CYCLIC PHOSPHATES OF FORMYCIN

OSAMU MAKABE,* AKIHIKO MIYADERA, MITSUHIRO KINOSHITA
and SUMIO UMEZAWA**

Department of Applied Chemistry, Faculty of Engineering, Keio University,
Hiyoshi, Kohoku-ku, Yokohama-shi, Japan

TOMIO TAKEUCHI

Institute of Microbial Chemistry, Tokyo, Japan

(Received for publication December 23, 1977)

The syntheses of N^1- and N^2-isopropylformycin (**10**, **11**), formycin 3′,5′-cyclic and 2′,3′-cyclic phosphate (**3**, **7**) and their *N*-methyl and *N*-isopropyl derivatives (**13**, **15**, **19**, **23**) are described. It was observed that substitution at N^1 or N^2 with a bulky alkyl group or cyclic phosphorylation of the ribose moiety made formycin resistant to adenosine deaminase.

The antibiotic formycin (**1**) was isolated by H. UMEZAWA and coworkers[1] from *Nocardia interforma* and found to have inhibitory activities against EHRLICH carcinoma, mouse leukemia L-1210, YOSHIDA sarcoma, HeLa cells, *Mycobacteria* and *Xanthomonas oryzae* as well as some antiviral activity.[1,2,3] Formycin is of particular interest since it is a *C*-nucleoside[4,5] analog of adenosine. The structure of formycin has been shown by X-ray analysis[4] and the total synthesis of formycin B has been achieved by ACTON *et al.*[5] The structural resemblance of formycin to adenosine suggests that formycin may act as a substrate to a number of adenosine-like enzymes such as adenosine kinase, adenosine deaminase, *etc.* Several formycin derivatives including N^7-methylformycin,[6] N^1- and N^2-methylformycin,[7] formycin anhydronucleosides,[8,9] have been synthesized for the biological investigation. In view of the biological importance of the analogs[11,12] of adenosine 3′,5′-cyclic phosphate (cAMP), it is of further interest to study some cyclic phosphate derivatives of formycin. The present paper presents the syntheses of formycin 3′,5′-cyclic phosphate (cFMP) (**3**), 2′,3′-cyclic phosphate (**7**) and their *N*-alkyl derivatives (**13**, **15**, **19**, **23**) and their biological activities.

Syntheses

Our initial attempts to prepare cFMP by several methods similar to those for cAMP did not give satisfactory results. The synthesis was achieved by use of trichloromethyl phosphonic acid dichloride (TPAD), a phosphorylating agent recently reported by MARUMOTO *et al.*[13] Reaction of formycin with this reagent in triethyl phosphate followed by chromatography gave formycin 5′-trichloromethyl phosphonate (**2**) in 72% yield. Hydrolysis of this intermediate by treatment with potassium *t*-butoxide afforded cFMP (**3**) in 66% yield.

Formycin 2′,3′-cyclic phosphate (**7**) was synthesized by the following reaction sequence. 2′,3′-*O*-Isopropylideneformycin (**4**)[8,9,10] was treated with benzoyl chloride in pyridine to give the tetrabenzoyl derivative (**5**) in 92% yield. Deisopropylidenation was effected by 98% formic acid. The deisopropylidenated product (**6**) was phosphorylated with phosphorous oxychloride in pyridine followed by hydroly-

* Present address: Research Laboratories, Meiji Seika Kaisha, Morooka-cho, Kohoku-ku, Yokohama, 222 Japan.

** Present address: Institute of Bio-organic Chemistry, Nakahara, Kawasaki, 211 Japan.

sis with saturated methanolic ammonia to afford formycin 2′,3′-cyclic phosphate (**7**).

N^1-Methyl and N^2-methylformycin (**8**, **9**) were prepared by reaction of formycin with methyl iodide and sodium ethylate according to the procedure of TOWNSEND *et al.*[7], yielding **8** and **9** in a ratio of 20 : 75 (total yield 95%).

Chart 1.

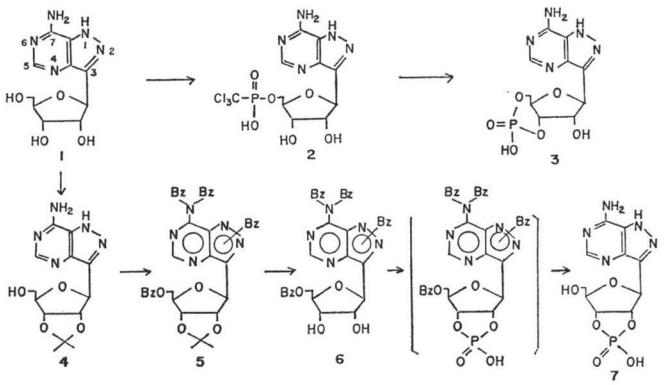
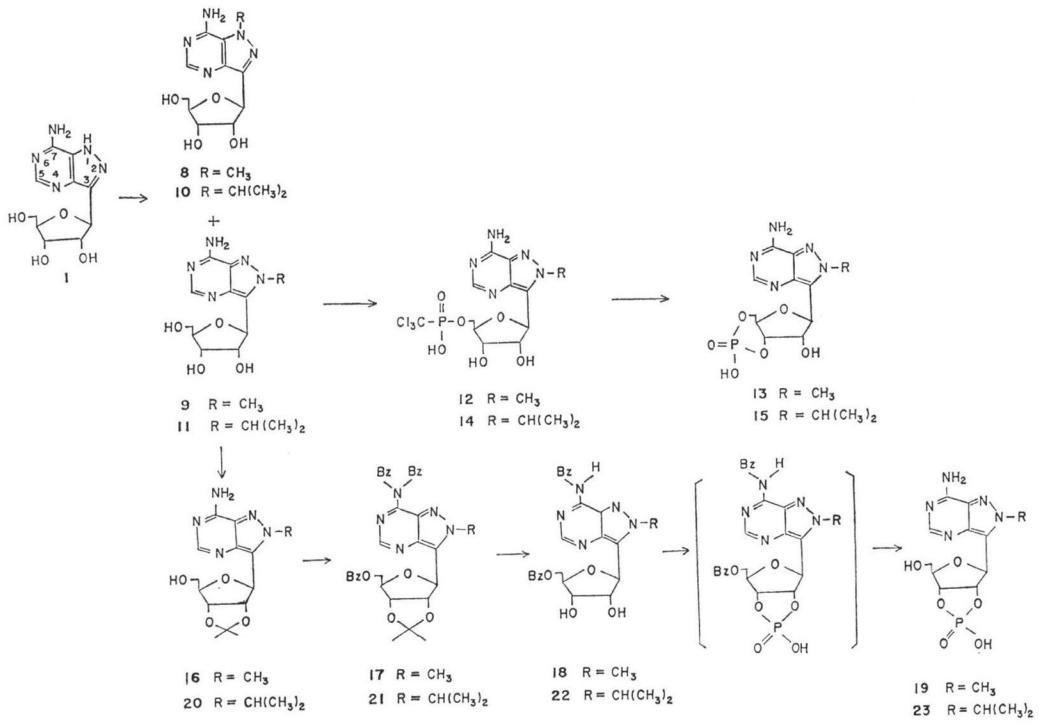


Chart 2.



N^1-Isopropyl- and N^2-isopropylformycin (**10**, **11**) were prepared by treatment of formycin with isopropyl iodide and sodium hydride in dimethylformamide, yielding **10** and **11** in a ratio of 6 : 87 (total yield 93%).

3′,5′-Cyclic phosphates (**13**, **15**) of N^2-alkylformycins (**9**, **11**) were prepared by use of TPAD in a similar manner as described above.

2′,3′-Cyclic phosphates (**19**, **23**) were prepared from **9** and **11**, respectively. Isopropylidenation gave **16** and **20** and their benzoylation gave tribenzoyl derivatives (**17**, **21**).

Deisopropylidenation followed by phosphorylation and hydrolysis afforded the 2′,3′-cyclic phosphates (**19**, **23**).

Structural Assignments

The structures of N^1- and N^2-isopropylformycins (**10**, **11**) were confirmed by their UV and PMR spectra. Their UV data were consistent with those of N^1- and N^2-methylformycin reported by TOWNSEND *et al.*[7] We synthesized also N^1-methyl and N^2-methylformycin according to the methods of TOWNSEND *et al.*[7] and studied their PMR in comparison with those of N^1- and N^2-isopropylformycin. We observed a characteristic difference in the anomeric proton signals of N^1- and N^2-alkylderivatives: δ 5.01 for **10**, δ 5.00 for N^1-methylformycin, δ 5.23 for **11** and δ 5.22 for N^2-methylformycin.

The structures of cyclic phosphates were deduced from PMR spectral data, paper electrophoresis, and hydrolysis studies. The 3′,5′-cyclic phosphates (**3**, **13**, **15**) showed coupling constants $J_{1',2'} < 1.0$ Hz which were consistent with that of cAMP[14] and their signals of *H*-3′, *H*-5′ and *H*-5″ were observed at significantly lower fields than those of formycin, indicating their 3′,5′-diester structures.

In the 2′,3′-cyclic phosphates (**7**, **19**, **23**), the signals of *H*-2′ and *H*-3′ shifted to lower fields than in formycin, but no shift of signals of their *H*-5′ and *H*-5″ was observed. Furthermore, their PMR spectra had close similarity to adenosine 2′,3′-cyclic phosphate.[15]

The mobilities of all cyclic phosphates (**3**, **13**, **15**, **7**, **19**, **23**) in paper electrophoresis (3,500V, 10 minutes, pH 7.5) were slower than those of formycin 2′-, 3′- and 5′-monophosphates (dianions) and similar to those of adenosine 3′,5′-cyclic and 2′,3′-cyclic phosphates (monoanions).

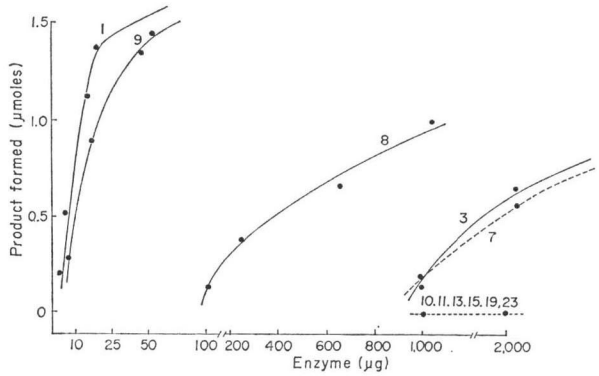
Hydrolysis of formycin 3′,5′-cyclic phosphate (**3**) with 1N barium hydroxide (100°C, 15 minutes) gave formycin 3′- and 5′-monophosphates when judged by TLC with isopropylalcohol - 28%NH_4OH - H_2O (7: 1: 2), whereas hydrolysis of formycin 2′,3′-cyclic phosphate (**7**) with 1 N HCl (100°C, 15 minutes) or 0.5 N NaOH (35°C, 12 hours) gave formycin 2′- and 3′-monophosphates. These hydrolysis patterns were in agreement with the cyclic structures assigned.

Biological Results

The activities of Takadiastase adenosine deaminase (*Aspergillus oryzae*, 17.1 u/mg) and calf intestinal mucosa adenosine deaminase (purchased from Sigma Chemical Company, 2 u/mg) to deaminate formycin and its derivatives were tested by the method in a previous paper.[9] Compounds tested are formycin (**1**), formycin 3′,5′-cyclic phosphate (**3**), formycin 2′,3′-cyclic phosphate (**7**), N^1- and N^2-methylformycins (**8**, **9**), N^1- and N^2-isopropylformycins (**10**, **11**), N^2-methylformycin 3′,5′-cyclic phosphate (**13**), and 2′,3′-cyclic phosphate (**19**), N^2-isopropylformycin 3′,5′-cyclic phosphate (**15**), and 2′,3′-cyclic phosphate (**23**). The results are shown in Figs. 1 and 2.

Fig. 1. Deamination of **1**,**3**,**7**,**8**,**9**,**10**,**11**,**13**,**15**,**19**,**23** by Takadiastase adenosine deaminase.

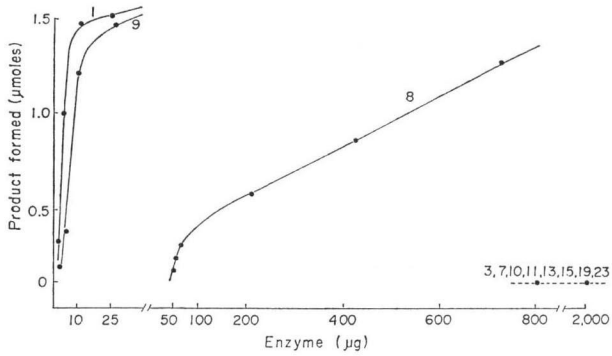
A reaction mixture (0.2 ml) containing 1.5 μmoles of a test compound, 5 μmoles of phosphate buffer (pH 6.6) and variable quantity of enzyme was incubated at 45°C for 60 minutes.



N^1-Methylformycin (**8**) and N^2-methylformycin (**9**) were found to be substrates but the former was deaminated much more slowly than the latter and formycin (**1**) (Figs. 1 and 2). N^1- and

N^2-isopropylformycins (**10, 11**) were not deaminated even by 100 times as much amounts of enzyme as required for complete deamination of formycin. It has been reported that bulky substituents such as thio and bromo in 8-position of adenosine (which corresponds to N^2 of formycin) prevent the action of adenosine deaminase.[16]

Fig. 2. Deamination of **1,3,7,8,9,10,11,13,15,19,23** by calf intestinal mucosa adenosine deaminase.
A reaction mixture (0.2 ml) containing 1.5 μmoles of a test compound, 5 μmoles of sodium phosphate (pH 7.4) and variable quantity of enzyme was incubated at 37°C for 60 minutes.



Formycin 3′,5′-cyclic and 2′,3′-cyclic phosphates (**3, 7**) were deaminated much more slowly than formycin and N^1- and N^2-methylformycins by the deaminase contained in Takadiastase, which has a broader spectrum than the deaminase of calf thymus.[17] N^2-Alkylformycin cyclic phosphates (**13, 15, 19, 23**) were not deaminate even by 100 times as much amount of enzyme as required for complete deamination of formycin. Calf intestinal mucosa adenosine deaminase, which has a high substrate specificity,[18] did not deaminate formycin cyclic phosphates (**3, 7, 13, 15, 19, 23**).

Thus cyclic phosphorylation or N^1 or N^2-substitution with a bulky group was found to make formycin resistant to adenosine deaminase.

Inhibition of rat brain cAMP phosphodiesterase (substrate concentration: 1 μM cAMP) by compounds **3, 13, 15** was tested in a modified method of RAMACHANDRAN[19] and FURUTANI.[20] Among these compounds, only cFMP (**3**) showed a similar degree of inhibition (ID_{50} of 100 mcg/ml) as theophylline.

There are a number of papers on inhibition of cAMP phosphodiesterase by a variety of substances. Methylxanthines[21,22] such as caffeine, theophylline, *etc.*, puromycin,[23] 4-(3,4-dimethoxybenzyl)-2-imidazolidinone,[24] papaverine,[25] and pyrimidines[12] were reported as inhibitors of the enzyme. Many 3′,5′-cyclic nucleotide derivatives were also reported for its inhibition.[12] MUNEYAMA *et al.*[26] reported that 8-thio-cAMP was less effective than theophylline and the addition of an alkyl group to the 8-thio moiety increased the inhibitory activity about 5 times. However, chemical derivation at the corresponding position of formycin (N^2 of pyrazolopyrimidine) caused a different result, N^2-derivatives such as N^2-methyl or N^2-isopropyl cFMP were less effective than cFMP. Since cFMP inhibited cAMP phosphodiesterase as strongly as theophylline, we thought that cFMP may also have some physiological effects.

In general cAMP and N^6-2′-*O*-dibutyryl cAMP, when added to the extracellular medium, inhibit the proliferation of both normal and malignant cell lines.[27,28,29] Then we tested the growth inhibition of L-1210 cells *in vitro* culture and in mice with formycin cyclic phosphates.

Cell culture of L-1210 and determination of cell growth were made by the method of HORI *et al.*[30] with minor modifications: (1) the cell line maintained *in vitro* culture was used; (2) EAGLES MEM medium containing 10% calf serum (v/v) was employed; (3) test materials were dissolved in the culture medium; (4) after 2 days of cultivation in the presence of test compounds, cells were collected,

and the protein content was measured spectrometrically by the LOWREY's method.

Fifty percent inhibition concentration of formycin and cFMP (**3**) were 1.4 mcg/ml and 8.3 mcg/ml, respectively.

The inhibition of cAMP phosphodiesterase by cFMP may explain, at least in part, its inhibitory effect on the growth of L-1210 cells *in vitro*. In the case of formycin, pseudofeedback inhibition of PRPP (phosphoribosylpyrophosphate) synthesis by FTP (formycin 5′-triphosphate) was reported to be responsible for its effect on growth of cells. Antitumor effect *in vitro* of cAMP was first reported by GERICKE and CHANDRA.[31] The above effect was stimulated by theophylline and epinephrine, so we wondered if cFMP has also some antitumor effect *in vivo*.

For the examination of the effect on L-1210 in mice, 1×10^5 tumor cells were intraperitoneally inoculated to mice BDF_1 (5 mice each group), and 2 hours thereafter, treatment was started by intraperitoneal injection of each cyclic phosphates and continued daily for 10 days. The mean survival of the untreated group was 8.6 days.

However, it was not effective even at 250 mcg/mouse/day.

Experimental

Formycin 5′-(trichloromethyl)phosphonate (**2**) NH_4 Salt.

Trichloromethylphosphonic acid dichloride (TPAD) (4.0 g, 17 mmol) was added dropwise to an ice-cold suspension of formycin (500 mg, 1.85 mmol) in triethyl phosphate (20 ml) under stirring. The stirring was continued at 0°C for 2 hours. After the reaction mixture was poured onto ice-water (100 ml) and the solution was maintained pH 3 by 1 N NaOH, the mixture was applied to a column of activated charcoal (5 g). The column was washed with water (500 ml) and eluted with EtOH - H_2O - 28%NH_4OH (9: 10: 1, 300 ml). The eluate was evaporated to 1/10 volume and applied to a column of DEAE-Sephadex (HCO_3^- form, 300 ml, packed with 0.05 M NH_4HCO_3). The effluent was fractionated into 18 ml's. Fraction Nos. 158~211 gave white powder of **2** NH_4 salt (625 mg, 72%). mp >272°C (dec.). $[\alpha]_D^{20}$ −32.5° (*c* 1.0, 0.18% $NaHCO_3$). UV λ_{max} (H_2O): 295 nm (ε 1.3×10^4), (0.1 N HCl): 234 (8.41×10^3), 296 (1.05×10^4), (0.1 N NaOH): 234 (1.72×10^4), 303 (8.14×10^3). PMR (D_2O): δ 8.15 (s, 1H, H-5), 5.40 (d, 1H, $J_{1',2'}$ = 6.5 Hz, H-1′), 4.9~4.3 (m, 5H, H-2′, H-3′, H-4′, H-5′, 5″). Found: C, 27.10; H, 3.59; N, 16.14%. Calcd for $C_{11}H_{12}O_6N_5PCl_3 \cdot NH_4 \cdot H_2O$: C, 27.32; H, 3.75; N, 17.38%.

Formycin 3′,5′-cyclic phosphate (**3**)

To a solution of **2** (100 mg, 0.21 mmol) in dry DMF (5 ml) in $KOBu^t$ (5 ml) was added at room temperature under stirring. After the stirring was continued for 4 hours, the reaction mixture was poured onto ice-water (20 ml). The solution was adjusted pH 3 with 1 N HCl and applied to a column of activated charcoal (1 g). The column was washed with water (100 ml) and eluted with EtOH - H_2O - 28% NH_4OH (9: 10: 1, 250 ml). The eluate was evaporated to dryness and the crude powder was dissolved in water (30 ml) and then applied to a column of DEAE-Sephadex (HCO_3^- form, 250 ml, packed with 0.05 M NH_4HCO_3). The effluent was fractionated into 12 ml's. Fraction Nos. 146~210 gave crude powder and crystallized from 50% EtOH - H_2O (at pH 2) gave **3** (48 mg, 66%). mp >239°C (dec.). $[\alpha]_D^{20}$ −50.0° (*c* 0.5, H_2O). UV λ_{max} (H_2O): 294 nm (ε 1.09×10^4), (0.1 N HCl): 233 (1.00×10^4), 295 (1.05×10^4), (0.1 N NaOH): 234 (1.63×10^4), 303 (8.11×10^3). PMR (D_2O): δ 8.20 (s, 1H, H-5), 5.55 (s, 1H, H-1′), 4.85~4.30 (m, 5H, H-2′, H-3′, H-4′, H-5′, 5″). Found: C, 33.52; H, 4.00; N, 19.13%. Calcd for $C_{10}H_{15}N_6O_6P \cdot 1.5\,H_2O$: C, 33.72; H, 4.24; N, 19.50%.

N^1 (or N^2), N^7, N^7, $O^{5'}$-Tetrabenzoyl-2′,3′-*O*-isopropylideneformycin (**5**)

Benzoyl chloride (0.76 ml, 6.51 mmol) was added dropwise to an ice-cold solution of 2′,3′-*O*-isopropylidene formycin[9] (**4**, 200 mg, 0.65 mmol) in dry pyridine (5 ml) and the mixture was stirred at room temperature for 4 hours. After the reaction mixture was poured into ice-cold saturated aqueous solu-

tion of sodium bicarbonate (20 ml), the solution was extracted with chloroform (10 ml × 3). The organic layer was washed with water (10 ml), 1 N HCl (10 ml × 2) and water (10 ml), dried ($MgSO_4$) and evaporated *in vacuo* to give a crude oil (810 mg). The crude oil was suitable for the next step. For analytical purpose a small sample of this crude oil was applied to a column of silica gel (20 : 1, benzene - ethyl acetate) to give a white powder. Recrystallization from acetone-petroleum ether to give colorless crystals of **5** (92% from **4**). mp 210~211°C. $[\alpha]_D^{20}$ −47.5° (*c* 1.0, $CHCl_3$). UV λ_{max} (MeOH): 237 nm (ε 3.8×10^4), 308 (1.4×10^4), (0.1 N HCl - MeOH): 238 (3.74×10^4), 307 (1.44×10^4), (0.1 N NaOH-MeOH): 229 (3.38×10^4). PMR ($CDCl_3$): δ 8.80 (s, 1H, H-5), 8.10, 7.50 (m, 20H, Ar), 5.60 (d, 1H, $J_{2',3'}=2.0$ Hz, H-2'), 5.55 (s, 1H, H-1'), 5.10 (m, 1H, H-3'), 4.60 (m, 3H, H-4', H-5', 5''), 1.65 and 1.40 (s, 3H, CH_3). Found: C, 67.74; H, 4.73; N, 9.45%. Calcd for $C_{41}H_{33}O_8N_5$: C, 68.04; H, 4.60; N, 9.68%.

N^1 (or N^2), N^7, N^7, $O^{5'}$-Tetrabenzoylformycin (**6**)

A solution of the above crude oil of **5** (810 mg) in 98% formic acid (80 ml) was kept at room temperature for 1 hour and then evaporated *in vacuo*. The resulting oil was applied to a column of silica gel (40 g, packed with benzene) and eluted with 5: 1 benzene - acetone. The main portion was evaporated to give white crystals of **6** (132 mg, 74% from **4**). mp 121~122°C. $[\alpha]_D^{20}$ −57.5° (*c* 1.0, $CHCl_3$). UV λ_{max} (MeOH): 236 nm (ε 3.75×10^4), 308 (3.73×10^4), (0.1 N HCl-MeOH): 238 (3.73×10^4), 308 (1.55×10^4), (0.1 N NaOH-MeOH): 229 (5.66×10^4). PMR ($CDCl_3$): δ 8.70 (s, 1H, H-5), 8.1, 7.4 (m, 20H, Ar), 5.42 (d, 1H, $J_{1',2'}=5.5$ Hz, H-1'), 4.88 (t, 1H, H-2'), 4.75~4.30 (m, 4H, H-3', H-4', H-5', 5''). Found: C, 66.46; H, 4.43; N, 10.02%. Calcd for $C_{38}H_{29}O_8N_5$: C, 66.76; H, 4.28; N, 10.25%.

Formycin 2',3'-cyclic phosphate (**7**)

Phosphorus oxychloride (0.04 ml, 0.44 mmol) was added to an ice-cold solution of **6** (300 mg, 0.44 mmol) in dry pyridine (5 ml) and the reaction mixture was stirred at 0°C for 1 hour. After the reaction mixture was poured into aqueous saturated NH_4HCO_3 (20 ml), the solution was maintained at pH 8 and the mixture was evaporated to dryness. The residue was suspended in dry pyridine (12 ml) and the mixture was filtered, washed with dry pyridine (12 ml). The filtrate and washings were combined and the solution was evaporated to dryness *in vacuo*. Then the resulting residue was treated with absolute methanol (100 ml) saturated with ammonia at room temperature for 2 days. After evaporation of the solvent, the residual syrup was dissolved in 50 ml water and the solution was extracted with ethyl acetate (25 ml × 3). The aqueous layer was evaporated to 1/5 volume and applied to a column of DEAE-Sephadex (HCO_3^- form, 50 ml, packed with 0.05 M NH_4HCO_3). The effluent was fractionated into 15 ml's. Fraction Nos. 99~195 gave colorless powder 107 mg (71%) of **7** (NH_4 salt). mp >200°C (dec.). $[\alpha]_D^{20}$ −6° (*c* 1.0, H_2O). UV λ_{max} (H_2O): 293.5 nm (ε 1.14×10^4), (0.1 N HCl): 233 (8.42×10^3), 295 (1.11×10^4), (0.1 N NaOH): 234 (1.70×10^4), 303 (8.38×10^3). PMR (D_2O): δ 8.10 (s, 1H, H-5), 5.50~4.90 (m, 3H, H-1', H-2', H-3'), 4.51 (m, 1H, H-4'), 4.05 (d, 2H, H-5', 5''). Found: C, 31.68; H, 5.09; N, 21.60%. Calcd for $C_{10}H_{15}O_6N_6P\cdot2H_2O$: C, 31.42; H, 5.01; N, 21.98%.

N^1-Methylformycin (**8**) and N^2-Methylformycin (**9**)

These compounds were prepared by the method of TOWNSEND *et al.*[7] The crystals in the reaction mixture were filtered off and washed with isopropylalcohol throughly to give a crude powder. Recrystallization from isopropylalcohol to gave **9**; mp 205~206°C (lit.[7] 205~206°C). $[\alpha]_D^{20}$ −100° (*c* 0.35, MeOH). PMR (DMSO-d_6): δ 8.07 (s, 1H, H-5), 5.22 (d, 1H, $J_{1',2'}=7.0$ Hz, H-1'), 4.63 (dd, 1H, $J_{1',2'}=7.0$ Hz, $J_{2',3'}=5.4$ Hz, H-2'), 4.15 (m, 2H, H-3', H-4'), 4.05 (s, 3H, N-CH_3). UV λ_{max} (MeOH): 317 nm (ε 8.48×10^3), 306 (1.32×10^4), 295 (1.13×10^4), 237 (6.04×10^3); (pH 1): 305 (1.10×10^4), 270 (5.36×10^3), 261 (5.50×10^3), 233 (1.01×10^4); (pH 11): 317 (9.12×10^3), 305 (1.34×10^4), 296 (1.12×10^4), 237 (6.19×10^3). Found: C, 46.98; H, 5.42; N, 24.76%. Calcd for $C_{11}H_{15}O_4N_5$: C, 46.98; H, 5.37; N, 24.91%.

The filtrate and washings were combined and evaporated to dryness. The resulting gum was dissolved in water (1 ml) and applied to a column of Dowex 50 × 2 (H^+ type, 3 ml). The column was washed with water (10 ml) and eluted with 1 N NH_4OH (15 ml). The eluate was evaporated to dryness.

The crude powder was dissolved in methanol (10 ml) and silica gel (100 mg) was added. The mixture was evaporated to dryness and chromatographed on a silica gel column (10 g, packed with 1: 1 chloroform - isopropylalcohol) and eluted with the same solvent system. The effluent was fractionated into 2 ml's. Fraction Nos. 11~21 gave colorless crystals of **9**, 49 mg (25.0%). Fraction Nos. 23~29 gave colorless foam of **8**, 42.8 mg (21.8%). This compound was identified with 1-methylformycin[7].

N^2-Methylformycin 5′-(trichloromethyl)phosphonate (**12**)

To a solution of **9** (100 mg, 0.356 mmol) in triethyl phosphate (3.56 ml), trichloromethyl phosphonic acid dichloride (840 mg, 3.56 mmol) was added dropwise at 0°C under stirring. After the stirring was continued for 2 hours under ice-cooling, the reaction mixture was poured onto ice-water (20 ml) and adjusted pH 3 by 1 N NaOH. The mixture solution was applied to a column of activated charcoal (1 g). The column was washed with water (75 ml) and eluted with 9: 10: 1 EtOH - H_2O - 28%NH_4OH (300 ml). The eluate was evaporated to dryness. The crude powder was dissolved in water (5 ml) and applied to a column of DEAE Sephadex (HCO_3^- form, 20 ml, packed with 0.03 M NH_4HCO_3). The elution was followed by the same solvent and fractionated into 15 ml's. Fraction Nos. 10~26 gave colorless powder of **12**, 148 mg (86.5%); mp >290°C (dec.). $[\alpha]_D^{20}$ −82° (*c* 1.0, H_2O). PMR (D_2O): δ 8.27 (s, 1H, H-5), 5.27 (d, 1H, $J_{1',2'}$=6.8 Hz, H-1′). 4.32 (s, 3H, CH_3). UV λ_{max}: (H_2O): 305 nm (ε 1.40×10^4), 295 (1.30×10^4). Found: C, 29.21; H, 3.24; N, 14.23%. Calcd for $C_{12}H_{14}O_6N_5PCl_3\cdot1/2HCl$: C, 30.04; H, 3.05; N, 14.60%.

N^2-Methylformycin 3′,5′-cyclic phosphate (**13**)

1 N KOBu^t (5 ml) in DMF (5 ml) was added to a solution of **12** (NH_4 salt, 120 mg, 0.23 mmol) in DMF (3 ml) at room temperature and the mixture was stirred for 4 hours. The reaction mixture was poured onto ice-water (20 ml). The pH of the solution was maintained at 3.0 with 1 N HCl and applied to a column of activated charcoal (1.4 g). The column was washed with water (60 ml) and eluted with 9: 10: 1 EtOH - H_2O - 28%NH_4OH (120 ml). After the eluate was evaporated to dryness, the crude powder was dissolved in water (1 ml) and chromatographed on a column of DEAE Sephadex (HCO_3^- form, 40 ml, packed with 0.05 M NH_4HCO_3) and eluted with the same solvent. The effluent was fractionated into 11 ml's. Fraction Nos. 41~69 gave a crude powder. This crude powder was dissolved in 5 ml water and adjusted to pH 2 with 1 N HCl. The solution was kept in a refrigerator for 24 hours to yield colorless needles of **13**, 80 mg (68%); mp >300°C. $[\alpha]_D^{20}$ −98.0° (*c* 0.37, 0.1 N NaOH). PMR (D_2O): δ 8.18 (s, 1H, H-5), 5.58 (s, 1H, H-1′), 4.40 (m, 6H, H-4′, H-5′, 5″, N-CH_3). UV λ_{max} (H_2O): 318 nm (ε 9.03×10^3), 305 (1.38×10^4), 295 (1.22×10^4), 238 (6.76×10^3); (0.1 N HCl): 303 (1.02×10^4), 271 (4.92×10^3), 260 (4.92×10^3), 230 (1.19×10^4); (0.1 N NaOH): 317 (8.44×10^3), 305 (1.28×10^4), 295 (1.12×10^4), 238 (5.36×10^3). Found: C, 38.47; H, 4.22; N, 20.17%. Calcd for $C_{11}H_{14}O_6N_5P$: C, 38.49; H, 4.11; N, 20.41%.

2′,3′-*O*-Isopropylidene-N^2-methylformycin (**16**)

To a solution of **9** (854 mg, 3.06 mmol), freshly fused *p*-toluenesulfonic acid (2.04 g, 15.3 mmol) in dry acetone (8.6 ml) was added 2,2-dimethoxypropane (3.74 ml, 30.6 mmol) and the mixture was stirred at room temperature for 4 hours. After the reaction mixture was poured into ice-cold aqueous saturated $NaHCO_3$ (50 ml), the solution was evaporated to dryness. The resulting mixture was suspended in pyridine (30 ml) and the precipitate was washed with pyridine (10 ml). The filtrate and the washings were combined and evaporated to dryness. The crude syrup was suitable for the next step. For analytical purpose, this crude syrup was dissolved in methanol (10 ml) and silica gel (1.3 g) was added and the mixture was evaporated to dryness. The resulting powder was placed on a column of silica gel (30 g, packed with 9: 1 benzene - methanol) and eluted with the same solvent system. The main portion was evaporated to yield a colorless powder and recrystallized from methanol gave colorless crystals of **16**, 810 mg (83%). mp 205~206°C. $[\alpha]_D^{20}$ −115° (*c* 1.0, DMSO). PMR (DMSO-d_6): δ 8.15 (s, 1H, H-5), 7.59 (br s, 2H, NH_2), 5.78 (dd, 1H, $J_{1',2'}$=4.0 Hz, $J_{2',3'}$=6.5 Hz, H-2′), 5.25 (d, 1H, $J_{1',2'}$=4.0 Hz, H-1′), 5.20 (br s, 1H, OH), 4.90 (dd, 1H, $J_{2',3'}$=6.5 Hz, $J_{3',4'}$=3.0 Hz, H-3′), 4.20 (s, 3H, N-CH_3), 4.15 (m, 1H, H-4′), 3.20 (d, 2H, H-5′,5″), 1.58 and 1.38 (s, 3H, CH_3). UV λ_{max} (H_2O): 317 nm (ε 7.32×10^3), 305 (1.12×10^4), 295 (9.90×10^3), 234 (5.95×10^3), (0.1 N HCl): 303 (9.61×10^3), 271 (4.79×10^3), 261 (4.86×10^3), 231 (1.05×10^4), (0.1 N NaOH): 317 (7.47×10^3), 305

(1.14×10^4), 295 (1.00×10^4), 236 (5.29×10^3). Found: C, 52.53; H, 5.98; N, 21.79%. Calcd for $C_{14}H_{19}O_4N_5$: C, 52.33; H, 5.96; N, 21.80%.

N^7,N^7,$O^{5'}$-Tribenzoyl-2',3'-*O*-isopropylidene-N^2-methylformycin (**17**)

Benzoyl chloride (0.34 ml, 2.93 mmol) was added dropwise into an ice-cold solution of **16** (126 mg, 0.39 mmol) in dry pyridine (3.2 ml) and the mixture was stirred at room temperature for 6 hours. After the reaction mixture was poured into ice-cold aqueous saturated solution of $NaHCO_3$ (25 ml), the solution was extracted with $CHCl_3$ (25 ml, 12.5 ml × 2). The organic layer was washed with water (20 ml), 1 N HCl (10 ml × 2), and water (10 ml), dried ($MgSO_4$) and evaporated *in vacuo* to give a crude syrup. This syrup was suitable for the next step. For analytical purpose this was dissolved in ethyl acetate (1 ml) and applied to a column of silica gel (5 g, packed with benzene) and eluted with 15 : 1 benzene - ethyl acetate. The main portion was evaporated to give a colorless powder of **17**, 201 mg (91%). mp 127~128°C. $[\alpha]_D^{20}$ −22.5° (*c* 1.0, $CHCl_3$). PMR ($CDCl_3$): δ 8.65 (s, 1H, H-5), 7.85, 7.45 (m, 15H, Ar), 6.20 (dd, 1H, $J_{1',2'}=4.0$ Hz, $J_{2',3'}=6.5$ Hz, H-2'), 5.47 (d, 1H, $J_{1',2'}=4.0$ Hz, H-1'), 5.08 (dd, 1H, $J_{2',3'}=6.5$ Hz, $J_{3',4'}=2.8$ Hz, H-3'), 4.30 (m, 3H, H-4', H-5', 5''), 4.25 (s, 3H, N–CH_3), 1.56 and 1.45 (s, 3H, CH_3). Found: C, 66.29; H, 5.02; N, 10.83%. Calcd for $C_{35}H_{31}O_7N_4$: C, 66.34; H, 4.94; N, 11.05%.

N^7,$O^{5'}$-Dibenzoyl-N^2-methylformycin (**18**)

A solution of **17** (195 mg, 0.31 mmol) in 85% formic acid (10 ml) was kept at room temperature for 6 hours. After removal of the solvent below 20°C and co-evaporated with ether, the resulting foam was chromatographed on a column of silica gel (4 g, packed with 7: 4 benzene - acetone). The elution was followed by the same solvent system and the main portion was evaporated to give a colorless powder of **18**, 111 mg (73.5%). mp 112~115°C. $[\alpha]_D^{20}$ −87.5° (*c* 1.0, $CHCl_3$). PMR ($CDCl_3$): δ 8.31 (s, 1H, H-5), 7.60 (m, 10H, Ar), 5.30 (m, 2H, H-1', H-2'), multiplet centered at 4.52 (m, 6H, H-3', H-4', H-5', 5'', OH, OH), 4.22 (s, 3H, N–CH_3). UV λ_{max} (MeOH): 323 nm (ε 1.20×10^4), (0.1 N HCl-MeOH): 283 (1.60×10^4), 334 (8.64×10^3), (0.1 N NaOH-MeOH): 345 (1.40×10^4). Found: C, 61.03; H, 4.90; N, 14.08%. Calcd for $C_{25}H_{23}O_6N_5$: C, 61.34; H, 4.74; N, 14.31%.

N^2-Methylformycin 2',3'-cyclic phosphate (**19**)

Phosphorus oxychloride (0.52 ml, 0.56 mmol) was added to an ice-cold solution of **18** (273 mg, 0.56 mmol) in dry pyridine (5.6 ml) and the reaction mixture was stirred at 0°C for 1 hour. After the reaction mixture was poured into aqueous saturated solution of NH_4HCO_3 (20 ml), the solution was adjusted to pH 8 and the mixture was evaporated to dryness. The residue was suspended in dry pyridine (12 ml) and filtered off, washed with dry pyridine (12 ml). The filtrate and the washings were combined and evaporated to dryness *in vacuo*. The crude syrup was dissolved in absolute methanol (100 ml) saturated with ammonia at 0°C and the solution was kept at room temperature for 2 days. After removal of the solvent, the resulting syrup was precipitated with 1: 10 methanol - diethyl ether. The precipitate was dissolved in water (2 ml) and applied to a column of DEAE Sephadex (HCO_3^- form, 100 ml, packed with 0.03 M NH_4HCO_3). The elution was followed by the above solvent and fractionated into 15 ml's. Fraction Nos. 65~100 gave a colorless powder of **19**, 116 mg (61%). mp 210°C (dec.). $[\alpha]_D^{20}$ −54.6° (*c* 0.4, H_2O). PMR (D_2O): δ 8.10 (s, 1H, H-5), 5.60~4.90 (m, H-2', H-3'), 5.49 (d, 1H, $J_{1',2'}=4.8$ Hz, H-1'), 4.27 (s, 3H, N–CH_3). UV λ_{max} (H_2O): 318 nm (ε 9.01×10^3), 305 (1.24×10^4), 295 (1.06×10^4), 237 (6.40×10^3), (0.1 N HCl): 306 (3.89×10^3), 271 (4.50×10^3), 262 (5.01×10^3), 231 (1.13×10^4), (0.1 N NaOH): 318 (8.20×10^3), 304 (1.23×10^4), 294 (1.01×10^4), 237 (5.10×10^3). Found: C, 39.60; H, 5.21; N, 25.18%. Calcd for $C_{11}H_{14}O_6N_5P\cdot NH_4$: C, 40.01; H, 5.46; N, 25.43%.

N^1-Isopropylformycin (**10**) and N^2-Isopropylformycin (**11**)

A solution of formycin (288 mg, 0.99 mmol) and 50% sodium hydride (67.3 mg, 1.40 mmol) in dry dimethylformamide (12 ml) was stirred at room temperature. After 1 hour, isopropyl iodide (0.37 ml, 3.24 mmol) was added and the reaction mixture was stirred vigorously at room temperature for 48 hours. After the removal of the solvent, the resulting residue was dissolved in water (15 ml) and the solution was washed with hexane (15 ml). The aqueous layer was applied to a column of Dowex 50W × 2 (H^+ type, 6 ml). The column was washed with water (24 ml) and eluted with 1 N NH_4OH (30 ml). The eluate was evaporated to dryness and the residue was dissolved in methanol

(1 ml) and chromatographed on a column of silica gel (15 g, packed with 2: 1 chloroform - isopropyl alcohol, 1×20 cm). The elution was followed by the above solvent system and fractionated into 2 ml's. Fraction Nos. 25~32 gave a colorless powder of **11** (243 mg). Recrystallization from ethyl acetate to give 222 mg (80.0%). mp 192.5~193.5°C. $[\alpha]_D^{20}$ −35.0° (*c* 1.0, DMSO). PMR (DMSO-d_6): δ 8.08 (s, 1H, H-5), 5.23 (d, 1H, $J_{1',2'}$=6.6 Hz, H-1'), 4.95 (m, 1H, >CH), 4.68 (dd, 1H, $J_{2',3'}$= 2.3 Hz, $J_{1',2'}$=6.6 Hz, H-2), 4.16 (dd, 1H, $J_{2',3'}$=2.3 Hz, $J_{3',4'}$=1.3 Hz, H-3'), 4.04 (m, 1H, H-4'), 3.70 (m, 2H, H-5', 5''), 1.60 and 1.52 (d, 3H, CH_3). UV λ_{max} (H_2O): 318 nm (ϵ 7.39×10³), 304 (1.09×10⁴), 295 (9.42×10³), 232 (7.01×10³); (0.1 N HCl): 306 (9.02×10³), 270 (4.91×10³), 262 (5.13×10³), 234 (9.15×10³), (0.1 N NaOH): 317 (7.55×10³), 305 (1.10×10⁴), 295 (9.20×10³), 237 (5.11×10³). Found: C, 50.23; H, 6.24; N, 22.50%. Calcd for $C_{13}H_{19}O_4N_5$: C, 50.48; H, 6.19; N, 22.62%.

Fraction Nos. 34~40 gave colorless foam of **10**, 17 mg. Recrystallization from methanol - acetone gave 14 mg (5.4%). mp 207°C. $[\alpha]_D^{20}$ −53.4° (*c* 0.5, MeOH). PMR (DMSO-d_6): δ 8.15 (s, 1H, H-5), 5.10 (m, 1H, >CH), 4.96 (d, 1H, $J_{1',2'}$=7.3 Hz, H-1'), 4.57 (dd, 1H, $J_{2',3'}$=2.5 Hz, $J_{1',2'}$=7.3 Hz, H-2'), 4.17 (dd, 1H, $J_{3',4'}$=1.5 Hz, $J_{2',3'}$=2.5 Hz, H-3'), 4.10 (m, 3H, H-4', H-5', 5''), 1.55 and 1.40 (d, 3H, CH_3). UV λ_{max} (H_2O): 315 nm (ϵ 3.60×10³), 301 (5.80×10³), 235 (6.9×10³), 231 (6.40×10³); (0.1 N HCl): 302 (5.93×10³), 235 (6.91×10³): (0.1 N NaOH): 314 (3.25×10³), 301 (5.96×10³), 293 (5.46×10³), 231 (5.03×10³). Found: C, 50.49; H, 6.12; N, 22.83%. Calcd for $C_{13}H_{19}O_4N_5$: C, 50.48; H, 6.19; N, 22.62%.

N^2-Isopropylformycin 5'-(trichloromethyl)phosphonate (**14**)

11 (136 mg, 0.44 mmol) was phosphonylated with trichloromethyl phosphonic acid dichloride (1.04 g, 4.40 mmol) in triethyl phosphate (5 ml) in a similar manner as described for the preparation of **12**. The aqueous solution (20 ml) of crude syrup (at pH 3) was passed through activated charcoal (1.4 g) and washed with water (60 ml). The elution was followed by 9: 10: 1 EtOH - H_2O - 28%NH_4OH (180 ml). After removal of the solvent, the crude powder was chromatographed on a column of DEAE Sephadex (HCO_3^- form, 40 ml, packed with 0.03 M NH_4HCO_3). The elution was followed by the same solvent and fractionated into 15 ml's. Fraction Nos. 17~45 gave a colorless powder of **14**, 191 mg (85%). mp >260°C (dec.). $[\alpha]_D^{20}$ −27.8° (*c* 1.0, H_2O). PMR (D_2O): δ 8.45 (s, 1H, H-5), 5.25 (d, 1H, $J_{1',2'}$=6.8 Hz, H-1'), 1.78 and 1.66 (d, 3H, CH_3). UV λ_{max} (H_2O): 304 nm (ϵ 1.15×10⁴). Found: C, 32.65; H, 4.43; N, 16.14%. Calcd for $C_{14}H_{19}O_6N_5PCl_3 \cdot NH_4$: C, 33.10; H, 4.52; N, 16.52%.

N^2-Isopropylformycin 3',5'-cyclic phosphate (**15**)

14 (NH_4 salt, 158 mg, 0.31 mmol) was cyclized with 1 N $KOBu^t$ (7 ml) in DMF (7 ml) at room temperature for 2 hours. After the reaction mixture was poured into ice-water (30 ml), the solution (at pH 3.0) was passed through activated charcoal (1.6 g) and washed with water (75 ml). The elution was followed by 9: 10: 1 EtOH - H_2O - 28%NH_4OH (150 ml) and evaporated to dryness. The crude powder was dissolved in minimum water and applied to a column of DEAE Sephadex (HCO_3^- form, 60 ml, packed with 0.03 M NH_4HCO_3) and eluted with the same solvent. The effluent was fractionated into 15 ml's. Fraction Nos. 48~71 gave **15**, 60.5 mg (50.0%). mp >300°C (dec.) $[\alpha]_D^{20}$ −82.3° (*c* 1.0, 0.1 N NaOH). PMR (D_2O): δ 8.20 (s, 1H, H-5), 5.52 (s, 1H, H-1'), 4.45 (m, 3H, H-4', H-5', 5''), 1.65 and 1.55 (d, 3H, CH_3). UV λ_{max} (H_2O): 318 nm (ϵ 8.80×10³), 305 (1.19×10⁴), 295 (1.05×10⁴), 237 (6.40×10³); (0.1 N HCl): 306 (3.65×10³), 270 (4.56×10³), 262 (4.88×10³), 231 (1.12×10⁴); (0.1 N NaOH): 318 (8.28×10³), 305 (1.10×10⁴), 295 (9.98×10³), 237 (5.13×10³). Found: C, 39.86; H, 5.13; N, 21.20%. Calcd for $C_{13}H_{17}O_6N_5P \cdot NH_4$: C, 40.21; H, 5.45; N, 21.64%.

N^2-Isopropyl-2',3'-isopropylideneformycin (**20**)

11 (240 mg, 0.78 mmol) was acetonated by 2,2-dimethoxypropane (0.95 ml, 7.8 mmol) in the presence of freshly fused *p*-toluenesulfonic acid (668 mg, 3.88 mmol) in acetone (4.2 ml) at room temperature for 4 hours. The reaction mixture was poured into cold aqueous saturated $NaHCO_3$ (12 ml) and the solution was evaporated to dryness. The residue was extracted with pyridine (25 ml). The pyridine layer was evaporated to give a colorless syrup. This syrup was suitable for the next step. For analytical purpose, this crude syrup was dissolved in methanol (10 ml) and silica gel (300 mg)

was added. The mixture was evaporated to dryness and the residue was applied to a column of silica gel (10 g, packed with 12: 1 ethyl acetate - methanol) and eluted with the same solvent system. The main portion was evaporated to give a colorless powder of **20** (218 mg, 80.4%). Recrystallization from methanol (1.5 ml) gave 156 mg (58%). mp 203°C. $[\alpha]_D^{20}$ −112.5° (*c* 1.0, DMSO). PMR (DMSO-d_6): δ 8.18 (s, 1H, H-5), 5.78 (dd, 1H, $J_{1',2'}=5.0$ Hz, $J_{2',3'}=6.0$ Hz, H-2'), 5.40 (d, 1H, $J_{1',2'}=5.0$ Hz, H-1'), 5.00 (m, 1H, >CH), 4.90 (dd, 1H, $J_{2',3'}=6.0$ Hz, $J_{3',4'}=2.0$ Hz, H-3'), 4.20 (m, 1H, H-4'), 3.39 (d, 2H, H-5', 5''), 1.43 (m, 12H, CH_3 of isopropyl and isopropylidene). UV λ_{max} (MeOH): 317 nm (ϵ 7.44×10^3), 304 (1.03×10^4), 294 (9.38×10^3), 233 (6.85×10^3); (0.1 N HCl): 306 (8.95×10^3), 271 (4.88×10^3), 262 (5.20×10^3), 235 (9.35×10^3); (0.1 N NaOH): 317 (7.63×10^3), 304 (1.15×10^4), 293 (9.32×10^3), 237 (5.23×10^3). Found: C, 54.74; H, 6.14; N, 20.17%. Calcd for $C_{16}H_{23}O_4N_5$: C, 55.00; H, 6.63; N, 20.05%.

N^7,N^7,$O^{5'}$-Tribenzoyl-N^2-isopropyl-2',3'-*O*-isopropylideneformycin (**21**)

20 (100 mg, 0.29 mmol) was benzoylated with benzoyl chloride (0.25 ml, 2.15 mmol) in dry pyridine (2 ml) at room temperature for 6 hours. After the reaction mixture was poured into cold aqueous saturated $NaHCO_3$ (15 ml) and extracted with chloroform (15 ml × 2), the organic layer was dried ($MgSO_4$) and evaporated to give a colorless syrup. This syrup was suitable for the next step. For the analytical purpose, this syrup was chromatographed on a column of silica gel (4 g, packed with 15: 1 benzene - ethyl acetate) and eluted with the packed solvent. The main portion was evaporated to give colorless crystals of **21**. Recrystallization from acetone gave 176 mg (90%). mp 180~181°C. $[\alpha]_D^{20}$ −35.0° (*c* 1.0, $CHCl_3$). PMR ($CDCl_3$): δ 8.62 (s, 1H, H-5), 7.95, 7.41 (m, 15H, Ar), 6.20 (dd, 1H, $J_{1',2'}=4.0$ Hz, $J_{2',3'}=6.0$ Hz, H-2'), 5.40 (d, 1H, $J_{1',2'}=4.0$ Hz, H-1'), 5.04 (m, 2H, H-3', >CH), 4.38 (m, 3H, H-4', H-5', 5''), 1.52 (m, 12H, CH_3 of isopropyl and isopropylidene). UV λ_{max} (MeOH): 323 nm (ϵ 1.3×10^4). Found: C, 67.21; H, 5.36; N, 10.48%. Calcd for $C_{37}H_{35}O_7N_5$: C, 67.16; H, 5.33; N, 10.58%.

N^7,$O^{5'}$-Dibenzoyl-N^2-isopropylformycin (**22**)

21 (100 mg, 0.13 mmol) was deisopropylidenated with 85% formic acid (3 ml) at room temperature for 6 hours. After removal of the solvent below 30°C, the residue was chromatographed on a column of silica gel (5 g, packed with 7: 4 benzene - acetone) and eluted with above solvent. The main portion was evaporated to give a colorless powder of **22**, 51.0 mg (75%). mp 110~112°C. $[\alpha]_D^{20}$ −77.5° (*c* 1.0, $CHCl_3$). PMR ($CDCl_3$): δ 8.50 (s, 1H, H-5), 8.21, 7.85, 7.55 (m, 10H, Ar), 5.22 (m, 5H, H-1', H-2', >CH, OH, OH), 4.54 (m, 4H, H-3', H-4', H-5', 5''), 1.55 (m, 6H, CH_3). Found: C, 62.91; H, 5.45; N, 13.53%. Calcd for $C_{27}H_{27}O_6N_5$: C, 62.66; H, 5.26; N, 13.53%.

N^2-Isopropylformycin 2',3'-cyclic phosphate (**23**)

To a solution of **22** (233 mg, 0.45 mmol) in dry pyridine (4.7 ml) phosphorus oxychloride (0.41 ml, 0.45 mmol) was added dropwise at 0°C under stirring. After the stirring was continued for 1 hour, the reaction mixture was poured into cold aqueous saturated NH_4HCO_3 (15 ml) and the solution was evaporated to dryness. The residue was extracted with pyridine (15 ml × 2) and the pyridine layer was evaporated. The resulting gum was dissolved in methanol (70 ml) saturated with ammonia at 0°C and the solution was kept at room temperature for 36 hours. After removal of the solvent, the residue was precipitated with 1: 10 methanol - diethyl ether. The precipitate was dissolved in minimum water and applied to a column of DEAE Sephadex (HCO_3^- form, 90 ml, packed with 0.03 M NH_4HCO_3). The elution was followed by the same solvent and fractionated into 15 ml's. Fraction Nos. 77~110 gave a colorless powder of **23** (NH_4 salt), 99 mg (59%). $[\alpha]_D^{20}$ −25° (*c* 1.0, H_2O). PMR (D_2O): δ 8.10 (s, 1H, H-5), multiplet centered at 5.30 (m, 4H, H-1', >CH, H-2', H-3'), 4.43 (m, 1H, H-4'), 4.00 (m, 2H, H-5', 5''), 1.65 and 1.55 (d, 3H, CH_3). UV λ_{max} (H_2O): 318 nm (ϵ 8.92×10^3), 305 (1.28×10^4), 295 (1.11×10^4), 237 (6.52×10^3); (0.1 N HCl): 306 (3.89×10^3), 271 (4.81×10^3), 262 (4.92×10^3), 231 (1.09×10^4); (0.1 N NaOH): 318 (8.33×10^3), 305 (1.18×10^4), 295 (1.02×10^4), 237 (5.06×10^3). Found: C, 40.17; H, 5.46; N, 21.27%. Calcd for $C_{13}H_{17}O_6N_5P\cdot NH_4$: C, 40.21; H, 5.45; N, 21.64%.

Deamination of formycin derivatives by adenosine deaminase of calf intestinal mucosa

A reaction mixture (0.2 ml) containing 1.5 μmoles of a test compound, 5 μmoles of sodium phosphate (pH 7.4) and a described quantity of the adenosine deaminase was incubated at 37°C for

60 minutes. The reaction was stopped by adding 0.2 ml of methanol. After centrifugation at 3,000 rpm (=75 mm) for 10 minutes, 40 μl of the supernatant was spotted on filter paper (Toyo Roshi No. 51) and subjected to high voltage electrophoresis at 3,500 V for 20 minutes. Compounds were localized on the paper with UV light and the appropriate areas were cut and extracted with 2 ml of 0.1 N HCl at 37°C for 30 minutes. The optical density of the extract was determined at 295 nm (303 nm in the case of N-alkyl compounds).

Deamination of formycin derivatives by adenosine deaminase of Takadiastase

It was performed in a similar manner as described above except that the phosphate buffer (pH 7.4) was changed to acetate buffer (pH 6.6).

Acknowledgement

We wish to thank Prof. H. UMEZAWA, Institute of Microbial Chemistry, for helpful discussion.

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) TAKEUCHI, T.; J. IWANAGA, T. AOYAGI & H. UMEZAWA: Antiviral effect of formycin and formycin B. J. Antibiotics, Ser. A 19: 286~287, 1966
3) SUHADOLNIK, R. J.: Nucleoside Antibiotics. Chapter 9. Pyrazolo-pyrimidine Nucleosides and Coformycin. Wiley-Interscience, New York, pp. 354~389, 1970
4) KOYAMA, G.; K. MAEDA, H. UMEZAWA & Y. IITAKA: The structural studies of formycin and formycin B. Tetrahedron Lett. 1966: 597~602, 1966
5) ACTON, E. M.; K. J. RYAN, D. W. HENRY & L. GOODMAN: Synthesis of the nucleoside antibiotic formycin B. J. Chem. Soc., Chem. Commun. 1971: 986~988, 1971
6) KUNIMOTO, T.; T. WAKASHIRO, I. OKAMURA, T. ASAJIMA & M. HORI: Structural requirements for formycin activity. J. Antibiotics 21: 468~470, 1968
7) TOWNSEND, L. B.; R. A. LONG, J. P. MCGRAW, D. W. MILES, R. K. ROBINS & H. EYRING: Pyrazolopyrimidine nucleosides. V. Methylation of the C-nucleoside antibiotic formycin and structural elucidation of products by magnetic circular dichroism spectroscopy. J. Org. Chem. 39: 2023~2027, 1974
8) ZEMLICKA, J.: Formycin anhydronucleosides. Conformation of formycin and conformational specificity of adenosine deaminase. J. Amer. Chem. Soc. 97: 5896~5903, 1975
9) MAKABE, O.; M. NAKAMURA & S. UMEZAWA: Cyclonucleoside derivatives of formycin. J. Antibiotics 28: 492~495, 1975
10) 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
11) DRUMMOND, G. I. & D. L. SEVERSON: Biological actions of cyclic AMP analogs. Ann. Rep. in Med. Chem. 1970, Chapter 21, pp. 215~226, 1970
12) SIMON, L. N.; D. A. SHUMAN & R. K. ROBINS: The chemistry and biological properties of nucleotides related to nucleoside 3',5'-cyclic phosphates. *in* Advan. Cyclic Nucleotide Res. Vol. 3, P. GREENGARD & G. A. ROBISON, *Eds.*, Raven Press, New York, 1973
13) MARUMOTO, R.; T. NISHIMURA & M. HONJO: A new method for synthesis of nucleoside 3',5'-cyclic phosphates. Cyclization of nucleoside 5'-trichloromethylphosphonates. Chem. Pharm. Bull. 23: 2295~2300, 1975
14) JARDETZKY, C. D.: Proton magnetic resonance of nucleotides. IV. Ribose conformation. J. Amer. Chem. Soc. 84: 62~66, 1962
BLACKBURN, B. J.; R. D. LAPPER & I. C. P. SMITH: A proton magnetic resonance study of the conformations of 3',5'-cyclic nucleotides. J. Amer. Chem. Soc. 95: 2873~2878, 1973
15) LAPPER, R. D. & I. C. P. SMITH: A ^{13}C and ^{1}H nuclear magnetic resonance study of the conformations of 2',3'-cyclic nucleotides. J. Amer. Chem. Soc. 95: 2880~2884, 1973
16) SIMON, L. N.; R. J. BAUER, R. L. TOLMAN & R. K. ROBINS: Calf intestine adenosine deaminase. Substrate specificity. Biochemistry 9: 573~577, 1970
17) WOLENDEN, R.; T. K. SHARPLESS & R. ALLAN: Substrate binding by adenosine deaminase. J. Biol. Chem. 242: 977~983, 1967
18) CODDINGTON, A.: Some substrates and inhibitors of adenosine deaminase. Biochim. Biophys. Acta 99: 442~451, 1965

19) RAMACHANDRAN, J.: A new simple method for separation of adenosine 3′,5′-cyclic monophosphate from other nucleotides and its use in the assay of adenyl cyclase. Anal. Biochem. 43: 227~239, 1971
20) FURUTANI, Y.; M. SHIMADA, M. HAMADA, T. TAKEUCHI & H. UMEZAWA: Reticulol, an inhibitor of cyclic nucleotide phosphodiesterases. Agric. Biol. Chem. 41: 989~993, 1977
21) SUTHERLAND, E. W. & T. W. RALL: Fractionation and characterization of a cyclic adenosine ribonucleotide formed by tissue particles. J. Biol. Chem. 232: 1077~1091, 1958
22) BUTCHER, R. W. & E. W. SUTHERLAND: Adenosine 3′,5′-phosphate in biological materials. J. Biol. Chem. 237: 1244~1250, 1962
23) APPLEMAN, M. M. & R. G. KEMP: Puromycin. A potent metabolic effect independent of protein synthesis. Biochem. Biophys. Res. Comm. 24: 564~568, 1966
24) DALTON, C.; J. B. QUINN, C. R. BURGHARDT & H. SHEPPARD: Investigation of the mechanism of action of the lipolytic agent 4-(3,4-dimethoxybenzyl)-2-imidazolidinone (Ro 7–2956). J. Pharm. Exp. Ther. 173: 270~276, 1970
25) AMER, M. S. & W. E. KREIGHBAUM: Cyclic nucleotide phosphodiesterases. Properties, activators, inhibitors, structure-activity relationships, and possible role in drug development. J. Pharm. Sci. 64: 1~37, 1975
26) MUNEYAMA, K.; R. J. BAUER, D. A. SHUMAN, R. K. ROBINS & L. N. SIMON: Chemical synthesis and biological activity of 8-substituted adenosine 3′,5′-cyclic monophosphate derivatives. Biochemistry 10: 2390~2395, 1971
27) BÜRK, R. R.: Reduced adenyl cyclase activity in a polyoma virus transformed cell line. Nature 219: 1272~1275, 1968
28) RYAN, W. L. & M. L. HEIDRICK: Inhibition of cell growth *in vitro* by adenosine 3′,5′-monophosphate. Science 162: 1484~1485, 1968
29) FRANK, W.: Cyclic 3′,5′-AMP and cell proliferation in culture of embryonic rat cells. Exp. Cell Res. 71: 238~241, 1972
30) HORI, M.; E. ITO, T. TAKEUCHI & H. UMEZAWA: Inhibitory effects of antitumor substances on growth and glycolysis of YOSHIDA rat sarcoma cells. J. Antibiotics, Ser. A 16: 1~6, 1963
31) GERICKE, D. & P. CHANDRA: Inhibition of tumor growth by nucleoside cyclic 3′,5′-monophosphates. Hoppe-Seyer's Z. Phusiol. Chem. 350: 1469~1471, 1969