Analogs of Tetrahydrofolic Acid XXII

Effects of Modification of the Bridge Between the Pyrimidyl and Phenyl Moieties of 2-Amino-5-(3-anilinopropyl)-6-methyl-4-pyrimidinol on Inhibition of Dihydrofolic Reductase and Thymidylate Synthetase III

By B. R. BAKER and JAMES K. COWARD

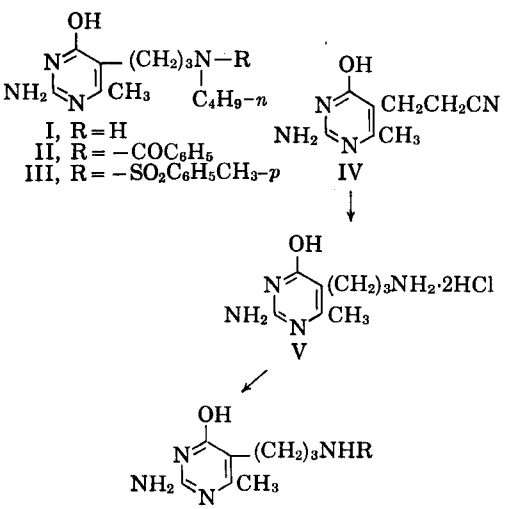
The key intermediate for these studies, 2-amino-5-(3-aminopropyl)-6-methyl-4-pyrimidinol dihydrochloride (V), was synthesized readily in three steps from ethyl acetoacetate by cyanoethylation, condensation with guanidine, and catalytic reduction of the nitrile group in the presence of hydrochloric acid. The conversion of V to the benzamidopropyl derivative (VI), the *p*-tolylsulfonamidopropyl derivative (XII), and related amides under carefully controlled conditions proceeded in good yield. The sulfonamide (XII) was a good inhibitor of thymidylate synthetase, being 30 times more effective on this enzyme than dihydrofolic reductase. A cross-over in specificity was noted with the benzamido derivative (VI).

STUDIES ON the inhibition of dihydrofolic reductase and thymidylate synthetase have shown (2) that the pyrimidyl analog (XV) of tetrahydrofolic acid is an inhibitor, even though XV does not contain the carboxy-L-glutamate moiety of the substrate. A further study showed that the $-SO_2N(CH_2)_3-$ bridge of III and the $-CON(CH_2)_3-$ bridge of II also allowed the respective phenyl groups to complex with these two enzymes, even though the bridge was one atom longer than the $-NH(CH_2)_3-$ bridge of the standard inhibitor (XV) (1). The emergence of these two new bridges suggested that a simpler synthetic route to related compounds (VI, XII) be investigated. This new synthesis afforded compounds VI–XIII, and this paper reports the synthetic methods and the enzymic evaluation of the compounds.

DISCUSSION

The *n*-butylaminopropyl pyrimidine (I) used in the earlier bridge study (1) requires six steps for its synthesis from ethyl acetoacetate in an over-all yield of about 6% (3) *via* 2-acetamido-4-hydroxy-6-methyl-5-pyrimidinepropionaldehyde (4, 5). The key intermediate, 2-amino-5-(3-aminopropyl)-6-methyl-4-pyrimidinol dihydrochloride (V), has now been synthesized in three steps from ethyl acetoacetate in an over-all yield of about 32% *via* ethyl 2-acetyl-4-cyanobutyrate (6) and the pyrimidylpropionitrile (IV) (7). Catalytic reduction of the nitrile group of IV proceeded in good yield only if acid was present to protonate the amine groups of V. The reduction was best performed in 85% aqueous ethanol in the presence of two equivalents of hydrochloric acid using Adams catalyst. In 95% alcohol, the dihydrochloride (V) precipitated from solution, which coated the catalyst and prevented the reduction from going to completion; although the nitrile (IV) was not completely soluble in 85% alcohol, the dihydrochloride (V) was soluble, and reduction proceeded smoothly in 70–80% yields.

Considerable difficulty was encountered in finding



VI, R = $-COC_6H_5$
VII, R = $-COCH_2C_6H_5$
VIII, R = $-COCH_3$
IX, R = $-COC_6H_4CH_2OC_2H_5$-*p*
X, R = $-COC_6H_4NO_2$-*p*

XI, R = $-COC_6H_4NH_2$-*p*-
XII, R = $-SO_2C_6H_4CH_3$-*p*
XIII, R = $-SO_2C_4H_9$-*n*
XIV, R = $-SO_2CH_3$
XV, R = C_6H_5

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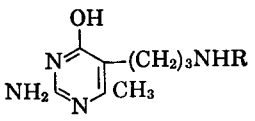
The authors acknowledge the technical assistance of Miss Karen Smith and Miss Shirley Herrmann with the enzyme assays in Table II.

TABLE I.—PHYSICAL CONSTANTS OF PYRIMIDYL ANALOGS



Compd.	R[a]	M.p., °C.	Method	% Yield	Anal. Calcd.	Anal. Found
VI	—COC_6H_5	265–270 dec.	*A*[b]	81	C, 62.9	C, 62.8
			C	91	H, 6.34	H, 6.50
					N, 19.6	N, 19.2
VII	—$COCH_2C_6H_5$	264–265 dec.	*C*[c]	85	C, 62.2[d]	C, 61.8
					H, 6.85	H 6.70
					N, 18.5	N, 18.3
VIII	—$COCH_3$	225–228	*B*	37	C, 53.6	C, 53.3
					H, 7.19	H, 7.07
					N, 25.0	N, 25.0
IX	—$COC_6H_4CH_2OEt$-*p*	260–261 dec.	*C*[e]	47	C, 62.8	C, 62.5
					H, 7.02	H, 6.86
					N, 16.3	N, 16.3
X	—$COC_6H_4NO_2$-*p*	320–324 dec.	*C*[b]	91	C, 54.4	C, 54.1
					H, 5.17	H, 5.28
					N, 21.1	N, 20.9
XI	—$COC_6H_4NH_2$-*p*	275–276 dec.	*D*[f]	40	C, 59.8	C, 59.6
					H, 6.36	H, 6.40
					N, 23.2	N, 23.3
XII	—$SO_2C_6H_4CH_3$-*p*	131–140[g]	*C*	64[g]	C, 53.6	C, 53.4
					H, 5.99	H, 5.99
					N, 16.7	N, 16.4
XIII	—$SO_2C_4H_9$-*n*	208–210	*C*[h]	41	C, 47.7	C, 47.4
					H, 7.33	H, 7.32
					N, 18.5	N, 18.4

[a] All compounds had the expected infrared and ultraviolet spectra. [b] Recrystallized from 2-methoxyethanol–water. [c] Recrystallized from methanol. [d] Hemihydrate. [e] Recrystallized from ethanol–water. [f] See under *Experimental*. [g] Amorphous, but one spot on TLC with benzene–methanol (3:1). [h] Recrystallized from water.

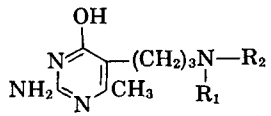
suitable conditions for selective acylation of the amine dihydrochloride (V) to the benzamide (VI) or the toluenesulfonamide (XII). Normally nonaqueous systems are preferable for selective monoacylation of a diamine by controlling the ratio of acylating agent to amine. In aqueous systems, an excess of acylating agent usually is employed to compensate for hydrolysis of the acylating agent, and at times it can be difficult to adjust the stoichiometry for selective acylation. Attempts to acylate V selectively in dipolar aprotic solvents, such as *N,N*-dimethylformamide or dimethylsulfoxide in the presence of *N*-methylmorpholine or triethylamine, proceeded in only trace yields due to the insolubility of the dihydrochloride. Furthermore, even though the ratio of *p*-tolysulfonyl chloride to V was 1:1, only a low yield of a ditosyl derivative could be isolated. Although aqueous systems are usually less preferable, it was necessary to find conditions to operate in aqueous systems so that V and its free base were in solution. The usual Schotten-Baumann conditions gave no acylated products, and only hydrolysis of the acid chlorides occurred.

An efficient aqueous system was finally devised. The dihydrochloride (V) was dissolved in two equivalents of 3 *N* aqueous sodium hydroxide, then acetone was added to 70% concentration. When 1.5 equivalents of benzoic anhydride was added to this system, the benzamido derivative (VI) rapidly precipitated in 81% yield. Although this procedure was satisfactory for anhydrides, it was not adequate for acid chlorides since the pH dropped more drastically from the evolved hydrochloric acid, thus protonating unreacted amine (V) so that it would not react. The reaction did proceed smoothly if to the 70% aqueous acetone solution 1.5 mole-equivalents of sodium carbonate was added prior to the addition of 1.5 mole-equivalents of benzoyl chloride; in this case, the benzamido derivative (VI) was obtained in 91% yield. Other acyl derivatives prepared by this method are listed in Table I.

When V was treated with a 5:1 ratio of acetic anhydride, the product was not the expected acetamide derivative (VIII), but further acetylation occurred on the 2-amino group to give a diacetyl derivative in 53% yield, as shown by its typical ultraviolet spectra (9). Selective deacetylation on the 2-amino group was accomplished by short reflux with methanolic butylamine (8) to give the desired acetamido derivative (VIII) in good yield.

Attempts to prepare the methanesulfonamide (XIV) with methanesulfonyl chloride, methanesulfonyl fluoride, or methanesulfonic anhydride gave water-soluble products which were methanesulfonate salts rather than the methanesulfonamide (XIV); these salts were distinguished readily from the desired XIV by the absence of an —SO_2— band at 1360 cm.$^{-1}$ which is present in sulfonamides. Fortunately, the use of *n*-butanesulfonyl chloride gave 41% of the *n*-butanesulfonamide (XIII).

TABLE II.—INHIBITION OF DIHYDROFOLIC REDUCTASE AND THYMIDYLATE SYNTHETASE BY



Compd.	R_1	R_2	Dihydrofolic Reductase[a] mM Concn. Inhibitor	Dihydrofolic Reductase[a] % Inhibitor	Dihydrofolic Reductase[a] Inhibitor: Substrate Ratio[c]	Thymidylate Synthetase[b] μM Concn. M-FAH$_4$[d]	Thymidylate Synthetase[b] mM Concn. Inhibitor	Thymidylate Synthetase[b] % Inhibition	Thymidylate Synthetase[b] Inhibitor: Substrate Ratio[e]	Synthetase: Reductase Ratio[f]
XV[g]	C_6H_5	H	0.60[h]	43	130	25.7	0.62[i]	50	50	0.39
						51.4	1.2[i]	50	50	
I[j]	C_4H_9-*n*	H	6.0	0	>4000	25.7	4.5	50	350	<0.087
II[k]	C_4H_9-*n*	C_6H_5CO—	0.19[h]	50	32	51.4	1.0[i]	0	>150	>4.7
VI	H	C_6H_5CO—	0.48[h]	50	80	51.4	1.5[i]	0	>230	>2.9
VII	H	$C_6H_5CH_2CO$—	0.94[h]	50	160	51.4	0.60[i]	0	>90	>0.56
VIII	H	CH_3CO—	1.3[h]	50	220	51.4	13	50	500	2.3
IX	H	*p*-$EtOCH_2C_6H_4CO$—	0.62[h]	50	100	51.4	1.5[i]	43	66	0.66
X	H	*p*-$NO_2C_6H_4CO$—	0.35[h]	50	58	51.4	1.1[i]	0	>170	>2.9
XI	H	*p*-$NH_2C_6H_4CO$—	0.77[h]	50	130	51.4	1.5[i]	14	>230	>1.8
III[k]	C_4H_9-*n*	*p*-$CH_3C_6H_4SO_2$—	0.19[h]	50	32	51.4	0.15[i]	26	17	0.50
XII	H	*p*-$CH_3C_6H_4SO_2$—	0.60[h]	20	400	51.4	0.28[i]	50	11	0.027
XIII	H	*n*-$C_4H_9SO_2$—	6.0[h]	45	1300	51.4	4.6[i]	50	180	0.014

[a] Dihydrofolic reductase from pigeon liver was prepared and assayed with 6 μ*M* dihydrofolate and 12 μ*M* TPNH in 0.05 *M* Tris buffer at pH 7.4 as previously described (2). [b] Thymidylate synthetase from *Escherichia coli* B was prepared and assayed with 80 μ*M* 2′-deoxyuridylate, *dl*-tetrahydrofolate, magnesium chloride, and formaldehyde in 0.05 *M* Tris buffer at pH 7.4 as previously described (2), except that cells were broken by passage of a suspension of 1 Gm. per 1 ml. of buffer through a French press (1). [c] Estimated ratio of concentrations of inhibitor to dihydrofolate required for 50% inhibition. [d] M-FAH$_4$ = 5,10-methylene-*dl*-tetrahydrofolate. [e] Estimated ratio of concentrations of inhibitor to the active *l*-isomer of 5,10-methylenetetrahydrofolate required for 50% inhibition. [f] Ratio of inhibitor:substrate for 50% inhibition of thymidylate synthetase to inhibitor:substrate for 50% inhibition of dihydrofolic reductase. [g] Enzyme data from *Reference 2*. [h] Enzyme assay with 10% *N*,*N*-dimethylformamide present. [i] Enzyme assay with 5% 2-methoxyethanol present. [j] Enzyme data from *Reference 3*. [k] Enzyme data from *Reference 1*.

EXPERIMENTAL

Melting points were determined in capillary tubes in a Mel-Temp block, and those below 230° are corrected. Infrared spectra were determined in KBr pellets with a Perkin-Elmer 137B spectrophotometer. Ultraviolet spectra were determined with a Perkin-Elmer 202 spectrophotometer. Thin-layer chromatograms were run on Brinkmann Silica Gel G, and spots were detected by iodine vapor.

2 - Amino - 4 - hydroxy - 6 - methyl - 5 - pyrimidylpropylamine Dihydrochloride (V).—A mixture of 3.56 Gm. (20 mmoles) of IV, 250 mg. of platinum oxide catalyst, 200 ml. of 85% aqueous ethanol, and 1.46 Gm. of 12 *N* hydrochloric acid was shaken with hydrogen at 2–3 Atm. for about 18 hr. when reduction was complete. The catalyst was removed by filtration, and the colorless filtrate was spin-evaporated *in vacuo*. The residue was dissolved in about 4 ml. of water, and several volumes of acetone were added. The product gradually crystallized from the solution as white crystals, m.p. 298–300° dec.; yield, 3.57–4.17 Gm. (70–80%); $\lambda_{max.}^{pH\ 1}$ 265 mμ; $\lambda_{max.}^{pH\ 7}$ 268, shoulder at 285 mμ; $\lambda_{max.}^{pH\ 13}$ 280 mμ; $\nu_{max.}$ 3380, 3240 (NH, OH); 2040 (NH^+); 1690 cm.$^{-1}$ (C=NH^+).

Anal.—Calcd. for $C_8H_{16}Cl_2N_4O$: C, 37.7; H, 6.33; N, 21.9. Found: C, 37.5; H, 6.30; N, 21.7.

2 - Amino - 5 - (3 - benzamidopropyl) - 6 - methyl-4-pyrimidinol (VI).—To a solution of 255 mg. (1 mmole) of V in 0.67 ml. of 3 *N* aqueous sodium hydroxide and 0.50 ml. of water was added 3.5 ml. of acetone. To the magnetically stirred solution was immediately added 339 mg. (1.5 mmoles) of benzoic anhydride in one portion. After being stirred for about 18 hr. in a stoppered flask, the product was collected on a filter and washed with water; yield, 232 mg. (81%), m.p. 265–268° dec. Recrystallization from 2-methoxyethanol by addition of water gave white crystals, m.p. 265–270° dec., with some softening at 150–170°; $\nu_{max.}$ 3400, 3300 (NH); 1660, 1640, 1600, 1550 (C=O, C=C, NH, pyrimidine); 782, 690 cm.$^{-1}$ (CH of benzoyl); $\lambda_{max.}^{pH\ 13}$ 280 mμ. (See Table I for analytical data; compounds prepared by this method are listed under method *A* in this table.)

2 - Acetamido - 5 - (3 - acetamidopropyl) - 6 - methyl-4-pyrimidinol.—Addition of 0.50 ml. (5 mmoles) of acetic anhydride to a solution of free base of V, as described for the preparation of VI, gave on the evaporation of the acetone, 140 mg. (about 50%) of product, m.p. 240–242°. Although this material was homogeneous on TLC, combustion analysis showed that it was partially an acetate salt. The product was dissolved in water and the pH adjusted to about 7 with 0.1 *N* sodium hydroxide. The product was collected and recrystallized from water to give white crystals, m.p. 244–246°; $\nu_{max.}$ 3500, 3450, 3300 (NH, OH); 1640, 1625, 1570 cm.$^{-1}$ (C=O, NH, pyrimidine); $\lambda_{max.}^{pH\ 1}$ 246, 263 mμ; $\lambda_{max.}^{pH\ 5}$ 246, shoulders at 252, 267 mμ; $\lambda_{max.}^{pH\ 13}$ 247, 277 mμ. The ultraviolet spectrum clearly showed that the compound was a 2-acetamido-4-pyrimidinol (9). Although this compound did not give acceptable combustion values, it could be smoothly converted to VIII.

Anal.—Calcd. for $C_{12}H_{18}N_4O_3 \cdot 0.75\ H_2O$: C, 51.5; H, 7.02; N, 20.0. Found: C, 51.2; H, 6.68; N, 19.8.

If the aqueous reaction mixture was neutralized before crystallization, then a mixture of the 2-acetamido-4-pyrimidinol and VIII was obtained; since VIII could be prepared from this mixture, the following preparative procedure for VIII was employed.

2 - Amino - 5 - (3 - acetamidopropyl) - 6 - methyl-4-pyrimidinol (VIII).—To a magnetically stirred solution of 765 mg. (3 mmoles) of V in 2.0 ml. of 3 *N* sodium hydroxide and 1.5 ml. of water was added 10.5 ml. of acetone, followed by 1.5 ml. (15 mmoles) of acetic anhydride. After being stirred for several hours, the mixture was neutralized to pH 7 with 0.1 *N* sodium hydroxide, then spin-evaporated *in vacuo* to about 10 ml. After several hours at 5°, the mixture was filtered, and the product was washed with a small amount of cold water. Recrystallization from water gave 470 mg. (about 60%) of crystals, m.p. 223–235° dec., which were a mixture of VIII and its N-2 acetyl derivative as shown by TLC.

A solution of 266 mg. of this mixture in 10 ml. of methanol and 0.5 ml. of *n*-butylamine was refluxed for 2 hr., then spin-evaporated *in vacuo*. After trituration of the residue with petroleum ether, it was recrystallized from 4 ml. of water to give white crystals, m.p. 225–228°, which were uniform on TLC, m.p. 225–228°; yield, 142 mg. (37% based on V); $\nu_{max.}$ 3450, 3350, 3140 (NH, OH); 1660, 1640, 1620, 1550 cm.$^{-1}$ (C=O, NH, pyrimidine); $\lambda_{max.}^{pH\ 1}$ 269 mμ; $\lambda_{max.}^{pH\ 5}$ 276, shoulder at 293 mμ; $\lambda_{max.}^{pH\ 13}$ 281 mμ. (This is listed as method *B* in Table I along with analytical data.)

2 - Amino - 6 - methyl - 5 - (3 - phenylacetamidopropyl) - 4 - pyrimidinol (VII).—To a magnetically stirred solution of 255 mg. (1 mmole) of V in 0.67 ml. of 3 *N* aqueous sodium hydroxide and 0.50 ml. of water was added 159 mg. (1.5 mmoles) of anhydrous sodium carbonate. As soon as solution was complete, 3.5 ml. of acetone was added, followed by 0.20 ml. (1.5 mmoles) of phenylacetyl chloride. The mixture was stirred for about 18 hr. in a stoppered flask, during which time the product separated. The product was collected on a filter and washed with aqueous acetone; yield, 255 mg. (85%), m.p. 252–257° dec. Recrystallization from methanol gave white crystals, m.p. 264–265° dec.; $\nu_{max.}$ 3300 (NH, OH); 1660 (C=O); 1650, 1625, 1590, 1530 cm.$^{-1}$ (NH, C=C, pyrimidine). (This is listed as method *C* in Table I along with analytical data.)

2 - Amino - 5 - [3 - (p - aminobenzamido)propyl]-6-methyl-4-pyrimidinol (XI).—A mixture of 731 mg. (2 mmoles) of X, 100 ml. of 85% aqueous ethanol, 0.34 ml. of 12 *N* hydrochloric acid (4 mmoles), and 100 mg. of 5% palladium charcoal was shaken with hydrogen at 2–3 Atm. until reduction was complete (about 3 hr.). The filtered solution was spin-evaporated *in vacuo*. The residue was suspended in water, and sufficient 3 *N* hydrochloric acid was added to complete solution. To the solution was added excess ammonia water; then it was concentrated by spin-evaporation *in vacuo* until the product began to separate. The product was collected on a filter and washed with water; yield, 294 mg. (49%), m.p. 265–272° dec. Recrystallization from 50% aqueous 2-methoxyethanol afforded the analytical sample, m.p. 275–276° dec.; $\nu_{max.}$ 3400, 3300 (NH, OH); 1680 (C=O);

1630, 1620, 1570, 1550, 1510 cm.$^{-1}$. (NH, C=C, pyrimidine.) (See Table I for analytical data.)

RESULTS AND DISCUSSION

It was previously reported that the *N*-butylbenzamidopropyl pyrimidine (II) was an effective inhibitor of dihydrofolic reductase, but not of thymidylate synthetase (1). Similar results have now been found (Table II) with the benzamidopropyl pyrimidine (VI) without the *N*-butyl group, although VI is somewhat less effective on dihydrofolic reductase than II. Lengthening of the chain by one more carbon atom, as in the phenylacetamidopropyl pyrimidine (VII), led to a further loss of binding to dihydrofolic reductase; that is, VI was about twice as effective as the standard anilinopropyl pyrimidine (XV), but VII was about equally effective as XV.

Since the possibility existed that the carbonyl group of VI or VII was binding to the enzyme rather than the phenyl ring, the acetamidopropyl pyrimidine (VIII) was synthesized and evaluated. Surprisingly, VIII was about one-half as effective as the anilinopropyl pyrimidine (XV), showing that the carboxamide group also can bind in place of a phenyl group. Thus, the benzamido compound (VI) may be binding to dihydrofolic reductase either through the carbonyl group or the phenyl ring or both. Although the benzamido compound (VI) is about three times as effective as the acetamido compound (VIII), the difference could still be because the carbonyl group bearing a phenyl substituent (VI) is a better electron acceptor than the carbonyl group bearing a methyl substituent (VIII). Since previous evidence (3) has indicated that the phenyl group may bind to dihydrofolic reductase as an electron acceptor in a charge-transfer complex, introduction of *p*-nitro on the phenyl ring (X) should tighten, and the *p*-amino group (XI) should weaken binding, compared to the parent benzamido derivative (VI). This was indeed the case, but the effects were relatively small. Of the three amides (VI–VIII), only the acetamido derivative (VIII) was soluble enough to reach the 50% inhibition concentration for thymidylate synthetase; VIII was somewhat less effective than the butylaminopropyl pyrimidine (I) which previously was felt to have only pyrimidine binding to this enzyme (3). VI and VII were two and five times, respectively, less effective than the anilinopropyl pyrimidine (XV) on this enzyme.

The tosylamidopropyl pyrimidine (XII) was an effective inhibitor of thymidylate synthetase, being four times better than the standard anilinopropyl pyrimidine (XV). A similar activity has been previously reported with the *N*-butyl analog (III) (1). Surprisingly, the tosylamidopropyl pyrimidine (XII) was twelvefold less effective on dihydrofolic reductase than the *N*-butyl derivative (III). No rational explanation for this phenomenum has emerged yet; the tosylamido group has a pKa too high to be appreciably ionized at pH 7.4. Therefore, this difference is not due to ionization. The possibility of a hydrophobic contribution by the *n*-butyl group to binding is unlikely, since a similar increment should have occurred with the two benzamido derivatives, II and VI.

The relative inactivity of the benzamidopropyl pyrimidine (VI) on thymidylate synthetase compared to the tosylamido pyrimidine (XII) was surprising since the bridge length is the same and the carbonyl group of VI certainly offers less steric hindrance than the sulfone group of XII. One possible explanation was that the sulfone group of XII rather the phenyl was binding to thymidylate synthetase. To check this point, the *n*-butylsulfonamidopropyl pyrimidine (XIII) was synthesized and evaluated. Since the tosylamido derivative (XII) binds to thymidylate synthetase 14 times better than the butylsulfonamido derivative (XIII), it is unlikely that the sulfonyl group contributes appreciable direct binding. A similar difference was previously observed with the binding of the *N*-butylbenzamide (II) and the *N*-butyltosylamide (III) (1). In the latter case, it was suggested that part of the difference may be due to the electron-donating properties of the methyl group, since the phenyl ring might bind to thymidylate synthetase in a charge-transfer complex where the phenyl is an electron donor (3, 10)—just the opposite proposed for binding of this group to dihydrofolic reductase.

Although the *p*-toluamide analog of VI was not available, the *p*-ethoxymethylbenzamide (IX) was available from another study; the ethoxymethyl group of IX should be an electron donor, but less effective than a methyl group. When tested for inhibition of thymidylate synthetase, IX was greater than three times as effective as the benzamido derivative (VI), being about equal to the anilinopropyl pyrimidine (XV).

Since the ethoxymethyl group of IX made the phenyl group bind better to thymidylate synthetase, the *p*-aminobenzamido derivative (XI) was synthesized for enzymic evaluation; the *p*-amino group is about the most powerful electron donor available (11). The *p*-aminobenzoyl derivative was not so effective as the *p*-ethoxymethyl derivative (IX); therefore, the increase in binding caused by the *p*-ethoxymethyl group of IX compared to VI does not appear to be due to its electron-donor properties.

Regardless of the mechanism of binding of the compounds to the two enzymes in Table I, an important cross-over specificity exists. (*a*) The tosylamidopropyl pyrimidine (XII) is thirty times more effective on thymidylate synthetase than on dihydrofolic reductase. (See column 3 under *Thymidylate Synthetase*, Table II.) (*b*) The benzamidopropyl pyrimidine (VI) is greater than three times more effective on dihydrofolic reductase than thymidylate synthetase; how much less effective VI is on thymidylate synthetase cannot be determined due to the lack of sufficient solubility of VI to reach a point where inhibition of thymidylate synthetase can be detected.

The utilization of these new bridges, such as in VI and XII, for the synthesis of potential active-site-directed irreversible inhibitors (12) of dihydrofolic reductase and thymidylate synthetase is currently under investigation. The synthetic sequence is relatively short, and the preattachment of covalent-linking groups (12) to benzoic acid or benzenesulfonyl chloride should be readily feasible.

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Segregation Kinetics of Particulate Solids Systems III

Dependence on Agitation Intensity

By MORRIS D. FAIMAN* and EDWARD G. RIPPIE

Studies of the segregation behavior of particulate systems indicate the simultaneous occurrence of both segregation and mixing. The specific rate constants for these processes are calculated from statistical data and are shown to be a function of the particulate velocities. The results suggest the analogy of mechanical agitation in these idealized systems to thermal motion in molecular systems. Arrhenius-type plots of first-order rate constants *versus* the square of the reciprocal particulate velocities make possible the prediction of segregation behavior at various agitation intensities.

IN PREVIOUS reports (1, 2), the segregation occurring in particulate systems of steel and glass spheres has been characterized partially. The over-all process results in a separation of particles of different physical properties and is a function of density, particle size, and the size of the container in which the segregation occurs.

The present work indicates the manner in which energy input affects both the segregation and mixing processes which are shown to occur simultaneously.

Donald and Roseman (3, 4), in experiments with a drum mixer, have reported that increased mixer speed reduced the final degree of mixing. The results reported here suggest that this may not always be the case, but that the segregation characteristics of granulations can be expected to vary with the physical properties of their constituents. The mechanical agitation of the systems under study shows a marked similarity to the thermal motion of molecular systems. The specific forward and reverse rate constants, representing segregation and mixing, respectively, have been evaluated from statistical data for the over-all process of segregation. These rate constants, when plotted as a function of the particulate energies, substantiate the analogy of agitation to thermal motion.

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* Present address: University of Kansas, Lawrence.

THEORY

Segregation has been shown to proceed by apparent over-all first-order kinetics to an equilibrium state in which the rates of mixing and unmixing balance (1). This suggests that both mixing and segregation, which are shown to occur simultaneously here at the particulate level, are first-order processes and that the observed rate constant, k_0, is the sum of the constant for segregation, k_1, and that for mixing, k_2. The process may be considered analogous to a reversible chemical reaction in which both forward and reverse steps occur simultaneously. The over-all process, if followed, will reflect the predominance of one reaction step (forward or reverse) over the other. A system of particles following this behavior (5) can be represented by

$$A \underset{k_2}{\overset{k_1}{\rightleftarrows}} B$$

Here, A represents the concentration of mixed particles and B the concentration of those unmixed. The rate equation for this system is given by

$$dA/dt = -k_1A + k_2B \qquad \text{(Eq. 1)}$$

The concept of concentration used here relates to the relative number of spheres which may be classified as mixed or unmixed on the basis of their immediate neighbors. Thus, particles within a sample are considered unmixed in so far as they exceed their mean proportion of the total system. The remainder of particles within the sample are