



methoxyl groups at C-7 and C-4' were also confirmed by the corresponding NOESY spectrum. Enzyme hydrolysis (almond emulsin) of a small amount of **1** afforded a substance, identified by optical rotation ( $[\alpha]_D^{24} -28^\circ$ )<sup>7</sup> and spectroscopic methods<sup>7,8</sup> as (-)-7-*O*-methylecoumol, indicating that the absolute configuration of C-3 is *S* ( $\alpha$ -OH).<sup>9</sup> All of these data demonstrated that compound **1** was (-)-7-*O*-methylecoumol 5-*O*- $\beta$ -D-glucopyranoside.

The UV and IR spectra of **2** were very similar to those of **1**. Its <sup>1</sup>H and <sup>13</sup>C NMR spectra were also similar to those of **1**, except that a disaccharide unit was suggested by the pair of anomeric proton resonances at  $\delta$  4.86 (1H, d, *J* = 7.3 Hz) and  $\delta$  4.36 (1H, brs) and anomeric carbon resonances at  $\delta$  101.6 and 101.0. Upon enzyme hydrolysis of **2**, (-)-7-*O*-methylecoumol was identified by optical rotation<sup>7</sup> and spectroscopic methods.<sup>7,8</sup> The molecular formula, C<sub>30</sub>H<sub>38</sub>O<sub>15</sub>, was inferred from the HRFABMS ( $[M - H]^-$  *m/z* 637.2128), and it was also supported by <sup>13</sup>C NMR and DEPT spectroscopy. The HMBC experiment showed a long-range correlation between C-5 ( $\delta$  160.1) and the anomeric proton ( $\delta$  4.86), revealing the site of glycosidation to be the 5-OH of (-)-7-*O*-methylecoumol. The observation of NOE between H'' ( $\delta$  4.86) and H-6 ( $\delta$  6.50) in NOESY and NOEDS spectra supported this result. The <sup>13</sup>C NMR chemical shifts of the carbohydrate moiety of **2** were comparable with literature values for the flavonoid rutinose.<sup>6,10</sup> The site of glucose and rhamnose linkage was further supported by the glycosylation shift (ca. 6.4 ppm) of C-6'' in glucose, also revealed by a long-range correlation between C-6'' ( $\delta$  67.1) and the anomeric proton ( $\delta$  4.36) of rhamnose in the HMBC spectrum. The *J*<sub>G1,G2</sub> value of 7.3 Hz further indicated that the glucosyl moiety was connected to the aglycone by a  $\beta$ -linkage.<sup>5</sup> The structure of compound **2** was therefore characterized as (-)-7-*O*-methylecoumol 5-*O*- $\beta$ -rutinoside.

The molecular formula of compound **3** was confirmed as C<sub>30</sub>H<sub>38</sub>O<sub>15</sub> from the HRFABMS. NMR and other spectroscopic data were nearly identical with those of **2**; the only difference was that the site of glucose and rhamnose linkage was rha1→glc2, suggested by the glycosylation shift (ca. 3.0 ppm) of C-2'' in glucose, also revealed by a long-range correlation between C-2'' ( $\delta$  76.3) and the anomeric proton ( $\delta$  5.12) of rhamnose in the HMBC spectrum. The <sup>13</sup>C NMR chemical shifts of the carbohydrate moiety of **3** were very similar to literature values for the flavonoid neohesperidoside.<sup>6,11</sup> The *J*<sub>G1,G2</sub> value of 7.4 Hz further indicated that the glucosyl moiety was connected to the aglycone by a  $\beta$ -linkage.<sup>5</sup> Therefore, compound **3** was determined to be (-)-7-*O*-methylecoumol 5-*O*- $\beta$ -neohesperidoside.

To date, only three ecoumol-type homoisoflavanones have been isolated from *Eucomis bicolor* Bak. (Liliaceae),<sup>7</sup> *Caesalpinia sappan* L. (Leguminosae),<sup>8</sup> and *Scilla draconmontana* Hilliard and Burt (Hyacinthaceae).<sup>12</sup> Compounds **1–3** were the first reported glycosides having an ecoumol-type homoisoflavanone as the aglycone.

Compounds **1–3** were tested for in vitro antitumor activities against P388 (mouse leukemia) and A-549 (human pulmonary adenocarcinoma). No positive activities were observed.

## Experimental Section

**General Experimental Procedures.** Melting points were determined on an Electrothermal 9200 micro melting point apparatus and are uncorrected. Optical rotations were recorded with a Perkin-Elmer model 241 polarimeter. UV and IR spectra were measured on a Shimadzu UV-1601 instrument and on a Perkin-Elmer 983 spectrometer, respectively. All

NMR spectra were run on a Bruker DRX-400 instrument operating at 400 MHz for <sup>1</sup>H and 100 MHz for <sup>13</sup>C, using standard pulse sequences. Chemical shifts are reported on the  $\delta$  scale in parts per million, downfield from TMS. Carbon multiplicities were determined from DEPT-135 and DEPT-90 experiments. All 2D NMR spectra were recorded using pulsed field gradients. <sup>1</sup>H–<sup>1</sup>H correlations were observed in double quantum filtered (DQF) COSY and TOCSY experiments. One-bond <sup>13</sup>C–<sup>1</sup>H correlations were observed in a HMQC experiment. Long-range <sup>13</sup>C–<sup>1</sup>H correlations were observed in HMBC experiments. FABMS spectra were obtained on a JEOL JMS DX-303HF mass spectrometer. Column chromatography was performed on Si gel (Marine Chemical Factory, Qingdao, People's Republic of China), Sephadex LH-20 (Pharmacia), and RP-18 (Shimadzu). Other conditions were as previously described.<sup>13</sup>

**Plant Material.** The bulbs of *O. caudatum* Ait. were collected from Jilin Province, People's Republic of China, and the plant was identified by Dr. Tao Wu. After collection, the bulbs were allowed to dry at ambient temperature for about one week and were then crushed and immediately extracted. A voucher specimen (No. SIOC-Bio-1999102802) was deposited in the State Key Laboratory of Bio-organic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai, People's Republic of China.

**Extraction and Isolation.** The dried and crushed bulbs of *O. caudatum* (7.8 kg) were extracted three times with 95% EtOH under reflux for 3 h each time, solvent was removed under reduced pressure, and the residue was dissolved in hot water. This residue was left in a refrigerator overnight and then filtered. The filtrate was partitioned with CH<sub>2</sub>Cl<sub>2</sub> and *n*-BuOH, successively. The *n*-BuOH extract (352.2 g) was concentrated and subjected to Si gel column chromatography eluting with CHCl<sub>3</sub>–MeOH (25:1) followed by MeOH to yield 13 fractions. Fraction 5 (28.6 g) was subjected to Si gel (CHCl<sub>3</sub>–MeOH, 10:1) and Sephadex LH-20 (MeOH) chromatography and was purified by HPLC (RP<sub>18</sub>, 4  $\mu$ m, 280 nm, MeOH–1% acetic acid, 25:75; **1**: *t*<sub>R</sub> = 18.75 min; **2**: *t*<sub>R</sub> = 13.22 min; **3**: *t*<sub>R</sub> = 12.45 min) to give **1** (34 mg), **2** (26 mg), and **3** (20 mg), respectively.

**(–)-7-*O*-Methylecoumol 5-*O*- $\beta$ -D-glucopyranoside (**1**):** white needles (MeOH), mp 198–199 °C;  $[\alpha]_D^{24} -18.8^\circ$  (*c* 0.01, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 200 (4.25), 224 (4.45), 282 (3.86) nm; IR  $\nu_{\max}^{KBr}$  3497, 3476, 1659, 1614, 1566, 1513, 1471, 1170–1000 cm<sup>–1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz)  $\delta$  7.14 (2H, d, *J* = 8.7 Hz, H-2',6'), 6.85 (2H, d, *J* = 8.7 Hz, H-3',5'), 6.51 (1H, d, *J* = 2.3 Hz, H-6), 6.29 (1H, d, *J* = 2.3 Hz, H-8), 4.88 (1H, d, *J* = 7.2 Hz, H-1''), 3.96 (1H, d, *J* = 7.2, H-2 $\alpha$ ), 3.92 (1H, d, *J* = 7.2, H-2 $\beta$ ), 3.81 (3H, s, 7-OMe), 3.73 (3H, s, 4'-OMe), 3.72 (1H, m, H-6''), 3.45 (1H, m, H-6''), 3.38 (1H, m, H-5''), 3.33 (1H, m, H-2''), 3.31 (1H, m, H-3''), 3.16 (1H, m, H-4''), 2.78 (2H, brs, H-9); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 100 MHz)  $\delta$  192.3 (C-4), 165.5 (C-7), 163.5 (C-8a), 160.1 (C-5), 158.0 (C-4'), 131.5 (C-2',6'), 127.2 (C-1'), 113.3 (C-3',5'), 104.1 (C-4a), 101.7 (C-1''), 97.4 (C-6), 95.5 (C-8), 77.4 (C-5'), 76.1 (C-3''), 73.3 (C-2''), 72.0 (C-3), 71.1 (C-2), 69.8 (C-4'), 60.7 (C-6''), 59.9 (4'-OMe), 55.8 (7-OMe), 38.5 (C-9); HRFABMS *m/z* 491.1550  $[M - H]^-$  (calcd for C<sub>24</sub>H<sub>27</sub>O<sub>11</sub>, 491.1553).

**(–)-7-*O*-Methylecoumol 5-*O*- $\beta$ -rutinoside (**2**):** white needles (MeOH), mp 207–208 °C;  $[\alpha]_D^{24} -15.5^\circ$  (*c* 0.01, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 202 (4.14), 224 (4.42), 281 (3.90) nm; IR  $\nu_{\max}^{KBr}$  3496, 3472, 1659, 1614, 1567, 1512, 1470, 1170–1000 cm<sup>–1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz)  $\delta$  7.14 (2H, d, *J* = 8.8 Hz, H-2',6'), 6.86 (2H, d, *J* = 8.8 Hz, H-3',5'), 6.50 (1H, d, *J* = 2.2 Hz, H-6), 6.30 (1H, d, *J* = 2.2 Hz, H-8), 4.86 (1H, d, *J* = 7.3 Hz, H-1''), 4.36 (1H, brs, H-1''), 3.96 (1H, d, *J* = 7.3 Hz, H-2 $\alpha$ ), 3.92 (1H, d, *J* = 7.3 Hz, H-2 $\beta$ ), 3.80 (3H, s, 7-OMe), 3.73 (3H, s, 4'-OMe), 3.70–3.05 (m, other sugar protons), 2.78 (2H, brs, H-9), 0.98 (3H, d, *J* = 6.1 Hz, H-6''); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 100 MHz)  $\delta$  192.4 (C-4), 165.4 (C-7), 163.6 (C-8a), 160.1 (C-5), 157.9 (C-4'), 131.6 (C-2',6'), 127.2 (C-1'), 113.3 (C-3',5'), 104.1 (C-4a), 101.6 (C-1''), 101.0 (C-1''), 98.2 (C-6), 95.5

(C-8), 76.0 (C-5''), 76.5 (C-3''), 73.5 (C-2''), 72.0 (C-3 and C-4''), 71.2 (C-2), 70.8 (C-3'''), 70.6 (C-2'''), 70.0 (C-4''), 68.5 (C-5'''), 67.1 (C-6''), 60.0 (4'-OMe), 55.8 (7-OMe), 38.5 (C-9), 18.0 (C-6'''); HRFABMS  $m/z$  637.2128  $[M - H]^-$  (calcd for  $C_{30}H_{37}O_{15}$ , 637.2132).

(-)-7-*O*-Methylecoumol 5-*O*- $\beta$ -neohesperidoside (3): white needles (MeOH), mp 212–213 °C;  $[\alpha]_D^{24} -17.2^\circ$  ( $c$  0.01, MeOH); UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ) 201 (4.18), 225 (4.44), 281 (3.91) nm; IR  $\nu_{max}^{KBr}$  3491, 3478, 1659, 1614, 1567, 1512, 1470, 1170–1000  $cm^{-1}$ ;  $^1H$  NMR (DMSO- $d_6$ , 400 MHz)  $\delta$  7.14 (2H, d,  $J = 8.8$  Hz, H-2',6'), 6.88 (2H, d,  $J = 8.8$  Hz, H-3',5'), 6.53 (1H, d,  $J = 2.4$  Hz, H-6), 6.30 (1H, d,  $J = 2.4$  Hz, H-8), 5.01 (1H, d,  $J = 7.4$  Hz, H-1'), 5.12 (1H, brs, H-1''), 3.96 (1H, d,  $J = 7.2$  Hz, H-2 $\alpha$ ), 3.92 (1H, d,  $J = 7.2$  Hz, H-2 $\beta$ ), 3.82 (3H, s, 7-OMe), 3.75 (3H, s, 4'-OMe), 3.90–3.10 (m, other sugar protons), 2.78 (2H, brs, H-9), 1.19 (3H, d,  $J = 6.2$  Hz, H-6'');  $^{13}C$  NMR (DMSO- $d_6$ , 100 MHz)  $\delta$  192.3 (C-4), 165.4 (C-7), 163.4 (C-8a), 160.0 (C-5), 157.9 (C-4'), 131.5 (C-2',6'), 127.2 (C-1'), 113.3 (C-3',5'), 104.1 (C-4a), 100.5 (C-1''), 100.4 (C-1'''), 97.3 (C-6), 95.4 (C-8), 77.4 (C-3''), 76.8 (C-5''), 76.3 (C-2''), 71.9 (C-3 and C-4''), 71.2 (C-2), 70.4 (C-3'''), 70.5 (C-2'''), 69.8 (C-4''), 68.2 (C-5'''), 60.6 (C-6''), 59.9 (4'-OMe), 55.7 (7-OMe), 38.5 (C-9), 18.1 (C-6'''); HRFABMS  $m/z$  637.2130  $[M - H]^-$  (calcd for  $C_{30}H_{37}O_{15}$ , 637.2132).

**Enzyme Hydrolysis of Compounds 1–3.** A solution of each compound (1–3, 10 mg) in HOAc–NaOAc buffer solution (pH 5.0, 10 mL) was treated with  $\beta$ -glucosidase (almond emulsin, G4511, Sigma Co., 8 mg) and kept stirring at 38 °C for 3 days. After addition of a small amount of  $CHCl_3$  and warming for a while, the total mixture was filtered. The filtrate was extracted with  $CHCl_3$ , and the  $CHCl_3$  extract was evaporated under reduced pressure to give the product, which was purified by preparative TLC (*n*-hexane–EtOAc, 5:1) to furnish (-)-7-*O*-methylecoumol, identified by optical rotation<sup>7</sup> and spectroscopic methods.<sup>7,8</sup>

**Biological Tests.** The *in vitro* antitumor activity assays were carried out following the methods of Nakamura et al.<sup>14</sup>

**Acknowledgment.** This work was supported by the Ministry of Science and Technology of China (96-901-05-266). We thank Prof. Jian Ding and Dr. Dong Xiao (Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, People's Republic of China) for assistance with biological tests.

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NP010466A