Distribution of Lipids in Embryonic Axis, Bran–Endosperm, and Hull Fractions of Hulless Barley and Hulless Oat Grain

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Content and composition of lipids in the embryonic axis, bran–endosperm, and hull fractions of hulless barley and hulless oat caryopses were determined as a basis for genetic improvement of the oil content of barley. Total lipid content of "Prilar Hulless" barley was 3.2% and "James Hulless" oats was 7.2%. Total lipid content of embryo, endosperm, and hull fractions of barley was 19.6, 2.8, and 2.4%, respectively, and of oats was 21.2, 7.1, and 4.4%, respectively. Barley had a higher percentage of linoleic acid (18:2) than oats, although linoleic was the predominant unsaturated fatty acid in the embryonic axis and bran–endosperm fractions in all lipid classes of both. Palmitic acid was the most abundant fatty acid in the glycolipid and phospholipid classes of barley hulls and the neutral lipid and glycolipid classes of oat hulls.

The lipid content of barley (*Hordeum vulgare* L.) is less than half that of oats (*Avena sativus* L.) (Price and Parsons, 1975), and the range in oil concentration among barley varieties and selections appears to be much narrower (Parsons and Price, 1974) than for oats (Brown and Craddock, 1972).

Over 90% of the oil in a maize (*Zea mays* L.) caryopsis is located in the large embryo. Increased oil content in maize is achieved through hybridization and selection for increased proportion of embryo weight to total seed weight (Hopkins, 1898). The embryo in barley and oat caryopses is small and represents a small percentage of total seed weight. Although the embryonic tissues of barley (McLeod and White, 1961) and oats (Youngs et al., 1977) contain about 15% lipid, dry weight basis, they make a relatively small contribution to total grain lipid. The relatively high total lipid content of oats compared to barley indicates the presence of considerably more lipids in the endosperm fraction of the oat seed. Genetic improvement in barley lipids will require an increase in the amount of lipid in the endosperm fraction since the thinness of the barley hull along with the small size of the embryo severely limits an increase in the total lipid content in their fractions.

This study was developed to provide information on the content and composition of lipids in the embryonic axis, bran–endosperm, and hull fractions of "Prilar Hulless" barley and "James Hulless" oats as a basis for genetic improvement of the oil content of barley.

MATERIALS AND METHODS

The caryopsis of a small grain plant is surrounded by the lemma and palea which form the hull. In hulled barley the lemma and palea become fused to the developing caryopsis through secretion of natural plant mucilages. In hulled oats the hull is not fused to the caryopsis but completely envelopes it. The so-called hulless varieties of small grains have hulls, but fusion or tight envelopment does not occur and the caryopsis separates readily from the hull when threshed. The "hulless condition" thus greatly facilitates the separation of complete, intact hulls from the caryopsis without requiring mechanical action that may cause intermixing of seed parts. In this study, spikes of "Prilar Hulless" barley and panicles of "James Hulless" oats were carefully hand harvested. Two hundred grams of grain of each variety were used in this study. The caryopses were removed from the hulls and the hulls were clipped at their attachment point. The embryonic axes were then removed from the bran–endosperms with a sharp needle under 3× magnification. Embryonic axes, bran–endosperms, and hulls were weighed separately to determine weight distribution, and then ground separately in a Udy mill to pass a 0.6-mm screen. Total lipids of each seed fraction were extracted and purified and separated into classes by silicic acid column chromatograph as described previously (Price and Parsons, 1974). Information on column and operating conditions of the gas–liquid chromatograph and the preparation of methyl esters for GLC analyses were also described previously (Price and Parsons, 1974).

Table I. Weight Distribution, Lipid Content, and Lipid Distribution in Three Grain Fractions of Barley and Oats

	barley, % ("Prilar Hulless")	oat, % ("James Hulless")
weight distribution		
embryonic axis	3.0	2.4
bran–endosperm	90.2	84.5
hull	6.8	13.1
lipid content		
whole grain	3.2	7.2
embryonic axis	19.6	21.2
bran–endosperm	2.8	7.1
hull	2.4	4.4
lipid distribution		
embryonic axis	17.9	7.2
bran–endosperm	77.1	84.7
hull	5.0	8.1

Table II. Lipid Composition of Three Grain Fractions

	barley ("Prilar Hulless"), % of total lipid	oat ("James Hulless"), % of total lipid
embryonic axis		
neutral lipid	75.8	87.4
glycolipid	6.4	3.8
phospholipid	17.8	8.8
bran–endosperm		
neutral lipid	64.4	56.9
glycolipid	12.5	21.4
phospholipid	23.1	21.7
hull		
neutral lipid	75.9	66.9
glycolipid	18.2	27.6
phospholipid	5.9	5.5

RESULTS

Total lipid in the whole grain (embryonic axis + bran–endosperm + hull) of "Prilar Hulless" barley was 3.2% and in "James Hulless" oats, 7.2% (Table I). Weight distribution, lipid content, and lipid distribution

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Table III. Fatty Acid Composition of Neutral Lipid, Glycolipid, and Phospholipid Classes of Barley and Oats[a]

cultivar	fatty acid								
	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0
Neutral Lipid									
"Prilar" hulless									
embryonic axis	Tr	0.2	19.5	Tr	0.6	20.4	49.7	9.6	Tr
bran-endosperm	Tr	0.2	20.6	0.1	1.3	16.6	56.8	4.4	Tr
hull	1.7	5.3	26.1	2.0	5.4	21.5	30.6	5.0	2.4
"James" hulless									
embryonic axis	Tr	0.1	17.1	0.6	0.6	32.1	45.8	3.7	
bran-endosperm	Tr	0.3	16.4	0.1	1.5	36.1	43.9	1.7	Tr
hull	0.6	3.4	32.4	1.7	4.7	18.2	27.6	8.8	2.6
Glycolipid									
"Prilar" hulless									
embryonic axis	Tr	0.2	17.4	Tr	3.9	14.7	40.6	23.2	
bran-endosperm	Tr	0.2	17.9	Tr	2.1	5.9	68.3	5.6	
hull	2.9	6.2	41.9	Tr	7.3	15.3	17.1	9.3	
"James" hulless									
embryonic axis	0.6	1.6	19.9	0.5	3.7	19.1	45.2	10.0	
bran-endosperm	Tr	0.3	20.1	Tr	2.0	17.4	56.4	3.3	
hull	1.3	2.2	36.7	0.1	4.4	10.0	22.2	23.1	
Phospholipid									
"Prilar" hulless									
embryonic axis	Tr	0.1	17.5	0.1	0.2	16.3	56.4	9.4	
bran-endosperm	Tr	0.6	30.4	Tr	1.5	12.2	53.3	2.0	
hull	0.3	1.6	37.6	1.4	2.2	14.0	32.4	10.5	
"James" hulless									
embryonic axis	Tr	0.2	19.0	1.2	0.3	25.2	51.0	3.1	
bran-endosperm	Tr	1.1	28.8	0.1	1.7	18.5	48.6	1.2	
hull	0.5	1.2	32.3	0.1	2.2	14.8	36.6	12.3	

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

of the three kernel fractions are also presented there. The difference in total lipid content between these two species lay primarily with the higher lipid level in the oat bran–endosperm, 7.1% vs. 2.8% (Table I).

Lipid composition varied some between the two species and among the grain fraction (Table II). Neutral lipids were predominant in all fractions. Glycolipids were a minor component in the embryonic axes of both species, but the percentage of this class increased markedly in the other fractions, especially in oats. The phospholipid content of barley embryonic axes was higher than in oats, but the two species were very similar in the bran–endosperm and hull fractions.

GLC analyses of the three lipid classes are presented in Table III. The fatty acids ranged from C_{12} to C_{20}. Linoleic acid (18:2) was the principal fatty acid of the embryonic-axis and bran–endosperm fractions in all three lipid classes. Linoleic acid accounted for more than half of the fatty acids of the bran–endosperm glycolipids of both oats and barley. Oleic (18:1) and palmitic (16:0) were the other prominent fatty acids. Oleic and palmitic acids were each present at the 20% level in the neutral lipid class of the embryonic axis of Prilar barley, whereas in James oats oleic and palmitic acid represented 32.1 and 17.1%, respectively. Palmitic acid was the major fatty acid in the hull fraction and the hull fraction had the most balanced distribution of fatty acids. The polyunsaturated acid, linolenic (18:3), was detected in considerable amounts in the embryo and hull fractions of both species. Small amounts of lauric (12:0), myristic (14:0), palmitoleic (16:1), and stearic (18:0) were also detected. Arachidic (20:0) was present in measurable amounts only in the neutral lipid class of the hull fraction of the two grains.

DISCUSSION

Several studies have been reported on the content and composition of barley and oat lipids. Most of those for barley (Johannson, 1976; Parsons and Price, 1974; Price and Parsons, 1974, 1975; Welch, 1975) and oats (Johannson, 1976; Welch, 1975; Youngs and Puskulcu, 1976) have been directed to analyses of whole grain samples. They have not dealt with the concentration and composition of lipids in grain fractions. McLeod and White (1961) fractionated the kernels of "Proctor" barley in two ways. They used a pearling machine to remove successive layers of the kernel from the hull inward. This method of separation did not give botanically homogenous fractions. The pearling produced a larger number of fractions than we utilized in this study and they extracted the lipids with diethyl ether, a procedure that is unsatisfactory for the removal of bound lipids. They employed a second method which divided the Proctor kernels into two fractions, embryo and remaining grain, and extracted the lipids with diethyl ether. They reported that the embryos contained 15.3% lipid, dry weight basis, and this represented 30.0% of lipid distribution. The three fractions analyzed in the study reported here were subjected to chloroform/methanol/water extraction which provides for nearly complete extraction of neutral lipids, glycolipids, and phospholipids. In our study, the embryonic axis contained 19.6% lipid, dry weight basis, and 17.9% of lipid distribution. Their fatty acid analyses by reverse-phase chromatography were run on whole grain lipids and compare generally to that reported for several barleys (Price and Parsons, 1974, 1975; Parsons and Price, 1974).

The recent study of oat lipids (Youngs et al., 1977) used the cultivars Dal (high lipid content) and Froker (medium lipid content) as test objects. Dehulled oat kernels, groats, and kernels fractionated into hulls, bran, endosperm, scutellum, and embryonic axis were extracted with diethyl ether and water-saturated 1-butanol. Their analyses of more kernel fractions (finer subdivisions) different extraction system and nonclassification of lipids again makes comparison difficult. The variety Froker had 6.8% lipid in the groat which makes it comparable to the 7.2% in the whole grain of James Hulless. The hulls of Froker con-

tained 2.6% lipid, endosperm 6.2%, and the average for scutellum plus embryonic axis was 19.7%. This is in good agreement with our data from James Hulless; hulls 4.4%, endosperm 7.1%, and embryo 21.2%. The fatty acid analyses of Dal and Froker were run on free lipids (mainly neutral lipids) and bound lipids (mainly glycolipids and phospholipids). The neutral lipids in the endosperm, embryonic axis, and scutellum of Dal oats had a fatty acid distribution similar to that of James Hulless (Table III) and reported previously for Chief oats (Price and Parsons, 1975). Oats have lower amounts of the polyunsaturated fatty acids, linoleic and linolenic, than barley (Table III) and (Price and Parsons, 1975) and are higher in saturated palmitic acid. If the amount of unsaturated acid present can be directly related to oil quality, barley is superior to oats in this aspect.

The results of this study suggest that genetic improvement in the lipids of barley need only be quantitative as it relates to oats. The hull and bran–endosperm of barley contain less lipid than comparable fractions of oats. The barley hull is much thinner and lighter than the oat hull, so the opportunity for a substantial increase in percentage distribution in this fraction is severely limited. The barley kernel is heavier and has a larger bran–endosperm than that of oats. It is to this fraction that attention will be directed to achieving an increase in the lipid content of barley. Nuclear magnetic resonance spectroscopic (NMR) analyses (Price, unpublished data) of over 17 000 entries in the U.S. Department of Agricultural World Collection of Barley indicate that genetic increase in lipid content to 5% may be possible. If so, the caloric content and nutritional value of barley can be increased and its competitive stance with other feed grains improved.

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On Chemical Problems of the Mechanism of Action of the Chlorothiobenzamides

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Both the direct and the photoinduced oxidation reactions of chlorine-substituted thiobenzamides (**1b–3e**) have been investigated. Asymmetric chlorophenylthiadiazoles (**4b–4e**) were identified as the principal transformation products. Oxidation of 2,6-dichlorothiobenzamide (**1e**) with nitrous acid unexpectedly afforded the corresponding 2,6-dichlorophenyl mustard oil (**3e**), which has a strongly enhanced herbicidal action.

Chlorine-substituted benzoic acid derivatives are among the most important systemic-action leaf and root herbicides (Linser, 1956). 2,6-Dichlorothiobenzamide (**1e**) especially has achieved great importance because of its excellent biological properties. The mechanism of action of these compounds is attributed primarily to the rapid formation of the corresponding nitriles (Wegler, 1977), which are known to display a strong herbicidal activity. This also explains the stronger action of 2,6-dichlorothiobenzamide (**1e**) relative to 2,6-dichlorobenzamide. The present work attempts to characterize the possible biologically active transformation products with the aid of the direct and the photoinduced oxidation reactions of the thiobenzamides (**1a–1e**). Determination of the herbicidal activity of these compounds should provide information about whether perhaps products other than nitriles (**2a–2e**) are responsible for the activity of the thiobenzamides (**1a–1e**) in the environment.

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MATERIALS AND METHODS

Chemicals. Thiobenzamide (**1a**), *p*-chlorobenzamide, and *o*-chlorobenzonitrile (**2b**) were purchased from E. Merck, Germany, while 2,6-dichlorobenzonitrile (**2e**) was obtained from Ega-Chemie. *o*-Chlorothiobenzamide (**1b**), *m*-chlorothiobenzamide (**1c**), and 2,6-dichlorothiobenzamide (**1e**) were prepared from the corresponding nitriles (**2b** and **2e**) by reaction with hydrogen sulfide in pyridine, while *p*-chlorothiobenzamide was prepared from *p*-chlorobenzamide with phosphorus pentasulfide in toluene.

Preparation of the Thiadiazoles (4a–4d). Twenty milliliters of a 10% solution of hydrogen peroxide was added drop by drop to a stirring suspension of 4 g of the relevant thiobenzamide (**1a–1d**) in 100 mL of 5% HCl at room temperature. After an hour the yellow precipitate

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