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# Potential Anticancer Agents.[1] LXIX. Tetrahydroquinazoline Analogs of Tetrahydrofolic Acid. IV. The Synthesis of 5,8-Dideaza-5,6,7,8-tetrahydroaminopterin

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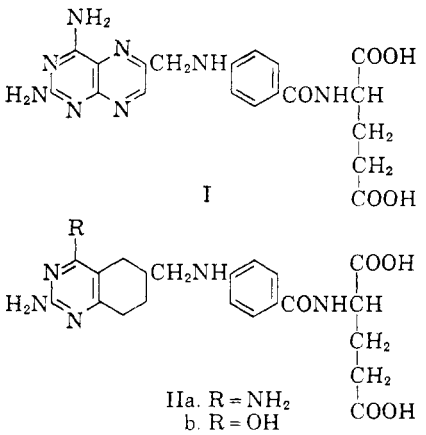
The direct fusion of 4-oxocyclohexanecarboxaldehyde dimethyl acetal (III) with cyanoguanidine provided the key intermediate, 2,4-diacetamido-5,6,7,8-tetrahydro-6-quinazolinecarboxaldehyde (XV), necessary for the synthesis of 5,8-dideaza-5,6,7,8-tetrahydroaminopterin (IIa). The conversion of XV to IIa was accomplished by the reductive alkylation of *p*-aminobenzoyl-L-glutamic acid with XV, followed by hydrolysis of the blocked intermediate XVIII to give the crystalline and chromatographically homogeneous IIa.

In the first paper[4] of this series, a biological rationale was presented for the preparation of folic acid analogs in which the N-5–nitrogen atom of tetrahydrofolic acid was replaced by a methylene group. In view of the strong antifolic activity of aminopterin[5] (I), the 4-amino analog of folic acid, it appeared that an aminopterin analog lacking the N-5–nitrogen might exhibit even greater antifolic activity. In addition, Kisliuk[6] has recently shown that tetrahydroaminopterin is a more potent antifolic agent than aminopterin itself in certain bacterial systems



I



IIa. R = $NH_2$
b. R = OH

(*S. faecalis* and *P. cerevisiae*). A compound which possesses all of these modifications of the folic acid structure is 5,8-dideaza-5,6,7,8-tetrahydroaminopterin (IIa), and its preparation is the subject of this paper.

In another paper in this series,[7] some approaches to the synthesis of 5,8-dideaza-5,6,7,8-tetrahydroaminopterin (IIa) were described. Earlier attempts to prepare a suitable 6-substituted derivative of 2,4-diamino-5,6,7,8-tetrahydroquinazoline as a precursor for IIa were unsuccessful. The synthesis of such an intermediate, 2,4-diacetamido-5,6,7,8-tetrahydro-6-quinazolinecarboxaldehyde (XV), and its subsequent conversion to the aminopterin analog (IIa), has been successfully accomplished.

The key intermediate for the preparation of the aldehyde (XV) was the previously described 4-oxocyclohexanecarboxaldehyde dimethyl acetal (III).[8] Two methods were explored for the conversion of III to XV, proceeding through the acetal XIVa. In the longer sequence, compound III was first treated with ethyl formate to give a 62% crude yield of the 2-formyl ketone (IV). Reaction of IV with hydroxylamine in pyridine afforded the crude isoxazole (V), which, without further purification, was converted to the 2-cyano ketone (VI) with methanolic sodium methoxide. This α-cyano ketone synthesis utilized the method developed by Johnson and co-workers[9] and gave an over-all yield of 30% of crude VI from III. The conversion of cyclohexanone (VII) to 2-cyanocyclohexanone (X) in a 34% yield *via* the isoxazole (IX) served as a model for the preparation of VI. Both cyano ketones VI and X possessed a nitrile doublet at 4.45 and 4.55 μ in the infrared, characteristic of this class of compounds.[10]

It has been shown[11,12] that α-cyano ketones

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

(5) A review of folic acid antagonists is found in *The Vitamins*, W. H. Sebrell, Jr., and R. S. Harris, Vol. III, Academic Press, Inc., New York, 1954, p. 149.

(6) R. L. Kisliuk, *Nature*, **188**, 584 (1960).

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

(8) J. DeGraw, L. Goodman, and B. R. Baker, *J. Org. Chem.*, **26**, 1156 (1961).

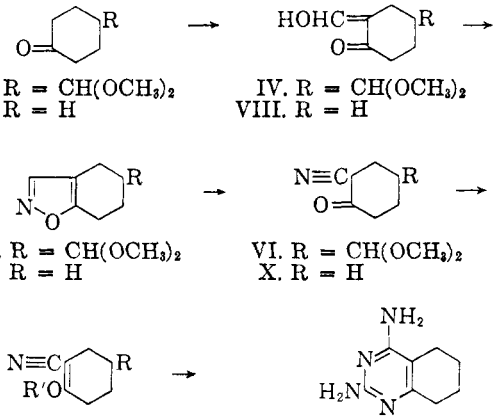
(9) W. S. Johnson, J. Petersen, and C. Gutsche, *J. Am. Chem. Soc.*, **69**, 2942 (1947); W. S. Johnson and W. Shelberg, *J. Am. Chem. Soc.*, **67**, 1745 (1945).

(10) L. J. Bellamy, *J. Chem. Soc.*, 4487 (1954).

(11) P. Russell and G. Hitchings, *J. Am. Chem. Soc.*, **73**, 3763 (1951).

(12) B. Chase and J. Walker, *J. Chem. Soc.*, 3518 (1953).

(as such) do not condense with guanidine to form pyrimidine heterocycles but do condense in the form of their enol ethers. Initially 2-cyanocyclohexanone (X) was used as a model and was converted to the isobutyl enol ether (XIa) in 73% yield according to the procedure of Chase and Walker.[12] These authors cyclized the enol ether with guanidine in methanolic sodium methoxide at 160° for two hours, but obtained only a 6% yield of 2,4-diamino-5,6,7,8-tetrahydroquinazoline (XII). This cyclization was repeated in this work, but was carried out at 150° for fifteen hours in a stirred autoclave to give a 48% yield of 2,4-diamino-5,6,7,8-tetrahydroquinazoline (XII).



III. R = $CH(OCH_3)_2$ IV. R = $CH(OCH_3)_2$
VII. R = H VIII. R = H

V. R = $CH(OCH_3)_2$ VI. R = $CH(OCH_3)_2$
IX. R = H X. R = H

XIII. R = $CH(OCH_3)_2$, R′ = $C_2H_5$
XIa. R = H, R′ = $CH_2CH(CH_3)_2$
b. R = H, R′ = $C_2H_5$

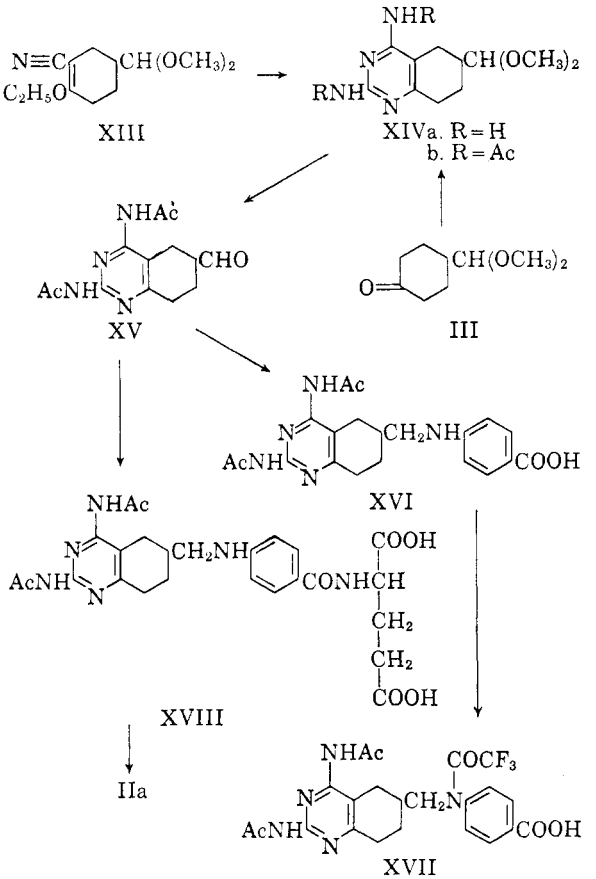
XII

The use of isobutyl alcohol and *p*-toluenesulfonic acid in boiling toluene with water separation,[12] as in the case of 2-cyanocyclohexanone, was unsuccessful when applied to the 2-cyanoketo acetal VI. However, 2-cyanocyclohexanone (X) could be smoothly converted to the ethyl enol ether XIb by heating with triethyl orthopropionate. The application of this procedure to VI afforded the enol ether XIII as an oil whose infrared spectrum showed the expected intense conjugated nitrile and olefinic bands. When XIII was allowed to react with guanidine hydrochloride and sodium methoxide in methanol at 150° for fifteen hours, an 11% yield of the purified diamino acetal XIVa was obtained. The low yield of XIVa is probably a reflection of the purity of XIII, which was obtained from a sequence where the intermediates V and VI were crude oils and the formyl compound IV was distilled but not otherwise purified.

Since the over-all yield of XIVa from the keto acetal III was only about 3%, a better method was sought. It was found that merely heating the keto acetal III with a small excess of cyanoguanidine at 180–185° gave a 17% yield of XIVa[13]; the temperature of 180–185° was found to be quite critical for this reaction.

The diamino acetal (XIVa) was readily acetylated by acetic anhydride at 100° for twenty minutes to give the 2,4-diacetamido acetal (XIVb) in yields of 70–75%. This blocked acetal could be selectively hydrolyzed to the blocked aldehyde XV by 90% formic acid at room temperature. This procedure was similar to that used in the preparation of folic acid[14] and 5,8-dideazatetrahydrofolic acid (IIb).[8] However, the increased lability of the 4-acetamido group required considerable experimentation to perfect the hydrolysis conditions.

The incorporation of the *p*-aminobenzoyl glutamic acid moiety was approached from two separate pathways, only one of which was successful. When the aldehyde XV was reductively aminated



with *p*-aminobenzoic acid, the 2,4-diacetamido-5,8-dideazapteroic acid (XVI) was obtained in yields of only 20–35% after recrystallization. Attempts to improve this yield by proceeding through a Schiff's base intermediate were fruitless, since loss of *N*-acetyl was observed when the aldehyde was refluxed with *p*-aminobenzoic acid in ethanol. It was then demonstrated by vapor phase chromatography that the aldehyde (XV) was 20% de-

(13) We are indebted to Dr. E. J. Modest, Children's Cancer Research Foundation, Boston, Mass., for referring us to the cyanoguanidine fusion method described in U. S. Patent No. 2,517,824.

(14) M. Sletzinger, D. Reinhold, J. Grier, M. Beachem, and M. Tishler, *J. Am. Chem. Soc.*, 77, 6365 (1955).

acetylated by refluxing it in absolute ethanol for one hour.

The acid (XVI) was treated with trifluoroacetic anhydride at room temperature to give the completely blocked crystalline acid XVII in a 45% yield. The conversion of XVII to its acid chloride and subsequent condensation with diethyl L-glutamate, however, failed to give a material that could be hydrolyzed to the desired aminopterin analog IIa.

Direct reductive alkylation of *p*-aminobenzoyl-L-glutamic acid with the blocked aldehyde XV proved to be a more fruitful approach to the desired compound IIa. Initial attempts to carry out the alkylation in 2-methoxyethanol at 35° with platinum oxide catalysis gave a product which had apparently suffered considerable deacetylation, as shown by elemental analysis. When the reaction was repeated in glacial acetic acid, the stoichiometric amount of hydrogen was absorbed in three to four hours, affording the intermediate XVIII as a clear gum which could be occasionally isolated as a partially crystalline solid. When the intermediate XVIII was hydrolyzed by mild alkali followed by acidification with hydrochloric acid, the desired 5,8-dideaza-5,6,7,8-tetrahydroaminopterin (IIa) was obtained as a crystalline precipitate.

Initially, a crystalline, chromatographically homogeneous analytical sample was obtained from a hot water extract of the crude hydrolysis product. In later runs it was found that careful acidification of the alkaline hydrolysate to *p*H 4.8 with hydrochloric acid yielded a product of suitable purity, but overacidification led to considerable contamination by the hydrochloride salt. Material which showed varying degrees of hydration could be obtained according to the method of isolation, as is discussed more fully in the Experimental section. This ability to form several hydrated species was also shown by 5,8-dideazatetrahydrofolic acid (IIb).[8] Some preliminary data on the behavior of 5,8 - dideaza - 5,6,7,8 - tetrahydroaminopterin (IIa) and the previously reported 5,8-dideazatetrahydrofolic acid (IIb)[4] in bacterial systems have been obtained. The compounds were tested for their antifolic acid activity by Dr. R. L. Kisliuk[15] in the *P. cerevisiae* (ATCC 8081) and *S. faecalis* (ATCC 8043) bacterial systems. In the former system the tetrahydrofolic analog IIb is less than half as inhibitory as aminopterin (I) whereas the tetrahydroaminopterin analog IIa possesses about eight times the activity of aminopterin. In *S. faecalis*, compound IIb has only 1/30 the activity of aminopterin, while IIa is about six times as active as aminopterin.

It would appear from the above results that the presence of the 4-amino group may be of much greater importance in obtaining strong folic acid inhibition than alterations induced about the N-5–N-10 portion of the folic acid molecule, designed to directly interfere with the one-carbon transfer sites.[16]

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## EXPERIMENTAL[17]

*2-Hydroxymethylene-4-dimethoxymethylcyclohexanone* (IV). To an ice-cold mixture of 4.2 g. (78 mmoles) of sodium methoxide in 90 ml. of ether was added 8.0 g. (46 mmoles) of the keto acetal III[8] followed by the slow addition of 5.92 g. (8 mmoles) of ethyl formate. The mixture was stirred at 0–5° for 15 min., heated at reflux overnight, then was poured into 150 ml. of ice water. The separated ether layer was extracted with two 25-ml. portions of ice-cold 5% sodium hydroxide. The alkaline extract was washed with 75 ml. of ether and then was adjusted to *p*H 6–7 with glacial acetic acid. The turbid solution was saturated with sodium chloride and was extracted with one 100-ml. and two 75-ml. portions of ether. The combined solutions were dried over magnesium sulfate, filtered, and evaporated *in vacuo* to give 5.80 g. (62%) of an oil, which was distilled at reduced pressure; a main fraction, 3.34 g. (36%), being collected at 90–95°/0.25 mm. The crude and redistilled product possessed the same infrared spectrum; $\lambda_{max(\mu)}^{film}$ 6.05–6.30 (chelated carbonyl and hydroxymethylene), 8.75–8.90 and 9.25–9.45 (acetal (C—O—C).

*5-Dimethoxymethyl-4,5,6,7-tetrahydrobenzisoxazole* (V). To a solution of 3.30 g. (16.5 mmoles) of the hydroxymethylene ketone (IV) in 13 ml. of pyridine was added 1.2 g. (17 mmoles) of hydroxylamine hydrochloride in 13 ml. of methanol. The solution was refluxed for 1.5 hr. and evaporated nearly to dryness *in vacuo*. The residue was partitioned between 80 ml. of water and 30 ml. of dichloromethane, and the separated aqueous layer was extracted with three 15-ml. portions of dichloromethane. The combined dichloromethane solutions were dried over magnesium sulfate, filtered, and evaporated to dryness *in vacuo*. Small portions of toluene were added and evaporated in succession until all pyridine was removed, to give 2.4 g. (74%) of a sirup, which was used directly in the next step $\lambda_{max(\mu)}^{film}$ 6.10 and 6.20 (C=C, C=N), 8.85 and 9.40 (acetal C—O—C).

*2-Cyano-4-dimethoxymethylcyclohexanone* (VI). To a solution of 2.2 g. (11 mmoles) of the isoxazole (V) in 8 ml. of benzene was added a solution of 1.32 g. (24 mmoles) of sodium methoxide in 11 ml. of methanol, and the resulting solution was allowed to stand at room temperature for 2 hr. The solution was poured into 100 ml. of ice water and extracted with 20 ml. of ether. The ether layer was separated and extracted with 20 ml. of ice-cold 5% sodium hydroxide. All aqueous layers were combined, washed with 20 ml. of ether, and adjusted to *p*H 7–8 with glacial acetic acid. The turbid solution was saturated with sodium chloride and extracted with four 20-ml. portions of dichloromethane. The combined dichloromethane extracts were dried over magnesium sulfate, filtered, and evaporated to dryness *in vacuo*, to give 1.44 g. (66%) of an oil, identified as VI by its

(16) For a review of folic acid and one-carbon metabolism, see F. M. Huennekens and M. J. Osborn in *Advances in Enzymology*, Vol. 21, Interscience Publishers, Inc., New York, 1959, p. 369.

(17) Boiling 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. The solvent systems used were: A, *n*-butyl alcohol–acetic acid–water (4:1:5); B, benzene–methanol–water (2:6:1); C, dimethylformamide–water (6:4); D, *n*-butyl alcohol–acetic acid–water (5:2:3).

n rared spectrum; $\lambda_{max(\mu)}^{film}$ 3.00 (enolic OH), 4.45 and 4.55 (≡N), 5.77 (C=O), 5.97 (C=C), 8.85 and 9.40 (acetal C—O—C).

*2-Cyanocyclohexanone* (X). This model sequence was carried out in the same manner as for the synthesis of VI. Cyclohexanone was condensed with ethyl formate to give 2-hydroxymethylenecyclohexanone (VIII)[18] in a 64% yield, b.p. 80–84°/14 mm., and this was converted to 4,5,6,7-tetrahydrobenzisoxazole (IX) in an 89% yield as a crude sirup. The crude isoxazole was treated with sodium methoxide to give a 60% yield of 2-cyanocyclohexanone (X), identified by its infrared spectrum[10]; the over-all yield from cyclohexanone was 34%. 2-Cyanocyclohexanone (X) has been prepared by Meyer[19] by another method.

*1-Cyano-2-isobutoxy-1-cyclohexene* (XIa). This compound was prepared in a 73% yield from 2-cyanocyclohexanone (X) using isobutyl alcohol and *p*-toluenesulfonic acid in refluxing toluene with water separation, according to the procedure of Chase and Walker.[12] The liquid product had b.p. 88–93°/0.3 mm. (lit.[12] b.p. 89–90°/0.4 mm.); $\lambda_{max(\mu)}^{film}$ 4.45 (conjugated C≡N), 6.10 (C=C), 9.90 (C—O—C).

*2,4-Diamino-5,6,7,8-tetrahydroquinazoline* (XII). To a solution of 5.60 g. (0.104 mole) of sodium methoxide in 50 ml. of methanol was added 5.1 g. (54 mmoles) of guanidine hydrochloride and 9.1 g. (51 mmoles) of the isobutyl enol ether (XIa). The solution was heated at 150° for 15 hr. in a stirred autoclave, cooled, and evaporated to dryness *in vacuo*, and the residue was partitioned between 50 ml. of water and 50 ml. of ether. The solid that separated was collected by filtration, washed with water and ether, and dried to give 4.0 g. (48%), m.p. 240–242°; $\lambda_{max(\mu)}^{KBr}$ 2.90, 3.00, 3.15 ($NH_2$), 6.0–6.15 ($NH_2$); $\lambda_{max(m\mu)}^{EtOH}$ 285 (ε 6575); $\lambda_{max(m\mu)}^{0.1N\ HCl}$ 275 (ε 6850).

*1-Cyano-2-ethoxy-4-dimethoxymethyl-1-cyclohexene* (XIII). To 5.0 g. (25.4 mmoles) of the cyano ketone (VI) was added 18 ml. of triethyl orthopropionate, and the solution was heated to boiling. The ethanol and ethyl propionate were distilled as the reaction proceeded, 4.8 ml. of distillate being collected at 80–100° (theory was 4.4 ml.). The residual solution was evaporated to dryness *in vacuo* (70°/5 mm.), giving 5.8 g. (100%) of a sirup identified as XIII by its infrared spectrum; $\lambda_{max(\mu)}^{film}$ 4.52 (conjugated C≡N), 6.10 (C=C), 8.85 and 9.3–9.5 (acetal C—O—C).

*2,4-Diamino-5,6,7,8-tetrahydro-6-quinazolinecarboxaldehyde dimethyl acetal* (XIVa). *A. From the enol ether nitrile* (XIII). To a solution of 2.59 g. (48 mmoles) of sodium methoxide in 25 ml. of methanol was added 2.27 g. (24 mmoles) of guanidine hydrochloride and 4.72 g. (21 mmoles) of the enol ether nitrile (XIII). The mixture was heated at 150° for 15 hr. in a steel bomb and was evaporated to dryness *in vacuo*. The residue was dissolved in 25 ml. of water and the solution extracted with 20 ml. of ether, then with with three 40-ml. portions of chloroform. The combined chloroform extracts were dried over potassium carbonate, filtered, and evaporated to dryness *in vacuo* to give 1.6 g. of a glassy residue. The residue was dissolved in 25 ml. of hot benzene–chloroform (4:1) and allowed to stand for an hour to give 560 mg. (11%) of tan crystals. Two recrystallizations from ethanol gave an analytical sample, m.p. 202.5–204.0°; $\lambda_{max(\mu)}^{KBr}$ 2.85, 2.95, 3.10 ($NH_2$), 8.6, 9.1, 9.5 (acetal C—O—C); $\lambda_{max(m\mu)}^{EtOH}$ 283 (ε 7325); $\lambda_{max(\mu)}^{0.1N\ HCl}$ 273 (ε 7375). The product moved as a single spot in solvent systems A and B, with $R_{Ad}$ 1.08 and 1.13, respectively.

*Anal.* Calcd. for $C_{11}H_{18}N_4O_2$: C, 55.4; H, 7.61; N, 23.5. Found: C, 55.5; H, 7.49; N, 23.1.

*B. From the keto acetal* (III). To a 500-ml. resin flask was added 125.0 g. (0.727 mole) of the ketoacetal (III) and 70.0 g. (0.830 mole) of cyanoguanidine. The mixture was heated at 180–185° (internal temperature) for 2 hr. with stirring; water was evolved during the process. The viscous mass was cooled to 160° and then 300 ml. of boiling water was added. The resulting solution was allowed to stand for 10 min. and the aqueous supernatant was decanted from some insoluble resin. The residual resin was extracted twice more with boiling water in the same manner. The combined aqueous extracts (900 ml.) were extracted with five 300-ml. portions of warm chloroform, and the combined choroform extracts were dried over magnesium sulfate, filtered, and evaporated to dryness *in vacuo*, giving 65.0 g. of product. This latter residue was triturated with 150 ml. of hot chloroform and chilled overnight to give 19.5 g. of yellowish crystals, m.p. 195–200°. The original water-insoluble resin was extracted with two 300-ml. portions of boiling chloroform to give another 9.9 g. of crystals, m.p. 195–200°. The total yield was 29.4 g. (17%) of crystalline XIVa, equal in all respects to material prepared by method *A*.

*2,4-Diacetamido-5,6,7,8-tetrahydro-6-quinazolinecarboxaldehyde dimethyl acetal* (XIVb). To 10 ml. of acetic anhydride was added 2.0 g. of the diamino acetal (XIVa), and the mixture was stirred at 100° for 20 min., complete solution resulting after 10 min. The solution was evaporated to dryness *in vacuo*, dissolved in 4 ml. of hot benzene, and diluted with 10 ml. of ether. The solution was chilled overnight, causing the deposition of white crystals, which were collected, washed with 5 ml. of ether, and dried to give 1.78 g. of product. A second crop of 0.18 g. was obtained by evaporation of the mother liquors to dryness and recrystallization of the residue from 3.5 ml. of benzene–ether (2:5); total yield, 1.96 g. (73%). An analytical sample was obtained by a similar recrystallization of material from another run; it had m.p. 140° (partially melts and resolidifies), 156.5–157.5°; $\lambda_{max(\mu)}^{Nujol}$ 3.10, 3.15 (NH), 5.80, 5.95 (amide C=O), 8.80, 9.10, 9.45 (acetal C—O—C); $\lambda_{max(m\mu)}^{EtOH}$ 228 (ε 24,400), 282 (ε 7775). The compound moved as a single spot in solvent system B, with $R_{Ad}$ 1.50.

*Anal.* Calcd. for $C_{15}H_{22}N_4O_4$: C, 55.9; H, 6.88; N, 17.4. Found: C, 56.3; H, 6.86; N, 17.1.

*2,4-Diacetamido-5,6,7,8-tetrahydro-6-quinazolinecarboxaldehyde* (XV). A solution of 13.0 g. of the diacetamido acetal (XIVb) in 40 ml. of 90% formic acid was allowed to stand for 4.25 hr. at room temperature, then was evaporated to dryness at room temperature and 1 mm. The sirupy residue was dissolved in 40 ml. of ethyl acetate and diluted with an excess of ether to give a gummy precipitate. The supernatant was decanted from the gum and chilled overnight, to give 1.5 g. of white crystals. The gum was redissolved in a minimum amount of ethyl acetate, followed by continued addition of ethyl acetate until a brown gum separated. The ethyl acetate supernatant was decanted, diluted with an excess of ether, and chilled overnight to give 5.6 g. of white crystals. The brown gum was dissolved in 10 ml. of hot acetic anhydride, diluted with 10 ml. of ether, and chilled overnight to give another 1.2 g. of tan crystals; all three crops, total 8.3 g. (75%), were shown to be pure XV by paper chromatographic and infrared comparison. Recrystallization of material from another run from acetic anhydride gave an analytical sample, m.p. 185.0–186.5°; $\lambda_{max(\mu)}^{Nujol}$ 3.02 (NH), 5.78 (aldehyde C=O), 5.95 (amide C=O); $\lambda_{max(m\mu)}^{EtOH}$ 228 (ε 22,400), 282 (ε 7500). The material moved as a single spot in solvent system B, with $R_{Ad}$ 1.44.

*Anal.* Calcd. for $C_{13}H_{16}N_4O_3$: C, 56.5; H, 5.84; N, 20.3. Found: C, 56.3; H, 5.98; N, 19.5.

An alternate crystalline form, m.p. 161–164°, which differed from the higher melting material in infrared spectrum, but was chromatographically equivalent, was occasionally obtained; $\lambda_{max(\mu)}^{Nujol}$ 3.15, 3.20 (NH), 3.65 (CH of CHO), 5.78 (aldehyde C=O), 5.90, 5.95 (amide C=O).

*p*-[(*2,4-Diacetamido-5,6,7,8-tetrahydro-6-quinazolinyl*)*methylamino*]*benzoic acid* (XVI). To 25 ml. of 2-methoxyethanol was added 280 mg. of platinum oxide, 2.1 g. (7.6 mmoles) of the diacetamidoaldehyde (XV), and 1.05 g. (7.6 mmoles) of *p*-aminobenzoic acid. The mixture was treated with one atmosphere of hydrogen at 35° and absorbed 70% of the theoretical amount of hydrogen after about 18 hr. The product, which had crystallized from the solution,

(18) P. Plattner, P. Treadwell, and C. Scholz, *Helv. Chim. Acta*, **28**, 771 (1945).

(19) R. E. Meyer, *Helv. Chim. Acta*, **16**, 1291 (1933).

was collected by filtration. It was freed of the catalyst by dissolving it in 20 ml. of boiling dimethylformamide and filtering the solution. The dimethylformamide filtrate was diluted with 20 ml. of water and chilled overnight to give 1.00 g. (33%) of crystalline material. An analytical sample, m.p. 265.5–267.0°, was obtained by recrystallization from dimethylformamide–water of material from another run; $\lambda_{max(\mu)}^{Nujol}$ 2.95, 3.10, 3.20 (NH), 5.9–6.0 (C=O of amide and carboxyl), 11.85 (*p*-disubstituted phenyl); $\lambda_{max(m\mu)}^{DMF}$ 294 ($\epsilon$ 26,000), shoulder 305 ($\epsilon$ 24,800); after hydrolysis of the acetyl groups by heating 30 min. at 100° in 0.1*N* sodium hydroxide, $\lambda_{max(m\mu)}^{pH\ 13}$ 284 ($\epsilon$ 22,600) and $\lambda_{max(m\mu)}^{pH\ 1}$ 224 ($\epsilon$ 25,500), 281 ($\epsilon$ 10,200 308 ($\epsilon$ 11,300). The compound moved as a single spot at $R_{Ad}$ 1.41 in solvent system C.

*Anal.* Calcd. for $C_{20}H_{23}N_5O_4$: C, 60.4; H, 5.83; N, 17.62. Found: C, 60.1; H, 6.09; N, 18.30, 18.36.

*p-{N-[(2,4-Diacetamido-5,6,7,8-tetrahydro-6-quinazolinyl)-methyl]-2,2,2-trifluoroacetamido}benzoic acid* (XVII). To 10 ml. of trifluoroacetic anhydride cooled to 0–5° was added 330 mg. of XVI, and the mixture was stirred to effect complete solution. The solution darkened, so a small amount of Norit was added and the stirring was continued for 2.5 hr. The carbon was removed by filtration, and the nearly colorless filtrate was evaporated to dryness *in vacuo* at room temperature. The residue was dissolved in 3 ml. of acetone, and a little ether was added to precipitate a dark gum. This was separated by decantation followed by addition of excess ether to the clear supernatant to give a white precipitate, which was collected, washed with ether, and dried, to give 154 mg. of product. A second crop of 31 mg. crystallized from the mother liquors upon standing, to give a total of 185 mg. (45%). The second crop served as the analytical sample and had m.p. 240–242°; $\lambda_{max(\mu)}^{Nujol}$ 3.15 (NH), 5.85–5.95 (C=O of carboxyl, NAc, and $NCOCF_3$), 8.30 and 8.60 ($CF_3$), 11.70 (*p*-disubstituted phenyl). The material traveled as a single spot in solvent system B, $R_{Ad}$ 1.20.

*Anal.* Calcd. for $C_{22}H_{22}F_3N_5O_5$: C, 53.6; H, 4.50; N, 14.2. Found: C, 53.6; H, 4.57; N, 13.9, 14.2.

*5,8-Dideaza-5,6,7,8-tetrahydroaminopterin* (IIa). To 30 ml. of glacial acetic acid was added 3.00 g. (10.9 mmoles) of the diacetamidoaldehyde (XV), 2.90 g. (10.9 mmoles) of *p*-aminobenzoyl-L-glutamic acid, and 600 mg. of platinum oxide. The mixture was treated with one atmosphere of hydrogen at room temperature and absorbed the theoretical amount of hydrogen in 3.5 hr. The catalyst was removed by filtration and the dark filtrate was concentrated to dryness at 50–60°/1 mm. The sirupy residue was extracted with two 100-ml. portions of hot (90–100°) water, followed by decantation each time from the dark, insoluble material. The combined aqueous extracts were chilled overnight, and the aqueous supernatant was decanted from the clear gum that separated. The gum was washed with water and dried *in vacuo* to give 2.65 g. (46%) of the crude diacetamido intermediate (XVIII); $\lambda_{max(\mu)}^{Nujol}$ 3.00, 3.20 (NH), 5.85, 6.02 (C=O of amide and carboxyl), 6.60 (amide), 11.95 (*p*-disubstituted phenyl).

The 2.65 g. of XVIII (see above) was dissolved in 75 ml. of 0.4*N* sodium hydroxide, and the solution was heated 30 min. on the steam bath. The hot solution was carefully acidified with 6*N* hydrochloric acid until a dark, gummy precipitate separated and adhered to the flask. The supernatant (*p*H 5–6) was rapidly decanted and upon cooling gave a light, crystalline precipitate which was collected, washed with water, and dried to give 0.64 g. of product (precipitate A). The filtrate was further acidified to *p*H 3–4 to give additional, white, crystalline precipitate, which was collected, washed, and dried to afford 0.92 g. of material shown to contain some of the hydrochloride salt by paper chromatography. Reprecipitation of the second crop by dissolving it in base and adjusting the *p*H to 4–5 gave 0.70 g. of hydrochloride-free material (precipitate B); total yield of IIa, 1.34 g. (28% from the starting aldehyde). Material obtained from another run was extracted with boiling water and the extract cooled, to give a small amount of a white, crystalline solid, m.p. 240–260° (dec. with effervescence) (precipitate C). All of the analytical samples moved as a single spot in solvent system D, with $R_{Ad}$ 1.16; the hydrochloride moved at $R_{Ad}$ 0.97 in the same system. All samples likewise exhibited the same general spectral properties; $\lambda_{max(\mu)}^{Nujol}$ 3.07, 3.20 (NH), 5.95–6.10 (C=O of amide and carboxyl), 11.95 (*p*-disubstituted phenyl); $\lambda_{max(m\mu)}^{pH\ 1}$ 220 ($\epsilon$ 30,300), 267 ($\epsilon$ 10,800), 295 (shoulder, $\epsilon$ 7500); $\lambda_{max(m\mu)}^{pH\ 13}$ 278 ($\epsilon$ 24,200).

*Anal.* (Precipitate A) Calcd. for $C_{21}H_{26}N_6O_5 \cdot H_2O$: C, 54.77; H, 6.13; N, 18.25. Found: C, 54.92; H, 6.47; N, 18.56.

(Precipitate B) Calcd. for $C_{21}H_{26}N_6O_5 \cdot 1/2\ H_2O$: C, 55.8; H, 6.02; N, 18.6. Found: C, 55.7; H, 5.96; N, 18.2.

(Precipitate C) Calcd. for $C_{21}H_{26}N_6O_5 \cdot 2\ H_2O$: C, 52.71; H, 6.32; N, 17.56. Found: C, 52.98; H, 6.46; N, 17.88.

In a large-scale run, a 48% yield of IIa from the blocked aldehyde (XV) was obtained, and by precipitation at *p*H 4.8 the product was obtained free of its hydrochloride salt.

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