Tentative Molecular Mechanism for Germination Stimulation of *Striga* and *Orobanche* Seeds by Strigol and Its Synthetic Analogues

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Analysis of the bioactivity of strigol analogues revealed that the CD part of these molecules is primarily responsible for germination stimulation of seeds of parasitic weeds. Preparation and bioassays of a "reduced" analogue of GR24, in which the enol ether carbon double bond has been replaced by a carbon single bond, showed that this double bond is essential for stimulatory activity. A molecular mechanism for germination stimulation is proposed which can account for the bioactivity of all strigol analogues. This mechanism involves addition of a nucleophilic species in a Michael fashion, followed by elimination of ring D. Reactions of GR24 with nucleophiles support the suggested mechanism, but replacement of ring D by other leaving groups resulted in loss of bioactivity. A structural model stimulant containing the essential functional groups and steric requirements of substituents is proposed.

INTRODUCTION

The process of germination in seeds of root-parasitic weeds differs from that in most other seeds as it requires an exogenous stimulant. Valuable information has accumulated concerning the conditions necessary for the germination process of *Striga* and *Orobanche* seeds (Worsham, 1987). The seeds need a postharvest ripening, a conditioning period under warm-moist conditions, and a minimum contact time with an exogenous germination stimulant. Furthermore, there is an optimum temperature for conditioning and stimulation. Light may influence the initial stages of the actual germination, and also pH and moisture effects have been reported. Conditioning of seed of parasitic weeds can be considered the initial stage of germination, which will progress to a stage where exogenously applied stimulant is required to start the subsequent series of events in the process (Visser, 1989). At present, however, the actual role of the germination stimulant is unknown, attributable mainly to the limited knowledge of the chemical nature of germination stimulants produced by host plants and their response reaction. Visser (1989) suggested that the germination stimulant could act as a promoter or activator of a particular reaction or could act at the level of gene expression by promoting the synthesis of some essential enzyme(s). In discussing the possible role of stimulants in the germination process one should realize that different compounds may differ in their mode of action. For example, ethylene (Eplee, 1975) and its precursor ethephon (Babiker and Hamdoun, 1983) may act in a manner different from that of strigol—a natural germination stimulant (Cook et al., 1966, 1972)—and its synthetic analogues (Johnson et al., 1976, 1981; Hassanali, 1984; Mangnus et al., 1992a-c; Mangnus and Zwanenburg, 1991, 1992; Pepperman et al., 1982; Vail et al., 1990). Recently, it was shown that strigol analogue GR24 (Figure 1) stimulates ethylene production of *Striga hermonthica* seeds and that its activity as germination stimulant was reversed by a competitive inhibitor of ethylene action (Jackson and Parker, 1991). Germination of *Striga forbesii* seeds was also stimulated by GR24; however, these seeds did not respond to exogenous ethylene or its competitive inhibitor.

In this paper the present knowledge on germination stimulation by strigol and its analogues is analyzed and used to develop a tentative molecular mechanism for germination stimulation and a structural model for germination stimulants. In addition, some experiments to support the proposed molecular mechanism will be described.

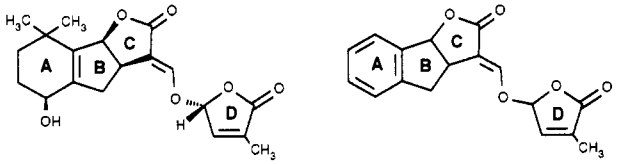

Figure 1. Structures of strigol and GR24.

MATERIALS AND METHODS

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. 7719). Thin-layer chromatograms (TLC) were run on plastic-supported silica gel 60 plates (0.2-mm layer, F_{254}, Merck art. 5735) or glass-supported silica gel 60 plates (0.25-mm layer, F_{254}, Merck art. 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. 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. An improved synthesis of strigol analogue GR24 has been described in a separate paper [Mangnus et al., 1992a; see also Johnson et al. (1981)].

5-(Benzoyloxy)-3-methyl-2(5H)-furanone (4). Triethylamine (1.4 mL, 0.01 mol) was added to a solution of benzoic acid (1.22 g, 0.01 mol) in acetone (20 mL) with stirring at room temperature. After 30 min, the yellow solution was cooled to 0 °C and 5-bromo-3-methyl-2(5*H*)-furanone (2.00 g, 0.011 mol; Mangnus et al., 1992a) was added. The dark brown mixture was heated at reflux for 30 min, and then water was added at room temperature. The mixture was extracted with dichloromethane (2×), and the combined organic layers were washed with water (1×),

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dried (Na_2SO_4), filtered, and concentrated. The crude product was obtained as a brown solid (2.11 g, 97%) and was already rather pure. The crude product was dissolved in hot methanol and, while still hot, treated with activated carbon. After filtration of the hot solution, cooling of the filtrate resulted in crystallization of product **4**: mp 104.5–105.5 °C; ^1H NMR ($CDCl_3$) δ 2.04 (m, CH_3), 7.02–7.23 (m, OCHO + =CH), 7.36–7.73 (m, 3 Ar H), 8.00–8.21 (m, 2 Ar H); IR (KBr) ν 1782 (C=O, benzoyl), 1731 (C=O, furanone), 1660 (C=C, furanone), 1598 (Ar) cm^{-1}; MS (EI^+) 218 (M^+), 105 (100%, $C_6H_5CO^+$), 97 (furanone$^+$), 77 (100%, $C_6H_5^+$). Anal. Calcd for $C_{12}H_{10}O_4$: C, 66.05; H, 4.62. Found: C, 65.90; H, 4.69.

3-(Hydroxymethyl)-3,3a,4,8b-tetrahydroindeno[1,2-b]furan-2-one (**8**). *n*-Butyllithium (1.6 M in hexane; 14 mL, 22 mmol) was quickly added to a solution of diisopropylamine (3.1 mL, 22 mmol) in tetrahydrofuran with stirring at −78 °C under nitrogen. Then a solution of lactone **7** (3.48 g, 20 mmol; Mangnus et al., 1992a) in tetrahydrofuran (40 mL) was gradually added with stirring at −78 °C. The temperature was raised to −20 °C, and the α-lithiated lactone was treated with formaldehyde vapors [paraformaldehyde (5 g) is heated to 150 °C to generate formaldehyde, and the vapors are carried by a nitrogen flow into the reaction mixture]. After all of the paraformaldehyde had been consumed, stirring was continued for 30 min. The reaction was quenched by the addition of 10% hydrochloric acid. Tetrahydrofuran was removed in vacuo, and the residue was dissolved in water and dichloromethane. The mixture was filtered to remove paraformaldehyde and the filtrate was extracted with dichloromethane (3×). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by flash chromatography [silica gel, hexane/ethyl acetate (1:1)]. This afforded unreacted lactone **7** (R_f = 0.33, 389 mg, 11%), α-hydroxymethyl lactone **8** (R_f = 0.16, 2.72 g, 67%), and α-methylene lactone **9** (R_f = 0.24, 163 mg, 4%).

α-Hydroxymethyl lactone **8**: mp 87–91 °C (white solid); ^1H NMR ($CDCl_3$) δ 2.30–3.47 (m, H_3 + H_{3a} + 2H_4), 3.63–4.11 (m, CH_2OH), 5.83 (d, J = 7.5 Hz, H_{8b}), 7.02–7.48 (m, 4 Ar H); IR ($CHCl_3$) ν 3700–3100 (OH), 1750 (C=O, lactone) cm^{-1}.

α-Methylene lactone **9**: mp 174–176 °C (white solid; recrystallized from diethyl ether); ^1H NMR ($CDCl_3$) δ 2.80–3.97 (m, H_{3a} + 2× H_4), 5.69 and 6.24 (2d's, J = 2.5 Hz, $=CH_2$), 7.00–7.53 (m, 4 Ar H); IR (CCl_4) ν 1773 (C=O, lactone) cm^{-1}; MS (EI^+) 186 (M^+), 142 ($M - CO_2$)$^+$, 141 [100%, ($M - CO_2H$)$^+$], 115 (C_9H_7)$^+$. Anal. Calcd for $C_{12}H_{10}O_2$: C, 77.40; H, 5.41. Found: C, 77.16; H, 5.43.

3-[[(2,5-Dihydro-3-methyl-2-oxo-5-furanyl)oxy]methyl]-3,3a,4,8b-tetrahydroindeno[1,2-b]furan-2-one (**10**). A solution of hydroxymethyl lactone **8** (204 mg, 1 mmol) in tetrahydrofuran (5 mL) was gradually added to a suspension of sodium hydride (30 mg, 1.2 mmol) in tetrahydrofuran (15 mL) with stirring at 0 °C under nitrogen. Stirring was continued for 30 min at 0 °C, and then the mixture was cooled to −60 °C. 5-Bromo-3-methyl-2(5*H*)-furanone (200 mg, 1.1 mmol) in tetrahydrofuran (5 mL) was gradually added, and then the mixture was allowed to warm slowly to room temperature. After the mixture was stirred for 16 h, tetrahydrofuran was removed in vacuo and the residue was dissolved in dichloromethane and water. The aqueous layer was acidified with diluted hydrogen chloride and extracted with dichloromethane (3×). The combined organic layers were washed with saturated sodium chloride, dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by flash chromatography [silica gel, hexane/ethyl acetate (1:1)] to give **10** (95 mg, 32%) as a mixture of diastereomers. Another fraction contained starting lactone (115 mg, 56%). The product crystallized from dichloromethane/diisopropyl ether: mp 140–143 °C (colorless crystals); ^1H NMR ($CDCl_3$) δ 1.96 (m, CH_3), 2.39–3.60 (m, H_3 + H_{3a} + 2H_4), 3.85–4.28 (m, CH_2O), 5.70–5.99 (m, H_{8b} + OCHO), 6.75–6.85 (m, =CH), 7.07–7.59 (m, 4 Ar H); IR ($CHCl_3$) ν 1770 (broad, C=O, lactone and 3-methylfuranone), 1605 (C=C, 3-methylfuranone) cm^{-1}; MS (CI^+) 301 ($M + 1$)$^+$ 187 (100%, [M-*O*-(3-methylfuranone)]$^+$, 97 (3-methylfuranone)$^+$. Anal. Calcd for $C_{17}H_{16}O_5$: C, 67.99; H, 5.37. Found: C, 68.00; H, 5.37.

3-[(Phenylthio)methylene]-3,3a,4,8b-tetrahydroindeno[1,2-b]furan-2-one (**11**). A solution of GR24 (0.150 g, 0.5 mmol) in ethanol (5 mL) was gradually added to a solution of sodium thiophenolate (0.5 mmol) in ethanol (5 mL) with stirring at room temperature under nitrogen. Stirring was continued for 16 h at room temperature. Then ethanol was removed in vacuo, and the residue was dissolved in diluted aqueous sodium chloride and ethyl acetate. The aqueous layer was extracted with ethyl acetate (2×). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by flash chromatography [silica gel, petroleum ether/ethyl acetate (5:1)] and afforded **11** as the main product (73 mg, 50%). An analytically pure sample was obtained by crystallization from diisopropyl ether: mp 121–122 °C (white solid); ^1H NMR ($CDCl_3$) δ 2.97–4.01 (m, 2× H_4 + H_{3a}), 5.93 (d, J = 8 Hz, H_{8b}), 6.70–7.53 (m, 9 Ar H), 7.64 (d, J = 2.5 Hz, =CHS); IR (KBr) ν 1728 (C=O, lactone), 1608 (C=C, thioenol ether) cm^{-1}; MS (EI^+) 294 (100%, M^+), 116 (C_9H_8)$^+$. Anal. Calcd for $C_{18}H_{14}O_2S$: C, 73.44; H, 4.79; S, 10.89. Found: C, 73.04; H, 4.80; S, 11.10.

3-[(Benzylthio)methylene]-3,3a,4,8b-tetrahydroindeno[1,2-b]furan-2-one (**12** *and* **14**). α-Toluenethiol (0.112 g, 0.9 mmol) was added to a suspension of sodium hydride (22 mg, 0.9 mmol) in anhydrous tetrahydrofuran (10 mL) with stirring at room temperature under nitrogen. Immediately hydrogen evolved and a white solid was formed. After the mixture had stirred at room temperature for 30 min, a solution of GR24 (0.268 g, 0.9 mmol) in tetrahydrofuran (5 mL) was quickly added. After 1 h of stirring, water and ethyl acetate were added; the aqueous layer was saturated with sodium chloride and extracted with ethyl acetate (2×). The combined organic layers were washed with saturated sodium chloride, dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by flash chromatography [silica gel, petroleum ether/ethyl acetate (3:1)] and gave **12** as the main product (188 mg, 61%). As a byproduct the cis isomer **14** was obtained (2 mg, <1%).

Trans isomer **12**: mp 108–110 °C (white powder); R_f [petroleum ether/ethyl acetate (1:1)] = 0.64. ^1H NMR ($CDCl_3$) δ 2.86–3.90 (m, H_{3a} + 2 × H_4), 4.00 (s, SCH_2Ph), 5.80 (d, J = 8 Hz, H_{8b}), 7.00–7.54 (m, 9× Ar H), 7.45 (m, =CHS); IR (KBr) ν 1730 (C=O, lactone), 1610 (C=C, enethiol ether) cm^{-1}; MS (EI^+) 308 (M^+), 217 (M − $PhCH_2$)$^+$, 91 [100%, ($PhCH_2$)$^+$]. Anal. Calcd for $C_{19}H_{16}O_2S$: C, 74.00; H, 5.23; S, 10.40. Found: C, 73.65; H, 5.22; S, 10.47.

Cis isomer **14**: R_f [petroleum ether/ethyl acetate (1:1)] = 0.55; ^1H NMR ($CDCl_3$) δ 2.70–3.95 (m, H_{3a} + 2 × H_4), 3.95 (s, SCH_2-Ph), 5.87 (d, J = 8 Hz, H_{8b}), 6.93 (m, =CHS), 7.00–7.55 (m, 9 × Ar H).

3-[[(p-Methoxybenzyl)amino]methylene]-3,3a,4,8b-tetrahydroindeno[1,2-b]furan-2-one (**13**). A solution of *p*-methoxybenzylamine (35 mg, 0.25 mmol) in methanol (1 mL) was gradually added to a solution of GR24 (75 mg, 0.25 mmol) in methanol (2 mL) with stirring at 0 °C. Stirring was continued for 16 h at room temperature. The reaction was monitored by TLC [petroleum ether/ethyl acetate (1:1)]. Not all GR24 had reacted, and extra *p*-methoxybenzylamine (7 mg, 0.2 equiv) was added. After an additional 2 h of stirring, all GR24 had reacted (TLC) and methanol was removed in vacuo. Ethyl acetate was added to the residue, and the insoluable precipitate was filtered off and washed with ethyl acetate. Attempts to recrystallize **13** (a light brown solid; 55 mg, 69%) failed: mp 152–155 °C; ^1H NMR ($CDCl_3$) δ 2.72–3.90 (m, H_{3a} + 2H_4), 3.71 (s, OCH_3), 4.25 (d, J = 5.5 Hz, NCH_2), 4.80–5.29 (m, NH), 5.77 (d, J = 8 Hz, H_{8b}), 6.70–7.53 (m, 8 Ar H + =CHN); IR (KBr) ν 3210, 3190 (NH), 1700 (C=O, lactone), 1650 (C=C, enamine), 1600 and 1510 (Ar) cm^{-1}; MS (EI^+) 321 (M^+), 121 [100%, (*p*-methoxybenzyl)$^+$]. Anal. Calcd for $C_{20}H_{19}NO_3$: C, 74.75; H, 5.96; N, 4.36. Found: C, 74.48; H, 6.11; N, 4.43.

3-[[(p-Tolylsulfonyl)oxy]methylene]-3,3a,4,8b-tetrahydroindeno[1,2-b]furan-2-one (**17**). A solution of *p*-toluenesulfonyl chloride (0.96 g, 5 mmol) in tetrahydrofuran (10 mL) was gradually added to a suspension of potassium salt **16** (1.20 g, 5 mmol; Mangnus et al., 1992a) in tetrahydrofuran with stirring at 0 °C under nitrogen. Stirring was continued for 3 h at room temperature. The reaction was monitored by TLC [petroleum ether/ethyl acetate (2:1)], and after 3 h, all *p*-toluenesulfonyl chloride had reacted. Tetrahydrofuran was removed in vacuo, and the residue was dissolved in dichloromethane and water. The aqueous layer was extracted with dichloromethane (2×). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The crude product was obtained as a white solid (1.64 g, 92%) and was

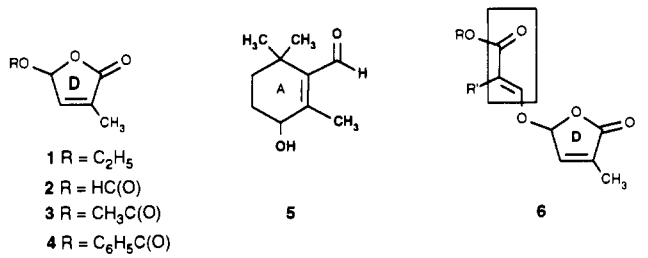


Figure 2. Structures of some strigol analogues.

already rather pure. Purification by flash chromatography [silica gel, petroleum ether/ethyl acetate (1:2), followed by pure ethyl acetate] was troublesome and afforded only a small amount of pure **17** (0.53 g, 30%). (Due to poor solubility in ethyl acetate, the product sticks at the top of the column.) An analytically pure sample was obtained by recrystallization from a large volume of ethyl acetate: mp 166–174 °C (white solid); ^{1}H NMR ($CDCl_3$) δ 2.43 (s, CH_3), 2.63–3.63 (m, 2 H_4), 3.67–4.07 (m, H_{3a}), 5.89 (d, J = 7.5 Hz, H_{8b}), 7.00–7.53 (m, 6 Ar H), 7.60 (d, J = 2.5 Hz, =CHO), 7.82 (d, J = 8 Hz, 2 Ar H); IR (KBr) ν 1750 (C=O, lactone), 1670 (C=C, enol ether), 1590 (Ar), 1390 (SO_2O) cm^{-1}; MS (EI$^+$) 356 (M^+), 185 [100%, $(M-TosO)^+$], 184 $(M-TosOH)^+$, 155 $(Tos)^+$, 91 $(PhCH_2)^+$. Anal. Calcd for $C_{19}H_{16}O_5S$: C, 64.03; H, 4.53. Found: C, 64.07; H, 4.56.

3-[[(5,5-Dimethyl-3-oxo-cyclohexen-1-yl)oxy]methylene]-3,3a,4,8b-tetrahydroindeno[1,2-b]furan-2-one (**18**). For the preparation of **18** from potassium salt **16** (Mangnus et al., 1992a) and 3-chloro-5,5-dimethylcyclohex-2-en-1-one (Chorvat et al., 1978) the procedure described for **17** was followed; however, the solvent, tetrahydrofuran, was replaced by dimethylformamide. The crude product was purified by flash chromatography [silica gel, petroleum ether/ethyl acetate (2:1)] to give **18** as a white solid (yield 30%). An analytically pure sample was obtained by recrystallization from ethyl acetate/petroleum ether: mp 125–127 °C; ^{1}H NMR ($CDCl_3$) δ 1.13 (s, 2 CH_3), 2.23 (s, CH_2C=O), 2.44 [s, $CH_2C(O)$=C], 2.90–3.75 (m, 2 H_4), 3.82–4.22 (m, H_{3a}), 5.60 (s, =CHC=O), 5.95 (d, J = 7.5 Hz, H_{8b}), 7.06–7.66 (m, 4 Ar H + =CHO); IR (KBr) ν 1740 (C=O, lactone); 1680 (C=O, dimedon), 1660 (C=C, dimedon); 1630 (C=C, enol ether) cm^{-1}; MS (EI$^+$) 324 (M^+), 185 $(M-C_8H_{11}O_2)^+$, 67 [100%, $C_4H_3O)^+$]. Anal. Calcd for $C_{20}H_{20}O_4$: C, 74.06; H, 6.21. Found: C, 74.51; H, 6.36.

Biological Activity. Aqueous solutions with the strigol analogues in concentrations varying between 10 and 0.01 mg/L, containing 1–0.001% acetone (v/v), respectively, as cosolvent, were prepared. These "stimulant solutions" were evaluated for stimulatory activity using seeds of *S. hermonthica* (Del.) Benth. (harvested in Sudan in 1987) and *Orobanche crenata* Forsk. (harvested in Egypt in 1988) in essentially the same bioassay as described by Parker et al. (1977). For a detailed description of the bioassay see Mangnus et al. (1992b).

RESULTS AND DISCUSSION

Much information about the mode of action of germination stimulants is based on observations with strigol and its analogues (Figure 2). An analysis of the bioactivity of so-called GR compounds (Johnson et al., 1976, 1981; Hassanali, 1984) reveals that the actiphore of strigol and its analogues, i.e., the part of the stimulant molecules which is primarily responsible for the bioactivity, resides in the CD part of the molecules. In contrast with these observations, other authors (Pepperman et al., 1982; Vail et al., 1990) reported a high activity for some A-ring analogues and for 5-ethoxy-2(5*H*)-furanone (**1**). However, these results were not reproducible, and 1 year later Pepperman et al. (1982) no longer observed activity with freshly prepared **1**. We prepared some alkoxycarbonyl furanones, viz. **2**–**4** (Laboratorios Bago S.A., 1981). However, 5-(formyloxy)- (**2**) and 5-acetoxy-2(5*H*)-furanone (**3**) were too unstable to assay the stimulatory activity. 5-(Benzoyloxy)-2(5*H*)-furanone (**4**) was stable but was completely inactive

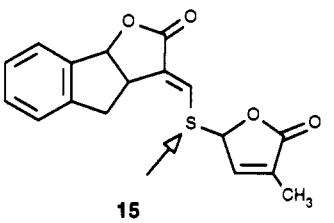
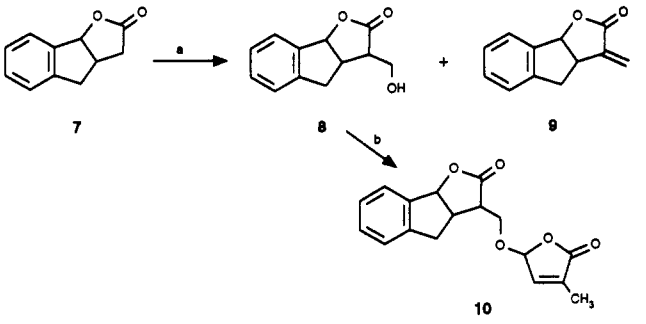


Figure 3. Structure of a bioisostere of GR24.

Scheme I. Synthesis of Reduced GR24



[a] 1. Lithium diisopropylamine; formaldehyde. 2. H^+. [b] Sodium hydride; 5-bromo-3-methyl-2(5H)-furanone.

as a germination stimulant for *S. hermonthica* and *O. crenata* seeds. This observation, in combination with those mentioned above, leads to the conclusion that the D ring alone is incapable of inducing germination.

Recently, the most active A-ring analogue, **5**, described by Pepperman et al. (1982), has been reinvestigated (Vail et al., 1990; Mangnus and Zwanenburg, 1991) and found to be completely inactive. This means the earlier conclusion, that the actiphore of strigol and its analogues resides in the CD part of the molecule, still holds.

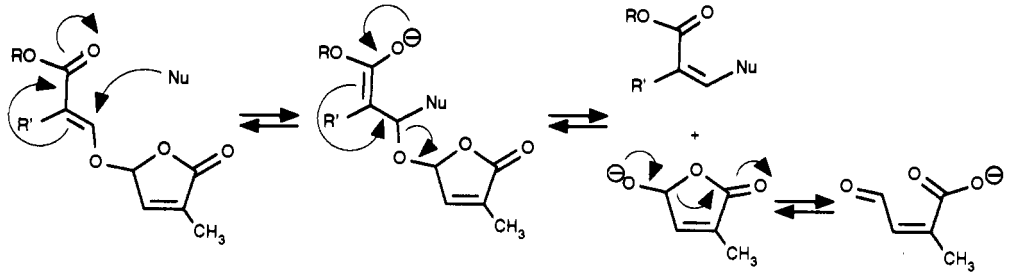
From results obtained with strigol analogues without ring C, viz. **6** (Mangnus et al., 1992c), it is evident that ring C is also not essential. Apparently, a structure consisting of a 3-methylfuranone connected to a carboxylic ester group via an enol ether linkage (cf. structure **6**) is sufficient for germination stimulant activity. So far, only compounds **6** with R = CH_3 and R′ = $(CH_2)_n$Ph (n = 0, 1, or 2) were investigated. Preliminary experiments with corresponding carboxylic acids (**6**, R = H) show that these are not active. It should be noted that α,β-unsaturated esters without any substituent at the α position (**6**, R′ = H) are still active (unpublished results).

To establish the importance of the α,β-unsaturated ester moiety (see box in Figure 2) for bioactivity, a compound was prepared in which the enol ether carbon double bond has been replaced by a carbon single bond. This "reduced" analogue of GR24, viz. **10**, was prepared from lactone **7** (Mangnus et al., 1992a) as depicted in Scheme I. Hydroxymethylation of lactone **7** was accomplished with base and formaldehyde vapors (Grieco, 1975) which also gave a small amount of α-methylene lactone **9** as an interesting byproduct. Hydroxymethyl lactone **8** was then coupled to 5-bromo-3-methyl-2(5*H*)-furanone (Mangnus et al., 1992a) using sodium hydride as the base. The desired analogue **10** was obtained as a mixture of several diastereomers in modest yield (32%).

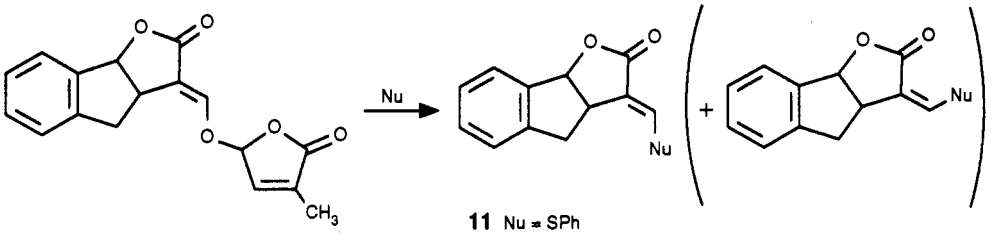
Evaluation of the biological activity of compound **10** using seeds of *S. hermonthica* and *O. crenata* revealed that this reduced analogue of GR24 does not show any germination activity. Apparently, the carbon double bond present in the C–D connecting enol ether unit is essential for the stimulatory activity.

A molecular mechanism that could account for the importance of this enol ether carbon double bond involves

Scheme II. Tentative Molecular Mechanism for Germination Stimulation by Strigol and Its Analogues



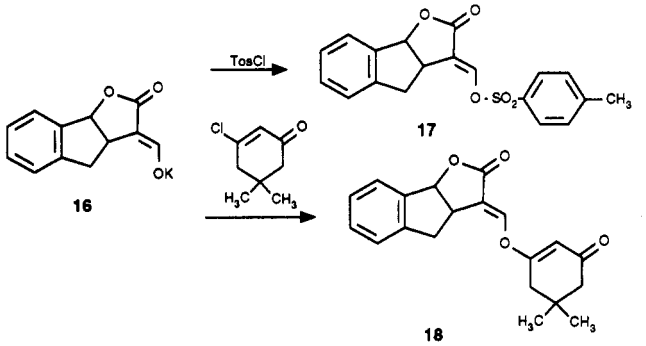
Scheme III. Reaction of GR24 with Nucleophiles



GR24

11 Nu = SPh
12 Nu = SCH_2Ph
13 Nu = $NHCH_2Ph(p\text{-}OMe)$
14 Nu = SCH_2Ph

Scheme IV. Synthesis of GR24 Analogues with Good Leaving Groups

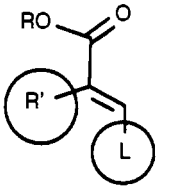


16 **17** **18**

addition of a nucleophilic species, present at the receptor site, in a Michael fashion, which is followed by subsequent elimination of ring D, as is illustrated in Scheme II. In this tentative molecular mechanism ring D serves in fact as a leaving group in the addition–elimination process. The ultimate result is that the ABC part of the stimulant is covalently bonded to the receptor, a chemical change that may be responsible for triggering germination. This mechanism explains the bioactivity of all strigol analogues, including the one lacking ring C, i.e., compound **6**. Supportive evidence for the proposed molecular mechanism in Scheme II was obtained from experiments of GR24 with nucleophiles (Scheme III). Reactions of GR24 with benzenethiolate, α-toluenethiolate, and *p*-methoxybenzylamine led to compounds **11**, **12**, and **13**, respectively. In these products the nucleophile has in fact replaced ring D in GR24.

With the mechanism depicted in Scheme II the bioactivity of bioisostere **15** (Figure 3), in which the enol ether oxygen in GR24 is replaced by sulfur (Mhehe, 1987; Mangnus and Zwanenburg, 1991), can be rationalized, because this replacement does not affect the essence of the mechanism.

The inherent instability of strigol and its analogues under alkaline conditions can also be understood by the mechanism shown in Scheme II. Now hydroxide ions serve as the nucleophile, and elimination of ring D will occur. This degradative reaction will deactivate the stimulant. Under acidic conditions this breakdown is less severe (Babiker et al., 1988).



I

Figure 4. Structural model stimulant.

Compound **9**, which was obtained as a byproduct during the preparation of compound **10**, has been assayed for stimulatory activity and was found to be inactive. This may be an indication of the importance of ring D. Attempts were made to replace ring D by another good leaving group (Scheme IV). An obvious choice was the tosylate function. Analogue **17** was readily prepared from potassium salt **16** (Mangnus et al., 1992a) and tosyl chloride. The initial test with compound **17** using seeds of *S. hermonthica* was very encouraging, and a moderate germination percentage was obtained. However, this result could not be reproduced. Another analogue of GR24 with a good leaving group as substitute for ring D (**18**) has a carbonyl function in a comparable position as in the D ring of GR24. However, **18** was also completely inactive as a germination stimulant. We also evaluated the products of the reactions of GR24 with nucleophiles, but compounds **11**–**13** were all inactive. It may be concluded that a simple replacement of ring D by a good leaving group does not meet all requirements for the role of this structural unit in strigol and its analogues. It is reported that changes in this ring, e.g., placing the methyl group at the adjacent C atom, have a considerable effect on the bioactivity (Hassanali, 1984). Apparently, ring D needs to fit precisely at the receptor site.

From the results presented in this paper it may be concluded that (i) a potential germination stimulant should be able to undergo an addition–elimination reaction with a nucleophilic group or function at the receptor site, as depicted in Scheme II, and (ii) a structural model stimulant I (Figure 4) must fulfil spatial requirements with regard to a steric function of the group R′ and must contain a good leaving group L, which also must meet spatial requirements. The latter requirement needs further elaboration, but it is already evident that besides the basic

chemical reactivity, there will be strict steric constraints for L. Design of new germination stimulants on the basis of the tentative mechanism and the structural model stimulant I is now under active investigation.

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