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Manipulation of Galactolipid Fatty Acid Composition with Substituted Pyridazinones

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ABSTRACT

The fatty acid composition of the major lipids of the chloroplast membranes, the mono- and digalactosyl diglycerides, can be definably altered with various substituted pyridazinones. Galactolipid fatty acid composition of wheat (*Triticum aestivum* L.) can be altered so that there is a decrease in linolenic acid accompanied by an increase in linoleic acid without a shift in the relative proportion of saturated to unsaturated fatty acids; the fatty acid composition can be shifted toward a higher proportion of saturated fatty acids; or the fatty acid composition of the monogalactosyl diglycerides can be altered in preference to the digalactosyl diglycerides. Also, the light-mediated parallel accumulation of chlorophyll and linolenic acid can be separated with a substituted pyridazinone. The substituted pyridazinones may be useful tools in clarifying the role the galactolipids and their component fatty acids play in the structure and function of chloroplast membranes in higher plants.

Our knowledge of membrane lipid composition remains dominated by information derived from studies with animal cells or microorganisms. The most predominant lipids in the membranes of animal cells and microorganisms are the phospholipids, particularly phosphatidylcholine and phosphatidylethanolamine. By appropriately manipulating the genetic characteristics of microorganisms (7, 14, 15, 18), or by altering the culture conditions for the growth of microorganisms (14, 15, 18) or mammalian cells (13), one can, within empirically defined limits, selectively control the phospholipid composition of the membrane matrix, as well as the fatty acid composition of the phospholipids. Such studies have provided valuable insights into the role phospholipids and their component fatty acids play in the structure and function of membranes.

Information on the overall lipid composition of plant cells and on the location of lipids within cells has been summarized by Hitchcock and Nichols (12). As in microorganisms and animal cells the major lipids of plant mitochondrial, plasma, and tonoplast membranes are phospholipids. Plant chloroplast membranes, differ from most other membranes in that the major portion of the nonpigmented lipids of the chloroplast are the linolenic acid-rich galactolipids, with sulfolipids and phospholipids present as the minor lipid constituents.

Many attempts have been made to determine the functions and biosynthetic pathways of the galactolipids (1, 5, 8, 12, 17). Attention has been directed toward determining the synthesis of both the galactose and the fatty acid moieties. Galactolipid metabolism is strongly modified by environmental conditions. The light-mediated greening of etiolated tissues is concomitant with an increase in galactolipids and accompanying linolenic acid. Magnesium or nitrogen deficiency causes a reduction of linolenic acid and galactolipids of higher plants. Temperature also affects the fatty acid composition of the membrane lipids of plants, animals, and microorganisms. The lower the growth temperature, the greater the content of polyunsaturated fatty acids. For plants, the temperature effect on fatty acid composition is more pronounced for phospholipids in nonphotosynthetic tissue and is accompanied by a variety of other metabolic changes.

We reported earlier that major effects of San 6706 were inhibition of photosynthesis and interference with chlorplast pigment development (10) and inhibition of the formation of chloroplast polar lipids (11). The inhibition in formation of polar lipids was related to an increase in linoleic acid accompanied by a decrease in linolenic acid of the polar lipids. We suggested that San 6706 might act by preventing the formation of chloroplast membranes, resulting from inhibition of the formation of the lipoidal constituents, namely, the polar lipids, Chl, and carotenoids necessary for chloroplast membrane formation.

The polar lipids from leaf tissue are predominantly galactolipids (5, 8, 12, 17). Previous results suggested that San 6706 and other substituted pyridazinones might be useful tools with which to alter the fatty acid composition of galactolipids.

The present paper reports alteration of the fatty acid composition of galactolipids with substituted pyridazinones (Fig. 1). Pyridazinone effects on Chl accumulation are also reported in order to demonstrate separation of Chl and linolenic acid accumulation in the light.

MATERIALS AND METHODS

Chemicals. Technical grades of pyrazon [5-amino-4-chloro-2-phenyl-3(2*H*)-pyridazinone], San 9785 [4-chloro-5-(dimethylamino)-2-phenyl-3(2*H*)-pyridazinone], San 9774 [5-amino-4-chloro-2(α,α,α-trifluoro-*m*-tolyl)-3(2*H*)-pyridazinone], San 6706 [4-chloro-5-(dimethylamino)-2-(α,α,α-trifluoro-*m*-tolyl)-3(2*H*)-pyridazinone], norflurazon [4-chloro-5-(methylamino)-2-(α,α,α-trifluoro-*m*-tolyl)-3(2*H*)-pyridazinone], and San 113-410 H [4-chloro-5-(methylamino)-2-phenyl-3(2*H*)-pyridazinone] were used in all studies.

Plants. Wheat seeds (*Triticum aestivum* L., cv. Arthur) were germinated in 9-cm Petri dishes on three layers of filter paper. Herbicides were dissolved in acetone and the filter papers were impregnated with 1 ml of the acetone solution. After the solvent evaporated, 10 ml of distilled H_2O were added to give the desired inhibitor concentration. Seeds were planted directly on the moist papers and germinated for 4 days in a controlled environment chamber in a 16-hr photoperiod in which day temperature was 27 ± 1 C and night temperature was 21 ± 1 C. Light intensity from a combination of cool-white fluorescent and incandescent bulbs was 2,600 ft-c at dish level.

Analytical Procedures. Lipids were extracted and recovered from 1 g of lyophilized shoot tissue according to the procedures

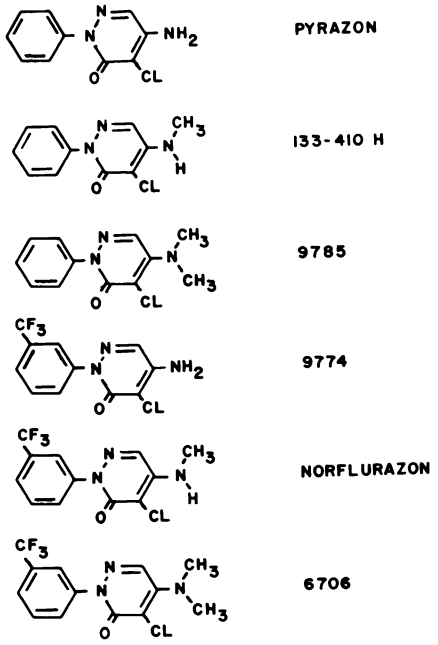

FIG. 1. Structure of six phytotoxic substituted pyridazinones.

of Folch *et al.* (9). Polar lipids were separated from nonpolar lipids by the rubber membrane dialysis method of Bottcher *et al.* (4). The polar lipids were further divided by TLC on Silica Gel G, using chloroform-methyl alcohol-acetic acid-water (85:15:10:2, v/v) as the developing solvent. The mono- and digalactosyl diglycerides were located, after visualization with iodine vapor, by co-chromatography with authentic standards. After evaporation of the iodine, the appropriate bands were removed, and the galactolipids were extracted from the silica gel with chloroform-methyl alcohol (2:1 v/v). The silica gel was removed by centrifugation and the galactolipids were saponified with alcoholic KOH as outlined by Burchfield and Storrs (6). Methyl esters of the resulting fatty acids were prepared with boron trifluoride in methyl alcohol by the method of Metcalfe and Schmitz (16) for gas chromatography as previously described (11) except that a 1.83-m glass column packed with 10% SP-216-PS on 100/120 mesh Supelcoport[1] (Supelco, Inc. Bellefonte, Pa.) was used in the present studies. Heptadecanoic acid was included in all samples as an internal standard. Throughout the procedure samples were maintained, as nearly as was possible, under a nitrogen atmosphere to minimize oxidation of the highly unsaturated lipids. A nitrogen atmosphere was extremely important during spotting and drying of the thin layer plates. Preliminary data accumulation and reduction was achieved with an Autolab System IV computing integrator (Spectra-Physics, Parsippany, N. J.).

Quantitative comparisons of the action of the pyridazinones on Chl content were made on 4-day-old shoots grown under the conditions described below. Shoot tissues of 12 seedlings were combined, homogenized, and the Chl content determined by the method of Arnon (2).

RESULTS AND DISCUSSION

Data in Tables I and II confirm the hypothesis that substituted pyridazinones are useful tools with which to alter the fatty acid composition of galactolipids. The galactolipids from control shoots show the expected linolenic acid-rich fatty acid composition, based on a previous report of fatty acid composition of wheat chloroplasts (see page 71, ref. 12). Pyrazon, the least substituted pyridazinone, did little to alter this normal fatty acid complement. The remaining five more highly substituted pyridazinones presently evaluated caused marked alteration in the fatty acid composition of both the mono- and digalactosyldiglycerides. Analysis of the data in Tables I and II (Table III) shows that there are potentially important differences in the effects exerted by the pyridazinones. San 9785 altered galactolipid fatty acid composition with respect to the relative content of linoleic and linolenic acid. San 9774, Norflurazon, San 6706, and San 133-410 H produced this alteration to varying lesser degrees, but these compounds also shifted the ratio of saturated to unsaturated fatty acid in the galactolipids. San 9774 preferentially altered the fatty acid composition of the monogalactosyl diglycerides.

The pyridazinones also exhibited differential effects on Chl accumulation (Table IV). Pyrazon and San 9785 accumulated Chl to nearly control levels. In contrast, San 9774 and San 133-410 H reduced Chl levels by approximately 50% while San 6706 and Norflurazon caused essentially complete prevention of Chl

Table I. *Effect of Substituted Pyridazinones on Fatty Acid Composition on Monogalactosyl Diglycerides of Wheat Shoots*

The data are the average of three separate experiments.

Compound (0.1 mM)	Fatty Acid Composition				
	C16	C18	C18:1	C18:2	C18:3
	% by weight				
Control	15	3.3	4.3	19	58.9
Pyrazon	17.2	5.3	6.1	21.1	50.2
San 9774	42.1	16.2	11.8	20.1	8.9
San 9785	21.1	5	5.6	53.9	14.1
Norflurazon	42.9	11.8	11.8	21.1	12.6
San 6706	37.5	11.7	12	24.7	14.5
San 113-410 H	38	8.5	12.7	19.7	21.1

Table II. *Effect of Substituted Pyridazinones on Fatty Acid Composition of Digalactosyl Diglycerides*

The data are the average of three separate experiments.

Compound (0.1 mM)	Fatty Acid Composition				
	C16	C18	C18:1	C18:2	C18:3
	% by weight				
Control	21.1	6.2	3.9	10.1	58.3
Pyrazon	23.8	3.6	4.5	13.8	54.3
San 9774	27.7	6.6	6.6	14.7	44
San 9785	22.8	5	5.7	51.1	15.4
Norflurazon	37.5	7.2	9.6	29.4	16.3
San 6706	36.5	8.6	7.6	27.8	19.8
San 133-410 H	34.2	7.4	5.9	17.8	34.7

Table III. *Effect of Substituted Pyridazinones on Relative Fatty Acid Composition of Galactolipids from Wheat Shoots*

Compound (0.1 mM)	Monogalactosyldiglyceride		Digalactosyldiglyceride	
	ΣS/U[1]	18:2/18:3	ΣS/U	18:2/18:3
	ratio			
Control	0.22	0.32	0.27	0.17
Pyrazon	0.29	0.42	0.38	0.25
San 9774	1.50	2.3	0.53	0.33
San 9785	0.35	3.82	0.39	3.32
Norflurazon	1.21	1.67	0.81	1.80
San 6706	0.97	1.70	0.82	1.40
San 133-410 H	0.87	0.93	0.71	0.5

[1] Total saturated/total unsaturated fatty acids.

[1] Mention of a trade name or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exlusion of other products that may also be suitable.

Table IV. *Effects of Substituted Pyridazinones on Chl Levels in 4-day-old Wheat Shoots*

The data are the average of nine determinations from three separate experiments.

Compound (0.1 mM)	Chl Content
	μg/g dry wt
Control	545
Pyrazon	471
San 9774	227
San 9785	521
San 6706	5
Norflurazon	22
San 133-410 H	221

accumulation. Bartels and Hyde (3) suggest that Chl is synthesized in San 6706-treated plants but is photooxidized upon exposure to light as a result of San 6706 inhibition of carotenoid synthesis.

It is well known (12) that during the greening of etiolated tissue there is a light-induced increase in galactolipids and accompanying linolenic acid, paralleled by a rapid rise in Chl. Comparison of the data in Tables I, II, III, and IV reveals that San 9785 caused a marked reduction in accumulation of linolenic acid in the galactolipids, while Chl accumulated to control levels. Thus, the lightmediated parallel accumulation of Chl and linolenic acid may be circumvented with San 9785.

Our data taken collectively demonstrate that the fatty acid composition of galactolipids can be definably manipulated in a higher plant with various substituted pyridazinones. Likewise, Chl content may also be manipulated. We suggest that the substituted pyridazinones will be valuable tools for use in clarifying the role galactolipids and their component fatty acids play in the structure and function of chloroplast membranes. Also, elucidation of the mechanism by which the pyridazinones exert their effects should provide valuable insight into the factors regulating the synthesis of unsaturated fatty acids and the ratio of saturated to unsaturated fatty acids in the membrane galactolipids.

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