Flavonoid Constituents from *Spiraea brahuica*

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Sparins A–C (**1**–**3**, resp.), three new flavonoids, were isolated from the $CHCl_3$ subfraction of the EtOH extract of the whole plant of *Spiraea brahuica*, along with 3′,7-di-*O*-methylquercetin (**4**) and luteolin 7-*β*-D-glucopyranoside (**5**), reported for the first time from this species. The structures of the new compounds were elucidated by spectroscopic techniques including MS and 2D-NMR spectroscopy.

Introduction. – The genus *Spiraea* belongs to the family Rosaceae and comprises well over 90 species growing as shrubs in the temperate region of the Northern hemisphere and in Eastern Asia [1]. Various species of *Spiraea* are used as effective medicines for the treatment of inflammation and malaria [2]. In China, the fruits and roots of various *Spiraea* species are used as diuretic and detoxicant agents, and also for the treatment of cough, headache, and toothache [2][3]. One of the species of the genus *Spiraea* is *Spiraea brahuica* which is distributed in Asia and abundantly grows in the Ziarat Valley of the Balochistan Province of Pakistan. No phytochemical or pharmacological work has so far been carried out on this species. The chemotaxonomic and ethnopharmacological significance of the genus *Spiraea* prompted us to undertake phytochemical studies on *S. brahuica*. As a result, we isolated three new flavonoids named sparin A (**1**), sparin B (**2**), and sparin C (**3**), along with 3′,7-di-*O*-methyl quercetin (=rhamnazin; **4**), and luteolin 7-*β*-D-glucopyranoside (**5**), reported for the first time from this species (*Fig.*).

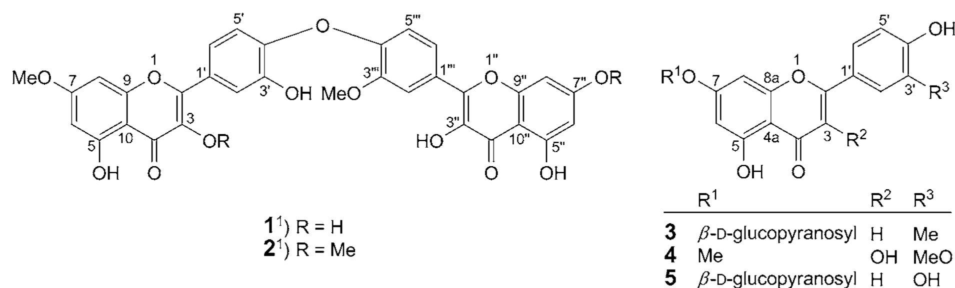

Figure. *Compounds* **1**–**5** *isolated from* Spiraea brahuica

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

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Results and Discussion. – The EtOH extract of the whole plant of *S. brahuica* BOISS. was suspended in H_2O and successively extracted with hexane, $CHCl_3$, AcOEt, and BuOH. The $CHCl_3$-soluble subfraction was subjected to a series of chromatographic separations to yield compounds **1**–**5**. All of these gave a blue-green color with ethanolic $FeCl_3$ solution for phenols and also a positive *Shinoda* test for flavonoids.

Sparin A (**1**) was obtained as yellow needles. The HR-FAB-MS of **1** showed a quasimolecular-ion peak $[M+H]^+$ at m/z 615.1130 consistent with the molecular formula $C_{32}H_{23}O_{13}$. The IR spectrum showed the presence of OH (3429 cm^{-1}), a conjugated CO (1668 cm^{-1}), a conjugated olefinic bond (1623 cm^{-1}), and aromatic moieties (1540, 1500 cm^{-1}). The UV spectrum exhibited the characteristic absorption maxima for a flavonoid at 214, 265, and 344 nm [4]. A bathochromic shift of 32 nm of band-II on addition of $AlCl_3$ indicated that position 5 of the flavonoid had a free ionizable phenolic OH group [5]. The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra showed 32 well resolved signals comprising two Me and ten CH groups, and 20 quaternary C-atoms (*Table 1*). The signals at δ(C) 177.4 and 177.3, 149.5 and 147.5, 138.2 and 137.9 and 105.3 and 104.4 were typical of C(4), C(2), C(3), and C(10) of two 3-O-bearing flavonoid moieties. The signals of ten O-bearing aromatic C-atoms were observed at δ(C) 137.9–165.6 (*Table 1*). The two MeO groups resonated at δ(C) 55.9 and 55.8. The ^{1}H-NMR spectrum showed two signals at δ(H) 13.6 and 14.1 revealing the presence of free OH groups at C(5) and C(5″), respectively. It further showed separate signals for two units of a monomethyl ether of quercetin including *meta*-coupled H-atoms at δ(H) 6.60 and 6.57 (each *d*, $J = 2.1$ Hz, 1 H) as well as δ(H) 6.86 and 6.75 (each *d*, $J = 1.8$ Hz, 1 H). The signals of a trisubstituted ring *B* were observed at δ(H) 8.62 (*d*, $J = 2.1$ Hz, 1 H), 8.12 (*dd*, $J = 8.4$, 2.1 Hz, 1 H,), and 7.43 (*d*, $J = 8.4$ Hz, 1 H). Another set of signals of a trisubstituted aromatic ring was observed at 8.28 (*d*, $J = 2.1$ Hz, H–C(2‴)), 8.18 (*dd*, $J = 8.4$, 2.1 Hz, H–C(6‴), and 7.35 (*d*, $J = 8.4$ Hz, H–C(5‴). Comparison of the ^{13}C-NMR spectra of **1** with those reported for quercetin (=2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one) showed downfield shifts of C(4′) (δ(C) 150.2) and C(4‴) (δ(C) 150.3), providing evidence for the combination of two quercetin units through dehydration of the phenolic groups at C(4′) and C(4‴). Both the MeO groups at δ(H) 3.86 and 3.75 (2*s*) showed ^3J correlations, with C(7) (δ(C) 165.4) and C(3‴) (δ(C) 148.1) confirming their presence at C(7) and C(3‴), respectively. The dimeric structure of sparin (**1**) was fully supported by its HMBC features (*Table 1*).

Sparin B (**2**) was obtained as yellow needles. The HR-FAB-MS of **2** showed a quasimolecular-ion peak $[M+H]^+$ at m/z 643.1446 consistent with the molecular formula $C_{34}H_{27}O_{13}$. The UV and IR spectra were similar to those of **1**. The ^{13}C-NMR spectra (broad band and DEPT; *Table 1*) showed 34 signals comprising four Me and ten CH groups, and 20 quaternary C-atoms. The spectrum was similar to that of **1**, except for the presence of four MeO groups at δ(C) 61.3, 55.9, 55.8, and 55.7. The ^{1}H-NMR spectrum also showed close resemblance to that of **1**, except for the presence of signals of four MeO groups at δ(H) 4.18 (*s*, 3 H), 3.86 (*s*, 3 H), and 3.75 (*s*, 6 H). Sparin B (**2**) is therefore the di-*O*-methyl ether of **1**. The presence of MeO groups at C(3) (δ(C) 141.3), C(7) (δ(C) 165.5), C(7″) (δ(C) 165.6), and C(3‴) (δ(C) 148.1) was confirmed by HMBC cross-peaks (*Table 1*). Further HMBC features were in complete agreement with the assigned structure of sparin B (**2**).

Table 1. *^{13}C- and ^{1}H-NMR Data* (125 and 500 MHz, resp.; C_5D_5N), *and Important HMBC Features of Compounds* **1** *and* **2**[1]). δ in ppm, J in Hz.

	1			**2**		
	δ(C)	δ(H)	HMBC	δ(C)	δ(H)	HMBC
	Rhamnetin			3,7-Di-O-methylquercetin		
C(2)	149.5		–	148.8	–	–
C(3)	137.9	–	–	141.3	–	–
C(4)	177.4	–	–	177.4	–	–
C(5)	161.8	–	–	161.8	–	–
H–C(6)	97.9	6.57 (*d*, *J* = 2.1)	C(5), C(7), C(8), C(10)	97.9	6.57 (*d*, *J* = 2.1)	C(5), C(7), C(8), C(10)
C(7)	165.4	–	–	165.5	–	–
H–C(8)	91.9	6.60 (*d*, *J* = 2.1)	C(6), C(7), C(9), C(10)	91.9	6.60 (*d*, *J* = 2.1)	C(6), C(7), C(9), C(10)
C(9)	156.9	–	–	156.9	–	–
C(10)	105.3	–	–	105.3	–	–
C(1′)	123.3	–	–	123.4	–	–
H–C(2′)	116.6	8.62 (*d*, *J* = 2.4)	C(1′), C(2), C(3′), C(4′), C(6′)	116.6	8.61 (*d*, *J* = 2.1)	C(1′), C(2), C(3′), C(4′), C(6′)
C(3′)	148.4	–	–	147.1	–	–
C(4′)	150.2	–	–	149.5	–	–
H–C(5′)	116.6	7.43 (*d*, *J* = 8.4)	C(1′), C(3′), C(4′), C(6′)	116.6	7.43 (*d*, *J* = 8.4)	C(1′), C(3′), C(4′), C(6′)
H–C(6′)	121.1	8.12 (*dd*, *J* = 8.4, 2.4)	C(1′), C(2), C(2′), C(4′), C(5′)	121.1	8.12 (*dd*, *J* = 8.4, 2.1)	C(1′), C(2), C(2′), C(4′), C(5′)
MeO–C(3)	–	–	–	61.3	4.18 (*s*)	C(3)
MeO–C(7)	55.8	3.75 (*s*)	C(7)	55.8	3.75 (*s*)	C(7)
	Isorhamnetin			Rhamnazin		
C(2″)	147.5	–	–	147.5	–	–
C(3″)	138.2	–	–	138.1	–	–
C(4″)	177.3	–	–	177.3	–	–
C(5″)	162.4	–	–	162.4	–	–
H–C(6″)	99.2	6.75 (*d*, *J* = 1.8)	C(5″), C(7″), C(8″), C(10″)	99.2	6.74 (*d*, *J* = 1.8)	C(5″), C(7″), C(8″), C(10″)
C(7″)	165.6	–	–	165.6	–	–
H–C(8″)	94.4	6.86 (*d*, *J* = 1.8)	C(6″), C(7″), C(9″), C(10″)	94.4	6.86 (*d*, *J* = 1.8)	C(6″), C(7″), C(9″), C(10″)
C(9″)	157.4	–	–	157.4	–	–
C(10″)	104.4	–	–	104.4	–	–
C(1‴)	123.8	–	–	123.3	–	–
H–C(2‴)	112.5	8.28 (*d*, *J* = 2.1)	C(1‴), C(2″), C(3‴), C(4‴), C(6‴)	112.5	8.28 (*d*, *J* = 2.1)	C(1‴), C(2″), C(3‴), C(4‴), C(6‴)
C(3‴)	148.1	–	–	148.1	–	–
C(4‴)	150.3	–	–	150.2	–	–
H–C(5‴)	116.6	7.35 (*d*, *J* = 8.4)	C(1‴), C(3‴), C(4‴), C(6‴)	116.6	7.36 (*d*, *J* = 8.4)	C(1‴), C(3‴), C(4‴), C(6‴)
H–C(6‴)	122.8	8.18 (*dd*, *J* = 8.4, 2.1)	C(1‴), C(2″), C(2‴), C(4‴), C(5‴)	122.8	8.17 (*dd*, *J* = 8.4, 2.1)	C(1‴), C(2″), C(2‴), C(4‴), C(5‴)
MeO–C(7″)	–	–	–	55.7	3.75 (*s*)	C(7″)
MeO–C(3‴)	55.9	3.86 (*s*)	(3‴)	55.9	3.86 (*s*)	C(3‴)

Sparin C (**3**) was obtained as yellow needles and gave a positive *Molish* test for a flavonoid glycoside. The HR-FAB-MS showed a quasimolecular-ion peak $[M+H]^+$ at *m/z* 447.1285 consistent with the molecular formula $C_{22}H_{23}O_{10}$. The IR spectrum was similar to that of **1**. The UV spectrum showed absorption maxima at 215, 263, and 342 nm. A bathochromic shift of 15 nm of band-II indicated the presence of a free ionizable phenolic OH group at position 5. The ^{13}C-NMR sprectra (broad band and DEPT; *Table 2*) showed 22 signals comprising one Me, one CH_2, and eleven CH groups, and nine quaternary C-atoms. The signals at δ(C) 162.5, 104.3, 182.9, and 105.3 were typical for a flavonoid moiety. The O-substituted aromatic C-atoms resonated at δ(C) 166.2, 165.1, 158.7, and 155.3, while an aromatic *C*-methyl was observed at δ(C) 16.8. The signal at δ(C) 98.8 could be assigned to the anomeric C-atom of a hexose moiety, while its other O-bearing C-atoms appeared in the range δ(C) 78.9 – 61.7. The ^{1}H-NMR spectrum (*Table 2*) showed *meta*-coupled H-atoms of ring *A* at δ(H) 6.98 and 6.83 (each *d*, $J=2.1$ Hz, 1 H). The signals of a disubstituted ring *B* were observed at δ(H) 7.88 (*d*, $J=2.1$ Hz, 1 H), 7.49 (*dd*, $J=8.4$, 2.1 Hz 1 H), and 7.26 (*d*, $J=8.4$ Hz, 1 H). The Me group resonated at δ(H) 2.48 (*s*). The signal of H–C(3) was observed at δ(H) 6.92 (*s*) and the anomeric H-atom at δ(H) 5.80 (*d*, $J=7.5$ Hz). The larger coupling constant allowed us to assign β-configuration to the hexose moiety. The CH–O H-atoms were observed at δ(H) 4.41 – 4.19, while the CH_2–O H-atoms appeared at δ(H) 4.55 and 4.42 – 4.45. Acid hydrolysis of sparin C (**3**) gave 3′-

Table 2. *^{13}C- and ^{1}H-NMR Data* (125 and 500 MHz, resp.; C_5D_5N), *and HMBC Features of Compound* **3**. δ in ppm, *J* in Hz.

	δ(C)	δ(H)	HMBC
C(2)	165.1	–	–
H–C(3)	104.3	6.92 (*s*)	C(2), C(4), C(5), C(1′)
C(4)	182.9	–	–
C(4^a)	105.3	–	–
C(5)	158.7	–	–
H–C(6)	99.1	6.83 (*d*, $J=2.1$)	C(4a), C(5), C(7), C(8)
C(7)	166.2	–	–
H–C(8)	94.6	6.98 (*d*, $J=2.1$)	C(4a), C(6), C(7), C(8a)
C(8a)	162.5	–	–
C(1′)	122.3	–	–
H–C(2′)	125.4	7.88 (*d*, $J=2.1$)	C(1′), C(2), C(3′), C(4′), C(6′)
C(3′)	124.2	–	–
C(4′)	155.3	–	–
H–C(5′)	115.4	7.26 (*d*, $J=8.4$)	C(1′), C(3′), C(4′), C(6′)
H–C(6′)	128.1	7.49 (*dd*, $J=8.4$, 2.1)	C(1′), C(2), C(2′), C(4′), C(5′)
H–C(1″)	98.8	5.80 (*d*, $J=7.5$)	C(7), C(2″), C(3″), C(5″)
H–C(2″)	78.1	4.34 – 4.35 (*m*)	–
H–C(3″)	74.5	4.36 – 4.38 (*m*)	–
H–C(4″)	70.1	4.19 – 4.22 (*m*)	–
H–C(5″)	78.9	4.39 – 4.41 (*m*)	–
CH_2(6″)	61.7	4.42 – 4.45 (*m*),	–
		4.55 (*dd*, $J=10.2$, 1.8)	C(5′), C(4′)
Me–C(3′)	16.8	2.48 (*s*)	C(3′)

methylapigenin and a glycone which could be identified as D-glucose by the sign of the optical rotation and comparison of the retention time of its Me_3Si ether with a standard in gas chromatography (GC). The anomeric H-atom at δ(H) 5.80 showed ^3J correlation with C(7) (δ(C) 166.2) allowing us to position the glucose moiety at C(7). The Me location could be assigned to C(3′) (δ(C) 124.2) based on ^2J and ^3J correlations (*Table 2*). Further HMBC features were in complete agreement with the assigned structure of sparin C (**3**) as 3′-methylapigenin 7-β-D-glucopyranoside.

The known compounds were identified as 3′,7-di-*O*-methylquercetin (**4**) [6] and luteolin 7-β-D-glucopyranoside (**5**) [7] by comparison of physical and spectral data with those reported in the literature.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 230–400 mesh; *E. Merck*, Darmstadt, Germany). Vacuum liquid chromatography (VLC): SiO_2 (230–400 mesh; *E. Merck*). TLC: SiO_2 *60* F_{254} plates (*E. Merck*). GC: *Schimadzu* gas chromatograph (*GC-9A*); 3% *OV-1*-silanized *Chromosorb W* column; column temp. 180°; injection port and detector temp. 275–300°; flow rate 35 ml/min; flame-ionization detector. M.p.: *Gallenkamp* apparatus; uncorrected. Optical rotation: *Jasco-P-2000* polarimeter. UV Spectra: *Hitachi-UV-3200* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Jasco-302-A* spectrometer; in KBr; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker-AMX-500* instrument; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. EI-MS: *Finnigan-MAT-312* mass spectrometer. HR-FAB-MS: *Jeol-JMS-HX-110* mass spectrometer; glycerol as matrix.

Plant Material. The whole plant *Spiraea brahuica* BOISS. (Rosaceae; 40 kg) was collected from the Ziarat Valley of Balochistan Province of Pakistan and identified by Prof. Dr. *Rasool Bakhsh Tareen*, plant taxonomist, Department of Botany, University of Balochistan, Quetta, where a voucher specimen has been deposited with the herbarium (Voucher specimen No. SB.RBT.08.BUH).

Extraction and Isolation. The shade-dried whole-plant material (40 kg) was extracted with EtOH (3 × 50 l, 10 d each) at r.t. The combined extract was concentrated at r.t. to yield a residue (700 g), which was suspended in H_2O (1.5 l) and successively extracted with hexane (80 g), $CHCl_3$ (100 g), AcOEt (55 g), and BuOH (40 g). The $CHCl_3$-soluble subfraction (50 g) was subjected to VLC (hexane, hexane/$CHCl_3$, $CHCl_3$, and $CHCl_3$/MeOH of increasing polarity); *Fractions A–J*. *Fr. E* (3 g; obtained with $CHCl_3$/MeOH 9:1) was subjected to CC (SiO_2, $CHCl_3$ and $CHCl_3$/MeOH of increasing polarity): *Frs. E.1–E.5*. *Fr. E.3* (40 mg; obtained with $CHCl_3$/MeOH 93:7) was a semi-pure compound which was resubjected to CC (SiO_2, $CHCl_3$/MeOH 93:7): **4** (35 mg). *Fr. E.4* (44 mg; obtained with $CHCl_3$/MeOH 88:12) was resubjected to CC (SiO_2, $CHCl_3$/MeOH 9:1) binary mixture of compounds (32 mg); final purification was achieved by prep. TLC ($CHCl_3$/MeOH 88:12) to furnish **1** (13 mg) and **2** (15 mg). *Fr. F* (35 mg, obtained with $CHCl_3$/MeOH 8.6:1.4) was a binary mixture CC (SiO_2, $CHCl_3$/MeOH 88:12 and 86:14) afforded **3** (13 mg) and **5** (17 mg), resp.

Sparin A (= *3,5-Dihydroxy-2-{3-hydroxy-4-[2-methoxy-4-(3,5,7-trihydroxy-4-oxo-4*H*-1-benzopyran-2-yl)phenoxy]phenyl}-7-methoxy-4*H*-1-benzopyran-4-one*; **1**): Yellow needles. M.p. 324–325°. IR (KBr): 3429, 1668, 1623, 1540, 1500. UV (MeOH): 344 (4.1), 265 (4.0), 214 (1.8). ^1H- and ^{13}C-NMR: *Table 1*. EI-MS: 316 (75, [*M* − 3‴-methoxy-5″,7″-dihydroxyflavonol]$^+$), 301 (6), 287 (16), 273 (18), 245 (15), 217 (9), 167 (9), 153 (20), 151 (19), 135 (100), 121 (19), 107 (21), 92 (25), 77 (35), 69 (33), 55 (25). HR-FAB-MS (pos.): 615.1130 ([*M* + H]$^+$, $C_{32}H_{23}O_{13}^+$; calc. 615.1138).

Sparin B (= *2-{4-[4-(3,5-Dihydroxy-7-methoxy-4-oxo-4*H*-1-benzopyran-2-yl)-2-methoxyphenoxy]-3-hydroxyphenyl}-5-hydroxy-3,7-dimethoxy-4*H*-1-benzopyran-4-one*; **2**): Yellow needles. M.p. 290–291°. IR (KBr): 3435, 1670, 1626, 1543, 1500. UV (MeOH): 345 (4.2), 264 (3.9), 214 (1.4). ^1H- and ^{13}C-NMR: *Table 1*. EI-MS: 330 (13, [*M* − 3‴,7″-dimethoxy-5″-hydroxyflavonol]$^+$), 316 (100), 301 (10), 287 (15), 273 (16), 245 (11), 217 (9), 167 (10), 153 (18), 151 (19), 135 (30), 121 (14), 109 (16), 95 (14), 77 (20), 69 (38), 55 (32). HR-FAB-MS (pos.): 643.1446 ([*M* + H]$^+$, $C_{34}H_{27}O_{13}^+$; calc. 643.1451).

Sparin C (= *3′-Methylapigenin 7-β-D-Glucopyranoside* = *7-β-D-Glucopyranosyloxy)-5-hydroxy-2-(4-hydroxy-3-methylphenyl)-4*H-*1-benzopyran-4-one*; **3**): Yellow needles. M.p. 254–255°. IR (KBr): 3425, 1667, 1629, 1538, 1500. UV (MeOH): 342 (4.1), 263 (3.9), 215 (1.3). ^{1}H- and ^{13}C-NMR: *Table 2*. EI-MS: 284 (100, [M – glucose]$^+$), 269 (11), 256 (10), 230 (9), 153 (33), 152 (24), 134 (13), 111 (15), 96 (8), 78 (12), 69 (10), 55 (7). HR-FAB-MS (pos.): 447.1285 ([M + H]$^+$, $C_{22}H_{23}O_{10}^+$; calc. 447.1291).

Acid Hydrolysis of **3**. Sparin C (**3**; 8 mg) in 10% HCl soln. was refluxed for 40 min. The cooled mixture was extracted with AcOEt. The residue recovered from the org. phase crystallized from MeOH (m.p. 136–138°) and was identified as 3′-methylapigenin by comparison of physical and spectral data with those reported in [8]. The aq. phase was concentrated, and the sugar was identified as D-glucose by the sign of its optical rotation ($[\alpha]_D = +52.8$) and co-TLC (BuOH/AcOEt/AcOH/H_2O 6:1:1:1, visualization with aniline phthalate reagent) with an authentic sample of D-glucose. The sugar was further confirmed by comparing the retention time of its Me_3Si ether with that of a standard sample in GC [9][10].

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