that both the reduced zeins and ASG of all the grains studied must consist of proteins of mol wt 22 000 and 24 000. This fact supports the close relationship among corn, teosinte, and Trip. However, the variations among the amino acid compositions, electrophoretic patterns, and molecular weights of the albumins, zeins, ASG's and AIG's strongly suggest that teosintes are much more closely related to corn than to the Trip. Furthermore, the presence of more doublet bands in the polyacrylamide gel electrophoresis patterns of the corn zeins and ASG's compared to those of the teosinte might indicate more complex protein composition and, therefore, later evolutionary origin of corn. These observations are consistent with other genetic and morphological evidence that modern teosinte is closely related to the ancestor of corn but that *Tripsacum*, although a close relative, lies on a divergent evolutionary branch among the Tripsacinae.

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Carbohydrates, Polyphenols, and Lignin in Seed Hulls of Different Colors from Turnip Rapeseed

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The carbohydrates, polyphenols, and lignins in seed hulls from turnip rapeseed, *Brassica campestris*, of different colors and in hulls from white mustard, *Sinapis alba*, were studied. Twelve low molecular weight carbohydrates were identified of which eight were quantified. The amount of polysaccharides in the yellow hulls is higher and the amount of Klason lignin is lower than in the dark hulls. The relative carbohydrate compositions of the polysaccharides were rather similar. Oxidative degradation of kraft-cooked (heating with aqueous sodium hydroxide–sodium sulfide) samples (methylated and hexadeuteromethylated) showed that dark hulls have a high polyphenol content and light hulls a low content; lignin contents, however, are about the same.

Rapeseed (*Brassica napus* L.) and turnip rapeseed (*Brassica campestris* L.), the most important oilseed crops grown in Sweden, usually have dark hulls which form about 15–20% of the seeds. Recently, plant breeders have obtained yellow-hulled seeds which have higher oil and protein content and lower crude fiber content compared with dark-hulled seeds. They have thinner hulls and the cells, especially the palisade cells, are smaller and the embryos are heavier (Jönsson and Bengtsson, 1970; Stringam et al., 1974).

The dark-hulled seeds give a darker oil which needs to be decolorized before it is used for food products. Rapeseed meal obtained after oil extraction is today an important fodder product because of its high content of proteins and well-balanced amino acid composition. Rapeseed protein concentrate (Anjou and Fecske, 1974) and rapeseed protein isolate (Gillberg and Törnell, 1977) may be useful for human consumption. These are greyish when prepared from dark-hulled seeds but are lighter and more attractive when prepared from yellow-hulled seeds.

Polysaccharide fractions from rapeseed hulls have been studied (Aspinall, 1974) with paper chromatography (PC) and the structure of the pectin from rapeseed hulls has

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Table I. Fractionation of Turnip Rapeseed and White Mustard Hulls[a]

Cultivar	A	B	C	D	E
Brassica campestris					
Bele	3.3	4.5	1.1	2.0	1.5
Lute	3.7	4.1	2.6	2.5	2.5
SV. 72/60029	4.9	4.9	5.7	2.7	2.9
Sinapis alba					
Sv. 67/670	3.2	2.6	6.7	2.0	1.2

[a] A = chloroform fraction, water-soluble material: B = neutral fraction, C = basic fraction (as chlorides), D = acidic fraction, and E = benzene–ethanol (2:1) fraction. Yields are given as percent of the dry weight of hulls.

been investigated by Aspinall and Jiang (1974). A study on the carbohydrate constituents and the lignin content in hull and cotyledon fractions of rapeseed was reported recently (Tollier and Guilbot, 1974) and an investigation has been made of the chemical and physical properties of carbohydrate fractions of mustard and rapeseed hulls by Vose (1974). Apparently little is known about rapeseed lignin and polyphenols. Paper chromatography of alcoholic extracts of seed coats of *Brassica campestris*, both after acid and after alkaline hydrolysis, revealed the presence of several unknown phenolic acids, indicating the possibility that a condensed tannin occurs (Durkee, 1971).

To explain the nature of the chemical differences between dark and yellow hulls, we have now examined the carbohydrates, lignin, and polyphenols in dark, intermediately colored, and yellow hulls from turnip rapeseed and, for comparison, in seed hulls of white mustard.

EXPERIMENTAL SECTION

Plant Material. Turnip rapeseed (*Brassica campestris* L., summer type) of the cultivars Bele (dark hulls), Lute (60% yellow hulls), and Sv. 72/60029 (yellow hulls) and white mustard (*Sinapis alba* L.) of the cultivar Sv. 67/670 (yellow hulls) were grown under similar conditions and obtained from the Swedish Seed Association, Svalöv. Hulls were separated from the embryos by hand after crushing the seeds in a mortar and milled.

Extractions. The hulls (3.00 g) were extracted with 80% ethanol in order to remove low molecular weight substances and the extracts, after evaporation, were separated into chloroform-soluble material (A) and water-soluble material, and then further fractionated into neutral (B), basic (C, as chlorides), and acidic (D) fractions as described earlier (Theander and Åman, 1976). Only the B fractions were studied further, but the increase of the basic fraction (C) from the dark to yellow hulls (including the white mustard) is notable. The residues were extracted with benzene–ethanol (2:1) (v/v, 4 × 25 ml, 40 min) on an ultrasonic bath to remove the remaining lipophilic material. The results are presented in Table I.

General Methods. Concentrations were performed at reduced pressure at bath temperatures not exceeding 40 °C. Paper chromatograms, using the systems (v/v) (a) ethyl acetate–acetic acid–water (3:1:1) and (b) pyridine–ethyl acetate–water (2:8:1), and paper electrophoresis, using 0.5 N sodium acetate–acetic acid buffer (pH 4.5) at 1500 V for 2 h, were run on Whatman No. 1 papers. The components were detected with *p*-anisidine hydrochloride, silver nitrate–sodium hydroxide, or resorcinol–hydrochloric acid.

Carbohydrates and Klason Lignin Analysis. Low molecular weight carbohydrates in the neutral fraction (B) were analyzed quantitatively by GLC (Table II) as trimethylsilyl ethers as previously described (Theander and Åman, 1976). The components were confirmed with PC (solvent a). All monomeric compounds were further identified with GLC–MS as trimethylsilyl ethers, and glycerol, arabinitol, galactitol, and *myo*-inositol, also as alditol acetates (Lonngren and Svensson, 1974).

Table II. Low Molecular Weight Carbohydrates in Turnip Rapeseed and White Mustard Hulls[a]

Carbohydrate	Turnip rapeseed cultivars			White mustard
	Bele	Lute	Sv. 72/60029	Sv. 67/670
Arabinitol[b]	0.01	0.01	0.01	0.01
Fructose[b]	0.08	0.07	0.06	0.11
Glucose[b]	0.06	0.05	0.05	0.08
Galactitol[b]	0.02	0.01	Trace	0.08
myo-Inositol[b]	0.01	0.01	0.01	0.01
Sucrose[c]	2.85	2.33	2.81	1.22
Raffinose[c]	0.16	0.13	0.08	0.02
Stachyose[c]	0.45	0.50	0.41	0.18
Σ low mol wt carbohydrates	3.66	3.11	3.43	1.64

[a] Yields are given as percent of the dry weight of hulls. [b] Determined on a 3% OV-1 column. [c] Determined on a 3% OV-17 column.

Table III. Carbohydrate and Klason Lignin Content in Turnip Rapeseed and White Mustard Hulls

	Turnip rapeseed cultivars			White mustard
	Bele	Lute	Sv. 72/60029	Sv. 67/670
Carbohydrate constituents[a]	26.0	33.1	38.1	38.4
Rhamnose[b]	2.5	2.1	3.9	4.0
Fucose[b]	1.5	1.0	1.9	1.0
Arabinose[b]	32.4	33.1	39.9	25.4
Xylose[b]	8.0	8.2	9.8	7.0
Mannose[b]	3.6	3.0	2.9	5.4
Galactose[b]	12.6	9.8	6.9	17.9
Glucose[b]	39.5	42.8	34.7	39.3
Klason lignin[a]	36.1	17.8	7.9	7.9

[a] Given as percent of dry, 80% ethanol extracted and benzene–ethanol (2:1) extracted hulls. Uronic acids have not been quantified. [b] Given as relative percent of neutral carbohydrates in extracted hulls after hydrolysis.

The polysaccharides in the benzene–ethanol extracted residue (1.0 g) were hydrolyzed (treatment in 12.0 M H_2SO_4 at room temperature for 2 h and reflux for 6 h after dilution to 0.358 M H_2SO_4), neutralized, reduced, acetylated, and analyzed for their neutral carbohydrates as alditol acetates with GLC and for Klason lignin (Bethge et al., 1971). The neutral carbohydrates in the hydrolysate were also confirmed with PC (solvent b). The results are presented in Table III. The uronic acids in the hydrolysate were analyzed with paper electrophoresis and PC (solvent a).

Lignin and Polyphenol Analysis. Oxidative characterization was done as described by Erickson et al. (1973a). This method comprises an alkaline degradation of the extracted plant material (heating with aqueous sodium hydroxide–sodium sulfide, "kraft cook"), methylation of the degradation products with dimethyl sulfate, and a two-step oxidative degradation (alkaline permanganate–periodate; alkaline hydrogen peroxide). The resulting aromatic carboxylic acids are methylated with diazomethane and the major esters are determined by GLC.

Methylation with hexadeuteriodimethyl sulfate (Merck) was carried out as described for dimethyl sulfate (Erickson et al., 1973a). The relative abundances of the trideuterio-

Table IV. Methyl Esters Obtained by Oxidative Degradation of Kraft-Cooked Turnip Rapeseed and White Mustard Hulls[a]

Methyl esters	Turnip rapeseed cultivars						White mustard
	Bele		Lute		Sv. 72/60029		Sv. 67/670
1	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
2	9.4	10.1	7.2	6.1	3.7	3.9	2.7
3	1.6	1.7	2.4	1.7	2.1	2.7	<1.1
4	1.1	1.0	1.0	0.8	<0.8	<0.9	<0.4
5	3.7	3.5	2.3	2.0	<0.3	<0.3	<0.3
6	0.3	0.3	0.2	0.3	0.2	0.2	0.2
7	0.6	0.6	0.4	0.5	0.5	0.5	0.4
8	<0.3	<0.3	<0.3	<0.1	<0.2	<0.2	<0.2

[a] Yields in milligrams per gram of plant material.

and hexadeuteriomethyl derivatives were measured on respective molecular ions recorded at 70 eV, accelerating voltage 3000 V, in a gas chromatograph–mass spectrometer equipped with a Becker-Ryhage separator. Temperatures used were: separator, 270 °C; source, 150 °C. Gas chromatographic conditions are as described under oxidative degradation (Erickson et al., 1973a).

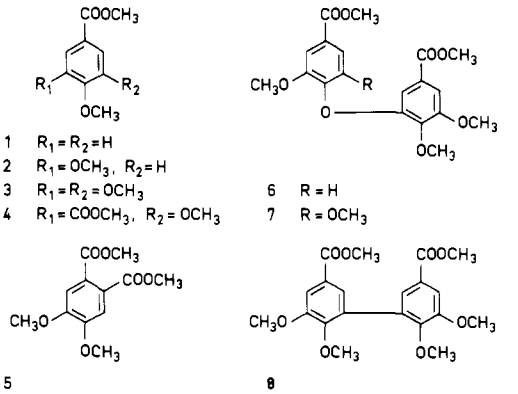
RESULTS AND DISCUSSION

Carbohydrates. Sucrose (2.3–2.9%) and stachyose (0.41–0.50%) are the predominant low-molecular carbohydrates in the neutral fraction (B) from turnip rapeseed hulls. There were varying amounts (0.05–0.16%) of raffinose, fructose, and glucose, mostly about 0.01% of arabinitol, galactitol, and *myo*-inositol, and traces of galactose, galactinol (D-galactosyl-*myo*-inositol), and digalactosylglycerol. Glycerol was also detected in all extracts but since glycerol tris(trimethylsilyl)ether is very volatile, quantitative determination was not attempted. All these low molecular weight carbohydrates in turnip rapeseed hulls (3.1–3.7%) except glycerol and arabinitol have been found previously in higher yields in the meal (Theander and Åman, 1976). The same low molecular weight carbohydrates were found in mustard seed hulls but generally in lower yields. The results are summarized in Table II.

In the hydrolysate of benzene–ethanol extracted rapeseed hulls there was an increase in the content of neutral carbohydrates, which contained arabinose, xylose, galactose, and glucose as main constituents, as the color diminished (Table III), but the relative compositions of the neutral carbohydrate constituents in the hydrolysates were similar. White mustard gave amounts of neutral carbohydrates in the hydrolysate similar to those obtained from yellow hulls of turnip rapeseed, but a greater relative content of galactose and a lesser amount of arabinose. Relatively large amounts of galacturonic acid and small amounts of glucuronic acid were detected by paper electrophoresis and PC in both turnip rapeseed and white mustard hulls.

Lignin and Polyphenols. The values for Klason lignin presented in Table III suggest a much higher lignin content in the hulls of the darker cultivars than in Sv. 72/60029 or in white mustard. However, the Klason method for the determination of lignin can lead to erroneous results. Materials other than lignin may remain as insoluble residues after the hydrolysis of carbohydrates with mineral acid. Oxidative characterization provides a better estimate of the amount of lignin present (Erickson et al., 1973a).

The results of the oxidative degradation applied to turnip rapeseed and white mustard hulls are presented in Table IV. Total ester yields are considerably higher for the darker cultivars (Bele and Lute) than for the lighter (Sv. 72/60029) and the reference white mustard. This is in agreement with the Klason lignin values given in Table III. A closer examination shows, however, that yields of methyl tri-*O*-methylgallate (**3**) and the dimeric esters **6–8** are similar for all samples, while yields for methyl veratrate (**2**) and dimethyl metahemipate (**5**) are considerably increased with the dark hulls. It must be noted that in the degradation of gymnosperm (Erickson and Miksche, 1974a) and angiosperm (Erickson et al., 1973b,c) lignins the yield of **5** is always small compared with that of **2** and **7**. High relative yields of **5** are specific for materials containing polyphenols in addition to lignin (Andersson et al., 1973). Polyphenols also give substantial yields of **2**, but none of the dimeric esters **6–8**.



With the exception of ester **1** (methyl *p*-anisate), the degradation products arising from lignin and polyphenols can be differentiated since the aromatic nuclei of lignins contain one (guaiacylpropane units) or two (syringylpropane units) methoxyl groups, while the nuclei in polyphenols giving aromatic carboxylic acids are alkyl-substituted catechols. When the methylation preceding oxidative degradation is carried out with hexadeuteriodimethyl sulfate, an incorporation of two trideuteriomethyl groups can be expected for catechol structures while only one trideuteriomethyl group will be found in guaiacyl structures or syringyl structures. Oxidative degradation of a trideuteriomethylated sample containing both lignin and polyphenols should thus give esters with one trideuteriomethyl group from the lignin constituent and esters with two trideuteriomethyl groups from the polyphenol constituent. The relative proportions of the trideutero and hexadeutero compounds may then be determined by MS.

This method (cf. Erickson and Miksche, 1974b,c) was applied to the present problem. The abundance of hexadeuterio-**2**, -**3**, -**4**, and -**5** in mole percent of total ester (tri- plus hexadeutero) is shown in Table V. Esters **6** and **8** were found to contain only one trideuteriomethyl group, while ester **7** had two such groups; they are thus solely derived from lignin.

These results enabled yields for esters **2–5** to be recalculated as lignin derived and polyphenol derived (Table VI). In accordance with the preliminary assessment of Table IV referred to above, it is evident that Bele and Lute

Table V. Methyl Esters 2, 3, 4, and 5 Derived from Catechol Units (Mole Percent of Total Yields)

Methyl esters	Turnip rapeseed cultivars		
	Bele	Lute	Sv. 72/60029
2	81	66	7
3	3	7	3
4	51	35	4
5	94	91	44

Table VI. Methyl Esters 2, 3, 4, and 5 Derived from Polyphenols and Lignin[a]

Methyl esters	Turnip rapeseed cultivars		
	Bele	Lute	Sv. 72/60029
(A) From Lignin			
2	1.9	2.3	3.5
3	1.6	2.0	2.3
4	0.5	0.6	0.9
5	0.2	0.2	0.2
(B) From Polyphenols			
2	7.9	4.4	0.3
3	0.1	0.1	0.1
4	0.6	0.3	0
5	3.4	2.0	0.1

[a] Yields in milligrams per gram of plant material.

cultivars have high contents of polyphenols, Bele containing nearly twice as much as Lute (esters **2**, **4**, and **5**). The low yield of **3** indicates negligible amounts of hydrolyzable tannins (yielding gallic acid on hydrolysis). Very little tri-*O*-methyl gallate (**3**) is derived from *O*-methoxycatechol structures and this material may have been formed by sulfide ion promoted cleavage of some aromatic methoxyl groups during the alkaline hydrolysis preceding oxidative degradation (Sarkanen et al., 1963). White mustard and Sv. 72/60029 hulls contain very little polyphenols. The lignin content shows a moderate increase going in the opposite direction (dark → yellow).

The major chemical difference between yellow-hulled and dark-hulled seeds is thus in the polyphenol (condensed type) content.

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d-α-Tocopheryl Poly(ethylene glycol) 1000 Succinate. Acute Toxicity, Subchronic Feeding, Reproduction, and Teratologic Studies in the Rat

Walter J. Krasavage* and Clarence J. Terhaar

The acute oral LD_{50} of *d*-α-tocopheryl poly(ethylene glycol) 1000 succinate (TPGS) and either of its moieties, poly(ethylene glycol) 1000 and *d*-α-tocopheryl acid succinate, is >7000 mg/kg for young adult rats of both sexes. When tested in 2-day old neonates, mechanical injury at the time of gavage made calculation of an LD_{50} impractical. However, the data did indicate that TPGS is no more toxic than the materials from which it was made and is somewhat less toxic in female rats than in males. When incorporated in the diet and fed for 90 days in concentrations of 0.002, 0.2, and 2.0% to male and female rats, TPGS had no adverse effects on body weight gain, food consumption, hematology, organ weights, serum chemistries, or micromorphology. When these animals were bred for two litters of a one-generation reproduction study, the reproductive indexes of the treated groups were unaffected and the offspring developed normally. A separate teratology study in which pregnant females ingested 0.002, 0.2, and 2.0% TPGS in the diet during the period of organogenesis revealed no congenital abnormalities attributable to TPGS.

d-α-Tocopheryl poly(ethylene glycol) 1000 succinate (Eastman Vitamin E TPGS) (TPGS) is a water-soluble form of vitamin E prepared from crystalline *d*-α-tocopheryl acid succinate by esterification of the acid group with poly(ethylene glycol) having an average molecular weight of 1000. It is a pale yellow waxy substance which provides 260 mg of *d*-α-tocopherol per g (387 IU) and it forms a

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