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Short Communication

Identification of enzymatic degradation products from synthesized glucobrassicin by gas chromatography–mass spectrometry

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ABSTRACT

Synthesized glucobrassicin, an indole glucosinolate present in rape, was submitted to exogenous enzymatic degradation with commercial myrosinase at two different pH values. Organic products were analysed after silylation by gas chromatography using a thermoionic detector. Three products (3-indolemethanol, 3-indoleacetonitrile and 3,3'-diindolylmethane) were identified by comparison with the retention times of silylated authentic materials and by gas chromatography–mass spectrometry. Two different degradation schemes were proposed according to the pH conditions: 3-indoleacetonitrile was obtained at acidic pH and 3,3'-diindolylmethane at neutral pH. The synthetic glucobrassicin thus behaved in the same manner as the natural product.

INTRODUCTION

Glucosinolates are an important class of compounds widely distributed in all crucifer plants [1–3]. Much work has been devoted to the rape: the meal remaining after oil extraction can be fed to livestock, but in limited amounts as it contains some of these glucosinolates. During processing of seeds, they are broken down by an endogenous enzyme, myrosinase (E.C. 3.2.3.1) to give, in particular, organic isothiocyanates, nitriles, oxazolidinethiones and thiocyanate ion [1–3]. These derivatives have been found to be harmful when consumed by humans and animals [1–3]. Thyroid, liver and kidney diseases are known to occur in monogastric animals [1–5].

Nowadays, new varieties named double low rapeseed (00 rapeseed), which contain less glucosinolates, are cultivated. However, this genetic improvement has affected only the aliphatic fraction. The indole glucosinolates (Fig. 1) represent the major part of the total glucosinolate content [6].

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It has been shown that they are susceptible to thermal degradation leading to the formation of thiocyanate ions [6–8]. Nitriles have also been obtained in rape seeds and meals [9,10]. Enzymatic degradation of natural glucobrassicin has been described [11]. Nevertheless, the separation and purification of indole glucosinolates are very tedious [12] and it seems that complete degradation schemes are to be found only by using pure compounds. We took advantage of a recently developed synthesis of glucobrassicin [13] to make an exogenous enzymatic degradation study of this glucosinolate at acidic and neutral pH.

EXPERIMENTAL

Materials

Glucobrassicin was synthesized as described by Viaud and Rollin [13] with a slight modification: the intermediate 3-(2-nitrovinyl)indole was prepared by the condensation of indole and 1-dimethylamino-2-nitroethylene [14]. 3-Indoleacetonitrile and pyridine were obtained from Merck, myrosinase from Sigma and bis(trimethylsilyl)trifluoroacetamide (BSTFA) from Pierce. 3-Indolemethanol was obtained by sodium borohydride reduction of the corresponding aldehyde [15].

To prepare 3,3'-diindolylmethane, 3-indolemethanol hydrate (1 g) (Aldrich) and distilled water (50 ml) were placed in a 100-ml flask equipped with a condenser and heated with magnetic stirring for 18 h under reflux. The solid was dissolved in ethyl acetate and the solution was dried (magnesium sulphate), filtered and concentrated *in vacuo*. Recrystallization from methanol afforded an orange solid (490 mg; m.p. 162–163°C; lit. [16] m.p., 164–165°C). ^{1}H NMR (300 MHz, DMSO-d_6), δ (p.p.m.): 4.12 (s, 2H, CH_2); 6.90 (dd, 2H, H_5, $J_{H_5-H_6}$ = 7.1 Hz); 7.02 (dd, 2H, H_6); 7.11 (d, 2H, H_2, J_{H_2-NH} = 1.5 Hz); 7.31 (d, 2H, H_7, $J_{H_6-H_7}$ = 8.2 Hz); 7.51 (d, 2H, H_4, $J_{H_4-H_5}$ = 7.9 Hz); 10.72 (m, 2H, NH). ^{13}C NMR (62 MHz, DMSO-d_6), δ (p.p.m.): 20.87 (C_{10}); 111.25 (C_7); 114.14 (C_3); 117.97 (C_5); 118.62 (C_4); 120.67 (C_6); 112.71 (C_2); 127.13 (C_8); 136.33 (C_9).

Enzymatic hydrolysis of glucobrassicin

Glucobrassicin (10 mg) was weighed into a 10-ml tube. Buffer solution (5 ml; pH 3 or 7) and a few milligrams of myrosinase were then added. The homogenized solution was kept at 37°C for 4 h. Organic products were extracted with hexane (2 × 5 ml). The extracts were concentrated under a slow flow of nitrogen and the residue was silylated with 50 μl of pyridine and 50 μl of BSTFA at room temperature. Analysis was performed after 5 min.

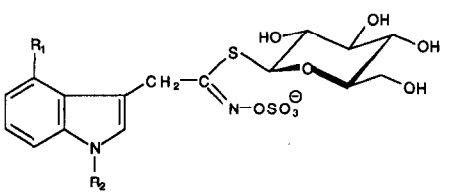
Gas chromatographic conditions

Gas chromatographic analysis was carried out on a Delsi DI-700 gas chromatograph with thermoionic detection (TID). A fused-silica column (25 m × 0.32 mm I.D.) with a 0.2-μm coating of SE-30 was used. The carrier gas (helium) pressure was 0.8 bar; the splitting ratio was 1:50. The oven temperature was programmed from 100 to 280°C at 5°C min^{-1}. The injector and detector temperatures were 320°C.

Gas chromatography–mass spectrometry

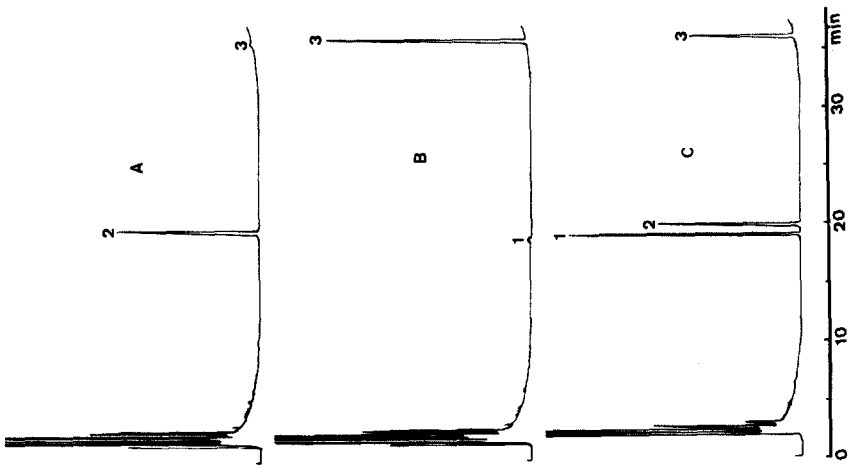
The instrument used was an Intersmat IGC 121 M gas chromatograph linked via a direct inlet to a Micromass VG 16-F mass spectrometer. The column was a 25 m × 0.22 mm I.D. fused-silica capillary column with a 0.25-μm coating of BP-1. The injection port temperature was 280°C, helium was used as the carrier gas at a pressure of 1.0 bar and the oven temperature was programmed from 130 to 300°C at 4°C min^{-1}. Positive-ion electron impact mass spectra were recorded at 70 eV. Spectra were swept from 20 to 700 u at 0.5 s per decade.

Mass spectra of silylated reference products are



$R_1 = R_2 = H$		glucobrassicin
$R_1 = OH$	$R_2 = H$	4-hydroxyglucobrassicin
$R_1 = OCH_3$	$R_2 = H$	4-methoxyglucobrassicin
$R_1 = H$	$R_2 = OCH_3$	neoglucobrassicin

Fig. 1. Major indole glucosinolates in rapeseed.



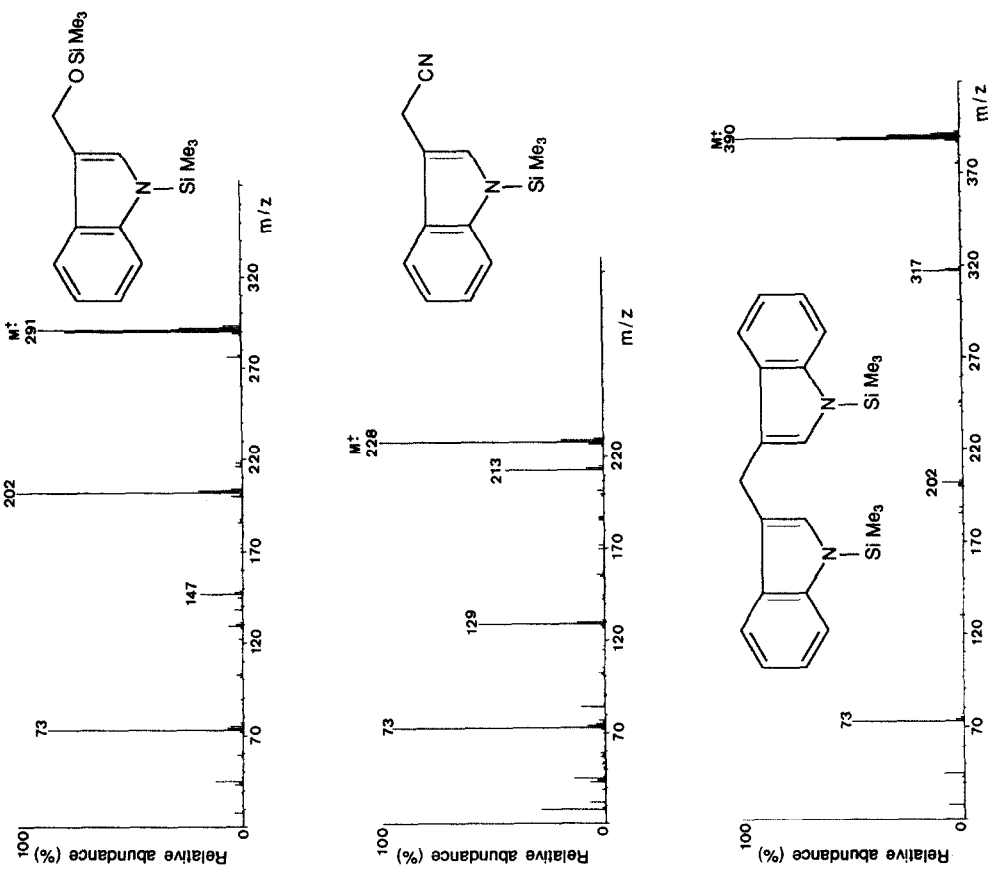


Fig. 2. Mass spectra (70 eV) of putative silylated reference products. Me = Methyl.

Fig. 3. Gas chromatograms of (A) enzymatic degradation products of glucobrassicin at pH 3; (B) enzymatic degradation products of glucobrassicin at pH 7; (C) a mixture of pure silylated products. For conditions, see Experimental. Peaks: 1 = 3-indolemethanol; 2 = 3-indoleacetonitrile; 3 = 3,3'-diindolylmethane.

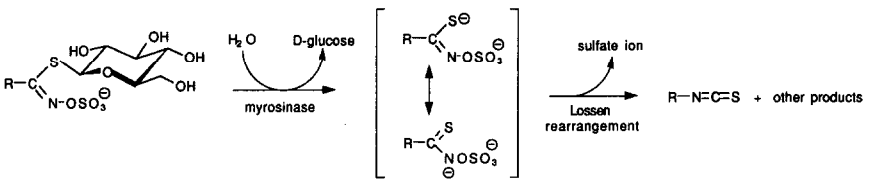


Fig. 4. General breakdown of glucosinolates.

given in Fig. 2. (The mass spectrum of the trimethylsilyl derivative of 3-indolemethanol differs considerably from that in the *Wiley/NBS Registry of Mass Spectral Data* [17]).

RESULTS AND DISCUSSION

Gas chromatograms of the trimethylsilylated derivatives of enzymatic degradation products of glucobrassicin are shown in Fig. 3. Hydrolysis products at pH 3 and 7 are shown in Fig. 3A and B, respectively, followed by a mixture of pure silylated products (Fig. 3C). Identification was confirmed by gas chromatography–mass spectrometry.

As can be seen, two different mechanisms were evidenced in the enzymatic degradation of glucobrassicin: at pH 3, the only detected product was 3-indoleacetonitrile; at pH 7, the predominant compound (>98%) was 3,3'-diindolylmethane, with a trace (<2%) of 3-indolemethanol.

Unambiguously, 3,3'-diindolylmethane comes from the self-condensation of the corresponding alcohol, certainly via a mechanism described previously [18]. It is well documented that myrosinase (thioglucoside glucohydrolase) cleaves the S–glucose bond to give, after desulphation, an isothiocyanate (Fig. 4), commonly observed with aliphatic glucosinolates [1–3].

With glucobrassicin, this compound has never been evidenced despite many trials [8]. (Nevertheless, in recent work dealing with the degradation of neoglucobrassicin, the corresponding isothiocyanate was evidenced under particular experimental conditions [19]). It appears that it is unstable and immediately yields 3-indolemethanol and thiocyanate ion.

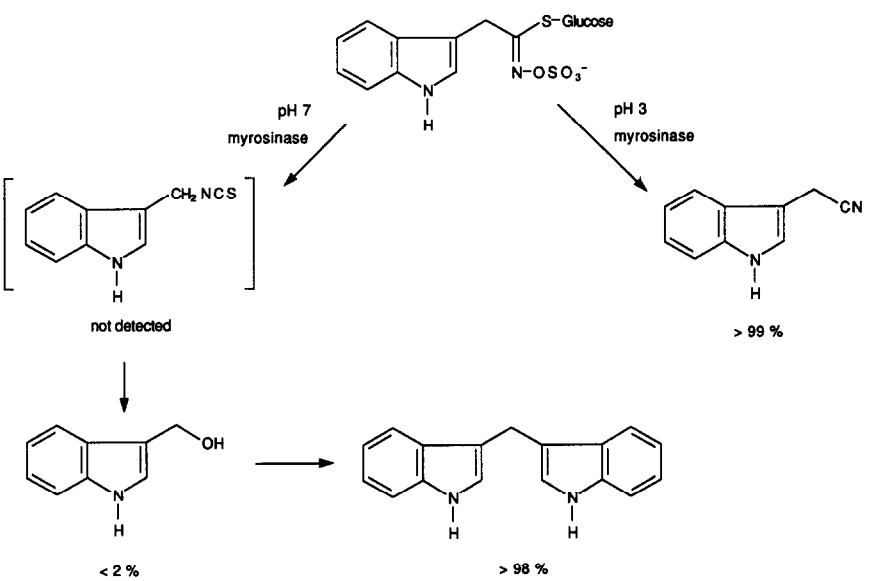


Fig. 5. Enzymatic hydrolysis of synthesized glucobrassicin.

A general scheme of the enzymatic degradation of synthesized glucobrassicin is given in Fig. 5.

In conclusion, during enzymatic hydrolysis, the synthesized glucobrassicin showed the same behavior as the natural product [11].

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REFERENCES

1 H. L. Tookey, C. H. van Etten and M. E. Daxenbichler, in I. E. Liener (Editor), *Toxic Constituents of Plant Foodstuffs*, Academic Press, New York, 2nd ed., 1980, p. 103.
2 C. H. Van Etten and H. L. Tookey, in M. Rechcigl, Jr. (Editor), *CRC Handbook of Naturally Occurring Food Toxicants*, CRC Press, Boca Raton, FL, 1983, p. 15.
3 G. R. Fenwick, R. K. Heaney and W. J. Mullin, *CRC Crit. Rev. Food Sci. Nutr.*, 18 (1983) 123.
4 M. Vermorel, R. K. Heaney and G. R. Fenwick, *J. Sci. Food Agric.*, 44 (1988) 321.
5 M. Vermorel, M. J. Davicco and J. Evrard, *Reprod. Nutr. Dev.*, 27 (1987) 57.
6 B. A. Slominski and L. D. Campbell, *J. Sci. Food Agric.*, 40 (1987) 131.
7 B. A. Slominski and L. D. Campbell, *J. Sci. Food Agric.*, 47 (1989) 75.
8 R. McDanell, A. E. M. McLean, A. B. Hanley, R. K. Heaney and G. R. Fenwick, *Food Chem. Toxicol.*, 26 (1988) 59.
9 B. A. Slominski and L. D. Campbell, *J. Chromatogr.*, 454 (1988) 285.
10 C. Gardrat and J. L. Coustille, *J. High Resolut. Chromatogr.*, 12 (1989) 626.
11 R. Gmelin and A. I. Virtanen, *Suom. Kemistil. B*, 34 (1961) 15.
12 A. B. Hanley, C. L. Curl, G. R. Fenwick and R. K. Heaney, *Cruciferae Newsl.*, 9 (1984) 66.
13 M. C. Viaud and P. Rollin, *Tetrahedron Lett.*, 31 (1990) 1417.
14 G. Büchi and C. P. Mak, *J. Org. Chem.*, 42 (1977) 1784.
15 R. M. Silverstein, E. E. Ryskiewicz and S. W. Chaikin, *J. Am. Chem. Soc.*, 76 (1954) 4485.
16 E. Leete and L. Marion, *Can. J. Chem.*, 31 (1953) 775.
17 F. W. MacLafferty and D. S. Stauffer, *The Wiley/NBS Registry of Mass Spectral Data*, Vol. 3, Wiley, New York, p. 2606.
18 J. N. Bemiller and W. Colilla, *Phytochemistry*, 11 (1972) 3393.
19 A. B. Hanley, K. R. Parsley, J. A. Lewis and G. R. Fenwick, *J. Chem. Soc., Perkin Trans. 1*, (1990) 2273.