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TWO NEW COMPOUNDS FROM *ARTEMISIA DRACUNCULUS* L.

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ABSTRACT

Two new compounds, 7-methoxycoumarin (I) and 7-hydroxyartemidin (II) were isolated from the ethanol/water (50:50, V/V) extract of *Artemisia drucunculus* L. leaves. Structures of the isolated compounds were elucidated on the basis of spectral data (^{1}H NMR and MS). It is shown that compound I is devoid of anticoagulation activity in male Albino rabbits in contrast to the same activity observed in rabbits using the crude extract of the leaves.

Key words: *Artemisia drucunculus,* Compositae, Coumarins, Isocoumarins

INTRODUCTION

One of the widely used natural sources in Persian traditional medicine is tarragon (*Artemisia drucunculus L.)*. This table vegetable, which is cultivated around Tehran province, has long been used in folk medicine as a natural food cure for cleaning and diluting of blood and treatment of dizziness and headache. As part of our research on the anticoagulant natural products, two new compounds were isolated from the ethanol/water extract of tarragon leaves. This paper reports structure elucidation of these new compounds and the anticoagulant activity of the most abundant component of the extract in rabbits.

MATERIAL AND METHODS

^{1}HNMR spectra were recorded at 80 MHz and ^{13}CNMR were recorded at 200 MHz on Varian spectrometers. The mass spectra were recorded at ionization potential of 70 ev. Silica gel for column chromatography was 70-230 mesh (Merck) and for thin layer chromatography (TLC) and preparative TLC was 60F 254 (Merck). All other chemicals used throughout this investigation were of the best analytical grade available and were purchased from Aldrich (UK) or Merck (Germany).

***Plant material*:** Tarragon was purchased from the farmlands around Tehran. The plant material was dried at room temperature, far from sunlight. The leaves were powdered and stored in closed container at 4°C pending further investigation.

Isolation of 7-methoxycoumarin and 7-hydroxyartemidin from aqueous extract: Tarragon leaf powder (100 g) was mixed with a mixture of ethanol and water (50:50, V/V) and the mixture was kept at room temperature overnight. The mixture was then filtered through cheesecloth. The extraction was repeated two more times using the same solvent system. The combined filtrates were concentrated by boiling to a volume of 50 ml. The resulting aqueous tarragon extract (50 ml) was extracted four times with chloroform in a separatory funnel and the combined chloroform solution was concentrated under reduced pressure at 50°C to a volume of 5 ml. The concentrated chloroform solution was kept at room temperature overnight. The crystals formed were then separated and rinsed with cold chloroform solution. Eight mg of crystals were obtained from 10 g of the tarragon powder. Crystals were then subjected to successive column chromatography on silicagel using petroleum ether, followed by gradual reduction in petroleum ether content and addition of equal volume of diethylether and then by diethylether followed by gradual increase of chloroform content to a final 50:50 mixture (V/V) as eluent and 10 fractions were collected. The 5th fraction was re-chromatographed on preparative TLC to give compounds I and II.

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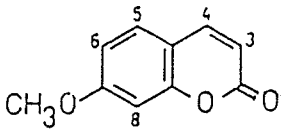
7-methoxycoumarin (I): UV ($CHCl_3$): $\lambda_{max.}$ 325 nm; MS m/z: 176 (M^+), 148[M-CO]$^+$, 133[M-(CO+Me)]$^+$; IR (cm^{-1}), 1710, 1620, 1550, 1499, 1459; ^{13}CNMR: C-2 (162.5), C-3(112.1), C-4(143.22), C-5(128.6), C-6(112.6), C-7(160.7), C-8(100.5), C-9(155.5), C-10(112.5) and CH_3(55.5); ^{1}HNMR (Table 1.)

7-hydroxyartemidin (II): UV (CHCl3): $\lambda_{max.}$ 270 nm; MS m/z: 216 (M^+, base peak), 201[M-15]$^+$,188[M-CO]$^+$,161[M-(-CH=CH-H_2CH_3)]$^+$, 133[M-(-CH=CH-CH2CH3+CO)]$^+$;IR(cm^{-1}) 3400, 3020, 2970, 1760, 1660, 1465; ^{1}HNMR (Table 1.)

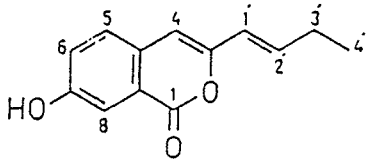
Animal treatments: Albino rabbits (1.4-1.6 kg) purchased from Hesarak, (Karaj) and they were kept and treated with compound I as described previously (2).

RESULTS AND DISCUSSION

The concentrated chloroform extract from the ethanol-water extract of tarragon leaves gave crystals that were separated and dissolved in chloroform. Successive chromatography of the chloroform solution led to the isolation of eight fluorescent compounds, which were further, purified by preparative TLC. Structures of two of these compounds were established according to combined spectral data. The most abundant constituent (I) was visualized on TLC as a purple fluorescent spot under UV light (360 nm). The UV, IR, ^{1}HNMR and ^{13}CNMR data led to the identification of this compound as 7-methoxycoumarin. The EI-mass spectrum revealed the empirical formula $C_{10}H_8O_3$ with the molecular ion peak (M^+) at 176 and fragment ions at 148 and 133 mass units corresponding to [M-CO]$^+$ and [M-(CH_3-OH)]$^+$, respectively (1). The ^{1}HNMR spectrum displayed a singlet at 8 3.8 assignable to the methoxy group at C_7. The ^{1}HNMR spectrum also confirmed the presence of H-3, H-4, H-5, H-6 and H-8. In addition, the ^{13}CNMR spectrum displayed the presence of 10 different carbon atoms in compound I. Compound II was visualized on TLC as a bluish spot under UV light. The mass spectrum revealed the empirical formula $C_{13}H_{12}O_3$ with the base peak ion at m/z 216 for M^+ and the fragment ions at 161 and 133 mass units corresponding to [M-(-CH=CH-CH_2CH_3)]$^+$ and [M(-CH=CH_2CH_3+CO)]$^+$, respectively, which strongly identified compound II as an isocoumarin 1. Comparison of ^{1}HNMR data of II with the corresponding data for 8-hydroxy-artimidin agreed with the substitution of the hydroxy group at C-7 and the butenyl group at C-3. In addition the occurrence of peaks at 1.11(t), 2.30(dq), 6.68(t) and 6.06(t) and 6.06(d), corresponding to 4′-H, 3′-H, 2′-H and 1′-H, confirmed the presence of butenyl group in compound II (1).



Compound 1



Compound 2

Anticoagulation activity of 7-methoxy-coumarin (I): Effects of 10 days treatment of male Albino rabbits with compound I on the plasma PT values, before and after treatments are presented in Table 2. As it is evident from this table, compound I most probably is devoid of anticoagulation activity. Our previous study in rat has indicated that the ethanol/water extract of tarragon leaves possess anticoagulation activity (2). Based on these data (Table 2) it may be concluded that either the anticoagulation activity of tarragon ethanol/water extract is not related to compound I (the most abundant constituent of the extract) or the purified compound I lacks the natural environment of the ethanol/water extract and consequently it is facing absorption obstacles.

Table 1. ^{1}HNMR of compounds I and II (δ values)

	I	II
H-3	6.22(1H, d, J=9.6 Hz)	---
H-4	7.67(1H, d, J=9.6 Hz)	6.24(1H, s)
H-5	7.36(1H, d, J=9.6 Hz)	6.94(1H, d, J=9.3 Hz)
H-6	6.87(1H, dd, J=7.6, 2.6 Hz)	6.86(1H, d, J=9.3 Hz)
H-8	6.82(1H, s)	
CH_3	3.87(3H, s)	
H-1		6.06(1H, d, J=7.2 Hz)
H-2	---	6.68(1H, t, J=7.2, 6.5 Hz)
H-3	---	2.28(2H, dq, J=7.2, 12 Hz)
H-4	---	1.11(3H, t, J=12 Hz)
OH	---	11.04(1H, s)

Table 2. Plasma PT values of the control and treated rabbits with compound I

Experimental groups	PT ratio (INR)	
	Before treatment	After treatment
Control	1.02±0.03	1.01±0.03
2 mg/kg/day	0.98±0.02	0.96±0.02
4 mg/kg/day	1.00±0.03	1.00±0.04

The results are presented as means±SEM.

Therefore, further research is required to clarify this point. Despite this observation, compound I have shown cytotoxicity, using the shrimp bioassay (3), with a LD_{50} value of 110 $\mu g.ml^{-1}$

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