THE STRUCTURE OF A NEW ANTIBIOTIC, CHROMOXYMYCIN

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Summary: Based on ^{1}H- and ^{13}C-NMR spectral data, the planar structure of chromoxymycin, a new antitumor antibiotic, has been determined as shown in Fig. 1.

Chromoxymycin (1) is an antitumor antibiotic produced by Streptomyces rubropurpureus No. 6362. It is active against mouse P-388 leukemia and B-16 melanoma and exhibits antimicrobial activity against Gram-positive bacteria[1]. We wish to report herein the structural elucidation of 1 accomplished mainly based on ^{1}H- and ^{13}C-NMR spectral analysis.

The physicochemical properties of 1 were as follows; yellow needles, m.p. 85 °C(dec.), $[\alpha]_D^{23}$ +291°(c 0.675, H_2O), $C_{49}H_{60}N_4O_{14} \cdot 4H_2O$, Anal. found: C 58.56, H 6.85, N 5.54%, calcd.: C 58.79, H 6.85, N 5.60%, SIMS m/z 929 (MH)$^+$, IR ν_{max}^{KBr} 3400, 2950, 1650, 1560 cm^{-1}, UV λ_{max} (H_2O) 276 nm(ε 36200), 340(sh, 7400), UV λ_{max} (H_2O + HCl) 272(37100), 330(sh, 7500), UV λ_{max} (H_2O + NaOH) 243(25500), 278(32500), 410(2900).

Fig. 1   Structures of Chromoxymycin (1) and Hedamycin (2)
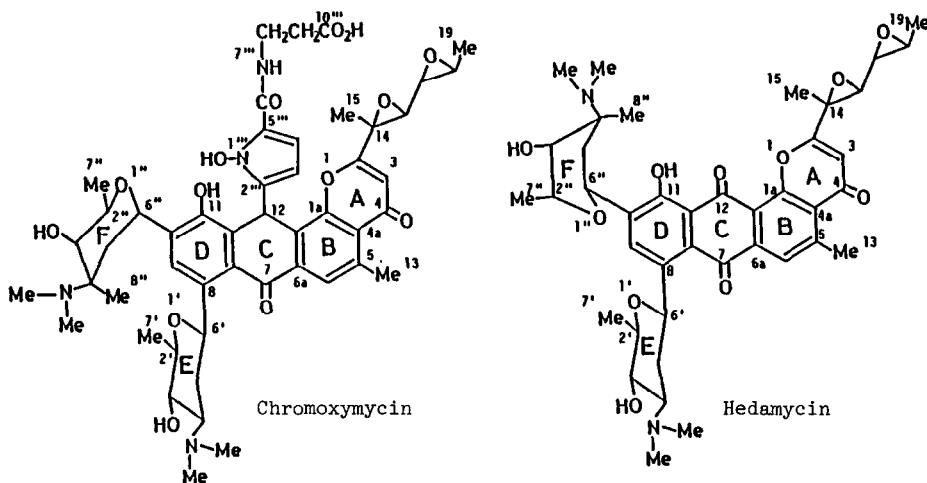
The ^{13}C-NMR spectrum of $\underline{1}$ (100 MHz, d_5-pyridine, Table 1) revealed the following functional groups which accounted for 54 non-exchangeable protons, CH_3 X6, CH_3-N X4, CH_2 X4, CH X1, CH-N or CH-O X10, C-N or C-O X2, CH= X5, C≡ X13, carboxylic acid and/or amide X2 (162.3 and 174.6 ppm) and ketone X2 (179.5 and 187.0). Therefore, $\underline{1}$ possesses 6 exchangeable protons.

Detailed analysis of the ^1H-NMR spectrum of $\underline{1}$ (400 MHz, d_5-pyridine, Table 2) accomplished by the aid of conventional proton spin decouplings, difference spectral method, 2D-COSY and NOE experiments revealed the partial structures (a)-(c) shown in Fig. 2 which are reminiscent of hedamycin $(\underline{2})$[2] (Fig. 1). The proton chemical shifts and coupling constants of $\underline{1}$, however, are different from those of $\underline{2}$ in some respects, in particular, the chemical shift of H-16 (3.11 ppm in $\underline{1}$ vs. 3.57 ppm in $\underline{2}$).

Sequin et al. reported[3] that H-16 in the diepoxide chain resonates at a higher field in the chromone type derivatives than in the anthraquinone-pyrone type compounds such as $\underline{2}$. Thus it is suggested that the chromophore moieties connected to the diepoxide chain in $\underline{1}$ and $\underline{2}$ are different from each other.

Table 1.   100 MHz ^{13}C-NMR data of chromoxymycin (in d_5-pyridine)

1a	154.9,s(153.4)*	12a	130.8,s(129.2)	2"	68.8,d	***	
2	166.3,s(164.7)	13	23.4,q ***	3"	71.1,d	***	
3	108.7,d(108.1)	14	58.8,s(58.9)	4"	64.9,s	***	
4	179.5,s(178.2)	15	14.8,q ***	5"	34.0,t	***	
4a	125.0,s(123.4)	16	65.1,d ***	6"	70.7,d	***	
5	138.7,s(137.5)	17	56.6,d ***	7"	18.0,q	***	
6	124.6,d(123.3)	18	52.5,d ***	8"	14.8,q	***	
6a	136.8,s(135.3)	19	17.9,q ***	4"-NMe	37.2,q(X2) ***		
7	187.0,s(185.2)	2'	78.4,d ***	2'"	132.4,s(130.5)		
7a	127.5,s(125.8)	3'	72.4,d ***	3'"	98.7,d(96.9)		
8	133.6,s(132.1)	4'	68.6,d ***	4'"	107.2,d(105.3)		
9	** (121.4,d)	5'	32.7,t ***	5'"	121.5,s(119.6)		
10	** (134.7,s)	6'	76.8,d ***	6'"	162.3,s(160.0)		
11	156.2,s(154.0)	7'	19.9,q ***	8'"	35.9,t	***	
11a	131.8,s(129.9)	4'-NMe	40.6,q(X2) ***	9'"	36.6,t	***	
12	31.6,d(29.5)			10'"	174.6,s(173.0)		

*in d_6-DMSO.   **Obscured by the solvent peaks.   ***not assigned.

Table 2.   400 MHz ^1H-NMR data of chromoxymycin (in d_5-pyridine)

H-3	6.45,s	H-5'	1.64,ddd	H-8"	1.20,s
H-6	8.14,s		(9.0/9.0/13.0)	4"-NMe	2.76,s
H-9	8.40,s		2.83,dd.br	H-3'"	5.37,d(4.0)
H-12	6.91,s		(2.0/13.0)	H-4'"	6.90,d(4.0)
H-13	3.08,s	H-6'	5.80,d.br(9.0)	H-7'"	10.66,t(6.0)
H-15	2.20,s	H-7'	1.71,d(6.0)	H-8'"	3.96,dddd(6.0/
H-16	3.11,d(5.0)	4'-NMe	2.51,s		6.5/6.5/13.0)
H-17	3.09,dd(2.0/5.0)	H-2"	4.48,dq(1.0/6.5)		4.16,dddd(6.0/
H-18	3.13,dq(2.0/5.0)	H-3"	3.86,s.br		6.5/6.5/13.0)
H-19	1.47,d(5.0)	H-5"	2.74,dd(3.0/11.5)	H-9'"	2.83,ddd(6.5/
H-2'	3.93,dq(6.0/9.0)		3.31,d.br(11.5)		6.5/16.0)
H-3'	3.65,dd(9.0/9.0)	H-6"	5.80,dd(3.0/3.0)		2.97,ddd(6.5/
H-4'	3.50,ddd	H-7"	1.63,d(6.5)		6.5/16.0)
	(2.0/9.0/9.0)				

Furthermore, the values of chemical shifts and coupling constants of the ring F in 1 differ from those of the corresponding moiety with a boat conformation in 2. The coupling constants between H-2'' and H-3'' (1.0 Hz), and H-6'' and two protons of H-5'' (3.0, 3.0 Hz) in 1, show that they are in <u>gauche</u> relationships. Furthermore, NOEs were observed with H-9 (8.40 ppm), H-2'' (4.48), H-3'' (3.86), 4''-NMe$_2$ (2.76) and one proton of H-5'' (2.74) upon irradiation of H-8'' (1.20) (indicated by open arrows in Fig. 2). Thus, the ring F in 1 is concluded to take a chair

Fig. 2   Partial structures of (1)

conformation as shown in Fig. 2. The very small coupling constant between H-2'' and H-3'' (1.0 Hz) is explicable in terms of the antiperiplanar relationships of these protons and oxygen substituents[4].

The structure of the chromophore part of 1 was analyzed based on ^{13}C-{^{1}H} long range selective proton decoupling (LSPD) experiments carried out in d$_6$-DMSO. Irradiation of H-3 (6.19 ppm), H-6, (7.65), H-9 (7.78), H-13 Me (2.78), H-15 Me (0.87), H-6' (5.12) and H-6'' (5.27) revealed the relationships between these irradiated protons and carbons separated by two or three bonds as shown in Fig. 3. These experiments proved the structural similarity of the chromophore moiety between 1 and 2. Unlike to 2, however, irradiation of a singlet at 6.24 ppm (H-12) changed the six carbon resonances shown in Fig. 3 in addition to two carbons which locate in the side chain (<u>vide infra</u>). This isolated proton (6.24 ppm) is assigned to a non-oxygenated sp^3 methine proton because of the chemical shift of the carbon (31.6 ppm) bearing this proton. Therefore, 1 possesses the partial structure shown in Fig. 3 with the C-12 quinone carbonyl carbon in 2 being replaced by a non-oxygenated methine carbon. The presence of a free hydroxy group at C-11 was verified by deuterium induced upfield shift[5] of this carbon (0.1 ppm).

The remaining unknown moiety, C$_8$H$_9$N$_2$O$_4$, attached to C-12 of the chromophore contains the partial structure of -XH-CH$_2$CH$_2$CO- (H-7''' 10.66, H-8''' 3.96 and 4.16, H-9''' 2.83 and 2.97 ppm, C-8''' 35.9, C-9''' 36.6 ppm, in d$_5$-pyridine, see Fig. 4). The proton chemical shifts of X-H (H-7''') and H-8''' methylene and the carbon chemical shift of C-8''' suggested that this methylene is connected to an acylated amino group. In agreement with this, amino acid analysis of an acid hydrolyzate of 1 proved the presence of a β-alanine moiety in 1. Since the two carbonyl carbons in the unknown moiety, which exhibited deuterium induced upfield
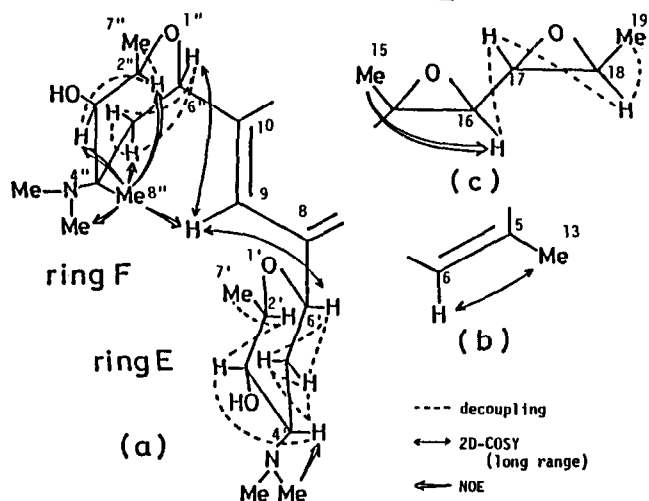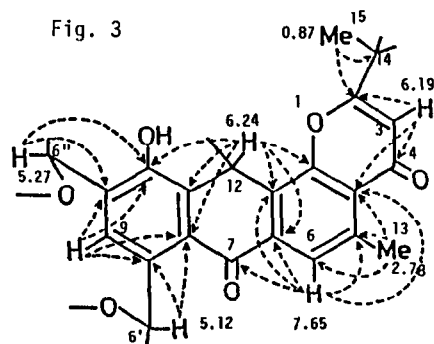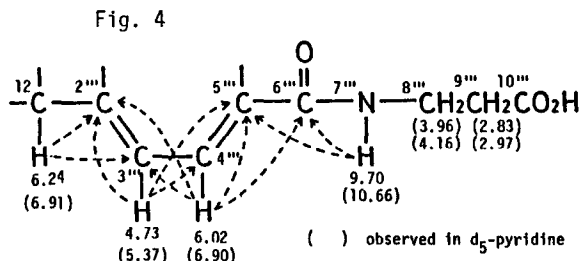
Fig. 3

shifts[5] (0.1 ppm), have been explained as described above and only one amide proton due to H-7''' was observed in the ^{1}H-NMR spectrum of $\underline{1}$, the carbonyl function in the β-alanine moiety must be a free carboxylic acid.

Thus, the -CO-β-alanine part must be connected to C-12 through the still unknown moiety comprising C_4H_3NO which accommodates two protonated carbons at 96.9 and 105.3 ppm and two quaternary carbons at 119.6 and 130.5 ppm observed in the ^{13}C-NMR spectrum taken in d_6-DMSO. In the ^{1}H-NMR spectrum of $\underline{1}$, two proton signals were observed as an AX quartet (in d_6-DMSO, d 4.73 and 6.02 ppm, J=4.0Hz) leaving the final exchangeable proton. In order to analyze this remaining moiety, LSPD experiments were carried out in d_6-DMSO as shown in Fig. 4. Irradiation at 9.70 ppm (7'''-NH) collapsed the carbon signals at 160.0 ppm (C-6''', amide carbonyl) and 119.6 (C-5''') affording the evidence that C-5''' is combined to the amide carbon (C-6'''). Since irradiation at 6.02 (H-4''') collapsed the resonances at 160.0 (C-6''') and 119.6 (C-5'''), the protonated $\underline{sp}^2$ carbon at 105.3 (C-4''') must be combined to C-5'''. In addition, changes of the resonances at 96.9 (C-3''') and 130.5 (C-2''') were also observed. Furthermore, long range couplings were observed between H-3'''(4.73 ppm) and C-2''', C-4''' and C-5''', and between H-12 (6.24 ppm) and C-2''' and C-3'''. These LSPD experiments established the linkage between C-12 to C-6''' as shown in Fig.4.

Now that there remain only one each of N, O and exchangeable H in $\underline{1}$, they are combined to form the N-hydroxy pyrrole moiety.

Based on these experimental results, the planar structure of $\underline{1}$ has been established as shown in Fig. 1. To the best of our knowledge, chromoxymycin is the first natural product with one of the quinone carbonyls being reduced to combine to a branched chain in the anthraquinone-pyrone type compounds represented by pluramycins[6], kidamycin[7] and hedamycin[2].

Fig. 4

( ) observed in d_5-pyridine

References and Footnotes

1) Isolation and biological properties of chromoxymycin will be reported elsewhere.

2) U. Sequin et al., Helv. Chim. Acta, 60, 896 (1977), U. Sequin, Tetrahedron Lett., 761 (1978), U. Sequin et al., Tetrahedron, 34, 3623 (1978)

3) A. Fredenhagen et al., Chimia, 35, 334 (1981)

4) H. Booth, Tetrahedron Lett., 411 (1965)

5) H. Nakayama et al., Tetrahedron Lett., 22, 5217 (1981), D. Gagraire et al., J. C. S. Chem. Comm., 509 (1977)

6) K. Maeda et al., J. Antibiotics, 9A, 75 (1956)

7) N. Kanda, J. Antibiotics, 24, 599 (1971), N. Kanda, ibid. 25, 557 (1972), M. Furukawa et al., Tetrahedron, 31, 2989 (1975)

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