METABOLISM OF GLUCOSINOLATES

Birthe Bjerg[1], Piotr W. Kachlicki[2], Lone Melchior Larsen[1]
and Hilmer Sørensen[1]

[1]Chemistry Department, Royal Veterinary and Agricultural University,
40 Thorvaldsensvej, DK-1871 Frederiksberg C, Denmark.

[2]Polish Academy of Science, Institute of Plant Genetics,
Strzeszyńska 34, 60-479 Poznań, Poland.

ABSTRACT

The metabolism of glucosinolates has been investigated, including the properties of thioglucoside glucohydrolase EC 3.2.3.1 (myrosinases). New column chromatographic techniques have been used for group separation and sample preparation prior to HPLC analysis of amino acids and glucosinolates. Pattern and content of glucosinolates in the samples have been determined by HPLC of intact glucosinolates. The properties of myrosinase isoenzymes have been investigated by traditional methods of enzyme analysis, enzyme kinetics and FPLC.

Glucosinolates are biosynthetically formed in the plants from structurally related amino acids. Tracer experiments with different ^{14}C-labelled precursors have been used in these studies. For Brassica species especially methionine, tryptophan and phenylalanine are important precursors which in complicated processes are transformed into glucosinolates by multi enzyme complexes. These processes involve (a) and/or (b)-(g):

(a) chain-lengthening via incorporation of C-2 units in the α-keto acid chain elongation process
(b) formation of terminal double bond in glucosinolates derived from methionine ± elimination of the methylthio group
(c) oxidation of the methylthio group into a methylsulphinyl group (with (R)-configuration only) or a methylsulphonyl group
(d) hydroxylation at C-2 in the glucosinolate side chain. Both (2S)- and (2R)-configuration are known
(e) hydroxylation/oxidation of aromatic side-chains
(f) introduction of methoxy-, sulpho- or glycoside groups in the side chains
(g) acylation in the thioglucose part with e.g. cinnamoyl derivatives.

Glucosinolates are catabolized in the plants and amines and γ-glutamyl derivatives thereof are in some cases found as products. It is also found that myrosinases occur with glucosinolates in all of the hitherto investigated glucosinolate-containing plants. Myrosinases catalyze in vitro the transformation of glucosinolates into several different products whereas reliable informations on such processes in vivo are difficult to obtain

INTRODUCTION

Glucosinolate metabolism is of utmost importance owing to the many diverse interests associated with glucosinolate containing plants. Examples are plant-insect relations (Larsen et al., 1985; Holm et al., 1985), plant-fungal relations (Buchwaldt et al., 1985), possibilities of changing the content and composition of glucosinolates in oilseed rape and other crops by plant breeding (Møller et al., 1985), and especially the effects glucosinolates and their degradation products have on animals (Bjerg et al., 1987a). The pungency, flavor and many undesirable toxic manifestations of different crucifers are associated with glucosinolates, affecting, e.g., the technically and economically important oils and proteins from oilseed rape. These properties of both glucosinolates and degradation products thereof are responsible for much of the interest in glucosinolates and thus the metabolism of glucosinolates (Figure 1).

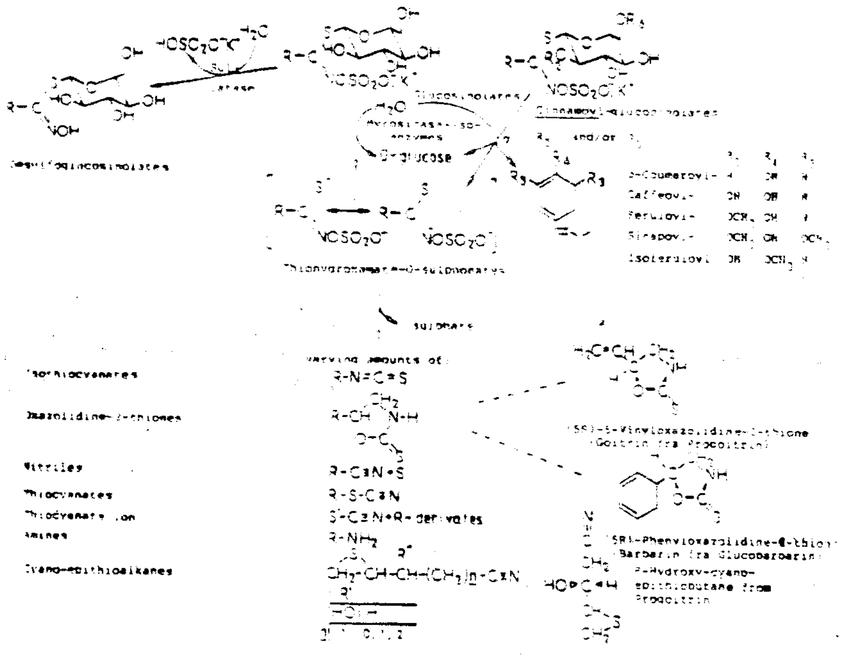


Figure 1. Structures and names of glucosinolates, desulfoglucosinolates and other glucosinolate degradation products in vitro.

OCCURRENCE AND METABOLISM OF GLUCOSINOLATES

Glucosinolates co-occur with myrosinase isoenzymes (thioglucoside glucohydrolase EC 3.2.3.1) in all of the plants of the order Capparales hitherto investigated (Dahlgren, 1975; Rodman, 1981). A restricted number of other plants have been occasional reported as containing glucosinolates but some of these reports have been seriously questioned (Larsen et al., 1983; Bjerg et al., 1987b).

CAPPARALES (in the botanical broad sence)	Brassicaceae (Cruciferae)
	Capparaceae
	Resedaceae
	Moringaceae
	Tropaeolaceae
	Tovariaceae
	Limnanthaceae
	Bretschneideraceae
	Salvadoraceae
	Pentadiplandraceae
	Gyrostemonaceae
	Bataceae

The metabolism of glucosinolates comprises their formation (biosynthesis) and degradation (catabolism) (Figure 2).

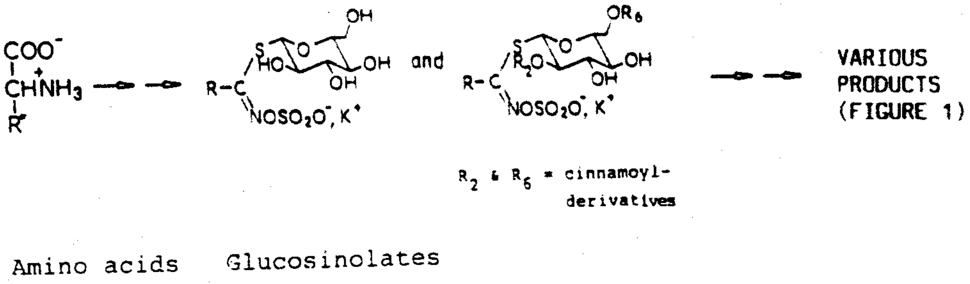


Figure 2. Biosynthesis of glucosinolates from amino acids and catabolism to various products.

Biosynthesis of glucosinolates is only known to occur in plants (vide supra) and with amino acids as precursors in complicated processes which can involve one or more of the processes (a) to (g) as mentioned in the abstract (Møller et al., 1985). Direct incorporation of the amino acid precursors into the corresponding glucosinolate with preservation of the amino acid nitrogen has been shown to occur in some few cases. Desulfoglucosinolates (Figure 1), thiohydroximic acids and aldoximes (Figure 3) are known intermediates but lots of informations on the biosynthesis are not available (Møller et al., 1985).

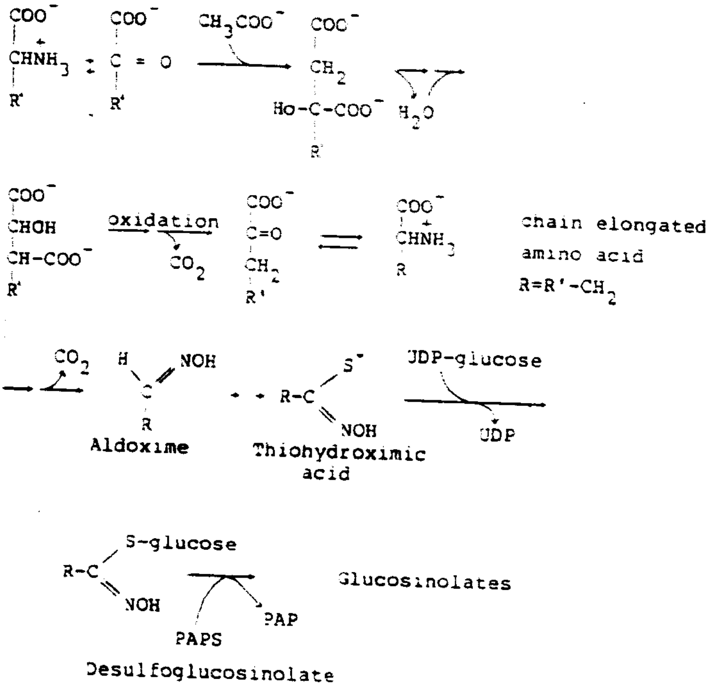


Figure 3. Illustration of biosynthetic steps involved in biosynthesis of glucosinolates.

Catabolism of glucosinolates can occur in myrosinase-catalyzed reactions in vitro resulting in several well known products (Figure 1). These reactions occur fast when glucosinolates and myrosinases are mixed in the presence of water, e.g. in autolysis processes (Olsen and Sørensen, 1981). In the intact plant tissues myrosinases and glucosinolates seem to be separated but an active catabolism and biosynthesis occur in plants (vide infra). Fungal thioglucosidases (Petroski and Kwolek, 1985) and most likely different types of enzymes in the digestive tract of animals catalyze glucosinolate degradation (Eggum et al., 1985). These degradation and/or absorption studies of glucosinolates in/and from the digestive tract of different animals have been continued (Bjerg et al., 1987a). The results obtained show that glucosinolates have a fast turnover and metabolism in the animals. However, we need to realize, that our knowledge of the metabolism in animals of the glucosinolates and/or their degradation products absorbed in the blood is limited and much more research in this field is required.

GLUCOSINOLATE METABOLISM IN VIVO

Investigations of the metabolism of glucosinolates in vivo require that we avoid ulceration and mixing of myrosinases and glucosinolates (vide supra). Special tracer techniques based on ^{14}C- and/or ^{13}C-labelled precursors are needed (vide infra). Information can also be obtained from investigations of the types and concentrations of the metabolites in the plants if reliable and sensitive methods of analysis are used (Bjerg et al., 1984; Bjerg et al., 1987c). Based on these methods the change in content of glucosinolates, amino acids, amines and related products have been followed during germination and development of several plant species. The plants included in these studies have comprised: Sinapis arvensis L., S. alba L. cv. Savor, Brassica nigra (L.) Koch cv. Junius, B. campestris L. cv. Candle, B. napus L. cv. Erglu and cv. Gulliver, 7 cultivars of B. oleracea L. and some other wild growing plants.

Figure 4 shows results from investigations of the change in the concentrations of glucosinolates in seed and seedlings of S. alba and B. nigra.

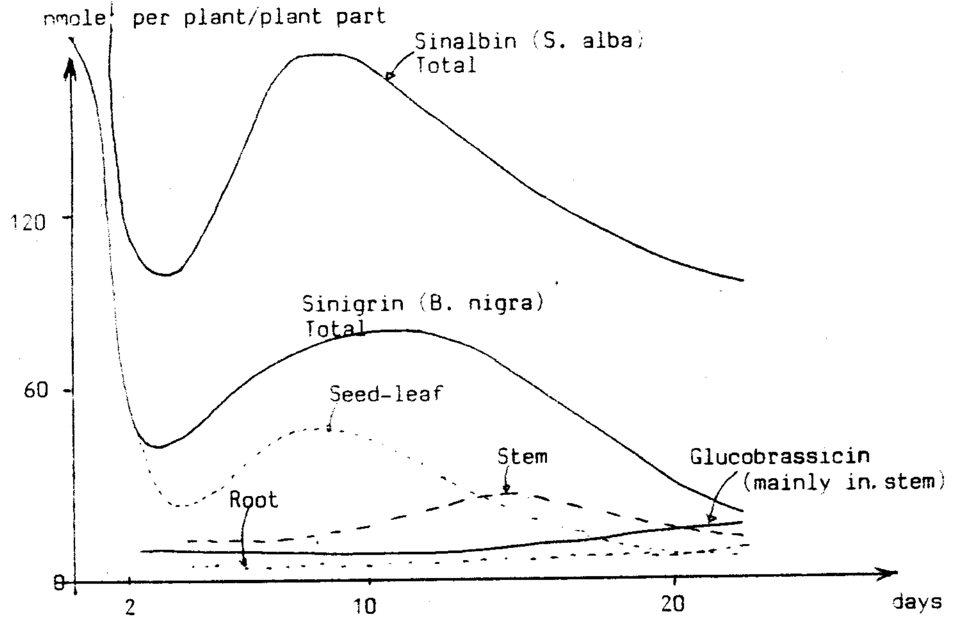


Figure 4. Concentrations of glucosinolates in seed and different parts of S. alba and B. nigra seedling.

The glucosinolates are degraded during germination and appreciable biosynthesis occurs after few days of germination. Of the indol-3-ylmethylglucosinolates (Figure 7), 4-hydroxyglucobrassicin occurs especially in seeds and seed-leaves. 4-Methoxyglucobrassicin reached the highest concentration in 14 days old seed-leaves, and neoglucobrassicin reached the highest concentration in stems and roots of 4 to 7 days old plants. The results obtained with the other investigated Brassica species (vide supra) followed the same trend. Several glucosinolates are, however, accumulated in these plants (Bjerg et al., 1987c) which gives a much more complicated picture than shown for sinigrin in B. nigra (Figure 4).

Of the various possible catabolic products of glucosinolates (Figure 2), it has been found that several plants accumulate biogenic amines as shown in Figure 5.

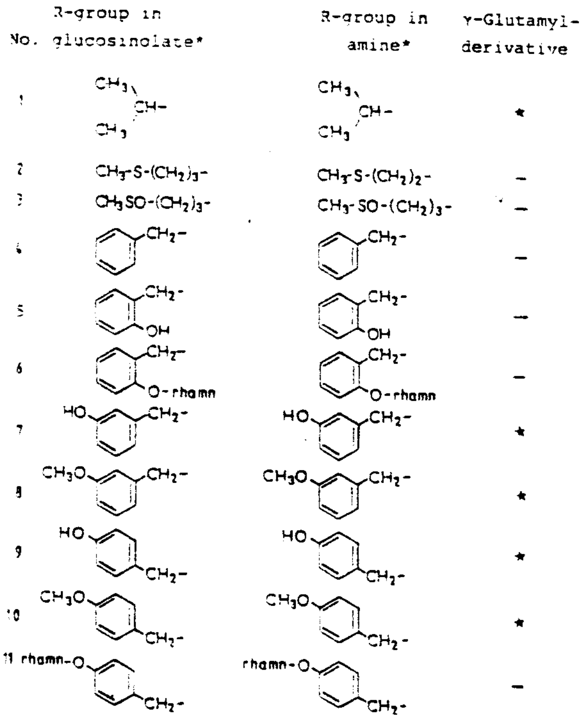


Figure 5. Biogenic amines formed from amino acids with glucosinolates as intermediates. * For structures of glucosinolates, structurally related amines and eventually γ-glutamyl-derivative, see Figure 6.

The biogenetic relationship between glucosinolates and amines as well as further transformations of the amines (Figure 6) have been the subject for several investigations (Olsen and Sørensen, 1980; Larsen et al., 1984).

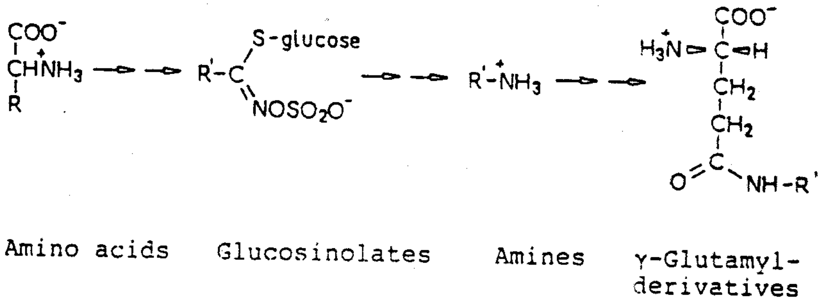


Figure 6. Amino acids as precursors for glucosinolates which can be transformed into amines and γ-glutamyl-derivatives thereof.

Apart from the above mentioned results, only little information is available on other possible in vivo transformations (vide infra) corresponding to these known as in vitro products of myrosinase-catalyzed glucosinolate hydrolysis (Figure 1).

Sulfatase-catalyzed glucosinolate hydrolysis is also a well known in vitro process widely used for analytical purposes (Bjerg et al., 1987c). The sulfatases have not been found as constituents of plants, whereas desulfoglucosinolates (Figure 1) are known intermediates in the biosynthesis of glucosinolates (Figure 3).

Myrosinases are thus the enzymes which have attracted special interest in relation to glucosinolate catabolism. Recently new and highly efficient methods of analysis have been developed for studies of these enzymes (Buchwaldt et al., 1986). Thereby it has also been possible to obtain additional information on myrosinase isoenzymes, and it gives possibilities for more careful studies of the complicated myrosinase kinetics which seem to involve an allosteric mechanism (Petroski and Kwolek, 1985) in addition to a possible ascorbic acid effect on the enzymes.

Biosynthesis of glucosinolates occurring in seeds of oilseed rape (Brassica napus cv. Gulliver and cv. Quinta) have been investigated with use of different ^{14}C-labelled precursors and the cotton-thread technique. Previously it has been shown that glucosinolates are present in the seeds before the compounds giving the major contribution to the seed weight (Rahman et al., 1986). It is also known that the siliques are photosynthetic organs which produce carbon assimilates not only for their own growth but also for the seed they contain. It was, however, found important to have additional informations on the site of the glucosinolate biosynthetic steps (Figure 3) and the sequence and details about the biosynthesis of individual rapeseed glucosinolates (Figure 7).

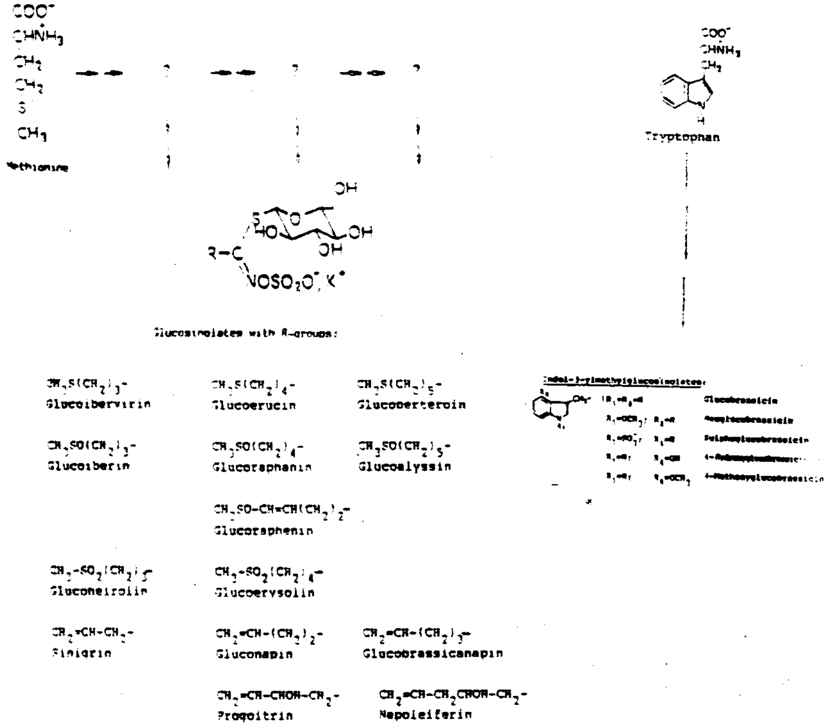


Figure 7. Glucosinolates biosynthetically derived from methionine and tryptophan.

The experiments performed have comprised supply of the precursors immediately before the silique. In another series of experiments the precursors were supplied immediately before the leaf. The incorporation of ^{14}C into the glucosinolates have been investigated with the precursors: 2-^{14}C-acetate; U-^{14}C-methionine; U-^{14}C-aspartic acid; U-^{14}C-UDP-glucose and U-^{14}C-glucose. Both total activity and specific activity (activity per umole of glucosinolate) have been determined using preparative HPLC and the techniques described elsewhere (Bjerg and Sørensen, 1987).

In accordance with the biosynthetic sceme (Figure 3) acetate was a most efficient precursor for the rapeseed glucosinolates (Figure 7; Bjerg et al., 1987c), followed by UDP-glucose and methionine. An appreciable lower efficiency was obtained with aspartic acid and especially glucose. The best results were obtained by supply of the precursors immediately before the silique, except for 2-^{14}C-acetate where supply immediately before the leaf gave better results. The use of unripe siliques (20-30% DM of the seeds) was preferable to that of later development (more than 55-60% DM of the seeds). Most of the glucosinolates and activity in the glucosinolates were present in the seeds but minor amounts were also found in the siliques.

Table 1. Relative activity in total rapeseed glucosinolates from biosynthesis using different ^{14}C-precursors.

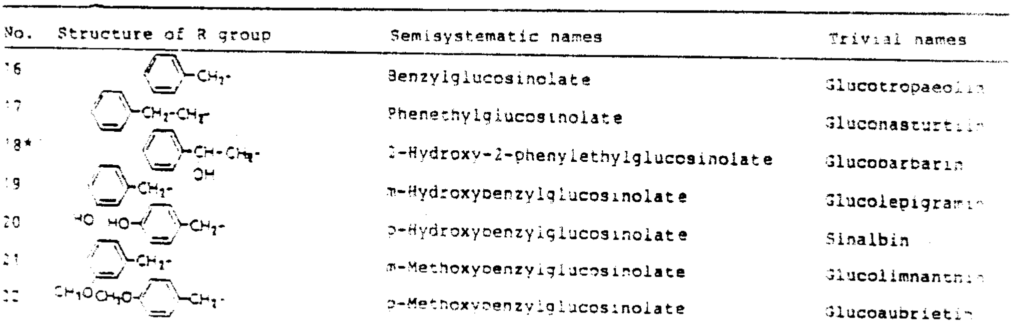
	U-^{14}C- UDP-gluc	2-^{14}C- Acetate	U-^{14}C- Met	U-^{14}C- Asp	U-^{14}C- Glucose
Supply immediately before the silique	330	100	203	113	30
Supply immediately before the leaves	100	243	-	86	17

Table 2. Specific activity (dpm in relation to glucosinolate concentration) from preparative HPLC and use of different precursors.

	Precursor		
Glucosinolate	U-^{14}C-Asp	2-^{14}C-Acetat	U-^{14}C-Met
Progoitrin	3.9	59.1	67.8
Glucoraphanin	10.8	198.0	200.0
Gluconapin	7.4	123.6	298.2
Glucobrassicanapin	6.8	46.0	51.9

The preliminary results presented in Table 2 show a higher specific activity in glucoraphanin than in gluconapin which have a much higher specific activity than progoitrin. If the glucosinolates are produced by catalysis of the same multi enzyme complex, the results indicate that the hydroxylation occur relatively late in the biosynthetic sequence.

Figure 8 shows the structures and names for some glucosinolates biosynthetically derived from the amino acids phenylalanine or tyrosine. In Limnanthes douglasii, the glucosinolates Nos. 19 and 21 are accumulated together with the corresponding amines and γ-glutamylderivatives thereof (Figure 5 and 6). This was found of interest to consider as examples of both m-hydroxylation and glucosinolate metabolism in vivo (Figure 6).



No.	Structure of R group	Semisystematic names	Trivial names
16		Benzylglucosinolate	Glucotropaeolin
17		Phenethylglucosinolate	Gluconasturtiin
18*		2-Hydroxy-2-phenylethylglucosinolate	Glucobarbarin
19		m-Hydroxybenzylglucosinolate	Glucolepigramin
20		p-Hydroxybenzylglucosinolate	Sinalbin
21		m-Methoxybenzylglucosinolate	Glucolimnanthin
22		p-Methoxybenzylglucosinolate	Glucoaubrietin

Figure 8. Structures and names of some glucosinolates biosynthetically derived from phenylalanine of tyrosine.

Table 3 shows results obtained by supply of the following precursors to leaves of L. douglasii: 2-^{14}C-acetate; U-^{14}C-aspartate; U-^{14}C-tyrosine; U-^{14}C-Phenylalanine.

Table 3. Counts per min. relative to applied precursors and amount of plant material for compounds isolated from preparative HPLC.

	Precursor				
	2-^{14}C-	U-^{14}C-	U-^{14}C-	U-^{14}C-	U-^{14}C-
Compound	Acetate	Asp	Glucose	Tyr	Phe
Glucolepigramin	74	156	644	360	2.693
Glucolimnanthin	160	170	2.303	589	7.355
m-Hydroxybenzylamine	37	57	178	59	890
m-Methoxybenzylamine	81	122	1.112	116	3.897
γ-Glu-m-Hydroxybenzylamine	196	185	144	74	357
γ-Glu-m-Methoxybenzylamine	245	416	237	155	407

The results show that phenylalanine is far the best precursor for biosynthesis of these m-hydroxylated and m-methoxylated compounds (Table 3). The in vivo metabolism of glucosinolates and structurally related biogenic amines (Figure 5 and 6) is also revealed by these experiments, and confirm previous observations (Olsen and Sørensen, 1980; Larsen et al., 1984; and refs. cited therein).

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