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NEUTRAL LIPIDS FROM THE LEAVES OF EUPHORBIA HELIOSCOPIA LINN.

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Abstract: The neutral lipids from the leaves of *Euphorbia helioscopia* Linn. have been separated into hydrocarbons, wax esters, triterpenoidal esters, hydroxyesters, free fatty alcohols and free sterols. The wax esters were composed of lauric, 1.35; myristic, 5.24; palmitic, 39.30; stearic, 13.27; oleic, 15.66; linoleic, 2.30; arachidic, 19.14; behenic acids 3.80 and higher fatty alcohols. Octacosyl alcohol and β-sitosterol were confirmed both in the free and esterified form. Heptacosane and triterpenoidal acetate C_{32} $H_{52}O_2$ were isolated from the hydrocarbon fraction and the terpenoidal ester fraction respectively.

Euphorbia helioscopia Linn. is locally known as Gandi Buti. It is a common field weed in spring in India and Pakistan. The medicinal uses of this plant as a purgative,[1] in the treatment against influenza[2] and other physiological properties[3] have been recorded in the literature. Earlier work on the species described the presence of a saponin,[1] drying oil,[4] hydroxyphenylglycine and dihydroxyphenylglycine.[5] Ikram *et al.*[6] isolated an aliphatic alcohol, a triterpenoid and a steroid from this species growing in Peshawar valley while Vololueva[7] studied the flavonoids occurring in this plant. The biological activity,[3] the preferential response of this plant to herbicides[8] and the probability of controlling its growth attracted our attention. The present communication deals with the fatty matter present in the leaves of this plant.

Results and Discussion

The hexane extract of the air dried leaves of *Euphorbia helioscopia* gave a solid brownish mass (2.25%, m.p. 50-70°) which on TLC analysis showed eight components having the R_f values as shown in Table 1. Crystallisation of this mass from hexane gave a white amorphous powder (m.p. 75-6° R_f 0.12) which was previously designated as helioscopiol.[6] Column chromatography and subsequent PLC was employed to resolve the mother liquor into TLC uniform fractions. The percentage distribution of these components and their chemical nature is given in Table 1.

In analogy to other plant waxes,[9] the free alcohols constitute a considerable percentage of the hexane extract of the leaves (Table 1). The more insoluble long chain ones crystallise out from the extract on cooling. The amorphous powder m.p. 75-6° is comparable to saturated long straight chain aliphatic alcohols in its IR and TLC analysis. The alcohols

TABLE 1. COMPOSITIONS OF THE NEUTRAL LIPIDS AND R_f VALUES OF THE CONSTITUENTS.

R_f	Percentage of the constituent	Class of compounds
0.85	7.50	Hydrocarbons
0.65	27.00	Wax esters
0.57	5.00	Polar wax esters
0.40	8.50	Triterpenoid
0.35	1.50	Unidentified
0.30	6.00	Hydroxy esters
8.12	40.00	Alcohols
0.05	2.20	Sterols

obtained from the column chromatography of the mother liquor are mainly unsaturated ones. Octacosyl alcohol which has also been isolated from other *Euphorbia* species[10] could also be obtained by crystallisation of the total alcoholic fraction. Further work on alcoholic fraction is in progress.

All the fractions with R_f 0.65, 0.57, 0.40 and 0.30 show carbonyl and ester bands in their IR spectra. They may be comprised of various combinations of saturated and unsaturated monocarboxylic, dicarboxylic, hydroxy acids etc. and saturated and unsaturated aliphatic alcohols, terpenoidal alcohols of steroidal alcohols or a glycol etc. To have a better understanding of these combinations all these fractions are handled separately.

The IR spectrum of the fraction with R_f 0.65 m.p. 57-61° shows in addition to the C-H strechings as in long chain hydrocarbons, strong bands at 1745, 1240, 1180 and 1040 cm^{-1}. In the NMR spectrum a triplet at δ 4.02 (CH_2-O in an ester) broad signals at δ 2.75 and 2.25 (CH_2 α to the carbonyl) and a strong peak

at δ1.32 $(CH_2)_n$ in long chain normal aliphatic compounds) and an overlapping triplet at δ 0.85 revealed it as the wax esters. The presence of unsaturation is indicated by low intensity signals between δ 5.15 and 5.50 (vinyl protons) and at δ 1.9 (allylic protons). Similarly the low intensity multiplet between δ 4.45 - 4.65 due to an ester of cyclohexanol and a singlet at δ 0.58 due to a methyl group attached to a tertiary carbon atom indicate the presence of some sterol or terpenoidal alcohol esters. This hypothesis is confirmed by saponification of the total fraction and resolving the unsaponifiable into the steroidal (R_f 0.05) and the non steroidal fraction (R_f 0.12). The non steroidal fraction behaves similar to the free alcoholic fraction. Octacosyl alcohol can be obtained from the latter fraction by repeated crystallisation. The acidic components of this fraction are esterified by methanolic HCl[11] and compare with the normal fatty acids in TLC analysis. The GLC of these methyl esters and the hydrogenated methyl esters is given in Table 2. They are monocarboxylic acid esters normally found in plant waxes. Among the saturated monocarboxylic acids palmitic acid is the major constituent (39.30%). Lauric acid (1.35), myristic acid (5.24%) stearic acid (13.27%) arachidic acid (19.14) and behenic acid (3.8%) are also present. Among the unsaturated, oleic acid (15.6%) and the linoleic acid (2.3%) were estimated and confirmed by their hydrogenation.

The IR spectrum (KBr) of the third fraction (R_f 0.57,m.p. 62-7°) is similar to that of the previous fraction, but the peaks exhibit varying intensities and is termed as polar wax. Saponification of this fraction gives higher percentage of the unsaponifiable (58.2%) and a lower percentage of the acidic flaction (41.8%) indicating that these esters are either composed of lower molecular weight fatty acids or dicarboxylic acids. The R_f value (TLC) of the methyl esters of this fraction lies in between the normal monocarboxylic acid esters and the dicarboxylic acid esters. The probability of hydroxy acids can be ruled out on the basis of TLC analysis and IR spectrum of this fraction. The unsaponifiable from this fraction behaves similar to the non steroidal fraction from wax esters.

The fraction (R_f 0.40) although uniform in TLC analysis is most probably a mixture of homologues as suggested by its m.p. (135-65°). The IR spectrum (KBr) of this fraction completely overlaps the one produced in the literature[6] for the triterpenoid.Repeated crystallisation of this fraction gives a constant melting crystalline compound which on the basis of its NMR and Mass spectrum proved to be an acetate of a triterpenoidal alcohol. However, contrary to a previous report[6] this fraction can be saponified to give fatty acids (22.5%) and the alcohols (77.5%) having the R_f of free alcohol mixture.

The pale oily liquid (R_f 0.03) shows in its IR in addition to other ester bands a weak hydroxyl band. Under the conditions used for previous esters its saponification is achieved only partially. The acidic fraction after methylation, shows a mixture of two classes of acids the comparatively unpolar having the R_f of polar wax acid esters and the polar that of hydroxy acid esters. The unsaponifiable behaves similar to the non steroidal fraction of the wax.

The IR spectrum of the least polar fraction (R_f 0.85 m.p. 55-60°) shows only C-H streching due to CH_2 and CH_3 at 2940 and 2710 cm^{-1}, CH bending (scissoring) due to CH_2 at 1470 cm^{-1} and C-H (skeletal) in $(CH_2)_n$ ($n \geq 4$) at 730 and 720 cm^{-1} indicating the mixture to consist of straight chain hydrocarbons. A weak band at 1380 cm^{-1} attributed to C-H bending in CH_3-C[12] shows that the mixture contains a very low percentage of methyl groups. Lack of any vinyl proton in the NMR spectrum and the argentation $AgNO_3$ TLC[13] of mixture further confirms saturated nature of this fraction. Similar hydrocarbon mixtures

TABLE 2. FATTY ACID COMPOSITION OF THE WAX ESTERS.

No. of C atoms	Saturated acid	Unsaturated acid	Percentage
C_{12}	Lauric acid	-	1.35
C_{14}	Myristic acid	-	5.24
C_{16}	Palmitic acid	-	39.30
C_{18}	Stearic acid	-	13.27
		Oleic acid	15.66
		Linoleic acid	2.30
C_{20}	Arachidic acid	-	19.14
C_{22}	Behenic acid	-	3.80

have been isolated from other *Euphorbia* species[14] Heptacosane can be isolated from this mixture by repeated crystallisation.

The sterol fraction (R_f 0.05) eluted from the column is homogenous in TLC analysis, but shows a m.p. 128-32°. Repeated crystallisation gives pure β-sitosterol also isolated from *Euphorbia caparissias*[14]. The same sterol is isolated also from the unsaponifiable by a similar procedure. The IR spectrum of the sample overlaps the one produced in literature.[15] The NMR and Mass spectra also confirm it to be β-sitosterol. The mixed m.p. with an authentic sample shows no depression.

Experimental

The melting points were determined on Fisher-Johns melting point apparatus and are uncorrected TLC sheets Woelm Precoated silica gel. F 254/366 were developed in hexane ether (90:10) as the developing solvent until or unless otherwise stated. UV light, iodine or spraying with ceric ammonium sulphate-sulphuric acid was used for visualisation of the spots. Glass plates carrying 1 mm thick layer of Kieselgel HF 254+366 (E.Merck) and activated at 140° for 2 hr were used for preparative layer chromatography. Silica gel 60 (E.Merck) was used for the column chromatography.

Collection and Extraction of the Leaves. Mature plants from the Canal Bank, Lahore were harvested during the month of March. They were allowed to dry in shade and the leaves were separated. The air dried powdered leaves (200 g) were extracted thrice by soaking for 48 hr in freshly distilled hexane. The combined extracts were charcoaled and concentrated to give a brownish white mass (4.5 g, 2.25%) m.p. 50-70°.

Resolution of the Components by Column Chromatography. The solid obtained above (2.0 g) was refluxed in hexane (28 ml) and then allowed to stand overningt. A white amorphous powder (400 mg, 20%, R_f 0.12, m.p. 75-6°) separated out. The clear solution was charged to a column of silica gel (50 g) and eluted with hexane (300 ml) to give the hydrocarbons (R_f 0.85, 150 mg, 7.5%). Wax esters (R_f 0.65, 260 mg, 13.0%) were eluted with hexane-benzene (80:20, 500 ml) and a mixture of wax esters (R_f 0.65) and polar wax esters (R_f 0.57, 300 mg) with another portion of the same eluant (300 ml). A further quantity of this mixture (80 mg) was eluted by hexane-benzene (60:40, 500 ml). The triterpinoidal esters (R_f 0.40, 150 mg, 7.5%) could be obtained by elution with benzene (250 ml). Further elution with benzene (250 ml) gave a mixture (50 mg) of the triterpenoidal esters and two minor constituents. A hydroxy ester (R_f 0.30, 120 mg, 6.0%) was eluted with benzene ethyl acetate (97:3, 100 ml) and alcohols (R_f 0.12, 400 mg, 20%) with the same solvent (150 ml).

The sterol (R_f 0.05, 45 mg, 2.2%) was eluted with the same eluant (200 ml). The remaining resinous mass (45 mg, 2.25%) was obtained by percolating the column finally with ethyl acetate. The mixture of wax and the polar wax esters was separated further by PLC by developing the plates in hexane-ether (95:5) to give the wax esters (280 mg) and polar wax esters (100 mg). Similarly the terpenoidal esters (20 mg) and the polar constituents (30 mg) were obtained by developing the PLC plates in hexane-ether (90:10).

Isolation of Octacosyl Alcohol. The alcoholic fraction (R_f 0.12) obtained as amorphous powder (400 mg) and from the column chromatography (400 mg) were mixed togeter and crystallised from boiling hexane repeatedly to give crystals (120 mg, 15.0.%) m.p. 82-3°, acetate m.p. 73-4° IR- (KBr) : 3125 and 1065 cm^{-1} (OH), 2950, 2860, 1400, 730 and 720 cm^{-1} (C-H); NMR (XL 100, $CDCl_3$): δ 0.88 (3H, t, j = $7H_z$ C-28); δ1.28 (52 H,S, C-2 to2-27): δ 3.62 (2H, , t, j = $7H_z$ C-l); MS (Probe): 410 M^+ (10%), 392 (M^+-18, 100%) 364 (M^+-46, 75%) 350 (M^+-60, 5%); 336 (M^+-74, 15%) 321 (M^+-89, 8%) 307 (M^+-103, 10%) etc. etc.

Isolation of β-sitosterol. The steroidal fraction (R_f 0.05) weighing 45 mg and melting between 128-32° was crystallised from alcohol to give needles (20 mg, 44.5%) m.p. 137-9°, acetates m.p. 133-4° and the m.p. with an authentic sample of β-sitosterol 138-9°. IR-(KBr) 3300, 1060 cm^{-1} (OH), 2950, 1485, 1390 (CH). NMR (XL-100, $CDCl_3$): δ 0.64 (3H, S, C-18), 0.84 (3H, S, C-19) 1.0 (3H, S) δ 3.5 (1H, m, C-3); 5.35 (1H, m, C-6), MS (Probe): 414 M^+ (100%), 396 (M^+ -18, 32%), 381 (M^+-33, 15%).

Isolation of Heptacosane. The hydrocarbon fraction (R_f 0.85) was crystallised thrice from chloroform methanol to give shining flakes (60 mg, 8.7%) m.p. 59-60°. IR (Oil Film): 2940, 2710 cm^{-1} (C-H stretchings); 730 and 720 cm^{-1} (C-H skeletal), NMR (60 MH_z $CDCl_3$) : δ 0.85 (6H, t, J= $6H_z$ terminal CH_3) and 1.25 (50 H, S, $(CH_2)_n$ in normal chain compounds). Elementary analysis; found C, 84.90, H, 14.19; $C_{27}H_{56}$ requires C, 85.17, H, 14.83%.

Isolation of Terpenoidal Acetate. A portion of the fraction (R_f 0.40, m.p. 135-65°) was repeatedly crystallised from boiling methanol to give white needless m.p. 195-7°. IR (KBr): 3080, 2950, 1740, 1645, 878 cm^{-1} NMR (XL 100,$CDCl_3$): β 4.68 (1H, q, J= 1.5 H_z H in CH_2 (C-CH_3). δ 4.56 (1Hq, j= 1.5 Hz, H in CH_2 = C- CH_3). δ 2.03 (3H, S, CH_3 in CH_3-CO), δ 1.68 (3H, d, J= 1.5 Hz, CH_3 in CH_2 = C -CH_3 and singlets at δ, 1.26, 1.03, 0.94, 0.84, 0.79 each equivalent to 3H. MS (Probe)468 M^+ (70%), 453 (M^+-15, 15%), 408 (M^+ -60, 75%), 365 (M^+-103, 70%).

Saponification of the Wax. The wax esters R_f 0.65 (360 mg) were taken in 1 N ethanolic potassium hydroxide (20 ml) and refluxed for 4 hr. The reaction mixture was diluted with ether (150 ml) and washed thrice with distilled water. The extract was dried over anhydrous Na_2SO_4 and evaporated to give the unsaponifiable as a solid mass (205 mg).

Resolution of the Unsaponifiable. The unsaponifiable (205 mg) was charged to glass plates having 1 mm thick layer of silica gel and developed in hexane ether (70:30). The zones were visualised with 2,4-dichlorofluorescein, scrapped off and eluted with chloroform. The non-steroidal and the steroidal portions weighed 188 mg and 12 mg respectively. Repeated crystallisation of the former fraction from boiling hexane gave crystals of octacosylalcohol m. p. 82-3° (32 mg, 15.5%) which remained undepressed when mixed with octacosyl alcohol isolated from the free alcohol. The latter fraction m.p. 124-32° on crystallisation gave β-sitosterol (5 mg, 41.6%) m.p. and mixed m.p. with an authentic sample 138-9°.

Liberation and Esterification of Wax Acids. The combined soap solutions from the saponification of wax were acidified with 2 N sulphuric acid, and the liberated acids extracted with ether. The ether extract on drying and evaporation gave a semi-solid mass (150 mg, 43.0%) which was taken in anhydrous methanol containing 5% hydrochloric acid (10 ml) and refluxed for 2 hr. After usual work up the product was filtered over silica gel (5 g) to give pure, colourless semi-solid esters (140 mg), which behaved as a single spot in TLC analysis.

Hydrogenation of the Methyl Esters. Purified methyl esters (70 mg) in methanol (100 ml) and palladium chloride (20 mg) were shaken in a Paar Hydrogenator for 4 hr. The pressure of hydrogen gas was 40 lb/□". Removal of the catalyst and the solvent gave solid esters (70 mg).

GLC of the Methyl Esters. Methyl esters of the fatty acids were analysed on a Pye Unicam 104 Instrument with a FID. A glass column (1.5 m. long, 0.6 mm O.D) filled with chromosorb A.W. 60-70 mesh having a coating of 10% DEGS and maintained at 180° was used for this separation. Nitrogen 50 ml/mm was used as the carier gas.

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