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CHEMICAL COMPOSITION OF VARIOUS EUPHORBIA SPECIES FOR INDUSTRIAL APPLICATIONS

Part I. Latex of *Euphorbia cauducifolia*

Muhammad Nazir, Waqar Ahmad, Naeem Akhtar Rabi and Shafiq Ahmad Khan

PCSIR Laboratories, Lahore-16

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The latex of *Euphorbia cauducifolia* was resolved into different classes of compounds. The hydrocarbons (1.2 %) as determined by GLC ranged from heneicosane (C_{21}) to dotriacontane (C_{32}) with the highest percentage of nonacosane(C_{29}). The esters were composed of fatty acids and straight chain and cyclic alcohols. The fatty acids ranged from lauric (C_{12}) to behenic (C_{22}) with the highest percentage of myristic acid (C_{16}) (58.73). Glutinol was isolated from the alcoholic fraction. The possible use of this latex is discussed.

Key words: Euphorbiaceae, *Euphorbia cauducifolia,* Latex, Hydrocarbons, Lipids, Glutinol.

INTRODUCTION

Latex producing or lactiferous plants are acquiring greater importance as resources for renewable energy [1]. The members of plant family Euphorbiaceae can easily grow on waste lands with enough sunshine and limited rainfall. They are amenable to biogenetic or agronomic changes and easy to tap for the latex. More than a hundred species belonging to Euphorbiaceae are known to grow in Pakistan, mostly in arid and waste lands of Sind, Baluchistan and Punjab [2].

Euphorbia cauducifolia is a tall, armed dense, erect and stout bush with thick cylindrical leafless green branches. A large plantation exists on rocky hillocks and grounds at Drigh Road, Manghopir and Band Murad in the Karachi area [3]. Earlier workers have reported the presence of anthocyanins in red cyathia [4], cauducifolin and Jolkinolide-A in the fresh root barks [5] and the occurrence of free amino-acids and sugars in the defatted seeds of this plant [6]. Attempts have also been made to use euphorbia resin as a substitute for commercial resins [7]. Recently 3-epi-cyclolaudenol, cyclolaudenol, cycloartenol and methyl-3-oxoursolate have been isolated from the latex of this species [8].

The present work was undertaken with the view to determining the chemical composition of the latex and to explore its possible applications. This communication, therefore, deals with the hydrocarbons, fatty acids and the triterpenoidal alcohols isolated from the latex of this species.

EXPERIMENTAL

Thin layer chromatography and preparative layer chromatography were carried out on glass plates coated with silica gel (Kieselgel 60 G F254, E.Merck). The plates were developed either in hexane-ether (90:10) or in chloroform. The spots were visualised by UV, iodine and finally with aqueous ceric sulphate-sulphuric acid. Silica gel (Kieselgel-60, 70-230 mesh, ASTM, E.Merck) was used for column chromatography. Infrared spectra were recorded on Beckman Acculab-10 specrtophotometer. NMR spectra were recorded on a Hitachi High Resolution spectrometer Model R-24B. Deuterochloroform was used as a solvent with tetramethyl silance as internal standard.

The plant was collected from Karachi area and shipped to Lahore. The latex oozed out after the thorns were removed. This latex was dropped into ethyl acetate when two layers were formed. The organic layer after drying over anhydrous sodium sulphate was evaporated and a pale resinous mass was obtained.

The stems were chopped and the inner pulp extracted with ethyl acetate. This extract, after drying and evaporation also gave a semi-solid resinous mass which was added to the one from latex.

Column chromatography of latex. The extract (2.5 g) was refluxed with hexane(30 ml) and allowed to stand overnight. The clear solution was charged to silica gel (120 g, ϕ 2 cm) and eluted with hexane, hexane-ether, ether and finally with chloroform-methanol. The fractions were monitored by thin layer chromatography.

Gas liquid chromatography of hydrocarbons. The hydrocarbon fraction was analysed on a Pye Unicam 104 Series instrument equipped with a flame ionisation detector. A glass column 1.5m long, 6 mm O.D., filled with 3 % Dexsil 300 quoated on chromosorb (80-100) was used for separation. The column, the injector and the detector were maintained at 250, 275 and 300°, respectively. Nitrogen 50ml/min was used as the carrier gas. The identification was made by comparison of the retention time and co-injection of standard samples. The area under each peak represented the concentration of that individual in the mixture.

Saponification of wax. The wax esters (Rf 0.73, 0.61, 0.39) weighing 100 mg were refluxed with IN methanolic potassium hydroxide (10 ml) and benzene (10 ml) for 4 hr. The reaction mixture was cooled and diluted with ether (100 ml). After washing the ether layer with distilled water (3 x) to remove the soaps, it was dried and evaporated to give the unsaponifiable fraction (70 mg).

Liberation and esterification of fatty acids. The combined soap solution was acidified with 2N-sulphuric acid. The liberated acids were extracted with ether, dried as usual and freed of solvent. The semi solid brownish residue was taken in methanol containing 0.5 % hydrochloric acid (5 ml) and refluxed for 2 hr. The esters were extracted with hexane, washed with water, saturated sodium biocarbonate solution and dried. Removal of solvent gave slightly coloured semi-solid esters (30 mg).

Gas liquid chromatography of esters. The instrumentation and the procedure employed were the same as for hydrocarbons. A glass column (1.5 m long, 6 mm 0.D.) filled with chromosorb A.W., 60-70 mesh, having a coating of 10 % DEGS, maintained at 200° was used for this separation. The temperature of the injector and the detector were held at 225 and 250° respectively.

Isolation of glutinol. The least polar alcoholic fraction (0.7 g) was recrystallised from boiling hexane to give glutinol (0.42 g); m.p. 200-2°, IR: 3400-3200 (broad) and 1040 cm^{-1} (OH); 2850, 2825, 1140, 1370 cm^{-1} (C-H). NMR; δ 5.3 -5.8 (m, 1H, olefinic), 3.2-3.6(m, 1H C_3-axial), 1.17, 1.12, 1.09, 1.03, 0.96, 0.86 (s, each 3H) and 1.00 (s, 6H). Acetate m.p. 198°. MS(Probe) *m/e* (rel. int. %): 468 (M^+ $C_{32}H_{52}O$, 10), 408 (8), 393 (4), 274 (100), 259 (60), 205 (18), 173 (14), 134 (23), 109 (21), 95 (23).

RESULTS AND DISCUSSION

The latex is a milky white suspension and has a total solid content of 15 %. In order to avoid secondary transformations, especially the enzymatic ones, the latex was dropped directly into ethyl acetate, which dissolved the organic matter (9.5 % of the latex) leaving behind the cellulosic matter.

Thin layer chromatography of the latex coagulum and extract from pulp was identical in different solvents, hence the coagulum and the extract were mixed together for further investigations. The Rf values, distribution of class compounds and their probable nature are given in Table 1.

The hydrocarbon fraction. The hydrocarbon fraction (Rf 0.77) was eluted with hexane in an 1.2 % yield. It was a white semi-solid. IR spectrum showed only -CH stretchings at 2900, 720, and 710 cm^{-1} indicating predominently straight, long-chain hydrocarbons. However, the NMR spectrum showed, in addition to a large signal at 1.25 ppm, small singlets at 1.00, 0.85, 0.73 and 0.65 ppm which means that not only straight chain but also hydrocarbons with angular methyl groups were present.

The composition of the hydrocarbon-fraction (Table 2) was determined by gas liquid chromatography. The *n*-alkanes ranged from docosane (C_{22}) to dotriacontane (C_{32}) with a maximum of nonacosane (C_{29}). The distribution of the individual hydrocarbons was in the natural order though the maximum was lower than *E. Helioscopia* hydrocarbons [9] by two carbon atoms.

Table 1. Contribution of various class compounds in the latex of *E. cauducifolia.*

R_f	Eluent	Contribution by weight (%)	Nature of fraction
0.77	Hexane	1.2	Hydrocarbons
0.73	Hexane:ether 97.5 : 2.5	6.0	Esters of fatty alcohols with fatty acids
0.61	"	0.8	Esters of terpenoidal alcohols with fatty acids
0.39	"	1.6	Easters of steroidal alcohols with fatty acids
0.30	Hexane:ether 90 : 10	3.6	Terpinoidal alcohol
0.24	"	40.0	Mixture of fatty alcohols and terpenoidal alcohols
0.19	"	2.0	Terpenoidal alcohol containing cyclopropyl grouping
0.09		1.5	Sterol
	Chloroform: Methanol 1 : 1	43.3	Resinous mass

Table 2. The amount of individual fatty acids and hydrocarbons as determined by gas liquid chromatography (%)

Carbon chain length	Acids	Hydrocarbons
12	2.49	–[1]
14	13.71	–
16	58.73	–
$18_{(0)}$[2]	6.63	–
$18_{(1)}$[2]	7.47	–
$18_{(2)}$[2]	4.67	–
20	3.87	–
21	–	2.99
22	3.16	2.90
23	–	14.00
24	–	5.26
25	–	11.37
26	–	7.09
27	–	10.53
28	–	7.46
29	–	16.49
30	–	8.21
31	–	8.40
32	–	4.70

–[1] Not detected.

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

The ester fraction. The ester fraction was eluted with 2.5 % ether in hexane. It was a mixture of three classes of compounds having Rf values 0.73, 0.61 and 0.39. In addition to the -CH stretchings it showed additional strong bands at 1700 and 1245 cm^{-1} when examined spectroscopically. The fraction was separated by preparative layer chromatography into TLC pure fractions.

The least polar fraction (Rf 0.73) showed in its NMR a very strong singlet at 1.28 ppm ($-CH_2^-$ in a straight chain), a triplet centered at 0.85 ($J=6H_z$, terminal methyl adjacent to a methylene), a triplet centered at 2.25 (J=6Hz, methylene linked to an oxygen atom). Small singlets at 0.5, 0.35, and 0.30 ppm were also observed. The data showed that the fraction consists mainly of straight chain alcohols esterified with straight chain acids. Nevertheless, the presence of some unsaturated and some cyclopropyl ring compounds can not be excluded because of the signals at higher fields.

The middle fraction (Rf 0.61) showed, in addition to most of the above signals, singlets at 0.85, 0.90, 1.00 ppm (angular methyls at tertiary carbon atoms); multiplet at 4.40 – 4.80 ppm (cyclohexanol esterified) and multiplet at 5.20 – 5.60 ppm (olefinic protons in ring systems and/or in straight chains). These data indicated that this fraction comprised the terpenoidal alcohols esterified with straight chain fatty acids.

The most polar fraction (Rf 0.39) had and NMR spectrum similar to the middle fraction (Rf 0.61). However, the angular methyl groups were more pronounced. On the basis of TLC of the terpenoidal alcohols and the sterols, this fraction was designated as sterols esterified with straight chain fatty acids.

For the recovery of fatty acids, the ester fractions were combined together and were converted into methyl esters as detailed in experimental. The composition of these acids was obtained by gas-liquid chromatography of the methyl esters and is shown in Table 2. The fatty acids ranged from lauric acid (C_{12}) to behenic acid (C_{22}). Myristic acid (C_{16}) formed the major part (58.73 %) of this saponifiable fraction.

The alcoholic fraction. The alcoholic fraction (45.6 %) was eluted with 10 % ether in hexane. It was a mixture of three components with Rf 0.30, 0.24 and 0.19. This fraction was further separated by preparative layer chromatography into TLC-pure fractions.

The first fraction with Rf 0.30 was a white solid which on crystallization from hexane gave needles m.p. 202° (lit. 207°) [10]. This alcohol was acetylated to give mono acetate m.p. 188° (lit. 192°) [10]. The m.p., IR and NMR data [11] are in agreement with glutinol which has been isolated from *E. royleana.* [12].

The second alcoholic fraction (Rf 0.24) was a solid with a low melting range (65-80°). The IR and NMR spectra showed that it was a mixture of aliphatic and terpenoidal alcohols. Further work on this fraction is in progress and will be reported later.

The third alcoholic fraction (Rf 0.19) has a melting point 110-125°. The NMR of this fraction showed the terpenoidal nature, as in glutinol, and two doublets at 0.1 and 0.2; 0.38 and 0.48 ppm. This fraction may be a mixture of cyclolaudenol and cycloartenol as determined by previous investigators [8].

The resinous/polar fraction. The non-crystalline resinous mass (43.3 %) was eluted with chloroform-methanol (1:1). The IR and NMR data of these compounds showed them to contain more than one functional group, i.e. alcoholic and ester groups. Further work on these materials is in progress and will be reported later.

The quality of the latex from *E. cauducifolia* as evaluated here is similar to that reported for other species e.g

E. lathyris [13] or *E. coerulescens* [14] containing hydrocarbons, esters, mono-functional and poly functional terpenoids. The quantity of available latex seems to be small but it can be increased by analogy to *Hevea brasiliensis* whose yield was raised from 400 lb/acre/year to 24,000 lb/acre/year [14]. The use of latex for burning in the furnaces does not need any treatment except drying in the sun. However, because of its chemical composition, it cannot be a good transport fuel. In order to obtain a better quality fuel, the latex may be processed over certain zeolite catalysts [15].

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