Adenanthusone, a New Ursane Type Nortriterpenoid from Isodon adenanthus

by Wei Xiang^a)^b), Qi-Shi Song^a), Hong-Jie Zhang^c), Rong-Tao Li^b), Zhi Na^a), and Han-Dong Sun^{*b})

^a) Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Kunming, Yunnan 650223, P. R. China

^b) State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650204, P. R. China

(tel: 086-871-522-3251; fax: 086-871-521-6343; e-mail: hdsun@mail.kib.ac.cn)

^c) Program for Collaborative Research in the Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy (M/C877), College of Pharmacy, the University of Illinois at Chicago, 833 S. Wood St., Chicago, IL 60612, USA

A new ursane-type nortriterpenoid, adenanthusone (= $(11\alpha,12\alpha)$ -4-demethyl-11,12-epoxy-3,13-dihydroxy-2-oxoursa-3,20(30)-diene-28-oic acid γ -lactone; **1**) was isolated from *Isodon adenanthus*. Its structure was determined by NMR spectra and X-ray crystallographic diffraction analysis. The biogenetic implication of the nortriterpene is discussed.

1. Introduction. – *Isodon adenanthus* (DIELS) KUDO (Labiatae), a widely distributed perennial plant in southwestern China, has been used in Chinese traditional folk medicine to treat enteritis and dysentery [1]. Similar to other *Isodon* genus plants, *I. adenanthus* was shown to be a fertile source of *ent*-kaurenoids, which frequently exhibited antitumor and antibacterial activities [2-4]. Besides the *ent*-kaurenoids, triterpenoids were found to be another type of rich metabolites in the *Isodon* plants, but new triterpenoids were rarely isolated from the *Isodon* plants in previous investigations [5-7]. Our recent study on the triterpene from the acetone H₂O 7:3 extract of the whole plant of *I. adenanthus* led to the discovery of an unprecedented 4-demethyl-ursane-type nortriterpenoid, adenanthusone (1) (*Fig. 1*). According to the biogenesis,



Fig. 1. Structures of adenanthusone (1) and some reported triperpenoids, i.e., of 2-5, from I. adenanthus

^{© 2004} Verlag Helvetica Chimica Acta AG, Zürich

adenanthusone (1) could be derived from the $(2\alpha, 3\beta, 4\beta, 11\alpha, 12\alpha)$ -11,12-epoxy-2,3,13,24-tetrahydroxyurs-20(30)-ene-28-oic acid γ -lactone (2) [7]. This report describes the structure elucidation and possible biogenetic implication of 1.

2. Results and Discussion. – Adenanthusone (1) was isolated as optically active colorless flake crystals. The HR-EI-MS showed a molecular ion at m/z 466.27038, in agreement with the molecular formula $C_{29}H_{38}O_5$ (calc. 466.27192), requiring eleven double-bond equivalents. Further spectral data of 1 (¹H- and ¹³C-NMR (*Table 1*), ¹H,¹H-COSY (*Table 2*), HMQC, HMBC (*Table 2*), and NOESY (*Fig. 2*) were consistent with the structure of the γ -lactone of (11 α ,12 α)-4-demethyl-11,12-epoxy-3,13-dihydroxy-2-oxoursa-3,20(30)-dien-28-oic acid.

Table 1. *NMR Data for Adenanthusone* (1). Recorded in C_5D_5N at 100 (¹³C) and 400 MHz (¹H), resp. δ in ppm, J in Hz.

	$\delta(C)$	$\delta(H)$		$\delta(C)$	$\delta(H)$
CH ₂ (1)	52.8 (<i>t</i>)	2.39 $(d, J = 16.1, H_{\beta}),$ 3.07 $(d, J = 16.1, H_{\alpha})$	CH ₂ (16)	23.1 <i>(t)</i>	1.83 (m, H_{β}) , 1.36 (m, H_{a})
C(2)	193.2 (s)	() / u)	C(17)	45.3 (s)	
C(3)	146.4 (s)		H - C(18)	61.1 (<i>d</i>)	1.85 (d, J = 12.3)
C(4)	129.8 (s)		H - C(19)	36.2 (d)	2.67(m)
H-C(5)	48.2(d)	2.26 (overlap)	C(20)	152.0 (s)	
$CH_{2}(6)$	20.6(t)	1.04 (m, H_{β}) , 1.42 (overlap, H_{α})	CH ₂ (21)	32.0(t)	1.57 (m , H_a), 1.94 (overlap, H_β)
$CH_{2}(7)$	30.5 (t)	1.04 (overlap, H_{β}), 1.29 (<i>m</i> , H_{α})	CH ₂ (22)	33.4 (t)	1.85 (overlap, H_{β}), 2.22 (<i>m</i> , H_{α})
C(8)	41.9 (s)		Me(23)	13.4(q)	2.02 (s)
H-C(9)	48.3 (d)	1.96 (overlap)			
C(10)	40.4(s)		Me(25)	15.5(q)	0.94 (s)
H - C(11)	54.2 (d)	3.19 (dd, J = 2.0, 4.0)	Me(26)	20.4(q)	1.08 (s)
H - C(12)	56.4(d)	3.05 (d, J = 4.0)	Me(27)	16.2(q)	1.15 (s)
C(13)	88.5 (s)		C(28)	178.2 (s)	
C(14)	41.3 (s)		Me(29)	16.5(q)	1.40 $(d, J = 6.3)$
$CH_2(15)$	27.1 (t)	1.69 (overlap, H_{β}),	$CH_{2}(30)$	108.4(t)	4.77 (br. s), 4.84 (br. s)
		1.03 (overlap, H_a)			

The ¹H- and ¹³C-NMR (including DEPT) spectra of **1** indicated the presence of a COOH group (δ (C) 178.2), an α,β -conjugated keto group (δ (C) 193.2 (s), 146.4 (s), and 129.8 (s)), an exocyclic CH₂=C group (δ (H) 4.84 (br. s) and 4.77 (br. s); δ (C) 152.0 (s) and 108.4 (t)), 4 tertiary Me (δ (H) 2.02, 1.15, 1.08, and 0.94; δ (C) 20.4, 16.2, 15.5, and 13.4), 1 secondary Me (δ (H) 1.40 (d, J = 6.3), δ (C) 16.5), 7 CH₂ (δ (C) 52.8, 33.4, 32.0, 30.5, 27.1, 23.1, and 20.6), 6 sp³ CH groups (δ (C) 61.1, 56.4, 54.2, 48.3, 48.2, and 36.2), 4 quaternary C-atoms (δ (C) 45.3, 41.9, 41.3, and 40.4), and 1 quaternary C–O moiety (δ (C) 88.5). An epoxy group (δ (H) 3.19 (dd, J = 2.0, 4.0 Hz) and 3.05 (d, J = 4.0 Hz); δ (C) 54.2 and 56.4) was established by the ¹H- and ¹³C-NMR, HMQC, and ¹H, ¹H-COSY data. The epoxy ring, 2 C=O, and 2 olefin moieties accounted for five sites of unsaturation, and the lack of NMR signals for further unsaturated functionality indicated the presence of 6 additional rings in **1**.

In the HMBC experiment with **1**, the presence of long-rang correlations of $CH_2(1)$ ($\delta(H)$ 2.39, 3.07) to C(25) (Me, $\delta(C)$ 15.5), C(5) (CH, $\delta(C)$ 48.2), C(2) (O=C, $\delta(C)$ 193.2), and C(3) (=C, $\delta(C)$ 146.4), and of CH₃(23) ($\delta(H)$ 2.02) to C(3) and C(4) (=C, $\delta(C)$ 146.4 and 129.8) assigned the α,β -conjugated keto group to C(2), C(3), and C(4), with the =O at C(2). The C=C bond consisting of two quaternary C-atoms further suggested an OH group at C(3) and only a Me group at C(4). The long-range correlations of H(18) ($\delta(H)$ 1.85) to C(12) (O-CH, $\delta(C)$ 56.4), C(13) (O-C, $\delta(C)$ 88.5), C(14) (C, $\delta(C)$ 41.3), C(17) (C, $\delta(C)$ 45.3), C(19) (CH, $\delta(C)$ 36.2), C(20) (=C, $\delta(C)$ 152.0), C(28) (OC=O, $\delta(C)$ 178.2), and C(29) (Me, $\delta(C)$ 16.5) revealed the

	¹ H, ¹ H COSY	HMBC		¹ H, ¹ H COSY H	HMBC C
Н	Н	С	Н		
CH ₂ (1)	CH ₂ (1)	C(25), C(10), C(5), C(3), C(2), C(9)	H-C(19)	H-C(18), Me(29)	C(18), C(20)
H-C(5)	CH ₂ (6)	not observed	CH ₂ (21)	$CH_2(21), CH_2(22)$	overlap
$CH_2(6)$	$H-C(5), CH_2(6), CH_2(7)$	overlap	CH ₂ (22)	$CH_{2}(21), CH_{2}(22)$	C(17), C(18), C(20)
CH ₂ (7)	$CH_2(6), CH_2(7)$	C(5), C(6), C(9), C(14)	Me(23)		C(3), C(4), C(5)
H-C(9)	H-C(11)	C(1), C(5), C(10), C(11), C(14), C(25), C(26)	Me(25)		C(1), C(5), C(9), C(10)
H-C(11)	H-C(9), H-C(12)	C(9), C(12)	Me(26)		C(7), C(8), C(9), C(14)
H-C(12)	H-C(11)	C(9), C(11), C(14)	Me(27)		C(8), C(13), C(14), C(15)
CH ₂ (15)	CH ₂ (15), CH ₂ (16)	C(13), C(14), C(16), C(27)	Me(29)	H-C(19)	C(18), C(19), C(20)
CH ₂ (16)	$CH_2(15), CH_2(16)$	overlap	CH ₂ (30)		C(19), C(20), C(21)
H-C(18)	H-C(19)	C(12), C(13), C(14), C(16), C(17), C(19), C(20), C(28), C(29)			

Table 2. Correlations in the ¹H, ¹H COSY and HMBC Plots of Adenanthusone (1)

second C=O at C(28), which lactonized to C(13), the three-membered epoxy ring at C(11) and C(12), and the exocyclic $CH_2=C$ group at C(30) and C(20). The relative configuration of **1** was determined by the analysis of the NOESY plot (*Fig. 2*) and by X-ray crystallographic diffraction (see below).

Since compound **1** could be recrystallized to suitable flake crystals from MeOH/ CHCl₃ 1:1 solution, an X-ray crystallographic diffraction analysis was carried out¹). The X-ray structure of **1** (*Fig. 3*) confirmed the elucidated structure and configuration. Furthermore, the X-ray structure evidenced the preferred conformation of seven carbocyclic units (A – G) in the solid state as follows: the epoxy ring G was found to be an equilateral triangle, the five-membered-lactone ring F to be an envelope, the sixmembered rings A and C to be half-chairs, and rings B, D, and E to be chair conformations; the ring junctions of A/B, B/C, C/D, and E/F were *trans*, while the ring

¹) Crystal data: C₂₉H₃₈O₅, *M* 466.62, orthorhombic system, space group: P2₁₂₁₂₁, *a* = 7.691 (1) Å, *b* = 8.237 (1) Å, *c* = 38.417 (2) Å, *V* = 2433.8 (2) Å³, *Q*_{calc} = 1.273 g/cm³, *Z* = 4, crystal size: 0.10 × 0.40 × 0.60 mm. The crystal was used for X-ray measurements on a *MAC-DIP-2030K* imaging plate area diffractometer with Mo-Kα radiation and a graphite monochromator, the space between the crystal and the imaging plate *d* was 100 mm. The maximum 2θ value was set at 50°. The total number of independent reflections measured was 2699, of which 2030 were considered to be observed (|F|² ≥ 8σ|F|²). The 29 non-H-atoms were located directly; repeated cycling with least-squares refinement and difference *Fourier* maps were used to identify other non-H-atom positions. The positions of all H-atoms were achieved by calculation and difference *Fourier* maps. Final discrepancy indices were *R*_f = 0.057, *R*_w = 0.057 (*w* = 1/σ/|F|²). The crystal structure has been deposited at the *Cambridge Crystallographic Data Centre* and allocated the deposition number CCDC 236726. Copies of the data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/ conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge, CB21EZ, UK; e-mail: deposit@ccdc.cam.ac.uk; fax: + 441223336033).



Fig. 2. Selected NOE correlations for compound **1**



Fig. 3. X-Ray crystallographic structure of 1

junction of D/E was *cis*. The X-ray crystallographic diffraction analysis of **1** provided a referential configuration for the analogues, which were separated as amorphous powder and lack X-ray crystallographic data [8].

From *I. adenanthus*, ursolic acid and other ursane-type triterpenoids, *i.e.*, **2**–**5**, were also isolated [7]. Considering the structures of these derivatives, especially of $(2\alpha, 3\beta, 4\beta, 11\alpha, 12\alpha)$ -11,12-epoxy-2,3,13,24-tetrahydroxyurs-20(30)-en-28-oic acid γ -lactone (**2**), a biosynthetic pathway was proposed for **1** (*Scheme*).

Scheme. Postulated Biogenesis of 1



This work was supported by *Yunnan Natural Science Foundation* (project No. 2000C0099M). The authors are grateful to Professors *Yang Lu, Peng Cao*, and *Qi-Tai Zheng* (Institute of Materia Medica, The Chinese Academy of Medical Sciences, Beijing 100050, P. R. China) for the measurement of data of the X-ray crystallographic diffraction.

Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh; Qingdao Marine Chemical Factory, P. R. China) and D101 resin (*Tianjin Agricultural Chemical Co. Ltd.*, P. R. China). Fractions were monitored by TLC (silica gel). Optical rotations: *SEPA-300* polarimeter. UV Spectra: *UV-210A* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Bio-Rad FTS-135* spectrometer; KBr pellets; in cm⁻¹. ¹H- and ¹³C-NMR Spectra: Bruker AM-400 spectrometer; for ¹H,¹H COSY, ROESY, HMQC, and HMBC experiments, *DRX-500* spectrometer; (D₅)pyridine as solvent and SiMe₄ as internal standard; δ in ppm, *J* in Hz. MS: *VG Auto-Spec-3000* magnetic sector instrument; in *m*/z (rel. %).

Plant Material. The whole plants of *I. adenanthus* were collected from the west mountain of Kunming, Yunnan Province, P. R. China, in October 2002. The identity of the plant material was verified by Prof. *Xi-Wen Li*, and a voucher specimen (KIB 02-01-11 Li) has been deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Chinese Academy of Sciences, P. R. China.

Extraction and Isolation. The aerial parts (3.6 kg) of *I. adenanthus* were extracted with Me₂CO/H₂O 7:3 and partitioned between AcOEt and H₂O. The org. layer was dried to give 39 g of extract, which was absorbed on *D101* resin (200 g), eluting with 75% MeOH, to afford, after evaporation, an oxygenated diterpene and triterpene portion (28 g), which was subjected to CC (silica gel (300 g), gradient petroleum ether/acetone mixtures 9:1, 8:2, 7:3, 6:4, 5:5): *Fractions 1–5. Fr. 2* was resubjected to CC reversed-phase C^{18} , MeOH/H₂O 4:6: *adenanthusone* (1). M.p. 295–296°. [α]₂₀²⁰ = +102.6 (MeOH, c = 0.19).

REFERENCES

- Delectis Florae Reipublicae Popularis Sinicae Agendae Academiae Sinice Edita, 'Flora Reipublicae Popularis Sinicae 66', Science Press, Beijing, 1977, p. 416.
- [2] Y. L. Xu, H. D. Sun, D. Z. Wang, T. Iwashita, H. Komura, M. Kozuka, K. Naya, I. Kubo, *Tetrahedron Lett.* 1987, 28, 499.
- [3] Y. H. Wang, Y. Z. Chen, H. D. Sun, Z. W. Lin, Acta Sci. Nat. Uni. Sunnyatseni 1998, 37, 122.

Helvetica Chimica Acta - Vol. 87 (2004)

[4] B. Jiang, H. Yang, M. L. Li, A. J. Hou, Q. B. Han, S. J. Wang, S. H. Li, H. D. Sun, J. Nat. Prod. 2002, 65, 1111.

[5] H. D. Sun, Y. L. Xu, B. Jian, Diterpenoids from Isodon Species, Science Press, Beijing, 2001, p. 1.

[6] W. Xiang, Q. B. Han, S. H. Li, Z. Na, H. D. Sun, *Acta Botanica Sinica* 2003, 45, 1383.
[7] B. Jiang, Q. B. Han, W. Xiang, Z. W. Lin, H. D. Sun, *Acta Botanica Yunnanica* 2002, 24, 663.

[8] G. F. Lai, Y. F. Wang, C. H. Lu, J. X. Cao, Y. L. Xu, S. D. Luo, Helv. Chim. Acta 2003, 86, 2136.

Received July 13, 2004