Potentiometric titration of IIb (0.1668 g./100 ml. of 50% ethanol) with 0.0995 N NaOH gave a pK_a of 6.75. The starting reagent, Ib (36.2 mg./100 ml. of water, titrated with 0.025 N NaOH), had $pK_{a_1} = 4.36$ and $pK_{a_2} = 8.92$.

Anal. Calcd. for $C_{12}H_{10}N_2O_3$: C, 62.60; H, 4.38; N, 12.17; mol. wt., 230.2. Found: C, 62.34; H, 4.49; N, 12.17; mol. wt., 232.8.

The cyclization reaction was repeated as above except that 40 ml. of 1,1,2,2-tetrachloroethane was used as the solvent and the refluxing time was reduced to 2 hr. This was the preferable method of preparation, as IIb was more soluble in the boiling solvent and crystallized in 76% yield upon filtering and cooling. Cyclization was effected also in polyphosphoric acid, but the yield of IIb was low.

2-(Anilinomethyl)-7,7a-dihydro-7a-methyl-1H-pyrrolo-[1,2-*c*]imidazole-1,3,5(2H,6H)-trione (IIc).—This compound was prepared by condensing IIa with equivalent quantities each of formaldehyde and aniline according to the procedure described previously[3]: yield 65%. After recrystallization from ethanol, it melted at 152–152.5°; $\lambda_{max}^{CH_3OH}$ 287, 240.5 mμ (ε 1757, 11,410); $\lambda_{max}^{CH_3OH-HCl}$ 282, 239.5 mμ (ε 796, 7871); $\lambda_{max}^{CH_3OH-KOH}$ 286.5, 238 mμ (ε 1782, 12,708).

Anal. Calcd. for $C_{14}H_{15}N_3O_3$: C, 61.53; H, 5.53. Found: C, 61.73; H, 5.70.

In a similar manner **2-(anilinomethyl)-7,7a-dihydro-7a-phenyl-1H-pyrrolo[1,2-*c*]imidazole-1,3,5(2H,6H)-trione** (IId) was prepared from IIb in 86% yield. After recrystallization from ethanol, it melted at 150–151°; $\lambda_{max}^{CH_3OH}$ 287, 267, 240 mμ (ε 1871, 1073, 13,414); $\lambda_{max}^{CH_3OH-HCl}$ 281.5, 267, 263, 239.5 mμ (ε 547, 471, 584, 5165); $\lambda_{max}^{CH_3OH-KOH}$ 287, 231 mμ (ε 1841, 21,228).

Anal. Calcd. for $C_{19}H_{17}N_3O_3$: C, 68.05; H, 5.11. Found: C, 68.25; H, 5.10.

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Synthesis and Biological Activity of 9-β-D-Ribofuranosyl-6-hydroxylaminopurine[1]

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The simple derivative of adenine, 6-hydroxylaminopurine,[2] has been found to induce mitotic inhibition and nuclear degeneration of mouse Sarcoma 180 cells, but not normal embryo skin fibroblasts, over a concentration range of 10^{-6} to 10^{-4} M.[3] When administered intraperitoneally to rats and mice at a single dose of 500 mg./kg., or at lower dosages over prolonged periods, 6-hydroxylaminopurine proved to be toxic (LD_{50} for a single dose, 470 mg./kg.).[4]

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(2) A. Giner-Sorolla and A. Bendich, *J. Am. Chem. Soc.*, **80**, 3932 (1958).

(3) J. J. Biesele, unpublished results.

(4) A. Giner-Sorolla, Ph.D. Thesis, Cornell University, 1958; *Dissertation Abstr.*, **20**, 1148 (1959).

Since 6-hydroxylaminopurine was inhibitory to several mouse tumors and leukemias (Table I) and was toxic, 9-β-D-ribofuranosyl-6-hydroxylaminopurine (II) was synthesized. It was found to induce a complete inhibition of mouse leukemia P815, but was ineffective on solid tumors. Mice survived the daily administration of 500 mg. of II for 2 weeks and a dose of 250 mg. for 3 weeks.

TABLE I

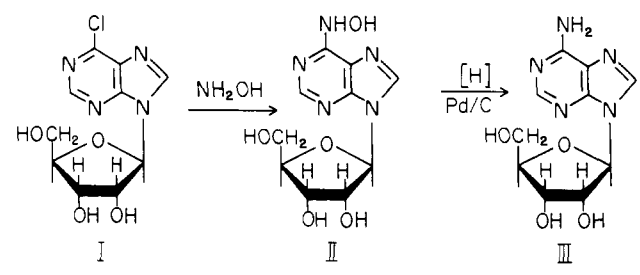
EFFECT OF SOME HYDROXYLAMINOPURINE DERIVATIVES ON MOUSE TUMORS AND LEUKEMIA[a]

	Dose, mg./kg./day	Result
6-Hydroxylaminopurine		
Sarcoma 180	125	−
Mouse carcinoma E0771	125	+
Mouse carcinoma E0771	250	+
Mouse carcinoma C1021	125	+
Ridgway osteogenic sarcoma	125	−
Ridgway osteogenic sarcoma	250	±
Mouse leukemia P815	250	+
Mouse leukemia P815 resistant to 6-mercaptopurine	150	±
Mouse leukemia P815 resistant to 6-mercaptopurine	40	−
9-β-D-Ribofuranosyl-6-hydroxylaminopurine (II)		
Sarcoma 180	62.5	−
Mouse carcinoma E0771	125	−
Ridgway osteogenic sarcoma	125	−
Mouse leukemia B82	125	±
Mouse leukemia B82	62.5	−
Mouse leukemia P815	150	+++
Mouse leukemia P815 resistant to 6-mercaptopurine	135	+
Mouse leukemia P815 resistant to 6-thioguanine	90	+
Mouse leukemia P388 resistant to 6-thioguanine	90	+

[a] The agents were administered intraperitoneally in physiological saline containing 0.5% carboxymethylcellulose for 2 weeks. −, no effect; ±, slight inhibition; +, moderate inhibition; +++, complete inhibition. Data courtesy of Dr. K. Sugiura and C. C. Stock of the Division of Experimental Chemotherapy.

The synthesis of 9-β-D-ribofuranosyl-6-hydroxylaminopurine (II) was achieved in 90% yield by treatment of 9-β-D-ribofuranosyl-6-chloropurine (I) with an excess of hydroxylamine in ethanol. Compound II was catalytically hydrogenated to adenosine (III) in 93% yield (Scheme I). Attempts to prepare II from either

SCHEME I



6-mercapto- or 6-methylmercaptopurine riboside upon interaction with hydroxylamine were unsuccessful; hypoxanthine or inosine were the reaction products.

Experimental Section[5]

9-β-D-Ribofuranosyl-6-hydroxylaminopurine (II).—9-β-D-Ribofuranosyl-6-chloropurine[6] (I, 2.83 g., 10 mmoles) was dissolved in an ethanol solution of hydroxylamine (350 ml., prepared as indicated in ref. 2), heated at 50° for 6 hr., and then kept at 25° overnight. The crystalline product which deposited was collected and washed with a little cold water and then with ethanol to yield 2.58 g. (90%), m.p. 210–212°. Upon recrystallization from methanol, colorless thin needles, m.p. 218°, $[\alpha]^{25}D$ −57° (*c* 0.5, water), were obtained.

Anal. Calcd. for $C_{10}H_{13}N_5O_5$: C, 42.40; H, 4.59; N, 24.73. Found: C, 42.19; H, 4.86; N, 24.54.

The product (II) gave positive $FeCl_3$ and phosphomolybdate tests, both indicative of the hydroxylamino function. II was recovered unchanged after 3 hr. boiling in water (10% solution) and after heating the same solution at 125° in an autoclave for 1 hr. The solubility of II was 6.9 g./l. of water at 25 ± 1°.

Ultraviolet Spectral Properties.—Compound II exhibited at pH 1.4, λ_{max} 265 mμ (ϵ 17.7 × 10^3); at pH 6.7 (phosphate buffer), λ_{max} 265 mμ (ϵ 14.3 × 10^3); at pH 12.2, λ_{max} 252 mμ,[7] shoulder at 310 mμ; at pH 1.4, λ_{min} 232 mμ (ϵ 3.31 × 10^3); at pH 6.7, λ_{min} 230 mμ (ϵ 4.65 × 10^3); and at pH 12.2, λ_{min} 230 mμ; pK_{a_1} = 3.1 ± 0.1, pK_{a_2} = 9.7 ± 0.1.[8]

Hydrogenation of II.—9-β-D-Ribofuranosyl-6-hydroxylaminopurine (II, 40.0 mg., 0.14 mmole) was dissolved in 95% aqueous ethanol (10 ml.), 5% platinum-on-charcoal catalyst (25 mg.) was added, and the suspension was hydrogenated at 1 atm. at 25°. After uptake of the theoretical volume of H_2, the suspension was filtered, the catalyst was washed with a little ethanol, and the combined filtrates were evaporated to dryness under reduced pressure. The residue was washed with 1 ml. of ethanol and a crystalline product was obtained (34.2 mg., 93%), m.p. 232–234°. The product, which no longer gave the $FeCl_3$ and phosphomolybdate tests, was identified as adenosine (III) by its mixture melting point, ultraviolet spectra at different pH values, and from R_f values in several solvent systems.

(5) Ultraviolet absorption spectra were determined with a Cary recording spectrophotometer, Model 11. Paper chromatograms were run by the ascending method on Schleicher and Schuell No. 1 paper in the following solvent systems: water saturated with 1-butanol; 1-butanol saturated with water (with or without 10% ammonia); 1-butanol–formic acid–water (77:10:13, v./v.). Melting points were taken in a Thomas–Hoover Unimelt apparatus and were corrected. The microanalysis was carried out by Spang Microanalytical Laboratory, Ann Arbor, Mich.

(6) G. B. Brown and V. S. Weliky, *J. Biol. Chem.*, **204**, 1019 (1953); supplied by Cyclo Chemical Corp., Los Angeles, Calif.

(7) The instability of II in alkali prevented precise determination of ϵ.

(8) For comparison, the corresponding values for 6-hydroxylaminopurine determined by titration are pK_{a_1} = 3.80, pK_{a_2} = 9.83, and pK_{a_3} > 12.

N-Substituted Ureidobis(1-aziridinyl)phosphine Oxides

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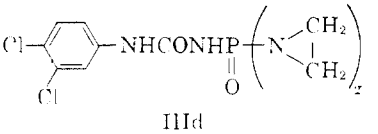
Although some N-substituted ureidobis(1-aziridinyl)-phosphine oxides, $RNHC(O)NHP(O)[NCH_2CHR']_2$ (III), have been reported recently,[1,2] neither a satisfactory method to obtain these difficultly accessible substances has existed so far, nor were the previously prepared representatives of this class sufficiently identified. It has been found that compounds of the general structure III are highly potent chemosterilants,[3] comparable in their biological activity with 2,2,4,4,6,6-hexakis(1-aziridinyl)cyclotriphospha-1,3,5-triene(Apholate) and related compounds.[4]

We have found a convenient method to obtain N-arylureidophosphoryl dichlorides, $RNHC(O)NHP(O)Cl_2$ (II), the precursors of III, by treating isocyanatophosphoryl chloride (I) *in situ* with various (especially aromatic) amines. Equimolar amounts of phosphorus pentachloride and ethyl carbamate in a small volume of ethylene chloride solvent gave I.[5] This solution was utilized directly for the addition reaction with amines without prior removal of the solvent and vacuum distillation. The distillation of I results in partial polymerization and therefore reduction in yield. The direct use of the crude I solution with various amines causes the desired intermediate II to be obtained in higher yields, based on the amount of phosphorus pentachloride employed, and avoids completely the use of the large volume of ether previously necessary for conducting the amine addition reaction.[1]

Table I compiles compounds of the general formula II which have been prepared and utilized for further reaction with aziridines to obtain compounds of structure III. Surprisingly, it has been found that the conversion of II to III can be conducted in aqueous sodium hydroxide solution at −5 to 15°. This method is a distinct advantage over the method in benzene or other anhydrous solvents using tertiary bases as hydrogen chloride acceptors, since it produces a more easily purified crude III free of phosphorus-bonded, ionizable, chlorine-containing contaminants.

The method proved to be especially useful in the synthesis of N-3,4-dichlorophenylureidobis(1-aziridinyl)phosphine oxide (IIId) which was the subject of intensive experimentation.



IIId

The aqueous sodium hydroxide method as well as the benzene–triethylamine method was also used in the preparation of the propyleniimine derivative IIIi, another powerful insect chemosterilant of low mammalian toxicity.

In Table II the N-substituted ureidobis(1-aziridinyl)-phosphine oxides of the general structure III which were prepared are given. While most of these compounds do not dissolve readily in ordinary organic solvents, dimethylformamide is suitable in some cases for recrystallization. However, sufficient recovery of large quantities of these chemicals from this solvent is difficult to achieve. Compound IIId, synthesized several times in larger amounts by applying the aqueous alkali method, was obtained in sufficient purity by

(1) A. A. Kropacheva, G. I. Derkach, L. P. Zhuravleva, N. V. Sasonov, and A. V. Kirsanov, *Zh. Obshch. Khim.*, **32**, 5, 1540 (1962).

(2) Z. P. Papanastassiou and T. J. Bardos, *J. Med. Pharm. Chem.*, **5**, 1000 (1962).

(3) Biological data was obtained by Pesticides Research Group, E. R. Squibb Co., Division of the Olin Mathieson Chemical Corp. A comprehensive publication covering these data will appear separately.

(4) R. Rätz and C. Grundmann, U. S. Patent 2,858,306 (Oct. 28, 1958); R. Rätz, E. Kober, C. Grundmann, and G. Ottmann, *Inorg. Chem.*, **3**, 757 (1964).

(5) A. V. Kirsanov and M. S. Marenets, *J. Gen. Chem. USSR*, **31**, 1496 (1961).