Phytochemistry 70 (2009) 211–215

Contents lists available at ScienceDirect

Phytochemistry

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

Fabacyl acetate, a germination stimulant for root parasitic plants from *Pisum sativum*

Xiaonan Xie [a,b], Kaori Yoneyama [a], Yuta Harada [a], Norio Fusegi [a], Yoichi Yamada [c], Satoshi Ito [d], Takao Yokota [e], Yasutomo Takeuchi [a], Koichi Yoneyama [a,*]

[a] *Weed Science Center, Utsunomiya University, 350 Mine-machi, Utsunomiya 321-8505, Japan*
[b] *United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu, Tokyo 183-8509, Japan*
[c] *Faculty of Education, Utsunomiya University, 350 Mine-machi, Utsunomiya 321-8505, Japan*
[d] *Faculty of Engineering, Utsunomiya University, 7-1-12 Yoto, Utsunomiya 321-8585, Japan*
[e] *Department of Biosciences, Teikyo University, 1-1 Toyosatodai, Utsunomiya, 320-8551, Japan*

ARTICLE INFO

Article history:
Received 6 November 2008
Accepted 8 December 2008
Available online 18 January 2009

Keywords:
Pisum sativum
Fabaceae
Orobanche minor
Orobanchaceae
Strigolactone

ABSTRACT

A germination stimulant, fabacyl acetate, was purified from root exudates of pea (*Pisum sativum* L.) and its structure was determined as *ent*-2′-epi-4a,8a-epoxyorobanchyl acetate [(3a*R*,4*R*,4a*R*,8b*S*,*E*)-4a,8a-epoxy-8,8-dimethyl-3-(((*R*)-4-methyl-5-oxo-2,5-dihydrofuran-2-yloxy)methylene)-2-oxo-3,3a,4,5,6,7,8,8b-decahydro-2*H*-indeno[1,2-*b*]furan-4-yl acetate], by 1D and 2D NMR spectroscopic, ESI- and EI–MS spectrometric, X-ray crystallographic analyses, and by comparing the ^{1}H NMR spectroscopic data and relative retention times (RR_t) in LC–MS and GC–MS with those of synthetic standards prepared from (+)-orobanchol and (+)-2′-epiorobanchol. The ^{1}H NMR spectroscopic data and RR_t of fabacyl acetate were identical with those of an isomer prepared from (+)-2′-epiorobanchol except for the opposite sign in CD spectra. This is the first natural *ent*-strigolactone containing an epoxide group. Fabacyl acetate was previously detected in root exudates of other Fabaceae plants including faba bean (*Vicia faba* L.) and alfalfa (*Medicago sativa* L.).

1. Introduction

The seeds of root parasitic plants, *Striga* and *Orobanche* spp., germinate only when they perceive chemical signals released from potential host roots (Parker and Riches, 1993; Joel et al., 1995). Among various plant secondary metabolites that have been shown to induce seed germination of root parasites (Bouwmeester et al., 2003), strigolactones are the most active, inducing germination at as low as 10 pM, and also acting as host recognition signals for symbiotic arbuscular mycorrhizal fungi (Akiyama et al., 2005). In addition to these known functions in rhizosphere communications, another function of strigolactones (or their metabolites) as plant hormones inhibiting shoot branching has recently been unveiled (Gomez-Roldan et al., 2008; Umehara et al., 2008).

So far, nine strigolactones have been isolated from the root exudates of various plant species, but several novel strigolactones remain to be characterized (Rani et al., 2008). In our previous study on the characterization of strigolactones from root exudates of the Fabaceae, a novel strigolactone was detected as a major constituent of the root exudates of *Pisum sativum*. This strigolactone was also detected in root exudates of *Astragalus sinicus*, *Arachis hypogaea, Cicer arietinum*, *Medicago sativa*, and *Vicia faba,* and thus is regarded as one of the major strigolactones of Fabaceae plants (Yoneyama et al., 2008). Gomez-Roldan et al. (2008) also identified this novel strigolactone from *P. sativum* root exudates. In this paper, we describe the isolation and structural elucidation of a novel germination stimulant fabacyl acetate (**1**) for root parasitic plants from garden pea (*Pisum satvum*) root exudates.

2. Results and discussion

Pea plants were grown hydroponically with root exudates collected as described previously (Xie et al., 2008a; Yoneyama et al., 2008). The root exudates were subjected to solvent partitioning to give a neutral EtOAc fraction. This was purified by silica gel column chromatography using *n*-hexane–EtOAc gradients as eluants. Two distinct stimulant activities, inducing >60% germination at 10,000-fold dilution, were eluted in the 30–50% and 60–80% EtOAc fractions (Fig. 2). The 60–80% EtOAc fractions were combined and purified to give orobanchol (**2**). The 30–50% EtOAc fractions, which were found to contain **1** and orobanchyl acetate (**3**) by LC–MS analysis, were combined and successively subjected to silica gel column chromatography and reversed phase HPLC to yield pure **1** and **3**.

* Corresponding author. Tel.: +81 28 649 5152; fax: +81 28 649 5155.
E-mail address: yoneyama@cc.utsunomiya-u.ac.jp (K. Yoneyama).

0031-9422/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2008.12.013

Table 1
NMR spectral data for compound **1**.

No.	d^1H (mult., *J* Hz)	d ^{13}C	DEPT and HMQC	^{1}H–^{1}H COSY	NOESY	HMBC
1						
2		169.9	C			
3		110.0	C			
3a	3.21 (*ddd*, 7.3, 2.9, 2.0)	44.0	CH	H-4, H-8b	H-4, H-8b	C-2, C-3, C-4
4	5.32 (*d*, 5.0)	81.8	CH	H-3a	H-3a, H-5, H-10, H-3‴	C-3, C-4a, C-1″, C-2″
4a		71.9	C			
5	1.80–1.91 (*m*)	22.4	CH_2	H-6	H-4	
6	1.30–1.47 (*m*)	16.3	CH_2	H-5, H-7		
7	1.02–1.05, 1.30–1.47 (*m*)	35.6	CH_2	H-6		
8		31.9	C			
8a		71.0	C			
8b	4.96 (*d*, 7.3)	81.0	CH	H-3a	H-3a, H-9	C-4, C-4a, C-8a
9	1.22 (*s*)	24.3	CH_3		H-8b	C-7, C-8, C-8a, C-10
10	1.16 (*s*)	25.7	CH_3		H-4	C-7, C-8, C-8a, C-9
1′						
2′	6.10 (*t*, 1.2)	99.7	CH	H-3′	H-6′, H-7′, H-3″	
3′	6.91 (*t*, 1.6)	140.8	CH	H-2′	H-2′, H-3″	
4′		136.3	C			
5′		170.1	C			
6′	7.43 (*d*, 2.0)	150.4	CH		H-2′	
7′	2.02 (*t*, 1.5)	10.7	CH_3		H-3′, H-6′	C-2′
1″		169.7	C			
2″	2.03 (*s*)	20.7	CH_3		H-4, H-2′, H-3″	

Compound **1** exhibited a pseudo molecular ion, $[M + H]^+$, in the HR–ESI–TOF–MS spectrum, at *m/z* 405.1542 (calcd. for $C_{21}H_{25}O_8$, 405.1549) corresponding to a molecular formula of $C_{21}H_{24}O_8$. ESI–MS analysis of **1**, affording the sodium adduct ion at *m/z* 427 $[M + Na]^+$, supported this molecular formula. The CID spectrum of **1** indicated that the $[M + Na]^+$ ion was converted with loss of AcOH $[M + Na - AcOH]^+$ to the ion at *m/z* 367 and further loss of the D ring $[M + Na - AcOH - D\ ring]^+$ to *m/z* 270 (data not shown). These data indicated that **1** has a structure either similar to orobanchyl acetate (**3**) or its isomer but with an extra oxygen atom.

The ^{1}H and ^{13}C NMR spectroscopic data (Table 1) established similarities between **1** and **3** (Matsuura et al., 2008; Xie et al., 2008b). The downfield shift of H-4 [δ^H 5.32 (1H, *d*)] in **1** indicated that the acetyloxyl group was attached to C-4. The resonances of H-4, H-3a, H-8b in compound **1** were shifted (ca. 0.3–0.6 ppm) as compared to those in **3**. Furthermore, the ^{13}C chemical shifts of C-4a and C-8a in compound **1** appeared at δ 71.9 and 71.0, respectively, clearly demonstrating that these were sp^3 carbons. These observations, as well as the presence of an extra oxygen atom in the molecule, substantiated the presence of an epoxide ring between C-4a and C-8a. Thus **1**, named fabacyl acetate, is likely to be 4a,8a-epoxyorobanchyl acetate, the structure of which is well supported by the HMQC, HMBC, and NOE analyses (Table 1).

X-ray diffraction analysis of **1** led to the final crystallographic model shown in Fig. 3, indicating that the absolute stereochemistry of **1** should be as either depicted (Fig. 1) or its antipode. It should

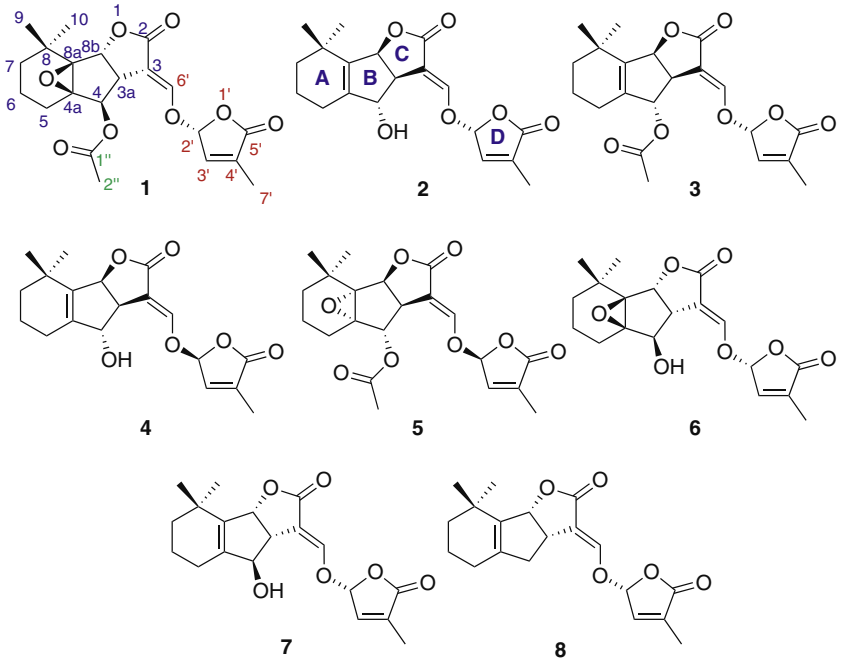

Fig. 1. Structures of fabacyl acetate (**1**), (+)-orobanchol (**2**), (+)-orobanchyl acetate (**3**), (+)-2′-epiorobanchol (**4**), (+)-2′-epi-4a,8a-epoxyorobanchyl acetate (**5**), fabacol (*ent*-2′-epi-4a,8a-epoxyorobanchol, **6**), *ent*-2′-epiorobanchol (**7**), and *ent*-2′-epi-5-deoxystrigol (**8**).

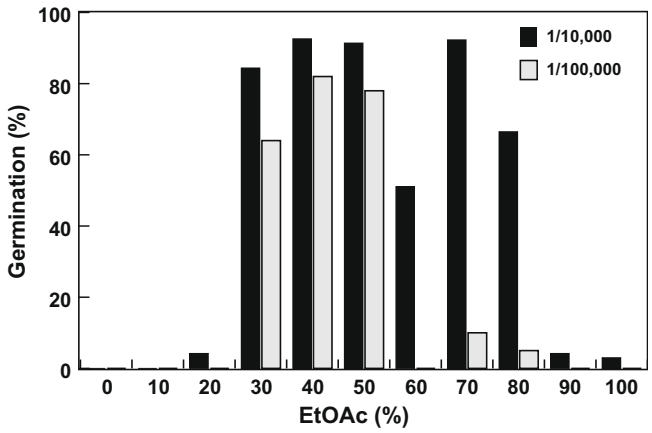

Fig. 2. Distribution of germination stimulation activity of *Pisum sativum* root exudate on *Orobanche minor* seeds after silica gel column chromatography. Germination stimulation activity of samples was examined at 10,000- and 100,000-fold dilutions.

be noticed that the epoxide and the acetyloxyl groups are oriented on the same side of the plane of ring B. The oxygen atom in the epoxide group is likely to be responsible for the ca. 0.1 ppm downfield shift of the methyl protons H-9.

To determine its absolute stereochemistry, we prepared four stereoisomers of **1** were prepared with respect to the epoxide and C-2′from (+)-orobanchol (**2**) and (+)-2′-epiorobanchol (**4**). One of the isomers, 2′-epi-4a,8a-epoxyorobanchyl acetate (**5**), was eluted with the same retention times as **1** in both LC–MS and GC–MS analyses. As further support, the ^{1}H NMR spectrum of **5** was identical to that of **1**. However, to our surprise, **5** showed a positive circular dichroism (CD), in contrast with the natural **1** which had a negative CD, leading us to conclude that **1** was the enantiomer of **5**. Consequently, the chemical structure of **1** was determined as *ent*-2′-epi-4a,8a-epoxyorobanchyl acetate [(3a*R*,4*R*,4a*R*,8b*S*,*E*)-4a,8a-epoxy-8,8-dimethyl-3-(((*R*)-4-methyl-5-oxo-2,5-dihydrofuran-2-yloxy)-methylene)-2-oxo-3,3a,4,5,6,7,8,8b-decahydro-2*H*-indeno[1,2-*b*]-furan-4-yl acetate] (Fig. 1). This strigolactone is quite unique in that it carries an epoxide group and the configuration of rings ABC is opposite that of known strigolactones (e.g., **2**).

Compound **1** was about 10-fold more active than **3**, inducing 80% germination of *O. minor* at 0.1 nM and 1 nM, respectively. This finding indicates that the unique stereochemistry of **1** comprising an α-oriented C ring, a β-epoxide group, and a β-acetyloxyl group increases the biological activity. This study also supports the earlier finding that the C-2′*R* configuration is important for germination stimulation activity (Thuring et al., 1997; Sugimoto et al., 1998; Reizelman et al., 2000).

3. Concluding remarks

Compound **1** was previously detected in root exudates of several legumes and is one of the major strigolactones in the Fabaceae plants (Yoneyama et al., 2008). Although fabacol (**6**) is expected to occur in these plant species, we could not detect it so far. This strigolactone is perhaps very unstable and/or its level is low. Applying general concepts on the biosynthesis of strigolactones, the occurrence of **1** suggests that *ent*-2′-epiorobanchol (**7**) and its precursor *ent*-2′-epi-5-deoxystrigol (**8**) exist (Rani et al., 2008). Further study is needed to clarify the biosynthetic pathway of *ent*-strigolactones along with their roles in the host recognition by root parasitic plants and by AM symbionts, and regulation of shoot branching (Gomez-Roldan et al., 2008; Umehara et al., 2008).

4. Experimental

4.1. General procedures

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ (δ_H 7.26, δ_C 77.0) on a JEOL Lambda 400 spectrometer. The standard pulse sequence and phase cycling were used for HMQC, HMBC, and NOE spectra. CD spectra were recorded with a JASCO J-720 W spectropolarimeter in MeCN. EI–GC–MS spectra were obtained with a JEOL JMX-500 and a JOEL JMS-Q1000GC/K9 using a DB-5 (J&W Scientific, Agilent) capillary column (4 or 5 m × 0.25 mm) with He carrier gas (3 ml min^{-1}). The operating conditions were the same as reported earlier (Yokota et al., 1998). ESI–LC–MS analyses were performed using a Quattro LC tandem MS instrument from Micromass (Manchester, UK). LC–MS analytical conditions were essentially the same as in (Sato et al., 2003, 2005). Column chromatography (CC) was conducted on silica gel (Wakogel C-300, Wako Pure Chemical Industries, Japan).

Synthetic standards of (+)-orobanchol (2) and (+)-2′-epiorobanchol (4) were generous gifts of Emeritus Professor Kenji Mori (The University of Tokyo, Japan). The other chemicals used, which were of analytical grade, and HPLC solvents were obtained from Kanto Chemical Co. Ltd. and Wako Pure Chemical Industries Ltd.

4.2. Plant material

Orobanche minor Sm. seeds were collected from mature plants that were parasites of red clover grown in the Watarase basin of

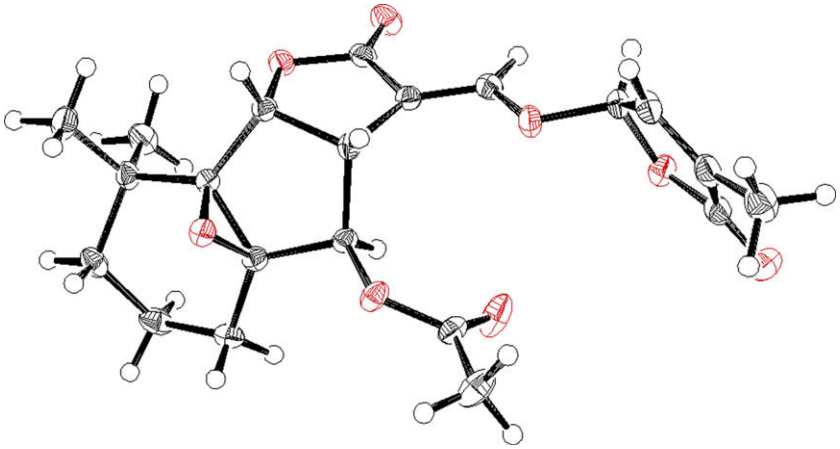

Fig. 3. X-ray structure of fabacyl acetate (**1**).

Tochigi Prefecture, Japan. Seeds of pea (*Pisium sativum* cv. Akabana–Tsurunashi) were obtained from a local supplier.

4.3. O. minor seed germination assay

Germination assays using *O. minor* seeds were conducted as reported previously (Yoneyama et al., 2007, 2008). Each test solution, unless otherwise mentioned, contained 0.1% (v/v) acetone.

4.4. Hydroponic culture of pea and collection of root exudates

The seeds of *Pisum sativum* cv. Akabana–Tsurunashi were sown on a 5 cm layer of vermiculite in a plastic strainer (28 × 23 × 9 cm, W × L × H) and grown for 3 weeks in a growth room with a 14:10 h photoperiod at 120 μmol m^{-2} s^{-1} at 25:23 °C. Tap H_2O was supplied when needed. Then two of the strainers carrying about 50 seedlings each were transferred to a larger container (53.5 × 33.5 × 14 cm, W × L × H) containing 10 l of tap H_2O and 10 mM $CaCl_2$. Root exudates released into the culture medium were adsorbed on activated charcoal (Akiyama et al., 2005). The plants were grown for 5 weeks and the culture medium and activated charcoal were changed every 3–4 days. Five containers each carrying 2 strainers and thus about 500 seedlings were used in the experiment. The root exudates absorbed on charcoal were eluted with acetone. After evaporation of the acetone *in vacuo*, the residue was dissolved in 0.2 M K_2HPO_4 (50 ml, pH 8.3) and extracted with EtOAc (3 × 50 ml). The EtOAc extracts were combined, dried ($MgSO_4$), and concentrated *in vacuo.*

4.5. Isolation of strigolactones

The crude EtOAc extracts collected during a 5-week incubation were combined (411.2 mg) and subjected to silica gel CC using a gradient of *n*-hexane–EtOAc (100:0–0:100) as eluting solvent system to give fractions 1 through 11 (Fr. 1–11). Two major germination stimulant activities were eluted in Frs. 4–6 (*n*-hexane–EtOAc, 70:30–50:50, v/v) and 7–9 (*n*-hexane–EtOAc, 40:60–20:80, v/v). By ESI–LC–MS analyses (Sato et al., 2003), Frs. 4–6 were found to contain a novel strigolactone and orobanchyl acetate (**3**), with orobanchol (**2**) detected in Frs. 7–9. Frs. 4–6 were combined (191.3 mg) and subjected to silica gel CC using *n*-hexane–EtOAc (70:30) as eluting solvent system. The active fractions combined (101.4 mg) was purified by HPLC on an ODS column (Mightysil RP-18, 10 × 250 mm, 10 μm; Kanto Chemicals, Japan) eluted isocratically with MeCN–H_2O (4:6, v/v) at a flow rate of 3.0 ml min^{-1} to give pure **1** (17.45 mg, RR_t 60.1 min) and **3** (2.33 mg, RR_t 72.4 min).

4.6. Fabacyl acetate (*1*)

White crystals; m.p. 207–209 °C; $[\alpha]_D^{24.2}$ −59.67 (*c* 0.425, MeCN). UV λ_{max}^{MeCN} nm (logε): 238 (4.27). CD (MeCN; *c* 0.0013) λ_{max} (Δε) nm: 246 (−12.71), 210 (−18.41). IR (KBr) ν_{max} cm^{-1}: 1743, 1682, 1185 (C–O), 1170 (C–O), 1011, 953. GC–EIMS, 70 eV, *m/z* (rel. int): 344 $[M-60]^+$ (1), 316 (1), 307 (3), 265 (1), 247 (21), 229 (6), 205 (3), 189 (4), 97 (100). For ^1H and ^{13}C NMR spectroscopic assignments, see Table 1.

Crystal data for **1**: $C_{21}H_{24}O_8$, *M* = 404.20, monoclinic, space group P2(1), *a* = 9.9661(7), *b* = 8.2604(6), *c* = 11.9435(8) Å, *β* = 92.5790(1), *U* = 982.20(12) $Å^3$, *Z* = 2, D_c = 1.378 g cm^{-3}, *μ* = 0.106 mm^{-1}, Mo-Kα radiation, *λ* = 0.71073 Å, *T* = 100 K, 5641 determined 3319 independent [$I > 2\sigma$ (*I*)], *R* = 0.0289, wR^2 = 0.0790. CCDC No.696228 contains the supplementary crystallographic data for **1**. This data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

4.7. Preparation of 4a,8a-epoxyorobanchyl acetate (5)

(+)-Orobanchol (2) (550 μg) and (+)-2′-epiorobanchol (4) (850 μg) were separately treated with m-chloroperbenzoic acid (MCPBA, 2 mg) in CH_2Cl_2 (100 μl) to afford epoxides (confirmed by LC–MS by monitoring the transition of *m/z* 385 > 288). The crude epoxides were partially purified by silica gel CC (*n*-hexane–EtOAc) and converted to the corresponding acetates as in the literature (Frischmuth et al., 1991), with the crude product purified by silica gel CC (*n*-hexane–EtOAc, 4:6, v/v) and preparative ODS–HPLC (MeCN–H_2O, 6:4, v/v). The RR_t of the isomers prepared from (+)-orobanchol (2) were 10.7 min (isolated yield < 50 μg) and 13.2 min (120 μg), whereas those from (+)-2′-epiorobanchol (4) were 9.9 min (170 μg) and 12.1 min (380 μg) in LC–MS (Mightysil RP-18, MeOH–H_2O, 6:4, v/v). The molecular formulae of both compounds were confirmed by HR–ESI–TOF–MS analyses (data not shown). The RR_t of fabacyl acetate (**1**) was 12.1 min and the same as that of an isomer prepared from (+)-2′-epiorobanchol (4). The four isomers were also separated by CG/MS but their RR_t values were very similar (11.0–11.2 min).

^1H NMR spectrum was obtained for the minor isomer prepared from (+)-orobanchol (2) because of its scarcity. The ^1H NMR spectra of the other three isomers were very similar and the only differences were observed for the chemical shifts of H-3a, H-4, and H-8b. The isomers in which the epoxide group and the acetyloxyl group are oriented on the same side, the protons H-3a, H-4, and H-8b resonated at *δ* 3.21, 5.32, and 4.96 ppm, respectively. By contrast, the isomer in which one of the two groups was oriented on the opposite side, the protons H-3a, H-4, and H-8b were at *δ* 3.32, 5.27, and 5.25 ppm, respectively.

4.8 (+)-2′-Epi-4a,8a-epoxyorobanchyl acetate (*5*)

1H NMR (400 MHz, $CDCl_3$) δ: 1.16 (3H, *s*, 8-Me), 1.22 (3H, *s*, (8-Me), 1.02–1.05 and 1.30–1.47 (2H, *m*, 7-CH_2), 1.30–1.47 (2H, *m*, 6-CH_2), 1.80–1.91 (2H, *m*, 5-CH_2), 2.02 (3H, *t*, *J* = 1.5 Hz, 7′-Me), 3.21 (1H, *ddd*, *J* = 7.3, 2.9 and 2.0 Hz, 3a-H), 4.96 (1H, *d*, *J* = 7.3 Hz, 8b-H), 5.32 (1H, *d*, *J* = 5.0 Hz, 4-H), 6.10 (1H, *t*, *J* = 1.2 Hz, 2′-H), 6.91 (1H, *t*, *J* = 1.6 Hz, 3′-H), 7.43 (1H, *d*, *J* = 2.0 Hz, 6′-H). UV λ_{max}^{MeCN} nm (log ε): 238 (4.26). CD (MeCN; *c* 0.00013) λ_{max} (Δε) nm: 245.5 (12.56), 210 (18.55). HR–ESI–TOF–MS $[M+H]^+$ *m/z* 405.1490 (calcd. for $C_{21}H_{25}O_8$, 405.1549). GC–EIMS, 70 eV, *m/z* (rel. int): 344 $[M-60]^+$ (1), 316 (1), 307 (2), 265 (1), 247 (22), 229 (7), 205 (3), 189 (3), 97 (100).

Acknowledgements

We are grateful to Emeritus Professor Kenji Mori (The University of Tokyo) for his generous gift of (+)-orobanchol and (+)-2′-epiorobanchol. This work was supported by a Grant-in-Aid for Scientific Research (1820810) from Japan Society for the Promotion of Science (JSPS). We thank Dr. Y. Jikumaru (RIKEN) for HR–ESI–TOF–MS measurements.

Appendix A. Supplementary material

The CD spectrum of fabacyl acetate (**1**) and its enantiomer **5** are supplied in the supplementary material. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2008.12.013.

References

Akiyama, K., Matsuzaki, K., Hayashi, H., 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 435, 824–827.

Bouwmeester, H.J., Matusova, R., Zhongkui, S., Beale, M.H., 2003. Secondary metabolite signalling in host-parasitic plant interactions. Curr. Opin. Plant Biol. 6, 358–364.

Frischmuth, K., Samson, E., Kranz, A., Welzel, P., Meuer, H., Sheldrick, W.S., 1991. Routes to derivatives of strigol (the witchweed germination factor) modified in the 5-position. Tetrahedron 7, 9793–9806.

Gomez-Roldan, V., Fermas, S., Brewer, P.B., Puech-Pages, V., Dun, E.A., Pillot, J.-P., Letisse, F., Matusova, R., Danoun, S., Portais, J.-C., Bouwmeester, H., Bécard, G., Beveridge, C.A., Rameau, C., Rochange, S., 2008. Strigolactone inhibition of shoot branching. Nature 455, 189–194.

Joel, D.M., Steffens, J.C., Matthews, D.E., 1995. Germination of weedy root parasites. In: Kigel, J., Galili, G. (Eds.), Seed Development and Germination. Marcel Dekker, New York, pp. 567–597.

Matsuura, H., Ohashi, K., Sasako, H., Tagawa, N., Takano, Y., Ioka, Y., Nebuta, K., Yoshihara, T., 2008. Germination stimulant from root exudates of *Vigna unguiculata*. Plant Growth Regul. 54, 31–36.

Parker, C., Riches, C.R., 1993. Parasitic Weeds of the World: Biology and Control. CAB International, Wallingford.

Rani, K., Zwanenburg, B., Sugimoto, Y., Yoneyama, K., Bouwmeester, H.J., 2008. Biosynthetic considerations could assist the structure elucidation of host plant produced rhizosphere signaling compounds (strigolactones) for arbuscular mycorrhizal fungi and parasitic plants. Plant Physiol. Biochem. 46, 617–626.

Reizelman, A., Scheren, M., Nefkens, G.H.L., Zwanenburg, B., 2000. Synthesis of all eight stereoisomers of the germination stimulant strigol. Synthesis 13, 1944–1955.

Sato, D., Awad, A.A., Chae, S.H., Yokota, T., Sugimoto, Y., Takeuchi, Y., Yoneyama, K., 2003. Analysis of strigolactones, germination stimulants for *Striga* and *Orobanche*, by high-performance liquid chromatography/tandem mass spectrometry. J. Agric. Food Chem. 51, 1162–1168.

Sato, D., Awad, A.A., Takeuchi, Y., Yoneyama, K., 2005. Confirmation and quantification of strigolactones, germination stimulants for root parasitic plants *Striga* and *Orobanche*, produced by cotton. Biosci. Biotechnol. Biochem. 69, 98–102.

Sugimoto, Y., Wigchert, S.C.M., Thuring, J.W.J.F., Zwanenburg, B., 1998. Synthesis of all eight stereoisomers of the germination stimulant sorgolactone. J. Org. Chem. 63, 1259–1267.

Thuring, J.W.J.F., Nefkens, G.H.L., Zwanenburg, B., 1997. Asymmetric synthesis of all stereoisomers of the strigol analogue GR24. Dependence of absolute configuration on stimulatory activity of *Striga hermonthica* and *Orobanche crenata* seed germination. J. Agric. Food Chem. 45, 2278–2283.

Umehara, M., Hanada, A., Yoshida, S., Akiyama, K., Arite, T., Takeda-Kamiya, N., Magome, H., Kamiya, U., Shirasu, K., Yoneyama, K., Kyozuka, J., Yamaguchi, S., 2008. Inhibition of shoot branching by new terpenoid plant hormones. Nature 455, 195–200.

Xie, X., Yoneyama, K., Kusumoto, D., Yamada, Y., Takeuchi, Y., Sugimoto, Y., Yoneyama, K., 2008a. Sorgomol, germination stimulant for root parasitic plants, produced by *Sorghum bicolor*. Tetrahedron Lett. 49, 2066–2068.

Xie, X., Yoneyama, K., Kusumoto, D., Yamada, Y., Yokota, T., Takeuchi, Y., Yoneyama, K., 2008b. Isolation and identification of alectrol as (+)-orobanchyl acetate, a novel germination stimulant for root parasitic plants. Phytochemistry 69, 427–431.

Yokota, T., Sakai, H., Okuno, K., Yoneyama, K., Takeuchi, Y., 1998. Alectrol and orobanchol, germination stimulants for *Orobanche minor*, from its host red clover. Phytochemistry 49, 1967–1973.

Yoneyama, K., Yoneyama, K., Takeuchi, Y., Sekimoto, H., 2007. Phosphorus deficiency in red clover promotes exudation of orobanchol, the signal for mycorrhizal symbionts and germination stimulant for root parasites. Planta 225, 1031–1038.

Yoneyama, K., Xie, X., Sekimoto, H., Takeuchi, Y., Ogasawara, S., Akiyama, K., Hayashi, H., Yoneyama, K., 2008. Strigolactones, host recognition signals for root parasitic plants and arbuscular mycorrhizal fungi, from Fabaceae plants. New Phytol. 179, 484–492.