Structure of a New Acylated Flavonoid Glycoside, Euryanoside, from Flowers of *Eurya japonica* THUNB.

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A new acylated flavonoid glycoside, named euryanoside (1), has been isolated together with known compounds, halleridone (4) and cornoside (5), from male flowers of *Eurya japonica* THUNB. (Theaceae). The structure of 1 has been established to be apigenin 5-*O*-α-L-rhamnopyranosyl-(1→2)-(6″-*O*-acetyl)-β-D-glucopyranoside, based on lines of chemical and spectral evidence. In addition, the first identifications of 4 and 5 from the Theaceae are also reported.

Keywords *Eurya japonica*; Theaceae; flower; acylated flavonoid glycoside; euryanoside; halleridone; cornoside

Leaves and fruits of *Eurya japonica* THUNB. (hisakaki in Japanese) (Theaceae), a Chinese crude drug (Ling-mu in Chinese; Reiboku in Japanese), have been used in China as an anodyne for rheumatism, as a remedy for swelling, and as an external hemostatic for traumatic bleeding, *etc.*[1] A number of flavonoids (anthocyanins,[2a] flavone and flavonol glycosides[2b]), as well as a few isoprenoids (betulinic acid and β-sitosterol),[2b,3] have been identified from fruits, leaves, and barks of *E. japonica*, but no phytochemical study on flowers of this plant has appeared to date. This paper deals with the isolation and structure elucidation of a new acylated flavonoid glycoside and two known cyclohexenone derivatives from male flowers of the plant.

After chromatographic and high pressure liquid chromatographic (HPLC) separation of the ethyl acetate–methanol (2:1) extract, a new acylated glycoside named euryanoside (**1**) and two cyclohexenone derivatives (**4** and **5**) were isolated. The isolated cyclohexenones, **4** and **5**, were identified as halleridone[4] (=rengyolone[5]) and cornoside,[5,6] respectively (see Experimental).

Euryanoside (**1**), $C_{29}H_{32}O_{15}$, mp 195—196 °C, $[\alpha]_D$ −75.1° (pyridine), showed bands due to an ester and a conjugated carbonyl group (1725 and 1630 cm^{-1}, respectively) in the infrared (IR) spectrum. The negative ion fast atom bombardment mass (FAB-MS) spectrum of **1** gave a molecular ion $[(M-H)^-]$ at *m/z* 619 and two significant fragment peaks at *m/z* 473 [619(M − H) − 146 (deoxyhexose unit)] and at *m/z* 269 [473 − 204 (monoacetyl hexose unit)]. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** showed signals ascribed to a secondary methyl (δ 1.07, d, J=6.1 Hz), an acetyl methyl (δ 1.87, s), two anomeric protons (δ 5.18, d, J=1.2 Hz and δ 5.40, d, J=6.4 Hz), and seven aromatic protons.

Acidic hydrolysis of **1** with 10% H_2SO_4–EtOH (1:1) yielded apigenin (**3**) as an aglycone and one mol each [judged by gas liquid chromatography (GLC)] of L-rhamnose and D-glucose (assumed to be of L- and D-configurations, respectively) as sugar components. On enzymic hydrolysis with hesperidinase, **1** afforded a partial

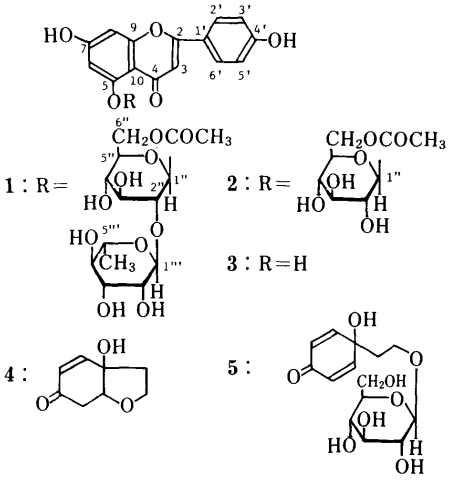

Chart 1

TABLE I. ^{13}C-NMR Spectral Data for **1**, **2**, and **3** (100.5 MHz, DMSO-d_6, δ_C, ppm, from TMS)[a]

Carbon No.	1	2	3
Aglycone			
C-2	162.13	161.13	163.66
C-3	105.69	105.60	102.76
C-4	175.55	176.78	181.65
C-5	158.62	158.30[b]	161.07
C-6	98.97	104.36	98.74
C-7	160.43[c]	162.53	164.04
C-8	96.17	98.32	93.87
C-9	156.98	158.20[b]	157.22
C-10	107.18	108.16	103.61
C-1′	121.23	121.12	121.10
C-2′,6′	127.79	128.01	128.37
C-3′,5′	115.77	115.83	115.88
C-4′	160.09[c]	160.70	161.37
(6-*O*-Acetyl)-glucose			
C-1″	96.96	103.69	
C-2″	76.59	73.47	
C-3″	76.40	75.45	
C-4″	70.23	69.76	
C-5″	73.25	73.94	
C-6″	62.77	63.23	
$OCO\underline{C}H_3$	20.25	20.52	
$O\underline{C}OCH_3$	170.02	170.20	
Rhamnose			
C-1‴	99.54		
C-2‴	70.39		
C-3‴	69.76		
C-4‴	72.06		
C-5‴	68.53		
C-6‴	17.85		

a) Assignments were made with the aid of INEPT and ^{13}C–H COSY experiments. *b*, *c*) Assignments may be interchanged in each column.

hydrolysate, *i.e.*, a monoglycoside (**2**), $C_{23}H_{22}O_{11}$, mp 188—190 °C, $[\alpha]_D$ −69.9° (pyridine). The monoglycoside (**2**) exhibited an ester carbonyl band at 1720 cm^{-1} in the IR spectrum and an acetyl methyl signal (δ 2.07, s) in the ^{1}H-NMR spectrum.

The apigenin glycoside (**1**) showed absorption maxima at 263 and 330 nm in the ultraviolet (UV) spectrum. The changes (see Experimental) caused by addition of three typical test reagents ($AlCl_3$, MeONa, and AcONa)[7] suggested that in **1**, the 7- and 4′-OH groups on apigenin are both in free forms and only the 5-OH group on apigenin is bound with the sugar (=the disaccharide) moiety through a glycosidic linkage. This structural feature of **1** was further substantiated by the following carbon-13 nuclear magnetic resonance (^{13}C-NMR) study. All A- and C- ring carbons of the apigenin glycosides (**1** and **2**) resonated upfield (C-2, C-4, C-5, and C-7) or downfield (C-3, C-6, C-8, C-9, and C-10) (by *ca.* 0.2—6.1 ppm) from the corresponding carbons of apigenin (**3**) (Table I). These glycosylation shifts between apigenin (**3**) and apigenin glycosides **1** and **2** are consistent with the corresponding reported shifts between luteolin and luteolin 5-*O*-glucoside.[8] These lines of spectral and chemical evidence suggest that **1** can be assigned as apigenin 5-*O*-L-rhamnosyl-(monoacetyl)-D-glucoside.

The position of an acetyl group on the glucosyl moiety in **1** (and also in **2**) was determined as follows. In the ^{1}H-NMR spectra, the glucosyl methylene (6″-H_2) of **1** [δ 4.02 (1H, dd, J=11.7, 6.2 Hz) and 4.31 (1H, dd, J=11.7, 1.7 Hz)] and **2** [δ 4.18 (1H, dd, J=11.9, 6.7 Hz) and 4.34 (1H, dd, J=11.9, 2.0 Hz)] resonated downfield from the corresponding signals for usual glucosides. Furthermore, in the ^{13}C-NMR study (Table I), C-6″ and C-5″ of **1** (δ 62.77 and 73.25 ppm, respectively) and **2** (δ 63.23 and 73.94 ppm, respectively), due to the (acetyl)-glucosyl moiety, were respectively shifted downfield (C-6″) and upfield (C-5″) (acylation shifts), compared with those reported for a usual glucosyl residue.[8] These NMR studies proved the presence of a (6-*O*-acetyl)-D-glucoside moiety in **1** (also in **2**).

Information concerning the interglycosidic linkage in the disaccharide part of **1** was obtained as follows. In the ^{13}C-NMR spectrum, the (acetyl)-glucosyl 2″-carbon (C-2″) of **1** resonated at δ 76.59 ppm downfield (by 3.12 ppm) from the corresponding carbon signal (δ 73.47 ppm) for **2**, whereas the (acetyl)-glucosyl anomeric carbon signal (δ 96.96 ppm) of **1** appeared upfield (by 6.73 ppm) from that (δ 103.69 ppm) of **2**. This ^{13}C-NMR study reveals that the 2″α-OH group on the inner (6-*O*-acetyl)-D-glucoside was connected with terminal L-rhamnose by an ether bond *via* the anomeric hydroxyl of the rhamnose. This structural feature was also substantiated by the following investigation. The nuclear Overhauser effect correlation spectroscopy (NOESY) spectrum of **1** gave an intense cross peak between 2″β-H (on the inner sugar residue) and the anomeric proton (1‴-H) of the terminal rhamnosyl moiety.

Finally, the anomeric configuration of the glycosidic linkages in **1** was determined by the following ^{1}H- and ^{13}C-NMR spectral and optical rotational studies. The anomeric proton doublets with large J-values of **1** (δ 5.40, J=6.4 Hz) and **2** (δ 4.79, J=7.3 Hz), due to the (acetyl)-glucosyl moiety, proved the presence of a (6″-*O*-acetyl)-β-D-glucopyranoside moiety in **1** (also in **2**). In the ^{13}C-NMR spectrum of **1**, the anomeric carbon (C-1‴) with large ^{13}C-H coupling constant (J_{C-H}=172.1 Hz), due to terminal rhamnoside, was indicative of the presence of α-L-rhamnopyranoside in **1**.[9] The α-anomeric configuration of the rhamnoside was further corroborated by the difference in molecular rotation ($\Delta[M]_D$ = −134.4°) between **1** and **2**.[10] Based on these lines of accumulated evidence, the structure for euryanoside (**1**) is defined as apigenin 5-*O*-α-L-rhamnopyranosyl-(1→2)-(6″-*O*-acetyl)-β-D-glucopyranoside.

To our knowledge, flavone 5-*O*-glycosides such as euryanoside (**1**) have previously been found only rarely in the plant kingdom. Apart from halleridone[4] (=rengyolone[5]) and cornoside,[5,6] this is the first report of their identification from the Theaceae.

Experimental

All melting points were determined on a Yanagimoto micro-apparatus and are uncorrected. IR spectra were run with a JASCO A-302 instrument. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100.5 MHz) spectra were measured with a JEOL JNM-GX400 spectrometer with dimethylsulfoxide-d_6 (DMSO-d_6) as a solvent and tetramethylsilane (TMS) as an internal standard. Negative ion FAB-MS were obtained from a JEOL JNM-DX300 spectrometer under the following conditions: accelerating voltage, 2—3 kV; matrix, triethanolamine; collision gas, Xe. Optical rotations were determined on a JASCO DIP-140 digital polarimeter. GLC was carried out on a Shimadzu GC-7AG gas chromatograph under the following conditions: column, 1.5% SE-52 on Chromosorb WAW DMCS (2 m × 3 mm i.d.); detector, hydrogen flame ionization detector; column temperature, 180 °C; carrier N_2 gas, 30 ml/min. For column chromatography and thin layer chromatography (TLC), Kieselgel 60 (Merck; 230—400 mesh) and precoated silica gel plates (Merck HF-254) were used, respectively. Preparative HPLC was carried out on a Waters instrument with a M 6000A pump, a U6K septumless injector, and a series R-401 differential refractometer. A micro-bonded silica-packed column (Waters μ-Porasil; 7.8 mm × 30 cm) with $CHCl_3$–AcOEt (3:7) and a reversed-phase ODS column (Waters μ-Bondapack-C_{18}; 7.8 mm × 30 cm) with H_2O were respectively used with an eluant flow of 3 ml/min. Hesperidinase from *Penicillium* species was purchased from Sigma Chem. Co. (Lot. No. 102F-0659).

Plant Material Male flowers of *E. japonica* were collected at the campus of Setsunan University (Faculty of Pharmaceutical Sciences, Hirakata, Osaka, Japan) in April 1988 and identified by one of us (H. M.). A voucher specimen is deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Setsunan University.

Extraction and Isolation of 1, 4, and 5 The air-dried male flowers (195 g) were extracted three times (0.6 l each) with AcOEt–MeOH (2:1) at room temperature for three weeks. The combined extract was concentrated to dryness under reduced pressure and the residue (18.8 g) was chromatographed on silica gel (300 g), eluting gradually with $CHCl_3$–MeOH (20:1) and the lower phase of $CHCl_3$–MeOH–H_2O (7:3:1). The less polar fraction obtained with the former eluent was further purified by preparative TLC and preparative HPLC separation to give **4** (25 mg), colorless oil, $[\alpha]_D$ −0.5° (MeOH, c=1.0). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3360, 3000, 1680, 1070. Optical rotational value and IR ($CHCl_3$), EI-MS, ^{1}H-NMR, and ^{13}C-NMR spectral data of **4** were consistent with those published for halleridone[4] (=rengyolone[5]). The polar fraction (1.8 g) obtained with the latter eluent was subjected to Sephadex LH-20 column chromatography with MeOH as the eluant. Fractions containing **5** (0.56 g) and **1** (0.5 g) were eluted in that order. The fraction containing **5** was further purified by reversed-phase HPLC separation to give **5** (92 mg), a white powder, $[\alpha]_D$ −11.4° (MeOH, c=0.28). IR ν_{max}^{KBr} cm^{-1}: 3350, 2860, 1665, 1620, 1070. Optical rotational value and IR (KBr), negative ion FAB-MS, ^{1}H-NMR, and ^{13}C-NMR spectral data of **5** were coincident with those reported for cornoside.[5,6]

Euryanoside (1) Pale yellow needles of mp 195—196 °C (MeOH), $[\alpha]_D$ −75.1° (pyridine, c=0.47). UV λ_{max}^{MeOH} nm (log ε): 263 (4.22), 330 (4.32). +NaOMe: 270, 320, 380; +$AlCl_3$: 263, 330; +AcONa: 270, 305, 340. IR ν_{max}^{KBr} cm^{-1}: 3350, 2900, 1725, 1630, 1600, 1355, 1260, 1085, 1050, 835. Negative ion FAB-MS m/z [%]: 619 [(M−H)$^-$, 90], 577 [(M−H−42)$^-$, 18], 473 [(M−H−146)$^-$, 9], 269 [(M−H−146−204)$^-$, 100]. ^{1}H-NMR δ:

1.07 (3H, d, J=6.1 Hz, 6‴-H_3), 1.87 (3H, s, $OCOCH_3$), 3.0—3.8 (8H, m, 2″-H, 3″-H, 4″-H, 5″-H, 2‴-H, 3‴-H, 4‴-H, 5‴-H), 4.02 (1H, dd, J=11.7, 6.2 Hz), 4.31 (1H, dd, J=11.7, 1.7 Hz) (6H″-H_2), 5.18 (1H, d, J=1.2 Hz, 1‴-H), 5.40 (1H, d, J=6.4 Hz, 1″-H), 6.49 (1H, d, J=2.0 Hz, 6-H), 6.54 (1H, s, 3-H), 6.61 (1H, d, J=2.0 Hz, 8-H), 6.91 (2H, d, J=8.8 Hz, 3′-, 5′-H_2), 7.87 (2H, d, J=8.8 Hz, 2′-, 6′-H_2). ^{13}C-NMR: given in Table I. *Anal.* Calcd for $C_{29}H_{32}O_{15}\cdot 1/2H_2O$: C, 55.32; H, 5.28. Found: C, 55.07; H, 5.37.

Acidic Hydrolysis of 1 A solution of **1** (30 mg) in 10% H_2SO_4–EtOH (1 : 1, 6 ml) was refluxed for 6 h, then poured into ice-water to afford a poduct as a precipitate, which was collected by filtration and dried. Recrystallization of the product from MeOH furnished pale yellow needles of **3** (8.5 mg) which was identical with authentic apigenin by direct comparison [TLC, IR (KBr), ^{1}H- and ^{13}C-NMR]. The aqueous layer of the hydrolysate was neutralized with Amberlite IRA-410 resin and evaporated to dryness under reduced pressure. The residue was identified as one mol each of glucose and rhamnose by paper partition chromatography [iso-PrOH–*n*-BuOH–H_2O (7 : 1 : 2) as a developing solvent system and aniline hydrogen phthalate for detection], TLC [EtOH–28% NH_4OH–H_2O (20 : 1 : 4) as a developing solvent system and 1% $Ce(SO_4)_2$ in 10% H_2SO_4 for detection], and GLC (as the corresponding trimethylsilyl ethers).

Enzymic Hydrolysis of 1 A suspension of **1** (50 mg) and hesperidinase (500 mg) in EtOH–H_2O (1 : 1, 6 ml) was stirred at 37 °C for 20 h, then poured into H_2O, and extracted with AcOEt. The AcOEt layer was dried over $MgSO_4$ and evaporated to dryness. The residue (47 mg) was recrystallized from aqueous MeOH to afford **2** (27 mg), pale yellow needles of mp 188—190 °C (dec.), $[\alpha]_D$ −69.9° (pyridine, c=0.91). IR ν_{max}^{KBr} cm^{-1}: 3300, 2900, 1720, 1630, 1600, 1065. Negative ion FAB-MS m/z [%]: 473 [(M−H)$^-$, 72], 431 [(M−H−42)$^-$, 2], 269 [(M−H−204)$^-$, 37]. ^{1}H-NMR δ: 2.07 (3H, s, $OCOCH_3$), 3.26 (1H, t, J=8.4 Hz, 4″-H), 3.33 (1H, t, J=8.4 Hz, 3″-H), 3.40 (1H, dd, J=8.4, 7.3 Hz, 2″-H), 3.61 (1H, ddd, J=8.4, 6.7, 2.0 Hz, 5″-H), 4.18 (1H, dd, J=11.9, 6.7 Hz), 4.34 (1H, dd, J=11.9, 2.0 Hz) (6″-H_2), 4.79 (1H, d, J=7.3 Hz, 1″-H), 6.65 (1H, s, 3-H), 6.70 (1H, d, J=2.3 Hz, 6-H), 6.75 (1H, d, J=2.3 Hz, 8-H), 6.92 (2H, d, J=8.9 Hz, 3′-, 5′-H_2), 7.89 (2H, d, J=8.9 Hz, 2′-, 6′-H_2). ^{13}C-NMR: given in Table I.

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