# Synthesis, Structural Characterization, and Biological Evaluation of All Four Enantiomers of Strigol Analogue GR7

Eddy M. Mangnus and Binne Zwanenburg*

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

All four optically pure enantiomers of strigol analogue GR7 were prepared by starting from commercially available, pure enantiomers of "Corey's lactone". The separation of the diastereomers in the crude products was readily accomplished by column chromatography on silica. The absolute configuration of the four enantiomers was determined from X-ray analysis in combination with $R_f$ values of the products and the configuration of the starting lactones. Biological evaluation revealed significant differences between the four enantiomers in stimulation of germination of parasitic weed seeds.

## INTRODUCTION

Parasitic weeds of the genera *Striga* and *Orobanche* cause severe reductions of the yields of graminaceous and leguminous crops in tropical and semitropical areas of the eastern hemisphere (Musselman, 1987; Parker, 1986; Ramaiah, 1987). Biological studies revealed that germination of seeds of these parasitic weeds is triggered by a chemical species exuded by roots of a host plant (Brown, 1965). In 1972 a compound, which stimulated germination of *Striga* seeds, was isolated from root exudate of cotton (*Gossypium hirsutum* L.) and identified by Cook et al. (1966, 1972). The discovery of this natural germination stimulant, named strigol 1, opened a new way for parasitic weed control in which germinating agents are used to induce seed germination in the absence of suitable host plants (Johnson et al., 1976). The germinated seeds cannot develop further and will die after a short period. This process of suicidal germination can be used, at least in principle, to reduce the number of viable parasitic weed seeds in the soil before the desired crop is planted (Eplee, 1975). The natural germination stimulant (+)-strigol (1) (see Figure 1) is less suitable for this control of weed pests because of its instability in alkaline soil and its complicated structure, which make its synthetic production complex and uneconomical (Johnson et al., 1976). Therefore, several investigators synthesized analogues of strigol with simpler structures, with the anticipation that the biological activity could be largely retained (Johnson et al., 1981; Mangnus et al., 1987; Mangnus and Zwanenburg, 1991; Pepperman et al., 1982; Zwanenburg et al., 1986). Highly potent strigol analogues are compounds **2** and **3**, commonly known as GR7 and GR24, respectively (see Figure 1).

Thus far, only scarce attention has been paid to the influence of the stereochemistry on the activity of germination stimulants. Heather et al. (1976) reported that (±)-strigol was much more active than its (±)-4′-epimer. Recently Hauck and Schildknecht (1990) separated the enantiomers of (±)-strigol and found significant differences in their biological activities. Connick and Pepperman (1981) separated and evaluated the diastereomers of GR7 and observed a slightly lower germination stimulant activity for the higher melting diastereomer (for *Striga asiatica* seeds: 50% germination at $10^{-6}$ M vs 62% germination at $10^{-7}$ M for the lower melting diastereomer). Thus far, no homochiral stereomers of strigol analogues have been prepared and evaluated.

The present study describes the synthesis, structural characterization, and biological evaluation of all four enantiomers of strigol analogue GR7.

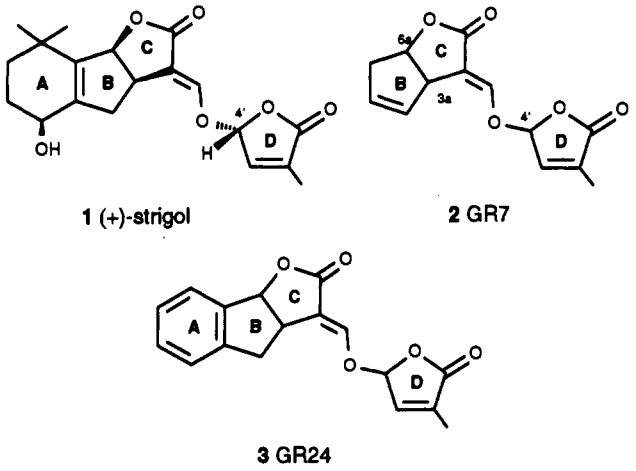


**Figure 1.** Structures of 1–3.

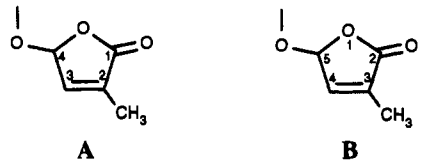

**Figure 2.** Atom numbering scheme for ring D according to butenolide (A) and furanone nomenclature (B).

## MATERIALS AND METHODS

**Nomenclature.** In this section we have adopted Chemical Abstracts Service nomenclature for all compounds. As a consequence the furanone system of naming is used for the butenolide group (ring D). Note that this method of naming uses an atom numbering different from that of butenolide nomenclature, which is used in the text (see Figure 2).

**Synthesis.** *General Remarks.* Melting points were measured with a Reichert Thermopan microscope and are uncorrected. IR spectra were recorded on a Perkin-Elmer 298 infrared spectrophotometer. $^1$H NMR spectra were recorded on a Varian EM 390 (90 MHz, CW) or a Bruker AM-400 (400 MHz, FT) spectrometer with TMS as internal standard. For mass spectroscopy a double-focusing VG 7070E was used. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. "Flash" chromatography was carried out at a pressure of ca. 1.5 bar using silica gel 60H (Merck Art. No. 7719). Thin-layer chromatograms (TLC) were run on plastic-supported silica gel 60 plates (0.2-mm

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layer, $F_{254}$, Merck Art. No. 5735) or glass-supported silica gel 60 plates (0.25-mm layer, $F_{254}$, Merck Art. No. 5715).

*(3aR,6aR)-3-(Hydroxymethylene)-3,3a,6,6a-tetrahydro-2H-cyclopenta[b]furan-2-one* (**5a**). A solution of potassium *tert*-butoxide (0.90 g, 8 mmol) in tetrahydrofuran (20 mL; freshly distilled over lithium aluminum hydride) was gradually added to a mixture of methyl formate (0.5 mL, 8 mmol) and (3a*S*,-6a*R*)-3,3a,6,6a-tetrahydro-2*H*-cyclopenta[*b*]furan-2-one (**4a**) (0.87 g, 7.0 mmol; $[\alpha]^{20}_D$ +104° (*c* 0.7, MeOH); purchased in optically pure form from Fluka) in anhydrous tetrahydrofuran (15 mL) with stirring at 0 °C under nitrogen. Stirring was continued at room temperature for 20 h. The reaction was monitored by gas–liquid chromatography (GLC), and after 20 h, still 12% of the starting material **4a** was present in the reaction mixture. After the addition of an extra amount of methyl formate (0.1 mL, 1.6 mmol) and potassium *tert*-butoxide (0.11 g, 1.0 mmol) and stirring for an additional 2 h, the amount of starting material was reduced to 7% (GLC analysis). Tetrahydrofuran was removed in vacuo, and the residue was dissolved in a mixture of water and dichloromethane. The mixture was acidified using potassium hydrogen sulfate and then extracted three times with dichloromethane. The combined organic layers were dried ($Na_2SO_4$), filtered, and concentrated. Unreacted starting material was then removed by washing the crude product three times with small amounts of cold diethyl ether. The hydroxymethylene compound **5a** was obtained as a white solid (0.70 g, 66%) and was used without further purification: mp 108–111 °C [lit. 107–110 °C for racemic material (Johnson et al., 1981)].

*(3aS,6aS)-3-(Hydroxymethylene)-3,3a,6,6a-tetrahydro-2H-cyclopenta[b]furan-2-one* (**5b**). The hydroxymethylene compound **5b** was prepared from (3a*R*,6a*S*)-3,3a,6,6a-tetrahydro-2*H*-cyclopenta[*b*]furan-2-one (**4b**) ($[\alpha]^{20}_D$ −104° (*c* 0.7, MeOH); purchased in optically pure form from Fluka) according to the same method as described for compound **5a**. Yield 68%; mp 108–111 °C.

*(3aR,6aR,5'R)-3-[[(2,5-Dihydro-3-methyl-2-oxo-5-furanyl)-oxy]methylene]-3,3a,6,6a-tetrahydro-2H-cyclopenta[b]furan-2-one* (**2a**) *and Its 5'S Epimer* (**2b**). Hydroxymethylenolactone **5a** (0.38 g, 2.5 mmol) was added to a solution of potassium *tert*-butoxide (0.30 g, 2.5 mmol) in anhydrous tetrahydrofuran (25 mL) with stirring at room temperature under nitrogen. Immediately the potassium salt of **5a** precipitated, and after 15 min of stirring at room temperature, 5-bromo-3-methyl-2(5*H*)-furanone (**6**) (0.53 g, 3.0 mmol; Johnson et al., 1981) was added. After 20 h of stirring at room temperature, tetrahydrofuran was removed in vacuo and the residue was dissolved in a mixture of water and dichloromethane. The organic layer was separated, and the aqueous layer was extracted twice with dichloromethane. The combined organic layers were washed with water, dried ($Na_2SO_4$), filtered, and concentrated. The crude product, obtained as a red oil, was purified using flash chromatography (silica gel, diisopropyl ether/ethyl acetate 4:1) to afford two diastereomeric products. The fast moving diastereomer **2a** (0.22 g, 36%; $R_f$ = 0.21, diisopropyl ether/ethyl acetate 4:1) was obtained as a white solid, and crystallization from dichloromethane/diethyl ether afforded analytically pure **2a**: colorless plates; mp 133–135 °C; $[\alpha]^{22}_D$ +339° (*c* 1.06, $CH_2Cl_2$); $^1H$ NMR ($CDCl_3$) $\delta$ 2.00 (m, 3 H, $CH_3$), 2.71 (m, 2 H, $CH_2$), 4.10 (m, 1 H, $C_{3a}H$), 5.12 (m, 1 H, $C_{6a}H$), 5.67 (m, 2 H, C*H*=C*H*), 6.25 (m, 1 H, OC*H*O), 6.98 [m, 1 H, C*H*=C($CH_3$)], 7.39 (m, 1 H, =C*H*O); IR ($CHCl_3$) $\nu$ 1785 (C=O), 1745 (C=O), 1684 (C=CO) $cm^{-1}$; MS ($EI^+$) 248 $(M)^+$, 97 [100%, $(butenolide)^+$].

The slow moving diastereomer **2b** (0.28 g, 45%; $R_f$ = 0.13, diisopropyl ether/ethyl acetate 4:1) was obtained as a slightly yellow oil and failed to crystallize: $[\alpha]^{22}_D$ +174° (*c* 1.44, $CH_2Cl_2$); IR, $^1H$ NMR, and mass data were the same as for compound **2a**.

*(3aS,6aS,5'S)-3-[[(2,5-Dihydro-3-methyl-2-oxo-5-furanyl)-oxy]methylene]-3,3a,6,6a-tetrahydro-2H-cyclopenta[b]furan-2-one* (**2c**) *and Its 5'R Epimer* (**2d**). For the coupling of hydroxymethylenolactone **5b** and 5-bromo-3-methyl-2(5*H*)-furanone (**6**) the same procedure as for the synthesis of compounds **2a** and **2b** was used. After the chromatographic purification, again two diastereomers were obtained. Fast moving diastereomer **2c**: yield 35%; colorless plates; mp 134–135 °C; $[\alpha]^{22}_D$ −341° (*c* 1.03, $CH_2Cl_2$); IR, $^1H$ NMR, and mass data were the same as for compound **2a**. Slow moving diastereomer **2d**: yield 40%; pale yellow oil; $[\alpha]^{22}_D$ −179° (*c* 1.44, $CH_2Cl_2$); IR, $^1H$ NMR, and mass data were the same as for compound **2a**.

*Enantiomeric Purity.* The absolute configuration of compound **2a** was determined by an X-ray diffraction analysis (Bosman et al., 1992). Through this, the absolute configuration of the other three isomers was also ascertained (see Results and Discussion).

$^1H$ NMR (400 MHz) analysis of racemic mixtures of GR7 diastereomers with chiral shift reagent Eu(hfc)$_3$ revealed a downfield shift for all resonances, with a prominent 1:1 splitting of the signals for the acetal proton in ring D (OC*H*O) and for the enol ether proton (=C*H*O). $^1H$ NMR (400 MHz) spectra of pure compounds **2a–d** displayed comparable shifts on treatment with Eu(hfc)$_3$, but, in contrast to the racemates, no splitting of signals was observed. No signals of the epimers were detected in these spectra, indicating an enantiomeric purity of at least 98%.

**Biological Evaluation.** *Seeds.* Seeds of *Striga hermonthica* (Del.) Benth. and *Orobanche crenata* Forsk. were harvested in Sudan in 1987 and in Egypt in 1988, respectively, and were stored in the dark at room temperature until used in germination tests.

*Preparation of Test Solutions.* Of a compound to be tested an amount of 10 mg was weighed very accurately, 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 with 1 and 0.01 mg/L of test compound and 0.1 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) and Triton X-100 (1% v/v) 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 25–50 seeds/disk) in Petri Dishes, covered with a second glass fiber filter paper disk, wetted with water, and stored in the dark for 14 days at 23 °C for *Orobanche* and 27 °C for *Striga* seeds. Then the conditioning water was removed and replaced by 100 $\mu$L of test solution/disk. After incubation for 4–7 days in the dark at the indicated temperatures, the germination percentage was determined under a microscope. Seeds were considered to be germinated if the radicle protruded through the seed coat.

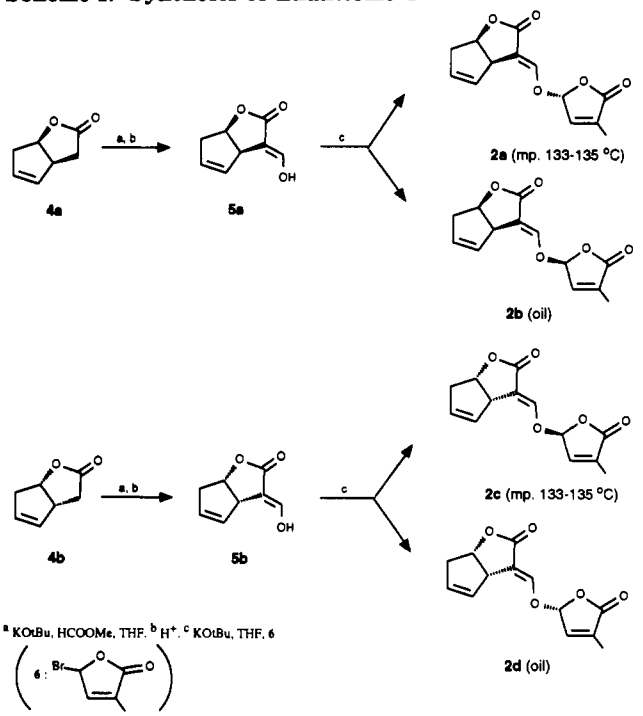
In each test series aqueous solutions with 0.1 and 0.001% (v/v) acetone were used as negative controls. Test solutions of the stimulant GR24 (concentrations of 1 and 0.01 mg/L) were used as positive controls. Tests were replicated three times, and in each test the germination percentages were determined on at least 10 separate disks.

## RESULTS AND DISCUSSION

The synthesis of GR7 was accomplished following in essence the method described by Johnson et al. (1976) which involves the coupling of bicyclic lactone **4** with a suitable butenolide **6** via an enol ether linkage. The bicyclic lactone **4**, also known as Corey's lactone, is commercially available in both enantiomeric forms, **4a** and **4b**, respectively. This enables the synthesis of all four enantiomers of GR7, as is outlined in the Scheme I.

Coupling of enantiomer **4a** with butenolide **6** leads to a diastereomeric mixture of **2a** and **2b**, which are epimeric at C-4'. It was found practical for this small-scale preparation to isolate the hydroxymethylenolactone **5a**, instead of using its potassium salt, obtained in the hydroxymethylation reaction, directly. The epimers **2a** and **2b** could readily be separated by chromatography on silica gel. This method is preferred over that described by Connick and Pepperman (1981), who used crystallization and preparative high-performance liquid chromatography (HPLC) to separate the racemic diastereomers of GR7. After chromatographic purification, the "fast moving" epimer **2a** was crystallized; however, all attempts to obtain crystals from the "slow moving" compound **2b** were unsuccessful. The same sequence of reactions was per-

**Scheme I. Synthesis of Enantiomers**




formed for enantiomerically pure bicyclolactone **4b**, leading to crystalline product **2c** (this is the antipode of **2a**) and oily compound **2d** (antipode of **2b**).

The enantiomeric purity of the four compounds prepared was determined by 400-MHz $^1$H NMR analysis using chiral shift reagent Eu(hfc)$_3$. The spectra obtained were compared with that of racemic GR7 under the same circumstances. All four compounds **2a–d** were found to have an enantiomeric purity of >98%; in no case could signals of the antipode be detected.

To establish the absolute configuration of the four compounds **2a–d**, an X-ray diffraction analysis of **2a** was undertaken (Bosman et al., 1992). This analysis reveals that compound **2a** has the same absolute configuration at C-3a, C-6a, and C-4′ as natural (+)-strigol. On the basis of the absolute configuration of **2a** the stereochemistry of the other three can readily be deduced, because **2c** is the enantiomer of **2a** (cf. starting lactone **4** and optical rotation: −341° for **2c** and +339° for **2a**) and **2b** and **2d** are their respective epimers at C-4′. The correct absolute stereochemistry of all four stereomers is depicted in Scheme I.

Connick and Pepperman (1981) assigned the relative configuration 3a(*R*),6a(*R*),4′(*S*) to the racemic diastereomer with the highest $R_f$ value on TLC. The results presented above show that the enantiomer with the highest $R_f$ value has the 3a(*R*),6a(*R*),4′(*R*) configuration. This implies that assignment of the relative stereochemistry by Connick and Pepperman, which was based on comparison of melting points and $R_f$ values of GR7 diastereomers with those of (±)-strigol and (±)-4′-epistrigol, is incorrect. The lack of correlation between physical parameters of GR7 diastereomers and those of strigol and 4′-epistrigol may be due to the large effect of the A ring of strigol—in particular the hydroxyl function at the A ring—on these parameters.

The ability of all four enantiomers to stimulate germination was investigated for seeds of *S. hermonthica* and *O. crenata*. The results and those obtained with mixtures of diastereomers of GR7 and GR24 are collected in Tables I and II. The data in Tables I and II reveal that there is a significant difference in stimulant activity between the four enantiomers. It is obvious that compound **2a** is the most active isomer, and at a concentration of 0.01 mg/L it is the only isomer that displayed appreciable activity for both seed types. On the other hand, compound **2c** (the antipode of **2a**) showed no activity at 0.01 mg/L and only limited activity at 1 mg/L. At a concentration of 1 mg/L compound **2d** gave the same germination results as obtained with **2a**, whereas the other two isomers, **2b** and **2c**, are less active. This clearly reveals that the configuration at C-4′ is more important, with respect to germination stimulant activity, than the configuration at the other chiral centers, C-3a and C-6a. This supports the hypothesis that the active site of strigol and strigol analogues resides in the C–D part of the molecule (Hassanali, 1984).

**Table I. Germination Percentages for Seeds of *S. hermonthica* after Exposure to Solutions (1 and 0.01 mg/L) of GR7 Enantiomers 2a–d, GR7, and GR24 (Mixture of Stereomers)[a]**

| sample | % germination ± $t_{0.05}(s/n^{1/2})$ at 1 mg/L | % germination ± $t_{0.05}(s/n^{1/2})$ at 0.01 mg/L |
|---|---|---|
| **2a** | 52.2 ± 5.2 | 30.0 ± 4.1 |
| **2b** | 37.4 ± 4.9 | 2.9 ± 1.4 |
| **2c** | 16.0 ± 5.2 | 0.3 ± 0.4 |
| **2d** | 52.5 ± 4.4 | 1.2 ± 0.8 |
| GR7 (mixture of stereomers) | 50.6 ± 5.2 | 7.2 ± 1.9 |
| GR24 (mixture of stereomers) | 61.3 ± 5.2 | 68.2 ± 4.9 |
| control (no stimulant) | 1.6 ± 1.3 | 2.2 ± 1.6 |

[a] Germination percentages given are the mean of three replicate tests. In each test the percentage was determined at least 10 times by counting the number of germinated *Striga* seeds in a sample of 25 seeds.

**Table II. Germination Percentages for Seeds of *O. crenata* after Exposure to Solutions (1 and 0.01 mg/L) of GR7 Enantiomers 2a–d, GR7, and GR24 (Mixture of Stereomers)[a]**

| sample | % germination ± $t_{0.05}(s/n^{1/2})$ at 1 mg/L | % germination ± $t_{0.05}(s/n^{1/2})$ at 0.01 mg/L |
|---|---|---|
| **2a** | 76.6 ± 2.4 | 22.6 ± 2.4 |
| **2b** | 26.0 ± 3.0 | 0.5 ± 0.5 |
| **2c** | 3.1 ± 1.2 | 0.0 ± 0.0 |
| **2d** | 76.4 ± 3.2 | 1.4 ± 0.8 |
| GR7 (mixture of stereomers) | 69.1 ± 3.6 | 10.5 ± 2.4 |
| GR24 (mixture of stereomers) | 78.4 ± 3.7 | 13.3 ± 2.9 |
| control (no stimulant) | 0.0 ± 0.0 | 0.0 ± 0.0 |

[a] Germination percentages given are the mean of three replicate tests. In each test the percentage was determined at least 10 times by counting the number of germinated *Orobanche* seeds in a sample of 25 seeds.

The differences in activities shown in Tables I and II, in particular those between enantiomers **2a** and **2c**, are more pronounced than the slight difference between diastereomers of GR7 observed by Pepperman et al. (1982). This smaller difference observed by these authors can easily be understood, because their lower melting diastereomer with the slightly higher activity is actually a 1:1 mixture of the most active and least active enantiomers, **2a** and **2c**. Their higher melting diastereomer consists of a mixture of enantiomers with intermediate activity, **2b** and **2d**.

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