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N^5-(4-HYDROXYBENZYL) GLUTAMINE, 4-HYDROXYBENZYLAMINE AND 4-HYDROXYBENZYLGLUCOSINOLATE IN *SINAPIS* SPECIES

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Key Word Index—*Sinapis alba*; *S. arvensis*; Cruciferae; 4-hydroxybenzylglucosinolate; sinalbin; 4-hydroxybenzylamine; N^5-(4-hydroxybenzyl) glutamine; γ-glutamyl-4-hydroxybenzylamine; glucosinolate catabolism; amine metabolism; chemotaxonomy.

Abstract—N^5-(4-hydroxybenzyl) glutamine has been isolated from *Sinapis alba* L. and *S. arvensis* L. The identification is based on data obtained by HPLC, paper chromatography, high voltage electrophoresis, UV and NMR spectroscopy of the amide and its degradation products. The amide occurs together with 4-hydroxybenzylamine and sinalbin in both seeds and seedlings of the *Sinapis* species. This co-occurrence is briefly discussed in relation to chemotaxonomy, glucosinolate catabolism and amine metabolism.

INTRODUCTION

Glucosinolates occur in all members of the order Capparales and in a few other plant families [1, 2]. They are of special interest in connection with chemotaxonomy [2, 3] and nutritive value of Cruciferous plants [4]. Much is known about their structure, properties [5] and biosynthesis [6], whereas only little information is available on glucosinolate catabolism [5]. The co-occurrence of 4-hydroxybenzylglucosinolate (sinalbin; **1**) and 4-hydroxybenzylamine (**2**) in *S. alba* has been described [7] as well as the co-occurrence of other glucosinolates and their structurally related amines in other plants [8–10]. The present work is a continuation of our previously described investigations of amines biosynthetically derived from glucosinolates (ref. [10] and refs. cited therein). We here report on the isolation and identification of N^5-(4-hydroxybenzyl)glutamine (γ-glutamyl-4-hydroxybenzylamine; **3**), its occurrence in *Sinapis* species and its possible relation to the glucosinolate catabolism.

RESULTS AND DISCUSSION

Extracts containing the low MW constituents present in seeds and seedlings of *S. alba* and *S. arvensis* were prepared by well established methods which secure efficient and immediate inactivation of myrosinases (thioglucoside glucohydrolase, EC 3.2.3.1). The extracts were separated by ion-exchange chromatography into three groups: cations including amines, neutral plus acidic amino acids and glucosinolates plus other anions. Analysis using PC, HVE, HPLC and an amino acid analyser revealed the presence of appreciable amounts of an unknown neutral amino acid (**3**). Isolation of **3** was performed by column chromatography, prep. PC, prep. HVE and recrystallization from water. R_f-values in 2D-PC and the reaction of **3** with ninhydrin were as found for γ-aminobutyric acid, and its mobility in HVE at pH 1.9 was similar to that of aspartic acid. Hydrolysis in diluted hydrochloric acid transformed **3** into a 1:1 mixture of glutamic acid and **2**. From these results and the retention times on HPLC and the amino acid analyser columns **3** was characterized as the γ-glutamyl derivative of 4-hydroxybenzylamine, i.e. N^5-(4-hydroxybenzyl)glutamine. The structure of **3**, previously described as a constituent of *Fagopyrum esculentum* Moench [11] was confirmed by UV and NMR spectroscopy and comparison with corresponding results obtained with authentic **2** [10, 12]. In *F. esculentum*, **3** co-occurs with **2** but in this plant it has nothing to do with glucosinolate catabolism, since glucosinolates are not present.

The glucosinolate (**1**) and the amine (**2**) were also isolated from both *Sinapis* species by well established methods [10]. R_t for **3** in HPLC [13] was 15 min compared to 10 min for tyrosine and 23 min for tryptophan. R_t for **3** on the amino acid analyser was between that obtained for leucine and tyrosine. Group separation of low MW constituents in extracts from 0.5 g plant samples, HPLC analysis and quantitative determinations of aromatic amino acids, **2** and **1** have been described previously [13]. The results are presented in Table 1.

Consideration of the structures of **1**, **2** and **3** shows that they may be metabolically interrelated. It is likely that **3** is a catabolic product of **2** but the enzymes required for this transformation and the possible involvement of myrosinases in the transformation of **1** into **2** have not yet been shown. However, the results now obtained support previously reported observations which indicate that amines are catabolic products of glucosinolates in some plants [8–10]. The co-occurrence of **1**, **2** and **3** in *Sinapis* are, furthermore, of chemotaxonomic interest, especially in connection with the occurrence of **1** in *Brassica* and *Sinapis* species [3] and in investigations on possible contamination of rapeseed with *S. arvensis* [14] since **2** and **3** have not been found in *Brassica* species. The HPLC method of analysis [13] is especially efficient in the detection of **1**, **2** and **3**.

EXPERIMENTAL

Plant material. Seeds of *S. alba* (white mustard) were obtained from Trifolium Silo A/S, DK-2630 Tåstrup, Denmark. Seeds of

Table 1. Concentration of 4-hydroxybenzylamine, N^5-(4-hydroxybenzyl)glutamine and 4-hydroxybenzylglucosinolate in *S. alba* and *S. arvensis*

	Concentration (μmol/g plant material)*			
	Sinapis alba		*Sinapis arvensis*	
Compound	Seeds	Seedlings (2 days)	Seeds	Seedlings (2 days)
4-Hydroxybenzylamine	5	7	1	1
N^5-(4-hydroxylbenzyl)glutamine	3	4	0.2	0.2
4-Hydroxybenzylglucosinolate	82	15	100	27

*Determined by HPLC and amino acid analysis as described in Experimental; for seedlings calculated per gram of freeze-dried plant material.

S. arvensis were collected from plants growing in their natural habitat. Seedlings were grown in the greenhouse in vermiculite at 25°; at harvest they were freeze-dried and stored at −20°, until extractions were carried out.

General methods. These have been described elsewhere [9]. PC was performed on Whatman No. 1 paper in solvents: (1) *n*-BuOH–HOAc–H_2O (12:3:5); (2) PhOH–H_2O–12 M NH_4OH (120:30:1) (w/v/v). HVE was carried out on Whatman 3MM paper in buffer systems: (1) pH 1.9 HOAc–HCO_2H–H_2O (4:1:45), 1 hr at 3.2 kV and 90 mA; (2) pH 6.5 pyridine–HOAc–H_2O (25:1:500), 30 min at 5 kV and 90 mA.

Isolation procedure. Homogenization (100 g *S. alba* seedlings; 2 days old), extraction and ion-exchange chromatography were performed as previously described [10, 13]. The pyridine eluate from the strongly acidic ion exchanger was taken to dryness leaving an evaporation residue containing neutral and acidic amino acids. Isolation and purification of **3** from this residue was performed by prep. PC is solvent (1), prep. HVE at pH 1.9 and finally recrystallization from H_2O resulting in 47 mg colourless crystals of **3**.

Hydrolysis of **3** (5 mg) in boiling 2 M HCl (10 ml; 2 hr) resulted in an equimolar mixture of glutamic acid and **2**. PC, HVE and colour reaction with ninhydrin for **2** and glutamic acid as well as the UV and ^{1}H NMR spectra for **2** were as described elsewhere [10, 12]. UV, ^{1}H and ^{13}C NMR spectra of **3** revealed the 4-hydroxybenzylamine part of **3** [10, 12] and confirmed finally the structure of **3** as N^5-(4-hydroxybenzyl) glutamine. The ^{1}H NMR spectrum of **3** in D_2O exhibited signals at the following δ values: 7.2 (2H, *d*), 6.8(2H, *d*), 4.2(2H, *s*), 3.3(1H, *t*), 2.3(2H, *m*), 1.9(2H, *m*).

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