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STRUCTURE ELUCIDATION OF RESTRICTICIN, A NOVEL ANTIFUNGAL AGENT FROM *PENICILLIUM RESTRICTUM*

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The structures and absolute stereochemistry of restricticin (1), its N,N-dimethyl derivative (4) and desglycyl hydrolysis product (3), isolated from *Penicillium restrictum*, have been determined on the basis of spectroscopic evidence. They belong to a new class of broad spectrum antifungal agents representing substituted tetrahydropyranyl ethers incorporating triene and glycyl ester side chains.

In our search for novel antifungal agents, we isolated restricticin (1), a minor component (4) and a desglycyl hydrolysis product (3), from both solid and liquid fermentations of *Penicillium restrictum*. We report here mainly on the structure determination and absolute stereochemistry of these three components on the basis of spectroscopic methods. The fermentation, isolation and biological properties of this new class of antifungal agents will be reported elsewhere[1].

Results and Discussion

Structure Determination. HR-EIMS of the major component **1**, gave an empirical formula of $C_{19}H_{31}NO_4$ (calc. *m/z* 337.2253, found *m/z* 337.2251) which formed a mono-TMS derivative on silylation and a monoacetate derivative **2**, $C_{21}H_{33}NO_5$ (calc. *m/z* 379.2357, found *m/z* 379.2342) on acetylation. The molecular formula was supported by ^{13}C NMR analysis of ^{1}H decoupled, APT and HETCOR data in CD_2Cl_2, which indicated 19 carbons comprising the following carbon types: 3 x CH_3, 1 x CH_3O, 2 x CH_2, 1 x CH, 2 x CH_2X, 3 x CHX, 5 x CH=, 1 x C=, 1 COX (see Table 1) implicating 29 carbon bound protons. The UV spectrum in methanol indicated maxima at

286(28,682), 275(36,453) and 265(28,007) nm, typical of a triene chromophore and the IR showed a strong absorbance at 1747 cm^{-1}, suggesting the presence of an ester or lactone functionality. The IR of the monoacetate **2** had an additional carbonyl band at 1661 cm^{-1}, suggesting that **2** is an acetamide and, therefore, **1** is an amine. During the isolation, or subsequently on base hydrolysis, **1** degraded to a substance **3**, designated restrictinol[2], having the empirical formula $C_{17}H_{28}O_3$ by EI-MS (calc. *m/z* 280.2038, found *m/z* 280.2030), confirmed by ^{13}C NMR analysis (see Table 1)

Table 1. ^{13}C NMR Assignments for **1**, **3** and **4** in CD_2Cl_2[a]

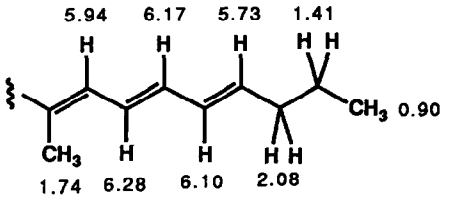
Assignment	1		4	3
C1'	85.7	(d, 143)	85.7 (d)	87.0 (d)
C2'	69.5	(d, 150)	69.1 (d)	68.1 (d)
C3'	81.9	(d, 147)	81.9 (d)	84.7 (d)
C4'	32.8	(d, 125)	32.8 (d)	32.2 (d)
C5'	71.1	(t, 146)	71.1 (t)	71.3 (t)
C1	11.9	(q, 131)	11.9 (q)	12.4 (q)
C2	133.7	(s, -)	133.6 (s)	134.6 (s)
C3	129.6	(d, 142)	129.8 (d)	129.5 (d)
C4	126.2	(d, 153)	126.3 (d)	126.3 (d)
C5	134.3	(d, 150)	134.3 (d)	134.3 (d)
C6	131.0	(d, 148)	131.0 (d)	131.0 (d)
C7	136.1	(d, 150)	135.9 (d)	135.9 (d)
C8	35.3	(t, 126)	35.2 (t)	35.3 (t)
C9	22.8	(t, 127)	22.8 (t)	22.8 (t)
C10	13.8	(q, 125)	13.8 (q)	13.8 (q)
C11	10.9	(q, 121)	10.9 (q)	11.0 (q)
C12	56.5	(q, 141)	56.5 (q)	56.2 (q)
C13	173.9	(s, -)	169.9 (s)	
C14	44.3	(t, 137)	45.0 (t)	
$-N(CH_3)_2$	-		60.6 (q) 60.6 (q)	

[a]Chemical shifts are given in ppm relative to the solvent peak at 53.8 ppm as internal standard. Multiplicities and $^1J_{CH}$ (in Hz) are given in parentheses and are abbreviated as follows: s=singlet, d=doublet, t=triplet, q=quartet.

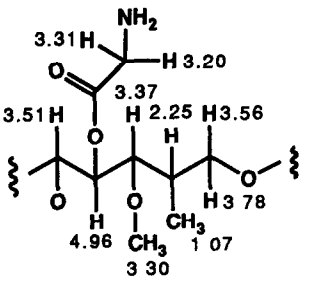
and no longer retaining the ester band at 1747 cm^{-1} in the IR. The ^{13}C NMR signals at 44.3 (triplet) and 173.9 (singlet) ppm were no longer present, suggesting the facile hydrolysis of a glycyl ester moiety in **1**.

On the basis of $^1J_{CH}$ values of **I** (Table 1), analysis of the 'gated' coupled spectrum allowed assignment of the CH-X resonances at 69.5, 81.9 and 85.7 ppm as

CH-O methines ($^1J_{CH}$ = 150, 147 and 143 Hz respectively) the CH_2-X resonance at 44.3 ppm ($^1J_{CH}$ = 137 Hz) as CH_2-N and the CH_2-X resonance at 71.1 ppm ($^1J_{CH}$ =146 Hz) as CH_2-O methylenes. Analysis of the other carbon and proton data including COSY, long-range COSY, ^1H-^1H double resonance and HETCOR experiments, led to the assignment of the partial structures X and Y.

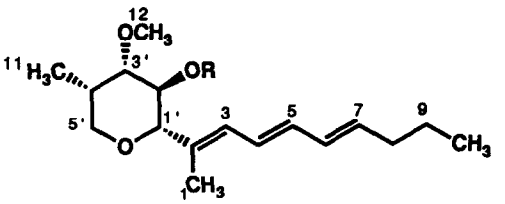


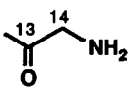
X

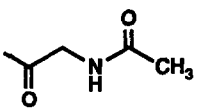


Y

Of particular interest was the proton doublet of doublets at δ4.96 in Y, which was vicinally coupled to the methine protons at δ3.37 and δ3.51. The diagnostic downfield shift of approximately 1 ppm for the δ4.96 resonance by comparison with that in 3



1 R = 

2 R = 

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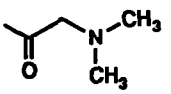
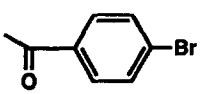
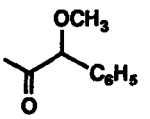
4 R = 

5 R = 

6 R = 

(see Table 2), allowed its assignment as the glycyl ester CH-O proton. The magnitude of the vicinal coupling constants in Y and the presence of only 4 oxygens in 1, of which two were assigned to the ester and one to the methoxyl groups, suggested the

presence of a tetrahydropyranyl ether in a chair conformation (see **1**). The MeO group could thus be located at C3' and the triene side at C1'.

Table 2. ^{1}H NMR Assignments of Restricticin (**1**) and Restrictinol (**3**) in CD_2Cl_2[a]

Proton	(1)		(3)	
Assignment	δ_{ppm}	mult (*J* in Hz)	δ_{ppm}	mult (*J* in Hz)
1'-H	3.51	d(9.6)	3.43	d(9.3)
2'-H	4.96	dd(9.6, 9.6)	3.56	dd(9.2, 9.2)
14-CH_2N	3.20	d(18.0)	-	
	3.31	d(18.0)	-	
3'-H	3.37	dd(5.2, 9.5)	3.22	dd(5.3, 9.0)
3'-OCH_3	3.30	s	3.37	s
4'-H	2.25	m	2.19	m
4'-CH_3	1.07	d(7.2)	1.01	d(7.1)
5'-H_a[b]	3.56	dd(2.4, 11.8)	3.58	dd(2.6, 11.7)
5'-H_e[b]	3.78	dd(1.8, 11.7)	3.77	dd(1.6, 11.7)
2-CH_3	1.74	d(1.2)	1.79	d(1.4)
3-H	5.94	dd(1.4, 10.8)	6.09	dd(1.3, ~10.6)
4-H	6.28	dd(10.8, 14.3)	6.36	dd(11.0, 14.5)
5-H	6.17	dd(10.3, 14.2)	6.22	dd(10.5, 14.5)
6-H	6.10	ddt(10.3, 14.6, 1.5)	6.12	ddt(10.4, 14.8, 1.4)
7-H	5.73	dt(14.5, 7.0)	5.74	dt(14.6, 7.2)
8-H_2	2.08	ddt(~1, 7.5, 7.5)	2.08	ddt(1.4, 7.2, 7.2)
9-H_2	1.41	tq(7.3, 7.3)	1.42	tq(7.3, 7.3)
10-H_3	0.90	t(7.3)	0.90	t(7.3)

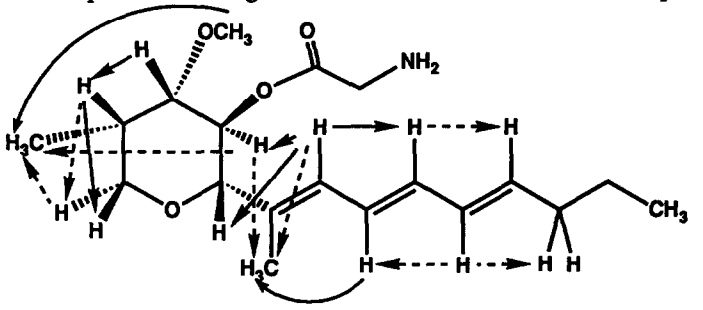
[a]Chemical shifts are given in δ_{ppm} relative to the solvent peak at δ5.32 as internal standard. J_{HH} values (in Hz) are given in parentheses and multiplicities are abbreviated as in Table 1.
[b]Subscripts a and e refer to the axial and equatorial protons at C5' respectively.

Corroboration of the structure was obtained from EI-MS where cleavage between the C1'-C2' and C5'-O bonds in **3** is observed giving rise to two major ions at *m/z* 165 [$^+$HO-CHC(CH_3)=(CH)$_5$$C_3H_7$] and *m/z* 115 [$CH_2$=C($CH_3$)CH($OCH_3$)CH=OH$^+$] which comprised the total structure (M^+ 280). The corresponding fragments in **1** were observed at *m/z* 165 and 97 after losing glycine (M^+ - $C_2H_5NO_2$ = *m/z* 262) from the molecular ion.

HR-EIMS of the minor component (**4**), gave the empirical formula $C_{21}H_{35}NO_4$ (calc. *m/z* 365.2566, found *m/z* 365.2558) which did not silylate. ^{1}H and ^{13}C NMR

comparison with **1** indicated an additional 6-proton singlet at δ2.22 and a methyl carbon resonance at 60.6 ppm respectively (see Table 1). The intense ion at *m/z* 58 [CH_2=NMe_2] and prominent peak at *m/z* 262 [$C_{17}H_{26}O_2$; calc. *m/z* 262.1933, found *m/z* 262.1917] resulting from loss of the ester in the EI-MS, readily confirmed the proposed structure **4**, incorporating a N,N-dimethylglycyl ester group.

Relative stereochemistry. Analysis of the proton vicinal couplings of both components **1** and **4** and restrictinol (**3**) (Table 2) allowed assignment of the relative stereochemistry and chair conformation of the pyranose ring. Equatorial substituents are indicated at C1', C2' and C3' whereas the methyl group at C4' is axial. The ***E,E*** geometry of the disubstituted diene portion of the triene chromophore also follows readily from coupling constant considerations, but the ***E*** or ***Z*** assignment for the methyl substituted olefin was not unequivocal. Verification of the ***E*** olefin configuration and the relative stereochemistry was obtained via a pure absorptive mode 2D NOE experiment using a mix time of 0.5 sec and an equilibration delay of 3



⟶ = Strong NOESY Correlation
⇢ = Medium NOESY Correlation

sec. The NOE experiment also allowed unequivocal assignment of the axial and equatorial protons at C5' which could not be assigned based solely on the small vicinal couplings with H4' (see Table 2).

Absolute stereochemistry: The chiral exciton coupling method predicts that when two chromophores exhibiting strong $\pi \rightarrow \pi^*$ interactions are located in chiral positions with respect to each other, the orientation between the two chromophores will determine the sign of the first CD band of the CD spectrum[3]. Since restricticin contains only one chromophore, a second chromophore was introduced into **3** in the form of the *p*-bromobenzoate ester of the alcohol at C2', in order to apply this method. Although this compound is easily prepared, it is unstable to both concentration and heat, and extreme care was taken to obtain the pure compound and maintain its integrity during spectral analysis.

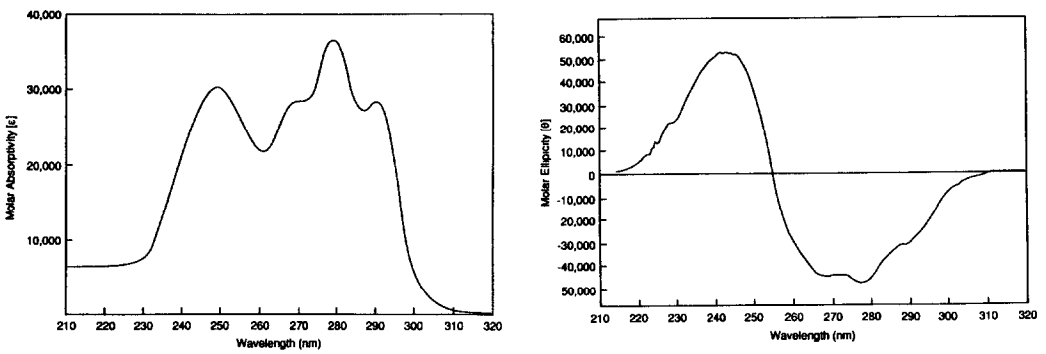
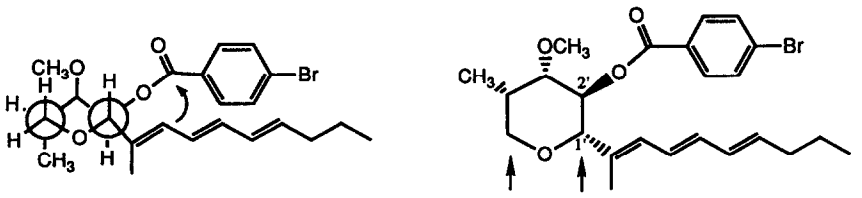


Figure 1. UV (left) and CD (right) traces of restrictinol *p*-bromo benzoate derivative (**5**) in dioxane.

The CD spectrum showed a negative first CD band centered at 278 nm and a positive second CD band at 244 nm as seen in Figure 1. This indicated a counterclockwise orientation between the two chromophores corresponding to a C1'(*S*) and C2'(*R*) stereochemistry. Knowing the relative stereochemistry of all centers C1'-C4', the absolute stereochemistry at the two centers C1' and C2' defines the absolute stereochemistry of the molecule as depicted in **5**.



5

A second independent analysis of the absolute configuration of restrictinol was undertaken using Trost's O-methylmandelate ester methodology[4]. The (*R*)- and (*S*)-O-methylmandelate esters of restrictinol were each prepared and the differential aryl anisotropy effect was observed upon analysis of their ^{1}H-NMR spectra. The phenyl shielding effects (see Table 3) were especially noticeable for the 3'-H (δ_R - δ_S = 0.16

Table 3. ^1H NMR Assignments of Restrictinol O-Methylmandelate Derivatives in $CDCl_3$[a]

Position	(*S*) - Mandelate (**6b**)	(*R*) - Mandelate (**6a**)
1'-H	3.54	3.52
2'-H	5.08	5.10
3'-H	3.24	3.40
4'-H	2.22	2.22
5'-H_a	3.56	3.56
5'-H_e	3.80	3.80
3'-OCH_3	2.96	3.19
2-CH_3	1.79	1.72

[a] Chemical shifts (300 MHz) are given in δ_{ppm} relative to solvent peak at δ7.24 as internal standard.

ppm), the 3'-OMe ($\delta_R - \delta_S$ = 0.23 ppm), and the 2-Me ($\delta_R - \delta_S$ = -0.07 ppm) resonances. These results are consistent with the accepted mandelate conformation if restrictinol possesses the *R* absolute stereochemistry for the C2' secondary alcohol.

Biological activity. Restricticin (**1**) was observed to display broad activity against both yeast and filamentous fungi and is fungistatic against *Candida albicans* and *Aspergillus* species[1]. Minimum inhibitory concentrations (MIC's in μg/ml), recorded on Kimmig agar at the lowest concentration of **1** showing no growth, were in the range 0.5-8.0 for 6 out of 14 *Candida* species/strains, 2.0 and 4.0 for 2 *Cryptococcus neoformans* strains and 2.0 for *Penicillium italicum*. Comparison of the activities of restricticin with its N,N-dimethyl derivative (**4**) and restrictinol (**3**), suggested that the glycine ester moiety was essential for activity. Restrictinol had no activity whereas the activity of **4** was severely reduced.

The novel structures reported here represent a new structural class of broad spectrum antifungal agents. They appear to be derived via mixed polyketide and amino acid biosynthetic pathways.

Experimental Section

The IR absorption spectra were obtained with a Perkin-Elmer model 1750 Infrared Fourier Transform Spectrophotometer using a multiple internal reflectance cell (MIR, ZnSe) on neat 10-20 μg samples. The UV absorption spectra were measured with a Beckman DU-70 Spectrophotometer. The CD spectra were obtained on an AVIV

Model 62DS circular dichroism spectrometer. Electron impact mass spectra (EI-MS) were obtained on a Finnigan MAT-212 mass spectrometer.

NMR spectra were recorded on Varian XL-400 or XL-300 NMR spectrometers. ^1H NMR spectra were recorded at 400 MHz (for **1**) and 300 MHz (for **2-6**) in CD_2Cl_2 and $CDCl_3$ at ambient temperature using the solvent peaks at δ5.32 and δ7.24 respectively, as internal references downfield of tetramethylsilane (TMS) at zero ppm. ^{13}C NMR spectra were recorded at 100 MHz (for **1**) and 75 MHz (for **2-5**) in CD_2Cl_2 at ambient temperature where chemical shifts are given in ppm downfield of TMS using the solvent peak at 53.8 ppm as internal reference. Proton-proton chemical shift correlation spectra (COSY) were recorded in CD_2Cl_2 using the standard pulse sequence[5]. Proton-carbon chemical shift correlation spectra (HETCOR) were recorded in CD_2Cl_2 (20 mg/0.5 ml) using the standard pulse sequence[6]. The 512 x 2K data set was accumulated in 128 increments with 576 transients for each value of t_1. The delay time between transients was 1 sec and the experiment was optimized for $^1J_{CH}$=140 Hz.

Prior to NOE experiments, a dilute solution of **1** (2 mg/0.5 ml) in CD_2Cl_2 was degassed using three freeze-thaw cycles under vacuum. The pure absorptive mode 2D NOE experiments were obtained by the Hypercomplex method of States, Haberkorn and Ruben[7]. 1K x 1K data sets were accumulated in 64 increments with 160 transients for each value of t_1. The mix time was 0.5 sec and the delay time between scans was 3 sec.

Restricticin (1). UV (MeOH) λ_{max} (ε) 286(28,682), 275(36,453), 265(28,007) nm; IR ν_{max} 1747 cm^{-1}; MS *m/z* (rel int) 337 (M^+, 7), 305 (4), 262 (100), 247 (12), 233 (14), 231 (48), 220 (30), 219 (18), 177 (25), 121 (20), 115 (48), 85 (46), 71 (46), 55 (88); ^1H NMR (400 MHz, CD_2Cl_2) see Table 2; ^{13}C NMR (100 MHz, CD_2Cl_2) see Table 1.

N-Acetyl restricticin (2). Restricticin (5.2 mg) was taken up in anhydrous pyridine (1 mL) and 10 drops of acetic anhydride added. The reaction was maintained at room temperature for 10 min and taken to dryness *in vacuo*. The concentrate was partitioned between CH_2Cl_2 (1 mL) and H_2O (1 mL) and the CH_2Cl_2 layer concentrated and chromatographed on silica gel (5 mL) using EtOAc/hexanes 75:25 followed by 100% EtOAc. The acetamide eluted with 100% EtOAc and was concentrated to yield **2** (1.7 mg). IR ν_{max} 1752, 1661 cm^{-1}; MS *m/z* (rel int) 379 (M^+, 2), 262 (46), 231 (26), 220 (18), 219 (10), 177 (16), 161 (20), 154 (16), 121 (16), 115 (34), 100 (68), 95 (36), 85 (58), 83 (36), 72 (100), 71 (48), 55 (76); ^1H NMR (400 MHz, CD_2Cl_2) δ 0.90 (3*H*, t, *J* = 7.3 Hz, 10-H_3), 1.06 (d, *J* = 7.1 Hz, 4'-CH_3), 1.41 (tq, *J* = ~7.3 Hz, 9-H_2), 1.75 (d, *J* = 1.2 Hz, 2-CH_3), 1.93 (s, OAc), 2.08 (br dt, *J* = 7.2, 7.2 Hz, 8-H_2), 2.27 (m, 4'-H), 3.31 (s, 3'-OCH_3), 3.39 (dd, *J* = 5.3, 9.5 Hz, 3'-H), 3.53 (d, *J* = 9.6 Hz, 1'-H), 3.57 (dd, *J* = 2.2, 11.8 Hz, 5'-H_a), 3.75 (1H, dd, *J* = 4.5, 18.3 Hz, 14-CH_2N), 3.79 (dd, *J* = 1.7, 11.7 Hz, 5'-H_e), 4.03 (1H, dd, *J* = 5.6, 18.3 Hz, 14-CH_2N), 4.97 (t, *J* = 9.6 Hz, 2'-H), 5.74 (dt, *J* = 14.5, 7.2 Hz, 7-H), 5.85 (br.s, N-H), 5.95 (dd, *J* = 1.3,10.6 Hz, 3-H), 6.10 (ddt, J = 10.1, 14.6, 1.3 Hz, 6-H), 6.20 (dd, *J* = 10.2, 14.3 Hz, 5-H), 6.27 (dd, *J* = 10.7, 14.4 Hz, 4-H).

Restrictinol (3). UV (MeOH) λ_{max} (ε) 284(35,854), 275(45,658), 265(35,294), 201(7,983) nm; IR ν_{max} 3420 cm^{-1}; MS *m/z* (rel int) 280 (M^+, 34), 262 (6), 231 (4), 177 (8), 165 (38), 144 (24), 115 (92), 107 (26), 95 (26), 93 (36), 85 (40), 83 (62), 81

(33), 79 (38), 71 (44), 67 (32), 57 (26), 55 (100); ^{1}H NMR (300 MHz, CD_2Cl_2) see Table 2; ^{13}C NMR (75 MHz, CD_2Cl_2) see Table 1.

N,N-Dimethyl restricticin (4). UV (MeOH) λ_{max} (ε) 287(39,890), 275(50,842), 266(38,938), 205(12,124) nm; IR ν_{max} 1750 cm^{-1}; MS *m/z* (rel int) 365 (M^+, 6), 262 (29), 231 (12), 220 (8), 104 (10), 102 (10), 85 (24), 71 (36), 58 (100); ^{1}H NMR (300 MHz, CD_2Cl_2) δ 0.90 (t, *J* = 7.3 Hz, 10-H_3), 1.07 (d, *J* = 7.1 Hz, 4'-CH_3), 1.41 (tq, *J* = ~7.3 Hz, 9-H_2), 1.75 (br s, 2-CH_3), 2.07 (br dt, *J* = 7.3, 7.3 Hz, 8-H_2), 2.22 (s, N(CH_3)$_2$), 2.25 (m, 4'-H), 2.97 (1H, d, *J* = 16.4 Hz, 14-CH_2N), 3.11 (1H, d, *J* = 16.4 Hz, 14-CH_2N), 3.31 (s, 3'-OCH_3), 3.38 (dd, *J* = 5.1, 9.6 Hz, 3'-H), 3.53 (d, *J* = 9.6 Hz, 1'-H), 3.57 (dd, *J* = 2.5, ~12 Hz, 5'-H_a), 3.78 (dd, *J* = 2.0, 11.7 Hz, 5'-H_e), 4.97 (t, *J* = ~9.5 Hz, 2'-H), 5.72 (dt, *J* = 14.5, 7.0 Hz, 7-H), 5.96 (br d, *J* = 11.1 Hz, 3-H), 6.10 (m, 6-H), 6.18 (m, 5-H), 6.27 (dd, *J* = 10.6, 14.1 Hz, 4-H); ^{13}C NMR (CD_2Cl_2) see Table 1.

Restrictinol *p*-bromobenzoate ester (5). A modification of the mixed anhydride procedure of Jouin *et al.*[8] was used to form **5**. Restrictinol (46 mg, 0.164 mmol) was dissolved in CH_2Cl_2 (5 mL) and added to a solution of *p*-bromobenzoic acid (54 mg, 0.27 mmol), triethyl amine (93 μl, 0.67 mmol), and 4-(dimethylamino) pyridine (2mg, 0.016 mmol) in ether (5 mL). While stirring under a N_2 atmosphere and cooling in an ice bath, isopropenyl chloroformate (36 μl, 0.33 mmol, Aldrich) was added by syringe with concommitant formation of a white precipitate. The reaction was allowed to warm to RT and after 15 h no starting material was observed by TLC (hexane/EtOAc restrictinol R_f =0.4, **5** R_f=0.9). MeOH (4 mL) was added and the reaction was stored at -80°C. A portion (2 mL out of 15 mL) of the reaction mixture was concentrated to dryness *in vacuo* and chromatographed on a 5 mL silica gel 60, 230-400 mesh column using hexane/EtOAc 90:10 as the mobile phase. Fractions 11-15 (1 mL fractions) were combined based on TLC (hexane/EtOAc 90:10 on silica), and concentrated to yield 7.9 mg. This preparation was a single spot via TLC before concentration, however, a number of other TLC spots were observed upon re-evaluation. ^{1}H NMR and UV strongly suggested that the triene portion of the molecule was decomposing.

Rechromatography of the decomposed 7.9 mg preparation under the same conditions yielded 0.5 mg of a preparation that, stored in dilute solution and -80°C, remained stable long enough to obtain MS, ^{1}H NMR, IR, UV and CD data. UV (Dioxane, Figure 1) λ_{max} (ε) 248(30,160), 269(28,512), 279(36,805), 290(28,539) nm; IR ν_{max} (neat) 1724 cm^{-1}; MS *m/z* 462/464 (M^+, 1:1); ^{1}H NMR (400 MHz, CD_2Cl_2) δ 0.87 (t, *J* = 7.3 Hz, 10-H_3), 1.12 (d, *J* = 7.1 Hz, 4'-CH_3), 1.38 (tq, *J* = ~7.3 Hz, 9-H_2), 1.77 (d, *J* = 1.2 Hz, 2-CH_3), 2.03 (br m, 8-H_2), 2.31 (m, 4'-H), 3.30 (s, 3'-OCH_3), 3.52 (dd, *J* = 5.3, 9.5 Hz, 3'-H), 3.66 (d, *J* = 9.5 Hz, 1'-H), 3.63 (dd, *J* = 2.3, 11.8 Hz, 5'-H_a), 3.83 (dd, *J* = 1.7, 11.8 Hz, 5'-H_e), 5.17 (t, *J* = 9.6 Hz, 2'-H), 5.61 (dt, *J* = 14.5, 7.2 Hz, 7-H), 5.90 (dd, *J* = 1.3, 11.1 Hz, 3-H), 5.91 (dd, *J* = 10.6, 14.5 Hz, 5-H), 5.99 (ddt, *J* = 10.8, 14.8, 1.4 Hz, 6-H), 6.18 (dd, *J* = 11.0, 14.4 Hz, 4-H), 7.57 (d, *J* = 8.6 Hz), 7.82 (d, *J* = 8.6 Hz).

Restrictinol (*R*)- and (*S*)-O-methylmandelates (6a, 6b) The Trost procedure[1b] was used for the preparation of the O-methyl mandelates. Oxalyl chloride (58 μl, 0.66 mmol) was added with formation of a precipitate to an ice bath cooled solution of DMF (70 μl, 0.90 mmol) in acetonitrile (5 mL). (*R*)- or (*S*)-O-methylmandelic acid (100 mg, 0.60 mmol) was added to the above suspension.

Restrictinol (18 mg, 0.064 mmol) was dissolved in pyridine (1 mL) and was added to the reaction. After 30 min the reaction was diluted with ether (40 mL) and the solution was washed with 5% aqueous $CuSO_4$ (20 mL) to remove pyridine. The organic solvents were remove *in vacuo* and the residue was chromatographed on silica gel (hexane/ether). The (*R*)-O-methylmandelate ester (**6a**) was obtained in 77% isolated yield (21 mg) and the (*S*)-O-methyl mandelate ester (**6b**) in 69% yield (19 mg). IR (neat) 1756, 1735, 1455 cm^{-1}; UV (dioxane) λ_{max} (ε) 269 (21000), 278 (28000), 290 (22000) nm. ^1H NMR ($CDCl_3$) see Table 3.

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