Available online at www.sciencedirect.com
ScienceDirect
Phytochemistry 69 (2008) 427–431
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Isolation and identification of alectrol as (+)-orobanchyl acetate, a germination stimulant for root parasitic plants

Xiaonan Xie [a,b], Kaori Yoneyama [a], Dai Kusumoto [a], Yoichi Yamada [c], Takao Yokota [d], 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] *Department of Biosciences, Teikyo University, 1-1 Toyosatodai, Utsunomiya 320-8551, Japan*

Received 23 May 2007; received in revised form 12 July 2007
Available online 5 September 2007

Abstract

Alectrol, a germination stimulant for root parasitic plants, was purified from root exudates of red clover (*Trifolium pratense* L.) and identified as a strigolactone, (+)-orobanchyl acetate [(3a*S*,4*S*,8b*S*,*E*)-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-octahydro-2*H*-indeno[1,2-*b*]furan-4-yl acetate], by 1D and 2D NMR spectroscopy and ESI- and EI-MS spectrometry. Orobanchyl acetate afforded an $[M–42]^+$ ion in EI-MS and thus had been recognized as an isomer of strigol. Orobanchyl acetate was detected in root exudates of soybean (*Glycine max* L.) and cowpea (*Vigina unguiculata* L.) along with orobanchol.

Keywords: *Trifolium pratense*; Leguminosae; *Orobanche minor*; Orobanchaceae; Alectrol; Orobanchyl acetate; Strigolactone

1. Introduction

Seed germination of root parasitic plants, *Striga*, *Orobanche,* and *Alectra* in the family Orobanchaceae, is induced by germination stimulants produced by and released from roots of host and some non-host plants (Parker and Riches, 1993; Joel et al., 1995). At least three different classes of plant secondary metabolites, dihydroquinones, sesquiterpene lactones, and strigolactones, have been shown to induce seed germination of root parasites (Bouwmeester et al., 2003). Among these germination stimulants, strigolactones appear to be widely distributed in the plant kingdom and thus play pivotal roles in the interactions between root parasites and host plants; these compounds are important host-recognition signals for arbuscular mycorrhizal fungi with which >80% of land plants form symbiotic relationships (Akiyama et al., 2005; Akiyama and Hayashi, 2006).

Alectrol was originally isolated as a germination stimulant of *Striga gesnerioides* and *Alectra volgelii* from root exudates of *Vigna unguiculata* (Müller et al., 1992), and then as a stimulant of *O. minor* from root exudates of *Trifolium pratense* (Yokota et al., 1998). Some other plant species including soybean (*Glycine max* L.) were found to produce alectrol as one of the major strigolactones (Yoneyama et al., 2006). The structure (**1**) originally proposed for alectrol was, however, proven to be incorrect by chemical synthesis (Mori et al., 1998). Wigchert et al. (1999) then suggested an alternative structure for it (**2**) which has not been confirmed to date.

In this paper we describe the isolation and identification of orobanchyl acetate (**3**), a novel germination stimulant for root parasitic plants from red clover (*Ttrifolium*

* 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 © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2007.07.017

pratense) root exudates. This is the actual compound previously referred to as alectrol (**1**).

2. Results and discussion

Red clover was grown hydroponically and root exudates collected as described previously (Yoneyama et al., 2001, 2007). The root exudates were subjected to solvent partitioning to give a neutral EtOAc fraction. This was purified by a silica gel column chromatography eluted with *n*-hexane–EtOAc. Two major stimulant activities eluted in the 40% and 70% EtOAc fractions were found to contain two compounds, respectively, by LC/MS analysis; the 70% EtOAc fraction gave orobanchol as its purified product. The first compound, supposedly alectrol (**1** or **2**), eluted in the 40% EtOAc fraction was purified to homogeneity by HPLC with ODS and ODS-CN column chromatographies.

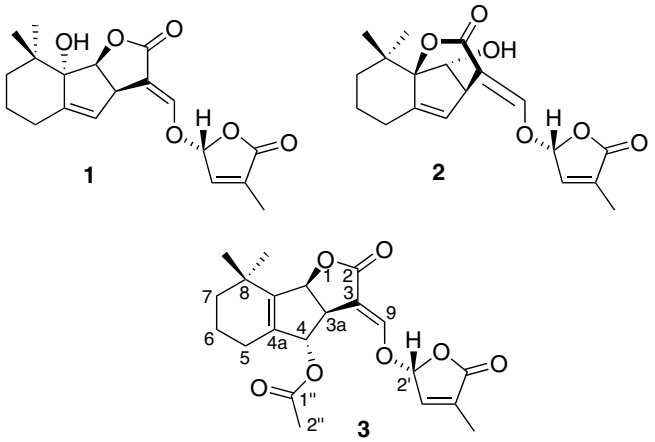

Fig. 1. Structures of alectrol proposed by Müller et al. (**1**) and Wigchert et al. (**2**), and orobanchyl acetate (**3**).

The ESI-MS analysis of "alectrol" afforded the sodium adduct ion at *m/z* 411 $[M+Na]^+$ along with the potassium adduct ion at *m/z* 427 $[M+K]^+$. This demonstrated that the molecular weight of "alectrol" was 388 rather than 346. The TOF/MS analysis also confirmed this (data not shown). In addition, the CID spectrum of "alectrol" indicated that the $[M+a]^+$ ion was converted with loss of AcOH to $[M+Na–AcOH]^+$ ion at *m/z* 351 and $[M+Na–AcOH–D\ ring]^+$ at *m/z* 254 (data not shown). Such evidence indicated that "alectrol" should be strigyl acetate previously identified from cotton (Sato et al., 2005) or its isomer. The R_t of "alectrol" (15 min) in ODS-HPLC (MeOH–H_2O, 6:4, v/v) was distinct from that of strigyl acetate (R_t, 12 min), suggesting that "alectrol" was isomeric to strigyl acetate.

The amount of the purified "alectrol" sample was enough to obtain the ^1H and ^{13}C NMR spectroscopic data for structural elucidation. Although it was not clear from the earlier studies (Müller et al., 1992; Yokota et al., 1998), acetyl methyl protons [δ_H 2.04 (3H, *s*, H_3-C2")] were clearly observed. The other signals in the ^1H NMR spectrum of "alectrol" were very similar to those of orobanchol except for the downfield shift of H-4 [δ_H 5.74 (1H, *s*)] by ca 1.0 ppm, clearly indicating that the 4-α-hydroxyl group of orobanchol is acetylated. The α-orientation of the acetyloxyl group was assigned since the H-4 appeared as a singlet and hence the dihedral angle between H-4 and H-3a was ca. 90°. The ^{13}C NMR, HMQC, HMBC and NOE analyses

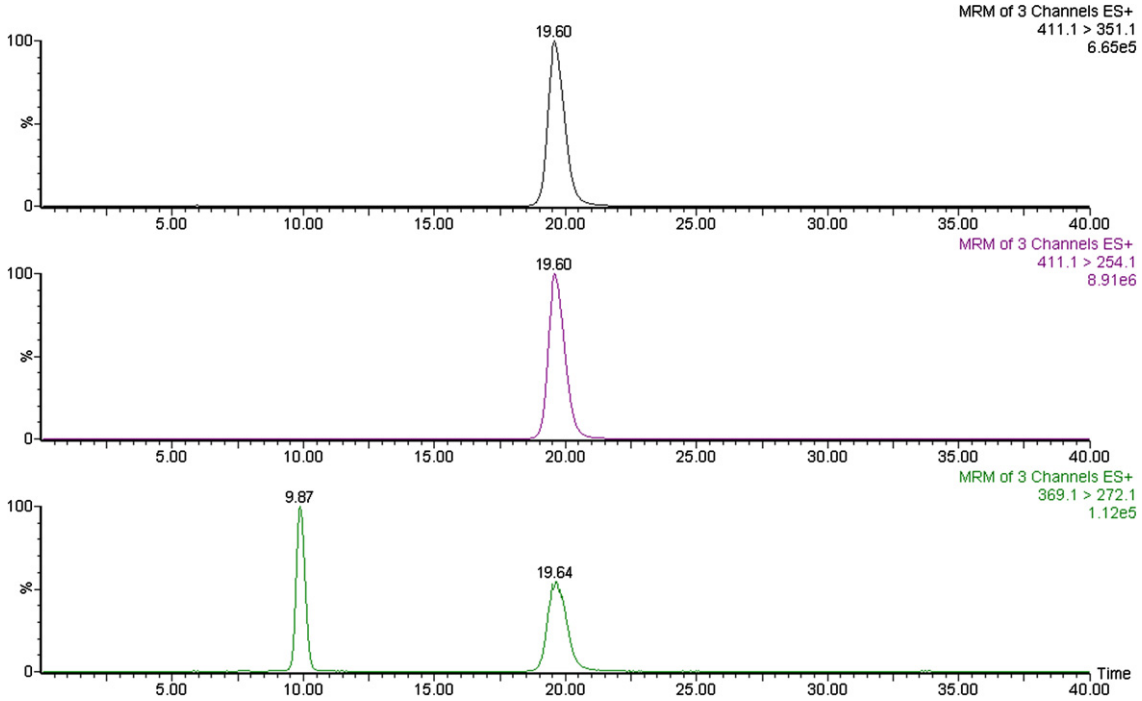

Fig. 2. Three-channel MRM chromatogram of red clover root exudates.