 1-methyladenine

treatment. Fertilized eggs were washed three times with seawater. More than 95% of fertilized eggs normally divided around 120 min after the initiation of the 1-methyladenine treatment. The inhibitory activity of the bacterial culture broth and each fraction purified from it was determined by adding a small number of fertilized eggs to serially diluted sample solutions within 10 min after insemination and observing the first mitotic cell division. The MeOH solution of the sample to be tested was added to the suspensions of fertilized eggs to give the final concentrations of MeOH less than 0.2% in seawater. MeOH at these concentrations had no effect on the first mitotic division.

Extraction and Isolation. To the fermentation broth (600 mL) of a *Streptomyces* sp. was added an equal volume of *i*-PrOH. The *i*-PrOH suspension was separated from the mycelial cake and supernatant by centrifugation. The supernatant was filtered and the filtrate was concentrated under reduced pressure to afford an aqueous solution, which was extracted three times with EtOAc. The EtOAc layer was concentrated to a small volume under reduced pressure below 30 °C and dried over anhydrous Na_2SO_4. The crude extract (630 mg) was subjected to silica gel column chromatography (1.5 cm i.d. × 85 cm) using 20−80% EtOAc in hexane. The biologically active fraction was rechromatographed on ODS using MeOH−H_2O (9:1) as eluent to afford a mixture of **1** and **2** (5 mg).

BE-52211 B (1) and BE-52211 C (2): pale yellow oil; $[\alpha]^{25}_D$ −38° (*c* 0.008, MeOH); UV (MeOH) λ_{max} (log ϵ) 224 (4.41), 238 (sh) (4.37) nm; IR (film) ν_{max} 3330, 1653, 1626, 1607 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; (+)FABMS *m*/*z* 356 $[M + H]^+$; (−)FABMS *m*/*z* 354 $[M - H]^-$; (+)ESIMS *m*/*z* 356 $[M + H]^+$, 378 $[M + Na]^+$, 394 $[M + K]^+$.

Preparation of MTPA Esters of 1 and 2. To a solution of the mixture of **1** and **2** (2 mg) in dry pyridine (200 μL) was added (−)-MTPA chloride (15 μL), and the solution was stirred at room temperature overnight. After removal of the solvent, the reaction mixture was purified by short silica gel column chromatography (5−20% EtOAc in hexane) to afford a mixture of (*S*)-MTPA esters **1a** and **2a** (1 mg). In the same way, by using (+)-MTPA chloride, the mixture of **1** and **2** (2 mg) was converted into a mixture of (*R*)-MTPA esters **1b** and **2b** (1 mg). **1a**: ^1H NMR ($CDCl_3$, 500 MHz) δ_H 0.94 (1H, m, H-4a), 0.97 (1H, m, H-4b), 1.04 (1H, m, H-3a), 1.07 (1H, m, H-3b), 1.14 (3H, br s, H_3-19), 1.75 (2H, m, H-5), 1.99 (3H, br s, H_3-20), 2.19 (3H, s, NAc), 2.32 (3H, s, H_3-21), 3.24 (1H, m, H-16a), 3.31 (1H, m, H-16b), 3.55 (3H, s, MTPA OCH_3), 3.58 (1H, m, H-1a), 4.00 (1H, m, H-1b), 4.47 (1H, m, H-2), 5.04 (1H, br d, *J* = 10.4 Hz, H-18a), 5.06 (1H, br d, *J* = 17.1 Hz, H-18b), 5.09 (1H, br t, *J* = 7.3 Hz, H-6), 5.39 (1H, br, NH), 5.90 (1H, m, H-17), 6.03 (1H, br s, H-8), 6.94−7.03 (2H, overlap, H-11 and H-12), 7.00 (1H, br s, H-14), 7.35−7.55 (5H, overlap, MTPA phenyl protons). **2a**: ^1H NMR ($CDCl_3$, 500 MHz) δ_H 0.94 (1H, m, H-4a), 0.97 (1H, m, H-4b), 1.04 (1H, m, H-3a), 1.07 (1H, m, H-3b), 1.16 (3H, br s, H_3-19), 1.75 (2H, m, H-5), 1.83 (3H, br d, *J* = 7.3 Hz, H-18), 1.96 (3H, br s, H_3-20), 2.19 (3H, s, NAc), 2.35 (3H, s, H_3-21), 3.55 (3H, s, MTPA OCH_3), 3.58 (1H, m, H-1a), 4.00 (1H, m, H-1b), 4.47 (1H, m, H-2), 5.09 (1H, br t, *J* = 7.3 Hz, H-6), 5.39 (1H, br, NH), 5.74 (1H, m, H-17), 5.99 (1H, br s, H-8), 6.36 (1H, br d, *J* = 11.6 Hz, H-16), 6.94−7.03 (2H, overlap, H-11 and H-12), 7.11 (1H, br s, H-14), 7.35−7.55 (5H, overlap, MTPA phenyl protons). **1b**: ^1H NMR ($CDCl_3$, 500 MHz) δ_H 1.17 (1H, m, H-3a), 1.22 (1H, m, H-3b), 1.13 (3H, br s, H_3-19), 1.34 (1H, m, H-4a), 1.37 (1H, m, H-4b), 1.78 (2H, m, H-5), 2.00 (3H, br s, H_3-20), 2.05 (3H, s, NAc), 2.32 (3H, s, H_3-21), 3.24 (1H, m, H-16a), 3.31 (1H, m, H-16b), 3.48 (1H, m, H-1a), 3.49 (3H, s, MTPA OCH_3), 3.74 (1H, m, H-1b), 4.47 (1H, m, H-2), 5.04 (1H, br d, *J* = 10.4 Hz, H-18a), 5.06 (1H, br d, *J* = 17.1 Hz, H-18b), 5.09 (1H, br t, *J* = 7.3 Hz, H-6), 5.22 (1H, br, NH), 5.90 (1H, m, H-17), 6.03 (1H, br s, H-8), 6.94−7.03 (2H, overlap, H-11 and H-12), 7.00 (1H, br s, H-14), 7.35−7.55 (5H, overlap, MTPA phenyl protons). **2b**: ^1H NMR ($CDCl_3$, 500 MHz) δ_H 1.17 (1H, m, H-3a), 1.22 (1H, m, H-3b), 1.14 (3H, br s, H_3-19), 1.34 (1H, m, H-4a), 1.37 (1H, m, H-4b), 1.78 (2H, m, H-5), 1.83 (3H, br d, *J* = 7.3 Hz, H-18), 1.97 (3H, br s, H_3-20), 2.05 (3H, s, NAc), 2.35 (3H, s, H_3-21), 3.48 (1H, m, H-1a), 3.49 (3H, s, MTPA OCH_3), 3.74 (1H, m, H-1b), 4.47 (1H, m, H-2), 5.09 (1H, br t, *J* = 7.3 Hz, H-6), 5.22 (1H, br, NH), 5.74 (1H, m, H-17), 5.99 (1H, br s, H-8), 6.36 (1H, br d, *J* = 11.6 Hz, H-16), 6.94−7.03 (2H, overlap, H-11 and H-12), 7.11 (1H, br s, H-14), 7.35−7.55 (5H, overlap, MTPA phenyl protons).

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