Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 339 (2004) 891–895
Carbohydrate RESEARCH

Note

# Two new flavonol glycosides from *Gymnema sylvestre* and *Euphorbia ebracteolata*

Xin Liu,[a,b] Wencai Ye,[b,*] Biao Yu,[a,*] Shouxun Zhao,[b] Houming Wu[a] and Chuntao Che[c]

[a]*State Key Laboratory of Bio-organic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 354 Fenglin Road, Shanghai 200032, China*
[b]*Department of Natural Product Chemistry, China Pharmaceutical University, Nanjing 210009, China*
[c]*School of Chinese Medicine, The Chinese University of Hong Kong, Hong Kong, China*

Received 28 October 2003; accepted 24 December 2003

**Abstract**—Two new flavonol glycosides, namely kaempferol 3-*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside (**1**) and quercetin 3-*O*-6″-(3-hydroxyl-3-methylglutaryl)-β-D-glucopyranoside (**2**), have been isolated from the aerial parts of *Gymnema sylvestre* and *Euphorbia ebracteolata*, respectively. Their structures were determined on the basis of chemical and spectroscopic methods.


*Keywords*: Flavonol glycoside; Kaempferol 3-*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside; Quercetin 3-*O*-6″-(3-hydroxyl-3-methylglutaryl)-β-D-glucopyranoside; *Gymnema sylvestre*; *Euphorbia ebracteolata*

*Gymnema sylvestre* (Retz.) Schult. (Asclepiadaceae) is widespread in southern China, India, and Vietnam. Its leaves, called 'Gur-mar' in India, are well known for their sweet taste suppressing activity[1] and are used for the treatment of diabetes mellitus and in food additives against obesity.[2] To explore the abundant resources of *G. sylvestre* in China, isolation of bioactive compounds has been carried out on samples collected in the Guangxi Autonomous Region of China. In this process, a new flavonol glycoside, kaempferol 3-*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside (**1**) was isolated, along with four known flavonoids, kaempferol 3-*O*-robinobioside (**5**),[3] rutin (**7**),[4,5] quercetin 3-*O*-robinobioside (**8**),[6] and tamarixetin 3-*O*-robinobioside (**9**),[7] which were isolated for the first time from this genus.

*Euphorbia ebracteolata* Hayata (Euphorbiaceae) has been used as a folk medicine in China for a long time. Recently two casbane diterpenoids have been isolated from this plant.[8] Herein we report the isolation and structure elucidation of a new flavonol glycoside, namely quercetin 3-*O*-6″-(3-hydroxyl-3-methylglutaryl)-β-D-glucopyranoside (**2**), from the upper part of this plant. Four known flavonoids, kaempferol 3-*O*-2″-galloyl-β-D-glucopyranoside (**3**),[9,10] kaempferol 3-*O*-rutinoside (**4**),[11] quercetin 3-*O*-β-D-glucopyranoside (**6**),[4,12] and rutin (**7**), were also isolated, for the first time, from this genus.

Compound **1**, $[\alpha]_D^{21}$ −31.1° (*c* 0.48, MeOH), was obtained as yellow needles. Its molecular formula was determined as $C_{33}H_{40}O_{20}$ by $^{13}C$ NMR spectroscopy and HRESIMS data (*m*/*z* 779.2020 for [M+Na$^+$]). Positive results of the HCl–Mg reaction and the Molish reaction indicated it to be a flavonoid glycoside. Acid hydrolysis of **1** provided kaempferol as the aglycone, and glucose, rhamnose, and galactose were identified through GC–MS analysis of their alditol peracetate derivatives.[13]

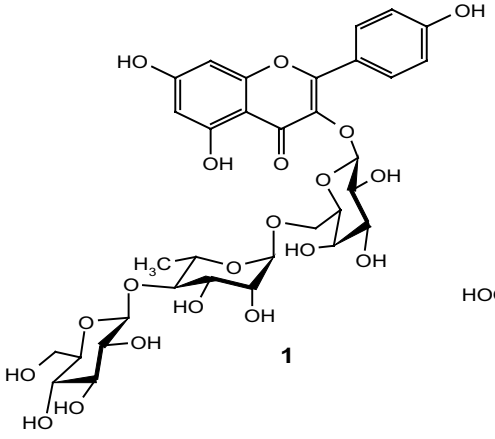
Compound **1** was proved to be a C-3-substituted monodesmosidic flavonol glycoside by comparing its $^{13}C$ NMR spectrum with references.[14] 2D NMR methods, that is, HMBC, HMQC, DQF-COSY, and

* Corresponding authors. Tel.: +86-21-6416-3300-1407; fax: +86-21-6416-6128; e-mail: byu@mail.sioc.ac.cn

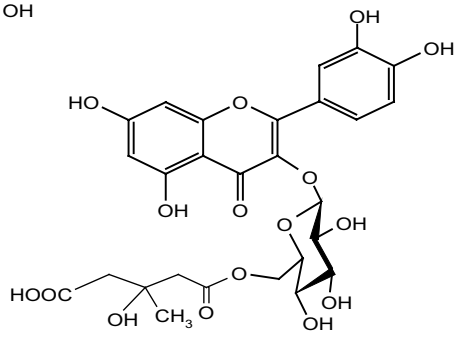
0008-6215/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2003.12.017

TOCSY, were then applied in determining the sequence of the trisaccharide residue. HMBC correlations were found between the rhamnopyranosyl methyl protons ($\delta$ 1.16, s) and two carbon atoms ($\delta$ 82.3, 66.8) assignable to the C-4 and C-5 of the rhamnose. The downfield C-4 signal at $\delta$ 82.3 was typical of such an atom that has undergone a glycosidation. HMBC correlations were also found between the rhamnopyranosyl anomeric proton ($\delta$ 4.42, s) and the methylene carbon ($\delta$ 65.4) assignable to the C-6 of the galactose, and between C-4 of the rhamnose and the anomeric proton ($\delta$ 4.34, d, $J$ 7.8 Hz) of the glucose residue. Corresponding carbon signals ($\delta$ 104.6, 74.6, 76.7, 70.2, 77.0, and 61.3), together with proton signals of the same spin system given by the TOCSY spectrum, proved the existence of a terminal glucopyranosyl unit. The remaining six carbon signals of the saccharide part, together with proton signals of the same spin system given by TOCSY spectrum, were in good agreement with a galactopyranosyl unit with C-6 substituted by a rhamnopyranosyl unit.[3,7,14,15] Because of the spin–spin relaxation time ($T_2$), the height of the carbon signals belonging to the galactose residue were significantly lower than those of the rhamnose and glucose residues, which were more flexible than the galactose residue. Glycosidation shifts of the rhamnopyranosyl C-4 ($\delta$ 72.3 → 82.3, $\Delta = 10$ ppm), C-3 ($\delta$ 71.0 → 70.7, $\Delta = -0.3$ ppm), and C-5 ($\delta$ 68.7 → 66.8, $\Delta = -0.9$ ppm) were observed by comparing with the $^{13}$C NMR data of kaempferol 3-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-galactopyranoside (**4**), which also supported the glucopyranosyl-(1 → 4)-rhamnopyranoside linkage.[3] Therefore, compound **1** was unambiguously determined as kaempferol 3-*O*-β-D-glucopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 6)-β-D-galactopyranoside.

Compound **2**, $[\alpha]_D^{23}$ 11.7° (*c* 0.31, MeOH), was obtained as a yellow amorphous powder. Its molecular formula was determined as $C_{27}H_{28}O_{16}$ by $^{13}$C NMR spectroscopy and by HRESIMS data (*m*/*z* 631.1210 for [M+Na$^+$]). Positive results of the HCl–Mg reaction and the Molish reaction indicated it to be a flavonoid glycoside. Its $^1$H NMR spectrum showed the presence of one chelated hydroxyl group ($\delta$ 12.4, s, 5-OH), three ABX protons belonging to a 3,4-disubstituted phenyl group ($\delta$ 7.50, 1H, s, H-2′; $\delta$ 6.82, 1H, d, $J$ 8.8 Hz, H-5′; $\delta$ 7.51, 1H, br, $J$ 8.8 Hz, H-6′), two protons typical of a 5,7-disubstituted ring A ($\delta$ 6.39, 6.19, each 1H, s, H-8 and 6), and an anomeric proton representing a glycosyl unit ($\delta$ 5.39, d, $J$ 6.8 Hz). These data, together with those of the $^{13}$C NMR spectroscopy, indicated the presence of a quercetin and a hexose unit. After acid hydrolysis, quercetin was obtained as the aglycone, and glucose was identified through GC–MS analysis of its alditol peracetate derivative. By comparing its $^{13}$C NMR spectrum with that of quercetin 3-*O*-β-D-glucopyranoside[5] and quercetin 3-*O*-6″-(3-hydroxyl-3-methylglutaryl)-β-D-galactopyranoside,[16] the glycosidation position was confirmed to be at C-3, and the 3-methyl-3-hydroxylglutaroyl group was assigned at the 6-OH of the glucose moiety. The acylation shift of the glucopyranose C-6 ($\delta$ 61.1 → 63.3, $\Delta = 2.2$ ppm) and C-5 ($\delta$ 77.6 → 74.3, $\Delta = -3.3$ ppm) was observed. Therefore, compound **2** was determined as quercetin 3-*O*-6″-(3-hydroxyl-3-methylglutaryl)-β-D-glucopyranoside.




1




2

## 1. Experimental

### 1.1. Materials and methods

Melting points were determined with an X-4 melting point apparatus (Taike Instruments Co.) and are uncorrected. Optical rotations were taken with a Perkin–Elmer 241 polarimeter. Infrared spectra were obtained using a Perkin–Elmer 16 PC FTIR spectrometer. $^1$H, $^{13}$C, and 2D NMR spectra were recorded in pyridine-$d_5$ on an AVANCE-500 (Bruker) and INOVA-600 (Varian) spectrometer. Chemical shifts were expressed in $\delta$ (ppm) referenced to solvent. FABMS was determined on a Finnigan MAT TSQ7000 and Bruker

**Table 1.** NMR data for compound **1**[a]

| No | $^{13}$C NMR $\delta$ (ppm) | Multiplicity[b] | $^{1}$H NMR $\delta$ (ppm), $J$ (Hz) | HMBC |
|---|---|---|---|---|
| 2 | 156.7 | s | | H-2′,6′ |
| 3 | 133.5 | s | | H-1″ |
| 4 | 177.5 | s | | |
| 4a | 104.0 | s | | H-6,8 |
| 5 | 161.3 | s | | H-6 |
| 6 | 99.0 | d | 6.20 (1H, s) | H-8 |
| 7 | 164.6 | s | | H-6,8 |
| 8 | 93.9 | d | 6.42 (1H, s) | H-6 |
| 8a | 156.6 | s | | H-8 |
| 1′ | 121.0 | s | | H-3′,5′ |
| 2′ | 131.1 | d | 8.05 (1H, d, $J$ 8.6 Hz) | H-3′,6′ |
| 3′ | 115.2 | d | 6.87 (1H, d, $J$ 8.6 Hz) | H-2′,5′ |
| 4′ | 160.1 | s | | H-2′,3′,5′,6′ |
| 5′ | 115.2 | d | 6.87 (1H, d, $J$ 8.6 Hz) | H-3′,6′ |
| 6′ | 131.1 | d | 8.05 (1H, d, $J$ 8.6 Hz) | H-2′,5′ |
| *Gal* | | | | |
| 1″ | 102.3 | d | 5.30 (1H, d, $J$ 7.7 Hz) | H-2″ |
| 2″ | 71.2 | d | 3.57 (1H) | H-4″ |
| 3″ | 73.1 | d | 3.42 (1H) | H-2″ |
| 4″ | 68.1 | d | 3.61 (1H) | H-5″,6″ |
| 5″ | 73.6 | d | 3.56 (1H) | H-6″ |
| 6″ | 65.4 | t | 3.23 (1H, dd, $J$ 9.5, 6.2 Hz) | H-5″,1‴ |
| | | | 3.62 (1H) | |
| *Rha* | | | | |
| 1‴ | 100.0 | d | 4.42 (1H, s) | H-6″ |
| 2‴ | 70.2 | d | 3.48 (1H) | H-1‴ |
| 3‴ | 70.7 | d | 3.56 (1H) | H-1‴,4‴,5‴ |
| 4‴ | 82.3 | d | 3.37 (1H) | H-4‴,5‴,1⁗ |
| 5‴ | 66.8 | d | 3.47 (1H) | H-1‴,4‴,6‴ |
| 6‴ | 17.9 | t | 1.16 (3H, s) | H-4‴,5‴ |
| *Glc* | | | | |
| 1⁗ | 104.6 | d | 4.34 (1H, d, $J$ 7.8 Hz) | H-1‴,2⁗ |
| 2⁗ | 74.6 | d | 2.99 (1H) | H-3⁗ |
| 3⁗ | 76.7 | d | 3.15 (1H) | H-2⁗ |
| 4⁗ | 70.2 | d | 3.02 (1H) | H-3⁗ |
| 5⁗ | 77.0 | d | 3.55 (1H) | H-4⁗ |
| 6⁗ | 61.3 | q | 3.44, 3.67 (1H, d, $J$ 10.9 Hz) | H-4⁗ |

[a] (DMSO-$d_6$).
[b] d = doublet; s = singlet; q = quartet; t = triplet.

APEX FTMS instruments, respectively. HRESIMS spectra were determined on FTMS-7 instrument (Bruker Daltonics). GC experiments were carried out on an HP-1 TCD instrument (Hewlett–Packard). Open column chromatography was carried out using D101 macroreticular resin (Tianjin Pesticide Co., China), silica gel (200–300 mesh, Qingdao Marine Chemical Co., China), octodecyl Si gel (40–60 μm, E. Merck) as well as Sephadex LH-20 (Pharmacia) as stationary phases. TLC was conducted on Silica Gel 60 $F_{254}$ S plates (Qingdao, China).

### 1.2. Plant material

The leaves of *G. sylvestre* R. Br. were collected in October 1996 from Beihai city, Guangxi Autonomous Region of China, and authenticated by Dr. Qin Min-Jan of China Pharmaceutical University. Voucher specimens were deposited in the herbarium of China Pharmaceutical University. The leaves of *E. ebracteolata* were collected in 1999 from Tongling city, Anhui province of China, and authenticated by Prof. Liu Xiao-Long of Anhui High Technical Secondary School of Chinese Traditional Medicine. Voucher specimens were deposited in the herbarium of China Pharmaceutical University.

### 1.3. Extraction and isolation

The air-dried leaves of *G. sylvestre* (Samples of *E. ebracteolata* were treated with the same procedure) were extracted through filtration with 95% EtOH. The EtOH extract was evaporated to dryness. The residue was dissolved in water and fractionated by successive

**Table 2.** NMR data for compound **2**[a]

| No | $^{13}$C NMR $\delta$ (ppm) | Multiplicity[b] | $^{1}$H NMR $\delta$ (ppm), $J$ (Hz) | HMBC |
|---|---|---|---|---|
| 2 | 156.5 | s | | H-2′,6′ |
| 3 | 133.1 | s | | H-1″ |
| 4 | 177.3 | s | | |
| 4a | 104.1 | s | | H-6,8 |
| 5 | 161.2 | s | | H-6 |
| 6 | 98.8 | d | 6.19 (1H, s) | H-8 |
| 7 | 164.1 | s | | H-6,8 |
| 8 | 93.7 | d | 6.39 (1H, s) | H-6 |
| 8a | 156.3 | s | | H-8 |
| 1′ | 121.6 | s | | H-2′,5′,6′ |
| 2′ | 116.2 | d | 7.50 (1H, s) | H-6′ |
| 3′ | 144.8 | s | | H-2′,5′ |
| 4′ | 148.5 | s | | H-2′,5′,6′ |
| 5′ | 115.2 | d | 6.82 (1H, d, $J$ 8.8 Hz) | H-6′ |
| 6′ | 121.1 | d | 7.51 (1H, br d, $J$ 8.8 Hz) | H-2′,5′ |
| *Glc* | | | | |
| 1″ | 100.8 | d | 5.39 (1H, d, $J$ 6.8 Hz) | |
| 2″ | 74.1 | d | 3.25 (1H) | H-3″ |
| 3″ | 76.3 | d | 3.23 (1H) | H-2″,4″ |
| 4″ | 70.1 | d | 3.11 (1H) | |
| 5″ | 74.3 | d | 3.30 (1H) | H-3″ |
| 6″ | 63.3 | t | 3.90 (1H, dd, $J$ 12.0, 6.8 Hz) | |
| | | | 4.12 (1H, d, $J$ 12.0 Hz) | |
| *Acyl.* | | | | |
| 1‴ | 170.1 | s | 5.39 (1H, d, $J$ 6.8 Hz) | H-2‴,1″ |
| 2‴ | 45.4 | t | 2.27 (1H, d, $J$ 14.4 Hz) | |
| | | | 2.39 (1H, d, $J = 14.4$ Hz) | |
| 3‴ | 68.9 | s | | H-2‴,4‴ |
| 4‴ | 45.2 | t | 2.23 (1H, d, $J = 15.2$ Hz) | H-2‴ |
| | | | 2.29 (1H, d, $J = 15.2$ Hz) | |
| 5‴ | 172.7 | s | | H-4‴ |
| 6‴ | 27.3 | q | 1.00 (3H, s) | H-2‴,4‴ |

[a](DMSO-$d_6$).
[b]d = doublet; s = singlet; q = quartet; t = triplet.

extraction with petroleum ether, EtOAc, and *n*-BuOH to give a petroleum ether-soluble fraction, an EtOAc-soluble fraction, and an *n*-BuOH-soluble fraction. The EtOAc-soluble fraction was then successively subjected to silica gel, ODS, and LH-20 columns to afford compounds **1**, **5**, **7**, **8**, and **9** (or compounds **2**, **3**, **4**, **6**, and **7** from *E. ebracteolata*).

### 1.4. Acid hydrolysis

HCl (1 N) was added to a MeOH (1:1 in volume) solution of **1** (or **2**). The mixture was heated under reflux for 2 h. The solution was extracted with EtOAc. The EtOAc solution was subjected to a silica gel column and washed with a gradient of 99:1–9:1 $CHCl_3$–$CH_3OH$. Yellow needles were obtained and were identified to be kaempferol (quercetin for **2**) by comparing with an authentic sample using high-performance thin-layer chromatography (HPTLC). The water layer was neutralized; and alditol peracetate derivatives were prepared for GC–MS examination.

Compound **1**: $C_{33}H_{40}O_{20}$, yellow needles, mp 207–209 °C; $[\alpha]_D^{22}$ −31.1° (*c* 0.48, MeOH); $UV_{max}$ (MeOH): 268, 349 nm; IR $\nu_{max}$ (KBr) cm$^{-1}$: 3410, 2925, 1656 (C=O of the aglycon), 1606, 1502, 1446, 1361, 1178, 1070, 1026; FABMS (*m*/*z*): 779 [M+Na$^+$]; HRESIMS (*m*/*z*): Calcd for $C_{33}H_{40}O_{20}Na$ [M+Na$^+$] 779.2005; found 779.2020. $^1$H and $^{13}$C NMR (DMSO-$d_6$) data are listed in Table 1.

Compound **2**: $C_{27}H_{28}O_{16}$, yellow amorphous powder, mp 146–148 °C; $[\alpha]_D^{23}$ 11.7° (*c* 0.31, MeOH); $UV_{max}$ (MeOH): 256, 358 nm; IR $\nu_{max}$ (KBr) cm$^{-1}$: 3416, 2987, 2913, 1721 (C=O), 1714 (C=O), 1652 (C=O of the aglycon), 1606, 1504, 1447, 1359, 1203, 1086, 1014; FABMS (*m*/*z*): 609 [M+H$^+$]; HRESIMS (*m*/*z*): Calcd for $C_{27}H_{28}O_{16}Na$ [M+Na$^+$] 631.1269; found 631.1210. $^1$H and $^{13}$C NMR (DMSO-$d_6$) data are listed in Table 2.

## References

1. Kapoor, L. D. *CRC Handbook of Ayurvedic Medicinal Plants*; CRC: Boca Raton, FL, 1990. pp 200–201.

2. Porchezhian, E.; Dobriyal, R. M. *Pharmazie* **2003**, *58*, 5–12.
3. Brasseur, T.; Angenot, L. *Phytochemistry* **1986**, *25*, 563–564.
4. Beck, M.-A.; Häberlein, H. *Phytochemistry* **1999**, *50*, 329–332.
5. Markham, K. R.; Tenai, B.; Stanley, R.; Geiger, H.; Mabry, T. J. *Tetrahedron* **1978**, *34*, 1389–1397.
6. Elliger, C. A.; Eash, J. A.; Waiss, A. C., Jr. *Biochem. Syst. Ecol.* **1992**, *20*, 268.
7. Ahmed, A. A.; Ishak, M. S.; Micheal, H. N.; EI-Ansari, M. A.; EI-Sissi, H. I. *J. Nat. Prod.* **1991**, *54*, 1092–1093.
8. Xu, Z. H.; Sun, J.; Xu, R. S.; Qin, G. W. *Phytochemistry* **1998**, *49*, 149–151.
9. Grzegora, P.; Jaromir, B. *Planta Med.* **1996**, *62*, 95–96.
10. Isobe, T.; Ito, N.; Noda, Y. *Phytochemistry* **1980**, *19*, 1877.
11. Markham, K. R.; Ternai, B.; Stanley, R. *Tetrahedron* **1978**, *34*, 1389–1397.
12. Bennini, B.; Chulia, A. J.; Kaouadji, M.; Thomasson, F. *Phytochemistry* **1992**, *31*, 2483–2486.
13. Fox, A.; Morgan, S. L.; Gilbart, J. *Analysis of Carbohydrates by GLC and MS*; CRC: Boca Raton, FL, 1988. pp 87–117.
14. Webby, R. F.; Markham, K. R. *Phytochemistry* **1990**, *29*, 289–292.
15. Salah, H. B.; Jarraya, R.; Martin, M.-T.; Veitch, N. C.; Grayer, R. J.; Simmonds, M. S. J.; Damak, M. *Chem. Pharm. Bull.* **2002**, *50*, 1268–1270.
16. Wald, B.; Galensa, R.; Herrmann, K.; Grotjahn, L.; Wray, V. *Phytochemistry* **1986**, *25*, 2904–2905.