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

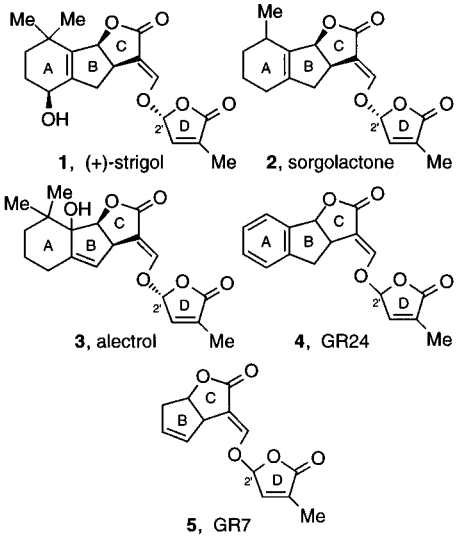

Figure 1. Structures of strigolactones **1**−**3** and analogues **4** and **5**.

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full stereocontrol 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.* ^{1}H-NMR (100 MHz) and ^{1}H-NMR (400 MHz) spectra were recorded on Bruker AC 100 and Bruker AM-400 spectrometers, respectively (Me_4Si as internal standard). All coupling constants are given as ^3J in hertz, unless indicated otherwise. For mass spectra a double-focusing VG7070E mass spectrometer was used. GC/MS spectra were run on a Varian Saturn 2 GC/MS ion-trap system. Separation was carried out on a fused-silica capillary column (DB-5, 30 m × 0.25 mm). Helium was used as carrier gas, and electron impact (EI) was used as ionization mode. GLC was conducted with a Hewlett-Packard HP 5890 gas chromatograph, using a capillary column (25 m) of HP-1 and nitrogen (2 mL/min, 0.5 atm) as the carrier gas. Melting points were measured with a Reichert Thermopan microscope and are uncorrected. Elemental analyses were performed at the Department of Micro-analysis of this laboratory. CD spectra were recorded using a Jasco J600 spectrophotometer.

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 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. The syntheses of chlorolactones **13** and *ent* **13** (Thuring *et al.*, 1995) and 5-bromo-3-methyl-2(5*H*)-furanone (**8**) (Mangnus *et al.*, 1992a) were reported previously. Bicyclic enone **9** was prepared following essentially the procedure according to Ramaiah (1984).

*2-[(Ethoxycarbonyl)methyl]-3-oxo-2,3,4,5,6,7-hexahydro-1H-indene-2-carboxylic Acid Ethyl Ester (**10**).* A solution of bicyclopentenone **9** (2.00 g, 14.7 mmol) in DMF (5 mL) was gradually added to a solution of diethyl carbonate (7.1 mL, 58.7 mmol) and sodium hydride (1.29 g, 32.3 mmol) in DMF (15 mL) with stirring at 65 °C. After 1 h of stirring at 65 °C, a solution of ethyl bromoacetate (3.67 g, 22.0 mmol) in DMF (5 mL) was gradually added. After 2 h of stirring at the same temperature, the reaction mixture was neutralized with glacial acetic acid. The mixture was concentrated *in vacuo*, and the residue was dissolved in a mixture of diethyl ether and water. The aqueous layer was extracted with diethyl ether (three times), and the combined organic layers were washed with water, dried ($MgSO_4$), and concentrated *in vacuo* to give crude **10**, which was sufficiently pure for further reactions. Purification by flash chromatography (SiO_2, hexane/ethyl acetate 9:1) provided pure **10** (3.55 g, 82%) as a yellowish oil: ^{1}H NMR ($CDCl_3$, 100 MHz) δ 1.15−1.31 (2t, 6H, J = 7.1 Hz, 2 CH_3), 1.71 (m, 4H, 2 $=CCH_2CH_2$ A-ring), 2.16 (m, 2H, $=CCH_2$ A-ring), 2.37 (m, 2H, $=CCH_2$ A-ring), 2.45 and 3.29 (AB, 2H, ^2J = 17.1 Hz, CH_2CO_2Et), 2.54 and 3.24 (AB, 2H, ^2J = 17 Hz, CH_2 B-ring), 4.02−4.26 (m, 4H, 2 CH_2CH_3); IR (CCl_4) ν 1750−1700 (several peaks, C=O), 1650 (C=C) cm^{-1}; MS [EI, *m/z*, rel intensity (%)] 294 ([M]$^+$, 33), 249 ([$C_{14}H_{17}O_4$]$^+$, 46), 220 ([$C_{12}H_{12}O_4$]$^+$, 100), 148 ([$C_{10}H_{12}O$]$^+$, 46), 91 ([C_7H_7]$^+$, 21); HRMS/EI, *m/z* calcd for $C_{16}H_{22}O_5$ 294.1467, found 294.14672 ± 0.00088.

*3-Oxo-2,3,4,5,6,7-hexahydro-1H-inden-2-yl-acetic Acid (**11**).* This compound was prepared as described for the synthesis of GR24 **4** (Mangnus *et al.*, 1992a). Starting from diester **10** (4.55 g, 15.0 mmol), crude **11** (2.29 g, 80%, GC purity 99%) was obtained as a brownish oil. For characterization a small sample was triturated in diisopropyl ether to give **11** as a white solid: mp 115−116 °C; ^{1}H NMR ($CDCl_3$, 100 MHz) δ 1.70 (m, 4H, 2 $=CCH_2CH_2$ A-ring), 2.10−2.97 (m, 9H, 2 $=CCH_2$ A-ring, CH_2 B-ring, CH B-ring, CH_2COOH), 11.0 (br s, 1H, COOH); IR (KBr) ν 3300−2500 (br, OH), 1735 (COOH), 1660 (C=O), 1630 (C=C) cm^{-1}; MS [CI, *m/z*, rel intensity (%)] 294 ([M + 1]$^+$, 100), 177 ([$C_{11}H_{13}O_2$]$^+$, 85), 149 ([$C_{10}H_{13}O_2$]$^+$, 69), 134 ([$C_9H_{10}O$]$^+$, 19), 91 ([C_7H_7]$^+$, 31).

3,3a,4,5,6,7,8,8b-Octahydroindeno-[1,2-b]furan-2-one (**7**). *Procedure a:* To a cooled (−78 °C) solution of **11** (806 mg, 4.15 mmol) in dichloromethane (25 mL) under nitrogen was gradually added DIBALH (8.7 mL of a 1 M solution in hexane) using a syringe. After 5 min of stirring at −78 °C, the mixture was quenched with 20% H_2SO_4 (15 mL) and allowed to warm up to room temperature. The aqueous phase was extracted with dichloromethane (three times). After drying ($MgSO_4$), the solvent was evaporated *in vacuo* and the residue was subjected to flash chromatography (SiO_2, hexane/ethyl acetate 4:1) to give **7** (472 mg, 64%) as a slightly brown oil, which solidified on standing. An analytical sample was obtained by crystallization from hexane/ethyl acetate to give **7** as white crystals: mp 38.5−40 °C; ^{1}H NMR ($CDCl_3$, 100 MHz) δ 1.66 (m, 4H, H_6 and H_7), 2.04 (m, 4H, H_5 and H_8), 2.27 (dd, 1H, ^2J = 17.6 Hz, J 5.1 Hz, H_3), 2.84 (dd, 1H, ^2J = 17.6 Hz, J 10.1 Hz, H_3), 2.21−2.61 (m, 2H, H_4), 3.09 (m, 1H, H_{3a}), 5.30 (br d, 1H, J = 7.0 Hz, H_{8b}); IR (CCl_4) ν 1775 (C=O) cm^{-1}; HRMS/EI, *m/z* calcd for $C_{11}H_{14}O_2$ 178.0994, found 178.09935 ± 0.00084.

Procedure b: Keto acid **11** (0.20 g, 1.0 mmol) was dissolved in 15 mL of a 0.13 M $CeCl_3 \cdot 7H_2O$ methanol solution (2 equiv), and $NaBH_4$ (0.16 g, 4.0 mmol) was slowly added with stirring at room temperature. After 20 min of stirring, the reaction mixture was quenched with 20% H_2SO_4 (pH 1−2) and filtered over hyflo. Ethyl acetate (25 mL) was added to the filtrate, and the organic phase was washed with saturated $NaHCO_3$ (three times), dried ($MgSO_4$), and concentrated *in vacuo* to give pure **7** (145 mg, 79%) as a slightly brown oil, which solified on standing. The analytical data were in complete agreement with those obtained in procedure a.

*3-(4-Methyl-5-oxo-2,5-dihydrofuran-2-yloxymethylene)-3,3a,4,,5,6,7,8,8b-octahydroindeno[1,2-b]furan-2-one (rac **6a** and rac **6b**).* To a stirred suspension of NaH (46 mg, 1.90 mmol) in diethyl ether (10 mL) was gradually added tricyclic lactone **7** (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 solvent was removed *in vacuo.* The thus obtained sodium salt **12** was dissolved in DMF (10 mL). A solution of bromofuranone **8** (333 mg, 1.87 mmol) in DMF (3 mL) was gradually added at −50 °C under nitrogen. After 17 h of stirring at room temperature, the mixture was quenched with acetic acid (0.5 mL) and the solvent was removed *in vacuo.* The residue was dissolved in 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 ($MgSO_4$), and concentrated *in vacuo.* The crude product was purified using flash chromatography (SiO_2, hexane/ethyl acetate 2:1) to afford two diastereomeric products. Fast moving diastereomer *rac* **6a** (166 mg, 32%) and slow moving diastereomer *rac* **6b** (145 mg, 28%) were obtained as white solids. Analytical samples were obtained by recrystallization from 2-propanol.

rac **6a**: mp 148−150 °C; R_f 0.34 (hexane/ethyl acetate 1:1); ^{1}H NMR ($CDCl_3$, 100 MHz) δ 1.62 (m, 4H, H_6, H_7), 1.83−2.90 (m, 9H, 2 $=CCH_2$ A-ring, CH_3, 2 H_4), 3.52−3.75 (m, 1H, H_{3a}), 5.32 (d, 1H, J 7.5 Hz, H_{8b}), 6.16 (m, 1H, OCHO D-ring), 6.93 (m, 1H, =CH D-ring), 7.43 (d, 1H, ^4J = 2.5 Hz, =CHO); MS [EI, *m/z*, rel intensity (%)] 302 ([M]$^+$, 0.2), 205 ([$C_{12}H_{13}O_3$]$^+$, 18.9), 97 ([$C_5H_5O_2$]$^+$, 100). Anal. Calcd for $C_{17}H_{18}O_5$: C, 67.54; H, 6.00. Found: C, 67.86; H, 5.94.

rac **6b**: mp 179−184 °C; R_f 0.24 (hexane/ethyl acetate 1:1); ^{1}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, 66.72; H, 5.91.

3-[6(S)-Methyl-5-oxo-4-oxa-tricyclo[5.2.1.0^{2,6}]dec-8-en-3(R)-yloxymethylene]-3,3a(R),4,5,6,7,8,8b(S)-octahydroindeno[1,2-b]-furan-2-one (**14a**) *and Its 3a(S),8b(R) Diastereomer* (*ent* **14b**). 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 thus obtained sodium salt **12** was dissolved in DMF (10 mL). A solution of chlorolactone **13** (308 mg, 1.55 mmol) in DMF (3 mL) was gradually added at room temperature under nitrogen. After 17 h of stirring, the mixture was quenched with acetic acid (0.5 mL) and the solvent was removed *in vacuo*. The residue was dissolved in 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 ($MgSO_4$), and concentrated *in vacuo*. The crude product was purified using flash chromatography (SiO_2, hexane/ethyl acetate 3:1) to afford two diastereomeric products. The 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.

14a: mp 210−212 °C; $[\alpha]_D$ +169° (*c* 0.4, $CHCl_3$); R_f 0.51 (hexane/ethyl acetate 1:1); ^{1}H NMR ($CDCl_3$, 100 MHz) δ 1.58 (s, 3H, C$\underline{H}_3$), 1.62−1.72 (m, 6H, $\underline{H}_{10}$, 2 =CCH$_2$C$\underline{H}_2$ A-ring), 1.97−2.80 (m, 6H, 2 =CC$\underline{H}_2$ A-ring, C$\underline{H}_2$ B-ring), 2.70 (dd, 1H, J = 4.1 Hz, J < 1 Hz, $\underline{H}_2$), 2.89 (m, 1H, $\underline{H}_7$), 3.23 (m, 1H, $\underline{H}_1$), 3.62 (m, 1H, $\underline{H}_{3a}$), 5.24 (d, 1H, J < 1 Hz, $\underline{H}_3$), 5.29 (br d, 1H, J = 7.2 Hz, $\underline{H}_{8b}$), 6.25 (m, 2H, $\underline{H}_8$ and $\underline{H}_9$), 7.35 (d, 1H, ^4J = 2.5 Hz, =C$\underline{H}$O); MS [EI, *m*/*z*, rel intensity (%)] 368 ([M]$^+$, 1.7), 302 ([$C_{17}\underline{H}_{18}O_5$]$^+$, 0.8), 206 ([$C_{12}H_{14}O_3$]$^+$, 13.2), 163 ([$C_{10}H_{11}O_2$]$^+$, 84.2), 97 ([$C_5H_5O_2$]$^+$, 100), 66 ([$C_5H_6$]$^+$, 13.0). Anal. Calcd for $C_{22}H_{24}O_5$: C, 71.72; H, 6.57. Found: C, 71.61; H, 6.58.

ent **14b**: mp 206−208.5 °C; $[\alpha]_D$ −227° (*c* 0.4, $CHCl_3$); R_f 0.37 (hexane/ethyl acetate 1:1); ^{1}H NMR ($CDCl_3$, 100 MHz) δ 1.57 (s, 3H, C$\underline{H}_3$), 1.62−1.72 (m, 6H, $\underline{H}_{10}$, 2 =CCH$_2$C$\underline{H}_2$ A-ring), 1.96−2.80 (m, 6H, 2 =CC$\underline{H}_2$ A-ring, C$\underline{H}_2$ B-ring), 2.73 (dd, 1H, J = 4.1 Hz, J < 1 Hz, $\underline{H}_2$), 2.89 (m, 1H, $\underline{H}_7$), 3.22 (m, 1H, $\underline{H}_1$), 3.61 (m, 1H, $\underline{H}_{3a}$), 5.23 (d, 1H, J < 1 Hz, $\underline{H}_3$), 5.28 (br d, 1H, J = 7.0 Hz, $\underline{H}_{8b}$), 6.25 (m, 2H, $\underline{H}_8$ and $\underline{H}_9$), 7.33 (d, 1H, ^4J = 2.6 Hz, =C$\underline{H}$O); mass data were the same as for **14a**. Anal. Calcd for $C_{22}\underline{H}_{24}O_5$: C, 71.72; H, 6.57. Found: C, 71.57; H, 6.54.

3-[6(R)-Methyl-5-oxo-4-oxa-tricyclo[5.2.1.0^{2,6}]dec-8-en-3(S)-yloxymethylene]-3,3a(R),4,5,6,7,8,8b(S)-octahydroindeno[1,2-b]-furan-2-one (***14b***) *and Its 3a(S),8b(R) Diastereomer* (*ent* ***14a***). These compounds were prepared in the same way as described for **14a** and *ent* **14b**, starting from tricyclic lactone **7** (274 mg, 1.54 mmol) and chlorolactone *ent* **13** (307 mg, 1.54 mmol). Yields were 216 mg, 38%, of fast moving diastereomer *ent* **14a** as white solid and 135 mg, 24%, of slow moving diastereomer **14b** as a white solid. Both compounds were crystallized from hexane/ethyl acetate to obtain analytically pure samples.

ent **14a**: mp 212.5−213 °C; $[\alpha]_D$ −178° (*c* 0.4, $CHCl_3$); ^{1}H-NMR and mass data were the same as for compound **14a**. Anal. Calcd for $C_{22}H_{24}O_5$: C, 71.72; H, 6.57. Found: C, 71.67; H, 6.46.

14b: mp 208−209.5 °C; $[\alpha]_D$ +233° (*c* 0.4, $CHCl_3$); ^{1}H-NMR and mass data were the same as for compound *ent* **14b**. Anal. Calcd for $C_{22}H_{24}O_5$: C, 71.72; H, 6.57. Found: C, 71.65; H, 6.48.

3-[4-Methyl-5-oxo-2,5-dihydrofuran-2(R)-yloxymethylene]-3,3a(R),4,,5,6,7,8,8b(S)-octahydroindeno[1,2-b]furan-2-one (***6a***). Fast moving cycloadduct **14a** (169 mg, 0.46 mmol) was dissolved in *o*-dichlorobenzene (40 mL) and heated at 180 °C for 6 h. The solvent was removed *in vacuo*. The residue was purified by flash chromatography (SiO_2, hexane/ethyl acetate 3:1) to give **6a** (42 mg, 30%) as a solid. An analytical sample was obtained by crystallization from hexane/ethyl acetate: mp 150.5−155 °C; $[\alpha]_D$ +281° (*c* 0.1, CH_2Cl_2); R_f 0.34 (hexane/ethyl acetate 1:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.60−1.67 (m, 4H, $\underline{H}_6$, $\underline{H}_7$), 1.96−2.03 (m, 3H, =CC$\underline{H}_2$ A-ring), 2.03 (m, 3H, C$\underline{H}_3$), 2.19 (m, 1H, =CC$\underline{H}_2$ A-ring), 2.34 (br d, 1H, ^2J = 16.6 Hz, $\underline{H}_4$), 2.73 (dd, 1H, ^2J = 16.6 Hz, J = 8.7 Hz, $\underline{H}_4$), 3.64 (m, 1H, $\underline{H}_{3a}$), 5.32 (d, 1H, J = 7.5 Hz, $\underline{H}_{8b}$), 6.15 (m, 1H, OC$\underline{H}$O D-ring), 6.92 (m, 1H, =C$\underline{H}$ D-ring), 7.42 (d, 1H, ^4J = 2.6 Hz, =C$\underline{H}$O); 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-furan-2(S)-yloxymethylene]-3,3a(S),4,,5,6,7,8,8b(R)-octahydroindeno[1,2-b]furan-2-one (ent ***6a***). This compound was prepared in the same way as described for **6a**, starting from *ent* **14a** (250 mg, 0.68 mmol). Yield was 73 mg, 36%, of *ent* **6a** as a white solid. An analytical sample was obtained by crystallization from hexane/ethyl acetate: mp 153.5−155 °C; $[\alpha]_D$ −285° (*c* 0.1, CH_2Cl_2); ^{1}H-NMR and mass data were the same as for compound **6a**. Anal. Calcd for $C_{17}H_{18}O_5$: C, 67.54; H, 6.00. Found: C, 67.36; H, 6.00.

*3-[4-Methyl-5-oxo-2,5-dihydrofuran-2(S)-yloxymethylene]-3,3a(R),4,,5,6,7,8,8b(S)-octahydroindeno[1,2-b]furan-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%, of **6b** as a white solid. An analytical sample was obtained by crystallization from hexane/ethyl acetate: mp 179.5−183.5 °C; $[\alpha]_D$ +161° (*c* 0.4, CH_2Cl_2); R_f 0.24 (hexane/ethyl acetate 1:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.57−1.67 (m, 4H, $\underline{H}_6$, $\underline{H}_7$), 1.96−2.01 (m, 3H, =CC$\underline{H}_2$ A-ring), 2.03 (m, 3H, C$\underline{H}_3$), 2.19 (m, 1H, =CC$\underline{H}_2$ A-ring), 2.33 (br d, 1H, ^2J = 16.6 Hz, $\underline{H}_4$), 2.70 (dd, 1H, ^2J = 16.6 Hz, J = 9.0 Hz, $\underline{H}_4$), 3.62 (m, 1H, $\underline{H}_{3a}$), 5.32 (d, 1H, J = 7.7 Hz, $\underline{H}_{8b}$), 6.13 (m, 1H, OC$\underline{H}$O D-ring), 6.93 (m, 1H, =C$\underline{H}$ D-ring), 7.43 (d, 1H, ^4J = 2.6 Hz, =C$\underline{H}$O); mass data were the same as for **6a**. Anal. Calcd for $C_{17}\underline{H}_{18}O_5$: C, 67.54; H, 6.00. Found: C, 67.32; H, 6.00.

3-[4-Methyl-5-oxo-2,5-dihydro-furan-2(R)-yloxymethylene]-3,3a(S),4,,5,6,7,8,8b(R)-octahydroindeno[1,2-b]furan-2-one (ent ***6b***). This compound was prepared in the same way as described for **6a**, starting from *ent* **14b** (170 mg, 0.46 mmol). Yield was 43 mg, 31%, of *ent* **6b** as a white solid. An analytical sample was obtained by crystallization from hexane/ethyl acetate: mp 180−184 °C; $[\alpha]_D$ −150° (*c* 0.3, CH_2Cl_2); ^{1}H-NMR and mass data were the same as for compound **6a**. Anal. Calcd for $C_{17}H_{18}O_5$: C, 67.54; H, 6.00. Found: C, 67.14; H, 5.99.

Biological Activity. *Seeds.* Seeds of *S. hermonthica* (Del.) Benth. [from *Sorghum bicolor* (L.) Moench] and *O. crenata* Forsk. (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.

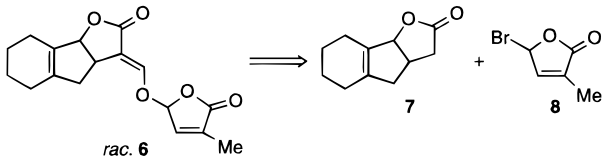
Preparation of Test Solutions. A compound to be tested was weighed out very accurately to the amount of 2.5 mg, dissolved in 5 mL of acetone p.a., and diluted with demineralized water to 25 mL. Aliquots of this stock solution were further diluted with water to obtain test solutions containing 1, 0.1, 0.01, and 0.001 mg/L test compound and 0.2, 0.02, 0.002, and 0.0002% (v/v) acetone, respectively.

Bioassays. For surface sterilization seeds of *S. hermonthica* were subsequently exposed to 70% (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.

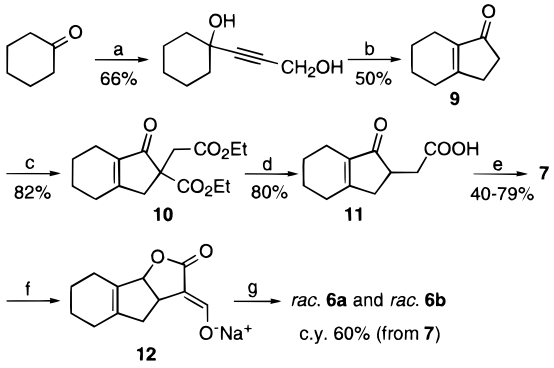
For conditioning the sterilized seeds were spread on glass fiber filter paper disks (8-mm diameter; approximately 30−70 seeds per disk) in Petri dishes, each containing 2 disks (*Striga*) or 4 disks (*Orobanche*), wetted with water, and stored in the dark for 14 days at 20 °C for *Orobanche* seeds and 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. Test solutions of the stimulant GR24 (concentrations of 1, 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

Scheme 1. Retrosynthetic Analysis of DMSL *rac* 6



Scheme 2. Synthesis of DMSL Diastereomers *rac* 6a and *rac* 6b



a) propargyl alcohol (1.5 equiv.), *n*-BuLi (3.3. equiv.) b) MeOH, H_2SO_4
c) 1. NaH (2 equiv.), $(EtO)_2CO$ 2. $BrCH_2CO_2Et$ d) HOAc, HCl
e) DIBALH or $NaBH_4/CeCl_3.7H_2O$ f) NaH, HCO_2Et g) **8**

particular of *S. hermonthica*, varies considerably from test to test. All tests were performed at least in duplicate, and in each test the germination percentages were determined on 6 disks (*Striga*) or 12 disks (*Orobanche*) per treatment.

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 **11** 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 $NaBH_4$ 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 $NaBH_4$ (4 equiv) in the presence of $CeCl_3 \cdot 7H_2O$ (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 *exo* selectivity as was deduced from the observed ^3J-coupling constants between H_3 and H_2 in the ^{1}H-NMR spectrum [*cf*. Thuring *et al*. (1995)].

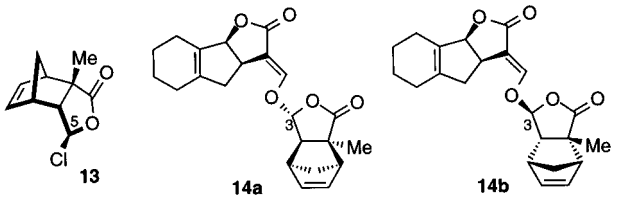
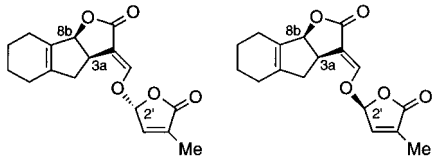


Figure 2. Structures of latent D-ring **13** and Diels−Alder adducts **14a,b**.

Table 1. Cycloreversion of Adducts 14a,b and Their Enantiomers



6a, 3a(R),8b(S),2'(R) **6b**, 3a(R),8b(S),2'(S)

entry	adduct	product	yield (%)	$[\alpha]_D$[a]
1	**14a**	**6a**	30	+281°
2	**14b**	**6b**	42	+160°
3	*ent* **14a**	*ent* **6a**	36	−285°
4	*ent* **14b**	*ent* **6b**	31	−150°

[a] $[\alpha]_D$ of products after cycloreversion. For details, see Materials and Methods.

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 ^{1}H-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; Heather *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

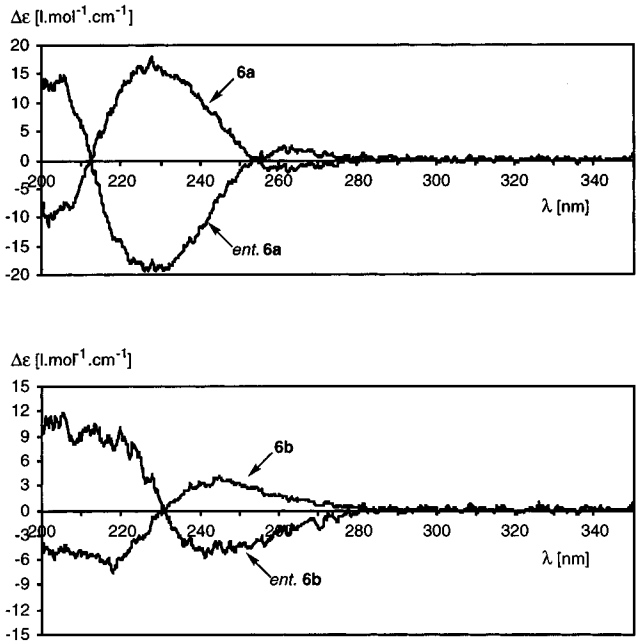


Figure 3. CD spectra of DMSL stereoisomers **6a** (*c* 19.8 μM), **6b** (*c* 24.8 μM), *ent* **6a** (*c* 19.8 μM), and *ent* **6b** (*c* 29.8 μM) using acetonitrile as the solvent.

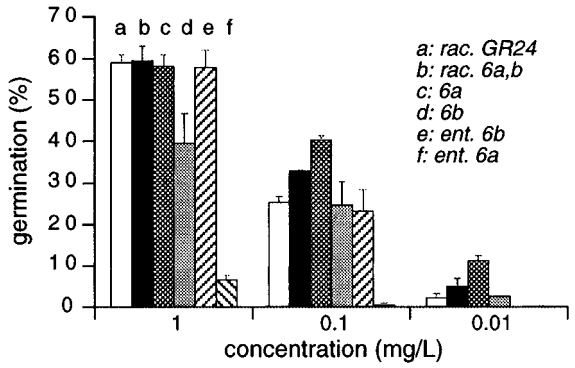


Figure 4. Germination percentages for seeds of *O. crenata* after exposure to different concentrations of DMSL stereoisomers **6**. The data presented are the mean ± SE of three replicate tests.

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

Table 2. Germination Percentages for Seeds of *S. hermonthica* after Exposure to Solutions (0.1 and 0.001 mg/L) of DMSL Enantiomers 6 and the Corresponding Diasteromeric Mixture[a]

entry	compound	configuration at C-2′	% germination ± SE at a concentration of 10^{-1} mg/L	% germination ± SE at a concentration of 10^{-3} mg/L
1	**6a**	*R*	63.2 ± 2.6	42.5 ± 1.2
2	**6b**	*S*	61.0 ± 3.5	0.0 ± 0.0[c]
3	*ent* **6a**	*S*	22.8 ± 4.6	0.7 ± 0.7[c]
4	*ent* **6b**	*R*	56.3 ± 4.8	1.6 ± 0.8
5	*rac* **4**[b]	*R*/*S*	47.1 ± 3.9	33.2 ± 2.2
6	*rac* **6**[b]	*R*/*S*	56.0 ± 1.0	28.3 ± 7.4

[a] Data presented are the mean ± SE of one representative experiment. [b] Equimolar mixture of two racemic diastereomers. [c] Not significantly different from aqueous control (without stimulant).

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 $c_{1/2max}$ (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 $C_{3a}C_{8b}$ and $C_{2'}$ on the bioactivity can be established by comparison of the activities of **6b** and *ent* **6b** (entries 2

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 $C_{2'}$ (Mangnus and Zwanenburg, 1992b). Bergmann *et al.* (1993) concluded from the relative activities of four stereoisomers of strigol that the absolute stereochemistry at $C_{2'}$ 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 $C_{3a}C_{8b}$ is of considerable importance for the stimulatory activity (*O. crenata*) as compared to that of DMSL (**6**) (Thuring *et al.*, 1996).

Evaluation of all available data regarding the germination stimulatory activity of several optically pure analogues derived from (+)-strigol reveals that 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 configuration at $C_{2'}$ (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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