NEW 20-MEMBERED LACTONES, IRUMANOLIDES I AND II, PRODUCED BY A MUTANT OF STREPTOMYCES

Sir:

Irumamycin (**1**)[1], a 20-membered ring macrolide antibiotic active against phytopathogenic fungi, is produced by *Streptomyces subflavus* subsp. *irumaensis* AM-3603. The previous biosynthetic studies of irumamycin[2] showed the incorporation of ^{13}C-labeled acetate and propionate into the aglycone of irumamycin. In order to elucidate the biosynthetic pathway to irumamycin after the formation of aglycone, mutants blocked in the biosynthesis of the antibiotic were generated by the treatment of the irumamycin-producing strain *S. subflavus* subsp. *irumaensis* AM-3603 with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. A mutant, strain FN-114, was obtained which produced a mixture of two new 20-membered lactones named irumanolides I (**2**) and II (**3**). This paper describes the fermentation, isolation and characterization of the two compounds.

The lactone complex was produced in a 30-liter jar fermentor containing 20 liters of a medium consisting of 2% glycerol, 0.4% glucose, 1% soybean meal and 0.3% NaCl (pH 7.0 prior to sterilization). After inoculation, the culture was incubated at 27°C with agitation (250 rpm) and aeration (10 liters/minute) for 67 hours.

The harvested fermentation broth (15 liters) was extracted with ethyl acetate. After evaporation of the extract,the residue was washed with *n*-hexane, and subjected to column chromatography on silica gel with benzene - acetone (5: 1) as development solvent. The fractions giving a single spot on silica gel TLC were collected and concentrated to give 0.75 g of **2** and 1.75 g of **3** as white amorphous powders.

Physicochemical properties of **2** and **3** are listed in Table 1. The molecular formula of **2** was determined to be $C_{34}H_{54}O_7$ by high resolution mass spectrometry; observed, molecular ion *m/z* 574.385; calcd. *m/z* 574.386. The molecular formula of **3** was shown to be $C_{34}H_{56}O_8$; observed, molecular ion *m/z* 592.398; calcd. *m/z* 592.397. Elemental analyses and mass spectra of **2** and **3** suggest the absence of 3-*O*-carbamoyl-2-deoxyrhamnosyl moiety present in irumamycin molecule. The structures of **2** and **3** were de-

Table 1. Physicochemical properties of irumanolides I (**2**) and II (**3**).

	2	**3**
Appearance	White amorphous powder	White amorphous powder
MP (°C)	125	145
$[\alpha]_D^{25}$ (*c* 1, $CHCl_3$)	+140°	+133°
Elemental analysis (%) C	70.65	68.89
Elemental analysis (%) H	9.37	9.62
Molecular formula	$C_{34}H_{54}O_7$ (MW 574)	$C_{34}H_{56}O_8$ (MW 592)
Mass *m/z* M^+	574.385	592.398
UV λ_{max}^{MeOH} nm (ε)	232 (11850)	End absorption
IR ν_{max}^{KBr} cm^{-1}	3450, 2970~ 2920, 1700, 1670	3440, 2960~ 2920, 1705

Table 2. ^{13}C NMR chemical shifts for irumanolides I (**2**), II (**3**) and irumamycin (**1**).

Carbon No.	Chemical shift δ (ppm)		
	2	**3**	**1**
C- 1	173.8 s[a]	173.9 s	173.8 s
2	43.6 t	43.6 t	43.6 t
3	94.3 s	94.3 s	94.4 s
4	35.3 t	35.2 t	35.4 t
5	117.2 d	117.2 d	117.2 d
6	133.4 s	133.3 s	133.3 s
7	80.3 d	80.3 d	80.3 d
8	135.1 s	135.0 s	135.2 s
9	129.9 d	129.9 d	129.7 d
10	27.2 t	27.3 t	27.2 t
11	26.4 t	26.5 t	26.1 t
12	37.5 t	37.4 t	35.6 t
13	75.1 d	75.1 d	82.6 d
14	135.9 d	135.7 d	134.6 d
15	135.1 d	135.2 d	134.6 d
16	42.2 d	42.2 d	42.3 d
17	78.1 d	77.9 d	77.8 d
18	34.6 d	34.8 d	34.9 d
19	82.1 d	82.2 d	81.9 d
20	32.9 d	32.8 d	32.1 d
21	39.7 t	36.2 t	36.1 t
22	31.4 d	32.8 d	30.7 d
23	147.9 d	77.7 d	66.4 d
24	135.4 s	48.5 d	64.6 s
25	203.4 s	217.3 s	211.5 s
26	30.5 t	37.2 t	28.9 t
	19.2 q	19.2 q	19.2 q
	19.2 q	17.4 q	17.9 q
	17.1 q	16.0 q	17.3 q
	16.4 q	14.4 q	17.1 q
	11.5 q	12.3 q	16.0 q
	10.8 q	10.8 q	13.0 q
	8.8 q	7.6 q	10.8 q
	5.5 q	5.7 q	7.4 q
			5.6 q

[a] Multiplicities in the off-resonance: s; singlet, d; doublet, t; triplet, q; quartet.

duced from the ^{13}C NMR analysis. Table 2 shows chemical shifts of ^{13}C NMR spectra of **2** and **3** together with those of **1**[3] for comparison. The signals at δ 98.7 due to an anomeric carbon (C-1′) and at δ 64.6 and 66.4 due to epoxide carbons (C-24 and 23), which are observed in the spectrum of **1**, were not seen. On the contrary, olefinic carbons at δ 147.9 (tertiary, C-23) and δ 135.4 (quaternary, C-24) appeared in **2**. The carbon bonded to oxygen with a signal at δ 77.7 (tertiary, C-23) and the methine carbon at δ 48.5 (tertiary, C-24) were observed in **3**. Signals of the other carbons of **2** and **3** remained virtually unchanged as compared with those of **1**, with respect to carbons 1 to 22. These data suggest that irumanolides I and II possess the fundamental carbon skeleton of the aglycone of irumamycin, as proposed in Fig. 1. Irumanolides I and II have weak activity against *Sclerotinia cinerea* and *Piricularia oryzae* (Table 3).

Fig. 1. Structures of irumamycin (**1**), irumanolides I (**2**) and II (**3**).

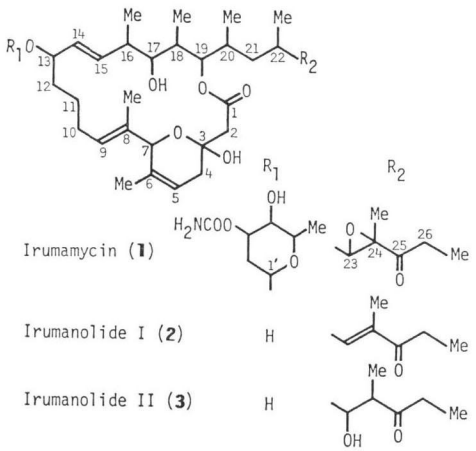

Table 3. Antifungal spectra of irumanolides I (**2**) and II (**3**).

Test organism	MIC (μg/ml)[a]	
	2	**3**
Candida albicans	>100	>100
Saccharomyces sake	>100	>100
Cryptococcus neoformans	>100	>100
Aspergillus niger	>100	>100
Sclerotinia cinerea	25	50
Piricularia oryzae	1.56	50
Botrytis cinerea	>50	>50
Mucor racemosus	>50	>50
Microsporum gypseum	>50	>50
Trichophyton interdigitale	50	50

[a] Potato - glucose agar, 27°C, 44 hours.

The time course study of irumanolide production revealed that component **3** was accumulated earlier than **2** in the culture broth. Further, when added to a culture of the parent strain AM-3603, compounds **2** and **3** were bioconverted to irumamycin in the presence of 80 μg/ml of cerulenin, an inhibitor of *de novo* aglycone biosynthesis.[4,5] These results indicate that irumamycin was biosynthesized from irumanolide II *via* irumanolide I, followed by attachment of sugar moiety and epoxidation.

Successful use of aglycones has been reported for the biosynthesis[5~8] and the elaboration of new derivatives[9] of 14- and 16-membered macrolide antibiotics. Experiments are now in progress using irumanolides I and II and the producing mutant for clarifying detailed biosynthetic mechanisms and regulation of irumamycin production.

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References

1) ŌMURA, S.; Y. TANAKA, A. NAKAGAWA, Y. IWAI, M. INOUE & H. TANAKA: Irumamycin, a new antibiotic active against phytopathogenic fungi. J. Antibiotics 35: 256~257, 1982
2) ŌMURA, S.; A. NAKAGAWA & Y. TANAKA: New macrocyclic antibiotics, irumamycin and hitachimycin (stubomycin). *In* Trends in Antibiotic Research. *Eds.* H. UMEZAWA, A. L. DEMAIN, T. HATA & C. R. HUTCHINSON, pp. 135~145, Japan Antibiotics Res. Assoc., Tokyo, 1982
3) ŌMURA, S.; A. NAKAGAWA & Y. TANAKA: Structure of a new antifungal antibiotic, irumamycin. J. Org. Chem. 47: 5413~5415, 1982
4) ŌMURA, S.: Cerulenin. *In* Methods in Enzy-

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mology. *Ed.* J. M. LOWENSTEIN, Vol. 72, pp. 520～532, Academic Press, 1981

5) ŌMURA, S.; N. SADAKANE & H. MATSUBARA: Bioconversion and biosynthesis of 16-membered macrolide antibiotics. XXII. Biosynthesis of tylosin after protylonolide formation. Chem. Pharm. Bull. 30: 223～229, 1982
6) HUNG, P. P.; C. L. MARKS & P. L. TARDREW: The biosynthesis and metabolism of erythromycins by *Streptomyces erythreus*. J. Biol. Chem. 240: 1322～1326, 1965
7) FURUMAI, T. & M. SUZUKI: Studies on the biosynthesis of basic 16-membered macrolide antibiotics, platenomycins. III. Production, isolation and structures of platenolides I and II. J. Antibiotics 28: 783～788, 1975
8) ŌMURA, S.; C. KITAO & H. MATSUBARA: Isolation and characterization of a new 16-membered lactone, protylonolide, from a mutant of tylosin-producing strain, *Streptomyces fradiae* KA-427. Chem. Pharm. Bull. 28: 1963～1965, 1980
9) ŌMURA, S.; N. SADAKANE, Y. TANAKA & H. MATSUBARA: Chimeramycins: New macrolide antibiotics produced by hybrid biosynthesis. J. Antibiotics 36: 927～930, 1983