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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 prominent 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 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 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 Beilstein 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 (Wisssembourg, 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: Dichlo-ro-methane 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-Tetrahydronindene(1,2-b)furane 2-one (rac 4) and 5-bromo-3-methyl-2(5H)-furanone (5) were prepared following published methods (Mangnus et al., 1992a). The synthesis of chloralactones 6 and ent 6 was reported previously (Thurung et al., 1995).

For the resolution of rac 4 mixture crystalline cellulose triacetate (80 g, Nuchar Luka, Buchs, Switzerland) was used. The enantiomers of 4 and ent 4 were determined by HPLC analysis using an Spectra Physics PS8700 HPLC apparatus (Spectra Physics, Eindhoven, The Netherlands), a chiral Baker Chiral OD-H column (eluent hexane/ethanol 60:40 (v/v), flow rate 0.5 mL/min), and an LKB 2138 UVicord S UV–vis detector (254 nm) (Pharmacia Biotech., Roosendaal, The Netherlands).

Chromatographic Resolution of 3,3a,4,8b-Tetrahydroindeno[1,2-b]furan-2-one (General Procedure). Potassium tert-butoxide (131 mg, 1.17 mmol) was added to a solution of lactone 4 (185 mg, 0.66 mmol) and methyl formate (1.6 mL) 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 ethyl/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)-yl-oxymethylene)-3,3a(R),4,8b(S)-tetrahydronindene(1,2-b)furane 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 2a (yield = 27%). Analytically pure samples were obtained by crystallization from hexane/ethyl acetate.

2b obtained as colorless crystals; mp 133.5–134.5 °C; [α]D +466° (c 0.25, CHCl₃). Anal. Calc. For C₁₇H₁₄O₅S: C, 68.12; H, 4.67. 1H-NMR and mass data were the same as for compound 2a.

Determination of Enantiomeric Purity. 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 the resonances of the endo proton signal for the enol ether proton (−CH=O), amounting to 0.35 ppm. 1H-NMR (400 MHz) spectra of pure compounds 2a and ent 2a,b displayed comparable shifts on treatment with Eu(hfc)₃, but in contrast to the racemates, no splitting of signals was observed, indicating an enantiomeric purity of at least 98%.

3-(6-S)-Methyl-5-oxo-4-oxatricyclo[5.2.1.0³,6]dec-8-en-3(R)-yl-oxymethylene-3,3a(R),4,8b(R)-tetrahydronindene(1,2-b)furane 2-one (7a) and Its 3a(S),8b(R) Diastereomer (7b). To a stirred suspension of NaH (139 mg, 3.48 mmol) in diethyl ether (10 mL) was gradually added a solution of rac 4 (300 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 obtained sodium salt was dissolved in DMF (10 mL). A solution of chloralactone 6 (372 mg, 1.87 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 (10 mL) and ethanol (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 ethyl/ethyl acetate 4:1) to give a white solid, and the residue was obtained as an analytically pure sample after crystallization from hexane/ethyl acetate.

7a: mp 174–177 °C; [α]D +276° (c 0.1, CHCl₃); Rₜ 0.48 (hexane/ethyl acetate 1:1); 1H NMR (CDCl₃, 400 MHz) δ 2.05 (3H, CH₃), 3.10 (dd, 1H, J = 16.9 Hz, J = 3.1 Hz), 3.42 (dd, 1H, J = 16.9 Hz, J = 9.3 Hz), 3.94 (m, 1H, H₃a), 5.96 (d, 1H, J = 7.9 Hz, H₃b), 6.17 (m, 1H, OCHO D-ring), 6.96 (m, 1H, =CH D-ring), 7.23–7.36 (m, 3H, Ar H), 7.48 (d, 1H, J = 2.5 Hz, =CHO), 7.50 (d, 1H, J = 7.9 Hz, Ar H); mass data were the same as for 2a.

7b: mp 192.5–194.4 °C; [α]D +33° (c 0.1, CHCl₃); Rₜ 0.36 (hexane/ethyl acetate 1:1); 1H NMR (CDCl₃, 400 MHz) δ 1.48 (3H, CH₃), 1.73 (m, 2H, CH₂), 2.78 (dd, 1H, J = 3.7 Hz, J = 1.8 Hz, H₂), 2.91 (m, 1H, H₃a), 3.05 (dd, 1H, J = 16.8 Hz, J = 3.5 Hz, CH₂ B-ring), 3.24 (m, 1H, H₃b), 3.42 (dd, 1H, J = 16.8 Hz, J = 8.8 Hz, CH₂ B-ring), 3.93 (m, 1H, H₃c), 5.24 (d, 1H, J = 1.8 Hz, H₃d), 5.95 (d, 1H, J = 7.9 Hz, H₃e), 6.26 (m, 2H, H₃f and H₃g).
H₂O), 7.21–7.53 (m, 4H, Ar H), 7.39 (d, 1H, J = 2.8 Hz, –CHO); mass data were the same as for 7a. Anal. Calcd for C₂₂H₂₀O₅: C, 72.52; H, 5.53. Found: C, 71.91; H, 5.47.

2-(5-methyl-2-oxo-4-oxatricyclic[5.2.1.0²⁶]dec-8-en-3(5)-yloxyxymethylene)-3a(R),4,8b(S)-tetrahydroindeno[1,2-b]furan-2-one (ent 7b) and its 3a(S),8b(R)-Diastereomer (ent 7a). These compounds were prepared in the same way as for racemic 5-bromofuranone (rac 4) in an analogous manner and at indicated temperatures, the germination percentage was determined under a microscope. Seeds were considered to be germinated if the radical protruded through the seed coat.

RESULTS AND DISCUSSION

Synthesis. To achieve the resolution of GR24 (2), two synthetic approaches were considered. The first approach (Scheme 1) involves the separation of tricyclic lactone rac 4 into its enantiomers, followed by formylation and coupling of the individual enantiomers with racemic 5-bromofuranone (5) in an analogous manner as described previously for the racemic preparation of GR24 (Mangnus et al., 1992a).

Tricyclic lactone rac 4 was chromatographically resolved using cellulose triacetate (CTA) as the chiral stationary phase. It has been demonstrated that a wide variety of racemic γ- and δ-lactones can be separated by column chromatography on the chiral phase CTA, on both analytic and preparative scales (Francotte and Lohmann, 1987). In the present case 500 mg of rac 4 was resolved by “flash” chromatography at a pressure of ca. 1.2 bar, using 50 g of CTA and 95% ethanol as the eluent, in a total recovery of enantiopure tricyclic lactones 4 and ent 4 of 68%. The enantiopurity of 4 and ent 4 was assessed by HPLC, using cellulose carbamate as the chiral stationary phase. The diastereomeric mixtures obtained after formylation and coupling of 4 and ent 4 with 5 (Scheme 1) were both separated by flash chromatography (SiO₂) to give 2a, ent 2b and 2b, and ent 2a (ee values > 98%) in total isolated yields of 59% and 60%, respectively. The ee values were determined by ¹H NMR using the chiral shift reagent Eu(hfc)₃.
In the second approach the complete resolution of GR24 (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 GR24 (2), which have the same [alpha]D values as those prepared by the route depicted in Scheme 1. It was essential to control the reaction temperature and time carefully to avoid concomitant epimerization at C2.

Determination of Absolute Configuration. It was attempted to establish the absolute configuration of the GR24 (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 GR24 (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 GR24 2a,b and ent 2a,b was assayed using seeds of S. hermonthica and O. crenata. In each bioassay a diastereomeric mixture of GR24 was included as a positive control. In preliminary experiments the concentration-dependent activity range (GR24) 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 GR24 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 GR24 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
C₃₃C₈₈-epimer 2b and the C₂₂-epimer ent 2b. The data in Table 1 (entries 2 and 4) reveal in the case of S. hermonothica a slight preference for the correct stereochemistry at C₂₂. In contrast, the data obtained for O. crenata (Figure 3) suggest that for this species the absolute configuration at C₃₃C₈₈ is more important for the stimulatory activity than the configuration at C₂₂. This result is in contrast with data obtained from a comparative study of GR7 stereoisomers, which revealed a more profound role of the C₂₂-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 three-dimensional 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 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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