Bartanol and Bartallol, Novel Macrodiolides from *Cytospora* sp. ATCC 20502

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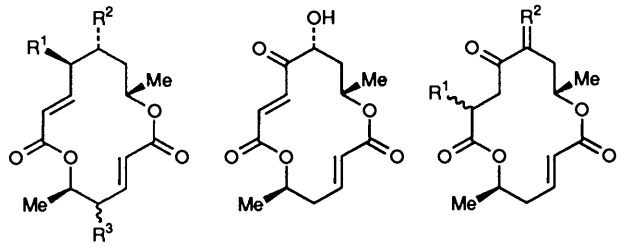
Two new macrodiolides, bartanol **10** and bartallol **11** have been isolated from a *Cytospora* sp. and their structures established by a detailed study of their high field ^1H and ^{13}C NMR spectra. Unlike the other 14-membered ring macrodiolides isolated from this source, bartanol and bartallol have a novel rearranged 13-membered macrocyclic ring. The conformations of the macrocyclic ring have been deduced from the analyses of difference NOE spectra and ^1H–^1H coupling constants.

Colletodiol **1**, a macrodiolide containing a 14-membered ring was originally isolated from the plant pathogen, *Colletotrichum capsici*.[1,2] Subsequently a number of related macrodiolides, colletoketol **2**, colletallol **3** and colletol **4** were isolated and



1 $R^1 = R^2 = OH$, $R^3 = H$
3 $R^1 = OH$, $R^2 = R^3 = H$
4 $R^1 = R^3 = H$, $R^2 = OH$
7 $R^1 = R^3 = OH$, $R^2 = H$

2

5 $R^1 = H$, $R^2 = O$
6 $R^1 = OH$, $R^2 = H$, OH

their structures elucidated.[3] The full stereochemistries of these metabolites have been established by a combination of chiroptical methods,[3] X-ray crystallography,[4] and total synthesis.[5] More recently colletoketol was isolated (as grahamimycin A) from culture filtrates of *Cytospora* sp. ATCC 20502 which displayed antibiotic activity. The co-metabolites, grahamimycins A_1 **5**, and B **6** were also reported.[6] These metabolites are now seen to be members of a larger group of macrodiolide metabolites from fungi and actinomycetes.[7] Recently a further member of the family, clonostachydiol **7**, which displays anthelmintic properties has been isolated from the fungus *Clonostachys cylindrospora*.[8] This group of metabolites has been the subject of numerous synthetic studies over the past 10 years.[5,9,10]

Biosynthetic studies have established that colletodiol **1** is polyketide derived and is formed *via* C_6 and C_8 hydroxy acids of tri- and tetra-ketide origins, respectively.[11,12] In the course of studies[13] on the later stages of colletodiol biosynthesis in *Cytospora*, extracts of the culture filtrate were examined for the presence of minor metabolites which might provide further information on the biosynthetic pathway. The isolation and characterisation of these minor metabolites is now reported.

Results and Discussion

Extracts from culture filtrates of *Cytospora* were purified by flash chromatography to give the major metabolites, colletodiol

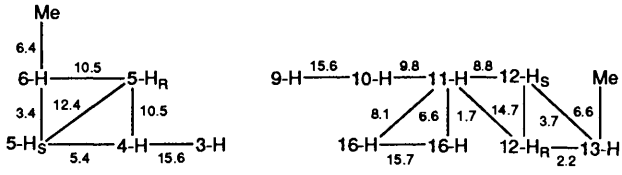
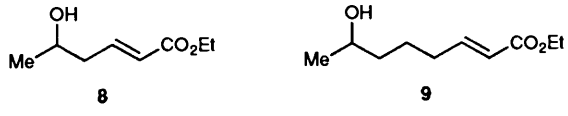


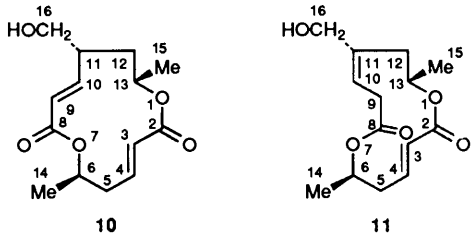
Fig. 1 Observed coupling constants (Hz) for bartanol **10**



8 **9**

and colletoketol. The fractions eluting between colletodiol and colletoketol were further purified by preparative normal phase HPLC. This led to the isolation of colletallol **3** and colletol **4** which have not previously been reported as metabolites of *Cytospora*. 5-Hydroxyhex-2-enoic and 7-hydroxyoct-2-enoic acids were isolated as their ethyl esters **8** and **9**, respectively. The former corresponds to the triketide moiety of the macrodiolides **1**–**6**. The latter is clearly related to the tetraketide derived portion of the macrodiolides but no metabolite with the oxidation level of **9** has yet been reported.

Two further metabolites were isolated and detailed spectroscopic studies have established the structures **10** and **11** for



10 **11**

these metabolites to which we have assigned the trivial names bartanol and bartallol, respectively. The molecular formula $C_{14}H_{20}O_5$ of bartanol was determined by high resolution CI mass spectrometry. The ^1H and ^{13}C NMR spectra (Table 1) indicated a close similarity to colletodiol **1** except that the methine signals due to the hydroxy substituents at C-10 and -11 were replaced by a two-proton multiplet centred at δ_H 3.55 which correlated with a methylene carbon at δ_C 65.0. Analysis

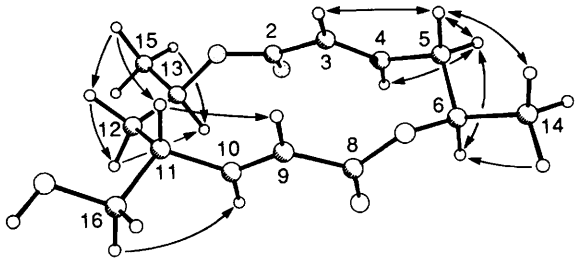


Fig. 2 Energy minimised conformation [15] of bartanol and observed NOEs

of proton–proton couplings in the 270 MHz ^{1}H NMR spectrum and cross peaks in the ^{1}H–^{1}H COSY NMR spectrum established the proton connectivities shown in Fig. 1. The C_6-hydroxy acid derived moiety found in colletodiol is clearly seen to be present in bartanol. The proton connectivities confirm that the remainder of the molecule is derived from a C_8 moiety containing a *trans*-α,β-unsaturated lactone (δ_H 5.73 and 6.41, *J* 15.6 Hz; δ_C 127.8, 156.7 and 167.4). The signal at δ_H 2.64 assigned to 11-H shows correlations to the olefinic signal assigned to 10-H at δ_H 6.41, the mutually coupled methylene hydrogens at δ_H 3.53 and 3.58, and to mutually coupled methylenes at δ_H 1.51 and 2.08. Decoupling of 11-H confirmed these correlations. The latter methylene is further coupled to a methine at δ_H 5.03, a chemical shift consistent with its position in a lactone moiety, and the methine in turn is coupled to a methyl doublet at δ_H 1.28. This establishes the 13-membered dilactonic structure **10** for bartanol.

The relative stereochemistry shown was assigned on the basis of NOE experiments and is consistent with the overall conformation established by X-ray crystallography [4] and NOE studies [14] for colletodiol. This overall conformation has been shown by X-ray studies to be adopted by other related compounds.[10,13] The conformation of bartanol and the observed NOEs are summarised in Fig. 2. Enhancements are observed between the 14-Me and 5-H_R. Irradiation of 5-H_R enhances 3-H whereas irradiation of 5-H_S enhances 4-H. The observed coupling constants are consistent with the conformation in that 5-H_R shows large (antiperiplanar) couplings to both 6- and 4-H whereas 5-H_S shows smaller (gauche) couplings. These NOEs and couplings are entirely consistent with those observed in colletodiol [1,14] and confirm that the conformations in this part of the ring are the same. The important enhancements seen in the remainder of the molecule are between the 15-Me and 11-H, between 11-H and 9-H, and between the 16-methylene and 10-H. These establish the spatial relationships shown in Fig. 2, in particular the stereochemistry at C-11 as *R*, and are further confirmed by the observed coupling constants. 12-H_R Shows very small couplings to both 13- and 11-H; consistent with mutual dihedral angles approaching 90° whereas the intermediate couplings between 12-H_S and 13- and 11-H agrees with the required dihedral angles of *ca.* 5 and 125°, respectively. 11-H Shows a large coupling to 10-H. This overall conformation has been shown to correspond to a minimised energy structure obtained by modified MM2 calculations.[15] The absolute configuration assigned to bartanol has not been established independently but is assumed to be the same as for its major co-metabolites.

In addition to bartanol, smaller amounts of a related metabolite bartallol were isolated. This has been assigned structure **11** on the basis of its ^{1}H and ^{13}C NMR spectra (Table 1 and Fig. 3). The ^{13}C NMR spectrum showed the presence of only three protonated olefinic carbons, three saturated methylenes, a hydroxymethyl group as in bartanol, and one conjugated and one non-conjugated lactone carbonyl

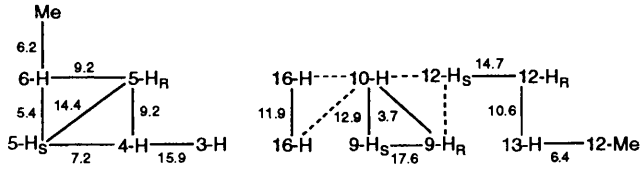


Fig. 3 Observed coupling constants and COSY correlations for bartallol **11**

Table 1 Chemical shifts in the ^{1}H and ^{13}C NMR spectra of bartanol **10** and bartallol **11**

	δ_H [a]			δ_C [a]	
H	**10**	**11**	C	**10**	**11**
			C-2	165.6	165.6
3-H	5.84	5.78	C-3	124.1	126.4
4-H	6.53	6.64	C-4	142.5	142.6
5-H_R	2.13	2.22	C-5	34.3	39.3
5-H_S	2.47	2.56			
6-H	5.22	5.25	C-6	69.6	69.3[b]
			C-8	167.4	171.8
9-H	5.73		C-9	127.8	33.1
9-H_R		2.93			
9-H_S		3.24			
10-H	6.41	5.75	C-10	156.7	122.7
11-H	2.64		C-11	40.15	137.0
12-H_R	1.51	2.12	C-12	40.9	36.5
12-H_S	2.08	2.48			
13-H	5.03	5.25	C-13	68.4	69.6[b]
14-H_3	1.36	1.33	C-14	20.5	21.4
15-H_3	1.28	1.36	C-15	19.7	20.7
16-H_2	3.53	4.07	C-16	65.0	68.8
	3.58	4.12			

[a] $CDCl_3$ solution. [b] May be interchanged.

(δ_C 165.6 and 171.8) consistent with the allylic alcohol structure **11** in which the 9,10 double bond in bartanol has migrated to the 10,11 position. This assignment has been confirmed by analysis of the observed couplings in the ^{1}H NMR spectrum and from correlations in the ^{1}H–^{1}H and ^{1}H–^{13}C COSY NMR spectra, (Fig. 3, Table 1). Again the anticipated couplings and correlations for the C_6 moiety were evident. The mutually coupled 9-methylene hydrogens at δ_H 2.93 and 3.24 are coupled to the olefinic 10-H resonance at δ_H 5.75. This, in turn, shows a correlation in the COSY spectrum due to an allylic coupling to the 16-methylene signal centred at δ_H 4.10 and to the 12-H_S at δ_H 2.48, which shows a geminal coupling to the signal at δ_H 2.12. The resonance at δ_H 2.12, but not that at 2.48, is coupled to the 13-H at δ_H 5.25 which, in turn, couples to the 15-Me doublet at δ_H 1.36. In addition, a homo-allylic correlation and very small couplings are observed

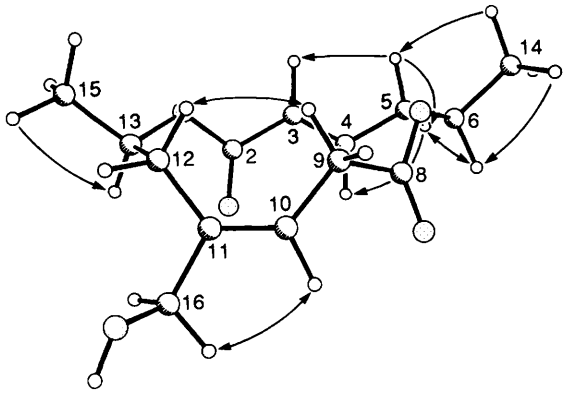
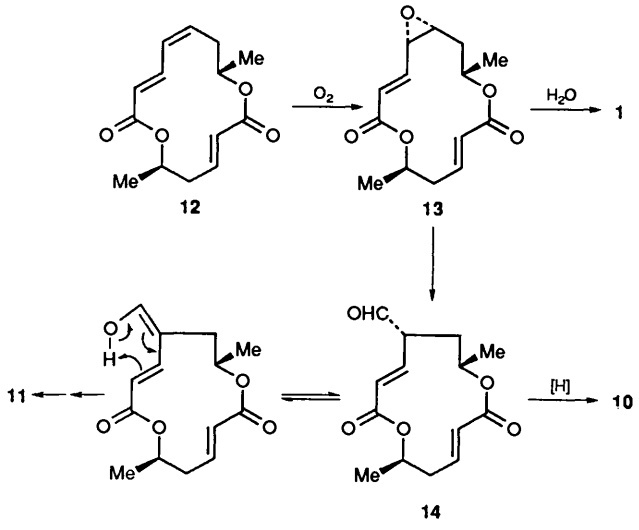


Fig. 4 Energy minimised conformation[15] of bartallol and observed NOEs



Scheme 1 Proposed pathway for formation of bartanol **10** and bartallol **11**

between the 9- and 12-H_S at δ_H 2.93 and 2.48, respectively. These couplings and correlations establish structure **11** for bartallol.

The absolute configurations at C-6 and -13 are assumed to be *R* by analogy with the isolated co-metabolites. The 10*E* double bond geometry has been assigned on the basis of examination of models and the observed couplings. The energy-minimised conformation[15] shown in Fig. 4 is again consistent with the general conformation adopted by molecules in this series. Attempts to find a conformation which would allow a 10*Z* double bond without significant angle strain or steric clashes proved difficult whereas the 10*E* geometry is readily accommodated. The couplings observed are entirely consistent with this conformation. In particular 12-H_S and 9-H_R are orthogonal to the plane of the 10,11 double bond, consistent with their observed homoallylic correlation. In addition the conformation requires a dihedral angle of 90° between 12-H_S and 13-H which is consistent with the lack of observed vicinal coupling between them.

Further evidence for the overall structure and solution conformation was provided by NOE studies in deuteriochloroform. Enhancements similar to those for bartanol were observed for the signals due to the C_6-moiety to confirm the similar conformation in this part of the molecule. The 15-Me shows a NOE to 12-H_S, whereas 12-H_R and 9-H_S show mutual enhancements. Irradiation of the olefin methine 10-H results in enhancement of 9-H_R and, crucially, both of the 16-methylene hydrogens to confirm the assignment of the double bond as *E*. In deuteriochloroform solution, 10-H and 3-H have very similar chemical shifts (δ_H 5.75 and 5.78, respectively). However, in hexadeuteriobenzene they are resolved (δ_H 5.99 and 5.73 respectively). Repeating the NOE studies in the latter solvent confirmed the NOEs from 16- to 10-H. The similarity of the proton–proton couplings confirmed that no change of overall conformation occurs on change of solvent.

The structures assigned to bartanol and bartallol are consistent with recent biosynthetic work[13] which has established that colletodiol is biosynthesised *via* stereospecific epoxidation of the triene **12** to give the epoxide **13** followed by an enzyme mediated hydrolysis as shown in Scheme 1. It may be envisaged that a concerted rearrangement of the epoxide **13** would give rise to the aldehyde **14** which on reduction would give bartanol directly with the correct absolute stereochemistry. Migration of the double bond into conjugation with the aldehyde prior to reduction would account for the formation of bartallol as indicated in Scheme 1. To the best of our knowledge, bartanol and bartallol represent the first example of macrolides or macrodiolides in which the ring size has been established by a ring-contraction process on a preformed macrocycle.

Experimental

All organic solvents were distilled prior to use; light petroleum refers to the fraction with the boiling range 40–60 °C. M.p.s were determined using a Kofler hot-stage apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 881 IR spectrometer. Optical density was measured using a Philips PU8800 UV–VIS spectrometer operating at 630 nm. Optical rotations were measured using a Perkin-Elmer 241MC polarimeter. Mass spectra were recorded using a Kratos MS9 spectrometer with VG ZAB ion source and electron impact (EI) or a VG Autospec BQ for CI spectra with ammonia as the ionising gas.

^1H and ^{13}C NMR spectra were recorded on JEOL JNM GX-270 and JNM GX-400 spectrometers with deuteriochloroform as solvent, unless otherwise stated, using tetramethylsilane as the internal standard. NMR chemical shifts are given in δ values downfield of tetramethylsilane (*J* values in Hz). Flash chromatography was carried out using silica gel (40–63 μm, Kieselgel 60, Merck, and 220–440 mesh, Kieselgel 60, Fluka). Preparative thin layer chromatography (TLC) was carried out on sheets precoated with silica gel (Merck 60 F_{254}). Bands were

visualised by UV light or *via* staining with ammonium molybdate (10% w/v in 2 mol dm^{-3} sulfuric acid). High performance liquid chromatography (HPLC) was carried out using Gilson model 303 pumps fitted with 25 cm^3 pump heads. A Gilson model 803C pressure gauge and a Gilson model 811 dynamic mixer were used along with a Rheodyne 7125 injection valve fitted with a 1 cm^3 loop. Fractions were detected using a SpectroMonitor 3000 variable wavelength detector set at 240 nm. Gradient control was carried out using an Apple IIe personal computer with Gilson gradient manager software.

Culture Details.—*Cytospora* sp. ATCC 20502 was stored on Agar slopes (Oxoid CM 139) at 4 °C. Large-scale fermentations were carried out using potato dextrose broth, prepared by homogenising peeled potato (70 g) with glucose (20 g) and deionised water (1 dm^3). Seed cultures are prepared by adding 100 cm^3 of potato dextrose broth to 500 cm^3 conical flasks. These were sterilised at 121 °C, 15 psig for 15 min. Each flask was then inoculated with a spore suspension in sterile deionised water, prepared directly from an agar slope. The seed flasks were then grown at 26 °C at 200 rpm on an orbital shaker for 96 h.

Large-scale fermentations were carried out on sterile potato broth, in 100 cm^3 aliquots in 500 cm^3 conical flasks. Each flask was inoculated with a 10 cm^3 aliquot of seed culture, then grown at 26 °C at 200 revolutions per min (rpm) on an orbital shaker for 144 h.

Isolation and Characterisation of Metabolites.—The mycelia was removed from the crude fermentation broth by vacuum filtration. The filtrate was then extracted with 3 equal volumes of ethyl acetate, the extract was dried ($MgSO_4$) and concentrated *in vacuo* to give a brown viscous liquid.

The crude extract was then purified by flash chromatography, with gradient elution from light petroleum to ethyl acetate in five steps. Colletoketol and colletodiol were isolated as the main fractions. [TLC mobile phase ether–methanol–methylene chloride (40:4:56) colletoketol R_f 0.75 colletodiol R_f 0.2]. A band of minor metabolites eluting between the main colletoketol and colletodiol fractions was also isolated. A 2 dm^3 fermentation typically yielded 200 mg of colletoketol, 700 mg of colletodiol and 100 mg of mixed fractions. These fractions were further purified by preparative HPLC on a Dynamax™ Macro HPLC column packed with silica gel (8 μm, 250 mm × 22.5 mm i.d.) and fitted with a pre-column packed with silica (8 μm, 50 mm × 22.5 mm i.d.). The column was eluted with a gradient of isopropyl alcohol (IPA) in methylene dichloride which was maintained at 1.5% IPA for 60 min, then increased to 4% IPA over 90 min. The flow rate was 22 cm^3 min^{-1} and the UV detector was set at 240 nm and a sensitivity of 2.0 Aufs. The following fractions were collected. (*a*) Colletoketol (2 mg) eluted after 25 min and identified by comparison of its ^1H NMR spectrum with an authentic sample. (*b*) Ethyl 7-hydroxyoct-2-enoate (1 mg) eluted at 33 min; δ_H 1.2 (3 H, d, *J* 6.2, 8-H_3), 1.29 (3 H, t, *J* 7.2, CH_3CH_2), 1.5 (4 H, br m, 5-H_2 and 6-H_2), 2.22 (2 H, m, 4-H_2), 3.81 (1 H, m, 7-H), 4.18 (2 H, q, *J* 7.2, CH_3CH_2), 5.83 (1 H, d, *J* 15.6, 2-H) and 6.96 (1 H, dt, *J* 7.0 and 15.6, 3-H); δ_C 166.17, 148.86, 121.57, 67.84, 60.19, 38.58, 32.06, 24.20, 23.61 and 14.27; *m/z* 185 ($M^+ - 1$, 1.1%), 168 (13), 140 (38), 125 (54), 114 (69), 95 (98), 81 (62), 68 (55), 55 (46) and 45 (100). (*c*) Ethyl 5-hydroxyhex-2-enoate (5 mg) eluted at 37 min; δ_H 1.24 (3 H, d, *J* 6.2, 6-H_3), 1.29 (3 H, t, *J* 7.1, CH_3CH_2), 2.37 (2 H, dd, *J* 6.4 and 7.1, 4-H), 3.97 (1 H, m, 5-H), 4.19 (2 H, q, *J* 7.1, CH_3CH_2), 5.91 (1 H, d, *J* 15.8, 2-H) and 6.97 (1 H, dt, *J* 15.8 and 7.5, 3-H); δ_C 166.32, 144.89, 123.97, 66.73, 60.32, 41.81, 23.20 and 14.24; *m/z* 143 ($M - 15$, 1.3%), 125, 114 (100), 86 (99), 68 (50) and 45 (59). Fractions (*d*) and (*e*) eluting at 70 and 81 min gave respectively colletol (8 mg), and colletallol (5 mg) which were identified by comparison of their ^1H NMR spectra with those previously reported.[3] Fraction (*f*) eluting at 89 min gave bartanol **10** (8 mg) (6R,11R,13R)(3E,9E)-11-*hydroxymethyl*-6,13-*dimethyl*-1,7-*dioxacyclotrideca*-3,9-*diene*-2,8-*dione*) as a colourless oil $[\alpha]_D^{21}$ +32.3 (*c* 1.92, $CHCl_3$) (Found: M^+, 286.1661. $C_{14}H_{20}O_5 + NH_3$ requires *M*, 286.1655); ν_{max}/cm^{-1} 3412, 1722 and 1652; δ_H 1.28 (3 H, d, *J* 6.6, 15-H_3), 1.36 (3 H, d, *J* 6.4, 14-H_3), 1.51 (1 H, ddd, *J* 14.7, 2.2 and 1.7, 12-H_R), 2.08 (1 H, ddd, *J* 14.7, 8.8 and 3.7, 12-H_S), 2.13 (1 H, ddd, *J* 12.4, 10.5 and 10.5, 5-H_R), 2.47 (1 H, ddd, *J* 12.4, 5.4 and 3.4, 5-H_S), 2.64 (1 H, m, 11-H), 3.53 (1 H, dd, *J* 15.7 and 6.6, 16-H), 3.58 (1 H, dd, *J* 15.7 and 8.1, 16-H), 5.03 (1 H, ddq, *J* 6.6, 3.7 and 2.2, 13-H), 5.22 (1 H, ddd, *J* 10.5, 6.4 and 3.4, 6-H), 5.73 (1 H, d, *J* 15.6, 9-H), 5.84 (1 H, d, *J* 15.6, 3-H), 6.41 (1 H, dd, *J* 15.6 and 9.8, 10-H) and 6.53 (1 H, ddd, *J* 15.6, 10.5 and 5.4, 4-H); *m/z* (CI, NH_3), 286 ($M^+ + 18$, 100%). Fraction (*g*), eluted at 97 min, gave bartallol (**11**) (2 mg) (6R,13R)(3E,10Z)-11-*hydroxymethyl*-6,13-*dimethyl*-1,7-*dioxacyclotrideca*-3,10-*diene*-2,8-*dione*) as a colourless oil; $[\alpha]_D^{21}$ −26.5 (*c* 0.27, $CHCl_3$); ν_{max}/cm^{-1} 3745, 1730, 1720, 1655 and 1601; δ_H 1.33 (3 H, d, *J* 6.4, 14-H_3), 1.36 (3 H, d, *J* 6.4, 15-H_3), 2.12 (1 H, dd, *J* 14.7 and 10.6, 12-H_R), 2.22 (1 H, ddd, *J* 14.4, 9.2 and 9.2, 5-H_R), 2.48 (1 H, dd, *J* 14.9 and < 1.0, 12-H_S), 2.56 (1 H, ddd, *J* 14.4, 7.2 and 5.4, 5-H_S), 2.93 (1 H, dd, *J* 17.6 and 3.7, 9-H_R), 3.24 (1 H, dd, *J* 17.6 and 11.9, 9-H_S), 4.07 (1 H, d, *J* 11.9, 16-H), 4.12 (1 H, d, *J* 11.9, 16-H), 5.25 (2 H, m, 6-H and 13-H), 5.75 (1 H, dd, *J* 11.9 and 3.7, 10-H), 5.78 (1 H, d, *J* 15.9, 3-H), 6.64 (1 H, ddd, *J* 15.9, 9.2 and 7.2, 4-H); $\delta_H(C_6D_6)$ 0.96 (3 H, s, 14-H_3), 1.06 (3 H, s, 15-H_3), 1.67 (1 H, m, 5-H_R), 1.78 (1 H, m, 5-H_S), 1.80 (1 H, dd, *J* 14.2 and 1.0, 12-H_S), 2.18 (1 H, dd, *J* 14.2 and 10.5, 12-H_R), 2.64 (1 H, dd, *J* 17.3 and 1.0, 9-H_R), 3.14 (1 H, dd, *J* 17.3 and 12.0, 9-H_S), 3.84 and 3.95 (each 1 H, d, *J* 13.0, 16-CH_2), 5.04 (1 H, m, 6-H), 5.39 (1 H, dqd, *J* 10.5, 6.4 and 1.0, 13-H), 5.73 (1 H, d, *J* 16.1, 3-H), 5.99 (1 H, dd, *J* 13.2 and 1.0, 10-H) and 6.62 (1 H, ddd, *J* 16.1, 9.3 and 6.7, 4-H); *m/z* 268 (M^+, 3.4%), 251 (1), 226 (7), 206 (5), 138 (41) and 113 (100) (Found: M^+, 268.3109. $C_{14}H_{20}O_5$ requires *M*, 268.1311). Colletodiol **1** (3 mg) eluted after 136 min.

Acknowledgements

The SERC is thanked for the award of studentships (K. H. and J. A. O'N.) and financial support from Schering Agrochemicals is gratefully acknowledged.

References

1 J. F. Grove, R. N. Speake and G. Ward, *J. Chem. Soc. C*, 1966, 230.
2 J. MacMillan and R. J. Pryce, *Tetrahedron Lett.*, 1968, 5497.
3 J. MacMillan and T. J. Simpson, *J. Chem. Soc., Perkin Trans. 1*, 1973, 1487.
4 D. Seebach, E. Hungerbuhler and R. Amstutz, *Helv. Chim. Acta*, 1981, **64**, 1793.
5 P. Schnurrenberger, E. Hungerbuhler and D. Seebach, *Tetrahedron Lett.*, 1984, **25**, 2209.
6 S. Gurusiddaiah and R. C. Ronald, *Antimicrob. Agents Chemother.*, 1981, **19**, 1953.
7 *Macrolide Antibiotics*, ed. S. Omura, Academic Press, New York, 1984, p. 538.
8 S. Grabley, P. Hammann, R. Thiericke, J. Wink, S. Phillips and A. Zeeck, *J. Antibiot.*, 1993, **46**, 343.
9 See, *e.g.*, T. Wakamatsu, S. Yamada, Y. Ozai and Y. Ban, *Tetrahedron Lett.*, 1985, **26**, 1989; L. R. Hills and R. C. Ronald, *J. Org. Chem.*, 1985, **50**, 470; K. Ohta and O. Mitsunobu, *Tetrahedron Lett.*, 1991, **32**, 517; F. J. Dommerholt, L. Thijs and B. Zwanenburg, *Tetrahedron Lett.*, 1991, **32**, 1495; 1499.
10 G. E. Keck and J. A. Murry, *J. Org. Chem.*, 1991, **56**, 6606.

11 J. MacMillan and M. W. Lunnon, *J. Chem. Soc., Perkin Trans. 1*, 1976, 184.
12 T. J. Simpson and G. I. Stevenson, *J. Chem. Soc., Chem. Commun.*, 1985, 1822.
13 J. A. O'Neill, T. J. Simpson and C. L. Willis, *J. Chem. Soc., Chem. Commun.*, 1993, 738.
14 G. I. Stevenson, Ph.D. Thesis, University of Edinburgh, 1988.
15 F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, C. Caulfield, G. Chang, T. Hendrickson and W. C. Still, *J. Comput. Chem.*, 1990, **11**, 440.

Paper 4/01077I
Received 22*nd February* 1994
Accepted 18*th April* 1994