NEW AUREOLIC ACID ANTIBIOTICS

II. STRUCTURE DETERMINATION

MITSUO KOENUMA, YOHKO YOSHIMURA, KOICHI MATSUMOTO
and YOSHIHIRO TERUI

Shionogi Research Laboratories, Shionogi & Co., Ltd.,
Fukushima-ku, Osaka 553, Japan

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Structure determination using NMR spectroscopy of new aureolic acid analogues, demethylchromomycins A_2 and A_3 and demethylolivomycins A and B produced by *Streptomyces aburaviensis* PA-39856, is described.

The preceding paper[1] reported the isolation of new aureolic acid analogues produced by *Streptomyces aburaviensis* PA-39856. The present report clarifies the structures of the new analogues named demethylchromomycins A_2 and A_3 (**1** and **2**) and demethylolivomycins A and B (**3** and **4**).[1]

Detailed NMR studies[2~4] on aureolic acid analogues, chromomycins A_2 and A_3 (**5** and **6**) and olivomycins A and B (**7** and **8**),[5,6] led to the assignments of the ^1H and ^{13}C signals confirming the structures of the known analogues. As shown in Fig. 1, the known analogues, **5**, **6**, **7** and **8**, had two methoxy groups. One of them belonged to aglycon and the other to the α-D-chromose A (*i.e.*, α-D-olivomose) unit of the disaccharide moiety. The ^{13}C and ^1H signals were assigned.[2] The new analogues, however, had only a single methoxy group,[1] which exhibited an ^1H or ^{13}C signal corresponding to the methoxy group of the aglycon[2]: δ_H 3.52~3.53 (s) and δ_C 59.4~59.5 (q). This indicated that the new analogues did not have the sugar unit, 2,6-dideoxy-4-*O*-methyl-D-*lyxo*-hexopyranose (*i.e.*, D-chromose A or D-olivomose) in the saccharide moiety, but they did retain the acyl groups (*i.e.*, isobutyryl and/or acetyl groups). Molecules, **1**, **2**, **3** and **4** could be characterized from the differences in composition of the acyl groups, as well as in the aglycon (*i.e.*, 7-CH_3 or 7-H).[1] This situation is similar to that of the known antibiotics, **5**, **6**, **7** and **8**. These findings suggested that the new antibiotics might be demethyl analogues of the known antibiotics, with respect to the methoxy group of the D-chromose A unit. ^{13}C Data support this supposition; data for **1**, **2**, **3** and **4** are listed in Table 1 together with those[2] for the known analogues.

The ^{13}C data of **1** are identical with that of **5**, except for some signals ascribable to the new unit in question (see Table 1). The identical ^{13}C signal behavior found for the aglycon moiety, trisaccharide moiety and inner component of the disaccharide moiety clearly indicates that the structural difference between **1** and **5** is in an outer unit of the disaccharide moiety (see Fig. 1). The ^{13}C signals[2] observed for the outer unit of **5** were δ 96.0 (d), 33.3 (t), 66.7 (d), 82.1 (d), 67.7 (d), 17.2 (q) and 62.1 (q), in accordance with the carbon number (see Fig. 1), while those of **1** are δ 96.0 (d), 32.5 (t), 66.0 (d), 71.5 (d), 67.2 (d) and 16.9 (q). The composition of the signals indicates that the new unit is also 2,6-dideoxyhexopyranose, although it lacks the methoxy signal. Accompanying the disappearance of the methoxy signal, δ 62.1 (q), significant shifts were found for the corresponding carbon signals, except for the anomeric carbon signal, δ 96.0 (d). Since demethylation of the methoxy group is known to cause a large upfield shift of the carbon directly connected to the group and small shifts on neighboring car-

Table 1. ^{13}C Chemical shifts[a] of demethylchromomycins A_2 and A_3, demethylolivomycins A and B, and related analogues.

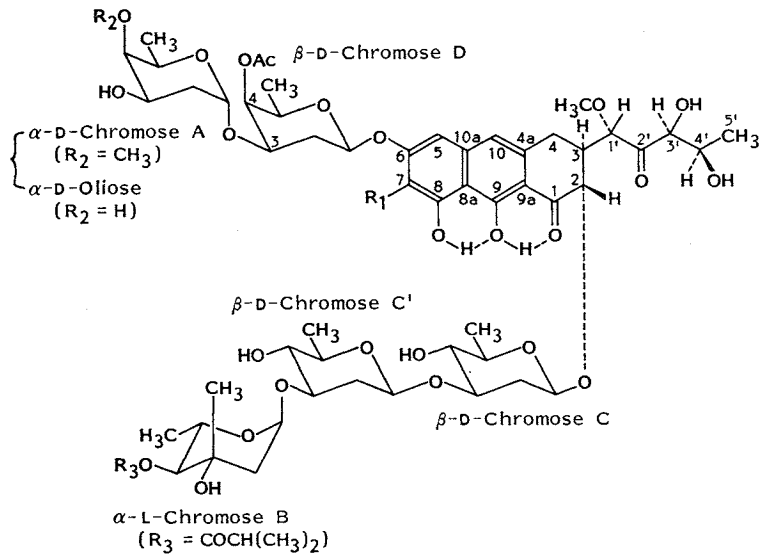
	1	5	2	6	3	7	4	8
Aglycon								
C-1	202.8	202.9	202.7	202.7	202.8	202.9	202.9	202.9
C-2	77.0	77.0	77.0	77.0	77.0	76.8	76.9	76.9
C-3	43.4	43.4	43.4	43.4	43.1	43.1	43.1	43.1
C-4	27.3	27.3	27.3	27.3	27.4	27.4	27.4	27.4
C-5	101.8	101.8	101.7	101.6	103.4	103.4	103.4	103.4
C-6	160.1	160.1	160.0	160.0	161.2	161.2	161.2	161.1
C-7	112.1	112.1	112.0	111.9	102.4	102.4	102.4	102.4
C-8	165.8	165.8	165.7	165.8	166.2	166.0	166.0	166.0
C-9	156.6	156.6	156.5	156.6	160.0	159.9	159.9	159.9
C-10	117.4	117.5	117.5	117.4	117.3	117.3	117.4	117.4
C-4a	135.5	135.5	135.4	135.4	136.9	136.9	136.9	136.9
C-8a	108.6	108.6	108.5	108.6	108.7[b]	108.6[b]	108.6[b]	108.6[b]
C-9a	108.6	108.6	108.5	108.6	109.0[b]	108.8[b]	108.8[b]	108.8[b]
C-10a	139.0	139.0	138.9	138.9	141.3	141.3	141.3	141.3
7-CH_3	8.3	8.3	8.3	8.3	—	—	—	—
C-1′	82.4	82.1	82.4	82.4	82.2	82.1	82.2	82.1
C-2′	211.9	211.9	212.0	211.9	211.8	211.8	211.8	211.8
C-3′	79.1	79.1	79.1	79.0	79.1	79.1	79.1	79.1
C-4′	68.4	68.5	68.4	68.4	68.5	68.5	68.5	68.5
C-5′	19.8	19.8	19.8	19.9	19.8	19.8	19.8	19.8
1′-OCH_3	59.5	59.5	59.5	59.5	59.4	59.4	59.4	59.4
D-Chromose D								
C-1	98.0	98.0	97.9	97.9	97.7	97.6	97.6	97.6
C-2	33.5	33.5	33.5	33.3	33.4	33.3	33.4	33.4
C-3	70.8	70.8	70.7	70.6	70.9	70.9	70.9	70.9
C-4	68.4	68.4	68.4	68.3	68.4	68.4	68.3	68.4
C-5	70.3	70.3	70.2	70.2	70.2	70.2	70.2	70.2
C-6	16.9	16.9	17.0	16.9	16.8	16.8	16.8	16.8
CH_3CO	20.7	20.7	20.7	20.7	20.7	20.7	20.7	20.7
CH_3CO	171.7	171.7	171.7	171.7	171.7	171.6	171.7	171.7
D-Chromose A[c] (D-Olivose)[d]								
C-1	96.0	96.0	95.8	95.8	96.0	96.1	95.9	96.1
C-2	32.5	33.3	32.5	33.3	32.4	33.3	32.4	33.3
C-3	66.0	66.7	66.0	66.6	66.0	66.6	65.9	66.6
C-4	71.5	82.1	71.4	82.1	71.4	82.1	71.4	82.1
C-5	67.2	67.7	67.3	67.6	67.2	67.5	67.2	67.6
C-6	16.9	17.2	17.0	17.2	16.9	17.2	16.9	17.2
OCH_3	—	62.1	—	62.1	—	62.1	—	62.1
D-Chromose C								
C-1	101.0	101.0	101.0	101.0	101.0	101.0	100.9	101.0
C-2	37.8	37.8	37.8	37.8	37.7	37.7	37.7	37.7
C-3	81.8	81.8	81.8	81.9	81.7	81.7	81.7	81.7
C-4	75.8	75.8	75.8	75.8	75.8	75.8	75.8	75.8
C-5	72.7	72.7	72.7	72.7	72.7	72.7	72.6	72.7
C-6	18.2	18.2	18.2	18.1	18.2	18.2	18.2	18.2
D-Chromose C′								
C-1	99.7	99.8	99.8	99.8	99.7	99.7	99.7	99.7
C-2	37.3	37.2	37.2	37.2	37.2	37.2	37.2	37.2
C-3	78.5	78.3	78.3	78.4	78.3	78.3	78.2	78.1
C-4	75.6	75.5	75.5	75.5	75.5	75.5	75.5	75.5
C-5	72.9	73.0	72.9	72.9	72.9	72.9	72.9	72.9
C-6	17.9	18.0	17.9	18.0	17.9	17.9	17.9	17.9

Table 1. (Continued)

	1	5	2	6	3	7	4	8
L-Chromose B								
C-1	96.1	96.0	96.0	96.1	96.0	96.0	95.9	95.7
C-2	44.2	44.2	44.0	44.0	44.1	44.1	44.0	44.0
C-3	70.8	70.8	70.7	70.6	70.8	70.7	70.6	70.6
C-4	79.5	79.5	79.8	79.8	79.4	79.4	79.8	79.8
C-5	67.2	67.2	67.1	67.1	67.1	67.1	67.1	67.1
C-6	17.9	17.9	17.8	17.9	17.9	17.8	17.8	17.8
3-CH_3	23.1	23.1	23.0	23.0	23.0	23.0	23.0	23.0
CH_3C	19.0	19.1	20.9	20.9	19.0	19.0	20.9	20.9
CH_3CHCO	34.7	34.8	—	—	34.7	34.7	—	—
CO	177.7	177.7	171.7	171.6	177.7	177.6	171.6	171.6

[a] For measurement conditions, see the Experimental section.
[b] May be interchanged in each vertical column.
[c] Available for the known analogues, **5**, **6**, **7** and **8**.
[d] Available for the new analogues, **1**, **2**, **3** and **4**.

Fig. 1. Structure of demethylchromomycins A_2 and A_3, demethylolivomycins A and B, and related analogues.



Demethylchromomycin A_2	**(1)**	$R_1=CH_3$	$R_2=H$	$R_3=COCH(CH_3)_2$
Demethylchromomycin A_3	**(2)**	$R_1=CH_3$	$R_2=H$	$R_3=Ac$
Demethylolivomycin A	**(3)**	$R_1=R_2=H$		$R_3=COCH(CH_3)_2$
Demethylolivomycin B	**(4)**	$R_1=R_2=H$		$R_3=Ac$
Chromomycin A_2	**(5)**	$R_1=R_2=CH_3$		$R_3=COCH(CH_3)_2$
Chromomycin A_3	**(6)**	$R_1=R_2=CH_3$		$R_3=Ac$
Olivomycin A	**(7)**	$R_1=H$	$R_2=CH_3$	$R_3=COCH(CH_3)_2$
Olivomycin B	**(8)**	$R_1=H$	$R_2=CH_3$	$R_3=Ac$

bons,[7] our supposition is supported by the data. The identical δ value of the anomeric carbon found between **1** and **5** and that of carbons belonging to the inner unit (*i.e.*, β-D-chromose D unit) indicate that **1** and **5** have similar situations around the interglycoside linkage of the disaccharide[2]; *i.e.*, the new sugar unit also belongs to the 2,6-dideoxy-α-D-series and is connected to the inner unit with an

α,1→3-bond. A small shift, $\Delta\delta$ −0.8, found on the C-2 carbon of the outer unit is ascribable to the difference in the substituent having an axial orientation on the C-4 (*i.e.*, OH in **1** or OCH_3 in **5**). When the configuration of the substituent on the C-4 is changed, a marked downfield shift should be caused on the C-2 carbon.[8] A marked upfield shift would occur on the anomeric carbon (C-1), when the configuration of the substituent on the C-3 or C-5 carbon is changed.[8] The shift behavior observed for the C-1 and C-2 carbons of the outer unit indicates retention of the configuration seen in **5**. Thus, we concluded that the new sugar unit is 2,6-dideoxy-α-D-*lyxo*-hexopyranose. The sugar, 2,6-dideoxy-D-*lyxo*-hexopyranose (*i.e.*, D-oliose), is known to be a component of the saccharide moiety of aureolic acid (mithramycin A).[9]

Next, the ^{13}C signals of **2**, **3** and **4** were compared with those of **6**, **7** and **8**, respectively. Each of the cases show shift behavior quite similar to that found in the comparison between **1** and **5** (see Table 1). This indicates that **2**, **3** and **4** are also demethyl analogues of **6**, **7** and **8**, respectively. Relative configurations in the outer sugar unit (*i.e.*, α-D-oliose unit) were confirmed by the vicinal coupling constants, $^3J_{H,H}$ values, observable in 400 MHz ^1H spectra. For example, the ^1H data in $CDCl_3$ at 24°C on the α-D-oliose unit of **2** was: 5.14 (J=2.5 and 2.0, 1-H), 1.75~1.79 (m, 2-H_{eq} and 2-H_{ax}), ~4.00 (m, 3-H), 3.64 (J=2.5 and 0.8, 4-H), 3.93 (J=0.8 and 6.8, 5-H), 1.27 (J=6.8, 6-H, *i.e.*, 5-CH_3). The J values are in Hz.

Thus, the structures of the new analogues were concluded to be those shown in Fig. 1.

Experimental

Descriptions of the materials used are reported in the preceding paper.[1]

^{13}C NMR spectral data were recorded with a Varian XL-100-12 spectrometer operating at 25.160 MHz. A mixed solvent system, $CDCl_3$ - CD_3OH (2:1), and an internal reference, TMS, were used in the measurements. The concentration and temperature used were *ca.* 140 mg/ml and 60°C, respectively. The observational error for δ values based on the digital resolution was *ca.* ±0.1 ppm.

^1H NMR data were recorded with a Varian XL-100-12 spectrometer operating at 100.058 MHz and with XL-400 spectrometer operating at 399.948 MHz. The temperature used for the 100 MHz ^1H measurements[1] and that for the 400 MHz ^1H measurement were 30°C and 24°C, respectively. Concentrations were *ca.* 10 mg/ml. Digital resolutions were *ca.* 0.3 Hz.

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