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The bioconversion of 5-deoxystrigol to sorgomol by the sorghum, *Sorghum bicolor* (L.) Moench

Noriko Motonami, Kotomi Ueno, Hitomi Nakashima, Saki Nomura, Masaharu Mizutani, Hirosato Takikawa, Yukihiro Sugimoto *

Graduate School of Agricultural Science, Kobe University, Rokkodai, Nada, Kobe 657-8501, Japan

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ABSTRACT

Strigolactones, important rhizosphere signalling molecules and a class of phytohormones that control shoot architecture, are apocarotenoids of plant origin. They have a structural core consisting of a tricyclic lactone connected to a butyrolactone group via an enol ether bridge. Deuterium-labelled 5-deoxystrigol stereoisomers were administered to aquacultures of a high sorgomol-producing sorghum cultivar, *Sorghum bicolor* (L.) Moench, and conversion of these substrates to sorgomol stereoisomers was investigated. Liquid chromatography–mass spectrometry analyses established that 5-deoxystrigol (5-DS) and *ent*-2′-*epi*-5-deoxystrigol were absorbed by sorghum roots, converted to sorgomol and *ent*-2′-*epi*-sorgomol, respectively, and exuded out of the roots. The conversion was inhibited by uniconazole-P, implying the involvement of cytochrome P450 in the hydroxylation. These results provide experimental evidence for the postulated biogenetic scheme for formation of strigolactones, in which hydroxylation at C-9 of 5-DS can generate sorgomol.

1. Introduction

Strigolactones are apocarotenoids of plant origin with a structural core consisting of a tricyclic lactone (the ABC-ring) that connects to the D-ring of butyrolactone via an enol ether bridge (López-Ráez et al., 2009). They have been shown to play dual roles in the rhizosphere as host detection signals for root parasitic plants (Zwanenburg et al., 2009) and arbuscular mycorrhizal fungi (Akiyama et al., 2010). In addition to their important roles as rhizosphere signalling compounds, strigolactones were also shown to act as plant hormones, regulating shoot architecture by inhibiting the outgrowth of axillary buds (Gomez-Roldan et al., 2008; Umehara et al., 2008). The first strigolactone, strigol (**1**) (Scheme 1), was isolated as a germination stimulant for the root parasitic weed *Striga lutea* in 1966 from root exudates of cotton (*Gossypium hirsutum* L.) (Cook et al., 1966). It was not until 1992 that sorgolactone (**2**), a compound with a structure similar to strigol (**1**), was isolated from root exudates of the sorghum, *Sorghum bicolor* (L.) Moench. (Hauck et al., 1992). Soon thereafter, the same authors reported the isolation of another germination stimulant, alectrol (**3**), from root exudates of the cowpea, *Vigna unguiculata* (L.) Walp. (Müller et al., 1992). The molecular structures of compounds **1** and **2** were confirmed by total synthesis (Brooks et al., 1985; Sugimoto et al., 1998), and a detailed analysis of spectroscopic data elucidated the genuine structure of compound **3** (Ueno et al., 2011). Several more strigolactones have been isolated from various root exudates, such as sorgomol (**4**), isolated from sorghum (Xie et al., 2008), and it is expected that many novel strigolactones have yet to be characterized (Yoneyama et al., 2009).

Traditionally, strigolactones were thought to be sesquiterpene lactones (Butler, 1995). However, a biochemical approach using isoprenoid biosynthetic inhibitors and several mutants indicated that the ABC C_{14} skeleton of the strigolactones is derived from carotenoids by oxidative cleavage through action of carotenoid cleavage dioxygenase enzymes (Matusova et al., 2005). Recently, it was discovered that two carotenoid cleavage dioxygenase (CCDs), CCD7 and CCD8, key enzymes in the biosynthesis of a shoot-branching inhibiting signal (Schwarz et al., 2004), play pivotal roles in the biosynthesis of strigolactones (Gomez-Roldan et al., 2008; Umehara et al., 2008). In addition, an *in vitro* approach demonstrated the conversion of β-carotene to carlactone (**5**) by sequential reactions catalysed by D27, CCD7, and CCD8 (Alder et al., 2012). Carlactone (**5**) already contains the D-ring and the enol ether bridge characteristic of strigolactones, and can likely be converted to 5-deoxystrigol (5-DS) (**6**), the strigolactone with the least structural modification, through a few more oxidation steps (at C-18 and C-19), followed by formation of the B and C rings. Therefore, although not yet identified in plants, carlactone

* Corresponding author. Tel./fax: +81 78 803 5884.
E-mail address: yukihiro@kobe-u.ac.jp (Y. Sugimoto).

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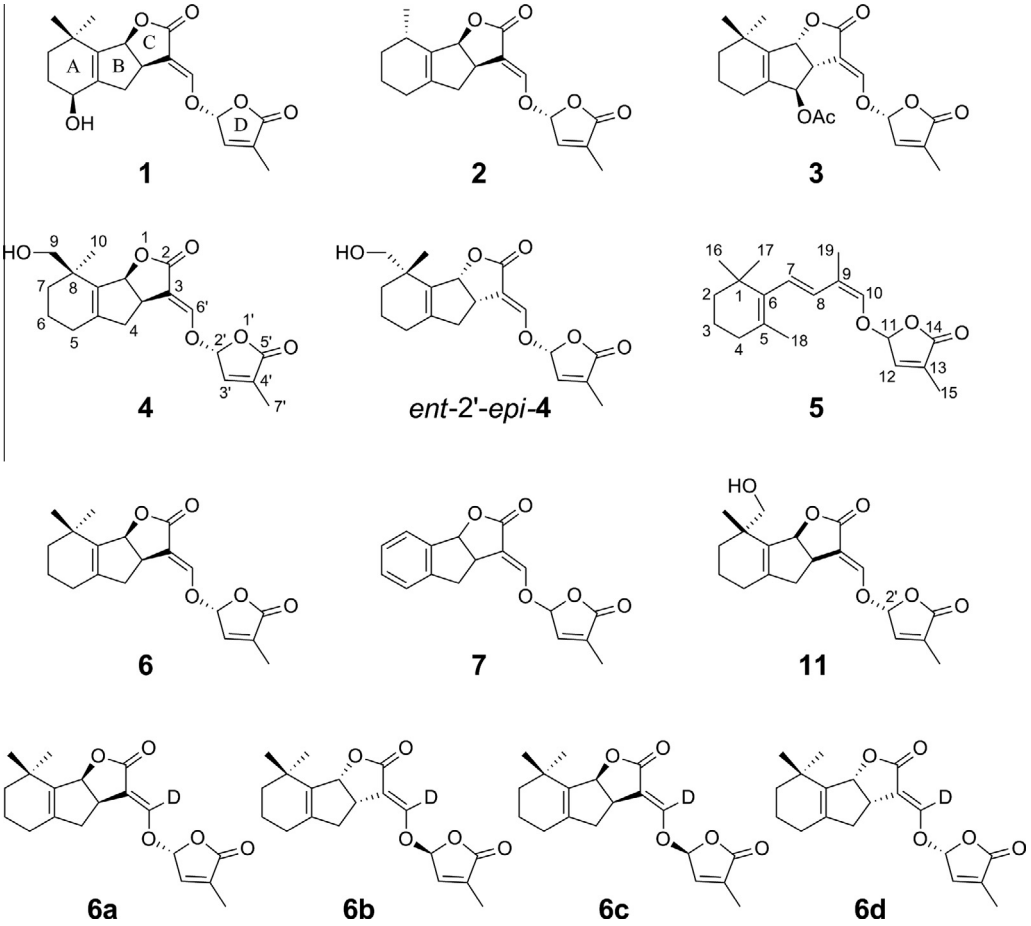

Scheme 1. Chemical structures of naturally occurring strigolactones and synthetic strigolactones. Compounds **1**–**4**, *ent*-2′-*epi*-**4**, **6** and **6a**–**d** are enantiomerically pure, whereas **5** and **11** are a racemic mixture. GR27 (**7**) is a racemic and diastereomeric mixture.

(**5**) is a probable intermediate in the biosynthetic pathway of strigolactones.

With respect to its structure, modifications of 5-DS (**6**) through hydroxylation, oxidation, decarboxylation, and esterification can generate all of the strigolactones that have been identified to date. However, no studies have described the biological conversion of 5-DS (**6**) to other strigolactones. Thus, the current study was undertaken to further investigate strigolactone biosynthesis by demonstrating the bioconversion of 5-DS (**6**) to sorgomol (**4**). In this regard, a high sorgomol-producing sorghum cultivar was selected, to which stereoisomers of deuterium-labelled 5-DS (**6a**–**d**) were supplied in the presence or absence of fluridone, a carotenoid biosynthesis inhibitor. The strigolactones derived from the labelled substrates were analysed by liquid chromatography–mass spectrometry (LC–MS). To gain insight into the hydroxylation mechanisms, the precursor administration experiments were also conducted in the presence of uniconazole-P, a cytochrome P450 inhibitor, or prohexadione, a 2-oxoglutarate-dependent dioxygenase (2OGD) inhibitor.

2. Results and discussion

2.1. Selection for high sorgomol-producing sorghum cultivars

Sorghum cultivars, designated NM01–NM21, were grown hydroponically, and the *Striga hermonthica* seed germination-inducing activity of each of the culture filtrates was evaluated. NM16 induced the greatest amount of germination, followed by NM01, NM17, and NM05. Other cultivars induced little or no germination (Table 1). LC–MS analyses established not only semi-quantitative, but also qualitative, differences in the strigolactones found in the culture filtrates. NM01 and NM12 exuded only 5-DS (**6**), while NM07 exuded both 5-DS (**6**) and sorgolactone (**2**), although the amount of the latter was limited and fluctuated. NM16 and NM17 exuded 5-DS (**6**) and sorgomol (**4**). The constant, high exudation of sorgomol (**4**) with a limited accumulation of 5-DS in the NM16 culture filtrates suggested that this cultivar had an enhanced ability to produce strigolactones, and an increased capacity to convert 5-DS (**6**) to sorgomol (**4**). Accordingly, NM16 was selected as the plant material for subsequent studies of the bioconversion of 5-DS (**6**) stereoisomers. Prior to precursor administration experiments, the absolute configuration of sorgomol (**4**) and 5-DS (**6**) exuded by NM16 was determined to be those of compounds **4** and **6**, respectively, based on their respective chromatographic behaviours on a chiral HPLC.

2.2. The preparation of [6′-D]5-DS stereoisomers

Stereoisomers of [6′-D]5-DS (**6a**–**d**) were prepared as shown in Scheme 2. Tricyclic lactone (**8**) was formylated with a deuterium-labelled methyl formate in the presence of NaH, and the subsequent coupling with bromo-3-methyl-2(*5H*)-furanone (**10**) produced [6′-D]5-DS (**6**) as a racemic and diastereomeric mixture. In the ^{1}H NMR spectrum of the labelled [6′-D]5-DS (**6**), a singlet signal at δ 7.43 ppm (H-6′) was not observed. The EI mass spectrum showed that the labelling ratio of [6′-D]5-DS (**6**) was greater than