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Biological activity and receptor-binding of ecdysteroids and the ecdysteroid agonists RH-5849 and RH-5992 in imaginal wing discs of *Spodoptera exigua* (Lepidoptera: Noctuidae)

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Ecdysone, nuclear hormone receptor, imaginal discs, Lepidoptera, ecdysteroid assay

Abstract. The effects of 20-hydroxyecdysone (20E), ponasterone A (PoA) and both dibenzoylhydrazine-based ecdysteroid agonists RH-5849 and RH-5992, were tested on in vitro-cultured imaginal wing discs of last-instar larvae of the beet armyworm, *Spodoptera exigua* (Hübner). In each case, the response was qualitatively similar to that induced by 20E, although the concentrations required to induce response varied widely. The EC_{50} of both ecdysteroids 20E and PoA to elicit wing disc evagination in 50% of the discs was 89.8 and 2.57 nM, respectively; for both nonsteroid agonists RH-5849 and RH-5992 this was 870 and 11.7 nM, respectively. Binding competition studies using whole imaginal wing discs, showed that I_{50} of 20E, PoA, RH-5849 and RH-5992 to displace 50% of [^{3}H]PoA-binding was 290, 7, 1100 and 33 nM, respectively, which is in the same order as the respective concentration to elicit a biological response.

INTRODUCTION

In previous studies, the imaginal discs of *Drosophila*-larvae have particularly been used to investigate the ecdysteroid action in imaginal discs. However, in all cases discs were mass-isolated. Although the ecdysteroid receptor (EcR) has already been partially characterized (Bidmon & Sliter, 1990; Cherbas et al., 1991; Koelle et al., 1991; Imhof et al., 1993), a direct comparison with bioassay studies of individual discs is difficult.

At present, substitued 1,2-dibenzoylhydrazines as RH-5849 and RH-5992 are indicated as the first nonsteroidal ecdysteroid agonists exhibiting a potent larvicidal activity against Lepidoptera. In our previous studies (Smagghe & Degheele, 1993, 1994a,b), RH-5992 possessed a significantly higher potency than RH-5849 against different larval stages of *Spodoptera exigua* (Hübner), and differences in pharmacokinetics and metabolism could not elucidate the shift in toxicity.

In continuation of our study, this study aimed to provide evidence for the intriguing question: Is the biological activity of both ecdysteroids 20-hydroxyecdysone (20E) and ponasterone A (PoA) and both ecdysteroid agonists RH-5849 and RH-5992 correlated with their affinity to bind EcRs? To investigate this, we have developed two assays. In a first assay, the in vitro-biological activity of the compounds was determined in terms of their ability to induce wing disc evagination in a wing disc bioassay that has been established for *S. exigua*. In another assay, we have measured EcR-binding affinities in individual discs. Next to the study of Terentiou et al. (1993) working with *Calliphora*-imaginal discs, this is the first report using whole individual imaginal discs for binding study. In this way, we have performed relative competition binding studies determining

concentration dependent displacement curves of bound [^{3}H]PoA by unlabelled competitors, both ecdysteroids and ecdysteroid agonists.

MATERIALS AND METHODS

Compounds

20E was purchased from Sigma Co. Unlabelled PoA and [25,26 ^{3}H]PoA (spec. act. 134 Ci/mmol) were a generous gift of R. Lafont (Ecole Normale Supérieure, Paris, France), and K. Richter and L. Sobek (University of Jena, Jena, Germany), respectively. Technical compound of both ecdysteroid agonists was kindly obtained from G.R. Carlson (Rohm and Haas Co., Spring House, PA, USA).

The chemical purity of ecdysteroids was established by RP-HPLC-analysis (Bondapak C_{18}, particle size 10 µm, 7.8 × 300 mm, Waters Ass.; methanol : water 4 : 6, v/v; 1.2 ml/min). Concentrations were spectrometrically determined at 242 nm.

Insects

All stages of the beet armyworm, *S. exigua*, were cultured under standard laboratory conditions at 23 ± 2°C, 75 ± 5% R.H. and a 16L : 8D photoperiod, as previously described (Van Laecke & Degheele, 1991).

Measurement of ATP in imaginal wing discs

ATP-measurements were performed using a Biocounter 2500 (Lumac). The amount of ATP present in sonicated mesothoracical imaginal wing discs was determined from a previously determined standard curve.

Imaginal wing disc bioassay

The mesothoracical imaginal wing discs originating from last-instar larvae of *S. exigua* at the very end of the feeding period, just before receiving their natural 20E-pulse were used. Larvae were briefly surface-sterilized in 70% ethanol before being water-anesthetized. Dissected discs were cultured at 5 discs in 1 ml modified Grace's medium [Grace's medium (Gibco), with added fetal bovine serum (15%, v/v, TechGen), 30% bovine serum albumine solution (3% v/v, Sigma) and gentamycine sulfate (50 µg/ml, Sigma)]. Culture plates were kept at 25°C and 97 ± 2% R.H. to prevent evaporation. For each compound at least 6 different concentrations were tested and for each concentration at least 30 discs. Suitable concentrations of both ecdysteroids were made in methanol, and of both nonsteroid agonists in dimethylsulfoxide and no more than 1 µl was added to 1 ml medium. An equivalent volume was added to control cultures. After 48 h, the discs were inspected whether the treatment could induce evagination in the discs tested. The minimum requirement for a positive result was completion of the first phase of evagination (Mandaron, 1980) in which epidermal folds develop inside the epidermal sac. Dose-response curves were drawn by eye through the evagination percentages plotted on a log dose-axis. For estimation of EC_{50}-values, evagination percentages were subjected to Polo-PC (LeOra Software, 1987) since chi-square fit test allowed probit transformation.

Receptor-binding assay

Individual mesothoracical wing discs from 2-day old last-instar larvae were dissected and collected in modified Grace's medium at 4°C until analysis.

Competition binding studies were performed applying 6 nM [^{3}H]PoA with increasing concentrations of unlabelled competitor. For each treatment, duplicate aliquots of 100 µl medium + 20 imaginal discs were added to [^{3}H]PoA and unlabelled competitor after evaporation of the solvent by freeze-drying and incubated for 1.5 h at 25°C.

After incubation, bound and free radioligand were separated by placing the discs on a Whatman GF/A glass fiber filter and washing 5 times with 1 ml ice-cold medium. The amount of radioactivity (discs + filter) was determined after being shaken overnight using 1 ml tissue-solubilizer Luma-solve and 10 ml Lipoluma (both Lumac LSC) by a Kontron liquid scintillation counter (LKB). [^{3}H]PoA-binding displacement curves were drawn by eye through the percentages plotted on a log dose-axis.

RESULTS

Establishment of imaginal wing disc bioassay

In a preliminary assay, the effect of fetal calf serum was tested. Although fetal serum was required for imaginal wing disc growth, discs remain alive for several days in the

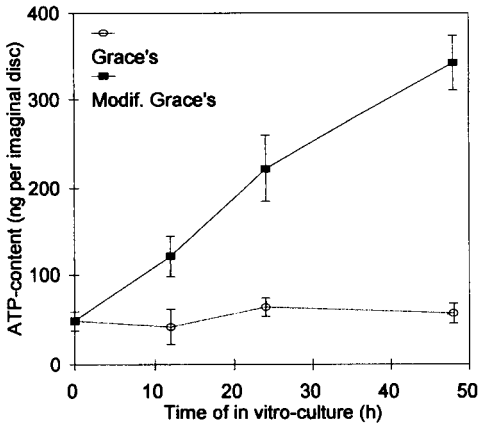

Fig. 1. ATP-content of imaginal discs cultured in different media for up to 48 h at 25°C.

absence of serum. The ATP-content of discs increased 6-fold during 48 h of cultivation in the presence of serum (± 6 ng/imaginal disc per h); in the absence of serum, the amount of ATP per disc remained approximately constant (Fig. 1). The evagination scores were also much lower as compared to those of serum-containing cultures.

In another series culturing discs originating from last-instar larvae of different ages, discs from 1- and 2-day-old last instars showed no noticeable evolution in the absence of hormone, even when cultured for extended periods in vitro. Discs from 3-day-old last-instars showed slow development, however, evagination was only seen in a few cases; discs from 4- and 5-day-old last-instar larvae scored much easier since nearly all discs showed signs of tracheole migration, elongation of wing tissues and evagination accompanied with an increase of volume within 48 h of cultivation. These discs developed normally, as observed in vivo. This agrees with the 20E-pattern, since the pulse of endogenous titre appears around day 3 into the last instar (own unpublished data). Thus, discs of 2-day-old last-instars were used, and the discs scored after 48 h of incubation. So, we found this established bioassay to be a convenient method to measure the ecdysteroid response of the discs.

Biological activity of ecdysteroids and ecdysteroid agonists

Fig. 2 shows the biological activity curves of the 4 compounds tested. Their respective concentration which induced evagination in 50% of the discs tested (= EC_{50}) is given in Table 1. Since the obtained dose-response curves of the 4 compounds were significantly parallel, the activity of the 4 compounds was specified by the position of its curve relative to that of PoA: that of 20E, RH-5849 and RH-5992 is displaced by a factor of about 35, 340 and 5 as compared to that of PoA. The potency profile of the 4 compounds was PoA > RH-5992 > 20E > RH-5849.

The biological response curves of PoA and both ecdysteroid agonists show a sharp profile. For these compounds a 7.6–15.4-fold increase of concentration was required to increase wing disc evagination from 10 to 90% (Table 1). For 20E the observed curve was not so sharp since R was 21.9 (Table 1), but was still evident.

Although the concentrations required to induce the response varied widely, in each case the response was qualitatively similar. At relatively high biologically active

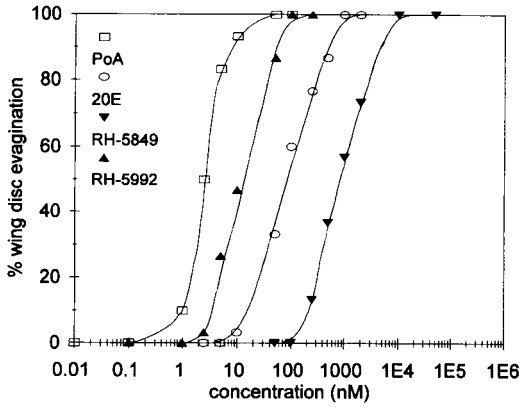

Fig. 2. Dose-response curves of PoA, 20E, RH-5849 and RH-5992 on evagination in *S. exigua*-imaginal wing discs.

concentrations of the 4 compounds, high numbers of discs showed evagination within 48 h. However, the development was clearly promoted within the wing pouches so that the resultant disc was generally small in size. Moreover, at these high concentrations wing disc tissues began to degenerate already after they had developed for 3–4 days in culture. At lower effective doses, most of the discs developed to a similar size as they normally reach in vivo; further on, such treated discs evaginated by bursting through the pouch membrane and then grew forming a wing-like shape.

TABLE 1. Biological potency [a] and receptor-binding affinity [b] of PoA, 20E, RH-5849 and RH-5992 in in vitro-cultured imaginal discs of *S. exigua*.

Compound	ED_{50} (nM) [a]	R [c]	I_{50} (nM) [b]	R [c]
PoA	2.57 (2.01–3.20)	7.6	7	188
20E	89.8 (66.6–118.4)	22	310	275
RH-5849	870 (682–1110)	15.4	1,100	200
RH-5992	11.7 (8.9–15.6)	13.7	33	251
RH-5992	11.7 (8.9–15.6)	13.7	33	251

[a] Biological activity is expressed as 50% wing disc evagination. The 95% confidence intervals are indicated in parentheses.

[b] Receptor-binding affinity is expressed as 50% displacement of bound [^{3}H]PoA. I_{50}-values are derived from the eye-drawn [^{3}H]PoA-binding displacement curves presented in Fig. 3.

[c] R is defined as the ratio of ligand concentrations needed to give 90 and 10% of full response.

Establishment of receptor-binding assay

In a first preliminary experiment, most of the amount of unbound radioactivity was removed after 5 washes with ice-cold medium. Whatman GF/A glass fiber filters without pre-coating were used according to the results of Terentiou et al. (1993).

In an another preliminary assay, imaginal wing discs were incubated with [^{3}H]PoA, followed by HPLC-analysis (as described in Materials and Methods) of methanolic extracts of a dry-frozen aliquot of 100 µl medium + 20 discs after 1.5 h of incubation. Data indicated no significant metabolites were formed and the original [^{3}H]PoA-concentration remained high, providing evidence that [^{3}H]PoA was stable during 1.5 h of incubation. However, we have to take in account that the assays were performed only after this short time of incubation and may be with an insufficient amount of tissue mass. Nevertheless,

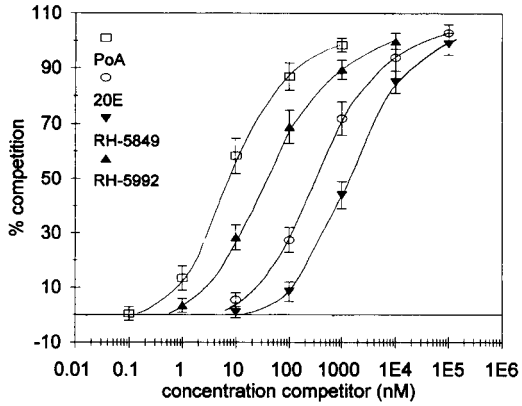

Fig. 3. [^{3}H]PoA-binding displacement curves of PoA, 20E, RH-5849 and RH-5992. Aliquots of 100 µl medium + 20 discs were incubated with 6 nM [^{3}H]PoA under equilibrium binding conditions of 1.5 h at 25°C with various concentrations of competitors. Maximal binding is shown as 0%, and total competition (= unspecific binding only) is expressed as 100%.

our data agree with those from Chihara et al. (1972) using *Drosophila*-imaginal discs and from Terentiou et al. (1993) using *Calliphora*-leg discs.

In a separate assay, a preliminary time course using 6 nM [^{3}H]PoA indicated equilibrium of binding appeared to be attained after 1.5 h of cultivation at 25°C (not presented).

Receptor-binding assay

Concentration-dependent displacement curves of the different compounds to bind receptors in competition with [^{3}H]PoA (Fig. 3) indicate that the potency range of the 4 compounds corresponds with that for the wing disc assay. The I_{50}-value (Table 1) represents that concentration of unlabelled competitor giving 50% displacement of bound [^{3}H]PoA, and yielded 7, 310, 1100 and 33 nM for PoA, 20E, RH-5849 and RH-5992, respectively. In addition, an increase of concentration of approximately 200-fold was required to increase displacement of [^{3}H]PoA from 10 to 90% for all compounds (Table 1).

CONCLUSIONS AND DISCUSSION

The experiments reported here clearly show both ecdysteroids and ecdysteroid agonists are necessary to initiate and sustain evagination of isolated *S. exigua*-wing discs. Imaginal evagination has previously been obtained with several ecdysteroids (Oberlander, 1969; Chihara et al., 1972; Agui & Fukaya, 1973; Oberlander et al., 1973; Fristrom & Yund, 1976; Riddiford, 1976; Blais & Lafont, 1980; Mandaron, 1980; Terentiou et al., 1993). Likewise, premature induction of the in vivo-wing disc development by RH-5849 and RH-5992 in last-instar larvae of *S. exigua* has been seen in own previous assays, and hyperecdysteroid effects were observed at relatively high doses.

In this study, the potency of the 4 compounds as expressed by their EC_{50}-value, ranged as follows: PoA > RH-5992 > 20E > RH-5849. Summarizing, RH-5849 was 10 and 74-fold less potent than 20E and RH-5992, respectively; 20E was 35-fold less potent than PoA. For both ecdysteroids, a similar potency profile has previously been found in various insect species (Oberlander, 1969; Chihara et al., 1972; Agui & Fukaya, 1973; Fristrom & Yund, 1976; Riddiford, 1976; Cherbas et al., 1980; Terentiou et al., 1993). A similar different potency of the prototype compound RH-5849 as compared to ecdysteroids was observed by Wing (1988), Wing & Ramsay (1989), Silhacek et al. (1990) and Spindler-Barth et al. (1991) finding that 20E was 4 to 100 times more potent than RH-5849 in vitro. For

RH-5992 no exact data in vitro have been published yet. Nevertheless, there seemed to be a good correlation between the activity of the compounds tested to elicit evagination in *S. exigua*-imaginal wing discs and that in other in vitro-systems from a wide variety of insects.

In in vivo-assays, both nonsteroids have been reported to induce a premature and lethal larval moult in *S. exigua*-larvae reflecting an ecdysteroid mimicking activity; however, RH-5992 was 75 times more potent by topical application and 19 to 44 times by ingestion of treated feeding than RH-5849 (Smagghe & Degheele, 1993, 1994a,b). A comparison with the concentrations required to elicit evagination in 50% of the discs in vitro, shows that RH-5992 was 74-fold more potent than RH-5849, suggesting there might be a general correlation between their in vivo and in vitro-activity. In comparison with ecdysteroids, Wing (1988), Wing et al. (1988) and Wing & Aller (1989) found 20E to be more active than RH-5849 in vitro; however in in vivo-assays the nonsteroid was much more active which may be attributed to its rapid pharmacokinetic properties and higher metabolic stability as compared to 20E.

In this study it is also demonstrated that the difference in 50% displacement of bound [^{3}H]PoA by unlabelled PoA and 20E is about 40-fold, which agrees well with the ability of these ecdysteroids to induce evagination in cultured *S. exigua*-wing discs. This difference is rather similar to that reported by Terentiou et al. (1993) for the induction of imaginal evagination and 50% competition by either PoA and 20E in imaginal discs of *Calliphora vicina*. Likewise, I_{50} of both nonsteroids corresponded well with their respective biologically effective concentrations (= EC_{50}). This relationship has also been noted in previous studies when the affinities of PoA and 20E in binding assays using K_c-cells and imaginal discs of *Drosophila* correlated well with the respective biologically active concentration (Fristrom & Yund, 1976; Yund et al., 1978; Yund & Osterbur, 1985). Likewise, binding assays with *Chironomus tentans*-cells (K.-D. Spindler et al., 1994, pers. comm.) indicated RH-5849 and RH-5992 to be approximately 9-fold less and 50-fold more potent than 20E to displace 50% of bound [^{3}H]PoA, which corresponded with their relative potency on endochitinase production. With *Galleria mellonella*-nuclear EcR-extracts, RH-5849 also competed 3.5-fold less effectively with [^{3}H]PoA for ecdysteroid binding sites than 20E (Sobek et al., 1993). Likewise, the EC_{50}-values of both nonsteroid agonists on in vitro-disc evagination in *S. exigua* noted in this paper certainly relate to the K_D-values obtained for binding of both compounds to EcR derived from *Plodia interpunctella*-cells (G.R. Carlson et al., 1994, pers. comm.). So, it is indicated that imaginal disc evagination and the displacement curves are a true approximation of the EcR-binding affinity for ecdysteroids and nonsteroid agonists. In this way, this study shows that parallel studies of biological activity and competition receptor-binding with individual imaginal discs enable us to evaluate the activity of ecdysteroids and ecdysteroid agonists.

Direct comparison of dose-dependent displacement curves (Fig. 3) and of wing disc evagination (Fig. 2) shows that the biological response is sharper than receptor-binding. In this way, it may be expected that wing disc evagination is qualitatively induced, as suggested by Cherbas et al. (1980) and Terentiou et al. (1993). So, the biological response of ecdysteroids may be switched on by very small changes in ecdysteroid concentration meaning that a threshold level of hormone is required for response, but once this level is reached, only a full response is possible. Otherwise, their receptor-binding displacement curves show a more concentration dependent pattern. In this way, our data agree with Terentiou et al. (1993), suggesting the biological response to be a switch turned on by the

hormone while receptor-binding follows Michaelis-Menten kinetics. For both ecdysteroid agonists, biological activity and receptor binding curves possess similar differences in their immediacy of changes affirming their ecdysteroid mimicking activity by direct binding on EcRs.

In general, it is shown that a different binding affinity of the EcRs for both ecdysteroids and ecdysteroid agonists assists in clarifying their different potency. This agrees with the concept that structure and biochemical binding properties of EcR may vary among insect species (Bidmon & Sliter, 1990). In addition, the presence of EcR-isoforms has recently been affirmed (Talbot et al., 1993). Likewise, Yao et al. (1992, 1993) determined that the activity of ecdysteroids may depend on heterodimer formation with Ultraspiracle. Further research performing receptor analysis of whole imaginal discs determining K_D-values and the number of binding sites and evaluating the binding model to be single or multiple binding site, is in progress in our laboratory. Likewise, direct comparison of the current biological activity and receptor-binding data with imaginal discs of other insect species and with parallel in vivo-toxicity data, is required in order to elucidate the different potency of nonsteroid agonists, particularly.

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REFERENCES

AGUI N. & FUKAYA M. 1973: Effects of moulting hormones and prothoracic glands on the development of wing discs of the cabbage armyworm (Mamestra brassicae L.) in vitro (Lepidoptera: Noctuidae). *Appl. Entomol. Zool.* **8**: 73–82.

BIDMON H.J. & SLITER T.J. 1990: The ecdysteroid receptor. *Invert. Reprod. Devel.* **18**: 13–27.

BLAIS C. & LAFONT R. 1980: In vitro differentiation of Pieris brassicae imaginal wing discs: effects and metabolism of ecdysone and ecdysterone. *Wilhelm Roux's Arch.* **188**: 27–36.

CHERBAS L., YONGE C.D., CHERBAS P. & WILLIAMS C.M. 1980: The morphological response of R_c-H cells to ecdysteroids: hormonal specificity. *Wilhelm Roux's Arch.* **189**: 1–15.

CHERBAS L., LEE K. & CHERBAS P. 1991: Identification of ecdysone response elements by analysis of the Drosophila Eip28/29 gene. *Genes Dev.* **5**: 120–131.

CHIHARA C.J., PETRI W.H., FRISTROM J.W. & KING D.S. 1972: The assay of ecdysones and juvenile hormones on Drosophila imaginal discs in vitro. *J. Insect Physiol.* **18**: 1115–1123.

FRISTROM J.W. & YUND M.A. 1976: Characteristics of the action of ecdysones on Drosophila imaginal discs cultured in vitro. In Maramorosch K. (ed.): *Invertebrate Tissue Culture.* Academic Press, New York, pp. 161–178.

IMHOF O.M., RUSCONI S. & LEZZI M. 1993: Cloning of a Chironomus tentans cDNA encoding a protein (cEcRH) homologous to the Drosophila melanogaster ecdysteroid receptor (dEcR). *Insect Biochem. Molec. Biol.* **23**: 115–124.

KOELLE M.R., TALBOT W.S., SEGRAVES W.A., BENDER M.T., CHERBAS P. & HOGNESS D.S. 1991: The Drosophila EcR gene encodes an ecdysone receptor, a new member of the steroid receptor superfamily. *Cell* **67**: 59–77.

LEORA SOFTWARE 1987: *POLO-PC, User's Guide to Probit or Logit Analysis.* LeOra Software Inc., Berkeley, California, pp. 1–23.

MANDARON P. 1980: Drosophila imaginal disc development in vitro. In Kurstak E. & Maramorosch K. & Dübendorfer A. (eds): *Invertebrate Systems in Vitro.* Elsevier, Amsterdam, pp. 149–165.

OBERLANDER H. 1969: Effects of ecdysone, ecdysterone, and inokosterone on the in vitro initiation of metamorphosis of wing discs of Galleria mellonella. *J. Insect Physiol.* **15**: 297–304.

RIDDIFORD L.M. 1976: In vitro action of ecdysone and juvenile hormone on epidermal commitment in the tobacco hornworm. In Kurstak E. & Maramorosch K. (eds): *Invertebrate Tissue Culture*. Academic Press, New York, pp. 213–222.

SILHACEK D.L., OBERLANDER H. & PORCHERON P. 1990: Action of RH-5849, a nonsteroidal ecdysteroid mimic, on Plodia interpunctella (Hübner) in vivo and in vitro. *Arch. Insect Biochem. Physiol.* **15**: 201–212.

SMAGGHE G. & DEGHEELE D. 1993: Toxicity, pharmacokinetics, and metabolism of the first nonsteroidal ecdysteroid agonist RH-5849, on Spodoptera exempta (Walker), Spodoptera exigua (Hübner), and Leptinotarsa decemlineata (Say). *Pestic. Biochem. Physiol.* **46**: 149–160.

SMAGGHE G. & DEGHEELE D. 1994a: Action of a novel nonsteroidal ecdysteroid mimic, tebufenozide (RH-5992), on insects of different orders. *Pestic. Sci.* **42**: 85–92.

SMAGGHE G. & DEGHEELE D. 1994b: The significance of pharmacokinetics and metabolism to the biological activity of RH-5992 (tebufenozide) in Spodoptera exempta, Spodoptera exigua and Leptinotarsa decemlineata. *Pestic. Biochem. Physiol.* **49**: 226–234.

SPINDLER-BARTH M., TURBERG A. & SPINDLER K.-D. 1991: On the action of RH-5849, a nonsteroidal ecdysteroid agonist, on a cell line from Chironomus tentans. *Arch. Insect Biochem. Physiol.* **16**: 11–18.

SOBEK L., BOHM G.-A. & PENZLIN H. 1993. Ecdysteroid receptors in last instar larvae of the wax moth Galleria mellonella. *Insect Biochem. Molec. Biol.* **23**: 125–129.

TALBOT W.S., SWYRYD E.A. & HOGNESS D.S. 1993: Drosophila tissues with different metamorphic responses to ecdysone express different ecdysone receptor isoforms. *Cell* **73**: 1323–1337.

TERENTIOU P., BLATTMANN M., BRADBROOK D., KÄUSER G. & KOOLMAN J. 1993: Biological activity and receptor-binding of ecdysteroids in imaginal discs of Calliphora vicina: a comparison. *Insect Biochem. Molec. Biol.* **23**: 131–136.

VAN LAECKE K. & DEGHEELE D. 1991: Detoxification of diflubenzuron in the larvae of the beet armyworm (Spodoptera exigua) (Lepidoptera: Noctuidae). *Pestic. Biochem. Physiol.* **40**: 181–190.

WING K.D. 1988: RH-5849: A nonsteroidal ecdysone agonist: effects on a Drosophila cell line. *Science* **241**: 467–469.

WING K.D. & ALLER H.E. 1989: Ecdysteroid agonists as novel insect growth regulators. In Casida J.E. (ed.): *Pesticides and Alternatives*. Elsevier, Amsterdam, pp. 251–257.

WING K.D. & RAMSAY J.R. 1989: Other hormonal agents: ecdysone agonists. *Progress and Prospects in Insect Control*. BCPC Monograph No 43: 107–118.

WING K.D., SLAWECKI R.A. & CARLSON G.R. 1988: RH-5849, a nonsteroidal ecdysone agonist: effects on larval Lepidoptera. *Science* **241**: 470–472.

YAO T.-P., FORMAN B.M., SEGRAVES W.A., ORO A.E., MCKEOWN M. & EVANS R.M. 1992: Drosophila ultraspiracle modulates ecdysone receptor function via heterodimer formation. *Cell* **71**: 63–72.

YAO T.-P., FORMAN B.M., JIANG Z., CHERBAS L., CHEN J.-D., MCKEOWN M., CHERBAS P. & EVANS R.M. 1993: Functional ecdysone receptor is the product of EcR and Ultraspiracle genes. *Nature* **366**: 476–479.

YUND M.A. & OSTERBUR D.L. 1985: Ecdysteroid receptors and binding proteins. In Kerkut G.A. & Gilbert L.I. (eds): *Comprehensive Insect Physiology Biochemistry and Pharmacology. Vol. 7*. Pergamon, Oxford, pp. 473–490.

YUND M.A., KING D.S. & FRISTROM J.W. 1978: Ecdysteroid receptors in imaginal discs of Drosophila melanogaster. *Proc. Natl. Acad. Sci. USA* **75**: 6039–6043.