

## Fungal Products. Part XVIII.<sup>1</sup> <sup>13</sup>C Nuclear Magnetic Resonance Spectrum and Biosynthesis of Colletodiol

By Martin W. Lunn and Jake MacMillan,\* School of Chemistry, The University, Bristol BS8 1TS

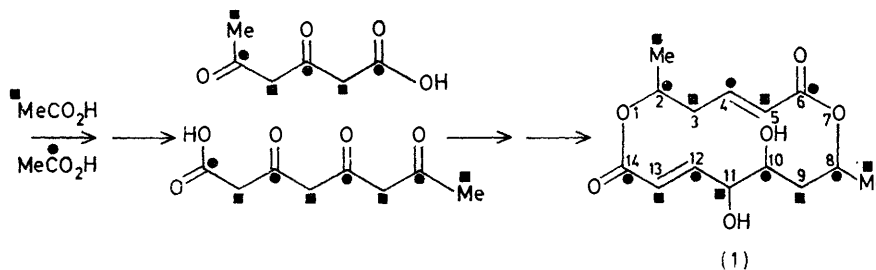
In the <sup>13</sup>C n.m.r. spectrum of colletodiol (10,11-dihydroxy-2,8-dimethyl-1,7-dioxacyclotetradeca-4,12-diene-6,14-dione) (1), the resonances have been assigned by correlation with <sup>1</sup>H n.m.r. assignments from irradiation experiments at single frequencies in the range of proton resonances. Spectra of colletodiol derived by incorporation of [1-<sup>13</sup>C]- and [2-<sup>13</sup>C]-acetate showed <sup>13</sup>C enrichments consistent with a biosynthetic pathway from the union of a triketide and a tetraketide component.

COLLETODIOL, a metabolite of the plant pathogen, *Colletotrichum capsici*, possesses the structure (1).<sup>2,3</sup> The following <sup>13</sup>C n.m.r. studies have established the unexceptional biogenesis of this fungal product from acetate and indicate the pathway shown in the Scheme.

Since the chemical shifts of all the protons in colletodiol (1) have been previously assigned<sup>2</sup> from <sup>1</sup>H n.m.r. spectra, the <sup>13</sup>C resonances could be assigned by using the graphical method described by Birdsall *et al.*<sup>4</sup> (Table I). Plots of the <sup>13</sup>C peak frequencies as a

resonances of the 6- and 14-carbonyl carbon atoms remained constant in each irradiation experiment, providing an internal check.

For incorporation studies the fungus was cultured as described by MacMillan and Simpson<sup>3</sup> except that mycelial growth was supported on a pad of glass wool. This arrangement facilitated the replacement of the original culture medium, after the production of colletodiol had ceased, by a medium containing only glucose and labelled acetate. In this way the previously



SCHEME Biosynthesis of colletodiol (1)

function of single frequencies in the range of proton resonances gave straight lines of similar slopes by least-squares fit (FOCAL program). Care was taken to ensure that the irradiating frequencies were within the range where they were proportional to the residual coupling. Data from four experiments were used except where the irradiating frequency caused apparent complete decoupling of a particular <sup>13</sup>C resonance. In such cases the data for the <sup>13</sup>C peak at that frequency were too inaccurate to be included. The intersections of the straight line plots for each <sup>13</sup>C peak gave values (Table I) which were in good agreement with those previously determined<sup>2</sup> from the <sup>1</sup>H n.m.r. spectra. Clear-cut assignments of individual <sup>13</sup>C resonances could therefore be made with the proviso that the assignments may be reversed for similar pairs of carbon atoms from each carbon chain. These assignments were entirely consistent with the literature values for <sup>13</sup>C chemical shifts and with conventional proton decoupling data. The results from the graphical method were also self-consistent. For example the <sup>13</sup>C chemical shifts derived from the intersections of the plots were in excellent agreement (Table I) with those measured directly from the proton-decoupled <sup>13</sup>C n.m.r. spectra. The <sup>13</sup>C

reported<sup>3</sup> yield of colletodiol was increased and the related dilactones,<sup>3</sup> colletol, colletal, and colletoketol, were formed in insignificant amounts.

Preliminary feeds with sodium [2-<sup>14</sup>C]acetate showed that glucose was required in the replacement medium; in its absence the yields of colletodiol and the percentage incorporation were very low. Following the approach of Holker *et al.*,<sup>5</sup> the amount of <sup>13</sup>C necessary for feeding was calculated by determining the overall dilution in feeds of [2-<sup>14</sup>C]acetate. When a replacement medium containing 5  $\mu$ Ci of sodium [2-<sup>14</sup>C]acetate, 500 mg of sodium acetate, and 50 g of glucose per litre was used, the overall dilution factor was 19, corresponding to a <sup>13</sup>C enrichment of 4.7% for 90% <sup>13</sup>C-labelled acetate.

The <sup>13</sup>C enrichments at individual positions in [1-<sup>13</sup>C]- and [2-<sup>13</sup>C]-acetate-derived samples of colletodiol (1) were calculated as described by Holker *et al.*<sup>5</sup> However the results (Table 2) are expressed as enrichment factors (*i.e.* as the factor by which the natural <sup>13</sup>C abundance has been increased) and not as <sup>13</sup>C abundance in excess (*i.e.* the difference between enriched and natural <sup>13</sup>C abundance) as used by Holker *et al.*<sup>5</sup> The observed labelling pattern is in complete agreement with that required by the Scheme.

<sup>3</sup> J. MacMillan and T. J. Simpson, *J.C.S. Perkin I*, 1973, 1487.

<sup>4</sup> B. Birdsall, N. J. M. Birdsall, and J. Feeney, *J.C.S. Chem. Comm.*, 1972, 316; B. Birdsall and J. Feeney, *J.C.S. Perkin II*, 1972, 1643.

<sup>5</sup> J. S. E. Holker, R. D. Lapper, and T. J. Simpson, *J.C.S. Perkin I*, 1974, 2135.

<sup>1</sup> Part XVII, J. R. Bearder, V. M. Frydman, P. Gaskin, I. K. Hatton, W. E. Harvey, J. MacMillan, and B. O. Phinney, preceding paper.

<sup>2</sup> J. MacMillan and R. J. Pryce, *Tetrahedron Letters*, 1968, 5497.

TABLE I  
Assignment of  $^{13}\text{C}$  resonances of colletodiol (1) from  $^1\text{H}$  chemical shifts

Proton assignment	$\delta_{\text{H}}$		$\delta_{\text{C}}$		Carbon assignment
	Graphical method	$^1\text{H}$ n.m.r. spectra	Graphical method	$^{13}\text{C}$ n.m.r. spectra	
2-Me	1.50 <sup>a</sup>	1.38	20.4 <sup>a</sup>	20.4	2-Me } <sup>c</sup> 8-Me } <sup>c</sup>
8-Me	1.37 <sup>a</sup>	1.38	18.2 <sup>a</sup>	18.2	
2	5.43 <sup>a</sup>	5.30	68.7 <sup>a</sup>	68.7	2 } <sup>c</sup> 8 } <sup>c</sup>
8	5.22 <sup>a</sup>	5.16	68.3 <sup>a</sup>	68.3	
3	2.57 <sup>a</sup>	2.50, 2.21	41.2 <sup>a</sup>	41.2	3 9
9	1.83 <sup>a</sup>	2.00, 1.5	36.3 <sup>a</sup>	36.2	
4	6.92 <sup>b</sup>	6.70	147.1 <sup>b</sup>	146.9	4 } <sup>c</sup> 12 } <sup>c</sup>
12	7.08 <sup>b</sup>	6.73	144.3	144.2	
5	5.83 <sup>b</sup>	6.72	125.7 <sup>b</sup>	125.7	5 } <sup>c</sup> 13 } <sup>c</sup>
13	6.04 <sup>b</sup>	6.14	123.5 <sup>b</sup>	123.5	
10	3.84 <sup>b</sup>	3.63	71.8 <sup>b</sup>	71.8	10 11
11	4.12 <sup>b</sup>	4.06	73.8 <sup>b</sup>	73.8	
				166.9	6 } <sup>c</sup> 14 } <sup>c</sup>
				165.3	

<sup>a</sup> Least-squares fit from 4 points. <sup>b</sup> Least-squares fit from 3 points. <sup>c</sup> Assignments of these pairs may be reversed without altering the biochemical conclusions.

TABLE 2  
 $^{13}\text{C}$  N.m.r. data for colletodiol (1) derived from  $[1-^{13}\text{C}]$ - and  $[2-^{13}\text{C}]$ -acetates

Carbon	$\delta_{\text{C}}$	Computer-listed intensities			Normalise to natural abundance		$^{13}\text{C}$ Enrichment factor	
		Natural	$[1-^{13}\text{C}]$ Acetate derived	$[2-^{13}\text{C}]$ Acetate derived	$[1-^{13}\text{C}]$ Acetate derived	$[2-^{13}\text{C}]$ Acetate derived	$[1-^{13}\text{C}]$ Acetate derived	$[2-^{13}\text{C}]$ Acetate derived
2	68.7	33.3	7.30	24.7	116.0	33.3		
8	68.3	29.5	7.74	24.3	123.0	32.8	3.8	
2-Me	20.4	24.2	1.74	56.2	27.6	75.7		2.7
8-Me	18.2	25.3	1.73	53.7	27.5	72.5		2.6
3	41.2	24.2	1.94	60.4	30.8	81.5		2.6
9	36.2	24.2	1.44	59.0	22.9	79.5		3.5
4	146.9	34.0	7.20	25.2	114.0	34.0	3.4	
12	144.2	37.0	7.90	26.2	125.0	35.4	3.5	
5	125.7	33.5	1.41	83.9	22.4	113.0		5.0
13	123.5	34.1	1.79	79.2	28.4	107.0		3.8
6	166.9	12.5	2.26	7.70	35.9	10.4	3.5	
14	165.3	9.8	3.62	8.42	57.5	11.4	5.0	
10	71.8	26.0	6.62	23.8	105.0	32.1	3.3	
11	73.8	29.3	1.62	72.6	25.7	98.0		3.8

#### EXPERIMENTAL

For general experimental procedures see ref. 3.

$^{13}\text{C}$  N.m.r. Determinations.—The  $^{13}\text{C}$  spectra were obtained for solutions in  $\text{CDCl}_3$  with  $\text{Me}_4\text{Si}$  as internal standard by use of a JEOL PFT-100 instrument operating at 25.2 MHz in the Fourier Transform mode. Sweep widths of 6.25 KHz with 4 096 data points were used throughout to give  $^{13}\text{C}$  chemical shifts with an accuracy of  $\pm 1.52$  Hz ( $\pm 0.06$  p.p.m.). A pulse width of 16  $\mu\text{s}$ , 'tilt angle' of 45°, and repetition time of 0.73 s were used. Off-centre proton-decoupled spectra were obtained by irradiation at 7.17 and 12.15 p.p.m. downfield from  $\text{Me}_4\text{Si}$  and at 2.84 and 7.8 p.p.m. upfield from  $\text{Me}_4\text{Si}$  at constant power.

Culture Methods.—The medium and conditions described by MacMillan and Simpson<sup>3</sup> were used with the following modification. The vessels were packed with glass wool so that the mycelial pad was supported on the surface of the medium.

$^{14}\text{C}$ -Enriched Colletodiol.—Ten flat culture vessels each containing 200 ml of medium were inoculated with a spore suspension of *C. capsici*. After 12 days the medium was decanted and extracted with chloroform. The crude extract (235 mg) was purified by p.l.c. on silica gel developed with ethyl acetate-light petroleum (3:1). Colletodiol (115 mg) recovered from the band at  $R_F$  0.3 was identical (m.p., n.m.r., g.l.c., and mass spectrum) with an authentic specimen.

In five of the above vessels the mycelium was re-incubated with replacement medium (200 ml per vessel) containing glucose (50 g l<sup>-1</sup>), sodium  $[2-^{14}\text{C}]$ acetate (5  $\mu\text{Ci}$  l<sup>-1</sup>), and unlabelled sodium acetate (500 mg l<sup>-1</sup>). After 10 days, colletodiol was isolated as before and crystallised to constant radioactivity ( $6.71 \times 10^6$  disint. min<sup>-1</sup> mmol<sup>-1</sup>; yield 45 mg).

Sodium  $[1-^{13}\text{C}]$ Acetate Feed.—By the same procedure as for sodium  $[2-^{14}\text{C}]$ acetate, the first incubation gave

unlabelled colletodiol (60 mg). Incubation of the mycelium from five vessels with replacement medium containing glucose (50 g l<sup>-1</sup>) and [90%-1-<sup>13</sup>C]acetate (500 mg l<sup>-1</sup>) gave [1-<sup>13</sup>C]acetate-derived colletodiol (18 mg).

*Sodium [2-<sup>13</sup>C]Acetate Feed.*—From an identical experiment with [90%-2-<sup>13</sup>C]acetate, unlabelled colletodiol (112

mg) and [2-<sup>13</sup>C]acetate-derived colletodiol (55 mg) were obtained.

We thank the S.R.C. for a research studentship (to M. W. L.) and Dr. M. Murray for the <sup>13</sup>C n.m.r. spectra.

[5/1343 Received, 7th July, 1975]

---