

- Province [J]. *Gansu Agric Sci Technol* (甘肃农业科技), 1992, 6: 38-39.
- [2] Zhou X H, Ryoji K, Kazuhiro O, et al. Oleanane and ursane glucosides from *Rubus steies* [J]. *Phytochemistry*, 1992, 31 (11): 3642-3644.
- [3] Takashi S, Takashi T, Osamu T, et al. β -Glucosyl esters of 19 α -hydroxyursolic acid derivatives in leaves of *Rubus steies* [J]. *Phytochemistry*, 1984, 23(12): 2829-2834.
- [4] Jia Z J, Liu X Q, Liu Z M. Triterpenoids from *Sanguisorba alpina* [J]. *Phytochemistry*, 1993, 32(1): 155-159.

A novel lupane triterpene from seeds of *Ziziphus jujuba* var. *spinosa*

HE Feng¹, PAN Qin^{2*}, MIN Zhi-da¹

(1. China Pharmaceutical University, Nanjing 210038, China; 2. Research Center, Tianjin Zhongxin Pharmaceuticals, Tianjin 300457, China)

Abstract: **Objective** To study the chemical constituents from the seeds of *Ziziphus jujuba* var. *spinosa*, which is used as a sedative herbal medicine in China. **Methods** The constituents were separated and purified by silica gel column. Their structures were elucidated based on their physicochemical properties and spectral analysis. **Results** Six compounds were obtained from 95% EtOH extracts in the seeds of *Z. jujuba* var. *spinosa*. They were identified as 2 α , 3 β -dihydroxy-lup-20(29)-en-28-oic acid methyl ester (I), β -sitosterol (II), betulin (III), betulinic acid (IV), hexadexanoic acid 2, 3-dihydroxypropyl ester (V), and daucosterol (VI). **Conclusion** Compound I is a novel lupane triterpene named as alplitolic acid methyl ester, compound V is obtained from the seeds of *Z. jujuba* var. *spinosa* for the first time.

Key words: the seeds of *Ziziphus jujuba* Mill. var. *spinosa* (Bunge) Hu ex H. F. Chow; lupane triterpene; alplitolic acid methyl ester (2 α , 3 β -dihydroxy-lup-20(29)-en-28-oic acid methyl ester)

酸枣仁中的一种新羽扇豆烷型三萜

何峰¹, 潘勤^{2*}, 闵知大¹

(1. 中国药科大学, 江苏 南京 210038; 2. 天津中新药业研究中心, 天津 300457)

摘要: **目的** 研究酸枣仁 *Ziziphus jujuba* var. *spinosa* 的化学成分。 **方法** 利用硅胶柱色谱进行分离纯化, 通过理化方法及光谱分析鉴定其结构。 **结果** 从酸枣仁乙醇提取液中得到 6 个化合物, 分别鉴定为 2 α , 3 β -dihydroxy-lup-20(29)-en-28-oic acid methyl ester (I)、 β -谷甾醇(II)、白桦酯醇(III)、白桦酯酸(IV)、1-十六烷酸甘油酯(V)、胡萝卜苷(VI)。 **结论** 化合物 I 为新化合物, 命名为罗珠子酸甲酯(alplitolic acid methyl ester), 化合物 V 为首次从酸枣仁中分离得到。

关键词: 酸枣仁; 羽扇豆烷三萜; 罗珠子酸甲酯

中图分类号: R284.1

文献标识码: A

文章编号: 0253-2670(2006)02-0168-04

The seeds of *Ziziphus jujuba* Mill. var. *spinosa* (Bunge) Hu ex. H. F. Chow are used as a sedative medicine in China. There were many published papers which described the isolation methods of new saponins^[1,2], flavonoids^[3], and alkaloids^[4,5]. Here, we present the isolation and structure elucidation of a new lupane triterpene, 2 α , 3 β -dihydroxy-lup-20(29)-en-28-oic acid methyl ester

(I) named as alplitolic acid methyl ester. Five known compounds β -sitosterol (II), betulin (III), betulinic acid (IV), hexadexanoic acid 2, 3-dihydroxypropyl ester (V), daucosterol (VI) have also been isolated. Compound V was obtained from *Ziziphus jujuba* var. *spinosa* for the first time. The structures of these compounds were elucidated by the means of MS, NMR and physical constant.

1 Experiment

1.1 Apparatus used

Melting points were determined by an X_4 apparatus. IR spectra were obtained by a Shimadzu Ftr—8400s spectrometer in KBr-disk. $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, DEPT, $^1\text{H-}^1\text{H COSY}$, HMQC, and HMBC were recorded with a Bruker AV 400 instrument, with TMS as an internal standard. ESI-MS were recorded in a Quattro Micro™ API instrument. EI-MS was recorded in a JEOL JMS D—300 instrument and HRFTICRMS was measured on a Bruker APEX—II spectrometer. Silica gel (200—300 meshes) was purchased from Qingdao Marine Chemical Factory. TLC were performed on HPTLC plates (Alltech).

1.2 Plant material

The seeds of *Z. jujuba* var. *spinosa* (11.0 kg) were collected, from Jixian, Tianjin of China in November 2002 and were identified by Professor Qin Minjian, Department of Natural Resource, China Pharmaceutical University. A retained specimen (Specimen No. z-007-2) has been deposited in Tianjin Zhongxin Pharmaceuticals, Tianjin, China.

1.3 Extraction and isolation

The seeds of *Z. jujuba* var. *spinosa* were extracted with 95% EtOH ($3 \times 20\text{ L}$). After removal of the solvent under reduced pressure, the extract was dissolved in water and extracted with petroleum, EtOAc, and *n*-BuOH, respectively. The EtOAc extract (60 g) was fractioned by silica gel column (200—300 meshes, 500 g) with gradient elution of $\text{CHCl}_3\text{-CH}_3\text{OH}$ and purified by silica gel column (200—300 meshes) eluted with petroleum-EtOAc to yield **I** (12 mg), **II** (15 mg), **III** (5 mg), **IV** (1.89 g), **V** (9 mg) and **VI** (10 mg).

2 Results and discussion

Compound **I**, mp 248—251 °C, colorless needles in MeOH, ESI-MS m/z 487.4 $[\text{M} + \text{H}]^+$ and a sodium molecular ion $[\text{M} + \text{Na}]$ at m/z 509.716 3 (calcd. 509.716 1) in the HRFTICRMS, corresponding with a molecular formula of $\text{C}_{31}\text{H}_{50}\text{O}_4$. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3 387, 3 070, 2 947, 1 724, 883. The molecular formula $\text{C}_{31}\text{H}_{50}\text{O}_4$ im-

plies seven degrees of unsaturation. It gave positive response to Libermann-Burchard reaction, that suggested that it should be triterpene. The $^1\text{H-NMR}$ spectrum showed five angular methyl groups [δ 0.98 (s, $\text{C}_{23}\text{-H}_3$), 0.94 (s, $\text{C}_{27}\text{-H}_3$), 0.89 (s, $\text{C}_{26}\text{-H}_3$), 0.87 (s, $\text{C}_{25}\text{-H}_3$), 0.78 (s, $\text{C}_{24}\text{-H}_3$)], and one allyl group [δ 1.66 (s, $\text{C}_{29}\text{-H}_3$); 4.71, 4.58 (each br s, $\text{C}_{30}\text{-H}_2$)]. The $^{13}\text{C-NMR}$ and DEPT showed 31 carbon signals which included one carbonyl carbon [δ 176.64 (s) in $^{13}\text{C-NMR}$] and one methoxy group [δ 51.25 (q) in $^{13}\text{C-NMR}$ and δ 3.64 (s) in $^1\text{H-NMR}$]. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ were submitted in Table 1.

Table 1 $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ Data of betulinic acid and compound **I** in CDCl_3

No.	Betulinic acid (100 MHz) ^[6]		Compound I	
	C	C	H	
1	39.0	46.77 (t)	2.02 (dd, $J=12.0, 4.8$) 0.82 (t, $J=12.0$)	
2	27.6	69.25 (d)	3.64 (m)	
3	78.2	83.91 (d)	2.94 (d, $J=12.0$)	
4	39.0	39.18 (s)	—	
5	55.5	55.47 (d)	0.77 (m)	
6	18.4	18.28 (t)	1.48 (m), 1.34 (m)	
7	34.5	34.21 (t)	1.34 (m)	
8	40.8	40.75 (s)	—	
9	50.7	50.49 (d)	1.30 (m)	
10	37.3	38.58 (s)	—	
11	21.0	21.00 (t)	1.43 (m)	
12	25.6	25.40 (t)	1.69 (m), 1.00 (m)	
13	38.2	38.20 (d)	2.15 (m)	
14	42.5	42.45 (s)	—	
15	30.8	30.61 (t)	1.87 (m), 1.33 (m)	
16	32.6	32.16 (t)	2.20 (m), 1.37 (m)	
17	56.3	56.55 (s)	—	
18	47.1	46.97 (d)	2.96 (t, $J=11.2$)	

Because of the long-range correlations showed between the carbonyl carbon and the methoxy group in HMBC spectrum (Fig. 1), the methoxy group was connected to the carbonyl carbon. The $^{13}\text{C-NMR}$ spectra of **I** was in a good agreement with those of betulinic acid^[6] except the C-28 and the carbons in the ring A (Table 1). The IR absorption of **I** at $3\,387\text{ cm}^{-1}$ showed the presence of hydroxyl groups. Comparing with the molecular formula and degrees of unsaturation, we know that there are two hydroxyl groups in it [δ 69.25 (d) and 83.91 (d) in $^{13}\text{C-NMR}$]. With the HMBC spectrum (Fig. 1), we concluded that the two hy-

droxyl groups connected to the C-2 and C-3, which was also been proved by ^1H - ^1H COSY (Fig. 2). From the above, the structure of **I** was lup-20 (29)-en-28-oic acid, 2, 3-dihydroxy-, methyl ester. In ^1H -NMR spectrum, δ 0.82 (1H, t, $J=12.0$ Hz, H-1a) and δ 2.02 (1H, dd, $J=12.0$, 4.8 Hz, H-1b) showed the C₂-OH was α configuration, and δ 2.94 (1H, d, $J=12.0$ Hz, H-3) showed the C₃-OH was β configuration (Fig. 3). From the above evidence, **I** was elucidated as 2 α , 3 β -dihydroxy-lup-20 (29)-en-28-oic acid methyl ester (Fig. 4).

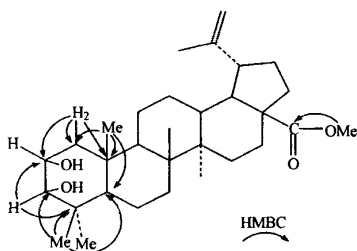


Fig. 1 HMBC Correlations in compound **I**

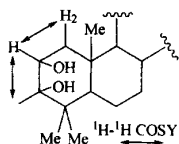


Fig. 2 ^1H - ^1H COSY Correlations in compound **I**

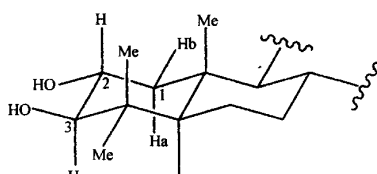
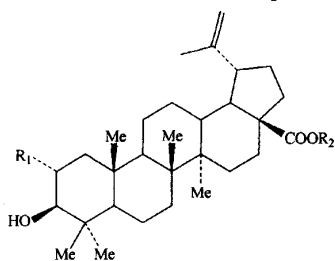


Fig. 3 Structure of ring **A** in compound **I**



Compound **I**: $\text{R}_1=\text{OH}$, $\text{R}_2=\text{CH}_3$

Betulinic acid: $\text{R}_1=\text{R}_2=\text{H}$

Fig. 4 Structures of compound **I** and betulinic acid

Compound **III**, mp 262–265 °C, colorless-needles in MeOH, it gave positive response to Libermann-Burchard reaction. ESI-MS m/z 442 $[\text{M}+\text{H}]^+$. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3 370, 3 090, 1 650, 885. ^1H -NMR (CDCl_3) δ 0.74, 0.81, 0.95, 0.96, 1.00 (3H \times 5, s, 23, 24, 25, 26, 27-Me), 1.66 (3H, s, 30-Me), 3.31; 3.77 (2H, each d, $J=10.8$ Hz,

H-28), 4.56; 4.66 (2H, s, H-29), 3.16 (1H, dd, $J=11.2$, 6.4 Hz, H-3). ^{13}C -NMR (CDCl_3) δ 38.69 (C-1), 27.36 (C-2), 78.90 (C-3), 38.84 (C-4), 55.28 (C-5), 18.28 (C-6), 34.23 (C-7), 40.91 (C-8), 50.40 (C-9), 37.15 (C-10), 20.82 (C-11), 25.21 (C-12), 37.30 (C-13), 42.71 (C-14), 27.04 (C-15), 29.17 (C-16), 47.77 (C-17), 47.77 (C-18), 48.76 (C-19), 150.45 (C-20), 29.75 (C-21), 33.96 (C-22), 27.97 (C-23), 15.33 (C-24), 16.08 (C-25), 15.96 (C-26), 14.74 (C-27), 60.49 (C-28), 19.06 (C-29), 109.66 (C-30). Comparing to the reference^[6], **III** was identified as betulin.

Compound **IV**, mp 287–289 °C, colorless needles in MeOH, it gave positive response to Libermann-Burchard reaction. ESI-MS m/z 455.2 $[\text{M}-\text{H}]^+$. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3 450, 1 686, 1 650, 875. ^1H -NMR (CDCl_3) δ 0.74, 0.81, 0.93, 0.95, 0.96 (3H \times 5, s, 23, 24, 25, 26, 27-Me), 1.68 (3H, s, 30-Me), 4.60; 4.72 (2H, each d, $J=12.0$ Hz, H-29), 3.16 (1H, dd, $J=11.2$, 6.4 Hz, H-3). ^{13}C -NMR (CDCl_3) δ 38.71 (C-1), 27.39 (C-2), 79.01 (C-3), 38.85 (C-4), 55.35 (C-5), 18.28 (C-6), 34.32 (C-7), 40.69 (C-8), 50.52 (C-9), 37.21 (C-10), 20.85 (C-11), 25.50 (C-12), 38.40 (C-13), 42.43 (C-14), 30.56 (C-15), 32.16 (C-16), 56.31 (C-17), 46.89 (C-18), 49.28 (C-19), 150.40 (C-20), 29.70 (C-21), 37.02 (C-22), 27.98 (C-23), 15.32 (C-24), 16.10 (C-25), 16.02 (C-26), 14.68 (C-27), 180.70 (C-28), 19.36 (C-29), 109.66 (C-30). Comparing to the reference^[6], **IV** was identified as betulinic acid.

Compound **V**, mp 68–72 °C, white powder, EI-MS m/z 299 $[\text{M}-\text{CH}_2\text{OH}]^+$, 239 $[\text{M}-\text{C}_3\text{H}_7\text{O}_3]^+$, 134 $[\text{M}-\text{C}_{14}\text{H}_{28}]^+$, 74 $[\text{M}-\text{OOC}_{15}\text{H}_{31}]^+$, 57 $[\text{C}_4\text{H}_9]^+$, 43 $[\text{C}_3\text{H}_7]^+$. ^1H -NMR (CDCl_3) δ 4.19 (1H, dd, $J=11.6$, 4.8 Hz), 4.13 (1H, dd, $J=11.6$, 6.0 Hz), 3.91 (m), 3.68 (1H, dd, $J=11.2$, 4.0 Hz), 3.58 (1H, dd, $J=11.2$, 6.0 Hz), 2.33 (2H, t, $J=7.6$ Hz), 1.61 (2H, m, $J=7.6$ Hz), 1.4–1.2 (24H, m), 0.86 (3H, t, $J=6.8$ Hz). ^{13}C -NMR (CDCl_3) δ 174.32

(s), 70.26 (d), 65.16 (t), 63.31 (t), 34.14 (t), 31.90 (t), 30—29 (t), 14.08 (q). Comparing to the reference^[7], V was identified as hexadecanoic acid 2, 3-dihydroxypropyl ester.

Compound II, mp 139—140 °C, was isolated as colorless needle, and compound VI, mp 292—295 °C, was isolated as colorless powder. They were identified as β -sitosterol and daucosterol, respectively after the determination of the mixed melting point and Rf vs authentic samples.

Acknowledgement: Authors are grateful for the support of Tianjin Zhongxin Pharmaceuticals.

References:

- [1] Yoshikawa M, Murakami T, Ikebata A, et al. Biocative saponins and glycosides X. on the constituents of *Zizyphi Spinosi Semen*, the seeds of *Ziziphus jujuba* Mill var. *spinosa* Hu; structures and histamine release-inhibitory effect of jujubosides A₁ and C and acetyljujuboside B [J]. *Chem Pharm Bull*, 1997, 45(7): 1186-1189.
- [2] Matsuda H, Murakami T, Ikebata A, et al. Bioactive saponins and glycosides XN. structure elucidation and immunological adjuvant activity of novel protojubenin type triterpene bisdesmosides, protojubenosides A, B, and B₁, from the seeds of *Ziziphus jujuba* Mill var. *spinosa* (*Zizyphi Spinosi Semen*) [J]. *Chem Pharm Bull*, 1999, 47(12): 1744-1748.
- [3] Cheng G, Bai Y J, Zao Y Y, et al. Flavonoids from *Ziziphus jujuba* Mill var. *spinosa* [J]. *Tetrahedron*, 2000, 56(45): 8915-8920.
- [4] Han B H, Park M H, Han Y N. Cyclic peptide and peptide alkaloids from seeds of *Zizyphus vulgaris* [J]. *Phytochemistry*, 1990, 29(10): 3315-3319.
- [5] Park M H, Sun D Y, Han B H. Absolute configuration of a cyclopeptide alkaloid, sanjoinine-G₁, from *Zizyphus vulgaris* var. *spinosa* [J]. *Phytochemistry*, 1996, 43(3): 701-704.
- [6] Mochammad S, Kazuo Y, Ryoji K. ¹³C nuclear magnetic resonance of lupine-type triterpenes, lupeol, betulin and betulinic acid [J]. *Chem Pharm Bull*, 1980, 28(3): 1006-1008.
- [7] Yuan J, Hu W Y. Studies on ¹³C-NMR and ¹H-NMR spectra of 3'-long straight-chain saturated carboxylic acid [J]. *J Yunnan Univ; Nat Sci*, 1992, 14(4): 370-372.

络石藤中的三萜类化合物

谭兴起^{1,2}, 陈海生², 周密², 张岳²

(1. 解放军第九八医院, 浙江 湖州 313000; 2. 第二军医大学药学院, 上海 200433)

摘要:目的 对络石藤中的三萜类化合物进行分离鉴定。方法 采用硅胶、反相硅胶、Sephadex LH-20 等柱色谱方法进行分离, NMR 等波谱学方法进行结构鉴定。结果 从络石藤中分离鉴定 8 个三萜类化合物: 络石苷 F (trachelosperoside F, I), 络石苷 B-1 (trachelosperoside B-1, II), 络石苷 D-1 (trachelosperoside D-1, III), 络石苷 E-1 (trachelosperoside E-1, IV), 3 β -O-D-glucopyranoside quinovic acid (V), 3 β -O- β -D-glucopyranoside 'quinovic acid 27-O- β -D-glucopyranosyl ester (VI), 3 β -O- β -D-glucopyranoside cincholic acid 27-O- β -D-glucopyranosyl ester (VII), 络石苷元 B (trachelosperogenin B, VIII)。结论 I 为新化合物, 其余均为首次从该植物中分离得到。

关键词: 络石藤; 三萜; trachelosperoside F

中图分类号: R284.1

文献标识码: A

文章编号: 0253-2670(2006)02-0171-04

Triterpenoids from canes with leaves of *Trachelospermum jasminoides*

TAN Xing-qi^{1,2}, CHEN Hai-sheng², ZHOU Mi², ZHANG Yue²

(1. No. 98 Hospital of PLA, Huzhou 313000, China; 2. College of Pharmacy, Second Military Medical University, Shanghai 200433, China)

Abstract: **Objective** To study the triterpenoids from the canes with leaves of *Trachelospermum jasminoides*. **Methods** The compounds were separated and purified by column chromatography with silica gel, RP-C₁₈, Sephadex LH-20 and identified by IR, MS, NMR, and 2D-NMR. **Results** Eight triterpenoids were identified as: trachelosperoside F (I), trachelosperoside B-1 (II), trachelosperoside D-1 (III), trachelosperoside E-1 (IV), 3 β -O-D-glucopyranoside quinovic acid (V), 3 β -O- β -D-glucopyranoside 'quinovic acid 27-O- β -D-glucopyranosyl ester (VI), 3 β -O- β -D-glucopyranoside cincholic acid 27-O- β -D-glucopyranosyl ester (VII), trachelosperogenin B (VIII). **Conclusion** I was a new compound, the others were first isolated from this plant.

收稿日期: 2005-05-20

基金项目: 湖州市自然科学基金资助项目 (2004-20)

作者简介: 谭兴起 (1972-), 男, 土家族, 医学博士, 湖北省巴东县人, 2004 年毕业于第二军医大学, 主要从事天然药物化学研究工作。

Tel: (0572) 7223107 E-mail: Tanxq@sohu.com