and in the proliferating cartilage (PC) we found about 0.05 in the fully mineralized areas (MI; CC). This can be interpreted as showing that the Mg content in the unmineralized regions is enriched (10-fold) in relation to the Ca content. Such a Mg enrichment in the prestages of apatite formation was also found in calcification experiments with cultures of the bacterium Matruchotii[11]. In the same region of the tendon where Mg is enriched a maximum in APase activity occurs[6]. So one may speculate that Mg has a regulating function for the enzyme activity which produces the phosphate as well as for mineralization and crystal growth[7]. CO_2 might act in the same way. Casciani[12] has found in the very first stages of enamel formation mainly CO_3^{2-} instead of phosphate. Boyde[13] assumes that the early mineral is rich in CO_3^{2-} or even contains $CaCO_3$. The fact that CO_2 is enriched in the collageneous system of turkey tibia tendon as well as in the collagen-free enamel may indicate that the same controlling functions exist in different hard tissues.

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(E,E)-10,12-Hexadecadienal: A component of the female sex pheromone of the spiny bollworm, *Earias insulana* (Boisd.) (Lepidoptera: Noctuidae)[1]

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Summary. *(E,E)*-10,12-Hexadecadienal has been identified as a component of the sex pheromone of the female spiny bollworm moth, *Earias insulana*, by gas-chromatographic, electroantennographic and microchemical studies of abdominal tip extracts and entrained volatiles from female moths.

The spiny bollworm, *Earias insulana*, is an important cotton pest in Africa and the Mediterranean region, extending as far east as India and S.E. Asia. The use of traps baited with virgin female moths in monitoring attacks by this insect has already been reported[2], and identification of the female sex pheromone was undertaken to provide a synthetic attractant for this purpose.

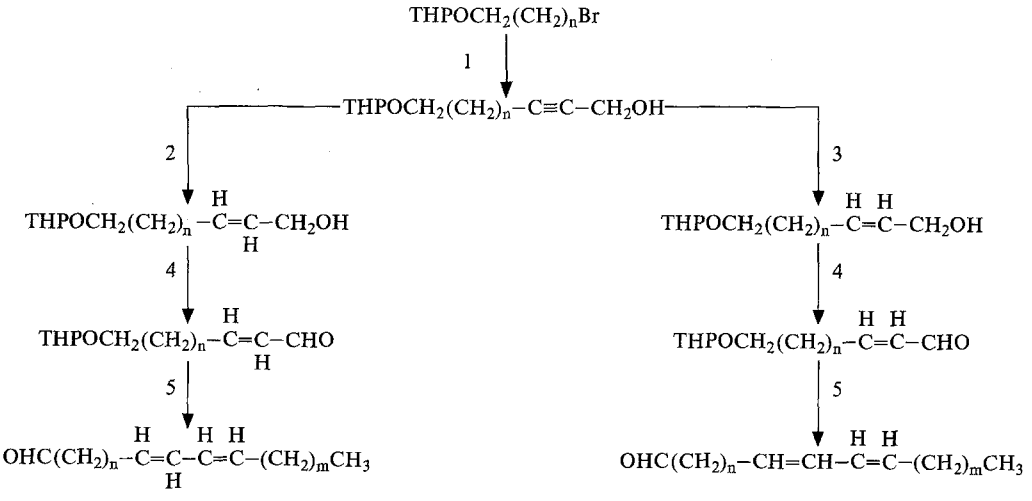
Materials and methods. Pupae were received from Israel, Malawi and the Ivory Coast, and were incubated at 25 °C under constant light until adult emergence. Virgin female moths were then maintained at 20–25 °C on a 12 h: 12 h reversed light/dark cycle, and their abdominal tips were clipped 3 h into the scotophase on the 2nd night after emergence[3]. The excised tips were extracted with ether or hexane for 15 min at room temperature, and the extracts were filtered and concentrated before analysis. Active material was also obtained by collection on charcoal of volatiles emitted by a virgin female moth[4].

Gas-chromatographic (GC) instrumentation and packed columns were as described previously[5]. High resolution GC analysis was carried out on SGE 'D' grade support-coated open tubular (SCOT) columns as follows: Carbowax 20M (51 m × 0.5 mm inner diameter) temperature programmed from 100 to 180 °C at 20 °C/min and then isothermal; SE 30 (39 m × 0.5 mm inner diameter) programmed from 100 to 160 °C at 20 °C/min and then isothermal. GC analyses combined with simultaneous recording of electroantennographic (EAG) responses from the male moth to the column effluent were carried out as described by Moorhouse et al.[6], and EAG responses to synthetic compounds 'puffed' directly over the male moth's antenna were recorded as described previously[7].

The natural pheromone component, after GC collection from Apiezon L[8], and synthetic compounds were treated with tetracyanoethylene (TCNE) in dichloromethane at room temperature for 1.5 h and then analyzed by GC[8]. Monounsaturated alcohols were prepared by standard acetylenic routes and oxidized to the corresponding aldehydes with buffered pyridinium chlorochromate (PCC) in dichloromethane[9]. Wittig reaction of *(E)*-2-pentenal with the triphenylphosphonium salt of the tetrahydropyranyl (THP) ether of 11-bromo-1-undecanol gave *(Z,E)*- and *(E,E)*-11,13-hexadecadienal after deprotection and oxidation (Hall et al.[10]). Reaction of sorbyl acetate with the Grignard reagent from the THP ether of 10-bromo-1-decanol in tetrahydrofuran (THF), catalysed by lithium tetrachlorocuprate, gave *(E,E)*-12,14-hexadecadienal after deprotection and oxidation[11]. The 9,11- and 10,12-hexadecadienals were prepared by means of a general route to conjugated dienes outlined in the scheme.

Any of these hexadecadienals could be isomerized to a mixture of all 4 geometric isomers (ca. 61% *E,E;* 18% *Z,E;* 18% *E,Z;* 3% *Z,Z*) by heating with 0.5% thiophenol for 60 min at 110 °C[12], and the pure *E,E* isomers were obtained by LC on silica gel impregnated with 20% silver nitrate. The isomeric compositions of the hexadecadienals were most conveniently determined by GC analysis on the liquid crystal stationary phase, diethyl 4,4′-azoxydicinnamate[13]. Configurational assignments were based on a) mode of synthesis, b) selective reaction of the *E,E* isomers with

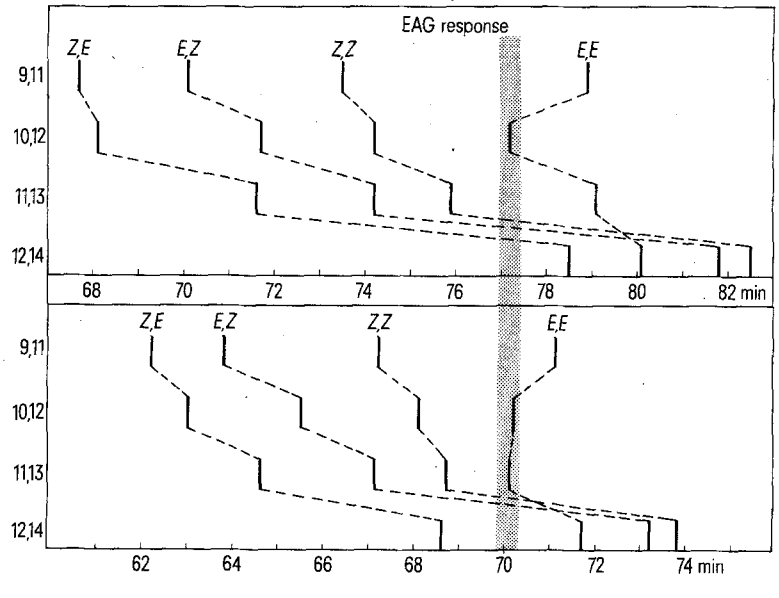
Scheme. Synthesis of 9,11- (n = 7, m = 3) and 10,12- (n = 8, m = 2) hexadecadienals



Reagents: 1. $HC{\equiv}C{-}CH_2OH/LiNH_2/NH_3/THF$; 2. $LiAlH_4/THF$; 3. H_2/Lindlar catalyst/quinoline/EtOH; 4. MnO_2/benzene; 5. $CH_3(CH_2)_mCH_2PPh_3{}^+Br^-$/NaH/DMSO/THF, HCl/MeOH, PCC/sodium acetate/CH_2Cl_2.

TCNE (see below), c) the proportions present in equilibrated mixtures of geometric isomers, d) the order of elution from silver nitrate impregnated silica gel, and e) literature data on homologous compounds[10,11].

Results and discussion. A single biologically-active component was detected when abdominal tip extracts or entrained volatiles from female moths were examined by linked GC-EAG using packed GC columns, and this was assumed to be a component of the female sex pheromone. Equivalent chain lengths based on the retention temperatures of n-alkyl acetates (ECL's) for this compound were 16.4 (Carbowax 20M), 16.3 (CHDS), 14.5 (SE 30) and 14.7 (Apiezon L). This GC behaviour suggested that the compound could be a C_{16} aldehyde with 2 double bonds in conjugation (ECL's of (E,Z)-9,11-hexadecadienal on the above columns were 16.1, 16.0, 14.2 and 14.5 respectively). Yields of the pheromone component were less than 1 ng per tip and could not be improved by clipping the female moths at different ages or at different times into the scotophase. Further identification was therefore based mainly on comparisons of the GC behaviour and EAG activity of the natural pheromone component and a range of synthetic compounds. When EAG responses of a male moth to the *Z* and *E* isomers of 7-, 9-, 10-, 12- and 13-hexadecenal were recorded, the maximum responses were to the 10-, 11- and 12- positional isomers, and there was little distinction between geometrical isomers (responses to 5 ng at source in mV: *(Z)*-7, 0.54; *(E)*-7, 0.37; *(Z)*-9, 0.24; *(E)*-9, 0.22; *(Z)*-10, 1.25; *(E)*-10, 1.54; *(Z)*-11, 2.44; *(E)*-11, 2.42; *(Z)*-12, 2.54; *(E)*-12, 2.56; *(Z)*-13, 0.50; *(E)*-13, 1.13). This data suggested that the pheromone component was a 10,12-hexadecadienal[14], and comparison of the GC behaviour of the natural pheromone component and synthetic 9,11-, 10,12-, 11,13- and 12,14-hexadecadienals on high resolution SCOT columns provided further evidence for this supposition. The only synthetic isomer with the same



Retention times for synthetic hexadecadienal isomers and the natural pheromone component on Carbowax 20M (upper) and SE 30 (lower) SCOT columns; peak width at half height 0.6–0.8 min.

retention characteristics on both Carbowax 20M and SE 30 SCOT GC columns was *(E,E)*-10,12-hexadecadienal (figure). The presence of an *E,E* conjugated diene system was also indicated by the observed reaction of the natural pheromone component with TCNE, since, on similar treatment of synthetic hexadecadienals, the *E,E* isomers reacted but the *Z,E*, *E,Z* and *Z,Z* isomers were unchanged[8,10].

Synthetic *(E,E)*-10,12-hexadecadienal elicited an EAG response from the male moth comparable to that to the natural pheromone component, and it has been shown to attract male *E. insulana* moths to traps in the field[15,16]. *(E,E)*-10,12-Hexadecadienal has not been found previously as a moth sex pheromone component, although the corresponding alcohol and the *E,Z* isomer of the aldehyde have recently been detected in extracts of the pheromone glands of female silkworm moths, *Bombyx mori*[17].

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Methanic fermentation in the digestive tract of a xylophagous insect: *Oryctes nasicornis* L. larva (Coleoptera; Scarabaeidae)

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Summary. Strict anaerobic conditions and the production of methane have been demonstrated in the proctodeum in larvae of *Oryctes nasicornis* L., a xylophagous coleopteran. In ruminants, the breakdown of cellulose by extracellular symbiotic organisms is complete and leads to the formation of by-products which may act as substrates for methanogenic bacteria.

Recently there has been a large amount of research on methanogenic bacteria because of their economic importance and also because of their very characteristic metabolism[1]. They are found in anaerobic conditions in the soil and in the flora of the gastrointestinal tract of herbivorous mammals, where they are capable of producing methane. Coleoptera Scarabaeidae larvae, which feed on lignin and cellulose litter, have a proctodeal dilatation inhabited by numerous bacteria. We demonstrate here that anaerobic conditions and production of methane occur in this particular intestinal segment of *Oryctes nasicornis* L. larvae.

Material and methods. The insects were reared in La Minière (Versailles INRA[2]). 30 larvae were fed on their natural food (decomposing sawdust) at 28 °C, the optimum growth temperature. The experiment was repeated using 30 larvae which had been fed on pure α-cellulose (Sigma) for 3 weeks.

The midgut and the hindgut were dissected and the electric potential of the contents was readily measured using a potentiometer with a platinum electrode and a fixed calomel reference electrode.

Methane was investigated by gas chromatography. The gas chromatography analyses were carried out using 2.5 m × 2.5 mm columns packed with porapak Q (80–100 mesh). The peaks were obtained using a Perkin-Elmer chromatograph (model 881) with a nitrogen flow of 25 ml/mn (at room temperature 25 °C, detector at 250 °C). These results were verified using a mass spectrometer CH_5 Varian M.A.T. connected with a chromatograph Girdel 3000. The column and the conditions were the same as above, but the carrier gas was helium (20 ml/mn). An attempt was made to detect methane in the midgut and proctodeal dilatation of the larvae. The study was made on 1 group of 15 larvae fed on their natural food and a 2nd group fed on pure α-cellulose. Samples were taken as follows: the digestive tract was rapidly exposed by dissection of the larvae and the mesenteron and the proctodeal dilatation was removed. Each was placed in a 15 ml air-tight bottle. With the aid of a needle pushed through the rubber stopper the intestinal segment was opened to liberate its contents and an aliquot of the atmosphere of the bottle was analyzed. The quantity of methane present was calculated from the peak obtained by gas chromatography read against a reference curve of different air and methane mixtures.

A series of experiments was conducted to detect any methane given off from a whole animal. 5 larvae, fed on sawdust, were placed in air-tight 500 ml bottles and kept