Stereostructure of Amphotericin A

PAWEŁ SOWIŃSKI, TADEUSZ BIESZCZAD, JAN PAWLAK
and EDWARD BOROWSKI

Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdańsk, 80-952 Gdańsk, Poland

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The stereostructure of amphotericin A was established on the basis of NMR studies which contained DQF COSY, ROESY, 1D TOCSY, HSQC and HMBC experiments.

Amphotericin A, a tetraene macrolide antibiotic, is a minor component (10%) accompanying amphotericin B in the antibiotic complex produced by *Streptomyces nodosus*[1,2]. Both components of the amphotericin complex, isolated in the pure crystalline forms, were reported to exhibit antifungal activity[1,2]. The gross structure of amphotericin A, elucidated by MS and ^{1}H NMR, has been published[3,4]. In this paper we report our NMR studies of amphotericin A which resulted in the assignment of its stereostructure.

Results and Discussion

Amphotericin A (**1**), Fig. 1, was transformed into its 3′-*N*-acetyl methoxycarbonylmethylamide derivative (**2**), Fig. 1, which facilitated the purification process. The NMR studies of **2**, consisting of DQF-COSY, ROESY, 1D TOCSY, HSQC and HMBC experiments, resulted in full proton and carbon assignments, which are listed in Tables 1 and 2, respectively.

The vicinal coupling constants were measured from the 1D-^{1}H NMR spectrum, 1D-TOSCY experiments and, in a few cases, from the fine structure of the DQF-COSY cross-peaks.

The analysis of the DQF-COSY spectrum of **2** revealed all the connectivities within three structural blocks: C-2 ~ C-12, C-14 ~ C-37 and the sugar moiety (Fig. 1). These blocks were connected based upon the HMBC experiment which displayed long-range heteronuclear connectivities. Thus, the blocks C-2 ~ C-12 and C-14 ~ C-37 were connected *via* C-13 due to couplings H-12/C-13 and H-14/C-13. The chemical shift of H-37 (5.63 ppm) associated with the H-2/C-1 and H-37/C-1 correlation revealed that the C-2 ~ C-12 fragment was connected *via* the lactone bond to C-37. The location of the *N*-acetylmycosaminyl substituent at C-19 was pointed out by the H-1′/C-19 correlation.

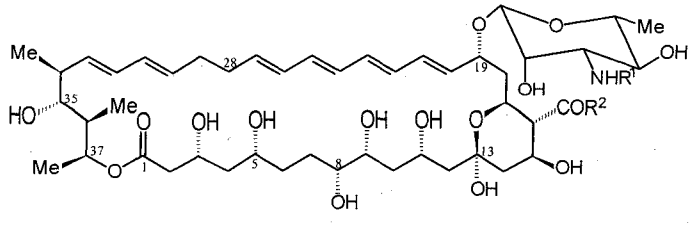
Thus, the gross structure of amphotericin A was confirmed as **1** which is shown in Fig. 1.

The geometries of the tetraene and diene chromophores, assigned as 20E, 22E, 24E, 26E, and 30E, 32E, respectively, were pointed out by the following set of vicinal coupling constants: $J_{20,21}=15.0$ Hz, $J_{22,23}=14.6$ Hz, $J_{24,25}=15.0$ Hz, $J_{26,27}=15.0$ Hz, $J_{30,31}=15.0$ Hz, $J_{32,33}=15.0$ Hz.

The data required for the assignment of the geometry of the C-27 ~ C-30 fragment could not be extracted from NMR spectra measured in the solvent system pyridine-d_5 - methanol-d_4 9:1 due to strong overlapping resonances of olephinic protons H-27 and H-30 as well as four protons of the C-28 and C-29 methylene groups.

Fortunately, the ^{1}H NMR spectrum of **2** measured in methanol-d_4 showed in the region of interest by the very well resolved proton resonances of H-27 ($\delta=5.72$ ppm) and H-30 ($\delta=5.58$ ppm). This subsequently allowed *via* 1D-TOCSY experiments for the assignment of chemical

Fig. 1. The structure of amphotericin A (**1**) and its 3′-*N*-acetylamphotericin A methoxycarbonylmethylamide (**2**).



1 $R^1=H$, $R^2=OH$
2 $R^1=CH_3CO$, $R^2=NHCH_2COOCH_3$

Table 1. ^{1}H NMR data of 3′-*N*-acetylamphotericin A methoxycarbonylmethylamide (**2**).

No.	δ (ppm)	Coupling partner (*J*, Hz)	ROE to proton	No.	δ (ppm)	Coupling partner (*J*, Hz)	ROE to proton
2a	2.62	2b (15.0), 3 (4.5)	2b, 3, 4a	19	4.98	18a (3.8), 18b (~0), 20 (7.7)	18a, b, 20, 21, 1′
2b	2.72	2a (15.0), 3 (8.8)	2a, 4b	20	6.24	19 (7.7), 21 (15.0)	11, 17, 19, 22
3	4.67	2a (4.5), 2b (8.8), 4a (4.4), 4b (9.0)	2a, 4a 5	21	6.50	20 (15.0), 22 (10.6)	19, 23
4a	1.82	3 (4.4), 4b (14.0), 5 (4.0)	2a, 3, 4b, 5	22	6.38	21 (10.6), 23 (14.6)	9, 11, 20, 24
4b	1.92	3 (9.0), 4a (14.0), 5(8.4)	2b, 4a	23	6.21	22 (14.6), 24 (10.4)	21, 25
5	4.13	4a (4.0), 4b (8.4), 6a (3.8), 6b (8.1)	3, 7a, 28a, b	24	6.31	23 (10.4), 25 (15.0)	9, 22, 26
6a	1.86	5 (3.8), 6b (12.0), 7b (~9.0)	6b, 7a, 8	25	6.09	24 (15.0), 26 (10.3)	23, 27
6b	2.03	5 (8.1), 6a (12.0)	6a, 8	26	6.14	25 (10.3), 27 (15.0)	7b, 24, 28a, b
7a	1.98	7b (14.2), 8 (3.0)	5, 6a, 7b, 8	27	5.65	26 (15.0)	25
7b	2.14	6a (~9.0), 7a (14.2), 8 (9.4)	7a, 9, 26	28a	2.18		5, 26
8	3.82	7a (3.0), 7b (9.4), 9 (3.5)	6a, 6b, 7a, 10a	28b			
9	4.15	8 (3.5), 10a (4.5), 10b (9.8)	7a, 11, 22, 24	29a	2.10		31
10a	1.87	9 (4.5), 10b (14.0), 11 (5.6)	8, 10b, 12a	29b			
10b	2.05	9 (9.8), 10a (14.0), 11 (9.6)	10a, 12b	30	5.64	31 (15.0)	32
11	4.97	10a (5.6), 10b (9.6), 12a (5.6), 12b (9.9)	9, 20, 22	31	6.13	30 (15.0), 32 (10.2)	29a, b, 33
12a	1.86	11 (5.6), 12b (13.8)	10a, 12b, 14a	32	6.29	31 (10.2), 33 (15.0)	30, 34, 36, 37
12b	2.03	11 (9.9), 12a (13.8)	10b, 12a, 14b	33	5.74	32 (15.0), 34 (8.5)	31, 35, 36, Me34
14a	1.73	14b (13.6), 15 (11.5)	12a, 14b, 16	34	2.61	33 (8.5), 35 (7.7), Me34 (6.8)	32, 36, 37, Me34
14b	2.51	14a (13.6), 15 (4.2)	12b, 14a, 15	Me34	1.23	34 (6.8)	33, 34, 35
15	5.09	14a (11.5), 14b (4.2), 16 (10.1)	14b, 17	35	3.49	34 (7.7), 36 (4.0)	33, 36, Me34, Me36
16	2.79	15 (10.1), 17 (10.1)	14a, 18a	36	2.19	35 (4.0), 37 (3.0), Me36 (6.9)	32, 33, 34, 35, 37, Me36
17	5.01	16 (10.1), 18a (9.0), 18b (7.0)	15, 18b, 20, 2′	Me36	1.09	36 (6.9)	35, 36, Me37
18a	2.26	17 (9.0), 18b (13.5), 19 (3.8)	16, 18b, 19	37	5.63	36 (3.0), Me37 (6.4)	32, 34, 36, Me37
18b	2.70	17 (7.0), 18a (13.5), 19 (~0)	17, 18a, 19, 1′	Me37	1.36	37 (6.4)	37, Me36
				1′	5.08	2′ (~0)	18b, 19, 3′, 5′
				2′	4.43	1′ (~0), 3′ (3.3)	17
				3′	4.53	2′ (3.3), 4′ (9.5)	1′, 5′
				4′	3.92	3′ (9.5), 5′ (9.3)	Me5′
				5′	3.70	4′ (9.3), Me5′ (6.0)	1′, 3′, Me5′
				Me5′	1.48	5′ (6.0)	4′, 5′
				Gly Ha	3.94	Gly Hb (18)	Gly Hb
				Gly Hb	4.48	Gly Ha (18)	Gly Ha
				OMe	3.65		
				N-Ac	2.01		

Table 2. ^{13}C chemical shifts of 3′-*N*-acetylamphotericin A methoxycarbonylmethylamide (**2**).

Carbon No.	δ [ppm]	Carbon No.	δ [ppm]	Carbon No.	δ [ppm]	Carbon No.	δ [ppm]
1	71.4	15	66.1	29	32.5	3′	56.2
2	43.7	16	59.2	30	131.7	4′	72.1
3	68.0	17	67.1	31	131.5	5′	74.3
4	44.4	18	38.1	32	130.5	Me5′	18.4
5	70.7	19	77.0	33	135.6	NH-Ac	
6	47.2	20	135.0	34	41.8	CO	171.2
7	30.0	21	131.0	35	77.6	Me	22.7
8	74.4	22	132.5	36	40.6	GlyOMe	
9	74.0	23	132.6	37	71.5	CH2	41.2
10	41.1	24	132.8	Me34	17.3	CO	160.8
11	68.0	25	131.5	Me36	12.5	OMe	52.0
12	35.8	26	133.0	Me37	17.0	COOR	174.0
13	98.1	27	134.0	1′	98.8		
14	45.5	28	32.5	2′	70.9		

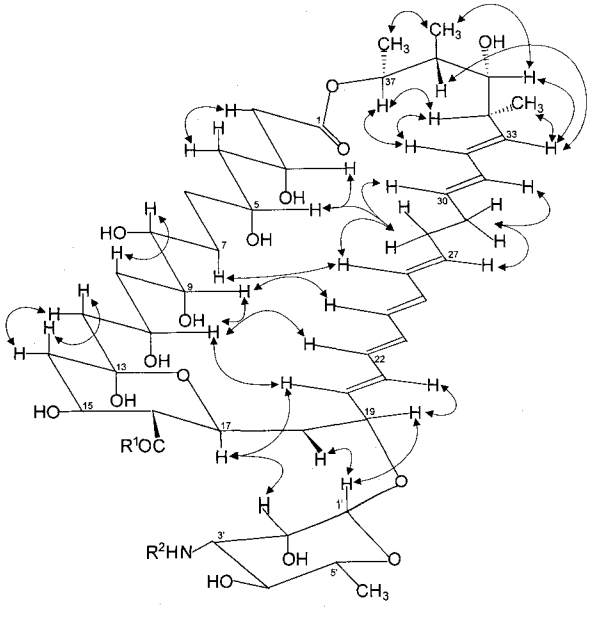
shifts of H-26 ($\delta=6.16$ ppm), H-28a ($\delta=2.20$ ppm), H-28b ($\delta=2.27$ ppm), H-29a ($\delta=2.12$ ppm), H-29b ($\delta=2.23$ ppm) and H-31 ($\delta=6.01$ ppm).

The 2D-NOESY spectrum of **2** measured in methanol-d_4 revealed that the C-28 methylene protons exhibited NOE's to H-26, H-27 and H-30 while C-29 methylene protons showed NOE's to H-27, H-30 and H-31. This assignment associated with vicinal coupling constants $J_{27,28a}=6.8$ Hz, $J_{27,28b}=6.8$ Hz, $J_{29a,30}=5.4$ Hz and $J_{29b,30}=7.4$ Hz pointed out the transoidal geometry of the C-27$\sim$C-30 fragment. It should be mentioned that the difference between $J_{29a,30}$ and $J_{29b,30}$ could imply that the planes of tetraene and diene chromophores were slightly deviated from ideal coplanarity.

The chair conformation of the glycosidically bound mycosamine (Fig. 2), belonging to the D-series established earlier[3,4], was pointed out by a set of vicinal coupling constants: $J_{1',2'}\sim 0$ Hz, $J_{2',3'}=3.3$ Hz, $J_{3',4'}=9.5$ Hz, $J_{4',5'}=9.3$ Hz. Thus the H-3', H-4' and H-5' were found to be in axial positions while the H-2' was equatorial. The axial position of 1'-H resulted from strong H-1'/H-3' and H-1'/H-5' ROE's (Table 1). This pointed out the β-configuration of the glycosidic bond.

The conformation of the C-13$\sim$C-19 fragment, (Fig. 2), was derived as follows: The proposed chair conformation of the hemiketal ring (C-13$\sim$C-17) resulted from the vicinal coupling constants: $J_{14a,15}=11.5$ Hz, $J_{15,16}=10.1$ Hz and $J_{16,17}=10.1$ Hz indicating axial positions of the appropriate protons, while the H-12a/H-14a and H-12b/H-14b ROE's defined equatorial position of the C-12 substituent (Fig. 2). This assigment was further supported by the strong ROE's: H-14a/H-16 and H-16/H-18a observed in the ROESY spectrum. The geometry of the C-17$\sim$C-19 fragment was reflected by $J_{17,18a}=9.0$ Hz, $J_{17,18b}=7.0$ Hz, $J_{18a,19}=3.8$ Hz and $J_{18b,19}\sim 0$ Hz. The configuration of the C-13$\sim$C-19 fragment was then correlated with that of the mycosaminyl substituent by the H-1'/H-19, H-1'/H-18b and H-2'/H-17 ROE's. Thus the absolute configuration of the C-13$\sim$C-19 fragment was established as 13*R*, 15*S*, 16*R*, 17*S* and 19*R*.

Fig. 2. The stereostructure of 3'-*N*-acetylamphotericin A methoxycarbonylmethylamide with diagnostic ROE's depicted as biderectional arrows.



The conformation of the C-13$\sim$C-19 fragment associated with H-17/H-20 and H-19/H-21 ROE's allocated the C-12 equatorial substituent of the hemiketal ring above the tetraene chromophore plane.

The conformation of the C-8$\sim$C-12 fragment was established based upon the vicinal coupling constants: $J_{9,10b}=9.8$ Hz and $J_{11,12b}=9.9$ Hz and the ROE's H-9/H-11 and H-8/H-10a which pointed out "full stretched" conformation of this fragment. Consequently, the fragment C-8$\sim$C-12 was found to be allocated above the tetraene chromophore plane due to the ROE's: H-11/H-20, H-11/H-22 and H-9/H-24.

The C-2$\sim$C-5 fragment was found to adopt also "full stretched" conformation reflected by vicinal coupling constants: $J_{2b,3}=8.8$ Hz, $J_{3,4b}=9.0$ Hz and $J_{4b,5}=8.4$ Hz and ROE's: H-2b/H-4b and H-3/H-5.

The vicinal coupling constants $J_{5,6a}=3.8$ Hz, $J_{5,6b}=8.1$ Hz, $J_{6a,7b}\sim 9.0$ Hz, $J_{7a,8}=3.0$ Hz and $J_{7b,8}=9.4$ Hz pointed out the relative allocation of the fragments C-2$\sim$C-5 and C-8$\sim$C-12.

Thus the C-2$\sim$C-12 fragment was found to adopt a "full stretched" conformation and was allocated above the planes of the tetranene and diene chromophores. This assignment was in full agreement with the observed H-11/H-20, H-11/H-22, H-9/H-24, H-7b/H-26 and H-5/H-28ab ROE's which are depicted in Fig. 2.

The absolute configuration of C-2$\sim$C-12 fragment was assigned as 3*R*, 5*S*, 8*R*, 9*S* and 11*R* based upon correlation with C-13$\sim$C-17 fragment by H-12a/H-14a and H-12b/H-14b ROE's.

The geometry of the C-33$\sim$C-37 fragment was derived from vicinal coupling constants: $J_{33,34}=8.5$ Hz, $J_{34,35}=7.7$ Hz, $J_{35,36}=4.0$ Hz, $J_{36,37}=3.0$ Hz associated with the ROE's H-33/H-35, H-33/H-36, H-35/Me36 and Me36/Me37 observed in ROESY spectrum of **2**. The absolute configuration of this fragment was deduced as follows. The C-2$\sim$C-12 fragment was found to be allocated above the planes of the tetrane and diene chromophores. This fact determined the condition for closing the macrolac-

tone ring which required that the C-35, C-36 and C-37 had to be located also above the planes of the diene and tetraene chromophores. This requirement could be covered by only one enantiomer of the C-33~C-37 fragment. Thus the absolute configuration of the C-33~C-37 fragment was assigned as 34*S*, 35*R*, 36*R* and 37*S*.

Experimental

Amphotericin A (1)

Amphotericin A in pure, crystalline form with $E^{1\%}_{1cm}$= 1.350 at 304 nm (methanol), was supplied by E.R. Squibb and Sons, Princeton, N.J., U.S.A.

3'-*N*-Acetylamphotericin A Methoxycarbonylmethylamide (2)

This derivative was obtained by the procedures previously described[5,6]. The products were purificated by flash chromatography on MN-Kieselgel with the solvent system chloroform - methanol - water, 5 : 0.5 : 0.05 (v/v) and 5 : 1 : 0.1 (v/v).

NMR Spectra

Spectra were recorded with a Varian Unity 500 Plus spectrometer in the solvent system, pyridine-d_5 - methanol-d_4 9 : 1 (v/v), with a sample concentration of 10 mg/ml. The spectra of ^{13}C and 1D-^1H were collected with standard parameters. 2D-^1H spectra were measured in phase sensitive mode with a spectral width of 4723.7 Hz.

Double-quantum filtered COSY spectra were acquired in 4094 × 800 matrix and were processed in 8 K × 4 K matrix (digital resolution 1.0 Hz and 2.0 Hz).

ROESY spectra were acquired with a mix time of 0.4 seconds in 2048 × 386 matrix with 16 accumulations per increment in 2 K × 1 K matrix (digital resolution 2.0 Hz and 4.1 Hz).

HSQC and HMBC spectra were performed with pulse field gradients. The HSQC spectrum was acquired in phase sensitive mode. The spectral windows for ^1H and ^{13}C axes were 3399.6 Hz and 12574.7 Hz, respectively. Data were collected in 1024 × 200 matrix and processed in 1 K × 1 K matrix. The HMBC spectrum was acquired in absolute value mode with broadband decoupling. The spectral windows for ^1H and ^{13}C axes were 3399.6 Hz and 12574.7 Hz, respectively. Data were collected in 832 × 256 matrix and processed in 1 K × 1 K matrix.

^1H NMR spectra were also recorded with methanol-d_4 solvent.

1D-TOCSY spectra were recorded with tophat shaped pulse (100~300 ms) with a mix time of 0.005~0.090 seconds.

NOESY spectrum was acquired with mix time 0.3 seconds, with spectral width of 4096.3 Hz, in 2048 × 341 matrix and was processed in 2 K × 1 K matrix.

Acknowledgements

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