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SOME GLUCOSINOLATES OF *FARSETIA AEGYPTIA* AND *FARSETIA RAMOSISSIMA*

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Key Word Index—*Farsetia aegyptia*; *F. ramosissima*; Cruciferae; glucosinolates; isothiocyanates; aroma volatiles.

Abstract—Air-dried leaves of *Farsetia aegyptia* and *F. ramosissima* have been analysed for their glucosinolates; the former was shown to contain at least six but chiefly allylglucosinolate, whilst the latter contains at least five but mainly but-3-enylglucosinolate with some 4-(methylthio)butylglucosinolate. Without the addition of extraneous thioglucosidase enzyme, both species gave predominantly nitrile degradation products of glucosinolates; but if extra enzyme were added, corresponding isothiocyanates became the major products instead. Varying the pH from the natural level for the plant also considerably affected the ratios of glucosinolate products.

INTRODUCTION

Many plants of the Cruciferae contain glucosinolates which are precursors of strongly odorous, pungent isothiocyanates formed by the action of the enzyme thioglucoside glucohydrolase EC 3.2.3.1. (myrosinase) on the glucosinolate substrate when the plant tissue is ruptured. As part of our continuing studies of glucosinolate degradation we analysed two species of the crucifer *Farsetia* (Old World, Saharo–Sindian) from the Western Sahara (Mauritania), namely *F. aegyptia* Turra 1765 (not *aegyptiaca*) and *F. ramosissima* Hochstetter *ex* Fournier 1864 (= *F. stylosa* R. Brown 1826). The only previously reported examination of a *Farsetia* species for its glucosinolate content was as long ago as 1955, when Kjaer and Gmelin found 4-(methylthio)butylglucosinolate in *F. clypeata* [1], so this genus is perhaps due for a more detailed study in this respect.

Two methods of analysis were applied, the main one being the method normally used in this laboratory which is based on the Likens and Nickerson apparatus [2]; this method has been employed successfully before with crucifers such as *Nasturtium officinale* [3] and *Lepidium sativum* [4]. The other method, intended to confirm these results, was that employed by Cole in her recent extensive study of the glucosinolates of many Cruciferae [5]. Both these methods detect only those glucosinolates which yield volatile degradation products, so the very few which do not will not be determined by either of these procedures. The normally accepted method of analysis for glucosinolates involves adding extra external (usually mustard) myrosinase to the system under study and carrying out the enzymic reaction in buffer solution at optimum pH for the enzyme. Further objectives of this work were thus to compare analytical data obtained by this standard procedure with those obtained more naturally relying only on the endogeneous enzyme of the plant, but both with the pH of the system still controlled to optimum and also without any such control at the natural pH of the plant. This latter is, of course, the genuine, natural situation (autolysis). These investigations formed part of our study of factors affecting glucosinolate decomposition.

RESULTS AND DISCUSSION

Table 1 lists the volatile aroma components obtained from both species of *Farsetia* using the more sensitive modified Likens and Nickerson procedure [3, 4] (data arbitrarily selected for analyses carried out with added myrosinase enzyme at the optimum pH for the enzyme). It can be seen that as well as glucosinolate products some other normal aroma components are also produced, but in Cruciferae these become very much the minor contributors and generally for the *Farsetia* species studied they comprised only about 10% at the most of the sample extracts. All identifications given in Table 1 were made from the mass spectra obtained by GC–MS analysis of the sample extracts. Summaries of the mass spectra of the glucosinolate products only (as relevant to this study) are given in Table 2, and those reported before compare well, e.g. [4, 6, 7]. Retention times quoted in Table 1 agree exactly with those determined for standard compounds where these were available, and these components are indicated in the table by (†).

Table 3 lists only the glucosinolate degradation products from the two species of *Farsetia* obtained both

Table 1. Volatile components from *Farsetia aegyptia* and *F. ramosissima*

GLC peak No.	Compound	*F. aegyptia* Mass spectra*	*F. aegyptia* t_R (min)	*F. aegyptia* % Rel. abund.	*F. ramosissima* Mass spectra*	*F. ramosissima* t_R (min)	*F. ramosissima* % rel. abund.
1	†Cyclohexane	xxx	1.5	0.5	xxx	1.5	1.0
2	†Benzene	xxx	2.0	tr	xxx	2.0	0.5
3	†Carbon tetrachloride	xxx	2.5	tr	xxx	2.5	tr
4	†Butanone	xxx	3.0	1.5	xxx	3.0	1.0
5	†Ethanol	xxx	3.5	1.5	xxx	3.5	1.0
6	†Chloroform	xxx	5.3	tr	xxx	5.3	tr
7	†2-Methylpropan-1-ol	xxx	6.0	tr	xxx	6.0	tr
8	2-Methylbutan-1-ol	xxx	6.2	tr	xx	6.3	tr
9	†Toluene	xxx	6.5	1.0	xxx	6.5	2.0
10	Unknown		7.6	tr			
11	†But-3-enonitrile	xxx	9.6	13.0	xxx	9.6	tr
12	†Pyridine	xxx	9.8	tr	xxx	9.8	tr
13	Unknown					10.5	tr
14	Pent-4-enonitrile	xxx	10.9	1.0	xxx	10.9	8.5
15	*sec*-Butyl isothiocyanate	xxx	11.0	tr			
16	Unknown		11.2	tr		11.2	tr
17	Unknown		11.5	tr			
18	Dimethylpyrazine				xxx	11.5	tr
19	†Allyl isothiocyanate	xxx	12.1	59.0	xxx	12.1	tr
20	†Allyl thiocyanate	xxx	12.8	‡			
21	2-Methylbutyl isothiocyanate	x	13.0	tr			
22	But-3-enyl isothiocyanate	xxx	13.6	2.5	xxx	13.6	77.5
23	Unknown		13.9	tr			
24	Unknown		14.1	tr			
25	†Benzaldehyde	xxx	15.8	tr	xxx	15.8	2.0
26	Unknown					16.0	tr
27	β-Ocimene	xx	16.0	tr			
28	Tolualdehyde	xxx	17.0	0.5	xxx	17.0	1.0
29	Piperitone	x	17.3	0.5			
30	2-Hydroxy-3-phenylpropionitrile	x	17.5	tr	x	17.5	tr
31	4-(Methylthio)-butyronitrile	xxx	18.0	4.5			
32	5-(Methylthio) pentanonitrile				xxx	19.0	1.5
33	Unknown		20.0	0.5			
34	†Phenylacetonitrile	xxx	20.2	tr	xxx	20.1	tr
35	Unknown		20.5	tr			
36	3-(Methylthio)propyl isothiocyanate	xxx	21.5	7.5			
37	Unknown		22.0	1.0			
38	Unknown					24.0	tr
39	†3-Phenylpropionitrile	xxx	26.0	tr	xxx	26.0	tr
40	†Benzyl isothiocyanate	xxx	27.0	tr			
41	4-(Methylthio)butyl isothiocyanate				xxx	30.0	1.5
42	†2-Phenethyl isothiocyanate	xxx	32.5	tr	xxx	32.5	tr
43	Unknown		34.0	0.5			
44	Unknown					35.0	tr
45	Unknown					40.0	tr

*xxx Positive identification due to perfect, or near perfect, mass spectra; xx reasonably certain identification due to good mass spectra; x tentative identification due to poor mass spectra.

†Reference compound t_R agreed exactly.

‡Only produced under certain circumstances—see text.

tr = trace.

Table 2. Mass spectra of glucosinolate degradation products from two species of *Farsetia*

But-3-enonitrile	*m/e*	41	39	67	40	38	52	66		
	% rel. int.	100	50	35	25	20	15	10		
Pent-4-enonitrile	*m/e*	41	81	39	54	52	53	40	38	
	% rel. int.	100	40	31	21	4	3	2	2	
sec-Butyl isothiocyanate	*m/e*	41	56	57	115	86	55			
	% rel. int.	100	60	40	30	25	25			
Allyl isothiocyanate	*m/e*	99	41	39	72	45	40	38	71	98
	% rel. int.	100	98	79	30	10	10	8	3	2
Allyl thiocyanate	*m/e*	41	99	39	72	45	40	38	71	
	% rel. int.	100	92	89	47	15	13	10	5	
2-Methylbutyl	*m/e*	41	39	72	69	55	129	114		
isothiocyanate	% rel. int.	100	90	30	29	28	3	2		
But-3-enyl isothiocyanate	*m/e*	72	113	39	41	55	53	54	85	
	% rel. int.	100	77	35	26	25	8	8	5	
2-Hydroxy-3-phenyl-	*m/e*	91	147	65	92	39	51			
propionitrile	% rel. int.	100	14	9	8	6	3			
4-(Methylthio)butyronitrile	*m/e*	61	115	47	62	54	68	75	40	
	% rel. int.	100	32	22	17	15	8	5	2	
5-(Methylthio)-	*m/e*	61	82	129	54	47	55	75	114	
pentanonitrile	% rel. int.	100	23	22	16	14	10	3	2	
Phenylacetonitrile	*m/e*	117	90	116	91	89	51	118	77	
	% rel. int.	100	35	28	20	8	5	3	2	
3-(Methylthio)propyl	*m/e*	41	43	39	61	101	72	91	115	147
isothiocyanate	% rel. int.	100	65	48	44	41	26	14	5	2
3-Phenylpropionitrile	*m/e*	91	43	131	65	51	77			
	% rel. int.	100	28	20	18	10	7			
Benzyl isothiocyanate	*m/e*	91	149	92	57	55	117			
	% rel. int.	100	15	7	4	2	2			
4-(Methylthio)butyl	*m/e*	61	115	41	44	55	72	87	85	161
isothiocyanate	% rel. int.	100	58	50	38	38	19	10	10	5
2-Phenethyl isothiocyanate	*m/e*	91	163	105	77	92	51	50		
	% rel. int.	100	20	9	9	7	7	5		

Table 3. Relative and absolute amounts of glucosinolate degradation products formed by two species of *Farsetia* under various conditions

	F. aegyptia				*F. ramosissima*					
	With added enzyme		Without added enzyme		With added enzyme		Without added enzyme		Autolysis (at natural pH)	
	µg/g	%	µg/g	%	µg/g	%	µg/g	%	µg/g	%
But-3-enonitrile	177	15.1	721	74.1	tr	tr	tr	tr	tr	tr
Allyl isothiocyanate	729	68.1	266	23.4	tr	tr	tr	tr	tr	tr
Pent-4-enonitrile	12	1.0	10	1.0	87	9.8	1522	75.3	1990	93.3
But-3-enyl isothiocyanate	30	2.8	tr	tr	768	86.9	374	15.0	150	5.7
4-(Methylthio)butyronitrile	50	5.0	10	1.0						
3-(Methylthio)propyl isothiocyanate	101	8.6	5	0.5						
5-(Methylthio)pentanonitrile					13	1.5	192	9.5	21	1.0
4-(Methylthio)butyl isothiocyanate					15	1.7	tr	tr	tr	tr
Phenylacetonitrile	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
Benzyl isothiocyanate	tr	tr	tr	tr						
3-Phenylpropionitrile	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
2-Phenethyl isothiocyanate	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
sec-Butyl isothiocyanate	tr	tr	tr	tr						
*(2-Methylbutyl isothiocyanate)	tr	tr								
*(2-Hydroxy-3-phenylpropionitrile)	tr	tr			tr	tr				

*Tentative identification.

tr = trace.

with and without the addition of external (mustard seed) enzyme, when analysed at the optimum pH for the enzyme and using the modified Likens and Nickerson apparatus [3, 4]. In addition for *F. ramosissima* only, results are also given for the products of genuine autolysis, i.e. completely natural decomposition at the natural pH of the plant without the addition of any external enzyme. Products from a common glucosinolate precursor are bracketed together in the table. Benzaldehyde, which may be formed from benzylglucosinolate, is not however listed in this table due to lack of positive proof of this origin. Allyl thiocyanate is not listed either, since this was only obtained (in amounts up to 5%) from *F. aegyptia* samples (enzyme treated) which were not submitted to the Likens and Nickerson procedure, but which were simply allowed to incubate at room temperature and were then extracted in the cold with ether.

For clarity, percentages quoted in Table 3 relate to total glucosinolate products alone, unlike the percentage relative abundances given in Table 1 which relate to the whole system, including components not of glucosinolate origin. Absolute amounts of glucosinolate products formed (Table 3) were determined by standard injections into the gas chromatograph of pure reference compounds where available, and extrapolated logically to the other similar components. These figures allow for the recovery of the sampling procedure (91%).

From Table 3 it can be seen that *F. aegyptia* contains eight glucosinolates although two are only tentatively identified, whilst *F. ramosissima* contains only six including one tentative assignment. The major glucosinolate of the former species is allylglucosinolate (>80%) whilst that of the latter is but-3-enylglucosinolate (>90%). There is thus a significant difference between the two species in this respect.

With both *F. aegyptia* and *F. ramosissima* the addition of extra myrosinase generally encourages the formation of degradation products from minor glucosinolates, hence aiding their detection. This is seen for *F. aegyptia* in particular, and therefore this approach is to be recommended, but solely for glucosinolate identification purposes since totally inaccurate results are then obtained with respect to the relative amounts of the degradation products. This can be seen for both species where under more natural conditions without the addition of external enzyme, nitriles were major components, whether or not the pH of the system was controlled, but when extra enzyme was added isothiocyanates became the main products. The results for *F. aegyptia* show this particularly clearly (Table 3) where although the absolute amount of allylglucosinolate products remains approximately constant (906 and 987 μg/g), when no extra enzyme is added about 74% of but-3-enonitrile is produced whilst when enzyme is added about 68% of the corresponding isothiocyanate is produced instead.

Even artificially controlling the pH of the natural (endogenous) enzymic process affects the results. This can be seen in Table 3 for the major glucosinolate of *F. ramosissima* where even more nitrile is produced, at the expense of isothiocyanate, when the reaction occurs at the natural pH of the plant (determined as 5.91) compared with the optimum pH for the enzyme (6.96 employed in this project). However, the total amount of glucosinolate products formed remains constant, within experimental error. Glucosinolate degradation is, of course, very susceptible to slight pH variations, with nitrile formation being dominant at low pH and isothiocyanate at higher pH values. Hence the results just mentioned are explicable on that basis alone. Furthermore, for *F. aegyptia*, in the absence of added enzyme, it was found that if incubation was carried out at pH 7.5 instead of the usual 6.96, then the amount of allyl isothiocyanate formed changed from *ca* 23% to *ca* 95%.

In the past it has been generally accepted that at pH levels greater than 5 only isothiocyanates could be formed from enzymic decomposition of glucosinolates. The results described here are clearly in serious conflict with this view, and although pH certainly affects the isothiocyanate/nitrile ratio, nitriles can still be very much the major products even at pH 6.96, provided only the endogenous enzymes are allowed to accomplish substrate decomposition. This latter is, of course, the important point, in that invariably the data on which the previous misconception had been based were gathered employing the principle of addition of extra enzyme during analysis. Furthermore, we found that even if the natural pH of the plant is employed, the addition of external enzyme still reverses the ratio of products formed, and for *F. ramosissima*, for example, but-3-enyl isothiocyanate was formed in relative amounts greater than 90% under these conditions (compare data in Table 3).

The ratio of nitrile to isothiocyanate obtained on enzymic glucosinolate degradation is hence very delicately balanced. Clearly, therefore, analysis of products must be accomplished under natural conditions for valid results, although to facilitate simple detection of precursor glucosinolates any device which might increase sensitivity, such as adding extra enzyme to ensure complete decomposition, is acceptable. However, from the absolute figures quoted in Table 3 this widely used method would not always seem to be effective (e.g. 906 against 987 μg/g for allylglucosinolate of *F. aegyptia*).

The results obtained using the method of analysis employed by Cole [5] were similar, but due to the lower sensitivity of that method lesser amounts of all products were obtained in all instances (usually far less than 10% of the absolute amounts obtained using the Likens and Nickerson apparatus). For this reason, none of the minor components reported in Table 3 was detected by this other method. In addition it tended to give a higher proportion of isothiocyanate than nitrile in all instances, even when no external enzyme was added. To exemplify these points (cf. Table 3), for *F. aegyptia* without added enzyme the method gave greater than 98% allyl isothiocyanate with traces of only but-3-enonitrile, 4-(methylthio)-butyronitrile and 3-(methylthio)propyl isothiocyanate. This represents only two of the six to eight glucosinolates detected by the main method. On addition of external enzyme only allyl isothiocyanate could be detected. The higher amounts of isothiocyanates in general produced by this method are undoubtedly due to carrying out incubation at an artificial pH of 7–8 rather than at the natural pH of the plant, for the reasons (and based on the observations) already detailed. In validation, it will be remembered that carry-

ing out a Likens and Nickerson extraction on *F. aegyptia* without added enzyme but at a pH of 7.5 gave about 95% of allyl isothiocyanate, and so the approximately 98% produced by the Cole method under similar circumstances is in agreement.

In summary, it is most important that the conditions employed for such analyses of glucosinolate products from Cruciferae are as natural and genuine as possible, under which circumstances it is generally observed that nitriles become the predominant products over the corresponding isothiocyanates. The significance of the natural formation of nitriles from Cruciferae has until now been considerably underestimated.

EXPERIMENTAL

Plant material. Plants were collected in Mauritania during December 1976 and hot air-dried. Two gatherings of *Farsetia ramosissima* were obtained from sand dunes *ca* 15 km W of Nouakchott and from a rocky slope on the Teguel plateau *ca* 15 km NNW of Atar. One gathering of *Farsetia aegyptia* was obtained from the Teguel plateau *ca* 19 km NNW of Atar. No appreciable differences were observed between analyses for the two *F. ramosissima* samples, nor between any analyses carried out in January 1977 and repeated in July 1977. Many replicate analyses were carried out in all instances, and quantitative results were reproducible within small experimental error.

Enzyme preparation. Approximately 2 g of a stable, myrosinase enzyme prepn was readily obtained by the method of ref. [8] from 100 g of Coleman's yellow mustard powder. Its activity was confirmed by its degradation of commercial sinigrin (allylglucosinolate).

Analysis by the Likens and Nickerson method. The apparatus and procedure used were those previously described in detail [9, 10] which have been employed successfully before with Cruciferae [3, 4]. Finely chopped, air-dried leaves (20 g) of the *Farsetia* samples were immersed in 200 ml of a buffer, pH 6.96 (or distilled water for the *F. ramosissima* autolysis experiment at the natural pH of the plant) and allowed to stand at room temp. for 1 hr either with or without the addition of mustard enzyme preparation (40 mg). Extraction with the Likens and Nickerson apparatus was carried out for 1 hr using triply distilled 2-methylbutane (35 ml) as solvent. Extracts thus obtained were employed for quantitative analyses, but for qualitative analysis the concn procedure previously described [3, 4, 9, 10] had to be carried out on the extracts.

Analysis by Cole's method [5]. The method described by Cole was followed exactly. In addition, when appropriate, enzyme prepn (15 mg) was added before the 1 hr incubation at 40°.

Gas chromatographic analysis. Analyses were carried out using a 1.5 m × 4 mm i.d. glass column packed with 10% Carbowax 20 M coated on 100–120 BSS mesh acid-washed Diatomite C. A Pye model 104 single column gas chromatograph with heated FID was used. A nitrogen carrier gas was employed (30 ml/min) and the temp. programme found to be most successful was a 16°/min rise from an initial setting for 5 min of 60° to a final setting of 200° for the remainder of the run. Typically 2 μl of extract were injected at an attenuation setting of 1000 (i.e. 1×10^{-9} A f.s.d.). Retention times were measured from the onset of the solvent peak and internal standards employed were allyl isothiocyanate and 3-phenylpropionitrile. Peak area measurements were accomplished manually (peak height × width at half-height). Absolute amounts of compounds produced were assessed by standard injections of pure samples of but-3-enonitrile, allyl isothiocyanate, allyl thiocyanate, phenylacetonitrile, benzyl isothiocyanate, 3-phenylpropionitrile and 2-phenethyl isothiocyanate, the data of which were extrapolated logically to the other compounds for which reference samples were not readily available. The recovery of the Likens and Nickerson sampling method was determined by submitting standard amounts of these same seven compounds to the whole procedure.

GC–MS analysis. Components in the conc extracts were identified by GC–MS using an AE1 model MS 30 instrument linked on-line to an AE1 model DS 50 data processing system. The same gas chromatography conditions were employed as already described (although using He carrier gas), separated components being passed via a heated membrane separator to the mass spectrometer. Significant mass spectrometry parameters were ionisation potential, 70 eV; ionisation current, 300 μA; source temp. 230°; resolution, 1500; scan speed, 3 s/decade (repetitive throughout run). The background subtraction facility and the retrospective single ion monitoring facility of the data system were extensively employed in evaluating the mass spectral results.

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