Note

The Rice Lamina Inclination-promoting Activity of Synthetic Brassinolide Analogues with a Modified Side Chain

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Brassinolide, a novel plant growth-promoting steroid lactone isolated from rape pollen,¹⁾ has been shown to possess a wide variety of biological activities in selected bioassays for auxin, gibberellin and cytokinin.^{2~5)} We have previously reported that brassinolide exhibited an extremely strong activity in a rice lamina inclination test,⁷⁾ and the method can be used as a simple and highly sensitive bioassay for brassinolide and related brassinosteroids.⁶⁾ Recently, new brassinosteroids have been isolated from several plants by Japanese scientists,^{8~13)} and their structures were elucidated by means of modern ultramicro-techniques. It should be emphasized that the

lamina inclination assay was employed by these scientists for isolation of new brassinosteroids, in particular, the 6-keto type of brassinosteroids were first detected from plants as a result of using this bioassay, in addition to the lactone type of new brassinosteroids.^{12,13)}

The response of the lamina joints to brassinosteroids seemed to be different from that of the bean second internode, the latter being originally used as the bioassay for isolation of brassinolide by the USDA group. Namely, the lamina inclination assay was highly sensitive to the 6-keto type of brassinosteroids as well as to the lactone types, whereas the bean bioassay was only susceptible to the lactone type of brassinosteroids in the limited bioassay time.

We continued chemical¹⁴⁾ and biological⁶⁾ studies on brassinosteroids in order to examine their potent activity in lamina inclination, and here we describe that the modification of the side chain of brassinolide (the lactone type) affects their activity on the lamina inclination of rice seedlings.

The bioassay procedure for the lamina inclination test was described in a previous paper.⁶⁾ The synthetic brassinolide analogues tested in this research were synthesized as already reported.^{15~18)}

The bioassayed results are summarized in Table I. Brassinolide (1) itself showed the strongest activity among the synthetic analogues (1~10). 28-Homobrassinolide (2) showed a similar activity to brassinolide, giving almost the same bending angles between laminae and sheaths at concentrations from 0.01 to 0.0001 $\mu\text{g/ml}$, indicating that the (24*S*)-methyl group in 1 can be replaced by (24*S*)-ethyl without any decrease of activity. The (22*S*,23*S*)-diol isomer (3) of 28-homobrassinolide (2) showed about one tenth the activity of 2. In the series of 28-norbrassinolides (4~7), only (22*R*,23*R*)-diol (4) with the correct side chain stereochemistry showed a rather strong activity; that is, when compared by their threshold concentrations (Fig. 1),

FIG. 1. Effect of Side Chain Structure of Brassinolide Analogues on the Lamina Inclination.

4 showed one tenth the activity of brassinolide, whereas the other three isomers (5~7) exhibited only a weak activity (0.5 $\mu\text{g/ml}$). Interestingly, both 23-deoxy- and 22,23-dideoxy-28-norbrassinolide (8 and 9) showed significant activity at 0.05 $\mu\text{g/ml}$, which was superior to that of their 22,23-dihydroxy-derivatives (5, 6 and 7) with the wrong stereochemistry. An analogue with no side chain (10) was only slightly active even at the high concentration of 50 $\mu\text{g/ml}$.

In a previous paper,¹⁴⁾ we mentioned that functional groups of ring A and B in brassinolide were very important for the manifestation of biological activity. From the above results, we believe that the side chain of brassinolide is also important, but some degree of modification is allowable for exhibiting activity on the lamina inclination of rice seedlings. Thus, the (22S,23S)-diol isomer (3) of 28-homobrassinolide (2) would show a rather strong activity, owing to a conformational change of the side chain which might make binding of the 22,23-vicinal diol moiety to the active site of the lamina joint possible, whereas the lack of 28-alkyl substituent in 28-norbrassinolides would result in difficulty of the diols with the wrong stereochemistry to fit with the active site.

Our further examination of the 6-keto type of brassinosteroids on the lamina inclination of rice seedlings will be reported in a forthcoming paper.

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