Stereostructure of Rimocidin

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(Received for publication April 4, 1995)

NMR studies of rimocidin, consisting of DQF-COSY, ROESY, HSQC, HMBC and 1D-TOCSY experiments, resulted in the assignment of the absolute configuration of the rimocidin chiral centers as 2*S*, 3*R*, 9*S*, 11*R*, 13*S*, 14*R*, 15*S*, 17*R* and 27*R*. The geometry of tetraene chromophore was found to be all-trans.

Rimocidin, a tetraene macrolide antibiotic, exhibiting antifungal activity, is produced by *Streptomyces rimosus*.[1] The structure of the carbon skeleton of rimocidin and the absolute configuration of its C-27 chiral center have been reported by COPE *et al.*[2] The gross structure of rimocidin, elucidated by MS, has been described.[3] In this paper we report our NMR studies of rimocidin, which resulted in the assignment of its stereostructure.

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

Results and Discussion

The NMR studies of **2**, consisting of DQF-COSY, ROESY, HSQC, HMBC and 1D-TOCSY experiments, enabled us to make full proton and carbon assignments, which are shown in the Tables 1 and 2, respectively. The $J_{H,H}$ coupling constants were measured from the 1D ^1H spectrum, 1D-TOCSY and in a few cases from the fine structure of the DQF-COSY cross-peaks.

The DQF-COSY spectrum of **2** displayed all expected connectivities within four structural blocks (Fig. 1) C-2∼C-4, C-6∼C-10, C-12∼C-30, and the N-acetylmycosamine moiety. These blocks were assembled based upon the HMBC experiment which showed long-range heteronuclear connectivities. Thus, the attachment of the blocks C-2∼C-4 and C-6∼C-10 to the C-5 keto group was revealed by H-4b/C-5 and H-6a/C-5 correlations. The blocks C-6∼C-10 and C-12∼C-30 were linked *via* C-11 based upon the H-10b/C-11 and H-12b/C-11 connectivities. The location of the lactone moiety resulted from the H-2/C-1 and H-27/C-1 correlations. Finally, the placement of the glycosidic bond at C-17 resulted from H-1′/C-17 connectivity. Thus, the gross structure of rimocidin was found to be identical to that reported earlier.[3]

The relative configuration of the fragment C-11∼C-17 was assigned as follows. The conformation of the C-11∼C-15 hemiketal ring was derived from the scalar couplings $J_{12a,13}$, $J_{13,14}$ and $J_{14,15}$, which were in a range from 10.5 Hz to 11.0 Hz. This pointed out the chair conformation of the hemiketal ring, which was supported by the H-12a/H-14 and H-13/H-15 ROE's. The antiperiplanar position of H-15 and H-16a was reflected by $J_{15,16a}=10.0$ Hz, while gauche conformation of H-16a, H-16b and H-17 was revealed by $J_{16a,17}=2.8$ Hz and $J_{16b,17}=5.6$ Hz.

The conformation of the mycosaminyl substituent, which belongs to the D-series was, established earlier[2], and results from the analysis of the vicinal coupling constants $J_{2',3'}=3.6$ Hz, $J_{3',4'}=9.8$ Hz and $J_{4',5'}=9.8$ Hz. This pointed out the chair conformation of the mycosaminyl substituent with H-3′, H-4′ and H-5′ in axial positions while H-2′ was found to be equatorial. The axial position of H-1′ and thereby the β-configuration of the glycosidic bond was derived from H-1′/H-3′ and H-1′/H-5′ ROE's. It should be mentioned that some ROE artefacts were observed in the ROESY spectrum of **2**. These were defined as relays[4] of which the formation pathway is given below the Table 1. The spatial relation between the mycosaminyl substituent and the aglycone, reflected by the H-1′/H-16b, H-1′/H-17 and H-2′/H-15

Table 1. ^{1}H NMR data of **2**.

No.	δ [ppm]	$J_{H,H}$ [Hz] (coupling partner)	ROE
2	2.56	10.8 (2″a), 3.2 (2″b), 10.8 (3)	2″b, Me2″, 4b
2″a	1.76	10.8 (2), 14.5 (2″b), 7.5 (Me2″)	2″b, Me2″, 3
2″b	2.21	3.2 (2), 14.5 (2″a), 7.5 (Me2″)	2, 2″a, Me2″
Me2″	0.99	7.5 (2″a), 7.5 (2″b)	2, 2″a, 2″b, 29ab
3	4.57	10.8 (2), 2.5 (4a), 10.5 (4b)	2″a, 4a
4a	2.60	2.5 (3), 14.7 (4b)	3, 4b, 22, 24
4b	2.81	10.5 (3), 14.7 (4a)	2, 4a, 6a, 6b
6a	2.39	17.3 (6b), 10.8 (7a), 4.3 (7b)	4b, 6b, 7b
6b	2.59	17.3 (6a), 4.5 (7a), 9.5 (7b)	4b, 6a, 7a, 8a
7a	1.67	10.8 (6a), 4.5 (6b), 13.0 (7b), 4.7 (8a), 10.0 (8b)	6b, 7b, 8a, 9, 22
7b	2.01	4.3 (6a), 9.5 (6b), 13.0 (7a), 10.5 (8a), 5.2 (8b)	6a, 7a, 8b
8a	1.30	4.7 (7a), 10.5 (7b), 14.0 (8b), 4.5 (9)	6b, 7a, 8b, 9
8b	1.52	10.0 (7a), 5.2 (7b), 14.0 (8a), 10.0 (9)	7b, 8a, 10b
9	4.49	4.5 (8a), 10.0 (8b), ~2 (10a), 10.8 (10b)	7a, 8a, 10a, 18, 20
10a	1.73	~2 (9), 14.2 (10b)	9, 10b
10b	1.84	10.8 (9), 14.2 (10a)	8b, 10a, 12b
12a	1.71	12.5 (12b), 11.0 (13)	12b, 14
12b	2.48	12.5 (12a), 5.0 (13)	10b, 12a, 13
13	5.08	11.0 (12a), 5.0 (12b), 10.5 (14)	12b, 14[a], 15
14	2.72	10.5 (13), 10.5 (15)	12a, 13[a], 15[b], 16a, 16b, 17[c]
15	5.18	10.5 (14), 10.0 (16a), 2.5 (16b)	13, 14[b], 16b, 17[d], 2′, 18
16a	2.08	10.0 (15), 15.0 (16b), 2.8 (17)	14, 16b, 17
16b	2.93	2.5 (15), 15.0 (16a), 5.6 (17)	14, 15, 16a, 17, 1′
17	4.91	2.8 (16a), 5.6 (16b), 8.4 (18)	14[c], 15[d], 16a, 16b, 1′, 18[e], 19
18	6.31	8.4 (17), 15.7 (19)	9, 15, 17[e], 19[f]
19	6.21	15.7 (18), 11.5 (20)	17
20	6.34	11.5 (19), 15.2 (21)	9
21	6.10	15.2 (20)	
22	6.12		4a, 7a
23	6.11	11.0 (24)	25
24	6.20	11.0 (23), 15.0 (25)	4a, 25[f], 26a
25	5.64	15.0 (24), 9.5 (26a), 5.2 (26b)	24[f], 26b, 27
26a	2.21	9.5 (25), 14.7 (26b), 9.5 (27)	24, 26b
26b	2.35	5.2 (25), 14.7 (26b), 2.5 (27)	25, 26a
27	5.14	9.5 (26a), 2.5 (26b), 5.5 (28a), ~0 (28b)	25, 26b, 28a, 28b, 29ab
28a	1.41	5.5 (27), 14.0 (28b), 7.0 (29ab)	26b, 27, 28b
28b	1.56	~0 (27), 14.0 (28a), 7.0 (29ab)	27, 28a
29ab	1.28	7.0 (28a), 7.0 (28b), 7.5 (Me30)	27, Me30
30Me	0.81	7.5 (29ab)	29ab
1′	5.23	~0 (2′)	2′, 3′, 4′[g], 5′, 16b, 17
2′	4.46	~0 (1′), 3.6 (3′)	1′, 3′[f], 4′[h], 5′[i], 15
3′	4.56	3.6 (2′), 9.8 (4′)	1′, 2′[f], 5′
4′	3.91	9.8 (3′), 9.8 (5′)	1′[g], 2′[h], 5′[f], 6′Me
5′	3.84	9.8 (4′), 6.3 (6′Me)	1′, 2′[i], 3′, 4′[f], 6′Me
6′Me	1.50	6.3 (5′)	4′, 5′
Gly-Ha	3.90	18.0 (Gly-Hb)	Gly-Hb
Gly-Hb	4.55	18.0 (Gly-Ha)	Gly-Ha
OMe	3.78		
N-Ac	1.98		

[a]: ROE/ROE/ROE 13/12b/12a/14, [b]: ROE/ROE/ROE 14/16a/16b/15, [c]: ROE/ROE/ROE 14/16a/16b/17, [d]: ROE/ROE 15/16b/17, [e]: ROE/HHT 17/19/18, [f]: Hartmann Hahn Transfer (HHT), [g]: ROE/HHT 1′/5′/4′, [h]: HHT/ROE/HHT 2′/3′/5′/4′, [i]: ROE/ROE 2′/1′/5′.

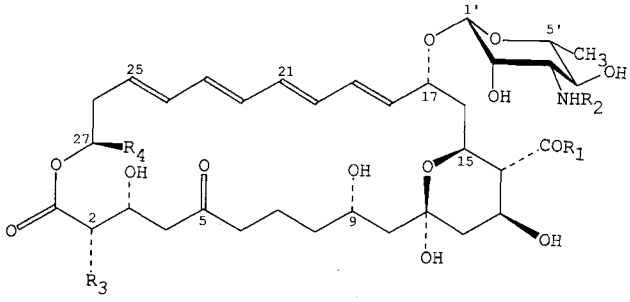
ROE's (Fig. 2), allowed for the assignment of the absolute configuration of the C-11~C-17 fragment as 11*R*, 13*S*, 14*R*, 15*S* and 17*R*.

The geometry of the three double bonds of the tetraene chromophore had been assigned as 18E, 20E and 24E based upon the vicinal coupling constants $J_{18,19}=15.7$ Hz, $J_{20,21}=15.2$ Hz and $J_{24,25}=15.0$ Hz. The vicinal coupling constant $J_{22,23}$ could not be measured due to the very small differences in chemical shifts of the H-21, H-22 and H-23. Thus, the geometry of the remaining double bond was proven indirectly. It was assumed that the geometry of the double bond between C-22 and C-23 was E. In the case ^{13}C resonances of C-21 and C-24 would have been shifted by 5 ppm upfield due to strong γ-shielding effect, as it was observed for vacidin A.[5] The ^{13}C chemical shifts of C-21 and C-24 were observed at 132.10 ppm and 133.73 ppm, respectively, which denied 22Z geometry. Thus, the all-trans geometry was assigned

Table 2. ^{13}C chemical shifts of **2**.

Carbon No.	[ppm]	Carbon No.	[ppm]	Carbon No.	[ppm]
1	172.91	14-CO	173.98	30	13.53
2	56.82	15	66.48	N-Acmycosaminyl	
2″	22.55	16	37.87	1′	98.30
2″Me	11.84	17	75.79	2′	70.64
3	68.28	18	137.51	3′	68.28
4	49.07	19	129.10	4′	72.62
5	209.81	20	133.73	5′	74.10
6	44.75	21	132.10	6′	18.18
7	19.80	22	132.91	3′-NHCOMe	
8	38.04	23	132.41	CO	171.51
9	68.20	24	133.73	Me	22.51
10	46.72	25	131.30	Gly-OMe	
11	97.92	26	38.10	CH_2	40.88
12	45.28	27	72.95	CO	171.51
13	66.04	28	37.10	OMe	51.91
14	58.78	29	18.23		

Fig. 1. The Structure of rimocidin (**1**) and its N-acetyl-methoxycarbonylmethylamido derivative (**2**).



Rimocidin (**1**): $R_1=OH$, $R_2=H$
N-acetylcarbomethoxymethylamidorimocidin (**2**):
$R_1=NHCH_2COOCH_3$, $R_2=Ac$
$R_3=\overset{2''}{C}H_2CH_3$, $R_4=\overset{28}{C}H_2CH_2CH_3$

for the rimocidin tetraene chromophore.

The spatial allocation of the C-11～C-15 hemiketal ring in relation to the tetraene chromophore plane (Fig. 2) was defined by H-15/H-18 and H-17/H-19 ROE's. Thus, the C-10, as an equatorial substituent of the hemiketal ring, is driven above the tetraene chromophore plane. The H-10b/H-12b ROE associated with $J_{9,10b}=10.8$ Hz and H-9/H-18 ROE determined the *S* configuration at C-9. The coupling pattern within the C-6～C-8 fragment combined with H-7a/H-9, H-7a/H-22 and H-6b/H-8a ROE's (Fig. 2) pointed out the conformation of this fragment and thereby allocated H-6a and H-6b. Consequently, the configuration of the C-2～C-4 fragment was correlated with that of C-6～C-10 based upon the H-4b/H-6a, H-4b/H-6b and H-4a/H-22 ROE's. Thus the scalar couplings, $J_{2,3}=10.8$ Hz and $J_{3,4b}=10.5$ Hz, along with H-2/H-4b ROE allowed us to assign the abolute configuration at C-2 and C-3 as 2*S* and 3*R*.

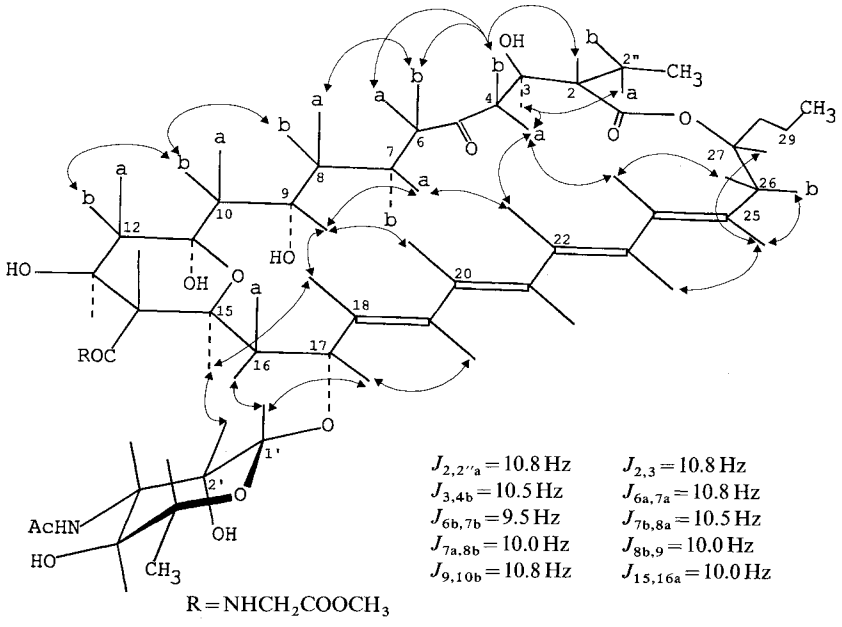
Finally, the absolute configuration at C-27, established earlier as $R^{2)}$, was confirmed by $J_{25,26a}=9.5$ Hz, $J_{26a,27}=9.5$ Hz and H-25/H-27 ROE.

Experimental

Rimocidin (1)

The sample of rimocidin was supplied by Tarchomin Pharmaceutical insutry, Polfa (Warsaw, Poland). The separation of rimocidin was carried out by counter-current distribution in chloroform - methanol - water, 2:2:1, v/v/v.[3] The rimocidin obtained exhibited $E_1^1=1025$ and 304 nm.

Fig. 2. The stereostructure of **2**.



$J_{2,2''a}=10.8$ Hz $J_{2,3}=10.8$ Hz
$J_{3,4b}=10.5$ Hz $J_{6a,7a}=10.8$ Hz
$J_{6b,7b}=9.5$ Hz $J_{7b,8a}=10.5$ Hz
$J_{7a,8b}=10.0$ Hz $J_{8b,9}=10.0$ Hz
$J_{9,10b}=10.8$ Hz $J_{15,16a}=10.0$ Hz

$R=NHCH_2COOCH_3$

The diagnostic ROE's are depicted as bidirectional arrows.

N-Acetylrimocidin

50 mg of rimocidin was N-acetylated by the procedure previously described.[6] Yield 45 mg. $E_1^1 = 1000$ at 304 nm.

N-Acetylmethoxycarbonylmethlamidorimocidin (**2**)

40 mg of N-acetylrimocidin was transformed into methoxycarbonylmethylamido derivative by the procedure previously described.[7] The product was purified by flash chromatography with chloroform-methanol-water, 10:1:0.1 v/v/v. Yield 30 mg of **2**. $E_1^1 = 985$ at 304 nm.

NMR Spectra

Spectra were recorded with a Varian Unity 500 Plus spectrometer with the solvent system, pyridine-d_5-methanol-d_4, 9:1 v/v. The sample concentration was 10 mg/ml.

1D spectra of ^{13}C and ^{1}H were collected with standard parameters. 2D ^{1}H spectra were acquired in phase sensitive mode with a spectral width of 4646.3 Hz.

Double Quantum filtered COSY spectra were acquired in 4096 × 512 matrix with 8 accumulations per increment and were processed in 4K × 2K matrix (digital resolution 2.27 Hz and 4.54 Hz).

ROESY spectra were acquired with mix time 0.35 seconds in 2048 × 387 matrix with 16 accumulations per increment and processed in 2K × 1K matrix (digital resolution 4.54 Hz and 9.08 Hz).

HSQC and HMBC spectra were performed with pulse field gradients. The HSQC spectrum was acquired in phase sensitive mode with broadband decoupling. The spectral windows for ^{1}H and ^{13}C axes were 4646.3 Hz and 20100 Hz, respectively. Data were collected in 1856 × 190 matrix and processed in 2K × 1K matrix. The HMBC spectrum was acquired in absolute value mode. The spectral windows for ^{1}H and ^{13}C axes were 4646.3 Hz and 26264 Hz, respectively. Data were collected in 2176 × 256 matrix and processed in 2K × 1K matrix.

1D-TOCSY spectra were acquired with tophat shaped pulse adjusted to 10 Hz selectivity. The mixing time was 80 ms. Digital resolution was 0.4 Hz.

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; as well as the Chemical Faculty of Technical University of Gdańsk.

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