New Glycosides from the Japanese Fern *Hymenophyllum barbatum*

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Thirteen glycosides and methyl (3R,5R)-5-hydroxy-(β-D-glucopyranosyl)-hexanoate were newly isolated from the Japanese fern *Hymenophyllum barbatum*, although our previous work revealed the isolation of hemiterpene glycosides, hymenosides A—J, from the same species. The structures of the newly isolated glycosides were elucidated by extensive two-dimensional (2D) NMR and/or chemical evidence. The structures of those aglycones were divided into four types, 2-methyl-but-2-ene-1,4-diol, 2-hydroxymethyl-but-2-ene-1,4-diol, 2-methylene-butyne-1,3,4-triol, and 3-hydroxy-5-hexanolid. The sugar moieties, which were acylated by phenylacetic acid derivatives, were also established by chemical and spectroscopic methods. Eight glycosides of the isolated compounds in the present investigation had a bitter or weakly pungent taste. It is clear that a phenylacetyl group attached to glucose or allose as an ester is necessary for the bitter taste.

**Key words** *Hymenophyllum barbatum*; hemiterpene glycoside; fern; pteridophytes; bitter taste

We have focused on the bioactive constituents and chemosystematics of bryophytes and pteridophytes, as well as the evolutionary relationship between terrestrial spore-forming green plants and algae using their characteristic chemical indicators.1—9)

Two different traditional views of the evolutionary relationships between bryophytes and pteridophytes have been reported.10) If ferns and bryophytes are indeed close to the main line of evolution of other vascular plants, then indicators of such links may be present in some chemical congruence. In our continuing chemosystematic research on cryptogamous plants, including bryophytes and pteridophytes, we reported that an acyclic bisbibenzyl characteristic of liverworts was present in the fern *Hymenophyllum barbatum*.4) The compound was considered to be a chemical fossil that could link bryophytes and ferns.

In the course of the investigation of bioactive substances of New Zealand pteridophytes and bryophytes, we found that some ferns contain a potent hot-tasting substance. This resulted in the isolation of (−)-polygodial from the New Zealand fern *Blechnum fluviatile*.11) Recently, we reported the isolation of hemiterpene glycosides, hymenosides A—J, which had a bitter or weakly hot taste, from the Japanese fern *Hymenophyllum barbatum*.1,2)

After the extraction of *H. barbatum* with methanol, the crude extract was partitioned between water and ethyl acetate, and then extracted with n-butanol. The n-butanol-soluble fraction was subjected to repeated chromatography on DIAION HP-20, silica gel, and Sephadex LH-20 columns, followed by preparative HPLC to yield thirteen glycosides, hymenosides K—W (1, 3—11, 15—17), together with methyl (3R,5R)-hydroxy-(β-D-glucopyranosyloxy)-hexanoate (19).2)

![Image](image-url)
Hymenoside K (1), [α]_D 18.5°, is a glucoside, as evidenced by the signals attributed to a β-glucopyranose unit in the 1H- and 13C-NMR spectra (Tables 1, 2). Actually, the 1H-NMR of 1 showed an anomic proton at δ 4.88 coupled with H-2' at δ 5.65 (brt, J=8 Hz). The coupling constant J_{H1,2'} value (δ, J=8 Hz) of H-1' amply demonstrated the β-configuration. The positive-FAB-MS spectrum gave a quasi-molecular ion peak at m/z 437 and its high resolution (HR)-FAB-MS resulted in the molecular formula C_{19}H_{26}O_{9}Na. The 1H-NMR spectrum of 1 showed two oxygenated methylenes at δ 4.11, 4.31 (each 1H, brd, J=12 Hz, H-1) and 4.43 (2H, brd, J=6 Hz, H-4), and a vinyl methyl group and an olefinic proton at δ 1.65 (3H, H-5) and 6.04 (1H, H-3). The presence of the aromatic ring was apparent from the absorption band at 283 nm (log ε, 3.47) in the UV spectrum of 1. The 1H-NMR spectrum further provided evidence for the presence of a 3,4-dihydroxyphenylacetyl group, namely, three protons at δ 6.93 (dd, J=8, 2 Hz, H-6'), 7.17 (d, J=8 Hz, H-5') and 7.38 (d, J=2 Hz, H-2') were assigned to the 1,3,4-tri-substituted aromatic ring, and AB type system protons at δ 3.79 and 3.84 (each, d, J=15 Hz, H-α) were assigned to an isolated methylene proton of the 3,4-hydroxyacetophenyl group. This is based on the methylene proton (H-α) being coupled with a carbonyl carbon signal at δ 171.6 through 1H-13C long-range; further coupling between the carbonyl carbon and H-2' proton signal at δ 5.65 were observed in its heteronuclear multiple bond correlation (HMBC) spectra (summarized in Fig. 1). It was apparent that the 3,4-dihydroxyphenyl group was esterified on C-2' of the glucopyranose. The acidic hydrolysis of 1 provided further evidence for the presence of the 3,4-dihydroxyphenyl group. The basic hydrolysis of 1 gave a glucoside, of which the 1H- and 13C-NMR spectral data were identical to those of 2 isolated from Ornithogalum montanum (Liliaceae). Further hydrolysis of 2 with 5% sulfuric acid afforded glucose, which was identified as (+)-glucose by GC-MS and HPLC equipped with a chiral detector. Accordingly, the structure of 1 was determined to be 2-(3,4-dihydroxyphenylacetyl)-β-D-glucopyranosyl (E)-2-methyl-but-2-en-4-ol.

The structures of hymenosides Q (3) and R (4) were deduced by comparing the spectral data with those of compound 1. While the HR-FAB-MS spectra gave the same molecular formula as that of 1, acylation shifted proton signals at δ 5.91 (t, J=10 Hz, H-3') of 3 and δ 4.75 (dd, J=12, 7 Hz, H-6') and 5.00 (dd, J=12, 2 Hz, H-6') of 4 were observed in the 1H-NMR spectra (Table 1). Assignment of the proton signals was performed by 1H-1H correlation spectroscopy (COSY) spectra of 3 and 4, confirming the presence of a 3,4-dihydroxyphenylacetyl group at C-3' of 3 and C-6' of 4. The structures of hymenosides Q and R were established as 3 and 4, respectively.

The structures of hymenosides S (5) and T (6) were again established by comparing the spectral data with those of 1. The 1H- and 13C-NMR spectra (Tables 1, 2) of 5 were similar to those of 1, except for the absence of the 3,4-dihydroxyphenylacetyl group, and for the appearance of two-proton signals at δ 7.39 (2H, d, J=8.5 Hz, H-2', 6') and 7.15 (2H, d, J=8.5 Hz, H-3', 5') in the 1H-NMR of 5. Positive FAB-MS of 5 afforded a quasimolecular ion peak at m/z 421.1503 (C_{19}H_{23}O_{9}Na), 16 mass units less than that of 1, confirming the presence of a 4-hydroxyphenylacetyl group. A correlation between H-2' and an ester carbonyl at δ 171.5 in the HMBC spectrum provided further evidence for the structure of 5. Thus, the structure of hymenoside S was established as 5. A positive HR-FAB-MS spectrum of 6 gave a quasimolecular ion peak at m/z 407.1333 (C_{19}H_{24}O_{9}Na), which was 14 mass units less than that of 5. Although the 1H-NMR of 6 gave an A_2B_2 type signal at δ 7.12 (2H, d, J=8.5 Hz, H-2', 6') and 8.23 (2H, d, J=8.5 Hz, H-3', 5'), the signal of the H-2' proton was not observed in its spectrum. This indicated the presence of a 4-hydroxybenzoyl group in 6. The benzoyl group at C-6' was clearly demonstrated from its HMBC spectrum, in which the 1H-13C long range coupling between the ester carbonyl at δ 166.4 and H-6' at δ 5.02 and 5.18 was observed. Thus, the structure of hymenoside T was established as 6.

The positive HR-FAB-MS of hymenoside V (7) showed a quasimolecular ion peak at m/z 449.1406, (C_{20}H_{28}O_{10}Na). The presence of an allopentose and (E)-2-methyl-but-2-ene-1,4-diol was apparent from the 13C- and 1H-NMR data (Tables 1, 2) of 7. The 1H-NMR spectrum exhibited signals of a caffeoyl group: i) at δ 6.53 and 7.73 (each 1H, d, J=16 Hz, H-α, β); ii) at δ 7.06 (1H, dd, J=8, 2 Hz, H-6'), 7.21 (1H, d, J=8 Hz, H-5'), and 7.52 (1H, d, J=2 Hz, H-2'). The acidic hydrolysis of 7 gave allose, identified by gas chromatography (GC)-MS. Analysis of the 1H-detected heteronuclear multiple quantum coherence (HMQCO) and HMBC spectra (summarized in Fig. 2) supports the structural assignment. In particular, the long range 1H-13C correlation of H- α and β with a carbonyl carbon at δ 167.0, and further correlation between the carbonyl carbon and H-4' at δ 5.61 (dd, J=10, 3 Hz) supported the position of the caffeoyl group at C-4' of allopentose. These chemical and spectral data led to the structure 7 for hymenoside V.

Compounds 8—11 were glycosides, as evident by the signals attributed in the 1H- and 13C-NMR spectra (Tables 1, 2) to an allose unit, which linked to an aglycone as the β-configuration (J_{H1,2'}=8 Hz).
The 1H-NMR spectrum of 9 was similar to that of cardiolan (12), except for the appearance of five signals due to a caffeoyl group at δ 6.70 (d, J=16 Hz, H-α), 7.08 (dd, J=8, 2 Hz, H-6″), 7.18 (d, J=8 Hz, H-5″), 7.51 (d, J=2 Hz, H-2″), and 8.01 (d, J=16 Hz, H-β) in the former. The basic hydrolysis of 9 afforded an alloprenylsamine, the 1H-NMR spectrum of which was identical to that of cardiolan (12) obtained from the fern *Cardiothamnus reniforme*, and also isolated from the present species. Acid hydrolysis of 9 gave a caffeic acid, confirming the presence of the caffeoyl group, and its position was apparent from the downfield shift of the H-3 proton (δ 6.49) in the 1H-NMR spectrum of 9. The 1H-NMR spectrum (Table 1) of hymenoside M (8) is similar to that of 9 due to only one acyl group observed in the 1H-NMR spectrum of 9. The acid hydrolysis of 8 gave 3,4-dihydroxyphenylacetic acid, identified by GC-MS analysis of its methyldialditol acetate. The 1H- and 13C-NMR (Tables 1, 2) except for proton signals of the sugar moiety of 8 showed an absorption band of an aromatic ring at 284 nm (log ε, 3.51). Since compound 17 was presumed to be a glycoside by the signals in the 1H-NMR data, the enzymatic hydrolysis was performed. This confirmed that the aglycone of 17 was (3R,5R)-3-hydroxy-hexanolide. The coinjection analysis of (3R,5R)-3-hydroxy-hexanolide by liquid chromatography (LC)-MS using a chiral column showed a retention time identical to that of the authentic sample. The authentic (3R,5R)-3-hydroxy-hexanolide has been prepared from the enzymatic hydrolysis of (3R,5R)-3-β-D-glucopyranosyloxy)-5-hexanolide (18), which was isolated from the same species, and its absolute structure was established by X-ray crystallographic analysis of its p-bromobenzoate derivative. The 1H- and 13C-NMR (Tables 1, 2) of 17 were similar to those of 18, except for the presence of signals due to an acyl group of 17. The signals at δ 3.74 (2H, s, H-α), 6.93 (1H, dd, J=8, 2 Hz, H-6″), 7.17 (1H, d, J=8 Hz, H-5″) and 7.38 (1H, d, J=2 Hz, H-2″) were ascribed to a 3,4-dihydroxyphenylacetyl group in the 1H-NMR spectrum of 17. The correlation of a carbonyl carbon at δ 171.0 between H-2′ at δ 5.52 (t, J=8 Hz), and the α-proton at δ 3.74 of the acyl group, was observed in the HMBC spectrum of 17. The above evidence indicated that hymenoside U (17) differed from (3R,5R)-3-β-D-glucopyranosyloxy)-5-hexanolide (18) by the replacement of a hydroxyl group at C-2′ by a 3,4-dihydroxyphenylacetoxyl group.

Compound 19 related to 17 and 18, was attributed to an artifact of 18. Actually, compound 18, which was isolated from the n-butanol-soluble fraction of this species, was easily converted to 19 in methanol with an anion exchange resin, Amberlite 120-B, the 1H-NMR spectrum of which was identical with that of methyl (3R,5R)-hydroxy-(β-D-glucopyranosyloxy)-hexanoate. The absolute configuration of 3R and 5R was apparent from the previous measurement of X-ray crystallographic analysis of 18. It was reported that (3S,5S)-5-hexanolide changed to methyl (3S,5S)-hydroxy-hexanoate in methanol. Compound 19 might be produced from 18 during the extraction with methanol.

The HMBC spectrum of 15. Thus, this indicated the presence of a 2-methylene-butane-1,3,4-triol as an aglycone of 15. The 1H-H spin coupling between H-1′ and 2′ showed 8 Hz in its 1H-NMR, as described earlier, confirming that the structure of 15 was assigned to the 1-O-β-allose of 2-methylene-butyrate-1,3,4-triol. An attempt to establish the absolute configuration of the secondary hydroxyl group at C-3 by X-ray crystallographic analysis of its p-bromobenzoate is in progress.

The structure of hymenoside W (16) was established by comparing its spectral data with those of hymenoside M (15). Particularly, the 13C-NMR spectrum (Table 2) of 16 was similar to that of 15, although the chemical shift of the signals due to a sugar moiety was different. The coupling pattern of the sugar moiety of 16 suggested the presence of a glucose. Actually, acidic hydrolysis of 16 afforded a glucose, whose alditol acetate was identified with a hexaacetate of glucitol by GC-MS analysis. Further analysis of HPLC with a chiral detector gave additional evidence of (+)-glucose. Accordingly, the structure of hymenoside W was 16.

Compound 17, named hymenoside U (m/z 465.1387, C16H21O8Na), was obtained as a viscous colorless oil. The UV spectrum of 17 showed an absorption band of an aromatic ring at 284 nm (log ε, 3.51). Since compound 17 was presumed to be a glycoside by the signals in the 1H-NMR data, the enzymatic hydrolysis was performed. This confirmed that the aglycone of 17 was (3R,5R)-3-hydroxy-hexanolide. The coinjection analysis of (3R,5R)-3-hydroxy-hexanolide by liquid chromatography (LC)-MS using a chiral column showed a retention time identical to that of the authentic sample. The authentic (3R,5R)-3-hydroxy-hexanolide has been prepared from the enzymatic hydrolysis of (3R,5R)-3-β-D-glucopyranosyloxy)-5-hexanolide (18), which was isolated from the same species, and its absolute structure was established by X-ray crystallographic analysis of its p-bromobenzoate derivative. The 1H- and 13C-NMR (Tables 1, 2) of 17 were similar to those of 18, except for the presence of signals due to an acyl group of 17. The signals at δ 3.74 (2H, s, H-α), 6.93 (1H, dd, J=8, 2 Hz, H-6″), 7.17 (1H, d, J=8 Hz, H-5″) and 7.38 (1H, d, J=2 Hz, H-2″) were ascribed to a 3,4-dihydroxyphenylacetyl group in the 1H-NMR spectrum of 17. The correlation of a carbonyl carbon at δ 171.0 between H-2′ at δ 5.52 (t, J=8 Hz), and the α-proton at δ 3.74 of the acyl group, was observed in the HMBC spectrum of 17. The above evidence indicated that hymenoside U (17) differed from (3R,5R)-3-β-D-glucopyranosyloxy)-5-hexanolide (18) by the replacement of a hydroxyl group at C-2′ by a 3,4-dihydroxyphenylacetoxyl group.

Compound 19 related to 17 and 18, was attributed to an artifact of 18. Actually, compound 18, which was isolated from the n-butanol-soluble fraction of this species, was easily converted to 19 in methanol with an anion exchange resin, Amberlite 120-B, the 1H-NMR spectrum of which was identical with that of methyl (3S,5R)-hydroxy-(β-D-glucopyranosyloxy)-hexanoate. The absolute configuration of 3R and 5R was apparent from the previous measurement of X-ray crystallographic analysis of 18. It was reported that (3S,5S)-5-hexanolide changed to methyl (3S,5S)-hydroxy-hexanoate in methanol. Compound 19 might be produced from 18 during the extraction with methanol.

Hymenosides G (13), H (14), K (1), Q (3), R (4), S (5), L (8), T (6), U (17) and (3R,5R)-3-β-D-glucopyranosyloxy)-5-

Fig. 3. Structure of Hymenoside L (8) and Its Long-Range Correlations in the HMBC Spectrum
hexanolide (18) had a bitter taste, but have not been yet evaluated quantitatively. The tasted compounds, and 2,5-
dihydroxyphenylacetic acid (homogentisic acid), have an
cid taste and their distribution in the plant kingdom is
known.16,17) It is suggested that the bitter taste is due to the presence of phenylacetic acid derivatives esterified with glu-
cose or allose, at least for the taste compounds isolated in the
present investigation. For example, hymenoside L (8) is the bitter taste, although cardanol (12) and hymenoside P
(11) don’t show any taste; thus, it is clear that a phenylacetyl
group is necessary for the bitter taste.

Table 1. (Continued) 1H-NMR Data of Hymenosides, T (6), V (7), L (8) and N (9) in Pyridine-d₅

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Measured at a) 600 MHz and b) 400 MHz. c) Overlapped signals.

Experimental

General Experimental Procedures TLC was carried out on silica gel
precoated glass plates with CHCl₃-MeOH (4:1 and 7:3 v/v) or
CHCl₃-MeOH-H₂O (15:6:1 and 65:35:5 v/v). Detection was per-
formed with Godin reagent. For normal-phase column chromatography (CC), silica
gel 60 (40—63 μm) was used. The mixture of CHCl₃-MeOH (1:1) was
used for column chromatography on Sephadex LH-20 as a solvent. Optical
rotations were recorded on a JASCO DIP-1000 automatic digital polarime-
ter. NMR spectra were recorded at 150 and 100 MHz for 1H, and at 600
and 400 MHz for 3H on a Varian UNITY 600 and JEOL ECP-400. The chemical
shifts are given in δ (ppm) with tetramethylsilane as an internal standard.
UV spectra were recorded in spectrophotometric-grade EtOH on a Hitachi U-
3900. IR spectra were measured on a JASCO FTIR-41. GC-MS spectra
were recorded on a Hewlett-Packard gas chromatograph with a 5972A mass selective detector. The temperature programming for GC-MS
analysis was performed from 50 to 250 °C at 8 °C/min, and isothermal at
250 °C for 5 min. The injection temperature was 250 °C. A fused silica col-
umn coated with DB-17 (30 m×0.25 mm i.d., film thickness 0.25 μm) was
used. Helium was used as the carrier gas at 1 ml/min.

Extraction and Isolation of Compounds Hymenophyllum barbatum
was collected in August 1997 in Kaito-gun, Tokushima. The air-dried and
mechanically ground powder (2.42 kg) was extracted with methanol for 1
month at room temperature to give a crude extract (459.2 g).

Part (70 g) of the n-butanol-soluble fraction (158.0 g) was chro-
mated on DIAION HP-20 using a CH₃OH-H₂O gradient, giving seven fractions. The third fraction (7.8 g) was rechromatographed on
Sephadex LH-20 to give a mixture of hymenosides K, L and N—U, which
were further purified by silica gel column chromatography using
CHCl₃-MeOH-H₂O followed by middle pressure liquid chromatography
(MPLC) to yield 11 hemiterpene glycosides, hymenosides K, L and N—U
which was further purified by silica gel column chromatography and MPLC chromatographed on Sephadex LH-20 to give a mixture of hymenoside V, copyranosyloxy-hexanoate (512). Table 2. 13C-NMR Data of Hymenosides K (1), P (11), M (15), W (16) and U (17)

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Table 2. 1<sup>1</sup>C-NMR Data of Hymenosides K (1), Q (3), R (4), S (5), T (6), V (7), L (8), N (9), O (10), P (11), M (15), W (16) and U (17)

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Measured a) in pyridine-d<sub>5</sub> at 600 MHz, b) in pyridine-d<sub>5</sub> at 400 MHz, c) in methanol-d<sub>4</sub> at 600 MHz. d) Overlapped signals.

Hymenoside K (1): Oil; positive HR-FAB-MS m/z: 437.1424 [M+Na]<sup>+</sup> (Calcd for C<sub>19</sub>H<sub>26</sub>O<sub>10</sub>Na: 437.1424); [m/z] 72<sup>c</sup> -18.5<sup>c</sup> (c=3.84, MeOH); IR (KBr) cm<sup>-1</sup>: 3252, 1730, 1607, 1447, 1281; UV λ<sub>max</sub> (EtOH) nm (log ε): 206 (4.31), 283 (3.47).

Hymenoside Q (3): Oil; positive HR-FAB-MS m/z: 437.1396 [M+Na]<sup>+</sup> (Calcd for C<sub>19</sub>H<sub>26</sub>O<sub>10</sub>Na: 437.1424); [m/z] 72<sup>c</sup> -16.7<sup>c</sup> (c=1.6, MeOH); IR (KBr) cm<sup>-1</sup>: 3323, 1727, 1525, 1287, 1079, 1040; UV λ<sub>max</sub> (EtOH) nm (log ε): 205 (4.40), 230 (3.75), 284 (3.48).

Hymenoside R (4): Oil; positive HR-FAB-MS m/z: 437.1463 [M+Na]<sup>+</sup> (Calcd for C<sub>19</sub>H<sub>26</sub>O<sub>10</sub>Na: 437.1424); [m/z] 72<sup>c</sup> -18.3<sup>c</sup> (c=1.2, MeOH); IR

(1, 164 mg; 8, 28 mg; 9, 88 mg; 10, 57 mg; 11, 40.0 mg; 3, 33 mg; 4, 12 mg; 5, 11 mg; 6, 6 mg; 17, 6 mg). The second fraction (5.3 g) was repeatedly chromatographed on Sephadex LH-20 to give a mixture of hymenosides M, W and 19, which was further chromatographed on silica gel using CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O, then repeatedly subjected to MPLC to give hymenoside M (15, 26 mg), W (16, 28 mg) and methyl (3R,5R)-5-hydroxy-β-D-glucopyranosylxyllo-hexaol (19, 807 mg). The fourth fraction (27 mg) was chromatographed on Sephadex LH-20 to give a mixture of hymenosides V, which was further purified by silica gel column chromatography and MPLC to yield hymenoside V (7, 5 mg).
Hymenoside S (5): Oil; positive HR-FAB-MS m/z: 421.1503 [M+Na]+
(Calcd for C19H26O11Na: 443.1529 [M]+); [α]D29 29.0° (c 1.15, MeOH); IR (KBr) cm−1: 3270, 1697, 1601, 1439. 1H-NMR (200 MHz, CD3OD) δ: 3.35 (1H, t, 3.9 Hz, H-6), 3.67 (s, OMe), 3.89 (2H, m, H-1), 4.23 (2H, m, H-4), 5.52 (2H, s, H-1), 6.49 (1H, d, J = 5.5 Hz, H-5), 7.17 (1H, d, J = 14.5 Hz, H-4), 8.22 (1H, d, J = 15.0 Hz, H-3), 8.31 (1H, d, J = 11.0 Hz, H-2), 8.40 (1H, d, J = 4.5 Hz, H-6), 8.59 (1H, d, J = 14.5 Hz, H-5), 8.68 (1H, d, J = 10.0 Hz, H-4).

Acid Hydrolysis of Hymenoside S (5) and N (9–11) Each solution of 9—11 (9; 17 mg; 10; 18.4 mg; 11; 8.8 mg) in 5% H2SO4 (1 ml) was heated under reflux for 3 h, then diluted with water, and extracted with EtOAc. The aqueous portion was passed through a short column on an ion exchange resin, DOWEX-1. The eluate was concentrated in vacuo, and the residue was dissolved in H2O (2 ml). Part of the solution was analyzed using the same method as described earlier. (+)-Allose was detected in the aqueous phase. Further identification was performed by coinjection with an authentic alditol acetate of allose.

Acid Hydrolysis of Hymenoside V (7) A solution of (4.9 mg) in 5% H2SO4 (2 ml) was heated for 3 h and then processed in the same manner as described above. A sample was subjected to GC-MS analysis to give methyl 3,4-dimethoxyphenylacetate. The identification was performed with conjection with an authentic sample.

Enzymatic Hydrolysis of Hymenoside U (17) A solution of 16 (5.53 mg) in H2O (1 ml) was added to a suspended solution of β-glucosidase (Nacalsu Tuske; β-glucosidase from sweet almond; 20 mg) in H2O (1 ml) and the mixture was incubated at 37°C overnight. The reaction mixture was evaporated. The residue was chromatographed on silica gel [CHCl3–CH2Cl2 (1:1, v/v)] to give 3(3R,5R)-3-hydroxy-5-hexanolide (0.2 mg).2) Acid Hydrolysis of Hymenoside W (16) A solution of (13 mg) in 5% H2SO4 (2 ml) was heated for 2 h, then the usual work-up afforded an aglycone. The mixture was passed through a short column on an ion exchange resin, DOWEX-1. The eluate was concentrated in vacuo, and the residue was dissolved in H2O (2 ml). Part of the solution was analyzed using the same method as described earlier. Caffeic acid was identified by GC–MS analysis of its TMS derivative and authentic sample.

Enzymatic Hydrolysis of Hymenoside U (17) A solution of 16 (5.53 mg) in H2O (1 ml) was added to a suspended solution of β-glucosidase (Nacalsu Tuske; β-glucosidase from sweet almond; 20 mg) in H2O (1 ml) and the mixture was incubated at 37°C overnight. The reaction mixture was evaporated. The residue was chromatographed on silica gel [CHCl3–CH2Cl2 (1:1, v/v)] to give 3(3R,5R)-3-hydroxy-5-hexanolide (0.2 mg).2)
References