ARIDICINS, NOVEL GLYCOPEPTIDE ANTIBIOTICS

III. PREPARATION, CHARACTERIZATION, AND BIOLOGICAL ACTIVITIES OF AGLYCONE DERIVATIVES

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The aglycone and two pseudoaglycones of aridicin A were prepared by selective hydrolysis and characterized, chemically and biologically. These new analogs demonstrate improved activities *in vitro* over the parent antibiotics against methicillin sensitive and resistant staphylococci. The major determinant of activity is the mannose substituent, the presence of which results in less potent compounds. The analogs have potent activity against enterococci.

In recent reports we described the isolation of the aridicin complex produced by *Kibdelosporangium aridum* (SK&F AAD-216, ATCC 39323), a member of a new genus of microorganisms[1,2]. The complex contains three major components which were shown to be novel members of the glycopeptide class of antibiotics[3], exemplified by vancomycin[4,5], teicoplanin[6], and ristocetin[7,8]. Initial degradation studies of aridicins A, B and C indicated that each contained mannose as the only neutral carbohydrate and a unique series of acidic glycolipids[9] containing a homologous series of acyl substituents. A similar series of glycolipids derived from glucosamine have recently been reported to be present in the teicoplanin complex[10,11]. The present study was undertaken to prepare for characterization and biological evaluation sufficient quantities of each of the aridicin aglycones in which the mannose and glycolipid were individually or collectively removed.

Materials and Methods

Chemical Methods

Analytical HPLC was performed on a Beckman 345 gradient HPLC system monitored at 220 nm with a Beckman Model 165 detector and an Altex CR1B integrator. Chromatography was run on a Beckman Ultrasphere ODS column (4.6×150 mm) fitted with a guard cartridge containing C-18 packing (Brownlee 18-GU). Phosphoric acid buffer (0.1 M) was prepared from Fisher HPLC grade phosphoric acid, 85% (6.77 ml/liter, adjusted to pH 3.2 with KOH). Acetonitrile was HPLC grade, (UV, Burdick and Jackson). Gradients were run from 18 to 33% acetonitrile at 1.5 ml/minute.

Isoelectric focusing was carried out on an LKB Multiphor using *Bacillus subtilis* for detection as previously described[12]. IR spectra were obtained using a Perkin-Elmer 299B spectrophotometer. UV spectra were determined using a Beckman DU-7 spectrophotometer. FAB-mass spectra were obtained on a VG-ZAB 1F-HF mass spectrometer equipped with a standard FAB ion source[13]. Thermogravimetric analyses (TGA) were carried out using a Perkin-Elmer TGS 2 instrument. Carbohydrate analyses and potentiometric titrations were carried out as previously described[2].

Preparation of Aglycone and Pseudoaglycones

Mannosyl-aglycone (II): A suspension of aridicin A (I, 1.2 g, 0.67 mmol) in 1,500 ml of ace-

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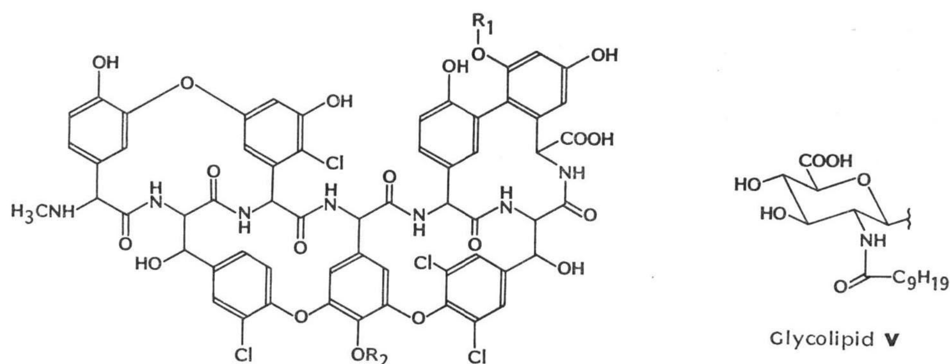

Compound	R_1	R_2
Aridicin A (I)	Mannose	Glycolipid
Mannosyl-aglycone (II)	Mannose	H
Pseudoaglycone (III)	H	Glycolipid
Aglycone (IV)	H	H

tonitrile - water (10%) was adjusted to pH 3 with HCl and refluxed for 48 hours until HPLC analysis indicated completion of hydrolysis. The mixture was lyophilized and the residue chromatographed on Whatman Prep 40 ODS-3 (40~60 μm) dry packed in a glass column (25×500 mm, Beckman-Altex) using 18% acetonitrile in 0.1 N phosphoric acid, pH 3.2. Fractions containing the product were combined and concentrated *in vacuo* to remove acetonitrile. The aqueous solution was poured onto a column (600 ml) of Amberlite XAD-7 (Rohm and Haas), and the column washed with water (2 liters) and eluted with 50% aqueous acetonitrile. The acetonitrile eluate was lyophilized to yield HPLC-homogeneous **II** (832 mg, 85% yield): mp >300°C (dec); IR ν_{max}^{KBr} 3400, 1660, 1610, 1590, 1510, 1460, 1430, 1390, 1300, 1230, 1180, 1150, 1120, 1060, 1010, 970 and 810 cm^{-1}.

Anal Calcd for $C_{65}H_{55}N_7O_{24}Cl_4 \cdot 6H_2O$: C 49.79, H 4.31, N 6.25, Cl 9.04; TGA 6.9.
Found: C 49.72, H 4.37, N 6.12, Cl 8.59; TGA 7.8.

Aglycone (**IV**): A sample of aridicin A (2.8 g, 1.6 mmol), was dissolved in DMSO (57 ml) and concentrated hydrochloric acid (3 ml) and heated at 100°C for 15 minutes. The solution was immediately cooled to room temperature and mixed with 400 ml of 10% acetonitrile in 0.1 M pH 3.2 phosphate buffer and filtered. The filtrate was chromatographed as above with a step gradient of acetonitrile (10~30%) in 0.1 M pH 3.2 phosphate buffer. Fractions containing aglycone **IV** (HPLC analysis) were pooled, concentrated *in vacuo*, and desalted as above on XAD-7 resin. The eluate was lyophilized to yield aglycone **IV** as a white powder (420 mg, 21% yield for **IV**, pseudoaglycone **III** is also produced in this reaction in 40% yield, total aglycone yield is 61%): mp >300°C (dec); IR ν_{max}^{KBr} 3400, 1660, 1610, 1590, 1510, 1460, 1430, 1390, 1300, 1240, 1150, 1080, 1060 and 1010 cm^{-1}.

Anal Calcd for $C_{59}H_{45}N_7O_{19}Cl_4 \cdot 6H_2O$: C 50.40, H 4.09, N 6.97, Cl 10.09; TGA 7.6.
Found: C 50.27, H 4.23, N 6.78, Cl 9.37; TGA 7.7.

Pseudoaglycone (**III**): Fractions from the above column containing **III** (HPLC analysis) were pooled and desalted on XAD-7 to yield **III** as a white powder (1.1 g, 40% yield): mp >300°C (dec); IR ν_{max}^{KBr} 3400, 2920, 1660, 1590, 1500, 1460, 1430, 1300, 1240, 1140, 1080, 1060 and 1010 cm^{-1}.

Anal Calcd for $C_{75}H_{72}N_8O_{25}Cl_4 \cdot 6H_2O$: C 51.91, H 4.88, N 6.46, Cl 8.17; TGA 6.2.
Found: C 52.16, H 4.97, N 6.21, Cl 7.77; TGA 6.0.

Antibiotics

Vancomycin, gentamicin, erythromycin, and clindamycin were purchased from Sigma Co. Samples of aridicin A and its aglycones were adjusted to pH 7 with sodium bicarbonate and lyophilized prior to biological evaluation.

Bacterial Strains

Strains used in sensitivity testing were recent clinical isolates from the Smith Kline & French Laboratories culture collection.

Antimicrobial Activity

The minimum inhibitory concentrations (MIC's, μg/ml) and *in vivo* mouse protection studies using *Staphylococcus aureus* HH 127 were carried out as previously described[1].

Results

Preparation and Characterization of Aglycones

In earlier work[2,13], aridicin A (I) was demonstrated to have an empirical formula of $C_{81}H_{82}N_8O_{30}Cl_4$ based on high resolution fast atom bombardment mass spectrometry (FAB-MS) measurements (Table 1), coupled with combustion and ^{13}C NMR data. In preliminary studies, acid hydrolysis of aridicin A in 0.1 N HCl was found to yield a complex mixture of aglycone and pseudoaglycone derivatives which differed widely in polarity as evidenced by HPLC retention times (Table 1)[9]. In order to obtain sufficient quantities of each of the derivatives for detailed spectral and biological evaluation, improved hydrolytic conditions were developed. Mild acid hydrolysis of aridicins A, B or C, or a mixture of the complex, at pH 3 in aqueous solution effected conversion in high yield to a single mannose-containing pseudoaglycone derivative **II**. The FAB mass spectrum of HPLC-purified **II** exhibited a cluster at m/z 1,458 $(M+H^+)$ which is 329 amu smaller than aridicin A and corresponds to a formula of $C_{65}H_{55}N_7O_{24}Cl_4$. This change, 329 amu ($C_{16}H_{27}NO_6$), is consistent with the loss of glycolipid **V** of molecular weight 347 (329 + one molecule of water). A detailed description of the isolation and characterization of the unique acidic glycolipid fragment **V** from aridicin A has been previously described[9]. As expected for a conversion involving the loss of a lipid containing fragment, the product was substantially more polar than aridicin A on reversed-phase HPLC analysis. The loss of an acidic function was also in line with the shift in isoelectric point from 3.8 to 5.1 and the loss of the lowest *pKa* value at 3.0.

Further chemical degradation to remove the mannose from **II** required more stringent hydrolytic conditions, HCl in dry DMSO. The absence of carbohydrate from this reaction product indicated it to be the true aglycone **IV** of formula of $C_{59}H_{45}N_7O_{19}Cl_4$ based on its high resolution FAB-MS

Table 1. Physical properties of aridicin A and its aglycone derivatives.

	Aridicin A (I)	Mannosyl-aglycone (II)	Pseudo-aglycone (III)	Aglycone (IV)
Formula	$C_{81}H_{82}N_8O_{30}Cl_4$	$C_{65}H_{55}N_7O_{24}Cl_4$	$C_{75}H_{72}N_8O_{25}Cl_4$	$C_{59}H_{45}N_7O_{19}Cl_4$
FAB-MS $(M+H^+)$	1,787.394	1,458	1,625	1,296.155
	(1,787.397)[a]			(1,296.160)[a]
pI	3.8	5.1	3.8	5.1
HPLC Retention time[b]	9.6	4.1	10.1	5.3
UV $\lambda_{max}^{CH_3CN\text{-}H_2O}$ nm ($E^{1\%}_{1cm}$)				
pH 2	280 (51)	281 (80)	281 (63)	281 (83)
pH 12	301 (73)	300 (136)	302 (94)	302 (140)
pKa[c]	3.0, 4.9, 7.4,	3.3, 7.1, 8.3,	3.0, 4.3, 7.4,	3.3, 7.1, 8.4,
	8.4, 10.0, 10.3	9.1, 10.0, 11.2	8.5, 9.9, 10.9	9.2, 10.1, 11.4
$[\alpha]_D^{25}$	$-66°$	$-3.1°$	$-17°$	$-11.8°$
	(c 0.3, H_2O)	(c 1, DMSO)	(c 1, DMSO)	(c 1, DMSO)

[a] Calculated values. [b] Minutes. [c] Acetonitrile - H_2O, 3: 7.

Table 2. Antibacterial activity of aridicin A and its aglycone derivatives.

Strains	MIC (μg/ml)						
	I	II	III	IV	Vanco-mycin	Genta-micin	Erythro-mycin
Staphylococcus aureus HH 127 (MS)[a]	1.6	1.6	0.8	0.4	1.6	3.1	0.4
S. aureus 910 (MR)[b]	3.1	1.6	0.4	0.4	1.6	6.3	>200
S. epidermidis 2479 (MS)	6.3	3.1	0.8	0.8	1.6	0.4	0.4
Streptococcus haemolyticus 651 (MR)	50	12.5	3.1	3.1	1.6	0.2	200
Enterococcus faecalis 34358	0.8	1.6	0.4	0.8	3.1	50	>200
Listeria monocytogenes 2255	0.8	1.6	0.4	0.8	1.6	0.8	≤0.2
Proteus mirabilis 444	>100	100	100	25	100	3.1	200
Escherichia coli 12140	>100	>100	>100	100	100	6.3	50
Klebsiella pneumoniae 4200	>100	>100	>100	>100	>100	0.4	100
Pseudomonas aeruginosa 63	>100	>100	>100	>100	>100	6.3	200
Serratia marcescens ATCC 13880	>100	>100	>100	>100	>100	6.3	200
Morganella morganii 179	>100	>100	>100	>100	>100	0.8	50
Providencia sp. 276	>100	>100	>100	>100	>100	12.5	>200
Enterobacter cloacae 31254	>100	>100	>100	>100	>100	1.6	200
Salmonella gallinarum 595	>100	25	100	25	25	1.6	12.5
Bacteroides sp.	16	>32	16	32	32	—	—
Clostridium difficile	0.125	2	0.125	2	2	—	>32

[a] MS: Methicillin sensitive strains.

[b] MR: Methicillin resistant strains.

(m/z 1,296.155, calculated $M+H^+$, 1,296.160)[13]. The mass difference of 162 amu between **II** and **IV** indicates that only one hexose unit (mannose) is present in pseudoaglycone **II**. The removal of a polar mannose moiety increased the HPLC retention time of **IV** relative to that of **II** (Table 1). Although **II** could be used as a precursor for preparation of **IV**, more efficient production of the aglycone could be achieved by hydrolysis of the more readily available parent antibiotic.

A third derivative **III**, containing the aridicin A glycolipid but not mannose was also observed in the aridicin A DMSO hydrolysate. It was also isolated by preparative HPLC and characterized by FAB-MS (Table 1). This mass corresponds to a formula of $C_{75}H_{72}N_8O_{25}Cl_4$ consistent with a loss of a single mannose ($C_6H_{12}O_6$) from aridicin A. As expected, the HPLC retention time became longer with the removal of the polar mannose moiety (Table 1). The overall yield for the production of aglycones **III** and **IV** was approximately 60%. The ratio of the two products could be varied by changing the reaction time.

The structural interrelationships between aridicin A and its aglycone derivatives are summarized in the figure[14,15]. The fact that two pseudoaglycones and one aglycone can be prepared from aridicin A is consistent with the two carbohydrate moieties being independently attached to the aglycone nucleus in the parent antibiotic. The three aglycone derivatives were further characterized as described in the experimental section and Table 1 on the basis of their IR and UV spectra and potentiometric titration data. Furthermore, each preparation was homogeneous (>95%) on HPLC analysis and, when corrected for water content by TGA analysis, gave acceptable combustion analyses.

Antimicrobial Activity

The hydrolysis products, in common with other glycopeptides, showed activity mainly against Gram-positive bacteria. The removal of the mannose and/or glycolipid constituents had a marked effect on *in vitro* potency and spectrum of activity (Table 2). Compounds lacking the mannose (**III**, **IV**) were more active than those containing it (**I**, **II**). The most active compound was the true aglycone **IV** comprising the aridicin nucleus. This compound was markedly more active than both the parent **I** and vancomycin against the staphylococci and also inhibited selected Gram-negative bacteria, in-

Table 3. Activity of aridicin A and its aglycone derivatives against anaerobic bacteria[a].

Compound	Strains (No. tested)			
	Bacteroides sp. (5)	*Fusobacterium* (1)	*Clostridium perfringens* (3)	*C. difficile* (6)
I	16 (16~32)	32	≤0.016 (≤0.016~0.031)	0.125 (<0.016~0.5)
II	>32 (32~>32)	32	0.031 (≤0.016~0.5)	2 (≤0.016~2)
III	16 (8~16)	16	≤0.016 (≤0.016~1.125)	0.125 (≤0.016~0.25)
IV	32 (16~32)	32	≤0.016 (≤0.016~0.5)	1.5 (≤0.016~2)
Vancomycin	32 (32)	32	0.5 (0.5~1.0)	2 (2~4)
Clindamycin	0.25 (0.25~>32)	0.25	0.125 (0.125 only)	>32 (>32 only)

[a] Data given as median and range of MIC, μg/ml.

Table 4. Activity of aridicin A and its aglycone derivatives against *Staphylococcus aureus* HH 127 in mouse protection tests.

Compound	ED_{50} (mg/kg)
I	3.5
II	7.6
III	5.0
IV	7.6
Vancomycin	1.56

cluding *Proteus mirabilis, Salmonella gallinarum* and *Escherichia coli* at MIC's of 25~100 μg/ml.

Aridicin A and its aglycones were all more active than vancomycin against *Enterococcus faecalis,* pseudoaglycone **III**, containing only the glycolipid, being the most active (MIC 0.4 μg/ml; vancomycin MIC 3.1 μg/ml). The parent antibiotic and **III** were also approximately 20-fold more active than vancomycin or the aridicin compounds lacking the glycolipid against the Gram-positive anaerobe *Clostridium difficile* (Tables 2 and 3). This trend was also observed with the Gram-negative anaerobe *Bacteroides fragilis* (Table 3).

In mouse protection studies, all compounds of the aridicin series proved effective in the therapeutic tests using *S. aureus* HH 127 (Table 4). Aridicin A was approximately two-fold more active than the other homologs.

Discussion

These studies have demonstrated the potential to significantly alter the *in vitro* spectrum and potency of the aridicin antibiotics. The glycolipid constituent apparently confers unexpected levels of potency against *E. faecalis* and *C. difficile*, relative to the activity against staphylococci. However, the mannose substituent was the strongest determinant of activity, its presence resulting in less active compounds (**I** and **II**). Overall, the true aglycone **IV** and the derivative containing a glycolipid **III** were the most active compounds, being generally four-fold more active than vancomycin. In cases where other glycopeptides have been deglycosylated, potency improved as with ristocetin[16,17], deteriorated as with vancomycin[18], or remained the same as with A35512B[19]. In one case, avoparcin[20], a natural homolog lacking a mannose had higher potency than the parent antibiotic, as observed in the aridicin series.

Recently, the preparation and biological characterization of an analogous series of aglycone derivatives of the teicoplanin complex was described[21]. The teicoplanins are structurally related to the aridicins in that they also contain a homologous series of glycolipids and a similar aglycone nucleus. However, the teicoplanin complex differs from the aridicins in that its glycolipids consist of *N*-acylglucosamines rather than *N*-acylaminoglucuronic acids. Furthermore a third carbohydrate, *N*-acetylglucosamine, is present in teicoplanin. The series of aglycone derivatives reported for teicoplanin result from sequential loss of glycolipid, mannose and, finally, *N*-acetylglucosamine. No analog such as **III** containing the glycolipid but lacking mannose was reported for teicoplanin. Attempts in our laboratories to prepare glycolipid-containing aglycones from either teicoplanin or from other glucosamine containing glycopeptides using the DMSO conditions were unsuccessful (CHUNG, S. K., Personal communication). The ability to prepare two pseudoaglycone derivatives (**II** and **III**) in the aridicin series requires that the rate of hydrolysis of the mannose and the glycolipid be comparable. Since the mannose and glycolipid moieties are attached to the same sites in teicoplanin and aridicin A, the inability to prepare a mannose-free pseudoaglycone from teicoplanin is presumably a consequence of a much greater rate of hydrolysis of its glycolipids relative to those in the aridicins. The differential rate is consistent with the general understanding of the high acid stability of the D-glucopyranuronosyl bond[22~24]. Similar biological results were reported for the teicoplanin aglycone analogs, *i.e.*, decreased activity against enterococci on loss of glycolipids and enhanced activity *in vitro* for the true aglycone. As with the aridicins, analogs lacking the mannose were two to four-fold more active than analogs containing mannose.

All of the aridicin derivatives were effective in mouse protection tests, although the changes in *in vitro* potency were not reflected *in vivo*. This may be due to differences in absorption, metabolism,

distribution or elimination.

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References

1) SHEARER, M. C.; P. ACTOR, B. A. BOWIE, S. F. GRAPPEL, C. H. NASH, D. J. NEWMAN, Y. K. OH, C. H. PAN & L. J. NISBET: Aridicins, novel glycopeptide antibiotics. I. Taxonomy production and biological activity. J. Antibiotics 38: 555~560, 1985
2) SITRIN, R. D.; G. W. CHAN, J. J. DINGERDISSEN, W. HOLL, J. R. E. HOOVER, J. R. VALENTA, L. WEBB & K. M. SNADER: Aridicins, novel glycopeptide antibiotics. II. Isolation and characterization. J. Antibiotics 38: 561~571, 1985
3) WILLIAMS, D. H.; V. RAJANANDA, M. P. WILLIAMSON & G. BOJESEN: The vancomycin and ristocetin group of antibiotics. Top. Antibiotic Chem. 5: 119~158, 1980
4) WILLIAMS, D. H.; M. P. WILLIAMSON, D. W. BUTCHER & S. J. HAMMOND: Detailed binding sites of the antibiotics vancomycin and ristocetin A: Determination of intermolecular distances in antibiotic/substrate complexes by use of the time-dependent NOE. J. Am. Chem. Soc. 105: 1332~1339, 1983
5) HARRIS, C.; H. KOPECKA & T. HARRIS: Vancomycin: Structure and transformation to CDP-1. J. Am. Chem. Soc. 105: 6915~6922, 1983
6) BARDONE, M. R.; M. PATERNOSTER & C. CORONELLI: Teichomycins, new antibiotics from *Actinoplanes teichomyceticus* nov. sp. II. Extraction and chemical characterization. J. Antibiotics 31: 170~177, 1978
7) WILLIAMS, D. H.; V. RAJANANDA, G. BOJESEN & M. WILLIAMSON: Structure of the antibiotic ristocetin A. J. Chem. Soc. Chem. Commun. 1979: 906~908, 1979
8) KALMAN, J. R. & D. H. WILLIAMS: An NMR study of the structure of the antibiotic ristocetin A. The negative nuclear Overhauser effect in structure elucidation. J. Am. Chem. Soc. 102: 897~905, 1980
9) JEFFS, P. W.; G. W. CHAN, R. D. SITRIN, N. HOLDER, G. D. ROBERTS & C. DEBROSSE: The structure of the glycolipid components of the aridicin antibiotic complex. J. Org. Chem. 50: 1726~1731, 1985
10) HUNT, A. H.; R. M. MOLLEY, J. L. OCCOLOWITZ, G. G. MARCONI & M. DEBONO: Structure of the major glycopeptide of the teicoplanin complex. J. Am. Chem. Soc. 106: 4891~4895, 1984
11) BARNA, J. C. J.; D. H. WILLIAMS, D. J. M. STONE, T. W. C. LEUNG & D. M. DODDREL: Structure elucidation of the teicoplanin antibiotics. J. Am. Chem. Soc. 106: 4895~4902, 1984
12) HENNER, J. & R. D. SITRIN: Isoelectric focusing and electrophoretic titration of antibiotics using bioautographic detection. J. Antibiotics 37: 1475~1478, 1984
13) ROBERTS, G. D.; S. A. CARR, S. ROTTSCHAEFER & P. JEFFS: Structural characterization of glycopeptide antibiotics related to vancomycin by fast atom bombardment mass spectrometry. J. Antibiotics 38: 713~720, 1985
14) JEFFS, P. W.; L. MUELLER, C. DEBROSSE, S. HEALD & R. FISHER: The structure of aridicin A. An integrated approach employing 2-D NMR, energy minimization and distance constraints. J. Am. Chem. Soc. 1986 (In press)
15) SITRIN, R. D.; G. W. CHAN, G. D. ROBERTS, C. DEBROSSE, L. MUELLER, L. WEBB, L. KILLMER & P. W. JEFFS: Degradation studies on the aridicin aglycone. Isolation and characterization of the constituent amino acids. J. Org. Chem. Submitted
16) NIELSEN, R. V.; F. HYLDIG-NIELSEN & K. JOCOBSEN: Biological properties of ristocetin-pseudo-aglycone. J. Antibiotics 35: 1561~1564, 1982
17) PHILIP, J. E.; J. R. SCHENCK, M. P. HARGIE, J. C. HOLPER & W. E. GRUNDY: The increased activity of ristocetins A and B following acid hydrolysis. Antimicrob. Agents Annu. 1960: 10~16, 1961
18) MARSHALL, F. J.: Structure studies on vancomycin. J. Org. Chem. 8: 18~22, 1965
19) DEBONO, M.; R. M. MOLLOY, M. BARNHART & D. E. DORMAN: A35512, a complex of new antibacterial antibiotics produced by *Streptomyces candidus*. II. Chemical studies on A35512B. J. Antibiotics 33: 1407~1416, 1980

20) McGahren, W. J.; R. A. Leese, F. Barbatschi, G. O. Morton, N. A. Kuck & G. A. Ellestad: Components and degradation compounds of the avoparcin complex. J. Antibiotics 36: 1671~1682, 1983
21) Malabarba, A.; P. Strazzolini, A. Depaoli, M. Landi, M. Berti & B. Cavalleri: Teicoplanin, antibiotics from *Actinoplanes teichomyceticus* nov. sp. VI. Chemical degradation: Physico-chemical and biological properties of acid hydrolysis products. J. Antibiotics 37: 988~999, 1984
22) Marsh, C. A.: Chemistry of D-glucuronic acid and its glycosides. *In* Glucuronic Acid. *Ed.*, E. J. Dutton, pp. 4~136, Academic Press, New York, 1966
23) Jones, J. K. N. & F. Smith: Plant gums and mucilages. Adv. Carbo. Chem. 4: 243~291, 1949
24) Bochkov, A. F. & G. E. Zaikov: Chemistry of the *O*-Glycosidic Bond. Pergamon Press, Oxford, 1979