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Alkanes from plants of the genus *Achillea*

RADOSAV PALIĆ,[1*] TIMOTHY I. EGLINTON,[2] BRYAN C. BENITEZ-NELSON,[2] GEOFFREY EGLINTON,[2] JASMINA VELIČKOVIĆ[1] and GORDANA STOJANOVIĆ[1]

[1]*Department of Chemistry, Faculty of Science, Ćirila i Metodija 2, 18000 Niš, Yugoslavia, and* [2]*Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA*

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The hydrocarbon fractions of three species of *Achillea* L. have been analysed by capillary gas chromatography (GC) and GC-mass spectrometry(GC-MS) and were shown to consist of the conventional, odd-carbon number dominant distributions of *n*-alkane homologues (C_{22}–C_{35}). *n*-Nonacosane (C_{29}) is the main compound (*ca.* 40%), and the carbon preference index (CPI) is high (*ca.* 11)

Key words: Achillea, n-alkanes, *n*-nonacosane.

The Genus *Achillea* L. includes about 100 species, spread mostly across the Euro-Asian Continent (the middle climate zone). In Serbia there are 24 species.[1] They have been used in traditional medicine for centuries. Chemical studies of these species have been concerned with essential oils,[2-8] sesquiterpenes,[9-11] alkamides[12] and flavonoides.[13] The present paper reports the content and composition of *n*-alkanes of three species of the *Achilea* genus: *A. lingulata, A. nobilis* and *A. crithmifolia*. *A. lingulata* is subcarpathian, *A. nobilis* is subpontiac, and *A. crithmifolia* is Pannonia-balkanic element of flora.

EXPERIMENTAL

Plant material. Plant material was collected in the blooming phase of vegetation. *A. critmifolia* was collected near Niš (location Selicevica), *A. lingulata* near Bosilegrad (location Milevska planina) and *A. nobilis* near Niš (location Kovanlucka Cuka Oblacina), all in Serbia. The leaves, flowers and green parts of the stems were air-dried for ten days at room temperature and kept in a cold and dark place until extracted.

Extraction and isolation. The dried plant material (200 g) was extracted with distilled petroleum ether (b.p. 40–70 °C) in a Soxhlet apparatus for 24 h. The extracts were evaporated to a small volume and dried for 2 h with anhydrous $MgSO_4$. After removal of $MgSO_4$, the extracts were evaporated under vacuum to constant weight. The extraction of *A. crithmifolia* gave 4.08 g (2.04%), *A. lingulata* 5.50 g (2.75%), and *A. nobilis* 6.12 g (3.06%) of residual extract. The extracts were separated on silica gel by CC (petroleum ether, b.p. 40–70 °C) into 8 fraction. The fractions were further characterized by

SiO_2 TLC using C_6H_6-EtOAc (9:1) as developer and visualization by treatment with 50% H_2SO_4. The fractions 3–5 were combined (on the basis of TLC), precipitated (×2) in Et_2O-Me_2CO and dried. The yields (based on the original extract) were 145 mg (3.55%, *A. crithmifolia*), 154 mg (2.80%, *A. lingulata*) and 132 mg (2.16%, *A. nobilis*).

Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GDC-MS). Each sample was transferred to a glass vial and made up to 4 ml in hexane (Fisher, GC Resolv.). The samples were vortexed for 90 s to completely dissolve the aliphatic material. Aliquots (25 μl) were then removed and diluted 10 fold with additional hexane in a GC autosampler vial. Each aliquot was analyzed by capillary gas chromatography. One aliquot was re-injected after adding *n*-tetracosane (86 ng/μl injection) and *n*-dotriacontane (94 ng/μl injection) standards (both high purity standards obtained from Applied Science Labs Inc., lot numbers 2185 and 2263 respectively).

Samples were injected (1 μl) into a Hewlett-Packard 5890 Series II gas chromatograph equipped with a HP 7673 autoinjector, a Gerstel programmable cooled injection system (CIS 3), electronic pressure control (He carrier gas flow of 1 ml min^{-1}), and two flame ionization detectors (FIC). Upon injection the samples were simultaneously introduced (Gerstel Dual Column Adapter) onto J&W DB-1 and DB-5 columns (both 60 m × 0.32 mm; film thickness, 0.25 μm). The GC oven was heated from 60 °C (1 min initial hold time) to 320 °C (50 min final time) at a rate of 6 °C min^{-1}. The CIS had an initial temperature of 30 °C (0.3 min hold time during solvent venting) and was heated at a rate of 12 °C s^{-1} to 350 °C (8 min final time) with solvent venting occuring during the first 0.3 min. GC-MS was performed using a HP 4890 series II gas chromatograph interfaced to a VG Fisions Autospec-Q double focussing mass spectrometer. A DB-5 capillary column was used with identical GC conditions to these above, with the exception than on-column injection was used to introduce the sample. The mass spectra were acquired at ≈ 50 eV across a scan range of 50–550 amu at a rate of ≈ 1 scan/s.

RESULTS AND DISCUSSION

The content and composition of alkanes of three species of *Achillea* genus: *A. lingulata, A. nobilis* L. and *A. critmifolia* were investigated by dual column, high-resolution GC. Identification was achieved by the coinjection of authentic hydrocarbon standards with independent confirmations by GC-MS. The results are summarized in Table I. Figure 1 shows partial GC traces (DB-5 stationary phase) of the alkane fractions from the three *Achillea* species. The samples contain relatively simple mixtures of hydrocarbons dominated by *n*-alkanes.

TABLE I. Percent composition of the *n*-alkane fractions of *Achillea* species

Alkanes	Carbon No.	*A. lingulata*	*A. nobilis*	*A. crithmifolia*
Docosane	22	0.34	0.09	0.12
Tricosane	23	6.13	1.88	2.78
Tetracosane	24	1.33	0.46	0.66
Pentacosane	25	13.25	4.84	8.31
Hexacosane	26	1.32	0.82	1.13
Heptacosane	27	20.06	17.15	20.84
Octacosane	28	2.45	2.83	2.95
Nonacosane	29	34.04	46.80	36.42

TABLE I. Contd.

Alkanes	Carbon No.	*A. lingulata*	*A. nobilis*	*A. crithmifolia*
Triacontane	30	2.11	2.92	2.62
Hentriacontane	31	16.43	19.58	20.98
Dotriacontane	32	0.56	0.74	1.24
Tritriacontane	33	1.59	1.44	1.66
Tetratriacontane	34	0.03	0.16	0.04
Pentatriacontane	35	0.35	0.29	0.26

The *n*-alkane distributions of these three species of *Achillea*[3] are odd carbon dominant, maximising at C-29 with a high CPI of *ca.* 11 and ACL of *ca* 28.5 (Table II). For comparison the principal *n*-alkanes of the *Satureja* genus[14] are *n*-C_{29} and *n*-C_{31} with a CPI of ≈5.5, providing some chemotaxonomic basis for distinguishing the different genera.

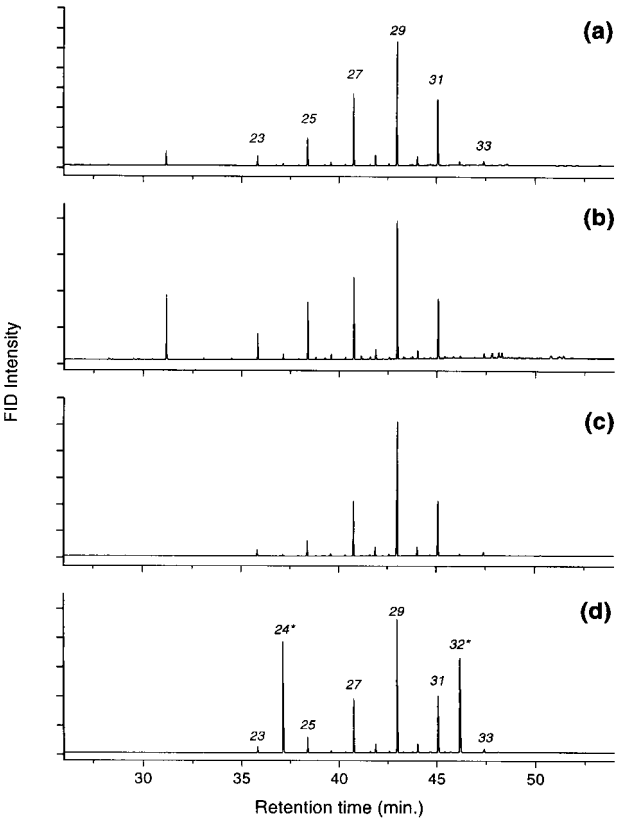

Fig. 1. Partial gas chromatograms (DB-5 stationary phase, FID detection) of *Achillea* alkane fractions: (a) *A. crithmifolia;* (b) *A. lingulata;* (c) *A. nobilis;* (d) *A. nobilis* co-injected with authentic C_{24} and C_{32} *n*-alkane standards. The *n*-alkane carbon numbers are indicated in italics. An asterisk * denotes an authentic standard.

TABLE II. Carbon Preference Index (CPI) and Average Chain Length (ACL) calculated using n-C_{22} to n-C_{35}

Sample	CPI	ACL
A. lingulata	11.28	28.00
A. nobilis	11.46	28.79
A. crithmifolia	10.27	28.55

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И З В О Д

АЛКАНИ ИЗ БИЉАКА РОДА *Achillea*

РАДОСАВ ПАЛИЋ,[1] TIMOTHY I. EGLINTON,[2] BRYAN C. BENITEZ-NELSON,[2] GEOFFREY EGLINTON,[2] ЈАСМИНА ВЕЛИЧКОВИЋ[1] и ГОРДАНА СТОЈАНОВИЋ[1]

[1]*Одсек за хемију, Филозофски факултет, Ћирила и Методија 2, 18000 Ниш,* Yugoslavia и [2]Department of Marine Chemistry and Geochemistry, Woods Hole Oceanoraphic Institution, Woods Hole, Massachusetts 02543, USA

Угљоводонична фракција три врсте рода *Achillea* L. анализирана је капиларном гасном хроматографијом (GC) и гасно-масеном спектрометријом (GC-MS). У испитиваним фракцијама доминирају n-алкани с непарним бројем С атома (C_{23}-C_{35}). n-Нонакозан је најзаступљенија компонента код све три испитиване врсте: *A. lingulata* (34,04%), *A. nobilis* (46,80%) и *A. crithmifolia* (36,42%). Угљенични преференцијални индекс (CPI) је висок: *A. lingulata* (11,28), *A. nobilis* (11,46) и *A. crithmifolia* (10,27). Просечна дужина низа је: A. lingulata (28,00), *A. Nobilis* (28,79) и *A. crithmifolia* (28,55).

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