[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, SCHOOL OF MEDICINE, YALE UNIVERSITY]

The Synthesis and Properties of Some Selenopurines and Selenopyrimidines

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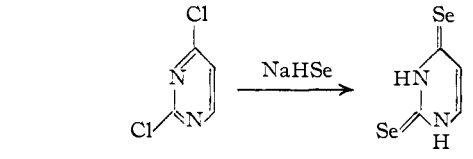
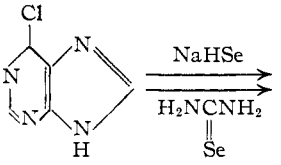
6-Selenopurine, 6-methylselenopurine, 2-selenouracil, 2,4-diselenouracil and 2-selenothymine were synthesized. The ultraviolet spectra of the above compounds and of their sulfur and oxygen analogs were measured. Acidic dissociation was found to increase from the oxygen to the sulfur to the selenium analogs. The results were found to be compatible with increasing polarization in passing from carbonyl to thiocarbonyl to selenocarbonyl bonds.

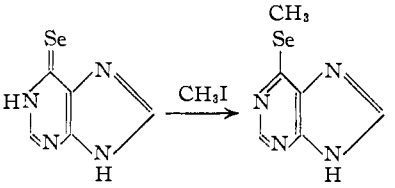
In recent years there has been considerable interest in the synthesis of sulfur analogs of naturally occurring purine and pyrimidine bases. Some of these compounds proved to be of considerable interest in the chemotherapy of cancer. For example, 6-mercaptopurine[2] and 6-methylmercaptopurine[3] were found to have powerful clinically useful antileukemic activity, while in the pyrimidine group, 2-thiouracil was found to produce transient improvement in chronic granulocytic leukemia.[4]

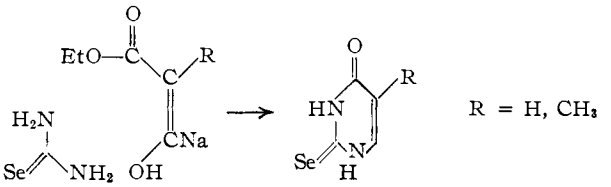
It was noted that the most useful analogs of purine and pyrimidine bases were those in which the size of the new atom or group introduced is closely similar to that of the group or atom replaced.[5] Since the radius of double-bonded selenium (1.07 Å.) is close to that of double-bonded sulfur (0.94 Å.),[6] a series of selenopyrimidines and selenopurines was synthesized. These compounds would be sterically almost identical with their sulfur analogs; however, their electron distribution would be expected to be rather different, the carbon–selenium double bond being polarized to a greater extent than the carbon–sulfur double bond.[7]

The following reaction schemes were utilized









6-Selenopurine was prepared by the addition of sodium hydroselenide to 6-chloropurine; this would be a useful method for introducing radioactive selenium into this molecule. Reaction of 6-chloropurine with selenourea produced a 92% yield of 6-selenopurine; this method was similar to the synthesis of 6-mercaptopurine used by Bendich.[5] The intermediate selenouronium salt could not be isolated. The syntheses of 2-selenouracil and 2-selenothymine resemble the syntheses of the thio compounds with selenourea being used instead of thiourea.[8,9] It should be noted that the relative lack of stability of the selenium compounds introduces certain special problems. Thus, direct light and prolonged heating in aqueous solution will cause the precipitation of colloidal selenium. The dry selenium compounds are quite stable if stored in darkness. Sulfur and selenium analogs were found to have identical crystal structures, often rather different from those of their oxygen analogs.

Ultraviolet Spectra.—It has been shown previously[7] that in a series of analogous ureides, thioureides and selenoureides, the wave lengths of maximum absorption increased in passing from carbamyl to thiocarbamyl to selenocarbamyl compounds. This was attributed to the increasing importance of the activated states $\overset{\oplus}{C}-\overset{\ominus}{B}$ (where B = O, S, Se) as the oxygen of the carbonyl group is replaced first by sulfur and then by selenium. As the energy difference between the activated state and the ground state decreases, absorption then shifts toward the visible. It is possible that the greater stabilization of the activated states of the thiocarbonyl and selenocarbonyl compounds might be attributed to the availability of more than eight orbitals in which electrons could be accommodated.

Investigation of the ultraviolet spectra of analogous pyrimidines, thiopyrimidines and selenopyrimidines, and of analogous oxypurines, thiopurines and selenopurines confirmed the wave length shifts previously observed.

Since some of the selenium compounds deposited trace amounts of selenium on standing in aqueous solution, the spectra were determined in absolute ethanol, except where otherwise indicated.

(1) Squibb Postdoctoral Fellow, 1955–1956.

(2) G. H. Hitchings and C. P. Rhodes, *Ann. N. Y. Acad. Sci.*, **60**, 183 (1954).

(3) B. E. Hall, personal communication to J. H. Burchenal, *Current Research in Cancer Chemotherapy*, Report No. 4, 15 (1956).

(4) J. Bernard, *et al.*, *Sang*, **23**, 629 (1952).

(5) A. Bendich, P. J. Russell and J. J. Fox, THIS JOURNAL, **76**, 6073 (1954).

(6) L. Pauling, "The Nature of the Chemical Bond," sec. ed., Cornell University Press, Ithaca, N. Y., 1948, p. 164.

(7) H. G. Mautner and W. D. Kumler, THIS JOURNAL, **78**, 97 (1956).

(8) H. L. Wheeler and L. M. Liddle, *Am. Chem. J.*, **40**, 547 (1908).

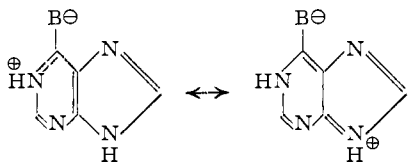
(9) H. L. Wheeler and D. F. McFarland, *ibid.*, **43**, 19 (1910).

TABLE I

Compd. (in abs. ethanol)	λmax, mμ		εmax		pK_a
Uracil	258		7,980		9.45[a]
2-Thiouracil	271		13,710		7.75[b]
2-Selenouracil	315		12,610		7.18
2,4-Dithiouracil	284	361	21,570	8,770	6.4
2,4-Diselenouracil	314	400	16,070	10,040	5.5
Thymine	264		8,640		
2-Thiothymine	275		15,360		
2-Selenothymine	314		16,260		
Hypoxanthine	249		8,100		8.94[c]
6-Mercaptopurine	330		19,180		7.77[c]
6-Selenopurine	361		14,550		7.33
	345[d]		9,440		
6-Methoxypurine	252[e]				
6-Methylmercapto-purine	289		17,820		
6-Methylseleno-purine	226 300	9440	16,900		

[a] P. A. Levene, L. W. Bass and H. S. Simms, *J. Biol. Chem.*, **70**, 229 (1926). [b] H. N. Christensen, *ibid.*, **160**, 425 (1945). [c] A. Albert and D. J. Brown, *J. Chem. Soc.*, 4935 (1954). [d] Determined in 0.02 *M* phosphate buffer (*p*H 7.0). [e] S. F. Mason, *J. Chem. Soc.*, 2071 (1954).

Methylation of hypoxanthine produced a negligible change in the wave length of maximum absorption, but methylation of 6-mercaptopurine produced a hypsochromic shift of 41 mμ, while methylation of 6-selenopurine gave rise to a hypsochromic shift of 61 mμ. Methylation would make impossible resonance favoring the activated states previously dis-



B = O, S, Se

cussed, and would produce hyspochromic shifts parallelling the importance of the activated states $\overset{\oplus}{C}-\overset{\ominus}{X}$ in the non-methylated compounds. These results are compatible with the assignment of ketonic rather than enolic structures to the compounds discussed here.

Dissociation Constants.—Greater contribution of the form $\overset{\ominus}{B}-C=\overset{\oplus}{N}H$ should make the selenium compounds more acidic than their sulfur analogs, which in turn should exceed the acidity of the oxygen compounds. The expected pK_a changes were observed.

Discussion

The results agree with the postulate that the polarizability of the carbonyl, thiocarbonyl and selenocarbonyl bonds in a series of analogs increases as one descends the periodic table.

Use of a copper-dependent polyphenol oxidase system[10] showed 6-mercaptopurine and 6-selenopurine to be powerful chelating agents, exceeding the complexing ability of hypoxanthine and comparable to that of 8-hydroxyquinoline. Biologic tests are presently in progress and will be reported elsewhere.

Acknowledgments.—We wish to express our thanks to the Squibb Institute for Medical Research for the grant of a postdoctoral fellowship and to Dr. Arnold D. Welch for his encouragement during the course of this investigation.

(10) P. B. Hagen and H. G. Mautner, unpublished results.

Experimental

2,4-Diselenouracil.—A solution of 3.2 g. (0.139 mole) of sodium shavings in 140 cc. of absolute ethanol was chilled in an ice-bath. Hydrogen selenide generated by the addition of water to aluminum selenide[11] was bubbled through the solution for 3 hours. Into the dark red liquid was placed 5.0 g. (0.0338 mole) of 2,4-dichloropyrimidine.[12] The mixture was heated to reflux for 3 hours, then 200 cc. of water was added and the solution was cooled in ice and filtered. Addition of 15 cc. of glacial acetic acid to the clear, red filtrate resulted in the separation of an orange precipitate which was washed with water, dried, and recrystallized from absolute ethanol. The product separated in the form of glittering orange-red needles melting at 208–209° dec.[13] A yield of 2.5 g. (31%) was obtained.

Anal.[14] Calcd. for $C_4H_4N_2Se_2$: C, 20.18; H, 1.69; N, 11.77. Found: C, 20.30; H, 1.85; N, 11.90.

6-Selenopurine. (a) **Using Sodium Hydroselenide.**—A solution of 0.16 g. (0.00695 mole) of sodium shavings in 15 cc. of absolute ethanol was chilled and saturated with hydrogen selenide, the whole system being kept under nitrogen. After 6 hours 0.5 g. (0.00324 mole) of 6-chloropurine (Francis Earle Laboratories) and 20 cc. of ethanol were added and the mixture was heated to reflux for 18 hours. At the end of that period 30 cc. of water was added and a small amount of black solid was removed by filtration. The filtrate was chilled and acidified with 3 cc. of acetic acid. The orange precipitate which separated was recrystallized from hot water, yielding coarse, light orange prisms melting at 280–282°. A yield of 0.54 g. (76%) was obtained.

(b) **Using Selenourea.**—A mixture of 0.8 g. (0.00518 mole) of 6-chloropurine and 0.65 g. (0.00529 mole) of selenourea in 15c c. of absolute ethanol was heated to reflux for 1 hour. The orange precipitate which had separated was filtered off and washed with water. The material weighed 1.03 g. (92%). It was dissolved in 90 cc. of warm 2% sodium carbonate solution. After cooling, the solution was acidified with acetic acid. Light orange crystals melting at 281.0–281.5° separated, the yield of recrystallized material being 0.65 g.

Anal. Calcd. for $C_5H_4N_4Se\cdot H_2O$: C, 27.66; H, 2.79; N, 25.81. Found: C, 27.85; H, 2.76; N, 25.60.

6-Methylselenopurine.—In a solution of 5.1 cc. of 0.43 *N* sodium hydroxide was placed 0.47 g. (0.00217 mole) of 6-selenopurine and 0.135 cc. (0.00217 mole) of methyl iodide was added. The mixture was stirred at room temperature for 1 hour, at which time the *p*H of the solution had dropped to 6. After the addition of 4 cc. of water the mixture was warmed and filtered to remove a small quantity of black solid. The clear, yellow filtrate was treated with acetic acid and refrigerated. After a few hours delicate, bright yellow needles began to separate; a yield of 0.3 g. (65%) of product melting at 193–194° was obtained.

Anal. Calcd. for $C_6H_6N_4Se$: C, 33.82; H, 2.84; N, 26.29. Found: C, 33.60; H, 2.70; N, 26.34.

2-Selenouracil.—A mixture of 2.5 g. of freshly prepared, crude sodium ethylformyl acetate[8] and 1.13 g. (0.00924 mole) of selenourea in 15 cc. of water was left to stand at room temperature for 3 hours and was then heated in a steam-bath for 25 minutes. The mixture was filtered to remove a small quantity of metallic selenium. The filtrate was cooled and acidified with acetic acid. A yield of 0.85 g. (53%) of pink needles separated. Recrystallization from absolute ethanol yielded 0.5 g. of delicate, faintly cream-colored needles melting at 235.5–236.0° dec.

Anal. Calcd. for $C_4H_4ON_2Se$: C, 27.44; H, 2.30; N, 16.00. Found: C, 27.92; H, 2.50; N, 16.10.

(11) G. R. Watkins and R. Shutt in W. C. Fernelius, "Inorganic Syntheses," Vol. II, McGraw-Hill Book Co., Inc., New York, N. Y., 1946, p. 184.

(12) N. Whittaker and T. S. G. Jones, *J. Chem. Soc.*, 1565 (1951).

(13) All m.p.'s are uncorrected; the m.p.'s of most of the selenium compounds depended somewhat on the rate of heating and the temperature at which the samples were added.

(14) Microanalyses were performed at the Huffman Microanalytical Laboratories, Wheatridge, Colo.

2-Selenothymine.—A solution of 2.0 g. of crude sodium ethylformyl propionate,[9] 1.62 g. (0.0132 mole) of selenourea and 0.43 g. of sodium in 30 cc. of absolute ethanol was heated to reflux for 3 hours. The solution was evaporated to dryness under aspirator suction. The dark red residue dissolved easily in 8 cc. of water. The solution was filtered and the filtrate acidified with acetic acid. After refrigeration 0.8 g. of purple solid separated, only part of which dissolved when treated with 15 cc. of boiling ethanol. Chilling the ethanol solution resulted in the separation of 0.1 g. of coarse, pale yellow needles which melted at 228.5–229.5°.

Anal. Calcd. for $C_5H_6ON_2Se$: C, 31.76; H, 3.20; N, 14.82. Found: C, 32.08; H, 3.31; N, 14.60.

2,4-Dithiouracil.—A modification[15] of the method of Wheeler and Liddle[8] was used. A solution of 1.6 g. of sodium shavings in 70 cc. of absolute ethanol was saturated with hydrogen sulfide. Then 2.5 g. (0.0169 mole) of 2,4-dichloropyrimidine was added and the mixture was permitted to reflux for 5 hours. Acidification yielded 1.2 g. (48%) of the desired product which was recrystallized from boiling water.

2-Thiouracil, 2-Thiothymine.—The syntheses of Wheeler and Liddle[8] and of Wheeler and McFarland[9] were utilized. The products were purified by recrystallization from absolute ethanol.

Ultraviolet Spectra.—A Beckman model DU spectrophotometer with quartz cells was utilized for all measurements. Solutions were made up in volumetric flasks from weighed quantities of the compounds.

Dissociation Constants.—The pK_a's were determined potentiometrically using a Beckman model G or a Cambridge *p*H meter. In 100 cc. of carbon dioxide-free water dissolved 0.0005-mole samples of the compounds investigated. They were then titrated with 0.050 *N* sodium hydroxide. To 2,4-dithiouracil and 2,4-diselenouracil, which are extremely insoluble in water, equimolar quantities of 0.050 *N* sodium hydroxide were added and the solutions back-titrated with 0.050 *N* hydrochloric acid. All determinations were made in duplicate.

NEW HAVEN, CONN.

(15) Analogous to S. B. Greenbaum and W. L. Holmes, THIS JOURNAL, **76**, 2899 (1954).

[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

Synthesis of 2-Thiocytosines and 2-Thiouracils

BY CALVERT W. WHITEHEAD AND JOHN J. TRAVERSO

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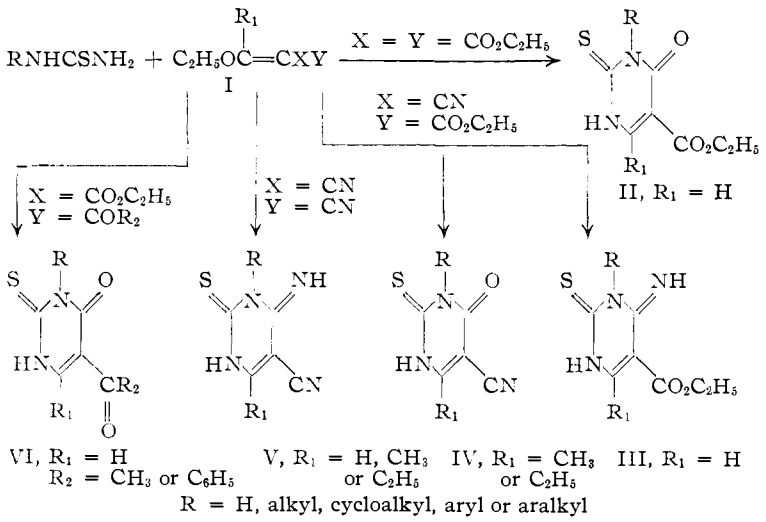
Forty-six new 2-thiouracils and 2-thiocytosines were prepared by the condensations of thioureas with ethoxymethylene derivatives of diethyl malonate, ethyl cyanoacetate, ethyl benzoylacetate, ethyl acetoacetate and malononitrile. These thiopyrimidines were then converted by known procedures to other new pyrimidines.

In previous papers[1-3] the reactions of ureas with ethoxymethylene derivatives of diethyl malonate, ethyl cyanoacetate and ethyl oxalacetate were shown to yield carbethoxypyrimidines. In this present work, ethoxymethylene derivatives of this general type were allowed to react with thioureas to yield 5-cyano-, 5-keto- and 5-carbethoxy-2-thiouracils and thiocytosines. These pyrimidines were prepared for evaluation as anticancer and antiviral agents.



Diethyl ethoxymethylenemalonate reacted with N-alkyl- and N-arylthioureas to give excellent yields of 5-carbethoxy-2-thiouracils (II). Equally good yields of 5-carbethoxy-2-thiocytosines (III) and 5-cyano-2-thiocytosines (V) were obtained from the above thioureas with ethyl ethoxymethylenecyanoacetate and ethoxymethylenemalononitrile, respectively. The 5-cyano-6-methyl-2-thiocytosines (V, R_1 = CH_3) were obtained from 1-ethoxyethylidinemalononitrile and the 5-cyano-6-ethyl-2-thiocytosines (V, R_1 = C_2H_5) from 1-ethoxypropylidinemalononitrile. Condensations of thioureas with ethoxymethyleneacetoacetate yielded 5-acyl-2-thiouracils (VI, R_2 = CH_3) and with ethyl ethoxymethylenebenzoylacetate yielded 5-benzoyl-2-thiouracils (VI, R_2 = C_6H_5). Reactions of these ethoxymethylene derivatives were found generally applicable to all the thioureas tried. Thus, preparation of thiopyrimidines having desired functional groups in the 5-position was particularly convenient. The ethoxymethylene derivatives used in these condensations were prepared by known reactions of orthoesters with appropriate active methylene compounds.

The reactions with unsymmetrical N-alkyl- and N-arylthioureas could supposedly yield 2-thiopyrimidines with the alkyl or aryl groups in either the 1-position or the 3-position on the ring. The structure of representative 2-thiouracils and 2-thiocytosines and the position of substituent groups were

(1) C. W. Whitehead, THIS JOURNAL, **74**, 4267 (1952).
(2) C. W. Whitehead, *ibid.*, **77**, 5867 (1955).
(3) R. G. Jones and C. W. Whitehead, *J. Org. Chem.*, **20**, 1342 (1955).