A Benzofuran Glycoside and an Acetylenic Acid from the Fungus *Laetiporus sulphureus* var. *miniatus*

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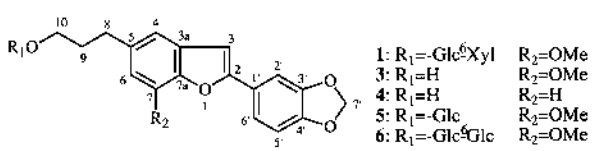
Received October 23, 2000; accepted December 5, 2000

A new benzofuran glycoside, masutakeside I (1) and a new C_{10} acetylenic acid, masutakic acid A (2) were isolated from the fruiting bodies of the fungus *Laetiporus sulphureus* var. *miniatus*. Their structures were established by spectroscopic and chemical methods. The known compounds 3—6 were also obtained and identified as egonol, demethoxyegonol, egonol glucoside and egonol gentiobioside. Some of these compounds exhibited cytotoxicity against Kato III cells.

Key words *Laetiporus sulphureus* var. *miniatus*; masutakeside I; masutakic acid A; Polyporaceae

As a part of a research program aimed at the discovery of biologically active compounds from fungi, we have previously reported the isolation and structure elucidation of five lanostane triterpenoids, named versisponic acids A—E and seven lanostanol glycosides, designated as laetiposides A—G from the fruit bodies of *Laetiporus versisporus* (LLOYD) IMAZ. (Polyporaceae).[1,2] We have recently initiated a chemical study of *Laetiporus sulphureus* (FR.) MURR. var. *miniatus* (JUNGH.) IMAZ. (Polyporaceae), which grows on the needle-leaf tree and is distributed throughout Japan.[3] An earlier chemical constituent study of this fungus resulted in the isolation of a ceramide and ergosterol.[4] The EtOH extract of the fresh fruiting bodies of the fungus was separated by reversed-phase HPLC with 40—70% MeOH to give two new compounds, masutakeside I (**1**) and masutakic acid A (**2**), in addition to five known compounds. The structures of the known compounds, egonol (**3**), demethoxyegonol (**4**), egonol glucoside (**5**), egonol gentiobioside (**6**),[5,6] and 2-(3,4-dihydroxyphenyl)-2,3-dihydro-7-hydroxy-3-hydroxymethyl-5-benzofuranpropanol,[7] were determined by a combination of spectroscopic analysis and comparison with reported data.

Masutakeside I (**1**) was obtained as an amorphous powder. The molecular formula was established as $C_{30}H_{36}O_{14}$ based on the molecular ion at *m/z* 643.1970 $[M+Na]^+$ in the high-resolution (HR)-FAB-MS, requiring 13 degrees of unsaturation. The IR absorption maxima at 3395, 1605 and 1520 cm^{-1} and the absorbance at 210, 233 and 278 nm in the UV spectrum suggested the presence of aromatic rings. The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectrum revealed signals for all 30 carbons, consisting of one methoxyl, eight sp^2 quaternary carbons, six sp^2 methines, four sp^3 methylenes (two oxygenated), and 11 signals due to a pentose unit and a hexose unit (Table 1). The ^1H-NMR and ^1H–^1H correlation spectroscopy (^1H–^1H COSY) spectra of **1** exhibited signals for a propanol group at δ 4.25 (1H, m), 3.70 (1H, m), 2.86 (2H, m) and 2.04 (2H, m), two *meta*-coupled doublets at δ 7.11 (1H, d, J=1.2 Hz) and 6.83 (1H, d, J=1.2 Hz), an ABX-type signal set at δ 7.56 (1H, d, J=1.6 Hz), 7.54 (1H, dd, J=8.0, 1.6 Hz), and 6.94 (1H, d, J=8.0 Hz), and an independent olefinic proton at δ 7.16 (1H, s). An isolated methylene signal at δ 6.00 (2H, s), which correlated with the carbon signal at δ 102.0 in the ^1H-detected multiple quantum coherrence spectrum (HMQC), was suggestive of the presence of a methylenedioxy moiety in **1**. The units described above were connected by the ^1H-detected heteronuclear multiple bond correlation spectrum (HMBC) and by rotating frame nuclear Overhauser and exchange spectroscopy (ROESY). Thus, H_2-8 (δ_H 2.86) showed in the HMBC spectrum a two-bond connectivity with C-5 (δ_C 138.5), and three-bond connectivities with C-4 (δ_C 113.1) and C-6 (δ_C 108.4). Further, H-4 (δ_H 7.11) showed long-range correlation to C-3a (δ_C 131.6) and C-7a (δ_C 143.0). In turn, the C-2 (δ_C 156.2), C-3a and C-7a carbons displayed HMBC correlation with H-3 (δ_H 7.16). A nuclear Overhauser effect (NOE) interaction was observed between H-6 (δ_H 6.83) and the methoxyl group (δ_H 3.95). Hence, a benzofuran unit having a propanol at C-5 and a methoxyl group at C-7 was evident. The long-range correlation from H_2-7′ (δ_H 6.00) to C-3′ (δ_C 148.7) and C-4′ (δ_C 148.5) further confirmed the involvement of C-3′ and C-4′ in a methylenedioxy ether linkage. Key NOE interactions between H-3 (δ_H 7.16) and H-2′ (δ_H 7.56) and H-6′ (δ_H 7.54) led to the assignment of the aglycone moiety of **1**, 5-(3-hydroxypropyl)-7-methoxy-2-(3,4-methylenedioxyphenyl) benzofuran [egonol (**3**)].[5,6] Acid hydrolysis of **1** afforded **3**, besides D-glucose and D-xylose confirmed by specific rotation using chiral detection in HPLC analysis.[8] Furthermore, the coupling constants observed for the anomeric protons at δ 5.02 (1H, d, J=7.4 Hz) and δ 4.82 (1H, d, J=8.0 Hz) in the ^1H-NMR spectrum of **1** indicated a β-glycosidic linkage for both the glucopyranose and xylopyranose units. The sugar sequence was established by the glycosylation shift rule,[9] and



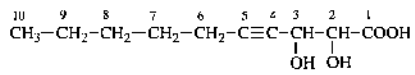


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Chart 1

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Table 1. ^{1}H- and ^{13}C-NMR Data for Compounds **1** and **2** in Pyridine-d_5

Position	**1**		Position	**2**	
	^{13}C	^{1}H, mult (*J* in Hz)		^{13}C	^{1}H, mult (*J* in Hz)
2	156.2 s		1	175.0 s	
3	101.4 d	7.16 s	2	76.6 d	5.04 (d, 4.4)
3a	131.6 s		3	65.6 d	5.50 (dd, 4.4,1.6)
4	113.1 d	7.11 (d, 1.2)	4	80.8 s	
5	138.5 s		5	85.6 s	
6	108.4 d	6.83 (d, 1.2)	6	19.0 t	2.11 (dt, 1.6, 7.2)
7	145.4 s		7	28.6 t	1.34 (quint, 7.2)
7a	143.0 s		8	31.1 t	1.21 (quint, 7.2)
8	32.8 t	2.86 m	9	22.3 t	1.08 (sext, 7.2)
9	32.5 t	2.04 m	10	14.0 q	1.21 (t, 7.2)
10	68.9 t	3.70 m			
		4.25 m			
1′	125.2 s				
2′	105.8 d	7.56 (d, 1.6)			
3′	148.7 s				
4′	148.5 s				
5′	109.1 d	6.94 (d, 8.0)			
6′	119.5 d	7.54 (dd, 8.0, 1.6)			
7′	102.0 t	6.00 s			
MeO	56.1 q	3.95 s			
Glc 1	104.8 d	4.82 (d, 8.0)			
2	75.3 d	4.05 (dd, 8.8, 8.0)			
3	79.1 d	4.22 (dd, 9.4, 8.8)			
4	72.6 d	4.24 (dd, 9.4, 8.5)			
5	77.2 d	4.09 (ddd, 8.5, 5.5, 1.9)			
6	70.0 t	4.31 (dd, 11.4, 5.5)			
		4.86 (dd, 11.4, 1.9)			
Xyl 1	106.1 d	5.02 (d, 7.4)			
2	75.3 d	4.06 (dd, 8.7, 7.4)			
3	79.1 d	4.16 (dd, 8.7, 8.5)			
4	72.6 d	4.24 (ddd, 9.7, 8.5, 2.7)			
5	67.2 t	3.69 (dd, 11.3, 9.7)			
		4.35 (dd, 11.3, 2.7)			

HMBC experiments. The downfield-shifted ^{13}C-NMR resonance observed at δ 70.0 among the sugar units could be assigned to C-6 of glucose. Further, long-range correlations were observed between H-1 (δ_H 4.82) of glucose and C-10 (δ_C 68.9) of the aglycon, and H-1 (δ_H 5.02) of xylose and C-6 (δ_C 70.0) of glucose, indicating the presence of a primeverose moiety (–Glc^{6}–Xyl). Accordingly, **1** was formulated as egonol primeveroside.

Masutakic acid A (**2**) was obtained as an amorphous powder, and the molecular formula was established as $C_{10}H_{16}O_4$ on the basis of HR-CI-MS [*m/z* 201.1149 $(M+H)^+$, Δ+2.2 mmu]. The ν_{max} at 2220 cm^{-1} in the IR spectrum of **2** was consistent with the presence of a triple bond. This was supported by the DEPT spectrum, which revealed the presence of one methyl group, four methylenes, two oxymethines [δ_C 76.6 (d), and δ_C 65.6 (d)], one acetylenic functionality [δ_C 85.6 (s), and δ_C 80.8 (s)], in addition to a carboxyl group [δ_C 175.0 (s)]. The ^{1}H-NMR and COSY spectra showed the presence of a 1,2-diol group at δ 5.50 (1H, dd, *J*=4.4, 1.6 Hz), 5.04 (1H, d, *J*=4.4 Hz), and a pentyl group (H_2-6—H_3-10). HMBC correlations between H-2 (δ_H 5.04) and C-1 (δ_C 175.0), and C-4 (δ_C 80.8), and between H_2-6 (δ_H 2.11) and C-5 (δ_C 85.6), C-4 (δ_C 80.8), and C-7 (δ_C 28.6) indicated that the acetylene group could be placed between C-3 and C-6. Accordingly, **2** was formulated as 2,3-dihydroxy-dec-4-ynoic acid. To the best of our knowledge, this is only the second report of the isolation of an acetylenic compound containing a C≡C–CH(OH)–CH(OH)–COOH unit from a fungal source.[10)]

Compounds **1** and **3**—**6** were tested for cytotoxic activities against Kato III cells.[11,12)] Compounds **3**, **4** and **5** showed *in vitro* cytotoxicity with IC_{50} values of 28.8, 27.5, and 24.9 μg/ml, respectively (positive control, hinokitiol 0.6 μg/ml).

Experimental

Optical rotations were taken on a JASCO DIP-1000 polarimeter. IR and UV spectra were recorded on JASCO FT-IR 5300 and Hitachi U-3000 spectrometers, respectively. NMR spectra were recorded on a Varian UNITY 600 spectrometer in C_5D_5N solution. NMR experiments included ^{1}H–^{1}H COSY, DEPT, HMQC, HMBC, and ROESY. Coupling constants (*J* values) are given in Hertz (Hz). HR-FAB-MS and HR-CI-MS were measured on a JEOL AX-500 mass spectrometer. Kieselgel 60 (230—400 mesh, Merck) was used for column chromatography, and silica gel 60F-254 (Merck) for TLC.

Plant Material The fruiting bodies of *Laetiporus sulphureus* var. *miniatus* were collected in Tokushima prefecture, in autumn 1998. The voucher specimen (TB3019) is deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

Extraction and Isolation The fresh fruiting bodies (0.55 kg) were extracted with 70% EtOH at room temperature for 6 weeks. The ethanolic extract (57.5 g) was partitioned between H_2O and EtOAc. The water layer was passed through an Amberlite XAD-2 column. After the column was washed with water, the adsorbed materials were eluted with 100% MeOH. The MeOH eluate (5.0 g) was chromatographed on HPLC (Develosil Lop ODS, 40—100% MeOH) to give 20 fractions. Fractions 13 and 14 were subjected to HPLC (YMC, ODS S-5, 37—40% CH_3OH) to give egonol gentiobioside (**6**, 7.4 mg) and masutakic acid A (**2**, 7 mg), respectively. Fractions 17, 18 and 25 were purified by preparative HPLC (YMC, ODS S-5, 58—60% CH_3OH) to afford egonol glucoside (**5**, 10 mg) from fr. 17, masutakeside I (**1**, 40 mg) from fr. 18, and demethoxyegonol (**4**, 10 mg) and 2-(3,4-dihydroxyphenyl)-2,3-dihydro-7-hydroxy-3-hydroxymethyl-5-benzofuranpropanol (10 mg) from fr. 25. Fraction 26 was subjected to HPLC (YMC, ODS S-5, 72% CH_3OH) to give egonol (**3**, 7.4 mg).

Masutakeside I (**1**): An amorphous powder, $[\alpha]_D^{25}$ −19.9° (*c*=2.6, MeOH). UV (MeOH) λ_{max} (log ε) 210 (4.49), 233 (4.46), 278 (4.17) nm. FT-IR (film) ν_{max} 3395, 1605, 1520 cm^{-1}. FAB-MS *m/z*: 643 $[M+Na]^+$, 659 $[M+K]^+$. HR-FAB-MS *m/z*: 643.1970 (Calcd for M^++Na, $C_{30}H_{36}O_{14}Na$: 643.2003).

Masutakic Acid A (**2**): An amorphous powder, $[\alpha]_D^{25}$ −13.2° (*c*=0.7, MeOH). FT-IR (film) λ_{max} 3346, 2220, 1728, 1105 cm^{-1}. CI-MS *m/z*: 201 $[M+H]^+$, 183 $[M+H-H_2O]^+$. HR-CI-MS *m/z*: 201.1149 (Calcd for M^++H, $C_{10}H_{17}O_4$: 201.1127).

Acid Hydrolysis of 1 A solution of compound **1** (10 mg) in 5% H_2SO_4–dioxane (1 : 1) was heated at 100 °C for 2 h. The reaction mixture was diluted with H_2O, and then neutralized with Amberlite IRA-35 and evaporated *in vacuo* to dryness. The reaction mixture was diluted with H_2O, and extracted with EtOAc. The EtOAc layer was purified by column chromatography on silica gel by elution with CH_2Cl_2–MeOH (30 : 1) to afford **3** (3 mg), which was confirmed by ^{1}H-NMR data and by co-HPLC (ODS, 65% MeOH) with an authentic sample. The identification of and the D or L configurations of the sugars were determined using refraction index (RI) detection (Waters 410) and chiral detection (Shodex OR-1) by HPLC {Shodex RSpak NH_2P-50 4E column, CH_3CN–H_2O–H_3PO_4 (95 : 5 : 1), 1 ml/min, 47 °C} by comparison with authentic sugar (10 mmol of D-Glc and D-Xyl). The sugar portion from compound **1** gave peaks for D-(+)-Xyl at 9.10 min and D-(+)-Glc at 20.7 min.

Acknowledgment We are grateful to Mr. T. Ohashi, mycologist, Tokushima Prefectural Tomioka Nishi High School for confirming the identity of the fungus.

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