

## Naphthoquinones from the Roots of *Onosma argentatum* Hub.-Mor. (Boraginaceae)

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*Onosma argentatum* Hub.-Mor. is an endemic species growing in Turkey. In this study, the roots of *O. argentatum* were extracted with n-hexane-dichloromethane (1:1). The extract was separated on a silica gel column eluting with n-hexane-ethyl acetate by gradient elution. New naphthoquinone compounds ( 3 known and 1 new) were isolated and their structures were elucidated by spectroscopic methods as deoxyshikonin, acetyl shikonin, 3-hydroxy-isovaleryl shikonin, and 5,8-*O*-dimethyl acetyl shikonin.

**Key Words:** *Onosma argentatum*, natural naphthoquinones, shikonin derivatives, Boraginaceae, 5,8-*O*-dimethyl acetyl shikonin.

### Introduction

The family Boraginaceae is represented by about 100 genera and 2000 species in tropical and temperate regions.<sup>1</sup> The roots of some genera belonging to Boraginaceae are rich in naphthoquinone derivatives. *Lithospermum*, *Arnebia*, *Alkanna* and *Onosma* species contain shikonin or alkannin derivatives.<sup>2-5</sup>

The genus *Onosma* has about 150 known species. <sup>6</sup> Some *Onosma* species (*Onosma sericeum* Willd., *O. microcarpum* Steven ex D.C.) are used for the treatment of wounds in rural areas in Turkey.<sup>7</sup> *O. argentatum* is an endemic species growing in Turkey.<sup>8</sup> Its roots are used for wound healing and burns in the rural parts of İlyca district (Erzurum, Turkey). Recently, our studies revealed that the extract of *O. argentatum* showed high antioxidant and antimicrobial activities.<sup>9</sup>

A survey of the literature revealed that there have been no phytochemical studies dealing with *Onosma* species in Turkey. We herein report the isolation and characterization of naphthoquinonic compounds from *O. argentatum*.

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## Experimental

**Instruments:** NMR spectra were recorded on a Varian 200 MHz instrument in CDCl<sub>3</sub> using TMS as internal standard. EI-MS spectra were recorded on a Thermo-Finnigan and Fisons model MD 800 mass spectrometer. Column chromatography was performed on silica gel 60 (0.063-0.200 mm, Merck) and preparative TLC was performed with silica gel F<sub>254</sub> plates (20 x 20 cm, 0.5 mm, Merck).

**Materials:** The roots of *O. argentatum* were collected from Dilimli village (İlyca district, Erzurum province, Turkey) in July 2002. It was identified by Dr. Yusuf Kaya (Assistant Professor in the Department of Biological Sciences, Atatürk University, Turkey). A voucher specimen was deposited in the Herbarium of the Faculty of Sciences (Atatürk University) (ATA), (ATA 9729).

**Extraction and Isolation.** The roots (400 g) of *O. argentatum* were powdered and extracted with a (1000 mL) x 3 n-hexane-dichloromethane mixture (1:1) under reflux for 3 h for each extraction. The combined extracts were evaporated under reduced pressure to give concentrated extract (8 g; yield: 2%). The extract (8 g) was subjected to silica gel column chromatography. Elution was performed with a n-hexane-ethyl acetate mixture with gradient elution. Similar fractions determined by TLC were combined. Deoxyshikonin, acetyl shikonin, 3-hydroxy-isovaleryl shikonin, 5,8-*O*-Dimethyl acetyl shikonin were obtained in that order. Preparative TLC was used to obtain pure compounds.

**Deoxyshikonin** (2-(4-methyl-pent-3-enyl)-5,8-dihydroxy-naphthalene-1,4-dione) (**1**) (8 mg). Red oil. <sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>) δ 12.65 (s, 1H, phenolic OH), 12.49 (s, 1H, phenolic OH), 7.21 (s, 2H, H-6 and H-7), 6.85 (t, 1H, H-3, J=1.2 Hz), 5.14 (tm, 1H, H-3', J=7.1 Hz), 2.64 (dt, 2H, H-1', J=7.2, 1.2 Hz), 2.30 (q, 2H, H-2', J=7.2 Hz), 1.70 (d, 3H, H-5', J=1.1 Hz), 1.58 (s, 3H, H-6'). **EI-MS** (m/e) 272.1 (M<sup>+</sup>, 30), 229.0 (15), 216.0 (18), 204.0 (54), 69.0 (100). <sup>1</sup>H-NMR is in agreement with data given in the literature.<sup>10</sup>

**Shikonin acetate (Acetyl shikonin)** [(+)-Acetic acid 1-(5,8-dihydroxy-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)-4-methyl-pent-3-enyl ester] (**2**) (20 mg). Dark red solid. <sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>) δ 12.56 (s, 1H, phenolic OH), 12.40 (s, 1H, phenolic OH), 7.16 (s, 2H, H-6 and H-7), 6.98 (d, 1H, H-3, J=1.0 Hz), 6.01 (ddd, 1H, H-1', J=7.0, 4.6, 1.0 Hz), 5.11 (tm, 1H, H-3', J=6.0 Hz), 2.65-2.42 (m, 2H, H-2'), 2.14 (s, 3H, H-2''), 1.68 (s, 3H, H-5'), 1.56 (s, 3H, H-6'). <sup>13</sup>C-NMR (50 MHz, CDCl<sub>3</sub>) δ 178.0 (C-4), 176.6 (C-1), 169.6 (C-1''), 167.5 (C-8), 167.0 (C-5), 148.2 (C-2), 136.0 (C-4'), 132.8 (C-6), 132.6 (C-7), 131.5 (C-3), 117.7 (C-3'), 111.8 (C-9), 111.6 (C-10), 69.5 (C-1'), 32.9 (C-2'), 25.6 (C-5'), 20.8 (C-2''), 17.9 (C-6'). **EI-MS** (m/e) 330.1 (M<sup>+</sup>, 3), 270.3 (100), 255.2 (55), 219.2 (88), 191.2 (12), 83 (30). <sup>1</sup>H-NMR is in agreement with data given in the literature.<sup>10</sup> The assignment of carbons was written according to the literature.<sup>11</sup>

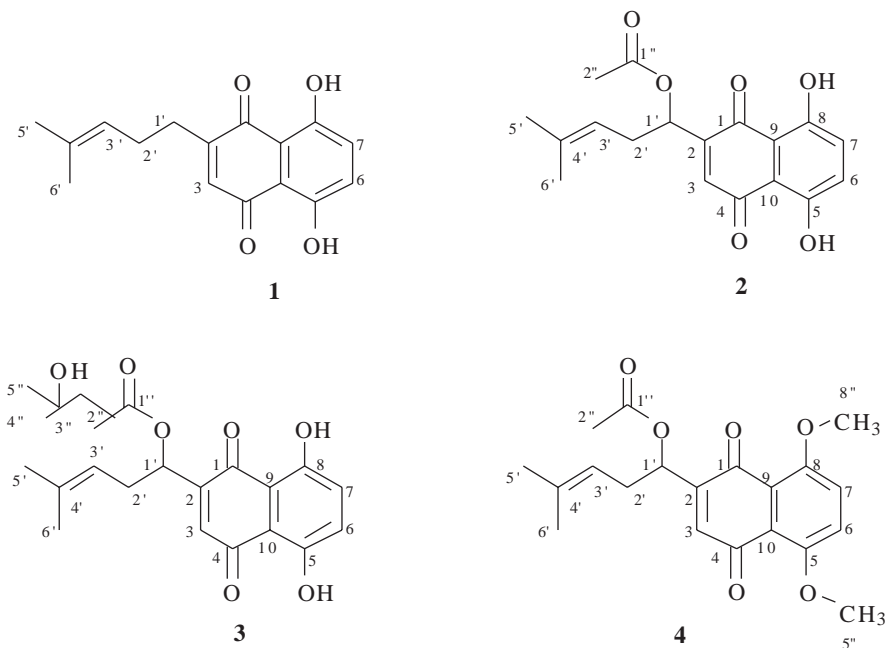
**3-Hydroxy-isovaleryl shikonin** [(+)-3-Hydroxy-3-methyl-butyric acid 1-(5,8-dihydroxy-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)-4-methyl-pent-3-enyl ester] (**3**) (25 mg). Dark red oil. <sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>) δ 12.59 (s, 1H, phenolic OH), 12.40 (s, 1H, phenolic OH), 7.17 (s, 2H, H-6 and H-7), 7.02 (bs, 1H, H-3), 6.08 (dd, 1H, H-1', J=6.5, 4.7 Hz), 5.11 (bt, 1H, H-3', J=7.3 Hz), 3.25 (bs, 1H, OH), 2.59 (s, 2H, H-2''), 2.72-2.38 (m, 2H, H-2'), 1.69 (s, 3H, H-5'), 1.59 (s, 3H, H-6'), 1.30 (s, 3H, H-4'' or H-5''), 1.29 (s, 3H, H-4'' or H-5''). <sup>13</sup>C-NMR (50 MHz, CDCl<sub>3</sub>) δ 176.1 (C-1 or C-4), 175.3 (C-1 or C-4), 171.5 (C-1''), 168.7 (C-5 or C-8), 168.2 (C-5 or C-8), 147.5 (C-2), 136.2 (C-4'), 133.2 (C-6 or C-7), 133.0 (C-6 or C-7), 131.3 (C-3), 117.7 (C-3'), 111.9 (C-9 or C-10), 111.6 (C-9 or C-10), 69.8 (C-1' or C-3''), 69.1 (C-1' or C-3''), 46.6 (C-2''), 32.8 (C-2'), 29.2 (C-4'' or C-5''), 29.1 (C-4'' or C-5''), 25.7 (C-5'), 17.9 (C-6'). <sup>1</sup>H-NMR is in agreement

with data given in the literature.<sup>12</sup> Carbon assignments in <sup>13</sup>C-NMR spectra were done by comparison with a similar system in the literature.<sup>11</sup>

**5,8-O-Dimethyl shikonin acetate (5,8-O-Dimethyl acetyl shikonin)** [(+)-Acetic acid 1-(5,8-dimethoxy-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)-4-methyl-pent-3-enyl ester] (**4**) (10 mg). Dark orange oil. <sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>) δ 7.30 (s, 2H, H-6 and H-7), 6.65 (bs, 1H, H-3), 5.90 (ddd, 1H, H-1', J=7.0, 4.6, 1.2 Hz), 5.11 (bt, 1H, H-3', J=7.4 Hz), 3.95 (s, 6H, 2x OMe), 2.64-2.39 (m, 2H, H-2'), 2.09 (s, 3H, H-2''), 1.67 (s, 3H, H-5'), 1.56 (s, 3H, H-6'). <sup>13</sup>C-NMR (50 MHz, CDCl<sub>3</sub>) δ 184.5 (C-1 or C-4), 183.3 (C-1 or C-4), 169.5 (C-1''), 154.0 (C-5 or C-8), 153.0 (C-5 or C-8), 148.2 (C-2), 135.6 (C-4'), 133.3 (C-6), 120.5 (2C, Ar-C), 120.1 (Ar-C), 118.1 (C-3'), 69.7 (C1'), 56.9 (C5'' or C8''), 56.8 (C5'' or C8''), 32.8 (C2'), 25.8 (C5'), 20.9 (C-2''), 17.9 (C-6'). **EI-MS** (m/e) 358.1 (M<sup>+</sup>, 18), 316.1 (100), 301 (42), 207 (40), 115 (15), 77 (12). <sup>1</sup>H-NMR and <sup>13</sup>C-NMR are in agreement with data given in the literature.<sup>13</sup>

## Results and Discussion

n-Hexane-CH<sub>2</sub>Cl<sub>2</sub> extracts of *O. argentatum* were subjected to chromatographic separation by a gradient solution with hexane-EtOAc. Similar fractions were collected and combined. As a result of repeated TLC experiments, 4 compounds **1-4** were purified (Figure).



Figure

Characterization of compounds **1-4** was performed by extensive NMR studies plus EI-MS. In the <sup>1</sup>H-NMR spectra of **1-3**, characteristic phenolic OH protons of **1-3** are seen as broad singlets between δ 12.40 and δ 12.65 ppm. H<sub>6</sub> and H<sub>7</sub> protons of **1-4** are seen as singlets between δ 7.16 and 7.30 ppm. H<sub>3</sub> protons of compounds **1-4** arise between δ 6.65 and 6.98 ppm. In the <sup>1</sup>H-NMR spectra of **1-4**, the peaks belonging to side chains were similar to each other. Olefinic protons of 4-methyl-pent-3-en-1-yl groups in **1-4** give triplet-like signals between δ 5.11 ppm and δ 5.14 ppm. Characteristic methyl singlets of 4-methyl-pent-3-en-1-yl groups are seen at δ 1.67-1.70 ppm and δ 1.56-1.59 ppm. In addition, <sup>13</sup>C-NMR and mass

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spectra confirmed the structures. Racemic synthesis and characterization of dimethoxy shikonin/alkannine acetate **4** were previously reported by Torii et al.<sup>13</sup> The literature indicated that (+)-**4** was isolated for the first time from nature.

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