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Professor Josep Coll Toledano
On the Occasion of his 70th Birthday**

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New Pseudoguaiane Derivatives from *Inula aschersoniana* Janka var. *aschersoniana*Antoaneta Trendafilova^{a,*}, Milka Todorova^a, Viktoriya Genova^a, Pavletta Shestakova^a, Dimitar Dimitrov^b, Milka Jadranin^c and Slobodan Milosavljevic^d^aInstitute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria^bNational Museum of Natural History, Bulgarian Academy of Sciences, 1000 Sofia, Bulgaria^cCenter for Chemistry, Institute for Chemistry, Technology and Metallurgy, University of Belgrade, 11000 Belgrade, Serbia^dFaculty of Chemistry, University of Belgrade, 11000 Belgrade, Serbia

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The aerial parts of *Inula aschersoniana* Janka var. *aschersoniana* afforded parthenolide, diepoxycostunolide, inusoniolide, chrysosplenol C and four new pseudoguaiane-type sesquiterpenoids. Their structures were determined using spectral methods and relative stereochemistry by NOESY correlations.

Keywords: *Inula aschersoniana*, Sesquiterpene lactones, Pseudoguaiane derivatives.

Inula aschersoniana Janka {synonyms *I. verbascifolia* (Janka) subsp. *aschersoniana* (Janka) Tutin, *I. candida* (L.) Cass. subsp. *aschersoniana* (Janka) Hayek, *I. conyza* DC. subsp. *verbascifolia* Nyman, *I. macedonica* Hausskn.}, family Asteraceae, is a subendemic species in the Balkan peninsula and Anatolia with areas of distribution in Bulgaria, Greece, Macedonia, and Turkey [1a]. There are three varieties of *I. aschersoniana* in Bulgaria: *madarense*, *macedonica* and *aschersoniana* [1b,1c]. A literature survey revealed the isolation of four sesquiterpene lactones from this species: parthenolide [2a,2b], 8 β -hydroxy-11 α ,13-dihydro eremanthin [2a], diepoxycostunolide and inusoniolide [2b], while for *I. verbascifolia* subsp. *methanea* of Greek origin are reported 9 β -hydroxy parthenolide, four esters of the latter, two guaianolides, inusoniolide and its 4-*O*-dihydroderivative [2c-2e].

The phytochemical investigation of *I. aschersoniana* Janka var. *aschersoniana* afforded parthenolide [3], diepoxycostunolide [2b], inusoniolide [2b], chrysosplenol C [4] and four new pseudoguaiane derivatives (1-4). Compounds forming two well defined TLC spots were isolated (samples A and B) by PTLC. The HRESIMS of A displayed a quasimolecular peak at m/z 349.20085 [M+H]⁺ corresponding to the molecular formula C₂₀H₂₈O₅. ¹H and ¹³C NMR spectra (Table 1) exhibited the characteristic signals for angeloyloxy and seneciolyloxy groups. Further, duplicate signals for one secondary (δ_H 1.05/1.06, d, 7.3 Hz) and one tertiary (δ_H 1.13/1.15 s) methyl groups, one exomethylene (δ_H 5.65, 6.24/5.65, 6.25), and a proton geminal to the ester residue (δ_H 5.35/5.40, dd, J 9.0 and 1.5 Hz), all of them in ratio 1:1.5 were observed. Obviously the studied sample A is an inseparable mixture of two esters (1 and 2) of one sesquiterpenoid. Analysis of the COSY spectrum showed the correlations H-3/H-2/H-1/H-10/H-9/H-8/H-7/H-6 and H-10/H-14. Connection of C-3 to C-6 through quaternary carbons C-4 and C-5 was established by HMBC cross-peaks of C-4 (δ_C 214.72/214.25) with H-3, H-2' and H-15, and C-5 (δ_C 51.15) with H-1, H-2', H-10 and H-15. The position of a COOH group at C-12 was suggested from the lack of corresponding protons and the correlation between C-12 (δ_C 171.14) with H-13 and H-13'. Finally, the pseudoguaiane skeleton was confirmed by the ¹H-¹H correlations observed in the TOCSY spectrum of sample A which displays correlations throughout the entire coupling network. The

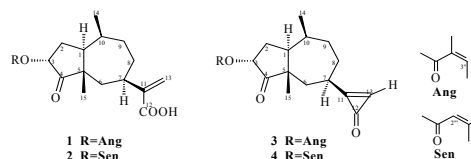


Figure 1: Structures of compounds 1-4

Table 1: NMR spectral data of compounds 1 and 2 in CDCl₃.

No	δ_H (mult., J Hz) 1/2	δ_C 1/2	HMBC (C→H)	NOESY
1	2.54 ddd (5.4, 7.0, 13.5)	43.09/43.12	2, 3, 5, 9, 10, 14, 15	2', 7, 10
2	2.44 ddd (9.0, 13.5, 13.5)	29.73/29.86	1, 3	3, 15
2'	1.72 ddd (1.5, 7.0, 13.5)	-	-	1
3	5.35/5.40 dd (1.5, 9.0)	70.87/71.47	2'	15
4	-	214.72/214.25	3, 2', 15	-
5	-	51.15	1, 2', 10, 15	-
6	2.34/2.35 dd (3.1, 14.4)	37.32/37.40	1, 15	7
6'	1.68 m*	-	-	15
7	2.75 m	36.32/36.29	6, 13, 13'	1, 6, 8
8	1.88 m*	31.16/31.07	6, 10	-
8'	1.60 m	-	-	-
9	1.70 m* (2H)	33.86	8', 10, 14,	-
10	2.05 m	34.64/34.61	1, 2, 8, 14	1
11	-	146.02	6, 8', 13	-
12	-	171.14	13, 13'	-
13	6.24/6.25 s	124.84	-	-
13'	5.65 s	-	-	-
14	1.05/1.06 d (7.3)	16.89	-	15
15	1.13/1.15 s	21.61/21.68	-	2, 3, 14
Angeloyl				
1''	-	167.27	3, 5''	-
2''	-	127.33	-	-
3''	6.10 qq (7.3, 1.2)	138.95	4'', 5''	-
4''	1.97 dq (7.3, 1.2)	15.93	-	-
5''	1.90 dq (1.2, 1.2)*	20.58	-	-
Seneciyl				
1'''	-	165.73	3, 4'''	-
2'''	5.73 brs	115.32	4'''', 5'''	-
3'''	-	158.47	-	-
4'''	2.17 d (1.2)	20.42	-	-
5'''	1.90 d (1.2)*	27.51	-	-

*Assignment determined by HSQC and HMBC. *overlapped signals.

overall analysis of the spectral data of A confirmed the pseudoguaiane structure of 1 and 2 with a C-3 connected ester side chain, C-4 carbonyl and C-12 carboxyl groups. The literature survey revealed that the obtained compounds were 3-acyloxy derivatives of damsinic acid [5a]. The relative configuration of 1 and 2 was determined by NOESY correlations. Assuming, on biogenetic grounds, a β -orientation of the C-7 side chain and NOESY (Table 1) cross peaks of H-7/H-1, H-1/H-10, H-15/H-3 and H-15/H-14 revealed a β -disposition of the C-10 and C-5 methyl

groups, an α -orientation of the C-3 ester residue and a *trans*-fusion of the 5/7-membered rings in the pseudoguaiane structure. The newly isolated compounds were identified as 3 α -angeloyloxy- and 3 α -seneciolyoxydamsinic acids. It is worthy of note that only eudesmane sesquiterpene acids have been isolated from *Inula* species so far [5b].

Table 2: NMR spectral data of compounds **3** and **4** in CDCl₃.

No	δ_H (mult., J Hz) 3/4	δ_C 3/4	HMBC (C→H)	NOESY
1	2.51 m	44.70/44.74	2', 6', 9', 9', 14, 15	2', 7, 10
2	2.34 m*	30.15/30.12	1	
2'	1.82/1.86 ddd (1.4, 6.9, 14.6)			1
3	5.21/5.25 dd (1.4, 9.0)	71.83, 71.24	2'	2, 15
4	-	215.81/215.41	2, 2', 3, 6, 15	
5	-	52.04/52.08	1, 2, 2', 6, 6', 7, 15	
6	2.32 m*	38.46/38.62	1, 7, 8, 8', 9, 10, 15	
6'	1.96 m*			
7	3.21 m	36.78/36.86	6, 6', 8, 8', 9', 13	1
8	2.11 m*	26.89	6, 6', 7, 9, 9'	
8'	1.96 m*			
9	1.96 m*	32.23/32.20	7, 14	
9'	1.59 m			13, 14, 15
10	2.13 m*	32.96/32.91	1, 2, 6, 8, 9', 14	
11	-	157.53	7	
12	-	173.80	7, 13	
13	8.45/8.46 d (0.9)	147.51/147.62	7	9', 14, 15
14	0.99/1.00 d (7.3)	17.24/17.26	1, 9	9, 13
15	1.04/1.05 s	19.02	1, 6, 6'	3, 9', 13
Angeloyl				
1''	-	166.81	3	
2''	-	127.10		
3''	6.12 qq (7.3, 1.2)	139.36		
4''	1.97 dq (7.3, 1.2)	15.96		
5''	1.90 dq (1.2, 1.2)*	20.57		
Senecioly				
1'''	-	165.34	3	
2'''	5.68 brs	115.04		
3'''	-	158.90		
4'''	2.17 d (1.2)	27.54		
5'''	1.90 d (1.2)*	20.43		

*Assignment determined by HSQC and HMBC. *overlapped signals

The HRESIMS of sample **B** showed a quasimolecular peak at m/z 331.19050 [M+H]⁺ corresponding to a molecular formula C₂₀H₂₆O₄. Comparison of ¹H and ¹³C NMR data of **B** (Table 2) with those of **A** (Table 1) established close similarity. Sample **B** also contained two components (**3** and **4**), the same as sample **A**. The main difference between **A** and **B** in their ¹H NMR spectra is related to the C-7 side chain. Instead of signals for geminal vinyl protons H-13 and H-13', a signal for an olefinic proton appeared as a pair of doublets at δ 8.45/8.46 ppm, with $J = 0.9$ Hz. The IR absorptions at 1830 and 1580 cm⁻¹, the presence of a carbonyl function (δ_C 173.80) and its β -effect on the chemical shift of the olefinic proton confirmed the presence of a cyclopropanone ring. The relative stereochemistry of **3** and **4** was determined from the correlations in NOESY spectrum (Table 2). These compounds were named aschersonianone **A** (**3**) and aschersonianone **B** (**4**). To the best of our knowledge, only few compounds with a cyclopropanone ring have been isolated from

natural sources – two acids (alutacenoic acids **A** and **B**) from fungi [5c] and four sesquiterpene derivatives (3 eremophilanes and one eudesmane) from *Telekia speciosa* [5d] and *I. linearifolia* [5e].

Experimental

General: IR, Bruker Tensor 27 FT-IR spectrometer; NMR, Bruker Avance II+ 600 NMR spectrometer; HRESIMS, Agilent 6210 LC/ESI TOF System; CC, Silica gel 60(230–400 mesh); TLC, silica gel 60 F₂₅₄ plates; compounds were visualised by spraying with conc. H₂SO₄ followed by heating.

Plant material: Wild growing *I. aschersoniana* was collected in July 2013 from eastern Rhodope Mts in Bulgaria. A voucher specimen (SOM169980) has been deposited in the Herbarium of the Institute of Biodiversity and Ecosystem Research, Sofia, Bulgaria.

Extraction and isolation: Dried and powdered plant material (41 g) was defatted with *n*-hexane and then successively extracted by cold maceration with CHCl₃. After filtration, the solvent was evaporated under vacuum at low temperature (40°C) to give the crude extract (2 g), which was submitted to CC on silica gel. Elution with a mixture CHCl₃/acetone with increasing polarity afforded 6 fractions (TLC monitoring). Four main TLC spots were observed. PTLC (silica gel, *n*-hexane/diethyl ether, 1:1) of portions of Fr. 2 (528 mg) and Fr. 3 (205 mg) afforded parthenolide (47.4 mg) and diepoxycostunolide (10 mg), respectively. PTLC (silica gel, *n*-hexane/Et₂O, 1:1) of Fr. 4 (5 mg) gave 1.1 mg of inusoniolide. Further, 1/2 (5.0 mg) and 3/4 (2.2 mg) were isolated from Fr. 5 (100 mg) by PTLC (*n*-hexane/acetone, 2:1). PTLC (*n*-hexane/acetone 2:1) of Fr. 6 (45 mg) afforded chrysosplenol (20 mg).

3 α -Angeloyloxydamsinic acid (**1**) and 3 α -seneciolyoxydamsinic acid (**2**) (mixture)

Colorless oil.

IR (film) ν_{max} cm⁻¹: 1756 (C=O, 5-membered ring), 1717, 1695 (C=O, ester), 1651 (C=C).

¹H (600 MHz, CDCl₃) and ¹³C NMR (150 MHz, CDCl₃): Table 1.

HRESIMS (positive mode) m/z : 349.20085 [M+H]⁺ (calculated for C₂₀H₂₈O₅, 349.20088).

Aschersonianone **A** (**3**) and aschersonianone **B** (**4**) (mixture)

Colorless oil.

IR (film) ν_{max} cm⁻¹: 1830, 1580 (cyclopropanone), 1753 (C=O, 5-membered ring), 1718 (C=O, ester), 1650 (C=C).

¹H (600 MHz, CDCl₃) and ¹³C NMR (150 MHz, CDCl₃): Table 2.

HRESIMS (positive mode) m/z : 331.19050 [M+H]⁺ (calculated for C₂₀H₂₆O₄, 331.19030)

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