

Acknowledgement

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Surface Waxes of Indian Mangrove Plants

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PLANT surfaces have been studied at regular intervals¹ since the early work of Aveguin in 1841 and Brongniart² in 1834. The last 15 years has seen considerable interest in plant surfaces and much work on the chemical composition has been recorded and reviewed³⁻⁵. One major stimulus for the study of plant wax constituents has been their possible use as chemotaxonomic criteria. The relative distribution of homologous series of wax components may act as a fingerprint for the individual species though Stransky and Streibl⁶ consider that the application of these distribution patterns for taxonomy purposes may be problematical. Tulloch⁷ summarised the taxonomic significance of leaf surface waxes and concluded that the waxes have been helpful in some plants, e.g. Crassulaceae, but not in other plants, e.g. Lignosae and Herbaceae. The plants examined in the present study grow in mangrove areas of the Sundarban region of West

Bengal, India, where they develop a peculiar physiology to cope with their unusual surroundings. Three of the plants, *Carapa moluccensis*, *Carapa obovata* (Meliaceae) and *Excoecaria agallocha* (Euphorbiaceae), belong to the order Geraniales and the other three, *Bruguiera gymnorhiza* and *Kandelia rheedii* (both Rhizophoraceae) and *Sonneratia apetala* (Sonneratiaceae), to the order Myrtiflorae. It was hoped that a study of the surface waxes of these plants might be useful taxonomically, or might highlight the common features amongst the plants which could be related to their habitat, or suggest meaningful conclusions regarding the metabolic pathways under extreme extrinsic conditions.

Both *Carapa* species and the *Kandelia* species are believed to possess medicinal activity⁸. *Bruguiera gymnorhiza* contains cyanidin chloride⁹, *Sonneratia apetala* contains β -sitosterol, ursolic acid, gibberellin A₃, 3,3',4-trimethoxy-4'-hydroxydiphenic acid dilactone and 3,3'-dimethoxy-4,4'-dihydroxydiphenic acid dilactone^{10,11}. *Excoecaria agallocha* contains taraxeryl acetate, taraxerone, taraxerol, β -amyrin, β -amyrenyl acetate, β -amyrone, 3-epi- β -amyrin, epitaraxerol, friedelin, cycloartenol, β -sitosterol, behenic acid, exocarol and agacol¹²⁻¹⁴.

Experimental

Fresh leaves (6 g) of *Carapa obovata* were extracted with cold chloroform (15 ml) for 15 sec¹⁵. The chloroform was removed under reduced pressure yielding a residue (40 mg). The residue was refluxed with 10% methanolic potassium hydroxide (20 ml) for 4 hr. The reaction mixture was extracted with petroleum ether (60-80°) (30 ml \times 3). The unsaponifiable matter (25 mg) was separated by tlc on silica gel using 10% diethyl ether in petroleum ether (40-60°) as the eluent. The hydrocarbon fraction was extracted from the silica gel with chloroform. After the excess chloroform has been removed, the hydrocarbons were analysed directly by glc. The alcohol fraction (10 mg) was dissolved in pyridine (1 ml) and acetic anhydride (200 μ l) and the mixture allowed to stand overnight. Water (3 ml) was added to the reaction mixture and the resultant solution was extracted with diethyl ether. The acetates were then purified by tlc on silica gel before analysis by glc. The acidic fraction, which consisted of free acids and acids which were originally present as wax esters, was esterified and analysed by glc. By following the same procedure the other plants were extracted.

Gas liquid chromatography conditions: GLC analyses were performed on a Pye 104 dual Flame Ionisation detector instrument using 5 ft \times $\frac{1}{8}$ inch columns. For methyl esters of fatty acids, alkyl acetates of alcohols and hydrocarbons, 1.5% OV17 on Gas Chrom Z (80-100 mesh) with temperature programming from 150 to 300°/min was used.

Results and Discussion

The hydrocarbons from the *Geraniales* plants each show some deviation from the most common

NOTES

TABLE 1—HYDROCARBONS FROM MANGROVE PLANTS

Chain length	<i>C. moluccensis</i>	<i>C. obovata</i>	<i>E. agallocha</i>	<i>B. gymnorhiza</i>	<i>K. rheedii</i>	<i>S. apetala</i>
n [*] 14	3.6	2.7	T†	8.9	4.8	T
br 15	T	—	—	—	—	—
n 15	T	4.1	T	—	1.0	—
br 16	T	T	—	—	—	T
n 16	2.3	14.8	5.2	T	3.5	T
br 17	T	—	T	1.1	—	1.0
n 17	4.4	16.5	6.4	2.4	1.6	T
br 18	T	7.0	3.6	—	—	2.4
n 18	4.4	2.8	6.4	1.7	8.1	T
n 19	4.8	1.6	15.7	4.7	2.4	2.9
br 20	1.1	1.9	5.1	1.7	4.5	7.0
n 20	3.7	12.7	3.2	2.2	6.4	T
br 21	1.0	—	T	—	T	4.3
n 21	5.0	3.5	6.5	T	6.4	T
br 22	1.0	—	—	1.1	—	5.0
n 22	6.1	T	3.8	2.5	2.9	T
br 23	1.0	—	3.8	4.7	6.7	5.5
n 23	6.8	4.6	4.8	32.5	2.4	1.0
br 24	1.0	—	—	—	—	6.7
n 24	6.1	4.6	6.4	2.5	3.2	1.3
br 25	1.0	—	2.5	T	—	5.6
n 25	7.8	6.1	5.9	3.2	2.5	T
n 26	6.9	—	—	4.3	4.5	9.2
n 27	7.6	4.2	6.8	6.0	8.1	7.0
n 28	7.6	10.1	—	—	22.2	13.2
br 29	—	—	18.9	9.6	—	8.0
n 29	6.9	2.5	—	8.6	6.4	—
n 30	3.6	T	—	2.2	2.4	9.5
n 31	6.1	T	—	—	—	5.0
						5.4

K. rheedii contains 2.9% br.C₂₄.

* n = normal

br = branched

† T = trace.

TABLE 2—FATTY ACIDS FROM MANGROVE PLANTS

Chain length	<i>C. moluccensis</i>	<i>C. obovata</i>	<i>E. agallocha</i>	<i>B. gymnorhiza</i>	<i>K. rheedii</i>	<i>S. apetala</i>
n 13	1.8	T	11.1	T	T	2.1
n 14	3.5	27.0	3.7	44.0	3.5	5.4
br 15	5.7	T	1.9	T	T	4.5
n 15	4.2	1.9	1.7	T	1.8	7.7
n 16	14.9	3.1	3.6	11.2	6.7	8.3
br 17	10.0	T	4.8	1.9	T	7.5
n 17	1.8	1.9	1.3	1.5	2.1	6.5
n 18	11.6	3.4	1.1	2.5	3.7	6.1
n 19	9.0	3.1	4.5	1.5	1.7	6.1
n 20	5.3	3.1	5.4	2.5	13.4	5.2
n 21	5.0	3.1	6.9	1.3	2.3	7.3
n 22	5.2	3.1	7.8	3.7	9.8	7.3
br 23	1.5	3.1	T	T	T	T
n 23	2.5	3.1	6.9	T	T	7.7
n 24	18.0	5.7	21.3	19.4	29.5	6.1
br 25	T	3.1	T	—	4.6	T
n 25	T	6.1	5.2	3.7	4.7	2.2
n 26	T	7.7	2.7	4.7	3.9	T
n 27	T	—	1.7	—	2.1	—
n 28	T	21.5	3.5	T	5.1	4.0

All plants have a trace of n C₁₂, br C₁₄, br C₁₆, br C₂₁, br C₂₄, n C₂₆, n C₂₈, n C₃₁ except *S. apetala* which has 1.8% n C₁₂ and 3.9% br C₃₁; *B. gymnorhiza* which has 2.2% br C₁₉; *E. agallocha* which has 4.6% n C₃₀; *K. rheedii* which has 5.1% n C₂₉.

dicotyledonous alkane distribution which shows odd chain predominance and a unimodal type⁴. *Carapa moluccensis* shows almost no odd-even alternation which is more like a petroleum hydrocarbon mixture, or like the hydrocarbons from seeds¹⁶. However, the hydrocarbons of *C. moluccensis* show a much larger chain length distribution, C₁₄–C₃₁,

than petroleum with C₂₆ and C₂₇ as the major components. The hydrocarbons of *C. obovata* and *E. agallocha* show trimodal distributions with maxima at C₁₇, C₂₁ and C₂₈ for the former and C₁₉, C₂₃ and C₂₈ for the latter. This finding of a major even-chain length hydrocarbon has been determined by glc retention data alone. It is hoped

TABLE 3—FATTY ALCOHOLS FROM MANGROVE PLANTS

Chain length	<i>C. moluccensis</i>	<i>C. obovata</i>	<i>E. agallocha</i>	<i>B. gymnorrhiza</i>	<i>K. rheedii</i>	<i>S. apetala</i>
br 12	7.5	8.0	—	—	—	—
n 12	14.5	12.0	T	T	T	2.1
br 13	—	2.3	T	—	—	18.2
n 13	7.4	3.2	T	5.7	4.6	1.0
br 14	3.7	—	—	1.4	2.0	4.1
n 14	4.2	1.0	1.0	2.1	16.3	1.3
br 15	1.0	24.0	1.0	1.0	—	—
n 15	10.2	2.9	3.3	5.8	5.1	3.9
br 16	6.0	—	T	—	T	—
n 16	4.2	T	T	1.2	1.8	T
br 17	T	T	—	1.5	T	—
n 17	1.2	1.0	54.0	32.4	19.7	45.3
n 18	1.3	1.3	—	1.2	2.3	1.0
br 19	—	2.6	3.4	2.1	1.7	—
n 19	5.5	2.6	3.8	25.6	9.6	3.2
n 20	3.5	T	2.3	T	2.3	2.3
n 21	2.4	T	2.0	2.5	1.8	3.1
n 22	2.5	7.2	2.0	2.2	2.0	1.5
n 23	2.4	2.7	2.3	T	1.1	2.7
n 24	8.0	3.9	4.8	2.6	5.6	2.7
n 25	2.3	13.7	1.7	1.0	—	2.3
br 26	4.0	—	2.0	T	10.0	—
n 26	T	4.5	2.3	T	—	—
n 27	—	—	2.9	11.7	9.7	—

C. moluccensis has br C₁₃ 4.6%, br C₂₈ 3.5%.

C. obovata has n C₁₁ 5.8%, br C₂₇ 1.2%.

E. agallocha has n C₁₁ 4.6%, n C₂₈ 3.6%, n C₃₀ 2.5%.

K. rheedii has br C₂₅ 4.1%.

S. apetala has br C₃₀ 5.1%.

that further work with glc/mass spectrometry will confirm this unexpected finding.

E. agallocha is a member of the Euphorbiaceae and it is interesting to note that the relative proportions of the C₂₇, C₂₉ and C₃₁ hydrocarbons in this plant are similar to those found for *Jatropha curcas*, *Croton melanocarpus*¹⁷ and *Euphorbia aphylla*¹⁸, though they are the predominant alkanes for these latter three. However, *E. aphylla* does have significant quantities of even chain length hydrocarbons (7 to 8% of total).

The hydrocarbons of the two members of the Rhizophoraceae show widely differing patterns with *B. gymnorrhiza* showing a very marked trimodal distribution with maxima at C₁₉, C₂₈ and C₂₉. *K. rheedii* also shows a trimodal distribution with maxima at C₂₀, C₂₃ and C₂₈. Both these Rhizophoraceae plants show a significant amount of branched chain components. *S. apetala* hydrocarbons show a very smooth distribution similar to *C. moluccensis* and reminiscent of *Eucalyptus largifolens* as reported by Herbin and Robins¹⁹. One of the Myrtiflorae plants, *K. rheedii*, also shows this unusual situation where the major hydrocarbon has an even number of carbon atoms. Herbin¹⁷ has shown, for *Solandra grandiflora*, that the hydrocarbon distribution changes as the plant ages and one of us²⁰ has observed similar changes for oat plants (*Avena sativa*). It may be that the observation of C₂₈ as the major hydrocarbon in three of the six plants of the Sundarban region indicates a

modification of the metabolism brought about by extreme climatic and soil conditions.

The acids of all the plants range from C₁₃ to C₃₀ and in common with many tropical lipids have high proportions of saturated acids. Acids have rarely been considered from taxonomic viewpoint probably because these acids in the range C₁₃ to C₁₈ are similar to the acids directly involved in the plant's main metabolism within the leaf. As with the hydrocarbons, the acids of the two *Carapa* species show considerable differences, i.e. *C. obovata* has two major components C₁₄ and C₂₈ with a small amount of branched component of the longer chain length, C₂₂ and C₂₄, whereas the branching in *C. moluccensis* is of C₁₆ and C₁₇ chain length. The acids of *E. agallocha* have C₂₄ as the major component in contrast to the previous report of C₂₂, though the latter finding was for plants from a different geographic site¹².

The acids of the two Rhizophoraceae show that the C₂₄ is the major component of the acids with a chain length longer than C₂₀. In *K. rheedii*, it is the major component of all the acids whilst in *B. gymnorrhiza* it is second to myristic acid. *S. apetala* shows much more branching than the others with the exception of *C. moluccensis*.

The distribution of the alcohols does not mirror the distribution for the acids suggesting that the rapid inter-conversion of acids and alcohols noted by Schlenk²¹ in animal tissue does not apply in

plants. Tulloch⁷ believes that alcohols can be used as a taxonomic criterion and it is clear from these six plants that each has its own characteristic distribution. The two *Carapa* species are distinguishable by the large amounts of branched chain components whilst the *E. agallocha* has a branched C₁₇ alcohol as the major component. This latter alcohol is a major component of the alcohols from the three members of the Myrtiflorae.

It appears that the waxes of plants grown in the mangrove areas of the *Sundarban* contain a number of unusual components which may be due to the plants habitat. It is clear that each plant wax is a distinct fingerprint for that species and could be used as taxonomic criteria. It is hoped later to compare these waxes with the waxes of plants from the same genera which grow under more normal conditions but it was felt necessary to examine first the waxes of mangrove plants to see if they had any unusual features.

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Bromatometric Determination of Some Aromatic Sulphones

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KOPPESCHAAR'S method¹ in which an acidified solution of the organic compound is treated with an excess of a standard solution of bromate-bromide and the excess bromine determined iodimetrically using starch as indicator was used for the determination of several organic compounds². These include aromatic compounds with ring substituents which strongly influence *ortho* and/or *para* bromination. Pence³, while determining resorcinol by the above method, allowed the reaction mixture to stand for 5 min after the addition of potassium iodide and titrated the liberated iodine with standard thiosulphate. Redman, Weith and Brock⁴ studied the application of the method to phenol. In the case of *meta*-cresol Fox and Barker⁵ allowed the reaction mixture to stand for 30 min after the addition of potassium iodide. Callin and Henderson⁶ tested for free bromine in the reaction mixture using starch-potassium iodide paper. Francis and Hill⁷ carried out extensive investigations on the estimation of the *meta* isomers among several disubstituted derivatives of benzene and reported that the directive influence of hydroxyl and amino groups are so great in comparison to those of other groups that in aqueous solution when one of them is present in the benzene ring, all available *ortho* and *para* positions are substituted quantitatively with bromine. Day and Taggart⁸ used the excess method for the analysis of twenty-five phenols and aromatic amines and claimed good results.

Recently, we reported the bromatometric determination of hydroxy carbonyl compounds^{9,10}, amino carbonyl compounds¹¹, *p*-aminosalicylic acid¹² and some phenols and alkyl phenols¹³. In the present investigation we report the results obtained in determining the aromatic sulphones bromatometrically, since there seems to be no report in the literature on the bromatometric determination of these compounds.

Experimental

The aromatic sulphones used were synthesised by the literature methods. Hydroxy sulphones were prepared by subjecting the respective arensulphonates to the Fries rearrangements. The arensulphonates were prepared by treating phenols with sulphenyl chlorides by the usual Schotten-Baumann method. The amino sulphones were prepared by the reduction of the corresponding nitro derivatives.

Known weights of the compounds were dissolved in 20% (v/v) aqueous acetic acid and made up to required volume with distilled water. Aliquots of