Flavonol Glycosides from the Flowers of *Aconitum paniculatum*

Gelsomina Fico,*,† Alessandra Braca,† Anna Rita Bilia,‡ Franca Tomè,§ and Ivano Morelli†

Dipartimento di Chimica Bioorganica e Biofarmacia, Università di Pisa, Via Bonanno, 33, 56126 Pisa, Italy, Dipartimento di Scienze Farmaceutiche, Università di Firenze, Via Gino Capponi 9, 50121, Firenze, Italy, and Dipartimento di Biologia, Università di Milano, Via Celoria, 26, 20133 Milano, Italy

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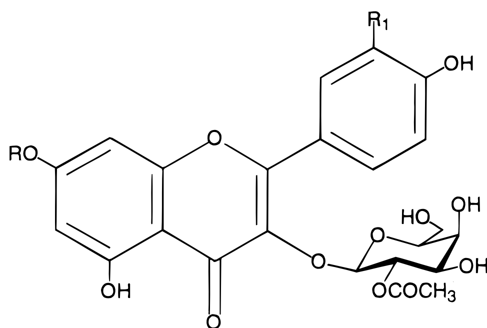
Three new acetylated flavonol glycosides—kaempferol 3-*O*-β-(2′′-acetyl)galactopyranoside (**1**), kaempferol 3-*O*-β-(2′′-acetyl)galactopyranoside-7-*O*-α-arabinopyranoside (**2**), and quercetin 3-*O*-β-(2′′-acetyl)galactopyranoside-7-*O*-α-arabinopyranoside (**3**)—were isolated from the flowers of *Aconitum paniculatum*. Their structures were elucidated by 1D and 2D NMR studies (^{1}H−^{1}H COSY, HSQC, HMBC) as well as by HPLC-MS.

Some *Aconitum* species (Ranunculaceae) are known to possess medicinal properties and are used as important remedies in Asian medicine.[1,2] The *Aconitum* genus is a source of diterpene alkaloids, and studies on such compounds have been extensively performed on Asian and European species. Preliminary investigations of roots and seeds of *A. paniculatum* showed the presence of the alkaloids talatisamine, paniculatine, and paniculamine.[3−5] Few studies about the other secondary metabolites, such as flavonoids, are present in the literature.[6−10] This prompted us to investigate flavonoid constituents of the flowers of *A. paniculatum* Lam., a plant common and widespread in the Italian Alps.[11] In this paper, we report the isolation and structure elucidation of three new acetylated flavonol glycosides, kaempferol 3-*O*-β-(2′′-acetyl)-galactopyranoside (**1**), kaempferol 3-*O*-β-(2′′-acetyl)galactopyranoside-7-*O*-α-arabinopyranoside (**2**), and quercetin 3-*O*-β-(2′′-acetyl)galactopyranoside-7-*O*-α-arabinopyranoside (**3**) from the polar extracts of the plant's flowers.



1 R = R$_1$ = H
2 R = ara, R$_1$ = H
3 R = ara, R$_1$ = OH

The dried flowers of *A. paniculatum* were successively extracted with *n*-hexane, $CHCl_3$, $CHCl_3$−MeOH (9:1), and MeOH. Compounds **1**−**3** were separated as pure components by Sephadex LH-20 column and reversed-phase HPLC from the $CHCl_3$−MeOH (9:1) and MeOH extracts.

Table 1. ^{13}C and ^{13}C DEPT NMR Spectral Data of Compounds **1**−**3** (CD_3OD, 200 MHz)

	1		**2**		**3**	
carbon	δ$_C$	DEPT	δ$_C$	DEPT	δ$_C$	DEPT
2	158.4	C	157.8	C	158.6	C
3	136.7	C	136.0	C	136.5	C
4	179.5	C	179.6	C	179.2	C
5	163.1	C	163.7	C	162.8	C
6	99.8	CH	99.9	CH	100.0	CH
7	165.8	C	164.3	C	164.6	C
8	94.7	CH	94.5	CH	94.6	CH
9	158.4	C	158.2	C	158.8	C
10	107.1	C	107.7	C	106.8	C
1′	122.8	C	122.6	C	123.0	C
2′	132.2	CH	132.0	CH	117.3	CH
3′	116.2	CH	116.4	CH	146.5	C
4′	161.5	C	161.0	C	148.8	C
5′	116.2	CH	116.4	CH	116.1	CH
6′	132.2	CH	132.0	CH	132.0	CH
3-*O*-gal-1′′	101.3	CH	101.4	CH	101.5	CH
2′′	73.1	CH	73.2	CH	73.0	CH
3′′	74.3	CH	74.0	CH	73.9	CH
4′′	70.3	CH	70.5	CH	69.9	CH
5′′	77.3	CH	77.0	CH	76.8	CH
6′′	62.0	CH_2	62.1	CH_2	62.2	CH_2
COO	172.6	C	172.4	C	172.0	C
CH_3	21.3	CH_3	21.2	CH_3	21.1	CH_3
7-*O*-ara-1′′′			100.6	CH	101.2	CH
2′′′			72.1	CH	72.0	CH
3′′′			74.5	CH	74.7	CH
4′′′			68.5	CH	68.9	CH
5′′′			66.3	CH_2	66.8	CH_2

Compound **1** was isolated as a yellow amorphous powder, with molecular formula $C_{23}H_{22}O_{12}$ deduced from the $[M + 1]^+$ peak at *m*/*z* 491 in the HPLC-MS and supported by ^{13}C and ^{13}C DEPT spectra. Its UV spectrum exhibited characteristic absorbance bands of flavonols at 258 and 353 nm. The ^{1}H and ^{13}C NMR spectra (Table 1) revealed signals of a flavonoid with glycosidic and acetyl groups. The ^{1}H NMR signals due to the aglycon [δ 6.17 (1H, d, $J = 1.8$ Hz, H-6), 6.35 (1H, d, $J = 1.8$ Hz, H-8), 6.88 (2H, d, $J = 8.8$ Hz, H-3′ and H-5′), 8.00 (2H, d, $J = 8.8$ Hz, H-2′ and H-6′)] showed the characteristic pattern of kaempferol derivatives, namely, a 2H AA′ and a 2H XX′ system. The ^{1}H NMR spectrum also revealed the presence of one glycosyl moiety, an anomeric proton at δ 5.44 (1H, d, $J = 7.8$ Hz), and one acetyl group, δ 2.13 (3H, s), which correlated in the HSQC spectrum, respectively, with signals at δ 101.3 and 21.3. The results of ^{1}H−^{1}H COSY and 2D HOHAHA experiments compared with those derived

* To whom correspondence should be addressed. Tel.: +39-050-44074. Fax: +39-050-43321. E-mail: fico@farm.unipi.it.
† Università di Pisa.
‡ Università di Firenze.
§ Università di Milano.

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from ^{13}C NMR spectrum allowed identification of the sugar moiety as β-galactopyranoside. The HPLC-MS peak at m/z 287 $[M - (203) + 1]^+$ suggested that the acetyl group was linked to the sugar unit. The lower field shift of H-2″ [δ 5.26 (1H, t)] and C-2″ (δ 73.1) signals indicated the bonding site of the acetyl group. Unequivocal information was obtained from the HMBC spectrum. The important correlations between the acetyl carbonyl group (δ 172.6) and the H-2″ signal and of C-3−H-1″ (δ 136.0 and 5.44) revealed the acetyl group to be at C-2″ and the β-galactopyranosyl moiety at C-3. These spectral data established **1** to be the new natural compound kaempferol 3-*O*-β-(2″-acetyl)galactopyranoside.

The molecular formula of compound **2** ($C_{28}H_{30}O_{16}$) was determined from its HPLC-MS, ^{13}C NMR (Table 1), and ^{13}C DEPT data. The UV spectrum (λ_{max} 267 and 348 nm) was very similar to **1**, but its ^1H NMR spectrum showed different sugar signals compared with **1**. The HPLC-MS spectrum of **2** revealed peaks at m/z 621 $[M - H]^+$, 489, 416, and 285 suggesting the presence of one more pentose moiety in respect to compound **1**. The nature of the pentose unit was easily determined as a α-arabinopyranoside from the chemical shifts, multiplicity of the signal, absolute values of the coupling constant, and the magnitude in the ^1H NMR and $^1H-^1H$ COSY spectra as well as ^{13}C NMR data. The HMBC spectrum indicated that C-3 and C-7 were the glycosylation sites. Connectivities were observed between δ 5.51 (1H, d, $J = 7.8$ Hz, H-1″) and 135.8 (C-3) and between δ 5.02 (1H, d, $J = 6.8$ Hz, H-1‴) and 164.3 (C-7). The linkage position of the acetyl group was confirmed to be H-2″ based on lower field shifts of the ^1H and ^{13}C NMR signals, respectively, at δ 5.29 (1H, t) and 73.2 and from the HMBC correlation H-2″−CO (δ 171.4). The structure of compound **2** was therefore characterized as kaempferol 3-*O*-β-(2″-acetyl)galactopyranoside-7-*O*-α-arabinopyranoside.

Compound **3** was determined to have a molecular formula of $C_{28}H_{30}O_{17}$. When **2** was used as a reference compound in the analysis of **3**, close similarities were observed between their sugar moieties, although the chemical shifts of the aglycons were different. The ^1H NMR aglycon signals δ 6.43 (1H, d, $J = 1.8$ Hz, H-6), 6.71 (1H, d, $J = 1.8$ Hz, H-8), 6.93 (1H, d, $J = 8.5$ Hz, H-5′), 7.57 (1H, dd, $J = 7.6$ and 1.8 Hz, H-6′), and 7.75 (1H, d, $J = 7.5$ Hz, H-2′) were consistent with a 3,7-glycosylated quercetin derivative. Thus, **3** was determined to be quercetin 3-*O*-β-(2″-acetyl)galactopyranoside-7-*O*-α-arabinopyranoside.

Experimental Section

General Experimental Procedures. Melting points (uncorrected) were determined with a Kofler apparatus. UV spectra were recorded in MeOH using a HP 1090L instrument with a diode array detector, in the range 200−590 nm. NMR spectroscopic measurements were performed on a Bruker Advance-600 (600 MHz) apparatus (^1H, $^1H-^1H$ COSY, HOHAHA, HSQC, HMBC) operating at 600.13 MHz and on AC-200 (200 MHz) instruments (^{13}C and ^{13}C DEPT) operating at 50.1 MHz. All the 1D and 2D NMR experiments were performed with an AV600 computer using a Win-NMR software package. Chemical shifts were expressed in δ (ppm) referring to the solvent peaks, δ_H 3.31 and δ_C 49.0 for CD_3OD. Optical rotations were measured on a Perkin-Elmer 241 polarimeter equipped with a sodium lamp (589 nm) and a 1-dm microcell. HPLC-MS (positive and negative mode) were obtained from a HP 1090L instrument with a diode array detector, managed by a HP 9000 workstation interfaced with a HP 1100 MSD API-electrospray. Column chromatography was performed over Sephadex LH-20 (Pharmacia); HPLC separations were conducted on a Shimadzu LC-8A series pumping system equipped with a Waters R401 refractive index detector and with a Waters μ-Bondapak C_{18} column and Shimadzu injector. TLC were obtained on silica 60 F_{254} gel-coated aluminum sheets. Spots were visualized by spraying and subsequent heating with a solution of $Ce(SO_4)_2/H_2SO_4$ and NTS−PEG.

Plant Material. The flowers of *A. paniculatum* were collected in Val di Rabbi, Trento, Italy, at 1200 m above sea level during the late summer of 1998, and were identified by Prof. F. Tomè of the Dipartimento di Biologia, University of Milano, where a voucher specimen (voucher no. Ap-101) has been deposited at its Herbarium.

Extraction and Isolation. The dried powdered flowers (60 g) of *A. paniculatum* were defatted with *n*-hexane and successively extracted with $CHCl_3$, $CHCl_3$−MeOH (9:1), and MeOH, each solvent for three times to give 0.8, 0.3, 3, and 6 g of residues, respectively. The $CHCl_3$−MeOH residue was chromatographed on Sephadex LH-20, using MeOH as eluent, to obtain 24 fractions of 10 mL. TLC similar fractions were combined together to give 13 groups (eluent: *n*-BuOH−CH_3-COOH−H_2O, 60:15:25). Crystallization of group 13 from MeOH afforded compound **1** (20 mg). The methanolic residue was chromatographed on Sephadex LH-20, using MeOH as eluent, to obtain 41 fractions of 12 mL, combined together into seven groups according to TLC separations (eluent: *n*-BuOH−CH_3COOH−H_2O, 60:15:25). Groups 4 and 5 were submitted to reversed-phase HPLC on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.5 mL min^{-1}) with MeOH−H_2O (40:60) to yield, respectively, **2** (t_R = 19 min, 29 mg) from the first and **3** (t_R = 15 min, 10 mg) from the last.

Kaempferol 3-*O*-β-(2″-acetyl)galactopyranoside (1): yellow powder; mp 158−162 °C; $[\alpha]^{25}_D$ −43.3° (*c* 0.1, MeOH); UV (MeOH) λ_{max} nm 258, 353; ^1H NMR (600 MHz, CD_3OD), δ 2.13 (3H, s, $COCH_3$), 3.52 (1H, dd, $J = 9.0$, 4.0 Hz, H-3″), 3.65 (1H, m, H-5″), 3.66 (1H, dd, $J = 12.0$, 2.0 Hz, H-6″a), 3.74 (1H, dd, $J = 4.0$, 2.5 Hz, H-4″), 3.86 (1H, dd, $J = 12.0$, 5.0 Hz, H-6″b), 5.24 (1H, dd, $J = 9.0$, 7.5 Hz, H-2″), 5.44 (1H, d, $J = 7.8$ Hz, H-1″), 6.17 (1H, d, $J = 1.8$ Hz, H-6), 6.35 (1H, d, $J = 1.8$ Hz, H-8), 6.88 (2H, d, $J = 8.7$ Hz, H-3′, 5′), 8.00 (2H, d, $J = 8.7$ Hz, H-2′, 6′); ^{13}C NMR (200 MHz, CD_3OD), Table 1; HPLC-MS m/z 491 $[M + H]^+$, 287 $[M - (\text{acetyl} + \text{gal}) + H]^+$; *anal.* C 56.30%, H 4.54%, O 39.16%, calcd for $C_{23}H_{22}O_{12}$, C 56.33%, H 4.52%, O 39.15%.

Kaempferol 3-*O*-β-(2″-acetyl)galactopyranoside-7-*O*-α-arabinopyranoside (2): yellow amorphous powder; mp 182−186 °C; $[\alpha]^{25}_D$ −34.4° (*c* 0.06, MeOH); UV (MeOH) λ_{max} nm 267, 348; ^1H NMR (600 MHz, CD_3OD) δ 2.14 (3H, s, $COCH_3$), 3.54 (1H, dd, $J = 9.0$, 4.0 Hz, H-3″), 3.60 (1H, m, H-5″), 3.63 (1H, dd, $J = 12.0$ and 4.0 Hz, H-5‴a), 3.69 (1H, dd, $J = 12.0$, 2.0 Hz, H-6″a), 3.75 (1H, dd, $J = 12.0$, 5.0 Hz, H-6″b), 3.79 (1H, dd, $J = 4.0$, 2.5 Hz, H-4″), 3.80 (1H, dd, $J = 9.0$, 2.5 Hz, H-3‴), 3.91 (1H, dd, $J = 9.0$, 6.8 Hz, H-2‴), 3.98 (1H, dd, $J = 12.0$, 2.0 Hz, H-5‴b), 4.03 (1H, m, H-4‴), 5.02 (1H, d, $J = 6.8$ Hz, H-1‴), 5.25 (1H, dd, $J = 9.0$, 7.5 Hz, H-2″), 5.52 (1H, d, $J = 7.8$ Hz, H-1″), 6.45 (1H, d, $J = 1.8$ Hz, H-6), 6.73 (1H, d, $J = 1.8$ Hz, H-8), 6.93 (2H, d, $J = 8.0$ Hz, H-3′, 5′), 8.12 (2H, d, $J = 8.0$ Hz, H-2′, 6′); ^{13}C NMR (200 MHz, CD_3OD), Table 1; HPLC-MS m/z 621 $[M - H]^+$, 489 $[M - \text{ara} - H]^+$, 416 $[M - (\text{acetyl} + \text{gal}) - H]^+$, 285 $[M - (\text{ara} + \text{acetyl} + \text{gal}) - H]^+$; *anal.* C 53.96%, H 4.90%, O 41.14%, calcd for $C_{28}H_{30}O_{16}$, C 54.02%, H 4.86%, O 41.12%.

Quercetin 3-*O*-β-(2″-acetyl)galactopyranoside-7-*O*-α-arabinopyranoside (3): yellow amorphous powder; mp 160−164 °C; $[\alpha]^{25}_D$ −17.3° (*c* 0.1, MeOH); UV (MeOH) λ_{max} nm 258, 354; ^1H NMR (600 MHz, CD_3OD) δ 2.20 (3H, s, $COCH_3$), 3.52 (1H, dd, $J = 9.0$, 4.0 Hz, H-3″), 3.60 (1H, m, H-5″), 3.65 (1H, dd, $J = 12.0$, 4.0 Hz, H-5‴a), 3.71 (1H, dd, $J = 12.0$, 2.0 Hz, H-6″a), 3.76 (1H, dd, $J = 12.0$, 5.0 Hz, H-6″b), 3.82 (1H, dd, $J = 4.0$, 2.5 Hz, H-4″), 3.83 (1H, dd, $J = 9.0$, 2.5 Hz, H-3‴), 3.90 (1H, dd, $J = 9.0$, 6.8 Hz, H-2‴), 4.00 (1H, dd, $J = 12.0$, 2.0 Hz, H-5‴b), 4.02 (1H, m, H-4‴), 5.04 (1H, d, $J = 6.8$ Hz, H-1‴), 5.28 (1H, dd, $J = 9.0$, 7.5 Hz, H-2″), 5.50 (1H, d, $J =$

7.8 Hz, H-1″), 6.43 (1H, d, $J = 1.8$ Hz, H-6), 6.71 (1H, d, $J = 1.8$ Hz, H-8), 6.93 (1H, d, $J = 7.6$ Hz, H-5′), 7.57 (1H, dd, $J = 7.6$, 1.8 Hz, H-6′), 7.75 (1H, d, $J = 7.6$ Hz, H-2′); ^{13}C NMR (200 MHz, CD_3OD), Table 1; HPLC-MS *m/z* 637 $[M - H]^+$, 505 $[M - ara - H]^+$, 432 $[M - (acetyl + gal) - H]^+$; *anal.* C 52.61%, H 4.78%, O 42.62%, calcd for $C_{28}H_{30}O_{17}$, C 52.67%, H 4.74%, O 42.60%.

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