MACROLIDE ANTIBIOTICS M-4365 PRODUCED BY *MICROMONOSPORA*

II. CHEMICAL STRUCTURES

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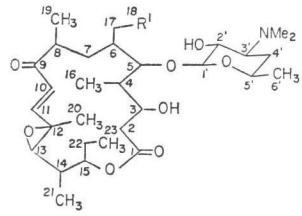
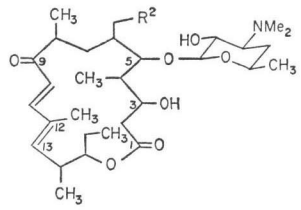
By physicochemical analyses and chemical procedures, the structures of a series of basic 16-membered macrolide antibiotics, M-4365 A_1, A_2, A_3, G_1, G_2 and G_3 were elucidated, and it was found that M-4365 A_1, G_1 and G_2 were novel, while M-4365 A_2, A_3 and G_3 were identical with rosamicin, juvenimicins A_4 and B_1, respectively.

As reported in the foregoing paper[1], a macrolide antibiotic complex M-4365 produced by *Micromonospora capillata* MCRL 0940 was composed of at least six components designated M-4365 A_1, A_2, A_3, G_1, G_2 and G_3. The present paper is concerned with the structure elucidation of these components (structures shown in Fig. 1.**). By this study, M-4365 A_1, G_1 and G_2 were found to be novel antibiotics belonging to the 16-membered basic macrolide antibiotic group, but M-4365 A_2, A_3 and G_3 were identified with rosamicin[2] (juvenimicin A_3), juvenimicins A_4 and B_1[3], respectively.

Fig. 1. Structures of M-4365 antibiotics.



M-4365 A_1 : R^1 = -CH_3
M-4365 A_2 : R^1 = -CHO
M-4365 A_3 : R^1 = -CH_2OH



M-4365 G_1 : R^2 = -CH_3
M-4365 G_2 : R^2 = -CHO
M-4365 G_3 : R^2 = -CH_2OH

In the elucidation of the chemical structures, comparative NMR and mass spectral analyses based on the data shown in Table 1 (^{1}H-NMR signals) and Table 2 (diagnostic mass spectral fragmentation pattern) played important roles. Of necessity, the other physicochemical data reported in the foregoing paper were optionally referred to.

From the UV spectra, components of the G-group with λ_{max} at 283 nm were assumed to have an α, β, γ, δ-dienone structure, while those of the A-group possessing λ_{max} at 240 nm have an epoxy-enone structure. A dienone structure of the G-group components was also supported by IR absorptions at 1685~1680 cm^{-1} (conjugated ketone) and two absorptions at 1630 and 1585~1595 cm^{-1} (conjugated double bond). In contrast, the A-group components showed IR absorptions at 1690 (conjugated ketone) and 1615~1620 cm^{-1} (conjugated double bond).

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** Structures of M-4365 components were referred to in Japan Kokai 75-160,494 (Dec. 25, 1975), 75-160, 481 (Dec. 25, 1975), 76-7,188 (Jan. 21, 1976) and 76-36,471 (Mar. 27, 1976).

Table 1. ^{1}H-NMR signals of M-4365 components

Assignment	A_1	A_2	A_3	G_1	G_2	G_3
CH_3-18	0.84, 3H,t,J=8	—	—	0.89, 3H,t,J=8	—	—
CH_3-23	0.90,3H,t,J=8	0.89,3H,t,J=8	0.91,3H,t,J=8	0.95,3H,t,J=8	0.96,3H,t,J=8	0.95,3H,t,J=8
CH_3-16,19 21,6′	1.0~1.4, 12H	1.05~1.35, 12H	1.05~1.35, 12H	1.02~1.36, 12H	1.05~1.40, 12H	1.06~1.35, 12H
CH_3-20	1.43,3H,s	1.43,3H,s	1.45,3H,s	1.81,3H,s	1.81,3H,s	1.92,3H,s
N-$(CH_3)_2$	2.28,6H,s	2.27,6H,s	2.35,6H,s	2.33,6H,s	2.37,6H,s	2.35,6H,s
H-5	3.73,bd,J=10	3.71,bd,J=10	obscure	3.79,bd,J=10	3.74,d,J=10	obscure
H-3	3.92,bd,J=10	3.92,bd,J=10	obscure	3.81,bd,J=10	3.92,bd,J=11	obscure
H-1′	4.23,d,J=7	4.22,d,J=7	4.34,d,J=7	4.30,d,J=7	4.27,d,J=7	4.35,d,J=7
H-15	4.88,bt	4.88,bt	4.90,bt	4.72,bt	4.78,bt	4.77,bt
H-13	2.83,d,J=9	2.83,d,J=9	2.82,d,J=9	5.64,d,J=10	5.71,d,J=10	5.69,d,J=10
H-10	6.37,d,J=16	6.37,d,J=16	6.41,d,J=16	6.28,d,J=16	6.32,d,J=16	6.31,d,J=16
H-11	6.55,d,J=16	6.55,d,J=16	6.58,d,J=16	7.24,d,J=16	7.32,d,J=16	7.31,d,J=16
-CHO	not observed	9.64,s	not observed	not observed	9.67,s	not observed

Table 2. Diagnostic mass spectral fragment ions in M-4365 components [*m/e* and relative intensity (%)]

Fragment ion	A_1 $C_{31}H_{53}NO_8$	A_2 $C_{31}H_{51}NO_9$	A_3 $C_{31}H_{53}NO_9$	G_1 $C_{31}H_{53}NO_7$	G_2 $C_{31}H_{51}NO_8$	G_3 $C_{31}H_{53}NO_8$
M^+	567 (70)	581 (51)	583 (58)	551 (63)	565 (28)	567 (71)
$[M-H_2O]^+$	549 (6)	563 (10)	565 (10)	533 (6)	547 (5)	549 (5)
$[M-CO]^+$	—	553 (18)	—	—	537 (10)	—
$[Agl+O]^+$	409 (14)	423 (10)	425 (11)	393 (6)	407 (3)	409 (5)
Agl^+	393 (82)	407 (48)	409 (60)	377 (75)	391 (38)	393 (74)
$[Agl-H_2O]^+$	375 (65)	389 (32)	391 (24)	359 (7)	373 (14)	375 (7)
$[Agl-CO]^+$	—	379 (8)	—	—	363 (6)	—
$[Agl-2H_2O]^+$	—	—	373 (13)	—	—	357 (6)
$[DS+O]^+$	174 (100)	174 (59)	174(100)	174 (45)	174 (72)	174 (70)
DS^+	158 (100)	158 (100)	158 (99)	158 (69)	158 (100)	158 (100)

M^+: molecular ion, Agl: aglycone ion without a glycoside oxygen, DS: desosamine ion without a glycoside oxygen.

Throughout all the components, a few common features in NMR and MS were observed, *i.e.* a singlet of six protons at δ 2.27~2.37 and a doublet of a proton at δ 4.22~4.35 (J=7) in the NMR and intensive fragment peaks at *m/e* 174 and 158 in the MS. The above data suggested that there may be desosamine, as an aminosugar moiety common to all components. When M-4365 G_2 was hydrolysed in 6 N HCl, D-desosamine was actually isolated and identified by direct comparison with an authentic sample prepared from narbomycin. Therefore, it was concluded that all components had a D-desosamine moiety.

Taking account of the above information, structural elucidation of M-4365 components was started. Among the six components, M-4365 A_2 was first identified with rosamicin, because the optical and chemical properties of M-4365 A_2 were entirely identical with those of rosamicin. The structure of M-4365 A_2 gave a clue for the assignment of the NMR signals and mass ion peaks which were finally assigned as described in Tables 1 and 2, respectively.

As mentioned above, M-4365 G_2 was assumed to have an α, β, γ, δ-dienone chromophore. Further the IR spectrum showed the presence of a hydroxyl (3460 cm^{-1}), an aldehyde (2720 and 1725 cm^{-1}) and a lactone carbonyl (1740 cm^{-1}) and NMR showed the existence of one primary, four secondary, one tertiary methyl groups and three olefinic (later ascribed to protons at C-10, C-11 and

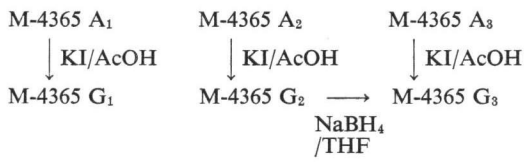
C-13) and one aldehyde proton together with signals due to desosamine. Comparative mass spectrometry of G_2 with A_2 suggested the structure of G_2. One oxygen difference found in aglycone peaks (*m/e* 391 for G_2 and *m/e* 407 for A_2) could be attributed to the difference between the chromophore of G_2 (dienone) and A_2 (epoxyenone). Thus, G_2 was assumed to be the deepoxy derivative of A_2. If so, A_2 could be converted into G_2 by the method reported by TSUKIURA, *et al.*[5] As expected, when A_2 was treated with potassium iodide in acetic acid, A_2 gave G_2 in 60% yield, thus confirming the structure of G_2.

Components A_3 and G_3 showed similar NMR signals and mass spectral fragmentation patterns, except for the following points. ^{1}H-NMR signals due to a tertiary methyl (δ 1.45 for A_3 and δ 1.92 for G_3) and a proton at C-13 (δ 2.82 for A_3, δ 5.69 for G_3) were greatly different in A_3 and G_3. However, this difference was readily understood, by taking into consideration the difference between the chromophores of A_3 and G_3 (an epoxyenone for A_3, a dienone for G_3). This difference was also observed in the mass spectral patterns. Though the fragmentation took place similarly in A_3 and G_3, the fragment ion peaks of G_3 were always less than those of A_3 by one oxygen unit (*m/e* 16), except for the ions due to the sugar moiety. Thus, G_3 was assumed to be a de-epoxide of A_3. This assumption was chemically proved, as A_3 was successfully converted into G_3 by the method of TSUKIURA, *et al.* mentioned above. Comparing the mass spectral fragment peaks of A_3 with A_2 and G_3 with G_2, it was assumed that A_3 and G_3 had no an aldehyde moiety, because (M − CO) and (aglycone − CO) peaks observed in A_2 and G_2 were lacking in A_3 and G_3. This consideration was also supported by the NMR spectra of A_3 and G_3, in which no signals due to an aldehyde proton were observed. It was further noticed that, in spite of the absence of an aldehyde function, no appearance of a new methyl signals was observed. This phenomenon together with the fact that the M^+ and the aglycone peaks of A_3 or G_3 were larger by two mass units than those of A_2 or G_2, respectively, suggested that A_3 and G_3 might contain a carbinol function in place of an aldehyde function. To confirm this suggestion, the reduction of G_2 to G_3 was attempted. And, as expected, the reduction of G_2 in tetrahydrofuran with sodium borohydride gave G_3. Thus, the structures of A_3 and G_3 were elucidated.

Components A_1 and G_1 showed many similarities in their NMR spectra and mass spectral fragmentation patterns, except for the signals or fragment peaks resulting from the chromophores (an epoxyenone for A_1 and a dienone for G_1). Thus, great differences were observed in the NMR signals due to the tertiary methyl (δ 1.43 for A_1, δ 1.81 for G_1) and a proton at C-13 (δ 2.83 for A_1, δ 5.64 for G_1), and in the mass spectral fragmentation pattern, G_1 always showed the peaks less than A_1 by one oxygen unit (*m/e* 16), except for the ions due to the sugar moiety. Thus, as in the case of G_2 and G_3, M-4365 G_1 was assumed to be a deepoxy derivative of A_1. This assumption was verified by successful chemical conversion of A_1 to G_1 carried out in analogy with the de-epoxidation of A_2. Comparing NMR signals of A_1 with A_2 and G_1 with G_2, it was evident that A_1 and G_1 lacked the signal due to an aldehyde proton, but, instead, showed one additional methyl signal. Mass spectral peaks also suggested that A_1 and G_1 lacked an aldehyde function, because (M − CO) and (aglycone − CO) peaks were not observed for these components. Considering that molecular weights of A_2 and G_2 were larger by 14 mass units than those of A_1 and G_1, respectively, and taking account of the chemical correlations between A_2, A_3, G_2 and G_3, M-4365 A_1 and G_1 were concluded to be deoxo-derivative of A_2 and G_2, respectively. Chemical conversion of A_1 to G_1 was successful as already mentioned, but A_1 or G_1 has not been chemically correlated with the other components.

Thus, the structures of all components were elucidated and are summarized in Fig. 1. The chemical correlation of these antibiotics is shown in Chart 1. The structures of A_3 and G_3 were identical with those recently proposed for juvenimicins A_4 and B_1.[3] Though the direct comparison was not made, the identity of respective pairs of antibiotics was established from the properties reported.

Chart 1. Chemical correlation of M-4365 components



As reported in the foregoing paper, A_1 and G_1 were bioactive in spite of the absence of an 18-aldehyde function. This represents a novel observation for basic 16-membered macrolide antibiotics.* The $-CH_2CH_3$ function as an intermediate in the formation of the $-CH_2$-CHO function was demonstrated in platenomycin biosynthesis[7] and in the biosynthesis of rosamicin, a labelled butyric acid was incorporated into the rosamicin skeleton as a precursor of the $-CH_2$-CHO function.[8] Therefore, M-4365 A_1 and G_1 with an 18-methyl function would be expected to be the biosynthetic intermediates of A_2 and G_2, respectively. M-4365 A_3 and G_3 with an 18-carbinol function in place of an aldehyde function were also bioactive as experienced in relomycin.[9] They may also be important substances in the biosynthesis of these macrolides.

Experimental

General method

Melting points were not corrected. The following instruments were used for measurements: Hitachi EPI-32 IR spectrometer, Hitachi-323 UV spectrometer, JEOL PS-100 spectrometer for 100 MHz (^{1}H-NMR was measured in $CDCl_3$ solution with TMS as an internal standard), Hitachi RMU-6M mass spectrometer. Thin-layer chromatography was performed on silica gel GF_{254} (Merck) plate with thickness of 0.25 mm and spots were visualized by spraying with 40% H_2SO_4 followed by heating.

Conversion of M-4365 A group antibiotics to M-4365 G group antibiotics

General procedure: To the solution of M-4365 A (100 mg) in acetic acid (3 ml), potassium iodide (150 mg) was added. The solution was stirred at 75°C for 3 hours. Then the reaction mixture was poured onto ice water. The solution was then adjusted to pH 8.5 with aqueous $NaHCO_3$ solution and extracted with ethyl acetate. After washing the ethyl acetate layer with 2% sodium thiosulfate and water, the solvent layer was dried over anhydrous sodium sulfate and evaporated *in vacuo* to yield a yellowish powder. The resulting powder was chromatographed over silica gel using the solvent system of $CHCl_3$ - CH_3OH - 7% NH_4OH (10 : 2 : 5, lower layer). From the eluate a de-epoxy product was obtained in a yield shown in Table 3. All products were identified with the natural products by TLC, IR, MS, $[\alpha]_D$ and NMR.

The conversion of an aldehyde to a carbinol in M-4365 G_2

M-4365 G_2 (30 mg) was dissolved in tetrahydrofuran (7 ml) and sodium borohydride (3 mg) was added. The solution was stirred for 3 hours at room temperature and concentrated *in vacuo*. The reaction mixture was diluted with water and extracted with ethyl acetate. The ethyl acetate extract was evaporated to dryness and the residue was chromatographed on preparative TLC plates of silica gel using a solvent system of $CHCl_3$ - ethanol (4 : 1). The UV quenching band on the TLC plate was gathered and extracted with acetone, and the extract was evaporated. The residue was recrystallized from benzene - hexane to give 9 mg of reduction product, m.p. 100~102°C. The product was identified with M-4365 G_3 by TLC, IR, MS and NMR.

Isolation of D-desosamine by 6 N hydrochloric acid hydrolysis of M-4365 G_2

M-4365 G_2 (60 mg) was hydrolysed with 6 N HCl (3 ml) under reflux for 2 hours. The reaction

* In our experience, 3-O-propionyl-5-O-mycaminosyl platenolide I showed no activity at all.[6]

solution was filtered and the filtrate was washed with chloroform and evaporated until hydrochloric acid was thoroughly removed. The residual product was diluted with water and was adsorbed on Dowex 50×W (5 ml). The eluate with 0.5 N HCl was concentrated *in vacuo* and passed through a Sephadex LH-20 (5 ml) column with water. The main eluate was evaporated to dryness to afford D-desosamine hydrochloride (8 mg), m.p. 82~84°C, $[\alpha]_{D}^{22}+44°$ (*c* 0.1, H_2O). This compound was identified with the authentic sample prepared from narbomycin by TLC, IR and $[\alpha]_D$.

Table 3. The yield of the de-epoxidation.

Starting material	De-epoxy-product	Yield (%)
A_1	G_1	52
A_2	G_2	60
A_3	G_3	19

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