The First Total Syntheses of Prenyllicoflavone A and Licoflavone A and Biological Evaluation as Inhibitors of Bone Resorption Pits Formation

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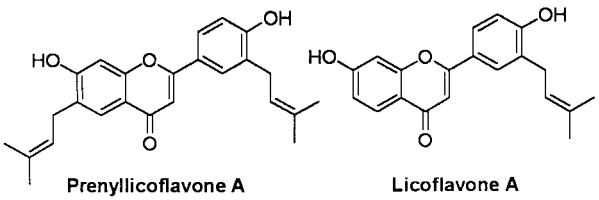
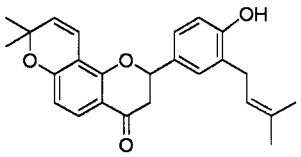
The roots and stolons of *Glycyrrhiza Glabra* L., a perennial plant of Leguminosae, are one of the most important crude drugs from ancient times. Glycyrrhizin (GL), the major oleanane-type triterpene saponin in licorice roots, is used in large quantities as a well known sweetener.[1] On the other hand, not only GL but also many flavonoids have been isolated from the underground parts of *G. glabra*.[2] Isoliquiritigenin glycosides (ILG) are the major flavonoids responsible for the yellow color of licorice roots. Some bioactive flavonoids have been isolated from commercial licorice and their structures were elucidated on the basis of spectroscopic evidence. In recent years, the number of reports referring to the biological activity of licorice constituents has been dramatically increased, and either flavonoids or isoflavonoids were identified as the active principles.[3,4]





We recently reported the first total synthesis of (±)-shinflavanone, which has flavonoids skeleton.[5] We also reported that (±)-shinflavanone possesses high inhibitory activity of bone resorption pits formation by osteoclast cell as compared to herbimycin A. Continuously, our research in this field has been focused on the syntheses of the other structurally related natural products and evaluation of their inhibition ability of osteoclast cell activity to generate potent anti-osteoporosis agents.

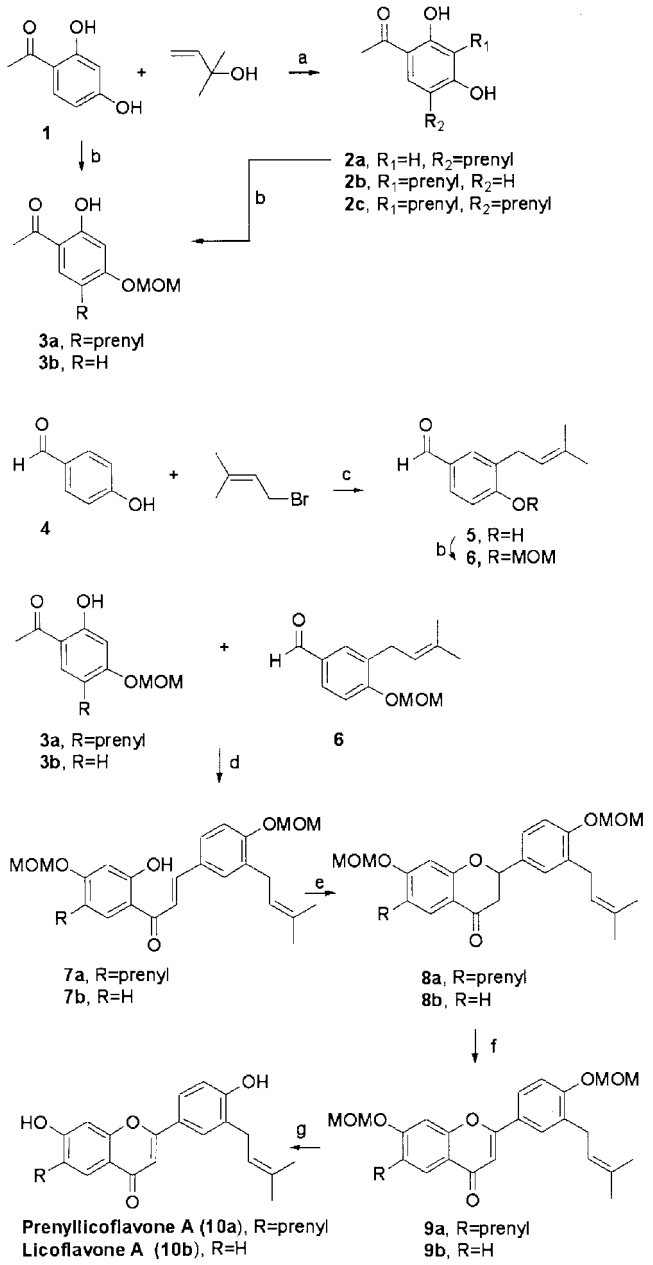
In this report, we reveal the first total syntheses of prenyllicoflavone A and licoflavone A, which have similar structures to shinflavanone, and inhibition abilities of bone resorption pits formation by osteoclast-like cell induced by 1α, 25-dihydroxyvitamine D_3.

Preparation of the prenyllicoflavone A (**10a**) began with the isoprenylation of 2,4-dihydroxyacetophenone (**1**) with 2-methyl-but-3-en-2-ol and boron trifluoride-etherate in dioxane. After separation by flash column chromatography, **2b** and **2c** were used[6] for the syntheses of (±)-shinflavanone and its analogues as was already reported by us. 5-C-Prenylresacetophenone (**2a**) and 2,4-dihydroxyacetophenone (**1**) were protected using methoxymethyl chloride and K_2CO_3 to obtain MOM-protected compounds **3a** and **3b** in 81% and 98% yields respectively. 2-Hydroxy-4-methoxymethyloxy-5-(3-methyl-2-butenyl)acetophenone (**3a**) and 2-hydroxy-4-methoxymethyloxyacetophenone (**3b**) was condensed with 4-methoxymethyloxy-3-(3-methyl-2-butenyl)benzaldehyde (**6**) in the presence of concentrated alcoholic alkaline solution to afford the corresponding chalcone **7a** and **7b** in 77% and 52% yields respectively. Subsequent cyclization with dilute alkaline solution provided flavones **8a** and **8b** in 90% and 60% yields respectively. The resulting flavones **8a** and **8b** were treated with LDA and seleninic anhydride in THF at −78 °C to provide flavones **9a** and **9b** in 20% and 17% yields respectively. Finally, demethoxymethylation of **9a** was achieved by using 3 N HCl/THF for 10 min at reflux to give the desired final natural product prenyllicoflavone A (**10a**) in 52% yield. The demethoxymethylation of **9b** by using the same condition generated another natural product licoflavone A (**10b**) in 25% yield. The spectroscopic data of the synthesized natural products **10a**[7a] and **10b**[7b] were identical with those of the naturally occurring prenyllicoflavone A and licoflavone A.[2]

The synthesized natural products 1**0a** and **10b** were assayed for their ability to inhibit the resorption pits formation by osteoclast-like cell (OCL) induced by 1α, 25-dihydroxyvitamine D_3.[8-10]

As shown in Table 1, the anti-osteoporosis activity of synthesized natural products **10a**, **10b**, appears to be weaker as compared with (±)-shinflavanone. The IC_{50} value of (±)-shinflavanone was 0.70 μg/mL as reported by us.[5] There is no report about the osteoporosis related activity with prenyllicoflavone A **10a** and licoflavone A **10b**.

In conclusion, we firstly synthesized prenyllicoflavone A and licoflavone A and found these compounds have inter-



Scheme 1. (a) BF_3OEt_2, dioxane; (b) methoxymethyl chloride, K_2CO_3, acetone; (c) 10% KOH/H_2O; (d) 50% KOH/H_2O, EtOH; (e) Na_2CO_3/H_2O, EtOH; (f) LDA, seleninic anhydride, THF; (g) 10% HCl/H_2O, THF.

Table 1. Effects of Bone Resorption Inhibitors on Pits Formation and Their Cytotoxicity on Osteoclastic Cells[a]

Compound	Numbers of Pits (inhibition %)		
	0.11 μg/mL	0.33 μg/mL	1.00 μg/mL
Prenyllicoflavone A (**10a**)	395 (n.s.)	380 (7.32%)	203 (49.3%)
Licoflavone A (**10b**)	400 (n.s.)	360 (12.20%)	215 (46.25%)
Control	402	410	400

[a]All the values are stated as the mean of at least three determinations.

mediate potency as the inhibitors of bone resorption pits formation by OCL induced by 1α, 25-dihydroxyvitamine D_3.

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References and Notes

1. Gibson, M. R. *J. Nat. Prod.* **1978**, *41*, 348.
2. Kitagiwa, I.; Chen, W. Z.; Hori, K.; Harada, E.; Yasuda, N.; Yoshikawa, M.; Ren, J. *Chem. Pharm. Bull.* **1994**, *42*, 1056.
3. Okada, K.; Tamura, Y.; Yamamoto, M.; Inoue, Y.; Takagaki, R.; Takahashi, K.; Demizu, S.; Kajiyama, K.; Hiraga, Y.; Kinoshita, T. *Chem. Pharm. Bull.* **1994**, *37*, 2528.
4. (a) Hatano, T.; Yasuhara, T.; Miyamoto, K.; Okuda, T. *Chem. Pharm. Bull.* **1988**, *36*, 2286. (b) Tawata, M.; Yoda, Y.; Aida, K.; Shindo, H.; Sasaki, H.; Chin, M. *Planta Med.* **1990**, *56*, 259. (c) Hatano, T.; Fukuda, T.; Miyase, T.; Noro, T.; Okuda, T. *Chem. Pharm. Bull.* **1991**, *39*, 1238.
5. Suh, H.; Lee, S.; Kim, N.; Han, J.; Kim, J. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1433.
6. Jain, A. C.; Pyare, L.; Seshadri, T. R. *Tetrahedron* **1970**, *26*, 2631.
7. (a) **10a** [synthetic prenyllicoflavone A]: a yellowish viscous liquid; *Rf* 0.30 (SiO_2, 75% EtOAc-Hexane); ^{1}H-NMR (300 MHz, acetone-d_6) δ 1.75 (s, 3H), 1.77 (s, 9H), 3.39 (d, 4H, J = 7.3 Hz), 5.36 (m, 2H), 6.57 (s, 1H), 6.93 (d, 1H, J = 8.6 Hz), 6.96 (s, 1H), 7.56 (dd, 1H, J = 2.1, 8.6 Hz), 7.79 (d, 1H, J = 2.2 Hz), 7.83 (s, 1H) ^{13}C-NMR (75 MHz, acetone-d_6) δ 17.4, 25.4, 28.1, 29.5, 103.3, 104.9, 115.6, 117.8, 122.2, 123.6, 124.1, 126.6, 126.8, 128.0, 129.2, 133.6, 133.9, 157.6, 158.7, 162.2, 164.5, 177.3, HRMS (EI) m/z: Found: 390.18285 (Calculated for $C_{25}H_{26}O_4$ M^+): 390.18318. (b) **10b** [synthetic licoflavone A]: a yellowish viscous liquid; *Rf* 0.32 (SiO_2, 75% EtOAc-Hexane); ^{1}H-NMR (300 MHz, acetone-d_6) δ 1.79 (s, 6H), 3.38 (d, 2H, J = 7.8 Hz), 5.38 (t, 1H), 6.43 (s, 1H), 6.45 (s, 1H), 6.86 (d, 1H, J = 7.8 Hz), 7.38 (s, 1H), 7.41 (d, 1H, J = 8.6 Hz), 7.82 (d, 1H, J = 8.6 Hz), 7.87 (d, 1H, J = 8.6 Hz) ^{13}C-NMR (75 MHz), acetone-d_6) δ 17.4, 25.4, 29.1, 103.4, 106.3, 117.2, 117.8, 123.2, 124.5, 126.6, 127.9, 128.3, 133.9, 161.6, 161.8, 162.2, 164.0, 177.2, HRMS (EI) m/z: Found: 322.12098 (Calculated for $C_{20}H_{18}O_4$ M^+): 322.12054.
8. **Resorption Pit Assay and Quantitation of Pits**: Drops of the osteoclast-like multinucleated cell population[9,10] were added on bone slices placed in a 96-well culture dich with and without samples. After incubation for 48 h, bone slices were placed for 30 min in 1 M NaOH and cleaned by ultrasonication to remove adherent cells. The bone slices were then stained with Mayers hematoxylin solution (hematoxylin, 1 g/L; $NaIO_3$, 0.2 g/L; $AlNH_4(SO)_4 \cdot 12H_2O$, 50 g/L; CH_3COOH, 7.5 g/L; pH 2.8) for 50 sec, washed with distilled water, cleaned by ultrasonication, and finally air dried. Resorption pits visualized by Mayers hematoxylin staining were identified by ight microscopy with a X5 objective lens. Using an image analysis sofrware (Image-pro plus, Media Cybernetics, MD, USA), the numbers of pits were counted.
9. Takahasi, N.; Akatsu, T.; Udagawa, N.; Sasaki, T.; Yamaguchi, A.; Moseley, J. M.; Martin, T. J.; Suda, T. *Endocrinology* **1988**, *123*, 2600.
10. Takami, M.; Woo, J.-W.; Takahashi, N.; Suda, T.; Nagai, K. *Biochem. Bioph. Res. Co.* **1997**, *237*, 111.
11. Sugawara, K.; Hmada, M.; Hosoi, S.; Tamaoki, T. *Anal. Biochem.* **1998**, *255*, 204.