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ARTICLE

Polyphenols, Including the New Peapolyphenols A−C, from Pea Root Exudates Stimulate *Orobanche foetida* Seed Germination

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Three new polyphenols, named peapolyphenols A−C, together with an already well-known polyphenol and a chalcone (1-(2,4-dihydroxyphenyl)-3-hydroxy-3-(4-hydroxyphenyl)-1-propanone and 1-(2,4-dihydroxyphenyl)-3-(4-methoxyphenyl)propenone) were isolated from pea root exudates. They were found to strongly stimulate *Orobanche* and *Phelipanche* species seed germination. Interestingly, only peapolyphenol A, 1,3,3-substituted propanone, and 1,3-disubstituted propenone had specific stimulatory activity on *O. foetida*, excluding any other *Orobanche* or *Phelipanche* species tested. This species specificity is relevant, as *O. foetida* does not respond to the synthetic strigolactone analogue GR24, commonly used as a standard for germination assays. As characterized by spectroscopic methods, peapolyphenols A−C proved to be differently functionalized polyphenols with hydroxy and methoxy groups on both the aromatic rings and the propyl chain.

KEYWORDS: Parasitic weeds; *Orobanche*; *Phelipanche*; seed germination; pea root exudates; polyphenols; peapolyphenols A−C

INTRODUCTION

Orobanche and *Phelipanche* species (the broomrapes) are parasitic plants, some of which represent serious weed problems and cause severe yield reduction of many important crops (*1*−*3*). The numerous efforts made so far have only slightly alleviated the broomrape problems, which rather has appeared to increase both in intensity and in bitterness (*1*−*3*). The main obstacle for long-term management of broomrape-infested fields is the durable seedbank, which may remain viable for decades. Although broomrape seedbanks usually give rise to a very low annual germination percentage, they need to be curbed whenever a susceptible host is grown in the infested field (*1*, *4*).

Pest control is a major problem in agriculture. It relies almost completely on chemicals, but many of them have been or will be soon withdrawn from market for toxicological and environmental reasons. This increases the need for novel, effective, and environmentally compatible alternatives. Natural product based pesticides offer many advantages, such as low environmental risks, high target selectivity, and novel mechanism of action, and show reduced risks for humans and nontarget organisms (*5*). With this perspective, different approaches have been put forward to develop natural pesticides to control *Orobanche* and *Phelipanche*. We recently isolated phytotoxic metabolites from fungi pathogens for *P. ramosa* infecting different crops and tested their effect on *Orobanche* and *Phelipanche* seed germination (*6*, *7*). In particular, fusaric and dehydrofuaric acids (*6*), neosolaniol monoacetate, and seven macrocyclic trichothecenes were isolated as potent inhibitors of *P. ramosa* from *Fusarium* species, *F. compactum*, and *Myrothecium verrucaria*, respectively (*7*). Interesting results were also obtained by testing two microbial metabolites as fusicoccins and ophiobolin A to induce the suicidal seed germination of nine *Orobanche* and *Phelipanche* species, including those resistant to a well-known chemical germination stimulant, namely the synthetic strigolactone analogue GR24 (*8*, *9*). Attention has also been focused on the isolation and identification of germination stimulants, including the metabolites recently isolated from fenugreek (*Trigonella foenum-graecum* L.) (*10*, *11*). Three different classes of plant secondary metabolites, dihydrosorgoleone, sesquiterpene lactones, and strigolactones (*12*), are known to induce seed germination of these parasites, with strigolactones showing the strongest activity. Different strigolactones were isolated from host and nonhost *Orobanche*, *Phelipanche*, and *Striga* plants (*12*, *13*), including sorgomol (*14*), isolated from root exudates of sorghum (*Sorghum bicolor* L.), and fabacyl acetate (*15*), isolated from root exudates of pea (*Pisum sativum* L.). In the root exudates of the same plant well-known strigolactones, namely didehydroorobanchol, orobanchol, orobanchyl acetate, and 5-deoxystrigol, were also identified (*16*).

We recently reported the isolation from pea root exudates of two strigolactone-like compounds (peagol and peagoldione) showing a selective stimulation of *Orobanche* seed germination (*17*).

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Table 1. ^{1}H and ^{13}C NMR Data of Peapolyphenols A−C (**2-4**)[a,b]

	2[c]		**3**		**4**	
position	δ(C) m[d]	δ(H)	δ(C) m[c]	δ(H)	δ(C) m[c]	δ(H)
C=O[e]	202 s		72.6 d	4.97 d (*J* = 5.0)	67.8 d	4.61 d (*J* = 3.4)
C-α	78.4 d	5.22, dd (*J* = 7.4 and 3.4)	76.8 d	3.97 ddd (*J* = 9.7, 5.0, and 2.5)	72.2 d	3.94 dd (*J* = 9.7 and 3.4)
C-β	41.2 t	2.98 dd (*J* = 14.2 and 3.4)	38.2 t	2.80 dd (*J* = 14.2 and 2.5)	77.8 d	4.95 d (*J* = 9.7 Hz)
		2.52 dd (*J* = 14.2 and 7.4)		2.48 dd (*J* = 14.2 and 9.7)		
1	128.9 s		107.5 s		104.0 s	
2	131.6 d	6.93 d (*J* = 8.1)	157.0 s		156.7 s	
3	116.1 d	6.66 d (*J* = 8.1)	107.9 d	6.44 br s	103.4 d	6.28 d (*J* = 2.2)
4	156.8 s		158.0 s		160.0 s	
5	116.1 d	6.66 d (*J* = 8.1)	115.9 d	6.42 br d (*J* = 8.1)	109.8 d	6.45 dd (*J* = 8.4 and 2.2)
6	131.6 d	6.93 d (*J* = 8.1)	129.7 d	7.30 d (*J* = 8.1)	132.6 d	7.16 d (*J* = 8.4)
1′	105.0 s		132.1 s		131.0 s	
2′	165.0 s		131.3 d	7.00 d (*J* = 8.4)	130.2 d	7.31 d (*J* = 8.5)
3′	100.0 d	6.39 br s	122.1 d	6.68 d (*J* = 8.4)	116.0 d	6.83 d (*J* = 8.5)
4′	165.6 s		156.0 s		158.6 s	
5′	109.9 d	6.45 br d (*J* = 8.6)	122.1 d	6.68 d (*J* = 8.4)	116.0 d	6.83 d (*J* = 8.5)
6′	134.5 d	7.74 d (*J* = 8.6)	131.3 d	7.00 d (*J* = 8.4)	130.2 d	7.31 d (*J* = 8.5)
MeO	56.0 q	3.86 s	55.7 q	3.81 s		

[a] The chemical shifts are in δ values (ppm) from TMS; *J* values are given in Hz. [b] 2D ^{1}H, ^{1}H (COSY), ^{13}C, and ^{1}H (HSQC) NMR experiments delineated the correlations of all the protons and the corresponding carbons. [c] Recorded in $CDCl_3/CD_3OD$, 95/5. [d] Multiplicities were assigned by DEPT spectra. [e] This carbon in peaphenols B and C is CH−OH.

In this paper we describe the isolation, structural elucidation, and biological characterization of three new polyphenols, named peapolyphenols A−C, isolated from pea root exudates together with the well-known polyphenol 1-(2,4-dihydroxyphenyl)-3-hydroxy-3-(4-hydroxyphenyl)-1-propanone and the chalchone 1-(2,4-dihydroxyphenyl)-3-(4-methoxyphenyl)propenone. Their structures were determined by extensive use of NMR and MS techniques.

MATERIALS AND METHODS

General Experimental Procedure. ^{1}H NMR spectra were recorded in CD_3OD at 600 and 400 MHz, on Bruker (Karlsruhe, Germany) spectrometers. ^{13}C NMR spectra were recorded in CD_3OD (unless otherwise noted) at 150, 100, and 75 MHz using the same instruments. The solvent (CD_3OD) was used as internal standard. Carbon multiplicities were determined by DEPT experiments (*18*). DEPT, COSY-45, HSQC, HMBC, and NOESY experiments (*18*) were performed using Bruker microprograms. HRESIMS and ESIMS spectra were recorded on a Waters Q-TOF Micro (Milford, MA) instrument. Analytical and preparative TLC was performed on silica gel (Kieselgel 60 F_{254}, 0.25 and 0.50 mm, respectively, Merck, Darmstadt, Germany) or reverse phase (Whatman, KC18 F_{254}, 0.20 mm, Maidstone, U.K.) plates; the spots were visualized by exposure to UV light or by spraying first with 10% H_2SO_4 in methanol and then with 5% phosphomolybdic acid in ethanol, followed by heating at 110 °C for 10 min. For column chromatography (CC), Kieselgel 60, 0.063−0.200 mm (Merck, Darmstadt, Germany), was used.

Production of Pea Root Exudates. Seeds of pea cv. Messire were sterilized in 4% sodium hypochlorite containing 0.02% (v/v) Tween 20, rinsed with sterile water, and then germinated in pots filled with sterile perlite watered with Hoagland nutrient solution modified to a one-fourth strength of phosphorus. A total of 1500 pea plants were cultivated. Pea plants that were 15 days old were transferred from the perlite to flasks filled with 150 mL of sterile distilled water, allowing the release of the root exudate over 2 days. Five plants were fitted per flask, with 300 flasks used in total. The hydroponically collected root exudates released, 45 L, were immediately frozen at −80 °C and then lyophilized at −20 °C.

Extraction and Purification of Peapolyphenols. The dry powder (22 g) of lyophilized root exudates (45 L) was dissolved in distilled water (400 mL) and extracted with EtOAc (3 × 400 mL). The organic extracts were combined, dried (Na_2SO_4), and evaporated under reduced pressure. The extract (285 mg) showed a high stimulatory activity on the seed germination of four broomrape species, namely *Phelipanche aegyptiaca* (Pers.) Pomel (syn. *O. aegyptiaca* Pers.), *Orobanche crenata* Forsk., *O. foetida* Poir. and *O. minor* Sm. The extract was purified by silica gel column chromatography with $CHCl_3$/*i*-PrOH (9/1) as eluent, yielding nine homogeneous fraction groups. The residues of fractions 2−4 showed strong stimulatory activity on germination of the mentioned broomrape species. The residue (32.7 mg) of fraction 1 was identified as pisatin. The residue (120 mg) of the second fraction was further purified by preparative TLC on silica gel (eluent $CHCl_3$/*i*-PrOH (9/1)), yielding eight fractions. The residue of fraction 1 (19.4 mg) was a further amount of pisatin. Fractions 4 and 6 yielded peagol and peagoldione, respectively, as previously reported (*17*). The residue (6.0 mg) of the seventh fraction was further purified by silica gel TLC (eluent $CHCl_3$/*i*-PrOH (9:1)), yielding the two compounds 1-(2,4-dihydroxyphenyl)-3-hydroxy-3-(4-hydroxyphenyl)-1-propanone and 1-(2,4-dihydroxyphenyl)-3-(4-methoxyphenyl)propenone (**1** and **5**, 0.8 and 0.6 mg, R_f 0.44 and 0.59, respectively). The residue (5.0 mg) of the eighth fraction was further purified by silica gel TLC (eluent $CHCl_3$/*i*-PrOH (9/1)) to yield peapolyphenol A (**2**, 0.8 mg, R_f 0.41). The residue (35.0 mg) of the third fraction of the initial column was further purified by silica gel TLC (eluent $CHCl_3$/*i*-PrOH (9:1)), yielding eight fractions. The residue (3.5 mg) of the eighth fraction was further purified by silica gel TLC (eluent $CHCl_3$/MeOH (9:1)), yielding peapolyphenols B and C (**3** and **4**, both 1.2 mg, R_f 0.27 and 0.25, respectively).

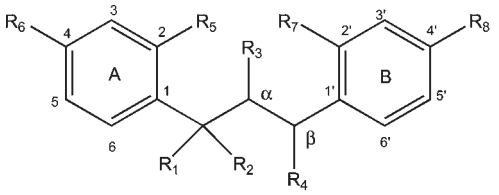
Table 2. HMBC Data of Peapolyphenols A−C (**2**−**4**)

C	**2**	**3**	**4**
CH−OH		H-6	H-6
C-α		H_2-β	H-β
C-β		H-2′,6′	CH−OH, H-α, H-2′,6′
C-1	H-2, H-3	H-3	
C-2		H-6, H-3	CH−OH, H-3, H-6
C-4			H-6
C-3			H-5
C-1′	H_2-β	H_2-β	H-3′,5′
C-2′			H-β
C-4′		H-2′,6′, H-3′,5′	H-2′,6′
C-6′			H-β

Peapolyphenols A−C (2−4). 2 is obtained as a homogeneous oil, and **3** and **4** are obtained as amorphous solids. For ^{1}H and ^{13}C NMR spectra, see **Tables 1** and **2**. HRESIMS (+): **2**, *m*/*z* 289 $[M + H]^+$, 311.0905 (calcd for $C_{16}H_{16}NaO_5$ 311.0895, $M + Na]^+$; **3**, *m*/*z* 313.1061 [calcd for $C_{16}H_{18}NaO_5$ 313.1052, $M + Na]^+$, 603 $[2M + Na]^+$; **4**, *m*/*z* 293 $[M + H]^+$, 315.0836 (calcd for $C_{16}H_{16}NaO_6$ 315.0845, $M + Na]^+$.

2′,4′-Dihydroxy-4-methoxychalchone (5). 5 was obtained as an amorphous solid. ^{13}C NMR ($CDCl_3/CD_3OD$, 95/5): δ 193.0 (s, C=O), 166.0 (s, C-4), 165.0 (s, C-2), 163.0 (s, C-4′), 143.7 (d, C-β), 133.2 (d, C-6), 130.8 (d, C-2′,6′), 128.0 (s, C-1′), 124.5 (d, C-α), 116.3 (d, C-3′,5′), 108.8 (s, C-1), 108.4 (s, C-5), 99.6 (d, C-3).

Broomrape Seed Germination Bioassay. The biological activity of pea root exudates and its organic extract, chromatographic fractions, and

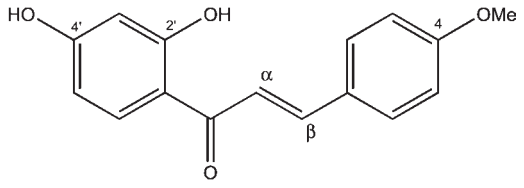


1 $R_1+R_2=O$, $R_3=R_7=H$, $R_4=R_5=R_6=R_8=OH$

2 $R_1+R_2=O$, $R_3=R_6=R_7=OH$, $R_4=R_5=H$, $R_8=OMe$

3 $R_1=R_3=R_5=R_8=OH$, $R_2=R_4=R_7=H$, $R_6=OMe$

4 $R_1=R_3=R_4=R_5=R_6=R_8=OH$, $R_2=R_7=H$



5

Figure 1. Structures of peapolyphenols A−C (**2**−**4**), 1-(2,4-dihydroxyphenyl)-3-hydroxy-3-(4-hydroxyphenyl)-1-propanone, and 1-(2,4-dihydroxyphenyl)-3-(4-methoxyphenyl)propenone (**1** and **5**).

pure metabolites were assayed on *P. aegyptiaca*, *O. foetida*, *O. minor*, and *O. crenata* seeds. For this purpose, seeds were surface-sterilized with formaldehyde, rinsed thoroughly with sterile distilled water, and dried in a laminar air flow cabinet. Approximately 100 seeds of each broomrape species were placed separately on disks of 1.5 cm diameter glass fiber filter paper (GFFP) moistened with 120 μL of sterile distilled water and conditioned in a 10 cm sterile Petri dish in the dark at 20 °C for 10 days. GFFP disks were then transferred onto a sterile sheet of paper to remove the water and again transferred to a new 10 cm sterile Petri dish. Tested samples were dissolved in methanol, and then the solution was diluted with sterile distilled water (final concentration of 0.7% of MeOH). Aliquots of of each fraction (100 μL) were applied in three replicated dishes containing the conditioned seeds. Seeds were incubated in the dark at 20 °C for 7 days to promote germination. Sterile distilled water (0.7% of MeOH) was used as a negative control. The synthetic strigolactone analogue GR24 was used as a positive control at a concentration of 10^{-5} M (*19*). Seeds with an emerged radicle through the seed coat were scored as germinated using a stereoscopic microscope at 30× magnification, and the percentage of germination was established for each dish.

RESULTS AND DISCUSSION

The organic extract of pea root exudates, showing a high stimulatory activity on seed germination of four broomrape species, namely *P. aegyptiaca*, *O. crenata*, *O. foetida*, and *O. minor*, was purified by a combination of CC and preparative TLC as detailed in Materials and Methods. This purification process allowed the isolation of two new strigolactone-like compounds named peagol and peagoldione (*17*), pisatin (3-methoxy-6*H*-[1,3]-dioxolo[5,6]benzofuro[3,2-*c*][1]benzopyran-6a(12aH)-ol), a very well-known phytoalexin belonging to the pterocarpon subgroup of isoflavonoids (*20*), and five other metabolites as a homogeneous amorphous oil and solids (**1**−**5**; **Figure 1**). As suggested by preliminary ^{1}H and ^{13}C NMR data, the last compounds appear to be related compounds belonging to chalcones and polyphenols, well-known groups of natural compounds occurring also in plants and as microbial metabolites (*20*). However, two of them (**1** and **5**) appeared to be a polyphenol and a chalcone already reported (*21*, *22*). The first one (**1**) was identified as 1-(2,4-dihydroxyphenyl)-3-hydroxy-3-(4-hydroxyphenyl)-1-propanone (β-hydroxy-DHP); it was previously isolated from the licorice root (*Glycyrrhiza glabra*) together with other polyphenols and chalcones and some of their glucosides (*21*). Licorice is an herb commonly used in cancer treatment, despite little understanding of the biological activity of its metabolites (*21*). A recent study demonstrated that β-hydroxy-DHP induced Bcl-2 phosphorylation, apoptosis, and G2/M cell cycle arrest in breast and prostate tumor cells, similarly to the action of a more complex (MW >800) antimicrotubule agent used clinically (*21*). However, comparison of the ^{1}H and ^{13}C NMR spectra of **1** with those reported (*21*) showed small differences in chemical shifts, probably due to the different solvents used to record the spectra, and a substantially different coupling between H-β and H-α', while the analogous coupling of the other α-proton and the geminal coupling are similar. The protons H-β and H-α, resonating as two double doublets at δ 5.04 and 2.77, had coupling constants of 6.97 and 4.07 Hz and of 14.2 and 6.97 Hz, respectively, while the literature values are 13.0 and 3.0 Hz and 16.3 and 13.0 Hz (*21*). This could suggest a different stereochemistry at C-β in **1** with respect to the β-hydroxy-DHP and therefore their enantiomeric relation. Unfortunately, since the total amounts of **1** and **2**−**5** were not sufficient, no effort was made to determine their stereochemistry. Moreover, the β-hydroxy-DHP stereochemistry was not previously determined (*21*). The chalcone **5** was identified as the 2′,4′-dihydroxy-4-methoxychalcone, previously isolated together with some flavonoids from *Oxytropis falcata*, which is a wild growing Leguminosae plant mainly distributed in the Qinghai-Tibet Plateau of China (*22*).

The ^{1}H NMR data of **5** showed little difference from those reported (*23*), as the spectrum was recorded in D_2O/DMSO-d_6 1/1 at 200 MHz, while the ^{13}C NMR data are reported here for the first time.

Peapolyphenol A (**2**; **Figure 1**) showed a molecular weight of 288, as deduced by HRESIMS, which, in comparison to that of **1**, was consistent with a presence of a methoxy group attached to one of the two aromatic rings A and B. Comparison of the ^{1}H and ^{13}C NMR spectra of **2** and **1** (**Table 1**) showed, in addition to the expected signals of the methoxy group at δ 3.86 (^{1}H) and 56.0 (^{13}C), a different substitution pattern of the two aromatic rings and of the 1-propanone chain. In fact, in **2**, as also shown by COSY and HSQC spectra (*18*), the aromatic ring protons attached to the ketone group and the β-carbon (ring A and B, respectively) generated signal systems typical of *p*-hydroxy- and 2-hydroxy-4-methoxy-substituted benzene rings, respectively (**Table 1**) (*24*). Furthermore, an ABX spin system was observed in the ^{1}H NMR spectrum as three double doublets (J = 7.4 and 3.4 Hz, J = 14.2 and 3.4 Hz, and J = 14.2 and 7.4 Hz) at δ 5.22, 2.98, and 2.52. COSY and HSQC spectra indicated that such a system stems from H-α and H_2-β, therefore identifying a partial structure with the hydroxyl group on the C-α and not on the C-β as in **1**. Hence, the ^{13}C signals resonating at δ 78.4 and 41.2 were assigned to C-α and C-β, respectively (*25*). This was consistent with the coupling between C-1′ and H_2-β observed in the HMBC spectrum (**Table 2**) (*18*). The methoxy group was located at C-4′ for the coupling between the MeO with both H-3′ and H-5′. observed in the NOESY spectrum (**Table 3**) (*18*).

The above data assigned structure **2** to peapolyphenol A. It was consistent with all the other couplings observed in the HMBC and NOESY spectra and with the sodium clustered $[M + Na]^+$ and pseudomolecular $[M + H]^+$ ions observed at m/z 311.0905 and 289 in the HRESIMS spectrum. Therefore, peapolyphenol A can be formulated as 2-hydroxy-3-(2-hydroxy-4-methoxyphenyl)-1-(4-hydroxyphenyl)propanone.

Table 3. NOE Data of Peapolyphenols A−C (**2**−**4**)

2		3		4	
obsd	effect	obsd	effect	obsd	effect
CH−OH		CH−OH	H-α	CH−OH	H-6
H-α	H-2	H-α	CH−OH, H_2-β, H-2′	H-α	H-2′, H-6′
H_2-β	OMe, H-2	H_2-β	H-α, H-2′	H-β	H-2′, H-6′
H-1		H-1		H1	
H-2	H-3, H-α,H_2-β	H-2		H-2	
H-3	H-2	H-3	MeO	H-3	
H-4		H-4		H-4	
H-5	H-6	H-5	H-6, MeO	H-5	H-6
H-6	H-5	H-6	H-5	H-6	CH−OH, H-5
H-2′		H-2′	H-α, H_2-β	H-2′	H-α, H_2-β, H-3′
H-3′	MeO	H-3′		H-3′	H-2′
H-4′		H-4′		H-4′	
H-5′	MeO, H-6′	H-5′		H-5′	H-6′
H-6′	H-5′	H-6′	H-α, H_2-β	H-6′	H-α, H_2-β, H-5′
MeO	H_2-β, H-3′, H-5′	MeO	H-3, H-5		

Peapolyphenol B (**3**; **Figure 1**) showed a molecular weight of 290, as deduced by HRESIMS, which was consistent with two more hydrogen atoms with respect to **2**. In fact, comparison of their ^{1}H and ^{13}C NMR spectra (**Table 1**) showed the absence of the ketone group and the presence of a further secondary hydroxylated carbon at C-1 of the alkyl chain, with a pattern typical of 1,2-propanediol. A doublet (J = 5.0 Hz), a doublet of double doublets (J = 9.7, 5.0, and 2.5 Hz), and two double doublets (J = 14.2 and 2.5 Hz and J = 14.2 and 9.7) were present at δ 4.97, 3.97, 2.80, and 2.48. They were assigned, on the basis of the couplings observed in the COSY and HSQC spectra, to HC-1 of the propanediol chain, to H-α, and to CH_2-β, respectively (*24*). The HSQC spectrum also allowed the assignment of the signals observed at δ 72.6, 76.8, and 38.2 to C-1 of the propanediol chain, C-α and C-β, respectively (*25*). The presence of 1,2-propanediol attached to the head and the tail of rings A and B, respectively, was supported by the HMBC couplings observed (**Table 2**) between the C-1 of the alkyl chain and H-6, the C-β and H-2′,6′ and the C-1′ and H_2-β. We also observed NMR evidence of an *ortho-para* and a *para*-substitutionfor the ring A and B, respectively. The location at C-4 of the methoxy group, resonating at δ 3.81 (^{1}H) and 55.7 (^{13}C), was supported by the coupling observed in the NOESY spectrum (**Table 3**) between this group and both H-3 and H-5. These results indicated that the structure **3** can be assigned to peapolyphenol B. Such a structure was also consistent with all the other couplings observed in the HMBC and NOESY spectra and the sodium dimer $[2M + Na]^+$ and self-clustered $[M + Na]^+$ ions observed at m/z 603 and 313.1061 in the HRESIMS spectrum. Peapolyphenol B can be formulated as 1-(2-hydroxy-4-methoxyphenyl)-3-(4-hydroxyphenyl)propane-1,2-diol.

Peapolyphenol C (**4**, **Figure 1**) showed a molecular weight of 292 as deduced by HRESIMS. As clearly inferred from its ^{1}H and ^{13}C NMR spectra (**Table 1**), this was consistent with an extra oxygen atom and the lack of the methoxy group with respect to **3**. The COSY and HSQC spectra showed similar substitution patterns of the A and B rings. Therefore, the two rings are ortho,para- and para-hydroxylated, respectively. The ^{1}H and ^{13}C spectra also showed signals typical of a 1,2,3-propanetriol moiety. In fact, the double doublet (J = 9.7 and 3.4 Hz) resonating at δ 3.94 and assigned to H-α coupled in the COSY spectrum with two doublets (J = 9.7 Hz and J = 3.4 Hz) at δ 4.95 and 4.61, which were attributed to H-β and the C-1 of the propanetriol chain (*24*). The couplings observed in the HSQC spectrum allowed the assignment of the signals at δ 77.8, 72.2, and 67.8 to C-β, C-α, and C-1 of the same chain (*25*). On the basis of these results, structure **4** could be assigned to peapolyphenol C. This structure was confirmed by the HMBC couplings observed (**Table 2**) between C-1 of the propanetriol chain and H-6, C-2, and the proton at C-1 of the propanetriol chain, C-β and H-2′,6′, and C-2′ and C-6′ with H-β. The location of the hydroxyl group on ring A at C-2 and C-4 was supported by NOESY couplings (**Table 3**) between H-5 and H-6 and of this latter atom with the proton of the C-1 of the propanetriol chain. Structure **4** was also supported by all the other couplings observed in the HMBC and NOESY spectra and by the sodium clustered $[M + Na]^+$ and pseudomolecular $[M + H]^+$ ions observed in the HRESIMS spectrum at m/z 315.0836 and 293. Peapolyphenol C can be formulated as 1-(2,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)propane-1,2,3-triol.

The effects observed in the NOESY spectra (**Table 3**) of the polyphenols A−C suggested for all of them a flexible bended structure in which rings A and B interact.

The configuration of bioactive natural products provides essential information for both the total synthesis and the molecular mode of action. In some cases the configuration has been determined by X-ray analysis or synthetic work. Unfortunately, polyphenols **2**−**4**, obtained as oils in very small amounts, are resistant to crystallization and at least two of them (**3** and **4**) are laborious to synthesize because of the number of chiral centers they contain. The same features prevent the application of Mosher's method for the determination of the absolute configuration of secondary hydroxylated carbons based on a combination of chemical and spectroscopic techniques (*26*−*28*). NOE-based methods in combination with molecular mechanics calculations have been proposed for configuration assignments of flexible molecules, particularly for macrocyclic compounds. However, it is still very difficult to assign the stereochemistry of highly flexible carbon chains because of the presence of multiple conformers, in which minor populations often make disproportionately large contributions to NOE intensity and, occasionally, lead to contradictory distance constraints. This is the case for polyphenols **3** and **4**, for which the NOESY effects (**Table 3**) detected between the protons of different moieties only suggest a bended conformation. Furthermore, new methods developed for stereochemical determinations of acyclic and small organic compounds, respectively based on carbon−proton spin-coupling constants (*29*) and on the analysis of chiroptical properties of the Cotton effects present in their circular dichroism spectra, in turn confirmed by means of $[\alpha]_D$ ab initio calculations (*30*) and residual dipolar couplings (*31*) were not applicable to **3** and **4** because of the very small amount available and for their scant water solubility.

Pea root exudates can potentially induce the germination of all species tested in this work, as do the crude pea root exudates (*32*) and the ethyl acetate organic extract of root exudates (*17*). Pea crop is highly damaged by *O. crenata* infection; however, there is little or no infection by *O. foetida*, *O. minor*, and *P. aegyptiaca*, thus causing the suicidal germination of these three important parasitic weeds (*33*). Polyphenols **1**, **2**, and **5** induced seed germination of *O. foetida* when tested at a 10^{-3} M level, with reduced activity at 0.5×10^{-3} M and none at lower concentrations. No activity was observed on *P. aegyptiaca*, *O. crenata*, or *O. minor* (**Table 4**). Polyphenols **3** and **4** did not show any activity on any of the species tested. The GR24 stimulatory effect generally assumed for all broomrape species (*19*) is clarified in this work, confirming that GR24 is not effective on some broomrape species such as *O. foetida*. The activity of **1**, **2**, and **5** on *O. foetida* seed germination appears to be very important, as no germination stimulant for this species is known, apart from the recently isolated peagol and peagoldione (*17*). This is in agreement with specialization

Table 4. Percentage of Germination of *P. aegyptiaca*, *O. crenata*, *O. foetida*, and *O. minor* Seeds When Exposed to Pea Root Exudates, Its Organic Extract, and Polyphenols **1**−**5**[a]

compd	concn	broomrape seed germination (%)			
		P. aegyptiaca	*O. crenata*	*O. foetida*	*O. minor*
control (distilled water)		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
control + GR24	10^{-5} M	85.3 ± 0.9[c]	79.2 ± 3.2[c]	3.3 ± 0.9	89.0 ± 1.5[c]
crude pea root exudate	0.03 g of pea root/mL	73.3 ± 4.4[c]	59.3 ± 3.0[c]	58.7 ± 1.9[c]	70.7 ± 1.6[c]
pea root exudate organic extract	100 mg/L	85.7 ± 2.3[c]	68.3 ± 4.3[c]	65.7 ± 2.3[c]	77.0 ± 1.5[c]
1	10^{-3} M	0.0 ± 0.0	0.0 ± 0.0	34.2 ± 0.7[c]	0.0 ± 0.0
	0.5×10^{-3} M	0.0 ± 0.0	0.0 ± 0.0	26.0 ± 0.7[c]	0.0 ± 0.0
	10^{-4} M	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	10^{-5} M	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2	10^{-3} M	0.0 ± 0.0	0.0 ± 0.0	11.5 ± 0.9[c]	0.0 ± 0.0
	0.5×10^{-3} M	0.0 ± 0.0	0.0 ± 0.0	4.1 ± 0.6[b]	0.0 ± 0.0
	10^{-4} M	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	10^{-5} M	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
3	10^{-3} M	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	0.5×10^{-3} M	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	10^{-4} M	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	10^{-5} M	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
4	10^{-3} M	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	0.5×10^{-3} M	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	10^{-4} M	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	10^{-5} M	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
5	10^{-3} M	0.0 ± 0.0	0.0 ± 0.0	36.5 ± 3.0[c]	0.0 ± 0.0
	0.5×10^{-3} M	0.0 ± 0.0	0.0 ± 0.0	10.3 ± 0.4[b]	0.0 ± 0.0
	10^{-4} M	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	10^{-5} M	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
LSD		10.3	12.2	4.6	5.6

[a] Distilled water was used as a negative control, and the synthetic strigolactone GR24 (10^{-5} M) was used as a positive control. 0.7% methanol was added to all treatments in order to dissolve the residual oil and also was added to positive and negative controls to allow valid comparisons. Percentages of germination data were calculated from 100 seeds. Analysis of variance was applied to replicate data on each treatment. Data are the means ± se. Significant differences at $P < 0.01$ and 0.001 indicated respectively by b and c were identified by comparing each treatment with the negative control by using Dunnett's test. The least significant difference between means in each broomrape species is indicated by the LSD value.

on host recognition by *Orobanche* and *Pheliphanche* (*32*), supporting that it could be mediated by unique combinations between the kind and amount of strigolactones exuded by each host plant.

In conclusion, three new polyphenols out of five were isolated from pea root exudates. Although they have previously been reported as plant metabolites, 1-(2,4-dihydroxyphenyl)-3-hydroxy-3-(4-hydroxyphenyl)-1-propanone (β-hydroxy-DHP) and 2′,4′-dihydroxy-4-methoxychalcone were isolated for the first time from *P. sativum*. They all belong to polyphenols which can be considered a combination of the shikimate and acetate pathways, which generate stilbenes and chalcones. The latter are very well-known precursors of flavonoids (a group of compound omnipresent in the plant kingdom (*20*, *34*−*36*)) and also as microbial metabolites such as cavoxin, the main toxin produced by *Phoma cava* isolated from chestnut (*37*). Isoflavonoids were diffused essentially among leguminous. Rearranged and polysubstituted isoflavonoids represent the subgroup of pterocarpans as medicarpina, found in alfalfa (*Medicago sativa* L.) (*38*) and in chickpea (*Cicerum arietinum* L.) seeds (*39*), and pisatin found in pea (*P. sativum*), which are plant defense compounds (phytoalexins) together with the closely related maackiain (*20*). Therefore, the relatively large amount of pisatin isolated from pea root exudates in the present study was somehow expected. In fact, biosynthetic studies demonstrated the excellent incorporation of chalcones and isoflavones into pisatin in *P. sativum* (*40*) as well as in related pterocarpans in *Medicago sativa* (*38*).

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