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THE FLAVONOID GLYCOSIDES OF THE LEAVES OF VIBURNUM FURCATUM BLUME

By
Tetsuo Iwagawa, Tomoaki Taguchi, Takahiro Maezono
and Tsunao Hase*

(Received Sep. 6, 1983)

Abstract
The flavonoid components of the leaves of Viburnum furcatum Blume were investigated. Two flavonoid glycosides were isolated, and identified as isoquercitrin (quercetin 3-O-β-D-glucoside) 1 and kaempferol 7-O-α-L-rhamnoside-3-O-β-D-glucoside 2 by spectral and chemical means.

Introduction
Viburnum furcatum Blume is a large shrub found in the mountains of Japan and its leaves are remarkably bitter. Previous investigation of the plant described that the structure of furcatin, non-bitter phenol glycoside, which had been proposed to be ß-vinylphenyl 6-O-apiosyl-(1 → 6)-β-D-glucoside was revised to ß-allylphenyl 4-O-β-D-apio-D-furanosyl-(1 → 6)-β-D-glucoside [1]. Very recently, three new bitter iridoid glycosides together with twelve known compounds have been isolated [Hase, T., Muanza-Nkongolo, D. and Iwagawa, T., unpublished results].

We have now examined the flavonoid glycosidic constituents of V. furcatum B.. The ethyl acetate soluble portion of the methanolic extract of the fresh leaves was fractionated by silica gel and polyamide column chromatographies to give compounds 1 and 2.

Results and Discussion
Compound 1 was crystallized as yellow prisms, mp 180-181⁰, with a molecular formula C₂₁H₂₆O₁₂ • 1/2 H₂O. It gave a reddish purple color on reduction with magnesium and hydrochloric acid and a positive Molish test. The UV spectrum had absorption maxima at 256 nm (ε 25000) and 357 nm (ε 20000). In addition, the UV spectra of 1 in methanol and methanol-sodium acetate were similar to those of rutin [2], which indicated that the glycosidic linkage in 1 was located at 3-position. The IR spectrum contained absorption bands of a hydroxyl group at 3200 cm⁻¹, a conjugated carbonyl at 1660 cm⁻¹ and a phenyl group at 1605, 1570 and 1510 cm⁻¹. Two doublets at δ 6.46 (1H, J = 2 Hz) and 6.52 (1H, J
\( = 2 \text{ Hz} \) in the \(^1\text{H} \) NMR spectrum were due to the protons at C-6 and C-8-positions, respectively. Signals at \( \delta \) 7.12 (1H, \( d, J = 8 \text{ Hz} \)), 7.88 (1H, \( dd, J = 2 \) and 8 Hz) and 8.29 (1H, \( d, J = 2 \text{ Hz} \)) were characteristic for a 3, 4-disubstituted B ring. These data suggested that compound 1 was isoquercetin 3-O-glycoside.

On acetylation with acetic anhydride-pyridine, compound 1 yielded an octa-acetate 3, mp 174-175\(^\circ\), \( \text{C}_{27}\text{H}_{36}\text{O}_{20} \). The \(^1\text{H} \) NMR spectrum of the acetate indicated the presence of four alcoholic acetoxyl groups at \( \delta \) 1.89-2.08 (3H x4, s) and four phenolic acetoxyl groups at \( \delta \) 2.34-2.47 (3H x4, s).

The IR and \(^1\text{H} \) NMR spectra of 1 and 3 were identical with those of isoquercitrin and its acetate, respectively [3]. Compound 1 was also isolated from \( V. \text{ urceolatum} \) Sieb. and Zucc [4].

Compound 2 was isolated as yellow needles, mp 260-261\(^\circ\) with a molecular formula \( \text{C}_{27}\text{H}_{30}\text{O}_{15} \cdot 1.5 \text{ H}_2\text{O} \). It gave a dark green color with ferric chloride solution and a red color with magnesium-hydrochloric acid. The IR spectrum showed absorption bands of a hydroxyl groups at 3300 cm\(^{-1}\), a conjugated carbonyl at 1660 cm\(^{-1}\) and a phenyl group at 1600 cm\(^{-1}\).

On acetylation with acetic anhydride-pyridine, compound 2 gave a nona-acetate 4, mp 136-137\(^\circ\), \( \text{C}_{45}\text{H}_{48}\text{O}_{24} \). The \(^1\text{H} \) NMR spectrum of the acetate indicated the presence of seven alcoholic acetoxyl groups at \( \delta \) 1.92-2.18 (3H x7, s) and two phenolic acetoxyl groups at \( \delta \) 2.32 and 2.44 (3H each, s). The signals corresponding to C-6 and C-8 protons appeared as an AB system at \( \delta \) 6.75 and 7.08 (\( J = 2 \text{ Hz} \)), respectively. An \( A_2B_2 \) system at \( \delta \) 7.21 and 8.02 (\( J = 10 \text{ Hz} \)) revealed the presence of a \( p \)-substituted phenyl group.

Hydrolysis of 2 with sulfuric acid gave kaempferol, mp 271-273\(^\circ\) whose IR and \(^1\text{H} \) NMR spectra were in accord with those of an authentic sample. D-Glucose and L-rhamnose as sugars were identified by paper chromatography.

The positions of the sugars were determined as followed. Methylation of 2 by the Purdie method followed by hydrolysis with Kiliani mixture gave a dimethylate 5, mp >300\(^\circ\), [M]\(^+\) at \( m/z \) 314. The \(^1\text{H} \) NMR spectrum of 5 showed signals due to two methoxyl groups at \( \delta \) 3.69 and 3.83 (3H each s), the latter resonance of which suggested that one of the methoxyl groups was located at C-5 position. In the UV spectrum of 5 the absorption maximum at 258 nm in band II suffered bathochromic shift of 7 nm with sodium acetate, indicating the presence of a free hydroxy group at C-7 position. The presence of the remaining methoxyl group at C'-4 position was suggested by bathochromic shift of 23 nm in band I (357 nm) with sodium methoxide. Thus, compound 5 must be 4', 5-di-O-methoxy-kaempferol [5]. The two glycoses therefore should be located at C-3 and C-7 positions.

Hydrolysis of 2 with partially deactivated Nariaginae [6] afforded astragalin (kaempferol 3-O-\( \beta \)-D-glucoside) which were established by co-paper chromatography [two solvent systems: \( n \)-BuOH-\( \text{OH} \)Ac-H\( _2\)O (4 : 1 : 5) and 15\% \( \text{OH} \)Ac]. The above results showed that compound 2 was kaempferol 7-O-L-rhamnoside-3-O-D-glucoside.

To establish the anomeric configurations of two sugars, compound 2 was persilylated...
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with hexamethyldisilazane and trimethylsilylchlorosilane to give a silyl ether 6. The α-linkage of the L-rhamnose and the β-linkage of the D-glucose were assigned from the presence of the anomeric protons at δ 5.35 (1H, br s) and 6.02 (1H, d, J = 7 Hz) in the 1H NMR spectrum of the silyl ether.

Therefore, compound 2 should be kaempferol 7- O-α- L-rhamnoside-3- O-β- D-glucoside [7]. This is the first example isolated from Viburnum species.

Experimental

Extraction and isolation. Plant material was collected in the northern highlands of Kagoshima prefecture and identified by Dr. S. Sako. The fresh leaves of V. furcatum B. (12.1 Kg) were extracted with MeOH (146 l x 2). The combined MeOH solns were concentrated to dryness to afford a dark green residue (1.068 Kg). The residue was diluted with H2O and extracted with Et2O. The aq. soln was extracted with EtOAc to give a residue (61 g). Part of the residue (28 g) was subjected to column chromatography on Si gel with CHCl3-MeOH (80 : 20) to yield a yellow residue (162 mg), which was chromatographed on a column of polyamide with MeOH-H2O (50 : 50) to give isoquercitrin 1 (20 mg), yellow prisms from MeOH, mp 180-181°, UV λmax nm (ε): 256 (25000), 357 (20000); λmax Ref. NAc nm: 267, 365; λmax Ref. NaOAc nm: 272, 400; IR νmax cm⁻¹: 3200, 1660, 1605, 1570, 1555, 1510; 1H NMR (CD3OD): δ 6.06 (1H, W 8 Hz), 6.46, 6.52 (1H each, d, J = 2 Hz), 7.12 (1H, d, J = 8 Hz), 7.88 (1H, dd, J = 2 and 8 Hz), 8.29 (1H, d, J = 2 Hz); Mg+HCl: (+); Molish test: (+). (Found: C, 53.46; C, 4.57%. Calc. for C21H20O12•1/2H2O: C, 53.27; H, 4.34%).

Elution with CHCl3-MeOH (70 : 30) gave a yellow residue (2.6 g). Column chromatography of the residue on polyamide with MeOH-H2O (50 : 50) gave kaempferol 7- O-α- L-rhamnoside-3- O-β- D-glucoside 2 (225 mg), yellow needles or prisms from MeOH-H2O, mp 260-261°, UV λmax nm (ε): 228 (sh, 19500), 249 (sh, 17400), 267 (26000), 350 (24000); λmax Ref. NAc nm: no shift; λmax Ref. NaOAc nm: 241, 273, 389; IR νmax cm⁻¹: 3300, 1660, 1600, 840, 810; 1H NMR (C6D6N): δ 1.62 (3H, s, 5Hz), 6.80, 6.97 (1H each, d, J = 9 Hz), 7.32, 8.52 (2H each, d, J = 9 Hz); FeCl3 test: (dark green); HCl+Mg: (+); Molish test: (+). (Found: C, 51.98; H, 5.10%. Calc. for C27H30O15•1.5 H2O: C, 52.17; H, 5.35%).

Acetylation of 1. Compound 1 was acetylated with acetic anhydride-pyridine to give
prisms 3 from EtOH, mp 174-175°, IR ν\text{max}\text{ cm}^{-1}: 1780, 1760, 1630, 1510; ^1H NMR (CDCl₃): δ
1.89, 1.97, 2.00, 2.08 (3H each, s), 2.34 (3H × 3, s), 2.47 (3H, s), 6.86, 7.32 (1H each, d, J = 2 Hz), 7.40, 8.10 (1H each, d, J = 7 Hz), 7.96 (1H, s). (Found: C, 55.29; H, 4.44%. Calc. for
C₃₇H₃₆O₂: C, 55.04; H, 4.46%.)

Acetylation of 2. Treatment of 2 (23 mg) with acetic anhydride-pyridine gave needles 4 (23 mg) from EtOH, mp 136-137°, IR ν\text{max}\text{ cm}^{-1}: 1765, 1630, 1505; ^1H NMR (CDCl₃): δ
1.23 (3H, d, J = 7 Hz), 1.92, 1.98, 2.00, 2.02, 2.04, 2.09, 2.18, 2.32, 2.44 (3H each, s), 6.75, 7.08 (1H each, d, J = 2 Hz), 7.21, 8.02 (2H each, d, J = 10 Hz). (Found: C, 55.10; H, 4.89%. Calc. for
C₄₅H₄₈O₄: C, 55.53; H, 4.98%.)

Hydrolysis of 2. To a soln of 1 (43 mg) in MeOH (2 ml) was added 2M H₂SO₄ (2 ml) and the mixture was refluxed at 5° for 4 hr. The reaction soln was diluted with H₂O. The resulting precipitate was recrystallized from MeOH to give needles (12 mg), mp 271-273°, IR ν\text{max}\text{ cm}^{-1}: 3300, 1660, 1610, 1560, 1500; ^1H NMR (C₅D₅N): δ 6.71, 6.77 (1H each, d, J = 2 Hz), 7.23, 8.47 (2H each, d, J = 9 Hz). The IR and ^1H NMR spectra were in good agreement with those of an authentic sample of kaemferol. The aq. soln was neutralized with excess of BaSO₄, the precipitate was filtered off, and the filtrate was evaporated to dryness in vacuo. The paper chromatography of the residue showed the presence of L-rhamnose and D-glucose (solvent system: EtOAc-pyridine-H₂O-HOAc, 5 : 5 : 3 : 1).

Methylation of 2. A soln of 2 (35 mg) in DMF (1 ml) was treated with Ag₂O (200 mg) and MeI (0.8 ml) and stirred at 5° for 4 days. After the usual work-up, the crude product was chromatographed on Si gel with CHCl₃ to give a permethylate (35 mg), IR ν\text{max}\text{ cm}^{-1}: 1630, 1610, 1515, 840; ^1H NMR (CDCl₃): δ 1.24 (3H, d, J = 6 Hz), 3.46, 3.55, 3.60, 3.83, 3.90, (3H × 9, s), 6.42, 6.80 (1H each, d, J = 2 Hz), 6.96, 8.17 (2H each, d, J = 10 Hz).

Hydrolysis of the permethylate. The permethylate (35 mg) was hydrolyzed with Kiliain mixture (1 ml) at 110° for 4 hr. The resulting precipitate was recrystallized from EtOH to give 4’, 5-di-O-methylkaempferol 5 (11 mg), mp > 300°, UV λ\text{max}\text{ nm (e): 258 (39900), 305 (22600), 357 (59300) ; λ\text{max}\text{NaOAc nm: 265, 301, 363 ; λ\text{max}\text{NaOMe nm: 273, 380 ; IR ν\text{max}\text{ cm}^{-1}: 3600-3000, 1650, 1610, 1560, 1500, 820 ; ^1H NMR (C₅D₅N): δ 3.69, 3.83 (3H each, s), 6.35, 6.87 (1H each, d, J = 2 Hz), 7.05, 8.41 (2H each, d, J = 8 Hz); MS m/z: 314 [M]+.

Enzymatic partial hydrolysis of 2. To a soln of 1 (50 mg) in H₂O (25 ml), pH to 6.7 by dilute alkali, was added partially deactivated Nariaginae soln [5] (5 ml). After the mixture had been left to stand for 5 days at 45°, the crude crystals were deposited. The identification of the aglycone as astragalin (kaempferol 3-O-β-D-glucosyl) was performed by co-paper chromatography [solvent systems: n-BuOH-HOAc-H₂O (4 : 1 : 5): Rf 0.86; 15% AcOH: Rf (0.42)].

Persilylation of 1. Me₃SiNHSiMe₃ (1 ml) and Me₃SiCl (1 ml) were added to a soln of 1 (42 mg) in pyridine (0.5 ml) and the mixture was stirred at room temp. for 10 min. Excess of the solvent and the reagents was removed in vacuo and the residue was extracted with CCl₄. Removal of the solvent gave a silylether 6, ^1H NMR (CCl₄): δ 1.29 (3H, m), 3.47-4.11 (10H, m), 5.35 (1H, br s), 6.02 (1H, d, J = 7 Hz), 6.49 6.88 (1H each, d, J = 2 Hz), 7.02, 8.15 (2H each, d, J = 9 Hz).
References