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GLUCOSINOLATES OF NINE CRUCIFERAE AND TWO CAPPARACEAE SPECIES

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Key Word Index—Cruciferae; Capparaceae; glucosinolates.

Abstract—Autolysis products of nine species of the Cruciferae and two species of the Capparaceae were analysed by high sensitivity GC/MS. Four of the Cruciferae species were examined for glucosinolates for the first time. One new glucosinolate, 9-methylthiononylglucosinolate, was identified in *Arabis purpurea* and many known glucosinolates were identified for the first time in previously studied plant species. 5-Methylthiopentylglucosinolate appears to be characteristic of the genus *Alyssum*.

INTRODUCTION

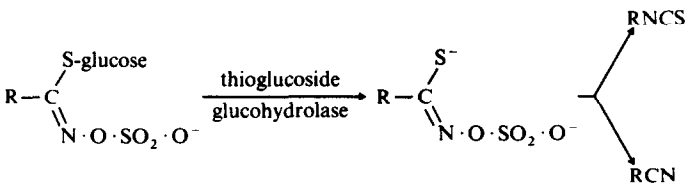
The first authoritative review listing the occurrence of glucosinolates in various plant species was published by Kjaer in 1960 [1] and has been up-dated at regular intervals [2–7]. A recent survey of the glucosinolates of a range of 79 Cruciferae plants was reported by Cole in 1976 [8]. However, many species have yet to be studied for their glucosinolate content and some which have been previously examined need reappraisal using more modern analytical methodology and instrumentation. Here we describe a survey of some Cruciferae and Capparaceae species collected from Spain, Cyprus and Thailand, some of which were examined for glucosinolates for the first time.

RESULTS AND DISCUSSION

During autolysis of plants or seeds any glucosinolates present undergo enzymic degradation as shown in Scheme 1 with the formation of two main products, isothiocyanates and nitriles. Other products are possible from certain glucosinolates in certain experimental conditions, but none was observed in the present study. Thus, identification of a particular isothiocyanate and/or nitrile in an autolysis medium is a direct indication of the nature of the glucosinolate precursor and the amounts of the products is a direct quantification of glucosinolate content in the plant or seed system being studied. In this study the autolysates were assayed by gas chromatography, so that only those glucosinolates which liberate sufficiently volatile products were determined. However, since the majority of glucosinolates produce volatile products few would be excluded using this procedure.

Table 1 gives details of the glucosinolates identified in the eleven plant species studied. The first nine species listed are all members of the family Cruciferae, whilst the other two belong to the Capparaceae. For each glucosinolate the absolute concentration (μg/g) is given, together with its percentage of the total glucosinolate content. All identifications are based on the mass spectra of isothiocyanate and/or nitrile degradation products obtained on GC/MS of samples of plant or seed autolysates. With one exception, all the spectra have been published previously [9–12], so they are not reported here. The mass spectrum of 9-methylthiononyl isothiocyanate obtained in this work agreed with spectra of the lower homologues (up to the 8-methylthiooctyl derivative) which have been previously reported [9], and comprised the following major peaks: *m/z* (%) 61 (100), 55 (96), 41 (91), 29 (47), 67 (34), 69 (32), 72 (23), 45 (19), 231 (M^+, 12).

None of the *Alyssum* or *Arabis* species analysed, which are endemic to Cyprus, has been studied before. However, in many other *Alyssum* species which have been examined [1, 8] 5-methylthiopentylglucosinolate was found to be a common constituent and the present results agree with these findings. It is interesting that this glucosinolate was not detected in any of the other species studied here nor was it detected in any of the 79 Cruciferae investigated by Cole [8] except *Alyssum saxitale* and *Berteroa incana*. Previously, Kjaer *et al.* had reported it from *Berteroa* [13], but that origin apart, this particular glucosinolate would appear to be characteristic of the *Alyssum* genus. We found three other glucosinolates in *Alyssum* for the first time, namely the 4-methylthiobutyl-, 6-methyl-



Scheme 1. Enzymic degradation of glucosinolates.

Table 1. Distribution and concentration of glucosinolates in nine species of Cruciferae and two species of Capparaceae

Plant species	Part of plant*	Origin†	Glucosinolates §, μg/g (%)													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
Alyssum chondrogynum B. L. Burtt	S	Cy								6 (11)	49 (89)					
Alyssum troodi Boiss.	S	Cy									103 (4)	2203 (92)	81 (4)			
Arabis kennedyae Meikle	S	Cy				44 (0.2)			923 (5)			438 (2)	16555 (82)			
Arabis purpurea Sibth. et Sm.	S	Cy											528 (28)	1328 (72)		
Cardaria draba (L.) Desv.	P	Sp		81 (7)	306 (28)	13 (1)	69 (6)			618 (57)						
Diplotaxis erucoides (L.) DC.	P	Sp				1794 (94)	18 (1)								37 (2)	49 (3)
Lobularia maritima (L.) Desv.	P	Sp				4 (1)	374 (70)	75 (14)				71 (13)			3 (1)	7 (1)
Sinapis arvensis L.	P	Sp				39 (6)	16 (2)									593 (92)
Sisymbrium orientale L.	P	Sp					1194 (82)								61 (4)	199 (14)
Cleome viscosa L.	P	Th	28 (100)													
Gynandropsis gynandra (L.) Briq.	P	Th	14 (100)													

*S = seed, P = plant.

†Cy = Cyprus, Sp = Spain, Th = Thailand.

§Glucosinolates: 1 = methyl, 2 = butyl, 3 = 1-methylpropyl, 4 = allyl, 5 = but-3-enyl, 6 = pent-4-enyl, 7 = 3-methylthiopropyl, 8 = 4-methylthiobutyl, 9 = 5-methylthiopentyl, 10 = 6-methylthiohexyl, 11 = 7-methylthioheptyl, 12 = 9-methylthiononyl, 13 = benzyl, 14 = 2-phenethyl.

thiohexyl- and 7-methylthioheptyl-derivatives. Allyl- and 6-methylthiohexyl-glucosinolates were found in *Arabis* species for the first time. More significant was the detection of 9-methylthiononylglucosinolate in *Arabis purpurea* which, to the best of our knowledge, is the first identification of this particular glucosinolate in nature, although its occurrence is not unexpected bearing in mind that all the lower ω-methylthioalkylglucosinolates (down to 2-methylthioethyl-) have been previously reported from various Cruciferae. However, the sulphoxide of this particular glucosinolate was characterized by Kjaer and Gmelin in 1956 [14] in another *Arabis* species, *A. alpina*.

The detection of 7-methylthioheptylglucosinolate in *Arabis kennedyae*, *A. purpurea* and *Alyssum troodi* (Table 1) is worthy of comment. At one time it was considered possible that 7-methylthioheptyl- and 8-methylthiooctyl-glucosinolates and their derivatives (i.e. *S*-oxides) were characteristic of a well-defined group of taxa centred on *Arabis* [15]. MacLeod and Islam then found the parent (reduced) glucosinolates in *Nasturtium officinale*, but suggested that the compounds might still be restricted to the tribe Arabideae [16]. Subsequently, however, they could not be found in other members of the tribe [8, 11], and their reported occurrence remained limited to a few species of *Arabis*, *Sibara* and *Nasturtium*. It is therefore noteworthy that both *Arabis* species studied in this project contained 7-methylthioheptylglucosinolate, although curiously neither showed the higher homologue. *Arabis kennedyae* in particular possessed an extremely high level of this compound (over 1 % of the seed content). However, its occurrence in *Alyssum troodi* in small amount now indicates that it is not entirely limited to the tribe Arabideae, although it is still a rare glucosinolate.

Allyl- and 4-methylthiobutyl-glucosinolates were identified in small amount in *Cardaria draba*. Our results agree with a previous investigation of this plant [8], although the quantities detected are much greater (particularly of the latter; 618 μg/g compared with *ca* 7 μg/g). Three other constituents, butyl-, 1-methylpropyl- and but-3-enyl-glucosinolates, were identified in significant amounts (Table 1) and are reported for the first time from this plant. Neither we nor Cole [8] were able to detect the previously reported 4-methylsulphinylbutyl isothiocyanate [17].

Diplotaxis erucoides has also been investigated previously although the data are in conflict, with 4-methylthiobutyl- [8] and allyl-glucosinolates [18], each being reported as the major constituent. Our results agree with those of Schultz and Gmelin [18], allylglucosinolate being present. Three other constituents, but-3-enyl-, benzyl- and 2-phenethyl-glucosinolates, were also found and are reported for the first time from this plant (Table 1).

Lobularia maritima has been shown to contain but-3-enyl- (main constituent) and 2-phenethyl-glucosinolates [8]. Our results agree, but again rather greater amounts were determined and four additional compounds were identified: allyl-, pent-4-enyl-, 6-methylthiohexyl- and benzyl-glucosinolates (Table 1).

Whilst Schultz and Gmelin detected allylglucosinolate in *Sinapsis arvensis* [18], Cole found only but-3-enylglucosinolate [8]. In our analysis both these compounds were found, but another derivative, 2-phenethylglucosinolate, was the major constituent.

Most species of *Sisymbrium* analysed during early studies were found to contain mainly allyl- and/or ethyl-glucosinolates [1]. *S. austriacum*, however, was shown to contain 2-hydroxyisopropylglucosinolate following the identification of its degradation product 4-methyl-2-oxazolidinethione [19]. This type of product is formed spontaneously by the cyclization of 2-hydroxyalkyl (or alkenyl) isothiocyanates. Subsequently, all *Sisymbrium* species examined by Cole produced 4-methyl-2-oxazolidinethione [8]. In our analysis of *S. orientale*, although the minor glucosinolates detected agree with those previously reported [8], namely benzyl- and 2-phenethyl-glucosinolates, we did not detect any oxazolidinethione, and the major component was but-3-enylglucosinolate.

The two members of the Capparaceae examined were both found to contain only methylglucosinolate. Despite careful study, no other glucosinolate was detected. This is in general agreement with data reported in 1955 for these species and for other members of this family [20]. It is interesting that methylglucosinolate, which is characteristic of the Capparaceae, is either absent from, or very rare in, members of the Cruciferae (there are some disputed occurrences).

EXPERIMENTAL

Plant material. Seeds of the Cruciferae species were collected in Cyprus during 1979 from plants growing in the vicinity of Mt. Olympus. Plants and seeds were characterized locally by experts at the Forestry Department of the Ministry of Agriculture and Natural Resources. Cruciferae plants were collected in Spain during 1979 from the vicinity of Castellon and were characterized by a local expert. These identifications were subsequently confirmed in London. Capparaceae plants were collected in Thailand during 1980 from the area of Khao Napha near the Nakorn Sawan-Chiengmai Asian Highway. These too were characterized locally.

Sample preparation: (a) Seeds, (typically 500 mg) were crushed in a ball mill and defatted with hexane. Autolysis was carried out in dist. H_2O for 1 hr at room temp., and products were extracted with isopentane (2-methylbutane). Centrifugation facilitated separation of the two layers. Extracts were concd under a gentle stream of N_2 to a final vol. of 50 μl. (b) Plants. Plant material (typically 50 g) was air-dried, crushed to a powder, and defatted with Et_2O. Autolysis was carried out in dist. H_2O for 1 hr at room temp., and products were extracted into isopentane (2-methylbutane) using a Likens and Nickerson apparatus [21] as modified by MacLeod and Cave [22]. Extracts were concd by low temp. vacuum dist. to a final vol. of 1 ml.

Analysis by gas chromatography. Samples were examined by routine GC using a Pye-Unicam model 104 or model 204, both equipped with heated FID. The column mainly used was a 1.5 m × 4 mm i.d. glass column packed with 10% Carbowax 20M coated on 100–120 BSS mesh acid-washed Diatomite C. With a carrier gas (N_2) flow of 30 ml/min, temp. programme was 60° for 5 min followed by an increase at 12°/min to 180° for the remainder of the run. Injector and detector temps. were 240°. Injection vol. varied but was in the range 1–5 μl. Quantification of results was based on standardization of the system on known amounts of ref. compounds (i.e. a range of representative isothiocyanates and nitriles).

Identification of components by GC/MS. A Kratos MS 25 instrument was used, equipped with a DS 50S data processing system. The same GC conditions as above were employed, but with He as carrier gas. The all-glass jet separator interface was operated at 250°. MS conditions were ionization potential, 70 eV; ionization current, 300 μA; source temp., 200°; resolution, 1500;

scan speed, 3 sec/decade (repetitive throughout run). The background subtraction facility and the retrospective single ion monitoring facility of the GC/MS data system were extensively employed in evaluating the MS results. A certain amount of MPM work was also performed.

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