TABLE II

In Vitro ANTIMICROBIAL ACTIVITY, MIN INHIB CONCN, μg/ml

Compd	SG[a]	ST	SA	SAG	BS	EC	AF	CA
1	0.1	<0.01	<0.01	0.1	1	1	10	10
3		1	100		100	10		1
5	1	1	1	1	0.1	1	100	>100
6	1	1	0.1	1	1	1	10	10
7	1	1	1	0.1	1	10	100	100
8	10	10	1	1	1	10	10	10
12		100	10		1	100		100
14		>100	100		100	>100		>100
15	>100	>100	>100	>100	>100	>100	>100	>100
16	100	100	10	100	10	100	100	>100
17	100	100	>100	100	100	>100	1	10
18	1	10	1	1	1	10	100	100

[a] SG = *Salmonella gallinarum*, ST = *S. typhimurium*, SA = *Staphylococcus aureus*, SAG = *Streptococcus agalactiae*, BS = *Bacillus subtilis*, EC = *Escherichia coli*, AF = *Aspergillus fumigatus*, CA = *Candida albicans*.

Salmonella choleraesuis infection in mice. In this test **1** and **5** were the most active of the series, but less active than **17** and **18**. The α-halogenated derivatives **3**, **4**, **6**, and **8** showed less *in vivo* antibacterial activity. Thus, the enhanced *in vitro* activity did not extend to an *in vivo* system. Replacement of a 5-nitro-2-furyl with a 5-nitro-2-thienyl group also decreased *in vivo* activity. Insufficient data are available, at present, to discuss the effect of the vinyl group on the toxicity of the series.

Surprisingly, **5** showed appreciable anticoccidial activity against *Eimeria acervulina* and *E. maxima* in chickens when administered in the feed at high levels (0.033%). The activity fell off rapidly at lower levels (0.0165, 0.0085, and 0.004%). This was particularly true with *E. acervulina* where activity was measurable only at 0.033%. Compound **5** also gave measurable protection against a mixed infection of *E. adenoeides* and *E. melagrimitis* in turkeys. This pattern of activity is in contrast to that of **18** which showed no anticoccidial activity at any level.

Experimental Section[8]

Starting Materials.—β-(5-Nitro-2-furyl)acrolein,[5] β-(5-nitro-2-furyl)-α-bromoacrolein,[6] β-(5-nitro-2-thienyl)acrolein,[4] β-(5-nitro-2-thienyl)-α-bromoacrolein,[4] and hydroxylamines,[2] not commercially available, were prepared by the methods described in the literature.

α-[2-(5-Nitro-2-heteroaryl)vinyl- or 1-bromovinyl]-*N*-substituted Nitrones. Method A.—The *N*-alkylhydroxylamine·HCl (0.01 mole) was added portionwise to a warm soln of β-(5-nitro-2-furyl)acrolein (0.01 mole) in abs EtOH (20 ml) contg $NaHCO_3$ (0.015 mole) and stirred overnight at room temperature. The mixture was worked up in the usual manner[2] and the results are shown in Table I.

Method B.—The same procedure as above was used except that 5 molar equiv of hydroxylamino alcohol oxalate was used.

Method C.—A mixture of β-(5-nitro-2-furyl)acrolein (0.005 mole) and the N-substituted hydroxylamine (0.005 mole) in C_6H_6 (20 ml) (**13**, **14**, **15**) or in a mixture (64 ml) of THF–EtOH (5 : 2) (**16**) was refluxed 0.5 hr using a Dean–Stark water separator. After cooling, the solid was filtered off and recrystd to afford the orange–red to red nitrones (Table I).

Acknowledgment.—We are indebted to W. E. Meredith, D. R. Filson, J. R. Challey, and C. A. Johnson for the biological data.

(8) Melting points were taken in open capillary tubes using a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich. Evaporation of solvents was done under reduced pressure using a rotary evaporator

Novel Substrate of Adenosine Deaminase[1a]

HOWARD J. SCHAEFFER,*[1b] S. GURWARA, ROBERT VINCE, AND S. BITTNER

Department of Medicinal Chemistry, State University of New York at Buffalo, Buffalo, New York 14214

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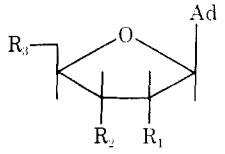
The structural features that determine whether a compound will be a substrate or an inhibitor of an enzyme are often difficult to untangle. For calf intestinal mucosal adenosine deaminase, it is well known that a variety of changes can be made in the carbohydrate moiety of adenosine analogs without loss of substrate activity.[2] For example, in addition to the 9-β-D-ribo configuration of adenosine (**1**), it is known that 9-β-D-arabino-, -xylo-, and -lyxofuranosyladenines (**2**, **3**, **4**) are all substrates of adenosine deaminase.[2] Furthermore, the 2′- and 3′-OH groups of adenosine do not play a critical role in substrate activity for it has been found that 2′-deoxy-, 3′-deoxy-, and even 2′,3′-dideoxyadenosine (**5**, **6**, **7**) undergo deamination with adenosine deaminase.[2] The 5′-OH group of adenosine and its analogs does, however, play a special role in the deamination reaction since the 5′-deoxynucleosides of adenine (**8**) do not undergo deamination with adenosine deaminase unless a properly positioned OH group is present at C-3′ as in 9-(5-deoxy-β-D-xylofuranosyl)adenine (**9**) such that the 3′-OH group can assume the function of the 5′-hydroxyl group of adenosine.[2a,d,f]

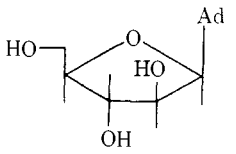
Based on these observations, we decided to synthesize an acyclic analog of adenosine containing several functional groups which appear to be important for substrate activity. The compound selected for preparation was 9-(2-hydroxyethoxymethyl)adenine (**10**).

(1) (a) This work was supported by Grant T-337D from the American Cancer Society and by a Public Health Service Training Grant 5-T1-GM-00555 from the Division of Medical Sciences, Bethesda, Md. (b) Wellcome Research Laboratories, Burroughs Wellcome & Co., Research Triangle Park, North Carolina 27709.

(2) (a) R. H. Shah, H. J. Schaeffer, and D. H. Murray, *J. Pharm. Sci.*, **54**, 15 (1965). (b) J. G. Cory and R. J. Suhadolnik, *Biochemistry*, **4**, 1729 (1965). (c) A. Coddington, *Biochim. Biophys. Acta*, **99**, 442 (1965). (d) J. L. York and G. A. LePage, *Can. J. Biochem.*, **44**, 331 (1966). (e) S. Frederiksen, *Arch. Biochem. Biophys.*, **113**, 383 (1966). (f) A. Bloch, M. J. Robins, and J. R. McCarthy, Jr., *J. Med. Chem.*, **10**, 908 (1967). (g) B. M. Chassy and R. J. Suhadolnik, *J. Biol. Chem.*, **242**, 3655 (1967). (h) D. H. Murray, J. Prokop, and A. Bloch, *J. Pharm. Sci.*, **58**, 1275 (1969).



1. $R_1, R_2, R_3 = OH$
5. $R_1 = H; R_2; R_3 = OH$
6. $R_2 = H; R_1; R_3 = OH$
7. $R_1, R_2 = H; R_3 = OH$
8. $R_1, R_2 = OH; R_3 = H$



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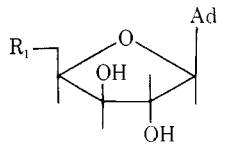
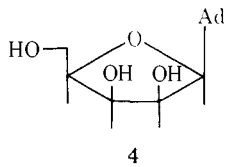
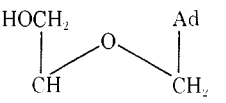


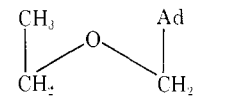
3. $R_1 = OH$
9. $R_1 = H$



4



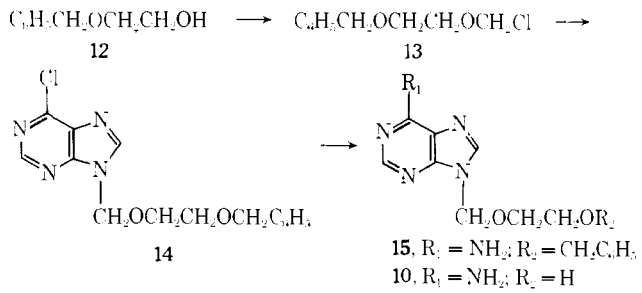
10



11

Furthermore, in order to assess the importance of the terminal OH of the 9 substituent of **10** on substrate activity, the deoxy derivative, 9-ethoxymethyladenine (**11**) was prepared.

The synthesis of **10**, which contains a glycosidic linkage, was initiated by converting 2-benzyloxyethanol into **1**-benzyloxy-2-chloromethoxyethane. Condensation of the chloro ether **13** with 6-chloropurine followed by reaction of the condensation product with methanolic NH_3 gave the protected adenine derivative (**15**). Catalytic hydrogenolysis of the benzyl group of **15** gave the desired 9-(2-hydroxyethoxymethyl)adenine (**10**). The 6-methylamino analog was prepared by allowing the 6-chloro derivative to react with $MeNH_2$ followed by catalytic hydrogenolysis of the benzyl group to give 6-methylamino-9-(2-hydroxyethoxymethyl)purine. Finally, the synthesis of 9-ethoxymethyladenine (**11**) was accomplished by a similar procedure in which 6-chloropurine was condensed with chloromethyl ethyl ether followed by conversion with methanolic NH_3 into the adenine derivative.



12 → 13

14

15. $R_1 = NH_2; R_2 = CH_2C_6H_5$
10. $R_1 = NH_2; R_2 = H$

Enzymatic evaluation of **10** with adenosine deaminase revealed that it was a substrate with a K_m[3] of 1.3×10^{-4} M whereas the V_{max} for **10** is only 1.4% that of adenosine. However, when the deoxy analog **11** was evaluated, it did not exhibit substrate activity and was, in fact, a relatively weak reversible inhibitor[4] of adenosine deaminase. Compound **10** can assume a conformation which is superimposable with the conformation of adenosine with respect to the adenine group, C-1′, ether oxygen, C-4′, C-5′, and the 5′-OH. Presumably, it is through this conformation that **10** complexes to the enzyme to act as a substrate of adenosine deaminase. Thus, based on the observation of the special role played by the 5′-OH for substrate activity in certain adenosine analogs, it has been possible to prepare an acyclic analog of adenosine with substrate activity for calf intestinal mucosal adenosine deaminase.

(3) For comparison, the K_m of adenosine is 3.0×10^{-5} M.
(4) The $(I/S)_{0.5}$ of **11** is 3.1.

Experimental Section[5]

9-Benzyloxyethoxyethyladenine (15).—A mixt of 2-benzyloxyethanol (9.88 g, 0.065 mole) and trioxymethylene (2.00 g, 0.022 mole) in dichloroethane (10 ml) was satd with dry HCl over a period of 2.5 hr with cooling (0°). The resulting mixt was dried ($MgSO_4$) and filtered and the solvent evapd *in vacuo*. A white oil was obtained which could not be distd *in vacuo* because of its decompn to $PhCH_2Cl$. The product (12.1 g, 93%) showed a distinctive etheric absorption in the ir. To a soln of 6-chloropurine (4.63 g, 0.03 mole) and Et_3N (3.33 g, 0.033 mole) in DMF (80 ml) was added the crude 1-benzyloxy-2-chloromethoxyethane (6.00 g, 0.03 mole). An exothermic reaction took place and Et_3NHCl immediately pptd. The reaction mixt was kept at room temp for 24 hr and filtered. The filtrate was evapd *in vacuo*, and the oily residue was chromatographed on silica gel (150 g, 100–200 mesh, column 29.5 mm id). The column was eluted with $CHCl_3$, and 55 100-ml fractions were collected. The solvent was then changed to 1% MeOH in $CHCl_3$ and another 15 100-ml fractions were collected. Eluent fractions 17–59 were combined and the solvent was evapd *in vacuo* to give an oil (**14**) which could not be crystd; yield, 4.2 g (44%).

A portion of the oily 6-chloro-9-benzyloxyethoxymethylpurine (250 mg, 0.78 mmole) was dissolved in 20 ml of methanolic NH_3 (20%) and heated in a stainless steel bomb at 95 ± 2° for 20 hr. On cooling, a white precipitate formed which was collected by filtration and recrystd from EtOH and then from *i*-PrOH; yield of **15**, 210 mg (90%); mp 180–181°. *Anal.* ($C_{15}H_{19}N_5O_2$) C, H, N.

9-Hydroxyethoxymethyladenine (10).—9-Benzyloxyethoxymethyladenine (400 mg, 1.34 mmoles) was dissolved in AcOH (100 ml) contg 200 mg of 5% Pd/C. The soln was hydrogenated for 20 hr, under an initial pressure of 4.2 kg/cm². The catalyst was filtered off over a Celite pad and the AcOH evaporated *in vacuo*. The remaining oil was washed several times with $CHCl_3$ and dried *in vacuo*. The crude product (150 mg, 54%) was recrystd twice from *i*-PrOH-C_6H_{14}, then from Me_2CO-C_6H_{14}, and then from Me_2CO, mp 198–199°. *Anal.* ($C_8H_{11}N_5O_2$) C, H, N.

6-Methylamino-9-(benzyloxyethoxymethyl)purine·HCl.—6-Chloro-9-(benzyloxyethoxymethyl)adenine (700 mg, 2.20 mmoles) was dissolved in EtOH (10 ml) and 15 ml of $MeNH_2$ in H_2O (40%) was added. The mixt was heated in a stainless-steel bomb for 20 hr at 95 ± 2°. The clear soln was concd *in vacuo* and the remaining material was dissolved in *i*-PrOH. Addition of dry Et_2O caused the pptn of $MeNH_2$·HCl which was filtered off. To the filtrate concd HCl (0.5 ml) was added, then the heavy ppt filtered off. The methylamino compound (0.65 g, 84%) was recrystallized from *i*-PrOH, mp 124–125°. *Anal.* ($C_{16}H_{20}ClN_5O_2$) C, H, N, Cl.

6-Methylamino-9-(2-hydroxyethoxymethyl)purine·HCl.—6-Methylamino-9-(benzyloxyethoxymethyl)purine·HCl (400 mg, 1.15 mmoles) was dissolved in glacial HOAc (100 ml) contg 200 mg of 5% Pd/C. The mixt was hydrogenated for 20 hr under an initial pressure of 4.2 kg/cm². The catalyst was removed by filtration through a Celite pad and the HOAc was evapd *in vacuo*. The remaining oil was crystd from *i*-PrOH contg 5%

(5) The melting points, unless noted otherwise, were taken in open capillary tubes on a Mel-Temp block and are uncorrected. All analytical samples had ir and uv spectra compatible with their assigned structures and moved as a single spot on tlc on Brinkman silica gel. When analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. The analyses were performed by Galbraith Microanalytical Laboratories, Knoxville, Tenn.

HCl. The product (0.17 g, 60%) was recryst'd from *i*-PrOH, mp 300–304° dec. *Anal.* ($C_7H_{14}N_5O_2Cl$) C, H, N, Cl.

6-Chloro-9-(ethoxymethyl)purine.—To a suspension of 3.85 g (25.0 mmoles) of 6-chloropurine in 75 ml of DMF contg 8.0 ml (55.0 mmoles) of Et_3N was added 2.50 g (27.5 mmoles) of chloromethyl ethyl ether. After stirring the reaction mixt for 36 hr at 55–60°, it was cooled in ice and filtered. The filtrate was concd *in vacuo* to give a syrup which was chromatographed on 150.0 g of silica gel (200 mesh, column 3.0 cm i.d.) with a solvent of $CHCl_3$–MeOH (9.5:1) to give the crude product; yield, 3.12 g (56.3%), mp 72–75°. The analytical product was obtained by recrystg the crude material from Et_2O; yield, 2.26 g (40.8%); mp 79–81°. *Anal.* ($C_8H_9ClN_4O$) C, H, Cl, N.

9-Ethoxymethyladenine.—A soln of 0.64 g (3.0 mmoles) of 6-chloro-9-(ethoxymethyl)purine in 35.0 ml of 25% MeOH–NH_3 was heated at 60–70° for 18 hr in a bomb. The reaction mixt was evapd to dryness and the residue was extd with hot Me_2CO (2 × 15 ml). The Me_2CO ext was evapd *in vacuo* to give the crude product; yield 0.53 g (91.4%); mp 184–188°. One recrystn from Me_2CO and one from MeOH gave the pure product; yield, 0.49 g (84.5%); mp 190–192° (188° softens). *Anal.* ($C_8H_{11}N_5O$) C, H, N.

Reagents and Assay Procedures.—Adenosine deaminase (Type I, calf intestinal mucosa) was purchased from the Sigma Chemical Co. The enzyme experiments were performed at pH 7.6 in 0.05 *M* phosphate buffer at 25°. The K_m was determined by the procedure of Lineweaver and Burk.[6] The assay for the study of reversible inhibitors has previously been described[2a] and is a modification of the procedure of Kaplan[7] based on the work of Kalckar.[8]

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Xanthoquininic Acid Derivatives

LEONARD M. RICE,* BHAGVANDAS S. SHETH, THEODORE B. ZALUCKY, AND J. W. WHEELER

College of Pharmacy and Department of Chemistry, Howard University, Washington, D. C. 20001

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Some 4-aminoquinolines have a high activity in the treatment of bronchial asthma[1a–c] and malaria, as well as having antiarrhythmic[2a–c] properties. Compound I, R = CH_3, X = 2, has antiasthmatic, antihistaminic, and antiarrhythmic activity but no antimalarial activity, whereas I, X = CCCC(C), R = C_2H_5, and chloroquine have good antimalarial activity. The latter also has antiasthmatic and antiarrhythmic activity. Thus as part of an earlier study we were interested in the modification of the quinoline nucleus to produce compds of type IIc and IId.

The starting point in our synthesis was quininic acid (IIa) which was demethylated to xanthoquininic acid IIb[3] and converted into its Et ester. This phenol was then subjected to the Mannich reaction to produce the 5-substituted derivs of type IIc. The 6-dialkyaminoalkoxy derivs IId were prepared from the Na salt of the phenol with the appropriate dialkylaminoalkyl halides.

(1) (a) C. F. Geschickter, *Southern Med. J.*, **48**, 497 (1955). (b) P. Blanc, *Praxis*, **5**, 127 (1962). (c) R. C. Young, A. J. Murry, C. Carr, and K. A. Harden, *J. Nat. Med. Ass.*, **57**, 189 (1965).
(2) (a) D. M. Aviado, private communication, 1968. (b) M. A. Silver and D. M. Aviado, *Exp. Parasitol.*, **24**, 152 (1969). (c) J. W. Lawson, *J. Pharmacol. Exp. Ther.*, **160**, 22 (1968).
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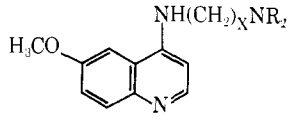
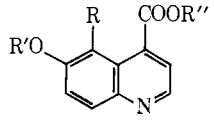
During this work we treated 6-hydroxyquinoline-4-carboxylic acid Et ester with Br in AcOH. This readily



I

a, R = R″ = H; R′ = CH_3
b, R = R′ = H



II

c, R = secondary amino CH_2; R′ = H; R″ = C_2H_5
d, R = H; R′ = dialkylaminoalkyl; R″ = C_2H_5

introduced one Br atom and the product pptd out as the bromide·HBr. When boiled in H_2O this compd lost HBr and gave the free base. Introduction of the Br atom at the 5 position was confirmed by the pmr spectrum which exhibited two AB quartets in the aromatic region with $J_{2,3}$ = 4.3 Hz and $J_{7,8}$ = 9.2 Hz consistent with other quinoline derivatives.[4]

All compds prepared are listed in Table I. When evaluated for antiarrhythmic,[2a–c] antimalarial,[5] and antiasthmatic[1a–c] activity none of the compds showed any appreciable activity.

Experimental Section

Elemental microanalyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. Melting points of hydrates were taken on a Fisher-Johns block, those of nonhydrates in a Thomas-Hoover capillary type apparatus. Melting points are corrected. Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values.

6-Hydroxyquinoline-4-carboxylic Acid, Xanthoquininic Acid.—Quininic acid (6-methoxyquinoline-4-carboxylic acid, mp 275–278°, 101.6 g, 0.5 mole) was refluxed in 300 ml of HI, sp gr 1.7, for 12 hr. The reflux mixt was concd to 0.5 its vol, dild several times its vol with H_2O, made alk with 10% NaOH, treated with Norit A, and filtered. With stirring and cooling, the filtrate was made slightly acid with AcOH. Crystn started and was completed on standing in the refrigerator overnight. The pale yellow material was filtered, resuspended in H_2O, boiled for a few min, and filtered hot. The microcryst residue (88 g, 93%) was nearly colorless, mp 339–342° dec (lit. mp 320° dec.)[3] On recrystn from DMF–H_2O, or redissolving in alk and repptg with AcOH, a colorless product was obtained which decompd at 340–342°. If placed in the oil bath at 300° decompn points as high as 347–348° were obtained. *Anal.* Calcd for $C_{10}H_7NO_3$: C, H, N.

6-Hydroxyquinoline-4-carboxylic Acid, Et Ester.—Xanthoquinic acid (94.5 g, 0.4 mole) was placed in a 1-l. flask, and a mixt of 450 ml of abs EtOH and 50 ml of concd H_2SO_4 was added. The whole was refluxed for 8 hr, most of the EtOH was removed at the aspirator, and the viscous dark red-brown mixt was poured with stirring into 1 l. of ice H_2O. With cooling and stirring, the mixt was neturalized with 20% NaOH and placed in the refrigerator overnight. The pale yellow ppt was filtered and washed co-

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(5) T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967).