ISOLATION AND STRUCTURES OF MONO- AND DI-DEACETYL CHROMOMYCIN ANTIBIOTICS 02-3D AND 02-3G FROM *STREPTOMYCES AVELLANEUS*

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During the course of a screening program for new antitumor antibiotics from microorganisms using a unique assay system, we have found that *Streptomyces avellaneus* 02-3 produced new chromomycin antibiotics, 02-3D and 02-3G in addition to several known related antibiotics. In this paper, we wish to report the isolation and structural studies of 02-3D and 02-3G.

We utilized the phenomenon of DNA intercalation as an assay system. A sample was incubated with 100 ng of pBR322 ccc form DNA for 30 minutes at 37°C and then subjected to 0.7% agarose gel electrophoresis. Concentrations of actinomycins as low as 0.001 μg/ml were used as a positive control. Decrease in motility of the plasmid DNA in the presence of test samples was judged as a positive result.

The stock culture of *S. avellaneus* 02-3 was inoculated into a seed medium (starch 1.0%, Polypepton 1.0%, molasses 1.0% and meat extract 1.0%) in a 500-ml Erlenmeyer flask and incubated at 27°C for 4 days. The seed culture (100 ml) was transferred to 10 liters of a production medium (glycerol 0.8%, starch 0.8%, soybean meal 0.3%, fish meal 0.8% and $CaCO_3$ 0.2%) in 500-ml Erlenmeyer flasks and incubated at 27°C for 3 days.

A culture filtrate (9.5 liters) was adjusted to pH 2.0 with 6 N HCl, passed through a column of Diaion HP-20, washed with 50% aq MeOH and eluted with MeOH. The active fraction was concentrated under reduced pressure to 1 liter and the aqueous residue was extracted with EtOAc. The separated organic layer was evaporated, chromatographed on silica gel and developed with $CHCl_3$-MeOH systems (50:1 and 25:1) containing 1% volume of AcOH to give two active fractions. The former fraction was concentrated and applied to preparative silica gel TLC using $CHCl_3$-MeOH (10:1) added with 1% AcOH. This procedure enabled us to separate a new active compound (02-3D) from chromomycins A_2 and A_3, deacetylchromomycin A_3, aburamycin C and olivomycin C. The band of Rf value 0.45 corresponding to 02-3D was extracted with MeOH, concentrated and chromatographed on a Sephadex LH-20 column eluted with MeOH. The active fraction was concentrated and finally applied to preparative HPLC. The conditions were as follows: Senshu Pak ODS-5251-N (2×25 cm) column; solvent CH_3CN-H_2O (2:3) containing 1% AcOH; flow rate 9 ml/minute; detection by UV absorption at 280 nm. 02-3D was eluted at 28 minutes, concentrated to 1/3 volume, extracted with EtOAc, concentrated and subjected to Sephadex LH-20 column chromatography developed with MeOH. After concentration, 02-3D was obtained as a yellow powder (10 mg).

The latter fraction was concentrated and applied to preparative silica gel TLC using $CHCl_3$-MeOH (6:1) added with 1% AcOH. The band of Rf value 0.40 was extracted with MeOH, concentrated and chromatographed on a Sephadex LH-20 column eluted with MeOH. The active fraction was concentrated and finally applied to preparative HPLC. The condition were almost the same as described above except for the composition of the solvent being changed to CH_3CN-H_2O (35:65) containing 1% AcOH. 02-3G was eluted at 35 minutes. After the same procedure as just described, 15 mg of 02-3G was obtained as a yellow powder.

The physico-chemical properties of 02-3D and 02-3G were as follows. 02-3D: MP 180~184°C; $[\alpha]_D^{20}$ +55.6° (*c* 0.1, MeOH); UV λ_{max}^{MeOH} nm (ε) 230 (26,900), 280 (50,800), 318 (9,100), 331 (7,100), 414 (10,600); IR ν_{max} (KBr) cm^{-1} 3420, 1722, 1629; FAB-MS *m/z* 1,139 (M−H, corresponding to the molecular formula $C_{55}H_{80}O_{25}$).

02-3G: MP 181~186°C (dec); $[\alpha]_D^{20}$ +21.1° (*c* 0.1, MeOH); UV λ_{max}^{MeOH} nm (ε) 230 (26,600), 280 (46,800), 318 (9,000), 331 (7,600), 416 (9,800); IR ν_{max} (KBr) cm^{-1} 3410, 1718, 1625; FAB-MS *m/z* 1,097 (M−H, corresponding to the molecular formula $C_{53}H_{78}O_{24}$), analysis; Calcd: C 57.91, H 7.15, O 34.93, Found: C 57.99, H 7.23, O 34.98.

The structures of 02-3D and 02-3G were finally determined by ^1H and ^{13}C NMR spectral analyses and by comparison with related compounds, chromomycin A_3[1~3] and K-2[4,5] as shown in Fig. 1.

The procedure for the structural determination of 02-3D was as follows: Upon comparison of ^{13}C

Table 1. ^{13}C NMR data of 02-3D, 02-3G and related compounds.

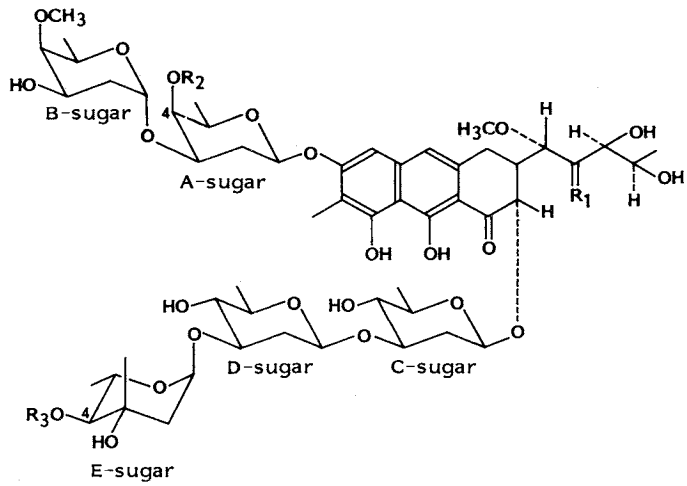
	02-3D ($CDCl_3$)	02-3G (CD_3COOD)	K-2 ($CDCl_3$-CD_3OD)	Chromomycin A_3 ($CDCl_3$-CD_3OD)
Aglycone				
C-1	202.5	203.7	203.8	202.7
C-2	77.2	77.8	77.8	77.0
C-3	43.1	43.7	42.4	43.4
C-4	27.2	27.9	27.7	27.3
C-5	101.3	102.3	101.7	101.6
C-6	159.9	160.3	160.0	160.0
C-7	111.7	112.2	111.6	111.9
C-8	164.8	165.0	165.5	165.8
C-9	156.2	156.6	156.5	156.6
C-10	117.3	118.1	117.2	117.4
C-4a	135.4	136.3	136.7	135.4
C-8a	108.5	108.7[a]	108.5[a]	108.6
C-9a	108.5	109.0[a]	108.8[a]	108.6
C-10a	138.8	139.4	139.0	138.9
7-CH_3	8.2	8.5	8.2	8.3
C-1′	81.8	83.1	78.6[b]	82.4
C-2′	211.8	212.3	72.2[b]	211.9
C-3′	78.8	79.3	74.0[b]	79.0
C-4′	68.3	69.1	70.2[b]	68.4
C-5′	19.7	20.0	19.7	19.9
1′-OCH_3	59.3	59.9	60.6	59.5
A-sugar				
C-1	97.7	98.4	98.1	97.9
C-2	32.5	33.1	32.7	33.3
C-3	73.3	73.8	73.4	70.6
C-4	66.8	68.2	67.3	68.3
C-5	71.5	72.0	71.6	70.2
C-6	16.9	17.1	16.9	16.9
$\underline{C}H_3CO$	—	—	—	20.7
$CH_3\underline{C}O$	—	—	—	171.7
B-sugar				
C-1	96.0	96.6	96.1	95.8
C-2	33.2	33.5	33.5	33.3
C-3	66.4	67.3	66.7	66.6
C-4	81.8	82.5	82.1	82.1
C-5	67.3	68.2	67.5	67.6
C-6	17.1	17.2	17.1	17.2
OCH_3	62.1	62.3	62.1	62.1
C-sugar				
C-1	100.8	101.7	100.8	101.0
C-2	37.6	38.2	37.7	37.8
C-3	81.8	82.3	81.9	81.9
C-4	75.5[a]	76.5	75.8	75.8
C-5	72.4[b]	72.7	72.6	72.7
C-6	17.9[c]	18.5[c]	18.2	18.1
D-sugar				
C-1	99.7	100.5	99.8	99.8
C-2	36.9	37.3	37.2	37.2
C-3	77.7	77.8	78.6	78.4
C-4	75.1[a]	76.2	75.6	75.5
C-5	72.7[b]	73.1	72.8	72.9
C-6	17.9[c]	18.2[c]	18.0	18.0

Table 1. (Continued)

	02-3D ($CDCl_3$)	02-3G (CD_3COOD)	K-2 ($CDCl_3$-CD_3OD)	Chromomycin A_3 ($CDCl_3$-CD_3OD)
E-sugar				
C-1	95.7	96.2	96.5	96.1
C-2	43.7	43.9	43.6	44.0
C-3	70.4	72.0	71.5	70.6
C-4	79.5	80.1	79.6	79.8
C-5	66.8	68.6	68.4	67.1
C-6	18.1[c]	18.1	18.0	17.9
3-CH_3	22.7	22.2	22.1	23.0
$\underline{C}H_3CO$	21.0	—	—	20.9
$CH_3\underline{C}O$	171.6	—	—	171.6

[a,b,c] Assignments in any column may be exchanged.

Fig. 1. Structures of 02-3D, 02-3G and their related compounds.



02-3D	R_1=O	R_2=H R_3=$COCH_3$
02-3G	R_1=O	R_2=R_3=H
Chromomycin A_3	R_1=O	R_2=R_3=$COCH_3$
K-2	R_1=OH, H	R_2=R_3=H

NMR spectral data (Table 1), 55 out of the 57 signals of chromomycin A_3 were identified in the spectrum of 02-3D. Two signals absent in 02-3D were ascribed to one acetyl group. This result was supported by the decrease in molecular weight of 02-3D by 42 units compared to chromomycin A_3. Furthermore, the signal derived from 4A-H, which was found at 5.15 ppm in the ^1H NMR spectrum of chromomycin A_3, was found at 3.73 ppm with 02-3D. The assignments of the key signals were made by ^{13}C-^1H COSY[6], ^1H-^1H COSY[7,8] and homonuclear Hartman-Hahn (HOHAHA)[9] (data not shown). These results clearly showed that 02-3D is the A-sugar deacetyl derivative of chromomycin A_3.

The structure of 02-3G was determined as follows: Comparison of the ^{13}C NMR spectral data (Table 1) showed that 53 signals of 02-3G were identified with those of chromomycin A_3 and that four absent signals were assignable to two acetyl groups. In addition, signals derived from the sugars in 02-3G coincided with those of K-2 which is a dideacetyldihydro derivative of chromomycin A_3. These results showed that 02-3G is dideacetyl chromomycin A_3.

02-3D and 02-3G inhibited the growth of P388 murine leukemia cells at the IC_{50} value of 12 and 0.60 μg/ml, respectively. On the other hand, they could be detected at concentrations of 0.1 and 0.01 μg/ml, respectively, by our assay system using intercalation with DNA. Consequently, this assay system is a sensitive one and may enable the

detection of compounds which have not been discovered by other assay systems.

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