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Terpenoid Precursors of Strigol as Seed Germination Stimulants of Broomrape (*Orobanche ramosa*) and Witchweed (*Striga asiatica*)

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Abstract. Strigol and some of its synthetic precursors and analogs are known to be germination stimulants for broomrape (*Orobanche ramosa*) and witchweed (*Striga asiatica*). Fifteen synthetic terpenoids, similar in structure to one of the four rings of the strigol molecule, were evaluated in two bioassays as seed germination stimulants with broomrape, and nine were found to be active. Five of the more active compounds contained ester groups. Whereas the study was intended primarily to evaluate forced germination of broomrape by aqueous solutions, the results are almost qualitatively identical for broomrape and witchweed. Monocyclic compounds with chemical structures similar to two of the rings of strigol have now been shown to possess significant bioactivity as germination stimulants.

Broomrape (*Orobanche ramosa*) and witchweed (*Striga asiatica*) are extremely destructive parasitic weeds, and heavy infestations can reduce or even eliminate some crop yields (Lolas 1986, Musselman 1980, Musselman 1987, Parker 1986, Shaw et al. 1962). Seeds of these parasitic weeds generally remain dormant in the soil until germination is stimulated by the release of a chemical signal from a host plant (Brown 1965, Riopel 1983, Shaw et al. 1962, Whitney 1978). Unfortunately, the chemical signal is often produced by the root of a developing crop plant. As a result the parasite germinates and generally is in an excellent position to attach itself to the host from which the signal originated. Broomrape is a root parasite of some broadleaf crops in temperate and semiarid regions, whereas witchweed primarily attacks cereal crops and is of greatest concern in the semiarid tropics. Control methods for these weeds include cultural procedures, hand-weeding, use of resistant or trap crops (involves use of plants whose exudates cause germination of the parasite but are not parasitized), and conventional biological and chemical approaches. Under many circumstances a more effective method to eradicate or reduce these weeds is to apply synthetic stimulants to the soil that force these weed seeds to germinate. This process is usually termed "suicidal germination," because with no host present the parasite dies before producing seed or the emerging weeds are destroyed with herbicides.

Despite much effort, little is known about the chemical nature of the stimulants produced by host plants. However, strigol (Fig. 1), a natural product, isolated from the root exudates of cotton (*Gossypium hirsutum*), a nonhost, has proven to be particularly effective in stimulating the germination of witchweed seed (Cook et al. 1972). Through the synthesis and evaluation of numerous analogs and precursors of strigol, many chemicals effective in stimulation of seeds of witchweed and broomrape have been discovered (Hassanali 1984, Johnson and Rosebery 1977, Johnson et al. 1976, Johnson et al. 1981, Pepperman et al. 1982, Saghir 1979, Vail et al. 1985, Zwanenburg 1986).

There are several reviews on chemical germination stimulants for parasitic angiosperms (Musselman 1980, Worsham 1987). Recently, the first germination stimulant for witchweed from the root exudate of a natural host, sorghum (*Sorghum bicolor*), was identified (Chang et al. 1986). This compound, the dihydroquinone form of sorgoleone, is structur-

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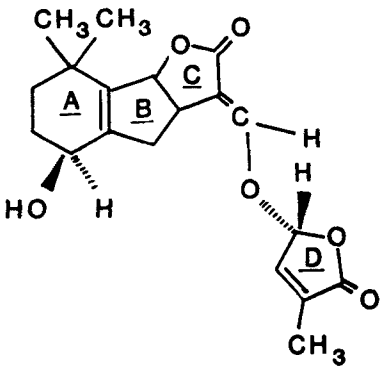


Fig. 1. Absolute configuration of (±)-strigol. Rings marked for identification purposes.

ally related to four minor components (three active), that have now been identified in droplets exuded from root hairs of sorghum. These compounds are hydrophobic and show limited but significant soil mobility (Netzly et al. 1988).

The total synthesis of strigol offers the synthetic chemist many opportunities to devise and prepare terpene-like compounds in the construction of the A and A–B rings of strigol. Four of these terpenoids were found to be active in germination of witchweed seed (Pepperman et al. 1982). In order to develop improved weed control procedures based on the concept of suicidal germination, this study was extended to include a comparison of the effects of selected synthetic terpenoids on both broomrape and witchweed seed. Advantage has been taken to include terpenoids of varying structures.

Materials and Methods

General Procedures for Surface Sterilization and Bioassay

Broomrape and witchweed seeds were surface sterilized in undiluted Sporicidin (Sporicidin Co., Washington, DC, USA) in a small vial with a sonicator probe inserted in the solution to enhance penetration of the disinfectant solution. After 3 min, seeds were rinsed with water three times to remove the Sporicidin. Seeds were conditioned in water in 125-ml flasks and stored in the dark for 2–3 weeks at 22°C for broomrape and 27°C for witchweed. Broomrape seeds were usually conditioned for a longer period, in order for the positive controls to produce a sufficient level of germination. Positive controls were GR-24 (100 ppm, see Fig. 2) for broomrape and strigol (10 ppm) for witchweed. Water was used as a negative control.

For each bioassay, approximately 50 seeds were placed in a well of a Falcon multiwell plate (Becton Dickson Co., Lincoln Park, NJ, USA) with the test solution. Tests were carried out in the dark at the same temperatures that the seeds were conditioned and were replicated a minimum of three times. The broomrape assays required 7 days, and the witchweed assays took only

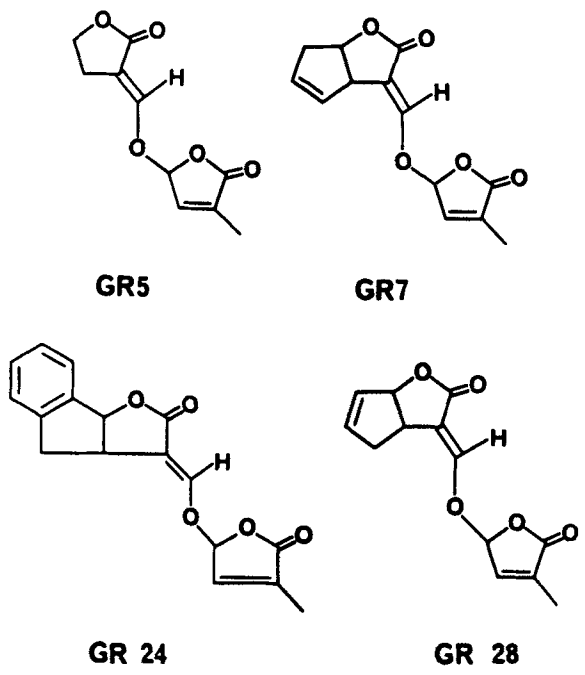


Fig. 2. Structures of some of the more promising GR compounds.

24 h for the seed to respond to a predetermined concentration of GR-24 or strigol, respectively. Germination was considered to occur when the radicle protruded through the seed coat.

Germination Stimulants

The terpenoids used in this study were synthesized and purified by previously published procedures from several strigol synthetic schemes (Brooks et al. 1985, Dailey 1987, Dailey and Vail 1985, Heather et al. 1976, Pepperman and Blanchard 1985). In bioassay 1, a saturated solution of all the compounds was prepared in water only, and this stock solution was diluted several times with water. In our earlier trials with broomrape more concentrated solutions of the terpenoids were not effective as germination stimulants. However, the possibility also exists that little or none of some of the terpenoids dissolved and was present in these solutions.

In bioassay 2, the compounds were first dissolved in dimethylsulfoxide (DMSO) and diluted with water to make the stock solutions. These stock solutions for compounds IV, V, VI, VIII, and XIV contained about 3% of the terpenoid and 33% DMSO. The stock solution for compounds XI and XV contained about 1% of the terpenoid and 50% DMSO. Three 10-fold dilutions with water were made with these solutions for the assays.

Results and Discussion

The *Orobanche* and *Striga* genera contain many species, and prior studies on chemical stimulation of seed germination are generally scattered between the more economically important of these parasites.

Table 1. Terpenoids active as seed germination stimulants for broomrape and witchweed.[a]

Sample[b]	% broomrape germinated—bioassay 1 dilutions			% witchweed germinated—bioassay 1 dilutions		
	1×10^{-4}	1×10^{-6}	1×10^{-8}	1×10^{-4}	1×10^{-6}	1×10^{-8}
II	40	7	0	54	33	7
IV	19	6	0	42	35	7
IX	3	0	0	13	0	0
X	44	0	0	20	0	0
XII	27	0	0	33	0	0
XIV	34	0	0	0	0	0

[a] See Materials and Methods for details on bioassay 1. Nine other compounds tested were inactive under these conditions. Values are the means of assays that were replicated a minimum of three times. Controls were as follows: (1) water only—no seed germinated for both species; (2) strigol at 10 ppm germinated 50% of the witchweed seed; and (3) GR-24 at 100 ppm germinated 86% of the broomrape seed.
[b] See Tables 3 and 4 for structures of the 15 terpenoids evaluated.

The effects of both natural and synthetic stimulants on the seeds of these genera have been the subject of several recent reviews (Riopel 1983, Vail et al. 1985, Worsham 1987). There are known compounds that produce similar results with regard to promotion or inhibition of germination; however, except for studies with strigol and its analogs, there is only limited consistency of results within the species of these genera or on comparison of one genus with the other. These factors have been noted previously and it has been suggested "that strigol may be a representative of a new class of plant hormones" and that other biological effects of strigol should be studied (Cook et al. 1972).

It was known that the compounds to be tested as germination stimulants were hydrophobic, and solutions of known concentrations would be difficult to obtain without the use of a carrier. The purpose of the carrier is to obtain solution of the compound prior to dilution with water. Carriers were used in closely related work, but the carrier often caused significantly higher germination even after the samples were well diluted with water (Pepperman et al. 1982). We considered it more useful to test all compounds in the absence of a carrier (results reported in Table 1) and to perform limited studies in the presence of a carrier (see Table 2). Compounds in Table 2 are primarily those found to be inactive in water only and were found to be active at only relatively high concentrations. Structures of the terpenoids and a summary of the results from Tables 1 and 2 are provided in Tables 3 and 4.

Table 2. Selected terpenoids active as seed germination stimulants for broomrape in the presence of DMSO.[a]

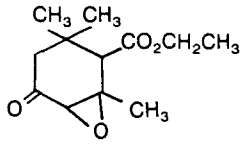
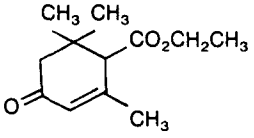
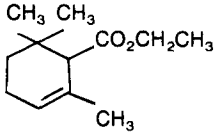
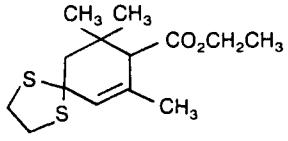
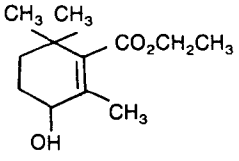
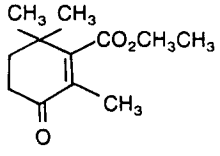
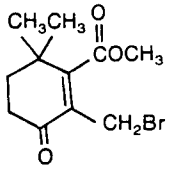
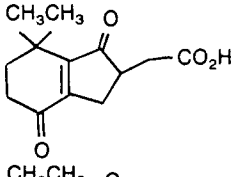
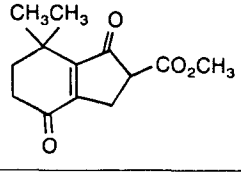
Sample[b]	% broomrape germinated—bioassay 2 dilutions			
	Initial concentration (%)	1×10^{-1}	1×10^{-2}	1×10^{-3}
V	3	81	71	0
VI	3	20	58	33
XV	1	73	29	0

[a] See Materials and Methods for details on bioassay 2. Compounds IV, VIII, XI, and XIV were found to be inactive in this bioassay. Values are the means of assays that were replicated a minimum of three times. Under these conditions none of these compounds were active as germination stimulants for witchweed and further dilutions to 1×10^{-12} failed to stimulate the germination of these seeds. Controls were as follows: (1) water only—no seed germinated for both species; (2) strigol at 10 ppm germinated 95% of the witchweed seed; and (3) GR-24 at 100 ppm germinated 70% of the broomrape seed.
[b] See Tables 3 and 4 for structures of the 7 terpenoids evaluated.

Strigol, Strigol Analogs, and Precursors, and Their Use as Seed Germination Stimulants

Many strigol analogs were synthesized as soon as the structure of strigol was established (Fig. 1). The analogs are called GR compounds (Fig. 2) and many were evaluated as germination stimulants for broomrape and witchweed (Heather et al. 1976, Johnson and Rosebery 1977, Johnson et al. 1976, Pepperman et al. 1982, Saghir 1979). Several of these analogs were found to be active in germination of both broomrape and witchweed. GR-5, -7, -18, -24, and -28 are the two-ring, three-ring, and four-ring analogs that have received the most attention. GR-18 is an isomer of GR-24 with the aromatic ring (and double bond) located one carbon further away from the lactone C-ring. The structural similarities of the GR compounds and strigol that merit comment include the butenolide ring structure (D-ring of strigol) joined to another cyclic lactone (C-ring of strigol) by a methyleneoxy bridge. However,

Table 3. Summary of active compounds.

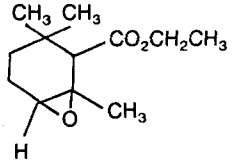
Sample and compound name	Structure	Activity	
		Broomrape	Witchweed
II. Ethyl 2,3-epoxy-4-oxo-2,6,6-trimethylcyclohexane-1-carboxylate		Yes	Yes
IV. Ethyl 4-oxo-2,6,6-trimethylcyclohex-2-ene-1-carboxylate		Yes	Yes
V. Ethyl 2,6,6-trimethylcyclohex-2-ene-1-carboxylate		Yes	No
VI. Ethyl 7,9.9-trimethyl-1,4-dithiospiro[4.5] dec-6-ene-8-carboxylate		Yes	No
IX. Ethyl 3-hydroxy-2,6,6-trimethylcyclohex-1-ene-1-carboxylate		Yes	Yes
X. Ethyl 2,6,6-trimethyl-3-oxocyclohex-1-ene-1-carboxylate		Yes	Yes
XII. Methyl 2-(bromomethyl)-6,6-dimethyl-3-oxo-cyclohex-1-ene-1-carboxylate		Yes	Yes
XIV. 1,4-Dioxo-7,7-dimethyl-4,5,6,7-tetrahydro-2-indanacetic acid		Yes	No
XV. Methyl 1,4-dioxo-7,7-dimethyl-4,5,6,7-tetrahydroindan-2-carboxylate		Yes	No

with the exception of our prior work (Pepperman et al. 1982, Vail et al. 1985), the contributions of the structural features of the aliphatic terpenoid A-ring of strigol has received little or no attention.

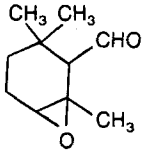
The strigol synthesis requires 12 to 20 steps depending on the synthetic procedures used for the synthesis. Synthesis of the analogs generally requires fewer steps, but the butenolide precursor for

Table 4. Summary of inactive compounds.

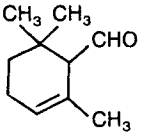
I. Ethyl 2,3-epoxy-2,6,6-trimethylcyclohexane-1-carboxylate



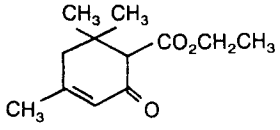
III. 2,3-Epoxy-2,6,6-trimethylcyclohexane-1-carboxaldehyde



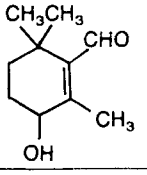
VII. α-Cyclocitral or 2,6,6-trimethylcyclohex-2-ene-1-carboxaldehyde



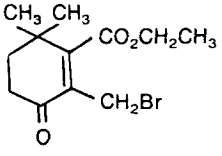
VIII. Ethyl 2-oxo-4,6,6-trimethylcyclohex-3-ene-1-carboxylate



XI. 3-Hydroxy-2,6,6-trimethylcyclohex-1-ene-1-carboxaldehyde



XIII. Ethyl 2-(bromomethyl)-6,6-dimethyl-3-oxocyclohex-1-ene-1-carboxylate



the D-ring of strigol or its analogs requires six steps by itself. There is no question that strigol and its analogs are highly effective germination stimulants for broomrape and witchweed, but the syntheses of these compounds are difficult and expensive and require many different types of chemical plant equipment. In order to use chemical stimulation of seed germination as a practical means of weed control, a more economical and readily available chemical system is needed.

The results from these studies demonstrate that nine of 15 terpenoids from various strigol synthetic schemes are active in the stimulation of broomrape. These nine compounds vary slightly in structure from the four terpenoids previously found to be effective in the germination of witchweed (Pepperman et al. 1982). Most of these active compounds from both sides share the trimethylcyclohexene structure (or are very similar).

The more active terpenoids in the previous work with witchweed (Pepperman et al. 1982) were aldehydes with epoxy (III) or hydroxy (XI) groups, and both compounds were more effective with DMSO as a carrier. In addition, compound XI was found to be unstable and oxidized to the corresponding acid during storage. In many of the biologically active terpenoids in the present work, the chemically active aldehyde group is replaced with the chemically stable ester group. Five of the terpenoids containing ester groups (compounds II, V, VI, X, and XV) caused germination of 40% or more of the broomrape seed.

The similarity of the qualitative results reported in Table 1 suggest that broomrape and witchweed seed react similarly to the terpenoids in the test solutions. These test solutions were prepared from saturated aqueous solutions with no carrier present. When DMSO was used as carrier to dissolve the terpenoids, the similarity of results was not obtained. Whereas samples V, VI, and XV in DMSO (see Table 2) produce fairly high levels of germination for broomrape seed, none of the seven terpenoids in DMSO were effective in the stimulation of witchweed. This is somewhat surprising, but the use of a carrier, such as DMSO in studies of this type, introduces a variable which is not presently understood.

The very high biological activity of strigol and some of its analogs suggests that these relatively large and complex molecules are uniquely structured to initiate the biochemical reaction involved in stimulating the germination of broomrape and witchweed seed. The mechanism of this reaction is not known, but it is noteworthy that a simple molecule, such as ethylene, accomplishes the same feat with witchweed seed (Egley and Dale 1970), but not with broomrape (Johnson et al. 1981). Some of the butenolides or D-ring precursors of strigol have been found to be active in the germination of both broomrape and witchweed (Johnson and Rosebery 1977, Johnson et al. 1976). However, these butenolides are expected to be susceptible to alkaline hydrolysis or degradation during storage (Hassanali 1984, Johnson et al. 1981, Pepperman et al. 1982), and variable results have been reported in assays with witchweed (Pepperman et al. 1982). Therefore, numerous compounds with chemical structures similar to both the A- and D-ring of strigol have now been shown to possess significant bioactivity with regards to the stimulated or forced germination of witchweed and broomrape seed.

It may be more than coincidence that the synthetic compounds reported in this article as promoting germination all have limited water solubility. Indeed, the vast majority of effective synthetic stimulants previously reported also have limited water solubility. These findings parallel the recent isolation of the first natural germination stimulants of witchweed found in sorghum exudate (Chang et al. 1986, Netzly et al. 1988), also hydrophobic compounds. In the case of sorghum, the germination stimulants are substituted benzohydroquinones that are easily oxidized to the inactive quinones. The chemical instability of the active hydroquinone and its low water solubility limits the effective area of

activity—a condition that is ideal for witchweed germination and propagation (Netzly et al. 1988). These substituted hydroquinones are structurally different from the terpenoids, and it is not clear whether similar promotion pathways for germination are involved. However, the germination stimulation by hydrophobic compounds serves to emphasize their potential biological importance as a strategy of host/parasite communication wherein seeds germinate in response to molecules with very limited soil mobility. Utilization of host signals of this type insures that a significant number of parasite seeds will germinate close to the host root surface, thus enhancing survival potential for these obligate parasites.

Seed Activation and Summary of Concerns Relating to Sample Preparation

Studies relating to the preparation of witchweed seed for bioassays, including the use of hypochlorite bleach, have been recently reviewed (Worsham 1987). Hypochlorite bleach can stimulate the spontaneous germination of witchweed seed. For this reason we avoided its use and found that an antibiotic, Sporicidin, is effective in surface sterilization of both broomrape and witchweed seed. The use of a chemical, such as sodium hypochlorite, that is expected to penetrate the seed coat during seed preparation introduces a variable that may produce unpredictable results whether that chemical is removed by further washing or not.

The primary objective of this work was to evaluate 15 terpenoids as seed germination stimulants for broomrape and witchweed. Satisfactory bioassays were selected for broomrape. The witchweed seed were evaluated with the same solutions and conditions. The terpenoids evaluated possess rather low solubilities in water and therefore saturated solutions were prepared and diluted for use in most of the bioassays. Whereas sample preparation can be readily reproduced, the absolute concentration of the germination stimulants is not known for the data in Table 1.

Acetone and DMSO were used as carriers in previous evaluations of terpenoids with witchweed, and a number of relevant points were discussed that are of importance in the present study. DMSO appeared to be the better carrier, but its use generally increased the germination of witchweed seed (Pepperman et al. 1982). Also, it is likely that minute droplets of the terpenoids are formed as the DMSO carrier solution is added to the large volume of water required in the bioassay. The effective concentrations of the terpenoid or other compounds as a stimulant are therefore in doubt whenever carriers are used with compounds of low water solubility. These compounds, though difficult to thoroughly evaluate in the laboratory, may be most effective in field applications, since their soil mobility is partially limited by their low solubility in water.

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