

Table 1. Substrate specificity for C-9 aldehyde formation by a homogenate of cucumber fruits

Substrate	Relative activities*	
	<i>trans</i> -2, <i>cis</i> -6-Nonadienal (1)	<i>trans</i> -2-Nonenal (2)
Linoleic acid	0	100
$\gamma$ -Linolenic acid	0	30
Linoleic acid	0	0
Methyl linoleate	0	4
Trilinolein	0	0
Linolenic acid	100	0
Methyl linolenate	6	0
Trilinolenin	0	0

\* Activities are expressed relative to the amounts of 1 and 2 produced from linolenic and linoleic acids, respectively.

or palmitic acid by the homogenate. It is concluded that compounds with a *cis*-1,*cis*-4-pentadiene system and a free carboxyl group act as substrate [2-4].

## EXPERIMENTAL

**Plant material.** Cucumber (*Cucumis sativus* L. cv Kurumecho-jitsuochiai) fruits grown in a greenhouse were used from April to May, 1976.

**Preparation of essential oil and quantitative analysis of C 9 aldehydes.** About 300 g of fr. cucumber fruits were used. The procedures were the same as described previously [2]. For substrate specificity, mid-ripening fruits were blended with substrate and then C-9 aldehydes were extracted and analysed by the same methods [2]. Homogenate without substrate was used as control. The C-9 aldehyde producing activity was obtained from the amounts of 1 and 2 formed with substrate minus the amounts in a control.

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## FATTY ACID COMPOSITION OF FERN SPORE LIPIDS

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**Key Word Index**—Lycopodiaceae; Filicales; spores; fatty acids; chemotaxonomy.

**Abstract**—Fatty acid composition of lipids isolated from spores of different fern groups show differences between the families whereas species variations within the families are smaller. As in seed fats, the spore lipids are mainly triglycerides, with the exception of *Osmunda* where free fatty acids accumulate. The spore lipids contain as major components oleic, linoleic, and palmitic acid although those of the sporophylls contain C-20 polyunsaturated acids.

## INTRODUCTION

Fern spores are haploid cells which are produced in large numbers during the diploid phase of the life cycle. They are, with the exception of the rare occurrence of adventitious bodies, the only organs of propagation in ferns. In this respect they are analogous to the seeds of higher plants. Like seeds they are equipped with a protective cover, the exine, to overcome periods of unfavourable conditions and contain storage material as energy source for germination and early development. In fern spores, reserve substances are mainly lipids [1-7] or proteins [8]. There is no report on carbohydrates as storage material. Although the lipid composition of fern sporophytes is well documented [9-15], only few data are available concerning the lipids of spores [3, 4, 7]. The fatty acid

composition of some lipid storing fern spores were therefore examined so that comparisons could be made with the fatty acids of the sporophytes.

## RESULTS AND DISCUSSION

The total amount of lipids and their fatty acid composition in spores of 17 fern species and one species of the Lycopodiales are shown in Table 1. The amount of lipids differs considerably, i.e. constituting 4% of the weight in *Ceratopteris thalictroides* and up to 79% in *Polypodium meyenianum*. A relatively high content is characteristic of spores of the two *Polypodium* species and for those of the Schizaeaceae. The small quantity in spores of *Ceratopteris* and *Osmunda* suggests that in these spores other sub-

Table 1. Fatty acid composition of lipids from fern spores (% total fatty acids)

Compound	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	n.i.	mg lipids	
										100 mg	spores
Lycopodiaceae <i>Lycopodium annotinum</i> L.	0.6	2.1	40.9	—	43.2	12.1	1.1	—	—	42	
Osmundaceae <i>Osmunda regalis</i> L.	tr	22.7	1.8	2.4	19.4	46.0	5.7	—	1.9	14	
Schizaeaceae <i>Anemia phyllitidis</i> (L.) Sw.	tr	18.2	1.4	5.0	38.3	35.4	tr	tr	1.7	56	
<i>Lygodium japonicum</i> Sw.	tr	11.7	0.6	2.7	43.6	40.7	0.6	—	tr	66	
<i>L. scandens</i> (L.) Sw.	tr	12.1	1.1	2.5	39.1	45.7	tr	—	tr	69	
<i>L. volubile</i> Sw.	tr	16.6	0.9	2.7	55.2	24.6	tr	—	—	66	
Polypodiaceae <i>Pteris longifolia</i> L.	tr	14.9	tr	2.8	75.1	5.2	tr	0.6	1.4	39	
<i>Anthyrium filix femina</i> (L.) Roth	—	8.1	0.7	1.0	45.2	45.0	tr	tr	—	32	
<i>Dryopteris filix mas</i> (L.) Schott	tr	8.0	0.4	2.0	63.7	25.9	tr	tr	—	50	
<i>D. hirtipes</i> (Blume)	tr	12.7	tr	1.5	50.2	35.5	tr	tr	—	42	
<i>D. oreopteris</i> (Ehrh.)	tr	12.6	2.7	2.9	42.2	39.6	tr	tr	—	36	
<i>D. spinulosa</i> (Müll.)	0.3	7.2	1.2	0.8	45.2	44.3	0.7	0.4	—	42	
<i>Polystichum lonchitis</i> (L.) Roth	—	9.8	tr	2.0	50.4	37.8	tr	tr	—	35	
<i>Polypodium crassifolium</i> L.	tr	19.9	tr	2.4	67.1	8.9	tr	tr	1.7	77	
<i>P. meyenianum</i> Hook	tr	27.1	tr	1.3	66.0	5.5	tr	tr	—	79	
<i>Pityrogramma argentea</i> (Willd.)	—	20.3	tr	2.6	63.0	12.5	—	1.5	—	62	
<i>Ceratopteris thalictroides</i> (L.) Brongn.	tr	21.5	tr	6.1	60.5	11.8	—	—	—	4	

n.i. = not identified

tr = trace, less than 0.5%

stances than lipids are the storage material. The major components of the lipids are triglycerides. The only exception are the spores of *Osmunda*: apart from a small amount of triglycerides, free fatty acids accumulate. Since all spores that accumulate triglycerides are viable for months and even years, this may be one reason for the short viability of *Osmunda* spores.

The fatty acid composition of the spore lipids is similar to the distribution and quantity of the common fatty acids in seed fats [16]. The major acids are palmitic, stearic, oleic and linoleic acid. In each species unsaturated acids predominate. Except for arachidic acid and a few fatty acids which were not identified but were present in very small quantities, no uncommon fatty acid was found. In all species the portion of oleic and linoleic acid is high (70–90%), where oleic acid predominates. In *Pteris longifolia* oleic acid represents 75%. The portions of palmitic acid vary between 2 and 27%. In nearly all species linolenic acid is present in small amounts.

A comparison of the fatty acid composition in spores with that in sporophylls, which contain substantial amounts of C-20 polyenoic acids [4, 9, 12, 14] and C-21–30 fatty acids [12, 15], shows clearly that the lipid metabolism is shifted during sporogenesis into producing triglycerides and fatty acids typical for storage lipids. In this respect the Filicales resemble the angiosperms where the fatty acid composition of seeds is different from that of vegetative tissue [16].

**Lycopodiaceae.** *Lycopodium annotinum*, the only representative examined, contains, apart from oleic acid, a high amount of hexadecenoic acid but no stearic acid. This pattern which was also found in other *Lycopodium* species [16] is typical for this family.

**Osmundaceae.** Spores of *Osmunda regalis* are exceptional: they contain chloroplasts, have a high water content, are only viable for a short time, and accumulate free fatty acids. The fatty acids in this spore differ from those of all other species, linoleic acid being the dominant fatty acid, followed by palmitic acid and oleic acid. The amount of linolenic acid may be due to the presence of chloroplasts.

**Schizaeaceae.** The fatty acid pattern of the four species investigated is relatively uniform although there is a distinct variation between the 3 *Lygodium* species.

**Polypodiaceae.** (sensu Wettstein) Fatty acid distribution within this group is rather heterogeneous. Unique is the fact that oleic acid constitutes the major component, being present in relatively high amounts. In this case, linoleic acid content is diminished. In some *Dryopteris* species the proportions of fatty acids more closely resemble those of the Schizaeaceae than the Polypodiaceae. The two *Polypodium* species studied have very similar patterns.

Although this investigation has revealed certain similarities in the fatty acid pattern from spores within taxonomic groups, the data are not sufficient to establish a phylogenetic relationship between these families.

#### EXPERIMENTAL

Fertile fronds were collected from plants grown either in the greenhouse at the university of Gießen or Ulm or in their natural environment in the surrounding of Ulm. Spores were being shed as the fronds dried. They were sieved through sheets of lens paper to separate them from the remnants of sporangia and were stored at about 5°.

**Separation of lipids.** Spores (100 mg) were homogenized in a dismembrator with 1 mm glass beads using  $\text{CHCl}_3$ –MeOH (2:1) as solvent. Water-soluble impurities were removed using the method of Folch [17]. The lipid extract was esterified with  $\text{BF}_3$  according to the procedure described elsewhere [18].

**GLC.** Fatty acid methyl esters were analysed on 10% DEGS on Chromosorb packed in an all glass column (150 cm × 2 mm) at 200°. Identifications were made by comparison of  $R_f$  with authentic standards. For quantitative estimation, pentadecanoic acid was added as internal standard.

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## GALACTOLIPIDS AND PHOSPHOLIPIDS OF ORANGE PEEL AND JUICE CHROMOPLASTS

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**Key Word Index**—*Citrus sinensis*; Rutaceae; orange; chromoplasts; chloroplasts; galactolipids; phospholipids.

**Abstract**—Chromoplasts from yellow orange (*Citrus sinensis*) fruit peel contain monogalactosyl diglycerides, (MGDG), digalactosyl diglycerides (DGDG) and phosphatidyl glycerol (PG) in amounts similar to those found in chloroplasts from green fruit peel. Juice chromoplasts contain relatively little MGDG and no DGDG with high levels of phosphatidyl choline and phosphatidyl ethanolamine but no PG.

### INTRODUCTION

Chromoplasts are found within mature citrus fruits in the flavedo (the outer, colored layer of the peel) and in the juice. Although both chromoplast types are rich in carotenoid pigments, their history is different. Flavedo chromoplasts originate from green photosynthetically active chloroplasts, whereas juice chromoplasts develop from the pigment-less plastids of juice vesicles. The purpose of the present study was to determine whether the different developmental origin of the two kinds of chromoplasts is reflected in their lipid composition.

### RESULTS AND DISCUSSION

Table 1 shows the important differences between flavedo and juice chromoplasts and, on the other hand, the great similarity between flavedo chromoplasts and chloroplasts. Flavedo chromoplasts contain monogalactosyl diglycerides, (MGDG), digalactosyl diglycerides (DGDG) and phosphatidyl glycerol (PG) in amounts similar to those found in mature green flavedo chloroplasts. Juice chromoplasts contain some MGDG, little or no DGDG and no PG. Juice chromoplasts extracts

revealed an additional major spot which moved about 0.1  $R_f$  unit behind MGDG (in the GL detection TLC system) and reacted in addition to iodine vapour and with the sugar reagents anthrone (green) and *p*-anisidine phosphate (purple-gray) but not with the periodate-Schiff reagent. Juice chromoplasts contained phosphatidyl choline (PC), phosphatidyl ethanolamine (PE) as their only major phospholipid (PL) components.

Chromoplasts are known to evolve either directly from non-differentiated pigment-less plastids or from chloroplasts which are transformed in senescing tissues into chromoplasts [1]. Juice chromoplasts apparently belong to the first, while flavedo chromoplasts belong to the latter type.

Flavedo chromoplasts which have lost all their chlorophyll and photosynthetic membranes [2, 3] nevertheless retain considerable amounts of chloroplast membrane lipids. Chromoplasts of daffodil petals which have recently been found to be rich in DGDG, MGDG and PG may also be developmentally linked to chloroplasts [4]. However, juice chromoplasts also contain some MGDG, suggesting a more complex relationship between the origin of chromoplasts and their lipid composition.