

Highly oxygenated sesquiterpenes in *Artemisia alba* Turra



Milka Todorova^a, Antoaneta Trendafilova^{a,*}, Kalina Danova^a, Luke Simmons^c, Evelyn Wolfram^b, Beat Meier^b, Rainer Riedl^c, Luba Evstatieva^d

^a Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

^b Zurich University of Applied Sciences, Institute of Biotechnology, Phytopharmacy, CH-8820 Wädenswil, Switzerland

^c Zurich University of Applied Sciences, Institute of Chemistry and Biological Chemistry, Center of Organic and Medicinal Chemistry, CH-8820 Wädenswil, Switzerland

^d Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

ARTICLE INFO

Article history:

Received 19 August 2014

Received in revised form 14 November 2014

Available online 23 December 2014

Keywords:

Artemisia alba

Asteraceae

Sesquiterpenes

Germacranes

Eudesmane

Guaiane

Oplopane

ABSTRACT

Ten new sesquiterpene alcohols of which seven germacranes, a eudesmane, a guaiane and an oplopane were isolated from the aerial parts of *Artemisia alba* Turra. Their structures and relative stereochemistry were elucidated by spectral methods (¹H and ¹³C NMR, COSY, HSQC, HMBC, NOESY, and MS). In addition, the known 7-hydroxycadin-4-en-3-one, centaureidin and axillarin were found for the first time in the studied species.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Many species of the *Artemisia* genus have been widely used for their curative properties in traditional medicine (Abad Martínez et al., 2012). Research has confirmed the antimalarial, cytotoxic, antihepatotoxic, antibacterial, antifungal and antioxidant activities of many representatives of the genus (Tan et al., 1998; Bora and Sharma, 2011).

However, the high number of species (over 500) and moreover their morphological resemblance, make this genus a taxonomically complex one (Oberprieler et al., 2007; Maggio et al., 2012). Numerous phytochemical studies have been conducted on different *Artemisia* species leading to the isolation and characterization of over 260 chemical compounds (Abad Martínez et al., 2012). Terpenoids, sterols, flavonoids, sesquiterpene lactones and coumarins have been isolated with particular attention devoted to the sesquiterpene lactones and essential oils (Abad Martínez et al., 2012; Teixeira Da Silva et al., 2004).

Artemisia alba Turra (synonyms – *A. lobelii* All., *A. biasoletiana* Vis., *A. suavis* Jord., *A. incanescens* Jord., *A. camphorata* Vill.) is a widespread species in the southern parts of Europe (Tutin et al., 1976). Its aerial parts have been traditionally utilized as a stomach

digestive and tonic in the form of a decoction (Rigat et al., 2007). Studies have mainly been focused on the essential oil composition (Radulović and Blagojević, 2010; Maggio et al., 2012; Dordević et al., 2013). Few reports are available concerning the non-volatile components of this species: santonin (Janot and Gautier, 1935), nerolidol derivatives (Appendino et al., 1985), hydroxydavanone, artalbic, artemiric and (6S*,7S*,10R*)-6,10-dimethyl-7,10-epoxycoc-ta-11-enoic acids, coumarins and flavonoids from the aerial parts (Maggio et al., 2011, 2013; Dordević et al., 2013), and a sesquiterpene-coumarin ether from the roots (Greger et al., 1982). In addition, three reports considering its synonyms *A. lobelii*, *A. biasoletiana* and *A. incanescens* described the isolation of davanone type sesquiterpenes (Tešević et al., 2004), flavonoids and coumarins (Barberá et al., 1986; Marco et al., 1987; Tešević et al., 2004).

The aim of the present work was to study terpenoid constituents of this species.

2. Results and discussion

The air dried aerial parts of *A. alba* were initially defatted with hexane and exhaustively extracted with CHCl₃. The chloroform extract was then subjected to Sephadex LH-20 and silica gel column chromatography (CC), as well as to procedures for purification (CC and prep. TLC) yielding 13 compounds. Ten new sesquiterpenoids **1–10** (Fig. 1) in addition to the known

* Corresponding author. Tel.: +359 29606144.

E-mail address: trendaf@orgchm.bas.bg (A. Trendafilova).

7-hydroxycadin-4-en-3-one (**11**) (Marco et al., 1996), and the flavonoids centaureidin (Long et al., 2003) and axillarin (Barberá et al., 1986) were identified.

The HRESIMS of compound **1** indicated a quasi-molecular ion peak at m/z 275.1614 $[M+Na]^+$ corresponding to a molecular formula $C_{15}H_{24}O_3$ and 4 degrees of unsaturation. Three of them were accounted for two exomethylene double bonds (δ_C 111.8/156.0 and δ_C 114.9/146.1) and one trisubstituted double bond (δ_C 131.4/134.7, δ_H 5.09) deduced from the NMR spectra (Table 1). The residual carbon resonances in the ^{13}C NMR spectrum were classified as four methylenes (δ_C 30.1, 32.5, 36.0 and 42.5), two oxymethines (δ_C 70.5 and 76.1), one methine (δ_C 33.6), one hydroxymethyl (δ_C 65.7) and one olefinic methyl (δ_C 17.1), respectively. The remaining degree of unsaturation suggested a monocyclic ring system of compound **1**. Further, the COSY, HSQC and HMBC experiments allowed determining of **1** as a germacra-1(10),4(15),11(13)-triene (δ_H 5.09, H-1, 4.97/5.10, H-13/13' and 5.16/5.31, H-15/15') containing hydroxyl groups at C-2 (δ_H 4.54), C-5 (δ_H 4.25) and C-12 (δ_H 4.07). The NOESY experiment did not allow exact determination of the relative stereochemistry at the chiral centers due to the absence of substituents at the carbons next to C-7, as well as the overlapping of the H-6, H-7 and H-9 (δ_H 2.09–2.11) and H-1 and H-13 (δ_H 5.09–5.10) signals. The cross peaks between H-5 and H-3 (δ_H 1.76) on one hand, and H-2/H-3 (δ_H 2.76), H-2/H-14, and H-2/H-15 on the other, gave an information only on their *syn* α - or *syn* β -orientations, respectively (Fig. 2). The stereochemistry was deduced as follows. The coupling of H-2 with one equatorial ($J_{2,3eq} = 4.0$ Hz) and two axial protons ($J_{2,3ax} = 11.6$ Hz, $J_{1,2} = 10.1$ Hz) showed the equatorial orientation of the C-2 hydroxyl group. The *trans* endocyclic C-1/C-10 double bond was shown by the H-2/H-14 NOE correlation as well as by the *anti* disposition of H-1 and H-2. Further, the NOE interaction H-5/H-3 (δ_H 1.76) indicated that these protons were *syn* diaxial, i.e. the C-5 OH group was equatorial. The side chain at C-7 was placed in β -position on biogenetic ground. Unfortunately, it was impossible to distinguish the two stereoisomers with $2\beta,5\alpha$ or $2\alpha,5\beta$ hydroxyl groups. The comparison of the spectral data of **1** with literature data of

tanacetol B, isolated from *Tanacetum vulgare* (Appendino et al., 1983) showed that both compounds differed in their C-7 side chain and C-2 substituent only. Thus, the conformation of the 10-membered ring and the configuration at the corresponding chiral centers in **1** were accepted to be the same as these of tanacetol B. The stereochemistry of the latter was proved by X-ray of tanacetol-B-monoacetate (Gallery et al., 1983). Therefore, **1** was assumed to be $2\beta,5\alpha,12$ -trihydroxygermacra-1(10),4(15),11(13)-triene.

The structure of compound **2** was established by comparison of its 1H and ^{13}C NMR data (Table 1) with those of **1**. The upfield chemical shifts of the H-14, H-1 and H-2 signals in the 1H NMR spectrum indicated that **2** differed from **1** in the presence of oxygen containing functions at C-1 and C-10. The signals at $\delta_{H/C}$ 2.74/70.3 and $\delta_{H/C}$ 1.33/61.4 confirmed the presence of an C-1/C-10 epoxy ring. Further, an acetyl group (δ_H 2.11 s and δ_C 171.4 and 21.0) was established at C-12 by the downfield shift of the C-12 protons (δ_H 4.60 and 4.68). The downfield chemical shifts of the H-5 and C-5 signals (δ_H 4.56 brs and δ_C 87.6) and one proton singlet at δ_H 8.32 confirmed the presence of a hydroperoxy group at this position in **2**. The remaining signals were assigned by the COSY, HSQC and HMBC experiments. The established structure of **2** was in agreement with a molecular formula $C_{17}H_{26}O_6$, deduced from the HRESIMS (a quasi-molecular ion peak at m/z 349.1632 $[M+Na]^+$). The relative stereochemistry at the chiral centres in **2** was deduced from the coupling constants of the corresponding proton signals as well as from the NOESY experiments and the Dreding model (Fig. 2). Thus, the α -oriented H-7 (assumed biogenetically) correlated with H-14 and H-15 (δ_H 5.25), the H-2 with H-14 and H-15 (δ_H 5.32), i.e. they were also α -oriented. The large vicinal coupling constant $J_{1,2} = 10.2$ Hz supported the *trans* diaxial disposition of H-1 and H-2. Further, the observed H-1/H-3 (δ_H 1.97), H-1/H-6 (δ_H 1.89), and H-5/H-3 (δ_H 1.97) NOE correlations indicated their β -disposition. Thus, **2** was identified as 12-acetoxy-1 α ,10 β -epoxy-5 α -hydroperoxy-2 β -hydroxygermacra-4(15),11(13)-diene.

The HRESIMS of compound **3** indicated a quasi-molecular ion peak at m/z 269.1751 $[M+H]^+$, corresponding to the molecular formula $C_{15}H_{24}O_4$, i.e. one oxygen atom more than in **1**. The 1H and

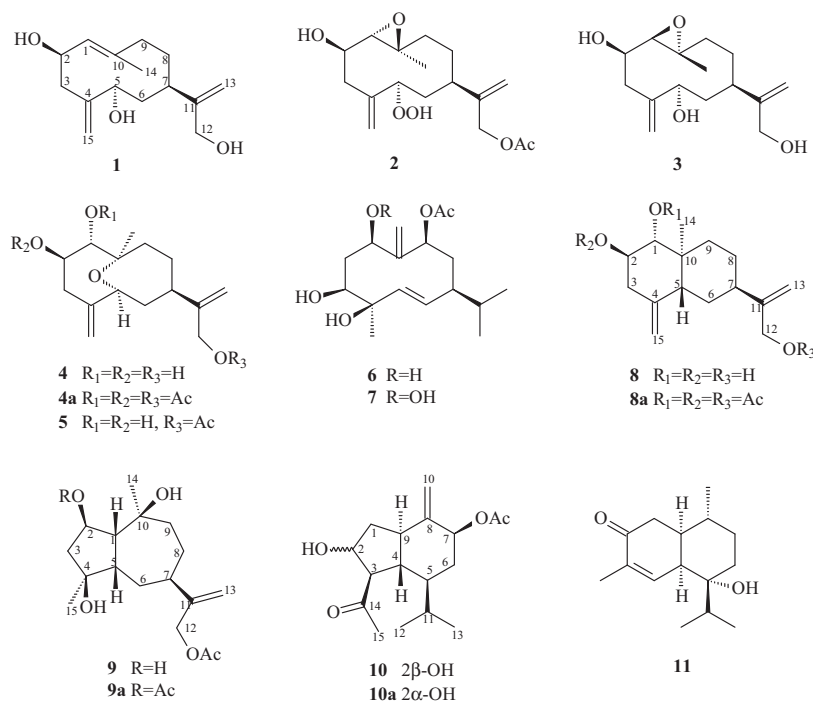


Fig. 1. Structures of compounds **1–11**.

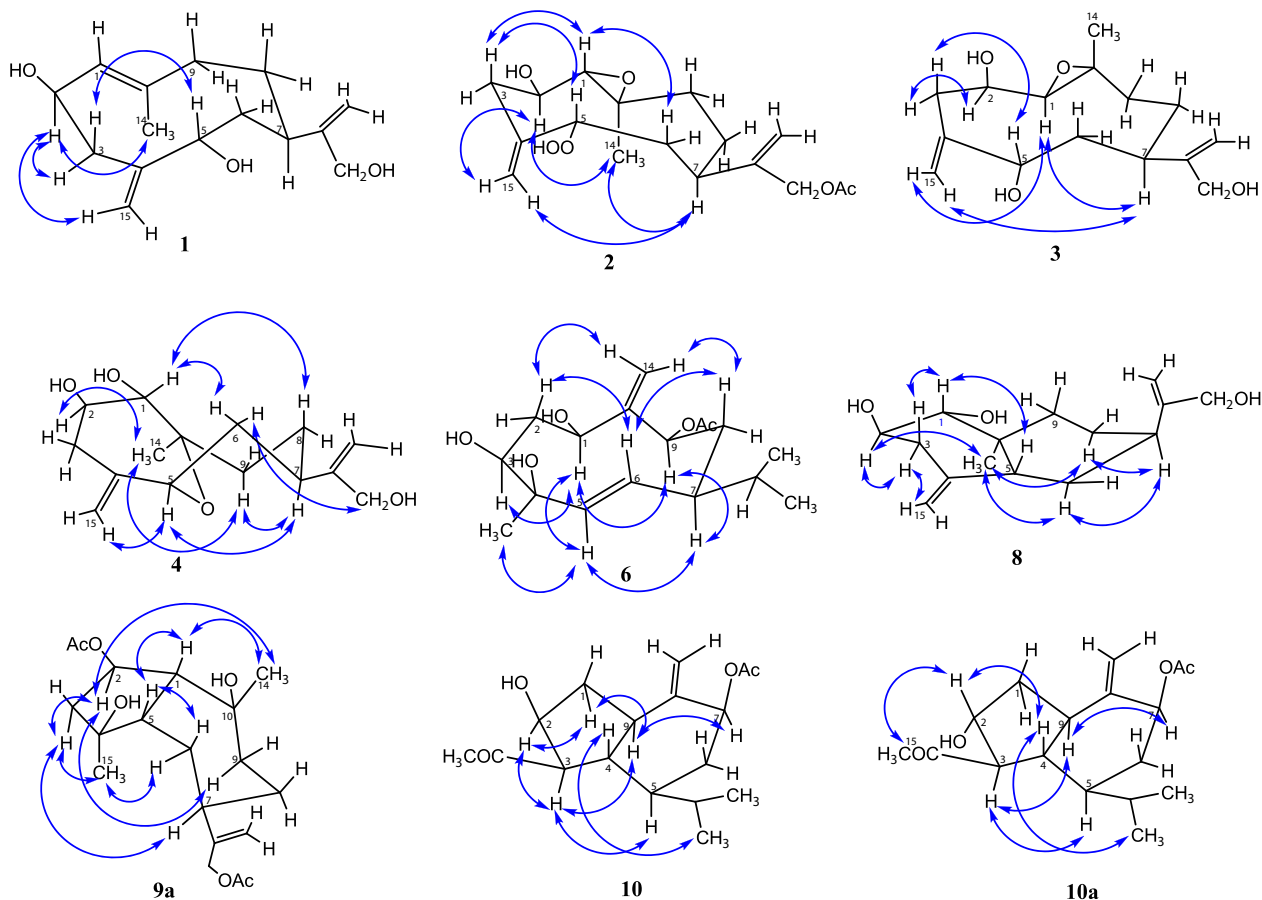


Fig. 2. Important NOE correlations in the structures of compounds **1–4**, **6**, **8**, **9a**, **10** and **10a**.

^{13}C NMR data of **3** (Table 1) were very similar to those of **1** with exception of the C-1/C-10 double bond signals which were replaced by epoxy ring signals (δ_{H} 3.20, H-1 and δ_{C} 63.9/60.2, C-1/C-10). Hence, **3** was 1,10-epoxy-2,5,12-trihydroxygermacra-4(15),11(13)-diene. On the other hand, the comparison of the spectral data of **3** with those of **2** showed some differences in the chemical shifts and coupling constants of the H-1 and H-2 signals. Thus, the H-1 and H-2 signals in **3** were downfield shifted at δ_{H} 3.20 and 4.68, respectively. The observed vicinal coupling constants ($J_{1,2} = 3.7$ Hz, $J_{2,3\text{eq}} = 6.6$ Hz and $J_{2,3\text{ax}} = 11.0$ Hz) indicated different stereochemistry at the C-1 and/or C-2 chiral centers. The NOESY experiment (Fig. 2) exhibited H-7/H-1, H-7/H-15 (δ_{H} 5.42), H-1/H-15 (δ_{H} 5.24) correlations indicating their *syn* α -orientation. The small coupling $J_{1,2} = 3.7$ Hz showed α -axial/ α -equatorial orientation of the corresponding H-1 and H-2 unlike the same protons in **2**. The cross NOE peaks of H-2/H-3 (δ_{H} 2.90) and H-3 (δ_{H} 1.76)/H-5 indicated the β -orientation of H-5. Thus, compound **3** was identified as 1 β ,10 α -epoxy-2 β ,5 α ,12-trihydroxygermacra-4(15),11(13)-diene.

Compounds **4** and **5** can be considered as a pair of compounds with similar structures based on their NMR spectra (Table 2). Compound **4** has the same molecular formula ($\text{C}_{15}\text{H}_{24}\text{O}_4$, $[\text{M}+\text{Na}]^+$, 291.1572) and ^1H NMR spectrum similar to that for the above discussed germacrane **3**. It contained signals for two exomethylene groups (δ_{H} 4.77 s and 4.88 s, H-15 and H-15'; δ_{H} 4.89 s and 5.00 s, H-13 and H-13'), one hydroxymethyl group (δ_{H} 4.07 brs, 2H, H-12), and a methyl group (δ_{H} 1.15 s) attached to a carbon bearing an oxygen function (δ_{C} 81.6). The ^1H NMR spectrum exhibited signals for three protons (δ_{H} 3.77, 3.81 and 4.45, H-1, H-2 and H-5, respectively) linked to oxygenated carbon atoms (δ_{C} 75.4, 72.4

and 78.6, C-1, C-2 and C-5, respectively) which positions were determined by COSY and HMBC experiments. Further, acetylation of **4** afforded compound **4a**, the ^1H NMR spectrum (Table 2) of which contained signals for 3 acetoxy groups. Their place at C-1, C-2, and C-12 was confirmed by a downfield shift of the corresponding signals (δ_{H} 5.35, 5.19, and 4.58) as compared with those in the spectrum of **4**. The molecular formula $\text{C}_{15}\text{H}_{24}\text{O}_4$ of **4**, deduced from the HRESIMS corresponded to 4 degrees of unsaturation – two double bonds, a cyclodecane ring and an ether bridge. The C-5–C-10 connectivity of the latter was confirmed by the HMBC and the chemical shifts of the corresponding proton and carbon signals. The observed NOE correlations between H-7/H-9 (δ_{H} 1.37), H-7/H-5, H-9 (δ_{H} 1.37)/H-14 and H-14/H-2 (Fig. 2) revealed their α -orientation. The coupling constant $J_{1,2} = 8.7$ Hz showed the *trans* disposition of H-1 and H-2. All these data and the inspection of the Dreiding model showed that the ether bridge is situated below the plane of the molecule, the C-2 hydroxyl function is β -equatorial, while the C-1 hydroxyl group is α -equatorial. Compound **4** was identified as 5 α ,10 α -epoxy-1 α ,2 β ,12-trihydroxygermacra-4(15),11(13)-diene.

Compound **5** was found to be a 12-acetoxy derivative of **4**. A three proton singlet at δ_{H} 2.10 together with signals at δ_{C} 170.7 and 20.8 showed the presence of an acetyl group. The downfield shift of H-12 signal at δ_{H} 4.58 similarly to the corresponding signal in **4a** showed that C-12 OH group was acetylated in compound **5**. The presented structure was supported by the HRESIMS ($\text{C}_{17}\text{H}_{26}\text{O}_5$, $[\text{M}+\text{Na}]^+$ 333.1689), ^1H and ^{13}C NMR, COSY, HSQC and HMBC. The relative stereochemistry of **5** was assumed to be like that of **4**, based on the same coupling constants observed for the corresponding protons and NOESY experiment. Accordingly, **5**

Table 1
¹H and ¹³C NMR data, and HMBC correlations of **1–3** in CDCl₃.

Position	1			2			3		
	δ_{H} mult. (J Hz)	δ_{C}	HMBC ^a	δ_{H} mult. (J Hz)	δ_{C}	HMBC ^a	δ_{H} mult. (J Hz)	δ_{C}	HMBC ^a
1	5.09 m*	131.4		2.74 d (10.2)	70.3	2, 3, 9	3.20 d (3.7)	63.9	2, 9
2	4.54 ddd (4.0, 10.1, 11.6)	70.5	3, 10	3.61 ddd (4.5, 10.2, 13.0)	70.5		4.68 ddd (3.7, 6.6, 11.0)	67.1	1, 3, 10
3	1.76 brt (11.6)	42.5	2, 4	2.91 dd (4.5, 13.0)	39.2	2, 5	2.90 dd (6.6, 14.0)	34.7	
	2.76 brdd (4.0, 11.6)		2, 4, 5	1.97 brt (13.0)		2, 5	1.76 ddd (1.6, 11.0, 14.0)		2, 4, 5
4	–	146.1		–	140.5		–	144.5	
5	4.25 brs ($w_{1/2}$ 9.4)	76.1	4, 7	4.56 brs ($w_{1/2}$ 9.5)	87.6		4.30 brd (5.6)	74.5	
6	1.91 ddd (5.0, 9.4, 14.2)	32.5	4, 5, 7, 11	1.89 dt (15.0, 2.3)	29.3	11	2.05 brd (15.1)	34.4	7, 11
	2.09 m*		5, 11	2.24 ddd (5.2, 9.5, 15.0)		4, 5, 7, 11	2.31 ddd (5.6, 10.0, 15.1)		5, 7, 11
7	2.11 m*	33.6	5, 6, 11, 12, 13	2.43 m	33.3		2.19 brt (10.0)	34.8	6, 8, 9, 12
8	1.47 m*	30.1	7, 10	1.59–1.70 m (2H)	30.8	7	1.52 dddd (4.9, 4.0, 10.0, 14.6)	34.6	7, 9, 10, 11
	1.51 m*		7				1.64 m		
9	2.11 m*	36.0		1.30 m	34.6		1.08 td (13.4, 4.9)	41.2	1, 7, 8, 10, 14
	2.20 m		10, 14	2.06 ddd (3.2, 7.0, 14.0)			2.07 ddd (3.5, 4.0, 13.4)		1, 7, 8, 10
10	–	134.7		–	61.4		–	60.2	
11	–	156.0		–	150.2		–	157.0	
12	4.07 brs (2H)	65.7	7, 11, 13	4.68 d (14.0)	66.0	11, 13, CO	4.13 brs (2H)	66.4	7, 11, 13
				4.60 d (14.0)		11, 13, CO			
13	4.97 brs	111.8	7, 11, 12	5.06 s	111.4	7, 12	4.99 s	110.6	7, 11, 12
	5.10 brs*		7, 11, 12	5.11 s		7, 11, 12	5.08 s		7, 11, 12
14	1.67 s	17.1	1, 10	1.33s	18.6	1, 9, 10	1.59 s	16.1	1, 9, 10
15	5.16 s	114.9	3, 4, 5	5.25 s	117.3	3, 5	5.24 brs	112.0	3, 5
	5.31 s		3, 4, 5	5.32 s		3, 5	5.42 brs		
OAc				2.11 s	21.0	CO (Ac)			
					171.4				
OOH				8.32 s					

^a Long-range ¹H–¹³C HMBC correlations are from the stated hydrogen(s) to the indicated carbon.

* Overlapped signals.

Table 2¹H and ¹³C NMR data, and HMBC correlations of **4**, **4a** and **5**.

Position	4 (CD ₃ OD)			4a (CDCl ₃)			5 (CDCl ₃)		
	δ_{H} mult. (J Hz)	δ_{C}	HMBC ^a	δ_{H} mult. (J Hz)	δ_{C}		δ_{H} mult. (J Hz)	δ_{C}	HMBC ^a
1	3.77 d (8.7)	75.4	2, 3, 10, 14	5.35 d (8.5)	73.2		3.77 d (8.5)	75.6	3, 10, 14
2	3.81 dt (8.7, 4.9)	72.4	1, 4, 10	5.19 dt (8.5, 4.3)	72.7		3.90 dt (8.5, 4.9)	71.6	4
3	2.85 dd (4.9, 14.0)	40.5	1, 2, 4, 5, 15	2.93 dd (4.3, 14.2)	36.5		2.94 dd (4.9, 14.0)	39.7	
	2.26 dd (4.9, 14.0)		1, 2, 4, 5, 15	2.37 dd (4.3, 14.2)			2.30 dd [*] (4.9, 14.0)		4
4	–	151.7		–	148.9		–	149.6	
5	4.45 dd (8.5, 10.1)	78.6	4, 6, 10	4.55 dd (8.5, 9.7)	76.5		4.47 brdd (8.5, 10.2)	77.4	4
6	2.29 brdd (8.5, 14.3)	44.9	7, 8, 11	2.38 brdd (8.5, 14.2)	42.5		2.32 m [*]	42.9	4
	1.88 ddd (10.7, 10.1, 14.3)		4, 5, 7, 8	1.99 ddd (9.7, 10.7, 14.2)			1.80 m [*]		4, 5, 7, 8
7	2.40 brt (10.7)	39.7 [*]	5, 6, 8, 11, 12, 13	2.47 brt (10.7)	37.7		2.45 brt (11.0)	38.6	11
8	1.77 m	33.6	6, 7, 9, 10, 11	1.77 [*] m	31.9		1.85 m [*]	32.2	
	1.71 m		6, 7, 9, 10	1.71 dddd (2.7, 8.7, 10.7, 14.1)			1.62 m		
9	1.37 ddd (4.4, 12.4, 14.4)	39.7 [*]	1, 7, 8, 10, 14	1.45 ddd (8.7, 10.1, 14.5)	36.9		1.47 ddd (4.3, 12.8, 14.4)	37.8	
	2.10 dt (14.4, 4.4)		7, 8, 10, 14	1.77 [*] m			2.07 dt (14.4, 4.0)		
10	–	81.6		–	79.0		–	79.3	
11	–	156.8		–	149.3		–	149.5	
12	4.07 brs (2H)	65.2	7, 11, 13,	4.58 brs (2H)	66.1		4.58 brs (2H)	66.1	7, 11, 13, CO (Ac)
13	4.89 s	107.8	7, 11, 12	5.00 s	111.0		4.97 s	110.8	7, 12
	5.00 s		7, 11, 12	5.01 s			5.01 s		7, 11, 12
14	1.15 s	24.7	1, 9, 10	1.26 s	24.8		1.21 s	24.2	1, 9, 10
15	4.77 s	111.4	3, 4, 5	4.85 s	112.1		4.81 s	111.5	3, 5
	4.88 s		3, 4, 5	4.90 s			4.90 s		3
OA				2.03 s	20.9		2.10 s	20.8	CO (Ac)
					170.9			170.7	
				2.05 s	21.0				
					170.2				
				2.11 s	21.1				
					169.6				

^a Long-range ¹H–¹³C HMBC correlations for compounds **4** and **5** are from the stated hydrogen(s) to the indicated carbon.^{*} Overlapped signals.

was determined as 12-acetoxy-5 α ,10 α -epoxy-1 α ,2 β -dihydroxygermacra-4(15),11(13)-diene.

The HRESIMS of compound **6** showed a quasi-molecular ion peak [M+Na]⁺ at *m/z* 335.1824, corresponding to a molecular formula C₁₇H₂₈O₅. The ¹H and ¹³C NMR data of **6** (Table 3) revealed the presence of an exomethylene group (δ_{H} 5.33 and 5.45), a disubstituted double bond (δ_{H} 5.27 d and 5.30 dd, δ_{C} 133.6 and 131.8), a methyl group attached to a carbon bearing an oxygen function (δ_{H} 1.34 s), an isopropyl group (2 CH₃ at δ_{H} 0.85 d, and 0.88 d) and an acetoxy residue (δ_{H} 2.02 s, δ_{C} 172.4) linked to a secondary carbon atom (δ_{H} 4.45 brd, δ_{C} 79.9). In addition, signals for two carbinolic protons (δ_{H} 3.86 brs and 3.70 brd) and two oxygen bearing carbon atoms (δ_{C} 73.6 and 77.4) were registered. The positions of the described functional groups were assigned using COSY, HSQC, and HMBC correlations. All these data revealed a germacra-5,10(14)-diene structure of **6** with hydroxyl groups at C-1, C-3, C-4 and an acetoxy residue at C-9. The large coupling constant (15.3 Hz) between the endocyclic olefinic protons H-5 and H-6 indicated a *trans* C-5/C-6 double bond. Unfortunately, the determination of the relative stereochemistry from the NOESY experiment was questionable because of the overlapping of the H-5 and H-6 signals in the spectra recorded in CDCl₃. Better separation of the two signals was achieved when C₆D₆ was used as a solvent. Then, the observed correlations H-7/H-9, H-9/H-1, H-1/H-3, H-1/H-5, H-5/H-15 in the NOESY spectrum (Fig. 2) indicated their *syn* α -orientation in a “crown” type conformation of the 10-membered ring, hence β -configuration of all substituents and the isopropyl group. In addition, the NOEs between H-2 (δ 2.56) and H-6, H-2 (δ 2.56) and H-14 (δ 5.45), H-8 (δ 1.73) and H-6, H-8 (δ 1.73) and H-14 (δ 5.33) confirmed the proposed relative stereochemistry of **6**. From the above data compound **6** was identified as 9 β -acetoxy-1 β ,3 β ,4 β -trihydroxygermacra-5,10(14)-diene.

According to the HRESIMS the structure of **7** ([M+Na]⁺ 351.1769, C₁₇H₂₈O₆) contained one more oxygen atom than **6**. The ¹H and ¹³C NMR data of **7** (Table 3) were very similar to those

of **6**. However, the downfield shift of H-1 at δ_{H} 4.17 and C-1 at δ_{C} 86.6 ppm indicated the presence of a hydroperoxy group in **7** (instead of OH as it was determined in **6**). This was confirmed by a chemical transformation of **7** to **6** with the use of Ph₃P. Therefore, compound **7** was identified as 9 β -acetoxy-1 β -hydroperoxy-3 β ,4 β -dihydroxygermacra-5,10(14)-diene. All spectral data (COSY, HSQC, HMBC and NOESY) were in agreement with the proposed structure of **7**.

Compound **8** gave a quasi-molecular ion peak by the HRESIMS at *m/z* 275.1625 [M+Na]⁺ consistent with a molecular formula of C₁₅H₂₄O₃ and indicative for 4 degrees of unsaturation. Two of them were attributed to two exomethylene double bonds (δ_{C} 149.8/109.6 and δ_{C} 147.1/107.3) deduced from the signals in the ¹³C NMR and HSQC spectra of **8** (Table 3). The residual carbon resonances in the ¹³C NMR spectrum were also classified to be one quaternary carbon (δ_{C} 39.3), two methines (δ_{C} 34.2 and 41.8), two oxygenated methines (δ_{C} 70.8 and 83.6), four methylenes (δ_{C} 22.0, 24.7, 32.2 and 42.8), one methyl (δ_{C} 9.7), and one hydroxymethyl (δ_{C} 64.2), respectively. Thus, the remaining two degrees of unsaturation suggested a bicyclic ring system in **8**. The three-proton singlet for a methyl group at δ_{H} 0.73 and one-proton doublet at δ_{H} 1.93 (H-5) supported a eudesmane structure. The observed couplings between the signals for carbinolic protons at δ_{H} 2.99 and 3.49, allylic methylene protons at δ_{H} 2.00 and 2.55 and exomethylene protons at δ_{H} 4.59 and 4.83 in the COSY spectrum supported the C₁–C₂–C₃–C₄–C₁₅ connectivity in the structure of compound **8**. On the other hand, cross peaks in the COSY spectrum between the olefinic proton signals at δ_{H} 5.00 and 5.24 with the signals at δ_{H} 2.62 and 4.03 confirmed the presence of a hydroxyisopropenyl function C₇–C₁₁(C₁₂)–C₁₃. The two methylene groups at C-6 and C-8 adjacent to H-7 were assigned also from the COSY correlations. The acetylation of **8** to **8a** confirmed the presence of three hydroxyl groups at C-1, C-2 and C-12. The carbinolic protons were downfield shifted and signals for three acetyl groups (δ_{H} 1.99, 2.05 and 2.07/ δ_{C} 170.5, 170.7, 170.8) were observed in the NMR

Table 3
¹H and ¹³C NMR data, of **6–8** and **8a** and HMBC correlations of **6–8**.

Position	6 (CDCl ₃ [C ₆ D ₆])			7 (CDCl ₃)			8 (CD ₃ OD)			8a (CDCl ₃)	
	δ_{H} mult. (J Hz)	δ_{C}	HMBC ^a	δ_{H} mult. (J Hz)	δ_{C}	HMBC ^a	δ_{H} mult. (J Hz)	δ_{C}	HMBC ^a	δ_{H} mult. (J Hz)	δ_{C}
1	3.86 [3.78] brs	73.6	2, 3, 9, 10, 14	4.17 dt (5.6, 1.8)	86.6	2, 3, 9, 10, 14	2.99 d (9.3)	83.6	2, 3, 9, 10, 14	4.87 d (10.0)	80.6
2	1.80 [2.01] dt (14.8, 2.5)	40.8	3	1.70 ddd (1.8, 5.6, 15.0)	37.7	1, 3, 4	3.49 ddd (5.6, 9.3, 12.2)	70.8	1	4.96 ddd (5.9, 10.0, 12.2)	71.6
	2.48 [2.56] ddd (4.1, 10.8, 14.8)		1, 3, 4, 10	2.27 ddd (1.8, 11.0, 15.0)		3, 4, 10					
3	3.70 [3.50] brd (10.8)	77.4	4	3.74 dd (1.8, 11.0)	76.5	1	2.55dd (5.6, 12.2) 2.00 brt (12.2)	42.8	1, 2, 4, 5, 15 1, 2, 4, 5, 15	2.72dd (5.9, 12.2) 2.15 brt (12.2)	39.6
4	–	74.3		–	74.2		–	147.1		–	144.1
5	5.27 [4.80] d (15.3)	133.6	4, 6, 7	5.36 d (15.3)	135.1	4, 6, 7	1.93 brd (12.5)	41.8	4, 6, 10, 14	2.05 [*]	41.5
6	5.30 [5.37] dd (10.4, 15.3)	131.8	4, 5, 7	5.38 dd (9.5, 15.3)	131.1	4, 7	1.67 ddd (5.5, 12.5, 13.4) 1.86 brd ⁺ (13.4)	24.7	5, 7, 10, 11	1.64 ddd (5.4, 12.4, 13.4) 1.83 m ⁺	24.8
7	1.88 [*] [1.56] m	48.8	6, 9	2.00 m	48.1		2.62 brs	34.2		2.59 brs	34.6
8	2.12 [1.94] ddd (2.3, 3.3, 12.0)	41.5	6, 7, 9, 10	2.11 ddd (2.2, 3.0, 12.0)	43.0	6, 7, 9, 10	1.77 dddd (4.2, 5.6, 12.6, 13.4)	22.0	7, 9, 10, 11	1.70 tt (13.5, 4.9) 1.83 m ⁺	22.0
9	1.87 [*] [1.73] q (12.0) 4.45 [4.37] brd (12.0)	79.9	6, 7, 9, 10 1, 7, 8, 10, 14, CO (Ac)	1.78 q (12.0) 4.75 dd (2.2, 12.0)	79.6	6, 7, 9, 10 1, 8, 10, 14, CO (Ac)	1.88 m ⁺ 1.36 td (13.4, 4.2) 1.71 dt (13.4, 5.6)	32.2	1, 7, 8, 10, 14 5, 7, 8, 10, 14	1.35 dt (13.4, 3.5) 1.42 td (13.4, 3.5)	31.6
10	–	158.2		–	153.4		–	39.3		–	39.7
11	1.55 [1.34] dqq (6.7, 6.7, 6.7)	32.2	6, 7, 8, 13	1.57 dqq (5.6, 6.8, 6.8)	32.7	6, 7, 8, 12, 13	–	149.8		–	144.4
12	0.85 [0.77] d (6.7)	19.7	7, 11, 13	0.85 d (6.8)	19.1	7, 11, 13	4.03 s (2H)	64.2	7, 11, 13	4.52 s (2H)	66.8
13	0.88 [0.80] d (6.7)	20.5	7, 11, 12	0.88 d (6.8)	20.5	7, 11, 12	5.00 s 5.24 s 0.73 s	109.6	7, 12 7, 11, 12 1, 5, 9, 10	5.08 s 5.27 s 0.84 s	110.4
14	5.33[5.21] brs 5.45 [5.45] brs	116.1	1, 8, 9, 10 1, 8, 9, 10	5.42 brs 5.46 brs	117.6	1, 9, 10 1, 9, 10		9.7			11.6
15	1.34 [1.24] s	26.8	3, 4, 5	1.38 s	26.5	3, 4, 5	4.59 s 4.83 s	107.3	3 3	4.66 s 4.92 s 1.99 s	114.5
OAc	2.02 [1.54] s	21.5 172.4	CO (Ac)	2.08 s	21.6 171.8	CO (Ac)					21.1 170.5 21.0 170.7 20.9 170.8

^a Long-range ¹H–¹³C HMBC correlations are from the stated hydrogen(s) to the indicated carbon.

^{*} Overlapped signals.

spectra (Table 3). The relative stereochemistry of **8** was determined via NOESY experiments (Fig. 2). Assuming an α -configuration of H-7, as it is in all natural eudesmanes isolated from higher plants, the NOE correlations between H-7/H-8 (δ_{H} 1.77), H-8 (δ_{H} 1.77)/H-14, H-2/H-14 and H-2/H-3 (δ_{H} 2.55) indicated that these protons were α -oriented. The correlations between H-1/H-3 (δ_{H} 2.00) and H-1/H-5 suggested their β -disposition. Hence, **8** was confirmed as 1 α ,2 β ,12-trihydroxyeudesma-4(15),11(13)-diene.

Further, a sesquiterpene **9** bearing an acetoxy group (δ_{H} 2.06, δ_{C} 20.7 and 172.5) in the isopropenyl moiety (δ_{H} 4.56 s, H₂-12, δ_{H} 5.01 s and 5.03 s, H₂-13, δ_{C} 67.3, C-12 and 111.8, C-13) (Table 4) similarly to **2** and **5**, was isolated. According to the calculated 4 degrees of unsaturation (C₁₇H₂₈O₅, HRESIMS, [M+Na]⁺ 335.1823) this compound should be bicyclic. The data from COSY, HSQC and HMBC were in accordance with a compound of guaiane type containing two methyl groups (δ_{H} 1.19, s and 1.37, s) attached to oxygen bearing carbon atoms (δ_{C} 81.8 and 75.6, respectively) and a hydroxyl group at C-2 (δ_{H} 4.11 ddd, δ_{C} 75.4). Further, the H-2 coupled with C-3 methylene (δ_{H} 1.78 dd and 2.02 dd) and C-1 methine (δ_{H} 2.58 dd) protons. The latter interacted in addition only with H-5 at 2.32 ppm. The information from the NOESY spectrum recorded in CD₃OD was insufficient for the determination of the relative stereochemistry of **9**, because of the overlapping of some signals. Later on this compound was acetylated to **9a**. The downfield shift of H-2 and a signal for additional acetyl methyl group in the NMR spectrum in C₆D₆ (Table 4) confirmed the presence of a hydroxyl group at C-2. Assuming α -configuration of H-7, the cross peaks of H-7/H-3 (δ_{H} 2.04), H-3 (δ_{H} 2.04)/H-2, H-2/H-14, H-3 (δ_{H} 2.04)/H-15, and H-15/H-6 (δ_{H} 1.51) (Fig. 2) in the NOESY spectrum revealed their *syn* α -orientation. Further, the NOE interactions between H-6 (δ_{H} 1.85), which should be β -oriented and H-5, and H-5/H-1 established the β disposition of these protons as well as the *cis* fusion of 5- and 7-membered rings in the discussed compound. Besides the observed NOE between H-3 (δ_{H} 2.04) and H-7, the additional NOE interaction of H-2 and H-9 (δ_{H} 1.46) supported the presented in Fig. 2 non-planar conformation of the described structure. As can be seen, the plane of the 7-membered ring is almost perpendicular to the plane of the 5-membered ring. An analogous compound, guaidiol, has been previously isolated from *Curcuma zedoaria* [Syu et al., 1998] and its relative stereochemistry was proved by X-ray.

The molecular formula of compound **10** C₁₇H₂₆O₄ was deduced from HRESIMS [M+Na]⁺ 317.1729. According to the NMR spectra (Table 4) and comparison with literature data (Joseph-Nathan et al., 1989; Marco et al., 1993; Arciniegas et al., 2003) **10** was defined as an oplopane derivative. An isopropyl moiety (δ_{H} 0.67 d, 0.91 d and 1.43 m), an acetyl group (δ_{H} 2.26 and δ_{C} 209.6) and two oxygenated secondary carbons (δ_{H} 4.59 and 5.18, δ_{C} 73.2 and 73.3) at C-2 and C-7, respectively, were determined by COSY, HSQC and HMBC spectra. During NMR experiments in the spectrum of **10** new signals appeared. Detailed analysis of the spectral data of the mixture revealed the formation of compound (**10a**) with the same planar structure as that of **10**. The significant difference in the multiplicity of H-2 and H-3 is probably due to the change of the stereochemistry at C-2 and/or C-3. The NOESY experiment (Fig. 2) revealed C-4/C-9 *trans*-connection (H-4 β and H-9 α) and β -orientation of C-3, C-5 and C-7 substituents in both compounds (**10** and **10a**). Further, the β disposition of the C-2 hydroxyl group in **10** was assumed on the basis of the observed H-2/H-3 NOE interaction, while its α orientation in **10a** was confirmed by the cross peaks between H-2/H-4 and H-2/H-15 in the NOESY spectrum. Thus, **10a** was found to be a C-2 epimer of **10**. Recently, the oplopane **10a** was reported as a natural compound, isolated from *Artemisia gmelinii* [Zeng et al., 2014]. It is worth to note that oplopanes are quite rare and in the *Artemisia* genus they have been

reported as constituents of *A. sieberi* (Marco et al., 1993) and *A. gmelinii* (Zeng et al., 2014) only.

Artemisia is one of the biggest genera in the Asteraceae family. As discussed above, unlike many other *Artemisia* species characterized by the presence of sesquiterpene lactones, research conducted so far on the European taxa of *A. alba* has shown that this species is free of sesquiterpene lactones (Appendino et al., 1985; Tešević et al., 2004; Maggio et al., 2011, 2013; Dordević et al., 2013). The present work led to the isolation of compounds of the germacrene, guaiane, eudesmane, cadinane and oplopane skeletal types. As can be seen (Fig. 1), the identified components are biogenetically related and could be described as derivatives of germacrene A (**1–5**, **8** and **9**) and germacrene D (**6**, **7**, **10** and **11**). Although these skeletal types of sesquiterpenoids are not unusual for the *Artemisia* genus, compounds **1–10** have never been established up to now according to our knowledge. It should be noted that with exception of the isolated irregular artalbic and artemiric acids, acyclic nerolidol and davanone derivatives have been reported for this species so far (Appendino et al., 1985; Tešević et al., 2004; Maggio et al., 2011, 2013). The isolation of only cyclic sesquiterpenoids distinguished our sample from the previously studied ones.

3. Experimental

3.1. General experimental procedures

Optical rotations were determined on a Perkin Elmer 341 polarimeter. IR spectra were obtained on a Bruker Tensor 27 FT-IR spectrometer. ¹H and ¹³C NMR spectra were recorded on a Bruker Avance II+ 600 NMR spectrometer with operating frequency 600 MHz (¹H) and 150 MHz (¹³C), using the residual solvent signal (δ 7.26 in ¹H and δ 77.00 in ¹³C for CDCl₃, δ 3.31 in ¹H and δ 49.05 in ¹³C for CD₃OD and δ 7.16 in ¹H and δ 128.39 in ¹³C for C₆D₆) as a reference. The chemical shifts (δ) are expressed in ppm and coupling constants (*J*) in Hz. The 1D and 2D NMR (¹H and ¹³C NMR, COSY, HSQC and HMBC) spectra were recorded using the standard Bruker pulse sequence. HRESIMS were recorded on Agilent 6530 Accurate Mass QTOF detector. Column chromatography was carried out on Silica gel 60 (230–400 mesh, Merck) and Sephadex LH-20 (Farmacia Fine Chemicals AB). Thin-layer chromatography (TLC) on Silica gel 60 F₂₅₄ plates (Merck) was used for preparative TLC and to check the purity of the compound. Spots were visualised by spraying with concentrated H₂SO₄ followed by heating.

3.2. Plant material

A. alba was cultivated in Bulgaria using seeds originated from Turkey. The aerial parts were collected in August 2012 in full flowering stage. The species (*A. alba* Turra) was identified by Dr. L. Evstatieva from the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences. A voucher specimen (SOM – 167590) has been deposited in the Herbarium of the Institute of Biodiversity and Ecosystem Research, Sofia, Bulgaria.

3.3. Extraction and isolation

Air-dried and finely powdered aerial parts of *A. alba* (85 g) were successively extracted with hexane (3 × 1 L) and then with CHCl₃ (3 × 800 mL) at room temperature. After filtration, the solvents were evaporated under vacuum at low temperature (40 °C) to give: hexane (1.3 g) and CHCl₃ (5.5 g) extracts. The CHCl₃ extract was dissolved in CH₃OH and then submitted to CC on Sephadex LH-20. Elution with the same solvent afforded three fractions. Fr. 2 (1.85 g) was further subjected to CC on silica gel, eluting with n-hexane/Et₂O (from 10:1 to 0:100 gradient) and 10 fractions (2/

Table 4
¹H and ¹³C NMR data of **9**, **9a**, **10** and **10a** and HMBC correlations of **9**, **10** and **10a**.

Position	9 (CD ₃ OD)			9a (C ₆ D ₆)		10 (CDCl ₃)		10a (CDCl ₃)		10/10a
	δ_{H} mult. (J Hz)	δ_{C}	HMBC ^a	δ_{H} mult. (J Hz)	δ_{C}	δ_{H} mult. (J Hz)	δ_{C}	δ_{H} mult. (J Hz)	δ_{C}	
1	2.58 dd (6.9, 10.4)	61.9	2, 3, 5, 6, 8, 9, 10	2.94 dd (6.6, 9.5)	58.1	2.35 ddd (5.1, 7.0, 12.3)	38.5	1.76 m [*]	37.9	2, 3, 9
2	4.11 td (6.9, 4.6)	75.4	10	5.23 ddd (2.8, 6.6, 8.4)	77.5	1.60 td (12.3, 8.3)		1.92 m [*]		2, 3, 9
3	1.78 dd (4.6, 13.8)	50.3	1, 2, 4, 5	1.68 [*]	48.3	4.59 ddd (7.0, 8.3, 9.5)	73.2	4.22 dd (3.1, 6.2)	76.5	
	2.02 dd (6.9, 13.8)		1, 5	2.04 dd (8.2, 14.8)		3.05 t (9.5)	59.9	2.70 dd (3.0, 10.1)	66.1	2, 4, 5, 14
4	–	81.8		–	81.4	2.02 ddd (9.5, 11.1, 12.0)	51.5	1.79 td [*] (10.9, 10.1)	51.1	3, 8, 9, 14
5	2.32 ddd (5.5, 5.9, 10.4)	50.4	2, 4, 6	2.23 dt (9.5, 5.5)	50.5	1.39 tt (11.1, 2.7)	46.3	1.52 tt (11.1, 2.9)	46.3	
6	1.71 m	33.3	5, 7	1.51 brdd (5.5, 14.8)	33.5	1.98 ddd (2.7, 5.2, 11.9)	32.2	1.98 m [*]	32.2	5, 7, 8
	1.99 ddd (5.9, 8.7, 14.6)		4	1.85 ddd (5.5, 8.6, 14.8)		1.24 m		1.24 m		
7	2.10 brt (8.7)	40.2		1.78 brt (8.6)	39.7	5.18 dd (5.2, 11.7)	73.3	5.24 (5.2, 11.7)	74.1	6, 8, CO(Ac)
8	1.62 m	31.5		1.38–1.43 [*]	30.8	–	146.6	–	147.2	
	1.76 m [*]			1.68 [*]						
9	1.72 m	39.5	8	1.38–1.43 [*]	38.4	1.86 ddd (5.1, 12.3, 12.0)	45.3	2.40 td (12.4, 5.3)	46.8	1, 4, 8
	1.76 m [*]			1.46 m						
10	–	75.6		–	73.8	4.68 brs	101.6	4.64 s	101.6	7, 9
						4.79 brs		4.77 brs		7, 8, 9
11	–	150.8		–	150.3	1.43 m	29.6	1.43 m	29.6	5, 6, 12, 13
12	4.56 brs (2H)	67.3	7, 11, 13, CO (Ac)	4.50 s (2H)	66.2	0.67 d (7.0)	15.2	0.65 d (7.0)	15.2	5, 11, 13
13	5.01 s	111.8	7, 12	4.89 s	110.9	0.91 d (7.0)	21.9	0.90 d (7.0)	21.9	5, 11, 12
	5.03 s		7, 12	4.97 s						
14	1.37 s	24.8	1, 9, 10	1.08 s	32.4	–	209.6	–	209.2	
15	1.19 s	31.3	3, 4	1.04 s	25.1	2.26 s	32.4	2.30 s	30.7	3, 14
OAc	2.06 s	20.7	CO(Ac)	1.67 s	20.8	2.14 s	21.1	2.13 s	21.1	7, CO (Ac)
		172.5			170.2		170.4		170.1	
				1.69 s	21.3					
					170.7					

^a Long-range ¹H–¹³C HMBC correlations are from the stated hydrogen(s) to the indicated carbon.

^{*} Overlapped signals.

1–2/10) were collected (TLC control). Fr. 2/5 afforded compounds **1** (2 mg), **2** (3 mg), **5** (6 mg), **6** (3 mg), **7** (10 mg), **10** (5 mg) and **11** (7 mg) after CC and PTLC using for elution n-hexane/Et₂O mixtures in an appropriate ratio of the two components. Further, **4** (19 mg), **9** (5 mg), **3** (11 mg), **8** (7 mg) were isolated from Fr. 2/8 by CC and PTLC using for elution CHCl₃/Me₂CO mixtures in an appropriate ratio. Fr. 3 afforded two flavonoids – centaureidin (20 mg) and axillarin (15 mg). Standard acetylation (Ac₂O-pyridine) (Appendino et al., 1983) of **4** (6 mg), **8** (4 mg) and **9** (2 mg) afforded the corresponding acetates **4a** (3.8 mg), **8a** (2.2 mg) and **9a** (1 mg).

3.3.1. 2β,5α,12-Trihydroxygermacra-1(10),4(15),11(13)-triene (**1**)

Colorless oil. $[\alpha]_D^{25} -29.1$ (c 0.93, CHCl₃); IR (film) ν_{\max} 3332, 3082, 2925, 2855, 1726, 1672, 1643 cm⁻¹; ¹H and ¹³C NMR (CDCl₃), see Table 1; HRESIMS m/z 275.1614 [M+Na]⁺ (calcd for C₁₅H₂₄O₃Na, 275.1623).

3.3.2. 12-Acetoxy-1α,10β-epoxy-5α-hydroperoxy-2β-hydroxygermacra-4(15),11(13)-diene (**2**)

Colorless oil. $[\alpha]_D^{25} -14.0$ (c 0.14, CHCl₃); IR (film) ν_{\max} 3435, 3019, 2916, 2849, 1731, 1462, 1216 cm⁻¹; ¹H and ¹³C NMR (CDCl₃), see Table 1; HRESIMS m/z 349.1632 [M+Na]⁺ (calcd for C₁₇H₂₆O₆Na, 349.1627).

3.3.3. 1β,10α-Epoxy-2β,5α,12-trihydroxygermacra-4(15),11(13)-diene (**3**)

Colorless oil. $[\alpha]_D^{25} -5.0$ (c 0.11, CHCl₃); IR (film) ν_{\max} 3389, 3086, 2929, 2857, 1716, 1646, 1647, 1246 cm⁻¹; ¹H and ¹³C NMR (CDCl₃), see Table 1; HRESIMS m/z 269.1751 [M+H]⁺ (calcd for C₁₅H₂₅O₄, 269.1753).

3.3.4. 5α,10α-Epoxy-1α,2β,12-trihydroxygermacra-4(15),11(13)-diene (**4**)

Colorless oil. $[\alpha]_D^{25} -18.9$ (c 0.97, CH₃OH); IR (film) ν_{\max} 3340, 2890, 1700, 1620 cm⁻¹; ¹H and ¹³C NMR (CD₃OD), see Table 2; HRESIMS m/z 291.1573 [M+Na]⁺ (calcd for C₁₅H₂₄O₄Na, 291.1572).

3.3.5. 1α,2β,12-Triacetoxy-5α,10α-epoxygermacra-4(15),11(13)-diene (**4a**)

Colorless oil. $[\alpha]_D^{25} -0.003$ (c 0.38, CHCl₃); IR (film) ν_{\max} 2890, 1730, 1625, 1220 cm⁻¹; ¹H and ¹³C NMR (CDCl₃), see Table 2; HRESIMS m/z 417.1880 [M+Na]⁺ (calcd for C₂₁H₃₀O₇Na, 417.1884).

3.3.6. 12-Acetoxy-5α,10α-epoxy-1α,2β-dihydroxygermacra-4(15),11(13)-diene (**5**)

Colorless oil. $[\alpha]_D^{25} -8.0$ (c 0.06, CHCl₃); IR (film) ν_{\max} 3474, 2918, 2850, 1733, 1654, 1619 cm⁻¹; ¹H and ¹³C NMR (CDCl₃), see Table 2; HRESIMS m/z 333.1689 [M+Na]⁺ (calcd for C₁₇H₂₆O₅Na, 333.1678).

3.3.7. 9β-Acetoxy-1β,3β,4β-trihydroxygermacra-5,10(14)-diene (**6**)

Colorless oil. $[\alpha]_D^{25} -31.2$ (c 0.43, CHCl₃); IR (film) ν_{\max} 3437, 2958, 2929, 2873, 1718, 1648, 1258 cm⁻¹; ¹H and ¹³C NMR (CDCl₃), see Table 3; HRESIMS m/z 335.1824 [M+Na]⁺ (calcd for C₁₇H₂₈O₅Na, 335.1834).

3.3.8. 9β-Acetoxy-1β-hydroperoxy-3β,4β-dihydroxygermacra-5,10(14)-diene (**7**)

Colorless oil. $[\alpha]_D^{25} -16.0$ (c 1.19, CHCl₃); IR (film) ν_{\max} 3435, 3018, 2960, 2875, 1726, 1437, 1246 cm⁻¹; ¹H and ¹³C NMR (CDCl₃), see Table 3; HRESIMS m/z 351.1769 [M+Na]⁺ (calcd for C₁₇H₂₈O₆Na, 351.1784).

3.3.9. 1α,2β,12-Trihydroxyeudesma-4(15),11(13)-diene (**8**)

Colorless oil. $[\alpha]_D^{25} -22.4$ (c 0.81, CH₃OH); IR (film) ν_{\max} 3320, 2900, 1625, 1430 cm⁻¹; ¹H and ¹³C NMR (CD₃OD), see Table 3; HRESIMS m/z 275.1625 [M+Na]⁺ (calcd for C₁₅H₂₄O₃Na, 275.1623).

3.3.10. 1α,2β,12-Triacetoxyeudesma-4(15),11(13)-diene (**8a**)

Colorless oil. ¹H and ¹³C NMR (CHCl₃), see Table 3; HRESIMS m/z 401.1947 [M+Na]⁺ (calcd for C₂₁H₃₀O₆Na, 401.1940).

3.3.11. 12-Acetoxy-2β,4β,10β-trihydroxyguai-11(13)-ene (**9**)

Colorless oil. $[\alpha]_D^{25} -5.5$ (c 0.52, CH₃OH); IR (film) ν_{\max} 3420, 2950, 2920, 2850, 1730, 1220 cm⁻¹; ¹H and ¹³C NMR (CD₃OD), see Table 4; HRESIMS m/z 335.1823 [M+Na]⁺ (calcd for C₁₇H₂₈O₅Na, 335.1834).

3.3.12. 2β,12-Diacetoxy-4β,10β-dihydroxyguai-11(13)-ene (**9a**)

Colorless oil. $[\alpha]_D^{25} -18.0$ (c 0.1, CH₃OH); ¹H and ¹³C NMR (C₆D₆), see Table 4; HRESIMS m/z 377.1944 [M+Na]⁺ (calcd for C₁₉H₂₉O₆Na, 377.1935).

3.3.13. 7β-Acetoxy-2β-hydroxyoplophenone (**10**) and 7β-Acetoxy-2α-hydroxyoplophenone (**10a**)

Colorless oil. IR (film) ν_{\max} 3448, 3095, 2958, 2918, 2873, 2850, 1734, 1711, 1659, 1424, 1241 cm⁻¹; ¹H and ¹³C NMR (CDCl₃), see Table 4; HRESIMS m/z 317.1742 [M+Na]⁺ (calcd for C₁₇H₂₆O₄Na, 317.1729).

Acknowledgments

The authors are thankful for the financial support of Swiss National Science Foundation (SNSF), Switzerland and Ministry of Education and Science, Bulgaria in the framework of the Bulgarian-Swiss Research Programme (BSRP, Grant No. IZEBZO_142989; DO2-1153).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.phytochem.2014.12.008>.

References

- Abad Martínez, M.J., Del Olmo, L.M.B., Ticona, L.A., Benito, P.B., 2012. The *Artemisia* L. genus: a review of bioactive sesquiterpene lactones. *Stud. Nat. Prod. Chem.* 37, 43–65.
- Appendino, G., Gariboldi, P., Nano, G.M., 1983. Tanacetols A abd B, non-volatile sesquiterpene alcohols from *Tanacetum vulgare*. *Phytochemistry* 22, 509–512.
- Appendino, G., Gariboldi, P., Menichini, F., 1985. Oxygenated nerolidol derivatives from *Artemisia alba*. *Phytochemistry* 24, 1729–1733.
- Arciniegas, A., Perez-Castorena, A.-L., Reyes, S., Contreras, J.L., Romo de Vivar, A., 2003. New oplopane and eremophilane derivatives from *Robinsonia gerberifolia*. *J. Nat. Prod.* 66, 225–229.
- Barberá, O., Marco, J.A., Sanz, J.F., Sánchez-Parareda, J., 1986. 3-Methoxyflavones and coumarins from *Artemisia incanescens*. *Phytochemistry* 25, 2357–2360.
- Bora, K.S., Sharma, A., 2011. The genus *Artemisia*: a comprehensive review. *Pharm. Biol.* 49, 101–109.
- Dordević, S., Stanisavljević, D., Ristić, M., Milenković, M., Veličković, D., Stojičević, S., Zlatković, B., 2013. Chemical, antioxidant and antimicrobial analysis of the essential oil and extract of *Artemisia alba* Tura. *Dig. J. Nanomater. Bios.* 8, 1377–1388.
- Gallery, M., Chiary, G., Viterbo, D., 1983. The structures of tanacetols A C₁₇H₂₆O₄ and B C₁₉H₃₀O₅, two new sesquiterpene alcohols from *Tanacetum vulgare* L. *Acta Cryst.* C39, 758–761.
- Greger, H., Haslinger, E., Hofer, O., 1982. Naturally occurring sesquiterpene-coumarin ethers. Part 2. Albartin, a new sesquiterpene-coumarin ether from *Artemisia alba*. *Monatsh. Chem.* 113, 375–379.
- Janot, M.M., Gautier, J., 1935. Some *Artemisia* species from Persia and their santonine content. *Bull. Sci. Pharmacol.* 42, 404–408.
- Joseph-Nathan, P., Villagomez, J.R., Rojas-Gardida, M., Roman, L.U., Hernandez, J.D., 1989. Minor oplopanes from *Senecio mexicanus*. *Phytochemistry* 28, 2397–2401.
- Long, C., Sauleau, P., David, B., Lavaud, C., Cassaboiss, V., Ausseil, F., Massiot, G., 2003. Bioactive flavonoids of *Tanacetum parthenium* revisited. *Phytochemistry* 64, 567–569.
- Maggio, A., Rosselli, S., Brancaccio, C.L., Sadfer, M., Spadaro, V., Bruno, M., 2011. Artablic acid, a sesquiterpene with an unusual skeleton from *Artemisia alba* (Asteraceae) from Sicily. *Tetrahedron Lett.* 52, 4543–4545.

- Maggio, A., Rosselli, S., Bruno, M., Spadaro, V., Raimondo, F.M., Senatore, F., 2012. Chemical composition of essential oil from Italian populations of *Artemisia alba* Turra (Asteraceae). *Molecules* 17, 10232–10241 (references cited therein).
- Maggio, A., Rosselli, S., Brancazio, C.L., Spadaro, V., Raimondo, F.M., Bruno, M., 2013. Metabolites from the aerial parts of the Sicilian population of *Artemisia alba*. *Nat. Prod. Commun.* 8, 283–286.
- Marco, J.A., Barberá, O., Sánchez-Parareda, J., 1987. Flavonol and coumarin glycosides from *Artemisia incanescens*. *J. Nat. Prod.* 50, 774–775.
- Marco, J.A., Sanz-Cervera, J.F., Sancenon, F., Jakupovic, J., Rustaiyan, A., Mohamadi, F., 1993. Oplopanone derivatives monoterpene glycosides from *Artemisia sieberi*. *Phytochemistry* 34, 1061–1065.
- Marco, J.A., Sanz-Cervera, J.F., Morante, M.D., García-Lliso, V., Vallès-Xirau, J., Jakupovic, J., 1996. Tricyclic sesquiterpenes from *Artemisia chamaemelifolia*. *Phytochemistry* 41, 837–844.
- Oberprieler, C., Himmelreich, S., Vogt, R., 2007. A new subtribal classification of the tribe Anthemideae (Compositae). *Willdenowia* 37, 89–114.
- Radulović, N., Blagojević, P., 2010. Volatile profiles of *Artemisia alba* from contrasting serpentine and calcareous habitats. *Nat. Prod. Commun.* 5, 1117–1122.
- Rigat, M., Bonet, M.A., Garcia, S., Garnatje, T., Vallès, J., 2007. Studies on pharmaceutical ethnobotany in the high river Ter valley (Pyrenees, Catalonia, Iberian Peninsula). *J. Ethnopharmacol.* 113, 267–277.
- Syu, W.-J., Shen, C.-C., Don, M.-J., Ou, J.-C., Lee, G.-H., Sun, C.-M., 1998. Cytotoxicity of curcuminoids and some novel compounds from *Curcuma zedoaria*. *J. Nat. Prod.* 61, 1531–1534.
- Tan, R.X., Zheng, W.F., Tang, H.Q., 1998. Biologically active substances from the genus *Artemisia*. *Planta Med.* 64, 295–302.
- Teixeira Da Silva, J.A., Yonekura, L., Kaganda, J., Mookdasanit, J., Nhut, D.T., Afach, G., 2004. Important secondary metabolites and essential oils of species within the Anthemideae (Asteraceae). *J. Herbs Spices Med. Plants* 11, 1–46.
- Tešević, V., Milosavljević, S., Vajs, V., Janačković, P., Jović, D.L., Vujisić, L., 2004. Tetrahydrofuran-type sesquiterpenes from *Artemisia lobelii* All. var. *canescens* (DC.) Briq. and *Artemisia lobelii* All. var. *biasoletiana* (Vis.) K. Maly. *Biochem. Syst. Ecol.* 32, 525–527.
- Tutin, T.G., Persson, K., Gutermann, W., 1976. *Artemisia* L. In: Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A. (Eds.), *Flora Europaea*, vol. 4. Cambridge University Press, Cambridge, pp. 178–186.
- Zeng, W.Z., Quesheng, Zhang, Q.Y., Liang, H., 2014. Two new oplopane sesquiterpenes from *Artemisia gmelinii* Web. ex Stechm. *Chin. Chem. Lett.* 25, 1153–1156.