Journal of Chemistry, Vol. 45 (1), P. 110 - 113, 2007

CHEMICAL CONSTITUENTS OF *ZIZYPHUS SATIVA* GAERTN FRUITS

II - TRITERPENOID ACIDS

Received 5 June 2006

NGUYEN THI HOANG ANH[1], TRAN VAN SUNG[1] AND L. WESSJOHANN[2]

[1]*Institute of Chemistry, Vietnamese Academy of Science and Technology*

[2]*Leibniz Institute of Plant Biochemistry, Halle (Saale), Germany*

SUMMARY

Three terpenoid acids have been isolated from an ethyl acetate extract of Z. sativa fruits. Their structures were established as betulinic, oleanolic and maslinic acids by means of MS, NMR spectroscopic data and by comparison with reported data.

I - INTRODUCTION

Some species of *Zizyphus* genus (Rhamnaceae) have been used for treatment of biliousness, chronic bronchitis, blood diseases or as analeptic and expectorant [1]. Tomoda [2] reported the isolation of D-glucose, D-fructose, oligosaccharide and polysaccharide from a water-soluble fraction of *Zizyphus jujuba*. On the study of pharmacological active principles in the fruits of *Z. jujuba* three p-coumaroylates of alphitolic acid were also obtained [1]. In a previous publication [3] we reported the separation and structural elucidation of alphitolic acid, 3-O-p-*trans*-coumaroylalphitolic acid and 3-O-p-*cis*-coumaroylalphitolic acid from *Zizyphus sativa* fruits. This paper concerns the isolation and structure determination of three triterpene acids. Theirs structures have been elucidated as betulinic acid (**1**), oleanolic acid (**2**) and maslinic acid (**3**) by using spectroscopic methods.

II - EXPERIMENTAL

1. Plant material

The fruits of *Z. sativa* were bought in the traditional medicine market in Hanoi, May 2005. A voucher specimen Nr. H.11 was deposited in the Herbarium of Institute of Chemistry.

2. Instruments and Chemicals

NMR: Varian Unity 300; MS: AMD 402; for analytical purposes: Merck TLC aluminium sheets silica gel 60 F_{254} (layer thickness 0.2 mm) were used. Silica gel Merck 60 (0.040 - 0.063 mm) is used for column chromatography.

3. Extraction and Isolation

Air dried and powdered fruits of *Z. sativa* (2 kg) were extracted with EtOH : H_2O (95: 5) at room temperature. The ethanol extract was concentrated under *vacuum* and aq. soln. was extracted with *n*-heptan, EtOAc and *n*-BuOH, successively. The solvent were evaporated in *vacuum* to afford *n*-heptan (2 g) EtOAc (7 g) and *n*-BuOH (24 g) extracts.

The EtOAc extract was separated by chromatography on silica gel, eluting gradient with CH_2Cl_2 and MeOH to furnish 24 fractions, which were combined according to TLC

monitoring.

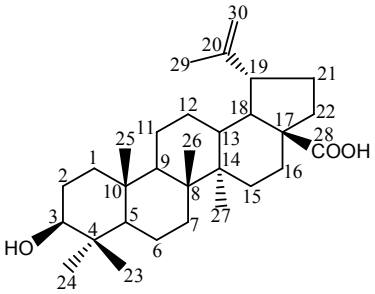
The fifth fraction (1.2 g) was further purified over a sephadex LH-20 column with methanol as eluant to yield 10 fractions. The combined fractions 2, 3 and 4 (342 mg) were rechromatographed over a flash silica gel column, eluting with *n*-hexane : EtOAc (8 : 2) to furnish 20mg of compound **1** and 42 mg of compound **2** as white powder. Negative ESI-MS of **1**: *m/z* 455 $[M-H]^-$ and of **2**: *m/z* 455 $[M-H]^-$.

The combination of fractions 10 and 11 from the first column of ethyl acetate extract was further purified over a flash silica gel column, eluting with *n*-hexane : EtOAc (7 : 3) giving 49 mg of compound **3**. Positive ESI-MS: *m/z* 495 $[M + Na]^+$.

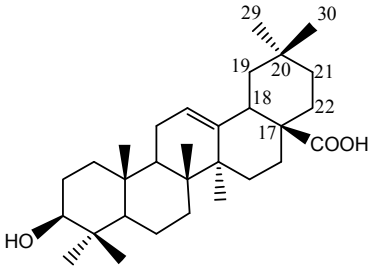
III - RESULTS AND DISCUSSION

Compound **1** was isolated as white powder and showed molecular ion peak at *m/z* 455 $[M-H]^+$ in the negative ESI-MS, corresponding the molecular formula of $C_{30}H_{48}O_3$. Its NMR spectral data revealed five tertiary methyl signals as singlet and an isopropenyl group [δ_H 4.72, 4.59 (each *d,* J = 1 Hz, 1 H); δ_C 109.3 t (C-29) and δ_H 1.69 s (3 H); δ_C 19.2 q (C-30)]. Furthermore, one oxygenated methine group (δ_H 3.0 m, δ_C 78.7 d) due to C-3 and a carboxyl group (δ_C 178.9) have been observed. These spectral data together with the presence of 30 carbon signals in the molecule of **1** confirmed that this compound possesses typical lupane-type triterpene. By exact comparison its spectral data with those of betulinic acid in the literature [4] led to conclusion that structure of **1** is betulinic acid. Recent investigation showed that betulinic acid and its derivatives have anti-HIV, anti-tumor and anti-inflammatory activities.

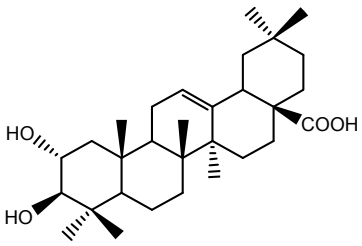
Compound **2**, an white powder, has the same molecular ion peak at *m/z* 455 $[M-H]^+$ as compound **1** in the negative ESI-MS, $C_{30}H_{48}O_3$. This compound is also a triterpene acid with 30 carbon in the ^{13}C-NMR spectrum, including one carboxyl group (δ_C 181.0). The further spectral analysis showed that it contained seven tertiary methyl groups, an olefinic proton (δ_H 5.26) of trisubstituted double bond (δ_C 121.7 d, 143.2 s) and an oxygenated methine group (δ_H 3.19m, δ_C 78.0 d). Consequently, the structure of **2** was elucidated as oleanolic acid. This compound has been already isolated many times from different plants, such as *Myrica rubra* [5], *Salvia officinalis*, *Boschniakia rossica*, *Liquidambar orientalis*,... It showed anti-inflammatory, anti-tumor,....



1: Betulinic acid



2: Oleanolic acid



3: Maslinic acid

Compound **3**, an white powder, indicated molecular ion peak at *m/z* 472 $[M-H]^+$ in the negative ESI-MS. Its NMR spectra and those of compound **2** are very similar. They showed also that the structure of **3** is a α^{12}-oleanene type triterpene. Besides the signals, which also exist in the spectra of compound **2** such as seven tertiary methyl groups, a trisubstituated double bond (δ_H 5.28, δ_C 121.7 d, 143.5 s), an oxygenated methine group (δ_H 2.95d, 9.3 Hz, δ_C 83.1, H-3) appeared in the spectra of **3** one oxygenated methine group more (δ_H 3.64 m, δ_C 68.2) due to C-2. Finally, the structure of **3** was established as maslinic acid by comparison with

Table 1: ^{1}H-NMR data of compounds **1 - 3** [$CDCl_3$: CD_3OD (10 : 1), 300 MHz)]

H	**1**	**2**	**3**
H-2			3.64
H-3	3.0 m	3.0 m	2.95 d (9.3)
H-12		5.26	5.28
CH_3-groups	0.75 s (3H)	0.78	0.79
	0.82 s (3H)	0.80	0.91
	0.94 s (3H)	0.91	0.93
	0.95 s (3H)	0.93	0.97
	0.97 s (3H)	0.94	1.14
	1.69 s (3H)		1.15
			1.16

Table 2: ^{13}C-NMR data of compounds **1** - **3** [$CDCl_3$: CD_3OD (10 : 1), 75 MHz]

C	**1**	**2**	**3**
1	38.7 (CH_2)	38.2	45.7
2	26.9 (CH_2)	27.2	68.2 (CH)
3	78.7 (CH)	78.0	83.1
4	38.6 (C)	38.8	39.0
5	55.2 (CH)	54.8	55.0
6	18.2 (CH_2)	17.9	18.2
7	34.2 (CH_2)	32.3	32.3
8	40.6 (C)	38.2	38.9
9	50.4 (CH)	47.2	47.3
10	37.1 (C)	36.5	37.9
11	20.8 (CH_2)	22.5	22.8
12	25.5 (CH_2)	121.7 (CH)	121.7 (CH)
13	38.2 (CH)	143.2 (C)	143.5 (C)
14	42.3 (C)	41.2	41.5
15	30.5 (CH_2)	26.1	27.4
16	32.2 (CH_2)	22.9	22.8
17	56.1 (C)	47.0	46.1
18	46.9 (CH)	40.8	40.9
19	49.1 (CH)	45.9 (CH_2)	45.7
20	150.5 (C)	30.1 (C)	30.4

C	1	2	3
21	29.6 (CH_2)	33.3	33.6
22	32.0 (CH_2)	32.1	32.3
23	27.8 (CH_3)	27.4	28.3
24	15.3 (CH_3)	15.0	16.5
25	16.0 (CH_3)	14.6	16.2
26	15.8 (CH_3)	16.2	16.6
27	14.6 (CH_3)	25.2	25.6
28	178.9 (C)	180.1	180.4
29	109.3 (CH_2)	32.3	32.7
30	19.2 (CH_3)	22.8 (CH_3)	23.2

the published data [6, 7]. It is a common substance from many species as *Crataegus oxycantha*, *Chamaenerion angustifolium*,...

REFFERENCES

1. A. Yagi, N. Okamura, Y. Haraguchi, K. Noda, I. Nishioka. Chem. Pharm. Bull., 26, No. 6, P. 1798 - 1802 (1978).
2. M. Tomoda, M. Takahashi, S. Nakatsuka. Chem. Pharm. Bull., 21, P. 707 (1973).
3. N. T. Hoang Anh, T. Van Sung, L. Wessjohann. Vietnamese Journal of Chemistry, Vol. 44, No. 6, P. 787 - 790 (2006).
4. M. Sholichin, K. Yamasaki, R. Kasai, Osamu Tanaka. Chem. Pharm. Bull., 28, No. 3, P. 1006 - 1008 (1980).
5. Y. Yaguchi, N. Sakurai, M. Nagai, T. Inoue. Chem. Pharm. Bull., 36, No. 4, P. 1419-1424 (1988).
6. H. Kojimo. Phytochemistry, 25, P. 729 - 733 (1986).
7. T. Furuya. Phytochemistry, 26, P. 715 - 719 (1987).