16-MEMBERED LACTONE COMPOUNDS FROM IZENAMICINS-PRODUCING MICROORGANISM

KENICHI YASUMURO*, MITSUYOSHI SHIBAZAKI, TOSHIO SASAKI, HARUMITSU IMAI, HIROSHI YAMAGUCHI, KENICHI SUZUKI and MOTO MORIOKA

Drug Serendipity Research Laboratories, Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd., 1-1-8 Azusawa, Itabashi-ku, Tokyo 174, Japan

YUKIHIRO TAKEBAYASI

Molecular Chemistry Research Laboratories, Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd., 21, Miyukigaoka, Tsukuba, Ibaraki 305, Japan

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Previously, we reported a series of 16-membered macrolide antibiotics, designated izenamicins, produced by *Micromonospora* sp. YS-02930K[1]. In the course of screening for intermediates of izenamicins, we obtained five 16-membered lactone compounds and examined the biosynthetic pathways of izenamicins B_2 and B_3 after lactone formation. In this paper we describe the isolation, characterization and bioconversion of lactone moieties.

In our studies of intermediates of izenamicins, we investigated the fermentation products of izenamicins-producing microorganism, which showed neutral lipophilic properties and UV 280 nm absorptions associated with dienone system, to obtain precursors of izenamicins.

Micromonospora sp. YS-02930K was cultivated on a rotary shaker at 28°C for 9 days in 500-ml Erlenmeyer flasks, each containing 60 ml of medium consisting of 5% corn starch, 2% dry yeast, 0.2% $MgSO_4 \cdot 7H_2O$, 0.15% $CaCO_3$, and 0.001% $CoCl_2 \cdot 6H_2O$. The broth filtrate (3 liters) was adjusted to pH 7 and extracted with equal volume of EtOAc. The organic layer was concentrated and successively subjected to silica gel column chromatography on Wakogel C-200 (Wako Pure Chemical) using $CHCl_3$ - MeOH (19 : 1) as eluent. Each fraction was monitored by TLC on Kieselgel $60F_{254}$ (Merck) employing $CHCl_3$ - MeOH (9 : 1). The lactone moieties containing fractions were combined and finally purified by HPLC on Inertsil ODS (GL science) using 30% MeCN as effluent to afford 15-B (**1**; 92.3 mg) 15-C (**2**; 29.8 mg), M1-A-3 (**3**; 6.4 mg), M1-A-4 (**4**; 15.5 mg), and M1-A-5 (**5**; 9.5 mg), respectively.

Structure elucidations of these compounds were based on spectroscopic analyses, mainly NMR and MS spectra. Physico-chemical properties of these compounds were summarized in Table 1.

The major compound **1** was identified as 23-hydroxyprotylonolide, since the ^{13}C NMR spectrum was identical to that of 23-hydroxyprotylonolide reported previously[2], except for the assignment of C-3 and C-6 which were revised by interpretation of 2D NMR spectra. The assignments of ^{13}C NMR spectra were listed in Table 2. **1** showed the same optical rotation as reported[3] ($-17.3°$ observed for **1**; $-15.4°$ as reported), which was chemically transformed from tylosin. So, **1** was the same absolute stereochemistry as protylonolide.

The molecular formula of **2** was deduced from MS and NMR, containing one additional oxygen in comparison with that of **1**. The ^{13}C NMR spectrum of **2** was quite similar to that of **1**, except a oxymethine signal at 67.6 ppm where methylene signal at 22.6 ppm was observed in **1** (Table 2). The structure of **2** was finally determined by an extensive 2D NMR experiment as 19,23-dihydroxyprotylonolide.

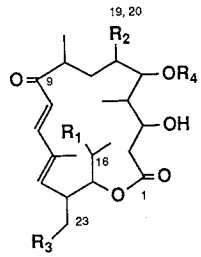
The structures of **3**, **4**, and **5** were also determined

Table 1. Physico-chemical properties of 16-membered lactone compounds.

	1	**2**	**3**	**4**	**5**
MW	410	426	396	410	410
Molecular formula	$C_{23}H_{38}O_6$	$C_{23}H_{38}O_7$	$C_{22}H_{36}O_6$	$C_{23}H_{38}O_6$	$C_{23}H_{38}O_6$
UV λ_{max} nm (ε)	284 (20,000)	285 (16,000)	282 (16,000)	283 (20,000)	283 (17,000)
$[\alpha]_D^{25}$ (c)	$-17.3°$ (0.5)	$-1.0°$ (1)	$-14.1°$ (0.1)	$-86.7°$ (0.1)	$-16.8°$ (0.1)
IR ν_{max} KBr cm^{-1}	3430, 2970, 2930, 1710, 1590, 1140, 990	3430, 2970, 2930, 1710, 1590, 1180, 990	3470, 2970, 2940, 1700, 1600, 1180, 990	3440, 2970, 2930, 1710, 1590, 1180, 980	3430, 2970, 2930, 1720, 1590, 1180, 990

UV spectra and optical rotations were measured in MeOH.

Fig. 1. Structures of 16-membered lactone compounds and izenamicins B_2 and B_3.



	R_1	R_2	R_3	R_4
1	H	CH_2CH_3	OH	H
2	H	$CH(OH)CH_3$	OH	H
3	H	CH_3	OH	H
4	OH	CH_2CH_3	H	H
5	H	$CH(OH)CH_3$	H	H
6	H	CH_2CHO	OH	Desosamine
7	H	CH_3	OH	Desosamine

Table 2. ^{13}C NMR data of 16-membered lactone compounds in $CDCl_3$ (δ, ppm).

Carbon	1	2	3	4	5
1	174.4	175.0	174.5	175.2	174.2
2	39.3	39.7	39.0	40.6	39.5
3	66.8	67.1	66.9	67.8	66.9
4	39.8	40.2	39.8	41.5	40.6
5	72.6	72.0	76.9	73.2	74.8
6	38.1	41.1	31.4	39.8	41.9
7	32.6	29.5	33.5	33.9	28.7
8	44.9	45.4	45.1	46.6	45.7
9	204.5	206.0	205.0	206.8	204.0
10	118.6	118.8	118.8	119.7	118.1
11	147.7	149.2	147.6	149.7	148.4
12	135.7	136.1	135.6	135.3	133.4
13	141.6	143.6	141.7	147.7	146.1
14	46.9	47.6	46.9	36.1	38.8
15	75.0	75.9	75.2	81.4	78.7
16	25.2	25.8	25.2	65.6	24.6
17	9.5	9.9	9.5	20.3	9.6
18	9.2	9.0	9.0	9.9	9.0
19	22.6	67.6	17.5	23.8	71.0
20	11.6	21.8	—	12.1	21.4
21	17.6	17.8	17.6	18.0	18.1
22	13.0	13.3	12.9	13.2	12.9
23	62.0	62.1	61.8	15.8	16.1

as 19-decarbonyltylonolide, 16-hydroxyprotylonolide, and 19-hydroxyprotylonolide, respectively, based on the comparison of ^1H and ^{13}C NMR spectra (Table 2) with those of **1** and the extensive 2D NMR experiments.

Among these 16-membered lactone compounds, **2**, **3**, and **4** are new compounds. **1** and **5** were previously obtained by microbial transformation from protylonolide[2], and **1** was also obtained by chemical transformation from tylosin[3]. However, this was the first evidence that **1** and **5** were isolated from fermentation products.

All five compounds were inactive against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Micrococcus luteus*, and *Candida albicans* at the concentration of 1 mg/ml by paper disc assay.

From the point of biosynthesis, we proposed that **1** and **3** were precursors of izenamicins B_3 (**6**) and B_2 (**7**), respectively. To examine biosynthetic pathways of **6** and **7**, bioconversion experiments, using novel fatty acid synthesis inhibitor cerulenin[4], were carried out.

Micromonospora sp. YS-02930K was cultured in a izenamicins production medium (5% corn starch, 2% dry yeast, 0.1% amylase, 0.15% $CaCO_3$, 0.002% $MgSO_4 \cdot 7H_2O$, 0.001% $CoCl_2 \cdot 6H_2O$). To the culture medium 40 μg/ml of cerulenin was added initially and at a 24-hour interval to prevent the *de novo* synthesis of the lactone moieties. After 72 hours of cultivation, 50 μg/ml of **1** was added and the cultivation was continued for further 86 hours, while monitoring izenamicins production by HPLC. As the result, **1** was converted to **6** in the yield 11%, so we concluded that **1** was an intermediate of **6**.

Bioconversion of **3** was carried out on the same condition mentioned above except 300 μg/ml of **3** was added as the substrate. In this case, only a little amount of **3** was converted to **7**, while much of **3** remained in the medium because of the poor production of **7** by *Micromonospora* sp. YS-02930 K. Recently, PUARL *et al.* proposed that 19-decarbonyl rosamicin would be generated from rosamicin by oxidation at C-20 and successive decarbonylation[5]. Though there may exist a pathway where **7** is produced from **6** as is the case with rosamicin, our studies demonstrated another pathway that 19-decarbonyl skeleton was formed before glycosylation.

The results of the studies described here revealed the biosynthetic pathway of izenamicins and will be useful for the studies for production of izenamicin B_3.

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