Irreversible Enzyme Inhibitors. CLXVII.[1,2] Thymidine Phosphorylase. X.[3] On The Nature and Dimensions of the Hydrophobic Bonding Region. II

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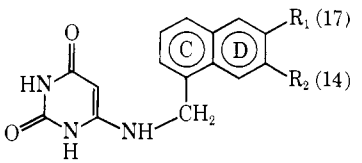
6-(1-Naphthylmethylamino)uracil (1) and three of its derivatives substituted on the naphthalene ring with 7-chloro (**2**), 6,7-dichloro (**3**), and 6,7-dimethyl (**4**) were synthesized for evaluation as inhibitors of *Escherichia coli* B thymidine phosphorylase. The appropriate α-tetralone (**12**) was condensed with triethyl phosphonoacetate, then dehydrogenated to the naphthalene-1-acetic esters (**14**) with *o*-chloroanil. Reaction with hydrazine gave the crystalline hydrazides (**15**) which were degraded with NOCl to the corresponding substituted 1-naphthylmethylamines (**17**); the latter were condensed with 6-chlorouracil to give the inhibitors **1–4**. These four compounds were excellent inhibitors of thymidine phosphorylase being complexed 1900–5800-fold better to the bacterial enzyme than the substrate, 5-fluoro-2′-deoxyuridine (FUDR); the best inhibition was observed with **2**.

The dimensions of the hydrophobic bonding region of *Escherichia coli* B thymidine phosphorylase were previously determined with 29 derivatives of 6-anilinouracil and 6-benzylaminouracil where substituents were placed on the benzene ring; from these studies emerged the map of the hydrophobic bonding region shown in Figure 1.[3] One of the predictions from this map was that 6-(α-naphthylmethylamino)uracil (**1**) should be an excellent inhibitor by hydrophobic complexing with areas C and D. Whether position 14 or 17 was hydrophobic was less certain, although it appeared that one of these two positions was hydrophobic; that position 14 was hydrophobic, but position 17 was not, has now been established by synthesis and evaluation of **2–4**. The results are the subject of this paper.[5]

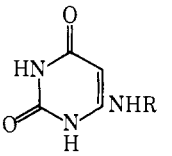


1, $R_1 = R_2 = H$
2, $R_1 = H$, $R_2 = Cl$
3, $R_1 = R_2 = Cl$
4, $R_1 = R_2 = CH_3$

Enzyme Results.—Insertion of the D ring (Figure 1) on 6-benzylaminouracil (**5**) resulted in **1** which gave 30-fold better binding (Table I); this increment in binding is the same order when the D ring is inserted on 6-anilinouracil (**6**) to give **7**, thus verifying that the D ring complexes to a flat hydrophobic area.[3]

Insertion of 7-chloro group (**2**) on **1** gave a further threefold increment in binding indicating that position 14 (Figure 1) is hydrophobic. The corresponding 6,7-dichloro (**3**) and 6,7-dimethyl (**4**) derivatives were no more effective than **2**, indicating that position 17 was not a hydrophobic area. 6-(7-Chloro-1-naphthylmethyl)aminouracil (**2**) is the most powerful inhibitor of *E. coli* B thymidine phosphorylase yet observed; **2** was complexed to the enzyme 5800-fold more effectively than the substrate, FUDR.

TABLE I
INHIBITION[a,b] OF THYMIDINE PHOSPHORYLASE BY



No.	R	μM concn for 50% inhibn	$([S]/[I])_{0.5}$[c]
1	1-Naphthyl-CH_2-	0.21	1900
2	7-Cl-1-naphthyl-CH_2-	0.079	5800
3	6,7-Cl_2-naphthyl-CH_2-	0.11	3700
4	6,7-Me_2-naphthyl-CH_2-	0.10	4000
5	$C_6H_5CH_2$	6.2[d]	65
6	C_6H_5	40[d]	10
7	2-Naphthyl	2.2[d]	180
8	2,3-$Cl_2C_6H_3$	0.35[d]	1100

[a] The technical assistance of Maureen Baker and Julie Leseman is acknowledged. [b] Thymidine phosphorylase was a 45–90% $(NH_4)_2SO_4$ fraction from *E. coli* B prepared and assayed with 400 μM 2′-deoxy-5-fluorouridine (FUDR) in arsenate–succinate buffer (pH 5.9) containing 10% DMSO as previously described.[5] [c] Ratio of 400 μM FUDR to concentration of inhibitor giving 50% inhibition. [d] Data from ref 3.

A striking species difference in the hydrophobic binding area between the enzyme from *E. coli* B and Walker 256 rat tumor has been observed; both **1** and **8** were 1100-fold less effective on the tumor enzyme than the bacterial enzyme.[6] Contrariwise, 5-benzyluracil was an excellent inhibitor of the Walker 256 and rat liver enzymes with an $([S]/[I])_{0.5}$ ratio of 60, but was 300-fold less effective on the *E. coli* B enzyme; such species differences in binding to a hydrophobic bonding region adjacent to the active site have been previously observed with dihydrofolate reductase.[7]

Chemistry.—The 6-(1-naphthylmethyl)aminouracils (**1–4**) were synthesized by condensation of 6-chlorouracil with the appropriate 1-naphthylmethylamines (**17**);[3,8] the parent 1-naphthylmethylamine was commercially available, but its derivatives (**17**) were synthesized by the sequence shown in Scheme I. The

(1) This work was supported in part by Grant CA-08695 from the National Cancer Institute, U. S. Public Health Service.

(2) For the previous paper of this series see B. R. Baker and N. M. J. Vermeulen, *J. Med. Chem.*, **13**, 82 (1970).

(3) For the previous paper on this enzyme see B. R. Baker and W. Rzeszotarski, *ibid.*, **11**, 639 (1968).

(4) NSF trainee on Grant No. GZ-796.

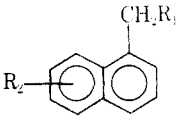
(5) For the possible chemotherapeutic utility of a tissue-specific blockade of this enzyme see (a) B. R. Baker, *J. Med. Chem.*, **10**, 297 (1967), paper LXXV of this series; and (b) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," John Wiley & Sons, New York, N. Y., p 80.

(6) B. R. Baker and J. L. Kelley, manuscript in preparation.

(7) B. R. Baker, *J. Med. Chem.*, **10**, 912 (1967), paper XCVII of this series.

(8) A. Paul and D. Sen, *Indian J. Chem.*, **2**, 212 (1964).

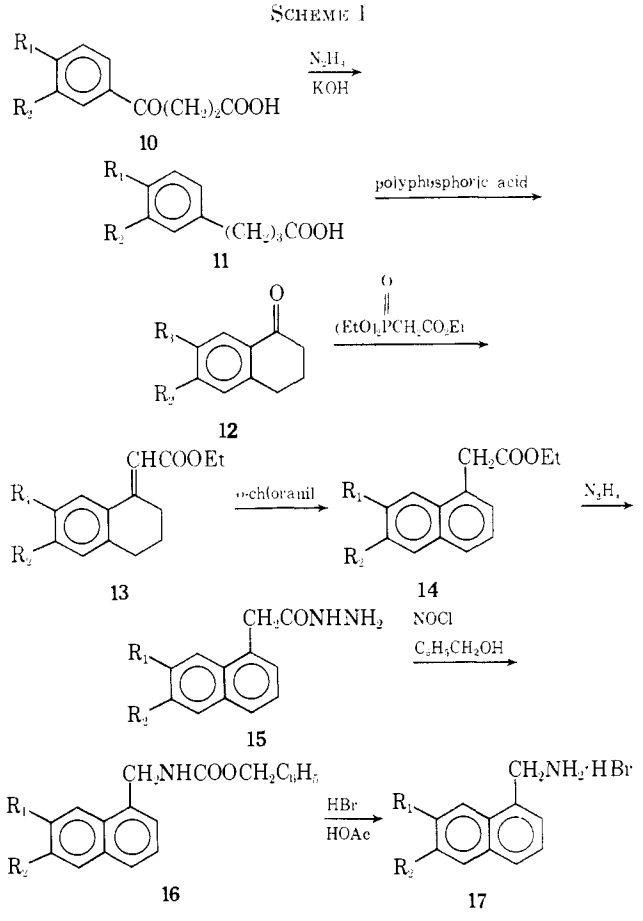
TABLE II
PHYSICAL CONSTANTS OF



No.	R_2	R_1	Method (hr)	Yield, %	Mp, °C	Formula[a]
1	H	NHU[b]	C (20)	14[c]	326 dec	$C_{15}H_{18}N_3O_2$
2	7-Cl	NHU[b]	C (24)	15[d]	322 dec	$C_{15}H_{12}ClN_3O_2 \cdot 0.5H_2O$
3	6,7-Cl_2	NHU[b]	C (48)	12[e,f]	>360	$C_{15}H_{11}Cl_2N_3O_2 \cdot 0.25HOAc$
4	6,7-Me_2	NHU[b]	C (72)	13[d]	337 dec	$C_{17}H_{17}N_3O_2$
15a	7-Cl	$CONHNH_2$	A	85,[g] 20[h]	199–200	$C_{12}H_{11}ClN_2O$
15b	6,7-Cl_2	$CONHNH_2$	A	88,[g] 24[i]	193–194	$C_{12}H_{10}Cl_2N_2O$
15c	6,7-Me_2	$CONHNH_2$	A	64,[g] 37[i]	162–163	$C_{14}H_{16}N_2O$
15d	7-Me	$CONHNH_2$	A	50,[g,k] 18[i,l]	163–164	$C_{13}H_{14}N_2O$
17a	7-Cl	$NH_2 \cdot HBr$	B	50	262 dec[m]	$C_{11}H_{10}ClN \cdot HBr$
17b	6,7-Cl_2	$NH_2 \cdot HBr$	B	65	272 dec[m]	$C_{11}H_9Cl_2N \cdot HBr$
17c	6,7-Me_2	$NH_2 \cdot HBr$	B	45	261 dec[m]	$C_{13}H_{15}N \cdot HBr$

[a] All gave analysis for C, H, and N within 0.4% of theory. [b] NHU = 6-uracilamino. [c] Recrystallized from HOAc. [d] Recrystallized from HOAc–H_2O. [e] Recrystallized from DMF. [f] Another sample was recrystallized from HOAc–H_2O for analysis. [g] Yield of **12** to **13**. [h] Recrystallized from EtOH. [i] Recrystallized from MeOH–H_2O. [j] Recrystallized from C_6H_6–hexane. [k] 27% of **12** recovered. [l] Conversion to **17d** did not give a crystalline product. [m] Recrystallized from EtOH–Et_2O.

SCHEME I



a, $R_1 = Cl$; $R_2 = H$
b, $R_1 = R_2 = Cl$
c, $R_1 = R_2 = CH_3$
d, $R_1 = CH_3$; $R_2 = H$

aroylpropionic acids (**10**)[9–11] were reduced with N_2H_4 and KOH in ethylene glycol[12] to **11**. Cyclization of **11** with P_2O_5 in H_3PO_4[13] gave the α-tetralones (**12**). Wittig condensation of **12** with triethyl phosphonoacetate and NaH in dimethoxyethane[14] afforded the α,β-unsaturated esters (**13**) as a mixture of *cis* and *trans* isomers. Dehydrogenation of **13** with *o*-chloranil[15] in boiling $PhCH_3$ afforded the 1-naphthylacetic esters (**14**) which were isolated as their hydrazides (**15**). The hydrazides were subjected to Curtius rearrangement with NOCl[16] in C_6H_6 containing benzyl alcohol; the resultant carbobenzoxy derivatives (**16**) were not purified, but subjected directly to cleavage by anhydrous HBr–HOAc[17] to the crystalline 1-naphthylmethylamine hydrobromides (**17**).

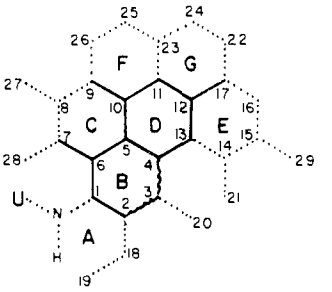


Figure 1.—A proposed map of the hydrophobic bonding region of *E. coli* B thymidine phosphorylase: ——, hydrophobic interaction; ····, no hydrophobic interaction; ∿∿, unknown. From ref 3.

Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Each analytical sample had ir and uv spectra compatible with its structure and was essentially homogeneous on tlc on Brinkman silica gel GF. The following solvent systems for tlc were used: A, 9:1 C_6H_6–petroleum ether

(9) E. B. Barnett and F. G. Sanders, *J. Chem. Soc.*, 434 (1933).
(10) S. Skraup and E. Schwamberger, *Ann.*, **462**, 135 (1928).
(11) E. A. Steck, R. P. Brundage, and L. Fletcher, *J. Am. Chem. Soc.*, **75**, 1117 (1953).
(12) Huang-Minlon, *ibid.*, **68**, 2487 (1946).
(13) A. J. Birch, R. Jaeger, and R. Robinson, *J. Chem. Soc.*, 582 (1945).
(14) H. Takamatsu, S. Umemoto, T. Shimizu, and A. Kagemoto, *Yakugaku Zasshi*, **85**, 975 (1965); *Chem. Abstr.*, **64**, 5016a (1966).
(15) E. A. Braude, A. G. Brook, and R. P. Linstead, *J. Chem. Soc.*, 3569 (1954).
(16) J. Honzl and J. Rudinger, *Collect. Czech. Chem. Commun.*, **26**, 2333 (1961).
(17) D. Ben-Ishai, *J. Org. Chem.*, **19**, 82 (1954).

(bp 60–110°); B, 5:3 C_6H_6–HOAc. All analytical samples gave combustion values within 0.4% of theory.

γ-(3,4-Dichlorophenyl)butyric Acid (11b).—Huang-Minlon reduction[12] of **10b** (prepared by ref 11 at 100°) gave 83% of crude product, bp 140–144° (0.1 mm), mp 48–64°, that was suitable for the next step. Three recrystallizations from EtOH–H_2O gave white crystals, mp 62–65°. *Anal.* ($C_{10}H_{10}Cl_2O_2$) C, H.

6,7-Dichloro-1-tetralone (12b).—To 184 g of polyphosphoric acid was added 18.4 g of P_2O_5 followed by 18.4 (78 mmoles) of **11b**. The mixture was stirred at 100° for 30 min, then cooled to 50° and diluted with 500 ml of ice water. The product was collected on a filter, washed with water, and recrystallized from EtOH–H_2O; yield 11.9 g (69%) of white crystals, mp 104–108°, tlc in solvent A. *Anal.* ($C_{10}H_8Cl_2O$) C, H, Cl.

Synthesized in the same way were **12d** [bp 77° (0.1 mm), mp 35° (lit.[9] mp 35°)], **12a** (mp 93–95°, lit.[18] mp 94°), and **12c** [bp 84–90° (0.05 mm), mp 44–46°]. *Anal.* ($C_{12}H_{14}O$) C, H.

6,7-Dichloronaphthyl-1-acethydrazide (15b) (Method A).—To a stirred suspension of 3.7 g (92 mmoles) of 60% suspension of NaH in mineral oil in 200 ml of dimethoxyethane (previously dried with molecular sieves) protected from moisture was added 18.8 g (84 mmoles) of triethyl phosphonoacetate. After being stirred at ambient temperature for 45 min, H_2 evolution was complete. A solution of 12 g (56 mmoles) of **12b** in 12 ml of dimethoxyethane was added, then the mixture was refluxed for 24 hr. The cooled reaction mixture was diluted with 400 ml of H_2O and extracted twice with $CHCl_3$. The combined extracts were washed with H_2O, dried with $MgSO_4$, and evaporated *in vacuo*. Distillation gave 13.1 g (88%) of **13b**, bp 127–130° (0.07 mm), as a colorless oil; tlc in solvent A showed one major, one minor, and two trace spots. The ir and nmr spectra were in agreement with this structure.

A solution of 14.9 g (61 mmoles) of *o*-chloranil and 15.7 g (56 mmoles) of **13b** in 700 ml of PhMe was refluxed for 4 hr; during this time the solution turned red, then orange. The solution was evaporated *in vacuo* and the residue dissolved in 700 ml of $CHCl_3$. The solution was washed successively with three 400-ml portions of 0.1 *N* KOH and two 150-ml portions of H_2O, then evaporated *in vacuo* leaving crude **14b** with the proper uv, ir, and nmr spectra.

A mixture of the crude **14b**, 350 ml of 85% $N_2H_4 \cdot H_2O$, and 110 ml of EtOH was refluxed for 4 hr, then evaporated to a small volume *in vacuo*. The solid was collected on a filter and washed extensively with H_2O. The crude product was stirred with 1.5 l. of 0.3 *N* HCl and 1 l. of MeOH until solution was essentially complete. The filtered solution was carefully made slightly basic with 5% $NaHCO_3$. The solid was collected on a filter, washed with H_2O, then recrystallized from MeOH–H_2O with the aid of charcoal; yield 3.56 g (24%), mp 198° on a Kofler–Heizbank since a melting point taken in the usual fashion showed no definite melting point due to decomposition. See Table II for additional data and other compounds prepared in this way.

6,7-Dichloro-1-naphthylmethylamine Hydrobromide (17b) (Method B).—A solution of 1.49 g (2.2 mmoles) of $C_6H_5CH_2OH$ in 270 ml of C_6H_6 was dried with molecular sieves, then 2.93 g (11 mmoles) of **15b** was added. The stirred suspension was saturated with NOCl. After being stirred 30 min at ambient temperature (N_2 evolved), the solution was refluxed 4.5 hr, then evaporated *in vacuo*. To the residual crude **16b** dissolved in 20 ml of HOAc was added a solution of 4.8 g of HBr gas in 40 ml of HOAc; CO_2 evolution was complete in 15 min and the product began to separate. The mixture was diluted with several volumes of Et_2O, then the product was collected on a filter and washed with Et_2O; yield, 2.18 g (65%) of crystals, mp 283–284 dec, suitable for the next step. For analysis a sample was recrystallized from EtOH–Et_2O; see Table II for additional data and other compounds prepared by this method.

6-(6,7-Dichloro-1-naphthylmethylamino)uracil (3) (Method C).—A mixture of 2.10 g (6.8 mmoles) of **17b**, 0.564 g (6.8 mmoles) of NaAc, 0.75 g (5.2 mmoles) o 6-chlorouracil,[3] and 78 ml of H_2O was refluxed with stirring for 48 hr. The hot solution was filtered and the product washed with H_2O; yield 0.82 g (45%). Recrystallization from DMF gave 0.22 g (12%) of product, mp >360°; tlc in solvent B showed one spot. See Table II for additional data and other compounds prepared by this method.

(18) J. v. Braun, A. Rohmer, H. Jungman, F. Zobel, L. Brauns, O. Bayer, A. Stuckenschmidt, and J. Reutter, *Ann.*, **451**, 1 (1926).

6-Fluorotetracyclines[1]

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The reaction of 11a-halotetracyclines with liquid HF results in the replacement of 6-OH with F. When 11a-chloro-6-demethyltetracycline is treated with HF, the two possible 6-F stereoisomers were isolated. A tentative assignment of stereochemistry is given. When either 7,11a-dichloro-6-demethyltetracycline or 7,11a-dichlorotetracycline is treated with HF, only the more stable 6α-F isomers were isolated. In the latter case some previously reported 6-methylene derivative was also isolated.

The lability of the 6-OH in the tetracycline molecule to both acid[2] and base degradation[3] has thwarted past efforts at successful replacement of this group with other than H.[4] Recently we have found that reaction of 11a-chloro-6-demethyltetracycline (**1**) in liquid HF yielded a mixture of two 6-fluoro stereoisomers, **2a,b** (Chart I). Dehydrochlorination of either of these stereoisomers afforded the same 5a(6)-anhydro-6-fluoro-6-demethyltetracycline (**3**). An nmr of this

(1) A preliminary report of this work was given at the First Northeast Regional Meeting of the American Chemical Society, Boston, Mass., Oct 1968.

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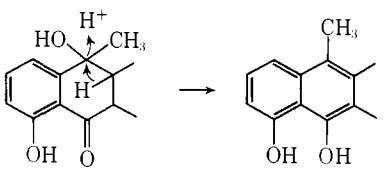
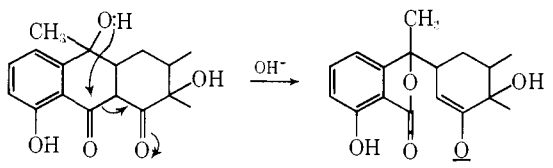


(3) Base treatment of tetracycline yields isotetracycline.



(4) (a) J. R. D. McCormick, E. R. Jensen, P. A. Miller, and A. P. Doerschuk, *J. Am. Chem. Soc.*, **82**, 3381 (1960); (b) C. R. Stephens *et. al.*, *ibid.*, **80**, 5324 (1958).