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Note

New flavonoid glycosides from *Aconitum naviculare* (Brühl) Stapf, a medicinal herb from the trans-Himalayan region of Nepal

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Abstract—Three new flavonoid glycosides, 3-*O*-[β-D-glucopyranosyl-(1→3)-(4-*O*-*trans*-*p*-coumaroyl)-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl]-7-*O*-[β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl]kaempferol, 3-*O*-[β-D-glucopyranosyl-(1→3)-(4-*O*-*trans*-*p*-coumaroyl)-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl]-7-*O*-[β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl]quercetin and 7-*O*-[β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl]quercetin were isolated from the aqueous extract of the aerial parts of *Aconitum naviculare*. Their structures were elucidated by spectral analysis (HRAPI-TOF MS, ^{1}H, ^{13}C NMR, HMQC, HMBC, DFQ-COSY, ROESY and TOCSY).

Keywords: *Aconitum naviculare*; Ranunculaceae; Manang; Flavonoid glycosides

Aconitum naviculare (Brühl) Stapf (Ranunculaceae), commonly called Ponkar, is a biennial medicinal herb of alpine grassland [>4000 m above sea level (asl)] found in the trans-Himalayan region of Nepal (Manang, Mustang and Dolpa districts). The whole plant is used by mountain people against colds, fevers and headaches (Shrestha, personal observation), and in Tibetan folk medicine, it is also used as a sedative, analgesic and febrifuge.[1] According to the Manangi people's tradition, *A. naviculare* is the most effective of the various medicinal plants used. Although the whole plant is medicinally important, the Manangis collects only the aerial parts (stem, leaves and flowers) during the flowering season (September–October). About 1 g of air-dried plant material is boiled with two glasses of water for about 30 min, and the bitter concoction is drunk twice a day. Although the Manangis living in and outside Manang use *A. naviculare* extensively, it is not marketed. Due to its high effectiveness in traditional medicine, it may be a potential source of income for mountain people and a source of important natural compounds for pharmaceutical companies. Although the chemical constituents of plants of the genus *Aconitum* have been extensively studied,[2,3] there is only one paper regarding alkaloids[1] available, and there are no phytochemical studies reported on *A. naviculare*. The present study describes the isolation and characterization of three new flavonoid glycosides from the aerial parts of *A. naviculare*.

Three new flavonoid glycosides were identified from the aqueous extract obtained by partition with organic solvents of crude methanol extract (Fig. 1).

Compound **1**. The HRAPI-TOF MS spectrum showed a negative ion $[M-H]^-$ at *m/z* 1209.3555, indicating a molecular formula of $C_{54}H_{66}O_{31}$. The ^{1}H NMR spectrum showed the typical signal pattern for a kaempferol derivative (Table 1). A pair of doublets (δ 7.61, 6.31) with a coupling constant of 15.95 Hz revealed the presence of a *trans* olefinic double bond. Two more *ortho*-coupled doublets at δ 7.54 and 6.89 (J 8.0 Hz),

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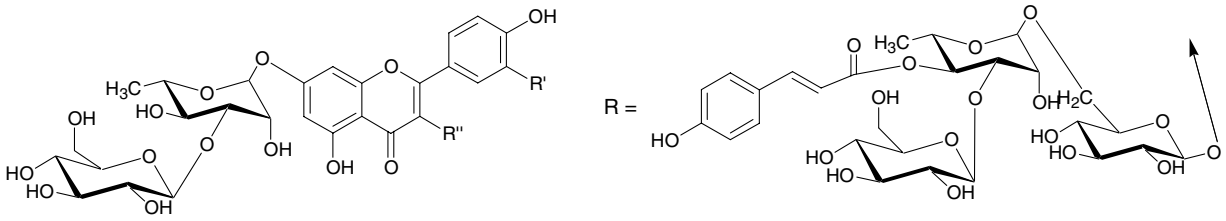

1: R' = H, R'' = R

2: R' = OH, R'' = R

3: R' = R'' = OH

Figure 1. Structure of flavonoid derivatives.

each integrating for two proton signals, showed a further *o*, *p*-disubstituted aromatic ring, indicating the presence of a coumaric acid residue. Complete structure assignments were achieved by HMQC, HMBC, DFQ-COSY, TOCSY and ROESY experiments and by comparison of spectral data previously published for similar compounds.[4–6] The anomeric signals of five sugar units were observed at δ 4.24 (J 8.0 Hz), 4.63 (J 0.8 Hz), 4.69 (J 7.8 Hz), 5.15 (J 7.5 Hz) and 5.59 (J 0.9 Hz). The HMQC, DQF-COSY and TOCSY data showed that sugar appeared as three β-D-glucopyranose units and two α-L-rhamnopyranose units. ROESY correlations were also observed between the anomeric proton signal of Rha^{I} (δ 5.59) and the doublets at δ 6.64 (H-8) and 6.50 (H-6). In addition, HMBC correlations were observed between H-1 of Rha^{I} and carbon resonance at δ 163.2 (C-7). These data revealed the linkage between Rha^{I} and the C-7 of the kaempferol unit. HMBC correlation was also observed between H-1 of Glc^{I} (δ 4.69) and the carbon resonance C-3 of Rha^{I} at δ 82.7, indicating that the sugar portion, which is linked to position 7 of the kaempferol moiety, was a β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl residue. The fact that a long-range correlation was observed between the proton signal at δ 5.15 (H-1 of Glc^{II}) and the carbon resonance at δ 135.3 (C-3), as well as the ROESY correlation between this signal at δ 5.15 and protons H-2′–6′ (δ 8.20), suggested that the second position of glycosidation is C-3 of the kaempferol unit. HMBC correlations between the H-1 of Rha^{II} (δ 4.63) and the C-6 of Glc^{II} (δ 68.8) and between the H-1 of Glc^{III} (δ 4.24) and the C-3 of Rha^{II} (δ 79.1) established that the residue was linked to position C-3 as a β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl unit. The HMBC correlation between the H-4 of Rha^{II} (δ 5.05) and the carbon resonance at δ 168.9 (C-9″) also revealed esterification of the coumaric acid at position 4 of this second rhamnose unit. Thus, **1** was identified as 3-*O*-[β-D-glucopyranosyl-(1→3)-(4-*O*-*trans*-*p*-coumaroyl)-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl]-7-*O*-[β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl]kaempferol.

Compound **2** was isolated as a yellow powder. The HRAPI-TOF MS spectrum showed a negative ion $[M-H]^-$ at m/z 1225.3515 corresponding to the molecular formula of $C_{54}H_{66}O_{32}$. The ^1H NMR spectrum showed the characteristic pattern of a glycosylated quercetin derivative. A pair of doublets (δ 7.59, 6.29) with a coupling constant of 15.5 Hz revealed a *trans* olefinic double bond. Two more *ortho*-coupled doublets at δ 7.50 (J 8.0 Hz) and 6.84 (J 8.9 Hz), each integrating for two proton signals, indicated a second *o*, *p*-disubstituted aromatic ring, suggesting a coumaric acid residue, as in **1**. Five sugar units were revealed by five signals of anomeric protons at δ 4.21 (J 7.6 Hz), 4.61 (J 0.9 Hz), 4.64 (J 0.8 Hz), 5.24 (J 8.0 Hz) and 5.51 (J 10.0 Hz), respectively. Exhaustive analysis of DQF-COSY, TOCSY and HMQC data determined sugar units as two β-D-glucopyranose and three α-L-rhamnopyranose units. The ROESY and HMBC data completed structure assignments, showing that compound **2** differed from **1** only due to the OH group in position 3′. This evidence led us to conclude that compound **2** was 3-*O*-[β-D-glucopyranosyl-(1→3)-(4-*O*-*trans*-*p*-coumaroyl)-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl]-7-*O*-[β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl]quercetin.

Compound **3** was isolated as a yellow powder. The HRAPI-TOF MS spectrum yielded a negative ion $[M-H]^-$ at m/z 609.1485, indicating a molecular formula of $C_{27}H_{30}O_{16}$. The ^1H NMR spectrum was consistent with the presence of a glycosylated quercetin derivative. Two proton signals were ascribable to anomeric protons at δ 5.58 (J 0.9 Hz) and 4.63 (J 8.0 Hz), respectively. Data obtained from DQF-COSY, NOESY and HMQC spectra established the sugars as one β-D-glucopyranose and one α-L-rhamnopyranose units. The glycosidation position was revealed both by the HMBC correlation between H-1 Rha (δ 5.58) and carbon resonance at δ 164.0 (C-7), and by the NOESY correlations between H-1 Rha and the doublet signals of H-6 (δ 6.46) and H-8 (δ 6.75) of the quercetin skeleton. Thus, compound **3** was identified as 7-*O*-[β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl]quercetin.

Table 1. ^{1}H and ^{13}C NMR data of compounds **1**–**3** in MeOD[a]

Position	**1**		**2**		**3**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	—	—	—	—	—	—
2	—	158.9	—	158.0	—	149.9
3	—	135.3	—	135.2	—	135.0
4	—	178.8	—	180.0	—	176.2
5	—	163.0	—	161.7	—	163.3
6	6.50 d (1.8)	100.8	6.43 d (1.5)	99.6	6.46 d (1.7)	100.3
7	—	163.2	—	162.4	—	164.0
8	6.64 d (1.8)	95.7	6.60 d (1.5)	94.2	6.75 d (1.7)	95.6
9	—	157.4	—	156.5	—	158.0
10	—	107.4	—	106.9	—	107.0
1′	—	121.8	—	133.8	—	124.6
2′	8.20 d (8.5)	132.7	7.89 d (1.6)	116.7	7.77 d (1.5)	116.2
3′	6.96 d (8.5)	116.3	—	144.6	—	148.0
4′	—	161.6	—	149.0	—	147.4
5′	6.96 d (8.5)	116.3	6.90 d (8.0)	115.7	6.89 d (7.8)	116.4
6′	8.20 d (8.5)	132.7	7.67 d (8.0; 1.6)	122.0	7.67 dd (7.8; 1.5)	122.4
RhaI (7)						
1	5.59 d (0.9)	99.7	5.51 d (1.0)	98.2	5.58 d (0.9)	99.9
2	4.37 m	71.0	4.30 m	69.6	4.31 m	71.2
3	3.99 m	82.7	3.93 m	80.9	3.95 m	83.3
4	3.67 m	71.0	3.60 m	71.0	3.66 m	71.8
5	3.71 m	71.3	3.65 m	69.0	3.70 m	71.5
6	1.31 d (6.0)	18.3	1.25 d (7.0)	18.1	1.28 d (6.0)	18.5
GlcI						
1	4.69 d (7.8)	105.7	4.64 d (8.0)	105.0	4.63 d (8.0)	106.4
2	3.41 m	75.5	3.36 m	77.1	3.28 m	75.2
3	3.50 t (9.0)	77.6	3.40 m	71.0	3.37 m	77.8
4	3.37 m	70.9	3.36 m	71.0	3.64 m	71.9
5	3.38 m	77.6	3.48 m	78.0	3.47 m	78.2
6	3.99–3.65 m	62.3	3.93 dd (12.0; 2.0)–3.72 dd (12.0; 5.0)	62.0	3.72–3.86 m	62.7
GlcII (3)						
1	5.15 d (7.5)	105.6	5.14 d (7.8)	105.8		
2	3.88 m	72.7	3.81 m	75.2		
3	3.69 m	75.0	3.69 m	73.7		
4	3.30 m	77.0	3.34 m	77.0		
5	3.20 m	77.5	3.61 m	71.0		
6	3.80–3.57 m	68.8	3.87 m–3.76 m	68.5		
RhaII						
1	4.63 d (0.8)	102.3	4.61 d (0.9)	101.9		
2	3.95 dd (8.8; 3.3)	71.7	3.95 m	71.8		
3	3.85 m	79.1	3.86 m	79.0		
4	5.05 dd (8.0; 8.2)	73.6	5.00 dd	72.6		
5	3.85 m	67.9	3.80 m	68.2		
6	0.98 d (6.5)	17.9	0.95 d (6.5)	18.0		
GlcIII						
1	4.24 d (8.0)	105.7	4.21 d (7.6)	105.2		
2	3.16 brt (9.0)	74.7	3.14 m	73.7		
3	3.31 m	77.5	3.26 m	77.2		
4	3.35 m	75.5	3.28 m	76.0		
5	3.26 m	71.0	3.30 m	71.0		
6	3.60 dd (11.2; 5.0)–3.80 dd (11.8; 2.0)	62.2	3.70–3.80 m	62.1		
Coumaric						
1	—	127.4	—	126.8		
2–6	7.54 d (8.0)	131.4	7.50 d (8.0)	129.8		
3–5	6.89 d (8.0)	117.1	6.84 d (8.9)	115.7		
4	—	161.3	—	161.1		
7	7.61 d (15.95)	147.0	7.59 d (15.5)	149.6		
8	6.31 d (15.95)	115.5	6.29 d (15.5)	114.1		
9	—	168.9	—	167.4		

[a] Assignments were confirmed by DFQ-COSY, HMQC and HMBC.

To our knowledge, **1**–**3** are reported here for the first time, as new, naturally occurring compounds from *A. naviculare*.

1. Experimental

1.1. General methods

Sephadex LH-20 was used for column chromatography. Silica gel plates were used for analytical TLC (E. Merck cat. 5715). Semipreparative HPLC was performed on a Gilson series 305 liquid chromatograph using a LiChrospher 100 RP-18 column (particle size 10 μm, 250 × 10 mm ID, E. Merck). The mobile phase was solvent A, 0.1% aq HCO_2H, and solvent B, AcCN. The flow rate was 6 mL/min. The separation gradient was from 75% to 45% of A in 25 min.

Analytical HPLC was performed on an Agilent 1100 series liquid chromatograph with a diode array UV–vis detector. A LiChrospher RP-18 column (particle size 5 μm, 250 × 4.6 mm ID) equipped with guard column was used. Chromatographic conditions were as follows: solvent A, 0.1% aq HCO_2H, solvent B, MeOH and solvent C, AcCN, in the following gradient elution.

Min	%A	%B	%C
0	80	10	10
10	50	25	25
30	50	50	0
35	50	50	0

NMR spectra in CD_3OD were obtained on a Bruker AMX-300 spectrometer operating at 300.13 MHz for ^1H NMR and 75.03 MHz for ^{13}C NMR. 2D experiments, ^1H–^1H DQF-COSY, ROESY, TOCSY and inverse-detected ^1H–^{13}C HMQC and HMBC spectra were performed using UXNMR software. Exact masses were measured on an API-TOF spectrometer (Mariner Biosystems). Samples were diluted in a mixture of 1:1 H_2O–AcCN with 0.1% NH_3 and directly injected at a flow rate of 10 μL/min.

1.2. Plant material

Aerial parts including stem, leaves, flowers and immature fruits were collected from Ladtar (4100 m asl, upper Manang) during the last week of September 2004. A voucher specimen was collected during a field survey and deposited at Tribhuvan University Central Herbarium (TUCH); identification was confirmed by Professor Ram Prasad Chaudhary of the Central Department of Botany, Tribhuvan University, Kathmandu.

1.3. Extraction and isolation

Fresh plant material was spread in a shaded, windy environment for 2 days, and was then packed in thin cotton bags and hung in the shade until it was dry. Dry material was then ground into pieces.

Air-dried powdered aerial parts (100 g) were exhaustively extracted in a Soxhlet apparatus with MeOH. The solvent was evaporated under reduced pressure (230–250 mbar) and a semisolid MeOH extract was obtained (8 g). The MeOH extract (about 5.0 g) was suspended in a mixture of 9:1 H_2O–MeOH (200 mL) and the pH was adjusted to pH 2 using 5% aq HCl. The solution was then partitioned first with $CHCl_3$ (5 × 50 mL) and then with EtOAc (5 × 50 mL). Solvents were removed under vacuum obtaining the $CHCl_3$ fraction CL-I (850 mg) and EtOAc fraction EA-I (330 mg). The pH of the residual aqueous layer was then adjusted to pH 8 using diluted NH_3. The aq layer was partitioned with $CHCl_3$ (5 × 50 mL) and EtOAc (5 × 50 mL). Solvents were removed under vacuum yielding the $CHCl_3$ fraction CL-II (250 mg) and EtOAc fraction EA-II (133 mg). The pH of the aqueous layer was then adjusted to pH 7.0, and the solvent was removed by freeze-drying, giving fraction AQ (3.3 g).

Fraction AQ was applied to a Sephadex LH-20 column (300 mL) and eluted with MeOH. The eluted fractions were analyzed by TLC and pooled on the basis of their chromatographic behaviour into eight groups (1–8). Further chromatographic steps on TLC (10:5:1 $CHCl_3$–MeOH–H_2O) and semipreparative HPLC yielded new compounds **1** (6.2 mg), **2** (7.8 mg) and **3** (4.8 mg).

The purity of the isolated compounds was checked by HPLC and found to be 97%. Retention times (t_R) were as follows: **1** 15.3 min, **2** 14.0 min, **3** 17.5 min.

1.4. 3-*O*-[β-D-Glucopyranosyl-(1→3)-(4-*O*-*trans*-*p*-coumaroyl)-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl]-7-*O*-[β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl]kaempferol (1)

Yellow amorphous powder; HRAPI-TOF MS: *m*/*z* 1209.3555 (calcd *m*/*z* 1209.3510 for $C_{54}H_{66}O_{31} - H$). ^1H and ^{13}C NMR data, see Table 1.

1.5. 3-*O*-[β-D-Glucopyranosyl-(1→3)-(4-*O*-*trans*-*p*-coumaroyl)-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl]-7-*O*-[β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl]quercetin (2)

Yellow amorphous powder; HRAPI-TOF MS: *m*/*z* 1225.3515 (calcd *m*/*z* 1225.3459 for $C_{54}H_{66}O_{32} - H$). ^1H and ^{13}C NMR data, see Table 1.

1.6. 7-*O*-[β-D-Glucopyranosyl-(1→3)-α-L-rhamnopyranosyl]quercetin (3)

Yellow amorphous powder; HRAPI-TOF MS: *m*/*z* 609.1485 (calcd *m*/*z* 609.1456 for $C_{27}H_{30}O_{16} - H$). ^{1}H and ^{13}C NMR data, see Table 1.

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References

1. Gao, L.; Wei, X.; Yang, L. *J. Chem. Res.* **2004**, 307–308.
2. Pelletier, S. W.; Mody, N. V. In *The Alkaloids*; Manske, R. H. F., Rodorigo, R. G. A., Eds.; Academic Press: New York, 1981; Vol. 17, pp 1–103.
3. Pelletier, S. W.; Mody, N. V.; Joshi, B. S.; Schramm, L. C. In *Alkaloids, Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Wiley: New York, 1983; Vol. 2, pp 206–462.
4. Agrawal, P. K.; Bansal, M. C. *Carbon-13 NMR of Flavonoids*; Elsevier: New York, 1989; pp 283–363.
5. Fico, G.; Braca, A.; De Tommasi, N.; Tomè, F.; Morelli, I. *Phytochemistry* **2001**, *57*, 543–546.
6. Diaz, J. G.; Ruiz, J. G.; Dias, B. R.; Gavin Sazatornil, J. A.; Herz, W. *Biochem. Syst. Ecol.* **2005**, *33*, 201–205.