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Structural diversity of strigolactones and their distribution in the plant kingdom

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Strigolactones (SLs) are plant secondary metabolites that were first identified as germination stimulants for the root parasitic weeds witchweeds (*Striga* spp.) and broomrapes (*Orobanche* and *Phelipanche* spp.). In the rhizosphere, SLs also promote root colonization by arbuscular mycorrhizal fungi. In plants, SLs as a novel class of plant hormones regulate various aspects of plant growth and development. Herein I discuss structural diversity of naturally occurring SLs and their distribution in the plant kingdom. © Pesticide Science Society of Japan

Keywords: strigolactone, germination stimulant, root parasitic plant.

Introduction

Strigolactones (SLs) are plant secondary metabolites that are structurally related to strigol (**1**), which was first identified as a germination stimulant for the root parasitic weed witchweed, *Striga lutea*.[1–3] SLs induce seed germination not only in hemiparasites *Striga* spp. but also in holoparasites broomrapes (*Orobanche* and *Phelipanche* spp.), both of the Orobanchaceae. These root parasitic weeds cause enormous damage to agricultural production all over the world, because appropriate and economically feasible control measures have not yet been developed. Furthermore, these weeds are gradually invading crop land in both developing and developed countries.[4,5] Extensive studies on the characterization of SLs produced by various plant species have demonstrated that most land plants produce and release not single but mixtures of SLs.[6]

The reason why plants produce and release SLs, which attract root parasitic weeds, has long been sought. In 2005, one SL, 5-deoxystrigol (5DS, **2**) was identified as a branching factor for important symbionts the arbuscular mycorrhizal (AM) fungi, with which more than 80% of land plants form symbiotic associations,[4,7] demonstrating that plants produce and release SLs to promote symbiotic associations with AM fungi which supply nutrients, especially phosphate. The Brassicaceae and Chenopodiaceae families and some legume plants like white lupin (*Lupinus albus*) are non-mycotrophic but still produce SLs,[8,9] indicating that SLs may have other important functions in plants. In 2008, SLs or their further metabolites were shown to be a novel class of plant hormone inhibiting shoot branching.[10,11] These findings attracted researchers to study the biological functions of SLs and they have been shown to be involved in the regulation of growth of lateral roots and root hairs,[12,13] secondary growth,[14] photomorophogenesis[15] and leaf senescence,[16,17] and to promote root colonization by root nodule bacteria.[18–21]

Approximately 25 natural SLs have been characterized from root exudates of various plant species.[22] Structural diversity in these natural SLs is closely related to biosynthesis, metabolism and biological functions of SLs. In this review, I discuss structural diversity of natural SLs and their distribution in the plant kingdom.

1. Natural SLs

The first reported SLs—strigol and its acetate, strigyl acetate (**3**)—were isolated from root exudate of cotton (*Gossypium hirsutum*) a non-host of *Striga* spp., as germination stimulants for *S. lutea*.[1] Therefore, there were some arguments that SLs were not true host-derived *Striga* germination stimulants. Then, strigol was isolated from root exudates of maize (*Zea mays*), sorghum (*Sorghum bicolor*) and proso-millet (*Panicum miliaceum*), genuine hosts of *Striga*.[23] We also analyzed root exudates of several maize cultivars, but did not detect strigol.[24] Jamil *et al.* reported that they could not detect any known SLs from root exudates of maize cultivars examined.[25] In addition to strigol, two new SLs structurally related to strigol, sorgolactone (**4**) and alectrol, were purified from root exudates of sorghum[26] and cowpea (*Vigna unguiculata)*,[27] respectively. Orobanchol,

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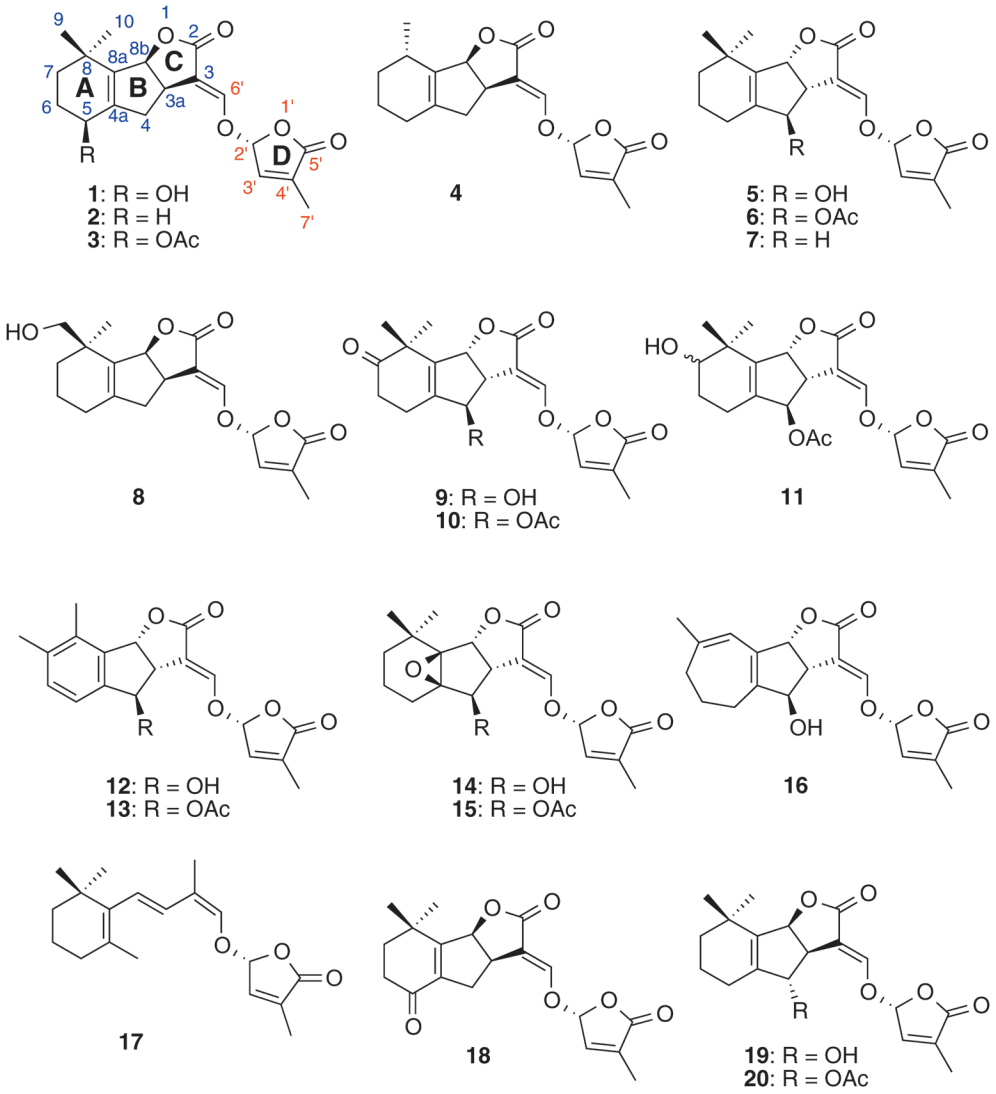

Fig. 1. Structures of canonical strigolactones and carlactone.

along with alectrol, was characterized as the first *Orobanche* germination stimulant from red clover (*Trifolium pratense*) root exudates.[28] This clearly demonstrates that SLs elicit seed germination in both *Striga* and *Orobanche* spp. Alectrol was later identified as orobanchyl acetate (**6**) but was not an isomer of strigol.[29,30] 5DS was originally isolated as a branching factor for AM fungi and was then shown to be produced by several plant species.[31] However, the retention of 5DS is very similar to that of its isomer 4-deoxyorobanchol (4DO, **7**) in both reversed phase and normal phase high performance liquid chromatography, and some plant species reported to produce 5DS may produce 4DO. Sorgomol (**8**) is an isomer of strigol and orobanchol and was isolated from sorghum root exudates.[32] Not only monocots but also dicots including white lupin and Chinese milk vetch (*Astragalus sinicus*) produce sorgomol.[9] The SLs 7-oxoorobanchol (**9**), 7-oxoorobanchyl acetate (**10**) and 7-hydroxyorobanchyl acetate (**11**) were isolated from root exudates of flax (*Linum usitatissimum*)[33] and were detected in root exudates from various plant species including cucumber (*Cucumis sativus*).[34] Solanaceae plants such as tobacco (*Nicotiana tabacam*) and tomato (*Solanum lycopersicum*) produce solanacol (**12**) and solanacyl acetate (**13**), unique SLs containing a benzene ring.[34,35] Fabacol (**14**) and fabacyl acetate (**15**), found in root exudate of pea (*Pisum sativum*), have an epoxide.[36] Medicaol (**16**), a putative didehydro-orobanchol isomer containing a seven-membered A ring, was recently identified from root exudates of barrel medic (*Medicago truncatula*).[37] Root exudates from red clover, tomato and tobacco were found to contain didehydro-orobanchol isomers, which differ from medicaol but with structures still to be elucidated. Putative desmethyl-orobanchyl acetate isomers, desmethyl-7-hydroxyorobanchyl acetate isomers, dihydro-orobanchol isomers and their derivatives have been detected from various plant species (Xie *et al.*, unpublished).

2. Structures and stereochemistry of naturally occurring SLs

Naturally occurring SLs (natural SLs) are classified into two groups based on their structures. SLs structurally related to strigol are called canonical (or classical) SLs in which the ABC ring moiety, a core structure, connects to a butenolide (D ring) *via* an enol–ether bridge. By contrast, in non-canonical SLs, the D ring connects to a variety of structures.[21] For example, carlactone (CL, **17**), a biosynthetic precursor for SLs, contains only the A ring of canonical SLs.[38]

Canonical SLs contain three asymmetric carbons (C-3a, C-8b and C-2′) but C-3a should be *cis* to C-8b as they are at bridge heads of the C ring. Therefore, canonical SLs without substituents on the AB ring (*e.g.*, 5DS and 4DO) consist of four stereoisomers. Since natural CL has an 11-*R* stereochemistry, which corresponds to the C-2′ in canonical SLs,[39] only 5DS with *β*-oriented C ring and 4DO with *α*-oriented C ring among four possible stereoisomers are natural SLs. The canonical SLs so far characterized have substituents on the A and/or B rings but no modifications on the CD ring moiety.[6,21]

Until the structure of fabacyl acetate was determined in 2009,[36] the first reported canonical SL containing an *α*-oriented C ring, all natural SLs were thought to have a *β*-oriented C ring and be derived from 5DS. In 2010, total synthesis of solanacol and solanacyl acetate confirmed that these SLs also carry an *α*-oriented C ring.[40] Ueno *et al.* revised the structures of orobanchol and orobanchyl acetate to have an *α*-oriented C ring.[41] This led to structural revisions of 7-oxoorobanchyl acetate and 7-hydroxyorobanchyl acetate. Consequently, natural canonical SLs can be divided into two types: orobanchol-type with *α*-oriented C ring and strigol-type with *β*-oriented C ring.[21,35] Strigol- and orobanchol-type SLs seem to be derived from 5DS and 4DO, respectively (Fig. 1).

We identified orobanchol, orobanchyl acetate and 4DO from rice root exudates.[35] Orobanchol-type SLs such as 4DO were detected in root exudates of bright yellow tobacco cultivar Tsukuba No. 1. In addition to orobanchol-type, strigol-type SLs were detected in root exudates of burley tobacco cultivar Michinoku No. 1.[35] A medicinal plant, dokudami (*Houttuynia cordata*), was found to produce strigol-type SLs and strigone (**18**), an oxidized metabolite of strigol, was first detected as a natural SL.[42] These results confirm that two biosynthetic pathways lead to two types of canonical SLs and suggest that some plant species produce either strigol- or orobanchol-type SLs as major SLs and the others produce both types of SLs.[21,35]

Canonical SLs are synthesized from *β*-carotene *via* the biosynthetic intermediate CL.[38,39,43] CL contains the A and D rings of canonical SLs but lacks the B and C rings. Oxidations at C-19 and C-18 and the subsequent ring closure appear to convert CL to 5DS or 4DO which are then modified independently.[38,43]

Allylic oxidations of 5DS at C-5 and C-4 produce strigol and 4-hydroxy-5DS (**19**), respectively, and an oxidation at a homoallylic position (C-9) gives sorgomol. Strigol is further oxidized to strigone, and sorgomol may be converted to sorgolactone after oxidation and subsequent decarboxylation. Although we grew a large number of sorghum plants (several different cultivars) and collected root exudates, sorgolactone has never been detected. Strigol and 4-hydroxy-5DS are acetylated to give strigyl acetate and 4-acetoxy-5DS (*ent*-2′-*epi*-orobanchyl acetate, **20**), respectively. Allylic oxidation of 4DO at C-4 affords orobanchol. The oxidation at C-7 of orobanchol gives 7-hydroxyorobanchol, which is further oxidized to 7-oxoorobanchol. The 7-hydroxyorobanchol may be converted to solanacol *via* dehydration, oxidation and migration of a methyl group. A seven-membered A ring may be formed before construction of the ABC ring structure. Orobanchol is converted to fabacol by epoxidation. Hydroxy-SLs are acetylated to corresponding acetoxy-SLs. It is intriguing that strigol-type isomers of orobanchol and orobanchyl acetate were detected,[35] while either strigol- or orobanchol-type SLs have been detected for other canonical SLs. For example, neither 5-hydroxy-4DO (orobanchol-type isomer of strigol) nor 9-hydroxy-4DO (orobanchol-type isomer of sorgomol) have been detected. Firstly, this is because that all orobanchol-type SLs, except for 4DO, are derived from orobanchol. Secondly, presence of the *β*-oriented C ring in 5DS may restrict directions and positions of oxidation on the A ring. However, orobanchol, orobanchyl acetate and a didehydro-orobanchol isomer were detected in red clover root exudates but not 4DO,[28] indicating that oxidation of 4DO to orobanchol proceeds rapidly. Alternatively,

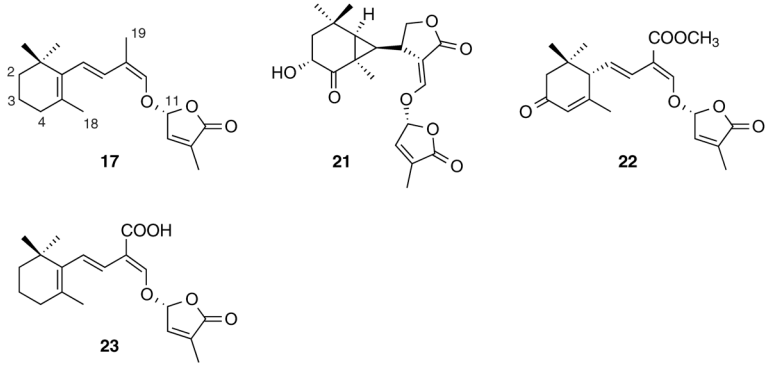

Fig. 2. Structures of non-canonical strigolactones.

orobanchol may be synthesized not from 4DO but directly from CL or hydroxy-CL in this plant.

3. Non-canonical SLs

Canonical SLs described in the previous section are derived from either 5DS or 4DO and contain the core structure, the ABC ring. Several novel germination stimulants that are structural distinct from canonical SLs have been characterized in root exudates from various plant species. Avenaol (**21**)[44] and heliolactone (**22**)[45] are two novel germination stimulants isolated from black oat (*Avena strigosa*) and sunflower (*Helianthus annus*), respectively, and are typical non-canonical SLs. Carlactonoic acid (CLA, **23**)[46] formed from CL by MAX1 oxidation has been detected in root exudates of various plant species including maize, sunflower, spikemoss (*Selaginella moellendorfii*) and poplar (*Populus* spp.). In addition to these non-canonical SLs, more than 10 novel germination stimulants structurally related to CL have been suggested. It is intriguing that root exudates from plant species producing these non-canonical SLs as major germination stimulants should exhibit strong germination stimulation activity to seeds of root parasitic plants but do not contain detectable levels of canonical SLs (Fig. 2).

4. Distribution of SLs in the plant kingdom

We analyzed SLs produced by various plant species and some results are listed in Table 1.

In angiosperms, rice and cucumber produce orobanchol-type SLs, while cotton and strawberry (*Fragaria×ananassa*) produce strigol-type SLs. By contrast, Chinese milk vetch is a producer of both types of SLs. Tobacco plants also produce both types of SLs, and the ratio of orobanchol- to strigol-type SLs significantly differ between cultivars. The burley tobacco Michinoku No. 1 produce both types of SLs at similar levels, while amounts of strigol-type SLs are only 1% that of orobanchol-type SLs in root exudates of bright yellow tobacco Tsukuba No. 1. In general, SL production is known to be promoted under phosphorus (P) and nitrogen (N) deficiencies.[47–49] It should be noted that in plant species producing both types of SLs, nutrient deficiencies, espe-

Table 1. Distribution of strigolactones in the plant kingdom

		Canonical strigolactones		Non-canonical strigolactones
		Orobanchol-type SL	Strigol-type SL	
Pteridophyte	*Selaginella moellendorffii*	4-deoxyorobanchol		CLA
Gymnosperm	*Pinus thunbergii*	orobanchol, orobanchyl acetate		CLA
	Ginkgo biloba	4-deoxyorobanchol, orobanchol, orobanchyl acetate		
Angiosperm	*Oryza sativa*	4-deoxyorobanchol, orobanchol, orobanchyl acetate, 7-oxoorobanchyl acetate		
	Pisum sativum	4-deoxyorobanchol, orobanchol, orobanchyl acetate, fabacol, fabacyl acetate		
	Solanum lycopersicum	4-deoxyorobanchol, orobanchol, solanacol, 7-hydroxyorobanchol		
	Cucumis sativus	4-deoxyorobanchol, orobanchol, orobanchyl acetate, 7-oxoorobanchol, 7-oxoorobanchyl acetate, 7-hydroxyorobanchol, 7-hydroxyorobanchyl acetate		
	Nicotiana tabacum	4-deoxyorobanchol, orobanchol, orobanchyl acetate, solanacol, solanacyl acetate	5-deoxystrigol, 4-hydroxy-5-deoxystrigol, 4-acetoxy-5-deoxystrigol	
	Astragalus sinicus	orobanchyl acetate	5-deoxystrigol, sorgomol	
	Gossypium hirsutum		strigol, strigyl acetate	
	Sorghum bicolor		5-deoxystrigol, strigol, strigyl acetate, sorgomol	
	Fragaria×ananassa		5-deoxystrigol, strigol, strigyl acetate	
	Lotus japonicus		5-deoxystrigol	methyl lotuslactonoate*
	Helianthus annuus			heliolactone, CLA
	Zea mays		5-deoxystrigol	methyl zealactonoate** CLA
	Populus	4-deoxyorobanchol		CLA, MeCLA

Strigolactones identified in root exudates are listed. CLA, carlactononic acid; MeCLA, methyl carlactonoate. *, ** Structures of these novel strigolactones will be reported elsewhere.

cially of P and N, more strongly affect production of one type of SL. In the case of tobacco, P deficiency increased production of strigol-type SLs by more than 1000-fold, whereas orobanchol-type SLs were unaffected. Similar results were obtained with Chinese milk vetch, in which P and N deficiencies promoted strigol-type SLs, 5DS and sorgomol, but level of orobanchyl acetate was unaffected.[50] These results suggest that production and exudation of strigol- and orobanchol-type SLs are regulated independently.

5. Transport of SLs from roots to shoots

Results from reciprocal grafting experiments using wild-type and SL biosynthetic and perception mutants of *Arabidopsis*, pea and *Petunia*, reveal that plant hormones inhibiting shoot branching are produced mainly in the roots and transported to shoots.[6,10,20,21] The most probable route for SL transport from roots to shoots is xylem and indeed Kohlen *et al.* detected orobanchol and other SLs in xylem sap from *Arabidopsis* and tomato.[51,52] However, no reports support xylem transport of SLs. We collected large amounts of xylem sap from various plant species including tomato and *Arabidopsis* but no signals attributable to known canonical or non-canonical SLs were detected by LC–MS/MS analyses.[53] Then, deuterated SLs, d_1-orobanchol and d_6-4DO, were fed to roots of rice plants and the shoots were harvested 2 and 20 hr after SL treatment. The SLs were detected in shoots harvested 20 hr after but not 2 hr after treatment.[53] These results strongly suggest that exogenous and endogenous SLs are transported from roots to shoots not through the xylem but through hypodermal passage cells as in *Petunia* where polar and asymmetric localization of an ABC transporter (PaPDR1) have been shown to mediate shootward SL transport as well as localized exudation into the rhizosphere.[54,55] Furthermore, aforementioned species-specific phenomena in SL production also occur in the transport of exogenous SLs from the roots to shoots, indicating that the transport is structure- and stereospecific.[56] For example, in rice plants, which produce orobanchol-type SL, only orobanchol-type SLs are transported from roots to shoots.[56] However, strigol applied to roots of SL biosynthetic rice mutant *d10* inhibited tiller bud outgrowth,[11] indicating that metabolites of SLs or other signaling compounds downstream of SLs—but not SLs themselves—are the true inhibitors of tiller bud outgrowth.

Conclusion

SLs were originally discovered as germination stimulants for root parasitic plants 50 years ago and are now recognized as important signaling compounds not only in the rhizosphere but also in plants. In the rhizosphere, the physicochemical and biological conditions significantly differ from those of bulk soil.[57] For example, pH in the rhizosphere is rather acidic and thus alkaline-unstable canonical SLs would endure longer than expected. In addition to canonical SLs, recently discovered non-canonical SLs were shown to be released into the rhizosphere, but their involvement in rhizosphere communications among soil organisms remains largely unknown. Therefore, further studies are needed to understand involvement of canonical and non-canonical SLs in chemical communications between plants and other organisms which have been exposed to these signaling compounds for more than 400 million years. In particular, some soil microorganisms may not only utilize SLs as cues of living plants nearby but also decompose and/or transform them to support their survival. It is therefore important to characterize novel SLs and elucidate their functions and action mechanisms to develop practical applications of SLs in plant production and crop protection. SLs and their agonists and/or antagonists, and biosynthetic inhibitors could be applied to regulate plant architecture, optimize AM symbiosis and control parasitic weeds.

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