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GLUCOSINOLATE HYDROLYTIC PRODUCTS GIVEN BY *SINAPIS ALBA*, AND *BRASSICA NAPUS* THIOGLUCOSIDASES

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Key Word Index—*Sinapis alba*; *Brassica napus*; Cruciferae; mustard seed; rapeseed; thioglucosidase; glucosinolate; isothiocyanate; nitrile; goitrin.

Abstract—Thioglucosidase prepared from rapeseed (*Brassica napus* cvs Zephyr and Bronowski) showed one major band in polyacrylamide gel and a high susceptibility to ascorbic acid activation. In contrast thioglucosidase from mustard (*Sinapis alba*) produced two major bands and was relatively unresponsive to ascorbic acid. Irrespective of the source, the thioglucosidase when incubated with heated *B. napus* cv Zephyr substrate produced a similar pattern of aglucones, identified by GLC, although the absolute amounts of the aglucones produced were different.

INTRODUCTION

TOXICITY of rapeseed meal is attributed to the presence of glucosinolate in the meal along with an enzyme myrosinase, or β-thioglucosidase[1]. When the seed is crushed the released enzyme and glucosinolate come in contact and, in the presence of moisture, hydrolysis occurs giving various toxic products including isothiocyanates, vinyloxazolidine-thione (goitrin) and nitriles. The hydrolysis by endogenous enzyme (autolysis) can be prevented by dipping the seed in boiling water for 2–3 min[2] or otherwise heating to inactivate the enzyme before crushing. Therefore, production of hydrolytic products from the glucosinolates of heated seed or meal requires incubation with an active thioglucosidase preparation. While it would seem logical to use for this purpose an enzyme extracted from rapeseed itself, it is difficult to obtain concentrates of satisfactory activity from rapeseed and an enzyme concentrate prepared from yellow mustard seed (*Sinapis alba*) is commonly employed.

The type and proportions of the hydrolytic products are influenced by the conditions of the hydrolysis including source of enzyme, pH, temperature age of seed and other factors[3,4]. However, under the usual conditions of intermediate pH range and temperature, incubation of heated rapeseed meal with mustard seed enzyme gives a high yield of goitrin and a relatively low yield of nitriles, whereas autolysis of the unheated meal or seed gives the reverse pattern.

Although several explanations of this difference have been offered[3], it is not ruled out that the thioglucosidases in mustard seed and rapeseed may be basically different in their

[1] BELL, J. M. and BELZILE, R. J. (1965) *Can. Dept. Agric. Publication*, Ottawa, 1257.
[2] EAPEN, K. E., TAPE, N. W. and SIMS, R. P. A. (1969) *J. Am. Oil Chemists' Soc.* **46**, 52.
[3] VAN ETTEN, C. H., DAXENBICHLER, M. E. and WOLFF, I. A. (1969) *J. Agric. Food Chem.* **17**, 483.
[4] TOOKEY, H. L. and WOLFF, I. A. (1970) *Can. J. Biochem.* **48**, 1024.

catalytic function. Disc electrophoresis[5,6] has shown that Crucifereae seeds vary greatly in the number of thioglucosidase isoenzymes they contain and a DEAE-Sephadex procedure has separated four subunits from mustard seed thioglucosidase[7].

In the present study we have prepared enzyme concentrates from *Sinapis alba* and from two varieties of rapeseed, *Brassica napus* cv Zephyr and Bronowski. The electrophoretic mobility of these preparations was studied by disc gel electrophoresis on polyacrylamide gel, and a comparison was made of the hydrolytic products the enzyme yielded when they were incubated with rapeseed meal prepared from heated seed. In view of reports that ascorbic acid increases the activity of thioglucosidases[6,8,9] incubations were conducted both in the presence and absence of this reducing agent.

RESULTS AND DISCUSSION

Enzymes from the two rapeseeds each produced a single major band, whereas the enzyme from mustard seed produced two major bands, close together but distinctly separated. In all cases the bands appeared on the gel at approximately the same distance from the origin. Results are similar but not identical to those of Henderson and McEwen[6] who found two major bands for an enzyme prepared by acetone extraction from *B. napus* cv Turret.

The band pattern of the *B. napus* enzymes became clearly visible, following incubation with sinigrin, only in the presence of ascorbic acid, whereas the band pattern of *S. alba* enzyme became rapidly visible regardless of the presence or absence of ascorbic acid. Doubling the amount of enzyme or lengthening the incubation period did not appreciably increase the density of the bands.

On the basis of electrophoretic pattern and susceptibility to ascorbic acid activation, the enzymes prepared from rapeseed and mustard were clearly not identical. However, it cannot be said that these differences necessarily reflect differences in the catalytic function of the enzymes. As pointed out by Henderson and McEwen[6] the low activity of rapeseed enzyme preparations could be due to difficulty in extraction, low activity to sinigrin or the influence of naturally occurring activators or inhibitors present in the seed but which may or may not be present in the isolated enzyme preparations.

Table 1 compares the quantitative yield of the glucosinolate aglucone products from *B. napus* cv Zephyr by the three enzymes, both with and without the addition of ascorbic acid. Data from autolysis of the unheated meal are included for comparison. Generally the yield of hydrolytic products was increased by the addition of ascorbic acid with the exception that the amounts of butenyl and pentenyl isothiocyanates given by autolysis were decreased. This reduction did not quantitatively account for the increase which occurred in the other aglucone products.

The role of ascorbic acid is not entirely clear. Ohtsuru and Hata[10] suggested that ascorbic acid induces conformational changes in the enzyme, exposing amino acid groups which are particularly responsible for enzyme activity. Of particular note here is that yields of the various aglucones given by the mustard seed enzyme were considerably increased by addition of ascorbic acid to the incubation mixture. This suggests that routine analysis

[5] MacGibbon, D. B. and Allison, R. M. (1970) *Phytochem.* **9**, 541.
[6] Henderson, H. M. and McEwen, T. J. (1972) *Phytochem.* **11**, 3127.
[7] Ohtsuru, M. and Hata, T. (1972) *Agr. Biol. Chem.* **36**, 2495.
[8] Nagashima, Z. and Uchiyama, M. (1959) *Bull. Agr. Chem. Soc. Japan* **23**, 555.
[9] Tsuruo, I. and Hata, T. (1967) *Agr. Biol. Chem.* **31**, 27.
[10] Ohtsuru, M. and Hata, T. (1973) *Agr. Biol. Chem.* **37**, 269.

of heated rapeseed meal, which employs mustard seed enzyme without the addition of ascorbic acid, may underestimate the amounts of isothiocyanates, nitriles and goitrin available from extant glucosinolates in the meal.

TABLE 1. PRODUCTION OF AGLUCONES FROM THE GLUCOSINOLATES OF *Brassica napus* CV ZEPHYR*

Hydrolytic product	*B. napus* cv Zephyr thioglucosidase		*B. napus* cv Bronowski thioglucosidase		*S. alba* thioglucosidase		Autolysis‡	
	−AA†	+AA†	−AA	+AA	−AA	+AA	−AA	+AA
1-Cyano-2-hydroxy-3-butene [Unsaturated nitrile]	0·09	0·30	0·10	0·35	0·25	0·47	1·40	2·10
1-Cyano-2-hydroxy-3,4-epithiobutane [(threo)EpisulphideA]	ND	ND	ND	ND	ND	ND	1·20	2·20
1-Cyano-2-hydroxy-3-4-epithiobutane [(erythro)EpisulphideB]	ND	ND	ND	ND	ND	ND	2·15	3·50
3-Hydroxypent-4-enethioamide [Thionamide]	ND	ND	ND	ND	ND	ND	0·44	1·02
5-Vinyloxazolidine-2-thione [Goitrin]	0·12	6·60	0·15	8·20	8·25	9·70	2·00	1·40
3-Butenylisothiocyanate	0·34	1·80	0·40	2·60	2·82	3·40	1·50	0·64
4-Pentenylisothiocyanate	ND	0·34	0·09	0·64	0·65	0·73	0·46	0·18

* Values are expressed as mg of the products per g defatted dry meal. Each value is the average of 3–4 determinations.

† −AA = without added ascorbic acid; +AA = with added ascorbic acid (2·5 mg/g defatted dry meal).

‡ Meal prepared from unheated seed incubated with citric acid-phosphate buffer, pH 7·0.

§ Not detected.

Of further note is that the characteristic pattern of hydrolytic products given by mustard seed enzyme, i.e. a high yield of goitrin relative to low yield of nitriles, was also characteristic of the rapeseed enzymes. This similarity supports the view that the thioglucosidases from the two species are much the same with respect to their catalytic function. It is possible that the difference between the patterns of aglucones liberated by the enzymes separated from the seed and by autolysis is due to the heat treatment of the seed prior to incubation with the liberated enzymes. Although the heating was mild, 100° for 2–3 min, it may have been sufficient to bring about changes in the glucosinolates subsequently reflected in altered hydrolytic products.

EXPERIMENTAL

Source material. Seeds of *B. napus* cv Zephyr (rapeseed) were obtained from Brett-Young Seeds Ltd., Winnipeg, Manitoba, and *B. napus* cv Bronowski and *S. alba* (yellow mustard) from Saskatoon Research Station, Canada Dept. of Agriculture, Saskatoon, Saskatchewan. Seeds of *B. napus* cv Zephyr which provided substrate meal for hydrolysis studies were immersed in boiling water for 2–3 min and dried in air below 50°.[2]

Enzyme preparation. Thioglucosidases were extracted with acetone according to the method of Schwimmer.[11] In each case the enzyme was finally prepared as a freeze dried powder.

Disc electrophoresis on polyacrylamide gel. The procedure used for both the separation and development of the isoenzyme pattern was as described by Henderson and McEwen[6] except that electrophoresis was for 6 hr at 2·0 mA rather than 1·5 hr at 4 mA.

Hydrolysis of rapeseed meals. 0·5 g meal, 1 ml H_2O and 1 ml buffer (citric acid–phosphate, pH 7·0) containing 10 mg of enzyme preparation were placed in a glass tube fitted with a screw cap. The cap was tightly closed, sealed with polyethylene and incubated 3 hr at 37° in a shaking H_2O bath. For the study of ascorbic acid activation the 1 ml H_2O added contained 1·26 mg ascorbic acid. In the case of samples of meal destined for isothiocyanate analysis 2 ml CH_2Cl_2 was included in the incubation mixture to trap the isothiocyanates released during hydrolysis.

Autolysis. 0·5 g meal (from raw unheated seed), 1 ml H_2O or 1 ml H_2O containing 1·26 mg ascorbic acid and 1 ml buffer (citric acid–phosphate pH 7·0) were added to the glass vial and the incubation carried out as for hydrolysis. Two ml CH_2Cl_2 was added as before when required.

[11] SCHWIMMER, S. (1961) *Acta. Chem. Scand.* **15,** 535.

GLC for isothiocyanates. After incubation, the mixture, already containing 2 ml CH_2Cl_2, was extracted by adding 30 ml of the solvent and shaking. The mixture was filtered by decanting through two layers of filter paper and the residue reextracted 2 × with 5 ml CH_2Cl_2. The pooled filtrates after the addition of a known amount of butylisothiocyanate were concentrated by evaporation to 0·5 ml. Four μl of the final sol was injected into the column of a Barber–Coleman Selectra gas chromatograph. The gas chromatographic procedure was that described by Youngs and Wetter[12] except that glass columns were substituted for the metal columns used by these authors.

GLC for nitriles and goitrin. Sample preparation was as above. The chromatographic procedure was that of Daxenbichler *et al.*[13] with the minor modifications that methyl stearate replaced methyl palmitate as an internal standard and the temp. programming of 4°/min up to a temp. of 215° was started immediately following injection of the sample.

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[12] YOUNGS, C. G. and WETTER, L. R. (1967) *J. Am. Oil Chemists Soc.* **44,** 551.
[13] DAXENBICHLER, M. E., SPENCER, G. F., KLEIMAN, R., VAN ETTEN, C. H. and WOLFF, I. A. (1970) *Anal. Biochem.* **38,** 373.