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FLAVONOL GLYCOSIDES OF *LIMNANTHES DOUGLASII*

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Key Word Index—*Limnanthes douglasii*; Limnanthaceae; flavonol glycosides; myricetin-3′-monomethyl ether; syringetin; 3-*O*-rutinoside-7-*O*-glucosides; 3-*O*-(rhamnosyl rutinosides).

Abstract—Eighteen flavonol glycosides were isolated from petal and leaf-stem of *Limnanthes douglasii*. There were six aglycones: kaempferol, quercetin, isorhamnetin, myricetin, syringetin and a new flavonol, myricetin 3′-methyl ether. Each occurred as the 3-rutinoside, 3-rhamnosylrutinoside and 3-rutinoside-7-glucoside.

INTRODUCTION

Limnanthes douglasii R. Brown var. *douglasii*, or meadow foam, is an attractive early spring annual of California and southern Oregon. Its flavonoids were examined as part of a chemotaxonomic study of the Limnanthaceae and the results are reported here. Very little previous work has been done on this family [1]; Bate-Smith [2] reported that hydrolyzed extracts of *L. douglasii* leaves yielded caffeic acid, kaempferol, quercetin, myricetin, cyanidin and delphinidin.

RESULTS

Eighteen flavonol glycosides were isolated from leaf and flower extracts of *L. douglasii* and identified. Four or five additional glycosides were consistently present in trace amounts but have not been identified. Aglycones were absent. The glycosides can be separated into three groups of six based on glycosylation.

The most prominent group behaved as diglycosides in aqueous solvents, and could be separated from each other using a $CHCl_3$ based solvent system. Each yielded glucose and rhamnose and a different aglycone on acid hydrolysis and gave a UV spectrum characteristic of a flavonol 3-*O*-glycoside. Four of the aglycones were kaempferol, quercetin, isorhamnetin, and myricetin. UV and NMR spectral data (see Table 1) for the corresponding four glycosides agreed completely with published data for 3-*O*-β-D-rutinosides.

The remaining two rutinosides were new. One had R_f and UV behaviour similar to isorhamnetin 3-*O*-rutinoside which suggested the presence of an *O*-methylated B-ring. The NMR of the trimethylsilyl derivative, indeed, showed the presence of two equivalent *O*-methyl groups, 3·84δ, and a singlet at 7·12δ which integrated for two protons. This singlet indicates equivalent protons on the B-ring. These data are consistent with a syringyl B-ring. Signals at 6·10δ and 6·41δ showed the expected *meta* splitting of 6-H and 8-H. The aglycone is, therefore, myricetin 3′,5′-dimethyl ether or syringetin. This was confirmed by direct comparison with authentic material from *Philydrum* [3]. Syringetin has a very limited natural distribution. Prior to its present discovery in *Limnanthes douglasii* (chromatographic and spectral data show that it occurs in several other *Limnanthes* species) it had been reported in *Lathyrus* [4], *Larix* [5, 6], *Soymida* [7] and *Philydrum* [3].

The second new rutinoside was similar to quercetin in its R_f and UV behaviour. The NMR spectrum of the trimethylsilyl derivative showed four aromatic protons, as in the case of syringetin rutinoside, but only one *O*-methyl group. The A-ring signals were very close to those for the other flavonols isolated from the plant (Table 1). The B-ring protons appeared as doublets at 7·20δ and 6·90δ and displayed *meta*-coupling. These results allow the assignment of the structure myricetin 3′-methyl ether to the aglycone. This is the first definitive report of this compound as a natural product; Niemann [5, 6] provisionally identified it earlier in *Larix*.

The second group of glycosides moved further in aqueous solvents than did the rutinosides which suggested a greater degree of glycosylation. Again,

Table 1. Proton chemical shift values* of TMS ethers of flavonol β-D-rutinosides of *Limnanthes douglasii*

Compound†	A-Ring 6	A-Ring 8	B-Ring 2′	B-Ring 6′	B-Ring 3′	B-Ring 5′	Carbohydrate Glc 1-H	Carbohydrate Rha 1-H	Carbohydrate Rha Me	Other
Km-3-*O*-Rut	6·08 d, *J* 2·5‡	6·36 *d*, *J* 2·5‡	(7·68) 2H, *d*, *J* 8·5			(6·78) 2H, *d*, *J* 8·5	5·80 *d*, *J* 7·	4·19 *d*, *J* 1	0·71	3·26, 3·83
Qu-3-*O*-Rut	6·08	6·36	7·30 *d*, *J* 2	7·34 *q*, *J* 2, 9·5		6·78 *d*, *J* 9·5	5·75	4·20	0·81	3·29, 3·80
Ir-3-*O*-Rut	6·08	6·38	7·45 *d*, *J* 2	7·27 *q*, *J* 2, 9	3·84 3H, *s*¶	6·77 *d*, *J* 9	5·84	4·20	0·72	3·25, 3·74
My-3-*O*-Rut	6·06	6·32	(7·08) 2H, *s*				5·81	4·22	1·00	3·29, 3·85
Lm-3-*O*-Rut	6·09	6·37	7·20 *d*, *J* 2	6·90 *d*, *J* 2	3·85 3H, *s*¶		5·89	4·23	0·81	3·28, 3·76
Sg-3-*O*-Rut	6·10	6·41	(7·12) 2H, *s*			(3·84) 6H, *s*¶	5·93	4·22	0·73	3·25, 3·75

* Expressed as δ in ppm relative to tetramethylsilane (0·00) in CCl_4.

† Compound abbreviations: Km—kaempferol; Qu—quercetin; Ir—isorhamnetin; My—myricetin; Lm—myricetin 3′-methyl ether; Sg—syringetin; Glc—glucose; Rha—rhamnose.

‡ Consistent values for H-6 and H-8.

§ Consistent values for glucose-1-H.

‖ Consistent values for rhamnose-1-H.

¶ *O*-Methyl groups

they were separated into individual components with a $CHCl_3$-based solvent system. Their UV spectra showed them to be flavonol 3-*O*-glycosides. Total hydrolyses gave the same six aglycones (see above) and, in each case, two equivalents of rhamnose and one equivalent of glucose. Partial acid hydrolysis of the triglycosides gave, in each case, the corresponding 3-*O*-rutinoside and rhamnose. Lack of material has prevented determination of the point of substitution of the terminal rhamnose but they can clearly be formulated as 3-(rhamnosyl rutinosides).

Table 2. R_fs for flavonol glycosides of *Limnanthes douglasii*

Compounds*	R_f (× 100) in Solvent 1	Solvent 2
Km-3-*O*-Rutinoside	32	49
Qu-3-*O*-Rutinoside	35	23
Ir-3-*O*-Rutinoside	35	63
My-3-*O*-Rutinoside	35	10
Lm-3-*O*-Rutinoside	38	33
Sg-3-*O*-Rutinoside	40	72
Km-3-*O*-Rha-Rut	59	33
Qu-3-*O*-Rha-Rut	62	20
Ir-3-*O*-Rha-Rut	63	49
My-3-*O*-Rha-Rut	60	10
Lm-3-*O*-Rha-Rut	66	27
Sg-3-*O*-Rha-Rut	68	61
Km-3-*O*-Rut-7-*O*-Glc	76	39
Qu-3-*O*-Rut-7-*O*-Glc	76	13
Ir-3-*O*-Rut-7-*O*-Glc	77	48
My-3-*O*-Rut-7-*O*-Glc	75	7
Lm-3-*O*-Rut-7-*O*-Glc	77	21
Sg-3-*O*-Rut-7-*O*-Glc	78	58

* Km—kaempferol; Qu—quercetin; Ir—isorhamnetin; My—myricetin; Lm—myricetin; 3′-methyl ether; Sg—syringetin; Rut—rutinoside.

† Solvent 1 = H_2O–*n*-BuOH–acetone–HOAc (16:2:1:1) on Polyamide DC-6.6. Solvent 2 = $CHCl_3$–isopropanol–butanone–HOAc (10:3:3:4) on polyamide DC-6·6 (two developments)

The third group of glycosides had a higher R_f than the 3-*O*-trioside group. Fractionation into six components was achieved as above. UV spectroscopy suggested these compounds to be flavonol 3,7-diglycosides. Total acid hydrolysis gave the same six aglycones, two equivalents of glucose and one equivalent of rhamnose. Partial acid hydrolysis yielded flavonol derivatives with UV spectra corresponding to 7-*O*-glycosides. Treatment with β-glucosidase produced the corresponding 3-*O*-rutinosides and glucose. These compounds must then be the flavonol 3-*O*-β-D-rutinoside 7-*O*-β-D-glucosides.

Separate extractions of petals and leaf–stem material of *L. douglasii* showed quantitative differences in flavonoid composition. The rutinosides occur in approximately equal concentrations in both types of tissue. The 3,7-diglycosides are nearly absent from petals while present in higher concentration in the rest of the plant. Conversely, the 3-*O*-rhamnosylrutinosides occur almost exclusively in petals with only trace amounts occurring in leaf–stem extracts.

Although Harborne [4] has shown that syringetin acts as a yellow flower pigment in *Lathyrus*, this is apparently not the case in *Limnanthes douglasii*. Some forms of *L. douglasii* have white petals

while others have yellow patterning. The white petaled forms contain concentrations of methoxylated flavonols, including syringetin, equal to those of the yellow petaled form.

EXPERIMENTAL

Plant material. Seed of *Limnanthes douglasii* R. Brown var. *douglasii* was obtained from the United States Department of Agriculture. The USDA accession number is 278170. Plants were grown in flats in the greenhouse under 16 hr days. *Ca.* 10000 petals were collected from these plants over a period of flowering of *ca.* 2 weeks. Leaf-stem material weighing 3·5 kg (fr. wt) was collected from field-grown plants just prior to flowering. Voucher specimens have been deposited in the UBC herbarium. Seed stock is also being maintained.

Extraction and chromatography. Petal and leaf-stem material were extracted, separately, with hot 90% MeOH. These extracts were concentrated *in vacuo* at 30°. The petal residue was taken up in a small amount of 50% EtOH and passed through a short column of SC-6 Polyamide. The eluent was concentrated *in vacuo* and the residue taken up in H_2O. This portion was chromatographed on a column of SC-6 Polyamide using a linear gradient 0–50% MeOH in H_2O. Treatment of the extract from stem-leaf material was treated the same except that trituration with Celite was performed prior to chromatography. Column fractions were repeatedly chromatographed (TLC) on DC-6·6 Polyamide in $CHCl_3$–MeOH–butanone–H_2O (55:22:20:3). An acidic variant of this system was also found useful: $CHCl_3$ isopropanol–butanone–HOAc (10:3:3:4). The system H_2O–*n*-BuOH–acetone–HOAc (16:2:1:1) was used to separate the glycoside types on SC-6·6 Polyamide; for R_f see Table 2.

Hydrolyses. Total hydrolyses were done with 0·1N HCl at 100° for 1 hr. Partial acid hydrolysis was done with 20% HOAc in H_2O at 100° for varying periods of time. Enzymic hydrolyses were done with emulsin overnight at room temp. in 0·1 N acetate buffer at pH 5·1.

UV and NMR. UV analysis was performed according to Mabry *et al.* [8]. For 3′-*O*-methylmyricetin-3-*O*-β-D-rutinoside absorption maximum are: 255, 266sh, 301, 361; for syringetin-3-*O*-β-D-rutinoside: 254, 268sh, 300, 361. NMR spectra were determined using tetramethylsilane as internal standard. Syringetin and limnanthetin rutinoside acetates were prepared using Ac_2O–NEt_3, a procedure which leaves the 5-OH group free. The product from syringetin rutinoside had m.p. 130–132°; that from limnanthetin rutinoside had m.p. 124–126°.

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REFERENCES

1. Hegnauer, R. (1966) *Chemotaxonomie der Pflanzen*, Vol. 4, pp. 391–392, Birkhasuser, Basel.
2. Bate-Smith, E. C. (1962) *J. Linn. Soc. (Bot.)* **58**, 39.
3. Bohm, B. A. and Collins, F. W. (1974) *Phytochemistry* in press.
4. Harborne, J. B. (1965) *Phytochemistry* **4**, 647.
5. Niemann, G. J. (1972) *Acta Bot. Neerl.* **21**, 549.
6. Niemann, G. J. (1973) *Phytochemistry* **12**, 2056.
7. Pardhasaradhi, M. and Sidhu, G. S. (1972) *Phytochemistry* **11**, 1520.
8. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, Heidelberg.

Note added in proof. After submission of this work we learned that Tynkavkin and co-workers have identified the 3-*O*-glucoside of 3′-methylmyricetin and the 3-*O*-rutinoside of syringetin from *Larix sibirica*. They assigned the trivial name "laricytrin" to 3′-methylmyricetin.

Tynkavkin, N. A., Medvedev, S. A. and Ivanov, S. Z. (1974) *Khim. Prir. Soed.* No. 2, 157.