Antifertility Triterpenoid Acids from *Kadsura angustifolia*[*]

Chen YeGao, Lin ZhongWen, Cao Lin, Sun HanDong, Qin GuoWei, and Xie YuYuan ([a]*Department of Chemistry, Yunnan Normal University, Kunming, Yunnan* 650092, *China*; [b]*Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, yunnan* 650204, *China*; [c]*Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai* 200031, *China*)

ABSTRACT **OBJECTIVE**: To isolate and characterize bioactive compounds from the stems of *Kadsura angustifolia*. **METHOD**: The compounds were extracted with solvent, isolated by column chromatography and identified by the spectroscopic methods. **RESULTS**: eight compounds and a mixture of two triterpenoid acids were isolated and identified as epi-anwuweizic acid (1), shikimic acid (2), (+)-catechin (3), poriferast-5-en-3β, 7α-diol (4), β-sitosterol (5), daucosterol (6), cerotic acid 1-monoglyceride (7) and behenic acid 1-monoglyceride (8), and mixiture of coccinic acid (9) and anwuweizonic acid (10). **CONCLUSION**: Mixture of 9 and 10 showed significant inhibitory activity against human decidual cells and rat luteal cells *in vitro*. Compounds 2~ 4, 7 and 8 were first reported in the family Schisandraceae, and compounds 1, 5, 6, 9 and 10 were isolated from *K. angustifolia* for the first time.

KEY WORDS *Kadsura angustifolia*, Schisandraceae, triterpenoid acids, antifertility

狭叶南五味子中的抗生育活性三萜酸

陈业高 林中文[2] 曹 霖[1] 孙汉董[2] 秦国伟[1] 谢毓元[1] (昆明 650092 云南师范大学化学化工学院制药与化工系; [1] 上海 200031 中国科学院上海药物研究所; [2] 昆明 650204 中国科学院昆明植物研究所)

摘要 目的: 从狭叶南五味子茎中分离鉴定生物活性成分。方法: 采用溶剂提取及柱色谱法进行分离, 光谱技术鉴定化合物的结构。结果: 分得 8 个已知化合物及两个三萜酸的混合物, 结构鉴定为表安五酸(epi-anwuweizic acid, 1), 莽草酸(shikimic acid, 2), 儿茶精((+)-catechin, 3), poriferast-5-en-3β, 7α-diol(4), β-谷甾醇(5), 胡萝卜甙(6), cerotic acid 1-monoglyceride(7), behenic acid 1-monoglyceride(8)及 coccinic acid(9)和安五酸(anwuweizonic acid, 10)的混合物。结论: 9 和 10 的混合物体外对人蜕膜细胞和鼠黄体细胞有显著抑制作用。化合物 2~ 4, 7 和 8 首次自五味子科植物中分得, 化合物 1, 5, 6, 9 和 10 首次分自本植物。

关键词 狭叶南五味子, 五味子科, 三萜酸, 抗生育活性

Kadsura angustifolia A. C. Smith (Schisandraceae) is an evergreen hana, indigenous to Yunnan Province, China. Its stems have been used in local folk medicine to promote blood circulation and to treat fracture and irregular menstruation[1]. Previously we reported four new dibenzocyclooctadiene lignans named angustifolin A~ D from the plant stems[2,3]. In the course of our search for antifertility natural products, we described the isolation and structure identification of eight known compounds and a mixture of two triterpenoid acids from the title plant in this paper.

The stems of *K. angustifolia* was extracted with 95% EtOH, and then fractionated into petroleum ether, ethyl acetate and n-butanol soluble fractions. The petroleum ether fraction was subjected to repeated column chromatography on silica gel to yield 1, 4, 5, 8 and mixture of 9 and 10, respectively. The EtOAc fraction yielded 2, 3, 6 and 7 in the same way. Their structures were identified as epi-anwuweizic acid (1)[4], shikimic acid (2)[5], (+)-catechin (3)[6], poriferast-5-en-3β, 7α-diol (4)[7], β-sitosterol (5)[8], daucosterol (6)[8], cerotic acid 1-monoglyceride (7), behenic acid 1-monoglyceride (8), and mixture of coccinic acid (9) and anwuweizonic acid (10)[9-12] respectively by various spectral studies and comparison with the literature. In antifertility assay, mixture of 9 and 10 showed 100% inhibitive rate against rat luteal cells at 20 μg/ml, and 98.5% inhibitive

陈业高, 男, 36 岁, 1998 年毕业于中国科学院上海药物研究所, 获天然药物化学博士学位。现为云南师范大学化学化工学院制药与化工系主任, 副教授。

rate to human decidual cells at 40 μg/ml *in vitro*. Compounds 2~ 4, 7 and 8 were first reported in the family Schisandraceae, and compounds 1, 5, 6, 9 and 10 were isolated from *K. angustifolia* for the first time.

Experimental

Plant materials were collected in April 1993 in Xichou County of Yunnan province, China and identified by Prof. Quan-An Wu, Kunming Institute of Botany, Chinese Academy of Sciences, where a voucher specimen (No. 9304016) is deposited.

Extraction and Isolation. —— The plant material (5.2 kg) were extracted with 95% EtOH (four times, each 10 L) at room temperature. The EtOH extract was evaporated in vacuum to yield a dark brown residue (300 g). H_2O (2.5 L) was added to the residue, and the resulting solution was extracted with petroleum ether, EtOAc and n-BuOH successively (four times, each 1.5 L). The petroleum ether extract (130g) was applied to a silica gel column, eluting with petroleum ether containing increasing amounts of Me_2CO. The fractious obtained from petrolemn ether-acetone (20: 1) elution were combined and subjected to repeated CC to yield 5 (200mg), and a mixture of 9 and 10 (25 g). The fractious obtained from petroleunm ether-acetone (10: 1) elution were combined and subjected to repeated CC to yield 1(70mg), 4 (15 mg), and 8 (15 mg). The EtOAc extract (100 g) was isolated in a similar manner to obtain 2 (200 mg), 3 (300 mg), 6 (500 mg) and 7 (60mg).

Epi-anwuweizic acid 1. —— mp 145- 146℃; IR v_{max}^{KBr} cm^{-1}: 3270, 3270~ 2500, 1695, 1637, 1458, 1371, 1261, 1076, 1032, 933; *m/z* 456([M]$^+$), 441 (base peak), 423, 412, 397, 301, 242, 215, 113, 95; δ_H(400MHz), see Table 1; δ_C, see Table 2.

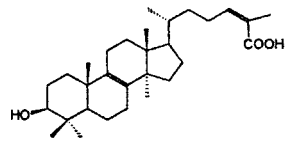
Table 1 ^{1}H NMR data of compounds 1, 9 and 10*

H	1(in C_5D_5N)	9(in $CDCl_3$)	10(in $CDCl_3$)
3	3.45*dd*(10, 5.2)		
11		5.19*d*(2.1)	
18	0.75*s*	0.68*s*	0.84*s*
19	1.11*s*	1.05*s*	1.07*s*
21	1.03*d*(6.2)	0.91*d*(6.8)	0.94*d*(6.2)
23	2.88, 2.76(each 1H, *m*)		
24	6.04*d*(7.4)	6.05*d*(7.0)	6.05*d*(7.0)
27	2.13*s*	1.87*s*	1.87*s*
28	1.04*s*	1.04*s*	1.06*s*
29	1.24*s*	1.07*s*	1.08*s*
30	0.94*s*	0.83*s*	0.89*s*

* *J*(Hz) in parentheses

Table 2 ^{13}C NMR data of compounds 1, 9 and 10

C	1(in C_5D_5N)	9(in $CDCl_3$)	10(in $CDCl_3$)
1	36.2*t*	36.5*t*	36.0*t*
2	28.8*t*	34.1*t*	34.8*t*
3	78.2*d*	217.6*s*	219.7*s*
4	39.6*s*	47.5*s*	48.0*s*
5	51.0*d*	51.8*d*	52.0*d*
6	18.8*t*	20.8*t*	20.3*t*
7	26.9*t*	26.0*t*	27.0*t*
8	134.5*s*	41.5*d*	133.2*s*
9	135.4*s*	148.6*s*	135.4*s*
10	37.5*s*	36.5*s*	38.0*s*
11	21.4*t*	116.8*d*	21.5*t*
12	31.4*t*	34.1*t*	31.9*t*
13	44.9*s*	45.0*s*	45.0*s*
14	50.2*s*	47.5*s*	50.2*s*
15	31.3*t*	33.4*t*	31.0*t*
16	28.6*t*	28.0*t*	28.0*t*
17	50.8*d*	50.2*d*	49.6*d*
18	16.1*q*	16.0*q*	16.0*q*
19	16.4*q*	23.0*q*	19.0*q*
20	36.6*d*	36.5*d*	37.5*d*
21	18.9*q*	19.0*q*	19.0*q*
22	36.5*t*	34.8*t*	36.5*t*
23	27.1*t*	26.5*t*	26.5*t*
24	142.6*d*	146.9*d*	146.9*d*
25	128.7*s*	126.0*s*	126.0*s*
26	170.7*s*	173.2*s*	173.2*s*
27	19.5*q*	20.9*q*	20.7*q*
28	24.5*q*	23.8*q*	24.5*q*
29	28.7*q*	29.0*q*	26.4*q*
30	21.5*q*	20.9*q*	21.5*q*



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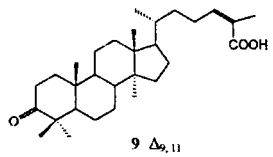

9 $\Delta_{9,11}$ 10 $\Delta_{6,9}$

Shikimic acid 2. —— UV λ_{max}^{MeOH} nm: 212.0; IR v_{max}^{KBr} cm^{-1}: 3483, 3386, 3222, 2850, 1682, 1647, 1454, 1387, 1294, 1276, 932, 862; *m/z*: 156(M^+-H_2O), 138, 120, 115, 114, 110, 109, 97, 96, 81, 69, 53(base peak); δ_H(400MHz; DMSO-d_6) 6.55 (d, 1H, *J* = 1.7Hz), 4.20 (br s, 1H), 4.80, 4.60 (br, s, 3H), 3.82 (m, 1H), 3.54 (dd, 1H, *J* = 10.0, 5.6 Hz), 2.

38(dd, 1 H, J = 16.0, 2.0 Hz), 2.00 (dd, 1H, J = 16.0, 3.2 Hz); δ_C(DMSO-d_6): 168.0 (C=O), 128.4(C-1), 138.7(C-2), 70.3(C-3), 66.8(C-4), 65.5(C-5), 29.9(C-6).

(+)-*Catechin* 3. —— FAB-MS m/z 291[M + H]$^+$; δ_H ($(CD_3)_2CO$) 4.52 (d, 1 H, J = 8.0 Hz), 3.96 (m, 1 H), 2.89 (dd, 1 H, J = 16.0, 5.6 Hz), 2.50(dd, 1 H, J = 16.0, 8.8Hz), 5.84(d, 1 H, J = 2.3 Hz), 6.00 (d, 1 H, J = 2.3 Hz), 6.88 (d, 1 H, J = 2.0 Hz), 6.77 (d, 1 H, J = 8.0 Hz), 6.72 (dd, 1 H, J = 8.0, 2.0 Hz).

Poriferast-5-en-3β, 7α-diol 4. —— mp 227~228℃; IR ν_{max}^{KBr} cm^{-1}: 3602, 3396, 1664, 1466, 1377, 1192, 1057, 951; m/z 430([M]$^+$), 412 (base peak), 394, 379, 312, 175, 161, 95, 69; δ_H(400 MHz; $CDCl_3$)5.59 (d, 1H, J = 4.9 Hz), 3.85 (m, 1 H), 3.55 (m, 1 H), 0.67 (s, 3 H), 0.98 (s, 3 H), 0.91 (d, 3 H, J = 6.5Hz), 0.79 (d, 3 H, J = 6.8 Hz), 0.81 (d, 3 H, J = 6.8Hz), 0.82(t, 3 H, J = 7.6 Hz); δ_C($CDCl_3$) 146.2*s*, 123.8*d*, 71.3*d*, 65.4*d*, 55.7*d*, 49.4*d*, 45.8*d*, 42.3*d*, 42.1*t*, 42.0*s*, 39.2*t* 37.5*d*, 37.4*s*, 37.0*t*, 36.1*d*, 33.9*t*, 31.4*t*, 29.2*d*, 28.3*t*, 26.0*t*, 24.3*t* 23.1*t*, 20.7*t*, 19.8*q*, 19.0*q*, 18.8*q*, 18.3*q*, 12.0*q*, 11.6*q*.

β-sitosterol 5. —— mp 136~137℃, m/z 414([M]$^+$), 396, 381, 354, 329, 303, 273, 255, 231, 213, 201, 187, 173, 161, 145, 133, 119, 107, 95. Identified by mixed melting point, co-TLC and comparison of IR spectrmn with that of authentic samples.[8]

Daucosterol 6. —— mp 296~298℃; FAB-MS: 577[M + H]$^+$; Identified by mixed melting point, co-TLC and comparison of IR spectrum with that of authentic samples.[8]

Cerotic acid 1-*monoglyceride* 7. —— m/z 470(M^+), 452(M^+-H_2O), 442, 424, 411, 396, 368, 351, 340, 323, 294, 134, 112, 98, 57(base peak); δ_H(400 MHz; $CDCl_3$+ CD_3OD) 4.04 (m, 2 H), 3.80 (m, 1 H), 3.55 (dd, 1 H, J = 11.6, 4.0 Hz), 3.47 (dd, 1 H, J = 11.6, 6.2 Hz), 2.26 (t, 2 H, J = 7.6 Hz), 1.53(m, 2 H), 1.18 (br s, 44 H), 0.79 (t, 3 H, J = 6.7Hz); δ_C($CDCl_3$+ CD_3OD) 174.3*s*(C-4), 69.9*d*(C-2), 65.0*t*(C-1). 63.2*t*(C-3), 34.1*t*(C-5), 31.8*t*(C-6), 29.0~29.5*t*(C-7~C-26), 24.8t (C-27), 22.5t (C-28), 13.9q (C-29).

Behenic acid 1-*monoglyceride* 8. —— m/z 414(M^+), 396(M^+-H_2O), 383, 368, 355, 340, 313, 299, 270, 257, 239, 134, 112, 98, 57 (base peak); δ_H(400 MHz; $CDCl_3$) 4.14(m, 2 H), 3.90 (m, 1 H), 3.67(dd, 1 H, J = 11.3, 3.3 Hz), 3.57 (dd, 1 H, J = 11.3, 5.6 Hz), 2.32 (t, 2 H, J = 7.6 Hz), 1.58 (m, 2 H), 1.23 (br s, 36 H), 0.85 (t, 3 H, J = 6.6 Hz).

Mixture of *Coccinic acid* 9 *and anwuweizonic acid* 10. —— IR ν_{max}^{KBr} cm^{-1} 2500~3500, 1693, 1633, 1458, 1373, 1263, 929; m/z 454([M]$^+$), 439, 421, 412, 408, 393, 342, 313, 271, 257, 248, 235, 205, 189, 175, 161, 95; δ_H (400 MHz), see Table 1; δ_C, see Table 2.

Antifertility Activity Assay. —— Both ovaries were excised from pseudopregnant rats. Corpora lutea were dissected out under a microscope, seeded at a density of 2~3 × 10^5 cells/well in 0.5 ml McCoy's 5A Medium, and supplemented with 30% BSA, benzylpenicillinium potasium 200 KIU/L, streptomycin 200 KIU/L. Human decidual cells were dispersed and seeded at 2~3× 10^5 cells/well in 0.5 ml FD Medium, and supplemented with 10% BSA, benzylpenicillinium potasium 200 KIU/L, streptomycin 200 KIU/L. Both kinds of cells described above were cultured at 37℃ in air with 5% CO_2 for 24 hours.

After cultured for 1~2 days, the two kinds of cells were exposed to two concentrations (20 μg/ml for rat lutel cells and 40 μg/ml for human decidual cells) of the various samples for 24 hours. Four wells were used for each dose, cell viability was assessed with typan blue dye. Inhibitive rates (%) of cells growing were assessed.

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