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

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Strigol and sorgolactone belong to the class of “strigolactones”, which are highly potent germination stimulants of seeds of the parasitic weeds Striga and Orobanche. The aim of the present work was to synthesize all four stereoisomers of demethylsorgolactone (6), which lacks the methyl group in the A-ring of naturally occurring sorgolactone, and to evaluate their activities in the stimulation of germination of Striga hermonthica and Orobanche crenata seeds. Two diastereomers of demethylsorgolactone (6) were prepared and resolved in the corresponding enantiomers. Bioassays revealed that the germination stimulatory activity of 6 is comparable to that of strigol and that there exist significant differences in activity among the individual stereoisomers.

Keywords: Striga; Orobanche; germination; demethylsorgolactone

INTRODUCTION

Parasitic weeds belonging to the genera Striga, Orobanche, and Alectra have an extremely devastating impact on several graminaceous and leguminous crops in tropical and semitropical areas of the eastern hemisphere (Musselman, 1987; Parker and Riches, 1993). These root parasites specifically interact with their host at four levels: (1) germination of parasitic seed; (2) initiation of haustorial development; (3) transfer of water and minerals; and (4) host responses to infection (Press et al., 1990). The first two mentioned events require host-derived signals as recognition cues. Especially, the stimulation of germination and the compounds that trigger this process have attracted much attention. The first naturally occurring germination stimulant, (+)-strigol (1) (Figure 1), was isolated from the root exudate of the false host cotton (Gossypium hirsutum L.) (Cook et al., 1966), and its structure was elucidated in 1972 (Cook et al., 1972). The absolute configuration was unambiguously determined several years later (Brooks et al., 1985).

It was not until 1992 that some germination stimulants closely related to strigol were identified in the root exudates of true hosts, viz. sorgolactone (2) (Hauck et al., 1992) from sorghum [Sorghum bicolor (L.) Moench], which is a host of several Striga species, and alectrol (3) (Müller et al., 1992) from cowpea [Vigna unguiculata (L.) Walp.], which is a host of Alectra species and Striga gesnerioides (Willd.) Vatke (Figure 1). Soon thereafter, strigol itself was shown to be the major Striga germination stimulant produced by maize (Zea mays L.) and proso millet (Panicum miliaceum L.) (Siame et al., 1993). Root exudates of Striga hosts contain a mixture of strigol, sorgolactone, and alectrol, albeit in different ratios. Recently, the collective name “strigolactones” was proposed for this class of compounds (Butler, 1995). So far, no germination stimulants from Orobanche hosts have been identified. However, it is assumed that structures closely related to the strigolactones are primary responsible for germination stimulation of Orobanche seeds. Structure–bioactivity relationship studies have been conducted to localize the bioactiphore and to design simpler analogues (Johnson et al., 1976, 1981; Vail et al., 1990; Zwanenburg et al., 1994). Especially, GR24 (4) and GR7 (5) (Figure 1) are highly potent synthetic strigol analogues. These analogues and the strigolactones 1–3 possess the same enol ether and CD fragments. It was concluded that the bioactiphore resides in this part of the molecule (Mangnus and Zwanenburg, 1992a). In this paper the synthesis and biological evaluation of a strigolactone analogue, which is structurally closely related to sorgolactone 2, viz. demethylsorgolactone (7) (DMSL), is reported. The primary aim of this work is to achieve a synthesis with
full stereorcontrol of all four stereoisomers of demethylsorgolactone and to evaluate their activities in the stimulation of germination of Striga hermonthica and Orobanche crenata seeds. In this manner further insight is gained on the stereochemical requirements of the stimulant to exert bioactivity.

MATERIALS AND METHODS

Nomenclature. The AUTONOM 1.0 program, provided by the Beilstein Institute and Springer Verlag, Weinheim, Germany, was used.

Synthesis. General Remarks. 1H-NMR (100 MHz) and 1H-NMR (400 MHz) spectra were recorded on Bruker AC 100 and Bruker AM-400 spectrometers, respectively (Me6Si as internal standard). All coupling constants are given as J in hertz, unless indicated otherwise. For mass spectra a double-focusing VG7070E mass spectrometer was used. GC/MS spectra were obtained on a Varian Star 3400 GC/MS 2020 spectrometer. Mass spectrometry was performed at the Department of Micro-analysis of this laboratory. CD spectra were recorded using a Jasco J 600 spectrophotometer.

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 aluminium hydride just before use. All other solvents were of analytical grade. Thin layer chromatography (TLC) was carried out on Merck precoated silicagel 60 F254 plates (0.25 mm). Spots were visualized with UV or using a molybdate spray. Flash chromatography (SiO2, hexane/ethyl acetate 9:1) was conducted with a Hewlett-Packard HP 5890 gas chromatograph. Spectra were run on a Varian Saturn 2 GC/MS system. Focusing VG7070E mass spectrometer was used. GC/MS were performed with a 6890 series, respectively (Me4Si as internal standard) and HRMS/EI, m/z calcd for C17H18O5. 6a

Analytical samples were obtained by recrystallization from a mixture of water and ethyl acetate. The aqueous phase was extracted with ethyl acetate (two times), and the combined organic layers were washed with water (two times), dried (MgSO4), and concentrated in vacuo.

To a cooled (17–30 °C) solution of GR24 4, 30% EtOH solution (2 equiv), 12 (306 mg, 1.72 mmol) in diethyl ether (5 mL) at room temperature under nitrogen. Ethyl formate (1.7 mL, 21 mmol) was added and stirring was continued for 15 h.

The crude product was purified using flash chromatography (SiO2, hexane/ethyl acetate 1:2) to afford two diastereomeric products. 3-(4-Methyl-5-oxo-2,5-dihydrofuran-2-yl)oxyethene (rac 6a) and rac 6b.

To a stirred suspension of NaH (46 mg, 1.90 mmol) in diethyl ether (10 mL) at room temperature, 13 (0.20 g, 1.0 mmol) was dissolved in 15 mL of a 0.13 M CeCl3/0.15M solution (2 equiv) and NaBH4 (0.16 g, 4.0 mmol) was slowly added stirring at room temperature. After 20 min of stirring, the reaction mixture was quenched with 20% H2SO4 (1 mL) and filtered over Celite. Ethyl acetate (25 mL) was added to the filtrate, and the organic phase was washed with saturated NaHCO3 (10 mL) and then dried (MgSO4), and concentrated in vacuo to give pure (145 mg, 79%) as a slightly brown oil, which solidified on standing. The analytical data were in complete agreement with those obtained in procedure a.

3-(4-Methyl-5-oxo-2,5-dihydrofuran-2-yl)oxyethene (rac 6a) and rac 6b.

A solution of 11 (0.20 g, 1.0 mmol) in 5 mL of acetone (145 mg, 79%) as a slightly brown oil, which solidified on standing. The analytical data were in complete agreement with those obtained in procedure a.

3-(4-Methyl-5-oxo-2,5-dihydrofuran-2-yl)oxyethene (rac 6a) and rac 6b.

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The crude product was purified using flash chromatography (SiO2, hexane/ethyl acetate 1:2) to afford two diastereomeric products. 3-(4-Methyl-5-oxo-2,5-dihydrofuran-2-yl)oxyethene (rac 6a, 6b, 6c, 6d).

To a stirred suspension of NaH (46 mg, 1.90 mmol) in diethyl ether (10 mL) at room temperature, 13 (0.20 g, 1.0 mmol) was dissolved in 15 mL of a 0.13 M CeCl3/0.15M solution (2 equiv) and NaBH4 (0.16 g, 4.0 mmol) was slowly added stirring at room temperature. After 20 min of stirring, the reaction mixture was quenched with 20% H2SO4 (1 mL) and filtered over Celite. Ethyl acetate (25 mL) was added to the filtrate, and the organic phase was washed with saturated NaHCO3 (10 mL) and then dried (MgSO4), and concentrated in vacuo to give pure (145 mg, 79%) as a slightly brown oil, which solidified on standing. The analytical data were in complete agreement with those obtained in procedure a.

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3-{[S]}-Methyl-5-oxo-4-oxa-tricyclo[5.2.1.0^2,6]decan-8-en-3 alpha-(R)-yl-oxy-methylene)-3,3a(R),4,5,6,7,8,8 b(S)-octahydroindeno[1,2-b]-furane-2-one (1a) and its 3a(S),8b(R) Diasteromer (ent 1b). To a stirred suspension of NaH (68.0 mg, 1.70 mmol) in diethyl ether (10 mL) was gradually added tricyclic lactone 7 (276 mg, 1.55 mmol) in diethyl ether (5 mL) at room temperature under nitrogen. Ethyl formate (1.7 mL, 21 mmol) was added and stirring was continued for 15 h. The solvent was removed in vacuo. The residue was obtained as a white solid, and recrystallization from hexane/ethyl acetate afforded analytically pure samples. Fast moving diastereomer 14a (203 mg, 36%) was obtained as a white solid, and crystallization from hexane/ethyl acetate afforded analytically pure 14a. The slow moving diastereomer ent 14b (261 mg, 46%) was obtained as a white solid, which gave an analytically pure sample after crystallization from hexane/ethyl acetate. 1.57(s,3H,CH$_3$),1.62 d 3.61(m,1H,H$_3$a),5.23(d,1H, H$_4$),1.96 - 2.80(m,6H,2 - C=C=CH$_2$ - A-ring), 1.96 - 2.33(br d, 1H, 2 - C=O - A-ring) for C$_{17}$H$_{18}$O$_5$: C, 67.54; H, 6.00. Found: C, 66.88; H, 5.97.

3-{[4-Methyl-5-oxo-2,5-dihydro-furanan-2(S)-yl-oxy-methylene]-3,3a(R),4,5,6,7,8,8b(S)-octahydroindeno[1,2-b]-furane-2-one (6a). This compound was prepared in the same way as described for 6a, starting from ent 14a (250 mg, 0.66 mmol). Yields were 73 mg, 36%, as a white solid. An analytical sample was obtained by crystallization from hexane/ethyl acetate: mp 153.5 - 155.5 °C; [α]$_D$ +285° (c 0.1, CH$_2$Cl$_2$); H-NMR and mass data were the same as for rac 6a. Anal. Calcd for C$_{17}$H$_{18}$O$_5$: C, 67.54; H, 6.00. Found: C, 66.88; H, 5.97.

3-{[4-Methyl-5-oxo-2,5-dihydro-furanan-2(S)-yl-oxy-methylene]-3,3a(R),4,5,6,7,8,8b(S)-octahydroindeno[1,2-b]-furane-2-one (6b). 6b was prepared in the same way as described for 6a, starting from 14b (226 mg, 0.61 mmol). Yield was 78 mg, 42%, as a white solid. An analytical sample was obtained by crystallization from hexane/ethyl acetate: mp 179.5 - 183.5 °C; [α]$_D$ +161° (c 0.4, CHCl$_3$); R$_f$ 0.24 (hexane/ethyl acetate 1:1); H-NMR (CDCl$_3$, 400 MHz) _δ_ (Del.) 1.56 - 1.66 (m, 4H, H$_6$) and mass data were the same as for 6a. Anal. Calcd for C$_{17}$H$_{18}$O$_5$: C, 67.54; H, 6.00. Found: C, 67.32; H, 6.00.

Bioassays. For surface sterilization seeds of S. hermonthica were subsequently exposed to 20%, (v/v) ethanol for 5 min and sodium hypochlorite (2% active chlorine) for 2 min with agitation. Seeds of _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.

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, each containing 2 disks (Orobanche) or 4 disks (Striga), 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 (Orobanche) or 3 mL per Petri dish (Striga). After incubation for 24 h (Striga) and for 5 days (Orobanche) in the dark at the 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 0.2% (v/v) acetone were used as the control. As positive controls gentier GR24 (concentrations of 0.1, 0.01, and 0.001 mg/L) were used as references, which enables a comparison among results obtained in different test series. These positive controls are important, since the response of seeds of parasitic weeds, in
RESULTS AND DISCUSSION

Synthesis of Racemic DMSL. Retrosynthetic analysis of DMSL (rac 6) leads to the key building blocks 7 and 8 (Scheme 1), which are coupled in the final step via an enol ether linkage. This strategy resembles that used for the synthesis of strigol and its analogues (Mangnus et al., 1992a, and references cited therein). Tricyclic lactone 7 was prepared using essentially the concept (Scheme 2) for the synthesis of the ABC fragment of strigol (MacAlpine et al., 1974, 1976).

Bicyclopentenone 9 was prepared in two steps from cyclohexanone via addition of the dianion of propargyl alcohol, followed by acid-induced in situ Rupe rearrangement and Nazarov-type electrocyclization (Ramaiah, 1984). Carboxylic acid 10 was obtained according to a procedure analogous to that described for GR24 (4) (Mangnus et al., 1992a). Attempted reduction of the ketone function by alkaline NaBH₄ to obtain 7 was not successful. The preferred reaction course was 1,4-reduction under these conditions. However, DIBALH treatment afforded the desired 1,2-reduction in a stereoselective fashion to give tricyclic lactone 7 in yields ranging from 40 to 64%. Better results were obtained using NaBH₄ (4 equiv) in the presence of CeCl₃·7H₂O (2 equiv) (Luche, 1978), which gave 7 in a reproducible yield of 79%. Coupling of 7 via formylation and subsequent reaction of the intermediate sodium enolate 12 with bromofuranone 8 (Mangnus et al., 1992a) provided DMSL 6 as a mixture of diastereomers rac 6a and rac 6b, which could readily be separated by chromatography on silica gel (Scheme 2).

Stereoselective Synthesis. Recently, the synthesis of the homochiral latent D-ring synthons, viz. 13 and ent 13 (Figure 2), was reported (Thuring et al., 1995).

Tricyclic lactone 7 was coupled via its sodium enolate 12 with chlorolactones 13 and ent 13 to give diastereomeric mixtures (ratio ca. 1:1) of 14a, ent 14b (cy 82%), and 14b, ent 14a (62%), respectively (Figure 2), which were separated by flash chromatography. These reactions were carried out in DMF as the solvent and proceeded with complete enantioselectivity as was deduced from the observed [3]r-coupling constants between H3 and H4 in the 1H-NMR spectrum (cf. Thuring et al. (1995)).

The thermal retro-Diels–Alder reaction of homochiral adducts 14a,b and ent 14a,b to give the corresponding enantiopure DMSL stereoisomers 6a,b and ent 6a,b was accomplished by heating these adducts in o-dichlorobenzene at 180°C. It was essential to control the reaction time and temperature carefully, since the stereocenter at C-2' is rather sensitive to epimerization. The results obtained from the cycloreversion are collected in Table 1. It should be noted that in none of these reactions was any epimerization observed.

The enantiopurity of DMSL stereoisomers 6a,b and ent 6a,b (Table 1) was determined by 400-MHz 1H-NMR analysis using chiral shift reagent Eu(hfc)₃. The spectra obtained were compared with those of the corresponding racemic mixtures under the same circumstances. All compounds 6a,b and ent 6a,b had an enantiopurity of >98% in no case could signals of the antipode be detected. It is thus demonstrated that this asymmetric synthesis affords excellent stereocontrol at C-2' of the D-ring. It should be noted that the employed strategy involving the use of a homochiral latent D-ring precursor has a much larger scope than the previously reported procedures (Brooks et al., 1985; Heath et al., 1976; Berlage et al., 1987; Samson et al., 1991; Mangnus and Zwanenburg, 1992b), which all comprise asymmetric synthesis of a particular ABC precursor.

Determination of Absolute Configuration. The correct absolute configurations of DMSL enantiomers 6a, ent 6a, 6b, and ent 6b were established by comparison of their circular dichroism (CD) spectra with those of the corresponding stereoisomers of strigol. The CD spectra of (+)- and (-)-strigol have been reported (Heather et al., 1976). More recently, Frischmuth et al. (1993) compared the CD curves of (+)- and (-)-strigol...
with those of their respective 2'-epimers. It was concluded that the sign of the Cotton effect around 270 nm could directly be correlated to the stereochemistry at C-2', a negative CD sign corresponding to the 2'R configuration. Hauck et al. (1992) observed that the CD spectrum of sorgolactone (2) is identical with that of naturally occurring (+)-strigol and inverse to the spectrum of the antipode (-)-strigol. On the basis of these data, it is justified to assign the absolute stereochemistry of the DMSL enantiomers 6 using their CD curves. These spectra, which are depicted in Figure 3 are nearly identical with those of the respective stereoisomers of strigol (Frischmuth et al., 1993).

The configuration at C-2', as deduced from the CD sign around 270 nm, was in all four cases in complete agreement with the expected stereochemistry, based on the chirality of the latent D-ring synthon (Figure 2). The stereochemistry of the ABC part was assigned (Table 1) by comparison of the shape of the CD curves with that of the corresponding stereoisomers of strigol.

**Biological Activity.** The germination stimulatory activity of all stereoisomers of DMSL (6a,b and ent 6a,b) was assayed using seeds of S. hermonthica and O. crenata. In each bioassay diastereomeric mixtures of GR24 and DMSL were included as positive controls. In preliminary experiments the concentration dependent activity range (GR24 and DMSL) of seeds of S. hermonthica has been established. Maximal germination percentages were obtained within the concentration range of 1 and 0.01 mg/L. Half-maximal activity was observed at approximately 0.001 mg/L (data not shown). Assessment of the relative bioactivity of the individual stereoisomers of DMSL was therefore established at an optimal concentration (0.1 mg/L) and at a sensitive concentration (0.001 mg/L). It was anticipated that the lower concentration should exhibit more profound differences. Relevant data are collected in Table 2. The same compounds were also tested for stimulant activity on O. crenata seeds at three concentrations, the results of which are shown in Figure 4.

Rac GR24 and rac DMSL exhibit similar bioactivities for seeds of S. hermonthica (entries 5 and 6, Table 2) and O. crenata (Figure 4). It should be added that none of these compounds showed stimulatory activity toward seeds of the related root parasite Striga gesnerioides (data not shown).

Thus, replacement of the aromatic A-ring present in GR24 by a cyclohexene fragment has only a marginal effect on the respective bioactivities. This conclusion is in agreement with previously performed comparative studies of strigol and its synthetic analogues. Hauck et al. (1992) found an activity of (+)-strigol on seeds of S. hermonthica of 10-9 M (concentration at half-maximal activity), which is almost the same as that of GR24 (4). Similarly, (+)-strigol and rac 4 are almost equally active (half-maximal activity at 10-7 M) toward stimulation of seed germination of O. crenata (Bergmann et al., 1993). Pepperman et al. (1987) have reviewed the biological activity of strigol and its analogues with respect to germination of seeds of several parasitic weed species. It was concluded that the activity of strigol is comparable to that of GR24.

Next, the influence of the stereochemistry on the bioactivities of DMSL enantiomers 6a,b and ent 6a,b was examined. Not unexpected, 6a, which possesses the "natural" absolute stereochemistry, is considerably more active than its optical antipode, ent 6a. The difference in activity toward seeds of S. hermonthica, expressed as C12max (concentration at half-maximal activity), is more than 100 (cf. entries 1 and 3; Table 2). For seeds of O. crenata this difference amounts to approximately a factor of 100 (Figure 4). The relative importance of the absolute configuration at the stereogenic centers C3,C6b and C2' on the bioactivity can be established by comparison of the activities of 6b and ent 6b (entries 2

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Configuration at C-2'</th>
<th>% Germination ± SE at a concentration of 10-1 mg/L</th>
<th>% Germination ± SE at a concentration of 10-3 mg/L</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>6a</td>
<td>R</td>
<td>63.2 ± 2.6</td>
<td>42.5 ± 1.2</td>
</tr>
<tr>
<td>2</td>
<td>6b</td>
<td>S</td>
<td>61.0 ± 3.5</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>3</td>
<td>ent 6a</td>
<td>S</td>
<td>22.8 ± 4.6</td>
<td>0.7 ± 0.7</td>
</tr>
<tr>
<td>4</td>
<td>ent 6b</td>
<td>R</td>
<td>56.3 ± 4.8</td>
<td>1.6 ± 0.8</td>
</tr>
<tr>
<td>5</td>
<td>rac 6a</td>
<td>R/S</td>
<td>47.1 ± 3.9</td>
<td>33.2 ± 2.2</td>
</tr>
<tr>
<td>6</td>
<td>rac 6b</td>
<td>R/S</td>
<td>56.0 ± 1.0</td>
<td>28.3 ± 7.4</td>
</tr>
</tbody>
</table>

* Data presented are the mean ± SE of one representative experiment.  * Equimolar mixture of two racemic diastereomers.  * Not significantly different from aqueous control (without stimulant).
and 4, Table 2; Figure 4). This reveals that these contributions are almost equal for both species, which is in contrast with the results obtained for the stereoisomers of GR7 (5), which indicate a more profound role of the configuration at C2 (Mangnus and Zwanenburg, 1992b). Bergmann et al. (1993) concluded from the relative activities of four stereoisomers of strigol that the absolute stereochemistry at C2 is of special importance to exhibit maximal stimulatory activity. However, it should be noted that the differences in activity are only marginal. A similar comparative study for all stereoisomers of GR24 (4) revealed that the stereochemistry at C3α-C8β is of considerable importance for the relative activities of four stereoisomers of strigol that et al. 1992b). Bergmann (stereoselective synthesis of all stereoisomers of DMSL (6) revealed that the stereochemistry at the absolute stereochemistry in the D-ring and BC part are both essential to exert a maximal effect. Generalizations suggesting a determining role of the correct configurations at C2 (Mangnus and Zwanenburg, 1992b; Bergmann et al., 1993) are not allowed, as these data represent special cases and obviously not a general trend. The combination of the configurations at all stereogenic centers determines the molecular shape, and this will govern the interaction with the receptor.

Concluding Remarks. A general route for the stereoselective synthesis of all stereoisomers of DMSL (6) has been achieved. It was shown that the stereochemistry at all stereogenic centers has a considerable influence on the stimulatory activity.

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LITERATURE CITED


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