New Eremophilane-Type Sesquiterpenoids, Eremoxylarins A and B from Xylariaceous Endophytic Fungus YUA-026

Yoshihito Shiono and Tetsuya Murayama

Department of Bioresource Engineering, Faculty of Agriculture, Yamagata University, Tsuruoka, Yamagata 997-8555, Japan

Reprint requests to Dr. Y. Shiono. Fax: +81-235-28-2820. E-mail: yshiono@tds1.tr.yamagata-u.ac.jp

Z. Naturforsch. **60b,** 885 – 890 (2005); received November 29, 2004

Two new eremophilane sesquiterpenes, eremoxylarins A and B, were isolated from the xylariaceous endophytic fungus YUA-026. Their structures were determined by spectroscopic methods. Eremoxylarins A and B showed antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Key words: Eremoxylarins A and B, Xylariaceae

Introduction

Endophytic fungi live inside of stems, roots and leaves of the host plant. These microorganisms have recently been recognized as rich source of structurally novel and biologically active secondary metabolites [1]. During our screening for bioactive compounds from endophytic fungi, three antimicrobial epoxycyclohexenone derivatives were isolated from unpolished rice fermented with xylariaceous endophytic fungus strain YUA-026 [2]. As a part of our continuing search for bioactive compounds from this fungus, we have isolated two eremophilane-type sesquiterpenoids, eremoxylarins A (**1**) and B (**2**). We describe in this paper the isolation and structural elucidation of compounds **1** and **2**, and their antimicrobial activity.

Results and Discussion

The purification of these metabolites was guided by their antimicrobial activity against *Pseudomonas aeruginosa* and intense blue characteristic coloration with vanillin-sulfuric acid solution on TLC plates. The fungus YUA-026 was stationarily cultured at 25 °C for 3 weeks in unpolished rice. Following fermentation, the MeOH extract of the moldy unpolished rice was concentrated and partitioned with EtOAc. The EtOAc extract was chromatographed on a column of silica gel. Two fractions obtained were chosen for further purification of ODS column chromatography to afford eremoxylarins A (**1**) and B (**2**).

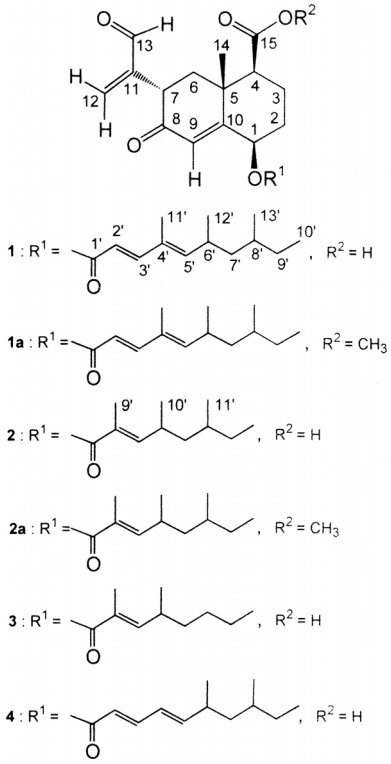


Fig. 1. The structures of eremoxylarins A (**1**) and B (**2**), methyl esters **1a** and **2a**, integric acid (**3**) and 07H239-A (**4**).

The molecular formula of **1**, $C_{28}H_{38}O_6$, was determined by HRFABMS measurement. The IR spectrum exhibited bands at 1733, 1718, 1685 and 1653 cm^{-1}, characteristic of multiple carbonyl groups. ^{13}C NMR

Table 1. ^{13}C (100 MHz) and ^{1}H (400 MHz) NMR Data for Eremoxylarins A (**1**) and B (**2**) (in CD_3OD)[a].

	1			**2**		
No.	δ_C (Mult.)	δ_H (Mult. *J* Hz)	HMBC (^{1}H to ^{13}C)	δ_C (Mult.)	δ_H (Mult. *J* Hz)	HMBC (^{1}H to ^{13}C)
1	75.1 d	5.49 (1H, br. s)	1', 3, 5, 9	75.6 d	5.49 (1H, br. s)	1', 3, 5, 9
2	31.6 t	α 1.77 (1H, m)	4, 10	31.6 t	α1.80 (1H, m)	1, 4
		β 2.26 (1H, m)	4, 10		β2.13 (1H, m)	1, 4, 10
3	22.1 t	2.09[b]		22.1 t	1.74 (1H, m)	4, 5
		2.32 (1H, t, 13.2)	1, 5, 15		2.26 (1H, m)	1, 4, 5, 15
4	55.3 d	2.44 (1H, dd, 13.2, 2.9)	5, 6, 14, 15	55.4 d	2.45 (1H, dd, 13.2, 2.9)	2, 5, 6, 14, 15
5	40.0 s			40.1 s		
6	45.1 t	2.09[b]		45.2 t	2.08 (1H, m)	4, 8, 10, 11, 14
		2.36 (1H, t, 14.5)	8, 10, 11		2.38 (1H, t, 14.7)	4, 8, 14
7	45.6 d	3.73 (1H, dd, 14.5, 5.5)	5, 6, 8, 11, 12, 13	45.8 d	3.75 (1H, dd, 14.7, 4.4)	5, 6, 8, 11, 12, 13
8	199.9 s			200.2 s		
9	131.0 d	6.02 (1H, s)	1, 5, 7	131.0 d	6.02 (1H, s)	1, 5, 7
10	162.5 s			162.7 s		
11	150.4 s			150.6 s		
12	138.4 t	6.29 (1H, s)	7, 11, 13	138.5 t	6.31 (1H, s)	7, 11
		6.42 (1H, s)	7, 11, 13		6.43 (1H, s)	7, 11, 13
13	195.5 d	9.49 (1H, s)	7, 11, 12	195.6 d	9.50 (1H, s)	7, 12
14	20.3 q	1.48 (3H, s)	4, 5, 6, 10	20.4 q	1.48 (3H, s)	4, 5, 6, 10
15	176.6 s			176.8 s		
1'	168.2 s			169.0 s		
2'	116.9 d	5.80 (1H, d, 15.6)	1', 4'	128.1 s		
3'	152.7 d	7.31 (1H, d, 15.6)	1', 5', 11'	151.4 d	6.54 (1H, dd, 10.3, 1.5)	1', 4', 5', 9'
4'	133.4 s			33.0 d	2.68 (1H, m)	2', 3', 5', 6', 10'
5'	151.4 d	5.70 (1H, d, 9.8)	6', 7', 11', 12'	46.0 t	1.13 – 1.24[b]	
					1.28 – 1.41[b]	
6'	32.8 d	2.69 (1H, m)	4', 5', 12'	34.6 d	1.28 – 1.41[b]	
7'	46.3 t	1.09 – 1.16[b]		32.0 t	1.13 – 1.24[b]	
		1.23 – 1.36[b]			1.28 – 1.41[b]	
8'	34.3 d	1.23 – 1.36[b]		12.5 q	0.87 (3H, t, 7.3)	6', 7'
9'	31.9 t	1.09 – 1.16[b]		13.6 q	1.87 (3H, d, 1.5)	1', 2', 3'
		1.23 – 1.36[b]				
10'	12.5 q	0.84 (3H, t, 7.3)	8', 9'	21.6 q	0.99 (3H, d, 6.8)	3', 4', 5'
11'	13.4 q	1.81 (3H, s)	3', 5'	21.1 q	0.86 (3H, d, 6.4)	5', 6', 7'
12'	22.2 q	0.97 (3H, d, 6.8)	5', 6', 7'			
13'	21.1 q	0.83 (3H, d, 6.8)	7', 8', 9'			

[a] Values in parentheses are coupling constants in Hz, [b] multiplicity patterns were unclear due to signals overlapping.

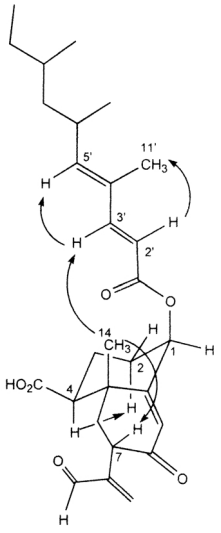
($\delta_C = 176.6$) of **1** and formation of a mono methyl ester (**1a**) by treatment with trimethylsilyldiazomethane supported the presence of a carboxyl group. Its UV spectrum showed an absorption maximum at 269 nm which suggested the presence of a conjugated dienoyl moiety. The gross structure of **1** was deduced from detailed analyses of ^{1}H and ^{13}C NMR data aided with 2D NMR experiments. A close inspection of the ^{13}C NMR (Table 1) and DEPT spectra of **1** showed carbon signals due to five methyls, five sp^3 methylens, five sp^3 methines (one of them bearing an oxygen atom), one sp^3 quaternary carbon, three sp^2 quaternary carbons, four sp^2 methines, one sp^2 methylene, one ester, one conjugated ketone, one carboxylic acid and one aldehyde. The ten unsaturation equivalents required by the molecular formula indicated this compound has two rings. The ^{1}H NMR and HMQC spectra of **1** showed proton signals due to one primary methyl [$\delta_H = 0.84$ (t, $J = 7.3$ Hz, 3H, 10'-H_3)], two secondary methyls [$\delta_H = 0.83$ (d, $J = 6.8$ Hz, 3H, 13'-H_3) and 0.97 (d, $J = 6.8$ Hz, 3H, 12'-H_3)], one tertiary methyl [$\delta_H = 1.48$ (s, 3H, 14-H_3)], one methyl attached to an olefinic carbon [$\delta_H = 1.81$ (s, 3H, 11'-H_3)], one disubstituted [$\delta_H = 5.80$ (d, $J = 15.6$ Hz, 1H, 2'-H) and 7.31 (d, $J = 15.6$ Hz, 1H, 3'-H)] and two trisubstituted double bonds [$\delta_H = 6.02$ (s, 1H, 9-H) and 5.70 (d, $J = 9.8$ Hz, 1H, 5'-H)], vinylidene protons [$\delta_H = 6.29$ (s, 1H, 12-H) and 6.42 (s, 1H, 12-H)], five methines [$\delta_H = 1.23 - 1.36$ (overlapped, 8'-H), 2.44 (dd, $J = 13.2$, 2.9 Hz, 1H, 4-H), 2.69 (m, 1H, 6'-H), 3.73 (dd, $J = 14.5$, 5.5 Hz, 1H, 7-H) and 5.49 (br. s, 1H, 1-H)]. Detailed analyses of the ^{1}H-^{1}H COSY spec-

Table 2. MIC of Eremoxylarins A (**1**) and B (**2**).

	MIC (μg / ml)	
Microorganism	**1**	**2**
Staphylococcus aureus	12.5	25
Pseudomonas aeruginosa	6.25	12.5
Candida albicans	>100	>100
Aspergillus clavatus	>100	>100

trum disclosed the proton connectivities of the following partial structures: 1-H to 2-H_2, 3-H_2 to 2-H_2 and 4-H, 6-H_2 to 7-H, 6'-H to 5'-H, 7'-H_2 and 12-H_3, 7'-H_2 to 8'-H, 8'-H to 13'-H_3, 9'-H to 8'-H and 10'-H_3. The HMBC correlations (Table 1) of 6-H to C-8 and C-10, 7-H to C-5, and 9-H to C-5 and C-7 supported the structure of cyclohexenone ring (C-5$\sim$C-10). The signal 1-H correlated with C-5 and C-9, signal of 2-H with C-10, and signal of 4-H with C-6 and C-14, suggesting thus giving rise to the connectivities of C-1 to C-10, C-4 to C-5 and C-5 to C-14. The correlations between 3-H and C-15, 4-H and C-15 established the position of carboxylic acid at C-4. The position of propenal group was assigned on the basis of HMBC correlations from 7-H to C-12, and 7-H to C-13. A combination of HMQC and HMBC experiments thus permitted the assignment of an eremophilane sesquiterpene skeleton substituted by a decadienoic acid. The HMBC correlations between 11'-H_3 to C-3' and C-5', suggested that there is a 4,6,8-trimethyldeca-2,4-dienoyl moiety in this molecule. In fact, acidic hydrolysis of **1** with 6 N HCl, followed by methylation with trimethylsilyldiazomethane afforded methyl 4,6,8-trimethyldeca-2,4-dienoate. Furthermore, the HMBC correlation between 1-H to C-1' and substantial downfield shift for 1-H revealed the location of the decadienoyl was at C-1. Geometries of two olefins at C-2' / C-3' and C-4' / C-5' of decadienoyl moiety were assigned as all *E* on the basis of the ^{1}H-^{1}H coupling constant ($J_{2',3'} = 15.6$ Hz) as well as NOE correlations between 3'-H and 5'-H, and 2'-H and 11'-H_3 (Fig. 2). These 2D NMR data led to the complete assignments of ^{1}H and ^{13}C signals of **1** as shown in Table 1. Thus the planar structure of eremoxylarin A was assigned as **1**.

The relative configurations of C-1, C-4, C-5, and C-7 in **1** was deduced from NOE experiments in MeOH-d_4. NOE correlations from 14-H_3 to 3'-H, 14-H_3 to 7-H indicated that 14-H_3, 7-H and ester side chain moiety at C-1 were all β-oriented. Furthermore, NOE correlations were observed from 4-H to 2-H α, suggesting that the carboxylic acid moiety was β-oriented. The configurations of the C-7 and C-4 were also supported by the coupling constants between 6-H_2 and 7-H, and



Selected NOE correlations are indicated.

Fig. 2. Relative stereochemistry of eremoxylarin A (**1**).

3-H_2 and 4-H, respectively. However, the configurations of methyl group at C-6' and C-8' and the absolute stereochemisry of **1** were left unassigned.

Eremoxylarin B (**2**) had a molecular formula of $C_{26}H_{36}O_6$, based on HRFABMS. The IR absorption bands at 1729, 1716, 1681 and 1648 cm^{-1} were very similar to those of **1**. The presence of a carboxyl group was also supported by the methylation of **2** with trimethylsilyldiazomethane giving a mono methyl ester **2a**. The ^{1}H and ^{13}C NMR data (Table 1) for **2** were very similar to those of **1** except for the signals of substitution group at C-1. Identification of the substitution group in **2** as 2,4,6-trimethyloct-2-enoyl was deduced from data of COSY, HMBC, and NOE experiments. In addition, hydrolysis of **2** with 6 N HCl, followed by methylation with trimethylsilyldiazomethane afford methyl 2,4,6-trimethyloct-2-enoate. The esterification site was confirmed by an HMBC correlation from H-1 to the ester carbonyl at $\delta_C = 169.0$. Based on the NOE difference experiments, the relative stereochemistry of the eremophilane sesquiterpene moiety in **2** was determined. The configuration of the trisubstituted $\Delta^{2'}$ double bond was assigned as *E* on the basis of the NOE correlation between 9'-H_3 and 4'-H. The configurations at C-4' and C-6' and the absolute stereochemistry of **2** remain uncertain.

Eremoxylarins A and B share the common structural feature with previously reported eremophilane sesquiterpenes, HIV-1 integrase inhibitor, integric acid (**3**) from *Xylaria* sp, and cytotoxic 07H239-A (**4**) from marine-derived xylariaceous fungus LL-07H239, re-

spectively [3, 4]. Integric acid and 07H239-A were esterified at C-1 with a 2,4-dimethyloct-2-enoyl moiety and a 6,8-dimethyldeca-2,4-dienoyl moiety, respectively. Some other fungal eremophilane-type metabolites, dendryphiellins A-G [5 – 7], eremophiline [8], KM-01 [9] and xylarenals A and B [10], also have been isolated.

The antimicrobial activity of eremoxylarins A (**1**) and B (**2**) against Gram-positive and -negative bacteria and against fungi was evaluated using the agar dilution methods. The results are listed in Table 2. The minimum inhibition concentration (MIC) of **1** and **2** was 12.5 μg/ml and 25 μg/ml against *Staphylococcus aureus*, and 6.25 μg/ml and 12.5 μg/ml against *Pseudomonas aeruginosa*, respectively. However, MIC of **1** and **2** against *Candida albicans* and *Aspergillus clavatus* were over 100 μg/ml, suggesting they had little or no activity against *C. albicans* and *A. clavatus*.

Experimental Section

General experimental procedures

Melting points (mp) data are uncorrected. Optical rotation was measured with a Horiba model SEPA-300 polarimeter, IR spectra were recorded with a JASCO J-20A spectrophotometer, and UV spectra were recorded with a Shimadzu UV mini-1240 instrument. Mass spectra were recorded with a JEOL JMS-700 instrument, and ^{1}H and ^{13}C NMR spectra were obtained with a JEOL EX-400 spectrometer. Chemical shifts are given on a δ (ppm) scale with TMS as an internal standard. Column chromatography was conducted on ODS (Fuji Silysia, Japan) and silica gel 60 (Kanto Chemical Co., Inc.). TLC was done on a precoated silica gel plate (Merck), and spots were detected by spraying 10% vanillin in H_2SO_4 followed by heating.

Isolation and cultivation of the fungus

Xylariaceous endophytic fungus YUA-026 was isolated from plant samples (twigs and petiols) collected in April, 2003 from Mt. Takadate, Yamagata, Japan [2]. Identification of this fungus was carried out at NCMIB Japan.

*Isolation of eremoxylarins A (**1**) and B (**2**)*

The strain YUA-026 was cultivated on sterilized unpolished rice (20 g / Petri dish×50) at 25 °C for 3 weeks. The moldy unpolished rice was soaked in MeOH. The MeOH was evaporated *in vacuo* and the aqueous concentrate was extracted with EtOAc. The EtOAc layer was subsequently dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue (10.5 g) was subjected to silica gel column chromatography with mixtures of *n*-hexane–EtOAc, and mixtures of EtOAc-MeOH to give fractions 1 through 13. Fractions 4 and 5 were combined (301 mg) and further chromatographed on ODS by eluting with mixtures of H_2O and MeOH to afford eremoxylarins A (**1**, 66 mg) and B (**2**, 52 mg).

*Eremoxylarin A (**1**)*

White powder. M.p. 131 – 133 °C. – $[\alpha]_D^{20}$ −38° (*c* 3.1, MeOH). – UV (MeOH) $\lambda_{max}(\lg\varepsilon) = 217$ nm (10.4), 269 nm (10.7). – IR (KBr): $\tilde{\nu} = 1733$, 1718, 1685, 1653, 1457 and 1340 cm^{-1}. – ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C {^{1}H} NMR (100 MHz, CD_3OD) data see Table 1. – HRMS (positive mode, FAB): $m/z = 471.2753$ [M+H$^+$] (calcd. for $C_{28}H_{39}O_6$: 471.2742). – MS (positive mode, FAB) $m/z = 471$ [M+H$^+$].

*Eremoxylarin B (**2**)*

White powder. M. p. 128 – 130 °C. – $[\alpha]_D^{20}$ +90° (*c* 2.0, MeOH). – UV (MeOH) $\lambda_{max}(\lg\varepsilon) = 221$ nm (10.0). – IR (KBr): $\tilde{\nu} = 1729$, 1716, 1681, 1648, 1457 and 1313 cm^{-1}. – ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C {^{1}H} NMR (100 MHz, CD_3OD) data see Table 1. – HRMS (positive mode, FAB): $m/z = 445.2591$ [M+H$^+$] (calcd. for $C_{26}H_{37}O_6$: 445.2590). – MS (positive mode, FAB) $m/z = 445$ [M+H$^+$].

*Methyl ester derivative of eremoxylarin A (**1**)*

Eremoxylarin A (**1**, 10 mg) was dissolved in a solution of MeOH, and trimethylsilyldiazomethane (2.0 M in diethylether, 0.05 ml), was added to the solution. The mixture was stirred at 0 °C for 5 min and evaporated to dryness. The residue (11 mg) was subjected to silica gel column chromatography with mixtures of *n*-hexane–EtOAc to afford a mono methyl ester (**1a**, 6.0 mg).

Monomethyl ester (**1a**): – IR (KBr): $\tilde{\nu} = 1735$, 1718, 1685, 1654, 1457 and 1288 cm^{-1}. – ^{1}H NMR (400 MHz, CD_3OD): $\delta = 0.85$ (d, $J = 6.4$ Hz, 3H, 8'-H_3), 0.86 (t, $J = 7.3$ Hz, 3H, 10'-H_3), 0.99 (d, $J = 6.9$ Hz, 3H, 12'-H_3), 1.12 – 1.18 (overlapped, 2H, 7'-H and 9'-H), 1.28 – 1.37 (overlapped, 3H, 7'-H, 8'-H, and 9'-H), 1.45 (s, 3H, 14-H_3), 1.77 (m, 1H, 2-H), 1.83 (m, 3H, 11-H_3), 1.85 (m, 1H, 3-H), 1.96 (m, 1H, 6-H), 2.12 (ddd, $J = 15.2$, 6.8, 4.4 Hz, 1H, 2-H), 2.32 (m, 1H, 3-H), 2.38 (t, $J = 14.2$ Hz, 1H, 6-H), 2.52 (dd, $J = 12.7$, 3.4 Hz, 1H, 4-H), 2.72 (m, 1H, 6'-H), 3.67 (s, 3H, O*Me*), 3.76 (dd, $J = 14.2$, 4.4 Hz, 1H, 7-H), 5.50 (br. s, 1H, 1-H), 5.71 (d, $J = 9.8$ Hz, 1H, 5'-H), 5.81 (d, $J = 15.6$ Hz, 1H, 2'-H), 6.03 (s, 1H, 9-H), 6.30 (s, 1H, 12-H), 6.43 (s, 1H, 12-H), 7.32 (d, $J = 15.6$ Hz, 1H, 3'-H), 9.49 (s, 1H, 13-H). – ^{13}C {^{1}H} NMR (100 MHz, CD_3OD): $\delta = 12.4$ (q, C-10'), 13.3 (q, C-11'), 20.3 (q, C-14), 21.1 (q, C-13'), 22.0 (q, C-12'), 22.1 (t, C-3), 31.6 (t, C-2), 32.0 (t, C-9'), 33.0 (d, C-6'), 34.5 (d, C-8'), 40.4 (s, C-5), 45.2 (t, C-6), 45.9 (d, C-7), 46.4

(t, C-7'), 53.0 (q, O*Me*), 55.5 (d, C-4), 75.2 (d, C-1), 116.9 (d, C-2'), 131.2 (d, C-9), 133.5 (s, C-4'), 138.6 (t, C-12), 150.6 (s, C-11), 151.5 (d, C-5'), 152.8 (d, C-3'), 162.4 (s, C-10), 168.4 (s, C-1'), 175.4 (s, C-15), 195.6 (d, C-13), 200.8 (s, C-8). – MS (EI): m/z (%) = 484 (13) [M^+], 275 (100), 214 (66), 193 (62), 111 (44), 95 (72).

*Methyl ester derivative of eremoxylarin B (***2***)*

Eremoxylarin B (**2**, 10 mg) was converted to a mono methyl ester (**2a**, 6.3 mg) by using a method similar to that in the case of compound **1**.

Mono methyl ester (**2a**): – IR (KBr): $\tilde{\nu}$ = 1731, 1710, 1697, 1685, 1457 and 1218 cm^{-1}. – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.85 (d, J = 6.4 Hz, 3H, 11'-H_3), 0.88 (t, J = 7.3 Hz, 3H, 8'-H_3), 0.99 (d, J = 6.3 Hz, 3H, 10'-H_3), 1.12 – 1.20 (overlapped, 2H, 5'- H and 7'-H), 1.29 – 1.34 (overlapped, 2H, 6'-H and 7'-H), 1.39 (ddd, J = 13.2, 10.3, 3.4 Hz, 5'-H), 1.45 (s, 3H, 14-H_3), 1.77 (m, 1H, 2-H), 1.80 (m, 1H, 3-H), 1.87 (s, 3H, 9'-H_3), 1.95 (dd, 1H, J = 14.2, 4.0 Hz, 6-H), 2.12 (ddd, 1H, J = 15.0, 6.8, 4.4 Hz, 2-H), 2.30 (m, 1H, 3-H), 2.38 (t, 1H, J = 13.2 Hz, 6-H), 2.53 (dd, 1H, J = 12.7, 3.4 Hz, 4-H), 2.69 (m, 1H, 4'-H), 3.67 (s, 3H, O*Me*), 3.73 (dd, 1H, J = 14.2, 4.4 Hz, 7-H), 5.49 (br. s, 1H, 1-H), 6.02 (s, 1H, 12-H), 6.31 (s, 1H, 12-H), 6.44 (s, 1H, 9-H), 6.53 (dd, J = 10.3, 1.5 Hz, 1H, 3'-H), 9.49 (s, 1H, 13-H). – ^{13}C {^{1}H} NMR (100 MHz, CD_3OD): δ = 12.5 (q, C-8'), 13.6 (q, C-9'), 20.4 (q, C-14), 21.1 (q, C-11'), 21.6 (q, C-10'), 22.0 (t, C-3), 31.6 (t, C-2), 32.0 (t, C-7'), 33.0 (d, C-4'), 34.7 (d, C-6'), 40.3 (s, C-5), 45.2 (t, C-6), 45.9 (d, C-7), 46.1 (t, C-5'), 52.9 (q, O*Me*), 55.5 (d, C-4), 75.5 (d, C-1), 128.2 (s, C-2'), 131.1 (d, C-9), 138.6 (t, C-12), 150.6 (s, C-11), 151.5 (d, C-3'), 162.4 (s, C-10), 169.0 (s, C-1'), 175.3 (s, C-15), 195.6 (d, C-13), 200.0 (s, C-8). – MS (EI): m/z (%) = 458 (9) [M^+], 274 (66), 167 (100), 153 (53), 105 (11), 83 (22).

*Preparation of methyl 4,6,8-trimethyldeca-4,6-dienoate (***1b***) from* **1**

A solution of **1** (10 mg) in 6 N HCl was heated at 110 °C for 6 h in a sealed tube. The resulting hydrolyzate was diluted with 5 ml of water and extracted with EtOAc and, after evaporation of the organic solvent, trimethylsilyldiazomethane (2.0 M in diethylether, 0.1 ml), was added to the residue at 0 °C. The mixture was stirred for 10 min, and, after evaporation, the residue was subjected to silica gel column chromatography to yield methyl 4,6,8-trimethyldeca-4,6-dienoate (**1b**, 3.2 mg).

Methyl 4,6,8-trimethyldeca-4,6-dienoate (**1b**): – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.82 (d, J = 6.4 Hz, 3H, 13-H_3), 0.84 (t, J = 7.3 Hz, 10-H_3), 0.97 (d, J = 6.8 Hz, 3H, 12-H_3), 1.08 – 1.18 (overlapped, 2H, 7-H and 9-H), 1.20 – 1.35 (overlapped, 3H, 7-H, 8-H and 9-H), 1.78 (d, J = 1.0 Hz, 3H, 11-H_3), 2.64 (m, 1H, 6-H), 3.75 (s, 3H, O*Me*), 5.64 (dd, J = 9.8, 1.0 Hz, 1H, 5-H), 5.69 (d, J = 5.6 Hz, 1H, 2-H), 7.31 (d, J = 6.1 Hz, 1H, 3-H). – ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$): δ = 11.3 (q, C-10), 12.3 (q, C-11), 19.1 (q, C-13), 21.1 (q, C-12), 30.1 (t, C-9), 30.9 (d, C-6), 32.3 (d, C-8), 44.4 (t, C-7), 51.4 (q, O*Me*), 115.0 (d, C-2), 131.1 (s, C-4), 149.0 (d, C-5), 150.2 (d, C-3), 168.1 (s, C-1). – MS (EI): m/z (%) = 224 (33) [M^+], 193 (18) [M^+-OCH_3], 165 (12) [M^+-OCH_3-CO], 153 (61), 127 (72), 125 (72), 93 (100), 69 (58).

*Preparation of methyl 2,4,6-trimethyloct-2-enoate (***2b***) from* **2**

Hydrolysis of **2** and methylation were carried out in the same way as described for **1b** to afford **2b** (4.0 mg).

Methyl 2,4,6-trimethyloct-2-enoate (**2b**): – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.82 (d, J = 6.3 Hz, 3H, 11-H), 0.85 (t, J = 7.3 Hz, 1H, 8-H), 0.98 (d, J = 6.4 Hz, 3H, 10-H), 1.10 – 1.18 (m, overlapped, 2H, 5-H and 7-H), 1.26 – 1.38 (m, overlapped, 3H, 5-H, 6-H and 7-H), 1.85 (d, J = 1.4 Hz, 3H, 9-H_3), 2.61 (m, 1H, 4-H), 3.73 (s, 3H, O*Me*), 6.51 (dd, J = 10.3, 1.4 Hz, 1H, 3-H). – ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$): δ = 11.2 (q, C-8), 12.5 (q, C-9), 19.0 (q, C-11), 20.4 (q, C-10), 30.0 (t, C-7), 30.9 (d, C-4), 32.3 (d, C-6), 44.2 (t, C-5), 51.7 (q, O*Me*), 125.9 (s, C-2), 148.6 (d, C-3), 168.9 (s, C-1). – MS (EI): m/z (%) = 198 (5) [M^+], 183 (2) [M^+-CH_3], 167 (3) [M^+-OCH_3], 141 (10) [M^+-OCH_3-C_2H_5], 119 (34), 105 (25), 91 (20).

Antimicrobial activity

This assay was performed as reported [2].

Acknowledgement

We thank Ms. Teiko Yamada of the Faculty of Agriculture at Tohoku University for HRMS measurements.

[1] R. X. Tan, W. X. Zou, Nat. Prod. Rep. **18**, 448 (2001).
[2] Y. Shiono, T. Murayama, K. Takahashi, K. Okada, S. Katohda, M. Ikeda, Biosci. Biotechnol. Biochem. **69**, 287 (2005).
[3] S. B. Singh, D. Zink, J. Polishook, D. Valentino, A. Shafiee, K. Silverman, P. Felock, A. Teran, D. Vilella, D. J. Hazuda, R. B. Lingham, Tetrahedron Lett. **40**, 8775 (1999).
[4] L. A. MacDonald, L. R. Barbieri, V. S. Bernan, J. Janso, P. Lassota, G. T. Carter, J. Nat. Prod. **67**, 1565 (2004).
[5] A. Guerriero, M. D'Ambrosio, V. Cuomo, F. Vanzanella, F. Pietra, Helv. Chim. Acta. **71**, 57 (1988).
[6] A. Guerriero, M. D'Ambrosio, V. Cuomo, F. Vanzanella, F. Pietra, Helv. Chim. Acta. **72**, 438 (1989).
[7] A. Guerriero, V. Cuomo, F. Vanzanella, F. Pietra, Helv. Chim. Acta. **73**, 2090 (1990).

[8] H. Fujimoto, E. Nakamura, E. Okuyama, M. Ishibashi, Chem. Pharm. Bull. **48**, 1436 (2000).
[9] S. Kim, M. Hatori, M. Ojika, Y. Sakagami, S. Marumo, Bioorg. Med. Chem. Lett. **6**, 1975 (1998).
[10] C. J. Smith, N. R. Morin, G. F. Bills, A. W. Dombrowski, G. M. Salituro, S. K. Smith, A. Zhao, D. J. MacNeil, J. Org. Chem. **67**, 5001 (2002).