

The present study showed that injectable amnion collagen promotes fibroblast attachment. The attachment rates for fibroblasts growing on collagen-coated dishes were higher than those growing on plastic dishes. No significant adverse effect from irradiated collagen was noted at irradiation dosages of 0.25 Mrads and 1 Mrads. When the irradiation dose increased to 2 Mrads, cell attachment rates were slower at 10, 20, and 40 min, but not lower than those on plastic dishes. At these high doses of irradiation (2 Mrads), cell attachment eventually reached the same level as that of cells growing on non-irradiated collagen-coated dishes. In contrast, with a tumor cell line (A431), attachment to the different substrates occurs at the same rate.

Further analysis has been presented concerning the proliferation of fibroblasts and A431 cells on plastic dishes, and non-irradiated and irradiated amnion collagen films. A431 cells were found to proliferate at approximately the same rate on all substrata, while human skin fibroblast cultured on collagen films (both irradiated and non-irradiated) grew faster than those cultured on non-collagen plastic dishes. No significant differences were noted for fibroblasts growing on the non-irradiated and various doses irradiated collagen films.

Thus, while normal fibroblast attachment and proliferation are promoted by collagen, no obvious effects were found on the attachment and growth of A431 cells. These results are consistent with the study by Klebe<sup>12</sup> and Liotta et al.<sup>13</sup>. Most untransformed mammalian cells require an appropriate surface for survival and growth in vitro, but not for the growth of their tumorigenic counterparts.

The cytotoxicity study showed no obvious toxic effect of gamma-irradiated collagen on fibroblast and A431 cell growth. Furthermore, gamma-irradiated amnion colla-

gen did not induce any observed morphological changes in cultured cells.

In light of these observations, we suggest that gamma-irradiated amnion collagen does not exhibit any adverse or toxic effects on fibroblasts and A431 cells. It allowed normal cell attachment, survival and growth. Monolayer cells cultured on gamma-irradiated and non-irradiated collagen eventually reached confluence at approximately the same time. We therefore suggest that low-dose gamma-irradiated collagen may prove to be an important biomaterial for plastic surgery.

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## (R)-(Z,E)-9,11-octadecadien-13-olide: An intriguing lactone from *Heliconius pacheus* (Lepidoptera)

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**Summary.** A fourteen-membered lactone, (R)-(Z,E)-9,11-octadecadien-13-olide, was isolated from extruded abdominal glands of a Neotropical, nymphalid butterfly, *Heliconius pacheus* (Lepidoptera). This compound was obtained from mature adults of both sexes, but was not detected in young adults or pupae.

**Key words.** Butterfly; neotropical; *Heliconius pacheus*; fourteen-membered lactone.

Pheromones are chemical compounds, secreted by an individual, which influence the behavior or development of other individuals of the same species<sup>2</sup>. Many pheromones have been identified, particularly from in-

sects. These include sex attractants, aphrodisiacs, alarm signals, trail markers, and substances with a variety of other activities<sup>3</sup>. An initial search for a new pheromone is usually based on a behavioral or electrophysiological

assay which guides the chemical isolation procedure. However, it has occasionally been useful simply to characterize the substances produced by a particular gland as an initial step<sup>3</sup>. Once the structure and stereochemistry of a unique glandular product is known, it becomes possible to synthesize the natural product, thereby making it more readily available for subsequent biological studies<sup>4</sup>. We report here the structure and absolute configuration of a macrocyclic lactone which is the major volatile component from the extruded, yellow abdominal glands of *Heliconius pachinus*.

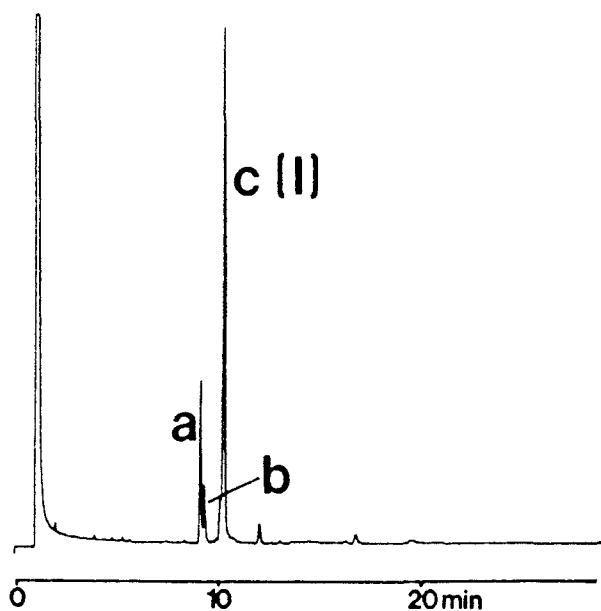
*Heliconius*, a large genus of New World tropical nymphalid butterflies has attracted biologists' attention for many years. Much of the research on these butterflies has concentrated on their taxonomy, ecology, morphology, and genetics<sup>5</sup>. While we have recently isolated and characterized several long-chain hydrocarbons from the abdominal glands of *Heliconius ismenius*, the chemistry of this genus has not been studied extensively<sup>6</sup>.

Five live adult *H. pachinus* females of a stock originating from the Osa Peninsula, Costa Rica, reared in tropical insectaries under semi-natural conditions, were used for the present study. Excised glands were immersed in distilled methylene chloride (300  $\mu$ l) for three days at 0 °C in the dark. After filtration, the extract was concentrated under a gentle stream of nitrogen to half its volume (150  $\mu$ l).

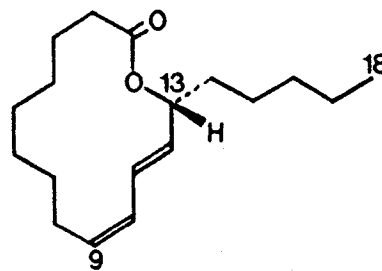
Gas liquid chromatographic (GLC) analyses showed that the extract contained three major volatile components **a**, **b**, and **c** in the relative proportions 18:7:75, respectively (fig.). By preparative GLC (3% OV-1, 3 mm  $\times$  1 m, oven temp. 170 °C), 10  $\mu$ g of a mixture of **a**

and **b** and 25  $\mu$ g of **c** were isolated. Capillary GC-FTIR examination of **c** revealed a normal ester or lactone absorption at 1747  $\text{cm}^{-1}$ . The presence of a conjugated diene chromophore in **c** was suggested by its ultraviolet spectrum ( $\lambda_{\text{max}}$  235 nm, in cyclohexane). A high resolution mass spectrum showed that the molecular formula of **c** is  $\text{C}_{18}\text{H}_{30}\text{O}_2$  (calcd.  $m/z$  278.2246, obsd. 278.2248)<sup>7</sup>. Compound **c** readily adsorbed 2 mol equivalents of  $\text{H}_2$  in the presence of Pd-C to give a saturated compound,  $\text{C}_{18}\text{H}_{34}\text{O}_2$ . The EI mass spectrum of this tetrahydro derivative showed the expected molecular ion at  $m/z$  282. Diagnostic fragment ions were observed at  $m/z$  264 (4%), resulting from the loss of the elements of water,  $m/z$  211 (6%), corresponding to the loss of a  $\text{C}_5\text{H}_{11}$  radical and  $m/z$  182 (9%), arising from the loss of  $\text{C}_5\text{H}_{11}\text{CHO}$ . This fragmentation pattern is analogous to that produced by the thirteen-membered lactone, octadecan-12-olide, a sample of which was synthesized from 12-hydroxy-octadecanoic acid by lactonization with 2,2'-dipyridyldisulfide<sup>8</sup> [ $m/z$  197 (12%) for loss of  $\text{C}_6\text{H}_{13}$ , 168 (10%) for loss of  $\text{C}_6\text{H}_{13}\text{CHO}$ ]. Thus, **c** would appear to be a fourteen-membered lactone with a  $\text{C}_5\text{H}_{11}$  group attached to the carbon  $\alpha$  to the ester oxygen. It remained to identify the placement of the conjugated double bonds.

Compound **c** gave the following  $^1\text{H}$ -NMR spectroscopic data (400 MHz,  $\text{C}_6\text{D}_6$ ):  $\delta$  6.69 (dd, 1H,  $J$ -15.2, 10.9 Hz), 6.09 (t, 1H,  $J$ -10.9 Hz), 5.60 (m, 1H), 5.54 (dd, 1H,  $J$ -15.1, 3.8 Hz), 5.46 (dt, 1H,  $J$ -10.9, 6.8 Hz), 2.43 (m, 2H), 2.21 (m, 1H), 1.88 (m, 1H), 1.74 (m, 1H), 1.55–1.05 (m, 16H), 0.93 (m, 1H), 0.84 (t, 3H,  $J$ -6.0 Hz). The conjugated olefinic multiplets were analyzed by exhaustive spin decoupling experiments. Irradiation of the carbinol proton  $\text{H}_{(13)}$  at  $\delta$  5.60 decoupled it from  $\text{H}_{(12)}$ , centered at  $\delta$  5.54, and caused the  $\text{H}_{(12)}$  double-doublet to collapse to a doublet with a splitting of 15.1 Hz, which is typical of *trans* coupling across a double bond. Thus, one *E* double bond must be adjacent to the carbon  $\alpha$  to the ester oxygen. Irradiation of one of the allylic methylene proton  $\text{H}_{(8)}$  at  $\delta$  1.88, decoupled  $\text{H}_{(9)}$  and caused the triplet-split-doublet centered at  $\delta$  5.46 to collapse to a 10.9 Hz doublet, a value typical of *cis* double bond coupling. Thus, the second double bond, between  $\text{C}_9$  and  $\text{C}_{10}$ , has the *Z* configuration, and compound **c** is (*Z*, *E*)-9,11-octadecadien-13-olide (**I**).



Gas chromatograph (glass capillary, 007-methylsilicone, 0.25 mm  $\times$  15 m, oven temperature 200 °C isothermal, helium as carrier gas) of an extract from extruded abdominal gland of *Heliconius pachinus* (female) showing one main component **c** (**I**) and minor components **a** and **b**.



(*Z*, *E*)-9,11-octadecadien-13-olide

Examination of the literature revealed that a lactone of this structure had been isolated as a constituent of the seed oil of *Monnina emarginata* (Polygalaceae), a plant native to Uruguay<sup>9</sup>. The absolute configuration of the single asymmetric carbon at C-13 of this seed oil lactone (coriolide) had been established as (S)-(+). The ORD spectrum of (S)-(+)-coriolide exhibits a plain positive curve between 600 and 255 nm (in MeOH). Because of the limited amount of the insect-derived lactone **I**, we could measure only a qualitative CD spectrum. Lactone **I** exhibited a simple negative CD curve between 250–220 nm (negative maximum, 235 nm) in MeOH. In order to make a direct comparison of the absolute configuration of the insect-derived lactone with that of coriolide, a small sample of coriolide was isolated from *M. emarginata* seeds<sup>10</sup>. The CD spectrum of this material, which showed a simple positive CD curve between 250–220 nm (positive maximum, 233 nm) in MeOH, clearly indicated that its configuration was the opposite of that in the *H. pachinus* lactone. Our insect component is therefore (R)-(Z,E)-9,11-octadecadien-13-olide. This is the first isolation of (R)-**I** from nature.

Fourteen-membered unsaturated lactones have been isolated previously from several coleoptera, including a eucalypt longicorn (*Phoracantha synonyma*) which contained (Z)-11-hydroxy-5-tetradecen-13-olide **II**, (Z,Z)-5,8-tetradecadien-13-olide **III**, and (Z)-5-tetradecen-13-olide **IV**<sup>11</sup>. Lactones **III** and **IV** have also been found in a flat grain beetle (*Cryptolestes pusillus*) and in *C. turcicus*, where they serve as aggregation pheromones<sup>12, 13</sup>. A saturated fourteen-membered lactone, 14-methyl-hexadecan-13-olide, has been characterized as an aggregation factor of an ant, *Iridomyrmex humilis*<sup>14</sup>. A more closely related compound, (12S, 13S)-(Z)-12-hydroxy-9-octadecen-13-olide, has been isolated from the seed oil of *Crepis conyzaeifolia*<sup>15</sup>. However, no fourteen-membered lactones appear to have been reported from lepidoptera.

The two minor constituents of this *H. pachinus* secretion, **a** and **b**, were isolated as a mixture by preparative GLC as described above (fig.). GC-MS analysis showed that both components possessed the same molecular formula, C<sub>18</sub>H<sub>32</sub>O<sub>2</sub>, and preliminary analyses of these data showed that they are both fourteen-membered lactones with one double bond in the ring system. The structural elucidation of these lactones is now in progress.

The average lactone content of a female was estimated to be circa 7 µg/insect. These constituents were also detected in the methylene chloride extract of a small piece of filter paper (2 × 5 mm) which had been touched gently for a few seconds to the extruded yellow gland itself. A considerable amount of these lactones must therefore be

present on the surface of the gland. Interestingly, the same three components were also detected from mature male's glands in about the same ratio as in females, although the amounts were only about one third of those found in females.

We could not detect these lactones in the glands of freshly emerged adults of either sex. We also examined the glandular constituents of several other *Heliconius* species, including *H. erato*, *H. sara*, *H. ismenius*, *H. charitonia*, *H. hecale*, *H. melpomene*, and *H. cydno*. With the exception of *H. cydno*, none of these species possessed **I**. This observation is consistent with the fact that *H. pachinus* is a very close relative of *H. cydno*, if not a race of that species. *H. cydno* and *H. pachinus* are completely inter-fertile<sup>16</sup>.

The biological significance of these lactones, present in mature adults of both sexes of *H. pachinus*, is not yet known. Further behavioral and ecological observations, however, should show whether these lactones play an important role in the life of this butterfly.

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