Isochromophilones I and II, Novel Inhibitors against gp120-CD4 Binding Produced by *Penicillium multicolor* FO-2338

II. Structure Elucidation

KEIICHI MATSUZAKI, HARUO TANAKA and SATOSHI ŌMURA*

School of Pharmaceutical Sciences, Kitasato University, and Research Center for Biological Function, The Kitasato Institute, Minato-ku, Tokyo 108, Japan

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The structures of isochromophilones I and II, new gp120-CD4 binding inhibitors isolated from a cultured broth of *Penicillium multicolor* FO-2338, were elucidated by NMR experiments. Both of compounds have an azaphilone skeleton substituted by a chlorine atom at C-5 and a side chain, 3,5-dimethyl-1,3-heptadien at C-3. Additionally, isochromophilone I has a γ-lactone ring, and isochromophilone II has 2-oxopropyl moiety instead of a γ-lactone ring.

In the course of screening of microbial metabolites for the inhibitory activities against gp120-CD4 binding, we discovered isochromophilones I and II, which were isolated from a cultured broth of *Penicillium multicolor* FO-2338[1,2] together with the structurally related known compounds ochrephilone (**3**)[3], sclerotiorin (**4**)[4] and rubrorotiorin[4]. In the preliminary analyses[1] of ^{1}H NMR spectra, isochromophilones I and II were suggested to be a complex of two isomers, Ia (**1a**) and Ib (**1b**), and IIa (**2a**) and IIb (**2b**), respectively, like TL-1 and TL-2[5] (Fig. 1.). After that, isochromophilone I was successfully separated by HPLC with a normal phase column. Consequently, a pure preparation of **1a** and a mixture of **1a** and **1b** were obtained. Though isochromophilone II also contained two isomers, **2a** and **2b**, they could not be separated.

In this paper, we wish to report the details of structure elucidation of **1a**, **1b**, **2a** and **2b**.

Structure Elucidation

Structure of Isochromophilone I

1a was obtained as yellow powder. The physicochemical properties of **1a** are as follows; $[\alpha]_D^{22}$ +368° (*c* 0.1, EtOH), UV λ_{max}^{EtOH} nm (ε): 256 (17,500), 273 (15,600), 340 (sh), 357 (12,000), 395 (15,200), 412 (15,700), 430 (sh) and 464 (sh); IR ν_{max}^{KBr} cm^{-1}: 1780 (C=O), 1720 (C=O), 1630 (C=O) and 1560 (C=C). Its EI-MS fragment patterns suggested that **1a** contains a chlorine atom. The

Fig. 1. Structures of isochromophilones I and II, ochrephilone and (+)-sclerotiorin.

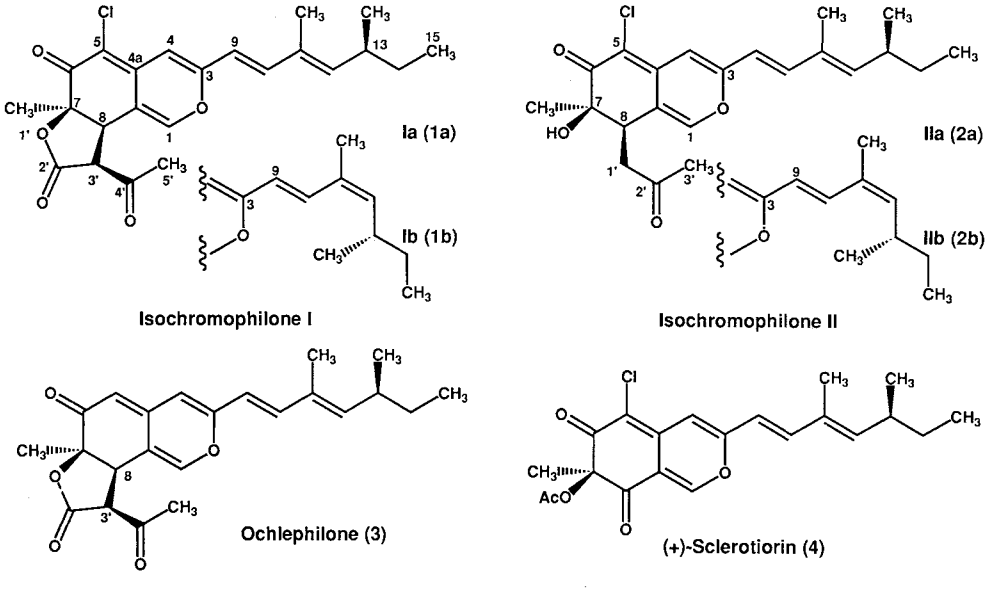


Table 1. ^{13}C and ^{1}H NMR spectral data of isochromophilones Ia (**1a**) and Ib (**1b**) and ochrephilone (**3**).

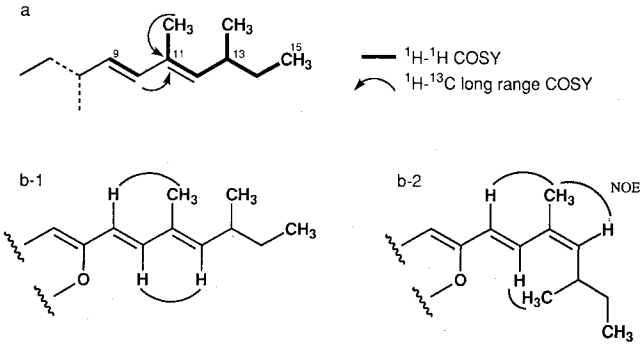
	1a		1b		3	
Number	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	146.4	7.44 1H s	145.9	7.54 1H s	147.0	7.40 1H s
3	158.8		158.4		157.1	
4	105.0	6.55 1H s	105.7	6.56 1H s	107.5	6.11 1H s
4a	140.8		140.8		144.7	
5	109.0		109.2		106.0	5.41 1H d J=0.7Hz
6	184.0		183.9		191.1	
7	83.3		83.3		82.8	
8	42.3	3.87 1H d J=12.0Hz	42.3	3.92 1H d J=12.0Hz	42.8	3.85 1H d J=12.2Hz
8a	113.2		113.2		113.9	
9	116.0	6.05 1H d J=16.0Hz	118.4	6.15 1H d J=16.0Hz	116.0	5.94 1H d J=15.8Hz
10	142.8	7.04 1H d J=16.0Hz	134.3	7.44 1H d J=16.0Hz	141.5	6.95 1H d J=15.8Hz
11	131.8		129.8		131.8	
12	148.5	5.67 1H d J=10.0Hz	145.9	5.53 1H d J=10.0Hz	147.5	5.62 1H d J=9.9Hz
13	35.0	2.46 1H m	34.1	2.47 1H m	35.0	2.45 1H m
14	30.0	1.38 1H m	30.0	1.38 2H m	30.1	1.27 2H m
15	11.8	0.86 3H t J=8.0Hz	12.0	0.85 3H t J=7.5Hz	11.9	0.85 3H t J=7.5Hz
7-CH_3	23.3	1.61 3H s	22.2	1.65 3H s	23.3	1.59 3H s
11-CH_3	12.3	1.82 3H d J=1.0Hz	12.3	1.91 3H d J=1.0Hz	12.3	1.80 3H s
13-CH_3	20.1	1.00 3H d J=6.5Hz	20.0	1.08 3H d J=6.5Hz	20.2	0.99 3H d J=8.6Hz
2'	168.1		168.0		168.5	
3'	57.2	3.81 1H d J=12.0Hz	57.2	3.78 1H d J=12.0Hz	57.3	3.78 1H d J=12.2Hz
4'	199.6		199.6		200.0	
5'	30.1	2.47 3H s	30.2	2.47 3H s	30.3	2.47 3H s

δ=ppm in $CDCl_3$.

molecular formula $C_{23}H_{25}O_5Cl$ of **1a** was derived from the high-resolution EI-MS (*m/z* 416.1396).

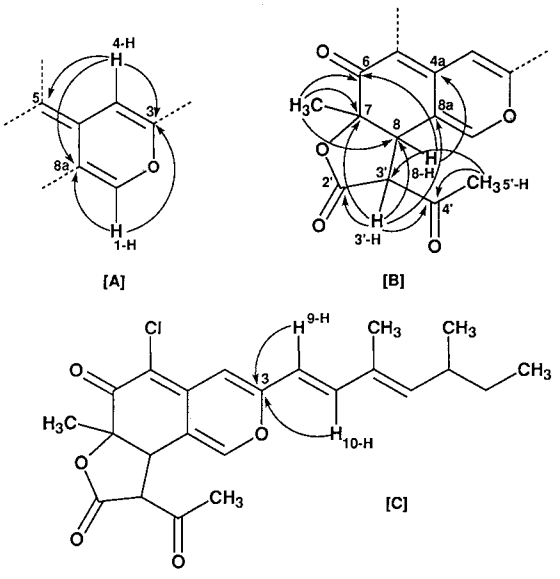
In the ^{1}H NMR spectrum of **1a**, the signals of five methyls (δ 0.86 t, 1.00 d, 1.61 s, 1.82 s, and 2.47 s), one methylene (δ 1.38 2H m) and three methines (δ 2.46 m, 3.81 d and 3.87 d) were observed. Two of methine protons appeared as a pair of coupled doublets at δ 3.87 and 3.81 (8-H and 3'-H, $J_{8,3'}=12.0$ Hz). In addition, in the olefinic proton region, two singlet signals at δ 7.44 (1-H) and δ 6.55 (4-H), one doublet signal at δ 5.67 ($J=10.0$ Hz, 12-H) and a pair of trans coupled signals at δ 6.05 and δ 7.04 (9-H and 10-H, $J=16.0$ Hz) were observed. The ^{13}C NMR spectrum showed nine quaternary carbon signals including an oxygenated carbon (δ 83.3), five olefinic carbons (δ 109.0, 113.2, 131.8, 140.8 and 158.8) and three carbonyl carbons (δ 168.1, 184.0, and 199.6). A heteronuclear multiple quantum coherence (HMQC) experiment revealed all of hydrogen-carbon connectivities (Table 1). In the ^{1}H-^{1}H COSY experiment, a large spin system, a triplet methyl proton signal at δ 0.86 (15-H) to a doublet methyl proton signal at δ 1.00 (13-CH_3), were observed. Additionally, a multiple methine proton signal at δ 2.46 (13-H) was correlated with a doublet olefinic proton signal at δ 5.67 (12-H) in which a long range coupling was observed to a methyl proton signal at δ 1.82 (11-CH_3) in this experiment. In ^{1}H-^{13}C long range COSY experiment, both of signals, an olefinic proton (10-H), and a methyl proton (11-CH_3) were correlated with a quaternary olefinic carbon signal at δ 131.4 (C-11). The above data suggested the presence

Fig. 2. Side chain of **1a** (a) and NOE data for **1a** (b-1) and **1b** (b-2).



of 3,5-dimethyl-1,3-heptadien as a side chain moiety (Fig. 2-a). The heteronuclear multiple-bond correlation (HMBC) experiment successfully revealed the presence of an azaphilone skeleton with a γ-lactone ring in **1a**. Thus, a singlet olefinic proton signal at δ 7.44 (1-H) which attached to an oxygenated olefinic carbon at δ 146.4, was correlated with an oxygenated quaternary olefinic carbon at δ 158.8 (C-3) which is bonded to C-1 through an oxygen atom, and a quaternary olefinic carbon at δ 113.2 (C-8a). Another singlet olefinic proton signal at δ 6.55 (4-H) was also coupled to C-3 and C-8a, and was correlated with a quaternary olefinic carbon at δ 109.0 (C-5). These data suggested the presence of a pyrone ring shown in Fig. 3 [A]. The azaphilone skeleton including a pyrone ring (Fig. 3 [B]) was elucidated from the following correlations. A singlet methyl proton signal at δ 1.61 (7-CH_3) was correlated with an oxygenated

Fig. 3. HMBC correlations for **1a**.



quaternary carbon signal at δ 83.3 (C-7), a conjugated carbonyl carbon signal at δ 184.0 (C-6), and a methine carbon signal at δ 42.3 (C-8). A methine proton signal at δ 3.87 (8-H) was correlated with a quaternary olefinic carbon signal at δ 140.8 (C-4a) and C-6. A γ-lactone ring with the azaphilone skeleton was confirmed by HMBC correlations of two proton signals. A methine proton signal at δ 3.81 (3'-H) was correlated with C-7, C-8 and C-8a in the azaphilone skeleton, and two carbonyl carbons, a lactone carbonyl carbon signal at δ 168.1 (C-2') and a ketone carbonyl carbon signal at δ 199.6 (C-4'). A low field shifted methyl proton signal at δ 2.47 (5'-H) was correlated with C-4' and a methine carbon signal at δ 57.1 (C-3'). The HMBC correlation between two olefinic protons, 9-H and 10-H, and C-3, were observed, the side chain moiety, 3,5-dimethyl-1,3-heptadien, was connected to C-3 position of the azaphilone skeleton. Finally, a quaternary olefinic carbon (C-5) must be attached to a chlorine atom because of its chemical shift (δ 109.0) and the molecular formula of **1a**. Therefore, the structure of **1a** was elucidated as shown in Fig. 1.

The preparation of the minor component, **1b**, contained the isomer **1a** as described above. The signals of the azaphilone and γ-lactone ring in ^{1}H and ^{13}C NMR of **1a** and **1b**, were overlapped. However, those of the side chain moieties were clearly separated (Table 1). The NOE experiments suggested that the isomers, **1a** and **1b**, have different partial structures as shown in Fig. 2 (b-1 and b-2). From these data, the structure of **1b** was elucidated as shown in Fig. 1.

Structure of Isochromophilone II

Isochromophilone II was obtained as yellow powder of a mixture of two isomers, IIa (**2a**) and IIb (**2b**). The molecular formula was established to be $C_{22}H_{27}O_4Cl$ by HREI-MS (*m/z* 390.1604 (M^+), calcd 390.1596). The UV spectrum was very similar to that of **1a** (UV λ_{max}^{EtOH} nm (ε): 250 (15,000), 318 (sh), 340 (13,000), 356 (14,300), 393 (14,300), 410 (16,500), 431 (12,400) and 464 (sh)). In the IR spectrum (IR ν_{max}^{KBr} cm^{-1}: 1715 (C=O), 1630 (C=O) and 1560 (C=C)), however, the absorption of γ-lactone carbonyl (ν_{max} 1780 cm^{-1} in **1a**) was not observed.

The ^{1}H and ^{13}C NMR spectra of **2a** and **2b** (Table 2) indicated the presence of the same side chain as those of **1a** and **1b**, and closely related azaphilone skeletons. The signals of **2a** and **2b** on the azaphilone skeletons were also overlapped. In the ^{1}H NMR, an AMX type coupling system, a methine proton signal at δ 3.42 (J=10.0 and 3.0 Hz, 8-H) and a pair of methylene proton signals at δ 2.37 (J=18.5 and 3.0 Hz, 1'-Ha) and δ 3.10 (J=18.5 and 10.0 Hz, 1'-Hb), was observed instead of a pair of AB type coupled methine proton signals in that of **1a**. In addition, in the ^{13}C NMR spectrum of **2a**, a carbonyl carbon signal which was observed at δ 168.0 (C-2') in that of **1a** disappeared. On the other hand, a methylene carbon signal at δ 41.3 (C-1') appeared instead of methine carbon signal at δ 57.2 (C-3' in **1a**). The HMBC experiments revealed that a singlet methyl proton signal at δ 2.06 (3'-H) was correlated with a ketone carbonyl carbon signal at δ 206.4 (C-2') and a methylene carbon signal at δ 41.3 (C-1'). It suggests that **2a** has a 2-oxopropyl moiety. This moiety is considered to attach to C-8 position of the azaphilone skeleton, because, in the HMBC experiments, a methine proton signal at δ 3.38 (8-H) was correlated with the signals of C-1', C-2', a conjugated carbonyl carbon signal at δ 191.4 (C-6), a hydroxylated quaternary carbon signal at δ 73.9 (C-7) and two olefinic carbon signal at δ 142.1 (C-4a) and δ 118.7 (C-8a). A methylene proton signal at δ 2.35 (1'-Ha) were correlated with the signal of C-8a, and another methylene proton signal at δ 3.08 (1'-Hb) were correlated with the signal of C-7 and C-8a. Finally, a chlorine atom must be on a quaternary olefinic carbon C-5 (δ 106.2) as same as **1a**. The ^{1}H-^{1}H COSY and HMBC correlations of **2a** were concluded as shown in Fig. 4. From the above data, the structures of **2a** and **2b** were determined as shown in Fig. 1.

Stereochemistry

Absolute Configuration of the Side Chain Moiety

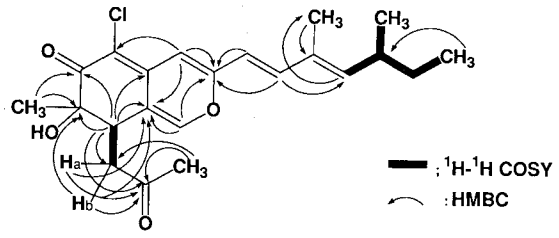
The degradation of **3** with 5% potassium hydroxide

Table 2. ^{13}C and ^{1}H NMR spectral data of isochromophilones IIa (**2a**) and IIb (**2b**).

	2a		2b	
Number	^{13}C	^{1}H	^{13}C	^{1}H
1	145.4	7.42 1H s	145.4	7.44 1H s
3	158.1		158.1	
4	104.9	6.47 1H s	105.6	6.48 1H s
4a	142.1		142.1	
5	105.6		105.6	
6	191.4		191.4	
7	73.9		73.9	
8	40.0	3.38 1H dd J=10.0,3.0Hz	40.0	3.42 1H dd J=10.0,3.0Hz
8a	118.7		118.7	
9	116.3	6.01 1H d J=15.5Hz	119.1	6.12 1H d J=15.5Hz
10	142.1	7.00 1H d J=15.5Hz	133.7	7.41 1H d J=15.5Hz
11	131.8		129.7	
12	147.7	5.56 1H d J=9.5Hz	145.3	5.49 1H d J=10.Hz
13	34.9	2.43 1H m	34.0	2.43 1H m
14	30.0	1.29 2H m	30.2	1.29 2H m
15	11.8	0.82 3H t J=7.5Hz	11.8	0.83 3H t J=7.5Hz
7-CH_3	26.7	1.28 3H s	26.1	1.31 3H s
11-CH_3	12.3	1.79 3H d J=1.0Hz	12.3	1.83 3H d J=1.0Hz
13-CH_3	20.2	0.97 3H d J=6.5Hz	20.1	0.99 3H d J=6.5Hz
1'	41.3	2.35 1H dd J=18.5, 3.0Hz	41.2	2.37 1H dd J=18.5, 3.0Hz
		3.08 1H dd J=18.5, 10.0Hz		3.10 1H dd J=18.5, 10.0Hz
2'	206.4		206.4	
3'	30.5	2.06 3H s	30.5	2.08 3H s

δ = ppm in $CDCl_3$.

Fig. 4. ^{1}H-^{1}H COSY and HMBC correlations for **2a**.



afforded a carboxylic acid (**5**), which was identified (+)-(2*E*,4*E*)-4,6-dimethyloctadienoic acid obtained from **4** by a similar manner. Thus, the configuration of C-13 of **1a**, **1b**, **2a**, **2b** and **3** was established to be *S*. It is also supported by comparison of ^{1}H and ^{13}C NMR data for the side chain moieties of them (Tables 1 and 2).

Relative Configuration of C-7, 8 and 3' of **1a**, **1b** and **3**

The stereochemistry on a γ-lactone ring of **1a** was determined by difference NOE experiments as follows. The observation of NOE between 7-CH_3 and 8-H indicated the *cis* diaxial relation between them. Additionally, the strong NOE between 1-H and 8-H was observed, suggesting that the orientation of both protons was near location. The coupling constant between 8-H and 3'-H (12.0 Hz) indicated that these two protons have trans diaxial configuration. The above difference NOEs are shown in Fig. 6. NOE experiments of **1b**, same correlations were observed. In the NOESY spectrum of **3**, the observation of cross peaks were agreed with those of **1a**. Additionally, the chemical shifts of a γ-lactone moiety in ^{1}H and ^{13}C NMR of **1a** were identical with those of **3** (Table 1). Consequently, **1a**, **1b** and **3** have the same related configuration on a γ-lactone moiety.

Relative Configuration of C-7 and 8 of **2a** and **2b**

In the NOESY spectrum of isochromophilone II, a mixture of IIa (**2a**) and IIa (**2b**), a cross peak, between 7-CH_3 and 8-H, was observed. It also indicates the *cis* diaxial relation between them like **1a** and **3**.

Discussion

The structures of isochromophilones I and II, gp120-CD4 binding inhibitors, were elucidated mainly by analyzing NMR spectral data. These have an azaphilone skeleton containing a chlorine atom at C-5 and 3,5-dimethyl-1,3-heptadien moiety at C-3 like (+)-sclerotiorin. Additionally, isochromophilone I has a γ-lactone ring like ochrephilone, while isochromophilone II has 2-oxopropyl moiety at C-8. The relative configurations of isochromophilones I and II were revealed to be identical with that of ochrephilone because the NOE correlations of isochromophilones I and II correspond to those of ochrephilone. It is also supported by the fact that ^{1}H and ^{13}C NMR data on the γ-lactone ring of isochromophilone I are identical with those of ochrephilone.

BIRCH *et al.*[6,7] and SETO *et al.*[3] revealed by using radioactive precursor and by using ^{13}C labeled acetate and methionine, respectively, that sclerotiorin and ochrephilone are biosynthesized from octaketide. It was found that the carbon skeleton of isochromophilone I is identical with that of ochrephilone, and that isochro-

Fig. 5. Alkaline degradation of **3**.

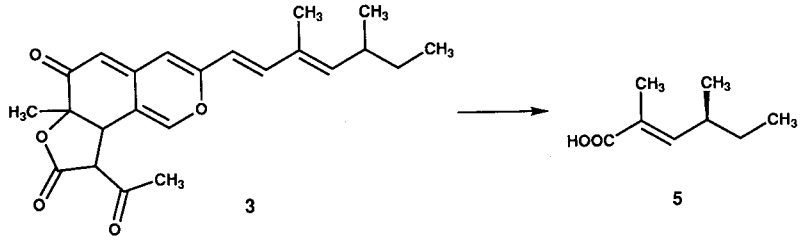
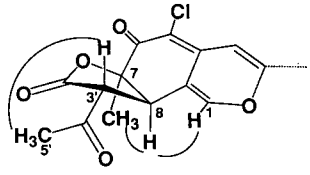


Fig. 6. NOE data and relative configuration of γ-lactone ring in **1a** and **1b**.



mophilone II has the carbon skeleton closely related to that of ochrephilone. Thus, isochromophilones I and II, ochrephilone and sclerotiorin should be biosynthesized *via* similar pathway. Consequently, the absolute configuration of C-7 of isochromophilones I and II and ochrephilone should be *R*.

Experimental

Isochromophilones I and II were obtained from a cultured broth of *P. multicolor* FO-2338 as described previously[2]. The UV and IR spectra were recorded on a Beckman model DU640 spectrophotometer and Perkin Elmer model 1650, respectively. ^{1}H NMR (270 and 400 MHz) and ^{13}C NMR (67.5 and 100 MHz) spectra were obtained on JEOL 270 EX and Varian XL-400 spectrometer. MS was obtained with JEOL model JMS-AX505 HA. Optical rotation was measured with Jasco DIP-370 polarimeter.

Degradation by Potassium Hydroxide of **4**

4 (170 mg) was dissolved in 10 ml of 5% potassium hydroxide and stirred for 90 minutes at room temperature. The reaction mixture was extracted with 10 ml of chloroform. The water layer was adjusted to pH 3.0 with 9% sulfuric acid and reextracted with 10 ml of petroleum ether. The organic extract was concentrated to dryness *in vacuo*. The extract was recrystallized with ethanol to give (+)- (2*E*,4*E*)-dimethyloctadienoic acid (40 mg).

4 was white powder, $[\alpha]_D^{22}$ +62° (*c* 0.2, EtOH), UV λ_{max}^{EtOH} nm (ε): 255 (3,700); IR ν_{max}^{KBr} cm^{-1}: 1780 (C=O), 1720 (C=O), 1630 (C=O) and 1560 (C=C); ^{1}H NMR (270 MHz, δ in $CDCl_3$): 10.95 (1H, bs, –COO*H*), 7.40 (1H, d, *J*=15.5 Hz, 3-*H*), 5.78 (1H, d, *J*=15.5 Hz, 2-*H*), 5.72 (1H, d, *J*=9.9 Hz, 5-*H*), 2.45 (1H, m, 6-*H*), 1.79 (3H, s, 4-C*H*$_3$), 1.38 (2H, m, 7-*H*), 0.99 (3H, d, *J*=6.6 Hz, 6-C*H*$_3$), 0.84 (3H, t, *J*=7.4 Hz, 8-*H*); ^{13}C NMR (67.5 MHz, δ in $CDCl_3$) :173.29 (s, C-1), 152.34 (s, C-3), 149.79 (d, C-5), 131.55 (s, C-4), 114.68 (d, C-2), 34.95 (d, C-6), 29.92 (d, C-7), 20.03 (q, 6-*C*H$_3$), 12.32 (q, 4-*C*H$_3$), 11.86 (q, C-8).

Degradation by Potassium Hydroxide of **3**

3 (50 mg) was dissolved in 5 ml of 5% potassium hydroxide and refluxed for 40 minutes. The reaction mixture was extracted with 10 ml of chloroform. The water layer was adjusted to pH 3.0 with 9% sulfuric acid and reextracted with 10 ml of petroleum ether. The organic extract was concentrated to dryness *in vacuo*. The extract was dissolved in chloroform and chromatographed on a silica gel column (1.2×8.0 cm) eluted with chloroform-methanol (49:1) to give (+)-(2*E*,4*E*)-dimethyloctadienoic acid (1.25 mg).

Acknowledgments

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