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SYNTHESIS AND NATURAL OCCURRENCE OF 8-METHYLTHIOOCTANONITRILE AND 9-METHYLTHIONONANONITRILE

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Key Word Index—*Nasturtium officinale*; *Matthiola bicornis*; *Cheiranthus allioni*; watercress; wallflower; stock; Cruciferae; aroma; 8-methylthiooctanonitrile; 9-methylthiononanonitrile; synthesis.

Abstract—The title compounds were synthesized and confirmed to be constituents of watercress (*Nasturtium officinale*) aroma. Neither they nor their S-oxides were present in seed extracts of *Matthiola bicornis* or *Cheiranthus allioni*, and it is possible that their glucosinolate precursors may be limited in natural occurrence to only three genera, namely *Arabis*, *Sibara* and *Nasturtium*.

INTRODUCTION

In a previous publication we described the discovery of two new compounds in an aroma extract of the green leaves of *Nasturtium officinale* R.Br. (watercress), namely 8-methylthiooctanonitrile and 9-methylthiononanonitrile [1]. Although the MS evidence supporting these identifications is very strong, for new compounds it is nevertheless necessary to synthesize them for final confirmation. This paper in part describes this work.

In addition these compounds are of chemotaxonomic interest which warrants a further search for them in related plants. Before our findings noted above the situation was as follows. The glucosinolate precursors of the two nitriles (7-methylthioheptyl- and 8-methylthiooctylglucosinolates) had been reported, but based on the discovery of the appropriate isothiocyanate degradation products [2]. They had only been located in *Arabis hirsuta* (L.) Scop. (a rock cress) [2], but the corresponding glucosinolates with S-oxide side chains (i.e. 7-methylsulphinylheptyl- and 8-methylsulphinyloctyl) had been found in *Sibara virginica* (L.) Rollins (a bitter cress) [3]. The latter plant was originally known as *Cardamine virginica* (L), but has since been renamed due to its close relationship botanically to the genus *Arabis* [4] (indeed the 'new' name *Sibara* is an anagram of *Arabis*). Kjaer and Schuster had surmised therefore that the presence of the uncommon C_7 or C_8 ω-methylthioalkyl (or S-oxide) side chain (unbranched) might be characteristic of a very well-defined collection of taxa [2]. The fact that glucosinolates with side chains conforming to this description have also been located in *N. officinale* R.Br. [1] need not necessarily diminish their chemotaxonomic significance (although it does render them less specific) in that this plant is closely related to both *Arabis* and *Sibara* in being one of the relatively few other members of the same tribe (Arabideae). The remaining genera of this tribe are *Cheiranthus* (wallflower), *Matthiola* (stock) and *Barbarea* (winter cress). The second objective of this project was thus to examine some other members of the Arabideae to ascertain whether these particular glucosinolates might be a characteristic chemotaxonomic feature of the tribe. With regard to specificity of these glucosinolates, it should be emphasized that although a number of glucosinolates have been reported with straight chain ω-methylthioalkyl substituents ranging from $MeS(CH_2)_3$– to $MeS(CH_2)_8$—inclusive (all biosynthesized from methionine, with chain elongation), the two longest chain members, under consideration here, have not been located in any of the very many other cruciferous plants examined for their glucosinolate content. This includes a recent study in which we did not find them amongst the glucosinolates of another cress; *Lepidium sativum* (L.) [5].

RESULTS AND DISCUSSION

Both nitriles were synthesized from the appropriate α,ω-dibromoalkanes. One possible approach involving firstly the replacement of one Br atom selectively by cyanide proved difficult despite a number of attempts using different reaction conditions, and generally mixtures of unchanged starting material and dinitrile with only small quantities of ω-bromonitrile were obtained, which were difficult to separate. The alternative approach involving initial careful monothiomethylation was more successful giving predominantly the required intermediate ω-methylthioalkyl bromide, from which the ω-methylthioalkylnitrile product could be obtained readily by treatment with cyanide. Both 8-methylthiooctanonitrile and 9-methylthiononanonitrile possessed strong, fresh aromas very reminiscent of radish and it is interesting to note that one of the chief flavour components of radish is the glucosinolate degradation product 4-methylthio-*trans*-3-butenyl isothiocyanate [6].

GLC of the two nitriles on a Carbowax 20 M column under a variety of conditions showed them to have exactly the same R_f as peaks in the extract of *N. officinale* chromatographed under the same conditions (see Table 1). In addition, the MS of the synthesized compounds proved identical (within instrumental variation) with the spectra previously obtained and reported for the relevant GLC peaks of a watercress extract [1].

Table 1. GLC retention times (mins) of the major volatile components of seed extracts of *Nasturtium officinale*, *Matthiola bicornis* and *Cheiranthus allioni*

Peak Number	70° (1 min)→(40°/min, 40° min)→180° (30 min)			180° isothermal			200° isothermal		
	N. officinale	*M. bicornis*	*C. allioni*	*N. officinale*	*M. bicornis*	*C. allioni*	*N. officinale*	*M. bicornis*	*C. allioni*
1			7.45			1.30			
2	7.85			1.40					
3			8.55			1.50			
4			9.60			2.05			1.30
5					2.20				
6					2.35				
7			10.3			2.70			1.65
8					3.00				
9					3.20				
10	11.4			3.70			1.90		
11		11.8	11.8		3.90	3.90		2.00	2.00
12		12.5	12.5		4.70	4.70		2.65	2.65
13		13.8			5.60			2.95	
14	14.2			6.15			3.20		
15		14.9	14.9		6.80	6.80		3.50	3.55
*16	15.8			7.40			3.85		
17		17.8	17.9		9.50	9.50		4.80	4.80
*18	19.1			10.6			5.30		
19			20.5			11.7			5.75
20	22.2	22.2		13.5	13.5		6.55	6.55	
21		24.0			15.1			8.00	
22			25.2			16.2			8.90
23	29.1			19.5			12.0		
8-methylthiooctanonitrile	15.8				7.40			3.85	
8-methylsulphinyloctanonitrile					30.7			14.4	
9-methylthiononanonitrile	19.1				10.6			5.35	
9-methylsulphinylnonanonitrile					35.1			16.5	

Summaries of these MS are as follows: 8-methylthiooctanonitrile; synthesized *m/e* (% rel. int.) 61 (100), 96 (64), 69 (22), 82 (21), 171 (18), 75 (13), 124 (10), 110 (8). Watercress *m/e* (% rel. int.) 61 (100), 96 (55), 69 (25), 171 (22), 82 (17), 124 (10), 75 (10), 110 (5). 9-methylthiononanonitrile; synthesized *m/e*, (% rel. int.) 61 (100), 96 (68), 69 (30), 82 (20), 185 (20), 110 (14), 138 (11), 75 (9), 170 (6). Watercress *m/e* (% rel. int.) 61 (100), 96 (65), 69 (35), 82 (20), 185 (15), 110 (10), 75 (8), 138 (5), 124 (5). It can be concluded therefore that the original identifications of the two new watercress aroma components are correct.

Table 1 gives in detail the GLC R_ts on a Carbowax 20 M column under a number of conditions of all the major peaks of seed extracts of *N. officinale*, *Matthiola bicornis* and *Cheiranthus allioni*. Seeds were used rather than the plants themselves since these are usually a richer source of the glucosinolates. Simple aqueous or isopentane extracts of the seeds gave rather weak samples, so steam distillation–solvent vapour extractions were performed using a Likens and Nickerson apparatus which has been modified and used extensively in similar work in this laboratory [1,5,7,8]. In this way concentrated samples could be obtained sufficient to show clearly by GLC all the previously determined components of watercress and also many peaks for both *M. bicornis* and *C. allioni*. The results for the 3 plants are shown together in Table 1 and peaks of different R_t have arbitrarily been assigned numbers for the purpose of this discussion. It must be emphasized that only the identities of watercress peaks numbers 10, 14, 16 and 18 are known (3-phenylpropionitrile, 2-phenethyl isothiocyanate, 8-methylthiooctanonitrile and 9-methylthiononanonitrile, respectively). It follows that, apart from these peaks, components of different samples but with the same R_t and listed together under the same peak number need not necessarily be chemically identical, although it is likely. It is interesting to note that *M. bicornis* and *C. allioni* do show a number of common features (peaks 11, 12, 15 and 17) and appear to be more closely similar to each other than either to *N. officinale*.

The results show quite clearly that neither of the two nitriles, peaks 16 and 18 (marked with asterisks), are present in the seed extract of *M. bicornis* or *C. allioni*. The possibility exists, however, that it might be the appropriate S-oxide glucosinolates which occur in these plants. The two ω-methylsulphinylalkylnitriles were prepared from the two ω-methylthioalkylnitriles by simple treatment with strong aqueous H_2O_2, and their R_ts are also quoted in Table 1. The GLC at 200° was carried out to obtain practical R_ts for these slowly eluting compounds. However, these components were not detected in any of the seed extracts studied. Although a number of minor peaks were obtained for the *M. bicornis* and *C. allioni* samples which are not quoted in the Table, none appeared in the vicinity of any of the standard compounds.

It can be concluded therefore that none of the glucosinolates in question occur in *M. bicornis* or *C. allioni*. It is not feasible that other glucosinolate degradation products would be given by these plants to the exclusion of any nitrile at all. It follows that the presence of the C_7 or C_8 ω-methylthioalkyl (or S-oxide) side chain (unbranched) is not a characteristic of member of the tribe

Arabideae and that on this basis *Nasturtium* is as closely related to *Arabis* and *Sibara* as the latter two are to each other, with *Matthiola* and *Cheiranthus* being less closely related to these three. It may be that this structural feature is restricted to the three genera in which it is presently known to occur. Should this prove to be the case this would set apart these genera from others as a very distinctive group and it might possibly indicate a specific common evolution.

EXPERIMENTAL

8-Methylthiooctyl bromide. Methanethiol (0.96 g, 0.02 M) was introduced into a soln of Na (0.46 g, 0.02 M) in MeOH (25 ml). This soln of MeSNa was added to 5.44 g (0.02 M) of 1,8-dibromooctane and the mixture refluxed with stirring for 3 hr. MeOH was removed on a rotary evaporator when NaBr was deposited. The remaining liquid was decanted and the solid washed several times with Et_2O. H_2O was added to the combined Et_2O washings and liquid, and Et_2O extraction performed. The Et_2O extract was dried and the solvent removed on a rotary evaporator. The residue was distilled under red. pres. to give 3.2 g (67%) of 8-methylthiooctyl bromide as a colourless, slightly viscous liquid, bp 122–4° (4.5 mm), n^{20} 1.5031, shown by GLC to be 95% pure. (Found: C, 45.08; H, 8.08; Br, 33.53; S, 13.28. $C_9H_{19}BrS$ requires C, 45.19; H, 8.01; Br, 33.41; S, 13.40%). IR (liq. film), MeS (960 cm^{-1}), CBr (650 cm^{-1}); MS, *m/e* (% rel. int.), 240 (21), 238 (21), 225 (5), 223 (5), 193 (14), 191 (14), 159 (34), 137 (3), 135 (3), 61 (100).

7-Methylthioheptyl bromide. Prepared as for the higher homologue above to give 62% of 7-methylthioheptyl bromide as a colourless liquid, bp 110–2° (5.0 mm), n^{20} 1.5028, shown by GLC to be 90% pure. (Found: C, 42.39; H, 7.50; Br, 35.25; S, 14.08. $C_8H_{17}BrS$ requires C, 42.67; H, 7.61; Br, 35.49; S, 14.24%). IR (liq. film), MeS (960 cm^{-1}); CBr (650 cm^{-1}); MS, *m/e* (% rel. int.), 226 (14), 224 (14), 211 (2), 209 (2), 179 (11), 177 (11), 145 (38), 123 (3), 121 (3), 61 (100).

9-Methylthiononanonitrile. NaCN (1.31 g, 0.026 M) partly dissolved in DMSO (15 ml) was added to 8-methylthiooctyl bromide (3.2 g, 0.0133 M). Mixing was carried out at 60° but since there was an exothermic reaction some cooling was necessary. The mixture was heated at 90° with stirring for 1.5 hr. On cooling and dilution with H_2O 2 layers separated. The mixture was extracted ×3 with Et_2O (20 ml), the Et_2O extract washed with 6N HCl (2 × 20 ml) and with H_2O (2 × 20 ml), and then dried. After removal of solvent on the rotary evaporator, the residue could be purified by fractional distillation but better purification was by means of an Al_2O_3 column and elution with hexane–Et_2O (1:1). 9-Methylthiononanonitrile was thus obtained in 65% yield (1.6 g) as a colourless liquid with a strong aroma reminiscent of radish, bp 278° (d), n^{20} 1.4^{764}, shown by GLC to be 98.5% pure. (Found: C, 64.59; H, 10.17; N, 7.45; S, 17.53. $C_{10}H_{19}NS$ requires C, 64.81; H, 10.33; N, 7.56; S, 17.30%). IR (liq. film), CN (2240 cm^{-1}), MeS (960 cm^{-1}); MS, quoted in text but accurate mass of M^+ = 185.123823 (0.000957 mass units deviation from $C_{10}H_{19}NS$).

8-Methylthiooctanonitrile. Prepared as for the higher homologue above to give 68% of 8-methylthiooctanonitrile as a colourless liquid, also with a radish-like aroma, bp 236° (d), n^{20} 1.4762, shown by GLC to be 95.5% pure. (Found: C, 63.36; H, 10.09; N, 8.00; S, 18.91. $C_9H_{17}NS$ requires C, 63.10; H, 10.00; N, 8.18; S, 18.72%). IR (liq. film), CN (2240 cm^{-1}), MeS (960 cm^{-1}); MS, quoted in text but accurate mass M^+ = 171.108104 (0.001032 mass units deviation from $C_9H_{17}NS$).

Examination of seeds. These were obtained from commercial suppliers (*N. officinale* from Suttons Seeds Ltd., Reading, England; *M. bicornis* and *C. allioni* from Stokes Seeds, St. Catherines, Ontario, Canada). *Ca* 30 g of seeds were ground in a mortar with 125 ml H_2O and submitted to steam distillation–solvent vapour extraction as previously described [1,5,7,8]. Isopentane was the best extracting solvent and 20 ml were used in extractions carried out over 3 hr. Further concentration (*ca* × 10) of extracts was achieved by low temp.–high vacuum distillation using the apparatus previously reported [1,5,7,8]. GLC analysis of samples was accomplished using a FID instrument and 2 m × 0.25 mm column packed with 10% Carbowax 20 M used at the various temps shown in Table 1. Detector temp. was 250°; injection port temp., 240°; N_2 flow rate *ca* 180 ml/min.

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