Asian Journal of Chemistry; Vol. 27, No. 3 (2015), 1063-1065

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2015.18240

Chemical Constituents of *Gleditsia sinensis* Thorns

YUN-XIA XIAN[1,2], HONG-LEI ZHOU[1], XIAO WANG[1,2], JIN-QIAN YU[2,*], ZHEN-JIA ZHENG[3] and BING-TIAN YANG[3]

[1]College of Pharmacy, Shandong University of Traditional Chinese Medicine, Jinan 250014, P.R. China
[2]Shandong Analysis and Test Center, Shandong Academy of Sciences, Jinan 250014, P.R. China
[3]Sanfeng Biological Engineering Technology Co., 203 Beiyuan Street, Jinan 250014, Shandong Province, P.R. China

*Corresponding author: Fax: +86 531 82964889; Tel: +86 531 82605319; E-mail: yujinqian87528@126.com

Received: 3 June 2014; *Accepted*: 29 October 2014; *Published online*: 19 January 2015; AJC-16725

Seven compounds were isolated from the thorns of *Gleditsia sinensis*, which were identified as daucosterol (**1**), β-sitosterol (**2**), (*E*)-3,3'-dimethoxy-4,4'-dihydroxystilbene (**3**), *trans*-coniferyl aldehyde (**4**), methyl syringate (**5**), *p*-hydroxyl-cinnamaldehyde (**6**), 4-amino-benzaldehyde (**7**). To our best of knowledge the compounds **3-7** were obtained from this plant for the first time.

Keywords: *Gleditsia sinensis* Thorns, *trans*-Coniferyl aldehyde, Isolation, Identification.

INTRODUCTION

Gleditsia sinensis LAM. (*G. sinensis*) (Leguminosae), a perennial shrub, is a traditional Chinese medicine widely distributed in mainland China. The thorns of *G. sinensis* have been used in traditional medicine for the treatment of swelling, suppuration, carbuncle and skin diseases[1]. However, phytochemical investigation on *Gleditsia sinensis* Thorns is very rare up to now and, to the best of our knowledge, with only four kinds of compounds having been reported, including flavones[2,3,4], terpenoids[4,5,6,7], phenolic acids[2,3] and steroids[7,8]. This prompted us to carry out further studies on this plant. In the course of our bioassay-guided isolation studies on the ethyl acetate extract of *G. sinensis* thorns, 7 known compounds, including daucosterol (**1**), β-sitosterol (**2**), (*E*)-3,3'-dimethoxy-4,4'-dihydroxystilbene (**3**), *trans*-coniferyl aldehyde (**4**), methyl syringate (**5**), *p*-hydroxyl-cinnamaldehyde (**6**), 4-aminoben-zaldehyde (**7**) were isolated and identified. In this study, compounds **3-7** (Fig. 1) were obtained from *Gleditsia sinensis* for the first time.

EXPERIMENTAL

1D and 2D NMR spectra were taken on a varian NMR system-600 NMR spectrometer. ESI-MS was obtained using an agilent 1100/MSG1946 mass spectrometer. Preparative HPLC was performed on a waters 600 pump, waters 600 controller and waters 996 photodiode array detector and a reversed-phase C_{18} column (YMC-Pack ODS-AU 20 × 250 mm, 10 μm) was employed. Column chromatography (CC) was undertaken over silica gel (200-300 mesh). TLC was carried out with glass plate precoated silica gel G. Spots were visualized under UV light and by spraying with 10 % H_2SO_4 in 95 % EtOH, followed by heating at 100 °C. Methanol used in preparative HPLC procedure was in HPLC grade and other solvents were of analytical grade.

General procedure: The dried-up, powdered thorns of *G. sinensis* (8 kg) were extracted three times under reflux with 95 % EtOH. The combined ethanolic solution was concentrated *in vacuo* to yield a dark brown residue (about 620 g). The concentrated extract was suspended in H_2O (about 3000 mL) and extracted successively with petroleum ether (PE, 60-90 °C), EtOAc (each 3 × 3000 mL). After evaporation of the solvent, the EtOAc fraction (230 g) was obtained. This resulting residue was separated over silica gel column eluted with gradient solvents of CH_2Cl_2-MeOH (100:0 → 1:1) to yield 10 fractions (designated as fraction 1-10) according to their TLC profiles. Compound **1** (100 mg) was obtained through recrystallization from fraction 9 (9 g). Fraction 4 (1 g) was subjected to column chromatography over silica gel with petroleum ether/EtOAc (100:0 → 1:1) and as eluants and further purified by the use of Sephadex LH-20 (MeOH-CH_2Cl_2 (1:1)), resulting in compound **2** (30 mg). Fraction 2 (0.63 g) was successively eluted over silica gel column with petroleum ether/EtOAc (100:0 → 1:1) to afford compound **3** (15 mg). Fraction 5 (9.5 g) was applied to silica gel column chromatography eluted with petroleum ether/EtOAc (100:0 → 1:1) to give nine subfractions (F5-1-F5-9). F5-3 (240 mg) was separated over silica gel column with petroleum ether/EtOAc (100:0 → 1:1) and purified by the use of Sephadex LH-20 (MeOH-CH_2Cl_2 (1:1)) to afford compound **4** (27 mg) and **5** (7 mg). F5-5 (110 mg)

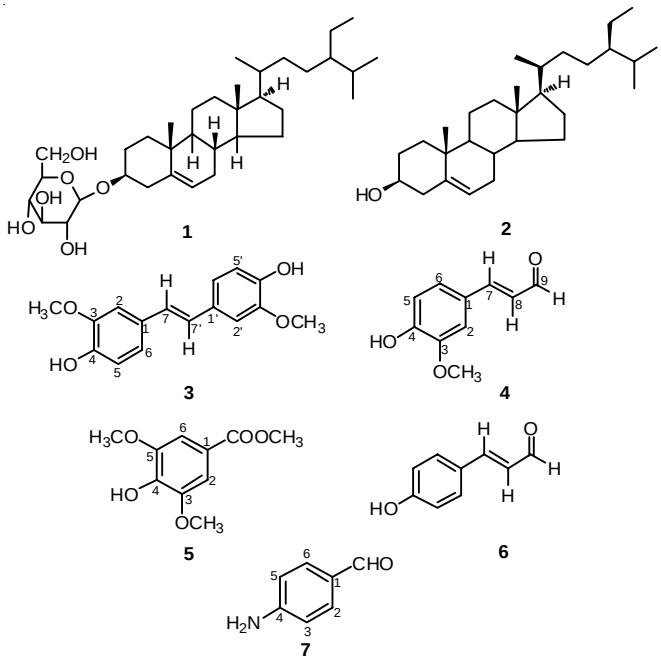

Fig. 1. Structures of compounds **1-7**

was applied to preparative HPLC system [mobile phase: CH_3OH/H_2O (55:45, v/v); flow rate: 3 mL min^{-1}; UV detection at 305 nm] resulting in the isolation of compound **7** (11.8 mg). F5-6 (70 mg) was applied to preparative HPLC system [mobile phase: CH_3OH/H_2O (55:45, v/v); flow rate: 3 mL min^{-1}; UV detection at 235 nm] resulting in the isolation of compound **6** (14.2 mg).

Daucosterol (1): White powder (CH_3OH). There is no fluorescence under ultraviolet light, 10 % sulfuric acid-ethanol colour in red. It was found to be identical with daucosterol by comparising with authentic sample by TLC.

β-Sitosterol (2): White needle crystal (acetone); positive Libermann-Burchard test and Salkowski test for steriols; 10 % sulfuric acid-ethanol colour in purple; ESI-MS (positive mode) *m/z*: 437.2 $[M + Na]^+$ ($C_{29}H_{50}O_2$). It was found to be identical with β-sitosterol on comparison with authentic sample by TLC.

(*E*)-3,3'-Dimethoxy-4,4'-dihydroxystilbene (3): Colourless powder (EtOAc); ESI-MS (positive mode) *m/z*: 273.1 $[M + H]^+$ ($C_{16}H_{16}O_4$); ^{1}H NMR (acetone, 600 MHz): 7.18 (1H, d, *J* = 1.8 Hz, H-2), 7.00 (1H, s, H-7), 6.99 (1H, dd, *J* = 1.8, 7.8 Hz, H-6), 6.81 (1H, d, *J* = 7.8 Hz, H-5), 3.04 (3H, s, -OCH_3); ^{13}C NMR (acetone, 150 MHz): 55.3 (q, -OCH_3), 109.0 (d, C-2), 115.0 (d, C-5), 119.8 (d, C-6), 126.1 (d, C-7), 130.1 (s, C-1), 146.2 (s, C-4), 147.7 (s, C-3).

***trans*-Coniferyl aldehye (4):** Yellowish needles ($CHCl_3$); ESI-MS (positive mode) *m/z*: 178.9 $[M + H]^+$ ($C_{10}H_{10}O_3$); ^{1}H NMR ($CDCl_3$, 600 MHz): 9.63 (1H, s, *J* = 7.8 Hz, -CHO), 7.41 (1H, d, *J* = 15.6 Hz, H-7), 7.12 (1H, dd, *J* = 1.2, 8.4 Hz, H-6), 7.07 (1H, d, *J* = 1.2 Hz, H-2), 6.96 (1H, d, *J* = 8.4 Hz, H-5), 6.60 (1H, dd, *J* = 7.8, 16.2 Hz, H-8), 3.94 (3H, s, -OCH_3); ^{13}C NMR ($CDCl_3$, 150 MHz): 193.8 (d, -CHO), 153.4 (d, C-7), 150.0 (s, C-4), 147.1 (s, C-3), 126.5 (s, C-1), 126.2 (d, C-8), 124.1 (d, C-6), 115.0 (d, C-5), 109.6 (d, C-2), 55.9 (q, -OCH_3).

Methyl syringate (5): Colourless needles ($CHCl_3$); ESI-MS (positive mode) *m/z*: 212.9 $[M + H]^+$ ($C_{10}H_{12}O_5$); ^{1}H NMR ($CDCl_3$, 600 MHz): 7.31 (2H, s, H-2, H-6), 5.95 (br. s, OH-4), 3.96 (6H, s, OCH_3-3, 5), 3.91 (3H, s, $COO\underline{CH_3}$); ^{13}C NMR ($CDCl_3$, 150 MHz): 166.9 (s, CO), 146.6 (2C, s, C-3, 5), 139.2 (s, C-4), 121.2 (s, C-1), 106.6 (2C, d, C-2, 6), 56.5 (2C, q, OCH_3-3, 5), 52.1 (q, $COO\underline{CH_3}$).

***p*-Hydroxyl-cinnamaldehyde (6):** Yellowish needles (CH_3OH); ESI-MS (positive mode) *m/z*: 148.8 $[M + H]^+$ ($C_9H_8O_2$); ^{1}H NMR (CD_3OD, 600 MHz): 9.56 (1H, d, *J* = 7.8 Hz, -CHO), 7.59 (1H, d, *J* = 16.2 Hz, H-7), 7.54 (2H, d, *J* = 8.4 Hz, H-2, 6), 6.84 (2H, d, *J* = 8.4 Hz, H-3, 5), 6.61 (1H, dd, *J* = 7.8, 16.2 Hz, H-8); ^{13}C NMR (CD_3OD, 150 MHz): 194.8 (d, -CHO), 161.1 (s, C-4), 154.6 (d, C-7), 130.6 (2C, d, C-2, 6), 124.9 (d, C-8), 115.7 (s, C-1), 110.0 (2C, d, C-3, 5).

4-Aminobenzaldehyde (7): Yellow powder (CH_3OH); ESI-MS (positive mode) *m/z*: 122.9 $[M + H]^+$ (C_7H_7ON); ^{1}H NMR (600 MHz, CD_3OD): 9.77 (1H, s, -CHO), 7.79 (2H, d, *J* = 0.6 Hz, H-2, 6), 6.92 (2H, d, *J* = 0.6 Hz, H-3, 5); ^{13}C NMR (150 MHz, CD_3OD): 191.4 (d, -CHO), 163.9 (s, C-4), 132.0 (2C, d, C-2, 6), 128.8 (s, C-1), 115.5 (2C, d, C-3, 5).

RESULTS AND DISCUSSION

Compound **1** was obtained as a white amorphous powder. It was unambiguously identified as daucosterol on the basis of its TLC profile.

Compound **2** was obtained as white needle crystal. The ESI-MS of **2** showed a molecular ion at *m/z* 414 corresponding to a molecular formula $C_{29}H_{50}O_2$. It was unambiguously identified as β-sitosterol on the basis of its TLC profile.

Compound **3** was isolated as colourless powder. It was determined to possess the molecular formula $C_{16}H_{16}O_4$ by its pseudomolecular ion peak at *m/z* 273.1 $[M + H]^+$ in the positive ESI-MS experiment. The ^{1}H NMR spectrum of compound **3** revealed the characteristic signals attributable to four aromatic protons, including an ABX spin system at δ_H 7.18 (d, *J* = 1.8 Hz), 7 (dd, *J* = 1.8, 7.8 Hz) and 6.18 (d, *J* = 7.8 Hz) and one olefinic proton signal at δ_H 7 (s) (Table-1). In addition, one methoxyl at δ_H 3.04 (3H, s) was also determined in the ^{1}H NMR spectrum. Compound **3** was established as (*E*)-3,3'-dimethoxy-4,4'-dihydroxystilbene[9] by comparision of its spectroscopic data (^{1}H and ^{13}C NMR, MS) with the literature values.

Compound **4** was isolated as yellowish needles. It was determined to possess the molecular formula $C_{10}H_{10}O_3$ by its pseudomolecular ion peak at *m/z* 178.9 $[M + H]^+$ in the positive ESI-MS experiment. The ^{1}H NMR spectrum of compound **4** revealed the characteristic signals attributable to five aromatic protons, including an ABX spin system at δ_H 7.12 (dd, *J* = 1.2, 8.4 Hz, H-6), 7.07 (d, *J* = 1.2 Hz, H-2) and 6.96 (1H, d, *J* = 8.4 Hz, H-5), two proton AB doublets at δ_H 7.41 (d, *J* = 15.6 Hz, H-7) and 6.60 (dd, *J* = 7.8, 15.6 Hz, H-8) and one -CHO group at δ_H 9.63 (d, *J* = 7.8 Hz, H-9). In addition, one methoxyl at δ_H 3.94 (3H, s) was also determined in the ^{1}H NMR spectrum. The coupling constants of $J_{7,8}$ and $J_{8,9}$ indicated that $\Delta^{7,8}$ was *trans* and the CHO group was supposed to be linked at C-8, which were supported by the long-range ^{1}H- ^{13}C correlations from δ_H 7.41 (d, *J* = 15.6 Hz, H-7) to δ_C 126.2 (C-8) and 193.8 (C-9), from δ_H 6.60 (d, *J* = 7.8, 16.2 Hz, H-8) to δ_C 153.3 (C-7) and 193.8 (C-9) and from δ_H 9.63 (d, *J* = 7.8 Hz, H-9) to δ_C 153.4 (C-7) and 126.2 (C-8) in the HMBC spectrum (Fig. 2).

TABLE-1
^1H AND ^{13}C NMR SPECTRAL DATA FOR COMPOUNDS **4** (IN $CDCl_3$) AND 6 (IN CD_3OD) (600/150 MHz)

Positions	**4**		**6**	
	δ_H	δ_C	δ_H	δ_C
1		126.5 (s)		115.7 (s)
2	7.07 (1H, d, 1.2)	109.6 (d)	7.54 (2H, d, 8.4, H-2, 6)	130.6 (d)
3		147.1 (s)	6.84 (2H, d, 8.4, H-3, 5)	110.0 (d)
4		150.0 (s)		161.1 (s)
5	6.96 (1H, d, 8.4)	115.0 (d)	6.84 (2H, d, 8.4, H-3, 5)	110.0 (d)
6	7.12 (1H, dd, 1.2, 8.4)	124.1 (d)	7.54 (2H, d, 8.4, H-2, 6)	130.6 (d)
7	7.41 (1H, d, 15.6)	153.4 (d)	7.59 (1H, d, 16.2)	154.6 (d)
8	6.60 (1H, dd, 7.8, 16.2)	126.2 (d)	6.61 (1H, dd, 7.8, 16.2)	124.9 (d)
9	9.63 (1H, d, 7.8)	193.8 (d)	9.56 (1H, d, 7.8)	194.8 (d)
OMe	3.94 (3H, s)	55.9 (q)		

Furthermore, $\Delta^{7,8}$ was proposed to be linked at C-8, which were supported by the long-range ^1H-^{13}C correlations from δ_H 7.41 (d, J = 15.6 Hz, H-7) to δ_C 126.5 (C-1), 109.6 (C-2) and 124.1 (C-6), from δ_H 6.60 (dd, J = 7.8, 16.2 Hz, H-8) to δ_C 126.5 (C-1), from δ_H 7.07 (d, J = 1.2 Hz, H-2) to δ_C 124.1 (C-6) and 153.4 (C-7) and from δ_H 7.12 (dd, J = 1.2, 8.4 Hz, H-6) to δ_C 109.6 (C-2) and 153.4 (C-7) in the HMBC spectrum (Fig. 2). Compound **4** was established as *trans*-coniferyl aldehyde[10] by comparision of its spectroscopic data (^1H and ^{13}C NMR, MS) with the literature values (Table-1).

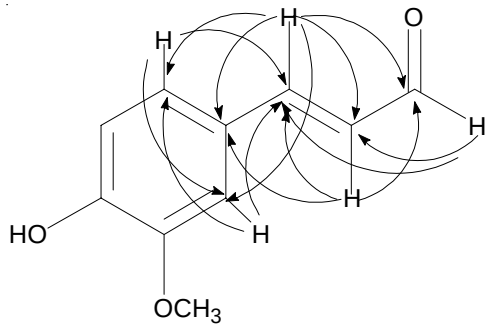

Fig. 2. Key HMBC correlations in compound **4**

Compound **5** was obtained as colourless needles. It was determined to possess the molecular formula $C_{10}H_{12}O_5$ by its pseudomolecular ion peak at *m/z* 212.9 $[M + H]^+$ in the positive ESI-MS experiment. Compound **5** was established as methyl syringate[11] by comparision of its spectroscopic data (^1H and ^{13}C NMR, MS) with the literature values.

Compound **6** was isolated as yellowish needles. It was determined to possess the molecular formula $C_9H_8O_2$ by its pseudomolecular ion peak at *m/z* 148.8 $[M + H]^+$ in the positive ESI-MS experiment. A close comparison of the ^1H and ^{13}C NMR data of **6** with those of **4** indicated that their structures are closely related (Table-1). Compound **6** was established as *p*-hydroxy-cinnamaldehyde[12] by comparision of its spectroscopic data (^1H and ^{13}C NMR, MS) with the literature values.

Compound **7** was obtained as yellow powder. It was determined to possess the molecular formula C_7H_7ON by its pseudomolecular ion peak at *m/z* 122.9 $[M + H]^+$ in the positive ESI-MS experiment. Compound **7** was established as 4-Aminobenzaldehyde[13] by comparision of its spectroscopic data (^1H and ^{13}C NMR, MS) with the literature values.

ACKNOWLEDGEMENTS

This work was sponsored by the Science and Technology Program of Shandong province and a research grant from Shandong Analysis and Test Center (CS201405).

REFERENCES

1. China Pharmacopoeia Committee, Pharmacopoeia of the People's Republic of China (the First Part), China Medical Science and Technology Press, Beijing, p. 166 (2010).
2. L. Zhou, D. Li, J. Wang, Y. Liu and J. Wu, *J. Nat. Prod*., **21**, 283 (2007).
3. W.H. Li, Q. Li, X.G. Wang and W.M. Zhao, *J. Northwest Univ*. (*Nat. Sci. Ed*.), **35**, 763 (2005).
4. Z. Xu, X.D. Zhao, Y.M. Wang, X.H. Chen and K.S. Bi, *J. Shenyang Pharm. Univ*., **25**, 108 (2008).
5. W.H. Li and J.X. Fu, *J. Hanzhong Teachers College*, **17**, 41 (1999).
6. W.H. Li, Q. Li, X.G. Wang and W.M. Zhao, *J. Northwest Univ*., **37**, 401 (2007).
7. J.-C. Lim, J.H. Park, M. Budesinsky, A. Kasal, Y.-H. Han, B.-S. Koo, S.-I. Lee and D.-U. Lee, *Chem. Pharm. Bull*. (Tokyo), **53**, 561 (2005).
8. D. Lu, Y.F. Xia, B. Tong, C.F. Zhang, R. Pan, H. Xu, X. Yang and Y. Dai, *Integr. Cancer Ther*., **13**, 446 (2012).
9. Z. Hajdú, E. Varga, J. Hohmann, A. Kálmán, G. Argay and G. Günther, *J. Nat. Prod*., **61**, 1298 (1998).
10. M. Miyazawa and M. Hisama, *J. Agric. Food Chem*., **51**, 6413 (2003).
11. J. Tan, S. Jiang and D. Zhu, *Nat. Prod. Res. Dev*., **17**, 267 (2004).
12. C. Luo and W.N. Zhang, *Chin. Tradit. Herbal Drugs*, **42**, 437 (2011).
13. S. Ghosal and D.K. Chakrabarti, *Phytochemistry*, **27**, 1339 (1988).