PLANT SUBSTANCES. XXXV.* ON NATURAL WAXES. XXI.** HIGHER ALKANES AND WAX ESTERS OF SOME LIVERWORTS

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Using gas chromatography the composition of two important groups of lipidic substances, *i.e.* n-alkanes and cerides, from five liverworts (*Hepaticae*) has been established. The range of the homologous series of n-alkanes is $C_{15}-C_{35}$ for the majority of the investigated samples. In *Calypogeia meylanii*, *Jungermania sphaerocarpa*, and *Pellia fabbroniana* n-C_{27} predominantes, while n-C_{31} is dominant in *Gymnocolea inflata* and *Mylia taylorii* which contain in addition the same amount of n-C_{25}. The homologous series of esters range from C_{34} to C_{54} but the prevalence of the dominant homologues is less distinct than in n-alkanes. C_{48} represents the main homologue in *Jungermania sphaerocarpa*, *Mylia taylorii* and *Pellia fabbroniana*, while in *Calypogeia meylanii* and *Gymnocolea inflata* the homologue C_{44} predominantes.

In connection with the study of the components of lower plants we also investigated liverworts (*Hepaticae*), which belong to plants from an separate class of *Bryophytae*. Investigations in this field take two directions. The first one concentrates on the major components of neutral character with the aim of solving their new structures[1-4], while the second direction leads to the identification of other components present in liverworts as minor components. As many of these substances are common not only to various liverwort species, but may also be found in liverworts belonging to various genera and even families, their study is of chemotaxonomic importance. In the present paper we describe the composition of two distinct groups of substances, *i.e.* higher saturated aliphatic hydrocarbons and aliphatic esters (cerides) in the following liverworts: *Calypogeia meylanii* (BUCH.), *Jungermania sphaerocarpa* HOOK. (syn. *Solenostoma sphaerocarpa* (HOOK.) STEPH.), *Gymnocolea inflata* (HUDS.) DUM., *Pellia fabbroniana* RADDI, and *Mylia taylorii* (HOOK.) GRAY.

The analyses of higher non-isoprenic saturated hydrocarbons have already been carried out for some time in plant material[5-7]; the composition and the ratio of higher n-alkanes was even proposed as a possible chemotaxonomic character for the classification of the plants[6,7], which,

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however, was later demonstrated as unsufficiently specific[8,9]. The analytical methods for higher n-alkanes and their simply branched isomers, especially their gas chromatographic analysis, are already well elaborated[7,10–13]. A different situation existed until recently in the analysis of wax esters (cerides) which could not be analysed by routine gas chromatography because of their relatively low volatility ($C_{30}-C_{60}$). Usually the ester fraction was first hydrolysed or trans-esterified, and then acid and neutral components were analysed separately. Recently we described a high temperature gas chromatography of cerides[14] which enabled the obtainnig of direct information on the composition of the esters in the ester fraction isolated mostly by column chromatography. We also made use of this method in this study.

EXPERIMENTAL

*Preparation of the material.** Carefully selected and purified (from mechanical impurities) liverwort (80 g) was dried at room temperature, ground in a ball-mill, and eventually extracted exhaustively with light petroleum (b.p. 40–60°C) at room temperature. The combined light petroleum extracts were concentrated on a rotatory vacuum evaporator and the residue (0·5 to 2·3 g, depending on the liverwort used) was adsorbed on a small amount of deactivated (10% of water) silica gel and put on the top of a column of the same adsorbent (100 g).

TABLE I

Composition of n-Alkanes[a] of the Liverworts Investigated (%)

No of C-at	Cal	Gym	Jun[b]	Myl	Pel	No of C-at	Cal	Gym	Jun[b]	Myl	Pel
15	tr	1·2	—	tr	—	27[d]	tr	—	—	—	—
16	0·6	tr	tr	1·2	tr	28	3·8	1·8	4·6	1·8	2·8
17	1·3	1·8	0·3	1·8	2·5	28[d]	0·6	—	—	—	—
18	0·6	0·3	0·7	1·8	1·2	29	15·3	12·3	17·8	5·3	22·2
18[c]	—	16·6	—	—	—	29[d]	0·4	—	—	—	—
19	1·3	1·0	2·1	2·1	1·4	30	2·1	1·8	3·0	2·1	1·4
20	0·6	1·9	3·7	1·9	1·4	30[d]	0·6	—	—	—	—
21	1·8	2·9	3·8	3·9	1·4	31	9·5	25·6	9·5	17·0	4·0
22	1·4	3·9	3·2	1·8	1·2	31[d]	17·2	—	—	—	—
23	3·8	4·2	6·1	6·6	2·8	32	1·4	1·2	1·3	2·4	1·0
24	2·4	1·9	3·3	2·1	1·4	32[d]	2·1	—	—	—	—
25	7·6	4·9	9·8	17·0	16·0	33	2·5	3·6	2·2	9·0	1·2
26	3·5	1·8	4·6	3·0	2·4	34	—	tr	tr	tr	—
26[d]	tr	—	—	—	—	35	tr	0·9	0·3	4·2	0·5
27	19·0	5·9	21·5	11·2	33·2						

[a] The amount of branched alkanes was usually 2·0–2·5%; in *Mylia taylorii* 4·0%. [b] „Chromatographic background". [c] Isoprenic hydrocarbon. [d] Homologous series of iso(anteiso)-alkanes.

* In the present work the extracts were prepared from botanically absolutely uniform liverwort samples.

Chromatographic separation. For column chromatography silica gel[15] of particle size 30–60 μ was used, which was partly deactivated by the addition of 10–13% of water. n-Alkanes were eluted from the column with light petroleum in the first fractions, while esters could be eluted with light petroleum containing 2–30% of benzene. Thin layer chromatography used for the control of the separation procedure on columns was carried out on silica gel G, Merck.

Gas chromatographic analyses were carried out on a PYE Series 104 Chromatograph, Model 24, provided with a dual column and detector (FI) system and the possibility of programmed heating. Columns of 0·4 × 150 cm size were filled with 2·5–3% of SE-30 G.C. Grade on Gas Chrom P (100–120 mesh). Chromatographic peaks were identified by means of standards with the utilisation of the linear relationship between the logarithm of the retention time and the number of carbon atoms, valid for homologous series. Quantitative evaluation of gas chromatograms was carried out without correction factors, on the basis of peak integrals; in the case of esters, where a sufficiently smooth zero line could not be achieved at higher temperatures, the height of single peaks was used as a quantitative parameter.

Transesterifications of esters were carried out with methanol and gaseous hydrogen chloride in sealed ampules in tetrachloromethane[16]. The homologous series of methyl esters of monocarboxylic acids and free alcohols were identified together by gas chromatography. Pairs of peaks were formed in which the first eluted peak belonged to the free alcohol and the second to the methyl ester of an acid having the same number of carbon atoms as its alcohol.

IR *spectrophotometry and mass spectrometry* served for preliminary determination of the limits of single groups of substances coming out of the chromatographic column. The IR spectra were measured on a Zeiss UR 10 spectrophotometer in tetrachloromethane, the mass spectra on a MS 902 AEI apparatus.

RESULTS AND DISCUSSION

n-Alkanes

In all five samples the homologous series had approximately the same range, $C_{17}-C_{33}$ (Table I). The hypothesis was emitted[7,8] that the predominance of odd n-alkanes over even ones is common in higher plants, while in lower plants (including *Pellia epiphylla* and *Fegatella conica* liverworts) the ratio of odd and even homologues seems to be almost equal. From this point of view the ratio of odd and even homologues in the $C_{15}-C_{22}$ range seem in the case of all the liverworts studied by us rather even, while in the case of higher homologues odd n-alkanes distinctly prevail. *Calypogeia meylanii* contains in addition to n-alkanes a relatively richer series of branched (iso- and anteiso-) isomers ($C_{26}-C_{32}$) (Table I). The mentioned paraffins, together with other alkanes monomethylated in the central part of the chain[17,18], often accompany normal alkanes, but in this case their frequent occurrence is exceptional. In the case of *Jungermania sphaerocarpa* the presence of another as yet unidentified mixture of hydrocarbons is evident; they form an asymmetric flat diffuse wave between n-C_{18} and n-C_{30}, as a "chromatographic background". This effect has been described several times for various plant extracts of lipidic character[17]. The fraction of n-alkanes from *Gymnocolea inflata*[19] contains a distinct amount

of an evidently isoprenic hydrocarbon with a retention time close to n-C_{18}. On the chromatogram of *Mylia taylorii* homologous series of branched alkanes are also well discernible, of which mention was made already in the case of *Calypogeia meylanii*. In the case of three of the five samples (*Calypogeia meylanii*, *Jungermania sphaerocarpa*, and *Mylia taylorii*) in the hydrocarbon fraction from chromatography isoprenoid sesquiterpenic olefins were also found, the composition of which will be described elsewhere.

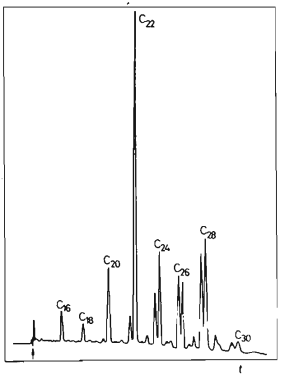

FIG. 1

Chromatogram of the Products of Transesterification of Cerides from *Jungermania sphaerocarpa*

Black waves belong to alcohols, unaccentuated waves to methyl esters. 3% SE-30, 150–260°C ($2°C/min^{-1}$).

Aliphatic wax esters (cerides)

They occur in all five samples to an approximately equal extent, $C_{36}-C_{52}$, with even homologues[10] distinctly prevailing (Table II). However, the difference in their quantita-

TABLE II

Composition of Cerides[a] of the Investigated Liverworts (%)

No of C-at	Cal	Gym	Jun	Myl	Pel	No of C-at	Cal	Gym	Jun	Myl	Pel
34	–	1·2	0·2	0·6	–	46	21·2	20·5	17·6	16·5	16·9
36	tr	2·5	1·0	2·1	tr	48	17·6	14·6	23·3	19·6	17·5
38	1·0	4·9	2·6	5·7	1·3	50	5·3	4·2	13·6	5·6	15·8
40	3·6	6·2	4·8	11·5	3·4	52	–	1·5	13·0	4·1	10·9
42	13·2	18·5	5·0	14·8	10·6	54	–	–	2·1	tr	4·3
44	33·3	22·6	13·3	17·1	16·2						

[a] Sum of odd homologues: *Calypogeia meylanii* 4%; *Gymnocolea inflata* 3%; *Jungermania sphaerocarpa* 3%; *Mylia taylorii* 2%; *Pellia fabbroniana* 3%.

tive ratio in the mentioned plants is not as great as in the case of n-alkanes. The amount of single homologous esters increases gradually until the maximum is reached in the $C_{44}-C_{50}$ range; then it drops slowly. In the IR spectrum the esters display typical absorption peaks at 1 175 and 1 735 cm^{-1}. In the case of esters of *Calypogeia meylanii* and *Jungermania sphaerocarpa* we carried out their transesterification and thus always obtained two homologous series in the reaction mixture: the methyl ester of monocarboxylic acids and free alcohols (Fig. 1). According to the results obtained earlier we suppose[14,16] that single chromatographic peaks of natural esters do not represent individual esters but that they contain a mixture of isomeric esters; for example the peak C_{40} would represent a mixture of esters $C_{15}CO.OC_{24}$, $C_{17}CO.OC_{22}$, $C_{19}CO.OC_{20}$, *etc.* Higher monocarboxylic acids were also present in the extracts in the form of acyls bound to plant sterols; the esters of this type were isolated from further fractions by column chromatography. The composition of these substances will be described later.

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REFERENCES

1. Benešová V., Samek Z., Herout V., Šorm F.: This Journal *34*, 582 (1969).
2. Benešová V., Samek Z., Herout V., Šorm F.: This Journal *34*, 1807 (1969).
3. Benešová V., Herout V.: This Journal *35*, 1926 (1970).
4. Benešová V., Sedmera P., Herout V., Šorm F.: Tetrahedron Letters *1971*, 2679.
5. Chibnal A. C., Piper S. H., Pollard A., Williams E. F., Sahai P. N.: Biochem. J. *28*, 2189 (1934).
6. Eglinton G., Hamilton R. J. in the book: *Chemical Plant Taxonomy* (T. Swain, Ed.), p. 187. Academic Press, London-New York 1963.
7. Douglas A. G., Eglinton G. in the book: *Comparative Phytochemistry* (T. Swain, Ed.), p. 57. Academic Press, London—New York 1966.
8. Stránský K., Streibl M., Herout V.: This Journal *32*, 3213 (1967).
9. Radler F.: Australian J. Biol. Sci. *18*, 1045 (1965).
10. Mazliak P. in the book: *Progress in Phytochemistry*, Vol. 1 (L. Reinhold, Y. Lifschitz, Eds), p. 49. Interscience — Wiley, London, New York, Sydney 1968.
11. Mold J. D., Stevens R. K., Means R. E., Ruth J. M.: Biochemistry *2*, 605 (1963).
12. Gelpi E., Schneider H., Doctor V. M., Tennison J., Oró J.: Phytochemistry *8*, 2077 (1969).
13. Jarolímek P., Wollrab V., Streibl M.: This Journal *29*, 2528 (1964).
14. Streibl M., Stránský K.: Fette, Seifen, Anstrichmittel *72*, 856 (1970).
15. Pitra J., Štěrba J.: Chem. listy *56*, 544 (1962).
16. Streibl M., Jiroušová J., Stránský K.: Fette, Seifen, Anstrichmittel *73*, 301 (1971).
17. Stránský K., Streibl M., Kubelka V.: This Journal *35*, 882 (1970).
18. Brieskorn C. H., Beck K.: Phytochemistry *9*, 1633 (1970).
19. Huneck S.: J. Hattori Bot. Laboratory *1970*, No. 33, 1.

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