Tetrahedron Letters 50 (2009) 6955–6958

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Peagol and peagoldione, two new strigolactone-like metabolites isolated from pea root exudates

Antonio Evidente [a,*], Mónica Fernández-Aparicio [b], Alessio Cimmino [a], Diego Rubiales [b], Anna Andolfi [a], Andrea Motta [c]

[a] Dipartimento di Scienze del Suolo, della Pianta, dell'Ambiente e delle Produzioni Animali, Università di Napoli Federico II, 80055 Portici, Italy
[b] Institute for Sustainable Agriculture, CSIC, Apdo. 4084, 14080 Córdoba, Spain
[c] Istituto di Chimica Biomolecolare del CNR, 80078 Pozzuoli, Italy

ARTICLE INFO

Article history:
Received 8 September 2009
Revised 18 September 2009
Accepted 23 September 2009
Available online 27 September 2009

Keywords:
Pea root exudates
Orobanche
Seed germination
Strigolactones
Peagol
Peagoldione

ABSTRACT

Two new strigolactone-like metabolites, named peagol and peagoldione, with germinative activity for root parasitic plants, were isolated from pea root exudates and were characterized by spectroscopic methods. Peagol was more active on *Orobanche foetida* and *Phelipanche aegyptiaca* seeds, while peagoldione was active on *P. aegyptiaca* only. Low activity was found on *Orobanche crenata* and *Orobanche minor*. Stimulatory activity of peagol on *O. foetida* seeds is most relevant as this species does not respond to the synthetic strigolactone analogue GR24, usually used as *Orobanche* germination standard.

Several *Orobanche* and *Phelipanche* species (broomrapes) are serious weed problems and cause severe yield reduction of many important crops.[1–3] Seeds of these parasitic weeds may remain dormant in the soil for many years until germination is stimulated by the release of a chemical signal with the roots of a host plant.[2] Pests control is a major problem in agriculture, which is almost completely dependent on the synthetic products; however, because of toxicological and environmental reasons, many of the currently used chemicals have been or will be soon withdrawn from the market.[4] This increases the need for novel, effective, and environmentally compatible alternatives. In general, natural product-based pesticides have low environmental risks, high target selectively, and novel mechanism of action, and show reduced risks for humans and no-target organisms.[5] In this perspective, different approaches are under investigation to develop natural product-based pesticides to control *Orobanche* and *Phelipanche*. Phytotoxic metabolites were isolated from fungi pathogenic for *Phelipanche ramosa* infecting different crops, and their effects on *Orobanche* and *Phelipanche* seed germination were evaluated.[4,6] Interesting results were obtained by testing microbial metabolites as fusicoccins and ophiobolin A to induce the suicidal seed germination of nine *Orobanche* and *Phelipanche* species, including those resistant to the well-known chemical germination stimulants.[7,8] Much attention has been focused on the isolation and identification of germination stimulants, including the metabolites recently isolated from the fenugreek (*Trigonella foenum-graecum* L.).[9,10] Three different class of plant secondary metabolites, dihydrosorgoleone, sesquiterpene lactones, and strigolactones[11] are known to induce seed germination of these parasites, with strigolactones showing the strongest activity. Different strigolactones were isolated from host and non-host *Orobanche*, *Phelipanche,* and *Striga* plants,[11,12] including sorgomol,[13] isolated from the root exudates of sorghum (*Sorghum bicolor* L.), and fabacyl acetate[14] from the root exudates of pea (*Pisum sativum* L.). Previously, the well-known strigolactones, namely didehydro-orobanchol, orobanchol, orobanchyl acetate, and 5-deoxy-strigol[15] were identified by LC/MS/MS in the root exudates of the same plant.

In this Letter, we report the isolation from pea root exudates and the structure and chemical characterization of two new strigolactone-like metabolites, namely peagol and peagoldione. Their activity stimulating *Orobanche foetida* and *Phelipanche aegyptiaca* seed germination is also defined.

Seeds of pea cv. Messire were sterilized in 4% sodium hypochlorite containing 0.02% (v:v) Tween 20, rinsed with sterile water, and germinated in pots filled with sterile perlite watered with Hoagland nutrient solution modified to a quarter-strength of phosphorous. Groups of 5 pea 15-day-old plants were transferred from the perlite to the flask filled with 150 ml of sterile distilled water,

* Corresponding author. Tel.: +39 081 2539178; fax: +39 081 2539186.
E-mail address: evidente@unina.it (A. Evidente).

0040-4039/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2009.09.142

allowing the release of the root exudate during two days. The hydroponically collected root exudates released in 45 L, were immediately frozen at −80 °C and then lyophilized at −20 °C. The resulting dry powder was extracted with EtOAc. The extract showed high stimulatory activity on the germination of seeds of four broomrape species, namely *P. aegyptiaca*, *Orobanche crenata*, *O. foetida*, and *Orobanche minor*. The extract (285 mg) was purified by a silica-gel column chromatography eluted with $CHCl_3$–*i*-PrOH, 9:1, yielding nine homogeneous fraction groups, the second-fourth of which showed strong stimulatory activity on germination of the above broomrape species. The residue (120 mg) of the second fraction was purified by a combination of preparative TLC on silica gel (eluent $CHCl_3$–*i*-PrOH, 9:1) and on reversed-phase (eluent EtOH–H_2O, 6:4), yielding two compounds, namely peagol and peagoldione (**1** and **2**, Fig. 1 and 1.2 and 1.8 mg). Their t_R was 31.2 and 27.8 min, respectively, as obtained by LC–MS analysis, carried out on a reversed-phase Luna 5 μ C18 using CH_3CN:H_2O (25:75 to 46:54, over 40 min, 0.5 mL/min).

Peagol exhibited a sodium clustered ion $[M+Na]^+$, in the HRESIMS spectrum, at *m*/*z* 385.0545 (calcd for $C_{17}H_{14}$ Na O_9, 385.0536), corresponding to the molecular formula $C_{17}H_{14}O_9$. The ^1H and ^{13}C NMR data (Table 1), compared with those reported for the strigolactones[12] mentioned above, showed a very similar structure for **1**, although interesting differences were noted, as confirmed by extensive study of the DEPT, COSY, HSQC, HMBC, and NOESY spectra.

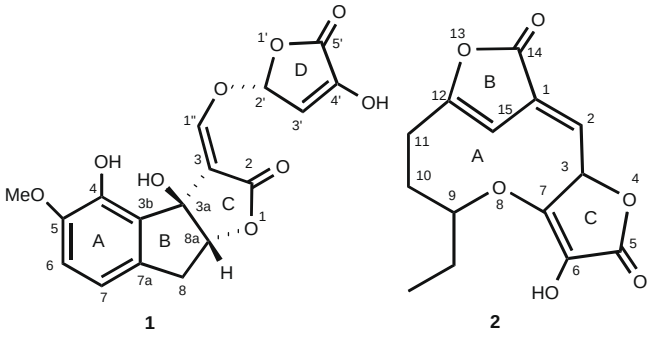

Figure 1. Structure of peagol (**1**) and peagoldione (**2**).

In fact, although the H-1″ signal has a correspondence, the H-3′ signal, as well as its geminal carbon (C-3′), appeared significantly upfield shifted. These results, together with the absence of the vinyl methyl group, corroborated the presence of a hydroxy group at C-4′. The latter is hydrogen bonded with the adjacent carbonyl lactone (C-5′), therefore generating a very stable pentacyclic ring, and consequently the broad singlet at δ 12.01.[16] The same happens between the hydroxy group at C-4 and the methoxy group at C-5, whose broad singlet probably overlapped with that of the hydroxy group at C-4′. Another striking difference is the presence of a typical ABX system in the ^1H NMR spectrum, noted for the first time in the strigolactone group, and attributed to the methylene (CH_2-8) of the 1,2,3,3,4-pentasubstituted cyclopentene ring and the adjacent proton (H-8a) of a secondary oxygenated carbon (C-8a). In fact, two double doublets (J = 14.2, 4.1 Hz and J = 14.2, 6.6 Hz) and a broad singlet resonated at δ 3.13, 2.92, and 5.21, respectively. The latter carbon (C-8a), bonded to the oxygen closing the furanone C ring and resonating at δ 72.8 in the ^{13}C NMR spectrum, is one of the two head-bridges carbons of the B/C ring junction, being the other one the quaternary oxygenated carbon (C-3a) observed at δ 92.0. This was confirmed by the significant coupling observed in the COSY spectrum between H-8a and the hydroxyl group at C-3a, which appeared as a broad singlet at δ 3.49. As expected, the latter coupled in the HMBC spectrum with its geminal carbon C-3a (Table 1). The penta-substituted cylopentenyl B ring was joined to an aromatic A ring as in solanacol, but it is a different tetra-substituted cycle. This was confirmed by the NOE effects and the couplings observed in the HMBC spectrum (Table 1). As expected, the two aromatic protons H-7 and H-6 coupled with the quaternary aromatic carbons C-5, and C-5 and C-4, respectively, in the HMBC spectrum. In the same spectrum, the couplings observed between H-7 and H-8β with C-8 and C-7, respectively, appear to be very important. This was further confirmed by the coupling observed between H-7 with both CH_2-8 protons in the NOESY spectrum. The same spectrum also showed the coupling between H-6 and the OMe. On the basis of these findings the structure **1** could be assigned to peagol. The relative stereochemistry attributed to peagol was deduced from the nOe effects reported in Table 1. Furthermore, the nOe effects also suggested for peagol a structure bent at the junction between B/C rings, as also confirmed by the inspection of Drieding model of **1** with H-8β pointing towards H-1″. From these results, peagol can be formulated as 3a,4-dihydroxy-3-(4-hydroxy-5-oxo-

Table 1
NMR data of peagol (**1**)

Position	δ^1H m, Hz	δ^{13}	DEPT & HSQC	HMBC	NOESY
1					
2		164.0	C	H-1″	
3		129.0	C	H-1″	
3a		92.0	C	H-8a, HOC-3a	
3b		110.0	C		
4		159.0	C	H-6	
5		159.0	C	H-7, H-6, OMe	
6	6.81, d, 8.4	113.4	CH		O-Me
7	7.05, d, 8.4	130.4	CH	H-8β	H-8α, H-8β
7a		127.0	C	H-6	
8α	3.13, dd, 14.2, 4.1	42.2	CH_2	H-7	H-7, H-8a
8β	2.92, dd, 14.2, 6.6				H-1″, H-7
8a	5.21, br s	72.8	CH	H-8β	H-1″, H-8α
1′					
2′	6.43, br s	104.0	CH	H-3′	
3′	6.43, br s	108.3	CH	H-2′	
4′		167.4	C		
5′		166.8	C	H-3′	
1″	7.59 br s	131.8	CH		H-8β, H-8a
OH	12.01 br s				
OMe	3.78 s	55.2	CH_3		H-6
HOC-3a	3.49 br s				

Table 2
NMR data of peagoldione (**2**)

Position	δ^1H m, Hz	δ^{13}	DEPT & HSQC	HMBC	NOESY
1		127.8	C		
2	7.26, dd, 9.4, 6.5	143.8	CH		H-3, H_2-10
3	6.16, d, 9.4	113.2	CH	H-15	H-2
5		162.9	C	H-3	
6		171.3	C		
7		154.9	C		
9	3.56 br s	72.3	CH	CH_3CH_2, CH_3CH_2, H-10′	
10	1.88, m	33.9	CH_2	H_2-11	H-2
10′	1.70, m				
11	2.70, dd, 9.6, 5.2	30.3	CH_2	H-15	H-15
11′	2.58, dd, 9.6, 6.9				H-15
12		154.9	C		
14		166.4	C	H-2, H-15, H_2-11	
15	6.01, d, 6.5	102.8	CH	H_2-11, H-3	H_2-11
CH_3CH_2	1.53 m	30.5	CH_2	CH_3CH_2	
CH_3CH_2	0.96, t, 7.1	9.8	CH_3		
OH	12.01 br s				

2,5-dihydro-furan-2-yloxymethylene)-5-methoxy-3,3a,8,8a-tetrahydro-1-oxa-cyclopenta[*a*]inden-2-one.

The comparison of its CD spectrum with those reported for strigolactone analogues[17] assigned to **1** the absolute stereochemistry as depicted in Figure 1. The spectrum revealed a negative band around 250 nm, typical of an *ent*-stereochemistry, and a negative CD around 270 nm consistent with a 2′-*R*-configuration.

Peagoldione had a sodium clustered ion $[M+Na]^+$, in the HRESIMS spectrum, at *m*/*z* 301. 0696 (calcd for $C_{14}H_{14}NaO_6$, 301.0688), corresponding to the molecular formula $C_{14}H_{14}O_6$. The inspection of its ^{1}H and ^{13}C NMR data (Table 2), also compared to those of **1**, suggested the presence of two furanone rings (B and C), which are joined by a tri-substituted olefinic group, a terminal ethyl residue, two methylene, and one methine groups.

In fact, the ^{1}H NMR spectrum (Table 2) showed the protons of the two tri-substituted furanones (B and C) as doublets at δ 6.01 (H-15, J = 6.5 Hz) and 6.16 (H-3, J = 9.4 Hz). They both coupled in COSY spectrum with the double doublet (J = 9.4 and 6.5 Hz) at δ 7.26 attributed to the proton (H-2) of the exocyclic tri-substituted olefinic group. These protons coupled in the HSQC spectrum with the carbons at δ 102.8, 113.2, and 143.8 corresponding to C-15, C-3, and C-2, respectively. This partial structure was confirmed by the couplings observed in the HMBC spectrum (Table 2) between H-15 and H-2 with C-14, and H-3 with C-15, while in the NOESY spectrum H-2 (Table 2) coupled with H-3. Furthermore, the ^{1}H NMR spectrum showed the presence of a broad singlet at δ 3.56, attributed to a proton (H-9) of a secondary oxygenated carbon (C-9, δ 72.3 in the ^{13}C NMR spectrum, Table 2) of the 1-ethylpropanoxy chain, which close the deca-membered ring A, also including the two furanone ones. In fact, this proton (H-9) resulted coupled in the COSY spectrum with the protons of both adjacent methylene groups CH_3CH_2 and CH_2-10, which in turn coupled with the terminal methyl group (CH_3CH_2) and the other methylene group CH_2-11. The latter represents the attaching point at C-12 of the B ring, while the oxygen (O-8) of the ethylpropanoxy chain close at C-7 of the C ring. This structure was confirmed by the coupling observed in the HMBC between H_2-11 with both C-14 and C-15, and H-15 with C-11, while in the NOESY spectrum (Table 2) H-15 coupled with H_2-11. On the basis of these results peagoldione can be formulated as 9-ethyl-6-hydroxy-4,8,13-trioxa-tricyclo[10.2.1.1.0*3,7*]pentadeca-1,6,12(15)-triene-5,14-dione. Furthermore, the significant nOe effect observed between H_2-10 and H-2 suggested, in agreement with an inspection of Drieding model of **2**, a bent conformation of the A ring with the CH_2-10 pointing towards C-2.

Pea crop is severely damaged by *O. crenata* infection.[3] Although little infected by other broomrape species, pea root exudates stimulate germination of other species such *P. aegyptiaca*, *O. foetida,* and *O. minor* besides *O. crenata*, causing suicidal germination.[18] Biological activity of the metabolites from pea root exudates was tested by following the standard germination bioassays.[9,10] Each root exudate fraction, and pure metabolite were dissolved in methanol and then solution-diluted with sterile distilled water (final concentration of 0.7% of methanol). Aliquots of 100 μl of each fraction were applied in three replicated dishes containing the conditioned seeds spread over discs of 1.5-cm diameter glass fiber filter paper (GFFP). The synthetic strigolactone analogue GR24 was used as a positive control at concentration 10^{-4} M.[19] The stimulatory activity of the pure metabolites obtained by purification of pea root exudates organic extract is reported in Table 3.

Peagol induced *P. aegyptiaca* and *O. foetida* seed germination when tested at 5×10^{-4} M but low activity was observed on *O. crenata* and *O. minor* (Table 3). Peagoldione tested at 2×10^{-3} M induced *P. aegyptiaca* seed germination only, with no activity on *O. crenata* or *O. minor*, and very little on *O. foetida* (Table 3). The GR24 stimulatory effect generally assumed for all broomrape species[19] is clarified in this work, which shows that GR24 is not effective on some broomrape species such as *O. foetida*. The activity

Table 3
Induction of germination of *Orobanche crenata*, *O. foetida*, *O. minor,* and *Phelipanche aegyptiaca* seeds by peagol and peagoldione, positive (GR24), and negative (0.7% MeOH in distilled water) control

Treatment/(concentration tested)	Broomrape seed germination (%)			
	O. crenata	*O. foetida*	*O. minor*	*P. aegyptiaca*
Peagol/(5×10^{-4} M)	7.0 b	27.4 a	2.7 b	42.3 b
Peagoldione/(2×10^{-3} M)	0.0 c	3.6 b	0.0 c	49.4 b
GR24/(10^{-4} M)	73.1 a	3.0 b	89.3 a	97.5 a
Negative control (distilled water)	0.0 c	0.0 c	0.0 c	0.0 c

All treatments contain 0.7% of methanol to allow comparisons.

of peagol on *O. foetida* seed germination appears to be relevant, as no germination stimulant for this species was known. Specificity of the activity of peagol and pegoldione is in agreement with specialization on host recognition by *Orobanche* and *Pheliphanche*,[18] supporting the fact that this specialization could be mediated by unique combinations between kind and amount of strigolactone exuded by each host plant.

In conclusion, we isolated from pea root exudates two new strigolactone-like metabolites that showed a lower and specific activity on different *Orobanche* and *Phelipanche* species. This is probably due to the unusual structures of **1** and **2** that differ from the well-known and the most active strigolactones isolated from the same plant. At this stage we cannot exclude that the strigolactones previously cited[14,15] could be present in the other chromatographic column fractions, whose purification is in progress.

Acknowledgments

The authors thank Mrs. Dominique Melck for recording NMR spectra. This work was supported by a Grant from the Italian Ministry of University and Research (MIUR) and by Spanish project AGL2008-01239. Contribution DISSPAPA N. 198.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.09.142.

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