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A new flavonol glycoside from *Catharanthus roseus*

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Abstract

A new flavonol glycoside was isolated together with two known flavonoids from the stems of *Catharanthus roseus* (Apocynaceae). Its structure was established as syringetin-3-*O*-robinobioside by means of UV, MS and NMR data, especially two dimensional experiments. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: *Catharanthus roseus*; Apocynaceae; Syringetin-3-*O*-robinobioside; Kaempferol-3-*O*-(2,6-di-*O*-α-L-rhamnopyranosyl-β-D-galactopyranoside); Quercetin-3-*O*-(2,6-di-*O*-α-L-rhamnopyranosyl-β-D-galactopyranoside)

1. Introduction

Catharanthus roseus (G. Don) is a herbaceous subshrub (Taylor & Farnsworth, 1975). This Madagascan periwinkle produces numerous indole alkaloids which have important therapeutic activities (Levêque, Wihlm, & Jehl, 1996). Only few phenolic compounds have been reported in this genus (Farnsworth, 1961; Daniel & Sabnis, 1978). Recently, Nishibe (Nishibe, Takenaka, Fujikawa, Yasukawa, Takido, Morimitsu et al., 1996) isolated and identified two flavonols trisaccharides of kaempferol (**1**) and quercetin (**2**) from leaves of *C. roseus*. We report here the isolation and structural determination of these two flavonoids from the stems of *C. roseus* beside a new glycoside of syringetin (**3**). This is the first report about isolation of syringetin in Apocynaceae.

2. Results and discussion

We have isolated from the stems and identified mauritianin or kaempferol-3-*O*-(2,6-di-*O*-α-L-rhamnopyranosyl-β-D-galactopyranoside) (**1**) (Yasukawa & Takido, 1987) and quercetin-3-*O*-(2,6-di-*O*-α-L-rhamnopyranosyl-β-D-galactopyranoside) (**2**) (Yasukawa, Sekine, & Takido, 1989). Their structures were assigned by comparison with literature.

Compound **3** was obtained as a yellow powder. The positive ion DCI(NH_3) mass spectrum exhibited a molecular ion peak at m/z 655 $[M + H]^+$. Other prominent peaks were observed at m/z 509 $[M\text{-}146 + H]^+$ and m/z 347 $[M\text{-}146\text{–}162 + H]^+$.

The UV spectrum of **3** in methanol showed two maxima at 252 (band II) and 357 (band I). A bathochromic shift of 69 nm was observed for band I after addition of NaOMe indicative of a free hydroxyl group in the 4′ position. The bathochromic shift of band II (22 nm) with NaOAc revealed the presence of a free hydroxyl group at C-7 in the A ring, whereas the absence of a shift after the addition of H_3BO_3 suggested that there was no free *ortho*-dihydroxyl group on ring B. This was confirmed by the bathochromic shift of band I (48 nm) with $AlCl_3$ which was unchanged on addition of HCl. From the UV spectral data, **3** was suggested to have a 5,7,4′-trihydroxy flavonol skeleton (Mabry, Markham, & Thomas, 1970).

The ^{1}H NMR spectrum (400 MHz, CD_3OD) indicated the presence of a methoxyl signal at δ 3.95 (6H, s, 3′-OMe and 5′-OMe), three aromatic proton signals at δ 6.23 (1H, d, H-6), 6.46 (1H, d, H-8) and 7.60 (2H, s, H-2′ and H-6′), which confirmed the syringetin 3-*O*-glycoside structure.

The ^{1}H NMR spectrum also supported the presence of one rhamnose and one galactose with the rhamnose

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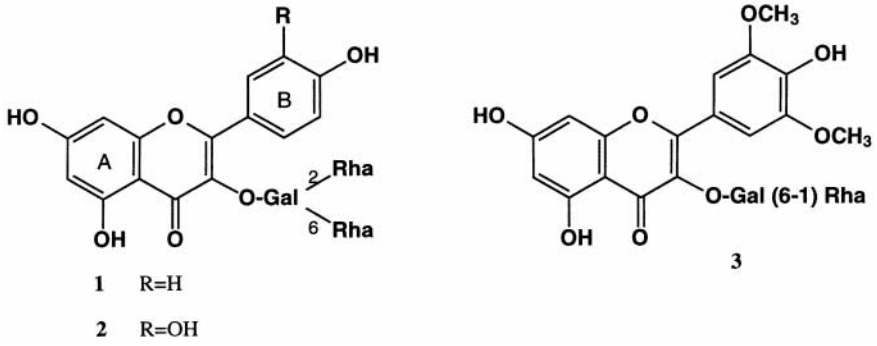

related signal at δ 4.56 (H-1, d, $J = 1.4$ Hz) and β-galactose H-1 at δ 5.42 (d, $J = 7.8$ Hz). The ^1H–^1H COSY experiment enabled the total assignment of the sugar protons. In the ^{13}C NMR spectral data, the C-6(gal) was downfield shifted at δ 67.4, indicating that glycosylation of the galactose unit by the rhamnopyranosyl took place on 6-hydroxyl. In the HMBC spectrum, a crosspeak between C-6(gal) and H-1(rha) established the linkage point between the two sugar moieties (see Table 1). In addition to this, a downfield shielding of C-2 indicated that position 3 was substituted by the glycosyl chain. This was confirmed by a crosspeak between H-1(gal) and C-3 of the aglycone.

Therefore **3** was characterized as syringetin-3-*O*-α-L-rhamnopyranosyl-(1-6)-β-D-galactopyranoside.

Table 1. ^{13}C and ^1H NMR assignments and ^1H–^{13}C long-range correlations of **3** by HMBC and HMQC in CD_3OD

Position	^{13}C	^1H	Cross-peaks in HMBC spectrum
Aglycone			
2	158.6		
3	135.6		
4	179.4		
5	163.1		
6	100.1	6.23 (d, 2.0)	95.1(8), 105.9(10), 163.1(5), 166.0(7)
7	166.0		
8	95.1	6.46 (d, 2.0)	100.1(6), 105.9(10), 158.5(9), 166.0(7)
9	158.5		
10	105.9		
1′	121.9		
2′/6′	108.4	7.60 (s)	108.4(6′/2′), 140.1(4′), 149.0(3′/5′), 158.6(2)
3′/5′	149.0		
4′	140.1		
MeO	57.5	3.95 (s)	149.0(3′), 149.0(5′)
Galactose			
1	104.3	5.42 (d, 7.8)	135.6(3)
2	73.2	3.79 (m)	104.3 (gal-1)
3	75.0	3.57 (dd, 9.7, 3.3)	
4	70.0	3.79 (br d, 2.5)	73.2 (gal-2), 75.7 (gal-5)
5	75.7	3.69 (br t, 6.1)	67.4 (gal-6)
6	67.4	3.77 (dd, 10.3, 3.6), 3.54 (dd, 10.3, 6.2)	75.7 (gal-5)
Rhamnose			
1	102.0	4.56 (d, 1.4)	67.4 (gal-6), 69.9 (rha-5), 72.4 (rha-3)
2	72.2	3.57 (m)	72.4 (rha-3), 73.9 (rha-4)
3	72.4	3.48 (dd,9.5, 3.4)	102.0 (rha-1)
4	73.9	3.25 (t, 9.4)	72.4 (rha-1), 69.9 (rha-5)
5	69.9	3.53 (m)	
6	18.4	1.17 (d, 6.2)	73.9 (rha-4), 69.9 (rha-5)

3. Experimental

3.1. General

^1H and ^{13}C NMR 400 and 100 MHz, respectively, using CD_3OD as solvent.

3.2. Plant material

C. roseus G. Don, cultivated plant, was collected in August 1997 in the southwest of France. A voucher specimen has been preserved in our laboratory.

3.3. Extraction and isolation

68 g of stems were ground to a fine powder then extracted successively with hexane (Soxhlet, 24 h) and with 50% aq. EtOH (reflux 3 h). The latter extract was concd in vacuo and submitted to a partitioning scheme (Van Wagenen et al., 1993). Briefly, the conc. aq. EtOH soln was partitioned successively with hexane and CH_2Cl_2. The aq. layer was concd in vacuo and further partitioned with EtOAc. The aq. layer was finally partitioned with BuOH. The BuOH extract (2.3 g) was subjected to MPLC on a RP-18® column using a linear gradient from H_2O to MeOH. The fraction containing **3** (52.6 mg), was finally purified by Visiprep® on RP-18® with a gradient of H_2O–MeOH to afford 12 mg of **3**.

3.4. Compound 3

Amorphous yellow powder; TLC (SiO_2 60): R_f 0.56 (AcOEt–HCO_2H–AcOH–H_2O, 100:11:11:26); Positive

DCIMS m/z 655 $[M + H]^+$, 509 $[M–Rha + H]^+$, 347 $[M–Rha–Gal + H]^+$; IR ν_{max}^{KBr} cm^{-1}: 3355, 1654, 1606, 1454; UV λ_{max}^{MeOH} nm: 252, 263 sh, 307 sh, 357; + NaOMe: 267, 328, 426; + NaOAc: 274, 312, 384; + NaOAc + H_3BO_3: 252, 263 sh, 307 sh, 360; + $AlCl_3$: 272, 308, 364, 405; + $AlCl_3$ + HCl: 273, 308, 364, 406.

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