Oviposition-deterring pheromone in *Rhagoletis cerasi* L.: Purification and determination of the chemical constitution

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Summary. An oviposition-deterring pheromone (ODP) of the European cherry fruit fly *Rhagoletis cerasi* L. was isolated from faeces using cellulose and several reverse phase TLC and HPLC procedures. The biological activity was evaluated by means of behavior tests and by electrophysiological recordings from tarsal contact chemoreceptors. The compound was structurally characterized as a N[15(β-glucopyranosyl)oxy-8-hydroxypalmitoyl]-taurine by spectroscopic means. The configurations of C-8 and C-15 of the fatty acid constituent remain to be established by synthetic work.

Key words. Tephritidae, *Rhagoletis cerasi* L.; oviposition-deterring pheromone; constitution; purification; electrophysiology; contactchemoreceptors; oviposition behavior; fast atom bombardment mass spectrometry; two-dimensional mass spectrometry; gas chromatography; mass spectrometry; ^{1}H-NMR spectroscopy.

Introduction

The European cherry fruit fly *Rhagoletis cerasi* L. lays one single egg into half-ripe cherries. Wiesmann[1] and later on Haefliger[2] demonstrated in the field that double or triple ovipositions into the same fruit did occur, but with a much lower frequency than would be expected by chance. These findings were confirmed later by Remund et al.[3]. Consequently, the hypothesis was put forward that the females mark the fruits, and it was speculated that the observed dragging of the ovipositor over the fruit surface after oviposition might be connected with the postulated marking procedure. Katsoyannos[4] first observed traces of a secretion possibly connected with the marking behavior of the fruit fly on artificial oviposition devices (wax domes). The marking trails from the oviposition substrate and the faeces were dissolved in water. Application of this raw extract to cherries, under field conditions, reduced the infestation rate up to 90%[5,6]. Further investigations by Hurter et al.[7] proved the significant chemical stability of this oviposition-deterring pheromone (ODP). Today, such pheromones are known to occur in at least 6 insect orders, 16 insect families and over 33 pest species. This new field of behavior-modifying chemicals of insects has been reviewed recently by Prokopy[8,9] and with respect to *Rhagoletis cerasi* by Boller[10].

The development of sensitive behavior tests in the laboratory by Boller and Hurter[11] and under semi-field conditions by Boller et al.[12], together with the possibility of measuring biological ODP activity in minute quantities with electrophysiological tools at the receptor level, as described by Städler and Katsoyannos[13] and Städler[14] provided the necessary bioassays for purification and elucidation of the chemical constitution of the ODP of the European cherry fruit fly.

Material and methods

Biological procedures. Fruit fly material. The ODP was produced by *R. cerasi* L. females collected as larvae from infested honeysuckle *(Lonicera xylosteum)* growing along Swiss highways. This mass-collection of *R. cerasi* material from the alternate wild host rather than from infested cherries was justified by the observation that both host races were producing the same ODP and recognized each other's pheromone (Boller and Katsoyannos, unpublished).

Flies used in the bioassays were mostly of the *Prunus avium* host race and were collected from cherries in Switzerland and Austria.

Collection of ODP. The procedures have been described in detail by Boller and Hurter[11]. Plexiglas cages 30 × 30 × 40 cm, lined with clean glass plates, were supplied with water and food, containing yeast hydrolysate and sugar[15]. The faeces on the flies deposited on the glass surfaces were scraped off with razor blades. The brown, sticky powder represented the raw material for the subsequent purification. It allowed storage at −20 °C until further use.

Evaluation of the biological activity of ODP. Four different approaches were available to monitor the presence and to evaluate the intensity of the ODP activity in test solutions: 1) the behavior test in the laboratory using artificial fruits[4,11]; 2) the semi-field test in large field-cages with natural host plants and fruits[12]; 3) the field test itself[5,6], and 4) the electrophysiological determination of ODP activity at the receptor level[14]. The results of simultaneously conducted behavioral test (1) and electrophysiological recordings (4) with the same ODP solutions correlated very well (3). Since for the recordings only 2–5 µl of the test solution is required, 3 orders of magnitude less than for the behavioral assay, the electrophysiological test method became more and more important as purification procedures progressed and quantities of test material decreased. The substance used in the final steps of identification was, however, tested under semi-field conditions (2) in order to verify and confirm the electrophysiological results.

Purification procedure. General. High quality solvents, including water (Merck, Darmstadt, FRG) were used throughout the experiments. Methanol was additionally distilled. Glassware, including rotary evaporators, were

previously treated in mucasol (Brand, Wertheim, FRG). The UV-detector was set at 260 nm to monitor the resolving capability of the HPLC-columns for substances to be separated from the biologically active sample.

Extraction of raw material. 7.3 g of collected fly faeces were sonicated in 75 ml of methanol for 10 min. The resulting suspension was agitated for another 4 h on a magnetic stirrer at 2 °C and finally centrifuged at 12,000 × g for 20 min. This last step was repeated twice with 40 ml of methanol each time. After concentration of the combined supernatants to 20 ml by rotary evaporation, 20 ml of ethanol was added. Precipitates formed after a few days, under refrigeration, were removed by centrifugation. The resulting crude extract contained 20–50 mg of dry matter/ml. It retained its biological activity when stored at −20 °C.

Liquid chromatography on cellulose column. The column, 50 × 600 mm containing methanol/ethanol (1:1), was prepared by slowly adding dry cellulose powder (type 123 A, Schleicher-Schuell, Dassel, FRG) to produce a final bed length of 400 mm. The crude extract (40 ml), was applied by a column inlet tube using a polyethylene syringe. The column was eluted with 1) 1400 ml of methanol/ethanol (1:1); 2) 1000 ml of methanol/water (1:1). Flow rate: 10–15 ml/min. Electrophysiological activity appeared between 1100 and 2000 ml of the elution volume. This fraction, concentrated by rotary evaporation to 0.5 ml was diluted with methanol to a total volume of 3.5 ml for further purification.

Preparative TLC on cellulose. The plates used were PSC cellulose 200/200 mm, thickness 0.5 mm, without fluorescent indicator (Merck, Darmstadt, FRG). In order to remove interfering material of the adsorbent, plates were preconditioned with water and methanol. They were loaded with 0.5 ml of the concentrated eluate from the cellulose column using a Linomat III apparatus (Camag, Muttenz, Switzerland). Development of the plates was carried out with ethanol/water (1:1). The cellulose layer was removed in strips of 0.1 R_f-units. They were separately eluted twice in 15 ml each of methanol under magnetic stirring for 15 min and centrifuged for another 15 min at 3000 × g. The combined supernatants were concentrated to 0.5 ml and tested for electrophysiological activity. It appeared repeatedly between 0.9 and 1.0 R_f-units.

HPLC on C-18-modified silicagel (steep gradient). The column used was: Ultrasphere-ODS 4.6 × 250 mm, 5 µm (Altex, USA). Mobile phase: 20% methanol in water to 100% methanol following a linear gradient of 5% methanol/min. Flow rate: 0.5 ml/min. Number of fractions: 30. Volume per fraction: 0.5 ml. For each run, the column was loaded with 0.1 ml of the methanolic solution from the thin layer purification step. The electrophysiological activity was recovered in the range of 7.5–9.0 ml of the elution volume. The combined active fractions were evaporated under vacuum to 0.1 ml and diluted with methanol to 0.5 ml.

TLC on C-12-modified silicagel. The plates used were: OPTI-UP C-12, 10 × 20 cm, layer 0.25 mm with fluorescent indicator (Antec, Beinwil, Switzerland). Each preconditioned plate was charged with the active compound dissolved in 0.1 ml of methanol, using the Linomat III apparatus. After developing in methanol, the layer was removed in strips of 0.1 R_f-units and eluted by suspension in 3 ml of methanol, agitation for 15 min and centrifugation for 20 min at 3000 × g. This desorption step was repeated once. The combined extracts were concentrated almost to dryness before adding 0.3 ml of methanol/acetonitrile (1:1). Electrophysiological activity was found in the R_f-range of 0.5–0.7.

HPLC on NH_2-modified silicagel. The column packing was: Lichrosorb-NH_2 4.0 × 250 mm, 5 µm (Merck, Darmstadt, FRG). Mobile phase: 100% acetonitrile to 100% water following a linear gradient of 6% water/min. Flow rate: 0.5 ml/min. Number of fractions: 40. Volume: 0.5 ml. The compound to be purified was put on the column in portions of 0.1 ml of the binary solvent mentioned in the preceding step. Compounds which stimulated the ODP receptor cell of the D-hairs of the prothorax tarsi eluted with pure water, at a volume between 12.5 and 15.0 ml. The solvent of the combined, active fractions was removed by lyophilization. For further purification, the residue was redissolved in 0.3 ml of 40% methanol in water.

HPLC on C-18-modified silicagel (smooth gradient). For this separation, the same HPLC procedure as before, but with a smoother gradient, was used: Mobile phase 40%

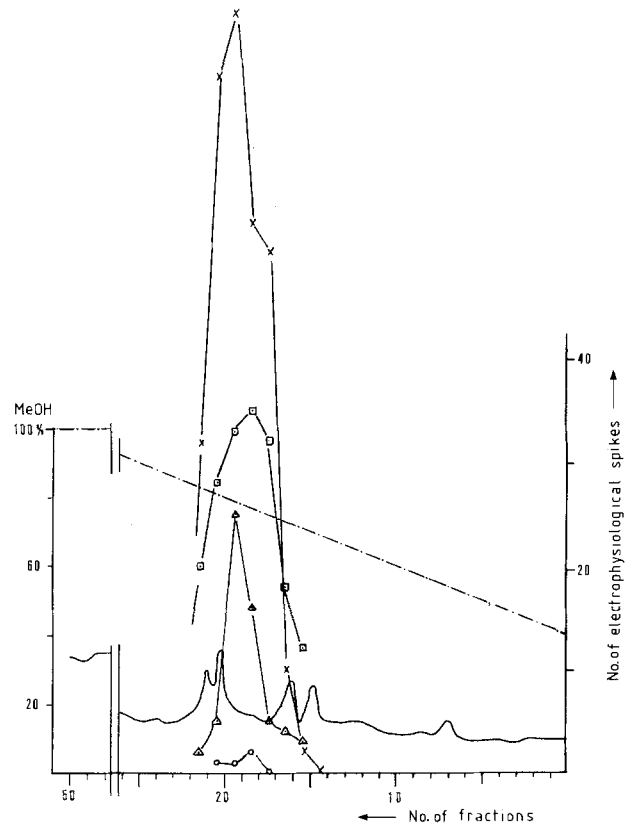


Figure 1. Elution profile and electrophysiological activity of purified ODP (abscissa: number of fractions). HPLC on C-18 modified silicagel (smooth gradient). Chromatographic conditions mentioned in 'Materials and methods'. The number of electrophysiological spikes (right scale) were counted during the 5th second of recording. Dilution of the final volume: □—□ 10^5; △—△, 10^6; ○—○, 10^7. x—x, FAB-MS signal of M-like ions (total of m/z 558 $[M+H]^+$, 580 $[M+Na]^+$, 602 $[M-H+2Na]^+$ and 575 $[M+NH_4]^+$) in arbitrary units. ——, UV absorption at 260 nm (arbitrary units). —·—·—, MeOH gradient in water (left scale).

methanol in water to 100% methanol, following a linear gradient of 2% methanol/min. Number of fractions: 50. After concentration to less than 0.1 ml (rotary evaporator), the residual solvent was transferred and completely removed in a conical vial (Reacti-vial, Pierce, Rockford, USA) under a stream of nitrogen. For investigations of the chemical constitution, the invisible residue was redissolved in 50 µl of distilled methanol. The elution profile of the electrophysiologically active fractions is given in figure 1.

Analytical procedure. Mass spectrometry. Fast atom bombardment mass spectrometry (FAB-MS) was carried out using a ZAB-HF instrument (VG Analytical Ltd, Manchester, UK) equipped with a saddle-field atom gun. Samples were dissolved in thioglycerol as a liquid matrix and bombarded with a beam of neutral xenon atoms of 10 keV kinetic energy. Accurate mass measurements at high resolution were made by peak-matching using polyethylene glycol as the liquid matrix as well as the internal mass standard. For two-dimensional mass spectrometry (FAB-MS/MS) in the mass-analyzed ion kinetic energy spectroscopy (MIKES) mode, the BE-configuration (B = magnetic, E = electric sector) of the ZAB spectrometer was used to select the ions of interest by adjusting the magnetic field strength to the proper fixed value (B operated as MS-I). Spontaneous dissociation within the field-free region between B and E (no addition of collision gas) was used to produce daughter ion spectra (MIKE spectra) of the mass-selected metastable parent ions by scanning the electrical field (E operated as MS-II). Gas chromatography combined with mass spectrometry (GC/MS) was performed on a Finnigan MAT (San Jose, Calif., USA) 5100 instrument (20 m × 0.3 mm SE 54 glass capillary column, 50–280 °C, 4 °C per min).

^{1}H-NMR spectrometry. ^{1}H-NMR spectra were obtained on a Bruker WM 400 Fourier transform spectrometer (at 400.1 MHz) equipped with a 5-mm sample head. The concentration of the sample solution was approximately 10^{-3} M in CD_3OD at 25 °C. Chemical shifts were related to the proton signal of CD_2HOD (δ = 3.30 ppm) as internal standard.

Detection of taurine by TLC. After cleavage of an ODP sample by methanolysis, taurine was detected in the reaction mixture by TLC using Silicagel 60 F 254 plates (Merck, Darmstadt, FRG) and Cl_2/KJ/N, N, N′, N′-tetramethyl-4, 4′-diaminodiphenylmethane as the spotting reagent (Solvent systems: n-butanol/99% acetic acid/water 8:3:1; n-propanol/25% aqueous ammonia, 8:2).

Degradation of ODP by methanolysis. Samples of ODP were reacted with 1.25 N anhydrous methanol/HCl at 80 °C for 12 h in a sealed tube. After evaporation the residue was treated with *bis*(trimethylsilyl)-acetamide (BSA, Pierce Chemicals, USA) at 60 °C for 1 h. The resultant mixture of trimethylsilyl derivatives was directly subjected to GC/MS analysis.

Synthesis of N-palmitoyltaurine. *N*-Palmitoyltaurine had to be prepared in two steps because of the insolubility of taurine in appropriate solvents. In a first step 1 mol of cystamine, $H_2NCH_2CH_2SSCH_2CH_2NH_2$, was reacted with 2 mols of palmitoyl chloride in pyridine at 0 °C. After evaporation and addition of water to the residue, the dipalmitoyl derivative was extracted into dichloromethane. In a second step the reaction product was oxidized to *N*-palmitoyltaurine with performic acid.

Results and discussion

Physical properties of ODP. The extremely polar character of the pheromone reported earlier by Hurter et al.[7] was confirmed. Since no absorption in the UV-region was observed for the ODP compound, it was impossible to correlate optical absorption with biological activity of chromatographic fractions. The only way of detection was by electrophysiological recordings or the more laborious behavior test, using much more of the active material.

The non-volatile character of the compound was demonstrated by the negative results of evaporation experiments (0.1 mbar, up to 120 °C). This was not only the case for the compound itself, but also for its methyl-, silyl- and acylderivatives. This peculiarity excluded investigations by conventional GC/MS methods.

Homogeneity of the biologically active substance obtained by TLC and HPLC. The FAB mass spectrum of the purified ODP shown in figure 2 indicated only one polar compound (m/z 557 daltons) associated with alkali metal ions. Its elution profile in the last purification step from the C-18-modified silicagel column (smooth gradient, fig. 1), appears to be correlated with very high electrophysiological activity. Fractions, concentrated to 50 µl of methanol, permitted 10^7-fold dilution before the limit of electrophysiological detection was obtained (threshold of ODP receptor cells).

Table 1. Biological activities of ODP treated cherries in field-cage experiments. Tests were conducted on July 27 and 28, 1985 at Wädenswil. Average values and SD of indicated visits are given. Standard solution was adjusted to 1 mg dry matter/ml MeOH

Test parameters	Treatment of test cherries			
	Untreated control	Crude ODP extract standard	Purified ODP	Natural ODP 1 female equivalent
No. of tests (visits)	16	49	48	18
A Visits with oviposition (%)	94	8	10	28
B Average duration of visits (s)	128 ± 29	38 ± 69	41 ± 64	36 ± 63
C Average time lapse between arrival on fruit and oviposition (s)	28 ± 32	63 ± 92	33 ± 36	65 ± 51
Irritation indices:				
D Visits with cleaning of body (%)	6	16	15	44
E Activity changes per 100 s	3 ± 1	16 ± 9	14 ± 8	14 ± 9
F Average number of visits per 100 s	< 0.5*	5 ± 5	7 ± 7	5 ± 4

* This statistic could not be evaluated in the test because untreated cherries had to be removed and be replaced by fresh fruits. The estimated value of 0.5 is based on value B.

Field desorption mass spectrometry (FD-MS) was unsuccessful and revealed neither the target compound nor any other additional substances. Therefore, this activity can be assigned with high probability to the prospective ODP molecule.

Biological activity of the pure isolated compound. The purified ODP was a very active stimulus for the tarsal ODP receptors. In order to prove that this compound was also active in the behavior of the fruit fly, tests under semi-field conditions with natural cherries were initiated.

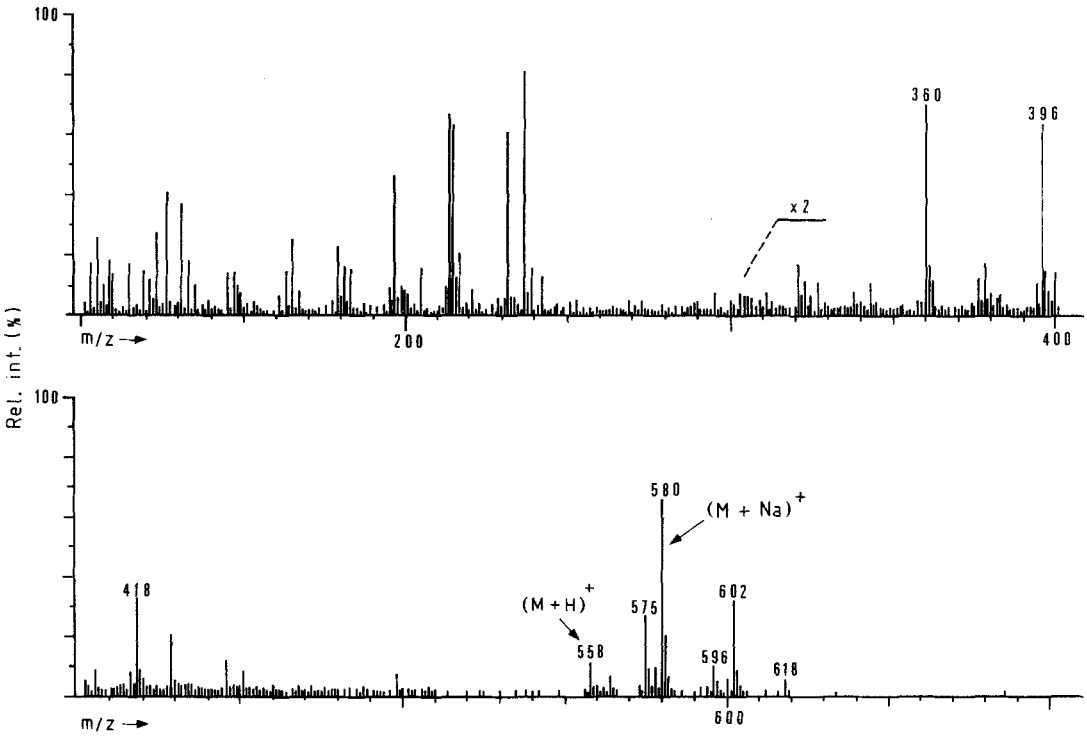


Figure 2. (+)FAB spectrum of purified ODP (**1**).

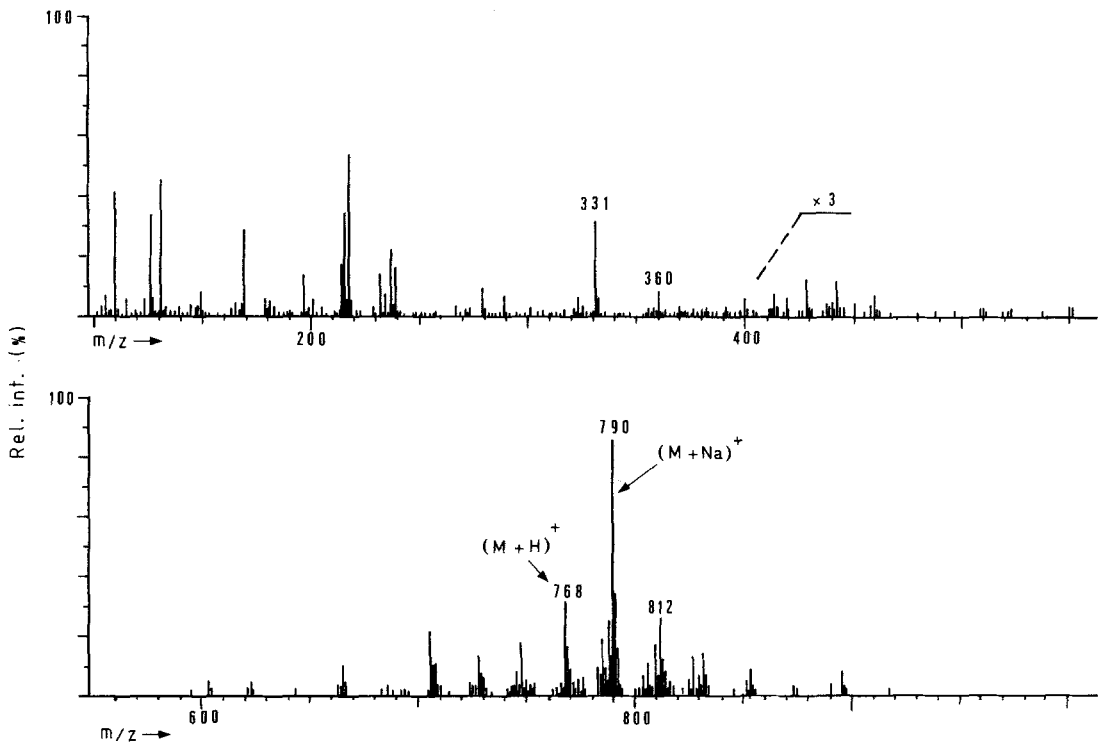


Figure 3. (+)FAB spectrum of pentaacetylated ODP (**1a**).

Since the quantity of the pure compound and its behavioral activity was unknown, we diluted the purified ODP solution with methanol so far that its electrophysiologically measured activity was comparable to the activity of the crude ODP extract from the fly feces containing 1 mg dry matter/ml methanol[11]. The results of the semi-field test are shown in table 1 where the purified ODP was tested against the ODP standard, natural ODP markings and untreated control cherries.

It is obvious that cherry fruit fly females produced different behavioral patterns, compared to those with control fruits, when in contact with cherries treated with the crude ODP standard, the purified ODP and natural ODP marks deposited by females. Details of these experiments are discussed by Boller et al.[12].

Spectroscopic measurements. Figure 2 shows a (+)FAB mass spectrum of purified ODP. In the mass range above m/z 550, clusters of ions spaced by 22 and/or 38 dalton increments appear at m/z 558, 580, 596, 602 and 618, indicating Na^+ and/or K^+ cationization of one common neutral species. Exchange of the metal ions for Li^+ by addition of lithium acetate identified the first ion of the series, m/z 558, as $[M+H]^+$ (mass increments of 6 instead of 22 or 38 daltons, respectively). The ion at m/z 575 was shown by MS/MS analysis to be an adduct with NH_4^+ (metastable decomposition $[M+NH_4]^+ \rightarrow [M+H]^+ + NH_3$). High-resolution measurements of the $[M+Na]^+$ ion, m/z 580, allowed the calculation of a molecular mass 557.2896 ± 0.0023 for the parent compound (**1** in scheme 1), suggesting an elemental composition $C_{24}H_{47}O_{11}NS$ (calculated mass 557.2870).

Evidence for a first structural element rich in oxygen was obtained by two-dimensional mass spectrometry (FAB-MS/MS) of the $[M+Na]^+$ adduct. Thus, the MIKES-spectrum of m/z 580 revealed a loss of a 162-dalton neutral particle, yielding a m/z 418 signal (already observed in the FAB spectrum) as the main daughter ion. A similar MS/MS-experiment on the $[M+H]^+$ ion, m/z 558, produced a corresponding signal 22 daltons lower, i.e. at m/z

Table 2. ^{1}H-NMR-parameters of ODP (**1**) and pentaacetylated ODP (**1a**) in CD_3OD

H[a]	δ (**1**)	δ (**1a**)[b]	Coupling constants J(Hz)	**1**	**1a**
1	4.31	4.70	J(1.2)	8.0	8.0
2	3.13	4.84	J(2.3)	9.0	9.5
3	3.33	5.24	J(3.4)	9.0	9.5
4	3.28	4.99	J(4.5)	9.0	10.0
5	3.23	3.84	J(5.6)	6.0	2.5
6	3.66	4.11	J(5,6′)	2.0	5.0
6′	3.84	4.25	J(6,6′)	12.0	12.5
X–CH_2	2.95	2.94	two triplets: $J(CH_2,CH_2) = 7$ Hz		
Y–CH_2	3.58	3.58			
Z–CO					
CH_2	2.18	2.18	triplet J = 7 Hz		
$(CH_2)_n$	c	c	c		
CH–OR	3.50	4.84	multiplet		
$(CH_2)_m$	c	c	c		
CHOR′	3.85	3.78	multiplet		
CH_3	1.16	1.11	doublet J = 6 Hz		

[a] RO–CH_2–CH–CH(OR)–CH(OR)–CH(OR)–CH–O– (6,6′ 5 4 3 2 1; O)

[b] The signals of the five acetyl methyl groups appear as singlets at 2.04, 2.01, 2.00 (2 ×), and 1.95 ppm. [c] The signals of the $(CH_2)_m$ and the (CH_2) groups absorb as multiplets at 1.6 and 1.45 to 1.25 ppm for **1**. For **1a** they are multiplets at 1.65 to 1.50 and 1.45 to 1.25 ppm.

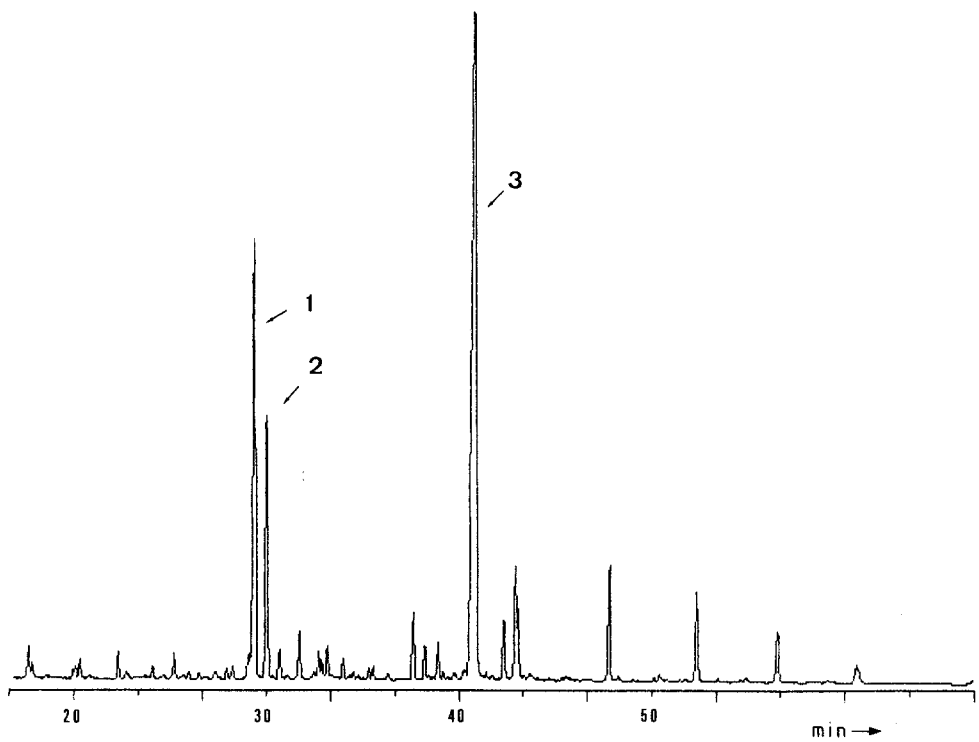


Figure 4. Reconstructed ion current gas chromatogram (RIC-GC) of silylated degradation products from methanolysis of ODP (see text for structural assignments).

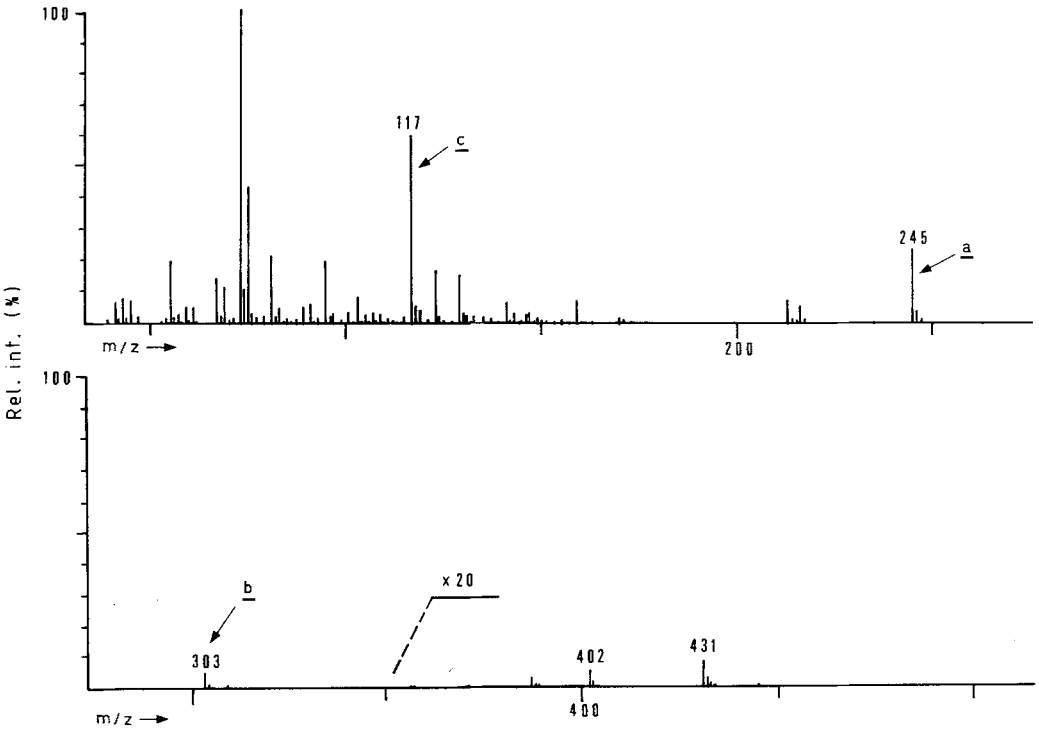


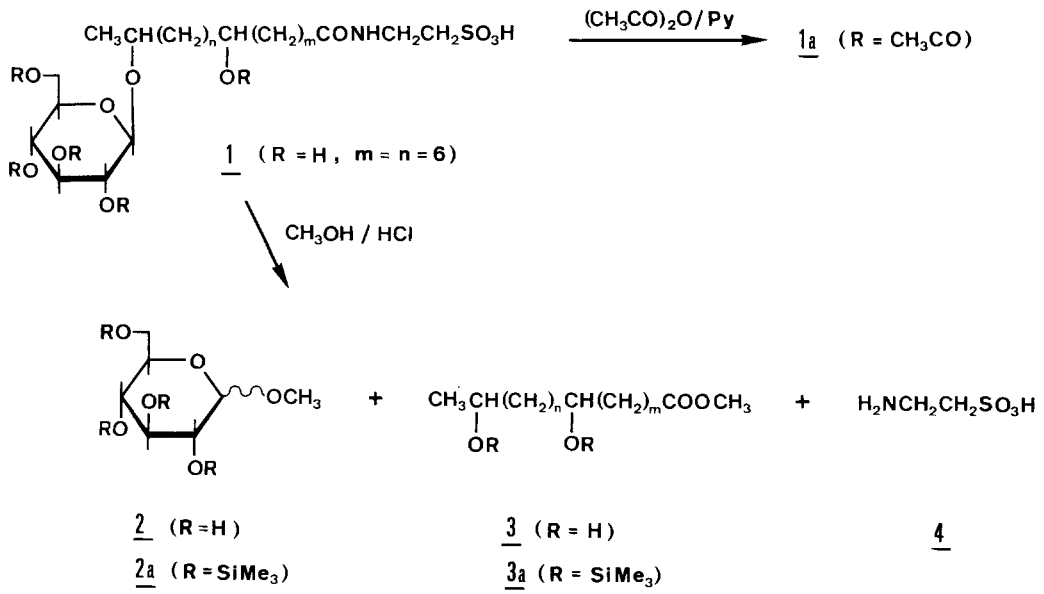
Figure 5. EI-MS spectrum of Bis-trimethylsilylether of methyl 8, 15-dihydroxypalmitate (**3a**).

396. In accordance with the well-known behavior of protonated glycosides these processes are likely to reflect the elimination of a hexose as an anhydrosugar fragment $C_6H_{10}O_5$. The presence of a hexose as a structural element in **1** was corroborated by peracetylation with acetic anhydride/pyridine (scheme 1). FAB-MS analysis of the crude reaction product **1a** (fig. 3) indicated for $[M+H]^+$ (m/z 768), $[M+Na]^+$ (m/z 790), and $[M-H+2Na]^+$ (m/z 812) invariably an uptake of 5 acetyl groups (mass increments 5 × 42 dalton). A new fragment at m/z 331, quite characteristic for pyranoside tetraacetates, indicated that, in accordance with expectations, 4 of the 5 acetyl groups had been incorporated in the sugar unit. Failure of acetylation in a separately conducted experiment in which only basic NH-groups react (methanol/acetic anhydride 4:1) required the assumption of one more free HO-group in addition to those of the hexose moiety. In support of this, the FAB-MS/MS of $[M+Na]^+$ (m/z 790) displayed fragment m/z 331 concomitantly with ions at m/z 438 and 460 which arise from the elimination of the tetraacetylated sugar unit with and without sodium (loss of $C_6H_5ONa(OAc)_4$ and $C_6H_6O(OAc)_4$, respectively). Identification of the hexose unit as glucose by chemical degradation through methanolysis (scheme 1) with GC/MS analysis of the silylated cleavage products as well as by ^{1}H-NMR on a concentrated sample of **1**, proved straightforward.

Peaks 1 and 2 in the reconstructed ion current gas chromatogram (RIC-GC, fig. 4) corresponded to the 2, 3, 4, 6-tetrakis(trimethylsilyl) ethers of the α- and β-anomeric methyl 1-*O*-glucopyranosides. A β-glucosidic linkage to the aglycone portion of the molecule in **1** was evident from the ^{1}H-NMR data (table 2).

As a second structural element an unbranched fatty acid with 16–18 C atoms and electronegative substituents both at the (ω-1) and an undetermined inner position of the chain was also deduced from the ^{1}H-NMR spectrum. Despite the high enrichment of the sample, signals of interfering compounds prevented the determination of the exact number of protons. The shifts of the signals in **1a** (table 2) clearly showed that the electronegative substituent within the center portion of the chain had to be the free hydroxyl group inferred from acetylation, rendering the (ω-1) function the site of glucosylation. GC/MS analysis of the products from methanolysis provided the lacking exact information on the chain length and the position of the free hydroxy group. The EI-MS (fig. 5) of the bis(trimethylsilyl) derivative **3a** (peak 3 in gas chromatogram, fig. 4) exhibited a highly instructive fragmention pattern in that pronounced α-cleavage products *a* ($TMS\text{-}O^+ = CH((CH_2)_6COOCH_3$, m/z 245), *b* ($(CH_3CH(O\text{-}TMS)\text{-}(CH_2)_6CH = O^+\text{-}TMS$, m/z 303) and *c* ($CH_3CH = O^+\text{-}TMS$, m/z 117) were observed. From these data, both a molecular mass of 302 and a $C_{17}H_{34}O_4$ composition of the unsilylated methyl ester **3**, i.e. the presence of a dihydroxy C_{16}-acid, was deduced. The fragments *a*, *b* and *c* placed the hydroxy substituents unequivocally in positions C-8 and C-15, thereby defining the intermediate sections of the chain as m = n = 6. The fatty acid as the central aglycone portion of the pheromone was, thus, characterized as a 8, 15-dihydroxypalmitic acid with unknown stereochemistry at its two chiral centers.

From these findings and from the $C_{24}H_{47}NO_{11}S$ formula of intact **1** an elemental composition $C_2H_6NO_3S$ (mass increment 124 dalton) was deduced for the third structural element (Z in table 2) still in question. An amide linkage between the carboxyl group of the fatty acid and an amino group of this structural unit accounts very well for the observed failure of N-acetylation. The ^{1}H-NMR



Degradation of ODP (**1**) by methanolysis.

spectrum of **1** (table 2) clearly showed a corresponding X-CH_2CH_2-Y moiety (X, Y = heteroatomic groups) which, when X = –NH–, must contain Y = SO_3H and, hence, comprise a taurine molecule. A taurine conjugate structure RCO-NH-$CH_2CH_2SO_3H$ was, indeed, supported by the ^{1}H-NMR spectrum of *N*-palmitoyltaurine, $C_{15}H_{31}CONHCH_2CH_2SO_3H$, which was prepared as a reference compound (triplet signals of X-CH_2CH_2-Y element having the same chemical shifts as **1**). Evidence in favor of taurine was also derived from an MS/MS experiment on *m/z* 360 present in the FAB-spectrum of **1** as a major fragment (fig. 2). It originates from the elimination of both oxygen functions (loss of glucose and H_2O) and, consequently, corresponds to an $[M+H]^+$ ion of a doubly unsaturated taurinate $C_{15}H_{27}CONHCH_2CH_2SO_3H$ (M = 359). The MIKES-spectrum of this ion displayed not only a distinct *m/z* 126 daughter ion representing protonated taurine $H_3N^+CH_2CH_2SO_3H$, but also the complementary acylium ion $C_{15}H_{26}C{\equiv}O^+$, *m/z* 235, produced by heterolysis of the amide bond. Taurine **4** itself was finally also identified as one of the degradation products of **1** after methanolysis (scheme) by TLC in two chromatographic systems.

Conclusion

The structural analysis revealed the pheromone molecule to contain a lipophilic core carrying, on both ends, highly polar peripheral units. Conjugation with taurine, a sulfonic acid with a high pK_a value, accounts for facile formation of anions and, thus, of salts, conveying high solubility in aqueous systems and, probably, relevant surface activity to the molecule. Its chemical constitution of a N[15(β-glucopyranosyl)oxy-8-hydroxypalmitoyl]-taurine (**1**, scheme), contains two chiral centers at C-8 and C-15 as the positions of oxygenation of the aliphatic chain. Configurational assignments will require further study, e.g. by determination of the biological activity of the four possible stereoisomers, to be synthesized separately. The biologically most active one will be the prime candidate for being identical with natural ODP.

Naturally-occurring compounds consisting of sugar and fatty acid constituents, such as the glycolipids known as cerebrosides and gangliosides are found in nerve cell membranes. Taurine as a further component of this kind of molecule opens many possibilities for speculation, because this β-aminosulfonic acid is known to be present in a variety of invertebrate and vertebrate tissues[16,17]. In mammalian brains it is considered to be a neurophysiologically important constituent[18]. The possibility that ODPs of other insect species also belong to the class of taurine derivatives is intriguing. An attempt to synthesize the 4 possible stereoisomers of the ODP and some of its more simple structural analogues has been initiated to provide the opportunity to study receptor specificity of the tarsal ODP receptor cell.

Acknowledgment. The authors thank Dr B.N. Green, VG Analytical Ltd, Manchester, for the high-resolution measurement of the pheromone, and Dr P. Koch, Ciba-Geigy AG, Basel, for the determination of taurine by TLC. The authors are also obliged to Prof. A. Eschenmoser of the Swiss Federal Institute of Technology at Zürich for critical advice, and to Mrs G. Schwarz of the Swiss Federal Research Station at Wädenswil for assistance in literature search. In addition, thanks are due to Mrs M. Manser, and Dr B. Katsoyannos for providing technical assistance in various stages of the project. Parts of this research has been supported by grants from Ciba Geigy AG and the Schweizerische Nationalfonds No. 3.476-0.83.

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0014-4754/87/020157-08$1.50 + 0.20/0

Short Communications

Permeability of arthrodial membrane to water: A first measurement using in vivo techniques

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Summary. Despite differences in surface morphology and fine structure, the permeabilities of untreated scorpion sternite and pleural cuticle to water are similar (0.69 versus 0.79 $\mu g \cdot cm^{-2} \cdot h^{-1} \cdot mm\ Hg^{-1}$). Hexane applied to pleural membrane increased its permeability 9-fold, but neither hexane nor chloroform:methanol had much effect on sternite permeability. When sternite cuticle was treated with 1.0 N KOH followed by chloroform:methanol, permeability increased about three times over control values. In contrast, cockroach pronotum, which is 17 times more permeable than scorpion sternite, exhibited a marked increase in permeability when treated with just hexane. In both the scorpion pleuron and cockroach pronotum, disruption of the lipid barrier caused by rubbing is partially responsible for the higher permeabilities observed following solvent treatment.
Key words. Arthrodial membrane; cockroach; cuticle; cuticular lipids; permeability; scorpion; water loss.

Arthrodial or 'intersegmental' membrane of arthropods occurs between true metameric segments, at the joints of appendages, and at the bases of hairs and bristles. Because the proteins in this cuticle do not tan, arthrodial membrane remains soft and flexible, permitting adjacent sclerotized segments to move relative to one another[1]. In most insects and arachnids, arthrodial membrane represents a relatively small, albeit critical, percentage of an individual's total surface area. However, during certain stages of reproduction or often following a large meal, arthrodial membrane unfolds and/or stretches to accomodate increased abdominal volume. At this time the surface area of arthrodial membrane can equal or exceed that of sclerotized ('hard') cuticle and, thus, becomes a potentially important site for transcuticular water loss.

Despite its functional importance, arthrodial membrane has received far less study than its counterpart, sclerotized cuticle. Filshie and Hadley's[2,3] ultrastructural examination of the dorsal sclerites and adjacent intersegmental membrane of the scorpion *Hadrurus arizonensis* is one of the few studies that have compared the two cuticle types. They found that the well-defined exocuticle of sclerite is absent in the intersegmental membrane. They also found differences in the fine structure of the epicuticle and in the pore/wax canal complex in intersegmental membrane. These differences might significantly alter the permeability of scorpion arthrodial membrane since the epicuticle is the site of the principal barrier to water efflux in most terrestrial arthropods. Until recently, however, it was not possible to test this experimentally.

Materials and methods. To address this question, we used miniature ventilated capsules that could be attached directly to the lateral pleuron of gravid female *H. arizonensis*. A capsule of similar design was used recently in conjunction with our transpiration monitor to measure electronically the permeability of cricket thorax in vivo[4]. With this system, dry air (0.5 $ml \cdot min^{-1}$) enters the capsule inlet tube, acquires moisture that is diffusing across the cuticle, and then exits via an outlet tube into an aluminum oxide sensor. The moisture content of the humidified air alters the impedance of the sensor. The signal is amplified and plotted on a millivolt recorder as a continuous tracing. Details of the system's design, operational characteristics, and calibration methods are given in Hadley et al.[5].

Test scorpions were cooled before being secured to a thin metal plate with strips of tape. The plate was then attached to a ring stand with the scorpion positioned on its side. The capsule was then lowered carefully until its base firmly contacted the lateral membrane. The capsule base was attached to the membrane using epoxy applied to the outer edge of the capsule. After drying, the entire unit was transferred to an insulated Plexiglas chamber into which air of desired temperature ($\pm$ 0.5 °C) was circulated. The temperature of the capsule was measured with a copper-constantan thermocouple connected to a BAT-12 thermometer. The inlet and outlet ends of the capsule were connected to the transpiration monitor and the system flushed of residual moisture. Readings were taken only after the tracing had stabilized (3–6 h). Similar in vivo determinations were made with the capsule attached to the ventral sclerite (= sternite) of *H. arizonensis* and to the pronotum of the cockroach *Periplaneta americana*.

Our comparison of sclerotized cuticle and arthrodial membrane included untreated cuticle (controls) and cuticle that had been lightly swabbed (Q-tips) with either a lipid solvent, KOH, or distilled water (shams). Scorpion surface lipids, extracted from regional washings (small hexane-soaked cotton balls held by forceps) or from dissected soft cuticle, were analyzed by conventional thin layer and gas chromatographic techniques[6]. The surface detail (SEM) and fine structure (TEM) of the cuticle were also determined for the pleural (arthrodial) membrane and sternite, as the previous ultrastructural study had investigated dorsal sclerites and adjacent intersegmental membrane.

Comparisons between control and treatment permeability mean