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Synthesis and Seed Germination Stimulating Activity of Some Imino Analogs of Strigolactones

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Strigolactones are germination stimulants for seeds of the root parasitic weeds, *Striga* and *Orobanche* spp. The imino analog of GR24 showed moderate germination stimulating activity against the seeds of *S. hermonthica*. The seed germination stimulating activity of some phenyliminoacetates and phenyliminoacetonitriles was also examined. The degree of activity of the phenyliminoacetate was less than that of the phenylacrylates. On the other hand, the degree of activity of the phenyliminoacetonitrile was comparable to that of the phenylacrylonitriles. Among the tested compounds, the 3-pyridyliminoacetonitrile showed higher activity against the seeds of *O. crenata* than GR24. These findings demonstrate that it is not always essential to have the Michael acceptor of the C–D ring junction moiety which has been proposed to react with nucleophilic species presented at the target site to enhance the activity.

Key words: strigolactone; seed germination stimulant; root parasitic weed

Parasitic weeds of the genera *Striga* and *Orobanche* cause severe damage to several gramineous and leguminous crops in tropical and subtropical areas.[1] The seeds of the parasites germinate only when stimulated by a chemical exuded from the roots of the host and some non-host plants. These chemicals include strigol (**1**), sorgolactone (**2**), alectrol (**3**) and orobanchol (**4**) (Fig. 1).[2] Their synthetic derivatives are also known to possess seed germination stimulation activity such as GR24 (**5**), Nijmegen 1 (**6**) and its phenyl derivative (**7**).[3] These germination stimulants are collectively referred to as strigolactones. 5-Deoxystrigol, one of strigolactones, is also known as a hyphal branching factor in arbuscular mycorrhizal fungi.[4] Carba-GR24 (**8**), in which the vinyl ether oxygen atom in GR24 has been replaced by a methylene function, is completely inactive in the stimulation of seeds of the parasitic weeds.[5] Based on these findings, a molecular mechanism for the stimulation of germination has been proposed by Mangnus and Zwanenburg,[6] as shown in Fig. 2. This mechanism involves the addition of a nucleophilic species presented at the target site in Michael fashion to the stimulant, with subsequent elimination of the D ring unit. This leads to covalent bonding of the ABC portion of the stimulant to the receptor, a chemical change that is claimed to be responsible for initiating or triggering germination.

Meanwhile, strobilurin A (**9**), which was isolated from mushroom, has been reported to have strong antifungal activity.[7,8] It was expected that, as methoxyacrylate **9** has an α,β-unsaturated carbonyl group in the molecule, it might work as a Michael acceptor at the target site in consideration of the structural similarity to strigolactones. However, as a result of the synthesis of strobilurin A analogs, for instance, kresoxim-methyl (**10**), which no longer has the enol ether group in its molecule, showed strong antifungal activity with the same mode of action as that of strobilurin A.[9] So the predicted reaction mechanism, *i.e.*, the Michael acceptor mechanism, is impossible in this case (Fig. 3). In the context of the structural modification from strobilurin A to kresoxim-methyl, it is interesting to examine if the imino analogs of strigolactones will show seed germination stimulating activity or not. Our interest in modifying the chemical structure to seek a higher active germination stimulant therefore led to the design, synthesis and bioassay of some imino analogs of strigolactones. We describe here the results of a study involving the discovery of phenyliminoacetonitriles as new germination stimulants.

Materials and Methods

Apparatus. ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ with a JNM-AL300 spectrometer (Jeol), using tetramethylsilane (TMS) as an internal standard, and chemical shifts are shown in δ (ppm). IR spectra were obtained with an IR-408 spectrometer (Shimadzu), and mass spectra were recorded with a QSTAR spectrometer

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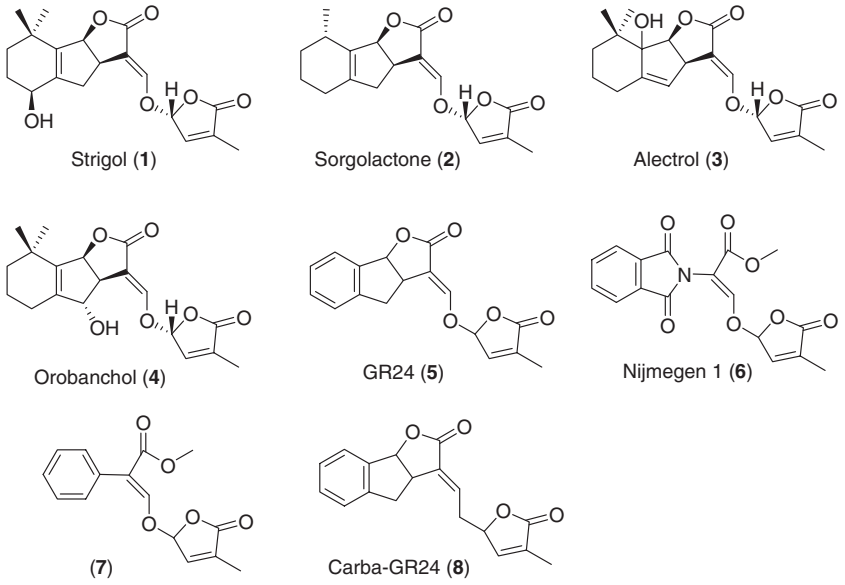

Fig. 1. Structures of Strigolactones and Their Synthetic Analogs.

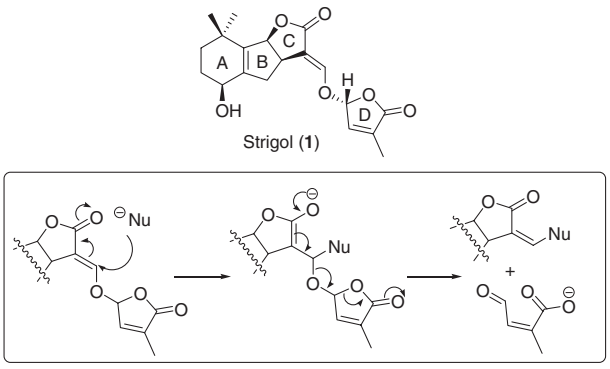

Fig. 2. Proposed Action Mechanism of Strigolactones.

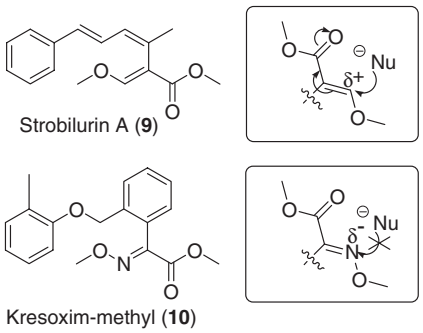

Fig. 3. Predicted Action Mechanism of Strobilurin A.

(Thermoquest). Melting point (mp) data were measured by MP-S3 apparatus (Yanaco) and are uncorrected. Silica gel 60N (Kanto Chemical) was used for column chromatography, and pre-coated silica gel plates (Kieselgel 60 F_{254}, Merck) were used for analytical thin-layer chromatography.

Synthesis of the compounds. GR24 (**5**),[10] Nijmegen 1 (**6**)[11] and its phenyl derivative (**7**)[12] were prepared by the reported procedure.

*3-(4-Methyl-5-oxo-2,5-dihydrofuran-2-yl)oxyimino-3a,4-dihydro-3H-indeno[1,2-b]furan-2(8bH)-one (**11**).* 3,3a,4,8b-Tetrahydroindeno[1,2-*b*]furan-2-one[10] (1.74 g, 10 mmol) was dissolved in DMF (36 ml), and the resulting solution was cooled to $-10\,^{\circ}$C. Potassium *t*-butoxide (1.34 g, 12 mmol) and then isoamyl nitrite (2.34 g, 20 mmol) were added to the solution at the same temperature. The mixture was gradually warmed to room temperature and kept for 2.5 h. The reaction mixture was diluted with dil. HCl (150 ml) and extracted with ethyl acetate (100 ml). The ethyl acetate layer was washed with water (2 times, each 100 ml), dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was chromatographed on silica gel (hexane/ethyl acetate = 10/1, v/v) to give a yellow solid of the corresponding oxime (1.38 g, 68%), mp 193–197 °C. IR ν_{max} (nujol) cm^{-1}: 1760 (s), 1650 (m), 1290 (s), 1245 (s), 1000 (s), 940 (s). NMR δ_H ($CDCl_3$): 3.24–3.61 (2H, m), 4.03–4.10 (1H, m), 6.06 (1H, d, $J = 7.5$ Hz), 7.25–7.55 (4H, m), 12.30 (1H, s). NMR δ_C ($CDCl_3$): 35.1, 38.2, 85.2, 124.9, 126.2, 127.4, 130.2, 137.9, 142.8, 149.6, 165.8.

To a cooled solution of the oxime (102 mg, 0.5 mmol) in DMF (3 ml) were added potassium *t*-butoxide (67 mg, 0.6 mmol) and 5-bromo-3-methylfuran-2(5*H*)-one[10] (106 mg, 0.6 mmol) at $-78\,^{\circ}$C while stirring. The mixture was warmed to room temperature and kept overnight. The reaction mixture was diluted with water (30 ml) and extracted with ethyl acetate (30 ml). The ethyl acetate layer was washed with water (2 times, each 30 ml), dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was chromatographed on silica gel (hexane/ethyl acetate = 10/1, v/v) to give a yellow

oil of **11** (50 mg, 33%). IR ν_{max} (nujol) cm^{-1}: 1770 (s, C=O), 1650 (w), 1330 (m), 1030 (m), 945 (s). NMR δ_H ($CDCl_3$): 2.04 (3H, s, CH_3), 3.18–3.62 (2H, m), 4.07–4.16 (1H, m), 6.06–6.10 (1H, m), 6.62–6.63 (1H, m), 6.98–6.99 (1H, m), 7.24–7.60 (4H, m). NMR δ_C ($CDCl_3$): 10.8, 35.4, 39.2, 85.6, 103.2, 125.1, 126.6, 128.0, 130.8, 135.9, 137.4, 140.3, 142.2, 154.2, 163.9, 170.9. HRESIMS m/z $[M + H]^+$: calcd. for $C_{16}H_{14}NO_5$, 300.0866; found, 300.0861.

*Methyl 2-[(4-methyl-5-oxo-2,5-dihydrofuran-2-yl)oxyimino]-2-phenylacetate (**12**).* To a cooled solution of (*EZ*)-2-hydroxyimino-2-phenylacetate (prepared from methyl phenylglutarate and hydroxylamine hydrochloride, 0.80 g, 4.5 mmol) in DMF (15 ml) were added potassium *t*-butoxide (0.55 g, 5.6 mmol) and 5-bromo-3-methylfuran-2(5*H*)-one (1.06 g, 6.0 mmol) at −60 °C while stirring. The mixture was warmed to room temperature and kept overnight. The reaction mixture was diluted with water (50 ml) and extracted with ethyl acetate (50 ml). The ethyl acetate layer was washed with water (2 times, each 50 ml), dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was chromatographed on silica gel (hexane/ethyl acetate = 10/1, v/v) to give a yellow oil of **12** (0.21 g, 17%) as an (*EZ*) mixture with regard to the CN double bond. NMR δ_H ($CDCl_3$): 1.95 and 1.99 (3H, s), 3.87 and 3.93 (3H, s), 6.47–6.54 (1H, m), 6.83–6.92 (1H, m), 7.39–7.59 (5H, m). HRESIMS m/z $[M + H]^+$: calcd. for $C_{14}H_{14}NO_5$, 276.0872; found, 276.0860.

Part of this mixture was further purified by silica gel column chromatography to give the pure *E*- and *Z*-isomers.

E-Isomer (**12E**): Yellow oil. IR ν_{max} (nujol) cm^{-1}: 1775 (s, C=O), 1725 (s, C=O), 1660 (m), 1340 (s), 1220 (s), 1080 (s). NMR δ_H ($CDCl_3$): 1.99 (3H, s, CH_3), 3.93 (3H, s, OCH_3), 6.47 (1H, m), 6.92 (1H, m), 7.39–7.59 (5H, m). NMR δ_C ($CDCl_3$): 10.6, 52.6, 102.5, 126.7, 128.7, 128.8, 129.0, 135.3, 140.9, 153.8, 162.9, 171.3.

Z-Isomer (**12Z**): Mp 98–102 °C. IR ν_{max} (nujol) cm^{-1}: 1770 (s, C=O), 1725 (s, C=O), 1300 (s), 1200 (m), 1160 (m), 950 (s). NMR δ_H ($CDCl_3$): 1.95 (3H, s, CH_3), 3.89 (3H, s, OCH_3), 6.54 (1H, m), 6.84 (1H, m), 7.41–7.44 (5H, m). NMR δ_C ($CDCl_3$): 10.6, 53.2, 103.0, 128.1, 128.4, 129.0, 130.2, 135.5, 140.5, 152.6, 163.3, 171.1.

*3-[(4-Methyl-5-oxo-2,5-dihydrofuran-2-yl)oxy]-2-phenylacrylo-nitrile (**13**).* To a stirred solution of phenylacetonitrile (5.0 g, 40 mmol) in methyl formate (30 ml) and DMF (10 ml), sodium hydride (60% in oil; 2.1 g, 50 mmol) was portionwise added at 0 °C. The resulting mixture was kept at room temperature overnight. The mixture was diluted with dil. HCl (30 ml) and extracted with dichloromethane (30 ml). The dichloromethane layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give a solid (2.7 g, crude 3-hydroxy-2-phenylacrylonitrile). NMR δ_H ($CDCl_3$): 7.20–7.78 (5H, m), 7.66 (1H, s), 11.00 (1H, s).

To a cooled solution of this crude 3-hydroxy-2-phenylacrylonitrile (1.34 g, 9.2 mmol) in DMF (20 ml), potassium *t*-butoxide (1.14 g, 12 mmol) and then 5-bromo-3-methylfuran-2(5*H*)-one (2.12 g, 12 mmol) were added at −20 °C while stirring. The mixture was warmed to room temperature and kept overnight. The reaction mixture was diluted with water (50 ml) and extracted with ethyl acetate (50 ml). The ethyl acetate layer was washed with water (2 times, each 50 ml), dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was chromatographed on silica gel (hexane/ethyl acetate = 10/1, v/v) to give a yellow oil of **13** (0.63 g, 28%). IR ν_{max} (nujol) cm^{-1}: 2040 (s, CN), 1760 (s, C=O). NMR δ_H ($CDCl_3$): 2.02–2.06 (3H, m), 6.21–6.22 (1H, m), 6.97–6.99 (1H, m), 7.03–7.70 (6H, m). NMR δ_C ($CDCl_3$): 10.6, 10.7, 100.0, 100.4, 117.9, 124.8, 125.4, 126.3, 127.4, 127.9, 128.6, 128.7, 128.9, 129.2, 129.4, 129.5, 129.9, 135.7, 136.2, 140.7, 141.2, 152.3, 152.4, 153.6, 170.0. HRESIMS m/z $[M + H]^+$: calcd. for $C_{14}H_{12}NO_3$, 242.0811; found, 242.0806.

*2-[(4-Methyl-5-oxo-2,5-dihydrofuran-2-yl)oxyimino]-2-phenyl-acetonitrile (**14**).* To a stirred solution of phenylacetonitrile (5.0 g, 40 mmol) in DMF (35 ml), potassium *t*-butoxide (9.0 g, 80 mmol) and isoamyl nitrite (9.4 g, 80 mmol) were added at 0 °C. The resulting mixture was kept at room temperature for 4 h. The reaction mixture was diluted with dil. HCl (30 ml) and extracted with ethyl acetate (30 ml). The ethyl acetate layer was washed with water (2 times, each 30 ml), dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give a solid (5.4 g, crude 2-hydroxyimino-2-phenylacetonitrile). NMR δ_H ($CDCl_3$): 7.42–7.53 (3H, m), 7.79–7.83 (2H, m), 9.07 (1H, s).

To a cooled solution of this crude 2-hydroxyimino-2-phenyl-acetonitrile (1.50 g, 10.3 mmol) in DMF (20 ml), potassium *t*-butoxide (1.27 g, 10.2 mmol) and then 5-bromo-3-methylfuran-2(5*H*)-one (2.24 g, 12.7 mmol) were added at −20 °C while stirring. The mixture was warmed to room temperature and kept overnight. The reaction mixture was diluted with water (50 ml) and ethyl acetate (50 ml). The ethyl acetate layer was washed with water (2 times, each 50 ml), dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was chromatographed on a silica gel (hexane/ethyl acetate = 10/1, v/v) to give a white solid of **14** (1.16 g, 47%), mp 158–161 °C. IR ν_{max} (nujol) cm^{-1}: 1760 (s, C=O), 1340 (s), 1205 (m), 950 (s). NMR δ_H ($CDCl_3$): 2.04–2.06 (3H, m), 6.58–6.59 (1H, m), 6.99–7.00 (1H, m), 7.47–7.57 (3H, m), 7.81–7.84 (2H, m). NMR δ_C ($CDCl_3$): 10.8, 102.7, 108.7, 126.8, 128.2, 129.2, 132.3, 135.6, 136.1, 140.1, 170.8. HRESIMS m/z $[M + H]^+$: calcd. for $C_{13}H_{11}N_2O_3$, 243.0764; found, 243.0751.

*N-[(4-Methyl-5-oxo-2,5-dihydrofuran-2-yl)oxy]nicotinimidoylcyanide (**15**).* In a similar manner, **15** was prepared from 2-hydroxyimino-2-(3-pyridyl)acetonitrile (0.68 g, 47%), mp 72–76 °C. IR ν_{max} (nujol) cm^{-1}: 1760 (s, C=O), 1660 (m), 1580 (m), 1340 (s), 1310 (s), 1200 (m), 1090 (s). NMR δ_H ($CDCl_3$): 1.89–1.92 (3H, m),

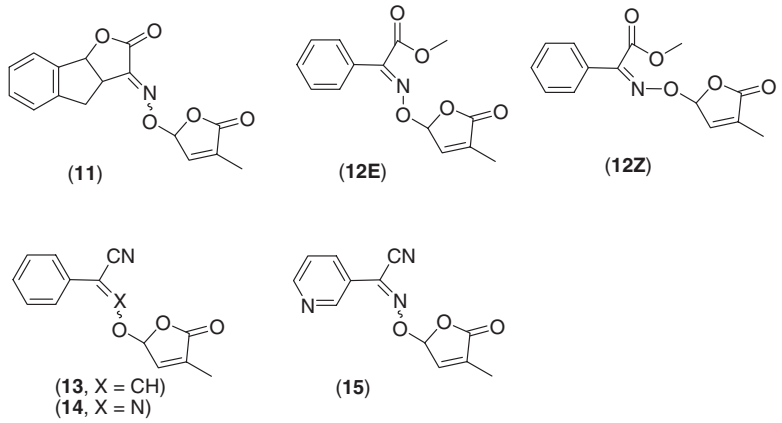

Fig. 4. Chemical Structure of the Synthesized Compounds.

6.47–6.53 (1H, m), 6.91–6.96 (1H, m), 7.24–7.38 (1H, m), 7.97–8.10 (1H, m), 8.60–8.64 (1H, m), 8.88–9.03 (1H, m). NMR δ_C ($CDCl_3$): 10.4, 31.0, 36.1, 102.6, 102.8, 107.7, 112.7, 123.2, 123.4, 123.6, 124.2, 132.8, 133.6, 135.3, 135.8, 135.9, 136.7, 139.8, 139.9, 147.4, 150.0, 152.3, 152.5, 162.2, 170.3, 170.4. HRESIMS m/z $[M + H]^+$: calcd. for $C_{12}H_{10}N_3O_3$, 244.0716; found, 244.0707.

Bioassay of the seed germination stimulating activity. *Striga hermonthica* seeds were collected from mature plants parasitizing sorghum at the Gezira Research Station in Sudan and provided by Prof. A. G. T. Babiker of Agricultural Research Corporation in Sudan. *S. gesnerioides* seeds were collected from mature plants parasitizing cowpea in Kano, Nigeria, and were provided by Prof. A. M. Emechebe of International Institute of Tropical Agriculture. *Orobanche crenata* seeds were collected from mature plants parasitizing vetch in Kfar Massarick, Israel, and provided by Dr. R. Vasey of Sheffield University, UK. *O. minor* seeds were collected from mature plants parasitizing red clover in the Watarase basin of Japan.

The seeds of these parasitic weeds were surface sterilized by immersion in 0.5% (w/v) NaOCl containing a few drops of Tween 20, and sonicated for 3 min in an ultrasonic cleaner, after having been rinsed 3 times with distilled water and surface-dried at 27 °C under a laminar hood. *S. hermonthica* and *S. gesnerioides* seeds were pretreated (conditioned) for 10 to 12 days at 30 °C on 8-mm glass fiber filter paper disks (*ca.* 50 seeds each) placed on distilled water-saturated filter paper. For each bioassay, distilled water and an *aq.* solution of GR24 were included as negative and positive controls, respectively. A dilution series were prepared for each sample. Each aqueous solution was assayed directly by applying 20-µl aliquots of the respective test solution to conditioned seeds on 8-mm disks. For those solutions containing an organic solvent, aliquots (20 µl each) of the test solution were applied to 8-mm disks of glass fiber filter paper which were then allowed to dry for 1 h at room temperature. A disk with conditioned seeds was placed on top of each treated disk and moistened with 40 µl of distilled water. The treated seeds were incubated at 30 °C and microscopically evaluated for their germination (radicle protrusion) 24 h later. *O. crenata* and *O. minor* seeds were conditioned at 23 °C for 6 days, treated with each test solution, incubated for 5 days at 23 °C, and then examined for germination.

Results

Synthesis of the imino analogs

The structures of the synthesized compounds are illustrated in Fig. 4. The tricyclic lactone, 3,3a,4,8b-tetrahydroindeno[1,2-*b*]furan-2-one, was converted into the corresponding oxime by nitrosation with isoamyl nitrite in a 68% yield. Coupling of this oxime with 5-bromo-3-methylfuran-2(5*H*)-one gave GR24 imino-analog **11** in a 33% yield, probably as an (*EZ*) mixture with regards to the CN double bond and used for the subsequent bioassay without further purification. Compounds **12–15** were smoothly obtained as a mixture of their geometrical isomers. The (*EZ*) isomers of **12** were separable by silica gel column chromatography. (*EZ*)-2-Hydroxyimino-2-phenylacetate prepared from methyl phenylglutarate and hydroxylamine hydrochloride contained geometrical isomers with regard to the CN double bond in a 3:1 ratio, this being estimated from each peak area of two OH groups in the ^{1}H-NMR spectrum. The signal of the major isomer appeared at δ_H 9.27 (1H, s, OH), while that of the minor isomer was at δ_H 8.52 (1H, s, OH). The lower chemical shift for the OH group of the major isomer is thought to have been due to intramolecular hydrogen bonding between the OH and CO groups in the molecule. Based on this observation, the geometry with regard to the CN double bond of the major isomer was assigned to be of (*Z*) configuration. The chromatographically purified (*Z*)-2-hydroxyimino-2-phenylacetate was reacted with 5-bromo-3-methylfuran-2(5*H*)-one to give a product whose ^{1}H-NMR pattern was completely consistent with that of **12Z**. Thus, the

Table 1. Seed Germination Stimulating Activity of Some Imino Analogs of Strigolactones

No.	Germination (%)							
	S. hermonthica		*S. gesnerioides*		*O. minor*		*O. crenata*	
	0.1 μM	10 μM	0.1 μM	10 μM	0.1 μM	10 μM	0.1 μM	10 μM
11	0.0	28.3 ± 7.2	0.0	0.0	0.0	0.0	0.0	1.7 ± 2.3
12E	0.0	4.7 ± 2.5	—	—	—	—	—	—
12Z	0.0	2.6 ± 3.4	—	—	—	—	—	—
13	8.5 ± 5.8	65.2 ± 11.0	0.0	0.2 ± 0.5	27.5 ± 13.5	70.8 ± 10.7	6.0 ± 3.2	46.3 ± 14.9
14	4.6 ± 2.2	46.9 ± 6.4	0.0	0.0	1.8 ± 2.6	72.2 ± 14.1	0.4 ± 0.9	28.3 ± 10.6
15	1.4 ± 1.3	63.7 ± 5.7	0.0	0.0	0.9 ± 1.9	35.2 ± 11.2	43.3 ± 8.5	51.6 ± 19.1
5	68.2 ± 5.2	66.9 ± 5.6	0.9 ± 1.3	0.5 ± 0.8	24.6 ± 10.4	57.8 ± 6.9	21.9 ± 6.8	32.2 ± 18.6
6	0.0	37.4 ± 3.3	—	—	—	—	—	—
7	0.0	24.2 ± 5.0	—	—	—	—	—	—

Each data value is the mean germination percentage ± standard error from five replicate tests. Compounds **12E**, **12Z**, **6** and **7** were tested only on *S. hermonthica*.

geometry with regard to the CN double bond in **12** was tentatively assigned without considering (*EZ*) isomerization during the reaction of the (*Z*)-hydroxyiminophenylacetate with 5-bromo-3-methylfuran-2(5*H*)-one. On the other hand, the isomers of **13**, **14** and **15** were not separable by column chromatography.

Structure–seed germination stimulating activity relationships

The imino analog of GR24 (**11**) showed moderate germination stimulating activity against the seeds of *S. hermonthica* in comparison with that of GR24 (**5**), as shown in Table 1. This finding encouraged us to design further imino analogs of strigolactones. We decided to make phenyliminoacetates and phenyliminoacetonitriles, as the corresponding phenylacrylates have been reported to possess moderate seed germination stimulating activity.[12] A bioassay of the synthesized compounds was conducted on the germination stimulating activity of the seeds of *S. hermonthica*, *S. gesnerioides*, *O. minor*, and *O. crenata*. The degree of activity of both geometrical isomers in phenyliminoacetate **12** was less than that of corresponding phenylacrylate **7**. On the other hand, phenylacrylonitrile **13** and phenyliminoacetonitrile **14** showed somewhat stronger activity than that of phenylacrylate **7**. The activity of compounds **13** and **14** was stronger than that of Nijmegen 1 (**6**), although **6** was only tested on *S. hermonthica*. Among the tested compounds, 3-pyridyliminoacetonitrile **15** had higher activity against *O. crenata* than that of GR24 (**5**). No compound showed seed germination stimulating activity toward *S. gesnerioides*.

Discussion

We firstly designed imino analog **11** of GR24, expecting that **11** would show seed germination stimulating activity, even if the enol ether group was converted into the imino ether group, just like kresoxim-methyl (**10**), as already described. As a result, imino analog **11** showed moderate germination stimulating activity toward the seeds of *S. hermonthica*. We then designed phenyliminoacetate **12** as the imino analog of **7**. We also designed phenylacrylonitrile **13** and phenyliminoacetonitrile **14** in order to examine if the seed germination stimulating activity was influenced or not by electronic factors concerning the C ring moiety in the molecule of strigolactones. Interestingly, the degree of activity of phenyliminoacetonitrile **14** was comparable to that of phenylacrylonitrile **13**. These findings demonstrate that it is not always essential to have the Michael acceptor moiety of the C–D ring junction in the molecule of strigolactones to enhance the seed germination stimulating activity. It thus appears that a critical structure fitting to the target site is one of the requisites for exhibiting seed germination stimulating activity. Moreover, 3-pyridyl derivative **15**, in which a nitrogen atom was introduced into the benzene ring in **14**, resulted in an increase in the degree of seed germination stimulating activity toward *O. crenata*. This also indicates the possibility of broadening the seed germination stimulating activity spectrum by chemical modification. A further study on designing new germination stimulants by this type of modification is in progress.

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