Neutral Lipids from *Euphorbia Helioscopia* Linn

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Summary: The neutral lipids were extracted with hexane in 2.8% yield and resolved into an acidic fraction (28.7%) and a neutral fraction (71.1%). The normal monocarboxylic acids (19.26%), the hydrocarbons (9.94%), the monohydric alcohols (35.53%) and the sterols (5.61%) were isolated from the acidic fraction and neutral fraction by column chromatography. The class compounds were analysed by gas liquid chromatography. Saturated and unsaturated fatty acids ranging from lauric (C_{12}) to cerotic (C_{26}) were present with palmitic acid (C_{16}) being predominent. The n-alkanes range from henocosane (C_{21}) to heptatriacontane (C_{37}) with hentriacontane (C_{31}) as the major product. The n-alkanols range from behenyl (C_{22}) to myricyl (C_{30}) with ceryl (C_{26}) as the maximum. β-Sitosterol was found to be the major component (97.35%) of the sterol fraction.

Introduction

Euphorbia helioscopia locally known as "gandi buti" grows in winter crops in the whole of Pakistan as an undesirable weed[1]. The plant possesses certain physiological properties which are associated mainly with the latex[2]. Its saponins, flavones[3] and other constituents[4 5 6] have been investigated by earlier workers. In our previous publication[7], the neutral lipids from the leaves had been investigated. The whole plant has now been selected for the present investigation.

Results and Discussion

The neutral lipids were extracted with hexane in 2.87% yield which is higher than what was found in the leaves (2.25%). The class compounds were the same as found in the extract from leaves, but in different concentrations. The present extract is richer in non-waxy components.

The extract was saponified to separate the acidic and neutral fractions. The neutral fraction was chromatographed on silica gel and various class compounds namely, hydrocarbons, alcohols, sterols were separated. All the classes have been dealt separately.

Acidic Fraction

The acidic fraction constituted 28.7% of the hexane extract. It was a dark brown solid and partially soluble in hexane. This fraction may contain, in addition to the normal fatty acids, hydroxy, keto or dicarboxylic acids which are usually found in the plant waxes. The entire fraction was esterified with BF_3-methanol and analysed by thin layer chromatography, whereupon it showed one major spot and some minor polar spots. The major spot corresponds to fatty acid esters and was isolated in 66.6% yield by column chromatography. The colourless semi-solid esters were analysed by gas liquid chromatography. On comparison of the retention times with standard samples, the acids were found to range from lauric (C_{12}) to cerotic (C_{26}) acids but the possibility of higher acids cannot be excluded. Certain peaks corresponded to unsaturated acids which were confirmed by the hydrogenation of this fraction. On the basis of equivalent chain length[9] (ECL), certain peaks were found to correspond to odd numbered acids as well. However, their concentration was very small. The composition as determined by gas liquid chromatography is given in Table-I. The unsaturated acids oleic, linoleic, linolenic and a C_{20}-unsaturated acid, most probably gadoleic acid, are derived from the semi-drying oil present in the seeds of this plant.

Hydrocarbon Fraction:

The hydrocarbon fraction was a white crystalline solid. TLC analysis of this fraction on silica gel plates impregnated with $AgNO_3$ showed mainly the saturated

nature of this fraction. Similar hydrocarbon fractions have also been isolated from other *Euphorbia* species[10] On gas liquid chromatographic analysis the fraction was resolved into 46 peaks. The chromatogram was well defined. The baseline remained stable and there was no indication of an envelope which is formed by the overlapping of unresolved components. The retention times of some of the peaks did not correspond to n-alkanes. However, their intensity was small and contributed only 6.94% of the total area under the chromatogram. These peaks may originate from iso or cyclic or unsaturated hydrocarbons. On the other hand some of the peaks corresponding to n-alkanes may contain some non-normal hydrocarbons e.g. pristane, phytane, squalene etc. Therefore, the n-alkanes were isolated by adsorption on molecular sieves and analysed by GLC. These n-alkanes range from C_{21} to C_{37}. This range is probably the most frequent n-alkane range occurring in plant waxes[11]. The overall composition of n-alkanes is given in Table-I. The concentration of C_{34} or C_{36} is only in traces. The odd numbered hydrocarbons predominate and C_{31} occurs as the maximum i.e. 56.3% of the hydrocarbon fraction. Such distributions are typical in plant waxes and sometimes may be used to characterise a certain plant wax.

Alcoholic Fraction:

The alcoholic fraction constitutes 50% of the unsaponifiable. The fraction is crystalline but chemically a mixture. For characterisation of the individual constituents, the mixture was acetylated and the n-alkanols isolated by urea adduction. These n-alkyl acetates formed 20.56% of this fraction and were analysed by gas liquid chromatography. Both odd and even numbered alcohols ranging from behenyl (C_{22}) to myricyl (C_{30}) exist in this mixture. However, the even numbered compounds predominate. Hexacosanol (C_{26}), which is usually a common constituent of plant waxes, both in free and esterified form, occurs as maximum (62.27%). The non adduct forming alcohol acetates might be unsaturated or branched or cyclic in structure. They form the major portion (79.46%) of the alcoholic fraction. Further work on this fraction is in progress.

Sterol Fraction:

The sterol fraction formed 7.92% of the unsaponifiable. It was crystalline but non homogeneous in melting point. β-Sitosterol which has been reported previously[7] could be obtained by crystallisation. The total fraction was acetylated. The acetate resolved into four components in GLC analysis. The retention times and the contribution of each component are given in Table-II.

Table-I The %age of individual acids hydrocarbons and alcohols as found by gas liquid chromatography

Carbon chain length	Acid	Hydrocarbon	Alcohol
12	3.979	–[1]	–
14	7.502	–	–
16	31.681	–	–
18(0)[2]	5.598	–	–
18(1)	8.020	–	–
18(2)	19.306	–	–
18(3)	4.425	–	–
20(0)	7.941	–	–
20(1)	1.761	–	–
21	–	0.117	–
22	6.527	0.109	0.130
23	–	0.445	–
24	2.884	0.310	0.528
25	–	1.117	0.158
26	0.379	0.487	62.274
27	–	7.047	0.513
28	0.071	0.421	29.879
29	–	15.820	Traces
30	0.890	6.515	6.515
31	–	56.303	–
32	–	0.488	–
33	–	11.422	–
34	–	Traces	–
35	–	2.953	–
36	–	Traces	–
37	–	1.373	–

1 not detected

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

Table-II The retention times and % occurrence of individual components in sterol fraction

S.No.	Rt in min.	% area	Identification
1.	21.05	0.09	
2.	33.92	0.23	
3.	43.45	2.31	
4.	56.09	97.35	β-sito sterol

Experimental

Collection of the Plant:

The mature plants were collected from the laboratory campus during the month of March. The plants were allowed to dry in shade when the mature seeds fell off. The remaining plants were chopped and ground.

Extraction of the Plant:

The ground plant (140 g) was Soxhlet-extracted with hexane for 24 hours. The greenish extract was treated with charcoal and the reddish solution freed of solvent to give a solid mass (3.9g, 2.8%).

Saponification of the Extract:

To a solution of the extract (3.8g) in toluene (20 ml), 2N ethanolic KOH (50ml) was added and the homogeneous mixture refluxed for 6 hr. The solvent was removed and the residue taken in water. The aqueous solution was extracted with ether (150 ml). The organic layer was washed thrice with water and the ethereal solution when dried over anhydrous sodium sulphate and evaporated gave the unsaponifiable (2.70 g, 71.1%).

Liberation of Fatty Acids:

The soap solution was acidified with 2N H_2SO_4, the liberated acids extracted with ether and the usual work up gave the semi solid material (0.66g, 28.70%).

Esterification of Acidic Fraction:

The acidic fraction obtained above (0.505g) was taken in a solution of boron trifluoride diethyl etherate in methanol (25 ml) and refluxed on water bath for two hours. Further work up gave the esters which on TLC analysis showed one major spot corresponding to methyl esters of fatty acids and other spots which were more polar than methyl esters but minor in concentration. The fatty acid esters were isolated by column chromatography on silica gel and amount to 0.3336 g which is 66.6% of the acidic fraction.

Resolution of the Unsaponifiable:

The unsaponifiable (1.64g) was charged to a column of silica gel (40 g, dia 2.5cm, height 25 cm). The hydrocarbon fraction (230 mg, 14.03%) was eluted with hexane (100 ml). Further elution with hexane-benzene (60:40, 500 ml) gave the oily material (30 mg 1.4%) which comprised of at least three types of constituents. Benzene (500 ml) eluted the alcohols (820 mg, 50.00%) and another portion of benzene (200 ml) gave the sterols C130 mg, 7.92;). More polar components (300 mg, 18.29%) were eluted with ethyl acetate (150 ml).

Separation of normal and branched/Cyclic Hydrocarbons:

A solution of the hydrocarbon fraction (153.6 mg) in cyclohexane (30 ml) was refluxed with molecular sieves (5A, 0.159cm pellets, activated in vac. at 250°C for 6 hours, 6g) for 72 hours. The sieves were filtered off and washed thrice with warm cyclohexane. They were Soxhlet extracted with benzene: propanol (1:2) for 4 hours. This extract, the filtrate and the washings were mixed together and evaporated to give a pale viscous liquid which was redissolved in hexane and passed over alumina to remove the polar impurities. A colourless semi-solid mass (13.6 mg, 8.85%) was obtained. The sieves were powdered, suspended in cyclohexane and digested with the dropwise addition of 24% HF (25 ml). The grey coloured aqueous solution was extracted with hexane, passed over alumina (3g), and evaporated to give colourless n-alkanes (135.9 mg 88.5%).

Acetylation of Alcoholic Fraction:

The alcoholic fraction (730 mg) was acetylated with acetic anhydride-pyridine at room temperature to yield a white solid (760 mg).

Isolation of n-Alkylacetates by Urea Clathrate:

The acetate mixture (330 mg) was taken in dry methanol (35 ml) and dissolved by warming. The turbidity which appeared on cooling was cleared by the addition of chloroform (5ml). Powdered urea (3.5g) was added to this solution, the mixture was stirred for one hour and then allowed to stand over night. The clathrate was filtered, washed with hexane and decomposed with hot water. The liberated n-alkyl acetates were extracted with ether, dried over Na_2SO_4 and freed of solvent. The yield was 73 mg (20.6%).

Gas Liquid Chromatography of Fatty Acid Methyl Esters:

The esters were analysed on Hewlett Packared Gas Chromatograph 5700A with a flame ionisation detector. A Soltec Ridkadenki Recorder and a Hewlett Packard Integrator 3373B were used for registering and measuring the peak areas respectively. Helium was used as the carrier gas. Hydrogen and air were used for the flame. The chart speed was 15 cm per hour. A stainless steel column 182cm x .32cm packed with 3% SP 2300 on 100/120 mesh supelcoport AWDMS was used for the separation. The oven temperature was programmed from 80-200° with a rise of 4° per minute and hold at final temperature till no more peaks appeared. The individual peaks were identified by comparison of retention times, equivalent chain length[8,9] and coinjection of standard samples.

Gas Liquid Chromatography of n-alkanes:

The instrumentation used was the same as for the fatty acid esters. A stainless steel column 300 cm x .32 cm packed with 3% dexsil 300 on 80/100 mesh chromosorb WAW (Supelco, Inc.) was used for the separations. The oven temperature was programmed from 150-300° and 2° rise per minute, and then run isothermally at 300° till no more peaks evolved. The identity of the constituents was confirmed by comparison of retention times and co-injecting the standard hydrocarbons. The percentage area of each component is given in Table-I.

Gas Liquid Chromatography of n-Alkyl Acetates:

The n-alkyl acetates were analysed using the above described equipment. A stainless steel column 182 cm x.32 cm packed with 3% OV-1 was used for these separations. The column was maintained isothermally at 250°. The temperature of injection port and the detector were 250° and 350° respectively. The n-alkyl acetates (10 mg) were dissolved in cyclohexane (0.05 ml) and 0.015 µl sample was injected on to the column.

G.L.C. analysis of the Sterol Fraction:

This fraction was acetylated and analysed just like the alcoholic fraction.

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