Based on the results from the aforementioned experiments, a reaction mechanism is thus proposed using sterculic acid as a substrate (Fig. 8). The endogeneous trace amount of chlorophyll acts as a photosensitizer to produce singlet oxygen (13). The singlet oxygen then attacks the cyclopropenoid fatty acid, such as sterculic acid, followed by degradative cleavage and molecular rearrangement as commonly seen in an organic reaction (14) and leads to 1-decyne formation. Other compounds were also formed and are identified as shown in Figure 8.

ACKNOWLEDGMENTS

Ken Hartman and Bob Longan provided information on 1-decyne and CPFA, respectively. Lynn Warren, Gail Womack, Michael Ma and David Duval provided technical assistance. John Fulcher helped to elucidate the mechanism, and George Pucak reviewed this manuscript.

REFERENCES

1. Deutschman, A.J., Jr., and I.S. Klaus, Anal. Chem. 32: 1809 (1960).
2. Official Methods of Analysis, 12th edn., Assoc. Off. Anal. Chem., Washington, DC, 1975, Method 3.107.
3. Ibid., Tentative Method Cd 8-5.
4. Smouse, T.H., B.D. Mookherjee and S.S. Chang, Chem. Ind. July: 1301 (1965).
5. Halphen, G., Analyst 22:326 (1897).
6. Fan, L.L., W.H. Barney and A. Wohlman, J. Food Protection 45:48 (1982).
7. Zarins, Z.M., R.K. Willich and R.O. Feuge, JAOCS 47:215 (1970).
8. Rayner, L.T., L.E. Brown and H.P. Dupuy, JAOCS 43:113 (1966).
9. Shenstone, F.S., and J.R. Vickery, Nature 190:168 (1961).
10. Hile, J.P., Fed. Reg. 43:43556 (1978).
11. Fan, L.L., A. Wohlman, B.J. Longan and J.Y. Tang, U.S. Patent 4,283,437 (1981).
12. Jefford, C.W., and C.G. Rimbault, ACS 100:20 (1978).
13. Chan, H.W.S., JAOCS 54:100 (1976).
14. Frimer, A.A., and A. Abraham, J. Org. Chem. 45:2334 (1980).

[Received November 4, 1982]

Phenolic Acids in Rapeseed and Mustard

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ABSTRACT

The compositions of free phenolic acids in rapeseed flours of diverse origin and white mustard were highly variable but represented less than 10% of the total phenolic acids. Phenolic acids released from hydrolysis of soluble esters constituted the major fraction, with Polish varieties having higher levels than a Canadian variety or white mustard. Yellow Sarson contained low levels of phenolic acids. Sinapic acid isomers constituted over 94% of the 13 phenolic acids found in the rapeseed varieties. Only traces of several phenolic acids appeared to be structurally bound to rapeseed and mustard proteins and carbohydrates.

INTRODUCTION

Phenolic acids and their derivatives are commonly occurring compounds in the plant world. Their presence in seeds causes a deterioration in the taste, odor and color of protein concentrates and prepared food products. Besides unfavorable organoleptic changes, oxidized phenolic compounds can bind with essential amino acids such as lysine or methionine, forming complexes which are unassimilable in the digestive tract of animals and man (1-3).

Preliminary investigations have demonstrated that rapeseed contained a wide range of phenolic acids (4-8). While sinapic acid, as a component of sinapine, was the major phenolic acid, the quantities and presence of other phenolic acids differed among these studies, depending on the method of analysis. Sosulski et al. (9) fractionated the phenolic constituents in canola flour into free phenolic acids, soluble esters and glycosides of phenolic acids, and insoluble-bound phenolic compounds. Phenolic acids in bound forms were released by acidic, alkaline and enzymatic hydrolysis prior to quantitation by gas liquid chromatography (GLC). Krygier et al. (10) refined these procedures by removing fatty acids and other contaminants from the hydrolysates and used thin layer chromatography (TLC), GLC and GLC-MS (mass spectrometry) to identify the major and minor phenolic constituents in each fraction. The flours of three cultivars were found to contain 6-98 mg/100 g of free phenolic acids, 768-1196 mg/100 g of phenolic acids from hydrolyzed esters and no phenolic acids in the residues (11). Sinapic acid represented 99% of the esterified phenolic compounds, minor components being *p*-hydroxybenzoic, vanillic, gentisic, protocatechuic, syringic, *p*-coumaric and ferulic acids. The *trans* isomers predominated but the *cis* forms of sinapic and ferulic acids occurred in most fractions.

The objective of the present investigation was to determine the phenolic composition of a wide range of rapeseed varieties as well as white mustard. The extraction procedure was modified to remove the residual lipids from the flours, and extracts were further purified by extraction with diethyl ether and monosodium carbonate.

MATERIALS AND METHODS

The varieties used in the present study were selected to represent a range in growth habit, seed color, erucic acid and glucosinolate levels (Table 1). The Polish varieties were grown at the Institute of Plant Breeding and Acclimatization, Pozen, Poland, and the remaining samples were supplied by the Department of Crop Science, Saskatoon, Canada. The intact glucosinolate content was determined by glucose release after enzymatic hydrolysis.

Flours for investigation of phenolic acids were prepared

TABLE I

Characteristics of the Rapeseed and Mustard Varieties

Species and variety	Origin	Growth habit	Seed coat color	Erucic acid (% of total fatty acids)	Total glucosinolates (mg/g flour)
Brassica napus					
Górczański	Poland	Winter	Dark	54.0	18.0
Bronowski	Poland	Summer	Dark	10.0	0.4
Start	Poland	Winter	Dark	1.5	3.0
Brassica campestris					
Candle	Canada	Summer	Brown-yellow	2.6	2.0
Yellow Sarson	India	Summer	Yellow	56.0	20.0
Brassica hirta (Sinapis alba)					
White mustard	–	Summer	Yellow	41.3	3.0

by defatting the seeds with hexane in a Soxhlet apparatus and then separating the hulls by differential sedimentation in pure hexane before desolventization. The flours, after removal of polar fat with trichloroethylene (TRI), were ground to a particle size of less than 0.25 mm.

The phenolic acids were extracted and fractionated by the modified Sosulski et al. (9) procedure illustrated in Figure 1. The defatted sample was extracted with hot 80% methanol until the extract failed to react with the Folin-Denis reagent. The extract was then evaporated to 50 mL under vacuum at 40 C in the presence of nitrogen, acidified with 1 N NCl to pH 2, centrifuged, and the clear supernatant was transferred to a continuous liquid-liquid extractor. Extraction with diethyl ether was carried out for 24 hr. The ether extract fraction containing free phenolic acids (F-1) was evaporated to dryness. The aqueous solution remaining after extracting free phenolic acids was hydrolyzed with 2 N NaOH for 4 hr at room temperature under nitrogen. The hydrolysate was then acidified to pH 2, centrifuged, and the phenolic acids were extracted continuously with diethyl ether for 24 hr. The obtained extract contained phenolic acids liberated from ester compounds (F-2). Alkaline hydrolysis of the dry solids after methanol extraction was accomplished in the same way. Bound phenolic acids (F-3) were separated from the hydrolysate by continuous extraction with diethyl ether as described above.

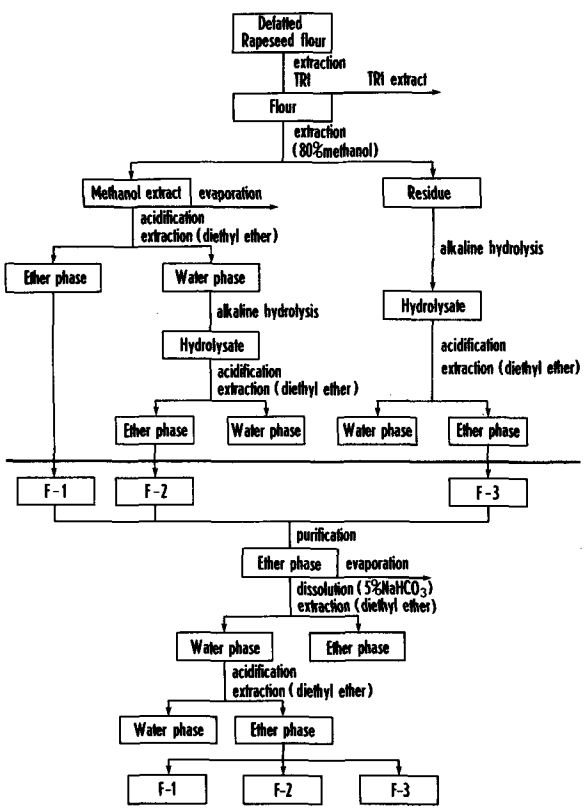

FIG. 1. Procedure for the fractionation of free, esterified and bound phenolic compounds and their hydrolysis to free phenolic acids.

Dry residues remaining after ether removal (F-1, F-2, F-3) were dissolved in 5% $NaHCO_3$ (pH 8), and extracted with diethyl ether, as described above. Ether extract did not contain any phenolic compounds but fatty substances were present. After removal of lipids, the water phase was acidified to pH 2.0 and extracted again with diethyl ether in order to separate phenolic acids.

Chromatographic analyses were made according to the procedure of Morita (12). An aliquot of solution was taken from each fraction, transferred into vials and evaporated to dryness under nitrogen. One hundred microliters of *N, O-bis* (trimethylsilyl) acetamide (Pierce Chemical Co.) were added to each vial, and the sample was allowed to react at room temperature for 24 hr.

Trimethylsilyl ethers of phenolic acids were chromatographed in a Pye Unicam gas chromatograph equipped with flame ionization detector. The 1.5 m X 0.4 cm glass column was packed with 1.5% SE-30 on 80-100 mesh Chromosorb W/HP. The flow rate for the carrier gas, nitrogen, was 60 cm^3/min, the temperatures of the detector and column were 280 C and 98-260 C, respectively, Δt being 6 C/min.

Phenolic acids were identified by comparing the retention times of the TMS derivatives with the values for retention times of TMS derivatives of standard acids. N-tetracosane was used as an internal standard. The contents of the phenolic acids are expressed as mg/100 g of flour on a dry weight basis.

RESULTS

Total content of free phenolic acids in rapeseed flours of the varieties Bronowski, Górczánski, Start and Candle was very similar (Table II), and ranged from 71.8 mg/100 g in Start flour to 59.9 mg/100 g in Bronowski flour. Considerably lower contents of free phenolic acids were found in Yellow Sarson flour and white mustard, in which the

TABLE II

Free Phenolic Acids in Flours (mg/100 g flour)

Phenolic acids	Rapeseed varieties					White Mustard
	Bronowski	Górczański	Start	Candle	Yellow Sarson	
Salicylic	1.5	0.5	1.5	3.1	0.1	tr
Cinnamic	1.0	0.5	0.8	tr	0.2	tr
p-Hydroxybenzoic	0.6	0.6	2.2	0.0	0.5	9.1
Vanillic	0.3	0.5	0.6	tr	0.9	0.5
Gentisic	0.2	0.7	0.8	0.2	tr	0.1
o-Coumaric	0.3	0.6	0.9	1.1	0.9	0.8
Protocatechuic	0.5	0.7	1.4	0.4	0.5	tr
Syringic	0.3	1.8	2.4	0.1	0.3	0.7
p-Coumaric	0.5	tr	3.0	tr	0.6	1.1
cis-Sinapic	5.8	9.7	10.1	10.1	3.2	tr
trans-Ferulic	1.8	3.2	4.7	0.9	1.2	2.1
trans-Caffeic	0.6	tr	1.8	tr	tr	3.1
trans-Sinapic	46.5	41.3	41.6	51.6	3.5	4.5
Total	59.9	60.1	71.8	67.5	11.9	22.0

TABLE III

Phenolic Acids Liberated from Esters (mg/100 g flour)

Phenolic acids	Rapeseed varieties					White Mustard
	Bronowski	Górczański	Start	Candle	Yellow Sarson	
Salicylic	1.0	0.7	0.9	1.0	0.9	2.5
p-Hydroxybenzoic	2.7	2.1	2.1	tr	tr	328.0
Vanillic	1.2	1.1	tr	0.5	0.9	1.7
Gentisic	0.2	0.1	tr	0.5	0.9	2.9
Protocatechuic	1.8	0.4	1.0	tr	tr	3.2
Syringic	2.3	1.8	tr	1.0	1.4	1.8
p-Coumaric	tr	tr	tr	0.8	0.8	1.5
cis-Caffeic	tr	2.1	tr	tr	tr	0.1
cis-Sinapic	63.9	68.3	95.8	98.9	44.5	0.0
trans-Ferulic	7.9	5.4	3.1	0.8	1.7	0.6
trans-Caffeic	tr	0.0	tr	tr	tr	22.5
trans-Sinapic	477.5	492.0	597.1	416.4	171.3	212.1
Total	558.6	574.0	700.0	520.0	222.4	576.9

TABLE IV

Phenolic Acids Liberated From Insoluble Residue (mg/100 g flour)

Phenolic acids	Rapeseed varieties					White Mustard
	Bronowski	Górczański	Start	Candle	Yellow Sarson	
Salicylic	0.3	0.2	0.1	0.2	0.2	0.1
p-Hydroxybenzoic	tr	0.0	0.0	tr	tr	1.1
Vanillic	0.1	0.0	tr	tr	tr	0.1
o-Coumaric	0.4	0.3	0.3	0.3	0.3	0.2
Syringic	0.2	0.1	0.2	0.1	0.1	0.1
p-Coumaric	0.7	0.8	0.8	0.7	0.7	0.3
cis-Sinapic	0.7	0.7	0.7	0.6	0.6	0.5
trans-Ferulic	0.9	0.8	0.9	0.5	0.5	0.4
trans-Sinapic	1.7	2.0	1.7	2.0	0.7	0.5
Total	5.0	4.9	4.7	4.4	3.1	3.3

TABLE V

Free, Liberated from Esters and Insoluble Residue Phenolic Acids in Rapeseed and Mustard Flour (mg/100g flour)

Phenolic acids	Rapeseed varieties					White mustard
	Bronowski	Górczański	Start	Candle	Yellow Sarson	
Free	59.9	60.1	71.8	67.5	11.9	20.0
Liberated from esters	558.6	574.0	700.0	520.0	222.4	576.9
Liberated from insoluble residue	5.0	4.9	4.7	4.4	3.1	3.3
Total	623.5	639.0	776.5	591.9	237.4	602.2

TABLE VI

Sinapic Acids in Rapeseed and Mustard Flours

Phenolic acids	Rapeseed varieties								White mustard	
	Bronowski		Górczański		Start		Candle		Yellow Sarson	
	(mg/100g)	(% total)	(mg/100g)	(% total)	(mg/100g)	(% total)	(mg/100g)	(% total)	(mg/100g)	(% total)
Free	52.3	87.3	51.0	84.8	51.7	72.0	61.7	91.4	6.7	56.3	4.5	20.4
Liberated from esters	541.5	96.9	560.3	97.6	692.9	99.0	515.4	99.1	215.8	97.0	212.1	36.8
Liberated from insoluble residue	2.4	48.0	2.7	55.1	2.4	51.1	2.6	59.1	1.3	41.9	1.0	30.0
Total	596.2	95.6	614.0	96.0	747.0	96.2	579.7	97.9	223.8	94.3	217.6	36.1

respective levels were 11.9 and 22.0 mg/100 g. Thirteen phenolic acids were found in all flours under study. They were predominated by the isomeric forms of sinapic acid (*trans* and *cis*) and *trans*-ferulic acid. The results for Candle and Yellow Sarson flours were similar to those reported by Krygier et al. (11).

Alkaline hydrolysis released twelve phenolic acids from ester linkages (Table III). The sinapic acid isomers were the predominant phenolic compounds in the rapeseed cultivars, representing over 95% of the total released acids. White mustard contained more *p*-hydroxybenzoic acid than *trans*-sinapic acid. Most of the other phenolic acids occurred in low concentrations, although significant quantities of ferulic acid were found in the Polish varieties.

The total contents of phenolic acids released from soluble esters was about ten times greater than was obtained in the free phenolic acid fraction (Tables II and III). The totals in the Polish varieties, Candle and white mustard ranged between 520 and 700 mg/100 g, which was about one-half the levels obtained by Krygier et al. (11) for Candle and Tower rapeseed. In this study, Yellow Sarson contained only 222 mg whereas Krygier et al. (11) found 768 mg/100 g flour for this variety. In the present study the purification steps were substantially greater than those applied by Krygier et al. (11), which may account for some of the difference.

The quantities of phenolic acids released from the residue insoluble in 80% methyl alcohol were low, ranging from 3.2 mg in Yellow Sarson to 5.0 mg/100 g in Górczański rapeseed flour (Table IV). Only nine phenolic acids were identified; sinapic acids predominated but the contents of *p*-coumaric and *trans*-ferulic acids were also significant.

The total contents of phenolic acids in the flours under study were similar in the Polish and Canadian varieties and white mustard (Table V). Only Yellow Sarson exhibited a substantially lower level of total phenolic acids. However, both white mustard and Yellow Sarson were low in sinapic acid content.

Based on several determinations, the standard deviations for total sinapic acid content of the flours were less than 10% of the means but the precision of the analytical procedures were much less for the minor phenolic acids. When consideration is given to the wide difference in the present values for Candle and Yellow Sarson and those reported by Krygier et al. (11), it is evident that much additional work is needed to improve and standardize the analytical procedures.

DISCUSSION

The isomeric forms of sinapic acid constituted 56 to 91% of the free phenolic acids and over 97% of the ester-bound phenolic acids in the *Brassica napus* and *campestris* cultivars (Table VI). The proportions of sinapic acids liberated from the insoluble residue represented 42-59% of the total acids. In white mustard, *p*-hydroxybenzoic acid was the major phenolic acid and sinapic acids represented only 36% of the total.

Sinapic acid occurs primarily as the choline ester, sinapine, in *Brassica* species (13), and is considered to be the principal cause of the bitter taste of rapeseed meal (14,15). The high levels but wide range in composition of the sinapic acid esters (216-692 mg/100 g flour) among the rapeseed flours indicate the need, and possibility, for reduction in the concentrations of the bitter component by plant breeding. In view of the results on sinapic acid content obtained with Yellow Sarson and white mustard flours, it appeared that there was a connection between sinapine content and

color of the seed coat. This result was not clearly demonstrated with the Candle variety which had been selected for its lighter seed coat color (Table I) but sinapic content was only slightly less than the other rapeseed cultivars.

Most of the phenolic acids were found in the free or esterified form in the rapeseed and mustard flours. The relative levels of phenolic acids released by hydrolysis of the residues were quite low. These insoluble acids were presumably bound to proteins or carbohydrates and could be released during subsequent processing or cooking to contribute adverse flavors or colors to food products. However, their low concentrations would suggest that solvent extraction procedures, such as those employed to produce protein concentrates and isolates, could be devised to remove most of the phenolic compounds present in the flours.

REFERENCES

1. Davies, A.M.C., V.K. Newby and R.L.M. Synge, J. Sci. Food Agric. 29: 33 (1968).
2. Subba Rau, B.H., K.V.R. Ramana and N. Singh, Ibid. 23: 233 (1972).
3. Van Sumere, C.F., T. Albrecht, A. Dedonder, H. de Pooter and I. Pe, in The Chemistry and Biochemistry of Plant Proteins, edited by T.B. Harborne and C.V. Van Sumere, Academic Press, London, 1975, p. 211.
4. Durkee, A.B., Phytochemistry 10: 1583 (1971).
5. Durkee, A.B., and J.B. Harborne, Ibid. 12: 1085 (1973).
6. Durkee, A.B., and P.A. Thivierge, J. Food Sci. 40: 820 (1975).
7. Kozlowska, H., M.A. Sabir, F.W. Sosulski and E. Coxworth, Can. Inst. Food Sci. Technol. J. 8: 160 (1975).
8. Rotkiewicz, D., H. Kozlowska and L. Swiatek, Hodowla, Aklimatyzacja i Nasiennictwo 20: 448 (1976).
9. Sosulski, F., R. Zadernowski and H. Kozkowska, in Analytical Chemistry of Rapeseed and Its Products, edited by J.K. Daun, D.I. McGregor and E.E. McGregor, Canola Council of Canada, Winnipeg, 1982, p. 153.
10. Krygier, K., F. Sosulski and L. Hogge, J. Agric. Food Chem. 30: 330 (1982).
11. Krygier, K., F. Sosulski and L. Hogge, Ibid. 30: 334 (1982).
12. Morita, H., J. Chromatogr. 71: 149 (1972).
13. Hegnauer, R., Chemotaxonomie Der Pflanzen, Band III, Dicotyledonae, I. Teil von Acanthaceae bis Cyrillacea, Birknauser Verlag Basel und Stuttgart, 1964.
14. Austin, F.L., and I.A. Wolff, J. Agric. Food Chem. 16: 132 (1968).
15. Clandinin, D.R., Poultry Sci. 40: 484 (1961).

[Received June 21, 1982]

Polymorphism and Transformation Energetics of Saturated Monoacid Triglycerides from Differential Scanning Calorimetry and Theoretical Modeling[1]

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ABSTRACT

Differential scanning calorimetry studies on saturated monoacid triglycerides were extended to include most odd and even chain lengths from tricaprylin (C_8) through tritriacontanoin (C_{30}). Two β'-forms were common with triglycerides C_{15} through C_{24}: shorter odd chain length triglycerides (C_9-C_{13}) exhibited only one β'-form; short even chain length triglycerides (C_8-C_{14}) exhibited three. Odd chain length C_{21} and C_{23} triglycerides showed two β-forms. Triglycerides of even chain lengths greater than C_{21} produced two α-forms. Apparent energies of phase excitation for α-form transformations (determined from scans at different heating rates) showed odd-even alternation for short chain lengths, but increased linearly with chain length above C_{14}, evidencing the importance of extended chain conformation and interactions as determinants of polymorph properties. Changes in melting point patterns, particularly for β' and β-forms, at C_{14} correlated with the change in apparent phase excitation energy. Comparisons of X-ray data with dimensions from space-filling models and agreement between observed entropies of fusion and values calculated for probabilistic models also emphasize the importance of extended chain conformation and suggest configurational differences, and possibly different polymorph conversion pathways for odd versus even chain length triglycerides.

[1]Presented in part at AOCS Meeting, Philadelphia, PA, September 1974.

INTRODUCTION

Nearly a half-century after Malkin (1) demonstrated that the multiple melting behavior of triglycerides is due to polymorphism, controversy still prevails regarding the structures of possible crystalline phases (2) and the mechanisms for transformation from one phase to another (3). Odd and even chain length saturated monoacid triglycerides are generally known to occur in α-forms (lowest melting), β'-forms (intermediate melting) and β-forms (highest melting) that exhibit distinct X-ray patterns and characteristic physical properties (4, 5), but an unequivocal molecular conformation is known for only the β-form (6). Difficulties inherent in growing single crystals of long-chain triglycerides and the instability of many α- and β'-forms still preclude their thorough characterization.

In a previous differential scanning calorimetry (DSC) study (7) of monoacid triglycerides, two β' endotherms were reported for even chain length samples tripalmitin through tribehenin. At the time, this finding contrasted with earlier work (8–10) except, perhaps, that of Lutton and Fehl (5), who occasionally found "artifacts" during differential thermal analysis of trimyristin. Further examination of this multiple melting behavior by DSC analysis of an extended series, tricaprylin through tritriacontanoin for even chain lengths and tripelargonin through tritrico-