STEREOSTRUCTURE AND NMR CHARACTERIZATION OF THE ANTIBIOTIC CANDIDIN

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(Received for publication February 22, 1993)

The stereostructure of the heptaene macrolide antibiotic candidin was established on the basis of NMR studies: ^{13}C, DQF-COSY, ROESY and C,H-COSY experiments. The absolute configuration of the candidin chiral centers were assigned as 3*R*, 5*S*, 10*R*, 11*R*, 13*R*, 15*S*, 16*R*, 17*S*, 19*S*, 34*S*, 35*R*, 36*R* and 37*S*.

Candidin, a heptaene macrolide antibiotic exhibiting antifungal activity, is a main component of the antibiotic complex produced by *Streptomyces viridoflavus*[1]. It belongs to the polyene macrolide antibiotics group of which over 200 representatives have been recognized and a considerable number have been characterized by their gross structures. However, only a limited number of works have reported the results of the elucidation of the absolute stereochemistry of this class of compounds. The stereostructures of amphotericin B[2] and roxaticin[3] were determined by X-ray diffraction. The ^1H NMR analysis combined with chemical degradation was applied for elucidation of the stereochemistry of lienomycin[4] and mycoticin[5]. The recent studies reporting the stereostructures of nystatin A1[6], vacidin A[7] and pimaricin[8], implemented with model studies of amphotericin B[9], have offered a general approach for the elucidation of the stereochemistry of polyene macrolides.

The physico-chemical properties and a gross structure of candidin have been already published[10]. In this paper we report the results of the NMR studies of the antibiotic candidin which resulted in the assignment of its absolute configuration.

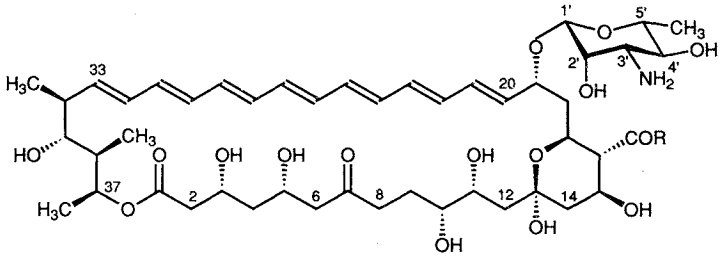
Results and Discussion

Candidin was isolated from the antibiotic complex by counter current distribution in the solvent system chloroform - methanol - borate buffer (pH 8.2) in a ratio of 2:2:1. The partition coefficient for candidin was $K=4.1$.

Candidin (Fig. 1) was subsequently transformed into the methoxycarbonylmethylamide derivative (**2**) which showed a higher solubility in the adopted NMR solvent systems. Candidin methoxycarbonylmethylamide (Cand-Gly-OMe) was purified by flash chromatography in the solvent system chloroform - methanol - water, 25:10:1 and analyzed by NMR spectroscopy.

The spectroscopic measurements were performed with the samples dissolved in two solvent systems, *i.e.* 10% methanol-d_4 in pyridine-d_5 and methanol-d_4 - pyridine-d_5 - dimethylsulfoxide-d_6, 2:2:1. These two

Fig. 1. The structure of candidin (**1**) and its methoxycarbonylmethylamido derivative (**2**).



R=OH; candidin (**1**)
R=$NHCH_2COOCH_3$; candidin methoxycarbonylmethylamide (**2**)

solvent systems were selected for their suitable differentiation of proton chemical shifts. Proton and carbon assignments for Cand-Gly-OMe listed in Tables 1, 2 and 3 were made based upon the DQF-COSY and C-H correlated experiments.

The DQF-COSY spectrum of Cand-Gly-OMe displayed all of the expected connectivities within five structural blocks designated as C-2 ~ C-6, C-8 ~ C-12, C-14 ~ C-20, C-33 ~ C-37 and the mycosamine moiety (Fig. 1). These connectivities resulted in the full proton assignments within all but the C-2 ~ C-6 block. Due to the symmetry of the C-2 ~ C-6 block and the fact that the lactone (C-1) and keto (C-7) carbonyl groups exhibited very similar proton shielding effects, the methylene protons attached to C-2 and C-6 could not be immediately assigned. This was ultimately possible through the analysis of the C-H correlation spectrum; the ^{13}C resonances at 43.1 and 51.2 ppm were assigned to C-2 and C-6, respectively, following an expected stronger deshielding effect induced by the keto group (C-7) against the lactone carbonyl (C-1). This allowed for the distinction of methylene protons attached to C-2 and C-6 and thereby completed the full proton assignment within the C-2 ~ C-6 block.

It should be mentioned that the connectivities within the heptaene chromophore could not be traced due to the strongly coupled spin system displayed by the corresponding protons. Nevertheless the all-*trans* geometry of the heptaene chromophore was assumed based upon the UV spectrum of candidin[11]. The five blocks were assembled on the basis of the following considerations. The 20-H and 33-H protons were identified as a part of the heptaene chromophore spin system, thus, the locations of C-14 ~ C-20 and C-33 ~ C-37 blocks were derived directly from 19-H ~ 20-H and 33-H ~ 34-H connectivities, respectively, as displayed by the DQF-COSY spectrum. The connection between the C-8 ~ C-12 and C-14 ~ C-20 blocks was reflected by the 12-Hb/14-Hb ROE, displayed by the ROESY spectrum. The C-8-end of the C-8 ~ C-12 block was found to be attached to the keto group (C-7) due to the chemical shifts of the C-8 methylene protons. The chemical shifts of the methylene protons attached to C-2 and C-6 indicated that they were located next to carbonyl groups at C-1 and C-7 originating from the lactone moiety and the keto group, respectively, as mentioned above. Thus, the only placement possible for the C-2 ~ C-6 fragment was between C-1 and C-7. Finally, the mycosamine moiety was located at C-19 based upon 1'-H/19-H ROE (Fig. 2).

Conformation $^4C_1$ of the mycosaminyl substituent, of which the D-configuration was earlier established[12], resulted from the following scalar couplings: $J_{2',3'}=3.5$ Hz; $J_{3',4'}=9.5$ Hz; $J_{4',5'}=9.5$ Hz, whereas the β-configuration of the glycosidic bond was assigned from dipolar couplings (ROE's) 1'-H/3'-H and 1'-H/5'-H observed in ROESY spectrum.

Thus, the mycosamine moiety could be used as an internal chiral reference for assignment of the

Table 1. ^{1}H NMR data of Cand-Gly-OMe in solvent system methanol-d_4-pyridine-d_5-DMSO-d_6, 2:2:1.

Proton	δ (ppm)	J (coupling partner) (Hz)	ROE to proton
2a	2.32	17.5 (2b), 9.5 (3)	2b, 4a
2b	2.13	17.5 (2a), 2.5 (3)	2a, 3, 4b[a]
3	4.37	9.5 (2a), 10.0 (4a), 2.7 (2b), 3.0 (4b)	2b, 4b, ol, ol[b]
4a	1.50	14.0 (4b), 10.0 (3), 10.0 (5)	4b, 2a, 5, 6a
4b	1.28	14.0 (4a), 3.0 (3), 3.0 (5)	4a, 3, 5, 2b[a]
5	4.48	10.0 (4a), 3.0 (4b), 10.5 (6a), 2.0 (6b)	4a, 4b, 6b, ol
6a	2.58	16.0 (6b), 10.5 (5)	4a, 6b
6b	2.20	16.0 (6a), 2.0 (5)	5, 6a
8a	2.79	18.5 (8b), 11.5 (9b), 4.0 (9a)	8b, 9a
8b	2.48	18.5 (8a)	8a, 9b, 10
9a	2.06	11.5 (10)	8a, 9b
9b	1.56	11.5 (9a), $\simeq$0 (10)	8b, 9a, 10[a], 11[a]
10	3.18	11.5 (9a), 2.0 (11), $\simeq$0 (9b)	8b, 12b, 9b[a], 11[a]
11	4.15	11.5 (12a), 1.5 (12b), 2.0 (10)	12b, ol, ol, 9b[a], 10[a]
12a	2.08	12.5 (12b), 11.5 (11)	12b
12b	1.54	12.5 (12a), 1.5 (11)	10, 11, 12a, 14b
14a	2.14	12.0 (14b), 4.5 (15)	14b, 15
14b	1.37	12.0 (14a), 11.5 (15)	12b, 14a, 16
15	4.44	4.5 (14a), 11.5 (14b), 10.0 (16)	14a, 17
16	2.21	10.0 (15), 10.0 (17)	14b
17	4.65	10.0 (16), 10.0 (18b), 1.5 (18a)	15, 20, 2′, 18a[a]
18a	2.58	14.5 (18b), 5.5 (19), 1.5 (17)	18b, 1′, 17[a]
18b	1.67	14.5 (18a), 10.0 (17), $\simeq$0 (19)	18a, 19
19	4.55	5.5 (18a), 9.0 (20), $\simeq$0 (18b)	18b, 21, 1′
20	6.15	9.0 (19), 15.5 (21)	
ol[a]			3, 3, 5, 11, 11, 17, 19
32	6.19	15.0 (33)	34
33	5.40	15.0 (32), 10.0 (34)	35, Me34, 36[a]
34	2.39	10.0 (33), 9.5 (35), 6.5 (Me34)	32, 37, Me34
35	3.22	9.5 (34), 2.5 (36)	33, 36, Me34, Me36
36	1.81	2.5 (35), 1.5 (37), 7.2 (Me36)	35, Me36, Me37, 33[a], 37[a]
37	5.40	1.5 (36), 6.5 (Me37)	34, Me36, Me37, 36[a]
Me34	1.12	6.5 (34)	33, 34, 35
Me36	1.02	7.2 (36)	35, 36, 37
Me37	1.20	6.5 (37)	36, 37
1′		$\simeq$0 (2′)	3′, 5′, 18a, 19
2′	4.02	$\simeq$0 (1′), 3.5 (3′)	17
3′	2.78	3.5 (2′), 9.5 (4′)	1′, 5′
4′	3.33	9.5 (3′), 9.5 (5′)	
5′	3.40	9.5 (4′), 6.0 (6′)	1′, 3′, 6′
6′	1.29	6.0 (5′)	5′
Gly-OMe			
$-CH_2-$	3.76, 4.26		
$COOCH_3$	3.72		

[a] Data for spectrum in solvent system pyridine-d_5-methanol-d_4, 9:1.
[b] Olefinc proton.

absolute configuration of the aglycone through the analysis of scalar and dipolar couplings.

The geometry of the C-13$\sim$C-20 fragment (Fig. 2) was derived from vicinal coupling constants $J_{14b,15}$, $J_{15,16}$, $J_{16,17}$ and $J_{17,18b}$ which were in a 10.0$\sim$11.5 Hz range indicating antiperiplanar positions of the 14-Hb, 15-H, 16-H, 17-H and 18-Hb protons. Thus, the hemiketal ring was found to adopt a chair conformation with substituents at C-13, C-15, C-16 and C-17 in equatorial positions. The 18-Hb/19-H

Table 2. ^{1}H chemical shifts of Cand-Gly-OMe in solvent system pyridine-d_6 - methanol-d_4, 9 : 1.

Proton	δ (ppm)	Proton	δ (ppm)	Proton	δ (ppm)
2a	2.59	12b	1.74	36	2.01
2b	2.35	14a	2.47	37	5.69
3	4.70	14b	1.70	Me34	1.24
4a	1.73	15	5.01	Me36	1.18
4b	1.49	16	2.68	Me37	1.37
5	4.79	17	5.19	1′	5.13
6a	2.75	18a	2.98	2′	4.51
6b	2.34	18b	1.93	3′	3.12
8a	2.68	19	4.90	4′	3.73
8b	1.81	20	6.43	5′	3.77
9a	2.43	ol	6.1～6.6	6′	1.51
9b	1.80	32	5.47	Gly-OMe	
10	3.39	33	5.46	$-CH_2-$	3.84, 4.49
11	4.48	34	2.60	$COOCH_3$	3.70
12a	2.41	35	3.37		

Table 3. ^{13}C chemical shifts of Cand-Gly-OMe in solvent system pyridine-d_5 - methanol-d_4, 9 : 1.

Carbon	δ (ppm)	Carbon	δ (ppm)	Carbon	δ (ppm)
1	171.6	14	45.8	Me36	12.2
2	43.1	15	65.9	Me37	16.9
3	67.5	16	58.8	12	43.7
4	43.4	17	65.9	13	98.6
5	67.5	18	37.6	Me34	18.5
6	51.2	19	75.4	1′	97.4
7	210.0	20～33	132～138	2′	70.5
8	42.0	34	43.4	3′	57.2
9	28.2	35	78.2	4′	73.2
10	73.5	36	40.6	5′	73.7
11	71.7	37	69.6	6′	18.1

and 19-H/20-H dihedral angles were estimated as 90° and 180°, respectively, from the appropriate coupling constants $J_{18b,19} \simeq 0$ and $J_{19,20} = 9$ Hz. This assignment was in full agreement with dipolar couplings 12-Hb/14-Hb, 14-Hb/16-H, 15-H/17-H, 18-Hb/19-H, 17-H/20-H and 19-H/21-H displayed by the ROESY spectrum.

The configurations of the C-13～C-20 fragment and the mycosamine moiety were correlated by 1′-H/19-H, 1′-H/18-Ha and 2′-H/17-H ROE's, which are depicted in Fig. 2. Thus, the absolute configurations of the five chiral centers within the C-13～C-20 fragment were established as 13*R*, 15*S*, 16*R*, 17*S* and 19*R*.

The conformation of the C-33～C-37 fragment (Fig. 3) resulted from the vicinal coupling constants $J_{33,34} = 10.0$, $J_{34,35} = 9.5$, $J_{35,36} = 2.5$ and $J_{36,37} = 1.5$ Hz as well as from dipolar couplings 34-H/35-H. 33-H/35-H, 33-H/36-H, 35-H/36-Me and 36-Me/37-Me which are depicted in Fig. 3. Comparison of the specific rotations of 2,5-dimethylheptane-1,4,6-triol, obtained from candidin and amphotericin B *via* ozonolysis followed by reduction with lithium borohydride, indicated that both degradation products were of the same absolute configuration. Thus, the absolute configurations of the four chiral centers of C-33～C-37 fragment were assigned as 34*S*, 35*R*, 36*R* and 37*S*.

The conformation of the C-2～C-6 the fragment (Fig. 4) was reflected by the antiperiplanar positions

Fig. 2. Stereostructure of the C-12~C-20 fragment of **2** and its spatial relation with the D-mycosaminyl substituent derived from listed coupling constants and ROE's depicted as bidirectional arrows.

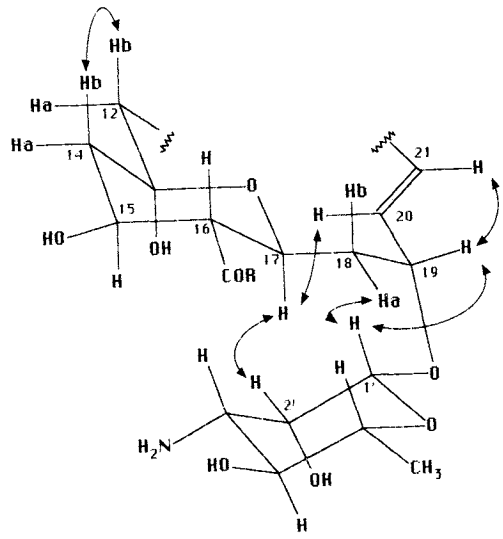


Fig. 3. Coupling constants and ROE's observed within the C-32~C-37 fragment of 2.

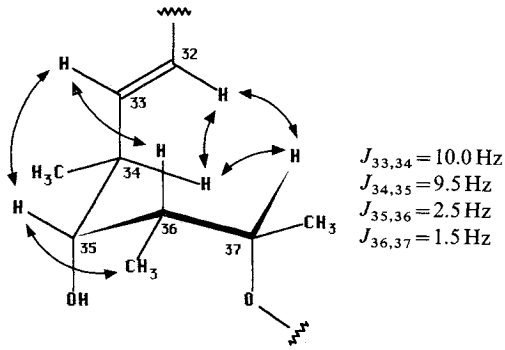


Fig. 4. Conformation of the C-2~C-6 fragment of 2 resulting from presented coupling constants and ROE's.

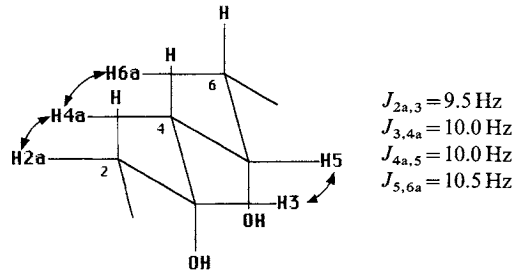
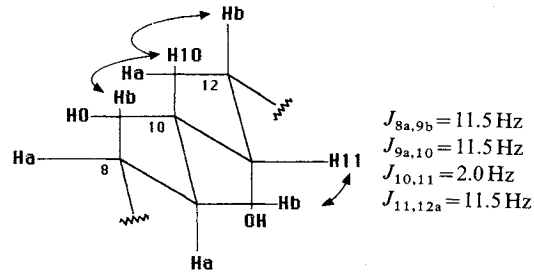


Fig. 5. Conformation of the C-8~C-12 fragment of 2 with diagnostic coupling constants and ROE's.



of the protons 2-Ha, 3-H, 4-Ha, 5-H and 6-Ha, which were derived from vicinal coupling constants $J_{2a,3}=9.5$, $J_{3,4a}=10.0$, $J_{4a,5}=10.0$ and $J_{5,6a}=10.5$ Hz as well as 2-Ha/4-Ha, 4-Ha/6-Ha and 3-H/5-H ROE's which are depicted in Fig. 4. In the remaining C-8~C-12 fragment (Fig. 5) three pairs of protons 8-Ha : 9-Hb, 9-Ha : 10-H and 11-H : 12-Ha were found to be in antiperiplanar positions, which resulted from the vicinal coupling constants value of around 11 Hz, while the dihedral angle between 10-H and 11-H was close to 90° due to $J_{10,11}=2.0$ Hz. This is fully confirmed by ROE's 8-Hb/10-H, 10-H/12-Hb and 9-Hb/11-H displayed by the ROESY spectrum.

The conformation of the C-2~C-12 fragment was deduced as follows. The geminal coupling constants

between protons at C-6 and C-8 were 16.0 Hz and 18.5 Hz, respectively, indicating that the dihedral angle between these methylene protons and the π-bond of the keto group at C-7 was around 90°[13].

Thus, the C-6 and C-8 methylene groups were found to possess a common symmetry plane on which was located the C-7-keto group. This allowed for correlation of the conformations of the C-2~C-6 and C-8~C-12 fragments, which resulted in the assignment of the relative configuration of the C-2~C-12 fragment. It became apparent from Dreiding model studies that only the full stretched conformation of the C-2~C-12 fragment would make it long enough to connect C-1 and C-13 in the candidin molecule.

The absolute configuration of the C-2~C-12 fragment was assigned as 3*R*, 5*S*, 10*R*, 11*R* upon correlation with the C-14~C-20 fragment *via* 12-H/14-H ROE (Fig. 2), as well as upon the fact that 3-H, 5-H and 11-H exhibited ROE's to the protons of the heptaene chromophore.

Experimental

Candidin (1)

Candidin was isolated from the antibiotic complex (candidin, candidoin and candidinin) supplied by Polfa (Poland), by means of counter current distribution with a Craig apparatus, Model 20 (123 elements). The solvent system was chloroform - methanol - 0.05 M borate buffer (pH 8.2), 2 : 2 : 1. The separation of the three components was completed after 500 transfers. From 4 g of starting material 800 mg of candidin was obtained.

Candidin Methoxycarbonylmethylamide (2)

Card-Gly-OMe was obtained by the procedure previously described[14]. The product was purified by flash chromatography on MN-Kieselgel (minus 0.08 mm) with the solvent system chloroform - methanol - water, 5 : 2 : 0.2 (v/v).

NMR Spectra

Spectra were recorded with a 300 MHz Varian spectrometer. The ^{1}H spectra were measured at the sample concentration 28 mg/ml in two solvent systems: methanol-d_4 - pyridine-d_5, 1 : 9 and methanol-d_4 - pyridine-d_5 - dimethylsulphoxide-d_6, 4 : 4 : 2.

^{13}C spectra DEPT and C,H-COSY were measured at the sample concentration 60 mg/ml in methanol-d_4 - pyridine-d_5, 1 : 9.

Double-quantum filtered COSY spectra were acquired in the phase-sensitive mode. Data were collected in a 1,024 × 256 matrix (24 scans for each increment) with a spectral width of 1,938 Hz and processed in 1 K × 1 K matrix. Exponential multiplication and Gaussian apodizaton (LB = 0.1, AF = 0.132, LB2 = 0.1, AF2 = 0.066) were applied prior to 2D Fourier transformation.

ROESY spectra were collected and processed with the same parameters as the DQ-COSY spectra. The spin-lock field value was 1.4 kHz within $\tau_{mix} = 0.4$ s.

C,H-COSY data were collected in two separate experiments with the partial H-H decoupling. The first spectrum (10,504 Hz and 742 Hz spectral windows for ^{13}C and ^{1}H respectively, 128 increments and 256 scans per increment) was processed in 2,048 × 512 matrix. The second spectrum, reflecting the correlation of the methylene groups region, was measured with sample concentration 90 mg/ml (4,574 Hz and 608 Hz spectral windows, 90 increments and 640 scans per increment) and processed in a 4,096 × 256 matrix.

Acknowledgments

This work was supported by the State Committee for Scientific Research, Warsaw, Poland (grant No. 406329101), and, in part, by the University of Camerino, Italy.

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