Phytochemistry, Vol. 22, No. 2, pp. 465–470, 1983.
Printed in Great Britain.

0031–9422/83/020465–06$03.00/0

SEED GLUCOSINOLATES OF FOURTEEN WILD *BRASSICA* SPECIES

PETER J. HORN and JOHN G. VAUGHAN*

Department of Biology, Queen Elizabeth College (University of London), Campden Hill Road, London W8 7AH, U.K.

(*Received* 15 *April* 1982)

Key Word Index—*Brassica* sp.; Cruciferae; chemotaxonomy; glucosinolates.

Abstract—Seed glucosinolates have been determined for 14 wild *Brassica* species, by micro-scale GC analysis of silylated derivatives. Of these, 12 were investigated for the first time. The majority of taxa exhibited high alkenylglucosinolate levels, although prop-2-enylglucosinolate appears to be generally absent. Other known methionine-derived glucosinolates predominate in *B. tournefortii*, *B. elongata* and *B. deflexa*. Phenylalanine-derived 4-hydroxybenzylglucosinolate is characteristic of section *Brassicaria* plants and represents the first finding of this glucosinolate in authenticated *Brassica* material.

INTRODUCTION

Within the Cruciferae, the taxonomy of the genus *Brassica* has probably attracted the greatest attention, mainly due to the outstanding economic importance of certain of its species. Bailey [1] has emphasized the difficulty in attempting a classification which includes both wild and cultivated species. The crop plants alone, with their vast multiplicity of forms, have represented such a confusing and anomalous situation, that their consideration has dominated work on this genus. Eventually resolved into six major species, their origins and taxonomy as based on morphological, cytogenetic and genomic inter-relationship studies have recently been reviewed [2]. Some cytogenetic studies, notably those of Mizushima [3], and most recently, Harberd [4, 5], have included the wild species and suspected relatives in other genera.

The occurrence and distribution of glucosinolates has been extensively studied in the *Brassica* crop species. However, the majority of work has related to the undesirability of a high glucosinolate content, rather than to any taxonomic interest. Nonetheless, the information may also offer a valuable opportunity for broadening the genetic base required for plant breeding programmes [6]. A recent comprehensive survey, based upon seed glucosinolate composition [7], supports and further elucidates the understanding of relationships within and between the six crop species. The potential value in using glucosinolates as chemotaxonomic markers has been stressed [8] and demonstrated with *B. juncea* (L.) Coss. and Czern. [9] and *B. oleracea* L. cultivars [10, 11], as well as in other genera [12].

Much of the taxonomy of the genus *Brassica* as a whole is still unresolved [13]. Additionally many of the wild species may have a potential for direct exploitation or as components of future plant breeding programmes; some are common, often vigorous weeds, especially in the Mediterranean region [13].

Considering the demonstrated utility of glucosinolate information, it is surprising that the wild species of *Brassica* have received so little attention; we are aware of only two reports on wild *Brassica* material. PC analysis of extracts from seed of *B. barrelieri* (L.) Janka indicated the presence of 5-vinyloxazolidinethione, a hydrolysis product of 2-hydroxybut-3-enylglucosinolate [14]. Another report briefly states that seed of *B. insularis* Moris has a glucosinolate pattern different from the rest of its wild relatives (*B. oleracea* L. *sensu-stricta* and wild 'sea-cabbage' species), although no further details were given [15].

Here we present a survey of 14 wild *Brassica* species based on their seed glucosinolate content. With the exception of the six crop species and the wild *B. balearica* Pers., the taxa broadly represent all of the morphological supra-specific groups indicated by the treatment of Schulz [16] and those subsequently [17, 18], as well as the cross-fertile cytodemes reported by Harberd [4]. The method employed enables direct and accurate simultaneous determination of all the glucosinolates present within a sample, by using GC of silylated derivatives. Thus, advantages are offered over previous methods which depend upon routine GC of glucosinolate hydrolysis products, with supplementary analysis, by PC for those products which are non-volatile (cf. ref. [12]). The results are discussed in the light of accepted biosynthetic information [20], since such an approach has been used successfully in studies of other genera [12].

RESULTS AND DISCUSSION

Table 1 shows the content of identified glucosinolates in the seed of 23 accessions of wild *Brassica* plants, representing 20 taxa and 14 species. The majority of taxa were analysed as single accessions because of the difficulty in obtaining viable seed. However, in some cases, viz. *B. barrelieri* subsp. *barrelieri*, *B. oxyrrhina* and *B. souliei*, we were able to obtain additional samples from a separate source. The botanical nomenclature used is that according to Salmeen [22], and the taxa are listed (Table 1) in a manner reflecting the order of discussion. Species are considered together if a natural grouping is apparent from

*Author to whom correspondence should be addressed.

Table 1. Distribution and concentration of glucosinolates in seed of fourteen *Brassica* species

Plant taxa†	Haploid chromosome number (*n*)‡	Glucosinolates* (%)								Total (mg/g)
		1	2	3	4	5	6	7	8	
B. repanda (Willd.) DC. subsp. *maritima* (Rouy) Heywood	10	—	—	—	—	—	—	100	—	25.8
B. desnottosii Emb. et Maire	10	—	—	—	8	—	–	92	—	45.2
B. gravinae Ten. var. *brachyloma* (Boiss. et Reut.) O. E. Schulz	20	—	—	2	35	—	—	63	—	118.4
B. elongata Ehrh. subsp. *elongata*	11	—	68	—	32	—	—	—	—	75.6
subsp. *integrifolia* (Boiss.) Breistr.	11	—	83	—	13	—	4	—	—	103.7
B. deflexa Boiss. subsp. *leptocarpa* (Boiss.) Hedge §	7	—	95	—	±	—	±	—	—	25.5
B. tournefortii Gouan §	10	94	—	—	5	—	±	—	—	13.9
B. insularis Moris	9	—	—	±	12	±	72	—	16	45.8
B. incana Ten.	9	—	—	79	—	±	21	—	—	50.2
B. oxyrrhina Cosson §	9	—	—	90	—	—	—	—	—	26.4
B. oxyrrhina Cosson	9	—	—	—	89	—	11	—	—	38.5
B. barrelieri (L.) Janka subsp. *barrelieri*	10	—	—	—	100	—	—	—	—	31.1
B. barrelieri (L.) Janka subsp. *barrelieri*	10	—	—	49	51	—	—	—	—	21.6
subsp. *sabularia* Brot. §	10	—	—	90	—	—	±	—	—	47.1
B. fruticulosa Cyrillo subsp. *fruticulosa*	8	—	—	96	3	1	—	—	—	91.3
subsp. *cossoniana* (Boiss. et Reut.) Maire	16	—	—	93	6	1	—	—	—	105.2
subsp. *glaberrima* (Pomel) Batt.	16	—	—	100	—	—	—	—	—	116.1
subsp. *mauritanica* (Cosson) Maire	16	—	—	94	1	—	5	—	—	82.0
subsp. *radicata* (Desf.) Pomel	16	—	—	82	14	—	4	—	—	110.0
B. maurorum Durieu	8	—	—	94	6	—	—	—	—	57.4
B. spinescens Pomel	8	—	—	98	2	—	—	—	—	40.1
B. souliei (Batt.) Batt.	11	—	—	—	88	—	12	—	—	37.5
B. souliei (Batt.) Batt.	11	—	—	—	71	—	29	—	—	54.8

*Glucosinolates: 1 = 3-methylsulphinylpropyl-; 2 = 3-methylsulphonylpropyl-; 3 = but-3-enyl-; 4 = 2-hydroxybut-3-enyl-; 5 = pent-4-enyl-; 6 = 2-phenylethyl-; 7 = 4-hydroxybenzyl-; 8 = benzyl-. 1–5 are methionine-derived; 6–8 phenylalanine-derived [20]. ±, Denotes trace amounts.

† Nomenclature according to Salmeen [22]. Duplicate results for *B. barrelieri* subsp. *barrelieri*, *B. oxyrrhina* and *B. souliei* are derived from separate accessions.

‡ Most recently accepted haploid chromosome numbers according to Gómez-Campo and Hinata [21].

§ Contained additional minor compounds not identifiable with the glucosinolates listed.

previous systematic studies [16–18, 22], or when a common cytodeme has been indicated [4]. If neither of these conditions apply, comparison is based upon any similarity of glucosinolate pattern. The individual glucosinolates are arranged in Table 1 according to their means of biogenesis. The first five are methionine-derived and listed in order of appearance within the biosynthetic sequence [20]. The last three glucosinolates are phenylalanine-derived and thought to follow separate biosynthetic pathways which are distinguished at a relatively early stage [20, 23]. The values are expressed as relative individual levels (%) and total concentration.

Although the absolute concentrations of glucosinolates, in part, reflect the genetically fixed aspect of a plant's biosynthetic capacity, such values are variable even at the population level and are very often significantly modified by environmental factors [24]. However, the relative proportions of the individual glucosinolates present are little affected in this way, remaining stable and consistently inheritable [6, 7, 11, 19, 25]. In addition, the resulting profiles may be further analysed biosynthetically [12], since the glucosinolates are ultimately derived from certain protein amino acids and formed by carbon-chain elongation, introduction of double-bonds, hydroxylation and oxidation of sulphur in the aglucone R-group [20, 23]. Consequently, a glucosinolate profile is subject to variation on three levels. The major distinction depends upon which amino acid is the initial precursor. Secondly, particular glucosinolates from within the variety, made possible by the subsequent modifications, will be selectively accumulated. Finally, the relative levels of each glucosinolate within a combination will vary. Since patterns may be altered by minor enzymatic or genetic changes, care is required in attaching importance to varying levels of compounds known to be closely related biosynthetically [12].

Seed of the *Brassica* crop species are characterized by the presence of predominantly alkenylglucosinolates [7, 8]. Prop-2-enyl, but-3-enyl, pent-4-enyl and hydroxy analogues of the last two, occur in varying proportions in seed of *B. oleracea* L., *B. juncea* (L.) Coss. and Czern., *B. carinata* Braun, *B. nigra* (L.) Koch, *B. campestris* L. and *B. napus* L. However, prop-2-enylglucosinolate has only been found in minute amounts, if at all, in material from the last two species [6, 7]. In the vegetative tissues of these six species, other glucosinolates may exist in significant amounts, but they are also largely methionine-derived [11, 24, 25]. Hereafter, the abbreviated notation GS is

used in place of the suffix 'glucosinolate'.

Our results clearly indicate that this characterization of the genus extends to the majority of the wild *Brassica* species. Combined levels of but-3-enyl-GS and 2-hydroxybut-3-enyl-GS are frequently in excess of 70%, in some cases making up the entire complement. The scarcity of pent-4-enyl-GS reflects the situation in the crop plants where this compound only occasionally constitutes a major component [7]. However, the complete absence of prop-2-enyl-GS, one of the first glucosinolates to be isolated [23], is surprising. The highly pungent prop-2-enylisothiocyanate, which is produced on hydrolysis, is readily observed in the mustards (*B. nigra* and *B. juncea*) as a major flavour component. Yet, it appears to be rare within the genus and restricted to the crop plants. Results presented by Cole [26], from an extensive survey of the Cruciferae, show that the co-existence of prop-2-enyl-GS and the other alkenylglucosinolates is uncommon, although the occurrence of either is generally frequent. Not all of the species investigated here have shown glucosinolate profiles typical of the *Brassica* genus. In these cases the presence of other glucosinolates at predominant levels in the seed is striking.

Glucosinolates of B. repanda, B. desnottosii, *and* B. gravinae

B. repanda, ($n = 10$), *B. desnottosii*, ($n = 10$) and *B. gravinae*, ($n = 10, 20$) exhibit high to exclusive levels of 4-hydroxybenzyl-GS, with correspondingly low levels of alkenyl-GS. Previous reports upon the occurrence of this glucosinolate have been restricted to the genus *Sinapis* [27, 28]. A report of this glucosinolate being found in rape seed (*B. napus* and *B. campestris*) [29] has since been shown to be incorrect [28]. Consequently our findings represent the first identification of 4-hydroxybenzyl-GS in thoroughly authenticated *Brassica* material. *B. repanda*, *B. desnottosii* and *B. gravinae* belong to the section *Brassicaria* (Godr.) Cosson, as used by Schulz [16] and recently maintained by Salmeen [22]. Morphologically, these plants form a discrete and very well-defined group within the genus, although their affiliations with the other *Brassica* species have never been clear [30]. They were not tested for cytodeme identity by Harberd [4], although they share the same chromosome number, $n = 10$. *B. gravinae* var. *brachyloma*, $n = 20$, is considered to be a spontaneous autotetraploid, since other infra-specific groups have $n = 10$ [21].

Not only do the glucosinolate data strongly support the discreteness of section *Brassicaria*, but they also raise further questions regarding their inter-relationships with other species. A recent study of seed coat characters [30] and earlier studies, more broadly based [31, 32], have suggested that these plants, along with *B. balearica*, are closely related to the genus *Sinapidendron*. This implies that their inclusion in *Brassica* may be of doubtful validity. The presence of 4-hydroxybenzyl-GS is so distinctive that it could be taken to favour an isolated position for these species, perhaps outside the genus; however, glucosinolate information for *B. balearica* and members of *Sinapidendron* is not presently available.

Any possible association of section *Brassicaria* with the genus *Sinapis* on the basis of glucosinolate profile similarity, is not borne out by morphology. Furthermore any close intra-generic affiliation between *Brassica* and *Sinapis* has been invalidated by protein studies [33] and by more general comparison of glucosinolate distribution (cf. ref. [28]). *B. gravinae* has some morphological characterististics which suggest, that of the three species in section *Brassicaria*, it bears the closest relationship to the other members of the genus [22, 30]. The presence of moderate alkenyl-GS levels in seed of *B. gravinae* may support this opinion.

Glucosinolates of B. elongata *and* B. deflexa

The two accessions of *B. elongata* ($n = 11$) and that of *B. deflexa* subsp. *leptocarpa* ($n = 7$) exhibit glucosinolate patterns dominated by 3-methylsulphonylpropyl-GS, with low to moderate levels of 2-hydroxybut-3-enyl-GS and some 2-phenylethyl-GS. Both species are confined in their distribution to the eastern Mediterranean and Middle East. On the basis of mainly morphological evidence they are placed in a reinstated section *Micropodium* DC. by Salmeen [22]. Viewed as the least natural grouping of those proposed, the section also includes *B. fruticulosa*, *B. maurorum* and *B. juncea*, (although the last species is well accepted as an amphidiploid derived from *B. campestris* and *B. nigra*). *B. deflexa* and *B. elongata*, respectively, occupy the lower and upper limits of the complete aneuploid chromosome series of *Brassica*, where $n = 7, 8, 9, 10, 11$. *B. elongata* has been allocated to a separate cytodeme, while *B. deflexa* was untested for cytodeme identity [4]. Since the latter species is the only *Brassica* with $n = 7$, it is unlikely to be cross-fertile with any other, although the plant is not morphologically outstanding [22, 34]. *B. elongata* is distinctive in exhibiting a long stipe (stalk) basal to the siliqua; this is considered to be a primitive characteristic [13]. *B. deflexa* may be viewed as primitive on the basis of its chromosome number, although this could reflect a derived status, reduced from a higher level. So, while the similarity of glucosinolate profile is unlikely to indicate a close relationship, both species appear to be chemically distinct from others with a primitive profile.

Experimental evidence supports a common origin of the ω-methylthioalkyl- and ω-alkenylglucosinolates, the latter arising from the former by elimination of the methanethiol group (or an oxidized equivalent), with double bond formation [8, 20]. Alternatively, successive oxidation of the R-group sulphur converts the ω-methylthioalkylglucosinolates into the corresponding ω-methylsulphinylalkyl- and ω-methylsulphonylalkyl-analogues. Thus the glucosinolate found in *B. elongata* and *B. deflexa* is biosynthetically related to prop-2-enyl-GS, via their mutual precursor 3-methylthiopropyl-GS. While these plants do not accumulate prop-2-enyl-GS, they do show synthesis of the higher homologues. This is apparent from the presence of trace to moderate amounts of 2-hydroxybut-3-enyl-GS. Consequently, compared to those species typified by high levels of alkenyl-GS, these plants are distinguished by their expression of perhaps only three additional enzyme systems [12].

Glucosinolates of B. tournefortii

This species has a seed glucosinolate profile dominated by 3-methylsulphinylpropyl-GS. As the immediate precursor of 3-methylsulphonylpropyl-GS, found in seed of *B. elongata* and *B. deflexa* discussed above, *B. tournefortii* ($n = 10$) may be chemically distinguished from these species by the absence of only one enzyme system [12].

The presence of 3-methylsulphinylpropyl-GS sets *B. tournefortii* apart from the other alkenyl-GS-bearing plants, but it does so by the operation of perhaps only two additional enzyme systems. *B. tournefortii* is a very widespread, and often aggressively weedy, species found from the Mediterranean region to India and naturalized in Scandinavia, U.S.A., New Zealand and Australia [22]. Once mistakenly considered a relative or progenitor of *B. campestris* (De Candolle, cited in ref. [35]), *B. tournefortii* shows a degree of polymorphism, but is not associated with either *B. deflexa* or *B. elongata* on morphological grounds. Salmeen [22] has placed this species in a reinstated section *Sinapistrum* Willkomm, with *B. barrelieri* and *B. oxyrrhina*. The latter two species have a seed glucosinolate pattern typical of the genus with but-3-enyl-GS and/or 2-hydroxybut-3-enyl-GS at very high levels, and are considered to be very closely related to each other [22].

B. tournefortii has a seed glucosinolate content which indicates its discreteness from other species, but the information cannot be taken to suggest an isolated position. However, the affiliation which this species has with *B. barrelieri* and *B. oxyrrhina* may not be as close as previously inferred [22]. Morphological parallelism is well-known within the Cruciferae, especially with respect to adult vegetative characters [13] and may reflect environmental pressures different to those affecting glucosinolate constitution [12].

With the exception of *B. insularis*, all the remaining taxa investigated exhibit glucosinolate profiles which are considered very characteristic of the genus [8], dominated by the four-carbon alkenyl compounds. The majority of variation depends upon the degree of hydroxylation, a final biosynthetic event, thought to be a single-step enzymatic conversion [20, 24]. Consequently, differential levels of but-3-enyl-GS and 2-hydroxybut-3-enyl-GS may reflect only slight genetic or enzymatic changes. In many cases low to moderate levels of 2-phenylethyl-GS were found in the seed. While this glucosinolate is not considered characteristic of the genus, its presence elicits little surprise since it is found in varying concentrations in the vegetative tissues of many *Brassica* crop plants [25, 36]. Derived from phenylalanine, 2-phenylethyl-GS is not very closely related biosynthetically to either benzyl-GS or 4-hydroxybenzyl-GS [20, 23].

Glucosinolates of B. insularis *and* B. incana

As known wild relatives of *B. oleracea* [15] these species are placed in the same cytodeme, along with many other $n = 9$ wild 'sea-cabbage' species. Members of *B. oleracea* contain minor to major levels of prop-2-enyl-GS and often exhibit a high degree of hydroxylation on the but-3-enyl-compound [7]. Neither *B. insularis* nor *B. incana* showed prop-2-enyl-GS in their seed. *B. cretica* Lamk., which is similarly related, we found to give moderate levels of prop-2-enylisothiocyanate during the screening of species by GC of hydrolysis products, [unpublished data]. Although *B. incana* gave a high level of but-3-enyl-GS, none of the hydroxylated analogue was detected. *B. insularis* gave a low level of 2-hydroxybut-3-enyl-GS with no other alkenyl-GS present, a high level of 2-phenylethyl-GS and some benzyl-GS. Such a high 2-phenylethyl-GS component is unusual. The presence of benzyl-GS in significant amounts is uncharacteristic [25], especially in co-existence with 2-phenylethyl-GS [23], although both are phenylalanine-derived. This result supports a previous comment regarding the unusual glucosinolate pattern of *B. insularis* seed [15], although a close relationship with *B. cretica*, which was also suggested, cannot be commented upon.

The wild relatives of *B. oleracea* often grow in permanently established local populations with considerable geographic isolation in some cases. This has led to little requirement for strict speciation and a lack of breeding barriers. So, despite co-fertility, variation of characters within this group of plants, which then remain fixed, is not surprising [4, 15]. Hence, while *B. incana* and *B. insularis* show little correlation of glucosinolate constitution, with each other or *B. oleracea*, such variation may be reflective of a wider trend, equalling the range of morphological differentiation apparent on a minor level within this group of plants.

Glucosinolates of B. oxyrrhina *and* B. barrelieri

B. oxyrrhina ($n = 9$) and *B. barrelieri* ($n = 10$) are very similar morphologically, forming a rosette of deeply dissected (saw-like) leaves from which the branched flowering stem is produced. Equally restricted to S. Spain, S. Portugal, N. Morocco and NW. Algeria, they have often been confused. *B. oxyrrhina* was treated as a subspecies of *B. barrelieri* until very recently [18]. However, the difference in chromosome number leading to inter-sterility, and their being placed in separate cytodemes [4], is considered to indicate specific status for each [22]. The glucosinolate patterns are not distinctive, but the accessions of each species reflect a similarly marked trend in hydroxylation activity. *B. barrelieri* shows but-3-enyl-GS and 2-hydroxybut-3-enyl-GS distributed at respective levels of: 0, 100; 49, 51; *ca* 90, 0. The two *B. oxyrrhina* accessions gave these glucosinolates at: *ca* 90, 0; 0, 88. Such a notable discontinuity in the presence or absence of hydroxylation is unexpected in either of these geographically restricted oligomorphic species. Since the conversion is possibly a relatively minor step, the maintenance of this disparity may be due to variation in localized populations. In general, the derivation of glucosinolate data from single accessions of taxa has been found taxonomically useful [12]. On an empirical basis, most species appear to exhibit consistent patterns in their major glucosinolates [12]. So, the profile differences determined here are not expected to indicate an ability for large intra-specific variation. Consequently, *B. barrelieri* and *B. oxyrrhina* appear to share a common, or very similar, complement of separate biogenetic compositions. If so close a relationship is justified, then the difference in chromosome number can be explained by an evolutionarily recent aneuploidy. This involves the loss or addition of one chromosome in whichever was the primary genome.

These species are considered to be close relatives of *B. tournefortii* according to Salmeen [22]. On morphological evidence, however, they also appear to bear a closer relationship to each other, than either does to *B. tournefortii*. The general and particular properties of the respective glucosinolate patterns serve to support this conclusion, while perhaps indicating a more ancient separation of *B. tournefortii* from *B. barrelieri* and *B. oxyrrhina*.

Glucosinolates of B. fruticulosa, B. maurorum *and* B. spinescens

B. fruticulosa ($n = 8, 16$) is considered to consist of eight subspecies [17, 22], five of which are reported on here; those with a double chromosome number, $n = 16$, are believed to be spontaneous autotetraploids [21]. Plants of this polymorphic species grow throughout the W. Mediterranean region, although some subspecies, e.g. subsp. *cossoniana*, are restricted to S. Spain and NW. Africa [18, 22]. *B. maurorum* ($n = 8$) and *B. spinescens* ($n = 8$) are both restricted to NW. Algeria and have been placed in the same cytodeme as *B. fruticulosa* [4]. *B. spinescens* is morphologically very distinct from the other two species, and is, therefore, rarely seen as being closely related to them [16, 22]. Sometimes this species has been confused with the wild relatives of *B. oleracea* [30], although the plants have a habit, which in detail, is singular within the genus. *B. spinescens* forms a much branched and very woody, perennating sub-shrub. The leaves are small and fleshy, and large pale-yellow flowers are produced on a series of fertile branches, rather than on a few flowering spikes. The petals show a distinct purple venation. Sterile branches produce clusters of smaller, more elongated leaves. The confusion with wild *B. oleracea* relatives may be due to *B. spinescens* having adopted similar morphological adaptations to the same cliff-dwelling habitat, especially in its leaf surface aspect and woodiness[37]. On the basis of juvenile characters, Gómez-Campo [13] has indicated that *B. spinescens* may be a relative of the *B. fruticulosa* group.

The glucosinolate patterns of the three species are very similar, dominated by but-3-enyl-GS with complementary levels of the hydroxylated analogue. This pattern is not unusual in the genus and is, for example, characteristic of certain *B. campestris sensu-lato* plants [7]. As a contributory factor, the pattern similarity supports the closeness of *B. fruticulosa* and *B. maurorum*. However, while the evidence may reflect a relationship between these two species and *B. spinescens*, no further indication of a more positive nature can be derived.

The absence of prop-2-enyl-GS from seed of *B. fruticulosa*, *B. maurorum* and *B. spinescens* further favours their distance from *B. nigra*, the only other $n = 8$ species in the genus [21]. *B. nigra* seed, black mustard, is well-known for its content of this glucosinolate, almost to the total exclusion of all others [7]. *B. nigra* constitutes a separate cytodeme [4] and exhibits some morphological characteristics which distinguish it from other *Brassica* species [16–18, 22].

Glucosinolates of B. souliei

B. souliei ($n = 11$) [syn. *B. amplexicaulis* (Desf.) Pomel] shares its chromosome number with *B. elongata* [21], although the plants are not closely related [22] and each constitutes a separate cytodeme [4]. *B. souliei* occupies a morphologically distinct and isolated position, largely due to its miniature habit, basally multi-branching, with zig-zag stem formation and pauciovulate siliquas [13, 16, 17, 22]. This last character may be viewed as being due to the reduced siliquas, which are in proportion to the rest of the plant but are unable to accomodate very many seeds. There is presumably a limit to the smallness of the seed itself. However, Schulz [16] used this character to place *B. souliei* in the same section as *B. nigra*. The latter species also has small siliquas, but out of proportion to its more usual tall, ascendent habit; thus, the conclassification is on a somewhat artificial basis. Salmeen [22] recently allocated *B. souliei* to a reassigned section *Nasturtiops* (Pomel) Salmeen, separated from other species. The glucosinolate patterns, shown by the two accessions of this species, are very similar, dominated by 2-hydroxybut-3-enyl-GS, with low to moderate levels of 2-phenylethyl-GS. The affiliations of this species are unknown and the glucosinolate data can only indicate a general *Brassica* trait.

EXPERIMENTAL

Plant material. Seed samples were obtained from the Crucifer Germplasm Collection, Madrid (Instituto Nacional de Investigaçiones Agrarias) via Professor C. Gómez-Campo [38]. Single samples of *B. barrelieri* and *B. oxyrrhina* were collected by Dr. O. Salmeen in Spain and a further accession of *B. souliei* and those of *B. insularis* and *B. elongata* subsp. *integrifolia* received from her own list. All accessions were grown to maturity in London, under glasshouse conditions, and additional experimental material harvested from these plants. Throughout their growth periods the plants were authenticated using the treatments of Schulz [16], Maire [17] and Tutin *et al.* [18]. Voucher specimens are held at Queen Elizabeth College, University of London.

Sample preparation. Seed was ground dry in a ball-mill and defatted with 10% *iso*-PrOH in *iso*-octane, followed by petrol (bp 40–60°). The meal was dried *in vacuo* and stored at *ca* 4° in sealed plastic vials. Samples were screened for the presence of significant levels of prop-2-enyl-GS, used as int. standard later, by routine GC of enzymatic hydrolysis products. Endogenous protein was inactivated by heating the meal at 120° for at least 4 hr. Crude myrosinase (*ca* 10 mg lyophilized Me_2CO-powder of *Sinapis alba* L. seed) was added to *ca* 50 mg meal, mixed with 125 μl H_2O and 1.0 ml hexane was added onto this in securely capped glass vials (vol. 1.75 ml). After 2 hr the hexane layer was analysed for prop-2-enylisothiocyanate. The column, 1.5 m × 4 mm i.d. glass, was packed with 10% PEG 20 M and N_2 carrier gas used at a flow-rate of 30 ml/min. The temp. programme employed was 130° for 12 min followed by an increase to 185° at 16°/min. The injector port temp. was 200° and the hydrogen-flame FID was used at 250°. Injection vol. was typically 8 μl. Presence of prop-2-enylisothiocyanate was determined by comparison with the RR_t of pure standard against butylisothiocyanate marker.

Direct analysis of glucosinolates by gas chromatography. Glucosinolates were extracted, purified and prepared according to ref. [19], using 20 mg columns of DEAE-Sephadex A-25 in Pasteur pipettes [39] and a purified sulphatase preparation [19]. Aq. extracts were frozen at −20° until required. The desulphoglucosinolates were analysed as their TMSi derivatives, employing pyridine, TMCS and MSHFBA (*N*-methyl-*N*-trimethylsilylheptafluorobutyramide) at 10:1:10, as silylating agents. Derivitization and sample loading were performed with a Perkin–Elmer MS-41 capsule-dosing system. The 1.5 m × 4 mm i.d. glass column was packed with 2% OV-7 and Ar carrier gas was used at a flow-rate of 40 ml/min. The temp. programme employed was 200° for 5 min, increasing to 275° at a rate of 5°/min. Injection port and hydrogen-flame FID temp. were 280°.

Quantification annd identification. Peak areas were integrated by an IBM-1130 computer (Fortran programme). Response ratio factors with respect to int. standard prop-2-enyl-GS had been calculated for some glucosinolates, as their desulpho derivatives [Heaney, R. K., personal communication]. When no response ratio factor had been evaluated the glucosinolates were assumed

to elicit the same response as the standard. Calibrated quantification was possible for but-3-enyl-GS, 2-hydroxybut-3-enyl-GS and benzyl-GS. Identification of peaks was carried out using RR_t with respect to prop-2-enyl-GS, and selective use of known reference samples of prepared desulphoglucosinolates. The presence of 4-hydroxybenzyl-GS, 3-methylsulphinylpropyl-GS and 3-methylsulphonylpropyl-GS was further investigated and verified by combined GC–CIMS. The method and means of spectral analysis have been reported by Eagles *et al.* [40] who arranged and performed the necessary GC–CIMS investigation.

Acknowledgements—The GC analysis was performed at the Food Research Institute, Norwich. We are indebted to the Director, Dr. R. F. Curtis, for making these facilities available. Further thanks are due to Dr. G. R. Fenwick and Mr. R. K. Heaney for their invaluable help, practical guidance and continued interest in this study, and who, with Mr. J. Eagles, arranged the GC–CIMS investigations. We are very grateful to Professor C. Gómez-Campo, Escuela T. S. Ing. Agrónomos, Universidal Politécnica, Madrid, and to Dr. O. Salmeen, Department of Botany and Microbiology, University of Kuwait, for the provision of seed samples. This work was funded by a S.E.R.C. studentship awarded to P.J.H.

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