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Antimicrobial Constituents from Fruits of *Ailanthus Altissima* SWINGLE

Chun-Chao Zhao, Jian-Hua Shao[1], Xian Li, Jing Xu, and Peng Zhang

Research Department of Natural Medicine, Shenyang Pharmaceutical University, Shenyang 110016, China and [1]School of Pharmaceutical engineering, Shenyang Pharmaceutical University, Shenyang 110016,China

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A new naturally occurring sterol, compound **5**, and six known stigmasterols were isolated from fruits of *Ailanthus altissima* Swingle by repeated column chromatography and RP-HPLC. Their structures were identified as, 5α-stigmastane-3,6-dione (**1**), 3β-hydroxystigmast-5-en-7-one (**2**), stigmast-5-ene-3β, 7α-diol (**3**), 6α-hydroxystigmast-4-en-3-one (**4**), 5α-stigmastane-3β, 6β-diol (**5**), stigmast-4-ene-3β, 6α-diol (**6**), stigmast-5-ene-3β, 7α, 20ξ-triol (**7**) by spectral analysis and comparison with the published data. These compounds have not been reported from genus *Ailanthus*, whereas compound **7** was identified by NMR for the first time. In addition, the 95% ethanol extract and compounds from the fruits of Ailanthus altissima SWINGLE were assayed for *in vitro* antimicrobial activity. The extract was potent active against the assayed bacteria while compounds **3** and **7** exhibited moderate activity.

Key words: *Simaroubaceae,* Fruits of *Ailanthus altissima* SWINGLE, Stigmasterol

INTRODUCTION

Ailanthus altissima Swingle (*Simaroubaceae*) is widespread in China. The fruits of this plant have been used for the antibacterial in Chinese traditional medicine. Some compounds including four bitter quassinoid glycosides (Shin *et al.*, 1984), five stigmasterols (Zhao *et al.*, 2003) have been isolated. Among them, stigmast-4-ene-3,6-dione had been reported to inhibit barley germination by 40% at a concentration of 3 mM, and also showed a moderate stimulatory effect on root growth at concentration below 0.1 mM (Pardo *et al.*, 2000). In this paper, we describe the isolation of seven stigmasterols from fruits of *A. altissima* Swingle as well as the identification of their structures by spectroscopic analysis and comparison with the published data.

MATERIALS AND METHODS

Instruments and reagents

Melting points were determined on Yanaco MP-S3 melting point apparatus and are uncorrected. EI-MS spectra was measured with a VG-5050E mass spectrometer. NMR spectra were recoreded on a Bruker ARX-300 NMR spectrometer using TMS as an internal standard. HPLC was Shimadzu CTO-6A model with ultraviolet detector. Silica gel for chromatography was produced by Qingdao Ocean Chemical Group Co. of China. All other chemicals and reagents were analytical grade.

Plant materials

The plant material of fruits of *Ailanthus altissima* Swingle was collected in Tanghe city, Henan Province, China, in July, 2002, and identified by Prof. Qishi Sun, Shenyang Pharmaceutical University, China. A voucher specimen (No. 20020703) is deposited in Research Department of Natural Medicine, Shenyang Pharmaceutical University, China.

Extraction and isolation

The air-dried fruits (9 kg) of *Ailanthus altissima* Swingle were extracted with 95% ethanol (90 L × 3) for 24 h at room temperature. Extracts were concentrated *in vacuo* to give a residue (170 g), which was subjected to column chromatographic separation on silica gel (700 g), gradiently eluted with petroleum ether-EtOAc. Subfraction 34 (100:14, 26 mg) was further purified by recrystallization from petroleum ether-acetone to give compound **5** (7 mg). Subfraction 58 (100:20, 231 mg) was rechromatographed

Correspondence to: Xian Li, Research Department of Natural Medicine, Shenyang Pharmaceutical University, Shenyang 110016, China
Tel: 86-24-23986476, Fax: 86-24-2384116
E-mail: Proflixian@163.com

over a silica gel column by a gradient elution with Petroleum ether-$CHCl_3$-MeOH to yield compound **7** (10:30:3, 6 mg). Subfraction 18 (100:4.5, 142 mg), 26 (100: 8.5, 426 mg) and 56 (100:16, 261 mg) were rechromatographed on a silica gel column by a gradient elution with petroleum ether-acetone to afford compound **1** (100:2, 35 mg), **3** (100:4, 12 mg), and **6** (100:7, 8 mg), respectively. A mixture was yielded from the subfraction 26 besides compound **3**. The mixture was chromatographed on RP-HPLC with MeOH-H_2O (92:18) to yield compound **2** (8 mg) and **4** (6 mg).

Compound **1**

Colorless needles (petroleum ether-acetone); m.p. 175-178°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ_H: 0.69 (3H, s, H-18), 0.96 (3H, s, H-19), 0.93 (3H, d, J = 6.2 Hz, H-21), 0.84 (3H, d, J = 6.6 Hz, H-26), 0.81 (3H, d, J = 6.8 Hz, H-27), 0.85 (3H, t, J = 7.0 Hz, H-29); ^{13}C-NMR (75 MHz, $CDCl_3$) data: see Table I.

Compound **2**

Colorless needles ($CHCl_3$-MeOH); m.p. 124-125°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ_H: 0.66 (3H, s, H-18), 1.20 (3H, s, H-19), 0.93 (3H, d, J = 6.3 Hz, H-21), 0.84 (3H, d, J = 7.1 Hz, H-26), 0.83 (3H, d, J = 6.5 Hz, H-27), 0.85 (3H, t, J = 7.0 Hz, H-29), 3.67 (1H, m, H-3), 5.69 (1H, s, H-6); ^{13}C-NMR (75 MHz, $CDCl_3$) data: see Table I.

Compound **3**

Colorless needles ($CHCl_3$-MeOH); m.p. 202-204°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ_H: 0.71 (3H, s, H-18), 1.02 (3H,

Table I. The ^{13}C-NMR data of compounds **1-7**

C	1	2	3	4	5	5a	6	6a	7
1	38.0	36.4	37.1	36.3	38.5	37.3	37.1	37.7	36.9
2	39.4	31.1	31.4	34.2	30.4	31.1	30.2	30.2	31.3
3	209.0	70.4	71.4	199.5	71.7	71.3	67.7	67.6	71.4
4	37.0	41.8	42.1	119.7	31.5*	32.3	122.9	130.0	42.0
5	57.5	165.3	146.3	158.7	47.4*	51.7	148.5	147.2	146.3
6	211.1	126.0	123.9	68.6	72.1	69.5	68.0	73.8	123.8
7	46.6	202.3	65.4	39.5	40.0	41.7	43.3	40.3	65.2
8	37.4	45.8	37.6	33.8	35.4*	34.3	34.3	31.0	37.4
9	53.5	49.9	42.3	53.8	54.3	53.8	54.9	55.1	42.4
10	41.2	38.3	37.5	39.0	35.4*	36.3	38.1	37.4	36.9
11	21.7	21.2	20.7	21.0	21.1	21.2	20.0	21.4	20.5
12	38.1	38.7	39.2	41.5	39.7	39.8	40.1	40.8	39.5
13	43.0	43.1	42.2	42.5	42.7	42.6	42.8	42.9	42.1
14	56.0	49.9	49.5	55.6	56.2	56.2	56.3	56.5	49.5
15	24.0	26.1	24.3	24.2	24.3	24.2	24.5	24.6	24.4
16	28.0	28.5	28.3	28.1	28.2	28.2	28.5	28.6	26.5
17	56.6	54.7	55.8	56.0	56.2	56.1	56.4	56.5	57.0
18	12.5	11.9	12.0	12.0	12.0	12.0	12.2	12.3	13.4
19	12.0	17.3	18.3	18.3	15.8	13.5	21.4	21.7	18.2
20	36.0	36.0	36.1	36.1	36.2	36.1	36.4	36.5	75.4
21	18.7	18.9	18.8	18.7	18.8	18.7	19.0	19.0	22.3
22	33.8	33.9	34.0	33.9	34.0	33.9	34.2	34.3	42.4
23	26.1	26.3	26.0	26.1	26.2	26.1	26.5	26.5	23.8
24	45.8	45.8	45.9	45.9	45.9	45.8	46.1	46.1	46.1
25	29.1	29.1	29.2	29.2	29.2	29.1	29.5	29.5	29.1
26	19.8	19.8	19.8	19.8	19.8	19.8	19.9	20.0	19.6
27	19.0	19.0	19.1	19.0	19.0	19.0	19.3	19.3	19.2
28	23.1	23.0	23.1	23.1	23.1	23.1	23.4	23.4	23.0
29	12.0	11.9	11.6	12.0	12.1	12.0	12.2	12.2	12.1

1-5 and **7** in $CDCl_3$, **6** in Pyridine-d_5; * Signals may be reversed.

s, H-19), 0.95 (3H, d, J = 6.2 Hz, H-21), 0.85 (3H, d, J = 6.6 Hz, H-26), 0.83 (3H, d, J = 6.5 Hz, H-27), 0.87 (3H, t, J = 7.1 Hz, H-29), 3.61 (1H, m, H-3), 3.88 (1H, br. s, H-7), 5.63 (1H, d- like, J = 4.8 Hz, H-6); ^{13}C-NMR (75 MHz, $CDCl_3$) data: see Table I.

Compound **4**

Colorless needles (petroleum ether-EtOAc); m.p. 201-203°C; H-NMR (300 MHz, $CDCl_3$) δ_H: 0.71 (3H, s, H-18), 1.18 (3H, s, H-19), 0.92 (3H, d, J = 6.4 Hz, H-21), 0.84 (3H, d, J = 6.6 Hz, H-26), 0.81 (3H, d, J = 6.8 Hz, H-27), 0.85 (3H, t, J = 7.0 Hz, H-29), 4.33 (1H, dd-like, J = 11.7, 4.6 Hz), 6.18 (1H, s); ^{13}C-NMR (75 MHz, $CDCl_3$) data: see Table I.

Compound **5**

Colorless needles (petroleum ether-acetone); m.p. 205-207°C; EI-MS (rel. int. %) *m/z* 432 ([M]$^+$, 5), 400 (11), 414 (78), 396 (5), 291 (1), 232 (32), 213 (27), 95 (75); ^{1}H-NMR (300 MHz, $CDCl_3$) δ_H: 0.69 (3H, s, H-18), 1.05 (3H, s, H-19), 0.91 (3H, d, J = 6.4 Hz, H-21), 0.84 (3H, d, J = 7.1 Hz, H-26), 0.83 (3H, d, J = 6.5 Hz, H-27), 0.85 (3H, t, J = 7.0 Hz, H-29), 3.65 (1H, m, H-3), 3.80 (1H, d-like, J=2.3 Hz, H-6); ^{13}C-NMR (75 MHz, $CDCl_3$) data: see Table I.

Compound **6**

Colorless needles ($CHCl_3$-MeOH); m.p. 256-258°C; EI-MS (rel. int. %) *m/z* 430 ([M]$^+$, 1), 412 (90), 394 (9), 287 (7), 271 (50), 253 (14), 229 (75), 95 (100); ^{1}H-NMR (300 MHz, pyridine-d_5) δ_H: 0.68 (3H, s, H-18), 1.05 (3H, s, H-19), 0.98 (3H, d, J = 6.2 Hz, H-21), 0.87 (3H, d, J = 6.2 Hz, H-26), 0.84 (3H, d, J = 6.7 Hz, H-27), 0.88 (3H, t, J = 7.0 Hz, H-29), 4.49 (1H, d-like, J = 8.8Hz, H-6), 4.66 (1H, m, H-3), 6.68 (1H, s, H-4); ^{13}C-NMR (75 MHz, pyridine-d_5) data: see Table I.

Compound **7**

Colorless needles ($CHCl_3$-MeOH); m.p. 286-288°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ_H: 0.88 (3H, s, H-18), 1.00 (3H, s, H-19), 1.29 (3H, s, H-21), 0.84 (3H, d, J = 7.1 Hz, H-26), 0.83 (3H, d, J = 6.5 Hz, H-27), 0.85 (3H, t, J = 7.0 Hz, H-29), 3.57 (H, m, H-3), 3.86 (1H, br. s, H-7), 5.61 (1H, d-like, J = 4.5Hz, H-6); ^{13}C-NMR (75 MHz, $CDCl_3$) data: see Table I.

RESULTS AND DISCUSSION

Seven compounds were isolated from an 95% ethanol extract of the air-dried fruits of *Ailanthus altissima* Swingle by repeated column chromatography and RP-HPLC.

Compound **1-4** were identified as 5α-stigmastane-3, 6-dione; 3β-hydroxystigmast-5-en-7-one; stigmast-5-ene-3β, 7α-diol; 6α-hydroxystigmast-4-en-3-one respectively by spectral analysis and comparison with the published data (Greca *et al.*, 1990).

Compound **5** was obtained as colorless needles from petroleum ether-acetone, m.p. 205-207°C. The EI-MS spectrum of **5** gave molecular ion peak [M]$^+$ at *m/z* 432, consistent with a composition $C_{29}H_{52}O_2$. The ^{1}H-NMR spectrum of **5** showed six methyl signals, very similar to that of compound **1**, except that two additional protons attached to oxyen-bearing cabons were found at δ 3.80 as a doublet, and at δ 3.65 as a multiplet. A comparison with stigmastane-3β, 6β-diol (**5a**) (Kimura *et al.*, 1995) suggested that both compounds had the same plane structure. The different stereochemistry at C-6 caused an downfield shift of the C-6 carbon in the ^{13}C-NMR spectrum of **5**. In conclusion, the compound **5** was identified as stigmastane-3β, 6β-diol, which was a product of refining low erucic acid rapeseed oil (Pierre *et al.*, 2003).

Compound **6** was obtained as colorless needles from $CHCl_3$- MeOH, m.p. 256-258°C. The EI-MS spectrum of **6** gave molecular ion peak [M]$^+$ at *m/z* 430, together with the ^{1}H- and ^{13}C-NMR spectral data of **6**, so that we can postulate that its molecular formula is $C_{29}H_{50}O_2$. In the ^{1}H-NMR spectrum of **6**, an olefinic proton resonated at δ 6.68 (1H, s, H-4) as a singlet, as well as three methyl doublets at δ 0.84 (3H, d, J = 6.7 Hz, H-27), 0.87 (3H, d, J = 6.2 Hz, H-26), 0.98 (3H, d, J = 6.2 Hz, H-21), a methyl triplet at δ 0.88 (3H, t, J = 7.0 Hz, H-29), and two methyl singlets

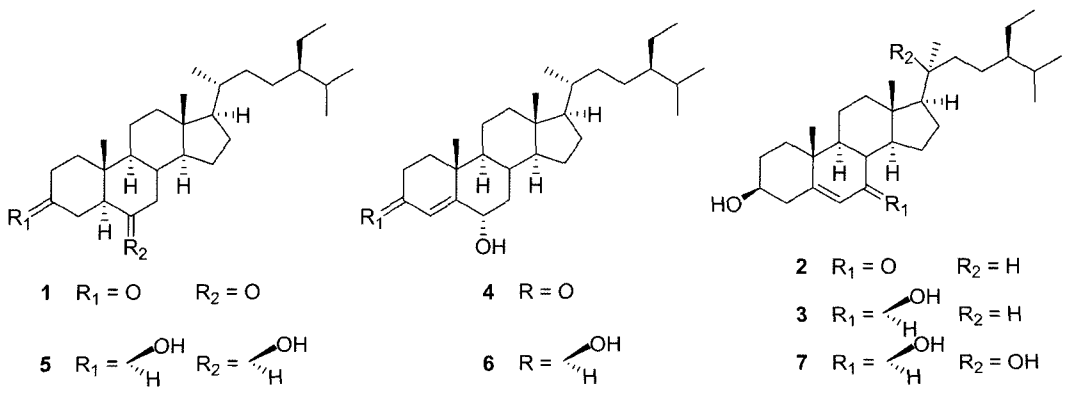

Fig. 1. Structures of compounds **1-7**

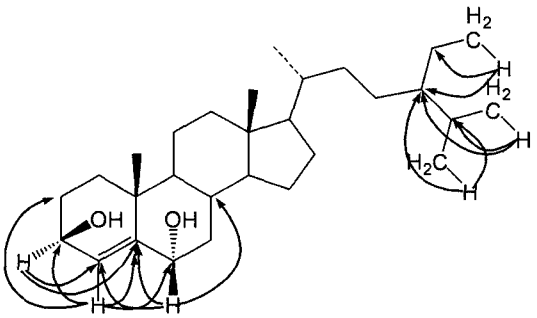

Fig. 2. Important HMBC correlations of **6**

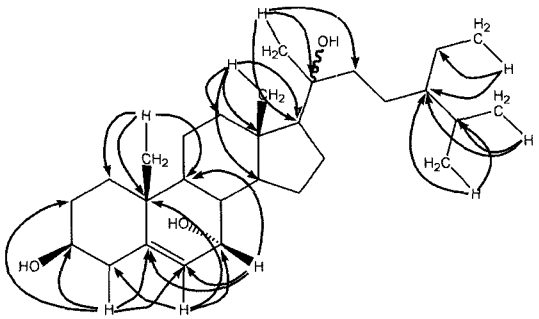

Fig. 3. Important HMBC correlations of **7**

Table II. The results of antibacterial activity of the extract and compounds **1-7**

Kind of bacteria	Inhibition circle/mm							
	extract	1	2	3	4	5	6	7
E. coli	7.40	-	-	14.12	-	-	-	14.00
S. aureus	7.07	-	-	13.20	-	-	-	13.24
P. aeruginosa	7.51	-	-	13.47	-	-	-	14.01
S. typhiuriun	6.87	-	-	-	-	-	-	-

at δ 0.68 (3H, s, H-18), 1.05 (3H, s, H-19). A comparison with stigmast-4-ene-3β, 6β-diol (**6a**) (Zhao *et al.*, 2003) suggested that both compounds had the same plane structure. The stereoisomeric 6α-hydroxy of compound **6** showed the H-19 proton shifted upfield 0.49 ppm, and the H-4 proton shifted downfield 0.68 ppm, due to the spatial effect of the 6-OH group. The different stereochemistry at C-6 caused an upfield shift of the C-4 and C-6 carbons and a downfield shift of C-5, C-8, and C-10 in the ^{13}C-NMR spectrum. In conclusion, the compound **6** was identified as stigmast-4-ene-3β, 6α-diol by extensive 1D, 2D-NMR technique (Fig. 2).

Compound **7** was obtained as colorless needles from $CHCl_3$- MeOH, m.p. 286-288°C. In the ^{1}H-NMR spectrum, an olefinic proton resonated at δ 5.61 (1H, d-like, J = 4.5 Hz, H-6), as well as two methyl doublets at δ 0.84 (3H, d, J = 7.1 Hz, H-26), 0.83 (3H, d, J = 6.5 Hz, H-27), a methyl triplet at δ 0.85 (3H, t, J = 7.0 Hz, H-29), and three methyl singles at 0.88 (3H, s, H-18), 1.00 (3H, s, H-19), 1.29 (3H, s, H-21). A comparison with compound **3** suggested that both compounds had the same plane structure, and the H-20 proton of compound **7** was replaced by a hydroxyl group due to H-21 proton becoming a singlet signal at δ 1.29 which shifted downfield 0.34 ppm. Furthermore, H-18 proton shifted downfield to δ 0.88 ppm, owing to the spatial proximity with the 20-OH group. Another independent supporting data was available from ^{13}C-NMR spectrum. Besides the chemical shifts at δ 71.2 (C-3), 65.3 (C-7), another oxygen bearing carbon signal can be observed at δ 75.4 (C-20). In the HMBC experiment, the long-range correlations between δ 1.29 (H-21) and δ 75.4 (C-20), 57.0 (C-17), as well as δ 0.88 (H-21) and δ 57.0 (C-17) indicated that H-20 was substituted by a hydroxyl group. In addition, δ 2.33 (H-4) presented long-range correlations with δ 31.3 (C-2), 71.4 (C-3), 146.3 (C-5), 123.8 (C-6) (Fig. 3). As a result, compound **7** was identified as stigmast-5-ene-3β, 7α, 20ξ-triol.

Investigation of antimicrobial activities of the extract and compounds **1-7** from the fruits of Ailanthus altissima SWINGLE was carried out using the filter paper method (Zheng *et al.*, 1989). The samples were dissolved in DMSO at a concentration of 1.2 mg/mL (extract) and 0.2 mg/mL (compound). The diameter of filter paper was 6 mm. We found that the extract was weakly active against *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. typhiuriun* while compounds **3** and **7** showed moderate activity against *E. coli*, *S. aureus*, and *P. aeruginosa*. The others had no inhibition (see Table II).

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REFERENCES

Greca, M. D., Monaco, P., and Previtera, L., Stigmasterols from *Typha Latifolia*. *J. Nat. Prod.*, 53, 1430-1435 (1990).

Kimura, Y., Akihisa, T., Yasukawa, K., Takido, M., and Tamura, T., Structures of five hydroxylated sterols from the seeds of Trichosanthes kirilowii MAXIM. *Chem. Pharm. Bull.*, 43,

1813-1817 (1995).

Pardo, F., Perich, F., Torres, R., and Monache, F. D., Stigmast-4-ene-3,6-dione an unusual phytotoxic sterone from the roots of *Echium vulgare* L. *Biochem. Sys. Ecol.,* 28, 911-913 (2000).

Pierre, L., Andre, G., Stephane, G., Pierre, J., Jean-Louis, S., and Constantin, B., Formation of modified fatty acids and oxyphytosterols during refining of low erucic acid rapeseed oil. *J. Agric. Food. Chem.,* 51, 4284-4290 (2003).

Shin, Y., Masami, I., Takakiko, T., Takahashi, T., and Matsushita, K., Constituents of *Ailanthus altissima* Swingle. Isolation and Structures of ShinJuglycosides A, B, C, and D. *Bull. Chem. Soc. Jpn.*, 57, 2496-2501 (1984).

Zhao, C. C., Wang, J.H., Li, W., Sha, Y., and Li, X., Studies on the chemical constituents of Fructus Ailanthi altissimae. *Chin. J. Med. Chem.*, 13, 211-214 (2003).

Zheng J. P., Wang G. B., Yao Pin Wei Sheng Wu Xue Ji Jian Yan Ji Shu [M]. Bei Jing: People's Medical Publishing House. (1989).