Pyrido[3,2-g]pteridines. 2. Synthesis and Growth-Inhibitory Evaluation of Some 10-Substituted 3H,10H-2,4-Dioxopyrido[3,2-g]pteridines (9-Azaisoalloxazines)†,‡

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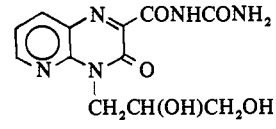
A number of 10-substituted 3H,10H-2,4-dioxopyrido[3,2-g]pteridines (9-azaisoalloxazines) were synthesized and evaluated for growth-inhibitory activity against selected microbial and mammalian cell culture systems *in vitro* and transplantable murine leukemia systems *in vivo*. Most of the agents tested showed significant inhibition of growth of KB (human epidermoid carcinoma) cells in culture; the 50% inhibiting dose (ID_{50}) for active compounds was in the range 0.1–3.0 μg/ml. Two compounds, **4j** and **7f**, were assayed for antimalarial activity and found to be inactive.

Recently we reported on the chemistry and growth-inhibitory activity of some 1H,3H-2,4-dioxopyrido[3,2-g]pteridines (9-azaalloxazines).[1] Now, in connection with our program on pyrido[3,2-g]pteridine derivatives for possible use in cancer chemotherapy, we wish to describe the preparation of some 9-azaisoalloxazines.

9-Azaisoalloxazines may be viewed as structural analogs of riboflavin.§ The synthesis of such agents for antitumor evaluation was suggested to us in part by the importance of riboflavin in growth and tissue repair in all animals.[2,3] A variety of riboflavin analogs have already been shown to inhibit the growth of experimental rodent tumors *in vivo*;[4-16] one known riboflavin analog and antagonist, galactoflavin,# has recently been used clinically in the management of patients with Hodgkin's disease, lymphosarcoma, and polycythemia vera.[17] All of the foregoing compounds contain a redox system equivalent to the functional N_1-C_{10a}-C_{4a}-N_5 oxidation–reduction system present in riboflavin. Since the biological activity of riboflavin analogs has been suggested[18] to be due to a change in redox potential, 9-azaisoalloxazines seem especially worthwhile to investigate; these compounds contain the functional oxidation–reduction system found in riboflavin and also incorporate an additional N atom (N_9) in a position to exert influence over the redox potential of the molecule. Of the handful of 9-azaisoalloxazines which have been reported to date,[19-22] we are unaware of any evaluation of their tumor growth-inhibitory activity.

The present report concerns 9-azaisoalloxazines carrying, primarily, terminal hydroxy- or aminoalkyl substituents at position 10 but having no substitution on the pyridine ring (ring A). Initially, we expected that the sequence 1 → 2 → 3 → 4 (route A, Scheme I) would be suitable for the preparation of the target compounds. Accordingly, 2-chloro-3-nitropyridine (**1**) was treated with 2 equiv of the appropriate amine in warm ethanol solution to give the corresponding aminonitro compound **2**; the various products obtained, **2a–j**, are indicated in Table I. The generally high yield of **2** reflects the ease of the reaction, which can be observed to proceed by the change in reaction solution color from the very pale yellow of **1** to the deep golden yellow of the *o*-nitroamine.

Catalytic reduction of **2** to **3** invariably gave dark red to black viscous oils from which no products could be solidified or characterized. In general, condensation of the crude reduction products with alloxan monohydrate under a variety of conditions gave very low yields of the corresponding azaisoalloxazines; however, these products were of such poor quality that clean samples could not be obtained from them. One exception to this generalization was the black oily reduction product of **2d** which, upon treatment with alloxan monohydrate in aqueous pH 4 solution,[21] gave **8**



8

rather than the tricyclic target compound. Another exception was the reduction product of **2j**, which with alloxan monohydrate in glacial AcOH in the presence of $B(OH)_3$ gave a 30% yield of the desired azaisoalloxazine **4j** (Table II).

It was hoped, in part from the example provided by the formation of **4j**, that the absence of OH or NH_2 groups in the aliphatic side chains might allow for a more effective condensation of the diamine intermediates with alloxan. The sequence 2 → 5 → 6 → 7 → 4 (route B, Scheme I) was thus adopted. Treatment of aminonitro compounds **2a–i** with Ac_2O in pyridine on the steam bath afforded the corresponding acetylated derivatives **5a–i** in good yields; these products are summarized also in Table I. The diamines **6a–i** were obtained by catalytic reduction of the appropriate acetylated aminonitro compounds. As indicated in Table III, some of these diamines were solids while others continued to be oils. In the latter instances, despite their inability to be solidified, the diamines were less susceptible to air oxidation and discoloration than their unacetylated counterparts and could be used directly for condensation with al-

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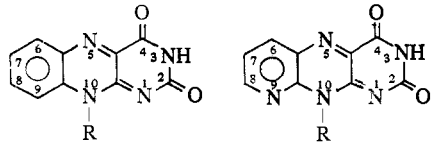
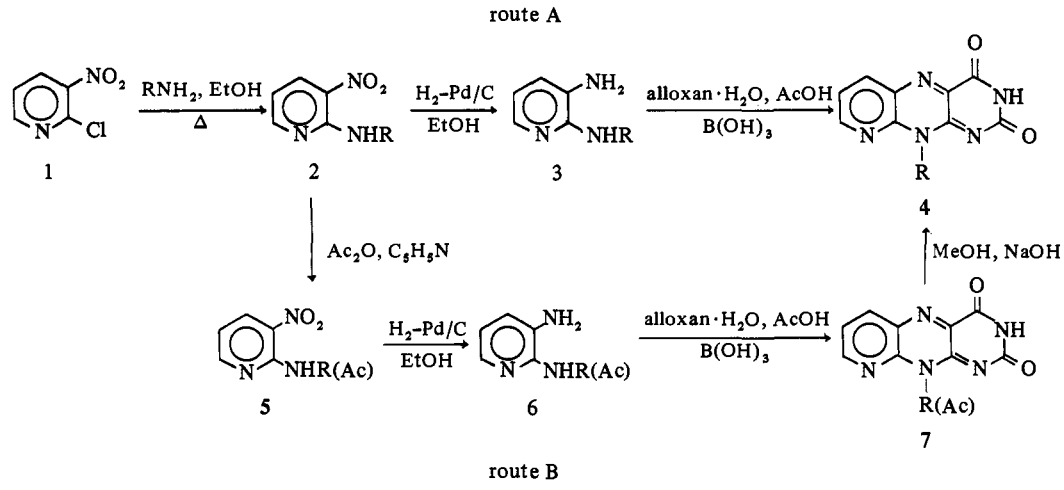
‡The use of alternate numbering systems in naming riboflavin and related compounds has resulted in some confusion in the literature. The Chemical Abstracts system assigns positions 5 and 10 to the pyrazine ring N atoms, whereas these locations are numbered 10 and 9, respectively, in the older literature. In the present report the Chemical Abstracts numbering sequence, shown below, for the benzo[g]pteridine and pyrido[3,2-g]pteridine ring systems is used exclusively and consistently. The convenient expression "9-azaisoalloxazines" for 10-substituted derivatives of the hypothetical 3H,10H-2,4-dioxopyrido[3,2-g]pteridine derives from the common use of "isoalloxazine" for 10-substituted derivatives of the analogous benzo compound.



§ Vitamin B_2; 7,8-dimethyl-10-(D-*ribo*-2,3,4,5-tetrahydroxypentyl)-isoalloxazine.

#7,8-Dimethyl-10-(D-*galacto*-2,3,4,5,6-pentahydroxyhexyl)isoalloxazine.

Scheme I



loxan. The condensation reactions were effected in glacial AcOH at 100° in the presence of 3 molar equiv of $B(OH)_3$. We earlier noted that $B(OH)_3$, which is of known[23] value in catalyzing the formation of alloxazines and isoalloxazines from alloxan and *o*-phenylenediamines, is equally as useful in the formation of azaalloxazines[1] and azaisoalloxazines.[24] The 9-azaisoalloxazines, **7a-i**, obtained from acetylated diamine intermediates are listed in Table II.

The ease with which 9-azaalloxazines are converted to carboxyureidopyrido[2,3-*b*]pyrazinones by alkaline hydrolysis[1] suggested that short reaction time and a minimum of H_2O be employed in the saponification of the acetyl-protected azaisoalloxazines. Treatment of the acetoxy compounds **7a-f** and 7i in warm MeOH with a small volume of 15% NaOH for 2-4 min afforded the corresponding deacetylated products **4a-f** and **4i** (also listed in Table II). This hydrolysis procedure was not effective for the acetamido compounds **7g** and **7h**, nor were these substances hydrolyzed by the action of boiling dilute or concentrated HCl for 2 hr.

The 9-azaisoalloxazines prepared in this study display properties typically associated with isoalloxazine derivatives.[25] They exist generally as orange-yellow microcrystalline solids, although some show a distinct green cast. When heated, they begin to darken gradually and finally melt into a black tar. Although only very slightly soluble in H_2O, highly dilute aqueous solutions at neutral pH are deep amber in color and exhibit an intense green fluorescence. No green fluorescence is observed in solutions at pH 1 or above pH 9. After a time, alkaline solutions gradually begin to show pale blue fluorescence; this can be attributed most probably to hydrolysis of the azaisoalloxazine to the carboxyureidopyrazinone.[1,26]

When sampled as KCl disks, the azaisoalloxazines showed in the ir the aromatic pyridine nucleus at 6.20-6.25 μ and two distinct carbonyl peaks of almost equal intensity at 5.84 and 6.00 μ. The acetoxy derivatives had an additional carbonyl absorption which appeared as a shoulder at 5.79 μ, and the acetamido derivatives **7g** and **7h** had a -CONH- peak at 6.10 μ. The main absorption of the azaisoalloxazine ring system in the visible region occurs at 425-427 nm with two distinct weaker absorptions at 401-407 and 443-448 nm. As noted by Rudy and Majer[19] for 10-*n*-propyl-9-azaisoalloxazine, the spectral pattern of these compounds is

Table I. 2-Substituted Amino-3-nitropyridines

Compd no.	R	Reaction time, hr	Yield, %	Purification solvent[a]	Mp, °C	Formula	Analyses
2a	CH_2CH_2OH	2.5	84	A	66-68	$C_7H_9N_3O_3$	C, H, N
2b	$(CH_2)_3OH$	3.5	100	A[b]	Oil	$C_8H_{11}N_3O_3$	C, H, N
2c	$CH_2CHOHCH_3$	2.5	92	A + B	55.5-57	$C_8H_{11}N_3O_3$	C, H, N
2d	$CH_2CH(OH)CH_2OH$	3.5	72	A[b]	Oil	$C_8H_{11}N_3O_4$	C, H, N
2e	$(CH_2)_5OH$	2.0	80	A + B	39-40	$C_{10}H_{15}N_3O_3$	C, H, N
2f	$(CH_2)_6OH$	3.5	93	C	35-36	$C_{11}H_{17}N_3O_3$	C, H, N
2g	$(CH_2)_5NH_2$	1.0	67[c]	B[d]	88-92	$C_{10}H_{16}N_4O_2$	C, H, N
2h	$(CH_2)_6NH_2$	1.0	82[e]	B[d]	57-58	$C_{11}H_{18}N_4O_2$	C, H, N
2i	$(CH_2)_3N(CH_2CH_2OH)_2$	2.0	75	A[b]	35-38	$C_{12}H_{22}N_4O_4$	C, H, N
2j	$C(CH_3)H(CH_2)_3NEt_2$	5.0	68	B[b]	Oil	$C_{14}H_{24}N_4O_2$	C, H, N
5a	CH_2CH_2OAc	2.0	72	A + B	102-104	$C_9H_{11}N_3O_4$	C, H, N
5b	$(CH_2)_3OAc$	2.0	84	A[b]	Oil	$C_{10}H_{13}N_3O_4$	C, H, N
5c	$CH_2CH(OAc)CH_3$	2.0	90	B	65-66.5	$C_{10}H_{13}N_3O_4$	C, H, N
5d	$CH_2CH(OAc)CH_2OAc$	2.0	73	B	78-80	$C_{12}H_{15}N_3O_6$	C, H, N
5e	$(CH_2)_5OAc$	2.0	60	C[b]	Oil[f]	$C_{12}H_{17}N_3O_4$	C, H, N
5f	$(CH_2)_6OAc$	2.0	70	C	32.5-33.5	$C_{13}H_{19}N_3O_4$	C, H, N
5g	$(CH_2)_5NHAc$	2.0	50	A + B	87-88	$C_{12}H_{18}N_4O_3$	C, H, N
5h	$(CH_2)_6NHAc$	2.0	75	A + B	83-85	$C_{13}H_{20}N_4O_3$	C, H, N
5i	$(CH_2)_3N(CH_2CH_2OAc)_2$	2.0	68	A[b]	Oil	$C_{16}H_{26}N_4O_6$	C, H, N

[a] Crystallized from the indicated solvent, except where otherwise noted. A = benzene; B = petroleum ether (bp 65-90°); C = petroleum ether (bp 30-60°). [b] Purified by silica gel chromatography. [c] A second product, *N,N'*-bis(3-nitropyridyl-2-)pentane-1,5-diamine, was isolated in 17% yield from this reaction; the material had mp 132-134° after crystallization from benzene-petroleum ether (bp 65-90°) mixture. *Anal.* ($C_{15}H_{18}N_6O_4$) C, H, N. [d] Initially purified by means of silica gel chromatography with $CHCl_3$. [e] A 14% yield of *N,N'*-bis(3-nitropyridyl-2-)-hexane-1,6-diamine, mp 134-135°, from benzene-petroleum ether (bp 65-90°), was also formed. *Anal.* ($C_{16}H_{20}N_6O_4$) C, H, N. [f] Melts at room temperature.

Table II. 10-Substituted 3*H*,10*H*-2,4-Dioxopyrido[3,2-*g*]pteridines (9-Azaisoalloxazines)

Compd no.	R	Yield, %	Crystallization solvent	Decomposition point, °C[a]	Formula	Analyses
7a	CH_2CH_2OAc	94	Dioxane-H_2O[b]	266–268	$C_{13}H_{11}N_5O_4$	C, H, N
7b	$(CH_2)_3OAc$	47	Dioxane-H_2O[b]	245–250	$C_{14}H_{13}N_5O_4$	C, H, N
7c	$CH_2CH(OAc)CH_3$	65	Dioxane-H_2O-Me_2CO[c]	266–268	$C_{14}H_{13}N_5O_4$	C, H, N
7d	$CH_2CH(OAc)CH_2OAc$	85	Dioxane-H_2O-Me_2CO[c]	255–257	$C_{16}H_{15}N_5O_6$	C, H, N
7e	$(CH_2)_5OAc$	60	Dioxane-H_2O[b]	298–302	$C_{16}H_{17}N_5O_4$	C, H, N
7f	$(CH_2)_6OAc$	95	Dioxane-H_2O[b]	283–284	$C_{17}H_{19}N_5O_4$	C, H, N
7g	$(CH_2)_5NHAc$	56	Dioxane-H_2O-Me_2CO[c]	300–301	$C_{16}H_{18}N_6O_3$	C, H, N
7h	$(CH_2)_6NHAc$	62	Dioxane-H_2O-Me_2CO[c]	308–312	$C_{17}H_{20}N_6O_3$	C, H, N
7i	$(CH_2)_3N(CH_2CH_2OAc)_2$	27	THF	215–217	$C_{20}H_{24}N_6O_6$	C, H, N
4a	CH_2CH_2OH	39	AcOH-H_2O[b]	270–271	$C_{11}H_9N_5O_3 \cdot 0.25H_2O$	C, H, N
4b	$(CH_2)_3OH$	36	MeOH-H_2O[b]	278–280	$C_{12}H_{11}N_5O_3$	C, H, N
4c	$CH_2CH(OH)CH_3$	62	Dioxane-H_2O[d]	295–300	$C_{12}H_{11}N_5O_3$	C, H, N
4d	$CH_2CH(OH)CH_2OH$	59	AcOH-H_2O[b]	266–267	$C_{12}H_{11}N_5O_4 \cdot 0.25H_2O$	C, H, N
4e	$(CH_2)_5OH$	65	MeOH-H_2O[b]	301–305	$C_{14}H_{15}N_5O_3$	C, H, N
4f	$(CH_2)_6OH$	78	Dioxane	281–282	$C_{15}H_{17}N_5O_3$	C, H, N
4i	$(CH_2)_3N(CH_2CH_2OH)_2$	37	MeOH	242–244	$C_{16}H_{20}N_6O_4 \cdot HCl \cdot 0.5H_2O$	C, H, N, Cl
4j	$C(CH_3)H(CH_2)_3NEt_2$[e]	30	C_6H_6[f]	183–185	$C_{18}H_{24}N_6O_2$	C, H, N

[a] Melts into a black tar. In general, these products begin to darken appreciably about 50° below the melting point. [b] 1:1 by volume. [c] 1:1:1 by volume. [d] 8:1 by volume. [e] Prepared by the direct condensation of the requisite diamine with alloxan monohydrate in glacial AcOH in the presence of $B(OH)_3$ at 100° for 1 hr. [f] Purified initially by silica gel chromatography with EtOH.

Table III. 2-Substituted Amino-3-aminopyridines

Compd no.	R	Yield, %	Crystallization solvent	Mp, °C	Formula	Analyses
6a	CH_2CH_2OAc	100[a]		Oil[b]	$C_9H_{13}N_3O_2$[c]	C, H, N[c]
6b	$(CH_2)_3OAc$	100[a]		Oil[b]	$C_{10}H_{15}N_3O_2$	
6c	$CH_2CH(OAc)CH_3$	69	C_6H_6	98–100	$C_{10}H_{15}N_3O_2$	C, H, N
6d	$CH_2CH(OAc)CH_2OAc$	100[a]		Oil[b]	$C_{12}H_{17}N_3O_4$[d]	C, H, N[d]
6e	$(CH_2)_5OAc$	100[a]		Oil[b]	$C_{12}H_{19}N_3O_2$	
6f	$(CH_2)_6OAc$	82	H_2O	85–87	$C_{13}H_{21}N_3O_2$	C, H, N
6g	$(CH_2)_5NHAc$	100[a]		Oil[b]	$C_{12}H_{20}N_4O$	
6h	$(CH_2)_6NHAc$	83	C_6H_6	129–130	$C_{13}H_{22}N_4O$	C, H, N
6i	$(CH_2)_3N(CH_2CH_2OAc)_2$	98[a]	Petroleum ether (bp 60–95°)	Oil	$C_{16}H_{28}N_4O_4$	C, H, N

[a] Crude yield. [b] Used directly for condensation with alloxan monohydrate. [c] Analysis is of the picrate salt ($C_9H_{13}N_3O_2 \cdot C_6H_3N_3O_7$), mp 163–165° from EtOH. [d] Analysis is of the HCl salt, mp 165–168° from EtOH.

similar to that of 10-alkylisoalloxazines, except that for the aza system the pattern is shifted about 15-20 nm to shorter wavelengths. The quantitative uv spectrum of **4e** is provided to illustrate the spectral features of the products listed in Table II: λ_{max}^{EtOH} ($\epsilon \times 10^{-3}$) 257.5 (33.4), 302 (3.4), 406 (shoulder, 9.5), 427 (13.0), and 445 nm (shoulder, 8.4).

Bioassay.** A number of 9-azaisoalloxazines were assayed for their ability to inhibit the growth of serially propagated KB (human epidermoid carcinoma) cells in culture according to a previously described procedure.[27] Of the 15 compounds evaluated, 12 showed significant growth-inhibitory activity against this malignant cell line (50% inhibiting dose of 3 μg/ml or less); the data are summarized in Table IV. While the acetylated side chain in 7a-**h** may or may not be hydrolyzed *in vitro* to the deprotected hydroxy or amino compound, the growth inhibition seen here appears to be due primarily to the azaisoalloxazine ring system since 10-*n*-propyl-9-azaisoalloxazine, which lacks hydroxyl or amino functions in the side chain, gives the same order of activity as the other tricyclic derivatives. The 10-propyl compound was originally prepared by Rudy and Majer[19] and was resynthesized by us in connection with this investigation.

In vitro assay of these compounds for their ability to inhibit the growth of selected cofactor-dependent bacteria and also of the fungus *Candida albicans* was also carried out as previously described.[28] The results are included in Table IV; only ID_{50} values of 40 μg/ml or less are provided. The data obviously do not correlate with any specific enzyme-pathway inhibition but instead indicate a general weak to moderate antimicrobial effect.

Compounds 7a, 7d, **7f**, and the 10-propyl derivative of Rudy and Majer were also evaluated for their ability to increase the mean survival time of BDF_1 and DBA_2 mice bearing the L1210 and P1534 lymphatic leukemias, respectively. Details of the assay procedures have been described.[29] At nontoxic dosages these compounds failed to show significant antitumor activity when administered intraperitoneally as suspensions in 10% polysorbate 80†† once daily for 4 days beginning 1 day after tumor implantation.

Compound 4j, which possesses an antimalarial-type side chain, was tested for antimalarial activity‡‡ against *Plasmodium berghei* in the mouse and *Plasmodium gallinaceum* in the chick.[30] In the mouse system, a nontoxic single dose of 40 mg/kg was inactive in increasing mean survival time of infected animals. A single dose of 320 mg/kg was toxic in the chicken; the compound was not tested at nontoxic dosages in this system. Also, compound **7f** was tested and found to be inactive in the *P. berghei*/mouse system at the three dosages used (40, 160, and 640 mg/kg).

**Bioassay studies were performed by the Laboratories of Microbiology and Cell Biology and the Laboratories of Experimental Therapeutics at The Children's Cancer Research Foundation.

††Marketed as Tween 80 by Atlas Chemical Industries, Inc., Wilmington, Del.

‡‡We thank Dr. Edgar A. Steck of the Walter Reed Army Institute of Research for arranging for the antimalarial assays and for providing us with the test data.

Table IV. ID_{50} Values for Some 9-Azaisoalloxazines against Selected Microbial Systems and Mammalian Cells in Culture

Compd no.	*S.f.*[b]	*L.a.*[c]	*L.a.*[d]	*L.c.*[e]	*Le.c.*[f]	*L.f.*[g]	*C.a.*[h]	KB[i]
4a		30	31	32	31		nt[j]	
4c		21	28	nt	33	22	nt	1.8
4d		26	28	25	31	18	nt	
4f	30	16	25	29	20	20	25	
4i		18	18	nt	30	19	nt	3.0
4j	35	3	3	30		2.3	30	0.1
7a		14	10	18		23	27	1.0
7b	30	23	22	29	20	20	25	3.0
7c		25	26	38	14	16	23	1.6
7d		20		20	7		28	1.0
7e	25	26	26	28	29	15	nt	1.5
7f	25	17	20	29	21	15	23	1.5
7g		25	28	nt	29	17	nt	2.5
7h		28	28	10	29	29	nt	1.0
4[k]	25	8	14	21	3	11		1.0

[a]Expressed as micrograms per milliliter. ID_{50} values in microbial systems greater than 40 μg/ml and in mammalian cell culture greater than 10 μg/ml have been omitted. [b]*Streptococcus faecium* ATCC no. 8043/folate dependent. [c]*Lactobacillus arabinosis* no. 17-5/pantothenate dependent. [d]*L. arabinosis* no. 17-5/niacin dependent. [e]*L. caseii* no. 7469/riboflavin dependent. [f]*Leuconostoc citrovorum* no. 8081/N^{10}-formyltetrahydrofolate dependent. [g]*L. fermenti* no. 9338/thiamine dependent. [h]*Candida albicans* CCRF Collection no. 60112. [i]Serially propagated mammalian cells derived from a human epidermoid carcinoma of the jaw and maintained in culture. [j]nt = not tested. [k]R = C_3H_7; 10-*n*-propyl-9-azaisoalloxazine previously synthesized by Rudy and Majer.[19]

Experimental Section§§

Ir spectra were determined with a Perkin-Elmer Model 137B spectrophotometer; uv absorption spectra were measured with Cary Model 11 and 15 spectrophotometers. Melting points were taken by the capillary method in a modified Wagner-Meyer melting point apparatus[31] at a rate of heating of 2°/min; decomposition points are not reproducible unless conditions are rigidly controlled.

Amination of 2-Chloro-3-nitropyridine. General Procedure for the Preparation of 2a-j. A solution of 2 molar equiv of the appropriate amine in absolute EtOH was added to a solution of 2-chloro-3-nitropyridine (1, Aldrich Chemical Co.) in EtOH. The mixture was stirred and warmed at 70° for the period of time indicated in Table I. The EtOH was evaporated, H_2O was added, and the solution or suspension was extracted with CH_2Cl_2 until the extracts appeared essentially colorless. Evaporation of the CH_2Cl_2 left either a solid, which was purified by crystallization, or an oil, which was purified initially by passage through a silica gel column and then by crystallization; the purification solvents are listed in Table I.

Acetylation of 2a-i. Preparation of 5a-i. The appropriate **2** was dissolved in a minimal volume of pyridine and an equimolar amount of Ac_2O was added. The solution was heated for 2 hr on the steam bath, then cooled, and poured into H_2O. The product was extracted into C_6H_6, and the extract was dried and evaporated to give the crude **5**, which was purified as indicated in Table I.

Reduction of 5a-i. Preparation of Diamines 6a-i. The nitro compound **5** was dissolved in a suitable volume of absolute EtOH and 200–400 mg of 5% Pd on C was added. The mixture was shaken under 3–4 atm of H_2 at room temperature. When the theoretical uptake of H_2 was achieved, the catalyst was separated and the EtOH was evaporated to give the crude product (Table III).

Condensation of Pyridinediamine Derivatives with Alloxan Monohydrate. Preparation of 7a-i and 4j. A solution of the diamine in glacial AcOH was added dropwise with stirring to a warm (60°) solution containing an equimolar quantity of alloxan·H_2O and a 2 *M* excess of $B(OH)_3$ in glacial AcOH. The reaction mixture was heated at 100° for 1–2 hr. When cool, the crude product was collected and purified as indicated in Table II. Alternatively, if after cooling the product remained in solution, H_2O was added to the AcOH solution to precipitate the material.

Deacetylation. Preparation of 4a-f and 4i. To a stirred hot suspension of the acetoxy compound in MeOH (0.5 g/30 ml) was added 15% NaOH (no more than 1 ml/0.5 g of compound/acetyl group). The suspended material dissolved within 0.5–2 min and heating and stirring were continued for another 2 min. Rapid cooling to room temperature, followed by neutralization with concentrated HCl, gave the crude product. Purification was accomplished as indicated in Table II.

2-Carboxyureido-3,4-dihydro-4-(2',3'-dihydroxypropyl)-pyrido[2,3-*b*]pyrazin-3-one (8). A solution of 5 mmol of **2d** in 30 ml of EtOH was shaken under H_2 in the presence of 5% Pd on C. The catalyst was separated, the EtOH was evaporated, and the dark oily residue was dissolved in 20 ml of H_2O. The aqueous solution was acidified with dilute HCl to pH 4 and 1.0 g of alloxan·H_2O was added. The solution was heated on the steam bath for 30 min, then cooled, and placed in the refrigerator. After several days, the precipitate was collected and crystallized from AcOH-H_2O (1:1 by volume) to give almost white needles, mp 220–225° dec. In alkaline solution, **8** exhibited pale blue fluorescence. The absorption spectrum of the material in H_2O or 95% EtOH closely resembled that of 2-carboxyureido-3,4-dihydropyrido[2,3-*b*]pyrazin-3-one.[1] *Anal.* ($C_{12}H_{13}N_5O_5$) C, H, N.

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§§Microchemical analyses were performed by Galbraith Microanalytical Laboratories, Knoxville, Tenn., and Werby Laboratories, Boston, Mass.; found values are within ±0.4% theory.

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Pyridine Nucleosides Related to 5-Fluorouracil and Thymine

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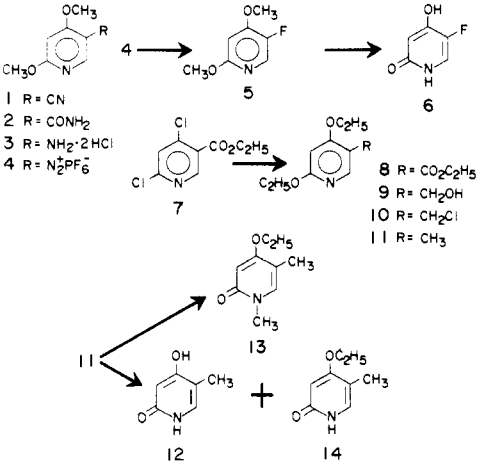
4-Hydroxy-5-fluoro-2-pyridone (5-fluoro-3-deazauracil, **6**) was synthesized from 2,4-dimethoxy-5-cyanopyridine (**1**) in a multistep procedure employing the intermediate, 2,4-dimethoxypyridine-5-diazonium hexafluorophosphate (**4**). Trimethylsilation of **6** and condensation with the appropriately protected halosugar gave (after deblocking) 5-fluoro-3-deazauridine (**28**) and 5-fluoro-2-deoxy-3-deazauridine (**19**) and its α anomer **23**. The ribonucleoside **28** was converted to 2-hydroxy-5-fluoro-1-(β-D-arabinofuranosyl)-4-pyridone ($O^2 \rightarrow 2'$)-cyclonucleoside (**29**), which was used in the determination of anomeric configuration. 4-Hydroxy-5-methyl-2-pyridone (3-deazathymine, **12**) was prepared from 2,4-dichloro-5-carbethoxypyridine (**7**) by a five-step procedure. Condensation of the trimethylsilyl derivative of **12** with the appropriately protected halosugar gave (after deblocking) 3-deazathymidine (**21**) and its α anomer **25**. Structure proof and anomeric configuration were determined from the uv, pK_a, nmr, and CD data. These compounds were not active as growth inhibitors of several cell lines in culture.

Because of a long standing interest in this laboratory in the synthesis of fluorinated pyrimidine nucleosides (see ref 1 for leading references) and the determination of their chemotherapeutic, pharmacological, and biochemical properties, it appeared worthwhile to expand our synthetic activities to include certain analogs in which there is an isosteric replacement of one of the nitrogen atoms in the pyrimidine ring. This paper reports our first effort along these lines: the synthesis of pyridine nucleoside analogs (3-deazapyrimidine nucleosides) of 5-fluorouracil and thymine.

There has recently been some interest in the synthesis and evaluation of the biological properties of 3-deazapyrimidine nucleosides. Currie and coworkers[2-6] have reported the preparation of 3-deazauridine, 3-deazacytidine, 3-deazaorotidine, and related nucleosides and have found marked cytotoxic activity of the uridine and cytidine analogs *in vitro* and *in vivo*. Other biochemical properties of 3-deazauridine and 3-deazacytidine have also been described, including their anti-RNA viral activity.[7]

The synthesis of 4-hydroxy-5-fluoro-2-pyridone (**6**), a necessary intermediate for the preparation of 5-fluoro-3-deazauracil nucleosides, was the crucial step in this synthetic sequence. After several preliminary unsuccessful attempts at ring closure of appropriately substituted acyclic precursors, it became obvious that substitution of fluorine on a preformed pyridine would be the method of choice. 2,4-Dimethoxy-5-cyanopyridine (1) was prepared from diethyl β-ketoglutarate according to the method of Taylor, *et al.*[8] This cyanopyridine 1 was then hydrolyzed by H_2O_2-NaOH to the amide **2** (Scheme I) which was converted *via* a Hoffmann hypochlorite rearrangement to the amine **3**. Introduction of the fluorine atom into the pyridine ring was accomplished with a Schiemann reaction, employing the diazonium hexafluorophosphate modification of Rutherford, *et al.*[9] 2,4-Dimethoxypyridine-5-diazonium hexafluorophosphate (**4**) was formed in 75% yield from the corresponding diazonium chloride and HPF_6. After thorough drying, 4 was decomposed at 250° to give 2,4-dimethoxy-5-fluoropyridine (**5**) in 31% yield.

Scheme I



The nmr of **5** revealed two aromatic doublets [τ 2.10 ($J_{6\text{-F}}$ = 2.9 Hz, H-6), 3.70 ($J_{3\text{-F}}$ = 5.8 Hz, H-3)] which supports the assigned structure. In addition, it indicates that no rearrangement had occurred, as any rearrangement would have produced a more complex spectrum.‡ The observation that $J_{3\text{-F}} > J_{6\text{-F}}$ was corroborated by similar observations made by Lyle and Taft[10] for 4-fluorolutidines and Rowbotham, *et al.*,[11] for methyl derivatives of 2-fluoropyridine.

Demethylation of **5** to give 4-hydroxy-5-fluoro-2-pyridone (**6**) was accomplished, albeit in low yield (11%), with 25% HCl at 145° for 4 hr.[12] Other attempts at demethylation including MeMgI,[13] BF_3-Ac_2O,[14] BBr_3-$CHCl_3$,[15] $Ph_2P^-Li^+$,[16] and NaI-HOAc[17] were unsuccessful. A marked change in the uv spectrum of **6** from pH 5 to 3 indicated an acidic proton with a pK_a of 4.6 ± 0.2. The nmr spectrum of **6** showed two doublets [τ 2.50 ($J_{6\text{-F}}$ = 6.2 Hz), 4.25 ($J_{3\text{-F}}$ = 8.0 Hz)], one of which disappeared (τ 4.25) upon addition of deuterium oxide. A similar effect was noted by Currie, *et al.*,[4] for 3-deazauridine, in which the H-3 proton under-

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‡The nmr spectra of compounds 1, 2, and 3 showed no coupling between H-3 and H-6 while Currie, *et al.*,[6] reported $J_{3\text{-}5}$ = 2.5 Hz for 3-deazauridine.