New Guaianolides and Xanthine Oxidase Inhibitory Flavonols from *Ajania fruticulosa*

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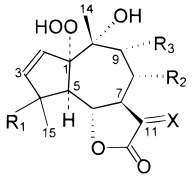
Two new guaianolides (**1** and **2**) were isolated from the aerial parts of *Ajania fruticulosa* along with a triterpene (α-amyrin), two plant sterols (*β*-sitosterol and daucosterol), four flavonols [santin (**3**), axillarin (**4**), centaureidin, and 5,7,4′-trihydroxy-3,3′-dimethoxyflavone], and five sesquiterpenes [ketoplenolide B, 9*β*-hydroxyeudesma-4,11(13)-dien-12-oic acid, 9*β*-acetoxyeudesma-4,11(13)-dien-12-oic acid, 1α,4α,10α-trihydroxy-9α-angeloyloxyguaia-2,11(13)-dien-12,6α-olide, and 3*β*,4α-dihydroxyguaia-11(13),10(14)-dien-12,6α-olide]. The structures of the new guaianolides were established as 1α-hydroperoxy-4*β*,8α,10α,13-tetrahydroxyguaia-2-en-12,6α-olide (**1**) and 1α-hydroperoxy-4α,10α-dihydroxy-9α-angeloyloxyguaia-2,11(13)-dien-12,6α-olide (**2**), respectively. Xanthine oxidase assays of all isolates revealed that santin (**3**) and axillarin (**4**) inhibited the enzyme with IC_{50} values of 36.5 and 36.0 *μ*M (that of allopurinol used as a positive control in the study was 24.2 *μ*M), respectively.

Ajania fruticulosa (Ledeb.) Poljak (Asteraceae), which grows mainly in the northwestern part of the People's Republic of China, has been used as a folk medicine for the treatment of appendicitis, tuberculosis, and emphysema. Previous phytochemical investigations of this species have demonstrated that it contains sesquiterpenes and phenolic compounds.[1,2]

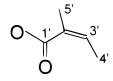
Xanthine oxidase is a key enzyme that catalyzes the oxidation of hypoxanthine to xanthine and of xanthine, in the presence of molecular oxygen, to yield uric acid and superoxide anions.[3] Therefore, inhibition of xanthine oxidase is an effective therapeutic approach for treating hyperuricemia that causes gout, kidney stones, and myocardial ischemia.[4] Since some flavonoids have been shown to be inhibitory against xanthine oxidase,[3,5] we have reinvestigated *A. fruticulosa* and have found two flavonols santin (**3**) and axillarin (**4**) as bioactive principles. Also found were two new guaianolides (**1** and **2**) together with 10 other plant constituents.

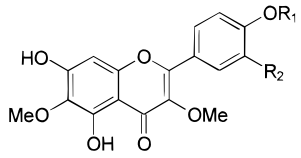
Repeated chromatography of a crude extract of the aerial parts of *A. fruticulosa* gave two new highly oxygenated guaianolides (**1** and **2**) and two xanthine oxidase inhibitory flavonols (**3**[6] and **4**[7]), as well as 10 other known phytochemicals [α-amyrin, *β*-sitosterol, daucosterol, centaureidin,[8] 5,7,4′-trihydroxy-3,3′-dimethoxyflavone,[9] ketoplenolide B,[2] 9*β*-hydroxyeudesma-4,11(13)-dien-12-oic acid,[10] 9*β*-acetoxyeudesma-4,11(13)-dien-12-oic acid,[11] 1α,4α,10α-trihydroxy-9α-angeloyloxyguaia-2,11(13)-dien-12,6α-olide,[2] and 3*β*,4α-dihydroxyguaia-11(13),10(14)-dien-12,6α-olide[12]]. The known compounds were identified by comparison of their physical and spectroscopic data with those reported in the cited literature and/or with authentic samples available in our laboratory.

Compound **1** was obtained as a white gum. The molecular formula $C_{15}H_{22}O_8$ was disclosed on the basis of high-resolution mass spectrometry. In the ^{1}H NMR spectrum of **1**, a pair of mutually coupled doublets (J = 6.0 Hz) at *δ* 5.85 and 5.82 was ascribed to a double bond in a five-membered ring, and another pair of double doublets (J = 10.5 and 7.0 Hz) at *δ* 3.67 and 3.59 was due to a hydroxymethyl group attached to a methine carbon. This observation, along with a pair of three-proton singlets at *δ* 1.10 and 1.35, suggested that lactone **1** was a guaianolide.[13] This hypothesis was confirmed by a set of 2D NMR experiments (COSY, NOESY, HMQC, and HMBC) which led to the unequivocal assignment of all ^{1}H and ^{13}C NMR resonances (Table 1). A hydroperoxy group at C-1 was revealed by the broadened singlet at *δ* 7.81 in the ^{1}H NMR spectrum and the nonprotonated carbon signal at *δ* 91.0 due to C-1 which showed long-range correlations with H-3 and H-14 in the HMBC spectrum.[14,15] Furthermore, 2,3-double-bond and 4,10-dihydroxy functionalities were indicated by the typical signals as in the case of 1α,4α,10α-



1 **2**

X	α–CH_2OH	CH_2
R_1	β–OH	α–OH
R_2	OH	H
R_3	H	



3 **4**

R_1	Me	H
R_2	H	OH

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Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1** and **2**[a]

	1 (in CD_3OD)		**2** (in $CDCl_3$)	
position	δ_H (J in Hz)	δ_C (DEPT)	δ_H (J in Hz)	δ_C (DEPT)
1		91.0 (C)		90.8 (C)
2	5.85 d (6.0)	136.0 (CH)	5.77 d (5.6)	134.2 (CH)
3	5.82 d (6.0)	141.4 (CH)	5.74 d (5.6)	139.1 (CH)
4		84.3 (C)		75.9 (C)
5	2.53 d (11.3)	66.2 (CH)	2.68 d (11.5)	65.2 (CH)
6	4.66 dd (11.3, 10.0)	81.0 (CH)	4.21 dd (11.5, 9.5)	78.2 (CH)
7	2.58 br ddd (10.7, 10.5, 10.0)	53.7 (CH)	3.22 m	38.6 (CH)
8α		71.5 (CH)	2.23 ddd (13.9, 3.5, 2.9)	31.0 (CH_2)
8β	3.89 ddd (10.7, 10.2, 4.3)		1.75 ddd (13.9, 11.8, 3.5)	
9α	2.47 dd (13.0, 10.2)	48.2 (CH_2)		
9β	1.83 dd (13.0, 4.3)		5.12 t (3.5)	82.3 (CH)
10		75.8 (C)		76.5 (C)
11	3.28 m	44.4 (CH)		138.0 (C)
12		178.5 (C)		166.6 (C)
13a	3.67 dd (10.5, 7.0)	58.1 (CH_2)	6.11 d (3.0)	120.6 (CH_2)
13b	3.59 dd (10.5, 7.0)		5.42 d (3.0)	
14	1.10 s	25.2 (CH_3)	1.31 s	23.1 (CH_3)
15	1.35 s	24.1 (CH_3)	1.01 s	22.6 (CH_3)
1′				169.5 (C)
2′				126.9 (C)
3′			6.02 qq (7.5, 1.5)	139.1 (CH)
4′			1.90 d (7.5)	20.3 (CH_3)
5′			1.80 br s	15.3 (CH_3)
-OOH	7.81 br s		7.40 br s	

[a] Assigned by COSY, NOESY, HMQC, and HMBC spectra.

Table 2. HMBC and NOESY Data of Compounds **1** and **2**

	1		**2**	
^{1}H	HMBC	NOESY	HMBC	NOESY
2	C-4, C-5	H-3, H-14	C-4, C-5	H-3, H-14
3	C-1, C-5	H-2, H-15	C-1, C-5	H-2, H-15
5	C-7	H-7, H-15	C-3, C-7	H-7
6	C-4, C-8	H-8, H-11, H-14	C-4, C-8	H-8β
7	C-5, C-11	H-5	C-5, C-9, C-11	H-5, H-8α
8α			C-6, C-7, C-9	H-7
8β	C-6, C-10	H-6, H-11, H-14	C-6, C-9, C-10	H-6, H-9
9α	C-8, C-10			
9β	C-7, C-8	H-14	C-1, C-7, C-1′	H-8β, H-14
11	C-8, C-13	H-6, H-8		
13	C-7, C-11		C-7, C-11	
14	C-1, C-9, C-10	H-2, H-6, H-8, H-9β	C-1, C-9, C-10	H-2, H-9
15	C-3, C-4, C-5	H-3, H-5	C-3, C-4, C-5	H-3
3′			C-1′	H-5′
4′			C-2′, C-3′	
5′			C-1′, C-2′, C-3′	H-3′

trihydroxyguaia-2,11(13)-dien-12,6α-olide.[13] The 8,13-dihydroxy groups were indicated by the chemical shift of H-8 (δ 3.89), and a pair of double doublets at δ 3.67 and 3.59 ascribable to the 11-hydroxymethyl group. Assuming the usual α-configuration for the isopropyl at C-7,[16] the stereochemistry of lactone **1** was established on the basis of the observed couplings (Table 1) and correlations in the NOESY spectrum. Specifically, the formulated configurations of C-1, C-10 and C-4∼C-8 were deduced from the NOE correlations of H-14 with H-2, H-6 and H-8, and of H-15 with H-3 and H-5 (but none between H-15 and H-6) (Table 2). The configuration of C-11 was based on the magnitude of the coupling ($J_{7,11}$ = 10.5 Hz)[16] and the NOE correlation between H-8 and H-11. In conclusion, the structure of compound **1** was determined as 1α-hydroperoxy-4β,8α,10α,-13-tetrahydroxyguaia-2-en-12,6α-olide.

Compound **2** was obtained as a colorless gum. The molecular formula was shown to be $C_{20}H_{26}O_8$ by its high-resolution mass spectrometry. The ^{1}H NMR spectrum of lactone **2** was similar to that of 1α,4α,10α-trihydroxy-9α-angeloyloxyguaia-2,11(13)-dien-12,6α-olide.[2] Furthermore, that compound **2** was also a guaianolide was evidenced from a pair of three-proton singlets at δ 1.01 and 1.31, and two exomethylene doublets at δ 5.42 (d, J = 3.0 Hz) and 6.11 (d, J = 3.0 Hz).[13] This proposal was reinforced by extensive 2D NMR analysis (COSY, NOESY, HMQC, and HMBC) allowing the exact assignment of all ^{1}H and ^{13}C NMR data for **2** (Table 1). An angeloyloxy group was indicated by the proton signals at δ 6.02 (1H, qq, J = 7.5, 1.5 Hz), 1.90 (3H, d, J = 7.5 Hz) and δ 1.80 (3H, br s) and a set of ^{13}C NMR resonance lines at δ 169.5 (C), 126.9 (C), 139.1 (CH), 20.3 (CH_3), and 15.3 (CH_3). The substitution pattern from C-1 through C-10, identical with that of 1α,4α,10α-trihydroxy-9α-angeloyloxyguaia-2,11(13)-dien-12,6α-olide, was disclosed by the downfield shifted triplet of H-9 at δ 5.12, and a pair of mutually coupled doublets (J = 5.6 Hz) at δ 5.74 and 5.77 attributable to the 2,3-double bond.[2,17] As in the case of lactone **1**, the presence of 1-hydroperoxy group of compound **2** was required by a slightly broadened singlet at δ 7.40 in the ^{1}H NMR spectrum and the C-1 resonance line at δ 90.8, which showed long-range correlations with the signals due to H-3 and H-9. The stereochemistry of lactone **2** was unequivocally assigned by the NOE correlations in the NOESY spectrum (Table 2). Therefore, the structure of compound **2** was determined as 1α-hydroperoxy-4α,10α-dihydroxy-9α-angeloyloxyguaia-2,11(13)-dien-12,6α-olide.

All isolates were tested for their inhibitory activity against xanthine oxidase at 50 μM, and those inhibiting the enzyme significantly (inhibition rate: >50%) were further tested to determine IC_{50} values. The flavonols **3** and **4** exhibited xanthine oxidase inhibitory activities with IC_{50} values of 36.5 and 36.0 μM, respectively. The IC_{50} value of allopurinol designated as a positive control in this work was 24.2 μM.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a DXP-118 apparatus. IR spectra were recorded in KBr disks on a Perkin-Elmer 577 instrument. All NMR experiments were performed on a JEOL JNM-A 500 FT-NMR spectrometer using TMS as the internal standard. EIMS and HREIMS experiments were run on VG-ZAB−HS and

JEOL JMX-HX110 mass spectrometers, respectively. Silica gel (200−300 mesh) for column chromatography and GF_{254} (30−40 μ) for TLC were produced by Qingdao Marine Chemical Factory, Qingdao, People's Republic of China. Sephadex LH-20 was from Pharmacia Biotech, Sweden. All other chemicals used in this study were of analytical grade.

Material. The aerial parts of *A. fruticulosa* was collected in July 1992 from Gaolan Mountain, near Lanzhou Railway Station, Gansu Province, People's Republic of China, and identified by Prof. G. L. Zhang. A voucher specimen (GLZ 92721) has been deposited in the Herbarium of Lanzhou University, Lanzhou 730000, People's Republic of China. Xanthine was purchased from Sigma, and cow's milk xanthine oxidase from Boehringer Mannheim.

Xanthine Oxidase Assay. The xanthine oxidase inhibitory activities of all isolates were tested spectrophotometrically with xanthine as the substrate by the method reported by Noro et al.[18] Xanthine oxidase inhibitory activity was expressed as $(1 - B/A) \times 100$, where A and B are the activities of the enzyme without and with the test material, respectively.

Extraction and Isolation. The air-dried plant material (3.2 kg) was pulverized, and then extracted three times with petroleum ether−Et_2O−MeOH (1:1:1) at room temperature. The extract was concentrated under reduced pressure to give a residue (102 g). This residue was dissolved in MeOH with occasional warming in a water bath (ca. 50 °C). The solution obtained was kept at −20 °C to precipitate lipids that formed. Filtration of the cooled solution followed by removal of the solvent in vacuo afforded a black gum (50 g) which was chromatographed over a silica gel column (200−300 mesh, 1200 g) using petroleum ether mixed with gradually increasing amounts of acetone to give α-amyrin (20 mg), β-sitosterol (102 mg), and daucosterol (88 mg) along with other five fractions (fractions 1−5). Fraction 2 (9 g) was subjected to gel filtration over a Sephadex LH-20 column eluting with $CHCl_3$−CH_3OH (1:1) to afford centaureidin[8] (17 mg) and 5,7,4′-trihydroxy-3,3′-dimethoxyflavone[9] (21 mg). Repeated chromatography of fraction 3 (15 g) over silica gel column using the mixture $CHCl_3$−CH_3OH (15:1) gave compounds **3**[6] (17 mg), **4**[7] (21 mg), and ketoplenolide B[2] (18 mg). By a combination of repetitive column chromatography over silica gel and gel filtration over Sephadex LH-20, fraction 4 (18 g) yielded 1α-hydroperoxy-4β,8α,10α,13-tetrahydroxyguaia-2-en-12,6α-olide (**1**, 13 mg), 1α-hydroperoxy-4α,10α-dihydroxy-9α-angeloyloxyguaia-2,11-(13)-dien-12,6α-olide (**2**, 11 mg), 9β-acetoxyeudesma-4,11(13)-dien-12-oic acid[10] (20 mg), 3β,4α-dihydroxyguaia-11(13),10(14)-dien-12,6α-olide[12] (24 mg), 9β-hydroxyeudesma-4,11(13)-dien-12-oic acid[11] (18 mg), and 1α,4α,10α-trihydroxy-9α-angeloyloxyguaia-2,11(13)-dien-12,6α-olide[2] (26 mg).

1α-Hydroperoxy-4β,8α,10α,13-tetrahydroxyguaia-2-en-12,6α-olide (1): white gum; $[\alpha]^{26}_D$ −38.9° (*c* 1.8, MeOH); IR (KBr) ν_{max} 3390 (br, OH), 1765 (γ-lactone), 1710, 1645 (weak), 1235, 1165 cm^{-1} (α,β-unsaturated ester); ^1H and ^{13}C NMR data are listed in Table 1; EIMS, *m*/*z* 330 $[M]^+$ (0.4), 312 $[M - H_2O]^+$ (1.0), 282 $[M - Me - OOH]^+$ (11), 218 (15), 165 (39), 97 (30), 70 (100); HREIMS, *m*/*z* 330.1302 $[M]^+$ ($C_{15}H_{22}O_8$ requires 330.1308).

1α-Hydroperoxy-4α,10α-dihydroxy-9α-angeloyloxyguaia-2,11(13)-dien-12,6α-olide (2): colorless gum; $[\alpha]^{26}_D$ +9.5° (*c* 0.13, MeOH); IR (KBr) ν_{max} 3395 (br, OH), 1770 (γ-lactone), 1710, 1640 (weak), 1236, 1160 cm^{-1} (α,β-unsaturated ester); ^1H and ^{13}C NMR data, see Table 1; EIMS, *m*/*z* 394 $[M]^+$ (0.5), 376 $[M - H_2O]^+$ (14), 360 $[M - H_2O_2]^+$ (11), 342 $[M - H_2O_2 - H_2O]^+$ (52), 278 (13), 260 (32), 217 (38), 149 (77), 83 (100); HREIMS, *m*/*z* 394.1616 $[M]^+$ ($C_{20}H_{26}O_8$ requires 394.1620).

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