Phytochemistry, 1980, Vol. 19, pp. 319–321. © Pergamon Press Ltd. Printed in England. 0031-9422/90/0201-0319 $02.00/0

DISTRIBUTION OF TRIGLYCERIDES IN *DICRANUM ELONGATUM*

PIRJO KARUNEN and HEIKKI MIKOLA

Department of Botany, University of Turku, SF-20500 Turku 50, Finland

(*Revised received* 4 *June* 1979)

Key Word Index—*Dicranum elongatum*; Dicranaceae; moss; triglycerides; senescence.

INTRODUCTION

In a previous investigation we reported that both the green and brown parts of the subarctic moss *Dicranum elongatum* Schleich were rich in lipids [1]. The great seasonal variation in the total lipid level in brown as well as green parts led us to suggest that the moss, including the brown part, contains storage lipids formed in early spring and utilized during summer and winter. Possibly the storage lipids also serve as an energy source for the onset of the regeneration process and subsequent formation of a new gametophyte from the cells of the brown part of the moss. In this investigation we characterize the triglyceride storage lipids in the green and brown parts of *D. elongatum*.

RESULTS AND DISCUSSION

It has previously been shown that several mosses, *Ceratodon purpureus, Dicranum montanum, Aulacomnium turgidum, Bryum tortifolium, Tortula ruralis* and *Fontinalis antipyretica* [2, 3–6] contain acetylenic fatty acids occurring exclusively in the triglycerides, or in small amounts in the more polar lipid fraction, i.e. phospho- and unidentified glycolipids. In *Ceratodon purpureus* the triglycerides could be fractionated into two portions, one containing *ca.* 90% acetylenic acyl groups and the other the common triglycerides [2].

In our present study of the neutral lipids of the subarctic moss *Dicranum elongatum* we found that the triglyceride fractions I and II, corresponding to the common and acetylenic triglycerides of *Ceratodon purpureus*, respectively, were completely separated on TLC only after having been twice developed. This suggested that the faster moving 'common' triglyceride fraction contained small amounts of slower moving fatty acyl groups. The analytical data (Table 3) indeed showed that the faster moving triglyceride fraction (hR_f 49) of *D. elongatum* contained some acetylenic acid, in addition to the common fatty acids. The slower moving fraction (hR_f 37) was composed mainly (*ca* 90%) of acetylenic acids and thus resembled the corresponding fraction of *Ceratodon purpureus* [2].

The green segment contained 24 mg fatty acids of triglycerides per g dry tissue (Table 1). The content in segment 0.5–2 cm was slightly higher and in segment 2–5 cm about 50% lower than in the green segment. The increase of triglycerides in segment 0.5–2 cm was related to the increase in the content of the faster moving triglyceride fraction. The content of the acetylenic triglyceride fraction, on the other hand, was highest in the green segment and decreased with increase in depth and age of the shoot (Table 1). As shown in Table 2 the increase in the content of the faster moving triglyceride fraction in segment 0.5–2 cm was largely due to an increase in acetylenic acids, and despite the decrease in the content of the acetylenic triglyceride fraction, the overall acetylenic acid content was highest in this segment.

Table 1. Content of triglycerides in green and brown segments of *Dicranum elongatum*

Segment (cm)	mg Fatty acids/g dry tissue Fraction I	Fraction II	Total
0–0.5	17.8 ± 0.20	6.4 ± 0.08	24.2
0.5–2	21.7 ± 0.41	5.0 ± 0.07	26.8
2–5	9.1 ± 0.36	2.9 ± 0.07	12.0

The pronounced increase in the acetylenic acid moiety in the faster moving triglyceride fraction in segment 0.5–2 cm was reflected in the fatty acid pattern of the fraction (Table 3). In the green segment the proportion of the acetylenic acids a18:3 (9, 12, 15-octadecatrien-6-ynoic acid) and a18:2 (9, 12-octadecadien-6-ynoic acid) was low, 2.8 and 0.4%, respectively. The major fatty acids were 18:2ω6 (20.5%), 18:3ω3 (32.6%) and 20:4ω6 (13.9%). In segment 0.5–2 cm the proportions of a18:3, a18:2 and 20:4ω6 increased and the proportions of 18:2ω6 and 18:3ω3 decreased. The same tendency was found in segment 2–5 cm. No great changes were found in the fatty acid pattern of the acetylenic triglyceride fraction in different portions of the shoot: in segments 0.5–2 and 2–5 cm the proportion of 18:3ω3 was slightly lower (0.4 and 0.7%, respectively) than in the green segment (1.5%), the proportion of a18:2 in segments 0.5–2 and 2–5 cm was slightly higher (3.5 and 5.6%, respectively) than in the green segment (3.1%) and the proportion of a18:3 was 93.1% in segment 0.5–2 cm, 88.0% in segment 2–5 cm, and 89.6% in the green

Table 2. Content of acetylenic acids of triglycerides in green and brown segments of *Dicranum elongatum*

Segment (cm)	μg Acetylenic acids/100 mg dry tissue Fraction I a18:2	Fraction I a18:3	Fraction II a18:2	Fraction II a18:3	Total
0–0.5	7	49	20	571	647
0.5–2	40	214	18	470	742
2–5	26	97	16	256	395

Table 3. Fatty acid composition of triglycerides in green and brown parts of *Dicranum elongatum*

	Percentage of total fatty acids			
	Fraction I			Fraction II
	Segment (cm)			
Acid	0–0.5	0.5–2	2–5	0–0.5
14:0	0.2±0.04	0.4±0.03	0.4±0.03	tr
15:0	0.5±0.03	0.4±0.02	0.4±0.02	tr
16:0	9.1±0.12	8.8±0.18	8.5±0.16	1.3±0.15
16:1	0.6±0.05	0.5±0.04	0.9±0.13	tr
17:0	0.3±0.02	0.1±0.04	0.2±0.02	tr
16:3ω3	0.5±0.05	tr	0.3±0.04	tr
18:0	1.2±0.02	0.9±0.03	1.2±0.13	0.8±0.04
18:1	7.4±0.13	7.2±0.46	8.1±0.23	0.4±0.07
18:2ω6	20.5±0.17	18.3±0.14	16.9±0.09	0.8±0.11
18:3ω6	1.4±0.01	1.7±0.03	2.1±0.02	tr
18:3ω3	32.6±0.37	24.3±0.18	19.3±0.29	1.5±0.22
18:4ω3	1.8±0.03	1.7±0.03	1.6±0.05	0.2±0.02
a18:2	0.3±0.03	1.7±0.07	2.8±0.11	3.1±0.02
a18:3	2.8±0.36	9.9±0.38	10.6±0.41	89.6±0.80
20:0	0.9±0.03	0.6±0.05	0.5±0.06	0.7±0.04
20:2ω6	0.2±0.02	0.2±0.01	0.2±0.01	tr
20:3ω6	1.1±0.03	2.2±0.04	2.1±0.03	tr
20:3ω3	1.2±0.15	1.1±0.04	1.2±0.05	tr
20:4ω6	13.9±0.13	16.4±0.22	17.8±0.18	0.7±0.09
20:4ω3	0.3±0.01	0.5±0.03	0.5±0.01	
20:5ω3	2.0±0.03	1.9±0.04	2.1±0.03	tr
22:0	0.3±0.02	0.2±0.04	0.2±0.04	0.2±0.04
24:0	0.1±0.04	tr	tr	0.2±0.07
Others	0.8	1.0	2.1	0.5

segment. Thus, the double bond + triple bond index of the triglycerides (fractions I + II) appeared to be slightly higher in the older parts of the moss shoot, i.e. 2.82, 2.86 and 2.87 in segments 0–0.5, 0.5–2 and 2–5 cm, respectively. This is surprising in consideration of the age of the shoot: segment 2–5 cm is 10–25 years old [1]. Evidently oxidation of storage lipids is not associated with senescence of the shoots of subarctic moss, as it is with the senescence of seeds [7, 8]. Because of the high degree of unsaturation, the triglycerides must be highly fluid and this may facilitate the metabolic utilization of these lipids at subzero temperatures.

EXPERIMENTAL

The tufts of *Dicranum elongatum* Schleich were collected in October 1978, while frozen and covered with snow, at the Kevo field station in Finnish Lapland (69° 45'N). The moss tufts were transferred under refrigeration by air to the Depart ment of Botany, University of Turku. The tufts were immediately cut into segments: (1) 0–0.5 cm, green segment; (2) 0.5–2 cm, light yellow-brown segment; (3) 2–5 cm, brown segment. The samples were cleaned of other plants and debris and stored for a few days at −20° until analysed. Both fr. and dry wts. (70°, overnight) were recorded. All values presented in this investigation are means of three independent experiments ± s.e.

Fresh moss tissue (1–2 g) was crushed with liquid N_2 and homogenized in ice-cold $CHCl_3$–MeOH (2:1). Lipid classes were separated on a Si gel G TLC (0.5 mm) using hexane–Et_2O–HOAc (90:10:1). The plates were developed to the height of 18 cm, dried under a stream of N_2 and developed in a second solvent system, hexane–Et_2O–HOAc (70:30:1) to a height of 10 cm. This double development was necessary for separation of the triglyceride fractions I and II, the latter having a lower R_f value and consisting predominantly of acetylenic acyl groups. IR spectra of the triglyceride fractions (in both $CHCl_3$ and CCl_4) were recorded. The triglyceride fraction with lower R_f value (fraction II) showed a small peak at 1335 cm^{-1} and absence of absorption at 2150 cm^{-1}, indicating the presence of a triple bond but one neither terminal nor near the carboxyl group [2]. Analysis of the component fatty acids of the triglycerides was carried out on glass capillary columns of different polarity as previously reported by Karunen [9]. For evaluating the degree of unsaturation of triglycerides the double bond index [10] and, accordingly, the triple bond index were calculated.

Acknowledgements—We thank the staff of the Kevo station for supplying the moss material, Mrs. Auli Alonen for laboratory assistance and the Academy of Finland for financial support.

REFERENCES

1. Karunen, P. and Kallio, P. (1976) *Rep. Kevo Subarctic Res. Stn.* **13**, 63.
2. Åndersson, B., Anderson, W. H., Chipault, J. R., Ellison, E. C., Fenton, S. W., Gellerman, J. L., Hawkins, J. M. and Schlenk, H. (1974) *Lipids* **9**, 506.
3. Anderson, W. H., Hawkins, J. M., Gellerman, J. L. and

Schlenk, H. (1974) *J. Hattori Bot. Lab.* **38**, 99.
4. Anderson, W. H., Gellerman, J. L. and Schlenk, H. (1975) *Lipids* **10**, 501.
5. Swanson, E. S., Anderson, W. H., Gellerman, J. L. and Schlenk, H. (1976) *Bryologist* **79**, 339.
6. Jamieson, G. R. and Reid, E. H. (1976) *Phytochemistry* **15**, 1731.
7. Spencer, G. F., Earle, F. R., Wolff, I. A. and Tallent, W. H. (1973) *Chem. Phys. Lipids* **10**, 191.
8. Harman, G. E. and Mattick, L. R. (1976) *Nature* **260**, 323.
9. Karunen, P. (1977) *Physiol. Plant.* **40**, 239.
10. Dogras, C. C., Dilley, D. R. and Herner, R. C. (1977) *Plant Physiol.* **60**, 897.

Phytochemistry, 1980, Vol. 19, pp. 321–322. © Pergamon Press Ltd. Printed in England. 0031-9422/80/0201-0321 $02.00/0

GERANYLGERANYL ESTERS IN NORWAY SPRUCE WOOD

RAINER EKMAN

Institute of Wood Chemistry and Cellulose Technology, Åbo Akademi, SF-20500 Åbo 50, Finland

(*Received* 6 *January* 1979)

Key Word Index—*Picea abies*; Pinaceae; Norway spruce; isoprenoid esters; geranylgeraniol; fatty acids.

Diterpenoids are considered to be biogenetically derived from geranylgeraniol and closely related compounds such as geranyllinalol which has been isolated from the oleoresin of Norway spruce, *Picea abies* (L.) Karst. [1]. Esters of isoprenoid alcohols in higher plants have been proposed to be located in the chloroplasts [2], but oligoprenyl esters of fatty acids have also been detected in the wood of silver birch [3, 4]. The occurrence of geranylgeranyl esters of fatty acids in the wood of coniferous trees has not been reported.

The presence of geranylgeranyl esters in the lipophilic extractives of Norway spruce wood was indicated by the following finding: saponification of the terpenoid ester fraction (TLC) gave sterols, triterpene alcohols, fatty acids and a diterpene alcohol, proved to be identical with an authentic sample of geranylgeraniol (GC–MS of the alcohol, its hydrogenation product and their TMSi ethers). Attempted GC–MS of the unhydrolysed fraction was not successful due to decomposition of the esters at the high temperatures required. Phytyl esters have been found to behave similarly [5]. However, hydrogenation of the fraction followed by GC–MS gave normal shaped peaks and mass spectra corresponding to phytanyl esters of saturated C_{16}–C_{20} fatty acids, the expected hydrogenation products of the geranylgeranyl esters assumed to be present. The GC–MS data for synthetically prepared phytanyl heptadecanoate and phytanyl 14-methylhexadecanoate (*anteiso*-heptadecanoate) were in complete agreement with those of the corresponding esters in the investigated sample.

Isolation of the geranylgeranyl esters was achieved by preparative TLC. Saponification of the fraction facilitated GC–MS identification of the fatty acid residues (Table 1). The high proportion of saturated (64%) and *anteiso* (40%) acids esterified with geranylgeraniol is noteworthy. Total fatty acids in Norway spruce wood are composed of the same fatty acids, but the contribution of saturated (10%) and *anteiso* (4%) acids is significantly lower [6]. The amount of geranylgeranyl esters was 0.7% of total

Table 1. Fatty acid composition of geranylgeranyl esters from *P. abies* wood

Fatty acid *	Percentage composition	ECL value BDS, 190°
anteiso 16:0	1.0	15.71
16:0	14.3	16.00
7–16:1	1.7	16.34
9-16:1	2.1	16.41
anteiso 17:0	22.2	16.72
17:0	2.0	17.00
iso 18:0	1.0	17.55
anteiso 18:0	1.9	17.72
18:0	3.6	18.00
9–18:1	5.3	18.32
11–18:1	5.4	18.39
anteiso 19:0	13.7	18.73
9,12–18:2	12.4	18.90
anteiso 9–19:1	1.5	18.97
5,9,12–18:3	4.0	19.16
20:0	4.4	20.00
11–20:1	2.2	20.29
11,14–20:2	1.2	20.87

* Analysed as the methyl ester.