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Cladobotric Acids A−F: New Cytotoxic Polyketides from a New Zealand *Cladobotryum* sp.

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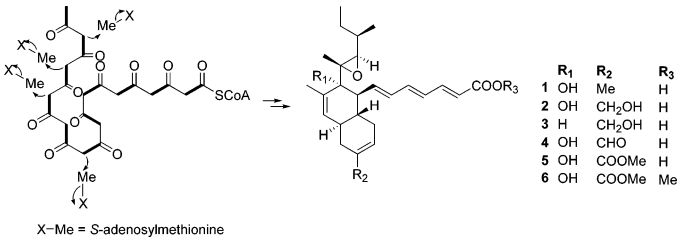

	R_1	R_2	R_3
1	OH	Me	H
2	OH	CH_2OH	H
3	H	CH_2OH	H
4	OH	CHO	H
5	OH	COOMe	H
6	OH	COOMe	Me

Cladobotric acids A−F (**1**−**6**), fungal-derived polyketides, were isolated from the fermentation broth of a New Zealand *Cladobotryum* species. Structures were determined by extensive spectral analysis and X-ray crystallography, and the polyketide origin of **1**−**6** was concluded from feeding experiments with ^{13}C-labeled precursors. The observed folding pattern for the polyketide chain is unusual for fungi. Cladobotric acids A−F (**1**−**6**) exhibited notable cytotoxicity against the murine P388 leukemia cell line and were also active against *Bacillus subtilis* and *Candida albicans*.

Introduction

The genus *Cladobotryum* comprises a variety of anamorphic fungal species parasitic on basidiomycetes, less frequently occurring on heterobasidiomycetes, ascomycetes, and plant litter.[1] Previous investigations have shown the capability of these fungi to produce a remarkable range of diverse, and often bioactive, natural products: cyclodepsipeptides containing nonproteinogenic amino acids,[2] a tetrasubstituted furan,[3] furopyridines,[4,5] and an azatricyclic phosphate ester,[6] have been reported.

Within the scope of our continuing search for new bioactive metabolites from New Zealand fungi, we investigated a strain of a *Cladobotryum* species (CANU E1042) isolated from a podocarp forest near Hokitika. After fermentation in SDY (Sabouraud dextrose yeast broth),[2] the extract of this fungus was cytotoxic against the P388 cell line (IC_{50} 8.69 μg/mL). Bioactivity-guided fractionation of the fermentation extract led to the isolation of six new metabolites, cladobotric acids A−F (**1**−**6**).

Results and Discussion

Using HRESIMS in combination with the ^{13}C NMR data, cladobotric acid A (**1**), the major component (9.13%) in the crude extract, was found to have the molecular formula $C_{26}H_{36}O_4$, indicating nine degrees of unsaturation. The structure was deduced by a detailed analysis of one-dimensional (1D) and two-dimensional (2D) NMR data (Table 1). The evaluation of the COSY and the TOCSY spectra, together with the long-range H, C-correlations, acquired using constant time inverse-detected gradient accordion rescaled long-range heteronuclear multiple bond correlation (CIGAR-HMBC) pulse sequence,[7] revealed the presence of a *sec*-butyl moiety, a trienoic acid, two trisubstituted double bonds, and three tertiary methyl groups. Analysis of the COSY and TOCSY data implied that the trienoic

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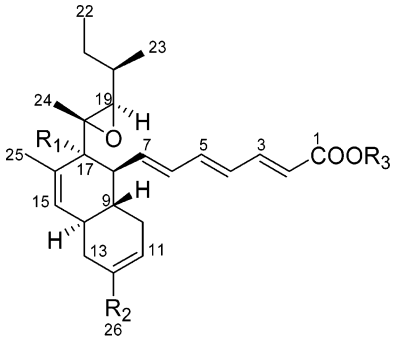
TABLE 1. 1D and 2D NMR Data (in $CDCl_3$ at 23 °C) for Cladobotric Acid A (1)

	^{1}H	^{13}C[a]		COSY	CIGAR		^{1}H	^{13}C[a]		COSY	CIGAR
1		169.7	(C)			14	2.05 (m)[b]	38.3	(CH)	9, 13b, 15	9, 11, 12, 26
2	5.64 (d, 15.5)	120.4	(CH)	3	1, 3, 4, 5	15	5.55 (br s)	132.2	(CH)	14, 25	9, 14, 17, 25
3	7.15 (dd, 15.5, 11.5)	146.2	(CH)	2, 4	1, 2, 4, 5	16		133.9	(C)		
4	6.14 (dd, 14.9, 11.5)	129.2	(CH)	3, 5	2, 3, 6	17		75.6	(C)		
5	6.54 (dd, 14.9, 10.9)	140.5	(CH)	4, 6	2, 3, 6, 7	18		65.1	(C)		
6	6.11 (dd, 14.7, 10.9)	133.6	(CH)	5, 7	4, 5, 7, 8, 9, 17	19	3.22 (d, 8.7)	63.8	(CH)	20, 21b	17, 18, 20, 21, 23
7	5.92 (dd, 14.7, 11.1)	136.8	(CH)	6, 8	4, 5, 6, 8, 9, 17	20	1.30 (m)[c]	34.2	(CH)	19, 21a, 23	19, 21, 22, 23
8	2.36 (dd, 11.9, 11.1)	59.0	(CH)	7, 9	6, 7, 9, 10, 17, 18	21a	1.59 (m)	27.7	(CH_2)	20, 21b, 22	19, 20, 22, 23
9	1.88 (m)[b]	38.0	(CH)	8, 10b, 14	8, 10, 11, 12, 13	21b	1.30 (m)[c]			21a, 22	
10a	1.90 (m)[b]	31.8	(CH_2)	10b, 11	8, 9, 11, 12	22	0.93 (m)[c]	11.2	(CH_3)	21a,b	20, 21
10b	1.48 (m)			9, 10a		23	0.94[c]	15.3	(CH_3)	20, 21b	19, 20, 21
11	5.35 (br s)	121.2	(CH)	10a, 26	9, 10, 26	24	1.41 (s)	15.7	(CH_3)		17, 18, 19
12		134.0	(C)			25	1.72 (s)	18.1	(CH_3)	15	14, 15, 16, 17
13a	2.02 (m)[b]	37.1	(CH_2)	13b	9, 11, 12, 26	26	1.65 (s)	23.4	(CH_3)	11	11, 12, 13
13b	1.77 (t, 14.7)			13a, 14							

[a] The number of attached protons was determined from HSQC-DEPT experiment. [b] Overlapping signals. ^{1}H NMR chemical shifts were determined from HSQC-DEPT experiment. [c] Overlapping signals.

acid and the two trisubstituted double bonds formed one extensive spin system, but the elucidation was complicated by the presence of overlapping signals (9-H/10a-H and 13a-H/14-H).



	R_1	R_2	R_3
1	OH	Me	H
2	OH	CH_2OH	H
3	H	CH_2OH	H
4	OH	CHO	H
5	OH	COOMe	H
6	OH	COOMe	Me
7	OH	Me	*p*-Br-Bzl

Further analysis using CIGAR-HMBC data and starting from the olefinic protons at δ 5.55 and 5.35 (15-H and 11-H, respectively) and the allylic proton at δ 2.36 (8-H) defined a tetradehydrodecalin system with double bonds at C-11/C-12 and C-15/C-16, two methyl groups at C-12 and C-16, a trienoic acid substituent at C-8, and two substituents at C-17 (δ 75.6), one of which must be oxygen. The key correlations were 15-H/C-17, 8-H/C-17, and 13a-H/C-11 (see Table 1). After the construction of this partial structure (C-1−C-17), just one degree of unsaturation was left unaccounted. The substructure attached to C-17 was elucidated starting from the methyl group C-24. This methyl group showed long-range H, C-couplings to C-17 as well as to the oxygenated carbons C-18 and C-19. The remaining *sec*-butyl residue was found to be attached to C-19, as indicated by HMBC correlations between 19-H and C-17,

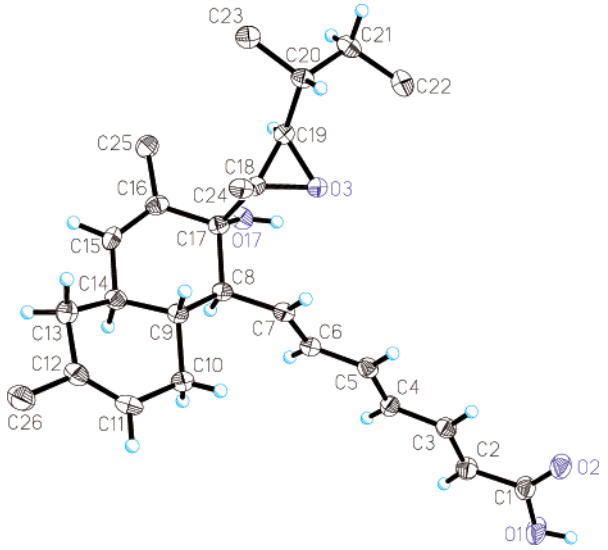


FIGURE 1. Perspective plot of cladobotric acid A (**1**). Two water molecules are contained in the crystal.

C-18, C-20, C-21 and the methyl group C-23. The large $^1J_{CH}$ coupling constant (170 Hz) between C-19 and 19-H and the chemical shifts of C-18 and C-19 suggested an epoxide at C-18/C-19, which completed the molecular formula requirements. This required a free hydroxyl group at C-17. The geometry of the double bonds C-2/C-3, C-4/C-5, and C-6/C-7 was assigned as *E* based on H,H-coupling constants of 15.5, 14.9, and 14.7 Hz, respectively. Thus, the gross structure of cladobotric acid A was elucidated as **1**. This structure was confirmed by X-ray crystallography (Figure 1). The relative configuration was determined to be 8*S**, 9*R**, 14*R**, 17*R**, 18*S**, 19*R**, and 20*R**, while the absolute configuration was subsequently determined to be 8*S*, 9*R*, 14*R*, 17*R*, 18*S*, 19*R*, and 20*R* by X-ray crystallographic analysis of the *p*-bromobenzyl ester of cladobotric acid A (**7**).

The molecular formula of cladobotric acid B (**2**) was found to be $C_{26}H_{36}O_5$. The NMR data were similar to those of cladobotric acid A (**1**; Tables 2 and 3), except that Me-26 was replaced by a hydroxymethyl group, as revealed by signals for diastereotopic methylene protons at δ 4.03 and 4.00 ($^2J_{H,H}$ = 12.7 Hz) in the ^{1}H NMR spectrum. These protons were correlated to an oxymethylene carbon at δ 67.1 in the HSQC

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TABLE 2. ^{1}H NMR Data (in $CDCl_3$ at 23° C) of Cladobotric Acids B−F (2−6)

	2	3	4	5	6
2	5.71 (d, 15.5)	5.80 (d, 15.1)	5.73 (d, 15.3)	5.68 (d, 15.3)	5.87 (d, 15.1)
3	7.21 (dd, 15.5, 11.5)	7.36 (dd, 15.1, 11.5)	7.22 (dd, 15.3, 11.5)	7.17 (dd, 15.3, 11.3)	7.29 (dd, 15.1, 11.5)
4	6.16 (m)[a]	6.23 (dd, 14.9, 11.5)	6.21 (dd, 14.6, 11.5)	6.18 (m)[a]	6.27 (dd, 15.1, 11.5)
5	6.59 (dd, 15.1, 10.7)	6.70 (dd, 14.9, 10.3)	6.60 (dd, 14.6, 10.9)	6.56 (dd, 15.1, 11.2)	6.64 (dd, 15.1, 10.7)
6	6.15 (m)[a]	6.14 (dd, 15.1, 10.3)	6.18 (dd, 14.6, 10.9)	6.16 (m)[a]	6.20 (dd, 15.1, 10.7)
7	5.95 (dd, 15.1, 11.5)	6.15 (dd, 15.1, 10.9)	5.95 (dd, 14.6, 11.4)	5.92 (dd, 14.7, 11.1)	5.95 (dd, 15.1, 11.1)
8	2.39 (t, 11.5)	2.33 (dt, 10.9, 6.7)	2.43 (t, 11.4)	2.39 (dd, 11.9, 11.1)	2.36 (dd, 12.3, 11.1)
9	1.97 (m)	1.73 (m)	2.03 (m)[a]	1.97 (m)[a]	1.97 (m)
10	1.99 (m), 1.55 (m)	2.07 (m), 1.51 (m)	2.34 (m), 1.91 (m)	2.19 (br d, 18.6), 1.67 (m)	2.20 (br d, 19.8), 1.68 (m)
11	5.66 (br s)	5.66 (br s)	6.77 (m)	6.92 (m)	6.92 m
13	2.22 (dd, 16.7, 0.8), 1.83 (dd, 16.7, 11.7)	2.20 (dd, 14.5, 2.0), 1.83 (m)	2.61 (br d, 16.4), 1.81 (m)	2.62 (br d, 15.9), 1.97 (m)[a]	2.61 (br d, 16.7), 1.95 (m)
14	2.09 (m)	1.96 (m)	2.03 (m)[a]	2.05 (m)	2.03 (m)
15	5.59 (s)	5.46 (s)	5.62 (br s)	5.61 (br s)	5.62 (br s)
17		1.79 (m)			
19	3.16 (d, 8.7)	2.47 (d, 8.7)	3.16 (d, 8.5)	3.20 (d, 8.7)	2.96 (d, 9.1)
20	1.31 (m)[a]	1.29 (m)[a]	1.30 (m)[a]	1.31 (m)[a]	1.28 (m)[a]
21	1.59 (m), 1.31 (m)[a]	1.65 (m), 1.29 (m)[a]	1.60 (m), 1.31 (m)[a]	1.60 (m), 1.33 (m)[a]	1.97 (m), 1.30 (m)[a]
22	0.93 (m)[a]	0.92 (m)[a]	0.93 (m)[a]	0.91 (m)[a]	0.92 (m)[a]
23	0.94 (m)[a]	0.95 (m)[a]	0.95 (m)[a]	0.94 (m)[a]	0.93 (m)[a]
24	1.40 (s)	1.28 (s)	1.39 (s)	1.39 (s)	1.35 (s)
25	1.72 (s)	1.72 (s)	1.74 (s)	1.74 (s)	1.72 (s)
26	4.03 (d, 12.7), 4.00 (d, 12.7)	4.04 (d, 13.1), 3.99 (d, 13.1)	9.43 (s)		
22-CO_2*Me*					3.73 (s)
26-CO_2*Me*				3.72 (s)	3.74 (s)

[a] Overlapping signals.

TABLE 3. ^{13}C Chemical Shift Data[a] (in $CDCl_3$ at 23 °C) of Cladobotric Acids B−F (2−6)

	2	3	4	5	6		2	3	4	5	6		2	3	4	5	6
1	170.2	171.7	169.8	169.6	167.5	11	123.3	123.3	150.4	139.2	139.3	20	34.2	34.6	34.2	34.1	34.3
2	120.1	119.1	120.4	120.5	120.5	12	137.2	137.5	141.6	130.2	130.1	21	27.7	27.5	27.7	27.8	27.8
3	146.6	147.0	146.3	146.2	144.7	13	32.7	33.1	28.4	31.3	31.3	22	11.2	11.1	11.2	11.1	11.2
4	129.2	128.0	129.6	129.6	129.3	14	37.9	37.9	37.9	37.6	37.7	23	15.4	15.3	15.3	15.3	15.3
5	140.7	142.1	140.4	140.4	140.1	15	132.1	129.3	131.3	131.5	131.8	24	15.6	15.5	15.6	15.6	15.4
6	133.7	130.2	134.0	133.9	133.4	16	132.9	132.4	134.6	134.6	134.3	25	18.1	23.0	18.0	18.1	18.1
7	136.9	141.8	136.2	136.1	136.5	17	75.6	53.2	75.6	75.6	75.3	26	67.1	67.1	193.8	167.6	167.6
8	58.8	48.8	58.6	58.6	58.7	18	64.3	61.5	64.2	64.8	62.6	22-CO_2*Me*				51.6	51.6
9	38.0	36.2	37.8	37.3	37.3	19	63.6	68.8	63.6	63.7	63.0	26-CO_2*Me*					51.6
10	31.4	31.4	32.7	32.1	32.1												

[a] The ^{13}C chemical shifts were determined from HSQC-DEPT and CIGAR experiments.

experiment. In the CIGAR experiment, cross peaks were observed between 26-H and C-11, C-12 and C-13, confirming that the hydroxymethyl group was attached at C-12.

^{1}H−^{1}H coupling patterns, supplemented with NOE observations from 1D ROESY experiments, established the relative configurations at C-8, C-9, C-14, C-18, and C-19 of **2.** A $^3J_{H\text{-}8,H\text{-}9}$ coupling constant of 11.5 Hz implied axial/axial positioning (trans) of 8-H and 9-H. The irradiation of 14-H and 13a-H enhanced the signals of 8-H, 10b-H, and 14-H/13b-H, respectively, requiring that 8-H, 10b-H, 13a-H, and 14-H be on the same face of the decalin moiety. As no NOE was observed between 9-H and 14-H, these data suggested a trans decalin system. Furthermore, no NOE was observed between 24-Me and 19-H, suggesting a trans configuration also for the epoxide ring. The identical chemical shift of C-17 in **1** and **2** suggested the same relative configuration at this stereogenic center for each compound. Thus, the relative stereochemistry of cladobotric acid B (**2**) is identical with that of **1**.

Cladobotric acid C (**3**) had a molecular formula of $C_{26}H_{36}O_4$, that is, one oxygen atom less than cladobotric acid B (**2**). The NMR data showed a close resemblance between **2** and **3**, with the most significant difference being a methine carbon at δ 53.2 (δ_H 1.79) instead of the oxygen-bearing quaternary carbon C-17 (δ_C 75.6) in **2** (Tables 2 and 3). The position of this methine carbon at C-17 in the structure of **3** was unequivocally established by long-range H and C couplings between δ_H 1.79 (17-H) and C-8, C-9, C-15, C-16, C-18, C-19, C-24, and C-25, showing **3** to be the 17-deoxy derivative of **2**. As was shown for **2**, the relative configuration of **3** was established as 8*S**, 9*R**, 14*R**, 17*S**, 18*R**, 19*R**, and 20*R** by the evaluation of coupling constants and NOEs. The apparent difference in configuration at C-17 and C-18 is due to the lower priority of the hydrogen substituent as compared to that of a hydroxyl.

Analysis of the HRESIMS and NMR data of **4** disclosed that this cladobotric acid was the formyl derivative of cladobotric acid A (**1**; Tables 2 and 3). In the ^{1}H NMR spectrum, a formyl singlet was observed at δ 9.43, correlating in the HSQC experiment with a carbonyl carbon at δ 193.8. The signals of 11-H (δ 6.77), C-11 (δ 150.4), and C-12 (δ 141.6) were significantly deshielded compared to those of **1** (11-H, δ 5.35; C-11, δ 121.2; C-12, δ 134.0). Together with long-range couplings between 26-H and C-12 and C-13, this indicated the formyl group attachment to be at C-12. Similar ^{1}H−^{1}H coupling

TABLE 4. ^{13}C NMR Analysis of Cladobotric Acid A (1) and Cladobotric Acid E (5) Biosynthesized in the Presence of Variously Labeled Precursors[a]

	1				**5**			
	[1,2-^{13}C]Ac,[b,c] J_{CC}, Hz	[1-^{13}C]Ac[d,e]	[2-^{13}C]Ac[d,e]	[Me-^{13}C]Met[d,e]	[1,2-^{13}C]Ac,[b,f] J_{CC}, Hz	[1-^{13}C]Ac[d,g]	[2-^{13}C]Ac[d,g]	[Me-^{13}C]Met[d,g]
1	75.1	14.1	0.9	0.8	74.8	11.1	0.8	0.7
2	75.1, 67.7	0.7	13.9	0.9	74.8, 68.6	0.7	11.0	0.5
3	67.7, 55.7	16.0	1.0	1.0	68.6, 56.1	13.6	1.0	0.9
4	68.5, 55.9	0.8	16.1	1.3	ND	0.8	12.9	0.9
5	68.5, 55.9	15.7	0.8	1.1	73.0, 56.2	11.7	0.9	0.9
6	ND	0.8	14.0	0.9	ND	1.0	14.0	1.1
7	78.4, 42.6	13.0	0.8	1.0	71.3, 44.4	11.0	0.9	1.0
8	42.6, 37.7, 36.2	0.7	13.9	0.9	44.4, 41.7, 37.1	0.9	12.1	1.0
9	ND	12.5	0.8	0.9	ND	10.1	0.9	1.0
10	41.1, 35.3	0.8	14.8	1.0	38.8, 34.4	1.0	14.3	1.0
11	72.1, 41.1	14.9	0.9	0.9	70.1, 38.8	12.5	0.9	0.9
12	ND	0.9	13.3	0.8	ND	1.4	16.1	1.1
13	35.7, 34.2	15.2	0.9	0.9	34.9, 34.0	12.6	1.0	0.9
14	ND	0.8	13.7	1.0	ND	1.1	13.8	0.9
15	70.6, 37.9	15.2	0.9	1.1	72.1, 41.5	9.9	0.9	0.9
16	ND	0.8	12.4	0.7	ND	1.0	14.4	1.0
17	44.7, 41.1, 36.2	11.1	0.8	0.9	45.1, 44.4, 38.6	8.8	0.7	0.8
18	41.1, 32.1	0.8	13.5	0.8	45.1, 32.6	1.0	12.7	0.8
19	44.3, 32.1	15.0	0.8	0.8	44.4, 32.6	12.2	0.9	0.9
20	44.3, 34.2	0.8	14.0	0.8	44.4, 34.2	1.1	13.2	0.8
21	34.9, 34.2	16.2	0.9	1.0	34.9, 34.2	12.9	0.9	0.9
22	34.9	0.9	15.3		34.9	1.3	14.5	
23				15.9				15.6
24				16.1				16.0
25				15.1				13.8
26				16.4				15.1
CO_2*Me*								11.5

[a] Ac, acetate; Met, *S*-methionine. [b] ND, not determined due to signal overlapping. [c] The 11.8% incorporation of [1,2-^{13}C]Ac was estimated by the comparison between the integrals of the natural ^{13}C abundance signal (1.1%) and the integrals of the coupling satellite signals. ^{13}C−^{13}C coupling constants were obtained from the ^{1}H-decoupled ^{13}C NMR spectrum of **1** biosynthesized in the presence of [1,2-^{13}C]Ac. [d] Enrichment factors for the labeled **1** and **5** were determined as the ratio between the relative peak heights [normalized to the average intensity of C-23, C-24, C-25, C-26, and C-26 methyl esters (only for **5**) for the acetate experiments and to the intensity of C-22 in the methionine experiment] observed for the labeled compounds and the relative peak heights observed in the natural abundance spectrum. [e] The 15.7, 15.7, and 17.5% incorporation of [1-^{13}C]Ac, [2-^{13}C]Ac, and [Me-^{13}C]*S*-Met, respectively, for the labeled **1** were estimated by the comparison between the average of the normalized peak heights observed for the labeled compounds and the average of the peak heights observed in the natural abundance spectrum (1.1%). [f] The 11.7% incorporation of [1,2-^{13}C]Ac and the ^{13}C−^{13}C coupling constants of **5** were estimated as described above in footnote *c*. [g] The 12.5, 15.0, and 15.8% incorporation of [1-^{13}C]Ac, [2-^{13}C]Ac, and [Me-^{13}C]*S*-Met, respectively, for the labeled **5** were estimated as described above in footnote *e*.

constants and NOE correlations to those of **1** and **2** were observed for **4**, suggesting again identical relative stereochemistry.

The NMR data of cladobotric acids E (**5**) and F (**6**) again indicated a close resemblance to **1**, **2**, and **4** (Tables 2 and 3). The ^{1}H NMR spectrum of compound **5** showed one additional signal for a methoxyl group (δ 3.72), while the spectrum of **6** contained two methoxyl signals (δ 3.73 and 3.74). Besides the signals for the methoxyl carbons, resonances for the carbonyl carbons (**5** and **6**: δ 167.6) were observed in the ^{13}C NMR spectrum of both compounds. In each case, long-range H and C correlations between 11-H/δ 167.6 (C-26) and between the methoxyl protons at δ 3.72 (**5**) and δ 3.74 (**6**), respectively, and C-26 and C-12 were observed, placing a methyl carboxylate grouping at C-12. The second methoxyl group of **6** showed long-range H, C-correlations to C-1 and C-2, indicating that the terminal acid group of the side chain (C-1) is also methylated. Similar ^{1}H−^{1}H coupling constants and NOE correlations to those of **1**, **2**, and **4** were observed for **5** and **6**, establishing identical relative stereochemistry for all compounds.

Cladobotric acids A−F (**1**−**6**) constitute the first examples of an unusual carbon skeleton for fungi, arising as the outcome of an intriguing ring closure of the polyketide chain. The related structures of mycoparasitic acids A and B (which are methylated at C-17) have been published, but the authors did not present any spectral evidence supporting the structures.[8] Related to the cladobotric acids are the antifungal polyketides hamigerone and dihydrohamigerone,[9] which also possess a substituted decalin moiety but differ in the methylation pattern as well as the nature of the side chain and the degree of oxidation.

The unprecedented structures of cladobotric acids A−F (**1**−**6**) prompted a study of their biosynthesis by incorporation experiments with ^{13}C-labeled precursors. From these experiments, only **1** and **5** could be isolated in sufficient quantity for the evaluation of the labeling patterns.

The ^{13}C spectrum of **1** produced in the presence of [1-^{13}C]-acetate showed strong enrichment for 11 carbon signals (on average 15.7% ^{13}C incorporation at all odd-numbered positions), while those labeled with [2-^{13}C]acetate also exhibited strong enrichment for 11 signals (on average 15.7% ^{13}C incorporation at all even-numbered positions; Table 4). The 2D INADEQUATE spectrum of **1** after the incorporation of [1,2-$^{13}C_2$]-acetate (11.8% enrichment) displayed ^{13}C−^{13}C couplings between 11 pairs of carbons, revealing the carbons originating from the same acetate unit. These data unambiguously disclosed

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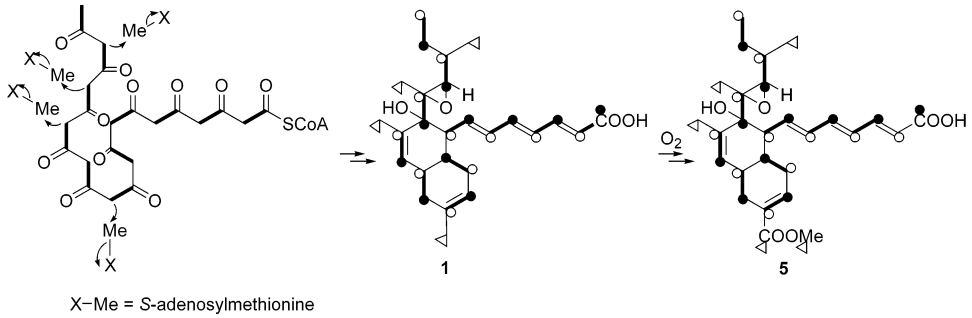
SCHEME 1. Biosynthesis of Cladobotric Acids A (1) and E (5) on the Basis of the Data from Feeding Experiments with ^{13}C-Labeled Acetate and ^{13}C-*S*-Methionine[a]



[a] The 11 intact acetate units, defined by 2D INADEQUATE measurements of **1** labeled with [1,2-$^{13}C_2$]acetate, are represented with bold lines. The positions of the 11 carbons enriched by [1-^{13}C]acetate are represented as filled circles, the 11 carbons enriched by [2-^{13}C]acetate are represented as unfilled circles, and the methyls enriched by [Me-^{13}C]-*S*-methionine are represented as triangles.

a C-1−C-22 polyketide chain assembled from 11 intact C_2 units (Scheme 1). Taking into account the labeling pattern of **1** obtained in the feeding experiments with [1-^{13}C]- and [2-^{13}C]-acetate as well as the presence of the C-1 carboxyl group, the position of the double bonds, the oxygen-bearing groups, and the implied sites of cyclization, C-21−C-22 was defined to be the starter unit and C-1−C-2 was defined to be the terminal unit of the polyketide chain. It is interesting to note that in the cladobotric acids, the cyclization of the polyketide chain does not follow the folding pattern usually observed in fungal polyketides.[10] The feeding experiments with acetate had accounted for the origin of 22 out of 26 carbons of **1** but left the origin of four methyl carbons (C-23, C-24, C-25, and C-26) uncertain. Feeding experiments with [Me-^{13}C]-*S*-methionine led to the strong enrichment of the signals of all four methyl carbons, strongly suggesting that their origin was by the methylation of the polyketide precursor with *S*-adenosylmethionine.

Since cladobotric acids A, B, and D−F (**1**, **2**, and **4**−**6**) differ mainly in the degree of oxidation of C-26, a stepwise oxidation of this carbon was presumed. In fact, analysis of the data from the feeding experiments of **5** confirmed an identical biosynthetic mechanism to that observed for **1** and showed that the C-26 carboxyl group is methionine-derived. Furthermore, these data disclosed that the methyl ester group of the C-26 carboxyl group of **5** is also derived from methionine (Scheme 1).

Compounds **1**−**6** showed cytotoxicity against murine P388 leukemia cells[11] with IC_{50} values of 6.58, 27.78, 19.37, 24.91, 1.41, and 15.57 μM, respectively. In an agar diffusion assay, all cladobotric acids exhibited antimicrobial activity at 100 μg/disc[12] against *Bacillus subtilis* with inhibition zones[13] of 9, 2, 9, 1, 4, and 1 mm, respectively, compounds **1**, **3**, and **5** were active against *Candida albicans* with inhibition zones of 5, 1, and 5 mm, respectively, compounds **1** and **5** were active against *Trichophyton mentagrophytes* with inhibition zones of 3 and 2 mm, respectively, and compound **5** was active against *Cladosporium resinae* with a 1 mm inhibition zone.

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(13) In the agar diffusion assay, the antimicrobial activity against all strains was measured as the radius of inhibition zone, excluding disc.

Experimental Section

Fungus. The fungus was isolated from a fallen twig in a podocarp forest near Hokitika, New Zealand. The fungus was identified as a *Cladobotryum* species on the basis of the characteristic microscopic features of short chains of oval, septate, hyaline conidia with a distinct basal scar, borne on phialides arranged in verticillate whorls. A voucher of the fungus was deposited in the culture collection of the School of Biological Sciences, University of Canterbury (CANU E1042). For chemical investigation, the fungus was cultured for 28 days in half-strength SDY[2] (6 × 500 mL) at 26 °C under static conditions.

Extraction. The mycelium was separated from the culture medium, macerated, and extracted with EtOAc (3 × 300 mL). The culture broth (3 L) was extracted with EtOAc (3 × 1.5 L). The combined EtOAc extracts were dried to yield a crude extract (1.5 g) that was then partitioned between petroleum ether and water, followed by partitioning between EtOAc and water. The resulting petroleum ether and EtOAc phases were taken to dryness (1.1 g and 270 mg of extract, respectively).

Isolation of Cladobotric Acids A−F (1−6). The petroleum ether extract was chromatographed on a semipreparative HPLC column (Phenomenex Luna C18, 10 × 250 mm, 5 μm) using isocratic conditions [water + 0.05% (v/v) TFA (A), MeCN (B); isocratic, 75% B; flow, 5 mL/min; UV detection at 210 nm]. Compounds **1** (124.2 mg), **2** (5.0 mg), **5** (22.8 mg), and **6** (3.8 mg) were eluted after 12.6, 6.2, 8.3, and 11.3 min, respectively.

The EtOAc extract was chromatographed on the same semipreparative HPLC column using gradient conditions (gradient, 0 min at 20% B and 30 min at 80% B; flow, 5 mL min^{-1}; UV detection at 210 nm). Compounds **1** (12.8 mg), **2** (10.0 mg), **3** (4.4 mg), **4** (2.3 mg), and **5** (12.5 mg) were eluted after 38.1, 21.5, 26.0, 25.0, and 30.3 min, respectively.

Cladobotric Acid A (1): Yellowish plates, 9.13% of the dry wt of the crude extract; $[\alpha]^{20}_D$ −87° (*c* 0.11, $CHCl_3$); mp 136−139 °C; UV (MeOH, ϵ) λ_{max} 302 (19 100); IR (film) ν_{max} 3153, 1699, 1230 cm^{-1}; for ^{1}H and ^{13}C NMR data and results from COSY, HSQC, and CIGAR experiments see Table 1; HRESIMS (*m*/*z*) calcd for $C_{26}H_{37}O_4$, 413.2692; found, 413.2693 $[M + H]^+$.

Cladobotric Acid B (2): Yellowish solid, 1.00% of the dry wt of the crude extract; $[\alpha]^{20}_D$ −56° (*c* 0.5, $CHCl_3$); UV [MeOH/CH_2Cl_2 (3:1), ϵ] λ_{max} 300 (19 050); IR (film) ν_{max} 3420, 1686, 1265 cm^{-1}; for ^{1}H and ^{13}C NMR data see Tables 2 and 3; HRESIMS (*m*/*z*) calcd for $C_{26}H_{37}O_5$, 429.2641; found, 429.2635 $[M + H]^+$.

Cladobotric Acid C (3): Yellowish solid, 0.29% of the dry wt of the crude extract; $[\alpha]^{20}_D$ −43.3° (*c* 0.39, $CHCl_3$); UV [MeOH/CH_2Cl_2 (3:1), ϵ] λ_{max} 302 (19 040); IR (film) ν_{max} 3396, 1684, 1269; for ^{1}H and ^{13}C NMR data see Tables 2 and 3; HRESIMS (*m*/*z*) calcd for $C_{26}H_{37}O_4$, 413.2692; found, 413.2686 $[M + H]^+$.

Cladobotric Acid D (4): Yellowish solid, 0.15% of the dry wt of the crude extract; $[\alpha]^{20}_D$ −53.5° (*c* 0.2, $CHCl_3$); UV [MeOH/CH_2Cl_2 (3:1), ϵ] λ_{max} 300 (19 037); IR (film) ν_{max} 3160, 1686, 1674, 1205 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 2 and 3; HRESIMS (*m*/*z*) calcd for $C_{26}H_{33}O_5$, 425.2328; found, 425.2330 $[M - H]^-$.

Cladobotric Acid E (5): Yellowish solid, 2.35% of the dry wt of the crude extract; $[\alpha]^{20}_D$ −41.5° (*c* 0.13, $CHCl_3$); UV [MeOH/CH_2Cl_2 (3:1), ϵ] λ_{max} 300 (19 046); IR (film) ν_{max} 3177, 1713, 1693, 1267 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 2 and 3; HRESIMS (*m*/*z*) calcd for $C_{27}H_{37}O_6$, 457.2590; found, 457.2585 $[M + H]^+$.

Cladobotric Acid F (6): Yellowish solid, 0.25% of the dry wt of the crude extract; $[\alpha]^{20}_D$ −57.4° (*c* 0.38, $CHCl_3$); UV [MeOH/CH_2Cl_2 (3:1), ϵ] λ_{max} 304 (19 057); IR (film) ν_{max} 3094, 1718, 1701, 1269 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 2 and 3; HRESIMS (*m*/*z*) calcd for $C_{28}H_{39}O_6$, 471.2747; found, 471.2741 $[M + H]^+$.

X-ray Structure Determination of 1. Cladobotric acid A (**1**) was crystallized from Et_2O. A plate crystal with dimensions of 0.9 × 0.4 × 0.07 mm^3 was used for data collection.

A Bruker-Nonius APEX II area detector system equipped with a nitrogen low-temperature gas-flow device was used to collect a full sphere of data with Mo Kα radiation. The data processing program SAINT[14] yielded 11 157 Bragg reflections of which 52% were unique. The structure solution, refinement, and resulting table and diagrams were all produced using the SHELXTL suite of programs.[15,16] The final *R* factor was 5.96%.

X-ray Determination of the Absolute Configuration of 1. The *p*-bromobenzyl ester of **1** (**7**) was prepared via an EDCI coupling reaction (EDCI, 12.46 mg, 0.065 mmol; HOBt, 10.13 mg, 0.075 mmol; DIPEA, 25.85 μL, 0.2 mmol; 5 mL of DCM) of **1** (20.6 mg, 0.05 mmol) and *p*-bromobenzyl alcohol (10.23 mg, 0.055 mmol). The reaction mixture was stirred for 1 day at room temperature and taken to dryness, and the solid obtained was partitioned between water (10 mL) and EtOAc (10 mL). The EtOAc layer was chromatographed on the same semipreparative HPLC column as described above using gradient conditions (0 min - 70% B, 14 min - 100% B, and 26 min - 100% B; flow, 5 mL min^{-1}; UV detection at 330 nm). Compound **7** (7.4 mg) was eluted after 17.52 min and subsequently crystallized from MeOH. A block crystal with dimensions of 0.4 × 0.4 × 0.2 mm^3 was used for data collection. Although poor crystal quality restricted the final *R* factor to 8.6%, this was definitively better than the 10.2% given for the enantiomeric structure.

Compound 7: Transparent block crystal; $[\alpha]^{20}_D$ −41.1° (*c* 0.74, $CHCl_3$); mp 130−132 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 0.93 (m, 6H, 22-H, 23-H), 1.28 (m, 2H, 21b-H, 20-H), 1.37 (s, 3H, 24-H), 1.49 (m, 1H, 10b-H), 1.61 (m, 1H, 21a-H), 1.65 (s, 3H, 26-H), 1.71 (s, 3H, 25-H), 1.78 (m, 1H, 13b-H), 1.91 (m, 2H, 9-H, 10a-H), 2.05 (m, 2H, 13a-H, 14-H), 2.34 (t, *J* = 11.5 Hz, 1H, 8-H), 2.96 (d, *J* = 8.8 Hz, 1H, 19-H), 5.12 (s, 2H, 1′-H), 5.35 (br s, 1H, 11-H), 5.57 (s, 1H, 15-H), 5.88 (d, *J* = 15.2 Hz, 1H, 2-H), 5.98 (dd, *J* = 14.8 Hz, *J* = 11.5 Hz, 1H, 7-H), 6.17 (dd, *J* = 14.8 Hz, *J* = 10.9 Hz, 1H, 6-H), 6.24 (dd, *J* = 14.9 Hz, *J* = 11.3 Hz, 1H, 4-H), 6.65 (dd, *J* = 14.9 Hz, *J* = 10.9 Hz, 1H, 5-H), 7.24 (d, *J* = 8.1 Hz, 2H, 4′-H, 6′-H), 7.32 (dd, *J* = 15.2 Hz, *J* = 11.3 Hz, 1H, 3-H), 7.48 (d, *J* = 8.1 Hz, 2H, 3′-H, 7′-H); ^{13}C chemical shift data ($CDCl_3$) δ 11.3 (C-22), 15.4 (C-23), 15.5 (C-24), 18.2 (C-25), 23.4 (C-26), 27.9 (C-21), 31.9 (C-10), 34.4 (C-20), 37.1 (C-13), 38.0 (C-9), 38.4 (C-14), 59.1 (C-8), 62.4 (C-18), 63.1 (C-19), 65.2 (C-1′), 75.4 (C-17), 119.8 (C-2), 121.2 (C-11), 122.2 (C-2′), 128.8 (C-4), 129.8 (C-4′, C-6′), 131.7 (C-3′, C-7′), 132.7 (C-15), 133.2 (C-16), 134.1 (C-12), 135.2 (C-5′), 137.8 (C-7), 139.9 (C-6), 140.9 (C-5), 145.5 (C-3), 166.7 (C-1); HRESIMS (*m*/*z*) calcd for $C_{33}H_{42}{}^{79}BrO_4$, 581.2266; found, 581.2272 $[M + H]^+$.

Biosynthetic ^{13}C Labeling. Feeding experiments were performed using [1-^{13}C]CH_3COONa, [2-^{13}C]CH_3COONa, [1,2-^{13}C]CH_3COONa, and [Me-^{13}C]-*S*-methionine (99% ^{13}C). The precursors were added to *Cladobotryum* cultures (200 mL) in the second week of growth (final concentration for labeled acetates was 1 mg/mL and for labeled *S*-methionine was 0.1 mg/mL). The fermentation was continued for 10 more days. Cladobotric acids A (**1**) and E (**5**) were then isolated as described above.

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Supporting Information Available: General experimental procedures, 1D and 2D NMR spectra of **1**−**7**, ^{13}C NMR spectra of **1** and **5** labeled with [1-^{13}C]acetate, [2-^{13}C]acetate, and [Me-^{13}C]-*S*-methionine, 2D-INADEQUATE spectrum of **1** labeled with [1,2-^{13}C]acetate, the crystal structure of **7**, tables of crystal data and structure refinement, and the full list of bond lengths and angles from the X-ray crystallographic study of **1** and **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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