Stereostructure of Perimycin A†

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The gross structure of perimycin A was revised: the position of the keto group was changed from C-13 to C-5. The stereostructure of perimycin A was established based upon NMR studies.

Perimycin (synonyms: fungimycin, NC-1968, aminomycin) is produced by *Streptomyces coelicolor* var. *aminophylus*[1]. It belongs to aromatic heptaene subgroup and exhibit antifungal activity.[1,2] Perimycin is a mixture of three active components designated as perimycin A, B and C[3]. The gross structure of perimycin A, elucidated by MS, has been reported[3].

In this paper we report our NMR studies of perimycin A, which led us to the assignment of its stereostructure. It was established at an early stage of our studies that the gross structure proposed for perimycin A[3] was wrong. Namely, the location of the keto group had to be changed from C-13 to C-5.

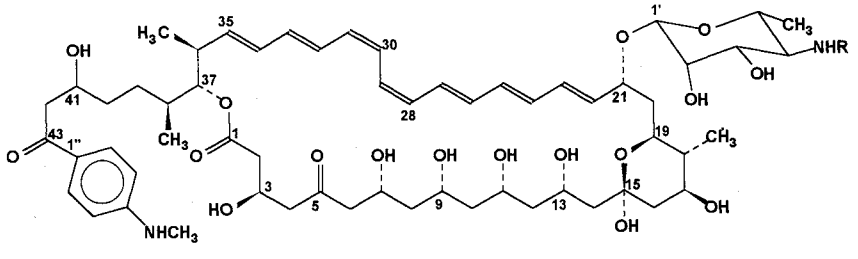
Results and Discussion

Perimycin A (**1**, Fig. 1) was transformed into its N-acetyl derivative (**2**, Fig. 1), which facilitated the purification process.

The NMR studies of N-acetylperimycin A , consisting of DQF-COSY, ROESY, 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 1D-^{1}H NMR spectrum and, in a few cases, from the phase structure of the DQF-COSY cross-peaks. The analysis of the DQF-COSY spectrum of N-acetylperimycin A revealed all the connectivities within five structural blocks: C-2~C-4, C-6~C-14, C-16~C-42, aromatic and sugar moieties (Fig. 1), separated by non protonated carbons C-1, C-5, C-15 and C-43 as well as by glycosidic bond. These blocks were assembled based upon the HMBC experiment which displayed long-range heteronuclear connectivities. Thus, the blocks C-6~C-14 and C-16~C-42 were connected *via* C-15 due to the couplings 14-H/C-15 and 16-H/C-15. The attachment of the aromatic moiety *via* C-43 to the C-16~C-42 fragment resulted from the 2″-H/C-43 and 42-H/C-43 con-

Fig. 1. The structure of perimycin A **1** and its N-acetyl derivative **2**.



Perimycin A (1) R=H
N-acetylperimycin A (2) R=CH_3CO

† This paper is dedicated to professor KOJI NAKANISHI to contribute in celebration of his 70th Birthday Anniversary.

Table 1. ^{1}H NMR data of N-acetylperimycin A.

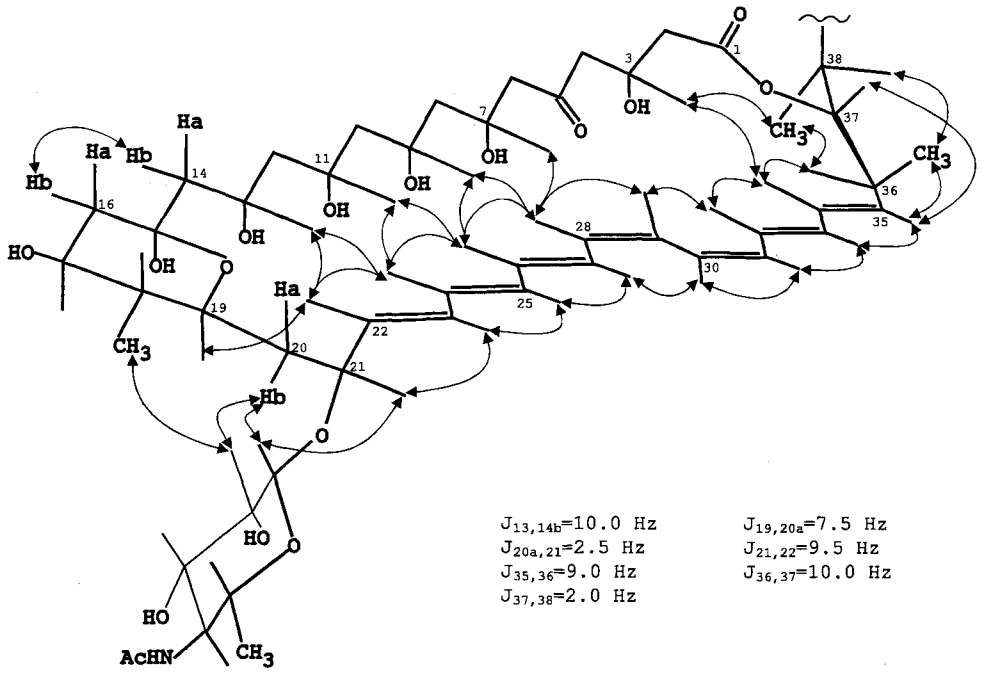
	δ (ppm)	J (coupling partner) (Hz)	ROE to proton
2a	2.62	15.0 (2b), 9.0 (3)	2b, 3
2b	2.87	15.0 (2a), 4.4 (3)	2a, 3
3	4.91	9.0 (2a), 4.4 (2b), 7.3 (4a), 6.1 (4b)	2a, 2b, 4a, 4b, 34, Me38
4a	2.79	7.3 (3), 17.1 (4b)	4b, 3
4b	3.03	6.1 (3), 17.1 (2a)	4a, 3
6a	2.50	17.0 (6b), 2.4 (7)	6b, 7, 8a
6b	2.78	17.0 (6a), 9.5 (7)	6a, 8b
7	4.67	2.4 (6a), 9.5 (6b), 3.0 (8a), 9.5 (8b)	6a, 8a, 9, 28, 29
8a	1.38	3.0 (7), 14.0 (8b), 3.0 (9)	6a, 8b, 7, 9
8b	1.70	9.5 (7), 14.0 (8a), 10.0 (9)	8a, 6b
9	4.18	3.0 (8a), 10.0 (8b), 3.0 (10a), 10.0 (10b)	7, 8a, 10a, 11, 26, 28
10a	1.36	3.0 (9), 3.0 (11), 13.4 (10b)	9, 10b (12b), 11
10b	1.59	10.0 (9), 13.4 (10a), 10.0 (11)	10a (12a)
11	4.23	3.0 (10a), 10.0 (10b), 3.0 (12a), 10.0 (12b)	9, 10a, 12a, 13, 26 (24)
12a	1.32	3.0 (11), 14.0 (12b), 2.5 (13)	11, 12b (10b), 13
12b	1.62	10.0 (11), 14.0 (12a), 9.5 (13)	12a, 14b
13	4.72	2.5 (12a), 9.5 (12b), 2.5 (14a), 10.0 (14b)	11, 12a, 14a, 22, 24 (26)
14a	1.71	2.5 (13), 14.4 (14b)	13, 14b
14b	1.89	10.0 (13), 14.4 (14a)	12b, 14a, 16b
16a	1.71	12.0 (16b), 10.5 (17)	16b, 18
16b	2.44	12.0 (16a), 5.0 (17)	14b, 16a, 17
17	4.10	10.5 (16a), 5.0 (16b), 10.5 (18)	16b, Me18, 19
18	1.57	10.5 (17), 11.0 (19), 6.5 (Me18)	M18, 16a, 17
19	4.45	11.0 (18), 7.5 (20a), ~3.0 (20b)	Me18, 17, 20b, 22
20a	1.83	7.5 (19), 15.4 (20b), 2.5 (21)	20b, 21
20b	2.42	~3.0 (19), 15.4 (20a), 6.0 (21)	Me18, 19, 20a, 21, 1′, 2′
21	4.78	2.5 (20a), 6.0 (20b), 9.5 (22)	20a, 20b, 22[a], 23, 1′
22	6.55	9.5 (21), 15.4 (23)	13, 19, 21, 23[d], 24
23	6.38	15.4 (22), 10.5 (24)	21, 22[d], 25
24	6.75	10.5 (23), 15.1 (25)	13, 22, 11
25	6.51	15.1 (24), 11.0 (26)	27
26	6.73	11.0 (25), 15.1 (27)	9, 11, 28
27	7.00	15.1 (26), 11.7 (28)	25, 30
28	6.55	11.7 (27), 11.5 (29)	7, 9, 26, 29
29	6.98	11.5 (28), 11.5 (30)	7, 28, 32
30	6.69	11.5 (29), 11.8 (31)	27, 31
31	6.22	11.8 (30), 11.8 (32)	30, 33
32	7.19	11.8 (31), 15.0 (33)	29, 34
33	6.32	15.0 (32), 10.8 (34)	31, 34[d], 35
34	6.43	10.8 (33), 15.1 (35)	3, 32, 33[d], 35[b], 36
35	5.56	15.1 (34), 9.0 (36)	33, 34[b], Me36, 37
36	2.55	9.0 (35), 10.0 (37), 6.5 (Me36)	34, 37[c], Me36, Me38
37	5.06	10.0 (36), 2.0 (38)	35, 36[c], 38, 39a, 39b, 40ab, Me36
38	1.89	2.0 (37), 6.5 (Me38)	37, Me36, Me38
39a	1.73		37, 41, Me38
39b	1.85		37, 41, Me38
40a,b	1.82	4.5 (41), 7.8 (41)	41
41	4.54	4.5, 7.8 (40a,b), 3.9 (42a), 8.0 (42b)	39a, 39b, 40ab, 42a, 42b, B
42a	3.15	3.9 (41), 15.6 (42b)	40ab, 41, 42b, B
42b	3.33	8.0 (41), 15.6 (42a)	40ab, 41, 42a, B
Me18	1.16	6.5 (18)	17, 18, 19, 20b, 2′
Me36	0.92	6.5 (36)	35, 36, 37, 38
Me38	1.01	6.5 (38)	3, 36, 38, 39a, 39b
NMe	2.72		A
1′	4.87	~0 (2′)	2′[d], 3′, 5′, 20b, 21
2′	4.40	~0 (2′), 3.0 (3′)	1′[d], 3′, 5′[e], 20b, Me18
3′	4.09	3.0 (2′), 10.0 (4′)	1′, 4′[d], 5′
4′	4.58	10.0 (3′), 10.0 (5′)	3′[d], 5′[d], 6′
5′	3.70	10.0 (4′), 6.0 (6′)	1′, 2′[e], 3′, 4′[d], 6′
6′	1.44	6.0 (5′)	4′, 5′
N-Ac	2.04		
	Aromatic protons		
2A	6.71	9.0 (B)	B, NMe
2B	8.10	9.0 (A)	A, 41, 42a, 42b

[a] Relay HHT/ROE 22-H/23-H/21-H.
[b] Relay HHT/ROE 34-H/33-H/35-H.
[c] Relay ROE/ROE/ROE 36-H/Me-36/35-H/37-H.
[d] HHT (Hartmann-Hahn Transfer).
[e] Relay HHT/ROE 2′-H/1′-H/5′-H.

Table 2. ^{13}C chemical shifts of N-acetylperimycin A.

Carbon No.	δ [ppm]	Carbon No.	δ [ppm]	Carbon No.	δ [ppm]	Carbon No.	δ [ppm]
1	170.97	17	69.04	32	128.16	2′	71.73
2	43.67	18	44.06	33	134.23	3′	72.96
3	63.99	18-Me	13.10	34	133.10	4′	54.57
4 (6)	51.43	19	69.92	35	137.84	5′	72.42
5	208.57	20	37.65	36	40.19	6′	18.58
6 (4)	51.37	21	77.69	36-Me	16.41	4′-NHCOCH$_3$	
7	67.61	22	137.79	37	78.46	CH$_3$CO–	23.12
8	43.98	23	130.04	38	33.97	CH$_3$CO–	171.07
9	72.96	24	134.55	38-Me	13.10	N-methylophenyl-	
10	44.43	25	132.78	39	30.82	–N–Me	29.38
11	73.67	26	135.41	40	35.70	1″	126.31
12	44.29	27	128.24	41	68.49	2″	131.15
13	69.36	28 (31)	130.63	42	46.01	3″	111.05
14	47.46	29	125.14	43	197.80	4″	154.47
15	97.79	30	124.94	N-Ac-perozaminyl-			
16	46.35	31 (28)	130.56	1′	98.98		

Fig. 2. The stereostructure of N-acetylperimycin A.



The diagnostic ROE's are depicted as bidirectional arrows.

nectivities. The chemical shift of H-37 (5.06 ppm), typical for an acyloxy proton, associated with 2-H/C-1 and 37-H/C-1 correlations revealed that the C-2~C-4 fragment was connected *via* lactone bond to C-37. Finally, the macrolactone ring was closed by linking *via* C-5 the C-2~C-4 and C-6~C-14 fragments based upon the 4-H/C-5 and 6-H/C-5 connectivities. The location of the N-acetylperosaminyl substituent at C-21 was pointed out by the 1′-H/C-21 correlation. Thus, the gross structure of perimycin A was reassigned as **1**, Fig. 1.

The geometry of heptaene chromophore, assigned as 22*E*, 24*E*, 26*E*, 28*Z*, 30*Z*, 32*E* and 34*E*, was pointed out by the following set of vicinal coupling constants: $J_{22,23}=15.4$ Hz, $J_{24,25}=15.1$ Hz, $J_{26,27}=15.1$ Hz, $J_{28,29}=11.5$ Hz, $J_{30,31}=11.8$ Hz, $J_{32,33}=15.0$ Hz and $J_{34,35}=15.1$ Hz. The presence of two cis double bonds was also reflected by a set of strong ROE's: 27-H/30-H, 28-H/29-H, 29-H/32H/-H and 30-H/31-H (Fig. 2).

The chair conformation of glycosidically bound perosamine (Fig. 2), of which belonging to the D-series

established earlier[4], was pointed out by a set of vicinal coupling constants: $J_{2',3'}=3.0$ Hz, $J_{3',4'}=10.0$ Hz, $J_{4',5'}=10.0$ 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 H-1' resulted from strong 1'-H/3'-H and 1'-H/5'-H ROE's. This pointed out the β-configuration of the glycosidic bond. Actually, this assignment could be accepted with the reservation that the presence of the "strange" 2'-H/5'-H ROE would be explainable. The origin of this "ROE" was defined as Relay mediated by Hartmann-Hahn Transfer (HHT) effect[5]. The HHT cross-peak 1'-H/2'-H distinguishable by its opposite phase to ROE, was observed in ROESY spectrum. Thus, the formation of the 2'-H/5'-H Relay of the HHT/ROE type can be shown on the following pathway: 2'-H/1'-H/5'-H. The more detailed discussion about the Relays observed in the ROESY spectra of polyene macrolides has been presented elsewhere[6].

The conformation of the C-15~C-21 fragment, Fig. 2, was derived as follows. The chair conformation of the hemiketal ring (C-15~C-19) resulted from the vicinal coupling constants: $J_{16a,17}=10.5$ Hz, $J_{17,18}=10.5$ Hz and $J_{18,19}=11.0$ Hz indicating axial positions of the appropriate protons. The geometry of the C-19~C-21 fragment was reflected by $J_{19,20a}=7.5$ Hz, $J_{19,20b}\sim 3.0$ Hz, $J_{20a,21}=2.5$ Hz and $J_{20b,21}\sim 6.0$ Hz. The configuration of the C-15~C-21 fragment was then correlated with that of perosaminyl substituent by the 1'-H/21-H, 1'-H/20-Hb, 2'-H/20b-H and 2'-H/18-Me ROE's. Thus, the absolute configuration of the C-15~C-21 fragment was established as 15*R*, 17*S*, 18*R*, 19*S* and 21*R*. The conformation of the C-15~C-21 fragment associated with 19-H/22-H and 21-H/23-H ROE's (Fig. 2) allocated the equatorial substituent of the hemiketal ring (C-14) above the heptaene chromophore plane.

Now, the conformation and the absolute configuration of the C-2~C-14, Fig. 2, will be discussed. The protons H-3, H-7, H-9, H-11 and H-13 exhibited very similar splitting pattern resulting from the fact that each of them was coupled to four protons of the adjacent methylene groups with two pairs of small and large scalar couplings, which were in a range of 3 Hz and 10 Hz, respectively. This regular alignment of the hydroxymethine protons, substantiated by the 7-H/9-H, 9-H/11-H and 11-H/13-H ROE's, strongly suggested full stretched conformation of the C-2~C-14 fragment. This conclusion was further supported by the fact that the hydroxymethine protons of the C-2~C-14 fragment exhibited strong ROE's (Fig. 2) to the appropriate olephinic protons. Finally, it became apparent from the Dreiding model studies that distance geometry requirement for closing macrolactone ring could be met only by the full stretched conformation of the C-2~C-14 fragment. The configurations of the C-2~C-14 and C-15~C-21 fragments were correlated *via* 14-Hb/16-Hb ROE. Thus, the absolute configuration of the C-2~C-14 fragment was assigned as 3*R*, 7*R*, 9*R*, 11*S* and 13*S*.

The conformation of the C-36~C-38 fragment was simply pointed out by scalar and dipolar couplings, which are depicted in Fig. 2. However, the absolute configuration of this fragment needed to be deduced. The C-2~C-14 fragment with full stretched conformation was allocated above the heptaene chromophore plane. Thus, in order to close the macrolactone ring the C-37 had to be driven above the chromophore plane. Such condition could be met by the only one enantiomer of the C-36~C-38 fragment and thereby its absolute configuration was assigned as 36*S*, 37*R* and 38*S*.

Unfortunately, the stereochemistry of the C-38~C-42 fragment could not be traced due to the strongly coupled five spin system of the protons at C-38, C-39 and C-40. Thus, the absolute configuration at C-41 remains undefined.

Experimental

Perimycin A (1)

The sample of perimycin complex was supplied by Tarchomin Pharmaceutical Industry, Polfa.

The separation and purification of perimycin A was carried out by flash chromatography on silica gel with the solvent system chloroform - methanol - water 5 : 1 : 0.1 v/v/v. Obtained perimycin A exhibited $E_1^1=1000$ at $\lambda=380$ nm. N-acetylperimycin A (**2**)

50 mg of perimycin A was N-acetylated by the procedure previously described[7]. Yield 45 mg.

The product was purified by HPLC. The separation conditions were as follows: HPLC Merck column 250 x 10 filled with LiChrosorb Si60 (7 μm); the solvent system chloroform - methanol - water 25 : 3 : 0.3; flow rate 6.25 ml/minute.

The injection size was 20 mg of the sample dissolved in 1.5 ml of the solvent system. The retention time of the N-acetylperimycin A was 9.8 minutes. Yield 12 mg.

NMR Spectra

Spectra were recorded with a Varian Unity 500 Plus spectrometer in solvents system pyridine-d_5 - methanol-d_4 9 : 1 v/v, with sample concentration 20 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 4634.5 Hz.

Double-quantum filtered COSY spectra were acquired

in 4096 × 700 matrix with 8 accumulations per increment and were processed in 4 K × 2 K matrix (digital resolutions 2.26 Hz and 4.52 Hz).

ROESY spectra were acquired with mix time 0.4 seconds in 2048 × 386 matrix with 16 accumulations per increment in 2 K × 1 K matrix (digital resolutions 4.6 Hz and 9.04 Hz).

HSQC and HMBC spectra were performed with pulse field gradients.

The HSQC spectrum was acquired in phase sensitive mode. The spectral windows for ^{1}H and ^{13}C axes were 4359 Hz and 20100 Hz, respectively. Data were collected in 1728 × 190 matrix and processed in 2 K × 1 K matrix.

The HMBC spectrum was acquired in absolute value mode with broadband decoupling. The spectral windows for ^{1}H and ^{13}C axes were 4359 Hz and 26264 Hz, respectively. Data were collected in 2048 × 256 matrix and processed in 2 K × 1 K matrix.

Acknowledgements

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