Fox, J. J., Cavalieri, L. F., and Chang, N. (1953), *J. Am. Chem. Soc. 75*, 4315.
Goldberg, I. H., and Rabinowitz, M. (1961), *Biochim. Biophys. Acta 54*, 202.
Hampton, A. (1961), *J. Am. Chem. Soc. 83*, 3640.
Hampton, A., and Magrath, D. I. (1957), *J. Am. Chem. Soc. 79*, 3250.
Hartung, W. H., and Simonoff, R. (1953), *Org. Reactions 7*, 263.
Kenner, G. W. (1957), *Ciba Found. Symp. Chem. Biol. Purines, 1957*, 312.
Lengyel, P., and Chambers, R. W. (1960), *J. Am. Chem. Soc. 82*, 752.
Levene, P. A., Bass, L. W., and Simms, H. S. (1926), *J. Biol. Chem. 70*, 229.
Michelson, A. M., and Cohn, W. E. (1962), *Biochemistry 1*, 490.
Paar, C. W. (1954), *Biochem. J. 56*, xxvii.
Reeves, R. E. (1950), *J. Am. Chem. Soc. 72*, 1499.
Shapiro, R., and Chambers, R. W. (1961), *J. Am. Chem. Soc. 83*, 3920.
Shugar, D., and Fox, J. J. (1952), *Biochim. Biophys. Acta 9*, 199.
Tener, G. M. (1961), *J. Am. Chem. Soc. 83*, 159.
Yu, C., and Allen, F. W. (1959), *Biochim. Biophys. Acta 32*, 393.

Pyrimidines. XII. 1-Substituted 5-Azauracils and Related Compounds*

DARRELL E. O'BRIEN, FRED BAIOCCHI, AND C. C. CHENG

From the Midwest Research Institute, Kansas City, Missouri

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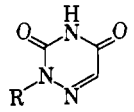
Base-catalyzed reactions of ethyl formate and 1-substituted biurets readily yielded 1-substituted 5-azauracils where the substituents were methyl, ethyl, cyclohexyl, benzyl, and furfuryl. The corresponding 5-azadithiouracils, where the substituents were methyl, cyclopentyl, cyclohexyl, and anisyl, were obtained from the reaction of thiocyanic acid and isocyanides. These compounds were synthesized in connection with the study of the relationship of 5-azauracils and 6-azauracils to the inhibition of pyrimidine biosynthesis.

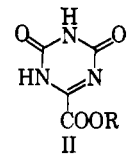
The carcinostatic activity of 6-azauridine (6-AzUR;[1] I. R = ribose) in certain types of leukemia, according to Handschumacher and co-workers (Jaffe *et al.*, 1957; Handschumacher and Pasternak, 1958; Handschumacher, 1960; Creasey and Handschumacher, 1961), is due to the blocking of the conversion of orotidylic acid



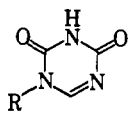
I

(OMP) to uridylic acid in certain types of human acute leukemic cells by 6-AzUR and its corresponding nucleotide, 6-azauridine-5′-monophosphate (6-AzUMP), through inhibition of OMP decarboxylase in *de novo* pyrimidine synthesis (Fig. 1). Although 6-AzUR and 6-AzUMP are potent inhibitors of the enzyme orotidylic decarboxylase, the inhibition is a competitive one because their effectiveness in blocking this conversion is, at least to a considerable degree, overcome by the accumulation of orotidylic acid in the cells.

Subsequent to our investigation on the derivatives of orotic acid (Daves *et al.*, 1961), the 5-aza analog of orotic acid (2,4-dihydroxy-5-azapyrimidine-6-carboxylic acid, or oxonic acid), isolated as its potassium salt (II. R = K, Moore and Thomas, 1918; Hartman and Fellig, 1955) as well as its decarboxylated derivative 5-azauracil (or oxadin, III. R = H, Hartman and Fellig, 1955), were prepared in our laboratories. Compound II (R = K) has now been found (Handschumacher,

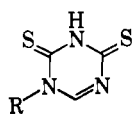


II



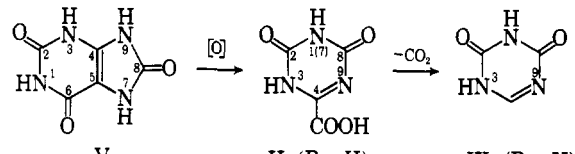
III

1962, 1963; A. D. Welch, personal communication) to interfere significantly with the conversion of orotate to orotydylic acid. Hence the oxonate inhibits the metabolic reversal of 6-AzUR (see Fig. 1), a fact which indicates that a combination of 6-AzUR (or 6-AzUMP) and oxonate can perhaps achieve significant carcinolytic effects. In order to understand the nature and effectiveness of oxonate in the role of pyrimidine biosynthesis, investigation of its closely related 1-substituted 5-azauracils and their thio analogs (IV) was therefore initiated as a logical extension of our orotic acid series.



IV

5-Azauracil (III. R = H) was obtained via 5-azaorotic acid (II. R = H) by the mild oxidation of uric acid (V). According to Brandenberger and Brandenberger (1954), the three nitrogen atoms in the triazine ring of II (R = H) originated from nitrogens 1 (or 7), 3, and 9 of V through one or more open-chain intermediates. Oxidation of 3- and 9-substituted uric acid under the



V II (R = H) III (R = H)

* The investigation was supported by a research contract (SA-43-ph-3025) from the Cancer Chemotherapy National Service Center, National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

[1] Abbreviations used in this paper: 6-AzUR, 6-azauridine; 6-AzUMP, 6-azauridine-5′-monophosphate; OMP, orotidylic acid.

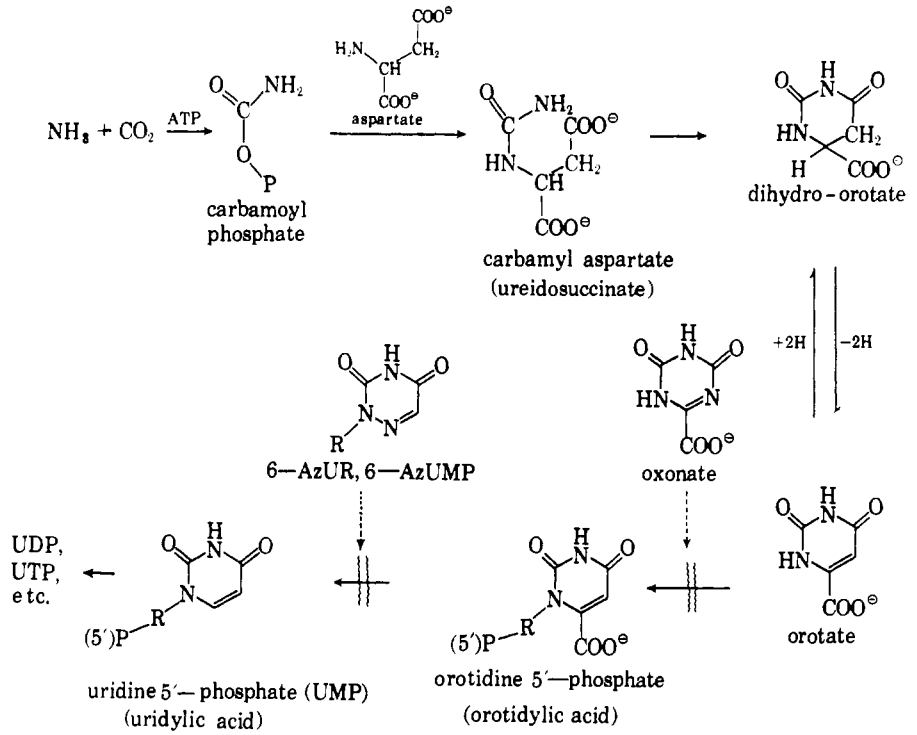
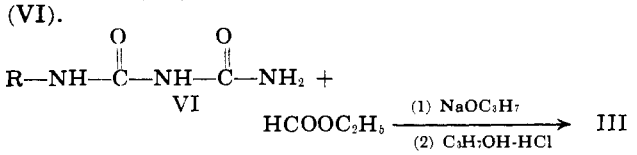


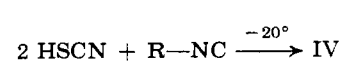
FIG. 1.—Biosynthesis of Pyrimidines

same conditions failed to yield the desired 1-substituted 5-azauracils (III). This was perhaps due to the instability of N-substituted *s*-triazines (even if formed) in aqueous base, the latter being the medium used for the oxidation of substituted uric acids.

1-Methyl-5-azauracil (III. R = CH_3) was prepared by the base-catalyzed reaction of ethyl formate and 1-methylbiuret (VI. R = CH_3) (Píškala and Gut, 1961). Because of the instability of 1-substituted 5-azauracils in aqueous base, it is important that the reaction be carried out in anhydrous conditions. It was found in our laboratory that when propanol was used as the solvent the reaction could be extended for the preparation of a number of 1-alkyl as well as 1-aralkyl 5-azauracils (III) from various 1-substituted biurets (VI).



The preparation of 1-substituted 5-azadithiouracils (IV) involves the reaction of two equivalents of thiocyanic acid and one equivalent of an isocyanide at −20° (Ugi and Rosendahl, 1961). The detailed experimental conditions have now been ascertained in our laboratory which resulted in the preparation of some 1-substituted 5-azadithiouracils (IV) for present investigation.



Although 1-substituted 5-azauracils and 1-substituted 5-azadithiouracils are structurally quite similar, their methods of preparation could not be used interchangeably; i.e., 1-substituted 5-azadithiouracils were not obtained by refluxing a mixture of ethyl formate, 1-substituted dithiobiuret, and sodium alcoholate in alcohol; cyanic acid and isocyanide under different conditions also failed to yield the desired 1-substituted 5-azauracils.

All the 1-substituted 5-azauracils possess a characteristic ultraviolet absorption maximum at *ca.* 245 mμ. The corresponding dithio analogs absorbed at 280–290 mμ.

Both the 1-substituted 5-azauracils and 1-substituted 5-azadithiouracils tend to form alcoholates rather readily with their environmental solvents. These alcoholates may be removed by drying the samples at 140° *in vacuo* for several hours.

The results of biological evaluation are presently under investigation and will be reported subsequently

EXPERIMENTAL AND RESULTS[2]

1-Furfurylbiuret.—A mixture of 19.4 g (0.2 mole) of furfurylamine and 29.6 g (0.2 mole) of nitrobiuret (Thiele and Uhlfelder, 1898) in 500 ml of water was heated at 85° for 2 hours. The resulting solution was then refluxed for 30 minutes, treated with charcoal, and filtered. On cooling, the filtrate deposited 22.6$_g$ (62% yield) of 1-furfurylbiuret, mp 151–153°. Recrystallization from water raised its melting point to 153–154°. The product showed no absorption in the ultraviolet region at 10 mg/liter (ethanol). Similar ultraviolet absorption properties for benzylbiuret (Bougault and Leboucq, 1930) were also observed in our laboratories.

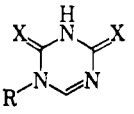
Anal. Calcd. for $C_7H_9N_3O_3$: C, 45.9; H, 5.0; N, 22.9. Found: C, 45.8; H, 5.2; N, 22.3.

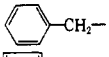
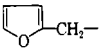
1-Substituted 5-Azauracils [1-Substituted 2,4(1 H-3 H)-dioxo-s-triazines].—To a solution of 2.80 g (0.122 g-atom) of sodium in 200 ml of propanol was added a solution of 0.122 mole of a 1-substituted biuret in 150 ml of hot propanol. The clear yellow solution was heated to reflux and 13.56 g (0.183 mole) of ethyl formate was added dropwise. The resulting mixture was refluxed with gentle stirring for 5 hours, and then was allowed to cool overnight. To the cooled mixture was added, with vigorous stirring, a freshly prepared solu-

[2] All melting points were taken on a Thomas-Hoover melting point apparatus. The ultraviolet absorption spectra were determined on the Beckman DK-2.

TABLE I

1-SUBSTITUTED 5-AZAURACILS AND 1-SUBSTITUTED 5-AZADITHIOURACILS



R	X	Formula	Yield (%)	Melting Point (°C)	Analyses Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	Ultraviolet Absorption (mμ) Ethanol λ_{max}	Ethanol log ε	pH 1 λ_{max}	pH 1 log ε
CH_3—	O[a]	$C_4H_5N_3O_2$	71	207–209	—	—	33.1	—[b]	—	33.4	243	3.28	243	3.61
C_2H_5—	O[a]	$C_5H_7N_3O_2$	72	133–135	42.5	5.0	29.8	42.2	5.4	30.0	243	3.38	244	3.68
	O[c]	$C_9H_{13}N_3O_2$	48	265–266	55.4	6.7	21.5	55.0	6.9	21.3	247	3.42	247	3.80
	O[d]	$C_{10}H_9N_3O_2$[e]	64	175–177	56.6	4.7	19.7	57.0	4.3	19.5	249	3.26	244	3.46
	O[d]	$C_8H_7N_3O_3$	42	186–187	49.7	3.7	21.7	49.8	3.9	21.4	—	—	240	3.36
CH_3—	S[f]	$C_4H_5N_3S_2$[g]	38	223–224	31.4	4.8	21.9	31.2	5.0	22.0	282	4.39	284	4.45
	S[h]	$C_8H_{11}N_3S_2$	62	190–191	45.1	5.2	19.7	45.4	5.5	19.9	248 290	4.14 4.15	276	3.32
	S	$C_9H_{13}N_3S_2$	64	202–204	47.5	5.8	18.5	47.5	5.8	18.3	248 288	4.05 4.17	251 280	4.07 4.19
CH_3—O— 	S	$C_{10}H_9N_3OS_2$[i]	56	202–203	48.3	5.1	14.1	47.9	5.0	13.8	290	4.32	282	4.39

[a] Starting material prepared from ethyl allophanate (Dains and Wertheim, 1920; Murray and Dains, 1934). [b] Prepared by Píškala and Gut (1961). [c] Starting material prepared by Bougault and Leboucq (1930). [d] Starting material prepared from nitrobiuret (Thiele and Uhlfelder, 1898; Davis and Blanchard, 1929). [e] Hemihydrate. [f] Starting material prepared by the method of Gautier (1869). [g] Mono-methanolate. [h] Starting material prepared from *N*-cyclopentyl formamide (Kost and Shvekhgeĭmer, 1950). [i] Mono-ethanolate.

tion of propanolic hydrogen chloride (containing 4.45 g [0.122 mole] of hydrogen chloride). The mixture was then refluxed for 15 minutes and filtered. Sodium chloride was washed with hot propanol and the combined washings and filtrate were evaporated to yield the desired 1-substituted 5-azauracil (see Table I). Purification was afforded by recrystallization from ethanol.

Cyclopentyl Isocyanide.—A solution of 76.3 g (0.675 mole) of *N*-cyclopentyl formamide in 340 ml of pyridine and 210 ml of heptane was treated with 62.1 g (0.405 mole) of phosphorus oxychloride by the method of Ugi and Meyr (1960). The pure cyclopentyl isocyanide, 36 g (56% yield), was collected by distillation at 48–49°/8 mm.

1-Substituted 5-Azadithiouracils [1-Substituted 2,4(1 H,3 H)-dithione-s-triazine].—The experimental conditions used were based on the information of Ugi and Rosendahl (1961): A solution of 11.82 g (0.2 mole) of thiocyanic acid in 200 ml of anhydrous ether was cooled to −20°. To the cold solution was added dropwise, with vigorous stirring, 0.1 mole of a substituted isocyanide. After the addition was complete, the solution was stirred at −20° ±2° for 2 hours; during this time the desired 1-substituted 5-azadithiouracil gradually precipitated from the reaction mixture. The product was filtered, washed with 3 × 50 ml of anhydrous ether, and recrystallized from ethanol or water (see Table I).

ACKNOWLEDGMENTS

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ADDED IN PROOF

It was found recently that 6-AzUR is not an inhibitor of OMP decarboxylase per se. It is active only after metabolic conversion to the 5′-phosphate derivative (letter from Dr. Handschumacher, October 7, 1963).

REFERENCES

Bougault, J., and Leboucq, J. (1930), *Bull. Soc. Chim. France 47* [4], 594.
Brandenberger, H., and Brandenberger, R. H. (1954), *Helv. Chim. Acta 37*, 2207.
Creasey, W. A., and Handschumacher, R. E. (1961), *J. Biol. Chem. 236*, 2058.
Dains, F. B., and Wertheim, E. (1920), *J. Am. Chem. Soc. 42*, 2303.
Daves, G. D., Baiocchi, F., Robins, R. K., and Cheng, C. C. (1961), *J. Org. Chem. 26*, 2755.
Davis, T. L., and Blanchard, K. C. (1929), *J. Am. Chem. Soc. 51*, 1801.
Gautier, A. (1869), *Ann. Chim. et Phys.* (France) *17*, [4], 215.
Handschumacher, R. E. (1960), *J. Biol. Chem. 235*, 2917.
Handschumacher, R. E. (1962), *Proc. Am. Assoc. Cancer Res. 3*, 326.
Handschumacher, R. E. (1963), *Cancer Res. 23*, 634.
Handschumacher, R. E., and Pasternak, C. A. (1958), *Biochim. Biophys. Acta 30*, 451.
Hartman, S. C., and Fellig, J. (1955), *J. Am. Chem. Soc. 77*, 1051.
Jaffe, J. J., Handschumacher, R. E., and Welch, A. D. (1957), *Yale J. Biol. Med. 30*, 168.
Kost, A. N., and Shvekhgeĭmer, G. A. (1950), *Vestn. Mosk. Univ. 5*, No. 9, *Ser. Fiz-Mat. i Estestven. Nauk*, No. 6, 51.
Moore, F. J., and Thomas, R. M. (1918), *J. Am. Chem. Soc. 40*, 1120.
Murray, J. A., and Dains, F. B. (1934), *J. Am. Chem. Soc. 56*, 144.
Píškala, A., and Gut, J. (1961), *Collection Czech. Chem. Commun. 26*, 2519.
Thiele, J., and Uhlfelder, E. (1898), *Ann. 303*, 93.
Ugi, I., and Meyr, R. (1960), *Ber. 93*, 239.
Ugi, I., and Rosendahl, K. (1961), *Angew. Chem. 73*, 656.