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COMPONENTS OF *Artemisia pontica*

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Chemical components from the aerial part of the Kazakhstan population of Artemisia pontica, *from which the rarely encountered flavonoids 7-O-methyl- and 4′,7-di-O-methyl-esters of apigenin were isolated for the first time, were identified. The complete chemical composition of the essential oil was established using GC-MS.*

Key words: *Artemisia pontica*, essential oil, monoterpenoids, GC-MS, 7-O-methyl- and 4′,7-di-O-methyl-esters of apigenin.

Artemisia pontica L. (Asteraceae) is a rather common Eurasian species of wormwood [1] that grows in Crimea, the European part of Russia, western Siberia, the Caucuses, and Middle Asia. The essential oil of this wormwood is known to have anti-inflammatory activity [2]. The isolation from wormwood growing in Bulgaria of sesquiterpene lactones has been reported [3, 4]. The chemical components of roots, ponticaepoxide [5] and sesquiterpene alcohol [6], and components of the essential oil from the aerial part of 14 different pontica wormwood samples collected in western Siberia were identified. The principal components of this essential oil obtained by steam distillation were camphor (up to 49.7%), borneol (up to 25.3%), bornylacetate (up to 21.6%), and 1,8-cineol (up to 19.7%) [7].

The chemistry of the Kazakh population of pontica wormwood has not been studied although this plant represents a significant natural resource. The present article contains data on the non-lactone components of this wormwood. A total of 61 compounds making up 99.7% of the total essential oil was isolated from the essential oil using steam distillation and GC-MS (Table 1). The principal components were 1,8-cineol (25.2%), α-thujone (23.2%), camphor (16.0%), and borneol (7.7%). One notable feature of the Kazakh population of this wormwood is the high α-thujone content (23.2%).

Wormwood typically contains various hydroxy- and methoxyflavones, the number of which can reach 17 in one species [8]. We studied the $CHCl_3$ extract of the air-dried aerial part of the investigated wormwood and separated it by chromatography over silica gel to isolate two yellow crystalline compounds of compositions $C_{16}H_{12}O_5$ and $C_{17}H_{14}O_5$, respectively (high-resolution MS). According to these data and PMR spectroscopy, they are mono- and dimethyl esters of apigenin (4′,5,7-trihydroxyflavone). Both spectra exhibit the same splittings for the doublets of H(6) and H(8) ($J_{6,8}$ = 2.2 Hz) and similar, but with double intensity, signals for protons of a *p*-disubstituted phenyl ring (H-2′ + H-6′ and H-3′ + H-5′, J_{AB} = 8.8 Hz). The ^{13}C NMR spectrum of the three monomethyl esters of apigenin have been reported [9] whereas the spectrum of the ester isolated by us corresponds to that of the 7-O-methyl ester (genkwanin) (**1**). This enabled this flavonoid derivative to be identified. Genkwanin was previously found in *Daphne meserium* and *Prunis serrata* [10] and in one Indian wormwood species *Artemisia sacrorum* [11] (according to [10]).

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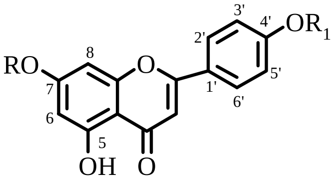
TABLE 1. Components in Essential Oil of *Artemisia pontica* L., % of Whole Essential Oil

RT	Component	Content	RT	Component	Content
3.730	Hexanal	0.1	14.698	Sabinaketone	0.4
4.873	*cis*-Salvene	0.3	14.887	Pinocarvone	0.5
6.730	Tricyclene	0.1	14.895	*cis*-Chrysanthenol	0.4
6.876	*α*-Thujene	0.1	**14.990**	Borneol	**7.7**
7.083	*α*-Pinene	0.1	15.463	Terpinen-4-ol	1.9
7.530	Camphene	1.8	15.695	*m*-Cymen-8-ol	0.3
7.714	Verbenene	0.1	15.884	*α*-Terpineol	0.3
7.899	Benzaldehyde	0.1	16.073	Myrtenol	0.6
8.340	Sabinene	0.3	16.477	*trans*-Piperitol	0.5
8.425	*β*-Pinene	0.1	16.535	Verbenone	0.2
8.543	Oct-1-en-3-ol	0.1	16.864	*trans*-Carveol	0.2
8.898	2,3-Dihydro-1,8-cineol	0.4	17.182	Bornylformate	0.3
8.973	*trans*-Herboxide	0.1	17.733	Cuminic aldehyde	0.3
9.232	Jomogi alcohol	0.2	17.723	Carvone	0.1
9.352	*α*-Phellandrene	0.2	18.119	Piperitone	0.5
9.487	*cis*-Herboxide	0.1	18.119	Piperitone epoxide	1.2
9.772	*α*-Terpinene	0.5	19.185	Bornylacetate	0.8
10.039	*p*-Cymol	2.0	19.314	Cuminic alcohol	0.1
10.292	**1,8-Cineol**	**25.2**	19.382	Thymol	0.2
10.482	Santolinic alcohol	0.2	19.623	Carvacrol	0.2
11.251	*γ*-Terpinene	0.6	21.291	*α*-Terpenylacetate	0.4
11.509	*trans*-Sabinene hydrate	0.2	21.428	Citronellylacetate	1.4
12.274	Terpinolene	0.2	22.666	*β*-Elemene	0.1
12.636	*cis*-Sabinne hydrate	0.3	22.528	*cis*-Jasmone	0.1
12.754	Linalool	0.2	23.525	Caryophyllene	0.1
12.944	*α*-Thujone	**23.2**	25.451	Germacrene D	0.2
13.283	*β*-Thujone	**2.7**	26.190	Germacrene A	0.1
13.426	*cis*-*p*-Menth-2-en-1-ol	1.2	28.330	Spatulenol	0.3
14.062	*trans*-Pinocarveol	0.2	28.485	Caryolphyllene oxide	0.1
14.089	*cis*-Sabinol	1.8	32.525	Chamazulene	1.6
14.242	**Camphor**	**16.0**			

Components with a content of at least 0.1% are given in bold.



1, 2

1: R = Me, R_1 = H

2: R = R_1 = Me

The second flavonoid was identified as 4′(O)-methylgenkwanin (**2**) using PMR spectra. The signals for H(6) and H(8) in the PMR spectrum practically do not change their positions upon adding the second methyl. The components of the AB-system formed by the side-chain protons shift to low field by 0.12-0.16 ppm. This proves the position of the second methoxy is C-4′. This flavonoid is also known and was previously observed in birch buds [10, 12].

The PMR spectra of **1** and **2** are not described in the literature available to us.

TABLE 2. ^{13}C NMR Spectra of **1** and **2** ($DMSO-d_6$, δ, ppm, 0 = TMS)

Atom	Compound		Atom	Compound	
	1	**2**		**1**	**2**
C-2	163.95 s	163.53 s	C-1′	120.95 s	122.62 s
C-3	102.89 d	103.62 d	C-2′	128.35 d	128.23 d
C-4	181.73 s	181.79 s	C-3′	115.83 d	114.48 d
C-5	157.09 s	157.15 s	C-4′	161.15 s	162.32 s
C-6	97.78 d	97.87 d	C-5′	115.83 d	114.48 d
C-7	165.0 s	165.09 s	C-6′	128.35 d	128.23 d
C-8	92.52 d	92.62 d	$\underline{C}H_3O$-C-7	55.86 q	55.91 q
C-9	161.08 s	161.08 s	$\underline{C}H_3O$-C-4′	-	55.44 q
C-10	104.54 s	104.62 s			

TABLE 3. PMR Spectra of **1** and **2** ($DMSO-d_6$, δ, ppm, J/Hz, 0 = TMS)

Atom	Compound	
	1	**2**
OCH_3-C4′	-	3.88 (3H, s)
OCH_3-C7	3.87 (3H, s)	3.87 (3H, s)
H-6	6.36 (1H, J = 2.2, d)	6.38 (1H, J = 2.2, d)
H-8	6.74 (1H, J = 2.2, d)	6.78 (1H, J = 2.2, d)
H-3	6.80 (1H, s)	6.9 (1H, s)
H-3′, H-5′	6.94 (2H, J = 8.8, d)	7.13 (2H, J = 8.8, d)
H-2′, H-6′	7.94 (2H, J = 8.8, d)	8.06 (2H, J = 8.8, d)
C5-OH	12.93 (1H, br.s)	12.89 (1H, br.s)
C4′-OH	10.27 (1H, br.s)	-

EXPERIMENTAL

Melting points were determined on a Boetius apparatus. IR spectra were recorded on an Avatar 360 instrument (Thermo Nicolet) in KBr disks; UV spectra, on a Specord UV-VIS instrument in ethanol solutions; NMR spectra, on a Bruker DRX-500 spectrometer (working frequency 500.13 MHz for ^1H, 125.76 MHz for ^{13}C). The solvent was $DMSO-d_6$ with TMS internal standard. Chemical shifts are reported on the δ scale. High-resolution mass spectra were recorded in a Finnigan MAT 8200 instrument with electron-impact ionization (70 eV).

GC-MS was performed on a Hewlett-Packard 5890/II gas chromatograph with a quadrupole mass spectrometer (HP MSD 5971) as a detector. We used a 30-m HP-5 quartz column (copolymer 5% diphenyl, 95% dimethylsiloxane) with internal diameter 0.25 mm and a stationary-phase film thickness of 0.25 μm. The percent composition of the essential oil was calculated using peak areas without correction factors. Qualitative analysis was based on a comparison of retention times and full mass spectra with the corresponding data for standard oil components and pure compounds and with Wiley 275 (275,000 mass spectra) library data and catalogs for the mass spectra [13, 14].

Column chromatography was performed over SiO_2 (KSK, 1:20); flash chromatography, over SiO_2 (Armsorbil, 100/160). TLC used Silufol plates with development by aqueous $FeCl_3$ (3%).

Starting raw material was the aerial part of *A. pontica* collected in mid-July (budding phase) 2003 in the Kent Karkaralin mountains of Karaganda region. It was air-dried without exposure to direct sunlight and ground to particles 2-5 mm in size.

Isolation and Analysis of Essential oil. Essential oil was a dark-blue thin liquid with a pleasant smell. It was isolated by steam distillation from raw material (80 g) for 2 h in a Clevenger apparatus. Yield, 0.16 g (0.2%).

The essential oil composition (Table 1) was determined by GC-MS.

Isolation of Flavones 1 and 2. Raw material (1 kg) was soaked with $CHCl_3$ (5 L). The extract was decanted after 3 h, filtered, and evaporated to dryness. The resulting resin (103 g) was dissolved in hot aqueous ethanol (0.5 L, 60%), cooled to room temperature, and extracted with $CHCl_3$ (0.5 L). Solvent was removed to afford a mixture of compounds (40 g), chromatography of which isolated successively **2** (0.02 g), mp 170-174°C (EtOAc) (lit. [10] mp 174°C) and **1** (0.04 g), mp 280-285°C (EtOAc) (lit. [10] mp 285-287°C) with elution by $CHCl_3$.

5,4′-Dihydroxy-7-methoxyflavone [genkwanin (1)]. IR spectrum (KBr, ν, cm^{-1}): 3267, 1666, 1605, 1590, 1435, 1376, 1342, 1162, 1118, 1016, 833, 819.

UV spectrum (λ, nm, log ε, EtOH): 206 (1.8), 329 (1.6). Mass spectrum (*m*/*z*, I, %): 284 (100) $[M]^+$, 255 (44), 241 (13), 227 (2), 212 (3), 167 (11), 138 (12), 128 (16), 95 (11), 69 (12), 32 (5), 28 (26).

^{13}C NMR spectrum (125.76 MHz, δ, ppm, TMS) appears in Table 2; PMR spectrum (500.13 MHz, δ, ppm, TMS), in Table 3.

5-Hydroxy-7,4′-dimethoxyflavone (4′,7-dimethyl ester of apigenin) (2). IR spectrum (KBr, ν, cm^{-1}): 2925, 2851, 1669, 1605, 1510, 1163, 1121, 1093, 1027, 1018, 947, 834, 819.

UV spectrum (λ, nm, log ε, EtOH): 210 (2.3), 269 (1.3), 326 (1.1). Mass spectrum (*m*/*z*, I, %): 298 (100), 269 (58), 255 (26), 240 (5), 218 (15), 166 (18), 149 (9), 138 (21), 135 (46), 97 (18), 95 (24), 82 (10), 69 (33), 43 (33), 28 (32).

^{13}C NMR spectrum (125.76 MHz, δ, ppm, TMS) appears in Table 2; PMR spectrum (500.13 MHz, δ, ppm, TMS), in Table 3.

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