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NEW ANTIBACTERIAL POLYACETYLENES FROM SUNFLOWER (*HELIANTHUS ANNUUS* L.) SEEDLINGS

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Abstract ― Three new C_{15} polyacetylenes **1**, **2**, and **3**, together with two known C_{15} polyacetylenes **4** and **5** were isolated from the seedlings of sunflower *Helianthus annuus* L. cv. Russia, and their structures were elucidated by spectroscopic data and chemical means. Compounds **1**, **2**, **4**, and **5** exhibited antimicrobial activity against *Staphyrococcus aureus* and especially compound **1** showed strong activity.

INTRODUCTION

Polyacetylenes have been found in many families of higher plants, such as Asteraceae, Araliaceae, and Umbelliferae.[1-3] It has been reported antibacterial,[4,5] antifungal,[5,6] and allelopathic activities.[7-9] In our previous research, C_{17} polyacetylenes from *Hedera rhombea* exhibited antimicrobial activity against the *Micrococcus luteus*.[4] We had been isolated C_{15} polyacetylenes, 8-(β-D-glucopyranosyloxy)-3-hydroxy-1,9,14-pentadecatriene-4,6-diyne termed “helian”, (*Z*)-3,8-dihydroxy-1,9,14-pentadecatriene-4,6-diyne (**4**), and (*Z*)-8-acetoxy-3-hydroxy-1,9,14-pentadecatriene-4,6-diyne (**5**) and reported for its plant growth activity on rice and cress seedlings.[10,11] However, to the best of our knowledge, effects on antimicrobial activity of C_{15} polyacetylenes have not been studied. In this paper, we describe the isolation and structure elucidation of new antibacterial polyacetylenes **1**~**3** from *H. annuus*, and the assessment of the antibacterial propaties of C_{15} polyacetylenes.

RESULTS AND DISCUSSION

The MeOH extract of the seedlings of *H. annuus* L. cv. Russia was partitioned between EtOAc and H_2O. The EtOAc-soluble portion was subjected to silica gel column chromatography, C_{18} Sep-Pak cartridges,

and reversed-phase HPLC to yield three new C_{15} polyacetylenes **1** (2.1×10^{-5}%), **2** (1.4×10^{-4}%), and **3** (6.5×10^{-5}%), together with two known C_{15} polyacetylenes, (*Z*)-3,8-dihydroxy-1,9,14-pentadecatriene-4,6-diyne (**4**) and (*Z*)-8-acetoxy-3-hydroxy-1,9,14-pentadecatriene-4,6-diyne (**5**). Compounds **4** and **5** had been previously isolated from *Grangea maderaspatana*.[12,13]

4 : $R_1 = R_2 = H$
5 : $R_1 = H$, $R_2 = Ac$

Figure 1. Chemical structures of **1~5**

(*Z*)-8-Acetoxy-1,2-epoxy-9,14-pentadecatriene-4,6-diyne (**1**), a pale yellow oil, $[\alpha]^{28}_{D}$ +68° (*c* 0.25, CH_2Cl_2) was assigned a molecular formula of $C_{17}H_{18}O_4$ by HREISMS [*m/z* 287.1287 $(M+H)^+$, Δ+0.4 mmu]. The UV absorptions (254, 270, and 286 nm) were in good agreement with published data of diacetylene, suggesting the presence of a conjugated diyne.[14] The IR spectrum (2362, 1745, 1654, and 1637 cm^{-1}) showed the presences of acetylene, ester carbonyl, ketone carbonyl, and olefin, respectively. The ^{13}C NMR data (Table 1) aided by the HMQC spectrum of **1** exhibited the signals due to a ketone carbonyl carbon at δ_C 183.0 (C-3), an acetoxy carbon at δ_C 169.3 and 20.8, four olefinic carbons at δ_C 138.1 (C-14), 137.0 (C-10), 123.0 (C-9), and 115.1 (C-15), four acetylene quaternary carbons at δ_C 84.5 (C-7), 76.5 (C-5), 72.7 (C-4), and 68.1 (C-6), an acetoxy-bearing carbon at δ_C 59.8 (C-8), a lower-field shifted methine carbon at δ_C 54.0 (C-2) and methylene carbon at δ_C 46.7 (C-1), and three methylene carbons at δ_C 31.7 (C-13), 28.2 (C-12), and 27.3 (C-11).

The ^1H NMR data (Table 2) showed signals for a proton attached to an acetoxy-bearing carbon at δ_H 6.16 (H-8), three olefinic protons at δ_H 5.79 (H-14), 5.72 (H-10), and 5.50 (H-9), two terminal olefinic protons at δ_H 5.02 (H-15a) and 4.98 (H-15b), three oxygen-bearing protons at δ_H 3.54 (H-2), 3.11 (H-1a), and 3.07 (H-1b), an acetoxy group at δ_H 2.10, and a methylene sequence at δ_H 2.17 (H-11), 2.07 (H-13), and 1.49(H-12). The *Z*-geometry of the double bond (C-9, 10) was deduced on the basis of the ^1H-^1H coupling constant ($J_{9,10}$=10.5 Hz). The chemical shifts of the oxymethine proton (δ_H 3.54), oxymethylene proton (δ_H 3.11 and 3.07), and carbons (δ_C 54.0 and 46.7) indicated the presence of a terminal epoxide

Table 1. ^{13}C NMR Data of compounds **1-3** in $CDCl_3$[a]

position	1	2	3
1	46.7, CH_2	9.3, CH_3	119.8, CH_2
2	54.0, CH	30.7, CH_2	131.8, CH
3	183.0, C=O	64.1, CH	64.4, CH
4	72.7, qC	83.4, qC	75.1, qC
5	76.5, qC	85.7, qC	70.7, qC
6	68.1, qC	69.9, qC	69.2, qC
7	84.5, qC	79.5, qC	76.6, qC
8	59.8, CH	17.7, CH_2	60.0, CH
9	123.0, CH	122.1, CH	124.0, CH
10	137.0, CH	132.9, CH	136.0, CH
11	27.3, CH_2	27.1, CH_2	27.2, CH_2
12	28.2, CH_2	28.7, CH_2	28.2, CH_2
13	31.7, CH_2	33.7, CH_2	33.1, CH_2
14	138.1, CH	139.0, CH	138.2, CH
15	115.1, CH_2	114.3, CH_2	115.0, CH_2
3-OAc	20.8, CH_3		20.8, CH_3
	169.3, qC		169.4, qC
8-OAc			20.9, CH_3
			169.4, qC

[a] δ_C in ppm.

comparing with those of (*Z*)-8-acetoxy-1-methoxy-3-oxoheptadeca-9-ene-4,6-diyne.[14] This finding was further supported by vicinal coupling constants of $J_{1a, 2}$ = 2.3 Hz and $J_{1b, 2}$ = 4.3 Hz. The chemical shift of H-2 (δ_H 3.54), which was at lower field by nearly 1 ppm compared to the value of typical epoxide protons, suggesting that the carbonyl carbon should be connected to C-2. This linkage was further confirmed by an HMBC correlation between H-1a (δ_H 3.11) and C-3 (δ_C 183.0). The higher-field shifted ketone carbonyl carbon (C-3) suggested that it was conjugated to a triple bond. This was also supported by chemical shift to lower frequency of the carbonyl peak (1654 cm^{-1}) in the IR spectrum.[14] An HMBC correlation of H-8 to OAc (δ_C 169.3) revealed the location of the acetoxy group at C-8. On the other hand, HMBC correlations of H-8 to C-4, C-5, C-6, and C-7 and H-9 to C-7 confirmed that the acetoxy-bearing carbon (C-8) was connected to an acetylenic carbon (C-7). Consequently, compound **1** was determined to be (*Z*)-8-acetoxy-1,2-epoxy-9,14- pentadecatriene-4,6-diyne.

Table 2. ^{1}H NMR Data of compounds **1-3** in $CDCl_3$[a]

position	1	2	3
1a	3.11, dd (5.8, 2.3)[b]	1.02, m	5.54, d (16.9)
1b	3.07, dd (5.8, 4.3)		5.34, d (11.1)
2	3.54, dd (4.3, 2.3)	1.75, m	5.85, ddd (16.9, 11.1, 5.9)
3		4.38, t (6.1)	5.90, d (5.9)
8	6.16, dd (8.6, 2.3)	3.15, m	6.11, d (8.8)
9	5.50, dd (10.5, 8.6)	5.39, dt (10.5, 8.8)	5.49, dd (10.6, 8.8)
10	5.72, ddt (10.5, 7.2, 2.5)	5.72, dt (10.5, 8.8)	5.66, dt (10.6, 7.6)
11	2.17, m	2.05, m	2.16, m
12	1.49, m	1.39, m	1.49, m
13	2.07, m	2.05, m	2.06, m
14	5.79, ddt (17.1, 10.2, 6.6)	5.81, ddt (17.1, 10.2, 6.9)	5.78 ddt (17.1, 10.3, 6.8)
15a	5.02, ddt (17.1, 1.7, 0.9)	4.99, ddt (17.1, 2.0, 1.6)	5.02, ddt (17.1, 1.8, 1.6)
15b	4.98, ddt (10.2, 1.7, 0.5)	4.94, ddt (10.2, 2.0, 1.0)	4.97, ddt (10.3, 1.8, 1.2)
3-OAc			2.10, s
8-OAc	2.10, s		2.08, s

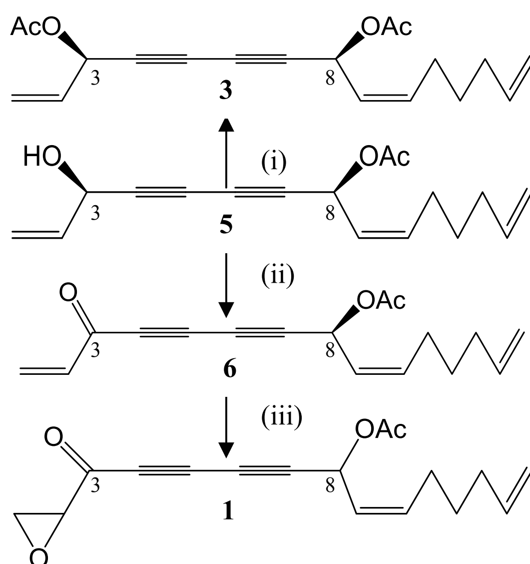
[a] δ_H in ppm, [b] ^{1}H-^{1}H coupling constants (J in Hz) in parentheses.

The molecular formula of **2** was assigned as $C_{15}H_{20}O$ by the HREIMS [m/z 215.1396 $(M-H)^+$, Δ-4.0 mmu] and the ^{13}C NMR data. In the ^{13}C NMR data (Table 1) aided by the HMQC and HMBC of **2**, signals due to four olefinic carbons at δ_C 139.0 (C-14), 132.9 (C-10), 122.1 (C-9), and 114.3 (C-15), four acetylene quaternary carbons at δ_C 85.7 (C-5), 83.4 (C-4), 79.5 (C-7), and 69.9 (C-6), an oxymethine carbon at δ_C 64.1 (C-3), five methylene carbons at δ_C 33.7 (C-13), 30.7 (C-2), 28.7 (C-12), 27.1 (C-11), and 17.7 (C-8), and a methyl carbon at δ_C 9.3 (C-1) were observed. The ^{1}H NMR spectrum (Table 2) showed signals for three olefinic protons, two terminal olefinic protons, two oxygen-bearing protons, four methylene protons, and a methyl proton. The partial structures of C-1−C-3 and C-8−C-15 could be deduced from consideration of the ^{1}H-^{1}H COSY of **2**. On the basis of this spectroscopic evidence, the structure of **2** was elucidated to be (*Z*)-3-hydroxy-9,14-pentadecatriene-4,6-diyne (**2**).

The molecular formula of **3** was assigned as $C_{19}H_{22}O_4$ by the HRESIMS [m/z 337.1431 $(M+Na)^+$, Δ+1.5 mmu] and the ^{13}C NMR data. The ^{13}C and ^{1}H NMR data (Tables 1 and 2) of **3** were partially similar to those of (*Z*)-8-acetoxy-3-hydroxy-1,9,14-pentadecatriene-4,6-diyne (**5**).[12,13] However, two signals of acetoxy carbons at δ_C 169.4, 20.8 and δ_C 169.4, 20.9 were observed in **3**. The ^{13}C NMR spectrum (Table 1) of **3** showed 19 carbon signals including four quaternary carbon signals (δ_C 76.6, 75.1, 70.7, and 69.2). Additionally, the ^{13}C NMR spectrum (Table 2) exhibited two acetoxy-bearing carbons at δ_C 60.0 and 64.4. The ^{1}H NMR spectrum of **3** showed characteristic signals for two protons at δ_H 6.11 and 5.90 attached to

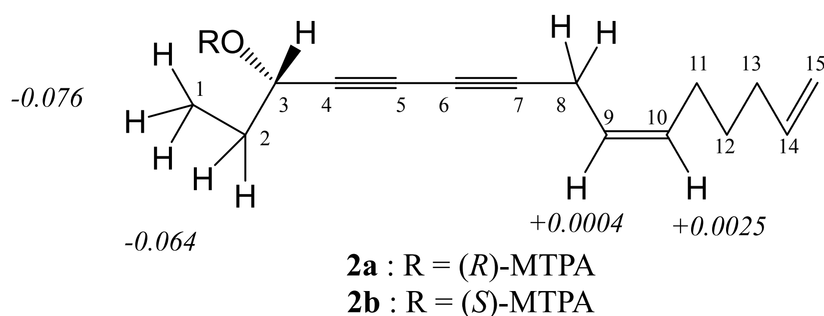
an acetoxy-bearing carbon and two acetoxy groups at δ_H 2.10 and 2.08. To establish the structure of **3**, compound **5** was acetylated by acetic anhydride in pyridine to give compound **3** (quant.). The $[\alpha]_D$ and ^{1}H NMR data of the synthetic product **3** were identical with those of natural compound **3**.

The structure of **1** was further confirmed by the synthesis of **1** from **5** by two steps as follows. First, compound **5** was oxidized by active MnO_2 in anhydrous CH_2Cl_2 to afford (*Z*)-8-acetoxy-3-oxopentadeca-1,9,14-triene-4,6-diyne (**6**) (87%). The structure of compound **6** was elucidated by spectroscopic data. Compound **6** was epoxidized by 3% H_2O_2 in acetone containing 1% Na_2CO_3 to give compound **1** (48%). Although synthetic compound **1** was a mixture of diastereomers, it was difficult to separate each isomer. Since the spectroscopic data of synthetic compound **1** was very similar to that of natural compound **1**, the gross structure of **1** was also confirmed by the derivatization of **1** from **5**.



Scheme 1. Derivatizations of **1** and **3** from **5**: (i) Ac_2O, Pyr, rt, 60 min, quant. (ii) MnO_2, CH_2Cl_2, rt, 60 min, 87%. (iii) 3 %H_2O_2, 1%Na_2CO_3, acetone, 0 °C, 60 min, 48%.

The absolute configuration of **2** was determined by modified Mosher's method[15] as follows. Compound **2** was treated with (*S*)- and (*R*)-α-methoxy-α-trifluoromethyl-phenylacetyl chloride (MTPA-Cl) in pyrindine-d_5 to give the (*R*)-MTPA ester derivative (**2a**) and (*S*)-MTPA ester derivataive (**2b**) of **2**. In the ^{1}H NMR spectrum of the (*S*)-MTPA ester (**2b**), proton signals assigned to H-1 and H-2 were observed at higher field than those of the (*R*)-MTPA ester (**2a**), while signals due to H-8, H-9, and H-10 in **2a** were shifted to a higher field than those in **2b**. Therefore, the absolute configuration at C-3 was concluded to be 3*S* (Figure 2).



2a : R = (*R*)-MTPA
2b : R = (*S*)-MTPA

Figure 2. Δδ Values [Δδ(in ppm)=δ_S-δ_R] obtained for the (*S*)- and (*R*)-MTPA esters (**2b** and **2a**, respectively) of **2**

The antibacterial activities of polyacetylenes against gram positive bacteria have been reported.[4,5] Therefore, new, known, and synthetic compounds **1-6** were tested antimicrobial activities against a gram positive bacterium *Staphylococcus aureus* by plate diffusion assay (Table 3). Compounds **1**, **2**, **4**, and **5** inhibited its growth, especially compound **1**, possessed an epoxide ring, showed strongest activity. Additionally, the structures have some hydroxy groups exhibited activities. These results suggested that the key sites for activity of a series of polyacetylenes from *H. annuus* are a free hydroxy group at C-3 and an epoxide ring.

Table 3. Antimicrobial activities of **1~6** against *Staphylococcus aureus*[a]

	Diameter of inhibition zone (mm)[b]					
Concentrations	**1**	**2**	**3**	**4**	**5**	**6**
50 μg	13	9	-[c]	-	10	-
100 μg	13	10	-	10	12	-

[a] inhibition zone (31 mm) of ampicillin (13 μg) as a positive control, [b] a paper disc (i. d. 8 mm), [c] no inhibition zone

EXPERIMENTAL

General Procedures.

Optical rotations were measured with a JASCO DIP-370 polarimeter. UV spectra were recorded on a HITACHI U-2000A spectrometer. IR spectra were recorded on a JASCO FT/IR-300 spectrometer. ^{1}H and ^{13}C NMR spectra were measured and recorded on a Bruker Avance 500 spectrometer in $CDCl_3$. The resonances of $CDCl_3$ at δ_H 7.26 and δ_C 77.0 were used as internal references for the ^{1}H and ^{13}C NMR spectra, respectively. HRESIMS and HREIMS were recorded on Waters Xevo Q-Tof, Waters Synapt G2 mass spectrometer, and JEOL JMS-T100LC.

Plant material.

Seeds of sunflower (*Helianthus annuus* L. cv. Russia) were spread evenly on moist vermiculite in trays and incubated at 25 °C in the dark for 7~10 days. Some of the seedlings (hypocotyls length, ca. 12 cm) were harvested, collected, and frozen at -30 °C until use. The other were illuminated by blue light (λ_{max} 445 nm, 1.90 μmol $m^{-2}s^{-1}$) for 1 h. The blue light-illuminated seedlings were also harvested, collected and frozen at -30 °C until use.

Extraction and Isolation of Compounds 1, 2, and 5.

The seedlings illuminated and not (2.3 kg) by blue light were homogenized in MeOH (1.5 L). The homogenate was filtered and allowed to dry *in vacuo* at 40 °C. The MeOH extracts were partitioned between EtOAc (100 mL×3) and H_2O (100 mL). The EtOAc-soluble portion (2.14 g) was subjected to silica gel column chromatography (ϕ2.4×35 cm) eluting with *n*-hexane/acetone (20:1 to 0:1) and then $CHCl_3$/MeOH (1:1 to 0:1) to separate into 28 fractions (EA-1~EA-28). Fraction EA-13 (18.2 mg) eluted with *n*-hexane/acetone (5:1) was applied to a C_{18} Sep-Pak cartridge (Waters, MeOH/H_2O, 3:2 to 1:0) to afford 10 fractions (EA13-1~ EA13-10). Fraction EA-13-2 (6.2 mg) eluted with MeOH/H_2O (3:2) was separated by reversed-phase HPLC [TSK-gel ODS-120A, TOSOH, ϕ7.8 mm×30 cm, flow rate 2.0 mL/min, MeCN/H_2O (3:7 to 1:0)] to give **1** (0.5 mg, t_R 35.1 min) and **5** (0.3 mg, t_R 36.9 min), respectively. Fraction EA-8 (9.2 mg) eluted with *n*-hexane/acetone, 10:1 was applied to a C_{18} Sep-Pak cartridge eluted with MeOH/H_2O (3:2 to 1:0) to give **2** (1.1 mg, EA8-4) eluted with MeOH/H_2O (4:1).

Extraction and Isolation of Compounds 3 and 4.

The seedlings (1.0 kg) were freeze-dried for five days before extract. The homogenate was filtered and allowed to dry *in vacuo* at 40 °C. The MeOH extracts were partitioned between EtOAc (100 mL×3) and H_2O (100 mL). The EtOAc-soluble portion (1.32 g) was subjected to silica gel column chromatography (ϕ1.0×35 cm) eluting with *n*-hexane/acetone (20:1 to 0:1) and then $CHCl_3$:MeOH (1:1 to 0:1) to separate into 19 fractions (EAII-1~EAII-19). Fraction EAII-7 (20.9 mg) eluted with *n*-hexane/acetone, 10:1 was separated by reversed-phase HPLC [TSK-gel ODS-120A, Tosoh, Japan, ϕ7.8 mm×30.0 cm, flow rate 2.0 mL/min, MeCN/H_2O (2:3 to 1:0)] to give **3** (0.7 mg, t_R 40.6 min). The ^{1}H and ^{13}C NMR data of EAII-15 eluted with *n*-hexane/acetone (3:1) was identical to (*Z*)-3,8-diacetoxy-1,9,14-pentadecatriene-4,6-diyne (**3**, 10.9 mg).

(*Z*)-8-Acetoxy-1,2-epoxy-9,14-pentadecatriene-4,6-diyne (1): Pale yellow oil; $[\alpha]_D^{28}$ +68 (*c* 0.25, CH_2Cl_2); IR (film) ν_{max} 2921, 2362, 1745, 1654, 1637, and 1227 cm^{-1}; UV (MeOH) λ_{max} (logε) 254 (4.2), 270 (4.2), and 286 (4.1) nm; ^{13}C and ^{1}H NMR (Tables 1 and 2); ESIMS (positive ion) *m/z* 287$(M+H)^+$;

HRESIMS (positive ion) *m/z* 287.1287 $(M+H)^+$, (calcd for $C_{17}H_{19}O_4$, 287.1283).

(*Z*)-3-Hydroxy-9,14-pentadecatriene-4,6-diyne (2): Colorless oil; $[\alpha]_D^{28}$ -20 (*c* 0.05, MeOH); IR (film) ν_{max} 3412, 2925, 2344, and 1637 cm^{-1}; UV (MeOH) λ_{max} (logε) 213 (4.0), 253 (3.5), 267 (3.5), and 283 (3.5) nm; ^{13}C and ^1H NMR (Tables 1 and 2); EIMS *m/z* 215 $(M-H)^+$; HREIMS *m/z* 215.1396 $(M-H)^+$, (calcd for $C_{15}H_{19}O$, 215.1436).

(*Z*)-3,8-Diacetoxy-1,9,14-pentadecatriene-4,6-diyne (3): Pale yellow oil; $[\alpha]_D^{28}$ +81 (*c* 0.21, CH_2Cl_2); IR (film) ν_{max} 2929, 2259, 1747, 1640, and 1221 cm^{-1}; UV (MeOH) λ_{max} (logε) 234 (4.1), 246 (4.1), and 260 (4.0) nm; ^{13}C and ^1H NMR (Tables 1 and 2); ESIMS *m/z* 337$(M+Na)^+$; HRESIMS (positive ion) *m/z* 337.1431 $(M+Na)^+$, (calcd for $C_{19}H_{22}O_4Na$, 337.1416).

Oxidation of 5.

Active MnO_2 (33.0 mg, 3.8×10^{-1} mmol) was added to a solution of compound **5** (5.2 mg, 1.91×10^{-2} mmol) in anhydrous CH_2Cl_2 (1.0 mL) and the mixture was stirred at room temperature for 60 min. The reaction mixture was filtered through Celite and the residue was washed with $CHCl_3$. The filtrate was evaporated to dryness *in vacuo* to give (*Z*)-8-acetoxy-3-oxopentadeca-1,9,14-triene-4,6-diyne (**6**, 4.5 mg, 87%): Pale yellow oil; ^1H NMR (500 MHz, $CDCl_3$): δ_H 6.56 (1H, d, *J* = 17.5 Hz, H-1a), 6.41 (1H, d, *J* =17.5 and 10.3 Hz, H-2), 6.24 (1H, d, *J* = 10.3 Hz, H-1b), 6.17 (1H, d, *J* = 8.8 Hz, H-8), 5.79 (1H, ddt, *J* = 17.1, 10.2, and 6.6 Hz, H-14), 5.72 (1H, dtd, *J* = 10.4, 7.7, and 2.7 Hz, H-10), 5.51 (1H, ddt, *J* = 10.3, 8.9, and 1.6 Hz, H-9), 5.02 (1H, ddt, *J* = 17.1, 2.0, and 1.6 Hz, H-15a), 4.98 (1H, ddt, *J* = 10.2, 2.0, and 1.3 Hz, H-15b), 2.18 (2H, m, H-11), 2.10 (3H, s, OAc), 2.07 (2H, m, H-13), and 1.50 (2H, m, H-12); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 177.3 (C-3), 169.4 (OAc), 138.1 (C-14), 137.6 (C-2), 136.8 (C-10), 134.7 (C-1), 123.3 (C-9), 115.1 (C-15), 83.3 (C-7), 75.2 (C-5), 74.0 (C-4), 68.3 (C-6), 59.9 (C-8), 33.1 (C-13), 28.2 (C-12), 27.3 (C-11), and 20.8 (OAc); HMBC correlations: ($CDCl_3$, H/C) 1a/2, 1a/3, 1b/2, 1b/3, 2/1, 2/3, 2/4, 8/5, 8/6, 8/7, 8/9, 8/10, 8/$CH_3\underline{C}O$, 9/11, 10/8, 11/9, 11/10, 11/12, 11/13, 12/10, 12/11, 12/13, 13/12, 13/14, 13/15, 14/12, 14/13, 15a/13, 15b/13, and $C\underline{H}_3CO$/$CH_3\underline{C}O$.

Epoxidation of 6.

To a solution of **6** (7.3 mg, 2.7×10^{-2} mmol) in acetone (500 μL) at 0 °C were added 3% H_2O_2 (225 μL, 2.7×10^{-1} mmol) and 1% Na_2CO_3 aq. (28.7 μL, 2.7×10^{-3} mmol) and the mixture was stirred at 0 °C for 60 min. The reaction mixture was partitioned between EtOAc (10 mL) and H_2O (10 mL) and then the EtOAc layer was dried with $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel TLC (*n*-hexane/acetone, 10:1) to give (*Z*)-8-acetoxy-1,2-epoxy-9,14-pentadecatriene-4,6-diyne (**1**, 3.8 mg, 48%).

Acetylation of 5.

Acetic anhydride (0.5 mL) was added to a solution of compound **5** (5.6 mg, 2.0×10^{-2} mmol) in pyridine (0.6 mL) and the mixture was stirred at room temperature for 60 min. After added toluene to remove pyridine it was evaporated to dryness *in vacuo* to give (*Z*)-3,8-diacetoxy-1,9,14-pentadecatriene-4,6-diyne (**3**, 6.5 mg, quant).

Preparation of the (*R*)- and (*S*)-MTPA Ester Derivatives of 2.

(R)-MTPA ester of 2 (2a): (*S*)-MTPA chloride (2 μL) was added to a solution of compound **2** (0.4 mg) in pyridine-d_5 (0.6 mL). After standing the reaction mixture at room temperature for 1day, the solution was evaporated to dryness under N_2 gas stream.

^{1}H NMR ($CDCl_3$): 0.9984 (3H, m, H-1), 1.3653 (2H, m, H-12), 1.8757 (2H, m, H-2), 2.0294 (4H, H-11 and H-13), 3.0189 (2H, m, H-8), 4.9272 (1H, ddt, J = 10.2, 2.2, and 1.0 Hz, H-15b), 4.9851 (1H, ddt, J = 17.1, 2.2, and 1.6, H-15a), 5.3720 (1H, m, H-9), 5.4997 (1H, m, H-3), 5.5095 (1H, m, H-10), and 5.7954 (1H, ddt, J = 17.1, 10.2, and 6.8 Hz, H-14).

(S)-MTPA ester of 2 (2b): (*R*)-MTPA chloride (2 μL) was added to a solution of compound **2** (0.4 mg) in pyridine-d_5 (0.6 mL). After standing the reaction mixture at room temperature for 1day, the solution was evaporated to dryness under N_2 gas stream.

^{1}H NMR (500 MHz, $CDCl_3$): 0.9221 (3H, m, H-1), 1.3652 (2H, m, H-12), 1.8117 (2H, m, H-2), 2.0293 (4H, H-11 and H-13), 3.0223 (2H, m, H-8), 4.9270 (1H, ddt, J = 10.0, 2.0, and 1.0 Hz, H-15b), 4.9850 (1H, ddt, J = 17.1, 2.0, and 1.5 Hz, H-15a), 5.3724 (1H, m, H-9), 5.5331 (1H, m, H-3), 5.5120 (1H, m, H-10), and 5.7955 (1H, ddt, J = 17.1, 10.0, and 6.7 Hz, H-14).

Antimicrobial test.

Antimicrobial activity against gram positive-bacterium *Staphylococcus aureus* KB210 was tested by plate diffusion assay using 8 mm paper disk. Compound solutions were prepared by dissolving each compound in acetone. Each adjusted solution was added in paper disk (10 and 20 μL) and paper disk were drying. The paper disks were set on the agar plate suspended *S. aureus*. After cultivating microorganisms for 24 h, the strength of antimicrobial activity was estimated by measuring the diameter length of inhibition zone (mm).

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