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# 3-Hydroxy-3-Methylglutaryl Flavonol Glycosides from *Oxytropis falcata*

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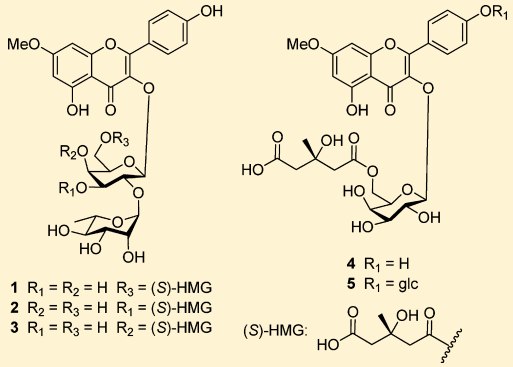
Supporting Information

**ABSTRACT:** Five new 3-hydroxy-3-methylglutaryl (HMG) flavonol 3-*O*-glycosides, named oxytroflavosides A–E (**1**–**5**), and two new rhamnocitrin 3-*O*-glycosides, oxytroflavosides F and G (**6** and **7**) were isolated from the n-BuOH-soluble fraction of an EtOH extract of *Oxytropis falcata* together with seven known kaempferol glycosides (**8**–**14**), of which six were isolated from the genus *Oxytropis* for the first time. The structures of these compounds were elucidated by spectroscopic techniques and chemical methods. The absolute configuration of HMG in compounds **1**–**5** was determined to be *S* through spectroscopic analysis of the mevalonamide obtained by amidation and reduction of the HMG moiety. Compounds **1**–**10** were evaluated for anti-inflammatory activities using lipopolysaccharide-induced RAW 264.7 cells, but none of them showed inhibitory effects on NO production.




*Oxytropis falcata* Bunge (Leguminosae), known as "King of Herbs" in Tibetan medicines, is mainly distributed at lake and river beaches, in ravines, on slopes, in steppe meadows, and in shrubberies throughout the Qinghai-Tibet Plateau in China. As a commonly used folk medicine, it is mainly used to treat arthritis, influenza, leprosy, and constipation. Modern pharmacological studies showed that its total flavonoids had good anti-inflammatory and analgesic activities in vivo,[1] and 2′,4′-dihydroxychalcone, a main constituent of this plant, exhibited significant inhibitory activity against NO production in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells[2] and antiproliferative activity against several cancer cells in vitro.[3] Chemical investigations of this plant were initiated in the past decade,[4] and flavonoids were shown to be major constituents. Isoflavans,[5] 24-hydroxyoleanane-type triterpenoids,[6] and *N*-benzoylindole analogues[7] have been reported from *O. falcata*. However, few details of chemical constituents of higher polarity have been published. In this study we report the isolation and identification of seven new rhamnocitrin 3-*O*-glycosides (**1**–**7**), five of which are 3-hydroxy-3-methylglutaryl (HMG)-moiety-conjugated compounds (**1**–**5**), from the n-BuOH-soluble fraction of an EtOH extract of the whole plant of *O. falcata*. Seven known kaempferol glycosides (**8**–**14**) were also isolated. Their anti-inflammatory activities against NO production in lipopolysaccharide (LPS)-induced RAW 264.7 cells were also described.

## RESULTS AND DISCUSSION

Compound **1** was isolated as an amorphous, yellow powder, and its molecular formula was determined to be $C_{34}H_{40}O_{19}$ by HRESIMS at *m*/*z* 753.2238 $[M + H]^+$ (calcd 753.2236). The IR spectrum showed typical absorption bands for OH (3408 $cm^{-1}$), carbonyl (1723 $cm^{-1}$), conjugated carbonyl (1656 $cm^{-1}$), and benzyl groups (1597 and 1499 $cm^{-1}$). The UV spectrum revealed absorption bands at 267 and 348 nm, suggesting a flavonol skeleton. The $^1H$ and $^{13}C$ NMR data exhibited signals characteristic of 5,7,4′-trisubstituted flavonol glycosides, with an $OCH_3$ [$^{13}C$: $\delta$ 56.1; $^1H$: $\delta$ 3.85 (s)] and two sugar units evidenced by signals of two anomeric carbons [$\delta$ 98.6 (C-1″), 100.6 (C-1‴)] and their corresponding anomeric protons [$\delta$ 5.58 (H-1″, d, $J$ = 8.0 Hz), 5.06 (H-1‴, s)]. The HMBC correlation between $\delta$ 3.85 ($OCH_3$) and $\delta$ 165.1 (C-7) placed the $OCH_3$ at C-7 of the aglycone. Acid hydrolysis of **1** yielded rhamnocitrin, i.e., 5,4′-dihydroxy-7-methoxyflavonol, by comparison of its NMR data with literature,[8] and two sugars. The sugars were identified as D-galactose and L-rhamnose by GC analysis, in which the retention times of derivatives of the sugar residues and standard sugars were compared, as described in the Experimental Section. The glycosidic linkage was established by the HMBC correlations between $\delta$ 5.58 (H-1″) and $\delta$ 132.9 (C-3) and between $\delta$ 5.06 (H-1‴) and $\delta$ 74.8 (C-2″), indicating that the galactose unit was located at C-3 of

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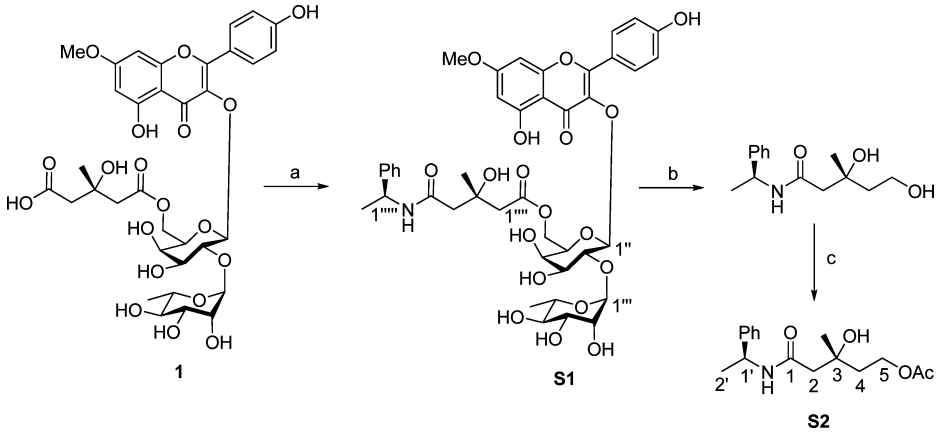
**Scheme 1. Determination of the Absolute Configuration of HMG Group in Compound 1**[a]

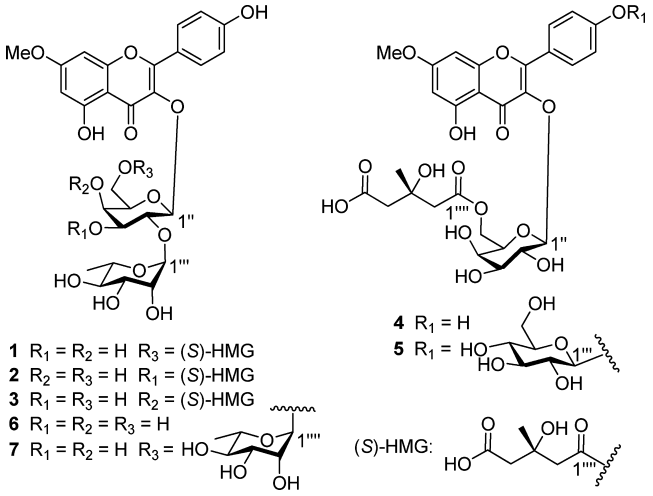



[a]Key: (a) (*S*)-1-phenylethylamine, DMF, $Et_3N$, PyBOP, HOBt; (b) $LiBH_4$, THF; (c) $Ac_2O$, pyridine.

aglycone and the rhamnose unit was attached to C-2 of galactose. The remaining signals in the $^1H$ and $^{13}C$ NMR spectra of **1** were identified as an HMG moiety by HMBC correlations between $\delta$ 2.37 (H-2a‴), 2.21 (H-2b‴) and $\delta$ 170.1 (C-1‴), between $\delta$ 2.23 (H-4‴) and $\delta$ 172.6 (C-5‴), and between $\delta$ 0.99 (H-6‴) and $\delta$ 68.7 (C-3‴), 45.3 (C-2‴), 45.2 (C-4‴). Characteristic HMG fragment ions appeared in the negative ESIMS spectrum.[9] The HMG substituent was placed at C-6 of galactose by HMBC correlations between $\delta$ 3.91, 4.00 (H-6 of galactose) and $\delta$ 170.1 (C-1‴). The absolute configuration of the chiral carbon in the HMG group was determined as follows (Scheme 1).[10] Amidation with (*S*)-1-phenylethylamine afforded compound **S1**. Reduction of **S1** with $LiBH_4$, followed by acetylation with $Ac_2O$, yielded 5-*O*-acetyl-1-[(*S*)-phenylethyl]mevalonamide (**S2**). Its $^1H$ NMR data were identical to those of (3*R*)-5-*O*-acetyl-1-[(*S*)-phenylethyl]-mevalonamide rather than the (3*S*) isomer.[11] Thus, **1** was characterized unambiguously as rhamnocitrin-3-*O*-[(*S*)-3-hydroxy-3-methylglutaryl-(1→6)]-[α-L-rhamnopyranosyl-(1→2)]-β-D-galactopyranoside, and it was named oxytroflavoside A.

Compound **2** had the same molecular formula as **1**, $C_{34}H_{40}O_{19}$, on the basis of HRESIMS. The UV, IR, $^1H$ NMR, and $^{13}C$ NMR data were similar to those of compound **1**. Acid hydrolysis also produced D-Gal, L-Rha, and rhamnocitrin. The 1D and 2D NMR spectra allowed the assignment of all signals, and the only difference between the two compounds was the location of the HMG moiety; it was placed at C-3, not C-6, of galactose in compound **2** on the basis of an HMBC correlation between $\delta$ 4.88 (H-3″) and $\delta$ 170.1 (C-1‴). The absolute configuration of the HMG group was also determined to be *S* using the method mentioned above. Consequently, compound **2** was characterized as rhamnocitrin-3-*O*-[(*S*)-3-hydroxy-3-methylglutaryl-(1→3)]-[α-L-rhamnopyranosyl-(1→2)]-β-D-galactopyranoside, and it was named oxytroflavoside B.

Compound **3** had a molecular formula of $C_{34}H_{40}O_{19}$, the same as compounds **1** and **2**, by HRESIMS. The $^1H$ and $^{13}C$ NMR data were again similar to those of compound **1**, indicative of a rhamnocitrin 3-*O*-diglycoside with an HMG substituent. Acid hydrolysis and coupling patterns of anomeric protons confirmed the presence of β-D-galactose and α-L-rhamnose, and their positons as determined by HMBC were the same as in compound **1**. A significant downfield shift of H-4 of galactose ($\delta$ 5.08) compared with that of compound **1** ($\delta$ 3.62−3.65) implied that the HMG substituent was at C-4 of




galactose, which was further supported by an HMBC correlation between $\delta$ 5.08 (H-4″) and $\delta$ 170.3 (C-1‴). The 3*S*-configuration of the HMG moiety was determined as described above. Thus, the structure of **3** was identified as rhamnocitrin-3-*O*-[(*S*)-3-hydroxy-3-methylglutaryl-(1→4)]-[α-L-rhamnopyranosyl-(1→2)]-β-D-galactopyranoside, and it was named oxytroflavoside C.

Compound **4** had a molecular formula of $C_{28}H_{30}O_{15}$, as deduced from HRESIMS. The $^1H$ and $^{13}C$ NMR data differed from those of **1** by the absence of a set of signals corresponding to one rhamnose, and this was consistent with the results of acid hydrolysis, which yielded only D-Gal and rhamnocitrin. Further analysis of the 1D and 2D NMR spectra and determination of the absolute configuration of the HMG group confirmed the structure of **4** to be rhamnocitrin-3-*O*-[(*S*)-3-hydroxy-3-methylglutaryl-(1→6)]-β-D-galactopyranoside, and it was named oxytroflavoside D.

Compound **5** had a molecular formula of $C_{34}H_{40}O_{20}$ by HRESIMS. The $^1H$ and $^{13}C$ NMR data were similar to those of **4**, but differed in the occurrence of only one sugar unit in **4**, and two sugar residues were observed in **5** on the basis of the signals of two anomeric carbons [$\delta$ 101.6 (C-1″), 100.1 (C-1‴)] and their corresponding anomeric protons [$\delta$ 5.35 (H-1″), 5.02 (H-1‴)]. Acid hydrolysis yielded D-glucose and D-galactose, determined by preparation of trimethylsilylated derivatives and comparison with standards in GC analysis. The β-anomeric configuration of each sugar was assigned from the large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coupling constant ($J$ = 7.5 Hz) of each anomeric proton. In addition, a blue shift of 25 nm for absorption band I in the UV spectrum compared with that of **4** implied the glycosylation of the C-4′ OH group,[12] which was supported by an HMBC correlation between $\delta$ 5.02 (glc H-1) and $\delta$ 159.3 (C-4′). Consequently, compound **5** was characterized as rhamnocitrin-3-*O*-[(*S*)-3-hydroxy-3-methylglutaryl-(1→6)]-*β*-D-galactopyranosyl-4′-*O*-*β*-D-glucopyranoside, and it was named oxytroflavoside E.

Compound **6**, $C_{28}H_{32}O_{15}$, had $^1$H and $^{13}$C NMR data similar to those of **1**, except that there were no signals for an HMG moiety. Analysis of the 2D NMR spectra combined with acid hydrolysis revealed compound **6** to be rhamnocitrin-3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside, and it was named oxytroflavoside F.

Compound **7**, $C_{34}H_{42}O_{19}$, had $^1$H and $^{13}$C NMR data that resembled those of **6**, except for the presence of an additional *α*-L-rhamnose unit, as was confirmed by the acid hydrolysis experiment. Furthermore, compared with the $^{13}$C NMR data of **6**, a downfield shift of 5.1 ppm for C-6″ as well as an upfield shift of 2.2 ppm for C-5″ indicated glycosylation of the C-6 OH of galactose. An HMBC correlation between $\delta$ 4.35 (H-1‴) and $\delta$ 65.2 (C-6″) confirmed the attachment. Thus, compound **7** was characterized as rhamnocitrin-3-*O*-*α*-L-rhamnopyranosyl-(1→6)-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-galactopyranoside, and it was named oxytroflavoside G.

The known compounds were identified as robinin (**8**),[13] kaempferol-3-*O*-robinobioside (**9**),[14] mauritianin (**10**),[15] astrasikokioside I (**11**),[13] kaempferol-3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranosyl-7-*O*-*α*-L-rhamnopyranoside (**12**),[13] kaempferol-3-*O*-[*β*-D-xylopyranosyl-(1→3)-*α*-L-rhamnopyranosyl-(1→6)]-*β*-D-galactopyranoside (**13**),[16] and kaempferol-3-*O*-[*β*-D-xylopyranosyl-(1→3)-*α*-L-rhamnopyranosyl-(1→6)]-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-galactopyranoside (**14**)[17] by comparison of the spectral data with those previously reported in the literature. Compounds **9**−**14** were isolated from the genus *Oxytropis* for the first time, and compound **8** was obtained from this plant for the first time.

The anti-inflammatory effects of compounds **1**−**10** were evaluated for the ability to inhibit NO production in LPS-induced RAW 264.7 cells,[18] but none of the compounds were active, with inhibition rates of lower than 5% at 50 $\mu$M. As a positive control, 2′,4′-dihydroxychalcone isolated from this plant inhibited NO production by 40.1% at a concentration of 50 $\mu$M, which agreed with the result in a previous study[2] of its inhibitory effect on NO production. It was reported that 2′,4′-dihydroxychalcone is one of the main constituents of this plant, accounting for 7.28 ± 0.13 mg/g in *O. falcata*[2] and 6.1% of its total flavonoids.[1] Thus, this compound could explain, in part, the in vivo anti-inflammatory activities of total flavonoids of *O. falcata* reported previously.[1] However, whether compounds **1**−**10** contribute to the anti-inflammatory activities of *O. falcata* will require further investigation using in vivo and other in vitro models.

The HMG group has been occasionally found conjugated in natural products such as flavonoids,[19] triterpenoids,[20] and steroids,[21] with the majority of them being flavonoid glycosides. An extensive literature survey showed that it tends to connect with C-6 of glucose or galactose. This study is the first report of the simultaneous isolation of three flavonol 3-*O*-glycoside isomers (**1**−**3**) with the HMG moiety located respectively at C-6, C-3, and C-4 of galactose. During the process of isolation we found that the amount of compound **1** was significantly greater than that of **2** and **3**, probably due to the lesser steric hindrance of C-6 compared with that of C-3 and C-4 of galactose. By detailed comparison of the $^{13}$C NMR data of compounds **1**−**3** with that of **6**, we can conclude that HMG esterification of the OH group generally leads to a downfield shift of 2−3 ppm for the carbon that HMG directly links to, as well as an upfield shift of 2−3 ppm for the adjacent carbons.

The determination of the absolute configuration of the HMG group was a key step in the process of structural elucidation. Generally, the methods designed to address this problem were all composed of three steps: reduction, amidation with (*S*)-1-phenylethylamine, and acylation with $Ac_2O$. The original method was to reduce the HMG ester with borane in the first step, unfortunately giving an unreasonable result of 3*R*-configuration for the HMG group.[11c] This was contradictory to the biosynthetic mevalonic acid pathway, in which naturally occurring HMG esters are formed via acylation of an OH group by (3*S*)-HMG-CoA. The unreasonable results were later revised to be 3*S*-configuration by replacing borane with $LiBH_4$ or $LiEt_3BH$.[11a,b] Although the latter method was reliable, its yield was low (10−30%) and, thus, was not applicable to natural compounds with only small amounts available. Recently, a refined method succeeded in enhancing the reaction yield to 70% simply by reversing the steps of reduction and amidation.[10] Considering the limited amounts of some compounds, the refined method was adopted in this study, and the *S*-configuration of the HMG groups in compounds **1**−**5** was determined unambiguously.

## EXPERIMENTAL SECTION

**General Experimental Procedures.** Optical rotations were measured with a Rudolph Research Analytical Autopol III automatic polarimeter. UV spectra were measured on a Shimadzu UV-260 spectrometer. IR spectra were recorded on a Nicolet Nexus 470 FT-IR spectrophotometer using KBr pellets. NMR spectra were recorded on a Bruker AVANCE III-400 or a Varian INOVA-500 instrument. Chemical shift values are given in $\delta$ (ppm) using the peak signals of solvent DMSO-$d_6$ ($\delta_H$ 2.50 and $\delta_C$ 39.5) as references, and coupling constants are reported in Hz. HRMS data were recorded on a Agilent QTOF 6538 or a Bruker APEX II FT-ICRMS spectrometer. ESIMS data were obtained using an Abseciex QSTAR spectrometer. EIMS data were measured on a Finnigan TRACE instrument. GC analysis was performed on an Agilent 6890N GC system (Agilent Co., USA). Column chromatography (CC) was performed on Diaion HP20 (200−300 mesh, Mitsubishi Chemical Co., Japan), MCI gel (20−45 $\mu$m, Fuji Silysia Chemical Co., Japan), ODS-A (50 $\mu$m, YMC Co. Ltd., Japan), Amberlite MB-3 (Organo, Tokyo, Japan), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Analytical and preparative TLC were carried out on precoated silica gel 60 F254 plates (Merck, Germany), and spots were visualized by 10% $H_2SO_4$/EtOH reagent. All solvents used for extraction and isolation were of analytical grade and purchased from Beijing Fine Chemicals (China) without further purification. All chemicals, unless otherwise noted, were purchased from J & K Co. Ltd. (China).

**Plant Material.** The whole plant of *O. falcata* was collected from Guide County, Qinghai Province, in August 2008, and was authenticated by Associate Professor Ying-Tao Zhang, Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University Health Science Center. A voucher specimen (No. 20080801) was deposited in the Herbarium of Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University Health Science Center.

**Extraction and Isolation.** The dried whole plant of *O. falcata* (4.0 kg) was pulverized and in turn percolated exhaustively with 95% EtOH and 50% EtOH. After concentration, the 50% EtOH extract (401 g) was successively partitioned with petroleum ether, $CHCl_3$, EtOAc, and


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Table 1. $^1$H NMR Spectroscopic Data of Compounds 1−7[a] in DMSO-$d_6$

| position | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| 6 | 6.35, d (2.0) | 6.39, d (2.0) | 6.36, d (2.0) | 6.35, d (1.5) | 6.37 br, s | 6.37, d (2.0) | 6.36, d (2.0) |
| 8 | 6.71, d (2.0) | 6.75, d (2.0) | 6.74 br, s | 6.72, d (1.5) | 6.76 br, s | 6.74, d (2.0) | 6.72, d (2.0) |
| 2′/6′ | 8.07, d (8.8) | 8.12, d (9.0) | 8.08, d (9.0) | 8.07, d (8.5) | 8.15, d (9.0) | 8.12, d (9.0) | 8.09, d (9.0) |
| 3′/5′ | 6.86, d (8.8) | 6.88, d (9.0) | 7.00, d (9.0) | 6.87, d (8.5) | 7.13, d (9.0) | 6.87, d (9.0) | 6.85, d (9.0) |
| OMe-7 | 3.85, s | 3.87, s | 3.86, s | 3.85, s | 3.86, s | 3.86, s | 3.86, s |
| 1″ | 5.58, d (8.0) | 5.78, d (7.5) | 5.67, d (7.5) | 5.31, d (7.5) | 5.35, d (7.5) | 5.66, d (8.0) | 5.57, d (8.0) |
| 2″ | 3.74−3.80[b] | 4.00, dd (10.0, 7.5) | 3.76, dd (9.5, 7.5) | 3.56, t (8.5) | 3.53, t (9.0) | 3.80, dd (9.5, 8.0) | 3.80, t (9.0) |
| 3″ | 3.62−3.65[b] | 4.88, dd (10.0, 3.0) | 3.81, m | 3.39, dd (10.0, 3.0) | 3.38−3.40[b] | 3.59 br, d (9.0) | 3.56−3.62[b] |
| 4″ | 3.62−3.65[b] | 3.89, d (3.0) | 5.08, d (3.0) | 3.63, d (3.0) | 3.64, d (2.5) | 3.65 br, s | 3.56−3.62[b] |
| 5″ | 3.62−3.65[b] | 3.51, t (6.5) | 3.63, t (6.0) | 3.60, t (6.0) | 3.61, t (6.5) | 3.36, t (5.5) | 3.56−3.62[b] |
| 6″ | 4.00, dd (11.2, 8.0)<br>3.91, dd (11.2, 4.0) | 3.39−3.43[b]<br>3.27, dd (10.5, 5.0) | 3.15, m | 3.98, dd (11.0, 7.0)<br>3.93, dd (11.0, 4.0) | 3.97, dd (11.5, 7.5)<br>3.90, dd (11.5, 5.0) | 3.43, dd (10.5, 6.0)<br>3.28, dd (10.5, 5.5) | 3.56−3.62[b]<br>3.23, dd (13.0, 9.5) |
| 1‴ | 5.06, s | 4.80, s | 4.98, s | | 5.02, d (7.5) | 5.06, s | 5.06, s |
| 2‴ | 3.74−3.80[b] | 3.59, br, s | 3.70 br, s | | 3.26, t (9.0) | 3.74 br, s | 3.74 br, s |
| 3‴ | 3.48, dd (9.2, 2.8) | 3.39−3.43[b] | 3.44, dd (9.5, 3.5) | | 3.38−3.40[b] | 3.47, dd (9.5, 3.0) | 3.50, dd (9.0, 3.0) |
| 4‴ | 3.14, t (9.2) | 3.12 br, t (9.0) | 3.11, t (9.5) | | 3.18, t (9.0) | 3.12, t (9.5) | 3.14, t (9.0) |
| 5‴ | 3.74−3.80[b] | 3.72, dq (9.5, 6.0) | 3.73, dq (9.5, 6.5) | | 3.31, m | 3.73, dq (9.5, 6.0) | 3.78, dq (9.0, 6.0) |
| 6‴ | 0.79, d (6.0) | 0.76, d (6.0) | 0.75, d (6.5) | | 3.69, dd (12.0, 1.5)<br>3.48, dd (12.0, 5.5) | 0.75, d (6.0) | 0.80, d (6.0) |
| 1⁗ | | | | | | | 4.35, s |
| 2⁗ | 2.37, d (14.0)<br>2.21, d (14.0) | 2.77, d (14.5)<br>2.62, d (14.5) | 2.64, d (13.5)<br>2.41, d (13.5) | 2.29, d (14.0)<br>2.18, d (14.0) | 2.22, d (13.0)<br>2.11, d (13.0) | | 3.34−3.35[b] |
| 3⁗ | | | | | | | 3.28, dd (9.0, 3.0) |
| 4⁗ | 2.23, s | 2.55, s | 2.16, d (14.0)<br>2.08, d (14.0) | 2.14, d (15.0)<br>2.02, d (15.0) | 2.04, d (15.0)<br>1.88, d (15.0) | | 3.08, t (9.0) |
| 5⁗ | | | | | | | 3.34−3.35[b] |
| 6⁗ | 0.99, s | 1.34, s | 1.21, s | 0.97, s | 0.92, s | | 1.05, d (6.0) |

[a]Measured at 400 MHz for **1** and 500 MHz for **2**−**7**. [b]Overlapping signals.

n-BuOH. The n-BuOH extract (136.0 g) was subjected to Dianion HP20 macroporous absorbent resin CC using an EtOH/$H_2O$ gradient (0, 20, 30, 50, 70, and 95% EtOH) to yield six fractions (I−VI). Fraction III (eluted with 30% EtOH, 8.9 g) was subjected to MCI gel CC with a gradient system of MeOH/$H_2O$ (0, 30, 50, 70, 100% MeOH) to afford five subfractions (Da−De). Further purification of Dc (2.3 g) by CC on Sephadex LH-20 (MeOH/$H_2O$, 1:1), on ODS-A (MeOH/$H_2O$, 1:4), and again on Sephadex LH-20 (MeOH/$H_2O$, 1:2) afforded compounds **5** (9 mg), **8** (13 mg), **10** (25 mg), **11** (22 mg), **12** (13 mg), **13** (16 mg), and **14** (27 mg). Fraction Dd (1.7 g) was subjected to repeated Sephadex LH-20 CC eluted with MeOH/$H_2O$ (1:2) to give compound **9** (18 mg). Fraction IV (eluted with 50% EtOH, 19.4 g) was subjected to MCI gel CC using the same elution system as fraction III to give a subfraction (eluted with 50% MeOH, 10.5 g), which was further separated on silica gel CC (EtOAc/EtOH/$H_2O$, 40:2:1−8:2:1) to yield five subfractions (Ga−Ge). Further purification of Ga (0.7 g), Gb (3.2 g), and Gd (2.1 g) through repeated Sephadex LH-20 CC (MeOH/$H_2O$, 1:1) and preparative TLC (plate: 20 × 20 cm, developing solvents: EtOAc/EtOH/$H_2O$/HCOOH, 16:2:1:1) afforded six compounds: **4** (16 mg) from Ga; **1** (320 mg), **3** (8 mg), and **2** (30 mg) from Gb; **7** (25 mg) and **6** (50 mg) from Gd.

*Oxytroflavoside A (**1**):* amorphous, yellow powder; $[\alpha]^{26}_D$ −78.3 (*c* 0.18, MeOH); UV (MeOH) $\lambda_{max}$ 267, 348 nm; IR (KBr) $\nu_{max}$ 3408, 2930, 1723, 1656, 1597, 1499, 1447, 1349, 1282, 1213, 1168, 1136, 1087 cm$^{-1}$; $^1$H NMR and $^{13}$C NMR (DMSO-$d_6$) data, see Tables 1 and 2; HRESIMS *m*/*z* 753.2238 [M + H]$^+$ (calcd for $C_{34}H_{41}O_{19}$, 753.2236).

*Oxytroflavoside B (**2**):* amorphous, yellow powder; $[\alpha]^{26}_D$ −68.8 (*c* 0.17, MeOH); UV (MeOH) $\lambda_{max}$ 267, 348 nm; IR (KBr) $\nu_{max}$ 3385, 2930, 1657, 1596, 1499, 1447, 1348, 1283, 1213, 1169, 1139, 1085 cm$^{-1}$; $^1$H NMR and $^{13}$C NMR (DMSO-$d_6$) data, see Tables 1 and 2; HRESIMS *m*/*z* 753.2238 [M + H]$^+$ (calcd for $C_{34}H_{41}O_{19}$, 753.2236).

*Oxytroflavoside C (**3**):* amorphous, yellow powder; $[\alpha]^{26}_D$ −80.0 (*c* 0.20, MeOH); UV (MeOH) $\lambda_{max}$ 267, 348 nm; IR (KBr) $\nu_{max}$ 3367, 2936, 1731, 1658, 1595, 1498, 1448, 1349, 1285, 1214, 1169, 1136, 1058, 1025 cm$^{-1}$; $^1$H NMR and $^{13}$C NMR (DMSO-$d_6$) data, see Tables 1 and 2; HRESIMS *m*/*z* 753.2235 [M + H]$^+$ (calcd for $C_{34}H_{41}O_{19}$, 753.2236).

*Oxytroflavoside D (**4**):* amorphous, yellow powder; $[\alpha]^{26}_D$ −17.1 (*c* 0.14, MeOH); UV (MeOH) $\lambda_{max}$ 268, 348 nm; IR (KBr) $\nu_{max}$ 3271, 2926, 1732, 1657, 1594, 1498, 1449, 1349, 1286, 1213, 1169, 1087, 1020 cm$^{-1}$; $^1$H NMR and $^{13}$C NMR (DMSO-$d_6$) data, see Tables 1 and 2; HRESIMS *m*/*z* 607.1668 [M + H]$^+$ (calcd for $C_{28}H_{31}O_{15}$, 607.1657).

*Oxytroflavoside E (**5**):* amorphous, yellow powder; $[\alpha]^{26}_D$ −76.5 (*c* 0.17, MeOH); UV (MeOH) $\lambda_{max}$ 268, 323 nm; IR (KBr) $\nu_{max}$ 3367, 2927, 1726, 1657, 1598, 1501, 1452, 1352, 1303, 1247, 1214, 1168, 1074 cm$^{-1}$; $^1$H NMR and $^{13}$C NMR (DMSO-$d_6$) data, see Tables 1 and 2; HRESIMS *m*/*z* 769.2187 [M + H]$^+$ (calcd for $C_{34}H_{41}O_{20}$, 769.2185).

*Oxytroflavoside F (**6**):* amorphous, yellow powder; $[\alpha]^{26}_D$ −84.2 (*c* 0.24, MeOH); UV (MeOH) $\lambda_{max}$ 267, 348 nm; IR (KBr) $\nu_{max}$ 3394, 2930, 1657, 1596, 1499, 1447, 1348, 1283, 1213, 1167, 1139, 1085 cm$^{-1}$; $^1$H NMR and $^{13}$C NMR (DMSO-$d_6$) data, see Tables 1 and 2; HRSIMS *m*/*z* 609.1811 [M + H]$^+$ (calcd for $C_{28}H_{33}O_{15}$, 609.1814).

*Oxytroflavoside G (**7**):* amorphous, yellow powder; $[\alpha]^{26}_D$ −86.2 (*c* 0.13, MeOH); UV (MeOH) $\lambda_{max}$ 267, 348 nm; IR (KBr) $\nu_{max}$ 3392, 2927, 1657, 1596, 1499, 1448, 1349, 1283, 1214, 1168, 1137, 1052 cm$^{-1}$; $^1$H NMR and $^{13}$C NMR (DMSO-$d_6$) data, see Tables 1 and 2; HRSIMS *m*/*z* 755.2400 [M + H]$^+$ (calcd for $C_{34}H_{43}O_{19}$, 755.2393).

**Acid Hydrolysis of Compounds 1−7 (ref 22).** Compounds **1**−**7** (each 1.0 mg) were hydrolyzed with 1 N HCl (1.0 mL) for 6 h at 100 °C. After cooling, the yellow precipitate was obtained by filtration and proved to be rhamnocitrin by NMR.

*Rhamnocitrin:* yellow solid; $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ 12.5 (1H, s, O*H*-5), 10.2 (1H, s, O*H*-4′), 9.50 (1H, s, O*H*-3), 8.09 (2H, d, *J* = 8.8 Hz, H-2′, H-6′), 6.94 (2H, d, *J* = 8.8 Hz, H-3′, H-5′), 6.75 (1H, d, *J* = 2.0 Hz, H-8), 6.36 (1H, d, *J* = 2.0 Hz, H-6), 3.87 (3H, s, OC*H*$_3$); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) $\delta$ 176.0 (C, C-4), 164.9 (C, C-7), 160.4 (C, C-5), 159.3 (C, C-4′), 156.1 (C, C-9), 147.2 (C, C-2), 135.9 (C, C-3), 129.5 (CH, C-2′, C-6′), 121.5 (C, C-1′), 115.4 (CH, C-3′, C-5′), 104.0 (C, C-10), 97.4 (CH, C-6), 92.0 (CH, C-8), 56.0 (CH$_3$, OCH$_3$).


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Table 2. $^{13}$C NMR Spectroscopic Data of Compounds 1−7[a] in DMSO-$d_6$

| position | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| 2 | 156.7 | 156.5 | 157.7 | 156.8 | 156.4 | 156.4 | 156.9 |
| 3 | 132.9 | 132.7 | 132.2 | 133.4 | 134.0 | 133.0 | 132.8 |
| 4 | 177.4 | 177.3 | 177.5 | 177.6 | 177.6 | 177.5 | 177.4 |
| 5 | 160.9 | 160.9 | 160.9 | 160.9 | 160.8 | 160.9 | 160.9 |
| 6 | 97.9 | 97.9 | 97.9 | 97.9 | 98.0 | 97.9 | 97.9 |
| 7 | 165.1 | 165.1 | 165.0 | 165.1 | 165.2 | 165.0 | 165.0 |
| 8 | 92.3 | 92.3 | 92.3 | 92.3 | 92.4 | 92.2 | 92.3 |
| 9 | 156.3 | 156.2 | 156.3 | 156.3 | 156.1 | 156.2 | 156.3 |
| 10 | 105.0 | 104.9 | 104.9 | 104.9 | 105.0 | 104.9 | 104.9 |
| 1′ | 120.7 | 120.6 | 119.5 | 120.4 | 123.5 | 120.7 | 120.3 |
| 2′/6′ | 130.9 | 130.9 | 130.5 | 130.9 | 130.6 | 130.9 | 130.9 |
| 3′/5′ | 115.1 | 115.1 | 115.6 | 115.2 | 115.8 | 115.1 | 115.3 |
| 4′ | 160.0 | 160.1 | 161.5 | 160.5 | 159.3 | 160.1 | 160.9 |
| OMe-7 | 56.1 | 56.1 | 56.1 | 56.1 | 56.1 | 56.1 | 56.1 |
| 1″ | 98.6 | 98.5 | 98.3 | 101.7 | 101.6 | 98.7 | 99.0 |
| 2″ | 74.8 | 73.2 | 76.2 | 71.0 | 71.0 | 75.1 | 74.8 |
| 3″ | 73.6 | 76.1 | 71.8 | 72.8 | 73.2 | 74.0 | 73.9 |
| 4″ | 68.7 | 65.2 | 70.5 | 68.2 | 67.9 | 68.5 | 68.6 |
| 5″ | 72.6 | 75.0 | 73.7 | 72.8 | 72.7 | 75.6 | 73.4 |
| 6″ | 63.0 | 59.5 | 59.9 | 63.0 | 62.6 | 60.1 | 65.2 |
| 1‴ | 100.6 | 101.0 | 100.7 | | 100.1 | 100.5 | 100.6 |
| 2‴ | 70.64 | 70.7 | 70.59 | | 73.2 | 70.64 | 70.7 |
| 3‴ | 70.56 | 70.4 | 70.63 | | 77.1 | 70.57 | 70.6 |
| 4‴ | 71.9 | 71.6 | 71.8 | | 69.6 | 71.8 | 71.9 |
| 5‴ | 68.2 | 68.6 | 68.2 | | 76.5 | 68.2 | 68.2 |
| 6‴ | 17.3 | 17.1 | 17.1 | | 60.6 | 17.2 | 17.3 |
| 1⁗ | 170.1 | 170.1 | 170.3 | 170.3 | 170.3 | | 100.1 |
| 2⁗ | 45.3 | 45.6 | 45.8 | 45.9 | 46.2 | | 70.4 |
| 3⁗ | 68.7 | 69.0 | 69.2 | 68.7 | 68.6 | | 70.6 |
| 4⁗ | 45.2 | 45.5 | 49.0 | 46.2 | 46.4 | | 71.9 |
| 5⁗ | 172.6 | 172.5 | 175.8 | 174.5 | 174.4 | | 68.3 |
| 6⁗ | 27.1 | 27.2 | 27.4 | 27.4 | 27.6 | | 18.0 |

[a]Measured at 100 MHz for **1** and 125 MHz for **2**−**7**.

The combined filtrate was neutralized by passing through an Amberlite MB-3 ion-exchange resin column. The $H_2O$ eluent was reduced to dryness and then analyzed by TLC over silica gel (EtOAc/EtOH/$H_2O$/HCOOH, 6:4:1:1) by comparison with authentic sugars. The sugar residue and 1.0 mg of L-cysteine methyl ester hydrochloride (TCI Co. Ltd.) were dissolved in 0.1 mL of anhydrous pyridine, and the resulting mixture was stirred at 60 °C for 2 h. After drying under vacuum, the reaction mixture was trimethylsilylated with 0.3 mL of HMDS/TMCS/pyridine (3:1:9, Sigma-Aldrich Co. Ltd.) at 60 °C for 1.5 h. The mixture was then concentrated and partitioned between *n*-hexane and $H_2O$. The hexane extract was analyzed by GC under the following conditions: instrument, Agilent 6890N GC; column, HP-5 (30 m × 0.32 mm × 0.25 μm); detector, FID; column temperature, 230 °C; detector temperature, 280 °C; injector tempetature, 260 °C; carrier gas, He (1.0 mL/min). The $t_R$ values (min) of standard D-Glc, L-Glc, L-Rha, and D-Gal derivatives prepared in a similar way were 12.51, 13.49, 8.95, and 13.63 respectively. D-Gal and L-Rha were detected from compounds **1**−**4**, **6**, and **7**, and D-Glc and D-Gal were identified from compound **5**.

**Determination of the Absolute Configuration of Compounds 1−5 (ref 10).** (*S*)-1-Phenylethylamine (19 μL, 150 μmol), $Et_3N$ (32 μL, 225 μmol), PyBOP (58.5 mg, 115 μmol), and HOBt (20.0 mg, 150 μmol) were added to a solution of compound **1** (56.5 mg, 75 μmol) in 0.5 mL of DMF under ice-cooling, and the mixture was stirred at room temperature for 9 h. The reaction was quenched with dilute aqueous HCl and then dried under vacuum to afford a yellow residue, which was purified by Sephadex LH-20 CC eluting with 50% MeOH to give amide **S1** (41.0 mg).

*Rhamnocitrin-3-O-{(3S)-hydroxy-3-methyl-5-[(S)-phenylethylamino]glutaryl-(1→6)}-[α-L-rhamnopyranosyl-(1→2)]-β-D-galactopyranoside (**S1**):* amorphous, yellow powder; $^1$H NMR ($CD_3OD$, 400 MHz) δ 8.10 (2H, d, *J* = 8.8 Hz, H-2′, H-6′), 7.19−7.31 (5H, m, $C_6H_5$-1⁗), 6.90 (2H, d, *J* = 8.8 Hz, H-3′, H-5′), 6.54 (1H, d, *J* = 2.0 Hz, H-8), 6.25 (1H, d, *J* = 2.0 Hz, H-6), 5.71 (1H, d, *J* = 7.6 Hz, H-1″), 5.21 (1H, s, H-1‴), 4.93 (1H, m, H-1⁗), 4.19 (1H, dd, *J* = 11.6, 7.6 Hz, H-6a″), 4.13 (1H, dd, *J* = 11.6, 4.4 Hz, H-6b″), 3.84 (3H, s, $OCH_3$), 2.26−2.42 (4H, m, H-2⁗, H-4⁗), 1.39 (3H, d, *J* = 6.8 Hz, H-2⁗), 1.08 (3H, s, H-6⁗), 0.95 (3H, d, *J* = 6.0 Hz, H-6‴); ESIMS *m*/*z* 856.227 $[M + H]^+$, 854.311 $[M - H]^-$.

$LiBH_4$ (14.0 mg, 643 μmol) was added to a solution of **S1** (37.0 mg, 43 μmol) in THF (0.5 mL) under ice-cooling. After stirring for 24 h at room temperature, the reaction was quenched with dilute aqueous HCl, and the resulting material was extracted with EtOAc. The product was then purified by preparative TLC (EtOAc/HCOOH, 20:1) to give a colorless oil, which was acetylated with $Ac_2O$ (25 μL, 227 μmol) in pyridine (100 μL). The mixture was stirred for 24 h at room temperature. The reaction mixture was then diluted with water, extracted with EtOAc, and concentrated to afford **S2** (7.5 mg) as a colorless oil. Its $^1$H NMR spectrum was consistent with that of (3*R*)-5-*O*-acetyl-1-[(*S*)-phenylethyl]mevalonamide, rather than the (3*S*) isomer reported in the literature.

*(3R)-5-O-Acetyl-1-[(S)-phenylethyl]mevalonamide (**S2**):* colorless oil; $^1$H NMR ($CDCl_3$, 400 MHz) δ 7.27−7.37 (5H, m, $C_6H_5$-1′), 6.11 (1H, d, *J* = 7.2 Hz, N*H*), 5.14 (1H, quintet, *J* = 7.2 Hz, H-1′), 4.23 (2H, t, *J* = 6.8 Hz, H-5), 2.41, 2.28 (each 1H, d, *J* = 14.4 Hz, H-2), 2.04, (3H, s, $CH_3COO$), 1.85 (2H, m, H-4), 1.50 (3H, d, *J* = 6.8 Hz, H-2′), 1.23 (3H, s, H-6); EIMS *m*/*z* (%) 293 ($M^+$, 2).


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The absolute configuration of the HMG moiety in compounds **2**–**5** (each 6.0 mg) was determined using the same method, and all the final products were proved to be (3*R*)-5-*O*-acetyl-1-[(*S*)-phenylethyl]-mevalonamide by comparison with $^1$H NMR data in the literature.

**Anti-inflammatory Effects on LPS-Induced RAW 264.7 Cells (ref 18).** The murine macrophage cell line, RAW 264.7 (ATCC, USA), was incubated in Dulbecco's modified Eagle's medium containing 0.1% sodium bicarbonate, 2 mmol/L L-glutamine, 100 U/mL penicillin G, 100 μg/mL streptomycin, and 10% fetal bovine serum and maintained at 37 °C in a humidified incubator containing 5% $CO_2$. After preincubation for 8 h, RAW 264.7 cells ($5 \times 10^5$/mL) were treated with test compounds (**1**–**10**) and 2′,4′-dihydroxychalcone (positive control) of 10 and 50 μM in a 96-well microplate (100 μL/well) for 4 h, followed by addition of LPS with a final concentration of 1 μg/mL. After stimulation with LPS for 24 h, NO levels in cell culture were measured using the Griess reaction described as follows: 50 μL of culture supernatant was mixed with an equal volume of Griess reagent (1% sulfanilamide and 0.1% *N*-[1-naphthyl]ethylenediamine dihydrochloride in 5% phosphoric acid). The absorbance was measured at 570 nm in a microplate reader, and the nitrite concentration was determined using sodium nitrite as a standard.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

The $^1$H and $^{13}$C NMR, $^1$H–$^1$H COSY, HSQC, and HMBC spectra of compounds **1**–**3** and $^1$H and $^{13}$C NMR spectra of compounds **4**–**7** are available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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