Epicuticular Wax of *Tamarix aphylla* L.

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Summary: Epicuticular wax from the leaves of *Tamarix aphylla* L. was extracted using *n*-hexane as a solvent and its yield was found to be 0.34 %. The wax constituents were characterized and identified by GC/MS and NMR spectroscopy. The major components were free fatty acids (60.45 %) and free alcohols (20.23 %) together with minor amounts of *n*-alkanes (2.33 %); *br*-alkanes (1.68 %); alkenes (0.30 %); aromatic hydrocarbon (0.34 %); esters (3.22 %); dihydroxy ester (2.11 %); aldehydes (5.37 %); benzofuranone (0.07 %) and oxirane (3.89 %).

Introduction

Tamarix aphylla L. is a fast growing evergreen tree belonging to the family Tamaricaceae which is widely distributed in Africa, Middle East and South Asia [1]. The aerial surfaces of all higher plants are covered by a layer of cuticular wax [2]. Epicuticular waxes cover the external side of the leaf epidermis of all higher plants [3]. Although the primary role of cuticular waxes is to prevent uncontrolled water loss, a more interesting function is their contribution to plant-insect interactions [4]. Surface waxes are mostly comprised of alicyclic and long chain aliphatic components that can be further classified according to their structure, functional groups and distribution of their dominant homologues. Hydrocarbons are one of the most ubiquitous wax class being present in almost all plant surface waxes in percentages varying from traces to over 50% of the whole wax [5]. Other compounds found in these waxes include aldehydes, ketones, acetates, β-diketones, primary and secondary alcohols, esters, different diterpenes and triterpenes including triterpene acetates [6-8]. Sterols have also been isolated from several waxes [8-10]. Many investigations have been made on epicuticular waxes of different plants but a very little work has been done on *Tamarix aphylla*. Surface extracts from the primary leaves of Castor bean were found to contain alkanes (C_{26}-C_{29}), primary alcohols (C_{22}-C_{38}), aldehydes (C_{26} and C_{28}), fatty acids (C_{20}-C_{34}) and triterpenoids (lupeol, β- and α-amyrin) [11]. The principle components of the wax from potato (*Solanum tuberosum*) leaves were very long chain include *n*-alkanes, 2-methyl alkanes and 3-methyl alkanes, primary alcohols, fatty acids and wax esters [12]. Quantity and composition of the wax on the leaves of salt cedar (genus *Tamarix*) is thought to be the basis for differences in sensitivity to herbicides [13]. In addition, the quantity and composition of the wax on leaf surfaces of tamarisk (*Tamarix*) varies during the season [14]. The present investigations were carried out for the characterization of epicuticular wax of *Tamarix aphylla* L.

Results and Discussion

The percentage yield of epicuticular wax from *Tamarix aphylla* leaves, extracted with *n*-hexane was 0.34 %. Various typical plant wax constituents were identified, including very long-chain alkanes, fatty acids, esters, primary alcohols and aldehydes. The approximate composition of the epicuticular wax with relative abundance of the constituents is given in Table-1.

Hydrocarbons

The alkane composition of epicuticuticular wax of *Tamarix aphylla* did not vary in relative abundance. The alkanes accounted for 4.01% of the total wax which included *n*-alkanes (58.10%) and *br*-alkanes (41.90%) ranging from C_{14} to C_{43}. The even-numbered homologues (71.82%) were more abundant as compared to odd-numbered homologues (28.18%). The dominating component among *n*-alkanes was tetradecane with 0.56% and among *br*-alkanes was eicosane-7-hexyl with 1.46% of the total wax. All of

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Table-1: Composition of epicuticular wax of *Tamarix aphylla* L.

Sr. No.	Compound Name	Type	$[M]^+$/Base Peak (m/z)	Retention Time(min.)	Area%
1	3-Ethenyl-5,5-dimethyl hexyl benzene	Alkenylalkylarene	216/104	3.172	0.34
2	Tridecanal	Alkanal	198/57	3.300	0.06
3	10-Methyleicosane	*br*-Alkane	296/57	3.393	0.09
4	2,3,5,8-tetramethyldecane	*br*-Alkane	198/57	3.570	0.13
5	Heptanal	Alkanal	114/44	3.841	0.04
6	Tetradecane	*n*-Alkane	198/57	3.974	0.56
7	Pentadecane	*n*-Alkane	212/57	6.022	0.23
8	5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2[4*H*]-Benzofuranone	Benzofuranone	180/111	7.120	0.07
9	Hexadecane	*n*-Alkane	226/57	8.770	0.20
10	Heptadecane	*n*-Alkane	240/57	12.183	0.18
11	Octadecane	*n*-Alkane	254/57	16.113	0.18
12	Nonadecane	*n*-Alkane	268/57	20.377	0.15
13	Hexadecanoic acid methyl ester	Alkylalkanoate	270/74	21.430	0.26
14	*n*-Hexadecanoic acid(Palmitic acid)	Alkanoic acid	256/73	23.135	16.05
15	Hexadecanoic acid ethyl ester	Alkylalkanoate	284/88	24.473	0.73
16	Eicosane	*n*-Alkane	282/57	24.797	0.16
17	9,12-Octadecadienoic acid methyl ester	Alkylalk-dienoate	294/67	28.739	0.39
18	11,14,17-Eicosatrienoic acid methyl ester	Alkylalk-trienoate	320/79	28.964	0.47
19	Heneicosane	*n*-Alkane	296/57	29.254	0.13
20	9,12-Octadecadienoic acid (Linoleic acid)	Alk-dienoic acid	280/67	30.716	25.15
21	9,12,15-Octadecatrioenoic acid	Alk-trienoic acid	278/79	30.910	14.83
22	7-Tetradecenal	Alkenal	210/55	31.116	5.27
23	2,3-dihydroxy-9,12-Octadecadienoic acid-propyl ester	Dihydroxyalkylalkanoate	354/67	31.747	2.11
24	Octadecanoic acid	Alkanoic acid	284/55	31.944	4.08
25	2-(9-Octadecenyloxy)ethanol	Alkenyloxyalkanol	312/55	33.001	0.47
26	1,19-Eicosadiene	Alk-diene	278/55	33.252	0.15
27	Tetratriacontane	*n*-Alkane	478/57	33.658	0.19
28	Pentatriacontane	*n*-Alkane	492/57	37.961	0.20
29	7-Hexyl-Eicosane	*br*-Alkane	366/57	38.547	1.46
30	3,13-Octadecadien-1-ol	Alk-dienol	266/55	40.013	0.39
31	9,12,15-Octadecatrien-1-ol	Alk-trienol	264/79	40.273	0.30
32	Eicosanoic acid	Alkanoic acid	312/43	40.495	0.34
33	Di-*n*-Octyl phthalate	Dialkylphthalate	390/149	47.351	1.37
34	Heptadecyloxirane	Oxirane	282/82	50.062	3.89
35	Triteracontane	*n*-Alkane	604/57	51.657	0.15
36	1-Tetracosanol (Lignoceric alcohol)	Alkanol	354/67	70.714	18.97
37	17-Pentatriacontene	Alkene	490/57	76.243	0.15
38	1-Hentetracontanol	Alkanol	592/57	79.887	0.10

the alkanes showed a base peak at m/z 57 in their mass spectra. The ^{1}H-NMR, ^{13}C-NMR and MS data of the isolated component confirmed the presence of a long chain *n*-alkane tetratriacontane (0.19 %) (**2**). The relative abundance of alkanes was less as compared to other classes of compounds present in *Tamarix aphylla* epicuticular wax.

The linear hydrocarbons are sometimes accompanied by small proportions of some monounsaturated hydrocarbons, common to many plants [2]. The alkenes and alk-dienes detected were 0.64% of the total wax and comprised of 17-pentatriacontene (0.15%) and 1,19-eicosadiene (0.15%). An aromatic hydrocarbon 3-ethenyl-5,5-dimethyl hexyl benzene) was also detected with 0.34%.

Esters

The esters detected were 5.33% of the total wax. The main esters were methyl, ethyl, dioctyl and dihydroxy propyl esters of even-numbered saturated and unsaturated hexadecanoic, octadecanoic, eicosanoic and phthalic acids. Methyl and ethyl estrers of fatty acids have also been reported from some eragrostoid grasses [15] and the roots of boraginaceous species with medicinal applications [16]. Methyl esters of saturated and unsaturated fatty acids (C_{16}, C_{18}) have been found to act as pheromones in some insect species, for example, worker bees [17]. Structures of this ester were clearly shown by GC/MS. Generally an ester with molecular formula RCO_2R^1 gives ions $[R^1\text{-}1]^+$ and $[CO_2\ R^1]^+$ indicating the alcohol chain length and $[RCO_2\ H_2]^+$

showing the acid chain length [18]. A C_{24} ester had a peak at m/z 113, showing that the alcohol component was octanol and a peak at m/z 167, showing that the acid component was phthalic acid. The above ester was identified as di-*n*-octyl phthalate (1.37%).

The presence of ethyl palmitate (0.73%) (**1**) was further confirmed by ^{1}H-NMR, ^{13}C-NMR and MS data of the isolated component. A dihydroxy ester α-glyceryl linoleate (2.11%) was also detected.

Carboxylic Acids

Free fatty acids were the most abundant constituents of *Tamarix aphylla* epicuticular wax making up 60.45% of the total wax out of which saturated fatty acids were 20.47% and unsaturated fatty acids were 39.98%. All fatty acids detected were even-numbered ranging from C_{16} to C_{20}. Palmitic acid (16.05% of the total wax) was dominent among saturated fatty acids and linoleic acid (25.15% of the total wax) among unsaturated fatty acids. Free aliphatic fatty acids are common components of leaf waxes but the composition and concentration of the acid fraction can be affected by environmental conditions [2].

Alcohols

Free primary alcohols are widespread components of plant waxes [2]. These are often prominent components of waxes from monocotyledons and dicotyledons. Different kinds of alcohols including saturated, unsaturated and alkenyloxy alcohols were found in *Tamarix aphylla* epicuticular wax. All were primary alcohols and accounted for 20.23%, with even-numbered homologues in major proportions. 1-Tetracosanol (18.97%) was major component among saturated alcohols, 3,13-octadecadien-1-ol (0.39%) was dominant among unsaturated alcohols and 2-(9-octadecenyloxy)ethanol (0.47%) was alkenyloxy alcohol.

Aldehydes, Benzofuranone and Oxirane

Epicuticular wax of *Tamarix aphlla* also contained saturated and unsaturated aldehydes (5.37%). Most abundant was an unsaturated aldehyde 7-tetradecenal (5.27%). Also were detected 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone (0.07%) and heptadecyloxirane (3.89%).

Experimental

Plant Material and Wax Extraction

6.1 Kg fresh leaves of *Tamarix aphlla* were collected from the Miani-Sahib Graveyard (Lahore) in October 2007 and was identified by Dr. Zaheer-ud-Din Khan, Plant Taxonomist and Chairperson of the Botany Department, GC University Lahore. The voucher specimen was deposited in Dr. Sultan Ahmed Herbarium, Botany Department, GC University Lahore, Voucher No. G.C Herb, Bot. 18. Epicuticular wax was extracted by immersing the plant sample in *n*-hexane for 90 secs. This procedure extracts only surface hexane-soluble compounds without disturbing the leaf interior [19]. *n*-hexane extract was filtered and concentrated on rotary evaporator. Recovered *n*-hexane was again used for the extraction of waxes from the same leaves for the same duration. The second *n*-hexane extract was also concentrated on rotary evaporator. Both extracts were combined. Total 20.76 g wax was obtained after evaporating the *n*-hexane.

Wax Analysis

The constituents of the epicuticular wax were characterized and identified by GC/MS and NMR spectroscopy. The small amount of wax was dissolved in redistilled GC-grade *n*-hexane and microfiltered (0.45 μ) to prepare diluted samples (1 μg / 250 mL). GC/MS analysis were performed on a Shimadzu GC/MS-QP2010A system in EI mode (70 eV) equipped with a split / splitless injector (250 °C), at a split ratio of 50/50 using DB-5MS column (30 m x 0.25 mm i.d., film thickness: 0002E25 μ m, J & W Scientific, Fulsom, CA, USA). Injection volume was 1 μL and electronic pressure programming was used to maintain a constant flow (0.67 mL/mins) of the Helium carrier gas. The oven temperature was programmed from 150 °C (4 mins) to 320 °C at a rate of 2 °C/mins and held at this temperature for 36 mins, with ion source temperature 200 °C and interface emperature 250 °C. The resulting data was processed using Shimadzu Lab Solution GC/MS Postrun Analysis software. The relative apparent percentage of each compound was determined by area normalization method (Figs. 1-3). Component identification was carried out using the NIST 147 and NIST 27 libraries.

The constituents of the wax were separated by column chromatography (silica gel 70-230 mesh)

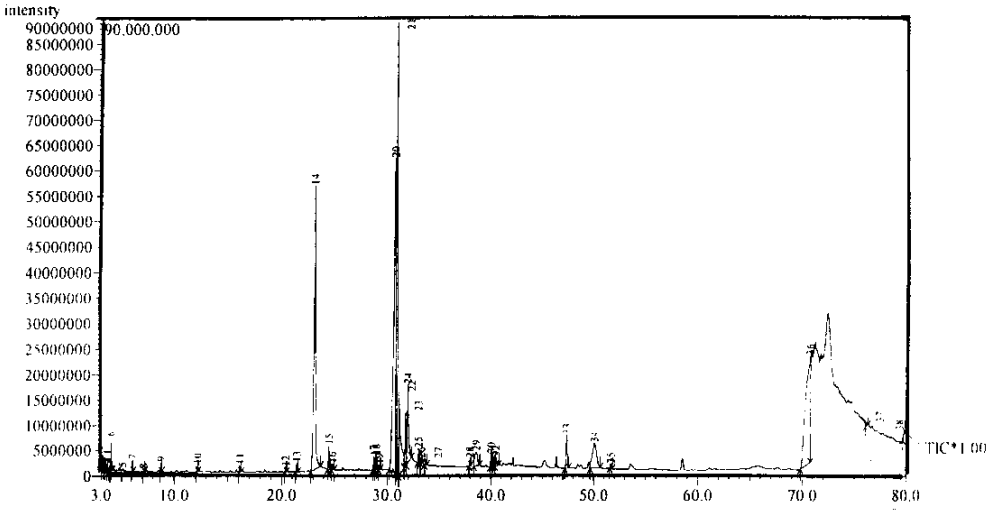


Fig. 1: Gas chromatogram of epicuticular wax of *Tamarix aphylla* L. numbering in the figure corresponds to that in the Table-1.

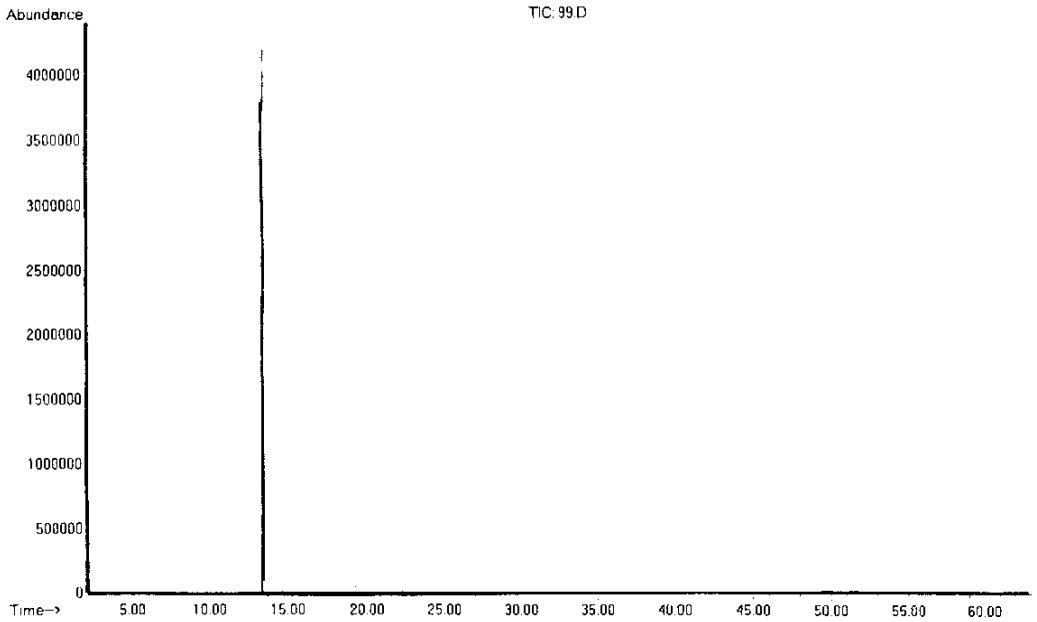


Fig. 2: Gas chromatogram of ethyl palmitate.

using *n*-hexane with increasing polarity of chloroform as an eluting solvent. Ethyl palmitate (**1**) and tetratriacontane (**2**) were eluted with *n*-hexane:chloroform (3:1) and *n*-hexane:chloroform (3.7:2) respectively, as single spots. Preliminary analysis of the constituents was carried out by TLC (Aluminium sheets 20x20 cm, 0.2 mm thick, silica gel 60 F_{254}, E-Merck), observed under UV lamp (254

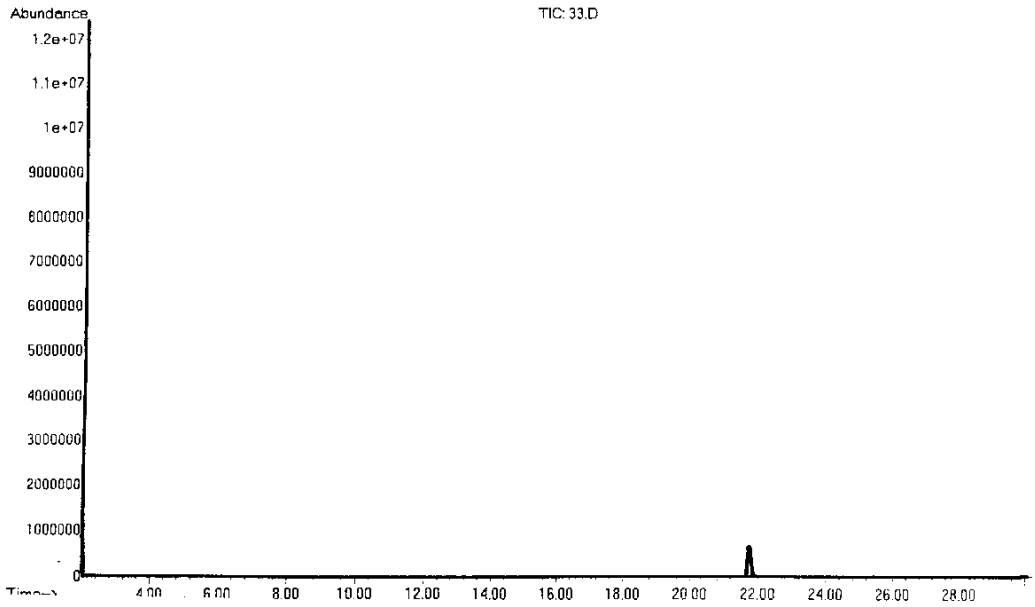
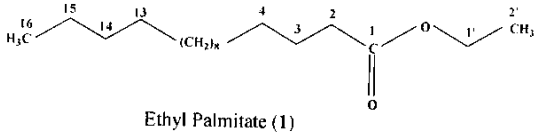


Fig. 3: Gas chromatogram of tetratriacontane.

and 366 nm) and treated with ceric sulphate as locating agent. Single spot fractions were further characterized by ^{1}H-NMR and ^{13}C-NMR. The NMR spectra of the isolated components were recorded on Bruker (300 MHz) spectrophotometer at room temperature using $CDCl_3$ as a solvent with TMS as an internal reference. The chemical shift values were reported in ppm (δ) units and the coupling constant J in Hz.

Ethyl Palmitate (**1**)

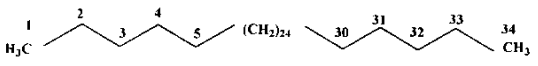
White solid; Mp 24-26 °C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 4.124 (q, J = 6.9 Hz, 2H, H-1'), 2.29 (t, J = 7.5 Hz, 2H, H-2), 1.609 (p, J = 6.9 Hz, 2H, H-3), 1.274 (br s, 24H, H-4—H-15), 1.253 (t, J = 6.9 Hz, 3H, H-2'), 0.89 (t, J = 6.9 Hz, 3H, CH_3-16); ^{13}C-NMR (300 MHz, $CDCl_3$): δ 173.78 (C-1), 60.40 (C-1'), 34.43 (C-2), 31.94 (C-14), 29.71-29.1 (C-4—C-13), 25.05 (C-3), 22.76 (C-15), 14.26 (C-2'), 14.10 (C-16); MS(GC) *m/z*: 284 $(M)^+$, 255, 239, 115, 101, 88, 73, 70, 57, 55, 45, 43,41 [20-22].



Ethyl Palmitate (1)

Tetratriacontane (**2**)

White solid; Mp 72-73 °C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.275 (br s, 64H, H-2—H-33), 0.900 (t, J = 6.9 Hz, 6H, CH_3-1, CH_3-34); ^{13}C-NMR (300 MHz, $CDCl_3$): δ 31.93 (C-3, C-32), 29.70 (C-5—C-30), 29.36 (C-4, C-31), 22.69 C-2, C-33), 14.11 (C-1, C-34); MS(GC) *m/z*: 478$(M)^+$, 311, 239, 225, 211, 197, 183, 155, 141, 127, 113, 99, 85, 71, 57, 43 [23-30].



Tetratriacontane (2)

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