Chemistry of Natural Compounds, Vol. 46, No. 1, 2010

IDENTIFICATION OF HYDROCARBONS FROM *Abies pindrow* LEAVES

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UDC 547.218

The genus *Abies* family Pinaceae consists of 51 species ranged mainly in temperate and boreal regions of the northern hemisphere, chiefly in mountainous regions [1]. The literature data on phytochemical and biological investigations of the genus of *Abies* revealed that up to now, 277 compounds were isolated from 19 plants of *Abies* species. The chemical constituents are mostly terpenoids, flavonoids, and lignans, together with minor constituents of phenols, steroids, and others. The crude extracts and metabolites have been found to possess various bioactivities, including insect juvenile hormone, antitumor, antimicrobial, antiulcerogenic, antiinflammatory, antihypertensive, antitussive, and CNS (central nervous system) activities [2].

Abies pindrow Royle, commonly known as west Himalayan fir, is a large evergreen tree growing up to 40–60 m tall with a trunk diameter of up to 2–2.5 m. It has a conical crown with level branches. In Pakistan, *Abies pindrow* Royle, known as partal or palundar, is widely distributed at elevations between 2000 and 3000 m throughout the western Himalayas from Afghanistan to Nepal [3]. *Pindrow* species of *Abies* is regarded as carminative, stomachic, astringent, expectorant, tonic, antispasmodic, and antiperiodic [4].

Phytochemical studies of the species resulted in the isolation of glucopyranoside, hydroxyflavanone, chalcone glycoside, bioflavonoids, flavonoids and pindrolactone [5], and pentacyclic triterpenoids [6]. Different extracts from the leaves of *Abies pindrow* Royle exhibited anti-inflammatory, analgesic, and hypnotic activities in rats, attenuated swim stress in mice, and produced hypotension in dogs. *Abies pindrow* Royle leaves also have an antiulcerogenic effect on the cold-restrained gastric ulcer model in rats [5]. A review of the literature revealed that no hydrocarbons have yet been reported from *Abies pindrow* Royle.

We have previously reported the fatty acid composition of the ethanol extract of the leaves of *Abies pindrow* Royle. A total of 11 fatty acids, including eight saturated and three unsaturated, was characterized [4].

The present investigation of aerial part (leaves) of *Abies pindrow* Royle describes the occurrence of long chain hydrocarbons. The presence of these hydrocarbons is detected by GC-MS and supported by FTIR. A careful look at the fragmentation pattern in the mass spectral data reveals the presence of saturated and unsaturated hydrocarbons. These hydrocarbons have been reported for the first time from *Abies pindrow* Royle.

The GC-MS of the hexane fraction revealed the presence of tricosane, eicosane, heneicosane, docosane, tetracosane, nonadecane, octadecane, 1-docosene, 1-octadecene, heptadecane, and 2,6,10,14-tetramethylhexadecane. The identity of these common hydrocarbons was made by comparison of these peaks with the standards by gas chromatography and confirmed by comparison of the fragmentation pattern with those of standard mass spectrum. FTIR analysis supported the structures of these hydrocarbons. The FTIR spectrum exhibits the diagnostic peaks relating to C-H stretching at 2956 cm^{-1}, 2923 cm^{-1}, and 2852 cm^{-1}, C-H bending (scissoring) at 1465 cm^{-1}, C-H methyl rocking at 1378 cm^{-1}, and long-chain methyl rocking at 722.9 cm^{-1}. These peaks verify the required data regarding the hydrocarbons.

The GC-MS study of major peaks revealed the presence of straight-chain saturated hydrocarbons. It showed the presence of 11 compounds. The GC-MS pattern showed 11 major peaks along with small peaks. All major peaks were detected as hydrocarbons by GC-MS at different retention times.

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 0009-3130/10/4601-0132

TABLE 1. Hydrocarbons Analyzed in the Leaves of *Abies pindrow*

Compound	Rt, min	%	Compound	Rt, min	%
Tricosane	16.995	41.2	Octadecane	06.907	2.8
Eicosane	10.588	23.8	1-Docosene	14.554	1.4
Heneicosane	12.624	11.5	Heptadecane	05.391	0.8
Docosane	14.679	6.2	1-Octadecene	10.463	0.8
Tetracosane	18.985	5.6	2,6,10,14-Tetramethylhexadecane	07.075	0.6
Nonadecane	08.663	3.1			

The molecular ion peaks and base peaks have been reported (Table 1). It showed the straight-chain nature of the compounds, which was confirmed by continuous removal of CH_2 units for all the higher hydrocarbons, the most common fragmentation path of these hydrocarbons.

The molecular ion peak of straight-chain, saturated hydrocarbons is always present, though of low intensity for long-chain compounds. The fragmentation pattern is characterized by a cluster of peaks, and the corresponding peaks of each cluster represent a C_nH_{2n+1} fragment and thus occur at *m/z* 14_{n+1}; this is accompanied by C_nH_{2n-1} fragments.

The saturated hydrocarbons (95.6%) were present in much greater proportion than unsaturated (2.2%) ones, and 2% hydrocarbon could not be detected. Tricosane, eicosane, heneicosane, docosane, tetracosane, nonadecane, and octadecane were found to be the major saturated hydrocarbons, and 1-docosene, and 1-octadecene as the predominant unsaturated hydrocarbons. Heptadecane and 2,6,10,14-tetramethylhexadecane were the next higher saturated hydrocarbons.

There are various methods for identifying the hydrocarbons of plants. Among them, GC-MS is one of the most commonly used techniques to determine the composition of the volatile oil of plants [7–9]. Not all organic compounds are suitable for direct GC-MS analysis due to their nonvolatile nature. Many important biological compounds, such as fatty acids, flavonoids, alkaloids, carbohydrates, amino acids, and terpenoids are polar and have limited volatility. The two main approaches adopted for the examination of analytes do not seem to satisfy the normal criteria of volatility for GC-MS. Either they are degraded under controlled conditions by pyrolysis to give characteristic volatile fragments, or they are derived from related compounds that are suitable for gas chromatography [10]. Low-molecular-weight hydrocarbons can be analyzed directly by GC-MS without volatile fragmentation.

The composition of hydrocarbons of *Abies pindrow* Royle is shown in Table1. As can be seen, tricosane (41.2%) and eicosane (23.8%) are the dominant hydrocarbons. A similarly higher content (0.1–18.720 ppm) of tricosane has been reported in plant species such as *Sambucus nigra* L., *Glycyrrhiza glabra* L., *Achillea millefolium* L., *Brassica oleracea var. gongylodes* L., *Pimenta dioica* (L.) Merr. *Pimenta racemosa* (Mill.) J. W. Moore, and *Tilia* sp by Duke [11]. However, low contents of tricosane (9.4%) and eicosane (6.6%) were found in *Ficus benghalensis* [7] and in *Achillea asplenifolia* (2.4%) [12].

A high content (0–120 ppm) of eicosane is also reported in species such as *Ageratum conyzoides* L., *Glycyrrhiza glabra* L., *Acacia farnesiana* (L.) Willd, *Anethum graveolens* L., *Brassica oleracea* var. *gongylodes* L., *Pimenta racemosa* (Mill.) J. W. Moore, and *Tilia* sp. [12].

Phytane-(2,6,10,14-tetramethylhexadecane), which constitute 0.6% of *Abies pindrow* Royle, is a diterpenoid alkane; it is used in organic geochemistry and in the chemistry of extraterrestrial materials as biological markers because of its stable isoprenoid skeleton and its structural relationship with terpanes and steroids. The nature of sources and the generation of phytane as biological markers are of considerable scientific interest. It has been assumed that this compound was derived mainly from the phytyl side chain of chlorophyll-a as well as from chlorophyll-b, bacteriochlorophyll-a, α- and γ-tocopherols, and carotenoid pigments [13].

Materials and Instruments. *Abies pindrow* Royle leaves collected from the Murree Hills (Punjab) were dried under shade at room temperature for two weeks. The specimen was identified and deposited in the herbarium of the Department of Biological Sciences, Quaid-i-Azam University, Islamabad.

The GC-MS analysis was performed on an Agilent 6890 N gas chromatography instrument coupled with an Agilent MS-5975 inert XL mass selective detector and an Agilent autosampler 7683-B injector (Agilent Technologies, Little Falls, NY, USA). A capillary column HP-5MS (5% phenylmethylsiloxane) with dimensions of 30 m × 0.25 mm i.d × 0.25 μm film thickness (Agilent Technologies, Palo Alto, CA, USA) was used. The initial temperature of 150°C was maintained for 2 min, raised to 230°C at the rate of 4°C/min, and kept at 230°C for 5 min. The split ratio was 1:50, and helium was used as a carrier

gas with a flow rate of 0.8 mL/min. The injector and detector temperatures were 240 and 260°C, respectively. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV in the scan range of 50–550 *m/z*.

FTIR spectra were obtained using a ThermoNicolet Avatar 330 FT-IR spectrometer controlled by OMNIC software (ThermoNicolet Analytical instruments, Madison, WI, USA) station with a deuterated triglycine sulfate (DTGS) detector and KBr optics. The sampling station was equipped with an overhead ATR accessory (Spectra-Tech, Shelton, CT) comprising transfer optics within the chamber through which infrared radiation is directed to a detachable ATR zinc selenide crystal mounted in a shallow trough for sample containment. A single-beam spectrum (4000–650 cm^{-1}) of the sample was obtained against air as a background at a resolution of 4 cm^{-1} and a total of 32 scans.

Extraction and Identification. The leaves of the plant were dried under shade and powdered (coarsely). The whole amount (2.5 kg approx.) was extracted with ethanol by percolation at room temperature for three times. The plant material was soaked for a week's period each time. The combined extract so obtained was evaporated at reduced pressure to afford a gummy residue (600 g). The residue was partitioned between ethyl acetate and water. The process was carried out five times. After separating both layers, the former ones were combined and evaporated under reduced pressure to get the ethyl acetate extract (205 g). The ethyl acetate extract was subjected to vacuum liquid chromatography, using silica gel (TLC grade), eluting with *n*-hexane, dichloromethane, methanol. The elution was carried out gradually by increasing the order of polarity. The fractions obtained by eluting the extract with *n*-hexane (100%) led to identification of 11 hydrocarbons.

ACKOWLEDGMENT

This research was supported by University of Sindh, Jamshoro, Sindh, Pakistan.

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