Synthesis and Biological Evaluation of the Strigol Analogue Carba-GR24

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Both geometrical isomers of the strigol analogue carba-GR24, (*E*)-3-[2-(4-methyl-5-oxo-2,5-dihydrofuran-2-yl)ethylidene]-3,3a,4,8b-tetrahydro-2*H*-indeno[1,2-*b*]furan-2-one (**1**) and **7** (*Z* isomer), were prepared separately. These analogues, in which the vinyl ether oxygen atom in GR24 has been replaced by a methylene function, are completely inactive for the stimulation of germination of seeds of the parasitic weeds *Striga hermonthica* and *Orobanche crenata*. Bioassays in which **1** was tested as an inhibitor of germination gave no indication as such.

Keywords: *Striga; Orobanche; germination; strigol analogue*

INTRODUCTION

Parasitic weeds of the genera *Striga* and *Orobanche* cause severe damage to graminaceous and leguminous crops in tropical and subtropical regions (Musselman, 1987; Parker and Riches, 1993). These parasitic species generally obtain their nutrients and/or energy from a host plant. A unique feature of these parasitic angiosperms is that they require a specific chemical signal produced by the host root to stimulate germination of their seeds. It has been demonstrated that root exudates of different *Striga* hosts contain one or more compounds of the class of "strigolactones" [this collective name has been introduced recently by Butler (1995)]. These strigolactones include strigol (Cook *et al*., 1972; Brooks *et al*., 1985; Siame *et al*., 1993), sorgolactone (Hauck *et al*., 1992), and alectrol (Müller *et al*., 1992) (Figure 1).

Extensive structure−activity studies revealed that the bioactiphore resides in the CD part of the molecule (Hassanali, 1984; Mangnus and Zwanenburg, 1992a; Zwanenburg *et al*., 1994; Mangnus *et al*., 1992a). Synthetic analogues, especially the so-called GR compounds GR24 and GR7 (Figure 1), received much attention (Johnson *et al*., 1976, 1981; Mangnus and Zwanenburg, 1991). These compounds were designed by systematically removing parts of the strigol skeleton. From the GR series, GR24 (Figure 1) is the most potent one, whose half-maximal activity is at 10^{-9} M (*Striga hermonthica*), which is comparable to that of strigol. Recently, we proposed a tentative molecular mechanism, which may account for the biological activity of strigol and its (synthetic) analogues (Mangnus and Zwanenburg, 1992b). This mechanism, which involves only the bioactiphoric CD part, is depicted in Scheme 1.

The enol ether unit plays a crucial role in this mechanism, since it enables the D ring to eliminate as is shown. Replacement of the enol ether oxygen atom by the bioisosteric methylene group will provide valuable, additional information about the bioactiphore. The strategy for the synthesis of carba-GR24 (**1**) was reported recently (Kishimba and Zwanenburg, 1994). The key step involves coupling of the ABC moiety **2** and the latent D-ring fragment **3a** (Scheme 2). The previously

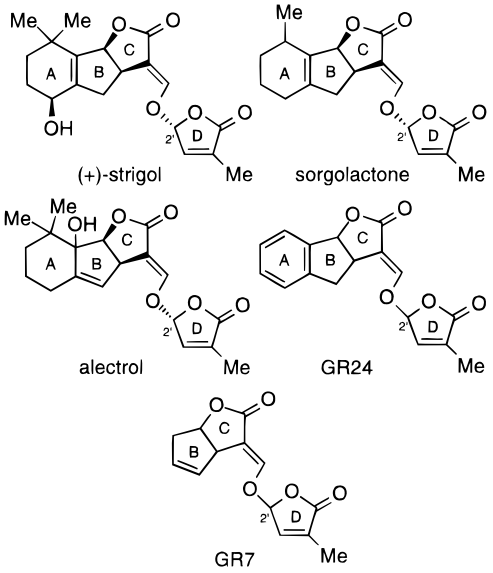
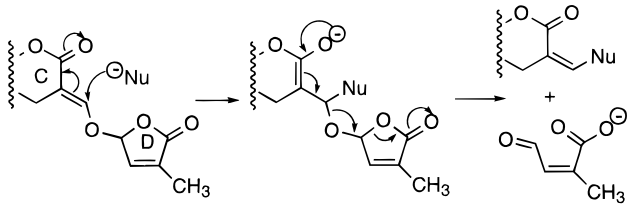


Figure 1. Strigolactones and some active analogues.

Scheme 1. Molecular Mechanism of Germination



described coupling using essentially the procedure of Tanaka (1980) proved to be rather problematic. Therefore, another method for the coupling was considered. The primary aim of this work is to establish the germination-stimulating or -inhibiting ability of carba-GR24.

MATERIALS AND METHODS

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

Synthesis. *General Remarks.* ^{1}H-NMR spectra (100 MHz) were recorded on a Bruker AC 100 spectrometer (Me_4Si as internal standard), and 400 MHz ^{1}H-NMR spectra were recorded on a Bruker AM-400 spectrometer (Me_4Si as internal

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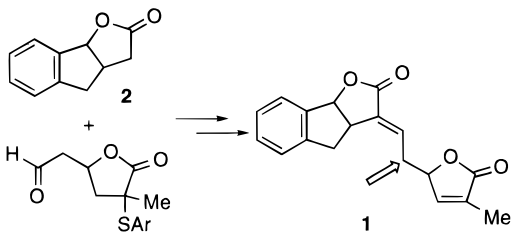
Scheme 2. Strategy for the Preparation of Carba-GR24 (1)



standard). Both instruments were from Brucker, Wissembourg, France. All coupling constants are given as ^3J in hertz, unless indicated otherwise. For mass spectra, a double-focusing VG7070E mass spectrometer from VG Analytical, Manchester, U.K., was used. GC−MS spectra were run on a Varian Saturn 2 GC−MS ion-trap system fromVarian Nederland BV, Houten, The Netherlands. Separation was carried out on a fused-silica capillary column (DB-5, 30 m × 0.25 mm). Helium was used as the carrier gas, and electron impact (EI) was used as the ionization mode. GLC was conducted with a Hewlett-Packard HP 5890 gas chromatograph, from Hewlett-Packard Nederland, using a capillary cross-linked methyl silicone gum column of 25 m length, 0.32 mm i.d., and 0.17 μm film thickness and nitrogen (2 mL/min, 0.5 atm) as the carrier gas. Melting points were measured with a Reichert Thermopan, Vienna, Austria, microscope and are uncorrected. Elemental analyses were performed at the Department of Microanalysis of this laboratory.

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, Darmstadt, Germany, precoated silica gel 60 F254 plates (0.25 mm), using the eluents indicated. Spots were visualized with UV light or by 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.

*3,3a,4,8b-Tetrahydroindeno[1,2-b]furan-2-one (**2**)* was prepared following a previously reported synthesis (Mangnus *et al.*, 1992b).

*{4-[(4-Chlorophenyl)sulfanyl]-4-methyl-5-oxotetrahydrofuran-2-yl}acetaldehyde (**3b**).* Precursor **4** was prepared similarly to 5-(2,2-diethoxyethyl)-3-methyl-3-(phenylthio)dihydrofuran-2-one as described by Kishimba and Zwanenburg (1994). Yield after chromatography (SiO_2, hexane/ethyl acetate, 3:1): 65% as a yellow oil. ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.22 (2t, 6H, J = 7.5 Hz, $(OCH_2CH_3)_2$), 1.47, 1.52 (2s, 3H, CH_3), 1.79−2.21 (m, 3H, $CH_2CH(OEt)_2$, CH_2), 2.35−2.60 (m, 1H, CH_2), 3.58 (m, 4H, $(OCH_2CH_3)_2$), 4.63 (m, 2H, C*H*), 7.28−7.57 (m, 4H, arom *H*), GC−MS (EI, *m*/*z*, relative intensity): 358, 360 ([M]$^+$, 4.1, 1.9), 313, 315 (5.4, 2.1), 215 (8.4), 241, 243 (3.0, 1.4), 169 (23.6), 143, 145 (13.7, 6.1), 125 (11.8), 103 (100), 75 (66).

A suspension of **4** (2.5 g, 7.0 mmol) in 0.5 N HCl (25 mL) was heated under reflux for 45 min. The cooled reaction mixture was extracted with CH_2Cl_2 (3 × 25 mL), and the organic extracts were washed with water (2 × 10 mL) and brine. Drying of the organic phase and evaporation of the solvent *in vacuo* gave **3b** (1.78 g, 90%) as an orange oil, which was almost pure. Purification by chromatography (Florisil, diethyl ether) afforded pure **3b** (1.21 g, 61%). Because of its instability, **3b** was characterized only by ^{1}H-NMR and used immediately for further reactions. ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.47, 1.51 (2s, 3H, CH_3), 1.89−2.97 (m, 4H, CH_2), 5.03 (m, 1H, C*H*), 7.15−7.54 (m, 4H, arom *H*), 9.70, 9.76 (2s, 1H, *H*C=O). Ratio of diastereomers: 2:1.

*3-{2-[4-[(4-Chlorophenyl)sulfanyl]-4-methyl-5-oxotetrahydrofuran-2-yl]-1-hydroxyethyl}-3,3a,4,8b-tetrahydro-2H-indeno[1,2b]furan-2-one (**5**).* A solution of tricyclic lactone **2** (835 mg, 4.80 mmol) in THF (10 mL) was added dropwise to a cooled (−78 °C) solution of LDA (4.80 mmol) in THF (15 mL) under N_2. The reaction mixture was stirred for 10 min, and then freshly prepared aldehyde **3b** (1.37 g, 4.80 mmol) in THF (5 mL) was added. Stirring was continued for 1 h at the same temperature. The reaction mixture was warmed to 0 °C, stirred for 1 h, and quenched with a saturated solution of NH_4Cl. Then THF was removed *in vacuo*. Extraction with ethyl acetate (3 × 20 mL) and washing of the combined organic extracts with water (2×) and brine followed by drying provided crude product **5**. Purification by flash chromatography (SiO_2, eluent gradient hexane/ethyl acetate, 3:1, 2:1, and then 3:2) gave seven fractions with different R_f values ranging from 0.17 to 0.062 (eluent hexane/ethyl acetate, 2:1), which all showed satisfactory ^{1}H-NMR data. Total yield of all collected, pure fractions: 1.46 g, 66%. From the fast moving fraction (R_f 0.17, hexane/ethyl acetate, 2:1), pure crystals (106 mg) could be obtained by crystallization from hexane/ethyl acetate. Mp: 192−195 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.48 (s, 3H, CH_3), 1.86 (dd, 1H, ^2J = 13.9 Hz, J = 9.0 Hz, CH_2 D ring), 2.00−2.11 (m, 2H, CH_2), 2.29 (t, 1H, J = 7.9 Hz, OC(O)C*H*), 2.55 (dd, 1H, J = 5.4 Hz, ^2J = 13.9 Hz, CH_2 D ring), 2.92 (d, 1H, ^2J = 16.4 Hz, CH_2 B ring), 3.17 (ddd, 1H, J = 7.9, 7.7, 7.6 Hz, C*H* BC rings), 3.33 (dd, 1H, J = 7.6 Hz, ^2J = 16.4 Hz, CH_2 B ring), 3.66 (s, 1H, exchanges in the presence of D_2O, O*H*), 4.18 (m, 1H, C*H*OH), 4.96 (m, 1H, C*H* D ring), 5.94 (d, 1H, J = 7.7 Hz, PhC*H*O), 7.27−7.49 (m, 8H, arom *H*). MS (CI, *m*/*z*, relative intensity): 459, 461 ([M]$^+$+1, 0.23, 0.05), 441, 443 (0.35, 0.13), 284, 286 (1.74, 0.80), 174 (13.2), 144, 146 (36.6, 14.2), 129 (37.6), 41 (100). Anal. Calcd for $C_{24}H_{23}ClO_5S$: C, 62.81; H, 5.05; S, 6.99. Found: C, 62.88; H, 4.98; S, 6.68.

*3-{2-[4-[(4-Chlorophenyl)sulfanyl]-4-methyl-5-oxotetrahydrofuran-2-yl]ethylidene}-3,3a,4,8b-tetrahydro-2H-indeno[1,2-b]furan-2-one [(Z-**6**) via the Mesylate of **5**.* Typical procedure for the mesylation of **5**: To a solution of pure aldol **5** (R_f 0.17, eluent hexane/ethyl acetate, 2:1) (50.1 mg, 0.11 mmol) in CH_2Cl_2 (3 mL) were added triethylamine (18.2 μL, 0.13 mmol) and then mesyl chloride (10.1 μL, 0.13 mmol) at 0 °C. The solution was stirred for 18 h before quenching with a saturated solution of $NaHCO_3$. The mixture was extracted with CH_2Cl_2 (2 × 10 mL), and the combined organic layers were washed with water (2 × 5 mL). Drying and evaporation of the solvent *in vacuo* afforded 46.0 mg (78%) of the desired mesylate, which was sufficiently pure for the next elimination reaction. ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.41 (s, 3H, CH_3), 1.81−3.48 (m, 7H), 3.08 (s, 3H, CH_3 mesyl), 4.69 (m, 1H, C*H* D ring), 5.22 (dt, 1H, J = 3.3, 9.2 Hz, C*H*OMs), 5.86 (d, 1H, J = 7.6 Hz, PhC*H*O), 7.34 (m, 8H, arom *H*). MS (CI, *m*/*z*, relative intensity): 536, 538 ([M]$^+$, 0.51, 0.21), 440, 442 (2.38, 1.28), 297 (1.16), 144, 146 (19.8, 10.2), 97 (8.15), 41 (100).

The crude mesylate (46.0 mg, 0.09 mmol) was treated with DBU (16.7 mg, 0.11 mmol) in CH_2Cl_2 (2 mL) at 0 °C. After 2 h of stirring at the same temperature, the solution was quenched with a saturated solution of $NaHCO_3$ and extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were washed with water (2 × 5 mL) and dried. Removal of the solvent *in vacuo* gave almost pure product *Z*-**6**, which was purified by chromatography (SiO_2, hexane/ethyl acetate, 2:1) to give 35 mg, 72% (based on starting aldol **5**), of (*Z*)-**6** as a white solid. ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.34 (s, 3H, CH_3), 1.92 (dd, 1H, J = 10.1 Hz, ^2J = 14.1 Hz, CH_2 D ring), 2.38 (dd, 1H, J = 5.5 Hz, ^2J = 14.1 Hz, CH_2 D-ring), 2.53−3.57 (m, 4H, 2CH_2), 3.78 (m, 1H, C*H* BC rings), 4.50 (m, 1H, C*H* D ring), 5.86 (d, 1H, J = 7.6 Hz, PhC*H*O), 6.36 (ddd, 1H, J = 6.9, 8.3 Hz, ^4J = 2.3 Hz, =C*H*), 7.19 (m, 8H, arom H). MS (EI, *m*/*z*, relative intensity): 440, 442 ([M]$^+$, 65.6, 26.9), 296 (7.2), 279 (35.2), 225 (34.6), 143, 145 (92.0, 20.7), 109 (43.3), 41 (100). HRMS/EI: *m*/*z* calcd for $C_{24}H_{21}{}^{35}ClO_4S$ 440.0849, found 440.0849.

*3-{2-[4-[(4-Chlorophenyl)sulfanyl]-4-methyl-5-oxotetrahydrofuran-2-yl]ethylidene}-3,3a,4,8b-tetrahydro-2H-indeno[1,2-b]furan-2-one [(E)-**6**].* Typical procedure starting from pure aldol **5** (R_f 0.17, eluent hexane/ethyl acetate, 2:1): To a solution of

aldol **5** (30.7 mg, 0.067 mmol) was added 29.9 mg (0.10 mmol) of 2-fluoro-1-methylpyridinium *p*-toluenesulfonate (95%, purchased from Aldrich), followed by triethylamine (20.3 mg, 0.20 mmol) at room temperature under N_2. The reaction mixture was stirred for 4 h, quenched with a saturated solution of $NaHCO_3$, and extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were washed with water (2 × 5 mL) and dried. Purification by flash chromatography (SiO_2, hexane/ethyl acetate, 3:1) gave two geometrical isomers, (*Z*)-**6** and (*E*)-**6**.

(*E*)-**6**: yield, 19.0 mg, 64% as a white solid; R_f 0.44 (eluent hexane/ethyl acetate, 1:1). ^{1}H-NMR ($CDCl_3$, 100 MHz): δ 1.44 (s, 3H, CH_3), 2.04 (dd, 1H, J = 10.2 Hz, ^2J = 13.8 Hz, CH_2 D ring), 2.50 (dd, 1H, J = 5.3 Hz, ^2J = 13.8 Hz, CH_2 D ring), 2.57−3.66 (m, 4H, 2CH_2), 3.85 (m, 1H, C*H* BC rings), 4.70 (m, 1H, C*H* D ring), 5.87 (d, 1H, J = 7.5 Hz, PhC*H*O), 6.68 (dt, 1H, J = 7.5, ^4J = 2.5 Hz, =C*H*), 7.30 (m, 8H, arom *H*). MS (EI, *m*/*z*, relative intensity): 440, 442 ([M]$^+$, 77.8, 32.1), 296 (19.0), 279 (10.7), 225 (42.5), 144, 146 (100, 38.2), 109 (53.6), 41 (65.8). HRMS/EI: *m*/*z* calcd for $C_{24}H_{21}{}^{35}ClO_4S$ 440.0849, found 440.0845.

(*Z*)-**6**: yield, 9.5 mg, 32% as a white solid; R_f 0.56 (eluent hexane/ethyl acetate, 1:1). ^{1}H-NMR and mass data were the same as described for the compound obtained by mesylation and elimination (*vide supra*).

*(E)-3-[2-(4-Methyl-5-oxo-2,5-dihydrofuran-2-yl)ethylidene]-3,3a,4,8b-tetrahydro-2H-indeno[1,2-b]furan-2-one (**1**)* was prepared similarly as described previously (Kishimba and Zwanenburg, 1994) starting from (*Z*)-**6** (150 mg, 0.34 mmol) as a mixture of diastereomers. Purification by chromatography (SiO_2, hexane/ethyl acetate, 2:1) afforded **1** (95 mg, 94%) as a white solid in a diastereomeric ratio of 1:1 (calculated from ^{1}H-NMR analysis). An analytical sample was obtained by crystallization from hexane/ethyl acetate. R_f: 0.26 (eluent hexane/ethyl acetate, 1:1). ^{1}H-NMR and mass data were in complete agreement with those reported previously. Anal. Calcd for $C_{18}H_{16}O_4$: C, 72.96; H, 5.44. Found: C, 72.69; H, 5.52.

*(Z)-3-[2-(4-Methyl-5-oxo-2,5-dihydrofuran-2-yl)ethylidene]-3,3a,4,8b-tetrahydro-2H-indeno[1,2-b]furan-2-one (**7**)* was prepared according to the procedure for the synthesis of **1** starting from (*Z*)-**7** (130 mg, 0.30 mmol) as a mixture of diastereomers. Yield after chromatography (SiO_2, hexane/ethyl acetate, 2:1): 79 mg, 90% as a white solid. Diastereomeric ratio: 1:1 as calculated from ^{1}H-NMR analysis. An analytical sample was obtained by crystallization from hexane/ethyl acetate. R_f 0.43 (eluent hexane/ethyl acetate, 1:1). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.75, 1.93 (2s, 3H, CH_3), 2.89−3.03 (m, 2H, =CCH_2 and CH_2 B ring), 3.34 (m, 1H, =CCH_2), 3.46−3.54 (m, 1H, CH_2 B ring), 3.84 (m, 1H, C*H* BC rings), 4.98, 5.02 (2m, 1H, =CC*H* D ring), 5.93 (d, 1H, J = 7.7 Hz, PhC*H*O), 6.35, 6.37 (2dt, 1H, J = 7.4 Hz, ^4J = 2.3 Hz, =C*H*), 6.98, 7.05 (2m, 1H, =C*H* D ring), 7.24−7.51 (m, 4H, arom *H*). MS (CI, *m*/*z*, relative intensity): 297 ([M + 1]$^+$, 5.6), 199 ([$C_{13}H_{11}O_2$]$^+$, 100), 97 ([$C_5H_5O_2$]$^+$, 70.3). Anal. Calcd for $C_{18}H_{16}O_4$: C, 72.96; H, 5.44. Found: C, 72.79; H, 5.37.

Biological activity. *Seeds*. Seeds of *S. hermonthica* (from *Sorghum bicolor* (L.) Moench) and *Orobanche crenata* (from *Vicia faba* L.) were harvested in Sudan in 1987 and in Egypt in 1991, 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 in 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 containing 1, 0.1, and 0.01 mg/L test compound and 0.1, 0.01, and 0.001% (v/v) acetone, respectively.

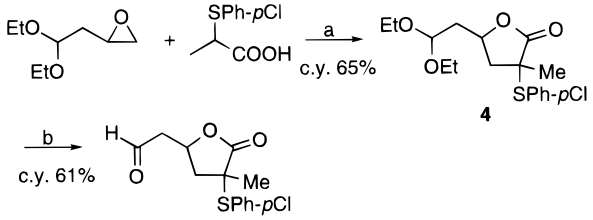
Bioassays. For surface sterilization, seeds of *S. hermonthica* and *O. crenata* were exposed to an aqueous solution of sodium hypochlorite (2% active chlorine) for 5 min with agitation. The seeds were then thoroughly rinsed with water and dried overnight.

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, 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. After incubation for 24 h (*Striga*) and 5 days (*Orobanche*) in the dark 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.

In each test series, aqueous solutions with 0.1, 0.01, and 0.001% (v/v) acetone were used as controls. Test solutions of the stimulant GR24 (concentrations of 1, 0.1, and 0.01 mg/L) were used as positive controls. All tests were performed in duplicate, and in each test the germination percentages were determined on 12 disks per treatment.

For full details of the bioassay, see Mangnus *et al*. (1992c).

Scheme 3. Synthesis of Latent D-Ring Synthon 3b[a]



[a] (a) (1) LDA (2 equiv); (2) aqueous tartaric acid; (3) SiO_2, Δ; (b) 0.5 N HCl, Δ.

RESULTS AND DISCUSSION

Synthesis. The key step in the synthesis of carba-GR24 (**1**) proceeds by coupling of tricyclic lactone **2** with an appropiate latent butenolide fragment (Scheme 2). It is essential to use this protected D-ring synthon, because the corresponding butenolide is too unstable to survive the coupling conditions. Previously the phenylsulfanyl group as protecting function in the D-ring synthon was used. During the revision of the coupling step, the (*p*-chlorophenyl)sulfanyl moiety was considered as an alternative, because this group would probably facilitate crystallization of intermediate products. The D-ring synthon **3b** was prepared essentially in the same manner as reported earlier, from 2-(2,2-diethoxyethyl)-oxirane and α-[(*p*-chlorophenyl)sulfanyl]propionic acid, and subsequent hydrolysis of the acetal function (Scheme 3). The aldehyde **3b** thus obtained is a rather unstable compound; its quality after chromatography (Florisil) varied to some extent.

Aldol condensation of aldehyde **3b** with ABC-fragment **2** gave product **5** as a mixture of diastereomers (Scheme 4). The subsequent dehydration step (Scheme 4) was not as straightforward as expected.

Conventional methods failed to give the desired result, e.g., *p*-toluenesulfonic acid catalysis, $Ac_2O-\Delta$, *p*-TosCl−DBU, $POCl_3$−HMPA−pyridine (Trost and Jungheim, 1980), DCC−CuCl (Miller, 1980; Alexandre and Rouessac, 1971). Only mesylation (MsCl, Et_3N) followed by elimination (DBU) gave the desired transformation, whereby the *Z* isomer (*Z*)-**6** was obtained as the main product. A photochemical isomerization of the exocyclic double bond to the *E* geometry could be accomplished; however, this is not recommended, since an extra step and an extra chromatographic separation are required. This step could indeed be avoided when aldol **5** was treated with 2-fluoro-1-methylpyridinium *p*-toluenesulfonate in the presence of Et_3N (Mukaiyama, 1979; Narasaka *et al*., 1984). Under these conditions, the *E* isomer (*E*)-**6** was formed predominantly. These findings provided a reason to investigate the dehydration reaction in more detail. Relevant results are collected in Table 1.

Scheme 4. Synthesis of (*E*)-6 and (*Z*)-6

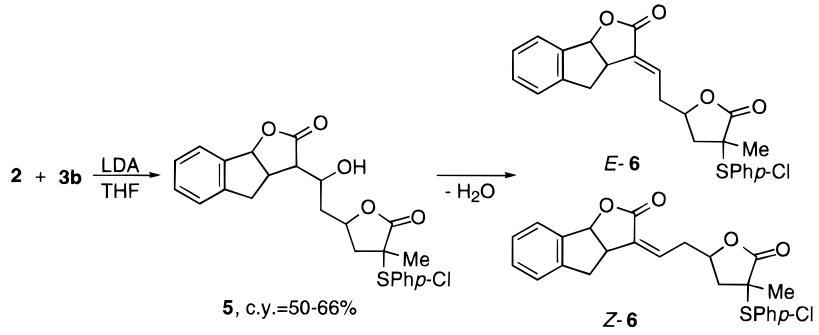
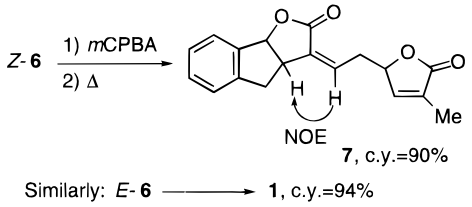


Table 1. Product Distribution after Exposure of Two Diastereomeric Compounds 5 to Different Elimination Conditions

R_f aldol **5**[a]	yield of **6**,[b] %	*E*:*Z* ratio[b]	yield of **6**,[c] %	*E*:*Z* ratio[c]
0.1	72	only *Z*	95	2:1
0.0	30	1:1	90	only *E*

[a] R_f value on TLC (SiO_2, eluent hexane/ethyl acetate, 2:1). [b] Prepared by mesylation, followed by DBU treatment. [c] Prepared by treatment with 2-fluoro-1-methylpyridinium *p*-toluenesulfonate and Et_3N.

Scheme 5. Oxidative Elimination of the Thioether Moiety



The choice of the starting diastereomer is an important factor for the yield of the reaction as well as its stereochemical outcome. Mesylation and subsequent treatment with DBU generally lead to the preponderant formation of the *Z* isomer, whereas reaction with 2-fluoro-1-methylpyridinium *p*-toluenesulfonate and Et_3N predominantly leads to the *E* isomer. The latter method gave a cleaner reaction and higher yields. Starting from a mixture of diastereomers, either method led, on a preparative scale, to the expected geometrical isomer (*E*)-**6** or (*Z*)-**6** in isolated yields ranging from 45 to 70%. These results indicate that the elimination reactions take place via different mechanisms. Amberg and Seebach (1990) suggested a *syn*-type mechanism for a similar base-induced elimination of a mesylate, which can be envisaged by deprotonation of the mesyl methyl group and a subsequent *syn* proton abstraction via a cyclic transition state. This explanation is in agreement with our observation that elimination of the corresponding tosylate under the same conditions does not take place. The formation of (*E*)-**6** by elimination of the *N*-methylpyridinium salt proceeds in a antiperiplanar fashion.

Finally, removal of the protecting thioether moiety was carried out by oxidation to the corresponding sulfoxides and their subsequent pyrolysis to give in high yields (*Z*)-carba-GR24 (**7**) and (*E*)-carba-GR24 (**1**), both as mixtures of two diastereomers (Scheme 5), which could not be separated by chromatography. The assignment of the *E*/*Z* geometry was based on their ^{1}H-NMR spectra. The signal of the exocyclic vinylic proton for the *E* isomer was about 0.3 ppm further downfield as compared to the corresponding signal for the *Z* adduct (anisotropic effect of the carbonyl). In addition, a NOE

Table 2. Germination Percentages for Seeds of *S. hermonthica* and *O. crenata* after Exposure to Solutions of 1 and 7[a]

	% germination ± SE			
	S. hermonthica		*O. crenata*	
compd	1 mg/L	0.01 mg/L	1 mg/L	0.1 mg/L
1	2.7±1.5 (37.7±3.5)[b]	2.9 ± 1.0 (55.1±2.6)[b]	0.0 ± 0 (59.9±3.2)[b]	0.0 ± 0 22.1 ± (1.4)[b]
7	2.1±0.6 (50.5 ± 4.2)[b]	3.7±0.8 (67.5 ± 0.4)[b]	0.0±0 (59.9 ± 3.2)[b]	0.0±0 22.1 ± (1.4)[b]

[a] Activities are indicated as germination percentages after treatment of the seeds with test solutions at 1 and 0.01 mg/L. Germination percentages given are the mean ± SE of two replicate tests. [b] The values in parantheses are the mean germination percentages for seeds tested under the same conditions and at the same time, with GR24 as stimulant.

was observed between the exocyclic vinylic proton and H_{3a} in the 2D-NOESY spectrum of the *Z* isomer (MM2 calculated distance 2.74 Å), which was absent for the *E* isomer (MM2 calculated distance 3.92 Å).

Biological Activity. The stimulatory activity of the carba-GR24 analogues **1** and **7** was assayed using seeds of *S. hermonthica* and *O. crenata*. The germination percentages are summarized in Table 2, together with those obtained for GR24 under the same conditions in the same bioassay. (This enables a comparison between results obtained in different test series, which is important, because the response of seeds of parasitic weeds, in particular of *S. hermonthica*, varies considerably from test to test). For the evaluation of the bioactivity, mixtures of diastereomers were used, which is acceptable because it was shown for GR24 that the activity of the most active diastereomer is hardly influenced by the presence of the less active diastereomer (Mangnus *et al.*, 1992b).

The germination results indicate that (*E*) and (*Z*)-carba-GR24 (**1** and **7**) have no effect upon seeds of *S. hermonthica* and *O. crenata*. This means that isosteric replacement of oxygen in GR24 by carbon causes complete loss of activity. This finding supports the tentative molecular mechanism for the stimulation of germination, as proposed in Scheme 1. These results can be explained by assuming either that both carba analogues do not fit properly in the receptor cavity for either steric or electronic reasons or that the affinity is sufficiently high, but it does not lead to a transduction of a signal. According to the proposed molecular mechanism (Scheme 1), essentially two events take place within the receptor site. The addition to the α,β-unsaturated system in a Michael fashion may still occur in the case of carba-GR24. The subsequent elimination reaction, however, is prevented, because the D ring is not a leaving group anymore. The essence of the elimination of the D ring remains to be established, but

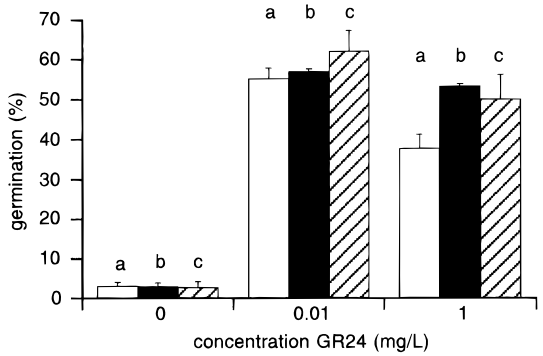


Figure 2. Effect of (*E*)-carba-GR24 (**1**) on the germination stimulatory activity of GR24. Preconditioned seeds of *S. hermonthica* were treated with (a) only GR24, (b) mixtures of (*E*)-carba-GR24 (0.01 ppm) and GR24, and (c) mixtures of (*E*)-carba-GR24 (1 ppm) and GR24 at the indicated concentrations. Data presented ± SE are from one representative experiment.

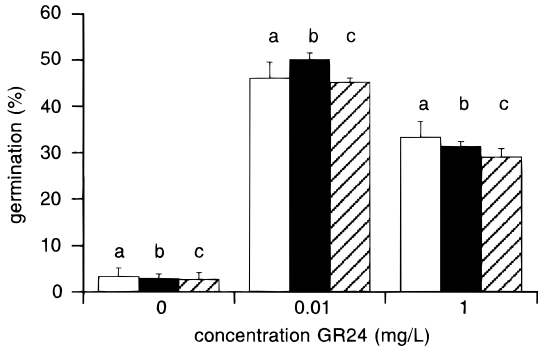


Figure 3. Effect of (*E*)-carba-GR24 (**1**) on the germination stimulatory activity of GR24. Preconditioned seeds of *S. hermonthica* were incubated with solutions of **1** for 24 h. After removal of **1**, the seeds were exposed to GR24 at the indicated concentrations: (a) seeds preincubated with aqueous control, followed by treatment with GR24; (b) seeds preincubated with **1** (0.01 ppm), followed by treatment with GR24; (c) seeds preincubated with **1** (1 ppm), followed by treatment with GR24. Data presented ± SE are from one representative experiment.

it may well be possible that the D ring, which is a highly reactive species, fulfills the function of a second messenger.

It may be hypothesized that carba-GR24 inhibits of the germination process by acting as a Michael acceptor in the same way as is proposed for GR24 (Scheme 1). However, the active site of the receptor is now being blocked, and an inhibitory effect is expected. It may also be argued that the inherent chemical reactivity of the α,β-unsaturated system in carba-GR24 does not play a role. In that case, competition of carba-GR24 and GR24 for the same receptor site with a comparable degree of affinity might still lead to a concentration-dependent inhibitory effect. In order to test these hypotheses, two types of experiments were conducted employing seeds of *S. hermonthica*. In both experiments, (*E*)-carba-GR24 (**1**) was used as a potential inhibitor. In the first experiment, preconditioned seeds of *S. hermonthica* were incubated in the presence of mixtures of GR24 and **1** at different concentrations. The results are depicted in Figure 2. In another experiment, preconditioned seeds of *S. hermonthica* were preincubated for 24 h in solutions containing different concentrations of **1**. The solution of **1** was then carefully removed, and the seeds were washed several times and then treated with different concentrations of GR24. The results are presented in Figure 3. From Figures 2 and 3, it may be concluded that (*E*)-carba-GR24 (**1**) does not exert any inhibitory effect. Apparently, the lack of stimulatory activity of **1** cannot be attributed to (irreversible) blocking of the receptor site through its Michael acceptor.

Concluding Remarks. An improved synthesis of (*Z*)- and (*E*)-carba-GR24 is presented. The biological data indicate that isosteric replacement of oxygen by carbon causes complete loss of biological activity. It should be emphasized that so far no strigol analogues were designed and assayed for inhibitory activity, because assays for stimulant activity are much easier to perform and to interpret than assays for inhibitor activity. These data, nevertheless, are essential since they may provide valuable information about the molecular basis of the germination process. It should be noted that compounds specific for inhibition of *Striga* and *Orobanche* seed germination would serve practical purposes in the control of these parasitic weeds (Butler, 1994, 1995).

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LITERATURE CITED

Alexandre, C.; Rouessac, F. Préparation de cétones α-éthyléniques et obtention de cétones α-cyclopropaniques par action des alcoylcarbodiimides sur les β- et γ-cétols. (Preparation of α,β-unsaturated and cyclopropyl ketones by the action of some alkyl carbodiimides on β- and γ-ketols). *Bull. Soc. Chim. Fr.* **1971**, 1837−1840.

Amberg, W.; Seebach, D. (E,E,R)-5-Alkyliden-2-*tert*-butyl-6-methyl-1,3-dioxan-4-one: herstellung aus (R)-hydroxybuttersaüre, cuprat-additionen und hydrolysen zu 3-hydroxycarbonsaüren mit chiralen sekundären alkylgruppen in 2-stellung ((E,R,R)-5-Alkylidene-2-*tert*-butyl-6-methyl-1,3-dioxan-4-ones: preparation from (R)-3-hydroxybutyric acid, cuprate addition, and hydrolyses to 3-hydroxycarboxylic acids with chiral secondary alkyl substituents in the 2-position. *Chem. Ber.* **1990**, *123*, 2413−2428.

Brooks, D. W.; Bevinakatti, H. S.; Powell, D. R. The absolute structure of (+)-strigol. *J. Org. Chem.* **1985**, *50*, 3779−3781.

Butler, L. G. Germination stimulants: state of the art after the Third International Workshop on *Orobanche*. In *Proceedings of the third international workshop on Orobanche and related Striga research*; Pieterse, A. H., Verkley, J. A. C., Ter Borg, S. J., Eds.; Royal Tropical Institute: Amsterdam, The Netherlands, 1994; pp 719−723.

Butler, L. G. Chemical communication between the parasitic weed *Striga* and its crop host. *ACS Symp. Ser.* **1995**, *582*, 158−166.

Cook, C. E.; Whichard, L. P.; Wall, M. E.; Egley, G. H.; Coggan, P.; Luhan, P. A.; McPhail, A. T. Germination stimulants II. The structure of strigol—a potent seed germination stimulant for witchweed (*Striga lutea* Lour). *J. Am. Chem. Soc.* **1972**, *94*, 6198−6199.

Hassanali, A. Strigol analogues: synthetic achievements and prospects. In *Striga: Biology and Control*; Ayensu, E. S., Doggett, H., Keynes, R. D., Marton-Lefevre, J., Musselman, L. J., Parker, C., Eds.; ICSU Press: Paris, 1984; pp 125−132.

Hauck, C.; Müller, S.; Schildknecht, H. A germination stimulant for parasitic flowering plants from *Sorghum bicolor*, a genuine host plant. *J. Plant Physiol.* **1992**, *139*, 474−478.

Johnson, A. W.; Roseberry, G.; Parker, C. A novel approach to *Striga* and *Orobanche* control using synthetic germination stimulants. *Weed Res.* **1976**, *16*, 223−227.

Johnson, A. W.; Gowda, G.; Hassanali, A.; Knox, J.; Monaco, S.; Razawi, Z.; Roseberry, G. The preparation of synthetic analogues of strigol. *J. Chem. Soc., Perkin Trans. 1* **1981**, 1734−1743.

Kishimba, M. A.; Zwanenburg, B. Synthesis of carba-strigol derivatives. *Recl. Trav. Chim. Pays-Bas* **1994**, *113*, 21−28.

Mangnus, E. M.; Zwanenburg, B. Design and synthesis of germination stimulants for seeds of *Striga* and *Orobanche* spp. In *Progress in Orobanche Research*; Wegmann, K., Musselman, L. J., Eds.; Eberhard-Karls-Universität: Tübngen, Germany, 1991; pp 157−166.

Mangnus E. M.; Zwanenburg, B. Synthesis and biological evaluation of A-ring analogues of the natural germination stimulant strigol. *Recl. Trav. Chim. Pays-Bas*, **1992a**, *111*, 155−159.

Mangnus, E. M.; Zwanenburg, B. Tentative molecular mechanism for germination stimulation of *Striga* and *Orobanche* seeds by strigol and its synthetic analogues. *J. Agric. Food Chem.* **1992b**, *40*, 1066−1070.

Mangnus, E. M.; van Vliet; L. A.; Vandenput, D. A. L.; Zwanenburg, B. Structural modifications of strigol analogues. Influence of the B and C rings on the bioactivity of the germination stimulant GR24. *J. Agric. Food Chem.* **1992a**, *40*, 1222−1229.

Mangnus, E. M.; Dommerholt, F. J.; de Jong, R. L. P.; Zwanenburg, B. Improved synthesis of strigol analogue GR24 and evaluation of the biological activity of its diastereomers. *J. Agric. Food Chem.* **1992b**, *40*, 1230−1235.

Mangnus, E. M.; Stommen, P. L. A.; Zwanenburg, B. A standardized bioassay for evaluation of potential germination stimulants for seeds of parasitic weeds. *J. Plant Growth Regul.* **1992c**, *11*, 91−98.

Miller, M. J. Isourea-mediated preparation of dehydro amino acids. *J. Org. Chem.* **1980**, *45*, 3131−3132.

Mukaiyama, T. New synthetic reactions based on the onium salts of aza-arenes. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 707−721.

Müller, S.; Hauck, C.; Schildknecht, H. Germination stimulants produced by *Vigna unguiculata* Walp cv Saunders Upright. *J. Plant Growth Regul.* **1992**, *11*, 77−84.

Musselman, L. J., Ed. *Parasitic Weeds in Agriculture. Vol. I. Striga*; CRC Press: Boca Raton, FL, 1987; 317 pp.

Narasaka, K.; Sakakura, T.; Uchimara, T.; Guédin-Vuong, D. Total synthesis of a macrocyclic pyrrolizidine alkaloid, (±)-integerrimine, utilizing an activable protecting group. *J. Am. Chem. Soc.* **1984**, *106*, 2954−2961.

Parker, C.; Riches, C. R. *Parasitic Weeds of the World: Biology and Control*; CAB International Press: Wallingford, Oxon, U.K., 1993; 332 pp.

Siame, B. A.; Weerasuriya, Y.; Wood, K.; Ejeta, G.; Butler, L. G. Isolation of strigol, a germination stimulant for *Striga asiatica*, from host plants. *J. Agric. Food Chem.* **1993**, *41*, 1486−1491.

Tanaka, K.; Tamura, N.; Kaji, A. A simple, stereocontrolled synthesis of α-alkylidene-γ-butyrolactones. *Chem. Lett.* **1980**, 595−598.

Trost, B. M.; Jungheim, L. N. 1-(Arylthio)cyclopropanecarboxaldehydes. Conjunctive reagents for secoalkylation. *J. Am. Chem. Soc.* **1980**, *102*, 7910−7925.

Zwanenburg, B.; Mangnus, E. M.; Thuring, J. W. J. F. Strigol analogues: design, synthesis and biological activity. In *Proceedings of the third international workshop on Orobanche and related Striga research*; Pieterse, A. H., Verkley, J. A. C., Ter Borg, S. J., Eds.; Royal Tropical Institute: Amsterdam, The Netherlands, 1994; pp 187−197.

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