DELTAMYCINS, NEW MACROLIDE ANTIBIOTICS. III
CHEMICAL STRUCTURES

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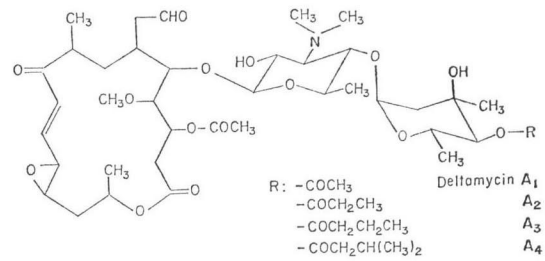
The structures of deltamycins A_1, A_2, A_3 and A_4 belonging to the basic macrolide family of antibiotics were determined mainly from their spectral properties. Deltamycin A_4 was identified as carbomycin A having an isovaleryl group on the mycarose moiety of the molecule. Deltamycin A_1, A_2 and A_3 possess similarities to the structure of deltamycin A_4, but they have acetyl, propionyl and *n*-butyryl group, respectively, in the place of isovaleryl group of deltamycin A_4. These structures were confirmed by chemical synthesis from deltamycin X (4″-O-deacyldeltamycin) and the corresponding acyl chlorides.

As described in the previous paper[1], the deltamycin complex was composed of four components designated as deltamycin A_1, A_2, A_3 and A_4. Their physicochemical properties suggested that they were macrolide antibiotics belonging to the carbomycin A group. The physicochemical properties of deltamycin A_4 were identical with those of carbomycin A[2]. The physicochemical properties of deltamycins A_1, A_2 and A_3 were similar to those of carbomycin A, but their Rf values on TLC and molecular ion peaks in mass spectra analysis were significantly different from those of carbomycin A, which suggested that they might be new antibiotics.

This paper describes the structure determination of the four components by spectroscopic analysis. In view of the following experimental evidence, the structures of the new antibiotics, deltamycin A_1, A_2 and A_3 were determined as shown in Fig. 1.

Fig. 1. Structure of deltamycins.



Mass spectral analyses for deltamycins with and without acetylation were accomplished. The mass spectra of the four intact components are shown in Fig. 2 and these diagnostic fragmentations are shown in Fig. 3. As shown in Fig. 2, most of the peaks are common to the four components and unique peaks are indicated by dotted lines. Peaks at *m/e* 174 (mycaminose), 300 (mycarosyl-mycaminoside), 395 (aglycone−CO), 423 (aglycone) and 740 (unit of mycarosyl-mycaminoside and the aglycone connecting with the disaccharide, an intense peak in the high mass region) were encountered in every

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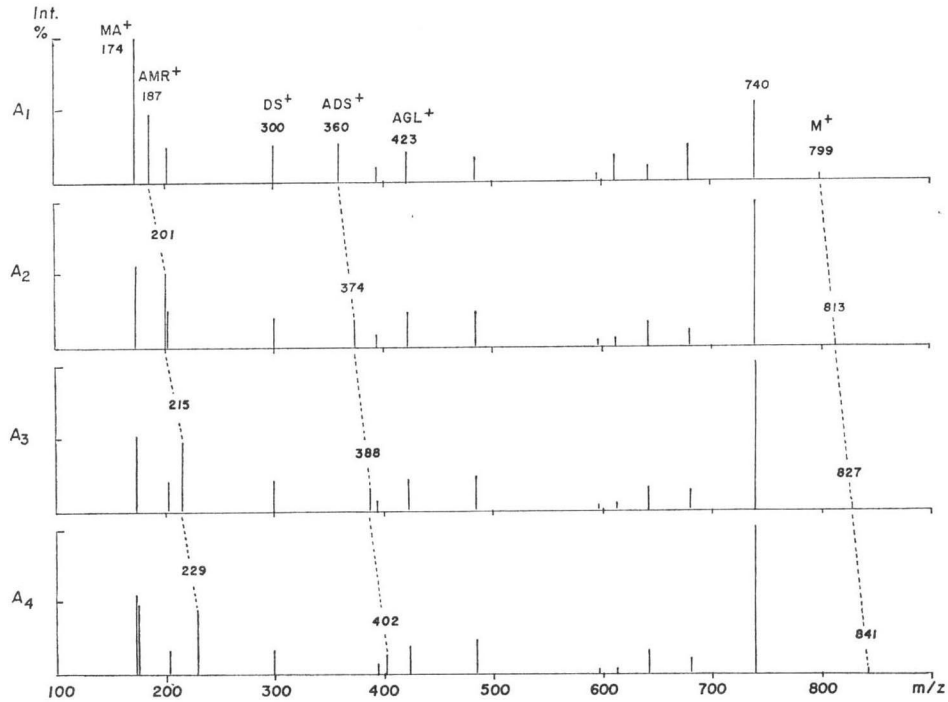
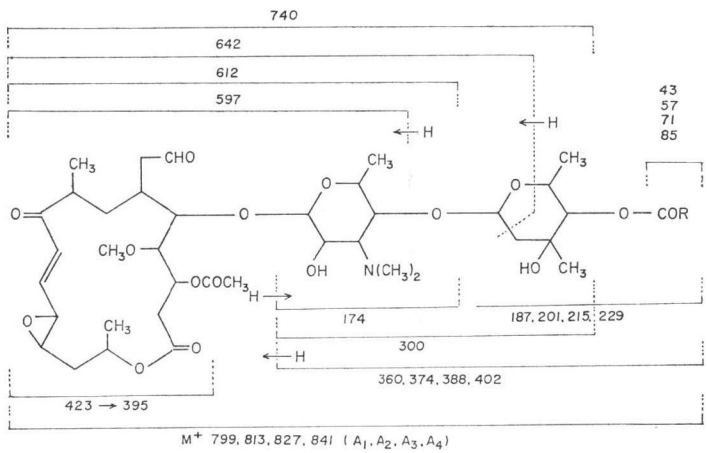
Fig. 2. Mass spectra of deltamycins.

MA: mycaminose AGL: aglycone AMR: acylmycarose
DS: disaccharide ADS: acyldisaccharide



components. On the other hand, molecular ion peaks of A_1, A_2, A_3 and A_4 were observed at *m/e* 799, 813, 827 and 841, respectively. These increments with 14 mass units showed that the molecular weights increase with one methylene chain in the order of A_1, A_2, A_3 and A_4. Similarly, a group of fragment ion peaks at *m/e* 360 (A_1), 374 (A_2), 388 (A_3) and 402 (A_4) and another group of those at *m/e* 187 (A_1), 201 (A_2), 215 (A_3) and 229 (A_4) displayed an identical trend. From analysis of the peaks at *m/e* 402 (isovalerylmycarosyl-mycaminoside) and 229 (isovalerylmycaroside) for A_4, it was assumed that the former represents a group of acylmycarosyl-mycaminoside and the latter the acylmycaroside moiety. Differences between each molecular ion peak of the intact components and the peak at *m/e* 740 became *m/e* 59 (A_1), 73 (A_2), 87 (A_3) and 101 (A_4), respectively. It is apparent that the peak at *m/e* 740 corresponds to the peak in which each acyloxy group *i.e.*

Fig. 3. Diagnostic fragmentations of deltamycins.



$-O-\overset{O}{\overset{\|}{C}}-C_nH_{2n+1}$ is removed from each molecular ion peak (A_1: n=1, acetoxyl; A_2: n=2, propionyloxyl; A_3: n=3, butyryloxyl; A_4: n=4, valeryloxyl).

The mass spectral pattern obtained from the intact deltamycins was also shown during examination of acetylated components. The four acetylated components were synthesized under mild conditions (acetic anhydride - dry acetone at room temperature) to give each 2′-O-acetate. The fragmentation pattern of acetylated deltamycin A_4 was identical with that of 2′-O-acetylcarbomycin A[3]. Mycaminose-containing peaks of acetylated components were 42 mass units (acetyl group) larger than the corresponding peaks of the intact ones. The mass spectral analyses of the two series indicated that the common structural units of the four deltamycin components are: the 16-membered macrocyclic lactone, which is the same as that of carbomycin A, mycarosyl-mycaminoside, and the fatty acid residue and that the essential difference in the structures exists in the fatty acid residue (acyl group) attached to the mycarose moiety. The acyl groups are proposed to be acetyl for A_1, propionyl for A_2 and butyryl for A_3 in place of isovaleryl for A_4.

CMR spectrometry was accomplished for the purpose of further confirmation of complete structural determination of the individual deltamycins. A correlation diagram of CMR spectra of the four components is shown in Fig. 4 and the chemical shift values of these components are shown in Table 1. Signal assignments were performed by means of chemical shift rules[5] and referring to the values of carbomycin A[4].

Chemical shift values of deltamycin A_4 were identical with those of carbomycin A. The spectra of A_1, A_2, A_3 and A_4 showed the presence of 39, 40, 41 and 42 carbon atoms, respectively. Some signals indicated by arrows and dotted lines were different from the other components and are as follows: A_1, 20.9 and 170.9 ppm; A_2, 9.5, 27.7 and 174.5; A_3, 13.8, 18.6, 36.3 and 173.6; A_4, 22.5 (double intensity), 25.4, 43.3 and 170.0. These signals were reasonably explained by the formula of $-O-\overset{O}{\overset{\|}{C}}-C_nH_{2n+1}$ the

Fig. 4. CMR Chemical shift correlation diagram of deltamycins.

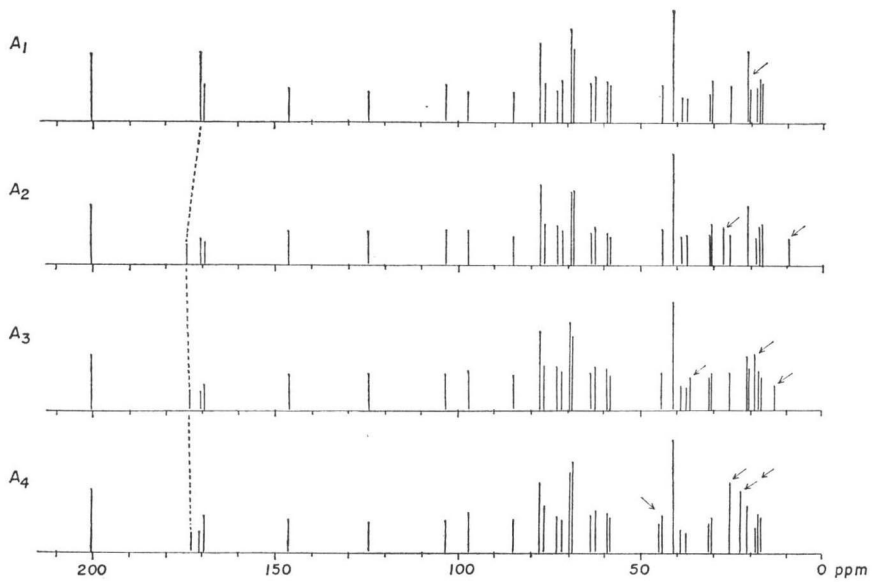


Table 1. ^{13}C Chemical shift values of deltamycins.

Position	Component				Carbomycin A[4]
	A_1	A_2	A_3	A_4	
C- 1	170.9	171.0	170.9	170.9	170.0
C- 2	37.3	37.6	37.5	37.4	37.2
C- 3	71.5	71.5	71.4	71.5	71.2
C- 4	77.4	77.3	77.5	77.7	77.2
C- 5	84.6	84.8	84.8	84.7	84.4
C- 6	30.5	30.7	30.7	30.5	30.4[a]
C- 7	31.0	31.2	31.2	30.9	31.0[a]
C- 8	39.1	39.3	39.2	39.1	39.0
C- 9	200.3	200.5	200.4	200.5	199.3
C-10	124.8	125.0	124.9	124.9	124.3
C-11	146.6	146.9	146.8	146.7	146.0
C-12	59.2	59.4	59.3	59.2	59.0[b]
C-13	58.1	58.3	58.2	58.1	57.8[b]
C-14	41.8	42.0	42.0	42.0	42.9[c]
C-15	68.7	69.0	69.2	68.9	68.5[d]
C-16	20.6	20.7	20.7	20.7	20.5
C-17	44.2	44.4	44.4	44.3	44.1[c]
C-18	200.3	200.5	200.4	200.5	199.3
C-19	17.1	17.3	17.3	17.2	17.1
C-20	169.7	169.9	169.7	169.9	168.9
C-21	20.9	20.7	21.0	20.7	20.8
C-22	62.3	62.5	62.5	62.5	62.1
C- 1′	103.4	103.6	103.4	103.4	103.1
C- 2′	68.7	69.0	69.2	68.9	68.5[d]
C- 3′	69.4	69.6	69.5	69.4	69.1[d]
C- 4′	76.1	76.3	76.2	76.2	76.0
C- 5′	72.9	73.2	73.1	73.1	72.7
C- 6′	18.7	18.9	18.9	18.7	18.7[e]
C- 7′	41.8	42.0	42.0	42.0	41.7
C- 8′	41.8	42.0	42.0	42.0	41.7
C- 1″	97.1	97.4	97.2	97.1	96.8
C- 2″	41.8	42.0	42.0	42.0	41.7
C- 3″	69.4	69.5	69.5	69.4	69.1
C- 4″	77.4	77.3	77.2	77.7	76.8
C- 5″	63.6	63.7	63.8	63.6	63.4
C- 6″	17.8	17.9	17.8	17.9	17.7[e]
C- 7″	25.3	25.5	25.6	25.4	25.4
C- 8″	170.9	174.5	173.6	173.0	172.1
C- 9″	20.9	27.7	36.3	43.3	43.1
C-10″		9.5	18.6	25.4	25.4
C-11″			13.8	22.5	22.3
C-12″				22.5	22.3

(δ ppm, 15.1 MHz, in $CDCl_3$)

[a~e] Assignments within any vertical column may be reversed[4].

Table 2. Characteristic PMR spectra of deltamycins.

Group	Component			
	A_1	A_2	A_3	A_4
21-$COCH_3$	2.17 (3H, s)	2.17 (3H, s)	2.17 (3H, s)	2.19 (3H, s)
7′,8′-$N(CH_3)_2$	2.53 (6H, s)	2.55 (6H, s)	2.70 (6H, s)	2.57 (6H, s)
22-OCH_3	3.51 (3H, s)	3.53 (3H, s)	3.54 (3H, s)	3.57 (3H, s)
10-H, 11-H	6.55~6.61 (2H, m)	6.56~6.64 (2H, m)	6.56~6.63 (2H, m)	6.64~6.71 (2H, m)
18-CHO	9.43 (1H, s)	9.45 (1H, s)	9.43 (1H, s)	9.55 (1H, s)

(δ ppm, 100 MHz, in $CDCl_3$)

same as in the case of the acyl group in the mass spectrometry. Thus, the respective acyl groups in A_1, A_2 and A_3 were definitely determined to be acetyl, propionyl and *n*-butyryl, instead of isovaleryl as in A_4. Coincidence of the assignment values except for the above acyl groups with the four components showed that the aglycone and the disaccharide are stereochemically the same as those of carbomycin A and the three structural units connect with each other in order of the aglycone, mycarosyl-mycaminoside and the acyl group.

PMR spectral analysis for deltamycins was carried out. In these spectra, characteristic signals are listed in Table 2. The characteristic signals which corresponded to the protons for aldehyde, vinyl, methoxyl, dimethylamino and acetyl were observed in every new macrolide. Therefore, it was found that a major part of the structures is common to the four components. Some signals which derived from the respective acyl groups were different. These signals are as follows: A_1, 2.13 ppm (3H, s, $-COCH_3$); A_2, approx. 1.18 (3H, t, J=approx. 7 Hz, $-CH_2CH_3$) and approx. 2.42 (2H, q, J=approx. 7 Hz, $-COCH_2CH_3$); A_3, 0.97 (3H, t, J=7 Hz, $-CH_2CH_3$), 1.40~1.80 (2H, m, $-CH_2CH_2CH_3$) and 2.40 (2H, t, J=7 Hz, $-COCH_2CH_2-$); A_4, 0.99 (6H, d, J=7 Hz, $-CH(CH_3)_2$), 1.50~1.70 (1H, m, $-CH_2CH(CH_3)_2$) and 2.24 (2H, d, J=7 Hz, $-COCH_2CH\langle$). Partial and characteristic differences among them could distinctly be determined such as acetyl in A_1, *n*-butyryl in A_3 and isovaleryl in A_4.

Deltamycin X has been reported as another deltamycin component and determined to be 4″-O-deacyldeltamycin by its physicochemical properties[6]. The structures of A_1, A_2, A_3 and A_4 were also confirmed by chemical synthesis of the four components from deltamycin X and the corresponding acyl chlorides.

On the basis of NMR and mass spectral data, as well as chemical synthesis, the structures of deltamycin A_1, A_2, A_3 and A_4 were unambiguously determined as shown in Fig. 1.

Experimental

General method:

Mass spectra of acetylated deltamycins were measured with a Hitachi RMU-7L mass spectrometer, mass spectra of intact components with a Hitachi RMU-6M mass spectrometer, PMR spectra with a JEOL JNM PS-100 spectrometer and CMR spectra with a Nichiden-Varian NV-14 spectrometer.

Deltamycin A_1, A_2, A_3 or A_4 from deltamycin X:

One hundred mg (0.13 m mole) of deltamycin X were dissolved in 2 ml of pyridine and 0.1 ml (0.82~1.41 m mole) of acyl chloride (acetyl, propionyl, *n*-butyryl or isovaleryl chloride) was added with ice-cooling and stirring. The reaction was allowed to continue for 2~3 hours in a range 0~5°C. To

the reaction mixture was added 5 ml of 1 M aqueous $NaHCO_3$ and the synthetic deltamycin component was extracted twice with 10 ml of benzene. After the benzene layer was washed twice with 3 ml of 0.1 M aqueous $NaHCO_3$, the solvent layer was extracted twice with 5 ml of 0.1 M acetate buffer (pH 2.0). The acidic aqueous layer was adjusted to pH 8.5 with 0.1 N aqueous NaOH and extracted twice with 3 ml of benzene. After the solvent layer was dried and evaporated off, it was chromatographed on a Sephadex LH-20 column with MeOH, fractions were monitored by TLC, collected, and concentrated to dryness. The residue was crystallized from benzene - *n*-hexane. Thus, 47 mg of 4''-O-acetate, 26 mg of 4''-O-propionate, 18 mg of 4''-O-*n*-butyrate or 21 mg of 4''-O-isovalerate was obtained in the respective experiments. Physicochemical properties of 4''-O-acetyl-, 4''-O-propionyl-, 4''-O-*n*-butyryl- or 4''-O-isovaleryl-deltamycin X were identical with those of deltamycin A_1, A_2, A_3 or A_4, respectively.

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