Synthesis of a Phthaloylglycine-Derived Strigol Analogue and Its Germination Stimulatory Activity toward Seeds of the Parasitic Weeds *Striga hermonthica* and *Orobanche crenata*

Gérard H. L. Nefkens, Jan Willem J. F. Thuring, Marco F. M. Beenakkers, and Binne Zwanenburg*

NSR−Center for Molecular Structure, Design and Synthesis, Department of Organic Chemistry, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands

The newly designed strigol analogue Nijmegen 1 (*rac* **7**) was prepared in high overall yield starting from *N*-phthaloylglycine. This relatively simple analogue exhibits high bioactivity in the stimulation of germination of seeds of the parasitic weeds *Striga hermonthica* and *Orobanche crenata*. Nijmegen 1 was resolved in its enantiomers **7** and *ent* **7** by using the homochiral latent D-rings *ent* **11** and *ent* **12**. The enantiomers **7** and *ent* **7** show significant differences in germination activity.

Keywords: *Striga; Orobanche; germination; strigol analogue*

INTRODUCTION

The devastating parasitic weeds *Striga* and *Orobanche* cause severe reductions in food crop yield of several graminaceous and leguminous crops in tropical and semitropical areas of the eastern hemisphere (Musselman, 1987; Parker and Riches, 1993). A strict requirement for the germination of the seeds of these parasitic weeds is exposure to a chemical substance that is usually present in the root exudate of a potential host plant (Press *et al*., 1990; Butler, 1995). An attractive control strategy for the eradication of infested fields is the concept of suicidal germination, *i.e.* introduction of a germination stimulating agent into the soil prior to sowing to induce germination of the parasitic seeds in the absence of a host plant (Eplee, 1975). The first known naturally occurring germination stimulant, (+)-strigol (**1**), was isolated from the root exudate of the false host cotton (*Gossypium hirsutum* L.) (Cook *et al*., 1966, 1972). Recently, (+)-strigol (**1**) was also identified in the root exudates of the *Striga* host plants maize (*Zea mays* L.) and proso millet (*Panicum miliaceum* L.) (Siame *et al*., 1993). In addition, some structurally closely related "strigolactones" (Butler, 1995) have been identified in the root exudates of other *Striga* hosts, *viz*. sorgolactone **2** (Hauck *et al*., 1992) and alectrol **3** (Müller *et al*., 1992).

However, strigolactones **1**−**3** (Figure 1) are not suitable for weed control purposes, because their structures are too complicated to allow synthesis in an economically feasible manner. Therefore, several studies aimed at synthetic analogues with a relatively simple structure but with high germination stimulatory activity (Johnson *et al*., 1976, 1981; Vail *et al*., 1990; Bergmann *et al*., 1993; Mangnus *et al*., 1992a; Zwanenburg *et al*., 1994). These studies mainly focused on the ABC-part of the strigolactones. In this part of the molecule a considerable structural variation is allowed to retain high biological activity. On the basis of these observations, a tentative molecular mechanism (Scheme 1), which accounts for the onset of the biochemical cascade leading to germination, has been proposed (Mangnus and Zwanenburg, 1992a). According to this mechanism the bioactiphore resides in the vinyl ether part of the D-ring.

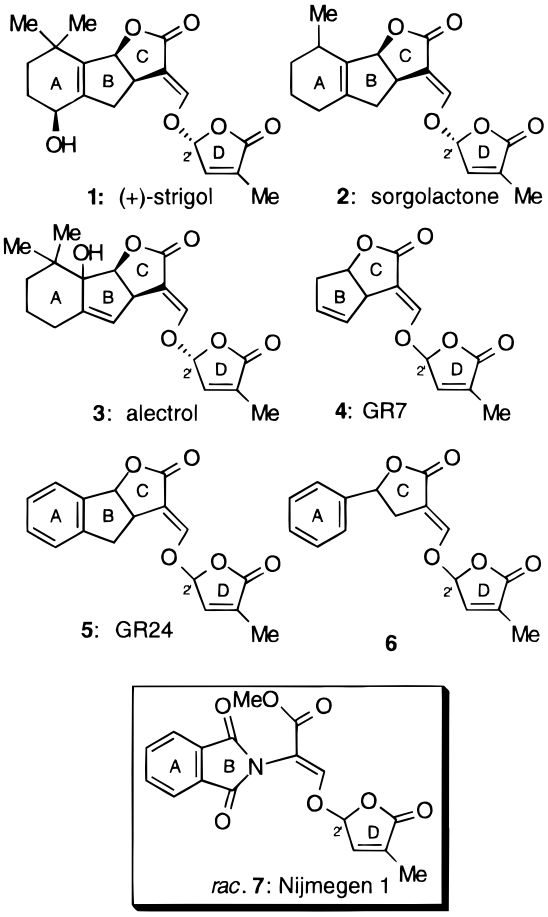


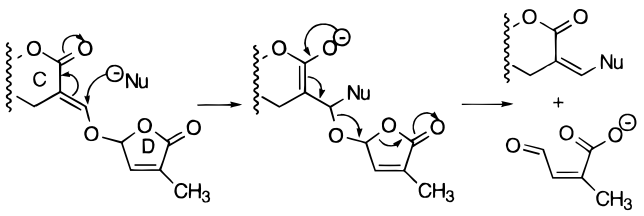
Figure 1. Strigolactones **1**−**3** and some active analogues.

Important examples of highly potent synthetic analogues include **4** (GR7) and **5** (GR24) (Johnson *et al*., 1981; Mangnus and Zwanenburg, 1992b; Mangnus *et al*., 1992b). The latter, having an aromatic A-ring, is especially highly relevant, as its stimulatory activity is comparable to that of strigol (Bergmann *et al*., 1993) and its preparation is much easier than that of strigol (Mangnus *et al*., 1992b). An even less complicated analogue is compound **6**, derived from γ-phenyl-γ-butyrolactone. This analogue, which lacks the B-ring, is almost as active as GR24 (Mangnus *et al*., 1992a). A

* Fax +31.24.3652929; e-mail zwanenb@sci.kun.nl

S0021-8561(96)00450-5 CCC: $14.00

Scheme 1. Proposed Molecular Mechanism Involved in Germination



problem associated with the analogues **4**−**6** is the presence of two stereogenic centers in these molecules with the consequence that during their syntheses mixtures of diastereomers are obtained, which can be separated only by tedious chromatography. The present paper describes the synthesis of phthaloylglycine-derived strigol analogue *rac* **7**, which we named Nijmegen 1, and the evaluation of the germination stimulatory activity toward seeds of *Striga* and *Orobanche* spp. This new germination stimulant contains only one chiral center, *viz*. in the D-ring. This compound was designed in such a manner that it contains the essential structural features for bioactivity (*vide supra*); that is, the essence of the molecular mechanism is not affected. Its planar phthalimido moiety is entirely divergent from that of the ABC-part of the strigolactones **1**−**3** and the GR analogues **4** and **5**.

MATERIALS AND METHODS

Nomenclature. We have used the AUTONOM 1.0 program, provided by the Beilstein Institute and Springer-Verlag, Weinheim, Germany.

Syntheses. *General Remarks.* ^{1}H-NMR (100 MHz) spectra were recorded on a Bruker AC 100 spectrometer (Me_4Si as internal standard), and 400 MHz ^{1}H-NMR spectra were recorded on a Bruker AM-400 spectrometer (Me_4Si as internal standard), both from Bruker (Wissembourg, France). All coupling constants are given as ^3J in hertz, unless indicated otherwise. For mass spectra a double-focusing VG7070E mass spectrometer from VG Analytical (Manchester, U.K.) was used. GLC was conducted with a Hewlett-Packard HP 5890 gas chromatograph, from Hewlett-Packard Nederland (Amstelveen, The Netherlands), using a capillary cross-linked methyl silicone gum column of 25 m length and 0.32 mm i.d., with 0.17 μm film thickness and nitrogen (2 mL/min, 0.5 atm) as the carrier gas. Melting points were measured with a Reichert Thermopan (Austria) microscope and are uncorrected. Elemental analyses were performed at the Department of Microanalysis of this laboratory.

Solvents were dried using the following methods: Dichloromethane was distilled from P_2O_5. Diethyl ether was distilled from NaH. Hexane was distilled from CaH_2. Tetrahydrofuran was distilled from lithium aluminum hydride just before use. All other solvents were of analytical grade. Thin layer chromatography (TLC) was carried out on Merck (Darmstadt, Germany) precoated silica gel 60 F254 plates (0.25 mm) using the eluents indicated. Spots were visualized with UV or using a molybdate spray. "Flash" chromatography was carried out at a pressure of *ca*. 1.5 bar, using Merck Kieselgel 60H. Column chromatography at atmospheric pressure was carried out using Merck Kieselgel 60.

The synthesis of chlorolactones **11** and *ent* **11** was reported previously (Thuring *et al*., 1995).

*Methyl 2-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)-3-oxopropionate (**9**).* To a cooled (−10 °C) solution of **8** (65.8 g, 300 mmol) in methyl formate (400 mL) were gradually added small pieces of sodium (6.90 g, 300 mmol), with mechanical stirring in a nitrogen atmosphere. Stirring was continued for 18 h until all sodium had dissolved. The reaction mixture was concentrated *in vacuo*, and to the residue was added a mixture of glacial acetic acid (25 mL) and 1 N HCl (50 mL). Crude **9** was obtained by extraction with dichloromethane (three times), drying ($MgSO_4$) and concentration *in vacuo*. Recrystallization from toluene gave pure **9** (59.3 g, 80%) as a pale yellow powder, with physical properties identical with those reported previously (Sheehan and Johnson, 1954).

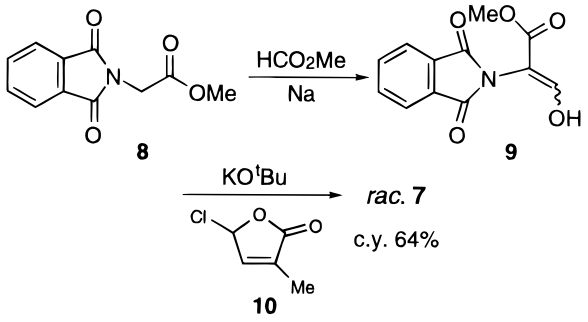
*Methyl 2-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)-3-[4-methyl-5-oxo-2,5-dihydrofuran-2(R)-yloxy]acrylate (rac **7**).* Potassium *tert*-butoxide (372 mg, 3.32 mmol) was added to a cooled (0 °C) and stirred solution of Sheehan aldehyde **9** (745 mg, 3.02 mmol) in DMF (10 mL) at room temperature under nitrogen. Then chlorofuranone **10** (480 mg, 3.62 mmol) in DMF (3 mL) was gradually added. The mixture was stirred at room temperature over a weekend. DMF was removed *in vacuo*, and the residue was dissolved in a mixture of water and ethyl acetate. The organic layer was separated, and the aqueous phase was extracted with ethyl acetate (two times). The combined organic layers were washed with water (two times), dried ($MgSO_4$), and concentrated *in vacuo*. The oily residue was triturated with diisopropyl ether. Almost pure *rac* **7** (660 mg, 64%) was isolated as a white solid by filtration and washing with diisopropyl ether. An analytical sample was obtained by recrystallization from 2-propanol. mp 151−152 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.97 (br s, 3H, C*H*$_3$), 3.78 (s, 3H, OC*H*$_3$), 6.17 (br s, 1H, OC*H*O), 6.90 (br s, 1H, =C*H*), 7.76 (m, 2H, 2 arom *H*), 7.90 (m, 3H, 2 arom *H* + =C*H*O); MS [EI, *m*/*z*, rel intensity (%)] 343 ([M]$^+$, 2.7), 246 ([$C_{12}H_8NO_5$]$^+$, 100), 97 ([$C_5H_5O_2$]$^+$, 59.3). Anal. Calcd for $C_{17}H_{13}NO_7$: C, 59.48; H, 3.82; N, 4.08. Found: C, 59.10; H, 3.85; N, 4.00.

*Methyl 2-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)-3-[6(S)-methyl-5-oxo-4-oxatricyclo[5.2.1.0^{2,6}]dec-8-en-3(R)-yloxy]acrylate (**12**).* Potassium *tert*-butoxide (149 mg, 1.33 mmol) was added to a stirred solution of Sheehan aldehyde **9** (302 mg, 1.22 mmol) in DMF (10 mL) at room temperature under nitrogen. Then chlorolactone **11** (265 mg, 1.33 mmol) in DMF (3 mL) was gradually added. The mixture was stirred at 55 °C for 7 days and then quenched with acetic acid (0.5 mL). DMF was removed *in vacuo*, and the residue was dissolved in a mixture of water and ethyl acetate. The organic layer was separated, and the aqueous phase was extracted with ethyl acetate (two times). The combined organic layers were washed with water (two times), dried ($MgSO_4$), and concentrated *in vacuo*. The crude product was purified by flash chromatography (SiO_2, hexane/ethyl acetate 2:1) to give **12** (143 mg, 29%) as a yellowish solid. An analytical sample was obtained by recrystallization from di*iso*propyl ether/ethyl acetate. mp 179.5−181 °C; $[\alpha]_D$ −22° (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.46 (s, 3H, C*H*$_3$), 1.65 (m, 2H, *H*$_{10}$), 2.62 (dd, 1H, J = 4.2 Hz, J < 1 Hz, *H*$_2$), 2.85 (m, 1H, *H*$_7$), 3.16 (m, 1H, *H*$_1$), 3.77 (s, 3H, OC*H*$_3$), 5.26 (d, 1H, J < 1 Hz, *H*$_3$), 6.17 (m, 1H, *H*$_9$), 6.28 (m, 1H, *H*$_8$), 7.77 (m, 2H, Ar *H*), 7.85 (s, 1H, =C*H*O), 7.91 (m, 2H, Ar *H*); MS [EI, *m*/*z*, rel intensity (%)] 409 ([M]$^+$, 0.6), 344 ([$C_{17}H_{14}NO_7$], 0.5), 247 ([$C_{12}H_9NO_5$], 43.6), 163 ([$C_{10}H_{11}O_2$], 78.2), 97 ([$C_5H_5O_2$], 100), 66 ([C_5H_6], 13.5). Anal. Calcd for $C_{22}H_{19}NO_7$: C, 64.54; H, 4.68; N, 3.43. Found: C, 64.52; H, 4.63; N, 3.48.

*Methyl 2-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)-3-[6(R)-methyl-5-oxo-4-oxatricyclo[5.2.1.0^{2,6}]dec-8-en-3(S)-yloxy]acrylate (ent **12**).* This compound was prepared in the same way as described for **12**, starting from Sheehan aldehyde **9** (601 mg, 2.43 mmol) and chlorolactone *ent* **11** (530 mg, 2.67 mmol). Yield: 286 mg, 29% of *ent* **12** as yellowish solid. Recrystallization from diisopropyl ether/ethyl acetate afforded analytically pure *ent* **12**. mp 179.5−181 °C; $[\alpha]_D$ +23° (*c* 0.2, $CHCl_3$). Anal. Calcd for $C_{22}H_{19}NO_7$: C, 64.54; H, 4.68; N, 3.43. Found: C, 64.50; H, 4.64; N, 3.47. ^{1}H-NMR and mass data were the same as for compound **12**.

*Methyl 2-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)-3-[4-methyl-5-oxo-2,5-dihydrofuran-2(R)-yloxy]acrylate (**7**).* Cycloadduct **12** (159 mg, 0.39 mmol) was dissolved in *o*-dichlorobenzene (40 mL) and heated at 180 °C for 7 h. The solvent was removed *in vacuo*. The residue was purified by flash chromatography (SiO_2, hexane/ethyl acetate 1:1) to give **7** (53 mg, 40%) as a colorless oil, which failed to crystallize. $[\alpha]_D$ +124° (*c* 0.15, CH_2Cl_2). ^{1}H-NMR and mass data were the same as for compound *rac* **7**.

Scheme 2. Preparation of *rac* 7



*Methyl 2-(1,3-dioxo-1,3-dihydroisoindol-2-yl)-3-[4-methyl-5-oxo-2,5-dihydrofuran-2(S)-yloxy]acrylate (ent **7**)* was prepared in the same way as described for **7**, starting from *ent* **12** (230 mg, 0.56 mmol). Yield: 43 mg, 31% of *ent* **7** as a colorless oil, which failed to crystallize. $[\alpha]_D$ −128° (*c* 0.15, CH_2Cl_2). ^{1}H-NMR and mass data were the same as for compound **7**.

Biological Activity. *Seeds.* Seeds of *Striga hermonthica* (Del.) Benth [from *Sorghum bicolor* (L.) Moench] and *Orobanche crenata* Forsk. (from *Vicia faba* L.) were harvested in Sudan in 1988 and in Egypt in 1991, respectively, and were stored in the dark at room temperature until use in germination tests.

Preparation of Test Solutions. A compound to be tested was weighed out very accurately to the amount of 10 mg, dissolved in 10 mL of acetone p.a., and diluted with demineralized water to 100 mL. Aliquots of this stock solution were further diluted with water to obtain test solutions containing 2, 1, 0.1, and 0.01 mg/L test compound and 0.2, 0.1, 0.01, and 0.001% (v/v) acetone, respectively.

Bioassays. For surface sterilization seeds of *S. hermonthica* and *O. crenata* were exposed to an aqueous solution of sodium hypochlorite (2% active chlorine) for 5 min with agitation. The seeds were then thoroughly rinsed with water and dried overnight.

For conditioning the sterilized seeds were spread on glass fiber filter paper disks (8-mm diameter; approximately 30−70 seeds per disk) in Petri dishes, wetted with water, and stored in the dark for 14 days at 20 °C for *Orobanche* seeds and at 30 °C for *Striga* seeds. Then the conditioning water was removed and replaced by 100 μL of test solution per disk. After incubation for 24 h (*Striga*) and 5 days (*Orobanche*) in the dark at indicated temperatures, the germination percentage was determined under a microscope. Seeds were considered to be germinated if the radical protruded through the seed coat.

In each test series aqueous solutions with 0.1, 0.01, and 0.001% (v/v) acetone were used as negative control. Test solutions of the stimulant GR24 (as a 1:1 diastereomeric mixture at concentrations of 1, 0.1, and 0.01 mg/L) were used as positive controls. All tests were performed in duplicate, and in each test the germination percentages were determined on 12 disks per treatment.

For full details of the bioassay, see Mangnus *et al.* (1992c).

RESULTS AND DISCUSSION

The key step in the synthesis of *rac* **7** involves coupling of aldehyde **9** with 5-chloro-3-methyl-2(5*H*)-furanone (**10**). This aldehyde **9** was prepared by condensation of methyl *N*-phthaloylglycinate (**8**) with methyl formate using metallic sodium (Scheme 2).

This procedure, which closely resembles that described by Schutz (1978), is superior to that originally reported by Sheehan and Johnson (1954). It should be noted that **9** is a stable, crystalline compound, which can be stored for several years. The coupling reaction with butenolide **10** (Scheme 2) proceeded in high yield, and purification was readily accomplished by recrystallization. It is important to note that only one geometrical isomer was obtained. The correct geometrical structure could not be deduced unambiguously by spectroscopic means, and therefore an X-ray diffraction analysis was undertaken (Beurskens *et al.*, 1994). The structure of **7** is depicted in Figure 2, showing that the *Z*-isomer was obtained.

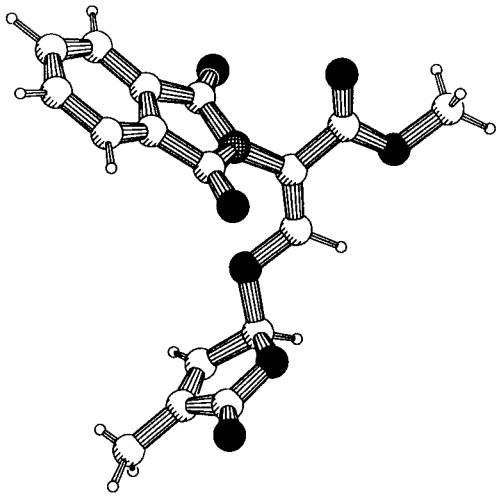


Figure 2. PLUTON-generated drawing of X-ray crystal structure of Nijmegen 1 (*rac* **7**).

Table 1. Germination Percentages for Seeds of *S. hermonthica* after Exposure to Aqueous Solutions of Strigol Analogues GR24 and Nijmegen 1 at Concentrations of 1 and 0.01 mg/L[a]

		% germination ± SE	
entry	stimulant	1 mg/L	0.01 mg/L
1	GR24 (**5**)	45.5 ± 13.5	52.5 ± 12.5
2	Nijmegen 1 (*rac* **7**)	40.3 ± 1.7	4.1 ± 0.9[b]

[a] Activities are indicated as germination percentages after treatment of the seeds with test solutions at 1 mg/L and 0.01 mg/L. Germination percentages given are the mean ± SE of two replicate tests. [b] Value is not significantly different from germination percentages obtained in the control (without stimulant)

Next, the preparation of the individual enantiomers of **7** was attempted, using enantiomerically pure tricyclic chlorolactones **11** and *ent* **11** as the D-ring precursors (Scheme 3). The stereoselective synthesis of **11** and *ent* **11** and their use in the preparation of the single isomers of strigol analogues has been reported recently (Thuring *et al.*, 1995).

The coupling reactions of Sheehan aldehyde **9** with **11** and *ent* **11** did not proceed as smoothly as was observed for the corresponding GR7 analogues (Thuring *et al.*, 1995). As a result of the relatively poor nucleophilicity of the enolate anion derived from **9**, a higher reaction temperature was required, which caused concomitant decomposition of **11** and *ent* **11**. The cycloreversion of **12** and *ent* **12** was performed in *o*-dichlorobenzene at 180 °C to give **7** and *ent* **7**, respectively, in moderate yields. The ee values of both enantiomers were >98%, as was determined by ^{1}H-NMR analysis using the chiral shift reagent Eu(hfc)$_3$.

Biological Evaluation. The germination stimulatory activity of Nijmegen 1 (*rac* **7**) was assayed using seeds of *S. hermonthica* and *O. crenata* spp. In each bioassay, GR24 was included as a positive control. The procedure enables a comparison between results obtained in different test series. This is important, since the response of seeds of parasitic weeds, especially *S. hermonthica*, varies considerably from test to test. In addition, the activities of enantiomers **7** and *ent* **7** were determined using seeds of *O. crenata* spp. The results

Scheme 3. Enantioselective Syntheses of 7 and *ent* 7

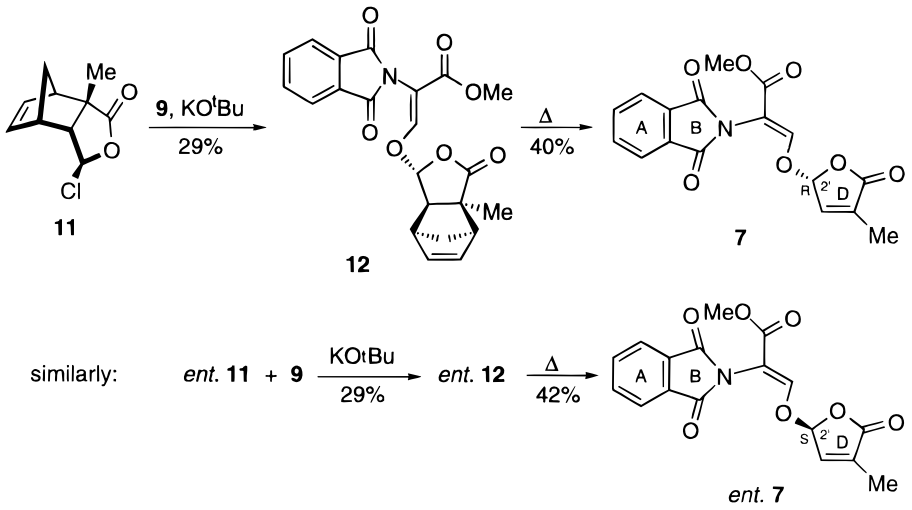


Table 2. Germination Percentages for Seeds of *O. crenata* after Exposure to Aqueous Solutions of Strigol Analogues GR24 and Nijmegen 1 at Concentrations of 2, 1, 0.1, and 0.01 mg/L[a]

		% germination ± SE			
entry	compound	2 mg/L	1 mg/L	0.1 mg/L	0.01 mg/L
1	GR24 (**5**)		62.5 ± 3.6	36.0 ± 10.6	3.4 ± 1.1
2	Nijmegen 1 (*rac* **7**)	58.3 ± 1.3	42.6 ± 1.8	5.6 ± 0.6	1.1 ± 0.6[b]
3	Nijmegen 1 (**7**)		54.8 ± 3.4	34.5 ± 2.2	
4	Nijmegen 1 (*ent* **7**)		26.9 ± 0.3	6.5 ± 1.3	

[a] Germination percentages given are the mean ± SE of two replicate tests. [b] Value is not significantly different from germination percentages obtained in the control (without stimulant).

are collected in Tables 1 and 2. It was beyond the aim of this study to establish complete dose−response curves, implying that the data obtained allow only an interpretation in a qualitative sense.

The data in Table 1 (*S. hermonthica* spp.) reveal that *rac* Nijmegen 1 exhibits considerable activity at the higher concentration of 1 ppm, whereas it is practically inactive at a concentration of 0.01 ppm. Similarly, in the stimulation of *O. crenata* spp. seeds, *rac* **7** has shown a bioactivity comparable to that of GR24 at higher concentrations (entry 1, Table 2). Comparison of the germination percentages exerted by enantiopure **7** and *ent* **7** (entries 2 and 3, Table 2) reveals that the former is considerably more active. Thus, the absolute stereochemistry at C-2′ in the D-ring should be the *R*-configuration to germinate a maximum number of seeds. This configuration is the same as in natural (+)-strigol. This result is in agreement with previous conclusions from comparative studies of the bioactivity of all stereoisomers of GR7 (Mangnus and Zwanenburg, 1992b) and of some stereoisomers of strigol (Bergmann *et al.*, 1993), namely, that the most active stereoisomer has the *R*-configuration at C-2′ in the D-ring.

From the results presented above, it may be concluded that phthaloylglycine-derived strigol analogue *rac* **7** is a potent germination stimulant of seeds of *S. hermonthica* and *O. crenata* spp. Moreover, optically active **7** with the "natural" configuration in the D-ring has a stimulatory activity comparable to that of GR24 for *O. crenata*. The charm of this particular stimulant is the fact that its racemic preparation is very simple and that it can be carried out without any chromatographic separation, which makes it an attractive compound for large-scale preparations and accordingly for use in the suicidal approach in the weed pest control. Moreover, the achiral "ABC"-part in *rac* **7** enables a rapid evaluation of the structural variation in the D-ring on the stimulatory activity. Research in this direction is in progress.

It should be noted that our newly developed asymmetric route allows for the first time the synthesis of a strigol analogue, which is only chiral at the D-ring. The ease of preparation and the high bioactivity of this new germination stimulant warrant further studies to evaluate its activity and stability under soil conditions. Activities in this direction are in progress.

From a mechanistic point of view we can conclude that a possible interaction of the ABC-fragment with a receptor site is sterically and electronically not highly demanding.

ACKNOWLEDGMENT

We thank Dr. A. G. T. Babiker and Dr. F. M. F. Zaitoun for supplying *Striga* and *Orobanche* seeds. We also thank H. Amatdjais, P. van Galen, and A. Swolfs for conducting elemental analysis, mass, and 400 MHz ^{1}H-NMR measurements.

LITERATURE CITED

Bergmann, C.; Wegmann, K.; Frischmuth, K.; Samson, E.; Kranz, A.; Weigelt, D.; Koll, P.; Welzel, P. Stimulation of *Orobanche crenata* seed germination by (+)-strigol and structural analogues. Dependence of constitution and configuration of the germination stimulants. *J. Plant Physiol.* **1993**, *142*, 338−342 and references cited therein.

Beurskens, G.; Smits, J. M. M.; Beurskens, P. T.; Beenakkers, M.; Nefkens, G.; Zwanenburg, B. Crystal and molecular structure of 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-3-(4-methyl-5-oxo-2,5-dihydro-furan-2-yloxy)acrylic acid methyl ester, $C_{17}H_{13}NO_7$. *J. Chem. Crystallogr.* **1994**, *24*, 643−646.

Butler, L. G. Chemical communication between the parasitic weed *Striga* and its crop host. *ACS Symp. Ser.* **1995**, *No. 582*, 158−166.

Cook, C. E.; Whichard, L. P.; Turner, B.; Wall, M. E.; Egley, G. H. Germination of witchweed (*S. lutea* Lour.): isolation and properties of a potent stimulant. *Science* **1966**, 1189−1190.

Cook, C. E.; Whichard, L. P.; Wall, M. E.; Egley, G. H.; Coggan, P.; Luhan, P. A.; McPhail, A. T. Germination stimulants II. The structure of strigol—a potent seed germination stimulant for witchweed (*Striga lutea* Lour.). *J. Am. Chem. Soc.* **1972**, *94*, 6198−6199.

Eplee, R. E. Ethylene: a witchweed seed germination stimulant. *Weed Sci.* **1975**, *23*, 433−436.

Hassanali, A. Strigol analogues: synthetic achievements and prospects. In *Striga; Biology and Control*; Ayensu, E. S., Doggett, H., Keynes, K. D., Marton-Lefevre, J., Musselman, L. J., Parker, C., Peckering, A., Eds.; ICSU Press; Paris, 1984; pp 125−132.

Hauck, C., Müller, S., Schildknecht, H. A germination stimulant for parasitic flowering plants from *Sorghum bicolor*, a genuine host plant. *J. Plant Physiol.* **1992**, *139*, 474−478.

Johnson, A. W.; Roseberry, G.; Parker, C. A novel approach to *Striga* and *Orobanche* control using synthetic germination stimulants. *Weed Res.* **1976**, *16*, 223−227.

Johnson, A. W.; Gowda, G.; Hassanali, A.; Knox, J.; Monaco, S.; Razawi, Z.; Roseberry, G. The preparation of synthetic analogues of strigol. *J. Chem. Soc., Perkin Trans. 1* **1981**, 1734−1743.

Mangnus E. M.; Zwanenburg, B. Tentative molecular mechanism for germination stimulation of *Striga* and *Orobanche* seeds by strigol and its synthetic analogues. *J. Agric. Food Chem.* **1992a**, *40*, 1066−1070.

Mangnus E. M.; Zwanenburg, B. Synthesis, structural characterization, and biological evaluation of all four enantiomers of strigol analogue GR7. *J. Agric. Food Chem.* **1992b**, *40*, 697−700.

Mangnus E. M.; van Vliet, L. A.; Vandenput, D. A. L.; Zwanenburg, B. Structural modifications of strigol analogues. Influence of the B and C rings on the bioactivity of the germination stimulant GR24. *J. Agric. Food Chem.* **1992a**, *40*, 1222−1229.

Mangnus E. M.; Dommerholt, F. J.; de Jong, R. L. P.; Zwanenburg, B. Improved synthesis of strigol analogue GR24 and evaluation of the biological activity of its diastereomers. *J. Agric. Food Chem.* **1992b**, *40*, 1230−1235.

Mangnus, E. M.; Stommen, P. L. A.; Zwanenburg, B. A standardized bioassay for evaluation of potential germination stimulants for seeds of parasitic weeds. *J. Plant Growth Regul.* **1992c**, *11*, 91−98.

Müller, S.; Hauck, C.; Schildknecht, H. Germination stimulants produced by *Vigna unguiculata* Walp cv Saunders Upright. *J. Plant Growth Regul.* **1992**, *11*, 77−84.

Musselman, L. J., Ed. *Parasitic Weeds in Agriculture. Vol. I. Striga*; CRC Press: Boca Raton, FL, 1987; 317 pp.

Parker, C.; Riches, C. R. *Parasitic Weeds of the World: Biology and Control*; CAB International Press: Wallingford, Oxon, U.K., 1993; 332 pp.

Press, M. C.; Graves, J. D.; Stewart, G. R. Physiology of the interaction of angiosperm parasites and their higher plant hosts. *Plant, Cell Environ.* **1990**, *13*, 91−104

Schutz, A. Totalsynthese von Penicillin-und Cephalosporinderivate mittels Vierkomponenten-kondensation und Mechanistische Studien. Doctoral Thesis, T.U. München, 1978.

Sheehan, J. C.; Johnson, D. A. The synthesis of substituted penicillins and simpler structural analogs. VIII. Phthalimidomalonaldehydic esters: Synthesis and condensation with penicillamine. *J. Am. Chem. Soc.* **1954**, *76*, 158−160.

Siame, B. A.; Weerasuriya, Y.; Wood, K.; Ejeta, G.; Butler, L. G. Isolation of strigol, a germination stimulant for *Striga asiatica*, from host plants. *J. Agric. Food Chem.* **1993**, *41*, 1486−1491.

Thuring, J. W. J. F.; Nefkens, G. H. L.; Schaafstra, R.; Zwanenburg, B. Asymmetric synthesis of a D-ring synthon for strigol analogues and its application to the synthesis of all four stereoisomers of germination stimulant GR7. *Tetrahedron* **1995**, *51*, 5047−5056.

Vail, S. L.; Dailey, O. D.; Blanchard, E. J.; Pepperman, A. B.; Riopel, J. L. Terpenoid precursors of strigol as seed germination stimulants of broomrape (*Orobanche ramosa*) and witchweed (*Striga asiatica*). *J. Plant Growth Regul.* **1990**, *9*, 77−83 and references cited therein.

Zwanenburg, B.; Mangnus, E. M.; Thuring, J. W. J. F. Strigol analogues: design, synthesis and biological activity. In *Proceedings of the Third International Workshop on Orobanche and Related Striga Research*; Pieterse, A. H., Verkley, J. A. C., Ter Borg, S. J., Eds.; Royal Tropical Institute: Amsterdam, The Netherlands, 1994; pp 187−197.

Received for review June 24, 1996. Revised manuscript received January 2, 1997. Accepted January 9, 1997.[⊗] These investigations were supported by the Netherlands Foundation of Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Research (NWO).

JF9604504

[⊗] Abstract published in *Advance ACS Abstracts*, April 1, 1997.