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Studies on strigolactone BC-ring formation: Chemical conversion of an 18-hydroxycarlactonoate derivative into racemic 4-deoxyorobanchol/5-deoxystrigol via the acid-mediated cascade cyclization

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ABSTRACT

Strigolactones are a group of apocarotenoids known as rhizosphere semiochemicals and phytohormones. Canonical strigolactone consists of a tricyclic lactone (ABC-rings) and a butenolide moiety (D-ring). Although strigolactone biosynthesis has been extensively studied, the process of BC-ring formation has not been elucidated to date. In this study, the chemical conversion of an 18-hydroxycarlactonoate derivative into racemic 4-deoxyorobanchol/5-deoxystrigol was achieved by the acid-mediated cascade cyclization. The acid-mediated BC-ring formation may offer inspiring suggestion for the currently unclear BC-ring formation in strigolactone biosynthesis.

Introduction

Strigolactone (SL) is the collective name originally given to the naturally occurring seed germination stimulants for root parasitic weeds [1]. SLs have received considerable attention in recent years because they have become recognized as signal substances in arbuscular mycorrhizal symbiosis [2] and as a new class of plant hormone regulating shoot branching [3,4]. Currently, SLs are recognized as apocarotenoids, and they are classified into canonical and non-canonical SLs according to their chemical structures. Figure 1 shows the representative structures of canonical and non-canonical SLs (e.g., strigol [5], orobanchol [6], heliolactone [7], and avenaol [8]). Canonical SLs have a tricyclic lactone portion (ABC-rings), whereas non-canonical SLs do not have a BC-ring system.

To date, the biosynthesis of SL has been extensively studied, and thus its outline has been largely clarified [9]. As shown in Figure 2, canonical SLs are biosynthesized from β-carotene via the common intermediates carlactone (CL) [9a] and carlactonoic acid (CLA) [9c]. Recently, the P450-mediated "stepwise" bioconversion of CLA into orobanchol in japonica rice was disclosed [9e]. The existence of a novel orobanchol synthase that can catalyze the "direct" conversion of CLA into orobanchol was also reported in cowpea and tomato plants [9f]. Furthermore, the P450-mediated bioconversion of CLA into 5DS in cotton was disclosed [9h]. These studies clearly suggested that oxidation at the 18-position of CLA is crucial for the biosynthesis of canonical SLs. However, except for that, the process of BC-ring formation affording the tricyclic lactone moiety of canonical SL has not been completely explained.

Results and discussion

As previously mentioned, it has been established that enzymatic hydroxylation at the 18-position of CLA is the most crucial step in the biosynthesis of canonical SL. It has also been established that the hydroxylated product, 18-hydroxy-CLA, must be the precursor for BC-ring formation affording 4-deoxyorobanchol (4DO) or 5-deoxystrigol (5DS) (Scheme 1). Similarly, it is agreed upon that 18-oxo-CLA, probably derived from 18-hydroxy-CLA via further oxidation, may also cyclize to furnish orobanchol. Although there is no doubt about the above outline, there has been no evidence providing considerable insight into the actual process of BC-ring formation, and thus no precise discussion on the mechanism of BC-ring formation. As such, we hypothesized that 18-hydroxy- and 18-oxo-CLAs are reactive enough to cyclize spontaneously to form BC-rings. Namely, these CLA derivatives may not be stable enough to be isolated and, without a trigger, can yield canonical SL skeleton. To verify this hypothesis, we envisaged to synthesize *t*-butyl *rac*-18-(methoxymethoxy)carlactonoate (**1**), an

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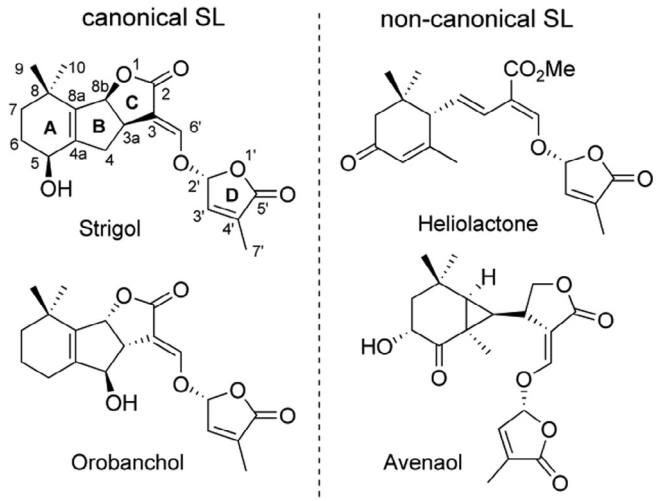


Fig. 1. Structures of canonical and non-canonical SLs.

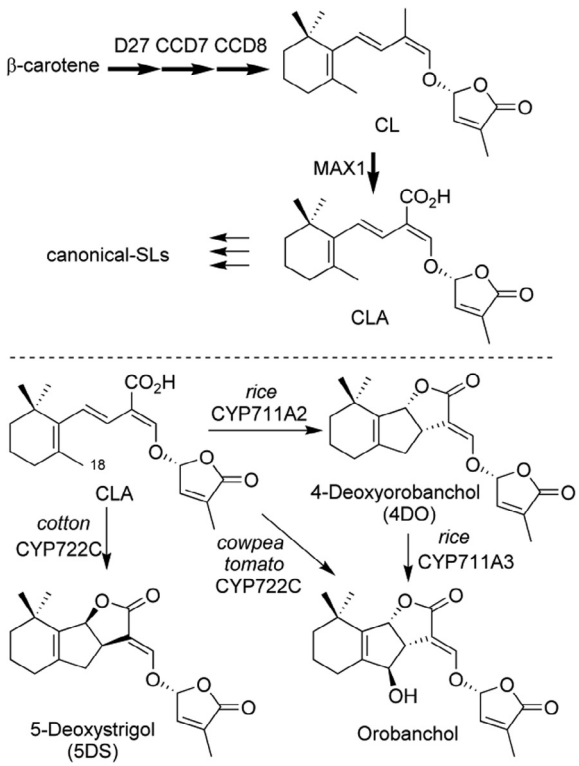
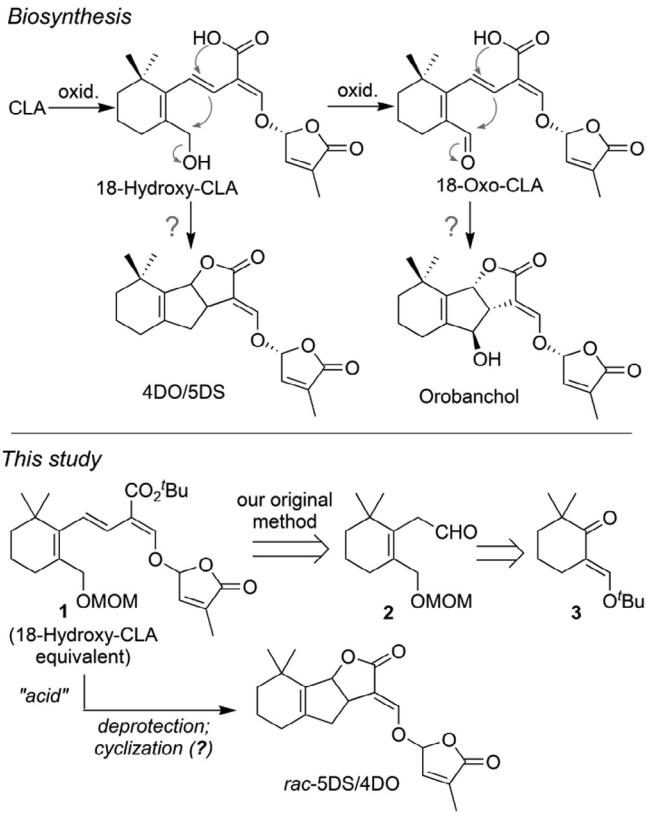


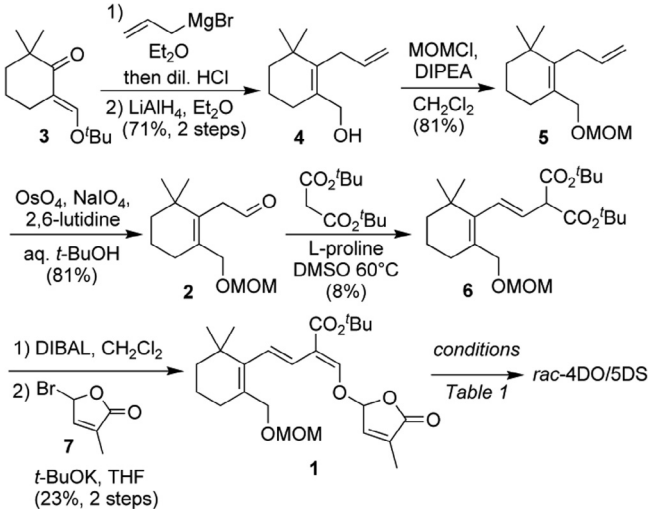
Fig. 2. Outline of canonical SL biosynthesis.

appropriate and more stable 18-hydroxy-CLA equivalent, as a substrate for "biomimetic" cyclization. If this hypothesis is correct, both methoxymethyl (MOM) ether and *t*-Bu ester linkages in **1** can be cleaved by treating with acid to afford 18-hydroxy-CLA (or its equivalent), which may then cyclize spontaneously "in a cascade manner" to furnish racemic 4DO/5DS under acidic conditions. The key compound **1** would be prepared from aldehyde **2** by employing our original method based on the Knoevenagel-type condensation [9f,10]. It was anticipated that aldehyde **2** would be prepared from the known compound **3** according to the conventional method.

As shown in Scheme 2, our synthesis of **1** commenced with the known ketone **3**, easily prepared from 2-methycyclohexanone [11]. The Grignard reaction between **3** and allylmagnesium bromide and



Scheme 1. Plausible biosynthetic formation of BC-ring and our plan for BC-ring formation.



Scheme 2. Synthesis of 1.

the subsequent acidic work-up afforded an aldehyde. This aldehyde was then treated with $LiAlH_4$ to give alcohol **4** (71%, 2 steps). Alcohol **4** was then protected as MOM ether to furnish **5** (81%). The oxidative cleavage of the side-chain double bond gave the desired aldehyde **2** in 81% yield. The Knoevenagel-type condensation between **2** and di-*t*-butyl malonate was not successful under the following previously developed conditions: di-*t*-butyl malonate, NaH, 1,4-dioxane, reflux [10a]. The reason for this failure was the elimination of the MOM-oxy group at the δ-position of the β,γ-unsaturated aldehyde **2** under strong basic conditions. Thus, this

condensation needed to be investigated again under milder conditions. Fortunately, in previous studies, it was demonstrated that this condensation could be mediated by an over-stoichiometric amount of proline (see Table S1 in SI) [12]. Therefore, proline-mediated condensation was re-investigated, and the desired Knoevenagel-type condensation product **6** was obtained in 8% yield under the following conditions: di-*t*-butyl malonate (3.0 eq.), L-proline (3.0 eq.), DMSO, 60 °C, 2 days. Note that the yield was lower at higher temperature probably due to the decomposition of **2** and/or **6**. It should be mentioned that the TBS-protected aldehyde was also prepared, but this did not stand up to the condensation reaction. Although the yield of this condensation is far from satisfactory, we continued with our studies. Diester **6** was reduced with DIBAL (2.0 eq.) in CH_2Cl_2 at −78 °C, and the resulting crude products were then treated with 5-bromo-3-methylfuran-2(5*H*)-one (**7**) and *t*-BuOK in THF to furnish the desired 18-hydroxy-CLA derivative **1** in 23% yield. This yield was less than moderate but similar to previous cases [10].

With the desired substrate **1** in hand, the critical acid-mediated BC-ring formation was attempted. Table 1 shows the representative results of these attempts. The substrate **1** contains an enol ether linkage, which is labile, so the first choice of acid was a weak Brønsted acid AcOH (pKa 4.8). However, AcOH did not promote not only the desired BC-ring formation but also deprotection of the MOM group (entry 1). Next, the stronger acid TFA (pKa 0.5) was examined. To our delight, the desired cyclized products *rac*-4DO/5DS were obtained by treatment with an excess amount of TFA (entry 3). However, it was revealed that the obtained *rac*-4DO/5DS contained other constitutional isomers, which would be discussed later. The acid-mediated BC-ring formation was also successfully performed by treatment with *p*-TsOH (pKa − 2.8), and the obtained *rac*-4DO/5DS contained only a small amount of the corresponding constitutional isomers (entries 5–7). The use of TESOTf, used for the conversion of *t*-butyl carlactonoate to CLA [9b,9f], was not effective for this reaction (entry 8). In the successful cases of the cyclization, no notable by-product with D-ring was detected by LC-MS analysis, even though the isolated yield of *rac*-4DO/5DS was rather low. This analysis was conducted on the crude products for the fragment ion at *m*/*z* 97 (D-ring). This result clearly suggested that the acid-mediated BC-ring formation might proceed in a cascade manner and not be interrupted. The acid-mediated cyclization afforded essentially the same amount of *rac*-4DO and *rac*-5DS, whereas the biosynthesis *in planta* generally gives canonical SLs in a diastereoselective manner.

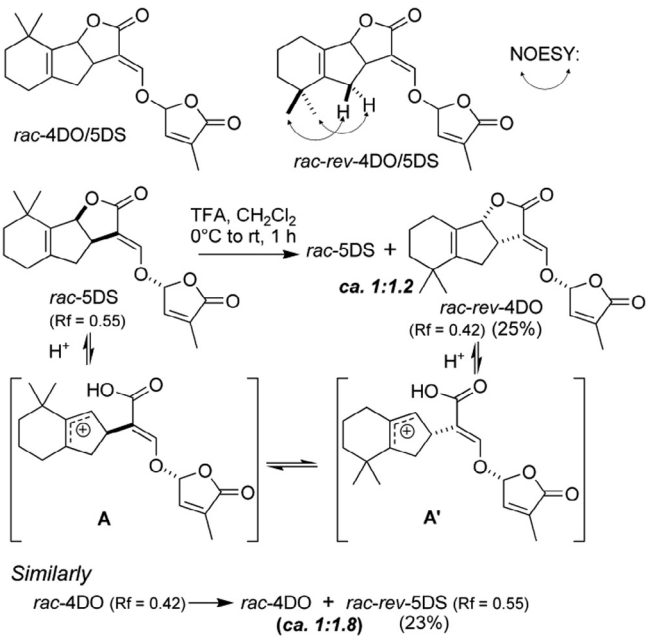
We were successful in demonstrating the feasibility of the "hypothetical" and "biomimetic" BC-ring formation in flask. However, there was an additional problem to be solved, because the obtained *rac*-4DO/5DS contained structurally quite similar compounds. This was particularly true under the conditions of entry 3, where the "counterfeit" isomers were more abundant than the "genuine" *rac*-4DO/5DS. On the basis of NMR analysis, we concluded that the structures of the counterfeits were constitutional isomers regarding the position of the *gem*-dimethyl group (Scheme 3) [13]. They were named "reverse" isomers and identified by the prefix "*rev*-" for convenience. The unambiguous determination of the relative stereochemistry of *rev*-4DO/5DS was difficult without using X-ray analysis. However, it could be assigned by the following experimental fact. To understand the reaction mechanism that result in *rev*-4DO/5DS, the purified *rac*-5DS [14] was exposed to the excess amount of TFA. Consequently, *rac*-5DS (Rf = 0.55, EtOAc/hexane = 1/1) was converted into a mixture of *rac*-5DS itself and "a *rev*-isomer" (Rf = 0.42). Both of these products were found to be virtually diastereomerically pure on the basis of NMR analysis. This fact suggested that the isomerization of "genuine" to "reverse" proceeded in a stereospecific manner. Scheme 3 shows the plausible reaction mechanism, where the acid-mediated elimination of the acyloxy group might proceed and generate the allylic cation **A**, which could isomerize to the corresponding regioisomer **A′**. Then, **A′** was captured by the carboxy group to furnish "a *rev*-isomer". Therefore, the structure of "a *rev*-isomer" could be estimated as *rac-rev*-4DO. Although the precise mechanism of isomerization of **A** into **A′** is uncertain, it may involve the formation of a cyclopentadiene-type intermediate. Note that the isomerization of *rac*-4DO into *rac-rev*-5DS was also successfully performed.

Next, the other 18-hydroxylated CLA analog **9** [methyl *rac*-18-(methoxymethoxy)carlactonoate] was prepared in the same manner, and then **9** was subjected to the acid-mediated BC-ring formation (Scheme 4). However, under similar conditions, the formation of *rac*-4DO/5DS was not detected, even though the substrate **9** had disappeared smoothly [15]. This result should be explained by the fact that methyl ester is less labile than *t*-butyl ester under acidic condition, but may also offer insight into the biosynthetic distinction between canonical and non-canonical SLs. Judging from their structural features, non-canonical SLs are considered to be derived from methyl carlactonoate (MeCLA) or its derivative (Fig. 1). Specifically, both *in planta* and in flask, the BC-ring formation does not proceed from MeCLA and its derivatives.

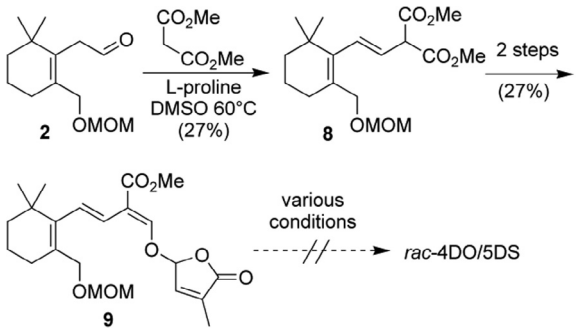
Table 1
Studies on the acid-mediated BC-ring formation.

Entry	Acid (eq.); Solvent; Temp.; Time	Yield (%)[a,b]
1	AcOH (>100); CH_2Cl_2; 0 °C to rt; 4 h	N.R.[c]
2	TFA (0.3); CH_2Cl_2; rt; 3 h	N.R.[c]
3	TFA (30); CH_2Cl_2; 0 °C to rt; 3 h	22–28 (1:1.5–1:2.5)[d]
4	*p*-TsOH (0.1); CH_2Cl_2; 0 °C to rt; 1 h	trace
5	*p*-TsOH (0.3); CH_2Cl_2; 0 °C to rt; 3 h	18 (>20:1)
6	*p*-TsOH (1.0); CH_2Cl_2; 0 °C to rt; 3 h	22 (>20:1)
7	*p*-TsOH (3.0); CH_2Cl_2; 0 °C to rt; 1 h	19 (>20:1)
8	TESOTf (5.0)[e]; CH_2Cl_2; 0 °C to rt; 1 h	Decomp.

[a] Combined yield.
[b] The ratio of *rac*-4DO/5DS to the corresponding constitutional isomers is given in parentheses.
[c] No reaction.
[d] The ratio was considerably fluctuated.
[e] The same amount of 2,6-lutidine was also added.

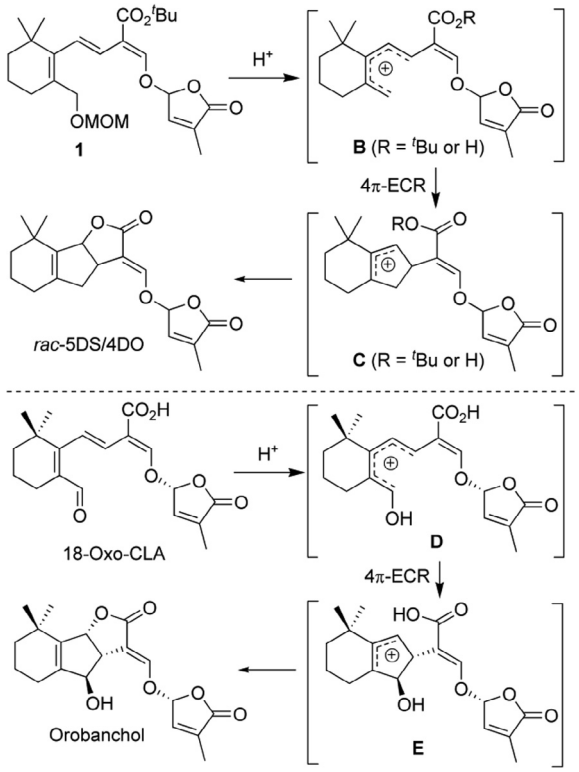


Scheme 3. Relationship between 4DO/5DS and *rev*-4DO/5DS.



Scheme 4. Synthesis of 9 and its cyclization.

We would like to mention the reaction mechanism of the BC-ring formation. Although the detail of BC-ring formation in biosynthesis is not certain, our results may offer considerable suggestion for it. We are speculating that the formation of *rac*-4DO/5DS "in flask" may procced as follows (Scheme 5). The pentadienyl cation-like intermediate **B** would be generated by acid treatment of **1**. Then, **B** might undergo a conrotatory 4π-electrocyclic reaction (ECR) yielding the allylic cation **C**, which should be captured spontaneously by the oxygen of carboxylate, affording *rac*-4DO/5DS. Note that the cation **B** can be generated not only via the deprotection of the MOM group and the subsequent dehydration but also via the direct elimination of the MOM-oxy group from **1**. According to our understanding, the biosynthesis of orobanchol from 18-oxo-CLA can be explained analogously by the sequence via **D** and **E**. Particularly, the 4π-ECR-based cascade cyclization, demonstrated herein, must be possible in biosynthesis. A similar 4π-ECR-based ring formation has been reported by Lin *et al.* in their gold-catalyzed cascade cyclization [16]. The possibility of the 4π-ECR-based cyclization was also suggested in the biosynthesis of zeapylanolactone, a novel non-canonical SL isolated from maize [17]. On



Scheme 5. Insight into the BC-ring formation mechanism.

the other hand, Chojnacka *et al.* were successful in synthesizing the ABC-ring portion of an SL-analog by the acid-mediated cascade cyclization, even though the A-ring of their substrate was benzene and there was no D-ring portion [18]. However, they did not mention 4π-ECR but proposed the nucleophilic addition-based mechanism for the cascade cyclization (see Scheme S1 in SI). As with the discussion on the reaction mechanism of the Piancatelli rearrangement (see Scheme S2 in SI) [19], the genuine mechanism may not be easily clarified and settled. However, putting the detailed mechanism aside, this study demonstrated successful construction of the whole skeleton of canonical SL from a synthetic analog of 18-hydroxy-CLA (**1**). Therefore, these results offer inspiring suggestion for the biosynthetic process. Essentially, the process of BC-ring formation in biosynthesis may be similar to the acid-mediated cascade cyclization described here. Despite no direct evidence, it seems reasonable to suppose that the 4π-ECR-based cyclization is the probable mechanism, rather than the nucleophilic substitution-based cyclization of 18-hydroxy-CLA to 4DO/5DS or the nucleophilic addition-based cyclization of 18-oxo-CLA to orobanchol (Scheme 1). Studies on the BC-ring formation mechanism based on DFT calculations are currently underway (see Fig. S1 in SI).

Conclusion

We have accomplished the chemical conversion of an 18-hydroxycarlactonoate derivative (**1**) into *rac*-4DO/5DS via the acid-mediated cascade cyclization. The BC-ring formation demonstrated "in flask" may be analogous to that in biosynthesis. Even though there is no particular evidence of the precise reaction mechanism, the results of this study may provide a basis for discussion on the unclarified BC-ring formation mechanism in biosynthesis. Our proposed mechanism can explain some of the unsolved problems in biosynthesis of canonical SLs. To gain additional insight into the BC-ring formation, studies on the synthesis of the 18-oxo-CLA derivative and its conversion into orobanchol are currently in progress and will be reported in due course.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2021.152922.

References

[1] L.G. Butler, I. In, in: Allelopathy, Organisms, Process, and Applications, American Chemical Society, Washington DC, 1995, pp. 158–168.
[2] K. Akiyama, K. Matsuzaki, H. Hayashi, Nature 453 (2005) 824–827.
[3] V. Gomez-Roldan, S. Fermas, P.B. Brewer, V. Puech-Pages, E.A. Dun, J.P. Pillot, F. Letisse, R. Matusova, S. Danoun, J.C. Portais, H. Bouwmeester, G. Becard, C.A. Beveridge, C. Rameau, S. Rochange, Nature 455 (2008) 189–194.

[4] M. Umehara, A. Hanada, S. Yoshida, K. Akiyama, T. Arite, N. Takeda-Kamiya, H. Magome, U. Kamiya, K. Shirasu, K. Yoneyama, J. Kyozuka, S. Yamaguchi, Nature 455 (2008) 195–200.
[5] (a) C.E. Cook, L.P. Whichard, B. Turner, M.E. Wall, G.H. Egley, Science 154 (1966) 1189–1190;
(b) C.E. Cook, L.P. Whichard, M.E. Wall, G.H. Egley, P. Coggon, P.A. Luhan, A.T. McPhail, J. Am. Chem. Soc. 94 (1972) 6198–6199.
[6] (a) T. Yokota, H. Sakai, K. Okuno, K. Yoneyama, Y. Takeuchi, Phytochemistry. 49 (1998) 1967–1973;
(b) K. Ueno, S. Nomura, S. Muranaka, M. Mizutani, H. Takikawa, Y. Sugimoto, J. Agric. Food Chem. 59 (2011) 10485–10490.
[7] K. Ueno, T. Furumoto, S. Umeda, M. Mizutani, H. Takikawa, R. Batchvarova, Y. Sugimoto, Phytochemistry. 108 (2014) 102–114.
[8] H.I. Kim, T. Kisugi, P. Kherkam, X. Xie, K. Yoneyama, K. Uchida, T. Yokota, T. Nomura, C.S.P. McErlean, K. Yoneyama, Phytochemistry. 103 (2014) 85–88.
[9] (a) A. Alder, M. Jamil, M. Marzorati, M. Bruno, M. Vermathen, P. Bigler, S. Ghisla, H. Bouwmeester, P. Beyer, S. Al-Babili, Science 335 (2012) 1348–1351;
(b) Y. Zhang, A.D.J. van Dijk, A. Scaffidi, G.R. Flematti, M. Hofmann, T. Charnikhova, F. Verstappen, J. Hepworth, S. van der Krol, O. Leyser, S.M. Smith, B. Zwanenburg, S. Al-Babili, C. Ruyter-Spira, H.J. Bouwmeester, Nat. Chem. Biol. 10 (2014) 1028–1033;
(c) S. Abe, A. Sado, K. Tanaka, T. Kisugi, K. Asami, S. Ota, H.I. Kim, K. Yoneyama, X. Xie, T. Ohnishi, Y. Seto, S. Yamaguchi, K. Akiyama, K. Yoneyama, T. Nomura, Proc. Natl. Acad. Sci. USA 111 (2014) 18084–18089;
(d) S. Al-Babili, H.J. Bouwmeester, Annu. Rev. Plant Biol. 66 (2015) 161–186;
(e) K. Yoneyama, N. Mori, T. Sato, A. Yoda, X. Xie, M. Okamoto, M. Iwanaga, T. Ohnishi, H. Nishiwaki, T. Asami, T. Yokota, K. Akiyama, K. Yoneyama, T. Nomura, New Phytol. 218 (2018) 1522–1533;
(f) T. Wakabayashi, M. Hamana, A. Mori, R. Akiyama, K. Ueno, H. Suzuki, H. Takikawa, M. Mizutani, Y. Sugimoto, Sci. Adv. 5 (2019) eaax9067;
(g) N. Mori, A. Sado, X. Xie, K. Yoneyama, K. Asami, Y. Seto, T. Nomura, S. Yamaguchi, K. Yoneyama, K. Akiyama, Phytochemistry 174 (2020) 112349;
(h) T. Wakabayashi, K. Shida, Y. Kitano, H. Takikawa, M. Mizutani, Y. Sugimoto, Planta 251 (2020) 97.
[10] (a) S. Yamamoto, T. Atarashi, M. Kuse, Y. Sugimoto, H. Takikawa, Biosci. Biotechnol. Biochem. 84 (2020) 1113–1118; (b) T. Wakabayashi, H. Shinde, N. Shiotani, S. Yamamoto, M. Mizutani, H. Takikawa, Y. Sugimoto, Nat. Prod. Res. Ahead of Print (2020) 1–8.
[11] For Konoevenagel condensation catalyzed by proline: (a) M. Rosenberger, C. Neukom J. Org. Chem. 47 (1982) 1782–1785;
(b) D.S. Garvey, G.J. Larosa, J.R. Greenwood, L.L. Frye, PCT Int. Appl. (2011) 15, 2011155983.
[12] (a) X. Yang, T. Fox, H. Berke, Chem. Commun. 47 (2011) 2053–2055;
(b) D.M. Nickerson, A.E. Mattson, Chem. Eur. J. 18 (2012) 8310–8314.
[13] One paper mentioned the generation of a *rev*-type compound during the synthetic studies on strigol: K. Frischmuth, E. Samson, A. Kranz, P. Welzel, H. Meuer, W.S. Sheldrick Tetrahedron 47 (1991) 9793–9806.
[14] We found that a mixture of 5DS and *rev*-5DS could be separated by careful column chromatography, whereas the separation of 4DO and *rev*-4DO was rather difficult.
[15] When treating **9** with TFA (excess) in CH_2Cl_2 at 0 to rt for 1 h, a considerable amount of by-product (compound **S1**) was isolated (see SI).
[16] C.C. Lin, T.M. Teng, C.C. Tsau, H.Y. Liao, R.S. Liu, J. Am. Chem. Soc. 130 (2008) 16417–16423.
[17] T.V. Charnikhova, K. Gaus, A. Lumbroso, M. Sanders, J.P. Vincken, A. De Mesmaeker, C.P. Ruyter-Spira, C. Screpanti, H.J. Bouwmeester, Phytochem. Lett. 24 (2018) 172–178.
[18] K. Chojnacka, S. Santoro, R. Awartani, N.G.J. Richards, F. Himo, A. Aponick, Org. Biomol. Chem. 9 (2011) 5350–5353.
[19] (a) G. Piancatelli, A. Scettri, S. Barbadoro, Tetrahedron Lett. 39 (1976) 3555–3558;
(b) M. D'Auria, Heyerocycles 52 (2000) 185–194;
(c) B.L. Yin, Y.L. Wu, J.Q. Lai, Eur. J. Org. Chem. 2009 (2009) 2695–2699;
(d) C. Piutti, F. Quartieri, Molecules 18 (2013) 12290–12310.