Identification of new odorants in Indian Cress (Tropaeolum majus L.), Structure-activity study on synthesized homologues

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IDENTIFICATION OF NEW ODORANTS IN INDIAN CRESS (*TROPAEOLUM MAJUS* L.); STRUCTURE-ACTIVITY STUDY OF SYNTHESIZED HOMOLOGUES

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Abstract

This paper reports on the analysis of Indian cress absolute (*Tropaeolum majus* L.). Indian cress is an edible cress species that belongs to the *Brassicale* order. Its absolute, obtained by extraction of the plant with a non-polar solvent (concrete) followed by treatment with ethanol, has never been studied. It was analyzed by GC-MS and GC-O in order to characterize its main volatile compounds and key odorants. This research made it possible to identify two new volatile compounds: *N*-benzyl *O*-ethyl thiocarbamate and *O,S*-diethyl thiocarbonate. To the best of our knowledge, both compounds were reported in cress extract for the first time. These molecules being of organoleptic interest, 27 homologues were further synthesized and evaluated. Among those new volatiles, *N*-phenyl thiocarbamates were shown to be particularly interesting for their savory and meaty odors.

Introduction

One of our main research goals is the study of natural matrices used in perfumery in order to identify new natural odorants with low perception thresholds. We particularly focus on organonitrogen and -sulfur compounds because their presence in a matrix, even in sub-ppb quantities, can provoke a significant effect on the organoleptic properties of the matrix [1–3].
With this intention, we analyzed Indian cress absolute. Indian cress (*Tropaeolum majus* L.) is an edible cress species that belongs to the *Brassicale* order. *Brassicales* are known to produce glucosinolates which are among the major precursors of sulfur- and nitrogen-containing molecules [4]. However, to our knowledge, the key volatile compounds of Indian cress absolute have never been studied. The absolute is a perfumery solvent material obtained by extraction of the plant with a non-polar solvent (concrete) and then treatment with ethanol.
In this work, Indian cress absolute was analyzed by GC-MS and GC-O. Two new volatile compounds were identified: *O,S*-diethyl thiocarbonate and *N*-benzyl *O*-ethyl

thiocarbamate. To the best of our knowledge, both compounds were reported in a cress extract for the first time. These molecules being of organoleptic interest, 27 homologues were further synthesized, then evaluated, and are presented in this paper.

Experimental

GC-MS. Analyses were performed as described by Breme *et al.* [5–7].

GC-Olfactometry. GC-Olfactometry analyses were performed using an Agilent gas chromatograph 4890D (Agilent) and an eight-way sniffing multiport device (8W-GC-O), recently designed by Berdagué *et al.* [8].

Headspace Solid-Phase MicroExtraction - Comprehensive Two-dimensional GC coupled to a Time-of-Flight Mass Spectrometer (HS-SPME-GC×GC-TOFMS). Analyses were performed as described by Breme *et al.* [6–7], using a 6890N chromatograph (Agilent) equipped with two separate ovens, a time-of-flight mass spectrometer LECO Pegasus 4D (LECO Corporation, St. Joseph, MI), and a cryogenic modulator (LECOQuad JetModulator).

Synthesis of Thiocarbamates. Thiocarbamates were obtained by addition of anhydrous alcohol (methanol, ethanol, or 1-propanol; 50 mmol) to alkyl isothiocyanate (10 mmol), as described by Breme *et al.* [5]. The corresponding thiocarbamates gave yields between 25 % and 96 % with a GC purity range of 97–99 %.

Synthesis of Thiocarbonates. Thiocarbonates were obtained by slow addition of *S*-alkyl chlorothioformate (*S*-methyl, *S*-ethyl or *S*-propyl; 4 mmol) to anhydrous alcohol (methanol, ethanol, or 1-propanol; 4 mmol) in the presence of pyridine (4 mmol) and dichloromethane (3 mL), as reported by Breme *et al.* [6]. Thiocarbonates were obtained in yields from 9 % to 93 % with GC purities between 90 % and 96 %.

Results

Identification of N-benzyl O-ethyl thiocarbamate. The GC-MS analysis of Indian cress absolute revealed the presence of a major unknown compound that represented 84 % of the Total Ion Current (TIC). This compound was isolated by silica gel fractionation and then analyzed by HR-MS and NMR. On the basis of the HR-MS results, an empirical formula was proposed as $C_{10}H_{13}NOS$ (*m/z* found 195.0717 u, *m/z* calculated 195.0718 u) (**Figure 1**) [5]. Moreover, the fragments (*m/z* 91.0543 u) and (*m/z* 106.0654 u) suggested the presence of a benzyl-NH group. In addition, NMR analysis (^{1}H and ^{13}C) showed the presence of an ethoxy group and demonstrated the co-existence of two rotamers due to a restricted rotation of a C–C bound. As a consequence, the structure of *N*-benzyl *O*-ethyl thiocarbamate was hypothesized and synthesized. The mass spectrum and linear retention indices of the synthetic *N*-benzyl *O*-ethyl thiocarbamate were identical to data obtained from Indian cress absolute and confirmed its identification. *N*-benzyl *O*-ethyl thiocarbamate had already been reported in an African *Brassicale* (*Pentadiplandra brazzeana*) [9], but was reported in cress for the first time. As this compound is rarely found in plants and was not detected in the Indian cress concrete, its origin was studied. Benzyl isothiocyanate is known as a major compound of Indian cress (concrete). As the absolute is obtained by addition of ethanol to the concrete, *N*-benzyl *O*-ethyl thiocarbamate was supposed to result from the addition of ethanol on benzyl isothiocyanate (**Figure 2**) [5]. Experiments performed on cress concrete confirmed this reaction. Consequently, *N*-benzyl *O*-ethyl thiocarbamate seemed to result rather from the absolute preparation conditions than from the plant metabolism.

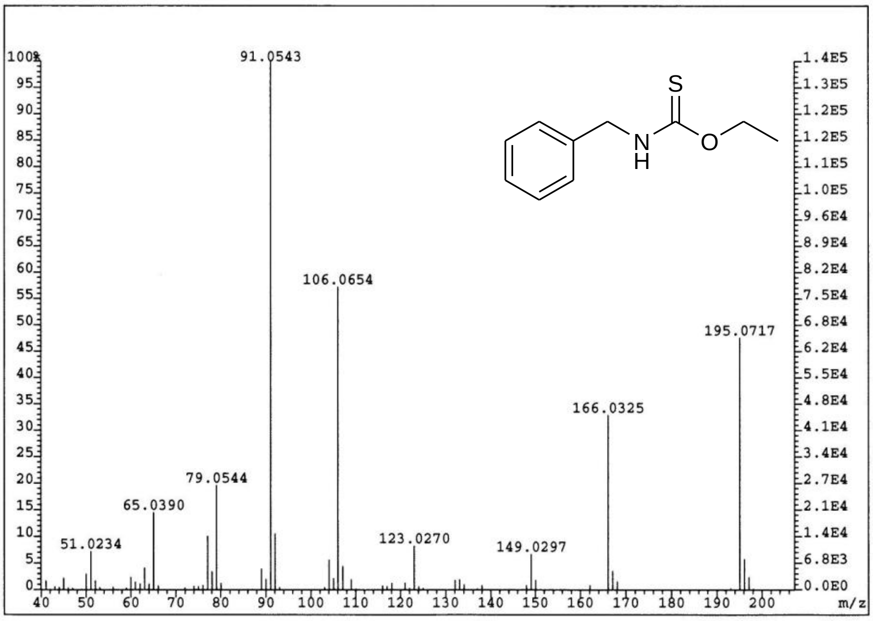


Figure 1. Mass spectrum of *N*-benzyl *O*-ethyl thiocarbamate identified in Indian cress absolute.

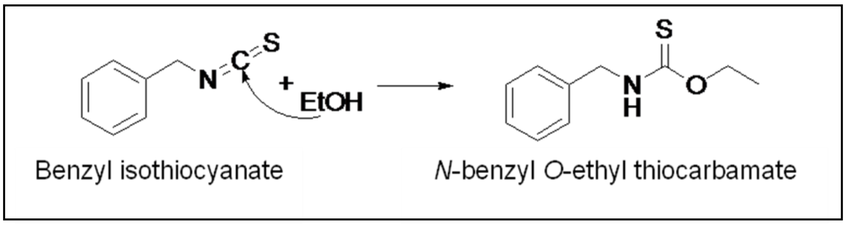


Figure 2. Formation of thiocarbamates in *Brassicales* during absolute preparation

8W-GC-O analysis of Indian cress absolute. Using the 8W-GC-O device recently designed by Berdagué *et al.* [8], Indian cress absolute was analyzed to characterize its key odorants. In this study, GC-O analysis was perfomed twice by eight judges. During the sniffing sessions, the sniffers were instructed (a) to signal each odor perceived by pressing a push button for as long as the odor lasted, (b) to describe the odors orally, and (c) to quantify their intensity on a five-point scale. The data were then compiled using the VIDEO-Sniff software by breaking the total olfactory signal (TOS, sum of the 16 individual aromagrams) up into olfactory classes that were defined during data processing, in order to highlight the key-odor zones of an aromagram. Using this data treatment, 44 odorants were characterized (**Figure 3**). Out of them, 22 were identified using comprehensive two-dimensional GC coupled to a time-of-flight-mass spectrometer (GC×GC-TOFMS). The main odoriferous compounds were identified as (*E*)-2-hexenal (**7**), diethyl trisulfide (**38**), an unknown fruity-sulfury compound (**15**), 1-octen-3-one (**18**), 1-octen-3-ol (**19**), 2-butylthiophene (**33**), 2-nonanone (**35**), and an unknown pyrazine (**46**) [7]. Most of them were reminiscent of fruity, green, mushroom, and sulfury nuances, which demonstrated the overall influence of sulfury and fruity notes in the olfactory profile of Indian cress absolute.

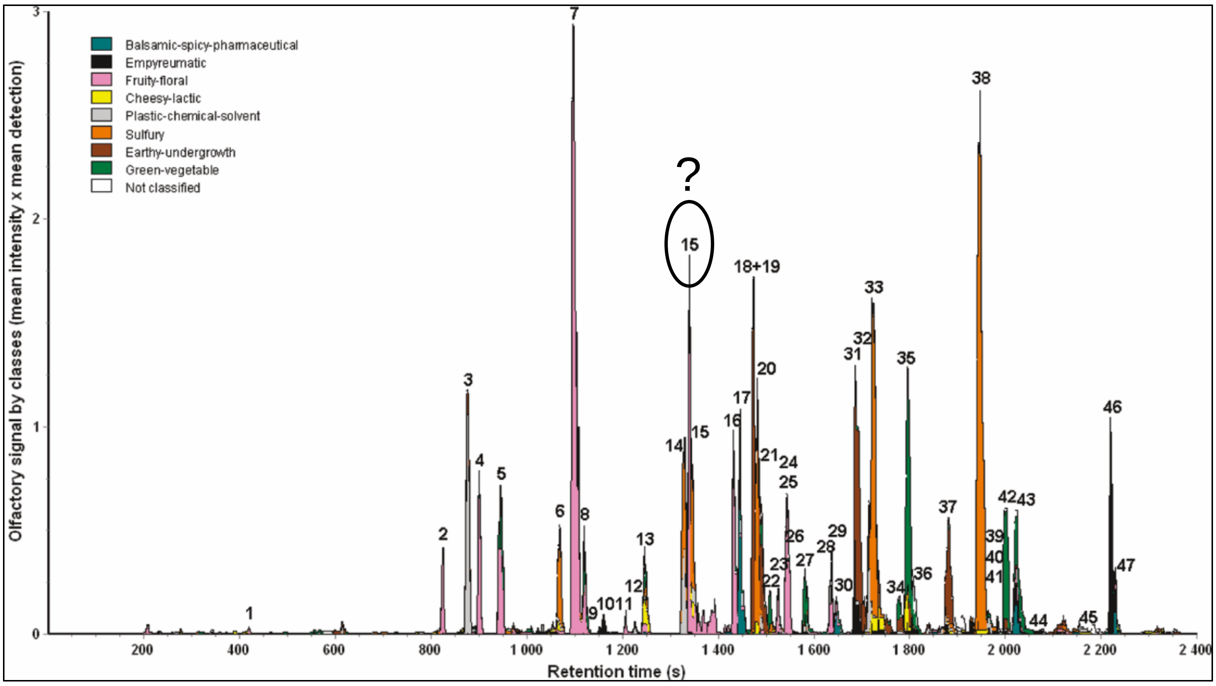


Figure 3. VIDEO-Sniff aromagram of Indian cress absolute, obtained with 16 judges. The breakdown of the mean total olfactory signal (TOS_{Int_Det}) into nine classes shows the odorant zones belonging to a given olfactory class. The compound **15** corresponds to *O,S*-diethyl thiocarbonate.

Identification of O,S-diethyl thiocarbonate. The key odorant **15** highlighted by 8W-GC-O was reminiscent of red fruit and slightly sulfury. This compound was detected by 15 out the 16 judges and presented a mean intensity of 4.3/5. Due to its appealing sensory properties, it had to be identified. However, compound **15** represented only 0.1 % of the TIC and could harldy be isolated by flash chromatography. Using GC×GC-TOFMS, a mass spectrum corresponding to this odorant was obtained. Its molecular mass was determined as *m/z* 134 u (**Figure 4**). Its isotopic peak pattern clearly demonstrated the presence of sulfur. Using preparative GC, this unknown odorant was isolated from the raw absolute and NMR (^{1}H and ^{13}C) was performed on the collected volatiles (3 mg). NMR proved the presence of a carbonyl group, as well as an ethoxy group, and an ethylthio group. On the basis of these data, the structure of *O,S*-diethyl thiocarbonate was proposed. It was synthesized and the data of the synthetic compound confirmed its identification in Indian cress absolute [8]. As far as we know, this compound was reported in cress for the first time. However, it has already been isolated in wine [10], as well as in pineapple [11]. Its origin remained unclear. It might either result form the plant metabolism, or be formed during the absolute preparation from the corresponding acid.

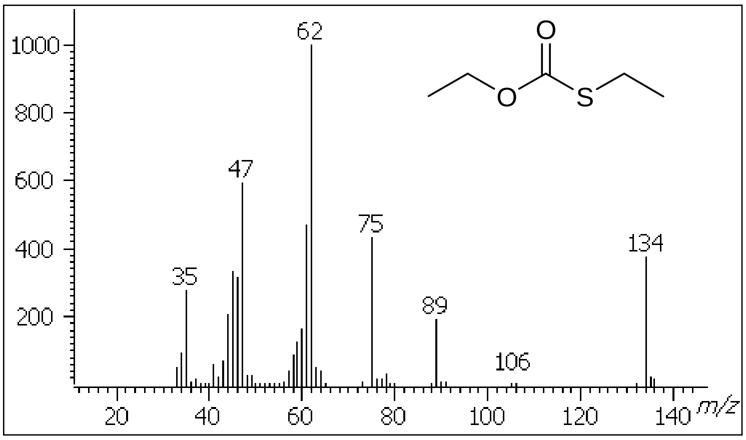
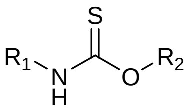
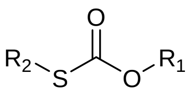


Figure 4. Mass spectrum of *O,S*-diethyl thiocarbonate identified in Indian cress absolute.

Table 1. Thiocarbamates and thiocarbonates synthesized.

	R_2	R_1	*LRI* HP-1	*LRI* Innowax	Olfactory evaluation by GC-O
Thiocarbamate 	methyl	ethyl	1046	1312	rubber, plastic, grilled
		allyl	1115	1948	garlic
		3-(methylthio)propyl	1556	2667	green, cress
		benzyl	1608	2766	green, faint odor
		phenyl	1536	2601	phenolic, geranium, green
		phenylethyl	1703	2827	mushroom
	ethyl	ethyl	1121	1878	grilled, a little bitter
		allyl	1195	1979	garlic, cooked garlic, onion
		3-(methylthio)propyl	1620	2696	green, cress, radish, pungent
		benzyl	1687	2784	green, cress, mild, faint odor
		phenyl	1592	2620	salty, bacon, smoked
		phenylethyl	1773	2847	mushroom, green, moss, wet forest
	propyl	ethyl	1219	1970	green, plastic
		allyl	1286	2069	spearmint
		3-(methylthio)propyl	1730	2770	green
		benzyl	1784	2868	green, floral, almond-like
		phenyl	1692	2690	smoked, sweet-vanilla
		phenylethyl	1875	2929	mushroom
Thiocarbonate 	methyl	methyl	767	1148	fruity, faint
		ethyl	829	1200	sulfury, pineapple
		propyl	926	1273	sulfury, main gas
	ethyl	methyl	825	1200	sulfury-fruity, pineapple
		ethyl	906	1240	fruity, red fruit, sulfury
		propyl	1001	1317	sulfury
	propyl	methyl	924	1280	alliaceous, fruity
		ethyl	1002	1325	red fruit, alliaceous
		propyl	1096	1399	sulfury, alliaceous

Synthesis of homologues of thiocarbamates and thiocarbonates. As *N*-benzyl *O*-ethyl thiocarbamate and *O,S*-diethyl thiocarbonate presented interesting sensory properties, homologues were synthesized. Six different isothiocyanates known for their presence in *Brassicale* plants were used for the synthesis of thiocarbamate homologues: allyl isothiocyanate, benzyl isothiocyanate, ethyl isothiocyanate, 3-(methylthio)propyl isothiocyanate, phenyl isothiocyanate, and phenylethyl isothiocyanate [4]. In total, 18 *O*-alkyl thiocarbamates were synthesized following a chemical pathway similar to that used for the synthesis of *N*-benzyl *O*-ethyl thiocarbamate. In addition, nine thiocarbonates were synthesized, as described by Breme *et al.* [6], by addition of alcohols on *S*-alkyl chlorothioformates. These 27 compounds were evaluated by GC-O, and their olfactory descriptors, as well as their linear retention indices (*LRI*) are listed in **Table 1**.
N-alkyl thiocarbamates were also evaluated by two flavorists from Robertet S.A. (Grasse, France). *N*-phenyl thiocarbamates were particularly appealing for the flavorists due to their savory nuances. They were tested in a meat flavor and were demonstrated to bring cooked pork nuances, and to be less fatty and greasy. As a result, they could be useful in the composition of meat aromas.

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