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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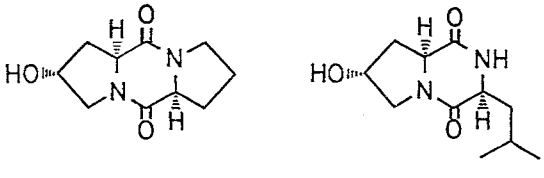
Summary. During screening of animal metabolites which induce drought resistance in plants, two diketopiperazines, cyclo(L-Hyp-L-Pro) (**1**) and cyclo(L-Hyp-L-Leu) (**2**), emerged as effective. When rice seeds were pretreated with the cyclic dipeptides (**1** and **2**) during their germination period, 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.

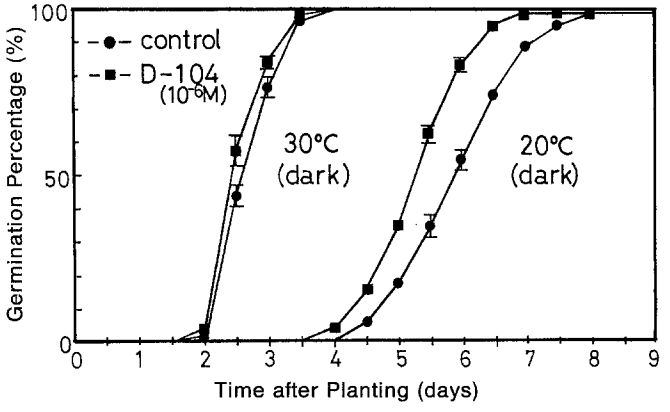


Figure 2. Effect of D-104 on rice germination. Each group was composed of three dishes containing 100 seeds of rice (*Oryza sativa* L. *japonica*) for which germination percentages were recorded every 12 h and mean values and their standard deviations were calculated.

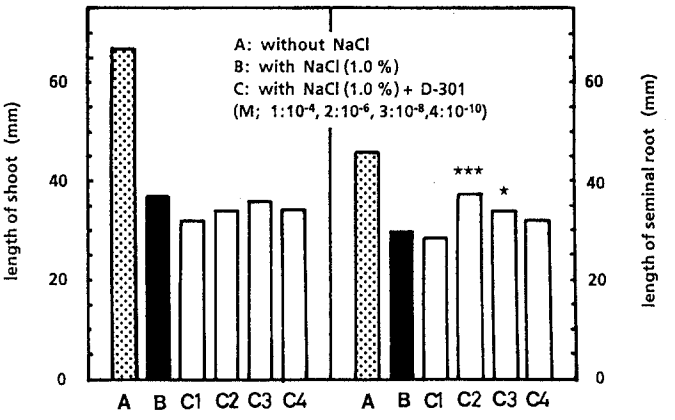


Figure 4. Effect of D-301 on rice seedlings under water stress caused by 1.0% NaCl. Lengths of both shoot and roots were measured 7 days after transplantation from germination dishes to water culture vessels (30 °C; 14 h daylight) with/without NaCl-solution. Only during the germination period (4 days), seeds were treated with corresponding test solutions (30 °C; 14 h daylight). Results of t-test between B and C groups are indicated thus: *** $p < 0.001$; * $p < 0.05$; $n = 25$.

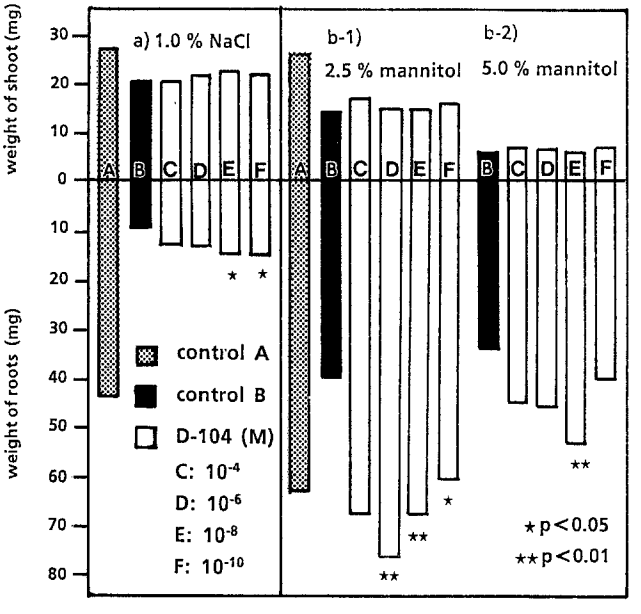


Figure 3. Effect of D-104 on rice seedlings under water stress caused by 1.0% NaCl (a), 2.5% mannitol (b-1) and 5.0% mannitol (b-2). Shoot and roots were blotted dry and their fresh weights were measured 7 days after transplantation from germination dishes to water culture vessels (30 °C; 14 h daylight) with/without NaCl or mannitol solution. The seeds were treated with D-104 test solutions only during the germination period (4 days) (30 °C; 14 h daylight). An intact control without NaCl or mannitol solution is shown as control A. Results of t-test between a control group (B) and a test group (C, D, E or F) are indicated thus: ** $p < 0.01$; * $p < 0.05$; $n = 25$.

Substance **1** and **2** both showed a significant effect on seedlings stressed by 2.5% mannitol (3.35 atm). Thus, as shown in figure 3, pretreatment with **1** (10^{-6}, 10^{-8} and 10^{-10} M) increased the weight of roots significantly by 88, 68 and 66%, respectively, in comparison with the control group. A similar significant effect on roots was observed even in the 5.0% mannitol (6.71 atm) treated test groups. In both cases, **1** did not affect the shoot weight. We also examined the effect of **1** on water stress caused by salt (1.0% NaCl; 3.84 atm). In this case, a similar significant effect on roots was observed along with a slight effect on the shoot weight. The NaCl stress seemed to cause a salt stress in addition to water stress, because the control value of root weight in the 1.0% NaCl-treated group was much less than that in the 5.0% mannitol-treated group, which had a higher osmotic pressure. Although D-301 (**2**) had similar effects on root-weights of rice seedlings as did D-104 (**1**) (data not shown), its effect on their lengths were clearer, as shown in figure 4; pretreatment with **2** (10^{-6} and 10^{-8} M) protected seminal roots from damage by salt stress to the extent of 47 and 21%, respectively; shoot length was not affected.

The mode of action of **1** and **2** remains unknown. However, the two diketopiperazines must be metabolized from collagen in animal skin. By analogy, we hypothesize that endogenous hydroxyproline-containing diketopirazine(s) may be formed from the hydroxyproline-rich glycoprotein laid down in the plant cell wall and afford a measure of resistance against some kinds of stress. Addition of exogenous **1** and **2** may be most effective under conditions of stress, because endogenous materials inducing stress-resistance might be particularly necessary in such cases.

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