Professor G. C. Lancini for discussions and encouragement.

References and Notes

(1) B. Cavalleri, R. Ballotta, V. Arioli, and G. C. Lancini, *J. Med. Chem.*, **16**, 557 (1973).
(2) B. Cavalleri, G. Volpe, and R. Pallanza, *Arzneim.-Forsch.*, **25**, 148 (1975).
(3) B. Cavalleri, R. Ballotta, and V. Arioli, *Arzneim.-Forsch.*, **25**, 338 (1975).
(4) B. Cavalleri, G. Volpe, V. Arioli, and G. C. Lancini, *Arzneim.-Forsch.*, in press.
(5) B. Cavalleri, G. Volpe, A. Ripamonti, and V. Arioli, *Arzneim.-Forsch.*, in press.
(6) B. Cavalleri, R. Ballotta, and G. C. Lancini, *J. Heterocycl. Chem.*, **9**, 979 (1972).
(7) G. C. Lancini, E. Lazzari, V. Arioli, and P. Bellani, *J. Med. Chem.*, **12**, 775 (1969).
(8) V. Arioli, R. Pallanza, S. Furesz, and G. Carniti, *Arzneim.-Forsch.*, **17**, 523 (1967).
(9) B. Cavalleri, R. Ballotta, and V. Arioli, *Chim. Ther.*, **6**, 397 (1971).

Synthesis and Anti-Herpes Simplex Activity of Analogues of Phosphonoacetic Acid

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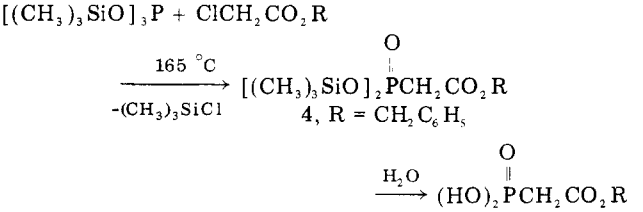
The synthesis of monoesters (P and C) of phosphonoacetic acid (PA) is given. The carboxyl esters were prepared by two methods: the reaction of chloroacetates with tris(trimethylsilyl) phosphite, followed by hydrolysis; and by the acid-catalyzed esterification of PA with the appropriate alcohol. P-Monoesters of PA were prepared either by the reaction of alkyl[bis(trimethylsilyl)] phosphite with benzyl chloroacetate followed by deprotection or by the reaction of dimethylphenyl phosphite with benzyl bromoacetate followed by hydrogenolysis. Three aryl- (alkyl-) phosphinic acid derivatives are reported. The above compounds were evaluated for anti-herpes activity against HSV-induced DNA polymerase and in animals infected with herpes dermatitis.

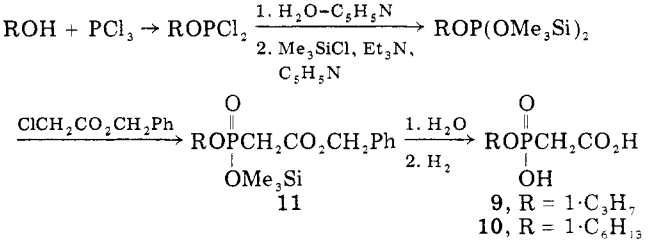
Since the original report of the suppression of herpes simplex virus (HSV) in rabbits by phosphonoacetic acid (1),[1] Gerstein et al.[2] have reported 1 to be equivalent to idoxuridine against an established herpes infection in rabbits. Phosphonoacetic acid has been shown to specifically inhibit HSV-induced DNA polymerase.[3] These promising early results with 1 encouraged a synthetic program to find an analogue of 1 with an improved therapeutic ratio. Prior to the start of the synthetic program, J. Mao (unpublished results) had found simple P-diesters of 1 [$(RO)_2P(O)CH_2CO_2H$] to be inactive against HSV-induced DNA polymerase. Therefore, the major effort was directed toward the synthesis of monoesters of 1. We wish to report some of the results from this study.

Chemistry. Simple alkylcarboxyl esters of 1 may be prepared by direct esterification of 1 with the appropriate alcohol and HCl.[4] Compounds **2** and **3** were prepared by this method. A more versatile method of preparation of carboxyl esters of 1 was suggested by a paper by Hata and Sekine.[5] These workers reported the reaction of tris(trimethylsilyl) phosphite [$(Me_3SiO)_3P$] and 5′-bis(trimethylsilyl) phosphite esters of nucleosides with diphenyl disulfide to give *S*-phenylphosphorothioates. A successful Arbuzov reaction with $(Me_3SiO)_3P$ and the appropriate chloroacetate would provide ready access to carboxyl esters of 1. When a solution of $(Me_3SiO)_3P$ and benzyl chloroacetate was heated to 165 °C, a vigorous reaction occurred as evidenced by the formation of chlorotrimethylsilane. Distillation of the pot residue yielded benzyl *P,P*-bis(trimethylsilyl)phosphonoacetate (**4**) in 83% yield (Scheme I). Treatment of the silyl ester **4** with H_2O yielded benzyl phosphonoacetate. The chloroacetates of two other alcohols were converted to the corresponding carboxyl esters of 1 (**6** and **7**) in a similar manner and these esters are listed in Table I. As this part of the work was being completed, Rosenthal et al.[6] and Hata et al.[7] reported the utility of $(Me_3SiO)_3P$ in Arbuzov reactions to yield readily hydrolyzable bis(trimethylsilyl)phosphonate esters.

Scheme I



Scheme II



Monoesters of phosphonic acids have been prepared by coupling phosphonic acids and alcohol with dicyclohexylcarbodiimide in refluxing THF.[8] Application of this method to the preparation of P-monoesters of 1 using benzyl phosphonoacetate and 1-propanol gave a mixture of benzyl *P*-propylphosphonoacetate and unreacted benzyl phosphonoacetate (10–20%). Debenzylation of the ester mixture gave a mixture of *P*-propylphosphonoacetic acid and 1 which was difficult to purify by crystallization. The coupling of benzyl phosphonoacetate and 1-hexanol gave a mixture of di- and monohexyl esters of *C*-benzyl phosphonoacetate. A cleaner method of preparation of *P*-propyl- and *P*-hexylphosphonoacetic acid (**9** and **10**, respectively) is outlined in Scheme II. Propyl phosphorodichloridite[4b] was hydrolyzed with 2 equiv of H_2O to give propyl phosphite, which was converted to propylbis(trimethylsilyl) phosphite with chlorotri-

Table I. Esters and Phosphinic Acid Analogues of Phosphonoacetic Acid

$R_1R_2P(=O)CH_2CO_2R_3$

Compd	R_1	R_2	R_3	Method of prepn	Mp, °C	Formula
2	OH	OH	1-C_3H_7	A		$C_5H_{11}O_5 \cdot 0.67H_2O$[a]
3	OH	OH	1-C_8H_{17}	A		$C_{10}H_{21}O_5P \cdot 0.50H_2O$[b]
5	OH	OH	$PhCH_2$	B		$C_7H_{11}O_5P \cdot NH_3$
6	OH	OH	c-C_6H_{11}	B		$C_8H_{15}O_5P \cdot NH_3$
7	OH	OH	*t*-Bu	B		$C_6H_{13}O_5P \cdot 2NH_3$
8	CH_3O	OH	H		183-186	$C_3H_7O_5P \cdot NH_3$
9	1-C_3H_7O	OH	H	C	155-156.5	$C_5H_{11}O_5P \cdot NH_3$
10	1-$C_6H_{13}O$	OH	H	C	178-190	$C_8H_{17}O_5P \cdot NH_3$
13	Ph	OH	H	D	120.5-123.0	$C_8H_9O_4P$
14	4-$CH_3OC_6H_4$	OH	H	D	165-166	$C_9H_{11}O_5P$
16	CH_3	OH	H			$C_3H_7O_3P \cdot NH_3$

[a] High-resolution mass measurements: measured, 183.0423; calcd for $C_5H_{12}O_5P$, 183.0423. [b] High-resolution mass measurements: measured, 253.1194; calcd for $C_{10}H_{22}O_5P$, 253.1205.

Scheme III

$PhOP(OCH_3)_2 + BrCH_2CO_2CH_2Ph \rightarrow CH_3OP(=O)(OPh)CH_2CO_2CH_2Ph \xrightarrow{H_2} CH_3OP(=O)(OH)CH_2CO_2H$

12

methylsilane and Et_3N in pyridine. The reaction of propylbis(trimethylsilyl) phosphite with benzyl chloroacetate proceeded cleanly to give benzyl *P*-propyl-*P*-(trimethylsilyl)phosphonoacetate (11, R = Pr). Conversion of the latter to *P*-propylphosphonoacetic acid was accomplished by treatment with H_2O, followed by hydrogenolysis. *P*-Methylphosphonoacetic acid was prepared by a slightly different method (Scheme III). Dimethylphenyl phosphite was allowed to react with benzyl bromoacetate to give the expected Arbuzov product (12) which underwent hydrogenolysis to give *P*-methylphosphonoacetic acid. Dephenylation of phosphonate esters by hydrogenolysis has been reported by Griffin and Burger.[9] The P-monoesters (*P*-methyl, *P*-propyl, and *P*-hexyl) of 1 (8–10, respectively) were characterized as their monoammonium salts.

The preparation of (hydroxyphenylphosphinyl)acetic acid (13) was achieved by the reaction of dimethyl phenylphosphonite with methyl chloroacetate, followed by hydrolysis of the resulting diester with aqueous HCl.[10] When this method was extended to the preparation of [hydroxy(4-methoxyphenyl)phosphinyl]acetic acid (14), the acid hydrolysis of the intermediate diester led to a mixture of two compounds, which from the NMR and TLC data appeared to be the expected product and 1. The preparation of the two target compounds (13 and 14) was accomplished by the method outlined in Scheme IV. Hydrolysis of 4-methoxyphenylphosphonous dichloride,[11] followed by silylation with chlorotrimethylsilane and Et_3N in pyridine, gave the bis(trimethylsilyl) ester 15 (R = 4-$MeOC_6H_4$). The silyl ester reacted cleanly with *tert*-butyl chloroacetate at 125 °C to give, after treatment with H_2O, *tert*-butyl [hydroxy(4-methoxyphenyl)phosphinyl]acetate (74% from 15, Ar = *p*-$CH_3OC_6H_4$). Heating the latter compound to 160 °C gave [hydroxy(4-methoxyphenyl)phosphinyl]acetic acid (14) in 76% yield.

The reaction of dimethyl methylphosphonite with methyl bromoacetate followed by acid hydrolysis gave [hydroxymethylphosphinyl]acetic acid (16). The acid was characterized as its ammonium salt.

Biological Results and Discussion. Compounds listed in Table I were evaluated for activity against

Scheme IV

$ArP(=O)(H)OH \xrightarrow[Et_3N,\ C_5H_5N]{Me_3SiCl} ArP(OMe_3Si)_2$ (15) $\xrightarrow[2.\ H_2O]{1.\ ClCH_2CO_2\text{-}t\text{-}Bu}$ $ArP(=O)(OH)CH_2CO_2\text{-}t\text{-}Bu \xrightarrow{160\ °C} ArP(=O)(OH)CH_2CO_2H$

13, Ar = C_6H_5
14, Ar = 4-$CH_3OC_6H_4$

HSV-induced DNA polymerase and against herpes dermatitis in mice. While the low-molecular-weight carboxyl esters of phosphonoacetic acid (2, 3, and 5–7) showed some activity in the enzyme test, only the propyl ester 2 was active in the herpes dermatitis test. Because of the high inhibitory action of 2 toward HSV–DNA polymerase, the suppression of herpes dermatitis may be due to the parent compound and not the result of metabolism of 2 to 1. Of the three P-monoesters of 1, only the hexyl ester 10 showed activity in vivo. As 10 was not active in the enzyme test, the ester is presumably converted to 1 which is the active drug. The three phosphinic acid derivatives (13, 14, and 16) were inactive in both tests (see Table II).

Experimental Section

The melting points were taken on a Thomas-Hoover apparatus and are not corrected. NMR spectra were recorded for intermediate and target compounds on a Varian T-60 and were consistent with the proposed structures. Analytical values for C, H, and N were within 0.4% of theory unless otherwise stated. Mass spectra were obtained on an MS-902 by direct insertion at 120–150 °C and the empirical formulas verified by the peak-matching technique.

Method A. Propyl Phosphonoacetate. A stream of HCl gas was passed through a solution of 5 g of 1 in 10 mL of 1-propanol for 0.5 h. The solution was refluxed for 4 h and the solvent evaporated at reduced pressure. The residue was dried at 0.5 mm to give 5 g of a viscous liquid. Anal. ($C_5H_{11}O_5 \cdot {}^2/_3H_2O$) C, H.

Method B. Benzyl Phosphonoacetate Monoammonium Salt. Tris(trimethylsilyl) phosphite [$(Me_3SiO)_3P$] was prepared in the following manner. References 5 and 6 give earlier references to $(Me_3SiO)_3P$. To 450 mL of pyridine (dried over KOH) was added 16.4 g (0.20 mol) of phosphorous acid. The mixture was cooled in an ice bath and 126 mL (0.90 mol) of dry Et_3N added, followed by the dropwise addition of 112 mL (0.90 mol) of chlorotrimethylsilane. The mixture was allowed to warm to room temperature gradually and was stirred overnight. Dry Et_2O (~500 mL) was added and the mixture filtered. The filtrate was concentrated on a rotary evaporator to remove the Et_2O and most of the pyridine. The residue was distilled to give 39.56 g (66%) of product, bp 67–68 °C (10 mm) [lit.[5] bp 86.5 °C (18 mm)]. The

Table II. Anti-Herpes Evaluation of Analogues of Phosphonoacetic Acid

Compd[a]	50% inhibitory concn to HSV-DNA polymerase type 2, μg/mL	Route of medication and dose[b]	Rank sum[c]		U values	
			Treated	Virus control	U_1	U_2
1	0.22	T, 2%	142.0[d]	68.0	13.0	87.0
		O, 800	132.0[d]	78.0	23.0	77.0
2	0.16	O, 100	126.0	84.0	29.0	71.0
		O, 500	111.5	98.5	43.5	56.5
		O, 1000	136.5[d]	73.5	18.5	81.5
		T, 2%	138.0[d]	72.0	17.0	83.0
3	0.26	O, 800	81.0	109.0	54.9	36.0
		T, 2%	110.0	100.0	45.0	55.0
5	40	O, 800	50.0	70.0	15.0	35.0
		T, 2%	105.0	105.0	50.0	50.0
6	49	O, 800	116.0	94.0	39.0	61.0
		T, 2%	98.0	112.0	57.0	43.0
7	3.9	O, 800	124.5	85.5	30.5	69.5
		T, 2%	121.5	88.5	33.5	66.5
8	19.9	O, 800	91.5	118.5	63.5	36.5
		T, 2%	116.0	94.0	39.0	61.0
9	200	O, 1000	106.0	104.0	49.0	51.0
		O, 500	121.0	89.0	34.0	66.0
		O, 100	106.5	103.5	48.5	51.5
		T, 2%	108.5	101.5	46.5	53.5
10	~166	O, 800	133.0[d]	77.0	22.0	78.0
		T, 2%	121.0	89.0	34.0	66.0
13	200	O, 800	85.0	125.0	70.0	30.0
		T, 2%	128.0	82.0	27.0	73.0
14	150	O, 800	90.0	120.0	65.0	35.0
		T, 2%	101.0	109.0	54.0	46.0
16	145	T, 2%	124.0	86.0	31.0	69.0

[a] Compound 1 was tested as the disodium salt. [b] In topical applications, a 2% aqueous solution was applied twice daily for 6 consecutive days. In oral medication, an aqueous solution, gavage, mg/kg/day, was given for 6 consecutive days as described in the Experimental Section. [c] A unique rating scale (unpublished work of Mr. E. T. Gade) was used in which the severity of the infection was quantitated by the day of occurrence of the following ordered parameters: death, paralysis, and lesion. The rank sum is based on the above and the Mann-Whitney U statistics[12] applied. A compound was then considered active if the treated groups showed significant differences from the virus control at the $p \leq 0.05$ level. [d] $p \leq 0.05$ level.

NMR spectra of samples of product [bp 67–68 °C (10 mm)] prepared in experiments using a 10% excess of chlorotrimethylsilane per phosphorus hydroxyl showed a doublet at δ 6.8 (J = 700 Hz) which is presumably due to P–H coupling in bis(trimethylsilyl) phosphite. These samples did not react with benzyl chloroacetate. The distilled product must also be free of pyridine in order to undergo a successful Arbuzov reaction.

A solution of 7.36 g (0.04 mol) of benzyl chloroacetate and 18.7 g (0.062 mol) of $(Me_3SiO)_3P$ was added to a flask equipped with a distillation head. The flask was heated gradually to 160–165 °C, at which temperature chlorotrimethylsilane began to distill. The distillation was complete within ~10 min and the solution held at 160–165 °C for a total of 1 h. The volatile material in the pot residue was removed by evaporative distillation [air bath temperature 120 °C (0.5 mm)]. The pot residue was evaporatively distilled at 140–150 °C (0.5 mm) to give 14.28 g (83%) of benzyl *P,P*-bis(trimethylsilyl)phosphonoacetate. To 5 g (0.0116 mol) of silyl ester was added 20 mL of H_2O. The mixture was concentrated at reduced pressure, the residue made alkaline with NH_4OH, and the solvent evaporated. The residue was crystallized from EtOH to give 1.20 g (41%) of the monoammonium salt of benzyl phosphonoacetate (no distinct melting point). Anal. ($C_9H_{14}NO_5P$) C, H, N.

Method C. *P*-Propylphosphonoacetic Acid Monoammonium Salt (9). A solution of 80 g (0.50 mol) of propyl phosphorodichloridite[4b] in 60 mL of Et_2O was added dropwise to a solution of 79 g (1.00 mol) of pyridine and 18 g (1.00 mol) of H_2O in 500 mL of Et_2O cooled with an ice bath. The mixture was allowed to warm to room temperature and stirred for 2 h. The mixture was filtered and the filtrate dried over $MgSO_4$ and concentrated at reduced pressure. The residue was dissolved in 500 mL of dry pyridine and chilled in an ice bath. To the chilled solution was added 208 mL (1.5 mol) of dry Et_3N followed by the dropwise addition of 189 mL (1.5 mol) of chlorotrimethylsilane. The mixture was stirred overnight at room temperature. The mixture was diluted with 500 mL of Et_2O and filtered and the filtrate concentrated at reduced pressure. The residue was distilled to give 50.34 g (37% from propyl phosphorodichloridite) of propylbis(trimethylsilyl) phosphite, bp 81.5–83.0 °C (10 mm). A mixture of the latter compound, 13.4 g (0.05 mol), and 9.2 g (0.05 mol) of benzyl chloroacetate was heated as described in method B at 165 °C to give after evaporative distillation [air bath temperature 140–160 °C (0.5 mm)] 12.77 g (74%) of benzyl propyl(trimethylsilyl)phosphonoacetate. The benzyl ester was treated with H_2O and NH_4OH and debenzylated with H_2–Pd/C to give, after crystallization from EtOH, 5.35 g [38% from benzyl propyl(trimethylsilyl)phosphonoacetate] of analytically pure monoammonium *P*-propylphosphonoacetate, mp 155–156.5 °C. Anal. ($C_5H_{14}NO_5P$) C, H, N.

Method D. [Hydroxy(4-methoxyphenyl)phosphinyl]acetic Acid (14). A solution of 23.65 g (0.123 mol) of 4-methoxyphenylphosphonous dichloride[11] in 25 mL of benzene was added dropwise to a solution of 20 mL of MeOH in 25 mL of benzene cooled in an ice bath. The solution was allowed to warm to room temperature and the solvent evaporated. The residue was added to 50 mL of H_2O containing 2 mL of 5% HCl. The mixture was refluxed for 3 h and then poured onto H_2O. The mixture was filtered and the precipitate crystallized from EtOAc to give 11.70 g (55%) of 4-methoxyphenylphosphonous acid,[13] mp 110.5–112.5 °C. A mixture of 17.35 g (0.101 mol) of 4-methoxyphenylphosphonous acid in 150 mL of dry pyridine was cooled in an ice bath and 42.1 mL (0.303 mol) of Et_3N added, followed by the dropwise addition of 38.2 mL (0.303 mol) of chlorotrimethylsilane. The reaction mixture was allowed to warm to room temperature gradually and was stirred overnight. The mixture was diluted with 200 mL of dry Et_2O and filtered, and the filtrate concentrated. The residue was distilled to give 20.53 g (64%) of bis(trimethylsilyl) 4-methoxyphenylphosphonite, bp 81–86 °C (50 μm). A solution of 20.50 g (0.0649 mol) of the above silyl ester and 9.75 g (0.065 mol) of *tert*-butyl chloroacetate was heated at 120–130 °C (oil bath) for 1.25 h. The chlorotrimethylsilane generated was allowed to distill from the reaction mixture. The

reaction mixture was evaporatively distilled to give 19.64 g (91%) of *tert*-butyl [(4-methoxyphenyl)trimethylsiloxyphosphinyl]-acetate, [air bath temperature 130 °C (50 μm)]. The latter compound was added to water and the solid filtered and dried to give 13.8 g [74% from bis(trimethylsilyl) 4-methoxyphenylphosphonite] of analytically pure *tert*-butyl [hydroxy(4-methoxyphenyl)phosphinyl]acetate, mp 125–129 °C dec (gas evolved). Anal. ($C_{13}H_{19}O_5P$) C, H. The latter compound, 12.85 g (0.049 mol), was heated at 160–165 °C (oil bath) for 1 h. The residue was crystallized from HOAc to give 7.94 g (76%) of analytically pure [hydroxy(4-methoxyphenyl)phosphinyl]acetic acid, mp 165–166 °C. Anal. ($C_9H_{11}O_5P$) C, H.

***P*-Methylphosphonoacetic Acid Monoammonium Salt.** Dimethylphenyl phosphite was prepared by the addition of 72.71 g (0.408 mol) of phenyl phosphorodichloridite[4b] to a solution of 26.11 g (0.816 mol) of MeOH and 64.46 mL (0.816 mol) of dry pyridine in 600 mL of dry Et_2O cooled in an ice bath. The mixture was allowed to warm to room temperature and stirred for 2 h. The mixture was filtered and the filtrate concentrated and distilled to give 44.80 g (59%) of dimethylphenyl phosphite, bp 96–98.5 °C (10 mm) [lit.[14] bp 96–97 °C (9 mm)]. A solution of 11.4 g (0.05 mol) of benzyl bromoacetate and 10.23 g (0.055 mol) of dimethylphenyl phosphite was heated at 130–140 °C (oil bath) for 5.25 h. The residue was evaporatively distilled to remove the volatile material. The pot residue, 14.8 g, was chromatographed on 300 g of Florisil. Elution with 2% MeOH–PhH gave 9.0 g of benzyl *P*-methyl-*P*-phenylphosphonoacetate. The triester, 9.0 g, was debenzylated with H_2–Pd/C to give 6.17 g (95%) of acid. Dephenylation with Pt–HOAc gave *P*-methylphosphonoacetic acid which was converted to its monoammonium salt with NH_4OH to give 2.74 g (60% for the last three steps) of the title compound, mp 183–186 °C. Anal. ($C_3H_{10}NO_5P$) C, H, N.

(Hydroxymethylphosphinyl)acetic Acid Monoammonium Salt. A solution of 11 g (0.081 mol) of diethyl methylphosphonite, prepared by a literature method,[13b] and 8.8 g (0.081 mol) of methyl chloroacetate was heated at 85 °C for 2 h. The residue was distilled to give 5.5 g (37%) of methyl (ethoxymethylphosphinyl)acetate, bp 103–105 °C (0.5 mm). The ester, 2.0 g, was hydrolyzed with 6 N HCl and the solvent evaporated. The oil was converted to the ammonium salt with NH_4OH and the salt crystallized from EtOH to give 1.2 g (70%) of product, mp 159–162 °C. Anal. ($C_3H_{10}NO_4P$) C, H, N.

Cutaneous Herpes Test in Mice. Female, 20-g CF mice, under light ether anesthesia, had a 20-mm^2 area of their back plucked free of hair. Herpes virus, type 2, ATCC, from Wi 38 infected cells ($10^{7.0}$ $TCID_{50}$/mL), was applied topically (0.05 mL) to the denuded skin and impregnated into the dermis with a 24-gauge sterile hypodermic needle. Herpes lesions or vesicles developed in 3–5 days. The lesions formed bands which extended over the denuded area. After 10 days the mice developed a paralysis which usually resulted in the death of the animal. The test was allowed to continue for a total of 17 days. Ten mice were used in each experiment. The mice that were treated topically had the drug applied to the site of infection as a 2% aqueous suspension 2 h after the virus was introduced into the skin and twice daily for 5 consecutive days. The drug was applied a total of 11 times. A single application of a 2% drug suspension delivered approximately 2 mg of material. Mice treated orally received the drug by gavage; the first medication (total dose) was administered 2 h after the virus was applied to the skin. Medication was continued twice daily (half the amount of drug given at each medication) for 5 consecutive days. The mice were medicated a total of 11 times during the course of the experiment.

DNA Polymerase Assay. HSV-induced DNA polymerase was purified by the method reported previously[3] and was assayed in a reaction mixture of 0.2 mL containing 10 μM dATP, dCTP, and dGTP and 2.5 μM [^{3}H]-TTP which was appropriately diluted with unlabeled TTP to give 880 counts/min per picomole, 10 μg of activated calf thymus DNA, 50 mM Tris–HCl buffer (pH 8.0), 3 mM $MgCl_2$, 100 mM KCl, and 1 mM dithiothreitol. The amount of enzyme used in each reaction was chosen to give a linear rate for at least 30 min at 37 °C. The reaction was terminated by the addition of 3 mL of cold 5% trichloroacetic acid–0.01 M sodium pyrophosphate. The acid-insoluble material was collected, washed on a glass fiber filter, and counted by the liquid scintillation method.

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References and Notes

(1) N. R. Shipkowitz, R. N. Bower, R. Appell, C. Nordeen, L. Overby, W. Roderick, J. Schleicher, and A. Von Esch, *Appl. Microbiol.*, **26**, 264 (1973).
(2) D. D. Gerstein, C. R. Dawson, and J. O. Oh, *Antimicrob. Agents Chemother.*, **7**, 285 (1975).
(3) J. C.-H. Mao, E. E. Robishaw, and L. R. Overby, *J. Virol.*, **15**, 1281 (1975).
(4) (a) G. M. Kosolapoff, "Organophosphorous Compounds", Wiley, New York, N.Y., 1950, p 160; (b) p 199.
(5) T. Hata and M. Sekine, *J. Am. Chem. Soc.*, **96**, 7363 (1974).
(6) A. F. Rosenthal, L. A. Vargas, Y. A. Isaacson, and R. Bittman, *Tetrahedron Lett.*, 977 (1975).
(7) T. Hata, M. Sekine, and N. Kagawa, *Chem. Lett.*, 635 (1975).
(8) A. Burger and J. J. Anderson, *J. Am. Chem. Soc.*, **79**, 3575 (1957).
(9) B. S. Griffin and A. Burger, *J. Am. Chem. Soc.*, **78**, 2336 (1956).
(10) H. G. Henning and G. Hilgetag, *J. Prakt. Chem.*, **29**, 86 (1965).
(11) J. A. Miles, M. T. Beeny, and K. W. Ratts, *J. Org. Chem.*, **40**, 343 (1975).
(12) S. Siegel, "Non-Parametric Statistics for the Behavior Sciences", McGraw-Hill, New York, N.Y., 1956, p 116.
(13) (a) G. M. Kosolapoff and L. Maier, "Organic Phosphorous Compounds", Vol. 4, Wiley-Interscience, New York, N.Y., 1972, p 362; (b) p 361.
(14) A. E. Arbuzov and L. V. Nesterov, *Bull. Acad. Sci. USSR, Div. Chem. Sci.*, 361 (1954); *Chem. Abstr.*, **49**, 9541e (1955).