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Chemistry

Insect pheromone biosynthesis: Stereochemical pathway of hydroxydanaidal production from alkaloidal precursors in *Creatonotos transiens* (Lepidoptera, Arctiidae)*

(pyrrolizidine alkaloids/biosynthetic mechanisms/stereochemistry/deuteriated alkaloids/sexual selection)

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ABSTRACT **The mechanism by which the moth *Creatonotos transiens* produces its male pheromone, (7*R*)-hydroxydanaidal, from heliotrine, an alkaloidal precursor of opposite (7*S*) stereochemistry, was investigated. Specifically deuteriated samples of heliotrine and epiheliotrine were prepared and fed to *C. transiens* larvae, and the steps in the biosynthetic process were monitored by gas chromatography/mass spectrometry. These analyses indicate that heliotrine is initially epimerized to (7*S*)-epiheliotrine by oxidation to the corresponding ketone followed by stereospecific reduction. The order of the subsequent steps is (*i*) aromatization of the dihydropyrrole ring, (*ii*) ester hydrolysis, and (*iii*) oxidation of the resulting primary alcohol to the final aldehyde. The ecological implications of this insect's ability (and the inability of another moth, *Utetheisa ornatrix*) to use representatives of two stereochemical families of alkaloids as pheromone precursors are discussed.**

That some insect species require plant alkaloids as biosynthetic precursors for their male courtship pheromones is well known (2, 3). Although these transformations appear unremarkable from an organic chemical viewpoint, few details of the underlying biosynthetic pathways have been elucidated (4–7). The conversion of monocrotaline (**1**, see Fig. 1) into hydroxydanaidal (**2**) in the Asian arctiid moth *Creatonotos transiens*, for example, requires only aromatization of a dihydropyrrole ring, ester hydrolysis, and oxidation of a primary benzylic alcohol to the corresponding aldehyde (8, 9). The observation that this process occurs with retention of the *R* configuration at the one asymmetric center (C-7) common to the alkaloidal precursor and the pheromonal product, although not at all surprising, led us to wonder how *Creatonotos* would process a related pyrrolizidine alkaloid with the opposite configuration (*S*) at C-7. Would the insect fail to recognize and metabolize a pyrrolizidine with the "incorrect" stereochemistry, would it utilize the new precursor to make the mirror image form of its pheromone (7*S*-**2**), or would it invert the configuration at C-7 to produce the original 7*R*-**2**? In an experiment in which *Creatonotos* larvae were fed heliotrine (**3**, a 7*S* alkaloid) as the only pyrrolizidine source, 7*R*-**2** was in fact produced, demonstrating that this insect can take up and metabolize alkaloids with *either configuration* at C-7 to make the *same* final product (8).

We now ask how this stereochemical result is achieved. Five mechanistic possibilities come quickly to mind, as summarized in Scheme I.

(*i*) There might be an oxidation of both the 7*R* and 7*S* precursors to a common C-7 ketone, which could then undergo stereospecific reduction to a 7*R* product.

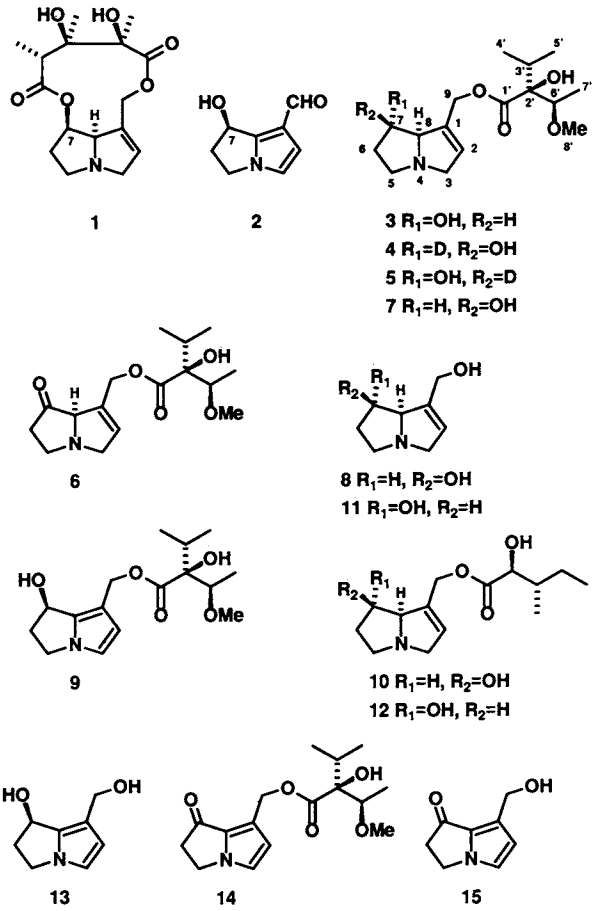

FIG. 1. Structures of compounds mentioned in the text. D, deuterium.

(*ii*) There might be selective oxidation of only the 7*S* precursor, followed by reduction to a 7*R* product.

(*iii*) There might be an S_N1 solvolysis of both precursors, followed by collapse of the cationic intermediate to a 7*R* product.

(*iv*) There might be selective S_N1 solvolysis of only the 7*S* precursor, followed by formation of the 7*R* product.

(*v*) There might be selective inversion of the 7*S* precursor by an S_N2 substitution mechanism to give a 7*R* product.

To study these mechanistic possibilities, we prepared two deuterium-labeled pheromone precursors: (7*R*)-7-deuterio-

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Abbreviation: EI, electron ionization.

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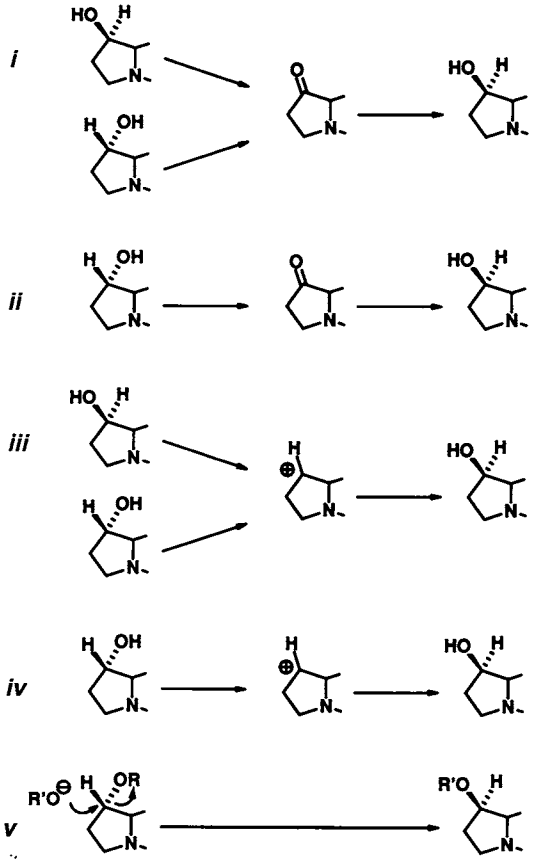


Scheme I

epiheliotrine (4) and (7*S*)-7-deuterioheliotrine (5). Table 1 summarizes the results of biosynthetic experiments to be anticipated from feeding of *Creatonotos* larvae with these labeled alkaloids, according to each of these five mechanisms. While no discrimination among pathways (*iii*, *iv*, and *v*) involving any of the nucleophilic substitution (S_N) mechanisms could be revealed, this set of pathways should be clearly distinguished from either of the oxidation/reduction mechanisms, *i* and *ii*.

MATERIALS AND METHODS

Instrumentation. ^1H and ^{13}C NMR spectra were obtained with Bruker WM 400, AC250P, and Varian XL-200 instruments, and tetramethylsilane or residual solvent protons were used as standards. Mass spectra (70 eV) were obtained with a VG 70/250 S mass spectrometer coupled to a Hewlett–Packard HP 5890 A gas chromatograph. Gas chromatographic analyses were carried out on a Carlo-Erba Fractovap 2101 gas chromatograph equipped with a flame ionization detector, using on-column injection and a 30-m CP-Sil-8-CB (internal diameter 0.32 mm, film thickness 0.25 μm, Chrompack, Middelburg, The Netherlands) fused silica column with hydrogen as carrier gas. For enantiomer separations, a 50-m Chirasil-L-Val (internal diameter 0.25 mm, Chrompack) fused silica column was used with helium as carrier gas. Column chromatography was performed on silica gel (230–400 mesh, Merck).

Table 1. Labeling patterns expected in hydroxydanaidal (2) after administration of labeled precursors

Mechanism	Deuterium retention, %	
	In 2 from 4	In 2 from 5
i	0	0
ii	100	0
iii, *iv*, or *v*	100	100

Feeding Experiments. *Creatonotos transiens* Walker larvae from an established laboratory culture originating from females collected in Indonesia and maintained on an artificial diet (10) were used. Alkaloids were fed to final instar larvae as their first meal after molting; 100 μl of a 1 μg/μl alkaloid solution in dichloromethane or methanol was applied to a 5-cm^2 piece of dandelion (*Taraxacum officinale*) leaf and offered after the solvent had evaporated. After the larvae had consumed the entire sample, they were fed with artificial diet until pupation.

Extraction of Whole Insects. Bodies of 12-h-old *Creatonotos* males were macerated in methanol/water (9:1, vol/vol), the solvent was removed, and the residue was taken up in 1 M sulfuric acid. Zinc was added to reduce any *N*-oxides, and the solution was extracted with chloroform, made alkaline, and extracted with chloroform as described by L'Empereur *et al.* (11). The chloroform solution was analyzed by gas chromatography/mass spectrometry (GC–MS). Pharate males (male pupae <12 h to eclosion) were macerated in 2 ml of dry dichloromethane. The mixture was filtered and the filtrate was immediately analyzed by GC–MS.

Extraction of Coremata. Hydroxydanaidal is produced in the *Creatonotos* male by two abdominal scent brushes called coremata. Coremata were removed from males 12 h after eclosion. They were macerated in 50 μl of dichloromethane and left for 30 min. After filtration through cotton, the extracts were analyzed by GC–MS or silylated at room temperature by the addition of 50 μl of *N,O*-bis-(trimethylsilyl)trifluoroacetamide (BSTFA). After 2 h, the mixture was concentrated under a stream of nitrogen, and the residue was taken up in dichloromethane for analysis.

Biosynthetic Experiments with *Utetheisa ornatrix*. For comparative purposes, we examined hydroxydanaidal biosynthesis in another arctiid moth, *Utetheisa ornatrix*, originally from Florida, which we maintain in culture in our Cornell laboratories. In this moth also, hydroxydanaidal is produced by the male, from a pair of coremata, as a courtship pheromone (4). The moth presumably derives hydroxydanaidal from pyrrolizidine alkaloid, since it fails to produce the pheromone when raised on an alkaloid-free diet (4). Three groups of *Utetheisa* larvae (n = 20–30 per group), raised on an alkaloid-free diet [PB diet (4)], were injected with monocrotaline, monocrotaline hydrochloride, or heliotrine in insect saline solution (12) (≈350–500 μg of alkaloid per larva). Larvae were held for pupation and adult emergence. Three days after emergence, the coremata of males were removed and placed in 0.5 ml of dichloromethane. These samples were analyzed for hydroxydanaidal by GC.

Synthesis of (*R*)- and (*S*)-Hydroxydanaidal. These enantiomers were prepared as previously described (9).

Synthesis of (8*R*)-5,6,7,8-Tetrahydro-3*H*-pyrrolizin-7-on-1-yl)methyl (2*S*,3*R*)-2-Hydroxy-2-(1-methoxyethyl)-3-methylbutanoate (6). A solution of 216 μl of dry dimethyl sulfoxide (2.8 mmol) in 0.75 ml of dichloromethane was added under a nitrogen atmosphere to 128 μl of oxalyl chloride (1.4 mmol). The resulting mixture was stirred for 10 min and cooled to −10°C. A solution of 200 mg of heliotrine (3) (0.62 mmol) in 3 ml of dimethyl sulfoxide was added, and the mixture was stirred for 15 min and cooled to −55°C. Subsequently, 893 μl of triethylamine (6.4 mmol) was added and the resulting mixture was allowed to warm to room temperature over 45 min. Water (6.4 ml) was added, followed by extraction with chloroform. The combined organic phases were washed with brine, dried over sodium carbonate, and concentrated under reduced pressure at 60°C. The crude product was immediately reduced as described below. ^1H NMR (200 MHz, $CDCl_3$): δ = 0.9 (m, 6H, H-4′ and H-5′), 1.15 (d, 3H, H-7′),

1.95 (sept, 1H, H-3'), 2.5 (m, 1H), 2.9–3.3 (m, 4H), 3.25 (s, 3H, H-8'), 3.6 (m, 1H), 3.65 (q, 1H, H-6'), 4.1 (bs, 1H, H-8), 4.85 (bs, 2H, H-9), 5.88 (bs, 1H, H-2). (For numbering system, see refs. 13 and 14.)

Synthesis of Deuteriated Heliotrine Diastereomers 4 and 5. To a stirred solution of crude **6** in 20 ml of methanol, 12 mg of sodium borodeuteride (0.3 mmol) was added at 0°C, followed by another 12 mg after 1 h. After 3 h, water was added and the mixture was stirred for 10 min. The water phase was extracted three times with chloroform, and the organic extract was dried with sodium carbonate and concentrated under reduced pressure. The pure deuterated compounds, **4** and **5**, could be obtained in 30% overall yield each from heliotrine, by column chromatography (silica gel; ethyl acetate/methanol/ammonia 50:50:1, vol/vol) followed by HPLC separation (25 cm Partisil, methanol with 0.5% triethylamine).

(7*R*)-7-Deuterioepiheliotrine (**4**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.87 (d, 3H, H-4' or H-5', $J_{3',4'(5')}$ = 6.9 Hz), 0.93 (d, 3H, H-4' or H-5', $J_{3',4'(5')}$ = 6.8 Hz), 1.15 (d, 3H, H-7', $J_{7'6'}$ = 6.1 Hz), 1.91–2.05 (m, 2H, H-6), 2.15 (sept, 1H, H-3'), 2.76 (ddd, 1H, H-5, $J_{5,5}$ = 12.3 Hz, $J_{5,6}$ = 8.7 Hz, $J_{5,6}$ = 5.9 Hz), 3.28 (m, 1H, H-5), 3.34 (s, 3H, H-8'), 3.46 (dd, 1H, H-3, $J_{3,3}$ = 15.8 Hz, $J_{3,2}$ = 6.0 Hz), 3.61 (q, 1H, H-6'), 3.96 (m, 1H, H-3, $J_{3,2}$ = 2.4 Hz), 4.14 (m, 1H, H-8, $J_{8,2}$ = 2.0 Hz), 4.70 (bd, 1H, H-9, $J_{9,9}$ = 12.4 Hz), 4.79 (d, 1H, H-9), 5.94 (bs, 1H, H-2).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.29 (C-7'), 16.38 (C-4'), 16.94 (C-5'), 31.92 (C-3'), 35.80 (C-6), 54.01 (C-5), 56.64 (C-8'), 62.57 (C-3),‖ 62.72 (C-9),‖ 70.22 (t, C-7), 78.07 (C-6'), 79.10 (C-8), 82.48 (C-2'), 131.67 (C-2), 132.43 (C-1), 175.00 (C-1').

Electron ionization (EI)-MS (70 eV): m/z = 43 (31), 53 (11), 59 (100), 67 (15), 80 (18), 93 (82), 94 (33), 95 (21), 137 (25), 138 (17), 139 (75), 140 (29), 157 (8).

Deuterium content: 97% (determined by NMR and MS).

(7*S*)-7-Deuterioheliotrine (**5**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.87 (d, 3H, H-4' or H-5', $J_{3',4'(5')}$ = 7.0 Hz), 0.95 (d, 3H, H-4' or H-5', $J_{3',4'(5')}$ = 6.7 Hz), 1.14 (d, 3H, H-7', $J_{7',6'}$ = 6.3 Hz), 1.92 (m, 1H, H-6, $J_{6,6}$ = 12.0 Hz, $J_{6,5}$ = 9.9 Hz, $J_{6,5}$ = 6.7 Hz), 2.08 (m, 1H, H-6, $J_{6,5}$ = 5.6 Hz, $J_{6,5}$ = 3.6 Hz), 2.19 (sept, 1H, 3'-H), 2.60 (dt, 1H, H-5, $J_{5,5}$ = 9.9 Hz), 3.35 (m, 1H, H-5), 3.36 (s, 3H, H-8'), 3.38 (m, 1H, H-3), 3.58 (q, 1H, H-6'), 3.86 (bd, 1H, H-3, $J_{3,3}$ = 16.0 Hz), 3.88 (bs, 1H, H-8), 4.64 (bd, 1H, H-9, $J_{9,9}$ = 12.4 Hz, $J_{9,2}$ = 1.0 Hz), 5.10 (d, 1H, H-9), 5.73 (bs, 1H, H-2).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.31 (C-7'), 16.41 (C-4'), 16.98 (C-5'), 31.79 (C-3'), 33.99 (C-6), 54.06 (C-5), 56.97 (C-8'), 61.81 (C-3),‖ 62.56 (C-9),‖ 74.80 (t, C-7), 78.47 (C-6'),** 79.81 (C-8),** 82.53 (C-2'), 127.11 (C-2), 136.10 (C-1), 174.90 (C-1').

EI-MS (70 eV): m/z = 43 (32), 53 (12), 59 (82), 67 (14), 80 (22), 93 (98), 94 (28), 95 (24), 137 (27), 138 (45), 139 (100), 140 (45), 157 (14).

Deuterium content: 90% (determined by NMR and MS).

Synthesis of (6,7-Dihydro-5*H*-pyrrolizin-7-on-1-yl)methyl (2*S*,3*R*)-2-Hydroxy-2-(1-methoxyethyl)-3-methylbutanoate (14). The procedure described by Culvenor *et al.* (15) was slightly modified. To a solution of 45 mg of heliotrine (0.14 mmol) in 50 ml of acetone, 80 mg of potassium permanganate (0.51 mmol) was added at room temperature. After stirring for 16 h, the dark solution was filtered through silica, 25 ml of methanol was added, and the resulting solution was warmed until it became colorless. The solvent was removed and the resulting oil was dried under reduced pressure, giving 22 mg (47% yield) of pure alkaloid.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.86 (d, 3H, H-4' or H-5', $J_{3',4'(5')}$ = 6.8 Hz), 0.91 (d, 3H, H-4' or H-5', $J_{3',4'(5')}$ = 7.0 Hz), 1.12 (d, 3H, H-7', $J_{7'6'}$ = 6.2 Hz), 1.90 (sept, 1H, H-3'), 3.06 (t, 2H, H-6), 3.22 (s, 3H, H-8'), 3.68 (q, 1H, H-6'), 4.27 (t, 2H, H-5), 5.31 (d, 1H, H-9, $J_{9,9}$ = 12.6 Hz), 5.36 (d, 1H, H-9), 6.52 (d, 1H, H-2, $J_{2,3}$ = 2.2 Hz), 6.94 (d, 1H, H-3).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.58 (C-7'), 17.02 (C-4'), 17.13 (C-5'), 33.01 (C-3'), 39.47 (C-6), 42.14 (C-5), 56.81 (C-8'), 58.71 (C-9), 78.72 (C-6'), 82.93 (C-2'), 117.40 (C-2), 118.79 (C-1), 123.03 (C-3), 129.92 (C-8), 174.60 (C-1'), 189.02 (C-7).

EI-MS (70 eV): m/z = 59 (43), 106 (14), 134 (100), 135 (51), 250 (9).

Synthesis of 1-Hydroxymethyl-6,7-dihydro-5*H*-pyrrolizin-7-one (15). A mixture of 10 mg of **14** in 1 ml of 0.5 M sodium hydroxide was stirred overnight and extracted with chloroform. The organic phases were dried over sodium sulfate and the solvent was removed on a rotary evaporator, leaving a colorless oil which proved to be pure by NMR analysis.

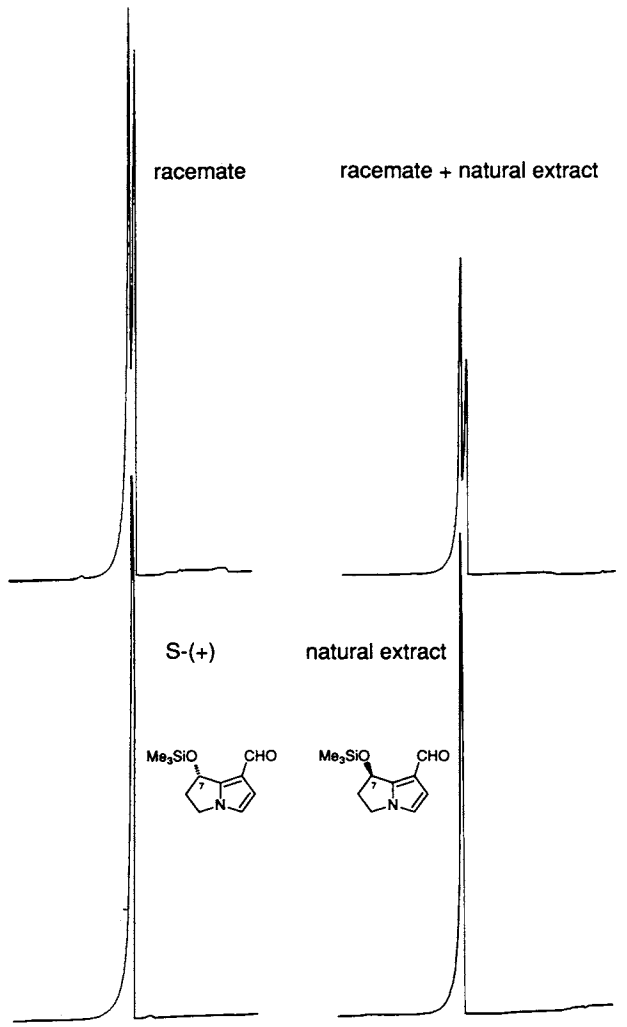


FIG. 2. Gas chromatographic resolution of *O*-trimethylsilyl hydroxydanaidal enantiomers on a 50-m Chirasil-L-Val-coated fused silica capillary column; identification of the second peak as that derived from *S*-(+)-hydroxydanaidal; confirmation by coinjection of the *R*-(−)-hydroxydanaidal produced from heliotrine by *Creatonotos transiens*.

‖Assignments are interchangeable.

Assignment of the signals at C-6' and C-8 was established by a deuterium shift of −0.07 ppm for the signal at 79.81 (C-8) compared to that of the signal from residual undeuteriated **3 in the sample, while the signal at 78.47 (C-6') showed no such shift. The earlier assignments for C-8 and C-6' (refs. 13 and 14) must therefore be reconsidered.

^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.11 (t, 2H, H-6, $J_{6,5}$ = 6.6 Hz), 3.7 (bs, 1H, OH), 4.32 (t, 2H, H-5), 4.75 (bs, 2H, H-9), 6.35 (d, 1H, H-3, $J_{3,2}$ = 1.8 Hz), 6.94 (d, 1H, H-2).

EI-MS (70 eV): m/z = 80 (12), 93 (14), 94 (12), 95 (11), 106 (19), 108 (12), 120 (16), 121 (21), 122 (78), 134 (11), 149 (29), 150 (26), 151 (100, M^+).

Synthesis of [(7*S*)-6,7-Dihydro-5*H*-pyrrolizin-7-ol-1-yl]methyl (2*S*,3*R*)-2-Hydroxy-2-(1-methoxyethyl)-3-methylbutanoate (9). This alkaloid was prepared according to the procedure of Mattocks (16).

^{1}H NMR (250 MHz, CD_3CN): δ = 0.79 (d, 3H, H-4′ or H-5′, $J_{3',4'(5')}$ = 6.8 Hz), 0.85 (d, 3H, H-4′ or H-5′, $J_{3',4'(5')}$ = 7.0 Hz), 1.03 (d, 3H, H-7′, $J_{7',6'}$ = 6.0 Hz), 2.14–2.35 (m, 2H, H-6), 2.68 (sept, 1H, H-3′), 3.18 (s, 3H, H-8′), 3.36 (bs, 1H, OH), 3.56 (q, 1H, H-6′), 3.86 (ddd, 1H, H-5, $J_{5,5}$ = 10.6 Hz, $J_{5,6}$ = 8.6 Hz, $J_{5,6}$ = 3.4 Hz), 4.03 (m, 1H, H-5, $J_{5,6}$ = 7.4 Hz, $J_{5,6}$ = 7.4 Hz), 5.05 (d, 1H, H-9, $J_{9,9}$ = 11.2 Hz), 5.12 (d, 1H, H-9), 5.13 (dd, 1H, H-7, $J_{7,6}$ = 6.4 Hz, $J_{7,6}$ = 2.4 Hz), 6.14 (d, 1H, H-2, $J_{2,3}$ = 2.4 Hz), 6.55 (d, 1H, H-3).

^{13}C NMR (100 MHz, CD_3CN): δ = 12.35 (C-7′), 17.11 (C-4′), 17.52 (C-5′), 33.47 (C-3′), 38.94 (C-6), 45.58 (C-5), 57.21 (C-8′), 61.75 (C-9), 67.11 (C-7), 79.67 (C-6′), 83.43 (C-2′), 110.62 (C-1), 114.14 (C-2),‖ 115.45 (C-3),‖ 139.70 (C-8), 176.23 (C-1′).

EI-MS (70 eV): m/z = 43 (34), 59 (100), 89 (12), 90 (16), 94 (11), 100 (10), 117 (39), 118 (22), 119 (18), 136 (95), 178 (8), 293 (2), 311 (5, M^+).

RESULTS AND DISCUSSION

To prepare the desired labeled biosynthetic precursors, heliotrine (**3**) was initially converted into the corresponding ketone (**6**) by Swern oxidation (17). Because of the liability of **6**, this intermediate was subjected to immediate reduction with sodium borodeuteride to give the target alkaloids, **4** and **5**.

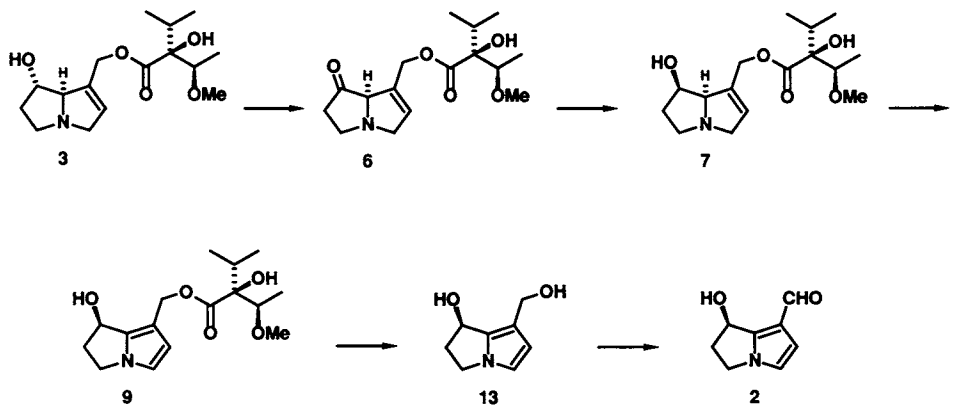
The deuterium-labeled alkaloids, as well as a variety of related compounds, were fed to *Creatonotos* larvae. Coremata were dissected from adult males and extracted with dichloromethane. After silylation, each extract was analyzed on a chiral capillary GC column. In all cases, only (7*R*)-(−)-hydroxydanaidal was observed (see Fig. 2), confirming our earlier report on the remarkable inversion of heliotrine-derived pheromone during this biosynthesis (8). In addition to the experiments on extracts from coremata, GC–MS analyses for alkaloids and alkaloid metabolites, carried out on bodies of adult males and on pupae, provided insight into the molecular details of the pathway from alkaloid to pheromone.

Mass spectrometric analyses of (7*R*)-(−)-hydroxydanaidal (**2**) derived from the isotopically labeled precursors, **4** and **5**, showed that the pheromone which retained the configuration of its precursor (**4**) also retained its deuterium, while the pheromone produced by stereochemical inversion of its precursor (**5**) lost its deuterium label. These results are uniquely compatible with mechanism *ii*. We may now ask at what stage in the alkaloid-to-pheromone transformation this selective redox reaction sequence occurs: is the 7*S* alkaloid directly inverted, is it an intermediate along the pathway, or is it 7*S*-hydroxydanaidal which undergoes this process?

GC–MS analyses of extracts of adults and of pupae have permitted the identification and examination of several intermediates along the biosynthetic pathway, and demonstrate that inversion at C-7 is the first step in the biosynthesis in the case of the 7*S* precursor. Thus, extracts from the bodies of adult males fed with 7-deuterioheliotrine (**5**) were found to contain a mixture of unchanged **5** and its unlabeled C-7 epimer, epiheliotrine (**7**) as the main alkaloids. These observations are in accord with those of Wink *et al.* (18), who fed heliotrine (**3**) itself to *Creatonotos* and found conversion to epiheliotrine (**7**). Bodies of animals fed with 7-deuterioepiheliotrine (**4**) showed only this alkaloid, still labeled. These results support the contention that the C-7 oxidation-reduction process occurs (when necessary) as the initial step in this pheromone biosynthesis.

What step follows this adjustment of C-7 stereochemistry? There are two possibilities: either the 7*R* alkaloid is hydrolyzed to its necine base or it is aromatized. In the case of epiheliotrine (**7**), hydrolysis would give the base retronecine (**8**); aromatization would give dehydroepiheliotrine (**9**). Interestingly, when **8** is fed to larvae, it is recovered largely as an alkaloid, creatonotine (**10**), resulting from reesterification of its primary hydroxyl group with (2*S*,3*S*)-2-hydroxy-3-methylpentanoic acid. [Creatonotine was identified independently in similar experiments by Hartmann *et al.* (19)]. Feeding of heliotridine (**11**) itself also yielded creatonotine (**10**), along with its C-7 epimer, epicreatonotine (**12**). Thus, free necine bases seem to be esterified prior to further processing.

We conclude that epiheliotrine (**7**) is aromatized to dehydroheliotrine (**9**), which is then hydrolyzed to dehydroretronecine (**13**) prior to oxidation to give **2**. The direct detection of these two aromatic intermediates (**9** and **13**) was expected to be difficult, since they decompose readily in solution, a fact we could easily confirm by using authentic samples of these compounds (15, 16). In fact, even the most careful experiments, involving maceration of adult bodies with dichloromethane for 5 min followed immediately by GC–MS analysis, failed to show the presence of these anticipated intermediates subsequent to feeding of **3**. Nevertheless, both **9** and **13** were found in extracts of pupae made just prior to eclosion. Interestingly, hydroxydanaidal (**2**) itself reaches a peak in the coremata *Anlagen* at this pupal stage (20). In summary, we conclude that the conversion of the 7*S* alkaloid, heliotrine (**3**), into the 7*R* pheromone, 7*R*-(−)-hydroxydanaidal (**2**), involves the sequence **3** → **6** → **7** → **9** → **13** → **2**, as shown in Scheme II.



Scheme II

We have also observed that ketone **6** is, in part, aromatized and hydrolyzed to give **14** and **15** *in vivo*. Although **14** and **15** were found in all our feeding experiments, these compounds failed to serve as precursors to **2**.

In plants, pyrrolizidine alkaloids often occur as *N*-oxides (21), and *Creatonotos* larvae may convert alkaloid free bases to *N*-oxides as well (19). In any case, the pathway we have described should be understood to include the possibility that it is the *N*-oxide that is actually the pheromone precursor.

In the tests with *Utetheisa*, hydroxydanaidal (**2**) was detected in coremata of males raised in the laboratory on a pyrrolizidine alkaloid-free diet (4) only after injection of monocrotaline or monocrotaline hydrochloride. The coremata of heliotrine-injected males were found to be hydroxydanaidal-free. *Utetheisa* in nature appears to have dietary access to pyrrolizidine alkaloids (monocrotaline, usaramine) with only the 7*R* configuration (22), and its inability to utilize heliotrine may be a reflection of its lack of "evolutionary exposure" to 7*S* alkaloids. Given these findings with *Utetheisa*, one wonders whether the ability of *Creatonotos* to utilize both *R* and *S* alkaloidal precursors is an indication that this insect actually obtains these stereoisomeric forms in its diet. Unfortunately, little is known about host plants of *Creatonotos* in nature.

Of further ecological interest, because of its remarkable similarity to the phenomenon in arctiids, is the production of alkaloid-derived pheromones in danaine butterflies (2, 3, 23). Some danaine species produce achiral pheromonal pyrrolizines (2, 3, 23), while others (*Euploea*) synthesize 7*R*-hydroxydanaidal. In the case of *Euploea sylvester* and *Euploea core*, adult males have access to both 7*R* and 7*S* alkaloids, but whether alkaloids from both stereochemical series can serve as pheromone precursors is as yet undetermined (24). Similarly, whether the producers of achiral pyrrolizine pheromones are able to utilize 7*S* and 7*R* alkaloids interchangeably for pheromone production, and whether they do in fact have access to both stereoisomeric precursors in nature, remains unknown.

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