STUDIES ON JUVENIMICIN, A NEW ANTIBIOTIC. II

ISOLATION, CHEMICAL CHARACTERIZATION AND STRUCTURES

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A series of new macrolide antibiotics was isolated from the culture filtrate of *Micromonospora chalcea* var. *izumensis*. The fat-soluble basic complex consisted of eight components which were named juvenimicin $A_1 \sim A_4$ and $B_1 \sim B_4$. Juvenimicin A_3 was found to be identical with rosamicin and the structures of four of the other components (JVM A_2, A_4, B_1 and B_3) have been elucidated. Juvenimicin A_2 has a methyl group at position 6 instead of the formylmethyl group of juvenimicin A_3. Juvenimicin A_4, B_1 and B_3 possess a hydroxyethyl group at position 6. Juvenimicin A_4 and B_1 differ from each other in the nature of the chromophore. Juvenimicin B_3 differs from juvenimicin B_1 in that a hydroxymethyl group is present at position 14 in the former.

The active fractions obtained from the culture filtrate of *Micromonospora chalcea* var. *izumensis*[1] were composed of fat-soluble basic antibiotics, juvenimicins (JVM), and neutral antibiotics, evernomicins (EVM).[2] Juvenimicin was purified by chromatography to afford five of the components JVM A_2, A_3, A_4, B_1 and B_3 as single entities. The antibiotics of the A and the B groups displayed absorption maxima at 241 and 283 nm, respectively.

This paper deals with the isolation, chemical characterization and structures of the juvenimicin components.

Isolation

The culture filtrate of *M. chalcea* var. *izumensis* was adjusted to pH 9 and extracted with ethyl acetate. The organic layer was extracted with hydrochloric acid (pH 3) and the aqueous layer was reextracted with ethyl acetate at pH 9. This extract was concentrated to afford a crude powder of JVM. The initial ethyl acetate extract on concentration gave crude EVM. The crude powder of JVM was chromatographed on a column of Sephadex LH-20. The active fractions obtained were shown to be comprised of eight metabolites by TLC on silica gel with a solvent system of $CHCl_3$ - MeOH - 7% NH_4OH (40:12:20, lower layer) and named JVM $A_1 \sim A_4$ and $B_1 \sim B_4$. The major components, JVM A_2, A_3, A_4, B_1 and B_3, were scraped from the TLC-plates and extracted with ethyl acetate at pH 9. The extracts were washed with water and concentrated to afford each component. The crude powder of EVM obtained from the initial ethyl acetate layer was purified by column chromatography on activated carbon and the active eluates were collected and concentrated to afford a white powder. The main component, EVM D, was isolated from the powder by TLC on silica gel.

Chemical Characterization

Each component of JVM behaves as a single spot on TLC and gives a positive color reaction with

DRAGENDORFF's reagent. They are, however, negative to FISCHBACH's carbomycin and erythromycin tests.[3] The juvenimicins are soluble in benzene, chloroform, ethyl acetate, dimethyl formamide, alcohols and acidic aqueous solvents but insoluble in petroleum ether, hexane and water.

The specific rotation of JVM A_2 is $[\alpha]_D^{24}$ + 17.9° (*c* 0.52, chloroform) and its UV spectrum in methanol displays a maximum at 241 nm (ε=14,900). From the elemental analysis and mass spectrum (*m/e* 553 (M^+)), the molecular formula $C_{30}H_{51}NO_8$ was assigned to JVM A_2. A 3,2′-diacetate was obtained from JVM A_2 by treatment with acetic anhydride in pyridine and its elemental analysis and mass spectrum confirmed the molecular formula assigned to JVM A_2. The IR spectrum of JVM A_2 is shown in Fig. 1.

The melting point of JVM A_3 is 120~125°C (decomp.) and its specific rotation $[\alpha]_D^{24}$ −17.6° (*c* 0.29, chloroform). A pKa′ of 8.4 (in 66% DMF) was obtained by the titration method. The UV spectrum of JVM A_3 in ethanol displays a maximum at 241 nm (ε=13,900) like that of JVM A_2. From the elemental analysis and mass spectral data *m/e* 581 (M^+), the molecular formula of JVM A_3 was established to be $C_{31}H_{51}NO_9$. This formula was in good accord with the data generated for JVM A_3 3,2′-diacetate.

The specific rotation of JVM A_4 is $[\alpha]_D^{24}$ −9.6° (*c* 0.5, chloroform). The UV spectrum of JVM A_4 in methanol displays a maximum at 241 nm (ε=13,700). From the elemental analysis and mass spectrum (*m/e* 583 (M^+)), the molecular formula of JVM A_4 was established to be $C_{31}H_{53}NO_9$. This formula is consistent with the data collected for JVM A_4 3,18,2′-triacetate. The IR spectrum of JVM A_4 is shown in Fig. 2.

Fig. 1. IR Spectrum of juvenimicin A_2

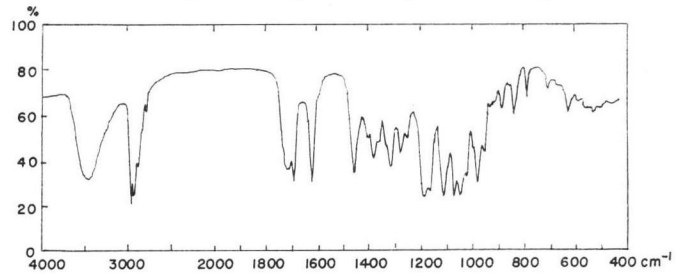
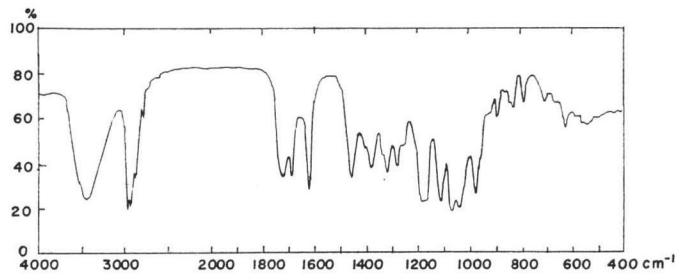


Fig. 2. IR Spectrum of juvenimicin A_4



The specific rotation of JVM B_1 is $[\alpha]_D^{24}$ +7.6° (*c* 0.5, chloroform). The UV spectrum of JVM B_1 in ethanol displays a maximum at 283 nm (ε=18,500) which is different from those of the components of the A group. From the elemental analysis and mass spectrum (*m/e* 567 (M^+)), the molecular formula of JVM B_1 was established to be $C_{31}H_{53}NO_8$. This formula is consistent with the data generated for JVM B_1 3,18,2′-triacetate. The IR spectrum of JVM B_1 is shown in Fig. 3.

Fig. 3. IR Spectrum of juvenimicin B_1

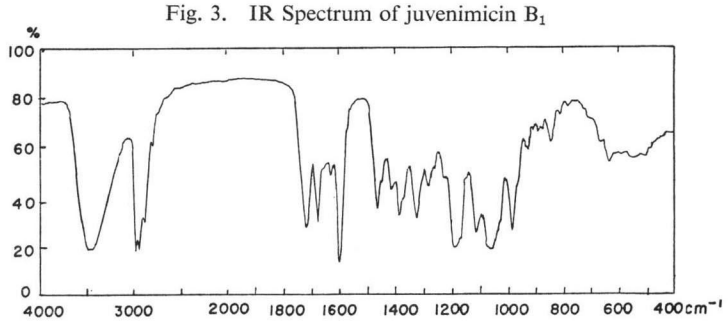
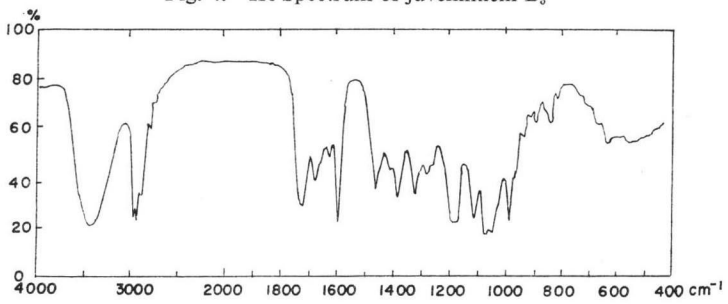


Fig. 4. IR Spectrum of juvenimicin B_3



The specific rotation of JVM B_3 is $[\alpha]_D^{24}$ +9.2° (*c* 0.5, chloroform) and its UV spectrum in methanol displays a maximum at 283 nm (ε=19,200). From the elemental analysis and mass spectrum (*m/e* 583 (M^+)), the molecular formula of JVM B_3 was established as $C_{31}H_{53}NO_9$. This formula was consistent with the data generated for JVM B_3 3,18,21,2′-tetraacetate. The IR spectrum of JVM B_3 is shown in Fig. 4. These data are summarized in Table 1.

Table 1. Physico-chemical properties of juvenimicins

Antibiotic	$[\alpha]_D^{24}$ (*c* 0.5) (Solvent)	UVλ_{max} nm(ε) (Solvent)	Analysis Found (Calcd.)	Molecular formula (MS (M^+))	TLC* (Rf)
JVM A_2	+17.9° ($CHCl_3$) −13.0° (MeOH)	241 (14,900) (MeOH)	C, 64.68; H, 9.44; N, 2.57 (C, 65.06; H, 9.28; N, 2.53)	$C_{30}H_{51}NO_8$ (553)	0.80
JVM A_3	−17.6° ($CHCl_3$) −31.2° (EtOH)	241 (13,900) (EtOH)	C, 63.22; H, 8.54; N, 3.02 (C, 64.08; H, 8.85; N, 2.41)	$C_{31}H_{51}NO_9$ (581)	0.70
JVM A_4	−9.6° ($CHCl_3$) −27.4° (MeOH)	241 (13,700) (MeOH)	C, 62.55; H, 9.03; N, 2.56 (C, 63.86; H, 9.16; N, 2.40)	$C_{31}H_{53}NO_9$ (583)	0.65
JVM B_1	+7.6° ($CHCl_3$) −3.0° (MeOH)	283 (18,500) (EtOH)	C, 64.76; H, 9.60; N, 2.35 (C, 65.66; H, 9.42; N, 2.47)	$C_{31}H_{53}NO_8$ (567)	0.50
JVM B_3	+9.2° ($CHCl_3$) −8.0° (MeOH)	283 (19,500) (MeOH)	C, 62.14; H, 8.70; N, 2.20 (C, 63.86; H, 9.16; N, 2.40)	$C_{31}H_{53}NO_9$ (583)	0.33

* Adsorbent; SiO_2, spot film (Tokyo Kasei), Solvent system; $CHCl_3$ - MeOH - 7%NH_4OH (40:12:20, lower layer), Detection; UV (258 nm) and conc. H_2SO_4. The Rf values of the other minor components are as follows: JVM A_1=0.85, JVM B_2=0.40, JVM B_4=0.25.

Reference Studies with Similar Macrolides

The basic macrolides[4] which display a maximum at 240~242 nm, cirramycin A_1[5] and B-58941 B[6a] are similar to JVM A_3 in both physico-chemical and biological properties. These antibiotics are distinguished from each other by TLC, mass and NMR spectra. The structure of cirramycin A_1 was elucidated.[5c,6]

Table 2. Mass spectra of juvenimicins (*m/e* (%))

Juvenimicin	M^+	M^+-S_1	$M^+-S_1-H_2O$	S_1*	S_2**		
A_3	581 (1.4)	407 (1.9)	389 (1.6)	174 (56)	158 (100)		116 (40)
A_2	553 (2.5)	379 (12)	361 (4.5)	174 (94)	158 (100)		116 (70)
A_4	583 (1.7)	409 (5.7)	391 (5.6)	174 (100)	158 (94)		116 (93)
B_1	567 (2.1)	393 (13)	375 (1.5)	174 (60)	158 (100)		116 (49)
B_3	583 (2.3)	409 (4.0)	391 (1.1)	174 (33)	158 (59)	118 (100)	116 (41)

The peaks showed in this table are of mass greater than *m/e* 116 and their relative intensities are more than 1%.

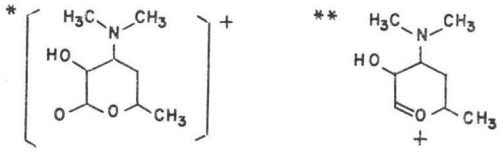


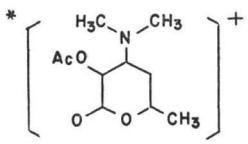
Table 3. Chemical shifts and coupling constants of juvenimicins
(100 MHz, $CDCl_3$, δ=ppm, J=Hz)

Protons	JVM A_3	JVM A_2	JVM A_4	JVM B_1	JVM B_3
H_{18}	9.69 (s, like)		3.70 (m, 2H)	3.70 (m, 2H)	3.73 (m, 2H)
H_{11}	6.55 (d, J=16)	6.54 (d, J=16)	6.54 (d, J=16)	7.28 (d, J=16)	7.34 (d, J=16)
H_{10}	6.36 (d, J=16)	6.38 (d, J=16)	6.36 (d, J=16)	6.28 (d, J=16)	6.29 (d, J=16)
H_{15}	4.84 (m)	4.84 (m)	4.84 (m)	4.95 (m)	4.97 (m)
H_1'	4.19 (d, J=7)	4.23 (d, J=8)	4.28 (d, J=7)	4.25 (d, J=7)	4.26 (d, J=7)
H_3	3.87 (d, like, J=10)	3.82 (d, like, J=10)	3.84 (d, like, J=10)	3.75 (d, like, J=10)	3.76 (d, like, J=10)
H_5	3.65 (d, like, J=10)	3.51 (d, like, J=10)	3.65 (d, like, J=9)	3.65 (d, like, J=10)	3.59 (d, like, J=10)
H_5'	3.42 (m)	3.44 (m)	3.48 (m)	3.52 (m)	3.53 (m)
H_2'	3.18 (q, J=7, 10)	3.20 (q, J=7, 10)	3.20 (q, J=7, 10)	3.21 (q, J=7, 10)	3.20 (q, J=6, 10)
H_{13}	2.78 (d, J=9)	2.76 (d, J=9)	2.78 (d, J=10)	5.83 (d, like, J=10)	5.83 (d, like, J=10)
H_3'	2.56 (m)	2.60 (m)	2.57 (m)	2.52 (m)	2.50 (m)
H_2	2.16~2.6 (m, 2H)	2.16~2.6 (2H)	2.15~2.6 (2H)	2.22~2.63(2H)	2.24~2.60 (2H)
N-dimethyl	2.22 (s, 3H×2)	2.24 (s, 3H×2)	2.24 (s, 3H×2)	2.27 (s, 3H×2)	2.26 (s, 3H×2)
H_{20}	1.40 (s, 3H)	1.40 (s, 3H)	1.40 (s, 3H)	1.80 (s, 3H)	1.79 (s, 3H)
sec.-methyl	1.1~41.19 (d, 3H×4)	1.09~1.20 (d, 3H×5)	1.12~1.21 (d, 3H×4)	1.15~1.21 (d, 3H×3)	1.07~1.20 (d, 3H×3)
H_{23}	0.88 (t, 3H)	0.87 (t, 3H)	0.88 (t, 3H)	0.86 (t, 3H)	0.91 (t, 3H)
H_{21}				0.98 (d, 3H, J=7)	3.62 (2H)

The fragment ions (*m/e* 174 (B^+) and 190) derived from mycaminose in cirramycin A_1 were found to correspond to the peaks of *m/e* 158 (B^+) and 174 in JVM A_3 (Table 2). This difference (174—158, and 190—174=16) was also observed with the parent peaks. In the NMR spectrum of cirramycin A_1, the signal assigned to the N,N-dimethyl group (2.50 ppm) is shifted up-field (2.22 ppm) in JVM A_3, whereas the signals due to the aglycone moiety showed very similar chemical shifts and splitting patterns (Table

Table 4. Mass spectra of juvenimicin acetates (*m/e* (%))

	M^+	M^+-60 (AcOH)	$M^+-60-44$ (CO_2)	M^+-216 (S_1)	$M^+-216-60$ (S_1) (AcOH)	$M^+-216-2\times60$	S_1*	S_2**
JVM A_3 3,2′-diacetate	665 (1.7)	605 (3.0)		449 (7.0)	389 (7.0)		216 (20)	200 (100)
JVM A_2 3,2′-diacetate	637 (11)	577 (1.6)	533 (2.4)	421 (61)	361 (35)		216 (48)	200 (100)
JVM A_4 3,18,2′-triacetate	709 (4.2)	649 (1.7)	605 (1.3)	493 (13.6)	433 (9.3)		216 (20)	200 (100)
JVM B_1 3,18,2′-triacetate	693 (11)	633 (1.7)	589 (1.7)	477 (36)	417 (8.5)	357 (5.9)	216 (32)	200 (100)
JVM B_3 3,18,21,2′-tetraacetate	751 (4.7)	691 (6.3)		535 (11)	475 (10)	415 (3.9)	216 (34)	200 (100)



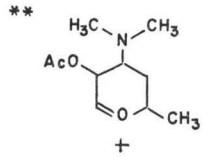


Table 5. Chemical shifts and coupling constants of juvenimicin acetates
(100 MHz, $CDCl_3$, δ=ppm, J=Hz)

Protons	JVM A_3 3,2′-diacetate	JVM A_2 3,2′-diacetate	JVM A_4 3, 18, 2′-triacetate	JVM B_1 3, 18, 2′-triacetate	JVM B_3 3,18,21, 2′-tetraacetate
H_{18}	9.57 (s, like)	—	4.07 (m, 2H)	4.06 (m, 2H)	4.09 (m, 2H)
H_{11}	6.66 (d, $J_{10,11}$ =16)	6.56 (d, J=16)	6.62 (d, J=16)	7.28 (d, J=16)	7.30 (d, J=16)
H_{10}	6.42 (d, $J_{10,11}$ =16)	6.40 (d, J=16)	6.40 (d, J=16)	6.24 (d, J=16)	6.26 (d, J=16)
H_{15}	4.60 (m)	4.75 (m)	4.68 (m)	4.78 (m)	4.78 (m)
H_1'	4.20 (d, $J_{1',2'}$ =7)	4.24 (d, $J_{1',2'}$ =8)	4.26 (d, $J_{1',2'}$ =8)	4.20 (d, $J_{1',2'}$ =8)	4.23 (d, $J_{1',2'}$ =8)
H_3	5.12 (d, like, $J_{2,3}$=10, $J_{3,4}$ <2)	5.18 (oc, $J_{3,4}$=1.5, $J_{2,3}$=3, 10)	5.03 (d, like, J=10)	5.10 (d, like, J=10)	5.06 (d, like, J=10)
H_5	3.50 (d, like, $J_{4,5}$=10, $J_{5,6}$ <2)	3.22 (q, $J_{4,5}$=7, $J_{5,6}$=3)	3.36 (d, like, J=9)	3.43 (d, like, J=8)	3.37 (d, like, J=8)
H_5'	3.44 (m)	3.45 (m)	3.45 (m)	3.43 (m)	3.44 (m)
H_2'	4.70 (q, $J_{2',3'}$ =10)	4.77 (q, $J_{2',3'}$ =10)	4.74 (q, $J_{2',3'}$ =10)	4.72 (q, $J_{2',3'}$ =10)	4.73 (q, $J_{2',3'}$ =10)
H_{13}	2.90 (d, $J_{13,14}$ =9)	2.78 (d, $J_{13,14}$ =10)	2.86 (d, $J_{13,14}$ =9)	5.78 (d, like, $J_{13,14}$=10)	5.82 (d, like, $J_{13,14}$=10)
H_3'	2.60 (m)	2.62 (m)	2.64 (m)	2.64 (m)	2.66 (m)
H_{21}	—	—	—	—	3.80~4.25 (m, 2H)
N-di-CH_3	2.24 (s, 3H×2)	2.25 (s, 3H×2)	2.24 (s, 3H×2)	2.23 (s, 3H×2)	2.24 (s, 3H×2)
H_{20}	1.46 (s, 3H)	1.46 (s, 3H)	1.45 (s, 3H)	1.79 (s, 3H)	1.80 (s, 3H)
Sec.-CH_3	1.06~1.2 (d, 3H×4)	0.98~1.23 (d, 3H×5)	1.04~1.21 (d, 3H×4)	0.95~1.20 (d, 3H×4)	0.98~1.19 (d, 3H×3)
H_{23}	0.84 (t, 3H)	0.86 (t, 3H)	0.85 (t, 3H)	0.92 (t, 3H)	0.92 (t, 3H)
3-OAc	2.04 (s, 3H)	1.98 (s, 3H)	1.98 (s, 3H)	2.01 (s, 3H)	2.02 (s, 3H)
2′-OAc	2.11 (s, 3H)	2.07 (s, 3H)	2.06 (s, 3H)	2.06 (s, 3H)	2.06 (s, 3H)
18-OAc	—	—	2.02 (s, 3H)	2.02 (s, 3H)	2.03 (s, 3H)
21-OAc	—	—	—	—	2.04 (s, 3H)

3). From these data it was assumed that the sugar moiety of JVM A_3 consists of a deoxy derivative of mycaminose. On acidic hydrolysis of JVM A_3, D-desosamine hydrochloride[7] was obtained.

The differences between cirramycin A_1 and JVM A_3 lie in their sugar moieties.

The mass spectrum of JVM A_3 3,2′-diacetate (Table 4) displays peaks at *m/e* 665 (M^+), 605 (M^+-60 (AcOH)), 449 (M^+-216 ($C_{10}H_{18}NO_4$)), 389 (449−60), 216 and 200 (base peak, $C_{10}H_{17}NO_3$). In the NMR spectrum, the proton signals at 3.87 ppm (d, like, J=10 Hz, H_3) and 3.18 (q, J=7, 10 Hz, H_2') in JVM A_3 shifted downfield to 5.12 and 4.70 ppm, respectively (Table 5) in its 3,2′-diacetate.

The data available for cirramycin A_1 was very helpful in determining the structure of JVM A_3. It was found that rosamicin[9] was identical with JVM A_3[8] in their physicochemical properties and antimicrobial activities.* The four other components of JVM are new macrolide antibiotics.

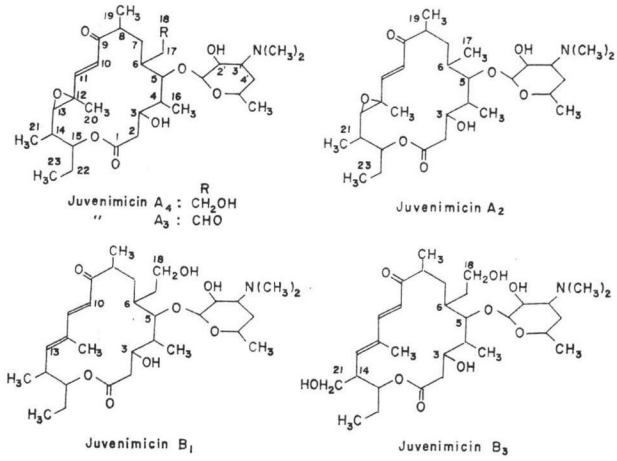
Chemical Structures of Juvenimicins

Juvenimicin A_3 consists of a sixteen-membered macrocyclic lactone and a dimethylamino sugar, desosamine, as shown in Chart 1. The chromophore of JVM A_3 is attributable to the grouping $O=C-CH=CH-\underset{\underset{Me}{|}}{C}(=O)-CH$. Since the UV maxima of JVM A_2 and A_4 are observed at 241 nm, the same as that of JVM A_3, they were assumed to have the above-mentioned group which was supported by the chemical shifts of H_{10}, H_{11} and H_{20} as shown in Table 3. On the other hand, the chromophore of JVM B_1 and B_3 was assumed to be $O=C-CH=CH-\underset{\underset{Me}{|}}{C}=CH$ on the basis of its UV maxima at 282 nm and the chemical shifts of H_{10}, H_{11} and H_{20}. In the mass spectral fragmentation pattern of basic macrolides, it has been known that the fragments derived from the dimethylamino sugar moiety show the strongest peaks. The fragments *m/e* 158 and 174 for JVM A_3 were also observed for JVM A_2, A_4, B_1 and B_3. They were, therefore, assumed to also have desosamine as their sugar moiety.

Juvenimicin A_4

The mass spectral fragmentation pattern of JVM A_4 is very similar to that of JVM A_3. The molecular ion (*m/e* 583) and the fragment peaks containing the aglycone moiety of JVM A_4, however, appear 2 mass units (2H) higher than the corresponding peaks of JVM A_3 as shown in Table 2, suggesting that the structural difference resides in the aglycone moiety. In the NMR spectrum of JVM A_4, no signal attributable to the 18-aldehyde group is observed and, instead, methylene signal is found at

Chart 1.



* It had been reported by KAWAMOTO *et al.*[10] that rosamicin was different from juvenimicin on the basis of the Rf values (paper chromatography), but no differences were observed by direct comparison.

3.70 ppm (m, 2H, $CH_2\underline{CH_2}$-O, H_{18}). Other signals are almost the same as those of JVM A_3 as shown in Table 3. Acetylation of JVM A_4 yielded a triacetate (M^+ *m/e* 709), while JVM A_3 gave a diacetate, indicating that JVM A_4 has one additional hydroxyl group in place of the aldehyde group in JVM A_3 as shown in Chart 1. For confirmation of the proposed structure, JVM A_3 was reduced with sodium borohydride, and the product obtained was identical in all respects with JVM A_4.

Juvenimicin B_1

The mass spectral fragmentation pattern of JVM B_1 is very similar to that of JVM A_4. The molecular ion (*m/e* 567) and fragment ions which contain the aglycone moiety of JVM B_1, however, appear at 16 mass units (O) lower than the corresponding peaks of JVM A_4 (Table 2). In consequence JVM B_1 was assumed to have a double bond in place of the epoxide group in JVM A_4. The partial structure, O=C-CH=CH-C(Me)=CH is also confirmed by the chemical shifts of H_{10}, H_{11}, H_{13} and H_{20} in JVM B_1 as shown in Table 3. The last three of these display down-field shifts relative to those of the corresponding protons of JVM A_4 (6.54 ppm→7.28, 2.78→5.83, 1.40→1.78). Such shifts may be attributed to the presence of a double bond at position 12 in JVM B_1. The structure of JVM B_1 (Chart 1) was established following its preparation from the interaction of JVM A_4 with potassium iodide.

Juvenimicin B_3

The mass spectral fragmentation pattern of JVM B_3 is very similar to that of JVM B_1. The molecular ion (*m/e* 583) and the fragment peaks containing the aglycone moiety of JVM B_3 appear at 16 mass units (O) higher than the corresponding peaks of JVM B_1 as shown in Table 2, suggesting that JVM B_3 has an additional oxygen in the aglycone moiety. In comparing the NMR spectrum of JVM B_3 with that of JVM B_1, it becomes apparent that the 14-methyl signal at 0.98 ppm in JVM B_1 has been replaced by a methylene signal at 3.62 ppm (m, 2H, H_{21}). Acetylation of JVM B_3 gave a tetraacetate (M^+ *m/e* 751), while JVM B_1 yielded a triacetate, indicating that JVM B_3 has an additional hydroxyl group in the aglycone moiety. An NMR spin-decoupling study and the down-field shift of H_{21} (3.80→4.25 ppm, m, 2H, Table 5) in JVM B_3 tetraacetate suggested the location of this additional hydroxyl group at position 21.

Juvenimicin A_2

The mass spectral fragmentation pattern of JVM A_2 is very similar to that of JVM A_3. However, the molecular ion (*m/e* 553) and the fragment peaks which contain the aglycone moiety of JVM A_2 appear at 28 mass units (C=O) lower than the corresponding peaks of JVM A_3 as shown in Table 2, suggesting that JVM A_2 is less one C=O group in the aglycone moiety. In the NMR spectrum of JVM A_2 there was no signal for an aldehyde group. In its place there was an additional secondary methyl signal with the remainder of the spectrum being almost the same as that of JVM A_3 as shown in Table 3. An NMR spin-decoupling study of JVM A_2 diacetate led to the following partial structure.

$\delta^{CDCl_3}_{ppm}$	2.25~2.55	5.18	1.80	3.22	1.68	0.98
	H	H	H	H	H	
—O—C_1—	C_2	—C_3—	C_4	—C_5—	C_6	—Me
	O H	OAc	Me_{16}	O		
(J=Hz)	(3.10)	(1.5)	(7)	(3)		

On irradiation of the proton at 1.68 ppm (H_6), the doublet methyl signal at 0.98 ppm became a singlet and the quartet at 3.22 ppm collapsed to a doublet. This establishes the point of attachment of this

Table 6. Biological activities of the juvenimicins in mice

Juvenimicins	Acute toxicity		Protection	
	Route	LD_{50} (mg/kg)	Route	ED_{50} (mg/kg)*
A_2	i.p.	200~400	s.c. p.o.	*ca.* 100 200
A_3	i.p. p.o.	200~400 >1,000	s.c. p.o.	2.81 218
B_1	i.p.	125~250	s.c. p.o.	5.81 81.2
B_3	i.p.	250~500	s.c. p.o.	5.0 >500

* Intraperitoneal infection with *Staphylococcus aureus* 308 A-1

additional secondary methyl group as position 6. On the basis of these observations, the structure shown in Chart 1 was proposed for JVM A_2.

Each of the components of the JVM complex was found to have interesting and characteristic structures. JVM A_2 lacks a formylmethyl group having instead a methyl group at position 6. In 16-membered macrolides it is known that the neutral macrolides such as chalcomycin,[11] neutramycin[12] and aldgamycins E and F[13] have a methyl group at position 6, but JVM A_2 is the first example among the basic macrolides. The side chain at position 6 of JVM A_4, B_1 or B_3 is comprised of a hydroxyethyl group. In the basic macrolides only relomycin[14] is known to have a hydroxyethyl group at this position. JVM B_3 may be the first example of a macrolide possessing a free 14-hydroxymethyl group. A neutral sugar is attached to the 14-hydroxymethyl group of tylosin,[15] relomycin, angolamycin,[16] chalcomycin, neutramycin and aldgamycins E and F.

The various components of JVM may afford valuable insight into the biosynthetic pathways leading to the macrolides and provide answers to the structure-activity relationships for the macrolide antibiotics.

Biological Activity

The components of JVM display antimicrobial activity against gram-positive bacteria, some of gram-negative bacteria and the others,[1] *in vitro*.

Table 6 shows the therapeutic effects (ED_{50})* and acute toxicities (LD_{50})** of the JVM components in mice. The oral protections afforded by JVM A_2 and A_3 were of the same whereas that of B_1 was superior to that of JVM A_3 despite its weak *in vitro* activity. JVM B_3 was not effective by oral administration. By subcutaneous administration all components with the exception of JVM A_2 displayed good activity at low dosage. It is believed that the activity of each component is a reflection of its lipophilicity. The acute toxicities of these antibiotics were somewhat more pronounced than that of macrolide antibiotics.

Experimental

Isolation of juvenimicins

The fermentation broth of *M. chalcea* var. *izumensis* (30 liters) was mixed with Hyflo Super-cel (750 g, Johns-Manville Co.) and filtered to afford broth filtrate (23.5 liters). The filtrate adjusted to

*, ** Data provided by Drs. K. TSUCHIYA, M. KONDO and H. YOKOTANI of this Division.

pH 9 was extracted with ethyl acetate (11 liters×2) after addition of sodium sulfate (2 kg) for salting-out. The extract was washed, dried and concentrated. The concentrate (1 liter) was extracted with dilute hydrochloric acid (3 liters, pH 3). JVM was transferred to the aqueous layer and EVM remained in the ethyl acetate layer. The aqueous layer adjusted to pH 9 was reextracted with ethyl acetate (1 liter×2). The washed extract was concentrated to give a crude powder of JVM (650 mg). The crude powder was chromatographed on a column of Sephadex LH-20 (150 ml, Pharmacia Co.) and eluted with ethyl acetate to afford a pale yellow powder (450 mg). The powder was purified by TLC on silica gel HF_{254} (Merck Co.) with the solvent system $CHCl_3$ - MeOH - 7% NH_4OH (40:12:20, lower phase). The major components were scraped from silica gel plates and extracted with ethyl acetate. The extracts were concentrated to give JVM A_2 (70 mg), A_3 (120 mg), A_4 (10 mg), B_1 (25 mg) and B_3 (60 mg), respectively.

The ethyl acetate layer (1 liter) remaining after washing with dilute hydrochloric acid was concentrated after washing with 2% sodium bicarbonate to afford a yellow powder of EVM (12 g). The powder (2 g) was chromatographed on a column of activated carbon and the active fraction was eluted with ethyl acetate. After concentration of the eluate a white powder of EVM (1.3 g) was obtained on addition of hexane. The powder was purified by TLC on silica gel HF_{254} with ethyl acetate. The main component was extracted with ethyl acetate and the extract was concentrated after washing with water to give pure EVM D (520 mg). EVM D; $[\alpha]_D^{22}$ −25.1° (*c* 1.0, MeOH), UV; λ_{max}^{MeOH} 286 nm ($E_{1cm}^{1\%}$ 19.2), $\lambda^{MeOH-0.1N\ NaOH}$ 298 nm ($E_{1cm}^{1\%}$ 83).

Anal. Calcd. for $C_{66}H_{99}O_{35}NCl_2$: C, 51.56; H, 6.49; N, 0.91; Cl, 4.61.
Found: C, 51.21; H, 6.49; N, 1.28; Cl, 4.66.

The IR spectrum and antimicrobial spectrum of this sample was in good accord with the corresponding data for everininomicin D.[2b]

Acetylation of JVM components

A solution of JVM components (120 mg each) in acetic anhydride (2 ml) and pyridine (2 ml) was allowed to stand at room temperature overnight. The reaction solution was poured into iced 2% sodium bicarbonate and extracted with ethyl acetate at pH 8.5. The extract was concentrated after washing with water and 2% sodium bicarbonate. The concentrated residue was purified by TLC on silica gel HF_{254} developed with C_6H_6 - Me_2CO (1:1). The main band was extracted with ethyl acetate and the extract was concentrated to dryness. Each acetyl derivatives was obtained as a foam (70~80 mg each).

JVM A_2 3,2′-diacetate: $[\alpha]_D^{24}$ +3.53° (*c* 0.51, MeOH), UV; λ_{max}^{MeOH} 240 nm (ε=14,800).

Anal. Calcd. for $C_{34}H_{55}NO_{10}$: C, 64.02; H, 8.69; N, 2.20.
Found: C, 63.95; H, 8.81; N, 2.07.

JVM A_3 3,2′-diacetate (rosamicin 3,2′-diacetate): $[\alpha]_D^{22}$ −17.5° (*c* 0.48, EtOH), UV; λ_{max}^{MeOH} 239 nm (ε=12,700).

Anal. Calcd. for $C_{35}H_{55}NO_{11}$: C, 63.14; H, 8.33; N, 2.10.
Found: C, 63.07; H, 8.92; N, 2.10.

JVM A_4 3,18,2′-triacetate: UV; λ_{max}^{MeOH} 240 nm (ε=13,600).

Anal. Calcd. for $C_{37}H_{59}NO_{12}$: C, 62.60; H, 8.38; N, 1.97.
Found: C, 62.51; H, 8.88; N, 2.01.

JVM B_1 3,18,2′-triacetate: $[\alpha]_D^{24}$ +30.6° (*c* 0.51, MeOH), UV; λ_{max}^{MeOH} 281 nm (ε=23,600).

Anal. Calcd. for $C_{37}H_{59}NO_{11}$: C, 64.05; H, 8.57; N, 2.02.
Found: C, 64.00; H, 9.01; N, 2.37.

JVM B_3 3,18,21,2′-tetraacetate: $[\alpha]_D^{23}$ −2.56° (*c* 0.39, MeOH), UV; λ_{max}^{MeOH} 281 nm (ε=22,200).

Anal. Calcd. for $C_{39}H_{61}NO_{13}$: C, 62.30; H, 8.18; N, 1.86.
Found: C, 62.08; H, 8.53; N, 2.06.

D-Desosamine hydrochloride

A solution of JVM A_3 (596 mg) in 3 N hydrochloric acid (20 ml) was refluxed for 2 hours. The filtered reaction solution was washed with chloroform and *n*-butanol to remove fat-soluble substances. The concentrated aqueous solution was applied to a column of Dowex 50 W × 8 (50~100 mesh, 20

ml) and eluted with 0.5 N and N hydrochloric acid (150 ml each). The eluate was passed through Amberlite IR-45 (20 ml), and concentrated to afford D-desosamine hydrochloride (α,β-anomer, 130 mg); $[\alpha]_D^{23}$ +50.6° (*c* 0.51, H_2O).

Anal. Calcd. for $C_8H_{17}NO_3 \cdot HCl$: C, 45.39; H, 8.57; N, 6.62.
Found: C, 45.08; H, 8.37; N, 6.60.

The IR and NMR spectra were in accord with those of D-desosamine hydrochloride.

Isolation of rosamicin

The culture filtrate of *M. rosaria* NRRL No. 3718 (27 liters) was extracted with ethyl acetate (1/1 v.×2) at pH 9 after addition of sodium chloride. (2.7 kg). The extract (57 liters) was washed with water (1/4 v.×2) and concentrated under reduced pressure. The concentrate (2.6 liters) was extracted with dilute hydrochloric acid (pH 2) and the aqueous layer was reextracted with ethyl acetate at pH 9. The ethyl acetate layer was concentrated after washing with water and diluted with hexane to give a crude powder (2.5 g). The crude powder was purified by TLC on silica gel HF_{254} with the solvent system of $CHCl_3$ - MeOH - 7% NH_4OH (40:12:20, lower layer). The concentrate was precipitated from hexane to afford a pure powder of rosamicin (910 mg); $[\alpha]_D^{23}$ −21.3° (*c* 0.5, $CHCl_3$), −32.3° (*c* 0.5, EtOH), UV; λ_{max}^{EtOH} 240 nm (ε=12,000).

Anal. Calcd. for $C_{31}H_{51}NO_9$: C, 64.08; H, 8.85; N, 2.41.
Found: C, 63.38; H, 8.85; N, 2.81.

MS: *m/e* 581 (M^+).

Sodium borohydride reduction of JVM A_3

To a solution of JVM A_3 (280 mg) in 80% MeOH - 0.2 M phosphate buffer (pH 7.1, 21 ml) was added sodium borohydride (30 mg) in 50% MeOH - 0.2 M phosphate buffer (pH 7.1, 1.4 ml) and the whole was stirred at room temperature for 1 hour. After addition of glycine-NaCl/NaOH buffer (pH 9.3, 200 ml) the reaction mixture was extracted with chloroform. The organic layer was concentrated after washing with water and the residue was purified by TLC on Silica gel HF_{254}. The JVM A_4 was obtained on addition of hexane (80 mg); $[\alpha]_D^{24}$ −29.9° (*c* 0.5, MeOH) and −7.4° (*c* 0.47, $CHCl_3$), UV; λ_{max}^{MeOH} 241 nm (ε=14,000).

Anal. Calcd. for $C_{31}H_{53}NO_9$: C, 63.86; H, 9.16; N, 2.40.
Found: C, 62.79; H, 8.89; N, 2.18.

The synthetic sample was identical in all respects with JVM A_4 by TLC, IR, mass and NMR spectra and antimicrobial activity.

Deepoxydation of JVM A_4

To a solution of JVM A_4 (200 mg) in acetic acid (2 ml) was added potassium iodide (300 mg) and the whole was stirred at 80°C for 30 minutes. The reaction mixture was added to iced water and extracted with ethyl acetate at pH 9. The extract was washed with 2% sodium thiosulfate and concentrated. JVM B_1 was obtained from hexane as colorless powder (44 mg); UV; λ_{max}^{EtOH} 282 nm (ε=16,100).

Anal. Calcd. for $C_{31}H_{53}NO_8$: C, 65.58; H, 9.41; N, 2.47.
Found: C, 64.02; H, 9.43; N, 2.28.

The synthetic sample was identical in all respects with JVM B_1 by TLC, IR, mass and NMR spectra and antimicrobial activity.

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