*Zusammenfassung.* In Ribosomen von Klapperschlangenleber ist das Verhältnis RNS/Protein 0,96, und das Molekulargewicht der meisten ribosomalen Proteine liegt zwischen 10 und $45 \times 10^3$ Daltons. Das durchschnittliche Molekulargewicht ist 22000 bzw. 26000 Daltons für die Proteine der 40 S bzw. der 60 S Untereinheit. Diese Daten weisen darauf hin, dass der erhöhte Proteingehalt der Ribosomen dieses Reptils gegenüber Ribosomen von Bakterien durch das Vorhandensein von Proteinen mit höherem Molekulargewicht bedingt ist.

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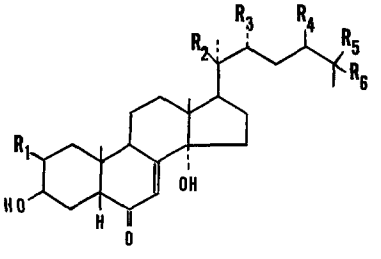
## Biological Activity of Insect Ecdysones and Analogues in vitro

The relationship between the structure of ecdysone analogues and their biological activity was investigated with in vivo bioassays in both Diptera [1] and Lepidoptera [2, 3]. However, some ecdysone analogues were converted to insect ecdysones in vivo [4]. Hence, the biological activity of some of the analogues may depend upon their conversion to active molecules in the insect [5]. The relationship between structure and activity should be examined in an in vitro system in which metabolism of the ecdysones is less likely to occur [6–8]. We have reported previously that continuous exposure to α-ecdysone and β-ecdysone had different effects on morphogenesis and cuticle deposition in imaginal disks in vitro [9–11]. Furthermore, fat body modified the action of the ecdysones [10–12]. We report here on relative activity of insect ecdysones and various analogues in vitro (Figure) in bioassays of wing disks of *Plodia interpunctella* (Hübner).

Wing disks from last-instar larvae were cultured as previously described [11]. The hormone analogues were tested as a continuous exposure in cultures of wing disks with and without fat body. Also, each analogue was tested as a 24-h pulse on cultures that did not contain fat




| Compound | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ |
|---|---|---|---|---|---|---|
| α-Ecdysone | OH | H | OH | H | $CH_3$ | OH |
| β-Ecdysone | OH | OH | OH | H | $CH_3$ | OH |
| Ponasterone A | OH | OH | OH | H | $CH_3$ | OH |
| Inokosterone | OH | OH | OH | H | $CH_2OH$ | H |
| Podecdysone A | OH | OH | OH | $C_2H_5$ | $CH_3$ | OH |
| 2-Deoxycrustecdysone | H | OH | OH | H | $CH_3$ | OH |
| 22,25-di-Deoxyecdysone | OH | H | H | H | $CH_3$ | H |

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Table I. Effects of continuous exposure to ecdysone analogues on wing disks in vitro

| Compound | Lowest concentration (μg/ml) required to produce response[a] | | | |
|---|---|---|---|---|
| | No fat body | | Fat body | |
| | Cuticle | Evagination | Cuticle | Evagination |
| α-Ecdysone | — | 0.1 (50%) | 10.0 (90%) | 0.1 (100%) |
| β-Ecdysone | 1.0 (17%) | 0.1 (90%) | 0.1 (100%) | 0.1 (100%) |
| Ponasterone A | — | 0.1 (50%) | 0.1 (25%) | 0.1 (100%) |
| Inokosterone | — | 0.1 (55%) | 0.1 (20%) | 0.1 (100%) |
| 2-Deoxycrustecdysone | 10.0 (15%) | 0.1 (100%) | 1.0 (45%) | 0.1 (100%) |
| 22,25-di-Deoxyecdysone | — | 1.0 (100%) | — | 1.0 (100%) |
| Podecdysone A | — | — | — | 1.0 (100%) |

[a] Percent response is given in parentheses. The concentrations tested were 0.1, 1.0, and 10.0 μg/ml.

Table II. Effects of a 24-h pulse of ecdysone analogue on wing disks cultured in vitro without fat body

| Compound | Cuticle (%) | Evagination (%) |
|---|---|---|
| α-Ecdysone | 0 | 0 |
| β-Ecdysone | 50 (2.0 μg/ml) | 100 (2.0 μg/ml) |
| Ponasterone A | 35 (0.1 μg/ml) | 65 (0.1 μg/ml) |
| Inokosterone | 0 | 15 (0.5 μg/ml) |
| Podecdysone A | 0 | 0 |
| 2-Deoxycrustecdysone | 25 (10.0 μg/ml) | 55 (10.0 μg/ml) |
| 22,25-di-Deoxyecdysone | 0 | 0 |

body. Both evagination of the disk and deposition of cuticle were scored in each test. Each test was run at least twice with a minimum of 20 disks. The ecdysone compounds were tested at concentrations of 0.1, 1.0, and 10.0 μg/ml of medium in each of the continuous exposure assays, and also at the most effective concentrations as determined in these tests and in earlier pulse experiments.

The effects of continuous exposure at the minimum effective concentrations are reported in Table I. The 24-h pulse data are shown in Table II. Exposure to solvent controls (10% or 50% ethanol) and a synthetic isomer, 22-iso ecdysone, had no effect in any of the assays.

The data show clearly that the mode of exposure to the hormone and the type of response examined influenced the relative activity of the test compound (Tables I and II). This difference was also observed when *Drosophila* disks were cultured in vitro. Here, opposite conclusions about the relative activity of α- and β-ecdysone were reached by workers who used either evagination[13] or cuticle[14] deposition as their bioassay. In our experiments we found that cuticle deposition was a better criterion of activity than evagination, but it was best to examine both responses. On the basis of both evagination and cuticle deposition in the three in vitro bioassay systems described, we suggest the following order of activity for the ecdysone analogues tested: β-ecdysone > ponasterone A > 2 deoxycrustecdysone > inokosterone > α-ecdysone > 22,25-dideoxyecdysone > podecdysone A.

Probably, because β-ecdysone, 2-deoxycrustecdysone and ponasterone A (all with 20-hydroxy groups) stimulate both cuticle deposition and evagination after a pulse treatment without fat body, they are acting as hormones per se. However, two other analogues (α-ecdysone and inokosterone) that required continuous exposure or the presence of fat body to become effective in our in vitro bioassays may be converted to more active forms. It is interesting that the addition of the ethyl group to the side chain of β-ecdysone produced a compound (podecdysone A) that was even less active that one (22,25 di-deoxyecdysone) without the 20-hydroxy group. On the basis of our study, the activity of ecdysone analogues may be easily affected by altering the side chain structure[15,16].

*Résumé.* Le développement des disques imaginaux isolés en culture in vitro en présence de β-ecdysone a été comparé au développement en présence des substances analogues.

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## DNA Degradation During Organ Storage

One of the critical areas in organ transplantation is the viability of the transplanted organ prior to re-establishing organ blood flow. Preservation of cadaver kidneys is needed to allow for preparation of the recipient for transplant surgery if the donor and recipient are in the same hospital, or to permit transport of the kidney to a suitable recipient in another hospital. For kidney transplantation, the organ is exposed to warm ischemia during removal from the donor and to cold ischemia during storage. Events occurring during these ischemic periods that prevent long term viability are not fully understood. Knowledge of the cellular defects that occur during storage will present areas to be modified for improving preservation. Long term organ survival may require an intact DNA memory bank. This study was designed to examine the effects of organ storage on the integrity of the nuclear DNA.

*Materials and methods.* The size of DNA molecules was examined by low shear viscometry[1]. Viscosity measurements allow evaluation of the size of high molecular weight DNA. Charles River strain male mice, 6–10 weeks old, were sacrificed; the kidneys flushed with 0.15 *M* NaCl to remove the blood; and the kidneys excized. One kidney was either stored at 37 degreees (warm ischemia) or at 0 degrees (cold ischemia) in 2 ml of 0.15 *M* NaCl. The contralateral kidney was taken as the control, and nuclei isolated immediately[2]. The nuclei were lysed overnight at 50 °C by 0.5% SDS and 5 m*M* EDTA (pH 6.2). After storage, nuclei were isolated from the kidney[2] and lysed overnight. The viscosity was then determined at 50 °C using a low shear viscometer[1] (Beckman Instruments, Palo Alto, California). The viscosity was stable over an 8-h period (16–24 h after lysis was initiated). The intrinsic viscosity determined for the lysed nuclei is, for practical purposes, dependent on the DNA, while the

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