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ON THE CONSTITUENTS OF THE LEAVES OF *WEIGELA CORAEENSIS*

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Abstract

From the leaves of *Weigela coraeensis* ursolic acid, β-sitosteryl β-D-glucoside, scopolin, secologanin dimethyl acetal and hyperin (quercetin-3-*O*-β-D-galactoside) have been isolated.

Introduction

Weigela coraeensis (Japanese name: hakoneutsugi) is an ornamental shrub and grows in the temperate zone of Japan. Although the family Caprifoliaceae is known as a rich source of iridoid glycosides[1], there seems to be no report on the constituents of *Weigela* species. We have now examined the methanolic extract of the leaves of *W. coraeensis*. Five compounds **1**, **2**, **3**, **4** and **5** have been isolated according to the isolation procedure shown Fig. 1.

Results and Discussion

Compound (**1**) was crystallized as prisms, mp 251-252° with a molecular formula $C_{30}H_{48}O_3 \cdot 1/4H_2O$. It gave a positive Liebermann-Burchard's reaction. The IR spectrum showed absorption bands for a hydroxyl group at 3400 cm^{-1} and a carboxyl group at 1690 cm^{-1}. The ^1H NMR spectrum indicated signals of a proton attached to a carbon bearing hydroxyl group at δ 3.46 (1H, *t*-like, $J=8$ Hz) and an olefinic proton at δ 5.50 (1H, *m*) besides those of typical triterpenoids at δ 0.8-2.4 (*m*). The characteristic fragmentations of the mass spectrum at *m/z* 456 $[M]^+$, 248, 203, 189 and 133, arising from a retro-Diels-Alder cleveage, suggested that compound **1** was either ursolic acid or oleanolic acid. The IR and ^1H NMR spectra

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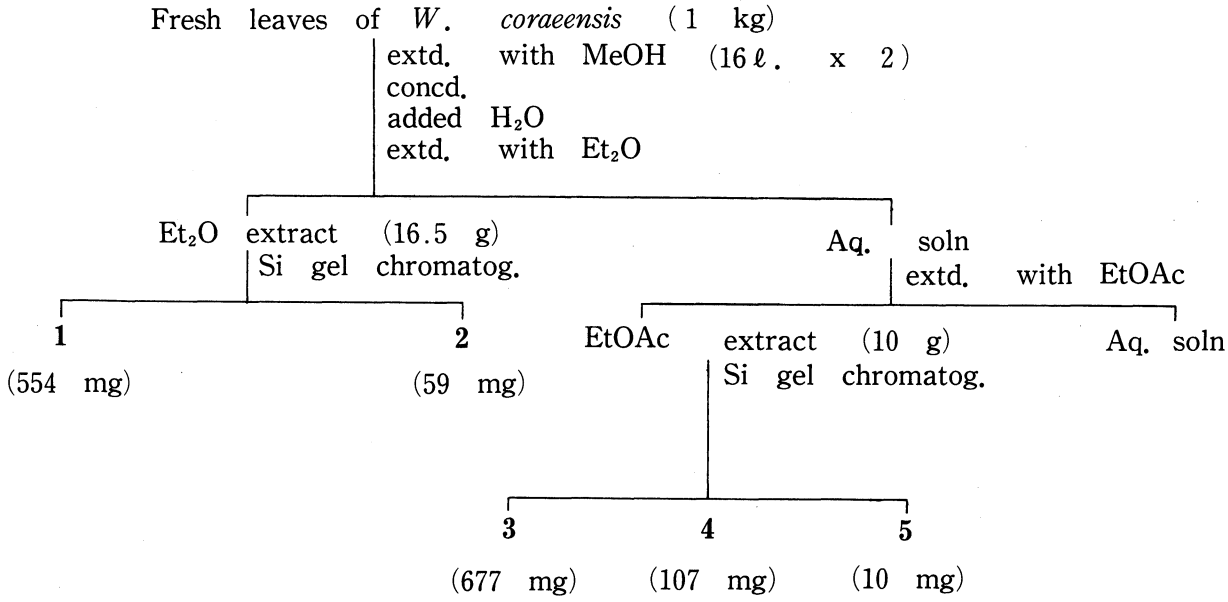


Fig. 1. Isolation procedure of the compounds

were identical with those of ursolic acid.

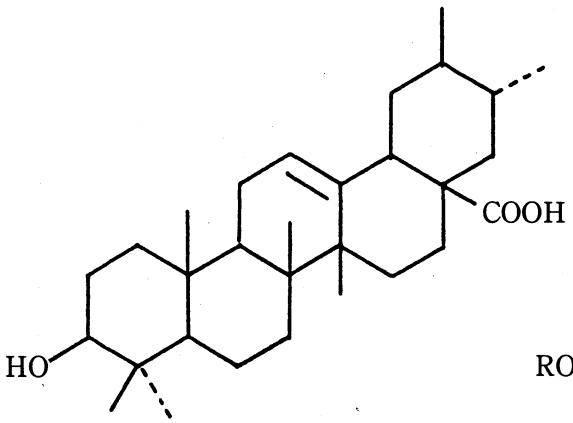
Compound (**2**) was isolated as a white mass, mp 263-264° with a molecular formula $C_{35}H_{60}O_6 \cdot 2/3H_2O$. The IR spectrum showed a strong hydroxyl absorption at 3400 cm^{-1}. The ^1H NMR spectrum showed a typical phytosterol skeleton at δ 0.55-2.36. On acetylation with acetic anhydride and pyridine, compound **2** gave needles (**6**), mp 171-172° with a molecular formula $C_{43}H_{60}O_{10}$. The ^1H NMR spectrum of the acetate indicated the presence of four acetoxyl groups at δ 1.98-2.06 (3H x 4, *s*). The above results suggested that compound **2** was β-sitosteryl β-D-glucoside. The spectral and physical data of **2** were in agreement with those of β-sitosteryl β-D-glucoside.

Compound (**3**) was an amorphous powder with a molecular formula $C_{19}H_{30}O_{11}$. Acid hydrolysis yielded D-glucose and a brown polymerized product like other iridoids. The UV absorption maximum at 234 nm (ε8080) and the IR absorption bands at 1700 and 1625 cm^{-1} were characteristic of a conjugated enol-ether system. The ^1H NMR spectrum showed signals due to a C-3 proton at δ 7.60 (*s*), vinylic protons at δ 5.00-5.40 (3H, *m*) and carbomethoxyl protons at δ 3.55 (3H, *s*) together with those of an anomeric proton at δ 5.27 (*d*, J=8 Hz). The above results suggested that compound **3** was a secoiridoid glucoside. This was confirmed by a

peak at m/z 165 in the mass spectrum characteristic of secoiridoid glycosides[2] . In the ^{1}H NMR spectrum, two singlets due to methoxyl protons at δ 3.26 (3H x 2) and signals assignable to a -CHCH$_2$CH- moiety at δ 1.79 (1H, *ddd*, J=6, 9 and 15 Hz) , 2.39 (1H, *ddd*, J=7, 7 and 15 Hz) and 4.62 (1H, *dd*, J=6 and 7 Hz) were observed. Acetylation of **3** with acetic anhydride and pyridine afforded a tetra-acetate (**7**) with a molecular formula $C_{27}H_{38}O_{15}$. Compound **3** was therefore assumed to be secologanin dimethyl acetal on the basis of the data described above. The physical and chemical properties of **3** were identical with those of an authentic sample[3] . Compond **3** would be formed during extraction process.

Compound (**4**) was needles, mp 216-220° with a molecular formula $C_{16}H_{18}O_9 \cdot 1\ 1/3H_2O$. Absorption bands at 1725, 1700, 1615 and 1565 and 1505 cm^{-1} in the IR spectrum suggested that compound **4** was a coumarin. Additional evidence for the presence of this carbon skeleton came from the ^{1}H NMR spectrum. The signals corresponding to C-3 and C-4 protons appeared as an AB system at δ 6.30 and 7.64 (J=9.5 Hz) . Two singlets at δ 7.01 and 7.43 (1H each) were attributable to C-8 and C-5 protons, respectively. The ^{1}H NMR spectrum also showed the presence of a methoxyl group at δ 3.74 (3H, *s*) and an anomeric proton at δ 5.64 (1H, W½ 12 Hz). Compound **4** was treated with acetic anhydride and pyridine to give a tetra-acetate (**8**), mp 169-169.8° with a molecular formula $C_{24}H_{26}O_{13}$. The above date suggested that compound **4** was scopolin. The spectral and physical data of **8** were identical with those of scoplin acetate[4] .

Compound (**5**) was yellow crystals, mp 238-240° and had a molecular formula $C_{21}H_{20}O_{12} \cdot H_2O$. The UV spectrum had absorption maxima at 257 nm (ε18000) and 359 nm (ε 15000). The IR spectrum showed absorption bands of a hydroxyl group at 3400 cm^{-1}, a conjugated carbonyl at 1655 cm^{-1} and a phenyl group at 1605 and 1500 cm^{-1}. The above data suggested that compound **5** was a flavonoid glycoside. An AB system at δ 6.72 (1H, J=2 Hz) and 6.77 (1H, J=2 Hz) in the ^{1}H NMR spectrum were due to C-6 and C-8 protons, respectively. Signals at δ 7.38 (1H, *d*, J=8 Hz) , 8.22 (1H, *dd*, J=2 and 8 Hz) and 8.58 (1H, *d*, J=2 Hz) were characteristic for a 3,4-disubstituted B ring. Acetylation of **5** with acetic anhydride and pyridine yielded (**9**) an octa-acetate with a molecular formula $C_{37}H_{36}O_{20}$. The ^{1}H NMR spectrum of the latter showed the presence of four alcoholic acetoxyl groups at δ 1.86-2.11 (3H x 4, *s*) and four phenolic acetoxyl groups at δ 2.28-2.41 (3H x 4, *s*) . Hydrolysis of **5** with 2N HCl afforded D-galactose and quercetin (**10**) , mp >300°, whose IR spectrum were identical with that of an authenic sample. The UV spectrum of **5** in methanol and methanol-sodium acetate were similar to those of rutin[5] , which indicated that the glycosidic linkage in **5** was located at 3-position. Therefore compound **5** should be hyperin (quercetin-3-*O*-β-D-galactoside) .



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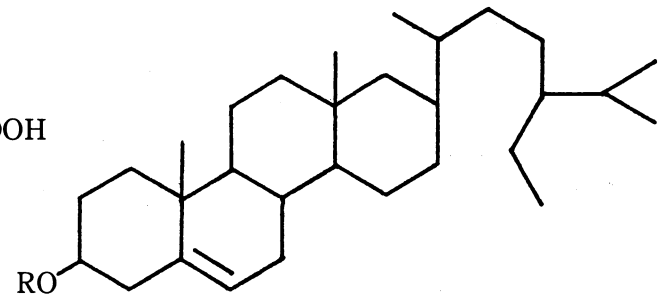
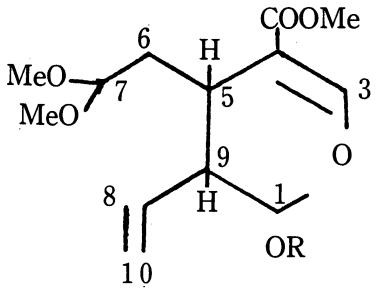


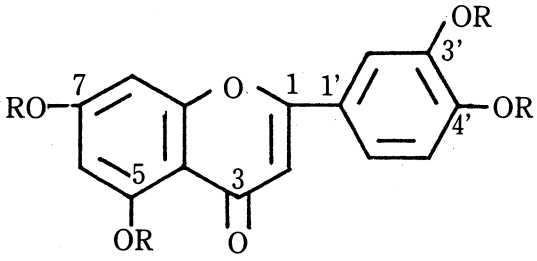
2 R=Glc

6 R=GlcAc$_4$



3 R=Glc

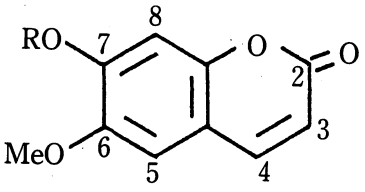
7 R=GluAc$_4$



5 R=H R'=Glc

9 R=Ac R'=GlcAc$_4$

10 R=R'=H



4 R=Glc

8 R=GlcAc$_4$

Experimental

Extraction and Isolation. Plant material was collected in the campus of Kagoshima University and identified by Drs. S. Higashi and M. Abe. The fresh leaves of *W.coraeensis* (2 kg) were extracted with MeOH (16 ℓ. x 2). After concentration of the combined MeOH solns, H_2O was added and the insoluble material was removed by filtration. The filtrate was extracted with Et_2O and then EtOAc. The Et_2O extract (16.5g) was chromatographed on a column of Si gel with $CHCl_3$-MeOH with increasing proportions of MeOH. Elution with $CHCl_3$ gave **1** (554mg). From the fractions eluted with $CHCl_3$-MeOH (9:1) **2** (39mg) was obtained. The EtOAc extract (10g) was subjected to CC on Si gel with $CHCl_3$-MeOH with increasing proportions of MeOH. Elution with $CHCl_3$-MeOH (92 : 8) afforded **3** (677mg). Compound **4** was obtained from the fractions eluted with $CHCl_3$-MeOH (9 : 1). The fractions eluted with $CHCl_3$−MeOH (17 : 3) gave **5** (10mg).

Ursolic acid **1.** Prisms from EtOH-H_2O, mp 251-252° (lit. [6] mp 285-288°) ; IR ν_{max}^{Nujol} cm^{-1} : 3400, 1690 ; ^1H NMR (100 MHz, C_5D_5N) : δ 0.8-2.4 (*m*) , 3.46 (1H, *t*-like, J=8 Hz), 5.50 (1H, *m*); MS *m/z*: 456 $[M]^+$, 248, 203, 189, 133. (Found: C, 78.05 ; H, 10.75%. Calc. for $C_{30}H_{48}O_3 \cdot 1/4\ H_2O$: C, 78.12 ; H, 10.60%.)

β-Sitosteryl β-D-glucoside **2.** A white mass from MeOH, mp 263-264° (lit. [7] mp 250-255°, 280-298°);IR ν_{max}^{Nujol} cm^{-1}: 3400, 1070, 1020 ; ^1H NMR (100 MHz, C_5D_5N); δ 0.55-2.36 (*m*), 3.66-4.58 (*m*, sugar H), 5.06 (1H, *d*, J=8 Hz, H-1'), *ca* 5.6 (*m*). (Found: C, 71.35 ; H, 10.37%. Calc. for $C_{35}H_{60}O \cdot 2/3\ H_2O$: C, 71.39 ; H, 10.50%.) Acetylation of **2** (19 mg) with Ac_2O and pyridine gave **6** (13 mg), needles from EtOH, mp 171-172° (lit. [7] mp 168-169°) : IR ν_{max}^{Nujol} cm^{-1}: 1750, 1220 ; ^{1}NMR (100 MHz, $CDCl_3$): δ 1.98, 2.00, 2.03, 2.06 (3H each, *s*). (Found: C, 69.05 ; H, 9.11%. Calc. for $C_{43}H_{60}O_{10}$: C, 69.32 ; H, 9.20%.)

Secologanin dimethyl acetal **3.** An amorphous powder, $[\alpha]_D$-58.8° (MeOH; *c* 0.2); UV λ_{max}^{MeOH} nm: 234 (ε 8080); IR ν_{max}^{film} cm^{-1}: 3400, 1700, 1625 ; ^1H NMR (100 MHz $CDCl_3$): δ 1.79 (1H, *ddd*, J=6, 9 and 15 Hz, H-6), 2.39 (1H, *ddd*, J=7, 7 and 15 Hz, H-6) , 2.83 (1H, *ddd*, J=6, 6 and 8 Hz, H-9), 3.26 (3H x 2, *s*, OMe) , 3.55 (3H, *s*, COOMe), 4.62 (1H, *dd*, J=6 and 7 Hz, H-7), 5.00-5.40 (3H, *m*) , 5.27 (1H, *d*, J= 8 Hz, H'-1), 5.79 (1H, *d*, J=6 Hz, H-1), 7.60 (1H, *d*-like, J=1 Hz, H-3) ; MS *m/z*: 403 $[M\text{-}MeO]^+$, 372, 171, 165, 139, 75. (Found: *m/z* 403.1609. Calc. for $C_{19}H_{30}O_{11}$-MeO: *m/z* 403.1604.) Comound **3** (7 mg) was treated with 2N HCl to give D-glucose which was confirmed by co-paper chromatography (solvent system: EtOAc -pyridine-H_2O-HOAc, 5 : 5 : 3 : 1). Acetylation of **3** (20 mg) with Ac_2O and pyridine yielded **7** (8 mg), an amorphous powder ; IR ν_{max}^{Nujol} cm^{-1}: 1755, 1710, 1630, 1220; ^1H

NMR (100 MHz, $CDCl_3$): δ 1.84, 1.95, 1.97, 2.04 (3H x 4, *s*). (Found: *m/z* 571.1994. Calc. for $C_{27}H_{38}O_{15}$-MeO: *m/z* 571.2025.)

Scopolin **4.** Needles from MeOH, 216-220°(lit. [6] mp 218°); IR ν_{max}^{Nujol} cm^{-1}: 3350, 1725, 1700, 1615, 1565, 1505 ; ^1H NMR (100 MHz, C_5D_5N) : δ 3.74 (3H, *s*, OMe), 3.88-4.60 (6H, *m*, sugar H), 5.64 (1H, *W*½ 12 Hz, H-1′), 6.30 (1H, *d*, *J*=9.5 Hz, H-3), 7.01 (1H, *s*, H-8), 7.43 (1H, *s*, H-5), 7.64 (1H, *d*, *J*=9.5 Hz, H-4). (Found: C, 50.90; 5.07%. Calc. for $C_{16}H_{18}O_9 \cdot 1\frac{1}{3}H_2O$: C, 50.79 ; H, 5.51%.) Acetylation of **4** (42 mg) with Ac_2O and pyridine gave **8** (30 mg), prisms form EtOH, mp 169-169.8° (lit. [6] mp 166° or mp 184-185°); IR ν_{max}^{Nujol} cm^{-1}: 1760-1740, 1620, 1570, 1510, 915, 890, 825 ; ^1H NMR (100 MHz, $CDCl_3$): δ 2.05, 2.06, 2.08, 2.14 (3H each, *s*); MS *m/z*: 522 $[M]^+$. (Found: C, 55.22; H, 5.08%. Calc. for $C_{24}H_{26}O_{13}$: C, 55.17; H, 5.02%.)

Hyperin **5.** Yellow crystals from MeOH, mp 238-240° (lit. [8] mp 238°; UV λ_{max}^{MeOH} nm: 257 (ε 18000), 359 (ε 15000); $\lambda_{max}^{MeOH\text{-}AcONa}$ nm: 269, 368 ; IR ν_{max}^{MeOH} cm^{-1}: 3400, 1655, 1605, 1550, 1500;^1H NMR (100 MHz, C_5D_5N): δ 4,18-4.98 (6H, *m*, sugar), 6.10 (1H, *d*, *J*=8 Hz, H-1′), 6.72 and 6.77 (1H each, *d*, *J*=2 Hz, H-6 and H-8), 7.38 (1H, *d*, *J*=8 Hz, H-5), 8.22 (1H, *dd*, *J*=2 and 8 Hz, H-6), 8.58 (1H, *d*, *J*=2 Hz, H-2). (Found: C, 52.10; H, 4.44%. Calc. for $C_{21}H_{20}O_{12} \cdot H_2O$: C, 52.47 ; H, 4.58%.) Acetylation of **5** (21 mg) with Ac_2O and pyridine gave **9** (20 mg), an amorphous powder; IR ν_{max}^{Nujol} cm^{-1}: 1775, 1750, 1210 ; ^1H NMR (100 MHz, $CDCl_3$): δ 1.86 and 1.96 (3H each, *s*), 2.11 (3H x 2, *s*), 2.28 (3H, *s*), 2.32 (3H x 2, *s*), 2.41 (3H, *s*). (Found: C, 55.56 ; H, 4.56%. Calc. for $C_{37}H_{36}O_{20}$: C, 55.50 ; H, 4.53%.) To a soln of **5** (6.3 mg) in MeOH (1 ml), was added 2N HCl (0.5 ml) and the mixture was refluxed for 4 hr. The reaction mixture was diluted with H_2O. The resulting precipitate was recrystallized from MeOH to give quercetin **10** (0.8 mg), yellow needles, mp >300°; IR ν_{max}^{Nujol} cm^{-1}: 3350, 1660, 1620, 1560, 1520. The Aq. soln was neutralized with Amberlite IRA-45 (3g) to give a residue. Paper chromatography of the residue showed the presence of D-glucose (solvent system: EtOAc-pyridine-H_2O-HOAc, 5: 5: 3: 1) .

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