# *N*-DEMETHYLVANCOMYCIN, A NOVEL ANTIBIOTIC PRODUCED BY A STRAIN OF *NOCARDIA ORIENTALIS*

## TAXONOMY AND FERMENTATION

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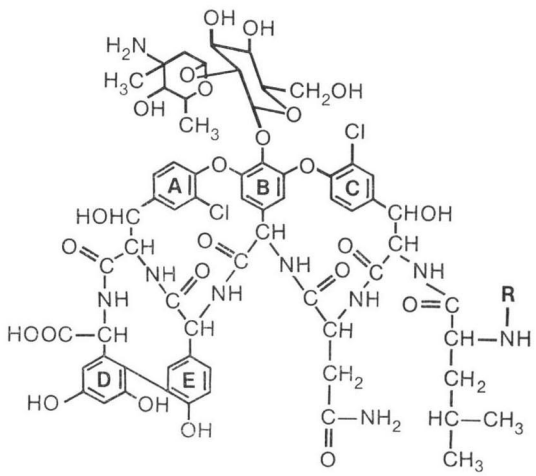
A novel vancomycin analog, *N*-demethylvancomycin, is produced by a soil isolate collected in Yucatan, Mexico. Taxonomic studies indicated this microorganism, designated NRRL 15232, is a strain of *Nocardia orientalis*. Unlike some glycopeptide antibiotics, virtually none of the *N*-demethylvancomycin synthesized remained bound to the cells of the producing culture. Antibiotic production was markedly depressed by the addition of orthophosphate to the fermentation medium. Enrichment of the medium with tyrosine, *p*-hydroxyphenylglycine, *p*-hydroxyphenylglyoxylic acid, or leucine, all putative precursors of the aglycone, stimulated the biosynthesis of *N*-demethylvancomycin.

During the process of screening actinomycetes for new antimicrobial substances, culture NRRL 15232 was isolated from a soil sample collected in Yucatan, Mexico. This isolate produced a novel vancomycin analog, *N*-demethylvancomycin (G. G. Marconi and A. H. Hunt, in preparation). The structures of *N*-demethylvancomycin and vancomycin are shown in Fig. 1. Soil isolate NRRL 15232 has been characterized as a strain of *Nocardia orientalis*. This paper describes the taxonomy and fermentation of NRRL 15232. The structure elucidation will be published elsewhere.

Fig. 1. Structures of vancomycin and *N*-demethylvancomycin.



Vancomycin R=$CH_3$
*N*-Demethylvancomycin R=H

### Materials and Methods

Cell Wall Analyses

Cell wall sugars were identified by the procedure of Lechevalier[1]. Diaminopimelic acid (DAP) isomers were determined by the method of Becker *et al.*[2].

Taxonomic Methods

Methods and media recommended by the International Streptomyces Project (ISP)[3], Berd[4], and Gordon *et al.*[5,6,7] were followed. Color names were assigned to reverse pigments on the basis of The Inter-Science Color Council-National Bureau of Standards (ISCC-NBS) Centroid Color Charts, standard sample No. 2106[8]. Spore mass color names were taken from the system of color wheels recommended by Tresner and Backus[9].

Fermentor Inoculum

NRRL 15232 was propagated on a medium containing precooked oatmeal 6.0%, yeast 0.25%, $K_2HPO_4$ 0.1%, $MgSO_4 \cdot 7H_2O$ 0.02%, KCl 0.02%, $FeSO_4 \cdot 7H_2O$ 0.0005% and agar 2.5%. Agar slope cultures were incubated at 30°C for 7 days, then lyophilized. Fermentor inoculum was prepared by introducing lyophilized pellets into wide-mouth Erlenmeyer flasks containing 50 ml of a medium composed of glucose 1.5%, dextrin 2.0%, soybean grits 1.5%, corn steep liquor 1.0%, yeast extract 0.1% and $CaCO_3$ 2.0%, pH 6.7. After incubation at 30°C for 48 hours on a rotary shaker, the resulting mycelial suspension was used to inoculate fermentors (1%, v/v).

Fermentors

Wide-mouth 250-ml Erlenmeyer flasks containing 50 ml of medium were incubated 4~6 days at 30°C on a gyrotatory shaker orbiting at 250 rpm in a circle with a diameter of 5.08 cm. Unless otherwise specified, the medium contained potato dextrin 3.0%, blackstrap molasses 2.0% and Bacto-Peptone (Difco Laboratories) 0.7% in deionized water, pH 6.8~7.0. This medium was designated DMP. Antibiotic activity was quantitated by a disc-plate assay employing *Micrococcus luteus*, ATCC 9341, as the test organism. Because the dose-response curves of *N*-demethylvancomycin and vancomycin were shown to be identical, a pure preparation of vancomycin served as the reference standard. The coefficient of variation of the assay was 3.07~3.43%. Qualitative evaluations were based on a TLC system using Darmstadt No. 5763 silica gel plates developed in a solvent system containing chloroform, methanol, ammonia, butanol and water (25: 50: 25: 25: 10). Chromatograms were bioautographed vs. *Bacillus subtilis*, ATCC 6633. The Rf value of *N*-demethylvancomycin in this system was 0.6. Supplementary qualitations were performed with a high performance liquid chromatography (HPLC) system specifically developed for use with glycopeptide fermentations (D. M. BERRY and L. D. BOECK, in preparation).

## Results and Discussions

### Taxonomy

Cultural Characteristics

NRRL 15232 grew well on the yeast dextrose agar and a variety of other solid media. Growth was frequently wrinkled and flaky. Aerial mycelia were in the gray (GY) or less frequently, the white (W) color series. Aerial hyphae, although abundantly produced, were not typical of *Streptomyces*. The reverse side was characterized by a distinctive dark brown color on certain media. This color was unaffected by changes in acidity. A reddish brown soluble pigment was elaborated into the agar. This cultural information is detailed in Table 1.

Morphological Characteristics

NRRL 15232 produced extensive substrate and aerial mycelia which, when viewed through a light microscope, appeared cobweb-like (Fig. 2). This morphology is characteristic of non-*Streptomyces* genera. Fig. 3 is an electron micrograph demonstrating the conidial chains. The spores are spherical to cylindrical in shape with smooth surface ornamentation. When grown under submerged shaken conditions, the hyphae break into short fragments. Neither sclerotia, sporangia, nor motile zoospores were observed.

Physiological Characteristics

Hydrolyzed whole cells contained *meso*-DAP, galactose, arabinose and ribose. These constituents represent a type IV cell wall. The whole-cell sugar pattern is type A. This combination of major cell wall constituents is indicative of the genus *Nocardia*[10].

Thin-layer chromatography of whole cell methanolysates by the method of MINNIKIN[11] indicated

Table 1. Cultural characteristics of NRRL 15232 and *N. orientalis* M43-05865 (type strain).

| Medium | | NRRL 15232 | M43-05865 |
|---|---|---|---|
| ISP No. 2 | G: | Abundant-wrinkled surface | Abundant-wrinkled surface |
| | R: | 59.d.Br (no pH change) | 72.d. OY |
| | Am: | Abundant: d light gray (GY) | Abundant: 2ba pale yellow (Y) |
| | Sp: | Reddish-brown | None |
| ISP No. 3 | G: | Abundant | Abundant |
| | R: | 89.p.Y | 89.p.Y |
| | Am: | Good: d light gray (GY) | Good: d light gray (GY) |
| | Sp: | None | None |
| ISP No. 4 | G: | Abundant | Abundant |
| | R: | 72.d.OY | 70.l.OY |
| | Am: | Abundant: b oyster white (W) | Abundant: b oyster white (W) |
| | Sp: | None | None |
| ISP No. 5 | G: | Abundant | Abundant |
| | R: | 59.d.Br (no pH change) | 70.l.OY |
| | Am: | Abundant: b oyster white (W) | Abundant: b oyster white (W) |
| | Sp: | Light brown | None |
| ISP No. 7 | G: | Abundant | Abundant |
| | R: | 65. br Black (no pH change) | 71.m.OY |
| | Am: | Abundant: 2ba pale yellow (Y) | Abundant: 2ba pale yellow (Y) |
| | Sp: | Dark reddish-brown | None |
| CZAPEK's solution | G: | Good | Good |
| | R: | 74.s. yBr | 70.l.OY |
| | Am: | Good: b oyster white (W) | Good: b oyster white (W) |
| | Sp: | Light brown | None |
| EMERSON's agar | G: | Abundant-wrinkled, flaky | Abundant-wrinkled, flaky |
| | R: | 74.s.yBr | 74.s.yBr |
| | Am: | Good: d light gray→ 2ba pale yellow (GY) | Good: 2ba pale yellow (Y) |
| | Sp: | None | None |
| Glucose - asparagine agar | G: | Abundant | Abundant |
| | R: | 75. deep yBr | 67.brill. OY |
| | Am: | Abundant: 2dc yellow gray (GY) | Abundant: 2ba pale yellow (Y) |
| | Sp: | Olive-brown | None |
| Glycerol - glycine agar | G: | Abundant | Abundant |
| | R: | 78. d. yB (no pH change) | 71. m. OY |
| | Am: | Abundant: 2dc yellow gray (GY) | Abundant: 2ba pale yellow (Y) |
| | Sp: | Olive-brown | None |
| Nutrient agar | G: | Good | Good |
| | R: | 70. l. OY | 70. l. OY |
| | Am: | Good: b oyster white (W) | Good: b oyster white (W) |
| | Sp: | None | None |
| Tap water agar | G: | Fair | Fair |
| | R: | 93. yellow gray | 93. yellow gray |
| | Am: | Poor | Poor |
| | Sp: | None | None |
| Yeast - dextrose agar | G: | Abundant-wrinkled, flaky | Abundant-slightly wrinkled |
| | R: | 68. s. OY | 68. s. OY |
| | Am: | Abundant: d light gray (GY) | Abundant: b oyster white (W) |
| | Sp: | None | None |

G: growth, R: reverse, Am: aerial mycelia, Sp: soluble pigment.

Fig. 2. Aerial mycelium of strain NRRL 15232 (on tap water agar, ×160).

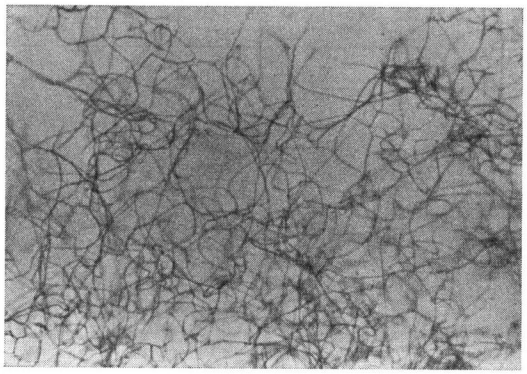
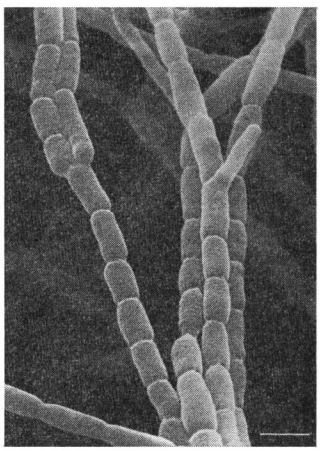


Fig. 3. Electron micrograph of the conidia of strain NRRL 15232 from a 14-day old culture grown on ISP No. 5.
Bar=1.0 μm.



that mycolic methyl esters were not present. Although *Nocardia* strains normally contain nocardomycolic acids, GOODFELLOW has noted that *N. orientalis* does not contain mycolic acids[12].

NRRL 15232 decomposed casein, tyrosine, xanthine, and hypoxanthine; hydrolyzed skim milk, starch, DNA, esculin, and hippurate; formed acid from arabinose, xylose, glucose, galactose, mannose, fructose, maltose, lactose, sucrose, trehalose, cellobiose, inulin, melizitose, methyl-D-glucoside, salicin, glycerol, erythritol, mannitol, and inositol; utilized acetate, citrate, lactate, malate, succinate, dextrin, and keratin; and remained viable after being incubated for 8 hours at 50°C. NRRL 15232 was resistant to lysozyme, rifampicin, cephalothin, erythromycin, benzylpenicillin, and streptomycin; produced catalase, phosphatase, and urease; liquefied gelatin; could not reduce nitrates to nitrites nor decompose adenine; would tolerate up to 8% NaCl; and grew at temperatures between 10 and 40°C. Melanoid pigments were not produced on Tryptone - yeast extract broth (ISP No. 1), or on slants of peptone - yeast extract - iron agar (ISP No. 6) or tryosine agar (ISP No. 7). The cells stained Gram-positive and were not acid-fast.

## Species Determination

The characteristics of NRRL 15232 placed it in the genus *Nocardia*. An examination of the published descriptions of 14 taxa of *Nocardia*[7] indicated it closely resembled *N. orientalis*. Simultaneous laboratory comparisons were therefore made with four *N. orientalis* strains from the Lilly culture

Table 2. Coefficient of similarity between NRRL 15232 and 18 reference strains.

| Culture | | Similarity coefficient | Culture | Similarity coefficient |
|---|---|---|---|---|
| NRRL 15232 | | 100 | *N. madurea* | 63 |
| M5-18215 | Authentic *N. orientalis* isolates in the Lilly culture collection | 95 | *N. brasiliensis* | 61 |
| M5-18260 | | 95 | *N. caviae* | 61 |
| C-392.2 | | 92 | *N. transvalensis* | 57 |
| M43-05865 | | 86 | *N. amarae* | 53 |
| *N. orientalis* (composite of 21 strains) | | 89 | *N. vaccinii* | 50 |
| *N. aerocolonigenes* | | 76 | *N. asteroides* | 47 |
| *N. hirsuta* | | 68 | *N. carnea* | 47 |
| *N. autotrophica* | | 65 | *N. pelletieri* | 39 |
| *N. dassonvillei* | | 63 | | |

Table 3. Comparison of NRRL 15232 properties with strains of *N. orientalis*.

| | NRRL 15232 | C-329.2 | M5-18215 | M5-18260 | M43-05865 | *N. orientalis*[a] |
|---|---|---|---|---|---|---|
| Acid-fastness | − | − | − | − | − | − |
| Aerial hyphae | + | + | + | + | + | + |
| Catalase | + | + | + | + | + | + |
| Cell wall type | IV | IV | IV | IV | IV | IV |
| Conidia | + | + | + | + | + | + |
| Decomposition of | | | | | | |
| Adenine | − | − | − | − | − | − |
| Casein | + | + | + | + | + | + |
| DNA | + | + | + | + | + | ND |
| Hypoxanthine | + | + | + | + | + | + |
| Tyrosine | + | + | + | + | + | + |
| Urea | + | + | + | + | + | + |
| Xanthine | + | − | + | + | − | − |
| Fragmentation | + | + | + | + | + | + |
| Gelatin liquefaction | + | + | + | + | + | ND |
| Gram stain | + | + | + | + | + | ND |
| Hydrolysis of | | | | | | |
| Esculin | + | + | + | + | + | + |
| Hippurate | + | + | + | + | + | + |
| Skim milk | + | + | + | + | + | ND |
| Starch | + | + | + | + | + | + |
| Melanoid pigmentation | − | − | − | − | − | ND |
| Morphology, cobweb | + | + | + | + | + | + |
| NaCl tolerance | 8 | 10 | 8 | 8 | 10 | ND |
| Nitrite from nitrate | − | − | − | − | + | − |
| Phosphatase | + | + | + | + | + | ND |
| Resistance to | | | | | | |
| Bacitracin | − | − | − | − | − | ND |
| Lysozyme | + | + | + | + | + | − |
| Novobiocin | − | − | − | − | − | ND |
| Benzylpenicillin | + | + | + | + | + | ND |
| Rifampicin | + | + | + | + | + | + |
| Spore color | GY∼W | GY∼Y | W∼Y | W∼Y | W∼Y | W∼Y |
| Spore shape, spherical to cylindrical | + | + | + | + | + | + |
| Spore surface, smooth | + | + | + | + | + | + |
| Survival at 50°C, 8 hours | + | + | + | + | + | + |
| Temperature range (°C) | 10∼40 | 10∼40 | 15∼40 | 15∼40 | 10∼40 | 10∼40 |
| Type A sugar pattern | + | + | + | + | + | + |
| Urease production | + | + | + | + | + | + |
| Utilization pattern of carbon compounds | + | + | + | + | + | + |
| Utilization of organic acids | + | + | + | + | + | + |

ND: Not done.

[a] These data represent a composite of information gathered from 21 species of *N. orientalis*[14].

collection. Similarity coefficients were calculated from the following equation[13]:

$$S=\frac{N_s^+ + N_s^-}{N_s^+ + N_s^- + N_d} \times 100$$

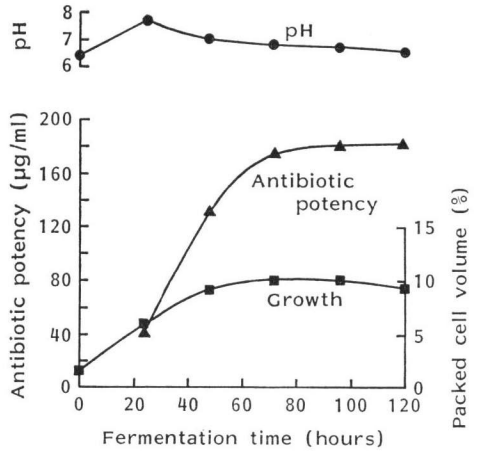
$N_s^+$ is the number of positive similarities, $N_s^-$ is the number of negative similarities, and $N_d$ is the number of dissimilarities. The coefficient of similarity (COS) between NRRL 15232 and strains of *N. orientalis* is high. The COS between NRRL 15232 and other *Nocardia* species is low (Table 2). The

Table 4. Effect of temperature on biosynthesis of *N*-demethylvancomycin.

| Temperature (°C) | Antibiotic potency[a] (μg/ml) |
|---|---|
| 25 | 90 |
| 28 | 155 |
| 30 | 180 |
| 32 | 188 |
| 34 | 197 |
| 37 | 116 |

[a] Maximum yields at temperatures above 32°C were achieved by 48 hours.

Fig. 4. Time course of *N*-demethylvancomycin biosynthesis by *N. orientalis* NRRL 15232.




similarities and differences between NRRL 15232 and other strains of *N. orientalis* are detailed in Table 3. Additional evidence confirming NRRL 15232 as a strain of *N. orientalis* was provided through a key devised by MISHRA *et al.*[14].

Based on the above data, NRRL 15232 is classified as a strain of *Nocardia orientalis* (Pittenger and Brigham) Pridham and Lyons, ATCC 19795. *N. orientalis* is recognized in the Approved List of Bacterial Names[15] and consequently is a validly published species.

## Fermentation

Because the level of antibiotic produced by NRRL 15232 in the original medium (GPM) was only 40~60 μg/ml, the fermentation was examined for modifications that would increase antibiotic productivity. As a preliminary step, the quantity of antibiotic present in the fermented broth was compared with the amount that remained associated with the biomass. Large percentages of another glycopeptide antibiotic, actaplanin, are normally bound to the producing culture but are released under either acidic or alkaline conditions[16]. Solvent extraction, acidification or alkalization of the biomass consistently recovered only trace amounts of antibiotic, indicating that >95% of the *N*-demethylvancomycin produced was released spontaneously into the fermentation broth. Fermentation media employed for the biosynthesis of other known glycopeptides were then compared with the GPM medium. Antibiotic productivity in each of these media was inferior to the productivity obtained with the GPM medium. Further manipulation of the original medium resulted in the DMP medium, in which antibiotic yields were 180~200 μg/ml. The DMP medium was used as the basis for all subsequent fermentation studies.

A typical time course of the fermentation in the DMP medium is shown in Fig. 4. The effect of temperature on antibiotic biosynthesis is shown in Table 4. Optimum yields were obtained at 32~34°C. The effect of various carbon sources on the biosynthesis of *N*-demethylvancomycin is shown in Table 5. Galactose supported higher yields than glucose or fructose. Only one disaccharide, maltose, produced similar yields. Glycerol and refined soybean oil produced equivalent yields, equal, or slightly superior to, galactose and maltose. The highest productivity was obtained with soluble starch and potato dextrin, with dextrin being slightly superior to soluble starch.

Orthophosphate is known to markedly affect the quantity of vancomycin synthesized by C329, a vancomycin-producing strain of *N. orientalis*[17]. Levels of $KH_2PO_4$ between 0.001 and 0.102 mg/ml are stimulatory while a level of 0.680 mg/ml reduces vancomycin biosynthesis by 98% without affecting the

Table 5. Effect of various carbohydrates and lipids on the biosynthesis of *N*-demethylvancomycin.

| Carbon source | Level tested (%) | Antibiotic potency ($\mu$g/ml) |
|---|---|---|
| None[a] | 0 | 58 |
| Glucose | 1 | 62 |
| | 2 | 81 |
| | 3 | 98 |
| | 4 | 90 |
| Fructose | 3 | 56 |
| Galactose | 3 | 128 |
| Maltose | 3 | 112 |
| Lactose | 3 | 56 |
| Sucrose | 3 | 65 |
| Potato dextrin | 2 | 138 |
| | 3 | 180 |
| | 4 | 156 |
| Soluble starch | 3 | 166 |
| Corn starch | 3 | 52 |
| Glycerol | 2 | 132 |
| | 3 | 97 |
| Corn oil | 3 | 76 |
| Refined soybean oil | 3 | 139 |
| Methyl oleate | 3 | 82 |

[a] DMP medium minus dextrin.

Table 6. Effect of putative precursors on the biosynthesis of *N*-demethylvancomycin.

| Putative precursor[a] | Antibiotic potency (% of control) |
|---|---|
| Control | 100 |
| Shikimic acid | 98 |
| L-Tyrosine | 114 |
| *p*-Hydroxyphenylglycine | 164 |
| *p*-Hydroxyphenylglyoxylic acid | 127 |
| L-Leucine | 118 |
| L-Asparagine | 97 |
| L-Glutamine | 104 |

[a] Added at medium make-up. Addition level was $5\times10^{-3}$ M.

growth of the producing organism. The *N*-demethylvancomycin fermentation is even more sensitive to phosphate regulation. No stimulation was observed by any level of phosphate enrichment while increases as small as $3\times10^{-4}$ M (0.05 mg/ml) depressed fermentation yields by 20%.

Very little information is available regarding the biosynthesis of glycopeptide antibiotics. One study employing $^{13}C$ and/or $^{2}H$ labelled precursors has elucidated the origin of the unusual aromatic amino acid residues present in the aglycone of vancomycin[18]. Inasmuch as *N*-demethylvancomycin differs from vancomycin only in the absence of the methyl function from the leucine residue, the biosynthetic pathways for the two antibiotics may be virtually identical. If so, tyrosine would be expected to precurse aromatic rings A, B, C, and E. Two of these same rings, B and E, should also be precursed by *p*-hydroxyphenylglycine (*p*-HPG). A third aromatic compound, *p*-hydroxyphenylglyoxylic acid (*p*-HPGA), is presumed to be involved in the transformation of tyrosine to *p*-HPG but its role has not been investigated. The effects of these and other putative precursors on the biosynthesis of *N*-demethylvancomycin is shown in Table 6. Shikimic acid did not affect productivity. Tyrosine increased productivity only 14% while *p*-HPG increased productivity 64%. Even *p*-HPGA increased productivity 27%, which indicates that it is involved in the biosynthesis of the aromatic rings. Leucine also stimulated antibiotic potency 18% but asparagine and glutamine did not significantly affect yields.

There is no information available regarding the point in vancomycin biosynthesis at which methylation of the leucine residue occurs. One possible explanation for the formation of *N*-demethylvancomycin therefore is *via* a pathway involving terminal demethylation of vancomycin. To test this hypothesis, vancomycin was pulsed into the fermentation at a level of 300 $\mu$g/ml at 24 hours and incubation was continued for an additional 120 hours. Samples were withdrawn at 24 hours intervals and evaluated by HPLC. There was no decrease in the vancomycin level present and no increase from the normal level of *N*-demethylvancomycin produced. These data suggest that NRRL 15232 cannot demethylate vancomycin and probably synthesizes *N*-demethylvancomycin *via* a mechanism that omits methylation of the leucine residue at what is presumed to be an earlier step in vancomycin biosynthesis. Conversely,

*N*-demethylvancomycin was pulsed into a vancomycin fermentation at the same level and time and monitored in the same manner. As before, there was no decrease in the level of the analog added and no increase in the level of the antibiotic normally produced, again suggesting that bioconversion had not occurred. More definitive investigation obviously requires the use of labelled compounds but the current results imply that *N*-methylation also is not the terminal step in vancomycin biosynthesis.

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## References

1) LECHEVALIER, M. P.: Identification of aerobic actinomycetes of clinical importance. J. Lab. Clin. Med. 71: 934~944, 1968
2) BECKER, B.; M. P. LECHEVALIER, R. E. GORDON & H. A. LECHEVALIER: Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole cell hydrolysates. Appl. Microbiol. 11: 421~423, 1964
3) SHIRLING, E. B. & D. GOTTLIEB: Methods of characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313~340, 1966
4) BERD, D.: Laboratory identification of clinically important aerobic actinomycetes. Appl. Microbiol. 25: 665~681, 1973
5) GORDON, R. E.; D. A. BARNETT, J. E. HANDERHAN & C. H. PANG: *Nocardia coeliaca*, *Nocardia autotrophica*, and the Nocardin strain. Int. J. Bacteriol. 24: 54~63, 1974
6) GORDON, R. E. & D. A. BARNETT: Resistance to rifampin and lysozyme of strains of some species of *Mycobacterium* and *Nocardia* as a taxonomic tool. Int. J. Syst. Bacteriol. 27: 176~178, 1977
7) GORDON, R. E.; S. K. MISHRA & D. A. BARNETT: Some bits and pieces of the genus *Nocardia*: *N. carnea*, *N. vaccinii*, *N. transvalensis*, *N. orientalis*, and *N. aerocolonigenes*. J. Gen. Microbiol. 109: 69~78, 1978
8) U. S. Department of Commerce. National Bureau of Standards. ISCC-NBS Centroid Color Charts Standard Sample No. 2106. 1958
9) TRESNER, H. D. & E. J. BACKUS: System of color wheels for streptomycete taxonomy. Appl. Microbiol. 11: 335~338, 1963
10) LECHEVALIER, M. P. & H. LECHEVALIER: Chemical composition as a criterion in the classification of aerobic actinomycetes. Int. J. Syst. Bacteriol. 20: 435~443, 1970
11) MINNIKIN, D. E.; L. ALSHAMAONY & M. GOODFELLOW: Differentiation of *Mycobacterium*, *Nocardia*, and related taxa by thin-layer chromatographic analysis of whole-organism methanolysates. J. Gen. Microbiol. 88: 200~204, 1975
12) GOODFELLOW, M. & K. P. SCHAAL: Identification methods for *Nocardia*, *Actinomadura* and *Rhodococcus*. *In* Identification Methods for Microbiologists. 2nd ed., *Ed.*, SKINNER F. A. & D. W. LOVELOCK, pp. 261~276, Society for Applied Bacteriology Technical Series No. 14. Academic Press, London and New York, 1979
13) KURYLOWICZ, W.; A. PASZKIEWICZ, W. WOZNICKA & W. KURATKOWSKI: Numerical Taxonomy of Streptomycetes. Polish Medical Publishers, Warsaw, 1975
14) MISHRA, S. J.; R. E. GORDON & D. A. BARNETT: Identification of nocardiae and streptomycetes of medical importance. J. Clin. Microbiol. 11: 728~736, 1980
15) SKERMAN, V. B. D.; V. MCGOWAN & P. H. A. SNEATH: Approved lists of bacterial names. Int. J. Syst. Bacteriol. 30: 225~420, 1980
16) BOECK, L. D. & W. M. STARK: Actaplanin, a complex of new glycopeptide antibiotics. Fermentation. *In* Developments in Industrial Microbiology. Vol. 25, p. 10.
17) MERTZ, F. P. & L. E. DOOLIN: The effect of inorganic phosphate on the biosynthesis of vancomycin. Can. J. Microbiol. 19: 263~270, 1973
18) HAMMOND, S. J.; M. P. WILLIAMSON, D. H. WILLIAMS, L. D. BOECK & G. G. MARCONI: On the biosynthesis of the antibiotic vancomycin. J. Chem. Soc., Chem. Commun. 1982: 344~346, 1982