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Asymmetric Synthesis of All Stereoisomers of the Strigol Analogue GR24. Dependence of Absolute Configuration on Stimulatory Activity of Striga hermonthica and Orobanche crenata Seed Germination

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All four optically pure stereoisomers of the strigol analogue GR24 were prepared via two different routes. In the first approach enantiopure ABC-fragments 4 were used as the chiral source, whereas in the alternative route both antipodes of the latent D-ring 6 were employed. Bioassays revealed significant differences in activity between the four stereoisomers in the stimulation of germination of the parasitic weeds Striga hermonthica and Orobanche crenata.

Keywords: Striga; Orobanche germination, GR24

INTRODUCTION

Parasitic weeds belonging to the genera Striga, Orobanche, and Alectra cause severe damage to several graminaceous and leguminous crops in tropical and semitropical areas of the eastern hemisphere (Musselman, 1987; Parker and Riches, 1993). Seeds of these parasitic weeds usually remain dormant in the soil, but after exposure to a specific germination stimulant, which is exuded by the roots of a suitable host, the seeds germinate and attach to the root system of the host (Press et al., 1990). The stimulation of germination, in particular the compounds by which this process is triggered, has received much attention. The most important naturally occurring germination stimulant, viz. (+)-strigol (1) (Figure 1), has been isolated from the root exudate of the false host cotton (Gossypium hirsutum L.) (Cook et al., 1966), and its structure was elucidated (Cook et al., 1972). The absolute configuration was unambiguously determined several years later (Brooks et al., 1985). Recently, it has been shown to be the major Striga germination stimulant produced by the true hosts maize (Zea mays L.) and proso millet (Panicum miliaceum L.) (Siame et al., 1993). It was demonstrated that the absolute stereochemistry of strigol 1 is of prime importance with respect to seed germination activity (Hauck and Schildknecht, 1990; Bergmann et al., 1993).

In a study to design simpler analogues of (+)-strigol (1) with optimal bioactivity, Johnson et al. (1976, 1981) have prepared the rather readily accessible synthetic strigol analogue GR24 (2) (Figure 1). Its racemic preparation and the separation of its diastereomers was improved considerably (Mangnus et al., 1992a; Nefkens et al., 1996). The seed germination stimulatory activity toward several parasitic weed species is within the same order of magnitude as that of strigol (Pepperman et al., 1987; Hauck et al., 1992; Bergmann et al., 1993). For these reasons GR24 (2) has found widespread applications, including its use as a positive control in bioassays of Orobanche and Striga seed germination (Mangnus et al., 1992b). So far, only little attention has been paid to the influence of the absolute stereochemistry of the synthetic strigol analogues on the biological activity. Recently, all four stereoisomers of GR7 (3), a strigol analogue lacking the A-ring, have been prepared and tested on seed germination activity (Mangnus and Zwanenburg, 1992). It was concluded that the correct absolute stereochemistry at C-2′ (R-configuration) is essential to exert maximal bioactivity.

The present paper deals with the asymmetric syntheses of all four stereoisomers of GR24 (2) and their germination activities on seeds of Striga hermonthica (Del.) Benth and Orobanche crenata Forsk.

MATERIALS AND METHODS

Nomenclature. The AUTONOM 1.0 program, provided by the Belstein Institute and Springer-Verlag (Weinheim, Germany) was used.

Syntheses. General Remarks. 1H-NMR (100 MHz and 400 MHz) spectra were recorded on Bruker AC 100 and Bruker AM-400 spectrometers, respectively (Me4Si as internal standard), both from Bruker (Wissembourg, France). All coupling constants are given as J in hertz, unless indicated otherwise. For mass spectra a double-focusing VG7070E mass spectrometer from VG Analytical (Manchester, U.K.) was used. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Elemental analyses were performed at the Department of Micro-analysis of this laboratory.

Solvents were dried using the following methods: Dichloromethane was distilled from P2O5. Diethyl ether was distilled...
from NaH. Hexane was distilled from CaH₂. 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 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.

Sodium hydride (60% in dispersion oil) was washed twice with hexane just before use. 3,3a,4,8b-Tetrahydroinden-1(2H)-furan-2-one (rac 4) and 5-bromo-3-methyl-2-(SH)-furanone (5) were prepared following published methods (Mangnus et al., 1992a). The synthesis of chloralactones 6 and 7 was reported previously (Thuring et al., 1995).

For the resolution of rac 4, a mixture of cellulose triacetate and silica gel 25–40 mesh (Merck) was used. The enantiopurities of 4 and 7 were determined by HPLC analysis using a Spectra Physics SP8700 HPLC apparatus (Spectra Physics, Eindhoven, The Netherlands), a chiral Baker Chiralcel OD-H column (eluent hexane/ethanol 60:40 (v/v), flow rate 0.5 mL/min), and an LKB 2138 Unicord UV–VIS detector (254 nm) (Pharma Biotech., Roozendaal, The Netherlands).

Chromatographic Resolution of 3,3a,4,8b-Tetrahydroinden-1(2H)-furanone (rac 4). The eluent CTA (50 g) was swollen before use by heating in EtOH/H₂O 95:5 (150 mL) at ca. 75 °C for 20 min. A glass column (i.d. 2.5 cm) was slurry-packed with this material, and the stationary phase was eluted with 95% EtOH at a pressure of ca. 1.2 bar. The racemic lactone rac 4 (500 mg), dissolved in 95% EtOH (3 mL), was chromatographically resolved, using 95% EtOH as the eluent. Fractions of ca. 5 mL were collected, their optical rotations were recorded, and they were analyzed for enantiomeric excess (ee) by chiral HPLC: yield: 170 mg (34%) of pure fast-moving enantiomer 4 (tR = 11.30 min) as a white solid; [α]D −107.0° (c 0.4, CHCl₃), ee > 98%.

The slow-moving enantiomer, ent 4 (170 mg, 34%) (tR = 12.18 min), was obtained as a white solid: [α]D −102.5° (c 0.4, CHCl₃), ee > 98%.

Formylation of 3a(R),8b(S)- and Coupling with 5-Bromo-3-methyl-2-(SH)-furanone 5 (General Procedure). Potassium tert-butoxide (131 mg, 1.17 mmol) was added to a solution of lactone 4 (185 mg, 1.07 mmol) and methyl formate (1.6 mmol) in THF (5 mL) with stirring at 0 °C under nitrogen. Stirring was continued for 18 h at room temperature, followed by the addition of a solution of furanone 5 (207 mg, 1.17 mmol) in THF (10 mL) at 0 °C under nitrogen. After 18 h of stirring, precipitated potassium bromide was removed by filtration. The filtrate was concentrated in vacuo, and the residue was dissolved in a mixture of water (5 mL) and chloroform (10 mL). The aqueous phase was extracted with chloroform (three times). The combined organic layers were dried (MgSO₄) and concentrated in vacuo. The crude yellow oil was purified by flash chromatography (SiO₂, disisopropyl ether/ethyl acetate 4:1) to give two separated diastereomeric products, 2a and ent 2b, as white solids.

3-[4-Methyl-5-oxo-2,5-dihydrofuran-2(R)-yloxymethylene]-3,3a(R),4,8b(S)-tetrahydroinden-1(2H)-furanone-2-one (2a) and its 2'S-Epimer (ent 2b). These compounds were prepared according to the general procedure, starting from lactone 4 (186 mg, 1.07 mmol) to give 2b (yield = 33%) and ent 2 (yield = 27%). Analytically pure samples were obtained by crystallization from hexane/ethyl acetate.

- 2b obtained as colorless crystals; mp 133.5–134 °C; [α]D −466° (c 0.25, CHCl₃). Anal. Calcd for C₁₂H₁₁O₃: C, 68.45; H, 4.73. Found: C, 68.31; H, 4.58.
- 2b: 1H NMR (CDCl₃, 400 MHz) δ 2.05 (3H, CH₃), 3.10 (dd, 1H, J = 16.9 Hz, J = 3.1 Hz, CH₃), 3.42 (dd, 1H, J = 16.9 Hz, J = 9.3 Hz, H₃), 3.94 (1H, H₃a), 5.96 (1H, J = 7.9 Hz, H₃b), 6.17 (1H, OCHO D-ring), 6.96 (1H, H₃, CH-D-ring), 7.23–7.36 (3H, 3H, CH₂, Ar H), 7.48 (1H, J = 2.5 Hz, =CHO), 7.50 (2H, J = 1.7 Hz, J = 7.5 Hz, Ar H); mass data were the same as for 2a.
- 2a: Anal. Calcd for C₁₂H₁₀O₃: C, 68.45; H, 4.73. Found: C, 68.31; H, 4.58.

3-[4-Methyl-5-oxo-2,5-dihydrofuran-2(R)-yloxymethylene]-3,3a(S),4,8b(R)-tetrahydroinden-1(2-b)-furanone-2-one (2b) and its 2’S-Epimer (ent 2a). These compounds were prepared according to the general procedure, starting from lactone ent 4 (186 mg, 1.07 mmol) to give 2a (yield = 33%) and ent 2 (yield = 27%). Analytically pure samples were obtained by crystallization from hexane/ethyl acetate.

- 2a obtained as colorless crystals; mp 152.5–154.5 °C; [α]D −446° (c 0.25, CHCl₃). Anal. Calcd for C₁₂H₁₀O₃: C, 68.45; H, 4.73. Found: C, 68.12; H, 4.67. 1H NMR and mass data were the same as for compound 2a.

Determination of Enantiopurities. 1H NMR (400 MHz) analysis of racemic mixtures of diastereomers 2a and 2b with optical shift reagent Eu(hfc)₃ (0.5 equiv) revealed a downfield shift of all resonances of the enantiomers, indicating the presence of a signal for the enol ether proton (―CH=O), amounting to 0.35 ppm.

1H NMR (400 MHz) spectra of pure compounds 2a and 2b displayed comparably shifts on treatment with Eu(hfc)₃, but in contrast to the racemates, no splitting of signals was observed, indicating an enantiopurity of at least 98%.

3-[4(S)-Methyl-5-oxo-4-oxatricyclo[5.2.1.0²,6]dec-8-en-3(R)-yloxymethylene]-3,3a(R),4,8b(S)-tetrahydroinden-1(2H)-furanone-2-one (7a) and Its 3a(S),8b(R) Diastereomer (7b). To a stirred suspension of [α]D −446° (c 0.25, CHCl₃). Anal. Calcd for C₁₂H₁₀O₃: C, 68.45; H, 4.73. Found: C, 68.12; H, 4.67. 1H NMR and mass data were the same as for compound 2a.

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1H NMR (400 MHz) spectra of pure compounds 2a and 2b displayed comparably shifts on treatment with Eu(hfc)₃, but in contrast to the racemates, no splitting of signals was observed, indicating an enantiopurity of at least 98%.
5.53. 1H-NMR and mass data were the same as for compound 5.45. 1H-NMR and mass data were the same as for compound chlorolactone 7a.

Compounds were prepared in the same way as described for 7a and 7b, starting from rac 4 (302 mg, 1.73 mmol) and chlorolactone ent 6 (379 mg, 1.91 mmol). The fast-moving diastereomer ent 7a (202 mg, 32%) was obtained as a white solid, and crystallization from diisopropyl ether/ethyl acetate afforded analytically pure ent 7a. The slow-moving diastereomer ent 7b (215 mg, 34%) was obtained as a white solid, which gave an analytically pure sample after crystallization from hexane/ethyl acetate.


ent 7b: mp 193–193.5 °C; [α]D +340° (c 0.1, CHCl3). Anal. Calcd for C22H20O5: C, 72.52; H, 5.53. Found: C, 72.17; H, 5.53. 1H-NMR and mass data were the same as for compound 7a.

Cycloreversion of Adducts 7a,b and ent 7a,b. A solution of the enantiopure cycloadduct 7d (93 mg, 0.26 mmol) in o-dichlorobenzene (40 mL) was heated at 180 °C for 14 h. The solvent was removed under vacuum. The residue was purified by flash chromatography (SiO2) to give analytically pure ent 7a. These compounds were prepared in the same way as described in the enantiopure cycloadduct 7a.

Vicia faba) seeds of Striga hermonthica (from Vicia faba L.) were harvested in Burkina Faso in 1994 and in Egypt in 1991, respectively, and were stored in the dark at room temperature until use in germination tests. Bioassays were carried out essentially following the procedure of Mangnus et al. (1992b) with minor modifications. Only analytically pure samples as judged from correct elemental analyses were used in these experiments.

Preparation of Test Solutions. A compound to be tested was weighed out very accurately to the amount of 2.5 mg, dissolved in 5 mL of acetone p.a., and diluted with demineralized water to 25 mL. Aliquots of this stock solution were further diluted with water to obtain test solutions containing 1, 0.5, 0.1, 0.05, and 0.001 mg/mL test compound and 0.2, 0.1, 0.02, 0.01, and 0.0002% (v/v) acetone, respectively.

Bioassays. For surface sterilization seeds of S. hermonthica [from Sorghum bicolor (L.) Moench] and Orobanche crenata (from Vicia faba L.) were harvested in Burkina Faso in 1994 and in Egypt in 1991, respectively, and were stored in the dark at room temperature until use in germination tests. Bioassays were carried out essentially following the procedure of Mangnus et al. (1992b) with minor modifications. Only analytically pure samples as judged from correct elemental analyses were used in these experiments.

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In the second approach the complete resolution of GR42 (2) was accomplished by formylation of rac 4, followed by coupling with the homochiral latent D-rings 6 and ent 6, respectively (Scheme 2).

The asymmetric syntheses of 6 and ent 6, together with their application in the preparation of all four homochiral diastereomers of GR7 (3), have recently been described (Thuring et al., 1995). The sequence as outlined in Scheme 2 gave the cycloadducts 7a and 7b and their corresponding enantiomers in diastereomeric ratios of approximately 1:1. These reactions proceeded with complete stereocontrol as no signals arising from the respective C3-epimers could be detected in the NMR spectra. Cycloreversion by heating the homochiral adducts of 7 in o-dichlorobenzene at 180 °C afforded the corresponding enantiopure stereoisomers of GR42 (2), which have the same $\left[\alpha\right]_{D}$ values as those prepared by the route depicted in Scheme 1. It was essential to control the reaction temperature and time carefully in order to avoid concomitant epimerization at C2.

Determination of Absolute Configuration. It was attempted to establish the absolute configuration of the GR42 (2) stereoisomers by comparison of their circular dichroism (CD) spectra with those of the corresponding stereoisomers of strigol 1, which have been reported (Heather et al., 1976; Frischmuth et al., 1993).

However, such a correlation is not reliable, because GR42 (2) has a different ABC-chromophore. Therefore, an X-ray diffraction analysis of 2a was undertaken (Moers et al., 1995) to establish its absolute configuration. A stereoview of 2a is depicted in Figure 2. With the absolute configuration of 2a known, the configuration of the remaining stereoisomers could be assigned on the basis of the synthetic sequence.

Biological Activity. The germination stimulatory activity of all stereoisomers of GR42 2a, b and ent 2a, b was assayed using seeds of S. hermonthica and O. crenata. In each bioassay a diastereomeric mixture of GR42 was included as a positive control. In preliminary experiments the concentration-dependent activity range (GR42) of seeds of S. hermonthica has been established. Maximal germination percentages were obtained within the concentration range 0.01–1 mg/L. Half-maximal activity was observed at approximately 0.001 mg/L (data not shown). The relative bioactivity of the individual stereoisomers of GR42 was therefore assayed at an optimal concentration (0.1 mg/L) and at a sensitive concentration (0.001 mg/L). It was anticipated that the latter should exhibit more profound differences. Relevant data are collected in Table 1.

The same stereoisomers were also tested for stimulant activity on seeds of O. crenata, using four concentrations. The results are shown in Figure 3. These data (Table 1; Figure 3) reveal that there is a significant difference in stimulatory activity among the four stereoisomers. For both parasitic species, the enantiomer of GR42 possessing the “natural” absolute stereochemistry, viz. 2a, is considerably more active than its optical antipode ent 2a. The difference in activity amounts to at least a factor of 100. The relative importance of the different stereogenic centers can be addressed by comparison of the bioactivities of the

![Figure 2](https://example.com/figure2.png)

**Table 1. Germination Percentages for Seeds of S. hermonthica after Exposure to Solutions (0.1 and 0.001 mg/L) of GR42 Enantiomers 2 and the Corresponding Racemic Mixture of Diastereomers rac 2.**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Configuration at C2</th>
<th>at 10^{-3} mg/L</th>
<th>at 10^{-3} mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2a</td>
<td>R</td>
<td>56.2 ± 4.6</td>
<td>32.5 ± 4.5</td>
</tr>
<tr>
<td>2</td>
<td>ent 2b</td>
<td>S</td>
<td>40.8 ± 2.5</td>
<td>0.7 ± 0.4p</td>
</tr>
<tr>
<td>3</td>
<td>ent 2a</td>
<td>S</td>
<td>4.0 ± 0.6</td>
<td>0.5 ± 0.5p</td>
</tr>
<tr>
<td>4</td>
<td>2b</td>
<td>R</td>
<td>54.0 ± 2.6</td>
<td>0.4 ± 0.4p</td>
</tr>
<tr>
<td>5</td>
<td>rac 2c</td>
<td>R/S</td>
<td>47.1 ± 3.9</td>
<td>33.2 ± 2.2</td>
</tr>
</tbody>
</table>

* The data presented ± SE are from one representative experiment. ** Not significantly different from aqueous control (without stimulant). † Equimolar mixture of two racemic diastereomers.
C₃aC₈b-epimer 2b and the C₂-epimer ent 2b. The data in Table 1 (entries 2 and 4) reveal in the case of S. hermonthica a slight preference for the correct stereochemistry at C₂. In contrast, the data obtained for O. crenata (Figure 3) suggest that for this species the absolute configuration at C₃aC₈b is more important for the stimulatory activity than the configuration at C₂. This result is in contrast with data obtained from a comparative study of GR7 stereoisomers, which revealed a more profound role of the C₂-configuration (Mangnus and Zwanenburg, 1992). This discrepancy indicates that one cannot attribute the configuration of a particular stereogenic center as solely relevant for the biological activity by disregarding the entire threedimensional structure. Because GR7 lacks the A-ring, its smaller BC-part is apparently more flexible with respect to interactions within the receptor cavity. This implies a less critical requirement for its absolute configuration in comparison with the ABC-fragment of GR24.

Concluding Remarks. Two routes for the asymmetric synthesis of all four stereoisomers of GR24 have been presented. The route that requires the homochiral latent D-rings 6 and ent 6 is generally applicable in the enantioselective synthesis of strigol analogues. The relative importance of the absolute configuration at the different stereogenic centers in GR24 for the germination stimulatory activity is has been assessed, indicating that the molecular shape is determined by the combination of all stereocenters.

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LITERATURE CITED


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