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IDENTIFICATION OF A POTENTIAL SEX PHEROMONE OF THE PROCESSIONARY MOTH, *THAUMETOPOEA PITYOCAMPA* (LEPIDOPTERA, NOTODONTIDAE)

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Summary: The major component of the active fraction from the *Thaumetopoea pityocampa* (Denis and Schiff.) virgin female extract has been identified as (Z)-13-hexadecen-11-ynyl acetate on the basis of spectroscopic and microchemical reactions data. This is the first report of an acetylenic structure found in the insect pheromone field.

Thaumetopoea pityocampa (Denis and Schiff.) (Lepidoptera, Notodontidae) is one of the most important defoliator pest of pine trees in all the Mediterranean countries. The occurrence of this moth in recreation and suburban areas has also health implications due to urticary substances, present in the caterpillar hairs, which can produce strong allergic reactions.[1]

In the present communication we report the isolation and characterization of the major component found in the extract from the sex pheromone producing glands of virgin females.[2] The crude extract from ca. 6000 specimens was obtained by maceration of the last two abdominal segments in nanograde benzene, dichloromethane or hexane, for 24 hrs. Filtration and concentration under argon afforded a residue which exhibited activity on EAG and field tests. Throughout the present work these same bioassays were used to monitor the different fractionation steps and some microchemical reactions described below. Removal of non-volatile components was brought about by short path bulb-to-bulb distillation (120ºC/0.1 torr). The distillate was purified by column chromatography on activated silica gel (70-230 mesh), the active fraction being eluted with a 92:8 pentane:diethyl ether mixture.

GLC analysis of this fraction showed a major active component, which was further purified by micropreparative GLC (3m x 1/8" glass column, 3% OV-101 on Chrom. W, 100-250ºC, 8ºC/min, N_2, 20 ml/min). The retention time of this active component was ascertained to be within the C_{16}-C_{18} range by comparison with those of authentic samples of saturated and unsaturated linear acetates, both on polar and non polar capillary and packed columns (50 m x 0.5 mm I.D., Carbowax 20M and SE-52 capillary columns, 3 m x 1/8", 15% FFAP or 3% OV-101, glass columns).

Hydrolysis of the above compound with an aqueous sodium hydroxide solution resulted in a complete loss of activity. However, the initial activity was recovered by acetylation, indicating that the presence of an acetate group was required to elicit a sexual attraction response. Likewise, hydrogenation on PtO_2/CH_3OH caused also the disappearance of the activity and afforded a compound which was fully identified as n-hexadecyl acetate by GLC and MS.[3]

High resolution MS (AEI, MS-9) of the active component indicated a molecular weight corresponding to a tri-unsaturated acetate ($C_{18}H_{30}O_2$: calc. 278.2246; found 278.2234). Other GC/EI-MS features (Ribermag R-10-10, 70 eV, fig. 1) revealed the presence of a base peak at m/z 94 in agreement with a C_7H_{10} fragment which retained the three unsaturations, and also the absence of the expected M-60 peak, usually found in the fragmentation of acetates. However, this fragmentation appeared in GC/CI-MS (CH_4: peaks at m/z 279 (M + 1, 100%) and 219 (M + 1-60, 73%). NH_3: peaks at m/z 296 (M + 18, 100%), 279 (M + 1, 26%) and 219 (M + 1-60, 1%)).

GC/FT-IR (Bruker Spektrometer IFS 113V) spectrum showed an absorption at 1740 cm^{-1}, which confirmed the presence of the acetate group, whereas absorption at 3030 cm^{-1} and the absence of a band at 980 cm^{-1} pointed out the occurrence of (Z)-double bond(s). Micro-ozonolysis[4] of the active compound (1 min, -70ºC, hexane) yielded a product with a molecular weight of 252 (GC/EI and CI/MS), assigned as a C_{13} di-unsaturated acetoxyaldehyde ($C_{15}H_{24}O_3$). This suggested the presence of a double bond at C_{13} position in the initial compound. A second minor ozonolysis product, which increased on prolonged treatment, exhibited a molecular weight of 224 (GC/EI and CI/MS).

Epoxydation with m-chloroperbenzoic acid in dichloromethane afforded two monoepoxides with the same fragmentation pattern (m/z 294 ($M^{+\cdot}$, 3%), 110 (75%) and 95 (100%)).[5] The fragment at m/z 110 ($C_7H_{10}O$) was in agreement with the occurrence of the same preferential cleavage as that aforementioned for the parent compound.

Hydrogenation over PtO_2/MeOH of the monoepoxides gave a single compound identified by GC/MS as 14-hydroxyhexadecyl acetate (EI: 271 (M-29, 100%),[14] 211 (M-29-60, 22%), 61 (8%); CI (NH_3): 301 (M + 1, 100%), 318 (M + 18, 51%), 283 (M + 1-18, 22%)). The specific formation of this alcohol and the data from MS, ozonolysis and epoxidation of the active compound strongly supported the hypothesis of a 13-ene-11-yne structure.

Final structure determination was made possible by FT-NMR on 8 μg of the active compound (Cameca, 250 MHz, 60 μl microcell) (fig. 2): δ_{CDCl_3} 1.02 (t, J=7.5 Hz), 2.05 (s), 2.33 (b), 4.06 (t, J=7 Hz), 5.41 (d, J=10 Hz) and 5.82 (m). Each one of the δ 5.41 and 5.82 absorptions correspond to one vinylic hydrogen, showing the existence of only one disubstituted dou-

Fig. 1

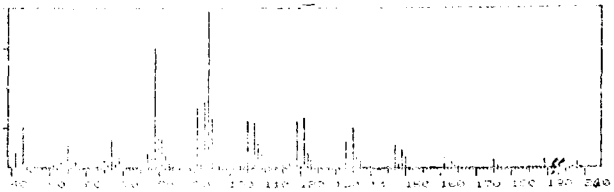


Fig. 2

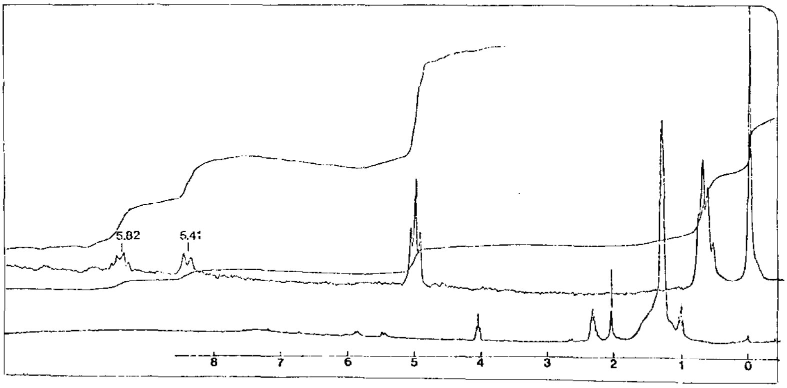


Fig. 3

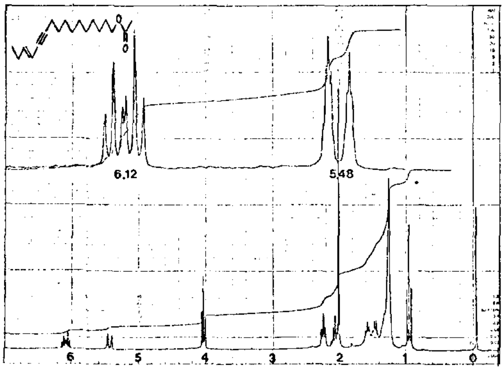
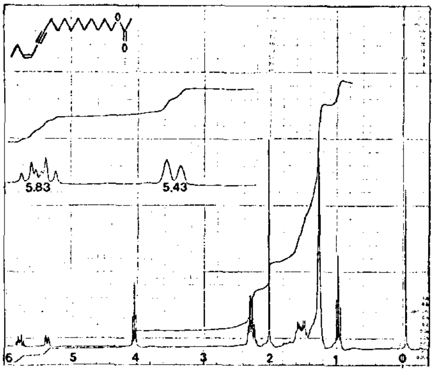


Fig. 4



ble bond with Z configuration. To account for three unsaturations and the chemical shift difference of these protons, the presence of a conjugated triple bond should be assumed. Irradiation at δ 2.35 collapsed the 1.02 triplet into a singlet and the δ 5.82 multiplet into a doublet (J=10 Hz). These results confirmed the occurrence of an AB system with only the A part coupled further to allylic protons. Moreover, the chemical shift of the triplet at δ 1.02 was in accordance with a homoallylic methyl group, confirming the existence of a $CH_3CH_2CH{=}CHC{\equiv}C$ moiety. Likewise, irradiation at δ 1.62 collapsed the 4.06 triplet into a singlet, pointing out a CH_2CH_2-$OCOCH_3$ partial structure.

Consequently, all the above data suggested the structure of Z-13-hexadecen-11-ynyl acetate (1) for the active component:

1

The proposed structure can explain the ozonolysis results, particularly the formation of 11-dodecynyl acetate as the secondary component (MW 224). In fact, this compound could be derived from the α,β-acetylenic aldehyde acetate (MW 252) initially formed, through oxidation and decarboxylation under the reaction conditions. This assumption was confirmed by comparison with the MS and GLC retention time on SE-30, 5% on GCQ, of an authentic sample. Furthermore, the same behaviour was observed in the ozonolysis of the synthetic material. The assignment was also confirmed by comparison of GLC, MS, IR and NMR features with those of two authentic samples of Z and E-13-hexadecen-11-ynyl acetates synthesized independently (figs. 3-4).[6,7]

Though a number of natural acetylenic compounds have been described,[15,16] this is the first report of a conjugated enyne structure in the insect pheromone field. However, we cannot exclude the possible presence in the sex pheromone secretion of allene-ene acetates, precursors of the structure reported herein. Studies along this line are in progress.

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