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Note

Structure–activity relationship of naturally occurring strigolactones in *Orobanche minor* seed germination stimulation

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Eleven naturally occurring strigolactones (SLs) were examined for their germination-stimulating activity on the seeds of a root parasitic plant *Orobanche minor* Sm. Based on their activity, SLs are classified into 3 groups (A–C). Group A, the most active germination stimulant, consists of 3 monohydroxy-SLs, orobanchol, 2′-epiorobanchol, and sorgomol, inducing >80% germination of *O. minor* seeds at 10 pM. Group B includes 5 SLs, which were *ca*. 10-fold less active than those in group A. The 3 least active SLs in group C were either more lipophilic or probably less stable than SLs in groups A and B. These results indicate that the germination-stimulating activity of SLs depends on the lipophilicity of the SL molecules and their stability also influences activity. Other structural features for germination-stimulating activity are also discussed. © Pesticide Science Society of Japan

Keywords: germination stimulant, *Orobanche*, root parasitic plants, *Striga*, strigolactone.

Introduction

Witchweeds (*Striga* spp.) and broomrapes (*Orobanche* and *Phelipanche* spp.) in the family Orobanchaceae are the two most devastating root parasitic weeds causing substantial crop losses throughout the world. Since they are intimately associated with their host roots and spend most of their life underground, effective and economically acceptable control measures have not yet been established.[1)] Seeds of these root parasites have special requirements for germination. The seeds should be kept in a wet environment at appropriate temperatures for several days to break dormancy. This pre-incubation period is called 'conditioning'. The conditioned seeds, however, will not germinate unless they are exposed to host-derived chemicals, termed 'germination stimulants', released from plant roots.[1)]

Among the germination stimulants identified to date, strigolactones (SLs) are the most potent and are distributed widely in the plant kingdom.[2)] In the rhizosphere, SLs induce not only seed germination of parasitic weeds but also spore germination and hyphal branching of symbionts arbuscular mycorrhizal (AM) fungi,[3, 4)] and thus SLs function as signals for both symbiosis and parasitism.[5)] In addition to these functions of SLs in the rhizosphere, SLs or their further metabolites act as a class of plant hormones inhibiting shoot branching.[6,7)] Therefore, it is important to clarify the structure-activity relationships of SLs in these three different functions to develop feasible control measures for root parasitic weeds without or with little effects on AM symbiosis and/or shoot architecture.[8,9)]

In the present study, naturally occurring SLs, including those isolated recently in our laboratory, were examined for their germination stimulation on the seeds of a root parasitic plant, *Orobanche minor* Sm., and the structure-activity relationships of SLs in the stimulation of *O. minor* seed germination are discussed.

Materials and Methods

1. Chemicals

(+)-Strigol, (±)-sorgolactone, and (±)-5-deoxystrigol were generous gifts from Emeritus Prof. Kenji Mori (The University of Tokyo, Tokyo, Japan), Prof. Yukihiro Sugimoto (Kobe University, Kobe, Japan), and Dr. Kohki Akiyama (Osaka Prefecture University, Sakai, Japan), respectively. GR24 was provided by Prof. Binne Zwanenburg (Radboud University Nijmegen, The Netherlands). The other SLs were purified from plant root exudates: orobanchol and orobanchyl acetate from red clover,[10,11)] 2′-epiorobanchol and solanacol from tobacco,[12)] sorgomol from sorghum,[13)] fabacyl acetate from pea,[14)] and 7-oxoorobanchyl acetate and 7-oxoorobanchol from flax.[15)] These SLs were dissolved in HPLC-grade acetone and used for germination tests.

2. Seeds and seed germination test

O. minor Sm. seeds were collected from mature plants that parasitized red clover (*Trifolium pratense* L.) grown in the Watarase basin of Tochigi Prefecture, Japan. Germination tests on *O. minor* seeds were conducted as reported previously.[16)] Surface-sterilized seeds, *ca.* 20 each, were placed on 6-mm glass fiber disks (What-

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Table 1. Germination-stimulating activity of strigolactones on *Orobanche minor* seeds

Strigolactones (Group)	Germination (%)				
	1 pM	10 pM	100 pM	1 nM	10 nM
Monohydroxy-SLs					
strigol (B)[a]	5±2.3	24±3.2	87±1.7	92±1.7	89±2.3
orobanchol (A)	23±1.8	80±2.3	91±2.1	92±2.2	90±2.0
2′-epiorobanchol (A)	74±2.1	86±2.5	95±1.4	95±0.9	96±1.4
sorgomol (A)	13±3.5	76±3.5	84±2.9	89±2.3	93±1.2
solanacol (B)	0	15±2.9	97±0.8	90±2.0	94±0.9
7-oxoorobanchol (C)	0	0	23±3.3	76±3.9	83±3.6
Acetates					
orobanchyl acetate (B)	0	18±3.5	95±2.7	93±1.9	97±1.8
7-oxoorobanchyl acetate (B)	0	12±2.3	84±3.3	95±2.0	97±0.9
fabacyl acetate (B)	3±1.0	21±2.5	77±1.7	88±1.2	90±1.9
More lipophilic SLs					
sorgolactone (C)[a]	0	0	12±2.3	67±3.4	92±2.9
5-deoxystrigol (C)[a]	0	4±1.7	31±3.4	87±1.7	88±2.3
GR24	0	0	0	0	11±0.8

Data are the means±S.E. (n=3). [a] Synthetic samples.

man GF/A) and *ca.* 90 disks were incubated in a 9-cm Petri dish lined with a sheet of filter paper (No. 2; Advantec, Tokyo, Japan) and wetted with 6 ml sterile Milli-Q water in the dark at 23°C for 7 days as a conditioning period during which the seeds became responsive to germination stimulants. Four disks carrying the conditioned seeds were then transferred to a 5-cm sterile Petri dish lined with filter paper and wetted with 650 μl test solution. Each test solution, unless otherwise mentioned, contained 0.1% (v/v) acetone. The Petri dishes were sealed, wrapped in aluminum foil, and placed in the dark at 23°C for 4–5 days. Seeds were considered germinated when the radicle protruded through the seed coat.

Results and Discussion

The structures of 11 naturally occurring SLs examined in this study are shown in Fig. 1. This is the first report on the comparison of germination-stimulating activities of the most naturally occurring SLs, including those identified recently. These SLs commonly contain a tricyclic lactone (ABC part) that connects to a butenolide group (D ring) *via* an enol ether bridge. All induced more than 80% germination of *O. minor* seeds at or below 1 nM. Based on their germination-stimulating activities on *O. minor* seeds, the SLs examined in this study were classified into three groups, A, B, and C (Table 1). The SLs in groups A, B, and C induced the high germination (∼80%) of *O. minor* seeds at 10 pM, 100 pM, and 1 nM, respectively. GR24, the most active synthetic analogue, elicited moderate (59±0.2 %) germination at 100 nM and thus was *ca.* 100-fold less active than naturally occurring SLs.

Group A, the most active germination stimulants, included three monohydroxy-SLs: orobanchol, 2′-epiorobanchol and sorgomol. Among these SLs, 2′-epiorobanchol was the most active stimulant on this root parasite and effected high germination

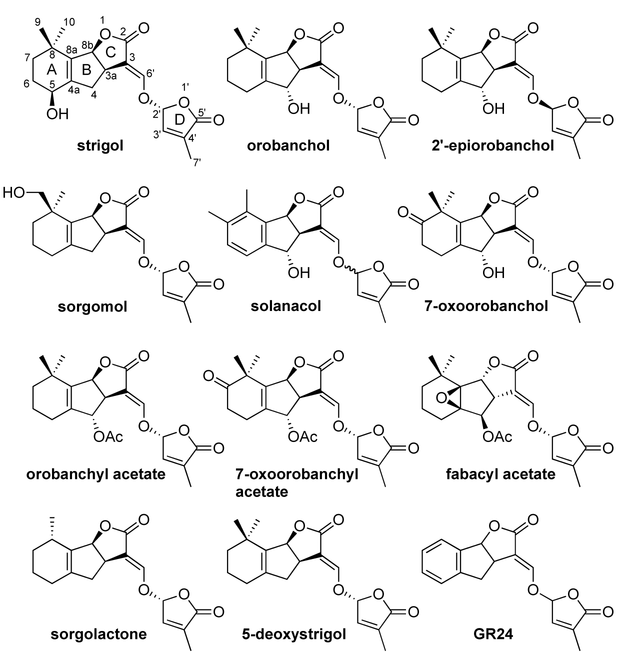

Fig. 1. Structures of naturally occurring strigolactones used in the study and the synthetic strigolactone GR24.

(74%) at as low as 1 pM. The two monohydroxy-SLs, strigol and solanacol, were *ca.* 10-fold less active than those in group A, displayed high activity (>80% germination) at 100 pM, and were thus included in group B. Among the monohydroxy-SLs examined in this study, 7-oxoorobanchol was the least active stimulant and exhibited high germination (76%) induction of *O. minor* seeds at 1 nM (group C).

Therefore, among the monohydroxy-SLs, a 100-fold difference was observed in their germination-stimulating potencies. These results indicate that, in general, the introduction of a hydroxyl group on the A/B ring moiety has a positive effect on germination-stimulating activity. Higher activities of orobanchol, 2′-epi-orobanchol, and sorgomol, as compared to strigol, suggest that the position of the hydroxyl group influences activity and the introduction of a hydroxyl group at C-4 (orobanchol and 2′-epi-orobanchol) or C-9 (sorgomol) is preferable than at C-5 (strigol). In particular, the presence of an α-hydroxyl group at C-4 seems to enhance germination-stimulating activity since solanacol, 5,7-dimethyl-4α-hydroxy-GR24,[17] was 1000-fold more active than GR24.[12] The relatively weak activity of 7-oxoorobanchol was due to its instability.[15]

C-2′ stereochemistry has been reported to be important for germination-stimulating activity. In the case of strigol, sorgolactone, and GR24, stereoisomers carrying the C-2′-(*R*) configuration were far more active than their (*S*) isomers.[18-21] In addition, all natural SLs, except for 2′-epiorobanchol, have the C-2′-(*R*) configuration. Although the C-2′ configuration of solanacol has not been established, it is likely that it has a 'natural' C-2′-(*R*) configuration; therefore, 2′-epiorobanchol is an exception and its extremely potent activity would also be attributable to the positive effect of the C-4α-hydroxyl group on germination-stimulating activity.

Acetates of monohydroxy-SLs, orobanchyl acetate, 7-oxo-orobanchyl acetate, and fabacyl acetate, induced ~80% germination of *O. minor* seeds at 100 pM and were classified into group B. As in the case of strigol and strigyl acetate,[22] acetylation of the hydroxyl group in orobanchol resulted in a 10-fold reduction of germination-stimulating activity, indicating that presence of a free hydroxyl group is preferable for higher activities.[11] By contrast, 7-oxoorobanchyl acetate was 10-fold more active than 7-oxoorobanchol probably due to improved stability by acetylation of the hydroxyl group. Therefore, the effect of acetylation on germination-stimulating activity varies with the stability of hydroxy-SL; acetates may be less active with more stable hydroxy-SLs, but more active with less stable hydroxy-SLs.

The least active SLs belonging to group C are sorgolactone, 5-deoxystrigol and 7-oxoorobanchol. Sorgolactone and 5-deoxystrigol are major SLs of sorghum and maize, hosts of *Striga* spp.[23] These two SLs lack oxygen-containing substituents on the A/B ring moiety, and are more lipophilic and would be chemically more stable than the SLs in groups A and B. These more stable SLs might be involved in the germination stimulation of *Striga* seeds in alkaline soils that are common in Sub-Saharan Africa.[24] The low germination-stimulating activity of 7-oxoorobanchol is attributable to its instability, as discussed before.

Accordingly, the germination-stimulating activity of naturally occurring SLs on *O. minor* seeds was found to depend on the lipophilicity of SL molecules, and their stability also influenced activity. In the present study, the germination-stimulating activities of SLs were examined on *O. minor* seeds in Petri dishes. The other *Orobanche* and *Striga* species may respond differently even in the Petri dish test. Furthermore, the intrinsic activity, stability, and amount of exudation should be taken into account to assess the contribution of each SL in the induction of seed germination in soil.

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