TABLE V.—ULTRAVIOLET ABSORPTION MAXIMA OF RUBBER CLOSURE INGREDIENTS

Rubber Stopper	Wavelength, mμ				
	Water	10% Ethanol	2% Benzyl Alcohol	50% *N,N*-Dimethylacetamide	50% Polyethylene Glycol 300
Natural					
Thiazole-type accelerator	312	312	312	322	322
	227	230	...	...	228
Thiuram-type accelerator	274	274	278	276	278
Dithiocarbamate reaction product	274	276	...	274	274
	...	250	...	257	253
Polyurethan					
Urethan monomer	...	...	...	288	287
Organo-metallic catalyst	272	274	272	274	274

vents from both the unlined polyurethan and natural rubber closures were qualitatively identified.

4. Teflon lined natural rubber stoppers, which were perforated several times by a hypodermic needle, still afforded some protection against sorption and considerable protection against leaching.

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Notes

Phosphorus-Nitrogen Compounds V. Some Guanidine and 2-Aminopyrimidine Derivatives

By LINDLEY A. CATES and NOEL M. FERGUSON

Five amidino(thio)phosphoramidates and six 2-pyrimidyl(thio)phosphoramidates were prepared for screening in four cancer test systems. Three of these derivatives were also tested against *Plasmodium berghei*. None of the compounds exhibited significant antineoplastic or antimalarial activity.

THE SYNTHESIS of phosphoramidopyrimidines was undertaken to further ascertain the effect of phosphorus bonding on the cytotoxic properties of heterocyclic amines. The preparation of similar pyridine-containing compounds was described in an earlier report in this series (1).

The rationale for antineoplastic activity by these derivatives originates from an hypothesis concerning the mechanism of action of cyclophosphamide. According to this theory, cyclophosphamide exists as an inactive transport form *in vivo* until the P-N bond is cleaved by the enzymatic action of phosphamidases or phosphatases to yield the alkylating ethylenimmonium ion (2). Thus, replacement of the bis(2-chloroethyl)amino group by an aminopyrimidine moiety might give rise to an antimetabolic activity of a more specific nature than would be realized by administration of the parent compound. More recent evidence (3), however, suggests that the conversion of cyclophosphamide *in vivo* into a cytostatically effective activation product is the result of a metabolic process dependent on the presence of oxygen and not the result of simple hydrolysis. At this time it can be stated that no direct proof of the postulated biochemical transformations of cyclophosphamide and related congeners is available.

Since guanidine and some of its derivatives are known to possess antiprotozoal properties (4) three of the amidino(thio)phosphoramidate intermedi-

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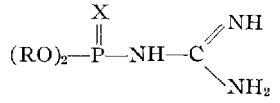
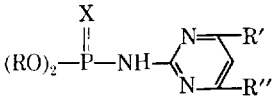
TABLE I.—AMIDINO(THIO)PHOSPHORAMIDATES



Compd.	R	X	M.p., °C.[a]	Formula	Anal., %[b] Calcd.	Found
I	Methyl	S	108–110	$C_3H_{10}N_3O_2PS$	C, 19.7 H, 5.5 N, 22.9	C, 19.8 H, 5.6 N, 23.3
II	Ethyl	S	87–88	$C_5H_{14}N_3O_2PS$	C, 28.4 H, 6.7 N, 19.9	C, 28.6 H, 6.8 N, 19.9
III	Ethyl	O	127–129	$C_5H_{14}N_3O_3P$	C, 30.8 H, 7.2 N, 21.5	C, 30.9 H, 7.4 N, 21.8
IV	*n*-Propyl	S	73–74	$C_7H_{18}N_3O_2PS$	C, 35.1 H, 7.6 N, 17.6	C, 35.2 H, 7.8 N, 17.4
V	*n*-Butyl	O	83–84	$C_9H_{22}N_3O_3P$	C, 43.0 H, 8.8 N, 16.7	C, 43.2 H, 8.8 N, 16.6
VI[c]	Phenyl	O	119	$C_{13}H_{14}N_3O_3P$	C, 53.6 H, 4.8 N, 14.4	C, 53.4 H, 4.8 N, 14.5

[a] All melting points were determined on a Fisher-Johns apparatus and are uncorrected. [b] Elemental analyses were run on a Fisher CHN analyzer. [c] Previously prepared by different methods [reported m.p. 118° (6, 8)].

TABLE II.—2-PYRIMIDYL(THIO)PHOSPHORAMIDATES



Compd.	R	X	R′	R″	M.p., °C.[a]	Formula	Anal., %[b] Calcd.	Found
VII	Phenyl	O	H	H	137–139	$C_{16}H_{14}N_3O_3P \cdot H_2O$	C, 55.6 H, 4.7 N, 12.2	C, 55.6 H, 4.7 N, 12.0
VIII	Phenyl	O	CH_3	CH_3	152–153	$C_{18}H_{18}N_3O_3P \cdot H_2O$	C, 57.9 H, 5.4 N, 11.3	C, 58.1 H, 5.4 N, 11.3
IX	Ethyl	S	OH	OH	149–150	$C_8H_{14}N_3O_4PS$	C, 34.4 H, 5.0 N, 15.0	C, 34.3 H, 5.1 N, 14.4
X	*n*-Propyl	S	OH	OH	108–109	$C_{10}H_{18}N_3O_4PS$	C, 39.1 H, 5.9 N, 13.7	C, 39.5 H, 6.0 N, 13.5
XI	*n*-Propyl	S	OH	NH_2	154–156	$C_{10}H_{19}N_4O_3PS$	C, 39.2 H, 6.2 N, 18.3	C, 39.5 H, 6.3 N, 18.3
XII	*n*-Propyl	S	OH	CH_3	127–128	$C_{11}H_{20}N_3O_3PS$	C, 43.3 H, 6.6 N, 13.8	C, 43.3 H, 6.7 N, 13.9

[a] All melting points were determined on a Fisher-Johns apparatus and are uncorrected. [b] Elemental analyses were run on a Fisher CHN analyzer.

ates, which contain the guanidino moiety, were incidentally tested for antimalarial activity.

The pyrimidine derivatives were prepared by two different methods: phosphorylation and condensation. Two of the products were synthesized by direct acylation using diphenyl phosphorochloridate, whereas several attempts at phosphorylation of halo- and hydroxy-substituted pyrimidines employing alkyl and aryl phosphorochloridates proved unsuccessful. This failure is attributed to the relatively high pK_b values of these pyrimidines. A recent report (5) indicates that halo-substituted 2-aminopyrimidines will, however, yield phosphoramidic dichlorides and phosphorimidic trichlorides

when the more reactive phosphorus pentachloride is employed.

As an alternate route pyrimidine synthetic methods involving double condensation between cyanoacetic, malonic and β-keto esters and substituted guanidines were employed. The amidinophosphoramidates and amidinophosphoramidothionates required in these condensations were obtained by the reaction between phosphorochloridates or phosphorochloridothionates and guanidine. The latter reactant was prepared *in situ* using guanidine hydrochloride and alcoholic potassium hydroxide. The synthesis of amidinophosphoramidates is somewhat controversial in that Shvachkin and Prokof'ev (6) reported that the preparation of diphenylphosphorylguanidine from phosphoryl chloride, phenol, and guanidine (7) could not be duplicated, and they synthesized the product using diphenylphosphorochloridate and guanidine. This procedure was also reported by Cramer and Vollmar (8), as well as an alternate method involving the treatment of guanidine hydrochloride in aqueous sodium hydroxide with diphenylphosphorochloridate in benzene. The guanidine hydrochloride–alcoholic potassium hydroxide process reported in this paper is considered to be a convenient and economical method for the synthesis of phosphoryl(thio)guanidines. Of the guanidine derivatives prepared, the ethyl and *n*-propyl amidinophosphoramidothionates were selected for pyrimidine condensation.

EXPERIMENTAL

Chemistry

Amidinophosphoramidates and Amidinophosphoramidothionates (Table I).—A solution of potassium hydroxide (0.2 mole) in 100 ml. of absolute ethanol was added in portions to guanidine HCl (0.2 mole) in 100 ml. of absolute ethanol at 0° with stirring. This temperature was maintained and the appropriate phosphorochloridate or phosphorochloridothionate (0.1 mole) was introduced dropwise with stirring. The reaction mixture was allowed to remain overnight and then spin evaporated over a steam bath to yield a white mass or oil. The residue was dissolved in hot benzene and ether was added to precipitate guanidine hydrochloride. The filtrate was again spin evaporated to yield a white mass or oil. Compounds I, II, and III were isolated by crystallizing from hot benzene. Compounds IV and V were obtained in pure form by washing an ethereal solution of the residue with 10% sodium hydroxide solution and water, drying over anhydrous calcium sulfate, and concentrating the ether filtrate. Known compound VI was purified according to a previously described method (6). All products were white crystalline solids.

2-Pyrimidylphosphoramidates (Table II).—A mixture of 2-aminopyrimidine or 2-amino-4,6-dimethylpyrimidine (0.2 mole) and diphenyl phosphorochloridate (0.1 mole) in 500 ml. of reagent dioxane was refluxed for 0.5 hr. and allowed to remain overnight. The reaction mixture was spin evaporated to yield a brown mass which was washed with petroleum ether and water and the residue dissolved in hot ethanol. After treating with activated charcoal, clouding with water, and cooling, the product formed as a white crystalline solid.

2-Pyrimidylphosphoramidothionates (Table II.)—The appropriate amidinophosphoramidothionate (0.05 mole) was refluxed for 1 hr. with sodium (0.25 mole), which was previously reacted with 300 ml. of absolute ethanol, and the reaction mixture was allowed to set overnight. A fivefold excess of diethyl malonate, ethyl cyanoacetate, or ethyl acetoacetate was added, the mixture refluxed for 2 hr., and allowed to remain overnight. The reaction mixture was concentrated by spin evaporation and any precipitate dissolved by addition of the least amount of water. Following filtration the solution was adjusted to pH 8 using 5% hydrochloric acid and a Corning pH meter. The resulting precipitate was collected and crystallized from ethanol–water.

Infrared Spectra

All starting materials and products were examined by means of a Beckman IR-8 spectrophotometer using a Nujol mull. The new guanidine derivatives showed the following characteristic absorptions, ν in cm.$^{-1}$: 3200 (NH), 1020–1060 (POCalkyl), and 1240 (P=O in compounds III and V). Some of the principal absorptions given by the pyrimidine derivatives were, ν in the cm.$^{-1}$: 1520–1580 (C=C, C=N in all compounds), 1240 (P=O in compounds VII and VIII), 1080–1090 and 1200 (POC aryl in compounds VII and VIII), 1020–1055 (POC alkyl in compounds IX–XII), and 3350 (NH_2 in compound XI). The lack of a free amino group (except, of course, in compound XI) indicates the correctness of the proposed structures, eliminating an alternative course of the condensations. No assignment of P–N absorption was attempted since this band, usually designated around 715 cm.$^{-1}$, falls in the skeletal structure absorption range. Derkach (9) reported the infrared spectra of some related phosphoramidates and assigned the 893–910 cm.$^{-1}$ range to P–N. This assignment may not be correct since a similar strong peak was noted in the compounds reported here but only in the case of the phenyl esters. The reliability of assigning P–N absorption in the region of 715 cm.$^{-1}$ has also been questioned by Bellamy (10) and Thomas (11).

Screening Data

Samples of the new compounds were submitted for antitumor testing against sarcoma 180, Lewis lung carcinoma, lymphoid leukemia L-1210, and KB cell culture.[1] Results of that testing which have been completed indicate that only compound I has a per cent T/C (ratio of tumor weight of test animals to control animals; expressed as per cent) of less than 50 when screened against sarcoma 180 and that at a dose of 500 mg./Kg. The most toxic member of the series was compound V which gave no survivors at a dose of 100 mg./Kg. The pyrimidine derivatives exhibited relatively low acute toxicities having lethal doses in excess of 250 mg./Kg. Five mice were infected with a lethal dose of *Plasmodium berghei* 3 days prior to administration of compounds I, III, and V in doses of 40, 160, and 640 mg./Kg.[2] At the higher dose level compound III (most active) extended the survival time by only 35%. Compounds I and III showed low acute toxicities and the relatively high toxicity of compound V was confirmed in these tests.

[1] Testing results furnished by the Cancer Chemotherapy National Service Center, Bethesda, Md.

[2] Testing results furnished by the Walter Reed Army Medical Center, Washington, D. C.

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Tracer and Radioactivation Studies on Tartar Emetic Impurities

By A. ALIAN, YEHIA M. DESSOUKY*, and R. SHABANA

The problem of contamination of tartar emetic with arsenic and lead was examined using the radioactive isotopes, ^{76}As and ^{209}Pb. Neutron activation and radiochemical separation methods have been applied for the determination of arsenic and lead concentrations throughout the different stages of tartar emetic synthesis. It has been shown that, while most of arsenic is eliminated during the synthesis process, the major portion of lead remains in the final product. It was also found that the two impurities can be eliminated by successive washing of the starting materials with 0.2 *M* nitric acid and water. Tartar emetic synthesized from purified starting materials conforms to the requirements in pharmacopeias.

ONE OF THE most important problems of the pharmaceutical industry is the production of synthetic drugs in a suitably pure condition. The classical methods of tracing impurities are either misleading, tedious, or inaccurate. Antimony potassium tartrate (tartar emetic) is the most commonly employed drug in the treatment of bilharziasis, schistosomiasis, leishmaniasis, filariasis, ascariasis, and other tropical diseases (1). It is one of the early drugs found to be active against trypanosomes (2). The drug is widely used in Africa, Asia, and Latin America because of the simplicity and easiness of its synthesis and acceptance among patients and physicians. It is still the drug of choice in the treatment of bilharziasis in Egypt as well as other countries where infections occur (3). Cases of toxicity and inconvenience of intravenous injection of this drug are reported, however, and have been attributed, at least partly to the presence of toxic impurities such as arsenic and lead (3, 4).

Most pharmacopeias in which tartar emetic is mentioned require certain specifications for arsenic and lead limits for the sake of its medical safety. These limits vary in the case of arsenic to not more than 200 p.p.m. in the U.S.P. (5), 10 p.p.m. in the E. P. (6), 8 p.p.m. in the B.P. (7), and in case of lead to not more than 10 p.p.m. in the E.P. and 5 p.p.m. in the B.P.

The quantitative determination of the two metals, particularly arsenic, at such low concentrations in the presence of antimony cannot be performed with accuracy by the conventional analytical methods (8–11). The utilization of radioactive isotopes in solving some production problems and in working up technical operations in the pharmaceutical industry has found its way only recently (12). Some studies have been published on the manufacture of pure tartar emetic (3, 13), but none of the authors have made use of radioactive isotope techniques.

The purpose of the present paper was to find an economical method to eliminate arsenic and lead and to produce tartar emetic in pure form. For tracing these two metals, the radioactive isotopes, ^{76}As and ^{209}Pb, have been used, while their concentrations in the starting materials and in the final products have been determined by a neutron radioactivation procedure.

EXPERIMENTAL

Materials.—Unless otherwise stated, all chemicals employed were analytical grade reagents. The radioactive isotopes ^{76}As and ^{209}Pb were prepared by irradiating spectrographically pure arsenic trioxide and lead. Arsenic trioxide was then dissolved in dilute hydrochloric acid, while lead was dissolved in dilute nitric acid. Standard and carrier solutions of arsenic and lead were prepared by dissolving inactive arsenic trioxide and lead in a similar manner.

Apparatus.—The instruments used were an EKCO scintillation assembly for counting the γ radiation of ^{76}As and a Philips counting instrument with an end window G.M. tube for measuring ^{209}Pb β-radiations.

Irradiations.—Samples to be irradiated were wrapped in thin aluminum sheets and were enclosed in aluminum cans which were then irradiated at a flux of about 1.3×10^{13} neutrons/cm.^{2}/sec. in the U.A.-RR-1, 2 MW reactor. For the radioactivation analysis of arsenic and lead, every sample was placed together with the standards in one can and exposures were of about 5 hr. duration.

Procedure.—All experiments on the study of arsenic and lead uptake were performed in 100-ml. conical flasks. For evaluating arsenic uptake on

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