n-Alkane Profile of *Argemone mexicana* Leaves

Indranil Bhattacharjee[a], Anupam Ghosh[a], Nandita Chowdhury[a], Soroj Kumar Chatterjee[a], Goutam Chandra[a,*], and Subrata Laskar[b]

[a] Microbiology Research Unit, Parasitology Laboratory, Department of Zoology, The University of Burdwan, Burdwan-713104, West Bengal, India. E-mail: goutamchandra63@yahoo.co.in
[b] Natural Product Laboratory, Department of Chemistry, The University of Burdwan, Burdwan-713104, West Bengal, India

* Author for correspondence and reprint requests

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An *n*-hexane extract of fresh, mature leaves of *Argemone mexicana* (Papaveraceae), containing thin-layer epicuticular waxes, has been analysed for the first time by TLC, IR and GLC using standard hydrocarbons. Seventeen long-chain alkanes (n-C_{18} to n-C_{34}) were identified and quantified. Nonacosane (n-C_{29}) was established as the *n*-alkane with the highest amount, whilst octadecane (n-C_{18}) was the least abundant component of the extracted wax fraction. The carbon preference index (CPI) calculated for the hydrocarbon sample with the chain lengths between C_{18} and C_{34} was 1.2469, showing an odd to even carbon number predominance.

Key words: Alkane, Epicuticular Wax, Carbon Preference Index

Introduction

Protecting coats, so-called waxes, present on the surface of plant parts have an almost universal occurrence. Their main functions are to protect plants from physical damage and, especially from the loss of water (Schoonhoven *et al.*, 1998), with long-chain hydrocarbons/alkanes and their derivatives present as principle chemical constituents (Baker, 1982). Alkanes from epicuticular waxes are used as indicators of taxonomic relations between plant species, and now attention has been directed towards the possibility of using their distribution as a means of establishing a chemotaxonomic system.

Argemone mexicana L. (Papaveraceae), commonly known as prickly poppy, is a common medicinal plant. The antibacterial potential of an alcoholic and aqueous extract of the plant leaves has been documented by Bhattacharjee *et al.* (2006). The petroleum ether extract of the plant exhibited larvicidal activity against laboratory-colonized and field-collected *Culex quinquefasciatus* larvae (Karmegam *et al.*, 1997). But neither the chemical composition nor the concentration of aliphatic compounds present in the thin-layer epicuticular wax of this plant have been determined so far. The aim of the present paper was to isolate only the alkane fraction by application of a nonpolar solvent (hexane) and to characterize the *n*-alkane profile of the epicuticular waxes of leaves of this plant by gas liquid chromatography (GLC).

Material and Methods

Wax extraction

Fresh, mature leaves of *A. mexicana* were harvested randomly during July 2008 from plants growing in the surroundings of Department of Zoology, University of Burdwan, India. 50 g freshly collected mature leaves of *A. mexicana* were rinsed with distilled water and dried on paper towelling. Leaves were then dipped in 2 L of cold *n*-hexane for 45 min, and surface wax was extracted at room temperature. The crude extract was filtered through Whatman filter paper no. 41 (Whatman, Maidstone, UK), and the solvent was removed under reduced pressure. The extract was then fractionated by preparative thin-layer chromatography (TLC) using carbon tetrachloride as mobile phase. The thin-layer chromatographic plates (thickness of 0.5 mm) were prepared with silica gel G (Merck, Mumbai, India) using a Unoplan coating apparatus (Shandon, London, UK). The single hydrocarbon band was identified through co-TLC studies with standard samples

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(Sigma, St. Louis, MO, USA). The band was eluted from the layer with chloroform; it showed no absorption of any detectable functional group in the infrared region. The presence of alkanes was further confirmed by argentometric TLC.

GLC analysis of surface wax

The purified hydrocarbon fraction was analysed directly by GLC on a Hewlett Packard (Palo Alto, CA, USA) model 5890 series II instrument fitted with an HP-1 capillary column (25 m × 0.01 mm internal diameter) and a flame ionization detector; nitrogen was used as carrier gas at a flow rate of 16.5 mL/min. Oven temperature was 170–300 °C at a 5 °C/min rise, initial period was 1 min, and the final period was 15 min. Components were characterized through co-TLC with authentic samples of *n*-alkanes (Sigma).

All solvents employed were of analytical grade and obtained from Merck (Mumbai, India).

Results and Discussion

Fig. 1 shows the GLC separation of the hydrocarbons of the *n*-hexane extract of the epicuticular wax of mature leaves of *A. mexicana*. Seventeen long-chain alkanes (n-C_{18} to n-C_{34}) were identified and quantified. The carbon preference index (CPI) calculated for the hydrocarbon sample with the chain lengths between C_{18} and C_{34} was 1.2469, showing an odd to even carbon number predominance.

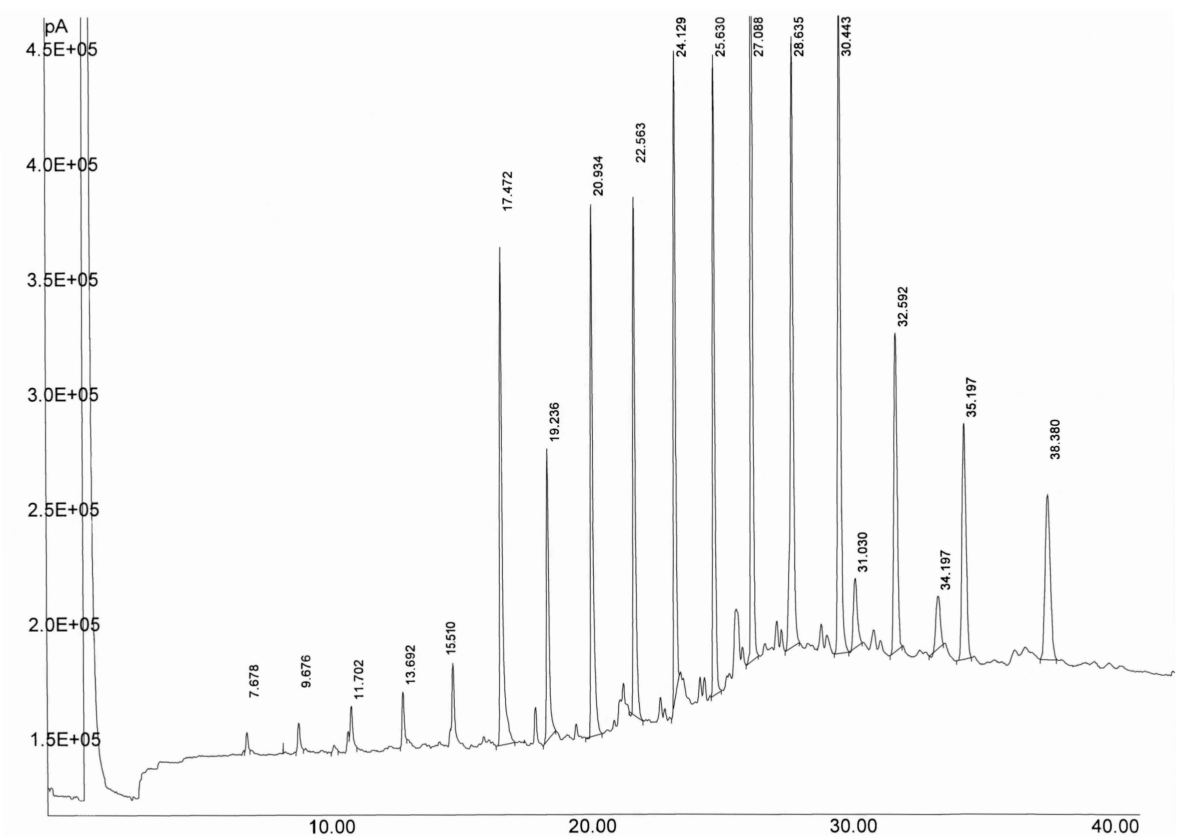

Fig. 1. Gas liquid chromatogram of the *n*-hexane extract of fresh, mature leaves of *A. mexicana.*

The surfaces of leaves, flowers, fruits, and non-woody stems are covered by a cuticle made of cutin and waxes (Jetter *et al.*, 2006). Among the cuticular wax layer, an intracuticular and an epicuticular layer can be distinguished according to the wax location inside the cutin matrix and exterior to it, respectively (Jeffree, 1986). Epicuticular waxes serve many physiological functions, including protection against UV light (Reicosky and Hanover, 1978) and moderation of gas exchange through stomata (Jeffree *et al.*, 1971). Besides, the epicuticular waxes form the true surface of the plant organs and, therefore, play an important ecological role in the interaction with insects (Müller, 2006) and pathogens (Carver and Gurr, 2006). There are significant differences in the individual and total *n*-alkane concentrations in plant leaves (Jetter *et al.*, 2000; Piasentier *et al.*, 2000; Barik *et al.*, 2004; Jetter and Schäffer, 2001). Higher alkanes are known to be one of the main components of cuticular waxes of plant leaves and stems. Moreover, in the homologous series the hydrocarbon fraction with an odd number of carbon atoms predominates considerably (Isidorov and Vinogorova, 2003; Buschhaus *et al.*, 2007). Further, Hellmann and Stoesser (1992), Dutton *et al.* (2000), and Piasentier *et al.* (2000) reported that heptacosane (n-C_{27}), hentriacontane (n-C_{31}), and tritriacontane (n-C_{33}) were the most prominent alkanes present in the epicuticular wax. However, our study produced different results as nonacosane (n-C_{29}) was established as the *n*-alkane with highest amount, whilst octadecane (n-C_{18}) was the least abundant (Table I).

Table I. Distribution of *n*-alkane constituents in the *n*-hexane extract of mature leaves of *A. mexicana*.

Peak	Compound		Retention time [min]	Amount (mol%)
1	Octadecane	(n-C_{18})	7.678	1.2080
2	Nonadecane	(n-C_{19})	9.676	1.4770
3	Eicosane	(n-C_{20})	11.702	1.6337
4	Heneicosane	(n-C_{21})	13.692	2.2129
5	Docosane	(n-C_{22})	15.510	1.0270
6	Tricosane	(n-C_{23})	17.472	7.1622
7	Tetracosane	(n-C_{24})	19.236	3.8396
8	Pentacosane	(n-C_{25})	20.934	6.9558
9	Hexacosane	(n-C_{26})	22.563	6.4799
10	Heptacosane	(n-C_{27})	24.129	7.4189
11	Octacosane	(n-C_{28})	25.630	7.5132
12	Nonacosane	(n-C_{29})	27.088	10.6814
13	Triacontane	(n-C_{30})	28.635	9.4555
14	Hentriacontane	(n-C_{31})	30.443	10.4134
15	Dotriacontane	(n-C_{32})	32.592	5.8923
16	Tritriacontane	(n-C_{33})	35.197	5.2423
17	Tetratriacontane	(n-C_{34})	38.380	4.8462

In conclusion, *A. mexicana* is a medicinal plant with established antimicrobial and mosquito-larvicidal property found in different parts of the world. The extraction of epicuticular wax by a nonpolar solvent made it possible to broaden greatly the list of compounds detected previously. This analysis also provides a basis for further research on interactions between leave surfaces of this plant and insect herbivores or pathogenic microorganisms.

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