

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

Sungeun Lee, Jae-Kon Kim, Jong Gun Lee, Jin-Soo Lee,[†] Moonjeong Leem,[‡]
Yong-Ho Chung,[†] Yeong-Ok Song,[‡] and Hongsuk Suh^{*}

Department of Chemistry and Chemistry Institute for Functional Materials, Pusan National University, Pusan 609-735, Korea

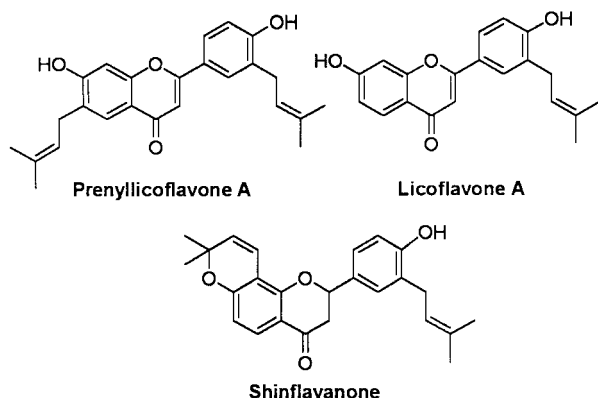
[†]Central Research Laboratory, Dong Wha Pharm. Ind. Co. Ltd., Anyang-Si, Anyang-Dong 189, Kyungki-Do 430-010, Korea

[‡]Department of Food Science & Nutrition, Pusan National University, Pusan 609-735, Korea

Received October 10, 2001

Keywords : Glycyrrhiza glabra, Flavonoid, Prenyllicoflavone A, Licoflavone A, Osteoporosis.

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 Iso-liquiritigenin 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 iso-flavonoids 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 prenylicoflavone A and licoflavone A, which have similar structures to shinflavanone, and inhibition abilities of bone resorption pits formation by osteoclast-like cell induced by

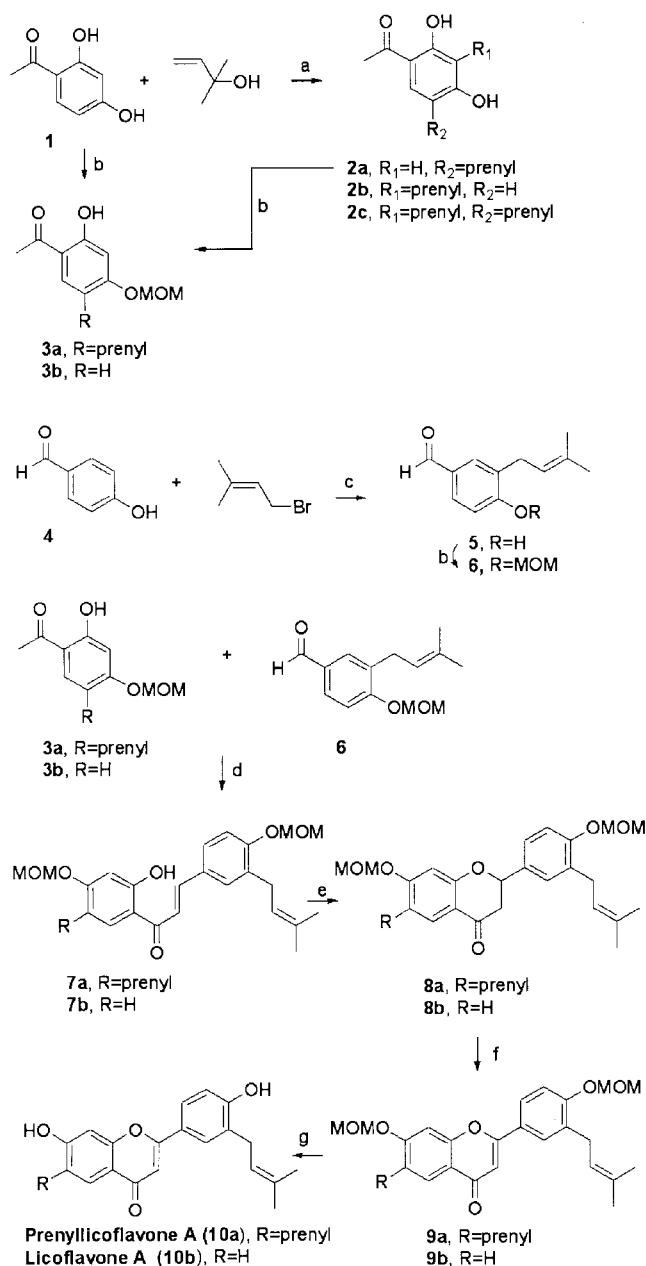
1 α , 25-dihydroxyvitamine D₃.

Preparation of the prenylicoflavone 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 used⁶ 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 K₂CO₃ to obtain MOM-protected compounds **3a** and **3b** in 81% and 98% yields respectively. 2-Hydroxy-4-methoxymethoxy-5-(3-methyl-2-butenyl)acetophenone (**3a**) and 2-hydroxy-4-methoxymethoxyacetophenone (**3b**) was condensed with 4-methoxymethoxy-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 prenylicoflavone 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 prenylicoflavone A and licoflavone A.²

The synthesized natural products **10a** and **10b** were assayed for their ability to inhibit the resorption pits formation by osteoclast-like cell (OCL) induced by 1 α , 25-dihydroxyvitamine D₃.⁸⁻¹⁰

As shown in Table 1, the anti-osteoporosis activity of synthesized natural products **10a**, **10b**, appears to be weaker as compared with (±)-shinflavanone. The IC₅₀ value of (±)-shinflavanone was 0.70 μ g/mL as reported by us.⁵ There is no report about the osteoporosis related activity with prenylicoflavone A **10a** and licoflavone A **10b**.

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



Scheme 1. (a) BF₃OEt₂, dioxane; (b) methoxymethyl chloride, K₂CO₃, acetone; (c) 10% KOH/H₂O; (d) 50% KOH/H₂O, EtOH; (e) Na₂CO₃/H₂O, EtOH; (f) LDA, seleninic anhydride, THF; (g) 10% HCl/H₂O, 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

^aAll 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-dihydroxyvitamin D₃.

Acknowledgment. The work was supported by grant No.

(2000-G-NB-01-C-06) of KISTEP.

References and Notes

- Gibson, M. R. *J. Nat. Prod.* **1978**, *41*, 348.
- Kitagiwa, I.; Chen, W. Z.; Hori, K.; Harada, E.; Yasuda, N.; Yoshikawa, M.; Ren, J. *Chem. Pharm. Bull.* **1994**, *42*, 1056.
- 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.
- (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.
- Suh, H.; Lee, S.; Kim, N.; Han, J.; Kim, J. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1433.
- Jain, A. C.; Pyare, L.; Seshadri, T. R. *Tetrahedron* **1970**, *26*, 2631.
- (a) **10a** [synthetic prenyllicoflavone A]: a yellowish viscous liquid; *R_f* 0.30 (SiO₂, 75% EtOAc-Hexane); ¹H-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) ¹³C-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 C₂₅H₂₆O₄ M⁺): 390.18318. (b) **10b** [synthetic licoflavone A]: a yellowish viscous liquid; *R_f* 0.32 (SiO₂, 75% EtOAc-Hexane); ¹H-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) ¹³C-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 C₂₀H₁₈O₄ M⁺): 322.12054.
- 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 dish 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; NaIO₃, 0.2 g/L; AlNH₄(SO)₄·12H₂O, 50 g/L; CH₃COOH, 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 light microscopy with a X5 objective lens. Using an image analysis software (Image-pro plus, Media Cybernetics, MD, USA), the numbers of pits were counted.
- Takahasi, N.; Akatsu, T.; Udagawa, N.; Sasaki, T.; Yamaguchi, A.; Moseley, J. M.; Martin, T. J.; Suda, T. *Endocrinology* **1988**, *123*, 2600.
- Takami, M.; Woo, J.-W.; Takahashi, N.; Suda, T.; Nagai, K. *Biochem. Bioph. Res. Co.* **1997**, *237*, 111.
- Sugawara, K.; Hmada, M.; Hosoi, S.; Tamaoki, T. *Anal. Biochem.* **1998**, *255*, 204.