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A NEW MELAMPOLIDE AND OTHER SECONDARY METABOLITES FROM *Artemisia baldshuanica*

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The composition of secondary metabolites in benzene and alcohol extracts of the epigeal part of Artemisia baldshuanica *Krasch. et Zarp. (Asteraceae) collected during the vegetative phase was investigated. A new melampolide, the structure of which was established based on spectral data and an x-ray crystal structure analysis, and known compounds were isolated.*

Keywords: *Artemisia baldshuanica* Krasch. et Zarp., terpenoids, sesquiterpene lactone, melampolide, baldshuanin, GC–MS study, PMR and ^{13}C NMR spectra, XSA.

Artemisia baldshuanica Krasch. et Zarp. (Asteraceae) is a subshrub sage species of height 60–100 cm that is indigenous to Central Asia [1]. It grows in Uzbekistan in the foothills of the Nuratin, Turkestan, Zarafshan, and Gissar Ranges [2]. The chemistry of this sage species is practically unstudied except for one report [3] in which only five constituents of the essential oil were identified (α- and β-pinenes, thujol alcohol, thujyl acetate, and thujyl valerate). It was also found that the juice of leaves exhibits anthelmintic properties [4].

Therefore, we performed a phytochemical investigation of the epigeal part of *A. baldshuanica* collected in Jizak Oblast of Uzbekistan at the beginning of May 2009 during the start of vegetation.

The composition of the benzene extract was studied by GC–MS. Table 1 presents the identified compounds and their percent content.

A total of 26 constituents comprising 95.59% of all observed was identified in the benzene extract. The main constituents were the hydrocarbons 5,5-dimethyl-1-ethyl-1,3-cyclopentadiene (24.41%), tetratriacontane (2.48), and nonacosane (2.23); the monoterpenoids 1,8-cineol (23.59), α-thujone (6.50), β-thujone (5.53), and camphor (4.76); the sesquiterpenoid chrysanthenone (11.80); and the sesquiterpene lactone ambrosine (4.54).

The observed tetraacontane, nonacosane, and 1-eicosanol are specific attractants for pollinating insects [5] whereas the volatile monoterpenoids α- and β-thujone, camphor, and 1,8-cineol are active inhibitors of seed growth and the growth of sprouts of surrounding annual plants [6]. The sesquiterpene lactone ambrosine (**1**) contains two activated double bonds (the exocyclic methylene of the γ-lactone ring and the α,β-unsaturated ketone) that are responsible for the high biological activity of **1**. Sesquiterpene lactones of similar structure have a bitter taste, are toxic for mammals, and protect the plants from consumption by herbivorous animals [7].

Chromatographic separation over a column of silica gel of the $CHCl_3$ extract obtained by work up of the alcohol extract isolated a new sesquiterpene lactone that we called baldshuanin (**2**).

Baldshuanin (**2**) consisted of transparent crystals, mp 178–179°C, $C_{15}H_{20}O_4$. The IR spectrum showed absorption bands for hydroxyl at 3388 cm^{-1}; aldehyde, 2762; γ-lactone carbonyl, 1764; and a double bond, 1669 and 1245.

Table 2 presents the details of the PMR and ^{13}C NMR spectra of **2**.

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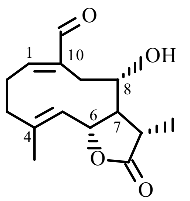
TABLE 1. Constituents of the Benzene Extract of the Epigeal Part of *Artemisia baldshuanica*

Constituent	RT, min	%
1,8-Cineol	4.08	23.59
Unidentified	5.11	0.41
β-Caryophyllene	5.15	Tr.
α-Thujone	5.16	6.50
β-Thujone	5.31	5.53
Chrysanthenone	5.43	11.80
Camphor	5.73	4.76
Unidentified	7.32	0.51
Unidentified	8.19	0.69
5,5-Dimethyl-1-ethyl-1,3-cyclopentadiene	8.32	24.41
Unidentified	9.73	0.20
Unidentified	9.81	1.04
2-Pinen-4-one (verbenone)	9.82	0.47
2-Ethylidene-6-methyl-3,5-heptadienal	9.91	0.80
Unidentified	11.62	0.32
Diisobutylphthalate	14.73	2.15
1-Propen-1,2,3-tricarboxylic acid tributyl ester	17.44	0.18
Citric acid tributyl ester	18.28	0.24
2,6,7,7a-Tetrahydro-1,5-dimethyl-1*H*-inden-3-carboxaldehyde	18.92	0.80
Valencene	18.95	0.58
Alloaromadendrene	18.96	0.32
cis-3a,4,5,6,7,7a-Hexahydro-2-(2′-propenyl)-1*H*-inden-1-one	18.97	Tr.
Ambrosine	18.98	4.54
Aromadendrene	18.99	0,30
Ledene (viridiflorene)	19.00	Tr.
cis-1,2-Diethyl-4-(1-methylethylidene)-cyclohexane	19.07	0.90
Linoleic acid	19.64	0.28
[1*S*-(1α,7α,8aβ)]-1,2,3,5,6,7,8,8a-Octahydro-1,4-dimethyl-7-(1-methylethenyl)azulene	19.90	2.14
Unidentified	20.63	0.51
1,2-Benzenedicarboxylic acid mono(2-ethylhexyl) ester	21.21	0.33
1-Eicosanol	21.69	0.26
Tetratriacontane	23.18	2.48
Unidentified	24.81	0.71
Nonacosane	25.30	2.23
Total		99.98

Tr.: traces.

An analysis of the spectral characteristics of the isolated sesquiterpene lactone **2** and a comparison with analogous spectra of the known melampolide (**3**) showed that they differed mainly in chemical shifts and spin–spin coupling constants of H-7, H-8, and H-9. Therefore, we assumed that the isolated lactone **2** was a geometric isomer of 8β-hydroxy-14-oxo-11β,13-dihydroacanthospermolide (**3**) [8]. Lactones **2** and **3** also differed in the aggregate state. Lactone **2** was crystalline whereas **3** was an oil.



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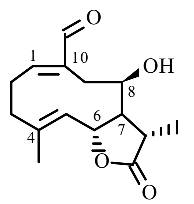

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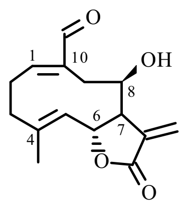

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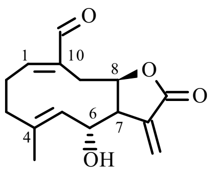

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TABLE 2. PMR and ^{13}C NMR Spectra of Baldshuanin (**2**), HMDSO 0.0 ppm

C atom	PMR (400 MHz, CDCl$_3$, ppm)	Splitting	SSCC (Hz)*	^{13}C NMR (77.23 MHz; CDCl$_3$, ppm)	DEPT
1	6.738	ddd	7.8 (2β); 6.7 (2α); 1.9 (3α)	159.62	CH
2α	2.319	dddd	12.0 (3β); 9.0 (2β); 9.0 (3α); 6.7 (1)	26.93	CH$_2$
2β	2.486	dddd	9.0 (2α); 8.5 (3α); 7.8 (1), 3.8 (3β)		
3α	2.259	dddd	12.3 (3β); 9.0 (2α); 8.5 (2β); 1.9 (1)	33.49	CH$_2$
3β	2.000	ddd	12.3 (3α); 12.0 (2α); 3.8 (2β)		
4				145.06	C
5	4.846	dq	10.4 (6); 1.4 (15)	126.14	CH
6	4.438	dd	10.4 (5); 9.8 (7)	71.98	CH
7	1.504	dddd	11.2 (11); 10.5 (8); 9.8 (6); 1.0 (9α)	56.24	CH
8	3.748	dddd	11.4 (OH); 10.5 (7); 5.3 (9β); 1.8 (9α)	77.33	CH
9α	2.403	ddd	15.8 (9β); 1.8 (8); 1.0 (7)	31.02	CH$_2$
9β	2.321	ddd	15.8 (9α); 5.3 (8); 1.1 (14)		
10				149.12	C
11	2.556	dq	11.2 (7); 7.0 (13)	41.50	CH
12				179.00	C
13	1.331	q	7.0 (11)	17.41	CH$_3$
14	9.364	d	1.1 (9β)	199.27	CH
15	1.809	d	1.4 (5)	16.58	CH$_3$
OH	5.220	d	11.4 (8)		

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*Protons with which a given proton is coupled are shown in parentheses.

TABLE 3. Torsion Angles (deg) in 10-Membered Rings of Melampolides **2**, **4**, and **5**

Compound	Angle at bond									
	–1–2–	–2–3–	–3–4–	–4–5–	–5–6–	–6–7–	–7–8–	–8–9–	–9–10-	–10–1–
2	–94.4	70.3	–86.9	158.4	–113.8	81.0	–65.7	–40.2	132.2	–1.2
4	–92	71	–90	157	–113	86	–64	–48	131	0
5	–90	73	–87	165	–121	65	–38	–65	136	–3

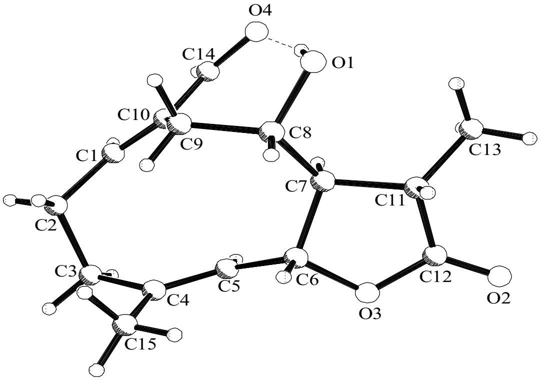

Fig. 1. Molecular structure and atomic numbering for **2** (intramolecular H-bond shown as a dashed line).

An x-ray structure analysis (XSA) of the obtained crystals was performed in order to establish unambiguously the structure of **2**. The molecular structure as determined by the XSA (Fig. 1) indicated that the isolated compound baldshuanin (**2**) was a *Z*,*E*-germacranolide-*trans*-6(7)-lactonized melampolide. The C8 hydroxyl in **2** and the C11 methyl had the α-orientation. The *trans*-fused lactone ring had the C-7α-envelope conformation (the other four ring atoms lay in a single plane within ±0.01 Å). The methyl (C15H$_3$) and aldehyde (HC14=O) groups in the ten-membered ring were mutually *trans*.

The aforementioned α-orientation of the C8 hydroxyl indicated that the isolated compound **2** was an epimer at asymmetric C8 of the known melampolide 8β-hydroxy-14-oxo-11β,13-dihydroacanthospermolide (**3**) [8].

The α-orientation of the C8 hydroxyl favored the formation of an intramolecular H-bond with the aldehyde (on C10). This was not observed for the β-orientation of the hydroxyl in **3**. The parameters of the intramolecular H-bond were distances O1…O4, 2.722(4); O4…H, 1.71 ; angle O1—H…O4, 163°. This H-bond had practically no effect on the conformation of the cyclodecadiene macrocycle. This could be seen by comparing the torsion angles of **2** and the related melampolides **4** and **5** [9, 11] (Table 3).

Z,*E*-Germacranolides (melampolides) are one of four types of sesquiterpenoid germacranes with 1(10)-*cis*, 4(5)-*trans*-configurations of double bonds in the 10-membered macrocycle. According to Samek and Harmatha [12], four types of macrocycle conformations with four types of configurations can occur in melampolides. They are designated by analogy with the conformations for bicyclic decalins that are produced if the 10-membered ring is stacked arbitrarily over atoms C5 and C10. The indices in symbols indicate the orientation of C14 and C15 (methyls) and the locations of the double bonds (C4=C5 and C1=C10) relative to the mean-square plane of the cyclodecadiene. The superscript denotes a position above; the subscript, below. The crystal of **2** has a chair–boat conformation for the cyclodecadiene ring with configurations ${}_1D_{14}$ and ${}^{15}D_5$. A comparison of the torsion angles of the macrocycle of **2** with the corresponding angles in the known melampolides alloschkuhriolide (**4**) [9] and schkuhriolide (**5**) [10, 11] with *trans*-6,7- and *cis*-7(8)-lactone rings, respectively (Table 3), indicates that three of these natural melampolides have practically the same shape of the macrocycle. According to our data and that in the literature (CCDC), the macrocycle has the chair–boat conformation with the ${}_1D_{14}$ and ${}^{15}D_5$ configurations in *trans*-6(7)- and *cis*-7(8)-lactonized melampolides. Based on the analysis, it can be noted that the chair–boat conformation of the macrocycle with the ${}_1D_{14}$ and ${}^{15}D_5$ configurations are typically found in *trans*-6(7)- and *cis*-7(8)-lactonized melampolides.

Thus, 26 known secondary metabolites were isolated and identified for the first time from the epigeal part of *A. baldshuanica* growing in Uzbekistan. The structure of the new sesquiterpene lactone baldshuanin (**2**) was established.

EXPERIMENTAL

General Comments. IR spectra were recorded on a Perkin–Elmer Model 2000 Fourier-spectrometer (KBr) and on a Nicolet Magna 4500 IR spectrometer (KBr). PMR and ^{13}C NMR spectra were recorded on a Unity-400+ spectrometer at operating frequency 400 and 100 MHz, respectively. Samples were prepared in $CDCl_3$ solution with HMDS internal standard (0 ppm). Spectra were recorded at room temperature on the δ-scale.

TLC analysis used Silufol UV-254 chromatographic plates with detection by I_2 and NH_3 vapor, a UV lamp at 254 and 365 nm, and vanillin solution (1%) in H_2SO_4 (conc.).

Plant Material. The species was determined by Cand. Biol. Sci. I. I. Mal′tsev of the Botanical Institute, Acad. Sci. Rep. Uzbekistan, by comparison of the collected samples with voucher herbarium specimens of *A. baldshuanica* stored in the Central Uzbekistan Herbarium (combined herbariums of Tashkent State University and the Botanical Inst., Acad. Sci. Rep. Uzb.).

GC–MS Analysis of Benzene-Extract Constituents. The air-dried epigeal part of *A. baldshuanica* was extracted with benzene at room temperature (1:10 material:extractant ratio), left overnight, and filtered. The resulting extract was analyzed by GC–MS. Mass spectra were recorded in an Agilent Technologist 5973 INERT GC–MS (HP ChemStation data processing system). Constituents were separated over a quartz capillary column (30 m × 0.25 mm) with grafted stationary phase 5% phenylmethyltrisiloxane at 75–280°C with heating rate 5°C/min. Constituents were identified by mass spectra and retention times using the Wiley GC/MS library. Table 1 presents the GC–MS data for constituents of the benzene extract.

Extraction and Isolation of Melampolide 2. The epigeal part of *A. baldshuanica* (2 kg) was extracted with alcohol (4×, 90%) by phase contact for 12 h. The combined extracts were condensed in a rotary evaporator to a volume of 1.5 L. The extract was worked up with hot water (0.5 L) and left overnight. The resulting extract was filtered from the precipitate of ballast substances. The filtrate was condensed in a rotary evaporator to a thick resin. The resin was mixed with KSK silica gel (1:1 w/w ratio) and placed on a column with a small amount of KSK silica gel and then separated by benzine, $CHCl_3$, EtOAc, and alcohol. The resulting extracts were evaporated.

The total $CHCl_3$ fraction (24.63 g) was placed on a column with KSK silica gel (1:10 fraction:silica gel ratio) and eluted by extraction benzine and a benzine:$CHCl_3$ mixture. Fractions of 100 mL (total 76 fractions) were collected.

Fractions 42–49 were combined and chromatographed again. Elution by benzine:$CHCl_3$ (1:3) isolated melampolide **2** as transparent crystals, mp 178–179°C, $C_{15}H_{20}O_4$. IR spectrum (film): 3388 (OH), 3002, 2975, 2943, 2858, 2762 (CHO), 1764 (γ-lactone C=O), 1669 (C=C), 1619, 1448, 1372, 1340, 1294, 1245 (C=C), 1221, 1160, 1131, 1077, 1046, 1049, 1019, 983, 955, 923, 873, 798, 756, 723, 599, 511. Table 2 lists the PMR and ^{13}C NMR data.

X-ray Crystal Structure Analysis. Crystals of **2** were obtained from EtOH solution by slow evaporation at room temperature. Single crystals were transparent and elongated prisms with $a = 7.406(1)$, $b = 13.536(3)$, $c = 14.056(3)$ Å, $V = 1409.1(5)$ Å^3, $\rho_{calcd} = 1.246$ g/cm^3, space group $P2_12_12_1$, $Z = 4$. Unit-cell constants were determined and refined using a crystal of size 0.8 × 0.2 × 0.5 mm on a CCD Xcalibur Ruby diffractometer (Oxford Diffraction) and Cu Kα-radiation (300 K, graphite monochromator). A three-dimensional dataset of reflections was obtained on the same diffractometer (scan range $4.5° \geq \theta \geq 60.0°$). Absorption was calculated semi-empirically using the SADABS program [13].

The structure was solved by direct methods using the SHELXS-97 program set. The structure was refined using the SHELXL-97 program [14]. All nonhydrogen atoms were refined by anisotropic full-matrix least-squares methods (over F^2). Coordinates of hydroxyl H atoms were found experimentally. Coordinates of the other H atoms were found geometrically and refined with fixed isotropic thermal factors $U_{iso} = nU_{eq}$, where n = 1.2 and U_{eq} is the equivalent isotropic thermal factor of the corresponding C atoms. The final agreement factor (R) over 1171 reflections [$I > 2\sigma(I)$] was 0.0393 (wR2 = 0.1067); over 1232 reflections, 0.0412 (wR2 = 0.1093). Data from the XSA were deposited in the Cambridge Crystallographic Data Centre (CCDC 818746).

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