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Identification of the Sex Pheromone of *Scrobipalpula absoluta*; Determination of Double Bond Positions in Triple Unsaturated Straight Chain Molecules by means of Dimethyl Disulphide Derivatization.

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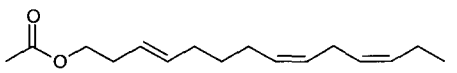
ABSTRACT: The sex pheromone of *Scrobipalpula absoluta* (Meyrick) (Lepidoptera: Gelechiidae) was identified as a 92:8 mixture of (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrienyl acetate (**1**) and (3*E*,8*Z*)-3,8-tetradecadienyl acetate (**2**) through mass spectrometric investigation of the dimethyl disulphide derivatives of excised sex pheromone glands. It is the first time that this method was used for triple unsaturated straight chain molecules. Compound (**2**) was identified as a new pheromone component. A synthetic mixture of the two identified compounds proved to be attractive in wind tunnel experiments.

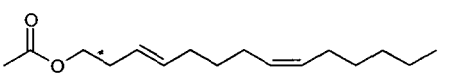
The tomato leafminer, *Scrobipalpula absoluta* (Meyrick) (Lepidoptera: Gelechiidae)[1], is at present considered to be the most important pest on tomatoes in South-America[2]. The larvae of this moth mine the leaves and stems of tomato plants and thus, cause considerable damage. The virgin females release a sex pheromone that strongly attracts conspecific males[3]. A synthetic sex pheromone, if available, could be applied for trapping male moths and, in this way could be helpful for establishing an Integrated Pest Management (IPM) program for this species.

Recently, a microscale random reduction procedure has been published for the identification of **1** as one of the constituents of the sex pheromone of *Scrobipalpuloides absoluta*[4,5]. A second, minor compound in the sex pheromone gland extract, has been detected but could not be identified[4]. These results prompted us to report on our independently obtained results in this area. In this paper, the identification of the minor component (3*E*,8*Z*)-3,8-tetradecadienyl acetate (**2**) as constituent of the sex pheromone of *S. absoluta*, as well as a new development in the identification of triple unsaturated linear pheromones, is presented.

Figure 1



1



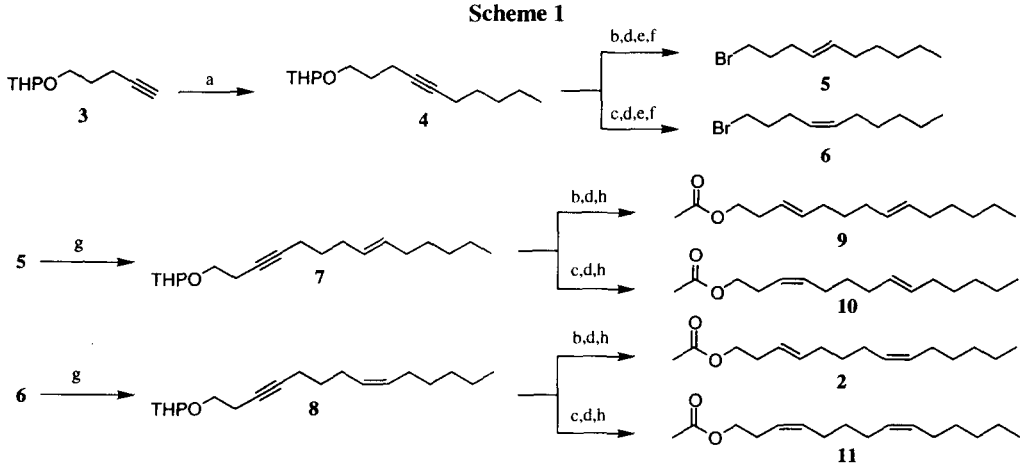
2

Derivatization of double bonds with dimethyl disulphide (DMDS) followed by analysis through mass spectrometry (MS) is an established technique for pinpointing the position of double bonds in straight-chain unsaturated molecules. This technique, although until now only applied for molecules with just one or two double bonds[6], proved to be also applicable for determining the exact location of three double bonds in triple unsaturated straight chain molecules. Preliminary examinations of a sex pheromone gland extract in hexane with GC-EAD[7] revealed that EAG active peaks only eluted during a short period of time.

GC-MS investigations of this part of the chromatogram gave evidence for two possible pheromone components (ratio 92:8). Both mass spectra of these two compounds had a small peak at *m/z* 61 indicating that they were acetates. The MS pertaining to the major peak showed an (M^+-60) fragment at *m/z* 190

indicating that this compound was a triple unsaturated tetradecyl acetate whereas the MS pertaining to the minor peak gave a (M^+-60) fragment at *m/z* 192 indicating that this compound was a double unsaturated tetradecyl acetate. GC Retention Index calculations showed that no conjugated double bonds were present in these molecules. DMDS derivatives[8,9] were prepared and subjected to detailed GC-MS studies. Straight chain DMDS derivatives with *n* double bonds, form (*n*-1) internal thio-ethers which give a unique MS fragmentation pattern from which the original double bond positions can be deduced. The MS of the DMDS derivative of the minor component with an M^+ at *m/z* 378[10] gave a fragmentation pattern that corresponded to a 3,8-tetradecadienyl acetate as the original molecule. To untangle the *E/Z* configuration of the double bonds, all four isomers of 3,8-tetradecadienyl acetate were synthesised in a stereo selective procedure as visualised in Scheme 1.

Scheme 1

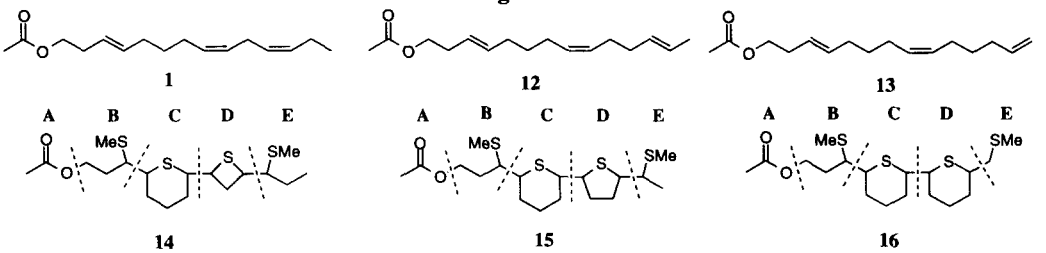


a) *n*-BuLi / THF, bromopentane / HMPA; b) $LiAlH_4$ / THF / diglyme, 140 °C; c) P-2 Ni / H_2 / EtOH; d) PTSA / H_2O / MeOH; e) *p*-TsCl / KOH / ether, 0 °C; f) LiBr / DMSO, 80 °C; g) Li—≡—OTHP , THF / HMPA; h) Ac_2O / AcOH, 60 °C.

The protected pentynol **3** was lithiated with *n*-BuLi in THF followed by alkylation with 1-bromopentane to give the protected alkynol **4** in 84% yield. After reduction of the triple bond with $LiAlH_4$ or catalytic with H_2, the molecules were deprotected with *p*-toluenesulfonic acid in a 10% H_2O in MeOH solution, tosylated, and converted into the bromoalkenes **5** and **6** in 52% and 79% yield respectively, based on **4**. These bromoalkenes reacted with the lithium salt of protected 3-butynol to give the protected compounds **7** and **8** in more than 80% yield respectively, based on **5** and **6**. Reduction and deprotection of **7** and **8** followed by acetylation gave all the stereo isomers **2** and **9** - **11**. The retention indices (RI's) of **2** (DB-1 and DB-WAX) accurately matched those obtained for the minor sex pheromone gland constituent. Moreover, electroantennography (EAG) measurements showed that **2** induced a significant larger response than the other three isomers **9** - **11** when exposed to the antennae of male *S. absoluta*.

From the MS fragmentation pattern of the DMDS derivative of the major compound (M^+ = 408[10]), double bonds at positions 3, 8 and 11 could be determined. The intensity of fragment E, originating from the ω-end of the molecule (Figure 2), is significant for the position of this double bond. To demonstrate this, molecules **1**, **12** an **13** (Figure 2) were synthesised, subjected to derivatization with DMDS and mass spectrometric analysis.

Figure 2



The molecules **1**, **12** and **13** will form DMDS derivatives **14**, **15** and **16** respectively. Fragmentation in the mass spectrometer of **14**, **15** and **16** will preferably occur at the positions as indicated with the dashed lines in figure 2. The intensities of the expected and obtained mass spectrometric fragments are given in table 1.

Table 1

			relative intensities (%)		
		m/z	3,8,11-14:Ac	3,8,12-14:Ac	3,8,13-14:Ac
specific	ABCDE	408	3.2	1.5	2
fragments	ABCDE - SMe	360/361	2.2	2	1.1
for all	ABCDE - 2x SMe	313	2.2	2.2	0.5
isomers	BCDE	348	0.6	-	0.6
	BCDE - SMe	300/301	2.4	0.9	1.8
	BCDE - 2x SMe	253	6.7	2.5	1.4
	ABC	247	4.6	2.3	2.3
	ABC - SMe	199	8.6	3.6	4
	CDE	261	30.2	7.6	13.3
	CDE - SMe	213	27.4	58.4	47.6
	AB	147	4.5	2.3	1.5
	AB - SMe	99	22.6	33.8	32.9
	BC	187	14	8.1	7.6
	BC - SMe	139	64.3	35.5	26.4
	DE	161	2.9	9.6	12.3
	DE - SMe	113	41.6	75.1	75.2
	B	87	100	96.1	71.2
specific	ABCD	319	-	-	-
fragments	ABCD - SMe	271	-	-	-
for	BCD	259	0.2	-	-
3,8,11-isomer	BCD - SMe	211	1.5	-	0.3
	E	89	13.1	7.2	4.9
specific	ABCD	333	-	0.3	-
fragments	ABCD - SMe	285	0.1	4.7	-
for	BCD	273	1	2.2	-
3,8,12-isomer	BCD - SMe	225	1.3	5	-
	E	75	15.2	100	8
specific	ABCD	347	-	-	-
fragments	ABCD - SMe	299	-	-	1.1
for	BCD	287	-	0.6	0.3
3,8,13-isomer	BCD - SMe	239	0.1	-	1.2
	E	61	47.6	50.6	100

Mass spectrometric fragments for DMDS derivatized tetradecatrienyl acetates. Relative intensities greater than 0.1% are given.

The presence of the fragments B (=AB - acetate), AB, CDE and CDE - SMe in the mass spectra of **14**, **15** and **16** pointed towards a double bond at position 3 in the original molecule. In the same way, the double bond at position 8 in the original molecules could be determined from the presence of the fragments BC, ABC, ABC - SMe, DE and DE - SMe in all the mass spectra of **14**, **15** and **16**. The relative intensities of the fragments E provide good evidence for the location of the omega double bond of the molecules. For molecules **15** and **16**, fragment E is the 100% peak in the mass-spectrum. The relative intensity of fragment E (*m/z* 89) of **14** is significantly higher in comparison to the relative intensities of *m/z* 89 of the molecules **15** and **16**. Its significance becomes more obvious if it is taken into account that the high relative intensity

fragment *m/z* 87, is predominantly responsible for the relative intensity of the fragment *m/z* 89 in case of compounds **15** and **16**, due to the presence of sulphur isotopes. Fragment *m/z* 61 is typical for acetates as well as DMDS derivatives and therefore also present in **14** and **15** in relative high intensities.

The *E/Z* configuration of the double bonds at positions 3 and 8 in **1** were assumed to be the same as **2** considering the results obtained with EAG. GC-RI calculations indicated **1** as the only possibility left with respect to the E/Z configuration of the double bond at position 11. The MS of **1** as well as **14** were identical to those obtained from the major sex pheromone component and its DMDS derivative, respectively.

The synthetic compound **1** elicited a large EAG response when exposed to the antennas of male *S. absoluta*. Wind tunnel experiments were performed in which males of *S. absoluta* were given a choice between the synthetic triple unsaturated acetate **1** and a 92:8 mixture of **1** together with the synthetic double unsaturated acetate **2**. In these experiments the latter proved to be more attractive.

In our opinion this extension of the DMDS method as described in this paper has distinct advantages over the also well known random reduction procedure[4,12]. The latter method is less suitable for the identification of the minor constituents of a sex pheromone having partially the same location and *E/Z* configuration of double bonds like in the present case. The reduction method further depends completely on the availability of all the positional and configurational mono unsaturated isomers of the linear tetradecenyl acetates[11] and is unsuitable for sex pheromones having constituents with different double bond positions and/or *E/Z* stereochemistry.

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5) The name *Scrobipalpuloides absoluta*[4] is used as a synonym for *Scrobipalpula absoluta.*
6) Vincenti, M.; Guglielmetti, G.; Cassani, G.; Tonini, C. *Anal. Chem.* **1987**, 59, 694-699.
7) GC-EAD: Gaschromatography on-line coupled to an electroantennography detector.
8) Schulz, S. *Thesis: Die Chemie der Duftorgane männlicher Lepidopteren*, University of Hamburg, 1987.
9) To a small airtight flask containing 20 sex pheromone glands in 200 µl of freshly distilled dimethyl-disulphide (DMDS) was added 5 µl of a 5% iodine solution in ether. The flask was heated for 16 hours at 50 °C. The reaction was quenched with a drop of saturated $Na_2S_2O_3$. After addition of a little NaCl the organic layer was separated, concentrated to ca. 2 µl and injected (splittles mode) into the GC-MS.
10) The molecular ion M^+ of a molecule with *n* double bonds can be calculated as follows: mass of the original molecule + (*n*-1) times the mass of Sulphur + twice the mass of the two remaining SCH_3 groups. In case of a double unsaturated molecule with a mass of 252 the observed M^+ will be: 252 + (2-1) x 32 + 94 = 378 and for a triple unsaturated molecule M^+ will be: 250 + (3-1) x 32 + 94 = 408.
11) IPO-DLO has over 250 straight-chain unsaturated pheromone compounds on stock. See also: Voerman, S. *Agric. Ecosystems Environ.* **1988**, 21, 31-41.
12) Yamaoka, R.; Tokoro, M.; Hayashiya, K. *J. Chromatogr.* **1987**, 399, 259-267. Yamaoka, R.; Fukami, H.; Ishii, S. *Agr. Biol. Chem.* **1976**, 40, 1971-1977.

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