Involvement of Diels–Alder reactions in the biosynthesis of secondary natural products: the late stage of the biosynthesis of the phytotoxins solanapyrones

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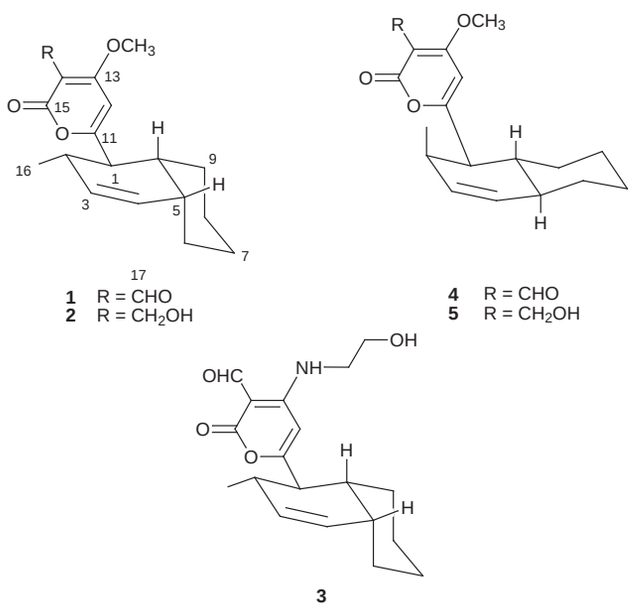
Advanced intermediates, prosolanapyrones I (**6**) and II (**7**) have been synthesized in deuterium labelled form and administered to cultures of *Alternaria solani*. Incorporation of [17,17,18,18,18-$^2H_5$]prosolanapyrone I (**6b**) afforded solanapyrones A (**1**) labelled at C-17 and C-18 with the expected integration in its ^2H NMR spectrum. Subsequently, [2,3,17,18,18,18-$^2H_6$]prosolanapyrone II (**7a**) was incorporated into solanapyrone A labelled, as expected, at C-2, C-3, C-17 and C-18. These results strongly support the involvement of a Diels–Alder reaction in the biosynthesis of solanapyrones. This is the first example of intact incorporation of diene-dienophile precursors into natural [4 + 2] adducts.

Introduction

Solanapyrones A (**1**), B (**2**) and C (**3**) are phytotoxins produced



by causal fungi of potato early blight *Alternaria solani*,[1] and also chick pea blight *Ascochyta rabiei*.[2] Their structures were elucidated by spectroscopic methods and chemical degradation,[1] and later by X-ray analysis.[2] The absolute configuration of **1** was determined by the CD exciton chirality method of its dibenzoate derivative.[3] Later, their diastereomers, solanapyrones D (**4**) and E (**5**), were isolated[4,5] and the absolute configuration of **4**[4,5] was determined in a similar manner to **1**.

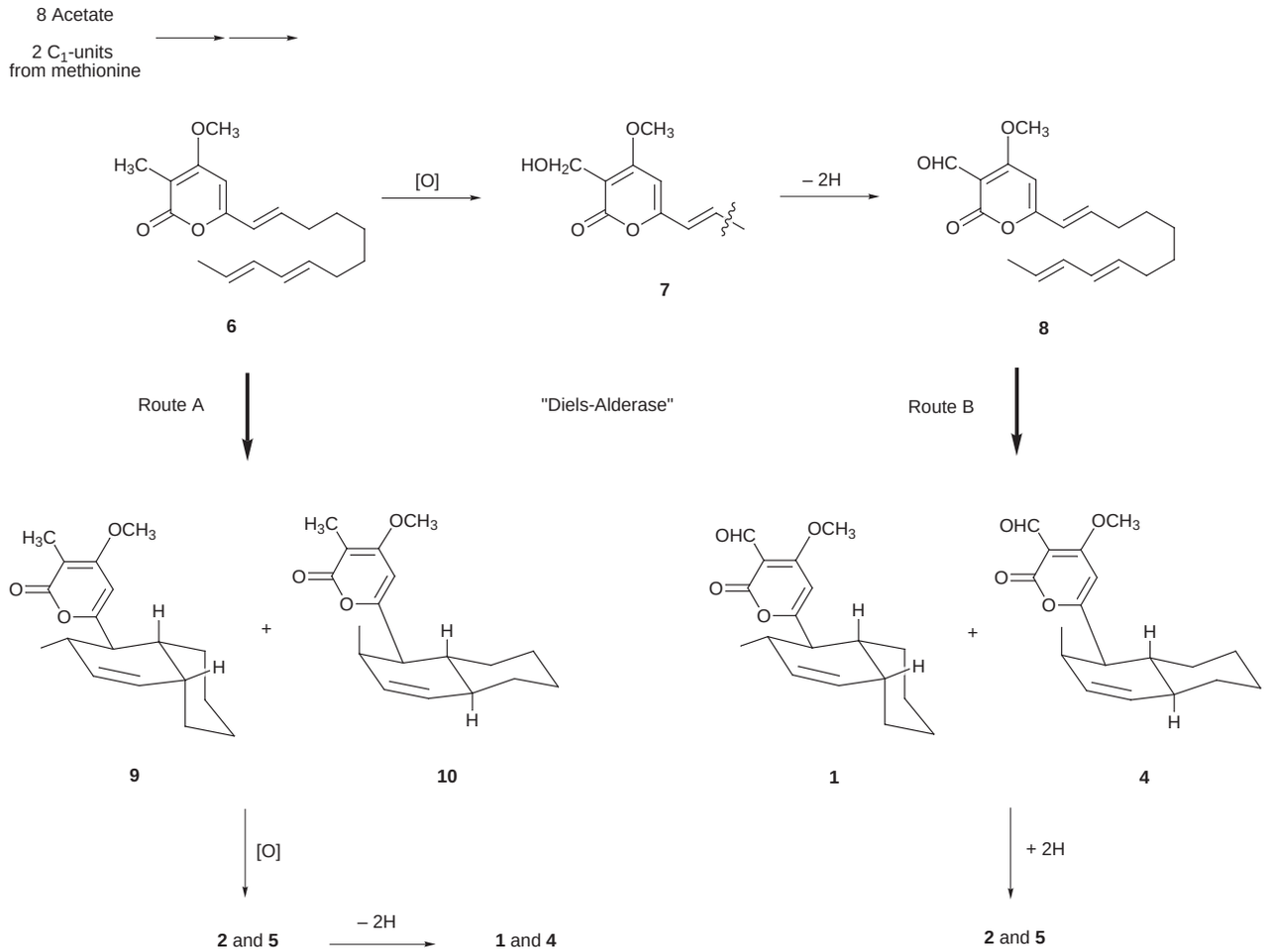
Biosynthetic studies of solanapyrones showed that the carbon skeleton was constructed from one acetate, seven malonates and two C_1-units from methionine *via* a polyketide pathway.[6] The co-occurrence of plausible diastereomeric adducts **1** and **4** strongly suggested that solanapyrones A and D are biosynthesized *via* a Diels–Alder reaction of the corresponding achiral triene precursor. There are a number of biological Diels–Alder adducts[7] including kuwanons,[8,9] manzamenones[10] and segolines[11] which may be biosynthesized *via* [4 + 2] cycloaddition of achiral precursors to afford chiral *endo*- and *exo*-adducts. To date, extensive studies to prove biological Diels–Alder reactions have been made. Solanapyrone biosynthesis, however, is the only proven example[12,13] of a biological Diels–Alder reaction. Here, we report the full details of a study to prove the involvement of an enzymatic Diels–Alder reaction in solanapyrone biosynthesis.

Results and discussion

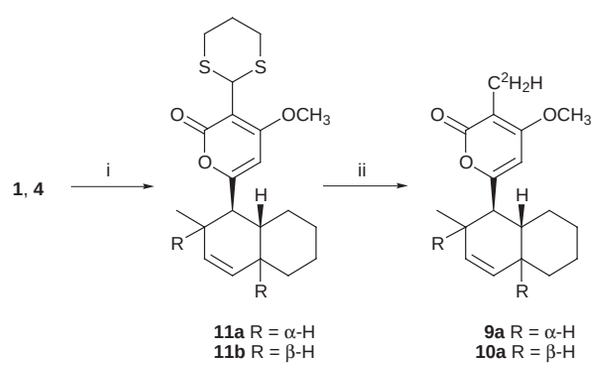
The occurrence of reduced solanapyrones B (**2**) and E (**5**) implies that the substituent at C-14 is oxidized in a stepwise manner ($CH_3 \rightarrow CH_2OH \rightarrow CHO$) after [4 + 2] cycloaddition (Scheme 1, Route A). To test this hypothesis, the plausible precursors **9**,**10** and their labelled forms **9a**,**10a** were synthesized from a mixture of **1** and **4** (Scheme 2). Efforts to detect **9** and **10** in the extracts of *A. solani* with the authentic samples were unsuccessful. Feeding experiments with [^2H]-labelled **9a** and **10a** showed no incorporation into **1** and **4**. The absence of **9** and **10** in the extract did not exclude the possibility that these compounds are intermediates, but the result of the feeding experiment gave additional support to the proposal that solanapyrones are biosynthesized *via* Route B (Scheme 1).

In order to confirm this observation, the timing of the oxidation at C-17 was next explored. L-[*Methyl*-$^2H_3$]methionine was efficiently incorporated into **2** (10% incorporation) and its ^{13}C NMR spectrum showed that the C-17 hydroxymethyl signal at 54.6 ppm was accompanied by an upfield shifted triplet (Δ 0.30 ppm, J_{CC} = 22.3 Hz) (Fig. 1). The integral ratio of methoxy to hydroxymethyl signals in the ^2H NMR spectrum was 3 : 1, and the most enriched molecular ion in the mass spectrum was the ($M^+ + 4$) peak. All the observations shown above indicate that the triene precursor **6** was oxidized to the aldehyde **8** which is then transformed by cycloaddition to give **1** and **4**. These aldehydes would then eventually be reduced to alcohols **2** and **5** (Scheme 1, Route B).

With this promising pathway proposed, we moved to the synthesis of [^2H]-labelled trienes **6a**, **6b** and **7a**. We essentially adopted the strategy which was used in our first total synthesis of **1**[14] and the synthesis of non-labelled material, as described



Scheme 1 Two possible solanapyrone biosynthetic pathways.



Scheme 2 *Reagents and conditions*: i, propane-1,3-dithiol, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0 °C (70%); ii, $Bu^n{}_3Sn^2H$, toluene, 80 °C (32%).

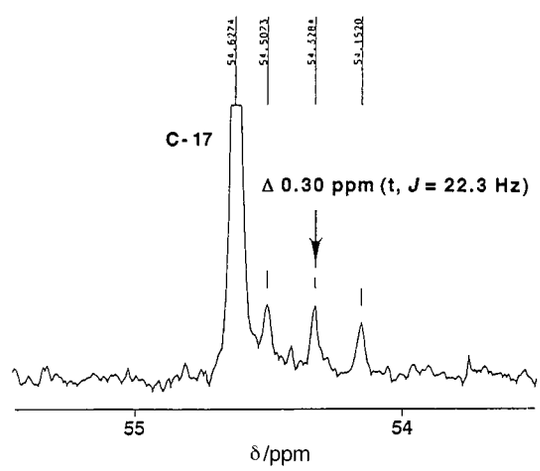


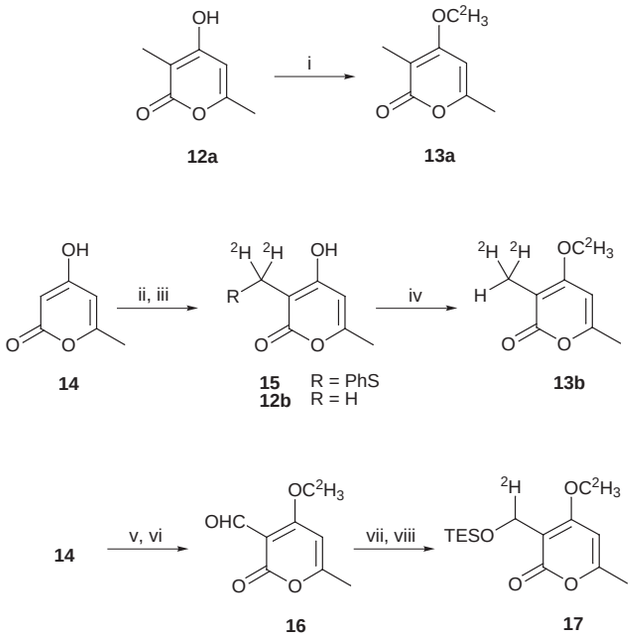
Fig. 1 Part of ^{13}C NMR spectrum of **1** from the feeding experiment with L-[*methyl*-$^2H_3$]methionine.

previously.[15] [^2H]-Labels on the *O*-methyl group were effectively introduced to the pyrone moiety by use of deuterated dimethyl sulfate or methyl toluene-*p*-sulfonate (Scheme 3). Introduction of the labels on C-17 was achieved by acid catalysed condensation with [$^2H_2$]paraformaldehyde or by formylation and subsequent reduction with NaB^2H_4. Thus the deuterated precursors **13a**, **13b** and **17** were prepared for elaboration into the target trienes **6a**, **6b** and **7a**.

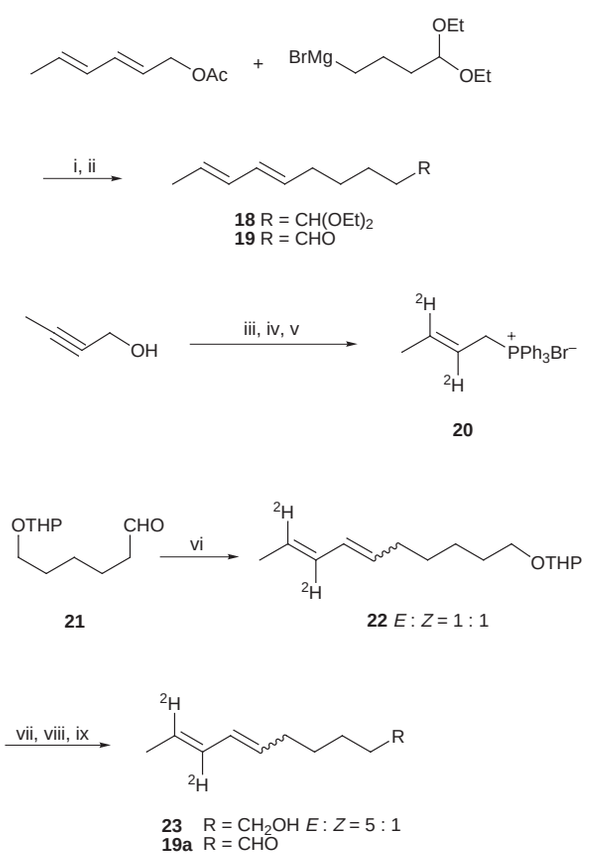
In the diene segment, non-labelled dienal **19** was synthesized by Li_2CuCl_4-catalysed cross coupling[16,17] between a Grignard reagent and sorbyl acetate (Scheme 4). The label on **19a** was introduced by the reduction of but-2-ynyl alcohol with $LiAl^2H_4$ followed by quenching with deuterium oxide. The but-2-en-1-ol thus obtained was transformed into **19a** *via* the Wittig reaction.

Aldol reaction of pyrones **13a**, **13b** and **17** with deuterated diene **19a** under optimized conditions[15] afforded aldol products **24a**, **24b** and **25** which were converted to the plausible triene precursors prosolanapyrones I (**6a**), (**6b**) and II (**7a**) (Scheme 5).

In order to establish the proposed pathway (Route B), the synthesized labelled precursors were fed to cultures of *A. solani*. Although administration of **6a** and **6b** showed poor incorporation (0.27 and 0.26%, respectively), ^2H NMR analysis of the resulting solanapyrone A from **6b** displayed signals at C-17 and OMe with the expected integration in a ratio of 1:4.3 [Fig. 2(a)]. Incorporation of the heavily deuterated **7a** was also low (0.37%) but four signals corresponding to the deuteriums at C-2, C-3, C-17 and OMe were observed with an integration in the ratio of 0.7:1:0.4:3 [Fig. 2(b)], essentially unchanged from



Scheme 3 *Reagents and conditions*: i, $(C^2H_3)_2SO_4$, K_2CO_3, butan-2-one (76%); ii, $(^2HC^2HO)_n$, PhSH, piperidine, AcOH (55%); iii, Raney Ni (W-2) (12%); iv, $(C^2H_3)_2SO_4$, K_2CO_3, butan-2-one (50%); v, C^2H_3OTs, K_2CO_3, DMF (86%); vi, Cl_2CHOMe, $TiCl_4$, CH_2Cl_2, 0 °C (57%); vii, NaB^2H_4, MeOH, 0 °C; viii, TESOTf, 2,6-lutidine, CH_2Cl_2, 0 °C (65%, over 2 steps).



Scheme 4 *Reagents and conditions*: i, Li_2CuCl_4, THF, −20 °C (48%); ii, $(CO_2H)_2$, THF (85%); iii, $LiAl^2H_4$, NaOMe, THF, reflux; iv, PBr_3, Py, ether, 0 °C; v, PPh_3, C_6H_6, reflux (over 3 steps, 28%); vi, **20**, Bu^{n}Li, 0 °C, THF, then **21** (60%); vii, PPTS, MeOH, 60 °C; viii, I_2, C_6H_6 (over 2 steps, 90%); ix, DMSO, $(COCl)_2$, CH_2Cl_2, −78 °C; Et_3N (96%).

that of the labelled precursor. To eliminate the possibility of a non-enzymatic Diels–Alder reaction of **7a**, the solanapyrone A obtained was converted to the α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) derivative **27**.[5] After adding

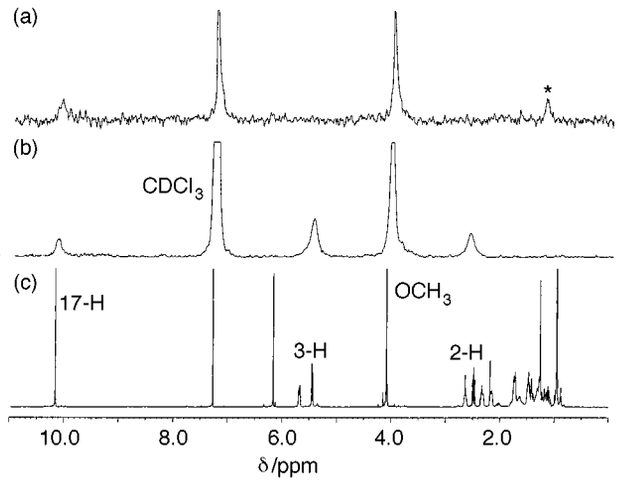
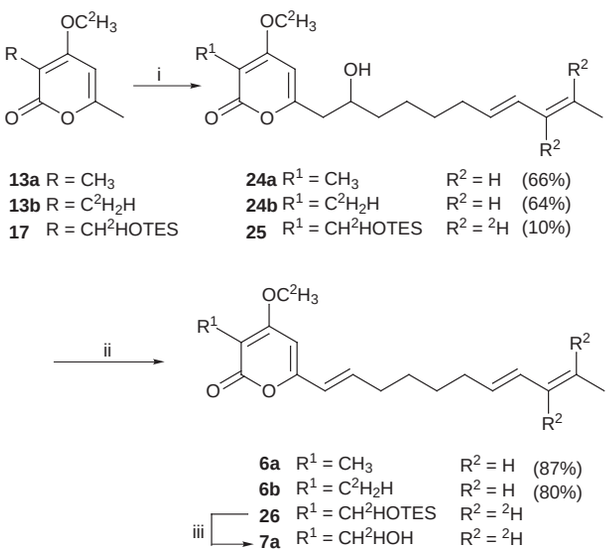
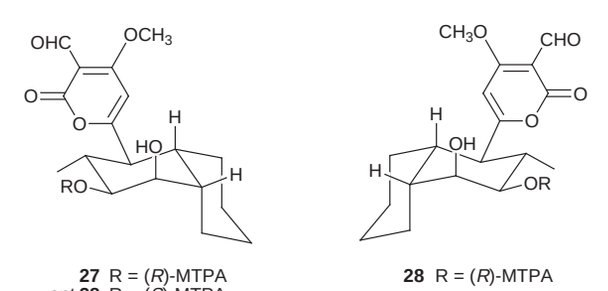


Fig. 2 (a) ^2H NMR spectrum of **1** derived from [17,17,18,18,18-$^2H_5$]-**6b**; (b) ^2H NMR spectrum of **1** derived from [2,3,17,18,18,18-$^2H_6$]-**7a**; (c) ^1H NMR spectrum of **1** (non-labelled). * The signal of Bu^{t}OH.



Scheme 5 *Reagents and conditions*: i, LHMDS (1.1–1.2 equiv.), THF, −78 °C, then **19a**; ii, TsCl, DMAP, CH_2Cl_2, 0 °C, then DBU; iii, TBAF, THF (83%, over 2 steps).



unlabelled *ent*-**28** to secure complete separation of diastereomers **27** and **28**, the two diastereomers were carefully separated and subjected to ^2H NMR analysis (Fig. 3). The spectra of **27** displayed the resonances at the same positions observed in labelled **1** whereas no signal was observed in that of **28**. Since we have already established the optical purities of solanapyrones to be 100% ee,[5] these data clearly showed that **6a**, **6b** and **7a** were incorporated into **1** *via* a biological Diels–Alder reaction. This is the first example of establishing the involvement of a Diels–Alder reaction in biosynthesis.

^2H NMR Analysis of solanapyrones B and E from the feeding experiments of **7a** revealed an interesting result. The spectrum of a 5 : 1 mixture of **2** and **5** from the experiment with **7a**

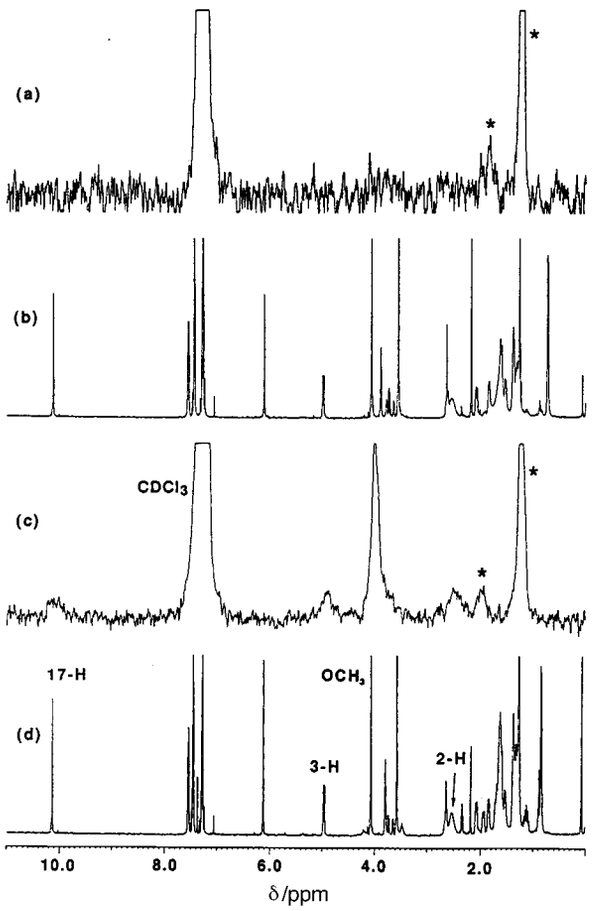


Fig. 3 (a) ^{1}H NMR spectrum of MTPA ester **27**; (b) ^{2}H NMR spectrum of **27** derived from **1** enriched with [2,3,17,18,18,18-$^2H_6$]-**7a**; (c) ^{1}H NMR spectrum of MTPA ester *ent*-**28**. (d) ^{2}H NMR spectrum of **28** derived from enriched **1**. * The signal of Bu^{t}OH.

showed that signals corresponding to the deuteriums at C-2 and C-3 were observed at 2.41 and 5.56 ppm† for **5** and at 2.53 and 5.44 ppm† for **2** in a nearly 1:1 integration [Fig. 4(a)]. The observation that 19% of **7** was converted to **2** and **5** in a ratio of 1:20 at 30 °C for 2 days in aqueous medium[15] indicates non-enzymatic cycloaddition of **7** producing **2** and **5** in the period of the feeding experiment (7 days). The data shown above reveal that enzymatic reaction of **7a** afforded [^{2}H]-labelled **2** and the non-enzymatic reaction gave [^{2}H]-**5** predominantly. This gave further support for the enzymatic Diels–Alder reaction.

Since we were interested in the alcohol dehydrogenase function of this enzyme which could oxidize **7** to **8** and further convert **8** to **1** and **4**, the absolute configuration at C-17 in **2** derived from the feeding experiment with L-[*methyl*-$^2H_3$]methionine was examined. The authentic material **2a** was synthesized by asymmetric reduction with Midland's chiral borane reagent[18] (Scheme 6). Reduction of **1** with NaB^2H_4 followed by re-oxidation with Dess–Martin periodinane gave **1a** with 77 atom% ^{2}H due to the isotopic effect in the oxidation. The labelled compound **1a** was then diastereoselectively reduced with the borane reagent prepared from (−)-α-pinene and 9-BBN to give **2a**. To determine the diastereoselectivity, **2a** was converted to the MTPA ester **29**. In the presence of a chiral shift reagent Eu(hfc)$_3$ (1.05 equiv.), ^{1}H NMR analysis of **29** was employed and showed that diastereotopic proton signals were observed at 5.90 and 5.96 ppm as doublets which accompanied the upper-shifted singlets. The substitution of deuterium at C-17 [Fig. 5(a) and upper traces] provides an explanation for

† The proton signals for **2** and **5** were unambiguously assigned on the basis of incorporation of sodium [1-^{13}C]- and [1,2-$^{13}C_2$]-acetate and analysis of the CH-COSY spectrum.[5]

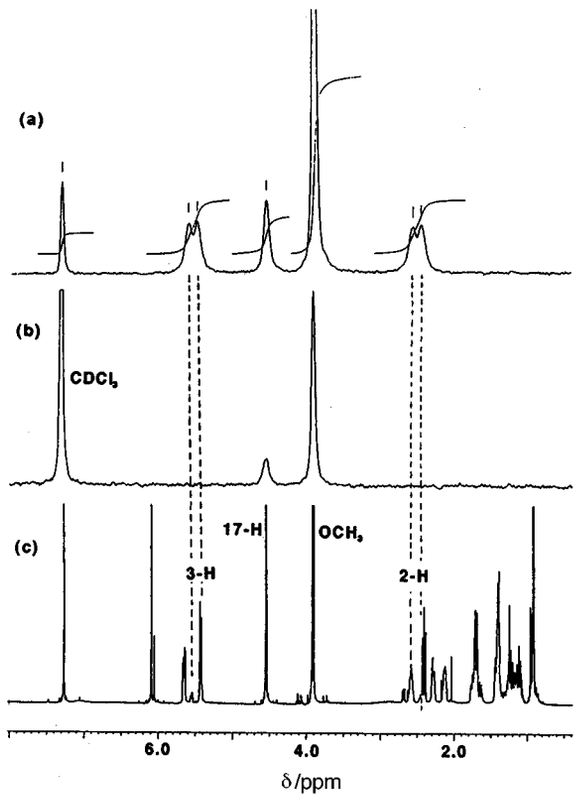
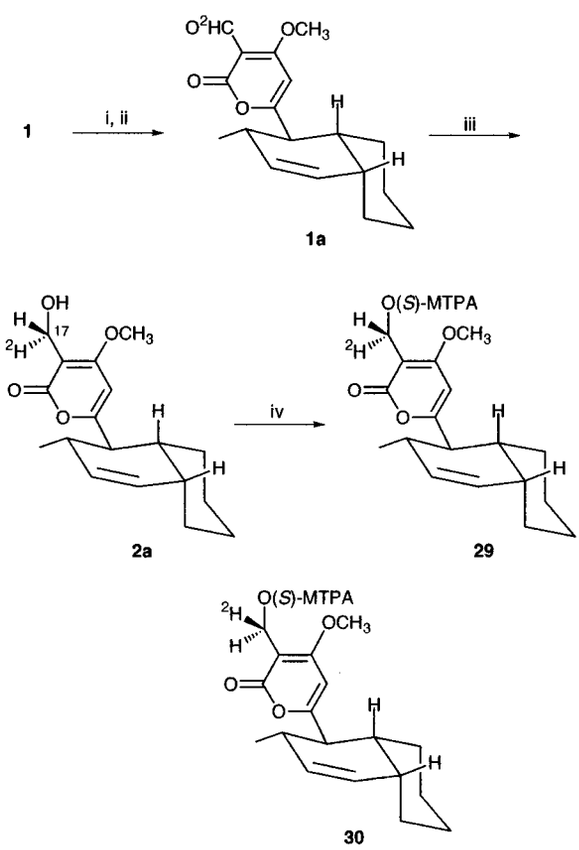


Fig. 4 (a) ^{2}H NMR spectrum of **2** and **5** derived from [2,3,17,18,18,18-$^2H_6$]-**7a**; (b) ^{2}H NMR spectrum of **2** and **5** derived from [17,17,18,18,18-$^2H_5$]-**6b**; (c) ^{1}H NMR spectrum of **2** and **5** (non-labelled).



Scheme 6 *Reagents and conditions*: i, NaB^2H_4, MeOH, 0 °C (82%); ii, Dess–Martin periodinane, CH_2Cl_2 (78%); iii, (−)-α-pinene, 9-BBN, THF, reflux; **1a** (57%); iv, (*S*)-MTPA, DCC, DMAP, CH_2Cl_2 (81%).

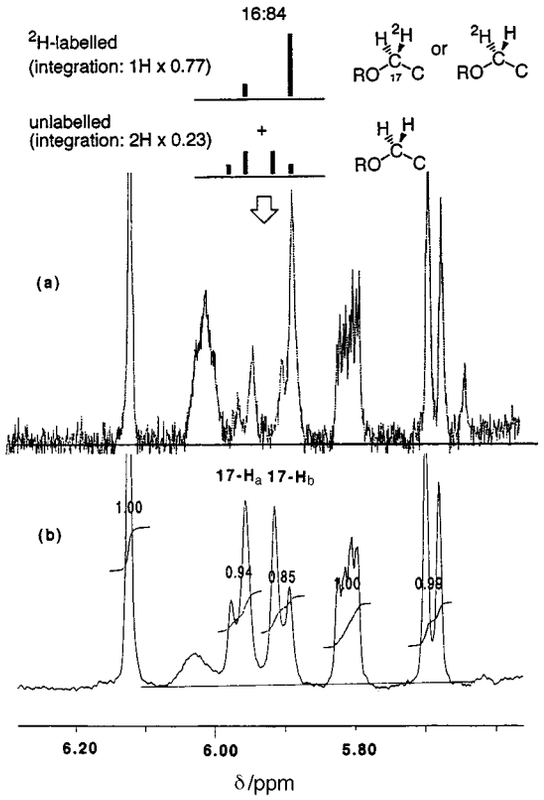


Fig. 5 (a) ^{1}H NMR spectrum of MTPA ester **29**; (b) ^{1}H NMR spectrum of **30** derived from **2** enriched with L-[*methyl*-^{2}H$_3$]methionine.

this. Based on the integration of the singlets (16 : 84), diastereoselectivity was calculated as 68% de. We assumed that the reduction proceeded from the *re*-face of the carbonyl group, as in the case of simple aromatic aldehydes,[18] to give the (17*S*)-isomer **29**. This occurs because the chiral trialkylborane reagent coordinates with the aldehyde group and not with the oxygen functionality on the pyrone. Solanapyrone B obtained from feeding experiments with L-[*methyl*-^{2}H$_3$]methionine was converted to its MTPA ester. In its ^{1}H NMR spectrum,‡ the integration of the upfield shifted signal was 10% larger than that of the downfield shifted one [Fig. 5(b)]. Thus, the absolute configuration of **30** is proposed to be 17*R* (Scheme 6) although it is necessary to confirm this by more direct methods such as the degradation of either biosynthetically derived **2** or synthetically deuterated **2** (or possibly chirally deuterated 3-hydroxymethyl-4-methoxy-2*H*-pyran-2-one) to glycolic acid derivatives and comparison of the NMR data with that of an authentic sample.

Experimental

IR Spectra were measured on a Hitachi 285 spectrophotometer, ^{1}H, ^{2}H and ^{13}C NMR spectra were obtained on JEOL EX-270 and Bruker AM-500 spectrometers for solutions in $CDCl_3$, mass spectra on JEOL DX-300 and 01SG-2 spectrometers. Column chromatography used Merck Kieselgel 60 silica gel (0.04–0.063 mm), and TLC was performed on Merck Kieselgel 60 F_{254}. Solvents were dried shortly before use with an appropriate drying agent. Anhydrous reactions were carried out under argon. Unless otherwise noted, starting materials were obtained from commercial suppliers and used without further purification. The following companies supplied deuterium labelled compounds: NaB^2H_4 (98 atom% ^{2}H), tributyltin deuteride (98 atom% ^{2}H) and [$^2H_6$]dimethyl sulfate (99 atom% ^{2}H) from Aldrich; ^2H_2O (99.8 atom% ^{2}H) and [$^2H_4$]methanol (99.8 atom% ^{2}H) from ISOTEC Inc.; [$^2H_2$]paraformaldehyde (99.3 atom% ^{2}H) from MSD Isotope; $LiAl^2H_4$ (99.3 atom% ^{2}H) from CEA; L-[*methyl*-^{2}H$_3$]methionine (98 atom% ^{2}H) from Cambridge Isotope Lab. Deuterium enrichment (atom%) of the compounds **13a**, **13b**, **15**–**17** and but-2-en-1-ol was estimated by comparison of the intensity of the molecular ion peak or the ion peak with highest mass. Incorporation (%) of the labelled precursors was calculated by comparison of the integration of the signals of the methoxy group and chloroform (internal standard, natural abundance 0.016%) in ^{2}H-NMR spectra of the labelled solanapyrones.

‡ In order to adjust the chemical shifts of the relevant protons, surrounding signals were utilized as internal references. Attempts to confirm the configuration at C-17 using ^{2}H NMR and addition of chiral shift reagents at varying concentrations were inconclusive because the resolution of the proton signals at C-17 obtained was only 0.06 ppm and minute differences in concentration of shift reagent can cause ambiguous assignments of the relevant signals in the ^{2}H NMR.

Organism and feeding experiment with sodium L-[*methyl*-^{2}H$_3$]-methionine and [^{2}H]-labelled synthetic compounds

The strain used in this experiment and fermentation conditions are as described previously. On the day (14–16 days) after inoculation of *Alternaria solani*, the solution of labelled materials was equally distributed into 500 ml flasks containing 150 ml of the medium. After further incubation (7–11 days), the work-up (as described previously)[5] yielded a mixture of solanapyrones. The ratio of the aldehydes (**1** and **4**) and the alcohols (**2** and **5**) varied. The amounts of administered substrate, solvent (volume), number of flasks, amounts of obtained solanapyrones and incorporation (%) are as follows: L-methionine, 490 mg, water (filtered through membrane filter (0.2 μm), 10 ml) × 2, 10%; **9a** and **10a**, 20 mg, ethanol (8 ml), × 4, **1** and **4** 46 mg, **2** and **5** 68 mg, 0%; **6a**, 35 mg, ethanol (14 ml) × 7, **1** and **4** 55 mg, **2** and **5** 74 mg, 0.27%; **6b**, 35 mg, ethanol (14 ml) × 7, **1** and **4** 22 mg, **2** and **5** 58 mg, 0.26%; **7a**, 12 mg, ethanol (6 ml) ×3, **1** 5 mg, **4** 1.5 mg, **2** and **5** 26 mg, 0.37%.

Thioacetalization of 1 and 4

To a solution of a mixture of **1** and **4** (119 mg, 0.39 mmol, **1** : **4** = 6 : 1) in CH_2Cl_2 (3 ml) was added propane-1,3-dithiol (0.08 ml, 86.5 mg, 0.8 mmol) and boron trifluoride–diethyl ether (0.15 ml, 170 mg, 0.56 mmol) at 3 °C. After stirring for 30 min, the mixture was further stirred at rt for 3 h. The reaction mixture was diluted with ether. The combined organic extracts were washed with saturated $NaHCO_3$, brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. Purification by preparative thin layer silica gel chromatography (hexane–EtOAc, 4 : 1) gave a mixture of **11a** and **11b** (107 mg, 70%) as a colourless oil; ν_{max}(KBr)/cm^{-1} 1700, 1630, 1550; δ_H (500 MHz, $CDCl_3$) 0.92 (3H, d, *J* 7.0, 16-H), 1.1–1.3 (6H, m), 1.70 (2H, m), 2.00 (1H, m, 5-H), 2.12 (2H, m, SCH_2CH_2), 2.27 (1H, m, 10-H), 2.39 (1H, dd, *J* 10.0, 11.7, 1-H), 2.57 (1H, m, 2-H), 2.8–3.2 (4H, m, SCH_2), 3.96 (3H, s, OCH_3), 5.42 (1H, td, *J* 1.8, 9.9, 3-H), 5.63 (1H, s, 17-H), 5.65 (1H, ddd, *J* 2.6, 5.6, 9.9, 4-H), 6.03 (1H, s, 12-H); major isomer **11a**: *m*/*z* (EI) 392.1473 (M$^+$, 12%, $C_{21}H_{28}O_3S_3$ requires 392.1481), 359 (10), 327 (15), 318 (100), 184 (68), 119 (59), 93 (88), 81 (77), 43 (92).

17-Deoxysolanapyrone B (9), E (10) and [17,17-^{2}H$_2$]-17-deoxysolanapyrone B (9a) and E (10a)

To a solution of a mixture of dithioacetal **11a** and **11b** (96 mg, 0.244 mmol) in benzene (1 ml) were added a trace amount of 2,2′-azobis(isobutyronitrile) and tributyltin hydride (0.9 ml, 1.0 g, 3.4 mmol). After stirring for 9 h at 80 °C, the mixture was concentrated *in vacuo*. Purification by silica gel column chromatography (hexane–EtOAc, 9 : 1) gave a mixture of **9** and **10** (23 mg, 32%) as a colourless oil; ν_{max}(KBr)/cm^{-1} 1680, 1630, 1550; δ_H (500 MHz, $CDCl_3$) 0.91 (3H, d, *J* 7.0, 16-H), 1.1–1.3 (3H, m), 1.40 (3H, m), 1.70 (2H, m), 1.92 (s, 3H, 17-H), 2.12 (1H, m, 5-H), 2.30 (1H, m, 10-H), 2.37 (1H, dd, *J* 9.9, 11.9, 1-H), 2.60

(1H, m, 2-H), 3.89 (3H, s, OCH_3), 5.43 (1H, br d, *J* 9.9, 3-H), 5.64 (1H, ddd, *J* 2.6, 5.0, 9.9, 4-H), 6.05 (1H, s, 12-H); major isomer **9** *m*/*z* (EI) 288.1735 (M^+, 57%, $C_{18}H_{24}O_3$ requires 288.1725), 233 (54), 180 (92), 139 (98), 83 (100), 41 (88).

[^{2}H]-Labelled compounds were synthesized as described above except using tributyltin deuteride. **9a** and **10a**: a colourless oil; δ_H (500 MHz; $CDCl_3$) 1.88 (1H, m); *m*/*z* (EI) 290.1849 (M^+, 49%, $C_{18}H_{22}{}^2H_2O_3$ requires 290.1851), 235* (62), 182* (82), 141* (96), 85* (100), 41 (91) (*: shifted by 2 mass units).

4-[$^2H_3$]Methoxy-3,6-dimethyl-2*H*-pyran-2-one (13a)

The above compound was synthesized as described previously[15] except for the use of [$^2H_6$]dimethyl sulfate. **13a**: white crystals, mp 87–89 °C (from $CHCl_3$) (lit.,[19] 82–83 °C); δ_H (270 MHz; $CDCl_3$) 3.88 (the signal was not observed in the deuterated material); *m*/*z* (EI) 157.0809 (M^+, 49%, $C_8H_7{}^2H_3O_3$ requires 157.0818), 129* (70), 114* (28), 86* (33), 43 (100) (*: shifted by 3 mass units); enrichment (98.5 atom% ^{2}H).

4-Hydroxy-6-methyl-3-phenylthio[$^2H_2$]methyl-2*H*-pyran-2-one (15)

The above compound was synthesized as described previously[15] except for the use of [$^2H_2$]formaldehyde. **15**: white crystals, mp 152–154 °C (from hexane–EtOAc) (lit.,[19] 151–153 °C); δ_H (270 MHz; CD_3OD) 3.95 (the signal was not observed in the deuterated material); *m*/*z* (FI) 250.0635* (M^+, 64%, $C_{13}H_{10}{}^2H_2O_3S$ requires 250.0633), 140* (76), 110 (100) (*: shifted by 2 mass units); enrichment (94.9 atom% ^{2}H).

3,6-[3,3-$^2H_2$]Dimethyl-4-hydroxy-2*H*-pyran-2-one (12b)

The above compound was synthesized as described previously.[19] **12b**: white crystals, mp 205–207 °C (from $CHCl_3$–MeOH) (lit.,[19] 209–211 °C); δ_H (270 MHz; CD_3OD) 1.81 (1H, m); *m*/*z* (EI) 142.0599* (M^+, 51%, $C_8H_7{}^2H_3O_3$ requires 142.0599), 114* (42), 85 (100), 58* (41), 43 (85) (*: shifted by 2 mass units).

3,6-[3,3-$^2H_2$]Dimethyl-4-[$^2H_3$]methoxy-2*H*-pyran-2-one (13b)

The above compound was synthesized as described previously[15] except for the use of [$^2H_6$]dimethyl sulfate. **13b**: white crystals, mp 85.5–87 °C (from $CHCl_3$); δ_H (270 MHz; $CDCl_3$) 1.90 (1H, m), 3.88 (the signal was not observed in the deuterated material); *m*/*z* (EI) 159.0925* (M^+, 82%, $C_8H_7{}^2H_3O_3$ requires 159.0944), 131* (100), 116* (39), 88* (48), 69 (63), 57 (81), 55 (74) (*: shifted by 5 mass units); enrichment (97.6 atom% ^{2}H).

[$^2H_3$]Methyl toluene-*p*-sulfonate

To a solution of toluene-*p*-sulfonyl chloride (27.1 g, 0.142 mmol) in THF (115 ml) were added [$^2H_4$]methanol (11.57 ml, 0.285 mmol) and 20% NaOH solution (69 ml) at 0 °C. After 3 h, the mixture was diluted with ether (250 ml) and water (200 ml) and extracted with ether (250 ml × 2). The combined organic extracts were washed with saturated aqueous NH_4Cl, then brine, dried over anhydrous Mg_2SO_4, filtered and concentrated *in vacuo*. The resultant [$^2H_3$]methyl toluene-*p*-sulfonate (22.6 g, 84%) was sufficiently pure and was used in the following methylation. δ_H (270 MHz; $CDCl_3$) 3.73 (the signal was not observed in the deuterated material); *m*/*z* (EI) 189.0527* (M^+, 29%, $C_8H_7{}^2H_3O_3$ requires 189.0539), 155* (36), 91* (100), 65* (19) (*: shifted by 3 mass units).

3-Formyl-4-[$^2H_3$]methoxy-6-methyl-2*H*-pyran-2-one (16)

The above compound was synthesized as described previously[15] except for the use of [$^2H_3$]methyl toluene-*p*-sulfonate. **16**: white crystals, mp 172–175 °C (from $CHCl_3$); δ_H (270 MHz; $CDCl_3$) 4.01 (the signal was not observed in the deuterated material); *m*/*z* (EI) 171.0604* (M^+, 3%, $C_8H_5{}^2H_3O_4$ requires 171.0611), 143* (70), 111 (72), 43 (100) (*: shifted by 3 mass units); enrichment (95.8 atom% ^{2}H).

(Triethylsilyloxy[^{2}H]methyl)-4-[$^2H_3$]methoxy-6-methyl-2*H*-pyran-2-one (17)

The above compound was synthesized as described previously[15] except for the use of [$^2H_2$]$NaBH_4$. **17**: white crystals, mp 111–112.5 °C (from $CHCl_3$); δ_H (270 MHz; $CDCl_3$) 3.83 (the signal was not observed in the deuterated material), 4.45 (1H, s); *m*/*z* (EI) 259.0925* ($M^+ - Et$, 100%, $C_8H_7{}^2H_3O_3$ requires 259.0944), 157* (62), 103 (24), 75 (40), 43 (29) (*: shifted by 4 mass units); enrichment (91.2 atom% ^{2}H).

(6*E*,8*E*)-Deca-6,8-dienal (19) *via* cross coupling

To a vigorously stirred mixture of Mg turnings (0.39 g, 16.2 mmol) was added THF (7.5 ml) containing a small piece of iodine and the mixture was refluxed for 2 h. A solution of 4-bromobutyraldehyde diethyl acetal (1.2 g, 5.3 mmol) was added dropwise over 20 min. The mixture was further refluxed for 2.5 h and the mixture was cooled to 0 °C. To a solution of 1-acetoxyhexa-2,4-diene (0.44 g, 3.15 mmol) in THF (14 ml) was added Li_2CuCl_4 (0.1 M THF solution, 1.14 ml, 0.114 mmol) and the mixture was cooled to −20 °C. A solution of Grignard reagent prepared above was added dropwise over 15 min and the reaction mixture was slowly warmed to 0 °C. After 5.5 h, saturated aqueous NH_4Cl (30 ml) was added and the mixture was extracted with ether three times. The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. Purification by silica gel chromatography (hexane–ether, 10 : 1) gave acetal **18** (342 mg, 48%) as a colourless oil; δ_H (500 MHz, $CDCl_3$) 1.20 (6H, t, *J* 7.1, OCH_2CH_3), 1.33–1.42 (4H, m, 3-CH_2, 4-CH_2), 1.61 (2H, m, 2-CH_2), 1.72 (3H, d, *J* 6.6, 10-CH_3), 2.06 (2H, m, 5-CH_2), 3.48 (2H, dq, *J* 7.1 and 9.4, OCH_2), 3.63 (2H, dq, *J* 7.1 and 9.4, OCH_2), 4.47 (1H, t, *J* 5.7, 1-H), 5.55 (2H, m, 6-H, 9-H), 6.00 (2H, m, 7-H, 8-H).

To a solution of acetal **18** prepared above (40 mg, 0.177 mmol) in THF (0.75 ml) was added 5% aqueous oxalic acid (0.51 ml) and the mixture was stirred for 40 h at ambient temperature. The reaction mixture was diluted with ether and water, and the aqueous phase was separated and extracted with ether twice. The combined organic extracts were washed with saturated aqueous $NaHCO_3$, then brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. Purification by silica gel chromatography (hexane–ether, 9 : 1) gave aldehyde **19**[15] (23 mg, 85%) as a colourless oil.

[2,3-$^2H_2$]But-2-enyltriphenylphosphonium bromide (20)

To a solution of NaH (1.676 g, 60% in oil, 41.9 mmol washed with *n*-hexane) in THF (40 ml) was added methanol (1.69 ml, 41.9 mmol) at 0 °C. After evolution of hydrogen had ceased, a solution of but-2-yn-1-ol (1.47 ml, 19.6 mmol) in THF (49 ml) was added and the mixture was stirred for 10 min. Then, $LiAl^2H_4$ (0.99 g, 23.5 mmol) was added and the mixture was refluxed for 3.5 h. After the mixture was cooled to 0 °C, deuterium oxide (5 ml) was added and the mixture was stirred for 5 min and the stirring continued for 5 min at rt. Following dilution with ether and water, the aqueous layer was extracted with ether twice. The combined organic extracts were washed with saturated aqueous NH_4Cl and brine, dried over anhydrous Na_2SO_4, filtered and carefully concentrated *in vacuo* under ice-cooled conditions to give crude volatile but-2-en-1-ol (1.28 g) as a colourless liquid; δ_H (270 MHz; $CDCl_3$) 5.6–5.9 (the signal was not observed in the deuterated material); *m*/*z* (EI) 73.0614* ($M^+ - H$, 76%, $C_4H_5{}^2H_2O$ requires 73.0622), 57* (100), 43 (44) (*: shifted by 2 mass units); enrichment (98.0 atom% ^{2}H).

To a solution of the above crude product in pyridine (0.5 ml) was added phosphorus tribromide (0.71 ml, 7.475 mmol) at

0 °C. After stirring for 10 min, the reaction mixture was diluted with ether and water, and the organic layer was washed with water, 1 M HCl, saturated aqueous sodium bicarbonate and brine. The extract was dried over anhydrous K_2CO_3 and anhydrous $MgSO_4$, filtered and carefully concentrated *in vacuo* under ice-cooled conditions to give crude volatile 1-bromobut-2-ene (1.50 g) as a colourless liquid; δ_H (270 MHz; $CDCl_3$) 5.6–5.9 (the signal was not observed in the deuterated material).

To a solution of the crude bromide in benzene (3.4 ml) was added triphenylphosphine (5.14 g, 19.6 mmol) in benzene (5.5 ml). After stirring for 2 h, the reaction mixture was cooled to rt and left overnight. The resulting phosphonium salt was collected by filtration to give **20** (2.15 g, 28% over 3 steps) as white crystals; δ_H (270 MHz; $CDCl_3$) 5.58–5.80 (the signal was not observed in the deuterated material).

(6*E*,8*E*)-1-(Tetrahydropyran-2-yloxy)-[8,9-$^2H_2$]deca-6,8-diene (22)

To a solution of phosphonium salt **20** (2.0 g, 5.0 mmol) in benzene (14 ml) was added dropwise *n*-butyllithium (1.6 M THF solution, 3.9 ml, 6.25 mmol) at 3 °C. After stirring for 30 min, aldehyde **21** (3.5 ml, 31.5 mmol prepared from hexanediol) in benzene (2.5 ml) was added. After the mixture was allowed to warm to ambient temperature and stirred for 5 h, saturated aqueous NH_4Cl (15 ml) was added. The mixture was extracted with ether. The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. Purification by silica gel chromatography (hexane–ether, 9 : 1) gave THP ether **22** (719 mg, 60%, (6*E*,8*E*) : (6*E*,8*Z*) = 1 : 1); δ_H (270 MHz; $CDCl_3$) 1.72 (2.4H, s, (*E*)-CH_3), 1.77 (0.6H, s, (*Z*)-CH_3), 5.26 (0.2H, d, *J* 10.6 and 7.9, (*Z*)-9-H), 5.58 (0.8H, dt, *J* 15.2 and 7.3, (*E*)-10-H), 5.96 (0.2H, d, *J* 10.6, (*Z*)-10-H), 6.00 (0.8H, d, *J* 15.2, (*E*)-9-H); *m*/*z* (EI) 240.2062* (M^+, 3%, $C_{15}H_{24}{}^2H_2O_2$ requires 240.2058), 156* (5), 138* (3), 123* (2), 95* (5), 85 (100), 70* (16), 57* (10) (*: shifted by 2 mass units).

(6*E*,8*E*)-[8,9-$^2H_2$]Deca-6,8-dienol (23)

To a solution of THP ether **22** (700 mg, 2.91 mmol) in ethanol (23 ml) was added pyridinium toluene-*p*-sulfonate (73 mg, 0.291 mmol). The mixture was stirred at 60 °C for 8 h before it was cooled and quenched with triethylamine (0.1 ml). The mixture was concentrated *in vacuo* and purification by silica gel chromatography (hexane–EtOAc, 96 : 4–4 : 1) gave dienol **23** (406 mg, 90%, (6*E*,8*E*) : (6*E*,8*Z*) = 1 : 1).

To a solution of dienol **23** (406 mg, 2.60 mmol) in benzene (5 ml) was added iodine (8 mg, 0.031 mmol). The mixture was stirred avoiding light for 24 h before it was quenched with saturated aqueous $Na_2S_2O_3$ (10 ml). After stirring for 15 min, the mixture was extracted with ether. The combined organic extracts were washed with brine (100 ml), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. Purification of the resultant oil by silica gel column chromatography (hexane–ether, 4 : 1) gave isomerized dienol **23** (349 mg, 86%, (6*E*,8*E*) : (6*E*,8*Z*) = 5 : 1) as a colourless oil; δ_H (270 MHz; $CDCl_3$) 1.72 (2.4H, s, (*E*)-CH_3), 1.77 (0.6H, s, (*Z*)-CH_3), 3.89 (the signal was not observed in the deuterated material), 4.51 (1H, s), 5.26 (0.2H, d, *J* 10.6 and 7.9, (*Z*)-9-H), 5.58 (0.8H, dt, *J* 15.2 and 7.3, (*E*)-10-H), 5.96 (0.2H, d, *J* 10.6, (*Z*)-10-H), 6.00 (0.8H, d, *J* 15.2, (*E*)-9-H); *m*/*z* (EI) 156.1495* (M^+, 13%, $C_{10}H_{162}H_2O$ requires 156.1483), 138* (100), 123* (46), 95* (46), 83* (33), 70* (54), 55 (18) (*: shifted by 2 mass units).

(6*E*,8*E*)-[8,9-$^2H_2$]Deca-6,8-dienal (19a)

The above compound was synthesized as described previously.[15] **19a**: a colourless oil; δ_H (270 MHz; $CDCl_3$) 1.72 (2.4H, s, (*E*)-CH_3), 1.77 (0.6H, s, (*Z*)-CH_3), 3.89 (the signal was not observed in the deuterated material), 4.51 (1H, s), 5.26 (0.2H, d, *J* 10.6 and 7.9, (*Z*)-9-H), 5.58 (0.8H, dt, *J* 15.2 and 7.3, (*E*)-10-H), 5.96 (0.2H, d, *J* 10.6, (*Z*)-10-H), 6.00 (0.8H, d, *J* 15.2, (*E*)-9-H); *m*/*z* (EI) 154.1331* (M^+, 24%, $C_{10}H_{14}{}^2H_2O$ requires 154.1327), 139* (6), 121* (6), 111* (23), 95* (29), 83* (100), 79* (22), 70* (50) (*: shifted by 2 mass units).

6-[(7*E*,9*E*)-2-Hydroxyundeca-7,9-dienyl]-4-[$^2H_3$]methoxy-3-methyl-2*H*-pyran-2-one (24a) and 6-[(7*E*,9*E*)-2-hydroxyundeca-7,9-dienyl]-4-[$^2H_3$]methoxy-3-[$^2H_2$]methyl-2*H*-pyran-2-one (24b)

The above compounds were synthesized as described previously.[15] **24a**: White crystals, mp 89–89.5 °C (from $CHCl_3$); δ_H (270 MHz; $CDCl_3$) 3.88 (the signal was not observed in the deuterated material); *m*/*z* (EI) 309.2040* (M^+, 5%, $C_{18}H_{23}{}^2H_3O_4$ requires 309.2019), 196* (4), 186* (8), 157* (100), 142* (10), 128* (9), 81 (13), 67 (10), 55 (15) (*: shifted by 3 mass units).

24b: White crystals, mp 89–89 °C (from $CHCl_3$); δ_H (270 MHz; $CDCl_3$) 1.90 (1H, m), 3.88 (the signal was not observed in the deuterated material); *m*/*z* (EI) 311.2151* (M^+, 7%, $C_{18}H_{21}{}^2H_5O_4$ requires 311.2144), 293* (8), 198* (12), 172* (11), 159* (100), 144* (24), 130* (14), 81 (34), 67 (26), 55 (36) (*: shifted by 5 mass units).

[*O*-C$^2H_3$]Prosolanapyrone I (6a) and [*O*-C$^2H_3$,17,17-$^2H_2$]-prosolanapyrone I (6b)

The above compound was synthesized using essentially the same procedure described previously.[15] **6a**: A colourless oil; δ_D (76.7 MHz; $CHCl_3$) 3.88 (3-^{2}H, s); *m*/*z* (EI) 291.1906* (M^+, 46%, $C_{18}H_{21}{}^2H_3O_3$ requires 291.1913), 236* (59), 196* (67), 183 (100), 170* (95), 157* (69), 142* (49), 81* (75), 67 (40), 55 (57) (*: shifted by 3 mass units). **6b**: A colourless oil; δ_H (270 MHz; $CDCl_3$) 1.90 (1H, m); δ_D (76.7 MHz; $CHCl_3$) 1.92 (2-^{2}H, s), 3.89 (3-^{2}H, s); *m*/*z* (EI) 293.2052* (M^+, 45%, $C_{18}H_{19}{}^2H_5O_3$ requires 293.2039), 238* (57), 198* (60), 185 (91), 172* (82), 159* (54), 144* (53), 81* (75), 67 (50), 55 (52), 41 (100) (*: shifted by 5 mass units).

3-[(Triethylsilyloxy[^{2}H]methyl)-6-[(7*E*,9*E*)-2-hydroxy[9,10-$^2H_2$]-undeca-7,9-dienyl]-4-[$^2H_3$]methoxy-2*H*-pyran-2-one (25)

The above compound was synthesized as described previously.[15] **25**: A colourless oil; δ_H (270 MHz; $CDCl_3$) 1.72 (2.4H, s, (*E*)-CH_3), 1.77 (0.6H, s, (*Z*)-CH_3), 3.89 (the signal was not observed in the deuterated material), 4.51 (1H, s), 5.26 (0.2H, d, *J* 10.6 and 7.9, (*Z*)-9-H), 5.58 (0.8H, dt, *J* 15.2 and 7.3, (*E*)-10-H), 5.96 (0.2H, d, *J* 10.6, (*Z*)-10-H), 6.00 (0.8H, d, *J* 15.2, (*E*)-9-H); *m*/*z* (EI) 413.2611* ($M^+ - Et$, 44%, $C_{22}H_{29}{}^2H_6SiO_5$ requires 413.2630), 259 (100), 157** (46), 103 (46), 83 (33), 75 (54), 69 (18), 57 (30) (shifted by * 6 or ** 5 mass units).

[*O*-C$^2H_3$,2,3,17-$^2H_3$]Prosolanapyrone II (7a)

The above compound was synthesized as described previously.[15] **7a**: A colourless oil; δ_H (270 MHz; $CDCl_3$) 1.72 (2.4H, s, (*E*)-CH_3), 1.77 (0.6H, s, (*Z*)-CH_3), 3.89 (the signal was not observed in the deuterated material), 4.51 (1H, s), 5.26 (0.2H, d, *J* 10.6 and 7.9, (*Z*)-9-H), 5.58 (0.8H, dt, *J* 15.2 and 7.3, (*E*)-10-H), 5.96–6.00 (2H, m, 1′-H and (*E*,*Z*)-9-H); δ_D (76.7 MHz; $CHCl_3$) 3.67 (3-^{2}H, s), 4.54 (1-^{2}H, s), 5.61 (1-^{2}H, s), 6.04 (1-^{2}H, s); *m*/*z* (EI) 310.2041* ($M^+ - H_2O$, 2%, $C_{18}H_{18}{}^2H_6O_4$ requires 310.2049), 292* (11), 235 (100), 195 (85), 182 (52), 169 (77), 156 (69), 136 (70), 81 (89) (*: shifted by 6 mass units).

[17-^{2}H]Solanapyrone A (1a)

To a solution of **1** (41 mg, 0.136 mmol) in MeOH (1.5 ml) was added NaB^2H_4 (10 mg, 0.239 mmol) at 3 °C. After the mixture was stirred for 1 h, the excess reagent was quenched with acetone. The volatile materials were removed *in vacuo*. Purification by silica gel chromatography ($CHCl_3$–EtOAc, 20 : 1) gave **2a** (34 mg, 82%) as a colourless oil: δ_H (270 MHz; $CDCl_3$) 4.56 (1H, s).

To a solution of **2a** (20 mg, 0.066 mmol) in CH_2Cl_2 (1 ml) was added Dess–Martin periodinane (37 mg, 0.099 mmol). The mixture was stirred at ambient temperature for 1 h, when saturated aqueous $NaHCO_3$ and saturated aqueous $Na_2S_2O_3$ were added. The resultant mixture was stirred for 10 min and then extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by preparative thin layer silica gel chromatography ($CHCl_3$–EtOAc, 4:1) gave **1a** (16 mg, 79%) as a colourless oil: δ_H (270 MHz; $CDCl_3$) 10.15 (0.23H, s).

(17*S*)-[17-^{2}H]Solanapyrone B (2a)

To a solution of (−)-α-pinene (43 mg, 0.31 mmol) in THF (1 ml) was added 9-borabicyclo[3.3.1]nonene dimer (0.05 ml, 34 mg, 0.13 mmol) and the reaction mixture was stirred under reflux for 2 h. The mixture was cooled to rt, and a solution of **1a** (7.5 mg, 0.027 mmol) in THF (0.4 ml) was added. The reaction mixture was refluxed for 1 h and cooled to rt. Isobutyraldehyde (0.027 ml, 0.297 mmol) was added, and after 5 min the volatiles were removed *in vacuo*. Under ice-cooled conditions, 2-aminoethanol (0.018 ml, 0.298 mmol) in ether (1 ml) was added and the resultant white precipitate was filtered through a Celite pad. The filtrate was concentrated *in vacuo*, and purification by preparative thin layer silica gel chromatography ($CHCl_3$–EtOAc, 4:1) gave **2a** (4.3 mg, 57%) as a colourless oil: δ_H (270 MHz; $CDCl_3$) 4.56 (1.23H, s).

(*S*)-MTPA ester (30)

To a solution of **2a** (4.3 mg, 0.014 mmol) in CH_2Cl_2 (0.3 ml) were added (*S*)-MTPA (5 mg, 0.021 mmol), 1,3-dicyclohexylcarbodiimide (5 mg, 0.024 mmol) and 4-dimethylaminopyridine (1 mg, 0.008 mmol). The mixture was stirred at ambient temperature for 1 h before being concentrated *in vacuo*. The residue was suspended in toluene and the undissolved materials were removed by filtration. The filtrate thus obtained was purified by preparative thin layer silica gel chromatography (hexane–EtOAc, 2:1) to give (*S*)-MTPA ester **27** (5.9 mg, 81%) as a colourless oil: δ_H (270 MHz; $CDCl_3$) 0.93 (3H, d, *J* 6.6, 16-H), 0.94–1.28 (3H, m), 1.28–1.49 (3H, m), 1.56–1.74 (2H, m), 2.10 (1H, m, 5-H), 2.26 (1H, m, 10-H), 4.95 (1H, t, *J* 10.6, 1-H), 2.61 (1H, m, 2-H), 3.55 (3H, d, *J* 1.3, $ArOCH_3$), 3.85 (3H, s, OCH_3), 5.20 (2H, s, 17-H), 5.42 (1H, td, *J* 2.0, 10.3, 3-H), 5.65 (1H, ddd, *J* 2.6, 4.6, 10.3, 4-H), 6.02 (1H, s, 12-H), 7.35–7.39 (3H, m, ArH), 7.52–7.57 (2H, m, ArH).

Similarly, solanapyrone B (2.5 mg, 0.008 mmol) obtained from the feeding experiment with L-[*methyl*-$^2H_3$]methionine was converted to the (*S*)-MTPA ester (3.6 mg, 83%).

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