Structures and Stereochemistries of New Compounds Related to Alternaric Acid

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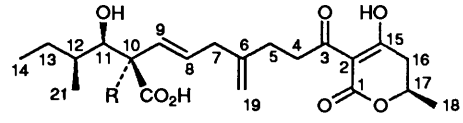
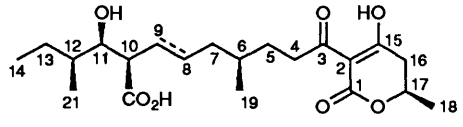
Three alternaric acid-related compounds, *viz.*, 10-deoxyalternaric acid **2**, 10-deoxy-6,19-dihydroalternaric acid **3**, and 10-deoxy-6,8,9,19-tetrahydroalternaric acid **4**, have been isolated from *Alternaria solani* which is a causal fungus of early blight disease on potato and tomato. The structures and stereochemistries of these compounds have been determined by spectral studies and chemical correlations. The structure–activity relationships of alternaric acid **1** and plausible biosynthetic routes from these compounds to alternaric acid **1** are also discussed.

Alternaria solani, a causal fungus of early blight disease on potato and tomato, produces several secondary metabolites including alternaric acid **1**,[1] macrosporin,[2] zinniol,[3] zinnolide,[4] and solanapyrones.[5] Alternaric acid **1** was isolated in 1949 by Brian and co-workers as an antifungal metabolite.[1a] After that, it was shown to contribute to disease development in the host by *A. solani* in a manner similar to the mode of action of the group of compounds classified as host-specific toxins, although all of the requirements as a primary disease determinant were not fulfilled.[6] This phytotoxin **1** was also shown to delay the occurrence of hypersensitive death of potato cells infected by an incompatible race of *Pytophthora infestans*.[7] Recently, we disclosed the determination of the complete stereochemistry, and achieved a total synthesis, of alternaric acid **1**.[8,9]

In this paper, we present further work on the isolation, structural elucidation, and determination of absolute configurations of three new metabolites, **2**, **3** and **4**, related to alternaric acid **1**.



1 R = OH (alternaric acid)
2 R = H



3 C-8,-9 -CH=CH-(*E*)
4 C-8,-9 $-CH_2CH_2-$

Results and Discussion

Isolation and Structure Elucidation.—The extraction and isolation of the metabolites from a culture filtrate of *Alternaria solani* were carried out as described in the Experimental section.

The structures of the new metabolites **2**, **3** and **4** were determined by chemical and spectroscopic methods, including 2D NMR experiments (^1H–^1H COSY and ^1H–^{13}C COSY).† In the case of compound **4**, all proton and carbon signals were assigned by ^1H–^1H COSY, DEPT, HMQC and HMBC experiments.‡ The new compounds and the derivatives were not obtained as single crystals which are suitable for X-ray analysis. However, the amounts of these metabolites were sufficient for chemical degradation, and the degradation products were necessary for us to investigate the structure–activity relationships. For these reasons, the absolute configurations were fully assigned on the basis of analyses of the spectral data, chemical degradations and chemical correlations. Key reactions for degradation involve the Dakin reaction[10] and Lemieux–Johnson oxidation.[11] First, the absolute configuration of compound **3** was revealed, and then those of compounds **2** and **4** were determined by chemical correlations with compound **3**.

From fast-atom-bombardment high-resolution mass spectrometry (FAB-HRMS, negative), the molecular formula of compound **3** was determined as $C_{21}H_{32}O_7$. The UV maximum at 273 nm and the signal due to a strongly hydrogen-bonded proton at δ_H 17.89 in the ^1H NMR spectrum showed the presence of a 3-acyl-4-hydroxy-5,6-dihydro-2-pyrone structure.[1d,12,13] The characteristic mass fragment at *m/z* 127 ($C_6H_7O_3^-$) also supported the presence of this moiety. The ^{13}C NMR spectrum exhibited the presence of the four CH_3, five CH_2, seven CH, and five quaternary carbons. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) of compound **3** were similar to those of alternaric acid **1**, except for the moieties around C-6, C-7, C-10 and C-19. Thus, in the ^1H NMR spectra, the doublet for the C-9 olefinic proton at δ_H 5.76 (*J* 15.5 Hz) in **1** is replaced by a double doublet in compound **3** [δ_H 5.38 (*J* 15.3 and 9.2 Hz)], and the doublet for the C-11 methine proton at δ_H 3.95 (*J* 2.3 Hz) in alternaric acid **1** is replaced by a double doublet in compound **3** [δ_H 3.85 (*J* 8.7 and 2.5 Hz)]. The triplet for the C-10 methine proton at δ_H 3.22 (*J* 8.9 Hz) was not observed in compound **1**. Furthermore, in the ^{13}C NMR spectrum, the C-10 atom of compound **3** is present as a methine group (δ_C 53.7). These data indicate that the hydroxy group at C-10 in alternaric acid **1** was changed to a methine hydrogen in compound **3**. Two broad singlets for C-19 exo-methylene protons (δ_H 4.86 and 4.83) and the corresponding carbon signals for C-6 and C-19 [δ_C 145.7 (C), 111.8 (CH_2)] in alternaric acid **1** are replaced by signals for a secondary methyl group in compound **3** (δ_H 1.61 and 0.91) [δ_C 32.7 (CH), 19.3 (CH_3)]. From the above results and an analysis of the ^1H–^1H and ^1H–^{13}C COSY spectra, the structure of compound **3** was determined as 10-deoxy-6,19-dihydroalternaric acid.

Since we failed to obtain compound **3** and derivatives as single crystals suitable for X-ray analysis, the stereochemistry of compound **3** was assigned by a combination of spectroscopic methods, chemical degradations, and interconversions. By

† COSY = 2D homonuclear chemical-shift correlation spectroscopy.
‡ DEPT = distortionless enhancement by polarization transfer; HMQC = 2D heteronuclear multiple quantum coherence; HMBC = 2D heteronuclear multiple bond coherence.

Table 1 ^{1}H NMR data (δ_H; 500 MHz; $CDCl_3$) of alternaric acid **1**, 10-deoxyalternaric acid **2**, 10-deoxy-6,19-dihydroalternaric acid **3** and 10-deoxy-6,8,9,19-tetrahydroalternaric acid **4** [δ_H (multiplicity, J/Hz)]

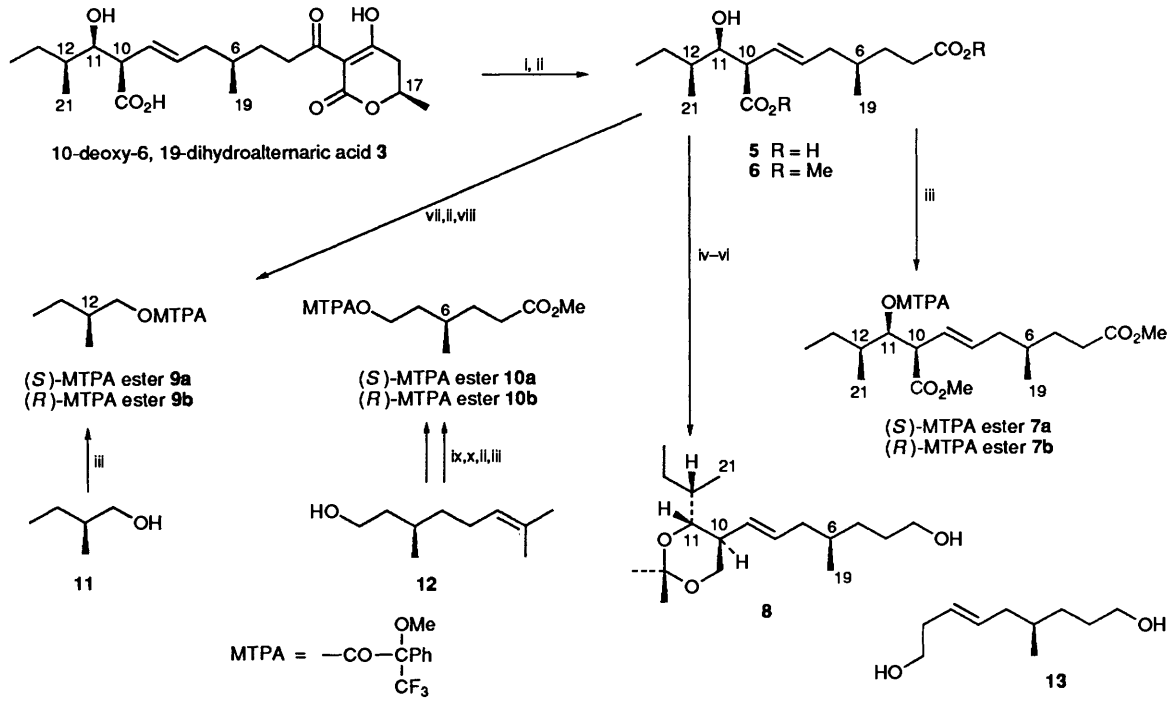
	1	2	3	4
4-H^a	3.25 (ddd, 14.1, 10.6, 5.2)	3.23 (ddd, 15.8, 9.2, 5.8)	3.06 (ddd, 15.3, 10.0, 4.4)	3.04 (ddd, 14.9, 10.4, 5.0)
4-H^b	2.94 (ddd, 14.1, 10.7, 5.4)	3.10 (ddd, 15.8, 9.3, 6.1)	2.96 (ddd, 15.3, 9.8, 4.9)	2.95 (ddd, 14.9, 9.6, 5.9)
5-H^a	2.44 (m)	2.39 (m)	1.63 (m)	1.51 (m)
5-H^b	2.27 (m)	2.32 (m)	1.52 (m)	1.65 (m)
6-H			1.61 (m)	1.52 (m)
7-H^a	2.85 (dd, 14.6, 6.6)	2.80 (d, 7.3)	2.07 (dt, 13.9, 6.9)	1.15 (m)
7-H^b	2.81 (dd, 14.6, 6.6)		1.97 (dt, 13.9, 6.9)	1.38 (m)
8-H^a	5.97 (dt, 15.5, 6.6)	5.70 (dt, 15.3, 7.3)	5.67 (dt, 15.3, 7.1)	1.36 (m)
8-H^b				1.36 (m)
9-H^a	5.76 (d, 15.5)	5.46 (dd, 15.3, 9.1)	5.38 (dd, 15.3, 9.2)	1.56 (m)
9-H^b				1.66 (m)
10-H		3.25 (t, 8.9)	3.22 (t, 8.9)	2.61 (m)
11-H	3.95 (d, 2.3)	3.84 (dd, 8.3, 2.9)	3.85 (dd, 8.7, 2.5)	3.63 (dd, 6.3, 5.0)
12-H	1.83 (m)	1.52 (m)	1.50 (m)	1.52 (m)
13-H^a	1.43 (m)	1.46 (m)	1.45 (m)	1.25 (m)
13-H^b	1.32 (m)	1.30 (m)	1.30 (m)	1.52 (m)
14-H_3	0.90 (t, 7.4)	0.90 (t, 7.3)	0.90 (t, 7.3)	0.91 (t, 7.4)
15-OH	17.86 (br s)	17.84 (br s)	17.89 (br s)	17.92 (br s)
16-H^a	2.69 (dd, 17.2, 9.1)	2.68 (dd, 17.6, 10.5)	2.67 (dd, 17.2, 10.0)	2.68 (dd, 17.5, 10.3)
16-H^b	2.65 (dd, 17.2, 5.7)	2.63 (dd, 17.6, 4.3)	2.62 (dd, 17.2, 4.1)	2.63 (dd, 17.5, 4.1)
17-H	4.56 (m)	4.54 (m)	4.53 (m)	4.54 (m)
18-H_3	1.47 (d, 6.2)	1.47 (d, 6.3)	1.46 (d, 6.3)	1.46 (d, 6.3)
19-H^a	4.86 (br s)	4.81 (br s)	0.91 (d, 6.4)	0.92 (d, 6.1)
19-H^b	4.83 (br s)	4.79 (br s)		
21-H_3	0.88 (d, 6.8)	0.87 (d, 6.6)	0.85 (d, 7.5)	0.94 (d, 6.6)

Table 2 ^{13}C NMR (125 MHz; $CDCl_3$) data (δ_C) of alternaric acid **1**, 10-deoxyalternaric acid **2**, 10-deoxy-6,19-dihydroalternaric acid **3** and 10-deoxy-6,8,9,19-tetrahydroalternaric acid **4**

	1^a	2	3	4
C-1	164.9	164.6	164.5	164.6
C-2	102.9	103.0	102.9	102.9
C-3	203.8	203.8	204.7	204.9
C-4	37.1	37.0	36.2	36.1^b
C-5	30.8	30.6	31.3	31.7
C-6	145.7	145.9	32.7	32.4
C-7	38.9	39.4	39.5	36.2^b
C-8	129.5	133.0	133.9	24.5
C-9	129.6	126.1	125.6	31.7
C-10	79.9	53.4	53.7	48.7
C-11	76.6	74.6	74.5	74.9
C-12	35.1	36.6	36.4	37.6
C-13	28.0	26.6	26.7	26.3
C-14	11.7	12.0	11.8	11.5
C-15	194.9	194.7	195.0	195.1
C-16	39.2	39.1	39.4	39.4
C-17	70.6	70.4	70.4	70.4
C-18	20.5	20.6	20.6	20.6
C-19	111.8	111.5	19.3	19.4
C-20	177.0	177.6	178.1	179.8
C-21	12.7	11.7	11.7	13.2

^{a} Ref. 13*b*. ^{b} The assignments are exchangeable.

Dakin oxidation[10] and subsequent treatment of the intermediate acid **5** with diazomethane, 10-deoxy-6,19-dihydroalternaric acid **3** was converted into the diester **6** as shown in Scheme 1. In this reaction, the C-10 epimer was not detected by ^{1}H NMR spectroscopy. The absolute configuration at C-11 in the diester **6** was established by use of an advanced Mosher's method.[14] Thus, the diester **6** was converted into its (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) ester **7a** and (*R*)-MTPA ester **7b**. The chemical-shift differences ($\Delta\delta = \delta_{7a} - \delta_{7b}$) of proton signals due to the diastereoisomers **7a** and **7b** in the ^{1}H NMR spectra (500 MHz; $CDCl_3$) indicate that the absolute configuration of the C-11 position of the diester **6** is *R* (Fig. 1). To determine the absolute configuration at C-10 in compound **6**, isopropylidene derivative **8** was prepared from the diester **6** in three steps (Scheme 1), though direct reduction of the diester **6** with lithium aluminium hydride gave diol **13** as a major product arising from retro-aldol-type cleavage followed by reduction. A pair of large coupling constants ($J_{10,11}$ 10.2 Hz, $J_{10,20ax}$ 10.9 Hz) for the *trans*-diaxial protons in the ^{1}H NMR spectrum of the isopropylidene derivative **8** indicate that the 1,3-dioxane ring has a chair conformation and that the relative configuration at C-10 and C-11 in compound **8** is *R*/*S* or *S*/*R* (Fig. 2). Since the absolute configuration at C-11 had already been determined as *R*, the absolute configuration at C-10 in the isopropylidene derivative **8** should be *S*. The stereochemistry at C-6 and C-12 in the diester **6** was determined by comparison of the (*S*)-MTPA esters (**9a**, **10a**) from **6** with synthetic samples (**9a**, **9b** and **10a**, **10b**). Thus, in order to cleave at the olefinic bond, the diester **6** was treated with osmium tetraoxide–sodium metaperiodate,[11] and subsequent reduction with sodium borohydride to give crude products. Esterification of the products by (*S*)-MTPACl gave the C(3)–C(8) fragment **10a** and an unexpected product, the C(11)–C(14) fragment **9a**. The product **9a** must have arisen *via* retro-aldol-type reaction of the diester **6** or its degradation product. On the other hand, MTPA esters **9** and **10** were synthesized from (*S*)-(−)-2-methylbutanol **11** and (*R*)-(+)-β-citronellol **12**, respectively (Scheme 1). The two diastereoisomers, (*S*)-MTPA ester **9a** and (*R*)-MTPA ester **9b**, were easily distinguished by ^{1}H NMR spectroscopy. The (*S*)-MTPA ester **9a** obtained from the diester **6** was correlated with the synthetic product **9a**, so the stereochemistry at C-12 is *S*. The two diastereoisomers, (*S*)-MTPA ester **10a** and (*R*)-MTPA ester **10b**, were also distinguishable by ^{1}H NMR spectroscopy. The (*S*)-MTPA ester **10a** obtained from the diester **6** was identical with the synthetic compound **10a**, so the stereochemistry at C-6 is *R*. The absolute configuration at C-17 in 10-deoxy-6,19-dihydroalternaric acid **3** was established by analysis of its CD spectrum. The CD spectrum curve of compound **3** is very similar to that of alternaric acid **1** as shown in Fig. 3. Since the determination of the absolute configuration at C-17 in alternaric acid **1** had been achieved by its degradation[1e] and total synthesis,[9] the stereochemistry at C-17 in 10-deoxy-6,19-



Scheme 1 Degradation of compound **3** and structure of products **5–13**. *Reagents and conditions:* i, 1 mol dm^{-3} NaOH, 30% H_2O_2; ii, CH_2N_2, Et_2O; iii, (*S*)- or (*R*)-MTPA, DMAP, DCC; iv, pyridine, Ac_2O, DMAP; v, $LiAlH_4$, THF; vi, $Me_2C(OMe)_2$, CSA, acetone; vii, OsO_4, $NaIO_4$, THF–water (1:1); then $NaBH_4$; viii, pyridine, (*S*)-MTPACl; ix, OsO_4, $NaIO_4$, THF–water (1:1); x, $NaClO_2$, 0.5 mol dm^{-3} NaH_2PO_4, H_2O_2, MeCN.

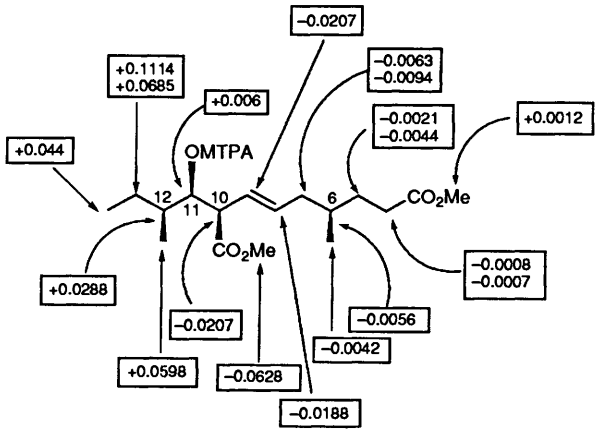


Fig. 1 Determination of absolute configuration at C-11 in the diester **6**; $\Delta\delta$-values obtained from (*S*)-MTPA ester **7a** and (*R*)-MTPA ester **7b** ($\Delta\delta = \delta_{7a} - \delta_{7b}$)

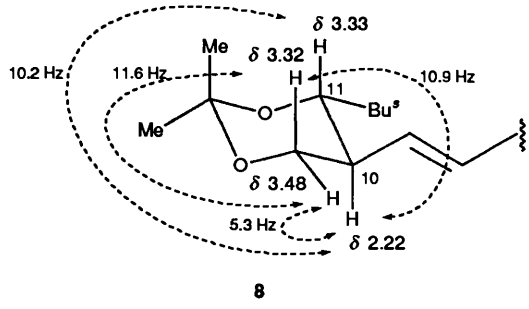


Fig. 2 Assignments of relative and absolute configurations at C-10/C-11 in the isopropylidene derivative **8** (270 MHz; C_6D_6)

dihydroalternaric acid **3** was expected to be the *R*-configuration. The CD spectra of compounds (*R*)-(−)-**15** and (*S*)-(+)-**15**, which were prepared from (*R*)-(−)-methyl 3-hydroxybutanoate and (*S*)-(+)-methyl 3-hydroxybutanoate, respectively (Scheme 2),[9,15] have a symmetrical maximum at ~260 nm (Fig. 3). Furthermore, in the CD spectrum of dicarboxylic acid **5**, there is no curve around 240–290 nm. These data indicate that the curve around 240–290 nm in the CD spectrum of 10-deoxy-6,19-dihydroalternaric acid **3** is due to the 3-acyl-4-hydroxy-5,6-dihydro-2-pyrone moiety, and that the absolute configuration of C-17 in compound **3** is *R*. From the data described above, the absolute stereochemistry of 10-deoxy-6,19-dihydroalternaric acid was determined as shown in structure **3**.

Compound **2** has the molecular formula $C_{21}H_{30}O_7$ from FAB-HRMS (negative). The UV maximum (274 nm), mass fragment (*m/z* 127, $C_6H_7O_3^-$) and the signal due to a strongly hydrogen-bonded proton at δ_H 17.84 in the ^1H NMR spectrum showed the presence of a 3-acyl-4-hydroxy-5,6-dihydro-2-pyrone structure.[1d,12,13] The ^{13}C NMR spectrum exhibited the presence of the three CH_3, six CH_2, six CH and six quaternary carbons. The ^1H NMR spectral data of compound **2** were very similar to those of alternaric acid **1**, except for the moiety around C-10. Thus, the doublet for C-9 olefinic proton in **1** is replaced by a double doublet in compound **2** [δ_H 5.46 (*J* 15.3 and 9.1 Hz)], and the doublet for C-11 methine proton in alternaric acid **1** is also replaced by a double doublet in compound **2** [δ_H 3.84 (*J* 8.3 and 2.9 Hz)]. Furthermore, the triplet for C-10 methine proton at δ_H 3.25 (*J* 8.9 Hz) was not observed for compound **1**. These facts indicate that the hydroxy group at C-10 in alternaric acid **1** is changed to a methine hydrogen in compound **2**. However, the two broad singlets at δ_H 4.79 and 4.81 due to the C-6 exo-olefinic moiety was observed in **2**. From these observations and an analysis of the ^1H–^1H COSY and ^1H–^{13}C COSY data, the structure of compound **2** is deduced to be 10-deoxyalternaric acid.

The stereochemistry of 10-deoxyalternaric acid **2** was elucidated by chemical correlation with 10-deoxy-6,19-dihydro-

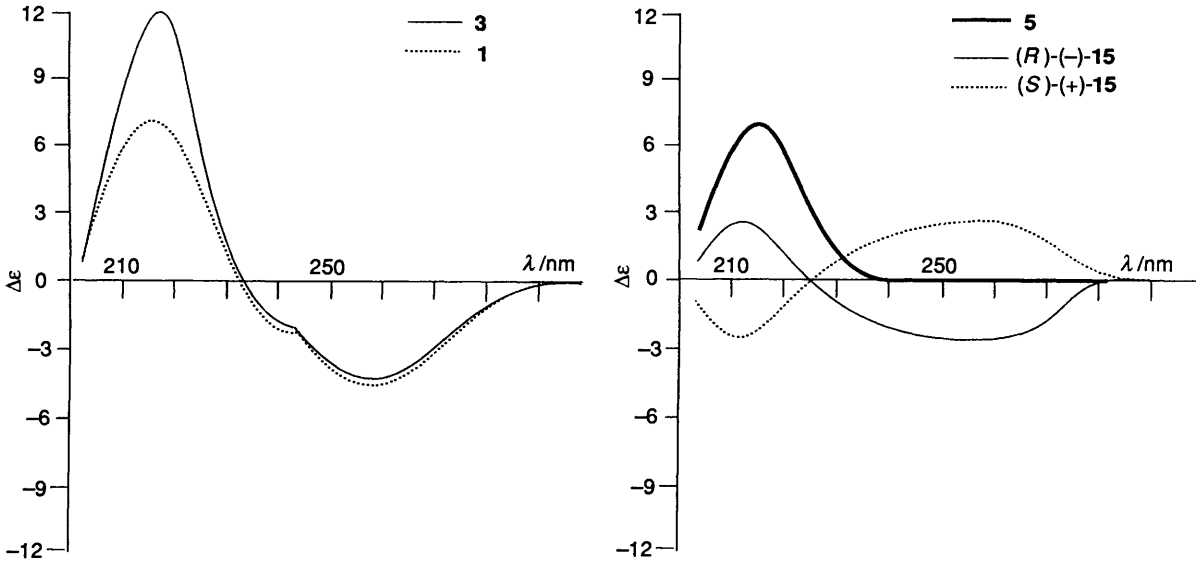
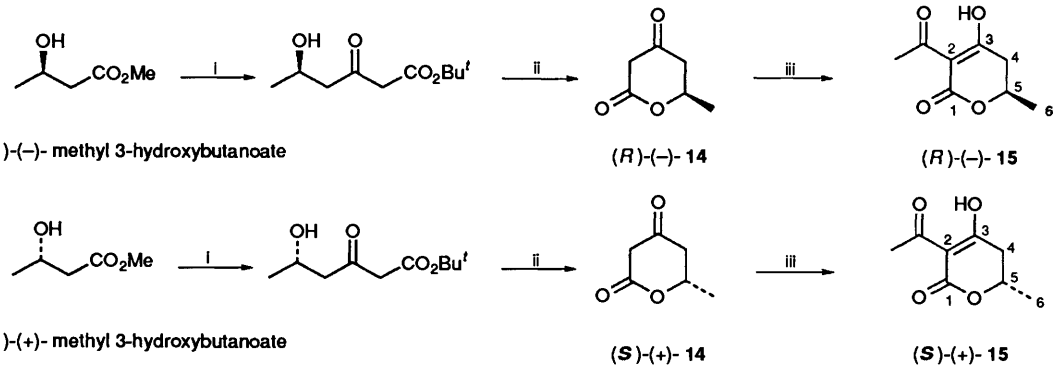


Fig. 3 CD spectra of compounds **1**, **3**, **5**, (*R*)-(−)-**15** and (*S*)-(+)-**15**



Scheme 2 Synthesis of compounds (*R*)-(−)-**15** and (*S*)-(+)-**15**. *Reagents and conditions:* i, Bu^{t}OAc, LiNPr$^i_2$ THF; ii, 1 mol dm^{-3} NaOH; then H$^+$; iii, AcOH, DCC, DMAP, CH_2Cl_2.

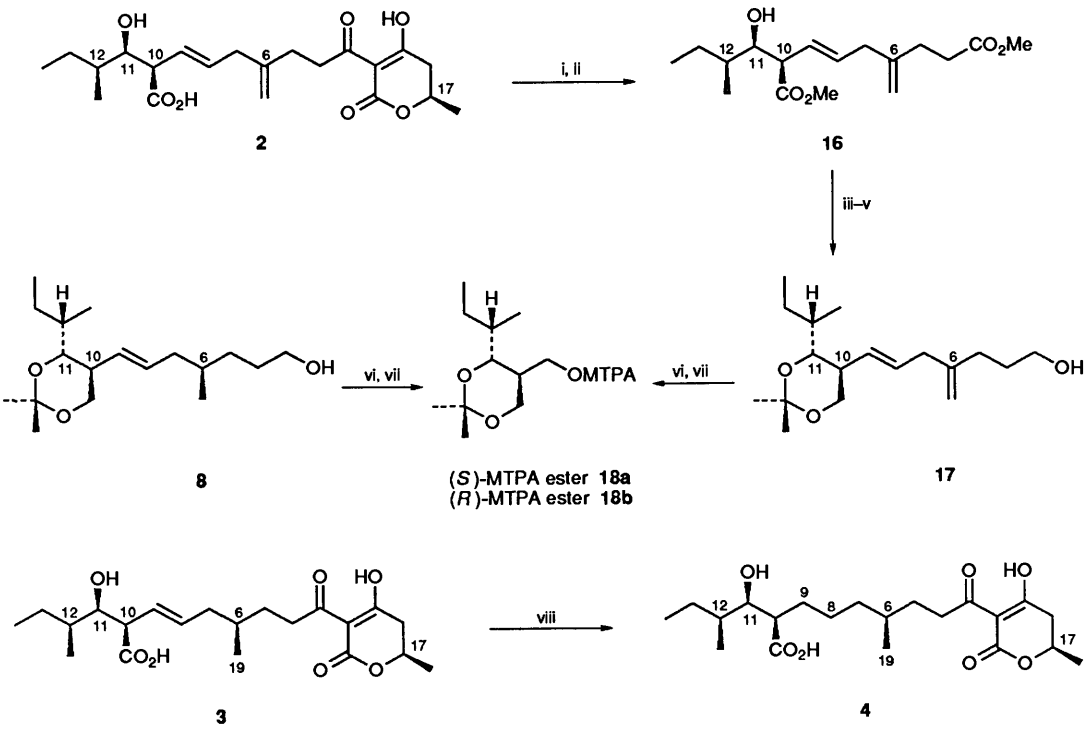
alternaric acid **3** and by analysis of its CD spectrum. The key intermediate, isopropylidene derivative **17**, was prepared from 10-deoxyalternaric acid **2** by the same way from 10-deoxy-6,19-dihydroalternaric acid **3** as was the isopropylidene derivative **8**, as shown in Scheme 3. At this stage, it was confirmed that the relative configuration at C-10 and C-11 in the isopropylidene derivative **17** was *R*/*S* or *S*/*R* by analysis of the coupling constants ($J_{10,11}$ 10.6) in the ^{1}H NMR spectrum. Both isopropylidene derivatives **8** and **17** were converted into (*S*)-MTPA ester **18a** through a two-step reaction. The ^{1}H NMR spectral data of the (*S*)-MTPA ester **18a** were differentiated from those of the (*R*)-MTPA ester **18b**. The ^{1}H NMR spectral data of (*S*)-MTPA ester **18a** from the isopropylidene derivative **17** were in fair agreement with those of the (*S*)-MTPA ester **18a** from the isopropylidene derivative **8**. These facts indicate that the absolute configurations at C-10, C-11 and C-12 in compound **2** must be *R*/*R*/*S* as shown in Scheme 3. Since the CD spectral curve of compound **2** was similar to that of compound **3**, the stereochemistry at C-17 in compound **2** is also *R* configuration.

Compound **4** has the molecular formula $C_{21}H_{34}O_7$ from FAB-HRMS (negative). The UV maximum (274 nm), mass fragment (*m*/*z* 127, $C_6H_7O_3^-$) and a signal due to a strongly hydrogen-bonded proton at δ_H 17.92 in the ^{1}H NMR spectrum showed the presence of 3-acyl-4-hydroxy-5,6-dihydro-2-pyrone structure.[1d,12,13] The ^{13}C NMR spectrum exhibited the presence of the four CH_3, seven CH_2, five CH, and five quaternary carbons. The ^{1}H NMR spectrum of compound **4** was similar to that of alternaric acid **1**. However, the signals due to the olefin moieties at C-6, C-8, C-9 and C-19 were lost and signals due to a secondary methyl group bonded to C-6 and two methylene groups at C-8 and C-9 were apparent for compound **4**. The hydroxy group at C-10 in alternaric acid **1** was changed to a methine hydrogen in compound **4**, as in structures **2** and **3**. These data indicate that compound **4** is 10-deoxy-6,8,9,19-tetrahydroalternaric acid. The complete assignments of the ^{1}H and ^{13}C NMR spectral data of compound **4** were achieved by measurements of 2D COSY, DEPT, HMQC and HMBC experiments, and are summarized in Tables 1 and 2.

In order to determine the absolute configuration of 10-deoxy-6,8,9,19-tetrahydroalternaric acid **4**, 10-deoxy-6,19-dihydroalternaric acid **3** was converted into compound **4** by catalytic hydrogenation with palladium–carbon. The optical rotation, $[\alpha]_D^{24}$ −9.1* (*c* 0.11 in EtOH) {natural, $[\alpha]_D^{24}$ −8.6 (*c* 1.98 in EtOH)}, and other spectral data of the product **4** were in good agreement with those of natural compound **4**. From these results and an analysis of its CD spectrum, we concluded that the absolute configurations of 10-deoxy-6,8,9,19-tetrahydroalternaric acid must be as depicted in structure **4**.

Phytotoxic Activity.—The effects of compounds **1**, **2** and **3** on the growth inhibition of tomato seedlings are summarized in Table 3. These results indicate that the phytotoxicity of

* Optical rotation values are in units of 10^{-1} deg cm^2 g^{-1}.



Scheme 3 Chemical correlations between compounds **2**, **3** and **4**. *Reagents and conditions:* i, 1 mol dm^{-3} NaOH, 30% H_2O_2; ii, CH_2N_2, Et_2O; iii, pyridine, Ac_2O, DMAP; iv, $LiAlH_4$, THF; v, $Me_2C(OMe)_2$, CSA, acetone; vi, OsO_4, $NaIO_4$, THF–water (1:1); then $NaBH_4$; vii, (*S*) or (*R*)MTPA, DMAP, DCC; viii, 10% Pd–C, H_2, EtOH.

Table 3 Effects of compounds **1**–**3** (25 ppm) on the growth inhibition of tomato seedlings (25 °C; 3 days; in the dark)

	Rate of growth inhibition (%)			
	1	**2**	**3**	Control
Hypocotyl	88	60	5.1	0
Root	94	89	23	0

alternaric acids depends on the oxidation levels of the molecules, and that the exo-methylene group at C-6 and the hydroxy group at C-10 in alternaric acid **1** play an important role in the phytotoxic activity. Very recently, the result of the same bioassay on the degradation products and the synthetic segments of alternaric acid suggested that the side-chain moiety and the 3-acyl-4-hydroxy-5,6-dihydro-2-pyrone moiety play different roles in the phytotoxic activity. Detailed results will be reported elsewhere.

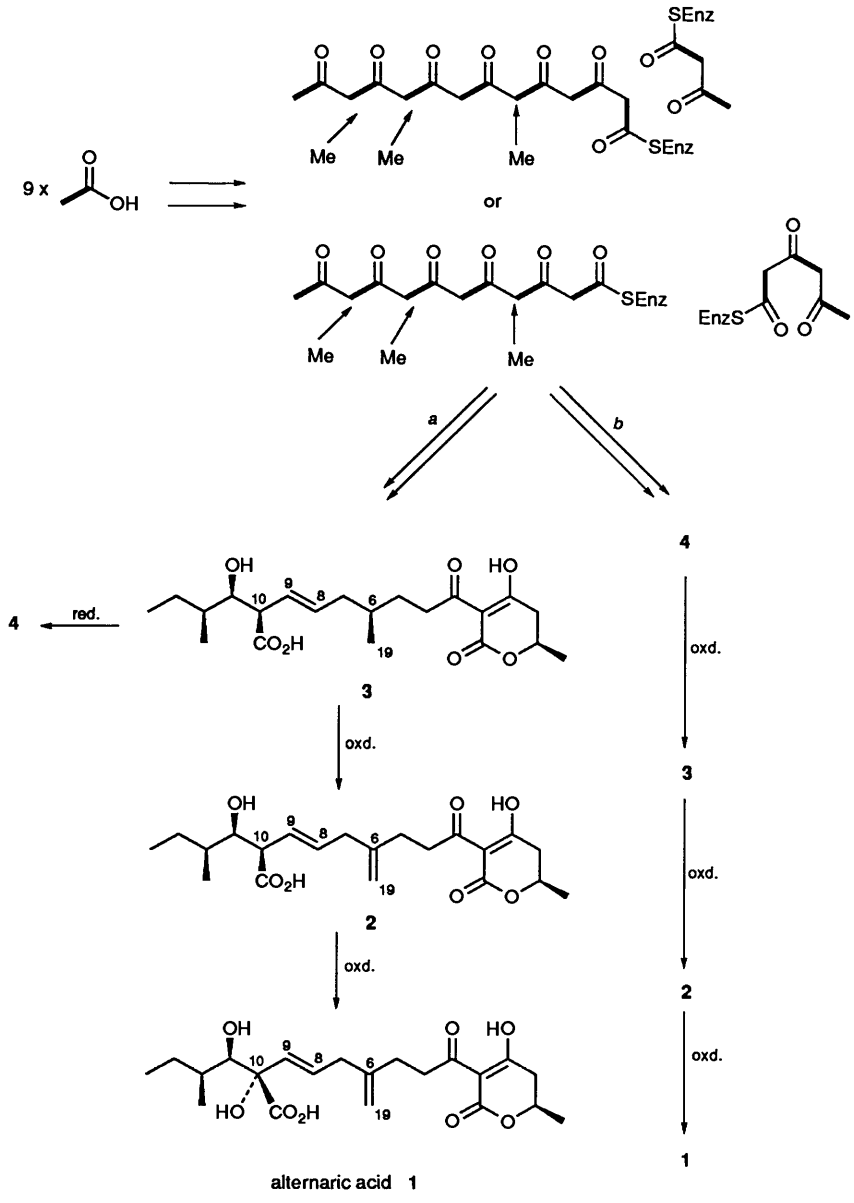
Biosynthesis.—From several feeding experiments,[13] the biosynthetic building units of alternaric acid **1** were established, and it was shown that compound **1** is biosynthesized by a condensation of two polyketide chains rather than from a single chain. However, the later aspects of the biosynthetic process have not been explored. The structure and stereochemistry of compounds **2**–**4** suggested that these compounds are precursors of alternaric acid **1**, and that compound **1** is biosynthesized *via* route *a* or *b* as shown in Scheme 4. Thus, on either route *a* or *b*, the methyl group at C-6 would be converted into an exo-methylene group as from compound **3** to compound **2**. After that, a hydroxy group is introduced at C-10, α to the carboxyl group, with retention of configuration.

Experimental

M.p.s were determined on a Yanaco Micro-melting Point Apparatus MP-30, UV spectra on a Hitachi U-3210 spectrophotometer, IR spectra on a Hitachi 285 spectrophotometer, ^1H and ^{13}C NMR spectra on Bruker AM-500 and JEOL EX-270 spectrometers for solutions of $CDCl_3$ or C_6D_6, with *J* values given in Hz, mass spectra on JEOL DX-300 and 01SG-2 spectrometers, optical rotations on a JASCO DIP-360 polarimeter, and CD spectra on a JASCO J-20A spectropolarimeter. Column chromatography used Merck Kieselgel 60 (0.04–0.063 mm). HPLC was performed with a Waters 600E system and 741 data module and a GL Science reversed-phase column (Inertsil ODS-2, 5 μm, 4.6 × 250 mm). All moisture-sensitive reactions were carried out under argon. Light petroleum refers to the fraction boiling in the range 30–70 °C. pH Values were measured by UNIV test paper (Toyo-Roshi).

Fermentation, Extraction and Isolation.—*Alternaria solani* A17 strain was cultured in Czapek Dox medium supplemented with 0.1% yeast extract, for 20 days at 25 °C in the dark. Culture filtrates (7.5 dm^3) were evaporated to 500 cm^3, acidified to pH 3–4 with 1 mol dm^{-3} hydrochloric acid, and extracted with $CHCl_3$ (500 cm^3 × 3). The extracts were washed with 5% aq. $NaHCO_3$ (300 cm^3 × 3), the combined alkaline aqueous layers were acidified, and extracted with ethyl acetate (300 cm^3 × 3). The acidic extracts were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue (1.9 g) was crystallized from benzene. After repeated recrystallizations, pure alternaric acid **1** was isolated (167 mg). On the other hand, the combined mother liquid was concentrated under reduced pressure to give an oily material. The residue (1.2 g) was chromatographed by HPLC [MeOH–0.2% aq. H_3PO_4 (8:2)] to give 10-deoxyalternaric acid **2** (167 mg), 10-deoxy-6,19-dihydroalternaric acid **3** (231 mg) and 10-deoxy-6,8,9,19-tetrahydroalternaric acid **4** (22 mg).

Compound **2**: crystals, m.p. 103–104.5 °C (from light petroleum–$CHCl_3$); $[\alpha]_D^{25}$ +15.1 (*c* 1.37 in EtOH); CD λ_{ext}/nm ($\Delta\varepsilon$) (EtOH) 216 (+11.0), 234 (0) and 266 (−3.9); λ_{max}(EtOH)/nm 213 (ε/dm^3 mol^{-1} cm^{-1} 11 000) and 274 (10 000);



Scheme 4 Plausible biosynthetic routes to alternaric acid **1**. oxd. = oxidation; red. = reduction.

ν_{max}(KBr)/cm^{-1} 3300, 2940, 1710, 1560, 1450, 1260, 1230 and 1070; δ_H(500 MHz; $CDCl_3$) see Table 1; δ_C(125 MHz; $CDCl_3$) see Table 2; *m/z* (FAB, negative, triethanolamine) 393.1899 (M^- − H. $C_{21}H_{29}O_7$ requires *m/z*, 393.1913).

Compound **3**: oil, $[\alpha]_D^{24}$ +28.6 (*c* 2.08 in EtOH); CD λ_{ext}/nm ($\Delta\varepsilon$) (EtOH) 216 (+11.9), 233 (0) and 260 (−3.9); λ_{max}(EtOH)/nm 215 (ε/dm^3 mol^{-1} cm^{-1} 9700) and 273 (11 000); ν_{max}(NaCl)/cm^{-1} 3400, 2940, 1720, 1570, 1440, 1260 and 1070; δ_H(500 MHz; $CDCl_3$) see Table 1; δ_C(125 MHz; $CDCl_3$) see Table 2; *m/z* (FAB, negative, triethanolamine) 395.2041 (M^- − H. $C_{21}H_{31}O_7$ requires *m/z*, 395.2070).

Compound **4**: oil, $[\alpha]_D^{24}$ −8.6 (*c* 1.98 in EtOH); CD λ_{ext}/nm ($\Delta\varepsilon$) (EtOH) 216 (+5.1), 227 (0), 241sh (−2.7) and 259 (−3.2); λ_{max}(EtOH)/nm 216 (ε/dm^3 mol^{-1} cm^{-1} 7000) and 274 (10 000); ν_{max}(NaCl)/cm^{-1} 3400, 2930, 1710, 1560, 1450, 1240 and 1060; δ_H(500 MHz; $CDCl_3$) see Table 1; δ_C(125 MHz; $CDCl_3$) see Table 2; *m/z* (FAB, negative, triethanolamine) 397.2260 (M^- − H. $C_{21}H_{33}O_7$ requires *m/z*, 397.2226).

Alkaline Degradation of 10-*Deoxy*-6,19-*dihydroalternaric Acid* **3**.—A solution containing compound **3** (40 mg, 0.102 mmol), 1 mol dm^{-3} aq. sodium hydroxide (0.96 cm^3) and 30% hydrogen peroxide (2 cm^3) was diluted with water (11 cm^3) and methanol (0.5 cm^3). The mixture was stirred for 4 h at room temperature. To the mixture was added further 30% hydrogen peroxide (1 cm^3). After 2 h, the solution was acidified with 2 mol dm^{-3} hydrochloric acid to pH 4 and extracted with diethyl ether (×6). The organic layers were washed with brine, dried over anhydrous $MgSO_4$, and evaporated to the residue.

To a solution of the residue in diethyl ether (0.5 cm^3) was added an excess of diazomethane in diethyl ether solution. The reaction mixture was left at 5 °C for 12 h. After concentration, the residue was subjected to silica gel flash chromatography (0.9 × 12 cm, silica gel; $CHCl_3$) to give the diester **6** (19 mg, 60%) as an oil, $[\alpha]_D^{23}$ +86.5 (*c* 1.17 in $CHCl_3$); ν_{max}(NaCl)/cm^{-1} 3500 and 1730; δ_H(270 MHz; $CDCl_3$) 5.60 (1 H, dt, *J* 15.2 and 6.9, 8-H), 5.34 (1 H, dd, *J* 15.2 and 9.2, 9-H), 3.82 (1 H, dd, *J* 9.2 and 2.3, 11-H), 3.70 (3 H, s, OMe), 3.66 (3 H, s, OMe), 3.19 (1 H, t, *J* 9.2, 10-H), 2.29 (2 H, m, 4-H_2), 2.04 (1 H, dt, *J* 14.0 and 6.7, 7-H^a), 1.90 (1 H, dt, *J* 14.0 and 6.9, 7-H^b), 1.24–1.69 (6 H, m, 5-H_2, 6-H, 12-H and 13-H_2), 0.90 (3 H, t, *J* 7.3, 14-H_3), 0.85 (3 H, d, *J* 6.3, 19-H_3) and 0.84 (3 H, d, *J* 6.6, 21-H_3); δ_C(68 MHz; $CDCl_3$) 174.5, 174.2, 133.4, 126.0, 74.4, 53.7, 51.9, 51.5, 39.6, 36.4, 32.5, 31.8, 31.3, 26.8, 19.0 and 11.8; FD-MS

m/z 315 ($M^+ + H$); EI-HRMS *m/z* 296.1995 ($M^+ - H_2O$. $C_{17}H_{28}O_4$ requires *m/z*, 296.1988).

(S)-*MTPA Ester* **7a** *of the Diester* **6**.—To a stirred solution of the diester **6** (1.5 mg, 4.8 μmol) in dry CH_2Cl_2 (0.4 cm^3) were added (*S*)-MTPA (6 mg, 25.6 μmol), 4-(dimethylamino)pyridine (DMAP) (1 mg, 8.2 μmol) and 1,3-dicyclohexylcarbodiimide (DCC) (6 mg, 27.8 μmol). After being stirred for 36 h at room temperature, the reaction mixture was filtered. The filtrate was diluted with diethyl ether and washed successively with saturated aq. $NaHCO_3$ and saturated aq. NH_4Cl, and dried over anhydrous $MgSO_4$. After concentration, the residue was subjected to HPLC [MeOH–water (9:1)] to give the MTPA ester **7a** (1.8 mg, 71%) as an oil, ν_{max}(NaCl)/cm^{-1} 1740; δ_H(500 MHz; $CDCl_3$) 7.55 (2 H, m, Ph), 7.40 (3 H, m, Ph), 5.66 (1 H, dt, *J* 15.3 and 7.3, 8-H), 5.57 (1 H, dd, *J* 10.4 and 1.7, 11-H), 5.31 (1 H, dd, *J* 15.3 and 9.7, 9-H), 3.66 (3 H, s, OMe), 3.50 (6 H, s, OMe × 2), 3.40 (1 H, t, *J* 10.0, 10-H), 2.33 (1 H, ddd, *J* 15.6, 9.4 and 6.1, 4-H^a), 2.27 (1 H, ddd, *J* 15.6, 9.2 and 6.4, 4-H^b), 2.04 (1 H, dt, *J* 13.9 and 6.7, 7-H^a), 1.91 (1 H, dt, *J* 13.9 and 7.4, 7-H^b), 1.67 (1 H, m, 12-H), 1.65 (1 H, m, 5-H^a), 1.52 (1 H, m, 6-H), 1.43 (1 H, m, 5-H^b), 1.38 (1 H, m, 13-H^a), 1.14 (1 H, m, 13-H^b), 0.92 (3 H, t, *J* 7.4, 14-H_3), 0.853 (3 H, d, *J* 6.8, 21-H_3) and 0.848 (3 H, d, *J* 6.6, 19-H_3); FD-MS *m/z* 531 ($M^+ + H$); EI-HRMS *m/z* 499.2311 ($M^+ - OCH_3$. $C_{26}H_{34}F_3O_6$ requires *m/z*, 499.2307).

(R)-*MTPA Ester* **7b** *of the Diester* **6**.—This compound was obtained in 56% yield from reaction of the diester **6** with (*R*)-MTPA by essentially the same procedure as for the preparation of diastereoisomer **7a**: δ_H(500 MHz; $CDCl_3$) 7.54 (2 H, m, Ph), 7.39 (3 H, m, Ph), 5.68 (1 H, dt, *J* 15.3 and 7.2, 8-H), 5.56 (1 H, dd, *J* 10.4 and 1.6, 11-H), 5.34 (1 H, dd, *J* 15.3 and 9.7, 9-H), 3.66 (3 H, s, OMe), 3.56 (3 H, s, OMe), 3.52 (3 H, s, OMe), 3.42 (1 H, t, *J* 10.0, 10-H), 2.33 (1 H, ddd, *J* 15.1, 9.5 and 6.2, 4-H^a), 2.27 (1 H, ddd, *J* 15.1, 9.2 and 6.5, 4-H^b), 2.05 (1 H, dt, *J* 14.0 and 6.9, 7-H^a), 1.92 (1 H, dt, *J* 14.0 and 7.1, 7-H^b), 1.64 (2 H, m, 5-H^a and 12-H), 1.53 (1 H, m, 6-H), 1.43 (1 H, m, 5-H^b), 1.27 (1 H, m, 13-H^a), 1.08 (1 H, m, 13-H^b), 0.88 (3 H, t, *J* 7.3, 14-H_3), 0.85 (3 H, d, *J* 6.7, 19-H_3) and 0.79 (3 H, d, *J* 6.8, 21-H_3); FD-MS *m/z* 531 ($M^+ + H$).

Acetonide **8** *from the Diester* **6**.—To a stirred solution of the diester **6** (15 mg, 47.5 μmol) in pyridine (1 cm^3) were added acetic anhydride (0.5 cm^3) and DMAP (6 mg, 49.2 μmol). After 6 h at room temperature, the reaction mixture was concentrated to dryness. The residue was diluted with diethyl ether and washed successively with 0.1 mol dm^{-3} hydrochloric acid, saturated aq. $NaHCO_3$ and brine, and dried over anhydrous $MgSO_4$. The organic solution was concentrated to afford crude material (18.1 mg).

To a solution of the crude product (18.1 mg) in dry tetrahydrofuran (THF) (1.8 cm^3) at 0 °C was added dropwise lithium aluminium hydride (7.8 mg). After 20 min, the reaction mixture was diluted with ethyl acetate, washed successively with 0.1 mol dm^{-3} hydrochloric acid and brine, and dried over $MgSO_4$. The organic layer was filtered and concentrated to afford an oil (18.1 mg), which was employed without further purification in the subsequent step.

To a solution of the crude triol (18.1 mg) in acetone (10.5 cm^3) were added, 2,2-dimethoxypropane (0.5 cm^3) and camphor-10-sulfonic acid (CSA) (catalytic amount). After 20 min at room temperature, the solution was quenched with saturated aq. $NaHCO_3$, and diluted with diethyl ether. After separation, the aqueous layer was extracted with diethyl ether (× 3). The combined organic phases were washed successively with 1 mol dm^{-3} hydrochloric acid and water, and dried over $MgSO_4$, filtered, and concentrated. Flash chromatography [0.9 × 12 cm, silica gel; hexane–diethyl ether (7:3)] of the residue gave acetonide **8** (8.3 mg, 58% for three steps) as an oil, $[\alpha]_D^{25}$ +41.4 (*c* 0.86 in $CHCl_3$); ν_{max}(NaCl)/cm^{-1} 3370; δ_H(270 MHz; C_6D_6) 5.07 (1 H, dt, *J* 15.2 and 7.3, 8-H), 4.61 (1 H, dd, *J* 15.2 and 9.0, 9-H), 3.48 (1 H, dd, *J* 11.6 and 5.3, 20-H^{eq}), 3.33 (1 H, dd, *J* 10.2 and 1.8, 11-H), 3.32 (1 H, dd, *J* 11.6 and 10.9, 20-H^{ax}), 3.06 (2 H, t, *J* 6.4, 3-H_2), 2.22 (1 H, m, 10-H), 1.62 (1 H, dt, *J* 13.9 and 6.6, 7-H^a), 1.45 (1 H, dt, *J* 13.9 and 6.9, 7-H^b), 0.92–1.33 (8 H, m, 4-, 5- and 13-H_2 and 6- and 12-H), 1.25 and 1.07 (each 3 H, each s, acetonide Me_2), 0.76 (3 H, d, *J* 6.3, 21-H_3), 0.66 (3 H, t, *J* 7.3, 14-H_3) and 0.50 (3 H, d, *J* 6.3, 19-H_3); δ_C(68 MHz; $CDCl_3$) 132.5, 127.8, 97.8, 74.2, 64.9, 63.3, 41.1, 40.1, 35.9, 32.8, 32.3, 30.2, 29.7, 26.4, 19.4, 19.0, 12.5 and 12.0; FD-MS *m/z* 299 ($M^+ + H$); EI-HRMS *m/z* 283.2271 ($M^+ - CH_3$. $C_{17}H_{31}O_3$ requires *m/z*, 283.2273).

(S)-*MTPA Esters* **9a** *and* **10a** *of Oxidation Products of the Diester* **6**.—To a solution of diester **6** (12 mg, 38.2 μmol) in THF (0.2 cm^3)–water (0.2 cm^3) were added 0.16 mol dm^{-3} aq. osmium tetraoxide (15 mm^3, 2.4 μmol) and sodium metaperiodate (18 mg, 84.1 μmol). The mixture was stirred for 5 h at room temperature. Sodium borohydride (9 mg) was added, and the mixture was stirred for another 1 h. The solution was diluted with water and acidified to pH 2 with 2 mol dm^{-3} hydrochloric acid. The solution was extracted with diethyl ether (× 3), and the combined organic layers were dried over anhydrous $MgSO_4$, filtered and evaporated to ~0.2 cm^3. The solution was treated with excess of diazomethane in diethyl ether for 2 days before being evaporated to ~0.2 cm^3. Without purification, the solution of the crude products was employed in the next step.

To the solution of the crude products were added pyridine (0.2 cm^3) and (*S*)-MTPACl (45.2 mg, 19.3 μmol). After being stirred for 3 h at room temperature, the mixture was evaporated to dryness, the residue was diluted with water, and the aqueous solution was extracted with diethyl ether (× 3). The combined organic solutions were washed successively with 0.1 mol dm^{-3} hydrochloric acid, saturated aq. $NaHCO_3$ and brine. The organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated. Flash chromatography [0.9 × 20 cm, silica gel; hexane–diethyl ether (9:1)] of the residue gave (*S*)-MTPA esters **9a** (1.0 mg, 9% for three steps) and **10a** (2.9 mg, 20% for three steps). *Compound* **9a** was an oil, ν_{max}(NaCl)/cm^{-1} 1750; δ_H(500 MHz; $CDCl_3$) 7.52 (2 H, m, Ph), 7.40 (3 H, m, Ph), 4.24 (1 H, dd, *J* 10.7 and 5.7, 11-H^a), 4.09 (1 H, dd, *J* 10.7 and 6.7, 11-H^b), 3.56 (3 H, s, OMe), 3.55 (3 H, s, Ome), 1.77 (1 H, m, 12-H), 1.41 (1 H, m, 13-H^a), 1.20 (1 H, m, 13-H^b), 0.91 (3 H, d, *J* 6.7, 21-H_3) and 0.90 (3 H, d, *J* 7.5, 14-H_3); EI-HRMS *m/z* 304.1299 (M^+. $C_{15}H_{19}F_3O_3$ requires M, 304.1286).

Compound **10a** was an oil, ν_{max}(NaCl)/cm^{-1} 1740; δ_H(500 MHz; $CDCl_3$) 7.51 (2 H, m, Ph), 7.41 (3 H, m, Ph), 4.36 (2 H, m, 8-H_2), 3.65 (3 H, s, OMe), 2.34–2.25 (2 H, m, 4-H_2), 1.75–1.45 (5 H, m, 5- and 7-H_2 and 6-H) and 0.90 (3 H, d, *J* 5.8, 19-H_3); FD-MS *m/z* 376 (M^+); EI-HRMS *m/z* 357.1522 ($M^+ - F$. $C_{18}H_{23}F_2O_5$ requires *m/z*, 357.1514).

Compound (S)-(+)-**15**.—To a solution of (*S*)-(+)-*tert*-butyl 5-hydroxy-3-oxohexanoate[16] (75 mg, 371 μmol) in methanol (3 cm^3) was added 1 mol dm^{-3} aq. sodium hydroxide (3 cm^3). The reaction mixture was stirred for 6.5 h at 70 °C, neutralized with 1 mol dm^{-3} hydrochloric acid, and evaporated under reduced pressure. The remaining water layer was extracted with ethyl acetate (× 3). The combined organic layers were washed with water, dried over Na_2SO_4, filtered, and concentrated to give keto lactone (*S*)-(+)-**14** (26 mg, 55%).

To a solution of lactone (*S*)-(+)-**14** (11 mg, 85.9 μmol) in dry CH_2Cl_2 (0.5 cm^3) were added acetic acid (6 mg, 100 μmol), DMAP (1 mg, 8.2 μmol) and DCC (17.7 mg, 85.9 μmol).[9,15] The

mixture was stirred for 48 h at room temperature, filtered, and evaporated to give a residue. The residue was subjected to preparative TLC [silica gel; $CHCl_3$–MeOH (95:5)] to give *compound* (*S*)-(+)-**15** (6.8 mg, 47%) as a powder, $[\alpha]_D^{23}$ +55.3 (*c* 0.55 in EtOH); ν_{max}(KBr)/cm^{-1} 3400 and 1710; δ_H(270 MHz; $CDCl_3$) 18.97 (1 H, s, OH), 4.53 (1 H, m, 5-H), 2.68 (1 H, dd, *J* 17.2 and 9.0, 4-H^{ax}), 2.65 (1 H, dd, *J* 17.2 and 4.0, 4-H^{eq}), 2.63 (3 H, s, 2-Ac) and 1.47 (3 H, d, *J* 6.6, 6-H_3); δ_C(68 MHz; $CDCl_3$) 200.7, 194.8, 163.9, 102.9, 69.9, 38.8, 26.0 and 20.1; EI-HRMS *m/z* 170.0580 (M^+. $C_8H_{10}O_4$ requires M, 170.0579).

(R)-(−)-**15**.—This compound was obtained in 40% yield from keto lactone (*R*)-(−)-**14** by essentially the same procedure as for the preparation of the enantioisomer (*S*)-(+)-**15**: $[\alpha]_D^{23}$ −49.4 (*c* 0.68 in EtOH).

Alkaline Degradation of 10-*Deoxyalternaric Acid* **2**.—By means of the reaction used in the conversion of 10-deoxy-6,19-dihydroalternaric acid **3** into the diester **6**, 10-deoxyalternaric acid **2** was converted into the diester **16** in 89% yield (for two steps) as an oil, $[\alpha]_D^{22}$ +85.8 (*c* 1.14 in $CHCl_3$); ν_{max}(NaCl)/cm^{-1} 3500 and 1740; δ_H(270 MHz; $CDCl_3$) 5.64 (1 H, dt, *J* 15.5 and 7.3, 8-H), 5.39 (1 H, dd, *J* 15.5 and 9.2, 9-H), 4.76 (2 H, br s, 19-H_2), 3.85 (1 H, ddd, *J* 9.2, 5.9 and 2.3, 11-H), 3.71 (3 H, s, OMe), 3.67 (3 H, s, OMe), 3.23 (1 H, t, *J* 9.2, 10-H), 2.75 (2 H, d, *J* 7.3, 7-H_2), 2.46 (2 H, t, *J* 7.3, 4-H_2), 2.31 (2 H, t, *J* 7.3, 5-H_2), 2.23 (1 H, d, *J* 5.9, 11-OH), 1.25–1.48 (3 H, m, 12-H and 13-H_2), 0.91 (3 H, t, *J* 7.3, 14-H_3) and 0.85 (3 H, d, *J* 6.6, 21-H_3); δ_C(68 MHz; $CDCl_3$) 175.9, 174.3, 146.1, 132.5, 126.5, 110.7, 74.4, 53.6, 52.0, 51.6, 39.5, 36.5, 32.3, 30.8, 26.8 and 11.8; EI-HRMS *m/z* 313.1998 (M^+ + H. $C_{17}H_{29}O_5$ requires *m/z*, 313.2015).

Acetonide **17** *from the Diester* **16**.—By use of the reaction used to convert the diester **6** into the acetonide **8**, the diester **16** was converted into acetonide **17** in 75% yield (for three steps) as an oil; $[\alpha]_D^{25}$ +20.6 (*c* 0.69 in $CHCl_3$); ν_{max}(NaCl)/cm^{-1} 3400; δ_H(270 MHz; $CDCl_3$) 5.55 (1 H, dt, *J* 15.8 and 6.6, 8-H), 5.12 (1 H, dd, *J* 15.5 and 9.2, 9-H), 4.78 (1 H, br s, 19-H^a), 4.74 (1 H, br s, 19-H^b), 3.70–3.66 (4 H, m, 3- and 20-H_2), 3.62 (1 H, dd, *J* 10.6 and 2.3, 11-H), 2.72 (2 H, d, *J* 6.6, 7-H_2), 2.46 (1 H, m, 10-H), 2.08 (2 H, d, *J* 6.3, 5-H_2), 1.70 (2 H, m, 4-H_2), 1.20–1.55 (3 H, m, 12-H and 13-H_2), 1.42 and 1.36 (each 3 H, each s, acetonide Me_2), 0.86 (3 H, t, *J* 7.3, 14-H_3), 0.85 (3 H, d, *J* 6.6, 21-H_3); δ_C(68 MHz; $CDCl_3$) 147.9, 131.4, 128.5, 110.3, 97.9, 74.2, 64.7, 62.7, 41.0, 39.6, 35.9, 32.2, 30.5, 29.6, 26.4, 19.0, 12.5 and 12.0; FD-MS *m/z* 297 (M^+ + H); EI-HRMS *m/z* 281.2081 (M^+ − CH_3. $C_{17}H_{29}O_3$ requires *m/z*, 281.2116).

(S)-*MTPA Ester* **18a** *from the Acetonide* **8**.—To a solution of the acetonide **8** (8 mg, 26.8 μmol) in THF (0.3 cm^3)–water (0.3 cm^3) were added 0.16 mol dm^{-3} aq. osmium tetraoxide (15 mm^3, 2.4 μmol) and sodium metaperiodate (18 mg, 84.1 μmol). The mixture was stirred for 23 h at room temperature. Sodium borohydride (9 mg) was added, and the mixture was stirred for a further 1 h. The solution was diluted with water, and extracted with diethyl ether (×3). The combined organic layers were washed with water, dried over $MgSO_4$, filtered, and evaporated to give a residue (5.2 mg). To a solution of the crude product (3.0 mg) in dry CH_2Cl_2 (0.4 cm^3) were added (*S*)-MTPA (28 mg, 120 μmol), DMAP (4 mg, 32.8 μmol) and DCC (15 mg, 72.8 μmol). The reaction mixture was stirred for 17 h at room temperature. The mixture was filtered and evaporated to give a residue. Flash chromatography [0.9 × 12 cm, silica gel; hexane–diethyl ether (9:1)] of the residue gave (*S*)-MTPA ester **18a** (2.3 mg, 45% for two steps) as an oil, ν_{max}(NaCl)/cm^{-1} 1750; δ_H(500 MHz; $CDCl_3$) 7.48 (2 H, m, Ph), 7.41 (3 H, m, Ph), 4.26 (1 H, dd, *J* 11.5 and 3.8, 9-H^a), 4.13 (1 H, dd, *J* 11.5 and 6.7, 9-H^b), 3.76 (1 H, dd, *J* 11.5 and 5.0, 20-H^{eq}), 3.672 (1 H, dd, *J* 10.0 and 2.3, 11-H), 3.668 (1 H, dd, *J* 11.5 and 9.6, 20-H^{ax}), 3.53 (3 H, s, OMe), 2.15 (1 H, m, 10-H), 1.55 and 1.33 (each 3 H, each s, acetonide Me_2), 1.44 (1 H, m, 12-H), 1.37 (1 H, m, 13-H^a), 1.27 (1 H, m, 13-H^b), 0.87 (3 H, d, *J* 6.7, 21-H_3) and 0.84 (3 H, t, *J* 7.4, 14-H_3); FD-MS *m/z* 419 (M^+ + H); EI-HRMS *m/z* 403.1729 (M^+ − CH_3. $C_{20}H_{26}F_3O_5$ requires *m/z*, 403.1733).

(S)-*MTPA Ester* **18a** *from the Acetonide* **17**.—In a similar manner, the acetonide **17** was converted into (*S*)-MTPA ester **18a** in 23% yield (for two steps).

Hydrogenation of 10-*Deoxy*-6,19-*dihydroalternaric Acid* **3**.—A solution of compound **3** (6.3 mg) and 10% palladium–carbon (10 mg) in EtOH (2.5 cm^3) was stirred vigorously under hydrogen. The solution was stirred for 2.5 h at room temperature, then filtered through Celite, and concentrated to afford a crude product. The product was chromatographed by HPLC [MeOH–0.2% H_3PO_4 (8:2)] to give compound **4** (1.2 mg, 19%), $[\alpha]_D^{24}$ −9.1 (*c* 0.11 in EtOH).

Bioassay (*Growth Inhibition of Tomato Seedlings*).—A methanolic solution (1 cm^3) containing a sample of a compound **1**–**3** (0.075 mg) was poured onto a sheet of filter paper (7 cm diameter, Toyo-Roshi No. 2) in a petri dish (9 cm diameter). A methanolic solution (1 cm^3) containing no sample was used as a control. After removal of the solvent, a solution of Tween-80 in deionized water (100 ppm; 3 cm^3) was added to the dish to make a 25 ppm solution of the sample. Tomato (Hikari) seedlings of uniform shape and size were placed on the filter paper and grown in the dark at 25 °C for 3 days.

Acknowledgements

We are grateful to Mr. K. Watanabe and Mrs. E. Fukushi in our department for the mass spectra, and to Dr. Tsuge, Dept. of Agriculture, Nagoya University for the A17 strain of *A. solani*.

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Paper 3/03096B
Received 1*st June* 1993
Accepted 1*st September* 1993