Cyclic Glycerol Acetals from the Abdominal Hair Pencil Secretion of the Male African Sugarcane Borer *Eldana saccharina* (Lepidoptera: Pyralidae)

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Four constituents of the hair pencil secretion of the male African sugarcane stalk borer, *Eldana saccharina,* having a molecular mass of 312 and peculiar EI mass spectra with an exceptionally abundant base peak at m/z 103, were isolated preparatively from an extract of the secretion. Using ^{1}H and ^{13}C NMR spectral analysis, these constituents were identified as five- and six-membered cyclic glycerol acetals of *Z*-9-hexadecenal, *viz. cis-* and *trans*-2-(*Z*-8-pentadecenyl)-4-hydroxymethyl-1,3-dioxolane, and *cis-* and *trans*-2-(*Z*-8-pentadecenyl)-5-hydroxy-1,3-dioxane. These compounds are related to the 2-alkenyl-4-hydroxymethyl-1,3-dioxolane dihydrogen phosphate esters, known to be the active constituents of the smooth muscle contracting acidic phospholipid (Darmstoff) which was isolated from the intestine of mammals. The presence of these acetals in the tail brush secretion of *E. saccharina* could possibly be the first evidence that compounds related to the active principle of Darmstoff, may also be present in the insect kingdom. The possibility that these four compounds or their dihydrogen phosphate esters might play a part in the eversion or retraction of the tail brushes of the male insect, is briefly discussed.

Introduction

In a recently completed investigation of the constituents of the wing gland and abdominal hair pencil secretions of the male African sugarcane borer, *Eldana saccharina* Walker, the results of which were reported in a preliminary note [1], a group of five compounds having the same molecular mass and similar mass spectra, remained unidentified, since their mass spectra afforded insufficient structural information. Although these components did not elicit any electroantennographic (EAG) response in gas chromatographic analyses with FID/EAG detection (GC-EAD), and therefore probably have no direct semiochemical function, their peculiar mass spectral properties prompted us to determine their structures.

Experimental

General

Pupae of the insect under investigation were sexed and the male pupae maintained in environmental chambers using a 12 h at 26 °C and 12 h at 18 °C temperature cycle. To prevent premature extrusion of the hair pencils, the emerged moths were exposed to constant light regime. About 24 h after the moths had emerged, they were anaesthesized with chloroform vapour for the collection of the hair pencil secretion. Applying slight pressure to the abdomen of a male resulted in a gradual extrusion of its abdominal hair pencils which were removed with a pair of ophthalmic scissors before they were fully everted and allowed to fall into a Reacti-Vial with dichloromethane (Merck, Residue Analysis Grade). The hair pencils of a thousand males were collected in a total volume of 10 ml of dichloromethane. The extract was filtered through a small sintered glass filter at 3000 r.p.m. in a centrifuge and the filtrate concentrated in a purified (activated charcoal) nitrogen atmosphere. To eliminate heavy waxes which were expected to hamper the isolation of individual components, this extract was subjected to a preliminary preparative gas chromatographic separation on a Perkin-Elmer 900 gas chromatograph fitted with a short packed column [1.7 m × 8 mm glass, 2.5% SE-30 on 60–80 mesh Chromosorb WAW DMCS, 15 ml H_2/min, effluent splitter 9:1, temperature programmed at 2 °C/min from 40–250 °C and held isothermally at 200 °C (5 min), 220 °C (5 min), 230 °C (5 min), 240 °C (10 min) and 250 °C (5 min)]. The effluent fractions were collected in 1 ml quantities of dichloromethane. Capillary gas

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chromatographic analysis of each fraction showed the compounds under investigation to be present in the fraction collected at 230–240 °C (10 min). Preparative isolation of the individual components in this fraction was carried out with the above gas chromatograph fitted with a wide-bore capillary column [35 m × 0.6 mm glass, coated with PS-255 at a film thickness of 2.0 µm, 15 ml H_2/min, temperature programmed at 12 °C/min from 40–200 °C and at 1 °C/min from 200–250 °C (hold)]. Four components were collected in stainless-steel needles, rinsed into Reacti-Vials with benzene-d_6 and transferred first to precision 1.7 mm sample tubes for preliminary recording of ^{1}H NMR spectra at 80 MHz on a Varian FT-80 NMR spectrometer and subsequently to 5 mm sample tubes for detailed ^{1}H and ^{13}C NMR analyses at respectively 300 MHz and 75 MHz on a Varian VXR-300 NMR spectrometer.

Analytical gas chromatographic analyses were carried out with a Carlo Erba 4160 gas chromatograph equipped with a flame ionization detector and fitted with a glass capillary column (40 m × 0.3 mm) coated with PS-255 at a film thickness of 1.0 µm. Helium was used as carrier gas at a linear velocity of 28.6 cm/s and the column was temperature programmed at 2 °C/min from 40–240 °C. EI and CI mass spectra were obtained by GC-MS analyses of the extract on Varian MAT 311 A, Finnigan 4510, and Carlo Erba QMD 1000 mass spectrometers, using the column and gas chromatographic parameters specified above.

The location of the double bond in the side chain of each of the isolated constituents was established by microozonolysis according to the method of Beroza and Bierl [2] and gas chromatographic identification of the alkanal derived from the terminal portion of the side chain.

2-(Z-8-Pentadecenyl)-4-hydroxymethyl-1,3-dioxolanes

A solution of 1-monooctanoyl-*rac*-glycerol (41 mg), *Z*-9-hexadecenal (45 mg) and toluene-4-sulfonic acid (1 mg) in benzene (2 ml) was refluxed for 2 h, traces of moisture in the reagents and the water formed in the condensation reaction being removed azeotropically. After cooling the reaction mixture, it was diluted with ether, washed successively with saturated aqueous $NaHCO_3$ and water, and dried on Na_2SO_4. Removal of the drying agent and evaporation of the solvent at reduced pressure, gave a viscous residue which was saponified to remove the protective ester group.

The product of the condensation reaction was dissolved in methanol (2 ml), treated with a solution of KOH (40 mg) in water (0.1 ml) and the reaction mixture stirred magnetically at room temperature for 24 h. The resulting deprotected acetals were extracted with ether, the extract washed free from alkali with water and dried on Na_2SO_4. The normal work-up procedure gave an oily residue (56 mg) which, according to GC-MS analysis, consisted of a practically pure mixture of four cyclic acetals, all of which showed a base peak at *m/z* 103.

2-(Z-8-pentadecenyl)-5-hydroxy-1,3-dioxanes

Condensation of 2-monohexadecanoylglycerol (50 mg) and *Z*-9-hexadecenal (45 mg) as described for the preparation of the dioxolanes above, gave a product which, according to ^{13}C NMR analysis, contained substituted dioxolanes as well as the expected substituted dioxanes. Removal of the protective ester group as described before, gave a mixture of cyclic acetals, containing the acetals present in the tail brush secretion with the dioxolanes as the major products.

As the substituted dioxolanes, unexpectedly, were the major constituents of this condensation, it was repeated in a NMR sample tube and the progress of the reaction followed by monitoring the resonance of the aldehyde proton. 2-Monohexadecanoylglycerol (100 mg) was suspended in a solution of *Z*-9-hexadecenal (44.8 g) in benzene-d_6 (600 µl) containing a catalytic quantity of *p*-toluenesulfonic acid and heated at 80 °C in a 5 mm NMR sample tube. After 5 min at 80 °C the reaction mixture which, due to the low solubility of the glyceride in the solvent, had been a suspension at the beginning of the experiment, became homogeneous. The ^{1}H NMR spectrum of the mixture showed that about 50% of the aldehyde had already been consumed at this stage. After a further 5 min at 80 °C the resonance of the aldehyde proton was barely visible and the reaction mixture was cooled to room temperature. NMR analysis showed that the *cis*- and *trans*-substituted dioxolanes were the major products of the condensation.

Removal of the protective ester group by saponification with KOH (100 mg) in $MeOH/H_2O$ gave

an oily product (51 mg) consisting of a mixture of the four cyclic acetals.

Results and Discussion

The base peak at m/z 103 in the EI mass spectrum of one of the five constituents under discussion, has a much lower relative abundance than that of the other four constituents. This constituent is, furthermore, only observed in the first two or three GC or GC-MS analyses with a new column. Apparently it is either sensitive to contaminants remaining in the column, or it elutes as such a broad peak as to be indistinguishable from the baseline hump in this part of the chromatogram, its elution as a sharp peak being dependent on very specific column properties or requirements that are only met in a new column. As in the case of the other four constituents, the mass spectrum of this compound contains very little structural information and it was clear that the compound's unpredictable gas chromatographic behaviour prohibited its isolation by preparative gas chromatography. Our efforts were therefore concentrated on the elucidation of the structures of the other four compounds.

Part of a typical electron impact (EI) total ion current chromatogram (TIC) of the abdominal hair pencil secretion of male *Eldana saccharina* in which these components appear at scan numbers 7323, 7393, 7513 and 7665, is shown in Fig. 1. The four compounds have virtually identical mass spectra and in a chemical ionization (CI) GC-MS analysis with methane as reactant gas, it was found that all of these components have a molecular

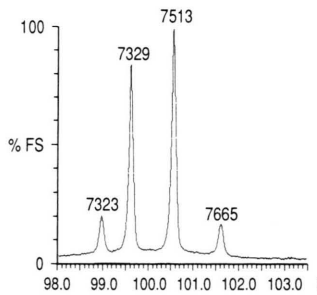


Fig. 1. Part of a total ion chromatogram (TIC) of the abdominal hair pencil secretion of male *Eldana saccharina*, showing the four constituents having a base peak at m/z 103. Column, 40 m × 0.3 mm I.D. glass, 1.0 µm PS 255; temperature programme, 40° to 240 °C at 2 °C/min.

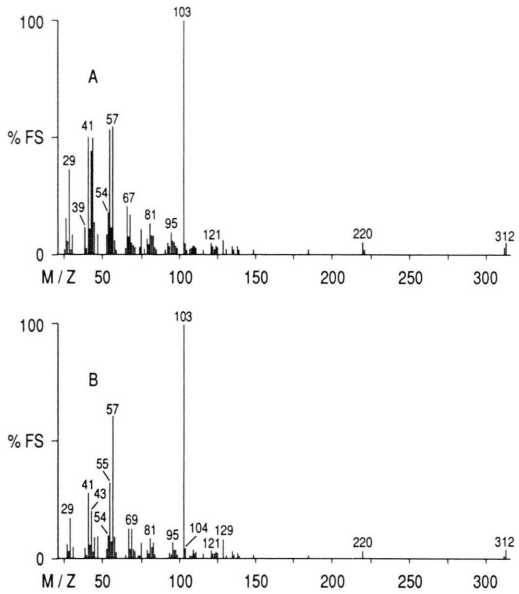


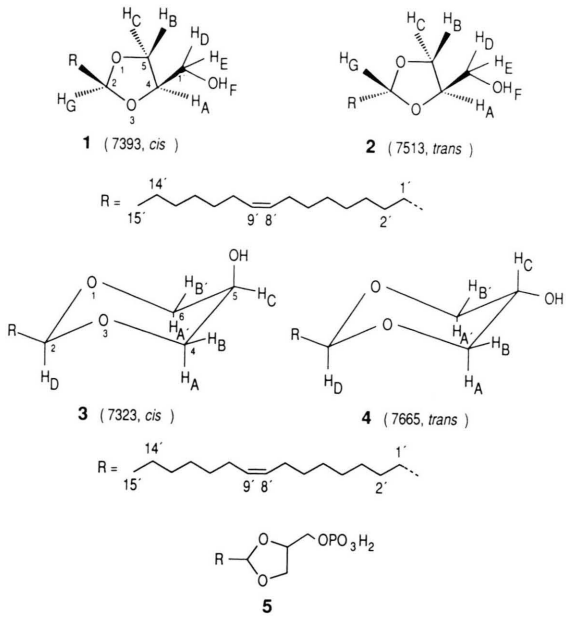
Fig. 2. EI mass spectra of components 7323 (A), and 7393 (B).

mass of 312. Two examples of the EI mass spectra of this group of compounds are shown in Fig. 2. As the mass spectra of some diethyl acetals have a strong peak at m/z 103 which can be ascribed to $(CH_3CH_2O)_2CH^+$, these compounds were initially presumed to be isomeric hexadecenal diethyl acetals. However, this structural assignment appeared to be untenable, as their spectra do not have a peak at m/z 267, attributable to the expected facile expulsion of an ethoxyl radical from the molecular ion at m/z 312. Based on the assumption that the ion at m/z 103 probably derives its exceptional stability from a cyclic structure, and that the ion at m/z 220 may be formed by a direct or stepwise loss of the elements of glycerol from the molecular ion, the presence of a cyclic acetal structure derived from glycerol was postulated.

As their mass spectra shed no further light on the structures of these compounds, they were isolated by preparative gas chromatography for NMR analysis. The resulting ^{13}C NMR data (Tables II und IV) can be reconciled with the presence in these compounds of an unbranched pentadecenyl group having a double bond with *Z* configuration in either position 7 or 8. As it is impossible to determine the position of a double bond near the middle of such a long-chain alkenyl group by

NMR, microozonolysis was employed to obtain this information. Using this method the alkenyl group was established to be 8-pentadecenyl in all four of the compounds.

The triplets at δ 4.838 (1 H) and δ 4.973 (1 H) in the spectra of components 7393 and 7513 respectively, can be attributed to the acetal function in a 2,4-disubstituted 1,3-dioxolane ring [3], the formation of which would involve two vicinal hydroxyl groups of, for example, glycerol, and the presence of two isomeric 1,3-dioxolanes can then be interpreted in terms of *cis* and *trans* substitution of the 5-membered ring. Information obtained from the ^{13}C NMR spectra of these two components (Table II), confirms the presence of three oxygen atoms in both compounds and to accommodate the previously mentioned base peak at *m/z* 103 in their mass spectra, the substituent in position 4 has to be a hydroxymethyl group. From a comparison with available ^1H and ^{13}C NMR spectral information on 2,4-disubstituted dioxolanes [3–5] the structures **1** and **2** were assigned to the constituents 7393 and 7513 respectively.



The relevant ^1H NMR information is given in Table I. The signal of the proton H_G of the *cis* iso-

Table I. ^1H NMR data for dioxolanes 7393 and 7513[a].

Proton[b]	Compound 7393 (*cis*)		Compound 7513 (*trans*)	
	δ_H [ppm]	*J* [Hz]	δ_H [ppm]	*J* [Hz]
H_A	3.786	$^3J_{AB}$ = 5.20	3.892	$^3J_{AB}$ = 7.10
		$^3J_{AC}$ = 7.23		$^3J_{AC}$ = 6.61
		$^3J_{AD}$ = 4.29		$^3J_{AD}$ = 4.18
		$^3J_{AE}$ = 5.17		$^3J_{AE}$ = 5.36
H_B	3.626	$^2J_{BC}$ = −8.06	3.425	$^2J_{BC}$ = −8.23
H_C	3.493		3.759	
H_D	3.413	$^2J_{DE}$ = −11.27	3.300	$^2J_{DE}$ = −11.58
		$^3J_{DF}$ = 5.48		$^3J_{DF}$ = 6.58
H_E	3.303	$^3J_{EF}$ = 5.97	3.231	$^3J_{EF}$ = 5.69
H_F	*ca.* 1.2		1.13	
H_G	4.838	$^3J_{G1'}$ = 4.80	4.973	$^3J_{G1'}$ = 4.80
CH_2 (1′)	1.76		1.76	
CH_2 (2′)	1.52		1.54	
CH_2 (6′+11′)	1.36[c]		–	
CH_2 (7′+10′)	2.13		2.13	
= CH (8′+9′)	5.54[d]		5.53[d]	
CH_2 (13′)	1.52[c]		–	
CH_2 (14′)	1.27[c]		–	
CH_3 (15′)	0.939	$^3J_{14',15'}$ = 6.68	0.937	$^3J_{14',15'}$ = 6.76
CH_2 envelope	1.22–1.46		1.22–1.46	

[a] At 300 MHz, benzene-d_6 as solvent, probe temperature 26 °C, residual aromatic protons of benzene-d_6 at δ 7.20 served as internal reference, chemical shifts and coupling constants involving protons A, B, C, D, E, and F were determined by simulation of the spectra using the program LAME [6] and are accurate within 0.002 ppm and 0.3 Hz respectively.

[b] Numbering as in the structures **1** (7393) and **2** (7513).

[c] Shifts determined in a ^1H-^1H correlation (COSY) experiment.

[d] Centre of coupling pattern.

mer is expected at higher field than the corresponding signal for the *trans* isomer [3, 4]. Thus the resonances at δ 4.838 and δ 4.973 in the respective spectra of these two components can be taken as evidence in favour of the above configurational assignment. This conclusion is substantiated by the observation of the proton H_A at δ 3.786 in the ^{1}H NMR spectrum of compound 7393 and at δ 3.892 for compound 7513, in accordance with the expected larger deshielding of this proton in the *trans* compound [4].

Borremans *et al.* [4] have found that, in general, the more deshielded of the two C-5 protons is *trans* with respect to the C-2 proton for the *cis* as well as *trans* isomers in a series of isomeric 4-halomethyl-2-methyl-1,3-dioxolanes, the only exceptions being the 4-iodomethyl- and 4-hydroxymethyl-substituted compounds of the *cis* series. Whereas the chemical shift differences between the C-5 protons are in the order of 0.4–0.7 ppm for compounds of the *trans* series, these differences are very small (0.02–0.06 ppm) for the *cis* compounds and assignment of the C-5 protons based on such small differences would seem to be somewhat risky. In the present investigation, in which benzene-d_6 was used as solvent, the chemical shift differences between the C-5 protons are 0.133 and 0.334 ppm for compounds 7393 and 7513 respectively.

If it is assumed that the above-mentioned rule also holds for a solution of the *cis* isomer 7393 in benzene-d_6, *i.e.* that the more deshielded C-5 proton is *trans* with respect to the C-2 proton, the signals at δ 3.626 and δ 3.493 can be assigned to the C-5 protons H_B and H_C respectively, in which case the observed coupling constants, $^3J_{AC}$ = 7.23 Hz and $^3J_{AB}$ = 5.20 Hz, are in line with the general trend $^3J_{syn} > {}^3J_{anti}$ for the *cis* compounds. The expected relationship $^3J_{syn} \leq {}^3J_{anti}$ [4] is observed for the *trans* compound 7513 ($^3J_{AC} < {}^3J_{AB}$). Using benzene-d_6 as solvent a chemical shift difference of 0.135 ppm is found for the proton H_G in the *cis* and *trans* isomers, *i.e.* slightly larger than the difference of 0.10 ppm found by Borremans *et al.* for the 2-methyl-substituted analogues in CS_2. In the *trans* compound the shift difference between H_B and H_C (Δδ = 0.334 ppm) is more pronounced than in the *cis* compound (Δδ = 0.133 ppm) in agreement with the observation of Borremans *et al.* (Δδ = 0.49 and 0.05 respectively).

Proton decoupled ^{13}C NMR spectra of components 7393 and 7513 reveal small but distinct ^{13}C chemical shift differences between these isomeric dioxolanes. The ^{13}C data given in Table II are in agreement with the assignments of Wedmid and Baumann [5] for similar *cis*- and *trans*-2-alkyl-4-hydroxymethyl-1,3-dioxolanes as well as with the assignment of *cis* and *trans* configuration respectively to the components 7393 and 7513 on the basis of the ^{1}H NMR data of these two compounds. In accordance with published ^{13}C NMR data [5] C-2 exhibits larger deshielding in the *cis* compound 7393 than in the *trans* compound 7513.

The observed δ_c values of *ca.* 27.64 for the allylic carbon atoms C-6 and C-9 in the side chain are typical for the *Z* configuration of the double bond in both compounds. For the *E* configuration these resonances are expected at about δ_c 32.5 [7].

The two remaining members of the group of four compounds under discussion elute at scan numbers 7323 and 7665 in the TIC given in Fig. 1. As a working hypothesis these compounds were presumed to be two isomeric 2,5-disubstituted 1,3-dioxanes, *i.e.* six-membered cyclic glycerol acetals of an aldehyde which, according to microozonolysis experiments and ^{13}C NMR data, had to be *Z*-9-hexadecenal.

The ^{1}H NMR spectrum of component 7323 was simplified be decoupling the C-5 proton at δ 2.97. The resulting spectrum contained an unchanged triplet at δ 4.308 (J = 5.13 Hz, 1 H) as well as two collapsed sets of splitting patterns at δ 3.3 and δ 3.8 which integrated for four protons. Due to the symmetrical nature of these patterns an AA′BB′ spectrum was simulated [6] to match the observed patterns in the decoupled spectrum. The fully coupled patterns were then simulated as the AA′BB′ part of an AA′BB′C spectrum. The resulting data (Table III) confirm the presence of a 2,5-disubstituted 1,3-dioxane moiety in component 7323. Since the following general trend has been observed for a wide range of 2-substituted 1,3-dioxanes: $^3J_{4ax,5ax}$ (12 Hz) > $^3J_{4eq,5ax}$ (5 Hz) > $^3J_{4ax,5eq}$ (2.6 Hz) > $^3J_{4eq,5eq}$ (1.3 Hz) [8], the relatively small values of $^3J_{AC}$ = 1.34 and $^3J_{BC}$ = 1.61 Hz can be accepted as an indication that H_C occupies an equatorial position on C-5.

The configuration of the substituents on C-2 of the dioxane ring was determined by Nuclear Overhauser Effect (NOE) determinations. NOE differ-

Table II. Comparison of ^{13}C chemical shift data[a] for dioxolanes 7393 and 7513 with published and simulated data.

Carbon atom[b]	Compound 7393[c] *(cis)*	*cis*-2-Alkyl-1,3-dioxolane [5]	Simulated[d]	Compound 7513[e] *(trans)*	*trans*-2-Alkyl-1,3-dioxolane [5]
2	105.23 d	105.3	105.1 ± 0.2	105.03 d	105.1
4	76.56 d	76.4	76.4 ± 0.2	76.33 d	76.3
5	66.60 t	66.5	66.7 ± 0.1	66.77 t	66.8
1′	34.43 t	34.0	34.0 ± 0.0	34.73 t	34.4
2′	24.45 t	24.0	24.1 ± 0.3	24.44 t	24.0
3′	29.36 t		29.3 ± 0.2	29.35 t	
4′	29.84 t[i]		29.2 ± 0.6	29.89 t[i]	
5′	30.14 t[ii]	29.8	29.4 ± 0.7	30.15 t[ii]	29.8
6′	27.63 t[iii]	27.3	27.3 ± 0.1	27.64 t[iii]	27.3
7′	130.16 d[iv]	129.9	130.1 ± 0.3	130.16 d[iv]	129.9
8′	130.20 d[iv]	129.9	130.1 ± 0.3	130.20 d[iv]	129.9
9′	27.66 t[iii]	27.3	27.3 ± 0.1	27.66 t[iii]	27.3
10′	30.15 t[ii]	29.8	29.4 ± 0.7	30.15 t[ii]	29.8
11′	29.89 t[i]		29.2 ± 0.6	29.93 t[i]	
12′	29.56 t		29.5 ± 0.2	29.57 t	
13′	32.14 t	32.0	32.0 ± 0.1	32.15 t	32.0
14′	23.03 t	22.7	22.7 ± 0.1	23.03 t	22.7
15′	14.29 q	14.1	14.0 ± 0.1	14.29 q	14.1
1″	63.50 t	63.5	63.0 ± 0.3	62.79 t	62.9

[a] At 75 MHz, benzene-d_6 with δ_c 128.0 as solvent and internal reference, probe temperature 26 °C, chemical shifts accurate to within ± 0.02 ppm, [i–iv] denote interchangeable assignments, multiplicities were determined by means of the APT technique.
[b] Numbering as in structures **1** (7393) and **2** (7513).
[c] Repetition time 0.8 s, pulse angle 45°, 196,000 transients accumulated.
[d] ^{13}C data were simulated by means of the Structure Estimation Option during online access to the INKA C 13 NMR databank in Karlsruhe, Germany.
[e] Repetition time 0.4092 s, pulse angle 45°, 274,000 transients accumulated.

Table III. ^1H NMR data for dioxanes 7323 and 7665[a].

Proton[b]	Compound 7323 *(cis)*		Compound 7665 *(trans)*	
	δ_H [ppm]	J [Hz]	δ_H [ppm]	J [Hz]
H_A, $H_{A'}$	3.318	$^2J_{AB}$ = −11.64 $^3J_{AC}$ = 1.34	3.16	$^2J_{AB}$ = −10.50 $^3J_{AC}$ = 10.41 $^4J_{AB'}$ = −0.27
H_B, $H_{B'}$	3.789	$^3J_{BC}$ = 1.61 $^4J_{BB'}$ = −2.97	3.992	$^3J_{BC}$ = 5.21 $^4J_{BB'}$ = −2.35
H_C	2.97		3.530	
H_D	4.308	$^3J_{D1'}$ = 5.13	4.318	$^3J_{D1'}$ = 5.08
CH_2 (7′ + 10′)	2.13		2.13	
=CH (8′ + 9′)	5.53[c]		5.53[c]	
CH_3 (15′)	0.937		0.946	$^3J_{14',15'}$ = 6.7
CH_2 envelope	1.06–1.47		1.07–1.47	
OH	–[d]		–[d]	

[a] At 300 MHz, benzene-d_6 as solvent, probe temperature 25 °C, residual aromatic protons of solvent at δ 7.20 served as internal reference, chemical shifts and coupling constants involving protons A, A′, B, B′, and C were determined by simulation of the spectra using the program LAME [6].
[b] Numbering as in structures **3** (7323) and **4** (7665).
[c] Centre of coupling pattern.
[d] Not observed.

ence spectra obtained on saturation of the C-2 proton (H_D) showed a NOE for the axial protons on C-4 (H_A) and C-6 ($H_{A'}$) only. As this is only possible with the C-2 proton (H_D) in the axial position, the *cis*-2,5-disubstituted 1,3-dioxane structure **3** could be assigned to component 7323. Similarly, the ^{1}H NMR spectrum of component 7665 contains two sets of widely spaced symmetrical splitting patterns which could be simulated as part of an AA'BB'C spectrum (Table III). The relatively large values of $^3J_{AC}$ = 10.41 and $^3J_{BC}$ = 5.21 Hz suggests that the proton on C-5 (H_C) has to occupy the axial position on this carbon atom. NOE difference spectra showed that also in this compound the proton on C-2 is in the axial position, as a NOE was observed once again between this proton and the axial protons H_A and $H_{A'}$ on C-4 and C-6 respectively. This component therefore possesses the *trans*-2,5-disubstituted 1,3-dioxane structure **4.** In both of these dioxanes a conformation is preferred with the alkenyl group on C-2 in an equatorial position.

In structure **3** the formation of stable intramolecular hydrogen bonds between the hydroxyl group and the two ring oxygen atoms might contribute to stabilize this conformation in which the hydroxyl is in an axial position. Most likely the presence of strong intramolecular hydrogen bonding in this isomer is also responsible for a gas chromatographic retention time which is much shorter than that of the isomer with its hydroxyl group in the equatorial position.

The ^{1}H NMR data for the two dioxanes 7323 and 7665 are summarized in Table III. In Table IV the ^{13}C NMR data for these compounds are compared with simulated and published data. The assignment of the *cis* configuration to compound 7232 is substantiated by the satisfactory agreement

Table IV. Comparison of ^{13}C chemical shift data[a] for dioxanes 7323 and 7665 with published and simulated data.

Carbon atom[b]	Compound 7232[c] *(cis)*	*cis*-2-Hexyl-1,3-dioxan-5-ol [16]	Simulated[d]	Compound 7665[e] *(trans)*
2	102.75	102.82	102.8 ± 0.0	102.17
4, 6	71.77	71.78	71.7 ± 0.2	71.72
5	64.08	64.03	63.9 ± 0.3/61.2 ± 0.2	–[f]
1′	35.42	34.88	34.9 ± 0.1	35.65
2′	24.20	23.90	24.1 ± 0.3	24.62
3′	29.36	29.16	29.3 ± 0.2	29.34
4′	29.85	–	29.2 ± 0.6	29.86
5′	30.15	–	29.4 ± 0.7	30.14
6′	27.63[i]	–	27.3 ± 0.1	27.64[i]
7′	130.14[ii]	–	130.1 ± 0.3	130.15[ii]
8′	130.21[ii]	–	130.1 ± 0.3	130.18[ii]
9′	27.66[i]	–	27.3 ± 0.1	27.64[i]
10′	30.15	–	29.4 ± 0.7	30.14
11′	29.85	–	29.2 ± 0.6	29.86
12′	29.57	–	29.5 ± 0.2	29.56
13′	32.14	31.78	32.0 ± 0.1	32.21
14′	23.03	22.59	22.7 ± 0.1	23.04
15′	14.30	14.06	14.0 ± 0.1	14.30

[a] At 75 MHz, benzene-d_6 with δ_c 128.0 as solvent and internal reference, probe temperature 25 °C, chemical shifts accurate to within ±0.03 ppm, [i–ii] denote interchangeable assignments.

[b] Numbering as in structure **3** (7323) and **4** (7665).

[c] Repetition time 0.4092 s, pulse angle 45°, 600,000 transients accumulated.

[d] ^{13}C NMR data were simulated by means of the Structure Estimation Option during online access to the INKA C13 NMR databank in Karlsruhe, Germany. (Now available from STN International.) The HOSE method used to encode structures does not provide for distinction between *cis* and *trans* substitution. However, data for both isomers are normally retrieved.

[e] Repetition time 0.4092 s, pulse angle 36°, 280,000 transients accumulated.

[f] Not observed. Expected at 61.2 ppm (INKA) or 61.55 ppm, using a synthesized short side chain model compound.

of its ^{13}C NMR-data with the data retrieved for *cis*-2-hexyl-1,3-dioxan-5-ol [16]. In accordance with the result obtained for the dioxolanes, C-2 exhibits a larger deshielding in the *cis*-dioxane.

Final confirmation of the structures assigned to these constituents of the abdominal hair pencil secretion of the insect was sought be selective synthesis of the dioxolanes and dioxanes using glycerol derivatives with appropriately protected hydroxyl groups. Acid-catalyzed condensation of *Z*-9-hexadecenal with 1-monooctanoyl-*rac*-glycerol, followed by saponification to remove the protective groups and isolation of the products under alkaline conditions, yielded a mixture of all four of the cyclic acetals with the two dioxolanes **1** and **2** as the major products (Fig. 3 A). However, a similar experiment in which 2-monohexadecanoylglycerol and *Z*-9-hexadecenal were used as starting compounds, did not give the expected *cis* and *trans* dioxanes as the major products, but produced a reaction product containing the four acetals in roughly the same quantitative ratio as before. The reaction time of approximately 2 h which is usually allowed for the condensation [5, 9], is probably unnecessarily long, allowing acid-catalyzed rearrangement, leading to a thermodinamically equilibrated mixture of the acetals, to take place. It has furthermore been found that the use of acid catalysts favours the formation of the dioxolanes [10]. The latter condensation was therefore repeated in a NMR sample tube in order to monitor the progress of the reaction. Due to the low solubility of 2-monohexadecanoylglycerol in benzene, the reaction mixture was not homogeneous at the start of the experiment. However, within 5 min at 80 °C the glycerol derivative had dissolved and the aldehyde resonance had substantially decreased. After a further 5 min at the same temperature, the condensation was practically complete.

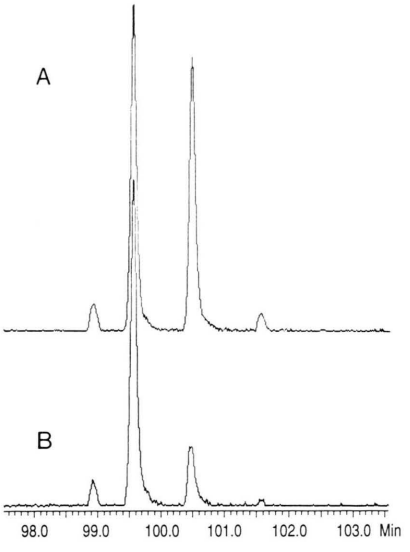


Fig. 3. Total ion chromatogram (TIC) of the products obtained in attempts to synthesize 2-(*Z*-8-pentadecenyl)-4-hydroxymethyl-1,3-dioxolanes (A), and 2-(*Z*-8-pentadecenyl)-5-hydroxy-1,3-dioxanes (B). Gas chromatographic conditions as in Fig. 1.

A detailed NMR analysis showed that substituted dioxolanes were the major products of the reaction. This was confirmed by a GC-MS analysis of the deprotected cyclic acetals. The resulting TIC is shown in Fig. 3 B. Although it might be possible to promote the formation of the dioxanes to a certain extent by using other reaction conditions than those which had to be used in the NMR sample tube, it seems unlikely that this synthetic route will produce dioxanes free from the isomeric dioxolanes. Nevertheless GC-MS and NMR analyses showed that these condensation reactions produced the four acetals identified in the tail brush secretion and proved their basic structures as far as the ring size, chain length, as well as the position and configuration of the double bond are concerned.

According to their ^1H NMR spectra, the acetals isolated from the tail brush secretion in benzene-d_6 contained small quantities of water, probably introduced during sample handling and dilution. Sealed off in NMR tubes the individual acetals did not show any sign of rearrangement over a period of more than three years at room temperature. Neither did heating the samples at 80 °C for a few hours have any effect. However, on addition at room temperature of *p*-toluenesulphonic acid to a sample of the *cis*-dioxane **3** containing 33% of the *cis*-dioxolane **1** and 5% of the *trans*-dioxolane **2,** the concentration of the dioxolanes decreased to give the pure dioxane **3** after 36 h. It is possible that this compound is favoured at room temperature as it is stabilized by the hydrogen bonding between the hydroxyl group and the ring oxygen atoms mentioned above. On heating the acidified sample at 80 °C for 2 h the signals of the dioxolanes reappeared and the dioxolane structures therefore

appear to be favoured at higher temperatures, in agreement with their preferential formation in the syntheses of the cyclic acetals in boiling benzene. In gas chromatographic analyses, direct solventless introduction, temperature-programmed introduction, and injection of extracts of the tail brush secretion produced virtually identical quantitative ratios of the four acetals, the only exception being the elution of a major, unidentified constituent along with the four acetals on injection of the tail brush extract on a new column. Such a gas chromatogram was included in our earlier paper [1] and this explains the difference between the elution profiles in the cyclic acetal region in our earlier paper and in Fig. 1. In the absence of acid the acetals appear to be stable as they were isolated by preparative gas chromatography and were collected in reasonably pure form after they had passed through a stainless-steel section of the outlet manifold of the gas chromatograph at 250 °C.

In 1949 Vogt [11] reported the isolation from horse intestine of an acidic phospholipid which he named Darmstoff, and which was capable of effecting smooth muscle contraction. On the basis of chromatographic evidence it was suggested that the active principle was a long-chain cyclic glycerol acetal phosphate **5** [12, 13]. Darmstoff was subsequently found in other mammalian and amphibian organs [14] and it was found to consist of a mixture of 2-alkyl- and 2-alkenyl-4-hydroxymethyl-1,3-dioxolanedihydrogen phosphate esters, the 2-alkyl and -alkenyl residues being derived primarily from hexadecanal and *Z*-9-octadecenal [15]. The smooth muscle contracting activity was shown to reside exclusively in the *Z*-9-octadecenal derivative. No long-chain dioxane derivatives were found in extracted material showing Darmstoff activity [15].

The four long-chain cyclic acetals isolated from the tail brush secretion of *Eldana* males are to the best of our knowledge the first evidence that compounds related to the active principle of Darmstoff may also be present in the insect kingdom. It is interesting to speculate on the possibility that these compounds or their dihydrogen phosphate derivatives, the isolation of which was not attempted in the present study, may play a part in the eversion or retraction of the tail brushes of the insect. It will therefore be interesting to find out whether other insects with similar pheromone disseminating structures produce the same or related compounds. The fact that Darmstoff is present in the gut of the mammals from which it had been isolated, raises the question as to whether the cyclic acetals in the tail brush secretion might not be picked up by the tail brush from the caudal part of the insect. This possibility appears to be unlikely, as care was taken to avoid this type of contamination of the tail brush secretion. The acetals were present in approximately the same relative concentration in tail brush material collected by different techniques from partly or fully everted tail brushes. Furthermore, extraction of the abdominal tips of females did not yield these acetals in detectable quantities.

Finally it has to be mentioned that the acetals may act as precursors for the release of *Z*-9-hexadecenal which could have, for example, a semiochemical function in the insect. This possibility is at present being investigated.

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