

腺花香茶菜中的三萜化合物

姜 北, 韩全斌, 项 伟, 林中文, 孙汉董*

(中国科学院昆明植物研究所植物化学与西部植物资源持续利用国家重点实验室, 云南 昆明 650204)

摘要: 对云南大理产腺花香茶菜 (*Isodon adenanthus*) 进行了系统的研究。从中分离得到了包括 1 个新三萜 (1) 在内的一系列乌索烷型三萜类化合物, 其结构通过现代波谱分析方法确定。活性筛选结果表明, 化合物 1 对肿瘤细胞株 K562, A549 以及 T24 具有一定的细胞毒活性。

关键词: 腺花香茶菜; 唇形科; 三萜; Isodonadenanthin; 细胞毒活性

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Triterpenoids from *Isodon adenanthus*

JIANG Bei, HAN Quan-Bin, XIANG Wei, LIN Zhong-Wen, SUN Han-Dong*

(State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany,
Chinese Academy of Sciences, Kunming 650204, China)

Abstract: Further investigation on the leaves of *Isodon adenanthus* afforded a new triterpenoid, isodonadenanthin (1), together with three known ones, ursolic acid, 2 α -hydroxyl-ursolic acid and 2 α , 24-dihydroxyl-ursolic acid. Structures of 1-4 were elucidated on the basis of their spectral properties. The cytotoxicities of the new compound against three kinds of human tumor cells K562, A549, and T24 were also tested. And 1 showed a certain antitumor activities toward all three kinds of cells.

Key words: *Isodon adenanthus*; Labiatae; Triterpenoids; Isodonadenanthin; Cytotoxicities

Isodon species were well known to be rich in *ent*-kaurane diterpenoids which have been verified to be the main bioactive constituents of the plants (Sun *et al*, 2001). Previous research on chemical constituents of *Isodon adenanthus* (Diels) Kudo had led to the isolation of three *ent*-kaurane diterpenoids (Xu *et al*, 1987; Lin *et al*, 1992; Wang *et al*, 1998). However, in our recent reinvestigation of this plant collected from a different place, in addition to several diterpenoids, four triterpenoids including a new one were isolated from *Isodon adenanthus*. In this paper, we wish to report the structural elucidation of those triterpenoids, as well as the results of bioactive tests for the new compound.

* To whom correspondence should be addressed. Tel.: 86 - 871 - 5223251

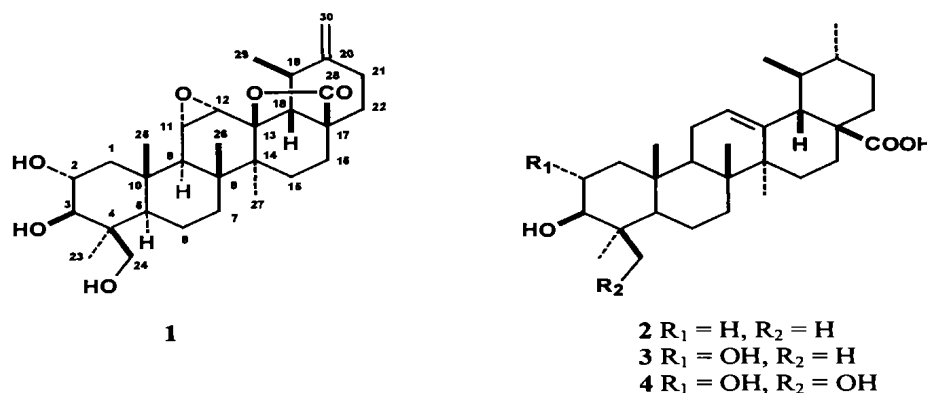
Fax: 86 - 871 - 5216343. E-mail: hdsun@mail.kib.ac.cn

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作者简介: 姜北 (1963 -) 男, 博士研究生, 主要从事植物化学的研究。

Results and discussion

Isodonadenanthin (**1**), white amorphous powder, showed no absorption in the UV spectrum. The HR-EIMS (obsd 500.3129, calcd 500.3138) indicated that **1** possessed the molecular formula of



$C_{30}H_{44}O_6$ which was further verified by signals for 30 carbons at the ^{13}C -NMR spectrum. The 1H and ^{13}C -NMR (DEPT) data showed the presence of five methyl groups, nine methylene groups (including an *exo*-methylene group and an oxygenated one), eight methine groups (including four oxygen-bearing ones) and eight quaternary carbons (including a carbonyl group at δ 177.39 and an oxygenated quaternary carbon at δ 88.01). Considering the correlation of biosynthesis between the triterpenoids (**2-4**) isolated from the same plant, **1** was presumed to be an ursane-type triterpenoid with seven rings as a basic skeleton. The presumption was identified by analysis of 2D-NMR spectra of **1**. From the 1H - 1H COSY, HMQC and HMBC spectra were clearly observed the correlations of an ursane-type triterpenoid with three hydroxyl groups at C-2, C-3 and C-24, respectively, while C-17 and C-13 was connected by an internal ester (C-28) (Table 1). Moreover, according to the correlations, there was an olefinic bond between C-20 and C-30 instead of the double bond between C-19 and C-29 as it used to be.

The relative configurations of **1** were achieved by the analysis of the NOESY spectrum, and the key correlations were shown in Fig. 1. According to the observation of the correlations between H-19 α with Me-27, and H-18 β with H-12 β and H-22 β , and no correlations between H-18 β with Me-27 from the NOESY spectrum, compound **1** should be no compound other than an ursane-type triterpenoid. Therefore, isodonadenanthin (**1**) was elucidated as 2 α , 3 β , 24-trihydroxy-11 α , 12 α -epoxy-urs-20(30)-en-28, 13 β -olide (Tommasi *et al*, 1998; Huang *et al*, 1999; Siddiqui *et al*, 2000).

Other three triterpenoids were determined to be ursolic acid, 2 α -hydroxyl-ursolic acid and 2 α , 24-dihydroxyl-ursolic acid, respectively, by their spectral properties, as well as the direct comparisons of the compounds **2-4** with the authentic samples on co-TLC.

Compound **1** was studied for its cytotoxicities against three kinds of human tumor cells K562, A549, and T24. And the 50% inhibitory concentration (IC_{50}) values of **1** equaled to 16.605 $\mu g/mL$, 38.135 $\mu g/mL$ and 9.020 $\mu g/mL$, respectively, while cis-platinum was used as a positive reference

substance with the IC_{50} values of 2.018 $\mu\text{g/mL}$, 11.940 $\mu\text{g/mL}$ and 1.155 $\mu\text{g/mL}$ correspondingly, suggesting that **1** possessed a certain antitumor activity toward all the cells.

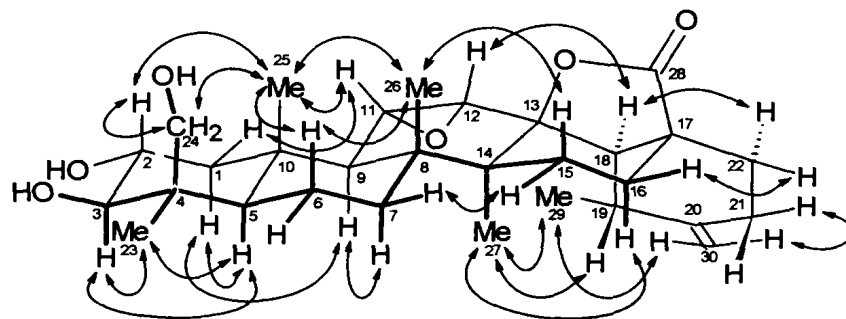


Fig. 1 The key ROESY correlations of **1**

Table 1 The correlations from the $^1\text{H}-^1\text{H}$ COSY and HMBC spectra of isodonadenanthin (**1**)

H	$^1\text{H}-^1\text{H}$ COSY	HMBC [*]	H	$^1\text{H}-^1\text{H}$ COSY	HMBC
	H	C		H	C
1	1, 2	(2), 3, 5, (10), 25	18	19	12, (13), 14, 16, (17), (19), 28, 29
2	1, 3, OH-2	n. o.	19	18, 29	(18), (20)
3	2, OH-3	(2), 23, 24	21	21, 22	17, (22), 30
5	6	(4), (6), (10), 24, 25	22	21, 22	16, (17)
6	5, 6, 7	overlap	23	—	3, (4), 5, 24
7	6, 7	overlap	24	24, OH-24	3, (4), 5, 23
9	11	1, 5, (8), (10), (11), 14, 25, 26	25	—	1, 5, 9, (10)
11	9, 12	(9)	26	—	7, (8), 9, 14
12	11	14	27	—	8, 13, (14), 15
15	15, 16	8, 13, (14), (16), 17, 27	29	19	18, (19), 20
16	15, 16	14, (15), (17), 18, 22, 28	30	30	19, (20), 21

* Two-bond correlations are indicated in parentheses; n. o. indicate no clear correlation with this proton.

Experimental

General Optical rotations were taken on a SEPA-300 polarimeter. IR spectra were measured on a Bio-Rad FTS-135 spectrometer with KBr pellets. UV spectra were obtained on a UV 210A spectrometer. MS were recorded on a VG Auto Spec-3000 spectrometer. 1D- and 2D-NMR spectra were run on a Bruker AM-400 and DRX-500 instrument with TMS as internal standard, respectively.

Plant Material The leaves of *I. adenanthus* were collected in Yangbi prefecture of Yunnan Province in September, 1997, and air-dried. The identity of plant material was verified by Prof. Xi-Wen Li, and a voucher specimen (KIB 97-10-07 Li) is deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Academia Sinica.

Extraction and Isolation The dried and powdered leaves (7.43 kg) were extracted with 70% Me_2CO and filtered. The filtrate was concentrated and extracted with petroleum ether and EtOAc successively. The residue (200 g) of

the EtOAc extract was applied to column chromatography over Si gel (200–300 mesh, 2.3 kg) column eluting with a CHCl_3 - Me_2CO (1:0–0:1) gradient system to yield fractions I–VIII. Fraction II was chromatographed over Si gel (petroleum ether-isopropanol 50:1) to produce **2** (650 mg). Fraction V was subjected to column chromatograph on Si gel repeatedly (petroleum ether-isopropanol 20:1 and CHCl_3 - MeOH 30:1), followed by MCI gel CHP-20 column (MeOH - H_2O 9:1) to yield **3** (73 mg). Fraction VI was further purified by repeated column chromatography over Si gel (CHCl_3 - MeOH 20:1 and cyclohexane-isopropanol 10:1) and RP-8 (MeOH - H_2O 7:3) to afford **1** (180 mg) and **4** (370 mg), respectively.

Isodonadenanthin (1), $\text{C}_{30}\text{H}_{44}\text{O}_6$, amorphous powder; $[\alpha]_D^{17} + 59.09^\circ$ (c 0.495, MeOH); UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: no absorption; IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3430 (br s), 2937, 2873, 1776, 1394, 1357, 1259, 1229, 1144, 1085, 1051, 1030, 996, 981, 936, 877; EIMS (70 eV) m/z (%): 500 [M^+] (23), 482 (8), 457 (5), 438 (5), 367 (1), 313 (3), 301 (5), 261 (36), 248 (22), 229 (12), 215 (16), 203 (39), 187 (100), 173 (36), 157 (20), 145 (35), 133 (38), 121 (50), 107 (67), 91 (56), 81 (51), 69 (61); FAB-MS m/z (%): 499 [$\text{M}-1$] (100); HREIMS m/z : 500.3129, calcd for $\text{C}_{30}\text{H}_{44}\text{O}_6$ 500.3138; ^1H NMR (500.13 MHz, $\text{C}_5\text{D}_5\text{N}$): δ 5.02 (1H, d, $J = 4.50$ Hz, OH-3 β), 4.78 (1H, s, H-30a), 4.69 (1H, s, H-30b), 4.52 (1H, d, $J = 4.65$ Hz, OH-2 α), 4.16 (1H, dd, $J = 3.05, 7.10$ Hz, OH-24), 3.67 (1H, dd, $J = 3.05, 11.10$ Hz, H-24a), 3.64 (1H, m, H-2 β), 3.34 (1H, dd, $J = 7.10, 11.10$ Hz, H-24b), 3.18 (1H, br d, $J = 3.62$ Hz, H-11 β), 2.93 (1H, d, $J = 3.62$ Hz, H-12 β), 2.88 (1H, dd, $J = 4.50, 9.45$ Hz, H-3 α), 2.74 (1H, m, H-19 α), 2.42 (1H, dt, $J = 5.55, 7.70$ Hz, H-16 α), 2.22 (2H, overlap, H₂-21), 2.07 (1H, dd, $J = 4.40, 12.48$ Hz, H-1 β), 1.76 (1H, d, $J = 11.95$ Hz, H-22 α), 1.74 (1H, overlap, H-18 β), 1.57–1.49 (3H, overlap, H₂-6 and H-15 β), 1.50 (1H, br s, H-9 β), 1.35–1.18 (3H, overlap, H-22 β , H-16 β and H-7 β), 1.23 (3H, d, $J = 6.25$ Hz, Me-29), 1.12–1.02 (2H, overlap, H-7 α and H-15 α), 1.10 (3H, s, Me-27), 1.06 (3H, s, Me-23), 1.01 (3H, s, Me-25), 0.93 (1H, overlap, H-1 α), 0.91 (3H, s, Me-26), 0.82 (1H, dd, $J = 3.30, 11.15$ Hz, H-5 α); ^{13}C NMR (100.6 MHz, $\text{C}_5\text{D}_5\text{N}$): δ 46.71 (t, C-1), 66.65 (d, C-2), 83.67 (d, C-3), 42.95 (s, C-4), 54.31 (d, C-5), 18.15 (t, C-6), 30.97 (t, C-7), 40.85 (s, C-8), 50.97 (d, C-9), 36.82 (s, C-10), 53.83 (d, C-11), 55.18 (d, C-12), 88.01 (s, C-13), 40.13 (s, C-14), 26.20 (t, C-15), 22.08 (t, C-16), 44.42 (s, C-17), 60.19 (d, C-18), 35.02 (d, C-19), 151.56 (s, C-20), 30.97 (t, C-21), 32.38 (t, C-22), 22.89 (q, C-23), 63.36 (t, C-24), 17.95 (q, C-25), 19.67 (q, C-26), 15.54 (q, C-27), 177.39 (s, C-28), 15.78 (q, C-29), 107.63 (t, C-30).

References:

- Huang PL, Wang LW, Lin CN, 1999. New triterpenoids of *Mallotus repandus* [J]. *J Nat Prod*, **62** (6), 891–892
- Lin ZW, Shen XY, Sun HD, 1992. The chemical constituents of *Rabdosia adenanthus* [J], *Acta Botanica Yunnanica* (云南植物研究), **14** (3): 343–344
- Siddiqui BS, Wahab A, Begum S, 2000. Two new pentacyclic triterpenoids from the aerial parts of *Lantana camara* Linn [J]. *Heterocycles*, **53** (3), 681–687
- Sun HD, Xu YL, Jiang B, 2001. Diterpenoids from *Isodon* Species (香茶菜属植物二萜化合物) [M]. Beijing: Science Press, 93–105
- Tommasi ND, Rastrelli L, Lauro MR, et al, 1998. Polyhydroxylated triterpenes from *Senecio pseudotites* [J]. *Phytochemistry*, **49** (4): 1123–1125
- Wang YH, Chen YZ, Sun HD, et al, 1998. The constituents of diterpenoids of *Isodon adenanthus* [J]. *Acta Scientiarum Naturalium Universitatis Sunyatseni* (中山大学学报), **37** (1), 122–124
- Xu YL, Sun HD, Wang DZ, 1987. Structure of adenanthin [J]. *Tetrahedron Letters*, **28** (5): 499–502