Defensive secretions of Nematinae larvae (Symphyta – Tenthredinidae)

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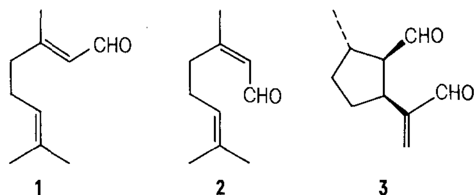
Summary. The composition of the defensive secretion of larvae of 8 Nematinae species is reported. 7 derivatives have been identified: benzaldehyde, (*E*)-2-hexenol, (*E*)-2-hexenal, (*E*)-4-oxo-2-hexenal, geranial, neral and *cis, trans*-dolichodial.

Various types of defensive mechanisms have been reported for Tenthredinidae larvae[3]. In particular, many larvae of the Nematinae subfamily possess well-developed medio-ventral abdominal glands[4]. These glands are eversible and emit an odoriferous, sometimes pungent, secretion that efficiently repels ants[5]. Since no data on the chemical nature of the active compounds of this group of insects were available, we decided to analyze the larvae secretion of 8 European Nematinae species. The larvae were collected in Belgium on different host-plants which are listed in the table. The secretions were obtained by gently pinching their abdomen. This induces the eversion of the glands, and droplets became visible on their surface. These droplets were then collected on bits of filter paper and stored in pentane or hexane at −30 °C. It was not possible to collect in this way the secretion of the small galicolous larvae of *Pontania proxima* from which total extracts in pentane were prepared. These solutions were analyzed by capillary GLC on 2 different liquid phases[6]. The volatile components identified are listed in the table.

Identification of benzaldehyde in *Pontania proxima* and *Nematus crassus* as well as of geranial (1) and neral (2), the stereoisomers of citral, in *Nematinus luteus*, is based on the odor and the retention times in comparison with authentic reference compounds (mixed injection in capillary GLC on 2 different stationary phases[6]).



GLC of the secretions of *Croesus varus* and *C. septentrionalis* revealed one and the same major volatile component. Its ^{1}H FTNMR shows the presence of 1 secondary methyl group (δ 1.09, 3 H, d, J = 6.5 Hz), 1 conjugated exomethylene group (δ 6.12, 1 H, d, J = 0.7 Hz and δ 6.28, 1 H, d, J = 1.5 Hz) and 2 aldehydic protons (δ 9.42, 1 H, d, J = 2.5 Hz and δ 9.54, 1 H, s). These data, together with the mass (M^+ at m/z 166) and UV spectra ($\lambda_{max}^{pentane}$ 213 nm), are compatible with those reported for *cis, trans*-dolichodial (3)[7] (relative configuration). Retention times comparison shows that this dialdehyde is also the major derivative of the secretion of *Nematus spiraeae*.

The secretion of *Nematus pavidus* and *N. melanaspis*, in contrast, are more complex. They are qualitatively identical, being constituted of 6 major derivatives, the relative proportions of which vary from sample to sample. 4 of the 6 components have been identified. The identification of (*E*)-2-hexenal[8], (*E*)-2-hexenol[9] and benzaldehyde[10] resulted from GC/MS measurements and was confirmed by mixed injection in capillary GLC with authentic samples. The mass spectrum of the 4th component (M^+ at m/z 112 (6), fragment ions at m/z 97 (1), 83 (100), 69 (4), 57 (20) and 55 (87)) agreed closely with that of (*E*)-4-oxo-2-hexenal[11,12]. The UV spectrum ($\lambda_{max}^{CH_3OH}$ 217 nm, $\varepsilon \sim 8.000$), recorded on a 30 µg sample isolated by preparative GLC[13], is also very similar to already published values[11,12]. To compare our material with an authentic sample of (*E*)-4-oxo-hexenal, we decided to synthesize this derivative following the procedure described by Pinder and Staddon[12]. The synthesis is based on the opening of 2-ethyl-2,5-dihydro-2,5-dimethoxyfuran. Despite our efforts, we were unable to obtain the desired aldehyde in this way. These failures may be compared with the conclusion of Hirsch and Szur[14] who found that direct conversion of α,α′-dimethoxydihydrofurans into (*E*)-enediones is not possible when 1 of the carbonyl groups of the enedione is an aldehyde. Nevertheless, in some cases, synthesis of (*E*)-enediones of this type is feasible starting from the corresponding(*Z*)-enedione[15].

Defensive compounds in the larvae secretions of 8 Nematinae species (%)

	(*E*)-2-hexenol	(*E*)-2-hexenal	(*E*)-4-oxo-2-hexenal	Benzaldehyde	Geranial	Neral	*cis, trans*-dolichodial	Host-plant
Croesus septentrionalis							100	*Alnus, Betula*
C. varus							100	*Alnus*
Nematinus luteus					89	11		*Alnus*
Nematus crassus (x)				83				*Populus*
N. melanaspis (x)	2	2	41	40				*Salix*
N. pavidus (x)	13	2	42	39				*Salix*
N. spiraeae (x)							90	*Aruncus*
Pontania proxima				100				*Salix*

(x), minor compound(s) remain unidentified.

A carefully controlled acid hydrolysis (H_2SO_4 0.005 M, 25 min, r.t.) of 2-ethyl-2,5-dihydro-2,5-dimethoxyfuran afforded(*Z*)-4-oxo-2-hexenal (94% purity by GLC), characterized by ^{1}H NMR[16] and MS[17]. However, all attempts to isomerize this derivative into the (*E*)-stereoisomer, either by acid or photochemical treatments always led to more or less complex mixtures. One of the components of these mixtures has the same MS (by GLC/MS) as that published for (*E*)-4-oxo-2-hexenal[17], as well as the same MS and retention time as our compound. This brings further arguments to our identification.

These preliminary results show that the defensive secretions of Nematinae larvae are chemically highly diversified. All compounds so far identified have already been reported as defense compounds from other groups of insects[18]. Works are in progress in our laboratory to determine if these allomones are synthesized de novo by the insects and which is the influence, if any, of the host-plant on the secretion components.

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Metabolism of C_{19}-steroids in testicular tissue of the newt *Triturus vulgaris*, during and after breeding

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Summary. Incubation studies with testicular homogenates of *Triturus vulgaris* showed that testosterone was quantitatively the most important metabolite of dehydroepiandrosterone and androstenedione during breeding. After breeding the recovery of testosterone declined. This suggests that 17β-hydroxysteroid dehydrogenase may be regulated for the needs of testicular androgen production.

The presence of $\Delta^5 3\beta$-hydroxysteroid dehydrogenase ($\Delta^5 3\beta$HSD), 17α-hydroxylase, 17–20 desmolase and 17β-hydroxysteroid dehydrogenase (17βHSD) has been shown in anuran and urodelan testis[1–7]. Furthermore 5α-reductase activity has been demonstrated in anuran testicular tissue[1,4,6,7].
Studies on seasonal variation of plasma steroid concentrations have shown that testosterone levels were maximal from December to March in *Rana esculenta*[8]. In *Bufo mauritanicus* both testosterone and 5α-dihydrotestosterone (5α-DHT) were high during breeding[9]. Incubation studies have indicated that testes of *Rana temporaria*[1,7], *Discoglossus pictus*[4] and *Bufo marinus, Rana catesbeiana* and *R. esculenta*[6] are capable of forming 5α-DHT in considerable amounts. The 5α-DHT/testosterone ratio seems to rise during the breeding season, 5α-DHT being the major testicular androgen from exogenous precursors[7]. However, in urodele *Amphibia* the major in vitro product of the testis has been found to be testosterone[2,3]. We wanted to study how the testicular androgen formation varies in the newt, *Triturus vulgaris* during the reproductive cycle.
Materials and methods. In southern Finland breeding of newts is in May. Their phenotype simultaneously changes to fit aquatic life and males turn colored. Breeding is completed during July. Newts used in the present study were caught at the end of May or at the beginning of June.
The animals were kept in groups of 50 individuals of both sexes in a half-filled aquarium with a bottom area of 15 dm^2, at +15 °C and exposed to a normal photoperiodic cycle. The dates of the experiments with number of animals and gross gonadosomatic indexes are given in table 1. The first males were sacrificed when the females were laying eggs (groups A and B). After breeding the water was reduced and newts were allowed to creep onto sand. The subsequent samplings (groups C–E) were made after the breeding when the phenotype of the newts started to turn to the terrestrial form. The last males (group F) were used after feeding for a month on natural living food. Testes were homogenized in frog-Ringer medium at pH 7.5 buffered by Trizma® (Sigma) and each of the homogenates (10% w/v) derived from 23–25 individuals were incubated as 5-ml portions at +15 °C for 30 min or 1 h, under oxygen with 14C-DHA (dehydroepiandrosterone, 0.12 µCi, 0.46×10^{-3} mmol/l) or 14C-androstenedione (4-androstene-3,17-dione, 0.12 µCi, 0.40×10^{-3} mmol/l) as the substrate. As indicated in the figure, NADP and/or NADPH (1.35 mmol/l) was used. An acetone denaturated homogenate was used as a control. The extraction and separation of steroids to give the free neutral and phenolic fractions and further studies on the neutral steroids by ascending TLC were carried out as described earlier[7,10]. Chromatographic separation in the first TLC was done by chloroform-diethylether, 3:1 (system A). All solvent systems used are described in the footnote to table 2. The radiochemical purity and identification of fractions obtained in the first TLC was confirmed by derivative formation, by repeated TLCs, by chromatography, or by recrystallizations to constant specific activity (c.s.a.) or to constant isotope ratio (c.i.r.)[11].
Results and discussion. Recovery of the ether extractable (free) radioactivity was 85.7 ± 4.4% for DHA and 86.2 ± 3.6% for androstenedione. Radioactivity recovered in the phenolic fraction amounted to 2.2 ± 0.2% after 30 min and 6.9 ± 1.5% after 1 h for both substrates. Recovery in the water phase was 5.7 ± 1.7%, which was significantly different from the control (2.4 ± 0.6%). Although the radioactivity in the phenolic steroid fraction increased with increasing incubation time, suggesting the possibility of estrogen formation, this fraction was not studied in more detail due to the low level of radioactivity present. The same applies to the water phase; the absolute amount of radioactivity remained too low for possible conjugates to be analyzed. Five peaks (fractions) numbered in the order of decreasing polarity could be recorded by scanning after the first TLC of each analysis from the free neutral steroid fraction (fig. 1).
The radioactive steroids found in TLC fractions were characterized as follows:
Fraction 1 was isopolar with 4-androstene-19-ol-3,17-dione (TLC systems A, D, I/see table 2, footnote). After reduction with $NaBH_4$ it behaved like authentic 4-androstene-3β,17β,19-triol (I,E).
Fraction 2 separated in further TLCs from authentic 4-androstene-11β-ol-3,17-dione and 4-androstene-17β-ol-3,11-dione. It was isopolar with 5-androstene-3β,17β-diol but in recrystallization the radioactivity separated from the carrier.