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IDENTIFICATION OF (3Z,6Z)-1,3,6-9,10-EPOXYHENEICOSATRIENE AND (3Z,6Z)-1,3,6-9,10-EPOXYEICOSATRIENE IN THE SEX PHEROMONE OF HYPHANTRIA CUNEA

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Summary: (3Z,6Z)-1,3,6-9,10-Epoxyheneicosatriene and (3Z,6Z)-1,3,6-9,10-epoxyeicosatriene were identified in the sex pheromone gland of the arctiid moth, *Hyphantria cunea*. Of the synthesized enantiomers, the (9*S*,10*R*)trienes were biologically active while the (9*R*,10*S*) forms were inactive.

Three compounds, (3Z,6Z)-3,6-9,10-epoxyheneicosadiene, (9Z,12Z)-9,12-octadecadienal and (9Z,12Z,15Z)-9,12,15-octadecatrienal, have previously been identified from the sex pheromone secretion of *Hyphantria cunea* Drury (Lepidoptera: Arctiidae)[1,2]. However, field activity of these compounds has never been reported. We have now identified two additional epoxides related to the above, which appear to be involved in pheromonal activity.

Larvae of *H. cunea* were collected from plum and cherry trees at Szabadszállás (Bács-Kiskun county), and Hódmezövásárhely (Csongrád county), Hungary, and reared through pupation on *Acer negundo* L. leaves. Ovipositor tips of 2 day old calling females were extracted in a minimum amount of hexane (1-5 µL per female).

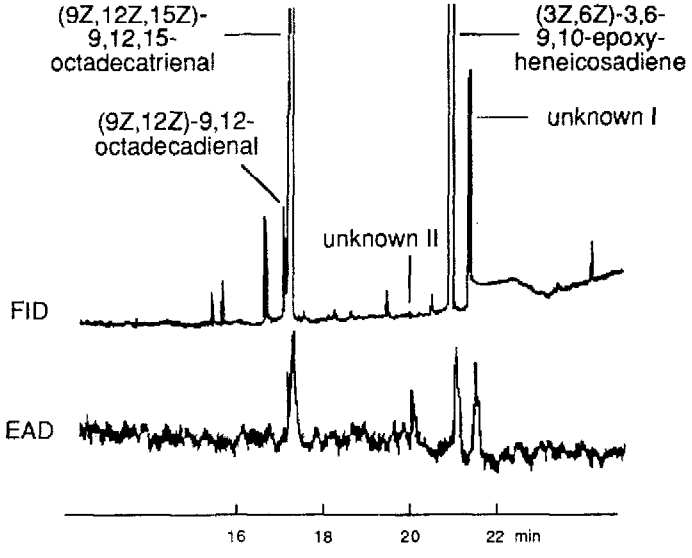


Figure 1. Gas chromatogram of *H. cunea* female sex gland extract on a 30-m SP-2340 (0.32 mm i.d., film thickness 0.2 µm) fused silica column. Program: 2 min at 40°C, 20°/min to 140°C, 5°/min to 225°C. Flame ionization (FID) and electroantennographic detector (EAD).

GC analysis with a 1:1 split between FID and the electroantennographic detector (EAD)[3] indicated the presence of 5 biologically active components (Fig. 1), the three previously identified and two unknowns, component I at about 15 % of the diene epoxide and with retention indices (RI) of 2289 and 2678, and II in trace amounts and with RI of 2185 and 2582 on SE 54 and SP-2340, respectively.

GC-MS analysis (Finnigan 4023, EI, 50 eV) was carried out on 25 m SE 54 using on-column injection, with a program of 2 min at 50°C, 20°/min to 140°C and 5°/min to 280°C. It confirmed the presence of the 3 published components.

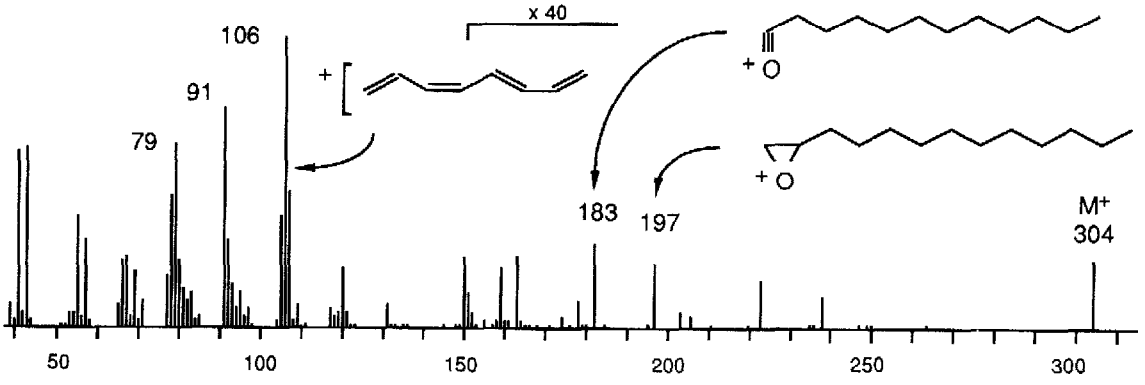


Figure 2. EI Mass spectrum of component I, identified as (3Z,6Z)-1,3,6-9,10-epoxyheneicosatriene.

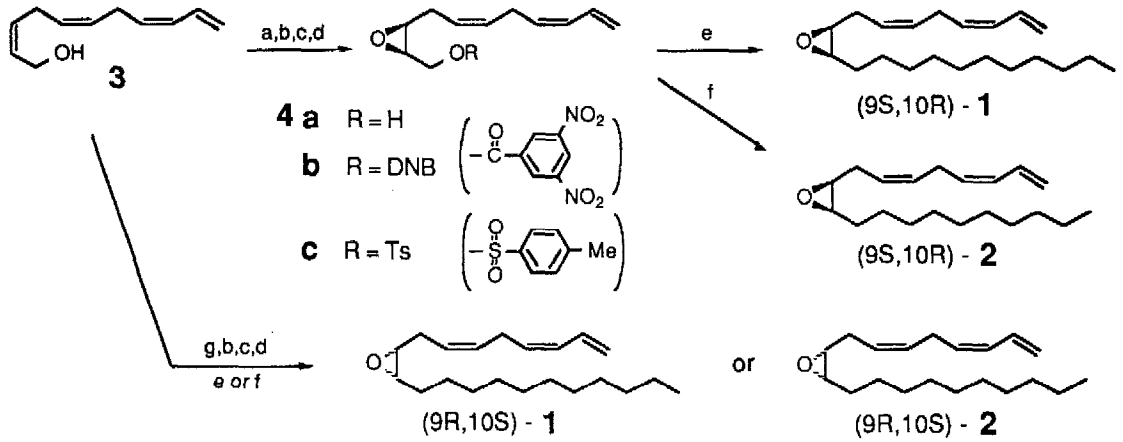
The mass spectrum of the unknown component I (Fig. 2) shows a molecular ion at m/z 304 with a series of highly unsaturated ions at m/z 79, 91 and 106. On comparison with the mass spectrum of (3Z,6Z)-3,6-9,10-epoxyheneicosadiene, this suggested a dehydrogenated analogue of the latter. Since the mass spectrum of component I contains signals at m/z 79, 91 and 106, as compared to m/z 79, 93 and 108 for (3Z,6Z)-3,6-9,10-epoxyheneicosadiene, an additional double bond in Δ^1-position was indicated, with the epoxy group still in $\Delta^{9,10}$-position. The retention difference between component I and (3Z,6Z)-3,6-9,10-epoxyheneicosadiene was the same as between (3Z,6Z,9Z)-1,3,6,9-nonadecatetraene, the sex pheromone of *Operophtera brumata* L.[4], and (3Z,6Z,9Z)-3,6,9-nonadecatriene on both columns, indicative of double bond conjugation for component I.

In order to test the above hypothesis, we treated synthetic (3Z,6Z,9Z)-1,3,6,9-nonadecatetraene with m-chloroperbenzoic acid, to obtain a lower homologue of the proposed structure. The reaction mixture contained three major peaks, one of which was highly active on the male *H. cunea* antenna. This component had a mass spectrum similar to component I with prominent ions at m/z 79, 91, 106, except that the apparent molecular ion was m/z 276 as expected for a (3Z,6Z)-1,3,6-9,10-epoxynonadecatriene.

The amount of component II was not sufficient for a complete mass spectrum. However, its RI and characteristic ions at m/z 79,91,106 suggested the C_{20} homologue of component I, i.e. 1,3,6-9,10-epoxy-eicosatriene.

We also detected small amounts of the following compounds in the female gland extracts, presumably of all *Z* configuration (RI on SE-54, characteristic ions): 3,6-9,10-epoxyeicosadiene (2153, m/z 79, 93, 108, 169, 183, 209, 223), 3,6,9-heneicosatriene (2068, m/z 79, 80, 93, 95, 108, 234, 290) and 1,3,6,9-heneicosatetraene (2100, m/z 79, 80, 91, 106).

Two enantiomers each of (3*Z*,6*Z*)-1,3,6-9,10-epoxyheneicosatriene (**1**) and (3*Z*,6*Z*)-1,3,6-9,10-epoxyeicosatriene (**2**) were synthesized as shown in the Scheme. The present synthesis followed the pathway previously developed for the synthesis of (3*Z*,6*Z*,9*S*,10*R*)-3,6-9,10-epoxyheneicosadiene[5]. Asymmetric epoxidation[6] of the tetraenol **3**[7] employing D-(-)-diethyl tartrate as the chiral auxiliary gave the epoxide **4a** (87-88% e.e.). Purification of **4a** was achieved by recrystallizing its 3,5-dinitrobenzoate (**4b**) to give pure **4b**, m.p. 33-34°C, $[\alpha]_D^{20}$ + 18.5° (c=0.33, $CHCl_3$). Hydrolysis of **4b** furnished pure **4a** (100% e.e.), which was converted to the corresponding tosylate (**4c**). Treatment of **4c** with lithium di(n-decyl)cuprate gave (9*S*,10*R*)-**1**, m.p. 14-15°C; n_D^{16} 1.4781; $[\alpha]_D^{16}$ -0.41° (c=1.97, $CHCl_3$). When **4c** was treated with lithium di-(n-nonyl)cuprate, (9*S*,10*R*)-**2** was obtained, m.p. 2-3°C; n_D^{16} 1.4787; $[\alpha]_D^{16}$ -0.57° (c=1.23, $CHCl_3$). By employing L-(+)-diethyl tartrate as the chiral auxiliary, the antipode of **4a** was prepared. Its 3,5-dinitrobenzoate , m.p. 33-34°C; $[\alpha]_D^{13}$ -18.2° (c=1.14 in $CHCl_3$), was further transformed to give (9*R*,10*S*)-**1**, $[\alpha]_D^{16}$ +0.43° (c=2.39, $CHCl_3$), and (9*R*,10*S*)-**2**, $[\alpha]_D^{16}$ +0.62° (c=2.34, $CHCl_3$).



Scheme. Synthesis of the epoxytrienes - Reagents: a) Ti (O*i*-Pr)$_4$, D-(-)-DET, *t*-BuOOH, MS 4A/CH_2Cl_2 (60%); b) DNBCl/C_5H_5N-Et_2O; recrystallization from Et_2O-pentane (20%); c) K_2CO_3/MeOH (93%); d) TsCl/C_5H_5N (87%); e) (*n*-$C_{10}H_{21}$)$_2$CuLi/Et_2O (77%); f) (*n*-C_9H_{19})$_2$CuLi/Et_2O (68%); g) Ti (O*i*-Pr)$_4$, L-(+)-DET, *t*-BuOOH, MS 4A/CH_2Cl_2.

In GC-MS, both enantiomers of **1** matched component I with respect to retention time on SE 54 and mass spectrum, except for minor deviations from co-eluting impurities. The enantiomers of **2** had the same retention times as component II and matching mass spectra in the lower mass range (m/z 41-131); in the upper mass range, amounts of II and ion intensities were too low for a valid comparison. All trienic epoxides showed considerable tailing, indicating some decomposition during GC. Therefore, actual amounts in the gland may be higher than indicated by our analysis.

In GC-EAD, synthetic (9*S*,10*R*)-**1** and (9*S*,10*R*)-**2** were highly active on the *H. cunea* male antenna whereas responses to the (9*R*,10*S*) enantiomers were at least 10 times lower. This corresponds with the biological activity of the (9*S*,10*R*) enantiomer of the diene epoxide already reported[1].

To summarize, our data show that the pheromone gland of *H. cunea* contains the following compounds (amounts in ng/female, * =EAD active): (3*Z*,6*Z*)-3,6-9,10-epoxyheneicosadiene (60)*, (3*Z*,6*Z*)-1,3,6-9,10-epoxyheneicosatriene (8)*, (3*Z*,6*Z*)-3,6-9,10-epoxyeicosadiene (0.4), (3*Z*,6*Z*)-1,3,6-9,10-epoxyeicosatriene (0.04)*, (3*Z*,6*Z*,9*Z*)-3,6,9-heneicosatriene (0.6), (3*Z*,6*Z*,9*Z*)-1,3,6,9-heneicosatetraene (0.06), (9*Z*,12*Z*)-9,12-octadecadienal (5)* and (9*Z*,12*Z*,15*Z*)-9,12,15-octadecatrienal (40)*. Since in the epoxides tested the (9*S*,10*R*) enantiomer was electrophysiologically active, we presume that this is the form present in the female and involved in pheromonal activity.

In preliminary field tests in Hungary, a mixture of the three (9*S*,10*R*) epoxides and the two aldehydes in the approximate proportions found in the female attracted males into traps in both test locations (Hódmezövásárhely and Lórév). On the other hand, the mixture of only (9*S*,10*R*)-**1** and (9*S*,10*R*)-**2**, or the ternary blend previously described were not attractive. Further optimization of the attractant is underway.

Unsaturated epoxides have been identified as sex pheromone components in other Lepidoptera, e.g. (3*Z*,6*Z*)-3,6-9,10-epoxyheneicosadiene[8], (3*Z*,6*Z*)-3,6-9,10-epoxyeicosadiene[9], (6*Z*,9*Z*)-6,9-3,4-epoxynonadecadiene[10], (3*Z*,9*Z*)-3,9-6,7-heptadecadiene and (6*Z*,9*Z*)-6,9-3,4-epoxyheptadecadiene[11]. To our knowledge, this is the first report of epoxytrienes in Lepidoptera pheromones.

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