Nonacosan-10-ol and \(n\)-Alkanes in Leaves of *Pinus pinaster*

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Abstract

In leaf cuticular wax of *Pinus pinaster*, content of nonacosan-10-ol is high (77.1% on average). \(n\)-Alkanes ranged from C\(_{18}\) to C\(_{35}\) with the most dominant C\(_{29}\) (24.8%). The carbon preference index (CPI\(_{total}\)) ranged from 3.1 to 5.6 (4.0 on average), while the average chain length (ACL\(_{total}\)) ranged from 14.0 to 17.0 (14.8 on average). Long-chain \(n\)-alkanes (\(n\)-C\(_{25-35}\)) strongly dominated (80.1%) over middle-chain \((n\)-C\(_{21-24}\) = 18.9%) and short-chain \((n\)-C\(_{18-20}\) = 0.9%) \(n\)-alkanes.

Keywords

*Pinus pinaster*, nonacosan-10-ol, \(n\)-alkanes, needles, waxes

Results and Discussion

Nonacosan-10-ol content is higher in spring (79.0%) than in autumn needles (75.2%). Mean value is 77.1%. Inside the subsection *Pinaster*, nonacosan-10-ol of *P. pinaster* is equal and is similar to nonacosan-10-ol of *P. halepensis* (77.1%), but higher than in wax of relic species, relic *P. heldreichii* (55.5%). \(n\)-Alkanes of *P. pinaster* ranged from C\(_{18}\) to C\(_{35}\), where C\(_{29}\) (24.8%, mean value) was the most dominant one (Figure 1; Table 1). Spring needles ranged from C\(_{18}\) to C\(_{35}\), while autumn needles ranged from C\(_{20}\) to C\(_{35}\). C\(_{29}\) was more abundant in spring (26.5%) than in autumn needles (23.0%). In *P. heldreichii*, this range is narrower, C\(_{18}\) to C\(_{32}\), with the most dominant C\(_{23}\).

The carbon preference index (CPI\(_{total}\)) of *P. pinaster* ranged from 2.6 to 5.6 (4.0 on average) (Table 1). Larger range was in spring needles. In *P. heldreichii*, it ranged from 0.8 to 3.1 (1.6 in average). All CPIs of *P. pinaster* (Table 1) exhibited odd/even predominance (OEP) (because CPI > 1 indicates OEP, CPI < 1 denotes OEP). The same situation was with *P. halepensis* which also belonged to subsection *Pinaster*.

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The average chain length (ACL total) in *P. pinaster* ranged from 14.0 to 17.0 (14.8 on average) (Table 1), where long-chain *n*-alkanes dominated (80.1%). Similar results were found in *P. halepensis*.18 In *P. heldreichii*, ACL total ranged from 20.9 to 26.5 (24.4 in average), where long-chain *n*-alkanes did not strongly dominate (middle chain: 37.9% and long chain: 49.6%).19

This study was done in small number of trees of *P. pinaster*. Full variability of nonacosan-10-ol and *n*-alkanes contents could be obtained in further population studies.

**Experimental**

**Plant Material**

Twigs with 1-, 2-, and 3-year-old needles from the lowest third of the full tree crown of several *P. pinaster* trees were collected in spring and autumn 2015 from Croatia, Island Korčula. The collected twigs were stored at –20°C prior to further needle analyses.

**Extraction of Needle Wax for the Investigation of Nonacosan-10-ol Content**

A concentrated sample of epicuticular wax was collected from each tree by immersing 3 g of needles in 10 mL of *n*-hexane (HPLC grade; Merck, Darmstadt) for 45 seconds. The samples were then dried under vacuum at 60°C, and aliquots of 1 mL of these samples were used to determine the nonacosan-10-ol content by gas chromatography-mass spectrometric (GC-MS) analysis.

**Extraction of Needle Wax for the Investigation of the n-Alkanes**

The concentrated extracts, obtained as described above, were chromatographed on small-scale columns using a Pasteur pipette filled with silica gel 60 (SiO₂, 0.2-0.5 mm; Merck) previously activated at –20°C. The wax samples were obtained by elution with 5 mL of hexane and stored at –20°C until further analysis.

**Gas Chromatography and Gas Chromatography-Mass Spectrometric Analyses of Needle Wax**

Gas chromatography and GC-MS analyses were performed using an Agilent 7890A GC equipped with an inert 5975C XL EI/CI mass selective detector and flame ionization detector (FID) connected by capillary flow technology 2-way splitter with make-up. A HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) was used. The GC oven temperature was programmed from 60°C to 315°C at a rate of 3°C/min and held for 15 minutes. Helium was used as the carrier gas at 16.255 psi (constant pressure mode). An auto-injection system (Agilent 7683B Series Injector) was employed to inject 1 μL of sample. The sample was analyzed in the splitless mode. The injector temperature and the detector temperature were 300°C. Mass
spectrometric data were acquired in the EI mode with scan range 30 to 550 m/z, source temperature 230°C, and quadrupole temperature 150°C; the solvent delay was 3 minutes.

**Identification of Needle Wax Components**

The components were identified based on their retention indices and comparison with reference spectra (Wiley and NIST databases) as well as by the retention time locking (RTL) method and the RTL Adams database. The retention indices were experimentally determined using the standard method of Van Den Dool and Kratz\(^27\) involving retention times of \(n\)-alkanes injected after the sample under the same chromatographic conditions. The relative abundance of the \(n\)-alkanes was calculated from the signal intensities of the homologs in the GC-FID traces.

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