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Chemical Constituents of the Leaves of *Staphylea bumalda*

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Abstract – Six compounds were isolated from the BuOH soluble fraction of the leaves of *Staphylea bumalda*. On the basis of spectral data, they were identified as atragalin (**1**), 2-methyl-5, 7-dihydroxy-chromone-7-*O*-β-D-glucopyranoside (**2**), isoquercitrin (**3**), nicotiflorin (**4**), kaempferol 3-neohesperidoside (**5**), kaempferol 3-*O*-[α-rhamno-pyranosyl-(1 → 4)-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside] (**6**), respectively.
Keywords – *Staphylea bumalda*, Staphyleaceae, chromone gylcoside, flavonoid glycosides

Introduction

Staphylea bumalda (Staphyleaceae) is widely distributed in Asia. The leaf and root of *Staphylea bumalda* have been used as anti-tussive and anti-inflammatory agents, respectively (Nanjing, 1999). We found one report on the chemical constituents of this plant (Morita *et al.*, 1968). To isolate other compounds, further work on the *n*-BuOH soluble fraction resulted in the isolation of a chromone glycoside and five flavonoid glycosides. These compounds were identified as atragalin (**1**), 2-methyl-5, 7-dihydroxychromone-7-*O*-β-D-glucopyranoside (**2**), isoquercitrin (**3**), nicotiflorin (**4**), kaempferol 3-neohesperidoside (**5**), kaempferol 3-*O*-[α-rhamnopyranosyl-(1→4)-rhamnopyranosyl-(1→6)-β-D-glucopyranoside] (**6**), respectively.

Materials and Methods

Instruments and reagents

Melting points were determined on a Fisher-Johns melting point apparatus and were uncorrected. NMR (Varian Gemini 200 and Bruker DPX 400) spectra (^{1}H-NMR taken at 200 and 400 MHz, and ^{13}C-NMR spectra taken at 100 and 50 MHz, respectively) were recorded in deuterated solvents using TMS as the internal standard. The FAB MS spectra were measured using an Autospec Micromass, UV spectra using a JASCO V-530 UV/Vis spectrophotometer, and IR spectra in a KBr disk using a Bio-Rad FTS-7. TLC work was carried out using plates coated with silica gel 60 F254 (Merck Co.). All solvents were routinely distilled prior to use. Silica gel and ODS column chromatography were performed on Merck silica gel 60 (70 – 230 mesh) and YMC gel (150 μm), respectively.

Plant materials

The leaves of *Staphylea bumalda* were collected at Mt. Samak in July 2003 and identified taxonomically with respect to morphology. A voucher specimen of the plant was deposited at the College of Pharmacy, Kangwon National University.

Extraction and isolation

The air-dried leaves (1.2 kg) were ground and extracted with hot MeOH three times, for 4 hours each time. The resultant extracts were combined and concentrated under reduced pressure to produce 270 g of the residue. This MeOH extract was suspended in 10 volumes of water and the partitioned successively with equal volume of *n*-hexane, $CHCl_3$ and *n*-BuOH, leaving a residual water soluble fraction. Each fraction was evaporated in vacuo to yield the residues of *n*-hexane fraction (fr.) (48 g), $CHCl_3$ fr., (8 g), and *n*-BuOH fr., (27 g).

The *n*-BuOH soluble fraction (27 g) was column chromatographed on a silica gel (300 g, 70 – 230 mesh, ψ 15 × 50 cm) using isocratic elution with $CHCl_3$-MeOH-Water (30 : 10 : 1), in order to divide the fraction into five sub-fractions (Fr. 1-Fr. 5).

Fr. 2 was re-chromatographed on silica gel column (120 g, 70 – 230 mesh, ψ 5 × 50 cm) to give compounds **1** (397.5 mg) and **2** (100 mg). Fr. 2 was re-chromatographed on ODS column (100 g, 150 μm, ψ 5 × 50 cm) by elution with MeOH-H_2O (50 : 50) to give compounds **3**

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(108.9 mg) and **4** (87.2 mg). Fr. 3 was re-chromatographed on silica gel column (150 g, 70 – 230 mesh, ψ 5 × 50 cm) by elution with $CHCl_3$-MeOH-Water (30 : 10 : 1) to give compounds **5** (1.4 g) and **6** (435.9 mg).

Compound 1 – Yellow powder, mp : 231~232°C, IR (KBr) ν_{max} 3400 (OH), 1680 (C=O), 1590, 1450 (C=C), 1070 (C-O) cm^{-1} : UV (MeOH) λ_{max} : 266, 348 nm; (NaOMe) 274, 321, 392 nm; (NaOAc) 274, 303, 370 nm; (NaOAc + H_3BO_3) 266, 346 nm; ($AlCl_3$) 233, 274, 306, 347, 399 nm; ($AlCl_3$ + HCl) 233, 274, 304, 350, 397 nm, ^{1}H-NMR (DMSO-d_6) : δ 12.70 (1H, s, 5-OH), 8.12 (2H, d, *J*=8.8 Hz, H-2', 6'), 6.96 (2H, d, *J*=8.8 Hz, H-3', 5'), 6.51 (1H, d, *J*=2.0 Hz, H-8), 6.28 (1H, d, *J*=2.0 Hz, H-6), 5.54 (1H, d, *J*=7.2 Hz, H-1"); ^{13}C-NMR (DMSO-d_6) : δ Table 1; FAM-MS : *m/z* 449 $[M+H]^+$

Compound 2 – White needles; mp : 235~236°C; IR (KBr) ν_{max} 3300 (OH), 1690 (C=O), 1550, 1460 (C=C), 1080 (C-O) cm^{-1} : UV (MeOH) λ_{max} : 228, 249, 255, 285, 313 nm; ^{1}H-NMR (DMSO-d_6) : δ 12.90 (1H, s, 5-OH), 6.72 (1H, d, *J*=2.2 Hz, H-8), 6.49 (1H, d, *J*=2.2 Hz, H-6), 6.33 (1H, s, H-3), 5.11 (1H, d, *J*=7.0 Hz, H-1"), 2.46 (3H, s, 2-methyl); ^{13}C-NMR (DMSO-d_6) : δ 182.11 (C-4), 168.49 (C-9), 162.99 (C-7), 161.24 (C-5), 157.53 (C-2), 108.38 (C-3), 105.11 (C-10), 99.99 (C-6), 99.54 (C-1'), 94.47 (C-8), 77.16 (C-3'), 76.34 (C-5'), 73.08 (C-2'), 69.58 (C-4'), 60.66 (C-6'), 20.13 (2-methyl); FAB-MS : *m/z* 355 $[M+H]^+$

Compound 3 – Yellow powder; mp : 225~226°C; IR (KBr) ν_{max} 3450 (OH), 1687 (C=O), 1560, 1460 (C=C), 1075 (C-O) cm^{-1} : UV (MeOH) λ_{max} : 257, 358 nm; (NaOMe) 272, 327, 408 nm; (NaOAc) 267, 365 nm; (NaOAc + H_3BO_3) 261, 378 nm; ($AlCl_3$) 275, 303 (sh), 342 (sh), 434 nm; ($AlCl_3$ + HCl) 270, 300, 361 (sh), 400 nm; ^{1}H-NMR (DMSO-d_6) : δ 12.71 (1H, s, 5-OH), 7.66 (2H, m, H-2', 6'), 6.92 (1H, d, *J*=8.8 Hz, H-5'), 6.49 (1H, d, *J*=1.8 Hz, H-8), 6.28 (1H, d, *J*=1.8 Hz, H-6), 5.53 (1H, d, *J*=7.4 Hz, H-1"); ^{13}C-NMR (DMSO-d_6) : δ Table 1; FAB-MS : *m/z* 465 $[M+H]^+$

Compound 4 – Yellow powder; mp : 183~184°C; IR (KBr) ν_{max} 3445 (OH), 1680 (C=O), 1550, 1465 (C=C), 1070 (C-O) cm^{-1} : UV (MeOH) λ_{max} : 266, 297 (sh), 348 nm; (NaOMe) 274, 326, 398 nm; (NaOAc) 268, 308 (sh), 354 nm; (NaOAc + H_3BO_3) 267, 306 (sh), 350 nm; ($AlCl_3$) 233 (sh), 274, 305, 353, 399 nm; ($AlCl_3$ + HCl) 232 (sh), 275, 303, 349, 397 nm; ^{1}H-NMR (DMSO-d_6) : 12.64 (1H, s, 5-OH), 8.06 (2H, d, *J*=8.8 Hz, H-2', 6'), 6.73 (2H, d, *J*=8.8 Hz, H-3', 5'), 6.48 (1H, d, *J*=1.8 Hz, H-8), 6.27 (1H, d, *J*=1.8 Hz, H-6), 5.39 (1H, d, *J*=7.0 Hz, H-1"), 4.45 (1H, s, H-1'''), 1.06 (3H, d, *J*=6.2 Hz, rhamnose methyl); ^{13}C-NMR (DMSO-d_6) : δ Table 1; FAB-MS : *m/z* 595 $[M+H]^+$

Table 1. ^{13}C-NMR data for compounds **1**, **3-6**

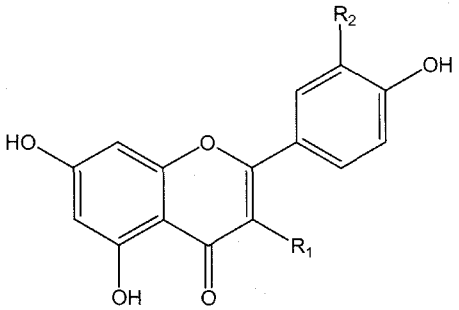
Carbon \ Compound	1	3	4	5	6
C-2	156.33	156.20	156.89	156.22	156.96
C-3	133.24	133.37	133.29	132.80	132.65
C-4	177.56	177.50	177.45	177.44	177.31
C-5	161.32	161.30	161.28	161.34	161.30
C-6	98.75	98.75	98.84	98.47	98.82
C-7	164.28	164.43	164.43	164.23	164.31
C-8	93.73	93.56	93.82	93.74	93.82
C-9	156.47	156.40	156.60	156.43	156.54
C-10	104.03	103.94	103.98	104.08	104.02
C-1'	120.97	121.57	120.97	121.00	121.02
C-2'	131.02	115.25	131.02	130.90	130.86
C-3'	115.14	144.91	115.10	115.10	115.10
C-4'	160.04	148.57	159.99	159.97	159.90
C-5'	115.14	116.32	115.10	115.10	115.10
C-6'	131.02	121.20	131.02	130.90	130.86
glucose					
C-1"	100.96	100.79	101.56	100.79	100.93
C-2"	74.24	74.06	74.21	77.33	75.61
C-3"	77.58	77.59	76.45	77.33	77.14
C-4"	69.93	69.96	69.98	70.66	71.87
C-5"	76.45	76.52	75.83	77.62	75.61
C-6"	60.86	60.92	66.94	60.86	66.93
rhamnose					
C-1			100.92	98.78	100.76
C-2			70.64	70.66	70.63
C-3			70.38	70.23	70.63
C-4			71.88	71.91	77.39
C-5			68.31	68.34	68.31
C-6			17.43	17.29	17.71
rhamnose					
C-1					100.52
C-2					70.63
C-3					70.37
C-4					71.87
C-5					68.31
C-6					17.32

Compound 5 – Yellow powder; mp : 176~177°C; IR (KBr) ν_{max} 3448 (OH), 1677 (C=O), 1545, 1485 (C=C), 1075 (C-O) cm^{-1} : UV (MeOH) λ_{max} : 280, 385 nm; (NaOMe) 280, 425 nm; (NaOAc) 280, 431 nm; (NaOAc + H_3BO_3) 287, 381 nm; ($AlCl_3$) 288, 417 nm; ($AlCl_3$ + HCl) 280, 390 nm; ^{1}H-NMR (DMSO-d_6) : δ 12.72 (1H, s, 5-OH), 8.12 (2H, d, *J*=9.0 Hz, H-2', 6'), 6.96 (2H, d, *J*

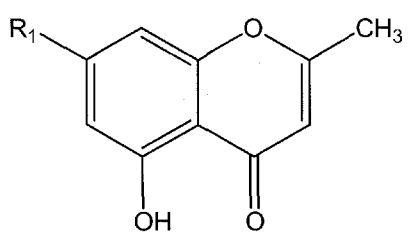
=9.0 Hz, H-3', 5'), 6.51 (1H, d, *J*=2.2H, H-8), 6.28 (1H, d, *J*=2.2 Hz, H-6) 5.74 (1H, d, *J*=7.0 Hz, H-1"), 5.16 (1H, s, H-1'''), 0.85 (3H, d, *J*=6.2 Hz, rhamnose methyl); ^{13}C-NMR (DMSO-d_6) : δ Table 1; FAB-MS : *m/z* 595 $[M+H]^+$

Compound 6 – Yellow powder; mp : 195~196°C; IR (KBr) ν_{max} 3340 (OH), 1680 (C=O), 1580, 1448 (C=C), 1080 (C-O) cm^{-1} : UV (MeOH) λ_{max} : 266, 349 nm; (NaOMe) 274, 325, 395 nm; (NaOAc) 267, 301(sh), 333 nm; (NaOAc + H_3BO_3) 265, 305, 347 nm; ($AlCl_3$) 232 (sh), 274, 304, 350, 398 nm; ($AlCl_3$ + HCl) 233 (sh), 275, 303, 347, 398 nm; ^{1}H-NMR (DMSO-d_6) : δ 12.72 (1H, s, 5-OH), 8.03 (2H, d, *J*=9.0 Hz, H-2', 6'), 6.96 (2H, d, *J* =9.0 Hz, H-3', 5'), 6.49 (1H, d, *J*=1.8 Hz, H-8), 6.28 (1H, d, *J*=1.8 Hz, H-6), 5.57 (1H, d, *J*=7.0 Hz, H-1"), 5.14 (1H, s, H-1'''), 4.40 (1H, s, H-1''''), 1.03 (3H, d, *J*=5.8 Hz, rhamnose methyl), 0.88 (3H, d, *J*=5.8 Hz, rhamnose methyl); ^{13}C-NMR (DMSO-d_6) : δ Table 1; FAB-MS : *m/z* 741 $[M+H]^+$

Acid hydrolysis of 1-6 – Each compound (5 mg) was refluxed with 5% H_2SO_4 (5 m*l*) in MeOH for 1h. The reaction mixture was then concentrated under reduced pressure to remove MeOH, diluted with H_2O and fractionated by EtOAc. Each EtOAc soluble fracton was concentrated and examined by tlc.



1. R_1 = *O*-Glucose, R_2 = H
3. R_1 = *O*-Glucose, R_2 = OH
4. R_1 = *O*-Rutinose, R_2 = H
5. R_1 = *O*-Neohesperidoside, R_2 = H
6. R_1 = *O*-α-rhamnosyl-(1→4)-rhamnosyl-(1→6)-β-glucopyranoside, R_2 = H



2. R_1 = *O*-Glucose

Each remaining aqueous layer was adjusted to pH 7 with $NaHCO_3$ and filtered. The filtrate was concentrated and examined by tlc.

As a result, **1** gave kaempferol and D-glucose, **2** gave norengenin and D-glucose, **3** gave quercetin and D-glucose, **4**, **5**, and **6** gave kaempferol, D-glucose, and L-rhamnose, respectively.

Results and Discussion

Compounds **1-3** were identified as astragalin (Agrawal, 1989; Lee *et al.*, 2004), 2-methyl-5, 7-dihydroxychromone-7-*O*-β-D-glucopyranoside (Gujral *et al.*, 1979; Tane *et al.*, 1990), isoquercitrin (Agrawal, 1989; Lee *et al.*, 2004), respectively, by comparison of their physical and spectral data with those of literature values.

Compound **4** was shown to be a kaempferol derivative from UV spectrum and the bathochromic shift resulting from the addition of NaOMe, NaOAc and $AlCl_3$ indicated the presence of free hydroxyl group at 4', 5, and 7 positions (Mabry *et al.*, 1970). The positive FAB-MS of **4** showed on ion $[M+H]^+$ at *m/z* 595. The ^{1}H-NMR spectrum of **4** exhibited signals due to the H-6 and H-8 position of flavonol ring at δ 6.27 (1H, d, *J*=1.8 Hz) and δ 6.48 (1H, d, *J*=1.8 Hz), respectively, H-2' and H-6' position at δ 8.06 (2H, d, *J*=8.8 Hz), H-3' and H-5' position at δ 6.73 (2H, d, *J*=8.8 Hz). The signals of anomeric protons of glucose and rhamnose revealed at δ 5.39 (d, *J*=7.0 Hz) and δ 4.45 (s), respectively. Acid hydrolysis of **4** afforded kaempferol, L-rhamnose and D-glucose identified by tlc analysis. In the ^{13}C-NMR spectrum, C-2 signal revealed at δ 156.89, which suggested that sugar moiety of **4** is attached to C-3 position of flavonol skeleton. The terminal sugar was determined as a rhamnose by lowfield chemical shift of glucose C-6 methylene at 66.94 (Agrawal, 1989). This was confirmed by the ^{1}H-^{1}H COSY, HMQC, and HMBC spectra.

Consequently, the structure of **4** was established as nicotiflorin [kaempferol-3-*O*-β-L-rhamnopyranosyl(1→6)-β-D-glucopyranoside]. The UV, IR, and NMR spectra of compound **5** was very similar to those of compound **4** except interglycosidic linkage points between two sugars. The interglycosidic linkage points were confirmed from its ^{1}H-^{1}H COSY, HMQC, and HMBC spectra as rhamnopyranosyl (1 → 2)-β-D-glucopyranoside.

Consequently, the structure of **5** was established as kaempferol-3-*O*-neohesperidoside [kaempferol-3-*O*-α-L-rhamnopyranosyl (1→2)-β-D-glucopyranoside] (Bacon *et al.*, 1975).

The positive FAB-MS of **6** showed on ion $[M+H]^+$ at

m/z 741. Acid hydrolysis of **6** afforded kaempferol, L-rhamnose and D-glucose identified by tlc analysis. The ^{1}H-NMR spectrum of **6** exhibited signals due to the H-6 and H-8 position of flavonol ring at δ 6.28 (1H, d, *J*=1.8 Hz) and δ 6.49 (1H, d, *J*=1.8 Hz), respectively, H-2' and H-6' position at δ 8.03 (2H, d, *J*=9.0 Hz), H-3' and H-5' position at δ 6.96 (2H, d, *J*=9.0 Hz). The ^{1}H-NMR spectrum also showed the presence of two rhamnoses and one glucose with rhamnose related signals at δ 5.14 (s, H-1'''), 4.40 (s, H-1''''), 1.03 (d, *J*=5.8 Hz, H-6'''), 0.88 (d, *J*=5.8 Hz, H-6''''), and β-glucose H-1'' at 5.57 (d, *J*=7.0 Hz). The chemical shift of C-2 revealed at δ 156.96 in ^{13}C-NMR spectrum showed that sugar moiety of **6** is attached to C-3 position of falvonol skeleton (Agrawal, 1989).

The glucose moiety was represented by the signals as in kaempferol 3-O-glucoside except that C-6 appeared 6.07 ppm downfield and C-5, 1.16 ppm upfield. Glycosylation of glucose by rhamnose at the 6-hydroxyl is thus evident. This was confirmed by ^{1}H-^{1}H COSY and HMBC spectra. The trisaccharide is therefore defined as a rhamnosylated rutinose. The second must be attached to the rhamnosyl portion of the rutinose. The signals of two rhamnosyl C-4 revealed at δ 71.87 and 77.39, respectively. This 5.52 ppm downfield shift is consistent with the additional rhamnose substituent being attached to the hydroxyl at C-4 of the rutinose rhamnose. This was confirmed by ^{1}H-^{1}H COSY, HMQC, and HMBC spectra. Based on these results and on values previously reported in the literature (Marbry *et al.*, 1970; Ararwal, 1989; Webby and Markham, 1990), compound **6** was identified as kaempferol 3-*O*-[α-rhamnosyl-(1→4)-rhamnosyl-(1→6)-β-D-glucopyranoside]. These compounds were isolated for the first time from this plant.

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