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Lambertellols A and B, Novel 3,4-Dihydronaphthalen-1(2*H*)-ones with Spiro-butenolide Produced by *Lambertella* sp.1346

Takanori Murakami, Yoshifumi Morikawa, Masaru Hashimoto,* Toshikatsu Okuno, and Yukio Harada

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

hmasaru@cc.hirosaki-u.ac.jp

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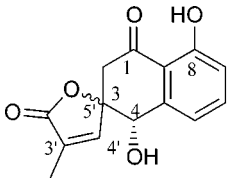
ABSTRACT



lambertellol A (1): 3*S*
lambertellol B (2): 3*R*

***Lambertella* sp. 1346 was found to produce lambertellols A (1) and B (2) carrying a novel dihydronaphthalen-1(2*H*)-ones with spiro-1-furan-2(5′*H*)-one. The spiro-lactone ring moiety of both 1 and 2 were easily migrated to afford lambertellin, a known metabolite of *Lambertella corni-maris*. The absolute stereochemistry of these compounds was established on the basis of CD spectrum after chemical derivatization.**

In 1990, Harada, one of the present authors, reported that discomycete *Monilinia fructigena* infection on apple fruits is sectored by *Lambertella* sp. 1346 under ambient atmosphere, which is recognized as so-called mycoparasitism.[1] This observation led to a hypothesis that some chemical substances of *L.* sp. 1346 may play an important roll in this mycoparasitism. The ensuing chemical investigation discovered lambertellols A (**1**) and B (**2**), a diastereomeric pair of unique 4,8-dihydroxy-2,3,4-trihydronaphthalene-1-one carrying spiro-butenolide at the C3 position. Now, we would like to report their structures, absolute stereochemistry, chemical properties, and some basic knowledge on their biosynthesis.

After *L.* sp. 1346 was cultured employing potato-sucrose medium for 5 days at 26 °C with shaking, isolation was performed with guidance of growth inhibition of spores using *Cochlibolus miyabeanus*[2] in place of *Monilinia* because of technical difficulty in preparing spores of *Monilinia*. The active fractions obtained right after elution from HPLC [Waters μ-bondasphere C18, 150 × 19 mm i.d. CH_3CN/H_2O (20:80, without TFA), 10 mL/min flow, t_R = 18 min (for **1**), 25 min (for **2**)] were colorless solutions, but gradual yellow colorization was observed. Lyophilization of each fraction after removal of acetonitrile keeping below 25 °C gave **1** and **2** with sufficient purity (>95% based on ^{1}H NMR) both as pale yellow amorphous powders. Recrystallization from $CHCl_3$−hexane afforded pure **1** (mp 161 °C, dec.) and **2** (mp 107 °C, dec.) both as colorless needles. However, when the recrystallized products were dissolved in MeOH, both solutions of **1** and **2** colorized gradually. Similar colorization was observed by adsorbing **1** and **2** each on silica gel. These conditions efficiently transformed them into the same red needles, which were identical to lambertellin (**3**) (mp, NMR, IR, and MS spectra). Lambertellin (**3**) was originally isolated from *Lambertella corni-maris*[3] and another fungus, *Ciboria gordonii* Funk.[4] The yields of **1** and **2** varied by culture (**1**, 2−15 mg; **2**, 5−20 mg from 4 L of

(1) Harada, Y.; Sasaki, M. *Rep. Tottori Mycol. Inst.* **1990**, *28*, 275−285.

(2) Nihei, K.; Itoh, H.; Hashimoto, K.; Miyairi, K.; Okuno, T. *Biosci., Biotechnol., Biochem.* **1998**, *62*, 852−857.

(3) Armstrong, J. J.; Turner, W. B. *J. Chem. Soc.* **1965**, 5927−5930.

10.1021/ol035889d CCC: $27.50
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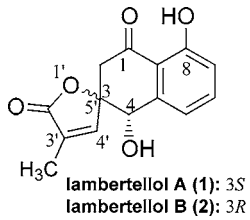
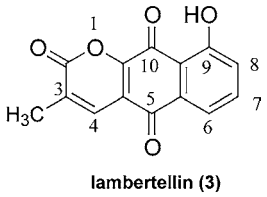
Table 1. NMR Spectral Data for Lambertellols A (**1**) and B (**2**), Triol **5**, and Dibenzoate **6** in $CDCl_3$

	lambertellol A (**1**)			lambertellol B (**2**)			triol **6**	dibenzoate **7**
position	δC	δH (*J* in Hz)	HMBC	δC	δH (*J* in Hz)	HMBC	δH (*J* in Hz)[a]	δH (*J* in Hz)
1	198.79		2	199.16		2	5.16 (4.4, 5.8)	6.52 (2.9, 5.8)
2	43.64	2.93 (17.1)		43.7	2.94 (17.5)	4	2.17 (3.9, 13.7)	2.47 (2.9, 14.7)
		3.22 (17.1)			3.19 (17.5)		2.26 (5.4, 13.9)	2.64 (5.8, 14.7)
3	87.37		2, 4, 4′	86.82		2, 4, 4′		
4	70.83	4.88	2, 5	71.91	4.88	2, 5	4.49	4.92 (6.4)[b]
4-OH		2.47			2.64			2.89 (6.4)[b]
4a	141.41		4, 6	141.58		4, 6		
5	118.40	7.10 (7.3)	4, 7	118.29	7.06 (7.8)	4, 7	6.66 (7.8)	not assigned
6	137.70	7.56 (7.3, 8.2)		137.75	7.55 (7.8, 8.3)		7.08 (7.8, 7.8)	7.54 (7.8, 7.8)
7	118.69	7.01 (8.2)	5, 8-OH	118.70	7.00 (8.3)	5, 8-OH	6.93 (7.8)	not assigned
8	162.68		6, 8-OH	162.79		6, 8-OH		
8-OH		12.08			12.09			
8a	115.02		4, 5, 7, 8-OH	114.72		4,5,7,8-OH		
2′	172.37		4′, 6′	172.34		4′, 6′		
3′	132.32		4′, 6′	132.54		4′, 6′	2.91 (m)	3.06 (m)
4′	147.74	6.97 (1.5)	6′	147.54	7.09 (1.5)	2, 4, 6′	1.86 (2.9, 13.7), 2.56 (3.9, 13.7)	1.83 (2.9, 13.2), 2.79 (3.4, 13.2)
3′-Me	10.76	1.97 (1.5)	4′	10.78	1.96 (1.5)		1.12 (7.3)	1.19 (7.3)

[a] Observed in CD_3OD. [b] Coupling with C4-OH and the signal at C4-OH disappeared upon addition of D_2O.

medium) due to this type of decomposition. In some cases, a considerable amount of **3** was obtained even though the crude extract exhibited a trace amount of **3** on HPLC. Fortunately, however, both **1** and **2** withstood the conditions used for the NMR measurements in $CDCl_3$.





The structural analysis of lambertellol B (**2**) was carried out as follows. The molecular formula of **2** was established as $C_{14}H_{12}O_5$ by EI-mass spectra. A characteristic fragment signal at m/z = 242 (M-18) suggested the existence of an alcohol function, which was also supported by a strong adsorption at 3490 cm^{-1} in the IR spectrum. The ^1H NMR spectrum displayed signals corresponding to a methyl (δ 1.96), three aromatic (δ 7.00, 7.06, and 7.55), an olefinic (δ 7.09), and a phenolic proton (δ 12.09) (see Table 1), which suggested a structure similar to **3**. In addition to these proton signals, an alcoholic (δ 2.64), a methylene (δ 2.94 and 3.19, AB), and a methine proton (δ 4.88, s) were observed. The ^{13}C NMR showed 14 carbons, the same number as **3**; however, the spectral profiles were totally different. Detailed HSQC and HMBC studies revealed almost of all correlations between protons and carbons, but the HMBC spectrum did not provide crucial information as to whether this compound was spiro-butenolide **2** or pyrone **4**.

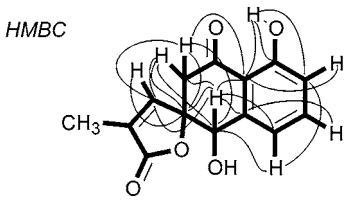


Figure 1. HMBC Correlation observed in **2**.

The chemical shift of the benzylic methine proton on C4 (4.88 ppm) suggested that this carbon was attached to alcohol or ether but not to the ester function. This interpretation is supported by the ^1H NMR of benzoate **7**, which was prepared for CD analysis (vide infra). The ^1H NMR of **6** showed the methine proton on C4 (attached to alcohol) at 4.92 ppm and the methine proton on C1 (attached to ester) at 6.52 ppm. Thus, pyrone **4** was eliminated, and we could reveal a novel dihydronaphthalen-1(2*H*)-one framework with spiro-butenolide **2**. Benzo[*g*]chromene **5** was also a candidate since **2** was easily transformed to **3** under mild condition. However, neither HMBC nor NOE experiments supported this structure as shown in Figure 2.

The ^1H and ^{13}C NMR spectra of **1** were quite analogous to those of **2** as shown in Table 1. Each corresponding signal appeared within differences of 0.17 (^1H) and 0.55 ppm (^{13}C). Furthermore, HMBC correlations of **1** were also almost same as those of **2**. These results suggest that **1** and **2** are diastereomeric to each other regarding the configurations at

(4) Poulton, G. A.; Bushnel, G. W.; Yun-Long, L. *Can. J. Chem.* **1992**, *70*, 2688.

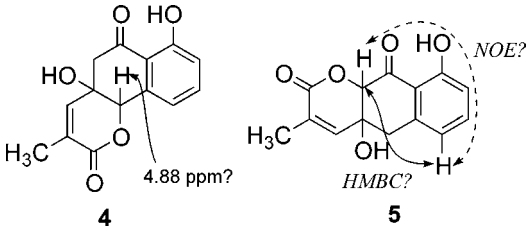


Figure 2. Eliminated structures **4** and **5**.

the C3 and C4 positions. Irradiation of a signal for the C4 proton of **1** induced NOEs at C2H, C5H, and C4′H. Similar NOEs for C2H↔C4H and C4H↔C5H were detected in **2**; however, that for C4H↔C4H′ was not detected. Accordingly, the relative stereochemistries of **1** and **2** could be established as (3*S**,4*S**) and (3R*,4*S**), respectively (Figure 3.).

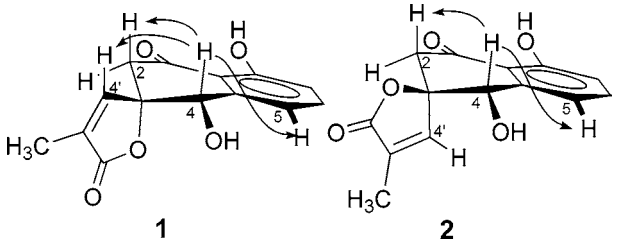
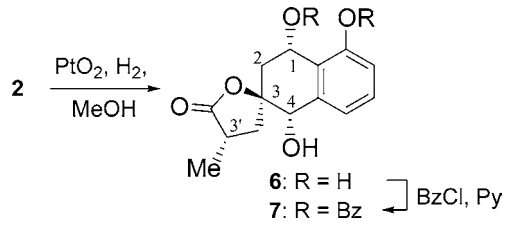


Figure 3. Stereochemistry and NOEs of **1** and **2**.

The absolute stereochemistries of **1** and **2** were studied next.[5] We supposed that the 3,4-dihydro-4-hydroxynaphthalen-1(*2H*)-one system caused the instability of the natural products. Thus, reduction of the C1-carbonyl group was attempted. Reduction of **2** proceeded to provide triol **6** in 55% yield by catalytic hydrogenation using PtO_2. The stereochemistry of **6** was established on the basis of detailed difference NOE experiments. Irradiation of a signal for C2H induced NOEs at C1H and C4H signals. These results revealed the cis relationship between C1 and C4 hydroxy groups. The stereochemistry of the C3′-methyl group was also established by observing NOEs for C2H↔C4′H and C4′H↔C3′CH_3. Triol **6** was stable enough for the next

Scheme 1



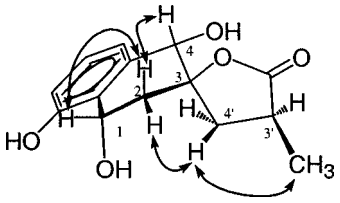


Figure 4. NOEs observed in **6**.

derivatization. Benzoylation under usual conditions proceeded at C1 and C8 hydroxy groups, giving dibenzoate **7** selectively.

The CD spectrum of **7** in MeOH gave a pair of typical exciton-split Cotton effects with a positive Cotton effect at 242 nm ($\Delta\epsilon_{242}$ +7.9) and negative one at 226 nm ($\Delta\epsilon_{226}$ −6.9), suggesting a positive chirality. The tetrahydronaphthalene moiety of **7** was unlikely to interact with the benzoyl group in the CD spectrum, since UV absorption of **6** around 230 nm was weak enough (log ϵ = 3.1 at 230 nm) compared to the benzoate **7** (log ϵ = 4.5 at 230 nm). However, one benzoyl group of **7** was attached to the C8 phenol function, which leaves an ambiguity in the conformational profile of this molecule. Thus, molecular modeling was performed. Conformation search[6] of **7** employing AM1 semiempirical molecular orbital method[7] found 12 local minimum conformations within 5 kcal/mol steric energy from that of global minimum conformation. All of the conformations provided by these calculations took type A or type B conformations about the dibenzoate moiety (Figure 5).[8] Accordingly, we

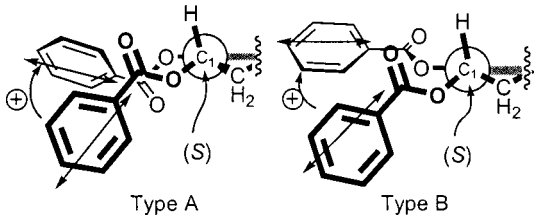


Figure 5. Typical conformations about the benzoate moiety of **7**.

were able to apply the dibenzoate rule[9] to assign the absolute stereochemistry of the newly introduced hydroxyl group at C1 as the (*S*)-configuration. The stereochemistry of C3 and C4 positions in the **2** was concluded to be (3*R*,4*S*)- by taking

(5) Unfortunately, single crystals suitable for X-ray crystarographic analysis could not be obtained.

(6) Conformation search was performed employing Pc Spartan Pro version 1.08 (Wavefunction, Inc: Irvine, CA).

(7) Dewar, M. J. S.; Zoebisch, E. G.; Hearly, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.

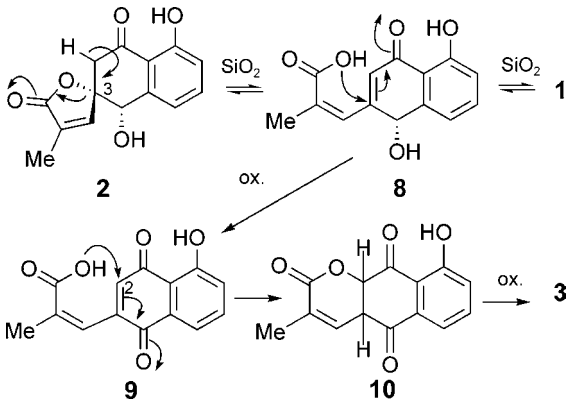
(8) When a MMFF molecular force field was employed, the geometries of the two benzoate chromopores were similar to those obtained by AM1 in all of the stable conformations, but the planes of two benzene rings have a tendency to be more parallelized.

(9) Koreeda, M.; Harada, N.; Nakanishi, K. *J. Chem. Soc., Chem. Commun.* **1969**, 548−549.

into account that the relationship of C1 and C4 alcohol functions was cis.

It is noteworthy that the two-dimensional silica gel TLC analysis revealed an interconversion between **1** and **2**. This tautomerism can be explained by *retro*-Michael reaction of the C3 carboxy group giving carboxylic acid **8** followed by *re*-Michael addition of the produced hydroxy group from the opposite site of the cyclohexane ring plane as shown in Scheme 2. These transformations should be reversible, and **1** can be converted to **2** in the same manner. On the basis of these considerations, we concluded that because the isomerization occurred at the C3 position, the absolute stereochemistry of **1** is (3*S*,4*S*)-. Both **1** and **2** might be produced nonenzymatically, and the real biosynthetic metabolite might be **8**.

Scheme 2



Formation of lambertellin (**3**) from **1** and **2** is discussed next. The intermediate **8** could be also susceptible to air oxidation, giving quinone **9**. Then, **9** is transformed into **3** by another intramolecular Michael addition at the C2 position, followed by air oxidation. So far, **8**−**10** have not been detected in our experiment. Our studies may indicate that **3** is an artifact in the case of *L.* sp. 1346. Actually, cultivation for long periods of time (22 days)[3] provided **3** as the major component (**1**:**2**:**3** = 7:14:79, based on the peak intensity at 280 nm), while HPLC analysis after 5 days indicated that **1** and **2** were the main metabolites involving trace amounts of **3** (**1**:**2**:**3** = 32:57:11) in contrast.[10]

Interestingly, when *L. corni-maris*, isolated in Aomori, Japan, was cultivated for 5 days under the same conditions, **3** was detected as the major component along with small amounts of **1** and **2**. (**1**:**2**:**3** = 7:15:78). Thus, *L. corni-maris* definitely produces **3** by another biosynthetic pathway. This biosynthetic difference between *L.* sp. 1346 and *Lambertella c.* has remained unclear and should be investigated in detail. We observed that *L. corni-maris* also brings about similar mycoparasitism against *Monilinia fructigena* on apple fruit.[1] This difference might provide clues for this phenomenon.

It was found that **1**−**3** all exhibited remarkable growth inhibition of spores of *Cochlibolus miyabeanus* (IC_{50} = 0.5, 0.5, 3.0 μg/mL, respectively). They also showed weak cytotoxity against P388 *murine leukemia* (IC_{50} = 12 μg/mL for **1**, 15 μg/mL for **2**, and 15 μg/mL for **3**). However, it is difficult to discuss their biological properties because of their instabilities.

As described, we have succeeded in obtaining unique metabolites **1** and **2** from mycoparasitic fungus *L.* sp. 1346 and found that both showed potent antifungal activity. Taking the ready interconversion between **1** and **2** into account, we propose that **8** might be the real genetic metabolite. Some of the metabolites may play important roles in mycoparasitism of *Lambertella* species against *Monilinia fructigena*.

Acknowledgment. We are grateful to Dr. Kaoru Yamada (Nagoya University) for cytotoxic assay. We would also like to thank Professor Akira Kawamura (Hunter College) for fruitful discussions about CD analysis and for reviewing the manuscript.

Note Added after ASAP Posting. There was an error in Figure 5 in the version posted ASAP December 17, 2003; the corrected version was posted December 19, 2003.

Supporting Information Available: Experimental procedure, physical data of **1** and **2**, and spectra of **1**−**3**, **6**, and **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL035889D

(10) These experiments could be performed only using static conditions, because cultutivation with shaking increased the viscosity of the media and the shaker became ineffective after 5 or 6 days. We employed the shaking conditions for production of **1** and **2**.