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Stereospecific reduction of the butenolide in strigolactones in plants

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ABSTRACT

Reductive metabolism of strigolactones (SLs) in several plants was investigated. Analysis of aquaculture filtrates of cowpea and sorghum each fed with four stereoisomers of GR24, the most widely used synthetic SL, revealed stereospecific reduction of the double bond at C-3′ and C-4′ in the butenolide D-ring with preference for an unnatural 2′*S* configuration. The cowpea metabolite converted from 2′-*epi*-GR24 and the sorghum metabolite converted from *ent*-GR24 had the methyl group at C-4′ in the *trans* configuration with the substituent at C-2′, different from the *cis* configuration of the synthetic H_2-GR24 reduced with Pd/C catalyst. The plants also reduced the double bond in the D-ring of 5-deoxystrigol isomers with a similar preference. The metabolites and synthetic H_2-GR24 stereoisomers were much less active than were the GR24 stereoisomers in inducing seed germination of the root parasitic weeds *Striga hermonthica*, *Orobanche crenata*, and *O. minor*. These results provide additional evidence of the importance of the D-ring for bioactivity of SLs.

1. Introduction

Strigolactones (SLs) are plant metabolites originally identified as seed germination stimulants of root parasitic weeds.[1] The SLs, a novel class of plant hormones, have been implicated in several physiological processes, including modulation of plant architecture[2–4] and regulation of the symbiotic association between plants and arbuscular mycorrhizal fungi.[5] Typically, canonical SLs contain a tricyclic lactone (ABC-ring) connected to a butenolide (D-ring) *via* an enol ether bridge. In addition to the canonical SLs, non-canonical SLs, such as heliolactone[6] and avenaol,[7] have been identified, in which B- and C-rings are unclosed. The configuration at 2′ of the D-ring is the same in all naturally occurring SLs, namely *R*. SLs and their analogues bearing the *R*-configured butenolide moiety showed enhanced biological activity, which highlights the importance of this stereochemical motif.[8,9] As significant physiological functions have been unveiled, the biosynthesis, perception, and signal transduction of SLs have attracted increasing attention. The SLs are derived from a carotenoid precursor.[10] A *cis-trans* isomerase, D27, catalyzes conversion of β-carotene to 9-*cis*-β-carotene, which is cleaved by CCD7 and CCD8 to give carlactone (CL).[11] In rice, two CYP711 enzymes catalyze two distinct steps, B–C ring closure of CL and a subsequent structural diversification step to give orobanchol.[12] An α/β hydrolase (D14, AtD14, DAD2, or RMS3), an F-box protein (D3, MAX2, PhMAX2, or RMS4) and a protein (D53 or SMXL6/7/8) have been identified as the components required for SL signal transduction.[13]

The level of plant hormones in plants is controlled not only by their biosynthesis but also through their catabolism. Bioactive plant hormones can be inactivated by many different mechanisms, including hydroxylation, side-chain cleavage, and glycosylation. For example, abscisic acid (ABA) is hydroxylated at the 8′ position. The unstable product is spontaneously converted to phaseic acid, which is biologically inactive.[14] A previous study reported regioselective and stereospecific hydroxylation in the A- and B-rings of GR24, a synthetic SL analog, by sorghum aquaculture. The hydroxy SL analogs were less active than GR24 in inducing germination of *Striga hermonthica* seeds.[15] Evidence indicates that the bioactiphore of SLs resides in the CD part of the molecule. Cleavage of the molecule into the ABC portion and the D-ring unit renders SLs inactive, whereas modifications in the A- and B-rings are tolerated.[16,17] Analyses of interactions between the SL receptor RMS3 and SL analogues indicated the importance of the D-ring in the bioactivity of SLs at a molecular level.[18] Accordingly, we searched plant aquaculture supplemented with GR24 for GR24-derived analogues with modification in the D-ring. The present report describes the isolation and identification of novel GR24 metabolites, in which the

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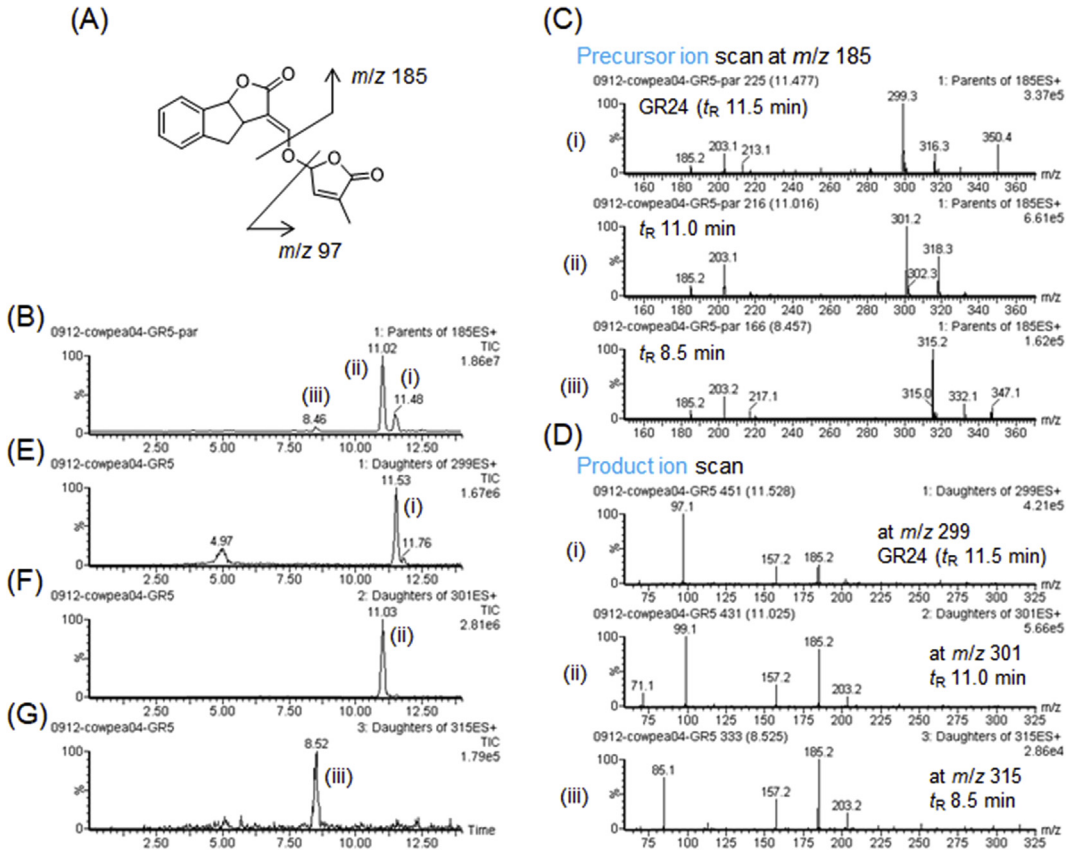

Fig. 1. Modification in the D-ring of GR24 (**1** + **2**) by cowpea. (A) Fragmentation of the protonated molecule of GR24 from electrospray ionization. (B) Chromatogram at *m/z* 185 from the precursor ion scan. Three peaks (i)–(iii) were detected. (C) Precursor ion spectra of peaks (i)–(iii) in chromatograms (B), (E), (F), and (G). (D) Product ion spectra of peaks (i)–(iii) in chromatograms (B), (E), (F), and (G). (E–G) Chromatograms in product ion scan at *m/z* 299 (E), *m/z* 301 (F), and *m/z* 315 (G). Samples applied to COSMOSIL 2.5C_{18}-MS-II column were eluted with 40–80% MeOH in H_2O (20 min, linear gradient).

double bond at C-3′ and C-4′ in the D-ring was reduced to a single bond, generated in plant aquaculture fed with the synthetic SL. The potency of these metabolites as a germination stimulant for the seeds of the root parasites *S. hermonthica*, *Orobanche crenata*, and *O. minor* was also determined.

2. Results and discussion

2.1. Search for GR24 metabolites in cowpea aquaculture

Aquaculture filtrates of cowpea fed with a racemic and diastereomeric mixture of GR24 (**1** + **2**) were analyzed by liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) using a precursor ion scan and product ion scan. In electrospray ionization (ESI), a protonated molecule ($[M+H]^+$) of GR24 at *m/z* 299 was cleaved to a fragment ion at *m/z* 185, corresponding to the ABC portion (Fig. 1A). The chromatogram from precursor ion scan analysis at *m/z* 185 showed peaks for GR24 derivatives at t_R 8.5 and 11.0 min in addition to a peak for GR24 at t_R 11.5 min (Fig. 1B). As shown in Fig. 1C, the mass spectrum of the compound at t_R 11.0 min showed a precursor ion at *m/z* 301, larger than that of GR24 at *m/z* 299 by 2 Da. In the product ion scan at *m/z* 301, a fragment ion at *m/z* 99 was detected instead of that of GR24 at *m/z* 97 [Fig. 1D(ii), Fig. 1F]. These mass spectral and chromatographic data suggest that the metabolite at t_R 11.0 min is a GR24 analog in which the double bond at C-3′ and C-4′ in the D-ring had been reduced. In contrast, the mass spectrum of the minor peak at t_R 8.5 min showed a precursor ion at *m/z* 315, 16 Da larger than that of GR24 [Fig. 1C(iii), Fig. 1G], suggesting that the compound is a hydroxylated GR24 analog. The absence of a fragment ion at *m/z* 97 or 99 in the product ion scan [Fig. 1D(iii)] supports a hydroxy group in the D-ring. However, production was too small for unambiguous structure elucidation; therefore, investigations focused on the major product at t_R 11.0 min.

2.2. Stereospecific reduction of the D-ring of GR24 by plants

Aquaculture of cowpea, sorghum, cotton, and rice converted a racemic and diastereomeric mixture of GR24 (**1** + **2**) to unidentified products, which were detected by LC–MS/MS analysis with a multiple reaction monitoring (MRM) channel at *m/z* 301.1 > 99. In the cowpea aquaculture filtrate, only a peak eluted at 8.0 min was detected, whereas two peaks at 8.0 min and 8.7 min were detected in culture filtrates from the other plants. Feeding experiments with each GR24 diastereomer revealed that the peak at 8.0 min was derived from *rac*-2′-*epi*-GR24 (**2**) (Fig. 2A), and that the peak at 8.7 min was derived from *rac*-GR24 (**1**) (Fig. 2B). Detailed feeding experiments using each of four stereoisomers of GR24 revealed that the reduction was stereospecific (Fig. 3A). The stereospecificity was confirmed by two independent experiments. Cowpea almost exclusively reduced 2′-*epi*-GR24 (**2a**), whereas sorghum, cotton, and rice reduced *ent*-GR24 (**1b**) and 2′-*epi*-GR24 (**2a**) with limited preference. However, reduction of their respective enantiomers **1a** and **2b** was negligible.

2.3. Isolation and identification of reduced analogs of GR24

2.3.1. Reduced product of 2′-epi-GR24 by cowpea

According to the method of Boyer et al.,[19] *rac*-2′-*epi*-GR24 (**2**) was treated with H_2 gas over a Pd/C catalyst to obtain the synthetic 3′,4′-dihydro product, *rac*-H_2-2′-*epi*-GR24 (**4**). Detailed NMR studies of **4** were conducted in C_6D_6 because of overlap of the H-4′ signal and one of