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THE FLAVONOID GLYCOSIDES OF THE LEAVES OF *VIBURNUM FURCATUM* BLUME

By

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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 *p*-vinylphenyl 6-*O*-apiosyl-(1 → 6)-*β*-D-glucoside was revised to *p*-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glycosides together with twelve known compounds have been isolated [Hase, T., Muanza-Nkongolo, D. and Iwagawa, T., unpublished results].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 compunds **1** and **2**.

Results and Discussion

Compound **1** was crystallized as yellow prisms, mp 180-181°, with a molecular formula $C_{21}H_{20}O_{12} \cdot 1/2\ H_2O$. 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 spctrum 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

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=2 Hz) in the ^{1}H NMR spectrum were due to the protons at C-6 and C-8-positions, respectively. Signals at δ 7.12 (1H, *d*, *J*=8 Hz), 7.88 (1H, *dd*, *J*=2 and 8 Hz) and 8.29 (1H, *d*, *J*=2 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°, $C_{37}H_{36}O_{20}$. The ^{1}H NMR spectrum of the acetate indicated the presence of four alcoholic acetoxyl groups at δ 1.89-2.08 (3H×4, *s*) and four phenolic acetoxyl groups at δ 2.34-2.47 (3H×4, *s*).

The IR and ^{1}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. urceolatum* Sieb. and Zucc [4].

Compound **2** was isolated as yellow needles, mp 260-261° with a molecular formula $C_{27}H_{30}O_{15} \cdot 1.5\ H_2O$. 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°, $C_{45}H_{48}O_{24}$. The ^{1}H NMR spectrum of the acetate indicated the presence of seven alcoholic acetoxyl groups at δ 1.92-2.18 (3H×7, *s*) and two phenolic acetoxyl groups at δ 2.32 and 2.44 (3H each, *s*). The signals corresponding to C-6 and C-8 protons appeared as an AB system at δ 6.75 and 7.08 (*J*=2 Hz), respectively. An A_2B_2 system at δ 7.21 and 8.02 (*J*=10 Hz) revealed the presence of a *p*-substituted phenyl group.

Hydrolysis of **2** with sulfuric acid gave kaempferol, mp 271-273° whose IR and ^{1}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°, $[M]^+$ at *m/z* 314. The ^{1}H NMR spectrum of **5** showed signals due to two methoxyl groups at δ 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 hydroxyl 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*-*β*-D-glucoside) which were established by co-paper chromatography [two solvent systems: *n*-BuOH-OHAc-H_2O (4 : 1 : 5) and 15% OHAc]. 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

with hexamethyldisilazane and trimethylsilylchlorosilane to give a silylether **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 ^{1}H NMR spectrum of the silylether.

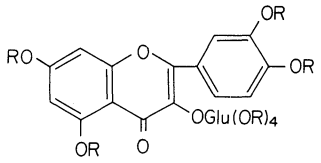
Therefore, compound **2** should be kaempferol 7-*O*-α-L-rhamnoside-3-*O*-β-D-glucoside [7]. This is the first example isolated from *Viburnum* species.

Experimental

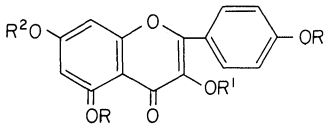
Extraction and isolation. Plant material was collected in the northern highlands of Kagoshima prefecture and identified by Dr. S. Sako. The fresh leaves of *V. furcatum* B. (12.1 Kg) were extracted with MeOH (146 $l\times2$). The combined MeOH solns were concentrated to dryness to afford a dark green residue (1.068 Kg). The residue was diluted with H_2O and extracted with Et_2O. 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 $CHCl_3$-MeOH (80 : 20) to yield a yellow residue (162 mg), which was chromatographed on a column of polyamid with MeOH-H_2O (50 : 50) to give isoquercitrin **1** (20 mg), yellow prisms froms MeOH, mp 180-181°, UV λ_{max}^{MeOH} nm (ε): 256 (25000), 357 (20000); $\lambda_{max}^{MeOH-NaOAc}$ nm: 267, 365; $\lambda_{max}^{MeOH-NaOMe}$ nm: 272, 400; IR ν_{max}^{nujol} cm^{-1}: 3200, 1660, 1605, 1570, 1555, 1510; ^{1}H NMR (C_5D_5N): δ 6.06(1H, $W_{1/2}$ 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 $C_{21}H_{20}O_{12}\cdot 1/2\ H_2O$: C, 53.27; H, 4.34%).

Elution with $CHCl_3$-MeOH (70 : 30) gave a yellow residue (2.6 g). Column chromatography of the residue on polyamide with MeOH-H_2O (50 : 50) gave kaempferol 7-*O*-α-L-rhamnoside-3-*O*-β-D-glucoside **2** (225 mg), yellow needles or prisms from MeOH-H_2O, mp 260-261°, UV λ_{max}^{MeOH} nm (ε): 228 (sh, 19500), 249 (sh, 17400), 267 (26000), 350 (24000); $\lambda_{max}^{MeOH-NaOAc}$ nm: no shift; $\lambda_{max}^{MeOH-NaOMe}$ nm: 241, 273, 389; IR ν_{max}^{nujol} cm^{-1}: 3300, 1660, 1600, 840, 810; ^{1}H NMR (C_5D_5N): δ 1.62 (3H, *d*, $J=5$ Hz), 6.80, 6.97 (1H each, *d*, $J=2$ Hz), 7.32, 8.52 (2H each, *d*, $J=9$ Hz); $FeCl_3$ test: (dark green); HCl+Mg: (+); Molish test: (+). (Found: C, 51.98; H, 5.10%. Calc. for $C_{27}H_{30}O_{15}\cdot 1.5\ H_2O$: C, 52.17; H, 5.35%.)

Acetylation of **1**. Compound **1** was acetylated with acetic anhydride-pyridine to give



1 R=H
3 R=Ac



2 R=H, R^1=Glu(OH)$_4$, R^2=Rham(OH)$_3$
4 R=Ac, R^1=Glu(OAc)$_4$, R^2=R=Rham(OAc)$_3$
5 R=Me, R^1=R^2=H
6 R=SiMe$_3$, R^1=Glu(OSiMe$_3$)$_4$, R^2=Rham(OSiMe$_3$)$_3$

prisms **3** from EtOH, mp 174-175°, IR ν_{max}^{nujol} cm^{-1} : 1780, 1760, 1630, 1510 ; ^1H NMR ($CDCl_3$) : 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 $C_{37}H_{36}O_{20}$: 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_{max}^{nujol} cm^{-1} : 1765, 1630, 1505 ; ^1H NMR ($CDCl_3$) : 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 $C_{45}H_{48}O_{24}$: C, 55.53 ; H, 4.98%.)

Hydrolysis of **2**. To a soln of **1** (43 mg) in MeOH (2 ml) was added 2M H_2SO_4 (2 ml) and the mixture was refluxed for 4 hr. The reaction soln was diluted with H_2O. The resulting precipitate was recrystallized from MeOH to give needles (12 mg), mp 271-273°, IR ν_{max}^{nujol} cm^{-1} : 3300, 1660, 1610, 1560, 1500 ; ^1H NMR (C_5D_5N) : 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 $BaSO_4$, 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-H_2O-HOAc, 5 : 5 : 3 : 1).

Methylation of **2**. A soln of **2** (35 mg) in DMF (1 ml) was treated with Ag_2O (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 $CHCl_3$ to give a permethylate (35 mg), IR ν_{max}^{film} cm^{-1} : 1630, 1610, 1515, 840 ; ^1H NMR ($CDCl_3$) : 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 Kiliani 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_{max}^{MeOH} nm (ε) : 258 (39900), 305 (22600), 357 (59300) ; $\lambda_{max}^{MeOH-NaOAc}$ nm : 265, 301, 363 ; $\lambda_{max}^{MeOH-NaOMe}$ nm : 273, 380 ; IR ν_{max}^{KBr} cm^{-1} : 3600-3000, 1650, 1610, 1560, 1500, 820 ; ^1H NMR (C_5D_5N) : 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 H_2O (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-glucosie) was performed by co-paper chromatography [solvent systems : n-BuOH-HOAc-H_2O (4 : 1 : 5) : *Rf* (0.86) ; 15% AcOH : *Rf* (0.42)].

Persilylation of **1**. $Me_3SiNHSiMe_3$ (1 ml) and Me_3SiCl (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 CCl_4. Removal of the solvent gave a silylether **6**, ^1H NMR (CCl_4) : 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).

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