range[40] of $\beta$-lactam structures and compared it with the kinetic parameters of their interaction with various enzymes (β-lactamases and peptidases), and the intrinsic chemical reactivity is shown not to be correlated with the enzymes' activities. It is proposed that the primary parameter that governs the biological action must be the goodness of fit of the β-lactam to the enzyme cavity, and this is in full agreement with the conclusions of our study.

The model proposed in our analysis also enables one to predict the possibility of restoring antibiotic properties to the inactive related structures and must help to arrive at a better understanding of the nature of the structural requirements, both geometric and electronic, that have to be controlled in the design of new therapeutic compounds in this field.

Note Added in Proof: Subsequent to the request of a referee, the analysis of Sulfazecin is presented here. The compound is a monobactam antibiotic (mainly active against Gram-negative bacteria) of the **20** type, the X-ray structure of which has been published.[41]   Due to the

20
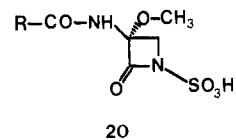
particular geometrical effects caused by the sulfur atom (bond lengths longer than those with the carbon), the resulting geometrical features of this novel antibiotic appear to be compatible with the 3-D requirements discussed in this paper (see view below). The β-lactam nitrogen is
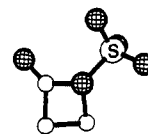
planar (the distance between the nitrogen and the plane of its three neighbors is 0.13 Å; the sum of the three valency angles around that atom is 357.7°). The distance between the oxygen of the amide group and the sulfur atom is 3.355 Å.

(40) Data on the intrinsic reactivity and some biochemical kinetic parameters of the structures mentioned in the present paper are available in ref 38.

(41) Kamiya, K.; Takamoto, M.; Wada, Y.; Asai, M. *Acta Crystallogr., Sect. B* **1981**, *B37*, 1626.

# Synthesis of Esters of Phosphonoformic Acid and Their Antiherpes Activity[1,2]

Jan O. Norén,* Erik Helgstrand,[3] Nils G. Johansson, Alfons Misiorny, and Göran Stening

Research and Development Laboratories, Astra Läkemedel AB, S-151 85 Södertälje, Sweden. Received March 23, 1982

Aliphatic and aromatic mono-, di-, and triesters of phosphonoformic acid (foscarnet) were synthesized. The triesters were prepared by the Michaelis–Arbuzov reaction and were hydrolyzed to di- and monoesters. The compounds were tested for antiviral activity on isolated herpes simplex virus type 1 (HSV-1) DNA polymerase, in a HSV-1 plaque reduction assay, and on a cutaneous HSV-1 infection in guinea pigs. None of the esters inhibited the activity of isolated HSV-1 polymerases. Monoesters with a free carboxylic group and diesters with an aromatic carboxylic ester function were active against the cutaneous herpes infection. Mono- and diesters with an aromatic phosphonic ester group also showed activity in the plaque-reduction assay. However, mono- and diesters with aliphatic carboxylic ester groups were inactive in all test systems. The results show that all three acidic groups of phosphonoformic acid must be free in order to get antiviral activity at the enzyme level. However, certain esters of this acid may be biotransformed to the acid itself to give antiherpes activity.

Herpes viruses induce a virus-specific DNA polymerase activity in infected cells.[4-6]   Therefore, this enzyme is a possible target for selective antiviral drugs. Phosphono-formic acid [(hydroxycarbonyl)phosphonic acid] trisodium salt (PFA) (INN, foscarnet sodium, **1**) and phosphono-

1
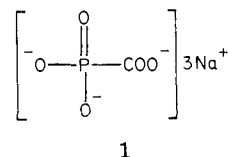
acetic acid (PAA) are selective inhibitors of DNA polymerases from several herpes viruses.[7-10] These compounds appear to interfere with the polymerase at a pyrophosphate binding site.[10,11]

(1) Part of this work was presented earlier. (a) Helgstrand, E.; Johansson, N. G.; Misiorny, A.; Norén, J. O.; Stening, G. 1st European Symposium on Organic Chemistry, Cologne, Germany, Aug 1979; abstract, p 368. (b) Norén, J. O.; Helgstrand, E.; Johansson, N. G.; Misiorny, A.; Stening, G.; Alenius, S.; Stridh, S.; Öberg, B. International Symposium on Medicinal Chemistry, 7th, Torremolinos, Spain, Sept 1980, abstr P 120. (c) Helgstrand, E.; Alenius, S.; Johansson, N. G.; Öberg, B. "Current Chemotherapy and Infectious Disease", Proceedings of the International Congress of Chemotherapy, 11th, and Interscience Conference on Antimicrobial Agents and Chemotherapy, 19th, Boston, Oct 1-5, 1979; Nelson, J. D.; Grossi, C., Eds.; American Society for Microbiology: Washington, DC, 1980; pp 1359-1361.

(2) Helgstrand, E.; Johansson, N. G.; Misiorny, A.; Norén, J. O.; Stening, G. European Patents 003275 and 003007, 1979.

(3) Present address: AB Draco, Box 1707, S-221 01 Lund, Sweden.

(4) Keir, H. M.; Gold, E. *Biochim. Biophys. Acta* **1963**, *72*, 263-276.

(5) Purifoy, D. J. M.; Lewis, R. B.; Powell, K. L. *Nature (London)* **1977**, *269*, 621-623.

(6) Jofre, J. T.; Schaffer, P. A.; Parris, D. S. *J. Virol.* **1977**, *23*, 833-836.

(7) Helgstrand, E.; Eriksson, B.; Johansson, N. G.; Lannerö, B.; Larsson, A.; Misiorny, A.; Norén, J. O.; Sjöberg, B.; Stenberg, K.; Stening, G.; Stridh, S.; Öberg, B.; Alenius, S.; Philipson, L. *Science* **1978**, *201*, 819-821.

(8) Reno, J. M.; Lee, L. F.; Boezi, J. A. *Antimicrob. Agents Chemother.* **1978**, *13*, 188-192.

(9) Overby, L. R.; Rabishaw, E. E.; Schleicher, J. B.; Reuter, A.; Schipkowitz, N. L.; Mao, J. C.-H. *Antimicrob. Agents Chemother.* **1974**, *6*, 360-365.

(10) Leinbach, S. S.; Reno, J. M.; Lee, L. F.; Isbell, A. F.; Boezi, J. A. *Biochemistry* **1976**, *15*, 426-430.

Table I. Phosphites[a]

$$R_1O{\diagdown}\atop{R_1O{\diagup}}P{-}OR_2$$

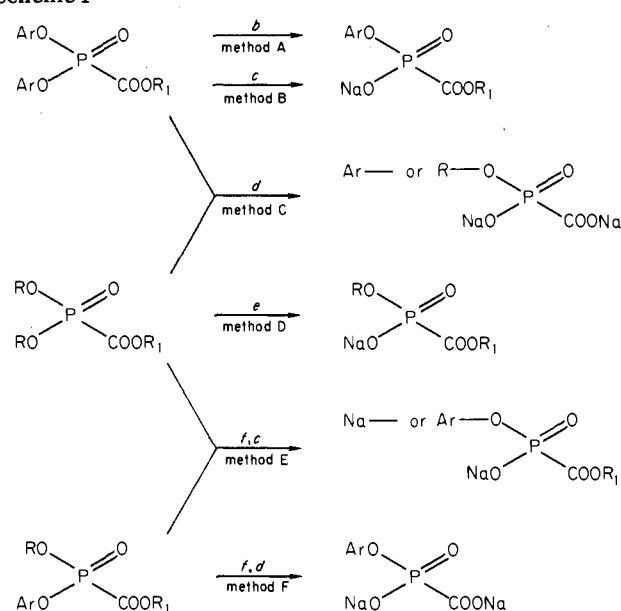| no.	R_1	R_2	bp, °C (mmHg)	$n^{25}D$	yield, %	formula	anal.[b]
4	CH_3	1-adamantyl	79–80 (0.01)	1.4996	50	$C_{12}H_{21}O_3P$	C, H, P
5	C_2H_5	$4\text{-}CH_3OC_6H_4$	92–96 (0.2)	1.4993	59	$C_{11}H_{17}O_4P$	C, H, P
6	C_2H_5	$4\text{-}ClC_6H_4$	102–104 (1.5)[c]	1.5047	43	$C_{10}H_{14}ClO_3P$	C, H, P, Cl
7	C_2H_5	$2,6\text{-}(CH_3)_2C_6H_3$	84–85 (0.01)		29	$C_{12}H_{19}O_3P$	d
8	C_2H_5	5-indanyl	140 (0.01)		29	$C_{13}H_{19}O_3P$	e
9	CH_3	$4\text{-}CH_3COC_6H_4$	128–130 (0.03)	1.5308	20	$C_{10}H_{13}O_4P$	C, H, P
10	C_2H_5	$4\text{-}CH_3COC_6H_4$	142–158 (0.02)	1.5194	21	$C_{12}H_{17}O_4P$	C, H; P[f]
11	C_2H_5	$3,4\text{-}Cl_2C_6H_3$	110 (0.02)	1.5188	18	$C_{10}H_{13}Cl_2O_3P$	C, H, Cl; P[g]
12	$i\text{-}C_3H_7$	C_6H_5	69–75 (0.02)[h]	1.4792	48	$C_{12}H_{19}O_3P$	C, H, P
13	$4\text{-}CH_3C_6H_4$	C_2H_5[i]	125 (0.01)	1.5368	69	$C_{16}H_{19}O_3P$	C, H, P

[a] Prepared by the method of Bentrude et al.[23] [b] Analyses within ±0.4% for indicated elements. [c] Cf. Petrov, K. A.; Evadakov, V. P.; Bilevich, K. A.; Radchenko, V. P.; Nifant'ev, E. E. *Zh. Obshch. Khim.* 1962, *32*, 920–923; *Chem. Abstr.* 1964, *58*, 2391h, gives bp 128–129 °C (7 mmHg), $n^{20}D$ 1.5050 [from $Et_2NP(OEt)_2$ and 4-chlorophenol]. [d] Contains 6% 2,6-dimethylphenol (by GLC) after two distillations. [e] Contains 10% 5-indanol (by GLC) after distillation. [f] P: calcd, 12.09; found, 11.35. [g] P: calcd, 10.94; found, 10.33. [h] Cf. Kamai, G., Kharrasova, F. M. *Tr. Kazan. Khim. Tekhnol. Inst.*, 1957, *23*, 122–126; *Chem. Abstr.* 1958, *52*, 9980i, gives bp 117–118 °C (10 mmHg), $n^{20}D$ 1.4825 (from $PhOPCl_2$ and i-PrOH). [i] Prepared by the method of Griffin and Burger.[24]

Herpes virus multiplication in cell culture is effectively inhibited by PFA[12] and PAA.[9] Topical treatment with PFA is also effective on cutaneous and genital herpes infections in guinea pigs[13,15] and herpes keratitis in rabbits.[14] PAA also has therapeutic effects on cutaneous herpes infections but causes a skin irritation in guinea pigs that is not seen with PFA.[15–20] Clinical studies with PFA are progressing and show positive results on herpes labialis.[21]

The effects of various pyrophosphate analogues on isolated HSV-1 DNA polymerase have been reported by Eriksson et al.[11]

In order to further analyze the structural requirements for antiherpes activity, we synthesized different esters of PFA and tested them in cell-free, cellular and animal test models. It was also of value to determine whether esters of PFA could be biotransformed to PFA during the conditions of the test systems. Since up to three ester functions can be introduced on the phosphonate and carboxylate groups of PFA, a wide range of structural variations are possible.

Chemistry. The (hydroxycarbonyl)phosphonic acid triesters were prepared by treating chloroformic acid esters

(11) Eriksson, B.; Larsson, A.; Helgstrand, E.; Johansson, N. G.; Öberg, B. *Biochim. Biophys. Acta*, 1980, *607*, 53–64.
(12) Schnürer, J.; Öberg, B. *Arch. Virol.* 1981, *68*, 203–209.
(13) Alenius, S.; Dinter, Z.; Öberg, B. *Antimicrob. Agents Chemother.* 1978, *14*(3), 408–413.
(14) Alenius, S.; Laurent, U.; Öberg, B. *Acta Ophthalmol.* 1980, *58*, 167–173.
(15) (a) Alenius, S.; Nordlinder, H. *Arch. Virol.* 1979, *60*, 197–206. (b) Kern, E. R.; Richards, J. T.; Overall, J. C., Jr.; Glasgow, L. A. *Antiviral Res.* 1982, *1*, 225–235.
(16) Alenius, S.; Öberg, B. *Arch. Virol.* 1978, *58*, 277–288.
(17) Kern, E. R.; Richards, J. T.; Overall, J. C., Jr.; Glasgow, L. A. *J. Infect. Dis.* 1977, *135*(4), 557–567.
(18) Gerstein, D. D.; Dawson, C. R.; Oh, J. O. *Antimicrob. Agents Chemother.* 1975, *7*, 285–288.
(19) Alenius, S. *Arch. Virol.* 1980, *65*, 149–156.
(20) Alenius, S., personal communication, 1981.
(21) (a) Wallin, J.; Lycke, E.; Lernestedt, J. O. *Curr. Chemother. Infect. Dis.* 1980, *11*, 1361–1362. (b) Helgstrand, E.; Flodh, H.; Lernestedt, J. O.; Lundström, J.; Öberg, B. in "Developments in Antiviral Therapy"; Collier, L. H.; Oxford, J., Eds.; Academic Press: New York, 1980; Chapter 5. (c) Wallin, J.; Lernestedt, J.-O.; Lycke, E. "Current Chemotherapy and Immunotherapy", Proceedings of the International Congress of Chemotherapy, 12th, Florence, July 19–24, 1981; Periti, P.; Graldroni Grassi, G., Eds.; American Society for Microbiology: Washington, DC, 1982; pp 1068–1069.

Scheme I[a]

[a] R = alkyl; R_1 = alkyl or aryl. [b] H_2O, $NaHCO_3$. [c] Amberlite IRC 50 (Na^+). [d] 2 equiv of NaOH. [e] NaJ/THF. [f] $(CH_3)_3SiBr$, 20 °C.

Scheme II

| 19, R = $i\text{-}C_3H_7$ | 70, R = $i\text{-}C_3H_7$ |
| 20, R = $n\text{-}C_4H_9$ | 71, R = $n\text{-}C_4H_9$ |
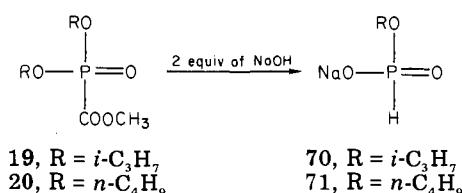
with phosphites containing at least one alkyl group (the Arbuzov reaction). All chloroformic acid esters are known compounds or were prepared by standard methods.[22]

The phosphites described in Table I were all prepared by known methods: phosphites with two alkyl groups (4–12) according to Bentrude et al.,[23] and the phosphite

(22) Zabik, M. J.; Scheutz, R. D. *J. Org. Chem.* 1967, *32*, 300–307.
(23) Bentrude, W. G.; Hansen, E. R.; Khan, W. A.; Min, T. B.; Rogers, P. E. *J. Am. Chem. Soc.* 1973, *95*(7), 2286–2293.

with two aryl and one alkyl group (13) according to Griffin and Burger.[24]

The preparation of the different sodium salts of mono- and diesters (Table III) from the corresponding triesters (Table II) is described in Scheme I. One (P) or two (P and C) ester groups can be removed selectively by aqueous hydrolysis (methods A–C in Scheme I). In order to hydrolyze the third ester group, strongly basic conditions are required.[25] Acidic hydrolytic conditions lead to decarboxylation.[25,26]

Steric factors also influence the route of the aqueous basic hydrolysis (method C). For example, the dimethyl (benzyloxycarbonyl)phosphonate (14) and diethyl (ethoxycarbonyl)phosphonate[25] were hydrolyzed to give the P-alkyl esters 50 and 51, respectively, whereas the diisopropyl (methoxycarbonyl)phosphonate (19) and di-*n*-butyl (methoxycarbonyl)phosphonate (20) on hydrolysis with sodium hydroxide decomposed and gave the phosphites 70 and 71, respectively (Scheme II). The resulting phosphites were compared with actual samples prepared by other methods.[27]

In method D, the strongly nucleophilic character of the iodide ion was used[28] to hydrolyze selectively one alkyl phosphonate group. The reaction was sensitive to steric hindrance. By this method, the complete hydrolysis of the dimethyl (benzyloxycarbonyl)phosphonate (14) required 72 h and the diethyl (ethoxycarbonyl)phosphonate[25] 120 h, whereas only 14% of the *P,P*-di-*n*-butyl ester 20 was hydrolyzed after 96 h.

In method E, treatment with $(CH_3)_3SiBr$ selectively cleaved the alkyl phosphonate group without affecting the carboxylic ester group. This is in agreement with other studies.[29] Subsequent hydrolysis of the silyl ester gave the corresponding sodium salts of diesters or monoesters. By method F, a phosphonic alkyl ester group was cleaved with $(CH_3)_3SiBr$, and the carboxylic ester group was subsequently hydrolyzed by sodium hydroxide.

Results and Discussion

The results of the plaque-reduction assay and of the treatment of cutaneous infections are shown in Table IV. In the cell-free assays, all tested esters of PFA were at least 30 times less potent as inhibitors of the HSV-1 DNA polymerase than PFA (1) itself (0.3 μM PFA gives 50% inhibition,[11] not shown in Table IV). Thus, for inhibition of HSV-1 DNA polymerase, the phosphonic and carboxylic groups must be free.

The esters that were active in the plaque-reduction assay are therefore probably biotransformed to PFA in the cells. The same should be true for the esters that are active in the cutaneous HSV-1 model. None of the esters were chemically hydrolyzed by the overlay medium to give more than 10% PFA under these conditions. Apparently, the skin of guinea pigs contains additional enzyme activities that are not present in Vero cells, since the monoalkyl P-esters 50–52 are active in the cutaneous HSV-1 system. The results can be rationalized in the following manner. The compounds with just one P-ester group (50–55 and 57) are more easily biotransformed to PFA than the compounds with one C-ester group (58–69). In both cases there

is also a difference between aryl and alkyl esters. The monoaryl P-esters (53–55 and 57) are active in the HSV plaque-reduction assay, as well as in the cutaneous system. The 2,6-dimethylphenyl P-ester (56) is probably inactive due to steric hindrance to hydrolysis. The corresponding monoalkyl P-esters (50–52) are active only in the cutaneous HSV-1 system. The monoaryl C-esters (64–69) are active only against the cutaneous HSV-1 infection, but the corresponding monoalkyl C-esters (58–63) are inactive in all systems.

The diesters with a C-aryl ester group (45–49) is an interesting series of compounds. These compounds show structure–activity relationships similar to the corresponding monoalkyl and monoaryl P-esters. Therefore, the C-ester group is likely to be hydrolyzed faster than the P-ester group.

The diesters with a C-alkyl group (39–44) are inactive in all testing systems, indicating that the C-alkyl group cannot be cleaved by the enzymes present (cf. 58–63). Triesters containing at least one aromatic P-ester group (31–38) were not chemically stable during the assay conditions, and one aromatic P-ester group was hydrolyzed to give the corresponding diester. The activities of these triesters and of the corresponding diesters are similar.

The results obtained show that all three acidic groups of PFA must be free in order to get an antiviral activity at the enzyme level. However, certain esters of PFA may be biotransformed to PFA to give antiherpes activity.

Experimental Section

Melting points were determined with a Büchi 510 melting point apparatus and are uncorrected. Refractive indexes were obtained with a Carl Zeiss refractometer. Infrared spectra were obtained on a Jasco IRA-1 spectrophotometer. ^{1}H NMR spectra were determined with a Varian EM 360 A instrument, and ^{31}P NMR spectra were determined with a JEOL FX-200 instrument. Me$_4$Si was used as internal standard for NMR spectra recorded in CDCl$_3$, and DSS served as standard in D$_2$O for the ^{1}H NMR spectra. H$_3$PO$_4$ (1%) in D$_2$O was used as external standard for the ^{31}P NMR spectra, and the spectra were determined at 80.76 MHz. Microanalyses were carried out by Analytische Laboratorien, 5270 Gummersbach, Elbach, Germany, and Novo Microanalytical Laboratory, DK-2880 Bagsvaerd, Denmark. The analyses are within ±0.4% for indicated elements. The commercially available phosphites and chloroformates were purchased from Aldrich—Europe Division, Belgium, or from Fluka AG, Switzerland.

To control the purity of mono- and diesters for phosphonoformic acid content, we used Polygram CEL 300 thin-layer chromatography plates from Macherey-Nagel, eluated with 1 M LiCl and developed by spraying with molybdate spray.[30] None of the esters contained more than 0.4% free PFA.

3,4-Dichlorophenyl chloroformate (2) was prepared by the method of Zabik and Schuetz[22] from 40.75 g (0.25 mol) of 3,4-dichlorophenol, 240 mL (0.46 mol) of 20% phosgene in toluene, and 31.5 g (0.26 mol) of *N,N*-dimethylaniline: bp 134 °C (20 mmHg); yield 46.4 g (82%). The product became slightly blue and crystallized in long needles, mp 51–53 °C. Anal. (C$_7$H$_3$Cl$_3$O$_2$) C, H, Cl.

4-(Ethoxycarbonyl)phenyl chloroformate (3) was prepared analogously to 2 from 49.9 g (0.3 mol) of ethyl 4-hydroxybenzoate, 40 mL (0.3 mol) of *N,N*-dimethylaniline, and 0.4 mol of a 20% solution of phosgene in toluene: bp 146–146.5 °C; n^{25}_D 1.5140; yield 54.4 g (79%). Anal. (C$_{10}$H$_9$ClO$_4$) C, H, Cl.

Diethyl [(4-Methoxyphenoxy)carbonyl]phosphonate (22). Triethyl phosphite (18.6 g, 0.12 mol) was heated at 125–130 °C in a flask with a reflux condenser, and 4-methoxyphenyl chloroformate (5) was added over 30 min. The reaction flask was further heated at about 120 °C for 1.5 h and left at room temperature overnight. The product was distilled: bp 174–178 °C

(24) Griffin, B. S.; Burger, A. *J. Am. Chem. Soc.* **1956**, *78*, 2337.

(25) Nylén, P. *Berichte* **1924**, *57*, 1023–1038.

(26) Warren, S.; Williams, M. R. *J. Chem. Soc. B* **1971**, 618–621.

(27) Nylén, P. *Sven. Kem. Tidskr.* **1936**, *48*, 2.

(28) Cremlyn, R. J. W.; Kenner, G. W.; Mather, J.; Todd, A., Sir *J. Chem. Soc.* **1958**, 528–530.

(29) McKenna, C. E.; Schmidhauser, J. *J. Chem. Soc., Chem. Commun.* **1979**, 739.

(30) Hanes, C. S.; Ischerwood, F. A. *Nature (London)* **1949**, *164*, 1107–1112.

Table II. Phosphonoformic Acid Triesters[a]

$$R_1O-\underset{\underset{OR_2}{|}}{\overset{\overset{O}{\|}}{P}}-COOR_3$$

no.	R₁	R₂	R₃	used[b] phos-phites	used[c] chloro-formate	bp, °C (mmHg)	n^{25}D	yield, %	formula	anal.[d]
14	CH_3	CH_3	$C_6H_5CH_2$			135–136 (0.02)	1.4997	90	$C_{10}H_{13}O_5P$	C, H, P
15	CH_3	CH_3	$i\text{-}C_3H_7$			90–92 (2.0)	1.4202	64	$C_6H_{13}O_5P$	C, H, P
16	CH_3	CH_3	$n\text{-}C_4H_9$			97–100 (1.0)	1.4269	81	$C_7H_{15}O_5P$	C, H, P
17	CH_3	CH_3	cyclohexyl		e, f	148–151 (1.4–1.8)	1.4554	64	$C_9H_{17}O_5P$	C, H, P
18	CH_3	CH_3	cyclopentylmethyl		e, g	150–154 (1.5–2.0)	1.4534	61	$C_9H_{17}O_5P$	C, H, P
19	$i\text{-}C_3H_7$	$i\text{-}C_3H_7$	CH_3			135–136 (13)	1.4211	74	$C_8H_{17}O_5P$	C, H, P
20	$n\text{-}C_4H_9$	$n\text{-}C_4H_9$	CH_3			85–105 (0.2)	1.4310	89	$C_{10}H_{21}O_5P$	C, H, P
21	CH_3	1-adamantyl	CH_3	4		153–155 (0.01)	1.4921	77	$C_{13}H_{21}O_5P$	C, H, P
22	C_2H_5	C_2H_5	$4\text{-}CH_3OC_6H_4$		e	174–178 (0.03)	1.4897	89	$C_{12}H_{17}O_6P$	C, H, P
23	C_2H_5	C_2H_5	$4\text{-}ClC_6H_4$		e	153–156 (0.01)	1.4949	90	$C_{11}H_{14}ClO_5P$	C, H, P
24	CH_3	CH_3	$4\text{-}CH_3C_6H_4$		e	131 (0.2)	1.4912	93	$C_{10}H_{13}O_5P$	C, H, P
25	CH_3	CH_3	$3,4\text{-}Cl_2C_6H_3$		2	164 (0.04)[h]		64	$C_9H_9Cl_2O_5P$	C, H, Cl, P
26	CH_3	CH_3	C_6H_5			125–127 (0.5)	1.4907	75	$C_9H_{11}O_5P$	C, H, P
27	CH_3	CH_3	$4\text{-}C_2H_5O_2CC_6H_4$		3	205–207 (0.05)[i]		88	$C_{12}H_{15}O_7P$	C, H, P
28	C_2H_5	C_2H_5	$4\text{-}C_2H_5O_2CC_6H_4$		3	190–192 (0.01)	1.4890	88	$C_{14}H_{19}O_7P$	C, H, P
29	CH_3	CH_3	$4\text{-}NO_2C_6H_4$			j		71	$C_9H_{10}NO_7P$	C, H, N, P
30	$i\text{-}C_3H_7$	$i\text{-}C_3H_7$	$4\text{-}NO_2C_6H_4$			k	1.4947		$C_{13}H_{18}NO_7P$	C, H, N; P[l]
31	C_2H_5	$2,6\text{-}(CH_3)_2C_6H_4$	CH_3	7		112–114 (0.005)	1.4959	81	$C_{12}H_{17}O_5P$	H, P, C[m]
32	C_2H_5	5-indanyl	CH_3	8		131–134 (0.001)	1.5104	65	$C_{13}H_{17}O_5P$	C, H, P[n]
33	CH_3	$4\text{-}CH_3COC_6H_4$	CH_3	9		k	1.5178		$C_{11}H_{13}O_6P$	C, H, P
34	C_2H_5	$4\text{-}CH_3COC_6H_4$	CH_3	10		k	1.5152		$C_{12}H_{15}O_6P$	C, H, P
35	C_2H_5	$4\text{-}CH_3OC_6H_4$	C_6H_5	5		k	1.5378		$C_{16}H_{17}O_6P$	C, H, P
36	C_2H_5	$4\text{-}ClC_6H_4$	C_6H_5	6		168–170 (0.001)	1.5377	72	$C_{15}H_{14}ClO_5P$	C, H, Cl, P
37	C_6H_5	C_6H_5	C_6H_5	o		k	1.5615		$C_{19}H_{15}O_5P$	H, P, C[p]
38	$4\text{-}CH_3C_6H_4$	$4\text{-}CH_3C_6H_4$	C_6H_5	13		k	1.5562		$C_{21}H_{19}O_5P$	C, H, P

[a] All compounds were prepared analogously to compound 22 (see Experimental Section) at a temperature ranging from 80 to 130 °C for 1 to 3 h. [b] Commercially available triphosphites where not indicated. [c] Commercially available chloroformates where not indicated. [d] Analyses are within ±0.4% for indicated elements. [e] Prepared according to Zabik and Scheutz.[22] [f] Cyclohexyl chloroformate: bp 84–88 °C (18–20 mmHg), n^{25}D 1.4587; cf. Saunders, J. H.; Slocombe, R. J.; Hardy. E. E. J. Am. Chem. Soc. 1951, 73, 3796–3797: bp 38–44 °C (2 mmHg); n^{20}D 1.4587. [g] Cyclopentylmethyl chloroformate: bp 82–84 °C (17–20 mmHg); n^{21}D 1.4541; cf. Dupuy, W. E.; Hudson, H. R. J. Chem. Soc., Perkin Trans. 2 1972, 1715: bp 28 °C (0.25 mmHg); n^{20}D 1.4522. [h] Solidifies to colorless crystals, mp 58–59 °C. [i] Solidifies to colorless crystals, mp 40–41 °C. [j] mp 89–91 °C (from toluene). [k] Not distilled. Purified by evaporation of excess starting materials at 100 °C (0.01 mmHg). [l] P: calcd, 9.35; found, 8.70. [m] C: calcd, 52.94; found, 52.31. [n] P: calcd, 10.89; found, 11.46. [o] $(PhO)_2POC_2H_5$ prepared according to Griffin and Burger.[24] [p] C: calcd, 64.41; found, 63.90.

Table III. Phosphonoformic Acid Mono- and Diesters

type I   type II   type III

no.	type	R_1	R_2	used triester	method[a]	mp, °C	yield, %	formula	anal.[b]
39	I	CH_3	$C_6H_5CH_2$	14	D[c]	120-122	82	$C_9H_{10}NaO_5P\cdot2/3H_2O$	C, H, Na, P, H_2O
40	I	$n\text{-}C_4H_9$	CH_3	20	D[d]	134-137	14	$C_6H_{12}NaO_5P$	C, H, Na, P
41	I	C_2H_5	C_2H_5	e	D[f]	103-105	76	$C_5H_{10}NaO_5P\cdot0.75H_2O$	C, Na, H_2O; H[g]
42	I	C_6H_5	C_2H_5	h	A	242-244	27	$C_9H_{10}NaO_5P$	C, H, Na, P
43	I	$2,6\text{-}(CH_3)_2C_6H_4$	CH_3	31	E	248 dec	45	$C_{10}H_{12}NaO_5P$	C, H, Na, P
44	I	5-indanyl	CH_3	32	E	240-241 dec	16	$C_{11}H_{12}NaO_5P$	C, H, Na, P
45	I	C_2H_5	$4\text{-}CH_3OC_6H_4$	22	D[f,i]	150-152 dec	90	$C_{10}H_{12}NaO_5P\cdot H_2O$	C, H, Na, P, H_2O[j]
46	I	CH_3	C_6H_5	26	D[f,i]	79-81 dec	95	$C_9H_8NaO_5P\cdot0.8H_2O$	C, H, Na, P, H_2O[j]
47	I	C_2H_5	$4\text{-}ClC_6H_4$	23	D[f,i]	153-156 dec	97	$C_9H_9ClNaO_5P\cdot0.75H_2O$	C, H, Cl, Na, P, H_2O[j]
48	I	C_6H_5	C_6H_5	37	B	>300	35	$C_{13}H_{10}NaO_5P$	C, H, Na, P[j]
49	I	$4\text{-}CH_3C_6H_4$	C_6H_5	38	A	247-248 dec	26	$C_{14}H_{12}NaO_5P$	H, Na, P; C[k]
50	II	CH_3		14	C[c]	>300	78	$C_2H_3Na_2O_5P$	C, H, Na, P
51	II	C_2H_5		e	C[c]	>300	30	$C_3H_5Na_2O_5P$	C, H, Na, P
52	II	1-adamantyl		21	F	>300	28	$C_{11}H_{15}Na_2O_5P$	C, H, Na, P
53	II	C_6H_5		37	C	>300	32	$C_7H_5Na_2O_5P$	C, H, Na, P
54	II	$4\text{-}CH_3C_6H_4$		38	C	>300	83	$C_8H_7Na_2O_5P$	C, H, Na, P
55	II	$4\text{-}CH_3OC_6H_4$		35	F	>300	60	$C_8H_7Na_2O_6P$	C, H, Na, P
56	II	$2,6\text{-}(CH_3)_2C_6H_3$		31	F	>300	78	$C_9H_9Na_2O_5P$	C, H, Na, P
57	II	5-indanyl		32	F	>300	72	$C_{10}H_9Na_2O_5P\cdot1/3H_2O$	C, H, Na, P, H_2O
58	III		C_2H_5	e	E[c]	>300	88	$C_3H_5Na_2O_5P\cdot1.5H_2O$	C, H, Na, P, H_2O[l]
59	III		$i\text{-}C_3H_7$	15	E	>300	90	$C_4H_7Na_2O_5P\cdot1.5H_2O$	C, H, Na, P, H_2O
60	III		$n\text{-}C_4H_9$	16	E	>300	92	$C_5H_9Na_2O_5P\cdot1.5H_2O$	C, H, Na, P, H_2O
61	III		$C_6H_5CH_2$	14	E[c]	>300	88	$C_8H_7Na_2O_5P\cdot1.5H_2O$	C, H, Na, P, H_2O[m]
62	III		cyclohexyl	17	E	>300	87	$C_7H_{11}Na_2O_5P\cdot0.5H_2O$	C, H, Na, P, H_2O
63	III		cyclopentylmethyl	18	E	>300	67	$C_7H_{11}Na_2O_5P\cdot H_2O$	C, H, Na, P, H_2O
64	III		C_6H_5	26	E	>300	88	$C_7H_5Na_2O_5P\cdot0.25H_2O$	C, H, Na, P, H_2O
65	III		$4\text{-}CH_3C_6H_4$	24	E	>300	87	$C_8H_7Na_2O_5P\cdot1/3H_2O$	C, Na, P, H_2O; H[n]
66	III		$4\text{-}CH_3OC_6H_4$	22	E	>300	85	$C_8H_7Na_2O_6P\cdot H_2O$	C, H, Na, P, H_2O
67	III		$4\text{-}ClC_6H_4$	23	E	>300	89	$C_7H_4ClNa_2O_5P\cdot0.4H_2O$	C, H, P, H_2O
68	III		$3,4\text{-}Cl_2C_6H_3$	25	E	>300	33	$C_7H_3Cl_2Na_2O_5P\cdot0.5H_2O$	C, H, P, H_2O
69	III		$4\text{-}C_2H_5O_2CC_6H_4$	28	E	116-120 dec	22	$C_{10}H_9Na_2O_7P\cdot2H_2O$	C, H, Na, P, H_2O

[a] See Experimental Section and Scheme I. [b] Analyses are within ±0.4% for indicated elements. TLC analysis for content of phosphonoformic acid on Polygram CEL 300 plates (see Experimental Section) showed that all esters contain <0.4% PFA. [c] See also West German Patent DT-OLS 243547, 1974. [d] Reaction time 96 h. [e] From diethyl (ethoxycarbonyl)phosphonate prepared according to P. Nylen.[25] [f] Reaction time 120 h. [g] H: calcd, 5.33; found, 4.75. [h] From diphenyl (ethoxycarbonyl)phosphonate prepared according to Takamizava, A.; Sato, Y. *Chem. Pharm. Bull.* 1964, 12(4), 398-403. [i] Contaminated with some NaI. Purified as in j. [j] Purified by HPLC (Lichrosorb 10 μ, RP-C_{18} 250 × 10 mm, 25% MeOH-H_2O). [k] C: calcd, 53.52; found, 52.42. [l] H_2O: calcd, 12.01; found, 11.45. [m] H_2O: calcd, 9.41; found, 8.87. [n] H: calcd, 2.65; found, 3.09.

Table IV. Antiviral Effects[a]

$$R_1O-\overset{\displaystyle O}{\underset{\displaystyle OR_2}{\overset{\|}{\underset{|}{P}}}}-COOR_3$$

no.	R$_1$	R$_2$	R$_3$	concn, μM, giving 50% inhibn of HSV-1 C42 plaque formation	effects[b] on cutaneous HSV-1 C42 infections in guinea pigs[c]
1	Na	Na	Na	74	active
50	CH$_3$	Na	Na	>500	active
51	C$_2$H$_5$	Na	Na	>500	active
52	1-adamantyl	Na	Na	>500	active
53	C$_6$H$_5$	Na	Na	79	active
54	4-CH$_3$C$_6$H$_4$	Na	Na	87	active
55	4-CH$_3$OC$_6$H$_4$	Na	Na	90	active
56	2,6-(CH$_3$)$_2$C$_6$H$_3$	Na	Na	>500	not active
57	5-indanyl	Na	Na	73	active
58-63	Na	Na	alkyl	>500	not active
64	Na	Na	C$_6$H$_5$	>500	active
65	Na	Na	4-CH$_3$C$_6$H$_4$	>500	active
66	Na	Na	4-CH$_3$OC$_6$H$_4$	>500	active
67	Na	Na	4-ClC$_6$H$_4$	250	active
68	Na	Na	3,4-Cl$_2$C$_6$H$_3$	d, e	active
69	Na	Na	4-C$_2$H$_5$O$_2$CC$_6$H$_4$	>500	active
45	C$_2$H$_5$	Na	4-CH$_3$OC$_6$H$_4$	165	active
46	CH$_3$	Na	C$_6$H$_5$	>500	active
47	C$_2$H$_5$	Na	4-ClC$_6$H$_4$	500	active
48	C$_6$H$_5$	Na	C$_6$H$_5$	52	active
49	4-CH$_3$C$_6$H$_4$	Na	C$_6$H$_5$	54	active[d]
39-41	alkyl	Na	alkyl	>500	not active
42-44	aryl	Na	alkyl	>500	not active
35	C$_2$H$_5$	4-CH$_3$OC$_6$H$_4$	C$_6$H$_5$	97	active[f]
36	C$_2$H$_5$	4-ClC$_6$H$_4$	C$_6$H$_5$	d, e	active[f]
37	C$_6$H$_5$	C$_6$H$_5$	C$_6$H$_5$	39[e]	active
38	4-CH$_3$C$_6$H$_4$	4-CH$_3$C$_6$H$_4$	C$_6$H$_5$	44[e]	active
14-21	alkyl	alkyl	alkyl	>500	not active
22-30	alkyl	alkyl	aryl	>500	not active
31-34	aryl	alkyl	alkyl	>500	not active

[a] All compounds were also tested in a polymerase assay (see antiviral evaluation under Experimental Section). [b] 30% or higher reduction of the cumulative score is described as active.[13] [c] Tested as 2% aqueous solutions (0.1% Tween 80, 10% glycerol w/w) where not indicated. [d] All cells killed at 500 μM concentration. At lower concentrations only cell-toxic effects were seen. [e] Dissolved in Me$_2$SO. Final concentration of Me$_2$SO 1% in the overlay. [f] Tested as 2% solution in Monash (10% glycerol, 45% *i*-PrOH, 45% H$_2$O).

(0.03 mmHg); n^{25}_D 1.4897; yield 25.8 g (89%). ^{1}H NMR (CDCl$_3$) δ 1.42 (t, CH$_3$), 3.78 (s, OCH$_3$), 4.13-4.63 (m, CH$_2$), 6.77-7.33 (aromatic); ^{31}P NMR (CDCl$_3$) δ 7.51 (quintet, J = 8 Hz); IR (neat) 1740 (CO), 1275, 1255, 1190, 1030 cm^{-1}. Anal. (C$_{12}$H$_{17}$O$_6$P) C, H. P.

Dimethyl [(4-Nitrophenoxy)carbonyl]phosphonate (29). 4-Nitrophenoxychloroformate (4.03 g, 0.02 mol) was added slowly to trimethyl phosphite (12.40 g, 0.1 mol) to maintain the reaction mixture at a gentle reflux. When the reaction was complete, the crystalline product precipitated. Excess trimethyl phosphite was evaporated, and the residue was recrystallized from 75 mL of toluene: mp 89-91 °C; yield 3.88 g (70.5%); ^{1}H NMR (CDCl$_3$) δ 4.08 (d, J = 11 Hz, CH$_3$), 7.45 (d, J = 11 Hz, aromatic), 8.38 (d, J = 11 Hz, aromatic); ^{31}P NMR (CDCl$_3$) δ 6.35 (quintet, J = 11 Hz). Anal. (C$_9$H$_{10}$NO$_7$P) C, H, N, P.

Sodium Phenyl (Ethoxycarbonyl)phosphonate (42). Method A. Diphenyl (ethoxycarbonyl)phosphonate (3.06 g, 0.01 mol) and sodium hydrogen carbonate (0.84 g, 0.01 mol) were stirred in water (10 mL) at room temperature for about 24 h. The water was evaporated, and the residue extracted with ethanol. The ethanol was evaporated, and the residue was washed with ether and recrystallized twice from 2-propanol to give colorless crystals: yield 0.67 g (27%); mp 242-244 °C; ^{1}H NMR (D$_2$O) δ 1.24 (t, J = 7 Hz, CH$_3$), 4.26 (quartet, J = 7 Hz, CH$_2$), 6.95-7.60 (m, C$_6$H$_5$); ^{31}P NMR (D$_2$O) δ 7.86 (s). Anal. (C$_9$H$_{10}$NaO$_5$P) C, H, Na, P.

Sodium Phenyl (Phenoxycarbonyl)phosphonate (48). Method B. Triphenyl (hydroxycarbonyl)phosphonate (37; 10.7

g, 0.03 mol) and Amberlite IRC 50 (Na$^+$ form; 50 g, wet weight, 0.09 mol equiv) were stirred in 100 mL of water at room temperature overnight. Ethanol was added, and the solution was filtered and evaporated in vacuo. The residue was dissolved in 25 mL of hot ethanol, filtered, and precipitated by the addn. of 300 mL of ether. The precipitate (5.11 g) was recrystallized twice from 2-propanol: mp >300 °C; yield 3.20 g (35%). A small amount was further purified by HPLC on a Lichrosorb 10 μ RP-C$_{18}$ 250 × 10 mm column with 25% MeOH-H$_2$O as eluent: ^{1}H NMR (D$_2$O) δ 7.25-7.69 (m, C$_6$H$_5$); ^{31}P NMR (D$_2$O) δ 8.73 (s). Anal. (C$_{13}$H$_{10}$NaO$_5$P) C, H, Na, P.

Methyl Disodium (Oxycarbonyl)phosphonate (50). Method C. Dimethyl (benzyloxycarbonyl)phosphonate (14; 6.20 g, 0.025 mol) was stirred in water (50 mL), and 50% aqueous NaOH (4.0 g, 0.05 mol) was added dropwise. The mixture was heated at reflux for 1 h, after which the solution was evaporated in vacuo. The product was redissolved in water (10 mL), and methanol (80 mL) was added slowly. The precipitate was filtered and dried: mp >300 °C; yield 3.59 g (78%); ^{1}H NMR (D$_2$O) δ 3.54 (d, J = 11 Hz, CH$_3$); ^{31}P NMR (D$_2$O) δ -3.07 (quartet, J = 11 Hz); IR (KBr) 1590 (CO), 1085 (PO$^-$), 1055 (POCH$_3$) cm^{-1}. Anal. (C$_2$H$_3$Na$_2$O$_5$P) C, H, Na, P.

Sodium Methyl (Benzyloxycarbonyl)phosphonate (39). Method D. Dimethyl (benzyloxycarbonyl)phosphonate (14; 3.66 g, 0.015 mol) and sodium iodide (2.25 g, 0.05 mol) were stirred in 25 mL of dry tetrahydrofuran for 3 days. The precipitate was filtered, washed with ether, and dried to give colorless, hygroscopic crystals: mp 120-122 °C; yield 23.15 g (82%); ^{1}H NMR (D$_2$O)

δ 3.67 (d, J = 11 Hz, CH$_3$), 5.28 (s, CH$_2$), 7.48 (s, C$_6$H$_5$); ^{31}P NMR (D$_2$O) δ 3.40 (quartet, J = 11 Hz). Anal. (C$_9$H$_{10}$NaO$_5$P·^{2}/$_3$H$_2$O) C, H, Na, P, H$_2$O.

Disodium (Ethoxycarbonyl)phosphonate (58). Method E. Diethyl (ethoxycarbonyl)phosphonate (1.20 g, 5.7 mmol) and bromotrimethylsilane (2.65 g, 17.2 mmol) were stirred at room temperature under an atmosphere of argon. After about 3 h, volatile components were evaporated in vacuo (1 mmHg), and the residue was added to 16 g of Amberlite IRC 50 (Na$^+$ form; 1.3 mequiv/g) in 25 mL of water. After 1.5 h, the ion exchanger was added to a column and eluted with another 25 mL of water. The combined aqueous layers (50 mL) were washed with diethyl ether and filtered, and the filtrate was evaporated in vacuo (3 mmHg) at room temperature. The residue was washed with ethanol, filtered, and dried: mp >300 °C; yield 1.88 g (88%). ^{1}H NMR (D$_2$O) δ 1.23 (t, J = 7 Hz, CH$_3$), 4.18 (q, J = 7 Hz, CH$_2$); ^{31}P NMR (D$_2$O) δ 3.42 (s). Anal. (C$_3$H$_5$Na$_2$O$_5$P·1.5H$_2$O) C, H, Na, P, H$_2$O.

4-Methoxyphenyl Disodium (Oxycarbonyl)phosphonate (55). Method F. Ethyl 4-methoxyphenyl (phenoxycarbonyl)-phosphonate (35; 16.8 g, 50 mmol) was stirred under an atmosphere of argon with bromotrimethylsilane (12.4 mL, 82 mmol) for 5 h. Excess bromotrimethylsilane was evaporated in vacuo (0.3 mmHg). The mixture was added dropwise to 100 mL of 1.00 M NaOH (0.10 mol) over 10 min. After stirring at room temperature for 4 h, the aqueous solution was extracted with 3 × 75 mL of ether and evaporated in vacuo. The residue was dissolved in 50 mL of water, and the crude disodium p-methoxyphenyl (oxycarbonyl)phosphonate was precipitated with 500 mL of ethanol: yield 12.1 g. It was contaminated with some trisodium (oxycarbonyl)phosphonate (PFA). The crude product was re-dissolved in water (50 mL), and ethanol (70 mL) was added slowly. The small amount of precipitate was discarded by filtration. Ethanol (400 mL) was added to the solution, and the new precipitate was collected: yield 11.1 g. An analytical sample was prepared by precipitation twice with ethanol from a water solution: mp >300 °C; yield 8.3 g (60%); ^{1}H NMR (D$_2$O) 7.24–7.29 (J = 8 Hz, aromatic), 7.08–7.12 (J = 9 Hz, aromatic), 3.96 (s, CH$_3$); ^{31}P NMR (D$_2$O) δ 1.25 (s). Anal. (C$_8$H$_7$Na$_2$O$_6$P) C, H, Na, P.

Isopropyl Sodium Phosphite (70). Diisopropyl (methoxy-carbonyl)phosphonate (19; 1.20 g, 0.005 mol) and NaOH (1.00 M, 10.00 mL, 0.01 mol) were stirred at room temperature for 1 h. The reaction mixture was freeze–dried overnight. The residue was dissolved in 7 mL of H$_2$O, and 90 mL of EtOH was added. The resulting precipitate was filtered off, and the filtrate was evaporated. The residue was dissolved in 10 mL of H$_2$O and freeze–dried: yield 0.44 g (60%); mp 125–127 °C; mmp with isopropyl sodium phosphite prepared according to Nylén[27] gave mp 126–128 °C (lit. mp 132–133 °C);[27] IR and NMR of the two samples were identical; ^{1}H NMR (D$_2$O) δ 1.25 (d, J = 6 Hz, CH$_3$), 4.34–4.51 (m, CH), 6.74 (d, J = 632 Hz, PH); IR (KBr) 3400, 2380, 1220, 1090, 980 cm^{-1}.

n-Butyl Sodium Phosphite (71). Di-n-butyl (methoxy-carbonyl)phosphonate (20; 2.52 g, 0.01 mol) was refluxed with NaOH (19.82 mL, 1.009 M, 0.02 mol) for 1 h. The workup procedure was the same as for isopropyl sodium phosphite (70): mp 184–185 °C; ^{1}H NMR (D$_2$O) δ 0.86–0.93 (t, CH$_3$), 1.27–1.67 (m, CH$_2$) 3.80–3.91 (m, OCH$_2$) 6.69 (d, J = 632 Hz, PH); IR (KBr) 3400, 2980, 2370, 1220, 1100, 1090, 1000 cm^{-1}.

Evaluation of Cellular Antiviral Activity. All the compounds in Table III and the compounds 14–30, 35, 37, and 38 in Table II were tested for antiviral activity in a cell-free HSV-1 DNA-polymerase assay,[11] in a HSV-1 plaque-reduction assay,[12] and on a cutaneous HSV-1 infection in guinea pigs.[13]

In the plaque-reduction assay, a monolayer of Vero cells infected with HSV-1 strain C 42 was used. The cells were incubated at 37 °C for 4–6 days with and, as a control, without the actual ester in the overlay. About 200 plaques were counted in the control. In order to determine the chemical stability of the esters during the conditions of the plaque assay, we incubated the esters for 1 to 3 days as solutions in the overlay medium at 37 °C in the absence of cells.

The therapeutic effect of topical treatment of cutaneous HSV-1 infection in guinea pigs was determined on a skin area on the back of the animal. This area was divided into four parts, and each part was inoculated with HSV-1 virus. Two of the infected areas were treated with a PFA ester and two with the vehicle alone. Treatment started 24 h after virus inoculation and consisted of two daily applications of a 2% aqueous solution of the ester (0.1% Tween 80, 10% glycerol) or the vehicle alone for 3 days. A score system described by Alenius et al.[13,16] was used to evaluate the effects. Two animals were used for each compound. All esters that gave at least 30% reduction of the score as compared to the control are described as active in Table IV. A quantitative evaluation of the activity of the esters in this model is in progress.