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AN HAUSTORIA INDUCER FOR THE ROOT PARASITE *AGALINIS PURPUREA*

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Key Word Index—*Agalinis purpurea*; Scrophulariaceae; *Lespedeza sericea*; Leguminosae; host recognition; soyasapogenol B.

Abstract—Host recognition by the angiosperm root parasite *Agalinis purpurea* occurs through the development of haustoria, organs specialized for the attachment and penetration of host roots. Axenic cultures of *Agalinis* do not develop haustoria, but low molecular weight compounds produced by host plants induce haustoria development. Structure–activity studies have shown that highest levels of haustoria induction are afforded by flavonoids bearing specifically substituted methoxyphenol functionality. This paper examines *Lespedeza sericea* to determine the extent of this structure–activity relationship among hosts of *Agalinis*. A pentacyclic triterpene has been isolated from *Lespedeza* roots as a haustoria inducer, and extensive NMR and mass spectral experiments led to its characterization as soyasapogenol B (3β,22β,24-trihydroxy-olean-12-ene). The activity of soyasapogenol B is lower than the phenolic inducers, and modification of hydroxyl substitutents on soyasapogenol B abolishes activity. This haustoria inducer appears to be preferentially secreted from the roots of *Lespedeza*, but not in quantities sufficient to account for all haustoria inducing activity exhibited by the crude root exudate.

INTRODUCTION

Angiosperm root parasites are a heterogeneous group of species distributed among eight families of flowering plants. Many, like *Agalinis purpurea*, are facultative parasites with no apparent host preference. Most of these parasites are capable of completing their life cycle in the absence of a host, although in nature they are invariably found attached to the roots of one or several hosts. Typically, these plants exert little physiological effect on their hosts. Others, like *Striga* spp. and *Orobanche* spp. are obligate parasites, and their physiological impact on crop species is often severe [1].

All known parasitic plants attach to and penetrate their host via a multicellular structure termed the haustorium. Grown axenically in agar, cultures of *Agalinis* develop very few, or no haustoria. However, in the presence of a host root, or host root exudate, the development of many haustoria is induced. The induction of haustoria development is therefore the first event of host recognition by *Agalinis* and the rapid, exogenously regulated development of haustoria provides an attractive system for developmental analysis. The cytological events of haustoria initiation, attachment, and development have been detailed by the studies of Baird and Riopel [2, 3].

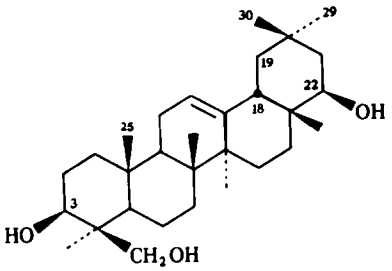
The biochemistry of haustoria induction has only recently been explored. Atsatt *et al.* [4] in *Orthocarpus purpurescens* (Scrophulariaceae), and Riopel and Musselman [5] in *Agalinis*, have shown that root exudates, extracts and a number of plant products are capable of inducing haustoria development. Because of its biochemical tractability, commercial availability and potent haustoria inducing activity, gum tragacanth has been used in the isolation and structural determination of the first known haustoria inducers [6, 7] called xenognosin A and B (**2** and **3**, respectively). In light of the structural similarity of these phenylpropanoid haustoria inducers, a number of xenognosin A analogs were synthesized to probe the structural specificity of haustoria induction [8]. The results showed that *m*-methoxyphenol functionality was a strict requirement for haustoria inducing activity in *Agalinis* [7].

However, gum tragacanth is derived from an exudate collected from the aerial portions of *Astragalus gummifer* (Leguminosae). The relevance of xenognosin A and B to host recognition by root parasites is uncertain, even though xenognosin A induces haustoria in other parasitic Scrophulariaceae: *Striga asiatica* [Riopel and Spivey, pers. comm.] and *Sopubia delphinofolia* [Sahai and Shivanna, pers. comm.]. We have examined *Lespedeza sericea*, a forage legume readily parasitized by *Agalinis*, to determine whether molecules analogous to xenognosin A are found in root exudate of this host plant, and whether these inducers are released into root exudate in concentrations sufficient to induce haustoria in *Agalinis*. This paper describes the isolation and structural determination of a pentacyclic triterpenoid haustoria inducer, **1**, from *Lespedeza* roots. Additional information is presented on the exudation of triterpenoid and flavonoid molecules from *Lespedeza* roots, and the ability of the triterpenoid inducer to account for haustoria induction by roots of *Lespedeza*.

RESULTS

Structural determination

^{1}H NMR of the purified haustoria inducer **1** from *Lespedeza* roots showed only five protons far enough



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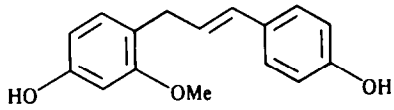

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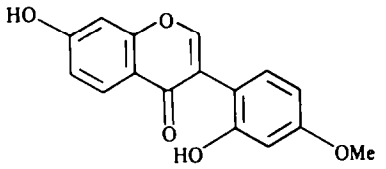

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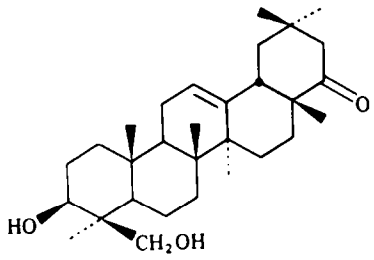

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downfield to be resolved at 360 MHz. Additionally, seven quaternary methyl singlets were apparent. The remaining signals appeared as an envelope of peaks encompassing the region of δ2.2–0.9. High resolution EIMS measured the molecular ion at 458.3757 (calc. 458.3760) giving a molecular formula $C_{30}H_{50}O_3$; calculation of rings plus double bonds [9] gave six degrees of unsaturation. A single olefinic proton (δ5.3, *t*, J = 2.5 Hz) and the seven quaternary methyls suggested a pentacyclic triterpenoid with one double bond. Intense EIMS fragment ions at *m/z* 224 and 234.1979 (calc. 234.1984, $C_{16}H_{26}O$) implicated a retro-Diels–Alder fragmentation of an olean-12-ene triterpene bearing two oxygens on the A-B ring fragment [10]. Three hydroxyl substituents were identified by deuterium exchange negative ion CIMS (ETOD, N_2O) by the appearance of a molecular ion at *m/z* 459 [$(M-H)^-$ with two exchanges]. Assignment of the 3β-hydroxyl followed from its ^{1}H NMR signal (δ3.45, *dd*, J = 5.4 Hz) and nearly ubiquitous presence in higher terpenes. Its proximity to the 24-hydroxyl was suggested by several lines of evidence. First, in positive ion CIMS (CH_4) an initial dehydration results in the apparent $[M+H]^+$ ion at *m/z* 441, which exhibits only one exchangeable hydroxyl by positive ion deuterium exchange [ETOD, *m/z* 443 $(M+D)^+$ +1 exchange]. Loss of the hydroxyl to generate the ion at *m/z* 441 apparently ties up the proximal hydroxyl in a form unexchangeable by CIMS. Secondly, the AB pattern of the H-24 protons showed the upfield doublet H-24 (δ3.5, *br d*, J = 11 Hz) to be considerably broadened by additional coupling. This small coupling was derived from a ^4J 'W type' coupling relationship between H-24 and H-5$_{ax}$, which was maintained by intramolecular hydrogen bonding between the 3β- and 24-hydroxyl substituents. Disruption of the hydrogen bonding with DMSO-d_6 and by acetylation eliminated the broadening of the upfield H-24 signal. Additionally, a 2.9% NOE of the 25-methyl was observed upon irradiation of the downfield (H-24', δ4.1, J = 11 Hz) signal of the H-24'AB pattern.

Placement of the third hydroxyl group was more difficult. ^{1}H NMR established its position as axial because the geminal proton appeared as a triplet (δ3.45, J = 4.5 Hz). Because H-18 appeared as a doublet of doublets, δ2.2, the 19 axial position was ruled out, leaving the 15, 16, 21 and 22 axial positions as possible sites for this substituent.

CADMS has previously been used to determine the structure of fragment ions generated by conventional ionization techniques [6, 11] by causing them to undergo further fragmentation by collision activated decomposition. Using CADMS, comparison of the triacetate of **1** with a 3-oxo-15-acetoxy-olean-12-ene analog ruled out the 15 axial position as a possible structure for the haustoria inducer. Both acetylated compounds give rise to intense *m/z* 276 ions arising from the retro-Diels–Alder fragmentation across the C ring. Subsequently, each loses acetic acid to give major fragment ions at *m/z* 216, generating a double bond at the site of oxidation. CAD fragmentation of this ion produces the daughter ion mass spectrum of the *m/z* 216 ion. Comparison of the daughter ion spectra obtained from the inducer **1** and the 15-acetoxy fragment isomer reveals that the spectra are identical until *m/z* 187; fragments at lower mass then begin varying by one or two mass units (Fig. 1). Therefore, the third hydroxyl group on the haustoria inducer **1** cannot reside at C-15. This was confirmed by observation of the retro-Diels–Alder fragment arising from the 22-ketoacetonide of **1**. Placement of a keto group close to the site of principal fragmentation, as at C-15, dramatically redirects the fragmentation pathway of this class of molecules [10]. An *m/z* 232 peak was obtained from the 22-ketoacetonide of **1**, thus restricting the position of oxidation to the 16, 21 or 22 axial sites.

To more specifically localize the third site of oxidation, a series of ^{1}H NMR NOE difference experiments were undertaken to assign the seven methyl resonances. In triterpenoids, methyl chemical shifts are highly sensitive to substituent effects, some of which have been tabulated and used in the structural elucidation of triterpenes [12]. Identification and correlation of methyl chemical shifts in the haustoria inducer would reveal which resonances are most affected by the presence of, and therefore the most proximal to, the site of oxidation. The NOE difference experiments were conducted with the 3β,24-phenylborate

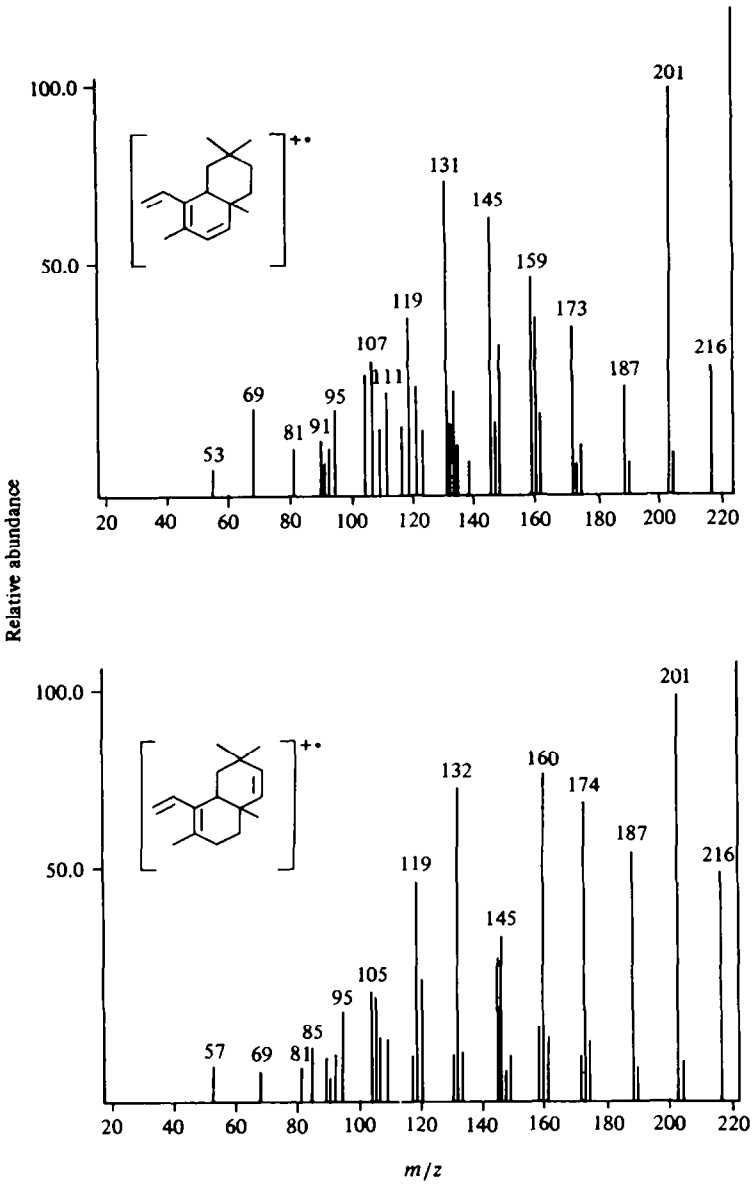

Fig. 1. Collisional decomposition spectra of soyaspogenol B triacetate (top) and 3-oxo-15-acetoxy olean-12-ene. The *m/z* 216 ions arising from electron impact ionization (70 eV, 200°) were subjected to collisionally activated decomposition (CAD) by collision with 1 μT argon to obtain the daughter ion spectra shown.

derivative of **1** to allow selective irradiation of signals which overlap in the underivatized compound. Key enhancements obtained in the NOE difference experiments and their assignments are listed in Table 1.

From the NOE difference spectra it was possible to identify Me-23, 25 and 28 unambiguously. Additionally, Me-27 and 30 were assigned from correlation data on the basis of their relatively invariant chemical shifts [12]. Me-29 and Me-26 were tentatively assigned to the signals at δ0.87 and 0.97, respectively. During the course of the work, it became clear that derivatization of the 1,3-diol system in **1** introduced small unexpected chemical shift changes in methyl signals distant from the A ring, and that therefore the predictive value of the correlation table was unreliable.

However, assignment of the methyl substituents permitted localization of the third hydroxyl to the E ring

Table 1. Nuclear Overhauser enhancement difference values for soyasapogenol B*

Signal irradiated		Signal enhanced		
assignment	δ	assignment	δ	% enhancement
H-18	2.2 (*dd*)	Me-28	1.05	1.3
H-24	3.17 (*br d*)	Me-23	1.02	0.6
H-24′	4.1 (*d*)	Me-25	0.93	1.9
Me-25	0.93 (*s*)	H-24′	4.1 (*d*)	4.9

* Pre-irradiation (selective 180°) of each signal was followed by a 90° observed pulse delayed by 0.7 sec. This spectrum (550–1000 transients) was acquired simultaneously with a spectrum in which one selective pulse was 3 ppm upfield of TMS, and the two spectra were computer subtracted to observe the enhancements.

through use of an NMR shift reagent. The shift reagent, $Eu(fod)_3$, induces concentration-dependent isotropic chemical shift changes in the vicinity of a complexation site such as an hydroxyl [13]. Tying up of the 3β,24-dihydroxy system in **1** with a borate ester effectively eliminated the 1,3-diol system as a site for complexation, as shown by the lack of chemical shift change for Me-23 and 25 (Fig. 2). The methyls exhibiting the largest chemical shift changes are those on the E ring: Me-28, 29 and 30; resonances assigned to Me-26 and 27 show only slight downfield shifts in response to added shift reagent. The relatively small chemical shift change experienced by Me-27 relative to the 29 and 30 methyls suggested that the third site of oxidation resides in the E ring (C-21 or C-22) rather than on the D ring at $C\text{-}16_{ax}$. Further resolution of the $Eu(fod)_3$ complexation site was more complicated. The axial hydroxyl would be α at C-22 and β at C-21. Ideally, then, if oxidation was at C-21 (β, axial), then Me-29 and 30 would be shifted downfield with C-30 shifted to the greatest extent. Conversely, in the case of oxidation at C-22 (α, axial), Me-28 and 30 would be expected to show the greatest change. The results shown in Fig. 2, however, show the resonances assigned as Me-28 and 29 experiencing the largest downfield shifts. The discrepancy in the results can probably be assigned to the angular dependence of shift reagent effects on chemical shift [14].

Two dimensional ^1H NMR correlation spectroscopy experiments (COSY) [15] provided direct evidence for the location of the third hydroxyl at C-22. The 22-keto-acetonide of **1** was generated to shift the protons α to the carbonyl downfield from the envelope of methylene resonances, allowing their assignment. The coupling of H-18 to H-19e and H-19a was also established. A 0.2 sec delay inserted into the COSY pulse sequence to favor observation of small couplings [16] resulted in observation of cross peaks arising from a four-bond coupling between H-19a and the higher field singlet of the E ring methyls. An analogous coupling also exists between Me-30 and one of the protons adjacent to the carbonyl, establishing its assignment as H-21a. The presence of a four-bond 'W-type' coupling between H-19e and the other methylene proton adjacent to the carbonyl, H-21e, establishes the site of oxidation at C-22 and so assigns the structure of the haustoria inducer as 3β,22β,24-trihydroxyolean-12-ene*. During the course of this work, Kitagawa *et al.* [19] revised the structure of the oleanene triterpene soyasapogenol B to this formula, on the basis of extensive chemical and X-ray crystallographic analyses. Our data, from a small-scale NMR and mass spectral approach is in good agreement with ref. [19] for soya-

*The work of Smith *et al.* [17] on soyasapogenol B supported a C-21 hydroxyl group whereas the solvolysis work of Cainelli *et al.* [18] supported a β-configuration but did not resolve the C-21 vs. C-22 assignment.

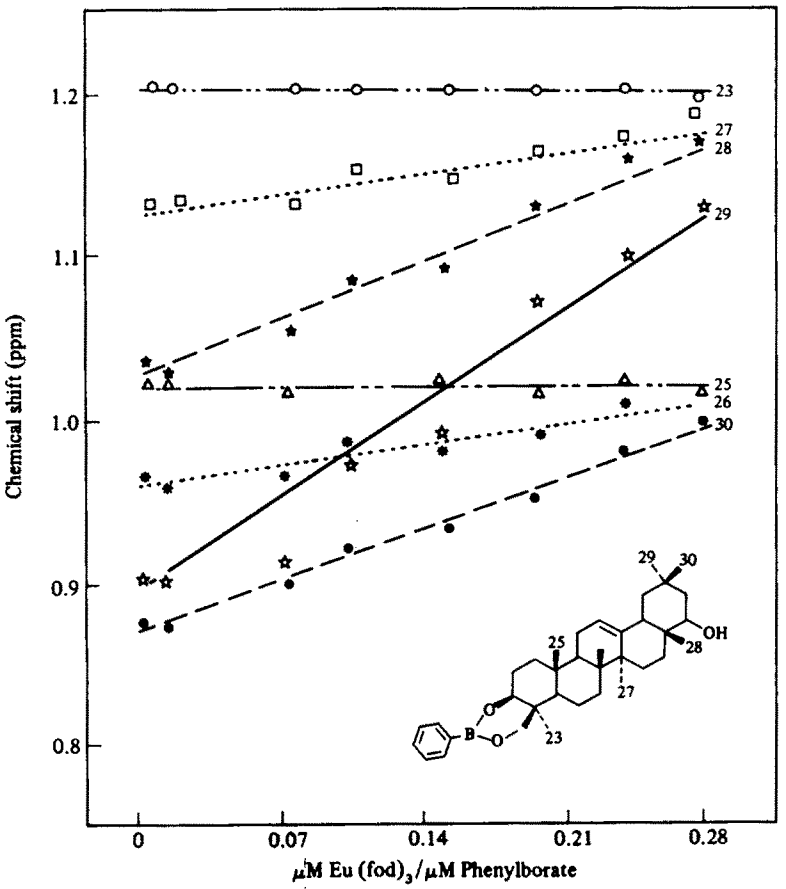

Fig. 2. Effect varying concentration of the NMR shift reagent $Eu(fod)_3$ on methyl resonances of soyasapogenol B 3β,24-phenyl borate. $Eu(fod)_3$ was dissolved in a minimal amount of acetone-d_6 and added to a 2 mg solution of soyasapogenol B phenyl borate in the same solvent. Spectra were obtained at 360 MHz.

sapogenol B and demonstrates the advantage of 2D-NMR to definitively assign overlapping or obscured resonances and to identify extremely small couplings.

Quantitation

The silica gel chromatogram of the total acetone-soluble root exudate components (Fig. 3) confirms the presence of the haustoria inducer, soyasapogenol B, in *Lespedeza sericea* root exudate. The identification of soyasapogenol B in root exudate based on its co-migration and similar charring behavior to purified material was confirmed by spiking an exudate sample with previously isolated soyasapogenol B for comparison by TLC and reversed phase HPLC. Intensification of the presumed band in TLC and co-elution in HPLC established the identity of this material.

Fig. 3. Thin-layer chromatogram of acetone-soluble components from *Lespedeza sericea* roots and root exudate. Lane 1: total acetone-soluble components from 15-day-old *Lespedeza* roots. Lane 2: purified soyasapogenol B. Lane 3: total acetone-soluble components from lyophilized *Lespedeza* root exudate. Lane 4: same as 3, spiked with soyasapogenol B. Arrow marks position of soyasapogenol E on chromatogram. TLC was 0.25 mm SiO_2 on aluminum backed plates, run in 5% MeOH in CH_2Cl_2 and developed with Ce_2SO_4 in 2 N H_2SO_4. Genistein, which reacts weakly with $CeSO_4$, appears at an R_f slightly below soyasapogenol B.

Soyasapogenol E, the 22-keto derivative of soyasapogenol B, appears as a minor component in the root extract, but in exudate appears to be present in levels equal to or exceeding soyasapogenol B. The major isoflavonoid of *Lespedeza sericea* roots, genistein 5,7,4′-trihydroxyisoflavone) is present in large amounts in the root, but is barely detectable by TLC in root exudate. Because the comparisons of TLC band intensity obtained with this visualization method are not reliable for quantitative comparisons, HPLC was used to quantitate the concentrations of three components in intact *Lespedeza* roots and in root exudate. Soyasapogenol B was analyzed to determine whether it is exuded in amounts sufficient to serve as a host recognition cue for *Agalinis purpurea*. Soyasapogenol E was quantitated to confirm the results from TLC suggesting that high levels of this molecule are exuded relative to soyasapogenol B. The isoflavone genistein, although it is inactive in haustoria induction, was analyzed to gain an estimate of levels of phenylpropanoids such as xenognosins A and B which might be expected in exudates of plants biosynthesizing molecules of this type.

The results of the HPLC quantitation are shown in Table 2. Although genistein accumulates in the root to levels comparable with soyasapogenol B, it is exuded at levels about two orders of magnitude lower than the soyasapogenols. In the root, the molar ratio of soyasapogenol B to soyasapogenol E is about 6:1 whereas in root exudate, soyasapogenol E is secreted in a slight molar excess over soyasapogenol B.

Activity

Water, 'conditioned' for several days by the presence of *Lespedeza* roots, will induce haustorial differentiation in *Agalinis* plants transferred from nutrient agar to the conditioned water [5]. From the data of Table 2 the maximum accumulation of soyasapogenol B to be expected after several days of exudation from *Lespedeza* roots is around 200 pmol. The lowest amount of soyasapogenol eliciting haustoria in the sensitive filter paper disk assay is about 20 nmol. Therefore it is unlikely that this inducer can account for the full range of haustoria induction by *Lespedeza* roots under these conditions.

Several derivatives of soyasapogenol B were bioassayed to probe the structure–activity relationships of the molecule and particularly to test whether a synthetic modification could increase the haustoria inducing activity. Oxidation of the 22-hydroxyl eliminates activity, and tying up of the A ring 1,3-diol system as an acetonide also abolishes activity. The latter case does not seem to be a simple solubility problem, since assaying with DMSO to enhance solubility does not affect the activity. From these results, it appeared that hydroxylation on both A and E rings is necessary but not a sufficient condition for activity. However, bioassay of a large number of oleanene and lupene triols and diols failed to disclose any possessing haustoria inducing activity. In particular, lupenetriol and longispinogenin, which bear 1,3-diol systems in addition to another hydroxyl on both the lupene and oleanene skeletons, do not show activity. The activity of soyasapogenol B must therefore be related to both the presence of the three hydroxyls and their location on the skeleton of the molecule.

Table 2. Quantitation of terpenoid and isoflavone levels in *Lespedeza* roots and root exudate*

	Soyasapogenol B	Soyasapogenol E	Genistein
Root extract			
pmol/root	291 ± 1.09	50.0 ± 0.238	304 ± 639
% dry weight of root	0.06	0.01	0.03
Root exudate			
pmol exuded/root/day	42.8 ± 0.115	56.7 ± 0.111	0.422 ± 0.0111
% dry weight of exudate	0.34	0.45	0.002

*Each figure represents the mean and standard error of three repetitions. The exudation pattern from the first 5 days of collection does not appear to differ from the second 5 days of collection. The relative levels of soyasapogenols and genistein within the root are not different at 15 days after germination, nor do they differ at 3 months of age. Soyasapogenols were not detected in shoot portions of seedlings. Variance in root size of 5-day-old plants was not taken into account in either the calculation of pmol/root or pmol exuded/root. The weight of filtered and lyophilized exudate of 375 plants was used to extrapolate to the figure of 3245 individuals used for exudate collection in the analysis of exudation/root.

DISCUSSION

The previously described haustoria inducers, xenognosin A (2) and xenognosin B (3) are phenolic molecules with rather strict molecular requirements for activity [7]. Both are products of phenylpropanoid metabolism and require a *m*-methoxyphenol substitution pattern for their activity. Smaller phenolic inducers such as vanillin and its isomers and *m*-methoxyphenol have considerably less activity but do induce haustoria. For these reasons the activity of soyasapogenol B, which is of totally different biosynthetic origin, was surprising.

Definite differences exist in the inducing activity of soyasapogenol B and the xenognosin-type molecules. Assayed by the filter paper disk method, 20 nmol of soyasapogenol B is the lowest quantity which will induce haustoria in *Agalinis*. In contrast, xenognosin A induces haustoria in the same assay at 1 nmol; more haustoria are also formed by this quantity of xenognosin A than are induced by soyasapogenol B at 20 nmol. Also, haustoria are not induced when soyasapogenol B is presented as a 10 μM solution, whereas xenognosin A retains high activity in solution assay [7]. Xenognosin B and *m*-methoxyphenol also exhibit much lower activity when tested in the solution assay rather than by filter paper disk [7].

The effects of chemical fractionation on the haustoria inducing activity of *Lespedeza* roots and gum tragacanth (from which xenognosins A and B were isolated) are also very different. Even in the early steps of tragacanth fractionation the activity is localized to two fractions corresponding to xenognosins A and B. In the fractionation of *Lespedeza* roots, the activity is generalized to a fairly non-polar fraction. Subsequent fractionation of this preparation results in a significant amount of activity in all fractions. By assaying at minimal concentrations required for activity, peaks of activity become evident; the oleanene triterpene described here represents one of these peaks. Preliminary evidence from attempts to reconstitute the full activity of the *Lespedeza* root extract suggests that several components may be required to achieve high levels of activity. The molecular entities involved are unknown at this point. They are slightly more polar than soyasapogenol B (as measured by reversed phase HPLC). Because their isolation at this stage remains at the microgram level, little more is known either of their structures or concentration ratios at which their effects with soyasapogenol B occur. Moreover, the presence of these components in *Lespedeza* root exudate is yet to be established.

The high activity, stringent molecular requirements and apparent lack of synergistic effects in the *m*-methoxyphenol substituted phenylpropanoids suggests that they may interact with some specific, primary receptor system for host recognition cues. The data for the pentacyclic triterpene soyasapogenol B show that: (a) a molecule of radically different structure can possess the same, although lower, activity; (b) soyasapogenol B possesses a fairly high degree of molecular specificity, and (c) its full activity may depend on simultaneous presentation with several other components present in *Lespedeza* roots. It is unclear therefore, whether the activity of soyasapogenol B results from a low affinity interaction with the same receptor for the *m*-methoxyphenol substituted inducers, or is a result of interactions with a separate receptor system.

The data presented here do not determine whether the selective nature of root exudation suggested by TLC (Fig. 3) and HPLC quantitation data (Table 2) represents an active aspect of root metabolism, or is rather a reflection of cellular compartmentalization of secondary metabolites and their passive leakage from the root. Bell and co-workers [20, 21] have shown a compartmentalization of plant chemical defenses in the cotton root, with triterpenoid synthesis occurring in the epidermis, proanthocyanidins in the hypodermis, sesquiterpenoids in the cortex and proanthocyanidins in the endodermis. If a spatial distribution of this kind is maintained in *Lespedeza sericea*, this could account for the differential exudation of relatively large quantities of epidermally localized soyasapogenol B and E, while the isoflavone genistein, occurring in large quantities in cells distal to the epidermis, would be represented at much lower levels in the root exudate.

Alternatively, the exudation of the soyasapogenols, which are probably not differentially localized in the root, could represent a more active process. According to the data in Table 2, by day 15 approximately 94% of the

soyasapogenol E synthesized in the root has been released into the root exudate. In contrast, only 69% of the soyasapogenol B synthesized in the root is found in root exudate. Thus, the differential secretion of soyasapogenol B and E may account for the observed ratio of soyasapogenol B to E within the root.

Atsatt [22] has proposed that, like herbivorous insects, parasitic seed plants may have evolved to utilize host defensive compounds as recognition cues. The biosynthesis of xenognosin A is induced to levels comparable with the pterocarpinoid phytoalexin pisatin in response to heavy metal stress in pea (*Pisum sativum*) [23, 24]. Xenognosin B (7,2'-hydroxy-4'-methoxyisoflavone) is a direct biosynthetic precursor to the phytoalexin (±)-medicarpin [25], although medicarpin itself does not induce haustoria in *Agalinis*. Although a possible defensive role for pentacyclic triterpenes in plants is less well established, the avenacins, esters of oleanane triterpenes found in oats, have been shown to be potent resistance factors to 'take all' disease caused by *Gaeumannomyces graminis* [26]. In their glycosylated form, the soyasapogenols are toxic and have been suggested [27] to have some role in the minimization of herbivory in leguminous seeds possessing these compounds.

Haustoria induction by pentacyclic triterpenes represents a novel activity for this class of molecules, and its generality is uncertain. It is significant for the understanding of haustoria induction in angiosperm root parasites that xenognosins A and B, and vanillin induce haustoria in *Striga asiatica*, whereas soyasapogenol B does not elicit haustorial differentiation in this economically important parasite. Until more host species are investigated, the significance of haustoria induction by either triterpenes or phenolic molecules will remain unresolved.

EXPERIMENTAL

Plant materials and bioassay. Lespedeza sericea was grown in vermiculite under greenhouse conditions. *Agalinis purpurea* was grown in sterile culture in 60 × 15 mm Petri plates on Murashige–Skoog medium as previously reported [5]. Bioassays for haustoria induction were carried out by spotting fractions on 0.5 cm filter paper discs and insertion of the discs into agar in which 2- to 3-week old *Agalinis* plants were growing [7].

Extraction and isolation. Three-month-old *Lespedeza* roots (400 g fr. wt) were removed from vermiculite and ground in a blender with 50% aq. MeOH (v/v). The resulting extract was filtered and concd *in vacuo* at 30° to remove MeOH and lyophilized. Extraction of 12 g of lyophilized root extract with Me_2CO and concn *in vacuo* at 30° yielded 2.5 g of a dark brown oil. The oil was dissolved in $CHCl_3$–MeOH–H_2O (7:13:8) and applied to droplet countercurrent chromatography in the same solvent (descending mode, organic phase mobile). Bioassay localized the highest activity to an early-eluting fraction (49 mg), which was further separated by flash column chromatography [28] on a 3 × 8 cm column eluted with a gradient of 100% CH_2Cl_2 to 20% MeOH–CH_2Cl_2. The most active fraction was purified further on a 2.5 × 5 cm silica gel column eluted with a 100% CH_2Cl_2 to 5% MeOH–CH_2Cl_2 gradient. A third silica gel column, 1.5 × 3 cm, eluted isocratically with 1.5% MeOH–CH_2Cl_2 followed by reverse phase HPLC using a Waters C-18 Radial Pac B column in 83% MeOH–H_2O (2 ml/min, detection at 214 nm) yielded 2 mg of active material crystallizing from MeOH (mp 260–261°).

Spectroscopy. High resolution electron impact MS was measured at 70 eV on a VG-7070, and chemical ionization MS were obtained on a Finnigan 3200. Deuterium exchange MS experiments were performed as described by Hunt and Sethi [29]. Collision activated decomposition MS (CADMS) were run on a Finnigan 4000 Q_3 instrument using a solid probe for EI ionization (70 eV) and 1 μT argon as collision gas in the second quadrupole [6, 30].

Proton nuclear magnetic resonance (^{1}H NMR) experiments were performed with a Nicolet 360 MHz instrument equipped with a 1280 computer and a 293B pulse programmer. Nuclear Overhauser enhancement difference experiments were performed on 2 mg samples as described in more detail elsewhere [31]. Lanthanide-induced shift ^{1}H NMR studies used tris-(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octandionato)europium [Eu(fod)$_3$], obtained from Aldrich Chem. Co. Experimental details of the 2D ^{1}H NMR experiments have been fully described elsewhere [15, 32].

Derivatizations. The 3β,24-phenylborate of **1** was produced by refluxing 2.2 mg of **1** with a 1.5 molar excess of phenylboronic acid in C_6H_6 for 12 hr with a Dean-Stark trap for H_2O removal. Workup involved concn *in vacuo* at 30°, partition between H_2O and Et_2O and purification of the organic phase on a 0.5 × 3 cm silica gel column eluted with CH_2Cl_2. The triacetate of **1** was formed by addition of 6 drops each of pyridine and Ac_2O to 1 mg of **1** with stirring for 3 hr. Residual pyridine was removed azeotropically with toluene *in vacuo* at 30°. Generation of the 3β,24-acetonide (dimethyl acetal) was accomplished by stirring 4 mg **1** in 2 ml dry Me_2CO with 200 μl of 2,2-dimethoxypropane and a catalytic amount of *p*-toluene sulphonic acid (*p*-TsOH) at room temp. for 4 hr. H_2O was then added, the mixture neutralized with Na_2CO_3, partitioned against Et_2O, and the organic phase was concd *in vacuo* and purified over a 1.5 × 4 cm silica gel column eluted with 100% CH_2Cl_2. The 22-ketoacetonide was formed by oxidation with pyridinium dichromate and a catalytic amount of pyridinium trifluoroacetate in dry CH_2Cl_2 at room temp. for 4 hr, following the method of ref. [33]. The acetonide was removed quantitatively by concn in MeOH soln with a trace of *p*-TsOH to give soyasapogenol E (22-oxo-3β,24-dihydroxyolean-12-ene, **4**).

Other analogs. A series of oleanene and leupene triterpenes was generously supplied by Dr. Henry Kircher, University of Arizona.

Quantitation. Seeds of *Lespedeza sericea* were surface sterilized by soaking in Captan (1 g/l) 1 hr, 35% clorox for 20 min, and washing with 1 N HCl and H_2O. For quantitation of components in *Lespedeza* roots, seeds were sown on moistened filter paper in plastic Petri dishes. At 15 days post-germination, roots (70 for isoflavone analysis; 210 for triterpenes) were dissected off, frozen and lyophilized. The dried tissue was then homogenized with 4 ml MeOH and further extracted × 3 with the same solvent. After filtration through glass wool and concn *in vacuo* at 30°, the extracts underwent droplet countercurrent chromatography in $CHCl_3$–MeOH–H_2O (7:13:8) run in the descending mode with the organic phase mobile. Fractions corresponding in retention time to the soyasapogenols and genistein (5,7,4'-trihydroxyisoflavone) were collected, pooled and concd *in vacuo*. Each fraction was then passed over a silica gel column 0.5 × 2 cm eluted isocratically with 8% MeOH–CH_2Cl_2. The eluate was then concd *in vacuo* and filtered prior to HPLC.

For quantitation of root exudate components, surface sterilized *Lespedeza* seeds were allowed to germinate and the seed coats were separated from the young plants. At 3 days post germination the root of each plant was inserted through Nitex mesh suspended over H_2O in a sterile Petri dish. In this way plants were supported by the mesh so that only the roots were allowed to come into contact with the water. Every 3 days for the next 9 days exudate was removed and replaced with sterile H_2O;

plates were discarded when signs of contamination appeared. Exudate collected in this way from 3200 plants, filtered through an 0.8 μ Millipore filter and lyophilized, yielded 185 mg dry exudate. The lyophilized exudate was extracted with MeOH and the resulting extract, after filtration and concn *in vacuo*, was chromatographed under conditions identical to those described above for root extracts.

HPLC was carried out on a Beckman gradient HPLC instrument with a Waters 5 μ C-18 column. For genistein analysis, detection was at 260 nm, in 65% MeOH–H_2O, flow rate 1 ml/min. Triterpenes were detected at 214 nm, in 84% MeOH–H_2O, flow rate 1 ml/min. Quantitation was performed with an Altex model CR1A programmer using a two-point external standard calibration program.

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