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Hydrocarbons in the surface wax of the leaves of *Alstonia scholaris* (Linn.) R.Br.

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ABSTRACT

A n-hexane extract of fresh, mature leaves of *Alstonia scholaris* (Linn.) R. Br containing a thin layer of epicuticular waxes, has been analysed for the first time by TLC, IR and GC using standard hydrocarbons. The leaves contained 18 identified long chain (C_{17} -C_{34}) n- alkanes. The predominant n-alkane was C_{31} (46.43%) and C_{33} (21.85%), while C_{29} (6.16%), C_{32} (4.28%), C_{25} (3.74%) were moderately abundant. The C_{17} (0.39%) and C_{22} (0.44%) n-alkanes were present only in minor amounts.

Key words: *Alstonia scholaris*, epicuticular waxes, leaves, hydrocarbons.

INTRODUCTION

The devil's tree also known as Dita bark tree or Chatim (*Alstonia scholaris*) is a large buttressed evergreen tree 12-25 m in height having leaves[1-7] arranged in whorl. Various parts of the tree is known to have magical healing and curative properties for a long time. It is used as good substitute for cinchona and quinine for the treatment of intermittent and remittent fevers[1]. An infusion of the bark is given in fever, dyspepsia, debility, skin diseases, liver diseases, diarrhea and dysentry[1]. A decoction of the young leaves is used in beri-beri and congestion of liver[2]. The leaves are also used for treatment of dropsy and ulcers[2]. It is well known that surface wax of the leaves of a plant plays an important role in affecting transportation and leaf surface properties although its composition may vary with environmental condition and also with the age of the plant[3-5]. The universal presence of normal alkanes as constituents of cuticular leaf wax is well established and their distributions are considered as taxonomic markers[6-11].But this approach has not always met with unambiguous success[3,4,12-14].Though the alkaloid composition of the leaves is known,but neither the chemical composition nor the concentration of the aliphatic compounds present in the thin epicuticular (surface) waxes which make the leaves shiny have been determined. The aim of this present study was to characterize the n-alkane profile of epicuticular waxes by TLC, IR spectroscopy and GC techniques.

MATERIAL AND METHODS

Plant material and chemicals

Fresh, mature (4-8 week old) leaves of *Alstonia scholaris* were harvested randomly during the rainy season (mid June to mid August, 2008) from in and around Burdwan ($23^{o}16^{/}$; 87^{o} $54^{/}$). All solvents employed were of analytical grade and purchased from E. Merck (India). Aluminium oxide F-20 grade of Alcoa, Francfurt, Germany was used and the silica gel 'G' was purchased from E. Merck (India).

Isolation and purification of the epicuticular leaf wax

Leaves were initially rinsed with distilled water and dried on paper towel. A sample (100 gm) of dried leaves was dipped in 1L of n-hexane for 45 mins at room temperature. The crude extract was passed through Whatman No. 41 filter paper and the solvent was removed under reduced pressure. The extract was further passed through a column of aluminium oxide and eluted with light petroleum. The eluent was fractioned by TLC on silica gel G layers (thickness 0.5 mm) which had been prepared using a Unopan coating apparatus (Shandon, London) with carbon tetrachloride as mobile phase. The single wax band was eluted from the layer with chloroform and subjected to Argentometric TLC[15] that showed no absorption of detectable functional groups by IR spectroscopy. The purified hydrocarbon was kept in the freeze for further analysis.

GC analysis

The purified hydrocarbon fraction was analysed directly by GC on a Hewlett Packard (HP; Palo Alto, CA, USA) model 5890 series II instrument with a HP-1 capillary column (25m × 0.01 mm i.d.) and a flame ionization detector. The oven temperature program was: initially 170°C held for a minute, then raised at 5°C /min to 300°C and finally held for 15 min. The carrier gas was nitrogen at a flow rate of 16.5mL/min. Components were characterized by co-elution with authentic n-alkane standards obtained from Sigma Chemical Co. (USA). The percentage composition of the n-hydrocarbon present was computed from the GC peak areas. The result of the analysis is tabulated in Table 1.

Table 1: Distribution of the hydrocarbon constituents of the Leaf Surface Waxes of *Alstonia scholaris*

n-alkanes (Carbon number)	Relative %
C_{17}	0.33
C_{18}	0.65
C_{19}	0.73
C_{20}	0.90
C_{21}	0.73
C_{22}	0.45
C_{23}	0.60
C_{24}	0.81
C_{25}	3.74
C_{26}	1.03
C_{27}	1.67
C_{28}	1.53
C_{29}	6.17
C_{30}	2.82
C_{31}	46.43
C_{32}	4.28
C_{33}	21.85
C_{34}	0.73
Total n-alkanes	95.45
Branched chain alkanes	4.55
Normal : Branched hydrocarbon	20.98 : 1
Odd : Even numbered hydrocarbons	6.23 : 1

RESULTS AND DISCUSSION

The GC separation of hydrocarbons in an n-hexane extract of the epicuticular wax of mature leaves of *Alstonia scholaris* identifies eighteen long chain alkanes (C_{17}-C_{34}). These alkanes have been quantified in Table 1 representing 95.45 % of the hydrocarbons present. The remaining 4.55% was made up of an unknown number of unidentified branched chain alkanes. C_{31} (46.43%) was the most abundant n-alkane and C_{17} (0.33%) was the least abundant n-alkane identified. C_{33} (21.84%) was fairly abundant and C_{29} (6.16%), C_{32} (4.28%) and C_{26} (3.74%) were moderately abundant in the thin epicuticular layer waxes in the fresh mature leaves of the tree. The exhibited n-alkane distribution pattern of the leaves of *A. scholaris* is very much conformity with that expected for higher plants, as the odd numbered n-alkanes in the range C_{25}- C_{33} are predominating.

The ratio of normal : branched hydrocarbon was found to be 20.98 : 1 and that of odd : even numbered hydrocarbon was found to be 6.23 : 1 (Table 1). Keeping all these above fact in consideration, it may be concluded that this is the characteristic feature of a higher plant[16,17].

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