Phytochemistry, Vol. 28, No. 8, pp. 2215–2216, 1989.
Printed in Great Britain.

0031-9422/89 $3.00 + 0.00
Pergamon Press plc.

TWO FLAVONOL GLYCOSIDES FROM *LYSIMACHIA FORTUNEI**

KEN YASUKAWA, HITOSHI SEKINE and MICHIO TAKIDO

Department of Pharmacy, College of Science and Technology, Nihon University, Tokyo 101, Japan

(*Received* 11 *January* 1989)

Key Word Index—*Lysimachia fortunei*; Primulaceae; whole plant; flavonol glycosides; quercetin 3-(2,6-dirhamnopyranosylgalactopyranoside); isorhamnetin 3-(2,6-dirhamnopyranosylgalactopyranoside).

Abstract—From the whole plant of *Lysimachia fortunei*, two new flavonol glycosides were isolated together with trifolin, hyperin, isorhamnetin 3-galactoside, rutin, quercetin 3-rhamnosyl(1→2)galactoside, isorhamnetin 3-robinobioside and mauritianin. The structures of the new compounds were established as quercetin 3-(2,6-dirhamnopyranosylgalactopyranoside) and isorhamnetin 3-(2,6-dirhamnopyranosylgalactopyranoside).

INTRODUCTION

Glycosides of kaempferol, quercetin and myricetin have already been isolated from the genus *Lysimachia*, Primulaceae (*L. vulgaris* [1, 2], *L. punctata* [3], *L. nummularia* [4], *L. japonica* [5], *L. clethroides* [5], *L. mauritiana* [6] and *L. vulgaris* var. *davurica* [7]). Mauritianin, which was isolated from *L. mauritiana*, has anti-tumour promoting activity on the two-stage carcinogenesis in mice [8]. In this paper we report the isolation and characterization of nine flavonol glycosides from the whole plant of *L. fortunei*, which is used in Chinese folk medicine for the treatment of bruises. Kaempferol 3-galactoside is reported for the first time in *Lysimachia* and quercetin 3-*O*-(2,6-di-*O*-rhamnopyranosylgalactopyranoside) and isorhamnetin 3-*O*-(2,6-di-*O*-rhamnopyranosylgalactopyranoside) are new compounds.

RESULTS AND DISCUSSION

The concentrated methanol extract prepared from the air-dried whole plant of *L. fortunei* was extracted successively with ethyl acetate and *n*-butanol. Three flavonol glycosides (**1**–**3**) were isolated from the ethyl acetate fraction and a further six flavonol glycosides (**4**–**9**) from *n*-butanol fraction by column chromatography.

Compound **1** was characterized as kaempferol 3-galactoside by hydrolysis to give kaempferol and galactose, UV spectral analysis and ^{13}C and ^{1}H NMR. Compounds **2**–**7**, were identified as hyperin, isorhamnetin 3-*O*-galactoside, rutin, quercetin 3-*O*-rhamnosyl(1→2)galactoside, isorhamnetin 3-*O*-robinobioside and kaempferol 3-*O*-(2,6-dirhamnosylgalactoside) (mauritianin) respectively by standard procedures and direct comparison with authentic samples.

Acid hydrolysis of **8** gave quercetin, galactose and rhamnose (TLC). The ^{13}C NMR of **8** in DMSO-d_6 also confirmed that it was a quercetin glycoside. The ^{13}C NMR shifts of the aglycone of **8** corresponded well to the shifts for quercetin, the only significant difference being an upfield shift of 3.5 ppm for the C-3. The ^{13}C NMR spectrum also showed that **8** had galactose and rhamnose in its structure, the only significant difference being upfield shifts of 4.0 and 5.3 ppm, respectively, for the C-2 and C-6 of galactose and downfield shifts of 3.2 and 2.0 ppm for the C-1 and C-5 of galactose (Table 1). These shifts are analogous to those reported [9] for a flavonol (2,6-di-rhamosylgalactoside). The structure of **8** is therefore quercetin 3-*O*-(2,6-di-*O*-α-rhamnopyranosyl-β-galactopyranoside).

Acid hydrolysis of **9** gave isorhamnetin, rhamnose and galactose (TLC). The ^{13}C NMR spectrum of **9** in DMSO-d_6 also confirmed that it was a isorhamnetin 3-glycoside, and the ^{13}C NMR shifts of the sugar units of **9** are in accord with the sugar units of mauritianin **7** and **8**. The structure of **9** is therefore isorhamnetin 3-*O*-(2,6-di-*O*-α-rhamnopyranosyl-β-galactopyranoside). Quercetin 3-rhamnosylrobinobioside [10] has already been isolated but in this case the rhamnose was not proved to be attached at the 2-position of robinobiose.

EXPERIMENTAL

Mps: uncorr. ^{1}H and ^{13}C NMR spectra were recorded at 100 and 25.5 MHz, respectively, chemical shifts are given in δ (ppm) with TMS at int. std. CC was carried out using Sephadex LH-20 (Pharmacia). TLC on Avicel SF (Funakoshi) was performed with *n*-BuOH–HOAc–H_2O (3:1:1).

Plant material. Lysimachia fortunei Maxim. was collected at Tsugaike, Nagano, Japan in the autumn of 1987.

Extraction and isolation. Dried whole plants (1.34 kg) were extracted with MeOH and the concd extract macerated with hot H_2O and filtered. The H_2O soln were extracted with EtOAc followed by *n*-BuOH. Yield: EtOAc extract (16.7 g) and *n*-BuOH extract (24.7 g).

The EtOAc extract (5 g) was then subjected to CC on Sephadex LH-20, using MeOH as eluent, to yield **1** (10 mg), **2** (450 mg) and **3** (5 mg). The *n*-BuOH extract (8 g) was analysed by the same method to yield **4** (15 mg), **5** (400 mg), **6** (10 mg), **7** (7 mg), **8** (10 mg) and **9** (8 mg).

*Part 4 in the series 'Studies of the constituents of genus *Lysimachia*'. For Part 3 see Yasukawa, K. and Takido, M. (1988) *Phytochemistry* **27**, 3017.

Table 1. ^{13}C NMR spectral data of sugar units of flavonol glycosides **3**, **5**–**9**

C		Compounds					
		3	**5**	**6**	**7**	**8**	**9**
galactose	1	102.4	99.2	102.3	99.2	99.2	99.2
	2	71.6	75.8	71.4	75.6	75.7	75.6
	3	73.5	74.0	73.4	73.9	73.9	73.9
	4	68.2	68.1	68.3	68.8	68.8	68.8
	5	75.9	75.8	74.0	73.9	73.8	73.9
	6	60.5	60.4	65.8	65.8	65.8	65.8
rhamnose	1		100.6	100.3	100.7	100.6	100.7
	2		70.9	70.9	70.9	70.9	70.9
	3		70.6	70.5	70.5	70.5	70.5
	4		72.3	72.2	72.2	72.2	72.2
	5		68.8	68.3	68.8	68.9	68.8
	6		17.2	17.7	17.2	17.2	17.2
rhamnose	1′				100.3	100.3	100.3
	2′				70.9	70.9	70.9
	3′				70.5	70.5	70.5
	4′				72.2	72.2	72.2
	5′				68.3	68.3	68.3
	6′				17.7	17.7	17.7

25.5 MHz, 90°, DMSO-d_6, TMS as internal standard.

Quercetin 3-O-(2,6-di-O-α-rhamnopyranosyl-β-galactopyranoside) (**8**). Recryst. (H_2O–MeCN) gave yellow needles, mp 185–188°. Analysis: calcd: $C_{33}H_{40}O_{21}$: C, 51.29; H, 5.22; found: C, 51.15; H, 5.30. Dark green with $FeCl_3$, pale red colour with Mg+HCl test. UV λ_{max}^{EtOH} nm: 258, 266 sh, 364. ^1H NMR (DMSO-d_6, 90°) δppm: 12.78 (1H, *s*, 5-OH), 7.65 (1H, *dd*, *J* = 1.9, 8.8 Hz, H-6′), 7.55 (1H, *d*, *J* = 1.9 Hz, H-2′), 6.87 (1H, *d*, *J* = 8.8 Hz, H-5′), 6.41 (1H, *d*, *J* = 1.9 Hz, H-8), 6.23 (1H, *d*, *J* = 1.9 Hz, H-6), 5.59 (1H, *d*, *J* = 7.3 Hz, galactosyl H-1), 5.12 (1H, *s*, rhamnosyl H-1), 4.46 (1H, *s*, rhamnosyl H-1′), 1.10 (3H, *d*, *J* = 6.3 Hz, rhamnosyl Me-6), 0.91 (3H, *d*, *J* = 6.3 Hz rhamnosyl Me-6′). ^{13}C NMR (DMSO-d_6, 90°) δppm: 177.3 (C-4), 164.0 (C-7), 161.3 (C-5), 156.4 (C-2, C-9), 148.2 (C-4′), 144.8 (C-3′), 133.0 (C-3), 121.9 (C-1′), 121.6 (C-6′), 116.1 (C-5′), 115.3 (C-2′), 104.2 (C-10), 98.8 (C-6), 93.6 (C-8). Chemical shifts of sugar units are given in Table 1.

Hydrolysis of **8**. Compound **8** (5 mg) treated with 2 M HCl at 100° for 3 hr gave an aglycone and sugars. The aglycone was identified as quercetin by direct comparison with an authentic sample. The sugars were identified as rhamnose and galactose by TLC.

Isorhamnetin 3-O-(2,6-di-O-α-rhamnopyranosyl-β-galactopyranoside) (**9**). Recryst. (H_2O–MeCN) gave yellow needles, mp 172–175°. Analysis: calcd: $C_{34}H_{42}O_{21}$: C, 51.91; H, 5.38; found: C, 51.76; H, 5.44. Dark green with $FeCl_3$, pale red colour with Mg+HCl test. UV λ_{max}^{EtOH} nm: 255, 268sh, 302sh, 360. ^1H NMR (DMSO-d_6, 90°) δppm: 12.78 (1H, *s*, 5-OH), 7.65 (1H, *dd*, *J* = 1.9, 8.8 Hz, H-6′), 7.55 (1H, *d*, *J* = 1.9 Hz, H-2′), 6.87 (1H, *d*, *J* = 8.8 Hz, H-5′), 6.41 (1H, *d*, *J* = 1.9 Hz, H-8), 6.23 (1H, *d*, *J* = 1.9 Hz, H-6), 5.59 (1H, *d*, *J* = 7.3 Hz, galactosyl H-1), 5.12 (1H, *s*, rhamnosyl H-1), 4.46 (1H, *s*, rhamnosyl H-1′), 3.91 (3H, *s*, 3′-Me), 1.10 (3H, *d*, *J* = 6.3 Hz, rhamnosyl Me-6), 0.91 (3H, *d*, *J* = 6.3 Hz, rhamnosyl Me-6′). ^{13}C NMR (DMSO-d_6, 90°) δppm: 177.4 (C-4), 164.0 (C-7), 161.3 (C-5), 156.6 (C-2, C-9), 147.2 (C-4′), 149.5 (C-3′), 132.9 (C-3), 121.5 (C-1′), 122.1 (C-6′), 114.1 (C-5′), 115.3 (C-2′), 104.3 (C-10), 98.9 (C-6), 93.7 (C-8), 56.3 (C-3′-OMe). Chemical shifts of sugar units are given in Table 1.

Hydrolysis of **9**. Compound **9** (3 mg) treated with 2 M HCl at 100° for 3 hr gave an aglycone and sugars. The aglycone was identified as isorhamnetin by direct comparison with an authentic sample. The sugars were identified as rhamnose and galactose by TLC.

Acknowledgements—We thank Miss A. Koizumi, and Dr T. Takido (Analytical Center, College of Science and Technology, Nihon University) for elemental analysis and NMR spectra.

REFERENCES

1. Rzadkowska-Bodalska, H. and Olechnowicz-Stepien, W. (1975) *Pol. J. Pharmacol. Pharm.* **27**, 345.
2. Prum, N., Pichon, P. and Raynaud, J. (1972) *Plant. Med. Phytother.* **6**, 267.
3. Mendez, J. (1970) *Experientia* **26**, 108.
4. Popov, V. I. (1967) *Tr. Leningrad. Khim.-Farm. Inst.* **21**, 221.
5. Yasukawa, K. and Takido, M. (1986) *Yakugaku Zasshi* **106**, 939.
6. Yasukawa, K. and Takido, M. (1987) *Phytochemistry* **26**, 1224.
7. Yasukawa, K. and Takido, M. (1988) *Phytochemistry* **27**, 3017.
8. Yasukawa, K., Takido, M., Takeuchi, M. and Nitta, K. (1988) *Plant Flavonoids in Biology and Medicine II: Biochemical, Cellular and Medicinal Properties* (Cody, V., Middleton, E. Jr., Harborne, J. B. and Beretz, A. eds), p. 247. Alan R. Liss, New York.
9. Lin, C. N., Arisawa, M., Shimizu, M. and Morita, N. (1982) *Phytochemistry* **21**, 1466.
10. Karl, C., Muller, G. and Pedersen, P. A. (1981) *Planta Med.* **41**, 96.