

Differences in glucosinolate degradation products related to aphid and *Brassicaceae* host plant myrosinases

Frédéric FRANCIS * ¹, Jean-Paul WATHELET ² & Georges LOGNAY ²

¹Unit of general and applied Zoology,

²Unit of general and organic Chemistry,

Gembloux Agricultural University,

Passage des Déportés 2, B-5030 Gembloux, Belgium

* to whom correspondence should be addressed : francis.f@fsagx.ac.be

Abstract

Studies on chemical ecology of host plant selection by herbivores were largely carried out to explain the diversity of secondary plant chemicals and host specialisation of herbivores. The glucosinolate – isothiocyanate system in Brassicaceae plants was considered as a plant defence and was known to influence the feeding behaviour of phytophagous insects such as the cabbage aphid, *Brevicoryne brassicae*. Purified myrosinases from the latter and from white mustard, *Sinapis alba*, were used to hydrolyse two glucosinolate compounds. Identification of the degradation products, mainly isothiocyanates, was performed by gas chromatography – mass spectrometry. While sinigrin hydrolysis gave identical isothiocyanates for insect and plant enzymes, *B. brassicae* myrosinase showed a particular activity toward sinalbin. A loss of an hydroxy group was observed for the two latter substrates related products when compared to the *S. alba* enzymatic specificity. Aphid and plant myrosinases have different properties and specificities which can be discussed in relation to a co-evolution approach.

Introduction

Glucosinolates (GLS) which occur in 11 dicotyledone are hydrolysed by β -thioglucosidases (E.C.3.2.3.1.), called myrosinases, in glucose, sulphate and an unstable intermediate, which rearranges to produce several products including isothiocyanates (ITC), nitriles, thiocyanates and oxazolidinethiones (Halkier and Du, 1997). The degradation products generally have pronounced biological effects as defence against generalist herbivore attacks but are also involved in host-plant recognition by specialized insects (Porter et al., 1991; Lamb, 1989).

The glucosinolate – isothiocyanate system in Brassicaceae plants was considered as a plant defence and was known to influence the feeding behaviour of phytophagous insects such as the cabbage aphid, *Brevicoryne brassicae*. The presence of myrosinase enzyme in herbivorous insects is an interesting feature of plant-insect co-evolution. Indeed, one of the crucifer pests, the cabbage aphid (*B. brassicae*), did not develop classical biochemical adaptation of generalist phytophagous insects such as inductions of GST detoxication enzymes (Francis, 1999). More than a plant GLS metabolism pathway, the aphid species evolved as its host plant by developing the glucosinolate-myrosinase system as defence against its predators by the use of the plant allelochemicals, the GLS.

In the present work, purified myrosinase from *B. brassicae* was used to investigate the degradation of pure glucosinolate substrates. White mustard, *Sinapis alba*, myrosinase was used as control to hydrolyse the glucosinolates.

Material and methods

Chemicals

Sinialbin was kindly supplied by Dr. Iori (Istituto sperimentale per le colture industriali, Bologna, Italy), pure sinigrin was purchased from Sigma (St.Louis-USA).

Purification of enzyme

Purification of *B. brassicae* myrosinase was performed from whole aphids homogenate by a three step protocol (ammonium sulphate selective precipitation, DEAE and Superdex chromatographies according to Francis et al. (2001).

Glucosinolate Hydrolysis Product analysis

The hydrolyses were performed as follows : 100 μ l of the enzymatic preparations (plant myrosinase from *S. alba*, crushed aphids (*B. brassicae*) and purified aphid-myrosinase at 0.35 U/ml) were added to 2 ml of 1 mM solution of each substrate in 20 mM phosphate buffer pH 6.8 and let for 1h at $30 \pm 0.2^\circ\text{C}$. After adding 100 μ l of a phenethyl-ITC (internal standard) ethanolic solution (2 mg/ml), the aqueous mixtures were extracted with 500 μ l of peroxide-free diethyl ether for 1 h at room temperature. To identify the hydrolysis products, the solutions were analysed by GC-MS on a Hewlett-Packard HP5972 Mass spectrometer coupled to a HP5890 series II gas chromatograph and their quantifications were undertaken by GLC-FID on a HP6890 gas chromatograph. The following chromatographic conditions were used : the molecule of interest were separated on a HP5-MS (5% phenyl-dimethylpolysiloxane) column (30m x 0.25mm, df=1 μ m); the temperature programme was from 40°C (1 min hold) to 180°C at $6^\circ\text{C}\cdot\text{min}^{-1}$ than to 280°C at $15^\circ\text{C}\cdot\text{min}^{-1}$; split-splitless injection at 250°C (splitless mode); carrier gas : He at $1.4\text{ ml}\cdot\text{min}^{-1}$ (average linear velocity : $33\text{ cm}\cdot\text{sec}^{-1}$). The MS spectra were obtained in the EI mode at 70eV (scanned mass range from 30 to 400 amu). The analytes were identified on the basis of their retention times and by interpretation of MS fragmentation patterns. The recorded spectra were finally compared to those of the Wiley275.L spectral library and to reference data obtained by MS analysis of reference isothiocyanates. For GLC-FID measurements, the detector was maintained at 250°C .

Results

While sinigrin was hydrolysed by both plant and aphid myrosinase into allyl-ITC, a loss of the hydroxy group of ITC from sinialbin degradation was observed with the aphid enzyme whereas plant thioglycosidase catalysed the production of conventional hydroxylated ITC (Figure 1).

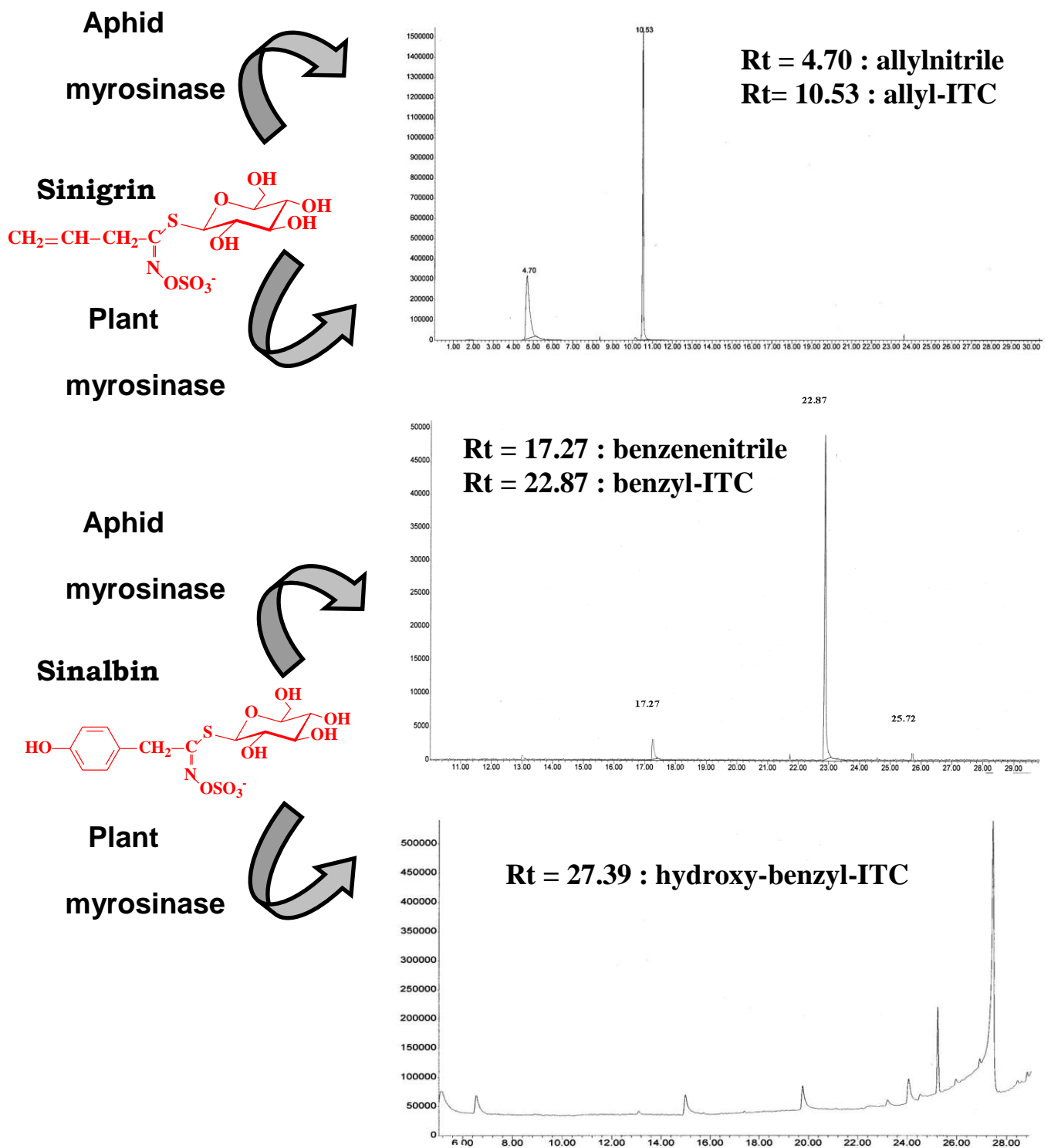


Figure 1 : Comparison of the pure glucosinolate degradation products using *Sinapis alba* and *Brevicoryne brassicae* myrosinases. Substrate formulae are presented in association to GC-MS chromatograms of diethyl-ether extracts of sinalbin and sinigrin degradation products obtained by plant and insect myrosinases.

Discussion

Little is known about the substrate specificity of myrosinase isoenzymes. Most of the studies on thioglucosidases only used sinigrin as substrate but myrosinase activity against some substrates such as progoitrin was already determined to be variable, depending on the tested isoenzyme. The hydrolysis products of two glucosinolates using both plant and aphid myrosinases were identified. A loss of the hydroxy group was observed for isothiocyanate from sinalbin in the presence of aphid purified thioglucosidase. Similar results were observed when crude aphid homogenate was used as source of enzyme. *S. alba* purified thioglycosidase was also used and catalysed the production of conventional hydroxylated isothiocyanate. The difference in the glucosinolate hydrolysis products was linked either to the insect myrosinase activity or to the reaction conditions. The latter was known to be important on the plant myrosinase activity: Wilkinson and colleague (1984) reported that ascorbate concentrations of 0.7-5.0 mM induced maximal activation of myrosinase in partially purified extracts of six crucifer species. Ascorbic acid does not participate in the reaction catalysed by mustard myrosinase (Ettlinger et al., 1961) nor is it involved in the association of the enzyme subunits. The activation appears to be due to a conformation change in the protein structure, leading to an enhanced reaction rate when the effector binding sites are occupied (Othsuru and Hata, 1973). Ascorbic acid was added in the reaction buffer of new trials on sinalbin hydrolysis. The recovery of the isothiocyanate hydroxy group led to the conclusion that ascorbic acid contributed to the liberation of 4-hydroxybenzyl ITC which has never been observed in the previously mentioned conditions. Following our observations, we hypothesise that ascorbic acid could prevent further alteration of 4-hydroxybenzyl ITC.

This biochemical study was performed in addition to previous biological and chemical observations on insect – cruciferous plant relations. The occurrence of the myrosinase system in the cabbage aphid was seen as a uncommon biochemical adaptation of *Brassica* herbivore towards its host plant. Indeed, only cruciferous feeding aphids have been shown to have myrosinase activity, the latter lacking in other aphid species such as *Macrosiphum avenae*, *Rhopalosiphum padi* and *Myzus persicae* (MacGibbon and Beuzenberg, 1978).

References

- Ettlinger, M.G., Dateo, G.P., Harrison, B.W., Mabry, T.J., and Thompson, C.P. (1961). Vitamin C as coenzyme : the hydrolysis of mustard oil glucosides. *Proc. Natl. Acad. Sci. USA* **47**, 1875-1880.
- Francis, F. (1999). Conséquences évolutives des relations entre le puceron et son prédateur en présence de substances allélochimiques chez les Brassicaceae. *Annales ANPP* **2**, 503-510.
- Francis, F., Lognay, G., Wathelet, J.P., and Haubruge, E. (2001). Effects of allelochemicals from first (Brassicaceae) and second (*Myzus persicae* & *Brevicoryne brassicae*) trophic levels on *Adalia bipunctata*. *J. Chem. Ecol.* **27**, 243-256.
- Halkier, B.A., and Du, L. (1997). The biosynthesis of glucosinolates. *Trends in Plant Science* **2**, 425-431.
- Lamb, R.J. (1989). Entomology of oilseed Brassica crops. *Ann. Rev. Entomol.* **34**, 211-229.
- MacGibbon, D.B., and Beuzenberg, E.J. (1978). Location of glucosinolate in *Brevicoryne brassicae* and *Lypaphis erysimi* (Aphididae). *NZ. J. Sci.* **21**, 389-392.
- Othsuru, M., and Hata, T. (1973). Studies on the activation mechanism of the myrosinase by L ascorbic acid. *Agr. Biol. Chem.* **37**, 2543-2548.
- Porter, J.R.M., Morton A.M., Kiddle G., Doughty K.J., and Wallsgrove, R.M. (1991). Variation in the glucosinolates content of oilseed rape (*Brassica napus* L.) leaves. Effect of leaf age and position. *Ann. Appl. Biol.* **118**, 461-467.
- Wilkinson, A.P., Rhodes, M.J.C., and Fenwick, R.G. (1984). Myrosinase activity of cruciferous vegetables. *J. Sci. Food Agric.* **35**, 543-552.