TABLE II (*Continued*)

Compound No.	Test system	Dose (mg./kg.)	Survivors	Animal wt. diff. (T − C)	Tumor wt. (mg.) or survival (days) Test	Control	Slope	ED_{50}[b]	% (T/C)
19	SA-180	500.0	0/6						
		125.0	6/6	−1.4	620	1395			44
		125.0	4/6	2.1	925	1372			67
	CA-755	100.0	8/10	−4.9	0	1423			0
	LE-1210	100.0	6/6	−3.7	13.0	9.3			139
20	SA-180	125.0	6/6	−1.5	258	1227			21
		125.0	2/7	2.2	600	600			
		125.0	0/6						
		125.0	0/6						
		93.0	6/6	−0.3	194	1372			14
		93.0	0/6						
		93.0	3/6	−2.7	285	1097			
		500.0	0/6						
		125.0	5/6	−6.2	400	1104			36
		125.0	4/6	−6.4	258	874			29
		125.0	6/6	1.5	258	1227			21
	KB	w.						L 1.0×10^0	
		w.					−1.29	3.0×10^{-1}	
21	SA-180	500.0	6/6	−2.5	355	1467			24
		500.0	3/6	−5.8	263	1104			
		500.0	2/6	−5.1	355	874			
		250.0	5/6	−2.0	716	1227			58
		250.0	5/7	0.1	542	600			90
	CA-755	200.0	5/10	−3.5	0	1586			
		100.0	7/10	−6.5	0	1345			0
	LE-1210	200.0	6/6	−2.7	10.7	9.3			115
	KB	w.						L 1.0×10^0	
		w.					−0.74	6.6×10^{-1}	

[a] Slope: change of response for each one-log change of dose. [b] ED_{50}: the dose that inhibits growth to 50% of control growth. For materials tested by weight (w. in dose column), ED_{50} is expressed in μg./ml.: L = less than; M = more than.

Jr., and members of the biochemistry committee of the Cancer Chemotherapy National Service Center for their helpful information. They are also indebted to Mrs. Margaret L. Rounds, Mr. John R. Gravatt, and Mr. Hal P. Van Fossen of our Institute for their valuable assistance in performing the analytical measurements.

Aminonucleosides. II.[1] 3′-Amino-3′-deoxyinosine and 3′-Amino-3′-deoxyadenosine 1-N-Oxide

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3′-Amino-3′-deoxyadenosine (I) from *Helminthosporium* sp. has been converted directly to 3′-amino-3′-deoxyinosine (II) enzymatically and has been oxidized to 3′-amino-3′-deoxyadenosine 1-N-oxide (III).

Recently we identified an antitumor agent from *Helminthosporium* sp. No. 215 as 3′-amino-3′-deoxyadenosine (I).[2] At least nine other "unusual" purine nucleosides have been obtained from natural sources: Angustmycin A,[3] nucleocidin,[4] cordycepin,[5] nebularin,[6] psicofuranine,[7] crotonoside,[8] puromycin,[9] the aminonucleoside[10] from puromycin and homocitrullylaminoadenosine.[11] Of the nine, seven (Angustmycin A, puromycin, purmomycin aminonucleoside, cordycepin, nebularine, crotonoside, and psicofuranine) have antitumor properties, six at nontoxic dose levels; for the others (nucleocidin and homocitrullylaminoadenosine), no data have been published.[12]

Undoubtedly for this reason, interest in chemically

(1) The U. S. Public Health Service (CA 6671) supported this investigation. It was presented in part before the 142nd National Meeting of the American Chemical Society, Atlantic City, N. J., Sept., 1962, p. 54Q.

(2) Part I in this series: N. N. Gerber and H. A. Lechevalier, *J. Org. Chem.*, **27**, 1731 (1962).

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(5) H. R. Bentley, K. G. Cunningham, and F. S. Spring, *J. Chem. Soc.*, 2301 (1951).

(6) G. B. Brown, M. P. Gordon, D. I. Magrath, and A. Hampton, "The Chemistry and Biology of Purines," G. E. W. Wolstenholme and C. M. O'Conner, Ed., Little, Brown and Company, Boston, Mass., 1957, p. 192; K. Isono and S. Suzuki, *J. Antibiotics* (Tokyo), **Ser. A**, **13**, 270 (1960).

(7) W. Schroeder and H. Hoeksema, *J. Am. Chem. Soc.*, **81**, 1767 (1959).

(8) J. Davoll, *ibid.*, **73**, 3174 (1951).

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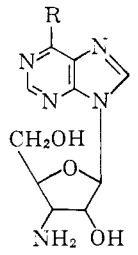
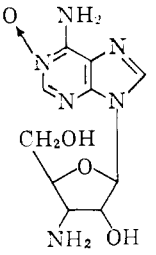
(10) B. R. Baker, J. P. Joseph, and J. H. Williams, *ibid.*, **77**, 1 (1955).

(11) N. M. Kredich and A. J. Guarino, *J. Biol. Chem.*, **236**, 3300 (1961).

(12) See references in L. H. Pugh and N. N. Gerber, *Cancer Res.*, **23**, 640 (1963).



I, R = -NH_2
II, R = -OH



III

prepared nucleosides has remained high,[13] in spite of the difficulties attending their synthesis. Syntheses of 3′-amino-3′-deoxypurine nucleosides have not been extensively published,[13,14] in spite of the conclusion of some authors that the 3-amino-3-deoxyribose moiety was essential for carcinostatic activity.[15] Probably this lack is due to the lengthy chemical synthesis of the required 3-amino-3-deoxyribose derivative.[16]

By a direct diazotization of I with excess nitrous acid in dilute mineral acid at 5 or 28° we were able to obtain small amounts of 3′-amino-3′-deoxyinosine (II); the main portion of I was recovered unchanged. Although II was obtained in low yields it was easily identified by its position on paper chromatograms. The structure of II was indicated by its ultraviolet absorption maximum at 250 mμ, similar to that of hypoxanthine, and its acid hydrolysis to hypoxanthine which was identified by paper chromatographic comparisons with authentic material in several solvent systems. Furthermore II was chromatographically identical with the enzymatically prepared crystalline II described below.

The stable, commercially available enzyme, takadiastase, contains a nonspecific adenosine deaminase; its purification from this source has been described.[17] However, we found purification unnecessary; I incubated in buffer with takadiastase was converted to II in high yield at a rate comparable to that of adenosine to inosine. The aminoinosine is moderately soluble in water, forms salts with both acids and bases, and may exist as an internal salt.

For the preparation of 3′-amino-3′-deoxyadenosine 1-N-oxide (III), I was oxidized with hydrogen peroxide in acetic acid at room temperature. The N-oxide, obtained in 30% yield, was very soluble in water. The structure of III was indicated by the similarity of its ultraviolet spectrum to that of adenosine 1-N-oxide[18] and was proved by analysis and acid hydrolysis to adenine 1-N-oxide and 3-amino-3-deoxyribose, which were identified by paper chromatographic comparisons with authentic materials in several solvent systems. The purification procedure, originally developed using paper chromatography, showed the presence of adenine, adenine 1-N-oxide, and two substituted adenine N-oxides as by-products as well as unchanged starting material. A 36-hr. reaction period was found to be optimum. Longer times increased the amounts and varieties of by-products without changing the conversion of I to III.

In streak dilution assay, II at 200 γ/ml., and III at 100 γ/ml. were inactive against a variety of bacteria and fungi.[19] The intraperitoneal acute toxicity of II in mice was greater than 400 mg./kg.; against the S_3A ascitic adenocarcinoma of mice it was inactive at 260 mg./kg. For the intravenous route the LD_{50} of III was 50 mg./kg. The N-oxide (III) was inactive against the ascitic adenocarcinoma when administered intraperitoneally at 12.8 mg./kg.[20]

Experimental[21]

3′-Amino-3′-deoxyadenosine (I).—This aminonucleoside was produced as before[2] with the following modifications. The *Helminthopsorium* sp. was maintained by biweekly transfer on potato dextrose agar[22] and stored at 28°. The medium for growth of inoculum consisted of 1% BYF no. 50[23] and 1% Cerelose; the production medium was 1% BYF no. 50, 1% Cerelose, and 0.1% ammonium chloride (pH 6.5), plus 0.25% calcium carbonate. After suction filtration the mycelium was discarded; the yield of I from the broth varied in 4 batches from 82–94 γ/ml. (average 88 γ/ml.) by paper chromatographic assay.[2]

The broth (10–20 l.) was neutralized to pH 7.0 with 10% hydrochloric acid (about 5 ml./l.) and then was applied at a rate of 1–1.5 l./hr. to a 500-ml. volume (measured in the H^+ form) IRC-50 ion-exchange resin column (about 7.5 × 16 cm.). The column had been converted previously to the hydrogen form, washed, then buffered with 2 l. of *N* sodium acetate solution. The column was washed with water (4 l.) and eluted with 2.8% aqueous ammonia (4 l.). The ammonia eluate was taken to dryness in a flash evaporator, and the residue dissolved in 10% acetic acid (150 ml.). This solution was filtered by gravity and made basic with ammonium hydroxide; a tan crystalline product was obtained after several days at 5° (50–70% recovery).

Purified I[2] melted at 271–273°; the mixture melting point with chemically synthesized I[14,24] was undepressed.

3′-Amino-3′-deoxyinosine (II). A. By Diazotization.—I (100 mg.) was dissolved in 10 ml. of 0.180 *M* hydrochloric acid (5 equiv.) at 0°, and 31 mg. of sodium nitrite (1.2 equiv.) in 1.2 ml. of water at 0° was added rapidly. After 2 hr. at 5° and 24 hr. at 28°, the reaction mixture was made basic with ammonium hydroxide and left 48 hr. at 5°. Half of the starting material precipitated unchanged; the filtrate was examined by paper chromatography in solvent B.[21] It contained, by comparison with authentic materials, adenine (about 30%), a trace of hypoxanthine, more unchanged starting material, no inosine or adenosine, and a spot b, R_{In} = 0.43.[25] Paper chromatography of a larger portion gave b as a band which was eluted with water

(13) See for example, B. R. Baker, J. P. Joseph, and R. E. Schaub (to American Cyanamid Co.), U. S. Patent 2,852,505 (Sept. 16, 1958); *Chem. Abstr.*, **53**, 8175*d* (1959); J. J. Fox, *Record Chem. Progr.*, **19**, 173 (1958); E. J. Reist, A. Benitez, W. W. Lee, B. R. Baker, and L. Goodman, *J. Org. Chem.*, **27**, 3279 (1962), and references in H. E. Skipper, J. A. Montgomery, J. R. Thompson, and F. M. Schabel, Jr., *Cancer Res.*, **19**, 425 (1959).

(14) B. R. Baker, R. E. Schaub, and H. M. Kissman, *J. Am. Chem. Soc.*, **77**, 5911 (1955).

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(17) N. O. Kaplan, "Methods in Enzymology," Vol. II, S. P. Colowick and N. O. Kaplan, Ed., Academic Press, Inc., New York, N. Y., 1955, p. 474.

(18) M. A. Stevens and G. B. Brown, *J. Am. Chem. Soc.*, **80**, 2759 (1958).

(19) H. A. Lechevalier and M. P. Lechevalier, private communications.

(20) L. H. Pugh, unpublished.

(21) All melting points were determined using the Kofler micro hot stage. All paper chromatography was carried out using the descending method with Whatman No. 1 paper which had been previously washed by descending chromatography with 2.8% aqueous ammonia and dried. Usually 20–50 γ of material was applied at the starting line, and after development the spots were located on a fluoroscopic papergram scanner similar to that described in R. J. Block, E. L. Durrum, and G. Zweig, "Paper Chromatography and Paper Electrophoresis," 2nd Ed., Academic Press, Inc., New York, N. Y., 1958, p. 66. The solvent systems employed were: A, 2.8% aqueous ammonia; B, water-saturated butanol; C, butanol–acetic acid–water (4:1:5 upper layer); D, 5% aqueous disodium hydrogen phosphate. Papers for solvent B were fumed with ammonia after the spots had been applied.

(22) The infusion from 300 g. of peeled potatoes, 20 g. of glucose, and 15 g. of agar per liter.

(23) A water soluble fraction of autolyzed brewers yeast sold by Amber Laboratories, Inc., 3456 N. Buffum St., Milwaukee 12, Wis.

(24) Kindly furnished by Dr. M. J. Weiss, Lederle Division, American Cyanamid Co.

(25) R_{In} is the distance traveled relative to inosine.

overnight.[26] The b solution had λ_{max} at 250 mμ. Hydrolysis of the b solution at 100° for 1 hr. with 0.1 of its volume of concentrated hydrochloric acid produced hypoxanthine identified by paper chromatographic comparison with authentic material in solvents B and C. The yield of b was 3–4% calculated from the ultraviolet absorption spectrum, and it was identical in paper chromatographic behavior with the authentic crystalline II prepared by method B.

In a second experiment 10 equiv. of 0.18 *M* hydrochloric acid, 1 equiv. of sodium bromide, and 5 equiv. of sodium nitrite were used, and the reaction mixture was stored at 5° in an amber bottle. The coupling test for diazonium salt with resorcinol was strong after 1 and 3 hr., weak after 5 hr., and negative after 23 hr. Other portions of the reaction mixture were removed and brought to pH 8 with 2.8% aqueous ammonia after 1, 3, and 23 hr. The supernatant solutions, examined chromatographically in solvent B, contained starting material and b, but no adenine, hypoxanthine, or inosine. The yields of b at 1, 3, and 23 hr. were 1.5, 2.8, and 1.8%, respectively.

B. By Enzymatic Deamination.—To 500 ml. of sterile 0.05 *M* phosphate buffer (pH 6.9) was added 2.5 g. of takadiastase (local drug store), 500 mg. of I (tan, as obtained, without additional purification), and 50 ml. of toluene. After 11 days at 28°[27] the reaction mixture was diluted to 1 l., separated from toluene, and applied to a 50-ml. volume buffered IRC-50 ion-exchange resin column at the rate of 250 ml./1.25 hr. The column was washed with 1 l. of water, then after standing overnight was eluted at the same slow rate with 2.8% aqueous ammonia. All of the ultraviolet absorbing material was found in the first 250-ml. fraction (by calculation from the optical density, the yield was 390 mg., 78%). This fraction was reduced to 50 ml. in the flash evaporator, neutralized to pH 7 with 2 *N* sulfuric acid (about 20 drops), and then reduced to 10 ml. in a vacuum oven. After cooling overnight the mixture was centrifuged, and the supernatant solution was removed to produce 375 mg. (75% yield) of faintly tan II, m.p. 260–263° after drying. Recrystallization from water–ethanol raised the m.p. to 261–264°; the mixture melting point with chemically synthesized II[24,28] was undepressed, λ_{max} 249 mμ ($E^{1\%}_{1cm}$ 475), $\lambda^{0.1\ N\ HCl}_{max}$ 253 mμ, $\lambda^{N_2O}_{max}$ 2.95 (OH), 3.25, 3.75, 6.05, 6.34, 6.55 (typical purine C=C and C=N), 7.7, 8.25, 9.15, 9.4, 9.75, and 10.4 μ. There was no distinct C=O peak.[29]

Anal. Calcd. for $C_{10}H_{13}N_5O_4$: C, 44.94; H, 4.90; N, 26.21. Found: C, 45.04, 44.94; H, 5.44, 4.99; N, 25.97, 26.04.[30]

In the first experiment adenosine was deaminated also as a control. To about 45 γ of adenosine and 3′-amino-3′-deoxyadenosine, each in 3.5 ml. of 0.05 *M* phosphate buffer in silica ultraviolet spectrophotometer cells, was added 5 mg. of takadiastase dissolved in 0.5 ml. of buffer. The rates of reaction as measured by optical density at 265 mμ/optical density at 250 mμ were very similar; both deaminations were essentially complete in 3.5 hr. and about half complete after 1 hr.

3′-Amino-3′-deoxyadenosine 1-N-Oxide (III).—To a solution of I (100 mg.) in glacial acetic acid (5 ml.) at room temperature was added 30% hydrogen peroxide (0.5 ml.). After 36 hr. at room temperature, the reaction mixture was applied in 350-λ aliquots to the starting lines of 15, 4 × 8 in., thin layer chromatography plates prepared from cellulose.[31] After drying, the plates were chromatographed 4.5 hr. in butanol–acetic acid–water (4:1:1), then dried overnight. The bands of III (R_f usually 0.1–0.25) fluoresced light blue when viewed with the ultraviolet light from the papergram scanner[21] and could be distinguished from dark bands of unchanged I and by-products. The bands containing III were scraped off the plate, ground in a mortar, then packed into a chromatography column and eluted with water until 60 ml. had been collected. The combined eluates from three such runs were reduced to 90 ml. in a vacuum oven, then were applied at a rate of 30 ml./hr. to a 10-ml. IRC-50 ion-exchange resin column (about 1 × 12 cm.) which had been converted previously to the hydrogen form, washed, then buffered with 50 ml. of 1 *N* sodium acetate. The column was washed with water (30 ml.) and eluted with 2.8% aqueous ammonia at the same rate. When the faint orange band was halfway down the column, collection of the 50-ml. fraction containing III began. This fraction was evaporated to dryness in the vacuum oven, and the residue was triturated with ethanol. Removal of the ethanol *in vacuo* left 110 mg. of white III, m.p. 225–230°, chromatographically homogeneous, R_f 0.70, 0.0, 0.18, and 0.65 in solvents A, B, C, and D, respectively. It could be recrystallized as needles, m.p. 236–239°, from aqueous ethanol, $\lambda^{neutral\ H_2O}_{max}$ 232, 260, 295 mμ ($E^{1\%}_{1cm}$ 1110, 236, 67), $\lambda^{acidic\ H_2O}_{max}$ 260 mμ ($E^{1\%}_{1cm}$ 344), $\lambda^{N_2O}_{max}$ 2.85, 3.1, 3.3, 3.8, 6.05, 6.3, 6.5, 6.8, 7.18, 7.6, 7.75, 8.0, 8.4, 8.85, 8.97, 9.2, 9.45, 9.7, 10.0–10.4, 11.3, 12.1, 13.2, 14.25 μ.

Anal. Calcd. for $C_{10}H_{13}N_5O_4$: C, 39.99; H, 5.39; N, 27.99. Found: C, 39.95, 40.08; H, 5.35, 5.48; N, 27.68, 27.88.[30]

One reaction mixture was allowed to stand 48 hr. Paper chromatography of an aliquot of it in solvent C revealed 6 spots: $R_{adenine\ N\text{-}oxide}$ 1.09, 1.0, 0.77, 0.61, 0.36, and 0.14. These 6 components were separated by preparative paper chromatography in solvents C and A, and the first two were identified as adenine and adenine 1-N-oxide, respectively, by paper chromatographic comparison with authentic materials[32] in solvents A and C. The second two components had the characteristic absorption spectrum of the adenine N-oxide chromophore (for example, $\lambda^{neutral\ H_2O}_{max}$ 232, 260 mμ, O.D. = 0.4, after 1:10 dilution 1.19; $\lambda^{acid\ H_2O}_{max}$ 260 mμ, O.D. 1.38) and were probably other glycosides of adenine 1-N-oxide formed by oxidative attack at the 3′-amino group. The fifth component was III, and for the sixth a hypoxanthine 1-N-oxide chromophore could be suggested on the basis of the ultraviolet spectrum ($\lambda^{neutral\ H_2O}_{max}$ 232, 248 mμ, O.D. 1.75, 1.53; $\lambda^{acid\ H_2O}_{max}$ 250 mμ, O.D. 0.33 after 1:10 dilution) and low mobility in solvent C.

Hydrolysis of 3′-Amino-3′-deoxyadenosine 1-N-Oxide. A. 3-Amino-3-deoxyribose.—I and III (5 mg.) were each heated in 1 ml. of 10% hydrochloric acid for 1 hr. at 100°. The resulting solutions were each applied at the starting lines of cellulose thin layer chromatography plates, and after drying, the plates were chromatographed in 2.8% aqueous ammonia for 3.5 hr. The portion of cellulose corresponding to R_f of 0.85–1.0 was scraped off, pulverized, and eluted in a small column with water until 2 ml. of eluate had been collected. Since aliquots of these solutions when paper chromatographed gave several spots with the aniline oxalate spray for reducing sugars, the remainder of the solutions each were purified by absorption on a 10-ml. volume IRC-50 buffered ion-exchange resin column and by elution with aqueous ammonia. The residues from the ammonia eluates each were redissolved in the original volume of water, and 25-λ aliquots of these solutions when paper chromatographed gave only one spot each (pink) with aniline oxalate; R_{ribose} 0.89 in butanol–acetic acid–water (4:1:1); $R_{glycerose}$ 0.58 in pyridine–ethyl acetate–acetic acid–water (5:5:1:3). In other solvents the R_{ribose} values of 3-amino-3-deoxyribose from I and III were also identical but the spots were less compact.

B. Adenine 1-N-Oxide.—Hydrolysis of III (1 mg. in 1 ml. of 10% HCl at 100° for 1 hr.) did not give adenine 1-N-oxide but a degradation product, probably 4-aminoimidazole-5-carboxamidoxime (see ref. 18) which was also obtained from adenine 1-N-oxide under identical conditions: R_f in solvents A and D, 0.63 and 0.61. The transient presence of adenine 1-N-oxide was demonstrated by hydrolysis in 1 *N* HCl at 100°, withdrawing samples for paper chromatography every 15 min. The zero time sample (which had stood 1 hr. at room temperature) and the 15-min. sample showed spots with the same mobility as adenine N-oxide in solvents A, C, and D. The spot of degradation product appeared at 15 min., and conversion to this product was complete at 30 min., both from III and from adenine 1-N-oxide under identical conditions.

(26) For the exact method see E. Lederer and M. Lederer, "Chromatography, a Review of Principles and Applications," 2nd Ed., Elsevier Publishing Co., New York, N. Y., 1957, p. 139.

(27) The reaction is complete 2–3 days after all the solid (I) has dissolved. In earlier experiments it was shown that for complete reaction, λ_{max} of an aliquot was 249 mμ, and optical density at 265 mμ/optical density at 250 mμ was 0.5 or less.

(28) L. Goodman, J. W. Marsico, and M. J. Weiss, *J. Med. Chem.*, **6**, 410 (1963).

(29) 9-α-L-Rhamnopyranosylhypoxanthine has a carbonyl peak at 1680 cm.$^{-1}$; see B. R. Baker and K. Hewson, *J. Org. Chem.*, **22**, 959 (1957).

(30) G. Robertson, Florham Park, N. J., and W. Manser, Zürich, Switzerland.

(31) MN-cellulose powder 300 distributed by Brinkmann Instruments, Inc., Great Neck, N. Y. Twenty grams of powder and 120 ml. of distilled water were mixed in a Waring blender for 3 min. The mixture was spread evenly on 5 clean glass plates with a bent rod and the plates were placed in an oven at 100° until dry, usually 15–30 min.

(32) Adenine 1-N-oxide was prepared by the method of M. A. Stevens, D. L. Magrath, H. W. Smith, and G. B. Brown [*J. Am. Chem. Soc.*, **80**, 2755 (1958)]. It did not decompose sharply as would be inferred from the literature value of 297–307° dec. but slowly darkened from 300–350° without losing the characteristic fine needle structure. The ultraviolet absorption spectra agreed with those published.

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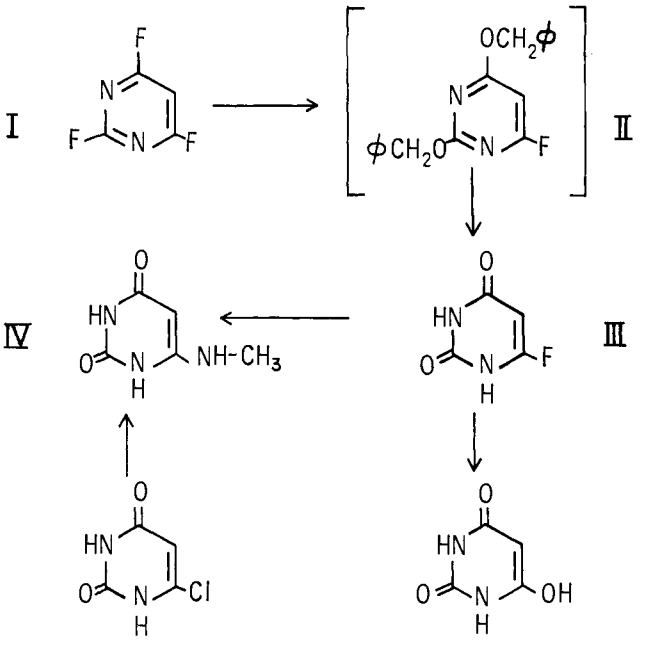
The synthesis of 6-fluorouracil from 2,4,6-trichloropyrimidine is described. The relative effects of a 5- and 6-halogeno atom on the ultraviolet absorption spectra and the apparent pK_a values of uracil are presented.

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**, 5157 (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).