Three New Hemiterpene Glycosides from *Ilex macropoda*

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Phytochemical studies of *Ilex macropoda* led to the isolation of three new hemiterpene glycosides, 4-β-D-glucopyranosyloxy-5-hydroxyprenyl caffeate (aohada-glycoside A), 5-caffeoyloxy-4-β-D-glucopyranosyloxy-prenyl alcohol (aohada-glycoside B), 4-(6-*O*-caffeoyl-β-D-glucopyranosyloxy)-5-hydroxyprenyl caffeate (aohada-glycoside C), together with betulin, acetyl ursolic acid, ilexosides XVII and XVIII, and 3,4,5-trimethoxyphenol β-D-5-*O*-caffeoyl-apiofuranosyl-(1→6)-β-D-glucopyranoside from the bark. Rotundioic acid, ursolic acid, ilexosides II and XXX, ziyu-glycoside I and rutin were also isolated from fresh leaves, and 3,5-dicaffeoylquinic acid and 3,4-dicaffeoylquinic acid from the wood.

Key words *Ilex macropoda*; hemiterpene glycoside; aohada-glycoside; ilexoside; caffeate; Aquifoliaceae

Ilex macropoda MIQ. (Japanese name: Aohada) is an Aquifoliaceous tree growing in Japan, Korea and China. Our detailed phytochemical studies have identified the constituents of fresh leaves, bark and wood of this tree, including three new hemiterpene glycosides. In this paper, we describe their isolation and structure determination.

From the MeOH extract of dried bark, three new hemiterpene glycosides, **1**—**3**, and a new phenolic glycoside, **4**, were isolated together with ilexosides XVII and XVIII,[1] betulin and acetyl ursolic acid.

1, named aohada-glycoside A, was formulated as $C_{20}H_{26}O_{11}$ by high-resolution (HR) FAB-MS. ^{1}H- and ^{13}C-NMR data indicated the presence of a caffeoyl and a β-D-glucopyranosyl moiety in the molecule (Table 1). Accommodation of the remaining signals, δ_C 58.4 [CH_2, δ_H 4.65 (2H, s)], 60.7 [CH_2, δ_H 5.12 (2H, d, J=6.7 Hz)], 71.5 [CH_2, δ_H 4.63 (1H, d, J=13.0 Hz), 4.89 (1H, d, J=13.0 Hz)], 123.5 [CH, δ_H 6.14 (1H, t, J=6.7 Hz)] and 142.0 (C), led to the formulation of a 4,5-dioxygenated-prenyl structure, and the signals at 60.7 and 123.5 were assigned for C-1 and C-2 on the basis of the coupling pattern. Other signals were assigned by nuclear Overhauser effect correlation spectroscopy (NOESY) as shown in Fig. 1. On alkaline methanolysis, **1** gave methyl caffeate and a deacyl compound, **1a**, which gave D-glucose on acid hydrolysis. Considering the down field shift of the proton signal for C-1, δ_H 5.12, and the correlation with the carboxyl carbon of the caffeoyl group, δ_C 167.3, in long-range ^{13}C–^{1}H shift correlation spectroscopy (COSY), the position of the caffeoyl group was determined to be at C-1. On the other hand, the anomeric proton signal of D-glucosyl group, δ_H 4.99 (d, J=7.6 Hz), showed correlation with the proton signals at 4.63 and 4.89 (H_2-4), indicating the glucosylation position to be at C-4 (Fig. 1). Thus, the structure of **1** was established as 4-β-D-glucopyranosyloxy-5-hydroxyprenyl caffeate.

2, named aohada-glycoside B, was given the same molecular formula as **1**, $C_{20}H_{26}O_{11}$, by HR-FAB-MS. ^{1}H- and ^{13}C-NMR data showed the presence of a caffeoyl, β-D-glucopyranosyl and 4,5-dioxygenated-prenyl group, indicating **2** to be a positional isomer of **1**. On alkaline methanolysis, **2** gave methyl caffeate and a deacyl compound which was identified as **1a**. Therefore, the structure of **2** was determined to be 5-caffeoyloxy-4-β-D-glucopyranosyloxyprenyl alcohol. 2D-COSY of **2** also confirmed this structure.

3, named aohada-glycoside C, was formulated as $C_{29}H_{32}O_{14}$ by HR-FAB-MS. The ^{1}H- and ^{13}C-NMR of **3** were similar to those of **1**, except for the signals of one more caffeoyl group and differences in the chemical shifts around C-6 of β-D-glucosyl. On alkaline methanoly-

Table 1. ^{13}C-NMR Data in C_5D_5N

	1	1a	2	3
1	60.7	58.3	58.3	60.6
2	123.5	131.3	134.7	123.8
3	142.0	138.3	132.6	141.8
4	71.5	72.3	71.3	71.5
5	58.4	58.4	60.1	58.4
Glc-1	104.0	103.8	103.8	103.9
Glc-2	75.2	75.2	75.2	75.0
Glc-3	78.6	78.6	78.6	78.3
Glc-4	71.6	71.7	71.6	71.4
Glc-5	78.5	78.5	78.5	75.4
Glc-6	62.7	62.8	62.7	64.5
Caf-1	126.9	—	126.8	125.8/126.8
Caf-2	115.8	—	115.8	115.8/115.7
Caf-3	150.5	—	150.6	150.5/150.4
Caf-4	147.7	—	147.7	147.7/147.6
Caf-5	116.8	—	116.7	116.7/116.7
Caf-6	122.1	—	122.2	122.1/122.1
Caf-7	146.0	—	146.1	146.0/146.0
Caf-8	114.8	—	114.7	114.8/114.8
Caf-9	167.3	—	167.4	167.3/167.7

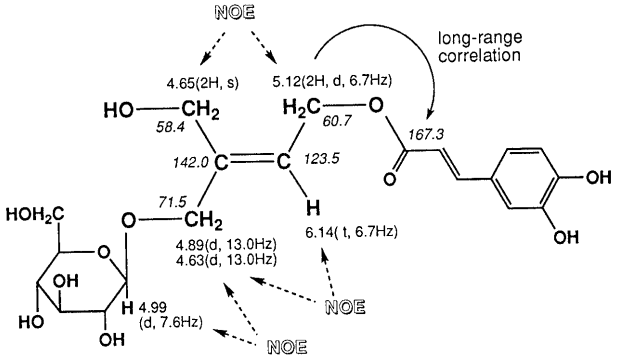

Fig. 1. ^{1}H and ^{13}C-(Italics) NMR Data and Diagnostic Correlations Observed in the Long-Range C–H COSY and NOESY of **1**

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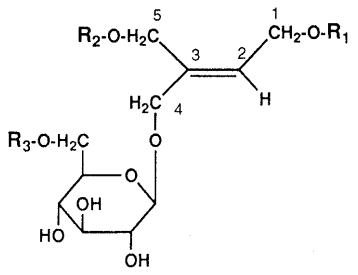
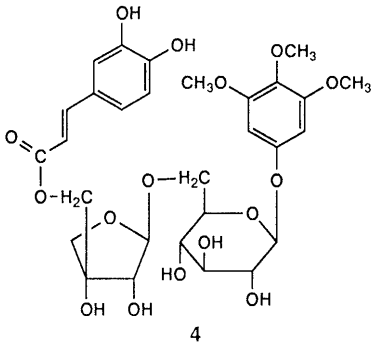


1 : R_1=Caffeoyl, R_2=R_3=H
2 : R_1=R_3=H, R_2=Caffeoyl
3 : R_1=R_3=Caffeoyl, R_2=H



4

Chart 1

sis, **3** gave methyl caffeate and the deacyl compound, **1a**. Considering the acylation shifts in ^{13}C-NMR,[2] the structure of **3** was determined to be 4-(6-*O*-caffeoyl-*β*-D-glucopyranosyloxy)-5-hydroxyprenyl caffeate.

4 was formulated as $C_{29}H_{36}O_{16}$ by HR-FAB-MS. The ^{1}H- and ^{13}C-NMR data revealed the presence of a caffeoyl [δ_C: 126.9 (C-1), 115.8 (C-2), 147.7 (C-3), 150.5 (C-4), 116.8 (C-5), 122.1 (C-6), 146.0 (C-7), 114.8 (C-8), 167.3 (C-9)], a 3,4,5-trimethoxyphenoxy [δ_C: 155.6 (C-1), 96.2 (C-2, 6), 154.5 (C-3, 5), 134.5 (C-4), 61.2 (4-OCH_3), 56.6 (3,5-OCH_3); δ_H: 6.44 (2H, s), 3.68 (3H, s), 3.78 (6H, s)] and two glycosyl groups assignable to D-glucose [δ_C: 103.1 (C-1), 74.9 (C-2), 77.9 (C-3), 71.5 (C-4), 76.9 (C-5), 68.4 (C-6)] and D-apiose [δ_C: 110.4 (C-1), 78.5 (C-2), 79.0 (C-3), 75.0 (C-4), 67.5 (C-5)]. On alkaline methanolysis, **4** gave methyl caffeate and a deacyl compound which was identified as 3,4,5-trimethoxyphenol *β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**4a**) by comparison of spectral data with those reported.[3] When compared with **4a**, **4** showed acylation shifts of −2.3 ppm for C-5″ and +1.3 ppm for C-3″. Therefore, the structure of **4** was determined to be the 5″-*O*-caffeoyl derivative of **4a**.

Constituents of other parts of this plant were also investigated. Rotundioic acid,[4] ilexoside XXX,[4] ziyu-glycoside I,[5] ursolic acid, ilexoside I[6] and rutin were isolated from the MeOH extract of fresh leaves collected in July. 3,5-Dicaffeoylquinic acid and 3,4-dicaffeoylquinic acid[7] were isolated from the MeOH extract of dried wood. Their structures were determined by comparison of their spectral data with reported values.

Few hemiterpenes have been reported so far. In general, isolation is difficult due to their volatility, unless they are polar such as tetraol from the fruits of *Cnidium monnieri*.[8] Aohada-glycosides are new members of this rare group.

Experimental

Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were recorded with a JASCO DIP-360 automatic polarimeter. The ^{13}C- and ^{1}H-NMR spectra were measured with a JEOL GSX-500 (500 MHz) spectrometer (multiplicity, s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet). Ultraviolet (UV) spectra were recorded on a Hitachi 323 spectrometer. Mass spectra were measured with a JEOL SX-102 spectrometer. Gas-liquid chromatography (GLC) and high-performance liquid chromatography (HPLC) were run on a Shimadzu GC-8A apparatus using an HR-1701 capillary column (0.3 mm i.d. × 30 m) and a Shimadzu LC-9A apparatus with a UV detector (Shimadzu SPD-6AV), respectively. The droplet countercurrent chromatography (DCC) apparatus consists of 200 column units of glass tubing (2.4 mm i.d., 60 cm long) connected by Teflon tubing (0.5 mm i.d.). The solvent system used was $CHCl_3$–MeOH–H_2O (4:4:3), with the upper layer as the mobile phase and the lower layer as the stationary one. The flow rate was 20 ml/h and 4 ml fractions were gathered in a fraction collector.

Isolation Fresh leaves (1.9 kg) collected in July in Han-nou, Saitama prefecture, were extracted twice with 4 l MeOH under reflux for 6 h. The extracts and then 10 l MeOH were passed over a column of activated charcoal (100 g) to obtain fraction M. The column was eluted with a mixture of MeOH and $CHCl_3$ (7:3, 10 l) to obtain fraction C–M. Fraction M was chromatographed on silica gel using $CHCl_3$ and MeOH. The fractions containing rotundioic acid and ursolic acid were collected and chromatographed on silica gel using *n*-hexane and EtOAc to give rotundioic acid (33 mg) and ursolic acid (105 mg). The fractions containing ilexoside XXX were collected and chromatographed on Sephadex LH-20 using MeOH and H_2O to give ilexoside XXX (915 mg). The fraction C–M was chromatographed on silica gel using $CHCl_3$ and MeOH and on Sephadex LH-20 using MeOH and H_2O to give ziyu-glycoside I (8 mg), ilexoside II (104 mg) and rutin (23 mg).

The air-dried bark (500 g) collected at the same time as the leaves was extracted twice, each time with 3 l MeOH under reflux. The extracts were evaporated under reduced pressure to a syrup. The syrup was chromatographed on silica gel using $CHCl_3$ and MeOH. The fractions containing triterpenes were collected and chromatographed on silica gel using *n*-hexane and EtOAc to give betulin (97 mg) and acetyl ursolic acid (82 mg). The fractions containing ilexosides were collected and chromatographed on Sephadex LH-20 using 80% MeOH and refined by HPLC (Shim-pac ODS, 90% MeOH) to give ilexoside XVII (20 mg) and XVIII (14 mg). The fractions containing hemiterpene glycosides were collected and chromatographed on Sephadex LH-20 using MeOH and H_2O and refined by DCC to give **1** (287 mg), **2** (48 mg), **3** (110 mg) and **4** (32 mg).

The dried wood (1 kg) collected at the same time as the leaves was extracted with 18 l MeOH under reflux for 6 h. The extract was evaporated under reduced pressure, and the residue was chromatographed on silica gel using $CHCl_3$ and MeOH, and on Sephadex LH-20 using 80% MeOH to give 3,5-dicaffeoylquinic acid (310 mg) and 3,4-dicaffeoylquinic acid (75 mg).

Aohada-glycoside A (1) A pale yellow amorphous powder, $[\alpha]_D$ −15° (c=1.0, MeOH). UV (MeOH) λ_{max} nm (log ε): 329 (4.31), 300 sh (4.18), 245 (4.07), 216 (4.24). ^{1}H-NMR (C_5D_5N) δ: 7.95 (1H, d, J=15.9 Hz, Caf-H-7), 7.62 (1H, d, J=1.8 Hz, Caf-H-2), 7.25 (1H, d, J=7.9 Hz, H-5), 7.19 (1H, dd, J=1.8, 7.9 Hz, Caf-H-6), 6.57 (1H, d, J=15.9 Hz, Caf-H-8), 6.14 (1H, t, J=6.7 Hz, H-2), 5.12 (2H, d, J=6.7 Hz, H_2-1), 4.99 (1H, d, J=7.6 Hz, Glc-H-1), 4.89 (1H, d, J=13.0 Hz, H-4), 4.65 (2H, s, H_2-5), 4.63 (1H, d, J=13.0 Hz, H-4), 4.56 (1H, dd, J=2.3, 11.9 Hz, Glc-H-6), 4.39 (1H, dd, J=5.5, 11.9 Hz, Glc-H-6). HR-FAB-MS m/z: 443.156 $[M+H]^+$. Calcd for $C_{20}H_{27}O_{11}$: 443.155.

Alkaline Methanolysis of 1 A mixture of **1** (75 mg) and 3% NaOMe in MeOH (10 ml) was refluxed for 30 min. The mixture was neutralized with 3% HCl and extracted with EtOAc. The extract was washed with water, dried over anhydrous Na_2SO_4 and evaporated. The residue was chromatographed on silica gel using 5% MeOH in $CHCl_3$ to obtain methyl caffeate (13 mg) which was identified by direct comparison with an authentic sample. The aqueous solution was evaporated and chromatographed on Sephadex LH-20 using 80% MeOH to obtain **1a** (26 mg).

Compound 1a A colorless amorphous powder, $[\alpha]_D$ −28° (c=1.0, MeOH). ^{1}H-NMR (C_5D_5N) δ: 6.30 (1H, t, J=6.1 Hz, H-2), 5.00 (1H, d, J=7.6 Hz, Glc-H-1), 4.89 (1H, d, J=12.2 Hz, H-4), 4.66 (2H, s, H_2-5),

4.62 (2H, d, J=6.1 Hz, H_2-1), 4.61 (1H, d, J=12.2 Hz, H-4). HR-FAB-MS m/z: 281.122 $[M+H]^+$. Calcd for $C_{11}H_{21}O_8$: 281.124.

Acid Hydrolysis of 1a Compd. **1a** (10 mg) was hydrolyzed with 3% HCl (5 ml) under reflux for 1 h. The reaction mixture was concentrated under reduced pressure and chromatographed on silica gel using 10% MeOH in $CHCl_3$ to obtain D-glucose (2.1 mg), $[\alpha]_D$ +38° (c=0.2, H_2O). Its trimethylsilyl ether was identified by comparison with an authentic sample on GLC.

Aohada-Glycoside B (2) A pale yellow amorphous powder, $[\alpha]_D$ −21° (c=1.0, MeOH). UV (MeOH) λ_{max} nm (log ε): 330 (4.27), 300 sh (4.15), 243 (4.06), 216 (4.23). ^{1}H-NMR (C_5D_5N) δ: 7.93 (1H, d, J=15.9 Hz, Caf-H-7), 7.60 (1H, d, J=1.8 Hz, Caf-H-2), 7.24 (1H, d, J=8.2 Hz, H-5), 7.16 (1H, dd, J=1.8, 8.2 Hz, Caf-H-6), 6.55 (1H, d, J=15.9 Hz, Caf-H-8), 6.45 (1H, t, J=6.7 Hz, H-2), 5.17 (2H, s, H_2-5), 4.97 (1H, d, J=7.6 Hz, Glc-H-1), 4.77 (1H, d, J=12.4 Hz, H-4), 4.67 (2H, d, J=6.7 Hz, H_2-1), 4.55 (1H, d, J=12.4 Hz, H-4), 4.56 (1H, dd, J=2.4, 11.9 Hz, Glc-H-6), 4.39 (1H, dd, J=5.2, 11.9 Hz, Glc-H-6). HR-FAB-MS m/z: 441.137 $[M-H]^-$. Calcd for $C_{20}H_{25}O_{11}$: 441.140.

Alkaline Methanolysis of 2 Compd. **2** (24 mg) was subjected to alkaline methanolysis in the same manner as **1** to obtain methyl caffeate (3.3 mg) and **1a** (9 mg).

Aohada-Glycoside C (3) A pale yellow amorphous powder, $[\alpha]_D$ −32° (c=1.0, MeOH). UV (MeOH) λ_{max} nm (log ε): 329 (4.60), 300 sh (4.47), 245 (4.35), 216 (4.49). ^{1}H-NMR (C_5D_5N) δ: 7.94, 7.97 (each 1H, d, J=15.9Hz, Caf-H-7), 7.57, 7.61 (each 1H, d, J=1.8 Hz, Caf-H-2), 7.21, 7.24 (each 1H, d, J=8.2 Hz, H-5), 7.12, 7.18 (each 1H, dd, J=1.8, 8.2 Hz, Caf-H-6), 6.55, 6.64 (each 1H, d, J=15.9 Hz, Caf-H-8), 6.17 (1H, t, J=6.7 Hz, H-2), 5.14 (2H, d, J=6.7 Hz, H_2-1), 5.09 (1H, dd, J=1.8, 11.9 Hz, Glc-H-6), 4.99 (1H, d, J=7.6 Hz, Glc-H-1), 4.92 (1H, d, J=12.2 Hz, H-4), 4.80 (1H, dd, J=5.5, 11.9 Hz, Glc-H-6), 4.68 (1H, d, J=12.2 Hz, H-4), 4.66 (2H, s, H_2-5). HR-FAB-MS m/z: 605.187 $[M+H]^+$. Calcd for $C_{29}H_{33}O_{14}$: 605.187.

Alkaline Methanolysis of 3 Compd. **3** (29 mg) was subjected to alkaline methanolysis in the same manner as **1** to obtain methyl caffeate (5.8 mg) and **1a** (7 mg).

3,4,5-Trimethoxyphenol β-D-5-*O*-Caffeoyl-apiofuranosyl-(1→6)-β-D-glucopyranoside (4) A pale yellow amorphous powder, $[\alpha]_D$ −55° (c=1.0, MeOH). UV (MeOH) λ_{max} nm (log ε): 330 (4.34), 300 sh (4.72). ^{1}H-NMR (CD_3OD) δ: 7.58 (1H, d, J=15.8 Hz, Caf-H-7), 7.05 (1H, d, J=2.0 Hz, Caf-H-2), 6.95 (1H, dd, J=2.0, 8.3 Hz, Caf-H-6), 6.77 (1H, d, J=8.3 Hz, Caf-H-5), 6.44 (2H, s, H-2, 6), 6.28 (1H, d, J=15.8 Hz, Caf-H-8), 5.00 (1H, d, J=2.3 Hz, Api-H-1), 4.80 (1H, d, J=7.6 Hz, Glc-H-1), 3.78 (6H, s, 3,5-OCH_3), 3.68 (3H, s, 4-OCH_3). ^{13}C-NMR (CD_3OD) δ: 155.6 (C-1), 96.2 (C-2), 154.5 (C-3), 134.5 (C-4), 154.5 (C-5), 96.2 (C-6), 61.2 (4-OCH_3), 56.6 (3,5-OCH_3), 103.1 (Glc-1), 74.9 (Glc-2), 77.9 (Glc-3), 71.5 (Glc-4), 76.9 (Glc-5), 68.4 (Glc-6), 110.4 (Api-1), 78.5 (Api-2), 79.0 (Api-3), 75.0 (Api-4), 67.5 (Api-5), 126.9 (Caf-1), 115.8 (Caf-2), 147.7 (Caf-3), 150.5 (Caf-4), 116.8 (Caf-5), 122.1 (Caf-6), 146.0 (Caf-7), 114.8 (Caf-8), 167.3 (Caf-9). HR-FAB-MS m/z: 641.209 $[M+H]^+$. Calcd for $C_{29}H_{37}O_{16}$: 641.208.

Alkaline Methanolysis of 4 Compd. **4** (31 mg) was subjected to alkaline methanolysis in the same manner as **1** to obtain methyl caffeate (3.0 mg) and **4a** (12 mg).

Compound 4a A pale yellow amorphous powder, $[\alpha]_D$ −54° (c=1.0, MeOH). ^{1}H-NMR (CD_3OD) δ: 6.46 (2H, s, H-2, 6), 4.97 (1H, d, J=2.3 Hz, Api-H-1), 4.80 (1H, d, J=7.3 Hz, Glc-H-1), 3.82 (6H, s, 3,5-OCH_3), 3.71 (3H, s, 4-OCH_3). ^{13}C-NMR (CD_3OD) δ: 155.9 (C-1), 96.4 (C-2), 154.8 (C-3), 134.6 (C-4), 154.8 (C-5), 96.4 (C-6), 61.3 (4-OCH_3), 56.7 (3,5-OCH_3), 102.9 (Glc-1), 74.5 (Glc-2), 77.5 (Glc-3), 71.2 (Glc-4), 76.6 (Glc-5), 68.6 (Glc-6), 110.5 (Api-1), 77.7 (Api-2), 80.3 (Api-3), 74.7 (Api-4), 65.2 (Api-5). Its physical properties and spectral data were the same as those reported.[6]

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