Total Synthesis of (−)- and (+)-Botryodiplodin and (+)- and (−)-Epibotryodiplodin

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The title compounds were prepared by the conjugate addition (d.e.>94 %) of LiMe-CNCu to the chiral isoprene units (+)-(2R)- and (−)-(2S)-benzyloxy-2,5-dihydrofuran-4-carboxaldehyde (1r and 1s) followed by methyllithium-attack on the mixture of aldehydes formed (3 and 4) and oxidation of the resulting mixture of alcohols (5 and 6) to give the ketones (−)-(2S,3R,4R)- and (−)-(2S,3R,4S)-4-acetyl-2-benzyloxy-3-methyltetrahydrofuran (7r and 8r) and (+)-(2R,3S,4S)- and (+)-(2R,3S,4R)-4-acetyl-2-benzyloxy-3-methyltetrahydrofuran (7s and 8s), respectively. The ketones were separated and hydrogenolytic removal of the benzyl group of 8r, 8s, 7r, and 7s gave the title compounds, respectively. Epimerisation of (−)-botryodiplodin with aqueous sodium hydrogencarbonate gave a (racemic) mixture of (−)- and (+)-boytryodiplodin and (+)- and (−)-epibotryodiplodin. Treatment of bovine serum albumin and L-lysine with (−)-botryodiplodin gave an orange gel and a dark-red solution, respectively.

This paper describes the experimental details for the synthesis of the two botryodiplodin enantiomers 10r and 10s and their epimers 9r and 9s. It also shows that (−)-botryodiplodin (10r) undergoes base-catalysed epimerisation at both carbons α to the carbonyl groups (ketone and aldehyde/hemiacetal), resulting in a racemic mixture containing all the title compounds. A preliminary account of part of this work has been reported.[1]

(−)-Botryodiplodin[2] (10r) was isolated from cultured *Botryodiplodia theobromae* Pat.[3] and later from other fungi[4,5] including *Penicillium roquefortii*, a species used in the ripening of blue-veined cheese.[6] Furthermore, epibotryodiplodin (9r) was suggested to be formed by treatment of botryodiplodin in ethyl acetate with sodium hydrogencarbonate.[5] The gross structure[7] of botryodiplodin was verified by synthesis, which also revealed the stereostructure.[8] The absolute configuration of natural (−)-botryodiplodin was established as (3R,4S) by conversion into an α-naphthyl derivative of known chirality.[9] The crystal structure of botryodiplodin has been determined via the 1-O-acetate.[6] A hypothesis for the biosynthesis of botryodiplodin via orsellinic acid was supported by ^{13}C tracer studies.[10]

Botryodiplodin shows various antimicrobial[4] and antileukemic[8] activities. It also causes the formation of pink-to-red stains on human skin.[2] Treatment of bovine serum albumin and L-lysine with botryodiplodin in water for a few hours causes the formation of an orange gel and a dark-red solution, respectively (*vide infra*). An increase in the number of accidental deaths among seals in some regions of Japan was related to forage infected with botryodiplodin-producing fungi.[5] The LD_{50} (mouse and rat) of botryodiplodin was found to be in the range 17–50 mg kg^{-1}.[5,11]

Botryodiplodin was a moderate mutagen in the *Salmonella typhimurium* (TA98) histidine reversion system without metabolic activation.[11] It also inhibits the multi

plication of rat hepatoma cells at non-toxic concentrations by affecting DNA, RNA and protein synthesis.[12] The ring-opened aldehydic tautomer of botryodiplodin was considered to be responsible for the formation of DNA-protein cross-links[13] in mammalian cells and for sister chromatid exchange in Chinese hamster V79 cells.[14] The reaction of botryodiplodin with various 2′-deoxynucleosides led to the formation of aminals, which was considered to emulate the first step in the formation of DNA–protein cross-links.[15]

Total syntheses of racemic botryodiplodin/epibotryodiplodin have been performed via Claisen rearrangement and Prins reaction processes of moderate-to-low stereoselection,[16-19] whereas ozonolysis of a *trans*-substituted cyclohexadiene (obtained by preparative GLC of a mixture of diastereomers) furnished racemic botryodiplodin free of its epimer.[8] Natural (−)-botryodiplodin has been prepared via a series of synthetic steps starting from the chiral antibiotic methylenomycin A.[9] Racemic fluorobotryodiplodin and fluoroepibotryodiplodin have recently been reported.[20] In a preliminary report[1] we outlined the synthesis of both enantiomers of botryodiplodin and epibotryodiplodin in high enantiomeric excess using diastereoselective conjugate addition of lithium methylcyanocuprate to the chiral α,β-unsaturated aldehydes 1r and 1s;[21] previous conjugate additions have been made mainly with chiral ketones, esters, lactones and aldimines.[22] The aldehydes 1r and 1s are chiral isoprenoid building units that were used for synthesis of complete norbornane skeletons by a Diels–Alder route[23] and for syntheses of several lignans.[24]

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Results

Synthesis. Conjugate addition of lithium methylcyanocuprate to the chiral aldehydes 1r[21] (and 1s, Scheme 1) gave, in the presence of *tert*-butyldimethylsilyl chloride[25] (TBDMS-Cl), the silyl enol ether 2r (and 2s) in 87 % yield after chromatography (in the absence of TBDMS-Cl the aldehydes 3r/4r were formed in 12 % yield from 1r). The ratio of olefinic isomers was approximately 20:1 according to NMR analysis. The ¹H NMR spectrum of crude 2r showed an acetal (H-2) doublet (5.6 Hz) at 5.00 ppm, which was assigned to a 2,3-*cis* diastereomer. Integration of the H-2 signals in the crude material showed that the 2,3-*trans* compound (2r) constituted ca. 97 % of the mixture and that therefore the LiMeCNCu reaction gave 2r (and 2s) with a diastereomeric excess (d.e.) of 94 %. Chromatographic purification of crude 2r gave material the NMR spectrum of which lacked the doublet at 5.00 ppm. The detection limit of 2,3-*cis* compounds in a *trans*/*cis*-mixture was determined to be ca. 0.3 % by integration of the ¹H NMR signal of the (anomeric) acetal proton in a sample of 7r/13r (99:1). Therefore, we conclude that purified 2r (and 2s) was obtained with a d.e. of >99.4 %.

The TBDMS group of 2r (and 2s) was removed by treatment with tetrabutylammonium fluoride trihydrate in tetrahydrofuran/acetic acid (19:1) which gave the epimeric aldehydes 3r/4r (and 3s/4s) in 91 % yield in the ratio 83:17. In an experiment where the acetic acid was added after the

fluoride, the mixture turned yellow and the yield dropped to 75 %.

Treatment of the aldehyde mixture 3r/4r (and 3s/4s) with methyllithium in ether at −70 °C gave a diastereomeric mixture of the alcohols 5r/6r (and 5s/6s) by chromatography in 88 and 2 % yield, respectively. The alcohol 5r (and 5s) was a 1:2 mixture of diastereomers according to NMR analysis. No attempt was made to determine the stereo-structures of the two compounds 5r. In contrast, 6r (and 6s) was a pure compound and a crystalline 3,5-dinitrobenzoate (11r) was obtained.

The alcohol mixture 5r/6r (and 5s/6s) was oxidised to the ketones 7r and 8r (and 7s/8s) in 90 and 3 % yield, respectively, using the Swern procedure[26] and in 59 and 11 % yield by barium manganate oxidation.[27] The ketones 7r and 8r were easily separated on silica gel. At equilibrium in 18 mM methanolic sodium methoxide, the ratio between 7r and 8r was ca. 5:1 as determined by GLC starting with pure 7r or 8r. Epimerisation of 7r and chromatography of the reaction mixture was used for the preparation of larger amounts of 8r.

Hydrogenolytic removal of the benzyl group in 7r (and 7s) gave, in 91 % yield, enantiomerically pure (e.e. >99.4 %; see above) (+)- (9r) [and (−)- (9s)] epibotryodiplodin, respectively. Similarly, 8r and 8s gave, in 96 % yield, enantiomerically pure natural (−)- (10r) [and (+)- (10s)] botryodiplodin. It is important that the hydrogenolysis leads to a pure product because chromatographic puri-
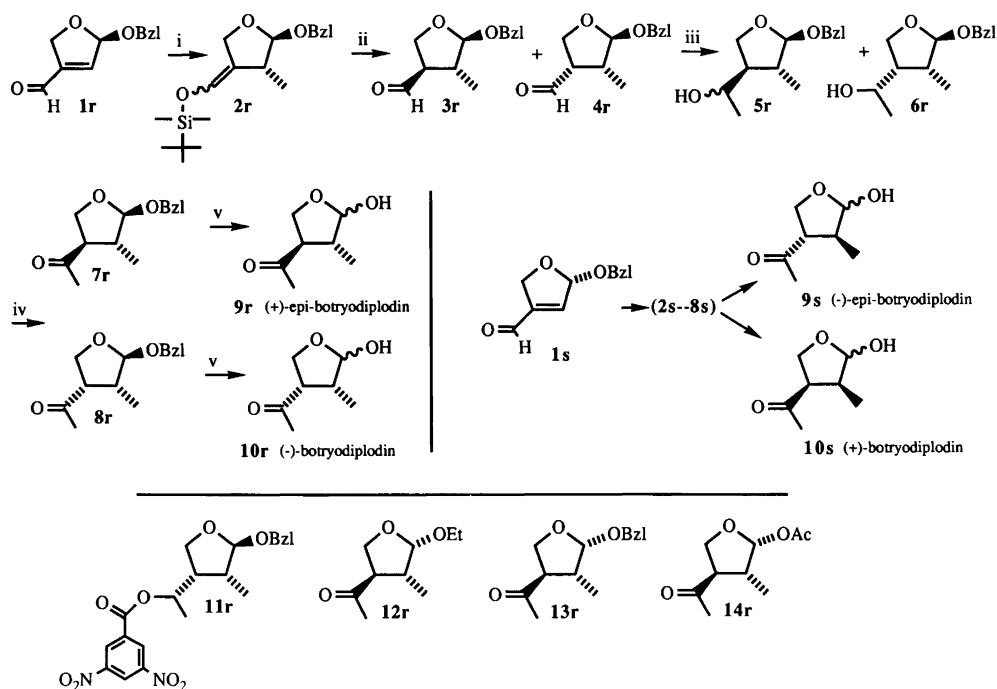
Scheme 1. (i) LiMeCNCu, t-BuMe₂SiCl, THF, −78→+23 °C; (ii) Bu₄NF·3H₂O, THF/HOAc (19:1); (iii) MeLi, Et₂O, −78→+23 °C; (iv) (COCl)₂, DMSO, (i-Pr)₂EtN, −60→+23 °C; (v) H₂, 1 atm, 10% Pd/C, DME/H₂O. (3/1).

fication is difficult due to the instability of both **9** and **10** on silica gel. When **7** and **8** were prepared by Swern oxidation, the purified ketones then reacted sluggishly with hydrogen, probably due to the presence of sulfur-containing impurities (emanating from dimethyl sulfoxide) that deactivate the catalyst. The ketones prepared by barium manganate oxidation[27] were, on the other hand, easily hydrogenolysed. Both **9** and **10** are rather volatile compounds, therefore, volatile solvents should be used in the final step in order to avoid losses of material in connection with solvent removal. With ethanol as the solvent for the hydrogenolysis of **7r**, part of the product reacted with ethanol to form the ethyl acetal **12r**. Chromatography of the reaction mixture gave 17% yield of **12r**; the remaining material was destroyed on the column. As an alternative to hydrogenolysis, acetolysis of **7r** (acetic acid, 90 °C) was tried which gave, after chromatography, the anomerised benzyl acetal **13r** (11 %) and epibotryodiplodin acetate **14r** (13 %).

Epimerisation reactions. It was suggested[5] that epibotryodiplodin was formed by base-catalysed (NaHCO$_3$) epimerisation of botryodiplodin during work-up of a crude isolate. We have investigated this possibility by exposing (−)-botryodiplodin (**10r**) to basic conditions (0.65 M aqueous NaDCO$_3$). We found that the rate of epimerisation (**10** → **9**) is low and therefore **9** is not likely to be formed by brief exposure of **10** to the basic aqueous conditions of the work-up procedure. Rather, **9** was formed during the fermentation process, possibly by enzymic action.

(−)-Botryodiplodin (**10r**) and sodium deuteriumcarbonate were dissolved in deuterium oxide and the solution (**10r**: 0.072 M; NaDCO$_3$: 0.65 M) was kept at room temperature in an NMR tube during the experiment. NMR spectra were recorded at intervals and the H-2 (anomeric) signals were integrated. The H-2 signal from **10** was a singlet (5.18 ppm) and initially the H-2 signals from **9** were a pair of doublets (5.35 and 5.05 ppm; 4.4 and 1.0 Hz) which slowly converted into a pair of singlets due to the gradual exchange of H-3 for deuterium (cf. Fig. 1). Therefore, the epimerisation at C-4 was more rapid than at C-3. All α-hydrogens were eventually substituted by deuterium (cf. Scheme 2), which simplified the ¹H NMR spectra. TLC on the reaction mixture (3 weeks) and freshly prepared **9r** were very similar, indicating that only few by-products had been formed (corroborated by ¹³C NMR spectrum of the same sample). This is in contrast with acid treatment as indicated by the rapid destruction of **9** and **10** by silica gel.
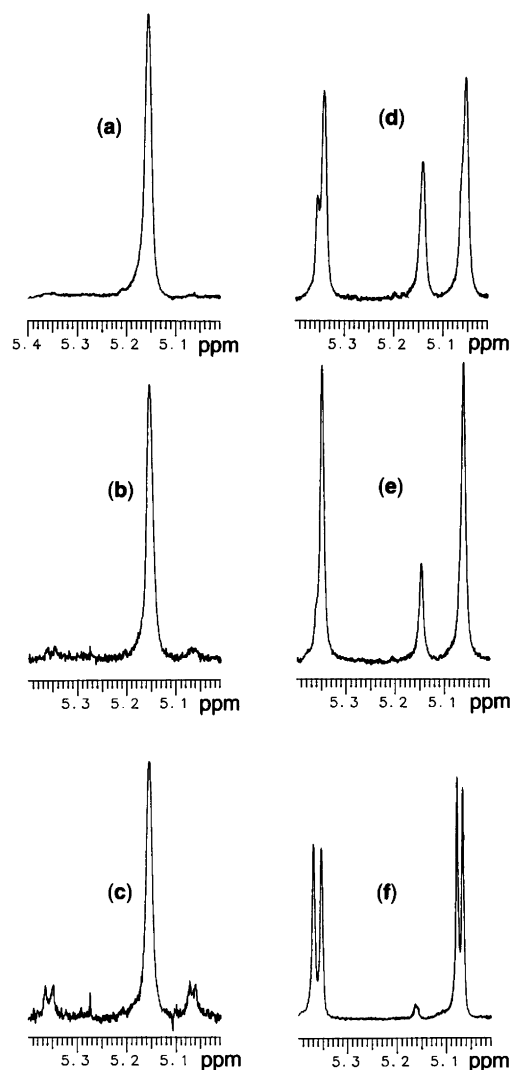

Fig. 1. The H-2 (anomeric) ¹H NMR signals of **10r** after treatment with NaDCO$_3$/D$_2$O: (a) pure **10r**; (b) after 60 min; (c) after 200 min; (d) after 6 days; (e) after 18 days; (f) **9r** containing a trace of **10r** (cf. Scheme 2 and the Experimental).

The approximate 9/10 ratios were found to be 1:25 (1 h), 1:3 (3.3 h), 6:1 (144 h), 6:1 (456 h). The fully equilibrated sample showed that **9** consisted of roughly equal amounts of the two anomers (Fig. 1). When NaDCO$_3$ was excluded, the 9/10 ratio was 1:5 after 144 h.

The optical rotation of the sample mixture at equilibrium (506 h) was +13°. This corroborates the finding above that
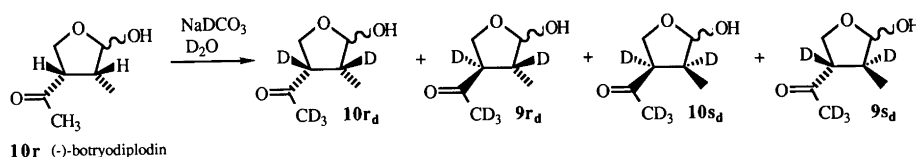
Scheme 2.

all α positions were subject to deuterium exchange, since epimerisation of **10r** α to the keto group only (→**9r/10r**) or α to the hemiacetal group only (→**9s/10r**) would give a rotation, at equilibrium, of ca. +64 and −84°, respectively (calculated for 6:1 mixtures of **9r/10r** and **9s/10r**, respectively; cf. Scheme 2). If no by-products were formed during the equilibrium (racemisation) process, the optical rotation would be zero. Therefore, the small positive value obtained (+13°) indicates that some by-products were formed.

Colour reactions. Several authors have reported that botryodiplodin causes the formation of pink-to-red stains on human skin. It occurred to us that the colour-forming reaction might be related to the Maillard reaction between proteins and sugars which gives browning products.[28] Botryodiplodin is sugar-like since it contains a furanosidic hemiacetal moiety. Freshly prepared (−)-botryodiplodin (**10r**) was added to a solution of bovine serum albumin (BSA). The mixture slowly attained an orange colour and after 6 h the initially clear solution had turned into an orange gel. The gel-formation might be a result of protein–protein cross-links, similar to the botryodiplodin-induced protein–DNA cross-links reported by Renauld et al.[15] The colour reaction was further investigated by treating L-lysine with **10r**. A pale pink colour developed over ca. 1 h and deepened to dark red (burgundy) over 6 h. In a similar investigation of the reaction between lysine and aldoses, it was found that lysine generated a yellow colour with glyceraldehyde and 2-amino-2-deoxyglucose.[29]

Experimental

Liquid chromatography purifications were performed in the gravity mode. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. IR spectra were recorded on a Perkin-Elmer 257 spectrometer. NMR spectra were recorded with a Varian XL-300 spectrometer; chemical shifts are relative to Me_4Si. Mass spectra were recorded on a modified Varian MAT 112 instrument. GLC was performed on a Varian 3700 instrument equipped with an RSL-300 intermediate polarity capillary column.

(−)-(2R,3R)-2-*Benzyloxy*-4-[(tert-*butyldimethylsilyloxy*)-*methylene]-3-methyltetrahydrofuran* **2r**. Methyllithium (50.0 mmol) in diethyl ether (33.3 ml) was added to an ice-cooled slurry of cuprous cyanide (4.48 g, 50.0 mmol) in tetrahydrofuran (160 ml). After 5 min the mixture was cooled (dry ice/acetone bath) and a solution of tert-butyldimethylsilyl chloride (7.53 g, 50.0 mmol) in tetrahydrofuran (38 ml) was added, followed by dropwise addition of a solution of **1r**[21] (8.00 g, 38.5 mmol) in tetrahydrofuran (118 ml). After 5 min, the reaction mixture was allowed to attain room temperature. Diethyl ether (170 ml) was added followed by aqueous ammonium sulfate (10 %, 85 ml). The aqueous phase was extracted with diethyl ether (2×85 ml) and the combined extract was washed with saturated aque-

ous sodium hydrogencarbonate (85 ml). Drying (Na_2SO_4) and evaporation of the solvent gave an oil which was chromatographed (SiO_2, heptane/EtOAc 30:1) to give **2r** (11.25 g, 87 %) as a 20:1 isomeric (*E:Z*) mixture: $[\alpha]_D^{25}$ −88° (c 0.89, $CHCl_3$); ^{1}H NMR data of the major (*E*) isomer ($CDCl_3$): δ 7.36–7.26 (m, 5 H, C_6H_5), 6.18 (dt, 1 H, *J* 2.5, 1.2 Hz, SiOCH), 4.85 (d, 1 H, *J* 1 Hz, H-2), 4.74, 4.50 (AB system, each 1 H, *J* 12.0 Hz, C*H*₂C_6H_5), 4.58 (ddd, 1 H, *J* 12.9, 2.4, 1.6 Hz, H-5), 4.52 (ddd, 1 H, *J* 12.9, 2.4, 1.2 Hz, H-5′), 2.71 (q with further couplings, *J* 7.2 Hz, H-3), 1.08 (d, 3 H, *J* 7.4 Hz, C*H*₃CH), 0.91 (s, 9 H, CH_3C), 0.12 (s, 6 H, CH_3Si); ^{13}C NMR: δ 138.1 (C_6H_5), 131.8 (=COSi), 128.2, 127.8, 127.4 (C_6H_5), 123.5 (C=CO), 108.7 (C-2), 68.6, 67.0 (C-5 and C_6H_5*C*H₂), 41.4 (C-3), 25.7, 19.4 (*C*H₃C and *C*H₃CH), 18.3 [(*C*H₃)₃C], −5.19, −5.24 (CH_3Si). ^{1}H NMR data of the minor (*Z*) isomer ($CDCl_3$): δ 6.24 (m, SiOCH), 4.87 (s, H-2), 4.73 (d, *J* 12.0 Hz, C*H*₂C_6H_5), 1.13 (d, *J* 6.7 Hz, C*H*₃CH). Anal. $C_{19}H_{30}O_3Si$: C, H.

(+)-(2S,3S)-2-*Benzyloxy*-4-[(tert-*butyldimethylsilyloxy*)-*methylene]-3-methyltetrahydrofuran* **2s**. Prepared as above (**2r**) but from **1s**:[21] $[\alpha]_D^{25}$ + 87° (c 0.89, $CHCl_3$); **2s** and **2r** had identical ^{1}H NMR data.

(+)-(2R,3S,4S/4R)-2-*Benzyloxy*-4-*formyl*-3-*methyltetra-hydrofuran* **3s/4s**. A solution of tetrabutylammonium fluoride trihydrate (3.39 g, 10.7 mmol) in tetrahydrofuran/acetic acid (19:1, 20 ml) was added to a solution of **2s** (1.80 g, 5.38 mmol) in tetrahydrofuran/acetic acid (19:1, 20 ml). After the reaction had been stirred for 1 h, toluene (10 ml) was added and the solvent was evaporated. The residue was dissolved in diethyl ether (50 ml) and washed with water (4×10 ml). The ether solution was dried (Na_2SO_4) and concentrated and the residue was chromatographed (SiO_2, heptane/EtOAc 5:1) to give a mixture (83/17) of **3s/4s** (1.08 g, 91 %) as an oil: $[\alpha]_D^{25}$ + 103° (c 1.0, $CHCl_3$); IR (CCl_4): 1727 cm⁻¹ (C=O); **3s** had: ^{1}H NMR ($CDCl_3$): δ 9.68 (d, 1 H, *J* 2.6 Hz, CHO), 7.37–7.25 (m, 5 H, C_6H_5), 4.87 (d, 1 H, *J* 1.1 Hz, H-2), 4.68, 4.42 (AB system, each 1 H, *J* 11.7 Hz, C*H*₂C_6H_5), 4.28 (dd, 1 H, *J* 8.9, 4.8 Hz, H-5), 4.16 (dd, 1 H, *J* 9.2, 7.9 Hz, H-5′), 2.66 (ddq, 1 H, *J* 7.0, 3.0, 0.9 Hz, H-3 or H-4), 2.57 (m, 1 H, H-4 or H-3), 1.13 (d, 3 H, *J* 7.0 Hz, CH_3); **4s** had: ^{1}H NMR: δ 9.79 (d, 1 H, *J* 1.8 Hz, CHO), 7.37–7.25 (m, 5 H, C_6H_5), 4.89 (s, 1 H, H-2), 4.71, 4.47 (AB system, 1 H each, *J* 11.8 Hz, C*H*₂C_6H_5), 4.35 (dd, 1 H, *J* 9.1, 6.5 Hz, H-5), 4.04 (dd, 1 H, *J* 9.3, 8.1 Hz, H-5′), 3.41 (q with further couplings, 1 H, *J* 7.3 Hz, H-4), 2.77 (quintet with further couplings, 1 H, *J* 7.5 Hz, H-4), 1.01 (d, 3 H, *J* 7.3 Hz, CH_3). Found: C 69.6; H 7.3. Calc. for $C_{13}H_{16}O_3$: C 70.9; H 7.3.

(−)-(2S,3R,4R/4S)-2-*Benzyloxy*-4-*formyl*-3-*methyltetra-hydrofuran* **3r/4r**. Prepared as above (**3s/4s**) but from **2r**. **3r/4r** had: $[\alpha]_D^{25}$ −102° (c 1.3, $CHCl_3$); **3r/4r** and **3s/4s** had identical ^{1}H NMR data; MS: 220 (*M*⁺), 219 (*M*⁺−H), 91 (C_7H_7⁺).

(−)-(2S,3R,4R)-2-Benzyloxy-4-[(1R/1S)-1-hydroxyethyl]-3-methyltetrahydrofuran **5r** and (−)-(2S,3R,4S)-2-benzyl-oxy-4-[(1R or 1S)-1-hydroxyethyl]-3-methyltetrahydrofuran **6r**. Compound **3r/4r** (5.29 g, 24.0 mmol) in diethyl ether (44 ml) was added dropwise to a cooled (dry ice/acetone bath) solution of methyllithium (26.4 mmol) in diethyl ether (61.6 ml). After 5 min, the reaction mixture was allowed to attain room temperature. Ammonium sulfate solution (10 %, 40 ml) was added. The aqueous phase was extracted with diethyl ether (2×20 ml). The ether solution was dried (Na₂SO₄) and concentrated. The residue was chromatographed (SiO₂, heptane/EtOAc 10:1 → 4:1) to give **5r**, as a mixture of diastereomers (1:2, oil, 5.00 g, 88 %) and **6r** (oil, 138 mg, 2 %). **5r** had: [α]_D²⁵ −117° (c 0.85, CHCl₃); ¹H NMR (CDCl₃): δ 7.38–7.26 (m, 5 H, C₆H₅), 4.81 (d, 2/3 H, J 1.0 Hz, H-2), 4.80 (d, 1/3 H, J 1.2 Hz, H-2), 4.74, 4.45 (AB system, each 1/3 H, J 11.7 Hz, C₆H₅CH₂), 4.72, 4.46 (AB system, each 2/3 H, J 11.8 Hz, C₆H₅CH₂), 4.13 (t, 1 H, J 8.8 Hz, H-5), 4.06 (dd, 1/3 H, J 8.8, 6.6 Hz, H-5′) 3.95 (dq, 1/3 H, J 6.3, 4.4 Hz, CH₃CHOH), 3.80–3.73 (m, 4/3 H, H-5′, CH₃CHOH), 2.34 (dq, 2/3 H, J 7.1, 3.7 Hz, H-3 or H-4), 2.14 (dq, 1/3 H, J 7.5, 3.6 Hz, H-3 or H-4), 1.95–1.84 (m, 1 H, H-3 or H-4), 1.17 (d, 2 H, J 6.4 Hz, CH₃), 1.15 (d, 1 H, J 6.4 Hz, CH₃), 1.11 (d, 2 H, J 7.4 Hz, CH₃), 1.09 (d, 1 H, J 6.9 Hz, CH₃). Anal. C₁₄H₂₀O₃: C, H. **6r** had: [α]_D²⁵ −125° (c 0.85, CHCl₃); ¹H NMR (CDCl₃): δ 7.38–7.27 (m, 5 H, C₆H₅), 4.84 (s, 1 H, H-2) 4.72, 4.45 (AB system, each 1 H, J 11.7 Hz, C₆H₅CH₂), 4.18 (t, 1 H, J 8.6 Hz, H-5), 3.94 (t, 1 H, J 8.8 Hz, H-5′) 3.82 (dq, 1 H, J 9.3, 6.1 Hz, CH₃CHOH), 2.55 (dq, 1 H, J 8.7, 6.6 Hz, H-4), 2.24 (quintet, 1 H, J 7.1 Hz, H-2), 1.28 (d, 3 H, J 6.1 Hz, CH₃CHOH), 0.93 (d, 3 H, J 7.4 Hz, CH₃), Anal. C₁₄H₂₀O₃: C, H.

(+)-(2R,3S,4S)-2-Benzyloxy-4-[(1R/1S)-1-hydroxyethyl]-3-methyltetrahydrofuran **5s** and (−)-(2R,3S,4R)-2-benzyl-oxy-4-[(1R or 1S)-1-hydroxyethyl]-3-methyltetrahydrofuran **6s**. Prepared as above (**5r** and **6r**) but from **3s/4s**. **5s** had: [α]_D²⁵ +118° (c 1.1, CHCl₃); IR (CCl₄): 3455 cm⁻¹ (OH); **5s** and **5r** had identical ¹H NMR data. **6s** had [α]_D²⁵ +125° (c 0.61, CHCl₃); IR (CCl₄): 3460 cm⁻¹ (OH); **6s** and **6r** had identical ¹H NMR data.

(−)-(2S,3R,4R)-4-Acetyl-2-benzyloxy-3-methyltetrahydrofuran (**7r**) and (−)-(2S,3R,4S)-4-acetyl-2-benzyloxy-3-methyltetrahydrofuran **8r**. (a) Oxalyl chloride (5.56 g, 43.8 mmol) was dissolved in dichloromethane (100 ml) and the mixture was cooled to −60 °C (dry ice/acetone bath). A solution of dimethyl sulfoxide (7.47 g, 95.6 mmol) in dichloromethane (27 ml) was added. After 10 min, a solution of **5r/6r** (4.70 g, 19.9 mmol) in dichloromethane (31 ml) was added and after an additional 15 min ethyl(diisopropyl) amine (17.99 g, 139 mmol) was added and the cooling bath was removed. When the reaction mixture had reached room temperature, water (50 ml) was added. The aqueous phase was extracted with dichloromethane (15 ml) and the combined extracts were washed with hydrochloric acid

(10 %, 50 ml) and water (2×50 ml), dried (Na₂SO₄) and concentrated. The residue was chromatographed (SiO₂, heptane/EtOAc 10:1) to give **7r** (4.18 g, 90 %) and **8r** (0.137 g, 3 %). **7r** had: [α]_D²⁵ −103° (c 1.5, CHCl₃); **7r** and **7s** had identical ¹H NMR data. Anal. C₁₄H₁₈O₃: C, H. **8r** had: [α]_D²⁵ −143° (c 0.45, CHCl₃); IR (CCl₄): 1712 cm⁻¹ (C=O); **8r** and **8s** had identical ¹H NMR data. Anal. C₁₄H₁₈O₃: C, H.

(b) The mixture **5r/6r** (1 g, 4.24 mmol) was dissolved in benzene (50 ml, 4 Å molecular sieve) and barium manga-nate²⁷ (10.86 g, 42.4 mmol) was added. The mixture was heated at reflux for 15 h, Celite (5 g) was added and the mixture was filtered through Celite. The residue was washed with ethyl acetate, the filtrate was concentrated, and the resulting material was chromatographed (SiO₂, heptane/EtOAc 9:1) to give **7r** (582 mg, 59 %) and **8r** (113 mg, 11 %).

(c) Compound **7r** (0.885 g, 3.78 mmol) was dissolved in methanolic sodium methoxide (0.019 M, 48 ml). After 1.5 h the reaction mixture was neutralised by the addition of Duolite (H⁺) resin and filtered. Ethyl(diisopropyl)amine (four drops) was added, the solvent was evaporated and the residue was chromatographed (SiO₂, heptane/EtOAc 9:1→4:1) to give **7r** (0.630 g, 71 %) and **8r** (0.131 g, 15 %).

(+)-(2R,3S,4S)-4-Acetyl-2-benzyloxy-3-methyltetrahydro-furan **7s** and (+)-(2R,3S,4R)-4-acetyl-2-benzyloxy-3-methyltetrahydrofuran **8s**. Prepared as above (**7r** and **8r**) but from **5s/6s**. **7s** had: [α]_D²⁵ +102° (c 0.72, CHCl₃); IR (CCl₄): 1717 cm⁻¹ (C=O); ¹H NMR (CDCl₃): δ 7.37–7.24 (m, 5 H, C₆H₅), 4.82 (d, 1 H, J 2.1 Hz, H-2), 4.71, 4.43 (AB system, 1 H each, J 11.9 Hz, C₆H₅CH₂), 4.24 (dd, 1 H, J 8.6, 7.4 Hz, H-5), 4.11 (t, 1 H, J 8.3 Hz, H-5′), 2.70 (dt, 1 H, J 7.9, 5.3 Hz, H-4), 2.60 (ddq, 1 H, J 7.1, 5.4, 1.7 Hz, H-3), 2.20 (s, 3 H, CH₃CO), 1.16 (d, 3 H, J 7.0 Hz, CH₃CH). **8s** had [α]_D²⁵ +143° (c 0.61, CHCl₃); ¹H NMR (CDCl₃) δ 7.38–7.27 (m, 5 H, C₆H₅), 4.87 (s, 1 H, H-2), 4.70, 4.46 (AB system, 1 H each, J 11.7 Hz, C₆H₅CH₂), 4.34 (t, 1 H, J 8.6 Hz, H-5), 3.98 (t, 1 H, J 8.7 Hz, H-5′), 3.66 (q with further coupling, 1 H, J 8.1 Hz, H-4), 2.67 (quintet, 1 H, J 7.2 Hz, H-3), 2.19 (s, 3 H, CH₃CO), 0.87 (d, 3 H, J 6.9 Hz, CH₃CH).

(+)-(2R/2S,3R,4R)-4-Acetyl-2-hydroxy-3-methyltetra-hydrofuran, (+)-epibotryodiplodin **9r**. Compound **7r** (100 mg, 0.43 mmol) was treated as in the preparation of **10r** to give **9r** (56 mg, 91 %): [α]_D²⁵ +87° (c 0.60, CHCl₃); **9r** and **9s** had identical ¹H NMR data. **9r** had: ¹³C NMR (DMSO-d₆): δ 208,1. 206,9 (CO), 103,7, 98,9 (C-2), 67.0, 65.7 (C-5), 57.9, 55.8 (C-4), 43.3, 41.5 (C-3), 29.6, 29.0 (CH₃CO), 16.7, 12.7 (CH₃CH).

(−)-(2R/2S,3S,4S)-4-Acetyl-2-hydroxy-3-methyltetrahydro-furan,(−)-epibotryodiplodin **9s**. Compound **7s** was treated as in the preparation of **10r** to give **9s**: [α]_D²⁵ +83° (c 0.69, CHCl₃). ¹H NMR (CDCl₃): δ 5.35 (d, 1/3 H, J 4.4 Hz, H-2), 5.05 (d, 2/3 H, J 1.0 Hz, H-2), 4.27 (t, 1/3 H, J 8.8

Hz, H-5), 4.21–4.11 (m, 4/3 H, H-5,5'), 3.92 (t, 1/3 H, J 8.2 Hz, H-5'), 3.12 (q with further coupling, 1/3 H, J 9.1 Hz, H-4), 2.90 (ddd, 2/3 H, J 7.8, 5.1, 2.7 Hz, H-4), 2.49–2.34 (m, 1 H, H-3), 2.28 (s, 2 H, CH$_3$CO), 2.20 (s, 1 H, CH$_3$CO), 1.12 (d, 3 H, J 7.4 Hz, CH_3CH).

(−)-(2R/2S,3R,4S)-4-Acetyl-2-hydroxy-3-methyltetrahydro-
furan, (−)-botryodiplodin 10r. Compound 8r (0.131 g, 0.560 mmol) was hydrogenated (H$_2$, 10% Pd/C, 33 mg) in dimethoxyethane/water (2:1, 3.9 ml) for 3 h. The catalyst was filtered off, fresh catalyst (66 mg) was added and the hydrogenation was continued for a further 6 h (8r prepared by barium manganate oxidation did not require a change of catalyst). The catalyst was filtered off and the solvent was evaporated to give 10r (77.5 mg, 96%) as an oil: $[\alpha]_D^{25}$ −69° (c 0.85, CHCl$_3$) {Lit.[4,9] $[\alpha]_D^{25}$ −70.12° (c 0.124, MeOH) and $[\alpha]_D^{25}$ −69.1° (c 0.13, MeOH)}. ^{1}H NMR data (CDCl$_3$): δ 5.18 (s, 1 H, H-2), 4.85 (br s, OH), 4.30 (t, 3/5 H, J 8.9 Hz, H-5), 4.09–3.99 (m, 7/5 H, H-5,5'), 3.67 (q 3/5 H, J 8.1 Hz, H-4), 3.42 (dt, 2/5 H, J 7.8, 2.9 Hz, H-4), 2.62 (quintet, 3/5 H, J 7.1 Hz, H-3), 2.52–2.42 (m, 2/5 H, H-3), 2.30 (s, 6/5 H, CH$_3$CO), 2.21 (s, 9/5 H, CH$_3$CO), 1.07 (d, 6/5 H, J 7.3 Hz, CH_3CH), 0.87 (d, 9/5 H, J 7.0 Hz, CH_3CH). ^{13}C (DMSO-d_6): δ 207.2, 207.0 (CO), 103.2, 98.8 (C-2), 65.2, 64.7 (C-5), 53.3, 52.4 (C-4), 42.1 (C-3), 30.4, 30.0 (CH$_3$CO), 12.3, 9.1 (CH$_3$CH).

(+)-(2R/2S,3S,4R)-4-Acetyl-2-hydroxy-3-methyltetrahydro-
furan,(+)-botryodiplodin 10s. Compound 8s was treated as above to give 10s: $[\alpha]_D^{25}$ +69° (c 0.65, CHCl$_3$); 10s and 10r had identical ^{1}H NMR data.

(−)-(2S,3R,4S)-2-Benzyloxy-4-[(1R or 1S)-1-(3,5-dinitro-
benzoyloxy)ethyl]-3-methyltetrahydrofuran 11r. 3,5-Dini-
trobenzoyl chloride (95 mg, 0.42 mmol) was added to a solution of 6r (49 mg, 0.21 mmol) in pyridine (1 ml, dried over KOH). After 80 min, the reaction mixture was concentrated and the residue was dissolved in chloroform (15 ml) and washed with water (5 ml), aqueous hydrochloric acid (10%, 5 ml) and saturated aqueous sodium hydrogencarbonate (5 ml) and dried (Na$_2$SO$_4$). The solvent was removed and the residue was chromatographed (SiO$_2$, heptane/EtOAc 1:7) to give crystalline 11r (66 mg, 74%): m.p. 104.5–106 °C (EtOH), $[\alpha]_D^{25}$ −71° (c 0.23, CHCl$_3$). ^{1}H NMR data (CDCl$_3$): δ 9.24 [t, 1 H, J 2.2 Hz, CH(CNO$_2$)$_2$], 9.12 (d, 2 H, J 2.1 Hz, CHCOO), 7.38–7.26 (m, 5 H, C$_6$H$_5$), 5.30 (dq, 1 H, J 10.2, 6.1 Hz, CH$_3$CHO), 4.89 (s, 1 H, OCHO), 4.74, 4.48 (AB system, each 1 H, J 11.8 Hz, C$_6$H$_5$CH_2), 4.16 (t, 1 H, J 8.4 Hz, H-5), 3.85 (t, 1 H, J 8.9 Hz, H5'), 3.02 (m, 1 H, H-4), 2.39 (quintet, 1 H, J 7.0 Hz, H-2), 1.49 (d, 3 H, J 6.1 Hz, CH_3CHO), 1.01 (d, 3 H, J 7.2 Hz, CH_3CH). Anal. C$_{21}$H$_{22}$N$_2$O$_8$: C, H.

(2S,3R,4R)-4-Acetyl-2-ethoxy-3-methyltetrahydrofuran 12r. Compound 7r (22 mg, 0.09 mmol) was hydrogenated (H$_2$, 10% Pd/C, 10 mg) in ethanol (1 ml) for 2.5 h. The catalyst was filtered off, the solvent was evaporated and the residue

was chromatographed (SiO$_2$, heptane/EtOAc 15:1) to give 12r (2.8 mg, 17%): ^{1}H NMR (CDCl$_3$): δ 4.93 (d, 1 H, J 4.6 Hz, H-2), 4.15 (t, 1 H, J 8.8 Hz, H-5), 3.91 (dd, 1 H, J 8.7, 7.2 Hz, H-5'), 3.72 (dq, 1 H, J 9.8, 7.1 Hz, CH$_3$CH_2O), 3.43 (dq, 1 H, J 9.8, 7.1 Hz, CH$_3$CH_2O), 3.06 (dt with further coupling, 1 H, J 9.7, 7.5 Hz, H-4), 2.41–2.33 (m, 1 H, H-3), 2.20 (s, 3 H, CH$_3$CO), 1.19 (t, 3 H, J 7.1 Hz, CH_3CH$_2$), 1.08 (d, 3 H, J 6.8 Hz, CH_3CH).

(2R,3R,4R)-4-Acetyl-2-benzyloxy-3-methyltetrahydrofuran (13r) and (2R,3R,4R)-4-acetyl-2-acetoxy-3-methyltetra-
hydrofuran 14r. Compound 7r (40 mg, 0.17 mmol) was dissolved in acetic acid (3 ml) and the mixture was stirred at 90 °C for 30 min. Evaporation of the solvent and chromatography of the residue (SiO$_2$, heptane/EtOAc 9:1) gave 13r (4.5 mg, 11%) and 14r (4.0 mg, 13%). 13r had: ^{1}H NMR (CDCl$_3$): δ 7.38–7.28 (m, 5 H, C$_6$H$_5$), 5.03 (d, 1 H, J 4.7 Hz, H-1), 4.74, 4.48 (AB system, 1 H each, J 12.2 Hz, C$_6$H$_5$CH_2), 4.19 (t, 1 H, J 8.9 Hz, H-5), 3.96 (dd, 1 H, J 8.4, 7.4 Hz, H-5'), 3.12 (dt, 1 H, J 9.6, 7.4 Hz, H-4), 2.40 (m, 1 H, H-3), 2.20 (s, 3 H, CH$_3$CO), 1.15 (d, 3 H, J 6.8 Hz, CH_3CH). 14r had: ^{1}H NMR (CDCl$_3$): δ 6.25 (d, 1 H, J 4.6 Hz, H-2), 4.30 (t, 1 H, J 8.9 Hz, H-5), 3.97 (t, 1 H, J 8.4 Hz, H-5'), 3.11 (q with further coupling, 1 H, J 9.4 Hz, H-4), 2.62–2.54 (m, 1 H, H-3), 2.22 (s, 3 H, CH$_3$CO), 2.08 (s, 3 H, CH$_3$CO), 1.08 (d, 3 H, J 6.9 Hz, CH_3CH).

Epimerisation of (−)-botryodiplodin 10r. Compound 10r (18.9 mg, 0.13 mmol) was dissolved in aqueous (D$_2$O) sodium deuteriumcarbonate (0.9 ml, 0.65 M; prepared by repeated addition/removal of D$_2$O to NaHCO$_3$). The solution was kept in an NMR tube and spectra were recorded. Integration of the anomeric (H-2) signals at intervals gave the 9/10 ratio: ca. 1:25 (1 h), 1:3 (3.3 h), 6:1 (144 h) and 6:1 (456 h) as shown in Fig. 1.

Treatment of bovine serum albumin (BSA) with (−)-botryo-
diplodin 10r. Compound 10r (2.0 mg) was dissolved in water (184 mg) and the mixture was added to bovine serum albumin (BSA, 3 mg). A colourless, clear solution was formed, which gradually changed colour. An orange gel was formed after 6 h.

Treatment of L-lysine with (−)-botryodiplodin 10r. L-Ly-
sine (1.0 mg) was dissolved in water and 10r (1.1 mg) was added. A colurless solution was formed, which gradually changed colour via pale pink (0.6 h) to dark (burgundy) red (6 h).

Treatment of BSA and L-lysine with a DMSO solution of 9r and 10r gave no colour change. Addition of approximately the same volume of water changed the colour to orange and dark red, respectively, within 6 h with both compounds.

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