Isolation of Proposed Intermediates in the Biosynthesis of Mycinamicins

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Some proposed intermediates in the biosynthesis of mycinamicins were isolated from the culture filtrate of *Micromonospora griseorubida* sp. nov and their structures were determined on the basis of their spectroscopic data.

Mycinamicins are sixteen-membered macrolide antibiotics produced by *Micromonospora griseorubida* sp. nov, and possess strong antibacterial activity against Gram-positive bacteria.[1—4] The biosynthetic pathway to the macrolactone system in such macrolide antibiotics has not yet been established. Recently, Hutchinson *et al.* reported that *N*-acetylcysteamine thioesters of (2*R*,3*R*)-3-hydroxy-2-methylpentanoic acid and (2*R*,3*R*)-5-hydroxy-2,4-dimethylhept-2-enoic acid were incorporated into tylactone (an aglycone of tylosin), and proposed a mechanism for carbon chain assembly in tylactone biosynthesis.[5] In our mutagenic studies on *Micromonospora griseorubida* sp. nov, we have obtained 5-hydroxy-4-methylhept-2-enoic acid (**1**), 7-hydroxy-6-methylnona-2,4-dienoic acid (**2**), and 9-hydroxy-8-methylundeca-4,6-diene-3-one (**3**), considered to be biosynthetic intermediates for formation of the macrolactone protomycinolide IV (**7**),[6] and we describe here the isolation and identification of these compounds.

Compound (**1**) was extracted with diethyl ether at pH 3 from the culture filtrate of a mutant which cannot produce the macrolactone, and was readily transformed into its methyl ester (**4**) by treatment with (trimethylsilyl)diazomethane in methanol–benzene (2:8). It was purified by preparative h.p.l.c. (YMC-Gel ODS; 5 μm) in MeOH–H_2O (4:6). Compounds (**2**) and (**3**) were extracted with ethyl acetate at pH 3 from the culture filtrate of a mycinamicin producer and the residue from the organic extract was separated by chromatography on silica gel. Treatment of the acid (**2**) with (trimethylsilyl)diazomethane yielded its methyl ester (**5**). Compounds (**5**) and (**3**) were purified by reversed-phase preparative h.p.l.c. and silica gel chromatography, respectively. The physicochemical properties of compounds (**3**)—(**5**), are given in Table 1. The ^1H and ^{13}C n.m.r. spectral data for these compounds are compared with those of the macrolide (**7**) in Tables 2 and 3, respectively.

The molecular formula of (**4**) was determined to be

Table 1. Physicochemical properties.

Compound	(4)	(5)	(3)
Appearance	Colourless oil	Colourless oil	Colourless oil
Formula	$C_9H_{16}O_3$	$C_{11}H_{18}O_3$	$C_{12}H_{20}O_2$
$[\alpha]_D^{25}$ (MeOH)	−45.9°	−5.2°	−6.3°
(*c*)	(1.01)	(1.00)	(0.86)
λ_{max}/nm (MeOH)	212	261	275
(log ε)	(4.12)	(4.29)	(4.37)
ν_{max}/cm^{-1}	3440, 1725, 1710, 1660	3460, 1720, 1640, 1620	3450, 1660 1635, 1600

Table 2. ^{1}H N.m.r. chemical shifts[a] ($CDCl_3$; δ values).

Proton	(4)	(5)	(3)	(7)
2				5.77 d
3				6.59 dd
4				2.52 qdd
5				3.29 dd
6				1.28 qdd
7				1.49 m
8			2.58 q	2.55 qd
10		5.83 d	6.12 d	6.22 d
11		7.28 dd	7.15 dd	7.10 dd
12	5.87 d	6.23 dd	6.24 dd	6.08 dd
13	6.95 dd	6.11 dd	6.15 dd	5.71 dd
14	2.44 qdd	2.37 qdd	2.38 qdd	2.30 qdd
15	3.49 ddd	3.42 ddd	3.43 ddd	4.65 ddd
16a	1.56 qd	1.55 qd	1.55 qd	1.82 qd
16b	1.40 qd	1.41 qd	1.43 qd	1.54 qd
17	0.97 t	0.96 t	0.97 t	0.94 t
18				1.12 d
19				1.00 d
20			1.11 t	1.18 d
21	1.09 d	1.09 d	1.10 d	1.09 d
OCH_3	3.74 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.

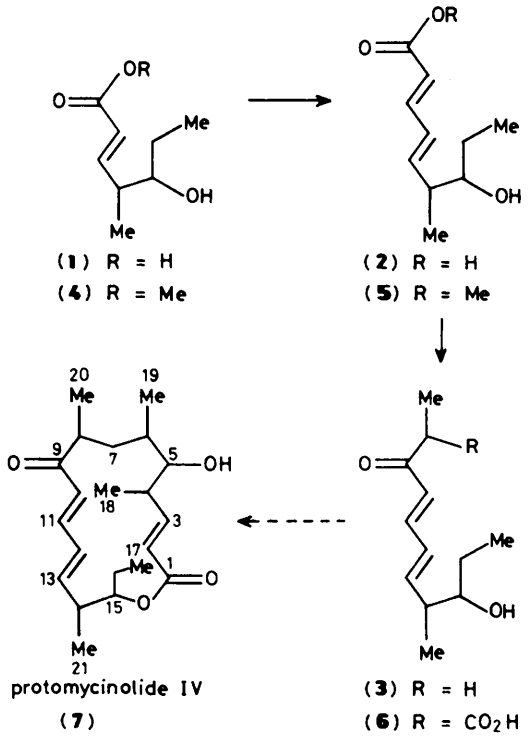

Table 3. ^{13}C N.m.r. chemical shifts[a] ($CDCl_3$; δ values).

Carbon	(4)	(5)	(3)	(7)
1				166.3 s
2				121.3 d
3				151.1 d
4				40.4 d
5				80.2 d
6				33.7 d
7				31.6 t
8			33.6 t	44.8 d
9		167.6 s	201.4 s	203.4 s
10		119.6 d	128.5 d	122.9 d
11	167.0 s	144.9 d	142.3 d	142.2 d
12	121.1 d	129.1 d	129.8 d	131.7 d
13	151.4 d	145.6 d	146.1 d	145.3 d
14	42.2 d	42.8 d	42.9 d	43.4 d
15	75.9 d	76.4 d	76.5 d	76.3 d
16	27.4 t	27.4 t	27.5 t	24.7 t
17	10.3 q	10.0 q	10.0 q	9.6 q
18				19.4 q
19				17.4 q
20			8.3 q	17.7 q
21	14.0 q	16.4 q	16.5 q	15.7 q
OCH_3	51.5 q	51.5 q		

[a] Recorded at 100 MHz; assignments were made on the basis of ^{1}H–^{13}C chemical shift correlated two-dimensional n.m.r.

$C_9H_{16}O_3$ from high resolution chemical ionization mass spectrometry (h.r.c.i.m.s.) [(*M* + H)$^+$, 173.1164; calc. for $C_9H_{17}O_3$: 173.1175]. The presence of an α,β-unsaturated ester was suggested by the u.v. absorption maximum at 212 nm (MeOH) and the i.r. spectrum showed unsaturated ester (1725, 1710, and 1660 cm^{-1}) and hydroxy (3440 cm^{-1}) absorption. Signals for two olefinic protons were observed at δ_H 5.87 (1H, d, *J* 16.1 Hz) and 6.95 (1H, dd, J_1 16.1, J_2 7.8 Hz) in the n.m.r. spectrum, which indicate *E* geometry. From these results, the compound was identified as methyl 5-hydroxy-4-methylhept-2-enoate (**4**).

The molecular formula of (**5**), $C_{11}H_{18}O_3$, was established by h.r.c.i.m.s. [(*M* + H)$^+$, 199.1336; calc. for $C_{11}H_{19}O_3$: 199.1334]. The u.v. spectrum suggested the presence of an α,β,γ,δ-unsaturated ester (261 nm). The i.r. spectrum showed unsaturated ester (1720, 1640, and 1620 cm^{-1}) and hydroxy (3460 cm^{-1}) absorption. The ^{1}H n.m.r. spectrum revealed the presence of two *C*-methyl, one *O*-methyl, one methylene, and two methine groups, and four olefinic protons. The coupling constants indicated *E*,*E*-geometry. The ^{13}C n.m.r. spectrum supported this conclusion. Accordingly, the compound was identified as methyl 7-hydroxy-6-methylnona-2,4-dienoate, (**5**), having a carbon chain two units longer than the ester (**2**).

Compound (**3**) was isolated from the fermentation broth as a neutral component. The molecular formula was determined to be $C_{12}H_{20}O_2$ from h.r.c.i.m.s. [(*M* + H)$^+$, 197.1536; calc. for $C_{12}H_{21}O_2$: 197.1538]. The u.v. absorption maximum (275 nm) suggested the presence of an α,β,γ,δ-unsaturated ketone. In the i.r. spectrum absorptions characteristic of an unsaturated ketone (1660, 1635, and 1600 cm^{-1}) and a hydroxy group (3450 cm^{-1}) were found. In the ^{1}H n.m.r. spectrum ethyl signals were observed at δ_H 1.11 (3H, t) and 2.58 (2H, q). The ^{1}H and ^{13}C n.m.r. spectra were similar to those of (**5**) except for signals due to a ketone carbonyl and an ethyl group. Therefore the compound was identified as 9-hydroxy-8-methylundeca-4,6-dien-3-one (**3**), which is considered to be the decarboxylation product of 9-hydroxy-2,8-dimethyl-3-oxoundeca-4,6-dienoic acid (**6**). An analogous

compound, 9-hydroxy-6,8-dimethylundeca-4,6-diene-3-one, has been obtained from a contaminated fermentation of a tylactone producer.[7]

These new compounds (**1**)—(**3**) are considered to be fragments of (**7**), corresponding to carbon atoms 11—15, 9—15, and 8—15 (with substituents), respectively. Their isolation may support the Hutchinson's hypothesis because they were isolated from the fermentation broth. In order to clarify further the biosynthesis of the macrolactone system in mycinamicins, we are attempting to isolate other biosynthetic precursors, and to establish the absolute configurations of these intermediates.

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