Mycinamicin Biosynthesis: Isolation and Structural Elucidation of Mycinonic Acids, Proposed Intermediates for Formation of Mycinamicins. X-Ray Molecular Structure of *p*-Bromophenacyl 5-Hydroxy-4-methylhept-2-enoate

Kenji Kinoshita,*[a] Satoshi Takenaka[b] and Mitsuo Hayashi[a]
[a] *Medicinal Research Laboratories, Toyo Jozo Co. Ltd., Ohito-cho, Shizuoka 410-23, Japan*
[b] *Development Division of Fermentation Technology, Toyo Jozo Co. Ltd., Ohito-cho, Shizuoka 410-23, Japan*

Mycinonic acids, proposed intermediates in the biosynthesis of the macrolide antibiotic mycinamicins, were isolated from the culture filtrate of *Micromonospora griseorubida* and their chemical structures were determined on the basis of their spectroscopic data. Their absolute configurations were confirmed by X-ray diffraction analysis and comparison of their total syntheses. Their possible role in the biosynthesis of mycinamicins is discussed.

Mycinamicins are 16-membered macrolide antibiotics produced by *Micromonospora griseorubida*, and comprises an aglycone and two sugars, desosamine and mycinose.[1-4] The first intermediate in the biosynthesis of mycinamicin aglycone is protomycinolide IV **5**,[5] which is assembled from three acetates and five propionates, as shown by labelling studies with radioactive precursors.[6] In a previous communication we reported on the isolation and chemical structure of 5-hydroxy-4-methylhept-2-enoic acid **11**, 7-hydroxy-6-methylnona-2,4-dienoic acid **2** and 9-hydroxy-8-methylundeca-4,6-dien-3-one **10**, considered to be biosynthetic intermediates in the formation of the macrolactone **5**.[7] On the basis of structural considerations we suggested that these compounds might be intermediates at an early stage in the biosynthetic pathway.

The biosynthesis of macrolides has been reasonably well studied for erythromycin and tylosin. After the formation of the lactone precursor, the aglycone, detailed knowledge of the chain-elongation mechanism in such macrolide lactone rings is still limited, and no intermediates in the chain assembly between the macrolactone and the precursors acetate, propionate and butyrate have yet been detected.[8] In general, in the biosynthesis of the basic carbon skeleton of macrolide antibiotics, polyketide secondary metabolites are constructed from small molecules, especially acetate, propionate and/or butyrate units, which are condensed by a head-to-tail mechanism. This head-to-tail sequence of reactions closely resembles the biosynthetic route to saturated fatty acids by the condensation of acetyl-CoA and malonyl-CoA. A basic difference between the two processes is that, with polyketide synthase enzymes, addition of the next building block may occur before a series of reduction–dehydration–reduction steps of the preceding unit is complete.[9-11] This process of condensation of simple building blocks is very complex and includes a whole array of consecutive reactions which are still rather poorly understood. Recently, important questions regarding the role of hypothetical intermediates in the biosynthesis of the macrolide antibiotics erythromycin and tylosin have been posed independently by both Cane[12] and Hutchinson and colleagues.[13] In their investigations, the ^{13}C-labelled forms of carbon-skeleton fragments, activated as the *N*-acetylcysteamine thioester, were fed to producing cultures of the relevant micro-organism, and were incorporated intact into the macrolide rings.

In our mutagenic studies on *M. griseorubida* we found that it produced new mycinamicin-related biosynthetic intermediates. These compounds have been named mycinonic acids I **1**, II **2**, III **3**, IV **4** and epimycinonic acid I **11**. In this paper we describe the isolation of these compounds, their physicochemical characteristics, and the elucidation of their absolute configuration, and discuss their possible roles in the biosynthesis of mycinamicins. This is the first successful isolation of early intermediates in the formation of macrolide antibiotics from fermentation broth (see Scheme 1).

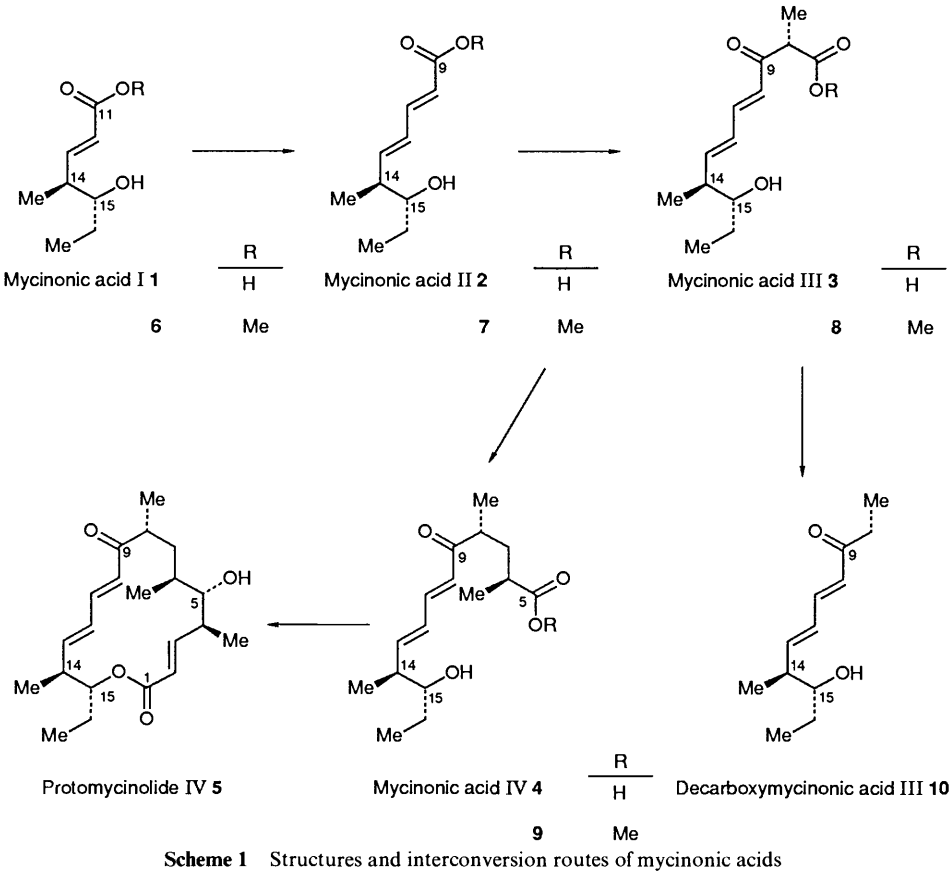
Results and Discussion

Structure Determination.—The natural products (the acids **1**–**4** and **11**) were isolated as their methyl derivatives **6**–**9** and **12** and the structural work was performed on the latter. The physicochemical properties of compounds **6**–**9** and **12** are given in Table 1. The molecular formulae of these compounds were established by high-resolution chemical-ionization mass spectrometry (HR-CIMS). The ^1H and ^{13}C NMR spectral data for these compounds are compared with those of macrolide **5** in Tables 2 and 3, respectively.

Methyl Mycinonate I **6**.—The UV spectrum suggested the presence of an α,β-unsaturated ester (210 nm). The IR spectrum showed unsaturated ester (1725, 1710 and 1660 cm^{-1}) and hydroxy group (3440 cm^{-1}) absorptions. Signals for two olefinic protons were observed at δ_H 5.88 (1 H, d, J 16.1 Hz) and 6.98 (1 H, dd, J_1 16.1, J_2 7.8 Hz) in the ^1H NMR spectrum, which indicate *E* geometry. From these results, the compound was identified as methyl 5-hydroxy-4-methylhept-2-enoate **6**.

Methyl Mycinonate II **7**.—The presence of an α,β,γ,δ-unsaturated ester was suggested by the UV absorption maximum at 261 nm, and the IR spectrum showed unsaturated ester (1720, 1640 and 1620 cm^{-1}) and hydroxy group (3460 cm^{-1}) absorptions. The ^1H NMR spectrum revealed the presence of two *C*-methyl [δ_H 0.96 (3 H, t, 17-H_3), 1.09 (3 H, d, 21-H_3)], one *O*-methyl [δ_H 3.74 (3 H, s)], one methylene [δ_H 1.55 (1 H, qdd, 16-H^a), 1.41 (1 H, qdd, 16-H^b)], and two methine groups [δ_H 2.37 (1 H, qdd, 14-H), 3.42 (1 H, qdd, 15-H)] and four olefinic protons [δ_H 5.38 (1 H, d, 10-H), 7.28 (1 H, dd, 11-H), 6.23 (1 H, dd, 12-H), 6.11 (1 H, dd, 13-H)]. The coupling constants indicated the all-(*E*) configuration of the diene moiety. The ^{13}C NMR spectrum supported this conclusion. Accordingly, the compound was identified as methyl 7-hydroxy-6-methylnona-2,4-dienoate **7**, having a carbon chain two units longer than that of the ester **6**.

Methyl Mycinonate III **8**.—The UV absorption maximum at 283 nm suggested the presence of an α,β,γ,δ-unsaturated ketone. In the ^1H NMR spectrum α-methyl-β-

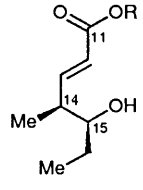


Scheme 1 Structures and interconversion routes of mycinonic acids

Table 1 Physicochemical properties

Compound	6	7	8	9	10	12
Formula	$C_9H_{16}O_3$	$C_{11}H_{18}O_3$	$C_{14}H_{22}O_4$	$C_{17}H_{28}O_4$	$C_{12}H_{20}O_2$	$C_9H_{16}O_3$
Appearance	oil	oil	oil	oil	oil	oil
HR-CIMS						
$[(M + H)^+, m/z]$	173.1165	199.1336	255.1589	297.2048	197.1536	173.1164
Calc.	173.1178	199.1334	255.1596	297.2066	197.1538	173.1178
(Formula)	($C_9H_{17}O_3$)	($C_{11}H_{19}O_3$)	($C_{14}H_{23}O_4$)	($C_{17}H_{29}O_4$)	($C_{12}H_{21}O_2$)	($C_9H_{17}O_3$)
$[\alpha]_D/^\circ$ (MeOH)	−8.1	−5.2	*a*	+1.0	−6.3	−45.9
(*c*)	(0.94)	(1.00)		(0.81)	(0.86)	(1.01)
λ_{max}/nm (MeOH)	210	261	283	280	275	212
(log ε)	(4.27)	(4.29)	(4.51)	(4.24)	(4.37)	(4.12)
ν_{max}/cm^{-1}	3440, 1725, 1710, 1660	3460, 1720, 1640, 1620	*a*	3470, 1735, 1660, 1635, 1595	3450, 1660, 1635, 1600	3440, 1725, 1710, 1660

[a] Insufficient material available.



Epimycinonic acid I

	R
11	H
12	Me
13	$CH_2COC_6H_4Br$-*p*

keto carboxyl signals were observed at δ_H 1.38 (3 H, d, 20-H) and 3.74 (1 H, q, 8-H). The ^1H and ^{13}C NMR spectra were similar to those of compound **7** except for signals due to a ketone carbonyl and an α-methyl-β-keto carboxy group. Therefore the compound was identified as methyl 9-hydroxy-2,8-dimethyl-3-oxoundeca-4,6-dienoate **8**. The acid **3** easily undergoes decarboxylation to 9-hydroxy-8-methylundeca-4,6-dien-3-one **10** in the isolation process.

Methyl Mycinonate **9**.—The UV and NMR spectra of **9** were similar to those of compound **8**. The presence of an α,β,γ,δ-unsaturated ketone was suggested by the maximum at 280 nm in the UV spectrum and the IR absorptions at 1660, 1635 and 1595 cm^{-1}. This was also evident from the ^1H NMR spectrum which showed characteristic signals of an unsaturated ketone at δ_H 6.17 (1 H, d, 10-H), 7.21 (1 H, dd, 11-H), 6.23 (1 H, dd, 12-H) and 6.18 (1 H, dd, 13-H). In the ^{13}C NMR spectrum there were no substantial differences between this compound and protomycinolide IV **5**, with the corresponding chemical shift of carbon atoms 9–15 (with substituent). The signals of the unsaturated ketone carbonyl and the ester carbonyl were observed at δ_C 203.1 (s, C-9) and 176.8 (s, C-5). From these results, the compound was concluded to be methyl 11-hydroxy-2,4,10-trimethyl-5-oxotrideca-6,8-dienoate **9**.

The Absolute Configuration of Mycinonic Acids I **1**, *II* **2**, *III* **3** *and IV* **4**—The structures of the methyl derivatives **6**–**9**, were

Table 2 ^{1}H NMR chemical shifts ($CDCl_3$; δ^a, mult.)

Proton	5	6	7	8	9	10	12
2	5.77 d						
3	6.59 dd						
4	2.52 qdd						
5	3.29 dd						
6	1.28 qdd				2.77 qd		
7a	1.49 m				2.11 ddd		
7b					1.35 ddd		
8	2.55 qd			3.74 q	2.52 qd	2.58 q	
10	6.22 d		5.38 d	6.22 d	6.17 d	6.12 d	
11	7.10 dd		7.28 dd	7.27 dd	7.21 dd	7.15 dd	
12	6.08 dd	5.88 d	6.23 dd	6.25 dd	6.23 dd	6.24 dd	5.87 dd
13	5.71 dd	6.98 dd	6.11 dd	6.24 dd	6.18 dd	6.15 dd	6.95 dd
14	2.30 qdd	2.43 qdd	2.37 qdd	2.37 qdd	2.39 qdd	2.38 qdd	2.44 qdd
15	4.65 ddd	3.47 ddd	3.42 ddd	3.44 ddd	3.43 ddd	3.43 qdd	3.49 ddd
16a	1.82 qdd	1.55 qdd	1.55 qdd	1.54 qdd	1.56 qdd	1.55 qdd	1.56 qdd
16b	1.54 qdd	1.43 qdd	1.41 qdd	1.43 qdd	1.42 qdd	1.43 qdd	1.40 qdd
17	0.94 t	0.97 t	0.96 t	0.97 t	0.97 t	0.97 t	0.97 t
18	1.12 d						
19	1.00 d				1.11 d		
20	1.18 d			1.38 d	1.16 d	1.11 t	
21	1.09 d	1.11 d	1.09 d	1.10 d	1.10 d	1.10 d	1.09 d
OMe		3.74 s	3.74 s	3.72 s	3.67 s		3.74 s

[a] Recorded at 400 MHz, with Me_4Si as internal standard; assignments were made on the basis of ^{1}H–^{1}H COSY spectra.

Table 3 ^{13}C NMR chemical shifts ($CDCl_3$; δ, mult.)

Carbon	5	6	7	8	9	10	12
1	166.3 s						
2	121.3 d						
3	151.1 d						
4	40.4 d						
5	80.2 d				176.8 s		
6	33.7 d				42.0 d		
7	31.6 t			171.2 s	36.7 t		
8	44.8 d			51.1 t	37.3 d	33.6 t	
9	203.4 s		167.6 s	195.1 s	203.1 s	201.4 s	
10	122.9 d		119.6 d	126.0 d	127.1 d	128.5 d	
11	142.2 d	167.0 s	144.9 d	144.3 d	142.9 d	142.3 d	167.0 s
12	131.7 d	121.6 d	129.1 d	129.5 d	129.8 d	129.8 d	121.1 d
13	145.3 d	150.7 d	145.6 d	147.9 d	146.6 d	146.1 d	151.4 d
14	43.4 d	42.1 d	42.8 d	42.9 d	43.0 d	42.9 d	42.2 d
15	77.3 d	76.1 d	76.4 d	76.5 d	76.5 d	76.5 d	75.9 d
16	24.7 t	27.4 t	27.4 t	27.6 t	27.5 t	27.5 t	27.4 t
17	9.6 q	10.0 q	10.0 q	10.0 q	10.0 q	10.0 q	10.3 q
18	19.4 q						
19	17.4 q				16.6 q		
20	17.7 q			13.2 q	17.7 q	8.3 q	
21	15.7 q	15.8 q	16.4 q	16.4 q	16.4 q	16.5 q	14.0 q
OMe		51.4 q	51.5 q	52.4 q	51.7 q		51.5 q

[a] 100 MHz ^{13}C NMR spectrum in $CDCl_3$ with solvent reference at 77.02 ppm. Assignments were made on the basis of ^{1}H–^{13}C chemical shift-correlated two-dimensional NMR spectra.

determined by a combination of physicochemical properties and the ^{1}H and ^{13}C NMR spectral data. The enantio-controlled synthesis of compounds **6** and **7** and the decarboxylation product **10** of mycinonic acid III **3** on the basis of the stereochemistry of mycinamicin[14,15] has been established by Takano *et al.*[16] Direct comparison of physicochemical properties and NMR spectra indicated that the natural products **6**, **7**, **10** and the synthetic substances are identical in all respects. Furthermore, the optical rotation and the chemical shifts and their coupling constants in the ^{1}H and ^{13}C NMR spectra of methyl mycinonate IV **9** were not recognized as differing significantly from those of compounds **6**–**8**. Consequently, compound **9** was presumed to maintain the absolute configuration of mycinamicin as well as compounds **6**–**8**. From these results, compounds **6**–**8** are considered to be fragment structure of protomycinolide IV **5**. Therefore, the absolute configuration of mycinonic acids I **1**, II **2** and III **3** were concluded to be (4*S*, 5*R*)-5-hydroxy-4-methylhept-2-enoic acid, (6*S*,7*R*)-7-hydroxy-6-methylnona-2,4-dienoic acid and (2*S*,8*S*,9*R*)-9-hydroxy-2,8-dimethyl-3-oxoundeca-4,6-dienoic acid, respectively.

Methyl Epimycinonate I **12**.—Compound **12** was isolated from the culture of a mutant strain which cannot produce the macrolactone. Its spectroscopic data (*e.g.*, ^{1}H and ^{13}C NMR spectra) showed a close similarity to those of methyl mycinonate I **6**. In our previous communication,[7] compound **12** was thought to be methyl mycinonate I **6**. On the other hand,

the optical rotation and 21-H_3 chemical shift in the ^{13}C NMR spectrum of compound **12** differ from those of compound **6** (**12**: $-45.9°$, δ_C 14.0; **6**: $-8.1°$, δ_C 15.8). In order to investigate the stereochemistry of epimycinonic acid I **11**, its *p*-bromophenacyl ester **13** was synthesized, and the absolute configuration of this ester **13** was determined by X-ray crystallography. The molecular structure and the stereoscopic drawing of ester **13** are shown in Fig. 1. The absolute configuration of epimycinonic acid I **11** was determined to be (4*S*,5*S*)-5-hydroxy-4-methylhept-2-enoic acid. Moreover, compound **12** was synthesized by Takano *et al.*[16] In its physicochemical properties and spectroscopic data, natural product **12** was identical with the corresponding synthetic substance.

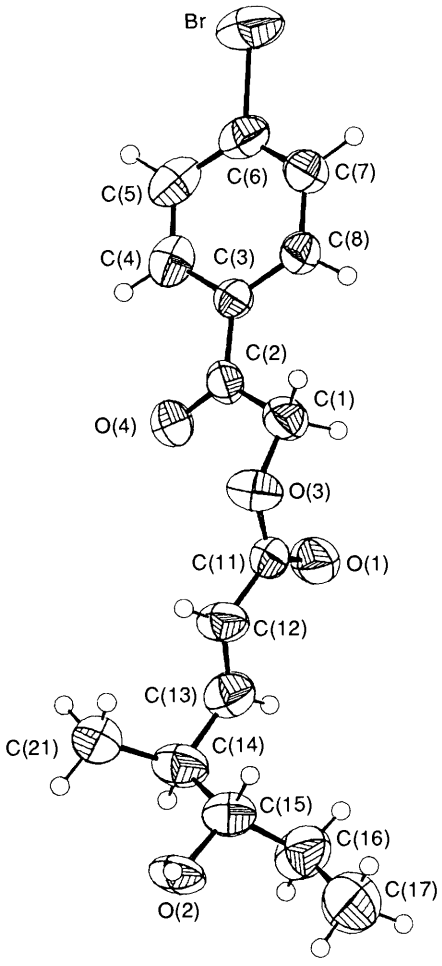
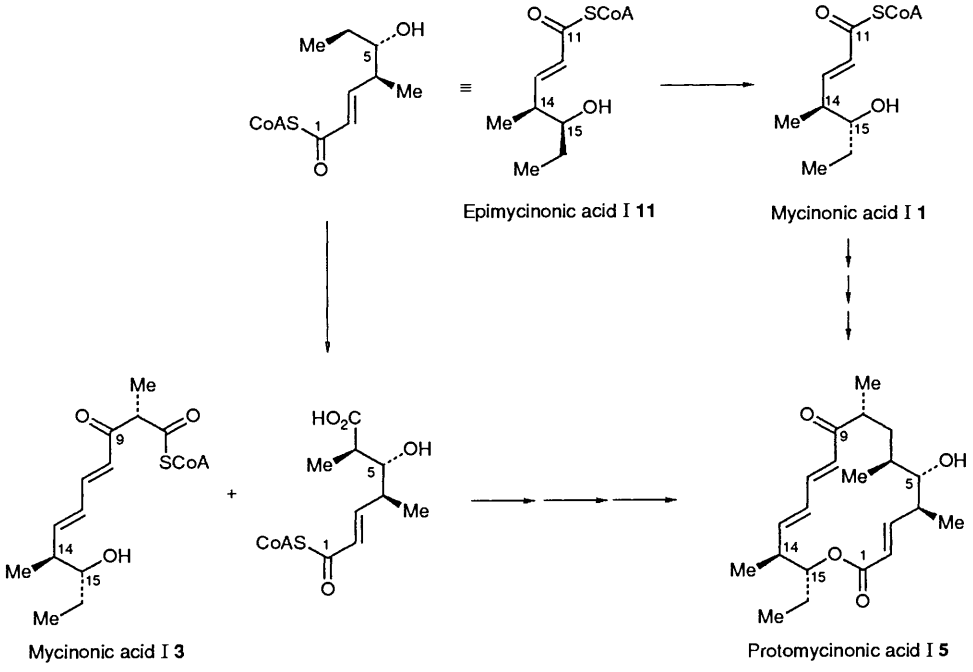


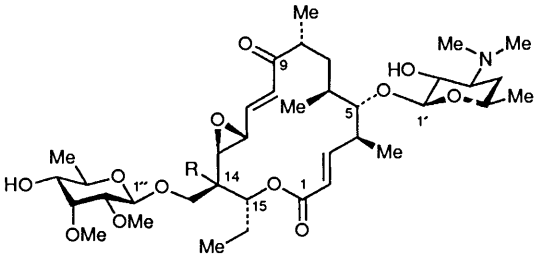
Fig. 1 Absolute configuration of *p*-bromophenacyl epimycinonate I, compound **13**

Mycinonic acids I **1**, II **2**, III **3** and IV **4** are interesting new intermediates of chain elongation into protomycinolide IV **5** isolated from the fermentation broth of *M. griseorubida*. According to these chemical structures, mycinonic acids are closely related to the group of mycinamicins. These new compounds **1**–**4** are considered to be fragments of protomycinolide IV **5**, corresponding to carbon atoms 11–15, 9–15, 8–15 and 5–15 (with substituents), respectively (Scheme 1). These results strongly suggest that the stereochemistry of the polyketide chain-elongation process must proceed systematically at each condensation stage. The discovery of these four compounds **1**–**4** may support the hypotheses regarding the biosynthesis of the macrolide antibiotics proposed by Cane and Yang[12] and Hutchinson's group.[13]

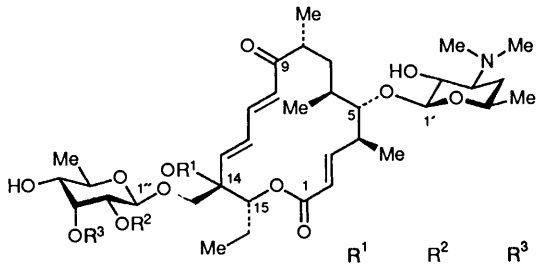
Epimycinonic acid I **11** was isolated from the culture filtrate of strain C-34-10. The absolute configuration at C-5 was *R* in mycinonic acid I **1**; in contrast it was *S* at C-5 in epimycinonic acid I **11**. However, the stereochemistry of compound **11** was equivalent to that of the fragment consisting of carbon atoms 1–6 in protomycinolide IV **5**. Normal fermentation products of the mycinamicin-producing strain contained components **1**–**4**, the major components being mycinamicins. Under the same conditions, fermentation of the mutant strain C-34-10 did not produce these compounds, but instead component **11** accumulated. Mycinamicin III **15**, IV **16**, V **17**, VI **18**, VII **19** and VIII **20**[17] biosynthetic intermediates were converted into mycinamicin II **14** more efficiently by this mutant.[18] Since its mutant products are blocked at an early stage in the chain assembly for protomycinolide IV **5** formation, we propose another chain-elongation mechanism (Scheme 2). In order to



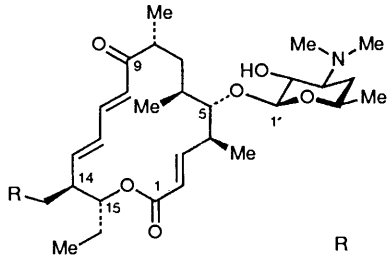
Scheme 2 Chain-elongation mechanism for mycinamicin



		R
Mycinamicin I		H
Mycinamicin II	**14**	OH



		R^1	R^2	R^3
Mycinamicin III	**15**	H	Me	H
Mycinamicin IV	**16**	H	Me	Me
Mycinamicin V	**17**	OH	Me	Me
Mycinamicin VI	**18**	H	H	H



		R
Mycinamicin VII	**19**	OH
Mycinamicin VIII	**20**	H

Scheme 3 Structures of mycinamicins

clarify further the biosynthesis of the macrolactone system in mycinamicins, attempted isolation of other biosynthetic intermediates and incorporation experiments are in progress.

Experimental

IR spectra were recorded with a Hitachi 260–50 spectrometer. UV spectra were determined with a Shimadzu UV-365 spectrophotometer. Optical rotations were measured with a Horiba SEPA-200 polarimeter. NMR spectra were recorded in $CDCl_3$ at room temperature on a JEOL JNM-GSX400 instrument at 400 MHz (^1H) and at 100 MHz (^{13}C), respectively. Chemical shifts are reported with Me_4Si as internal standard for ^1H spectra and solvent (referenced to Me_4Si) as internal standard for ^{13}C spectra. Finally, by using ^1H–^1H COSY, standard DEPT, and two-dimensional ^1H–^{13}C correlated spectroscopy, the earlier NMR assignments were extended to cover all proton and carbon resonances. HR-CIMS were taken with a JEOL JMS-D300 spectrometer. High-performance liquid chromatography (HPLC) was carried out with a Shimadzu LC-6A system, a YMC-GEL ODS 5 μ m stainless steel column (Yamamura Chemical Laboratory Corp., Kyoto, Japan), 150 × 4 mm i.d., and an SPD-6A variable-wavelength UV detector. The flow rate of the mobile phase [0.1 mol dm^{-3} NaH_2PO_4–MeOH–MeCN, (55:31:14)] was 0.8 cm^3 min^{-1} at 40 °C.

Fermentation Conditions and Mutant Strain.—The fermentation by the mycinamicin-producing strain *M. griseorubida* was carried out at 27 °C for 7 days under aeration at a rate of 20 dm^3 min^{-1} and agitation at 300 rpm in a 30 dm^3 jar fermenter containing production medium (20 cm^3; 7.0% dextrin, 0.5% glucose, 2.5% cotton meal, 0.5% soybean meal, 0.5% $CaCO_3$, 0.4% $MgSO_4 \cdot 7H_2O$, 0.1% K_2HPO_4, 0.0002% $CoCl_2 \cdot 6H_2O$, adjusted to pH 7.0). The medium was inoculated with 5.0% of its volume of a seed culture prepared as follows. The organism was first cultured for 2 days at 30 °C on a rotary shaker in a 150 cm^3 Erlenmeyer flask containing seed medium (20 cm^3; 1.0% dextrin, 1.0% glucose, 2.5% casamino acids, 0.5% yeast extract, 0.1% $CaCO_3$, adjusted to pH 7.0) and the culture (1.0% by volume) was then inoculated in the seed medium (1 dm^3) in a 5 dm^3 round flask and cultured for 2 days at 30 °C on a rotary shaker.

The mutant strain C-34-10 was isolated by treatment of the mycinamicin-producing strain with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Fermentation was carried out at 28 °C for 48 h in a shaken 150 cm^3 Erlenmeyer flask containing seed medium (20 cm^3). This seed culture was then inoculated (10.0% by volume) in a 500 cm^3 Erlenmeyer flask containing the production medium (80 cm^3) described above and cultured for 7 days at 27 °C on a rotary shaker.

The culture filtrates were assayed by analytical HPLC with detection at both 220 and 280 nm.

Isolation of Mycinonic Acids.—The culture filtrate (17 dm^3) of the mycinamicin producer was extracted at pH 9.0 with the same volume of chloroform. This organic layer contained mycinamicins. Then the aq. layer was extracted with diethyl ether at pH 3.0 and the organic phase was dried over Na_2SO_4. This extract was concentrated to afford the biosynthetic intermediates of mycinamicin as an oil. The crude material was dissolved in a small amount of methanol–benzene (20:80). Treatment of the acid components **1–4** with a solution of (trimethylsilyl)diazomethane in hexane for 30 min at room temperature resulted in the formation of its methyl esters **6–9** which were purified by reversed-phase preparative HPLC (YMC-GEL ODS; 5 μ m, 20 × 300 mm i.d.) in MeOH–water (40:60). The flow rate of the mobile phase was 0.8 cm^3 min^{-1}. Compound **10**, which is considered to be the decarboxylation product of mycinonic acid III **3**, was isolated at the same time. The yield of the four components **6–9** from the culture filtrate (17 dm^3) was 8 mg, 15 mg, 5 mg and 12 mg, respectively.

Table 4 Final atomic co-ordinates for *p*-bromophenacyl epimycinonate **13**

Atom	*x*	*y*	*z*
Br(A)	−0.033 6(1)	0.250 0	−0.520 4(2)
O(1A)	0.465 6(5)	0.056 5(2)	0.283(1)
O(2A)	0.456 6(6)	−0.131 8(2)	0.510(1)
O(3A)	0.316 4(5)	0.036 4(2)	−0.066(1)
O(4A)	0.147 4(6)	0.082 4(2)	0.171(1)
C(1A)	0.302 0(8)	0.075 4(2)	−0.160(2)
C(2A)	0.193 2(7)	0.096 4(2)	−0.025(2)
C(3A)	0.142 5(7)	0.134 2(2)	−0.145(2)
C(4A)	0.022 4(8)	0.151 0(2)	−0.056(2)
C(5A)	−0.028 2(8)	0.185 7(2)	−0.168(2)
C(6A)	0.040 1(9)	0.204 0(2)	−0.367(2)
C(7A)	0.156 2(9)	0.188 6(2)	−0.448(2)
C(8A)	0.208 0(7)	0.153 2(2)	−0.343(2)
C(11A)	0.403 5(8)	0.030 8(2)	0.169(2)
C(12A)	0.404 5(8)	−0.010 8(2)	0.242(2)
C(13A)	0.489 1(9)	−0.023 3(3)	0.450(2)
C(14A)	0.518(1)	−0.064 3(3)	0.543(2)
C(15A)	0.409 0(9)	−0.093 0(2)	0.448(2)
C(16A)	0.273 5(9)	−0.088 0(3)	0.569(2)
C(17A)	0.161(1)	−0.116 4(3)	0.460(2)
C(21A)	0.661(1)	−0.075 6(3)	0.462(2)
Br(B)	0.976 9(1)	−0.041 46(3)	0.019 0(2)
O(1B)	0.462 3(5)	0.156 7(2)	0.214(1)
O(2B)	0.444 8(6)	0.344 5(2)	−0.033(1)
O(3B)	0.622 8(5)	0.171 8(1)	0.557(1)
O(4B)	0.783 8(5)	0.124 3(2)	0.314(1)
C(1B)	0.629 1(8)	0.132 0(2)	0.643(2)
C(2B)	0.740 8(7)	0.111 1(2)	0.518(2)
C(3B)	0.792 3(6)	0.074 0(2)	0.636(1)
C(4B)	0.911 0(8)	0.057 3(3)	0.551(2)
C(5B)	0.967 2(8)	0.023 2(3)	0.656(2)
C(6B)	0.901 2(9)	0.005 0(2)	0.860(2)
C(7B)	0.784 8(8)	0.020 2(2)	0.952(2)
C(8B)	0.730 4(7)	0.055 1(2)	0.842(2)
C(11B)	0.541 2(7)	0.179 6(2)	0.323(2)
C(12B)	0.565 1(8)	0.219 5(2)	0.241(2)
C(13B)	0.493(1)	0.235 7(3)	0.034(2)
C(14B)	0.515(1)	0.276 6(3)	−0.070(2)
C(15B)	0.410 9(9)	0.305 4(2)	0.036(2)
C(16B)	0.263(1)	0.296 3(3)	−0.073(2)
C(17B)	0.157(1)	0.325 2(3)	0.031(2)
C(21B)	0.665(1)	0.291 1(3)	0.005(2)

Isolation of Methyl Epimycinonate I **12**.—Compound **11** was extracted at pH 3.0 with ethyl acetate (100 cm^3) from the culture filtrate (60 cm^3) of mutant strain C-34-10 blocked in its production of the macrolactone, and the extract was concentrated under reduced pressure. Treatment of the acid **11** with (trimethylsilyl)diazomethane yielded its methyl ester **12**. The mixture was concentrated under reduced pressure to give the crude product, which was purified by preparative HPLC. The yield of compound **12** from the culture filtrate (60 cm^3) was 45 mg.

p-*Bromophenacyl Ester* **13** *of Epimycinonic Acid I* **11**.—To a solution of compound **11** (60 mg) in MeCN (1 cm^3) were added triethylamine (60 mm^3) and *p*-bromophenacyl bromide (164 mg). The solution was refluxed for 2 h. After completion of the reaction, cold water (20 cm^3) was added to the reaction mixture, which was extracted with $CHCl_3$ (50 cm^3 × 2). The combined extracts were washed with saturated aq. NaCl (50 cm^3 × 2). After the extract had been dried over anhydrous Na_2SO_4 it was evaporated under reduced pressure. The residue was chromatographed over silica gel, and elution with benzene–EtOAc (10:1) afforded *p*-bromophenacyl 5-hydroxy-4-methylhept-2-enoate (**13**, 45 mg) as a powder.

* See Instructions for Authors, section 5.6.3, in the January issue.

X-*Ray Molecular Structure Analysis of Ester* **13**.—Crystal data: $C_{16}H_{19}BrO_4$, M = 355.23, monoclinic, space group $P2_1$, a = 9.721(2), b = 34.307(7), c = 4.921(3) Å, β = 96.41(3)°, V = 1633(1) $Å^3$, D_c = 1.45 g cm^{-3}, Z = 4, $F(000)$ = 728, μ(Cu-Kα) = 33.1 cm^{-1}, crystal dimensions ~0.5 × 0.2 × 0.1 mm.

The X-ray intensity data for compound **13** were collected on a Mac Science MXC18 automatic four-circle diffractometer using graphite-monochromated Cu-Kα radiation (λ = 1.541 78 Å) and a 15 kW rotating anode generator. All independent reflections collected within a range $2\theta \leqq 120°$ with ω–2θ scan technique and systematic absences (only 0, k, 0 when K = $2n + 1$ was observed) are consistent with the space group $P2_1$. The intensities of 2418 reflections were collected; 2161 non-zero reflections were used in the subsequent structure detemination and refinement. The intensity data were corrected for Lorentz and polarization effects, but not for absorption or secondary extinction.

Crystallographic calculations were performed using the CRYSTAN system of programs, run on a SUN3-80 computer. The structure was solved by direct methods, and the location of all non-hydrogen atoms of the two crystallographically independent molecules in the asymmetric unit were successfully revealed from the resulting *E*-map. The hydrogen atoms were subsequently located on a series of difference Fourier maps.

The structure was refined by full-matrix least-squares using anisotropic temperature factors for the non-hydrogen atoms and isotropic temperature factors for the hydrogen atoms. The weighting scheme was $w = 1/[\sigma^2(F) + 0.002(F_o)^2]$ and the final residual values were R = 0.058, R_w = 0.049 (R = 0.065, R_w = 0.055 for the opposite absolute structure). The final difference Fourier map contained no features in excess of 0.4 e $Å^3$.

The absolute configuration was investigated by inclusion of the anomalous scattering of the bromine, oxygen and carbon atoms in the structure-factor calculations. For each isomer the last part of the refinement was repeated on the inverted structure using the same data set. The residuals ratios (both unweighted and weighted) obtained for the two different inverted configurations were found to differ significantly from each other. The final non-hydrogen-atom co-ordinates and their standard deviations are given in Table 4.

The atomic scattering factors and anomalous dispersion parameters were taken from International Tables for X-ray Crystallography.[19] Tables of anisotropic thermal parameters, fractional co-ordinates for hydrogen atoms, and bond distances and angles are available from the Cambridge Crystallographic Data Centre.*

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