In Vitro Cytotoxic Activity of Some Glucosinolate-Derived Products Generated by Myrosinase Hydrolysis

Claudio Nastruzzi,[‡] Rita Cortesi,[‡] Elisabetta Esposito,[‡] Enea Menegatti,[‡] Onofrio Leoni,[§] Renato Iori,[§] and Sandro Palmieri*[,§]

Dipartimento di Scienze Farmaceutiche, Università di Ferrara, I-44100 Ferrara, Italy, and Istituto Sperimentale per le Colture Industriali, MRAAF, I-40129 Bologna, Italy

The effects of glucosinolates (GLs) (sinigrin, gluconapin, progoitrin, *epi*-progoitrin, sinalbin, glucotropaeolin, glucoerucin, glucocheirolin, and glucoraphenin) and their enzymatic hydrolysis-derived products (EHDPs) have been studied in controlling the proliferation of cancer cell lines. The results of this study indicate the following: (i) neither myrosinase nor intact GLs have any effect on tumor cell growth when used up to 36 U/mL and 500 μM, respectively; (ii) all EHDPs show a clear inhibition of human erythroleukemic K562 cell proliferative growth, which is particularly evident for EHDPs from sinigrin, glucotropaeolin, glucoerucin, and glucocheirolin (IC_{50} < 20 μM); (iii) the EHDP production by *in situ* or *pre-mix* procedures gives rather similar antiproliferative effects; and finally, (iv) the EHDPs from glucoraphenin are active toward several other tumor cells, viz. FL (murine erythroleukemic cells), Jurkat (human T-lymphoid cells), HeLa (human cervix carcinoma cells), H9 (human T-lymphoid cells), and H3-T1-1 cells (obtained by transfection of HeLa with a LTR-HIV-1-CAT plasmid).

Keywords: *Crucifers; glucosinolates; isothiocyanates; myrosinase; antitumor activity*

INTRODUCTION

Several authors report that a diet rich in cruciferous vegetables such as Brussels sprouts, cabbage, broccoli, turnip, and cauliflower may have important functions in reducing the risk of developing colorectal cancer in humans (Manousos et al., 1983; Cohen, 1987; Lee et al., 1989). In addition, other results, produced by both epidemiological studies (Graham and Mettlin, 1981) and pharmacological tests employing experimental animals exposed to carcinogens (Stoewsand et al., 1988), demonstrate the protective effect of the above-mentioned crucifer vegetables. These effects appear to be strictly correlated to the glucosinolate (GL) content of these vegetables and especially to the enzymatic hydrolysis-derived products (EHDPs), which become available after *in vivo* metabolization of GLs by endogenous or commensal microflora enzymes with myrosinase or myrosinase-like activity (Nugon-Baudon et al., 1988). In this regard, several authors report that GLs display only a scarce protective effect, whereas EHDPs are much more effective (Graham et al., 1978; Haensezel et al., 1980; Manousos et al., 1983; Lee et al., 1989), being often antinutritional or toxic compounds depending on the concentrations and exposure time (Fenwick et al., 1983).

Myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1), the agent responsible for GL hydrolysis, is a widespread enzyme, especially present in seeds and tissues of crucifers, although it has been found in several microorganisms (Greer and Deeney, 1959; Greer, 1962) and in the digestive systems of birds and ruminants (Lanzani et al., 1974; Marangosa and Hill, 1974). Myrosinase isolated from *Sinapis alba* is a glycopolypeptide that contains various thiol and disulfide groups, existing in multiple forms with about 18% of carbohydrates, principally hexoses (Bjorkman, 1976). The main isoenzyme isolated from seeds of *S. alba* consists of two identical subunits of molecular mass of 71.7 kg/mol and has a p*I* of 5.1 (Pessina et al., 1990). Myrosinases catalyze the hydrolysis of GLs to β-D-glucose, sulfate, and a series of sulfur- and nitrogen-containing compounds such as isothiocyanates, thiocyanates, thiones, and nitriles, depending on the substrate and reaction conditions used, such as the pH and the presence of particular compounds and ions (Gil and MacLeod, 1980; Uda et al., 1986a,b) (see Figure 1).

GLs are a class of approximately 100 secondary plant compounds (Kjaer and Skrydstrup, 1987) which are contained in the seeds, roots, stems, and leaves of plants belonging to 11 families of dicotyledonous angiosperms, of which the crucifers are certainly the most important. These thioglucosides have a common structure characterized by a side chain (R) constituted by aliphatic, aromatic, or heteroaromatic residues, which are synthesized *in vivo* from some amino acid precursors such as methionine, phenylalanine or tyrosine, and D,L-tryptophan, respectively, by chain lengthening and hydroxylation or oxidation pathways (Larsen, 1981).

Among the various biological activities ascribed to GLs and their EHDPs, the antitumoral action appears to be the most interesting. For this reason, therefore, it is worthwhile to clarify in detail how and why GLs are bioactive substances, given their evident implication in human health. The mechanisms by which these thiocompounds display cancer prevention is not completely understood. It seems that these minor dietary constituents inhibit carcinogenesis by neutralization of a wide number of carcinogens or by suppression of proliferation activity of neoplastic cells. GLs and EHDPs could prevent carcinogen molecules from reaching the target site or weaken the effects of genetic modification that occurred in the early stages of neoplastic transformation (Wattemberg, 1977, 1992) or may inhibit

* Address correspondence to this author at Via di Corticella 133, 40129 Bologna, Italy (telephone 0039 51 353598; fax 0039 51 374857).

[‡] Università di Ferrara.

[§] Italian Ministry of Agricultural, Food and Forestry Resources (MRAAF).

0021-8561/96/1444-1014$12.00/0

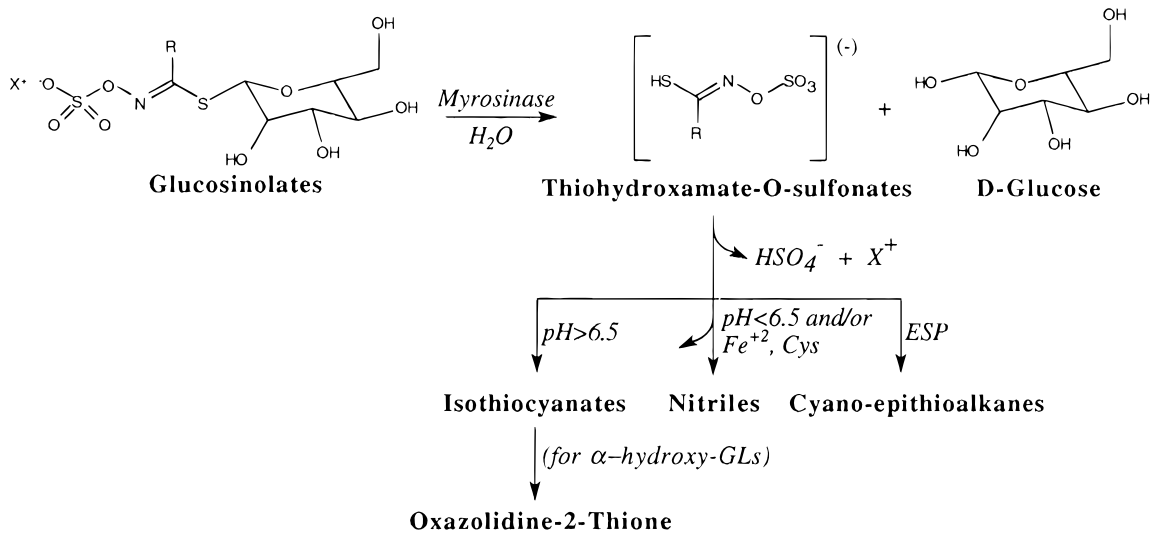


Figure 1. Scheme of the myrosinase-catalyzed hydrolysis of glucosinolates.

some important enzymes in the regulation of cell division cycle (Marx, 1993). The first process seems to be realized by induction and activation of quinone reductase, glutathione *S*-transferase (Zhang et al., 1992), and UDP-glucoronosyl transferase (Nugon-Baudon et al., 1990), which are important hepatic enzymes for protection against several carcinogens. Taking into account these findings and those regarding our recent studies on the myrosinase−GL system (Lazzeri et al., 1993; Leoni et al., 1991, 1993, 1994; Palmieri, 1994; Streicher et al., 1995), we studied the effect of some native GLs, namely sinigrin, gluconapin, progoitrin, *epi*-progoitrin, sinalbin, glucotropaeolin, glucoerucin, glucocheirolin, and glucoraphenin, and their EHDPs on *in vitro* cultured tumor cell lines.

The present work is therefore an attempt aimed to give information on a molecular basis, which to the best of our knowledge is still lacking, of a possible positive effect of GLs and their derived products in controlling cancer cell growth. In particular in this study, we evaluate the antiproliferative activity on *in vitro* cultured tumor cell lines of GLs and thioglucosidic compounds obtained after myrosinase-mediated hydrolysis.

MATERIALS AND METHODS

Myrosinase. The myrosinase used in the present study was isolated from ripe seeds of white mustard (*S. alba* L.). The isolation procedure was based essentially on Con A−Sepharose affinity chromatography and chromatofocusing techniques (Palmieri et al., 1986; Pessina et al., 1990). The myrosinase stock solution had a specific activity of ca. 60 U/mg of soluble protein. Myrosinase solution was stored at 4 °C in sterile distilled water until used.

Myrosinase Unit Definition. One myrosinase unit is defined as the amount of enzyme able to hydrolyze 1 μmol of sinigrin/min determined as reported by Palmieri et al. (1982).

Glucosinolates. The GLs used were sinigrin (2-propenylglucosinolate), gluconapin (3-butenylglucosinolate), progoitrin [(2*R*)-(2-hydroxy-3-butenyl)glucosinolate], *epi*-progoitrin [(2*S*)-(2-hydroxy-3-butenyl)glucosinolate], sinalbin [(*p*-hydroxybenzyl)glucosinolate], glucotropaeolin (benzylglucosinolate), glucoerucin [4-(methylthio)butylglucosinolate], glucocheirolin [3-(methylsulfonyl)propylglucosinolate], glucoraphenin [4-(methylsulfinyl)-3-butenylglucosinolate] (see chemical structure and origin in Table 1). They were purified according to the method proposed by Thies (1988) with some important modifications reported by Visentin et al. (1992), starting from ripe seeds of some crucifers (Figure 2). The HPLC analyses of desulfo derivatives (*Official Journal of the European Community*,

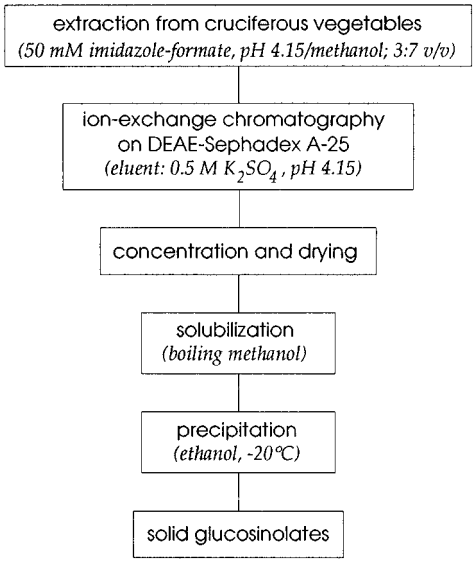


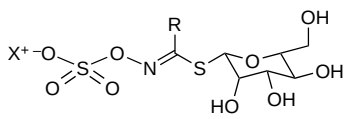
Figure 2. Flow sheet of GL isolation.

1990), coupled with polarographic determinations of total GL content (Iori et al., 1983), showed that the GLs used in this study were nearly homogeneous. Each GL was identified using NMR and, in some cases, also by mass spectrometry.

Production of EHDPs. The EHDPs were produced using free or immobilized myrosinase in 0.1 M phosphate buffer (pH 6.5) at 37 °C (Leoni et al., 1993, 1994). The reaction products, isothiocyanates (ITCs) and vinyloxazolidine-2-thiones (VOTs), were dried by a Büchi Model RE 121 rotary evaporator at 50−60 °C under vacuum and then solubilized in CH_2Cl_2. The solution was dried using anhydrous Na_2SO_4 and filtered, and then the EHDPs were concentrated in a rotary evaporator or under a nitrogen stream until the solvent was completely removed. Finally, the EHDPs were characterized by GLC and/or HPLC and NMR spectroscopy.

EHDPs Analyses. The GLC analyses of EHDPs were carried out using a Fison Carlo Erba Model Mega 5330 instrument equipped with a 30 m × 0.32 mm capillary column Restek Rtx 2330. The flow rate of the carrier gas (He) was 1.8 mL/min (split rate 1:80). The flow rates of the auxiliary gases (H_2 and air) were 25 and 300 mL/min, respectively. The column temperature was 40 °C at the start and 230 °C at the end with a rate of 10 °C/min; the temperature of the injector (split) and detector (FID) was 260 °C. The EHDPs were also analyzed by HPLC, using a Hewlett-Packard Model 1090L chromatograph equipped with a diode array as detector and a 200 × 4.6 mm column HP ODS Hypersil C18, 5 μm. The column was eluted for 22 min using a linear gradient (20−

Table 1. Origin and Structure of Tested GLs



GL (trivial name)	source (ripe seeds)	structure of R group
sinigrin	*Brassica carinata*	$CH_2{=}CHCH_2-$
gluconapin	*Brassica rapa* cv. Silla	$CH_2{=}CHCH_2CH_2-$
progoitrin	*Brassica napus* cv. Jet Neuf	$CH_2{=}CHCHOHCH_2-$ (2*R*)
epi-progoitrin	*Crambe abyssinica* cv. Belenzian	$CH_2{=}CHCHOHCH_2-$ (2*S*)
sinalbin	*Sinapis alba* cv. Maxi	*p*-$HOC_6H_4CH_2-$
glucotropaeolin	*Lepidium sativum* L.	$C_6H_5CH_2-$
glucoerucin	*Eruca sativa* M.	$CH_3SCH_2CH_2CH_2CH_2-$
glucoraphenin	*Raphanus sativus* cv. Pegletta	$CH_3SOCH{=}CHCH_2CH_2-$
glucocheirolin	*Cheirantus cheiri*	$CH_3SO_2CH_2CH_2CH_2-$

75%) of aqueous acetonitrile at 40 °C, with a flow of 2 mL/min. The chromatographic fractions were detected at 200 and 210 nm.

Partition Coefficient Determination of EHDPs. Four milliliters of an aqueous solution containing a known concentration of EHDP (ITC or VOT) was mixed at room temperature for several minutes with the same volume of *n*-octanol in a Mixxor apparatus (10 mL) purchased from Genex Co. (Gaithersburg, MD). The EHDP concentration in the aqueous phase was determined by HPLC before and after partition. The validation of the linearity of the analytical assay response in the range of experimental EHDP concentrations used was previously performed, and the amount of EHDPs dissolved in *n*-octanol was determined as difference from the original concentration. Finally, the partition coefficient was calculated as the ratio between the EHDP concentration in aqueous solution and that in organic solvent.

Spectroscopy. Nuclear magnetic resonance (^{1}H NMR) spectra of EHDPs were recorded on a Bruker AC-200 spectrometer after solubilization in $CDCl_3$, except in the case of EHDPs from sinalbin, for which acetone was used as solvent. Peak positions are given in parts per million downfield from tetramethylsilane as an internal standard.

Cell Lines and Culture Conditions. Cells lines for the present study were K562 (human erythroleukemic cells), FL (murine erythroleukemic cells), Jurkat (human T-lymphoid cells), HeLa (human cervix carcinoma cells), H9 (human T-lymphoid cells), and H3-T1-1 cells (obtained by transfection of HeLa with a LTR-HIV-1-CAT plasmid) (Caputo et al., 1993). Standard conditions for cell growth were a-medium (GIBCO, Grand Island, NY), 50 mg/L streptomycin, and 300 mg/L penicillin, supplemented with 10−15% fetal calf serum (FCS) (Flow Laboratories, Inc., McLean, VA) in 5% CO_2, 80% humidity, and 37 °C.

***In Vitro* Antiproliferative Assays.** The activity of EHDPs was tested using two different approaches. In the first one, the *in situ* method, 22.5 U of myrosinase was directly added to 1 mL of 10% FCS culture medium containing increasing concentrations of GLs (0.1−500 μM) to which cells were subsequently added (5×10^4 cells in 1.5 cm tissue culture dishes). Alternatively, in the *pre-mix* method, the myrosinase-catalyzed hydrolysis of GLs was previously carried out in 100 mM phosphate buffer (pH 7.4) at 37 °C. After 30 min of incubation, the time needed for the complete hydrolysis of GLs, the buffer solution was diluted with an equal volume of 2× cell culture medium to which cells were subsequently added. Cell growth determinations were performed by electronic counting with a Model ZF Coulter Counter (Coulter Electronics, Inc., Hialeah, FL). Assays were carried out in triplicate, and usually counts differed by <5%.

RESULTS AND DISCUSSION

Choice of GLs and Their Isolation. For this study we chose 9 GLs (see Table 1) of a group of 12 available in our laboratory following two criteria: (i) their abundance in plant material; (ii) their chemical structure and stability. Our choice allowed a comparative evaluation of three main groups of GLs, namely aliphatic, aromatic, and thioaliphatic. We selected two alkenyl (sinigrin and gluconapin), two hydroxyalkenyl (progoitrin and *epi*-progoitrin), two aromatic (sinalbin and glucotropaeolin) and, finally, three side chain sulfur-containing glucosinolates (glucoerucin, glucocheirolin, and glucoraphenin). This set of GLs was considered sufficient for attaining at least preliminary information both on the main objective of this study and, because of the side chain diversity, on a possible structure−activity relationship. For instance, with the couples sinigrin/gluconapin, progoitrin/*epi*-progoitrin, and sinalbin/glucotropaeolin, we could establish the influence of the aliphatic chain length, the aliphatic epimer structure, and the aryl hydroxyl para-substitution, respectively, on the activity of these compounds. Finally, considering glucocheirolin, glucoerucin, and glucoraphenin, it should also be possible to verify the effectiveness of side chain sulfur-containing GLs and the influence of the sulfur oxidation state on the antitumoral activity of the EHDPs.

The investigation of side chain sulfur-containing GLs appeared to be particularly interesting because ITCs and nitriles derived from these compounds have been reported as interesting molecules with a potential role in cancer chemoprevention and protection (Zhang et al., 1992; Kore et al., 1993).

Production of EHDPs and Their Characterization. The quality and quantity of EHDPs generated by myrosinase-catalyzed hydrolysis of GLs depend on many parameters, such as pH, presence of specific ions and molecules, temperature, and substrate structure (Figure 1). In particular, it is important to emphasize that at neutral pH the formation of ITCs or VOTs, in the case of progoitrins, is definitely favored instead of nitriles (Gil and MacLeod, 1980; Uda et al., 1986a,b). This means that in our experimental conditions (pH 7.4, 37 °C), the formation of ITCs (VOTs) is almost quantitative. This finding, further demonstrated by capillary GLC and HPLC analyses, was confirmed by NMR spectroscopy of the EHDPs of each GL (data not shown). These results show that in the above conditions ITCs are the main products of the enzymatic hydrolysis of GLs, with the exception of the final EHDPs of the alkenylhydroxy GLs progoitrin and *epi*-progoitrin, which are the (5*S*) and (5*R*) VOTs, respectively, deriving from the hydroxy-ITC cyclization process. This finding has been discussed by Leoni et al. (1994), who also report the NMR spectrum of VOT obtained by hydrolyzing *epi*-progoitrin with immobilized myrosinase.

Antiproliferative Activity of Glucosinolates and Myrosinase. When used separately for assaying their cytotoxic activity, neither myrosinase nor native GLs with different chemical structures showed any effects on tumor cell growth when added up to 36 U/mL and 500 μM, respectively (Figure 3). These data appear particularly important both because a suitable experimental control is available and because we can confirm the low cellular toxicity of native GLs reported also by several authors (Fenwick et al., 1983; Bell, 1984; Lazzeri et al., 1993).

Antiproliferative Activity of Glucosinolate Hydrolysis Products. To better evaluate the effect of EHDPs the enzyme-mediated hydrolysis of GLs was carried out by two alternative approaches, namely the *in situ* and *pre-mix* methods (see Materials and Meth-

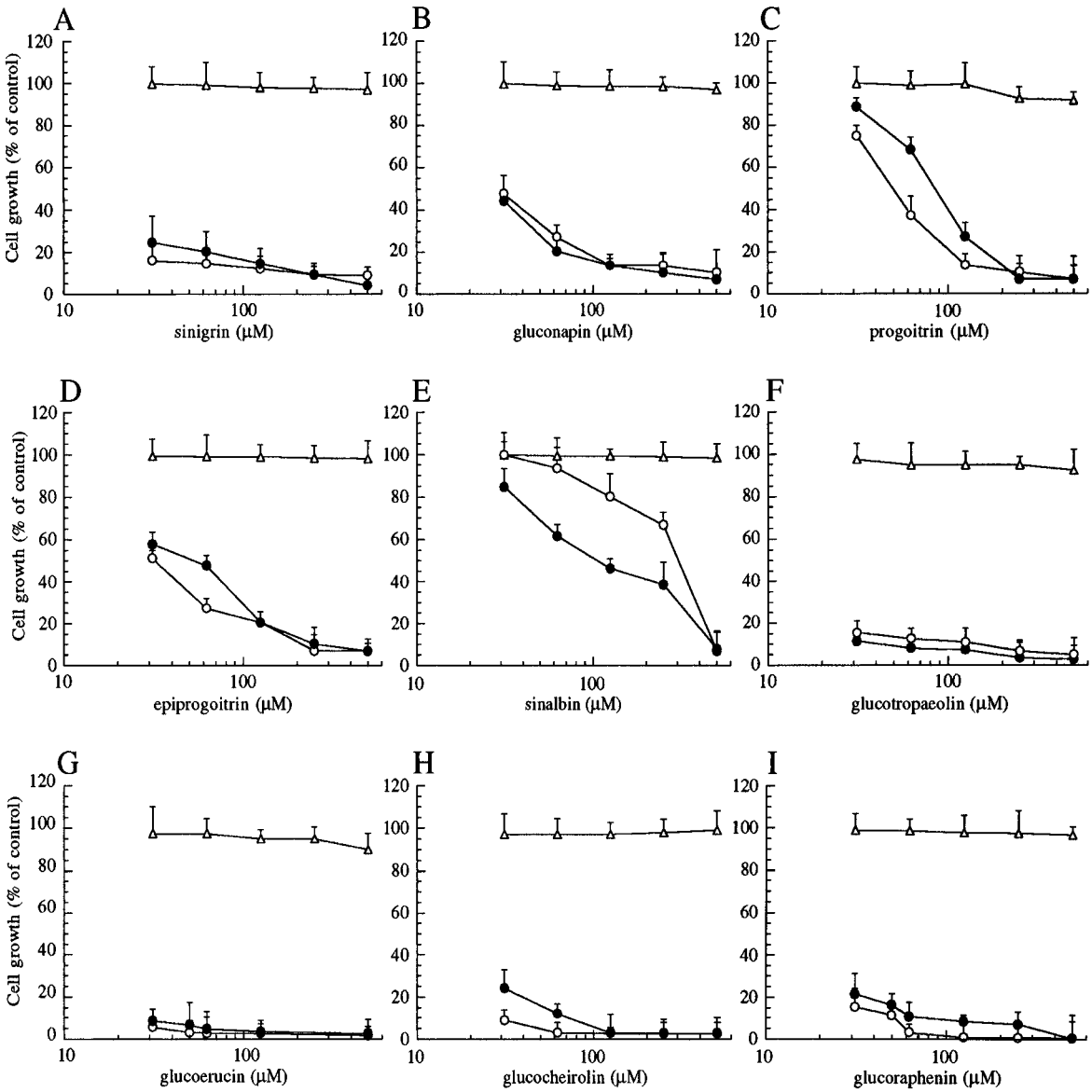


Figure 3. Effect of the high concentrations (30−500 μM) of glucosinolates (△) and their myrosinase-catalyzed hydrolysis products obtained following the *in situ* (○) or *pre-mix* procedures (●) (see Materials and Methods) on the growth of human erythroleukemic K562 cells. Data represent the average of four independent determinations, performed after 6 days of cells culture. (A) sinigrin; (B) gluconapin; (C) progoitrin; (D) *epi*-progoitrin; (E) sinalbin; (F) glucotropaeolin; (G) glucoerucin; (H) glucocheirolin; (I) glucoraphenin. See chemical structure and origin in Table 1.

ods). In the *in situ* method, the myrosinase-catalyzed reaction was performed in cell culture medium in direct contact to cells, whereas in the *pre-mix* procedure EHDPs were produced before addition to cells.

Figure 3 shows the antiproliferative effect both of GLs and their corresponding EHDPs on human erythroleukemic K562 cells. As it is clearly appreciable, in spite of the complete absence of activity demonstrated by native GLs, all of the EHDPs induce an evident inhibition of cell growth. With the aim to better evaluate the tumor cell growth inhibition activity of EHDPs, we made experiments using different GL concentration ranges between 30 and 500 μM (Figure 3) and between 0.1 and 15 μM (Figure 4). Considering the curve trends shown in Figure 4, it is evident that the EHDPs obtained from sinigrin and glucotropaeolin are the most active, especially if produced *in situ*. In fact, a concentration of 0.1 μM of these GLs hydrolyzed *in situ* gives inhibition around 50%. In this regard, it seems important to emphasize that the stoichiometry of the general hydrolytic reaction of GLs (Figure 1) establishes that the ITC final concentration, as weight/volume, is theoretically almost 30% of that of the starting GLs.

The cytotoxic effect of EHDPs can be easily estimated by evaluating the compound concentration required to cause a 50% inhibition of *in vitro* cell growth. The IC_{50} values of EHDPs, reported in Table 2, range between 0.1 and 320 μM. On the basis of these results, EHDPs can be divided into three groups reflecting their activity. The first group is constituted by the EHDPs from sinigrin, glucotropaeolin, and glucoerucin characterized by IC_{50} values lower than 5 μM; the second one includes EHDPs from glucocheirolin, glucoraphenin, and gluconapin (IC_{50} between 5 and 30 μM); and, finally, the third one includes the less active EHDPs with $IC_{50} >$ 30 μM, from *epi*-progoitrin, progoitrin, and sinalbin.

With the exception of sinalbin, both *in situ* and *pre-mix* strategies lead to almost superimposable antiproliferative effects, although slightly higher activity can be observed for the experiments made with the *in situ* strategy. This is quite evident, in different concentration ranges, for the EHDPs obtained from progoitrin,

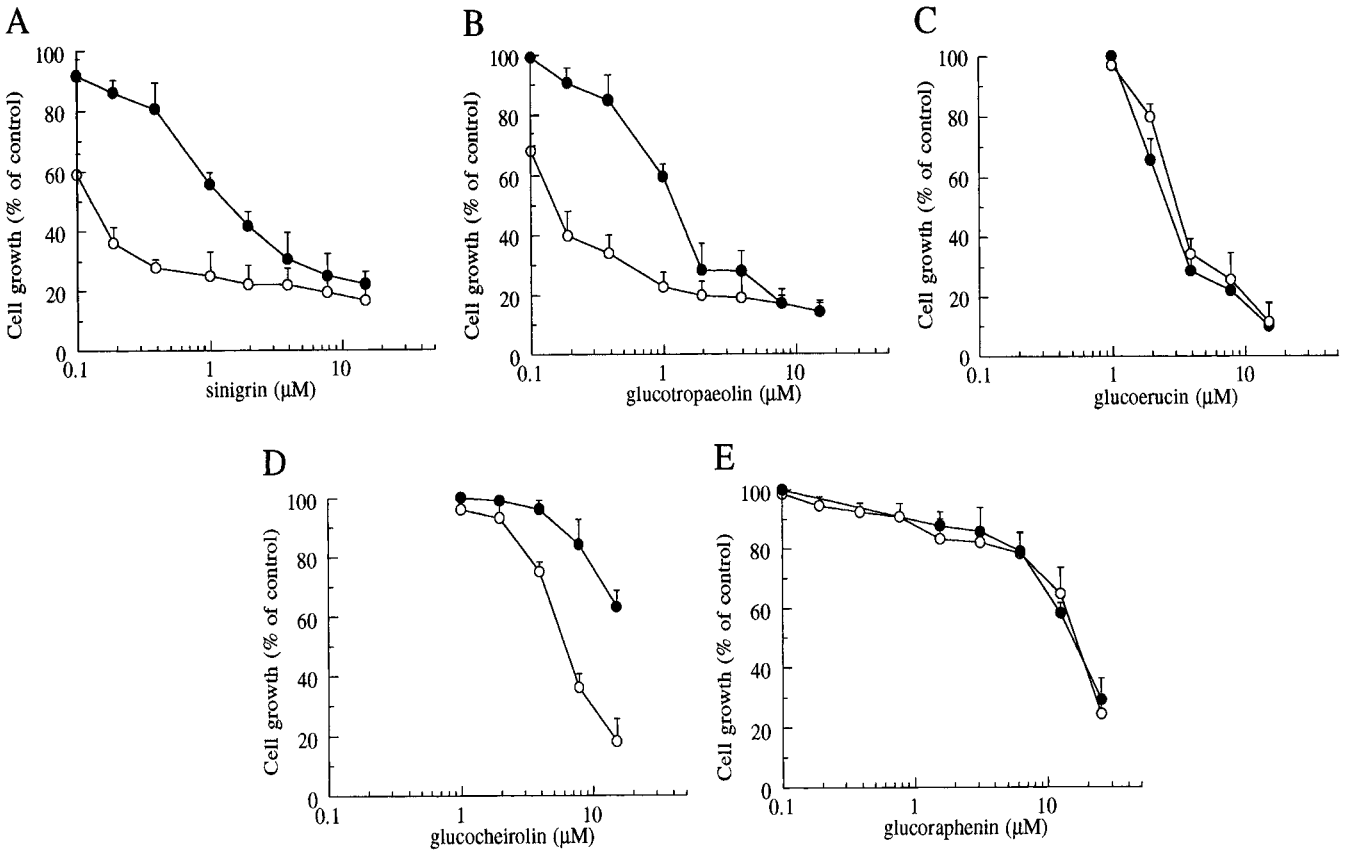


Figure 4. Effect of the low concentrations (0.1−15 μM) of myrosinase-catalyzed hydrolysis products obtained from sinigrin (A), glucotropaeolin (B), glucoerucin (C), glucocheirolin (D), and glucoraphenin (E) on the growth of human erythroleukemic K562 cells. Hydrolysis reactions were carried out following the *in situ* (○) or *pre-mix* procedures (●) described under Materials and Methods. Data represent the average of four independent determinations, performed after 6 days of cell culture.

Table 2. Effect of Myrosinase-Catalyzed Hydrolysis Products of Glucosinolates on Human Erythroleukemic K562 Cell Growth and Some of Their Properties

hydrolyzed compound	*in situ*[a] IC_{50}[b] (μM)	*pre-mix*[a] IC_{50}[b] (μM)	ITC partition coefficient	ITC retention time HPLC
sinigrin	<0.1	1.0	0.015	9.195
gluconapin	24.0	28.0	0.628	2.101
progoitrin	55.0[c]	95.0[c]	nd[d]	nd
epi-progoitrin	42.0[c]	60.0[c]	0.568[c]	2.165[c]
sinalbin	320.0	100.0	0.468	1.606
glucotropaeolin	0.1	1.5	0.006	12.313
glucoerucin	2.5	3.0	0.012	12.455
glucocheirolin	6.0	20.0	0.580	4.064
glucoraphenin	15.0	18.0	0.405	3.868

[a] The myrosinase-catalyzed hydrolysis of the indicated glucosinolate was performed following the *in situ* and *pre-mix* methods as indicated under Materials and Methods. [b] Inhibitory concentration 50%: compound concentration (μM) required to cause a 50% inhibition of *in vitro* growth of K562 cells. [c] These data have to be ascribed to (*R*) or (*S*) VOT. [d] nd, not determined.

epi-progoitrin (Figure 3C,D), sinigrin, glucotropaeolin (Figure 4A,B), and glucocheirolin (Figures 3H and 4D). In the case of progoitrin and *epi*-progoitrin, it is reasonable to suppose that the higher activity found when these GLs were hydrolyzed *in situ* is attributable to the effect of ITCs activity. These compounds, in fact, are produced in strict contact to their target(s), before further cyclization to VOT.

The activity of EHDPs produced *in situ* unequivocally demonstrates that myrosinase remains stable in tissue culture environments. Otherwise, many other enzymes are rapidly degraded in tissue culture conditions, due to the presence of many hydrolytic enzymes (i.e., serum proteinases). This finding confirms the extraordinary stability shown by this enzyme in both soluble and immobilized forms (Bjorkmann, 1976; Leoni et al., 1991). This property is typical of many glycoproteins, and in the case of myrosinase seems to be due to the protective action of the carbohydrate moiety, which is localized in the external part of the molecule.

In the case of sinalbin, the higher activity found when the *pre-mix* strategy is used could be explained only by admitting a further degradation of the *p*-hydroxybenzyl ITC to other more active compounds, which can be produced preferentially in *pre-mix* conditions in which the ITC cannot interact with the biological target(s). In this regard, it is known that the aromatic and indolyl ITCs are quite unstable in neutral or alkaline solutions. This seems to be particularly true for the *p*-hydroxybenzyl ITC, which on standing as in our *pre-mix* conditions could degrade to *p*-hydroxybenzyl alcohol and/or *p*-hydroxybenzyl thiocyanate (Tookey et al., 1980). Nevertheless, the NMR spectrum of the sinalbin EHDP(s), analyzed immediately after production, does not reveal the formation of any new degradation products. Other experiments aimed to clarify this aspect are still in progress.

Finally, to verify if EHDPs would be effective on other tumor cells, we tested the activity of glucoraphenin, taken as model compound, on cell lines of different origin and hystotype such as murine erythroleukemic FL, human T-lymphoid Jurkat, human cervix carcinoma HeLa, human T-lymphocyte H9, and human cervix carcinoma H3-T1-1, obtained by transfection of HeLa cells with LTR-HIV-1-CAT plasmid. We chose glucoraphenin because this GL generates an ITC having an intermediate activity and contains a sulfinyl group.

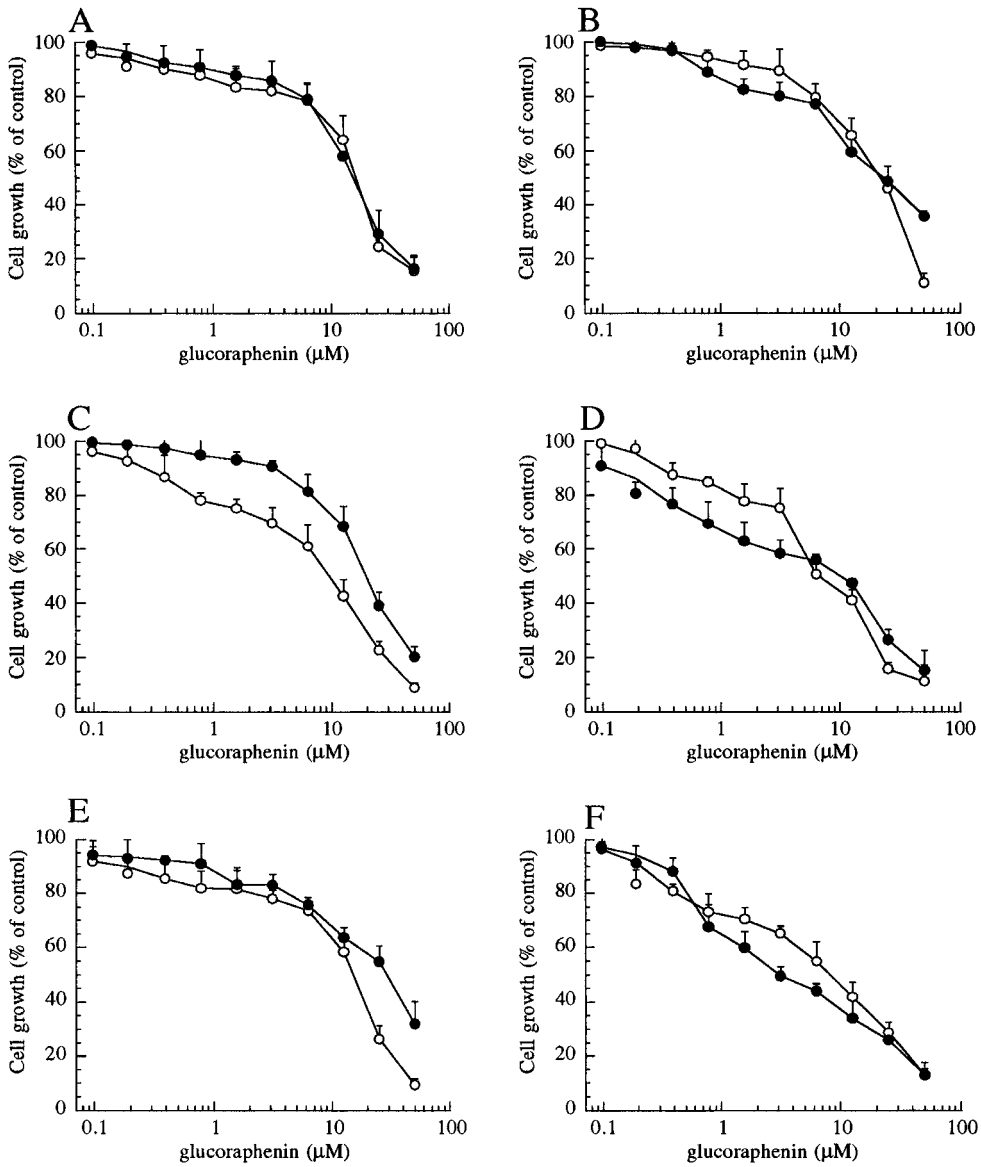


Figure 5. Differential antiproliferative activity of glucoraphenin hydrolysis products obtained by *in situ* (○) or *pre-mix* (●) procedures (see Materials and Methods) on human erythroleukemic K562 cells (A), murine erythroleukemic FL cells (B), human T-lymphoid Jurkat cells (C), human cervix carcinoma HeLa cells (D), human T-lymphocyte H9 cells (E), and human cervix carcinoma H3-T1-1 cells obtained by transfection of HeLa cells with a LTR-HIV-1-CAT plasmid (F). Data represent the average of three independent determinations.

Table 3. Effect of Myrosinase-Catalyzed Hydrolysis of Glucoraphenin on Different Tumor Cell Lines

cell line	*in situ*[a] IC_{50}[b] (μM)	*pre-mix*[a] IC_{50}[b] (μM)
K562	15	18
FLC	20	21
Jurkat	10	20
HeLa	6	10
H9	12	30
H3-T1-1	3	8

[a] The myrosinase-catalyzed hydrolysis of glucoraphenin was performed following the *in situ* and *pre-mix* method as indicated under Materials and Methods. [b] Inhibitory concentration 50%: compound concentration (μM) required to cause 50% cell growth inhibition.

Figure 5 shows that 4-(methylsulfinyl)but-3-enyl ITC displays a cytotoxic activity toward all of the cell lines tested without significant differences proved by the IC_{50} values reported in Table 3.

Structure−Activity Relationships. Although the limited number of GLs tested does not permit a definitive evaluation of the structure−activity relationship, this study seems to allow some preliminary conclusions on this aspect.

With the aim to find a rationale to our results and possibly a structure−activity relationship, we determined for all of the tested EHDPs their oil−water partition coefficient. We performed these analyses because this physical property is often strictly correlated with the pharmacological activity of many drugs (Lambert, 1993). The partition coefficient, being a measure of molecular lipophilicity or hydrophobicity, assumes in this case particular importance. In fact, due to the wide distribution in nature of lipids, which are also important constituents of biological membranes, it is not surprising that the oil−water partition coefficient is, in this case, a parameter of practical interest, providing information on the ability of EHDPs to pass through cell membranes. In this connection, the hydrophobicity of EHDPs could be one of the most important properties able to affect their bioactivity, especially in the case in which they have similar molecular structure. From the data of Table 2, in fact, it is evident that the most active ITCs

are characterized by low partition coefficients, independent from the group of GLs to which they belong.

Conclusions. The results of this study indicate that some GLs and EHDPs are able to inhibit tumor cells growth *in vitro*. In spite of these interesting findings, the mechanism by which the cytotoxic effect of EHDPs is developed must still be studied in detail and explained. Although it has been proposed that some ITCs inhibit protein synthesis and affect carbohydrate metabolism, the activity of these compounds seems to be mainly ascribed to the chemical reactivity of the ITC group, which can bind easily to proteins. In principle, some EHDPs could be considered as antimitotic substances, given their strong antiproliferative activity. In this regard, in the future it will be possible to produce a new generation of foods with good nutritional properties at the boundary with drugs, due to their increased content of a desired compound. In this view, within certain limits, GLs and EHDPs can be considered, in addition to be important phytochemicals in plant protection, good candidates for producing foods designed ("nutraceuticals") for preventing cancer.

LITERATURE CITED

Bell, J. M. Nutrients and toxicants in rapeseed meal: a review. *J. Anim. Sci.* **1984**, *58*, 996−1010.

Bjorkman, R. Properties and function of plant myrosinases. In *The Biology and Chemistry of the Cruciferae*; Vaugham, J. G., MacLeod, A. J., Jones, B. M. G., Eds.; Academic Press: London, 1976; pp 191−205.

Caputo, A.; Balboni, P. G.; Manservigi, R.; Bozzini, R.; Marconi, P. C.; Rossi, C.; Zucchini, S.; Barbanti-Brodano, G.; Grossi, M. P. Possible approaches of gene therapy of AIDS. *Minerva Biotecnol.* **1993**, *5*, 322−325.

Cohen, L. A. Diet and cancer. *Sci. Am.* **1987**, *257*, 42−48.

Gil, V.; MacLeod, A. J. The effects of pH on glucosinolate degradation by a thioglucoside glucohydrolase preparation. *Phytochemistry* **1980**, *19*, 2547−2551.

Graham, S.; Mettlin, C. Fiber and other constituents of vegetables in cancer epidemiology. In *Nutrition and Cancer. Aetiology and Treatment*; Newell, G. R., Ellison, N. M., Eds.; Raven Press: New York, 1981; pp 189−198.

Graham, S.; Dayal, H.; Swanson, M.; Mittleman, A.; Wilkinson, G. Diet in the epidemiology of cancer in the colon and rectum. *J. Natl. Cancer Inst.* **1978**, *61*, 709−714.

Greer, M. A. The natural occurrence of goitrogenic agents. *Recent Prog. Horm. Res.* **1962**, *18*, 187−212.

Greer, M. A.; Deeney, J. M. Antithyroid activity elicited by the ingestion of pure progoitrin, a naturally occurring thioglucoside of the turnip family. *J. Clin. Invest.* **1959**, *38*, 1465−1474.

Fenwick, G. R.; Heaney, R. K.; Mullin, W. J. Glucosinolates and their breakdown products in food and food plants. *CRC Crit. Rev. Food Sci.* **1983**, *18*, 123−201.

Haensezel, W.; Locke, F. B.; Segi, M. A case-control study of large bowel cancer in Japan. *J. Cancer Inst.* **1980**, *64*, 17−22.

Iori, R.; Leoni, O.; Palmieri, S. A polarographic method for the rapid determination of total glucosinolates and free glucose of cruciferous material. *Anal. Biochem.* **1983**, *134*, 195−198.

Kjaer, A.; Skrydstrup, T. Selenoglucosinolates synthesis and enzymatic hydrolysis. *Acta Chem. Scand.* **1987**, *B41*, 29−33.

Kore, A. M.; Gayland, F. S.; Matthew, A. W. Purification of the ω-(methylsulfinyl)alkyl glucosinolate hydrolysis products: 1-isothiocyanate-3-(methylsulfinyl)propane, 1-isothiocyanato-4-(methylsulfinyl)butane, 4-(methylsulfinyl)butanenitrile and 5-(methylsulfinyl)pentanenitrile from broccoli and *Lesquerella fendleri*. *J. Agric. Food Chem.* **1993**, *41*, 89−95.

Lambert, J. W. Modelling oil-water partioning and membrane permeation using reverse-phase chromatography. *J. Chromatogr. A* **1993**, *656*, 469−484.

Lanzani, A.; Piana, G.; Piva, G.; Cardillo, M.; Rastelli, A.; Jacini, G. Changes in *Brassica napus* progoitrin induced by sheep rumen fluid. *J. Am. Chem. Soc.* **1974**, *96*, 517−518.

Larsen, P. O. Glucosinolates. In *The Biochemistry of Plants*; Conn, E. E., Stumpf, P. K., Eds.; Academic Press: New York, 1981; Vol. 26, pp 59−70.

Lazzeri, L.; Tacconi, R.; Palmieri, S. In vitro activity of some glucosinolate and their reaction products toward a population of the nematode *Heterodera schactii*. *J. Agric. Food Chem.* **1993**, *41*, 825−829.

Lee, H. P.; Gourley, L.; Duffy, S. W.; Esteve, J.; Lee, J.; Day, N. E. Colorectal cancer and diet in the asian population−a case-control study among Singapore Chinese. *Int. J. Cancer* **1989**, *43*, 1007−1016.

Leoni, O.; Iori, R.; Palmieri, S. Immobilization of myrosinase on membrane for determining the glucosinolate content of cruciferous material. *J. Agric. Food Chem.* **1991**, *39*, 2322−2326.

Leoni, O.; Felluga, F.; Palmieri, S. The formation of 2-hydroxybut-3-enyl cyanide from (2*S*)-2-hydroxybut-3-enyl glucosinolate using immobilized myrosinase. *Tetrahedron Lett.* **1993**, *49*, 7967−7970.

Leoni, O.; Marot, C.; Rollin, P.; Palmieri, S. Preparation of (5*R*)-5-vinyloxazolidine-2-thione from natural *epi*-progoitrin using immobilized myrosinase. *Tetrahedron Asymm.* **1994**, *5*, 1157−1160.

Manousos, O.; Day, N. E.; Trichopoulos, D.; Gerevassilis, F.; Tzonou, A.; Polychronopoulou, A. Diet and colorectal cancer: a case-control study in Greece. *Int. J. Cancer* **1983**, *32*, 1−5.

Marangosa, A.; Hill, R. The hydrolysis and absorption of thioglucosides of rapeseed meal. *Proc. Nutr. Soc.* **1974**, *33*, 90A.

Marx, J. How cells cycle toward cancer. *Science* **1993**, *263*, 319−321.

Nugon-Baudon, L.; Szylit, O.; Raibaud, P. Production of toxic glucosinolate from rapeseed meal by intestinal microflora of rat and chicken. *J. Sci. Food Agric.* **1988**, *43*, 299−308.

Nugon-Baudon, L.; Rabot, S.; Szylit, O.; Raibaud, P. Glucosinolates toxicity in growing rats: interactions with the hepatic detoxification system. *Xenobiotica* **1990**, *20*, 223−230.

Official Journal of the European Community. Regulation (EEC) 1864/90, June Enclosure VIII, J. O. n. L170. **1990**, 27−34.

Palmieri, S. Bioactivity of some glucosinolates and their derived breakdown products obtained using immobilized myrosinase. *Proceedings of the International Euro Food Tox IV Conference*; 1994; pp 40−45.

Palmieri, S.; Leoni, O.; Iori, R. A steady state kinetics studies of myrosinase with direct ultraviolet spectrophotometric assay. *Anal. Biochem.* **1982**, *123*, 320−324.

Palmieri, S.; Iori, R.; Leoni, O. Myrosinase from *Sinapis alba* L.: a new method of purification for glucosinolate analyses. *J. Agric. Food Chem.* **1986**, *34*, 138−140.

Pessina, A.; Thomas, R. M.; Palmieri, S.; Luisi, P. L. An improved method for the purification of myrosinase and its physicochemical characterization. *Arch. Biochem. Biophys.* **1990**, *280*, 383−389.

Stoewsand, G. S.; Babish, J. B.; Munson, L. Protective effect of dietary Brussels sprouts against mammary carcinogenesis in Sprague-Dawley rats. *Cancer Lett.* **1988**, *39*, 199−207.

Streicher, H.; Thiem, J.; Rollin, P.; Iori, R.; Palmieri, S. Influence of the substrate sugar structure on myrosinase activity demonstrated by using native and synthetic glucotropaeolins. *Proceedings*, 9th International Rapeseed Congress, Cambridge, U.K.; 1995; Vol. 3, pp 958−960.

Thies, W. Isolation of sinigrin and glucotropaeolin from cruciferous seed. *Fat Sci. Technol.* **1988**, *8*, 311−314.

Tookey, H. L.; Van Etten, C. H.; Daxenbichler, M. E. Glucosinolates. In *Toxic Constitutents of Plants Foodstuff*, 2nd ed.; Liener, I. E., Ed.; Academic Press: New York, 1980; pp 103−142.

Uda, Y.; Kurata, T.; Arakawa, N. Effects of pH and ferrous ion on the degradation of glucosinolates by myrosinase. *Agric. Biol. Chem.* **1986a**, *50*, 2735−2740.

Uda, Y.; Kurata, T.; Arakawa, N. Effects of thiol compounds on the formation of nitriles from glucosinolates. *Agric. Biol. Chem.* **1986b**, *50*, 2741−2746.

Visentin, M.; Tava, A.; Iori, R.; Palmieri, S. Isolation and identification of *trans*-4-(methylthio)-3-butenyl glucosinolate from radish roots (*Raphanus sativus*). *J. Agric. Food Chem.* **1992**, *40*, 1687−1691.

Wattemberg, L. W. Inhibition of carcinogenic effects of polycyclic hydrocarbons by benzyl isothiocyanate and related compounds. *J. Natl. Cancer Inst.* **1977**, *58*, 395−398.

Wattemberg, L. W. Inhibition of carcinogenesis by minor dietary constituents. *Cancer Res.* **1992**, *52* (Suppl.), 2085s−2091s.

Zhang, G. Y.; Talalay, P.; Cho, C.; Posner, G. H. A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 2399−2403.

Received for review June 12, 1995. Accepted January 3, 1996.[⊗] This research was supported by the Italian Ministry of Agriculture, Food and Forestry Resources in the framework of the Project "Resistenze genetiche delle piante agrarie agli stress biotici ed abiotici" Subproject 17 and by AIRC.

JF9503523

[⊗] Abstract published in *Advance ACS Abstracts*, March 1, 1996.