COMMUNICATIONS

Neutral Lipids of Barley Grain

Fractionation of the neutral lipids of the grains of two barley (*Hordeum vulgare* L.) varieties, "Kearney" (winter type) and "Prilar" (spring type), by thin-layer chromatography produced 11 individual lipid classes. Gravimetric determination of each fraction showed the triglycerides to be the predominant class. Somewhat smaller amounts of 1,2-diglycerides, 1,3-diglycerides, free sterols, free fatty acids, sterol esters, hydrocarbons, and small amounts of four unknowns were present. Fatty acids present in the fractions ranged from lauric (C12:0) to arachidic (C20:0). Linoleic acid (C18:2) was the principal fatty acid in all the saponifiable fractions, excluding sterols, except unknown no. 4 in which oleic acid (18:1) was predominant.

Neutral lipids are the major lipid class in barley and other cereal grains. Their representation ranges from about 60 to 90% of the total lipid content of the caryopsis (Price and Parsons, 1975). The neutral lipid class is a complex group of compounds containing free fatty acids, glycerides, free sterols, and sterol esters.

This study is a continuation of research on the individual lipid classes of barley neutral lipids, glycolipids, and phospholipids. The intent of the research is to obtain detailed information on the fractions in each class. The information will provide a basis for monitoring changes in weight distribution and fatty acid content of the fractions when germinated seeds are subjected to changes in ambient temperature. The changes in lipid composition may be related to rate of cold acclimation and degree of winter hardiness.

MATERIALS AND METHODS

Whole grain samples of the barley varieties "Kearney" and "Prilar" were ground in a Udy cyclone mill to pass a 0.6-mm screen. Total lipids were extracted from a 25-g sample with 20 volumes of chloroform/methanol/water (1.0:1.0:0.9) using modification from the methods of Bligh and Dyer (1959), Floch et al. (1957), Weber (1970), and Atkinson et al. (1972) and were purified as described previously (Price and Parsons, 1974). The lipids were separated into classes by silicic acid column chromatography (Hirsch and Ahrens, 1958). The neutral lipid class was eluted with diethyl ether, and the solvent was removed by a rotary vacuum evaporator at 40 °C. The neutral lipids were then transferred to vials with petroleum ether, flushed with nitrogen, and stored at −20 °C.

The neutral lipids were separated by one-dimensional TLC on silica gel G coated glass plates (gel 0.25 mm thick) with the solvent system petroleum ether/diethyl ether/acetic acid (180:25:2). The individual neutral lipids, separated by TLC, were identified by cochromatography with authentic reference lipids (Applied Science Laboratories, State College, PA; Supelco, Bellefonte, PA; and Analabs, North Haven, CT) and from published R_f values (Lepage, 1967; Nichols, 1964). Preliminary work with developed TLC plates revealed no differences in number and size of fractions when visualized by exposure to iodine vapor or sprayed with 2′,7′-dichlorofluorescein. TLC plates developed throughout this study were treated with the latter reagent to avoid the unreliable GLC results induced by iodine. The developed TLC plates were sprayed with 2′,7′-dichlorofluorescein (Stahl, 1969) and the spots were observed under UV light. Individual spots were scraped into separate Erlenmeyer flasks containing 50 mL of methanol/diethyl ether (2:1). The lipids were separated from the silica gel by filtering through glass wool, with three additional washings by solvent, dried by a rotary vacuum evaporator at 35 °C, weighed, dissolved, transferred to vials, and stored at −20 °C.

Table I. Composition of the Neutral Lipids of "Kearney" and "Prilar" Barley[a]

neutral lipid[b]	"Kearney" mean[c]	"Kearney" SD	"Prilar" mean	"Prilar" SD
1. 1,2-diglycerides	8.6	0.60	8.1	0.58
2. 1,3-diglycerides	7.4	0.75	7.2	0.71
3. free sterols	9.4	0.66	10.1	0.69
4. unknown 1	0.9	0.29	0.7	0.22
5. unknown 2	1.2	0.51	1.0	0.44
6. unknown 3	1.4	0.41	1.5	0.46
7. free fatty acids	8.7	0.63	9.2	0.65
8. triglycerides	52.3	1.38	51.4	1.43
9. unknown 4	Tr[d]		Tr	
10. sterol esters	4.2	0.66	4.6	0.72
11. hydrocarbons	5.9	0.57	5.4	0.51

[a] Expressed as percent total neutral lipid. [b] Listed in ascending order from the origin on TLC plate. [c] Averages based on four replications of 10 TLC plates. [d] Trace.

Determination of the weight percent of the individual fractions was made after collecting the 11 spots on each of 10 plates with four replications (total of 40 plates). Aliquots of the individual fractions were converted to methyl esters (Metcalfe et al., 1966) for fatty acid analysis by GLC. Information on column and operating conditions of the GLC and the preparation of methyl esters for GLC analyses were described previously (Price and Parsons, 1974).

RESULTS

The solvent system used in the one-dimensional thin-layer chromatograph produced good separation of the individual lipids in the neutral lipid class of barley grain. A representative TLC of Kearney barley is presented in Figure 1. Chromatograms of the neutral lipids of Prilar barley were similar.

Eleven lipids were obtained by thin-layer separation and seven of them were identified. The composition of the neutral lipid class was similar for both barley varieties (Table I). Triglycerides were the predominant group in

Table II. Fatty Acid Composition of the Saponifiable Neutral Lipid Fractions of Kearney Barley[a]

fraction	fatty acid								
	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0
1,2-diglyceride	Tr[b]	0.6	17.9	0.1	2.3	11.7	62.0	5.4	
1,3-diglyceride	0.1	0.4	18.2	0.3	2.2	12.5	62.4	3.9	
unknown 1	1.5	3.5	21.8	0.3	4.9	19.7	43.8	3.9	0.6
unknown 2	0.9	8.8	16.4	3.5	10.5	18.6	36.9	3.0	1.4
unknown 3	0.6	0.7	18.7	0.1	9.4	21.0	44.6	4.7	0.2
free fatty acids	Tr	1.0	32.5	Tr	2.5	7.7	51.9	4.4	
triglycerides	Tr	0.2	17.4	0.1	1.0	22.7	53.4	5.2	
unknown 4	2.0	3.1	24.7	9.8	22.5	16.5	5.6	9.8	6.0

[a] Percent by weight calculated from peak areas of the gas chromatograms. Fatty acids are expressed as number of carbons/number of double bonds. [b] Trace.

Table III. Fatty Acid Composition of the Saponifiable Neutral Lipid Fractions of "Prilar" Barley[a]

fraction	fatty acid								
	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0
1,2-diglyceride	Tr	0.8	23.4	0.5	3.2	13.9	53.7	4.5	
1,3-diglyceride	Tr	0.3	23.6	0.2	2.3	13.3	56.5	3.8	
unknown 1	0.3	1.2	24.1	0.6	3.5	17.5	48.4	4.4	Tr
unknown 2	0.7	6.4	22.0	1.2	3.3	18.0	43.7	4.7	Tr
unknown 3	0.6	1.6	21.7	0.8	3.2	16.3	49.1	6.7	Tr
free fatty acids	0.3	1.3	37.1	0.3	4.0	9.3	46.3	1.4	
triglycerides	Tr	0.9	20.6	0.1	0.6	19.0	54.5	4.3	
unknown 4	0.7	3.6	26.4	2.8	13.6	38.0	10.2	1.3	3.4

[a] Percent by weight calculated from peak areas of the gas chromatograms. Fatty acids are expressed as number of carbons/number of double bonds.

gravimetric determinations, comprising over 50% of this lipid class. Smaller amounts of 1,2-diglycerides, 1,3-diglycerides, free sterols, free fatty acids, sterol esters, and hydrocarbons were present along with small amounts of four other fractions, identified as unknowns 1, 2, 3, and 4.

The fatty acid composition of the neutral lipids was similar for both varieties (Tables II and III). Fatty acids ranged from lauric (12:0) to arachidic (20:0). The major fatty acids detected were linoleic (18:2), palmitic (16:0), and oleic (18:1). Linoleic was the principal fatty acid in all fractions except unknown 4. In that fraction, oleic and palmitic acids were present in highest amounts. Linoleic acid was low, 5.6 and 10.2%, respectively, in "Kearney" and "Prilar". Arachidic was present in small amounts in the first three unknowns and in appreciable amounts in unknown 4, but totally absent in all other fractions. A previous study (Price and Parsons, 1979) revealed that arachidic is present in measurable amounts only in the neutral lipid class of the hull fraction of the barley caryopsis.

The hydrocarbon fraction, located on the solvent front of the TLC plate (Figure 1), is a collection of higher molecular weight materials. A sample of this fraction was esterified and analyzed by GLC. Three unidentified peaks were obtained with a high attenuation setting of the gas chromatograph. This would seem to indicate that very small amounts of neutral lipid are included in this mixture.

The two sterol fractions, free sterols and sterol esters, were identified but their composition was not determined. They represent about 15% of the total weight of the neutral lipids. A separate study on the composition of the two sterol groups will be reported later.

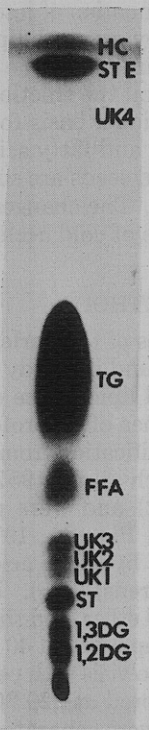

Figure 1. One-dimensional thin-layer chromatographic separation of neutral lipids from Kearney winter barley. Absorbent: silica gel G. The spots were identified as follows: 1,2DG, 1,2-diglycerides; 1,3DG 1,3-digylcerides; ST, free sterols; UK1, unknown 1; UK_2, unknown 2; UK3, unknown 3; FFa, free fatty acids; TG triglycerides; UK4, unknown 4; STE sterol esters; HC, hydrocarbons.

DISCUSSION

Neutral lipids represent about 70% of the total lipid content of barley caryopses and between 60 and 90% of total lipid in the caryopses of most other cereal grains (Price and Parsons, 1975). Unlike the phospholipids (Parsons and Price, 1979), the neutral lipids contain both saponifiable (free fatty acids and glycerides) and nonsaponifiable (sterols and sterol esters) fractions.

As presented in previous chromatograms (Parsons and Price, 1974; Price and Parson, 1974, 1975), the triglyceride fraction appeared to be the major entity in the neutral lipid class. Gravimetric analyses of the fractions in this study verified the triglycerides as representing over 50% of the total neutral lipid weight in both "Kearney" and "Prilar"

(Table I) (Figure 1). There was fairly uniform weight distribution among six other fractions, but none of them attained 11%.

Thin-layer chromatographic separation of the neutral lipids of seven cereal grains has been presented previously (Price and Parsons, 1975). The seven major fractions observed in this study on barley are found in the other six cereals as well. Triglycerides are the principal fraction in every instance. Variation among the seven cereals seems to exist only in numbers of minor fractions labeled unknowns. Sorghum is the widest variant in having two unknowns present in measurable amounts with a higher R_f value than the triglyceride fraction.

The identity of the four unknown fractions has not been established (Table I). They are different from the larger fractions in that they all contain a trace or measurable amounts of arachidic acid (20:0) (Tables II and III). The more slowly ascending unknowns (Figure 1), i.e., unknowns 1, 2, and 3, have a fatty acid distribution that is quite comparable to the other fractions. In these, linoleic (18:2) is the principal fatty acid and palmitic (16:0) is second in percent of total. Unknown 4, a rapidly ascending fraction, contains more higher molecular weight fatty acids, e.g., arachidic, and deviates considerably from the other fractions in fatty acid distribution. In unknown 4, oleic (18:1) is the principal fatty acid and only a relatively small amount of linoleic (18:2) is present.

LITERATURE CITED

Atkinson, J., Fowler, V. R., Garton, G. A., Lough, A. K., *Analyst (London)* **97**, 562 (1972).
Bligh, E. G., Dyer, W. J., *Can. J. Biochem. Physiol.* **37**, 911 (1959).
Folch, J., Lees, M., Sloane, G. H., *J. Biol. Chem.*, **226**, 497 (1957).
Hirsch, J., Ahrens, E. H., *J. Biol. Chem.*, **233**, 311 (1958).
Lepage, M., *Lipids* **2**, 244 (1967).
Metcalfe, L. D., Schmitz, A. A., Pelk, J. R., *Anal. Chem.* **38**, 514 (1966).
Nichols, B. W., in "New Biochemical Separations", James, A. T., Morris, L. J., Ed., Van Nostrand, New York, 1964, p 321.
Parsons, J. G., Price, P. B., *Lipids* **9**, 804 (1974).
Parsons, J. G., Price, P. B., *J. Agric. Food Chem.* **27**, 913 (1979).
Price, P. B., Parsons, J. G., *Lipids* **9**, 560 (1974).
Price, P. B., Parsons, J. G., *J. Am. Oil Chem. Soc.* **52**, 490 (1975).
Price, P. B., Parsons, J. G., *J. Agric. Food Chem.* **27**, 813 (1979).
Stahl, E., "Thin Layer Chromatography", Academic Press, New York, 1969, p 866.
Weber, E. J., *J. Am. Oil Chem. Soc.* **46**, 485 (1970).

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Residue Analysis of β-Naphthoxyacetic Acid and β-Naphthol on Field-Sprayed Tomatoes by High-Pressure Liquid Chromatography

Experiments were performed to determine the levels and fate of β-naphthoxyacetic acid (BNOA) and its postulated metabolite (β-naphthol) on tomato flowers, fruit, and leaves when a wettable powder formulation of 42 and 84 ppm active ingredient BNOA in water was sprayed on the tomato plant blossoms in the field, not taking care to avoid leaves and fruit. Residues of BNOA dropped to negligible levels 5–10 days after application and no β-naphthol residues were detected (<0.01 ppm). Even after three spray applications at 10-day intervals, no residue buildup was detected on the plant parts. The tomato leaves retained the BNOA for the longest period of time. However, 5–10 days after application, no significant residues remained on the leaves. Similar results were obtained when the entire plant was intentionally sprayed with BNOA. The harvest fruit in all experiments contained <0.01 ppm of BNOA and β-naphthol.

Various plant growth-regulating hormone-type chemicals such as β-naphthoxyacetic acid, 4-chlorophenoxyacetic acid, and 2,4-dichlorophenoxyacetic acid have been investigated as to their effects on tomatoes (Mann and Minges, 1948; Wittwer and Reath, 1952). Emphasis was on early market production rather than on growing the crop for processing.

Tomatoes, which might otherwise be lost due to poor pollination, are set, especially in the early part of the season, by BNOA. Short cloudy days, which lack sunlight and have cool nights, under 15 °C, are conditions that are unfavorable for flower pollination. BNOA stimulates the blossoms to set fruit and holds them on the plants.

The purpose of the present investigation was to determine the levels and fate of β-naphthoxyacetic acid (BNOA) and its postulated metabolite (β-naphthol), if any, on tomato flowers, fruit, and leaves when a wettable powder formulation of 42 and 84 ppm active ingredient BNOA in water was sprayed on the tomato plants in the field. The possibility exists that BNOA could be a useful chemical for assisting in the production of fresh early market tomatoes. Archer and Stokes (1978a) have previously published on the residue analysis of β-naphthoxyacetic acid and β-naphthol by high-pressure liquid chromatography

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