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DETERMINATION OF N-ALKANE CONSTITUENTS IN THE EPICUTICULAR WAX OF LEAVES AND YOUNG PODS OF *CASSIA ALATA* OFTEN INFESTED BY LARVAE OF *CATOPSILIA PYRANTHE*.

Dr. Irani Biswas*[1], Prof. Subrata Laskar[2], Prof. Ambarish Mukherjee[3]

[1]Department of Botany, M.U.C. Women's College, Burdwan – 713104 (W.B.), India.

[2](Retired), Department of Chemistry, The University of Burdwan, Burdwan –713104 (W.B.), India.

[3]Department of Botany, The University of Burdwan, Burdwan –713104 (W. B.), India.

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***Corresponding Author**
Dr. Irani Biswas
Department of Botany,
M.U.C. Women's College,
Burdwan – 713104 (W.B.),
India.

ABSTRACT

The present communication is a first time investigation on the n-alkane compositions of the epicuticular wax of leaves and young pods of *Cassia alata*, a leguminous medicinal plant often attacked by the larvae of the butterfly *Catopsilia pyranthe* (mottled emigrant butterfly) which voraciously feed upon the leaves and young pods of this plant during its flowering and fruiting time. The normal chained hydrocarbons (n-alkanes) in these plant parts ranged from C_{12}-C_{27}. The cumulative percentage of low volatile n-alkanes (C_{12}-C_{23}) showed much higher values than the medium chained n-alkanes (C_{24}-C_{27}) which perhaps recalls the characteristics of herbivore induced plant volatiles (HIPV). These are complex blends of different volatile organic compounds (VOC) including alkanes which are usually produced by host plants infested by herbivorous insects. The overall findings not only update the phytochemical database of surface lipid of the plant for the first time in terms of n-alkane analyses but might also provide certain cues towards host recognition of the plant in insect herbivory.

KEYWORDS: *Cassia alata*, epicuticular wax, n-alkane, host recognition cues.

INTRODUCTION

Cassia alata Linn. is a pantropical, ornamental, shrubby weed belonging to family Caesalpiniaceae which gregariously grows along the roadsides, wastelands, agricultural fields etc. In traditional medicine, they are used against various skin ailments like ringworm, psoriasis, dermatitis and eczema.[1,2] Chemical investigations are confined to anthraquinones, flavonoids and phenolic compounds only.[1,2] The leaves and immature seeds of young pods of *Cassia alata* are usually fed upon by the caterpillars of the butterfly *Catopsilia pyranthe* (mottled emigrant butterfly) which regularly visit the plant as a pollinator. It is a member of family Pieridae, sub-family Coliadinae (Lepidoptera: Rhopalocera). The butterfly preferably chooses the different species of *Cassia* including *C. alata* for oviposition and rearing of their larvae.[3] It has also been observed that the developmental stages of another related species of *Catopsilia* butterfly, *C. crocale* is found to be associated with the flowering and fruiting stages of *C. alata* plants.[4] It is well established that the plant epicuticular surfaces play a key role in affecting such behaviour of the insect after its landing[5,6] and the epicuticular layer mainly consists of a complex mixture of straight and branched chain alkanes, esters, aldehydes, alcohols and fatty acids.[7,8] Since there are reports that alkanes play interesting role in plant-insect interactions[9,10] and also serve as palatable larval feed for growth and development of herbivorous insect pests,[11,12] the present authors preliminarily investigate only the n- alkane composition of the epicuticular wax of the leaves and young pods of *C. alata* for the first time which not only update the phytochemical database in terms of surface lipid analyses but also pave the way for further investigation of the identified n-alkanes of *C. alata* as probable host recognition cues in insect herbivory.

2. MATERIALS AND METHODS

2.1. Plant materials and Chemicals: Fresh mature leaves of *C. alata* and green young pods were collected from specific sites within Burdwan district (23°14'24.75″ N and 87°52'02.54″E) during the middle of the month of October in the year 2015. Identification and authentication of the plants were done by Prof. Ambarish Mukherjee, Department of Botany, Burdwan University and the voucher specimens in their flowering and fruiting stages (Biswas CAFl.1, Biswas CAFr.2) have been deposited in the Departmental Herbarium bearing acronym BURD for future reference. All the solvents employed were of analytical grades purchased from E.Merck (Mumbai, India) and the standard hydrocarbon samples were purchased from Supelco, USA.TLC plates (0.5mm thickness) were prepared with silica gel G (Merck, India) using TLC apparatus with roller.

2.2. Wax extraction: 100g of fresh mature leaves and young green pods were collected in three replicates each during the month of October and dipped separately in 1 litre each of cold n-hexane for 45 minutes[13] at room temperature to extract the epicuticular wax. The wax extract was filtered through Whatman filter paper no. 40 and the solvent was removed under reduced pressure to obtain the dry wax. The wax was weighed in each case. The extracted wax in each case was fractionated by preparative TLC (thickness 0.5mm) with carbon tetrachloride as the mobile phase. The single hydrocarbon band (R_f = 0.85-0.90) was detected through co–TLC studies with standard n-alkane samples. The band was eluted with chloroform and it showed no absorption for any detectable functional group in the infrared region and the absence of alkenes was further confirmed by argentrometric TLC thus clearly indicating the presence of only alkanes. Weight of the hydrocarbons obtained from the respective epicuticular wax were also taken and expressed as percentage of the total extractable wax. The amount of crude wax and hydrocarbon obtained there from are presented in Table1. All the values are mean of three replicates and the results are expressed as standard error of mean.

2.3. GLC analysis of hydrocarbon samples

The purified hydrocarbon fractions obtained in each case were analyzed by GLC on a Hewlett Packard Gas Chromatograph ([HP] Palo Alto, CA, USA) Agilent 6890 N Series instrument fitted with a HP- 5 capillary column (30m x 0.25mm internal diameter) and a flame ionization detector with nitrogen as the carrier gas at a flow rate of 16.5 ml min^{-1}. The oven temperature was 170 – 300^0C at a 5^0C min^{-1} rise, the initial period was 1 min and the final period was 15 min. Volume of the gas injected was 1μl with a split ratio of 1: 20. Components were characterized by co-elution with standard n-alkane samples and they were quantified by comparison of their retention times with those of standard n-alkane samples. The n-alkane constituents were expressed as their relative percentages and the results were tabulated as Mean ± S.E. in Table 2.

3. RESULTS AND DISCUSSION

The amount of surface wax and percentages of hydrocarbons obtained from crude wax of leaves and young pods are presented in Table 1 and the gas liquid chromatographic (GLC) analyses of the respective hydrocarbons expressed as their relative percentages are presented in Table 2. A total of fifteen n-alkanes from C_{12} to C_{27} are identified which are present in variable amounts.

Table 1. Variation in amount of epicuticular wax and surface *n*-alkanes obtained from leaves and young pods of *Cassia alata*.

Parts of the plant	Leaves	Young green pods
Amount of surface wax (in mg/100g fresh wt.)	149 ± 0.21	55 ± 0.21
Obtained hydrocarbon (in %)	7.54	18.18
Other constituents of surface wax (in %)	92.46	81.82

All the values are expressed as Mean ± S.E, n=3.

Table 2. GLC analyses of the n-alkane constituents in the epicuticular wax of leaves and young pods of *Cassia alata* expressed as relative percentages.

Name of the n-alkanes	*Leaves	*Young pods
Dodecane (C_{12})	----	1.68 ± 0.02
Tridecane (C_{13})	12.49 ± 0.02	1.23 ± 0.02
Tetradecane (C_{14})	8.91 ± 0.03	----
Pentadecane (C_{15})	1.32 ± 0.01	0.27 ± 0.01
Hexadecane (C_{16})	5.84 ± 0.06	0.76 ± 0.06
Heptadecane (C_{17})	----	0.69 ± 0.04
Octadecane (C_{18})	0.93 ± 0.11	31.17 ± 0.11
Nonadecane (C_{19})	2.62 ± 0.12	7.17 ± 0.12
Eicosane (C_{20})	10.16 ± 0.12	----
Heneicosane (C_{21})	1.02 ± 0.18	38.66 ± 0.18
Docosane (C_{22})	1.32 ± 0.23	----
Tricosane (C_{23})	16.08 ± 0.12	3.96 ± 0.12
Tetracosane (C_{24})	----	5.47 ± 0.04
Pentacosane (C_{25})	----.	----
Hexacosane (C_{26})	3.43 ± 0.06	----
Heptacosane (C_{27})	----	1.16 ± 0.02
Total n-alkanes	64.12 ± 0.12	92.22 ± 0.14
Branched chain alkanes	35.88 ± 0.12	7.78 ± 0.14
Ratio of normal to branched hydrocarbons	1.79: 1	11.85:1
Ratio of Odd and Even numbered hydrocarbons (CPI value)	1.1:1	1.36:1

*All the values are expressed as Mean ± S.E, n=3. '----' means absent.

The percentages of hydrocarbon in leaves (7.54%) and young pods (18.18%) are in conformity with the variation in relative percentages of their hydrocarbon composition i.e. the total amount of n-alkanes are appreciably high in young pods (92.22%) than that of leaves (64.12%). The carbon preference index (CPI) values are found to be low being 1.1 in leaves and 1.36 in young pods and such low range of CPI values can be well correlated with certain plants associated with insect herbivory.[10, 14 15,16] The cumulative percentage of short chain low volatile n-alkanes (C_{12}-C_{23}) are higher in leaves (60.69%) as well as in pods (85.59%) as compared to those of the medium chained hydrocarbons (C_{24}-C_{27}) which are 3.43% and 6.63% in leaves and pods respectively. This recalls the characteristics of herbivore induced plant volatiles (HIPV) which are complex blends of a variety of volatile organic compounds (VOC) including alkanes that are usually produced by those parts of the host plants severely attacked by insect herbivores.[17] Likewise, Schoonhoven et al. (2005) suggested that low molecular weight hydrocarbons in the range C_9-C_{21} present in the lipid layer of plant cuticle surface act as chemical barrier to insect attack. Some legume species are also reported to produce HIPVs when oviposition & host feeding occur simultaneously and are produced in high doses both by damaged & undamaged plant parts.[19,20] On the other hand, the presence of n-alkanes like C_{18}, C_{21}, C_{23}, C_{24}, C_{26} and C_{27} in leaves and pods might suggest their role as oviposition stimulants since there are reports of such n-alkanes which have elicited oviposition responses in lepidopteran insects.[9,10,14]

4. CONCLUSION

The present study updates the phytochemical data of *C. alata* for the first time in terms of n-alkane constituents of surface lipids of its plant organs which are frequently attacked by herbivorous insects. Since there are reports as mentioned in the introductory part that the leaves of certain plants serve as palatable larval feed for growth and development of herbivorous insect pests like *Diacrisia oblique* and *D. casignatum*, so in this context the compositional variation of short and medium chained low volatile n-alkanes in leaves and young pods of *C. alata* may be looked upon as an important finding likely to be helpful in future investigation where such alkanes might prove their potential as host recognition cues for the larva of *Catopsilia pyranthe* (Linn.) which selectively feeds, breeds and develops upon these plants.

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