Flavonol Glycosides from *Paederia scandens* var. *mairei*

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Four kaempferol glycosides and five quercetin glycosides have been isolated from a methanolic extract of *Paederia scandens* var. *mairei* leaves and stems, in which in addition four unknown glycosides of kaempferol and quercetin are present in a trace. Nine flavonol glycosides including a new glycoside quercetin 3-O-rutinoside-7-O-xylosylglucoside (paederinin) were identified by PC, HPLC, UV spectral and NMR studies.

Introduction

Paederia scandens (Lour.) Merrill var. *mairei* (Léveillé) Hara belonging to Rubiaceae is well known to smell bad when the leaves was squeezed and also to contain iridoide glycoside such as paederoside and scandoside [1] and arubutin as phenolic glucoside [2]. In the present study, 13 glycosides of quercetin and kaempferol including a new glycoside were isolated from a methanolic extract of *Paederia* leaves and stems. Flavonoids in this plant are hereby reported for the first time.

Results and Discussion

A methanolic extract of *P. scandens* var. *mairei* leaves and stems was concentrated *in vacuo* and separated into Et_2O and EtOAc extracts and mother liquor. The Et_2O extract contained no flavonoid. The EtOAc extract and the mother liquor were fractionated by Polyamide C-200 CC and Polyclar AT CC followed by Sephadex LH-20 CC or G-15 CC and PC. As a consequence, 13 flavonol glycosides were isolated from the EtOAc extract and the mother liquor (Table I). The EtOAc extract gave 10 flavonol glycosides as follows: kaempferol 3-O-glucoside (astragalin) (**1**), 3-O-rutinoside (**2**), and 3-O-rutinoside-7-O-glucoside (**3**), and quercetin 3-O-glucoside (isoquercitrin) (**5**), 3-O-rutinoside (rutin) (**6**), and 3-O-rutinoside-7-O-glucoside (**7**), and also four unknown glycosides of kaempferol (**10**) and quercetin (**11, 12** and **13**). The mother liquor gave seven flavonol glycosides as follows: **3, 7,** kaempferol 7-O-glucoside (populnin) (**4**), and quercetin 7-O-glucoside (quercimeritrin) (**8**) and 3-O-rutinoside-7-O-xylosylglucoside (**9**) and two unknown glycosides of quercetin (**11** and **12**). The glycoside **9** has not previously been reported and provisionally named as paederinin. The separation of their flavonol glycosides was effected by HPLC as shown in Fig. 1. The t_R values of **1–9** were 24.30, 18.90, 5.22, 11.89, 16.07, 12.50, 4.65, 11.46 and 4.25. The main components of **2, 3, 5, 6, 7** and **9** were present in about the ratio 13:14:3:40:24:6. The UV spectra (band II) of **3, 4, 7, 8** and **9** on the addition of NaOAc showed almost no shift in contrast with those of **1, 2, 5** and **6** which showed a bathochromic shift of about 10 nm, due to the presence of a free C7 hydroxyl group. The spectral shifts (band I) of **1–3** and **4–9** on the addition of $AlCl_3$ and $AlCl_3$ + dilute HCl indicated that both groups have a free C5 hydroxyl group and in addition the latter group has

Table I. PC data for *Paederia* flavonoids.

Compound	UV	UV + NH_3	R_F values in I	II	III	IV
1	DP	LY	0.93	0.44	0.20	0.72
2	DP	LY	0.77	0.53	0.37	0.66
3	DP	LY	0.37	0.70	0.65	0.56
4	Y	LY	0.72	0.15	0.03	0.62
5	DP	Y	0.90	0.38	0.15	0.53
6	DP	Y	0.64	0.50	0.34	0.49
7	DP	Y	0.27	0.63	0.51	0.41
8	Y	Y	0.46	0.09	0.02	0.45
9	DP	Y	0.19	0.73	0.71	0.38
10	DP	LY	0.93	0.55	0.34	0.72
11	DP	Y	0.43	0.61	0.36	0.54
12	DP	Y	0.53	0.67	0.45	0.61
13	DP	Y	0.93	0.38	0.16	0.68

DP = dull purple, LY = lemon-yellow, Y = yellow.

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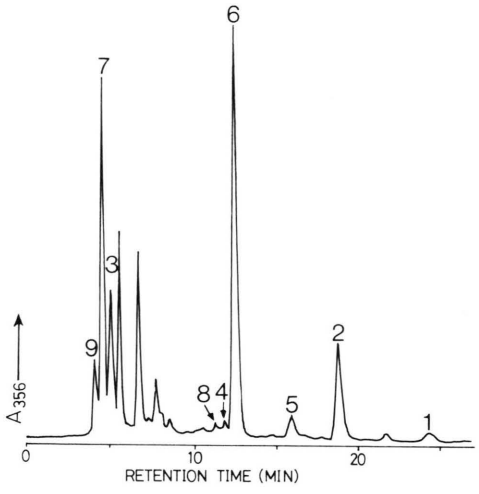

Fig. 1. HPLC separation of the flavonol glycosides of *P. scandens* var. *mairei* leaves and stems on a Finepak SIL C_{18}-5 column as described in the Experimental section. **1,** astragalin; **2,** kaempferol 3-O-rutinoside; **3,** kaempferol 3-O-rutinoside-7-O-glucoside; **4,** populnin; **5,** isoquercitrin; **6,** rutin; **7,** quercetin 3-O-rutinoside-7-O-glucoside; **8,** quercimeritrin; **9,** paederinin.

o-dihydroxyl group in the B ring. The crystals of **2, 3, 6** and **7** gave the analytical values and the NMR spectra just corresponding to those of the glycosides as expected.

It is noted that **3, 7** and a new glycoside **9** are found in *Paederia* leaves and stems. **3** and **7** have been hitherto reported only from *Equisetum palustre* [3] and *Nicotiana tabacum* [4], respectively. The presence of flavonol 3-O-rutinoside could be characteristic of the Rubiaceae including *Paederia* plant, because the 3-O-rutinosides of kaempferol and quercetin have been reported to be distributed in many plants of the Rubiaceae [5], and as well pelargonidin 3-O-rutinoside and cyanidin 3-O-rutinoside have been reported to distribute characteristically in Rubiaceous plants [6].

Experimental

Plant material and extraction

Fresh mature leaves and stems (6.7 kg) of *Paederia scandens* var. *mairei* were collected in the suburbs of Kumamoto from May to July. They were extracted thoroughly with MeOH (15 l) and concentrated *in vacuo* to *ca.* 3 l. The concentrate was extracted successively with petroleum ether (8 l), Et_2O (7 l) and EtOAc (7 l). The EtOAc extract and the mother liquor were used for isolation of flavonol glycosides.

Chromatography

The methanolic extract of *Paederia* leaves and stems was co-chromatographed with authentic samples on an HPLC column (Finepak SIL C_{18}-5, 4.6 × 150 mm) in a Tri Rotar-V HPLC (Japan Spectroscopic Co. Ltd.) using the isocratic solvent system $CH_3CN-H_2O-H_3PO_4$ (20:80:0.2) at a flow rate of 0.5 ml·min^{-1}. Flavonoids applied were quantified by measuring the A at 356 nm of the column effluent using a multi channel detector Multi-340. Likewise, sugars in the hydrolysate of **9** were co-chromatographed with authentic samples on an HPLC column (Finepak SIL NH_2-P and Finepak SIL NH_2-5, joined tandem) in a Tri Rotar-V HPLC using the isocratic solvent system CH_3CN-H_2O (85:15) at a flow rate of 1.5 ml·min^{-1}. The t_R values of glucose, xylose and rhamnose were 14.1, 8.2 and 6.7, respectively. The sugars applied were quantified by measuring the column effluent using a RID-300 detector. PC on Tôyô No. 51 C filter paper and TLC on Avicel SF cellulose plate (Funakoshi Pharmaceutical Co. Ltd., Tokyo) were carried out for separation of flavonol glycosides using the following solvent systems: I = *n*-BuOH−AcOH−H_2O (4:1:5), II = 15% AcOH, III = H_2O and IV = phenol−H_2O (4:1). 80% or 50% MeOH were used as the eluting solvent. CC was carried out using Polyclar AT, polyamide C-200 (Wako Chemical Co. Ltd., Tokyo) and Sephadex G-15 and LH-20.

Isolation of flavonol glycosides in the EtOAc extract

The EtOAc extract was evaporated to dryness. Yield was 12.33 g. This was applied on a polyamide C-200 column, which was previously equilibrated with H_2O, and eluted with H_2O (0.6 l), 30% MeOH (1 l), 50% MeOH (1 l) and 70% MeOH (1.5 l). The 70% MeOH eluate was separated into two fractions A and B-1 by a large scale PC using the solvent I. From the fraction A, **1, 5** and very small amount of **10** and **13** were separated by Avicel SF TLC using the solvent II, and from the B-1, **2** was separated as needles after passing through a Sephadex G-15 column which was previously

equilibrated with H_2O. The 50% and 30% MeOH eluates also were separated into two fractions of B-2 and C by a large scale PC using the solvent I. From the fraction B-2, needles of **2** were separated by the same way as above. Yield was 198 mg as a total of B-1 and B-2. The fraction C was dissolved in 50% EtOH, from which needles of **6** were separated. Yield was 220 mg. The aqueous eluate from a polyamide C-200 CC as above contained very small amount of **3, 7, 11** and **12,** which were separated by PC using the solvent I.

Isolation of flavonol glycosides in the mother liquor

The mother liquor obtained above was applied on a Polyclar AT column, which was previously equilibrated with H_2O, and eluted with H_2O and 70% MeOH. The aqueous eluate was then applied on a Sephadex G-15 column, which was previously equilibrated with H_2O, followed by a large scale PC using the solvent I. Thus, **3** (200 mg), **7** (1.0 g) and **9** (198 mg) were isolated in a powdery state, and also very small amounts of **11** and **12** obtained. The 70% MeOH eluate from a Polyclar AT CC was applied on a Sephadex LH-20 column, which was previously equilibrated with 70% MeOH, from which **4** and **8** were eluted with 70% MeOH. Small amounts of both glycosides were isolated respectively by Avicel SF TLC using the solvent II.

*Kaempferol O-glycosides (**1, 2, 3, 4** and **10**)*

1, 2, 3 and **4** were identified as the 3-O-glucoside (astragalin), the 3-O-rutinoside, the 3-O-rutinoside-7-O-glucoside and the 7-O-glucoside (populnin) of kaempferol, respectively, based on the agreement with PC (Table I), TLC, UV spectra and their spectral shifts with the chemicals such as NaOMe, $AlCl_3$, NaOAc and H_3BO_3 with those of authentic samples. Among those compounds, **2** obtained as the aqueous eluate from a Sephadex G-15 column was freeze-dried and dissolved in H_2O, from which **2** was crystallized as pale-yellow needles, m.p. 189–192 °C. Found: C, 54.24; H, 5.14%. Calcd. for $C_{27}H_{30}O_{15}$: C, 54.55; H, 5.09%. **3** (200 mg) separated by a large scale PC was crystallized as pale-yellow needles from MeOH after the treatment as ref. [3]. Yield was 60.2 mg. M.p. 238–241 °C. Found: C, 52.10; H, 5.24%. Calcd. for $C_{33}H_{40}O_{20}$: C, 52.38; H, 5.33%. The identity of **2** and **3** with kaempferol 3-O-rutinoside and kaempferol 3-O-rutinoside-7-O-glucoside was confirmed by ^1H and ^{13}C NMR. On complete acid hydrolysis, **1, 2, 3, 4,** and **10** gave kaempferol as aglycone. As the bound sugar, glucose was detected from the hydrolysates of **1** and **4,** and glucose and rhamnose from **2, 3,** and **10.** PC of aglycones and sugars as ref. [7]. **2** and **3** gave rutinose by H_2O_2-oxidative degradation, and on partial acid hydrolysis the former gave astragalin and the latter gave astragalin, kaempferol 3-O-rutinoside, populnin and kaempferol 3,7-O-diglucoside as the intermediates expected. The amount of **10** was too small for further detailed examination.

*Quercetin O-glycosides (**5, 6, 7, 8, 11, 12** and **13**)*

5, 6, 7 and **8** were identified as the 3-O-glucoside (isoquercitrin), the 3-O-rutinoside (rutin), the 3-O-rutinoside-7-O-glucoside and the 7-O-glucoside (quercimeritrin) of quercetin, respectively, based on the agreement with PC (Table I), TLC, UV spectra and their spectral shifts with the chemicals (as above) with those of authentic samples. Among these compounds, **6** was separated as needles, m.p. 193–194 °C. Found: C, 53.32; H, 5.28%. Calcd. for $C_{27}H_{30}O_{16}$; C, 53.12; H, 4.95%. **7** (1.0 g) eluted from PC was crystallized as yellow needles from MeOH after the treatment as ref. [3]. Yield was 300 mg. M.p. 205–208 °C. Found: C, 50.94; H, 5.24%. Calcd. for $C_{33}H_{40}O_{21}$: C, 51.30; H, 5.22%. The identity of **6** with rutin and **7** with quercetin 3-O-rutinoside-7-O-glucoside was confirmed by ^1H and ^{13}C NMR. On complete acid hydrolysis, **5, 6, 7, 8, 11, 12,** and **13** gave quercetin as aglycone. As the bound sugar, glucose was detected from the hydrolysates of **5** and **8,** and glucose and rhamnose from **6** and **7. 6** and **7** gave rutinose by H_2O_2-oxidative degradation and also the intermediates expected on partial acid hydrolysis. **11, 12,** and **13** were too small amount for further detailed examination.

*Quercetin 3-O-rutinoside-7-O-xylosylglucoside (paederinin) (**9**)*

For further purification, **9** (198 mg) separated from PC was placed again on a Sephadex G-15 column equilibrated with H_2O. The fractions containing **9** were pooled, freeze-dried and dissolved in hot EtOH, from which **9** was obtained as an

amorphous powder after evaporation. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.69 (1H, d, J = 8.06 Hz, H-6′), 7.63 (1H, s, H-2′), 6.93 (1H, d, J = 8.79 Hz, H-5′), 6.78 (1H, d, J = 1.37 Hz, H-6), 6.50 (1H, d, J = 1.37 Hz, H-8), 5.69 (1H, d, J = 6.59 Hz, −OCHO−), 5.53 (1H, br. s, OH), 5.20 (1H, br. s, OH), 5.13 (1H, d, J = 7.33 Hz, −OCHO−), 4.75 (1H, br. s, OH), 4.63 (1H, d, J = 7.32 Hz, −OCHO−), 4.42 (1H, s, −OCHO−), 3.78−3.06 (33H, m, aliphatic C−H and OH), 1.03 (3H, d, J = 5.87 Hz, $-CH_3$). ^{13}C NMR (100 Hz, DMSO-d_6) δ = 177.63 (1C, s, >C=O), 162.86 (1C, s, C-7), 161.023 (1C, d, J = 3.0 Hz, C-5), 156.53 (1C, s, C-2 or C-9), 156.03 (1C, d, J = 4.6 Hz, C-9 or C-2), 148.77 (1C, d, J = 6.6 Hz, C-4′), 144.99 (1C, s, C-3′), 133.31 (1C, s, C-3), 122.09 (1C, J = 170.9 Hz, C-6′), 121,13 (1C, d, J = 9.1 Hz, C-1′), 116.43 (1C, d, J = 160.2 Hz, C-5′), 115.38 (1C, d, J = 158.7 Hz, C-2′), 105.67 (1C, s, C-10), 104.54 (1C, d, (J = 140 Hz), −OCHO−), 100.58 (1C, d, J = 167.8 Hz, C-6 or −OCHO−), 100.0 (1C, d, J = 170.9 Hz, −OCHO− or C-6), 99.40 (1C, d, J = 163.3 Hz, −OCHO−), 98.23 (1C, d, J = 170.9 Hz, −OCHO−), 94.59 (1C, d, J = 172.4 Hz, C-8), 81.71 (1C, d, J = 148.0 Hz, >CHO−), 77.22 (1C, d, J = 138.9 Hz, >CHO−), 76.81 (1C, d, J = 142.0 Hz, >CHO−), 76.45 (1C, d, J = 141.9 Hz, >CHO−), 76.22 (1C, d, J = 143.4 Hz, >CHO−), 76.10 (1C, d, J = 143.4 Hz, >CHO−), 73.99 (1C, d, J = 138.8 Hz, >CHO−), 73.24 (1C, d, J = 143.4 Hz, >CHO−), 71.95 (1C, d, J = 141.9 Hz, >CHO−), 70.65 (1C, d, J = 138.3 Hz, >CHO−), 70.41 (1C, d, J = 145.0 Hz, >CHO−), 69.74 (1C, d, J = 144.5 Hz, >CHO−), 69.68 (1C, d, J = 144.5 Hz, >CHO−), 69.49 (1C, d, J = 140.4 Hz, >CHO−), 68.33 (1C, d, J = 135.8 Hz, >CHO−), 66.53 (1C, t, J = 140.3 Hz, $-CH_2O-$), 65.73 (1C, d, J = 143.5 Hz, $-CH_2O-$), 60.74 (1C, t, J = 140.4 Hz, $-CH_2O-$), 17.79 (1C, q, J = 125.1 Hz, $-CH_3$). On H_2O_2-oxidative degradation, **9** gave rutinose. On complete acid hydrolysis, **9** gave quercetin, glucose, rhamnose and xylose, of which the molar ratio was 1:2:1:1. The amount of quercetin was estimated spectrophotometrically at 373 nm, and the sugars were quantified by HPLC as described above. On partial acid hydrolysis, **9** gave the intermediate glycosides as expected. The UV spectrum of **9** and its spectral shifts with the chemicals as above agreed with those of quercetin having glycosidic linkage at 3 and 7 positions.

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