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단신 (Notes)

New Macrolactone Secondary Metabolites Produced from a Strain of *Cytophaga* sp.

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is the major cause of nosocomial infections which have become a serious clinical problem during the past decade.[1–3] Glycopeptides such as vancomycin and teicoplanin are currently the agents of choice for the treatment of MRSA infection.[4] However, they suffer from a number of drawbacks, including a relative high failure rate, poor tissue penetration, serious adverse effects (notably nephrotoxicity and neutropena) and unpredictable synergy with other antimicrobial agents.[5] In addition, the first isolation of a strain with a diminished susceptibility to vancomycin from hospitalized patents in Japan has been reported.[6] Therefore, with the increasing prevalence of MRSA and the prospect of multiple resistant strains, the need for alternative anti-MRSA antibiotics becomes more urgent. In the course of our screening program for new antibiotics against MRSA, we found that a bacterial strain belonging to *Cytophaga* species isolated from a loess collected in Korea produced four macrolactone class of secondary metabolites, compound **1**–**4** (*Fig.* 1).

The compound **1**–**4** are all colorless amorphous solid in their appearance and have pretty similar physic-chemical properties such as TLC behavior and their solubilities in several solvents. Four compounds show a same TLC behavior (Rf 0.67 on Si $60F_{254}$ in elution condition of CH_2Cl_2–MeOH (=10:1, v/v)) and could be isolated into each component only with HPLC (*Fig.* 2). The structures of four compounds were determined based on spectroscopic analysis (UV, ^{1}H-, ^{13}C-, DEPT, ^{1}H-^{1}H COSY, HMQC, HMBC, and FAB-MS). FAB-mass spectra of four compounds exhibit the same molecular ion peak, i.e. $[M+Na]^+$, and its mass value (m/z) is found 563.1. In the UV spectra, the compound **1** and **2** show the characteristic UV absorption maxima of a conjugated tetraene at 288, 301, 315 nm, and the compound **3** and **4** show those of a conjugated triene at 263, 279, 287 nm (*Fig.* 2).[7] These observation and spectroscopic data would strongly suggest that these compounds are most likely to be of isomeric relationship. The partial structures of compound **3** (A, B, C, E and D) were determined by its ^{1}H-^{1}H long range couplings in HOMOCOSY spectrum (*Fig.* 3), and the connection of the partial skeletons was clarified by the analysis of HMBC spectrum and ^{1}H-^{1}H long range couplings in HOMOCOSY spectrum as shown in *Fig.* 1. The H-21/C-1 HMBC correlation provides strong evidence on the existence of macrocyclic lactone ring. And the connection of the isolated double bond at C-9 and C-33 to the ring was confirmed by the analysis of the long-range correlations in ^{1}H-^{1}H COSY. The carbon multiplicities observed in DEPT spectrum of compound **3** meets the carbon types required for the proposed structure of compound **3**, i.e. two methyl carbons at 17 and 20 ppm, two terminal methylene carbons at 113 and 115 ppm, three quaternary carbons at 132, 145, 170 ppm, and five alcoholic carbons in range of 60–80 ppm (*Fig.* 4). The analysis of spectra of other compounds showed that four compounds comprise the same nucleus, i.e. macrocyclic lactone ring, at which the different aliphatic chains

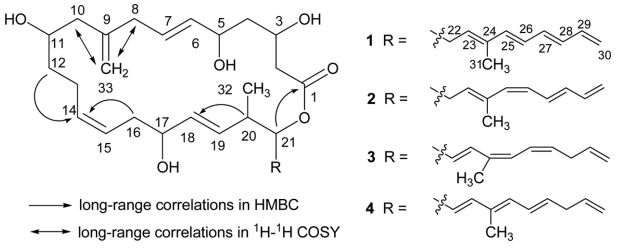

***Figure* 1.** Chemical structures of the macrolactones **1**–**4**.

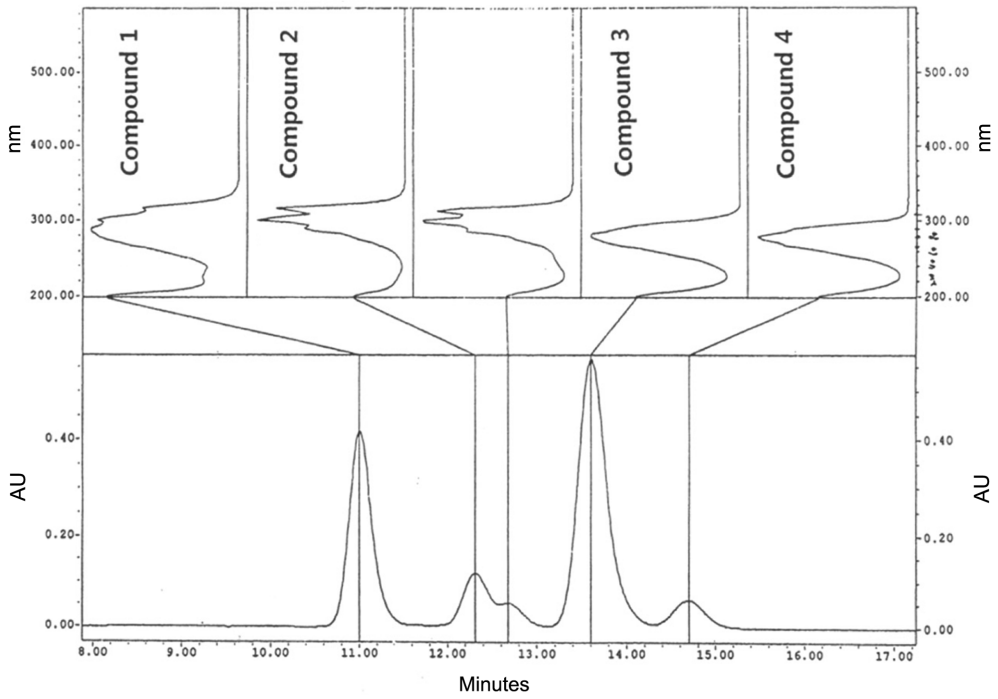

***Figure* 2.** HPLC profile and UV spectra of compound **1**–**4**.

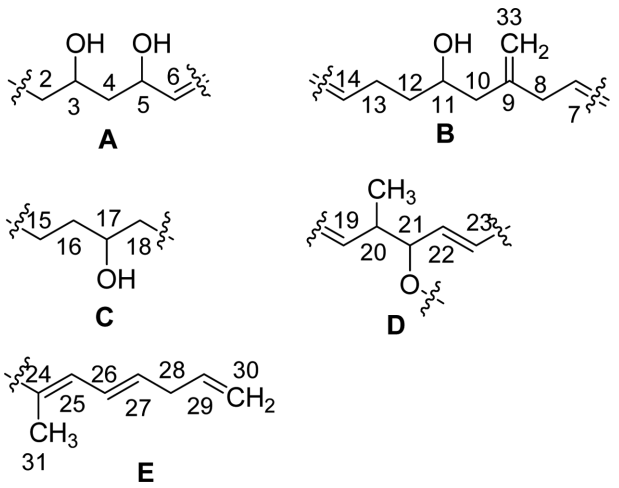

***Figure* 3.** The partial structures of compound **3** based on the analysis of ^{1}H-^{1}H long range couplings in HOMOCOSY spectrum.

are connected at C-21. This type of chemical structure is pretty different structure from the usual polyene macrolactones.[8] The assignment of signals from ^{1}H- and ^{13}C-NMR spectra of the nucleus of compound **3** are shown in *Table* 1, and the assignment of ^{1}H-NMR signals of the aliphatic chains of compound **1**–**4** is given in *Table* 2. One interesting observation to be worth to be mentioned is that the compound **3** under storage in refrigerator, dissolved in DMSO, converted to compound **1** in about 10 days, which was confirmed by HPLC and ^{1}H-NMR. This observation would be explained with tautomerization of compound **3** to **1**, and which process was proposed in *Fig*. 5. With the analysis of coupling constants of proton signals (*Table* 3) and with the aid of the putative isomerization pattern from compound **3** to **1**, the possible relative configuration of the double bonds along the chain, C-22~C-30 were determined (*Table* 3). Our intense literature survey showed that the compound **1** and **3** are identical to compound YL-02905-A and B, respectively which has been reported in a Japanese patent,[9] meanwhile the compound **2** and **4** were determined to be their respective new structural isomers. In the patent, the detection of these isomers was not reported, although the possibility of existence of other isomers was briefly mentioned. To clarify the entity of isomers **2** and **3** and accordingly their novelty, we examined the production of macrolactone isomers in three production media and their relative amounts by HPLC (*Table* 4). In A and B medium which we used in our experiment for main production, the isomer **2** and **4** are actually produced along with other two known isomers **1** and **3**, although the relative ratios of isomers produced are different and in relatively small amount (**2**: 6.0% and **4**: 9.2% in medium A; **2**: 22.0% and **4**: trace in medium B). However, in Y medium used by Japanese researchers in Yamanouchi Pharmaceutical Co., little or trace amounts of isomers **2** and **4** were detected. The relative proportion of four isomers was kept

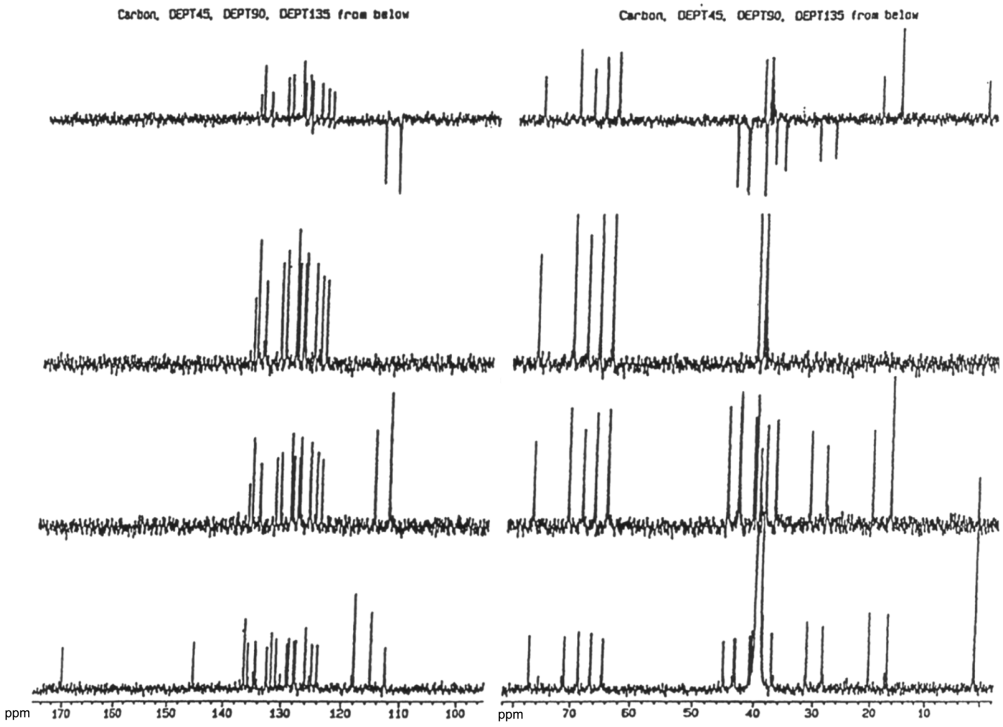

***Figure* 4.** DEPT spectrum of compound **3**.

***Table* 1.** ^{13}C (150 MHz)[a] and ^{1}H (600 MHz)[a] data of lactone ring of **3**

No.	C[b]	H[c]	No.	C[b]	H[c]
C1	170.17 s	–	C15	126.10 d	5.24 ddd
C2	43.32 t	2.29	C16	40.46 t	2.16
		2.35			2.02
C3	64.58 d	3.84	C17	71.25 d	3.84
C4	45.10 t	1.60	C18	134.59 d	5.37
		1.35	C19	131.06 d	5.37
C5	68.79 d	4.06	C20	40.84 d	2.35
C6	165.92 d	5.37	C21	77.46 d	5.17
C7	127.74 d	5.47	C31	20.41 q	1.86
C8	38.79 t	2.73 dd	C32	17.15 q	0.93
C9	145.48 s	–	C33	112.71 t	4.77 s
C10	43.17 t	2.17			4.73 s
		1.94	3-OH		3.79
C11	66.67 d	3.57	5-OH		4.68
C12	37.18 t	1.35	11-OH		4.25 d
C13	28.49 t	1.94	17-OH		4.68
C14	131.9 d	5.37			

[a]the spectra were taken in DMSO-d_6.
[b]150 MHz, chemical shifts in ppm, multiplicity.
[c]600 MHz, chemical shifts in ppm, multiplicity.

constant with little deviation through repeated isolation experiments. Considering the results above mentioned together, we concluded that two isomers (**2** and **4**) pro-

***Table* 2.** ^{1}H NMR data of aliphatic chains of **1**, **2**, **3**, and **4**

Compound	**1**	**2**	**3**	**4**
Number of carbon	Chemical shifts in ppm (δ_H, 600 MHz)			
21	4.70	4.70	5.17	5.11
22	2.30	2.30	5.64	5.57
23	5.30	5.30	6.81	6.79
25	5.99	6.65	6.29	5.92
26	6.09	6.36	6.53	6.56
27	6.49	6.32	5.47	5.70
28	6.31	6.36	2.95	2.88
29	6.43	6.36	5.83	5.85
30	5.29	5.29	5.05	5.08
	5.14	5.14	4.99	5.02
31	1.86	1.80	1.86	1.80

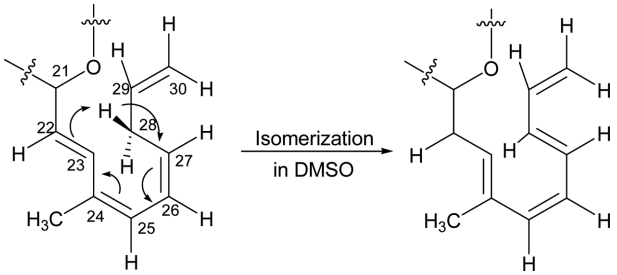

***Figure* 5.** The proposed isomerization of the compound **3** to **1** in DMSO.

duced in our culture media (especially in medium A) are new numbers of the family of macrocyclic secondary metabolites. The isomer, **1** and **2** possessing conjugated tetrene system in their aliphatic tail, interestingly show little bioactivity (MIC >200 ug/mL against *Staphylococcus aureus* KCTC 1927), while the isomer, **3** and **4** containing conjugated triene system separated by a methylene carbon at C-28 demonstrate moderate activity (MIC~10–20 ug/mL). This observation suggests that the conjugation pattern of double bonds along the aliphatic chain attached to the macrolactone at C-21 could play a critical role in antibacterial activity.

EXPERIMENTAL SECTION

Instruments

^{1}H- and ^{13}C-NMR spectra were taken on a Bruker DMX 600 NMR spectrometer using TMS as an internal standard. FAB mass spectra were recorded on a Krapos mass spectrometer using *m*-nitrobenzyl alcolhol as a matrix.

Fermentation and Isolation

The first seed-culture of the organism, prepared by incubation at 30 °C for 2 days on a reciprocal shaker (180 rpm) in Tryptic Soy Broth (TBS, Sigma, 50 mL) was inoculated into the same medium and cultured under the same culture condition for the second seed-culture. The production culture medium (20 liters) was chosen among the three media (*Table* 4), which was cultured on a jar fermentor for 4 days at 30 °C with an air flow of 2–3 liters per minute and agitation rate of 175 rpm. The harvested cells were extracted with MeOH, and the extract was chromatographed on a column packed with silica gel which was eluted with CH_2Cl_2–MeOH (30:1, v/v) to give a mixture of four macrolactone class of compounds **1**–**4**. Each of the components was finally purified by reverse-phase HPLC (Inertsil ODS-2, 5 μm, i.d. 4.6×150 mm: solvent system: CH_3CN–H_2O = 1:1 (v/v): detection: UV at 275 nm).

***Table* 4.** The ratios of isomers produced according to production media

Medium	Isomer (%)			
	1	**2**	**3**	**4**
A[a]	23.9	6.0	60.9	9.2
B[b]	32.5	22.0	45.5	trace
Y[c]	21.8	trace	77.1	1.1

[a]TSB, sigma.

[b]Lactose 1.6%, glucose 1.6%, soy bean flour 1.2%, malt extract 1.2%, casamino acid 1.2%, KH_2PO_4 0.2%, $MgSO_4$ 0.1% and trace amount of mineral salts solution ($CaCl_2{\cdot}2H_2O$ 5 ppm, $FeSO_4{\cdot}7H_2O$ 5 ppm, $MnSO_4{\cdot}4H_2O$ 5 ppm, $ZnSO_4{\cdot}7H_2O$ 5 ppm, $CuSO_4{\cdot}5H_2O$ 1 ppm and $CoCl_2{\cdot}6H_2O$ 1 ppm).

[c]Glucose 1.0%, potato starch 2.0%, yeast extract 0.5%, polypeptine 0.5% and $CaCO_3$ 0.4%.

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