Bioorganic & Medicinal Chemistry Letters 18 (2008) 4228–4231

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Spiroleptosphol isolated from *Leptosphaeria doliolum*

Masaru Hashimoto *, Taro Tsushima, Takanori Murakami, Masahiro Nomiya, Noboru Takada, Kazuaki Tanaka

Faculty of Agriculture and Life Science, Hirosaki University, 3-Bunkyo-cho, Hirosaki 036-8561, Japan

ARTICLE INFO

Article history:
Received 6 March 2008
Revised 11 May 2008
Accepted 15 May 2008
Available online 24 May 2008

Keywords:
Isolation
Structure determination
NMR spectra
CD spectra
Absolute stereochemistry
Degradation
Chiral GC

ABSTRACT

A novel γ-methylidene-spirobutanolide spirolephtoshol (**1**) was isolated from ascomycetous fungus *Leptosphaeria doliolum* as a cytotoxic compound. The relative structure was established by the NMR analysis involving the NOE experiments. Absolute structure of the bicyclic moiety was determined by chemical derivation followed by the CD analysis. The relative and absolute stereochemistry of the side chain was established by comparison of the ^{1}H NMR spectra and the chiral GC chromatograms of the degradation product with the synthetic samples.

In the course of studies investigating metabolites from fungi with unique ecologies, we isolated spiroleptosphol (**1**), a novel γ-methylidene-spirobutanolide, as a cytotoxic compound from saprophytic ascomycete *Leptosphaeria doliolum* isolated from mugwort stems.[1] Here, we report the structure of **1** involving its absolute stereochemistry.

Spiroleptosphol (**1**, Figure 1) was isolated as oil from the fermentation broth of *Leptosphareia doliolum* by culturing with potato-sucrose medium with shaking (110 rpm) for 14 days and the following extraction with ethyl acetate and silica gel column chromatography.[2] Biological assay revealed that **1** exhibited cytotoxicity against P388 murine leukemia (EC_{50} = 20 μg/mL). The EI mass indicated the molecular ion signal at m/z = 336.1905 suggesting its molecular formula as $C_{19}H_{28}O_5$.

This molecular formula was supported by observing 19 resonances in the ^{13}C NMR spectrum as shown in Table 1. The ^{1}H NMR spectrum provided 28 proton signals including three exchangeable protons. The COSY spectral analysis disclosed an (*E*)-3,5-dimethyl-1-heptenyl side chain attached to a methine carbon of which ^{1}H resonance was 3.45 ppm. The *E*-geometry of the double bond in the side chain was established based on a large coupling constants (J = 15.5 Hz). The triplet signals observed at 4.68 and 4.80 ppm were coupled each other with 2.3 Hz, suggesting an *exo*-methylene group. These signals were further coupled with a proton appeared at 5.16 ppm both in 2.3 Hz. Detailed analysis of HMBC and HSQC spectra suggested a 3-methylidene-2-oxaspiro[4.5]decan-8-en-1-one framework possessing hydroxy groups at C4, C6, and C7 positions. Strong adsorption at 1785 cm^{-1} in the IR spectrum indicated the existence of a butanolide ring in the molecule, which consisted with the above assumption. These results led us to propose the planar structure of **1**.

The stereochemistries of C4, C6, and C7 alcohol groups were studied employing tribenzoate **2**[3] prepared by a reaction with BzCl/DMAP in pyridine (Scheme 1). In the ^{1}H NMR of **2**, the C4-H, C6-H, and C7-H signals were shifted to higher frequency ($\Delta\delta$ = 1.08, 2.30, and 1.73 ppm, respectively), which confirmed the alcohol positions.[4] The coupling constant between C6-H and C7-H of **2** was 8.1 Hz, suggesting that these protons took *pseudo*-1,2-diaxial relationship. Irradiation of the signal for C6-H induced the NOEs at C4-H and C10-H to establish the relative stereochemistry around the spirobicyclic moiety. Only the 4*R**,5*R**,6*R**,7*R**,10*R** isomer satisfies these results.

The absolute configuration of **1** was investigated by the CD spectral analysis. Since tribenzoate **2** gave only broad positive Cotton curve, as shown in Figure 2, but not distinct exciton coupling probably due to cancellation by existence of more than three chromophores,[5] **1** was converted into dibenzoate **4** in order to simplify the CD spectrum.[6]

Hydrogenation using PtO_2 catalyst provided **3** as a single diastereomer.[7] The stereochemistry at C3 position was assigned by an NOE between C3-H and C4-H. Heating **3** with BzCl/DMAP in pyridine gave the desired **4**.[8] Sterically hindered C4-OH retained under this condition. The coupling constant between C6-H and

* Corresponding author. Tel./fax: +81 172 39 3782.
E-mail address: hmasaru@cc.hirosaki-u.ac.jp (M. Hashimoto).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.05.087

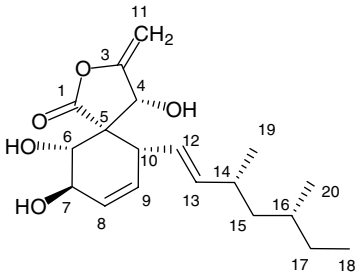
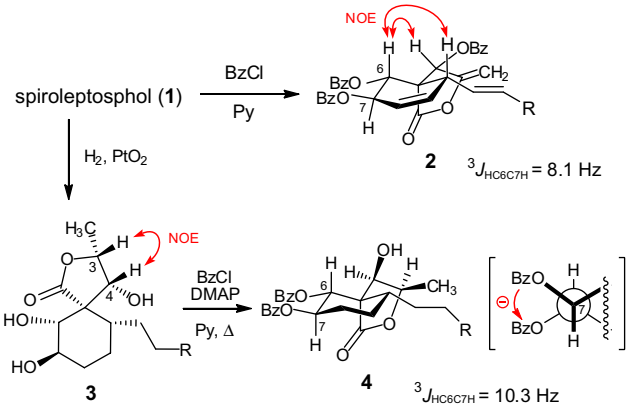


Figure 1. Structure of spiroleptosphol (**1**).

Table 1
NMR spectral data of **1** in $CDCl_3$

Position	^{13}C	^{1}H	HMBC
1	174.63	–	6, 10
3	157.11	–	4, 11
4	68.69	5.16 (dt, *6.5, 2.3*)	11
4-OH	–	3.36 (brd, *6.5*)	–
5	58.74	–	6, 9, 10
6	72.22	3.84 (brd, *6.8*)	4, 8
6-OH	–	4.40 (br)	–
7	70.00	4.71 (br)	6, 9
7-OH	–	4.39 (br)	–
8	127.73	5.75 (dt, *10.2, 2.8*)	10
9	129.78	5.58 (ddd, *1.6, 2.8, 10.2*)	10, 12
10	38.95	3.45 (dq, *8.4, 2.8*)	4, 8, 9, 12, 13
11	89.08	4.68 (t, *2.3*) 4.80 (t, *2.3*)	4
12	124.32	5.33 (dd, *8.4, 15.5*)	9, 10, 13, 14
13	141.67	5.43 (dd, *7.6, 15.5*)	10, 14
14	34.28	2.16 (m)	12, 13, 15
15	43.86	0.98 (ddd, *5.3, 8.0, 13.4*) 1.24 (ddd, *5.1, 8.6, 13.4*)	13, 14, 17, 19, 20
16	31.57	1.32 (m)	17
17	29.77	1.11 (m) 1.28 (m)	16, 18, 20
18	11.89	0.85 (t, *7.2*)	16, 17
19	20.86	0.91 (d, *6.7*)	13, 14, 15
20	19.04	0.80 (d, *6.3*)	15, 16, 17

^{1}H NMR spectrum was measured at 40 °C.



Scheme 1. Stereochemical determination of the bicyclic moiety.

C7-H in the ^{1}H NMR spectrum was 10.3 Hz, which is a typical value for 1,2-diaxial protons in cyclohexanes taking chair conformation. As expected, **4** gave a pair of exciton-split curve with negative Cotton effect at 238 nm ($\Delta\varepsilon_{238}$−18.9) and positive one at 222 nm ($\Delta\varepsilon_{222}$+10.9) in the CD spectrum in MeOH to indicate a negative chirality for the C6–C7 glycol function based on the 'dibenzoate rule'.[9] These results revealed the absolute stereochemistry around the bicyclic moiety to be 4*R*,5*R*,6*R*,7*R*,10*R* as depicted (see Figure 1).

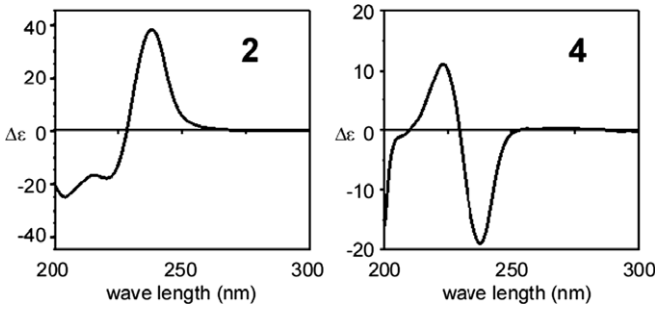
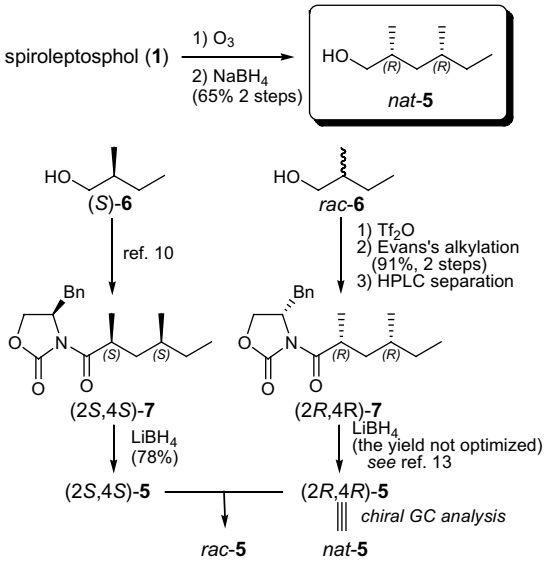


Figure 2. The CD spetra of tribenzoate **2** and dibenzoate **4**.



Scheme 2. Stereochemical determination of the side chain.

On the other hand, (2*R*,4*R*)-2,4-dimethylhexan-1-ol (*nat*-**5**) was obtained by ozonolysis of **1** followed by reductive workup as shown in Scheme 2. The relative stereochemistry of *nat*-**5** was estimated to be 2*R**,4*R** by ^{1}H NMR spectral comparison with those of the corresponding epimeric isomers in the literature.[10] Unfortunately, the reported optical rotation value of **5** was quite small ($[\alpha]_D^{24}$ + 3.7 for 2*R*,4*R*-isomer).[11] To make matters worse, vaporization of *nat*-**5** during purification process was not disregardable in such small scale to provide only approximately 6 mg of *nat*-**5** from

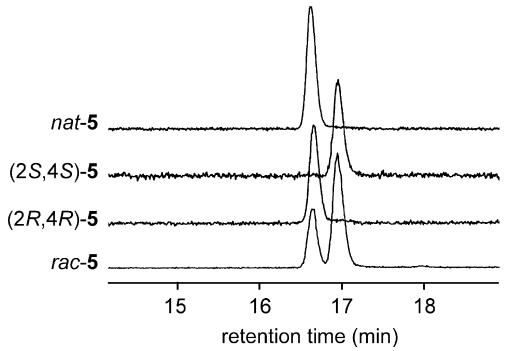
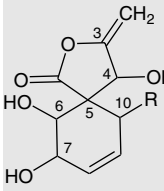


Figure 3. Total ion chromatograms of *nat*-**5**, (2*S*,4*S*)-**5**, (2*R*,4*R*)-**5**, and *rac*-**5** by GC-EIMS using chiral capillary column (RESTEC Rt-βDEXm™, 30 m, 0.25 mm ID) at 75° (constant temperature).

Table 2
Stereochemistries of 3-methylidene-2-oxaspiro[4.5]decan-1-ones in the literature

	Compound	C4	C5	C6	C7	C10	R	Producers
	Spiroleptosphol (this report)	*R*	*R*	*R*	*R*	*R*	(3*R*,5*R*)-(*E*)-3,5-dimethylhept-1-en-1-yl	*Leptosphaeria doliolum*
	Oxaspirol[15]	—[a]	—[a]	—[a]	—[a]	—[a]	(*E*,*E*)-3,5-dimethylhepta-1,4-dien-1-yl	*Rhodotorula glutinis T-110*
	Arthropsolide A[c16]	*S*	*R*[b]	*S*	*S*	*S*	(*E*)-but-2-en-1-yl	*Arthropsis truncata*
	Oxaspirodion[17]	—[d]	—[d]	*S**	keto form	*S**	(*E*)-but-2-en-1-yl	*Chaetomium subspirale*
	Paecilospirone[18]	*S**	*R**	*S**	keto form	*S**	(*E*)-but-2-en-1-yl	*Paecilomyces* sp.
	Massarigenin A[19]	*S**	*S**	*R**	*S**	*R**	methyl	*Massarina tunicata*
	Mycosporulone[20]	keto form	*S**	*R**	keto form	*S**	methyl	*Coniothyrium sporulosum*
	6-epi-5′-hydroxy- mycosporulone[21]	*S**	*R**	*R**	keto form	*R*	methyl	*Microsphareopsis* sp. FO-5050IV
	Rosigenin[e22]	*S**	*R**	*R**	keto form	*S**	methyl	*Mycospharella rosigena*

[a] Not described.
[b] The stereochemistry in the text was inconsistent with that in the figure in the literature.
[c] The C3 is sp^3, but its stereochemistry was not assigned.
[d] Not assigned.
[e] The C3 is sp^3, and its stereochemistry was assigned to be *S**.

of **1** (24 mg). This amount was insufficient to judge the absolute chemistry confidently based on its optical rotation.

We dissolved this subject by direct comparison of the chiral GC chromatograms. Prior to the analysis, we prepared both enantiomers (2*S*,4*S*)-**5** and (2*R*,4*R*)-**5** as well as racemate *rac*-**5**. The (2*S*,4*S*)-**5** was synthesized by the Organ's protocol *via* (2*S*,4*S*)-**7**.[10] The enantiomer (2*R*,4*R*)-**5** was obtained by the similar methodology but employing racemic alcohol *rac*-**6**[12] and enantiomeric Evans's auxiliary, (*S*)-4-benzyl-3-propionyloxazolidin-2-one.[13] Although the alkylation gave a 1:1 mixture of (2*R*,4*R*)-**7** and the corresponding (4*S*)-isomer, these were successfully separated by HPLC (Develosil 60, AcOEt:hexane = 7:93).[14] The racemate *rac*-**5** was readily prepared by combining (2*R*,4*R*)-**5** and (2*S*,4*S*)-**5**.

As the authentic samples in hand, these were analyzed by GCMS. It was found that a chiral capillary column (RESTEC Rt-βDEXm™, 30 m, 0.25 mm ID) was effective to distinguish these enantiomers with sufficient reproducibility (Figure 3). Both GC peaks provided signals at m/z = 112 $[M\text{-}H_2O]^+$, 101 $[M\text{-}(CH_2CH_3)]^+$, and 57 $[CH(CH_3)CH_2CH_3]^+$ with the same intensities to confirm the peak assignment. These results clearly proved that the sample derived from the natural product has 2*R*,4*R* configuration, establishing the (3*R*,5*R*)-(*E*)-3,5-dimethyl-1-heptenyl side chain attached to the C10 position.

As described, we achieved in disclosing the structure of spiroleptosphol (**1**) including its absolute configuration as depicted in Figure 1. Several 3-methylidene-2-oxaspiro[4.5]decan-1-one derivatives, oxaspirol,[15] arthropsolide A,[16] oxaspirodion,[17] paecilospirone,[18] massarigenin A,[19] mycosporulone,[20] 6-epi-5′-hydroxymycosporulone,[21] and rosigenin[22] have been isolated from basidomycetous and ascomycetous fungi. These exhibited various biological activities. Interestingly, the stereochemistries of this bicyclic unit are diverse in these compounds as shown in Table 2. Although two types of biogeneses for this moiety have been proposed, neither has reached to the final conclusion.[22,23] Further biological assays and biosynthetic studies of **1** are now under investigation in our laboratories. Taking high productivity (120 mg from 5.0 L of culture medium) into account, *Leptosphaeria doliolum* would produce sufficient amount of samples for these investigations.

Acknowledgments

We thank Mr. Junsuke Miyanishi and Ms. Yuki Yagihashi for their experimental assistance. Cyitotoxicity of **1** was determined by Dr. Kaoru Yamada of Nagoya University.

References and notes

1. Otani, Y.; Mikawa, T. *Mem. Natn. Sci. Mus. Tokyo* **1971**, *30*, 71.
2. Physical property of **1**: IR (film) 3360, 2960, 1785, 1680, 1200, 1100, 1065 cm^{-1}. $[\alpha]_D^{25}$ −230° (c = 1.10, $CHCl_3$). EIMS (rel. int.) m/z 336 (5.2, M^+), 318 (21, $[M-H_2O]^+$), 300 (7.6, $[M-2H_2O]^+$), 121(88), 69 (100), FDMS (rel. int.) m/z 359 (43, $[M+Na]^+$), 337 (100, MH^+), 336 (43, M^+), EIHRMS found m/z 336.1905. calcd for $C_{19}H_{28}O_5$; M^+: 336.1936.
3. Physical data of **2**: IR 2960, 1808, 1731, 1675, 1260, 1115, 1090, 710 cm^{-1}. ^1H NMR (400 MHz in $CDCl_3$) δ 0.84 (3H, d, J = 6.5), 0.85 (3H, t, J = 7.5), 0.97 (3H, d, J = 6.5), 1.06 (1H, ddd, J = 5.5, 9.3, 13.7), 1.14 (1H, dq, J = 13.4, 7.5), 1.31 (2H, m), 1.42 (1H, m), 2.27 (1H, m), 3.50 (1H, dq, J = 7.7, 2.6), 4.51 (1H, dd, J = 2.2, 3.2), 4.88 (1H, dd, J = 2.2, 3.2), 5.50 (1H, dd, J = 7.7, 15.5), 5.59 (1H, dt, J = 10.6, 2.6), 5.60 (1H, dd, J = 7.7, 15.5), 5.91 (1H, dt, J = 10.6, 2.6), 6.14 (1H, d, J = 8.1), 6.24 (1H, t, J = 2.2), 6.44 (1H, dt, J = 8.1, 2.6) 7.35–7.7 (12H, m), 7.99 (4H, m), 8.14 (2H, m), ^{13}C NMR (100 MHz, in $CDCl_3$) δ 11.22, 18.92, 21.12 (each CH_3), 29.99 (CH_2), 31.87, 34.50, 41.28 (each CH), 43.97 (CH_2), 56.42 (C), 71.82, 72.70, 70.43 (each CH), 90.60 (CH_2), 123.22, 125.51, 125.51, 128.26, 128.33. 128.50, 128.79, 128.84, 129.42. 129.77, 129.78, 129.85, 130.07, 132.97, 133.52, 134.14 (each CH), 152.69, 165.03, 165.22, 169.9, 165.97 (each C), ESIMS (rel. int.) m/z 1319 (7.5, $[2M+Na]^+$), 671 (100, $[M+Na]^+$), ESIHRMS found m/z 671.2606. calcd for $C_{40}H_{40}O_8Na$; $[M+Na]^+$.
4. Addition of D_2O led to serious spectral broadening in the ^1H NMR spectrum of **1**.
5. Ayer et al. investigated the stereochemistry of tri-*O*-benzoate of arthropsolide A in their structural studies. They judged its absolute stereochemistry based on a possitive cotten effect in the CD spectrum. Interestingly, they concluded the enantiomeric configuration for the C6 and the C7. In their report, the stereochemistry of arthropsolide A in the text did not accord with that in the figure (see Ref. 16).
6. We have not succeeded in preparing the 6,7-*O*-dibenzoate of **1** so far we examined. Benzoylation with BzCl in pyridine gave a mixture of monobenzoates, 4,6-*O*-dibenzoate, and tribenzoate **2**.
7. Physical data of **3**: IR (film) 3460, 2930, 1735, 1055 cm^{-1}. 1H NMR (400 MHz, acetone-d_6) δ 0.82 (3H, d, J = 7.4), 0.83 (3H, d, J = 6.5), 0.84 (3H, t, J = 6.7), 0.89–1.20 (5H, m), 1.22 (1H, dt, J = 13.4, 6.6) 1.29 (1H, m) 1.29 (3H, dd, J = 1.5, 6.3), 1.31–1.43 (3H, m), 1.63 (1H, ddt, J = 1.5, 7.5, 9.5), 1.74 (2H, m), 1.81 (1H, m), 1.88 (1H, dt, J = 12.7, 4.4), 3.32 (1H, dd, J = 2.9, 9.2), 3.83 (1H, d, 4.9), 3.91 (1H, dddd, J = 4.4, 4.9, 9.2, 11.3), 4.21 (1H, d, J = 2.9), 4.64 (1H, dd, J = 1.5, 5.4), 4.73 (2H, m), ^{13}C NMR (100 MHz, acetone-d_6) δ 11.51, 14.99, 19.97, 20.77 (each CH_3), 26.70 30.05 (each CH_2), 30.93 (CH), 31.37 (CH_2), 32.46 (CH), 32.87, 35.86 (each CH_2), 39.12 (CH), 45.46 (CH_2), 58.18 (C), 70.65, 77.19, 79.29, 81.59 (each CH), 177.46 (C), ESIMS (rel. int.) m/z 343 (100, $[M+H]^+$), 325 (24, $[M-H_2O+H]^+$), 260 (9), ESIHRMS found m/z 343.2503. Calcd for C $_{19}H_{35}O_5$; $[M+H]^+$: 343.2484.
8. Physical data of **4**: IR (film) 3460, 2930, 1730, 1230, 710 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.83 (3H, d, J = 6.6), 0.85 (3H, d, J = 6.7), 0.86 (3H, t, J = 7.3 Hz), 0.92, 1.06 (each 1H, m), 1.35 (3H, d, J = 6.5), 1.68 (1H, dt, J = 5.0, 11.3), 1.69 (1H, dt, J = 4.3, 10.0), 1.9–2.2 (3H, m), 2.42 (1H, dq, J = 12.7, 3.4), 4.53 (1H, quint, J = 6.5), 4.60 (1H, brd, J = 6.5), 5.60 (1H, d, J = 10.3), 5.73 (1H, dt, J = 4.7, 10.3 Hz), 7.31 (2H, t, J = 7.9), 7.36 (2H, t, J = 7.6), 7.44 (1H, brt, J = 7.9), 7.50 (1H, brt, J = 7.6), 7.85 (2H, brd, J = 7.6), 7.94 (2H, brd, J = 7.9 Hz), ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.27, 14.45, 19.70, 20.27, 24.85, 28.99, 29.28, 30.20, 30.30, 31.69, 35.18, 38.85, 44.59, 56.53, 72.50, 75.08, 78.04, 78.51, 128.20, 128.60, 128.64, 129.52, 129.80, 129.93, 132.77, 133.73, 165.52, 166.33, 175.13, ESIMS (rel. int.) m/z 573 (100, $[M+Na]^+$), 551 (15, $[M+H]^+$), ESIHRMS found m/z 551.3010. calcd for $C_{33}H_{43}O_7$; $[M+H]^+$: 551.3009.
9. Harada, N.; Nakanishi, K. *J. Am. Chem. Soc.* **1969**, *91*, 3989.
10. Organ, M. G.; Bilokin, Y. V.; Bratovanov, S. *J. Org. Chem.* **2002**, *67*, 5176.
11. White, J. D.; Johnson, A. T. *J. Org. Chem.* **1994**, *59*, 3347.
12. (*R*)-2-Methylbutanol was not available commercially. Organ et al. synthesized this compound from methyl (*S*)-(+)-3-hydroxy-2-methylpropionate through six steps. Since microgram scale was enough in our case, we adopted the described scheme providing desired (2*R*,4*R*)-7 directly.
13. Evans, D. A.; Ennis, M. D.; Mathre, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 1737.
14. The reduction of (2*R*,4*R*)-**7** was performed using 1.0 mg of the HPLC purified sample. The NMR spectrum of the product (2*S*,4*S*)-**5** was identical to that of (2*S*,4*S*)-**5**. Although the accurate amount of the product was not available due to the small scale, the amount was still enough for GC analysis.
15. Doi, J.; Hirota, A.; Nakagawa, M.; Sakai, H.; Isogai, A. *Agric. Biol. Chem.* **1985**, *49*, 2247.

16. Ayer, W. A.; Craw, P. A.; Neary, J. *Can. J. Chem.* **1992**, *70*, 1338.
17. Rether, J.; Erkel, G.; Anke, T.; Sterner, O. *J. Antibiot.* **2004**, *57*, 493.
18. Hirota, A.; Nakagawa, M.; Hirota, H. *Agric. Biol. Chem.* **1991**, *55*, 1187.
19. Oh, H.; Swenson, D. C.; Gloer, J. B.; Shearer, C. A. *J. Nat. Prod.* **2003**, *66*, 73.
20. Kaouadji, M. *J. Nat. Prod.* **1993**, *56*, 2189.
21. Fukami, A.; Taniguchi, Y.; Nakamura, T.; Rho, M.-C.; Kawaguchi, K.; Hayashi, M.; Komiyama, K.; Omura, S. *J. Antibiot.* **1999**, *52*, 501.
22. Albinati, A.; Brückner, S.; Camarda, L.; Gianluca, N. *Tetrahedron* **1980**, *36*, 117.
23. Ayer, W. A.; Craw, P. A. *Can. J. Chem.* **1992**, *70*, 1348.