COMPOSITION OF THE WAX OF *Sonchus Asper* VILL.

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Chromatographic and spectroscopic methods were employed for analysis of the plant wax of *Sonchus asper* VILL. The wax contains hydrocarbons (11·7%), esters (17·4%), triterpene acetates (16·1%), primary alcohols (11·1%), triterpenic alcohols (7·8%) and sterols (11·7%).

Sonchus asper VILL. (*Compositae*) is an annual herb. The plant is pounded and applied to wounds and boils[1]. In view of its medicinal properties a chemical analysis of the surface wax of the herb has been undertaken and the results being presented in this communication.

The neutral extract (2·1%) was chromatographed on a column of alumina (30-fold excess). The successive elution with hexane and benzene afforded following substances after further purification: A homologous series of n-alkanes (11·7% of the extract) ranged from C_{17}—C_{35} with a maximum occurrence of odd numbered members n-nonacosane (C_{29}), n-hentriacontane (C_{31}) and n-tritriacontane (C_{33}) (Table I). The predominance of odd members between C_{27}—C_{35} is preserved[2,3].

The subsequent group of compounds exhibited bands at 1176 and 1725 cm^{-1} in IR spectrum and contained aliphatic long-chain esters which were transesterified with methanol and gaseous hydrogen chloride[4]. Resulting products were gas chromatographed as methyl esters of the acids and free alcohols. Their record revealed that the original esters are formed by a mixture of normal acids (C_{12}—C_{32}) with the highest participation of C_{22}-homologue, esterified by a series of primary alcohols (C_{22}—C_{30}) with a maximum occurrence of hexacosanol (C_{26}), triacontanol (C_{30}), then by β-sitosterol (C_{29}) and probably by triterpene alcohols (C_{30}) (Table I). Even members were prevalent[5,6] as usual.

Triterpene acetates (16·1% of the extract) were obtained as crystals melting at 210–212°C. This fraction is not silylated (BSA) and moreover the GLC traces in BSA and in isobutane were identical showing the absence of free hydroxyl group. The identification of individual substances in the mixture was performed by combined gas chromatography–mass spectrometry, indicating the presence of eight compounds; three major ones having molecular weight of M^+ 468

corresponding to $C_{32}H_{52}O_2$ and possessing almost identical mass spectra. The other five minor products had molecular weight M^+ 408 corresponding to $C_{30}H_{48}$, the lower part of the spectra being identical with those of the three major components. These results indicate that the chromatographic fraction is a mixture of three isomeric acetates which probable loose a molecule of acetic acid to give the products of mass 408. The loss of acetic acid was thermal during GLC as only three compounds were separated by preparative TLC. Three major isomeric triterpene acetates were identified on the basis of mass fragmentation[7-9] pattern and by GLC comparison with those of authentic samples as (A) ψ-taraxasterol acetate (20·5%), (B) taraxasterol acetate (32·5%) and (C) lupeol acetate (38·9%).

The further group of substances (19·0% of the extract) represented on GLC analysis a homologous series of free primary alcohols (C_{22}—C_{28}, 58·6%) with maximum occurrence of hexacosanol and three free triterpenic alcohols lupeol, taraxasterol

TABLE I

Composition of n-Alkanes and Esters (from GLC analysis)

Number of C-atoms	n-Alkanes, %	Acids[a] from esters, %	Alcohols from esters, %
12—15	—	tr	—
16	—	12·3	—
17	tr	tr	—
18	tr	4·1	—
19	tr	tr	—
20	tr	17·1	—
21	tr	tr	—
22	tr	35·3	tr
23	tr	tr	tr
24	tr	2·1	tr
25	tr	tr	tr
26	tr	tr	52·3
27	6·8	tr	tr
28	5·8	6·5	tr
29	16·3	tr	[b]
30	11·4	9·1	[c]
31	28·1	tr	—
32	9·8	12·0	—
33	11·7	—	—
34	5·1	—	—
35	2·6	—	—

[a] As methyl esters; [b] β-sitosterol 7·4%; [c] triacontanol 12·2%, triterpenols 26·7%.

and ψ-taraxasterol (40·8%). The last eluted fraction (11·7% of the extract) consisted of hexacosanol (19·6%), β-sitosterol (42·1%), stigmasterol (31·6%) and campesterol (6·3%).

The results indicate that sterols occur in this herb as esters of normal acids (C_{12}—C_{32}) as well as in the free state, while triterpenols occur in free state and as acetates.

EXPERIMENTAL

Extract. Air-dried herb (500 g) from the campus of Shri Varshneya College, Aligarh (India), was extracted thrice with 5 l light petroleum (60—80°C) at boiling temperature. A total of 10·4 g (2.1%) purified neutral extract was obtained.

Chromatography. Column chromatography was done on activated alumina or silica gel. Thin layer chromatography was done on silica gel G. Elution of the plate with tetrachloromethane with 5% ethyl acetate gave spots of following R_F values: hydrocarbons 1·0; esters 0·9; triterpene acetates 0·82; free alcohols 0·51; sterols 0·33. Preparative TLC of triterpene acetates was performed on silica gel impregnated with 20% silver nitrate (plate 20 × 20 cm, 0·5 mm layer of the adsorbent). Gas chromatography of hydrocarbons and esters was done on PYE series 104 chromatograph model 124 with flame ionisation detectors, two columns packed with 3% SE-30 on Gas Chrom Z at programmed temperature 100—270°C (3°C . min^{-1}). Triterpene acetates were analyzed on a column packed with Dexsil 300 GC on 100—120 mesh Diatomite CQ at 260°C with helium as a carrier gas. For identification of homologues the usual graphical method was employed[10]. For a quantitative evaluation areas under the peaks have been calculated. The comparison of the retention values with those of standards was used for identification of the gas chromatographic peaks.

Spectroscopy. IR spectra were recorded on a Perkin–Elmer spectrometer using KBr discs. The ^{1}H-NMR spectrum was recorded on Varian spectrometer A-60 D in deuteriochloroform using tetramethylsilane as internal standard. The chemical shifts are expressed in the δ-scale. The mass spectra were measured in an AEI model MS 9 spectrometer in connection with the PYE series 104 chromatograph through a silicon rubber membrane separator. The mass measurement was facilitated by the use of an Instem Maxi data sastem.

Separation of the components from the extract. The basic column chromatography on alumina afforded three main fractions: the first one was eluted with hexane, the second with hexane and the last one with benzene. First fraction showed two spots on TLC plate (silica gel–silver nitrate 2%) and was rechromatographed over a column of silica gel which yielded n-alkanes (m.p. 62 to 63°C, acetone–benzene) and aliphatic esters (m.p. 67—69°C, acetone–benzene). The second fraction (acetates of triterpenic alcohols) was obtained on further elution with hexane. It exhibited prominent IR peaks at 1 730, 1 248 (acetate), 1 640 (C=C), 1 366, 1 388 cm^{-1} (geminal dimethyl group). ^{1}H-NMR spectrum gave the signals at 0·75, 0·81, 0·86, 0·96, 1·05, 1·27, 1·41 and 1·46 ppm, singlet at 2·03 ppm (—$OCOCH_3$) and multiplets at 4·29—4·6 ppm (>$CHOCOCH_3$), 4·59—4·69 ppm (>C=CH_2) and 4·76—4·92 ppm (>C=CH—) (ref.[11]). Suitable preparative separation of the mixture of triterpene acetates was accomplished on the TLC plate using hexane–benzene (3 : 2) as developer into three principal zones, (A) m.p. 232—233°C, (B) m.p. 248—249°C and (C) m.p. 216°C in the following respective R_F values: 0·52, 0·45 and 0·32. Mass spectra (*m/e*, rel. intensity) of individual triterpene acetates: A) ψ-Taraxasterol acetate 189(100), 203(35·8), 218 (67·8), 365(6·6), 393(6·4), 408(9·6), 453(11·8), 468 M^+(12·9). B) Taraxasterol acetate189(100), 203(31·7), 218(4·5), 365(9·5), 393(8·7), 408(13·3), 453(7·6), 468 M^+(16·4). C) Lupeol acetate 189(100), 203(19·8), 218(15·4), 365(9·1), 393(8·2), 408(15·8), 453(5·8), 468 M^+(11·4).

Third fraction eluted with benzene was found to be a mixture on the basis of TLC (silica gel–silver nitrate 2%). It was thus rechromatographed on alumina yielding the mixture of aliphatic and triterpenic alcohols. This mixture was converted into acetates and then spotted along with authentic specimens of triterpene acetates on TLC plate with silver nitrate for identification. Analogously, the comparison of the retention values on GLC of free alcohols with those of standards was done. On crystallisation from acetone–benzene the alcohols yielded hexacosanol m.p. 79°C. It exhibited bands at 1050, 3300 (OH) and 715, 725 cm^{-1} [$(CH_2)_n$] in the IR spectrum and was identified by determination of mixed m.p. (lit.[12] m.p. 79·5°C), on TLC with the authentic sample and by preparing its acetate m.p. 64°C (lit.[13] m.p. 65°C).

An other product of rechromatography was a mixture obtained on elution with light petroleum–benzene (2 : 3) which on GLC analysis represented hexacosanol and phytosterols. Repeated crystallisation from chloroform–methanol yielded β-sitosterol, m.p. 134—135°C, homogeneous on TLC (silica gel–silver nitrate 10%). It gave positive Liebermann–Burchard test and yellow colour with tetranitromethane. IR spectrum showed bands at 3400 (OH), 1630 (C=C) and 830 cm^{-1}. It was identified by mixed m.p., on TLC with the authentic sample and by preparing its acetate and benzoate (m.p. 122°C and 144°C respectively)[14].

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