Synthesis of Certain Hydroxy Analogues of the Antimalarial Drug Primaquine and Their in Vitro Methemoglobin-Producing and Glutathione-Depleting Activity in Human Erythrocytes

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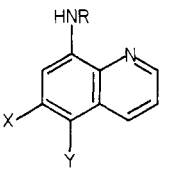
A number of hydroxy analogues of the antimalarial drug primaquine [8-[(4-amino-1-methylbutyl)amino]-6-methoxyquinoline] were synthesized and characterized by ^1H NMR and mass spectra. Several of the compounds were found to be active in forming methemoglobin in human erythrocytes, particularly in those from glucose-6-phosphate dehydrogenase (G6PD) deficient subjects. Decreased levels of glutathione (GSH) in G6PD-deficient erythrocytes were also found with compounds that were active methemoglobin formers.

Numerous compounds related to 8-aminoquinoline have been synthesized since World War II and studied for antimalarial activity in *Plasmodium vivax* malaria. Although primaquine has been used therapeutically for many years and is presently the drug of choice for elimination of exoerythrocytic forms, serious hemolytic problems result when it is administered to malaria patients whose erythrocytes are deficient in glucose-6-phosphate dehydrogenase (G6PD). Hemolysis of the erythrocytes in these patients is associated with oxidation of glutathione (GSH) to oxidized glutathione (GSSG), oxidation of hemoglobin (Hb) to methemoglobin (metHb), and Heinz bodies formation.[1] Fraser and Vesell[2] in their in vitro studies have reported that certain analogues of pentaquine decreased the GSH content of G6PD-deficient erythrocytes and oxidized the Hb to metHb. Recently a great deal of evidence has been presented on the role of oxidative agents in promoting increased methemoglobin production, as well as disulfide bond formation between GSH and erythrocytic membrane sulfhydryl groups, which are suggested to lead to the ultimate disintegration of the erythrocytes.[3]

Our initial studies demonstrated that inclusion of mouse liver microsomes and cofactors in the incubation mixtures containing primaquine and erythrocytes resulted in significantly increased levels of methemoglobin formation in both normal and G6PD-deficient erythrocytes.[4,5] These results suggested that oxidative metabolites, such as 5,6-dihydroxy-8-[(4-amino-1-methylbutyl)amino]quinoline (**4**) and 5,6-dihydroxy-8-aminoquinoline (**5**), rather than intact primaquine could be involved in metHb production. In fact, Dybing et al.[6] and Graham et al.[7] have presented evidence that dihydroxy compounds, such as α-Me-Dopa, and other catecholamines could be oxidized by cytochrome P-450 generated superoxide anion to quinoid reactive intermediates capable of oxidizing Hb to metHb.

With this rationale in mind, the synthesis and characterization of compounds with structures considered likely to result from metabolism of primaquine have been accomplished. These compounds would not only be used in our investigations on drug-induced hemolytic anemia, but they could also facilitate structural verification by comparison with the actual metabolites of primaquine.

In fact, some of these compounds have been identified as actual metabolites of primaquine. Baty et al.[8] have detected 6-methoxy-8-aminoquinoline (**8**) as a metabolite in man. We have reported studies on the dog and tentatively identified 8-[(4-amino-1-methylbutyl)amino]-6-hydroxyquinoline (**2**) and 5-hydroxy-6-methoxy-8-[(4-amino-1-methylbutyl)amino]quinoline (**3**) as metabolites in the urine.[4,9] Also our in vitro mouse liver microsomal studies with primaquine have resulted in identification of 5,6-dihydroxy-8-[(4-amino-1-methylbutyl)amino]quinoline (**4**) and its photooxidation product.[10] It should be pointed out that Baker et al.[11] have reported the presence in the rat plasma of 8-[3-carboxy-1-methylbutyl)amino]-6-methoxyquinoline as an oxidative deamination metabolite of primaquine, and Clark et al.[12] in their metabolic studies of primaquine by microorganism have identified 8-[(4-acetamido-1-methylbutyl)amino]-6-methoxyquinoline plus the above carboxy compound.



5′ 1′ 2′ 3′ 4′

1, X = OCH_3; Y = H; R = $CH_3CHCH_2CH_2CH_2NH_2$
2, X = OH; Y = H; R = $CH_3CH(CH_2)_3NH_2$
3, X = OCH_3; Y = OH; R = $CH_3(CH_2)_3NH_2$
4, X = Y = OH; R = $CH_3CH(CH_2)_3NH_2$
5, X = Y = OH; R = H
6, X = OCH_3; Y = OH; R = H
7, X = OH; Y = R = H
8, X = OCH_3; Y = R = H
9, X = OCH_3; Y = H; R = OH
10, X = Y = OCH_3; R = $CH_3CH(CH_2)_3NH_2$

Furthermore, preliminary screening of compounds **1** through **8** revealed that a few were significantly active with

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Table I. Methemoglobin Formation by Primaquine and Selected Analogues in Normal and G6PD-Deficient Human Erythrocytes[a]

	% methemoglobin formed with the following concn			
	0.05 mM		0.01 mM	
no.	normal	deficient	normal	deficient
1	1.0 ± 0.2	1.3 ± 0.3		
2	1.1 ± 0.3	1.7 ± 0.2	1.1 ± 0.7	1.2 ± 0.5
3	12.8 ± 1.2	36.0 ± 1.8	3.4 ± 1.6	10.1 ± 2.0
4	16.3 ± 1.1	36.4 ± 1.9	4.2 ± 1.0	7.5 ± 0.7
5	22.3 ± 1.6	40.5 ± 1.5	4.8 ± 0.9	10.2 ± 2.5
6	34.2 ± 2.5	43.8 ± 2.2	b	22.3 ± 2.0

[a] Values are means and standard error for five to eight experiments. [b] Not available. Also, no GSH are available on 6 at this time due to the inaccessibility of G6PD-deficient erythrocytes.

respect to metHb formation.[4] Therefore, in the present study we have assessed the in vitro toxicity and found that a select number of primaquine analogues are highly active in decreasing the GSH content and producing metHb in human erythrocytes.

Chemistry. The starting materials for the synthesis of compounds **3** through **6**, namely, 5,6-dimethoxy-8-aminoquinoline and 5,6-dimethoxy-8-[(4-amino-1-methylbutyl)amino]quinoline, were prepared as described by Elderfield.[13] Selective hydrolysis of the 5-methoxy group resulting in formation of **6** was achieved with a 48% hydrobromic acid solution, but a 53% hydrobromic acid solution was necessary to effect the hydrolysis of both methoxy groups to provide **5**.[14] Hydrolysis of the dimethoxy analogue **10** with a 53% hydrobromic acid solution gives **3** and **4**, but a longer reflux period was required to completely hydrolyze the methoxy groups to obtain **4**. The hydrobromide and sulfate salts of **3** were extremely hygroscopic and air sensitive. A stable crystalline chloroplatinate derivative was obtained. It should be pointed out that in aqueous media, **3** undergoes O-demethylation with facility presumably by a mechanism similar to other vinyllic ethers.[15] Demethylation of **1** in 47% hydriodic acid was achieved by procedures for similar 8-aminoquinoline derivatives.[16,17] The resulting hydriodide salt was difficult to recrystallize. It was converted to a sulfate salt, which was recrystallized from ethanol. Partial catalytic hydrogenation of 6-methoxy-8-nitroquinoline by a literature procedure[18] with minor modifications was used to prepare **9**. However, **9** is highly unstable in aqueous media and spontaneously breaks down to **8**, as confirmed by HPLC results.

Pharmacology. The in vitro metHb-forming activity of compounds **1** through **6** on normal and G6PD-deficient erythrocytes are compared in Table I. With the exception of **2**, all the hydroxy analogues (**3–6**) were significantly more active in producing metHb than the parent compound **1**, particularly in G6PD-deficient erythrocytes. Even at 0.01 mM, compounds **3** through **6** had some metHb-forming activity in G6PD-deficient erythrocytes. Compounds **3** and **4** displayed comparable activity with respect to metHb formation presumably because of the instability of **3**, which in aqueous media is readily converted to **4**, as confirmed by HPLC results. Compound **9** was not tested owing to its spontaneous conversion to **8** in aqueous media. In all the cases, erythrocytes deficient in G6PD exhibited greater metHb formation than normal erythrocytes. The effect of compound **1** and selected analogues on decreasing the GSH content is shown in Table II. Variation in the control data is due to the fact that the same subjects were unavailable for all the experiments. Compounds **1** and **2** had little effect on the GSH levels in either normal or G6PD-deficient erythrocytes. Compounds **3** through **5**, on the other hand, markedly decreased the GSH content in the G6PD-deficient erythrocytes and slightly depleted it in normal erythrocytes. These results suggest that the hydroxy analogues, rather than primaquine itself, play a crucial role in decreasing the GSH content and metHb production in human erythrocytes.

Experimental Section

Primaquine diphosphate and 6-methoxy-8-nitroquinoline were purchased from Aldrich Chemical Co. (Milwaukee, WI). 8-Amino-6-methoxyquinoline hydrobromide (**8**) and 8-amino-6-hydroxyquinoline (**7**) were obtained through the courtesy of Sterling-Winthrop Research Institute (Rensselaer, NY). Precoated silica gel 60G TLC plates were obtained from EM Reagents or prepared with the same adsorbent from Merck. Developing solvent systems were as follows: A, chloroform/methanol, 97:3; B, benzene/methanol, 1:1. High-performance liquid chromatography (HPLC) runs were carried out on a system consisting of a Model 6000A pump, a Model 440 UV detector, and a μBondapak C_{18} RP column from Waters Associates, interfaced with a Model 4200 computing integrator from Spectra-Physics (SP). Solutions of samples (1.0 mg/mL in absolute methanol) were applied and eluted isocratically at a flow rate (FR) of 1.0 or 2.0 mL/min with a solvent mixture consisting of water/methanol (1:1) and sodium 1-heptane sulfate (0.005 M). The retention times were determined by monitoring UV absorbance at 254 nm, and the relative peak areas were calculated by the (SP) integrator at a chart speed of 0.5 cm/min. Mass spectra were obtained on Finnigan Models 3200 and 4000 automated INCOS data acquisition GC/MS instruments in the electron-impact (EI) or chemical-ionization (CI) modes or on EAI Quad 300 quadrupole mass spectrometer coupled to a Varian 4000 gas chromatograph with a glass column (2 m × 2 mm, i.d.) packed with 3% OV-17 on 100–120 mesh Gas Chrom Q and operated at a N_2 flow rate of 30 mL/min and column temperature of 240 °C. Proton NMR spectra were recorded on a Varian 390 (90 MHz) instrument. Chemical-shift values are reported in parts per million (ppm) relative to DHO at 5 ppm or relative to $(CH_3)_4Si$ as an internal reference. NMR abbreviations used are as follows: s, singlet; d, doublet; triplet; dd, doublet of doublets; m, multiplet; br, broad. Melting points were taken by capillary tubes and are uncorrected. Elemental analyses were conducted by Midwest Micro Laboratories, Inc., and Analytical Research Laboratories Inc.

The syntheses of 5,6-dihydroxy-8-aminoquinoline dihydrobromide [**5**: 83% yield; mp 277–287 °C dec; TLC R_f (solvent system B) ~0.63; HPLC, a single peak at 2.2 min (FR = 2.0)], 5-hydroxy-6-methoxy-8-aminoquinoline[14] [**6**: mp 239–241 °C dec; TLC R_f (solvent system B) ~0.66], 5,6-dimethoxy-8-[(4-amino-1-methylbutyl)amino]quinoline[13] [**10**: mp 143–144 °C (lit.[13] mp 140–142 °C)], and 8-amino-6-hydroxyquinoline dihydrobromide (**7**)[19] were achieved according to the cited literature procedures.

Trifluoroacetyl derivatives of **8** and **9** were prepared by adding an excess of trifluoroacetic anhydride (TFAA, Regis Chemical Co., Morton Grove, IL) to a methylene chloride solution of **8** and **9** and heating for 10 min at 40 °C. Excess TFAA was decomposed by the addition of 10 μL of 1-butanol. Aliquots from the CH_2Cl_2

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Table II. Effect of Primaquine and Selected Analogues on GSH Levels in Normal and G6PD-Deficient Human Erythrocytes[a]

	control		GSH level with the following concn							
			0.05 mM				0.01 mM			
			normal		deficient		normal		deficient	
no.	normal: mg %	deficient: mg %	mg %	% control	mg %	% control	mg %	% control	mg %	% control
1	60.0 ± 4.6	49.7 ± 3.9	60.0 ± 4.6	100	47.4 ± 2.8	95				
2	66.9 ± 2.9	46.7 ± 1.9	63.4 ± 3.5	95	44.8 ± 1.9	96	63.8 ± 2.7	95	45.8 ± 1.9	98
3	65.3 ± 3.0	44.7 ± 3.1	64.1 ± 3.8	98	15.6 ± 2.5	35	64.1 ± 4.8	98	15.4 ± 2.1	34
4	66.0 ± 3.1	50.5 ± 4.0	63.3 ± 3.8	96	15.1 ± 1.8	30	64.9 ± 3.2	98	18.2 ± 1.9	36
5	62.2 ± 2.5	53.1 ± 4.1	58.9 ± 3.7	95	13.2 ± 1.2	25	61.4 ± 3.1	99	16.8 ± 2.4	32

[a] Values are means and standard errors for five to eight experiments.

layer were injected to obtain the GC/MS data in the EI mode: EIMS of the ditrifluoroacetyl derivative of **8**, *m/e* (relative intensity), 270 (10.71) [M], 201 (100) [M - CF_3], 186 (13.61 [201 - CH_3], 158 (41.41) [186 - CO], 130 (67.32) [150 - CO], 103 (15.18) [130 - HCN]; EIMS of the *N*-trifluoroacetyl derivative of **9**: *m/e* (relative intensity) 286 (41.49) [M], 217 (199) [M - CF_3], 202 (54.67) [217 - CH_3], 174 (27.03) [202 - CO], 118 (10.66), [174 - (HCO, HCN)].

6-Methoxy-8-(hydroxyamino)quinoline (9). To a suspension of 30 mg of platinum oxide in 10 mL of ethyl acetate, prereduced for 5 min under 12 psi of hydrogen, was added a solution of 6-methoxy-8-nitroquinoline (3.0 g, 15 mmol) in 70 mL of ethyl acetate, and the reduction was continued at 31 psi until the theoretical amount of hydrogen was taken up in about 1 h. After cooling in a freezer, the precipitate was collected and recrystallized from acetone, providing orange crystals, mp 124.5–125 °C dec, in 58% yield. The product was homogeneous on TLC using solvent system A, with R_f ~0.30, compared to 6-methoxy-8-nitroquinoline, R_f ~0.75. The HPLC of **9** gave a single peak at 4.02 min (FR = 1.0 mL/min), which was followed by an inseparably coalesced little shoulder. This peak had exactly the same retention time as that of 8-amino-6-methoxyquinoline (**8**):[20] ^{1}H NMR (acetone-d_6) δ 2.78 (1 H, s, NH), 3.91 (3 H, s, 6-OCH_3), 6.76 (1 H, *J* = 3 Hz, H-5), 7.02 (1 H, *J* = 3.0 Hz, H-7), 7.45 (1 H, dd, *J* = 4.2 and 7.5 Hz, H-3), 8.0 (1 H, s, OH), 8.18 (1 H, dd, *J* = 2 and 9 Hz, H-4), 8.62 (1 H, dd, *J* = 1.5, 4.8 Hz, H-2); EIMS, *m/e* (relative intensity) 190 (4.09) [M], 174 (18.09), 159 (2.40), 145 (13.58), 131 (7.01), 128 (5.53), 117 (5.17), 115 (2.90), 102 (2.47), 89 (3.01). Anal. ($C_{10}H_{10}N_2O_2$) C, H, N.

8-[(4-Amino-1-methylbutyl)amino]-6-hydroxyquinoline Sulfate (2). A solution of **1** (20 g, 44.0 mmol) in 400 mL of water was made alkaline with sodium hydroxide, and the free base was extracted with ether (4 × 50 mL). The combined ether extract was dried over Na_2SO_4 and evaporated in vacuo, leaving primaquine (11 g, 0.043 mmol) as a dark brown oil. The oil was dissolved in 100 mL of 47% hydriodic acid, and the solution was stirred under nitrogen at 90–97 °C for 13 h. The excess hydriodic acid was removed in vacuo (almost 50 mL), leaving a dark brown precipitate, which after refrigeration was collected and washed with ethyl acetate, giving dull orange crystals (23 g). This product was dissolved in 50 mL of ethanol and precipitated by gradual addition of 100 mL of ether, providing orange crystals of 8-[(4-amino-1-methylbutyl)amino]-6-hydroxyquinoline dihydriodide, mp 223–226 °C dec, in 50% yield. The dihydriodide salt on TLC (solvent system B) showed several impurities, which could not be removed by recrystallization from ethanol. This product was converted to the sulfate salt by dissolving the dihydriodide salt (13.0 g, 26 mmol) in 150 mL of ethanol and adding concentrated H_2SO_4 (1.5 mL, 27 mmol). The resulting precipitate was purified by dissolving it in 10 mL of water containing 4 drops of concentrated H_2SO_4 and precipitating by gradual addition of acetone and repeating this procedure twice. Finally the product was recrystallized from 100 mL of ethanol containing 1 drop of concentrated H_2SO_4, providing yellow-orange crystals, mp 155–157 °C dec, in 24.5% yield. It gave one spot on TLC with solvent system B, R_f ~0.31 compared to the R_f ~0.40 for **1**. The HPLC of **2** gave a single peak at 4.51 min (FR = 2.0 mL/min): ^{1}H NMR (D_2O) δ 1.60 (3 H, d, *J* = 6 Hz, 5′-CH), 2.08 (4 H, m, H-2′, H-3′), 3.34 (2 H, m, H-4′), 3.88 (1 H, m, H-1′), 6.81 (1 H, s, H-7), 7.95 (1 H, dd, *J* = 4.5 and 9.0 Hz, H-3′), 8.81 (1 H, dd, *J* = 2.0 and 9.0 Hz, H-4), 8.91 (1 H, dd, *J* = 1.5 and 5.7 Hz, H-2): EIMS, *m/e* (relative intensity) 245 (1.27) [M], 228 (1.54), 188 (1.57), 187 (14.28), 110 (62.06), 133 (10.45), 132 (13.40), 131 (23.45). Anal. ($C_{14}H_{19}N_3O{\cdot}H_2SO_4$) C, H, N.

5,6-Dihydroxy-8-[(4-amino-1-methylbutyl)amino]quinoline Trihydrobromide (4). A solution of diphosphate salt of **10** (20 g, 41.3 mmol) in 400 mL of water was made alkaline, and the free base was extracted with ether (4 × 50 mL). The combined ether extract was dried over K_2CO_3, and ether was evaporated, leaving an orange oil (10.0 g, 38.3 mol). The oil was dissolved in 135 mL of 10 M HBr and stirred under argon for 5 h to ensure complete hydrolysis of both OCH_3 groups. The excess HBr was distilled off under vacuum, leaving a solidified residue. The residue was stirred in 60 mL of warm ethanol and precipitated further by the addition of 100 mL of isopropyl ether. The product was collected after overnight refrigeration, washed with 50 mL of isopropyl ether/ethanol (3:2) and then with 50 mL of isopropyl ether, and dried over P_2O_5 in a vacuum dissicator. This resulted in 74% yield of **4** as yellow-brown crystals, mp 205–209 °C shrinks, 240 °C dec. TLC (solvent system B) of **4** gives a spot at R_f ~0.14 as compared to R_f ~0.46 for the starting material **10**. The HPLC of **4** gave two peaks, one at 2.2 min (FR = 2.0 mL/min), corresponding to **5** (1%), and a major peak at 3.2 min (FR = 2.0 mL/min), which represented **4**. ^{1}H NMR (D_2O) δ 1.54 (3 H, d, *J* = 7.5 Hz, 5-CH_3), 2.05 (4 H, m, H-2′, H-3′), 3.23 (2 H, m, H-4′), 3.82 (1 H, m, H-1′), 4.11 (3 H, s, 6-OCH_3), 7.60 (1 H, s, H-7), 7.82 (1 H, dd, *J* = 4.4 and 8.4 Hz, H-3), 8.90 (1 H, dd, *J* = 2, H Hz, H-4), 9.05 (1 H, dd, *J* = 1.6 and 5.4 Hz, H-2); EIMS, *m/e* (relative intensity), 261 (1.13), 244 (5.12), 217 (2.16), 203 (6.12), 202 (14.17), 177 (2.67), 176 (13.94), 175 (3.04), 161 (9.26), 147 (1.08), 131 (2.52), 130 (4.46), 110 (3.53), 108 (3.43), 103 (5.21), 82 (50.56), 81 (36.49), 80 (71.34), 79 (43.66). An elemental analysis indicated it was probably a hydrate. Anal. ($C_{14}H_{19}N_3O_2{\cdot}3HBr{\cdot}H_2O$) C, H, N. After block drying at 120 °C for 1 min: Anal. ($C_{14}H_{19}N_3O_2{\cdot}3HBr$) C, H, N.

5-Hydroxy-6-methoxy-8-[(4-amino-1-methylbutyl)amino]quinoline Trihydrobromide (3). A solution of diphosphate salt of **10** in 250 mL of water was made alkaline, and the free base was extracted with ether (4 × 50 mL). The combined ether extract was dried over K_2CO_3 and evaporated, leaving an orange oil (5 g, 17.3 mmol). This was dissolved in 70 mL of 10 M HBr and refluxed under argon at 100 °C for 30 min. Bubbling ceased after the first 15 min, indicating that the 5-OCH_3 group was hydrolyzed. A total of 60 mL of HBr was distilled off at reduced pressure, leaving a mash. Ethanol was added, and distillation was continued until the residual volume was 20 mL. An additional 40 mL of ethanol was added, and the dark red solution was placed in the freezer at −8 °C overnight to induce crystallization. Further crystal formation was effected by the slow addition of 120 mL of isopropyl ether with stirring under argon. This provided in 55% yield a brown crystalline product that was extremely hygroscopic and air sensitive. A second batch of 6 g was obtained as a brown hygroscopic powder. TLC (solvent B) showed the product at R_f ~0.17 plus a small amount of material at the origin. The HPLC of **3** (FR = 2.0 mL/min) showed three

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peaks, a small peak at 2.2 min (0.3%), corresponding to **5**, a peak at 3.2 min (60%), which had the same retention time as that of 4, and a third peak at 4.9 min (39.5%), which was **3**. After 15 min the same sample was applied on HPLC, and the same three peaks, but with different percentages, were observed, the peak at 2.2 min (1.5%), the peak at 3.2 min (85.5%), and the peak at 4.9 min (13.2%): ^{1}H NMR (acetone-d_6) δ 1.57 (3 H, d, J = 7.5 Hz, 5'-CH_3), 2.13 (4 H, m, 2'-H, 3'-H), 3.25 (2 H, 7, 4'-H), 3.94 (3 H, s, 6-OCH_3), 4.1 (1 H, m, 1'-H), 7.63 (1 H, s, H-7), 7.81 (1 H, dd, J = 4.5 and 8.5 Hz, H-3), 8.72 (1 H, dd, J = 2.1 and 9.0 Hz, H-4), 8.95 (1 H, dd, J = 1.5 and 5.4 Hz, H-2); EIMS, m/e (relative intensity), 275 (6.18) [M], 258 (1.98, 257 (0.98), 243 (1.16), 231 (1.11), 218 (3.06), 203 (4.50), 202 (6.51), 191 (7.02), 190 (7.88), 189 (10.03), 175 (8.75), 147 (8.88), 103 (4.53), 99 (4.98), 89 (93.90), 80 (100), 79 (48.47). A sulfate salt was also quite hygroscopic. A chloroplatinate derivative precipitated from an alcoholic solution of chloroplatinic acid. Two precipitations with DMF and ethanol gave a dark red crystalline powder that sinters at 270 °C. TLC (solvent system B) showed the product at R_f ~0.60 plus traces of contaminants at R_f ~0.76 and at the origin. Anal. ($C_{15}H_{21}N_3O_2 \cdot PtCl_2$) C, H, N.

Incubations. Glucose-6-phosphate dehydrogenase (G6PD), glucose 6-phosphate (G6P), NADPH, and glutathione (GSH) were purchased from Sigma Chemical Co. Blood specimens (30–40 mL) were obtained from normal and G6PD-deficient volunteers with sodium edetate as the anticoagulant. Washed erythrocyte suspensions (50%) were prepared in buffered dextrose saline solution as described previously[2] and incubated at 37 °C with compounds 1 through 6 individually. At the end of the 2-h incubation period, the reaction was stopped by placing the flasks in an ice bath for 5 min. The erythrocytes were separated by centrifugation at 1500g for 10 min at 5 °C. They were washed twice by resuspension in ice-cold buffered saline dextrose, recentrifugation, and finally resuspended to produce a 50% mixture. Aliquots (0.1 mL) were used for measurements of MetHb and GSH levels as described previously.[2]

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Registry No. 1, 90-34-6; 2, 80038-07-9; 2·H_2SO_4, 88563-35-3; 2·2HI, 88563-36-4; 3, 57695-07-5; 3·3HBr, 57514-28-0; 3·XH_2SO_4, 88563-38-6; 3 (chloroplatinate), 88563-39-7; 4, 87321-06-0; 4·3HBr, 88563-37-5; 5, 17605-92-4; 5·2HBr, 7505-74-0; 6, 67472-57-5; 7, 7402-16-6; 8, 90-52-8; 8 [bis(trifluoroacetyl) derivative], 88563-33-1; 9, 57742-99-1; 9 (*N*-trifluoroacetyl derivative), 88563-34-2; 10, 47136-26-5; 10·2H_3PO_4, 5443-73-2; 6-methoxy-8-nitroquinoline, 85-81-4; glutathione, 70-18-8.

Acetylenic Nucleosides. 3.[1] Synthesis and Biological Activities of Some 5-Ethynylpyrimidine Nucleosides

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Iodination of 1-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)uracil furnished the 5-iodo derivative (Ib), which, on treatment with (trimethylsilyl)acetylene in the presence of catalytic amounts of $(Ph_3P)_2PdCl_2$/CuI and subsequent deblocking, afforded 1-β-D-arabinofuranosyl-5-ethynyluracil (Ie). Condensation of the trimethylsilyl derivative of 5-(dibromovinyl)uracil with 3-*O*-acetyl-5-*O*-benzoyl-2-deoxy-2-azido-D-arabinofuranosyl chloride, followed by treatment with phenyllithium, gave 1-(2-deoxy-2-azido-β-D-arabinofuranosyl)-5-ethynyluracil (IIb). Condensation of 3-*O*-acetyl-5-*O*-benzoyl-2-deoxy-2-fluoro-D-arabinofuranosyl bromide with the trimethylsilyl derivative of 5-ethynylcytosine and subsequent removal of the protecting groups furnished 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-ethynylcytosine (IIIb). The structural assignment for IIb and IIIb was made by NMR and ORD spectra. Compounds Ie and IIIb inhibited the growth of leukemia L-1210 cells in culture by 50% at concentrations of 1.7×10^{-5} and 6×10^{-5} M, respectively. In addition, Ie and IIIb inhibited the replication of herpes simplex virus type I by 90% at concentrations of 2.8×10^{-5} and 5×10^{-5} M, respectively. Compound IIb did not show any antileukemic or antiherpes activity.

Previous studies[2] showed that the nature of the substituents at the C-5, as well as the C-2', position of the pyrimidine nucleosides are important factors in the determination of biological activity. As part of our program of the design and synthesis of potential anticancer and antiviral nucleosides, we have been concerned with the synthesis of nucleosides substituted with the ethynyl function, which, in its size, is similar to the methyl group, but its electronegative effect on the pyrimidine ring closely approaches that of fluorine.[3] This report describes the synthesis and preliminary biological evaluation of 5-ethynyl derivatives of 1-β-D-arabinofuranosyluracil (Ie), 1-(2-deoxy-2-azido-β-D-arabinofuranosyl)uracil (IIb), and 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)cytosine (IIIb).

Chemistry. Acetylation of 1-β-D-arabinofuranosyluracil[4,5] with acetic anhydride in pyridine, followed by iodination[6] with iodine monochloride in methylene chlo-

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