scribed in this paper, the solute mole fractions can be calculated readily and the DEC's of the solvent systems can be determined experimentally, accurately and quickly. The resulting graphs may be used for estimating the quantitative solubility of a solute in a solvent of a homologous series, for estimating the quantitative solubility of a solute in a solvent blend, for estimating the composition of a solvent blend required to dissolve a given amount of solute, for evaluating the efficiency of various solvents as coupling agents or ingredients of solvent blends, and for predicting the probable solvency properties of related solvents.

Thus, the use of dielectric constants presents an intriguing approach for the determination and correlation of solubility phenomenon.

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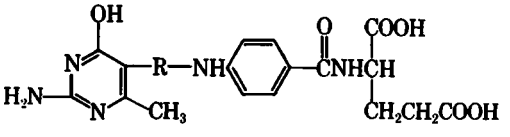
Analogs of Tetrahydrofolic Acid XI

Synthesis and Enzymic Evaluation of *p*-{[*N*-(2-Amino-4-hydroxy-6-methyl-5-pyrimidyl)carbamoyl methyl]amino}benzoyl-L-glutamic Acid

By B. R. BAKER and KRISHNA SACHDEV

Condensation of ethyl α-acetamidoacetoacetate (XII) with guanidine carbonate afforded 5-acetamido-2-amino-4-hydroxy-6-methylpyrimidine (X). Acid hydrolysis of X followed by reaction with bromoacetyl bromide gave 2-amino-5-(bromoacetamido)-4-hydroxy-6-methylpyrimidine (XI). Reaction of XI with aniline or *p*-aminobenzoyl-L-glutamic acid in dimethylsulfoxide resulted in formation of 2-amino-5-(anilinoacetamido)-4-hydroxy-6-methylpyrimidine (XIII) and the title compound (III), respectively. III was a poor inhibitor of both dihydrofolic reductase and 5,10-methylene-tetrahydrofolate dehydrogenase, showing inhibition of about the order obtained with *p*-aminobenzoyl-L-glutamic acid.

A PYRIMIDYL ANALOG (I) (2–4) of tetrahydrofolic acid has been found to bind to folic reductase five times better than the substrate folic acid (4), to bind to 5,10-methylene-tetrahydrofolate dehydrogenase about one-thirtieth as well as the substrate (1), and to bind to thymidylate synthetase one-sixth as well as the substrate (5); *dl*-5,10-methylene-tetrahydrofolate was the substrate for the latter two enzymes. In contrast, the analog (II) is only one-eightieth as effective as I as an inhibitor of folic reductase (1), but II binds somewhat better to 5,10-methylene-tetrahydrofolate dehydrogenase than does I (1). It was concluded that the basic *p*-amino group of



I, R = $-CH_2CH_2CH_2-$
II, R = $-CH_2CH_2CO-$
III, R = $-NHCOCH_2-$

the *p*-aminobenzoyl-L-glutamate moiety was probably one of the essential groups for binding to folic reductase, but not to 5,10-methylene-tetrahydrofolate dehydrogenase. It was therefore of interest to synthesize III for enzymic evaluation; III has a different bridge between the pyrimidyl and aminobenzoyl moieties, but still retains the basicity of the latter moiety.

A key intermediate in the synthesis of III (see Scheme I) was 2,5-diamino-4-hydroxy-6-methylpyrimidine (IX), a compound described only once in the literature (6). Bromination of 2-amino-4-

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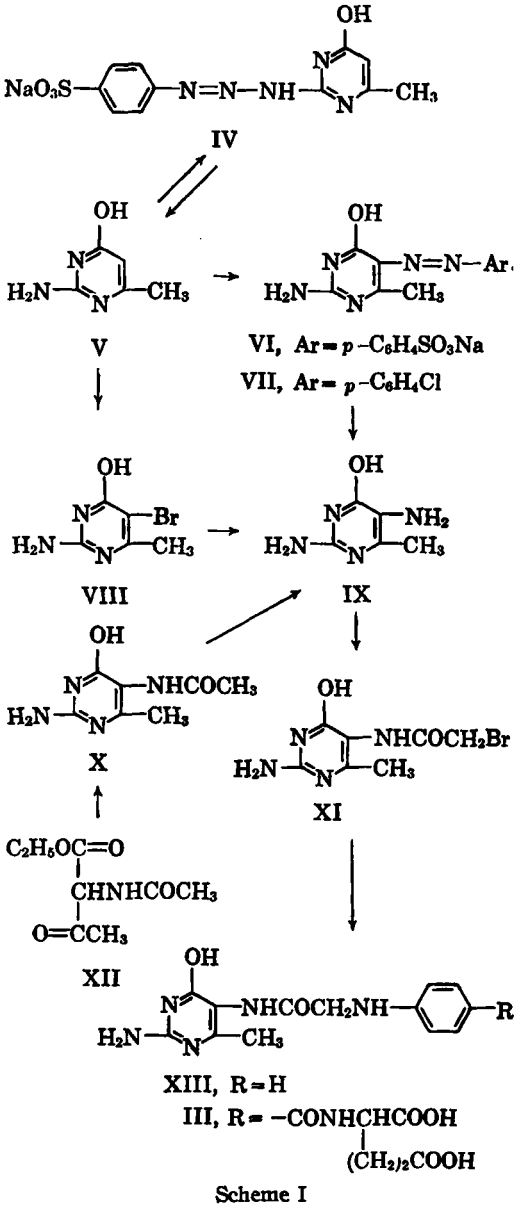

Scheme I

hydroxy-6-methylpyrimidine (V) according to Jaeger (6) gave a 98% yield of the 5-bromopyrimidine (VIII) as the hydrobromide salt. Reaction of VIII with concentrated ammonia water at 200° as previously described (6) gave only traces of the desired 5-aminopyrimidine (IX).

Todd, *et al.* (7), have introduced the 5-amino group in certain pyrimidines by coupling with a diazonium salt followed by reduction of the resultant azo derivative; although they made a number of 5-aminopyrimidines in this way, a number of other pyrimidines were merely surveyed to see if coupling with a diazonium salt to form an azo compound took place—as evidenced by color development. In the latter category was 2-amino-4-hydroxy-6-methylpyrimidine (V) which they described gave a color with *p*-chlorophenyl diazonium chloride attributed to formation of the azo dye (VII). We have investigated this reaction in more detail using the more stable diazonium salt from sulfanilic acid; coupling to V in sodium carbonate solution occurred with formation of an insoluble sodium salt, presumably with structure VI. When the insoluble azo dye was reduced in warm water with sodium hydrosulfite, the product did not agree in properties with the 5-aminopyrimidine (IX) prepared earlier from the 5-bromopyrimidine (VIII); the ultraviolet and infrared spectra indicated that a mixture of the starting material (V) and the desired 5-aminopyrimidine (IX) was obtained. That such was the case was readily verified by thin-layer chromatography by comparison of this mixture with IX (obtained from VIII) and V.

At first glance it would appear that V must have been carried along with the insoluble azo compound (VI). That such was not the case was shown by thin-layer chromatography; although two spots were present in the dye, one moved slower than V and one moved faster than V. It is clear that one spot must be the desired 5-azopyrimidine (VI); it is probable that the second spot was an azoaminopyrimidine (IV), which on reduction would regenerate V. Attempts to rearrange this supposed *N*-azo derivative (IV) to a 5-azo derivative (VI) in dilute acetic acid, according to the general procedure of Rosenhaus and Unger (8) were unsuccessful. It is clearly possible that other azo dyes mentioned by Todd, *et al.* (7), may also be mixtures of *N*-azo and 5-azo aminopyrimidines.

A simple and successful route to the 5-aminopyrimidine (IX) *via* X was finally found. Condensation of ethyl α-acetamidoacetoacetate (XII) (9) with guanidine carbonate in boiling ethanol gave the 5-acetamidopyrimidine (X) in 30–40% yield. Since this range of yields was obtained within 2 hours and did not increase with increasing reaction time, ethanolic cleavage of the keto ester (XII) was probably a competitive reaction (10). When the guanidine carbonate reaction with XII was run under noncleavage conditions by using *tert*-butyl alcohol (10) as the solvent, the yield of 5-acetamidopyrimidine (X) was increased to 72%.

Hydrolysis of the 5-acetamidopyrimidine (X) with boiling 6 *N* hydrochloric acid gave the desired 5-aminopyrimidine (IX) hydrochloride in 83% yield; the free base (IX) obtained from the hydrochloride was uniform on thin-layer chromatography and was identical with IX prepared from the 5-bromopyrimidine (VIII). When the 5-aminopyrimidine (IX) hydrochloride was re-

acted with excess bromoacetyl bromide in aqueous sodium bicarbonate suspension, the 5-(bromoacetamido)pyrimidine (XI) was obtained in 69% yield; although XI had no melting point, it was uniform on thin-layer chromatography and was clearly distinguishable from the starting material (IX) and had an infrared spectrum similar to X.

Displacement of bromide from the 5-(bromoacetamido)pyrimidine (XI) with excess aniline in dimethylsulfoxide at ambient temperature proceeded smoothly to the 5-(anilinoacetamido)-pyrimidine (XIII) in 73% yield. A similar reaction between XI and *p*-aminobenzoyl-L-glutamic acid to give the tetrahydrofolate analog (III) proceeded less effectively; partly due to the difficulty in isolation and purification of the product the yield was only 13%.

When the 5-(anilinoacetamido)pyrimidine (XIII) was assayed as an inhibitor of the dihydrofolic reductase from pigeon liver, no inhibition was observed up to a concentration of 0.5 m*M;* in contrast, 0.5 m*M* 2-amino-5-(3-anilinopropyl)-4-hydroxy-6-methylpyrimidine (1) gave 40% inhibition of this dihydrofolic reductase when 6 μM dihydrofolate and 9 μM reduced triphosphopyridine-nucleotide (TPNH) were employed (1). Similarly, III was a poor inhibitor of this dihydrofolic reductase, only 20% inhibition being observed at a concentration of 6 m*M;* this result contrasts with the 50% inhibition of this enzyme shown by 0.10 m*M* concentration of I.

When III was assayed as an inhibitor of the 5,10-methylene-tetrahydrofolate dehydrogenase from pigeon liver in the presence of 0.03 m*M dl*-5,10-methylene-tetrahydrofolate and 0.10 m*M* TPN (1), 50% inhibition was obtained at a concentration of 3.2 m*M;* this compares not too favorably with 1.1 m*M* of I and 0.47 m*M* of II required for 50% inhibition of this enzyme (1). In fact, III showed about the same order of inhibition of the two enzymes as *p*-aminobenzoyl-L-glutamic acid, indicating that the pyrimidyl moiety of III made no contribution toward the binding of the compound to either enzyme.

At this time an exact explanation of why III is not so good an inhibitor of dihydrofolic reductase or 5,10-methylene-tetrahydrofolate dehydrogenase as I or II cannot be given. Since III should have all the necessary binding groups for both of these enzymes, it is possible that this alternate side chain in III (compared to I) could cause some steric interference with enzyme binding in the region of —NHCO— group, or the —NHCO— group of the bridge could have an unfavorable influence on the pK's or tautomerism of the groups in the pyrimidine moiety that are necessary for binding. Whether III would be a better inhibitor than I and II of some of the other enzymes in folic cofactor area (2) remains to be determined.

EXPERIMENTAL

Melting points were determined in capillary tubes with a Mel-temp block; those melting points below 230° are corrected. Infrared spectra were determined in KBr disk with a Perkin-Elmer model 137B spectrophotometer. Ultraviolet and visible spectra were determined with a Perkin-Elmer model 202 spectrophotometer. Thin-layer chromatography (TLC) was performed on silica gel on glass plates with methanol as the developing solvent; spots were detected with iodine vapor. Concentration of all solutions was performed by spin-evaporation *in vacuo* at a bath temperature of 50–70°.

5 - Acetamido - 2 - amino - 4 - hydroxy - 6-methylpyrimidine (X).—Ethyl α-acetamidoacetoacetate (XII) was prepared by the reaction of phenyl diazonium chloride with ethyl acetoacetate followed by reductive acetylation as described by Pfister, *et al.* (9), in 76% over-all yield for the two steps. The crude, waxlike, but crystalline XII was employed for condensation with guanidine since better over-all yields were obtained; losses in recrystallization of the low melting XII were high.

A mixture of 0.93 Gm. (5 mmoles) of XII, 12 ml. of *tert*-butanol and 0.45 Gm. of guanidine carbonate was refluxed with magnetic stirring for 10 hours during which time the product separated. The mixture was evaporated and the residue triturated with 20 ml. of cold water. The product was collected on a filter and washed with ice-water; yield, 0.67 Gm. (72%), m.p. 305–308° dec. Recrystallization of an identical sample, prepared in ethanol as the reaction solvent, from ethanol gave white crystals of a hydrate, m.p. 308–310° dec.; $\lambda_{max.}^{EtOH}$ 227, 291 mμ; $\nu_{max.}^{KBr}$ 3300, 3100 (NH); 1700 (amide C=O); 1670–1630 (broad); 1580, 1530, 1510 cm.$^{-1}$ (NH, pyrimidine). The compound traveled as a single spot on TLC.

Anal.—Calcd. for $C_7H_{10}N_2O_2 \cdot H_2O$: C, 42.0; H, 6.05; N, 28.0. Found: C, 42.3; H, 5.91; N, 27.7, 28.0.

2,5 - Diamino - 4 - hydroxy - 6 - methylpyrimidine (IX) Hydrochloride.—A solution of 2.0 Gm. of X in 20 ml. of 6 *N* hydrochloric acid was refluxed for 30 minutes, then evaporated to dryness. The residue was triturated with methanol; yield, 1.6 Gm. (83%) of hydrochloride, m.p. 255–260° dec.; $\nu_{max.}^{KBr}$ 3330 (NH); 1705 (C=NH$^+$); 1680, 1540, 1480, 1460 cm.$^{-1}$ (NH, pyrimidine). This material was suitable for conversion to XI.

The free base (IX) crystallized when a concentrated aqueous solution of the hydrochloride was neutralized with sodium bicarbonate. The base (IX) is quite water soluble and had m.p. 270–275° dec.; $\lambda_{max.}^{EtOH}$ 245, 305 mμ; $\nu_{max.}^{KBr}$ 3400, 3300, 3200, 3100 (NH); 1680, 1640, 1580, 1550, 1530, 1500 cm.$^{-1}$ (NH, pyrimidine). This compound gave a single spot on TLC that moved slower than X, but was the same as IX prepared in traces from 2-amino-5-bromo-4-hydroxy-6-methylpyrimidine as described by Jaeger (6). Jaeger (6) recorded a m.p. of 275°, the yield being a "small amount."

2 - Amino - 5 - (bromoacetamido) - 4 - hydroxy-6-methylpyrimidine (XI).—To a stirred suspension of 1.41 Gm. (8 mmoles) of IX hydrochloride in

50 ml. of water containing 3.8 Gm. of sodium bicarbonate was added 3.03 Gm. (16 mmoles) of bromoacetyl bromide in one portion. The mixture was vigorously stirred for 7 hours at ambient temperature during which time the nature of the suspended material changed. The product was collected on a filter and washed with water, then ethanol; yield, 1.28 Gm. (60%) of white solid which partially melts about 255° but does not completely melt below 320°. This material was suitable for further reactions.

To the aqueous filtrate was added 1.00 Gm. of sodium bicarbonate and 1.3 Gm. of bromoacetyl bromide. After being stirred for 4 hours, the solution deposited an additional 0.10 Gm. (9%) of product identical with the first crop. A similar preparation was purified for analysis by two recrystallizations from absolute ethanol: white crystals, m.p. above 300°, that gave a strong halogen test; $\lambda_{max.}^{EtOH}$ 227, 291 mμ; $\nu_{max.}^{KBr}$ 3400, 3300–3000 (NH); 1690 (amide C=O); 1670–1630, 1580, 1540, 1500 cm.$^{-1}$ (NH, pyrimidine). This compound gave a single spot on TLC which moved faster than X and IX.

Anal.—Calcd. for $C_7H_9BrN_4O_2 \cdot {}^1/_3EtOH$: C, 33.3; H, 4.02; N, 20.3. Found: C, 33.2, 33.3; H, 3.92, 3.80; N, 20.0.

2 - Amino - 5 - (anilinoacetamido) - 4 - hydroxy-6-methylpyrimidine (XIII).—To a solution of 522 mg. (2 mmoles) of XI in 5 ml. of dimethylsulfoxide was added 0.37 ml. (4 mmoles) of aniline. After standing at ambient temperature in a stoppered flask for 2 days, the solution was diluted with 20 ml. of cold water. After 20 minutes in an ice bath, the mixture was filtered and the product washed with water; yield, 400 mg. (73%), m.p. 200–205° dec., that was soluble in 0.1 *N* hydrochloric acid, in contrast to XI. Two recrystallizations from aqueous ethanol gave white crystals of a hydrate, m.p. 210° dec.; $\nu_{max.}^{KBr}$ 3540, 3300, 3280 (NH, OH); 1720 (amide C=O); 1680–1580, 1530, 1490 (NH, C=C, pyrimidine); 763, 690 cm.$^{-1}$ (C_6H_5-). This compound could not be freed completely from solvated water. Analyses varied depending upon the drying conditions.

Anal.—Calcd. for $C_{13}H_{15}N_5O_2 \cdot 1{}^1/_2H_2O$: C, 52.6; H, 6.20; N, 23.5. Found: C, 52.7; 52.9; H, 6.12, 5.98; N, 23.1, 23.1.

Further drying at 100° in high vacuum over P_2O_5 gave the following analytical results:

Anal.—Calcd. for $C_{13}H_{15}N_5O_2 \cdot {}^1/_2H_2O$: C, 54.8; H, 5.75; N, 24.5. Found: C, 54.8; H, 6.04; N, 24.5.

p - {[N - (2 - Amino - 4 - hydroxy - 6 - methyl-5 - pyrimidyl)carbamoylmethyl]amino} benzoyl-L-glutamic Acid (III).—A solution of 0.783 Gm. (3 mmoles) of XI and 1.20 Gm. (4.5 mmoles) of *p*-aminobenzoyl-L-glutamic acid in 10 ml. of dimethylsulfoxide was allowed to stand in a stoppered flask at ambient temperature for 3 days. The solution was diluted with 40 ml. of water and adjusted to pH 3.8 with 1% aqueous sodium bicarbonate. The finely divided precipitate was collected by centrifugation and washed with water. The crude product was dissolved in the minimum of 1% aqueous sodium bicarbonate. After clarification by filtration, the solution was acidified with glacial acetic acid. The product was again collected by centrifugation and washed with water; yield, 0.175 Gm. (13%). Recrystallization from methanol gave a white powder that had no melting point and did not give a resolved infrared spectrum. Solvate was removed only after drying for 24 hours at 100° in high vacuum over P_2O_5. The analytically pure material then gave a negligible Bratton-Marshall test for diazotizable amine (4) and had $\lambda_{max.}^{pH\ 1}$ 280 mμ (ε 25,200), $\lambda_{max.}^{pH\ 8.4}$ 288 mμ (ε 24,400), $\lambda_{max.}^{pH\ 13}$ 282 mμ (ε 25,700).

Anal.—Calcd. for $C_{19}H_{22}N_6O_7$: C, 51.1; H, 4.93; N, 18.8; O, 25.1. Found: C, 50.9; H, 4.90; N, 19.0; O, 25.2.

No better yields were obtained when the dry *N,N*-dimethylformamide was employed as the solvent. When the reaction was run in dimethylsulfoxide at 80–90° for 5 hours, the isolated product (11%) was considerably darkened and had about 7% of Bratton-Marshall positive material after short treatment with hot 3 *N* hydrochloric acid (1).

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