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Identification of novel substituted fused aromatic compounds, meshimakobnol A and B, from natural *Phellinus linteus* fruit body

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Abstract—Novel 1*H*,6*H*-pyranyl[4,3-*c*][2]benzopyrane-1,6-diones, meshimakobnol A and B, were isolated from natural *Phellinus linteus* fruit body. The structure elucidation of these fused aromatic compounds was achieved by a spectroscopic method including the measurement of FG-HMBC with various delay times.

The phellinus species are well known as harmful fungi causing white pocket rot and sever plant disease in living trees in Western countries, but some species have been used as traditional medicines such as Kampo medicine in Oriental countries for thousands of years. From the end of the 20th century, mushrooms were found to possess anti-cancer activities.[1] Especially, activities of the water extract of *Phellinus linteus*, which is called 'meshimakobu' in Japan, have been thoroughly investigated in detail in South Korea and Japan, and the active principals were found to be the polysaccharides.[2] At the same time, many patents were applied to obtain larger amounts of effective polysaccharides from the cultured mycelia. Thus, most commercially available extracts of *P. linteus* used as supplements are produced from the cultured mycelia. However, consumers prefer natural ones over cultured ones, and the products 'including natural fruit body' are sometimes more expensive.

We are interested in the difference of components in the cultured mycelia, the cultured fruit body, and the natural fruit body, because there must be a difference in the minor components of not only polysaccharides but also the lower molecular weight compounds and there were few reports about the compounds from *P. linteus*, except for polysaccharides. Actually, there were some unambiguous differences between the HPLC chromatogram of the MeOH extracts of the cultured mycelia, the cultured fruit body, and the natural fruit body, and the extract of the natural *P. linteus* fruit body gave the characteristic peaks on the chromatogram recorded by the photodiode alley detector, which had UV absorption at 410–420 nm. In this letter, we describe the isolation and identification of the compounds with this feature named meshimakobnol A (**1**) and B (**2**) (Fig. 1) from the extract of the natural *P. linteus* fruit body.

The commercially available dried and powdered fruit body (70 g) was extracted with hot MeOH (500 mL × 3) and the solvent was removed to give the extract (2.5 g). The extract was separated by ODS column (MeOH–H_2O = 4:1) and HPLC (Nomura Chemical RP Aqueous, C30, MeOH–H_2O = 90:10) to give meshimakobnol A (**1**, 53 mg) and B (**2**, 7 mg) as a brown amorphous powder.

The molecular formula of meshimakobnol A (**1**) was given as $C_{20}H_{12}O_8$ from the high-resolution (HR) ESI-FT-ion cyclotron resonance (ICR) MS spectrum (m/z

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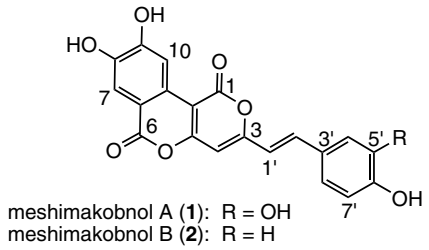

Figure 1. Structures of meshimacobunol A (**1**) and B (**2**).

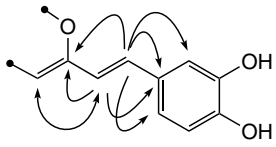

Figure 2. The partial structure of **1**. The arrows indicate the selected HMBC correlations. (H → C, $^{2,3}J = 8.3$ Hz).

403.04463 $[M+Na]^+$, calcd for $C_{20}H_{12}O_8Na$ 403.04244), and the elemental analysis of **1** (calcd for $C_{20}H_{12}O_8 \cdot 5/2H_2O$: C, 56.47; H, 4.00. Found: C, 56.97; H, 3.86) also supported the formula. The UV spectrum of **1** showed absorption bands at λ_{max} 417 (2.30×10^4) and 255 nm (2.07×10^4), which indicated the long conjugation. The IR spectrum of **1** showed the absorption bands of carbonyl (1690 cm^{-1}) and hydroxy groups (3400 cm^{-1}). In the ^1H NMR spectra of **1**, the signals of the ABX system of a tri-substituted benzene ring, a *trans*-olefine, and three singlet aromatic protons were observed. All signals in the ^{13}C NMR spectrum were sp^2 carbons, and 8 of them were methyne and the other 12 were quaternary carbons (Table 1). As the carbon signal at δ_C 161.3 was at the lowest field in these signals, the carbonyl group(s) was(were) supposed to be of the α-pyrone(s). By measurement of FG-HMQC and FG-HMBC[3] with usual delay time ($\Delta = 60$ ms: $J_{CH} = 8.3$ Hz), the connectivity of the trisubstituted benzene—the *trans*-olefine—another olefine was found (Fig. 2) but we could not determine further the structure from these data.

In order to find the number of OH groups, **1** was treated with acetic anhydride in pyridine to obtain the peracetate. The ^1H NMR spectrum of the resulting acetate[4] showed four singlet methyl signals at around δ 2.3, which implied four phenolic OH groups in **1**. Thus, the other four oxygen atoms in **1** were those of the α-pyrones. From this result and the usual HMBC correlations, we assumed the two candidate structures of **1** (Fig. 3).

For the next step, the measurement of FG-HMBC with the delay time at 200 ms ($J_{CH} = 2.5$ Hz)[5] and 400 ms ($J_{CH} = 1.25$ Hz)[6] was carried out in order to detect the longer range (4 and/or 5 bonds) C–H correlations. In this experiment, the correlations from δ 8.27 and δ 9.15 to δ_C 160.4 were detected. In the case of the structure B, the proton at δ 8.27 and the carbon at δ_C 160.4 were six bonds apart from each other and it was hard to detect $^6J_{CH}$ by these experiments. In the case of the structure A, the protons resonated at δ 8.27 and δ 9.15 were 5 and 4 bonds from the carbon at δ_C 160.4, respectively. As the 4 or 5 bonds correlations can be detected in such HMBC experiments with these parameters, the structure A in Figure 2 sufficiently represented all these data. Also no NOE was detected between the signals at δ 6.43, δ 8.27, and δ 9.15, in NOE difference spectra irradiating these signals, and this result did not support the structure B. Thus, we determined the structure of meshimakobnol A as a novel compound, 3-(2′-(3,4-dihydroxyphenyl)-*E*-ethenyl)-8,9-dihydroxy-1*H*,6*H*-pyrane[4,3-*c*][2]benzopyrane-1,6-dione (Fig. 1).

Table 1. ^1H and ^{13}C NMR spectral data of **1** and **2** (in pyridine-*d*5, ^1H: 500 MHz, ^{13}C: 125 MHz)

Pos.	**1**			**2**		
	δ_C (ppm)	δ_H (ppm), *J* (Hz) (each 1H)	HMBC correlations ($^{2,3}J_{CH} = 8.3$ Hz)	δ_C (ppm)	δ_H (ppm), *J* (Hz)	HMBC correlations ($^{2,3}J_{CH} = 8.3$ Hz)
1	160.4			160.3		
3	159.1			159.0		
4	99.2	6.43, s	3, 4a, 10b, 1′	99.3	6.50, 1H, s	3, 4a, 10b, 1′
4a	161.3			161.2		
6	159.8			159.7		
6a	112.8			112.7		
7	115.8	8.27, s	6, 6a, 8, 9, 10a	115.7	8.30, 1H, s	6, 6a, 8, 9, 10a
8	149.1[a]			149.1[a]		
9	155.7[a]			155.7[a]		
10	111.9	9.15, s	6a, 8, 9, 10b	111.9	9.17, 1H, s	6a, 8, 9, 10b
10a	128.3			128.2		
10b	100.0			100.0		
1′	115.8	6.83, d, 15.8	3, 4, 2′, 3′	115.8	6.86, 1H, d, 15.8	3, 4, 2′, 3′
2′	137.1	7.65, d, 15.8	3, 1′	136.5	7.64, 1H, d, 15.8	3, 1′
3′	127.9			127.0		
4′	115.4	7.63, d, 1.8	5′, 6′, 8′	130.2	7.62, 2H, d, 8.5	2′, 3′, 5′, 6′
5′	147.9			116.9	7.24, 2H, d, 8.5	3′, 4′, 6′
6′	150.0			161.0		
7′	116.9	7.29, d, 8.2	3′, 5′, 6′		See 5′	
8′	121.7	7.20, dd, 1.8, 8.2	2′, 4′, 6′		See 4′	

[a] The data may be exchangeable in the same vertical column (MHz).

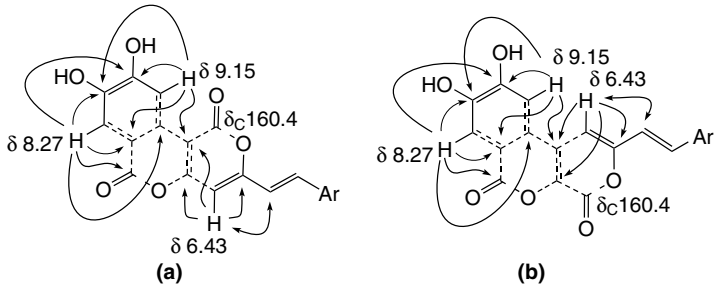

Figure 3. The structures assumed from usual FG-HMBC correlations. The arrows indicate the HMBC correlations ($^{2,3}J = 8.3$ Hz) from the proton signals at δ 6.43, δ 8.27, and δ 9.15. (H $\rightarrow$ C).

The molecular formula of meshimakobnol B (**2**) was given as $C_{20}H_{12}O_7$ from the HR-ESI-FT-ICR MS spectrum (m/z 387.04850 $[M+Na]^+$, calcd for $C_{20}H_{12}O_7Na$ 387.04802). The UV spectrum of **2** showed the absorption bands at 409 (1.48×10^4) and 250 nm (1.66×10^4) and the IR spectrum showed the absorption bands of ester and ketone carbonyl, and hydroxy groups. The ^1H and ^{13}C NMR spectra of **2** were quite similar to those of **1**, except for the signals due to a *para*-substituted benzene ring instead of those due to 1,2,4-tri-substituted one in **1**. As the chemical shifts of the signals and long-range CH correlations in FG-HMBC ($J_{CH} = 8.3$ Hz) due to the 8,9-dihydroxy-1*H*,6*H*-pyranyl[4,3-*c*][2]benzopyrane-1,6-dione moiety and *trans* olefine of **2** were quite identical with those of **1**, we determined the structure of meshimakobnol B (**2**) as novel 3-(2′-(4-hydroxyphenyl)-*E*-ethenyl)-8,9-dihydroxy-1*H*,6*H*-pyranyl[4,3-*c*][2]benzopyrane-1,6-dione.

Although the some synthetic compounds with 1*H*,6*H*-pyranyl[4,3-*c*][2]benzopyrane-1,6-dione skeleton were reported as coumarino(3,4:4′,3′)isocoumarins,[7] the structure elucidation of such natural fused aromatic compounds by spectroscopic methods is sometimes quite difficult, because small numbers of protons give us the limited information about C–H correlations and the carbon(s) without any correlations through the routine 2D NMR measurement. Meshimakobnols, the substituted 1*H*,6*H*-pyranyl[4,3-*c*][2]benzopyrane-1,6-diones, were the typical example, but we could determine the structures by measurement of FG-HMBC with appropriate delay times, which gave us sufficient information about the 4 and/or 5 bonds C–H correlations. One natural 3-methyl-8,9-dihydroxy-1*H*,6*H*-pyranyl[4,3-*c*][2]-benzopyrane-1,6-dione, phelligridin A, was recently determined by the interpretation of the ^1H, ^{13}C NMR, HMQC, and usual HMBC spectral data and the number of unsaturation.[8] However, the authors did not recognize the other possible structure corresponding to structure B in Figure 2 at all, and did not indicate any evidence for the connection of C1–C10b nor C4–C4a in their paper. This reported structure of phelligridin A might be correct, but meshimakobnol A (**1**) and B (**2**) should be the first properly identified natural 1*H*,6*H*-pyranyl[4,3-*c*][2]benzopyrane-1,6-diones. As meshimakobnol A (**1**) and B (**2**) were detected from only the natural fruit body of *P. linteus*, they could become the indicator discriminating between the commercially available *P. linteus* produced from natural source and that from a cultured one. Further investigation on the biological activities of meshimakobnol A (**1**) and B (**2**) is now in progress.

Acknowledgements

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References and notes

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3. Peracetate of **1**: FAB MS (m/z): 549 $(M+H)^+$. ^1H NMR (500 MHz, in $CDCl_3$, δ ppm): 2.31 (3H, s), 2.33 (3H, s), 2.34 (3H, s), 2.38 (3H, s), 6.39 (1H, s), 6.66 (1H, d, $J = 15.8$ Hz), 7.22 (1H, d, $J = 8.8$ Hz), 7.42 (1H, d, $J = 1.9$ Hz), 7.45 (1H, dd, $J = 1.9$, 8.8 Hz), 7.60 (1H, d, $J = 15.8$ Hz), 8.19 (1H, s), 8.91 (1H, s).
4. Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093–2094.
5. The spectra was recorded both on JNM alpha-500 spectrometer with a data size 1K (F2)$\times$256 (F1) points for the spectral width of 4800 Hz (^1H)$\times$8320 Hz (^{13}C) and 150 scans and on JNM ECA-800 spectrometer with a data size 2K (F2)$\times$512 (F1) points for the spectral width of 3300 Hz (^1H)$\times$14220 Hz (^{13}C) and 60 scans.
6. The spectrum was recorded on JNM ECA-800 spectrometer with a data size 4K (F2)$\times$1K (F1) points for the spectral width of 3400 Hz (^1H)$\times$14230 Hz (^{13}C) and 68 scans (ca. 40 h).
7. For example Darbarwar, M.; Sundaramurthy, V.; Rao, N. V. S. *Indian J. Chem.* **1973**, *11*, 637–640.
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