While these results do not indicate what effects may occur in the human, they do show that nitroglycerin is effective in the rabbit when ingested. The possibility of buccal absorption is eliminated because none of the material touched the surfaces of the mouth. Incidentally, these results indicate that most of the nitroglycerin contained in the granules was released.

It is interesting that the effect of the nitroglycerin contained in the granules was more prolonged than the effect of the equivalent amount of free nitroglycerin, shown by a comparison of the results from the groups given nitroglycerin powder and those from the groups given the granules. To produce the prolonged vasodilatation, the nitroglycerin must have been released gradually from the granules. It is possible that if small quantities of nitroglycerin are released in the alimentary tract over a period of hours, the degradation by the liver may be less than that found when a quantity is released at once. The hypothesis would account for the marked and prolonged dilatation observed when the nitroglycerin source was the granule, contrasted wth that observed when the source was free nitroglycerin.

REFERENCES

(1) Salter, W. T., "A Textbook of Pharmacology," W. B. Saunders Co., Philadelphia, Pa., 1952, p. 311.
(2) Sollmann, T., "A Manual of Pharmacology," W. B. Saunders Co., Philadelphia, Pa., 1957, p. 631.
(3) Souder, J. C., and Ellenbogen, W. C., DRUG STANDARDS, 26, 77(1958).

Phosphorus-Nitrogen Compounds IV. Some 2-Aminopyridine Derivatives

By LINDLEY A. CATES and NOEL M. FERGUSON

Seventeen phosphoramidates and phosphoramidothionates containing 2-aminopyridine moieties were synthesized as potential antineoplastic agents.

THE PREPARATION of phosphoramidates and phosphoramidothionates has been extended in this report to include 2-amino and C-5 substituted 2-aminopyridines. The synthesis of related compounds containing *p*-toluidine moieties was described in an earlier paper in this series (1).

Synthetic work similar to the P-N compounds herein reported has been previously carried out by Russian investigators. Arbuzov and co-workers (2) prepared dialkyl *N*-2-pyridylphosphoramidates by the reaction between dialkyl phosphorochloridates and 2-aminopyridine. These investigators also attemped the synthesis of the dimethyl and diethyl esters of *N*-2-pyridylphosphoramidothionic acid and obtained uncrystallizable masses in each case. Compounds III and IV (Table I) indicate the preparation of the dimethyl and di-*n*-propyl esters of the 5-methyl homolog of this acid. Zhmurova and Kirsanov (3) earlier synthesized diphenyl *N*-(5-nitro-2-pyridyl)phosphoramidate (compound XV) by means of a different procedure, *i.e.*, the reaction between triphenyl *N*-phenylphosphorimidate and 2-amino-5-nitropyridine.

The phosphorochloridate-amine method was employed for the synthesis of the compounds summarized in Table I. Thus, compound XV was prepared by refluxing diphenylphosphorochloridate and 2-amino-5-nitropyridine in reagent dioxane according to previously described procedures (1, 4).

The 2-aminopyridines employed in the synthesis of these P-N compounds are congeners of 6-aminonicotinamide, because the carbamyl radical in the 3-position is replaced by a hydrogen, bromine, or chlorine atom and by a methyl or nitro group. The antimetabolic activity of 6-aminonicotinamide (5, 6) and its value as an anticancer agent and drug adjunct has been well established (7–11). A number of pyridines related to those used in this report have been investigated for antileukemic activity (12), and an absence of good inhibitory effect was noted in mono-substituted derivatives, such as 2-aminopyridine and 3-picoline. This latter compound was also shown to be inactive by Goldin *et al.* (13). Most antineoplastic activity appears to reside in 2,5 disubstituted pyridines, and the cytotoxicity of such derivatives is exemplified by the effect of 2-amino-5-nitropyridine against trichomonal infections (14). It has also been reported that derivatives of 3- and 5-nitropyridine and pyrimidine inhibit *T. vaginalis in vitro;* whereas 5-nitropyridines and pyrimidines with a single substituent at C-2 inhibit both *in vitro* and *in vivo* (15).

In those pyridines exhibiting antitumor activity, however, toxicity for the host has been a limiting factor in therapy. One approach to increasing selective cytotoxicity has been the incorporation of alkylating agents in P-N compounds (*e.g.*, cyclophosphamide) and is based on the enzymatic influence of phosphamidases, which occur in higher concentration in neoplastic cells. The compounds reported in this paper represent a further attempt to include potential antimetabolic moieties in similar phosphoramide structures.

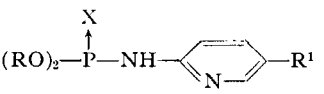
Samples of the compounds listed in Table I have been submitted to the Cancer Chemotherapy National Service Center for preliminary evaluation.

EXPERIMENTAL

Syntheses.—The phosphoramidates and phosphoramidothionates (Table I) were prepared by standard methods involving the reaction between

Received September 3, 1964, from the College of Pharmacy, University of Houston, Houston, Tex.
Accepted for publication October 14, 1964.
This investigation was supported by research grant CA-06820-02 from the National Cancer Institute, U. S. Public Health Service, Bethesda, Md.

TABLE I.—2-AMINOPYRIDINE DERIVATIVES



No.	R	X	R′	M.p., °C.[a]	Formula	Anal., %[b] P Calcd.	P Found	N Calcd.	N Found
I	Ethyl	O	H	87–8[c]	$C_9H_{15}N_2O_3P$	13.4	13.6	12.2	12.6
II	*p*-Tolyl	O	H	174–175	$C_{13}H_{13}N_2O_3P$	8.7	8.7	7.9	8.2
III	Methyl	S	CH_3	95–96	$C_8H_{13}N_2O_2PS$	13.3	13.5	12.1	12.3
IV	*n*-Propyl	S	CH_3	74–75	$C_{12}H_{21}N_2O_2PS$	10.7	10.9	9.7	9.4
V	Ethyl	O	CH_3	107–108	$C_{10}H_{17}N_2O_3P$	12.7	12.4	11.5	11.1
VI	*n*-Butyl	O	CH_3	oil	$C_{14}H_{25}N_2O_3P$	10.3	10.1	9.3	9.6
VII	Phenyl	O	CH_3	169	$C_{18}H_{17}N_2O_3P$	9.1	9.3	8.2	8.3
VIII	*o*-Tolyl	O	CH_3	164	$C_{20}H_{21}N_2O_3P$	8.4	8.2	7.6	7.9
IX	*p*-Tolyl	O	CH_3	151	$C_{20}H_{21}N_2O_3P$	8.4	8.5	7.6	7.8
X	Phenyl	O	Cl	149–150	$C_{17}H_{14}ClN_2O_3P$	8.6	8.6	7.8	7.6
XI	*o*-Tolyl	O	Cl	125–127	$C_{19}H_{18}ClN_2O_3P$	8.0	7.7	7.2	7.0
XII	Phenyl	O	Br	133–134	$C_{17}H_{14}BrN_2O_3P$	7.6	7.3	6.9	6.7
XIII	*p*-Tolyl	O	Br	152–153	$C_{19}H_{18}BrN_2O_3P$	7.2	7.4	6.5	6.3
XIV	*o*-Tolyl	O	Br	144–145	$C_{19}H_{18}BrN_2O_3P$	7.2	7.4	6.5	6.9
XV	Phenyl	O	NO_2	191[d]	$C_{17}H_{14}N_3O_5P$	...	...	...	...
XVI	*o*-Tolyl	O	NO_2	156–157	$C_{19}H_{18}N_3O_5P$	7.7	7.3	10.5	10.0
XVII	*p*-Tolyl	O	NO_2	216	$C_{19}H_{18}N_3O_5P$	7.7	7.9	10.5	10.3

[a] All melting points are uncorrected. [b] Analyses were performed by Schwarzkopf Microanalytical Laboratories, Woodside, N. Y. [c] Previously prepared by same method. [Reported m.p. 86–88°C. (2).] [d] Previously prepared by a different method. [Reported m.p. 188–190°C. (3).]

2-amino or substituted 2-aminopyridines and dialkyl and diaryl phosphorochloridates or dialkyl phosphorochloridothionates (2:1) at reflux temperature in inert solvents. Reaction mixtures were allowed to reflux for 1 to 4 hr. for all derivatives except compound IV. The reflux time was extended to a total of 43 hr. for this derivative because of the lower reactivity of the chloridothionate. Anhydrous ether was used as the solvent for compounds I–IX, and reagent dioxane was found more appropriate for compounds X–XVII.

Isolation.—Compounds I, III–VI, VIII, IX, XI, XII, and XV–XVII were isolated by evaporating the reaction mixture filtrates to yield oils or solids. Compounds II and VII were isolated from the precipitates formed in the reaction mixtures and washed with petroleum ether and water. Compound X was isolated as a solid, and compounds XIII and XIV were isolated as oils by precipitating the reaction mixture filtrates with dilute hydrochloric acid.

Purification.—All of the derivatives were crystalline white solids, except compound VI which was isolated as an analytically pure oil. Compounds I and V were recrystallized from water, compound III from petroleum ether, and compounds II, VII, X, and XII–XIV from ethanol–water. The oily residues containing compounds IV and XI were washed with petroleum ether and water to yield solids which were recrystallized from ethanol–water. Compound VI was washed with 10% sodium carbonate solution and water, dissolved in ether, and dried over anhydrous calcium sulfate. Filtration and removal of the ether *in vacuo* yielded the product as a pale yellow viscous oil. Compounds VIII and IX were washed with 10% sodium carbonate solution and water and recrystallized from ethanol. Compounds XV–XVII were washed with acetone and recrystallized from ethanol–water. The washing of the isolated brown solids with a small amount of acetone to give pale yellow residues is a useful step in the purification of these derivatives in quantity.

REFERENCES

(1) Cates, L. A., and Ferguson, N. M., THIS JOURNAL, **53**, 973(1964).
(2) Arbuzov, B. A., Zoroastrova, V. M., and Osipova, M. P., *Otd. Khim. Nauk*, **1961**, 2163; through *Chem. Abstr.*, **57**, 8536(1962).
(3) Zhmurova, I. N., and Kirsanov, A. V., *Zh. Obshch. Khim.*, **29**, 1687(1959); through *Chem. Abstr.*, **54**, 8688 (1960).
(4) Cates, L. A., and Jones, T. E., THIS JOURNAL, **53**, 691(1964).
(5) Johnson, W. J., and McColl, J. D., *Federation Proc.*, **15**, 284(1956).
(6) Johnson, W. J., and McColl, J. D., *Science*, **122**, 834(1955).
(7) Martin, D. S., Kligerman, M. M., and Fugmann, R. A., *Cancer Res.*, **18**, 893(1958).
(8) Shapiro, D. M., Dietrich, L. S., and Shils, M. E., *ibid.*, **17**, 600(1957).
(9) Martin, D. S., Dietrich, L. S., and Fugmann, R. A., *Proc. Soc. Exptl. Biol. Med.*, **103**, 58(1960).
(10) McColl, J. D., Rice, W. B., and Adamkiewicz, V. W., *Can. J. Biochem. Physiol.*, **35**, 795(1957).
(11) Shapiro, D. M., *Radiology*, **69**, 188(1957).
(12) Humphreys, S. R., *et al.*, *Cancer Res., suppl.* **22**, *part* **2**, **1962**, 483.
(13) Goldin, A., Venditti, J. M., and Kline, I., *ibid., suppl.* **21**, *part* **2**, **1961**, 27.
(14) Michaels, R. M., and Strube, R. E., *J. Pharm. Pharmacol.*, **13**, 601(1961).
(15) Michaels, R. M., *J. Protozool.*, **9**, 478(1962).