*Viscosic acid* (**5**). Oil (8 mg). UV $\lambda_{max}^{MeOH}$ nm: 215 (log $\varepsilon$ 4.3); IR $\nu_{max}^{CHCl_3}$ cm$^{-1}$: 2950, 2840, 2600 (*br* sh), 1695, 1620, 1445, 1380, 1100, 1050, 980, 820, 750; $^1$H NMR (400 MHz, $CDCl_3$) see text; MS (probe) 70 eV (rel. int.): 264 $[M]^+$ ($C_{16}H_{24}O_3$) (methyl ether of viscosic acid) (18), 250 $[M-CH_2]^+$ (16), 249 $[M-Me]^+$ (68), 233 (13), 203 (20), 156 (31), 117 (98).

*Epoxidation of costic acid methyl ester.* *p*-Chloroperbenzoic acid (10 mg) was added to costic acid methyl ester (5 mg) in 1 ml $CH_2Cl_2$ in the presence of $NaHCO_3$ (10 mg). The mixture was left at room temp. for 1 hr. The $^1$H NMR spectrum of the resulting compound was identical with that of compound **5**.

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# A FLAVONOL GLYCOSIDE FROM *LYSIMACHIA MAURITIANA*

KEN YASUKAWA and MICHIO TAKIDO

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

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**Key Word Index**—*Lysimachia mauritiana*; Primulaceae; mauritianin; kaempferol-3-*O*-(2,6-di-*O*-α-rhamnopyranosyl-β-galactopyranoside); flavonol glycoside.

**Abstract**—From the whole plant of *Lysimachia mauritiana*, a new flavonol glycoside (mauritianin) was isolated together with hyperin, kaempferol-3-*O*-robinobioside and kaempferol-3-*O*-α-rhamnopyranosyl-(1-2)-β-galactopyranoside. The structure of mauritianin was established as kaempferol-3-*O*-(2,6-di-*O*-α-rhamnopyranosyl-β-galactopyranoside).

## INTRODUCTION

Flavonol glycosides of the kaempferol, quercetin and myricetin type have already been isolated from the genus *Lysimachia*, a member of the Primulaceae (*L. vulgaris* [1, 2], *L. punctata* [3] and *L. nummularia* [4]). Initial chemical investigations of *L. mauritiana* led to the isolation and structure elucidation of sapogenins [5, 6]. In this paper we report the isolation and characterization of four flavonol glycosides from this plant. Hyperin and kaempferol-3-*O*-rhamnosyl galactosides are reported for the first time in *Lysimachia*; mauritianin is a new compound, which an anti-tumour promoter [7].

## RESULTS AND DISCUSSION

The concentrated methanol extract prepared from the air dried plant was extracted successively with ethyl acetate and *n*-butanol. One flavonol glycoside (compound **1**) was isolated on a Sephadex LH-20 column using water–methanol from the ethyl acetate fraction, and another three flavonol glycosides (compounds **2**–**4**) were isolated by the same method from the *n*-butanol fraction.

Compound **1**, yellow needles, mp 238.5–239°, gave positive ferric chloride and Mg + HCl tests and was identified as hyperin by direct comparison with an authentic sample.

Compound **2**, pale yellow needles, mp 198–200°, also gave positive ferric chloride and Mg + HCl tests. The acid hydrolysis products of **2** showed the presence of kaempferol and the two sugars, galactose and rhamnose, by TLC. **2** was subsequently identified as kaempferol-3-*O*-robinobioside by direct comparison with an authentic sample.

The similarity in the chromophore of compound **3** to that of compound **2** was established by comparison of their colour reactions, acid hydrolysis products and UV spectrum. $^{13}$C NMR also showed that **3** had galactose and rhamnose units in its structure, the only significant difference from **1** being an upfield shift of 4.1 ppm for the C-2″ of galactose and a downfield shift of 3.5 ppm for the C-1″ of galactose (Table 1). These shifts are analogous to those reported [8] when the C-2″ of galactose is rhamnosylated in a flavonol glycoside. The structure of compound **3** was therefore determined as kaempferol-3-*O*-α-rhamnopyranosyl-(1-2)-β-galactopyranoside.

Compound **4**, pale yellow needles, mp 202–204°, again gave positive ferric chloride and Mg + HCl tests. The acid hydrolysis products of **4** showed the presence of kaempferol, galactose and rhamnose on TLC. Kaempferol was identified by direct comparison with an authentic sample. The $^{13}$C NMR of **4** in DMSO-$d_6$ also confirmed that it was a glycoside of kaempferol. The $^{13}$C NMR shifts of the aglycone part of **4** corresponded well to the shifts for kaempferol, the only significant difference being an upfield shift of 2.4 ppm for the C-3. This shift is analogous to that reported [8] when the 3-hydroxy group is glycosylated in a flavonol glycoside. UV shifts on the addition of sodium acetate and aluminium chloride also showed the position of linkage between sugar and aglycone as C-3. The $^{13}$C NMR of **4** is similar to that of compounds **1**–**3**, the only significant difference being an upfield shift of 4.1 and 5.4 ppm, respectively, for the C-2″ and C-6″ of galactose because it is a rhamnosylated flavonol glycoside [9]. Thus, the structure of compound **4** (named mauritianin) was determined as kaempferol-3-*O*-(2,6-di-*O*-α-rhamnopyranosyl-β-galactopyranoside). The same trisaccharide has already been found in another kaempferol glycoside isolated from *Rhamnus nakaharai* but in this case the sugar is attached to the 4′-OH [9].

## EXPERIMENTAL

All mps are 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 as 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. L. mauritiana* Lam. was collected at Miura beach, Kanagawa, Japan in the autumn of 1983.

*Extraction and isolation.* Dried whole plant (1.5 kg) was extracted with MeOH and concd to a dark residue, which was then macerated with hot $H_2O$ and filtered. The $H_2O$ soln was extracted with EtOAc followed by *n*-BuOH. The EtOAc extract (2 g) was then subjected to CC on Sephadex LH-20, using $H_2O$–MeOH as eluant, to yield compound **1** (10 mg). The *n*-BuOH extract (10 g) was analysed by the same method, to yield compounds **2** (10 mg), **3** (20 mg) and **4** (102 mg).

*Hyperin.* Recrystallization ($H_2O$–MeCN) gave yellow needles, mp 238.5–239°. *Analysis:* calcd: $C_{21}H_{20}O_{12}\cdot H_2O$: C, 52.33; H, 4.76; found: C, 52.29; H, 4.60. Dark green colour after $FeCl_3$ reaction, pale red colour in Mg + HCl test. UV $\lambda_{max}^{EtOH}$ nm: 258, 270 sh, 293sh, 364. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3300 (OH), 2900 (C–H), 1655 (C=O), 1605 (C=C), 1060 (C–O). $^1$H NMR (DMSO-$d_6$) δ ppm: 12.65 (1H, *s*, OH-5), 7.60 (2H, *dd*, *J* = 2.0 Hz, *J* = 8.3 Hz, H-6′), 7.59 (1H, *d*, *J* = 2.0 Hz, H-2′), 6.86 (1H, *d*, *J* = 8.3 Hz, H-5′), 6.42 (1H, *d*, *J* = 1.9 Hz, H-8), 6.21 (1H, *d*, *J* = 1.9 Hz, H-6), 5.52 (1H, *d*, *J* = 7.3 Hz, galactosyl H-1). $^{13}$C NMR (DMSO-$d_6$, 90°) δ ppm: 177.6 (C-4), 164.2 (C-7), 161.3 (C-5), 156.5 (C-2, C-9), 148.4 (C-4′), 144.8 (C-3′), 133.8 (C-3), 121.9 (C-6′), 121.4 (C-1′), 116.4 (C-5′), 115.3 (C-2′), 104.2 (C-10), 98.8 (C-6), 93.6 (C-8). For chemical shifts of sugar units see Table 1.

*Kaempferol-3-O-robinobioside.* Recrystallization ($H_2O$–MeCN) gave pale yellow needles, mp 198–200°. *Analysis:* calcd: $C_{27}H_{30}O_{16}\cdot 3/2H_2O$: C,50.80; H,5.17; found; C,50.87; H,5.22. Dark green colour after $FeCl_3$ reaction, pink colour in Mg + HCl test. UV $\lambda_{max}^{EtOH}$ nm: 263, 301sh, 349. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3400 (OH), 2950 (C–H), 1660 (C=O), 1605 (C=C), 1060 (C–O). $^1$H NMR (DMSO-$d_6$) δ ppm: 12.58 (1H, *s*, OH-5), 8.00 (2H, *d*, *J* = 8.3 Hz, H-2′, H-6′), 6.89 (2H, *d*, *J* = 8.3 Hz, H-3′, H-5′), 6.40 (1H, *d*, *J* = 1.9 Hz, H-8), 6.23 (1H, *d*, *J* = 1.9 Hz, H-6), 5.56 (1H, *d*, *J* = 7.3 Hz, galactosyl H-1), 5.05 (1H, *s*, rhamnosyl H-1), 1.05 (3H, *d*, *J* = 5.4 Hz, rhamnosyl Me). $^{13}$C NMR (DMSO-$d_6$, 90°) δ

Table 1. $^{13}$C NMR spectral data of sugar units of compounds **1**–**4** (25.5 MHz, DMSO-$d_6$, TMS as internal standard)

| | 1 | 2 | 3 | 4 | |
|---|---|---|---|---|---|
| Galactose C-1″ | 102.6 | 102.2 | 99.1 | 99.2 | — |
| C-2″ | 71.5 | 71.5 | 75.6 | 75.6 | — |
| C-3″ | 73.6 | 73.4 | 74.1 | 73.7 | — |
| C-4″ | 68.2 | 68.4 | 68.2 | 68.3 | — |
| C-5″ | 75.9 | 73.9 | 75.9 | 74.0 | — |
| C-6″ | 60.4 | 65.9 | 60.4 | 65.8 | — |
| Rhamnose C-1 | — | 100.4 | 100.7 | 100.7 | 100.3 |
| C-2 | — | 71.0 | 70.9 | 70.9 | 70.9 |
| C-3 | — | 70.3 | 70.8 | 70.8 | 70.5 |
| C-4 | — | 72.6 | 72.3 | 72.3 | 72.3 |
| C-5 | — | 68.4 | 68.7 | 68.8 | 68.3 |
| C-6 | — | 17.6 | 17.2 | 17.2 | 17.7 |

ppm: 177.5 (C-4), 164.3 (C-7), 161.3 (C-5), 160.0 (C-4'), 156.6 (C-2, C-9), 133.8 (C-3), 130.9 (C-2', C-6'), 121.1 (C-1'), 115.4 (C-3', C-5'), 104.3 (C-10), 99.0 (C-6), 93.9 (C-8). Chemical shifts of sugar units are given in Table 1.

*Hydrolysis of* **2**. Compound **2** (3 mg) treated with 2 N HCl at 100° for 3 hr gave kaempferol, galactose and rhamnose. Kaempferol was identified by direct comparison with an authentic sample. The sugars were identified by TLC with authentic samples. $R_f$ = 0.44 and 0.30 (rhamnose and galactose $R_f$ = 0.44 and 0.30).

*Kaempferol-3-O-α-rhamnopyranosyl-(1-2)-β-galactopyranoside*. Recrystallization ($H_2O$–MeCN) gave pale yellow needles, mp 202–204°. *Analysis:* calcd: $C_{27}H_{30}O_{16}\cdot 9/5H_2O$: C, 50.73; H, 5.57; found: C, 50.44; H, 5.27. Dark green colour with $FeCl_3$ reaction, pink colour in Mg + HCl test. UV $\lambda_{max}^{EtOH}$ nm: 262, 300sh, 350. $\lambda_{max}^{EtOH+AlCl_3}$ nm: 272, 300, 320sh, 345, 395. $\lambda_{max}^{EtOH+NaOAc}$ nm: 263, 300sh, 349. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400 (OH), 2950 (C–O), 1660 (C=O), 1610 (C=C), 1060 (C–O). $^1$H NMR (DMSO-$d_6$) δ ppm: 12.55 (1H, *s*, OH-5), 8.07 (2H, *d*, *J* = 8.3 Hz, H-2', C-6'), 6.85 (2H, *d*, *J* = 8.3 Hz, H-3', H-5'), 6.39 (1H, *d*, *J* = 1.9 Hz, H-8), 6.17 (1H, *d*, *J* = 1.9 Hz, H-6), 5.65 (1H, *d*, *J* = 7.3 Hz, galactosyl H-1), 5.04 (1H, *s*, rhamnosyl H-1), 0.73 (3H, *d*, *J* = 5.4 Hz, rhamnosyl Me). $^{13}$C NMR (DMSO-$d_6$, 90°) δ ppm: 177.5 (C-4), 164.8 (C-7), 159.8 (C-4'), 156.5 (C-2), 156.0 (C-9), 132.9 (C-3), 130.6 (C-2', C-6'), 121.2 (C-1'), 115.1 (C-3', C-5'), 104.0 (C-10), 98.9 (C-6), 93.7 (C-8). Chemical shifts of sugar units are given in Table 1.

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

*Kaempferol-3-O-(2,6-di-O-α-rhamnopyranosyl-β-galactopyranoside*) (*mauritianin*). Recrystallization ($H_2O$–MeCN) gave pale yellow needles, mp 202–204°. *Analysis*: calcd: $C_{33}H_{44}O_{19}\cdot 9/2H_2O$: C,48.14; H,5.71; found: C,48.23; H,6.01. Dark green colour with $FeCl_3$ reaction, pink colour in Mg + HCl test. UV $\lambda_{max}^{EtOH}$ nm: 264, 300sh, 348. $\lambda_{max}^{EtOH+AlCl_3}$ nm: 274, 301, 343, 393. $\lambda_{max}^{EtOH+NaOAc}$ nm: 265, 300, 356. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400 (OH), 2950 (C–H), 1660 (C=O), 1610 (C=C), 1050 (C–O). $^1$H NMR (DMSO-$d_6$) δ ppm: 12.70 (1H, *s*, OH-5), 8.05 (2H, *d*, *J* = 8.3 Hz, H-2', H-6'), 6.88 (2H, *d*, *J* = 8.3 Hz, H-3', H-5'), 6.41 (1H, *d*, *J* = 1.9 Hz, H-6), 6.21 (1H, *d*, *J* = 1.9 Hz, H-8), 5.56 (1H, *d*, *J* = 7.3 Hz, galactosyl H-1), 5.05 (2H, *s*, rhamnosyl H-1, H-1'), 1.05 (3H, *d*, *J* = 5.4 Hz, rhamnosyl Me), 0.79 (3H, *d*, *J* = 5.4 Hz, rhamnosyl Me'). $^{13}$C NMR (DMSO-$d_6$, 90°) δ ppm: 177.5 (C-4), 164.0 (C-7), 161.3 (C-5), 159.7 (C-4'), 156.5 (C-2, C-9), 132.9 (C-3), 130.6 (C-2', C-6'), 121.3 (C-1'), 115.2 (C-3', C-5'), 104.2 (C-10), 98.9 (C-6), 93.8 (C-8). Chemical shifts of the sugar units are given in Table 1.

*Hydrolysis of* **4**. Compound **4** (5 mg) treated with 2 N HCl at 100° for 3 hr gave an aglycone and sugars. The aglycone was identified as kaempferol by direct comparison with an authentic sample. The sugars were identified as rhamnose and galactose by TLC.

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