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PENTACYCLIC TRITERPENOIDS AND STEROLS FROM SEVEN SPECIES OF MANGROVE

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Key Word Index—*Avicennia officinalis*; Avicenniaceae; *Acanthus illicifolius*; Acanthaceae; *Bruguiera gymnorhiza*; Rhizophoraceae, *Ceriops decandra*; Rhizophoraceae; *Derris trifoliata*; Fabaceae; *Rhizophora mucronata*; Rhizophoraceae; *Suaeda maritima*; Chenopodiaceae, pentacyclic triterpenoids, sterols.

Abstract—The isolation of pentacyclic triterpenoids from seven species of fresh mangrove leaves using a simple and rapid method is described. The leaves were homogenized using chloroform–methanol and the extract was diluted with water to precipitate out triterpenoids which were separated into neutral and acidic fractions. These were analysed by gas-liquid chromatography as acetyl and trimethylsilyl ether derivatives on a 3% OV-17 column. Sterols were isolated from the chloroform layer by preparative thin layer chromatography and were analysed by gas-liquid chromatography as their trimethylsilyl ether derivatives on a 3% OV-17 column. The triterpenoids found were α-amyrin, β-amyrin, lupeol, oleanolic acid and ursolic acid in most of the samples. Sterols found in all the samples were cholesterol, campesterol, stigmasterol, sitosterol and stigmast-7-en-3β-ol. Retention indices of the triterpenoids and sterols have been determined.

INTRODUCTION

Triterpenoids and sterols are generally extracted from plant sources using lengthy procedures. During the extraction of lipids from fresh mangrove leaves, using the method described by Bligh and Dyer [1], it was observed that a heavy white precipitate appears on diluting the chloroform–methanol extract with water. The precipitate was found to contain neutral and acidic pentacyclic triterpenoids. This observation prompted us to standardize the method leading to a short and rapid route to the isolation of pentacyclic triterpenoids from fresh leaves. Sterols were concentrated in the chloroform phase, which were also isolated and analysed. In the present communication seven species of mangrove plant leaves were studied for pentacyclic triterpenoid and sterol compositions.

RESULTS AND DISCUSSION

The lipid, sterol and triterpene contents of the fresh leaves from the seven species of plants examined are presented in Table 1.

The sterols and triterpenes were separated and the latter were fractionated into alcohols and acids and were analysed by GLC after derivatization. The sterol and triterpene compositions are presented in Table 2. The sterols were identified by comparison of retention indices with those reported. Triterpenes were identified by comparison of retention times with those of authentic standards, analysed under identical conditions of gas chromatography. Relative retention times and the retention indices of the triterpenes were determined and they are presented in Table 3.

Table 1 Lipid, sterol and triterpene contents* of seven species of mangrove leaves

Species	Lipid	Sterol	Triterpene
Avicennia officinalis	14 100	1000	1600
Acanthus illicifolius	6000	320	6100
Bruguiera gymnorhiza	13 200	1000	3000
Ceriops decandra	15 200	1700	11 100
Derris trifoliata	25 000	3000	3100
Rhizophora mucronata	6400	600	16 400
Suaeda maritima	3500	240	2400

*Expressed as μg/g of fresh leaves.

The results presented in Tables 2 and 3 indicate that there is a possibility of cross contamination of compounds with retention indices between 3400 and 3500, the silyl derivatives of sitosterol, stigmast-7-en-3β-ol, an unidentified neutral triterpene, β-amyrin and α-amyrin. The retention indices of the sterol-OTMS derivatives in the present study are in good agreement to those reported by Knights [2]. To confirm the identifications, an authentic mixture of β-amyrin and sitosterol was analysed as the silyl derivatives and discernible resolution was obtained. Among the other component sterols and terpenes the differences in the retention indices provided good resolution.

Table 1 indicates that *D. trifoliata* contains the highest proportion of sterols, whereas *R. mucronata* contained

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Table 2. Sterol and triterpene compositions (% w/w) of seven species of fresh mangrove leaves

	1*	2	3	4	5	6	7	RI†
Sterols								
Cholesterol	7.0	4.9	1.7	1.0	1.4	4.5	4.0	3253
Campesterol	9.2	19.8	22 7	7.5	7.6	15.0	11.9	3355
Stigmasterol	12.8	26.0	17.7	11.6	8.9	25.1	11.9	3385
Sitosterol	51.7	36.1	53.7	13.8	21.6	40.4	55.9	3440
Stigmast-7-en-3β-ol	19.3	13.2	4 2	66 1	60 5	15.0	16.3	3500
Triterpenes								
Unidentified alcohol	—	0.7	6 8	—	—	3 3	10.1	
β-Amyrin	14.7	2.3	17.0	—	9.7	5.7	50.6	
α-Amyrin	14.7	17.7	5.4	19.0	12.1	7.2	13.9	
Lupeol	27.0	19.0	14.6	49.8	78.2	10 8	25 4	
Betulin	—	—	—	—	—	22 5	—	
Unidentified acid	6.0	—	—	3 2	—	—	—	
Oleanolic acid	18.0	35.9	27.7	19.7	—	29.1	—	
Ursolic acid	19.6	24.4	28.5	8.3	—	21 4	—	

*1. *Avicennia officinalis*; 2. *Acanthus ilicifolius*; 3. *Bruguiera gymnorhiza*; 4. *Ceriops decandra*; 5. *Derris trifoliata*; 6. *Rhizophora mucronata*; 7. *Suaeda maritima*.

†Retention indices of sterol-OTMS derivatives

Table 3 Relative retention times* and retention indices of acetyl and trimethylsilyl derivatives of various triterpenes

	Relative retention times		Retention indices	
Triterpenes	Acetyl	Trimethyl-silyl	Acetyl	Trimethyl-silyl
Unidentified	0.94	0.92	3470	3411
β-Amyrin	1.00	1.00	3512	3454
α-Amyrin	1.14	1.06	3590	3482
Lupeol	1.33	1.20	3669	3541
Betulin	1.95	1.60	3890	3671
Unidentified acid†	1.86	1.51	3886	3660
Oleanolic acid	1.98	2.01	4009	3751
Ursolic acid	2.24	2 32	4065	3812

*Relative retention times were determined with respect to β-amyrin. Acetyl and trimethylsilyl derivatives were both analysed on 3% OV-17 column, oven temperatures being 290° and 284° respectively. Nitrogen flow rate was 60 ml/min in both the cases. Retention times of β-amyrin-OAc and β-amyrin-OTMS were 9.8 and 10.0 min respectively.

†Triterpene acids were methylated before derivatization.

the highest proportion of triterpenes. The sterol compositions presented in Table 2 indicate that the major component was sitosterol, except in *C. decandra* and *D. trifoliata*, which contained stigmast-7-ene-3β-ol as the major component. A study on the fresh mangrove leaf of *A. marina* by Wannigama *et al.* [3], indicated the presence of sitosterol as the major component sterol. Sterols from sterol esters of three species of mangrove leaves by Misra *et al.* [4], also indicated the presence of sitosterol as the major constituent. It is interesting to note that 28-isofucosterol was found in esterified form [4] in *A. ilicifolius*, *B. gymnorhiza* and *R. mucronata*, whereas the same sterol was absent in the free state in the leaves of the same three species in the present study. On the other hand, stigmast-7-en-3β-ol was present mostly in the free state, except in *R. mucronata* where it was present both in esterified form [4] and in the free state.

The common constituent triterpenes were α-amyrin, β-amyrin, lupeol, oleanolic acid and ursolic acid. Betulin was present only in *R. mucronata*, whereas *D. trifoliata* and *S. maritima* did not contain any triterpene acid (Table 2).

The use of GLC in the field of triterpene analysis is increasing rapidly [5]. The simple and rapid technique for the isolation of triterpenoids and their analysis by GLC,

as discussed in the present study, appears to be very suitable for fast screening of plant materials for triterpenoid and sterol constituents.

EXPERIMENTAL

Samples. Leaf samples were collected from Prentice Island, between latitudes 21.43° and 21.46° N and longitudes 88.18° and 88.19° E of the Sunderban mangrove forest, West Bengal, India.

Isolation of triterpenoids. Leaves were washed, cut into pieces, and lipids were extracted as in ref. [1]. A heavy white ppt appeared upon dilution of the pooled extracts with 10 volumes of H_2O. The lower $CHCl_3$ layer was withdrawn. The ppt was washed with a small volume of $CHCl_3$, dissolved in MeOH–$CHCl_3$ (2·1), dried and weighed.

Isolation of sterols. The pooled $CHCl_3$ extracts were dried, solvents removed and sterols were isolated by prep. TLC, as described by Mangold [6].

Colour reactions of sterols and triterpenoids. The Liebermann–Burchard [7, 8] test produced a greenish colour with sterols and a blue or violet colour with triterpenes. Differentiation of sterols and triterpenes by colour reactions was done according to Hashimoto [9]

Derivatization and GLC of sterols Sterols were silylated [10] and were analysed by GLC [11].

Separation of triterpene alcohols and acids. The terpene alcohols and acids were separated by saponification [11]. Terpene acids were methylated with CH_2N_2 [12].

Derivatization and GLC of triterpenoids. The triterpene alcohols and methyl esters of the acids were acetylated [13], aliquots were also silylated [10] and analysed by GLC. Retention indices were determined according to Knights [14]

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