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

Potential Anticancer Agents.[1] XLII. Tetrahydroquinazoline Analogs of Tetrahydrofolic Acid. III. An Improved Synthesis of 5,8-Dideaza-5,6,7,8-tetrahydrofolic Acid

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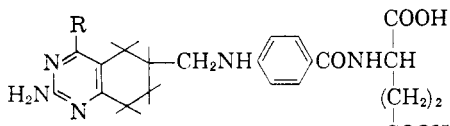
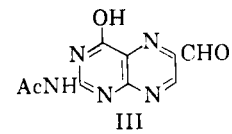
The conversion of 4-oxocyclohexanecarboxaldehyde dimethyl acetal (VIII) to 2-acetamido-5,6,7,8-tetrahydro-4-hydroxy-6-quinazolinecarboxaldehyde (XIV) provided the key intermediate for an improved synthesis of 5,8-dideaza-5,6,7,8-tetrahydrofolic acid (I). The transformation of XIV to I was accomplished either by the direct reductive alkylation of *p*-aminobenzoyl-L-glutamic acid with XIV, followed by hydrolysis, or by the preparation of *p*-[(2-acetamido-5,6,7,8-tetrahydro-4-hydroxy-6-quinazolinyl)methylamino]benzoic acid (XVIII) from XIV, followed by the condensation of the appropriately blocked XVIII with diethyl L-glutamate and a final hydrolysis to give the crystalline and chromatographically homogeneous I.

An earlier paper in this series[2] described the synthesis of 5,8-dideaza-5,6,7,8-tetrahydrofolic acid (I) and a later paper was concerned with approaches to the synthesis of 5,8-dideaza-5,6,7,8-tetrahydroaminopterin (II).[3] These approaches to the preparation of II were unsuccessful and a new synthetic scheme was devised for its preparation which, from a common intermediate, permitted an improved synthesis of I. The present manuscript describes this new preparation of I; it is anticipated that a later paper will describe the application of the synthetic scheme to the synthesis of II.



I. R = OH
II. R = NH_2

The key compound in the original synthesis of I was 2-amino-5,6,7,8-tetrahydro-4-hydroxy-6-quinazolinecarboxylic acid (XI)[2]; the corresponding 2,4-diamino-5,6,7,8-tetrahydro-6-quinazolinecarboxylic acid,[3] however, could not be converted to II. Sletzinger and co-workers[4] have reported an efficient synthesis of folic acid via the reductive alkylation of *p*-aminobenzoyl-L-glutamic acid (PABGA) with the pteridine aldehyde (III).



III

It seemed possible that analogous intermediates in the 5,8-dideaza-5,6,7,8-tetrahydroquinazoline series might lead to useful syntheses of both I and II. The preparation of the intermediates XIV and XV was accomplished; that of XIV and its conversion to I are described in this manuscript.

The reaction of a methanolic solution of butadiene (IV) with *N,N*-dibromobenzenesulfonamide was carried out as described by Petrov[5] and gave a 65% yield of 1-(bromomethyl)allyl methyl ether (V). The dehydrobromination of V with potassium hydroxide in diethylene glycol gave a 64% yield of 2-methoxybutadiene (VI), which was distilled from the reaction mixture as it formed. The Diels-Alder condensation of VI with acrolein was carried out in benzene solution at 160° for thirty minutes in a steel bomb[6] and gave a 75% yield of 4-methoxy-3-cyclohexene-1-carboxaldehyde (IX). This Diels-Alder reaction between an unsymmetrical diene and an unsymmetrical dienophile is known to give IX as the single isomer.[7] When relatively small amounts of the aldehyde IX were heated with dry methanol in the presence of a catalytic amount of ammonium chloride, an excellent yield of 4-oxocyclohexanecarboxaldehyde dimethyl acetal (VIII) resulted. The water produced in the formation of the acetal moiety was efficiently utilized in the hydrolysis of the enol ether of IX. In a larger scale preparation of VIII, the water formed in the initial reaction was insufficient to cleave the enol ether and it was necessary to add water deliberately after the acetal formation had proceeded.

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. SA-43-ph-1892. The opinions expressed in this paper are those of the authors and are not necessarily those of the Cancer Chemotherapy National Service Center. For the preceding paper in this series, *cf.* L. Goodman, L. O. Ross, M. O. Greene, J. Greenberg, and B. R. Baker, *J. Med. Pharm. Chem.*, in press.

(2) R. Koehler, L. Goodman, J. DeGraw, and B. R. Baker, *J. Am. Chem. Soc.*, **80**, 5779 (1958).

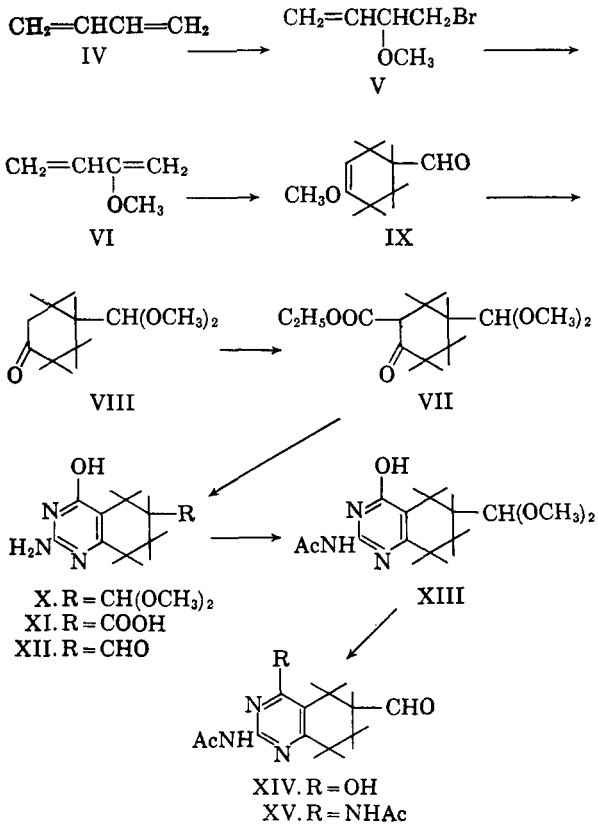
(3) J. DeGraw, L. Goodman, R. Koehler, and B. R. Baker, *J. Org. Chem.*, **24**, 1632 (1959).

(4) M. Sletzinger, D. Rheinhold, J. Grier, M. Beachem, and M. Tischler, *J. Am. Chem. Soc.*, **77**, 6365 (1955).

(5) A. Petrov, *J. Gen. Chem.* (*USSR*), **8**, 208 (1938); *Chem. Abstr.*, **32**, 5370 (1938).

(6) H. Fiesselmann, *Ber.*, **75B**, 881 (1942).

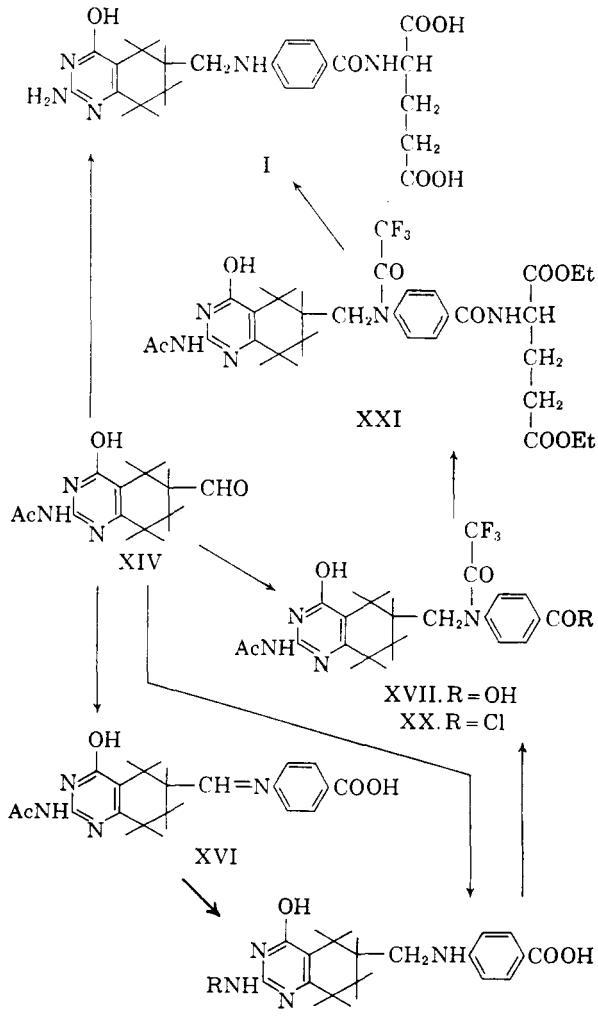
(7) H. L. Holmes, *Org. Reactions*, **4**, 63 (1948).



Carbethoxylation of the keto acetal VIII to give the β-keto ester VII was best accomplished with diethyl carbonate in ether solution using sodium hydride to form the anion of VIII. The yield was only 25%; low yields were noted by Hauser and Swamer[8] in their studies of the carbethoxylation of cyclohexanone. Efforts to improve the yield of VII by the use of ethyl chloroformate rather than ethyl carbonate or through the enamine of VIII were unsuccessful. The infrared spectrum of VII showed the presence of both the keto form and the chelated enol form of the β-keto ester.

Reaction of the crude keto ester VII with quanidine in methanolic sodium methoxide afforded the tetrahydroquinazoline acetal X as an easily purified solid. Treatment of X with acetic anhydride at 100° gave 2-acetamido-5,6,7,8-tetrahydro-4-hydroxy-6-quinazolinecarboxaldehyde dimethyl acetal (XIII) in good yield. The further reaction of XIII with 90% formic acid at room temperature[4] selectively hydrolyzed the acetal to generate the aldehyde function of XIV. A crystalline phenylhydrazone was prepared from XIV. The direct hydrolysis of the acetal X with dilute aqueous hydrochloric acid to the crystalline aldehyde XII was also carried out. A small-scale oxidation of XIV with aqueous hydrogen peroxide gave a mixture of the carboxylic acid XI and its N^2-acetyl derivative, as was shown by infrared spectra and paper chromatographic comparison with authentic materials. This experiment confirmed the orientation of the product IX of the Diels-Alder reaction,[7] as compound XI had been prepared unambiguously by another route.[2]

Attempts were made to alkylate reductively *p*-aminobenzoyl-L-glutamic acid (PABGA) with the acetylated aldehyde XIV using anhydrous formic acid or thiocresol as the reducing agent according to the procedures described by Sletzinger, *et al.*[4] Neither procedure was successful, which suggests that the aldehydes that can be employed as alkylating agents using these reagents must have carbonyl groups approximating the activity of aromatic aldehydes. The catalytic reductive alkylation of *p*-aminobenzoyl-L-glutamic acid with XIV using platinum oxide proceeded readily, however, and the product was hydrolyzed with dilute alkali to give the 5,8-dideazatetrahydrofolic acid I in 20% yield (from XIV) as a crystalline hydrate. Different samples of I recrystallized in different ways contained varying amounts of water of crystallization but all samples gave the same single spot when chromatographed on paper and



(8) F. W. Swamer and C. R. Hauser, *J. Am. Chem. Soc.*, **72**, 1352 (1950).

materials of a given degree of hydration had unique and reproducible infrared spectra.

When the aldehyde XIV was heated in ethanol with *p*-aminobenzoic acid (PABA), a good yield of the anil XVI was formed. The anil XVI was catalytically reduced over palladium-on-charcoal at 60–65° and gave a 55% yield of the recrystallized 5,8-dideazapteroic acid XVIII. The direct reductive alkylation of *p*-aminobenzoic acid with XIV in 2-methoxyethanol over platinum oxide gave a 40% yield of XVIII. The hydrolysis of XVIII to the free 5,8-dideazatetrahydropteroic acid XIX was readily accomplished by heating with dilute aqueous base.

Treatment of XVIII with trifluoroacetic anhydride gave good yields of a solid which had an infrared spectrum in agreement with that of XVII but which was not further purified. Gentle treatment of XVII with thionyl chloride gave the acid chloride XX which, without purification, was allowed to react with diethyl glutamate to give the diester XXI. Saponification of the diester XVI was accompanied by loss of the *N*-acetyl and *N*-trifluoroacetyl groups and gave a 39% yield of I (based on XVII) collected in two crops as the hemihydrate and as the hydrate with 2.5 moles of water. These samples agreed well in physical properties with the material isolated from the direct reductive alkylation of *p*-aminobenzoyl-L-glutamic acid. An effort to condense XVIII with diethyl glutamate by means of the dicyclohexyl carbodiimide procedure was unsuccessful.

As 5,8-dideaza-5,6,7,8-tetrahydrofolic acid (I), prepared from XIV, gave only one spot on paper chromatography[9]—in contrast to I prepared from 2 - amino - 6 - chloromethyl - 5,6,7,8 - tetrahydro-4-hydroxyquinazoline,[2] which was not uniform on paper chromatography—the suspicion that the earlier preparation of I may not have been pure was confirmed. The pure I at 50 mγ/ml. gave 50% inhibition of growth of *S. faecalis* on a Flynn folic acid medium containing 3 mγ of folic acid; thus, the earlier preparation, which had resisted attempted purification, had a purity of 15–25%. It follows that the physical constants for I recorded in this paper should supersede the old values.

EXPERIMENTAL[9]

1-(Bromomethyl)allyl methyl ether (V). To a flask equipped with a stirrer, a Dry Ice condenser and a drying tube, was added 680 ml. of reagent methanol. The flask was cooled to −10° and 720 ml. (8.7 moles) of butadiene (dried by passing over Drierite) was condensed. Then 2800 ml. of methanol was added and, with good stirring, 640 g. (2.03 moles) of *N,N*-dibromobenzenesulfonamide (Arapahoe Chemical, Inc.) was added over a 2.5-hr. period while maintaining the temperature at −8 to −12°. The solution was allowed to warm to room temperature. After being stirred for an additional 3.5 hr., the mixture was poured into 8 l. of water. The aqueous mixture was extracted with four 1-l. portions of pentane, the extract dried over magnesium sulfate, filtered, and distilled, to give 434 g. (65%) of product, b.p. 71–88° (62 mm.); λ_{max}^{film} (μ) 3.27, 7.05, 10.10, and 10.70 (CH of vinyl group), 6.05 (C=C), 9.02 (C—O—C). In an earlier run the product was distilled at atmospheric pressure, b.p. 140–142° (Petrov[5] gave b.p. 141–142° and yield of 54%).

2-Methoxybutadiene-1,4 (VI). To a hot (95–100°) solution of 42.0 g. (0.75 mole) of potassium hydroxide in 500 ml. of diethylene glycol in a flask equipped for distillation was added 79.0 g. (0.48 mole) of the bromo ether V over a period of 75 min. The temperature was slowly raised to 130° over a 2-hr. period and about 30 ml. of distillate was collected. Water (25 ml.) was added to the glycol solution and about 5 ml. of a steam distillate was collected. The combined distillate, which contained two layers, was separated and the aqueous phase discarded. The organic layer was dried over magnesium sulfate, leaving 30.5 g. of liquid which was distilled using a Vigreux column to give 25.5 g. (64%) of product, b.p. 74–76° (Petrov[5] used a different dehydrohalogenation technique and gave b.p. 74–74.5°); λ_{max}^{film} (μ) 3.30, 7.06. 10.13 and 10.75 (CH of vinyl group), 6.13 (C=C), 9.05 and 9.25 (C—O—C).

4-Methoxy-3-cyclohexene-1-carboxaldehyde (IX). A mixture of 9.5 g. (0.17 mole) of acrolein, 9.5 g. (0.11 mole) of 2-methoxybutadiene (VI), 29 ml. of benzene and 0.50 g. of hydroquinone was heated at 160° for 30 min. in a stainless steel bomb. After cooling to room temperature, the solution was transferred and distilled using a short Vigreux column to give 23.8 g. (75%) of IX, b.p. 80–85° (5 mm.) (Fiesselmann[6] reported a 75% yield, b.p. 94–95° (13 mm.)); λ_{max}^{film} (μ) 3.71 (aldehyde CH), 5.80 (C=O), 6.00 (C=C), 8.53 (C—O—C).

4-Oxocyclohexanecarboxaldehyde dimethyl acetal (VIII). To a solution of 9.90 g. (70.7 mmoles) of the aldehyde IX in 15 ml. of reagent methanol was added 0.20 g. of ammonium chloride. A vigorous, exothermic reaction resulted, after which the solution was heated at reflux for 1.5 hr. The solution was distilled from a short Vigreux column, yielding 10.25 g. (85%) of product, b.p. 92–96° (5 mm.). A portion of this disillate was redistilled for analysis, b.p. 79.5–80.0° (3 mm.); λ_{max}^{film} (μ) 5.80 (C=O), 8.85, 9.05, 9.30, 9.50 (C—O—C).

Anal. Calcd. for $C_9H_{16}O_3$: C, 62.8; H, 9.36. Found: C, 62.9; H, 9.19.

When the preparation of VIII was carried out on a preparative scale, it was necessary to add water deliberately to complete the hydrolysis of the enol ether. To a mixture of 446 g. (3.18 moles) of IX and 13.0 g. of ammonium chloride was added 900 ml. of reagent methanol. After the exothermic reaction subsided, the solution was heated on the steam bath for 2.5 hr., then was cooled to 40–50° and 39 ml. (2.2 moles) of water was added. The solution was stirred for 1.5 hr. without application of heat and was evaporated at 55° (2 mm.), leaving a residue that was filtered to remove ammonium chloride. Distillation of the residue from a Claisen flask gave 360 g. (65.5%) of VIII, b.p. 96–108° (1 mm.), n_D^{21} 1.4653, whose infrared spectrum agreed well with the above analytical sample. A run using 249 g. of IX gave 228 g. (74%) of VIII, b.p. 90–108° (1 mm.), n_D^{25} 1.4610.

Ethyl 5-formyl-2-oxocyclohexanecarboxylate dimethyl acetal (VII). A mixture of sodium hydride (12 mmoles) (0.52 g.

(9) 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. Generally, adenine was used as a standard and the spots were located relative to R_{Ad} 1.00. These solvent systems were used: A,[10] *n*-butyl alcohol–acetic acid–water (5:2:3); B, *N,N*-dimethylformamide-water (6:4); C,[11] *n*-butyl alcohol-ethanol-12*N* ammonium hydroxide–water (4:1:4); D,[12] *n*-butyl alcohol-acetic acid–water (4:1:5); E,[13] benzene-methanol-water (2:6:1); F,[14] 5% aqueous disodium hydrogen phosphate (no organic phase).

of a 54% suspension in mineral oil), 1.75 ml. (15 mmoles) of diethyl carbonate and 5 ml. of ether was prepared and to it was added 1.0 g. (6 mmoles) of the keto acetal VIII. The mixture was heated at reflux for 6.5 hr. and poured into 15 ml. of ice water. The organic layer was separated and extracted with 5 ml. of chilled (0°) 5% aqueous sodium hydroxide. The extract was combined with the original aqueous layer, the *p*H of the resulting solution adjusted to 7 to 8 with glacial acetic acid, the solution saturated with sodium chloride, and extracted with three 10-ml. portions of ether. The ether was dried over magnesium sulfate, filtered, and the filtrate evaporated *in vacuo*, leaving 0.40 g. (28%) of VII which was suitable for use in the next step; $\lambda_{max\ (\mu)}^{film}$ 5.72 (ester C=O), 5.80 (ketone C=O), 6.03 (chelated carbonyl), 6.17 (C=C), 8.15 and 8.25 (ester C—O—C), 8.80, 9.15 and 9.45 (acetal C—O—C).

2-Amino-5,6,7,8-tetrahydro-4-hydroxy-6-quinazolinecarboxaldehyde dimethyl acetal (X). To a solution of 1.57 g. (29 mmoles) of sodium methoxide in 26 ml. of reagent methanol was added 1.35 g. (14 mmoles) of guanidine hydrochloride and 2.10 g. (8.6 mmoles on a pure basis, but actually of unknown purity) of keto ester VII. The solution was heated at reflux for 4 hr. and was evaporated to dryness *in vacuo*. Water (10 ml.) was added to dissolve the residue, the solution was extracted with 5 ml. of ether and the ether extract discarded. The aqueous layer was adjusted to *p*H 7–8 with glacial acetic acid, giving a gummy precipitate which was separated by decantation. Water (5 ml.) was added to the precipitate and the suspension was stirred until the precipitate solidified. The solid was separated by filtration, washed with water and air dried to give 0.90 g. (44%) of product, m.p. >290°. This was recrystallized from 30 ml. of methanol to give the analytical sample, m.p. >300°; $\lambda_{max\ (\mu)}^{Nujol}$ 2.98, 3.20 (OH, NH), 6.02 (NH_2, pyrimidine ring), 6.20 (pyrimidine ring), 8.80, 9.20, and 9.43 (C—O—C); $\lambda_{max\ (m\mu)}^{pH\ 1}$ 226 (ε 9060), 262 (ε 7080); $\lambda_{max\ (m\mu)}^{pH\ 7}$ 270 (ε 4850), 284 (shoulder, ε 4050); $\lambda_{max\ (m\mu)}^{pH\ 13}$ 230 (ε 8270), 276 (ε 6360). On paper chromatography[9] in solvents A and F, the compound moved as a single spot with R_{Ad} 1.29 and 1.68, respectively.

Anal. Calcd. for $C_{11}H_{17}N_3O_3$: C, 55.2; H, 7.16; N, 17.6. Found: C, 55.0; H, 7.39; N, 17.4.

2-Amino-5,6,7,8-tetrahydro-4-hydroxy-6-quinazolinecarboxaldehyde (XII). The acetal X (0.300 g., 1.26 mmoles) was stirred for 1 hr. with 3 ml. of 0.5*M* hydrochloric acid on the steam bath. The solution was filtered and the filtrate was brought to *p*H 8 with saturated aqueous sodium bicarbonate. The solid was collected, washed, and dried, giving a material with m.p. >300°; $\lambda_{max\ (\mu)}^{KBr}$ 2.97 and 3.05 (NH, OH), 3.65 (aldehyde CH), 5.85 (C=O), 6.03–6.13 (NH_2, pyrimidine ring), 6.55 (pyrimidine ring, NH); $\lambda_{max\ (m\mu)}^{pH\ 1}$ 262 (ε 6950); $\lambda_{max\ (m\mu)}^{pH\ 7}$ 269 (ε 4850); $\lambda_{max\ (m\mu)}^{pH\ 13}$ 276 (ε 6050). On paper chromatography in solvent A, the compound moved as a single spot with R_{Ad} 1.14 and was easily distinguishable from X (R_{Ad} 1.29).

Anal. Calcd. for $C_9H_{11}N_3O_2 \cdot {}^3/_4H_2O$: C, 52.5; H, 6.18; N, 20.3. Found: C, 52.3, 52.5; H, 6.56, 6.35; N, 20.2, 20.4.

2-Acetamido-5,6,7,8-tetrahydro-4-hydroxy-6-quinazolinecarboxaldehyde dimethyl acetal (XIII). To 0.30 g. (1.26 mmoles) of the quinazoline acetal X was added 2 ml. of acetic anhydride and the mixture was heated on the steam bath for 10 min., complete solution resulting. The solution was evaporated to dryness *in vacuo* and the residue was dissolved in 8 ml. of chloroform. The chloroform solution was washed with 5 ml. of saturated aqueous sodium bicarbonate and 5 ml. of water and was dried over magnesium sulfate. After filtration, the solution was evaporated *in vacuo*, leaving 0.29 g. (83%) of product which was recrystallized from benzene to give 0.17 g. (49%) of crystalline material collected in two crops, m.p. 187–191° and 191–193°. The analytical sample was obtained by a second recrystallization from benzene, m.p. 188.0–189.5°; $\lambda_{max\ (m\mu)}^{KBr}$ 2.95 and 3.15 (NH, OH), 6.05 (amide C=O and pyrimidine ring), 6.37 (pyrimidine ring and NH), 8.05 (NAc), 8.85, 9.25, 9.50 (C—O—C); $\lambda_{max\ (m\mu)}^{C_2H_5OH}$ 243 (ε 11,700), 286 (ε 8750). On paper chromatography in solvent E the compund gave a single spot with R_{Ad} 1.83.

Anal. Calcd. for $C_{13}H_{19}N_3O_4$: C, 55.5; H, 6.81; N, 14.9. Found: C, 56.1; H, 6.74; N, 14.7.

2-Acetamido-5,6,7,8-tetrahydro-4-hydroxy-6-quinazolinecarboxyaldehyde (XIV). To 20 ml. of 90% formic acid was added 11.3 g. (40.2 mmoles) of the acetamido acetal XIII and the solution was allowed to stand at room temperature for 2 hr. Ether (400 ml.) was added, the solution was chilled, and the crystals filtered to give 7.5 g. of solid. The filtrate was evaporated to dryness *in vacuo* and the residue was extracted with 15 ml. of hot toluene. Evaporation of the toluene extract *in vacuo* yielded 0.80 g. more of product. The combined solids (8.3 g.) were recrystallized from 40 ml. of acetone to give three crops of XIV, totaling 6.45 g. (68%), m.p. 203–204°, 202–208° and 203–205°. From another run an analytical sample was obtained, m.p. 208.5–211.0°; $\lambda_{max\ (\mu)}^{KBr}$ 2.95 and 3.16 (NH, OH), 3.57 and 3.72 (aldehyde CH), 5.81 (C=O), 6.03–6.10 (amide C=O and pyrimidine ring), 6.22 and 6.40 (NH and pyrimidine ring), 8.05 (NAc). On paper chromatography in solvents D and E the compound moved as a single spot with R_{Ad} 1.46 and 1.32, respectively.

Anal. Calcd. for $C_{11}H_{13}N_3O_3$: C, 56.2; H, 5.57; N, 17.9. Found: C, 56.1; H, 5.95; N, 17.9.

By gentle heating, a solution of 0.200 g. (0.85 mmole) of the aldehydė XIV was dissolved in 5 ml. of 95% ethanol and to the solution was added 0.15 ml. (1.53 mmoles) of phenylhydrazine and 1 drop of glacial acetic acid. The solution was warmed for 15 min. at 50–60°, centrifuged warm to isolate the precipitate, the crystals washed with 5 ml. of 95% ethanol, and dried to give 0.17 g. (64%) of the phenylhydrazone of XIV. This was recrystallized with large losses from aqueous 2-methoxyethanol to give yellow crystals, m.p. 207–212° (the melting point was strongly dependent on the rate of heating); $\lambda_{max\ (\mu)}^{KBr}$ 2.87 and 3.05 (NH, OH), 6.02 (amide C=O and pyrimidine ring), 6.25 (pyrimidine ring), 6.35 (NH), 7.96–8.04 (NAc), 13.32 and 14.40 (monosubstituted phenyl); $\lambda_{max\ (m\mu)}^{Methyl\ cellosolve}$ 243 (ε 15,000), 253 (ε 13,600), 281 (ε 24,400). On paper chromatography in solvents A and E, the product moved as a single spot with R_{Ad} 1.60 and 1.66, respectively.

Anal. Calcd. for $C_{17}H_{19}N_5O_2$: C, 62.8; H, 5.89. Found: C, 62.2; H, 5.92.

p-[(2-Acetamido-5,6,7,8-tetrahydro-4-hydroxy-6-quinazolinyl)methyleneamino]benzoic acid (XVI). A solution of 0.40 g. (1.7 mmoles) of the *N*-acetyl aldehyde XIV, 0.24 g. (1.7 mmoles) of *p*-aminobenzoic acid, and 25 ml. of absolute ethanol was heated to boiling under a nitrogen atmosphere and the solvent slowly distilled until 18 ml. of distillate had been collected. The remaining solution was chilled and the solid filtered and washed with ethanol; yield, 0.43 g. (72%) of yellow solid. A portion (0.33 g.) of this was extracted with 20 ml. of hot 2-methoxyethanol, the insoluble portion separated by centrifugation, dissolved in 10 ml. of hot *N,N*-dimethylformamide, and the solution diluted with 1.5 ml. of water and chilled. The crystalline solid was filtered, washed with 2 ml. of *N,N*-dimethylformamide and with 10 ml. of ethanol, and dried to yield 0.10 g. (22%), decomposing in the range 200–240°; $\lambda_{max\ (\mu)}^{KBr}$ 2.95–3.20 (OH, NH), 2.90–4.05 (OH of COOH), 5.95–6.10 (C=O of carboxyl; amide C=O; and C=N), 6.25 (pyrimidine ring and NH), 7.85–8.15 (NAc and COOH), 11.90 (disubstituted phenyl), 12.85 (pyrimidine ring); there was no absorption at 10.5 μ characteristic of *p*-aminobenzoic acid; $\lambda_{max\ (m\mu)}^{Methyl\ cellosolve}$ 243 (ε 13,800), 318 (ε 22,900).

Anal. Calcd. for $C_{18}H_{18}N_4O_4$: C, 61.0; H, 5.12; N, 15.8. Found: C, 60.6; H, 5.37; N, 15.0.

p-[(2-Acetamido-5,6,7,8-tetrahydro-4-hydroxy-6-quinazolinyl)methylamino]benzoic acid (XVIII). *A. From the anil* XV. A solution of 4.0 g. (17.2 mmoles) of the *N*-acetyl aldehyde XIV, 2.36 g. (17.2 mmoles) of *p*-aminobenzoic acid and 40 ml. of absolute ethanol under nitrogen was heated at reflux for 3 hr. and was cooled to room tempera-

ture. After standing 30 min., the solid was filtered, washed with absolute ethanol and ether, and dried to give 5.8 g. (97%) of the crude anil XVI.

A vigorously stirred mixture of this crude anil, 0.58 g. of 5% palladium-on-charcoal, and 60 ml. of 2-methoxyethanol was shaken with hydrogen at 60–65° and atmospheric pressure. After 8 hr., 0.85 molar equivalent of hydrogen was absorbed and the warm mixture was filtered. The mixture of product and catalyst was suspended in 50 ml. of water and 6*M* ammonium hydroxide was added to adjust the *p*H to 9–10. The suspension was filtered twice through Celite and the vigorously stirred filtrate was adjusted to *p*H 4–5 with 6*M* hydrochloric acid. The precipitated solid was collected, washed with water, absolute ethanol and ether, and dried, leaving 4.2 g. (72%) of product. The crude solid was dissolved in 20 ml. of boiling *N,N*-dimethylformamide, the solution filtered, and 5 ml. of water added to the filtrate. After chilling, 2.95 g. (46%) of material was collected, m.p. 279–281°, and the mother liquors yielded a second crop of 0.15 g., giving a total yield from XIV of 49.6%. From a previous run an analytical sample was obtained, m.p. 276–277°; $\lambda_{max\ (\mu)}^{KBr}$ 2.95 and 3.12 (NH, OH), 3.78–4.05 (OH of COOH), 5.93–6.05 (C=O of carboxyl and acetyl), 6.20 (phenyl ring, pyrimidine ring and NH), 7.97–8.05 (NAc), 11.90 (disubstituted benzene), 12.90 (pyrimidine ring); $\lambda_{max\ (m\mu)}^{Methyl\ cellosolve}$ 226 (ε 15,200), 242 (ε 12,500), 252 (ε 10,500), 302 (ε 32,100). The product moved as a single spot in solvents B and C with R_{Ad} 1.25 and 0.43, respectively.

Anal. Calcd. for $C_{18}H_{20}N_4O_4.{}^1/_2H_2O$: C, 59.1; H, 5.71; N, 15.4. Found: C, 58.7, 59.0; H, 5.51, 5.39; N, 15.4.

B. From the aldehyde (XIV). A stirred mixture of 0.50 g. (2.1 mmoles) of aldehyde XIV, 0.30 g. (2.1 mmoles) of *p*-aminobenzoic acid, 0.05 g. of platinum oxide, and 10 ml. of 2-methoxyethanol was stirred with hydrogen at 30° and atmospheric pressure for 4 hr., 1 mole-equivalent of hydrogen being absorbed. The suspension was filtered and the product separated from the catalyst by extraction with 5 ml. of boiling *N,N*-dimethylformamide. Water (1 ml.) was added to the solution and, on chilling, 0.23 g. of product separated, with an infrared spectrum identical with that of the analytical sample of XVIII, described above. From the mother liquors was recovered a second crop of product, 0.07 g., giving a total yield of 40%.

p-[(5,6,7,8-Tetrahydro-4-hydroxy-6-quinazolinyl)methylamino]benzoic acid (XIX). A solution of 0.50 g. (1.40 mmoles) of the *N*-acetyl acid XVIII in 5.0 ml. of 1*M* aqueous sodium hydroxide was heated on the steam bath for 30 min. The solution was adjusted to *p*H 5 with 6*M* hydrochloric acid, filtered, and the precipitate washed with water and dried to give 0.39 g. (89%) of solid which did not melt at 300°. A portion of this (0.25 g.) was stirred with warm (40°) concentrated hydrochloric acid; the insoluble solid was collected and washed with water. The solid was dissolved in 5 ml. of 1*M* aqueous sodium hydroxide and precipitated by adjusting the *p*H to 4–5 with 6*M* hydrochloric acid. The purified solid was collected, washed with water and dried to give 0.15 g. (50%) of product, m.p. >300°; $\lambda_{max\ (\mu)}^{Nujol}$ 3.01 and 3.17 (NH), 3.66–3.90 (OH of COOH), 5.92 (C=O), 6.19 (pyrimidine and phenyl rings), 6.50 (pyrimidine ring), 11.96 (disubstituted phenyl), 12.91 (pyrimidine ring); lack of absorption near 8.0 μ indicated the loss of the N-acetyl; $\lambda_{max\ (m\mu)}^{pH\ 1}$ 226 (ε 21.200), 268 (ε 9800), 307 (ε 8640); $\lambda_{max\ (m\mu)}^{pH\ 13}$ 281 (ε 24,000). On paper chromatography in solvent C, the product moved as a single spot with R_{Ad} 0.22.

Anal. Calcd. for $C_{16}H_{18}N_4O_3.H_2O$: C, 57.8; H, 6.02; N, 16.9. Found: C, 57.6; H, 5.88; N, 16.6, 16.7.

p-{N-[(2-Acetamido-5,6,7,8-tetrahydro-4-hydroxy-6-quinazolinyl)methyl]-2,2,2-trifluoroacetamido}benzoic acid (XVII). A mixture of 2.0 g. (5.6 mmoles) of *N*-acetyl acid XVIII and 20 ml. of trifluoroacetic anhydride was heated under reflux for 35 min. The solution was evaporated to dryness *in vacuo*, the residue was stirred vigorously with 25 ml. of ether, filtered, and washed with ether. The solid was suspended in 20 ml. of water, stirred vigorously for 2 hr., filtered, washed with water, and dried to give 2.07 g. (82%) of solid which was not purified further and was not analytically pure, although it moved as a single spot in solvent E with R_{Ad} 1.67 and was free of XVIII. In the infrared it had $\lambda_{max\ (\mu)}^{Nujol}$ 3.12 (NH), 3.75–3.90 (OH of COOH), 5.81–5.88 (carboxyl C=O and CF_3CO), 6.0 (CH_3CO), 6.20 (phenyl and pyrimidine rings), 8.05 (NAc), 8.29 and 8.61 (CF_3), 11.96 (disubstituted benzene).

Anal. Calcd. for $C_{20}H_{16}N_4O_5F_3$: F, 12.6. Found: F, 13.4.

Diethyl N-{p-{N-[(2-acetamido-5,6,7,8-tetrahydro-4-hydroxy-6-quinazolinyl)methyl]-2,2,2-trifluoroacetamido}-benzoyl}glutamate (XXI). A mixture of 0.45 g. (1 mmole) of the *N*-trifluoroacetyl acid XVII and 4.5 ml. of thionyl chloride was heated under reflux for 20 min., giving a light yellow solution which was evaporated *in vacuo*. To the residue was added 5 ml. of dry 1,2-dichloroethane and the solution was again evaporated *in vacuo*. The solution in 1,2-dichloroethane and the evaporation were repeated twice more, giving a residue of the acid chloride XX.

A solution of 1.0 g. (4.2 mmoles) of diethyl L-glutamate hydrochloride[15] (m.p. 107.5–108.5°) in 10 ml. of chloroform was washed with 4.0 ml. of 10% aqueous potassium carbonate. The carbonate extract was washed with 5 ml. of chloroform which was combined with the original chloroform solution and the combined solutions dried over potassium carbonate. After filtration, the solution was evaporated *in vacuo*, leaving a residue of 0.67 g. (3.3 mmoles) of diethyl glutamate.

The diethyl glutamate, dissolved in 3 ml. of dry methylene chloride, was added to a solution of the acid chloride XX in 5 ml. of methylene chloride; the resulting solution was heated under reflux for 2 hr. protected from moisture, then allowed to stand overnight. The solution was washed with 5 ml. of 1% aqueous potassium carbonate and dried over potassium carbonate. After filtration, the solution was evaporated *in vacuo* to give a gum which solidified when it was stirred with water. The solid was filtered, washed with water, and dried to give 0.58 g. (91%) of crude product; $\lambda_{max\ (\mu)}^{Nujol}$ 3.15 (NH), 5.74 (ester C=O), 5.85 (C=O of CF_3CON), 5.98–6.01 (amide C=O and pyrimidine ring), 6.21 (phenyl and pyrimidine rings), 8.00 (NAc), 8.28 (ester C—O—C and CF_3), 8.64 (CF_3), 11.60 (disubstituted phenyl).

N-{p-[(2-Amino-5,6,7,8-tetrahydro-4-hydroxy-6-quinazolinyl)methylamino]benzoyl}glutamic acid (5,8-dideaza-5,6,7,8-tetrahydrofolic acid) (I). A. *From the diester* XXI. A stirred mixture of 0.57 g. (0.9 mmole) of the diester XXI (*cf.* above) and 40 ml. of 0.25*M* aqueous sodium hydroxide was heated on the steam-bath for 30 min., then adjusted to *p*H 3–4 with 1*M* hydrochloric acid. The precipitate was collected, washed with water and dried to give 0.29 g. (74%) of product. A portion of this (0.28 g.) was dissolved in 3 ml. of hot *N,N*-dimethylformamide and the solution, on chilling, gave 0.080 g. (20.4% calculated as the hemihydrate) of white crystals which darkened near 220°, softened near 240°, and vigorously decomposed above 260°; $\lambda_{max\ (\mu)}^{Nujol}$ 2.98 and 3.19 (NH), 3.80–3.92 (OH of COOH), 5.81 (carboxyl C=O), 5.95 (amide C=O), 6.19 (pyrimidine and phenyl rings), 6.56 (amide NH), 11.89 (disubstituted phenyl), 13.03 (pyrimidine ring); $\lambda_{max\ (m\mu)}^{pH\ 1}$ 224 (ε 20,100), 265 (ε 10,300); $\lambda_{max\ (m\mu)}^{pH\ 7}$ 290 (ε 20,100); $\lambda_{max\ (m\mu)}^{pH\ 13}$ 284 (ε 21,000). The product moved as a single spot in solvent A with R_{Ad} 1.17.

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

(11) A similar system was used by E. L. Hirst, L. Hough, and J. K. N. Jones, *J. Chem. Soc.*, 928 (1949).

(12) R. L. M. Synge, *Biochem. J.*, **48**, 429 (1951).

(13) Th. Wieland and W. Kracht, *Angew. Chem.*, **69**, 172 (1957).

(14) C. E. Carter, *J. Am. Chem. Soc.*, **72**, 1835 (1950).

(15) H. M. Chiles and W. A. Noyes, *J. Am. Chem. Soc.*, **44**, 1798 (1922); R. B. Angier, C. W. Waller, B. L. Hutchings, J. H. Boothe, J. H. Mowat, J. Semb, and Y. SubbaRow, *J. Am. Chem. Soc.*, **72**, 74 (1950).

Anal. Calcd. for $C_{21}H_{25}N_5O_6 \cdot {}^1/_2H_2O$: C, 55.8; H, 5.79. Found: C, 55.9; H, 5.90.

The mother liquors from the separation of the above material (0.080 g.) were diluted with 2.5 ml. of water and chilled, giving 0.100 g. (21.3% calculated as the hydrate with 2.5 moles of water), of crystalline solid which showed the same melting behavior as the hemihydrate (*cf.* above); λ_{max}^{Nujol} (μ) 2.98 (NH), 5.84–5.92 (carboxyl and amide C=O), 6.21 (pyrimidine and phenyl rings), 6.60 (amide NH), 11.95 (disubstituted phenyl), 13.10 (pyrimidine ring). The paper chromatographic behavior of the sample was identical with that of the hemihydrate.

Anal. Calcd. for $C_{21}H_{25}N_5O_6 \cdot 2{}^1/_2H_2O$: C, 51.6; H, 6.19; N, 14.3. Found: C, 51.7, 51.7; H, 5.89, 6.11; N, 14.2.

B. From the N-acetyl aldehyde XIV. A suspension of 0.47 g. (2.00 mmoles) of aldehyde XIV, 0.53 g. (2.00 mmoles) of *p*-aminobenzoyl-L-glutamic acid, 0.050 g. of platinum oxide, and 10 ml. of 2-methoxyethanol was vigorously stirred with hydrogen at 35° and atmospheric pressure for 3.5 hr., when 1 molar equivalent of hydrogen had been absorbed. The mixture was filtered, the filtrate was concentrated *in vacuo* to 3 ml., water (2 ml.) was added, and the solution was chilled. A black gum deposited and was removed by decantation of the supernatent liquid which was evaporated *in vacuo*, leaving 0.90 g. (93%) of residue. The residue was dissolved in 40 ml. of 0.1*M* aqueous sodium hydroxide, the solution heated on the steam bath for 30 min., filtered and the filtrate adjusted to *p*H 3–4 with 1*M* hydrochloric acid. The precipitate (0.60 g.) was stirred with 4.0 ml. of hot (100°) *N,N*-dimethylformamide and the insoluble, white crystalline material removed by filtration to give 0.033 g. (3.65% calculated as the hemihydrate) of solid which had the same melting behavior, infrared spectrum and paper chromatographic behavior as the hemihydrate isolated from procedure A (*cf.* above).

Anal. Calcd. for $C_{21}H_{25}N_5O_6 \cdot {}^1/_2H_2O$: C, 55.8; H, 5.79; N, 15.5. Found: C, 56.0, 55.9; H, 5.80, 5.83; N, 15.0, 15.2.

The *N,N*-dimethylformamide solution, after removal of the hemihydrate, was diluted with 4 ml. of water and chilled. The crystalline precipitate was washed with *N,N*-dimethylformamide, then water; yield 0.156 g. (16.5% calculated as the sesquihydrate) of solid whose melting and chromatographic behaviors were identical with those of the hemihydrate and whose infrared spectrum was almost identical with that of the compound containing 2.5 moles of water isolated from procedure A (*cf.* above).

Anal. Calcd. for $C_{21}H_{25}N_5O_6 \cdot 1{}^1/_2H_2O$: C, 53.6; H, 5.99; N, 14.9. Found: C, 53.8, 53.8; H, 6.21, 6.29; N, 14.7, 14.8.

Acid hydrolysis of I. A solution of 5 mg. of the hemihydrate of I (prepared by procedure A) in 5 ml. of 6*M* hydrochloric acid was heated at 100° for 1 hr. and was evaporated to dryness *in vacuo*. Water (1 ml.) and 2 drops of 10% aqueous sodium hydroxide were added to the residue and the *p*H was adjusted to 5 with 1*M* hydrochloric acid. The precipitate was separated by centrifugation and the supernatent liquid was removed. Both the solid (3.5 mg.) and the supernatent liquid were subjected to paper chromatography in solvent A. The solution showed the presence of glutamic acid (R_f 0.30) and the solid showed two spots at R_f 0.65 and 0.80 which lined up exactly with the spots from the product of a similar acid hydrolysis of the *N*-acetylpteroic acid XVIII.

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[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF THE UNIVERSITY OF KANSAS]

Condensation of Isocyanates with Reissert Compounds; Synthesis of an Analog of Lidocaine

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O-Benzoyl-*N*-phenylisoquinaldamide and *O*-benzoyl-*N*-(α-naphthyl)isoquinaldamide were prepared by the reaction of phenyl and 1-naphthyl isocyanate, respectively, with the lithium salt of 2-benzoyl-1,2-dihydroisoquinaldonitrile. Hydrolysis of the *O*-benzoyl derivatives gave *N*-phenylisoquinaldamide and *N*-(α-naphthyl)isoquinaldamide, respectively, plus benzoic acid. There was no analogous reaction between the isocyanates and the lithium salt of 1-benzoyl-1,2-dihydroquinaldonitrile.

Catalytic hydrogenation of *N*-phenylisoquinaldamide gave the tetrahydro derivative, and treatment of the latter compound with ethyl iodide afforded *N*-phenyl-2-ethyl-1,2,3,4-tetrahydroisoquinaldamide, an analog of the local anesthetic Lidocaine.

Although Reissert compounds, 1-acyl-1,2-dihydroquinaldonitriles (I) and 2-acyl-1,2-dihydroisoquinaldonitriles (II), are mainly noted for their ability to form aldehydes on acid-catalyzed hydrolysis, increased attention in recent years has been directed toward the use of such compounds in the synthesis of diverse quinoline and isoquinoline derivatives.[1-11] The present communication describes an extension of the latter area of work, one leading to the production of the *O*-acyl derivatives

(1) W. E. McEwen and R. L. Cobb, *Chem. Revs.*, **55**, 511 (1955).

(2) R. L. Cobb and W. E. McEwen, *J. Am. Chem. Soc.*, **77**, 5042 (1955).

(3) A. P. Wolf, W. E. McEwen, and R. H. Glazier, *J. Am. Chem. Soc.*, **78**, 861 (1956).

(4) F. D. Popp and W. E. McEwen, *J. Am. Chem. Soc.*, **79**, 3773 (1957).

(5) L. R. Walters, N. T. Iyer, and W. E. McEwen, *J. Am. Chem. Soc.*, **80**, 1177 (1958).

(6) F. D. Popp and W. E. McEwen, *J. Am. Chem. Soc.*, **80**, 1181 (1958).

(7) N. C. Rose and W. E. McEwen, *J. Org. Chem.*, **23**, 337 (1958).

(8) N. C. Rose, L. R. Walters, and W. E. McEwen, *J. Org. Chem.*, **23**, 341 (1958).