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Structural Requirements of Strigolactones for Germination Induction of *Striga gesnerioides* Seeds

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ABSTRACT: Strigolactones are highly potent germination stimulants for seeds of the parasitic weeds *Striga* and *Orobanche* spp. 4-Hydroxy-GR24 and 4-acetoxy-GR24 were prepared and their abilities to induce seed germination of *Striga gesnerioides* evaluated. Optically active (8b*R*,2′*R*)-isomers induced germination, although the racemic diastereomers were inactive. In contrast, the stereoisomer of GR24 with the same configuration induced negligible germination. Some stereoisomers of GR24 and its analogues acted as effective antagonists for induction of seed germination by cowpea root exudates. These results suggest that both an oxygenated substituent at C-4 and the configuration of the tricyclic lactone and the D-ring are essential structural requirements for induction of germination in *S. gesnerioides* seeds.

KEYWORDS: strigolactone, *Striga gesnerioides*, synthetic strigolactone GR24, partial agonist, antagonist

INTRODUCTION

The root parasitic weeds of the genera *Orobanche* and *Striga* (Orobanchaceae) severely reduce the yield of economically important crops in tropical and semitropical areas of the eastern hemisphere and the Mediterranean region.[1] The parasites are copious seed producers, and their life cycle is strongly linked to that of their hosts. The parasitic process begins with the germination of the weed seeds, which is induced by the presence of stimulants derived from the root exudates of the host and some nonhost plants.[2] Most of the germination stimulants isolated thus far possess the same basic skeleton and are collectively referred to as strigolactones, as first proposed by Butler.[3] Naturally occurring strigolactones include strigol,[4] sorgolactone,[5] alectrol,[6] orobanchol,[7] 5-deoxystrigol,[8,9] fabacyl acetate,[10] and solanacol[11,12] (Figure 1). Because the holoparasite *Orobanche* completely lacks chlorophyll and the obligate hemiparasite *Striga* can only partly assimilate, they are entirely dependent on their host to survive and develop. Therefore, an attractive approach for the control of parasitic weeds is to interfere with the intimate relationship between host and parasitic plants. Stimulating germination in the absence of a host plant will kill the germinated seeds and hence reduce the seed population in the soil.

This concept of suicidal germination[13,14] has attracted attention for the development of synthetic analogues of strigolactones with simpler structures and higher activity. As a result, a series of so-called GR compounds have been developed.[15] Among them, GR24 (**1**) is the most active and used worldwide for research into parasitic weeds such as *Striga hermonthica* and *Orobanche minor* as a reference compound in bioassays. However, the seeds of *Striga gesnerioides* do not respond to GR24[16] and germinate only by exposure to cowpea (*Vigna unguiculata*) root exudates or alectrol, which is the active constituent isolated from cowpea. Since the proposed structure for alectrol was disproven,[17] the structure of the strigolactone had remained obscure until alectrol was reisolated from cowpea root exudates as a germination stimulant for the seeds of *S. gesnerioides*,[18] and its structure was proposed as orobanchyl acetate.[19] Alectrol is much more effective than strigol in inducing germination of *S. gesnerioides* seeds,[6] suggesting that modification at C-4 in the B-ring with an acetoxy group is important for germination induction of *S. gesnerioides* seeds.

In the present study we evaluated the germination stimulatory activity of GR24 analogues with modification of the hydroxyl and acetoxy groups at C-4 of the GR24 molecule, named HO-GR24 (**2**) and AcO-GR24 (**3**), respectively, toward seeds of *O. minor*, *S. gesnerioides*, and *S. hermonthica*. To gain insight into the importance of stereochemistry for inducing *S. gesnerioides* seed germination, we also evaluated the potency of each stereoisomer of the synthetic strigolactones.

MATERIALS AND METHODS

Apparatus. ^{1}H and ^{13}C NMR spectra were taken in $CDCl_3$ with a JNM-AL300 spectrometer (JEOL, Ltd., Tokyo, Japan), using tetramethylsilane (TMS) as an internal standard. Chemical shifts are shown in δ (ppm). Mass spectra were obtained on a JMS-700 spectrometer (JEOL). Circular dichroism (CD) spectra were recorded with a J-805 spectropolarimeter (JASCO Corp., Tokyo, Japan). Optical rotation was recorded with a SEPA-300 (Horiba, Ltd., Kyoto, Japan) or DIP-1000 digital polarimeter (JASCO). Silica gel 60N (Kanto Chemical Co., Inc., Tokyo, Japan) or Wakogel C-200 (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was used for column chromatography, and precoated silica gel plates, Kieselgel 60 F_{254} (Merck, Darmstadt, Germany), were used for analytical thin layer chromatography (TLC).

Chemicals. GR24 (**1**) was provided by Prof. Binne Zwanenburg (Nijmegen University, Nijmegen, The Netherlands). HO-GR24 (**2**) was prepared from racemic α-hydroxylactone [(±)-(3a*S**,4*S**,8b*S**)-4-hydroxy-3,3a,4,8b-tetrahydro-2*H*-indeno[1,2-*b*]furan-2-one] using a previously reported method[20,21] as an epimer mixture (ca. 1:1) at C-2′. For the preparation of AcO-GR24 (**3**), acetic anhydride (1.07 g, 10.5 mmol) was added to a solution of **2** (330 mg, 1.05 mmol) in dry pyridine

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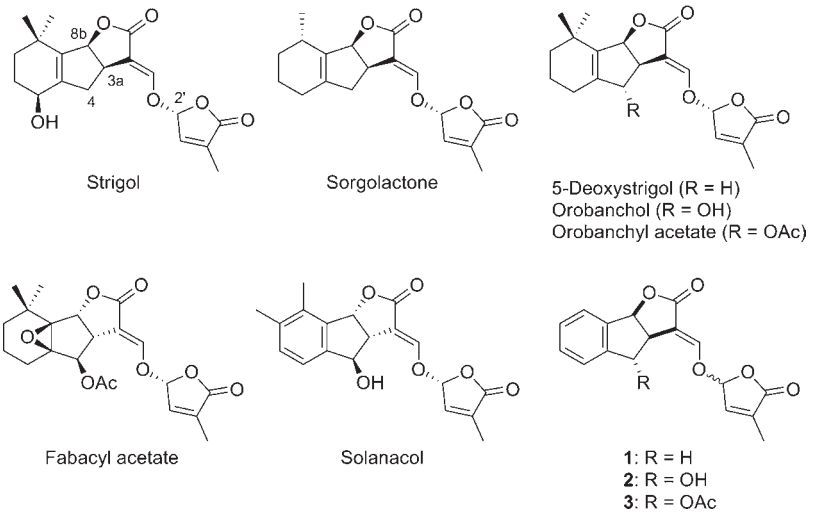

Figure 1. Structures of naturally occurring and synthetic strigolactones. Naturally occurring strigolactones are enantiomerically pure, whereas compounds **1**−**3** are racemic and diastereomeric mixtures. The true structure of solanacol has been reported by Chen et al.[12].

(5 mL). After stirring overnight at room temperature, the mixture was diluted with 3 M HCl solution and extracted with CH_2Cl_2. The organic layer was washed with water and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel to obtain **3** (305 mg, 82%) as a diastereomeric mixture, with HREIMS m/z 379.0797 $[M + Na]^+$ ($C_{19}H_{16}O_7Na$ requires 379.0794). Part of this mixture was further purified by silica gel column chromatography to obtain the pure fast-moving and slow-moving diastereomers of **3**. The fast-moving diastereomer was characterized as follows: ^{1}H NMR (300 MHz, $CDCl_3$), δ 2.04 (t, J = 1.5 Hz, 3H, 4′-Me), 2.07 (s, 3H, Ac), 3.85−3.90 (m, 1H, 3a-H), 6.12 (d, J = 7.5 Hz, 1H, 8b-H), 6.18 (br s, 1H, 2′-H), 6.46 (s, 1H, 4-H), 7.02 (t, J = 1.5 Hz, 1H, 3′-H), 7.41−7.62 (m, 5H, 5-, 6-, 7-, 8- and 6′-H); ^{13}C NMR (75 MHz, $CDCl_3$), δ 10.8, 21.2, 47.2, 78.9, 83.7, 100.6, 108.7, 126.5, 126.6, 130.6, 130.7, 135.8, 140.48, 140.52, 141.0, 152.9, 170.17, 170.21, 170.3. The slow-moving diastereomer was characterized as follows: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.07 (br s, 6H, 4′-Me and Ac), 3.86−3.91 (m, 1H, 3a-H), 6.12 (d, J = 7.5 Hz, 1H, 8b-H), 6.22 (br s, 1H, 2′-H), 6.39 (s, 1H, 4-H), 6.98−6.99 (t, J = 1.5 Hz, 1H, 3′-H), 7.41−7.62 (m, 5H, 5-, 6-, 7-, 8- and 6′-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 10.8, 21.1, 47.4, 78.9, 83.7, 100.0, 109.0, 126.4, 126.7, 130.58, 130.63, 136.4, 140.4, 140.5, 140.8, 151.7, 169.9, 170.0, 170.3.

(+)-Deoxystrigol was isolated as reported by Sugimoto and Ueyama.[9] From *Lotus japonicus* root (300 g), 5-deoxystrigol (1.1 μg) was isolated, and the weight was estimated using the molar absorbance coefficient.[22] (+)-Orobanchol was kindly supplied by Emeritus Professor Kenji Mori (The University of Tokyo, Tokyo, Japan). Acetylation of (+)-orobanchol was performed as follows: (+)-orobanchol (0.50 mg) was dissolved in acetic anhydride (0.5 mL), and a small quantity of trichloroacetic acid was added to the solution. The mixture was left for 3 days at 4 °C and then poured into H_2O (15 mL). The resulting mixture was extracted with EtOAc. The organic layer was washed with H_2O, dried over Na_2SO_4, and concentrated. The residual oil was purified by silica gel column chromatography with 35% EtOAc in hexane to give (+)-orobanchyl acetate (0.52 mg, 92%) as a colorless oil. Characterization was as follows: ^{1}H NMR (300 MHz, $CDCl_3$), δ 1.14 and 1.16 (s, each 3H, 8-Me_2), 1.41−1.52 (m, 2H, 7-H_2), 1.61−1.76 (m, 2H, 6-H_2), 1.88−1.98 (m, 2H, 5-H_2), 2.01 (m, 4′-Me), 2.06 (s, 3H, 2″-Me), 3.44 (ddd, J = 7.3, 2.6, and 1.9 Hz, 1H, 3a-H), 5.62 (d, J = 7.3 Hz, 1H, 8b-H), 5.80 (s, 1H, 4-H), 6.13 (m, 1H, 2′-H), 6.97 (m, 1H, 3′-H), 7.58 (d, J = 2.6 Hz, 1H, 6′-H); $[\alpha]_D^{28}$ +148.8 (c 0.054, $CHCl_3$).

Separation of GR24 Stereoisomers. A racemic and diastereomeric mixture of GR24 (**1**; 51 mg) was loaded onto the silica gel column and eluted with 30−35% EtOAc in hexane to obtain the fast-moving diastereomer (*rac*-**1a**; 21 mg; R_f 0.52, hexane/EtOAc = 1:1) as colorless needle-like crystals and eluted with 35−40% EtOAc in hexane to obtain the slow-moving diastereomer (*rac*-**1b**; 26 mg; R_f 0.46, hexane/EtOAc = 1:1) as a colorless oil. The relative configurations of these racemates were determined by comparisons of R_f values in TLC analysis.[23] Optical resolution of *rac*-**1a** and *rac*-**1b** was performed using HPLC with a 250 × 4.6 mm i.d., 5 μm, Chiralcel OJ (Daicel Chemical Ind., Ltd., Osaka, Japan) and 50% EtOH in hexane as the mobile phase at a flow rate of 0.5 mL/min. The objective compounds were detected by absorption at 237 nm. Compounds **1a**, *ent*-**1a**, **1b**, and *ent*-**1b** were eluted at 26, 30, 31, and 25 min, respectively. The absolute configurations of these isomers were determined by the sign of the optical rotation compared to those reported previously:[22,23] **1a**, $[\alpha]_D^{25}$ +366 (c 0.15, $CHCl_3$) {$[\alpha]_D$ +436 (c 0.25, $CHCl_3$)[23]}, CD (MeCN, c 0.000064) λ_{ext} ($\Delta\varepsilon$) 228 (+33.0), 205 (−4.2) nm; *ent*-**1a**, $[\alpha]_D^{25}$ −423 (c 0.11, $CHCl_3$) {$[\alpha]_D$ −446 (c 0.25, $CHCl_3$)[23]}, CD (MeCN, c 0.000057) λ_{ext} ($\Delta\varepsilon$) 230 (−32.6), 205 (+4.5) nm; **1b**, $[\alpha]_D^{25}$ +223 (c 0.17, $CHCl_3$) {$[\alpha]_D$ +273 (c 0.2, $CHCl_3$)[23]}, CD (MeCN, c 0.000051) λ_{ext} ($\Delta\varepsilon$) 237 (+9.0), 216 (−5.3) nm; *ent*-**1b**, $[\alpha]_D^{25}$ −341 (c 0.13, $CHCl_3$) {$[\alpha]_D$ −272 (c 0.2, $CHCl_3$)[23]}, CD (MeCN, c 0.000044) λ_{ext} ($\Delta\varepsilon$) 238 (−7.9), 215 (+6.2) nm.

Preparation of Stereoisomers of HO-GR24 and AcO-GR24. A 2′-epimeric mixture of (+)-4-hydroxy-GR24[20] was separated by silica gel column chromatography to give pure compounds **2a** and **2b**. The 2′-epimeric mixture of (−)-4-hydroxy-GR24[20] was also separated to give *ent*-**2a** and *ent*-**2b**. Their configurations were confirmed based on comparisons of their CD curves with those of the corresponding stereoisomers of GR24: **2a**, $[\alpha]_D^{24}$ +267 (c 0.804, $CHCl_3$); CD (MeCN, c 0.000027) λ_{ext} ($\Delta\varepsilon$) 230 (+24.7), 208 (+2.0) nm; *ent*-**2a**, $[\alpha]_D^{27}$ −264 (c 0.902, $CHCl_3$); CD (MeCN, c 0.000030) λ_{ext} ($\Delta\varepsilon$) 229 (−29.4), 208 (−2.7) nm; **2b**, $[\alpha]_D^{26}$ +177 (c 0.290, $CHCl_3$); CD (MeCN, c 0.000024) λ_{ext} ($\Delta\varepsilon$) 243 (+4.8), 211 (−3.1) nm; *ent*-**2b**, $[\alpha]_D^{27}$ −154 (c 1.10, $CHCl_3$); CD (MeCN, c 0.000036) λ_{ext} ($\Delta\varepsilon$) 238 (−6.5), 217 (+2.9) nm. Each stereoisomer of HO-GR24 (ca. 1 mg) was acetylated and purified as described above to obtain its corresponding AcO-GR24 stereoisomers.

Seeds. Seeds of *O. minor* Sm. were collected from mature plants parasitizing red clover (*Trifolium pratense* L.) in Japan. Seeds of *S. hermonthica* (Del.) Benth. were collected from mature plants parasitizing sorghum (*Sorghum bicolor*) and supplied by Professor Abdel Gabar Babiker (Sudan University of Science and Technology, Sudan). Seeds of *S. gesnerioides* (Willd.) Vatke were collected from mature plants

Table 1. Seed Germination Stimulating Activities (Percent) of Synthetic and Naturally Occurring Strigolactones toward Orobanchaceae Seeds[a]

	O. minor		*S. hermonthica*		*S. gesnerioides*	
compound	0.1 μM	10 μM	0.1 μM	10 μM	0.1 μM	10 μM
1	1.9 ± 1.2	62.9 ± 3.0	48.9 ± 4.9	50.5 ± 0.6	1.2 ± 1.2	0.9 ± 0.9
2	21.5 ± 2.9	63.1 ± 2.5	30.2 ± 15.3	59.5 ± 6.9	0.0	0.0
3	2.7 ± 0.5	77.7 ± 0.7	51.6 ± 9.8	55.2 ± 2.2	0.9 ± 0.9	1.6 ± 1.6
5-deoxystrigol	66.8 ± 3.1	76.2 ± 4.8	50.9 ± 1.5	45.3 ± 4.2	0.0	0.9 ± 0.9
orobanchol	71.9 ± 1.7	72.1 ± 3.1	26.5 ± 6.5	39.9 ± 8.3	0.0	0.0
orobanchyl acetate	7.6 ± 2.5	70.9 ± 1.3	10.9 ± 1.1	44.6 ± 2.3	0.0	0.0
cowpea root exudates[b]	55.8 ± 1.1		50.0 ± 3.8		25.0 ± 6.1	

[a] Data are shown as the mean ± SEM from three replicate tests. [b] Culture medium of cowpea seedlings, grown for 2 weeks.

parasitizing cowpea [*V. unguiculata* (L.) Walp.], and *S. gesnerioides* and cowpea seeds were supplied by Dr. Satoru Muranaka (International Institute of Tropical Agriculture, Nigeria).

Cowpea Root Exudates. Cowpea seeds, sown on moist glass fiber paper in Petri dishes, were covered with aluminum foil and incubated in the dark. The seedlings were transferred to test tubes and allowed to grow hydroponically in 40% Long Ashton nutrient solution[24] for 2−5 weeks. The plants were maintained in growth chambers at 28 °C with a 16 h photoperiod. Aquaculture filtrate was collected and stored at −20 °C until use.

Germination Bioassay. Parasitic weed seeds were surface sterilized by immersion in 0.5% (w/v) NaOCl containing a few drops of Tween 20 and sonication for 3 min in an ultrasonic cleaner. After three rinsings with distilled water and surface drying in a laminar hood, *S. hermonthica* and *S. gesnerioides* seeds were pretreated (conditioned) for 10−12 days on 8 mm glass fiber filter paper disks (ca. 50 seeds each) placed on distilled water-saturated filter paper. Aliquots (20 μL) of dilution series of aqueous solution of synthetic and naturally occurring strigolactones and cowpea root exudates were assayed by applying them to the conditioned *Striga* seeds on 8 mm disks. The treated seeds were incubated at 30 °C and microscopically evaluated after 24 and 48 h for germination (radicle protrusion) of *S. hermonthica* and *S. gesnerioides*, respectively. *O. minor* seeds were conditioned at 23 °C for 6 days, treated with test solutions as described above, incubated for 5 days at 23 °C, and then examined for germination. For the inhibition assay, conditioned *S. gesnerioides* seeds were concomitantly treated with cowpea root exudates and each of the stereoisomers at different concentrations under the same conditions as described above.

RESULTS AND DISCUSSION

Preparation and Seed Germination Stimulatory Activity of Strigolactones. A 2′-epimeric mixture of (±)-(3a*S**,4*S**,8b*S**)-4-hydroxy-GR24 (**2**) was prepared from racemic α-hydroxylactone with a 41% yield,[20] because GR24 (**1**) was used in racemic and diastereomeric mixtures. Most recently, an advanced synthetic procedure of 4-hydroxy-GR24 (aromatic orobanchol) and its acetate has been reported by Zwanenburg and co-workers.[25] They synthesized not only compound **2** but also the 4-hydroxy epimer of **2** [(3a*S**,4*R**,8b*S**)-4-hydroxy-GR24]. In this paper, we prepared (3a*S**,4*S**,8b*S**)-4-hydroxy-GR24 and termed it HO-GR24 (**2**), because we used this compound as a stereochemical mimic of orobanchol that has the (3a*S*,4*S*,8b*S*)-configuration in the tricyclic lactone. Compound **2** was acetylated with Ac_2O and pyridine to obtain AcO-GR24 (**3**) with an 82% yield. AcO-GR24 is regarded as a mimic of (+)-(3a*S*,4a*S*,8b*S*,2′*R*)-orobanchyl acetate; compound **3** has the (3a*S**,4*S**,8b*S**)-configuration in the tricyclic lactone.

GR24 induced seed germination of *O. minor* and *S. hermonthica* at concentrations of 0.1 and 10 μM, as shown in Table 1. The most extensively used strigolactone was more effective toward seeds of *S. hermonthica* than toward *O. minor*. AcO-GR24 was as active as GR24 toward *O. minor* and *S. hermonthica* seeds. Compared to GR24 and AcO-GR24, HO-GR24 was more active toward *O. minor* but less active toward *S. hermonthica* at the lower concentration, as reported previously.[25] The seeds of *O. minor* and *S. hermonthica* also responded to naturally occurring strigolactones. 5-Deoxystrigol was more effective toward *S. hermonthica* than orobanchol and orobanchyl acetate. Orobanchol was the most effective stimulant toward *O. minor* among the tested compounds. In contrast, *S. gesnerioides* seeds did not respond to any of the synthetic and natural strigolactones. The seeds were exclusively responsive to cowpea root exudates (Table 1), qin which Müller et al.[6] and Matsuura et al.[18] found alectrol to be a major stimulant. Although HO-GR24 and AcO-GR24 possess an oxygenated functional group in the B-ring, these synthetic strigolactones contain the aromatic A-ring without methyl groups and consist of racemic and diastereomeric mixtures, in contrast to naturally occurring strigolactones that are enantiomerically pure, suggesting that *S. gesnerioides* seeds have rigorous structural and stereochemical requirements for germination.

Preparation of Optically Active Synthetic Strigolactones. All naturally occurring strigolactones including alectrol are optically active. A previous paper demonstrated significant differences in stimulatory activity toward seed germination of *S. hermonthica* and *O. crenata* among eight stereoisomers of sorgolactone, although the seeds responded to any of the stereoisomers.[26] To obtain insight into the preferred configuration of synthetic strigolactones for *S. gesnerioides* germination, we prepared optically active GR24 derivatives (Figure 2) by optical resolution using chiral column chromatography or asymmetric synthesis.

Each stereoisomer of GR24 (**1a**, *ent*-**1a**, **1b**, and *ent*-**1b**) was obtained by optical resolution using an optically active column[22] after diastereomeric separation by column chromatography on silica gel. The absolute configurations of the GR24 stereoisomers were determined by comparison of their $[\alpha]_D$ values with those reported previously.[22,23] Four stereoisomers of HO-GR24 were synthesized using the same method reported previously.[20] The CD curves of HO-GR24 stereoisomers were comparable to those of the corresponding stereoisomers of GR24. These results indicated that the hydroxyl moiety at C-4 in strigolactones showed little influence on CD sign. Accordingly, (+)-GR24 (**1a**) and (+)-HO-GR24 (**2a**) have the configuration of (8b*S*,2′*R*), and (+)-2′-*epi*-GR24 (**1b**) and (+)-2′-*epi*-HO-GR24 (**2b**) have

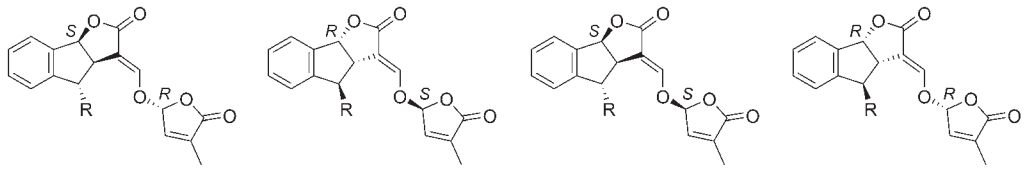

Figure 2. Structures of four stereoisomers of GR24 (**1a**, *ent*-**1a**, **1b**, and *ent*-**1b**), HO-GR24 (**2a**, *ent*-**2a**, **2b**, and *ent*-**2b**), and AcO-GR24 (**3a**, *ent*-**3a**, **3b**, and *ent*-**3b**).

Table 2. Seed Germination Stimulating Activities (Percent) of Isomers of GR24 (1), HO-GR24 (2), and AcO-GR24 (3) toward *Striga gesnerioides* Seeds[a]

	GR24 (**1**)		HO-GR24 (**2**)		AcO-GR24 (**3**)	
configuration (compounds)	0.1 μM	10 μM	0.1 μM	10 μM	0.1 μM	10 μM
8b*S*,2′*R* (**1**−**3a**)	0.0	0.0	0.0	0.4 ± 0.4	0.0	0.0
8b*R*,2′*S* (*ent*-**1**−**3a**)	0.0	0.3 ± 0.4	0.6 ± 0.6	2.3 ± 0.5	2.3 ± 1.4	4.5 ± 1.3
8b*S*,2′*S* (**1**−**3b**)	0.0	1.0 ± 1.2	0.0	0.7 ± 0.7	2.0 ± 1.2	2.5 ± 1.2
8b*R*,2′*R* (*ent*-**1**−**3b**)	0.0	0.7 ± 0.5	8.7 ± 2.8	19.0 ± 7.9	1.8 ± 1.0	5.7 ± 2.4

[a] Data are shown as the mean ± SEM from three replicate tests. Cowpea root exudates used as a positive control induced 35−40% seed germination in a simultaneous test.

the configuration of (8b*S*,2′*S*). To avoid complication, we do not describe the assignment of the configuration at C-3a of GR24 and its 4-substituted analogues because the assignment is changed by the substituent at C-4. Four stereoisomers of AcO-GR24 were prepared by acetylation of corresponding HO-GR24 stereoisomers with Ac_2O in the presence of trichloroacetic acid.

(8b*R*,2′*R*) Isomers Are Active in Inducing *S. gesnerioides* Seed Germination. The germination stimulatory activity of all synthetic strigolactone stereoisomers toward *S. gesnerioides* seeds was estimated. Cowpea root exudates were included as a positive control in the bioassay. The results of germination assays are shown in Table 2. Among the four stereoisomers of HO-GR24, *ent*-**2b** induced considerable seed germination in a dose-dependent manner. The acetylated compound *ent*-**3b** also induced seed germination, but with less than half the activity of compound *ent*-**2b**. The (2′*S*) isomers of HO-GR24 (*ent*-**2a** and **2b**) and AcO-GR24 (*ent*-**3a** and **3b**) acted as weak germination inducers, regardless of the configuration of the tricyclic system and acetylation of the hydroxyl group at C-4. The (8b*S*,2′*R*) isomers of HO-GR24 (**2a**) and AcO-GR24 (**3a**) and all stereoisomers of GR24 induced negligible seed germination. In contrast, all stereoisomers of GR24, HO-GR24, and AcO-GR24 induced seed germination of *S. hermonthica*, although the abilities varied among stereoisomers (data not shown).

Whereas all stereoisomers induced seed germination of *S. hermonthica*, only HO-GR24 and AcO-GR24 stereoisomers with the configuration (8b*R*,2′*R*), *ent*-**2b** and *ent*-**3b**, induced seed germination of *S. gesnerioides*. In contrast, the GR24 stereoisomer with the same configuration (*ent*-**1b**) did not induce *S. gesnerioides* seed germination. These results show that the oxygenated functional groups of strigolactones at C-4 are required for seed germination of *S. gesnerioides*. The absolute configurations of *ent*-**2b** and *ent*-**3b** are the same as those of fabacyl acetate and solanacol (8b*R*,2′*R*), but not those of strigol, sorgomol, or 5-deoxystrigol (8b*S*,2′*R*). Solanacol was isolated from root exudates of tobacco, which is a host plant of *S. gesnerioides*.[11] Coincidence of the absolute configuration of the naturally occurring strigolactone (solanacol) with the active synthetic strigolactones is a reasonable explanation for germination mechanism of parasitic plants induced by exposure to root exudates of host plants.

GR24 Effectively Suppresses Seed Germination of *S. gesnerioides* Induced by Cowpea Root Exudates. The optically active synthetic strigolactone *ent*-**2b** induced seed germination of *S. gesnerioides*. Nonetheless, compound **2** induced negligible seed germination (Table 1). These results imply that an antipode and/or diastereomer of *ent*-**2b** may prevent seed germination. To confirm whether inactive strigolactones work antagonistically to active strigolactones, we assayed the potency of the synthetic strigolactone stereoisomers in combination with cowpea root exudates for induction of *S. gesnerioides* seed germination.

Figure 3 shows the seed germination percentage of *S. gesnerioides* induced by cowpea root exudates with 0.1−10 μM stereoisomers of HO-GR24 and AcO-GR24. All stereoisomers except compound **2b** suppressed seed germination in a dose-dependent manner. The (2′*R*) isomers **2a**, **3a**, and *ent*-**3b** (Figure 3, open symbols) effectively suppressed germination compared to the 2′*S* isomers *ent*-**2a**, *ent*-**3a**, and **3b** (Figure 3, solid symbols). Compound *ent*-**2b** also suppressed seed germination, which implies that compound *ent*-**2b** is a partial agonist. The most effective antagonists among the four stereoisomers of HO-GR24 and AcO-GR24 were **2a** and **3a**, respectively. Compounds **2a** and **3a** have the configuration (8b*S*,2′*R*); that is, configurations at C-2′ in these compounds are the same as those of active stereoisomers, *ent*-**2b** and *ent*-**3b** (8b*R*,2′*R*). Thus, a strigolactone receptor of *S. gesnerioides* seems to prefer (2′*R*) strigolactone isomers as ligand compared to (2′*S*) isomers. However, a signal cascade for inducing seed germination may be activated when the hydroxyl group in the tricyclic lactone is located in precise space. All stereoisomers of GR24, **1a**, *ent*-**1a**, **1b**, and *ent*-**1b**, prevented seed germination more effectively than

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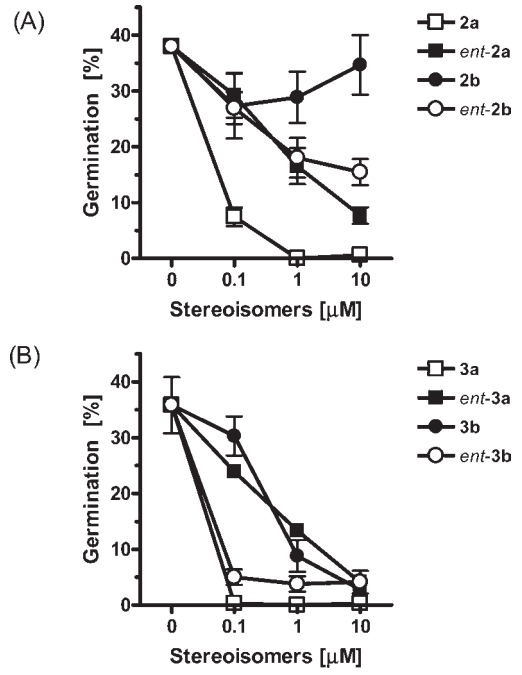

Figure 3. Germination-inducing activities of cowpea root exudates in combination with four stereoisomers of HO-GR24 (A), **2a** (open square), *ent*-**2a** (solid square), **2b** (solid circle), and *ent*-**2b** (open circle), or stereoisomers of AcO-GR24 (B), **3a** (open square), *ent*-**3a** (solid square), **3b** (solid circle), and *ent*-**3b** (open circle) at concentrations of 0.1, 1, and 10 μM. Data are shown as the mean ± SEM from five replicate tests.

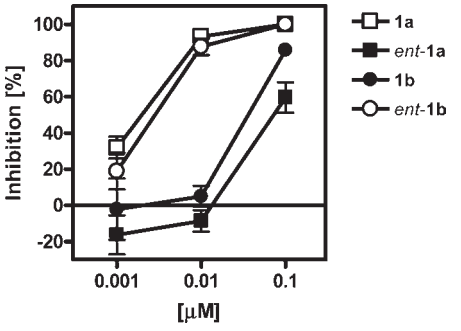

Figure 4. Inhibitory activities of GR24 stereoisomers, compounds **1a** (open square), *ent*-**1a** (solid square), **1b** (solid circle), and *ent*-**1b** (open circle), on *S. gesnerioides* seed germination induced by cowpea root exudates. Data are shown as the mean ± SEM from five replicate tests.

their 4-substituted analogues. Their inhibitory activity on seed germination induced by cowpea root exudates at lower concentrations than tested in Figure 3 is shown in Figure 4. (8b*S*,2′*R*)-GR24 (**1a**) was found to be the most effective inhibitor among the stereoisomers. This finding is consistent with the results that **2a** and **3a** are the most effective inhibitors among stereoisomers of HO-GR24 and AcO-GR24, respectively.

In summary, we clarified the structural and stereochemical requirements of synthetic strigolactones for seed germination of *S. gesnerioides*. To our knowledge, this study is the first to report GR24 inhibitory activity against seed germination of *S. gesnerioides* at concentrations that induce seed germination of *S. hermonthica* and *O. minor*. This knowledge will facilitate the development of not only specific suicidal germination inducers but also germination inhibitors of *S. gesnerioides* seeds. In addition, our findings on stereochemical requirements for *S. gesnerioides* seed germination provide impetus to reinvestigate the structure of alectrol, because the stereochemistry of the tentative structures proposed by Matsuura et al.[18] and Xie et al.[19] indicate strigolactones with inhibitory activity.

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