Biosynthesis of Pyrrolidine Alkaloid-Derived Pheromones in the Arctiid Moth, Creatonotos transiens: Stereochemical Conversion of Heliotrine

M. Wink*
Universität München, Pharmazeutische Biologie, Karlstraße 29, D-8000 München 2, Bundesrepublik Deutschland

D. Schneider
Max-Planck-Institut für Verhaltensphysiologie, D-8131 Seewiesen, Bundesrepublik Deutschland

L. Witte
Technische Universität Braunschweig, Institut für Organische Chemie, Hagenring 30, D-3300 Braunschweig, Bundesrepublik Deutschland

Z. Naturforsch. 43c, 737–741 (1988); received May 4/June 13, 1988

Creatonotos, Pyrrolizidine Alkaloids, Heliotrine, Male Pheromone, Hydroxydanaidal

In larvae and later developmental stages of Creatonotos transiens, which had been reared on the pyrrolizidine alkaloid 75-heliotrine, a new major metabolite was detected by capillary GLC. The structure of this metabolite was determined by GLC-MS (EI, CI-MS) and 13C NMR to be 7R-heliotrine and 7R-heliotrine-N-oxide. 7R-Heliotrine is likely to be the direct precursor for the pheromone R(-)-hydroxydanaidal.

Introduction

Larvae of the arctiid moths, Creatonotos transiens and C. gangis are polyphagous. When feeding on plants with pyrrolizidine alkaloids (PA), the insects accumulate and store PAs [1], and thus appear to gain chemical defence. In male insects, PAs induce the development of abdominal scent organs (coremata) [2]. Furthermore, the heterocyclic moiety of PAs is transformed into a dihydro-5H-pyrrolizine pheromone, hydroxydanaidal (I), which is secreted and dissipated by the coremata [3].

Hydroxydanaidal (I) possesses an asymmetric carbon atom (C-7), which is also present in its alkaloidal precursor. When PAs of the retronecine (7R) (II)-type are exploited for pheromone biosynthesis, the configuration at this center does not need to change. But 7S-heliotrine (III), which has the “wrong” configuration at C-7, can also serve as a precursor for

Abbreviations: GLC, gas-liquid chromatography; MS, mass spectrometry; EI, electron impact; CI, chemical ionization; TMS, trimethylsilyl; MSTFA, N-methyl-N-(trimethylsilyl)trifluoroacetamide; PA, pyrrolizidine alkaloids.

* New address: Institut für Pharmazie, Universität Mainz, Staudinger Weg 5, D-6500 Mainz.

Reprint requests to M. Wink and D. Schneider.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen 0341–0382/88/0900–0737 $ 01.30/0

Scheme. I = 7R-Hydroxydanaidal, II = retronecine, III = 7S-heliotrine, IV = 7R-heliotrine, V = callimorphine, VI = PA-N-oxide.
$R(-)$-hydroxydanaidal (I) [3, 4]. It was therefore assumed that 7S-heliotrine or a derivative undergoes a net inversion of the C-7 stereochemistry. We now determined the stage of pheromone biosynthesis at which the stereochemical conversion at C-7 presumably takes place.

**Materials and Methods**

Late instar larvae (L7) of *C. transiens* received purified 7S-heliotrine (commercially available from Corkwood Enterpr., Blakehurst, NSW Australia) as the sole PA-source (5 mg PA/larva) in an artificial diet [5]. Larvae, prepupae, pupae, and imagines were homogenized in 0.5 M HCl. Zinc powder was added to reduce the PA-N-oxides, the dominant alkaloidal form of PAs in *C. transiens* [6] to free PAs. After 3 h at room temperature the homogenate was made alkaline with 2 M NaOH and applied onto Chemelute columns (ICT, Frankfurt). Alkaloids were eluted with methylene chloride and analyzed by capillary gas-liquid chromatography (Fig. 1). GLC-conditions: GLC: Perkin Elmer 8500 equipped with flame ionization (FID) and nitrogen (PND) detectors. Column: DB-1 (J&W; ICT, Frankfurt), 30 m x 0.3 mm; carrier gas: Helium (91 kPa); split injection (1:20); injector temperature: 250 °C; detector temperature: 320 °C; oven temperature: 170–320 °C, 30 °C/min.

GLC-MS measurements were performed under similar conditions, employing a Finnigan MAT 4515 instrument [7]. NMR measurements were made on a Bruker AM 360.

**Results and Discussion**

Whereas the original 7S-heliotrine (III) resulted in a single GLC-peak, we detected two major peaks in alkaloid extracts from larvae and subsequent developmental stages (Fig. 1A, B). The abundance of the new compound increased in later stages and reached 43–66% in females and nearly 80% in males as compared to the amount of total pyrrolizidine alkaloids (= 100%) recovered (Table I).

**Table I. Metabolization of 7S-heliotrine (III) in *Creatonotos transiens.***

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>Percentage* of 7R-heliotrine in total PA (= 100%)**</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larvae (L7)</td>
<td>56</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Prepupae (1. d)</td>
<td>48</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Prepupae (2. d)</td>
<td>54</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Pupae (1d)</td>
<td>77</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Pupae (5d)</td>
<td>72</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>Pupae (8d)</td>
<td>77</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Imagines (1–3d)</td>
<td>79</td>
<td>66</td>
<td></td>
</tr>
</tbody>
</table>

* Mean of 2 animals (4 in case of imagines).

** Larvae obtained 5000 µg 7S-heliotrine each with their diet. About 670 µg PA were recovered from the animals (mean value of 32 animals), 742 µg from their faeces (9 animals). Whether the missing amounts were completely metabolized will be determined in further studies.
GLC-MS analysis (EI and CI-MS) of both GLC-peaks gave nearly identical mass spectra (Table II), indicating that the new compound was a stereo-isomer of 7S-heliotrine (III). Some minor metabolites could also be detected, one of which was found to be callimorphine (V), which had previously been identified as a PA-derived compound in the arctiids Tyria jacobaea, Arctica caja and Callimorpha dominula [8].

In order to see whether the new metabolite was the 7R(-)-isomer of heliotrine (IV), (i.e. the pheromone precursor with the correct stereochemistry at C-7) heliotrine and its isomer were hydrolyzed in 5% NaOH (1 h at 80 °C), extracted with ethylacetate, derivatized with MSTFA and finally analyzed by GLC and GLC-MS. Two distinct compounds could be separated by capillary GLC (Table II), i.e. the TMS-derivatives of retronecine (II) and of heliotridine, with identical mass spectra (Table II). TMS-derivatives were also produced from the necine acids, i.e. heliotrinic acid, of the molecules. They resulted in a single GLC-peak (Table II) which means that the stereochemical difference should be restricted to the pyrrolizidine nucleus.

Carbon-13 NMR analysis allowed us to decide whether the hydroxyl-group at C-7 had the R- or S-configuration:

13C NMR analysis of the heliotrine metabolite, isolated from ca. 500 animals fed with 7S-heliotrine (III) and of the original 7S-heliotrine (III), indeed shows that a net inversion of the C-7 stereochemistry took place (Fig. 2A, B): The signal for C-7 is at about 75 ppm for the S-configuration (Fig. 2A) and at 70 ppm for the R-type as determined in [9]. In a control experiment we oxidized 400 mg 7S-heliotrine (III) with pyridinium dichromat (PDC) (1 g PDC in 2 ml methylene chloride; 20 h at room temperature) to the corresponding ketone (which was not found as a metabolite in C. transiens). The product was purified on a chemelute column (s. above) and reduced with sodium borohydride in methanol. 13C NMR data (Fig. 2C) of this product clearly show that a racemization took place at C-7 resulting in a mixture of 7R- and 7S-heliotrine (III/IV), i.e. corresponding signals are present at 75 and 70 ppm. The origin and meaning of the signal at 74.5 ppm (in Fig. 2B, C) is not clear yet.

The NMR, GLC, and GLC-MS data clearly show that 7S-heliotrine is (in an as yet unknown process) metabolized to 7R-heliotrine by C. transiens, which then has the correct configuration for the biosynthesis of R(-)-hydroxydanaidal. The minor metabolite, callimorphine (V) also has the R-configuration, and is thus probably also derived from 7R-heliotrine. In Creatonotos, 7R- and 7S-heliotrine are present by up to 90% as their N-oxides (VI) [6]. To our knowledge, both 7R-heliotrine and 7R-heliotrine-N-oxide have not been described before and are new natural alkaloids.

In order to explain the capacity of Creatonotos to use a variety of PAs with either the common 7R- and/or even the less common 7S-configuration as precursors for the synthesis of 7R-hydroxydanaidal (I) [3], a possible conversion mechanism was postulated [4]. From our data presented in this communication it can be concluded that the conversion takes place at the level of the ester alkaloid and not at the level of hydroxydanaidal.

But the stereochemical conversion at C-7 of the heliotrine nucleus could be an even more general

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention index (RI*)</th>
<th>Mass spectral data of 5 fragments (relative abundance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7R-Heliotrine (IV)</td>
<td>2090</td>
<td>EI: M⁺ 313 (0.3); 156 (10); 138 (100) 93 (94)</td>
</tr>
<tr>
<td>7S-Heliotrine (III)</td>
<td>2105</td>
<td>EI: M⁺ 313 (0.3); 156 (12); 138 (100); 93 (85)</td>
</tr>
<tr>
<td>Callimorphine (V)</td>
<td>1963</td>
<td>EI: M⁺ 297 (1.2); 154 (13); 138 (59); 93 (100)</td>
</tr>
<tr>
<td>Retronecine-TMS (7R)</td>
<td>1603</td>
<td>EI: M⁺ 299 (9); 183 (85); 103 (35); 93 (100); 73 (82)</td>
</tr>
<tr>
<td>Heliotridine-TMS (7S)</td>
<td>1550</td>
<td>EI: M⁺ 299 (5); 183 (75); 103 (33); 93 (100); 73 (83)</td>
</tr>
<tr>
<td>Heliotrinic acid-TMS**</td>
<td></td>
<td>EI: 262 (14); 203 (26); 147 (41); 73 (99); 59 (100)</td>
</tr>
</tbody>
</table>

* Kovats retention index.
** 3-Methoxy-2-hydroxy-2-(1-methylethyl)-butanoic acid.
phenomenon which reaches beyond the *Creatonotos* case: Although hydroxydanaidal was isolated from a number of male danaine and arctiid Lepidoptera, only recently was it possible to determine the enantiomeric properties of this substance [10–12]. In all cases studied, it was found to be \( 7R(-) \)-hydroxydanaidal (I), and we suspect that the now detected PA-conversion also applies to these other species as well.

**Acknowledgements**

Financial support by the Deutsche Forschungsgemeinschaft, the Fonds der Chemischen Industrie (to M. W.) and the Max-Planck-Gesellschaft (to D. S.) are gratefully acknowledged. We thank Mrs. H. Söchting-Mayr, E. Roth, U. Schade, and M. Weyerer for technical assistance and R. Stadler (BSc) for recording the NMR spectra. Prof. Dr. E. Röder (Bonn) gave valuable information on NMR data of PAs.

Fig. 2. \(^{13}\)C NMR analysis of 7S-heliotrine and its metabolites. A. 7S-heliotrine; B. 7R-heliotrine*, the metabolite from *S. transiens*; C. racemization product of 7S-heliotrine in CDCl\(_3\).

\* ca. 80% 7R-heliotrine according to GLC-analysis.