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# THE CHEMICAL COMPOSITION OF VARIOUS EUPHORBIA SPECIES FOR INDUSTRIAL APPLICATIONS

## Part-II. Neutral Lipids of *Euphorbia cauducifolia*

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*Euphorbia cauducifolia* gave ethyl acetate - extractable material (10.4%) which was resolved into free fatty acids (0.207%), free alcohols (4.015%), hydrocarbons (0.36%), esters (0.812%), sterols (0.028%) and highly polar components (4.863%). The composition of various classes of compounds was determined by gas liquid chromatography of the individual fractions or derivatized fractions. The acids, both free and combined, ranged from lauric acid ($C_{12:0}$) to behenic acid ($C_{22:0}$) with palmitic acid ($C_{16:0}$) as the highest contributor in all fractions. The alcohols ranged from pentacosanol ($C_{25:0}$) to dotriacontanol ($C_{32:0}$) with the highest percentage of octacosanol ($C_{28:0}$). The hydrocarbons ranged from tetradecane ($C_{14:0}$) to pentatriacontane ($C_{35:0}$) with maxima at octadecane ($C_{18:0}$) and tritriacontane ($C_{33:0}$).

*Key words:* Euphorbiaceae, *Euphorbia cauducifolia,* Fatty acids, Fatty alcohols, Hydrocarbons.

## INTRODUCTION

*Euphorbia cauducifolia* is found abundantly in the sandy terrain of Sind [1]. Large plantations exist on rocky hillocks and grounds at Drigh Road, Mangopir and Band Murad in the Karachi area [2] including some areas of southern Baluchistan. The latex and the root extracts are used in the indigenous system of medicine and are believed to possess antitumour properties.

The chemical work, carried out previously, describes the isolation of two diterpenoids, jolkinolide and cauducifolin from the root bark of *E. cauducifolia* [3]. 3-Epicyclolaudenol, cycloartenol and 3-ketomethyl ursolate were isolated from the latex of this species [4]. Attempts were also made to use euphorbia resin as a substitute for commercial resins [5]. In our previous publication, we reported the composition of the hydrocarbon fraction, ester-fraction and the isolation of glutinol from the latex of this species [6]. The present paper describes neutral lipids from the whole plant *E. cauducifolia*.

## EXPERIMENTAL

Melting points were determined on a Fisher Johns melting point apparatus and are not corrected. Infrared spectra were recorded on Beckman Acculab 10 as smeer between sodium chloride plates unless otherwise stated. $^1$H NMR-spectra were recorded on a Hitachi Model R-24-B high resolution spectrometer. Carbon tetrachloride or deuterated chlorofrm and tetramethyl silane (TMS) were used as solvent and internal standard respectively. Thin layer chromatography (TLC) and preparative layer chromatography (PLC) were carried out on glass plates coated with silica gel (kieselgel 60 g $F_{254}$, E. Merck). The plates were developed in chloroform, unless otherwise stated. The spots were observed under UV light and/ or by placing in an iodine chamber and finally by spraying with sulphuric acid in methanol. Silica gel (Keiselgel 60, 70-230 mesh ASTM, E. Merck) was used for column chromatography.

*Extraction of the plant.* The plant was collected from Karachi in December 1985. The air dried ground stems (175 g) were extracted with ethyl acetate in a soxhlet extractor for 15 hr. The extract was allowed to stand overnight when a pale yellow material deposited and the material was filtered off (3.50g, 2.0%). The filtrate was freed from solvent on a rotary evaporator to give a semi-solid material (14.7g, 8.4%).

*Column chromatography of the semi-solid material.* The semi-solid material (3g) was refluxed with hexane: chloroform (93:7, 30 ml) and allowed to stand overnight. After the removal of a solid material (0.9g, 2.52%), the clear filtrate (containing 2.1g, 5.88%) was charged on to a column of silica gel (150g, $\phi$ 2.4 cm). The elution was carried out with increasing percentages of chloroform in heaxane, chloroform and finally with methanol. The fractions were monitored by TLC. The $R_f$ values, percentage contribution and the inference drawn are given in Table 1.

*Isolation of free acids.* The acid-containing fraction (0.6g) was dissolved in ether (100 ml) and stirred with 2.5%

aqueous potassium hydroxide (75 ml) for 1 hr. at room temperature. The layers were separated and the aqueous layer was acidified. The acids were extracted with ether, dried and freed of solvent (0.150g, 41.666%).

*Saponification of esters.* To a solution of wax esters (0.250g) in benzene (10 ml) 2N ethanolic potassium hydroxide (10 ml) was added and the mixture refluxed for 4 hr. The reaction mixture was cooled and taken up in ether which was washed 3x with distilled water to remove the solvent alcohol and the soaps. The ether layer on usual work-up gave the alcohols (0.165g, 65% of esters).

*Liberation of acids and their esterification.* 2N sulphuric acid was added dropwise to the above soap solution until a pH of 2 was achieved. The liberated acids were extracted with ether. The ether extract was worked up as usual and the residue (0.085g, 35%) was refluxed with methanol containing 5% hydrochloric acid for 2 hr. The reaction mixture was diluted with water and extracted with hexane. The organic layer was washed with water, saturated sodium bicarbonate solution, dried and evaporated to give the methyl esters (0.076g, 90%).

*Gas liquid chromatography (GLC) of methyl esters.* Gas liquid chromatography of methyl esters was carried out on a Pye Unicam 204 Series instrument equipped with a flame ionisation detector. A glass column filled with chromosorb A.W. (60-70 mesh) having a coating of 10% diethylene glycol succinate (DEGS) was used for the separation. The column, the injection port and the detector were maintained at 200, 220 and 250° respectively. The identification of individual peaks was made through comparison of the retention times with standard esters and co-injection of the markers. The area under each peak represented the quantity of that individual in the mixture.

*Gas liquid chromatography of fatty alcohol acetates.* GLC of the acetates was carried out on a Pye Unicam 104 Series instrument equipped with a flame ionisation detector. An SE 30 WCOT column (25 m) was used for the separation. The oven temperature was programmed from 150-297° at 14° per min. with an initial hold for 4 min and final hold till no more peaks appeared. The peaks were registered and integrated by a spectra physics recorder. The identification was made by comparing the retention times with standard fatty alcohol acetates and co-injection of the markers.

*Gas liquid chromatography of hydrocarbons.* The hydrocarbons were analysed using the same equipment column, programming and procedure as for the fatty alcohol acetates.

## RESULTS AND DISCUSSION

The fresh plant on drying left behind 18.9 to 22.4% of air dried wood which was extracted with ethyl acetate in a Soxhlet extractor. The extract, on standing, deposited pale solid material (2.0%) having a melting range from 50 to 80°. Thin layer analysis showed a less polar component ($R_f$ .24) and a polar component which did not move.

The clear ethyl acetate filtrate on removal of the solvent yielded a brownish material (8.4%) which was refluxed with 7% chloroform in hexane when another sediment (0.94%) could be obtained. This sediment was resolved into acidic (0.39%) and neutral (0.55%) fractions.

The clear chloroform hexane solution (7.46%) was resolved into various class compounds by column chromatography. The $R_f$ values, and the percentage contributions are given in Table 1. The chemical constituents of these classes of compounds are given below.

Table 1. Contribution of various classes of compounds in the ethyl acetate extract of *E. cauducifolia*.

| $R_f$ | Eluent | Contribution by weight % | Nature of the fraction |
|---|---|---|---|
| .77 | H :chl. 93:7 | 0.364 | Hydrocarbons |
| .73 | H :chl. 85:15 | 0.54 | Aliphatic esters |
| .61 | H :chl. 80:20 | 0.224 | Esters of fatty acids with cyclic alcohol. |
| .39 | H :chl 75:25 | 0.084 | Esters of fatty acids with cyclic alcohol. |
| .30 | H :chl 75:25 | 0.411 | Mixture of fatty alcohols plus cyclic alcohols |
| .24 | H :chl. 70:30 | 1.092 | Mixture of fatty alcohols plus cyclic alcohols |
| .20 | H :chl 70:30 | 0.392 | Cyclic alcohols |
| .19 | H :chl. 50:50 | 0.140 | Cyclic alcohols containing cyclopropyl ring |
| .09 | Chl | 0.028 | Sterol |
| | Methanol | 2.380 | Acids plus resinous mass |

H and Chl stand for hexane and chloroform respectively.

*Acids.* The acids were found to be present in free as well as esterified form. Because of the partial solubility of the total extract in ether solvent, the free acids could not be isolated in a single step and were, therefore, isolated by stirring etherial solution of various fractions with 2.5%

aqueous potassium hydroxide at room temperature for 1 hr. The alkaline layer from the first sediment did not show any precipitation on acidification.

The alkali treatment of the second sediment and acidification of the aqueous layer yielded acids (0.041%). These acids on the basis of TLC, IR and NMR were found to be fatty acids. They were converted to methyl esters and analysed by GLC. Their composition is given in Table 2. They ranged from lauric acid ($C_{12}$) to arachidic acid ($C_{20}$) with palmitic acid (46.69%) as the major contributor. The only unsaturated acid which could be detected was oleic acid ($C_{18:1}$) to an extent of 3.85%.

*Free acids also remained dissolved in hexane.* chloroform (93:7) and were eluted in the column chromatography along with the resinous material. They were isolated (0.166%), characterised as fatty acids, derivatized and analyzed as above. They also represent the same range as those of the second sediment (Table 2). Two unsaturated acids oleic acid ($C_{18:1}$) and linoleic acid ($C_{18:2}$) could be detected. This observation agrees well with the physical consistency and the solubility of the unsaturated fatty acids.

The major portion of the acids was present as esters of aliphatic and cyclic alcohols. Three types of esters

Table 2. Amount of individual fatty acids, alcohols and hydrocarbons as determined by gas liquid chromatography (%).

| Carbon chain length | Fatty acids | | | | Alcohols | | Hydrocarbons |
|---|---|---|---|---|---|---|---|
| | Free from | | Combined with | | | | |
| | Semiment | Resinous mass | Fatty alcohols | Cyclic alcohols | Free | Combined | |
| 12 | 2.91 | 14.67 | 0.33 | 3.85 | – | – | – |
| 14 | 31.13 | 12.82 | 3.08 | 8.65 | – | – | 0.62 |
| 15 | – | – | – | – | – | – | 0.17 |
| 16 | 46.69 | 20.38 | 62.14 | 47.73 | – | – | 3.06 |
| 17 | – | – | – | – | – | – | 0.23 |
| 18(0)[2] | 12.45 | 18.22 | 7.89 | 11.55 | – | – | 3.45 |
| 18(1) | 3.89 | 10.56 | 1.73 | 7.73 | – | – | – |
| 18(2) | – | 13.90 | – | – | – | – | – |
| 19 | – | – | – | – | – | – | 0.36 |
| 20 | 2.91 | 9.49 | 17.75 | 20.53 | – | – | 1.87 |
| 21 | – | – | – | – | – | – | 0.31 |
| 22 | – | – | 7.05 | – | – | – | 0.91 |
| 23 | – | – | – | – | – | – | 0.26 |
| 24 | – | – | – | – | – | – | 0.24 |
| 25 | – | – | – | – | – | 0.84 | 0.62 |
| 26 | – | – | – | – | 1.97 | 3.48 | 2.50 |
| 27 | – | – | – | – | 1.00 | 2.12 | 6.25 |
| 28 | – | – | – | – | 44.36 | 47.62 | 1.64 |
| 29 | – | – | – | – | 3.16 | 2.94 | 20.44 |
| 30 | – | – | – | – | 43.18 | 37.41 | 1.17 |
| 31 | – | – | – | – | 0.86 | – | 23.93 |
| 32 | – | – | – | – | 5.46 | 5.59 | 2.16 |
| 33 | – | – | – | – | – | – | 25.19 |
| 34 | – | – | – | – | – | – | 0.86 |
| 35 | – | – | – | – | – | – | 4.61 |

' Not detected.

( )[2] The figure in parenthesis denotes the number of double bonds in the molecule.

($R_f$ s. 73, .61 and .39) were eluted with hexane: chloroform mixtures (Table 1). The NMR-spectrum of the least polar fraction ($R_f$ .73, 0.504%) m.p. 50-60 showed a strong singlet at 1.28 ppm ($-CH_2-$ in a straight chain), a triplet at 0.85 ($CH_3-$ linked to a methylene). These signals confirm the fraction as fatty acid esters of fatty alcohols. Saponification of this fraction gave acids (0.176%) which were characterized, derivatized and analysed by GLC. These acids ranged from lauric ($C_{12}$) to behenic acid ($C_{22}$) with palmitic acid as the major component (62.14%) and minor quantities of oleic acid (1.73%).

The ester fraction ($R_f$ .61, 0.224%) m.p. 65-135$^o$ showed in NMR spectrum a strong singlet at 1.28 ppm ($-CH_2-$ in straight chain), small singlets at 1.00, 0.90 and 0.85, ppm ($CH_3-$ attached to a tertiary carbon atom) and multiplets at 4.40 to 4.80 ppm (esterified cyclohexanol) and 5.20 to 5.60 ppm (olefinic protons in ring as well as in straight chain).

The ester fraction ($R_f$ .39, 0.084%) showed NMR spectrum similar to the previous fractions. The angular methyl groups were more pronounced. The fraction on saponification gave acids (0.025%) which were characterized, derivatized and analysed. The acids showed a range from lauric ($C_{12}$) to arachidic acid ($C_{20}$) with the highest percentage of myristic acid 47.37% (Table 2).

*Alcohols.* Alcohols constituted the highest proportion of the ethyl acetate extract. They were present both in free as well as in esterified form. Due to the low solubility of the higher members, they precipitated on cooling the extract or by dissolving the extract in less polar solvents. Higher aliphatic alcohols are usually present in plant waxes but the occurrence of cyclic alcohols other than sterols is a less common phenomenon.

Free alcohols (1.080%) were obtained from the sediment of ethyl acetate extract by PLC. The isolated alcohol was crystalized out of methanol. The m.p., IR and NMR data of the alcohol and its acetate confirmed it to be octacosenol.

Free alcohols (0.90%) obtained from the hexane-chloroform sediment after removal of the acids showed three spots in TLC analysis. The IR and NMR of the mixture indicated mainly their cyclic nature. The fatty alcohols could have been recovered through repeated crystallization from boiling hexane when the fatty alcohols were supposed to stay in the mother liquor. In this way, only traces of fatty alcohols could be obtained.

Free alcohols were eluted from the column (2.446%) and they comprised four groups which could be detected in this layer chromatography ($R_f$ .30, .24, .20 and .19). The alcoholic fraction (Rf .30, yield 0.411%) was a mixture of straight chain and cyclic alcohols as seen from IR and NMR spectra of the fraction. It was crystallized from boiling ethanol. The crystals (0.132%) compared well in all respects with the previously isolated glutinol [7-9]. The residue from the mother liquor (0.279%) on the basis of IR and NMR was found to be *n*-alkanols. The fraction ($R_f$ .24, 1.092%) was also a mixture of straight chain and cyclic alcohols (IR and NMR). It was crystallized from chloroform-acetone at room temperature. The crystals were filtered off and the mother liquor evaporated to give fatty alcohols (0.656%).

These were mixed with the free alcohols from the previous fraction ($R_f$ .30) and acetylated with acetic anhydridepyridine at room temperature. The acetates on argentation TLC showed the presence of saturated acetates only. The acetates were analysed by GLC. The results given in Table 2 show that the alcohols range from hexacosanol ($C_{26}$) to dotriacontanol ($C_{32}$), with octacosanol ($C_{28}$) being in the highest percentage.

The combined alcohols were present in all the ester fractions but fatty alcohols were present only in the esters ($R_f$ .73) and were obtained by the saponification of this fraction (0.328%). They were acetylated and the acetates were anlysed. The results given in Table 2 show a range of pentacosanol ($C_{25}$) to dotriacontanol ($C_{32}$) with the maximum percentage of octacosanol ($C_{28}$). The even to odd ratio predominated very clearly.

*Hydrocarbons.* The fraction eluted with hexane: chloroform (Table 1) ($R_f$ .77, 0.36%) is semi-solid, having a melting range of 40-54$^o$. The IR spectrum showed only C–H– stretchings and could be a mixture of homologues. The fraction was resolved into less polar and polar sub-fractions in TLC analysis using hexane as the developing solvent. These spots were separated by PLC in yields of 0.277% and 0.118% respectively. The IR spectra of the less polar sub-fraction exhibited higher methylene to methyl ratio than in the polar sub-fraction. This indicated that the lower spot is branched/cyclic in nature.

The compositon of the less polar sub-fraction was determined by GLC (Table 2). The identified *n*-alkanes ranged from tetradecane ($C_{14}$) to pentatriacontane ($C_{35}$). with two maxima at octadecane ($C_{18}$) and tritriacontane ($C_{33}$). This composition differed from the hydrocarbon fraction of the latex [7] both qualitatively and quantitatively indicating that all the hydrocarbons in this plant are not being synthesized in the latex.

*Polar components.* The polar components form a substantial portion of the ethyl acetate extract. They separated out from the extract (1.24%) and were eluted from the column as resinous mass (2.50%). Chemically, they were

neutral but contained more than one functional group (IR spectrum). Further work on this fraction is in progress.

The ethyl acetate extract of the air dried plant yielded an organic material (10.4%) which was quite comparable to other euphorbia species, i.e. *E. neriifolia* Linn, 11.74% [10]; *E. antiquorum*, 9.74% [10]; or *Asclepias rotundifolia*, N.O. Asclepiadaceae, 10.9% [11]. Such plants were once deemed as possible sources of renewable energy [12]. Similarly *E. cauducifolia* may at least in time of need be considered as an "energy farm" candidate.

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