Notes

Folate Antagonists. 4. Antimalarial and Antimetabolite Effects of 2,4-Diamino-6-[(benzyl)amino]pyrido[2,3-*d*]-pyrimidines†

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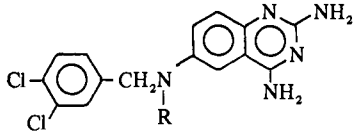
2,4-Diamino-6-[(3,4-dichlorobenzyl)amino]quinazoline (Ia),[2] 2,4-diamino-6-[(3,4-dichlorobenzyl)nitrosamino]-quinazoline (Ib),[3] and an array of related 2,4-diamino-6-[(aralkyl and heterocyclicmethyl)amino]quinazolines[2] and 2,4-diamino-6-[(aralkyl and heterocyclicmethyl)nitrosamino]quinazolines[3] exhibit interesting antimalarial, antifilarial, antitrypanosomal, and antimetabolite effects.[1-6] The present communication describes the synthesis and biological properties of several representative 2,4-diamino-6-[(benzyl)amino]pyrido[2,3-*d*]pyrimidines, including the 8-aza isosteres of Ia and Ib.

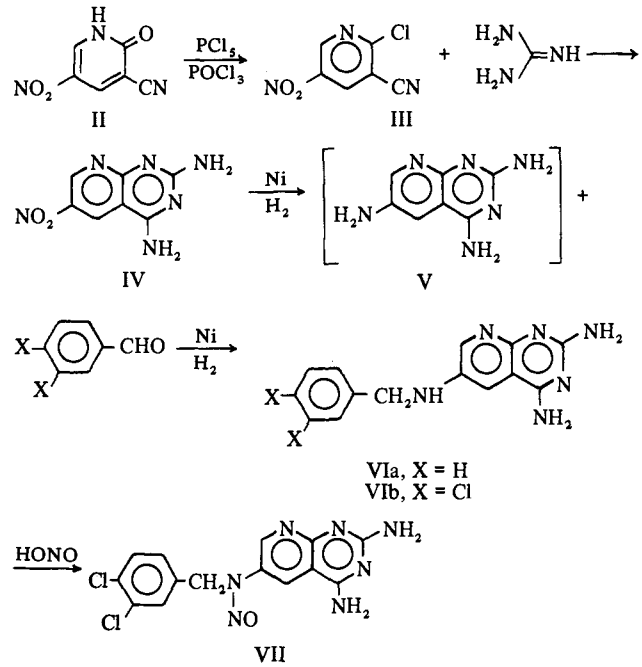


Ia, R = H
Ib, R = NO

The 2,4-diamino-6-[(benzyl)amino]pyrido[2,3-*d*]pyrimidines were prepd utilizing the route outlined in Scheme I: Thus the chlorination of 1,2-dihydro-5-nitro-2-oxonicotinonitrile (II)[7] with a mixt of PCl_5 and $POCl_3$ gave 2-chloro-5-nitronicotinonitrile (III) (78%).[7] Condensation of III with guanidine base afforded 2,4-diamino-6-nitropyrido[2,3-*d*]-pyrimidine (IV) (85%), which was hydrogenated over Raney Ni[8] at room temp in $CH_3O(CH_2)_2OH$ to provide 2,4,6-triaminopyrido[2,3-*d*]pyrimidine (V) *in situ*. Without admitting air, benzaldehyde or 3,4-dichlorobenzaldehyde was added and hydrogenation was continued to give 2,4-diamino-6-[(benzyl)amino]pyrido[2,3-*d*]pyrimidine (VIa) (50%) and 2,4-diamino-6-[(3,4-dichlorobenzyl)amino]pyrido[2,3-*d*]-pyrimidine (VIb) (58%), respectively. Nitrosation of VIb afforded 2,4-diamino-6-[(3,4-dichlorobenzyl)nitrosamino]-pyrido[2,3-*d*]pyrimidine (VII) (58%).

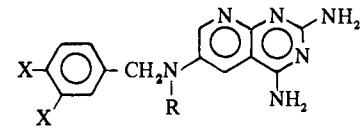
The 2,4-diamino-6-[(benzyl)amino]pyrido[2,3-*d*]pyrimidines (VIa, VIb, VII) were supplied to Dr. Paul E. Thompson and coworkers of these laboratories for evaluation against *Plasmodium berghei* in mice. As in previous work the drugs were administered continuously in the diet for 6 days to mice infected with a normal drug-sensitive strain of *P. berghei*.[4] Results (Table I) are expressed both in terms of the SD_{90} (daily dose required for 90% suppression of the parasitemia) and the quinine equiv *Q* (the ratio of the SD_{90} of quinine·HCl to the SD_{90} of the test substance under comparable exptl conditions). 2,4-Diamino-6-[(benzyl)-amino]pyrido[2,3-*d*]pyrimidine (VIa) and 2,4-diamino-6-[(3,4-dichlorobenzyl)amino]pyrido[2,3-*d*]pyrimidine (VIb) lacked significant antimalarial effects at daily doses of 325 and 171 mg/kg, respectively, and were therefore <20 times as potent as the corresponding quinazoline analog 2,4-diamino-6-[(3,4-dichlorobenzyl)amino]quinazoline (Ia).[2] 2,4-Diamino-6-[(3,4-dichlorobenzyl)nitrosamino]pyrido[2,3-*d*]-

Scheme I



†This is paper 26 of a series on antimalarial substances. For paper 25, see ref 1.

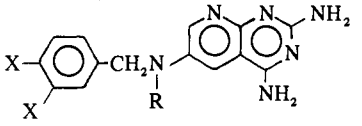
Table I. Oral Suppressive Antimalarial Effects of 2,4-Diamino-6-[(benzyl)amino]pyrido[2,3-*d*]pyrimidines and Reference Drugs against *Plasmodium berghei* in Mice



No.	X	R	SD_{90}, mg/kg per day,[a,b]	*Q*[c]
VIa	H	H	>325	<0.2
VIb	Cl	H	>171	<0.4
VII	Cl	NO	12	6.2
Ia base			8.5	8.8
Ib base			0.12	620
Pyrimethamine			0.28	270
Trimethoprim			120	0.6
Cycloguanil hydrochloride			2.1	35

[a] Compds given continuously in the diet of mice for 6 consecutive days. [b] All doses calcd as free base equiv. SD_{90} represents the daily dose (mg/kg) required for 90% suppression of the parasitemia in treated mice relative to control mice. The SD_{90} was estimated graphically using semi-log paper. [c] The quinine equiv *Q* is the ratio of the SD_{90} of quinine·HCl to the SD_{90} of the test substance under comparable exptl conditions.

Table II. Inhibitory Effects of 2,4-Diamino-6-[(benzyl)amino]pyrido[2,3-*d*]pyrimidines and Reference Drugs against *Strep. faecalis* R, *L. plantarum*, and *Strep. faecalis* A



			Concns, ng/ml, causing 50% inhibition			
			Strep. faecalis R		*L. plantarum*	*Strep. faecalis* A
No.	X	R	FA[a]	5-CHO-FAH$_4$[b]	None	FA[c]
VIa	H	H	32	300	5200	2820
VIb	Cl	H	50	>400	>4000	2800
VII	Cl	NO	29	>400	5800	2880
Ia			6	112	550	294
Ib			4	88	720	150
Pyrimethamine			4	3,100	590	680
Trimethoprim			12	70	74	284
Cycloguanil hydrochloride			8	11,400	480	560

[a] 0.4 ng/ml of FA. [b] 0.4 ng/ml of 5-CHO-FAH$_4$. [c] 500 ng/ml of FA.

pyrimidine (VII) displayed significant antimalarial effects (SD_{90} = 12 mg/kg per day, Q = 6.2), but the dose required was 100 times the SD_{90} of the quinazoline analog 2,4-diamino-6-[(3,4-dichlorobenzyl)nitrosamino]quinazoline (Ib).[3]

Antimetabolite studies with the 2,4-diamino-6-[(benzyl)amino]pyrido[2,3-*d*]pyrimidines (VIa, VIb, VII) utilizing *Streptococcus faecalis* R (*Strep. faecium* var. *durans*, ATCC 8043), *Lactobacillus plantarum* (ATCC 8014), and *Strep. faecalis* A were conducted as described previously.[2] Results with *Strep. faecalis* R (Table II) indicate that the pyrido[2,3-*d*]pyrimidines are somewhat less potent folic acid inhibitors than the corresponding quinazolines with either FA or 5-CHO-FAH$_4$ as substrates. However, the relatively small potency differential between Ib and VII against *Strep. faecalis* R correlates poorly with the large variance in antimalarial effects noted previously. The 2,4-diamino-6-[(benzyl)amino]pyrido[2,3-*d*]pyrimidines were also considerably less potent inhibitors of *L. plantarum* and the aminopterin-resistant *S. faecalis* A than the quinazoline isosteres Ia and b.

In contradistinction with 2,4-diamino-6-[(3,4-dichlorobenzyl)amino]quinazoline (Ia), 2,4-diamino-6-[(3,4-dichlorobenzyl)amino]pyrido[2,3-*d*]pyrimidine (VIb) also lacked appreciable effects against *Trypanosoma cruzi*[4] when administered by drug-diet to mice at a dose of 266 mg/kg per day for 14 days. It is concluded that the insertion of nitrogen at position 8 of the quinazoline ring results in a marked reduction in both antimalarial and antitrypanosomal effects.

Experimental Section ‡,§

2-Chloro-5-nitronicotinonitrile (III). 1,2-Dihydro-5-nitro-2-oxonicotinonitrile (II)[7] (9.1 g, 0.055 mole) was chlorinated with 23 g of PCl_5 and 11 ml of $POCl_3$ at 150° for 3 hr, and the reaction mixt was worked up utilizing the procedure of Fanta and Stein.[7] The crude product was recrystd from EtOH–H_2O (decolorizing charcoal) to give 7.9 g (78%) of product as pale tan needles, mp 120–122° (lit.[7] reports mp 121–122°).

2,4-Diamino-6-nitropyrido[2,3-*d*]pyrimidine (IV). 2-Chloro-5-nitronicotinonitrile (III) (7.9 g, 0.043 mole) was heated under reflux with guanidine base in EtOH for 2 hr. Guanidine base was prepd *in situ* by treating a soln of 8.2 g (0.045 mole) of guanidine hydrochloride in 80 ml of warm, dry EtOH with 2.0 g of Na in 55 ml of dry EtOH and removing the NaCl by filtration. The reaction mixt was then chilled, and the product was collected and recrystd from DMF–H_2O (decolorizing charcoal) to give 7.5 g (85%) of yellow needles, mp >395°. *Anal.* ($C_7H_6N_6O_2$) C, H, N.

2,4-Diamino-6-[(benzyl)amino]pyrido[2,3-*d*]pyrimidine (VIa). 2,4-Diamino-6-nitropyrido[2,3-*d*]pyrimidine (IV) (2.4 g, 0.012 mole) was suspended in 116 ml of $CH_3O(CH_2)_2OH$ and hydrogenated with Raney Ni[8] for 3 hr. The hydrogen uptake was 838 ml (2.99 moles/mole at 20°). Benzaldehyde (1.24 g, 0.012 mole) in 12 ml of $CH_3O(CH_2)_2OH$ was then added *via* the side arm, without admitting air, followed at once by 23 ml of AcOH. The suspended pale yellow solid dissolved to give a green soln. Hydrogenation was continued for 2.75 hr at room temp, during which time the H_2 uptake was 307 (1.10 mmoles/mole at 20°). The mixt was allowed to stand overnight under H_2 and filtered, and the light orange filtrate (with an intense green fluorescence) was concd *in vacuo*. The residue was crystd from EtOH–H_2O to give 2.9 g of the AcOH salt, which was suspended in 70 ml of boiling 50% EtOH and treated with 4 ml of concd NH_4OH. The mixt was chilled at 0° for 4 hr, and the product was collected, washed with 50% EtOH, and dried. Recrystn from 130 ml of 60% EtOH (decolorizing charcoal) afforded 1.5 g (50%) of the base as pale yellow laths, mp 299–300° dec, uv EtOH [λ_{max} ($\epsilon \times 10^{-3}$)] 209 (20.5), 225 sh (19.0), 254 (22.2), 286 (19.0), 403 nm (5.6). *Anal.* ($C_{14}H_{14}N_6$) C, H, N.

2,4-Diamino-6-[(3,4-dichlorobenzyl)amino]pyrido[2,3-*d*]pyrimidine (VIb). Utilizing the procedure described for the synthesis of 2,4-diamino-6-[(benzyl)amino]pyrido[2,3-*d*]pyrimidine (VIa), 4.1 g (0.02 mole) of 2,4-diamino-6-nitropyrido[2,3-*d*]pyrimidine (IV) was hydrogenated in 200 ml of $CH_3O(CH_2)_2OH$ over Raney Ni.[8] The H_2 uptake in the first stage was 1360 ml (2.84 moles/mole at 20°). To the resulting suspension was added without exposure to the air a soln of 3.5 g (0.02 mole) of recrystd 3,4-dichlorobenzaldehyde in 20 ml of warm $CH_3O(CH_2)_2OH$, followed by 40 ml of AcOH. Hydrogenation was continued at 45–50° for 5 hr during which time 485 ml (1.01 moles/mole at 20°) of H_2 was absorbed. The hydrogenation mixt was processed according to VIa to give 3.9 g (58%) of the base as yellow crystals from EtOH–H_2O, mp 321° dec (inserted at 270° with fairly rapid heating), uv EtOH [λ_{max} ($\epsilon \times 10^{-3}$)] 219 (27.3), 252 (22.2), 283 (19.0), 394 nm (6.9). *Anal.* ($C_{14}H_{12}Cl_2N_6$) C, H, N.

2,4-Diamino-6-[(3,4-dichlorobenzyl)nitrosamino]pyrido[2,3-*d*]-pyrimidine (VII). 2,4-Diamino-6-[(3,4-dichlorobenzyl)amino]-pyrido[2,3-*d*]pyrimidine (VIb) (2.2 g, 0.0065 mole) was dissolved in 13 ml of hot AcOH and treated successively with 65 ml of ice-cold DMF and 0.50 g (0.0072 mole) of $NaNO_2$ in 4 ml of H_2O. A yellow solid separated. The mixt was allowed to stand overnight at room temp, dild with 65 ml of H_2O, and made basic with 26 ml of concd NH_4OH. After 2 hr, the yellow solid that sepd was collected, washed with H_2O, stirred for 1 hr with a mixt of 80 ml of EtOH and 50 ml of 2 *N* NaOH, and dild with 250 ml of H_2O. The product was collected, washed successively with EtOH and H_2O, and dried for 4 hr at 100° *in vacuo*; yield, 1.4 g (58%), mp 326–328° dec (inserted at 280° with fairly rapid heating). *Anal.* ($C_{14}H_{11}Cl_2N_7O$) C, H, N.

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‡Melting points (uncorrected) were taken in open capillary tubes in a Townson and Mercer melting point apparatus.

§Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

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5'-*O*-Methyl Derivatives of 1-β-D-Arabinofuranosylcytosine and 1-β-D-Arabinofuranosyluracil

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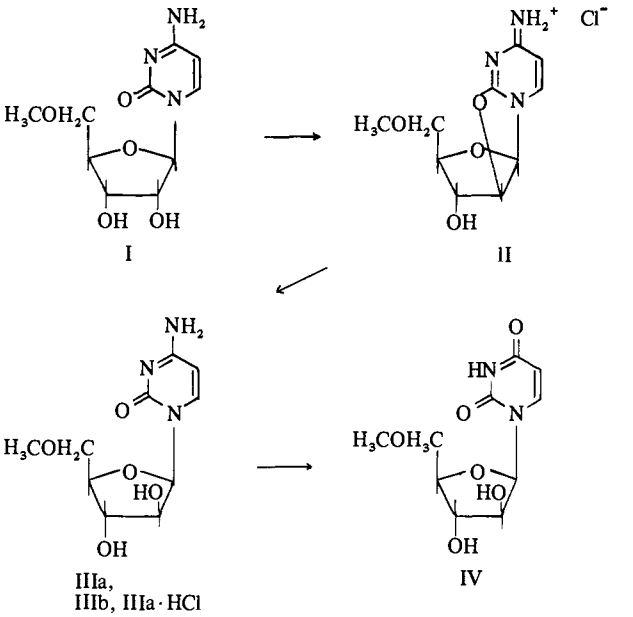
The potential utility as antimetabolites of analogs of 1-β-D-arabinofuranosylcytosine (ara-C) containing alkylated sugar hydroxyls has been discussed elsewhere,[1] and is further underlined by the recent demonstration of improved immunosuppressive and antileukemic activities exhibited by some 5'-acyl esters of ara-C.[2] We have undertaken the preparation of all possible *O'*-alkyl derivatives of ara-C, based on the observation that 1-substituted cytosine nucleosides undergo little or no ring N_3 alkylation in alkaline medium, rendering possible the preparation of the various *O'*-alkyl (methyl and ethyl) derivatives of cytidine and, by subsequent deamination, of uridine.[3,4]

The 2'-*O*-methyl and 3'-*O*-methyl derivatives of ara-U were reported by Codington, *et al.*,[5] but the methylation procedure employed was such that the products were also methylated on the ring N_3. For reasons discussed elsewhere,[1] such analogs are of limited interest as antimetabolites.

It occurred to us that an unambiguous synthesis of 5'-*O*-methyl-ara-C (III) might be feasible *via* the recently reported 5'-*O*-methylcytidine (I),[3,4] using the procedure of Kanai, *et al.*,[6] for the conversion of cytidine to ara-C *via* the 2,2'-anhydride. It was found that the conversion of I to II proceeded in almost 50% yield. The high alkaline lability of II ($t_{1/2}$ for hydrolysis at pH 10–11 about 1–2 min at room temp, the reaction being followed by the shift in λ_{max} from 262 to 271 nm and the decrease in optical density at 232 nm) suggested the use of milder conditions for this reaction. In fact, it was confirmed that hydrolysis in aqueous triethylamine simplified subsequent isolation of IIIa, which was recovered from II in 85% yield, and converted to the HCl salt.

Attempts to extend this procedure to the preparation of 3'-*O*-methyl-ara-C *via* 3'-*O*-methylcytidine were unsuccessful. A similar failure was encountered in attempts to prepare ara-C 3'-phosphate *via* cytidine 3'-phosphate. These findings suggest that the presence of a *cis*-glycol group is a prerequisite for the inversion at $C_{2'}$. Formation of an intermediate between chlorophosphoric acid and the *cis*-glycol grouping would then be followed by nucleophilic attack of the O_2 on $C_{2'}$, analogous to the mechanism proposed by Fox, *et al.*,[7] for the reaction of thiocarbonyldiimidazole with the *cis*-glycol grouping of 5-fluorouridine.

Conversion of III to 5'-*O*-methyl-ara-U (IV) profited from the observation of Notari, *et al.*,[8] on the high rate of deamination of ara-C relative to cytidine in acetate buffers and ascribed to intramolecular participation of the 2'-hydroxyl in ara-C. We have confirmed this observation and have found that, for preparative purposes, it is simpler to conduct the deamination reaction in 1 *M* AcOH on a water bath overnight, conversion of ara-C to ara-U under these conditions being quantitative. Application of this procedure to 5'-*O*-methyl-ara-C gave 5'-*O*-methyl-ara-U quantitatively.



It has been shown by Dr. M. Swierkowski that 5'-*O*-methyl-ara-C is fairly resistant to enzymatic deamination, using a highly active mouse kidney cytidine deaminase preparation which readily deaminated ara-C (*cf.* ref 1). This may account for the improved therapeutic activity noted by Gish, *et al.*,[2] for some 5'-acyl esters of ara-C.

Experimental Section

Melting points, uncorrected, were measured on a Boetius hot stage. Thin-layer chromatography made use of Merck HF_{254} silica gel, and paper chromatography was on Whatman No. 1. The following solvent systems were used with paper chromatography:[9] (A) *n*-BuOH saturated with saturated aqueous H_3BO_3; (B) isopropyl alcohol–1% H_3BO_3–NH_4OH (*d* 0.88), 7:2:1, v/v, using paper previously saturated with 1% H_3BO_3; (C) *n*-BuOH–AcOH–H_2O, 5:2:3, v/v; (D) isopropyl alcohol–NH_4OH (*d* 0.88)–H_2O, 7:1:2, v/v) (E) *n*-BuOH–H_2O, 84:16, v/v. Uv absorption spectra were run on a Zeiss (Jean) VSU-2P and on a Perkin-Elmer Model 450 recording instrument.

2,2'-Anhydro-5'-*O*-methylcytidine (II). To a suspension of 580 mg (2 mmoles) of 5'-*O*-methylcytidine hydrochloride (I),[3,4] in 70 ml of EtOAc, was added 6 ml of partially hydrolyzed phosphorus oxychloride ($POCl_3/H_2O$ = 1, mole/mole), the mixture was heated at the boiling point for 2.5 hr and then added to 250 ml of water (with ice), and the whole was stirred for 1 hr to completely hydrolyze the $POCl_3$. EtOAc was then removed under reduced pressure, and the aqueous phase deposited on a 43 × 2.6 cm column of Dowex 50W (H^+) 200–400 mesh. The column was washed with water until the effluent was neutral, and the product then eluted with 1.5 l. of 1 *M* pyridine–HCOOH buffer (pH 4). The eluate was brought to dryness, water was added to the residue, and it was again evaporated to dryness. This was repeated several times to remove traces of pyridine. The resulting glassy solid was dissolved in 20 ml of water and 1 *M* HCl added to give a strong acid reaction. The solution was brought to dryness, and the residue evaporated several times with water to remove excess HCl and evapd from 80% EtOH to give small, colorless needles, which, on recrystallization from 96% EtOH, gave 310 mg (48%) of the HCl salt of 2,2'-anhydro-5'-*O*-methylcytidine: mp 254–256°, dec at 257°; uv_{max} (pH 2–7) 231 nm (ε 9500), 263 (10,300). The product was chromatographically homogeneous in solvents A, C, and E (R_f 0.14, 0.65, 0.23) and gave a negative reaction with periodate.

1-(5-*O*-Methyl-β-D-arabinofuranosyl)cytosine (IIIa). A solution of 350 mg (1.27 mmoles) of II in 10 ml of 10% aqueous triethylamine was left overnight at room temp. The solution was brought to dryness, dissolved in 250 ml of water, and deposited on a 32 × 2.2 cm column of Dowex 50W (H^+), 200–400 mesh, which was washed with water until the effluent was neutral. The product was

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