for this compound; Virazole is the ICN Pharmaceuticals, Inc., trademark.
(5) P. C. Srivastava, R. W. Mancuso, R. J. Rousseau, and R. K. Robins, *J. Med. Chem.*, **17**, 1207 (1974).
(6) G. A. Ivanovics, R. J. Rousseau, M. Kawana, P. C. Srivastava, and R. K. Robins, *J. Org. Chem.*, **39**, 3651 (1974).
(7) P. C. Srivastava, A. R. Newman, T. R. Matthews, and R. K. Robins, *J. Med. Chem.*, **18**, 1237 (1975).
(8) P. C. Srivastava, G. A. Ivanovics, R. J. Rousseau, and R. K. Robins, *J. Org. Chem.*, **40**, 2920 (1975).
(9) K. Mizuno, M. Tsugune, M. Takada, M. Hayashi, K. Atsumi, K. Asano, and T. Matsuda, *J. Antibiot.*, **27**, 775 (1974). (A zwitterionic structure for bredinin has been proposed based on x-ray crystallographic studies.)
(10) E. Shaw, *J. Am. Chem. Soc.*, **80**, 3899 (1958).
(11) H. Guglielmi, *Justus Liebigs Ann. Chem.*, 1286 (1973).
(12) R. P. Panzica and L. B. Townsend, *J. Org. Chem.*, **36**, 1594 (1971).
(13) K. Suzuki and I. Kumashiro, U.S. Patent 3450693 (1969); *Chem. Abstr.*, **71**, 81698z (1969).
(14) The thin-layer silica gel chromatograms were sprayed with a dilute ethanolic solution of 2,3-dichloronaphthoquinone and exposed to ammonia. A red to purple color indicated the positive test for thioamide: M. B. Devani, C. J. Shishoo, H. J. Mody, and P. K. Raja, *J. Pharm. Sci.*, **63**, 1471 (1974).
(15) Compound **29** was conveniently synthesized in our laboratory by condensing the trimethylsilyl derivative (prepared by refluxing 5-fluoroimidazole-4-carboxamide in hexamethyldisilazane in the presence of ammonium sulfate catalyst) with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose in the presence of stannic chloride in dichloroethane. The deacetylation of the major fast-moving product, isolated by silica gel column chromatography, provided crystalline **29**, mp 159–160°: E. De Clercq, M. Luczak, J. C. Reepmeyer, K. L. Kirk, and L. A. Cohen, *Life Sci.*, **17**, 187 (1975); J. C. Reepmeyer, K. L. Kirk, and L. A. Cohen, *Tetrahedron Lett.*, 4107 (1975).
(16) B. Goz and W. H. Prusoff, *Annu. Rev. Pharmacol.*, **10**, 143 (1970).
(17) R. W. Sidwell and J. H. Huffman, *Appl. Microbiol.*, **22**, 797 (1971).
(18) D. G. Streeter, J. T. Witkowski, G. P. Khare, R. W. Sidwell, R. J. Bauer, R. K. Robins, and L. N. Simon, *Proc. Natl. Acad. Sci.* U.S.A., **70**, 1174 (1973).
(19) C. M. Smith, L. J. Fontenelle, H. Muzik, A. R. Paterson, H. Unger, L. W. Brox, and J. F. Henderson, *Biochem. Pharmacol.*, **23**, 2737 (1974).
(20) F. F. Snyder, J. F. Henderson, and D. A. Cook, *Biochem. Pharmacol.*, **21**, 2351 (1972).
(21) G. R. Revankar, T. R. Matthews, and R. K. Robins, *J. Med. Chem.*, **18**, 1253 (1975).
(22) R. S. Gordee and T. R. Matthews, *Antimicrob. Agents Chemother., 1967*, 378 (1968).
(23) The filtrate was found to be the mixture of **7** and the corresponding carboxamide compound **8** and was utilized for the isolation of **8**.

Synthesis and Anti-DNA Virus Activity of the 5′-Monophosphate and the Cyclic 3′,5′-Monophosphate of 9-(β-D-Xylofuranosyl)guanine

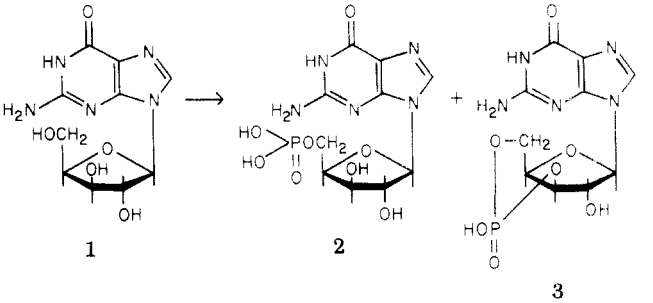
Ganapathi R. Revankar,* John H. Huffman, Robert W. Sidwell, Richard L. Tolman, Roland K. Robins, and Lois B. Allen

ICN Pharmaceuticals, Inc., Nucleic Acid Research Institute, Irvine, California 92715. *Received February 13, 1976*

9-(β-D-Xylofuranosyl)guanine (xylo-G) was converted chemically to the 9-(β-D-xylofuranosyl)guanine 5′-monophosphate (xylo-GMP) and 9-(β-D-xylofuranosyl)guanine cyclic 3′,5′-monophosphate (c-xylo-GMP). These compounds were tested against a variety of DNA viruses in tissue culture in parallel with 9-(β-D-arabinofuranosyl)adenine (ara-A). This evaluation revealed that xylo-G, xylo-GMP, and c-xylo-GMP were all moderately active but less effective than ara-A. When the four compounds were administered intracerebrally as a treatment for herpes virus, type 1 induced encephalitis in mice, c-xylo-GMP exhibited superior activity to that shown by the other three. When administered intraperitoneally, c-xylo-GMP was found to have a therapeutic index of about 4, which is less than that for ara-A (~30) in the same system.

Nucleosides possessing either D-arabinofuranose[1] or D-xylofuranose[2] in place of D-ribofuranose moieties have received increasing attention in recent years as antimetabolites. Of particular importance in antiviral and antitumor studies of such nucleoside analogues are 1-(β-D-arabinofuranosyl)cytosine (ara-C),[3] 9-(β-D-arabinofuranosyl)adenine (ara-A),[4] and 9-(β-D-xylofuranosyl)-adenine.[5] The observation that most of the nucleoside analogues must be converted to nucleotides[1,6] before they are biologically active, coupled with the increased water solubility of such nucleotides over the corresponding nucleosides,[7] has prompted considerable activity toward the synthesis and biological evaluation of phosphorylated compounds of the above class.[8]

The importance of guanine nucleotide metabolism in a variety of microbiological and mammalian systems has been comprehensively reviewed.[9] Antimetabolites have proved to be unique biochemical tools in probing enzymatic transformations. The biological resistance to purine and pyrimidine antimetabolites is ascribed to high levels of a deaminase[4b] or lack of enzymatic phosphorylation of the nucleosides.[10] This problem could be overcome by using 5′-monophosphates of the nucleosides. However, the free nucleotides at physiological pH carry two negative charges and in general[11] penetrate the cell as an intact nucleotide in very small amounts. The exogenous adenosine cyclic 3′,5′-monophosphate (c-AMP)[12a] and guanosine cyclic 3′,5′-monophosphate (c-GMP)[13] may exert specific biological effects of c-AMP or c-GMP, respectively, on the cell membrane[12b] and the metabolic pathways inside the cell. The interesting biological activity reported for 9-(β-D-xylofuranosyl)purines[14] suggested the synthesis of 9-(β-D-xylofuranosyl)guanine 5′-monophosphate and the cyclic 3′,5′-monophosphate as potential antiviral agents.



1 2 3

For the synthesis of 9-(β-D-xylofuranosyl)guanine (**1**), the method reported by Lee and co-workers[15] via the

Table I. Comparative in Vitro Antiviral Activity of Ara-A, Xylo-G, Xylo-GMP, and c-Xylo-GMP

Compd	Virus ratings[a]			Min KB cell cytotoxic dose, μg/ml
	Herpes virus[b] type 1	Herpes virus type 2	Vaccinia virus	
Ara-A	0.9	0.8	0.8	3.2
Xylo-G	0.6	0.2	0.5	10.0
Xylo-GMP	0.6	c	0.4	3.2
c-Xylo-GMP	0.5	0.4	0.5	32.0

[a] The virus rating (VR) was determined by comparing CPE development in drug-treated cells (T) and virus control cells (C). The CPE value (0-4) assigned to T for each drug level was subtracted from that of C, and the differences (C – T) were totaled. If partial toxicity was evident at any drug level, the C – T of that level was divided by 2. The sum of all C – T values was then divided by ten times the number of test cups used per drug level. [b] Minimum inhibitory concentrations for compounds in this experiment are as follows: ara-A, 3.2 μg/ml; xylo-G, 10 μg/ml; xylo-GMP, 10 μg/ml; and c-xylo-GMP, 32 μg/ml. [c] Not determined.

coupling of the trimethylsilyl derivative of 2-amino-6-chloropurine with 2,3,5-tri-*O*-acetyl-D-xylofuranosyl bromide followed by the conversion of the condensed product, 2-amino-6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-xylofuranosyl)purine, with mercaptoethanol in the presence of sodium methoxide in methanol was used. The enzymatic phosphorylation of **1** using nucleoside phosphotransferase (from *Pseudomonas trifolii*[16]) and *p*-nitrophenyl phosphate as the phosphate donor has been reported[17] to yield 9-(β-D-xylofuranosyl)guanine 5′-monophosphate (**2**), isolated as the ammonium salt. However, the direct chemical phosphorylation[8c,18] of **1** using phosphoryl chloride in trimethyl phosphate at −10° for 2 h followed by hydrolysis furnished a mixture of **2** and 9-(β-D-xylofuranosyl)guanine cyclic 3′,5′-monophosphate (**3**), which was separated by ion-exchange chromatography (Dowex 1) and isolated in free acid form. The purity of **2** and **3** was assured by the homogeneity in several thin-layer systems and on paper electrophoresis (phosphate buffer, pH 7.2, and borate buffer, pH 9.2).

Antiviral Evaluation. Inhibition of the virus-induced cytopathic effect (CPE) was used as the initial indicator of antiviral activity. CPE was observed in human carcinoma of the nasopharynx (KB) cells after infection with type 1 (HV/1) or type 2 (HV/2) herpes virus or vaccinia virus (VV). In this system, monolayers (18–24 h) of cells were exposed to 320 $CCID_{50}$ of virus and concentrations of each compound ranging in one-half log dilutions from 1000 to 1 μg/ml were added within 15 min. The degree of CPE inhibition and compound cytotoxicity were observed microscopically after 72 h of incubation at 37° and scored numerically in order to calculate a virus rating (VR) as previously described.[19] Significance of antiviral activity in terms of VR's has been assigned as follows: <0.5, slight or no activity; 0.5–0.9, moderate activity; and ≥1.0, marked activity. The results of a single experiment in parallel with ara-A are shown in Table I. Of the compounds tested, ara-A possessed the best activity against all viruses with the xylopurines having comparable slight to moderate antiviral activity against HV/1 and VV but none had appreciable activity against HV/2.

The moderate in vitro anti-DNA virus activity observed for this class of synthetic compounds led us to the evaluation of their efficacy as antiviral agents in vivo. Encephalitis was induced in 18–20-g Swiss mice by intracerebral (ic) inoculation of an LD_{75} of HV/1, strain 123.

Table II. Effect of Intracerebrally Administered Xylo-G, Xylo-GMP, c-Xylo-GMP, or Ara-A on Herpes Virus Type 1 Induced Encephalitis in Mice

Compd	Dosage, mg/kg	Survivors/total
	Experiment 1	
Saline	0.03[c]	6/24
Xylo-G	5.0	4/10
Xylo-GMP	1.25	4/10
c-Xylo-GMP	1.25	9/10[a]
Ara-A	3.2	5/9
	Experiment 2	
Saline		3/20
c-Xylo-GMP	1.25	6/10[b]
	0.31	5/10[b]

[a] Probability <0.01, Fisher's exact test. [b] Probability <0.05, Fisher's exact test. [c] ml.

Table III. Effect of Intraperitoneally[a] Administered c-Xylo-GMP on Herpes Virus Type 1 Induced Encephalitis in Mice

Drug dosage, mg/kg/day	Toxicity controls (survivors/total)	Infected (survivors/total)
Saline		2/21
104	4/5	2/10
52	5/5	5/10[b]
26	c	3/9[d]

[a] b.i.d. × 9, starting 4 h postvirus inoculation. [b] Probability (p) < 0.02, Fisher's exact test. [c] Not determined. [d] This dose extended survival by 2.4 days ($p < 0.02$, t test).

A single treatment with the maximum tolerated dose (MTD) of each drug was administered ic at 6 h postvirus inoculation.[8d] c-Xylo-GMP increased the number of surviving animals to the greatest extent with xylo-G, xylo-GMP, and ara-A being similar (see Table II, experiment 1). These observations suggest that the cyclic nucleotide could be the active form, since xylo-G and xylo-GMP would have the same potential for conversion to other nucleotides (a di- or triphosphate). The c-xylo-GMP activity was further verified in a second experiment with the compound being active at its MTD and MTD/4 (0.31 mg/kg). Thus, in this experiment c-xylo-GMP demonstrated a marked ability to protect mice from HV/1 encephalitic deaths with the compound having a therapeutic index (TI = MTD/minimum inhibitory dose) of ≥4. This activity stimulated an additional experiment to determine the effect of intraperitoneal administration of c-xylo-GMP on the encephalitic deaths. c-Xylo-GMP was found to have a narrow range of effectiveness (Table III). The only dose (52 mg/kg/day) which increased survivors caused a marked weight loss in both toxicity and infected animals. In addition, the lowest dose significantly increased survival time. The TI of about 4, observed for c-xylo-GMP, was much lower than that usually found for ara-A (~30)[8e] and probably resulted from the greater toxicity. Since c-xylo-GMP was more toxic and comparatively less active than other potential anti-DNA virus agents,[8c] no further studies have been planned.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Specific rotations were measured in a 1-dm tube with a Perkin-Elmer Model 141 automatic digital readout polarimeter. Nuclear magnetic resonance (^1H NMR) spectra were recorded at 60 MHz on a Hitachi Perkin-Elmer R-20A spectrometer in Me_2SO-d_6 as well as in D_2O–NaOD using DSS as an internal standard. Ultraviolet spectra (uv, s = shoulder) were recorded on a Cary Model 15 spectrophotometer. Elemental analyses were performed by

Galbraith Laboratories, Inc., Knoxville, Tenn., and the results are within ±0.4% of the theoretical values. Thin-layer chromatography was run on a silica gel F-254 (EM Reagents) plates and developed with either solvent system: A, 2-propanol–concentrated ammonium hydroxide–water (7:1:2, v/v); B, 2-propanol–concentrated ammonium hydroxide–water (5.5:1.0:3.5, v/v); C, 1-butanol–acetic acid–water (5:2:3, v/v); and D, acetonitrile–0.2 M aqueous ammonium chloride (7:3, v/v). Evaporations were carried out under reduced pressure with bath temperature below 30°.

9-(β-D-Xylofuranosyl)guanine 5′-Monophosphate (2) and 9-(β-D-Xylofuranosyl)guanine Cyclic 3′,5′-Monophosphate (3). To a solution of phosphorus oxychloride (2.9 ml) in freshly distilled trimethyl phosphate (20.0 ml) cooled in an ice–salt bath (−10°) was added 9-(β-D-xylofuranosyl)guanine[15] (1, 3.0 g, 0.0105 mol, dried at 80° for 15 h over P_2O_5 under vacuum). The contents of the stoppered flask was stirred at −10°. Within 25 min a clear solution was obtained. Stirring was continued for 2 h before the resulting, slightly orange-colored solution was poured into ice–water (150 ml) containing sodium hydrogen carbonate (2.5 g) with stirring and external cooling. The mixture was occasionally stirred in an ice bath for 1 h, and the pH was monitored at 5–6 by adding solid sodium hydrogen carbonate when needed. The pH stabilized solution was extracted with ether (3 × 50 ml) and the aqueous phase was concentrated in vacuo until salts began to crystallize. Enough water was added to achieve solution; the pH was adjusted to 6–7, before it was applied to a column containing Dowex 1 X2 (100–200 mesh, formate form, 100 ml). The resin was washed with water (3 l.) to remove unreacted 1 and the inorganic salts before using gradient elution (0.5 M formic acid to water). The eluent containing the product was pooled, frozen, and lyophilized to yield 2.95 g of a cream-colored solid which was found to be a mixture of **2** and **3**.

The above mixture was dissolved in water (25 ml) and the pH was checked (6–7) before it was rechromatographed on a freshly generated Dowex 1 X2 resin column (100–200 mesh, formate form, 100 ml). The column was first washed with water (2 l.) before the nucleotides were eluted using a gradient (0.25 M formic acid to water). The first fraction was pooled and concentrated to ~15 ml. Ethanol (50 ml) was added and refrigerated overnight. The white solid that separated was collected, washed with ethanol, and dried over P_2O_5. It was crystallized from aqueous ethanol as needles to yield 2.45 g (63.7%) of 9-(β-D-xylofuranosyl)guanine cyclic 3′,5′-monophosphate (3), mp >235° dec, which was homogeneous on paper electrophoresis and on paper chromatography (solvent A): $[\alpha]^{25}D$ −48.5° (c 1.0, water); ^1H NMR (D_2O) δ 7.98 (s, C_8H), 5.99 (s, $C_1'H$); uv λ max (pH 1) 255 nm (ε 13 460), 275 s (9400); λ max (pH 7) 252 nm (ε 15 140), 269 s (11 100); λ max (pH 11) 256–268 nm (ε 12 800). Anal. ($C_{10}H_{12}N_5O_7P \cdot H_2O$, 363.22) C, H, N.

The second fraction containing the homogeneous product was pooled and concentrated to ~3 ml. Ethanol (25 ml) was added and refrigerated overnight. The white amorphous solid that separated was collected, washed with ethanol, and dried over P_2O_5 at 60° to yield 0.35 g (8.0%) of 9-(β-D-xylofuranosyl)guanine 5′-monophosphate (**2**), mp >250° dec, which was homogeneous on paper chromatography (solvent A): $[\alpha]^{25}D$ −40.3° (c 1.0, water); ^1H NMR (Me_2SO-d_6–D_2O) δ 7.90 (s, C_8H), 5.85 (d, J = 2.5 Hz, $C_1'H$); uv λ max (pH 1) 255 nm (ε 12 700), 274 s (8600); λ max (pH 7) 252 nm (ε 13 800), 270 s (9700); λ max (pH 11) 256–267 nm (ε 11 960). Anal. ($C_{10}H_{14}N_5O_8P \cdot 3H_2O$, 417.26) C, H, N.

References and Notes

(1) (a) S. S. Cohen, *Prog. Nucl. Acid Res. Mol. Biol.*, **5**, 1 (1966); (b) R. J. Suhadolnik, "Nucleoside Antibiotics", Wiley-Interscience, New York, N.Y., 1970, p 123.

(2) (a) D. B. Ellis and G. A. LePage, *Can. J. Biochem.*, **43**, 617 (1965); (b) *Mol. Pharmacol.*, 1, 231 (1965).

(3) (a) G. E. Underwood, *Proc. Soc. Exp. Biol. Med.*, 111, 660 (1962); (b) H. E. Kaufman and E. D. Maloney, *Arch. Ophthalmol.*, **69**, 626 (1963); (c) R. D. Casey and R. R. Ellison, *Clin. Res.*, **13**, 337 (1965); (d) T. C. Hall, J. Griffiths, G. Watters, R. Baringer, and S. Katz, *Pharmacologist*, **10**, 171 (1968); (e) E. M. McKelvey and H. C. Kwaan, *Blood*, **34**, 706 (1969); (f) M. D. Dowling, Jr., I. H. Krakoff, and D. A. Karnofsky in "Chemotherapy of Cancer", W. H. Cole, Ed., Lea and Febiger, Philadelphia, Pa., 1970, p 1; (g) D. Prager, M. Bruder, and A. Sawitsky, *J. Pediat.*, **78**, 321 (1971).

(4) (a) F. M. Schabel, Jr., *Chemotherapy*, **13**, 321 (1968); (b) D. L. Chao and A. P. Kimball, *Cancer Res.*, **32**, 1721 (1972); (c) F. M. Schabel, Jr., and J. A. Montgomery in "Chemotherapy of Virus Diseases", Vol. 1, D. Bauer, Ed., Pergamon Press, Oxford, 1972, Chapter 4, p 231; (d) B. E. Juel-Jensen and F. O. MacCallum in "Herpes Simplex, Varicella and Zoster", J. B. Lippincott Co., Philadelphia, Pa., 1972; (e) L. T. Ch'ien, F. M. Schabel, Jr., and C. A. Alford, Jr., in "Selective Inhibitors of Viral Functions", W. A. Carter, Ed., CRC Press, Cleveland, Ohio, 1973, p 227; (f) for a review of preliminary results, see *J. Am. Med. Assoc.*, **230**, 189 (Oct 14, 1974).

(5) D. B. Ellis and G. A. LePage, *Cancer Res.*, **26**, 893 (1966).

(6) (a) G. A. LePage, Y. T. Lin, R. E. Orth, and J. A. Gottlieb, *Cancer Res.*, **32**, 2441 (1972); (b) W. H. Prusoff and B. Goz, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **32**, 1679 (1973).

(7) For the therapeutic use of nucleosides and the penetrability of phosphorylated compounds, see S. S. Cohen, *Biochem. Pharmacol.*, **24**, 1929 (1975).

(8) (a) R. W. Sidwell, L. B. Allen, J. H. Huffman, T. A. Khwaja, R. L. Tolman, and R. K. Robins, *Chemotherapy*, **19**, 325 (1973); (b) A. M. Mian, R. Harris, R. W. Sidwell, R. K. Robins, and T. A. Khwaja, *J. Med. Chem.*, **17**, 259 (1974); (c) G. R. Revankar, J. H. Huffman, L. B. Allen, R. W. Sidwell, R. K. Robins, and R. L. Tolman, *ibid.*, **18**, 721 (1975); (d) L. B. Allen, J. M. Thompson, J. H. Huffman, G. R. Revankar, R. L. Tolman, L. N. Simon, R. K. Robins, and R. W. Sidwell, *Antimicrob. Agents Chemother.*, **8**, 468 (1975); (e) L. B. Allen, J. H. Huffman, G. R. Revankar, R. L. Tolman, L. N. Simon, R. K. Robins, and R. W. Sidwell, *ibid.*, **8**, 474 (1975); (f) R. W. Sidwell, L. B. Allen, J. H. Huffman, G. R. Revankar, R. K. Robins, and R. L. Tolman, *ibid.*, **8**, 463 (1975); (g) G. A. LePage, S. R. Naik, S. B. Katakkar, and A. Khaliq, *Cancer Res.*, **35**, 3036 (1975); (h) R. A. Long, G. L. Szekeres, T. A. Khwaja, R. W. Sidwell, L. N. Simon, and R. K. Robins, *J. Med. Chem.*, **15**, 1215 (1972); (i) R. W. Sidwell, L. N. Simon, J. H. Huffman, L. B. Allen, R. A. Long, and R. K. Robins, *Nature (London)*, **242**, 204 (1973).

(9) C. I. Pogson, *Am. J. Clin. Nutr.*, **27**, 380 (1974).

(10) I. C. Caldwell, J. F. Henderson, and A. R. P. Peterson, *Can. J. Biochem.*, **45**, 735 (1967).

(11) W. Plunkett, L. Lapi, P. J. Ortiz, and S. S. Cohen, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 73 (1974).

(12) (a) G. A. LePage and E. M. Hersh, *Biochem. Biophys. Res. Commun.*, **46**, 1918 (1972); (b) P. V. Hauschka, L. P. Everhart, and R. W. Rubin, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 3542 (1972).

(13) J. W. Hadden, E. M. Hadden, M. K. Haddox, and N. D. Goldberg, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 3024 (1972).

(14) (a) T. Kaneko and G. A. LePage, *Cancer Res.*, **30**, 699 (1970); (b) G. A. LePage and S. R. Naik, *Ann. N.Y. Acad. Sci.*, **255**, 481 (1975).

(15) W. W. Lee, A. P. Martinez, L. Goodman, and D. W. Henry, *J. Org. Chem.*, **37**, 2923 (1972).

(16) K. Mitsugi, K. Komagata, M. Takahashi, H. Iizuka, and H. Katagiri, *Agric. Biol. Chem.*, **28**, 586 (1964).

(17) S. Suzaki, A. Yamazaki, A. Kamimura, K. Mitsugi, and I. Kumashiro, *Chem. Pharm. Bull.*, **18**, 172 (1970).

(18) M. Yoshikawa, T. Kato, and T. Takenishi, *Tetrahedron Lett.*, 5065 (1967).

(19) R. W. Sidwell and J. H. Huffman, *Appl. Microbiol.*, **22**, 797 (1971).