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SEED VOLATILES WITHIN THE FAMILY TROPAEOLACEAE

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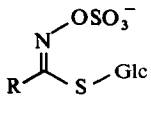
Key Word Index—Tropaeolaceae; seeds; volatiles; isothiocyanates; nitriles; chemotaxonomy.

Abstract—The volatile isothiocyanates arising from enzymic hydrolysis of the glucosinolates in 23 collections of seeds of 9 species of the genus *Tropaeolum* have been studied by chromatography of their thiourea derivatives; three patterns have been distinguished. GC–MS analysis of the volatiles from seeds of *T. cochabambae* and *T. peregrinum* permitted the identification of 9 and 17 individual volatile components, respectively.

INTRODUCTION

The South American family Tropaeolaceae is generally conceived as a uniform collection of about 90 species, all but a few grouped together in the genus *Tropaeolum* [1]. Chemically, the family attracts interest by its content of C_n:1 ($n \geqslant 20$) fatty acids along with isothiocyanate-producing glucosinolates (**1**), a combination that is encountered also within the small North American family Limnanthaceae and reminiscent also of the pattern within the Cruciferae. Isothiocyanates reported from *Tropaeolum* species comprise, besides the benzyl derivative (**2**), identified almost 80 years ago [2], the 2-propyl (**3**) and 2-butyl (**4**) esters, co-occurring in the seeds of *T. peregrinum* L. [3]. In addition, *Tropaeolum* species have been cited as sources of the glucosinolates (**1a**)–(**1d**) [4a].



1

R – N = C = S

1a, R = $[Me]_2 \cdot C(OH) \cdot CH_2$
1b, R = $3\text{-}(OH)C_6H_4 \cdot CH_2$
1c, R = $4\text{-}(OH)C_6H_4 \cdot CH_2$
1d, R = $4\text{-}(MeO)C_6H_4 \cdot CH_2$

2, R = $Ph \cdot CH_2$
3, R = $[Me]_2 \cdot CH$
4, R = $Et \cdot CH(Me)$
5, R = Et
6, R = $[Me]_2CH \cdot CH_2$

Popular ornamentals ('garden Nasturtium'), notably *T. majus*, are hybrids, mostly of unknown origin. Occasionally, garden seeds have found use in seasoning of food ('peasant's capers'). With this background, we have studied the pattern of volatile isothiocyanates in a series of authentic *Tropaeolum* seed specimens, collected in the wild in South America. In two cases, viz. *T. cochabambae* Buch. and *T. peregrinum* L., a more detailed analysis of the seed volatiles has been performed by GC–MS.

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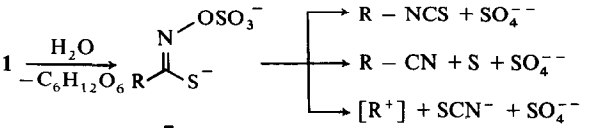
RESULTS AND DISCUSSION

In the presence of the enzyme myrosinase, recognized in seeds of *T. majus* and *T. peregrinum* about 85 years ago [5], glucosinolates (**1**) undergo hydrolysis to the aglucones (**7**) [4d], the further fate of which depends on



7 Scheme 1

their structure as well as on the prevailing conditions. Three frequently encountered degradative routes comprise (Scheme 1): rearrangement to isothiocyanates; fragmentation to nitriles; and production of carbonium ions, the further fate of which may vary from case to case. Hence, the essential oil fractions expectedly contain the genuine seed volatiles, supplemented by enzymically liberated products such as isothiocyanates, nitriles, and products derivable from carbonium ion intermediates.

Twenty-three collections of *Tropaeolum* made in the wild in Peru and mostly consisting of only a few seeds, representing nine species (Table 1), were surveyed for their contents of volatile isothiocyanates after enzymic hydrolysis by conversion of the mustard oils into thioureas and chromatographing these on paper, alone and in combination with authentic thioureas [6]. The results

Table 1. Volatile isothiocyanates in *Tropaeolum* species, identified by PC of the corresponding thiourea derivatives; the number of crosses signifies the relative amounts as estimated from the paper chromatograms

Species	No of collections	Volatile isothiocyanates Et (5)	$[Me]_2CH$ (3)	EtCH(Me) (4)	$Ph \cdot CH_2$ (2
T boliviense Loes.	1		++	+	+
T cochabambae Buch	2		+	+	+
T longiflorum Killip	1		+	++	+
T seemannii Buch	1		+	++	+
T tuberosum R & P	3		++	+	++
T. hjertingii Sparre, ined	1				+
T majus L *	3				+
T. minus L.	3				+
T peregrinum L	8	+	++	++	(+)

* Commercial cultivar specimen

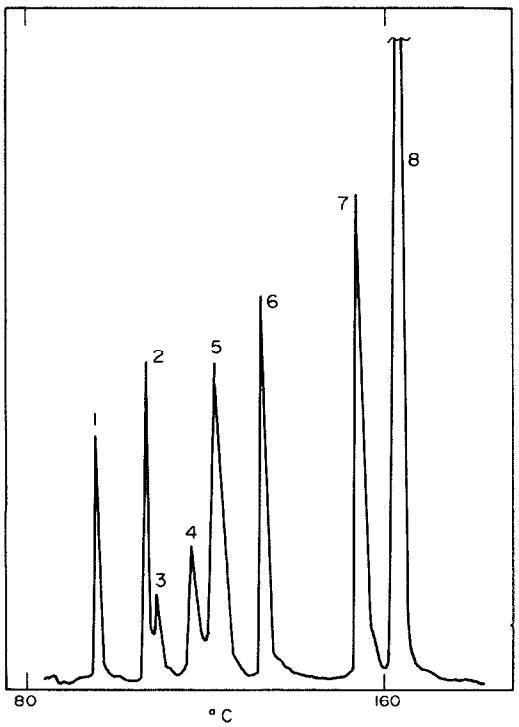


Fig. 1. GLC of volatiles from seeds of *Tropaeolum cochabambae* (cf. Table 2).

are presented in Table 1. Only minor variations were observed in the relative amounts of isothiocyanates within the various collections of a given taxon. Three patterns can be distinguished: the first five taxa encompass **3**, **4**, and **2** as their characteristic profile, whereas *T. hjertingii*, *T. minus* and *T. majus* are unique in producing **2** as the sole detectable, volatile isothiocyanate. Finally, *T. peregrinum* stands out by its consistent production of ethyl isothiocyanate (**5**), in addition to large quantities of **3** and **4** ,as well as trace amounts of **2**. The commercial cultivar '*T. majus*' is generally agreed to represent a hybrid of *T. peltophorum* Benth., *T. minus* and *T. majus* [1], the last itself presumably of hybrid character, with *T. hjertingii* and *T. minus* as putative parents. The isothiocyanate pattern is not at variance with this suggestion.

With the double purpose of evaluating the sensitivity

Table 2. Volatile constituents from seeds of *T. cochabambae*

Peak No. (Fig. 1)	MW	Structure	Ref.
1	101	$[Me]_2 \cdot CH \cdot NCS$ (**3**)	[8]
2	115	$Et \cdot CH(Me) \cdot NCS$ (**4**)	[8]
3	115	$[Me]_2 \cdot CH \cdot CH_2NCS$ (**6**)	[8]
4*			
5*			
6	117	$Ph \cdot CH_2 \cdot CN$	[7]
7	136	$4\text{-}(MeO)C_6H_4 \cdot CHO$†	
7	138	$4\text{-}(MeO)C_6H_4 \cdot CH_2OH$	[9]
8	149	$Ph \cdot CH_2 \cdot NCS$ (**2**)	[8]

* Structure unknown. † MS identical with that of an authentic specimen.

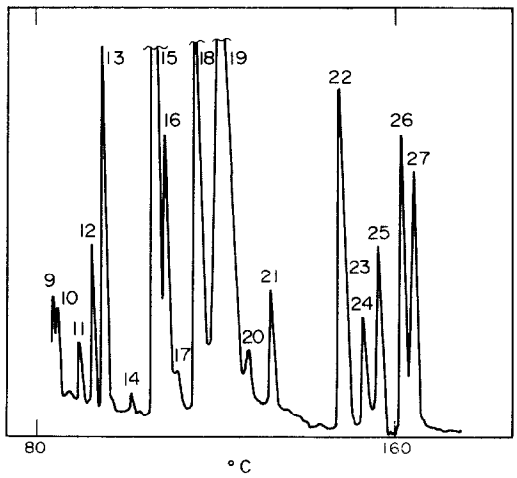


Fig. 2. GLC of volatiles from seeds of *Tropaeolum peregrinum* (cf. Table 3)

of the routine paper chromatographic method and of providing a more detailed picture of the volatiles in *Tropaeolum* seeds, two species, viz. *T. cochabambae* and *T. peregrinum*, were selected for GC–MS analysis. The total ion current tracings are presented in Figs. 1 and 2, respectively.

T. cochabambae (Fig. 1) displays a simple pattern of seed volatiles, quantitatively dominated by isothiocyanates. Structural assignment of the individual peaks, as presented in Table 2, rests solely on retention times and mass spectrometry. The small amount of 2-methylpropyl isothiocyanate (**6**), a congener of (**4**), was overlooked by paper chromatography, the corresponding

Table 3 Volatile constituents from seeds of *T. peregrinum*

Peak No. (Fig. 2)	MW	Structure	Ref
9	88	$Me \cdot CO_2Et$	[7]
10	104	$Me \cdot CH(OEt)OMe$	[7]
11	79	C_5H_5N (pyridine)	[7]
12	87	$Et \cdot NCS$ (**5**)	[8]
13	101	$[Me]_2CH \cdot NCS$ (**3**)	[8]
14	106	$C_6H_4[Me]_2$	[7]
15	115	$Et \cdot CH(Me) \cdot NCS$ (**4**)	[8]
16	115	$[Me]_2CH \cdot CH_2 \cdot NCS$ (**6**)	[8]
17	106	$Ph \cdot CHO$	[7]
18*,a			
19*,a			
19	108	$Ph \cdot CH_2OH$	[7]
20*			
21	117	$Ph \cdot CH_2CN$	[7]
22	152	$4\text{-}(MeO)C_6H_4 \cdot CH_2 \cdot OMe$†	
23	136	$4\text{-}(MeO)C_6H_4 \cdot CHO$‡	
24	138	$4\text{-}(MeO)C_6H_4 \cdot CH_2OH$	[9]
25	166	$4\text{-}(MeO)C_6H_4 \cdot CH_2 \cdot OEt$§	
26	149	$Ph \cdot CH_2 \cdot NCS$ (**2**)	[8]
27	147	$4\text{-}(MeO)C_6H_4 \cdot CH_2 \cdot CN$‖	

* Structure unknown, [a]identical with peaks nos. 4 and 5, cf. Table 2. † Identified by peaks at *m/e* (rel. intens. %): 152 (M^+, 44), 151 (26), 135 (12), 122 (13), 121 (100), 91 (8), 78 (6), and 77 (12). ‡ MS identical with that of an authentic specimen. § Identified by peaks at *m/e*: 166 (M^+, 35), 165 (9), 137 (18), 122 (21), 121 (100), 109 (14), 107 (8), and 77 (18). ‖ Identified by peaks at *m/e*: 147 (M^+, 100), 146 (39), 132 (32), 121 (8), 120 (8), 116 (20), and 104 (15).

thioureas possessing virtually identical R_f-values [6]. The supposedly isomeric constituents (peaks Nos. 4 and 5), exhibiting fragments indicative of an octene backbone remain unidentified. 4-Methoxybenzyl alcohol and anisaldehyde (peak No. 7), its oxidation product, most likely have their origin in the glucosinolate (**1d**).

T. peregrinum (Fig. 2) exhibits a somewhat more complex pattern of volatiles. Structural diagnoses of the individual components are presented in Table 3. Ethyl acetate, 1-ethoxy-1-methoxyethane, and pyridine, all very minor constituents, were unequivocally identified through their MS patterns [7]. Other volatiles, supposedly unrelated to the glucosinolate content, comprise a minor quantity of xylene and very substantial amounts of the isomeric unknowns discussed above. The origin of these volatiles is obscure.

Obviously derived from glucosinolates, the isothiocyanates (**2**)–(**6**) were identified upon comparison of their MS with those of authentic specimens [8]. Of similar derivation, benzyl cyanide was easily recognized by its MS, identical with that reported [7], whereas the second nitrile (peak No. 27), obviously a methoxybenzyl cyanide (cf. Table 3), has been formulated as the 4-methoxy isomer in view of the reported occurrence of the glucosinolate (**1d**) in *Tropaeolum* species [4a]. Because of agreement in the MS patterns with those recorded, reported, or expected for the 4-methoxy substitutes, the methoxybenzyl alcohol (peak No. 24), the corresponding methyl ether (peak No. 22, *cf.* Table 3), the methoxybenzaldehyde (peak No. 23), and, by inference, the methoxybenzyl ethyl ether (peak No. 25, cf. Table 3) all have been formulated as the 4-methoxy isomers. Benzyl alcohol (peak No. 19), identified through its MS, may conceivably arise similarly from benzylglucosinolate via the carbonium ion (Scheme 1); the trace of benzaldehyde (peak No. 17), recognized by its characteristic MS, may arise from oxidation of benzyl alcohol.

The *in vivo* synthesis of glucosinolates (**1**) is uniform, invariably requiring α-amino acids, $R\cdot[CH_2]_n\cdot CH(NH_3^+)CO_2^-$, as starting materials for the final products, $R\cdot[CH_2]_n\cdot C(S\cdot glucose):N\cdot OSO_3^-$ [4b]. In Cruciferae, Resedaceae, and, to a smaller extent, Capparaceae, protein amino acids ($n = 0$), as well as their homologized counterparts ($n \geq 1$), may serve as biosynthetic departure points, whereas the enzymic apparatus for homologizing amino acids seems absent, or far less well-developed, in other glucosinolate-producing families [4c]. By way of illustration, the isothiocyanates encountered within Tropaeolaceae can be formally accounted for in terms of α-aminobutyric acid, a common constituent of higher plants, supplemented with the protein amino acids valine, leucine, phenylalanine and tyrosine whereas derivatives of chain-elongated amino acids are strikingly absent.

It is hoped that access to additional species of the Tropaeolaceae, and hence to knowledge about their patterns of volatiles, may help to delineate both intra- and interfamilial affinities.

EXPERIMENTAL

PC-analysis of volatile isothiocyanates from Tropaeolum *seeds.* The finely ground powder from 2 to 5 seeds was suspended in a dilute phosphate buffer (pH 6.7, 8 ml); a few drops of a myrosinase-soln and a trace of ascorbic acid was added. After having been kept at 22° for 1 hr, the suspension was subjected to distillation through a short Vigreux-column. The receiver contained 2 ml of MeOH, saturated with NH_3. After standing for 1 hr at 22°, the soln in the receiver was conc to dryness *in vacuo*. The remaining thioureas were chromatographed on paper in H_2O-saturated $CHCl_3$ as previously described [6].

GC–MS analysis of volatiles from seeds of T. cochabambae *and* T. peregrinum. The enzymically hydrolyzed extract from 25 seeds, prepared as described above, was distilled through a short column; about 10 ml of distillate were collected. After saturation with NaCl, the aqueous phase was extracted × 3 with 2-ml portions of Et_2O. After drying, the Et_2O-phase was cautiously concentrated by distillation through a short column. The oily residue was applied to GC–MS analysis, performed on a Perkin-Elmer model 270 mass spectrometer, attached to a gas chromatograph equipped with a steel column (1.8 m × 3.2 mm), packed with 5% OV-101 on Chromosorb W (HP, 80–110 mesh). The operating conditions were: column temp. 80–230° at 10°/min; injector 200°; transfer lines and Watson-Biemann separator 250°; carrier gas He 40 ml/min; split ratio 4:1, ionization energy 70 eV; ion source temperature 200°.

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