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Structural Assignment

Securing Important Strigolactone Key Structures: Orobanchol and 5-Deoxystrigol

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Abstract: Strigolactones (SLs) constitute an important new class of plant hormones. Their isolation from natural resources, such as root exudates, is laborious and difficult. Therefore, synthetic SLs are needed to discover their (biological) properties. Such syntheses involve many steps. When repeating a published procedure for the synthesis of orobanchol, we noticed that the structure of the synthesized material was ambiguous. This structure was secured by means of X-ray analysis. An essential step in the synthesis, namely an allylic oxidation of the ABC scaffold, was significantly improved by using Pd/C and *tert*-butyl hydroperoxide (Corey's method). The second issue deals with the structure of the four stereoisomers of 5-deoxystrigol. The stereochemistry of these compounds was based on the use of Welzel's empirical rules for CD spectra. By means of X-ray analysis the stereochemistry of one of the stereoisomers was established unambiguously, thereby securing the configuration of all four isomers.

Introduction

Strigolactones (SLs) are new plant hormones that have received much attention in the recent literature.[1–6] In 1966, the first SL was isolated, and was named strigol (**1a**; Figure 1).[7a] It took about 20 years before its detailed structure was established.[7] The most notable biological property of strigol is its ability to germinate the seeds of the parasitic weeds *Striga* (witchweed) and *Orobanche* spp. (broomrape).[8] These weeds cause serious problems in agricultural food production in developing countries.[8,9] The roots of important food crops, such as maize, sorghum, and rice produce SLs, which induce germination of the seeds of the weeds. This produces a radicle, which attaches itself to the roots of the host plant. The parasite then takes nutrients from the host plant for its own development. As a consequence, the crop yields are lowered substantially, sometimes by up to 90 %.[9] Controlling the parasitic weeds is extremely difficult, and in fact no satisfactory method is currently available.[9]

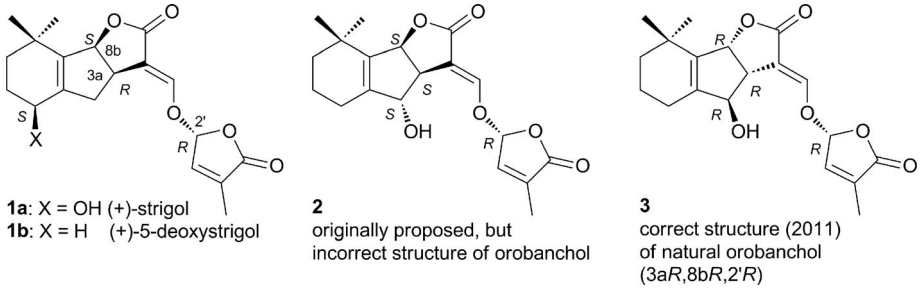

Figure 1. Structures of strigol, 5-deoxystrigol, and orobanchol.

The isolation of SLs from the root exudates of host plants is very difficult and laborious; the plants produce very small amounts of SL, about 15 pg/plant/day, and many other compounds are also present. In the last decade of the previous century, the isolation and structural elucidation of some new SLs was reported.[1,2,10,11] All SLs have three annulated rings, the so called ABC scaffold, which is connected to a butenolide ring (the D-ring) by an enol ether unit.[1,2] The structures of sorgolactone and strigol were confirmed by total synthesis,[1,2,12–14]

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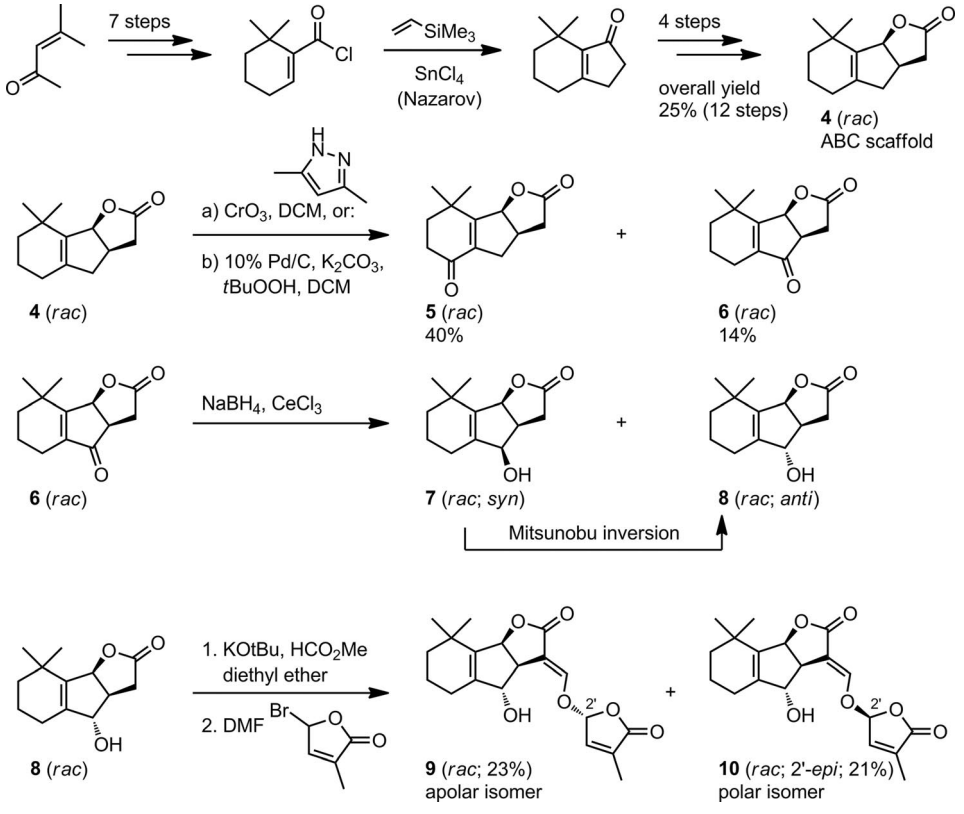
and the originally proposed structures of alectrol[11b,15] and orobanchol[15] were corrected about 20 years later.[1,17–18] This paper deals with the structure of orobanchol. This SL was isolated from red clover roots by Yokata et al.[15] Its structure was determined on the basis of spectroscopic analysis, and its stereochemistry was proposed based on an assumed analogy with the stereostructure of strigol (**1a**).[19] It should be noted, however, that chiroptical data were not made available. Mori et al.[19,20] synthesized orobanchol with the proposed structure (i.e., **2**), also in enantiopure form using a lipase-catalysed asymmetric acetylation as the key step.[21] Originally, structure **2** was assigned to the naturally occurring orobanchol.[15] However, this structure was shown to be incorrect; it actually has a stereoisomeric structure, namely **3**.[17] Several structural corrections of naturally occurring SLs have been reported.[1,16–18] However, the correction of the orobanchol structure has a serious impact on SL research, as this SL is one of the most abundant ones. The literature published before the structure change in 2011 refers to the old and incorrect structure (i.e., **2**), but not all researchers are aware of this, which may give rise to confusion.[1,13] The structure of racemic orobanchol assigned to the product synthesized by Mori et al.[19,20] is ambiguous. In this paper, the correct structures will be reported.

In addition, this paper deals with the stereochemistry of the four stereoisomers of 5-deoxystrigol (**1b**, 5-DES; for nomenclature, see refs.[1,6]).[14,22,23,28] Biosynthetically, (+)-5-deoxystrigol originates from carlactone,[24,25] and it plays a key role in SL research. Note that carlactone is not an SL, but is accepted as a precursor of SLs.[25] The assignment of the stereostructure of the 5-DES isomers relies on the application of the Welzel rule using circular dichroism (CD) spectroscopy.[26] Isomers with a positive Cotton effect in the 270 nm region have the (*S*) stereochemistry at C-2′, while those with a negative Cotton effect have the (*R*) configuration at C-2′. The Welzel rule is entirely empirical, and is based on correlation diagrams with structures of known absolute stereochemistry.[26a] The assignment of the stereochemistry at C-2′ of SLs seems to be consistent. However, for the assignment of the stereochemistry at C-2′ of carlactone, the Welzel rule does not apply.[24] The stereochemistry of SLs is a very important issue, and therefore it is relevant to unambiguously determine the absolute structure of one 5-DES stereoisomer by means of an X-ray diffraction analysis. The results are reported in this paper.

Results and Discussion

The synthetic outline of Mori et al.[20] for the preparation of racemic orobanchol and its 2′-epimer was essentially followed. The required starting material, ABC scaffold **4**, was synthesized in a 12-step sequence starting from mesityl oxide, as reported by Reizelman et al.[14] (Scheme 1). All steps were optimized for multigram quantities, resulting in an overall yield of ca 25 %. For the conversion of the ABC scaffold into orobanchol, two strategies can be envisaged: initial oxidation of the B-ring, followed by attachment of the D-ring; or alternatively, initial at-



Scheme 1. Optimized synthesis of racemic orobanchol epimers.

tachment of the D-ring, and then oxidation of the B-ring.[13] Mori et al.[20] used the first-mentioned route. The oxidation of the ABC scaffold was problematic, as it gave a mixture of A-ring product **5** (40 %; ref.[20] 75 %) and B-ring product **6** in a ratio of 3:1, and only a 15 % yield (ref.[20] 23 %) of the desired regioisomer (i.e., **6**). Moreover, the literature procedure[20] requires the use of 100 equiv. of toxic chromium trioxide, as well as 3,5-dimethylpyrazole. Therefore, an alternative procedure for this allylic oxidation was investigated. The method reported by Corey,[27] using palladium on charcoal in combination with *tert*-butyl peroxide and a catalytic amount of potassium carbonate, gave a similar ratio and yield of strigol precursor **5** and orobanchol precursor **6**. However, the procedure is far more attractive than the procedure by Mori in terms of workup and environmental impact.[18] The resulting 4-oxo-ABC unit (i.e., **6**) was subsequently subjected to a Luche reduction with $NaBH_4$/$CeCl_3$ to give a mixture of the corresponding alcohols (i.e., **7** and **8**); unwanted isomer **7**, with the OH group in a *syn* orientation relative to the C-ring, was the predominant isomer. The configuration of this alcohol was inverted using the standard Mitsunobu procedure to give the desired *anti*-alcohol (i.e., **8**).[14,20]

Having adjusted the stereochemistry of the hydroxy group at the B-ring to *anti* relative to the C-ring (**8**), the coupling with the D-ring was carried out in the usual manner, by potassium *tert*-butoxide mediated formylation with methyl formate, and coupling with bromobutenolide (Scheme 1). This coupling gave the two racemic diastereoisomers (i.e., **9** and **10**) in 23 and 21 % yield, respectively.

The conversion of ABC scaffold **4** into a mixture of orobanchol and its 2′-epimer described above essentially follows the route reported by Mori et al.[20] However, this paper by Mori contains disturbing mistakes concerning the identities of the structures of the racemic epimers. In the main text (p. 2202), the structure of the *low*-melting epimer (m.p. 170–172 °C) is reported to have structure **10**, which was substantiated by an X-ray analysis. In the experimental section (p. 2208) the *high*-melting epimer (m.p. 200–201 °C) is given the same structure **10**. Unfortunately, the crystallographic data at the Cambridge Structural Database do not give conclusive information, because the melting point of the compound that was subjected to X-ray analysis is not mentioned.

To solve this ambiguity, X-ray analysis was carried out for both epimers **9** and **10**. Our results clearly show that the *low*-melting epimer has structure **9**, while the *high*-melting compound is 2′-epimer **10** (Figure 2). We therefore conclude that the information in the experimental section of the Mori paper[20] is correct. The compound with the originally assigned structure (i.e., **2**) for the naturally occurring orobanchol (Figure 1) is present in racemate **9** with the low melting point, while the compound with the correct structure (i.e., **3**) is present in the high-melting racemic 2′-*epi*-orobanchol **10** (Figure 2). It should be noted that usually only one enantiomer of a racemate is pictured (see Scheme 1). This means that the correct structure of natural orobanchol, having the (*R*,*R*,*R*) configuration, is "hidden" in racemic 2′-epimer **10**. The name of natural orobanchol with the correct structure (i.e., **3**) is *ent*-2′-*epi*-orobanchol when (+)-strigol is taken as the parent compound. However, some SL researchers prefer to take the correct structure as the parent, meaning that the natural product is named orobanchol. These different names may lead to confusion.[1,13] For details of the naming issue, see ref.[1]

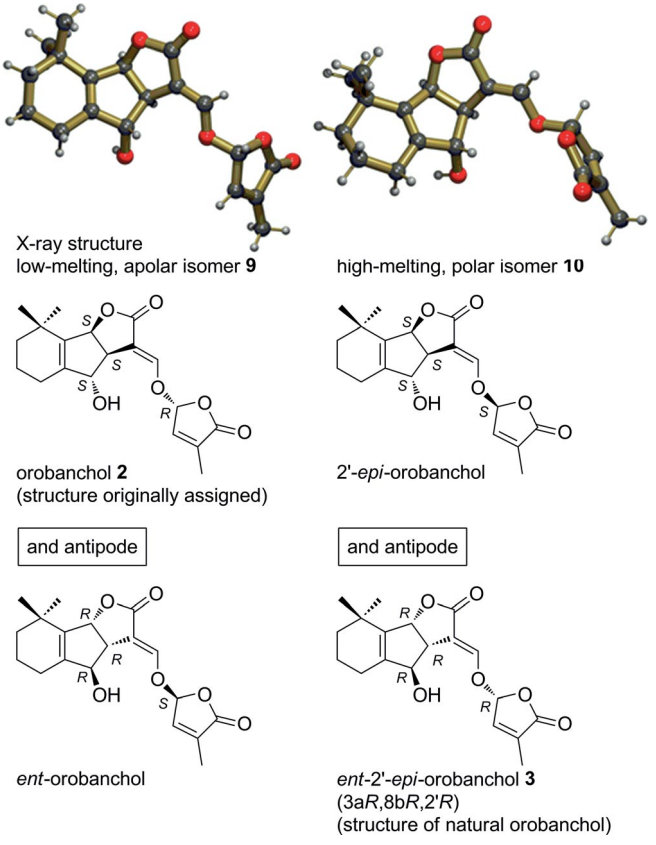
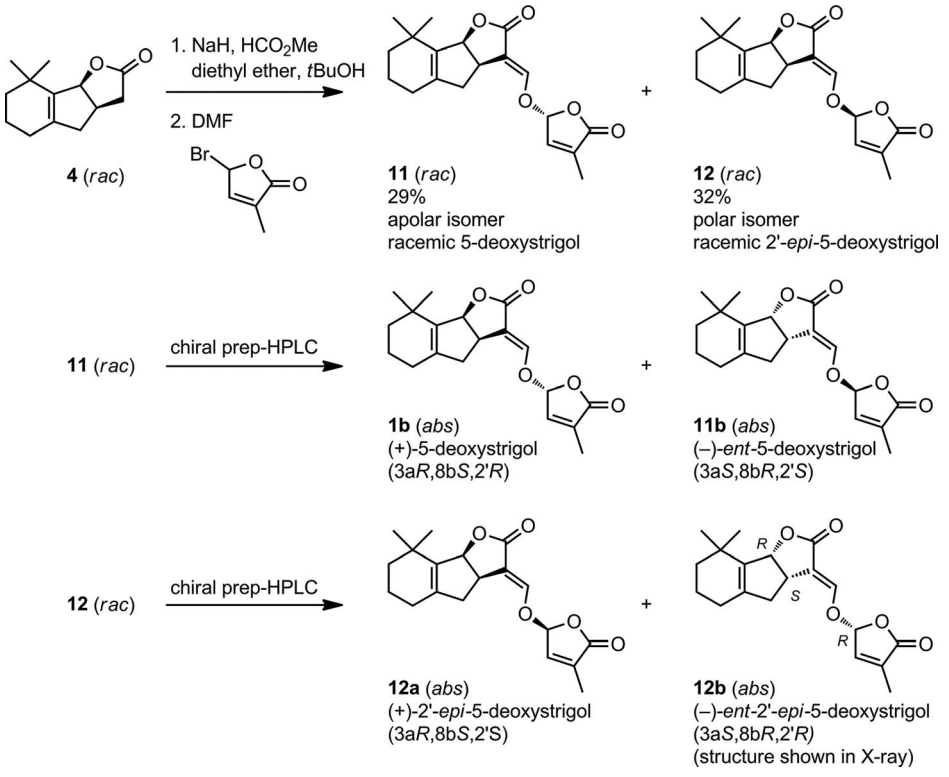


Figure 2. X-ray structures of racemic orobanchol epimers **9** and **10**, and correlation with natural orobanchol **3**.

The second stereochemical problem in this paper deals with securing the absolute configuration of all four stereoisomers of 5-deoxystrigol (**1b**). These four stereoisomers were prepared according to an optimized literature procedure,[14a] as shown in Scheme 2. Chiral separation[14a,22,28] of both the racemic diastereoisomers of 5-deoxystrigol was carried out using a Chiralpak AD HPLC column, and CD spectra of the four stereoisomers were recorded (Figure 3). One of these stereoisomers was subjected to X-ray diffraction analysis, which identified the structure of this compound as isomer **12b**. Gratifyingly, the X-ray structure (Figure 4) was in full agreement with the structure assigned by CD spectra applying Welzel's rule,[23,26,28] i.e., the (3a*S*,8b*R*,2′*R*) configuration, with a negative Cotton effect in the 270 nm region of the CD spectrum, thus corresponding to (–)-*ent*-2′-*epi*-5-deoxystrigol. Having determined the absolute configuration of stereoisomer **12b**, the remaining peaks in the chiral HPLC chromatograms could be attributed to the enantiopure 5-deoxystrigol stereoisomers **1b**, **11b**, and **12a**, respectively, using the CD correlation diagrams (Figure 3). We now have an unambiguous proof of the stereostructures of all four 5-deoxystrigols, which can reliably be used in plant research.



Scheme 2. Synthesis of racemic 5-deoxystrigol epimers **11** and **12** and their chiral separation.

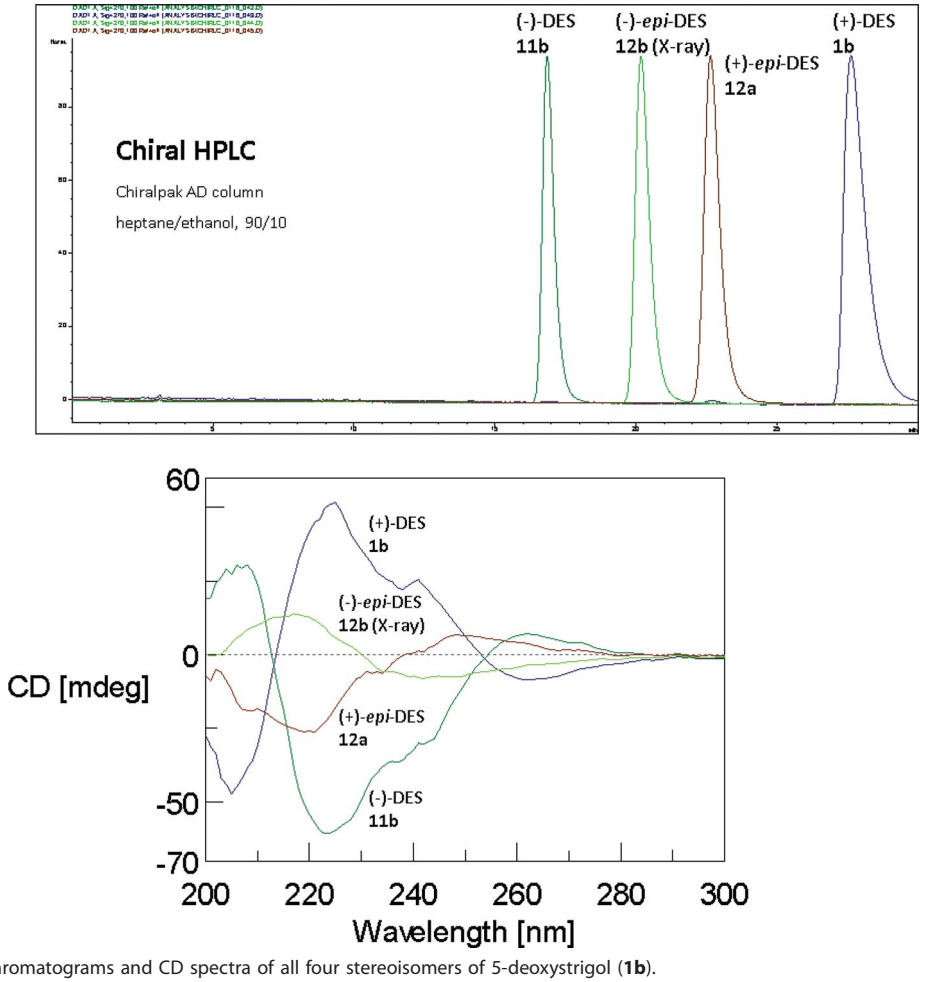


Figure 3. Chiral HPLC chromatograms and CD spectra of all four stereoisomers of 5-deoxystrigol (**1b**).

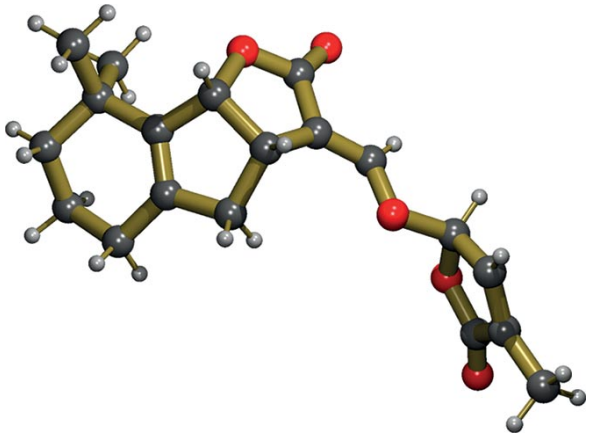


Figure 4. X-ray structure of (–)-*ent*-2′-*epi*-5-deoxystrigol (**12b**; second peak in chiral HPLC).

Conclusions

The stereochemistry of SLs is increasingly important for the understanding of the biological processes in which these new plant hormones are involved. The results described here remove any confusion about the structure of the synthetic orobanchol in ref.[20] By means of X-ray analyses, the structures were assigned unambiguously. The assignment of the stereostructures of all four 5-deoxystrigol isomers was confirmed by X-ray analysis of one of the isomers, together with circular dichroism and chiral HPLC. The reported syntheses of ABC scaffold **4**, orobanchols, and 5-deoxystrigols were optimized, and chiral compounds were prepared on a large enough scale to allow material to be supplied to biologists for wide application in SL plant research.

Experimental Section

General Information: Compounds **4**,[14] **7**,[20] **8**,[20] **11**,[14a] and **12**[14a] were prepared according to (optimized) literature procedures. All reactions were carried out under nitrogen using dry solvents and reagents. Reactions were monitored by thin-layer chromatography (TLC) using silica gel 60 F254 plates (Merck). Flash column chromatography: Silica gel 60, 20–45 μm (Grace). Column chromatography: Silica gel 60, 60–200 μm (Acros). LCMS-UV: Waters Iclass, PDA 220–320 nm, SQD2 ESI, pos/neg 100–800; column: Waters Acquity™ CSH C18, 50 × 2.1 mm, 1.7 m; temp.: 35 °C; flow: 0.6 mL/min; gradient: t_0 = 5 % A, $t_{1.5min}$ = 98 % A, $t_{2.0min}$ = 98 % A, post-time 0.5 min; eluent A: acetonitrile (with 0.1 % formic acid), eluent B: water (with 0.1 % formic acid). ^{1}H NMR (400 MHz): Bruker 400 MHz Ultra Shield; auto wobble; temperature 293 K. Melting points were recorded with a Büchi B-540 apparatus.

Oxidation of *rac*-(3a*R*,8b*S*)-8,8-Dimethyl-3,3a,4,5,6,7,8,8b-octahydro-2*H*-indeno[1,2-*b*]furan-2-one (4). Method a: 3,5-Dimethylpyrazole (9.32 g, 97.0 mmol) was added to a suspension of chromium(VI) oxide (9.70 g, 97.0 mmol) in CH_2Cl_2 (70 mL) at –20 °C. The reaction mixture was stirred at –20 °C for 30 min. A solution of *rac*-(3a*R*,8b*S*)-8,8-dimethyl-3,3a,4,5,6,7,8,8b-octahydro-2*H*-indeno-[1,2-*b*]furan-2-one (**4**; 200 mg, 0.970 mmol) in CH_2Cl_2 (6 mL) was added. The reaction mixture was stirred at –20 °C for 4 h, and then it was quenched with a solution of NaOH (8.80 g, 220 mmol) in water (44 mL). The layers were separated, and the organic layer was washed with water (40 mL), HCl (1 M aq.; 20 mL), and saturated aq. $NaHCO_3$ (20 mL). The organic phase was then dried with Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel using a gradient of heptane/EtOAc (80:20 to 40:60) to give, as the first eluting fraction, compound **6** (32 mg, 15 %), and, as the second eluting fraction, compound **5** (88 mg, 41 %). **Method b:** *tert*-Butyl hydroperoxide (70 % solution in water; 33.6 mL, 31.2 g, 242 mmol) was extracted with CH_2Cl_2 (200 mL), dried with Na_2SO_4, and partly concentrated (75 mL). ***CAUTION:*** Strong oxidant and potentially explosive! The solution was added to a mixture of K_2CO_3 (1.68 g, 12.1 mmol) and compound **4** (10.0 g, 48.5 mmol) in CH_2Cl_2 (250 mL) at 0 °C. Pd/C (10 %; 1.24 g) was added (gas evolution), and the reaction mixture was stirred at 0 °C overnight. Celite was added, and the mixture was filtered through Celite and concentrated. The crude material (19 g) was purified by column chromatography on silica gel using a gradient of heptane/EtOAc (70:30 to 50:50) to give, as the first eluting fraction, compound **6** (1.48 g, 14 %), and, as the second eluting fraction, compound **5** (4.22 g, 40 %).

***rac*-(3a*R*,8b*S*)-8,8-Dimethyl-3a,4,6,7,8,8b-hexahydro-2*H*-indeno[1,2-*b*]furan-2,5(3*H*)-dione (5):** $C_{13}H_{16}O_3$ (MW 220.26). Purity >95 % by ^{1}H NMR spectroscopy. The analytical data were identical to those reported in the literature.[20]

***rac*-(3a*R*,8b*S*)-8,8-Dimethyl-3,3a,5,6,7,8-hexahydro-2*H*-indeno[1,2-*b*]furan-2,4(8b*H*)-dione (6):** $C_{13}H_{16}O_3$ (MW 220.26). Purity >95 % by ^{1}H NMR spectroscopy. The analytical data were identical to those reported in the literature.[20]

***rac*-Orobanchol (9) and *rac*-2′-*epi*-Orobanchol (10):** Potassium *tert*-butoxide (786 mg, 7.00 mmol) was added portionwise to a solution of *rac*-(3a*R*,4*R*,8b*R*)-4-hydroxy-8,8-dimethyl-3,3a,4,5,6,7,8,8b-octahydro-2*H*-indeno[1,2-*b*]furan-2-one (**8**; 778 mg, 3.50 mmol) and methyl formate (1.08 mL, 1.05 g, 17.5 mmol) in diethyl ether (25 mL) at 0 °C. The mixture was stirred for 1.5 h, then it was cooled to –50 °C, and a solution of 5-bromo-3-methylfuran-2(5*H*)-one (1.08 g, 6.13 mmol) in DMF (12.5 mL) was added dropwise over 5 min. The reaction mixture was stirred at room temperature overnight, then it was poured into a mixture of water and saturated aq. $NaHCO_3$ (1:1; 100 mL), and extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with brine (3 × 50 mL), dried with Na_2SO_4, and concentrated. The residue (1.44 g) was purified by flash column chromatography on silica gel (80 g) using a gradient of heptane/EtOAc (70:30 to 100:0) to give, as the first eluting fraction, compound **9** (277 mg, 23 %), and, as the second eluting fraction, compound **10** (252 mg, 21 %).

***rac*-(3a*R*,4*R*,8b*R*,*E*)-4-Hydroxy-8,8-dimethyl-3-({[(*S*)-4-methyl-5-oxo-2,5-dihydrofuran-2-yl]oxy}methylene)-3,3a,4,5,6,7,8,8b-octahydro-2*H*-indeno[1,2-*b*]furan-2-one (9):** A sample obtained by flash column chromatography was recrystallized from diethyl ether. $C_{19}H_{22}O_6$ (346.37). LCMS-UV: purity >95 %; *m*/*z* = 347 [M + H]$^+$; t_R = 3.3 min. M.p. 168–170 °C (ref.[20] 170–172 °C). The analytical data were identical to those reported in the literature.[20]

***rac*-(3a*R*,4*R*,8b*R*,*E*)-4-Hydroxy-8,8-dimethyl-3-({[(*R*)-4-methyl-5-oxo-2,5-dihydrofuran-2-yl]oxy}methylene)-3,3a,4,5,6,7,8,8b-octahydro-2*H*-indeno[1,2-*b*]furan-2-one (10):** A sample obtained by flash column chromatography was recrystallized from heptane/EtOAc. $C_{19}H_{22}O_6$ (346.37). LCMS-UV: purity >95 %; *m*/*z* = 347 [M + H]$^+$; t_R = 3.3 min. M.p. 188–190.5 °C (ref.[20] 200–201 °C). The analytical data were identical to those reported in the literature.[20]

***rac*-5-Deoxystrigol (11) and *rac*-2′-*epi*-5-Deoxystrigol (12):** Sodium hydride (60 % in mineral oil; 1.66 g, 41.6 mmol) was added to a solution of *rac*-(3a*R*,8b*S*)-8,8-dimethyl-3,3a,4,5,6,7,8,8b-octahydro-

2*H*-indeno[1,2-*b*]furan-2-one (**4**; 8.58 g, 41.6 mmol) and methyl formate (12.8 mL, 12.5 g, 208 mmol) in diethyl ether (100 mL) at 0 °C. The reaction mixture was allowed to reach room temperature, and *tert*-butanol (3.98 mL, 3.08 g, 41.6 mmol) was added. After 4 h, *N*,*N*-dimethylformamide (50 mL) was added, and the mixture was cooled to –55 °C. A solution of 5-bromo-3-methylfuran-2(5*H*)-one (8.10 g, 45.8 mmol) in *N*,*N*-dimethylformamide (50 mL) was added dropwise over 30 min. The reaction mixture was allowed to reach room temperature and was stirred overnight. Et_2O (200 mL) and ice-cold water (200 mL) were added, and the layers were separated. The organic layer was washed with a solution of K_2CO_3 (2 g in 150 mL water), water (100 mL), and brine (3 × 50 mL), dried with Na_2SO_4, and concentrated. The residue (13.8 g) was purified by column chromatography on silica gel using a gradient of heptane/EtOAc (3:1 to 1:1) to give, as the first eluting fraction, *rac*-5-deoxystrigol (**11**), and, as the second eluting fraction, 2′-*epi*-5-deoxystrigol (**12**). Both compounds were recrystallized from Et_2O/heptane.

***rac*-(3a*R*,8b*S*,*E*)-8,8-Dimethyl-3-({[(*R*)-4-methyl-5-oxo-2,5-dihydrofuran-2-yl]oxy}methylene)-3,3a,4,5,6,7,8,8b-octahydro-2*H*-indeno[1,2-*b*]furan-2-one (11):** $C_{19}H_{22}O_5$ (330.37). Yield: 4.02 g (29 %). M.p. 141.5–143.0 °C (ref.[14a] 139–140 °C). The analytical data were identical to those reported in the literature.[14a]

***rac*-(3a*R*,8b*S*,*E*)-8,8-Dimethyl-3-({[(*S*)-4-methyl-5-oxo-2,5-dihydrofuran-2-yl]oxy}methylene)-3,3a,4,5,6,7,8,8b-octahydro-2*H*-indeno[1,2-*b*]furan-2-one (12):** $C_{19}H_{22}O_5$ (MW 330.37). Yield: 4.37 g (32 %). M.p. 143.5–145.0 °C (ref.[14a] 146–147 °C). The analytical data were identical to those reported in the literature.[14a]

Chiral HPLC of 5-Deoxystrigol Isomers: Equipment for preparative HPLC: Shimadzu LC8-A preparative pumps, Shimadzu SCL-10Avp system controller, Shimadzu SPD-10Avp UV/Vis detector; fraction collector: Gilson 215 Liquid Handler. Equipment for analytical HPLC: Agilent 1260 quart. pump; G1311C, auto sampler, ColCom; diode-array detector (DAD): Agilent G4212B, 220–320 nm and 220 nm. Samples of 25 mg/mL *rac*-5-deoxystrigol (**11**; total 2.7 g) and *rac*-2′-*epi*-5-deoxystrigol (**12**; total 3.2 g) in heptane/EtOH (1:1, heated) were separated in 100–150 runs into enantiomers **1b** and **11b**, and enantiomers **12a** and **12b**, respectively, by preparative HPLC on a Chiralpak AD normal-phase column (250 × 20 mm, 5 µ, 25 °C), eluting with isocratic heptane/EtOH (90:10, 18 mL/min), with UV detection at λ_{max} = 220 nm. The elution order was (see note below): (–)-*ent*-5-deoxystrigol (**11b**; 16.9 min; 1.05 g, 39 %), (–)-*ent*-2′-*epi*-5-deoxystrigol (**12b**; 20.2 min; 1.05 g, 39 %), (+)-2′-*epi*-5-deoxystrigol (**12a**; 22.6 min; 1.10 g, 33 %), (+)-5-deoxystrigol (**1b**; 27.7 min; 1.10 g, 33 %). Enantiomeric excesses were >99 %, as determined by analytical HPLC using a Chiralpak AD-H column (250 × 4.6 mm, 5 µm, 35 °C), eluting with isocratic heptane/EtOH (90:10, flow rate 1 mL/min; time 30 min) and UV-DAD detection at 220–320 nm and 220 nm. Purities were >97 % based on LCMS-UV, t_R = 5.4 min; m/z = 331 $[M + H]^+$ for $C_{19}H_{22}O_5$ (330.37). ***Note:*** The elution order of the enantiomers is different on an Astec cellulose DMP chiral column (hexane/MeOH/methyl-*tert*-butyl ether, 6:1:1), see ref.[28] Similarly for a Daicel Chiralpak IB column (heptane/EtOH, 97:3), see ref.[14b]

(3a*S*,8b*R*,*E*)-8,8-Dimethyl-3-({[(*R*)-4-methyl-5-oxo-2,5-dihydrofuran-2-yl]oxy}methylene)-3,3a,4,5,6,7,8,8b-octahydro-2*H*-indeno[1,2-*b*]furan-2-one (12b): $C_{19}H_{22}O_5$ (330.37). Yield from chiral preparative HPLC: 1.05 g (39 %). Enantiomeric excess >99 %. LCMS-UV: purity >98 %; m/z = 331 $[M + H]^+$; t_R = 5.4 min. A sample (13 mg) was recrystallized from EtOAc/heptane (1:8, 2 mL, 2 weeks) and subjected to X-ray diffraction. M.p. 131–132 °C. The analytical data were identical to those reported in the literature.[14b]

Circular Dichroism 5-Deoxystrigol Isomers: CD spectra of **1b**, **11b**, **12a**, and **12b** were recorded for samples in acetonitrile (LCMS grade) with a JASCO J-815 CD spectrophotometer.

Data for 1b: CD (c = 45 µM, MeCN): λ_{ext} ($\Delta\varepsilon$) = 263 (–8.5), 225 (+51.6), 205 (–47.3) nm.

Data for 11b: CD (c = 40 µM, MeCN): λ_{ext} ($\Delta\varepsilon$) = 262 (+7.1), 223 (–60.7), 208 (+30.5) nm.

Data for 12a: CD (c = 40 µM, MeCN): λ_{ext} ($\Delta\varepsilon$) = 248 (+6.7), 221 (–26.4) nm.

Data for 12b: CD (c = 70 µM, MeCN): λ_{ext} ($\Delta\varepsilon$) = 242 (–8.2), 217 (+13.8) nm.

X-ray Diffraction Analysis: For single-crystal X-ray diffraction, crystals were cut to size, mounted on a Mitagen Microloop using high-viscosity oil, and shock frozen to 208 K using liquid nitrogen. Intensity data were collected at 208 K. The measurements were carried out using a Nonius Kappa CCD single-crystal diffractometer (φ- and ω-scan mode) using graphite-monochromated Mo-K_α radiation. Diffraction images were integrated using Eval14.[29] Intensity data were corrected for Lorentz and polarization effects. A semiempirical multiscan absorption correction was applied (SADABS[30]). The structures were solved using SHELXT.[31] Refinement was carried out by standard methods: refinement against F^2 of all reflections with SHELXL-2014. All non-hydrogen atoms were refined with anisotropic temperature factors. The positions of the hydrogen atoms were initially determined from a difference Fourier map, and were subsequently, when possible, replaced by hydrogen atoms at calculated positions and refined riding on the parent atoms. CCDC 1053254 (for high-melting orobanchol **10**), 1053255 (for low-melting orobanchol **9**), and 1053256 [for (–)-*ent*-2′-*epi*-5-deoxystrigol **12b**] contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre.

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