Antiecdysteroid activity of brassinosteroids

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Summary. We report the discovery of the first antiecdysteroids which belong to a comparatively new class of plant growth regulators, the brassinosteroids. These compounds bind competitively to ecdysteroid receptors partially purified from larvae of the blowfly *Calliphora vicina* and inhibit biological responses to 20-hydroxyecdysone, the active form of the molting hormone.

Key words. Ecdysteroid; brassinosteroid; hormone antagonist.

Steroids with signal functions are found in all three kingdoms of eucaryotic organisms – fungi, plants and animals[2]. The discovery that plants possess growth regulators with a steroidal structure has only been made recently[3] and since then a series of brassinosteroids have been isolated. They possess striking structural similarities to the ecdysteroids[4,5] (fig. 1). These similarities suggest that brassinosteroids and ecdysteroids share a common progenitor hormone which might be the ancestor of all steroid hormones existing today[6]. To see whether the analogy between brassinosteroids and ecdysteroids even extends to the recognition by ecdysteroid receptors we investigated the binding of two brassinosteroids and their synthetic precursors to ecdysteroid receptors from the blowfly, *Calliphora vicina.*

Results and discussion. Isolation, purification, and characterization of nuclear ecdysteroid receptors has been described in detail recently[7]. Binding of the brassinosteroids was studied by means of a competitive binding assay using the radiolabeled ecdysteroid ponasterone A as a high affinity receptor ligand. The synthetic brassinosteroids 22S,23S-homobrassinolide and 22S,23S-homocastasterone[5] (fig. 1) as well as the tetra-acetates of these compounds, which served as synthetic precursors, clearly competed with (^{3}H)ponasterone A for the binding to ecdysteroid receptors. Figure 2 shows competition curves for homocastasterone, homobrassinolide and the ecdysteroids 20-hydroxyecdysone and ecdysone. An equilibrium dissociation constant (K_D) of 5×10^{-6} M for the homocastasterone-receptor complex could be estimated from the competition curves. This K_D is of the same order of magnitude as the K_D for the molting hormone secreted by the endocrine gland, ecdysone, which is 2×10^{-6} M. Brassinosteroids bound to ecdysteroid receptors could be displaced again by an elevation of the (^{3}H)ponasterone A concentration, demonstrating the competitive nature of the binding.

It has been independently shown by two research groups that 22S,23S-homobrassinolide inhibits the evagination of imaginal discs of the dipteran, *Phormia terra-novae*, in vitro[8] and that it causes a delay in the imaginal molt when fed to the last larval instar of the cockroach, *Periplaneta americana*[9]. Our results show that these effects can be explained by the competitive displacement of 20-hydroxyecdysone from its receptor binding site. 22S,23S-homocastasterone, however, which was the most effective competitor in the binding assay, did not disturb the imaginal molt when fed to cockroaches[9]. We suppose that the lack of effect in the in vivo system is due to metabolism of homocastasterone by enzymes normally acting on ecdysteroids. This would lead to a rapid decline of the

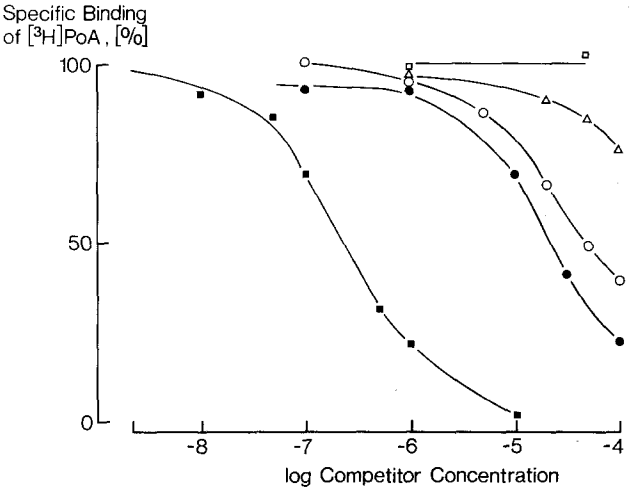

Figure 2. Competition curves for the binding of 20-hydroxyecdysone (■), ecdysone (●), 22S,23S-homocastasterone (○), 22S,23S-homobrassinolide (△) and azadirachtin (□) to ecdysteroid receptors.
A purified preparation of ecdysteroid receptors from nuclei of third instar larvae of *Calliphora* was incubated with 8 nM (^{3}H)ponasterone A in the presence of increasing concentrations of the foregoing unlabeled competitors. After equilibrium had been achieved, specific binding of (^{3}H)ponasterone A was assayed. The amount of (^{3}H)ponasterone A specifically bound in the absence of any competitor was defined as 100%. For further experimental details see ref. 7.

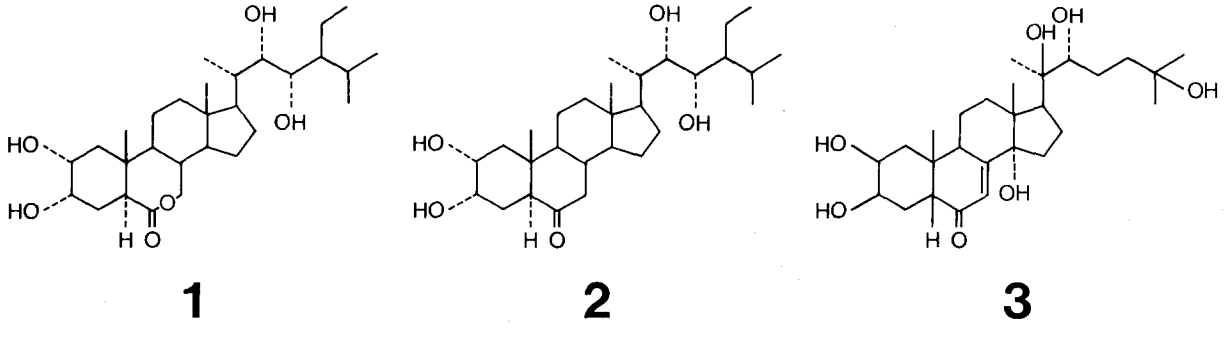

Figure 1. Chemical structures of 22S,23S-homobrassinolide (**1**), 22S,23S-homocastasterone (**2**) and 20-hydroxyecdysone (**3**).

effective concentration of this compound. When investigated in the imaginal disc evagination assay, castasterone, a compound closely related to 22S,23S-homocastasterone, turned out to be the most effective inhibitor of the biological response to 20-hydroxyecdysone[8]. This is in accordance with our finding that the absence of the seven-membered ring B lactone structure, which constitutes the only structural difference between 22S,23S-homocastasterone and 22S,23S-homobrassinolide, favors the binding to ecdysteroid receptors. In view of the large diversity of brassinosteroids, it should be rewarding to test further compounds with the aim of acquiring further insight into their structure-activity relationship.

On the basis of molt-inhibiting effects, it has been recently suggested that azadirachtin, a tetranortriterpenoid compound and natural insecticide isolated from the Neem tree, might also act as an antiecdysteroid[10]. This hypothesis lacks experimental support so far, since we were unable to find an interference of azadirachtin with the binding of ecdysteroids to their receptors (fig. 2). However, azadirachtin appears to act on other parts of the ecdysteroid hormone system[11].

We hope that our study will stimulate further research on the interaction of brassinosteroids and the ecdysteroid hormone system. Did this interaction play a role in the co-evolution of insects and plants? Above all, antiecdysteroids with even higher antagonistic activity may remain undiscovered among the group of brassinosteroids. Those antiecdysteroids could be highly valuable tools as ligands for receptor studies and as potential insecticides for target-directed and safer pest control.

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Naturally occurring heterocycles inducing drought resistance in plants[1]

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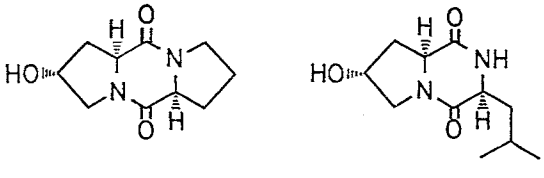
Summary. During screening of animal metabolites which induce drought resistance in plants, two diketopiperazines, cyclo(L-Hyp-L-Pro) (**1**) and cyclo(L-Hyp-L-Leu) (**2**), emerged as effective. When rice seeds were pretreated with the cyclic dipeptides (**1** and **2**) during their germination period, the resulting seedlings showed significant resistance to water-stress caused by 0.5–1.5% NaCl solution or 2.5–5.0% mannitol solution.
Key words. Diketopiperazines; hydroxyproline; drought resistance; plant growth regulators.

Since Bonner suggested the possibility of a 'chemical cure of climatic lesions'[2,3], many attempts have been made to prevent by this means a decrease of agricultural yield resulting from abnormal environments[4]. Older treatments, such as hardening, are clearly effective in curing desiccated plants[5,6] but have the disadvantage that they damage plants under normal conditions. Accordingly, we have screened certain animal metabolites, which can induce drought resistance in plants but which suffer from no such disadvantages. We have already reported[1] that the tricyclic diketopiperazine, L-hydroxyprolyl-L-proline anhydride (**1**) (named as D-104), and the bicyclic diketopirazine, L-hydroxyprolyl-L-leucine anhydride (**2**) (named as D-301), were isolated from rabbit skin and that they act as germination promoters. Thus, as shown in figure 2, the effect of **1** at 20 °C was marked and reproducible. However, its promoting effect on rice germination at 30 °C (its optimal temperature) was very weak or non-existent. Similar observation was also obtained in a case of **2** (data not shown). The normalizing effect of **1** and **2** at low temperature occurred without any changes in cellular size. It is well known[7] that proline accumulation occurs generally in plants suffering from climatic lesions, such as abnormal temperature or water deficiency. If this phenomenon is involved in the normalizing effect of the diketopiperazines (**1** and **2**) on the germination of low-temperature stressed rice, then both compounds (which include hydroxyproline in their structures) may also induce drought resistance by a similar mechanism.

To test this hypothesis, it seemed best to treat experimental plants with **1** or **2** at the germination stage, when concentration control of the test material is easiest. Accordingly, rice seeds were immersed in the test solutions during the first four days of the germination period prior to transplantation into water culture vessels containing NaCl or mannitol solutions to raise osmotic pressure. Two control groups, in which seedlings germinated in pure water were subsequently cultured with or without a solute causing higher osmotic pressure, were compared with the test groups.



1 (D-104) **2 (D-301)**

Figure 1. Hydroxyproline-containing diketopiperazines from rabbit skin.