top of a Celite column (215 × 35 ml.) prepared with 40 g. of Celite 545 and 40 ml. of water-saturated butanol.[8] The column was developed with water-saturated butanol and 20-ml. fractions were collected automatically. The nucleoside appeared in fractions 8–14 and was maximal at fraction 11. No other material appeared through fraction 37. The combined fractions (8–14) were evaporated *in vacuo* to give 121 mg. (70% recovery) of pure nucleoside XIII as a colorless glass; λ_{max}^{KBr} 2.93 μ (OH, NH), 6.09, 6.15, 6.35 μ (NH_2, C=C, C=N), 8.85, 9.15 μ (C–O–C and C–O–H). The over-all yield from 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-6-deoxy-L-talofuranose (XI) was 19%.

(C) Characterization.—A sample of the nucleoside, contaminated with ammonium formate, that was obtained from the ion exchange column was dissolved in 10% aqueous methanol and treated with an excess of 10% methanolic picric acid. The picrate was collected on a filter and recrystallized from water, then the nucleoside was regenerated with Dowex 2 (CO_3) as described previously to give a white foam, $[\alpha]^{27}D$ −35° (0.84% in H_2O). This material was chromatographically pure in solvents A, B and C,[15] but could not be completely freed of solvents without decomposition.

Anal. Calcd. for $C_{11}H_{15}N_5O_4$: C, 47.0; H, 5.38; N, 24.9. Found: C, 45.8; H, 5.98; N, 22.3.

2,6-Diacetamido-9-(2′,3′,5′-tri-O-benzoyl-6′-deoxy-α-L-talofuranosyl)-purine (XV).—A solution of freshly prepared XIV (from 2.00 g. (3.86 mmoles) of XI) in 50 ml. of dry xylene was added to a stirred suspension of 1.81 g. (3.86 mmoles) of chloromercuri-2,6-diacetamidopurine[14] and 2.05 g. of Celite in 130 ml. of xylene previously dried by distillation. After being refluxed with stirring for 3.5 hours, the mixture was processed in the usual way[2,14]; yield 2.47 g. (92%) of crude XV as a glass.

2,6-Diamino-9-(6′-deoxy-α-L-talofuranosyl)-purine (XVI).—A solution of 2.40 g. of crude XV in 60 ml. of methanol and 5.4 ml. of 1 *N* methanolic sodium methoxide was refluxed for 3 hours. After being acidified with acetic acid, the solution was evaporated to dryness *in vacuo* and the resultant residue was partitioned between 60 ml. each of water and chloroform. The aqueous solution was evaporated to dryness *in vacuo*. The residue (1.90 g.) was converted to the picrate (0.50 g.) and regenerated with Dowex 2 (CO_3)[14] as described for the preparation of XIII; yield 0.32 g. (29% based on XI) of an amorphous glass; λ_{max}^{KBr} 2.98 μ (OH, NH), 6.10, 6.25 μ (NH_2, C=C, C=N). This was essentially pure XVI with a trace of a 2,6-diaminopurine as shown by paper chromatography.[15]

The crystalline hydrochloride was prepared by the addition of 2 drops of 2 *N* hydrochloric acid to a solution of 0.24 g. of the base in 3 ml. of alcohol. The solid which separated was recrystallized from ethanol by addition of a little ethanolic hydrogen chloride to give white crystals, m.p. 212–213° dec., $[\alpha]^{28}D$ −19° (0.25% in H_2O); λ_{max}^{KBr} 2.98 μ (OH, NH), 5.94 μ (=NH_2^+), 6.07, 6.26 μ (NH_2, C=C, C=N).

Anal. Calcd. for $C_{11}H_{16}N_6O_4 \cdot HCl$: C, 39.7; H, 5.16; N, 25.2. Found: C, 39.8; H, 5.56; N, 25.3.

Both the free base and the hydrochloride gave a major spot with R_{ad} 0.97 in solvent A and a trace spot of 2,6-diaminopurine at R_{ad} 0.48. The hydrochloride consumed 0.91 mole of periodate in 15 minutes and 1.01 moles in 24 hours, thus confirming the furanose structure.

Acknowledgments.—The authors are indebted to Dr. Peter Lim for infrared interpretations, to Dr. Lloyd K. Moss and staff for paper and column chromatography and to O. P. Crews, Jr., and staff for large-scale preparation of certain intermediates

MENLO PARK, CALIF.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL SCIENCES, STANFORD RESEARCH INSTITUTE]

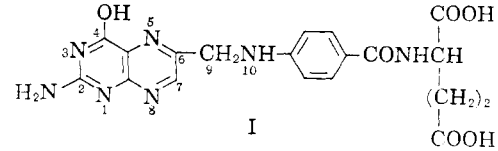
Potential Anticancer Agents.[1] IX. Tetrahydroquinazoline Analogs of Tetrahydrofolic Acid. I

BY RUTH KOEHLER, LEON GOODMAN, J. DEGRAW AND B. R. BAKER

RECEIVED MAY 26, 1958

Condensation of 2,4-dicarbomethoxycyclohexanone with guanidine led to an excellent yield of 2-amino-5,6,7,8-tetrahydro-4-hydroxyquinazoline-6-carboxylic acid (VI) which, *via* its *n*-butyl ester, could be reduced in good yield to 2-amino-5,6,7,8-tetrahydro-4-hydroxy-6-hydroxymethylquinazoline (IX). Compound IX was converted to 2-amino-6-chloromethyl-5,6,7,8-tetrahydro-4-hydroxyquinazoline (X) which, in turn, was condensed with N-(*p*-aminobenzoyl)-L-glutamic acid to give the tetrahydrofolic acid analog 5,8-dideaza-5,6,7,8-tetrahydrofolic acid (XI). The acid VI was converted to a variety of amides and esters and, *via* the acid hydrazide and acid azide, to the 6-amino compound XX. The latter compound was acylated conventionally to yield an amide and a sulfonamide.

Folic acid (I), one of the important B vitamins, serves as a precursor for the biogenetic synthesis of the cofactor, tetrahydrofolic acid conjugate. This latter, in turn, serves as both a formyl and hydroxymethyl transfer agent in a variety of biological systems. A large amount of work has been done on the synthesis of potential folic acid antagonists and a few active compounds have been found,[2] the best known of which are the clinically useful aminopterin, the 4-amino analog of folic acid (I), and amethopterin, the 4-amino-10-methyl analog of I. Both of these compounds, in microbiological systems, function by non-competitive blocking of the reduction of folic acid (I) to tetrahydrofolic acid, but their effects are competitively reversed by tetrahydrofolic acid or its formyl derivative.[3,4] Recent research has indicated that both the N_5 and N_{10} nitrogens of tetrahydrofolic acid are



I

involved in one-carbon transfer reactions. It is

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, and is in collaboration with the Sloan-Kettering Institute for Cancer Research. This paper was presented in part at the 133rd Meeting of the American Chemical Society, San Francisco, Calif., April 18, 1958; see Abstracts, page 84-N. For the preceding paper in this series *cf.* E. J. Reist, L. Goodman and B. R. Baker, THIS JOURNAL, **80**, 5775 (1958).

(2) A more complete discussion of folic acid antagonists is found in "The Vitamins," W. H. Sebrell, Jr., and R. S. Harris, Vol. III, Academic Press, Inc., New York, N. Y., 1954, p. 149.

(3) A. D. Welch and C. A. Nichol, *Ann. Rev. Biochem.*, **21**, 633 (1952).

(4) W. Jacobson, *J. Physiol. (London)*, **123**, 618 (1954).

probable[5-8] that the action of tetrahydrofolic acid (or a conjugate) in its cofactor cycle involves four steps: (1) the one-carbon fragment is accepted at the N_5-nitrogen, (2) an N_5–N_{10} bridge is formed, (3) the N_5-linkage is broken with formation of either N_{10}-hydroxymethyl- or N_{10}-formyltetrahydrofolic acid, and (4) the one-carbon fragment is transferred to a substrate with concurrent regeneration of the cofactor, tetrahydrofolic acid. Thus, if the N_5-nitrogen of tetrahydrofolic acid were blocked or replaced with a methylene group, an antagonist might be obtained which would block step 1 and therefore the succeeding steps. Such a compound is 5,8-dideaza-5,6,7,8-tetrahydrofolic acid (XI) and its synthesis is the subject of this paper.

Cyanoethylation of diethyl malonate was carried out in *t*-butyl alcohol using sodium methoxide as catalyst and gave a 96% yield of 4,4-dicarbethoxypimelonitrile (II), a substantial improvement over the procedure of Bruson and Riener.[9] Hydrolysis and simultaneous decarboxylation of II with 6 *N* hydrochloric acid gave 4-carboxypimelic acid (III) in quantitative yield. Esterification of III by the Clinton–Laskowski method[10] afforded dimethyl 4-carbomethoxypimelate (IV) in 88% over-all yield for the three steps. This synthesis is far superior both in yield and simplicity to the synthesis of IV from malonic ester and formaldehyde (which proceeded in 43% over-all yield) described by Openshaw and Robinson.[11]

Openshaw and Robinson[11] have described the Dieckman cyclization of the triester IV to 2,4-dicarbomethoxycyclohexanone (V) in 88% yield using metallic sodium in benzene. Commercial sodium methoxide has now been demonstrated to be a useful base for the cyclization in benzene and, although lower yields were obtained with it than those reported by Openshaw and Robinson, it was much more conveniently employed for the large amount of V that was required. Yields of 57–61% of V were obtained consistently. Similar results were found with sodium hydride. For small-scale preparations, the keto diester V could be extracted from the reaction mixture with cold 3% sodium hydroxide; since on a large scale this treatment led to extensive cleavage, the ketone V was isolated by distillation.

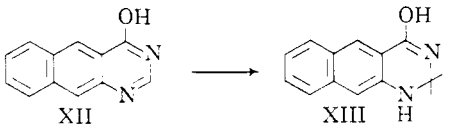
Condensation of 2,4-dicarbomethoxycyclohexanone (V) with guanidine proceeded readily using methanolic sodium methoxide to give a quantitative yield of 2-amino-5,6,7,8-tetrahydro-4-hydroxyquinazoline-6-carboxylic acid (VI) after saponification with sodium hydroxide. In some preparations there was no trace of the methyl ester VII in the crude reaction product before saponification, as shown by the absence of infrared absorption at 5.78 μ; in other preparations of VI a mixture of ester VII and acid VI resulted and was converted to the acid VI by saponification. The water formed in the condensation reaction was obviously responsible for the ester saponification accompanying condensation. The acid VI showed a strong carbonyl absorption at 5.90 μ. The ketoester V also was condensed, in good yield, with urea and thiourea. The products of these reactions will be discussed in a forthcoming publication.

Esterification of the 6-carboxylic acid VI with *n*-butyl alcohol in the presence of *p*-toluenesulfonic acid proceeded smoothly in 88% yield to the crystalline butyl ester VIII. The simple derivatives of VI showed low solubility in the common organic solvents and the *n*-butyl ester VIII was prepared with the expectation that it would be more soluble in the organic solvents employed in subsequent hydride reactions.

Initial attempts to reduce the ester VIII to the 6-hydroxymethylquinazoline IX with lithium aluminum hydride in tetrahydrofuran were inconclusive. Reduction with the sodium borohydride–aluminum chloride combination in diglyme (two moles of aluminum chloride per mole of VIII were required, presumably as a result of complexing of the halide by VIII) as described by Brown and Subba Rao[12] gave excellent yields of IX when the

(5) A. Miller and H. Waelsch, *J. Biol. Chem.*, **228**, 397 (1957).
(6) J. C. Rabinowitz and W. E. Pricer, Jr., THIS JOURNAL, **78**, 5702 (1956).
(7) J. M. Peters and D. M. Greenberg, *J. Biol. Chem.*, **226**, 329 (1957).
(8) R. L. Kisliuk, *ibid.*, **227**, 805 (1957).
(9) H. A. Bruson and T. W. Riener, THIS JOURNAL, **65**, 23 (1943).
(10) R. O. Clinton and S. C. Laskowski, *ibid.*, **70**, 3135 (1948).
(11) H. T. Openshaw and R. Robinson, *J. Chem. Soc.*, 912 (1946).
(12) H. C. Brown and B. C. Subba Rao, THIS JOURNAL, **77**, 3164 (1955).

time and temperature were controlled. Too high a temperature or too long a reaction time resulted in reduction of the pyrimidine ring, as shown by the decreased intensity of ultraviolet absorption near 270 mμ. It seems probable that this situation is analogous to that noted[13] in the lithium aluminum hydride reduction of the quinazoline ring system (XII → XIII). Too low a temperature



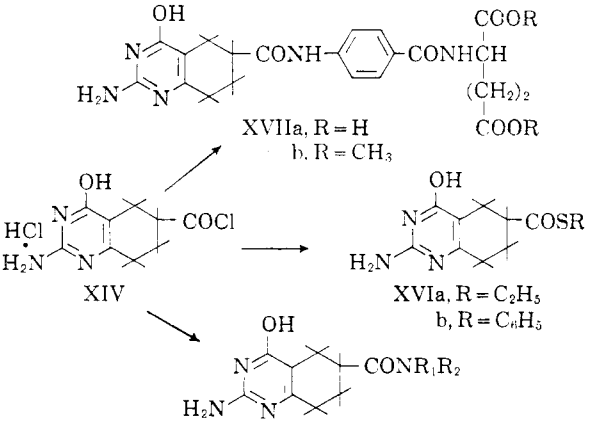
XII XIII

for the reduction resulted in the recovery of some ester (VIII). Using the proper conditions the alcohol IX was obtained in 75–85% yields.

A number of attempts were made to prepare the *p*-toluenesulfonate ester of IX, which was regarded as a logical precursor of XI. When equimolar quantities of IX and *p*-toluenesulfonyl chloride were allowed to react in pyridine, a 10–15% yield of the desired tosylate ester was isolated, accompanied by a large amount of a colored solid which rapidly changed to a red gum on exposure to air. When a large excess of the sulfonyl chloride was used, the unstable solid was the sole product. It is most probable that the unstable solid resulted from sulfonylation at the 2-amino group. In an effort to block the 2-amino group selectively, the alcohol IX was converted to its 2-acetamido-6-acetoxymethyl derivative with acetic anhydride in pyridine. Although the ease of cleavage of the 2-acetamido group was anticipated, it was surprising that treatment of the diacetate with cold methanolic ammonia led to more rapid cleavage of the 2-acetamido group than of the O-acetate.

The carbinol IX was converted to the 6-chloromethylquinazoline X by refluxing thionyl chloride containing 1.2 moles of pyridine per mole of IX. This amount of pyridine appeared to be quite critical; an excess of pyridine led to extensive decomposition, while insufficient pyridine gave incomplete reaction. The chloride X gave a low yield of condensation product with *p*-chloroaniline when the reactants were refluxed in butyl Cellosolve for long periods. The product was difficult to purify; self-condensation of X evidently was a competing reaction, as shown by the presence of highly insoluble by-products. The reaction of X with N-(*p*-aminobenzoyl)-L-glutamic acid was conducted under similar conditions and gave a much better yield of product, the tetrahydrofolic acid analog of XI. The infrared spectrum of the product showed the expected functional groups and the di-substituted phenyl; the ultraviolet spectrum of XI resembled that of tetrahydrofolic acid.[14] The product resisted a variety of attempts at further purification. These included Celite chromatography and ion exchange chromatography. A number of solvent systems were investigated in an effort to establish the homogeneity of the product, but extensive streaking resulted in all the systems tried, probably due to the high insolubility of the compound. Accordingly, the analytical data serve as the only criterion of purity of XI and these are not completely satisfactory. When XI was subjected to hydrolysis by heating in hydrochloric acid, glutamic acid was formed, as detected by paper chromatography. In view of the thorough removal of *p*-aminobenzoyl-L-glutamic acid from the product, this is additional evidence in favor of structure XI.

The 6-carboxylic acid VI was converted to a variety of interesting compounds (XV, XVI and XVII) by way of its acid chloride hydrochloride



XVIIa, R = H
b, R = CH_3

XIV

XVIa, R = C_2H_5
b, R = C_6H_5

XVa, $R_1 = R_2 = H$
b, $R_1 = R_2 = C_2H_5$
c, $R_1 = H$, $R_2 = 4\text{-}ClC_6H_4$
d, $R_1 = H$, $R_2 = 2\text{-}ClC_6H_3$
e, $R_1 = H$, $R_2 = 3,4\text{-}Cl_2C_6H_3$
f, $R_1 = H$, $R_2 = 3\text{-}CF_3C_6H_4$
g, $R_1 = H$, $R_2 = 4\text{-}FC_6H_4$

XIV. The acid chloride hydrochloride XIV was prepared with thionyl chloride containing a small amount of pyridine; since it was an unstable material no attempt was made to purify it, but it was used immediately in further reactions.

The amides XVb-g were prepared in dry acetone using an excess of the amine; the simple amide XVa was prepared by passing dry ammonia into an ether suspension of the acid chloride hydrochloride XIV.

The two thioesters XVIa and b were prepared in good yield by adding the acid chloride hydrochloride XIV slowly to a large excess of the appropriate mercaptan dissolved in dry pyridine.

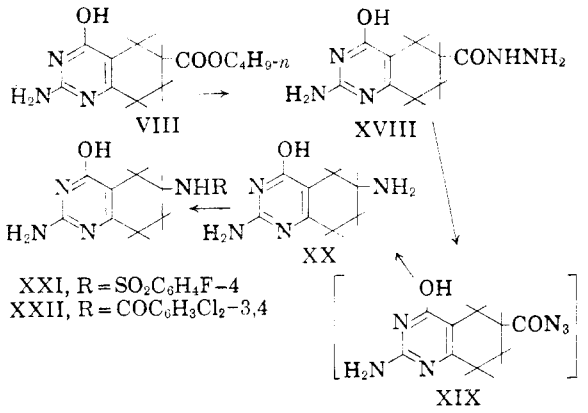
The interesting dipeptides XVIIa and b were prepared by the slow addition of acid chloride hydrochloride XIV to a pyridine solution of N-(*p*-aminobenzoyl)-L-glutamic acid or its dimethyl ester, respectively. The yields of compounds XVIIa and b were low, largely as a result of purification difficulties. The acid chloride hydrochloride XIV in pyridine solution tended to self-condense rapidly and it was difficult to separate the self-condensation products from the desired amides XVIIa and b. Compound XVIIa is structurally very similar to the tetrahydrofolic acid analog XI, differing only in the oxidation level of C9; accordingly, amides XVIIa and b represent especially interesting compounds for biological testing.

The 6-amino compound XX was prepared from the 6-carboxazide XIX by Curtius rearrangement in warm, dilute hydrochloric acid. The azide XIX,

(13) A. Etienne and M. LeGrand, *Compt. rend.*, **229**, 220 (1949).

(14) B. L. O'Dell, J. M. Vandenbelt, E. S. Bloom and H. H. Pfiffner, THIS JOURNAL, **69**, 250 (1947).

an unstable solid, was prepared from the 6-carboxhydrazide XVIII and since it could not be purified, it was rearranged *in situ*. The over-all



yield of 2,6-diamino-5,6,7,8-tetrahydro-4-hydroxyquinazoline (XX) from the hydrazide XVIII was 67%. The amine XX was isolated from the rearrangement mixture as its picrate (a mixture of the mono- and dipicrate), which was in turn converted to the amine XX dihydrochloride with concentrated hydrochloric acid. Treatment of the amine XX with *p*-fluorobenzenesulfonyl chloride and 3,4-dichlorobenzoyl chloride in the presence of aqueous sodium bicarbonate led to good yields of the sulfonamide XXI and benzamide XXII, respectively.

Experimental[15]

4,4-Dicarbethoxypimelonitrile (II).—Acrylonitrile (53 g., 1 mole) was added dropwise with stirring to a solution of diethyl malonate (80 g., 0.5 mole), sodium methoxide (1.10 g., 0.02 mole) and 100 ml. of *t*-butyl alcohol, the temperature being maintained at 30–35° by occasional cooling. Addition was completed in 50 minutes. The reaction was stirred 2 hours more, then allowed to stand overnight at room temperature. Dilute hydrochloric acid was added with stirring and cooling to give *p*H 3. The mixture was poured over 500 g. of ice, filtered, and washed with water. A sample of the wet product dried *in vacuo* indicated that the dry yield was 129 g. (96%), m.p. 63.5–64° (lit.[9] m.p. 62°); λ_{max}^{KBr} (μ) 4.45 (CN), 5.80 (C=O), 8.27–8.40 (ester C–O–C).

4-Carboxypimelic Acid (III).—A mixture of wet 4,4-dicarbethoxypimelonitrile (II) (128.2 g., dry wt., 0.48 mole) and 6 *N* hydrochloric acid (550 ml.) was refluxed for 20 hours. After concentration, the residue was extracted with acetone. The acetone extracts, filtered from ammonium chloride, were concentrated to dryness to give 108.5 g. (114% yield) of III, m.p. 114–115° (lit.[11] m.p. 113–114°). The excess weight resulted from incomplete removal of acetone; λ_{max}^{KBr} (μ) 3.75–3.80 (carboxyl OH), 5.75 (acetone C=O), 5.90 (carboxyl C=O).

Dimethyl 4-Carbomethoxypimelate (IV).—A mixture of the preceding solvent-wet 4-carboxypimelic acid (III) (107 g., 0.52 mole), absolute methanol (149.8 g.), ethylene dichloride (468 ml.) and concentrated sulfuric acid (4.7 ml.) was refluxed for 53 hours. The water layer, which had separated as the reaction proceeded, was removed; the remaining organic layer was washed with water, then with saturated sodium bicarbonate solution, and again with water. After concentration, the crude product was distilled to give 92.8 g. of IV, b.p. 118–120° (0.05 mm.) (lit.[11] b.p. 162° (12 mm.)); λ_{max}^{film} (μ) 5.76 (ester C=O), 7.29 (CH_3), 8.00 and 8.36 (ester C–O–C). The over-all yield of IV from malonic ester was 88%.

2,4-Dicarbomethoxycyclohexanone (V).—A mixture of 400 g. (1.86 moles) of dimethyl 4-carbomethoxypimelate (IV), 100 g. (1.86 moles) of sodium methoxide and 1900 ml. of anhydrous benzene was refluxed with stirring for 6 hours. A solution of 200 ml. of glacial acetic acid in 2500 ml. of water was added with stirring to the cooled reaction mixture. The layers were separated, the aqueous layer extracted with benzene (3 × 250 ml.) and the combined benzene extracts dried over magnesium sulfate. Concentration of the dried benzene extracts *in vacuo* afforded 322.7 g. of a light yellow liquid, which after fractionation through a Widmer column gave 197.8 g. (57%) of product, b.p. 135–138° (5 mm.), that solidified in the receiver; 44.4 g. of IV was recovered by further distillation, b.p. 134–135° (1 mm.).[19]

When the above reaction was carried out as above using only 20.0 g. (0.093 mole) of the triester IV, the cooled reaction mixture was added to a cold solution of 45 ml. of glacial acetic acid in 320 ml. of water. The layers were separated and the aqueous layer was extracted with benzene (5 × 30 ml.). The combined extracts were washed once with 30 ml. of water and then with ice-cold 3% sodium hydroxide solution (5 × 30 ml.). Each basic extract was added immediately to a solution of 20 ml. of acetic acid in 100 ml. of water. The combined acidic solutions were extracted with benzene (6 × 30 ml.) and the benzene extracts were washed with 30 ml. of water and concentrated *in vacuo* to give 12.1 g. (61%) of product, m.p. 41–43° (lit.[11] m.p. 40–44°); λ_{max}^{EtOH} 255 mμ (ϵ 10,010); λ_{max}^{KBr} (μ) 5.76 (ester C=O), 6.03 (chelated C=O), 6.17 (conjugated C=C), 8.25 (ester C–O–C).

When sodium hydride (as a 27.2% suspension in mineral oil) was used as the condensing agent, the yield of V was 58%, m.p. 42–43°.

2-Amino-5,6,7,8-tetrahydro-4-hydroxyquinazoline-6-carboxylic Acid (VI).—A solution of 2.14 g. (0.01 mole) of 2,4-dicarbomethoxycyclohexanone (V), 1.53 g. (0.016 mole) of guanidine hydrochloride and 1.8 g. (0.032 mole) of sodium methoxide in 32 ml. of methanol was refluxed for 3 hours, then allowed to stand overnight. Addition of 7 ml. of 50% sodium hydroxide gave a homogeneous solution which was refluxed for 75 minutes. Acidification with acetic acid gave a white precipitate which was collected on a filter, washed with water and dried; yield 2.19 g. (100%), m.p. >300°. An analytically pure sample of VI was obtained in 81% yield after purification of the above product by dissolving in saturated sodium bicarbonate solution and reprecipitating with dilute hydrochloric acid. On paper chromatography, the product showed a single spot at the origin in solvents A and C and moved as a single spot with R_{Ad} 1.82 in solvent B. In the ultraviolet it had $\lambda_{max}^{pH\ 1}$ (mμ) 225 (ϵ 9250), 262 (ϵ 7030); $\lambda_{max}^{pH\ 14}$ (mμ) 230 (ϵ 8360), 277 (ϵ 6180). In the infrared it had λ_{max}^{Nujol} (μ) 2.97, 3.16 (OH, NH), 5.90 (carboxyl C=O), 6.08, 6.54 and 6.63 (substituted pyrimidine ring).

Anal. Calcd. for $C_8H_{11}N_3O_5$: C, 51.7; H, 5.26; N, 20.1. Found: C, 51.6; H, 5.50; N, 19.7.

2-Acetamido-5,6,7,8-tetrahydro-4-hydroxyquinazoline-6-carboxylic Acid.—The N-acetyl derivative of VI was prepared by heating a mixture of 3.0 g. (14.3 mmoles) of VI and 60 ml. of acetic anhydride at 135–150° for 3 hours. The resulting mixture was poured onto 100 g. of ice and the solution which formed was filtered. The filtrate was concentrated, *in vacuo*, to 30 ml., and 2.74 g. (76%) of product, m.p. >300°, was obtained. A portion (0.50 g.) was recrystallized from 50 ml. of methyl Cellosolve, yielding 0.17 g.; λ_{max}^{KBr} (μ) 3.17 and 6.35 (NH), 5.92 (carboxyl C=O), 6.0–6.1, 6.2–6.4 (amide and substituted pyrimidine ring).

Anal. Calcd. for $C_{11}H_{13}N_3O_4$: C, 52.6; H, 5.22; N, 16.7. Found: C, 52.5; H, 5.47; N, 16.1, 16.5.

2-Amino-6-carbo-*n*-butoxy-5,6,7,8-tetrahydro-4-hydroxyquinazoline (VIII).—A suspension of 2-amino-5,6,7,8-tetrahydro-4-hydroxyquinazoline-6-carboxylic acid (VI) (0.21

(15) Boiling points and melting points are uncorrected; the latter were obtained with the Fisher–Johns apparatus. Paper chromatography was done by the descending technique on Whatman No. 1 paper and the spots were detected by visual examination under ultraviolet light. Adenine was used as a standard and the spots were located relative to R_{Ad} 1.00. These solvent systems were used: A,[16] methyl Cellosolve–water (9:1); B,[17] 5% aqueous Na_2HPO_4 (no organic phase); C,[18] *n*-BuOH–HOAc–H_2O (5:2:3).

(16) A. E. Bender, *Biochem. J.*, **48**, xv (1951) (Proc. Biochemical Society).

(17) C. E. Carter, THIS JOURNAL, **72**, 1835 (1950).

(18) D. M. Brown, A. Todd and S. Varadarajan, *J. Chem. Soc.*, 2388 (1956).

(19) This reaction was performed by Mr. R. R. Spencer.

g., 0.001 mole) and *p*-toluenesulfonic acid monohydrate (0.30 g., 0.0015 mole) in 15 ml. of *n*-butyl alcohol was heated 1.25 hours while the alcohol was distilled slowly from the reaction mixture. Saturated aqueous sodium bicarbonate solution (10 ml.) and benzene (10 ml.) were added to the residue. The layers were separated. The top layer, containing suspended solid, was washed with sodium bicarbonate solution and water. It was then concentrated to dryness to give 0.27 g., the theoretical yield, of VIII, m.p. 264–265°. Two recrystallizations from hot methyl Cellosolve gave 0.04 g. of VIII, m.p. 273.5–274.5°; λ_{max}^{KBr} (μ) 3.02, 3.27 (NH), 5.80 (ester C=O), 6.07, 6.70 (pyrimidine ring), 8.51 (ester C–O–C). Paper chromatography in solvent A showed a single spot with R_{Ad} 1.48.

Anal. Calcd. for $C_{13}H_{19}N_3O_3$: C, 58.9; H, 7.22; N, 15.8. Found: C, 58.9; H, 6.98; N, 15.9.

In a larger run, the yield of material suitable for further transformations was 1.51 g. (88%), m.p. 265–266°.

2-Amino-5,6,7,8-tetrahydro-4-hydroxy-6-hydroxymethylquinazoline (IX).—A slurry of 6.0 g. (22 mmoles) of *n*-butyl ester VIII in 70 ml. of purified diglyme (the dimethyl ether of diethylene glycol) was added with stirring to a mixture of 6.06 g. (45.6 mmoles) of aluminum chloride and 5.16 g. (0.138 mole) of sodium borohydride in 90 ml. of diglyme. The addition required 35 minutes during which the temperature was maintained at 20–22°. The mixture was stirred at room temperature (22–25°) for 65 minutes more and then was poured onto about 100 g. of ice. The resulting slurry was made strongly acid by the slow addition of 6 ml. of concentrated sulfuric acid in order to decompose the complex hydrides. The *p*H was adjusted to 5 with 46 ml. of 10% sodium hydroxide and the clear solution was evaporated to dryness *in vacuo* at 60°. The residue was powdered and was extracted continuously for 10 hours with 800 ml. of dry methanol in a Soxhlet apparatus. The methanol extract was evaporated *in vacuo*, yielding 9.77 g. of the alcohol IX as its crude sulfate salt.

The crude salt was dissolved in 66 ml. of water and the solution was filtered. The filtrate was adjusted to *p*H 9 with saturated aqueous sodium carbonate solution and the resulting mixture was chilled, yielding 3.78 g. (86%) of the alcohol IX. An analytical sample, m.p. >300°, was obtained by recrystallization from water (1 g./70 ml.). On paper chromatography in solvents A, B and C, the product moved as a single spot with R_{Ad} 1.00, 1.51 and 1.00, respectively. In the ultraviolet it had $\lambda_{max}^{pH\ 1}$ (mμ) 263 (ϵ 7630), $\lambda_{max}^{pH\ 7}$ (mμ) 270 (ϵ 5300), $\lambda_{max}^{pH\ 14}$ (mμ) 277 (ϵ 6690). In the infrared it had λ_{max}^{KBr} (μ) 2.93 and 3.25 (NH, OH), 6.10, 6.25, 6.50 (substituted pyrimidine ring); there was no absorption near 5.80 μ (ester C=O).

Anal. Calcd. for $C_9H_{13}N_3O_2$: C, 55.4; H, 6.71; N, 21.5. Found: C, 55.0; H, 6.84; N, 21.6.

The picrate of the alcohol IX was prepared by mixing hot aqueous solutions of IX and picric acid. It was recrystallized from water (1 g./250 ml.), m.p. 209–211°. In the infrared it showed a strong band at 5.90 μ (C=N$^+$H).

Anal. Calcd. for $C_{15}H_{16}N_6O_9$: C, 42.5; H, 3.80. Found: C, 42.5; H, 4.08.

2-Acetamido-6-acetoxymethyl-5,6,7,8-tetrahydro-4-hydroxyquinazoline.—A mixture of 0.45 g. (2.3 mmoles) of IX, 3 ml. of dry pyridine and 1.5 ml. of acetic anhydride was heated at 85° for 1 hour, complete solution resulting. Upon addition of 20 ml. of water to the mixture and chilling of the resulting solution, 0.42 g. (64%) of product, m.p. 207.5–210°, precipitated. This was recrystallized from 10 ml. of methyl Cellosolve to yield 0.35 g. with unchanged melting point. It moved as a single spot in solvent C with R_{Ad} 1.45. In the infrared it had λ_{max}^{KBr} (μ) 3.20 and 6.38 (NH), 5.76 (O-acetate C=O), 6.05, 6.25, 6.40 (N-acetate C=O and pyrimidine ring), 8.07 (ester C–O–C).

Anal. Calcd. for $C_{13}H_{17}N_3O_4$: C, 55.9; H, 6.14; N, 15.1. Found: C, 55.9; H, 6.23; N, 14.8.

2-Amino-5,6,7,8-tetrahydro-4-hydroxy-6-(*p*-toluenesulfonyloxymethyl)-quinazoline.—A solution of 0.21 g. (1.1 mmoles) of *p*-toluenesulfonyl chloride in 1.6 ml. of reagent pyridine was added dropwise, with stirring, to an ice-cold suspension of 0.20 g. (1.0 mmoles) of IX in 1.5 ml. of reagent pyridine. The mixture was stirred at 0° for 1.3 hours and was then poured into 25 ml. of ice and water. The yellow precipitate which formed was collected and dried, and weighed 0.05 g. (14% yield), m.p. 195–197.5°. The crude product was recrystallized from a methyl Cellosolve–water mixture to give 0.04 g. (80% recovery), m.p. 212–213.5°; λ_{max}^{KBr} (μ) 3.05, 3.25 (NH), 6.05, 6.70 (pyrimidine ring), 7.37 and 8.53 (sulfonate ester), 12.26 (*p*-di-substituted benzene ring).

Anal. Calcd. for $C_{16}H_{19}N_3O_4S$: C, 55.0; H, 5.48. Found: C, 55.6; H, 5.95.

When a large excess of tosyl chloride was used, the product was an unstable yellow solid which contained none of the tosylate ester described above.

2-Amino-6-chloromethyl-5,6,7,8-tetrahydro-4-hydroxyquinazoline (X).—Thionyl chloride (80 ml.) was added slowly to an ice-cold mixture of 5.00 g. (26 mmoles) of alcohol IX and 2.5 g. (31 mmoles) of dry pyridine. The addition required 15 minutes and the mixture then was refluxed for 130 minutes, complete solution resulting. The reaction mixture was concentrated *in vacuo* to one-fourth its volume and the residue was poured with stirring onto 200 g. of ice. The resulting mixture was filtered and the filtrate was adjusted to *p*H 6–7 by the slow addition of 10% NaOH to the chilled solution. The precipitate which formed weighed 4.32 g. (79%); it was chromatographically homogeneous and was suitable for use in the next reaction. A portion was recrystallized from methyl Cellosolve (1.4 g./liter) and had m.p. 287–288.5°; λ_{max}^{KBr} (μ) 2.98, 3.23 (NH), 6.0–6.15, 6.70 (pyrimidine ring). On paper chromatography it moved as a single spot in solvent C.

Anal. Calcd. for $C_9H_{12}N_3OCl$: C, 50.6; H, 5.66; Cl, 16.6. Found: C, 50.5; H, 5.76; Cl, 16.1, 16.2.

N-[{*p*-[(2-Amino-5,6,7,8-tetrahydro-4-hydroxy-6-quinazolinyl)-methyl]-amino}-benzoyl]-L-glutamic Acid (5,8-Dideaza-5,6,7,8-tetrahydrofolic Acid) (XI).—A suspension of 0.54 g. (2.5 mmoles) of the chloromethyl compound X, 2.0 g. (7.6 mmoles) of N-(*p*-aminobenzoyl)-L-glutamic acid, 5.0 ml. of butyl Cellosolve, and a trace of sodium iodide was heated to reflux, at which point complete solution resulted. The solution then was refluxed for a total of 13 hours. Ether (100 ml.) was added to the cooled reaction mixture and the resulting suspension was filtered and the residue washed thoroughly with ether. The solid was stirred in 5 ml. of 1 *N* sodium hydroxide; it slowly dissolved. The solution was treated with Norit, filtered and the filtrate adjusted to *p*H 6 with 1 *N* hydrochloric acid. The resulting solid, 0.78 g. (69%), had m.p. 188–210°. A small portion (*ca.* 0.15 g.) of the solid was dissolved in 10 ml. of saturated aqueous sodium bicarbonate, the solution treated with Norit and filtered, and the filtrate adjusted to *p*H 5 with 1 *N* hydrochloric acid. The resulting solid was extracted with three 5-ml. portions of hot (80°) pyridine. Ether (50 ml.) was added to the combined, filtered extracts, yielding a tan solid which was washed thoroughly with water and dried, m.p. 199–202°. In the ultraviolet it had $\lambda_{max}^{pH\ 1}$ 268 mμ (ϵ 17,100), $\lambda_{max}^{pH\ 7}$ 276 mμ (ϵ 22,100), $\lambda_{max}^{pH\ 14}$ 279 mμ (ϵ 21,600); in the infrared it had λ_{max}^{KBr} (μ) 2.94 (NH), 5.90 (carboxyl C=O), 6.0–6.1, 6.55–6.65 (pyrimidine ring and amide), 11.95 (*p*-di-substituted phenyl). No satisfactory paper chromatographic solvent system could be found.

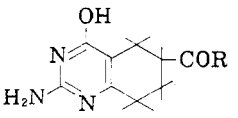
Anal. Calcd. for $C_{21}H_{25}N_5O_6$: C, 56.9; H, 5.68; N, 15.79; O, 21.65. Found: C, 57.1; H, 5.55; N, 14.06, 14.20; O (Unterzaucher), 22.05, 22.12.

Repeated determinations, even on different samples, failed to improve the nitrogen analysis.

Approximately 10 mg. of purified product XI was heated at 100° for 1 hour in 2 ml. of 4 *N* hydrochloric acid. The hydrolysate was subjected to paper chromatography in solvent C and L-glutamic acid was detected by ninhydrin spray. It appeared at the same place (R_{Ad} 0.53) as authentic L-glutamic acid.

2-Amino-6-(*p*-chloroanilinomethyl)-5,6,7,8-tetrahydro-4-hydroxyquinazoline.—A suspension of 0.54 g. (2.54 mmoles) of the chloromethyl compound X, 0.95 g. (7.5 mmoles) of *p*-chloroaniline, 5.0 ml. of butyl Cellosolve and a trace of sodium iodide was heated under reflux for 15 hours. Ether (100 ml.) was added to the cooled reaction mixture and the light brown solid was filtered and washed thoroughly with ether and with water, yielding 0.16 g. (21%) of product. This was dissolved in 1 *N* hydrochloric acid and the suspension was centrifuged to remove insoluble materials. The solution was neutralized with aqueous sodium bicarbonate and the resulting precipitate was filtered and washed thoroughly with water. It was dissolved in 6 ml. of hot methyl Cello-

TABLE I



Compound	R	Yield,[a] %	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
XVb	NEt_2	35	59.1	7.57	21.2	59.3	7.66	21.1
XVc	4-ClC_6H_4NH[b]	32[e]	56.6	4.71	17.7	56.4	4.92	18.0
XVd	2-ClC_6H_4NH	34	53.4[c]	5.08	16.6	53.4	4.90	17.1, 17.3
XVe	3,4-$Cl_2C_6H_3NH$	64	49.8[d]	4.18	15.5	49.8	4.38	15.5
XVf	3-$CF_3C_6H_4NH$	55	53.2[d]	4.47	15.5	53.0	4.93	15.7
XVg	4-FC_6H_4NH	50	56.2[c]	5.35	17.5	55.7	5.41	16.5

[a] Yield of recrystallized product; no attempt was made to obtain optimum recovery. The compounds failed to melt below 300°. All compounds had infrared spectra typical of the system in XV, including proper phenyl bands in the 12- to 15-μ region. [b] % Cl, calcd. 11.1, found 11.2. [c] The calculated values are for the monohydrate. [d] The calculated values are for the hemihydrate. [e] On a large-scale run the yield of chromatographically homogeneous product was 77%.

solve, the solution was decolorized with Norit, diluted with water, and chilled; yield 0.02 g. (3%) of purified product, m.p. 229–231° with darkening at 190°, which traveled as a single spot in solvent C with R_{Ad} 1.38. In the infrared it had $\lambda_{max}^{KBr}(\mu)$ 3.20 and 6.68 (NH), 6.0–6.15, 6.55–6.70 (pyrimidine ring), 12.26 (*p*-di-substituted phenyl).

Anal. Calcd. for $C_{15}H_{17}ClN_4O$: C, 59.1; H, 5.62; N, 18.4. Found: C, 59.2; H, 5.78; N, 19.0.

2-Amino-6-chloroformyl-5,6,7,8-tetrahydro-4-hydroxyquinazoline Hydrochloride (XIV).—Thionyl chloride (16.4 g., 0.13 mole) was added dropwise to 0.90 g. (4.3 mmoles) of 2-amino-5,6,7,8-tetrahydro-4-hydroxyquinazoline-6-carboxylic acid (VI). Pyridine (0.35 g., 4.4 mmoles) was added to the suspension, which then was stirred at room temperature for 3.75 hours. After addition of 30 ml. of anhydrous ether, the mixture was cooled and the crystalline precipitate XIV which formed was filtered and washed with 20 ml. of anhydrous ether. The material, protected from moisture, was used immediately without further treatment.

2-Amino-5,6,7,8-tetrahydro-4-hydroxyquinazoline-6-carboxamide (XVa).—The acid chloride XIV, prepared from 0.50 g. (2.4 mmoles) of the 6-carboxylic acid VI by the procedure described above, was suspended in 30 ml. of anhydrous ether and anhydrous ammonia was bubbled into the suspension during 20 minutes. The pink solid which formed was collected by filtration, washed with 10 ml. of ether and then with two 10-ml. portions of water, and dried to yield 0.55 g. (110%) of crude product which contained some inorganic material. The solid was heated, with stirring, in 10 ml. of saturated aqueous sodium bicarbonate and the resulting solid was washed thoroughly with water and dried, yielding 0.43 g. (86%). This was dissolved in 40 ml. of cold 0.1 *N* hydrochloric acid, the solution filtered and the filtrate neutralized with aqueous sodium bicarbonate, yielding the analytical sample of XVa, m.p. > 300°. The material moved as a single spot in solvents A, B and C with R_{Ad} 0.80, 1.53 and 0.71, respectively. In the infrared it had $\lambda_{max}^{KBr}(\mu)$ 2.98 and 3.15 (OH NH), 6.02–6.08, 6.45–6.60 (pyrimidine ring and amide).

Anal. Calcd. for $C_9H_{12}N_4O_2 \cdot 1/2H_2O$: C, 49.8; H, 5.99; N, 25.8. Found: C, 49.9, 49.7; H, 5.56, 5.70; N, 26.2.

2-Amino-6-(*p*-chlorophenylcarbamoyl)-5,6,7,8-tetrahydro-4-hydroxyquinazoline (XVc).—The acid chloride XIV prepared from 0.20 g. (1.0 mmole) of the acid VI was added to a solution of 0.56 g. (4.3 mmoles) of *p*-chloroaniline in 6 ml. of dry acetone and the resulting slurry was stirred for 3 hours. After the addition of 5 ml. of ether, the mixture was filtered and the solid was washed thoroughly first with ether and finally with water, to yield 0.22 g. (70%), m.p. >300°. The solid was heated, with stirring, in 5 ml. of saturated sodium bicarbonate solution, filtered and washed with water. Finally, it was dissolved in 15 ml. of dimethylformamide, the solution filtered and the filtrate brought to the cloud point with water. On chilling, 0.10 g. (32%) of the analytical sample precipitated. On paper chromatography in solvent A it moved as a single spot with R_{Ad} 1.33. In the infrared it had $\lambda_{max}^{KBr}(\mu)$ 3.0 (NH), 6.0–6.1, 6.46–6.60 (amide, NH_2 and pyrimidine ring), 6.52 (amide NH), 12.03 (*p*-di-substituted benzene). The analytical figures are found in Table I along with the data for the other amides which were prepared by the same procedure.

N-{*p*-[(2-Amino-5,6,7,8-tetrahydro-4-hydroxy-6-quinazolinyl)-carbonylamino]-benzoyl}-L glutamic Acid (XVIIa).—The acid chloride XIV prepared from 0.42 g. (2 mmoles) of VI was added, in small portions, over a period of 30 minutes, to a solution of 0.53 g. (2 mmoles) of N-(*p*-aminobenzoyl)-L-glutamic acid in 4 ml. of reagent pyridine while the temperature was maintained at 0°. The solution was stirred an additional 2 hours at room temperature and, after standing overnight at room temperature, was added to 100 g. of ice. A fine, white solid precipitated and was separated by centrifugation. Its infrared spectrum indicated it to be VI. The filtrate was concentrated *in vacuo* to about 25 ml., and 0.74 g. (81.3%) of solid, m.p. 221–231°, was obtained by filtration. Recrystallization of the crude solid from 40 ml. of hot water gave 0.06 g. (16.3%) of solid, m.p. 221–223°; $\lambda_{max}^{pH\,1}$ 267 mμ (ϵ 25,000), $\lambda_{max}^{pH\,7}$ 269 mμ (ϵ 24,900), $\lambda_{max}^{pH\,14}$ 272 mμ (ϵ 26,400); $\lambda_{max}^{KBr}(\mu)$ 3.08 (NH), 5.93 (carboxyl C=O), 6.15–6.30 and 6.55–6.67 (pyrimidine ring), 11.75 (*p*-di-substituted phenyl).

Anal. Calcd. for $C_{21}H_{23}N_5O_7 \cdot H_2O$: C, 53.1; H, 5.31; N, 14.7. Found: C, 53.5; H, 5.41; N, 14.6.

Dimethyl N-(*p*-Aminobenzoyl)-L-glutamate.—A mixture of 10 ml. of reagent methanol and 0.30 g. (3.8 mmoles) of acetyl chloride was held at 0° for 10 minutes and to the chilled solution was added 1.00 g. (3.8 mmoles) of N-(*p*-aminobenzoyl)-L-glutamic acid followed by 1.0 ml. of acetyl chloride.[20] The solution was heated at reflux for 10 minutes and was concentrated to dryness *in vacuo*. Water (10 ml.) was added and the solution was adjusted to *p*H 8 with concentrated ammonium hydroxide. A gummy precipitate resulted which slowly solidified upon chilling. The solid material was filtered and dried, yielding 0.89 g. (80%), m.p. 109–111°. The crude solid was chromatographically homogeneous in solvent B with R_{Ad} 2.18. Recrystallization of 0.20 g. from an ethanol–ether mixture gave 0.11 g. (44%), m.p. 110–112.5°; $\lambda_{max}^{KBr}(\mu)$ 5.76 (ester C=O), 6.15 (amide C=O), 11.80 (*p*-di-substituted phenyl).

Anal. Calcd. for $C_{14}H_{18}N_2O_5$: C, 57.1; H, 6.12; N, 9.52 Found: C, 57.4; H, 6.30; N, 9.43, 9.31.

Dimethyl N-{*p*-[(2-Amino-5,6,7,8-tetrahydro-4-hydroxy-6-quinazolinyl)-carbonylamino]-benzoyl}-L-glutamate (XVIIb).—The acid chloride XIV prepared from 0.21 g. (1 mmole) of the 6-carboxylic acid VI was added to a solution of 0.29 g. (1 mmole) of dimethyl N-(*p*-aminobenzoyl)-L-glutamate in 2 ml. of reagent pyridine, an exothermic reaction resulting. After the dark reaction mixture had been stirred overnight it was diluted with 5 ml. of water and the resulting mixture was taken to dryness *in vacuo*. The residue was slurried successively with 28 ml. of water, 15 ml. of hot saturated aqueous sodium bicarbonate solution and 20 ml. of water, to yield 0.30 g. (61%) of crude product, m.p. 268–269°. The product had an infrared spectrum that differed only slightly from that of the analytical sample and showed a paper chromatographic behavior that differed slightly from that of the purified sample. The material was recrystallized first from a dimethylformamide–methanol mixture, m.p. 278.5–281°, and then from a dimethyl-

(20) This method has been used for the esterification of amino acids by B. R. Baker, J. P. Joseph and J. H. Williams, THIS JOURNAL, **77**, 1 (1955).

formamide–water mixture, m.p. 285–285.5°. The over-all yield was 47%. On paper chromatography in solvents A and C the recrystallized material moved as a single spot with R_{Ad} 1.34 and 1.60, respectively. In the infrared it had λ_{max}^{KBr} (μ) 3.00 (NH), 5.75 (ester C=O), 6.10, 6.55, 6.67 (pyrimidine ring and amide), 11.70 (*p*-di-substituted phenyl).

Anal. Calcd. for $C_{23}H_{27}N_5O_7$: C, 56.9; H, 5.61; N, 14.4. Found: C, 56.6; H, 5.63; N, 13.5.

Repeated nitrogen analyses, even on different samples, gave the same value, within analytical error.

Ethyl 2-Amino-5,6,7,8-tetrahydro-4-hydroxyquinazoline-6-thiolcarboxylate (XVIa).—The acid chloride XV prepared from 4.2 g. (20 mmoles) of VI was added in small portions to a well stirred solution of 3.4 g. (55 mmoles) of ethanethiol in 40 ml. of dry pyridine. The mixture was stirred overnight at room temperature, then was drowned in 600 ml. of ice-water. The brown solid was filtered and dried to yield 4.1 g. (80%) of product which was recrystallized from a mixture of 350 ml. of water and 175 ml. of methyl Cellosolve, yielding 3.3 g. (60%) of crystalline solid. The material darkened near 220° and softened near 260° but showed no definite melting point up to 300°. For further purification, 0.26 g. of the crystalline solid was stirred in 30 ml. of 0.1 *N* hydrochloric acid. A small amount of brown solid was separated and the filtrate was neutralized with aqueous sodium bicarbonate, yielding a white solid which was recrystallized once from 28 ml. of methyl Cellosolve and again from a methyl Cellosolve–water (6:10) mixture to give 0.07 g. (16%)[21] of white solid which seemed to sublime near 245° but did not melt below 295°. It moved as a single spot in solvent C with R_{Ad} 1.35. In the infrared it had λ_{max}^{KBr} (μ) 3.20 (NH), 5.93 (shoulder) (thiolester C=O), 6.1–6.25 and 6.65–6.70 (pyrimidine ring).

Anal. Calcd. for $C_{11}H_{15}N_3O_2S$: C, 52.2; H, 5.97; S, 12.7. Found: C, 52.0; H, 6.06; S, 12.3.

Phenyl 2-Amino-5,6,7,8-tetrahydro-4-hydroxyquinazoline-6-thiolcarboxylate (XVIb).—Compound XVIb was prepared as above using the acid chloride XIV from 4.62 g. (22 mmoles) of VI, 11.0 ml. (0.10 mole) of thiophenol and 44 ml. of pyridine. The crude yield was 5.55 g. (83.7%) of material which failed to melt by 300°. A small portion was purified by the procedure for XVIa, giving a 13% yield[21] of purified compound. In the infrared it had λ_{max}^{KBr} (μ) 3.23 (NH), 5.92 (thiolester C=O), 6.0–6.15, 6.50, 6.70 (pyrimidine ring), 13.40 and 14.55 (mono-substituted phenyl).

Anal. Calcd. for $C_{15}H_{15}N_3O_2S$: C, 59.8; H, 5.02; S, 10.6. Found: C, 59.6; H, 5.21; S, 10.2.

2-Amino-5,6,7,8-tetrahydro-4-hydroxyquinazoline-6-carboxhydrazide (XVIII).—A mixture of 1.00 g. (3.8 mmoles) of 2-amino-6-carbo-*n*-butoxy-5,6,7,8-tetrahydro-4-hydroxyquinazoline (VIII) and 4 ml. of 85% hydrazine hydrate was refluxed with stirring for 80 minutes. The mixture was evaporated to dryness *in vacuo* and the solid residue washed 3 times with 5 ml. of cold water; yield 0.84 g. (98%), m.p. >300°. Recrystallization of 0.20 g. from 30 ml. of dimethylformamide and 22 ml. of water with use of Darco gave 0.15 g. (74%). An analytical sample was prepared in 39% overall yield by a second recrystallization from 23 ml. of dimethylformamide and 20 ml. of water. Since no suitable solvent system for paper chromatography could be found and since the nitrogen analysis was low, it is not known whether the compound is impure or burns to a nitrogenous ash. In the infrared, compound XVIII had λ_{mux}^{KBr} (μ) 3.02 and 3.12 (NH), 6.0–6.2, 6.45–6.70 (amide NH_2 and pyrimidine ring).

Anal. Calcd. for $C_8H_{13}N_5O_2$: C, 48.3; H, 5.83; N, 31.4. Found: C, 48.3; H, 6.07; N, 29.8.

2,6-Diamino-5,6,7,8-tetrahydro-4-hydroxyquinazoline (XX) Dipicrate.—A solution of 1.04 g. (15.2 mmoles) of sodium nitrite in 20 ml. of water was added dropwise, with stirring, to an ice-chilled solution of 2.66 g. (11.9 mmoles) of 2-amino-5,6,7,8-tetrahydro-4-hydroxyquinazoline-6-carboxhydrazide (XVIII) in a mixture of 9.3 ml. of glacial acetic acid, 9.3 ml. of 5 *N* hydrochloric acid and 46 ml. of water. The addition required 10 minutes and the solution was stirred for an additional hour. The resulting solution of azide XIX was added dropwise with stirring to 50 ml. of a 1.5 *N* hydrochloric acid solution maintained at 55°. The addition required 10 minutes and the reaction mixture was stirred for an additional 45 minutes at 55°. The solution was adjusted to *p*H 7 with saturated aqueous sodium carbonate and the neutral solution was added to a solution of 5.0 g. (21.8 mmoles) of picric acid in 350 ml. of water, 4.94 g. of picrate precipitating. The infrared spectrum of the product made it likely that it contained some monopicrate. An analytical sample of the dipicrate was obtained by two recrystallizations of the crude product (above) from hot water. The material had no definite melting point; it slowly darkened and was completely decomposed on heating to 285°. In the infrared it λ_{max}^{KBr} (μ) 3.18–3.25 (NH, NH_3^+), 5.92 (C=$\overset{+}{N}$H), 6.13–6.21, 6.40–6.50 (pyrimidine aromatic rings) 6.40 and 7.50–7.60 (NO_2).

Anal. Calcd. for $C_{20}H_{18}N_{10}O_{15}$: C, 37.6; H, 2.81. Found: C, 37.8, 37.8; H, 3.15, 3.06.

The crude picrate from the above reaction was converted to the amine XX dihydrochloride (below), giving an overall yield of 67.2% from the hydrazide XVIII.

2,6-Diamino-5,6,7,8-tetrahydro-4-hydroxyquinazoline (XX) Dihydrochloride.—To a stirred suspension of 1.00 g. (2.44 mmoles) of 2,6-diamino-5,6,7,8-tetrahydro-4-hydroxyquinazoline (XX) dipicrate in 20 ml. of water and 50 ml. of benzene was added 2.6 ml. of concentrated hydrochloric acid. The benzene layer was separated and the aqueous layer was extracted with one 40- and three 20-ml. portions of benzene. The colorless aqueous layer was concentrated to dryness *in vacuo*, leaving 0.94 g. of residue, m.p. 275–277°. The crude product was dissolved in 60 ml. of dry methanol and the solution was filtered. To the filtrate was added 200 ml. of dry ether, which precipitated 0.54 g. (87%) of the dihydrochloride, m.p. 272–273°. It was homogeneous in solvents B and C with R_{Ad} 2.28 and 0.52, respectively. In the infrared it had λ_{max}^{KBr} (μ) 3.25–3.50 (NH_3^+ and CH), 5.93 (C=$\overset{+}{N}$H), 6.03, 6.50, 6.70 (NH_3^+ and pyrimidine ring).

Anal. Calcd. for $C_8H_{14}N_4OCl_2$: C, 38.0; H, 5.58; N, 22.1. Found: C, 38.2; H, 5.91; N, 22.0.

N-(2-Amino-5,6,7,8-tetrahydro-4-hydroxy-6-quinazolinyl)-*p*-fluorobenzenesulfonamide (XXI).—To a solution of 0.40 g. (1.6 mmoles) of 2,6-diamino-5,6,7,8-tetrahydro-4-hydroxyquinazoline (XX) dihydrochloride and 0.58 g. (7.0 mmoles) of sodium bicarbonate in 10 ml. of water was added, with stirring, 0.34 g. (1.7 mmoles) of *p*-fluorobenzenesulfonyl chloride. The mixture was stirred at room temperature for 21 hours and was filtered. The collected precipitate was washed thoroughly with water and dried, to yield 0.46 g. (87%) of crude product which had no definite melting point but began to decompose at 265°. Recrystallization of 0.34 g. of the crude material from a dimethylformamide–water mixture and with the use of Darco gave 0.29 g. (74% yield) of XXI. This was dissolved in 5 ml. of 1 *N* sodium hydroxide and the solution was decolorized with Norit and filtered. The filtrate was adjusted to *p*H 6–7 with 1 *N* hydrochloric acid, yielding 0.22 g. (57%), m.p. >300°; λ_{max}^{KBr} (μ) 3.00 (NH), 6.07–6.10, 6.50 (2-amino-4-hydroxypyrimidine ring), 7.59, 8.58 and 8.67 (SO_2N), 11.92 (*p*-di-substituted phenyl).

Anal. Calcd. for $C_{14}H_{15}N_4O_3SF$: C, 49.7; H, 4.46; N, 16.6. Found: C, 49.3; H, 4.27; N, 16.3.

N-(2-Amino-5,6,7,8-tetrahydro-4-hydroxy-6-quinazolinyl)-3,4-dichlorobenzamide (XXII).—To a solution of 0.40 g. (1.6 mmoles) of 2,6-diamino-5,6,7,8-tetrahydro-4-hydroxyquinazoline (XX) dihydrochloride and 0.58 g. (7.0 mmoles) of sodium bicarbonate in 10 ml. of water was added, with stirring, 0.36 g. (1.7 mmoles) of 3,4-dichlorobenzoyl chloride. The mixture was stirred at room temperature for 22 hours and was filtered. The collected precipitate was washed thoroughly with water and dried to yield 0.53 g. (95%) of crude product which failed to melt but began to decompose near 290°. The crude product was recrystallized twice from a dimethylformamide–water mixture and gave 0.37 g. (66%) of pure material, m.p. >300°. In the infrared it had λ_{max}^{KBr} (μ) 3.02, 3.17 (NH), 6.03–6.17, 6.45–6.53 (amide, NH, pyrimidine ring) 12.00 (tri-substituted phenyl).

Anal. Calcd. for $C_{15}H_{14}N_4O_2Cl_2 \cdot 1/2H_2O$: C, 49.7; H, 4.17; N, 15.5. Found: C, 49.6; H, 4.35; N, 15.8, 16.0.

Acknowledgments.—The authors are indebted

(21) No attempt was made to obtain optimum recoveries in the purification of the thiolesters.

to Dr. Peter Lim for infrared interpretations, Dr. Lloyd K. Moss and group for paper chromatography and Mr. O. P. Crews, Jr., and group for the large-scale preparation of intermediates.

Addendum.—The 5,8-dideaza-5,6,7,8-tetrahydrofolic acid (XI) at 80 mγ/ml. gave 50% inhibition of growth of *S. faecalis* on a Flynn folic acid medium containing 1mγ/ml. of folic acid. Compound XVIIa was inactive at 1γ/ml. We wish to thank Dr. Dorris Hutchison of Sloan-Kettering Institute for these data.

MENLO PARK, CALIF.

[CONTRIBUTION FROM THE BOUND BROOK LABORATORIES, RESEARCH DIVISION, AMERICAN CYANAMID CO.]

Synthesis of Carboxylic Acid Hydrazides and *s*-Triazoles of the Anthraquinone Series

BY ERWIN KLINGSBERG

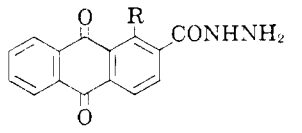
RECEIVED MAY 5, 1958

2-Anthraquinonecarboxylic acid hydrazide, its 1-chloro and 1-amino derivatives, and certain related diaroylhydrazines were prepared. Some of these compounds were converted to *s*-triazoles.

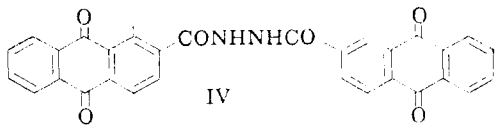
The present paper describes the preparation of certain 2-anthraquinonecarboxylic acid hydrazide derivatives, and the conversion of some of them to anthraquinonyl *s*-triazoles. Both classes of compounds are of interest by virtue of their relationship to anthraquinonyl oxadiazoles, which have recently acquired importance as vat dyestuffs.[1]

2-Anthraquinonecarboxylic Acid Hydrazides.—At the outset of this investigation, the preparation of the parent compound, 2-anthraquinonecarboxylic acid hydrazide itself, was undertaken. The reaction of 2-anthraquinonecarbonyl chloride with a large excess of hydrazine gave an apparently intractable product with a high indefinite melting point and a low nitrogen content. Ester hydrazinolysis under normal conditions was also discouraging; although 1-amino and 1,4-diamino-2-anthraquinonecarboxylic esters are reported to react normally with hydrazine,[2] ethyl 2-anthraquinonecarboxylate did not react, but was recovered unchanged after refluxing with hydrazine in methyl Cellosolve solution.

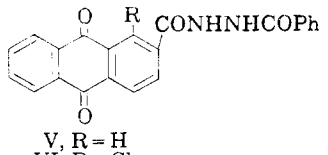
It was, found however, that in the absence of organic solvent the ester is reduced rapidly by aqueous hydrazine hydrate to the deep red hydroquinone. This is converted to the hydrazide, which is oxidized readily to a good yield of the desired product (I).[2a]



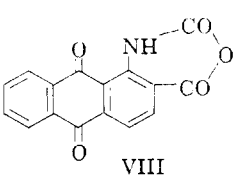
I, R = H
II, R = Cl
III, R = NH_2



IV



V, R = H
VI, R = Cl
VII, R = NH_2



VIII

It is not apparent why the carbethoxy group is more readily subject to nucleophilic attack when the anthraquinone system is in reduced, and probably anionic, form.

After the hydrazide I had been prepared and characterized, re-examination of the reaction between 2-anthraquinonecarbonyl chloride and hydrazine showed that the product was a mixture of a 55% yield of I with a 45% yield of bis-aroylhydrazine (IV). These are readily separable by crystallization from *o*-dichlorobenzene. It is noteworthy that so much diacylation occurs, despite the limited solubility of the acid chloride and the presence of a large excess of hydrazine. Under similar conditions, 1-chloro-2-anthraquinonecarbonyl chloride gives a good yield of the hydrazide II without a significant degree of diacylation.

2-Anthraquinonecarbonyl chloride and its 1-chloro derivative react with benzhydrazide to give the bis-aroylhydrazines V and VI.

For the preparation of the 1-amino hydrazide III, 3,4-phthaloylisatoic anhydride (VIII) was used. This compound was prepared readily by the phosgenation of 1-amino-2-anthraquinonecarboxylic acid, as described in the patent literature,[3] although it melts much higher than reported. It reacts with hydrazine to give III,[4] and with benzhydrazide to give VII.

2-Anthraquinonyl *s*-Triazoles.—Stollé[5] has described the conversion of dibenzoylhydrazine to the dichloroaldazine or "hydrazide dichloride," which reacts with aniline to give triphenyltriazole.

(1) U. S. Patents 2,464,831, March 22, 1949; 2,749,352, June 5, 1956; 2,759,948, August 21, 1956.

(2) P. V. Laakso, R. Robinson and H. P. Vandrewala, *Tetrahedron*, **1**, 103 (1957).

(2a) NOTE ADDED IN PROOF.—The author has now learned that the preparation of this compound from methyl 2-anthraquinonecarboxylate and hydrazine hydrate was reported by J. Shavel, Jr., F. Leonard, F. H. McMillian and J. A. King (*J. Am. Pharm. Assoc.*, **42**, 402 (1953)). There is no indication whether reduction to the hydroquinone was observed during hydrazinolysis.

(3) British Patent 719,193, November 24, 1954.

(4) U. S. Patent 2,717,898, September 13, 1955; British Patent 731,008, June 1, 1955; *cf.* ref. 2.

(5) R. Stollé, *J. prakt. Chem.*, **73**, 288 (1906); **75**, 416 (1907).