# ISOLATION AND STRUCTURE OF A NEW POLYETHER ANTIBIOTIC, OCTACYCLOMYCIN

SHINJI FUNAYAMA and SHIGEO NOZOE

Pharmaceutical Institute, Tohoku University, Aobayama, Sendai 980, Japan

CLAUDE TRONQUET†, YUMI ANRAKU, KANKI KOMIYAMA and SATOSHI ŌMURA*

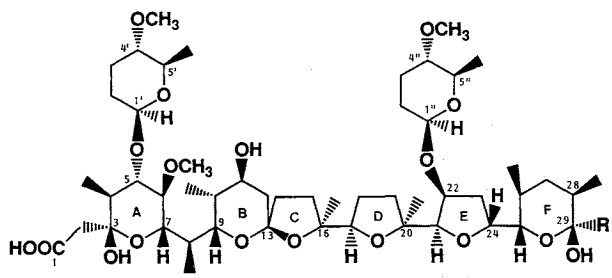
The Kitasato Institute and School of Pharmaceutical Sciences of Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108, Japan

(Received for publication May 14, 1992)

In the course of a screening program for novel antibiotics showing antitumor activity, a cyclic peptide antibiotic sohbumycin was isolated from the culture broth of *Streptomyces* sp. No. 82-85, which had been isolated from a soil sample collected in Kanagawa prefecture, Japan. The isolation and physico-chemical properties of sohbumycin and fermentation and taxonomy of the producing organism, *Streptomyces* sp. No. 82-85, was reported in the preceding paper.[1] Through careful fractionation of the fermentation broth from which sohbumycin was isolated, a new polyether antibiotic named octacyclomycin (**1**) was isolated which showed both cytocidal activity against B16 melanoma cells and antimicrobial activity against Gram-positive bacteria *in vitro*. The antibiotic showed no inhibitory activity against Gram-negative bacteria, yeast and fungi at the concentration of 500 $\mu$g/ml. This paper deals with the isolation and structure elucidation of octacyclomycin (**1**).

The fermentation broth (300 liters) was mixed with 15 kg of Hyflo Super-Cel (Johns-Manville Co., U.S.A.) and then filtered with a filter press. The brown filtrate (260 liters) was adjusted to pH 6.0 and extracted with EtOAc ($2 \times 150$ liters) and the combined EtOAc layers were concentrated to *ca.* 10 liters, washed with $H_2O$ (5 liters) and dried over $Na_2SO_4$ (anhydrous). Concentration of the EtOAc layer resulted in a brown oil. The brown oil was chromatographed over silica gel. Fractions which showed antimicrobial activity against *Micrococcus luteus* were collected and further chromatographed over silica gel to afford octacyclomycin Na salt (51.7 mg) as a colorless powder.

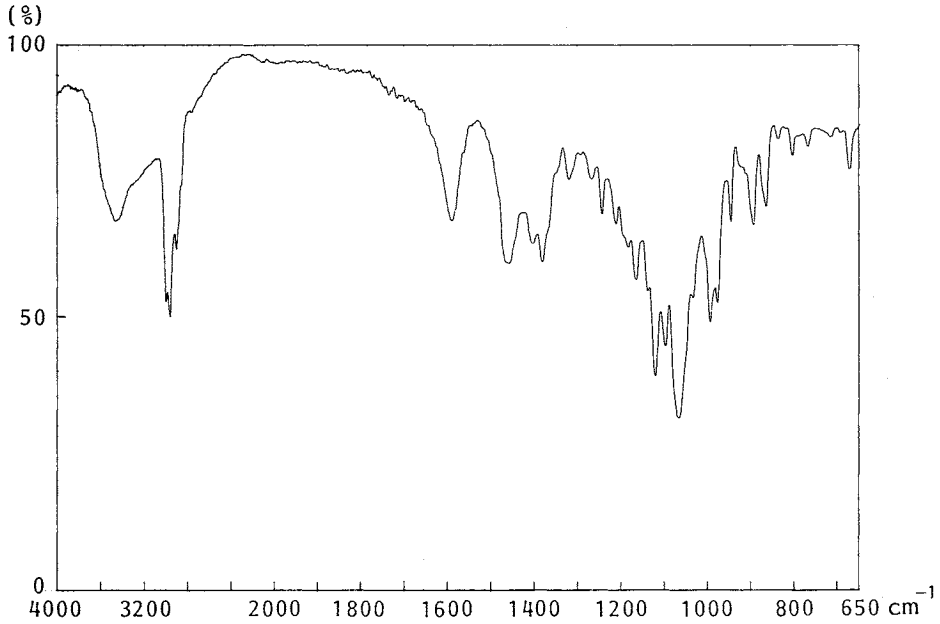
Physico-chemical properties of octacyclomycin (**1**) Na salt are listed on Table 1. The IR spectrum (Fig. 1) indicated the presence of hydroxyl (3460 $cm^{-1}$) and carboxylate (1589 $cm^{-1}$) functions in its structure. The $^1H$ and $^{13}C$ NMR spectral data summarized in Table 2 were obtained through $^1H$-$^{13}C$ two dimentional NMR spectrometry and 52 carbons and 83 hydrogens including $9 \times CH_3$, $13 \times CH_2$, $5 \times CH$, $3 \times OCH_3$, $14 \times OCH$, $2 \times C$–O, $3 \times O$–C–O, $2 \times O$–CH–O and $1 \times COO$ were observed. On the other hand, in the MS of octacyclomycin (**1**) Na salt (Fig. 2), 1,039 ($(M+1)^+$) was observed. By the combination of the MS data and $^1H$ and $^{13}C$ NMR spectral data, molecular formula and molecular weight of octacyclomycin (**1**) Na salt and its free acid were concluded to be $C_{52}H_{87}O_{19}Na$ (MW 1,038) and $C_{52}H_{88}O_{19}$ (MW 1,016), respectively. The functions shown in the $^1H$ and $^{13}C$ NMR spectra accounted for all the protons in **1** except for five exchangeable ones, which were ascribed to four free hydroxy functions and a carboxyl function. These physico-chemical and spectroscopical characteristics suggested that this compound was classified to be a polyether antibiotic. In the MS of octacyclomycin (**1**) Na salt,




**1** R = $CH_2OH$
**2** R = $CH_3$

† Present address: Sanofi Recherche Centre de Montpellier, Rue du P. J. Blayac, 34082 Montpellier, France.

Fig. 1. IR spectrum of octacyclomycin (**1**) Na salt.[a]




[a] Recorded on a Jasco model A-102 interferometer.

Table 1. Physico-chemical properties of octacyclomycin (**1**) Na salt.

| | |
|---|---|
| Appearance | Colorless powder |
| Optical rotation[a] | $[\alpha]_D^{24}$ +55.0° (*c* 0.02, $CHCl_3$) |
| TLC (silica gel) | $CHCl_3$-$CH_3OH$ (19:1) Rf 0.64 |
| Molecular formula | $C_{52}H_{87}O_{19}Na$ |
| MW | 1,038 |
| UV $\lambda_{max}$ | End absorption |
| IR $\lambda_{max}$ | Fig. 1 |
| FAB-MS | Fig. 2 |
| Color reaction: | |
| Positive | 50% $H_2SO_4+\Delta$ |
| Negative | Ninhydrin reagent, iodine |

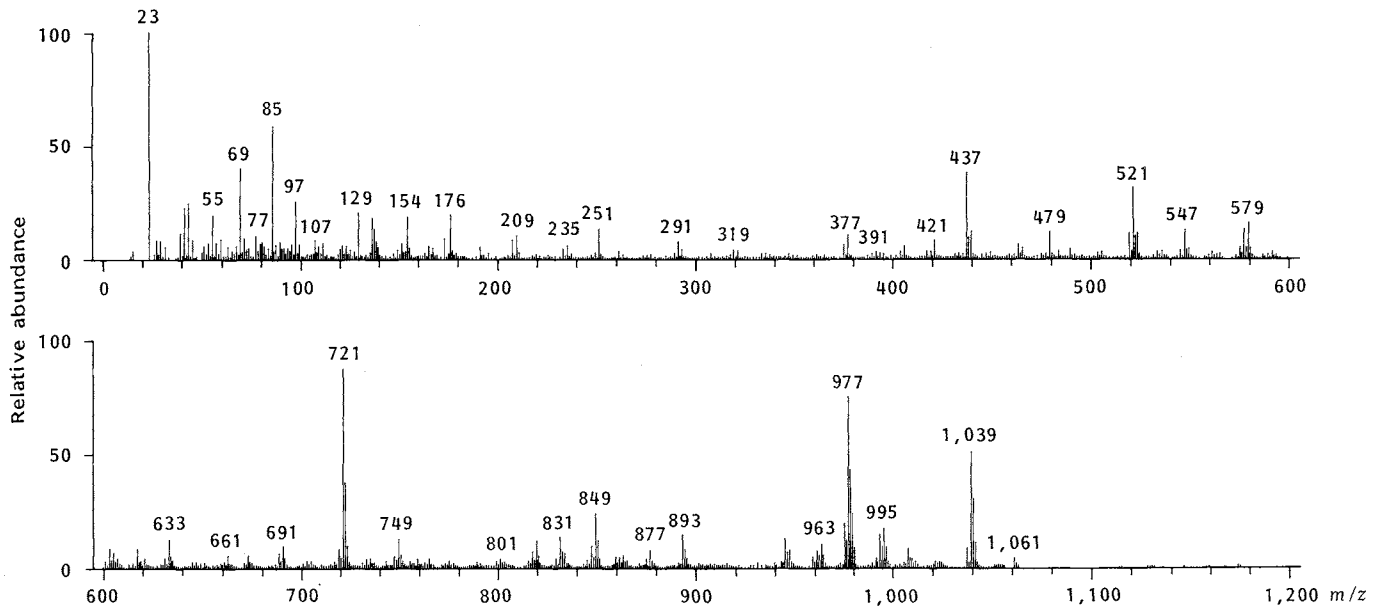
[a] Measured with a Jasco model DIP-370 digital polarimeter.

1,061 ($(M+Na)^+$) and 977 ($(M+1-62)^+$) were also observed. The existence of the peak 62 MU less than the corresponding metal-adduct molecular ion is common for polyether antibiotics possessing a $\beta$-hemiketal carboxylic acid group and is derived from $((M+1)-(CO_2+H_2O))^+$.[2]

In the $^{13}C$ NMR spectrum of **1**, two hemiketal signals ($\delta_C$ 97.7 and 98.1) and a ketal signal ($\delta_C$ 106.9) were observed together with two anomeric signals ($\delta_C$ 102.4 and 103.3) assignnable to two sugar moieties. Up to now, four polyether antibiotics, K-41B ($C_{54}H_{92}O_{20}$),[3] CP-91,243 ($C_{50}H_{84}O_{18}$), CP-91,244 ($C_{51}H_{86}O_{18}$) and UK-58,852 (**2**, $C_{52}H_{88}O_{18}$),[4] have been known to possess these moieties (two hemiketals, a ketal and two sugar moieties) in their structures. Because the established molecular formula ($C_{52}H_{88}O_{19}$) of octacyclomycin (**1**) is different from these known antibiotics, **1** was shown to be a new polyether antibiotic.

Because the $^1H$ and $^{13}C$ NMR spectral data of octacyclomycin (**1**) were similar to those of UK-58,852 (**2**), spectral data of **1** and **2** were compared carefully. The total number of carbons and hydrogens of octacyclomycin (**1**) were the same with those of **2** but **1** possesses an excess oxygen atom in its structure. Through further comparison of $^1H$ and $^{13}C$ NMR spectral data, it was shown that most signals including a carboxyl signal ($\delta_C$ 178.7), a methoxyl signal attributed to 6-$OCH_3$ ($\delta_C$ 60.3), C-2 signal ($\delta_C$ 45.3), signals attributed to 4-*O*-methylamicetose moieties and most of the methine and methylene signals were assigned straightforwardly (Table 2). Whereas the number of methyl signals of octacyclomycin (**1**) is 9 instead of 10 in UK-58,852 (**2**). Namely, the NMR spectra of **1** lack a methyl signal corresponding to 29-$CH_3$ in **2** ($\delta_C$ 26.06, $\delta_H$ 1.24). On the other hand, an additional methylene signal at $\delta_C$ 65.1 ($\delta_H$ 3.22 (1H, d, $J$=12 Hz) and 3.98 (1H, d, $J$=12 Hz))

Fig. 2. FAB mass spectrum of octacyclomycin (**1**) Na salt.[a]




[a] Obtained with a Jeol model JMS-AX500 mass spectrometer.

Table 2. $^{1}H$ and $^{13}C$ NMR chemical shifts of octacyclomycin (**1**) Na salt and UK-58,852 (**2**) in $CDCl_3$.

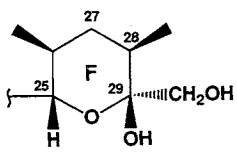
| Carbon | | **1** | | **2**[4] | |
|---|---|---|---|---|---|
| | Index | $\delta_C$[a] | $\delta_H$[a] | $\delta_C$[a] | $\delta_H$[a] |
| 1 | COOH | 178.7 | — | 179.19 | — |
| 2 | $CH_2$ | 45.3 | 2.20 | 45.74 | 2.11 |
| | | | 2.62 | | 2.47 |
| 3 | C | 98.1 | — | 97.16 | — |
| 4 | CH | 44.8 | 1.40 | 44.79 | 1.44 |
| 4-$CH_3$ | $CH_3$ | 12.6 | 1.03 | 12.40 | 0.99 |
| 5 | CH | 82.1 | 3.69 | 81.73 | 3.74 |
| 6 | CH | 82.8 | 3.08 | 82.58 | 3.13 |
| 6-$OCH_3$ | $CH_3O$ | 60.3 | 3.48 | 59.58 | 3.45 |
| 7 | CH | 67.9 | 3.72 | 67.39 | 3.65 |
| 8 | CH | 34.6[b] | 1.80 | 33.54 | 1.96 |
| 8-$CH_3$ | $CH_3$ | 10.9 | 1.05 | 11.04 | 1.01 |
| 9 | CH | 68.8 | 3.98 | 67.73 | 4.18 |
| 10 | CH | 33.7[b] | 1.85 | 33.66 | 1.73 |
| 10-$CH_3$ | $CH_3$ | 10.6 | 0.85 | 10.40 | 0.79 |
| 11 | CH | 69.9 | 3.96 | 70.19 | 3.88 |
| 12 | $CH_2$ | 33.6 | 1.69 | 33.90 | 1.60 |
| | | | 1.97 | | 1.87 |
| 13 | C | 106.9 | — | 107.59 | — |
| 14 | $CH_2$ | 39.2 | 1.59 | 38.96 | 1.67 |
| | | | 1.94 | | 1.92 |
| 15 | $CH_2$ | 33.3 | 1.65 | 33.48 | 1.69 |
| | | | 2.10 | | 2.01 |
| 16 | C | 84.4[c] | — | 84.55 | — |
| 16-$CH_3$ | $CH_3$ | 27.3 | 1.40 | 27.65 | 1.44 |
| 17 | CH | 82.5 | 3.46 | 82.43 | 3.50 |
| 18 | $CH_2$ | 28.5 | 1.47 | 26.85 | 1.42 |
| | | | 1.63 | | 1.63 |
| 19 | $CH_2$ | 32.3 | 1.40 | 32.31 | 1.41 |
| | | | 2.61 | | 2.37 |
| 20 | C | 83.5[c] | — | 84.20 | — |
| 20-$CH_3$ | $CH_3$ | 24.7 | 1.17 | 23.24 | 1.08 |
| 21 | CH | 87.2 | 3.81 | 87.08 | 4.01 |
| 22 | CH | 80.8 | 4.10 | 80.94 | 4.12 |
| 23 | $CH_2$ | 33.2 | 1.90 | 32.54 | 2.21 (2H) |
| | | | 2.29 | | |
| 24 | CH | 78.1 | 4.54 | 80.34 | 4.46 |
| 25 | CH | 74.8 | 3.82 | 73.05 | 3.90 |
| 26 | CH | 31.7 | 1.31 | 33.21 | 1.19 |
| 26-$CH_3$ | $CH_3$ | 16.7[d] | 0.82 | 17.48 | 0.82 |
| 27 | $CH_2$ | 35.3 | 1.33 (2H) | 36.54 | 1.28 |
| | | | | | 1.35 |
| 28 | CH | 36.1 | 1.42 | 39.97 | 1.38 |
| 28-$CH_3$ | $CH_3$ | 16.2[d] | 0.82 | 16.96 | 0.86 |
| 29 | C | 97.7 | — | 96.93 | — |
| 29-$CH_2OH$ | $CH_2$ | 65.1 | 3.22 | — | — |
| | | | 3.98 | | |
| 29-$CH_3$ | $CH_3$ | — | — | 26.06 | 1.24 |
| 1′ | CH | 102.4 | 4.71 | 102.41 | 4.65 |
| 2′ | $CH_2$ | 31.1 | 1.47 | 31.10 | 1.48 |
| | | | 1.89 | | 1.87 |
| 3′ | $CH_2$ | 27.3[e] | 1.34 | 27.39 | 1.25 |
| | | | 2.18 | | 2.13 |

Table 2. (Continued)

| Carbon | 1 | | | 2[4] | |
|---|---|---|---|---|---|
| | Index | $\delta_C$[a] | $\delta_H$[a] | $\delta_C$[a] | $\delta_H$[a] |
| 4′ | CH | 80.5[f] | 2.79 | 80.58 | 2.76 |
| 4′-$OCH_3$ | $CH_3O$ | 56.9[g] | 3.34 | 56.78 | 3.30 |
| 5′ | CH | 74.4[h] | 3.27 | 74.44 | 3.24 |
| 5′-$CH_3$ | $CH_3$ | 18.3[i] | 1.23 | 18.28 | 1.19 |
| 1″ | CH | 103.3 | 4.41 | 103.22 | 4.38 |
| 2″ | $CH_2$ | 30.6 | 1.47 | 30.62 | 1.51 |
| | | | 1.75 | | 1.75 |
| 3″ | $CH_2$ | 26.9[e] | 1.27 | 26.99 | 1.25 |
| | | | 2.18 | | 2.14 |
| 4″ | CH | 79.9[f] | 2.82 | 79.95 | 2.76 |
| 4″-$OCH_3$ | $CH_3O$ | 56.8[g] | 3.34 | 56.82 | 3.30 |
| 5″ | CH | 74.7[h] | 3.30 | 74.67 | 3.24 |
| 5″-$CH_3$ | $CH_3$ | 18.4[i] | 1.25 | 18.40 | 1.20 |

[a] NMR spectra were recorded on a Varian XL-400 instrument in $CDCl_3$ solution and the data were expressed in $\delta$ ppm from TMS.
[b~i] Assignments may be interchanged.

Fig. 3. Partial structure (F ring) and $^{13}C$ NMR assignments of nigericin.[5]




| Carbon | $\delta_C$ |
|---|---|
| 25 | 76.9 |
| 26 | 31.9 |
| 26-$CH_3$ | 17.0 |
| 27 | 37.2 |
| 28 | 36.8 |
| 28-$CH_3$ | 16.4 |
| 29 | 97.2 |
| 29-$CH_2OH$ | 67.2 |

was newly appeared in the NMR spectrum of **1**. Hypothesis of the existence of an excess oxygen atom in its F ring is supported by the similarity of the $^{13}C$ NMR spectral data of F ring with those of nigericin (Fig. 3)[5] especially the higher field shift of the signal attributed to C-28 position. Thus the one oxygen difference in their molecular formula between octacyclomycin (**1**) and UK-58,852 (**2**) was explained by the presence of a hydroxymethyl moiety in **1** in place of the methyl group in the molecule of **2** and the structure of octacyclomycin was concluded to be **1**.

Cytocidal activity and antimicrobial activity tests were performed as described previously.[6] Octacyclomycin (**1**) showed cytocidal activity against B16 melanoma cells and the $IC_{50}$ value was 0.23 μg/ml when the cells were exposed to the antibiotic for 3 days *in vitro*. On the other hand, **1** showed weak antimicrobial activity against Gram-positive bacteria such as *Staphylococcus aureus* KB 34 (FDA 209P) and *Micrococcus luteus* KB 40 (PCI 1001) at the concentration of 100 μg/ml, whereas *Bacillus subtilis* KB 27 (PCI 219) was not affected at this concentration. *B. subtilis* growth was incompletely inhibited at the concentration of 500 μg/ml. The antibiotic showed no activity against other microorganisms tested (*Xanthomonas oryzae* KB 88, *Candida albicans* KF 1, *Saccharomyces sake* KF 26, *Mucor racemosus* KF 223 (IFO 4581), *Piricularia oryzae* KF 180, *Aspergillus niger* KF 103 (ATCC 6275), *Escherichia coli* KB 8 (NIHJ), *E. coli* KB 176 (NIHJ JC-2), *Pseudomonas aeruginosa* KB 105 (P3), *Bacteroides fragilis* KB 169, *Mycobacterium smegmatis* KB 42 (ATCC 607) and *Acholeplasma laidlawii* PG 8 KB 174) at the concentration of 500 μg/ml.

Acknowledgments

This work was supported, in part, by Grants-in-Aid from the Ministry of Health and Welfare, the Ministry of Education, Science and Culture, Japan and by funds from Japan Keirin Association. The authors would like to thank Ms. A. HATANO, School of Pharmacy, Kitasato University for recording NMR spectra and Mr. K. KAWAMURA, Pharmaceutical Institute, Tohoku University for FAB-MS data.

## References

1) UMEZAWA, I.; C. TRONQUET, S. FUNAYAMA, K. OKADA & K. KOMIYAMA: A novel antibiotic, sohbumycin. Taxonomy, fermentation, isolation and

physico-chemical and biological characteristics. J. Antibiotics 38: 967~971, 1985

2) SIEGEL, M. M.; W. J. MCGAHREN, K. B. TOMER & T. T. CHANG: Applications of fast atom bombardment mass spectrometry and fast atom bombardment mass spectrometry-mass spectrometry to the madumycins and other polyether antibiotics. Biomedical and Environmental Mass Spectrometry 14: 29~38, 1987

3) TSUJI, N.; K. NAGASHIMA, Y. TERUI & K. TORI: Structure of K-41B, a new diglycoside polyether antibiotic. J. Antibiotics 32: 169~172, 1979

4) DIRLAM, J. P.; W. P. CULLEN, L. H. HUANG, T. H. NELSON, J. R. OSCARSON, L. PRESSEAU-LINABURY, E. J. TYNAN & E. B. WHIPPLE: CP-91,243 and CP-91,244, novel diglycoside polyether antibiotics related to UK-58,852 and produced by mutants of *Actinomadura roseorufa*. J. Antibiotics 44: 1262~1266, 1991

5) SETO, H. & N. OTAKE: The $^{13}C$ NMR spectra of polyether antibiotics and some empirical rules for structural studies of polyether antibiotics. Heterocycles 17: 555~580, 1982

6) KOMIYAMA, K.; S. FUNAYAMA, Y. ANRAKU, M. ISHIBASHI, Y. TAKAHASHI, T. KAWAKAMI & S. ŌMURA: A new antibiotic, okicenone. I. Taxonomy, fermentation, isolation and biological characteristics. J. Antibiotics 44: 814~818, 1991