

Experimental Section

Isobutyl N-Chloroethylcarbamate.—Ethyleneimine (7 g) in dry chloroform (20 ml) was added during 2 hr to a stirred solution of isobutyl chloroformate (21.5 g) in chloroform (150 ml) kept at 0–2°. The solution was then warmed to 30° and kept at this temperature for 3 hr. After removing the solvent under reduced pressure at 30° the residual oil was extracted with two 250-ml portions of petroleum ether (bp 30–40°). Distillation of the material contained in this extract afforded an oil, bp 76° (0.04 mm), yield 20 g.

Anal. Calcd for $C_7H_{14}ClNO_2$: C, 46.8; H, 7.9; Cl, 19.7; N, 7.8. Found: C, 46.8; H, 7.9; Cl, 19.2; N, 7.9.

Isobutyl N-Chloroethyl-N-nitrosocarbamate.—Sodium nitrite (20 g) in water (125 ml) was added during 1 hr to a stirred solution of the above carbamate (18 g) in formic acid (125 ml) keeping the temperature below 5°. After standing for 4 hr at 5°, the solution was diluted with water (125 ml) and extracted with two 250-ml portions of petroleum ether (bp 60–80°). On concentrating and distilling the dried ($CaCl_2$) solution, an oil, bp 74° (0.02 mm), was obtained, yield 15 g. It showed strong absorption at 1400 cm^{-1} (NO).

Anal. Calcd for $C_7H_{13}ClN_2O_3$: C, 40.3; H, 6.3; Cl, 17.0; N, 13.4. Found: C, 40.4; H, 6.6; Cl, 17.3; N, 13.0.

Abolition of Immunosuppressive Activity of 6-Mercaptopurine and Thioguanine by 8-Phenyl Substitution^{1a}

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Purine derivatives are the most promising immunosuppressive agents tested to date.² Their activity has been ascribed to the purine moiety which is capable of blocking the interconversion of nucleotides, particularly from inosinic acid to adenylic acid, thus interfering with the synthesis of nucleic acids.³ Although many studies on immunosuppressive activity have been carried out with 6-mercaptopurine (6-MP) and its S-substituted derivatives, 2-amino-6-mercaptopurine (TG) seems to be the only ring substituted 6-MP reported.^{2c-e} The present investigation details the synthesis of 2-amino-6-mercapto-8-phenylpurine (8-PTG) and the experimental data on the immunosuppressive studies of 8-PTG and of 6-mercapto-8-phenylpurine (8-PMP) which we synthesized previously.⁴

2,4-Diamino-6-hydroxy-5-benzamidopyrimidine, which was prepared by benzoylation of 2,4,5-triamino-6-hydroxypyrimidine in alkaline solution,⁵ was dehydrocyclized with polyphosphoric acid⁴ to the hydroxypurine. Treatment of the hydroxypurine with phos-

phorus oxychloride in the presence of N,N-diethyl-aniline afforded 2-amino-6-chloro-8-phenylpurine. Subsequent thiation of the chloro compound with thiourea in absolute ethanol gave a nearly quantitative yield of 8-PTG.

Modification of the mercapto group of 6-MP produced 6-alkyl-, 6-aryl-, 6-puriny-, and 6-imidazolyl mercaptopurines⁶ which retained or, in some cases, showed an enhancement of the immunosuppressive activity.^{2c-e,6} The only ring-substituted compound tested, TG, was found to be as potent as 6-MP but was more toxic.^{2c,d,7} The 8-phenyl substituted 6-MP and TG in our present studies showed no immunosuppressive property as measured by the inhibition of antibody production in mice. The activity of the S-substituted 6-MP is considered to be unsheathed only after *in vivo* hydrolysis or reduction with liberation of the free mercapto group.⁸ By this hypothesis, 8-PMP and 8-PTG should retain at least some of the activity because of the intact free mercapto group. The inactivity of 8-PMP and 8-PTG seems to show that a free 8 position is also essential for immunosuppressive activity.

All the active immune suppressors are also antitumor agents even though the reverse is not always true.^{2c} This indicates a certain duality of action which is displayed by some of the chemical agents, including 6-MP and TG. Various 8-substituted purines showed some degree of inhibition toward experimental animal tumors although most of them are only moderately active.^{6,9} Our present observation of the abolition of immunosuppressive activity of 6-MP and TG by 8-phenyl substitution is noteworthy. However, both 8-PMP and 8-PTG showed moderate inhibitory activity in the KB-line cell culture, ID_{50} at 30 and 20 $\mu\text{g}/\text{ml}$, respectively.¹⁰ These details will be reported with other antineoplastic data elsewhere. Substitutions on different positions in the 6-MP nucleus would be of interest for studies on the chemical structure-biological activity relationship and the pharmacodynamics of the immunosuppressive processes. Judicious choice of substitutions could also lead to derivatives of 6-MP and other purines, some of which might be of significant biological importance.

Experimental Section¹¹

2-Amino-6-hydroxy-8-phenylpurine.—To a mixture of 3 g of dry 2,4-diamino-6-hydroxy-5-benzamidopyrimidine⁶ and 25 g of P_2O_5 , cooled to 0°, was added 18 ml of 85% H_3PO_4 . The mixture was then heated to 160–170° and stirred for 1.5 hr. By this time, the slowly dissolving amidopyrimidine had gone into solution. After cooling to room temperature, the thick syrup was poured with vigorous stirring onto crushed ice. The precipitate was allowed to stand at 4° for 18 hr and was then filtered and washed thoroughly with water and ether. The crude product was recrystallized from 2.7 l. of 2 N HCl to give 2.7 g (64%) of orange

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(11) The elemental analyses were performed by Dr. C. K. Fitz, P. O. Box 115, Needham Heights, Mass. The infrared spectra were measured in potassium bromide disks, using a Perkin-Elmer 137B spectrophotometer.

crystals: mp $>350^\circ$; $\lambda_{\text{max}}^{0.1N\text{HCl}}$ 238, 266, 305 μ (E_{max} 13,900, 10,000, 15,900); $\lambda_{\text{max}}^{0.1N\text{NaCl}}$ 226, 274 (sh), 305 μ (E_{max} 13,550, 11,100, 16,300); $\lambda_{\text{max}}^{0.1N\text{NaOH}}$ 237, 318 μ (E_{max} 18,660, 17,200).

Anal. Calcd for $\text{C}_{11}\text{H}_9\text{N}_5\text{O} \cdot 0.5\text{H}_2\text{O} \cdot \text{HCl}$: C, 48.4; H, 4.1; N, 25.7; Cl, 13.0. Found: C, 48.2; H, 4.1; N, 26.2; Cl, 12.7.

2-Amino-6-mercapto-9-phenylpurine.—To 50 ml of POCl_3 , containing 5 ml of N,N-diethylaniline, was added 2.4 g of 2-amino-6-hydroxy-8-phenylpurine, and the mixture was heated under reflux for 5 hr. The POCl_3 was then removed *in vacuo*, and the residue was poured over crushed ice with vigorous stirring. The mixture was made strongly alkaline with 10 N KOH, allowed to stand for 20 min, and then shaken with two 100-ml portions of ether to remove the diethylaniline. The aqueous solution was acidified to pH 1 with concentrated HCl and cooled to 0° . The resulting precipitate was filtered and washed with water and acetone to give 2 g of product. The product was extracted from the crude material with 200 ml of absolute ethanol in a Soxhlet extractor for 24 hr. The ethanol solution was concentrated to about 30 ml, from which 1 g of light yellow solid, mp $>350^\circ$, was obtained upon dilution with 120 ml of water. The 2-amino-6-chloro-8-phenylpurine was thiated without further purification.

To 50 ml of absolute ethanol, containing 2 g of thiourea, was added 1 g of 2-amino-6-chloro-8-phenylpurine, and the mixture was heated under reflux for 1 hr with stirring. The reaction mixture which became a clear solution at the end of that time was cooled to 4° . The crystalline product was isolated by filtration and washed with ethanol to give 1 g of yellow solid. The crude product was recrystallized from 300 ml of 1 N HCl, to give 0.9 g (91%) of yellow crystals: mp $>350^\circ$; $\lambda_{\text{max}}^{0.1N\text{HCl}}$ 258, 275 (sh), 366 μ (E_{max} 14,650, 12,300, 16,000); $\lambda_{\text{max}}^{0.1N\text{NaOH}}$ 246, 264, 349 μ (E_{max} 18,150, 17,150, 20,200).

Anal. Calcd for $\text{C}_{11}\text{H}_9\text{N}_5\text{S}$: C, 54.3; H, 3.7; N, 28.8; S, 13.2. Found: C, 54.1; H, 3.9; N, 28.4; S, 13.3.

Biological Data.—A/JAX female mice weighing 18–20 g were used. The animals in groups of nine or ten, were sensitized and tested for antibody production by a modification of the method of Nathan, *et al.*^{2c} Sheep red blood cells stored in Alsever's solution were washed three times with 0.15 M pH 7.2 buffered saline solution and a 30% cell suspension was made. Each mouse was given 0.25 ml of the suspension intravenously. Thirteen days later, the mice were bled from the ophthalmic venous plexus and the sera were pooled for each group.

6-MP and TG, in doses of 75 and 2 mg/kg, respectively, were used as standards. The dose levels for 8-PMP and 8-PTG ranged from 2 to 150 and 2 to 75 mg/kg, respectively. All four purine derivatives, both as suspensions in Tween 80 and as solutions in physiological saline achieved by the addition of minimum amounts of 0.1 N NaOH, were administered daily by intraperi-

toneal injection for the first 4 days of the sensitization period.

Before titration for antibody content, the mouse antisera were heated at 56° for 30 min to inactivate the complement. Serial twofold dilutions of antisera were made in diluted normal rabbit serum (1:100 with buffered saline). To each 0.5 ml of diluted antiserum was added 0.05 ml of washed sheep red cells (3% in buffered saline). The antigen-antibody suspension was thoroughly mixed and allowed to stand at room temperature for 1 hr and then refrigerated for 18 hr. The hemagglutination titer was determined according to the method of Stavitsky.¹² The "antibody index" was calculated as described by Nathan, *et al.*^{2c} For each experiment, a group of uninjected mice was included as an additional control. Their sera were always uniformly negative. Sheep cell injected mice not receiving the purine derivatives were the positive controls from which the antibody indices were obtained. The results are listed in Table I.

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Heterocycles Containing (8-Hydroxy-5-methyl-7-quinoly)vinyl Groups¹

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The reactions of 7-formyl-5-methyl-8-quinolinol with various N-methylated pyridines, quinolines, and related heterocycles having an active methyl group adjacent to the nitrogen yielded the compounds reported in Table I. As merocyanines, they are strongly colored and show considerable changes of color as a function of solvent as well as a tendency toward solvation. They were prepared as model compounds of the open form of some photochromic chelating agents,² but are themselves only solvatochromic chelating agents.³

As compared to a series of similar derivatives of 5-formyl-8-quinolinol,⁴ these compounds were similarly rather toxic in cell culture tests, but only one showed any activity in the routine antitumor screen; this was compound 10 of Table I against Sarcoma 180.

The results of Cancer Chemotherapy National Service Center tests are given in Table II for those compounds that were most toxic.

Experimental Section

7-Formyl-5-methyl-8-quinolinol was prepared by Fiedler's method;⁵ the appropriate heterocycles for reaction with it were N-methylated, usually with methyl iodide, by standard procedures.⁶

The general preparation of the compounds in Table I consisted in refluxing, for 4 hr, 0.01 molar quantities of 7-formyl-5-methyl-8-quinolinol with the appropriate N-methyl compound in 60 ml of absolute methanol containing 0.8 ml of piperidine. After cooling the solution, the precipitated product was filtered, washed with ether, dried, and recrystallized from methanol or ethanol.

In general, the lower the basicity of the heterocycle the higher the yield, and for compounds 4 and 5, piperidine as a catalyst was

TABLE I
EFFECT OF MERCAPTOPYRINES ON ANTIBODY PRODUCTION

Compd	Dose, mg/kg	Vehicle ^a	Toxicity ^b	Antibody index
None	...	A	1/9	0.97
None	...	A	2/10	0.80
6-MP	75	A	3/9	0.63
6-MP	75	B	3/30	0.25
6-TG	2	A	4/10	0.00
6-TG	2	B	0/10	0.05
8-PMP	2	A	1/9	1.39
8-PMP	10	A	3/9	1.31
8-PMP	75	B	3/10	0.83
8-PMP	150	B	2/10	0.83
8-PMP	150	A	1/10	0.80
8-PTG	2	A	4/10	1.45
8-PTG	10	A	1/10	0.97
8-PTG	10	B	8/9	c
8-PTG	20	A	3/10	1.12
8-PTG	25	A	2/9	0.86
8-PTG	50	B	7/9	1.00
8-PTG	75	B	8/9	c

^a A = 13% Tween 80 in physiological saline. B = About 1 ml of 0.1 N NaOH was used to dissolve the purines in 6.5 ml of physiological saline. The final pH was 8.5–9.0. ^b Number of mice dead during experiment/number of mice used. ^c Insufficient quantity of serum for measurement.

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