Chemical Constituents of *Ailanthus triphysa*

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Two new compounds, 8(14),15-isopimaradiene-2α,3α,19-triol (1), and 6α,7β-dihydroxy-17(20)-*cis*-5α-pregna-16-one (2), together with four known compounds, a oxygenated rare phyllocladane, phyllocladan-16α,19-diol (3), kaempferol-3-*O*-β-*D*-galactopyranoside, kaempferol-3-*O*-α-*L*-rhamnopyranoside and scopoletin, were isolated from the leaves of *Ailanthus triphysa*. Structures of 1—3 were elucidated on the basis of spectroscopic data as compared with related compounds.

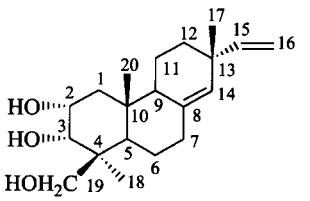
Keywords *Ailanthus triphysa*, isopimaradien, pregnane, phyllocladan

Introduction

The *Simarubaceae*, comprising about thirty genera, one hundred and fifty species, grows naturally in the torrid zone and subtropical zone. Four genera, about ten species are distributed in China. Five species and one variety of the four genera are used for medicinal materials. *Ailanthus* species are rich in bitter principle.[1] As part of our continuing study on chemical constituents of the genus *Ailanthus*, we examined the MeOH extract of the leaves of *Ailanthus triphysa*. A new diterpenoid, 8(14),15-isopimaradiene-2α,3α,19-triol (**1**), and a new pregnane, 6α,7β-dihydroxy-17(20)-*cis*-5α-pregna-16-one (**2**), together with four known compounds, phyllocladan-16α,19-diol (**3**), kaempferol-3-*O*-β-*D*-galactopyranoside,[2] kaempferol-3-*O*-α-*L*-rhamnopyranoside[2] and scopoletin[3] were obtained. Oxygenated phyllocladanes are rare in nature. The structure of **3** was determined by X-ray in the early literature without NMR data.[4] In this paper, we report the structure elucidation of two new compounds and assignment of the NMR data of **3**.

Results and discussion

Compound **1** was obtained as colorless needles, and has a molecular formula of $C_{20}H_{32}O_3$ by HREIMS (obsd: 320.2352, calcd: 320.2351). The IR (KBr) spectrum showed absorption bands at 3396 cm^{-1}(OH) and 1634 cm^{-1} (olefinic linkage). The ^{13}C and DEPT NMR spectra displayed the presence of three methyl carbons (δ 17.4, 23.2, 26.4), seven methylenes (δ 20.1, 23.0, 35.7, 37.2, 40.9, 65.7, 110.7), six methines (δ 49.6, 51.7, 67.2, 74.5, 130.3, 150.0) and four quaternary carbons (δ 38.4, 39.9, 45.4, 137.8). The ^1H NMR spectrum showed signals for a vinyl group [δ 4.89 (dd, J = 17.5, 1.4 Hz, 1H), 4.86 (dd, J = 10.6, 1.4 Hz, 1H), 5.76 (dd, J = 17.5, 10.6 Hz, 1H)], a trisubstituted double bond [δ 5.21 (s, 1H)] and four protons linked to oxygenated carbon [δ 3.91 (ddd, J = 7.5, 4.2, 1.8 Hz, 1H), 3.79 (d, J = 1.8 Hz, 1H), 3.70 (d, J = 11.2 Hz, 1H), 3.39 (d, J = 11.2 Hz, 1H)]. All these data suggested that **1** was either a trihydroxypimarene or trihydroxyisopimarene diterpenoid having two double bonds between C-8 and C-14, and between C-15 and C-16.[5-7] This was supported by the ^1H-^1H COSY spectrum which showed a correlation between H-15 [δ 5.76 (dd, J = 17.5, 10.6 Hz, 1H)] and H-16 [δ 4.86 and 4.89 (each 1H)] and HMBC spectrum which had long range coupling for H-14 [δ 5.21 (s)] to C-7 [δ 37.2 (t)], C-9 [δ 51.7 (d)] and C-15 [δ 150.0 (d)]; H-15 [δ 5.76 (dd, J = 17.5, 10.6 Hz)] to C-14 [δ 130.3 (d)], C-13 [δ 38.4 (s)] and C-17 [δ 26.4 (q)]; H-16 [δ 4.86 and



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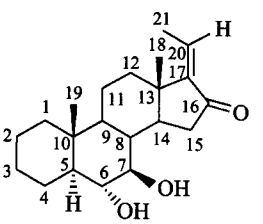

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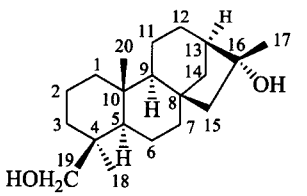

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Fig. 1 Structures of compounds **1**—**3**.

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4.89 (each 1H)] to C-13 [δ 38.4 (s)] and C-15 [δ 150.0 (d)].

Three hydroxy groups placed at C-2, C-3 and C-19, respectively, on the basis of the ^{1}H-^{1}H COSY spectrum which showed correlations between H-2 [δ 3.91 (ddd, J = 7.5, 4.2, 1.8 Hz, 1H)] and H-3 [δ 3.79 (d, J = 2.4 Hz, 1H)], H-2 and H-1 [δ 1.53—1.64 (m, 2H)], and HMBC spectrum with correlation for H-2 [δ 3.91 (ddd, J = 7.5, 4.2, 1.8 Hz, 1H)] to C-3 [δ 74.5 (d)]; H-3 [δ 3.79 (d, J = 2.4 Hz, 1H)] to C-1 [δ 40.9 (t)], C-4 [δ 45.4 (s)], C-5 [δ 49.6 (d) and C-2 (δ 67.2); H-19 [δ 3.70, 3.39 (d, J = 11.2 Hz, each 1H)] to C-18 [δ 23.2 (q)], C-4 [δ 45.4 (s)] and C-3 [δ 74.5 (d)]. The chemical shift value for C-8 (δ 137.8) assumed that **1** possessed an isopimar-8(14)-ene skeleton rather than its C-13 epimer since the latter showed a resonance at δ 141.0 for C-8.[6,8,9] The inference was supported by a NOESY experiment, and the relative stereochemistry of **1** was determined by a combination of coupling constant analyses and NOESY. The large coupling constant between H-2 and H-1 (J = 7.5 Hz) and the small coupling constant between H-2 and H-3 (J = 2.4 Hz) indicated that H-2 was in axial position and H-3 was in equatorial position. The signal at δ 0.81 (s, 3H, Me-20) showed NOE correlation with the signals at δ 3.91 (ddd, J = 7.5, 4.2, 1.8 Hz, 1H, H-2) and 3.70 (d, J = 11.2 Hz, 1H, H-19a), which indicated that Me-20, H-2 and H-19 had *cis*-relationship. NOE correlations of δ 1.83 (t, J = 7.8 Hz, 1H, H-9) with δ 1.55 (dd, overlap, 1H, H-5) and 2.01 (dt, J = 14.2, 5.3 Hz, 1H, H-7a), suggested their *cis*-relationship. The signal at δ 1.02 (s, 3H, Me-17) showed NOE correlation with the signal at δ 2.28 (dd, J = 14.2, 4.3 Hz, 1H, H-7b), which indicated their *cis*-relationship. On the basis of spectroscopic analysis and comparison with 7,15-isopimaradiene-2α,3β,19-triol {[α]$_D$ − 44 ($CHCl_3$)}[7] and its derivatives,[10] compound **1** was assumed to be isopimaradiene, and not *ent*- isopimaradiene. So the structure of **1** was elucidated as 8(14),15- isopimaradiene-2α,3α,19-triol {[α]$_D$ − 23 (*c* 0.5, CH_3OH)}.

Compound **2** showed a molecular formula of $C_{21}H_{32}O_3$ by HREIMS (obsd: 332.2362, calcd: 332.2351). The IR (KBr) spectrum showed absorptions at 3510 cm^{-1}(OH) and 1715, 1641 cm^{-1}(α,β-unsaturated ketone). The ^{1}H NMR spectrum showed two methyl protons as singlets [δ 0.85, 0.97 (s, each 3H)], one methyl proton as doublet [δ 1.81 (d, J = 7.5 Hz, 3H)], two oxymethines [δ 3.23—3.27 (m, 1H), 3.31—3.36 (m, 1H)], and one olefinic proton [δ 6.47 (q, J = 7.5 Hz, 1H)]. The ^{13}C and DEPT NMR spectrum displayed the presence of three methyl carbons (δ 13.1, 13.5, 17.6), seven methylenes (δ 20.6, 22.4, 28.2, 31.4, 35.8, 36.2, 37.8), seven methines (δ 33.8, 49.9, 50.5, 54.0, 75.3, 76.3, 129.1), two of which were oxygenated, three quaternary carbons (δ 37.3, 43.2, 147.8), and one carbonyl (δ 206.5). Compound **2** was supposed to possess a dihydroxy-17(20)-pregna-16-one skeleton, on the basis of comparison with those spectral data of 2β,3β-dihydroxy-17(20)-5α-pregna-16-one (*cis* and *trans*)[11] and 3β,4β-dihydroxy-17(20)-5α-pregna-16-one (*cis* and *trans*).[12] This was supported by ^{1}H-^{1}H COSY and HMBC. The ^{1}H-^{1}H COSY spectrum showed a correlation between H-20 [δ 6.47 (q, J = 7.5 Hz, 1H)] and H-21 [δ 1.81 (d, J = 7.5 Hz, 3H)]. The HMBC spectrum had long range coupling for H-20 and C-21 [δ 13.1 (q)], C-13 [δ 43.2 (s)], C-17 [δ 147.8 (s)] and C-16 [δ 206 (s)]; H-21 and C-20 [δ 129.1 (d)], C-17; H-15 [δ 2.16, 1.98 (dd, overlap, each 1H)] and C-16. The chemical shift values of C-15 (δ 37.8), C-16 (δ 206.5), C-17 (δ 147.8), C-18 (δ 17.6), C-20 (δ 129.0) and C-21 (δ 13.1) were more similar to those of 2β,3β-dihydroxy-17(20)-*cis*-5α-pregna-16-one (δ 37.9, 206.4, 148.0, 17.7, 129.0, 13.1)[11] and 3β,4β-dihydroxy-17(20)-*cis*-5α-pregna-16-one (δ 38.1, 206.7, 147.9, 17.6, 129.3, 13.2)[12] than those of 2β,3β- dihydroxy-17(20)-*trans*-5α-pregna-16-one (δ 39.5, 208.7, 148.4, 19.7, 130.0, 14.1).[11] So compound **2** was elucidated as a dihydroxy-17(20)-*cis*-pregna-16-one.

Two hydroxy groups positioned at C-6 and C-7, respectively, on the basis of the ^{1}H-^{1}H COSY spectrum with correlation between δ 3.23—3.27 (m, 1H, H-6) and 3.31—3.36 (m, 1H, H-7) and the HMBC spectrum with long range coupling for C-6 [δ 75.3 (d)] to H-7 and H-5 [δ 1.04—1.08 (m, 1H)], C-7 [δ 76.3 (d)] to H-6 and H-8 [δ 1.48—1.51 (m, 1H)]. The NOESY spectrum displayed the correlations between H-6 and H-19 [δ 0.85 (s, 3H)], which indicated that the configuration of H-6 was β. H-6 showed no correlation with H-7, which suggested the configuration of H-7 was α. H-7 showed correlation with H-5, which indicated that the configuration of H-5 was α. Therefore, the structures of **2** was elucidated as 6α,7β-dihydroxy-17(20)-*cis*-5α-pregna-16-one.

Compound **3** was determined by extensive analysis of its NMR spectra and comparison with other phyllocladan derivatives.[13] All the NMR data of **3** were assigned by its HMBC, HMQC and ^{1}H-^{1}H COSY spectra in experimental section.

Experimental

General

All melting points were measured on an XRC-1 micro melting point apparatus and uncorrected. Optical rotations were measured with a Horiba SEAP-300 spectropolarimeter in MeOH solution. IR spectra were obtained on a Bio-Rad FTS-135 infrared spectrophotometer. ^{1}H NMR, ^{13}C NMR and 2D-NMR spectra were recorded on Bruker AM-400 MHz and DRX-500 spectrometers with TMS as internal standard. MS data were recorded on a VG Autospec-3000 spectrometer.

Plant material

Leaves of *Ailanthus triphysa* were collected in Xishuangbanna County, Yunnan Province, China, in September 1999 and were identified by Prof. Cui J. Y., and a voucher specimen (Li Tingfei 001744) was deposited in the Herbarium of

the Department of Taxonomy, Kunming Institute of Botany, Chinese Acedemy of Sciences.

Extraction and isolation

Dried and powdered leaves (2 kg) were extracted with 95% MeOH three times at room temperature, and then the solvent was removed *in vacuo*. The residue was partitioned between H_2O and petroleum ether, EtOAc, respectively. The EtOAc extract (61 g) was subjected to column chromatography over silica gel eluted with $CHCl_3$-Me_2CO (from 100% $CHCl_3$ to 100% Me_2CO) to afford 12 fractions (F1-F12). Fractions F3—6 were repeatedly chromatographed over silica gel and then purified with $CHCl_3$-Me_2O (11:1 and 6:1) to yield **3** (43 mg), and **2** (34 mg), respectively. Fractions F7—9 were repeatedly chromatographed over silica gel eluted with $CHCl_3$-MeOH (16:1 and 12:1 to 11:1) and recrystallized (in MeOH) to afford **1** (28 mg).

1 Whiter crystals (CH_3OH), m.p. 207.5—209.5 ℃, $[\alpha]_{14}^{D}$ −23.0 (*c* 0.5, CH_3OH); ^{1}H NMR (CD_3OD, 500 MHz) δ: 1.53—1.64 (m, 2H, H-1), 3.91 (ddd, J = 7.5, 4.2, 1.8 Hz, 1H, H-2), 3.79 (d, J = 2.4 Hz, 1H, H-3), 1.55 (dd, overlap, 1H, H-5), 1.52—1.57 (m, 2H, H-6), 2.01 (dt, J = 14.2, 5.3 Hz, 1H, H-7α), 2.28 (dd, J = 14.2, 4.3 Hz, 1H, H-7β), 1.83 (t, J = 7.8 Hz, 1H, H-9), 1.51—1.55 (m, 2H, H-11), 1.35—1.38 (m, 1H, H-12α), 1.44—1.49 (m, 1H, H-12β), 5.21 (s, 1H, H-14), 5.76 (dd, J = 17.5, 10.6 Hz, 1H, H-15), 4.89 (dd, J = 17.5, 1.4 Hz, 1H, H-16a), 4.86 (dd, J = 10.6, 1.4 Hz, 1H, H-16b), 3.70 (d, J = 11.2 Hz, 1H, H-19a), 3.39 (d, J = 11.2 Hz, 1H, H-19b), 1.02 (s, 3H, Me-17), 1.08 (s, 3H, Me-18), 0.81 (s, 3H, Me-20); ^{13}C NMR (CD_3OD, 125 MHz) δ: 40.9 (t, C-1), 67.2 (d, C-2), 74.5 (d, C-3), 45.4 (s, C-4), 49.6 (d, C-5), 23.0 (t, C-6), 37.2 (t, C-7), 137.8 (s, C-8), 51.7 (d, C-9), 39.9 (s, C-10), 20.1 (t, C-11), 35.7 (t, C-12), 38.4 (s, C-13), 130.3 (d, C-14), 150.0 (d, C-15), 110.7 (t, C-16), 26.4 (q, C-17), 23.2 (q, C-18), 65.7 (t, C-19), 17.4 (q, C-20); IR (KBr) ν: 3396, 3078, 2936, 2869, 1634, 1447, 1427, 1405, 1317, 1272, 1199, 1144, 1077, 1045, 1001, 906 cm^{-1}; EIMS (70 eV) *m/z* (%): 320 (M^+, 20), 302 (15), 289 (17), 271 (60), 253 (16), 243 (14), 229 (5), 201 (8), 187 (25), 167 (29), 149 (42), 135 (71), 121 (100), 107 (61), 93 (51); HREIMS calcd for $C_{20}H_{32}O_3$ 320.2351, found 320.2352.

2 White powder, m.p. 197.5—199.5 ℃, $[\alpha]_D^{14}$ −124.1 (*c* 0.5, CH_3OH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.60—1.64 (m, 1H, H-1α), 0.99—1.04 (m, 1H, H-1β), 1.85—1.90 (m, 1H, H-2α), 1.12—1.17 (m, 1H, H-2β), 1.83—1.86 (m, 1H, H-3β), 1.43—1.49 (m, 1H, H-3α), 1.67—1.70 (m, 2H, H-4), 1.04—1.08 (m, 1H, H-5), 3.23—3.27 (m, 1H, H-6), 3.31—3.36 (m, 1H, H-7), 1.48—1.51 (m, 1H, H-8), 0.82—0.85 (m, 1H, H-9), 1.59—1.62 (m, 1H, H-11β), 1.34—1.37 (m, 1H, H-11α), 2.24—2.28 (m, 1H, H-12α), 1.60—1.63 (m, 1H, H-12β), 1.45—1.49 (m, 1H, H-14), 2.16 (dd, overlap, 1H, H-15a), 1.98 (dd, overlap, 1H, H-15b), 0.97 (s, 3H, Me-18), 0.85 (s, 3H, Me-19), 6.47 (q, J = 7.5 Hz, 1H, H-20), 1.81 (d, J = 7.5 Hz, 3H, Me-21); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 35.8 (t, C-1), 22.4 (t, C-2), 28.2 (t, C-3), 31.4 (t, C-4), 49.9 (d, C-5), 75.3 (d, C-6), 76.3 (d, C-7), 33.8 (d, C-8), 54.0 (d, C-9), 37.3 (s, C-10), 20.6 (t, C-11), 36.2 (t, C-12), 43.2 (s, C-13), 50.5 (d, C-14), 37.8 (t, C-15), 206.5 (s, C-16), 147.8 (s, C-17), 17.6 (q, C-18), 13.5 (q, C-19), 129.1 (d, C-20), 13.1 (q, C-21); IR (KBr) ν: 3510, 3401, 2929, 2852, 1715, 1641, 1449, 1377, 1151, 1062, 1039, 750 cm^{-1}; EIMS (70 eV) *m/z* (%): 332 (M^+, 100), 317 (87), 299 (22), 290 (57), 275 (15), 257 (7), 248 (24), 234 (61), 216 (11), 201 (7), 191 (21), 175 (21), 161 (20), 149 (36), 135 (45), 121 (52), 107 (45); HREIMS calcd for $C_{21}H_{32}O_3$ 332.2351, found 332.2362.

3 White needles, m.p. 192—194 ℃, $[\alpha]_D^{14}$ +7.0 (*c* 0.4, CH_3OH); ^{1}H NMR (CD_3OD, 500 MHz) δ: 0.80—0.84 (m, 1H, H-1α), 1.61—1.67 (m, 1H, H-1β) 1.51—1.53 (m, 2H, H-2), 1.80—1.82 (m, 1H, H-3β), 0.83—0.85 (m, 1H, H-3α), 0.95—0.98 (m, 1H, H-5), 1.59—1.61 (m, 1H, H-6α), 1.18—1.23 (m, 1H, H-6β), 1.61—1.64 (m, 1H, H-7β), 1.45—1.47 (m, 1H, H-7α), 1.07—1.10 (m, 1H, H-9), 1.52—1.56 (m, 1H, H-11α), 1.25—1.29 (m, 1H, H-11β), 1.65—1.67 (m, 2H, H-12), 1.68 (brs, 1H, H-13), 2.04—2.06 (m, 1H, H-14a), 1.00—1.02 (m, 1H, H-14b), 2.02 (d, J = 13 Hz, 1H, H-15a), 1.24 (d, J = 13 Hz, 1H, H-15b), 3.73 (d, J = 11 Hz, 1H, H-19a), 3.29 (d, J = 11 Hz, 1H, H-19b), 1.29 (s, 3H, Me-17), 0.88 (s, 3H, Me-18), 0.92 (s, 3H, Me-20); ^{13}C NMR (CD_3OD, 125 MHz) δ: 40.6 (t, C-1), 19.0 (t, C-2), 36.5 (t, C-3), 38.8 (s, C-4), 58.4 (d, C-5), 21.3 (t, C-6), 43.2 (t, C-7), 45.5 (s, C-8), 58.6 (d, C-9), 39.6 (s, C-10), 20.3 (t, C-11), 28.5 (t, C-12), 48.1 (d, C-13), 50.0 (t, C-14), 50.0 (t, C-15), 82.7 (s, C-16), 23.9 (q, C-17), 27.9 (q, C-18), 65.2 (t, C-19), 16.1 (q, C-20); IR (KBr) ν: 3311, 2929, 2855, 1485, 1450, 1373, 1142, 1044, 931 cm^{-1}; EIMS (70 eV) *m/z* (%): 306 (M^+, 5), 288 (9), 275 (74), 257 (100), 248 (39), 230 (4), 217 (26), 187 (15), 175 (25), 161 (15), 147 (24), 135 (30), 123 (62), 109 (48); HREIMS calcd for $C_{20}H_{34}O_2$ 306.2558, found 306.2565.

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