GERI-155, a New Macrolide Antibiotic Related to Chalcomycin

Sir:

In the course of screening new antibiotics from soil microorganisms, we have isolated GERI-155 as a new naturally occurring antibiotic. GERI-155 shows antimicrobial activities against Gram-positive bacteria. In addition, two known compounds, chalcomycin[1,2] and aldgamycin E[3,4], were also detected in the same culture broth. This communication describes the isolation, physico-chemical properties, structure and antibacterial activity of GERI-155 (Fig. 1).

The producing organism, *Streptomyces* sp. GERI-155, was isolated from a soil sample collected in Cheolwon-gun, Kangwon-do, Korea, and deposited at the Korea Research Institute of Bioscience and Biotechnology, Korean Collection for Type Cultures, under the accession number KCTC-0041BP.

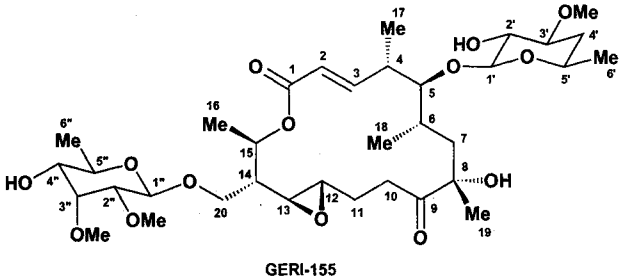
The spore suspension of *Streptomyces* sp. GERI-155 from agar slant culture was inoculated into a seed culture medium. The inoculated flask was shaken on a rotary shaker at 28°C for 48 hours. The seed culture (300 ml) was transferred to 30-liter fermenter containing 18 liters of the medium. The seed and production medium consisted of glucose 2%, soluble starch 1%, meat extract 0.1%, yeast extract 0.4%, soybean meal 2.5%, NaCl 0.2%, and K_2HPO_4 0.005%. The pH of the medium was adjusted to 7.3 before autoclaving. The fermentation was continued for 72 hours at 28°C with agitation at a rate of 200 rpm and aeration of 20 liters per minutes.

The culture filtrate was extracted with ethylacetate. The organic layer was evaporated to dryness *in vacuo* and the remaining oily residue was chromatographed on a silica gel column with 1 ~ 50% methanol in chloroform. The active eluate was concentrated and subjected to a Lobar column (Merck, Lichroprep Si 60, Art. 10401, Germany) and eluted with a gradient mixture of chloroform - acetone, from 10 : 1 to 1 : 1. Three active fractions were successively eluted containing aldgamycin E (fraction I), chalcomycin (fraction II), and GERI-155 (fraction III). Fraction III was further purified by Sephadex LH-20 column chromatography eluting with acetone, followed by final purification on HPLC using a reverse phase column (Senshu Pak C_{18}, i.d. 10 mm × 300 mm, Japan) eluting isocratically with 55% aqueous methanol. The active eluate was concentrated *in vacuo* and the resulting aqueous suspension was lyophilized to give 15.9 mg of GERI-155 as a white powder.

Physico-chemical properties of GERI-155 are summarized in Table 1. It is soluble in acetone, methanol, and chloroform, but insoluble in water or hexane. After TLC on silica gel 60 F_{254} (Merck) with chloroform - acetone (1 : 1), GERI-155 showed an Rf value of 0.21 whereas chalcomycin and aldgamycin E had Rf values of 0.25 and 0.44, respectively. GERI-155 was visualized as a red spot after spraying with vanillin/sulfuric acid reagent [3% (w/v) vanillin in MeOH - H_2SO_4 (97 : 2.4, v/v)] and heating to 80°C for 10 minutes, whereas chalcomycin and aldgamycin E gave purple and yellow spots, respectively. GERI-155 has a molecular formula $C_{35}H_{58}O_{14}$ determined by high resolution FAB-MS *m/z* 703.3923 $(M+H)^+$ (calcd, 703.3905) in combination with the ^{13}C NMR data. The UV maximum at 217 nm suggested the presence of α,β-unsaturated carbonyl group, also supported by the IR absorption band at 1700 cm^{-1}.

The structure elucidation of GERI-155 was completed by NMR spectroscopic studies. ^1H and ^{13}C NMR spectra of GERI-155 were very similar to those of the coisolated chalcomycin. In comparison of ^{13}C NMR data of GERI-155 with those of chalcomycin,[2] chemical shifts of all carbon signals except for C-9, C-10 and C-11 signals of GERI-155 were in agreement with those of chalcomycin. The carbon resonance of C-9 exhibited a down field shift from 199.4 ppm of α,β-unsaturated

Fig. 1. Structures of GERI-155 and chalcomycin.



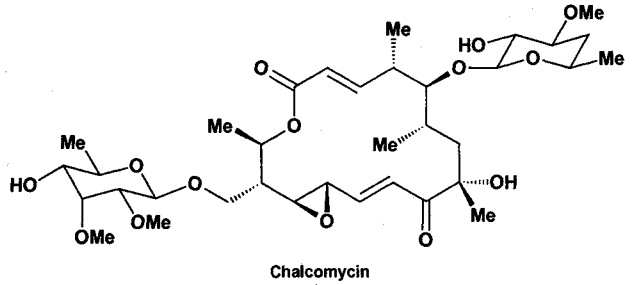


Table 1. Physico-chemical properties of GERI-155.

Appearance	White powder
MP (°C)	98.5 (dec)
$[\alpha]_D^{23}$ (MeOH)	−75.5° (*c* 0.1)
FAB-MS (*m/z*)	703 $(M+H)^+$
HR-FAB-MS (*m/z*)	
Found:	703.3923 $(M+H)^+$
Calcd:	703.3905
Molecular formula	$C_{35}H_{58}O_{14}$
UV (MeOH) λ_{max}^{MeOH}nm (ε)	217 (18,350), 290 (s)
IR($CHCl_3$) ν cm^{-1}	3580, 2950, 1700
TLC (Rf)*	0.21

* Merck, Kieselgel 60 F_{254}, Art. 5715: chloroform - acetone (1 : 1).

Table 2. ^{13}C and ^{1}H NMR data for GERI-155 in $CDCl_3$.

No.	^{13}C (150 MHz)	^{1}H (600 MHz)
1	165.6	—
2	121.1	5.86 d (15.2)
3	151.4	6.74 dd (15.2, 10.7)
4	41.8	2.75 ddq (10.7, 9.8, 6.0)
5	87.4	3.27 br d (9.8)
6	34.3	1.30 br dd (11.2, 5.9)
7	37.0	1.92 dd (14.9, 5.9), 1.89 dd (14.9, 11.2)
8	79.6	—
9	212.7	—
10	32.6	2.71 ddd (12.7, 12.7, 3.9), 2.14 ddd (12.7, 12.7, 4.8)
11	27.3	2.01 dddd (13.1, 12.7, 3.9, 3.4), 1.55 dddd (13.1, 12.7, 8.5, 4.8)
12	59.3	2.74 ddd (8.5, 3.4, 1.9)
13	58.0	2.83 ddd (8.8, 1.9)
14	48.6	1.35 dddd (10.8, 8.8, 3.4, 2.4)
15	69.8	5.33 dq (10.8, 5.8)
16	18.5	1.35 d (5.8)
17	18.3	1.24 d (6.0)
18	18.8	1.00 d (6.9)
19	28.2	1.38 s
20	67.1	4.16 dd (10.3, 3.4), 3.63 dd (10.3, 2.4)
1′	103.2	4.25 d (7.3)
2′	75.0	3.33 dd (8.8, 7.3)
3′	80.4	3.23 ddd (11.7, 8.8, 4.9)
4′	36.7	2.05 ddd (13.2, 4.9, 1.5), 1.23 ddd (13.2, 11.7, 11.2)
5′	67.8	3.49 ddq (11.2, 1.5, 6.0)
6′	20.9	1.23 d (6.0)
OMe-3′	56.7	3.42 s
1″	100.9	4.57 d (7.8)
2″	81.9	3.09 dd (7.8, 2.7)
3″	79.6	3.77 dd (3.0, 2.7)
4″	72.7	3.22 ddd (11.2, 8.8, 3.0)
5″	70.8	3.53 dq (8.8, 6.4)
6″	17.8	1.27 d (6.4)
OMe-2″	59.6	3.57 s
OMe-3″	61.7	3.62 s
OH-8	—	3.71 s
OH-2′	—	2.52 s
OH-4″	—	2.31 d (11.2)

Coupling constants (J values in Hz) are given in parentheses. TMS as internal standard.

Fig. 2. HMBC correlations of GERI-155.

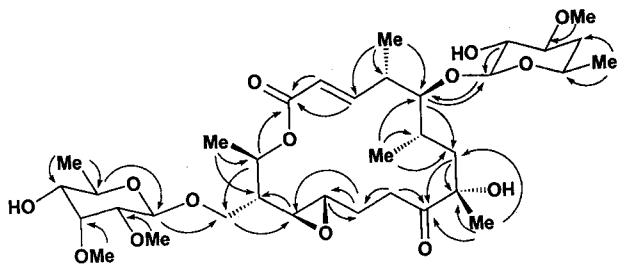


Table 3. Antibacterial activity of GERI-155.

Test organisms	MIC (μg/ml)[a]
Staphylococcus aureus giorgio	6.25
Staphylococcus aureus 209 P	6.25
Staphylococcus aureus SR 511	12.5
Micrococcus luteus ATCC 9341	0.78
Streptococcus pyogenes T 12 A	12.5
Streptococcus pyogenes 77 A	6.25
Streptococcus pyogenes 308 A	6.25
Streptococcus faecium MD 8b	0.78
Bacillus subtilus ATCC 6633	1.56
Bacillus cereus ATCC 9634	0.19
Bacillus anthracis	0.09
Escherichia coli 055	>50
Escherichia coli DC 0	>50
Escherichia coli DC 2	>50
Pseudomonas aeruginosa 9027	1.56
Pseudomonas aeruginosa 1592 E	0.19
Pseudomonas aeruginosa 1771 M	0.09
Salmonella typhimurium	0.09

[a] Agar dilution method using Mueller-Hinton agar.

ketone of chalcomycin to 212.7 ppm, and the C-10 and C-11 signals of GERI-155 appeared at 32.6 and 27.3 ppm, whereas those carbons of chalcomycin appeared at 124.5 and 145.8 ppm, respectively. From ^{13}C-NMR data and molecular formula, the structure of GERI-155 was speculated as to be hydrogenated analogue of chalcomycin at C-10,11 double bond. To confirm the NMR assignments (Table 2) and structure of GERI-155, ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY, DEPT, HMBC and spin decoupling spectra were measured. By analysis of ^{1}H-^{1}H correlation and coupling constants, the presence of partial structures of C-2 to C-7 and C-10 to C-16 portions and two sugar moieties of chalcose and mycinose was confirmed. By the HMBC correlations shown in Fig. 2, all connectivities of the partial structures and three quaternary carbons of C-1, 8 and 9, including the linkage positions of the sugar moieties were established. After the establishment of all ^{13}C NMR assignments of GERI-155 independently, we found that the assignments of C-6 (34.3 ppm) and C-7 (37.0 ppm) of GERI-155 were different from the reported assignments of chalcomycin[2], which were 36.7 and 33.9 ppm for C-6 and C-7, respectively. These assignments of chalcomycin should be revised. Based on the spectral data and the evidence of the coproduction of GERI-155 and chalcomycin, the structure of GERI-155 was determined to be 10,11-dihydrochalcomycin. Recently the absolute stereochemistry of chalcomycin was established by X-ray analysis.[5] The good similarity of ^{13}C NMR data of GERI-155 with chalcomycin except C-9, 10, and 11, together with similarity of the optical rotation value $[\alpha]_D = -75.5°$ (c 0.1, MeOH) of GERI-155 with the reported value [$-43.5°$ (c 1, EtOH)] of chalcomycin, suggested that the absolute stereochemistry of GERI-155 was the same as that of chalcomycin as depicted.

GERI-155 showed balanced antibacterial activity against both Gram-positive and Gram-negative bac-

teria except against *E. coli* as shown in Table 3. Its particularly strong antibacterial effect was found on *Bacillus anthracis*, *Pseudomonas aeruginosa* 1771 M, and *Salmonella typhimurium* among the strains tested.

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