anhydrous potassium carbonate. The drying agent was removed by filtration, the chloroform filtrate was evaporated to dryness *in vacuo*, and the residue was crystallized from chloroform. The material (VII) thus obtained weighed 21.0 g. (48%), m.p. 157–158°.

Anal. Calcd. for $C_{24}H_{18}BrN_3O$: C, 64.87; H, 4.08; Br, 17.99. Found: C, 64.86; H, 4.13; Br, 17.74.

N-(2-Chloroethyl)-4-(3-dibenzofuranylazo)-1-naphthylamine.—A suspension of 18.3 g. (0.1 mole) of 3-aminodibenzofuran[13] in a mixture of 200 ml. of water and 25 ml. of concentrated hydrochloric acid was cooled to 0° and diazotized by the addition of a solution of 6.9 g. (0.1 mole) of sodium nitrite in 50 ml. of water while maintaining the temperature at 0–5°. The yellow solution thus obtained was added slowly at 5–10° to a solution of 33.1 g. (0.1 mole) of 1-(2-bromoethyl)aminonaphthalene hydrobromide[4] in 1 l. of 95% ethanol containing 20 ml. of concentrated hydrochloric acid. The thick, purple reaction mixture was diluted with water to a volume of 3 l. and stirred for 3 hr. at 0–15°. The precipitate was collected, washed thoroughly with hot dilute hydrochloric acid, and dried *in vacuo* at 65° for 48 hr.; yield, 41 g. The crude salt was suspended in dilute sodium hydroxide solution, the base was extracted with chloroform, and the chloroform extracts were dried over anhydrous potassium carbonate. The drying agent was collected, the chloroform was removed *in vacuo*, and the residue was crystallized three times from chloroform. The product was obtained as orange-red needles, m.p. 170–171°.

Anal. Calcd. for $C_{24}H_{18}ClN_3O$: C, 72.09; H, 4.54; Cl, 8.87. Found: C, 71.89; H, 4.74; Cl, 8.95.

1-(2-Diethylaminoethyl)-6-[4-(2-diethylaminoethylamino)-1-naphthylazo]-1,2,3,4-tetrahydroquinoline Dihydrochloride (XI d).—Utilizing method V, 11.7 g. (0.03 mole) of N-(4-amino-1-naphthyl)-N-(2-diethylaminoethyl)-2,2,2-trifluoroacetamide monohydrochloride[5] was diazotized and coupled with 7.0 g. (0.03 mole) of N-(2-diethylaminoethyl)-1,2,3,4-tetrahydroquinoline. Hydrolysis of the crude trifluoroacetamide gave 4.0 g. (23% over-all) of product as maroon crystals, m.p. 195–198.5°.

Anal. Calcd. for $C_{31}H_{44}N_6 \cdot 2HCl$: C, 64.90; H, 8.08; N 14.65; Cl, 12.36. Found: C, 64.42; H, 8.20; N, 14.53; Cl, 12.27.

N-(2-Diethylaminoethyl)-1,2,3,4-tetrahydroquinoline.—A mixture of 111.0 g. (0.834 mole) of 1,2,3,4-tetrahydroquinoline,[6] 143.5 g. (0.834 mole) of 2-diethylaminoethyl chloride hydrochloride, 230 g. (1.67 mole) of anhydrous potassium carbonate, and 800 ml. of toluene was boiled under reflux for 17 hr. Upon cooling, the reaction mixture was stirred with 10% aqueous sodium hydroxide solution, the organic layer was separated, and the aqueous layer was extracted with ether. The hydrocarbon and ether solutions were combined, washed with water, and dried over anhydrous potassium carbonate. Volatile materials were removed on a steam bath and the residue was distilled *in vacuo* through a 30-cm. Vigreux column. The product was obtained as a pale yellow oil, b.p. 98–103° (0.2 mm.), $n^{25}D$ 1.5411; yield, 56 g. (29%).

Anal. Calcd. for $C_{15}H_{24}N_2$: C, 77.53; H, 10.41; N, 12.06. Found: C, 77.61; H, 10.47; N, 12.24.

N,N-Diethyl-2-(1-naphthylamino)acetamide.—To a suspension of 16.1 g. (0.33 mole) of 50% sodium hydride dispersion in oil in 200 ml. of toluene was added a solution of 47.8 g. (0.33 mole) of 1-naphthylamine in 200 ml. of toluene. The mixture was heated under reflux for 2 hr., during which time a solid separated. The mixture was cooled to room temperature and to it was added a solution of 50 g. (0.33 mole) of N,N-diethylchloroacetamide in 200 ml. of toluene. The mixture was heated under reflux for 21 hr. and cooled. Water was added cautiously, and the organic layer was separated and dried over sodium sulfate. Volatile materials were removed *in vacuo* on a steam bath and the residue was distilled under high vacuum through a 15-cm. Vigreux column. A majority of the distillate was low boiling and appeared to be unreacted 1-naphthylamine. A high boiling fraction weighing 7.5 g., b.p. 188–190° (0.3 mm.), was obtained which solidified in the receiver. Crystallization from ethanol gave 4.3 g. (5%) of colorless plates, m.p. 92–98°.

Anal. Calcd. for $C_{16}H_{20}N_2O$: C, 74.96; H, 7.86; N, 10.93. Found: C, 75.17; H, 7.79; N, 11.04.

3-[4-(2-Diethylaminoethylamino)-1-naphthylazo]pyridine Salt with 1/2 F. Wt. 2-(4,6-Disulfo-1,3,2-benzodioxastibiol-2-yloxy)-1-phenol-3,5-disulfonic Acid (XII).—A solution of 2.57 g. (0.005 mole) of 3-[4-(2-diethylaminoethylamino)-1-naphthylazo]pyridine trihydrochloride·3.25 hydrate in 20 ml. of water was added with stirring to a solution of 2.71 g. (0.003 mole) of stibophen in 30 ml. of water. A dark-colored oil separated. The supernatant liquid was decanted and the residue was triturated with methanol, whereupon the salt solidified. The salt was collected, washed with methanol, and dried *in vacuo* at 60° for 18 hr.; weight, 2.85 g. (83%), m.p. > 200°.

Anal. Calcd. for $C_{21}H_{25}N_5 \cdot 0.5\ C_{12}H_9O_{16}S_4Sb$: N, 10.32; S, 9.45. Found: N, 10.10; S, 9.01.

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Preparation and Antibiotic Properties of Some Phosphinylaminopenicillanic Acids and Phosphinothioylaminopenicillanic Acids[1]

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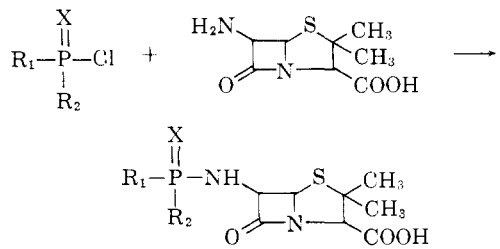
A novel class of N-substituted 6-aminopenicillanic acid derivatives exhibiting noteworthy inhibitory action against antibiotic-resistant strains of *Staphylococcus aureus* and a high degree of inertness toward penicillinase has been synthesized by the reaction of 6-aminopenicillanic acid with organophosphorus chlorides. This class consists of phosphinylaminopenicillanic acids (I, X = O) and phosphinothioylaminopenicillanic acids (I, X = S). In general, I with aryloxy groups attached to phosphorus are slightly more active *in vitro* against sensitive and resistant staphylococci than those with alkoxy groups on phosphorus, while the latter type of compounds are more effective *in vivo* in protecting mice against resistant staphylococcal infections.

An objective in the screening program of semisynthetic N-substituted 6-aminopenicillanic acids is the discovery of compounds which are effective against antibiotic-resistant strains of *Staphylococcus aureus*. The reaction of 6-aminopenicillanic acid (6-APA) with organophosphorus chlorides affords a novel series of

(1) Presented in part at the 144th National Meeting of the American Chemical Society, Division of Medicinal Chemistry, Los Angeles, California, April, 1963.

derivatives which exhibit noteworthy *in vitro* and *in vivo* activity against resistant staphylococcal strains. The general structure of these compounds is represented by I, in which X is oxygen (phosphinylaminopenicillanic acids) or sulfur (phosphinothioylaminopenicillanic acids) and R_1 and R_2 may consist of the following groups: R_1, R_2 = alkoxy, cycloalkoxy, or aryloxy; R_1 = aryl, R_2 = alkoxy or aryloxy; and R_1 = aryloxy, R_2 = dialkylamino.

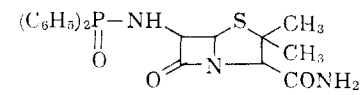
Preparative Method.—The phosphinylaminopenicillanic acids and phosphinothioylaminopenicillanic acids were synthesized by treating 6-APA with the appropriate chlorophosphate, phosphonyl chloride, or their respective thiono analogs. The organophosphorus chlorides were obtained commercially or prepared by known methods.[2] In some instances, the chloride intermediate was used directly following synthesis without isolation. The formation of I required a longer reaction time than acylation of 6-APA by acid chlorides[3];



I

a useful procedure consisted of running the reaction in aqueous acetone at pH 6–7.5 for 3–5 hr. at room temperature. This reaction medium was more advantageous than nonaqueous media; *e.g.*, in nitromethane 6-APA (solubilized by triethylamine) and diphenyl chlorophosphate rapidly formed a new, insoluble product devoid of biological activity.[4] The quality of I obtained depended upon the pH at which the product was extracted from the reaction solution. Solvent extraction at pH 5.5 was employed for I containing aryl or aryloxy groups, while the more polar compounds, such as the lower dialkoxyphosphinylaminopenicillanic acids, were satisfactorily recovered by solvent extraction at pH 3.5. The lower dialkoxyphosphinothioylaminopenicillanic acids could be extracted at either pH. Compounds of structure I were isolated conveniently as crystalline N-ethylpiperidine salts. Sodium, potassium, or calcium salts were prepared by freeze-drying an aqueous solution of the salt or by evaporating an ethyl acetate–methanol solution of the salt to dryness; the purity of these latter noncrystalline solids was assayed by the usual hydroxylamine colorimetric procedure for penicillin.[5]

6-Diphenoxyphosphinylaminopenicillanamide (II) was synthesized in a similar manner using 6-aminopenicillanamide[6] in place of 6-APA.



II

Biological Studies.—The antibacterial properties of I were investigated using standard methods described by English and McBride in previous papers from these Laboratories.[8] This class of compounds is primarily active against Gram-positive bacteria as illustrated by the spectrum of diphenoxyphosphinylaminopenicillanic acid in Table I. Activity against most organisms is lower than that of penicillin G, but significantly higher activity against resistant staphylococci is manifested. Tables II–IV summarize *in vitro* activity against sensitive and resistant staphylococcal strains for selected compounds. Minimum inhibitory concentrations (m.i.c.) of the same order of magnitude were obtained for other resistant strains of staphylococci (clinical isolates). The *in vivo* properties of I observed in mice experimentally infected with resistant *S. aureus* 400 are given in Table V. Approximately equivalent chemotherapeutic efficacies expressed as the median protective dose (PD_{50}) were obtained for infections with normal and resistant staphylococcal strains as shown in Table VI for diethoxyphosphinothioylaminopenicillanic acid.

The penicillinase resistance of I was demonstrated by the low rates of enzymatic hydrolysis of diethoxy-

TABLE I
ANTIMICROBIAL ACTIVITY *In Vitro*

Microorganism	Minimum inhibitory concentration[a,b] Diphenoxyphosphinylaminopenicillanic acid N-ethylpiperidine salt (4), μg./ml.	Penicillin G, μg./ml.
Staphylococcus aureus	1.3	0.03
Staphylococcus aureus 400[c]	2.5	>100
Streptococcus pyogenes	0.08	0.003
Streptococcus faecalis	2.5	.13
Diplococcus pneumoniae	5	.03
Erysipelothrix rhusiopathiae	0.2	.03
Aerobacter aerogenes	>100	50
Escherichia coli	>100	25
Proteus vulgaris	>100	25
Pseudomonas aeruginosa	>100	12.5
Salmonella typhosa	>100	12.5
Klebsiella pneumoniae	>100	3.12
Hemophilus influenza	6.3	0.78
Staphylococcus aureus + serum[d]	10	.06
Streptococcus pyogenes + serum[d]	2.5	.007

[a] Twofold serial dilution technique; m.i.c. read after 20-hr. incubation at 37°. [b] See footnote 7. [c] Strain resistant *in vitro* to high concentrations (>100 μg./ml.) of penicillin, streptomycin, oxytetracycline, chlorotetracycline, or tetracycline. [d] Brain heart infusion broth containing 20% human serum.

(2) G. M. Kosolapoff, "Organophosphorus Compounds," John Wiley and Sons, Inc., New York, N. Y., 1950.

(3) Y. G. Perron, W. F. Minor, C. T. Holdredge, W. J. Gottstein, J. C. Godfrey, L. B. Crast, R. B. Babel, and L. C. Cheney, *J. Am. Chem. Soc.*, **82**, 3934 (1960).

(4) (a) A. Cosmatos, I. Photaki, and L. Zervas, *Chem. Ber.*, **94**, 2641 (1961); (b) S. Wolfe, J. C. Godfrey, C. T. Holdrege, and Y. G. Perron, *J. Am. Chem. Soc.*, **85**, 1643 (1963).

(5) G. E. Boxer and P. M. Everett, *Anal. Chem.*, **21**, 670 (1949).

(6) B. K. Koe, *Nature*, **195**, 1200 (1962).

(7) All biological properties of I are reported on the basis of 100% purity. Data for N-ethylpiperidine salts of I (in Tables I, II, and V), for diphenoxyphosphinylaminopenicillanamide (II), and for standards (penicillin G or V) were determined assuming these compounds to be 100% pure. Data for all other preparations of I (in Tables III–VIII) were determined after correcting the weight of test sample taken to correspond to 100% purity.

(8) (a) T. J. McBride and A. R. English, in "Antibiotics Annual 1957–1958," Medical Encyclopedia, Inc., New York, N. Y., 1958, p. 493; (b) A. R. English and T. J. McBride, *Antibiot. Chemotherapy*, **8**, 424 (1958); (c) A. R. English and T. J. McBride, *Proc. Soc. Exptl. Biol. Med.*, **100**, 880 (1959).

TABLE II

ANTISTAPHYLOCOCCAL ACTIVITY *In Vitro* OF SOME PHOSPHINYLAMINOPENICILLANIC ACIDS AND PHOSPHINOTHIOYLAMINOPENICILLANIC ACIDS (CRYSTALLINE N-ETHYLPIPERIDINE SALTS)

	Compound I				Minimum inhibitory concentration[a]		
No.	R_1	R_2	X	$[\alpha]^{b}D°$	*S. aureus* μg./ml.	*S. aureus* with serum μg./ml.	*S. aureus* 400[c] μg./ml.
1	MeO	MeO	O	+197	10	10	10
2	EtO	EtO	O	+191	5	10	10
3	*n*-BuO	*n*-BuO	O	+166	5	10	5
4	PhO	PhO	O	+173	1.3	10	2.5[d]
5	*o*-MePhO	*o*-MePhO	O	+162	1.3	10	2.5
6	*p*-MePhO	*p*-MePhO	O	+149[e]	0.6	10	2.5
7	PhO	*n*-BuO	O	+174	2.5	10	5
8	PhO	Me_2N	O	+175	10	10	10
9	Ph	*o*-MePhO	O	+189[e]	2.5	10	5
10	MeO	MeO	S	+200	10	10	10
11	EtO	EtO	S	+197	10	>10	10

[a] See footnote 7. [b] *c* 1 in water; 23–25°. [c] Antibiotic-resistant strain (see Table I). [d] Corresponding m.i.c.-values for diphenoxyphosphinylaminopenicillananamide (II): *S. aureus*, 10; *S. aureus* + serum, >10; *S. aureus* 400, >10 μg./ml. [e] In ethanol.

phosphinylaminopenicillanic acid (**12**) and diethoxyphosphinothioylaminopenicillanic acid (**28**) as compared to that of penicillin G (Table VII).[9] These two compounds **12** and **28** were also completely refractory to penicillinase when tested against this enzyme by a modified Gots technique.[10]

Diethoxyphosphinylaminopenicillanic acid and diethoxyphosphinothioylaminopenicillanic acid were observed to induce the formation of penicillinase in three penicillinase-producing strains of *S. aureus* (including the resistant 376 and 400 strains) but not in two penicillin-sensitive strains, when the organisms were grown in the presence of 1–4 μg./ml. of the test compound.

Acute intravenous toxicities (LD_{50}) observed in mice for diethoxyphosphinylaminopenicillanic acid and for diethoxyphosphinothioylaminopenicillanic acid were approximately 900 mg./kg. and greater than 600 mg./kg., respectively.

In absorption studies in dogs, very low antistaphylococcal activity was detected in sera following oral administration of representative compounds. A comparison of serum levels measured as activity dilutions against *Streptococcus pyogenes* is presented in Table VIII for several compounds possessing *in vivo* activity.

Compounds in Table II did not exhibit antiviral activity against Newcastle disease virus, vaccinia, herpes simplex, poliomyelitis (Type II), or influenza A in a tissue culture test at a level of 10 mg./ml.[11]

Discussion

All phosphinylaminopenicillanic acids and phosphinothioylaminopenicillanic acids which we have examined are active *in vitro* against resistant staphylococci, so that this property is characteristic of compounds of structure I. The substitution of the acyl side chain in conventional penicillins by a noncarboxylic moiety does not necessarily confer activity against resistant staphylococcal strains. We observed that arylsulfonamidopenicillanic acids, such as benzenesulfonamidopenicillanic acid, *p*-fluorobenzenesulfonamidopenicillanic acid, *p*-toluenesulfonamidopenicillanic acid, and 2-naphthalenesulfonamidopenicillanic acid, gave m.i.c.-values of >100 μg./ml. for resistant *S. aureus* 376 and 400 strains.

The inhibitory activity of I toward resistant strains probably results from the inertness of this class of compounds to penicillinase (Table VII). Penicillinase resistance is also partly responsible for the constant m.i.c. obtained over wide variation of inocula sizes as shown in Table VI.

Since amide formation at C-3 carboxyl in benzylpenicillin imparts penicillinase resistance,[12] the effect of attaching both phosphinyl and amide groupings to 6-APA was studied. A representative compound, diphenoxyphosphinylaminopenicillanamide (II), however, is less active than the corresponding carboxylic acid (**4**); m.i.c.-values against normal *S. aureus* and resistant *S. aureus* 400 were 10 and >10 μg./ml., respectively, for II as compared to 1.3 and 2.5 μg./ml., respec-

TABLE III: ANTISTAPHYLOCOCCAL ACTIVITY *In Vitro* OF SOME PHOSPHINYLAMINOPENICILLANIC ACIDS (POTASSIUM SALTS)

	Compound I				Minimum inhibitory concentration[b]			
No.	R_1	R_2	X	Purity,[a] %	*S. aureus* μg./ml.	*S. aureus* with serum μg./ml.	*S. aureus* 376[c] μg./ml.	*S. aureus* 400[c] μg./ml.
12	EtO	EtO	O	70	6.3–12.5	6.3–12.5	12.5	12.5
13	*n*-BuO	*n*-BuO	O	80[d]	3.1	6.3	6.3	6.3
14	EtO	*n*-BuO	O	60	1.6	6.3	6.3	6.3
15	EtO	$ClCH_2CH_2O$	O	60	3.1	3.1	12.5	12.5
16	EtO	$C_8H_{17}O$[e]	O	80[d]	1.6	6.3	6.3	6.3
17	*p*-MeOPhO	*p*-MeOPhO	O	57	1.6	>100	6.3	6.3
18	3,5-Me_2PhO	3,5-Me_2PhO	O	72	1.6	50	12.5	6.3
19	PhO	*n*-PrO	O	54	1.6	6.3	6.3	6.3
20	PhO	*i*-PrO	O	51[d]	1.6	1.6	3.1	3.1
21	Ph	PhO	O	50	0.8	0.8	12.5	6.3
22	Ph	*o*-MePhO	O	61	.8	6.3	3.1	3.1
23	Ph	*m*-MePhO	O	54	1.6	6.3	6.3	6.3
24	Ph	*p*-MePhO	O	52	1.6	3.1	6.3	6.3
25	Ph	*o*-ClPhO	O	54	3.1	6.3	12.5	12.5
26	Ph	*m*-ClPhO	O	53	0.8	0.4	3.1	6.3
27	Ph	*o*-BrPhO	O	51	1.6	6.3	6.3	6.3

[a] Assayed by hydroxylamine colorimetric procedure. [b] See footnote 7. [c] Strains resistant to high concentrations (>100 μg./ml.) of penicillin and other antibiotics (see Table I). [d] Calcium salt. [e] C_8H_{17} = 2-ethylhexyl.

(9) J. F. Snell and L. H. Cheng, unpublished observations, using method of E. P. Abraham and G. G. F. Newton, *Biochem. J.*, **63**, 628 (1956).

(10) (a) J. S. Gots, *Proc. Soc. Exptl. Biol. Med.*, **60**, 165 (1945); (b) A. R. English, T. J. McBride, and H. T. Huang, *ibid.*, **104**, 547 (1960).

(11) C. D. Brandt, unpublished observations.

(12) D. E. Cooper and S. B. Binkley, *J. Am. Chem. Soc.*, **70**, 3966 (1948).

Table IV

Antistaphylococcal Activity *In Vitro* of Some Phosphinothioylaminopenicillanic Acids (Potassium Salts)

	Compound I				Minimum inhibitory concentration[b]			
No.	R_1	R_2	X	Purity,[a] %	*S. aureus* μg./ml.	*S. aureus* with serum μg./ml.	*S. aureus* 376[c] μg./ml.	*S. aureus* 400[c] μg./ml.
28	EtO	EtO	S	88	6.3–12.5	6.3–12.5	12.5	12.5
29	*n*-BuO	*n*-BuO	S	95[d]	3.1	100	6.3	6.3
30	$ClCH_2CH_2O$	$ClCH_2CH_2O$	S	74	1.6	12.5	1.6	3.1
31	PhO	PhO	S	54	0.8	50	1.6	1.6
32	Ph	MeO	S	85	.8	6.3	25	25
33	Ph	EtO	S	78	1.6	12.5	6.3	1.6
34	Ph	*n*-PrO	S	83	1.6	6.3	3.1	1.6
35	Ph	*i*-PrO	S	81	3.1	6.3	0.8	1.6
36	Ph	$ClCH_2CH_2O$	S	75	1.6	12.5	3.1	3.1
37	Ph	*n*-BuO	S	80	0.8	25	1.6	3.1
38	Ph	PhO	S	65	.8	12.5	1.6	1.6
39	Ph	*o*-MePhO	S	75	.8	50	12.5	12.5
40	Ph	*m*-MePhO	S	63	.8	25	1.6	1.6
41	Ph	*o*-ClPhO	S	78	1.6	50	3.1	3.1
42	Ph	*m*-ClPhO	S	71	0.8	50	3.1	3.1
43	Ph	*p*-MeOPhO	S	76	1.6	50	3.1	3.1
44	Ph	EtS	S	71	0.4	12.5	3.1	3.1

[a] Assayed by hydroxylamine colorimetric procedure. [b] See footnote 7. [c] Strains resistant to high concentrations (>100 μg./ml.) of penicillin and other antibiotics (see Table I). [d] Procaine salt.

Table V

In Vivo Activity of Some Phosphinylaminopenicillanic Acids and Phosphinothioylaminopenicillanic Acids Against Resistant *Staphylococcus aureus* 400 Infection in Mice[a]

	Compound I			Serum inactivation ratio *in vitro*[b]	PD_{50}	
No.	R_1	R_2	X		Oral, mg./kg.	Parenteral, mg./kg.
12	EtO	EtO	O	0–2	50–90	25–65[c]
13	*n*-BuO	*n*-BuO	O	2	100	40
14	EtO	*n*-BuO	O	4	70	40[c]
15	EtO	$ClCH_2CH_2O$	O	0	210	95
16	EtO	$C_8H_{17}O$[d]	O	4	[e]	50
4	PhO	PhO	O	8	120	100
17	*p*-MeOPhO	*p*-MeOPhO	O	>64	>400	>200
18	3,5-Me_2PhO	3,5-Me_2PhO	O	>32	>400	>200
19	PhO	*n*-PrO	O	4	400	110
20	PhO	*i*-PrO	O	0	110	45
45	Ph	*i*-PrO[f]	O	0	[e]	90
	Ph	ArO[g]	O	0–8	>400	75–140
10	MeO	MeO	S	0	200	100
28	EtO	EtO	S	0–2	120–140	100–120[c]
29	*n*-BuO	*n*-BuO	S	32	>400	>200
31	PhO	PhO	S	64	>400	>200
	Ph	RO[h]	S	2–8	>400	>200
37	Ph	*n*-BuO	S	32	>400	>200
	Ph	ArO[i]	S	16–64	>400	>200
44	Ph	EtS	S	32	>400	>200

[a] Oral and parenteral PD_{50} values of penicillins G and V for the resistant *S. aureus* 400 infection in mice are >800 mg./kg.; *in vivo* data refer to intraperitoneal infection with single dose treatment given after 0.5 hr. and PD_{50} calculated after 96 hr. [b] Serum inactivation ratio (s.i.r.) = m.i.c. for *S. aureus* with serum/m.i.c. for *S. aureus* in absence of serum (*cf.* Tables II–IV). [c] Against normal (antibiotic-sensitive) *S. aureus* infection in mice, the following oral and parenteral PD_{50} values were observed, respectively: **12**, 86–110 and 25–65; **14**, 75 and 50; **28**, 125 and 100. [d] C_8H_{17} = 2-ethylhexyl. [e] Not run. [f] Calcium salt (73% pure). [g] Compounds **21–27** in Table III; individual parenteral PD_{50} values were: **21**, 100; **22**, 75; **23**, 100; **24**, 140; **25**, 120; **26**, 100; **27**, 120. [h] Compounds **32–36** in Table IV. [i] Compounds **38–43** in Table IV.

tively, for **4**. This result is not unexpected, since converting benzylpenicillin to its amide also depressed biological activity against normal microorganisms.[12]

Chemotherapeutic activity of I in protecting mice against resistant *S. aureus* 400 infections (Table V) is correlated qualitatively with the *in vitro* serum inactivation ratio (s.i.r.) for the sensitive strain. The s.i.r. is the ratio of the m.i.c. determined in the presence of serum to the m.i.c. determined in the absence of serum. For phosphinothioylaminopenicillanic acids (I, X = S) only the alkoxy members afford significant protection; the latter have low s.i.r.-values (*e.g.*, 0–2 for I, R_1, R_2 = methoxy or ethoxy), while the inactive compounds have high ratios (16 or greater). An exception is the group of lower alkoxyphenylphosphinothioylaminopenicillanic acids (**32–36**) which exhibit low ratios (s.i.r., 0–8) but have no *in vivo* activity. For phosphinylaminopenicillanic acids (I, X = O) those showing interesting PD_{50}-values have s.i.r.-values of 0–8; again those with higher ratios are inactive. In either series the lower alkoxy members are the most active *in vivo*. In a separate study diethoxyphosphinothioylaminopenicillanic acid (**28**) administered either simultaneously or 0.5 hr. later with a high level of penicillin V

TABLE VI

In Vitro and *In Vivo* ACTIVITY OF 6-DIETHOXYPHOSPHINOTHIOYLAMINOPENICILLANIC ACID (**28**)

		in vitro activity		*in vivo* activity of **28** PD_{50} (in mice)	
Organism	Inocula dilution	M.i.c. for **28** μg./ml.	M.i.c. for penicillin G μg./ml.	Oral, mg./kg.	Parenteral, mg./kg.
S. aureus	10^{-3}[a]	6.3	0.02	125	100
	10^{-5}	3.1	.01		
	10^{-7}	3.1	.005		
S. aureus 376 resistant strain	10^{-3}[a]	6.3	>100		
	10^{-5}	6.3	6.3		
	10^{-7}	6.3	0.8		
S. aureus 400[b] resistant strain	10^{-3}[a]	6.3	>100	120	110
	10^{-5}	6.3	6.3		
	10^{-7}	3.1	6.3		
S. aureus K3 resistant strain	10^{-3}[a]	6.3	>100	120	70
	10^{-5}	3.1	1.6		
	10^{-7}	3.1	0.4		
S. aureus K4 resistant strain			[c]	135	130

[a] 10^{-3} is the standard inocula dilution used in our laboratories. Number of viable units at this dilution are: *S. aureus* 1.5×10^6; *S. aureus* 376, 2.4×10^6; *S. aureus* 400, 2.4×10^6; *S. aureus* K3, 2.1×10^6. [b] PD_{50} for penicillin G or V against *S. aureus* 400 in mice is greater than 800 mg./kg. orally or parenterally. [c] Not run.

TABLE VII

COMPARISON OF PENICILLINASE ACTION

	Relative rates of hydrolysis[a] (arbitrary units)	
Compound	Penicillinase from *S. aureus* 400	Penicillinase from *B. cereus*
Penicillin G	1.00	1.00
Diethoxyphosphinylamino-penicillanic acid (**12**)	[b]	0.11
Diethoxyphosphinothioylamino-penicillanic acid (**28**)	0.00	.05

[a] See footnote 9. [b] Not run.

I has little apparent effect on *in vitro* activity; except in the lower alkoxy compounds a phosphorus–sulfur linkage appears to increase the s.i.r., which is reflected in a reduction in *in vivo* activity.

Experimental

6-Dimethoxyphosphinothioylaminopenicillanic Acid N-Ethylpiperidine Salt (10).—A solution of 6-APA (4.32 g., 0.02 mole) in 200 ml. of 0.133 *M* disodium hydrogen phosphate was adjusted to pH 7.5 with *N* NaOH, mixed with 80 ml. of acetone, and cooled (ice bath). A solution of dimethyl phosphorochloridothionate (3.21 g., 0.02 mole) in 40 ml. of acetone was added rapidly with stirring. The pH was kept at 7.5 by periodic additions of dilute alkali. After stirring 4 hr. at room temperature, the solution was washed twice with 0.5 volumes of ether. The aqueous solution was mixed with 1 volume of ethyl acetate, stirred, and adjusted to pH 5.5 with *N* HCl. A second extraction at pH 3.5 was done in the same manner.[13] Each ethyl acetate extract was washed with 0.5 volume of water, dried (sodium sulfate), and adjusted to an apparent pH 8 with N-ethylpiperidine. The first extract yielded crystalline **10** (1.94 g.; m.p. 133–137° dec.) on concentrating to a small volume, while the second extract gave crystalline **10** (2.52 g.; m.p. 133–137°) after evaporating the solvent and triturating the residue with ether (total yield, 49%). For analysis a sample was recrystallized from ethyl acetate–hexane; m.p. 133–137° dec.; $[\alpha]^{24}D$ +200° (*c* 1, water); λ_{max} 5.65 μ (vs) in KBr.

Anal. Calcd. for $C_{17}H_{32}N_3O_5PS_2$: C, 45.02; H, 7.11; N, 9.27; P, 6.83; S, 14.14. Found: C, 45.09; H, 7.24; N, 9.29; P, 6.97; S, 14.54.

6-Diethoxyphosphinylaminopenicillanic Acid N-Ethylpiperidine Salt (2).—Diethyl chlorophosphate (17.3 g., 0.10 mole) and 6-APA (21.6 g., 0.10 mole) were made to react as described for **10**. The second ethyl acetate extract (pH 3.5) yielded 7.04 g. (15%) of crystalline **2** (m.p. 107–109° dec.). For analysis a sample was recrystallized from acetone; m.p. 107–109° dec.; $[\alpha]^{23}D$ +191° (*c* 1, water); λ_{max} 5.66 μ (vs) in KBr.

Anal. Calcd. for $C_{19}H_{36}N_3O_6PS$: C, 49.02; H, 7.80; N, 9.03; P, 6.65; S, 6.89. Found: C, 48.46; H, 7.95; N, 8.79; P, 6.77; S, 7.21.

6-Diethoxyphosphinothioylaminopenicillanic Acid. A. N-Ethylpiperidine Salt (11).—Diethyl phosphorochloridothionate (7.52 g., 0.04 mole) and 6-APA (8.64 g., 0.04 mole) were allowed to react as described for **10**. The first and second extracts afforded 4.69 g. and 1.02 g. of crystalline **11**, m.p. 102–106° dec., respectively (total yield, 30%). For analysis a sample was recrystallized from ethyl acetate–hexane; m.p. 102–106° dec.; $[\alpha]^{24}D$ +197° (*c* 1, water); λ_{max} 5.66 μ (vs) in KBr.

Anal. Calcd. for $C_{19}H_{36}N_3O_5PS_2$: C, 47.38; H, 7.54; N, 8.73; P, 6.43; S, 13.32. Found: C, 47.23; H, 7.75; N, 8.93; P, 6.53; S, 13.30.

B. Procaine Salt.—A cooled solution of 6-APA (10.0 g., 0.046 mole) dissolved in 100 ml. of acetone–water (1:1) by adjusting pH to 7 with 10% sodium hydroxide was treated with a solution of diethyl phosphorochloridothionate (10.0 g., 0.053 mole) in 40 ml. of acetone added with stirring. The reaction solution was maintained at 5–10° and at pH 7–7.3 for 3 hr., adding acetone

TABLE VIII

SERUM LEVELS IN DOGS AFTER ORAL ADMINISTRATION[a]

	Compound I			Dosage, mg./kg.,	Average serum activity dilutions (reciprocals) at hr. Assay organism: *Streptococcus pyogenes*					
No.	R_1	R_2	X	oral	0.5	1	2	3	5	7
12	EtO	EtO	O	20		1		1	1	1
28	EtO	EtO	S	80	10	2.5	0	0		0
				160	16	11	2.5	0		0
14	EtO	*n*-BuO	O	20		8	5	0.6	0	
46	PhO	PhO	O[b]	20	35	22	22	8	1.6	
20	PhO	*i*-PrO	O	20	7	12	4	2	0.2	
	Penicillin V			10	96	72	10	4.7	0	

[a] Single experiments involving five dogs per compound tested. [b] Potassium salt (50% pure).

did not result in a PD_{50} significantly different from that observed for **28** alone.

The structure–activity relationships for I can be summarized as follows. Inhibitory action *in vitro* against resistant staphylococci and the high degree of inertness to penicillinase appear to be nonspecific properties in respect to the R_1 and R_2 groups attached to phosphorus in I. Aryloxy groups increase *in vitro* activity slightly and promote higher serum levels than alkoxy groups. However, the s.i.r. also increases with number and complexity of the aryloxy groups, so that only compounds containing the simpler groups are active *in vivo*. Compounds in which R_1 and R_2 = lower alkoxy have slightly higher m.i.c.-values, low s.i.r.-values, and exhibit good *in vivo* properties. The nature of X in

(13) In the synthesis of I ethyl acetate extractions of the reaction solution were done routinely at pH 5.5 and pH 3.5; for compounds **10** and **11**, the former extraction can be omitted.

when necessary to keep the solution homogeneous. The solution was washed with ethyl acetate (100 ml.) and extracted at pH 3 with two 100-ml. portions of the same solvent. The combined extract was washed with 50 ml. of water. Fresh water (50 ml.) was added to the ethyl acetate solution, and the mixture was adjusted to pH 6.8 with saturated aqueous sodium carbonate. The last extraction was repeated with 35 ml. of water; the combined pH 6.8 aqueous solution was washed with ether (30 ml.) and evaporated briefly to remove solvent. The aqueous solution was cooled to 5° and treated with a cold solution of procaine hydrochloride (7.2 g.) in 8 ml. of water. After refrigerating the hazy solution for 1 hr., crystalline procaine salt was recovered by filtration, washed with cold water and ether, and dried: yield, 13.6 g. (49%); m.p. 139–143° dec.; $[\alpha]^{24}D$ +143° (*c* 1, 70% acetone); λ_{max} 5.66 μ (vs) in KBr.

Anal. Calcd. for $C_{25}H_{41}N_4O_7PS_2$: C, 49.65; H, 6.84; N, 9.27. Found: C, 49.58; H, 6.78; N, 9.23.

6-Diphenoxyphosphinylaminopenicillanic Acid N-Ethylpiperidine Salt (4).—Diphenyl chlorophosphate (13.5 g., 0.05 mole) and 6-APA (10.8 g., 0.05 mole) were made to react as described for **10**. The first ethyl acetate extract (pH 5.5) yielded 3.0 g. (11%) of crystalline **4**; m.p. 133–136° dec. For analysis a sample was recrystallized from acetone; m.p. 133–136° dec.; $[\alpha]^{24}D$ +173° (*c* 1, water); λ_{max} 5.64 μ (vs) in KBr.

Anal. Calcd. for $C_{27}H_{36}N_3O_6PS$: C, 57.74; H, 6.46; N, 7.48. Found: C, 57.91; H, 6.48; N, 7.42.

6-Bis-*p*-methylphenoxyphosphinylaminopenicillanic Acid N-Ethylpiperidine Salt (6).—Bis-*p*-tolyl chlorophosphate (14.8 g., 0.05 mole) and 6-APA (10.8 g., 0.05 mole) reacted as described for **4** yielded 3.4 g. (12%) of crystalline **6**; m.p. 128–130° dec.; $[\alpha]^{24}D$ +149° (*c* 1, ethanol); λ_{max} 5.66 μ (vs) in KBr.

Anal. Calcd. for $C_{29}H_{40}N_3O_6PS$: C, 59.07; H, 6.84; N, 7.13. Found: C, 58.99; H, 6.91; N, 6.91.

6-Phenoxyphenylphosphinothioylaminopenicillanic Acid N-Ethylpiperidine Salt.—Phenoxyphenylphosphinothioyl chloride, $C_6H_5PS(C_6H_5O)Cl$ [13.5 g., 0.05 mole; b.p. 131–135° (0.01 mm.); $n^{26}D$ 1.6190] and 6-APA (10.8 g., 0.05 mole) were allowed to react as described for **4** to give 10.4 g. (37%) of crystalline product. For analysis a sample was recrystallized from acetone; m.p. 106–111° dec.; $[\alpha]^{23}D$ +214° (*c* 1, water); λ_{max} 5.66 μ (vs) in KBr.

Anal. Calcd. for $C_{27}H_{38}N_3O_4PS_2$: C, 57.73; H, 6.46; N, 7.48. Found: C, 57.92; H, 6.70; N, 7.54.

6-Ethylthiophenylphosphinothioylaminopenicillanic Acid Potassium Salt (44).—A solution of 6-APA (4.32 g., 0.02 mole) in 30 ml. of water with the addition of solid potassium bicarbonate (4 g.) was treated with a solution of ethylthiophenyl phosphinothioyl chloride, $C_6H_5PS(C_2H_5S)Cl$ [4.72 g., 0.02 mole; b.p. 114–118° (0.02 mm.); $n^{25}D$ 1.6310] in 30 ml. of acetone. The solution was adjusted to pH 6 with 10% potassium bicarbonate (14 ml.) and stirred for 5 hr. (final pH 6.6). After clarification the solution was extracted with 2 volumes of ethyl acetate at pH 5.5. The extract was washed with 0.5 volume of water, dried, and adjusted to pH 8 with *N* methanolic potassium hydroxide. The solution was evaporated to dryness, and the residue was triturated with absolute ether to an amorphous solid, which was dried over phosphorus pentoxide under vacuum. The yield of **44** was 1.55 g. (12%); calculated chemical assay for $C_{16}H_{20}N_3O_5PS_2K$, 1310 sodium penicillin G units per mg.; found (hydroxylamine colorimetric method[5]), 935 units per mg.; purity, 71%.

6-Diphenoxyphosphinylaminopenicillanamide (11).—A solution of diphenyl chlorophosphate (6.7 g., 0.025 mole) in 50 ml. of acetone was added rapidly with stirring to a cooled solution of 6-aminopenicillanamide *p*-toluenesulfonate[6] (9.8 g., 0.025 mole) in a mixture of 50 ml. of 0.067 *M* disodium hydrogen phosphate (adjusted to pH 7.5) and 20 ml. of acetone. After stirring for 3 hr. at room temperature (pH kept at 7.5), the reaction solution was extracted with 1 volume of ethyl acetate. The extract was washed with one-half volume of water, three times with 0.5 volumes of 5% sodium bicarbonate, and again with water. Solvent was removed from the dried extract by evaporation, and the residue on trituration with benzene yielded 1.1 g. (10%) of crystalline **11**. For analysis a sample was recrystallized from acetone–hexane; m.p. 105–108°; $[\alpha]^{23}D$ +197° (*c* 1, chloroform); λ_{max} 5.60 (vs) and 5.99 μ (vs) in KBr.

Anal. Calcd. for $C_{28}H_{32}N_3O_5SP$: C, 53.68; H, 4.96; N, 9.39. Found: C, 53.37; H, 5.20; N, 9.14.

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Analogs of Tetrahydrofolic Acid. VIII.[1,2] Synthesis of N-[1-(2-Amino-4-mercapto-6-methyl-5-pyrimidyl)-3-propyl]-*p*-aminobenzoyl-L-glutamic Acid, a Mercapto Analog

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The title compound (II) has been synthesized in nine steps starting with ethyl acetoacetate *via* the key intermediates 2-acetamido-4-chloro-5-[2-(1,3-dioxolan-2-yl)ethyl]-6-methylpyrimidine (XIII) and 2-amino-5,6-dihydro-7-hydroxy-4-methyl-7H-thiopyrano[2,3-d]pyrimidine (XVIII). Conversion of 2-amino-5-[2-(1,3-dioxolan-2-yl)ethyl]-6-methyl-4-pyrimidinol (X) to the corresponding 4-pyrimidinethiol XV or 4-chloropyrimidine XI could not be accomplished due to the instability of the 5-side chain; in contrast, the N^2-acetyl of X, namely XII, underwent fast conversion to 2-acetamido-4-chloro-5-[2-(1,3-dioxolan-2-yl)ethyl]-6-methylpyrimidine (XIII) under mild conditions due to the greater reactivity and better solubility of XII compared to X. The key intermediate 2-amino-4-mercapto-6-methyl-5-pyrimidylpropionaldehyde (XX) existed almost completely in the hemiacetal form XVIII in neutral solution, but existed in the open chain aldehyde form XXII when converted to an anion at pH 13.

Since fifteen enzymes that use folic acid, tetrahydrofolic acid, or derivatives of tetrahydrofolic acid as substrates are known,[3-5] a program was initiated on analogs of tetrahydrofolic acid. The rationale[6] for, as

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