New Lanostanoid Glycosides from the Fruit Body of Laetiporus versisporus

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · MAY 1999

Impact Factor: 3.8 · DOI: 10.1021/np980456i · Source: PubMed

3 AUTHORS, INCLUDING:

Kazuko Yoshikawa
Tokushima Bunri University
93 PUBLICATIONS **1,475** CITATIONS

JOURNAL OF NATURAL PRODUCTS

Volume 62, Number 4 April 1999

Full Papers

New Lanostanoid Glycosides from the Fruit Body of *Laetiporus versisporus*

Kazuko Yoshikawa,* Kenji Matsumoto, and Shigenobu Arihara

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770-8514, Japan

Received October 16, 1998

Four new lanostanoid glycosides, 3β,7α-dihydroxy-24-methylene-lanost-8-en-21-oic acid 7-*O*-β-D-glucopyranoside (**1**), 3β,7α-dihydroxy-lanost-8,24-dien-21-oic acid 7-*O*-β-D-glucopyranoside (**2**), 7α-hydroxy-3-keto-lanost-8,24-dien-21-oic acid 7-*O*-β-D-glucopyranoside (**3**), and 7α-hydroxy-3-keto-24-methylene-lanost-8-en-21-oic acid 7-*O*-β-D-glucopyranoside (**4**) were isolated from the fruit bodies of *Laetiporus versisporus*. Compounds **1**−**4** were named laetiposides A−D, respectively. Their structures were established by extensive NMR experiments and chemical methods.

Laetiporus versisporus (*Polyporus calvatioides*) (Polyporaceae) grows on the dead trees in broad-leaf forests and is distributed throughout Japan.[1,2] A phytochemical study of the genus of *Laetiporus* (*Polyporus*) reported the isolation of polyporusterones A−G[3] and 9α-hydroxy-1,2,3,4,5,10,19-heptanorergosta-7,22-diene-6,9-lactone.[4] However, a systematic investigation of *L. versisporus* has not been reported. Therefore, it appeared of interest to investigate the high polar components of this fungus.

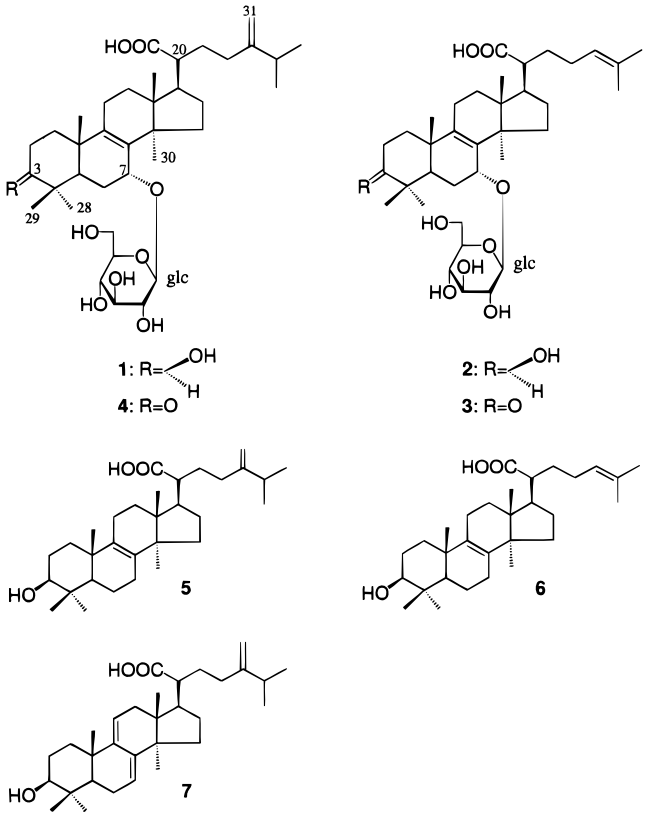
Four new lanostanol glycosides, laetiposides A (**1**), B (**2**), C (**3**), and D (**4**), along with eburicoic acid (**5**),[5] trametenolic acid (**6**),[5] sulfurenic acid,[6] and 15α-dihydroxytrametenolic acid[6] were isolated from the EtOH extract of the fruit bodies of *L. versisporus* by column chromatography and reversed-phase HPLC. We describe here the isolation and structure elucidation of laetiposides A−D (**1**−**4**) by various NMR techniques, including COSY, HMQC, HMBC, and ROESY experiments, and chemical degradation.

Results and Discussion

The main compound, laetiposide A (**1**), was obtained as a colorless needle, which gave a molecular ion peak at *m*/*z* 647 in its FABMS. This corresponds to a molecular formula $C_{37}H_{60}O_9$, requiring eight unsaturation equivalents. The IR spectrum of **1** showed broad absorption at 1700 cm^{-1} (carboxyl). The 37 carbon signals were observed in the ^{13}C NMR spectrum; these were sorted, by DEPT experiment, into seven methyl carbons; nine methylene carbons; six methine carbons, two of which had oxygen substituents; four sp^2 carbons, one of which was protonated; four sp^3 quaternary carbons; and a carboxyl carbon, in addition to the six signals of a hexose (Table 1). These data suggested **1** to be a tetracyclic triterpene containing two double-bonds (δ 156.0, 142.1, 134.5, and 107.0), one carboxyl (δ 178.8), two secondary hydroxy moieties (δ 77.8 and 72.2), and one glucopyranosyl moiety (δ 101.2, 78.5, 78.3, 74.6, 72.4, and 63.6). A combination of COSY and HMBC experiments enabled us to construct a 24-methylenelastanol skeleton as the aglycon of **1**. A carboxyl group was located at the C-21 position from HMBC correlation of H-20 at δ 2.64 to C-21 (Figure 1). A ^{13}C NMR spectral comparison of **1** with eburicoic acid (**5**)[5] showed that **1** differs structurally from **5** only in the B ring. One of two secondary hydroxy carbons (δ 72.2) was defined at C-7 from HMBC correlations of H-7 (δ 4.66, br s) to C-5 (δ 44.3), C-8 (δ 134.5), and C-9 (δ 142.1) (Figure 1). Further, the long-range coupling between the H-1 (δ 4.97) of the glucosyl unit and C-7 (δ 72.2) established that the glucosyl group is attached to C-7. The α-*O* function at C-7 position could be assigned from the broad singlet signal observed for H-7 and the NOE experiment (Figure 2). The acid hydrolysis of **1** with 5% H_2SO_4 afforded dehydroeburicoic acid (**7**)[5] and D-glucose, which was confirmed by specific rotation using chiral detection by HPLC

* To whom correspondence should be addressed. Tel.: (81) 88-622-9611. Fax: (81) 88-655-3051. E-mail: yosikawa@ph.bunri-u.ac.jp.

10.1021/np980456i CCC: $18.00
Published on Web 03/12/1999



analysis.[7,8] The coupling constant for the anomeric protons observed at δ 4.97 (J = 7.0 Hz) in the ^{1}H NMR spectrum of **1** indicated the glucose had a β configuration. Thus, the structure of **1** was formulated as 3β,7α-dihydroxy-24-methylene-lanost-8-en-21-oic acid 7-*O*-β-D-glucopyranoside.

Laetiposide B (**2**) gave a quasi molecular ion at *m*/*z* 633 [M − H]$^-$, 14 mass units (CH_2) less than that of **1**. Acid hydrolysis of **2** also yielded D-glucose, which has the β configuration by its coupling constant (J = 7.8 Hz) of the anomeric proton at δ 4.98. Comparison of physicochemical data of **2** with those of **1** revealed that the only difference was that **2** had a hydrogen at C-24 instead of a methylene. The carbon signals due to the side-chain part of **2** were in good agreement with those of trametenolic acid (**6**).[5] The connectivities of the COSY and HMBC experiments supported the assumed structure of **2**. Accordingly, **2** was formulated as 3β,7α-dihydroxy-lanost-8,24-dien-21-oic acid 7-*O*-β-D-glucopyranoside.

The FABMS of laetiposide C (**3**) gave a quasi molecular ion at *m*/*z* 631 [M − H]$^-$, 2 mass units less than that of **2**. A ^{13}C NMR spectral comparison of **3** with **2** showed that **3** differs structurally from **2** only in the A ring, namely, the presence of a carbonyl function at C-3 in **3** instead of a hydroxyl group in **2**. Indeed, in the ^{13}C NMR spectrum of **3**, a signal at δ 216.7 was observed. Long-range correlations, in the HMBC spectrum of **3**, observed for H_2-2 (δ 2.12 and 2.31), H-28 (δ 1.36), H-29 (δ 1.11), and C-3 (δ 216.7) indicated a carbonyl function at the C-3 position. Accordingly, **3** was formulated as 7α-hydroxy-3-keto-lanost-8,24-dien-21-oic acid 7-*O*-β-D-glucopyranoside.

The last compound, laetiposide D (**4**) showed the molecular formula $C_{37}H_{58}O_9$ from the elemental analysis data and the observation of 37 carbon signals in the ^{13}C NMR spectrum. Comparison of physicochemical data of **4** with those of **1** revealed that the only difference was that **4** had a carbonyl function at C-3 instead of a hydroxy group. Therefore, **4** was formulated as 7α-hydroxy-3-keto-24-methylene-lanost-8-en-21-oic acid 7-*O*-β-D-glucopyranoside.

Table 1. ^{13}C NMR Data for Compounds **1**−**4** and **6** (in C_5D_5N at 100 MHz)

position	1	2	3	4	6
C-1	35.4 t	35.3 t	35.2 t	35.1 t	36.1 t
2	28.5 t	28.5 t	34.2 t	34.2 t	28.7 t
3	77.8 d	77.7 d	216.7 s	216.8 s	78.0 d
4	39.1 s	39.1 s	46.8 s	46.8 s	39.5 s
5	44.3 d	44.2 d	43.9 d	43.8 d	50.9 d
6	23.6 t	23.4 t	24.7 t	24.7 t	18.7 d
7	72.2 d	72.0 d	71.9 d	71.9 d	26.6 t
8	134.5 s	134.5 s	135.1 s	135.1 s	135.2 s
9	142.1 s	142.1 s	140.2 s	140.2 s	134.6 s
10	38.5 s	38.5 s	38.0 s	37.9 s	37.4 s
11	21.6 t	21.4 t	21.3 t	21.2 t	21.3 t
12	29.6 t	29.4 t	29.3 t	29.2 t	29.4 t
13	45.6 s	45.4 s	45.4 s	45.4 s	44.9 s
14	50.4 s	50.2 s	50.4 s	50.3 s	49.9 s
15	29.4 t	29.2 t	29.5 t	29.5 t	30.9 t
16	27.8 t	27.6 t	27.9 t	27.6 t	27.5 t
17	47.8 d	47.7 d	47.8 d	47.8 d	47.7 d
18	16.6 q	16.5 q	16.7 q	16.6 q	16.3 q
19	18.0 q	17.8 q	17.7 q	17.7 q	19.4 q
20	49.5 d	49.3 d	49.3 d	49.4 d	49.0 d
21	178.8 s	178.8 s	178.7 s	178.8 s	178.6 s
22	31.8 t	33.2 t	33.3 t	31.7 t	33.3 t
23	32.7 t	26.6 t	26.7 t	32.7 t	26.7 t
24	156.0 s	124.9 d	124.9 d	155.9 s	124.9 d
25	34.2 d	131.6 s	131.7 s	34.2 d	131.6 s
26	21.9 q	25.6 q	25.8 q	21.9 q	25.8 q
27	22.0 q	17.6 q	17.7 q	22.0 q	17.7 q
28	28.6 q	28.5 q	27.2 q	27.2 q	28.6 q
29	16.9 q	16.7 q	21.3 q	21.2 q	16.4 q
30	25.7 q	25.6 q	25.5 q	25.5 q	24.5 q
31	107.0 t			107.0 t	
glc C-1	101.2 d	101.1 d	101.7 d	101.7 d	
2	74.6 d	75.2 d	75.1 d	75.1 d	
3	78.5 d	78.4 d	78.5 d	78.5 d	
4	72.4 d	72.4 d	72.4 d	72.4 d	
5	78.3 d	78.2 d	78.3 d	78.3 d	
6	63.6 t	63.6 t	63.5 t	63.5 t	

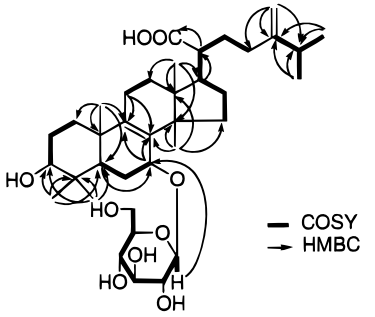


Figure 1. COSY and selected HMBC correlations of laetiposide A (**1**).

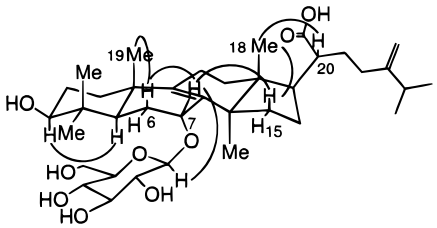


Figure 2. Selected NOE correlations of laetiposide A (**1**).

Experimental Section

General Experimental Procedures. Melting points were measured with a Yanagimoto micromelting point apparatus and were uncorrected. Optical rotations were obtained on a JASCO DIP-360 polarimeter. IR spectra were recorded on a JASCO FT/IR-5300. NMR spectra were recorded on a JEOL GSX-400 spectrometer in pyridine-d_5, using TMS as internal standard. NMR experiments included ^{1}H−^{1}H COSY, HMQC,

HMBC, and ROESY. Coupling constants (*J* values) are given in Hz. The FABMS (Xe gun, 10 kV, triethylene glycol as the matrix) was measured on a JEOL JMS−PX303 mass spectrometer. HPLC separations were performed with a Hitachi HPLC system (L-7100 pump, L-4000 UV).

Plant Material. The fruit bodies of *Laetiporus versisporus* were collected at Tokushima City, Japan, in autumn 1997. A voucher specimen (TB3001) is deposited in the Herbarium of Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan.

Extraction and Isolation. The fresh fruit bodies (1.3 kg) of *L. versisporus* were extracted with 70% EtOH at room temperature for 6 weeks. The EtOH extract (57.5 g) was partitioned between H_2O and EtOAc. The EtOAc-soluble portion (9.0 g) was repeatedly subjected to Si gel column chromatography with CH_2Cl_2−MeOH−H_2O (25:1:0−25:4:0.1) to afford five fractions (fractions 1−5). Fractions 2 (3.0 g) and 3 (1.0 g) were purified by preparative HPLC (ODS, 70−72% CH_3OH) to afford eburicoic acid (**5**, 135 mg), trametenolic acid (**6**, 65 mg), sulfurenic acid (15 mg), and 15α-dihydroxytrametenolic acid (30 mg). Fraction 5 (2.0 g) was further subjected to Si gel column chromatography with CH_2Cl_2−MeOH−H_2O (25:4:0.1) to give four fractions fractions 5-1−4). Fractions 5-2 (0.2 g) and 5-3 (0.21 g) were purified by preparative HPLC (ODS, 68% CH_3OH) to afford laetiposides C (**3**, 20 mg) and D (**4**, 35 mg) from fraction 5-2, and laetiposides A (**1**, 62 mg) and B (**2**, 10 mg) from fraction 5-3.

Laetiposide A (1): colorless needles, mp 276−277 °C; $[\alpha]^{25}_D$ +9.9° (*c* 2.0, MeOH); FT−IR (dry film) ν_{max} 3400 (OH), 1700 (C=O) cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) δ 0.98 (3H, s, Me-19), 1.01 (3H, s, Me-18), 1.03 (6H, d, J = 6.3 Hz, Me-26 and 27), 1.09 (3H, s, Me-29), 1.40 (3H, s, Me-30), 1.46 (3H, s, Me-28), 2.28 (1H, m, H-25), 2.50 (1H, q, J = 9.9 Hz, H-17), 2.64 (1H, td, J = 11.0, 3.0 Hz, H-20), 3.13 (1H, dd, J = 11.3, 4.0 Hz, H-3), 4.66 (1H, br s, H-7), 4.97 (1H, d, J = 7.0 Hz, H-1 of glc), 4.89 and 4.92 (each 1H, br s, H_2-31); ^{13}C NMR (C_5D_5N), see Table 1; FABMS *m/z* $[M - H]^-$ 647, $[M - H - glc - H_2O]^-$ 467; *anal.* C 68.77%, H 9.13%, calcd for $C_{37}H_{60}O_9$, C 68.49%, H 9.32%.

Laetiposide B (2): colorless needles, mp 232−234 °C; $[\alpha]^{25}_D$ +12.8° (*c* 0.7, MeOH); FT−IR (dry film) ν_{max} 3335 (OH), 1700 (C=O) cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) δ 0.98 (3H, s, Me-19), 1.03 (3H, s, Me-18), 1.09 (3H, s, Me-29), 1.38 (3H, s, Me-30),1.46 (3H, s, Me-28), 1.62 (3H, s, Me-27), 1.68 (3H, s, Me-26), 2.48 (1H, q, J = 9.9 Hz, H-17), 2.65 (1H, td, J = 11.0, 3.0 Hz, H-20), 3.14 (1H, dd, J = 11.3, 4.0 Hz, H-3), 4.67 (1H, br s, H-7), 4.98 (1H, d, J = 7.8 Hz, H-1 of glc), 5.33 (1H, m, H-24); ^{13}C NMR (C_5D_5N), see Table 1; FABMS *m/z* $[M - H]^-$ 633, $[M - H - glc - H_2O]^-$ 453; *anal.* C 67.97%, H 9.56%, calcd for $C_{36}H_{58}O_9$, C 68.11%, H 9.21%.

Laetiposide C (3): colorless needles, mp 179−181 °C; $[\alpha]^{25}_D$ +29.1° (*c* 1.9, MeOH); FT−IR (dry film) ν_{max} 3380 (OH), 1700 (C=O) cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) δ 0.89 (3H, s, Me-19), 1.02 (3H, s, Me-18), 1.11 (3H, s, Me-29), 1.36 (3H, s, Me-28), 1.37 (3H, s, Me-30), 1.62 (3H, s, Me-27), 1.68 (3H, s, Me-26), 2.46 (1H, q, J = 9.9 Hz, H-17), 2.64 (1H, td, J = 11.0, 3.0 Hz, H-20), 4.58 (1H, br s, H-7), 4.92 (1H, d, J = 7.7 Hz, H-1 of glc), 5.34 (1H, m, H-24); ^{13}C NMR (C_5D_5N), see Table 1; FABMS *m/z* $[M - H]^-$ 631, $[M - H - glc - H_2O]^-$ 451; *anal.* C 68.20%, H 8.78%, calcd for $C_{36}H_{56}O_9$, C 68.33%, H 8.92%.

Laetiposide D (4): colorless needles, mp 232−233 °C; $[\alpha]^{25}_D$ +33.3° (*c* 3.4, MeOH); FT−IR (dry film) ν_{max} 3380 (OH), 1700 (C=O) cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) δ 0.87 (3H, s, Me-19), 1.00 (3H, s, Me-18), 1.00 (6H, d, J = 6.5 Hz, Me-26 and 27), 1.09 (3H, s, Me-29), 1.33 (3H, s, Me-28), 1.37 (3H, s, Me-30), 2.28 (1H, m, H-25), 2.50 (1H, q, J = 9.9 Hz, H-17), 2.61 (1H, td, J = 11.0, 3.0 Hz, H-20), 4.55 (1H, br s, H-7), 4.88 (1H, d, J = 7.5 Hz, H-1 of glc), 4.88 and 4.91 (each 1H, br s, H_2-31); ^{13}C NMR (C_5D_5N), see Table 1; FABMS *m/z* $[M - H]^-$ 645, $[M - H - glc - H_2O]^-$ 465; *anal.* C 68.75%, H 9.18%, calcd for $C_{37}H_{58}O_9$, C 68.70%, H 9.04%.

Acid Hydrolysis of Laetiposide A (1). A solution of **1** (20 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 extracted with EtOAc. The EtOAc layer was subjected to Si gel column chromatography with CH_2Cl_2−MeOH (25:1) to give dehydroeburicoic acid (**7**, 10 mg) of mp 255−257 °C; $[\alpha]^{25}_D$ +11.5° (*c* 0.9, pyridine), whose UV, ^1H NMR, ^{13}C NMR, and EIMS data were consisted with literature values.[5] The aqueous layer was neutralized with Amberlite IRA-35 and evaporated in vacuo to dryness. The identification and the D or L configuration of sugar was determined by using RI detection (Waters 410) and chiral detection (Shodex OR-1) by HPLC [Shodex RSpak NH_2P-50 4E, CH_3CN−H_2O−H_3PO_4 (95:5:1), 1 mL/min, 47 °C] by comparison with an authentic sugar (10 mmol each of D-glc and L-glc). The sugar portion gave the peak of D-(+)-glc at 20.7 min.

Acid Hydrolysis of Laetiposides B−D (2−4). A solution of each compound **2**−**4** (each 2 mg) in 5% H_2SO_4−dioxane (1:1) was heated at 100° for 2.0 h. The reaction mixture was diluted with H_2O and then neutralized with Amberlite IRA-35 and evaporated in vacuo to dryness. The identification of the D or L configuration of the sugar was carried out in the same manner as described for **1** to give the peak of D-(+)-glc at 20.7 min from compounds **2**−**4**.

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

References and Notes

(1) Imazeki, R.; Hongo, T. *Coloured Illustrations of Fungi of Japan*; Hoikusha Press: , Japan, 1964; Vol. 2, p 148.
(2) Hiroe, I.; *Newest Applied Mushroom Science*; Ariake Press: , Japan, 1976; p 1239.
(3) Ohsawa, T.; Yukawa, M.; Takao, C.; Murayama, M.; Bando, H. *Chem. Pharm. Bull.* **1992**, *40*, 143−147.
(4) Ohta, K.; Yaoita, Y.; Matsuda, N.; Kikuchi, M. *Nat. Med.* **1996**, *50*, 179−181.
(5) Tai, T.; Akahori, A.; Shingu, T. *Phytochemistry* **1993**, *32*, 1239−1244.
(6) Batey, I. L.; Pinhey, J. T.; Ralph, B. J.; Simes, J. J. H. *Aust. J. Chem.* **1972**, *25*, 2511−2515.
(7) Yoshikawa, K.; Kimura, E.; Mimura, N.; Kondo, Y.; Arihara, S. *J. Nat. Prod.* **1998**, *61*, 786−790.
(8) Yoshikawa, K.; Satou, Y.; Tokunaga, Y.; Tanaka, M.; Arihara, S. *J. Nat. Prod.* **1998**, *61*, 440−445.

NP980456I