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3-HYDROXYPROPYLGLUCOSINOLATE, A NEW GLUCOSINOLATE IN SEEDS OF *ERYSIMUM HIERACIFOLIUM* AND *MALCOLMIA MARITIMA*

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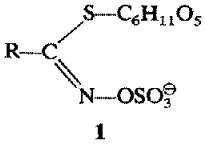
Key Word Index—*Erysimum hieracifolium; Malcolmia maritima*; Cruciferae; glucosinolate; 3-hydroxypropylglucosinolate.

Abstract—Glucosinolates from seed meals of *Erysimum hieracifolium* and *Malcolmia maritima* were treated with thioglucosidase (EC 3.2.3.1), and the resultant aglucon products investigated. A major product from *E. hieracifolium* was 3-hydroxypropyl isothiocyanate from the novel precursor 3-hydroxypropylglucosinolate. Other aglucon products were 3-methylsulfonylpropyl, 3-methylsulfinylpropyl, 3-methylthiopropyl, and allyl isothiocyanates. The aglucon products from *M. maritima* seed meal included 3-hydroxypropyl isothiocyanate, in addition to the previously known 3-methylsulfonylpropyl and 3-benzoyloxypropyl isothiocyanantes.

INTRODUCTION

Erysimum hieracifolium L. and *Malcolmia maritima* L. R.Br. are members of the Cruciferae (Brassicaceae) family. Seed from plants of this family consistently contain glucosinolates, a class of natural compounds that have the general structure (**1**).



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1a R = HO—$CH_2CH_2CH_2$—

1b R = C_6H_5—C(=O)—O$CH_2CH_2CH_2$–

Excellent reviews have been published regarding the distribution and chemistry of these compounds in seeds and plants of the Cruciferae; *ca* 80 compounds of this type, with variations in the *R* group, are known [1–3]. We report here the presence of a new compound, 3-hydroxypropylglucosinolate, in seeds of *M. maritima* and *E. hieracifolium*. *M. maritima* was previously investigated for glucosinolates by Kjaer and Gmelin [4] and by Schultz and Wagner [5, 6]. A seed material reported to be *E. hieracifolium* was also investigated [7], but its identity was corrected to *E. virgatum* Roth in subsequent reports [8, 9]. Because the taxonomy of *Erysimum* is rather complex [7], knowledge of the glucosinolates present may assist in solution of the classification problems in this genus.

RESULTS AND DISCUSSION

We have surveyed a number of cruciferous seed meals for hydrolytic products from glucosinolates, including two accessions from India positively identified as *E. hieracifolium*. Investigation of thioglucosidase-treated *E. hieracifolium* seed meal revealed a new component (by GLC on two columns). Subsequent GC–MS studies that included trimethylsilylation and ^{1}H NMR studies of the isolated aglucon portion eventually established its structure as 3-hydroxypropyl isothiocyanate. Presumably the compound arises through thioglucosidase hydrolysis of **1a**, a glucosinolate not previously recognized, although the related benzoylated glucosinolate (**1b**) is known. This benzoyl ester (glucomalcolmiin) described earlier [4] served as a convenient reference material in the present investigation. The finding of the free, unesterified form of the glucosinolate in *E. hieracifolium* prompted us to re-examine a sample of *M. maritima* seed meal. Earlier workers detected a minor component in *M. maritima* which they believed to be a new glucosinolate, but they did not identify it [5, 6]. Kjaer and Gmelin [4] described the small amount of an isothiocyanate they detected as 3-hydroxypropyl isothiocyanate, but they believed that the compound most likely arose in their preparations from hydrolysis of the benzoyl ester. We found large amounts of the two isothiocyanates previously reported [4], i.e. 3-methylsulfonylpropyl and 3-benzoyloxypropyl, and a smaller amount of 3-hydroxypropyl isothiocyanate (Table 1). We have not totally excluded the possibility that the small amount of 3-hydroxypropyl isothiocyanate in *M. maritima* was the result of inadvertent hydrolysis as believed by Kjaer and Gmelin [4]. Even if this should be the case, most of the benzoyl ester remains intact in our preparations from *M. maritima*, and we are confident therefore that 3-hydroxypropyl isothiocyanate from *E. hieracifolium* (handled in the same manner) is not the result of lysis of a benzoyl ester and does represent 3-hydroxypropylglucosinolate, because none of the benzoyl ester was found in *E. hieracifolium* preparations.

Table 1. Glucosinolate composition of *Erysimum hieracifolium* and *Malcolmia maritima* seed meals

R group of glucosinolate (GS)	% of defatted air-dried meal *Erysimum hieracifolium*	*Malcolmia maritima*
Allyl-	0.2	0
3-Methylthiopropyl-	0.1	0
3-Hydroxypropyl-	1.2	0.4
3-Methylsulfinylpropyl-	1.0	0
3-Methylsulfonylpropyl-	1.3	2.9
3-Benzoyloxypropyl-	0	2.4
Total GS	3.8	5.7
Total GS (calculated from glucose release)	3.7	6.0

The characterization of **1a** in this report is based on MS data obtained for the isolated 3-hydroxypropyl isothiocyanate and its TMSi derivative, and is substantiated by ^{1}H NMR measurements. The MS of the isolated 3-hydroxypropyl-NCS had M^+ as the base peak with major intense ions at *m/e* 99 $(M-H_2O)^+$, 72 $(CH_2NCS)^+$ and 59 $(C_3H_7O)^+$. The MS of its TMSi ether derivative allowed straightforward interpretation of ions: *m/e* 189 (M^+), 174 $(M-Me)^+$, 116 $(M-TMSi)^+$, 99 $(M-TMSiOH)^+$, 75 $(TMSiH_2)^+$, 73 $(TMSi)^+$ and 59 $(C_3H_7O)^+$. Ions found at *m/e* 146 and 145 could arise from migration of the TMSi moiety to the NCS group which would allow losses of *m/e* 43 $(C_2H_3O)^+$ and 44 $(C_2H_4O)^+$ from the M^+. Such a migration of TMSi groups to areas of high electron densities has been observed in the spectra of fatty acid derivatives [10].

The ^{1}H NMR spectrum obtained for the compound was consistent with the proposed structure in that two appropriate triplets centered at δ 3.8 ($J = 5.9$ Hz) and 3.69 ($J = 6.5$ Hz) were observed for the methylene protons (—CH_2—OH and —CH_2—NCS), in addition to the central methylene multiplet at δ 1.93, and the absence of Me protons, which confirms the location of the OH at the 3-position.

We conclude that the 3-hydroxypropylglucosinolate does occur as a natural glucosinolate in *E. hieracifolium* and probably also, although only *ca* 30% as much, in *M. maritima* seed. Glucosinolate composition of the two seeds is given in Table 1. The finding of the substituent-free OH located in the 3-position is of added interest because the hydroxylation in aglucons of this type is typically located at the 2-position, i.e. β to the sinolate carbon. Indeed, to our knowledge, the only other reported examples of 3-hydroxylated components of this type are from other *Erysimum* species [7] and from the leaves of the caper, *Capparis flexuosa* L. [8]. It should also be noted that an *Arabis* species has been reported to contain two 3-position keto components [11]. This fact may be suggestive of genetic similarities among these genera.

EXPERIMENTAL

Original *Erysimum* seed was obtained from a wild stand in India and was collected in March 1970. Some of this seed was later grown in a greenhouse and compared with authentic herbarium specimens of *E. hieracifolium* L. The *M. maritima* seed was supplied by Dr. Quentin Jones of the Beltsville Agricultural Research Center at Beltsville, MD.

Glucosinolate extraction and analysis. Seed samples were ground and defatted with petrol (pentane–hexane) in a Butt extraction apparatus. The air-dried defatted meals were added to boiling MeOH to inactivate the endogenous enzyme system and the intact glucosinolates were extracted by 3 extractions with MeOH–H_2O (7:3). The combined extracts were concentrated to a syrup and redissolved in a small vol. of H_2O. Following centrifugation to remove some insoluble materials, aliquots equivalent to 250 mg of the defatted seed meals were taken for the glucosinolate analyses presented in Table 1. Analyses were obtained by the combined procedures previously applied to cruciferous vegetables and seed [12–14]. The combined procedures describe the preliminary extraction of the glucosinolates and subsequent separation of extraneous materials by a 'mini' ion-exchange separation step, followed by thioglucosidase hydrolysis to allow GLC analysis of the resultant aglucons and a separate analysis for the enzymatically liberated glucose. Components were identified by GLC and, in addition, they were subjected to GC–MS analysis [15].

Isolation and identification of 3-hydroxypropyl isothiocyanate. Glucosinolates from 5 g samples of defatted meal were extracted into aq MeOH and subsequently hydrolysed by thioglucosidase, as described under glucosinolate extraction and analysis. Following extraction of the hydrolysate with CH_2Cl_2 (4×4 vol.), the combined extracts were concentrated to an oil. The components of the oil were separated by prep.-TLC on 2 mm thick Si gel plates with Et_2O as solvent. The separated bands were located by UV. The component (6 mg) determined to be 3-hydroxypropyl isothiocyanate, which migrated 1–2 cm behind the solvent front, was eluted from the adsorbent with Et_2O. Conversion of 3-hydroxypropyl isothiocyanate to the corresponding TMSi ether was accomplished in Py with a 2:1 mixture of hexamethyldisilazane and trimethylchlorosilane. MS of 3-hydroxypropyl isothiocyanate: *m/e* 117 (M^+, 100), 99 (54), 89 (16), 72 (77), 61 (16), 60 (55), 59 (95), 58 (30) and 57 (40). MS of TMSi ether of 3-hydroxypropyl isothiocyanate: *m/e* 189 (M^+, 5), 174 (42), 146 (23), 145 (15), 116 (100), 99 (26), 75 (16), 73 (26) and 59 (11). ^{1}H NMR spectra were obtained in $CDCl_3$ at 100 MHz.

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