The Synthesis of "Stretched-Out" Analogs of Lumazine, 6,7-Dimethyllumazine and 2-Amino-5,6,7,8-tetrahydro-6,7-dimethyl-4-pteridinone (1,2)

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The synthesis of pyrazino[2,3-*g*]quinazolin-2,4-(1*H*,3*H*)dione (**4**) and its 7,8-dimethyl derivative (**5**), as linear benzo-separated lumazines, is reported. Also described is the preparation of 2-amino-6,7,8,9-tetrahydro-7,8-dimethylpyrazino[2,3-*g*]quinazolin-4-one (**6**), as a linear benzo-separated analog of a synthetic cofactor for phenylalanine hydroxylase. All of the syntheses began with ethyl 2,4,5-triaminobenzoate (**8**) and proceeded through the appropriate derivatives of ethyl 6-aminoquinoxaline-7-carboxylate (**9**, **10**, and **11**) which were subsequently ring closed to **4**, **5**, and **6**.

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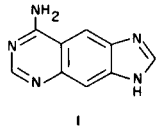
A very interesting approach to analyzing enzyme-substrate and cofactor relationships is being developed by Leonard and his co-workers (3). Their program involves the formal insertion of a benzene unit (actually four new carbon atoms) into the center of a number of naturally occuring purine derivatives to result in what they have designated as *linear*, *proximal*, and *distal* benzo-analogs (as represented by the adenine series **1-3**) (4). From these analog studies the Leonard group has demonstrated that such defined structural manipulations can, for example, (i) help establish the size and flexibility of enzyme binding sites, (ii) produce molecules with biologically useful fluorescence properties and (iii) enlighten the present understanding of the molecular interactions that occur when purine derivatives are required to participate in biological transformations (5-7).



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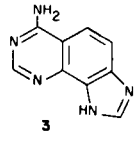

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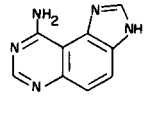

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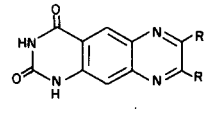
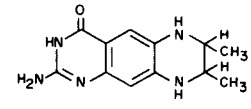
Prompted by these successes and the ubiquitous biological nature of the pteridine ring system (8), we became interested in the synthesis of similarly benzo-separated pteridines possessing functionality appropriate to various biologically important pteridines. Among the synthetic goals were pyrazino[2,3-*g*]quinazolin-2,4-(1*H*,3*H*)dione (**4**) and its 7,8-dimethyl derivative (**5**), as *linear*-benzo-separated lumazines (9,10) and 2-amino-6,7,8,9-tetrahydro-7,8-dimethylpyrazino[2,3-*g*]quinazolin-4-one (**6**), as a *linear*-benzo-separated analog of a synthetic cofactor (*i.e.*, **7**) for phenylalanine hydroxylase (12). To this end, the preparations of **4-6** are reported herein.



4, R = H
5, R = CH_3



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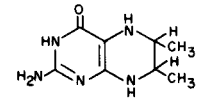

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Analogous to the synthetic objectives that confronted Leonard (13), three approaches can be envisioned to the benzo-separated pteridines: (i) fusion of a pyrimidine unit onto an appropriately substituted quinoxaline, (ii) construction of a pyrazine moiety onto a substituted quinazoline, or (iii) formation of both the pyrimidine and pyrazine rings from a tetrasubstituted benzene derivative. In view of the great deal of information available on quinazolines (14) the second method was initially attempted. However, many intractable problems were encountered when employing various quinazolin-2,4-(1*H*,3*H*)diones or 2-aminoquinazolin-4-ones as required by this method. Attention then turned to commencing with a tetrasubstituted benzene (method iii) from which quinoxalines disubstituted in their benzene portion (method i) could be derived for subsequent ring closure to the desired tricyclic analogs.

Thus, ethyl 2,4,5-triaminobenzoate hydrochloride (**8**) was prepared by a modification of a reported procedure (15,16) and reacted with glyoxal and butane-2,3-dione to give **9** and **10**, respectively. Reaction of **9** and **10** with urea then resulted in the desired **4** and **5**, respectively. On the other hand, catalytic hydrogenation of **10** yielded the tetrahydroquinoxaline derivative **11**, formally a tetrasubstituted benzene wherein the *o*-diamine is protected in the form of an incipient pyrazine moiety. Treatment of **11**

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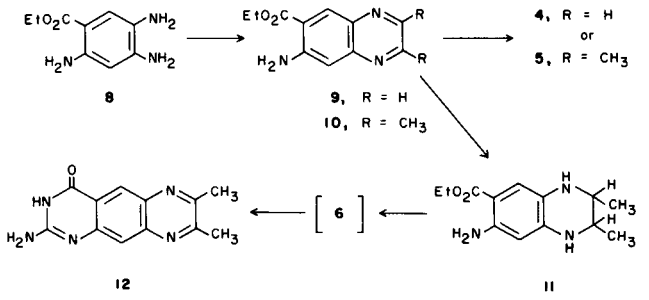
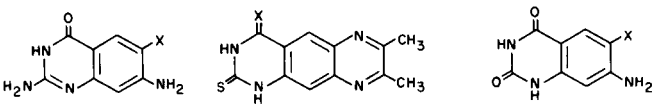
with freshly prepared chloroformamidine gave the analog **6** as an unstable material possessing a blue fluorescence. Oxidation of **6** with basic hydrogen peroxide produced the totally unsaturated tricyclic system **12**, a material with yellow-green fluorescence.



Compound **12** prepared by this route was compared (ir, tlc, fluorescence) with an authentic sample synthesized from 2,6,7-triaminoquinazolin-4-one (**13**) and butane-2,3-dione. It should be mentioned that **13** has been described by Leonard and Keyser (16) but only as an intermediate that was trapped by formic acid to result in their *linear*-benzoguanine. In our case it was not possible to conduct the catalytic hydrogenation of the precursor to **13** (*i.e.*, **14**) in the presence of butane-2,3-dione to achieve **12**. Consequently, **13** was isolated as its sulfate salt and, in a separate step, was condensed with butane-2,3-dione to produce **12**.

Attempts (i) at ring closing **8** to **13** and **10** to **12** with cyanamide, chloroformamidine, or guanidine, (ii) at ring closing **10** to **15** with thiourea, (iii) at reducing **12** to **6**, and (iv) at converting **5** into **16** with phosphorus pentasulfide



13, X = NH_2 **15**, X = O **17**, X = NO_2
14, X = NO_2 **16**, X = S **18**, X = NH_2

as alternative routes to **6** or its potential precursors failed. A reaction between **10** and chloroformamidine did occur to form a red paste, but nothing fruitful could be done with this material. Also, satisfactory reduction of **17** (15) to **18** for further reactions with glyoxal and butane-2,3-dione as alternative pathways to **4** and **5**, respectively, could not be achieved.

EXPERIMENTAL

All melting points were obtained on a Thomas-Hoover or a Mel-Temp melting point apparatus and are uncorrected. Infrared spectra were recorded on a Beckman AccuLab 3 spectrophotometer. The pmr spectra were determined at 60 MHz with a Varian EM-360 spectrometer and are reported in parts per million downfield from tetramethylsilane as an internal standard. The spin multiplicities are indicated by the symbols s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ.

Ethyl 2,4,5-Triaminobenzoate (**8**) as Its Hydrochloride.

A mixture of 1.0 g. (3.2 mmoles) of ethyl 2,4-diacetamido-5-nitrobenzoate (16) and 10 ml. of absolute ethanol was heated to 85° under nitrogen and mechanical stirring while dry hydrogen chloride gas was added. Such a process first dissolved the insoluble material and then resulted in precipitation of a new yellow product at which point the hydrogen chloride addition was continued for 20 minutes longer. This mixture was cooled to 0°, filtered at the aspirator under nitrogen, and the resulting solid washed twice with 20 ml. of cold diethyl ether. This solid was transferred to a Parr hydrogenation bottle to which 50 ml. of absolute ethanol and 0.1 g. of 5% palladium-on-charcoal was added. This mixture was shaken under 50 p.s.i. of hydrogen for 10 hours (or until the yellow color was discharged). The catalyst was removed by filtration and 5 ml. of hydrochloric acid was added to the filtrate. The resultant white hydrochloride was isolated by filtration, dried under nitrogen, and used directly in the preparation of **9**.

Ethyl 6-Aminoquinoxaline-7-carboxylate (**9**).

The hydrochloride of compound **8**, prepared as described above, was dissolved in 50 ml. of water to which 1 g. of sodium bisulfite and 3.2 mmoles (0.19 g.) of 40% aqueous glyoxal were added. This mixture was stirred at room temperature for 5 hours, neutralized with ammonium hydroxide solution, and, finally, extracted with chloroform (2 x 100 ml.). The extracts were combined, dried over anhydrous magnesium sulfate, and evaporated to dryness to give a yellow solid which was purified by recrystallization from petroleum ether (60-110°)-ethanol and then sublimed (140°/1 mm) to give 0.21 g. (0.96 mmole, 30%) of **9** as yellow needles, m.p. 141-142°; ir (potassium bromide): 3420 (NH_2), 1700 (C=O) cm^{-1}; pmr (deuteriochloroform): δ 1.45 (t, 3H, J = 3 Hz, CH_3), 4.40 (q, 2H, J = 3 Hz, CH_2), 5.70 (br s, 2H, NH_2), 7.10 (s, 1H, H-5), 8.51 (d, 1H, J = 1 Hz, H-2 or H-3), 8.60 (d, 1H, J = 1 Hz, H-2 or H-3), 8.65 (s, 1H, H-8).

Anal. Calcd. for $C_{11}H_{11}N_3O_2$: C, 60.82; H, 5.10; N, 19.34. Found: C, 60.86; H, 5.28; N, 19.36.

Ethyl 6-Amino-2,3-dimethylquinoxaline-7-carboxylate (**10**).

To the filtrate obtained from removing the palladium-on-charcoal catalyst in the preparation of **8** was added 50 ml. of water followed by 0.275 g. (3.2 mmoles) of butane-2,3-dione with stirring. The solution initially became bright red and upon further stirring a red solid precipitated. After 20 minutes of stirring, the mixture was neutralized with ammonium hydroxide, at which point the solid and the solution became bright yellow, and the mixture was refrigerated for 2 hours at 0°. Isolation of the yellow solid by filtration with subsequent drying and purification by recrystallization from petroleum ether (60-110°)-ethanol followed by vacuum sublimation (140°/1 mm) gave 0.28 g. (1.1 mmoles, 35.5%) of **10** as yellow needles, m.p. 198°; ir (potassium bromide): 3420 (NH_2) 1700 (C=O) cm^{-1}; pmr (deuteriochloroform): δ 1.38 (t, 3H, J = 3 Hz, ester CH_3), 2.60 (s, 6H, C-2 and C-3 CH_3), 4.31 (q, 2H, J = 4 Hz, CH_2), 5.84 (br s, 2H, NH_2), 6.95 (s, 1H, H-5), 8.55 (s, 1H, H-8).

Anal. Calcd. for $C_{13}H_{15}N_3O_2$: C, 63.66; H, 6.16; N, 17.13. Found: C, 63.55; H, 6.20; N, 17.10.

Pyrazino[2,3-*g*]quinazolin-2,4-(1*H*,3*H*)dione (**4**).

A finely ground mixture of 1.0 g. (4.6 mmoles) of **9** and 3.4 g. (56.6 mmoles) of urea was heated in an open flask to 198° by the use of an oil bath and under magnetic stirring. The solid mixture first melted, evolved a gas, and then resolidified. After 30 minutes at 198°, 20 ml. of water was added to the mixture and the mixture boiled. The insoluble tan solid was isolated by filtration, air dried, and recrystallized from aqueous dimethylsulfoxide using decolorizing charcoal to give 0.3 g. (1.4 mmoles, 30%) of **4** as a light tan, amorphous solid, m.p. > 400° dec.; ir (potassium bromide): 1720 (C=O) cm^{-1}; pmr (DMSO-d_6): δ 7.50 (s, 1H, H-10), 8.50 (s, 1H, H-5), 8.90 (d, 1H, J = 1 Hz, H-7 or H-8), 8.95 (d, 1H, J = 1 Hz, H-7 or H-8), 11.50 (br s, 2H, 2NH).

Anal. Calcd. for $C_{10}H_6N_4O_2$: C, 56.08; H, 2.82; N, 26.16. Found: C,

55.84; H, 2.93; N, 26.28.

7,8-Dimethylpyrazino[2,3-*g*]quinazolin-2,4-(1*H*,3*H*)dione (**5**).

In a procedure analogous to that for preparing **4**, 1.1 g. (4.5 mmoles) of **10** and 3.2 g. (53.3 mmoles) of urea were ground together and heated at 198° until gas no longer evolved and the resulting melt had resolidified (approximately 20 minutes). Following the same water work-up as used with **4**, the resulting solid was isolated by filtration, washed with 20 ml. of water and 50 ml. of chloroform, and recrystallized from aqueous dimethylsulfoxide using decolorizing charcoal to give 1 g. (4.1 mmoles, 92.1%) of **5** as a light tan, amorphous solid, m.p. 383-385° dec.; ir (potassium bromide): 1730 (C=O) cm^{-1}; pmr (DMSO-d_6): δ 2.60 (s, 6H, $2CH_3$), 7.32 (s, 1H, H-10), 8.19 (s, 1H, H-5), 11.28 (br s, 2H, 2NH).

Anal. Calcd. for $C_{12}H_{10}N_4O_2$: C, 59.50; H, 4.16; N, 23.13. Found: C, 59.32; H, 4.37; N, 23.16.

Ethyl 6-Amino-1,2,3,4-tetrahydro-2,3-dimethylquinoxaline-7-carboxylate (**11**).

A mixture of 1 g. (4 mmoles) of **10**, 0.77 g. of potassium bicarbonate, and 0.77 g. of 10% palladium-on-charcoal in 150 ml. of 95% ethanol was shaken under 45 p.s.i. of hydrogen for 30 hours. The catalyst was then removed by filtration, and the filtrate was evaporated to dryness to give a light brown oil. This oil was dissolved in 50 ml. of chloroform, the chloroform solution was dried over anhydrous magnesium sulfate, and the solution was evaporated to dryness. The oil which remained was triturated with petroleum ether (65-110°) to give a yellow solid which was isolated by filtration and recrystallized from petroleum ether (65-110°)-ethanol to give 0.59 g. (2.4 mmoles, 60%) of **11** as light yellow needles, m.p. 102-103°; ir (potassium bromide): 3410 (NH_2), 1720 (C=O) cm^{-1}; pmr (deuteriochloroform): δ 1.25 (m, 11H, 3 CH_3 and H-2 and H-3), 3.35 (q, 2H, J = 4 Hz, CH_2), 4.30 (m, 4H, NH_2 and 2 NH), 5.57 (s, 1H, H-5), 7.0 (s, 1H, H-8).

Anal. Calcd. for $C_{13}H_{19}N_3O_2$: C, 62.63; H, 7.68; N, 16.85. Found: C, 62.61; H, 7.88; N, 17.03.

2-Amino-6,7,8,9-tetrahydro-7,8-dimethylpyrazino[2,3-*g*]quinazolin-4-one (**6**).

A mixture of 0.5 g. (2 mmoles) of **11**, 0.8 g. (6.96 mmoles) of chloroformamidine hydrochloride and 0.5 g. of dimethylsulfone was heated in an oil bath, under nitrogen, at 180° for 20 minutes. A light yellow solid formed as hydrogen chloride gas was evolved. The solid was subsequently washed with diethyl ether (3 x 20 ml.) and then chloroform (3 x 20 ml.) to remove unreacted **11**. The desired material was then isolated by evaporation of the chloroform under a stream of nitrogen to give 0.17 g. (0.72 mmoles, 36%) of **6** as a hygroscopic and unstable compound which was used as obtained in the next reaction; ir (potassium bromide): 1710 (C=O) cm^{-1}.

2-Amino-7,8-dimethylpyrazino[2,3-*g*]quinazolin-4-one (**12**).

Refluxing a mixture of 0.17 g. (0.72 mmoles) of **6**, 5 ml. of 30% hydrogen peroxide solution, and 10 ml. of 2*N* sodium hydroxide solution for 2 hours gave 0.03 g. (0.14 mmoles, 19.4%) of a yellow solid following cooling, careful neutralization (20% acetic acid solution), and filtration of the refluxed mixture. This product was identical (ir; tlc using chloroform-methanol, 4:1, on silica gel; yellow-green fluorescence) to the **12** obtained in the following way.

To a boiling mixture of 2.18 g. (9.25 mmoles) of **14** (whose preparation is described below) in 100 ml. of water was added, portionwise, 9.22 g. (52.9 mmoles) of sodium dithionite. Each sodium dithionite addition was performed after the effervesence of the previous addition had subsided. This mixture was then refluxed for 15 minutes after the addition was complete, treated with charcoal and vacuum filtered into a mixture of 30 ml. of cold sulfuric acid and 100 ml. of ice. The resulting mixture was refrigerated for 6 hours; the white solid which formed was isolated by filtration and suspended in 40 ml. of water. To this suspension was added 2.4 g. of barium chloride and this new mixture was then heated, with stirring, on a steam bath for 30 minutes. The resulting solution was vacuum filtered (to remove the precipitated barium sulfate) into a mixture of 50 ml. of glacial acetic acid, 50 ml. of water, and 0.77 g. (9.25 mmoles) of butane-2,3-dione. This mixture was stirred on a steam bath for 30 minutes, cooled and carefully brought to neutrality with solid sodium bicarbonate. At this point, a solid formed which was isolated by filtration, washed with 50 ml. of water, 50 ml. of 95% ethanol, and, finally, 50 ml. of diethyl ether. The dried product was recrystallized from dimethylsulfoxide with decolorizing charcoal to give 1 g. (4.14 mmoles, 44.8%) of **12** as an off-yellow, amorphous powder, decomposition point 380°; ir (potassium bromide): 1690 (C=O) cm^{-1}; pmr (DMSO-d_6): δ 2.57 (s, 6*H*, 2 CH_3), 6.55 (br s, 2H, NH_2), 7.50 (s, 1H, H-10), 8.40 (s, 1H, H-5).

Anal. Calcd. for $C_{12}H_{11}N_5O \cdot 1/4H_2O$: C, 58.64; H, 4.71; N, 28.49. Found: C, 58.50; H, 4.56; N, 28.68.

2,7-Diamino-6-nitroquinazolin-4-one (**14**).

Method A.

A mixture of 1 g. (4.39 mmoles) of methyl 2-amino-4-chloro-5-nitrobenzoate (17), 1.17 g. (6.56 mmoles) of guanidine carbonate, and 2 g. of dimethylsulfone was ground to a fine powder. This mixture was heated in an open flask at 200° for 3 hours. Upon cooling the resulting solid mass was diluted with water and neutralized with glacial acetic acid to give **14** (0.92 g., 4.16 mmoles, 95%) which was isolated by filtration and washed, sequentially, with water, chloroform, ethanol, and ether and used directly in the preparation of **12** described above. The **14** obtained by this method was identical in all respects with that prepared in reference 16.

Method B.

Another approach to **14** that is a refinement of a reported (16) procedure involved placing 18 g. (58 mmoles) of ethyl 2,4-diacetamido-5-nitrobenzoate (16) into 75 ml. of absolute ethanol and mechanically stirring this mixture under nitrogen at 80° while anhydrous hydrogen chloride was added. The diacetamide first dissolved and shortly thereafter a yellow solid precipitated. The hydrogen chloride was added to this mixture for an additional 20 minutes. The mixture was then cooled to 0° and the solid isolated by filtration, washed with cold anhydrous diethyl ether, and dried under a stream of nitrogen to give ethyl 2,4-diamino-5-nitrobenzoate hydrochloride (15,16) in quantitative yield. This hydrochloride was immediately ground with 36 g. of dimethylsulfone and 6.89 g. (58 mmoles) of chloroformamidine hydrochloride and placed in an open flask. Following heating the mixture at 175° for 1 hour under nitrogen and mechanical stirring, it was cooled to approximately 70°, 100 ml. of water was then added and the solution carefully neutralized with solid sodium bicarbonate. The neutralized mixture was refrigerated for 1 hour and the resultant solid isolated by filtration, washed with methanol (2 x 100 ml.) and then diethyl ether (2 x 100 ml.) to give 10.9 g. (49 mmoles, 85%) of **14**, identical to that obtained by Method A (16).

Acknowledgement.

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REFERENCES AND NOTES

(1) A major portion of this investigation was sponsored by the U.S. Army Medical Research and Development Command, Contract Number DAMD17-78-C-8002.

(2) Preliminary accounts of this research were presented in part at (i) the 7th International Congress of Heterocyclic Chemistry, Tampa, FL, August 12-17, 1979, M1515B; (ii) the 179th National Meeting of the American Chemical Society, Houston, TX, March 23-28, 1980, ORGN 13; and (iii) the Second Chemical Congress of the North American Continent, Las Vegas, NE, August 24-29, 1980, ORGN 176.

(3a) N. J. Leonard, M. A. Sprecker and A. G. Morrice, *J. Am. Chem. Soc.*, **98**, 3987 (1976); (b) D. I. C. Scopes, J. R. Barrio and N. J. Leonard, *Science*, **195**, 296 (1977); (c) N. J. Leonard, *Heterocycles*, **12**, 129 (1979).

(4) See N. J. Leonard, A. G. Morrice and M. A. Sprecker, *J. Org. Chem.*, **40**, 356 (1975) for an explanation of the use of *linear, proximal, distal,* and benzo terms as prefixes in defining a particular analog.

(5) N. J. Leonard, D. I. C. Scopes, P. VanDerLijn and J. R. Barrio, *Biochemistry*, **17**, 3677 (1978).

(6) N. J. Leonard and G. E. Keyser, *Proc. Natl. Acad. Sci. USA*, **76**, 4262 (1979).

(7) P. VanDerLijn, J. R. Barrio and N. J. Leonard, *ibid.*, **75**, 4204 (1978); J. R. Barrio, F.-T. Liu, G. E. Keyser, P. VanDerLijn and N. J. Leonard, *J. Am. Chem. Soc.*, **101**, 1564 (1979).

(8) R. L. Blakley, "The Biochemistry of Folic Acid and Related Pteridines", North-Holland Publishing Co., Amsterdam, 1969.

(9) For leading references see (i) pages 133-135 of reference 8, (ii) G. Harzer and S. Ghisla in "Chemistry and Biology of Pteridines", R. L. Kisliuk and G. M. Brown, Eds., Elsvier/North Holland, New York, N.Y., 1979, pp. 37-42, and (iii) P. X. Iten, H. Märki-Danzig and C. H. Eugester, *ibid.*, pp. 105-109.

(10) Compound **5** can also be viewed as an "inside-out" lumichrome (11).

(11) A. J. Birch and C. J. Moye, *J. Chem. Soc.*, 412 (1957).

(12) J.-P. Hénichart, J.-L. Bernier, J.-L. Dhondt, M. Dautrevaux and G. Biserte, *J. Heterocyclic Chem.*, **16**, 1489 (1979).

(13) N. J. Leonard, A. G. Morrice and M. A. Sprecker, *J. Org. Chem.*, **40**, 356 (1979).

(14) For example, W. L. F. Armarego in "Fused Pyrimidines", Part I, D. J. Brown, Ed., Interscience, New York, N.Y., 1967.

(15) G. E. Keyser and N. J. Leonard, *J. Org. Chem.*, **44**, 2989 (1979).

(16) G. E. Keyser and N. J. Leonard, *ibid.*, **41**, 3529 (1976).

(17) S. W. Schneller and W. J. Christ, unpublished results.