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

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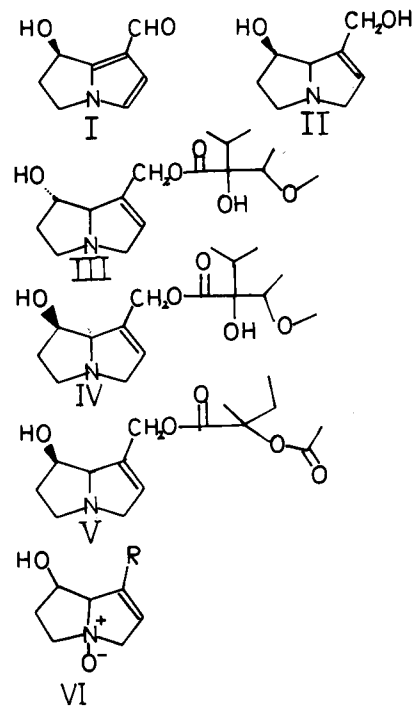
In larvae and later developmental stages of *Creatonotos transiens*, which had been reared on the pyrrolizidine alkaloid 7*S*-heliotrine, a new major metabolite was detected by capillary GLC. The structure of this metabolite was determined by GLC-MS (EI, CI-MS) and ^{13}C NMR to be 7*R*-heliotrine and 7*R*-heliotrine-N-oxide. 7*R*-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 (7*R*) (**II**)-type are exploited for pheromone biosynthesis, the configuration at this center does not need to change. But 7*S*-heliotrine (**III**), which has the "wrong" configuration at C-7, can also serve as a precursor for



Scheme. *I* = 7*R*-Hydroxydanaidal, **II** = retronecine, **III** = 7*S*-heliotrine, **IV** = 7*R*-heliotrine, **V** = callimorphine, **VI** = PA-N-oxide.

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.

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R(−)-hydroxydanaidal (**I**) [3, 4]. It was therefore assumed that 7*S*-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 7*S*-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 × 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.

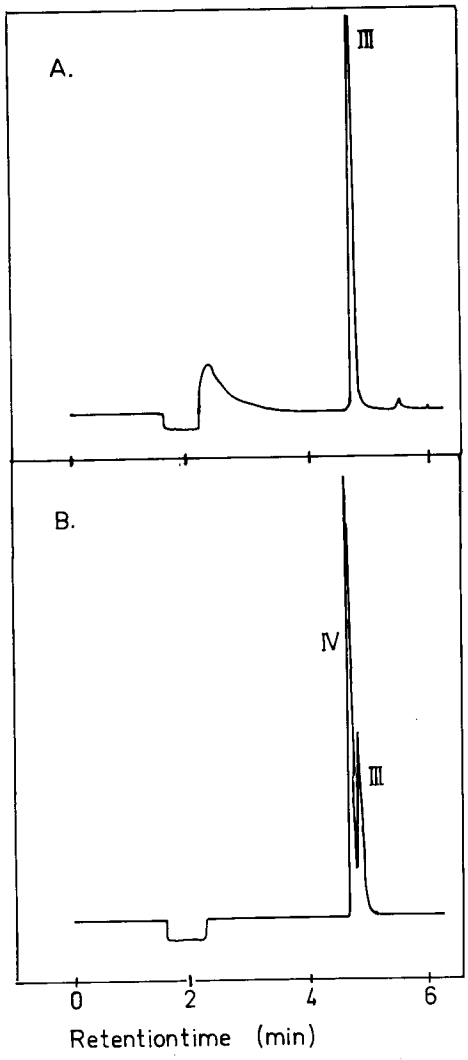


Fig. 1. Separation of 7*S*-heliotrine and its metabolites by capillary GLC. A. 7*S*-heliotrine; B. alkaloid extract from *C. transiens*, after application of 7*S*-heliotrine.

Results and Discussion

Whereas the original 7*S*-heliotrine (**III**) resulted in a single GLC-peak, we detected two major peaks in alkaloid extracts from larvae and subsequent developmental stages (Fig. 1 A, 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 7*S*-heliotrine (**III**) in *Creatonotos transiens*.

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

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

** Larvae obtained 5000 µg 7*S*-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 stereoisomer of 7*S*-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, Arctia caja* and *Callimorpha dominula* [8].

In order to see whether the new metabolite was the 7*R*(−)-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:

^{13}C NMR analysis of the heliotrine metabolite, isolated from *ca.* 500 animals fed with 7*S*-heliotrine (**III**) and of the original 7*S*-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 7*S*-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. ^{13}C NMR data (Fig. 2C) of this product clearly show that a racemization took place at C-7 resulting in a mixture of 7*R*- and 7*S*-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 7*S*-heliotrine is (in an as yet unknown process) metabolized to 7*R*-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 7*R*-heliotrine. In *Creatonotos*, 7*R*- and 7*S*-heliotrine are present by up to 90% as their N-oxides (**VI**) [6]. To our knowledge, both 7*R*-heliotrine and 7*R*-heliotrine-N-oxide have not been described before and are new natural alkaloids.

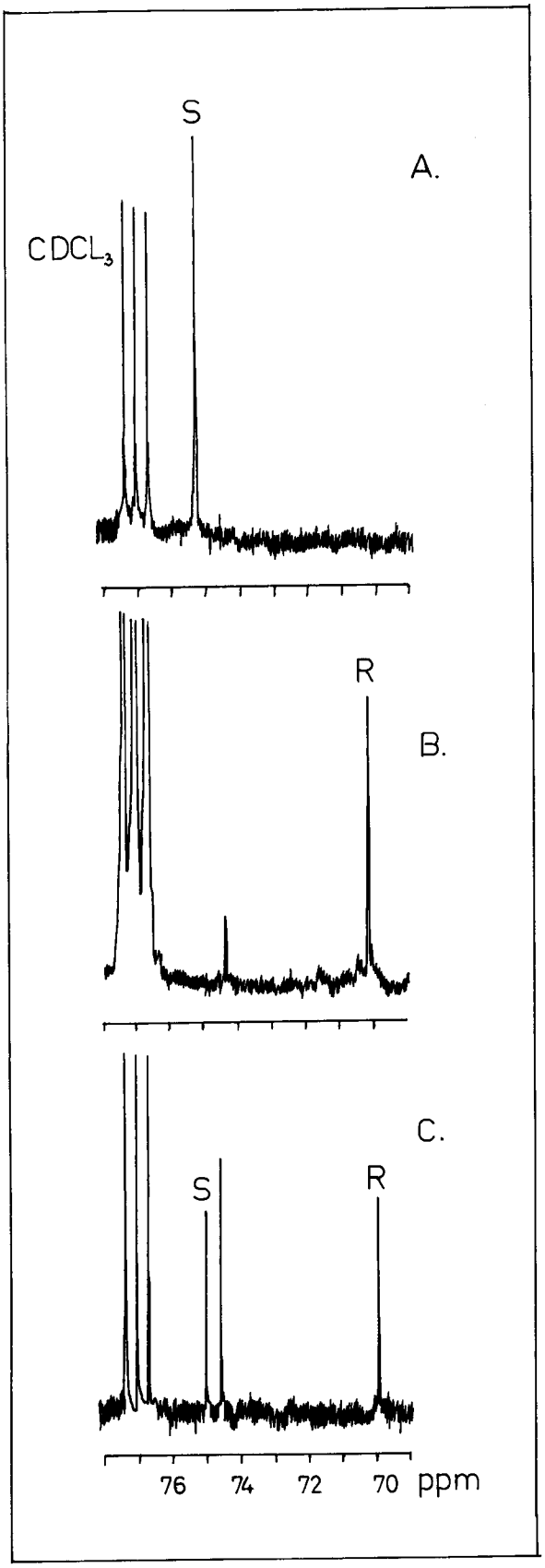
In order to explain the capacity of *Creatonotos* to use a variety of PAs with either the common 7*R*- and/or even the less common 7*S*-configuration as precursors for the synthesis of 7*R*-hydroxydanaidal (**I**) [3], a possible conversion mechanism was postulated [4]. From our data presented in this 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 II. GLC-MS analysis of 7*S*- and 7*R*-heliotrine and their TMS-derivatives.

Compound	Retention index (RI*)	Mass spectral data of 5 fragments (relative abundance)
7*R*-Heliotrine (**IV**)	2090	EI: M^+ 313 (0.3); 156 (10); 138 (100) 93 (94)
		CI: M^+ 314 (37); 148 (11); 140 (76); 138 (100)
7*S*-Heliotrine (**III**)	2105	EI: M^+ 313 (0.3); 156 (12); 138 (100); 93 (85)
		CI: M^+ 314 (100); 148 (27); 140 (28); 138 (46)
Callimorphine (**V**)	1963	EI: M^+ 297 (1.2); 154 (13); 138 (59); 93 (100)
Retronecine-TMS (7*R*)	1603	EI: M^+ 299 (9); 183 (85); 103 (35); 93 (100); 73 (82)
Heliotridine-TMS (7*S*)		EI: M^+ 299 (5); 183 (75); 103 (33); 93 (100); 73 (83)
Heliotrinic acid-TMS**		EI: 262 (14); 203 (26); 147 (41); 73 (99); 59 (100)

* 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 7*R*(−)-hydroxydanaidal (**I**), and we suspect that the now detected PA-conversion also applies to these other species as well.

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Fig. 2. ^{13}C NMR analysis of 7*S*-heliotrine and its metabolites. A. 7*S*-heliotrine; B. 7*R*-heliotrine*, the metabolite from *S. transiens;* C. racemization product of 7*S*-heliotrine in $CDCl_3$.

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

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