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B R I E F C O M M U N I C A T I O N S

ALKANES OF *Jurinea mollis*, A PANNONIAN SUBENDEMIC SPECIES

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The genus *Jurinea* Cass. (Asteraceae, subfamily Cichorioideae, tribe Carduae) includes from about 100 [1] to 200 species [2] natively distributed in Central Asia, Iran, Turkey, and the Mediterranean basin [3]. It is taxonomically a complex group of plants, with unsolved problems, especially within the species surrounding *Jurinea mollis* (L.) Reichb. [1] which spreads from Austria and Italy across Southeastern Europe to West Anatolia [4]. The later is described by Holub et al. [5] as a Pannonian subendemic species which expands into neighboring regions, especially into the Illyrian part of the Balkan Peninsula.

Nowadays, whenever classification of plants based on selected similarities and differences in morphology is problematic, revision studies of the families are supported by molecular phylogenetics [6]. Another approach usually employs secondary metabolites as marker compounds and is collectively termed chemotaxonomy. Variability in the profile of *n*-alkanes is very often used in chemotaxonomic investigations of many plants (e.g., [7] and references therein). Carbon preference index (CPI) and average chain length (ACL) are sometimes used as chemotaxonomic markers at the genus level [8]. We conducted a thorough literature survey which showed that *J. mollis* has never previously been chemically investigated. Furthermore, this is the first report on the composition of alkanes in *J. mollis*, as well as in the genus *Jurinea*, and the first chemical data in general for this species.

Plant Material, Extraction, and Isolation. Aerial parts of *J. mollis* were collected on 15th June 2010 from the Suva Planina Mountain, Southeastern Serbia. Plant material was air-dried at room temperature for two weeks and kept in a cold and dark place until extracted. Voucher specimens were deposited in the Herbarium of the Faculty of Science and Mathematics, University of Nis (No. AM1906).

Air-dried above-ground parts of *J. mollis*, cut into small pieces (500 g), were extracted using a mixture (3 L) obtained by mixing equal volumes of hexane, methanol, and diethyl ether for 15 days at room temperature, without direct exposure to light. The crude extract obtained after the removal of solvents (*ca.* 17 g) was subjected to "dry flash" column chromatography on silica gel 60 (40–63 μm) using *n*-hexane–diethyl ether mixtures of increasing polarity for elution to furnish 12 fractions. Fraction 1, *n*-hexane (100%), white crystals (300 mg), was analyzed by GC-MS and IR.

The results, which revealed the presence of *n*-, *iso*- and *anteiso*-alkanes in fraction 1, are presented in Table 1. Other fractions had no alkanes whatsoever.

Fraction 1 contains a mixture of hydrocarbons dominated by *n*-alkanes (C_{17}–C_{39}), the distribution of which reflects the conventional higher plant pattern of high CPI (17.31), average-chain-length (ACL) (29.85), and odd-numbered carbon dominance (maximum at *n*-C_{31}). CPI and ACL were calculated according to a modified formula given in [9]. In the same region of the chromatogram as the *n*-alkanes, C_{23}–C_{36} dominate among the *iso*-(2-methyl) and C_{24}–C_{30} among *anteiso*-(3-methyl) alkanes. The *iso*-alkanes have a predominant odd-numbered distribution, whereas only even-numbered hydrocarbons are detected among *anteiso*-alkanes. Similar patterns have been reported previously and reflect the biosynthetic pathways of these compounds [10]. The *anteiso*-alkanes originate mainly with a 2-methylbutanoyl (C_5)-CoA "starter", which is elongated by C_2 units and then decarboxylated, resulting in an even-numbered dominance. The *iso*-alkanes have an odd-numbered dominance because they are mostly biosynthesized with a 2-methylpropanoyl (C_4)-CoA "starter".

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TABLE 1. The Distribution and Abundance of *n*-, *anteiso*- and *iso*-Alkanes in *Jurinea mollis*

Compound	RI	%	Compound	RI	%
***n*-Alkanes**			***iso*-Alkanes**		
Heptadecane	1700	Tr.	2-Methyldocosane	2260	Tr.
Nonadecane	1900	Tr.	2-Methyltetracosane	2460	Tr.
Heneicosane	2100	0.6	2-Methylpentacosane	2560	Tr.
Docosane	2200	0.2	2-Methylhexacosane	2660	0.1
Tricosane	2300	4.7	2-Methylheptacosane	2760	0.1
Tetracosane	2400	0.3	2-Methyloctacosane	2860	Tr.
Pentacosane	2500	1.8	2-Methylnonacosane	2960	0.1
Hexacosane	2600	0.2	2-Methyltriacontane	3060	0.2
Heptacosane	2700	7.1	2-Methylpentatriacontane	3560	Tr.
Octacosane	2800	0.9	Odd max., C_{31}; even max., C_{28} and C_{30}.		
Nonacosane	2900	24.5	CPI (C_{23}–C_{36}), 1.5.		
Triacontane	3000	2.0	ACL (C_{23}–C_{36}): all 29.25, odd 29.30, even 29.18		
Hentriacontane	3100	44.2	***anteiso*-Alkanes**		
Dotriacontane	3200	1.6	3-Methyltricosane	2372	Tr.
Tritriacontane	3300	9.4	3-Methylpentacosane	2572	Tr.
Tetratriacontane	3400	0.2	3-Methylheptacosane	2772	0.1
Pentatriacontane	3500	1.2	3-Methylnonacosane	2972	Tr.
Hexatriacontane	3600	Tr.	Odd max., –; even max., C_{28}.		
Heptatriacontane	3700	0.2	CPI (C_{24}–C_{30}), 0.		
Octatriacontane	3800	Tr.	ACL (C_{24}–C_{30}): all 27.69, odd 0, even 27.69		
Nonatriacontane	3900	0.1			
Odd max., C_{31}; even max., C_{30}.					
CPI (C_{19}–C_{39}), 17.31.					
ACL (C_{19}–C_{39}): all 29.85, odd 29.86, even 29.66					

[a]CPI (carbon preference index) = (Σodd C from the range of carbon numbers listed in parentheses)/(Σeven C from the range of carbon numbers listed in parentheses).

[b]ACL (average chain length) = (Σ[Cj]× j)/ (Σ[Cj]) where j is the range of carbon numbers listed in parentheses and Cj is the relative concentration of the alkane containing j carbon atoms. Tr.: traces.

As we have already mentioned, alkanes from the taxon *J. mollis*, the genus *Jurinea*, as well as from the Asteraceae subfamily Cichorioideae, have never been investigated to date. Comparison of the *J. mollis* alkanes' distribution with some other Asteraceae species, e.g., from the genus *Achillea* (subfamily Asteroideae), with a CPI values in the range of 6.87 to 11 and an ACL from 20.18 to 28.5, and maximum at *n*-C_{29} [8, 11], could provide a chemotaxonomic basis for the discrimination of the subfamilies. A more comprehensive chemical investigation of other *Jurinea* species is undoubtedly necessary to achieve a proper evaluation of the utility of the alkanes' profiles as chemotaxonomic traits at any taxonomic level.

GC and GC-MS Analysis. The GC-MS analysis was performed using a Hewlett-Packard 6890N gas chromatograph. The gas chromatograph was equipped with a fused silica capillary column DB-5MS (5% phenylmethylsiloxane, 30 m × 0.25 mm, film thickness 0.25 μm, Agilent Technologies, USA) and coupled with a 5975B mass selective detector from the same company. The injector and interface were operated at 250° and 320°C, respectively. The oven temperature was raised from 70° to 315°C at a heating rate of 5°C/min and then isothermally held for 20 min. As a carrier gas, helium at 1.0 mL/min was used. The samples, 1 μL of the fraction 1 solutions in chloroform (10 mg/100 mL), were injected (three repetitions) in a pulsed split mode (flow 1.5 mL/min for the first 0.5 min, then set to 1.0 mL/min throughout the remainder of the analysis; split ratio 40:1). The mass selective detector was operated at the ionization energy of 70 eV, in the 35–500 amu range with a scanning speed of 0.34 s. GC (FID) analysis was carried out under the same experimental conditions using the same column as described for the GC-MS. The percentage composition was computed from the total ion chromatogram peak areas without the use of correction factors. Qualitative analyses of fraction 1 constituents were based on the coinjection of authentic hydrocarbon standards (*n*-alkanes) and by comparison of mass spectra with reference spectra (Wiley 6, NIST02 databases). Linear retention indices were calculated relative to retention times of C_{17}–C_{39} *n*-alkanes on the DB-5MS column [12]. The *anteiso*- and *iso*-alkanes were identified by comparing their retention data to published chromatograms [13], by comparison of mass spectra, and by their fragmentation patterns, which generally show prominent ions for $[M - C_2H_5]^+$ and $[M - C_3H_7]^+$, respectively [13].

The IR measurements (ATR-attenuated total reflectance) were carried out using a Thermo Nicolet model 6700 FTIR instrument (Waltham, USA). IR spectrum (neat, ν, cm^{-1}): 2954, 2916, 2848, 1462, 1431, 1377, 1155, 1048, 969, 860, 775, 729, 719, 619.

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