# DEOXYNYBOMYCIN FROM A *STREPTOMYCES*

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During the screening for new antibiotics, a new antibiotic was isolated in crystalline form from the cultured broth of a *Streptomyces*, isolated from a soil sample collected in Okinawa. The structural studies revealed that this antibiotic was deoxynybomycin[1], which had been already derived chemically from nybomycin[2] by RINEHART *et al.* In this communication, characteristics of the strain, production, isolation, properties of this antibiotic, and the structural determination are reported.

## Characterization of the Producing Strain

Colonies of the strain (the laboratory number: MB891-A1) on starch agar and calcium malate agar were examined microscopically. It belongs to the *Streptomyces*, forming well-branched substrate mycelia with long aerial mycelia in open spirals without whorl formation. The surface of the conidial spore has a hairy structure under scanning electron microscope. In the case of this strain, observation of the hairy structure by the conventional electron microscope is difficult. The cultural characteristics of the strain on various media are shown in Table 1. Utilization of carbohydrates on the PRIDHAM-GOTTLIEB's basal medium is as follows: rhamnose, glucose, mannose, galactose, fructose, starch, glycerol and inositol are utilized, yielding abundant growth; saccharose is slightly utilized; maltose and dextrin give varied results; arabinose, xylose, lactose, raffinose, inulin, salicin, sorbitol, mannitol and dulcitol are not utilized. As shown in Table 1, *Streptomyces* No. MB891-A1 belongs to a nonchromogenic type, the growth on various media is colorless to pale yellow, the aerial mycelium is white to light gray, the soluble pigment is generally none, and the proteolytic action is weak. Among known species of *Streptomyces*, the strain is most similar to *Streptomyces pseudogriseolus*.[3,4,5] However, several differences are found between characters of *Streptomyces* No. MB891-A1 and *Streptomyces pseudogriseolus* as shown in Table 2. Thus, *Streptomyces* No. MB891-A1 can be assigned to a new species named *Streptomyces hyalinum* n. sp. HAMADA *et* YOKOYAMA.

## Production, Isolation and Structure

A medium containing starch 1.0%, glucose 1.0%, meat extract 0.75%, peptone 0.75%, sodium chloride 0.3%, magnesium sulfate 0.1%, and trace of metal ions was used for the production of the antibiotic. The maximum production was observed in four to five days of a shaking culture at 30°C. The antibiotic was extracted with butyl acetate from the broth filtrate at pH 2.0. The extract was concentrated and charged on a silica gel column. After washing the column with benzene, the antibiotic was eluted with benzene - methanol (10 : 1). It was found that the eluate contained two active components. They were separated by silica gel column chromatography using chloroform and chloroform - methanol (10 : 1) as developing solvents.

Both of the isolated compounds had similar and characteristic UV absorptions. A compound eluted later was identified as nybomycin by direct comparison with an authentic sample kindly supplied by G. B. WHITFIELD of the Upjohn Company.

A compound eluted faster in the silica gel column chromatography (compound I) was crystallized as colorless needles from acetic acid-water. It does not melt up to 300°C. It is slightly soluble in concentrated hydrochloric acid and acetic acid, but hardly soluble or insoluble in water, methanol, ethyl acetate, chloroform, acetone, pyridine, dimethylformamide, and dimethylsulfoxide. It has molecular formula $C_{16}H_{14}N_2O_3$ (M.W. 282). Calcd.; C 68.07, H 5.00, N 9.92. Found: C 67.80, H 5.00, N 9.94. Parent peak of mass spectrum was m/e 282. The ultraviolet spectrum is shown in Fig. 1.

Table 1. Cultural characteristics of the strain No. MB891-A1

| | Growth | Aerial mycelim | Soluble pigment | Physiological properties |
|---|---|---|---|---|
| Glycerol nitrate agar, 27°C | Colorless to pale yellow [1½ Ca, Cream]; pale yellowish brown reverse | White to light brownish gray to light gray [5fe, Ashes] | Brownish | |
| Glucose-asparagine agar, 27°C | Poor, colorless | Thin, white to light brownish gray | None | |
| Calcium malate agar, 27°C | Colorless | White to brownish gray [3 fe, Silver Gray] | None | No transparent zone is observed around the growth |
| Peptone solution (containing 1.0 % of $NaNO_3$), 27°C | Colorless | None | None | No reduction of nitrate |
| Starch agar, 27°C | Poor, colorless | Thin, white to light brownish gray | None | Negative hydrolysis of starch |
| Tyrosine agar, 27°C | Colorless | White to gray [3 fe, Silver Gray] | None | Negative tyrosinase reaction |
| Potato plug, 27°C | Colorless to pale yellowish brown | Scant, white to gray | Brownish | |
| Nutrient agar, 27°C or 37°C | Colorless | None | None | |
| LOEFFLER's serum, 30°C | Colorless; when cultured at 37°C, the growth was not observed | None | None | No liquefaction of coagulated serum |
| Gelatin, 20°C | Colorless | White to gray | None | No liquefaction of gelatin |
| Skimmed milk, 30°C | Colorless; when cultured at 37°C, the growth was not observed | None | Faint brownish | No coagulation and peptonization is observed after about 20-day culture |
| Cellulose, 27°C | Colorless | None | None | No decomposition of cellulose |
| Yeast-malt agar (ISP), 27°C | Colorless to pale yellowish brown | Abundant, light gray [5 fe, Ashes] | None | |
| Oat meal agar (ISP), 27°C | Colorless | Thin, light gray | None | |
| Salts-starch agar (ISP), 27°C | Poor, hyaline | Thin, white | None | |
| Glycerol-asparagine agar (ISP), 27°C | Poor, colorless to pale yellow | Light gray [5 fe, Ashes to 5ih, Lead Gray] | None | |

The description in parenthesis [ ] follows the color standard shown in Color Harmony Manual of Container Corporation of America.

Table 2. Comparison of *Streptomyces* No. MB891-A1 with *Streptomyces pseudogriseolus*

| | MB891-A1 | *S. pseudogriseolus* |
|---|---|---|
| Surface of spore | hairy | smooth or spiny |
| Hydrolysis of starch | negative | strong |
| Liquefaction of gelatin | negative | weak to medium |
| Milk | no coagulation and peptonization completed in 15~21 days | coagulation and peptonization completed in 25~30 days |
| Utilization of carbohydrate: arabinose, lactose, salicin, mannitol and dulcitol | not utilized | utilized |
| Metabolites produced | nybomycin and deoxynybomycin | xanthomycin-like substance |

Fig. 1. Ultraviolet spectrum of deoxynybomycin (MeOH)

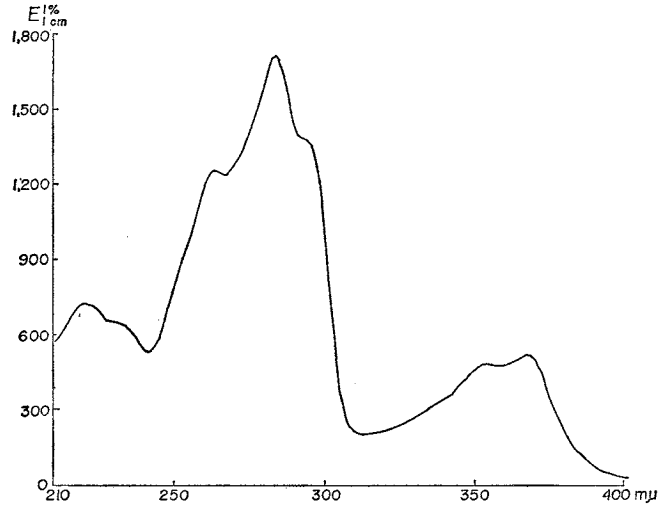
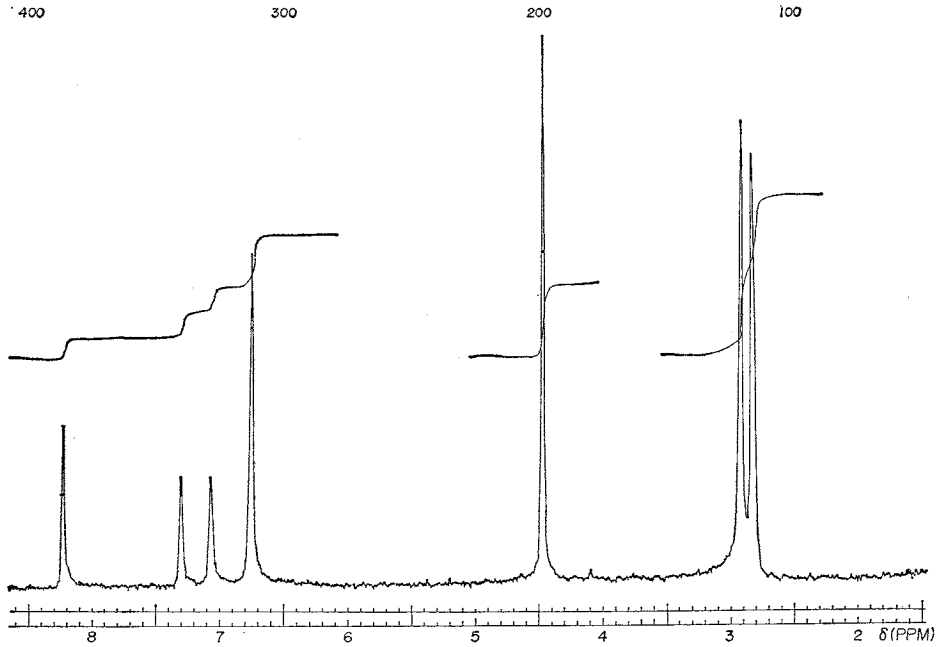



Fig. 2. The n.m.r. spectrum of deoxynybomycin (100 MHz, Deuterotrifluoroacetic acid)

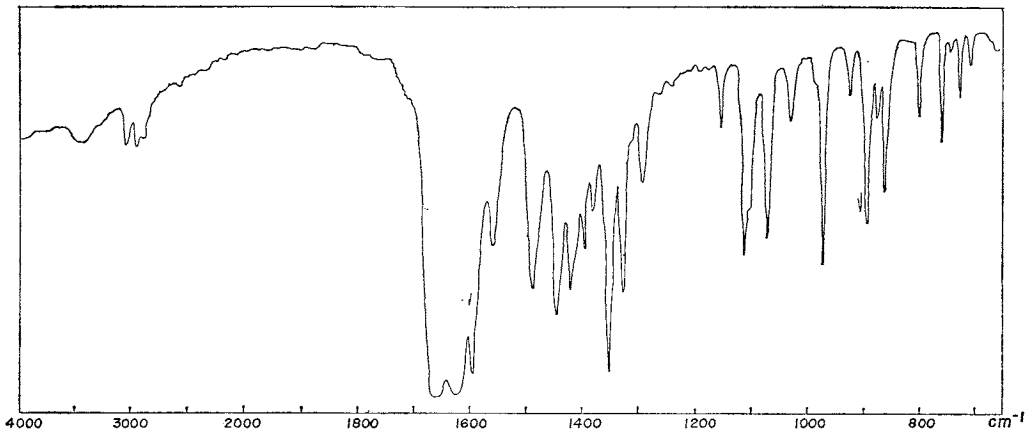


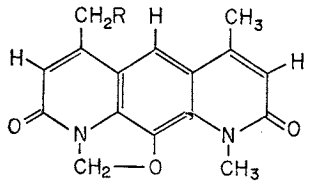

$\lambda_{max}^{MeOH}$ (log ε): 220 mμ (4.44), 264 mμ (4.56), 285 mμ (4.67), 294 mμ (4.59, shoulder), 353 mμ (3.08), and 368 mμ (3.11). The infrared spectrum is shown in Fig. 2. The NMR spectrum (Fig. 3) in deuterotrifluoroacetic acid shows three C-methyl groups. Two methyl signals at 2.82 and 2.91δ coupled with methine protons at 7.08 and 7.30δ, respectively, with small coupling constants less than 1.0 Hz. Irradiation at 2.82δ brought about 27.0 % increase of integrated area of a proton at 8.23δ, but 5.5 % decrease of that of a proton at 7.08δ. Whereas irradiation at 2.91δ brought about 32.5 % and 15.1 % increases of integrated areas of protons at 8.23 and 7.30δ, respectively.

From these results, compound I is deduced to be deoxynybomycin, which has a methyl group instead of the hydroxymethyl group of nybomycin.

The conclusion was confirmed by direct comparison of compound I and deoxynybo-

Fig. 3. Infrared spectrum of deoxynybomycin (KBr).



R=H, compound I
(deoxynybomycin)
R=OH, nybomycin

mycin, which was derived from nybomycin by refluxing with hydroiodic acid[1].

The antibacterial activities of deoxynybomycin are compared with nybomycin in Table 3. In general, deoxynybomycin has stronger activities than nybomycin.

Table 3. Antibacterial spectra of deoxynybomycin and nybomycin (Minimum inhibitory concentration on nutrient agar)

| Test organisms | mcg/ml | |
|---|---|---|
| | Deoxynybomycin (Compound I) | Nybomycin |
| *Staphylococcus aureus* FDA 209P | 1.25 | 20.0 |
| *Staphylococcus aureus* Terajima | 1.25 | 20.0 |
| *Staphylococcus aureus* Smith | 1.25 | 20.0 |
| *Staphylococcus aureus* 193 | 2.5 | 20.0 |
| *Staphylococcus aureus* 52-34 | 1.25 | 20.0 |
| *Sarcina lutea* PCI 1001 | 0.62 | 1.25 |
| *Micrococcus flavus* FDA 16 | 0.31 | 0.61 |
| *Bacillus cereus* ATCC 10702 | 0.62 | 10.0 |
| *Bacillus anthracis* | 0.31 | 5.0 |
| *Bacillus subtilis* NRRL B-558 | 0.31 | 1.25 |
| *Corynebacterium bovis* 1810 | 0.31 | 0.31 |
| *Escherichia coli* NIHJ | 10.0 | 5.0 |
| *Escherichia coli* K-12 | >20.0 | >20.0 |
| *Shigella flexneri* 1a (ew8) | >10.0 | >10.0 |
| *Pseudomonas aeruginosa* $A_3$ | >10.0 | >10.0 |
| *Pseudomonas fluorescens* | >10.0 | >10.0 |
| *Proteus vulgaris* OX19 | >10.0 | >10.0 |
| *Salmonella typhosa* | >10.0 | >10.0 |
| *Klebsiella pneumoniae* PCI 602 | 10.0 | 10.0 |
| *Mycobacterium smegmatis* ATCC 607 | 2.5 | 20.0 |
| *Mycobacterium phlei* | 1.25 | 10.0 |
| *Candida albicans* 3147 | >10.0 | >10.0 |

## References

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