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Diterpenes and norditerpenes from the roots of *Dorystoechas hastata*

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Five compounds, two diterpenoids ferruginol (**1**), and 6,7-didehydrosempervirol (**2**) and two norditerpenoids 17-hydroxycryptotanshinone (**3**), przewaquinone A (**4**) along with a new norditerpenoid cryptotanshinone 17β-oic acid (**5**) have been isolated from the roots of *Dorystoechas hastata* Boiss. et Heldr. ex Bentham (Lamiaceae = Labiatae) growing wild in Southwestern Turkey (Antalya).

1. Introduction

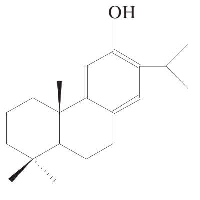
Dorystoechas hastata Boiss. et Heldr. ex Bentham (Lamiaceae) is a monotypic "single genus/single species" endemic plant growing only in the southwestern province of Turkey(Antalya). The plant is a woody shrub possessing an inflorescence which is an erect cylindrical spike with many flowered verticillasters (Davis 1971; Hedge 1971; Davis 1982). Its local name is "Çalpa" and it is used as an aromatic tea (Baytop 1984). The aerial parts of *D. hastata* have been studied for its essential oils (Meriçli and Meriçli 1986). In another study of the aerial parts two diterpenes, carnosol and rosmanol, and flavonoids, luteolin, luteolin 7-glucoside, and 6-methoxyluteolin 7-glucoside as well as caffeic acid and chlorogenic acid were isolated (Venturella et al. 1988). The essential oil composition of the stems and leaves and the flowering stems have been further studied (Başer and Öztürk 1992).

The present study was carried out with the roots of *D. hastata* and five compounds, one being new, were isolated. Two of the compounds were diterpenoids, ferruginol (**1**) (Cambie et al. 1971), and 6,7-didehydrosempervirol (**2**) (Gonzales et al. 1992), while the other three including the new compound were tanshinone type norditerpenes, 17-hydroxycryptotanshinone (**3**) (de la Torre et al. 1992), przewaquinone A (**4**) (Lee et al. 1991) and the new compound cryptotanshinone 17-oic acid (**5**). Tanshinones have been isolated mostly from *Salvia miltiorrhiza* Bunge (Tanshen). The plant has been known in China as a "Supergrade" medicinal herb since ancient times. Its extracts and isolated compounds have been tested for biological activities, and anti-spasmolytic, anti-arthritic, tonic, sedative and astringent activities have been established. The plant extract is also highly recommended in cases of hemorrhage, menstrual disorders and against miscarriages. In recent studies Tanshen exhibited significant improvement *in vitro* of the blood flow in the coronary circulatory system and reduction in myocardial infarctions (Lee et al. 1987). Tanshen also exhibited cytotoxicity against five human tumor cell-lines (Ryu et al. 1997). Due to these activities the compounds obtained from *D. hastata* should have biological importance, and after this first report their activities will be investigated.

2. Investigations, results and discussion

The dried and powdered roots (1 kg) of the plant were extracted with acetone until extinction in a Soxhlet apparatus: 83 g of residue was obtained. Half of this residue was fractioned on a silica gel column eluting with petroleum ether, followed by a CH_2Cl_2 gradient (increasing 1% increments). The collected fractions were controlled on TLC plates and similar fractions were combined. Fractions 1–56 were discarded as having only oily material, while fractions 56–77 yielded ferruginol (**1**) and 6,7-didehydrosempervirol (**2**). Fractions 78–98 contained a number of compounds in small amounts in a complex mixture, not even separable on a chromatotron plate, and therefore set aside. Fractions 99–111 yielded przewaquinone A (**4**) and the new compound cryptotanshinone 17β-oic acid (**5**) and fractions 112–121 yielded 17-hydroxycryptotanshinone (**3**). Other fractions were polar mixtures and contained no diterpenoids. They were set aside for possible future studies.

The new compound cryptotanshinone 17β-oic acid (**5**) was a dark orange colored amorphous compound with an optical rotation $[\alpha]_D^{27} = -25°$ (c = 0.5, $CHCl_3$). The mo-



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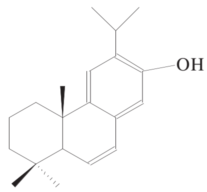

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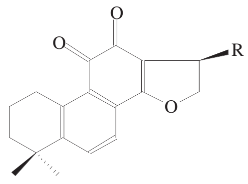

3 R=CH$_2$OH

5 R=COOH

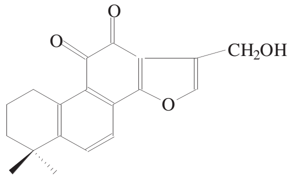

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Table: NMR data of cryptotanshinone 17β-oic acid

	^{1}H NMR	^{13}C NMR	COSY	NOESY
2H-1	3.22 t (6.5 Hz)	28.7 t	2H-2	H-3; H-2
2H-2	1.80 m	19.1 t	2H-1; 2H-3	H-3; H-1
2H-3	1.66 m	37.8 t	2H-2	H-1; H-2
4	–	36.4 s		
5	–	143.7 s		
6	7.52 d (7.8 Hz)	133.9 d	H-7	H-7
7	7.66 d (7.8 Hz)	122.5 d	H-6	H-6
8	–	128.4 s		
9	–	124.4 s		
10	–	152.4 s		
11	–	184.3 s		
12	–	175.7 s		
13	–	118.3 s		
14	–	143.7 s		
15	3.62 dd (6; 11 Hz)	34.9 d	H-16A; H-16B	H-16A; H-16B
HA-16	4.38 dd (6; 11 Hz)	81.8 t	H-15; H-16B	H-16B; H-15
HB-16	4.90 t (11Hz)	–	H-15; H-16A	H-16A; H-15
17	–	170.8 s		
18	1.32 s	1.8 q		
19	1.32 s	29.6 q		

lecular formula $C_{19}H_{18}O_5$ was calculated from HRMS (m/z 326.1171) and confirmed by ^{13}C NMR and APT (Attached Proton Test) spectral data. The ^{1}H NMR spectrum of **5** showed signals at δ 10.25 (1 H, s, COOH) an acid group, the aromatic protons at δ 7.52 (1 H, d, J = 7.8 Hz), 7.66 (1 H, d, J = 7.8 Hz) (H-6 and H-7 respectively), 4.89 (1 H, t, J = 6 and 11 Hz, H-16B), 4.38 (1 H, t, J = 11 Hz, H-16A), 3.62 (1 H, dd, J = 6, 11 Hz, H-15), 3.22 (2 H, t, J = 6.5 Hz, H_2-1), 1.80 (2 H, m, H_2-2), 1.66 (2 H, m, H_2-3), 1.24 (6 H, s, Me-18 and Me-19). The correlations between $C_1{-}C_3$, $C_6{-}C_7$ and between $C_{15}{-}C_{16}$ protons were established by COSY studies. ^{13}C NMR (APT), given in the Table, is in agreement with the suggested structure. The spectra are quite similar to those of compound **3** as well as the values given for it in the literature (de la Torre et al. 1992) with the exception of the lack of hydroxymethylene group signal at C-15, and the presence of an acid group in compound **5**. The NOESY experiment also indicated the placement of the acid at C-15 by giving NOESY between H-15 (δ 3.62) with H-16A (δ 4.38) and H-16B (δ 4.89). Since the angles between H-15 and H_2-16 differed slightly it was not possible to decide its stereochemistry. However from a close study of the Dreiding model β-stereochemistry for the acid group seems more likely. The AM-1 semiemprical method indicated the optimized geometry of the acid group as β (Fig. 1). Therefore we decided that compound 5 is cryptotanshinone 17β-oic acid.

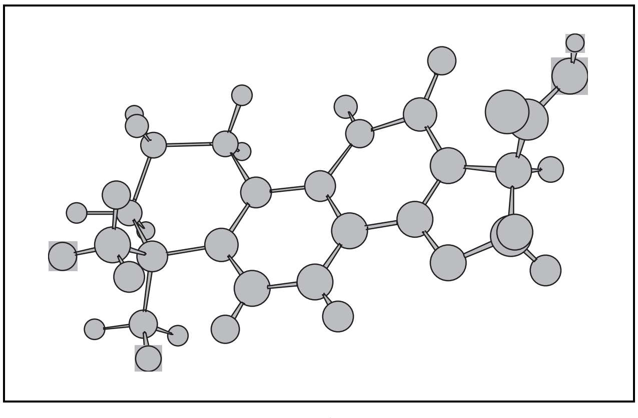

Fig.: Structure of cryptotanshinone 17β-oic acid

3. Experimental

3.1. Equipment

UV spectra were recorded in MeOH on a Shimadzu UV 1601 spectrophotometer. IR spectra were recorded on a Perkin-Elmer 983 spectrophotometer. Optical rotations were determined in an Opt. Act. Ltd. AA-5 polarimeter. ^{1}H NMR (400 and 500 MHz) and ^{13}C NMR (125 MHz) were recorded on Varian and Bruker instruments respectively. EIMS and HRMS were recorded on a Zapspec instrument.

3.2. Plant material

The roots of *Dorystoechas hastata* Boiss. et Heldr. ex Bentham were collected from southwestern Turkey (Antalya) and identified by Prof. Dr. Ertan Tuzlac (Marmara University, Faculty of Pharmacy), and a voucher specimen is deposited in the Herbarium of the Faculty of Pharmacy, Istanbul University ISTE 54478.

3.3. Extraction and isolation

The dried and powdered roots of the plant (1 kg) were extracted with acetone in a Soxhlet apparatus until extinction. The acetone solution was evaporated to dryness under vacuum 83 g of a residue was obtained. Half of this residue (42 g) was added at the top of a silica gel column eluting with petroleum ether, and gradients of CH_2Cl_2 up to 100% followed by EtOH. Collected fractions were checked on TLC plates and similar ones were combined. Fractions 1–56 were oily and discarded, fractions 56–77 yielded ferruginol (**1**, 300 mg) and 6,7-didehydrosempervirol (**2**, 22 mg), fractions 78–98 were a complex mixture having a number of compounds in small amounts and were set aside. Fractions 99–111 yielded przewaquinone A (**4**, 30 mg) and cryptotanshinone 17β-oic acid (**5**, 22 mg). From fractions 112–121 17-hydroxycryptotanshinone (**3**, 27 mg) was isolated. Other fractions (122–178) were more polar mixtures and as no diterpenoid was established on TLC plates, they were left aside for future studies.
The known compounds were identified by comparing their spectral data (^{1}H and ^{13}C NMR) with literature values. The structure of the new compound cryptotanshinone 17-oic acid (**5**) was established mainly by spectral data [^{1}H, ^{13}C NMR (APT), COSY, HETCOR and NOESY] and comparing its spectra to those of compound **3**.

3.4. Cryptotanshinone 17 β-oic acid (*5*)

$[\alpha]_D^{27} = -25°$ (c = 0.5); UV λ_{max}^{MeOH} (log ε) nm: 445 (0.1), 351 (0.15), 263 (1.2), 214 (1.15); IR ν_{max}^{CHCl3} cm^{-1}: 3447, 2960, 2931, 2869, 1687, 1654, 1622, 1558, 1460, 1419, 1406, 1365, 1331, 1301, 1196, 1170, 1140, 1033, 992, 936, 846, 823, 754, 665; ^{1}H NMR ($CDCl_3$) (see text and Table); ^{13}C NMR ($CDCl_3$) (see Table 1). EIMS (rel. int.) at m/z 326 $[M]^+$ (2), 296 $[M\text{-}2 \times Me]^+$; (82), $[253\ M{-}C_3H_4O_2 + H]^+$ (100), 235 (10), 171 (12), 128 (10), 83 (5). HRMS: 326.1171 $C_{19}H_{18}O_5$ (calc. 326.1154).

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