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FIRST TOTAL SYNTHESIS AND CYTOTOXICITY OF NATURALLY OCCURRING LESPEDEZOL E_1

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Lespedeza, a genus of the Fabaceae family, is widely distributed in Asia, Australia, and North America. In the last several decades, many flavonoids and isoflavonoids isolated from *Lespedeza* species have been reported for a variety of biological and pharmacological activities, such as hepatoprotective [1], antioxidant [2], antinematode [3], anticoccidial [4], and antitumor activity [5–7]. Lespedeza E_1 (**1**), a C_8-geranylated isoflavonoid, was first isolated from the stem of *Lespedeza homoloba* in 1999 and was shown to exhibit antioxidant activity [2]. Recently, it was also isolated from *Ficus benjamina* and evaluated for its inhibitory activity on BACE1 [8]. In 2007, the methylated homologues of 1,8-*C*-geranyl-7-*O*-methylbiochanin A (**3**) and olibergin B were identified and isolated from the stems of *Dalbergia* species [9], but their biological activities have not been further evaluated.

Recent studies have shown that some natural geranylated flavonoid/isoflavonoid possess potent antitumor activities [10]. The development of convenient and efficient synthetic approaches for the preparation of large quantities of geranylated flavonoid/isoflavonoid and their analogues would be highly desirable for their biological studies. Here, we report the first total synthesis of the naturally occurring lespedezol E_1 and its methyl derivatives 5,7,4′-trimethyl-8-*C*-geranylisoflavone (**2**) and **3**.

In contemplating a general approach for the synthesis of C_8-geranylated isoflavanone natural products, we envisaged a simple protocol involving the Pd-catalyzed Suzuki coupling of geranyl boronic esters [11] with a C_8-iodo derivative of genistein. The latter is expected to be readily accessible based on our experience in the regioselective iodination of flavanoids [12, 13]. Our initial synthesis commenced with the fully methylated genistein **5** (Scheme 1, *A*). The Suzuki coupling reaction afforded **2** from regiospecific iodide **6**. However, attempted removal of the methyl groups without affecting the geranyl side chains proved unsuccessful. Thus, treatment of **2** in the presence of BCl_3 at 0°C for 30 min led to the formation of **3** in 85% yield, with demethylation occurring at the C_5-position in accordance with the literature precedent that BCl_3 selectively demethylates the methoxyl group ortho to a carboxyl group [14]. Further demethylation of **3** using BCl_3 or BBr_3 was unsuccessful because the geranyl group was unstable under an extended reaction time (> 30 min) and higher reaction temperature (> 0°C). The all MOM-protected isoflavone **7** was then prepared using MOMCl with the hope that the geranyl side chains would be preserved under the reaction conditions for the deprotection of the MOM groups. Therefore, geranylated isoflavone **9** was converted to the naturally occurring **1** in 81% yield (Scheme 1, *B*).

All these synthetic products and the unsubstituted genistein (**4**) itself were evaluated for their cytotoxicity *in vitro* against tumor cell lines, including K562, HepG2, HT-29, and MGC-803 (Table 1). The preliminary SAR study showed that the presence of a geranylated side chain on the isoflavonoid structure can enhance the cytotoxic activity of **1** by more than tenfold for K562, HepG2, and HT-29, and sixfold for MGC-803 when compared to that of **4**. A similar conclusion can be drawn when comparing the trimethylated product **2** with **5**. However, the 7,4′-dimethyl product **3** lost potency.

In summary, we have achieved the first and facile total synthesis of lespedezol E_1 starting from genistein in four steps with 47% overall yield. This approach allows the regioselective introduction of geranyl side chains in the natural isoflavonoid in good yield. Furthermore, a comparison of the cytotoxic activity of the geranylated isoflavonoid with its mother core showed that the geranyl is important to improve the antitumor activity *in vitro*. The synthesis of additional modified 1-analogues to establish SARs and generate an optimized antitumor lead is in progress.

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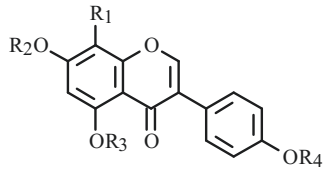
TABLE 1. Cytotoxicity *in vitro* of Lespedezol E_1 and Its Derivatives (IC_{50}, μM)*

Compound	K562	HepG2	HT-29	MGC-803
1	3.14 ± 0.52	19.58 ± 3.59	4.45 ± 1.87	8.30 ± 4.50
2	7.78 ± 2.16	25.19 ± 3.39	25.21 ± 2.16	16.72 ± 4.27
3	47.25 ± 6.78	>100	>100	>100
4	59.64 ± 6.14	>100	>100	48.88 ± 9.17
5	32.65 ± 2.24	87.04 ± 0.01	79.91 ± 4.12	>100
13	52.68 ± 6.85	>100	>100	>100
Camptothecin**	0.13 ± 0.04	>10	0.38 ± 0.03	0.58 ± 0.02

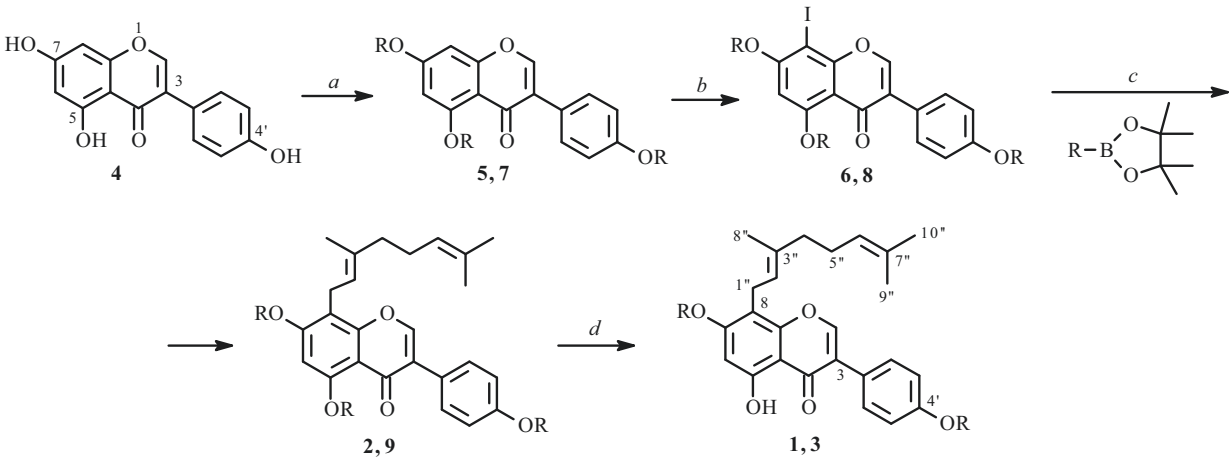
*Results are the average of three independent experiments, each performed in duplicate. Standard deviations were below ±10%.
**Reference compound.



1 - 5, 13

1 - 3: R_1 =

1: $R_2 = R_3 = R_4 = H$; **2:** $R_2 = R_3 = R_4 = CH_3$
3: $R_2 = R_4 = CH_3$, $R_3 = H$; **4:** $R_1 = R_2 = R_3 = R_4 = H$
5: $R_1 = H$, $R_2 = R_3 = R_4 = CH_3$; **13:** $R_1 = R_3 = H$, $R_2 = R_4 = CH_3$



4 **5, 7** **6, 8** **2, 9** **1, 3**

1: R = H; **2, 3, 5, 6:** R = CH_3; **7** – **9**: R = MOM

A: *a*. CH_3I, K_2CO_3, DMF, 40°C, 4 h, 95%; *b*. NIS, DMF, 70°C, 4 h, 94%; *c*. Pd(dppf)·Cl_2, Cs_2CO_3, DMF, 110°C, microwave, 2 h, 66–76 %; *d*. BCl_3, DCM, 0°C, 30 min, 85 %. **4→5→6→2→3**
B: *a*. MOMCl, 60% NaH, DMF, 0°C – r.t., 2 h, 87%; *b*. NIS, DCM, r.t., 2 h, 93%; *c*. Pd(dppf)·Cl_2, Cs_2CO_3, DMF, 110°C, microwave, 2 h, 71–75 %; *d*. 2M·HCl, MeOH/THF (5:1), 0–60°C, 81%. **4→7→8→9→1**

Scheme 1. Synthesis of compounds **2** and **3** (*A*) and compound **1** (*B*).

Lespedezol E_1 (1). Yellow solid. ^{1}H NMR (400 MHz, MeOD-d_4, δ, ppm, J/Hz): 1.52 (3H, s), 1.57 (3H, s), 1.79 (3H, s), 1.97 (2H, t, J = 7.2), 2.05 (2H, m, J = 7.6), 3.41 (2H, d, J = 7.2), 5.01 (1H, t, J = 6.8), 5.20 (1H, t, J = 6.8), 6.27 (1H, s), 6.84 (2H, d, J = 8.8), 7.37 (2H, d, J = 8.4), 8.12 (1H, s). ^{13}C NMR (100 MHz, MeOD-d_4, δ, ppm): 14.8, 16.2, 20.7, 24.4, 26.1, 39.3, 98.1, 104.8, 106.5, 114.8, 122.0, 122.1, 122.9, 123.9, 130.0, 130.6, 134.5, 153.4, 155.4, 157.3, 160.0, 161.8, 181.2. LR-MS(ESI) *m/z* 407.1 $[M + H]^+$.

5,7,4′-Trimethyl-8-*C*-geranylisoflavone (2). White solid. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.56 (3H, s), 1.62 (3H, s), 1.79 (3H, s), 1.97 (2H, t, J = 8.8), 2.14 (2H, m), 3.45 (2H, d, J = 7.2), 3.82 (3H, s), 3.94 (3H, s), 3.96 (3H, s), 5.05

(1H, t, J = 6.8), 5.17 (1H, t, J = 6.8), 6.42 (1H, s), 6.93 (2H, d, J = 8.8), 7.49 (2H, d, J = 8.8), 7.82 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 16.1, 17.6, 21.6, 25.6, 26.6, 19.7, 55.3, 55.8, 56.4, 91.9, 109.6, 109.9, 113.6, 121.8, 124.2, 124.5, 125.1, 130.3, 131.3, 135.4, 150.2, 156.5, 159.3, 159.8, 160.8, 176.0. HR-MS(ESI) *m/z* calcd for $C_{28}H_{33}O_5$ $[M + H]^+$ 449.2323, Found 449.2323.

5-Hydroxyl-7,4′-dimethyl-8-*C*-geranylisoflavone (3). Yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.57 (3H, s), 1.63 (3H, s), 1.79 (3H, s), 1.97 (2H, t, J = 7.2), 2.06 (2H, m), 3.42 (2H, d, J = 6.8), 3.84 (3H, s), 3.90 (3H, s), 5.05 (1H, t, J = 7.2), 5.16 (1H, t, J = 6.2), 6.42 (1H, s), 6.98 (2H, d, J = 8.0), 7.47 (2H, d, J = 8.0), 7.92 (1H, s), 12.94 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 16.0, 17.6, 21.3, 25.6, 26.6, 39.7, 55.3, 56.0, 95.2 105.7, 107.9, 114.1, 121.8, 123.0, 123.1, 124.2, 130.1, 131.3, 135.5, 152.8, 154.6, 159.7, 161.0, 162.9, 181.3. LR-MS(ESI) *m/z* 435.2 $[M + H]^+$.

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REFERENCES

1. S. M. Kim, K. Kang, E. H. Jho, Y. J. Jung, C. W. Nho, B. H. Um, and C. H. Pan, *Phytother. Res*., **25**, 1011 (2011).
2. T. Miyase, M. Sano, K. Yoshino, and K. Nonaka, *Phytochemistry*, **52**, 311 (1999).
3. J. M. Burke, N. C. Whitley, D. A. Pollard, J. E. Miller, T. H. Terrill, K. E. Moulton, and J. A. Mosjidis, *Vet. Parasitol*., **181**, 345 (2011).
4. J. M. Burke, J. E. Miller, T. H. Terrill, S. T. Orlik, M. Acharya, J. J. Garza, and J. A. Mosjidis, *Vet. Parasitol.*, **193**, 39 (2013).
5. L. Li, H. K. Wang, J. J. Chang, A. T. McPhail, D. R. McPhail, H. Terada, T. Konoshima, M. Kokumai, M. Kozuka, J. R. Estes, and K. H. Lee, *J. Nat. Prod*., **56**, 690 (1993).
6. T. Konoshima, M. Takasaki, M. Kozuka, H. Tokuda, H. Nishino, E. Matsuda, and M. Nagai, *Biol. Pharm. Bull.*, **20**, 865 (1997).
7. C. Ito, M. Itoigawa, H. T. Tan, H. Tokuda, X. Yang Mou, T. Mukainaka, T. Ishikawa, H. Nishino, and H. Furukawa, *Cancer Lett.*, **152**, 187 (2000).
8. J. Dai, D. Shen, W. Y. Yoshida, S. M. Parrish, and P. G. Williams, *Planta Med*., **78**, 1357 (2012).
9. S. I. Khalivulla, B. A. K. Reddy, D. Gunasekar, M. M. Murthy, T. P. Rao, A. Blond, and B. Bodo, *Nat. Prod. Commun.*, **2**, 1109 (2007).
10. V. Dominguez-Villegas, V. Dominguez-Villegas, M. L. Garcia, A. Calpena, B. Clares-Naveros, and M. L. Garduno-Ramirez, *Nat. Prod. Commun.,* **8**, 177 (2013).
11. Guillaume Dutheuil, Nicklas Selander, Kalman J. Szabo, and Varinder K. Aggarwal, *Synthesis*, **14**, 2293 (2008).
12. K. Lu, J. Chu, H. M. Wang, X. L. Fu, D. W. Quan, H. X. Ding, Q. W. Yao, and P. Yu, *Tetrahedron Lett.,* **54**, 6345 (2013).
13. H. M. Wang, Z. H. Yan, Y. N. Lei, K. Sheng, Q. W. Yao, K. Lu, and P. Yu, *Tetrahedron Lett.*, **55**, 897 (2014).
14. K. Q. Wu, L. H. Xu, L. L. Weng, and H. Zheng, *Chem. Res. Appl.*, **16**, 165 (2004).