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6-Hydroxyluteolin-7-*O*-β-D-[2-*O*-β-D-xylosylxyloside]: a novel flavone xyloxyloside from *Hebe stenophylla*

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Abstract

In the course of a chemotaxonomic study of New Zealand *Hebe* species, a novel flavone xylosylxyloside, 6-hydroxyluteolin-7-*O*-β-D-[2-*O*-β-D-xylosylxyloside] has been isolated and characterized from *Hebe stenophylla*. This compound and the accompanying 6-hydroxyluteolin-7-*O*-β-D-[2-*O*-β-D-xylosylglucoside] are the major and characteristic flavonoid components of *H. stenophylla*. Structure identification is based primarily on ^{1}H-, ^{13}C- and 2D-NMR evidence. This is the first recorded isolation of a flavone or flavonol glycoside containing a dixylosyl moiety.

Keywords: *Hebe stenophylla*; Scrophulariaceae; Flavonoid; 6-Hydroxyluteolin-7-*O*-β-D-[2-*O*-β-D-xylopyranosyl-xylopyranoside]; 6-Hydroxyluteolin-7-*O*-β-D-[2-*O*-β-D-xylopyranosyl-glucopyranoside]

1. Introduction

Hebe is New Zealand's largest genus of flowering plants with over 100 species. The most recent monograph (Allan, 1961) of this genus is almost 40 years old and now seriously out of date. For this reason a study of the distribution of flavonoids in this genus has been initiated as part of a multidisciplinary approach to better elucidate phylogenetic relationships and specific boundaries within the *Hebe* complex. Previous studies of the flavonoids in *Hebe* species are few, but include the finding of 6-hydroxyluteolin-7-glucoside and 7-glucuronide together with luteolin-7-glucuronide and chrysoeriol-7-glucuronide in *H. salicifolia* (Grayer, 1979), pectolinaringenin and sakuranetin in *H. cupressoides* (Perry & Foster, 1994), pectolinaringenin and hispidulin in *H. cupressoides* (Mitchell, 1993), and 6-hydroxyluteolin-7-xylosyl(1–2)glucoside and luteolin-4′-glucoside from *H. stricta* (Mitchell, 1993; Kellam, Mitchell, Blunt, Munro & Walker, 1993).

The research reported here details the structure elucidation of a novel flavone diglycoside, one of two major flavonoids that appear to be distinctive for *H. stenophylla*. A paper outlining the taxonomic status of *H. stenophylla*, including chemotaxonomic data, has been submitted to the New Zealand Journal of Botany.

2. Results and discussion

HPLC and 2D-PC screening of *H. stenophylla* and related species revealed that *H. stenophylla* is characterised by two major flavonoid glycosides, **1** and **2**. These were separated from other compounds by a combination of paper and column chromatography, monitored by HPLC. Both compounds gave the same absorption spectrum (λ_{max} 283, 345). Compound **1** proved to be chromatographically and spectrally (UV, NMR) identical with the 7-*O*-sambubioside of 6-hydroxyluteolin. This has been found only once previously, in *Hebe stricta* (Kellam, Mitchell, Blunt, Munro & Walker, 1993). The published NMR data

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were determined using pyridine-d_5, but are reported here again for comparison with data for **2** using DMSO-d_6 as solvent (see Table 1).

The absorption spectrum of **2** suggests that it is closely related to **1**. Indeed the ^{1}H-NMR spectrum exhibits aromatic proton resonances between δ 6.7 and 7.5 similar to those of **1** (Table 1) and therefore consistent with a 7-*O*-glycosylated 6-hydroxyluteolin formulation. One proton doublets at δ 5.18 and 4.56 with coupling constants of ca. 7 Hz, indicate the presence of two β-linked sugar units which were both shown to be xylose by acid hydrolysis followed by sugar analysis. The 6-hydroxyluteolin-*O*-dipentoside formulation was confirmed by electrospray low resolution MS which gave an $[M-H]^-$ peak at m/z 565.

A combination of ^{1}H-, ^{13}C-, ^{1}H, ^{1}H-COSY, ^{1}H, ^{13}C-TOCSY and HMBC NMR experiments led to the assignment of all ^{1}H- and ^{13}C-NMR signals for **1** and **2** as presented in Table 1. In particular, the three quaternary ^{13}C signals at 150.6, 149.8 and 148.8 ppm were assigned to C-7, C-4′ and C-9, respectively on the basis that (a) the H-5′ doublet centred at δ 6.90 exhibits long range H–C coupling to the 149.8 ppm signal, (b) H-1″ at δ 5.18 is long range coupled to the 150.6 ppm signal and (c) there are no proton signals long range coupled to the 148.8 ppm signal. The site of attachment of the first xylose to 6-hydroxyluteolin is thereby defined as the 7-hydroxyl group. That the terminal xylose is attached to the 2-hydroxyl of the first xylose is evidenced by the downfield shift of the C-2″ resonance to 82.1 ppm. This resonance was identified as that of C-2″ from its ^{1}H, ^{13}C-COSY detected cross correlation with H-2″ at δ 3.63, which in turn was recognised through its ^{1}H, ^{1}H-COSY cross correlation with H-1″ at δ 5.18. The proton and carbon spectra define both sugars as β-linked xylopyranosides (Markham, 1982). Accordingly, **2** is assigned the structure 6-hydroxyluteolin-7-*O*-β-D-[2-*O*-β-D-xylopyranosyl-xylopyranoside].

Compound **2** is not only novel, and therefore a potentially useful character for the identification of *H. stenophylla*, but it is also the first reported example of a flavone or flavonol glycoside containing a dixylosyl moiety (Williams & Harborne, 1994).

3. Experimental

3.1. Collection and preparation of plant material

Authentic *H. stenophylla* plant material was provided by the Museum of New Zealand. Samples were collected from wild populations, placed in press-sealed plastic bags with silica gel and couriered to Industrial Research. Upon arrival the plant material was oven dried at 100°C until crisp (30–60 min) and then ground using a mortar and pestle.

3.2. Extraction and isolation

Combined ground plant material (27.0 g) from several populations was extracted three times at ambient temperature in MeOH : H_2O, 4 : 1 (500 ml, overnight; 500 ml, 3 h; 200 ml, 3 h) with occasional shaking. Following each extraction the resultant solution was filtered through Whatman GF/C glass fiber paper, the filtrates combined, and reduced to a small volume (ca. 20 ml). To remove the high level of phenolic acids, the combined extract was applied to a microcrystalline cellulose (Merck) column (16 × 3 cm). The column was washed with H_2O : HOAc, 49 : 1 (3000 ml) and the flavonoids eluted with EtOH : H_2O, 4 : 1 (500 ml). The resultant solution was reduced in volume and applied to a polyamide (CC-6, Macherey-Nagel) column (5 × 51 cm). Flavonoids were chromatographed using a solvent gradient stepped from H_2O : HCOOH, 199 : 1 to MeOH : HCOOH, 199 : 1, fractions being analysed by HPLC. Combining the 80% and 90% MeOH fractions gave crude **1**. This fraction was reduced in volume and chromatographed by 1DPC. Elution of the major band with MeOH : H_2O, 4 : 1, reduction in volume and final clean-up on a Sephadex LH20 column (3 × 46 cm) using MeOH : H_2O : HCOOH, 60 : 39.5 : 0.5 as eluting solvent gave pure **1**. Compound **2** was purified in a similar manner by combining the 50% and the 60% MeOH fractions.

3.3. HPLC analysis

Fractions were analysed on an end capped RP-18 column (11.9 × 0.4 cm I.D.; 5 μm) using a Waters (Milford, MA, USA) 600E solvent controller, a Waters 996 photodiode array detector, a Jasco (Tokyo, Japan) 851-AS intelligent sampler and Millennium^{32} (Milford, MA, USA) version 3.05 software. Injection volume was 20 μl and elution was performed using a flow rate of 0.8 ml/min at 24°. The solvents, water adjusted to pH 2.5 with orthophosphoric acid (A) and acetonitrile (B), were mixed using a linear gradient starting with 100% A, decreasing to 91% over 12 min, to 87% over the next 8 min and to 67% over the next 10 min. After holding the solvent at this composition for 2 min, A was decreased to 57% over 10 min, and then held at this level until the end of the 60 min analysis. Spectral data for all peaks were accumulated in the range 220–400 nm, and chromatograms were plotted at 352 nm.

Table 1
NMR Data for **1** and **2** (DMSO-d_6; 300 MHz)

Position	6-Hydroxyluteolin-7-*O*-[2-xylosylglucoside] (**1**)		6-Hydroxyluteolin-7-*O*-[2-xylosylxyloside](**2**)	
	^{13}C (ppm)	^{1}H (δ)[a]	^{13}C (ppm)	^{1}H (δ)[a]
Aglycone				
2	164.4		164.3	
3	102.6	6.70s	102.4	6.70s
4	182.4		182.3	
5	146.3		146.4	
6	130.8		130.7	
7	150.9		150.6	
8	94.5	7.01s	94.2	6.91s
9	149.0		148.8	
10	106.2		106.2	
1′	121.6		121.5	
2′	113.5	7.41s[b]	113.4	7.43s[b]
3′	145.8		145.8	
4′	149.8		149.8	
5′	116.0	6.91d (J = 8.1 Hz)	116.0	6.90d (J = ca. 8 Hz)
6′	119.0	7.45d (J = 8.3 Hz)[b]	119.0	7.44d (J = 7.2 Hz)[b]
Glycosyl				
1″	99.8	5.16d (J = 7.2 Hz)	100.2	5.18 d (J = 6.8 Hz)
2″	82.6	3.59 m	82.1	3.63 m
3″	75.5	3.56 m	75.1	3.46 m
4″	69.2	3.27 m	68.8	3.48 m
5″	77.2	3.51 m	65.8	3.86d (J = 8.2 Hz); 3.43 m[b]
6″	60.6	3.78 d (J = 10.2 Hz); 3.53 m[b]	–	–
1‴	105.2	4.58d (J = 7.5 Hz)	105.1	4.56d (J = 7.5 Hz)
2‴	74.2	3.04 dd (8.1, 8.1)	74.1	3.04 m
3‴	76.0	3.16 m	76.0	3.16 m
4‴	69.4	3.27 m	69.3	3.27 m
5‴	65.8	3.59 m; 3.16 m	65.8	3.57 m; 3.12 m

[a] s: singlet; d: doublet; m: unresolved multiplet.
[b] Fine splitting not observed due to broadened peaks.

3.4. Paper chromatography

IDPCs were produced by applying ca. 2 ml of the crude extract to sheets of Whatman 3MM (46 × 57 cm). Development involved elution with *t*-butanol : acetic acid : water, 3 : 1 : 1 (TBA). Flavonoid bands were visible under UV (366 nm) as dark areas. 2DPCs were produced using TBA and 15% HOAc, and 1DPC sugar analyses using *n*-BuOH : Pyr : H_2O, 30 : 20 : 15. For further information see Markham (1992).

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References

Allan, H. H. (1961). In *Flora of New Zealand* (pp. 885–952). Wellington: Government Printer.
Grayer, R. L. (1979). Chemosystematic investigations in *Veronica* L. (Scrophulariaceae) and related genera. PhD thesis, Rijksuniversiteit of Leiden, Holland.
Perry, N. B., & Foster, L. M. (1994). *Planta Med*, *60*, 491.
Mitchell, K. A. (1993). Studies in New Zealand natural products. MSc thesis, University of Canterbury, New Zealand.
Kellam, S. J., Mitchell, K. A., Blunt, J. W., Munro, M. H. G., & Walker, J. R. L. (1993). *Phytochemistry*, *33*, 867.
Markham, K. R. (1982). In *Techniques of flavonoid identification* (p. 81). London: Academic Press.
Williams, C. A., & Harborne, J. B. (1994). In J. B. Harborne, *The flavonoids — advances in research since 1986* (p. 341). London: Chapman & Hall.
Markham, K. R. (1992). In *Techniques of flavonoid Identification* (pp. 16–52). London: Academic Press.