NMR STUDIES OF CHROMOMYCINS, OLIVOMYCINS, AND THEIR DERIVATIVES

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Detailed studies on the ^{13}C and ^{1}H NMR spectra of chromomycins A_2 and A_3, olivomycins A and B, and their derivatives clarified the assignment of many signals which had been unassigned or erroneously reported in the literatures. The revised assignments for chromomycin A_3 and olivomycin A include the assignment of a key ^{13}C signal used to discuss the saccharide linkage in question. Structure analyses based on the revised assignments support the α,1→3-bond between components of the disaccharide moiety in the molecules. Some general information useful for structure analysis of saccharides is also reported.

Chromomycins and olivomycins are structurally related antibiotics displaying antitumor properties and are classified as aureolic acid analogues.[1] Most of them consist of an aglycon and five sugars (*i.e.*, disaccharide and trisaccharide moieties). In an early stage of structural studies on these antibiotics,[2,3] an inter-glycoside linkage of the disaccharide moiety consisting of a D-chromose A unit and a D-chromose D unit was considered to be the α,1→4-bond because 2,6-dideoxy-3-*O*-acetyl-D-*lyxo*-hexopyranose (*i.e.*, 3-*O*-acetyl-D-oliose) was obtained after acid hydrolysis. The D-chromose D unit of the chromomycins and the acetyloliose unit of the olivomycins were considered to be the 2,6-dideoxy-3-*O*-acetyl-D-*lyxo*-hexopyranose connected to the anomeric carbon of the D-chromose A unit (*i.e.*, D-olivomose unit) at the C-4 oxygen and aglycon at C-1. The α-glycoside bond of the terminal D-chromose A was assigned using the data on rotatory contribution, $[M]_D^{24}$.[2] Reinvestigation[4,5] of the structure of chromomycin A_3 (**1**) and olivomycin A (**2**) by NMR, however, revised the structure from the α,1→4-bond to an α,1→3-bond with respect to the inter-glycoside linkage of the disaccharide moiety. The revised structure was confirmed by extensive circular dichroic (CD) studies[6] of the derivatives prepared by permethylation, hydrolysis and per-*p*-bromobenzoylation. Formation of the 2,6-dideoxy-3-*O*-acetyl-D-*lyxo*-hexopyranose in earlier studies was attributed to migration of the acetyl group under the reaction conditions.[6] The structure of the aglycon of chromomycins, chromomycinone, and that of olivomycins, olivin, were also determined;[1,7,8] chromomycinone was 7-methylolivin. Accordingly, the structures **1** and **2** shown in Fig. 1 seem to be appropriate for chromomycin A_3 and olivomycin A, respectively, at present.††

To investigate the structure or biosynthesis of a series of analogues, correct NMR signal assignment is essential. In the course of our studies on the structures of new aureolic acid analogues,[9,10] which are produced by *Streptomyces aburaviensis* PA-39856 together with the known analogues, **1**, **2**, chromomycin A_2 (**3**) and olivomycin B (**4**), we encountered difficulties in identifying the structure

† Deceased.

†† Note that some of chiral centers of the aglycon reported in refs 4 and 5 were erroneously represented (see ref 6).

when we assumed that the reported assignments[4,5] of the ^{13}C signals of **1** and **2** were correct. Doubt occurred as to assignment of a key signal used[4] to revise the inter-glycoside linkage. For these reasons, we carried out detailed NMR studies to permit unequivocal assignments.

The molecular sizes of the analogues are large. Generally, as the molecular size increases, the assignment becomes more difficult. Fortunately, however, partial hydrolysis[2,3] is known to give a variety of degradation products; some sugars are released with conservation of the original order of the saccharide sequence in the remaining portion. Using the partial hydrolysis method, we obtained six derivatives from **1**: A-D-CHR-C-C′ (**1a**), A-D-CHR-C (**1b**), D-CHR-C-C′-B (**1c**), D-CHR-C-C′ (**1d**), D-CHR-C (**1e**) and D-CHR (**1f**). A, B, C, D and CHR refer to the units, D-chromose A, L-chromose B, D-chromose C, D-chromose D, and chromomycinone, respectively; C′ also refers to the D-chromose C unit but the one farthest from the aglycon. Hydrolysis with 50% AcOH aqueous at 50°C for 1.5 hours gave the derivatives **1a**, **1b** and **1c**, the yields in % of those were 5.0, 5.7 and 13.3, respectively. The reaction at 65°C for 2 hours gave **1d**, **1e** and **1f** in 2.4, 11.6 and 9.6% yield, respectively. These derivatives were used to establish definite assignments of the ^{13}C signals, as described below.

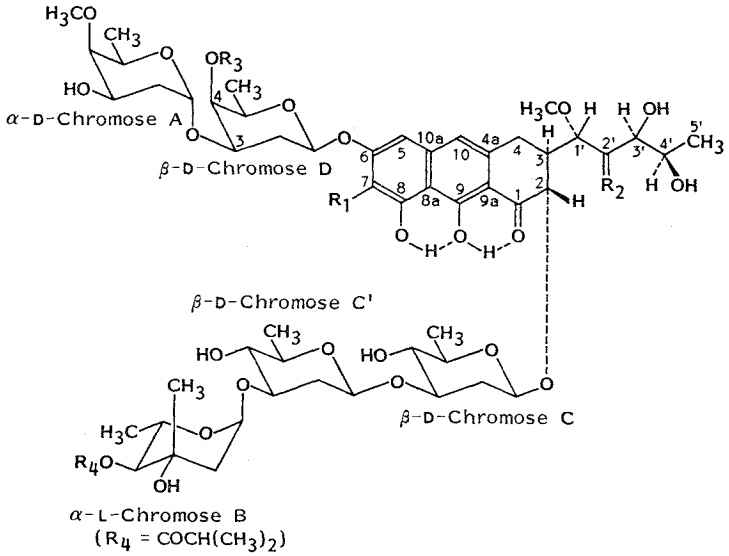
When the ^{13}C data of D-CHR-C-C′-B are compared with those of A-D-CHR-C-C′-B (**1**), the A unit signals should disappear and significant shifts of carbons α and β to the original point of attachment should be observed. The shifts observed are the deglycosylation shifts and the shifts with the reverse sign correspond to the so-called glycosylation shifts or glycosidation shifts.[11,12] The signals corresponding to γ- and δ-carbons in the D unit and those belonging to the other units should remain unchanged, as long as the skeletal conformation does not change. In the NMR spectrum of D-CHR-C-C′ the ^{13}C signals for the B unit are not observed and significant shifts are seen on the C′ unit carbons. The γ- and δ-carbon signals of C′ as well as the shifted α- and β-carbon signals can be assigned by their disappearance from the ^{13}C spectrum of D-CHR-C. The signals belonging to C are also assignable by their disappearance from the spectrum of D-CHR.

Most of the signals could be assigned from successive comparisons using all the hydrolysates. Helpful information from the other derivatives are described later. In using the successive comparison method, we needed to know whether the 4-acetoxy group of the D unit was conserved in the derivatives, since acetyl migration during hydrolysis to the monosaccharide has been reported.[2,3,6] Our ^{1}H data indicated its conservation for derivatives, **1a**~**1f**. Our ^{1}H analyses of **1**, **2** and the hydrolysates confirmed the assignments given for **1** and **2** in the literature[4,5] with a little revision for the signals of the anomeric and C-2-protons of the A and B units. As seen from the ^{1}H data listed in Table 1, the anomeric proton signal of the A or B unit was obvious from its disappearance from **1c** or **1a**. Spin-decoupling experiments on **1** at 400 MHz confirmed the listed assignment. Accordingly, the assignments of ^{13}C signals related to the revised ^{1}H signals also necessitate a revision.

A downfield chemical shift (δ 5.09) and coupling constants (3.0 and *ca.* 0.8 Hz) of the C-4 proton of the D unit in **1f**, which were confirmed by spin-decoupling experiments, clearly indicated the conservation of 4-acetoxy group having an axial orientation. Similar ^{1}H behavior found for the D unit signals of **1e**, **1d** or **1c**, indicated that the partial degradation without acetyl group migration were obtained under the reaction conditions applied.

Based on these methods of assigning the ^{13}C signals, we found the results given in Tables 2 and 3. In our ^{13}C spectroscopic study, a mixed solvent system ($CDCl_3$ - CD_3OH, 2 : 1) was mainly used because of good solubility for all analogues including the derivatives. The signals belonging to each sugar

Fig. 1. Structure of chromomycins, olivomycins and derivatives.



Chromomycin A_3	**(1)**	R_1=CH_3	R_2=O	R_3=R_4=Ac	
Olivomycin A	**(2)**	R_1=H	R_2=O	R_3=Ac	R_4=$COCH(CH_3)_2$
Chromomycin A_2	**(3)**	R_1=CH_3	R_2=O	R_3=Ac	R_4=$COCH(CH_3)_2$
Olivomycin B	**(4)**	R_1=H	R_2=O	R_3=R_4=Ac	
A-D-CHR-C-C′	**(1a)**	R_1=CH_3	R_2=O	R_3=Ac	
A-D-CHR-C	**(1b)**	R_1=CH_3	R_2=O	R_3=Ac	
D-CHR-C-C′-B	**(1c)**	R_1=CH_3	R_2=O	R_3=R_4=Ac	
D-CHR-C-C′	**(1d)**	R_1=CH_3	R_2=O	R_3=Ac	
D-CHR-C	**(1e)**	R_1=CH_3	R_2=O	R_3=Ac	
D-CHR	**(1f)**	R_1=CH_3	R_2=O	R_3=Ac	
Dihydrochromomycin A_2	**(3a)**	R_1=CH_3	R_2=H, OH	R_3=Ac	R_4=$COCH(CH_3)_2$
K-1	**(3b)**	R_1=CH_3	R_2=H, OH	R_3=Ac	R_4=H
K-2	**(3c)**	R_1=CH_3	R_2=H, OH	R_3=R_4=H	

unit were clear, and the deglycosylation α-shifts on the C-3 carbons and β-shifts on the C-2 and C-4 carbons were determined (see Table 3). The anomeric carbons also exhibited the typical ^{13}C behavior depending on the configuration; the anomeric carbon having the α-bond gave a relatively upfield δ value and a relatively large $^1J_{C,H}$ value (see Table 2). Although spin-lattice relaxation time, T_1, of some carbons reported in the literature[4] exhibited inconsistent values for the proposed structure of **1**, the T_1 evaluation based on our assignment supported the proposed saccharide sequence (see NT_1 values shown in Fig. 2). Comparison of the ^{13}C data of **4** with that of **1** showed that, including the sequence, the saccharide moieties of **1** and those of **4** were identical. Similarly, the saccharide sequence of **2** and of **3** were proved to be identical. The differences found for the B unit signals between **1** and **3** or that between **2** and **4** were ascribable to the difference in the 4-*O*-acyl group[2,3] (*i.e.* acetyl or isobutyryl group) of the B unit. When this difference was excluded, the ^{13}C data for the saccharide sequence was the same for **1**, **2**, **3** and **4**. This conclusion necessitates revision of the earlier proposed structures[1,2] for **3** and **4**, and confirms the revised structures[4,5] for **1** and **2**, as shown in Fig. 1.

The C-3 carbon of the D unit† of **1** in the literature[4] is assigned to the signal appearing at δ 82.3

† Note that the names of the sugar units used in the present report differ from those in refs 4 and 5; the D-chromose D unit (*i.e.*, the D unit in the simplified form) corresponds to the saccharide A in ref 4 or 5, and the units, A, C, C′ and B, correspond to the saccharides, B, C, D and E, respectively. Accordingly, C-3 D means the C-3 carbon of the D unit (*i.e.*, the C-3 carbon of the saccharide A in ref 4 or 5).

Table 1. ^{1}H NMR data[a] of chromomycin A_3 and its hydrolysates.

	1		1a		1c	
	δ_H	J (H, H)	δ_H	J (H, H)	δ_H	J (H, H)
Aglycon						
2-H	4.73	11.6 (2, 3)	4.70	11.6 (2, 3)	4.70	11.6 (2, 3)
3-H	2.60	*ca.* 12 (3, 4_{ax}), 11.6 (3, 2), 3.8 (3, 4_{eq}), 2.0 (3, 1′)	2.60~2.65		2.60~2.65	
4-H_{ax}	3.10	16.6 (4_{ax}, 4_{eq}), *ca.* 12 (4_{ax}, 3) 1.4 (4_{ax}, 10)	3.08	*ca.* 15 (4_{ax}, 4_{eq}), *ca.* 13 (4_{ax}, 3)	3.08	*ca.* 15 (4_{ax}, 4_{eq}), *ca.* 13 (4_{ax}, 3)
4-H_{eq}	2.67	16.6 (4_{eq}, 4_{ax}), 3.8 (4_{eq}, 3)	2.65	*ca.* 15 (4_{eq}, 4_{ax}), *ca.* 3 (4_{eq}, 3)	2.66	*ca.* 15 (4_{eq}, 4_{ax}), *ca.* 3 (4_{eq}, 3)
5-H	6.63	s	6.65		6.64	
10-H	6.75	1.4 (10, 4_{ax})	6.76		6.75	
7-CH_3	2.19	s	2.19		2.20	
8-OH	9.81	0.7 (8-OH, 9-OH)	9.82	0.4 (8-OH, 9-OH)	9.81	
9-OH	15.71	0.7 (9-OH, 8-OH)	15.70	0.4 (9-OH, 8-OH)	15.67	
1′-H	4.72	2.0 (1′, 3)	4.72	1.8 (1′, 3)	4.71	1.5 (1′, 3)
3′-H	4.23	5.5 (3′, 3′-OH), 2.0 (3′, 4′)	4.25	5.0 (3′, 3′-OH), 1.8 (3′, 4′)	4.23	5.0 (3′, 3′-OH), 2.0 (3′, 4′)
4′-H	4.37	9.8 (4′, 4′-OH), 6.4 (4′, 5′), 2.0 (4′, 3′)	4.38		4.37	
5′-H	1.38	6.4 (5′, 4′)	1.38	6.2 (5′, 4′)	1.38	6.2 (5′, 4′)
1′-OCH_3	3.53	s	3.52		3.52	
3′-OH	3.69	5.5 (3′-OH, 3′)				
4′-OH	2.00	9.8 (4′-OH, 4′)				
β-D-Chromose D						
1-H	5.22	10.0 (1, 2_{ax}), 2.3 (1, 2_{eq})	5.23	9.8 (1, 2_{ax}), 2.6 (1, 2_{eq})	5.21	9.6 (1, 2_{ax}), 2.4 (1, 2_{eq})
2-H_{ax}	2.22	12.5 (2_{ax}, 2_{eq}), 12.5 (2_{ax}, 3), 10.0 (2_{ax}, 1)	2.0~2.4		2.1~2.3	
2-H_{eq}	2.26	12.5 (2_{eq}, 2_{ax}), 2.3 (2_{eq}, 1), 2.0 (2_{eq}, 3)	2.0~2.4		2.1~2.3	
3-H	3.98	m	3.9~4.2		4.10	
4-H	5.18	3.5 (4, 3), 0.8 (4, 5)	5.18	2.8 (4, 3)	5.06	3.4 (4, 3)
5-H	3.82	6.8 (5, 6), 0.8 (5, 4)	3.83	6.5 (5, 6)	3.86	6.4 (5, 6)
6-H	1.29	6.8 (6, 5)	1.29	6.5 (6, 5)	1.32	6.4 (6, 5)
4-OAc	2.18	s	2.18		2.22	

α-D-Chromose A							
1-H	5.13[b]	2.2 (1, 2_{ax}), 2.2 (1, 2_{eq})	5.13	*ca.* 2 (1, 2_{ax}), *ca.* 2 (1, 2_{eq})		
2-H_{ax}	1.75~1.77[b]	m	1.75~1.83			
2-H_{eq}	1.75~1.77[b]	m	1.75~1.83			
3-H	3.98	m	3.9~4.2			
4-H	3.24	3.5 (4, 3), 0.8 (4, 5)	3.25	3.5 (4, 3)		
5-H	3.89	6.8 (5, 6), 0.8 (5, 4)	3.90	6.5 (5, 6)		
6-H	1.30	6.8 (6, 5)	1.30	6.5 (6, 5)		
4-OCH_3	3.61	s	3.61			
3-OH	1.95	10.8 (3-OH, 3)				
β-D-Chromose C						
1-H	5.10	10.0 (1, 2_{ax}), 2.0 (1, 2_{eq})	5.12	*ca.* 10 (1, 2_{ax}), *ca.* 2 (1, 2_{eq})	5.08	10.0 (1, 2_{ax}), 2.0 (1, 2_{eq})
2-H_{ax}	1.71	12.5 (2_{ax}, 2_{eq}), 10.0 (2_{ax}, 1), 9.5 (2_{ax}, 3)	1.6~1.8		1.6~1.8	
2-H_{eq}	2.49	12.5 (2_{eq}, 2_{ax}), 5.0 (2_{eq}, 3), 2.0 (2_{eq}, 1)	2.4~2.6		2.51	
3-H	3.60	m	3.6~3.7		3.68	
4-H	3.13	9.3 (4, 5), 9.0 (4, 3)	3.16	9.0 (4, 5), 9.0 (4, 3)	3.15	*ca.* 9 (4, 5), *ca.* 9 (4, 3)
5-H	3.31	9.3 (5, 4), 6.0 (5, 6)	3.39~3.40		3.32	6.3 (5, 6), 9 (5, 4)
6-H	1.36	6.0 (6, 5)	1.36	6.2 (6, 5)	1.36	6.3 (6, 5)
4-OH	4.53[c]	br s				
β-D-Chromose C′						
1-H	4.61	9.9 (1, 2_{ax}), 2.0 (1, 2_{eq})	4.66	9.8 (1, 2_{ax}), *ca.* 2 (1, 2_{eq})	4.60	10.0 (1, 2_{ax}), 1.8 (1, 2_{eq})
2-H_{ax}	1.70	12.5 (2_{ax}, 2_{eq}), 9.9 (2_{ax}, 1), 9.5 (2_{ax}, 3)	1.6~1.8		1.6~1.8	
2-H_{eq}	2.29	12.5 (2_{eq}, 2_{ax}), 5.0 (2_{eq}, 3), 2.0 (2_{eq}, 1)	2.0~2.5		2.31	
3-H	3.52	m	3.6~3.7		3.5~3.6	
4-H	3.13	9.3 (4, 5), 9.0 (4, 3), 2.0 (4, 4-OH)	3.16	9.0 (4, 5), 9.0 (4, 3)	3.15	*ca.* 9 (4, 5), *ca.* 9 (4, 3)
5-H	3.39	9.3 (5, 4), 6.0 (5, 6)	3.40~3.41		3.39	*ca.* 9 (5, 4), 6.3 (5, 6)
6-H	1.39	6.0 (6, 5)	1.38	6.2 (6,5)	1.39	6.3 (6, 5)
4-OH	4.02	2.0 (4-OH, 4)				
α-L-Chromose B						
1-H	5.03[b]	4.5 (1, 2_{ax}), 2.2 (1, 2_{eq})			5.01	4.0 (1, 2_{ax}), 2.4 (1, 2_{eq})
2-H_{ax}	2.01[b]	12.0 (2_{ax}, 2_{eq}), 4.5 (2_{ax}, 1)			2.04	
2-H_{eq}	2.06	12.0 (2_{eq}, 2_{ax}), 2.2 (2_{eq}, 1)			2.08	
4-H	4.63	9.3 (4, 5)			4.62	9.4 (4, 5)
5-H	3.99	9.3 (5, 4), 6.3 (5, 6)			3.99	9.4 (5, 4), 6.3 (5, 6)
6-H	1.25	6.3 (6, 5)			1.25	6.3 (6, 5)
3-CH_3	1.37	s			1.37	
4-OAc	2.15	s			2.15	
3-OH	2.34[c]	br s				

Table 1. (Continued)

	1d		1e		1f	
	δ_H	J (H, H)	δ_H	J (H, H)	δ_H	J (H, H)
Aglycon						
2-H	4.68	11.6 (2, 3)	4.71	12.0 (2, 3)	4.47	12.0 (2, 3)
3-H	2.58		2.55~2.65		2.65	*ca.* 13 (3, 4_{ax}), *ca.* 12 (3, 2), *ca.* 3 (3, 4_{eq}), 2.5 (3, 1′)
4-H_{ax}	3.08	*ca.* 16 (4_{ax}, 4_{eq}), *ca.* 12 (4_{ax}, 3)	3.10	*ca.* 17 (4_{ax}, 4_{eq}), *ca.* 13 (4_{ax}, 3)	3.06	*ca.* 17 (4_{ax}, 4_{eq}), *ca.* 13 (4_{ax}, 3)
4-H_{eq}	2.64	*ca.* 16 (4_{eq}, 4_{ax}), *ca.* 3 (4_{eq}, 3)	2.66	*ca.* 17 (4_{eq}, 4_{ax}), *ca.* 3 (4_{eq}, 3)	2.73	*ca.* 17 (4_{eq}, 4_{ax}), *ca.* 3 (4_{eq}, 3)
5-H	6.66		6.65		6.67	
10-H	6.76		6.75		6.82	
7-CH_3	2.20		2.19		2.20	
8-OH	9.83	0.7 (8-OH, 9-OH)	9.82	0.9 (8-OH, 9-OH)	9.63	1.0 (8-OH, 9-OH)
9-OH	15.70	0.7 (9-OH, 8-OH)	15.63		14.74	1.0 (9-OH, 8-OH)
1′-H	4.73	1.8 (1′, 3)	4.73	1.8 (1′, 3)	4.66	2.5 (1′, 3)
3′-H	4.23	4.8 (3′, 3′-OH), 1.8 (3′, 4′)	4.22		4.29	
4′-H	4.36		4.35		4.40	
5′-H	1.38	6.2 (5′, 4′)	1.38	6.6 (5′, 4′)	1.39	6.4 (5′, 4′)
1′-OCH_3	3.52		3.52		3.59	
3′-OH			3.67		3.60[c]	
4′-OH			3.67		3.81[c]	
β-D-Chromose D						
1-H	5.22	9.5 (1, 2_{ax}), 2.5 (1, 2_{eq})	5.22	9.6 (1, 2_{ax}), 2.3 (1, 2_{eq})	5.23	9.8 (1, 2_{ax}), 2.8 (1, 2_{eq})
2-H_{ax}	2.1~2.3		2.1~2.2		2.15	
2-H_{eq}	2.1~2.3		2.1~2.2		2.19	
3-H	4.08		4.08		4.08	
4-H	5.08	3.4 (4, 3), 0.8 (4, 5)	5.08	3.0 (4, 3), 0.8 (4, 5)	5.09	3.0 (4, 3), 0.8 (4, 5)
5-H	3.86		3.86	6.4 (5, 6)	3.86	6.4 (5, 6), 0.8 (5, 4)
6-H	1.33	6.7 (6, 5)	1.32	6.4 (6, 5)	1.33	6.4 (6, 5)
4-OAc	2.22		2.22		2.22	
β-D-Chromose C						
1-H	5.10	10.0 (1, 2_{ax}), 1.8 (1, 2_{eq})	5.17	*ca.* 10 (1, 2_{ax}), *ca.* 2 (1, 2_{eq})		
2-H_{ax}	1.6~1.8		1.70			
2-H_{eq}	2.50		2.57			
3-H	3.6~3.7		3.67~3.75			
4-H	3.15[d]	*ca.* 9 (4, 5), *ca.* 9 (4, 3)	3.15	9.0 (4, 5), 9.0 (4, 3)		
5-H	3.31	*ca.* 9 (5, 4), *ca.* 6.5 (5, 6)	3.35	9.0 (5, 4), 5.8 (5, 6)		
6-H	1.36	6.5 (6, 5)	1.35	5.8 (6, 5)		
β-D-Chromose C′						
1-H	4.65	9.8 (1, 2_{ax}), 2.0 (1, 2_{eq})				
2-H_{ax}	1.6~1.8					
2-H_{eq}	2.2~2.3					
3-H	3.6~3.7					
4-H	3.13[d]	*ca.* 9 (4, 5), *ca.* 9 (4, 3)				
5-H	3.40	*ca.* 9 (5, 4), 6.5 (5, 6)				
6-H	1.38	5.8 (6, 5)				

[a] In $CDCl_3$ at 24°C. J(H, H) values are apparent coupling constant; in Hz.
[b] Assignments given in ref 4 were revised as shown.
[c,d] Assignments may be interchanged in each vertical column.

Table 2. ^{13}C NMR data of chromomycins and olivomycins.

	1				3	4	2		
	δ_C[a]	δ_C[b]	δ_C[c]	J_{CH}[b]	(δ_C[c])	(δ_C[c])	δ_C[a]	δ_C[b]	δ_C[c]
Aglycon									
C-1	202.1	202.7	202.7	s	202.9	202.9	202.3	202.8	202.9
C-2	75.9	76.6	77.0	144[f]	77.0	76.9	75.9	76.8	76.8
C-3	43.8	43.1	43.4	130[f]	43.4	43.1	43.5	42.8	43.1
C-4	27.0[d]	27.1	27.3	131[f]	27.3	27.4	27.0[d]	27.2	27.4
C-5	100.8	101.1	101.6	162	101.8	103.4	102.9	102.8	103.4
C-6	159.6	159.6	160.0	s	160.1	161.1	160.8	160.8	161.2
C-7	111.6	111.6	111.9	s	112.1	102.4	102.0	102.2	102.4
C-8	165.3	165.4	165.8	s	165.8	166.0	165.6	165.6	166.0
C-9	156.1	156.2	156.6	s	156.6	159.9	159.6	159.6	159.9
C-10	117.1	117.5	117.4	162	117.5	117.4	117.1	117.3	117.3
C-4a	134.6	135.0	135.4	s	135.5	136.9	135.9	136.6	136.9
C-8a	108.0	108.1[e]	108.6	s	108.6	108.6[e]	108.1[e]	108.4[e]	108.6[e]
C-9a	108.0	108.2[e]	108.6	s	108.6	108.8[e]	108.4[e]	108.5[e]	108.8[e]
C-10a	138.4	138.6	138.9	s	139.0	141.3	140.8	141.1	141.3
7-CH_3	8.2	8.3	8.3	129	8.3	—	—	—	—
C-1′	82.0[d]	82.2	82.4	148[f]	82.1	82.1	81.6[d]	81.9	82.1
C-2′	211.2	212.1	211.9	s	211.9	211.8	211.0	212.0	211.8
C-3′	78.4	79.0	79.0	142[f]	79.1	79.1	78.2	79.0	79.1
C-4′	67.9	68.3	68.4	144	68.5	68.5	68.0	68.5	68.5
C-5′	20.5	19.8	19.9	128	19.8	19.8	20.5	19.7	19.8
1′-OCH_3	59.7	59.4	59.5	143	59.5	59.4	59.6	59.3	59.4
D-Chromose D									
C-1	97.4	97.4	97.9	162[f]	98.0	97.6	97.1	97.2	97.6
C-2	33.0	33.2	33.3	133[f]	33.5	33.4	32.9	33.2	33.3
C-3	69.9[d]	70.1	70.6	137[f]	70.8	70.9	70.1[d]	70.4	70.9
C-4	67.3	67.9	68.3	150	68.4	68.4	67.3	68.0	68.4
C-5	69.7	69.9	70.2	137[f]	70.3	70.2	69.8	70.0	70.2
C-6	16.8	16.9	16.9	128	16.9	16.8	16.8	16.8	16.8
CH_3CO	20.8	20.8	20.7	130	20.7	20.7	20.8	20.8	20.7
CH_3CO	170.9	171.6	171.7	s	171.7	171.7	170.9	171.6	171.6
D-Chromose A									
C-1	95.2[d]	95.3	95.8	168	96.0	96.1	95.4[d]	95.5	96.1
C-2	33.5	33.0	33.3	133[f]	33.3	33.3	33.5	33.0	33.3
C-3	65.9	66.3	66.6	146	66.7	66.6	65.9	66.4	66.6
C-4	81.5	81.4	82.1	140[f]	82.1	82.1	81.5	81.9	82.1
C-5	67.0[d]	67.4	67.6	140[f]	67.7	67.6	67.0[d]	67.4	67.5
C-6	17.3	17.2	17.2	128	17.2	17.2	17.3	17.1	17.2
OCH_3	62.3	62.1	62.1	142	62.1	62.1	62.4	62.1	62.1
D-Chromose C									
C-1	100.3	100.8	101.0	162[f]	101.0	101.0	100.3	100.8	101.0
C-2	37.5	37.6	37.8	131	37.8	37.7	37.5	37.6	37.7
C-3	82.3[d]	81.4	81.9	140[f]	81.8	81.7	82.3[d]	81.7	81.7
C-4	75.1	75.5	75.8	142[f]	75.8	75.8	75.1	75.5	75.8
C-5	72.3	72.5	72.7	140[f]	72.7	72.7	72.3	72.4	72.7
C-6	18.0	18.1	18.1	127	18.2	18.2	18.0	18.2	18.2
D-Chromose C′									
C-1	99.7	99.7	99.8	160	99.8	99.7	99.7	99.6	99.7
C 2	37.1	36.8	37.2	131	37.2	37.2	37.1	36.9	37.2
C-3	80.5	77.6	78.4	142	78.3	78.1	80.7	78.0	78.3
C-4	75.3	75.2	75.5	142[f]	75.5	75.5	75.3	75.1	75.5
C-5	72.2	72.7	72.9	140[f]	73.0	72.9	72.2	72.8	72.9
C-6	17.8	17.9	18.0	127	18.0	17.9	17.8	17.9	17.9

Table 2. (Continued)

	1				3	4	2		
	δ_C[a]	δ_C[b]	δ_C[c]	J_{CH}[b]	(δ_C[c])	(δ_C[c])	δ_C[a]	δ_C[b]	δ_C[c]
L-Chromose B									
C-1	97.0[d]	95.5	96.1	164	96.0	95.7	97.2[d]	95.7	96.0
C-2	43.8	43.7	44.0	130	44.2	44.0	43.9	44.0	44.1
C-3	70.6	70.5	70.6	s	70.8	70.6	70.6	70.6	70.7
C-4	79.6[d]	79.5	79.8	145[f]	79.5	79.8	79.4[d]	79.1	79.4
C-5	66.7	66.8	67.1	146	67.2	67.1	66.7	66.7	67.1
C-6	17.8	17.7	17.9	128	17.9	17.8	17.9	17.8	17.8
3-CH_3	23.0	22.7	23.0	126	23.1	23.0	23.0	22.7	23.0
CH_3C	20.9	21.0	20.9	130	19.1	20.9	18.9	19.0	19.0
CH_3C	—	—	—	—	19.1	—	19.0	19.1	19.0
CH_3CHCO	—	—	—	—	34.8	—	34.3	34.6	34.7
CO	171.4	171.6	171.6	s	177.7	171.6	177.5	177.5	177.6

[a] In $CDCl_3$ at 24°C, [b] In $CDCl_3$ - CD_3OH (2:1) at 24°C, [c] In $CDCl_3$ - CD_3OH (2:1) at 60°C.
[d] Assignments given in refs 4 and 5 were revised. [e] Assignments may be interchanged in each column.
[f] Approximate value; a part of the ^{13}C signals in ^1H-undecoupled spectrum overlapped.

in $CDCl_3$ at room temperature, and the low field δ value has been cited to support assignment of a saccharide linkage at the C-3. Surprisingly, however, our data showed that the δ value of the carbon is 69.9. The δ value of the corresponding signal in the mixed solvent system ($CDCl_3$ - CD_3OH, 2:1) at room temperature and that at 60°C were 70.1 and 70.9, respectively. In addition to the assignment of the signal, C-3 D, several signal assignments reported for **1** or **2** apparently differ from our assignments (see Table 2).† The assignment of the anomeric carbons, C-1 A and C-1 B, has been explained above. To confirm the validity of our assignment, other derivatives were prepared. First, deacylation was attempted to inspect the ^{13}C behavior of the D unit and B unit signals, because this method[13] has previously been used for assigning ^{13}C signals. Two acetyl groups are involved in **1**, while **3** has an acetyl group and an isobutyryl group. Compound **3** was chosen because it is easy to determine its deacylated site. The usual alkaline hydrolysis gave a deisobutyryl derivative, and not a simple deacetyl derivative. More vigorous hydrolysis of **3** gave various compounds, which were attributable to the reactivity of the carbonyl groups in the aglycon moiety. Next, the side-chain carbonyl of **3** was reduced using sodium borohydride[14], and dihydrochromomycin A_2 (**3a**) was obtained. Alkaline hydrolysis of **3a** gave a deisobutyryl derivative, K-1 (**3b**), and subsequently a deacetyldeisobutyryl derivative, K-2 (**3c**). The derivative, **3a**, was useful for discriminating the aglycon carbons from the sugar carbons, and the derivatives, **3b** and **3c**, were useful for confirming the carbons belonging to the B and D units. As can be seen in the ^{13}C data listed in Table 3, reduction of the side-chain carbonyl caused a significant shift on many carbons of the aglycon with little change on the sugar carbons, while the deisobutyrylation or the deacetylation selectively caused a shift on the B unit or D unit carbons, which strongly supported the validity of the assignment shown in the present study. Clearly, the reported assignments of the C-4 signal of the aglycon, δ 43.7, and that of the B unit, δ 67.0, were mistaken, since the result of successive comparisons using all the derivatives indicated clearly different signals: The corresponding δ values of the signals of **1** were 27.3 (C-4 CHR) and 79.8 (C-4 B), respectively, in the mixed solvent system. The difference in the solvent system or temperature had a little effect on the shift, and in the case of some contiguous signals, a change in the order of δ values

† See foot note of p. 55.

Table 3. ^{13}C Chemical shifts[a] of chromomycin A_3 (**1**), A_2 (**3**) and their derivatives.

	1	1a	1b	1c	1d	1e	1f	3	3a	3b	3c
Aglycon											
C-1	202.7	202.7	202.7	202.6	202.6	202.5	203.6	202.9	204.0	203.8	203.8
C-2	77.0	77.0	76.8	77.0	77.3	76.8	72.9	77.0	77.9	77.8	77.8
C-3	43.4	43.3	43.3	43.2	43.3	43.2	44.5	43.5	42.6	42.4	42.4
C-4	27.3	27.4	27.4	27.2	27.4	27.2	27.6	27.3	27.8	27.7	27.7
C-5	101.6	101.6	101.6	101.6	101.7	101.6	101.6	101.8	101.5	101.5	101.7
C-6	160.0	159.9	159.9	159.9	160.0	159.9	159.9	160.1	159.9	159.8	160.0
C-7	111.9	111.9	111.9	111.9	112.0	111.9	111.9	112.1	111.7	111.6	111.6
C-8	165.8	165.7	165.7	165.6	166.0	165.6	164.6	165.8	165.6	165.6	165.5
C-9	156.6	156.5	156.5	156.4	156.7	156.4	156.3	156.6	156.4	156.5	156.5
C-10	117.4	117.3	117.3	117.3	117.2	117.2	117.6	117.5	117.5	117.3	117.2
C-4a	135.4	135.5	135.5	135.4	135.8	135.3	135.7	135.5	136.6	136.6	136.7
C-8a	108.6	108.6	108.6	108.4[b]	108.8[b]	108.4	108.4[b]	108.6	108.4[b]	108.5[b]	108.5[b]
C-9a	108.6	108.6	108.6	108.5[b]	108.9[b]	108.4	107.9[b]	108.6	108.8[b]	108.8[b]	108.8[b]
C-10a	138.9	138.9	138.9	138.8	139.0	138.8	138.9	139.0	139.0	138.9	139.0
7-CH_3	8.3	8.3	8.3	8.3	8.3	8.2	8.3	8.3	8.3	8.2	8.2
C-1′	82.4	82.3	82.3	82.2	82.4	82.3	83.3	82.1	78.5[c]	78.6[c]	78.6[c]
C-2′	211.9	211.9	211.9	211.9	212.2	211.8	212.0	211.9	72.3[c]	72.2[c]	72.2[c]
C-3′	79.0	79.1	79.1	79.0	79.1	78.9	79.3	79.1	74.0[c]	73.9[c]	74.0[c]
C-4′	68.4	68.5	68.5	68.4	68.6	68.4	68.4	68.5	70.2[c]	70.2[c]	70.2[c]
C-5′	19.9	19.8	19.8	19.8	19.8	19.8	19.7	19.8	19.8	19.7	19.7
1′-OCH_3	59.5	59.4	59.4	59.4	59.4	59.4	59.7	59.5	60.5	60.5	60.6
D-Chromose D											
C-1	97.9	97.9	97.9	97.9	98.1	97.8	98.0	98.0	98.0	97.9	98.1
C-2	33.3	33.4	33.4	34.8	34.9	34.7	34.9	33.5	33.3	33.3	32.7
C-3	70.6	70.8	70.8	67.6	67.7	67.7	67.7	70.8	70.6	70.6	73.4
C-4	68.3	68.4	68.4	72.0	72.2	72.0	72.2	68.4	68.3	68.2	67.3
C-5	70.2	70.2	70.2	70.3	70.5	70.3	70.4	70.3	70.2	70.2	71.6
C-6	16.9	16.9	16.9	16.9	17.0	16.9	16.9	16.9	16.9	16.9	16.9
CH_3CO	20.7	20.7	20.7	20.8	20.8	20.8	20.8	20.7	20.7	20.7	—
CH_3CO	171.7	171.7	171.7	172.2	172.4	172.3	172.4	171.7	171.7	171.6	—

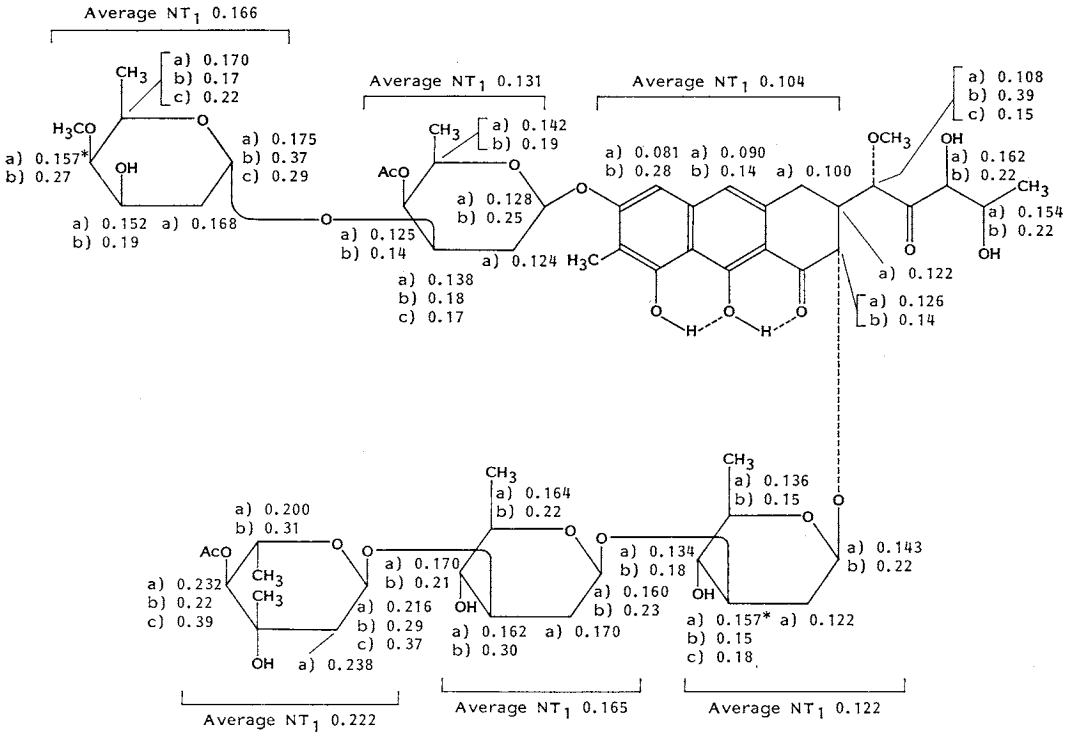
Table 3. (Continued)

	1	1a	1b	1c	1d	1e	1f	3	3a	3b	3c
D-Chromose A											
C-1	95.8	96.0	96.0	—	—	—	—	96.0	95.7	95.7	96.1
C-2	33.3	33.3	33.3	—	—	—	—	33.3	33.3	33.3	33.5
C-3	66.6	66.6	66.6	—	—	—	—	66.7	66.7	66.6	66.7
C-4	82.1	82.1	82.1	—	—	—	—	82.1	82.1	82.0	82.1
C-5	67.6	67.6	67.6	—	—	—	—	67.7	67.7	67.6	67.5
C-6	17.2	17.2	17.2	—	—	—	—	17.2	17.3	17.2	17.1
OCH_3	62.1	62.1	62.1	—	—	—	—	62.1	62.1	62.1	62.1
D-Chromose C											
C-1	101.0	101.1	101.1	101.0	101.2	101.1	—	101.0	100.8	100.7	100.8
C-2	37.8	37.7	39.6	37.7	37.9	39.5	—	37.8	37.8	37.8	37.7
C-3	81.9	81.9	71.9	81.7	81.8	71.9	—	81.8	82.0	82.0	81.9
C-4	75.8	75.8	78.0	75.7	75.9	77.9	—	75.8	75.8	75.7	75.8
C-5	72.7	72.7[b]	72.7	72.6	72.7[c]	72.6	—	72.7	72.7	72.6	72.6
C-6	18.1	18.2	18.0	18.2	18.2	18.0	—	18.2	18.0	18.1	18.2
D-Chromose C′											
C-1	99.8	99.9	—	99.6	99.9	—	—	99.8	99.8	99.8	99.8
C-2	37.2	39.6	—	37.1	39.8	—	—	37.2	37.2	37.2	37.2
C-3	78.4	71.6	—	78.3	71.6	—	—	78.3	78.3	78.6	78.6
C-4	75.5	77.4	—	75.4	77.5	—	—	75.5	75.6	75.6	75.6
C-5	72.9	72.8[b]	—	72.8	72.9[c]	—	—	73.0	72.9	72.8	72.8
C-6	18.0	17.8	—	17.9	17.8	—	—	18.0	18.0	18.0	18.0
L-Chromose B											
C-1	96.1	—	—	95.9	—	—	—	96.0	96.0	96.5	96.5
C-2	44.0	—	—	43.9	—	—	—	44.2	44.2	43.6	43.6
C-3	70.6	—	—	70.6	—	—	—	70.8	70.8	71.5	71.5
C-4	79.8	—	—	79.7	—	—	—	79.5	79.5	79.6	79.6
C-5	67.1	—	—	67.0	—	—	—	67.2	67.2	68.4	68.4
C-6	17.9	—	—	17.8	—	—	—	17.9	17.9	18.0	18.0
3-CH_3	23.0	—	—	23.0	—	—	—	23.1	23.1	22.1	22.1
CH_3C	20.9	—	—	20.9	—	—	—	19.1	19.1	—	—
CH_3CHCO	—	—	—	—	—	—	—	34.8	34.8	—	—
CH_3CO	171.6	—	—	171.7	—	—	—	177.7	177.7	—	—

[a] In $CDCl_3$ - CD_3OH (2 : 1) at 60°C.

[b,c] Assignments may be interchanged in each vertical column.

Fig. 2. ^{13}C NT_1 values (in s) of chromomycin A_3.



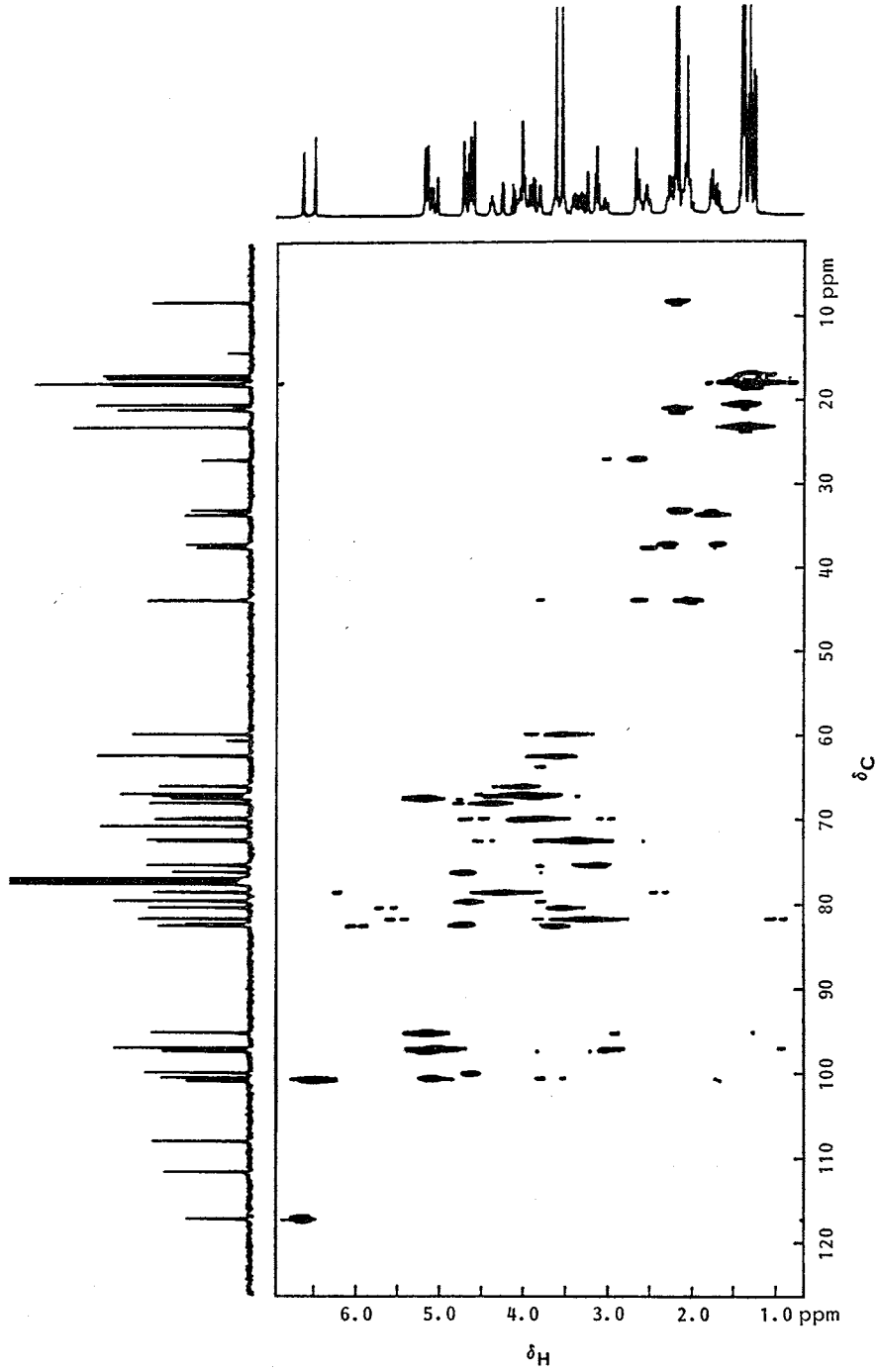
[a] Values obtained from 50 MHz spectra in $CDCl_3$ - CD_3OH (2 : 1), at 24°C. [b] Values reported in ref 4; in $CDCl_3$, 68 MHz, [c] Values in $CDCl_3$ rearranged on the basis of our assignment.

* Averaged value of the two components showing the identical chemical shift.

may have occurred depending upon the measurement conditions. To make the assignment in $CDCl_3$ more concrete, we measured the ^1H-^{13}C correlated two-dimensional NMR (HC COSY) spectra, one of which is shown in Fig. 3. Using the spectra, we confirmed the assignment listed in Table 2 and revised the reported assignment of the eight carbons (*i.e.* C-3 D, C-1 A, C-5 A, C-3 C, C-1 B, C-4 B, C-4 CHR and C-1′ CHR) and assigned the 16 carbon signals unassigned in the literature.[4] The same revision of the reported assignment[5] for **2** was also made based on data comparison. Note that the δ values of ^1H signals recorded at high concentration for the ^{13}C measurement slightly differ from the data listed in Table 1; the upfield shifts ($\Delta\delta$ $-0.13 \sim -0.22$) at the high concentration were observed. We also confirmed the ^1H assignment under the high concentration by the spin-decoupling experiments and analyzed the HC COSY spectra.

One of the merits of the successive comparison method using the derivatives is that the shift behavior dependent on the environment can be examined. This can be used to obtain information useful to assignment work and to conformational or configurational analysis. The acylation shifts and the glycosylation shifts found in the sugars are summarized in Table 4. Reflecting the differences in environment, significant differences were found for the acylation and the glycosylation shifts. Although the acylation shifts on the D unit carbons exhibit the expected behavior, the acylation α-shift on the C-4 of the B unit almost equals zero. The extremely small shift, which we sometimes encounter, is a weak point of the acylation method. To circumvent this weak point, a method using the methoxy-

Fig. 3. HC COSY spectrum of chromomycin A_3.



carbonylation shifts or the mesylation shifts has been proposed.[15] The extremely small α-shift is related to the conformational status. A crowd of bulky groups on C-3, C-4 and C-5 of the B unit prevent free rotation of the acyl group and bring about a preferred conformation in which a γ-effect[16,17] to the C-4 from the carbonyl or alkyl group of the acyl group is enhanced. The effect toward an

Table 4. Acylation shifts and glycosylation shifts found in sugar carbons[a].

Sugar unit	D	B	C	C′
Acetylation α-shift	+0.9 (C-4)	+0.2 (C-4)[b]		
Acetylation β-shift	−2.8 (C-3)	−0.9 (C-3)[b]		
	−1.4 (C-5)	−1.3 (C-5)[b]		
Isobutyrylation α-shift		−0.1 (C-4)		
Isobutyrylation β-shift		−0.7 (C-3)		
		−1.2 (C-5)		
Glycosylation α-shift	+3.0 (C-3)		+10.0 (C-3)	+6.8 (C-3)
Glycosylation β-shift	−1.5 (C-2)		−1.9 (C-2)	−2.6 (C-2)
	−3.7 (C-4)		−2.3 (C-4)	−1.9 (C-4)

[a] In ppm, in $CDCl_3$ - CD_3OH (2 : 1) at 60°C.
[b] Caluculated value using the data of **3b** and **1**.

upfield shift cancels out the downfield shift caused by an inductive effect of the acyl group. This is the reason for the extremely small α-shift. A similar conformation effect on the α-shift also exists in the case of the glycosylation shift. The glucosidation α-shift[12] is known to vary from a small one comparable with an absolute value of the β-shift to a large one over 10 ppm, in correlation with the conformational status. The α-shifts listed in Table 4 also offer good examples. It should be noted that a large glycosylation α-shift[4] of about 10 ppm does not always occur. When a large α-shift is observed, the conformation preferred would one in which a proton on the α-carbon (*i.e.*, α-proton) and a proton on the glycosyl C-1′ (*i.e.*, anomeric proton) have a nearly parallel relationship.[17,18] The preference of a nearly parallel relation between the α-proton and a glycosyl C-2′ carbon or that between the α-proton and a glycosyl ring-oxygen can be assumed when the α-shift is very small.[16,17] The α-shift observed for the C-3 C corresponds to the former case and that for the C-3 D to the latter case. Usually, however, the parallel conformation is an unstable form[19] and is not observed. On such an occasion, the intermediate value of the α-shift, similar to the value for the C-3 C′, is observed. This intermediate α-shift is also observable when all of the possible conformers have a similar stability. The shift behavior on glucosides or mannosides of alcohols were examined, and the characteristic α- and β-shifts were used to investigate the stereochemistry of the original alcohol.[11,12] The reported shift rule to deduce the absolute configuration of an alcohol is not applicable in the present case because the sugars have a polar group (*i.e.*, hydroxy or acetoxy group) on a position corresponding to the β-carbon, which influences the conformation about the glycoside bond and therefore affects the shift behavior through hydrogen bonding, electrostatic interaction, and steric interaction. The reported shift rule is for alcohols having nonpolar groups or no substituent on the β-carbon.

The conspicuous shift of some carbons caused by solvent effects also is related to the conformational considerations described above. Apart from the shift behavior, the positive α-shift and the negative β-shift caused by the glycosylation (*i.e.*, the shifts with reverse sign caused by the deglycosylation) could be used to assign the sugar carbons.

Among the ^1H NMR data listed in Table 1 the spin-spin interaction between the hydroxyl protons, 8-OH and 9-OH, attracted our attention, though absolute values of the coupling constants observed were small (0.4～1.0 Hz). The hydrogen bonds shown in Fig. 1 undoubtedly contribute to the occurrence of the spin coupling. The spin coupling behavior is probably useful for detection of specific hydrogen bonding system.

In conclusion, this study clarified the assignment of many signals which had been unassigned or

erroneously reported for chromomycins and olivomycins. Our findings should be useful for analyzing the structures of new analogues.

Experimental

Measurements of NMR Spectra

^1H NMR spectra were recorded with Varian XL-200 and XL-400 spectrometers, operating at 200.057 MHz and 399.948 MHz. The concentrations were *ca.* 10 mg/ml ($CDCl_3$ solution) and temperatures were 24°C. An internal standard, TMS, was used in the ^1H measurements and also in the ^{13}C measurements described below.

^{13}C NMR spectra in the mixed solvent system ($CDCl_3$ - CD_3OH, 2:1) at 60°C were recorded with a Varian XL-100-12 spectrometer operating at 25.160 MHz. The concentrations were *ca.* 140 mg/ml. ^{13}C Spectra and T_1 values in the mixed solvent at 24°C were measured with a Varian XL-200 spectrometer operating at 50.309 MHz. The T_1 values were determined by inversion recovery method using an exponential least-squares fit. Concentrations were *ca.* 140 mg/ml. HC COSY spectra were recorded with a Varian XL-400 spectrometer operating at 100.579 MHz for ^{13}C and at 399.948 MHz for ^1H. The solvent was $CDCl_3$, and the concentration and temperature were *ca.* 95 mg/ml and 24°C, respectively.

The observational error for the ^{13}C spectra was *ca.* $\pm$0.1 ppm and that for the spectra to determine $^1J_{C,H}$ values was *ca.* $\pm$1 Hz. The digital resolution for the ^1H spectra was *ca.* 0.3 Hz.

Materials

Isolation procedure of the aureolic acid analogues produced by *S. aburaviensis* PA-39856 is described elsewhere.[9] The procedures for preparing the derivatives are described here.

Dihydrochromomycin A_2 (**3a**), K-1 (**3b**), and K-2 (**3c**): To an ice-cooled solution of 3.2 g of chromomycin A_2 in 100 ml of dry MeOH, sodium borohydride (850 mg) was gradually added. After 45 minutes, dilute HCl was added and the mixture was poured into water. A crude extract with EtOAc was loaded on a preparative HPLC column, Lichroprep RP-18, and eluted with MeOH - 30 mM tartrate buffer (80:20, pH 3.5) to give 965 mg of **3a**; mp 173.5~174.5°C, *Anal* C 58.11, H 7.42 (calcd for $C_{59}H_{88}O_{26}$, C 58.40, H 7.31), $[\alpha]_D^{25}$ (*c* 1.00, EtOH) $-39.3\pm0.8°$.

The **3a** (780 mg) was dissolved in 160 ml of MeOH and treated with 5% KOH in MeOH (40 ml) at room temperature under N_2. After 50 minutes, the solution was neutralized with 44 ml of 5% aq oxalic acid at 4°C, and then concentrated and extracted with BuOH. The extract was washed twice with 1% aq NaCl and then with water. After purification with the preparative HPLC column, Lichroprep RP-18, using the eluting solvent, MeOH - H_2O - AcOH (80:20:0.1), gave 300 mg of **3b** and 360 mg of **3c**. The physico-chemical constants of **3b** and **3c** are: **3b**; mp 184.0~185.7°C, *Anal* C 54.47, H 7.27 (calcd for $C_{55}H_{82}O_{25}$, C 57.78, H 7.23), $[\alpha]_D^{25}$ (*c* 1.00, EtOH) $-5.2\pm0.4°$: **3c**; mp 181.7~182.5°C, *Anal* C 57.49, 7.32 (calcd for $C_{53}H_{80}O_{24}$, C 57.81, H 7.32), $[\alpha]_D^{25}$ (*c* 1.00, EtOH) $+77.9\pm1.2°$.

A-D-CHR-C-C′ (**1a**), A-D-CHR-C (**1b**), and D-CHR-C-C′-B (**1c**): A solution of chromomycin A_3 (1.2 g) in 50% AcOH aq (25 ml) was warmed, and the temperature was maintained at 50°C for 1.5 hours. After addition of water, the reaction mixture was extracted with EtOAc. The extract was washed well with water, 1% NaCl aq, and then water. After evaporation of the solvent, the residual materials were separated by chromatography using silica gel containing 10% water. Elution with $CHCl_3$ - MeOH - H_2O - EtOAc, changing the ratio from 2,000:50:3:2 to 2,000:100:6:2, gave 140 mg of **1c**, 100 mg of the nearly equimolar mixture of **1a** and **1b**, and 650 mg of starting material. The physico-chemical constants of **1c** are: MP 169.0~170.5°C, *Anal* C 57.94, H 6.88 (calcd for $C_{50}H_{70}O_{23}$, C 57.79, H 6.79), $[\alpha]_D^{25}$ (*c* 1.00, EtOH) $-77.4\pm1.2°$.

D-CHR-C-C′ (**1d**), D-CHR-C (**1e**), and D-CHR (**1f**): A solution of chromomycin A_3 (4 g) in 50% AcOH aq (80 ml) was warmed, and the temperature was maintained at 65°C for 2 hours. After addition of water, the reaction mixture was extracted with EtOAc and the extract was washed with water, 1% NaCl aq, and then water. Evaporation *in vacuo* gave 2.4 g of residue. Two-step silica gel chromatography of the residue was conducted using AcOH containing 1% oxalic acid and using

$CHCl_3$ - MeOH - H_2O - AcOH, with the ratio changing from 2,000 : 50 : 3 : 2 to 2,000 : 150 : 10 : 2, as eluting solvents, subsequent purification with HPLC using Lichroprep RP-18 and eluting solvent, MeOH - H_2O - AcOH (700 : 200 : 1), gave 70 mg of **1d**, 280 mg of **1e**, and 190 mg of **1**. Physicochemical constants of the derivatives are as follows: **1e**; mp 170.5~172.0°C (dec), *Anal* C 57.81, H 6.42 (calcd for $C_{35}H_{46}O_{16}$, C 58.16, H 6.42), $[\alpha]_D^{25}$ (*c* 1.00, EtOH) $-104.1\pm1.5°$: **1f**; mp 149.5~150.8°C (dec), *Anal* C 58.38, H 6.10 (calcd for $C_{29}H_{36}O_{13}$, C 58.78, H 6.12), $[\alpha]_D^{25}$ (*c* 1.00, EtOH) $-30.4\pm1.1°$.

References

1) BERLIN, YU. A.; M. N. KOLOSOV & L. A. PIOTROVICH: The stereochemistry of antibiotics of the aureolic acid group. Tetrahedron Lett. 1970: 1329~1331, 1970
2) MIYAMOTO, M.; Y. KAWAMATSU, K. KAWASHIMA, M. SHINOHARA, K. TANAKA, S. TATSUOKA & K. NAKANISHI: Chromomycin A_2, A_3 and A_4. Tetrahedron 23: 421~437, 1967
3) BERLIN, YU. A.; S. E. EPIPOV & M. N. KOLOSOV: Olivomycin and related antibiotics. XVIII. Structure of olivomycins A, B, C and D. Khim. Prir. Soedin 5: 567~572, 1969 [Chem. Abstr. 73: 25823r, 1970]
4) THIEM, J. & B. MEYER: Studies on the structure of chromomycin A_3 by ^1H and ^{13}C nuclear magnetic resonance spectroscopy. J. Chem. Soc. Perkin Trans. II 1979: 1331~1336, 1979
5) THIEM, J. & B. MEYER: Studies on the structure of olivomycin A and mithramycin by ^1H and ^{13}C nuclear magnetic resonance spectroscopy. Tetrahedron 37: 551~558, 1981
6) RICCIO, R. & K. NAKANISHI: Circular dichroic method for determining the position of glycosidic linkage of deoxy sugar moieties antitumor antibiotic chromomycin A_3. J. Org. Chem. 47: 4589~4592, 1982
7) BAKHAEVA, G. P.; YU. A. BERLIN, O. A. CHUPRUNOVA, M. N. KOLOSOV, G. YU. PECK, L. A. PIOTROVICH, M. M. SHEMYAKIN & I. V. VASINA: The stereochemistry of olivomycins. Chem. Commun. 1967: 10~11, 1967
8) HARADA, N.; K. NAKANISHI & S. TATSUOKA: A method for Determining the chirality of two aromatic chromophores and the absolute configurations of chromomycin A_3 and related antibiotics. J. Am. Chem. Soc. 91: 5896~5898, 1969
9) KOENUMA, M.; N. UCHIDA, K. YAMAGUCHI, Y. KAWAMURA & K. MATSUMOTO: New aureolic acid antibiotics. I. Screening, isolation, characterization and biological properties. J. Antibiotics 41: 45~52, 1988
10) KOENUMA, M.; Y. YOSHIMURA, K. MATSUMOTO & Y. TERUI: New aureolic acid antibiotics. II. Structure determination. J. Antibiotics 41: 68~72, 1988
11) KASAI, R.; M. SUZUO, J. ASAKAWA & O. TANAKA: Carbon-13 chemical shifts of isoprenoid-β-D-glucopyranosides and -β-D-mannopyranosides. Stereochemical influences of aglycone alcohols. Tetrahedron Lett. 1977: 175~178, 1977
12) SEO, S.; Y. TOMITA, K. TORI & Y. YOSHIMURA: Determination of the absolute configuration of a secondary hydroxy group in a chiral secondary alcohol using glycosidation shifts in carbon-13 nuclear magnetic resonance spectroscopy. J. Am. Chem. Soc. 100: 3331~3339, 1978 (errata; 102: 2512 & 7618, 1980)
13) DORMAN, D. E. & J. D. ROBERTS: Nuclear magnetic resonance spectroscopy. Carbon-13 spectra of some common oligosaccharides. J. Am. Chem. Soc. 93: 4463~4472, 1971
14) KUMAR, V.; W. A. REMERS & W. T. BRADNER: Preparation and antitumor activity of olivomycin A analogues. J. Med. Chem. 23: 376~379, 1980
15) TERUI, Y.; K. TORI & N. TSUJI: Esterification shifts in carbon-13 NMR spectra of alcohols. Tetrahedron Lett. 1976: 621~622, 1976
16) GRANT, D. M. & B. V. CHENEY: Carbon-13 magnetic resonance. VII. Steric perturbation of the carbon-13 chemical shift. J. Am. Chem. Soc. 89: 5315~5318, 1967
17) BEIERBECK, H. & J. K. SAUNDERS: A reinterpretation of beta, gamma, and delta substituent effect on ^{13}C chemical shift. Can. J. Chem. 54: 2985~2995, 1976
18) BOAZ, H.: Separable contribution of induction and polarization to the chemical shift. I. Symmetrical, saturated hydrocarbons having no internal rotation. Tetrahedron Lett. 1973: 55~58, 1973
19) LEMIEUX, R. U. & S. KOTO: The conformational properties of glycosidic linkages. Tetrahedron 30: 1933~1944, 1974