Antimicrobial Assay.—Pure 3′-amino-3′-deoxyinosine (II) at 200 γ/ml. in water was assayed by the streak dilution method on nutrient agar. After 20 hr. at 37° for the bacteria, and 60 hr. at 28° for the fungi, there was full growth of *Staphylococcus aureus* strains LeCompte and Valentin, *Pseudomonas aeruginosa* Ingant, *Sarcina lutea* ATCC 9341, *Escherichia, coli.* A.V. 2, *Bacillus subtilis* 5262, *B. cereus* ATCC 10702, *B. celbenina* CHP, *Aspergillus niger* 4823, *Candida albicans* Normande, *Penicillium* sp. 4847, and *Trichophyton mentagrophytes* 4806.

Pure 3′-amino-3′-deoxyadenosine 1-N-oxide (III) at 100 γ/ml. in water was assayed in the same way on no. 3 medium.[33] The bacteria were incubated 48 hr. at 37° and the fungi 72 hr. at 28°. There was no inhibition of *Sarcina lutea* 14, *Micrococcus lysodeikticus* 19, *Escherichia coli* 54, *Proteus vulgaris* 73, *Mycobacterium smegmatis* 607, *M. rhodochrous* ATCC 271, *Aspergillus niger* 13, *Penicillium notatum* 40, *Trichophyton mentagrophytes* 171, *Hensenula anomala*, and *Candida albicans* 204.

(33) Eight grams of Difco dehydrated nutrient broth, 10 g. of glucose, and 15 g. of agar per liter.

Acknowledgment.—We wish to thank Mrs. Eva M. Fekete for valuable technical assistance, Dr. H. A. Lechevalier, and Mrs. M. P. Lechevalier for testing the pure materials against microorganisms, and Miss L. H. Pugh for toxicity and antitumor studies.

Pyrimidines. II. Synthesis of 6-Fluorouracil[1]

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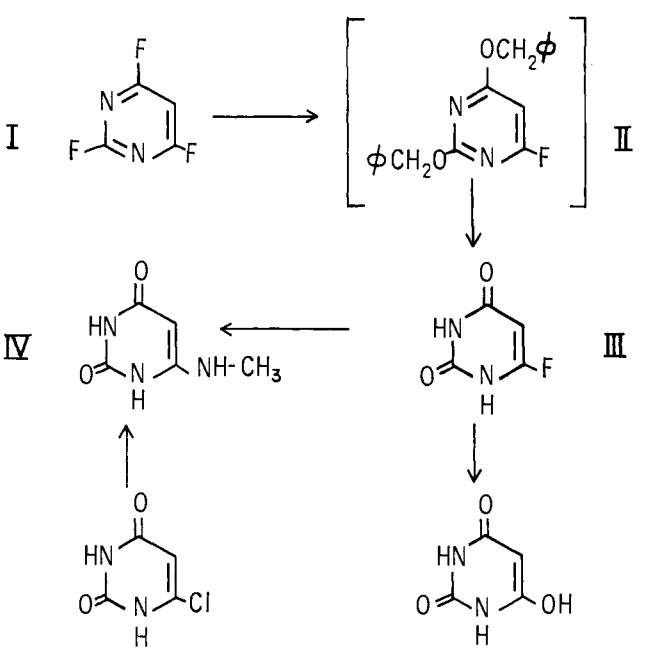
Received October 7, 1963

The synthesis of 6-fluorouracil from 2,4,6-trichloropyrimidine is described. The relative effects of a 5- and 6-halogeno atom on the ultraviolet absorption spectra and the apparent pK_a values of uracil are presented.

The pronounced chemotherapeutic activity of some 5-fluoropyrimidines and their nucleosides[3] prompted an investigation of the effect which a fluorine in the 6-position of certain pyrimidines might have on their biological activity. The synthesis of 6-fluorocytosine, 6-fluoroisocytosine, and various derivatives have been reported recently.[2] 6-Fluorocytosine was found to have only limited activity against certain fungi. However, since 5-fluorocytosine has a limited biological action[4] relative to that of 5-fluorouracil, due apparently to the lack of cytosine deaminases in many microbiological systems,[5] it was conceivable that 6-fluorouracil might be a better substrate than the cytosine analog. There is the possibility also that 6-fluorouracil (as a nucleotide) might enter the metabolic pathway and inhibit orotidylic acid decarboxylase as does 6-azauracil 5′-ribonucleotide.[6]

Previous attempts to synthesize 6-fluorouracil by the demethylation of 2,4-dimethoxy-6-fluoropyrimidine[7] or by deamination of 6-fluorocytosine[2] were unsuccessful because of the lability of 6-fluorouracil in an acidic environment. The synthesis of 6-fluorouracil has now been accomplished by the route previously suggested[8] (Chart I). Treatment of 2,4,6-trifluoropyrimidine[2,9] (I) with 2 moles of sodium benzyl oxide yielded a crude oil which was uncrystallizable. Attempts to purify the oil by fractional distillation have not been successful. The crude oil in ethanol solution with palladium on charcoal as catalyst was rapidly reduced with uptake of approximately 2 moles of hydrogen. The product (III) was isolated as an unstable white solid which always contained varying amounts of barbituric acid and usually one or two other minor contaminants. The quantity of barbituric acid was increased by attempted recrystallization. In aqueous solution, self-catalyzed acid degradation of III yielded barbituric acid. Even in the solid state, a slow decomposition occurred which could be lessened to some extent by storage under dry nitrogen. From stability studies conducted spec-

CHART I



[a] Although drawn in the carbonyl (lactam) form, the structures shown need not represent the true tautomeric state.

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service (Grant No. CA 03190-07). For the previous pertinent paper in this series, see ref. 2.

(2) I. Wempen and J. J. Fox, *J. Med. Chem.*, **6**, 688 (1963).

(3) For a brief review of some of these fluorine-containing antimetabolites, see R. E. Handschumacher and A. D. Welch in "The Nucleic Acids," Vol. III, E. Chargaff and J. N. Davidson, Ed., Academic Press, Inc., New York, N. Y., 1960, p. 498.

(4) J. Malbica, L. Sello, B. Tabenkin, J. Berger, E. Grunberg, J. H. Burchenal, J. J. Fox, I. Wempen, T. Gabriel, and R. Duschinsky, *Federation Proc.*, **21**, 384 (1962).

(5) J. Kream and E. Chargaff, *J. Am. Chem. Soc.*, **74**, 5137 (1952).

(6) See ref. 3, p. 514.

(7) J. P. Horwitz and A. J. Tomson, *J. Org. Chem.*, **26**, 3392 (1961).

(8) See ref. 2, footnote 19.

(9) H. Schroeder, E. Kober, H. Ulrich, R. Rätz, H. Agahigian, and C. Grundmann, *J. Org. Chem.*, **27**, 2580 (1962).

TABLE I
THE EFFECT OF HALOGEN SUBSTITUTION ON THE "APPARENT" pK_{a_1} VALUES[a]

	pK_{a_1}	ΔpK_{a_1}
Uracil	9.50[b]	
5-Fluoro-	7.98[c]	−1.52
5-Chloro-	7.95[d]	−1.55
5-Bromo-	8.05[d]	−1.45
5-Iodo-	8.25[d]	−1.25
6-Fluoro- (III)	4.03[e]	−5.47
6-Chloro-	5.67	−3.83

[a] All values were determined spectrophotometrically and are accurate to ±0.05 pH units unless otherwise noted. [b] Taken from ref. 17. [c] Berens and Shugar[16] give 8.00. [d] Value taken from ref. 16. [e] This value accurate to ±0.10 pH unit.

trophotometrically, it was found that compound III appeared to be stable in basic solution. Therefore, the potassium salt was prepared by titration of III in methanol with methanolic potassium hydroxide. The resulting white solid on recrystallization was obtained as prisms which, after drying, gave the correct analysis[10] for the potassium salt of 6-fluorouracil.[11] The recrystallized salt appears to be free of contaminants as demonstrated by thin layer chromatography and by paper ionophoresis. It is also stable on storage in the dry state.

In order to establish that the fluorine atom in compound III is located on position 6 (4) of the pyrimidine ring and not on 2, III was converted to a derivative which could also be derived from 6-chlorouracil.[12] Compound III was converted to 4-aminobarbituric acid[13] by heating with alcoholic ammonia. In contrast, 6-chlorouracil was found to be stable to ammonia even after prolonged treatment at an elevated temperature. Likewise, 6-chlorouracil, on brief treatment with hot dilute hydrochloric acid was practically not affected, whereas III under the same conditions was converted completely to barbituric acid. This lack of reactivity of 6-chlorouracil confirms a previous observation[14] on the stability of this compound to nucleophilic reagents. Replacement of the 6-chlorine atom was finally accomplished by use of a stronger nucleophile, methylamine, to yield the known 6-methylaminouracil[15] (IV). The same derivative (IV) was obtained by treatment of III with methylamine; the identity of the two derivatives was demonstrated by thin layer chromatography, paper ionophoresis, and ultraviolet and infrared absorption data to prove the structure of III as 6-fluorouracil.

Ultraviolet Absorption Spectra and Dissociation Constants.—The spectral pattern exhibited by III was similar to that demonstrated by 6-chlorouracil, but with an over-all hypsochromic shift of *ca.* 15 mμ. It is interesting to note that the spectral patterns of these two 6-halogenouracils are dissimilar to those exhibited by the 5-halogenouracils[16] or to that of uracil[17] itself. This dissimilarity is analogous to that already demonstrated[2] for the 6-fluoro- and 6-chlorocytosines and their respective 5-halogenocytosines.[17a] Similarly, comparison of the extinction coefficients reveals that the 6-halogenouracils have considerably higher ϵ_{max} values, particularly for the monoanionic species than either of their 5-halogeno analogs.

As expected, the spectral pattern of the 6-methylaminouracil (IV) very closely resembled that of 4-aminobarbituric acid except for a slight over-all bathochromic shift of the maxima due to the presence of the methyl group on the exocyclic amino group.[18]

The pK_{a_1} value for III of 4.03 is less than the respective value for 6-chlorouracil, 5.67 (see Table I). This increased acidity is attributed to the greater electronegativity of a fluorine *vs.* a chlorine atom with the resultant decrease in negativity of the proton accepting center(s) of the pyrimidine ring. Thus, 6-fluorouracil is a stronger acid than benzoic acid (pK_a 4.20) and in fact, is comparable in acidity to barbituric acid (pK_{a_1} 3.9)[19] itself.[20]

As can be seen from Table I, the presence of halogen atom in position 6 has a greater acid strengthening effect on the first dissociation constant (monoanion formation) of the unsubstituted uracil than it has in position 5. A similar relationship was observed[2] when the acidic dissociations of the 6- and 5-halogenocytosines were compared to that of cytosine. The pK_{a_2} value of 6-fluorouracil is *ca.* 13, while that for 6-chlorouracil is slightly less than 13, which indicates that formation of the dianion occurs with somewhat greater facility in 6-chlorouracil.

Microbiological Studies.[21]—A sample of the potassium salt of 6-fluorouracil was tested against 14 microorganisms (yeasts, molds, Gram-positive, and Gram-negative bacteria) by the agar cup-plate diffusion method at a concentration of 0.1 mg. and 0.01 mg./ml. in water and simultaneously compared with a 0.1 mg./ml. solution of 5-fluorouracil. Whereas 5-fluorouracil gave large inhibition zones against at least nine microorganisms, 6-fluorouracil was completely inactive at both concentrations tested against all of the test organisms.

Experimental[22]

6-Fluorouracil (6-Fluoro-2,4-pyrimidinedione) (III).—Sodium (4.60 g., 0.20 g.-atom) was converted to "sand" in 1500 ml. of boiling toluene and was treated slowly with 30 g. (0.28 mole) of benzyl alcohol. After all the sodium had reacted, the suspension was

(10) The authors wish to thank Dr. Al Steyermark of Hoffmann-La Roche, Inc., Nutley, N. J. for the microanalytical results on this compound.

(11) Attempts to regenerate pure 6-fluorouracil from the potassium salt were unsuccessful due to the instability of the free base at the low pH required for complete conversion.

(12) This compound was prepared by hydrolysis of 6-chloro-2,4-dimethoxypyrimidine as described in ref. 7.

(13) No attempt was made to isolate this product. Identity with authentic 4-aminobarbituric acid was established by spectrophotometric methods and purity by chromatographic means.

(14) H. C. Koppel, R. H. Springer, R. K. Robins, and C. C. Cheng, *J. Org. Chem.*, **26**, 792 (1961).

(15) F. E. King and T. J. King, *J. Chem. Soc.*, 726 (1947).

(16) K. Berens and D. Shugar, *Acta Biochim. Polonica*, **10**, 25 (1963).

(17) D. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, **9**, 199 (1952).

(17a) NOTE ADDED IN PROOF.—A comparative study of the spectra of 6- and 5-halogenouracils and -cytosines will appear in a forthcoming paper (I. Wempen and J. J. Fox, in press).

(18) D. J. Brown, E. Hoerger, and S. F. Mason, *J. Chem. Soc.*, 4035 (1955).

(19) J. J. Fox and D. Shugar, *Bull. Soc. Chim. Belges*, **61**, 44 (1952).

(20) Due to this similarity of pK_a values, separation of III from any contaminating barbituric acid could not be accomplished by the usual ion-exchange techniques; separation was achieved through the differential solubility of the potassium salts in aqueous methanol.

(21) The authors are indebted to Dr. Louis Kaplan of the Sloan-Kettering Institute and to Dr. Julius Berger of Hoffmann-La Roche, Inc., Nutley, N. J. for these results.

(22) All melting points were taken on a Thomas-Hoover capillary melting point apparatus and are corrected. Microanalyses were performed by the Galbraith Laboratories, Inc., Knoxville, Tennessee, and by Spang Microanalytical Laboratory, Ann Arbor, Michigan except where noted.

cooled, diluted with an additional 500 ml. of dry toluene, and added slowly to a solution of 13.4 g. (0.10 mole) of 2,4,6-trifluoropyrimidine; the temperature was kept at 10–15° during the addition. The suspension thinned as the reaction progressed, and when the addition was complete, the reaction mixture was allowed to stand overnight at room temperature. The gel-like precipitate was collected on a Celite pad on a large Büchner funnel and the clear filtrate evaporated *in vacuo* to an oil. The oil was poured into a large volume of heptane and any solid that formed was filtered and discarded. The heptane filtrate was evaporated *in vacuo* to an oil which was used directly for hydrogenation.

A. Potassium Salt of III.—An aliquot (1 g.) of crude oil in ethanol solution was reduced at atmospheric pressure with palladium on charcoal as a catalyst. After 2 moles of hydrogen was consumed, no further uptake occurred. The catalyst was removed by filtration through a Celite pad and the resultant colorless filtrate evaporated as rapidly as possible (<40°) to incipient precipitation. Any precipitate which formed was redissolved by addition of a minimal amount of methanol. To the well stirred solution was added slowly a 0.2 *N* methanolic solution of potassium hydroxide to pH of *ca.* 8.5–9. A white precipitate formed during this operation. The mixture was evaporated *in vacuo* almost to dryness and the residue crystallized from a minimum amount of hot 90% methanol. The slowly cooled solution deposited a crystalline precipitate which appeared as highly iridescent prisms under a polarizing microscope. The product exhibited a single spot when examined by paper ionophoresis and thin layer chromatography. The yield of potassium salt (monohydrate) was 330 mg., m.p. >300°. The water of hydration was removed by drying for 2 hr. at 100° *in vacuo*. Ultraviolet absorption properties: in *N* HCl (neutral species), maximum at 248 mμ, ϵ_{max} 8600; minimum at 224 mμ, ϵ_{min} 2330; at pH 6.5 (monoanionic species), maximum at 267 mμ, ϵ_{max} 13,070; minimum at 238 mμ, ϵ_{min} 1620; in *N* NaOH (dianionic species), maximum at 258 mμ, ϵ_{max} 7880; minimum at 239 mμ, ϵ_{min} 3325; spectrophotometrically determined pK_a values: pK_{a_1} = 4.03 ± 0.1 and pK_{a_2} = ~13.

Anal.[10] Calcd. for $C_4H_2FKN_2O_2$: C, 28.57; H, 1.20; F, 11.30; K, 23.25; N, 16.66. Found: C, 28.52; H, 1.55; F, 11.20; K, 23.09; N, 16.67.

B. Free Base.—Reduction of a 1-g. aliquot of II was carried out as outlined in part A, the catalyst removed, and the ethanol evaporated rapidly to dryness. The residue was triturated thoroughly with dry ether and filtered, then dried *in vacuo* and stored under dry nitrogen; yield, 260 mg. (62%), m.p. 245° dec. Paper ionophoresis showed the presence of three trace contaminants, one of which was barbituric acid. Attempted recrystallization of the crude product from various solvents led to increased amounts of degradation products. Analysis of the crude free base, however, revealed that the amount of impurities was quite small.

Anal. Calcd. for $C_4H_3FN_2O_2$: C, 36.93; H, 2.32; F, 14.61; N, 21.54. Found: C, 36.89; H, 2.70; F, 14.39; N, 21.45.

6-Methylamino-2,4-pyrimidinedione (6-Methylaminouracil) (IV). A. From 6-Fluorouracil (Free Base).—A solution of 100 mg. of III in 5 ml. of an aqueous solution of methylamine (40%) was refluxed for 20 min. At the end of this time, an aliquot of the solution, acidified to pH 1, showed a bathochromic shift from 248 mμ for starting material to 266 mμ. The solution was evaporated *in vacuo* and reconcentrated with ethanol several times until all of the amine had been removed. The residue was triturated thoroughly with water and finally given a brief wash with alcohol, then ether, yielding 70 mg. (65%), m.p. *ca.* 310° (efferv.) (lit.[15] m.p. 302° dec.). Paper ionophoresis of the crude product showed only a trace amount of barbituric acid as the sole impurity.

B. From 6-Chlorouracil.—A solution of 340 mg. of 6-chlorouracil[12] was refluxed with 40% aqueous methylamine for 3.5 hr. until an aliquot at pH 14 showed a shift from 275 to 266 mμ. The product was isolated as in part A; yield 230 mg. (59%), m.p. *ca.* 310° (efferv.). This product appeared identical with that obtained in part A when compared by paper ionophoresis, thin layer chromatography, and ultraviolet and infrared absorption data.

4-Aminobarbituric Acid. A. From 6-Fluorouracil.—A solution of 100 mg. of III in ethanol, previously saturated with ammonia at 0°, was heated in a sealed tube at 145° for 7 hr. The cooled tube was opened and the contents evaporated *in vacuo* to dryness. Ultraviolet absorption spectra of the residue (which contained inorganic salts) was identical with that of 4-aminobarbituric acid. Chromatography showed only one spot of the same R_f as an authentic sample of 4-aminobarbituric acid.

B. From 6-Chlorouracil.—An aliquot of 6-chlorouracil was subjected to a treatment identical with that described in A. Both ultraviolet spectra and chromatographic examination revealed that only a very small amount of 4-aminobarbituric acid had been formed.

Acid Degradation of 6-Fluorouracil. A. Isolation of Barbituric Acid.—An aliquot of the potassium salt of 6-fluorouracil (86 mg. monohydrate) was treated with *N* HCl at 100° for 0.5 hr. The solution was evaporated *in vacuo* and the residue reconcentrated with ethanol several times, filtered, and dried; yield, 41 mg. (69%), m.p. 245–248° (efferv.). A mixture melting point with an authentic sample of barbituric acid showed no depression. The identity of this product with barbituric acid was confirmed when compared by paper ionophoresis, thin layer chromatography, and by spectrophotometric methods.

B. Stability of Aqueous Solutions.—A series of very dilute solutions of 6-fluorouracil at varying pH values were prepared for spectrophotometric studies. The optical density (248 mμ) was read at zero time, repeated at 2.5 hr., and again in 24 hr. After 2.5 hr., the extinction coefficient of the compound at pH 1 had fallen 6% and after 24 hr. had dropped 23%. Paper ionophoresis showed barbituric acid to be the product of degradation. In contrast, the neutral (pH 6.5) or even basic (pH 14) solutions showed no appreciable change in values.

Spectrophotometric Studies.—Ultraviolet absorption data were determined with a Cary Model 15 recording spectrophotometer, and a Beckman Model DU spectrophotometer using buffers and techniques previously described.[17] The apparent pK_a values were determined spectrophotometrically by known methods.[17,19]

Chromatography.—The thin layer chromatograms were carried out at room temperature on glass coated with Silica Gel G prepared according to Stahl[23] and air-dried. The chromatograms were developed in butanol–water (86:14). The dried plates were placed in an iodine chamber and the pyrimidines visualized as brown spots.

Electrophoretic Experiments.—All studies were made with an E. C. electrophoresis apparatus.[24] Whatmann 3MM paper was employed in 0.1 *M* sodium borate solution, pH 9.2, 800 v., 2 hr. After the paper was dried, the pyrimidines were visualized under ultraviolet light.

Acknowledgment.—The authors wish to thank Dr. George Bosworth Brown for his warm and continued interest.

(23) E. Stahl, *Chemiker-Ztg.*, **82**, 323 (1958).

(24) Manufactured by E. C. Apparatus Co., Swarthmore, Pa.