Ajanoside, a Xanthine Oxidase Inhibitor with a Novel Skeleton from *Ajania fruticulosa*

Jun Cai Meng and Ren Xiang Tan*
Institute of Functional Biomolecules, State Key Laboratory of Pharmaceutical Biotechnology, School of Life Science, Nanjing University, Nanjing 210093, P. R. China

(Received August 21, 2000; CL-000790)

The structure of ajanoside, a xanthine oxidase inhibitor with a novel skeleton isolated from the aerial parts of *Ajania fruticulosa*, was determined to be [2,2-bis(4-hydroxy-3-methoxyphenyl)-4-oxo-1,3-dioxin-5-yl]methyl β-D-glucopyranoside on the basis of extensive spectroscopic analyses.

Ajania fruticulosa (Ledeb.) Poljak (Asteraceae), a medicinal plant distributed mainly in northwest of China, has been used by the local to treat appendicitis, tuberculosis and emphysema.[1] In the process of our continuous investigations of structurally novel and/or biologically active natural products of plant origin, the highly oxygenated sesquiterpenoides and xanthine oxidase inhibitory flavonoids have been characterized from the title species.[1,2] As a follow-up to the findings, a novel skeletal glycoside **1** (15 mg), exhibiting xanthine oxidase inhibitory activity in vitro, was isolated from the EtOAc extract of the aerial parts of title plant (3.2 kg) by repeated silica gel chromatography. We hereby wish to present the structure determination and bioassay evaluation of the new phytochemical.

Ajanoside (**1**) was obtained as a white gum, $[\alpha]_D^{25}$ +10.7° (*c* 0.5, MeOH). The molecular formula was disclosed by the molecular ion at *m/z* 536.1535 ($C_{25}H_{28}O_{13}$ requires 536.1530) in its high-resolution electron impact mass spectrum. The IR absorption band at 1675 cm^{-1} was ascribable to an α,β-unsaturated ester or ketone moiety. This observation, coupled with an olefinic singlet at δ 8.38 in the ^{1}H-NMR spectrum and the carbon resonance lines at δ 90.3 (C), 171.1 (C), 128.1 (C) and 155.0 (CH), led to the assumption of 2,2,5-trisubstituted 1,3-dioxin-4-one framework.[3] This hypothesis was subsequently confirmed by extensive 2D-NMR experiments (^{1}H–^{1}H COSY, HMQC and HMBC) allowing the unequivocal assignment of all ^{1}H- and ^{13}C-NMR resonances (Table 1). Furthermore, a carbon signal at δ 61.9 (CH_2) indicated the presence of an oxygenated methylene which was demonstrated to bond to C-5 by a pair of doublets at δ 4.33 and 4.51 in the ^{1}H-NMR spectrum, both being broadened by their allylic couplings with H-6 at δ 8.38. This assumption was reinforced by the long-range correlations of H-7 with C-4 and C-6 in the HMBC spectrum. Moreover, along with the anomeric doublet at δ 4.24 (*J* = 7.7 Hz, H-1''') in its ^{1}H-NMR spectrum, five methine [δ 102.7, 77.0, 76.6, 73.4 and 70.1] and a methylene [δ 61.1] carbon resonances disclosed the presence of a β-D-glucopyranosyl group which was identified by co-PC (paper chromatography) with authentic sample after acid hydrolysis. The strong cross peak between C-7 and H-1''' in the HMBC spectrum suggested that glucopyranosyl moiety was anchored on C-7. In addition, a six-proton singlet at δ 3.70 and two sets of aromatic proton signals [δ 6.81/6.79 (d, *J* = 2.0 Hz), 6.76/6.75 (d, *J* = 8.0 Hz), 6.73/6.72 (dd, *J* = 8.0, 2.0 Hz)] in the ^{1}H-NMR spectrum indicated the presence of a pair of 4-hydroxy-3-methoxyphenyl groups corresponding to the typical fragment at *m/z* 123 in the EI-MS formed due to the cleavage between C-2 and C-1'/C-1''. The positioning of the hydroxy and methoxy functions was further reinforced by the ^{13}C-NMR spectrum and 2D-NMR experiments (Table 1). Furthermore, the two aromatic residues could be attached on none but C-2, a quaternary carbon resonating at δ 90.3. This proposal was further supported by the discerned long-range correlation of C-2 with H-6, H-2', H-6', H-2'' and H-6'' in the HMBC spectrum. In conclusion, the structure of compound **1** was determined as [2,2-bis(4-hydroxy-3-methoxyphenyl)-4-oxo-1,3-dioxin-5-yl]methyl β-D-glucopyranoside, the first representative possessing a novel 2,2-diphenyl-5-methyl-1,3-dioxin-4-one skeleton.

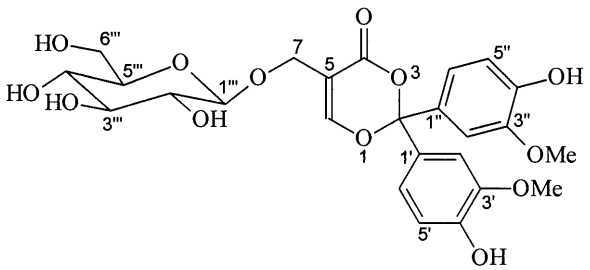

Figure 1. The structure of ajanoside **(1)**.

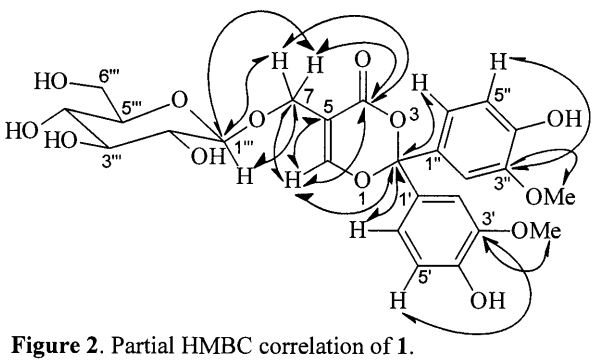

Figure 2. Partial HMBC correlation of **1**.

Xanthine oxidase is a key enzyme that catalyzes the oxidation of hypoxanthine to xanthine and of xanthine to yield uric acid and superoxide anions.[4] Therefore, inhibition of xanthine oxidase is an effective therapeutic approach for treating hyperuricemia that causes gout, kidney stones, and myocardial ischemia.[5] In vitro bioassay revealed that compound **1** inhibited xanthine oxidase as a competitive inhibitor (K_i = 21.3 μM) with IC_{50} values being 48.7 μM (the IC_{50} value of allopurinol used as positive control in the bioassay was 23.0 μM).

Table 1. NMR (400 MHz, DMSO-d_6) spectral data for **1**

C	δ_C (DEPT)	δ_H (J in Hz)	HMBC
2	90.3 (C)		H-6, H-2', H-2", H-6', H-6"
4	171.7 (C)		H-6, H-7
5	128.1 (C)		H-6, H-7
6	155.0 (CH)	8.38 br s	H-7
7	61.9 (CH_2)	4.33, 4.51 br d (14.0)	H-6, H-1"'
1'	130.9 (C)		H-5'
2'	110.7 (CH)	6.81 d (2.0)	H-6'
3'	147.5 (C)		H-2', H-5', -OCH_3
4'	146.7 (C)		H-2', H-5', H-6'
5'	115.4 (CH)	6.76 d (8.0)	
6'	119.2 (CH)	6.73 dd (8.0, 2.0)	H-2'
1"	130.9 (C)		H-5"
2"	110.6 (CH)	6.79 d (2.0)	H-6"
3"	147.5 (C)		H-2", H-5", -OCH_3
4"	146.7 (C)		H-2", H-5", H-6"
5"	115.4 (CH)	6.75 d (8.0)	
6"	119.1 (CH)	6.72 dd (8.0, 2.0)	H-2"
1"'	102.7 (CH)	4.24 d (7.7)	H-7, H-2"'
2"'	73.4 (CH)	3.02 dd (8.4, 7.7)	H-3"'
3"'	77.0 (CH)	3.13 t (8.4)	H-2"', H-5"'
4"'	70.1 (CH)	3.04 t (8.4)	H-3"'
5"'	76.6 (CH)	3.11 m	H-4"'
6"'	61.1 (CH_2)	3.50 br d (12.0)	
		3.71 dd (12.0, 4.4)	
CH_3	55.9	3.70 s	

Grants to RXT from the Natural Science Foundation of China (Nos. 39725033 and 39970083) are gratefully acknowledged.

References and Notes

1 H. Li, J. C. Meng, C. H. K. Cheng, T. Higa, J. Tanaka, and R. X. Tan, *J. Nat. Prod.*, **62**, 1053 (1999).
2 W. Z. Wang, R. X. Tan, Y. M. Yao, Q. Wang, and F. X. Jiang, *Phytochemistry*, **37**, 1347 (1994).
3 G. L. Lange, M. G. Organ, and M. R. Roche, *J. Org. Chem.*, **57**, 6000 (1992).
4 L. Costantino, G. Rastelli, and A. Albasini, *Eur. J. Med. Chem.*, **31**, 693 (1996).
5 T. Hayashi, K. Nagayama, M. Arisawa, M. Shimizu, S. Suzuki, M. Yoshizaki, N. Morita, E. Ferro, I. Basualdo, and L. H. Berganza, *J. Nat. Prod.*, **52**, 210 (1989).