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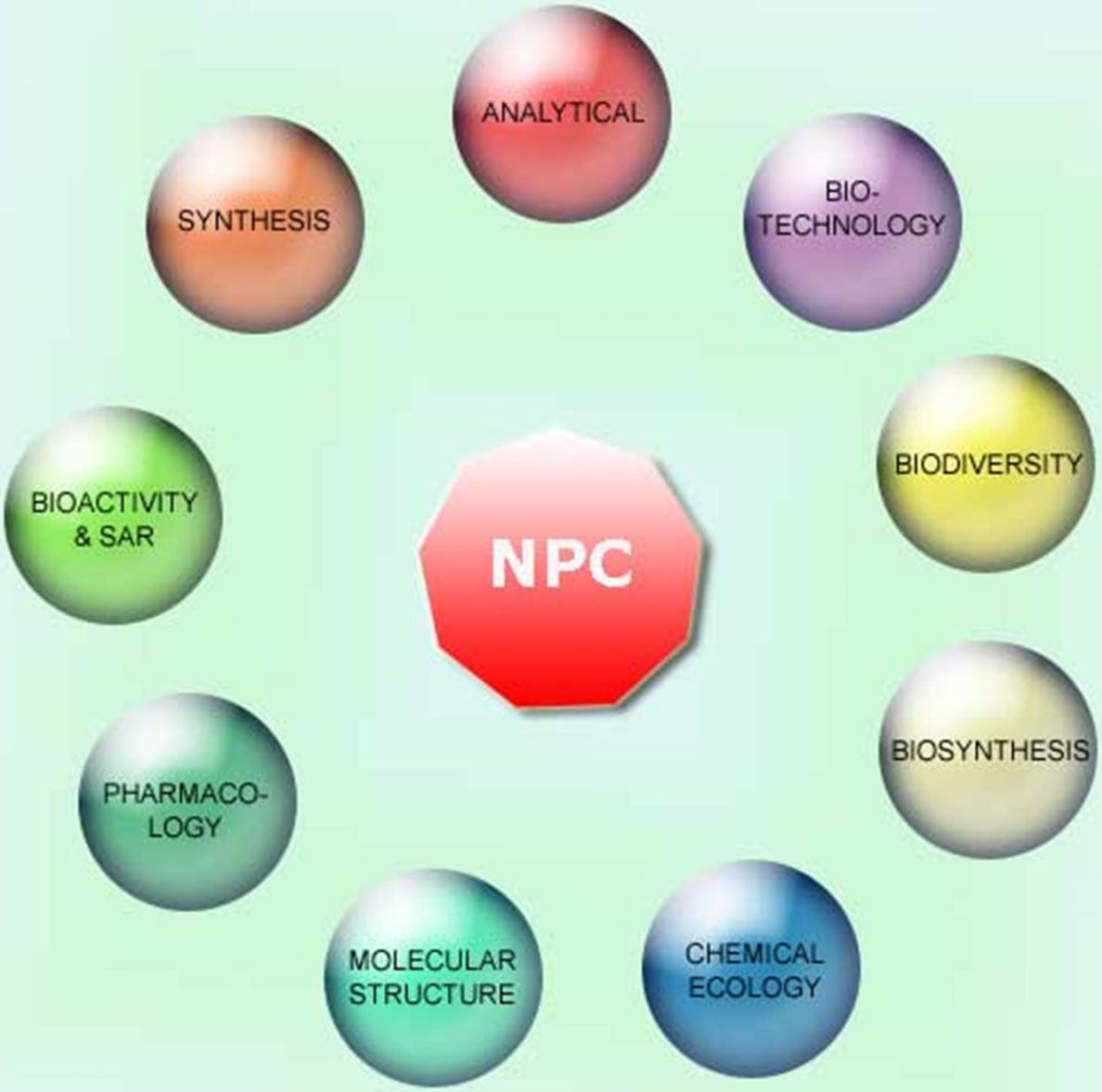


**This Issue is Dedicated to**
**Professor Josep Coll Toledano**
**On the Occasion of his 70th Birthday**

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# New Pseudoguaiane Derivatives from *Inula aschersoniana* Janka var. *aschersoniana*

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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], 8β-hydroxy-11α,13-dihydro eremanthin [2a], diepoxycostunolide and inusoniolide [2b], while for *I. verbascifolia* subsp*. methanea* of Greek origin are reported 9β-hydroxyparthenolide, 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 $C_{20}H_{28}O_5$. $^1H$ and $^{13}C$ NMR spectra (Table 1) exhibited the characteristic signals for angeloyloxy and senecioyloxy groups. Further, duplicate signals for one secondary ($\delta_H$ 1.05/1.06, d, 7.3 Hz) and one tertiary ($\delta_H$ 1.13/1.15 s) methyl groups, one exomethylene ($\delta_H$ 5.65, 6.24/5.65, 6.25), and a proton geminal to the ester residue ($\delta_H$ 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 ($\delta_C$ 214.72/214.25) with H-3, H-2' and H-15, and C-5 ($\delta_C$ 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 ($\delta_C$ 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

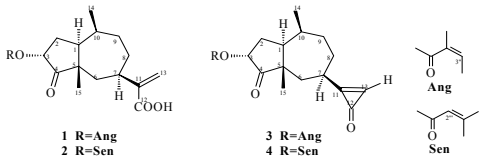


**Figure 1:** Structures of compounds **1-4**

**Table 1**: NMR spectral data of compounds **1** and **2** in $CDCl_3$.

| No | $\delta_H$ (mult.,J Hz) **1/2** | $\delta_C$[#] **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 | | |
| | Senecioyl | | | |
| 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 3α-angeloyloxy- and 3α-senecioyloxydamsinic 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 $CDCl_3$.

| No | $\delta_H$ (mult., J Hz) **3/4** | $\delta_C$[#] **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) | 147.57/147.62 | 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 | | |
| | Senecioyl | | | |
| 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 $C_{20}H_{26}O_4$. Comparison of $^1H$ and $^{13}C$ 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 ($\delta_C$ 173.80) and its β-effect on the chemical shift of the olefinic proton confirmed the presence of a cyclopropenone 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 cyclopropenone 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. linearii*folia[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 $F_{254}$ plates; compounds were visualised by spraying with conc. $H_2SO_4$ 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 $CHCl_3$. 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 $CHCl_3$/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/$Et_2O$, 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).

**3α-Angeloyloxydamsinic acid (1) and 3α-senecioyloxydamsinic acid (2) (mixture)**
Colorless oil.
IR (film) $\nu_{max}$ $cm^{-1}$: 1756 (C=O, 5-membered ring), 1717, 1695 (C=O, ester), 1651 (C=C).
$^1H$ (600 MHz, $CDCl_3$) and $^{13}C$ NMR (150 MHz, $CDCl_3$): Table 1.
HRESIMS (positive mode) *m/z*:349.20085 $[M+H]^+$ (calculated for $C_{20}H_{28}O_5$, 349.20088).

**Aschersonianone A (3) and aschersonianone B (4) (mixture)**
Colorless oil.
IR (film) $\nu_{max}$$cm^{-1}$: 1830, 1580 (cyclopropenone), 1753 (C=O, 5-membered ring), 1718 (C=O, ester), 1650 (C=C).
$^1H$ (600 MHz, $CDCl_3$) and $^{13}C$ NMR (150 MHz, $CDCl_3$): Table 2.
HRESIMS (positive mode) *m/z*:331.19050 $[M+H]^+$ (calculated for $C_{20}H_{26}O_4$, 331.19030)

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