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Heliolactone, a non-sesquiterpene lactone germination stimulant for root parasitic weeds from sunflower

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ABSTRACT

Root exudates of sunflower (*Helianthus annuus* L.) line 2607A induced germination of seeds of root parasitic weeds *Striga hermonthica*, *Orobanche cumana*, *Orobanche minor*, *Orobanche crenata*, and *Phelipanche aegyptiaca*. Bioassay-guided purification led to the isolation of a germination stimulant designated as heliolactone. FT-MS analysis indicated a molecular formula of $C_{20}H_{24}O_6$. Detailed NMR spectroscopic studies established a methylfuranone group, a common structural component of strigolactones connected to a methyl ester of a C_{14} carboxylic acid via an enol ether bridge. The cyclohexenone ring is identical to that of 3-oxo-α-ionol and the other part of the molecule corresponds to an oxidized carlactone at C-19. It is a carlactone-type molecule and functions as a germination stimulant for seeds of root parasitic weeds. Heliolactone induced seed germination of the above mentioned root parasitic weeds, while dehydrocostus lactone and costunolide, sesquiterpene lactones isolated from sunflower root exudates, were effective only on *O. cumana* and *O. minor*. Heliolactone production in aquacultures increased when sunflower seedlings were grown hydroponically in tap water and decreased on supplementation of the culture with either phosphorus or nitrogen. Costunolide, on the other hand, was detected at a higher concentration in well-nourished medium as opposed to nutrient-deficient media, thus suggesting a contrasting contribution of heliolactone and the sesquiterpene lactone to the germination of *O. cumana* under different soil fertility levels.

1. Introduction

Root parasitic weeds of the genera *Striga*, *Orobanche*, and *Phelipanche* adversely affect many important food crops. The germination of parasitic root weeds depends on chemical signals released from host and non-host plants into the rhizosphere. Since the identification of strigol (Cook et al., 1972), a series of analogous compounds have been isolated. Strigol and analogous compounds, generally known as strigolactones (SLs) (Butler, 1995), share a common structure composed of a tricyclic lactone (the ABC part) connected via an enol ether bridge to a methylfuranone group (the D-ring). SLs were recently identified as a hyphal branching factor for arbuscular mycorrhizal (AM) fungi and a new class of plant hormones that play a key role in the regulation of plant architecture (Ruyter-Spira et al., 2013). Recently, it was demonstrated that SLs were biosynthesized from carlactone (**1**) (Seto et al., 2014). The latter has a SL-like carbon skeleton and is synthesized from β-carotene catalyzed by sequential reactions of three biosynthetic enzymes: D27, carotenoid cleavage dioxygenase 7 (CCD7), and CCD8 (Alder et al., 2012).

The root holoparasitic plant *Orobanche cumana* presents a serious constraint to sunflower (*Helianthus annuus* L.) production in Southeast Europe, the Middle East, and Southwest Asia (Parker, 2013). The sesquiterpene lactones (STLs), dehydrocostus lactone (DCL, **2**) (Joel et al., 2011), costunolide (**3**), tomentosin, and 8-epixanthatin (Raupp and Spring, 2013), potent germination stimulants for *O. cumana* seeds instead of SLs, were isolated from sunflower root exudates. Yoneyama et al. (2011) reported the detection of LC–MS/MS signals corresponding to the SLs 5-deoxystrigol (5-DS, **4**) and alectrol (**5**) in sunflower root exudates, but the eluted fractions did not induce *O. minor* seed germination. Considering the biological importance of SLs and their ubiquitous distribution in the plant kingdom (Ruyter-Spira et al., 2013), the production of SLs or its related molecule(s) by sunflowers is logical. In a preliminary experiment and as described below, it was

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observed that sunflower root exudates induced the germination of *Striga hermonthica* seeds, whereas the commercially available STLs **2** and **3** did not. Failure of these STLs to induce germination of *S. hermonthica* led to probing sunflower root exudates for non-STLs with germination inducing activity. This paper reports the isolation and identification of a carlactone-like molecule named heliolactone (**6**) from sunflower root exudates, its germination inducing activity on the seeds of several root parasitic weeds, and the effects of phosphorus and nitrogen on its production.

2. Results and discussion

2.1. Isolation and structural determination of heliolactone (*6*)

Bioassay-guided purification of sunflower root exudates by silica gel column chromatography led to the separation of active fractions that induced germination of *S. hermonthica* seeds. Fraction 10 (40% EtOAc in hexane), the most active fraction, contained a novel germination stimulant with a molecular mass of 360 and a major fragment ion at *m/z* 97, characteristic of SLs, in LC–MS/MS analysis. The stimulant was purified with reversed-phase HPLC, followed by chiral HPLC to give a nearly pure compound. The FT-MS analysis of the stimulant established the sodium adduct ion at *m/z* 383.1457 $[M+Na]^+$ calculated for $C_{20}H_{24}O_6Na$. The ^1H NMR spectrum of the stimulant in $CDCl_3$ exhibited fourteen signals (Table 1). The presence of a methyl resonance at δ_H 2.04 and two methine signals at δ_H 6.15 and 6.95, typical of the D-ring in SLs such as alectrol (**5**) (Table 1) (Ueno et al., 2011; Nomura et al., 2013), indicated that the stimulant was analogous to SLs as predicted by a major fragment ion at *m/z* 97 in its mass spectrum, as well as germination inducing activity toward *S. hermonthica* seeds. The chemical shift of an oxygen-bearing olefinic methine resonance (δ_H 7.53), corresponding to the enol ether moiety adjacent to the carbonyl group in SLs, also supported the similarity of the stimulant to SLs. However, the signal appeared as a singlet in the stimulant, although the H-6′ resonance appeared as a doublet by coupling with H-3a in SLs. In addition, two olefinic methine signals (δ_H 6.24 and 6.25) and a methoxycarbonyl resonance (δ_H 3.75) were observed. These data suggested that the stimulant had a carlactone-like carbon skeleton in which a methyl group at C-19 was oxidized and methylated to a methoxycarbonyl group. The remaining three methyl signals (δ_H 0.96, 1.04, and 1.91), two non-equivalent methylene resonances (δ_H 2.09 and 2.34), and two methine signals (δ_H 2.57 and 5.91) were similar to those of the trimethylcyclohexenone ring in 9,10-dihydroxy-4,7-megastigmadien-3-one (**7**) (Greger et al., 2001) (Table 1) and 1′-deoxyabscisic acid (1′-deoxy-ABA, **8**) (Todoroki et al., 1995), thus suggesting the presence of a 3-oxo-ε-end group instead of a β-end group in carlactone (**1**) and β-carotene (see fig. 1).

In order to separate two partially overlapping olefinic methine resonances (H-7 and H-8) occurring in $CDCl_3$, ^1H and ^{13}C NMR spectra of the stimulant were measured in C_6D_6 (Table 2). The methine signals were successfully separated and detected at δ_H 6.46 and 6.40. The *E* configuration of the double bond (C-7 and C-8) was deduced from the coupling constants (both 15.9 Hz). A DIFNOE experiment also supported the partial structure; irradiation of the H-8 proton (δ_H 6.40) caused an NOE enhancement of the H-6 resonance (Fig. 2). The ^1H and ^{13}C NMR spectroscopic data corresponding to the six-membered ring of the stimulant were similar to those of isophorone (3,5,5-trimethylcyclohex-2-en-1-one, **9**) and acetylated 3-oxo-α-ionol β-D-glucoside (Pabst et al., 1992). Moreover, those corresponding to the enol ether bridge and the D-ring were very similar to those for 5-deoxystrigol (**4**), measured in the same solvent. The ^{13}C NMR signals corresponding to the diene moiety in the acyclic C_5 branched chain were comparable with those for carlactone measured in CD_2Cl_2 (Alder et al., 2012). The connections of the structural portions mentioned above were unambiguously confirmed by HMBC data (Fig. 2). The HMBC experiment confirmed that the H-10 resonance correlated with the C-8, C-9, C-11, and C-19 signals, the H-8 signal correlated with the C-6, C-7, C-10, and C-19 resonances, and the H-6 signal correlated with the C-7 and C-8 resonances. Attachment of the methoxy group to the carbonyl group was confirmed by the HMBC correlation of the methyl proton (19-OMe) signal to the C-19 signal. All ^1H and ^{13}C NMR resonances were assigned on the basis of the DEPT, DIFNOE, ^1H–^1H COSY, HMQC and HMBC data as shown in Table 2. The structure of the novel sunflower stimulant was therefore established to be (2*E*,3*E*)-methyl 2-[(4-methyl-5-oxo-2,5-dihydrofuran-2-yl)oxy]methylene}-4-(2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)but-3-enoate, and is assigned the name heliolactone (**6**).

Table 1
^1H NMR Spectroscopic data for heliolactone (**6**), norsesquiterpene **7**, and alectrol (**5**) in $CDCl_3$.

Position[a]	**6**	Position[a]	**7**[b]	Position (part)[a]	**5**[c]
2	2.09 d (16.5 Hz) 2.34 d (16.5 Hz)	2	2.09 d (16.4 Hz) 2.33 d (16.4 Hz)	7 (A)	1.40–1.48 m
				6 (A)	1.60–1.72 m
4	5.91 br.s	4	5.91 s	5 (A)	1.86–1.96 m
6	2.57 d (7.5 Hz)	6	2.55 d (9.0 Hz)		
7	6.25 d[d] (7.5 Hz)	7	5.71 dd (15.2, 9.0 Hz)	8b (BC)	5.62 d (7.3 Hz)
8	6.24 s[e]	8	5.62 dd (15.2, 5.5 Hz)	3a (BC)	3.45 ddd (7.3, 2.7, 1.7 Hz)
		9	4.30 m		
10	7.53 s	10	3.50 dd (11.0, 7.4 Hz) 3.69 dd (11.0, 3.5 Hz)	6′ (enol ether)	7.46 d (2.7 Hz)
11	6.15 m			2′ (D)	6.16 m
12	6.95 m			3′ (D)	6.95 m
16	0.96 s	11	0.96 s	9 (A)	1.13 s
17	1.04 s	12	1.03 s	10 (A)	1.16 s
18	1.91 d (1.1 Hz)	13	1.90 d (1.6 Hz)	4 (B)	5.74 s
19-OMe	3.75 s				
20	2.04 dd (1.5, 1.0 Hz)			7′ (D)	2.04 m
				2″ (AcO)	2.05 s

[a] The numbering and the ring position are shown in Figs. 1 and 2. The numbering of heliolactone (**6**) is based on the nomenclature of carotenoids (IUPAC, 1975) and the biosynthetic pathway of carlactone proposed by Alder et al. (2012).
[b] Reported by Greger et al. (2001).
[c] Reported by Ueno et al. (2011).
[d] ABX pattern, chemical shift shows the value from the inner two peaks because the outer two peaks were not detectable.
[e] AB pattern, chemical shift shows the value from the inner peak because the outer peak was not detectable.