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Isolation, structure elucidation, preparation, and biological properties of neolambertellin

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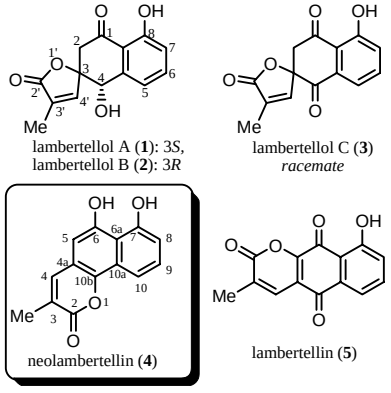
Abstract—Neolambertellin (**4**), a novel biosynthetic congener of lambertellol and lambertellin, was isolated. The structure was confirmed by successful preparation from lambertellol A, which involves a rearrangement of spiro-butenolide moiety.

Pathogenic fungi *Lambertella* sp. are sometimes parasitic on another filamentous fungi, *Monilinia* sp., on apple fruits.[1] This phenomenon is recognized as mycoparasitism,[2] and we have explored the metabolites responsible for this mycoparasitism. In these studies, we recently isolated unique spiro-butenolides, lambertellols A (**1**) and B (**2**) from the parasite *Lambertella* sp. 1346 as potent hyphal germination inhibitors of the host, *Monilinia* sp.[3] Our biosynthetic study revealed that *Lambertella* sp. 1346 incorporates ^{13}C-labeled acetate quite remarkably into both **1** and **2**,[4] exhibiting characteristic ion patterns in the mass spectra due to significant increase in isotopomers.[5] According to our assumption that labeled acetates are also incorporated at a similar level into biosynthetic congeners, we succeeded in disclosing labile biosynthetic congener lambertellol C (**3**) by an investigation that used broth extracts prepared under the labeling conditions with LC–ESIMS.[6] This letter reports another biosynthetic analogue, neolambertellin (**4**). The structure was proposed on the basis of the ^1H NMR and the mass spectra of **4**. The proposed structure of **4** was confirmed by chemical conversion from lambertellols, which involves unique rearrangement. Slow transformation of **4** into lambertellin (**5**)[7] was also observed in a dilute methanolic solution. Their mechanisms are also discussed.

Neolambertellin (**4**) was found at 14.3 min by monitoring the HPLC [Merck Lichrosphere RP18e 120 Å, 4 mm (ID) × 150 mm, H_2O/CH_3CN containing 0.1% TFA, 1.0 mL/min flow, 20–100% linear gradient for 30 min] at UV 375 nm as shown in Figure 1. This peak was detected as a trace at UV 350 nm, which we employed in the previous investigation. The direct HPLC analysis under above conditions indicated that this compound existed in the culture medium. The LC–ESIMS spectra of **4** displayed a characteristic distribution pattern of isotopomers when the sample was prepared after cultivation of *Lambertella* sp. 1346 under labeling conditions.[5] This suggested that **4** is a biogenetic analogue of other lambertellols. By preparing the sample under non-labeling conditions, the molecular weight was established to be 242 by observing the pseudo molecular ion signal at $m/z = 243$ (MH^+) together with the small signal at $m/z = 244$ due to $[MH+1]^+$ in the LC–ESIMS spectrum. The molecular formula, $C_{14}H_{10}O_4$, was established by measuring EIHRMS ($m/z = 242.0537$).



Keywords: *Iso*lambertellin mycoparasitism; Synthesis; Structure determination.
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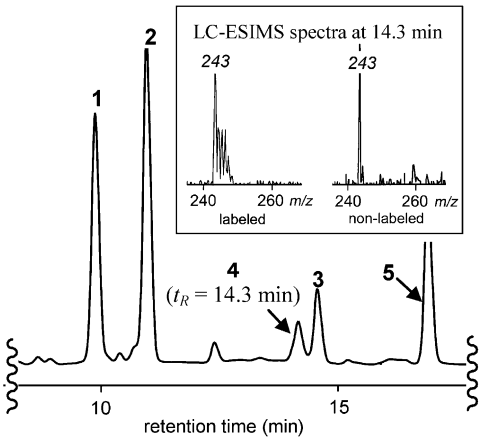


Figure 1. HPLC chromatogram detected at 375 nm and LC–ESIMS spectrum at 14.3 min.

The photodiode array (PDA) spectrum (Fig. 2) suggested that **4** has a different framework from that of either lambertellols or lambertellin (**5**). The ^{1}H NMR spectrum in CD_3OD displayed six resonances. The methyl signal appearing at 2.11 ppm was coupled with a methine proton at 7.66 ppm (J = 1.2 Hz). The intensity of the singlet at 6.72 ppm gradually decreased in CD_3OD, suggesting that this proton is located on the *ortho*-position of naphthol by slow keto–enol tautomerization. Also by considering biosynthetic similarity to other lambertellols, the structure of neolambertellin was proposed to be **4**. Structure **A** in Scheme 1 could not be eliminated at that stage despite its molecular weight being 18 greater than that of **4**. Mass spectrometry sometimes provides only dehydrated ions for some labile compounds, which might be considerable due to the formation of the pyrone system in this case. Since we cannot discuss deuterium exchangeable protons in CD_3OD, the ^{1}H NMR resonances might satisfy structure **A** in CD_3OD. Fortunately, our previous theoretical calculations suggest that structure **A** is unlikely to be observed because of easy tautomerization into structure **B**, based on theoretical calculations (HF-631G*).[6] Structure **B** was suggested to be 4.1 kcal/mol more stable than structure **A**. Further analysis utilizing ^{13}C resonances (e.g., HMBC, HMQC) could not be performed due to the quantitative limitation of **4** from *Lambertella* sp. 1346 (100 μg from 2.0 L of the cultured broth).

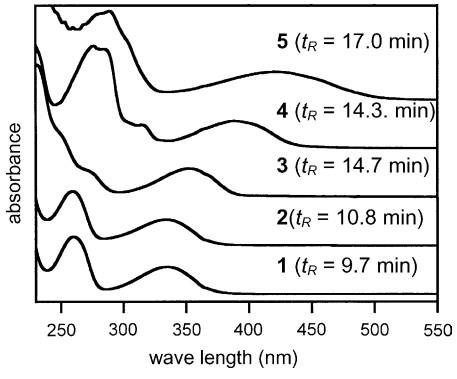
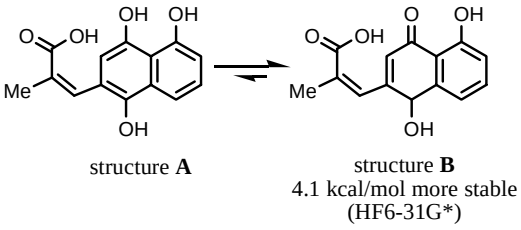


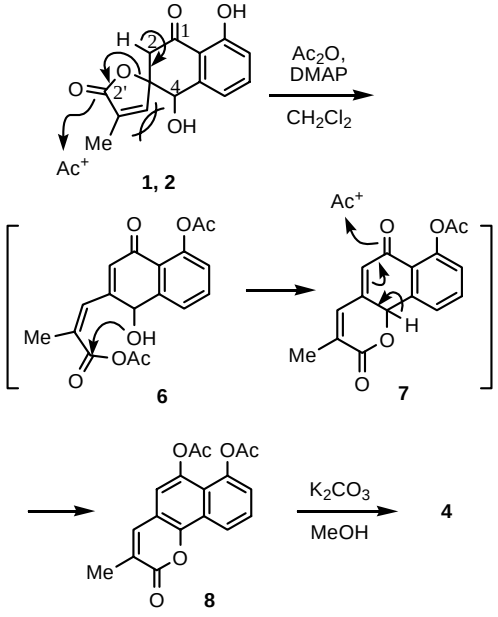
Figure 2. Photodiode array UV spectra of lambertellos A (**1**), B (**2**), C (**3**), neolambertellin (**4**), and lambertellin (**5**).



Scheme 1. Another possible structure A and its isomerization.

Fortunately, we succeeded in preparing **4** from both lambertellols **1** and **2** via the unique rearrangement shown in Scheme 2. Treatment of **1** with acetic anhydride (5 equiv) and *N*,*N*-dimethy-4-aminopyridine (5 equiv) in CH_2Cl_2 provided 6,7-di-*O*-acetyl-neolambertellin (**8**) at 59%.[8] Lambertellol B (**2**) also provided **8** with a similar yield. The structure of **8** could be fully assigned by ^{1}H and ^{13}C NMR as well as by the HMBC spectra. The IR spectrum suggested the absence of an OH function. The chemical shift for C2 (neolambertellin numbering) was observed at 161.6 ppm which also supports the pyrone ring.[9] This rearrangement can be explained by (i) acetylation of C8–OH (lambertellol numbering), (ii) acetylation of C2′ carbonyl oxygen (lambertellol numbering), which induced retro-Michael ring opening, affording acid anhydride **6**, (iii) pyrone ring formation to provide semiquinone **7**, and (iv) aromatization induced by the acetylation of C1 carbonyl oxygen. The sterically hindered C4–OH groups in **1** or **2** were not acetylated under these conditions.[10] Step ii in the rearrangement seemed to be essential for the rearrangement. Interestingly, other conditions such as acetic anhydride–pyridine gave only polar materials.

Treatment of **8** with potassium carbonate in methanol/CH_2Cl_2 at room temperature successfully provided neolambertellin (**4**) with a 96% yield.[11] The pyrone ring moiety was not hydrolyzed under these conditions as



Scheme 2. Preparation of neolambertellin (**4**).

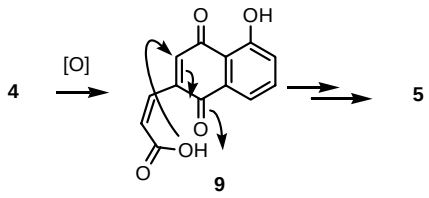
Table 1. NMR spectral data of natural and prepared **4** and **8**

Solvent:	Neolambertellin (**4**)					**8**		
	CD_3OD		DMSO-d_6 (prepared sample)			$CDCl_3$		
Position	δH (*J* in Hz)		δH (*J* in Hz)	δC	HMBC	δH (*J* in Hz)	δC	HMBC
	Natural	Prepared						
1	—	—	—	—	—	—	—	—
2	—	—	—	161.3	3-Me	—	161.6	4, 3-Me
3	—	—	—	125.3	4, 5, 3-Me	—	127.0	3-Me
4	7.66(*q*,1.2)	7.70(*q*, 1.2)	7.87(*q*,1.2)	140.0	5, 3-Me	7.52(*q*,1.2)	138.8	5, 3-Me
4a	—	—	—	115.6	—	—	114.8	—
5	6.72(*s*)	6.63(*s*)	6.78(*s*)	103.7	4	7.01(*s*)	117.4	4
6	—	—	—	150.5	5	—	141.3	5
6a	—	—	—	114.9	5,8,10	—	121.6	5, 8, 10
7	—	—	—	154.7	8, 9	—	145.3	8, 9
8	6.83(*dd, 1.0, 7.8*)	6.85(*dd, 1.0, 7.8*)	6.92(*dd, 1.0, 8.0*)	111.5	10	7.24(*dd, 1.0, 7.8*)	123.0	10
9	7.37(*dd, 7.8, 8.0*)	7.41(*t*,7.8)	7.47(*t*,8.0)	128.6	—	7.61(*dd, 7.8, 8.2*)	127.5	—
10	7.77(*dd, 1.0, 8.0*)	7.77(*dd, 1.0, 7.8*)	7.71(*dd, 1.0, 8.0*)	112.0	8	8.49(*dd, 1.0, 8.2*)	121.1	8
10a	—	—	—	125.1	9	—	126.0	9
10b	—	—	—	142.5	4,5,10	—	147.9	4, 5, 10
3-Me	2.11(*d*,1.2)	2.10(*d*,1.2)	2.12(*d*,1.2)	16.6	4	2.25(*d*,1.2)	17.2	4
6-OH	—	—	11.17	—	—	—	—	—
7-OH	—	—	11.17	—	—	—	—	—
CH$_3$CO	—	—	—	—	—	2.40 (*s*)	21.14	—
	—	—	—		—	2.40 (*s*)	21.18	—
CH$_3$*C*O	—	—	—	—	—	—	169.3	*CH*$_3$CO
	—	—	—	—	—	—	169.4	*CH*$_3$CO

described below. The HPLC retention time and PDA spectrum were identical with **4** directly isolated from *Lambertella*. The ^{1}H NMR spectra in CD_3OD of the sample thus obtained had good accordance with natural **4** despite a slight difference in their chemical shifts (Max $\Delta\delta$ was 0.09 ppm; see Table 1). These were probably caused by a small difference in the concentration of the trace acid in the solvent.

This achievement provides a sufficient quantity of **4** to measure the ^{13}C NMR, HSQC, and HMBC spectra. Dimethylsulfoxide-d_6 was used as the solvent for the NMR study because we have experienced decrement in intensity for C5 methine resonance (neolambertellin numbering) in CD_3OD. We could not distinguish between structures **4** and **A** based on the number of the exchangeable protons. All these appeared as a broad signal at 11.8 ppm and the integration of this peak was too inaccurate to estimate the number. Clues to lactone ring (or *seco*-acid) moiety were not still obtained with these measurements. The pyrone ring moiety was established by comparison of the chemical shift for C2 (neolambertellin numbering, 161.3 ppm[9]) with that of **8** (161.6 ppm in $CDCl_3$) to eliminate the possibility of structure **A** completely. Silica gel and the ODS chromatographic behavior of **4** also support its polarity. This led us to conclude the structure of neolambertellin to be as depicted in structure **4**.

We also observed slow decomposition of **4** into lambertellin **5** in a dilute methanolic solution (ca. 100 μg/mL). In fact, this decomposition made us difficult to obtain a pure sample of **4** from the culture broth. This was not remarkable at higher concentrations. Light or dissolved oxygen in the solvent provably promoted oxidation to form quinone **9**, which was further transformed into **5** as we have proposed in its biosynthesis (Scheme 3).[4]



Scheme 3. Degradation of **4** into **5**.

As described, we succeeded in disclosing a novel polyketide, neolambertellin (**4**), from the culture broth of mycoparasite *Lambertella* sp. 1346. The preliminary biological experiments revealed that **4** inhibits the effective hyphal germination of *Cochlibolus miyabeanus* (IC_{50} = ca. 100 μg/mL), but showed weak inhibition against the host, *Monilinia fructicola* (IC_{50} = ca. 1.0 mg/mL). These results suggest that **4** is not responsible for the mycoparasitism of *Lambertella* sp. against *Monilinia* sp. Further exploration of key substances of mycoparasitism from *Lambertella* is in progress in our laboratory.

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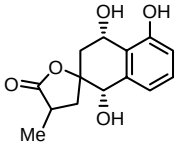
References and notes

1. Harada, Y.; Sasaki, M. *Rep. Tottori Mycol. Inst.* **1990**, *28*, 275.
2. Jeffries, P.; Young, T. W. K. *Interfungal Parasitic Relationships*; CABI International: Wallingford, 1994.
3. Murakami, T.; Morikawa, Y.; Hashimoto, M.; Okuno, T.; Harada, Y. *Org. Lett.* **2004**, *6*, 157.
4. Murakami, T.; Takahashi, Y.; Fukushi, E.; Kawabata, J.; Hashimoto, M.; Okuno, T.; Harada, Y. *J. Am. Chem. Soc.* **2004**, *126*, 9214.
5. Murakami, T.; Sasaki, A.; Fukushi, E.; Kawabata, J.; Hashimoto, M.; Okuno, T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2591.
6. Murakami, T.; Sasaki, A.; Fukushi, E.; Kawabata, J.; Hashimoto, M.; Okuno, T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2587.
7. Armstrong, J. J.; Turner, W. B. *J. Chem. Soc.* **1965**, 5927.
8. Lambetellol A (10.0 mg, 38.4 μmol) was stirred with DMAP (2.4 mg, 19.2 μmol) and Ac_2O (18 μL) in CH_2Cl_2 at room temperature for 20 min. The mixture was directly purified by silica gel column chromatography (AcOEt:Hex = 30:70) to give **8** as prisms (7.5 mg, 23.0 μmol, mp = 189 °C).
9. Pretsch, E.; Bühlmann, P.; Affolter, C. *Structure Determination of Organic Compounds: Tables of Spectral Data*; Springer Verlag: Berlin, 2000, p 139.
10. We experienced similar low reactivity in the C4–OH of stable analogue **I**. Only C1– and C8–OH were acylated (see Refs. 2 and 5).



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11. A solution of **8** (2.1 mg, 6.4 μmol) in a mixture of CH_2Cl_2 (0.2 mL) and MeOH (0.2 mL) was stirred with K_2CO_3 (0.5 mg) at room temperature for 3 h. The mixture was poured into dil HCl solution and extracted using AcOEt. The extracts were washed with brine, dried over $MgSO_4$, and then concentrated in vacuo to give **4** (1.6 mg, 6.2 μmol). The HPLC retention time and PDA spectrum of this sample were identical to those of the natural sample. The ^1H NMR spectrum in CD_3OD also resembled that of the natural compound.