formosus all failed to agglutinate tumor cells such as Ehrlich ascites cells, S-180 cells, AH109A cells and AS653 cells[11], and human, mouse, rat or chicken erythrocytes, *Xenopus laevis* skin extract agglutinated both tumor cells and human erythrocytes. Table 1 gives the reactivities of each fraction of the extract from *Xenopus laevis* separated by gel filtration on Sephadex G-75. S1 (Fr. No. 15–45) agglutinated human erythrocytes without showing any specificity for A, B and O blood groups[12], but had no effect on erythrocytes of other species and on tumor cells, whereas S2 (Fr. No. 46–72) and S3 (Fr. No. 73–110) preferentially agglutinated xenogenic tumor cells: S2 agglutinated tumor cells grown as ascites in ddY mice, such as Ehrlich and S-180 ascites cells, while S3 agglutinated only tumor cells maintained in Donryu rats, such as AH109A cells and AS653 cells. We have recently found that a lectin, lactose-binding protein, from eggs of *Xenopus laevis* strongly agglutinates various tumor cells as well as sialidase-treated human erythrocytes[13]. Agglutination of S-180 cells by the egg lectin was inhibited by D-fucose, L-arabinose, D-galactose and lactose. Since the agglutinability of Ehrlich ascites cells and S-180 cells by the egg lectin increases after brief treatment with sialidase, and since lectin-induced hemagglutination occurs only after digestion of erythrocytes by sialidase, it seems likely that the egg lectin recognizes terminal D-galactosyl residues. A lectin with similar specificity isolated from embryos and oocytes of *Xenopus laevis* has quite recently been described by Roberson et al.[10]. The sugar specificity of this lectin reported by Roberson et al. is similar to that of the egg lectin, and these lectins may be identical.

After S2 was separated by chromatography on a column of DEAE-cellulose, the lectin activity was found in a peak labelled Dlc (Fr. No. 50–90), which was homogeneous by electrophoresis on cellulose acetate in barbiturate buffer (pH 8.6) (fig. 2). The minimum quantity of Dlc required for agglutination of S-180 cells was 60 μg/ml, while that for intact human erythrocytes was 4000 μg/ml. Agglutination of S-180 cells by the Dlc fractions was inhibited by D-fucose, L-arabinose, D-galactose, lactulose and lactose (table 2), while hemagglutination by the lectin derived from S1 was inhibited by lactulose and lactose but not by D-fucose, L-arabinose and D-galactose[12].

Conclusion. Both Dlc (skin-derived) and the egg lectin agglutinate Ehrlich cells and S-180 cells (mouse tumor cells) but do not agglutinate AH109A or AS653 cells (rat tumor cells). Sialidase-treated human erythrocytes are very strongly agglutinated by the egg lectin and very weakly by Dlc.

Dlc seems to recognize not only the configurations of the 2-, 3- and 4-hydroxyl groups of the terminal sugar but also its β-D-glycosidic linkage, while the lectin derived from S1 seems to recognize only a β-glycosodic linkage containing a D-galactosyl end-group.

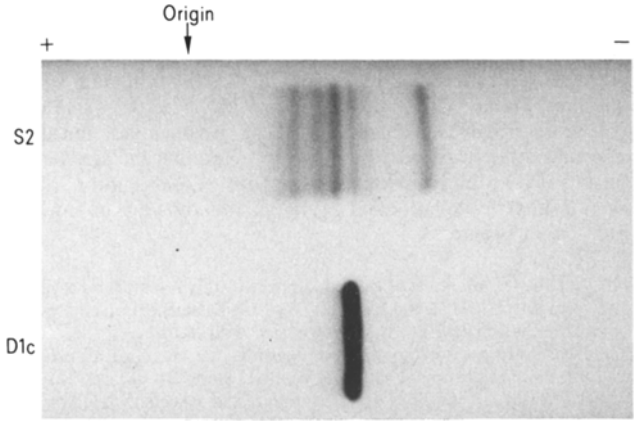

Figure 2. Cellulose acetate electrophoresis pattern of S2 and Dlc. Each sample (10 μg) was applied to the membrane and electrophoresis was carried out at a constant current of 0.8 mA/cm for 45 min.

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Stereochemical course of pheromone biosynthesis in the arctiid moth, *Creatonotos transiens*[1,2]

T.W. Bell, M. Boppré, D. Schneider and J. Meinwald

Department of Chemistry, State University of New York, Stony Brook (New York 11794, USA), Zoologisches Institut der Universität, D-8400 Regensburg (Federal Republic of Germany), Max-Planck-Institut für Verhaltensphysiologie, D-8131 Seewiesen (Federal Republic of Germany), and Department of Chemistry, Cornell University, Ithaca (New York 14853, USA), 10 August 1983

Summary. The biosynthetic conversion of a pyrrolizidine alkaloid (heliotrine, **IV**) to a male moth pheromone (hydroxydanaidal, **III**) is found to proceed with inversion of configuration at the remaining asymmetric center (C-7).

Dihydro-5H-pyrrolizines, such as danaidone (**I**), danaidal (**II**) and hydroxydanaidal (**III**), constitute an interesting group of compounds secreted by the males of certain Lepidoptera[3–6]. These substances, which must be biosynthesized from any of several plant alkaloids, such as heliotrine (**IV**) or monocrotaline (**V**)[3–6], have been demonstrated to function as pheromones in some cases[4,7,8]. In recent studies of 2 Asian species of the genus *Creatonotos* (Arctiidae), *C. gangis* L. and *C. tran-*

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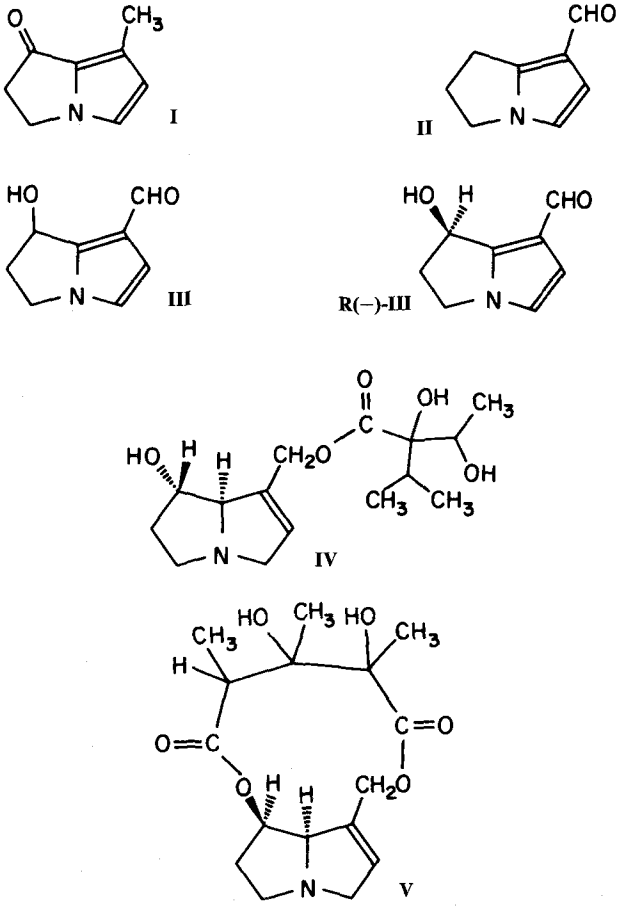
siens (Walker), we have shown that both the production of **III** and the development of male scent organs (coremata) are dependent on the presence of pyrrolizidine alkaloids (e.g. **IV** or **V**) in the larval diet[6,9].

In contrast to the achiral pheromones, **I** and **II**, hydroxydanaidal (**III**) posesses an asymmetric carbon atom (C-7), which is also present in its alkaloidal precursor. Since the simplest process for transformation of **IV** or **V** into **III** need not involve C-7, the configuration at this center might not be expected to change. We now find that, contrary to this straightforward prediction, *Creatonotos transiens* produces **R(−)-III** from heliotrine (**IV**), *with net inversion* of the C-7 stereochemistry.



Material and methods. C. transiens larvae were initially raised on leaves of *Taraxacum officinale* (Asteraceae), which lack pyrrolizidine alkaloids. Natural heliotrine was administered to 7th instar larvae by evaporating a methanol solution of 0.6–1.8 mg/larva on *Taraxacum* leaves, which were eagerly eaten. Normal feeding was resumed only after the alkaloid impregnated leaf was entirely ingested[10]. The coremata of the male moths were inflated by application of pressure on the abdomen, then severed from the moth. Organs thus prepared were preserved in sealed ampoules containing a small amount of carbon disulfide.

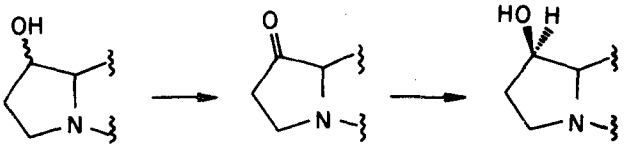
C. transiens coremata were extracted with chloroform or ethyl acetate (0.04–0.09 ml/corema). Evaporation of the extract gave a glassy residue, which was chromatographed on deactivated neutral alumina with CH_2Cl_2/methanol. Fractions containing pure hydroxydanaidal (7-hydroxy-6,7-dihydro-5H-pyrrolizine-1-carboxaldehyde, **III**) were combined and evaporated to yield a viscous oil, amounting to 9–23% of the crude extract by weight. Rotations were determined in ethanol using a Perkin-Elmer model 254 polarimeter.

Results and discussion. The hydroxydanaidal (**III**) isolated separately from samples comprising 81 and 54 coremata had the following weights and rotations, respectively: 1.8 mg, $[a]_D^{22} - 170 \pm 30°$ (c = 0.18); and 4.2 mg $[a]_D^{22} - 122 \pm 20°$ (c = 0.21). For comparison, authentic samples of **R(−)-III** and **S(+)-III** were synthesized from natural retronecine and heliotridine, respectively, following literature procedures[11,12]. The rotations of these substances in ethanol were as follows: **S(+)-III**, $[a]_D^{25}+137°$ (c = 0.73) (lit. [11] $[a]_D^{23}+135°$ (c = 0.65)); **R(−)-III**, $[a]_D^{25} - 140°$ (c = 0.74).

These data demonstrate that the biosynthesis of **III** from heliotrine (**IV**), which includes the heliotridine nucleus, occurs with net inversion at the asymmetric center (C-7). Preliminary results from our laboratories[13] suggest also that *C. gangis* produces **R(−)-III** both from heliotrine (**IV**, C-7(**S**)) and from monocrotaline (**V**, C-7(**R**)), the latter including the retronecine nucleus.

These biosynthetic observations are consistent with a number of different mechanisms. One possibility is that at some stage in the biosynthesis, the C-7 hydroxyl group is oxidized to a carbonyl group, which is then stereoselectively reduced to produce an alcohol with the **R** configuration (eq. 1). In this case, the configuration of **III** produced would be independent of the stereochemistry of its alkaloidal precursor.

Equation 1



Alternatively, it is conceivable that C-7 (**R**) alkaloids are converted to **III** without disturbing the C-7 oxidation state or its chirality, while C-7 (**S**) alkaloids are inverted at C-7 in the course of their transformation to **III**. Experiments designed to examine these questions, and also to determine the stereochemistry of **III** found in wild males of both *C. gangis* and *C. transiens* (whose foodplants and available alkaloids are unknown) are now in progress.

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2 Please address correspondence regarding the chemical aspects of this communication to either T.W. Bell (Stony Brook) or J. Meinwald (Ithaca); inquiries regarding biological aspects should be sent to M. Boppré (Regensburg) or D. Schneider (Seewiesen).

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