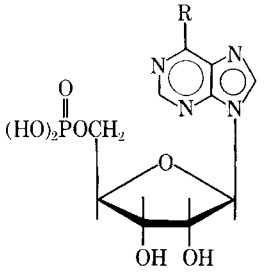
Irreversible Enzyme Inhibitors 98

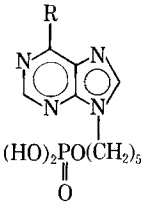
Hydrophobic Interactions with Succinoadenylate Kinosynthetase and 9-Substituted Adenines

By B. R. BAKER and EDWARD H. ERICKSON

Adenines substituted at the 9-position with *n*-butyl or benzyl groups were complexed about tenfold better to succinoadenylate kinosynthetase than 9-methyladenine. The same *n*-butyl or benzyl substituents on 6-mercaptopurine failed to show this hydrophobic interaction even though 5′-adenylate and thioinosinate are complexed equally well to the enzyme. It is proposed that either (*a*) 6-mercaptopurine can complex in only one way to the inosinate binding locus regardless of the 9-substituent, whereas adenine can complex to the inosinate locus as one of two rotomers dependent upon whether the 9-substituent is hydrophilic or hydrophobic, or (*b*) the adenines complex at the inosinate binding locus, but the 6-mercaptopurines complex at the cofactor (guanosine-5′-triphosphate) binding locus.

SUCCINOADENYLATE kinosynthetase is an anabolic enzyme that condenses inosinate (I) with L-aspartate to form succinoadenylate (II) where



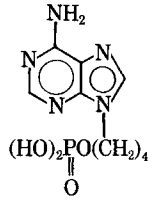


I, R = OH
II, R = L-NHCH(COOH)CH_2COOH
III, R = NH_2
IV, R = SH

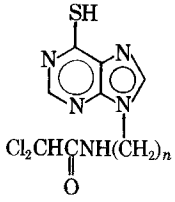
V, R = NH_2
VI, R = SH

guanosine-5′-triphosphate is used as the cofactor (1–4). The enzyme can be inhibited by 5′-adenylate (III) (3, 4) and by thioinosinate (IV) (4–7). The relative contribution of the functional groups of the ribofuranose moiety of III and IV to binding to the enzyme has been previously reported by Baker and Tanna (4, 8). The phosphate group contributed more than all the other oxygen functions combined; only a thirteenfold loss in binding occurred with the pentanol phosphates V and VI compared to III and IV, while the corresponding nucleosides were less than one-sixtieth as effective as III and IV.

In a further study (4, 9), the conformational aspects of binding of V and VI were considered. It was predicted that the butanol phosphate (VII) should bind better than the pentanol phosphate (V); the staggered ground state conformation of VII should position its phosphate group closer to the binding point for the phosphate of the nucleotide III than does the pentanol phosphate (V). If the dichloroacetamido group was simulating the binding of the phosphate group (10), it was predicted that IX should have a better ground state conformation for optimum binding than VIII (4, 9). The emergence of hydrophobic bonding (11) to such enzymes as adenosine deaminase (12), dihydrofolic reductase (13), thymidine phosphorylase (14), guanine deaminase (15), and others (11) suggested the possibility that V, VI, and VIII might have additional binding to succinoadenylate due to a hydrophobic interaction by part of the pentyl group. Therefore additional studies were performed on the mode of binding of the pentyl group of V, VI, and VIII; the results are reported in this paper, along with inhibition studies on VII and IX.



VII



VIII, $n = 5$
IX, $n = 3$

DISCUSSION

Enzyme Results—In Table I are listed the inhibition results with adenine-9-pentanol phosphate (V) and adenine-9-butanol phosphate (VII); note that the binding of the two compounds is essentially the same. If the phosphate group of V and VII is indeed being complexed to the same locus on the enzyme that complexes the 5′-phosphate of 5′-adenylate (III), then it is apparent that there is no

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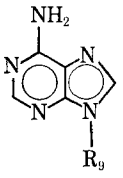
Previous paper: Baker, B. R., *J. Med. Chem.*, **10**, September(1967). Previous paper on this enzyme: see *Reference 9*.
The assistance of Ann Jaqua with the assays in Tables I–III is acknowledged.

TABLE I—INHIBITION OF SUCCINOADENYLATE KINOSYNTHETASE[a] BY



Compd.	R_9	m*M* Concn.	% Inhibition	Estimated Concn. for 50% Inhibition	Source
III	RP[b]	0.068	50	0.068[c]	NBC
V	$-(CH_2)_5OPO(OH)_2$	0.97	50	0.97[c]	(8)
VII	$-(CH_2)_4OPO(OH)_2$	1.05	50	1.05	Exptl.
X	$-CH_3$	5.8	50	5.8	(30)[d]
XI	$-C_4H_9$-*n*	0.75	50	0.75	CCNSC
XII	$-C_5H_{11}$-*i*	3.5	50	3.5	Exptl.
XIII	Cyclopentyl	3.0	33	6.0	Schaeffer
XIV	Cyclohexyl	0.25[e]	0	>1.0[f]	Montgomery
XV	$-C_6H_5$	0.5[e]	0	>2.0[f]	(31)[d]
XVI	$-CH_2C_6H_5$	0.51	50	0.51	Schaeffer
XVII	$-(CH_2)_2C_6H_5$	1.1	50	1.1	Exptl.
XVIII	$-(CH_2)_3C_6H_5$	1.7	50	1.7	Exptl.

[a] Succinoadenylate kinosynthetase was isolated from *E. coli* B and assayed with 30.6 μ*M* IMP, 50 μ*M* GTP, and 3.76 μ*M* L-aspartate in pH 7.4 Tris buffer in the presence of 10% *N*,*N*-dimethylformamide as previously described (8). [b] RP = 5-phospho-β,D-ribofuranosyl. [c] Data from *Reference 8*. [d] Prepared in this laboratory by W. F. Wood for another project by the method of Montgomery and Temple (25, 26). [e] Near maximum solubility. [f] Since 25% inhibition is readily detectable, the concentration for 50% inhibition is greater than four times that measured.

difference in the energy required for the butyl and pentyl group to assume the proper conformation; the ground state conformation of the alkylene group of V and VII is not necessarily staggered in aqueous solution as it is in nonaqueous solution (16), but may be slightly coiled due to internal hydrophobic bonding. Since it was found that 9-butyladenine (XI) can bind slightly better than the phosphate (V and VII) by a hydrophobic interaction of the *n*-butyl group with the enzyme, it is possible that V and VII show no phosphate binding of the 9-side chain, but only hydrophobic bonding of the alkyl portion.

The nature of the hydrophobic interaction of the enzyme with adenines bearing a hydrocarbon moiety at the 9-position was then investigated. Note that the 9-butyl group of XI gives an eightfold increment in binding over the 9-methyl group of X. Since the isoamyl (XII), cyclopentyl (XIII), and cyclohexyl (XIV) groups were complexed considerably poorer than *n*-butyl (XI), it appeared that chain branching of these types does not give a good fit to the hydrophobic region of this enzyme; these results are different from those observed with hydrophobic interactions with dihydrofolic reductase (13), where isoamyl is complexed better than *n*-butyl, cyclohexyl is equal to *n*-butyl, and cyclopentyl is poorer than *n*-butyl (17).

Possible hydrophobic interaction with succinoadenylate kinosynthetase by phenylalkyl groups on the 9-position of adenine was then investigated (Table I). The in-plane phenyl group of XV gave no useful amount of hydrophobic bonding. In contrast, the 9-benzyl group of XVI gave the best hydrophobic bonding in Table I, the increment in binding being elevenfold better than the 9-methyl of X. That maximum hydrophobic bonding by an aralkyl group was obtained with the 9-benzyl group of XVI was indicated by the poorer binding of the higher homologs, phenethyl (XVII) and phenylpropyl (XVIII). A further study that might lead to better inhibitors of this enzyme should be performed by substitution on the benzene ring of 9-benzyladenine (24).

Hydrophobic interaction was then investigated with 6-mercaptopurine bearing hydrocarbon groups at the 9-position (Table II). In contrast to the adenine series in Table I, the 9-butyl (XX) and 9-benzyl-6-mercaptopurine (XXIV) did not show detectable hydrophobic interaction. These results are indeed surprising in view of the facts that adenine and 6-mercaptopurine substituted by the 9-ribosyl phosphate moiety (III, IV) bind nearly identically—as do the two purines substituted by the pentanol phosphate moiety (V, VI).

These discrepancies indicated that 9-alkyladenines and 9-alkyl-6-mercaptopurines did not complex to the enzyme in the same way. Three possibilities were considered as follows.

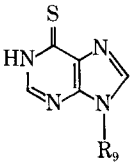
(*a*) Adenines bearing a 9-hydrocarbon group might be complexed to the enzyme locus that normally complexes the cofactor, guanosine-5′-triphosphate, whereas 6-mercaptopurines bearing a 9-hydrocarbon group might bind at the inosinate binding locus.

(*b*) The converse of (*a*) might be possible where the adenines bind at the inosinate locus and the mercaptopurines at the guanosine-5′-triphosphate locus.

(*c*) Both the adenines and the 6-mercaptopurines bind at the inosinate locus, but in different rotomeric configurations depending upon the 9-substituent.

To help differentiate between these explanations, the inhibition by guanines bearing a hydrocarbon group at the 9-position was investigated and the results are shown in Table III. Note that guanine substituted by 9-methyl (XXVI), 9-phenyl (XXIX),

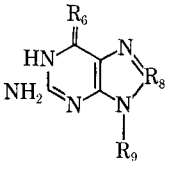
TABLE II—INHIBITION OF SUCCINOADENYLATE KINOSYNTHETASE[a] BY



Compd.	R_9	mM Concn.	% Inhibition	Estimated Concn. for 50% Inhibition	Source
IV	RP[b]	0.070	50	0.070[c]	Montgomery
VI	$-(CH_2)_5OPO(OH)_2$	0.83	50	0.83[c]	(8)
VIII	$-(CH_2)_5NHCOCHCl_2$	0.96[d]	19	4.0[e]	(9)
XIX	$-(CH_2)_5OH$	1.2[d]	30	2.8[e]	(9)
IX	$-(CH_2)_3NHCOCHCl_2$	1.0[d]	0	>4.0[e]	Exptl.
XX	$-C_4H_9$-*n*	1.0[d]	0	>4.0[e]	CCNSC
XXI	$-C_5H_{11}$-*i*	1.0[d]	0	>4.0[e]	CCNSC
XXII	Cyclohexyl	0.25[f]	0	>1.0[e]	Montgomery
XXIII	$-C_6H_5$	0.33[f]	0	>1.3[e]	CCNSC
XXIV	$-CH_2C_6H_5$	0.25[f]	0	>1.0[e]	CCNSC
XXV	$-CH_2C_6H_4COOH$-*p*	0.83	50	0.83[c]	(9)

[a] See Table I for assay conditions. [b] RP = 5-phospho-β,D-ribofuranosyl. [c] Data from *Reference 9*. [d] Maximum concentration allowing full light transmission. [e] Since 25% inhibition is readily detectable, the concentration for 50% inhibition is greater than four times that measured. [f] Near maximum solubility.

TABLE III—INHIBITION OF SUCCINOADENYLATE KINOSYNTHETASE[a] BY

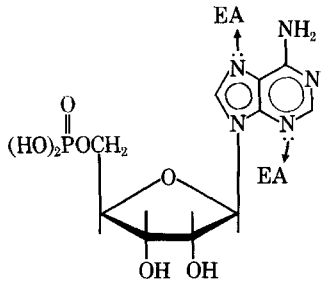


Compd.	R_6	R_8	R_9	mM Concn.	% Inhibition	Estimated Concn. for 50% Inhibition	Source
XXVI	O	CH	$-CH_3$	0.40	50	0.40	Robins
XXVII	O	CH	$-C_5H_{11}$-*n*	0.30[b]	0	>1.2[c]	Robins
XXVIII	O	CH	Cyclohexyl	0.50[b]	0	>2.0[c]	Robins
XXIX	O	CH	$-C_6H_5$	0.33	50	0.33	Robins
XXX	S	CH	$-C_6H_5$	0.30[b]	39	0.75	Robins
XXXI	O	CH	$-C_6H_4Cl$-*p*	0.38	50	0.38	Robins
XXXII	O	N	$-C_6H_4Cl$-*p*	0.05[b]	0	>0.20[c]	Robins
XXXIII	O	CH	$-CH_2C_6H_5$	0.39	50	0.39	Robins
XXXIV	O	N	$-CH_2C_6H_5$	0.35	50	0.35	Robins
XXXV	O	CH	$-CH_2C_6H_4Cl$-*p*	0.30[b]	29	0.63	Robins
XXXVI	O	CH	$-CH_2C_6H_4Cl$-*o*	0.32	50	0.32	Robins
XXXVII	O	CH	2-Furfuryl	0.4[b]	0	>1.6[c]	Robins
XXXVIII	O	C-C_6H_5	H	0.23	50	0.23	(13)
XXXIX	O	C-$(CH_2)_2C_6H_5$	H	0.25[b]	0	>1.0[c]	(13)
XL	O	C-$(CH_2)_3C_6H_5$	H	0.25[b]	0	>1.0[c]	(13)

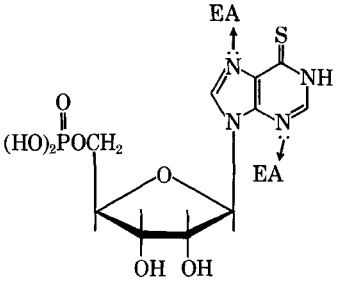
[a] The enzyme from *E. coli* B was prepared and assayed as previously described (8), except that 33 μM GTP and 122 μM IMP were employed. [b] Near maximum solubility. [c] Since 25% inhibition is readily detectable, the concentration for 50% inhibition is at least four times greater than that measured.

and 9-benzyl (XXXIII) have essentially the same binding, thus indicating that there is no hydrophobic bonding by these 9-substituted guanines and that guanines do not bind the same way as the adenines substituted by 9-methyl (X), 9-phenyl (XV), or 9-benzyl (XVI) groups (Table I). Furthermore, a variety of other related compounds such as 8-substituted guanines and 9-substituted 8-azaguanines failed to show hydrophobic bonding. Thus possibility (*a*) is eliminated, but (*b*) and (*c*) remain definite possibilities.

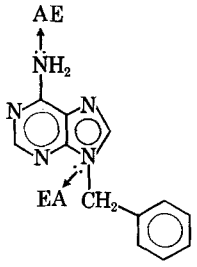
Partial support for possibility (*b*) can be gleaned by noting that 5′-adenylate (III) is a competitive inhibitor of inosinate (I) and a noncompetitive inhibitor of guanosine-5′-triphosphate (3); contrariwise, thioinosinate (IV) is a noncompetitive inhibitor of inosinate (I) but whether it is competitive with guanosine-5′-triphosphate was not determined (37). Although it would not be too difficult to determine whether thioinosinate (IV) is competitive with guanosine-5′-triphosphate, this has not been done, since the interpretation of binding based on competitive or noncompetitive observations is subject to numerous interpretations (38) besides a competitive inhibitor being one that resides on the throne of the active site.



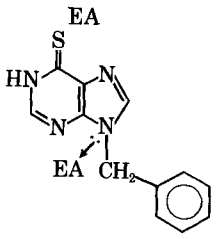
IIIa



IVa



XVIa



XXIVa

Possibility (*c*) merits explanation. Suppose 9-substituted adenines can complex to the inosinate (I) locus in more than one conformation; the particular binding rotomer could be dependent upon whether the 9-substituent is hydrophobic or hydrophilic. Such rotomeric binding conformations have been invoked to account for the binding of unnatural D-isomers to chymotrypsin (18, 19), to dihydrofolic reductase by 5- and 6-hydrophobically substituted pyrimidines (20–22), and for the binding of isoadenosine and isoadenylic acid to certain enzymes (22, 23).

Possibility (*c*) suggests that both the adenines and the 6-mercaptopurines complex with the inosinate (I) locus. If such were the case, then the NHC=S moiety of thioinosinate (IV) and the N=C—NH_2 moiety of 5′-adenylate (III) cannot be binding points to the enzyme, since the latter moiety is an electron donor at the 1-position and the former is an electron acceptor (7, 24); therefore different rotomer configurations for binding adenine would have to consider this difference in binding at the 1- and 6-positions of the purine. For example, suppose the purine ring of 5′-adenylate is complexed as rotomer III*a* where the N-7 and N-3 groups are electron donors to acceptor groups on the enzyme, AE. Thioinosinate could complex similarly through N-7 and N-3 of the purine as rotomer IV*a*. Suppose the hydrophobic bonding region of the enzyme can complex the benzyl group of 9-benzyladenine in conformation XVI*a*; the 6-amino group and 9-nitrogen could now be electron donors to the two acceptor groups on the enzyme in place of N-7 and N-3 of rotomer III*a*. In order for the 9-benzyl group attached to 6-mercaptopurine to bind in this hydrophobic region as depicted in XVI*a*, 9-benzyl-6-mercaptopurine would have to assume the XXIV*a* conformation. But rotomer XXIV*a* will bind poorly since the 6-thione group is a poor electron donor (24); hence, the binding point normally complexing N-3 in configuration III*a* will be lost. Other rotomers to account for the relative binding of 9-benzyladenine (XVI) and 9-benzyl-6-mercaptopurine (XXIV) can probably be visualized, but further experiments would be necessary to differentiate between the possibilities.

Since hydrophobic bonding cannot be detected with 9-benzyl-6-mercaptopurine, possibility (*c*) suggests that all the compounds in Table II complex with the purine as the same rotomer as thioinosinate, such as IV*a*. It follows that VI, VIII, XIX, and XXV should have their extra functional group complexed in the locus for binding the 5-phospho-D-ribosyl moiety of IV*a*. Since the dichloroacetamido group of IX (Table II) binds less effectively than VIII, it also follows that the dichloroacetamido group is not simulating phosphate binding with either possibility (*b*) or (*c*).

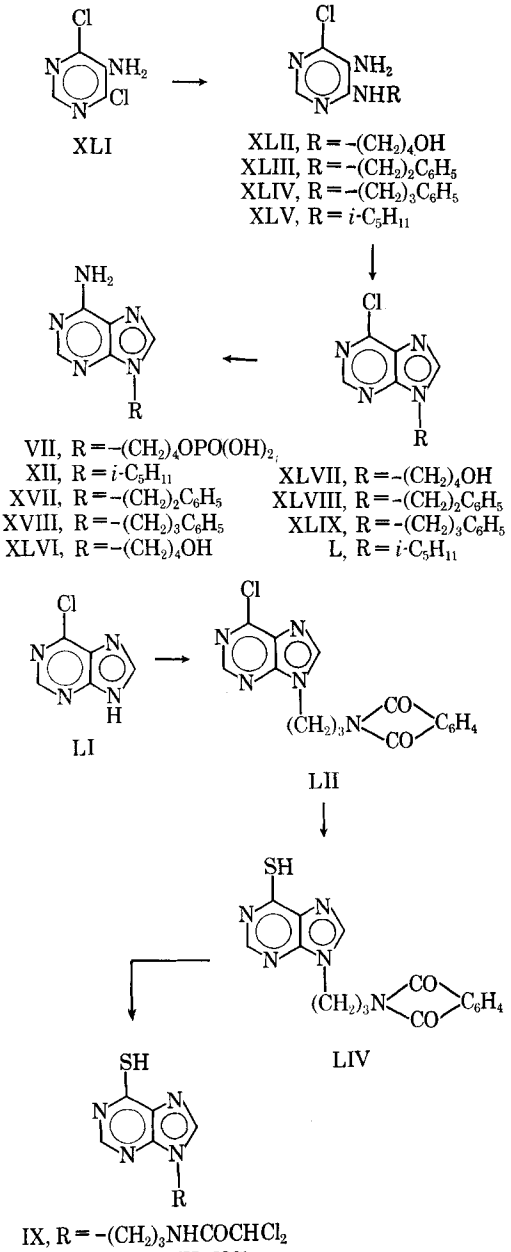
Chemistry[1]—Some of the compounds were available in this laboratory from other enzyme studies, as indicated by the references in the tables. The remainder of the compounds were synthesized as follows (Scheme I).

Reaction of 5-amino-4,6-dichloropyrimidine (XLI) with the appropriate amine in *n*-butanol containing triethylamine afforded the 6-alkylaminopyrimidines (XLII–XLV) by the elegant method of Montgomery and Temple (25, 26). Ring closure of the 6-alkylaminopyrimidines (XLII–XLV) with ethyl orthoformate in the presence of one equivalent of hydrogen chloride (27) afforded the corresponding 6-chloropurines (XLVII–L), which were not purified, but were directly converted to the adenines (XII, XVII, XVIII, XLVI) with ethanolic ammonia at 90° (25). The synthesis of XLVI has been previously described (28), as has the synthesis of XVII (32).

Treatment of 9-(4′-hydroxybutyl)adenine (XLVI) with polyphosphoric acid at 70°, as previously described for the higher homolog (V) (8), gave the butanol phosphate (VII) isolated as its barium salt; the barium salt of VII was converted to the zwitterion of VII with 0.1 *N* aqueous hydrochloric acid.

Alkylation of 6-chloropurine (LI) with *N*-(3-bromopropyl)phthalimide in dimethylsulfoxide in the presence of potassium carbonate (25, 29) afforded a 41% yield of LII; that alkylation had occurred on the 9-position was verified by conversion of LII to the 9-substituted-6-diethylaminopurine with its characteristic U.V. spectra (34–36). Reaction of LII with thiourea (25) smoothly proceeded to the 6-mercaptopurine derivative (LIV). The phthalyl group was cleaved with hydrazine in 2-methoxyethanol (9) and the resultant amine was

[1] The authors thank Drs. J. A. Montgomery, R. K. Robins, H. J. Schaeffer, and H. B. Wood, Jr., CCNSC, for some of the compounds mentioned in the tables.



Scheme I

isolated as its hydrochloride (LIII). Reaction of the free base of LIII with dichloroacetyl chloride in *N,N*-dimethylformamide in the presence of potassium carbonate afforded the desired dichloroacetamide (IX).

EXPERIMENTAL

Melting points were taken in capillary tubes on a Mel-Temp block and those below 230° are corrected. Infrared spectra were determined in KBr pellet with a Perkin-Elmer 137B or 337 spectrophotometer. Ultraviolet spectra were determined in 10% ethanol, unless otherwise indicated, with a Perkin-Elmer 202 spectrophotometer. Thin-layer chromatograms (TLC) were run on Brinkmann Silica Gel GF (unless otherwise indicated), and spots were detected by visual examination under ultraviolet light, except in the case of 6-mercaptopurines where iodine vapor was employed.

9H-Adenine-9-yl-butanol Phosphate (VII)—9-(4′-Hydroxybutyl)adenine (XLVI) (28) was prepared from XLI by the general methods described in the literature (25–27). To a solution of 1.54 Gm. of P_2O_5 in 2.06 Gm. of 85% H_3PO_4 at 66° was added 292 mg. (1.4 mmoles) of XLVI. After being heated in a bath at 66–73° for 18 hr., the solution was diluted with 15 ml. of water and heated on a steam bath for 30 min. The pH was carefully adjusted to pH 6.5 with hot saturated aqueous $Ba(OH)_2$. The barium phosphate was removed by filtration through a pad of diatomaceous earth.[2] The combined filtrate and aqueous washings were spin evaporated *in vacuo;* yield, 91 mg. of crude barium salt that moved as single spot on TLC with Brinkmann MN–polyamide–U.V. 254 in *n*-butanol–water–acetic acid (5:3:2). The barium salt was dissolved in 10 ml. of hot 0.1 *N* aqueous hydrochloric acid; the filtered solution was allowed to stand at 5° for about 18 hr. when crystallization of the zwitterion of VII was complete; yield, 50 mg. (14%) of white crystals that moved as a single spot on polyamide as above. For analysis the zwitterion was recrystallized once more from 0.1 *N* aqueous hydrochloric acid to give white crystals, m.p. 244–245°; $\nu_{max.}$ 3420 (NH); 3200–2600 (NH+); 1700 (C=NH+); 1225, 1050, 940 cm.$^{-1}$ (P—O); $\lambda_{max.}$ (pH 1): 263 mμ; (pH 13); 265 mμ.

Anal.—Calcd. for $C_9H_{14}N_5O_4P$: C, 37.6; H, 4.91; N, 24.4. Found: C, 38.1, 37.4; H, 5.38, 5.05; N, 23.8, 24.0.

9-(i-Amyl)adenine (XII)—A solution of 500 mg. (3.05 mmoles) of XLI, 280 mg. (3.22 mmoles) of isoamylamine, 15 ml. of *n*-butanol, and 1 ml. of triethylamine was refluxed for 17 hr. The solution was spin evaporated *in vacuo.* The residue was extracted with hot ethyl acetate, then the solution filtered to remove the insoluble triethylamine hydrochloride. Spin evaporation of the solution *in vacuo* left crude XLV as a brown oil which was dissolved in 12 ml. of ethyl orthoformate and treated with 0.4 ml. of 12 *N* aqueous HCl. After 18 hr. at ambient temperature, at which time the ultraviolet maximum at 297 mμ had shifted to 263 mμ, the solution was evaporated *in vacuo.* The residual L was dissolved in 15 ml. of ethanol previously saturated with ammonia at 0°, then the solution was heated in a Parr bomb at 90° for 18 hr. The solution was kept at −15° overnight, then the product was collected on a filter; yield, 201 mg. (33%), m.p. 136–141°, that gave one spot on TLC in *n*-butanol–acetic acid–water (10:5:1); additional material could be isolated from the filtrate. Recrystallization from ethanol gave white crystals, m.p. 140–142°; $\lambda_{max.}$ (1 *N* HCl): 252 mμ; (1 *N* NaOH): 264 mμ; $\nu_{max.}$ 3400, 3100 (NH); 1660, 1500 cm.$^{-1}$ (C=C, C=N, NH).

Anal.—Calcd. for $C_{10}H_{15}N_5$: C, 58.5; H, 7.37; N, 34.1. Found: C, 58.4; H, 7.50; N, 34.0.

[2] Marketed as Celite by Johns-Manville Corp., New York, N.Y.

9-(3-Phenylpropyl)adenine (XVIII)—This was prepared in 24% over-all yield from XLI as described for the preparation of XII, white crystals, m.p. 184–186°; $\lambda_{max.}$ (1 *N* HCl): 262 mμ; (1 *N* NaOH): 266 mμ.

Anal.—Calcd. for $C_{14}H_{15}N_5$: C, 66.4; H, 5.97; N, 27.7. Found: C, 66.5; H, 6.01; N, 27.6.

Similarly, 9-(4-phenylbutyl)adenine, m.p. 183–185°, was prepared from XLI in 33% over-all yield.

Anal.—Calcd. for $C_{15}H_{17}N_5$: C, 67.4; H, 6.41; N, 26.2. Found: C, 67.4; H, 6.55; N, 26.2.

9-Phenethyladenine (XVII)—This compound was prepared in 15% over-all yield from XLI; white crystals, m.p. 185–188°. [Lit. (32) m.p. 179–180°.] This compound was also prepared by direct alkylation of the sodium salt of adenine in *N,N*-dimethylformamide (33) to give a 36% yield, m.p. 183–186°; $\lambda_{max.}$ (1 *N* HCl): 262 mμ; (1 *N* NaOH): 266 mμ.

6-Chloro-9-(3′-phthalimidopropyl)purine (LII)—A mixture of 3.30 Gm. (21.3 mmoles) of LI, 130 ml. of dimethylsulfoxide, 2.9 Gm. (21 mmoles) of anhydrous potassium carbonate, and 5.40 Gm. (20.2 mmoles) of *N*-(3-bromopropyl)phthalimide was magnetically stirred at ambient temperature for 17 hr. The mixture was filtered. The filtrate was diluted with water, then the product was collected on a filter; yield, 3.02 Gm. (41%) that moved as a single spot on TLC in chloroform–ethanol (1:1) that was different from each starting material. Recrystallization of a sample from ethanol gave an analytical sample, m.p. 185–188°; $\nu_{max.}$ 1770, 1690 (phthalyl C=O); 1580, 1540 (C=C, C=N); 725, 712 cm.$^{-1}$ (C_6H_4); $\lambda_{max.}$ (EtOH): 267, 299 mμ.

Anal.—Calcd. for $C_{16}H_{12}ClN_5O_2$: C, 56.4; H, 3.72; N, 20.5. Found: C, 56.1; H, 3.52; N, 20.4.

The structure of LII was verified by reaction with diethylamine. A solution of 71 mg. (0.21 mmole) of LII, and 0.21 Gm. (3 mmoles) of diethylamine in 5 ml. of ethanol was refluxed for 90 min.; at this time TLC with petroleum ether–chloroform–ethanol (1:1:1) gave only a single spot which moved differently from starting material. That this was a 9-substituted 6-diethylaminopurine was shown by its $\lambda_{max.}$ (pH 1): 275 mμ; (pH 13): 280 mμ.

6-Mercapto-9-(3′-phthalimidopropyl)purine (LIV)—A mixture of 0.48 Gm. (6.3 mmoles) of thiourea, 1.09 Gm. (3.2 mmoles) of LII, and 50 ml. of ethanol was refluxed with magnetic stirring for 12 hr., during which time the product separated. The cooled mixture was filtered and the product was washed with ethanol; yield, 0.59 Gm. (53%), which was suitable for further transformation. Recrystallization of a sample from 2-methoxyethanol gave nearly white crystals, m.p. 310–315°; $\lambda_{max.}$ (pH 1): 327 mμ; $\nu_{max.}$ 2710 (acidic H); 1760, 1710 (phthalyl C=O); 1540, 1575, 1490 (C=C, C=N); 725, 718 cm.$^{-1}$ (C_6H_4). The compound moved as a single spot on TLC in chloroform–ethanol (1:1).

Anal.—Calcd. for $C_{16}H_{13}N_5O_2S$: C, 56.1; H, 3.41; N, 20.5. Found: C, 56.4; H, 3.62; N, 20.4.

6-Mercapto-9H-purine-9-ylpropylamine Hydrochloride (LIII)—A mixture of 2.51 Gm. (7.3 mmoles of LIV, 60 ml. of 2-methoxyethanol, and 1.38 ml. of 85% hydrazine hydrate was refluxed with magnetic stirring for 20 min., then diluted with 160 ml. of ethyl acetate and stored at 5° for 2 hr. The intermediate phthalamide was collected on a filter and washed with ethyl acetate. The solid was stirred with 80 ml. of 1 *N* aqueous hydrochloric acid at about 80° for 20 min. The phthalhydrazide containing considerable LIV was removed by filtration and washed with water. The phthalhydrazide containing LIV (1.47 Gm.) was retreated with hydrazine and aqueous hydrochloric acid as above. The two hydrochloric acid solutions were combined and spin evaporated *in vacuo*. Recrystallization from aqueous acetone gave 1.78 Gm. (86%) of nearly white crystals, m.p. 274–276°. A second recrystallization of a portion from aqueous acetone gave the analytical sample, m.p. 274–276°; $\lambda_{max.}$ (H_2O): 327 mμ; $\nu_{max.}$ 3100–2400 (NH, NH+); 1600, 1540 (C=C, C=N); no C=O near 1700 cm.$^{-1}$.

Anal.—Calcd. for $C_8H_{11}N_5S \cdot HCl \cdot H_2O$: C, 36.4; H, 5.35; N, 26.5. Found: C, 36.5; H, 5.22; N, 26.5.

9-(3′-Dichloroacetamidopropyl)-6-mercaptopurine (IX)—A solution of 442 mg. (1.8 mmoles) of LIII in 3.5 ml. of 1 *N* aqueous sodium hydroxide was spin evaporated *in vacuo*. The dry residue was suspended in 3.4 ml. of *N,N*-dimethylformamide, then cooled in an ice bath. To the magnetically stirred mixture was added 519 mg. of anhydrous K_2CO_3 followed by 0.70 ml. of dichloroacetyl chloride. After being stirred in the ice bath for 1 hr., the mixture was diluted with 50 ml. of water. The product was collected on a filter and washed with water; yield, 120 mg. (21%), m.p. 180–190°, which gave a single spot on TLC in *n*-butanol–acetic acid–H_2O (10:5:1). Recrystallization from aqueous acetone gave nearly white crystals, m.p. 203–205°; $\nu_{max.}$ 3400 (NH); 2600–2500 (acidic H); 1690 (amide C=O); 1590, 1575 cm.$^{-1}$ (C=C, C=N); $\lambda_{max.}$ (pH 1): 326 mμ; (pH 13): 312 mμ.

Anal.—Calcd. for $C_{10}H_{11}Cl_2N_5OS$: C, 37.5; H, 3.47; N, 21.8. Found: C, 37.2; H, 3.60; N, 21.8.

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Inhibitors of Thymidine Phosphorylase 7. Further Studies on Hydrophobic Bonding with Hydrocarbon Substituents on Acidic Uracils

By B. R. BAKER, MITSUTAKA KAWAZU, and J. D. MCCLURE

Replacement of the 6-methyl group of 6-methyluracil (II) by 6-trifluoromethyl gives a sevenfold increment in binding to thymidine phosphorylase presumably due to the increased acidity of the uracil. Similarly, replacement of the 6-methyl group by 6-methylsulfonyl gave a fifteenfold increment in binding. The binding of 6-methyluracil (II) was increased only 2.5-fold by introduction of a 5-phenylazo group. 6-(Trifluoromethyl)uracil (IV) was further substituted at the 5-position by six different alkyl, aryl, and aralkyl groups; the best inhibitor of this series was 5-phenylbutyl-6-(trifluoromethyl)uracil (XI) which was complexed tenfold better than IV and 67-fold better than 6-methyluracil. Comparison of 5-(phenylazo)uracils substituted with methyl, phenyl, benzyl, or *n*-amyl at the 6-position showed that the *n*-amyl derivative (XVI) gave the best binding; XVI was complexed 23-fold better than 6-methyl-5-(phenylazo)uracil (XII) and 58-fold better than 6-methyluracil.

THYMIDINE PHOSPHORYLASE is an enzyme that catalyzes phosphorolysis of thymidine and related 2′-deoxynucleosides to thymine (1) or the reverse conversion of thymine to thymidine (2), depending upon the stress of genetic or dietary deficiencies upon the cell line (3). The enzyme can also convert the anticancer agent, 5-fluorouracil, to its 2′-deoxyriboside (FUDR) or *vice versa* (3, 4). The chemotherapeutic utility for a tissue-specific inhibitor of thymidine phosphorylase has been previously discussed (5), and initial studies from this laboratory on the mode of binding of inhibitors to this enzyme have been reported (5–10).

It was previously noted that hydrophobic bonding to the enzyme could take place with alkyl or aralkyl groups on uracil at the 1-position (6) or the 5- and 6-positions (7, 8), for example, 6-benzyluracil (XV) was eighteenfold more effective than uracil (7) (Table I). Furthermore, binding of uracils could be made more effective when substituted with electron-withdrawing groups at the 5- or 6-position which increased the acidity of the uracil (9); for example, 5-bromouracil (III) was ninefold more effective than uracil (I). Finally, the best inhibitors were obtained by combining both phenomena, that is, hydrophobic bonding and increased acidity (10); for example, 5-bromo-6-benzyluracil (XVIII) was complexed to the enzyme fortyfold more effectively than the substrate, FUDR, and 150-fold more effectively than uracil (I) (Table I). Therefore, further studies on combinations of hydrophobic groups and electron-withdrawing groups attached to the 5- and 6-positions of uracil were initiated and are the subject of this paper.

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The technical assistance of Pepper Caseria with the assays in Table I is acknowledged.

DISCUSSION

It was previously reported (9) that the electron-withdrawing trifluoromethyl group at the 6-position (IV) of uracil gave a sevenfold increment in enzyme