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Cyclopropane Fatty Acids in Relation to Earliness in Spring and Drought Tolerance in Plants[1]

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P. J. C. KUIPER AND BEP STUIVER
Laboratory of Plant Physiological Research, Agricultural University, Wageningen, The Netherlands

ABSTRACT

Long chain cyclopropane fatty acids were observed in the sulfolipid fraction extracted from leaves of the early spring plants *Galanthus nivalis* L. and *Anthriscus silvestris* L. (Hoffm.). The content of cyclopropane fatty acids with 25 carbon atoms appeared to be clearly correlated with earliness in spring, and it ranged from 68% (*G. nivalis* L., snow drop) to 0.5% (wheat). Several long chain cyclopropane fatty acids were found in the drought-tolerant *Corynephorus canescens* (L.) P.B., exclusively in the phosphatidyl choline fraction.

Many lipids extracted from plant tissue show a characteristic fatty acid composition. Lignoceric acid, for instance, was found only in large quantities in the phosphatidyl choline fraction of grape roots (8) and bean roots while it was absent from the phosphatidyl choline fractions of tubers, leaves, and flower petals. Palmitoleic acid was only found in phosphatidyl glycerol extracted from leaves of alfalfa (9, 13), spinach (1), clover (16), potato, Dahlia, and wheat. It may well be a requirement of the photosynthetic apparatus (1, 16). Monogalactose diglyceride extracted from leaves was predominantly esterified with linolenic acid as was observed in leaves from alfalfa (9, 13), spinach (1), potato, Dahlia, wheat, rye and snow drop (*G. nivalis* L.). Lipids extracted from tubers of potato and Dahlia almost always contained more than 50% linoleic acid. In conclusion, several lipids showed a fatty acid composition which could be related to the plant organ from which the lipid was obtained.

On the other hand, fatty acid composition of some lipids could be correlated with an environmental adaptation. Such correlations were absent when varietal differences in salt tolerance (8) or cold hardiness (9) were compared, but, as reported below, striking correlations were observed between fatty acid composition and earliness of plant species in spring and between fatty acid composition and drought tolerance. The occurrence of long chain cyclopropane fatty acids in these groups of plants was of special interest.

MATERIALS AND METHODS

Leaves of snow drop (*Galanthus nivalis* L.) were collected in the laboratory garden in early March; leaves of cow parsley (*Anthriscus silvestris* Hoffm. [L.]) were collected in the forest near the laboratory, also in March, when the total plant did not exceed 25 cm, and in May. Leaves of rye (*Secale cereale* L. var. Petkuser), wheat (*Triticum estivum* L. var. Apollo), and English ryegrass (*Lolium perenne* L.) were harvested from plants grown in the greenhouse, when the plants were about 25 cm high. Leaves of the drought-tolerant species *Corynephorus canescens* (L.) P.B. were collected from sand dunes near Wageningen. This species can tolerate 50% dehydration of the leaves without injury (15).

The lipids were extracted from the leaves as described by Allen *et al.* (1) for spinach leaves and separated on a diethylaminoethyl cellulose column (1, 9, 14). Separation of the fractions of phosphatidyl glycerol, sulfolipid, and phosphatidyl inositol sometimes was incomplete, but doubtful test tubes were discarded. Purity of the lipids was checked by thin layer chromatography (14).

The lipids were deacylated with methanolic KOH (17). The fatty acids were methylated with 10% BCl_3 in methanol (12) and analyzed on a Victoreen gas chromatograph with a hydrogen flame ionization detector. The column was composed of 15% diethylene glycol succinate on Anakrom 60/70 as a stationary phase. Because of the wide range of fatty acids observed, from 12 carbon atoms (lauric acid) to 25 carbon atoms (cyclopropane fatty acid) in the hydrocarbon chain, the samples were run at two different temperatures, 180 and 200 C, to obtain an accurate analysis. Also, samples were run in different amounts, because the retention time of very long chain fatty acids was slightly affected by the amount of fatty acid methyl ester injected on the column. All fatty acid methyl esters were identified with known fatty acid methyl esters, or the retention times were compared with literature data (5–7). For determination of long chain unsaturated fatty acids, samples were analyzed by gas chromatography and the remaining part of the samples was dissolved in methanol and hydrogenated with palladium oxide as a catalyst for 6 hr at room temperature. After evaporation of the methanol the hydrogenated sample was analyzed by gas chromatography and a comparison was made between the original and the hydrogenated sample.

Cyclopropane fatty acids were detected after treatment of the fatty acid methyl ester sample with bromine in ether at 30 C (2). Ether and excess bromine were evaporated. Further identification of the cyclopropane fatty acid was carried out by strong hydrogenolysis of samples in glacial acetic acid at 50 C for 4 hr with palladium oxide as a catalyst (7), converting them to methyl-branched fatty acids which differ in retention time.

RESULTS AND DISCUSSION

Table I shows the fatty acid composition of the sulfolipid fraction of the leaves of the early spring plants studied. The

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Table I. *Fatty Acid Composition of the Sulfolipid Fraction of the Leaves of Early Spring Plants (Snow Drop, Cow Parsley, Rye, English Ryegrass, and Wheat) and of the Phosphatidyl Choline Fraction of the Drought-tolerant C. canescens (L.) P.B.*

Fatty Acid	Percentage of Total Fatty Acid					
	Sulfolipid fraction					Phosphatidyl choline fraction
	G. nivalis L.	*A. silvestris* Hoffm.	*S. cereale* L.	*L. perenne* L.	*T. estivum* L.	*C. canescens* (L.) P.B.
12:0	0.2	0.6	...	0.3	0.2	0.2
13:0	0.3	0.7	...	0.5	0.4	0.2
15:0	...	...	...	0.6	0.3	0.3
16:0	1.3	5.6	11.4	15.1	14.9	1.6
16:1	...	...	...	6.7	...	...
18:0	0.2	0.7	1.2	1.0	1.4	0.5
18:1	0.3	1.4	1.8	1.4	2.8	0.6
18:2	1.5	1.7	6.7	6.4	22.1	2.6
18:3	3.4	2.3	28.4	58.3	53.8	5.7
20:0	4.8	7.1	3.6	1.8	...	6.1
21:0	6.6	4.9	4.3	...	0.5	5.0
21:cycl.[1]	5.6	...	...	...	...	11.7
22:0	6.3	2.0	4.9	1.6	1.4	7.0
22:cycl.[1]	...	...	...	...	...	2.9
23:0	0.8	1.2	5.4	0.8	0.8	...
23:cycl.[1]	...	...	...	...	...	2.5
24:0	0.5	4.6	3.3	0.8	0.7	...
25:0	...	0.7	4.9	...	0.2	...
25:cycl.[1]	68.2	66.5	24.1	4.7	0.5	53.1

[1] Cyclopropane fatty acid.

sulfolipid fractions of the leaves of snow drop and of cow parsley were predominantly esterified with a long chain cyclopropane fatty acid containing 25 carbon atoms (25:cycl.). The content of this cyclopropane fatty acid appeared to be correlated with the earliness of the plants in spring and thus correlated with the capacity of the plants to grow at low temperature, because it was absent in the sulfolipid fraction of leaves of cow parsley collected in May.

This fatty acid was also present, but in small quantity, in rye (24%), English ryegrass (4.7%), and wheat (0.5%), grown in the greenhouse at 15 C. Rye starts its vegetative growth in early April, while English ryegrass and wheat appear later. No relation with winter hardiness was observed, since the varieties of rye and wheat, selected for equal winter hardiness, contained very different amounts of this cyclopropane fatty acid.

In addition to C_{25} cyclopropane fatty acid other long chain fatty acids, ranging from 20 to 25 carbon atoms, were present in small quantities (2 to 12%) in snow drop, cow parsley, and rye and in traces in English ryegrass and wheat. In snow drop another cyclopropane fatty acid was found (21:cycl.); also, small quantities of odd chain fatty acids (21:0, 23:0, and 25:0) were sometimes present.

Careful comparison of the chromatograms of the fatty acid samples of snow drop, cow parsley, and rye before and after hydrogenolysis of the sulfolipid fractions showed that the only unsaturated fatty acids present were oleic, linoleic, and linolenic acid (18:1, 18:2, and 18:3). Comparison of the fatty acid composition of the glyco-, phospho-, and sulfolipids of snow drop and rye showed that cyclopropane fatty acids were only found in the sulfolipid fractions of both species, and, in small amount, in the phosphatidyl inositol fraction of snow drop. Thus, these fatty acids were characteristic for the sulfolipid fraction of the early spring plants studied.

Cyclopropane fatty acids were also observed in the drought-tolerant species *C. canescens* (L.) P.B., but only in the phosphatidyl choline fraction. Four different cyclopropane fatty acids were noted, with 21, 22, 23 and 25 carbon atoms to a total content of 70%. They were identified by bromination (13) and by the observed shift in retention time after strong hydrogenolysis in glacial acetic acid (8).

In another sample of this grass, collected after a rainy period, the total amount of cyclopropane fatty acids was considerably less, about 20%, indicating that the amounts of cyclopropane fatty acids present in this lipid fraction might depend on the amount of water available to the plant. This aspect will be studied in more detail.

Cyclopropane fatty acids have been detected in the seed oil of *Dimocarpus longans* and of species of the Malvaceae (4) and in bacterial lipids (2, 4). From the ecophysiological viewpoint, the occurrence of these fatty acids in *Halobacterium* from the Dead Sea, Israel, is noteworthy (Y. Avi-Dor, private communication).

Lipids esterified with long chain cyclopropane fatty acids could contribute to the physiological adaptations of early spring plants and drought-tolerant plants in several ways. Chilling resistance in plants is correlated with a high flexibility of the mitochondrial membrane and with a high degree of unsaturation of the mitochondrial lipid (10, 11). Flexibility of membranes may depend on the physical state of the membrane lipid involved, and cyclopropane fatty acids will behave like unsaturated fatty acids in this respect. The "hydrophobic melting point" of lipids esterified with unsaturated fatty acids is lower than that of saturated lipids (3). The lipid esterified with cyclopropane fatty acid very likely will also show physiologically low hydrophobic melting points, allowing increased mobility of the hydrocarbon chain at low temperature in comparison with the saturated analogue. In this connection it is of interest that the cyclopropane fatty acid of cow parsley disappeared later in spring when the low temperature adaptation becomes less critical. The cyclopropane ring would be essential for the required liquid state of the hydrophobic part of the membrane lipid at low temperatures (3).

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