Improved Synthesis of Strigol Analogue GR24 and Evaluation of the Biological Activity of Its Diastereomers

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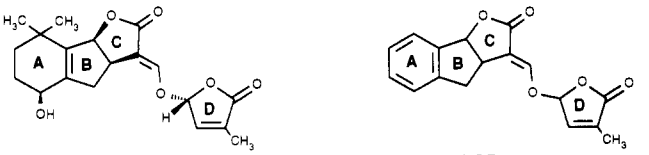
A high yield preparation procedure for the germination stimulant GR24 (2) is described. Improvements were introduced in the syntheses of the key building blocks 3 and 4. The tricyclic lactone 3 was prepared from indan-1-one in four steps. The major improvement was the introduction of the carboxymethyl function at the α-position of indan-1-one via an ethoxycarbonyl auxiliary group. The furanone moiety 4 was prepared in four steps in an overall yield of 75% using ethyl α-bromopropionate and oxirane as main starting materials. The final coupling afforded GR24 as a mixture of diastereomers, which were separated by chromatography. The separate diastereomeric racemates **2a** and **2b** were fully characterized, and the configuration was determined by X-ray analysis. Biological tests of the diastereomers demonstrated differences in their stimulatory activities for parasitic weed seeds.

INTRODUCTION

The parasitic weeds of the genera *Striga* and *Orobanche* cause severe damage to graminaceous and leguminous crops in tropical and subtropical areas (Musselman, 1987; Parker, 1986; Ramaiah, 1987). Seeds of these parasitic weeds only germinate when they are stimulated by a chemical signal exuded by the roots of the host plant (Brown, 1965). Suicidal germination of *Striga* and *Orobanche*, i.e., introduction of a germinating agent into soil to induce germination of the parasitic seeds before the desired crop is planted, is an attractive approach for controlling weed pests (Eplee, 1975). Strigol 1, which was isolated from the root exudate of cotton, is a very active germination stimulant (Cook et al., 1966, 1972). However, this compound is not suitable for weed control purposes, because of its limited stability under soil conditions and its complicated chemical structure, which makes its synthesis lengthy and uneconomic. For these reasons, Johnson et al. (1976, 1981) synthesized a series of strigol analogues which have a simpler structure and which retained their biological activity to a considerable extent. One of the most potent analogues is GR24, in which the A ring in strigol has been replaced by an aromatic ring. This compound 2, with the same main framework as the strigol molecule, is now being used worldwide to induce parasitic weed seed germination for in vitro experiments and as a reference compound in the evaluation of other strigol analogues (Zwanenburg et al., 1986; Mangnus and Zwanenburg, 1991).

In this paper we describe a modified and efficient synthesis of GR24 from inexpensive starting materials, which lends itself for multigram preparations. In addition, the two diastereomeric forms of GR24 are separated and evaluated for biological activity (Figure 1).



1 (+)Strigol 2 GR24

Figure 1. Structures of 1 and 2.

MATERIALS AND METHODS

Nomenclature. We have adopted Chemical Abstracts Service nomenclature for all compounds; ring D is named as a *dihydrofuranone*. In the older literature the name *butenolide* is used for this fragment, which has consequences for the atom numbering.

Synthesis. *General Remarks.* Melting points were measured with a Reichert Thermopan microscope and are uncorrected. IR spectra were recorded on a Perkin-Elmer 298 infrared spectrophotometer. ^{1}H NMR spectra were recorded on a Varian EM390 (90 MHz) spectrometer with TMS as internal standard. For mass spectroscopy a double-focusing VG 7070E was used. Flash chromatography was carried out at a pressure of ca. 1.5 bar using silica gel 60H (Merck art. no. 7719). Thin-layer chromatograms (TLC) were run on plastic-supported silica gel 60 plates (0.2-mm layer, F_{254}, Merck art. no. 5735) or glass-supported silica gel 60 plates (0.25-mm layer, F_{254}, Merck art. no. 5715).

Solvents were dried using the following methods: Dimethylformamide P.A. was dried on 4-Å molecular sieves. Tetrahydrofuran was distilled from lithium aluminum hydride just before use. Petroleum ether 60–80 and hexane were distilled from calcium hydride. Diethyl ether was predried over calcium chloride and then distilled from sodium hydride. Dichloromethane was distilled from phosphorus pentoxide. All other solvents used were of analytical grade.

Pure sodium hydride was obtained from a 60% dispersion in mineral oil by washing the dispersion several times with anhydrous hexane to remove the oil. To exclude contact of the sodium hydride with moist air, the washings were carried out in a continuous stream of dry nitrogen.

Ethyl 1-Oxo-2-(ethoxycarbonyl)-2-indanylacetate (8). A solution of indan-1-one (132 g, 1 mol) in dimethylformamide (0.2 L) was gradually added to a solution of diethyl carbonate (475 g, 4 mol) and sodium hydride (53 g, 2.2 mol) in anhydrous dimethylformamide (1.5 L) with stirring at 65 °C. Stirring was continued for 1 h at 65 °C. The reaction was monitored by TLC (eluent ethyl acetate/hexane 2:3). When the reaction was complete, a solution of ethyl bromoacetate (250 g, 1.5 mol) in dimethylformamide (0.2 L) was gradually added with stirring. After 1 h of stirring at 65 °C, the reaction mixture was neutralized with glacial acetic acid. The reaction mixture was then concentrated in vacuo (oil pump) to remove solvents and remaining starting materials. The residue was dissolved in a mixture of diethyl ether and water, and the aqueous layer was extracted with diethyl ether (3×). The combined organic layers were washed with water, dried ($MgSO_4$), filtered, and concentrated. Distillation of the crude product gave the diester 8 (235 g, 81%) as a colorless oil: bp 135–140 °C/0.02 mmHg; ^{1}H NMR ($CDCl_3$) δ 1.17 (t, J = 7 Hz, 6 H, CH_3), 2.70 and 3.27 (AB, 2 H, J = 18 Hz, CH_2COOEt), 3.12 and 3.83 (AB, 2 H, J = 18 Hz, indane CH_2),

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3.92–4.19 (2 × q, J = 7 Hz, 4 H, OCH_2CH_3), 7.13–7.78 (m, 4 Ar H); IR (neat) ν 1710–1730 (C=O, several), 1608, 1590 cm^{-1}.

1-Oxo-2-indanylacetic Acid (7). A solution of diester **6** (233 g, 0.8 mol) in a mixture of glacial acetic acid (250 mL) and 6 N aqueous hydrogen chloride (250 mL) was heated to reflux for 3 h. The reaction was monitored by TLC (eluent ethyl acetate/hexane 2:3). When the reaction was complete, the solution was cooled, diluted with water, and extracted with ethyl acetate (3×). The organic layers were washed with water, dried ($MgSO_4$), filtered, and concentrated. The residue, a pale yellow solid, was washed with a small amount of cold diethyl ether to afford acid **7** as a white solid (135 g, 87%). The product was sufficiently pure for further use: mp 148–149 °C [lit. 147–148 °C (Groves and Swan, 1951)]; ^1H NMR (acetone-d_6) δ 2.41–3.57 (m, 5 H, $ArCH_2CHCH_2COOH$), 7.21–7.65 (m, 4 Ar H); IR (KBr) ν 2900–2280 (broad, OH), 1735 (COOH), 1668 (C=O), 1604 (Ar) cm^{-1}.

Racemic 3,3a,4,8b-Tetrahydroindeno[1,2-b]furan-2-one (3). Lactone **3** was prepared from γ-keto acid **7** by reduction with sodium borohydride followed by cyclization catalyzed by acid as described by House et al. (1962). Yield: 90%.

Ethyl 2-(Phenylthio)propanoate (12). Benzenethiol (110 g, 1 mol) was added to a solution of sodium ethanolate in ethanol (from 23 g sodium in 500 mL of ethanol P.A.) with stirring at ambient temperature. Stirring was continued for 30 min. The solution of thiophenolate was then cooled to about 10 °C and ethyl 2-bromopropionate (**11**) (181 g, 1 mol) was added. To complete the reaction, stirring was continued at room temperature for an additional 15 min. The mixture was concentrated in vacuo, and the residue was dissolved in aqueous ammonium chloride solution and diethyl ether. The aqueous layer was extracted with diethyl ether (3×), and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated. The residue was distilled under reduced pressure to afford ethyl 2-(phenylthio)propanoate (**12**) (193 g, 92%) as a colorless oil: bp 93–95 °C/0.8 mmHg [lit. 159–161 °C/23.5 mmHg (Minamada et al., 1968)]; ^1H NMR ($CDCl_3$) δ 1.15 (t, J = 7 Hz, 3 H, OCH_2CH_3), 1.46 (d, J = 7 Hz, 3 H, CH_3), 3.74 (q, J = 7 Hz, $CHSPh$), 4.06 (q, J = 7 Hz, 2 H, OCH_2CH_3), 7.13–7.52 (m, 5 ArH).

Dihydro-3-methyl-3-(phenylthio)-2(3H)-furanone (13). A solution of freshly distilled diisopropylamine (1.01 mol, 142 mL) was quickly added to a mixture of *n*-butyllithium (2.5 M in hexane; 405 mL, 1.01 mol) and anhydrous tetrahydrofuran (200 mL) with stirring under nitrogen at 0 °C. Then ethyl 2-methyl-2-(phenylthio)propanoate (**12**) (210 g, 1 mol) was added in 20 min with stirring at 0 °C. Stirring was continued for 15 min at 10 °C. Then a solution of oxirane (52.9 g, 1.20 mol) in anhydrous tetrahydrofuran (100 mL) was added in small portions. After each addition, the mixture was allowed to react before the next portion was added (very exothermic reaction!). After the addition was completed, the temperature was slowly raised to room temperature in about 30 min. After the mixture was stirred for 20 h at room temperature, ice water was added and the mixture was acidified with 4 N aqueous hydrogen chloride to pH ≈2. The aqueous layer was extracted with diethyl ether (3×), and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated. The crude, crystalline product was purified by crystallization from diethyl ether to give **13** as pale yellow crystals (187 g, 90%): mp 59–60 °C [lit. bp 153–158 °C/0.9 mmHg (Dethy and Wood, 1980)]; ^1H NMR ($CDCl_3$) δ 1.52 (s, 3 H, CH_3), 2.37 (t, J = 6.5 Hz, CH_2CH_2O), 4.18 (t, J = 6.5 Hz, CH_2CH_2O), 7.16–7.62 (m, 5 ArH).

3-Methyl-2(5H)-furanone (10). Water was added to a mixture of dihydro-3-methyl-3-(phenylthio)-2(3H)-furanone (**13**) (187.2 g, 0.9 mol) in methanol (500 mL) until a clear solution was obtained (ca. 110 mL of water). Sodium periodate (213 g, 1 mol) was added in small portions with stirring at 40 °C. Stirring was continued until the reaction mixture was cooled to room temperature. The precipitate ($NaIO_3$) was filtered off, and diethyl ether was added to the filtrate until two layers were obtained. The aqueous layer was extracted with diethyl ether (3×). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated. The residue was dissolved in carbon tetrachloride (250 mL) and heated at reflux for 30 min to complete the intramolecular elimination. The solvent was removed in vacuo, and the residue was distilled under reduced pressure; 3-methyl-2(5H)-furanone (**10**) (80.3 g, 91%) was obtained as a colorless oil: bp 74–76 °C/3 mmHg [lit. 52 °C/1.5 mmHg (Johnson et al., 1981)]; ^1H NMR ($CDCl_3$) δ 1.89 (m, 3 H, CH_3), 4.74 (m, CH_2O), 7.20 (m, =CH).

5-Bromo-3-methyl-2(5H)-furanone (X = Br) (4). Bromide **4** was prepared from 3-methyl-2(5H)-furanone (**10**) and *N*-bromosuccinimide as described by MacAlpine et al. (1976).

Potassium Salt of Racemic 3-(Hydroxymethylene)-3,3a,4,8b-tetrahydroindeno[1,2-b]furan-2-one (15). Potassium *tert*-butoxide (2.24 g, 0.020 mol) was added in small portions to a solution of lactone **3** (3.10 g, 0.018 mol) and methyl formate (1.62 g, 0.027 mol) in anhydrous tetrahydrofuran (100 mL) with stirring at 0 °C under nitrogen. Stirring was continued at room temperature until all lactone had reacted (monitored by TLC; eluent ethyl acetate/hexane 3:7). Tetrahydrofuran was removed in vacuo, and the residue was stirred with anhydrous diethyl ether. The insoluble potassium salt was filtered off quickly, washed with a small amount of diethyl ether, and dried in an desiccator. The hygroscopic product **15** (4.11 g, 95%), obtained as a gray powder, was sufficiently pure for further use.

For full characterization a small quantity of the potassium salt was dissolved in water and potassium hydrogen sulfate was added until pH ≈1. The aqueous layer was extracted with dichloromethane (3×), and the combined organic layers were dried (Na_2SO_4), filtered, and concentrated giving racemic 3-(hydroxymethylene)-3,3a,4,8b-tetrahydroindene[1,2-*b*]furan-2-one as a white solid: mp 167–169 °C; ^1H NMR (DMSO) δ 2.84–3.55 (m, 2 × H_4), 3.75–4.04 (m, H_{3a}), 5.90 (d, J = 7.5 Hz, H_{8b}), 7.12–7.58 (m, 5 H, Ar H + =CHO); IR (KBr) ν 2900–3600 (OH), 1705 (C=O, lactone), 1610 (C=C, enol ether) cm^{-1}.

(3aR,8bS*,5'R*)-3-[[(2,5-Dihydro-4-methyl-5-oxo-2-furanyl)oxy]methylene]-3,3a,4,8b-tetrahydroindeno[1,2-b]furan-2-one (2a) and Its 5'S* Diastereomer 2b.* A solution of furanone **4** (0.38 g, 2.2 mmol) in anhydrous 1,2-dimethoxyethane (25 mL) was quickly added to a suspension of potassium salt **15** (0.48 g, 2 mmol) in 1,2-dimethoxyethane (50 mL) with stirring at 0 °C under nitrogen. Stirring was continued for 4 h at room temperature. Precipitated potassium bromide was removed by filtration. The filtrate was concentrated in vacuo, and the residue was dissolved in a mixture of water and chloroform. The aqueous layer was extracted with chloroform (2×). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The crude, mainly solid product was purified using flash chromatography (silica gel, diisopropyl ether/ethyl acetate 4:1) to afford two partly separated diastereomeric products (**2a** and **2b**) (0.4 g, 82%). The fast moving diastereomer **2a** (R_f = 0.18; diisopropyl ether/ethyl acetate 4:1) was crystallized from dichloromethane/hexane to give **2a** as colorless crystals: mp 156–157 °C; ^1H NMR ($CDCl_3$) δ 2.00 (m, 3 H, CH_3), 2.87–3.63 (m, 2 × H_4), 3.76–4.09 (m, H_{3a}), 5.93 (d, J = 7.5 Hz, H_{8b}), 6.17 (m, OCHO), 6.95 (m, =CH), 7.12–7.60 (m, 5 H, 4 Ar H + =CHO); IR (KBr) ν 1790 (C=O, 4-methylfuranone), 1732 (C=O, lactone), 1675 (C=C, enol ether), 1020, 865 cm^{-1}. Anal. Calcd for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 68.55; H, 4.76.

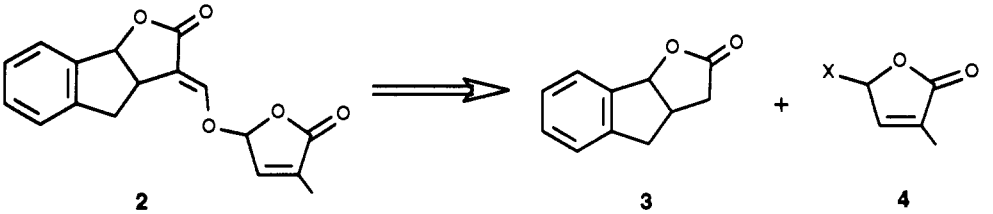
The slow moving diastereomer **2b** (R_f = 0.11; diisopropyl ether/hexane 4:1) was crystallized from dichloromethane/hexane to give **2b** as colorless crystals: mp 142–144 °C; ^1H NMR ($CDCl_3$) identical to ^1H NMR of the fast moving diastereomer **2a**; IR (KBr) ν 1790 (C=O, 4-methylfuranone), 1735 (C=O, lactone), 1670 (C=C, enol ether), 1010, 860, 830 cm^{-1}. Anal. Calcd for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 68.07; H, 4.73.

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

Preparation of Test Solutions. A compound to be tested was weighed out very accurately to the amount of 10 mg, dissolved in 10 mL of acetone P.A., and diluted with demineralized water to 100 mL. Aliquots of this stock solution were further diluted with water to obtain test solutions with 1 and 0.01 mg/L test compound and 0.1 and 0.001% (v/v) acetone, respectively.

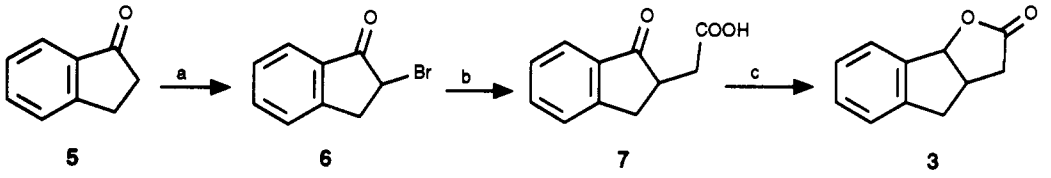
Bioassay. For surface sterilization seeds of *S. hermonthica* and *O. crenata* were exposed to an aqueous solution of sodium hypochlorite (2% active chlorine) and Triton X-100 (1% v/v) for 5 min with agitation. The seeds were then rinsed thoroughly with water and dried overnight.

Scheme I. Retrosynthetic Analysis of the GR24 Molecule



2 3 4

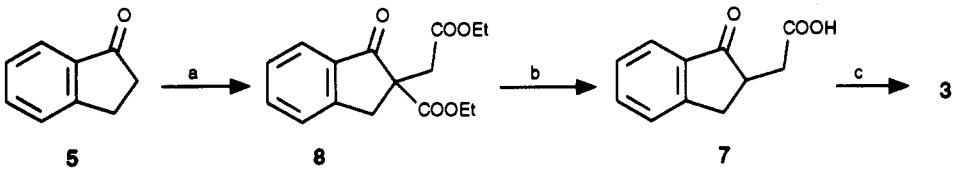
Scheme II. Johnson's Procedure for the Preparation of the Tricyclic Building Block 3



5 6 7 3

[a] Br_2, Et_2O, 10 °C. [b] 1. $(COOEt)_2CH^-Na^+$, C_6H_6, reflux; 2. KOH, EtOH, reflux; 3. 170 °C. [c] 1. $NaBH_4$, 0.2N NaOH; 2. pTosOH, C_6H_6, reflux.

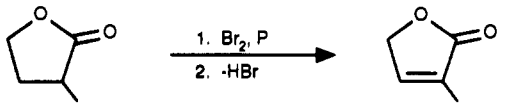
Scheme III. Improved Procedure for the Synthesis of the Tricyclic Building Block 3



5 8 7

[a] 1. $(EtO)_2CO$, NaH, DMF, 65 °C; 2. $BrCH_2COOEt$, 65 °C. [b] 6N HCl(aq.), HOAc, reflux. [c] 1. $NaBH_4$, 0.2N NaOH(aq.); 2. pTosOH, C_6H_6, reflux.

Scheme IV. Synthesis of 10 from α-Methyl-γ-butyrolactone



9 10

For conditioning the sterilized seeds were spread on glass fiber filter paper disks (8-mm diameter; approximately 25–50 seeds per disk) in Petri dishes, wetted with water, and stored in the dark for 14 days at 23 °C for *Orobanche* seeds and at 27 °C for *Striga* seeds. Then the conditioning water was removed and replaced by 100 μL of test solution per disk. After incubation for another 4–7 days in the dark at indicated temperatures, the germination percentage was determined under a microscope. Seeds were considered to be germinated if the radicle protruded through the seed coat.

In each test series aqueous solutions with 0.1 and 0.001% (v/v) acetone were used as negative control. Tests were replicated three times, and in each test the germination percentages were determined on at least 10 separate disks.

RESULTS AND DISCUSSION

Synthesis. Retrosynthetic analysis of structure 2 leads to the two key building blocks 3 and 4 (Scheme I). Therefore, the final steps in the synthesis of GR24 are formylation of the tricyclic lactone 3 followed by condensation with an appropriate furanone 4.

Johnson et al. (1981) prepared the tricyclic building block 3 starting from indan-1-one (5) according to literature procedures (Scheme II; Groves and Swan, 1951; House et al., 1962). The preparation of monobromo compound 6 is always accompanied by the formation of 2,2-dibromoindan-1-one. During scaling up of this reaction, it was found that the ratio mono- vs dibromoindan-1-one changes in favor of the dibromo compound. Increased HBr concentration may be responsible for the formation of more dibromoindan-1-one, but the addition of an HBr scavenging agent (viz., propylene oxide) had no effect on the ratio mono- vs dibromoindan-1-one.

Modifying the reaction conditions and using other bromination procedures did not result in a reduction of the amount of dibromo compound. Attempts to separate the two bromo compounds were also unsuccessful. Therefore, an alternative procedure for the synthesis of γ-keto acid 7 from indan-1-one was needed.

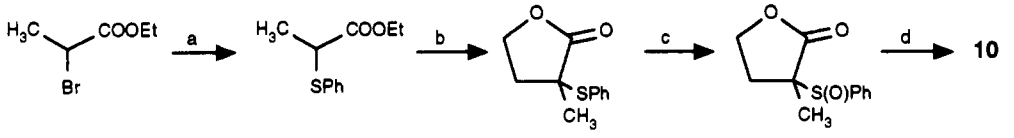
In the total synthesis of strigol the problem of introducing the required carboxymethyl side chain was solved by introduction of a ethoxycarbonyl (Heather et al., 1976; Samson et al., 1991) or ethoxalyl (MacAlpine et al., 1974) substituent which served the dual purpose of activating the position to be alkylated and protecting against dialkylation. As alkylating agent, methyl bromoacetate was used in all cases.

The ethoxycarbonyl method proved to work well also for the introduction of the required carboxymethyl side chain in indan-1-one (Scheme III). The ethoxycarbonyl auxiliary group was introduced in 5 using a 4-fold excess of diethyl carbonate and sodium hydride as base. The subsequent alkylation was performed with ethyl bromoacetate. By using 2 equiv of base during the introduction of the ethoxycarbonyl function, the alkylation step can be carried out in the same pot. An overall reproducible yield of 80–85% of 8 was obtained in this manner. Acid-catalyzed hydrolysis and subsequent decarboxylation afforded carboxylic acid 7 in 87% yield.

The conversion of γ-keto acid 7 into tricyclic lactone 3 was performed by sodium borohydride reduction, followed by acid treatment of the crude product to accomplish complete lactonization (Scheme III; House et al., 1962).

Several methods have been used for the preparation of the furanone synthon 4. Johnson et al. (1981) investigated a number of these methods, but most of them were unsuited for multigram preparations. They therefore used bromination and dehydrobromination of commercially

Scheme V. Alternative Synthesis for 10



11 12 13 14

[a] PhSNa, EtOH. [b] 1. LDA, THF; 2. oxirane; 3. H^+. [c] $NaIO_4$, $MeOH/H_2O$, 40 °C. [d] NBS, CCl_4, reflux.

Table I. Comparison of Physical Constants (R_f Values and Melting Points) of Diastereomers of Strigol and Analogues GR7 and GR24

compound	R_f[a]	R_f[b]	mp, °C
(±)-strigol	0.20		203–205
(±)-2′-epistrigol	0.32		178–180
(±)-high melting GR7	0.48	0.14	174–176
(±)-low melting GR7	0.53	0.21	143–145
3aR,6aR,2′R-GR7	0.53	0.21	133–135
3aR,6aR,2′S-GR7	0.48	0.14	oil
(±)-3aR,8bS,2′R-GR24 (**2a**)	0.52	0.18	156–157
(±)-3aR,8bS,2′S-GR24 (**2b**)	0.46	0.11	142–144

[a] Solvent system: chloroform/acetone 4:1. [b] Solvent system: diisopropyl ether/ethyl acetate 4:1.

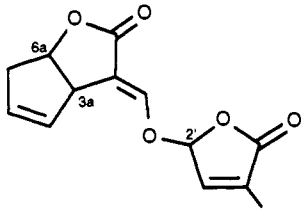


Figure 2. Structure of GR7.

available α-methyl-γ-butyrolactone (**9**) to prepare **10** (Scheme IV). Furanone **10** can readily be brominated at the 4-position with *N*-bromosuccinimide to give the desired furanone **4** (X = Br; MacAlpine et al., 1976).

Although this method is suitable for large-scale preparation, it has the disadvantage that the starting lactone **9** is expensive. We therefore developed a new method for the synthesis of **10** starting from inexpensive ethyl 2-bromopropionate (**11**) (Scheme V). Replacement of bromine in **11** by a phenylthio group could be accomplished on 1 to 3 mole scale in a very high yield (90–95%). Ester **12** was then deprotonated with lithium diisopropylamine (LDA) and treated with oxirane, which led to a very exothermic reaction. Subsequent acidification gave ethyl 4-hydroxy-2-methyl-2-(phenylthio)butanoate, which lactonized spontaneously to give lactone **13** in a yield of 90% (1 to 2 mole scale). Reactions of deprotonated α-phenylthiocarboxylates with epoxides have previously been described by Iwai et al. (1974) to prepare 5-substituted furanones. Instead of using an α-(phenylthio) *ester*, Iwai et al. used the free *carboxylic acid* and 2 equiv of LDA in the reaction with epoxides, because the ester gave unsatisfactory results. In our hands the ester gave better results than the corresponding free acid.

Oxidation of sulfur in **13** to the corresponding sulfoxide **14** and subsequent elimination of phenylsulfenic acid by thermolysis in refluxing carbon tetrachloride produced furanone **10** in a yield of 90–94%.

The overall yield of this sequence starting from **11** amounts to about 75%. The reactions can conveniently be performed on a 1 to 2 mole scale. Furanone **10** was brominated according to a literature procedure (MacAlpine et al., 1976). It is recommended to perform this bromination shortly before use, because of the limited stability of **4** (X = Br).

Coupling of the building blocks **3** and **4** was accomplished, as depicted in Scheme VI. During the formylation of **3**, potassium *tert*-butoxide was used as base instead of sodium methoxide which was used by Johnson et al. (1981). The potassium enolate **15**, which is very hygroscopic, was treated, without delay, with the bromofuranone **4** to give GR24 as a mixture of diastereomers **2a** and **2b**. This mixture of **2a** and **2b** could be separated by chromatography on silica gel. Both isomers have the *E* geometry, as was deduced from the ^1H NMR spectra. [The chemical shift of the enolate proton would show an upfield shift of ca. 0.6 ppm for the *Z* isomer (MacAlpine et al., 1976; Jonas et al., 1984).] This preference for the *E* geometry was also noticed by Johnson et al. (1981). A separation of diastereomers of GR24 has also been reported by Johnson et al. (1981). However, the melting points are entirely different from those found in the present work. Probably, Johnson et al. have separated the diastereomers of GR18, which is a regioisomer of GR24. The latter was confirmed by patents covering the same work (Johnson and Hassanali, 1981a,b).

The spectral characteristics of the diastereomers **2a** and **2b** are very similar, as was the case for (±)-strigol and (±)-2′-epistrigol (Heather et al., 1976) and for diastereomers of some other strigol analogues (Kendall et al., 1979; Connick and Pepperman, 1981). For this reason the relative stereochemistry of diastereomers of strigol analogues has often been determined by comparison of TLC mobilities and melting points of the diastereomers with those of (±)-strigol and (±)-2′-epistrigol (Table I). For example, on this basis the "slow moving", "high melting" diastereomer of strigol analogue GR7 (see Figure 2) was assigned the same relative stereochemistry as (±)-strigol (Connick and Pepperman, 1981). Recently, the absolute configuration of one optical pure enantiomer of GR7 was established by X-ray diffraction analysis (Bosman et al., 1992a; Mangnus and Zwanenburg, 1992), and it was shown that the structure assignment by Connick and Pepperman was incorrect. This lack of correlation between physical parameters of GR7 and strigol diastereomers may be mainly due to the presence of a hydroxyl group at the A ring of the strigol diastereomers. For that reason one would expect a better correlation between TLC mobilities of diastereomers of GR7 and those of GR24. In fact, it was found that for different solvent systems R_f values for GR7 and GR24 diastereomers were almost the same (Table I). On this basis the "fast moving" GR24 diastereomer **2a** was assigned the same relative stereochemistry as 3aR,-6aR,2′R-GR7 [and thus as in (±)-strigol] as shown in Scheme VI. This structure assignment based on R_f values was confirmed by an X-ray diffraction analysis of **2a** (Bosman et al., 1992b). Figure 3 shows a stereoview of molecule **2a** in the minimum overlap position.

Biological Activity. The stimulatory activity of both diastereomers was tested using seeds of *S. hermonthica* and *O. crenata*. The results are collected in Tables II and III. The data in Table II reveal that there is no significant difference between **2a** and **2b** at a stimulant concentration of 1 mg/L. However, at a concentration of 0.01 mg/L the

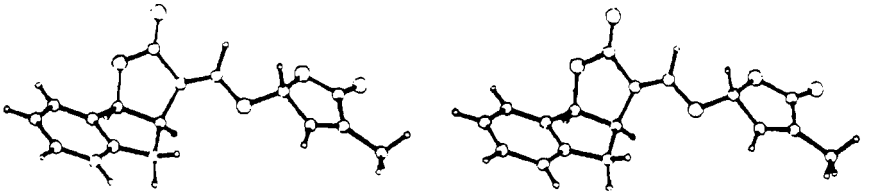
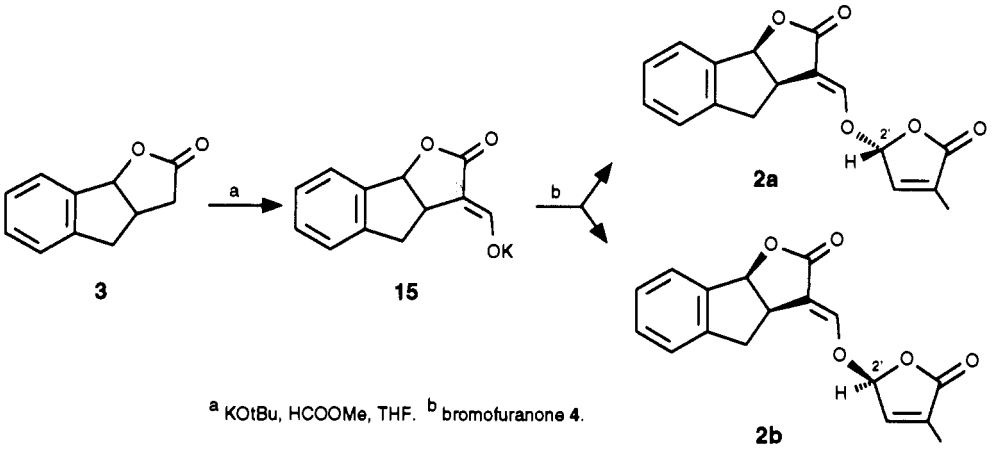


Figure 3. Stereoview of structure **2a** in the minimum overlap position.

Scheme VI. Synthesis of GR24 Diastereomers 2a and 2b from Building Blocks 3 and 4



[a] KOtBu, HCOOMe, THF. [b] bromofuranone 4.

Table II. Germination Percentages of Seeds of *S. hermonthica* after Exposure to Solutions of GR24 Diastereomers at Concentrations of 1 and 0.01 mg/L[a]

sample	% germination ± $t_{0.05}(s/n^{1/2})$ at 1 mg/L	% germination ± $t_{0.05}(s/n^{1/2})$ at 0.01 mg/L
high melting GR24 (**2a**)	22.4 ± 3.4	30.4 ± 3.2
low melting GR24 (**2b**)	25.5 ± 3.4	8.5 ± 1.8
mixture of diastereomers	27.3 ± 4.6	34.1 ± 4.7
control (no stimulant)	3.3 ± 1.5	4.6 ± 1.6

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

Table III. Germination Percentages for Seeds of *O. crenata* after Exposure to Solutions of GR24 Diastereomers at Concentrations of 1 and 0.01 mg/L[a]

sample	% germination ± $t_{0.05}(s/n^{1/2})$ at 1 mg/L	% germination ± $t_{0.05}(s/n^{1/2})$ at 0.01 mg/L
high melting GR24 (**2a**)	77.1 ± 2.9	12.0 ± 2.1
low melting GR24 (**2b**)	66.7 ± 3.2	3.5 ± 1.2
mixture of diastereomers	70.7 ± 3.4	7.6 ± 2.2
control (no stimulant)	0.2 ± 0.4	0.2 ± 0.4

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

low melting isomer **2b** is significantly less active than its diastereomer **2a**. A 1:1 mixture of diastereomers **2a** and **2b** is giving slightly better germination results than either individual diastereomer. It may be concluded that for S. *hermonthica* seeds it is not necessary to separate the diastereomers of GR24 to improve germination stimulation.

For *O. crenata* seeds diastereomer **2a** is significantly more active than the low melting isomer **2b** and even more active than the mixture of diastereomers at both concentrations. The activity of the mixture of **2a** and **2b** is almost the average of the activities of the individual diastereomers.

Although the germination percentages obtained with *O. crenata* seeds are different for both diastereomers of GR24, from a practical point of view it is desirable to use the mixture of diastereomers. The higher activity of **2a**, compared with a 1:1 mixture of **2a** and **2b**, is not spectacular and does not counterbalance the loss of material resulting from separation of the diastereomers. Moreover, chromatographic separation procedures are not desirable when multigram quantities of compound are needed.

For both types of seeds it can be concluded that the high melting diastereomer of GR24 has a somewhat higher activity than the low melting GR24. For diastereomers of GR7 it was found that the low melting diastereomer was the most active one (Pepperman et al., 1982). Although this might look contradictory, in fact this is what was expected, because (±)-high melting GR24 and (±)-low melting GR7 have the same relative stereochemistry as present in (±)-strigol.

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