BIOSYNTHESIS OF FORMYCIN

ROLE OF CERTAIN AMINO ACIDS IN FORMYCIN BIOSYNTHESIS

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(Received for publication September 20, 1974)

Working with *Streptomyces* sp. MA406-A-1, effects of addition of various amino acids on the production of formycin were examined. In replacement culture, it was confirmed that lysine, aspartate or glutamate stimulates the production of formycin and carbon molecules in these amino acids incorporated efficiently into formycin. It is estimated that biosynthesis of formycin in this organism is closely related to lysine metabolism but not to biosynthesis of purine nucleosides.

Formycin was originally isolated as an antitumor antibiotic from the culture filtrates of *Nocardia interforma*[1] and identified as 7-amino-3-β-D-ribofuranosyl-1H-pyrazolo-[4, 3d] pyrimidine[2~7]. This antibiotic, having been also found in cultured broth of *Streptomyces lavendulae*[8] or *S. gummaensis*[9], is one of microbial products which contain an unusual C-riboside linkage in their molecules. The other natural C-riboside products of microorganisms are formycin B[2,8,10], oxoformycin B[11,12], pseudouridine[13~15], showdomycin[16~18], pyrazomycin[19~22] and minimycin[23,24]. Formycin, formycin B and oxoformycin B are pyrazolopyrimidine nucleosides and structural analogs of adenosine, inosine and xanthosine, respectively. Formycin has been found to be active against Ehrlich carcinoma in mice, mouse leukemia L-1210, Yoshida rat sarcoma cells, HeLa cells, influenza virus A-1 in cells of chick choriallantoic membrane, *Xanthomonas oryzae*, *Mycobacterium* 607 and certain other microorganisms[1,10,26~30], whereas formycin B is active only against influenza virus A-1[25,29~31], tobacco mosaic virus[32], *X. oryzae*, *Pellicularia filamentosa* or *Pseudomonas dacunhae*[8,25,28] and oxoformycin B does not show significant antibiotic activity against mammalian tumor cells, viruses and many microorganisms but shows weak activity against *X. oryzae*[11,25].

There have been several investigations on the biosynthesis of these C-ribonucleosides. It was confirmed that pseudouridine and its 5′-nucleotide seem likely to occur by either uridine-pseudouridine interconversion through an intramolecular rearrangement in tRNA[33], enzymatic condensation by pseudouridylate synthetase of uracil and ribose 5-phosphate[14,15] or the formation of 1, 5-diribosyl-uracil[34]. The accumulation of pseudouridine at a high concentration in culture filtrates of *Streptoverticillium ladakanus*, in which the activity of the synthetase was not detectable, was also reported, indicating that the physiological role of the synthetase is to act as a degradative enzyme to hydrolyze pseudouridine to uracil and ribose[13]. This estimation is in agreement with the hypothesis which has been proposed from the experi-

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mental results with pyrimidine auxotrophs of *Escherichia coli*[35]. On the biosynthesis of showdomycin, the maleimide C-riboside antibiotic, it has been shown that C-2, C-3, C-4 and C-5 of 2-oxoglutarate serve as the precursor for C-2, C-3, C-4 and C-5 of the maleimide ring, respectively, and the ring, thus formed, will condense as an asymmetrical unit with D-ribose to form showdomycin[36~38]. These findings were the first case of the biosynthesis of a nucleoside antibiotic in which purine or pyrimidine nucleosides and/or nucleotides are not the precursors. Although the biosynthesis of pyrazomycin, a pyrazol derivative of the C-riboside antibiotic, still remained to be studied, it has been proposed that biosynthesis by *N. interforma* takes place *via* the following pathway: formycin B monophosphate→formycin monophosphate→formycin→formycin B→oxoformycin B[12] and it was suggested that none of free adenine, adenosine, adenylic acid or RNA-adenine is a direct precursor for the formycin family while ribose added exogenously is estimated to incorporate into the ribosyl moiety of formycin[39]. It is very interesting that the biosynthesis of showdomycin and formycin seem not to be related to purine or pyrimidine biosynthesis.

The present paper concerns the biosynthesis of formycin of *Streptomyces* sp. MA406-A-1 with special respect to the incorporation of carbon molecule of lysine into formycin moiety.

Materials and Methods

Organisms

Streptomyces sp. MA406-A-1, a formycin-producing strain, and *X. oryzae* were obtained from the Institute of Microbial Chemistry, Tokyo.

Media

MPY-medium (organic medium): maltose, 3%; Polypeptone, 3%; yeast extract, 1%; NaCl, 0.5%; KCl 0.05%; $MgSO_4 \cdot 7H_2O$, 0.05%; $ZnSO_4 \cdot 7H_2O$, 0.001% and M/15 phosphate buffer (pH 7), 10%. Before addition of the buffer, the mixture was neutralized with 2 N NaOH, then the buffer was added, followed by sterilization at 120°C for 20 minutes.

GA-medium (chemically defined medium): glucose, 3%; ammonium citrate, 1%; NaCl, 0.5%; KCl, 0.05%; $MgSO_4 \cdot 7H_2O$, 0.05%; $ZnSO_4 \cdot 7H_2O$, 0.001% and M/15 phosphate buffer (pH 7), 10%. Adjustment of pH and sterilization were carried out by the same methods as MPY-medium.

GL-medium (medium for replacement culture); glucose, 10 mM; lysine, 10 mM; NH_4Cl, 20 mM; $MgSO_4 \cdot 7H_2O$. 1 mM and phosphate buffer (pH 7.0), 0.1 M. This medium was used in replacement culture without sterilization.

Cultivation

The experiments with MPY-medium employed a seed culture prepared by 2 days' shaking at 27°C in the same medium. A 1-ml aliquot was inoculated into each SAKAGUCHI-flask (500-ml volume) containing 50 ml of medium and the cultivation was carried out at 27°C under reciprocal shaking (125 s.p.m.; 5.5 cm).

In the experiments with GA-medium, a seed culture was prepared by 2 days' shaking at 27°C with the modified GA-medium (GA-medium plus yeast extract, 0.2%) and the inoculation and cultivation (5 days) were carried out by the same methods as the experiments with MPY-medium. In the experiments with replacement culture, cells were harvested and washed three times with M/15 phosphate buffer (pH 7.0) by centrifugation (5°C; 8,000 r.p.m; 3 min.) and the cell-density in GL-medium was adjusted to a definite value corresponding to 0.5 of optical density at 590 mμ. Optical density was measured after 1/20 dilution. The replacement culture was performed at 27°C for 9 hours with vigorous shaking in an ERLENMEYER flask (500-ml volume) or a test tube (50-ml volume) containing 50 ml or 5 ml of GL-medium, respectively. When

addition of amino acid was made, the final concentration of each amino acid in the GL-medium was adjusted to 10^{-2}M.

Determination

Formycin was determined by a cylinder-plate method with *X. oryzae* as a test organism. Specific radioactivity of formycin, adenosine, guanosine or inosine was calculated by the following method. After the supernatant of replacement culture was acidified to pH 2 with conc. HCl it was mixed with active carbon and allowed to stand for several hours at room temperature. The active carbon was collected on the glass-filter, washed first with dil. HCl (pH 2) and then with water. Elution was made with 20% ethanol in 2 N NH_4OH, followed by evaporation to dryness at 40°C under vacuum. After the residue was dissolved in 25 mM NH_4Cl (adjusted to pH 10.5 with conc. NH_4OH), it was applied to a column of Dowex-1×4 (200~400 mesh; 7 mm×50 cm) in the chloride form. The elution was made at 50°C with 1 mM HCl in 25 mM NH_4Cl and the eluate was cut into each 10 ml. The optical densities in eluate at 230, 260 and 280 mμ were continuously recorded using a Hitachi UV-VIS Effluent Monitor Model-034. Radioactivities in the eluted fractions were measured in liquid scientillator of toluene solution consisting of 0.3% (w/v) of 2, 5-diphenyloxazol, 0.0023% (w/v) of 1, 4-bis [2-(5-phenyloxazolyl)]-benzene and 25% (v/v) of Triton-X. The specific radioactivity was determined from the radioactivity and the concentration of formycin or purine nucleosides calculated from the optical density in the eluate.

Reagent

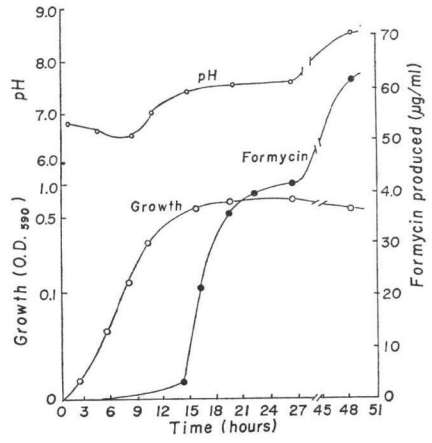
The reagents used for liquid scintillator were purchased from Wako Pure Chemical Co., Ltd. and ^{14}C-labeled compounds were from Daiichi Chemical Co., Ltd. All amino acids employed in the experiments are of L-form unless otherwise specified.

Results

Effect of Amino Acids on Production of Formycin

As shown in Fig. 1, formycin accumulated to the concentration of about 60 μg/ml after 48 hours in MPY-medium and the accumulation occurred at an interval of late logarithmic to stationary phase of growth. Although MPY-medium is an organic medium, preliminary experiments were made to assess whether Polypeptone or yeast extract in the medium is responsible for production of formycin. It was found that formycin was produced in maltose-Polypeptone medium (without yeast extract) at the same concentration as in MPY-medium, indicating the possibility of biosynthesis of formycin from certain amino acid(s) in Polypeptone.

Fig. 1. Time course of formycin production by *Streptomyces* sp. MA406-A-1 in MPY-medium
The mycelial growth was estimated by measuring the optical density at 590 mμ after 1/20 dilution of the cultured broth.



These results led us to examine the effects of adding various amino acids to a chemically defined medium (GA-medium) on the production of formycin. Stimulative effects were observed when lysine, asparagine or hydroxyproline was added. The results are

Table 1. Effect of certain amino acids on formycin production in growing culture with the synthetic medium (GA-medium)

Amino acid added (10^{-2}M)	Formycin produced (μg/ml)	Growth (O.D. 590mμ)
Glycine	5	0.24
Alanine*	5	0.22
Valine	5	0.42
Leucine	5	0.22
Isoleucine	5	0.20
Serine	11	0.28
Threonine*	5	0.28
Phenylalanine*	16	0.68
Tyrosine	13	0.28
Tryptophan	7	0.27
Cystine	5	0.22
Methionine	5	0.34
Proline	5	0.28
Hydroxyproline	19	0.61
Aspartate	5	0.24
Asparagine	45	0.72
Glutamate	6	0.28
Glutamine	7	0.23
Histidine	5	0.17
Lysine	55	0.85
Arginine	5	0.20
Ornithine	5	0.23
Citrulline	13	0.69
D-Lysine	5	0.24
None	8	0.20

* DL-Amino acid was added at a concentration of 2×10^{-2}M.

Table 2. Effect of certain amino acids on formycin production in replacement culture with G-medium*

Amino acid added (10^{-2}M)	Formycin produced (μg/ml)
Glycine	14
Serine	16
Lysine	36
Tyrosine	13
Citrulline	13
Isoleucine	13
Hydroxyproline	5
Phenylalanine**	11
Tryptophan	11
Aspartate	22
Asparagine	20
Glutamate	23
Glutamine	20
None	13

When 10^{-1}M phosphate buffer (pH 7.0) was used instead of G-medium, 12 μg/ml of formycin was produced (endogenous formation).

* Glucose, 10mM; NH_4Cl, 20mM; $MgSO_4\cdot7H_2O$, 1mM and phosphate buffer (pH 7.0), 0.1M.

** DL-Amino acid was added at a concentration of 2×10^{-2}M.

shown in Table 1. Since it was found, however, that these amino acids show their stimulative activity for both production of formycin and growth of the organism, it seemed required for the estimation of precursor(s) to repeat these experiments under conditions such as replacement culture where cell-growth does not take place but formycin is still produced. From the results shown in Fig. 1, the cells prepared from MPY-medium after incubation for 15~18 hours were estimated to be able to produce formycin even in a replacement culture. It was found, however, that cells obtained after 15 to 18 hours of incubation produce a small amount (10~15 μg/ml) of formycin, whereas cells from 10 to 12 hours of incubation produce an increased amount (35~40 μg/ml) of the antibiotic under the conditions described in Materials and Methods. Throughout the experiments with replacement culture, therefore, the washed cells were prepared after 10~12 hours of incubation in MPY-medium.

As shown in Table 2, it is very interesting that lysine, just as in GA-medium (Table 1), stimulates actively the production of formycin in replacement culture and that aspartate, asparagine, glutamate or glutamine are also stimulative to some extent. When, however, aspartate, asparagine, glutamate or glutamine was added with lysine to the medium, formycin production was not stimulated additively suggesting that lysine may act independently in some

Table 3. Specific stimulative effect of lysine on formycin production among various compounds related to lysine metabolism or structure of lysine (Replacement culture; G-medium*)

Compound added (10^{-2}M)	Formycin produced (μg/ml)
Lysine	41
D-Lysine	22
α, ε-Diaminopimelate**	13
α-Aminoadipate**	13
Pipecolate	13
Cadaverine	12
δ-Amino-*n*-valerate	17
Norleucine**	12
Norvaline**	12
Adipate	11
ε-Amino-*n*-caproate	13
n-Caproate	12
n-Capronaldehyde	15
None	14

* Components of G-medium are described in Table 2.

** DL-Form was added at a concentration of 2×10^{-2}M.

important role in the biosynthesis of formycin.

On the differences of the stimulative actions of certain amino acids between growing culture (GA-medium) and replacement culture, the details still remain to be studied.

Results in Table 3 show that, except for D-lysine, the substances which have been estimated to be closely related to lysine metabolism in micro-organisms cannot act in the place of L-lysine.

Isolation and Identification of Formycin from Replacement Culture

To assess the possibility that antibiotic(s) other than formycin is produced in replacement culture with the presence of lysine, the compound(s) which is active against *X. oryzae* should be isolated for identification. From culture filtrates of replacement culture, the antibiotic which is active against *X. oryzae* was isolated by the following procedure; treatment with active carbon, separation by column chromatography of Dowex-1 and Dowex-50, and repeated crystallization from water to obtain crystals (needle, colorless). The crystalline antibiotic, thus obtained, was identified as formycin on the basis of its identical characteristics with authentic formycin in melting point, NMR spectrum, IR and ultraviolet absorption, and fragmentation pattern in mass-spectrometry. It was also confirmed that neither formycin B nor any other antibiotic than formycin is accumulated in the culture filtrates of replacement culture.

Incorporation of Radioactivity into Formycin Moiety from ^{14}C-Labeled Amino Acids in Replacement Culture

Carbon sources of the medium for replacement culture are glucose and lysine and it was reasonable to examine the relative ratio of incorporation of radioactivity into the formycin molecule from ^{14}C-U-lysine and ^{14}C-U-glucose. As shown in Table 4, they incorporated efficiently into formycin molecule but not efficiently into the molecule of adenosine, guanosine or inosine which is accumulated in the filtrates of replacement culture.

These results suggest that *de novo* biosynthesis of formycin occurred during replacement culture while net synthesis of purine nucleosides did not and indicated the different mechanism of biosynthesis of formycin from those of normal purine nucleosides. It was also found that the incorporation of carbon atom from lysine into RNA and DNA extracted from the cells after the replacement culture was not detected, indicating that these nucleic acids may not be involved in the biosynthetic pathway of formycin. Since total specific activity of carbon molecule in formycin was 63% (total % of specific activity of carbon molecule in formycin

Table 4. Incorporation of radioactivity from lysine or glucose into formycin or nucleosides accumulated in GL-medium after replacement culture

Labeled compound used	Percent specific radioactivities* of C-atom in		
	Formycin	Adenosine	Guanosine and inosine**
^{14}C-U-Lysine	18.0	8.5	5.6
^{14}C-U-Glucose	44.8	—***	7.4

* $\%$ Specific radioactivity $= \frac{\text{Specific radioactivity of C-atom in formycin or nucleosides}}{\text{Specific radioactivity of C-atom in labeled compound}} \times 100$

** In the column chromatography (see Materials and Methods), both guanosine and inosine are eluted into the same fraction. On the basis of similarity of molar extinction coefficients of these nucleosides at 250 mμ, the molar concentration was calculated tentatively using the coefficient of guanosine.

*** Since accumulation of adenosine was not detectable, the calculation was impossible.

Table 5. Incorporation of certain amino acids into formycin in replacement culture*

Compound added	Percent specific activities** of C-atom in		
	Formycin	Adenosine	Guanosine and inosine***
^{14}C-U-Lysine	18.0	8.5	5.6
+Glycine	16.3	—****	4.3
+Glutamine	17.7	4.2	4.9
+Aspartate	14.5	8.1	6.4
Lysine			
+^{14}C-U-Glycine	7.4	0.8	2.7
+^{14}C-U-Glutamate	24.3	5.1	2.3
+^{14}C-U-Aspartate	15.4	0	3.4

* Where indicated, glycine, glutamine, glutamate or aspartate was added to GL-medium at a concentration of 10^{-2}M.

** Specific radioactivity was calculated as described in Table 4.

*** Concentration of guanosine and inosine was calculated as described in Table 4.

**** Since accumulation of adenosine was not detectable, the calculation was impossible.

arising from ^{14}C-U-glucose and ^{14}C-U-lysine), it was estimated that one third of the formycin accumulated should be produced *via* endogenous formation and, indeed, this endogenous formation was confirmed quantitatively in the medium from which glucose, lysine and NH_4Cl were omitted (Table 2).

As aspartate or asparagine, and glutamate or glutamine give also stimulus to production of formycin (Table 2), ratio of incorporation of radioactivities into formycin molecule from these ^{14}C-labeled amino acids were examined and results are shown in Table 5.

It is worth noting that carbon atom in glutamate and aspartate as well as lysine incorporated more efficiently into formycin than glycine which has been confirmed as a precursor of C-4 and C-5 of purine ring. It was also found that the ratio of the incorporation of lysine into formycin is not significantly affected by the addition of glutamine, aspartate or glycine indicating that lysine is closely related to the biosynthesis of formycin.

Discussion

On the biosynthesis of formycin in *N. interforma*, it has been reported that formycin may be formed from formycin 5'-monophosphate which is an aminated product of formycin B 5'-monophosphate[12] and that the major part of biosynthesis of the pyrazolopyrimidine ring in the formycin molecule does not seem to be related to purine biosynthesis[8,9]. However, the major part of the mechanism of formycin formation has remained obscure.

According to the data described in this paper, however, lysine seems to act an important role in biosynthesis of formycin in *Streptomyces* sp. MA406-A-1. It was also found that carbon molecules from aspartate and glutamate incorporated efficiently into formycin without affecting the ratio of lysine incorporation. Therefore, it can be estimated that formycin is formed *via* a novel pathway which is closely related to metabolism of these amino acids.

Acknowledgments

The authors express their appreciations to Dr. Y. SASAKI for his interest and encouragement, and to M. MUNEKATA, I. TAKAHASHI and T. KURODA for compling the raw experimental data. Their thanks are also due to Dr. T. NIIDA for his kind supply of the authentic formycin and formycin B. This investigation was supported in part by grant-in-aid from Association of Amino Acid and Nucleic Acid, Japan.

References

1) HORI, M.; E. ITO, T. TAKITA, G. KOYAMA, T. TAKEUCHI & H. UMEZAWA: A new antibiotic, formycin. J. Antibiotics, Ser. A 17: 96~99, 1964
2) KOYAMA, G.; K. MAEDA, H. UMEZAWA & Y. IITAKE: The structural studies of formycin and formycin B. Tetrahedron Letters 1966: 597~602, 1966
3) ROBINS, R.K.; L.B. TOWNSEND, F. CASSIDY, J.F. GERSTER, A.F. LEWIS & R.L. MILLER: Structure of the nucleoside antibiotics formycin, formycin B and laurusin. J. Heterocycl. Chem. 3: 110~114, 1966
4) PRUSINER, P.; T. BRENNAN & M. SUNDARALINGUM: Crystal structure and molecular conformation of formycin monohydrates. Possible origin of the anomalous circular dichroic spectra in formycin mono- and polynucleotides. Biochemistry 12: 1196~1202, 1973
5) WARD, D.C. & E. REICH: Conformational properties of polyformycin. A polyribonucleotide with individual residue in the syn conformation. Proc. Nat. Acad. Sci. 61: 1494~1501, 1968
6) TOWNSEND, L.B. & R.K. ROBINS: The mass spectra of formycin, formycin B and showdomycin carbon linked nucleoside antibiotics. J. Heterocycl. Chem. 6: 459~464, 1969
7) KRUGH, T.R.: Tautomerism of the nucleoside antibiotic formycin, as studied by C-13 nuclear magnetic resonance. J. Am. Chem. Soc. 95: 4761~4762, 1973
8) AIZAWA, S.; T. HIDAKA, N. ŌTAKE, H. YONEHARA, K. ISONO, N. IGARASHI & S. SUZUKI: Studies on a new antibiotic, laurusin. Agr. Biol. Chem. 29: 375~376, 1965
9) Japanese Patent No. 10,928, 1967 (Nippon Kayaku Co., Ltd.)
10) UMEZAWA, H.; T. SAWA, Y. FUKAGAWA, G. KOYAMA, M. MURASE, M. HAMADA & T. TAKEUCHI: Transformation of formycin to formycin B and their biological activities. J. Antibiotics, Ser. A 18: 178~181, 1965
11) ISHIZUKA, M.; T. SAWA, G. KOYAMA, T. TAKEUCHI & H. UMEZAWA: Metabolism of formycin and formycin B *in vivo*. J. Antibiotics 21: 1~4, 1968
12) SAWA, T.; Y. FUKAGAWA, I. HOMMA, T. WAKASHIRO, T. TAKEUCHI, M. HORI & T. KOMAI: Metabolic conversion of formycin B to formycin A and oxoformycin B in *Nocardia interforma*. J. Antibiotics 21: 334~339, 1968
13) UEMATSU, T. & R.J. SUHADOLNIK: Pseudouridine, isolation and biosynthesis of the nucleoside isolated from the culture filtrates of *Streptoverticillium ladakanus*. Biochemistry 11: 4669~4674, 1972
14) HEINRIKSON, R.L. & E. GOLDWASSER: Studies on the biosynthesis of 5-ribosyluracil 5'-monophosphate in *Tetrahymena pyriformis*. J. Biol. Chem. 239: 1177~1187, 1964

15) SUZUKI, T. & R. M. HOCHSTER: On the biosynthesis of pseudouridylic acid in *Agrobacterium tumefaciens*. Cand. J. Biochem. 44: 259~272, 1966
16) NISHIMURA, H.; M. MAYAMA, Y. KOMATSU, H. KATO N. SHIMAOKA & Y. TANAKA: A novel antibiotic, showdomycin. J. Antibiotics, Ser. A 17: 148~152, 1964
17) DARNALL, K.R.; L.B. TOWNSEND & R.K. ROBINS: The structure of showdomycin, a novel carbon-linked antibiotic related to uridine. Proc. Nat. Acad. Sci. 57: 548~553, 1967
18) NAKAGAWA, Y.; H. KANŌ, Y. TSUKADA & H. KOYAMA: Structure of a new class of C-nucleoside antibiotic, showdomycin. Tertrahedron Letter 1967: 4105~4109, 1967
19) GERZON, K.; R. H. WILLIAMS, H. HOEHN, M. GORMAN & D. C. DELONG: Pyrazomycin, a C-nucleoside with antiviral activity. Abs. 2nd Intern. Cong. Heterocyclic Chem.: C-30, 1969 (Montpellier, France)
20) WILLIAMS, R.H.; K. GERZON, M. HOEHN & D.C. DELONG: Pyrazomycin, a novel carbon-linked nucleoside. Abs. 158th National Meeting, Am. Chem. Soc., New York, 1969
21) SWEENY, M.J.; F. A. DAVIS, G. E. GUTOWSKY, R. L. HAMILL, D. H. HOFEMAN & G. A. POORE: Experimental antitumor activity of pyrazomycin. Cancer Res. 33: 2619~2623, 1973
22) SWEENY, M.J.; G.E. GUTOWSKY, G.A. POORE & R.L. HAMILL: Pyrazomycin, a new antitumor antibiotic. Proc. Am. Assoc. Cancer Res. 13: 108, 1972
23) KUSAKABE, Y.; J. NAGATSU, M. SHIBUYA, O. KAWAGUCHI, C. HIROSE & S. SHIRATO: Minimycin, a new antibiotic. J. Antibiotics 25: 44~47, 1972
24) SASAKI, K.; Y. KUSAKABE & S. ESUMI: The structure of minimycin, a novel carbon-linked nucleoside antibiotic related to β-pseudouridine. J. Antibiotics 25: 151~154, 1972
25) KUNIMOTO, T.; T. WAKASHIRO, I. OKAMURA, T. ASAJIMA & M. HORI: Structural requirements for formycin activity. J. Antibiotics 21: 468~470, 1968
26) UMEZAWA, H.; T. SAWA, Y. FUKAGAWA, I. HOMMA, M. ISHIZUKA & T. TAKEUCHI: Studies on formycin and formycin B in cells of EHRLICH carcinoma and *E. coli*. J. Antibiotics, Ser. A 20: 308~316, 1967
27) HENDERSON, J. F.; A. R. P. PATERSON, I. C. CALDWELL & M. HORI: Biochemical effects of formycin, an adenosine analogue. Cancer Res. 27: 715~719, 1967
28) SAWA, T.; Y. FUKAGAWA, Y. SHIMAUCHI, K. ITO, M. HAMADA, T. TAKEUCHI & H. UMEZAWA: Studies on formycin and formycin B phosphates. J. Antibiotics, Ser. A 18: 259~266, 1965
29) TAKEUCHI, T.; J. IWANAGA, T. AOYAGI & H. UMEZAWA: Antiviral effect of formycin and formycin B. J. Antibiotics, Ser. A 19: 286~287, 1966
30) TAKEUCHI, T.; J. IWANAGA, T. AOYAGI, M. MURASE, T. SAWA & H. UMEZAWA: Antiviral effect of formycin derivatives. J. Antibiotics, Ser. A 20: 297~298, 1967
31) ISHIZUKA, M.; T. SAWA, M. HORI, H. TAKAYAMA, T. TAKEUCHI & H. UMEZAWA: Biological studies of formycin and formycin B. J. Antibiotics 21: 5~12, 1968
32) KUMMERT, J.: Effect of antiviral substances on different plant host-virus systems. Meded. Rijiksfac. Landboun Wetensch. **33**: 1195~1201, 1968 (Chem. Abs. 72: 10227e, 1970)
33) JOHNSON, L. & D. SÖLL: *In vitro* biosynthesis of pseudouridine at the polynucleotide level by an enzyme extract from *Escherichia coli*. Proc. Nat. Acad. Sci. 67: 943~950, 1970
34) DLUGAJCZYK, A. & J.J. EILER: A comparison of the properties of synthetic 1, 5- and 1, 3-diribosyl-uracils with the natural presumed 1, 5-diribosyl-uracil. Biochim. Biophys. Acta 119: 11~19, 1966
35) BREITMAN, T.R.: Pseudouridylate synthetase of *Escherichia coli*, correlation of its activity with utilization of pseudouridine for growth. J. Bact. 103: 264~265, 1970
36) ELSTNER, E. F. & R. J. SUHADOLNIK: Nucleoside antibiotics. Biosynthesis of the maleimide nucleoside antibiotic, showdomycin, by *Streptomyces showdoensis*. Biochemistry 10: 3608~3614, 1971
37) ELSTNER, E.F. & R.J. SUHADOLNIK: Nucleoside antibiotic. Glutamic acid and acetate into the maleimide ring of showdomycin by *Streptomyces showdoensis*. Biochemistry 11: 2578~2584, 1972
38) ELSTNER, E.F. & R.J. SUHADOLNIK: Effect of changes in the pool of acetate on the incorporation and distribution of ^{13}C- and ^{14}C-labeled acetate into showdomycin by *Streptomyces showdoensis*. J. Biol. Chem. 248: 5385~5388, 1973
39) KUNIMOTO, T.; T. SAWA, T. WAKASHIRO, M. HORI & H. UMEZAWA: Biosynthesis of the formycin family. J. Antibiotics 24: 253~258, 1971