Phytochemistry, 1974, Vol. 13, pp. 2209 to 2213. Pergamon Press. Printed in England.

POLYUNSATURATED HYDROCARBONS FROM *POLYTRICHUM COMMUNE* SPORES*

PIRJO KARUNEN

Department of Botany, University of Turku, SF-2500 Turku 50, Finland

(*Received* 30 *March* 1974)

Key Word Index—*Polytrichum commune*, Musci, *n*-alkenes.

Abstract—*Polytrichum commune* Hedw. spores were found to contain the polyunsaturated hydrocarbons normal all-*cis*-6,9,12,15-heneicosatetraene (20·8 ± 0·5 μg/100 mg spores) and normal all-*cis*-3,6,9,12,15-heneicosapentaene (22·5 ± 1·2 μg/100 mg spores). *N*-alkanes were present only in minor amounts.

INTRODUCTION

IT HAS been repeatedly shown that mosses contain small amounts of polyunsaturated fatty acids such as arachidonic and eicosapentaenoic acids[1–10] which are characteristic of marine algae.[11] This, together with the fact that hydrocarbons appear to be metabolically derived by decarboxylation of fatty acids,[12] leads to the assumption that the hydrocarbon pattern of mosses consists of polyunsaturated *n*-alkenes such as are abundantly present in marine algae.[13–16] Earlier investigations of the aliphatic hydrocarbons of mosses have shown the presence of normal alkanes with chain lengths varying between 19 and 35 carbon atoms.[17–21] Fern (*Lycopodium*) sporophytes and their spores contain *n*-alkanes and monounsaturated *n*-alkenes[22] as do also the higher plants.[12] The present investigation

* Part III in the series "Studies on Moss Spores", III. For parts I and II see: *Annls Univ. turku.* Ser. A II. **51**, 1 (1972); *J. Exp. Botany* **24**, 1186 (1973).

[1] GELLERMAN, J. L. and SCHLENK, H. (1964) *Experientia*, **20**, 426.
[2] SCHLENK, H. and GELLERMAN, J. L. (1965) *J. Am. Oil Chem. Soc.* **42**, 504.
[3] NICHOLS, B. W. (1965) *Phytochemistry* **4**, 769.
[4] WAGNER, H. and FRIEDRICH, H. (1965) *Naturwissenchaften* **52**, 305.
[5] WOLF, F. T., CONIGLIO, J. G. and BRIDGES, R. B. (1966) *Biochemistry of Chloroplasts* (GOODWIN, T. W., ed.), pp. 187–194. Academic Press, New York.
[6] WAGNER, H. and FRIEDRICH, H. (1969) *Phytochemistry* **8**, 1603.
[7] KARUNEN, P. (1971) *Phytochemistry* **10**, 2811.
[8] KARUNEN, P. (1972) *Annls Univ. turku.* Ser. A II. **51**, 1.
[9] ANDERSON, W. H., GELLERMAN, J. L. and SCHLENK, H. (1972) *Lipids* **7**, 710.
[10] GELLERMAN, J. L., ANDERSON, W. H. and SCHLENK, H. (1972) *The Bryologist* **75**, 550.
[11] HITCHCOCK, C. and NICHOLS, B. W. (1971) *Plant Lipid Biochemistry*, Academic Press, New York.
[12] KOLATTUKUDY, P. E. (1970) *Lipids* **5**, 259.
[13] LEE, R., NEVENZEL, J., PAFFENHÖFER, G.-A., BENSON, A., PATTON, S. and KAVANAGH, T. (1970) *Biochim. Biophys. Acta* **202**, 386.
[14] BLUMER, M., MULLIN, M. and GUILLARD, R. (1970) *Marine Biol.* **6**, 226.
[15] BLUMER, M., GUILLARD, R. and CHASE, T. (1971) *Marine Biol.* **8**, 183.
[16] YOUNGBLOOD, W., BLUMER, M., GUILLARD, R. and FIORE, F. (1971) *Marine Biol.* **8**, 190.
[17] STRÁNSKY, K., STREIBL, M. and HEROUT, V. (1967) *Collection Czechoslov. Chem. Commun.* **32**, 1304.
[18] MARSILI, A. and MORELLI, I. (1970) *Phytochemistry* **9**, 651.
[19] MARSILI, A., MORELLI, I. and IORI, A. M. (1971) *Phytochemistry* **10**, 432.
[20] MARSILI, A., MORELLI, I., BERNARDINI, C. and PACCHIANI, M. (1972) *Phytochemistry* **11**, 2003.
[21] CORRIGAN, D., KLOOS, C., O'CONNOR, C. and TIMONEY, R. (1973) *Phytochemistry* **12**, 213.
[22] LYTLE, T. and SEVER, J. (1973) *Phytochemistry* **12**, 623.

shows the presence of *n*-alk-polyenes in the spores of *Polytrichum commune* and suggests that the metabolic pathways involved in the production of *n*-alk-polyenes in algae extend to some degree at least to the level of mosses in the plant kingdom.

RESULTS AND DISCUSSION

Analysis of the lipids of *Polytrichum commune* spores, showed the spores to have a small pigmented hydrocarbon fraction. In TLC analyses on silica gel G coated plates developed with hexane,[13] the following two major bands accompanying the carotene band were found: fraction 1, R_f 0·43 and fraction **2**, R_f 0·54. The band for saturated hydrocarbons at the solvent front was faint and not clearly detectable. After hydrogenation of the fractions, the R_f-values were shifted to 1·00, indicating that the fractions contained unsaturated hydrocarbons.

TABLE 1. STRUCTURES AND ANALYTICAL DATA OF POLYUNSATURATED HYDROCARBONS OF *Polytrichum commune* SPORES

Compound	MW (mass spec.) Before hydrogenation	MW (mass spec.) After hydrogenation	R_f on TLC plates Before hydrogenation	R_f on TLC plates After hydrogenation	Retention data Before hydrogenation			Retention data After hydrogenation	
					Apiezon L	FFAP	Δ I†	Apiezon L	FFAP
n-C 21:4*	288	296	0·43	1·00	2012	2249	237	2100	2100
n-C 21:5	286	296	0·54	1·00	2008	2300	292	2100	2100

* Notation of chain length: number of double bonds.
† Retention index on FFAP—retention index on Apiezon L.

The hydrocarbon fractions separated by TLC were further analysed by GLC using techniques that covered the C 14 to 28 range. The absence of longer chained hydrocarbons was checked by running the fractions on an OV-1 column at 270°. Each fraction appeared to contain only one component. If other components were present they were so only in trace amounts. This result was confirmed by analysing the fractions on a glass capillary column. The retention data taken before and after hydrogenation (Table 1) suggests that both hydrocarbons have a straight chain carbon skeleton with 21 carbon atoms. The assignments were verified in an analysis of the mass spectra of the products yielded by hydrogenation of the fractions. The two mass spectra were similar and characteristic of *n*-alkanes, displaying a series of prominent peaks at *m/e* 43, 57, 71, 85, 99 etc. Both fractions had a parent ion at *m/e* 296 corresponding to an empirical formula of $C_{21}H_{44}$. The Δ I-value for the unhydrogenated compound in fraction **1** is close to the value for a C21:4 *n*-alk-polyene and the ΔI-value for the compound in fraction **2** is close to the value for a C21:5 *n*-alk-polyene.[16] In the mass spectrum of the unhydrogenated compound in fraction **1** a parent ion at *m/e* 288 was observed, corresponding to an empirical formula of $C_{21}H_{36}$. Since hydrogenation yielded a product whose mass spectrum showed a shift of the parent ion to *m/e* 296, uptake of 8 hydrogens by 4 double bonds is indicated. In the mass spectrum of the unhydrogenated compound in fraction **2** a parent ion at *m/e* 286 was observed, corresponding to an empirical formula of $C_{21}H_{34}$ and indicating uptake of 10 hydrogens by 5 double bonds.

The bonds of the molecules were further characterized through UV and IR spectroscopy. At a concentration of 0·025 mg/ml in hexane, neither compound showed absorption maxima above 210 nm. Thus the double bonds in both compounds are presumed to be

non-conjugated. The IR spectra of both compounds showed the presence of aliphatic –Me and $-CH_2$ bands (2950-2855, 1461, 1452 and 1373 cm^{-1}), and significant absorption at 3010 cm^{-1} attributable to –CH stretching vibrations. The absorption maximum at 3010 cm^{-1} was weaker in the spectrum of the compound in fraction **1** (C21:4) than in the spectrum of the compound in fraction **2** (C21:5) and in both cases was weaker than the corresponding band of a C21:6 hydrocarbon.[14] A wide band centered at 695 cm^{-1} is attributable to out-of-plane bending of *cis*-double bonds. There was no absorption at 970–960 cm^{-1} where *trans*-double bonds absorb strongly. The extinction of the *trans*-double bond was sufficient to allow detection of a *trans*-single bond.[23] There was no absorption at 1950 cm^{-1} (allenes), 2100–2260 cm^{-1} (acetylenes), 3095–3075, 995–985, 915–905, or 1420–1410 cm^{-1} (vinyl group) in the IR spectra of either fraction. The absence of the doublet at 1600 and 1680–1620 cm^{-1} excludes, in conformity with the UV data, the possibility of conjugation of the double bonds. As the IR spectra of the compounds in fractions **1** and **2** are compared with the absorption maxima reported for other *n*-alk-polyenes,[14,16] their essential similarity is observed. On the basis of the above data, the structure of C21:4 is assigned to the compound in fraction **1** and C21:5 to the compound in fraction **2**. Both have methylene-interrupted double bonds, all with *cis* configuration.

Mass spectral evidence is used to establish the double bond position in the C21:4 and C21:5 molecules. The mass spectra of the compounds are compared with the fragmentation patterns of other polyenes,[14,16] as well with the published spectra of the all-*cis*-3,6,9,12,15,18-heneicosahexaene[13] and polyunsaturated fatty acids.[24,25] According to Blumer *et al.*[14] and Youngblood *et al.*[16] the positions of the terminal double bond in methylene-interrupted polyenes and polyunsaturated fatty acids can be determined from mass spectral evidence alone.

The major peaks in the mass spectra of both unhydrogenated compounds were at *m/e* 41 ($C_3H_5^+$), 55 ($C_4H_7^+$), 67 ($C_5H_7^+$), 79 (a C_6 fragment), 93 (a C_7 fragment), 119 and 133. These fragments are among the major fragments in the mass spectra of C21:6 hydrocarbons.[13] The shorter fragments up to *m/e* 93 are also prominent in the mass spectra of polyunsaturated fatty acids.[24,25] Also in the spectra of both compounds investigated the entire series of ions $Me(CH_2)_n(CH{=}CH)_3^+$ is present (*m/e* 107, 121, 135 etc).

The compound of fraction **1** has a prominent characteristic peak at *m/e* 150 corresponding to $Me(CH_2)_4(CH{=}CH)_3H^+$ and one at *m/e* 190 corresponding to the loss of a 7 carbon fragment having one double bond ($Me(CH_2)_4CH{=}CH_2$). These peaks are characteristic of ω 6 isomers of polyunsaturated fatty acids.[24,25] The range between the peak at *m/e* 190 and the molecular ion has only minor peaks, in conformity with the mass spectra of ω 6 isomers of polyunsaturated fatty acids.[24,25] These peaks are at *m/e* 203 (M-85), 217 (M-71), 231 and 234 (M-57 and M-54) of which M-71 (C_5H_{11}) fragments exhibit the most predominant loss. This pattern is in comformity with the fragmentation pattern of polyenes with a terminal double bond in 6-position.[14,16] Thus the investigated molecule is symmetrical and the structure of all-*cis*-6,9,12,15-heneicosatetraene can be unequivocally assigned to the compound present in fraction **1.**

The structure of the compound present in fraction **2** can be assigned on the basis of two series of peaks. One series is the above-mentioned, indicating that the molecule has a ter-

[23] NAKANISHI, K. (1962) *Infrared Absorption Spectra*. Holden Day, San Francisco.
[24] HOLMAN, R. T. and RAHM, J. J. (1966) *Progress in the Chemistry of Fats and Other Lipids* (HOLMAN, R. T., ed.), Vol. 9, pp. 13–90. Pergamon Press, Oxford.
[25] ZEMAN, A. and SCHARMAN, H. (1972) *Fette, Seifen, Anstrichmittel* **74**, 509.

minal double bond in 6-position. The other series is considered to be characteristic of $\omega 3$ isomers of polyunsaturated fatty acids[24,25] and of *n*-alk-polyenes with a terminal double bond in 3-position.[14,16] Thus the prominent peak at *m/e* 108 corresponds to $MeCH_2(CH{=}CH)_3H^+$ and a predominant loss of C_2(M-29) and C_5 (M-69) fragments. Other ions lost were M-15, M-43 and a cluster of ions, range M-54 to M-57. The fragmentation pattern of this molecule suggests that the two ends of the molecule are dissimilar, one end having the structure of $Me(CH_2)_4CH{=}CH$, the other end $MeCH_2CH{=}CH$. Thus the structure of the compound present in fraction **2** is restricted to all-*cis*-3,6,9,12,15-heneicosapentaene. The structure is identical to that of the C21:5 polyene found in marine benthic algae.[16] A C21:4 polyene, found in *Laminaria digitata* in small quantities, has not yet been strictly assigned.[16]

The C21:4 and C21:5 polyenes were present in the spores in about equal amounts: the content of the former was estimated at 20·8 ± 0·5 μg/100 mg spores, and the content of the latter at 22·5 ± 1·2 μg/100 mg spores. *N*-alkanes were present in the spores only in minor amounts and have not yet been identified. In higher plants a substantial variation in alkane/alkene content occurs in the morphologically different parts.[26] Such variation has also been confirmed in algae.[16] Therefore, an investigation is underway of the distribution of *n*-alkanes and *n*-alkenes in various organs of the moss *Polytrichum commune*.

EXPERIMENTAL

Material. The sporophytes of *Polytrichum commune* Hedw. were collected in October 1973 and stored in glass bottles at 20° and 40% r.h. The spores were released from their capsules and 100–500 μg were used in each analysis.

Extraction. The material was ground in a Potter–Elvehjelm homogenizer in an ice-bath using ice-cold C:M (2:1, v/v) mixture. The grinding was carried out in darkness for 15 min, during which time the spores were completely homogenized. The homogenate was filtered, using water aspiration, through a plug of defatted cotton and filter paper in the inverted filtration tube into a separatory funnel and then washed by the technique of Folch *et al.*[27] The chloroform layer was separated and transferred to an evaporation tube with capillary ending and evaporated to dryness below 35°. The residue was diluted in hexane.

TLC. The hydrocarbons were separated on plates coated with 0·5 mm of silica gel G using hexane.[13]

Hydrogenation. PtO_2 was used as the catalyst in the hydrogenation of the hydrocarbons. Hydrogenation was performed by bubbling hydrogen for 1 min into a methanol solution of the hydrocarbons. The tubes were then screw-capped and heated at 50° for 0·5 hr with frequent shaking.

Gas chromatography. The hydrocarbons were analysed in a Perkin–Elmer Model F 30 gas chromatograph equipped with dual glass columns (183 × 0·32 cm) containing 12·5% FFAP or 2·2% Apiezon L as stationary phase on a solid support Gas Chrom Q, 80/100 mesh. In the temperature programmed runs, the columns were held at 80° until the solvent had been eluted, after which the temperature was raised to 290° (Apiezon L) or 270° (FFAP) at the rate of 4°/min. In isothermic runs the temperature was 200° for the FFAP column and 210° for the Apiezon L column. The temperature of the injection block was held at 200°. Occasionally, the samples were also analysed at 270° on a column packed with 3% OV-1. The flow rate of the carrier gas (N_2) was 30 ml/min and its pressure 60 lbf/in^2. For identification, internal standard mixtures of *n*-alkanes and monounsaturated *n*-alkenes with carbon chain lengths ranging from 14 to 28 were used. Retention indices were measured.[28] The identification of the hydrocarbons made on the basis of results obtained with the packed columns was checked by running the samples in a Varian Aerograph Model 2100–20 gas chromatograph equipped with a glass capillary column (44 m × 0·31 mm i.d.) coated with FFAP. The column was held at 190°, the injection block at 210°. The carrier gas (N_2) flow rate was 0·72 ml/min.

Gas chromatography-mass spectrometry. Gc–Ms analyses were performed with an LKB 9000 instrument. The gas chromatograph was equipped with a 370 × 0·32 cm O.D. packed glass column containing 3% EGSS-X as a stationary phase. The column temperature was 170°. The temperature of the injection block was held at 200°. Operating conditions were: molecular separator temperature, 240°, ion source temp. 270°, and ionizing potential, 70 or 20 eV.

[26] STRÁNSKY, K. and STREIBL, M. (1969) *Collection Czechoslov. Chem. Commun.* **34**, 103.
[27] FOLCH, J., LEES, M. and STANLEY, G. H. S. (1957) *J. Biol. Chem.* **226**, 497.
[28] KOVATS, E. (1958) *Helv. Chim. Acta* **41**, 1915.

IR and UV spectrometry. IR spectra were measured in CCl_4 and UV spectra at a concn of 0·025 mg/ml in hexane.

Estimation of hydrocarbons. The hydrocarbon contents of the spores were estimated by co-chromatography with an internal standard *n*-C 20 hydrocarbon.

Acknowledgement—I am most appreciative of the financial support received from the Emil Aaltonen Foundation.