

## CHEMICAL CONSTITUENTS OF ZIZYPHUS SATIVA GAERTN FRUITS

### III - ALKALOIDS

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#### SUMMARY

Two alkaloids have been isolated from *Z. sativa* fruits besides butane-2,3-diol and glycerol. The structures of alkaloids were established as stepharine and 1,2-dimethoxy,5,6,6a,7-tetrahydro-4H-dibenzoquinoline by using 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, expectorant [1]. *Zizyphus* species have been known as a rich source of cyclopeptide alkaloids [2, 3]. In a previous article [4] we reported the separation and structural elucidation of triterpene acids from *Zizyphus sativa* fruits. This paper concerns the isolation and structural determination of two alkaloids and two common compounds. Their structures have been elucidated as stepharine (1), 1,2-dimethoxy,5,6,6a,7-tetrahydro-4H-dibenzoquinoline (2), butane-2,3-diol (3) and glycerol (4) by using spectroscopic methods.

#### II - EXPERIMENTAL

##### 1. Plant material

The fruits of *Z. sativa* were bought in the traditional medicine market in Hanoi, in 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<sub>254</sub> (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* (1.43 kg) were extracted with EtOH : H<sub>2</sub>O (95 : 5) at room temperature. The obtained ethanol extract was dissolved in 5% HCl solution and then extracted with *n*-hexane. The aqueous phase was neutralized with NaHCO<sub>3</sub> and then extracted with mixture of CHCl<sub>3</sub> : C<sub>2</sub>H<sub>5</sub>OH (2 : 1). After removing organic solvents, 3.9 g of alkaloid fraction has been obtained.

The alkaloid fraction was separated by chromatography on silica gel, eluting gradient with CHCl<sub>3</sub> and MeOH to furnish 10 fractions, which were combined according to TLC monitoring.

The combination of fractions 5, 6 and 7 (529

mg) was further purified over a flash silica gel column with  $\text{CHCl}_3$  : MeOH (95 : 5) as eluant to yield 7 fractions. The fourth fraction (93mg) was rechromatographed over a flash silica gel column, eluting with EtOAc : MeOH (45 : 10) to furnish 56 mg of compound **1**. The combined fractions 8 and 9 (153 mg) were purified over a flash silicagel by using a solvent system of EtOAc : MeOH (45 : 10) as eluant to yield 9mg of compound **2**.

21.5 g of BuOH extract was subjected in a dilation HP-20 column, eluted with water, MeOH :  $\text{H}_2\text{O}$  (1 : 1) and then with MeOH. After evaporation of solvents, 20 g, 1.14 g and 0.27 g of extracts have been obtained, respectively.

The fraction eluting with MeOH :  $\text{H}_2\text{O}$  (1 : 1) (1.14 g) was purified over a sephadex LH-20 column, eluting with MeOH to obtain 8 fractions. The third fraction (257mg) was chromatographed over a flash silica gel column, eluting with  $\text{CH}_2\text{Cl}_2$  : MeOH (90 : 10) to yield 28mg of **3** as mixture of two isomers **3a** and **3b**.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ): **3a**: 1.15 d (6.04) ( $\text{CH}_3$ ), 3.50q (6.04) (CH); **3b**: 1.12 d (6.4) ( $\text{CH}_3$ ), 3.78 q (6.4) (CH);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ ): **3a**: 19.3 ( $\text{CH}_3$ ), 72.5 (CH); **3b**: 16.9 ( $\text{CH}_3$ ), 70.8 (CH).

The fraction eluting with  $\text{H}_2\text{O}$  (20 g) was subjected in a flash silica gel column, eluting with  $\text{CHCl}_3$  and increasing amount MeOH to give 18 fractions. The combination of second and third fractions was rechromatographed over flash silica gel column, eluting with EtOAc : MeOH (90 : 10) to furnish 42 mg of **4**. Positive ESI-MS: 115  $[\text{M}+\text{Na}]^+$ ,  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}$ ): 3.4 - 3.7 m,  $^{13}\text{C-NMR}$  ( $\text{CD}_3\text{OD}$ ): 73.8 (CH), 64.3 ( $\text{CH}_2$ ).

### III - RESULTS AND DISCUSSION

The alkaloid fraction has been obtained from ethanol extract of *Z. sativa* fruits by usual method. Chromatographic separation of this fraction yielded two compounds. Compound **1** indicated positive reaction with Dragendorff reagent. It has a molecular ion peak at  $m/z$  298  $[\text{M} + \text{H}]^+$  in the positive ESI-MS spectrum, corresponding to the molecular formula  $\text{C}_{18}\text{H}_{19}\text{NO}_3$ . It seems that compound **1** contains

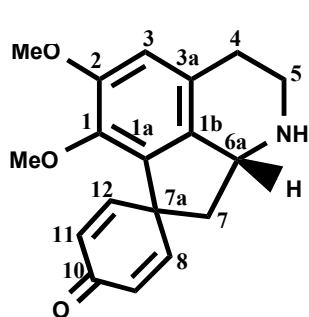
one nitrogen atom in the molecule, because its molecular weight was odd. Its  $^1\text{H-NMR}$  spectrum revealed signals of 18 protons, among them five resonanced in the olefinic and six in aliphatic ranges. Besides these signals, two-singlet of methoxy groups at  $\delta_{\text{H}}$  3.79, 3.58 and one signal of a heterogenated methine group at  $\delta_{\text{H}}$  4.38 have also been observed. The  $^{13}\text{C-NMR}$  spectrum of **1** indicated 19 carbons, containing 10 aromatic (110 - 160 ppm), one carbonyl at  $\delta_{\text{C}}$  188.1, one heterogenated methine at  $\delta_{\text{C}}$  58.4, two methoxy, three methylene and a quaternary carbons. The NMR and MS spectroscopic data suggested that **1** has one NH group in the molecule, but it did not appear in the  $^1\text{H-NMR}$  spectrum, because measured in  $\text{CD}_3\text{OD}$ . Finally, the structure of **1** was determined as stepharine by careful comparison of these data with the published data [5]. It is well known compound from *Stephania* species [6], but it was found in *Z. sativa* for the first time.

Compound **2** give a positive reaction with Dragendorff reagent. It revealed a molecular ion peak at  $m/z$  282  $[\text{M} + \text{H}]^+$  in the positive ESI-MS spectrum. This suggested that the molecule **2** possess one nitrogen atom, corresponding to the molecular formula  $\text{C}_{18}\text{H}_{19}\text{NO}_2$ . The  $^1\text{H-}$  and  $^{13}\text{C-NMR}$  spectra showed signals of two methoxy groups ( $\delta_{\text{H}}$  3.61, 3.86 and  $\delta_{\text{C}}$  56.4, 60.5), one nitrogenated methine group at  $\delta_{\text{H}}$  3.75,  $\delta_{\text{C}}$  54.6. Besides these, three methylene groups resonanced in the range from 2.1 to 3.5 ppm in  $^1\text{H-NMR}$  and at  $\delta_{\text{C}}$  29.1, 37.6, 43.7 in the  $^{13}\text{C-NMR}$  spectrum. Furthermore, 5 aromatic protons and 12 aromatic carbons have also been observed. The spectroscopic data of compound **2** are in good agreement with those of 1,2-dimethoxy,5,6,6a,7-tetrahydro-4H-dibenzoquinoline in [8, 9]. This compound was isolated previously from *Liriodendron tulipiferum* [8], but until now it was not found in *Z. sativa*.

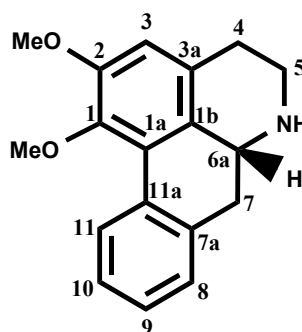
From butanol extract of *Z. sativa* two common compounds were isolated. They are butane-2,3-diol (**3**) and glycerol (**4**). Compound **3** was obtained as a mixture of two isomers with a ratio of about 3 : 1 by the integral of proton

signals in  $^1\text{H}$ -NMR spectrum. The positive ESI-MS spectrum of **3** showed a molecular ion peak at  $m/z$  113  $[\text{M} + \text{Na}]^+$ , giving the molecular formula  $\text{C}_4\text{H}_{10}\text{O}_2$ . Its  $^1\text{H}$  and  $^{13}\text{C}$ -NMR spectra indicated two sets of signals with identical coupling patterns but slightly different chemical shifts, supporting the assumption that **3** contains two isomers. The signals contain two methyl groups at  $\delta_{\text{H}}$  1.12 d (6.4), 1.15 d (6.04) and  $\delta_{\text{C}}$

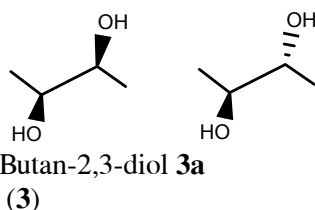
16.9, 19.3 and two methine groups, which coupled with methyl [ $\delta_{\text{H}}$  3.50 q (6.04), 3.78 q (6.4) and  $\delta_{\text{C}}$  70.8, 72.5]. The NMR and MS data revealed that **3** is butane-2,3-diol. The coupling constants suggested that the signals  $\delta_{\text{H}}$  1.15 and 3.50 belong to an isomer and  $\delta_{\text{H}}$  1.12, 3.78 belong to the other. The assignment (see experimental) was confirmed by comparison with the published data [10].



Stepharine (**1**)



1,2-dimethoxy,5,6,6a,7-tetrahydro-4H-dibenzoquinoline (**2**)



Butan-2,3-diol **3a**

**3b**

Glycerol (**4**)

Table 1:  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data of compound **1**, **2** ( $\text{CD}_3\text{OD}$ , 75 MHz)

Position	1		2	
	$^1\text{H}$	$^{13}\text{C}$	$^1\text{H}$	$^{13}\text{C}$
(1)	(2)	(3)	(4)	(5)
<b>1</b>	-	145.4 (C)	-	145.2 (C)
<b>1a</b>	-	135.1 (C)	-	127.4 (C)
<b>1b</b>	-	129.2 (C)	-	129.9 (C)
<b>2</b>	-	154.9 (C)	-	153.6 (C)
<b>3</b>	6.79 s (1H)	113.4 (CH)	6.77 s (1H)	113.0 (CH)
<b>4</b>	2.81 m (2H)	26.4 ( $\text{CH}_2$ )	2.6 - 3.0 m	29.1 ( $\text{CH}_2$ )
<b>5</b>	3.15 m (1H) 3.41 m (1H)	45.5 ( $\text{CH}_2$ )	2.6 - 3.0 m	43.7 ( $\text{CH}_2$ )
<b>6a</b>	4.38 dd (6.5, 10.0)	58.4 (CH)	3.75 dd (4.5, 13.7, 1H)	54.6 (CH)

(1)	(2)	(3)	(4)	(5)
<b>7</b>	2.28 dd (10.5, 12.0) 2.39 dd (6.5, 12.0)	48.1 (CH <sub>2</sub> )	2.6 - 3.0 m	37.6 (CH <sub>2</sub> )
<b>7a</b>	-	52.7 (C)	-	136.9 (C)
<b>8</b>	7.03 dd (2.9, 9.9)	152.7 (CH)	8.29 d (7.9, 1H)	129.2 (CH)
<b>9</b>	6.38 dd (1.9, 9.9)	128.7 (CH)		127.9 (CH)
<b>10</b>	-	188.1 (C=O)	7.20 - 7.28 m (3H)	128.8 (CH)
<b>11</b>	6.26 dd (1.9, 9.9)	127.7 (CH)	-	128.5 (CH)
<b>11a</b>	-	-	-	133.1 (C)
<b>12</b>	7.20 dd (2.9, 9.9)	156.1 (CH)	-	-
<b>1-OMe</b>	3.58 s (3H)	61.3	3.61 s (3H)	60.5
<b>2-OMe</b>	3.79 s (3H)	56.7	3.86 s (3H)	56.4

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