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IDENTIFICATION OF 1-METHOXYINDOLYL-3-METHYL ISOTHIOCYANATE AS AN INDOLE GLUCOSINOLATE BREAKDOWN PRODUCT

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Key Word Index—1-Methoxyindolyl-3-methyl isothiocyanate; glucobrassicin; neoglucobrassicin; HRMS; myrosinase; low water system.

Abstract—The putative indole glucosinolate enzymic hydrolysis product, 1-methoxyindolyl-3-methyl isothiocyanate has been detected for the first time using low and high resolution mass spectrometry.

INTRODUCTION

The enzymic hydrolysis of glucosinolates by the co-occurring enzyme myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) leads, after Lossen type rearrangement, to the formation of organic isothiocyanates which have pronounced biological and sensory properties [1]. For those glucosinolates which contain an indolic side chain, the degradation pathway is assumed to proceed in a similar fashion but the intermediate indolyl-3-methyl isothiocyanate is considered to be water-labile, rapidly hydrolysing to give the corresponding indolyl-3-carbinol (Fig. 1) [2, 3]. Some limited studies on other members of the class of indole glucosinolates have been carried out and some of the products have been shown to be similar to those formed in the case of the parent unsubstituted compound [3, 4]. The presence of an *N*-methoxy substituent, as occurs in the case of neoglucobrassicin (1-methoxyindolyl-3-methyl glucosinolate), is known to deactivate the 3 position of the indole ring [5] thus the proposed intermediate hydrolysis product, 1-methoxyindolyl-3-methyl isothiocyanate might be expected to be more stable than the unsubstituted compound. In addition, myrosinase is known to be a membrane-bound enzyme [6] and the presence of an interface and less free water may have consequences for product formation. A group of indolic phytoalexins have recently been isolated and characterized from fungally infected brassicaceous plants [7, 8] and these bear a striking structural similarity to indolyl-3-methyl isothiocyanates (Fig. 2).

It is, therefore, important to determine if the isothiocyanate can be formed from the corresponding indole glucosinolate, thereby confirming the postulated breakdown pathway. Furthermore, the isolation of such compounds may suggest a direct link between the indolic phytoalexins and indole glucosinolates. The occurrence of novel isothiocyanates may be important in the detection of trace amounts of indole glucosinolates using techniques developed by McLeod and co-workers for other glucosinolates [9] and finally these compounds may have biological significance given the anti-carcinogenic effects attributed to indole glucosinolates and their breakdown products [10, 11].

RESULTS AND DISCUSSION

TLC analysis (mobile phase, EtOAc–hexane 2:3) of the organic extracts from the hydrolysis of glucobrassicin in normal aqueous buffer afforded products with expected R_f values, by comparison with authentic samples, for indole-3-carbinol, 3,3-diindolylmethane and indole-3-acetonitrile. Neoglucobrassicin was treated similarly and the major product had a similar R_f to the unsubstituted acetonitrile. Examination by EIMS indicated that no isothiocyanates were present, however the major product appeared to be the acetonitrile. In the low water system where both enzyme and substrate were adsorbed onto celite prior to mixing in hexane saturated with water (see Experimental), the glucobrassicin incubation once more failed to give any indication of the formation of the isothiocyanate by mass spectrometry. In the case of neoglucobrassicin, however, a spectrum was obtained which was consistent with the proposed methoxy indole isothiocyanate, displaying the expected molecular ion at *m/z* 218 and two major fragment ions at *m/z* 160 and 129 (Fig. 3). High resolution accurate mass measurement confirmed the molecular formula as $C_{11}H_{10}N_2OS$ and gave formulae of $C_{10}H_{10}NO$ and C_9H_7N for the ions at *m/z* 160 and 129 respectively.

These results confirm, for the first time, that the enzymic hydrolysis of indole glucosinolates proceeds, as in the case of other glucosinolates, via the corresponding isothiocyanate. While indolyl-3-methyl isothiocyanate remains too unstable for characterization, even in a low water system, the less reactive 1-methoxy analogue can be detected, albeit in small amounts. This substantiates the hydrolysis mechanism proposed previously. A link between this neoglucobrassicin breakdown product and the phytoalexins recently isolated remains to be proven but is now more likely that at first thought. The possible biological consequences of the formation of such a reactive indolic compound *in vivo* remain uninvestigated.

EXPERIMENTAL

Mass spectrometry. EIMS (low resolution) were obtained on a Kratos MS30 mass spectrometer (Kratos Analytical, Manches-

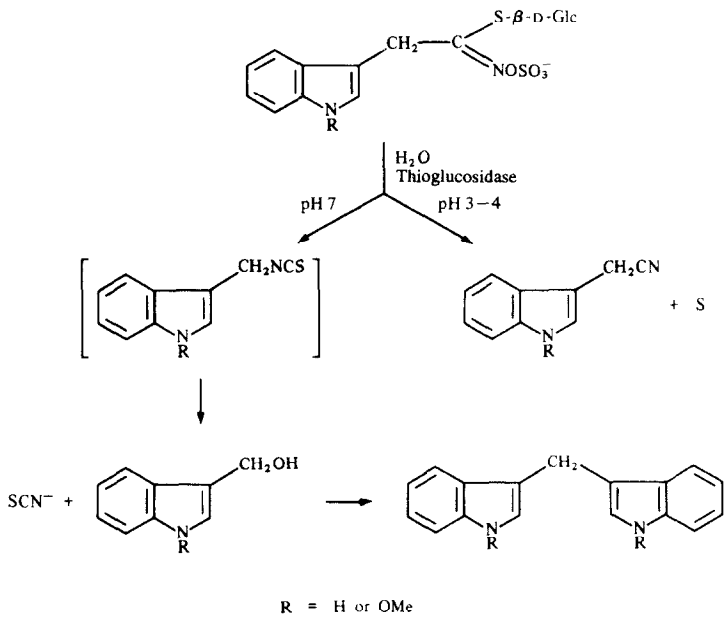

Fig. 1.

ter, U.K.) at a resolution of 1000 with a source temperature of 200° and an ionising potential of 70 eV. HR data were obtained on a Kratos MS80RFA mass spectrometer at a resolution of 7500 with similar source conditions.

Incubations. (a) *Aqueous.* Glucobrassicin (10 mg), purified as described previously [12, 13], was dissolved in aq. buffer (Tris–HCl, pH 7.4, 100 mM, 5 ml) and incubated with myrosinase [14] at 37° for 4 hr. After this time, hexane (10 ml) was added, the mixture shaken and the organic layer removed, dried (Na_2SO_4) and evapd to dryness *in vacuo* at room temp. The residue was taken up in EtOAc (100 μl) and an aliquot analysed by mass spectrometry.

(b) *Low water system.* Glucobrassicin (10 mg) or neoglucobrassicin (10 mg) was dissolved in aq. buffer (Tris–HCl, pH 7.4, 100 mM, 1 ml) and then added to celite (*ca* 200 mg). Both samples were treated identically with myrosinase, dried separately *in vacuo* at room temp. then the powders mixed together. Hexane presaturated with H_2O (5 ml) was added followed by a further aliquot of H_2O (5 μl). The suspension was stirred vigorously for 40 hr then filtered through glass wool, dried (Na_2SO_4) and evapd to dryness *in vacuo* at room temp. The residue was taken up in EtOAc (100 μl) and an aliquot examined by mass spectrometry.

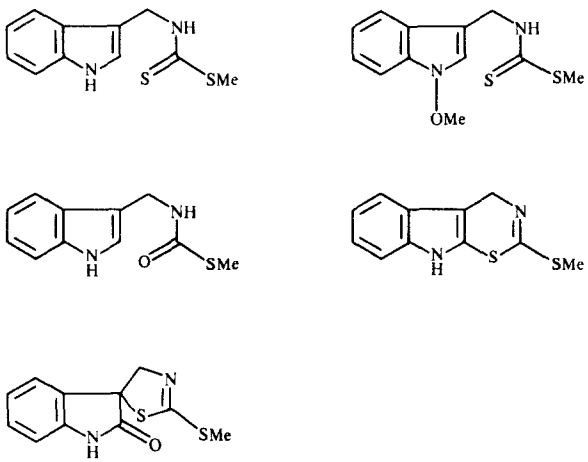

Fig. 2.

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REFERENCES

1. Fenwick, G. R., Heaney, R. K. and Mullin, W. J. (1983) *CRC Crit. Rev. Food Sci. Nutr.* **18**, 123.
2. Gmelin, R. and Virtanen, A. I. (1961) *Suomen Kemist.* **B34**, 15.
3. Gmelin, R. and Virtanen, A. I. (1962) *Suomen Kemist.* **B35**, 34.
4. Hanley, A. B., Belton, P. S., Fenwick, G. R. and Janes, N. F. *Phytochemistry* **24**, 598.
5. Acheson, R. M., Hunt, P. G., Littlewood, D. M., Murrer, B. A. and Rosenberg, H. E. (1978) *J. Chem. Soc. Perkin Trans I* 1117.
6. Björkman, R. (1976) in *The Biology and Chemistry of the Cruciferae* (Vaughan, J. G., MacLeod, A. J. and Jones, B. M. G., eds), pp. 191–205. Academic Press, London.

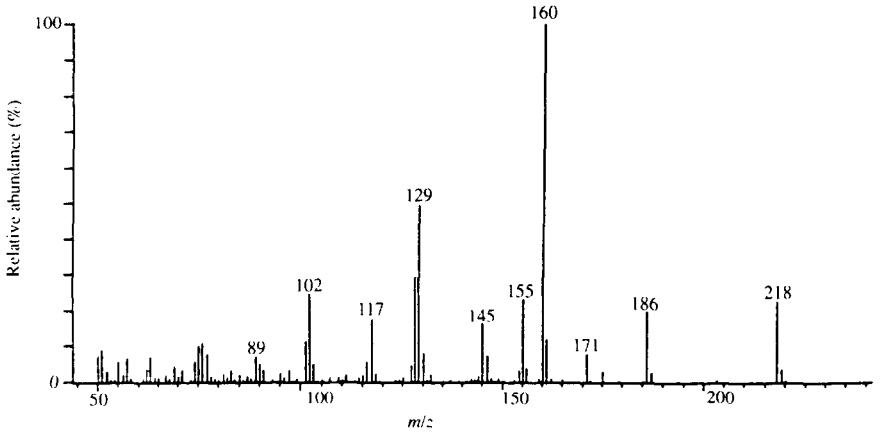

Fig. 3.

7. Takasugi, M., Moude, K., Katsui, N. and Shirata, A. (1987) *Chem. Letters* 1631.
8. Takasugi, M., Katsui, N. and Shirata, A. (1986) *J. Chem. Soc. Chem. Comm.* 1077.
9. MacLeod, A. J. (1976) in *The Biology and Chemistry of the Cruciferae* (Vaughan, J. G., MacLeod, A. J. and Jones, B. M. G., eds), pp. 307–330. Academic Press, London.
10. McDanell, R., McLean, A. E. M., Hanley, A. B., Heaney, R. K. and Fenwick, G. T. (1988) *Food Chem. Toxicol.* **26**, 59.
11. McDanell, R., McLean, A. E. M., Hanley, A. B., Heaney, R. K. and Fenwick, G. R. (1987) *Food Chem. Toxicol.* **25**, 363.
12. Hanley, A. B., Heaney, R. K. and Fenwick, G. R. (1983) *J. Sci. Food Agric.* **34**, 869.
13. Hanley, A. B., Curl, C. L., Fenwick, G. R. and Heaney, R. K. (1984) *Eucarpia, Cruciferae Newsletter* **9**, 66.
14. Heaney, R. K. and Fenwick, G. R. (1981) *Z. Pflanzenzeuchty* **87**, 89.