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Structure Elucidation of L-687,781, A New β-1,3-D-Glucan Synthesis Inhibitor.

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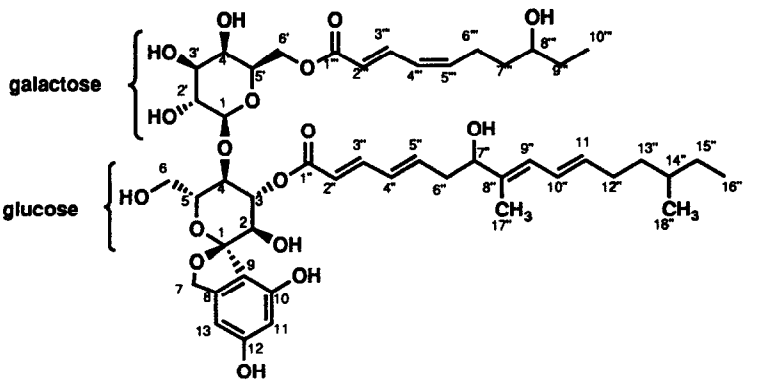
A new β-1,3 glucan synthesis inhibitor, L-687,781 is produced by the cultivation of *Dictyochaeta simplex* ATCC 20960 Through NMR, mass spectroscopy, and degradation studies the structure of L-687,781 was determined to be **1**, a novel member of the papulacandin family of antifungal agents L-687,781 exhibits potent *in vitro* antifungal activity as well as anti-Pneumocystis activity in a rat model

The recent increase in reports of opportunistic infections has pointed out the urgent need for new antifungal therapies [1] In the course of our efforts to discover novel antibiotics, we found that cultivation of a species of *Dictyochaeta simplex* produces L-687,781 (**1**), a new β-1,3 glucan synthesis inhibitor [2] L-687,781 and other β-1,3 glucan synthesis inhibitors were shown to be effective in treating AIDS related *Pneumocystis carinii* infections in a rat model [2,3] Herein, we report the structure determination of L-687,781 as a novel member of the papulacandin family of antifungal agents The structure was determined and the NMR assignments were made on the basis of 2D-NMR, mass spectroscopy, and degradation studies



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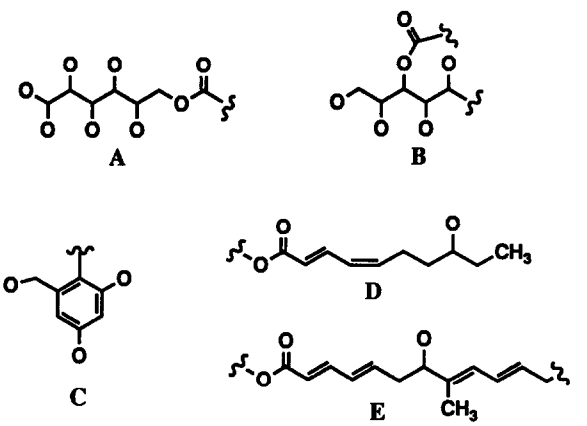
The molecular formula for L-687,781 was not immediately derivable from mass spectral measurements FAB-MS indicated a molecular weight of 902 for L-687,781 [(M+H) at m/z 903 and (M+Na) at m/z 925], but the peak was not intense enough for precise mass determination The ^{13}C-NMR spectra exhibited 45 distinct resonances with two double intensity peaks implying the presence of 47 carbons The APT spectra accounted for 57 carbon bound hydrogens With the assumption that the remainder of the

molecule contains only oxygen and exchangeable hydrogens, the three sets of data then suggest an empirical formula of $C_{47}H_{66}O_{17}$, with 9 exchangeable hydrogens.

Consistent with the presence of a diene and α,β–unsaturated esters, the UV spectrum exhibits maxima at 230, 240, and 262 nm and the IR spectrum shows a strong absorbance at 1700 cm^{-1} Structural subunits were constructed using 2D-NMR techniques. Fragments **A** through **E** were identified from short and long range $^1H,^1H$-COSY, $^1H,^1H$-relayed COSY[4], $^1H,^{13}C$-COSY, and 2D-J resolved ^1H spectra Fragments **A** and **B** are strongly suggestive of the presence of two sugar moieties Fragments **D** and **E** are likely subunits of acyl side chains



These structural features were reminiscent of the papulacandin family of compounds (*vide infra*) [5, 6] As shown in Table 1, a comparison of the ^1H-NMR assignments (CD_3OD) of papulacandin B[6] (**8**) and L-687,781 shows striking similarities. The ^{13}C-NMR assignments are also very similar

Table 1 Comparison of ^1H NMR (CD_3OD) data for L-687,781 and Papulacandin B[6] (δ in ppm, mult, J in Hz)

C no	Papulacandin B			L-687,781		
3'''	7 80	dd	11, 14	7 77	dd	13, 17
3''	7 25	dd	12, 16	7 27	dd	12, 17
11*	6 21	s		6 20	s	
13*	6 24	s		6 22	s	
11''	5 68	dt	8, 16	5 66	dt	8, 17
3	5.44	t	10	5 43	t	10
7	5 02	AB	12	5 03	AB	12
2	4 32	d	10	4 37	d	11
5'	3 65	t	5 5	3 68	t	7

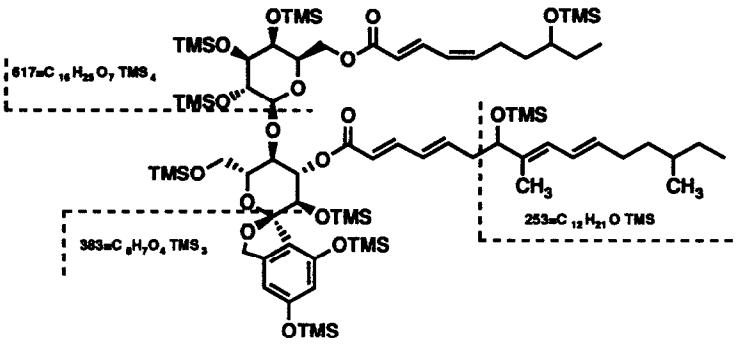
However, a major difference between papulacandin B and L-687,781 is detectable in the ^1H-NMR spectrum A resonance at 6 9 ppm (H-7''') is present in the spectrum of papulacandin B but absent in that of L-687,781 This implies the absence of a double bond in the C_{10} side chain of L-687,781 From the 2D NMR data, a full assignment of the ^1H and ^{13}C NMR data was carried out for L-687,781 (Table 2)

Table 2. NMR assignments of L-687,781 in ppm.

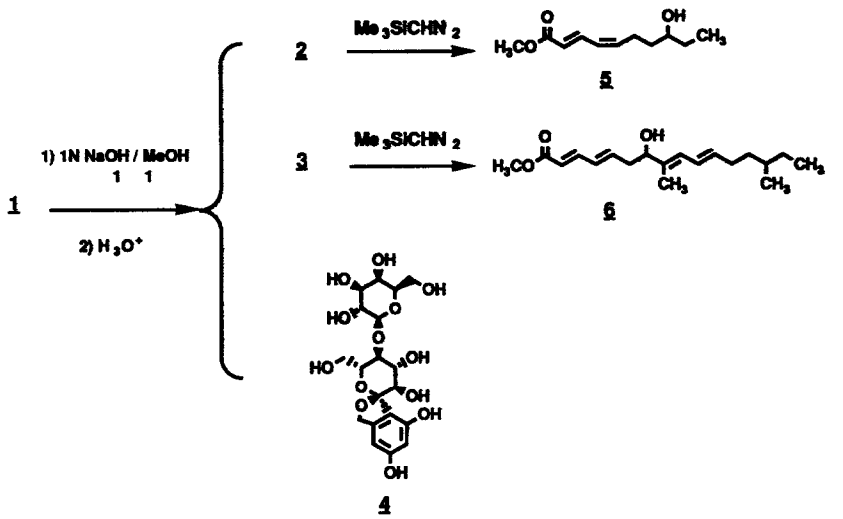
C no.	Cδ	Hδ	mult , *J* (Hz)
1	111 9	none	
2	71.8	4 37	d, 11
3	76 3	5 43	dd, 10, 11
4	77 7	3 94	
5	74 8	4 00	
6	61 5	4 00, 3 78	
7	73 9	5.03	AB q
8	145 5	none	
9	116 4	none	
10	161 6	none	
11	100 0	6 20	
12	154.5	none	
13	103.0	6 22	
1'	105 3	4 34	d, 8
2'	72 5*	3 46	
3'	70 4	3 75	br d, 5
4'	74 6*	3 46	
5'	74 0	3 68	ddd, 8, 5, 2
6'	64 9	4 29	dd, 12, 8
		4.12	dd, 12, 5
1"	169 0	none	
2"	121.6	5 92	d, 17
3"	146 0	7 27	dd, 17,13
4"	127 1	6 25	
5"	141.6	6 07	dt, 17, 8
6"	40 0	2 41	t, 8
7"	77 6	4 05	
8"	137 5	none	
9"	143 0	5 95	
10"	131 5	6 25	
11"	136 2	5 66	dt, 17, 8
12"	31 6	2 12	
13"	30 4	1 12	
14"	35 2	1 38	
15"	37 5	1.55	
16"	11 7	0 88	t, 8
17"	12 2	1 72	bs
18"	19 5	0 88	d, 7
1"'	168.4	none	
2"'	121 9	5 98	d, 16
3"'	141 3	7 78	dd, 17, 13
4"'	127 6	6 22	
5"'	127 1	5 95	
6"'	25 9	2 48	m
7"'	37 5	1 55, 1 20	
8"'	73 2*	3 43	
9"'	31 1	1 55, 1 12	
10"'	10 4	0.91	t, 8

* signals are interchangeable

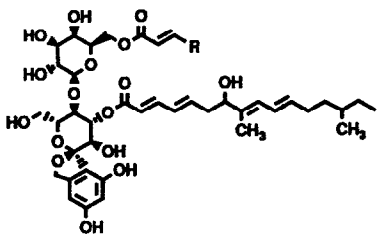
Mass spectral studies also confirmed the papulacandin-like structure for L-687,781 EI (TMS, deutero-TMS) spectra showed a peak at m/z 617, high resolution determined a composition of $C_{16}H_{25}O_7$ TMS_4 Traxler has shown that this glycosidic fragmentation differentiates the various papulacandins [6] L-687,781 contains two hydrogens more than papulacandin B in this fragment. Two other intense ions [m/z 253 = $C_{12}H_{21}O$ TMS by h r., and m/z 383 = $C_8H_7O_4$ TMS_3 by h r] define other portions of the molecule as indicated below.



To confirm the identity of the C_{10} side chain of L-687,781 as well as to confirm the absolute configuration of the spirocyclic core, L-687,781 was subjected to base hydrolysis The three products obtained from the hydrolysis, **2**, **3**, and **4**, exhibited widely different polarities and could be readily separated by partitioning with pH adjustments (see experimental) Acid **2** was esterified with trimethyl silyl diazomethane to ester **5** and the indicated trans, cis double bond relationship could be assigned from the observed coupling constants ($J_{2'''\text{-}3'''}$=18Hz, $J_{3'''\text{-}4'''}$=12Hz, $J_{4'''\text{-}5'''}$=12Hz) Acid **3** was esterified to methyl ester **6** which exhibited identical NMR and MS data to that reported [6] The polar core fragment **4** was isolated by LH-20 chromatography and exhibited identical ^{13}C NMR spectra and sign of optical rotation ($[\alpha]_D$=+20 1°, c=1, MeOH) as the literature values for the hydrolysis product from papulacandin B ($[\alpha]_D$=+30°, c= 25, MeOH) [7] The relative and absolute configurations of the spirocyclic core of papulacandins A, B, C, and D have been assigned based on X-ray crystallographic analysis of a derivatized spirocyclic degradation product [8] Based on this precedent, and the identical spectral properties of degradation product **4** with the literature values, the 10 diglycoside chiral centers were assigned as depicted The only ambiguities which were not addressed in this study concern the absolute configurations of the three side chain chiral centers



The papulacandins were first reported by Traxler *et al* [5,6,9] Papulacandins A (7), B (8) and C (9) all exhibit a spirocyclic diglycoside which contains ester linkages to two unsaturated fatty acids. Papulacandin D lacks the galactose and C_{10} ester moieties A related antifungal compound, chaetiacandin (10), has recently been reported in which the spirocycle is open [10, 11] Thus, L-687,781 is the sixth member of the papulacandin family to be isolated The papulacandins and the cyclic lipopeptide echinocandins are the only two classes of natural products which have been reported to be inhibitors of β-1,3-glucan synthesis.[12] This mode of action is being investigated as a target for antifungal therapy,[13] and further studies will determine whether an inhibitor such as L-687,781 will be useful in human therapeutics



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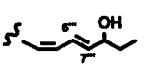
8 R =



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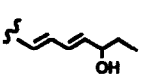

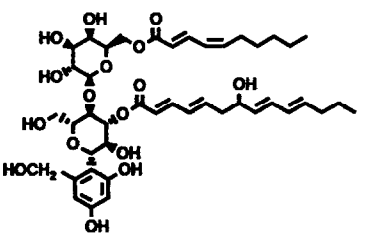

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Experimental

The NMR spectra were measured on a Bruker AM-250 equipped with an Aspect 3000 computer, or a Varian XL-300 Deuteriated methanol was used as the solvent and as the internal reference (δ 3.30 for ^{1}H, and 49 0 for ^{13}C) Mass spectra (70 eV EI and FAB) were recorded employing a Finnigan MAT-90 spectrometer

Degradation (1 to 2, 3, and 4)

L-687,781 (168mg, 186μmol) was dissolved in methanol (17ml) and an aqueous 1.0N NaOH solution (17ml) was added whereupon the reaction immediately turned bright red/orange. After 1hr only three products were observed by HPLC (Vydac RP C_{18}, 25cm x 4.6mm, 1 5ml / min, 50 min gradient from 100% A to 100% B where A = 0 1% TFA in water and B = 0 1%TFA in acetonitrile, UV detection at 215nm) The retention times in this HPLC system were· **1**, 28.2min, **2**, 16.1min; **3**, 30.9min, and **4**, 5.3min.

After 1 5hr 100ml of 0 5M potassium phosphate buffer (pH7 0) was added and crude **3** was extracted into CH_2Cl_2 (2 x 100ml). The organic phase was separated and the solvents were removed *in vacuo* to yield crude **3** as a yellow oil (55mg)

The aqueous layer was then acidified to pH 2 with 5N H_2SO_4, and extracted with CH_2Cl_2 (2 x 100ml) The CH_2Cl_2 layer was separated, back washed with water, and evaporated *in vacuo* to yield crude **2** as a yellow oil (46mg).

The acidified aqueous layer was then lyophilized and the solids triturated with MeOH (20ml) The MeOH solution was applied to a Sephadex LH-20 column (350ml) and eluted with MeOH Fractions containing the polar core **4** were combined and the solvents removed *in vacuo* to yield **4** ^{1}H-NMR (CD_3OD) 3.48-3.88 (11H, m), 4 26 (1H, d, J=11Hz), 4.44 (1H, d, J=8Hz), 5.02 (2H, AB q), 6.21 (1H, s), 6 24 (1H, s) ^{13}C-NMR (CD_3OD) 61.9, 62.6, 70 4, 72 5, 73 2, 73 8, 74 3, 74.7, 74 8, 77.0, 80 3, 100 1, 103 0, 105.0, 111.4, 116 4, 145.4, 154.4, 161 5

Esterification (2 to 5, and 3 to 6)

Crude **2** (50mg) was dissolved in 5ml of ether and 5ml of MeOH and 0 5ml of a 2N hexane solution of trimethyl silyl diazomethane (Aldrich) was added After 45min the solvents were evaporated *in vacuo* and the residue was dissolved in CH_2Cl_2 and filtered through silica gel. The solvents were removed to yield **5** (20mg) as a yellow oil ^{1}H-NMR ($CDCl_3$) 0.85 (3H, t, J=8Hz), 1.35-1 60 (4H, m), 2.42 (2H, q, J=8Hz), 3 40-3 50 (1H, m), 5.85 (1H, m), 5 86 (1H, d, J=18Hz), 6 14 (1H, t, J=12Hz), 7 22 (1H, dd, J=12, 18Hz)

Crude **3** (40mg) was treated as above to yield **6** (13mg) as a colorless oil ^{1}H-NMR ($CDCl_3$) 0.88 (3H, t, J=8Hz), 0.89 (3H, d, J=8Hz), 1.05-1 45 (5H, m), 1.73 (3H, s), 2 04-2 18 (2H, m), 2 45 (2H, t, J=11Hz), 3.73 (3H, s), 4.13 (1H, t, J=9Hz), 5 62-6 35 (6H, m), 7 25 (1H, dd, J=12, 14Hz)

References

1. Mills, J., Masur, H., Scientific American, 50-57, August 1990
2 VanMiddlesworth, F , Nallin-Omstead, M , Schmatz, D , Bartizal, K , Fromtling, R., Bills, G , Nollstadt, K , Honeycutt, S , Zweerink, M., Garrity, G , Wilson, K *J Antibiotics*, **1990,** *44*, 613-619
3 Schmatz, D , Romanchek, M ; Pitterelli, L , Fromtling, R , Schwartz, R , VanMiddlesworth, F , Wilson, K , Turner, M. *Proceedings of the National Academy of Sciences*, **1990,** *87*, 5950-5954
4 Bax, A J , Drobny, G *J Magn Reson* , **1985,** *61*, 306
5 Traxler, P , Gruner, J , Auden, J A L , *J Antibiotics* **1977,** *30*, 289-296
6 Traxler, P , Fritz, H , Fuhrer, H , Richter, W , *J Antibiotics*, **1980,** *33*, 967-978.
7 Traxler, P , Fritz, H , Richter, W , *Helv Chim Acta*, **1977,** *63*, 578-584
8 Rihs, G , Traxler, P , *Helv Chim Acta*, **1981,** *64* , 1533-1539
9 Traxler, P , Tosch, W , Zak, O , *J Antibiotics* , **1987,** *40*, 1146-1164.
10 Komori, T , Yamashita, M , Tsurumi, Y , Kohsaka, M , *J Antibiotics*, **1985,** *38*, 455-459
11 Komori, T , Itoh, Y., *J.Antibiotics* , **1985,** *38*, 544-546
12 Perez, P., Varona, R ; Garcia-Acha, I ; Duran, A , *FEBS Lett* , **1981,** *129*, 249-252
13 Tkacz, J , in *Emerging Strategies for Antibacterial and Antifungal Therapies*, J Sutcliffe and N Georgopapaddkuo (eds), **1991,** Chapman and Hall Co