Unequivocal Syntheses of 6-Methyl-
and 6-Phenylisoxanthopterin

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Although 6-methyl- (1) and 6-phenylisoxanthopterin (2) have previously been synthesized, the requirement of high purity necessary for immunological testing has necessitated our development of the first reported synthesis of these compounds by unequivocal methods. In the process of so doing four new pyrazines, ethyl 3-amino-5-chloro-6-methyl-2-pyrazinecarboxylate (11), N,N-dimethyl-N'-(6-chloro-3-cyano-5-phenylpyrazin-2-yl)methanimidamide (16), 2-amino-3-ethoxycarbonyl-5-phenylpyrazine 1-oxide (19), and ethyl 3-amino-5-chloro-6-phenyl-2-pyrazinecarboxylate (20) were synthesized. Four new pteridines, 7-methoxy-6-methyl-2,4-pteridinediamine (7), 7-methoxy-6-phenyl-2,4-pteridinediamine (17), 2-amino-7-ethoxy-6-methyl-4(3H)-pteridinone (12), and 2-amino-7-ethoxy-6-phenyl-4(3H)-pteridinone (21) have also been synthesized enroute to these isoxanthopterins.

Introduction.

Numerous investigations have shown pteridines to be involved in the immune response [2-8]. One investigator has postulated that certain pteridines may act as lymphokines, or regulators, in the immune response [9]. In the search for potential immunomodulators, we desired to synthesize 6-methylisoxanthopterin (1) and 6-phenylisoxanthopterin (2).
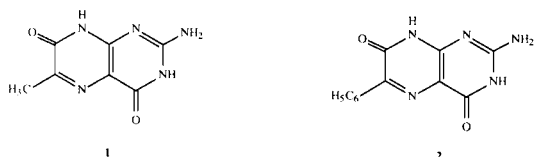
Previous syntheses of 1 and 2 are reported in the literature, but all were performed by Isay methods [10-14]. Since these compounds were destined for biological testing, we desired a synthetic approach which would assure that 1 and 2 would be synthesized in a high level of purity. An Isay synthesis involves a potentially ambiguous condensation between a 4,5-diaminopyrimidine and a dicarbonyl moiety which may lead to mixtures. Occasionally these mixtures have even been reported to be inseparable [15]. Additionally, previous syntheses were done in a period of time when supporting analytical data, now routinely available and which could provide adequate assurances of purity, were not available.

Failing to find any unequivocal syntheses for either 6-methyl- or 6-phenylisoxanthopterin, we decided to design an approach to these compounds modeled after Taylor's unequivocal pterin synthesis [15-17]. Taylor's synthesis forms the pyrazine ring first by a method similar to Jones' synthesis of pyrazines [18]. Taylor then unambig-

uously ring closes on the pyrazine with guanidine to form the pteridine ring. In this publication we present the first reported unequivocal syntheses of 6-methylisoxanthopterin (1) and 6-phenylisoxanthopterin (2).

Results and Discussion.

Following the procedure reported by Taylor et al. [17], 2-amino-3-cyano-5-methylpyrazine 1-oxide (5) (Scheme 1) was formed by a condensation of 2-oxopropanal 1-oxime (3) and aminomalononitrile p-toluenesulfonate (4). The starting material, 3 was obtained by a nitrosation of acetone [19], while 4 was obtained by nitrosation of malononitrile followed by reduction with aluminum amalgam [20]. Deoxychlorination of 5 following Taylor's approach [21] yielded 3-amino-5-chloro-6-methyl-2-pyrazinecarbonitrile (6). Addition of a free-radical inhibitor to the above reaction gave more consistent yields of a higher quality product.

Scheme 1
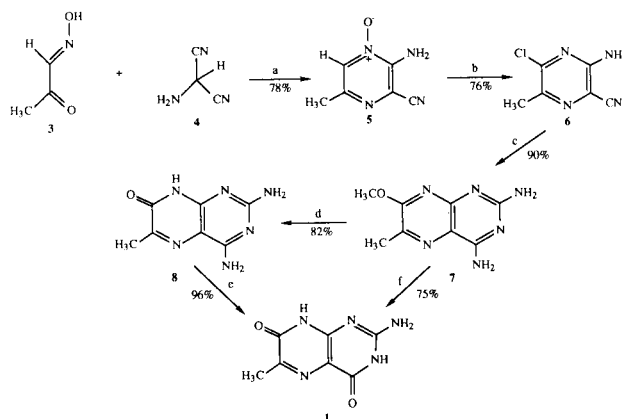

(a) (CH₃)₂CHOH, room temp., 18 h. (b) i. POCl₃, DMF, 80-90°; ii. H₂O (c) CNH(NH₂)₂, CH₃ONa, CH₃OH, reflux, 20 h. (d) i. 2% NaOH, reflux, 5 min.; ii. 6N HCl (e) i. 10% NaOH, reflux, 28 h.; ii. 6N HCl (f) i. 10% NaOH, reflux, 28 h.; ii. 6N HCl.

Ring closure of **6** with guanidine in sodium methoxide solution was successful, and 7-methoxy-6-methyl-2,4-pteridinediamine (**7**) was formed in 90% yield. We then were able to partially hydrolyze **7** to form 2,4-diamino-6-methyl-7(8H)-pteridinone (**8**) in 82% yield. To suppress hydrolysis at the C-4 position, a dilute solution of sodium hydroxide (2%) and short reflux times were required. In the purification of the crude product **8** by acid-base treatment, the purified product obtained was a fine, white precipitate which was nearly impossible to filter. Refluxing the product in the acidified solution after neutralization of the sodium hydroxide solution resulted in an increase in the overall crystal size allowing rapid filtration. The uv spectrum of the purified product displayed maxima at 216, 258, and 340 nm in 0.1 sodium hydroxide. The literature had reported maxima at 255 and 340 nm ($pH = 11$) for this substance [10].

Hydrolysis of 2,4-diamino-6-methyl-7(8H)-pteridinone (**8**) with 10% sodium hydroxide solution for 28 hours yielded the 6-methylisoxanthopterin (**1**) in 96% yield. Alternatively, **1** could be obtained in one step by refluxing **7** in a 10% sodium hydroxide solution. The overall yields of 6-methylisoxanthopterin (**1**) from the reaction of **3** and **4** were 40%. The uv spectrum of the purified showed maxima at 214, 256, and 340 nm in 0.1N sodium hydroxide. A previous preparation of **1** reported maxima at 253 and 336 nm in basic medium [10].

An alternative route to 6-methylisoxanthopterin (**1**) began with a condensation between 2-oxopropanal 1-oxime (**3**) and ethyl 2-amino-2-cyanoacetate (**9**) to form 2-amino-3-ethoxycarbonyl-5-methylpyrazine 1-oxide (**10**) [15] (Scheme 2). The p-toluenesulfonate salt of **9** was conveniently prepared by a reduction of ethyl 2-cyano-2-oximinoacetate with sodium dithionite [22]. Yields were

Scheme 2
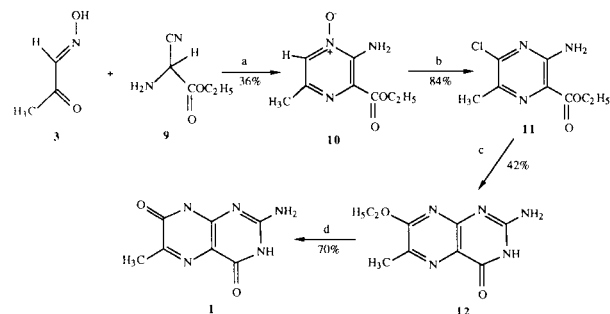
(a) CH₃OH, 35°, 24 h. (b) i. POCl₃, DMF, 80-90°; ii. H₂O (c) i. C₂H₅ONa, C₂H₅OH reflux, 3.5 h.; ii. CNH(NH₂)₂, C₂H₅ONa, C₂H₅OH, reflux, 146 h.; iii. pTsOH (d) i. 10% NaOH, reflux, 45 min.; ii. 6N HCl.

consistently low and comparable to that reported [15]. Deoxychlorination of **10** under Vilsmeier-Haack conditions yielded ethyl 3-amino-5-chloro-6-methyl-2-pyrazinecarboxylate (**11**) (84%). Ring closures of **11** with guanidine

in sodium ethoxide solution followed by acidic workup with dilute mineral acid yielded a mixture of 2-amino-7-ethoxy-6-methyl-4(3H)-pteridinone (**12**) and 6-methylisoxanthopterin (**1**). Our studies indicated that in order to avoid hydrolysis of the C-7 ethoxy group during workup, the amount of water used needed to be minimized. A 42% yield of **12** was obtained by merely adding p-toluenesulfonic acid monohydrate directly to the reaction mixture. Hydrolysis of **12** to form **1** was easily accomplished by briefly refluxing **12** in aqueous alkali. This second pathway to 6-methylisoxanthopterin (**1**) proved to be less desirable than the first method described above. The overall lyield of **1** from the condensation of **3** and **9** was only 9%.

The synthesis of 6-phenylisoxanthopterin (**2**) was carried out in a fashion similar to the two methods employed in the synthesis of its 6-methyl analog. However, synthesis of the requisite oxime, α-oxobenzeneacetaldehyde aldoxime (**13**) (Scheme 3) was initially more difficult than synthesis

Scheme 3
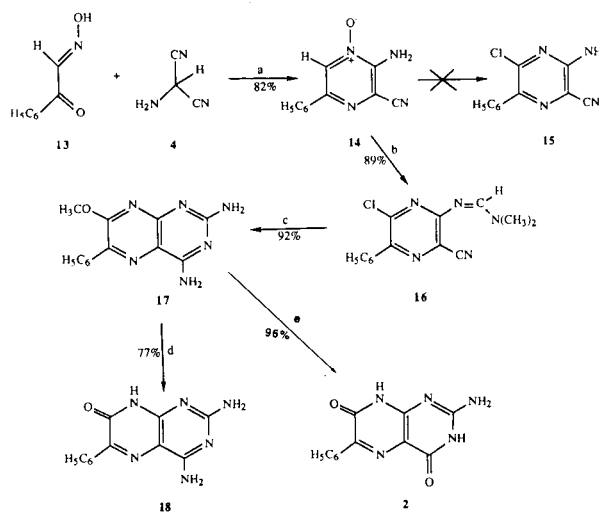
(a) i. (CH₃)₂CHOH, room temp., 5 h.; ii. sat. AcONa (aq) (b) i. POCl₃, DMF, 80-90°; ii. H₂O(c) i. CH₃ONa, CH₃OH, reflux, 1 h.; ii. CNH(NH₂)₂. CH₃ONa, CH₃OH, reflux, 21.5 h. (d) i. 1% NaOH, reflux, 35 min.; ii. 6N HCl (e) i. 10% NaOH, reflux, 168 h.; ii. 6N HCl.

of the analogous oxime of acetone. Preparations described in the literature for **13** were Claisen-like condensations between acetophenone and an alkyl nitrite [23-28]. In all these syntheses, the yields vary from low to merely modest. Touster [29] reviewed nitrosations of aceto- and propiophenone and noted that acetophenone consistently gave lower yields than propiophenone. Our search of the literature did produce one preparation described in a German patent [30] which was unique in that the author reports an 83% yield of the oxime. Adaptation of the procedure to laboratory scale, however, repeatedly gave yields of 10-15% in our hands. This patent did contain a reference to another method which was not cited in *Chemical Abstracts*. This method proved to be the most convenient

involving hydrolysis of ethyl benzoylacetate to the β-keto-acid followed by nitrosation and decarboxylation to yield 13 in 63% yield [31].

The published experimental procedure [17] for the condensation between α-oxobenzeneacetaldehyde (13) and aminomalononitrile p-toluenesulfonate (4) to form 2-amino-3-cyano-5-phenylpyrazine 1-oxide (14) lacks detail but implies that the reaction occurs in good yield under the conditions described for the condensation between 3 and 4. Attempts to condense 13 and 4 under such conditions resulted consistently in the product partially crystallizing out of the reaction mixture with significant contamination by p-toluenesulfonic acid. Variations in the ratio of the reactants and the solvent failed to reduce the ratio of the contaminating p-toluenesulfonic acid to 2-amino-3-cyano-6-phenylpyrazine 1-oxide (14) (2:1 as estimated from the ¹H-nmr). Suspecting this condensation to be acid-catalyzed, we feared that the salt formation could be tying up the catalyst thus preventing the reaction from going to completion. One additional mole of p-toluenesulfonic acid monohydrate was added to the reaction mixture and, surprisingly, resulted in a ratio of 0.33:1 for acid to product in the precipitate. The acid contaminant was eliminated by stirring a saturated sodium acetate solution into the crude reaction mixture, and then collecting the crystalline product by filtration. The crude product, 14, was isolated in 82% yield by this method, and the ¹H-nmr showed no trace of acid contamination.

Spectral data for the purified product failed to match the reported data [17]. The ¹H-nmr spectrum of our product showed the C-6 proton at δ 9.14 rather than at δ 8.70 as reported. The uv spectrum of our product in methanol revealed maxima at 206 and 282 nm with a shoulder at 217 nm. The literature reports 391 nm and 281 (sh) nm, respectively.

Deoxychlorination of 14 under Vilsmeier-Haack conditions failed to yield the expected 3-amino-5-chloro-6-phenyl-2-pyrazinecarbonitrile (15) as the product. Rather, the isolated product was identified as N,N-dimethyl-N'-(6-chloro-3-cyano-5-phenylpyrazin-2-yl)methanimidamide (16). The insolubility of this product in water made acid-or base-catalyzed hydrolysis quite difficult. We finally were successful in ring closing 16 by refluxing it in sodium methoxide and subsequently adding a methanolic guanidine solution to the reaction mixture to yield 7-methoxy-6-phenyl-2,4-pteridinediamine (17). An X-ray diffraction study of 17 revealed the amino groups to the strongly intermolecularly hydrogen bonded. The phenyl ring was found to be 37.6° out of plane with the pteridine ring.

Refluxing 17 in 1% aqueous sodium hydroxide solution successfully hydrolyzed the C-7 methoxy group forming 2,4-diamino-6-phenyl-7(8H)-pteridinone (18) in 77% yield. Partial hydrolysis of 7-methoxy-6-phenyl-2,4-pteridine-

diamine (17) was more easily accomplished than for its 6-methyl analogue, 7, due to the relatively slower hydrolysis of the C-4 amino group as will be shown below. Two previous preparations of 18 were found in the literature: one a Timmis [32] and the other an Isay synthesis [14]. Our uv spectrum for 18 reveals λ max at 362 and 272 nm with a minimum of 310 nm in aqueous alkali. These data match closely the data reported for the synthesis of 18 by Isay methods. Surprisingly, the uv spectrum reported for the product obtained by the presumedly unequivocal Timmis procedure are λ max 362 and 306 and λ min 310 and 271 nm!

Refluxing 17 in 10% aqueous sodium hydroxide formed 6-phenylisoxanthopterin (2), in 96% yield. This hydrolysis of 17 required very long reflux times--a minimum of 168 hours--which necessitated the use of Teflon vessels to avoid contamination of the product by silica. The overall yield of 6-phenylisoxanthopterin (2) from the condensation of 14 and 5 was 64% by this method.

We then sought to synthesize 2 by initially forming 2-amino-3-ethoxycarbonyl-5-phenylpyrazine 1-oxide (19) from a condensation of α-oxobenzeneacetaldehyde aldoxime (14) with ethyl 2-amino-2-cyanoacetate p-toluenesulfonate salt (9) (see Scheme 4). The product, 19, was then deoxygenated and chlorinated as described previously to form ethyl 3-amino-5-chloro-6-phenyl-2-pyrazinecarboxylate (20) in 74% yield after recrystallization. This product was then treated with sodium ethoxide initially, and then an ethanolic guanidine solution was added to the reaction mixture to effect the ring closure to form 2-amino-7-ethoxy-6-phenyl-4(3H)-pteridinone (21). The yield was, however, a disappointing 21%. Hydrolysis of the ethoxy group of 21 was easily accomplished by briefly refluxing 21 in a 10% aqueous sodium hydroxide solution giving 6-phenylisoxanthopterin (2) in 92% yield. The overall yield by this route was only 10%.

Scheme 4
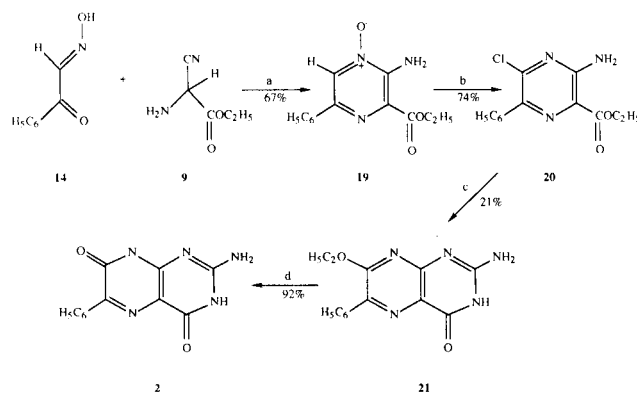
(a) $(CH_3)_2CHOH$, room temp., 32.5 h. (b) i. $POCl_3$, DMF, 80-90°; ii. H_2O (c) i. C_2H_5ONa, C_2H_5OH, reflux, 3.5 h.; ii. $CNH(NH_2)_2$, C_2H_5ONa, C_2H_5OH, reflux, 95 h.; iii. pTsOH (d) i. 10% NaOH, reflux, 25 min.; ii. 6N HCl.

Conclusions.

Two independent methods have been developed for the unequivocal synthesis of both 6-methyl- (**1**) and 6-phenylisoxanthopterin (**2**). In comparing the two independent pathways, the better method in the case of both pterins was *via* the cyanopyrazines rather than the ethoxycarbonylpyrazines. Ring closures on the cyanopyrazines with guanidine in alkoxide solutions provided significantly better yields than ring closures on ethoxycarbonylpyrazines. Both 6-methyl- and 6-phenylisoxanthopterin and their precursors were characterized by their uv, ir, and ^{1}H-nmr spectra, as well as their ^{13}C-nmr spectra in some cases. Discrepancies between our spectral data and the reported literature data were examined. The structural assignments reported in this paper were confirmed by X-ray crystallographic analysis of 7-methoxy-6-phenyl-2,4-pteridinediamine (**17**).

In the process of synthesizing **1** and **2**, two pteridinediamines, 7-methoxy-6-methyl- (**7**) and 7-methoxy-6-phenyl-2,4-pteridinediamine (**17**), and two pteridinones, 2-amino-7-ethoxy-6-methyl-4(3*H*)-pteridinone (**12**) and 2-amino-7-ethoxy-6-phenyl-4(3*H*)-pteridinone (**21**) have been synthesized for the first time. We have also reported the synthesis of four new pyrazines: ethyl 3-amino-5-chloro-6-methyl-2-pyrazinecarboxylate (**11**), *N,N*-dimethyl-*N'*-(6-chloro-3-cyano-5-phenylpyrazin-2-yl)methanimidamide (**16**), 2-amino-3-ethoxycarbonyl-5-phenylpyrazine 1-oxide (**19**), and ethyl 3-amino-5-chloro-6-phenyl-2-pyrazinecarboxylate (**20**).

EXPERIMENTAL

Melting points were taken on an Electrothermal capillary melting point apparatus and are uncorrected. Both ^{1}H- and ^{13}C-nmr spectra were obtained on a JEOL FX 90Q spectrometer at 89.6 and 22.5 MHz, respectively. Chemical shift values are expressed in parts per million (δ values) relative to tetramethylsilane as an internal standard. The ir spectra were obtained on a Matson Sirius 100 FT-IR instrument and the values of the absorptions from peak printouts are expressed in reciprocal centimeters (cm^{-1}). The uv spectra were recorded by a Hewlett Packard IR 100 spectrophotometer and plotted by a Hewlett Packard 7407A plotter. Elemental analyses were performed by Robertson Labs of Florham Park, New Jersey. Thin-layer chromatography (tlc) was done on silica gel 60 F-254 plates (E. M. Reagents) and eluted in the solvents as indicated. Detection of the components on the chromatograms was by fluorescence under long-wave uv light.

7-Methoxy-6-methyl-2,4-pteridinediamine (**7**).

To 1780 ml dry methanol, 27.2 g (1.18 g-atoms) of sodium metal was added. After the sodium had reacted completely, 41.18 g (0.431 mole) of guanidine hydrochloride was added to the methoxide solution, and the precipitated sodium chloride was removed by filtration. This methanolic guanidine solution was combined with 30.35 g (0.180 mole) of 3-amino-5-chloro-6-methyl-2-pyrazinecarbonitrile (**6**), and the mixture was refluxed 20 hours. The product crystallized out of the reaction mixture and was collected by filtration and dried *in vacuo* to yield 33.34 g (90%) of gold colored crystals. Recrystallization of 32.38 g of the crude product from 6200 ml of methanol yielded 23.31 g (72% recovery) of yellow crystals. Two additional recrystallizations yielded an analytically pure sample, mp

261° dec, tlc (2:1 1-propanol/1% aqueous ammonia) 0.70, (2:1 1-butanol/5*N* aqueous acetic acid) 0.49, (3% aqueous ammonium chloride) 0.27, (4% aqueous sodium citrate) 0.29; uv (0.1*N* sodium hydroxide): λ max, nm (log ϵ), 214 (4.70), 254 (4.15), 348 (3.91); uv (methanol): λ max, nm (log ϵ), 214 (4.59), 227 (sh), 256 (4.42), 278 (4.06), 348 (4.02); ir (potassium bromide): cm^{-1}, 3452 (w), 3341 (w), 3130 (w), 1620 (s), 1552 (s), 1448 (s), 1386 (m), 1333 (s), 1248 (m), 1222 (m), 1004 (w), 816 (w); ^{1}H-nmr (DMSO-d$_6$): δ 7.19 (bs, 2, NH_2), δ 6.31 (bs, 2, NH_2), δ 3.95 (s, 3, OCH_3), δ 2.40 (s, 3, CH_3); ^{13}C-nmr (DMSO-d$_6$): δ 162.74, δ 161.95, δ 160.59, δ 154.78, δ 137.73, δ 115.20, δ 53.73, δ 18.92.

Anal. Calcd. for C$_8$H$_{10}$N$_6$O: C, 46.59; H, 4.89; N, 40.76. Found: C, 46.43; H, 4.71; N, 40.45.

2,4-Diamino-6-methyl-7(8*H*)-pteridinone (**8**).

A 2% sodium hydroxide solution (200 ml) was heated to reflux, and 2.50 g (12.1 mmoles) of 7-methoxy-6-methyl-2,4-pteridinediamine (**7**) was added. After 5 minutes most of the solid had dissolved, and the solution was filtered hot to remove the small portion of undissolved material. The filtrate was immediately acidified while hot to pH 4 with 6*N* hydrochloric acid. After cooling to room temperature, the precipitated white crystals were collected by suction filtration and dried. The product was redissolved in 350 ml of 5% sodium hydroxide, and the resultant yellow solution was treated with 0.25 g of Norit. The mixture was suction filtered to remove the carbon, and the filtrate was acidified with 6*N* hydrochloric acid to pH 4 resulting in the formation of a fine, white precipitate. This acidified mixture was heated to boiling and stirred 30 minutes to allow formation of larger crystals. The mixture was then cooled first to room temperature and subsequently to ice-bath temperature. Filtration of the short white needles and drying *in vacuo* yielded 1.90 g (82%) of analytically pure product, mp >320°, tlc (2:1 1-propanol/1% aqueous ammonia) 0.69, (2:1 1-butanol/5*N* aqueous acetic acid) 0.38, (4:2:1 ethyl acetate/water/1-propanol) 0.59; uv (0.1*N* sodium hydroxide): λ max, nm (log ϵ), 216 (4.56), 222 (sh), 258 (4.10), 280 (sh), 340 (4.17); uv (0.1*N* hydrochloric acid): λ max, nm (log ϵ), 218 (4.38), 300 (4.12), 344 (4.13); ir (potassium bromide): cm^{-1}, 3477 (m), 3426 (m), 3332 (m), 3225 (m), 2926 (w), 2847 (w), 1621 (s), 1568 (m), 1501 (m), 1461 (m), 1397 (w), 1376 (w), 1266 (w), 1202 (w), 1160 (w), 1066 (w), 1007 (w), 943 (w), 800 (w); ^{1}H-nmr (DMSO-d$_6$): δ 6.91 (bs, 2, NH_2), δ 6.43 (bs, 2, NH_2), δ 2.24 (s, 3, CH_3).

Anal. Calcd. for C$_7$H$_8$N$_6$O: C, 43.74; H, 4.20; N, 43.74. Found: C, 43.67; H, 4.22; N, 43.52.

6-Methylisoxanthopterin (**1**) from 7-Methoxy-6-methyl-2,4-pteridinediamine (**7**).

A suspension of 1.00 g (4.85 mmoles) of **7** in 70 ml of 10% sodium hydroxide was heated forming a yellow solution, which was refluxed 28 hours. After the reflux period was completed, the solution was combined with 100 ml of boiling water, treated with 0.1 g Norit, and filtered. The filtrate was then acidified with 6*N* hydrochloric acid to pH 4 yielding a white precipitate. This suspension was boiled for 30 minutes. After this period of heating, the mixture was cooled initially to room temperature slowly and then refrigerated. The precipitate was filtered off and dried *in vacuo*. The crude product was dissolved in 100 ml of 10% sodium hydroxide and diluted with 300 ml of water to form a yellow solution which was treated with 0.1 g Norit and filtered hot. The filtrate was then acidified to pH 3.5-4 with 6*N* hydrochloric acid while hot which resulted in formation of a white precipitate. The mixture was heated to boiling and after boiling 30 minutes, the mixture was refrigerated. Once cooled, the crystals were collected by filtration, washed with water and then acetone, and dried *in vacuo* overnight to yield 0.70 g (75%) of **1** in an analytically pure state, mp >350°, tlc (2:1 1-propanol/1% aqueous ammonia) 0.50, (2:1 1-butanol/5*N* aqueous acetic acid) 0.39, (3% aqueous ammonium chloride) 0.37, (4% aqueous sodium citrate) 0.28; uv (0.1*N* sodium hydroxide): λ max, nm (log ϵ), 214 (4.66), 256 (3.96), 340 (4.10); uv (0.1*N* hydrochloric acid): λ max, nm (log ϵ), 216 (3.86), 292 (3.76), 338 (3.87); ir (potassium bromide): cm^{-1}, 3313 (m), 3160 (m), 2923 (m), 2774 (m), 1659 (s), 1601 (s), 1480 (w), 1386 (m), 1336 (w), 1238 (w), 1079 (w), 912 (w), 862 (w); ^{1}H-nmr (deuteriotrifluoroacetic acid): δ 2.86 (s, CH_3).

Anal. Calcd. for $C_7H_7N_5O_2$: C, 43.52; H, 3.65; N, 36.26. Found: C, 43.35; H, 3.67; N, 35.99.

6-Methylisoxanthopterin (1) from 2,4-Diamino-6-methyl-7(8*H*)-pteridinone (8).

A solution of 0.40 g (2.08 mmoles) of **8** in 30 ml of 10% hydrochloric acid was refluxed 28 hours. After this period of reflux, the solution was diluted with 100 ml of boiling water and treated with 0.04 g of Norit. The filtrate was acidified while hot to pH 4 with 6N hydrochloric acid and boiled 20 minutes before cooling to room temperature. The white crystals were collected by filtration and dried *in vacuo*. The crude product was redissolved in 50 ml of hot 10% sodium hydroxide, diluted with 150 ml of boiling water, and then reacidified while hot to pH 3.5-4 with 6N hydrochloric acid. After boiling 10 minutes, the mixture was refrigerated. The white crystals were then filtered out, washed first with water and finally with acetone, and dried *in vacuo* to yield 0.382 g (96%) of **9** which was identical in all respects to the sample obtained by the hydrolysis of 7-methoxy-6-methyl-2,4-pteridinediamine (**7**).

Ethyl 3-Amino-5-chloro-6-methyl-2-pyrazinecarboxylate (11).

A 1.95 g (0.086 mmole) sample of 2-amino-3-ethoxycarbonyl-5-methylpyrazine 1-oxide (**10**) was dissolved in 15 ml of *N,N*-dimethylformamide, and the solution was heated to 80°. To this solution, 3 ml (0.03 mole) of phosphorus oxychloride was added dropwise with stirring while maintaining the temperature between 80 and 90° with occasional cooling in a cold water bath. After addition of the phosphorus oxychloride was completed, the mixture was stirred while maintaining the temperature at 86° with a preheated oil bath. After stirring 10 minutes, the reaction mixture was poured into 150 ml of ice cold water, and allowed to stand overnight. The orange crystals were collected by filtration and dried *in vacuo* to yield 1.80 g (84%), mp 125-127°. The crude product was recrystallized from 20 ml 100% ethanol to yield 1.56 g (87% recovery) of yellow crystals. An additional recrystallization yielded an analytically pure sample, mp 127°C, tlc (9:1 chloroform/methanol) 0.68, (9:1 chloroform/acetone) 0.63, (95:5 chloroform/ethyl acetate) 0.38; uv (methanol): λ max, nm (log ε), 208 (4.07), 224 (4.04), 258 (4.17), 362 (4.00); ir (potassium bromide): cm^{-1}, 3469 (m), 3330 (m), 1692 (s), 1599 (s), 1515 (w), 1374 (w), 1358 (w), 1325 (w), 1305 (w), 1199 (w), 1124 (s), 990 (w); ^{1}H-nmr (DMSO-d$_6$): δ 7.44 (bs, 2, NH_2), δ 4.31 (q, J = 7.1 Hz, 2, OCH_2), δ 2.39 (s, 3, CH_3), δ 1.30 (t, J = 7.1 Hz, 3, CH$_2$CH_3); ^{13}C-nmr (DMSO-d$_6$): δ 165.46, δ 154.08, δ 149.27, δ 137.16, δ 120.86, δ 60.98, δ 20.17, δ 14.17.

Anal. Calcd. for $C_8H_{10}ClN_3O_2$: C, 44.56; H, 4.67; N, 19.49; Cl, 16.44. Found: C, 44.31; H, 4.53; N, 19.63; Cl, 16.47.

2-Amino-7-ethoxy-6-methyl-4(3*H*)-pteridinone (12).

Sodium metal (0.125 g, 5.48 mmoles) was reacted with 17 ml of anhydrous ethanol (dried first with calcium oxide and then with magnesium metal). When the reaction was finished, 0.851 g (3.95 mmoles) of ethyl 3-amino-5-chloro-6-methyl-2-pyrazinecarboxylate (**11**) was added to this sodium ethoxide solution, and the mixture was refluxed. After 3 ½ hours at reflux, 30 ml of an ethanolic guanidine solution was added to the reaction mixture, and refluxing continued. The ethanolic guanidine solution was formed by reacting 0.277 g (12.0 mmoles) of sodium metal with 30 ml of anhydrous ethanol. When the reaction was completed 0.915 g (9.58 mmoles) of guanidine hydrochloride was added to the ethoxide solution, and the precipitated sodium chloride was removed by filtration. After 146 hours reflux, the reaction mixture was acidified while hot to pH 4.0 by the addition of small portions of *p*-toluenesulfonic acid monohydrate. The reaction mixture was cooled in an ice bath and filtered. The precipitate was washed first with water (100 ml) and then acetone (50 ml) and dried *in vacuo* to yield 0.365 g (42%) of **12**. An analytical sample was obtained by refluxing the crude product overnight in 20 ml of 100% ethanol. After isolation of the solid by filtration and drying, the solid was dissolved in 100 ml of 2% sodium hydroxide and then quickly acidified to pH 4 with 6N hydrochloric acid.

The product was collected by filtration and dried *in vacuo*, mp >300°, tlc (2:1 1-butanol/5N aqueous acetic acid) 0.58, (2:1 1-propanol/1%

aqueous ammonia) 0.72, (4:2:1 ethyl acetate/water/propanol upper phase) 0.37; uv (0.1N sodium hydroxide): λ max, nm (log ε), 214 (4.66), 254 (4.06), 342 (4.02); ir (potassium bromide): cm^{-1}, 3475 (m), 3348 (m), 2993 (m), 1650 (s), 1597 (s), 1526 (s), 1432 (s), 1383 (s), 1344 (s), 1308 (m), 1215 (s), 1134 (m), 1027 (m), 917 (m), 864 (w), 806 (w); ^{1}H-nmr (DMSO-d$_6$): δ 7.23 (bs, 2, NH_2), δ 4.38 (q, J = 7.12 Hz, 2, OCH_2CH$_3$), δ 2.22 (s, 3, CH_3), δ 1.34 (t, J = 7.12 Hz, 3, OCH$_2$CH_3); ^{13}C-nmr (DMSO-d$_6$): δ 167.84, δ 158.95, δ 154.71, δ 129.69, δ 113.22, δ 62.11, δ 17.62, δ 14.28.

Anal. Calcd. for $C_9H_{11}N_5O_2$: C, 48.87; H, 5.01; N, 31.66. Found: C, 48.63; H, 5.20; N, 31.39.

6-Methylisoxanthopterin (1) from 2-Amino-7-ethoxy-6-methyl-4(3*H*)-pteridinone (12).

A 10% sodium hydroxide solution (25 ml) was combined with 0.50 g (2.3 mmoles) of **12** and then heated to reflux. After refluxing 45 minutes, the hot solution was acidified to pH 4 with 6N hydrochloric acid and refrigerated. After cooling several hours, the off-white crystals were collected by filtration, washed with water and acetone, and dried *in vacuo* overnight at 140° to yield 0.44 g (100%) of **1**. The isolated product was redissolved in 25 ml of hot 10% sodium hydroxide, treated with 0.05 g of Norit, and filtered hot. The filtrate was acidified while hot to pH 4 by the addition of 6N hydrochloric acid causing the product to precipitate out as fine white crystals. The mixture was then heated to boiling and boiled 30 minutes. After cooling in the refrigerator 1 hour the white crystals were collected by filtration, washed with water and acetone, and dried *in vacuo* overnight at 140° to yield 0.35 g (80% recovery) of purified **1** which was identical in all respects to the sample of **1** obtained by the full hydrolysis of 7-methoxy-6-methyl-2,4-pteridinediamine (**7**).

2-Amino-3-cyano-5-phenylpyrazine 1-Oxide (14).

A suspension of 37.69 g (0.253 mole) of α-oxobenzeneacetaldehyde aldoxime (**13**), 64.00 g (0.253 mole) of aminomalononitrile *p*-toluenesulfonate (**4**), and 48.13 g (0.253 mole) of *p*-toluenesulfonic acid in 600 ml of 2-propanol was stirred 5 hours at room temperature. The reaction mixture was then neutralized by the addition of 250 ml of an aqueous saturated sodium acetate solution and then refrigerated 1 hour. The yellow crystals were collected by filtration and washed with a little water, and then dried *in vacuo* yielding 43.78 g (82%) of **14**. The crude product was recrystallized from 3800 ml of methanol (cooled finally to -20°) to yield 28.55 g (65% recovery) of **14** after drying *in vacuo*. The mother liquor was evaporated to 400 ml, refluxed for 2 hours, cooled and filtered to yield 9.58 g (18%, 83% total recovery) of yellow crystals which were of similar purity as the first crop as judged by tlc, mp 216-218° dec, lit mp 214° [17]; tlc (9:1 chloroform/methanol) 0.56, (9:1 chloroform/acetone) 0.50, (95:5 chloroform/ethyl acetate) 0.33, (1:1 ethyl acetate/benzene) 0.35; uv (methanol): λ max, nm (log ε), 206 (4.17), 217 (sh), 282 (4.40); ir (potassium bromide): cm^{-1}, 3408 (w), 3280 (w), 3226 (w), 3142 (w), 2233 (w), 1629 (m), 1558 (w), 1486 (m), 1358 (w), 1246 (w), 1177 (m), 1030 (w), 903 (w); ^{1}H-nmr (DMSO-d$_6$): δ 9.14 (s, 1, C6-*H*), δ 8.2-7.8 (m, 4, NH_2, C$_6H_5$), δ 7.6-7.4 (m, 3, C$_6H_5$); ^{13}C-nmr (DMSO-d$_6$): δ 149.56, δ 142.31, δ 134.16, δ 131.33, δ 129.29, δ 128.84, δ 125.61, δ 115.09, δ 111.18.

***N,N*-Dimethyl-*N'*-(6-chloro-3-cyano-5-phenylpyrazin-2-yl)methanimidamide (16).**

In a preheated oil bath, 21 ml of dry *N,N*-dimethylformamide (spectral grade, stored over Molecular Sieve) was heated to 84°, and 2.90 g (0.014 mole) of 2-amino-3-cyano-5-phenylpyrazine 1-oxide (**14**) was added to form an orange solution. Addition of the first few drops of 4.5 ml of freshly distilled phosphorus oxychloride caused the temperature of the reaction mixture to rise rapidly to 95°. The reaction mixture was cooled to 82° with a cold water bath, and addition of phosphorus oxychloride was continued. Addition of the first milliliter of phosphorus oxychloride required cooling in a cold water bath to maintain the temperature between 80-90°. After 1 ml of phosphorus oxychloride had been added, the temperature was controlled solely by the rate of addition of phosphorus oxychloride without external cooling. After addition of the phosphorus oxychloride was completed, stirring of the reaction mixture continued

while the temperature was maintained between 80 and 90° by immersion of the reaction vessel in a preheated oil bath. During this period of stirring, yellow needles began to form in the reaction vessel. After 10 minutes of stirring, the reaction mixture was quenched in 220 ml of ice water. The product crystallized out immediately as yellow crystals, which were collected by filtration and dried *in vacuo* to yield 2.85 g (89%) of **16**. Recrystallization of the crude product from 800 ml of 100% ethanol yielded 2.12 g (75% recovery) of the desired product. Two subsequent recrystallizations were used to prepare an analytically pure sample; mp 202°, tlc (9:1 chloroform/methanol) 0.64, (9:1 chloroform/acetone) 0.64, (95.5 chloroform/ethyl acetate) 0.58, (1:1 benzene/ethyl acetate) 0.63; uv (methanol): λ max, nm (log ε), 210 (4.66), 316 (4.61), 376 (4.15); ir (potassium bromide): cm^{-1}, 2931 (w), 2229 (w), 1624 (s), 1529 (s), 1503 (s), 1453 (s), 1428 (s), 1395 (s), 1353 (s), 1189 (m), 1123 (m), 1093 (m), 970 (w), 889 (w); ^{1}H-nmr (DMSO-d$_6$): δ 8.67 (s, 1, *H*CNMe$_2$), δ 7.9-7.3 (m, 5, C$_6$*H*$_5$), δ 3.25 (s, 3, HCNMeC*H*$_3$), δ 3.15 (s, 3, HCNC*H*$_3$Me); ^{13}C-nmr (deuteriochloroform): δ 157.52, δ 157.17, δ 147.17, δ 145.51, δ 135.55, δ 129.38, δ 128.19, δ 121.17, δ 41.37, δ 35.48.

Anal. Calcd. for C$_{14}$H$_{12}$ClN$_5$: C, 58.85; H, 4.23; N, 24.51; Cl, 12.41. Found: C, 58.77; H, 4.35; N, 24.68; Cl, 12.58.

7-Methoxy-6-phenyl-2,4-pteridinediamine (17).

To 100 ml of dry methanol (spectral grade, stored over Molecular Sieve), 0.98 g (43 mmoles) of sodium metal was added. When the sodium had reacted completely, 10.00 g (35.0 mmoles) of N,N-dimethyl-N′-(6-chloro-3-cyano-5-phenylpyrazin-2-yl)methanimidamide (**16**) was added to the sodium methoxide solution, and the mixture was refluxed. After 1 hour reflux, 100 ml of a methanolic guanidine solution was added to the reaction mixture, and refluxing continued. The methanolic guanidine solution was formed by reacting 2.98 g (0.130 mole) of sodium with 100 ml of dry methanol, subsequently adding 9.97 g (0.104 mole) of guanidine hydrochloride, and removing the precipitated sodium chloride by filtration. After refluxing 21.5 hours, the reaction mixture was cooled to room temperature and filtered to yield 6.54 g (70%) of yellow crystals after washing with a little cold methanol and drying *in vacuo*. Evaporation of the filtrate to 75 ml allowed a second crop to be collected weighing 1.685 g (18%). Further evaporation yielded a third crop (0.356 g, 4%, 92% overall). All three crops were dissolved in 2 l of hot methanol and treated with 0.43 g of Norit. The mixture was filtered, and the filtrate was cooled to room temperature. The mixture was then seeded to induce crystallization and stored at -25° overnight. Filtration of the yellow needles and drying *in vacuo* yielded 3.76 g (44% recovery). The filtrate was further evaporated to 300 ml to yield a second crop weighing 0.95 g (11%, 55% overall recovery). Elution of a sample of the second crop on tlc showed the second crop to be of purity equal to that of the first. Recrystallization of the first crop one additional time provided an analytically pure sample, mp 255-256° dec, tlc (2:1 1-propanol/1% aqueous ammonia) 0.69, (2:1 1-butanol/5N aqueous acetic acid) 0.58, (4:2:1 ethyl acetate/water/1-propanol upper phase) 0.65; uv (methanol): λ max, nm (log ε), 208 (4.45), 272 (4.40), 294 (sh), 370 (4.25); ir (potassium bromide): cm^{-1}, 3492 (m), 3412 (m), 3278 (m), 3097 (m), 1634 (s), 1619 (s), 1583 (s), 1536 (s), 1433 (s), 1343 (s), 1244 (s), 1086 (w), 1042 (w), 976 (w), 819 (w), 790 (w); ^{1}H-nmr (DMSO-d$_6$): δ 8.2-8.0 (m, 2, C$_6$*H*$_5$), δ 7.5-7.3 (m, 5, C$_6$*H*$_5$, N*H*$_2$), δ 6.52 (bs, 2, N*H*$_2$), δ 4.00 (s, 3, OC*H*$_3$); ^{13}C-nmr (DMSO-d$_6$): δ 163.42, δ 162.35, δ 159.91, δ 154.65, δ 135.86, δ 135.58, δ 128.90, δ 128.50, δ 127.93, δ 116.28, δ 53.79.

Anal. Calcd. for C$_{13}$H$_{12}$N$_6$O: C, 58.20; H, 4.51; N, 31.33. Found: C, 57.99; H, 4.34; N, 31.33.

2,4-Diamino-6-phenyl-7(8H)-pteridinone (18).

7-Methoxy-6-phenyl-2,4-pteridinediamine (**17**) (0.145 g, 0.540 mmole) was added to 50 ml of boiling 2% sodium hydroxide, and then the reaction mixture was diluted with 50 ml of water. The mixture was stirred and refluxed 35 minutes until most of the starting material had dissolved. The mixture was filtered hot to remove the insoluble material, and the filtrate was acidified while hot to pH 3.5 by the addition of 6N hydrochloric acid to yield yellow crystals. The mixture was heated to boiling

and then allowed to cool to room temperature which increased the crystal size. The mixture was then refrigerated. Filtration, washing with water and then with acetone, and drying *in vacuo* yielded 0.112 g (77%) of **18** as yellow crystals. The crude product was dissolved in 200 ml of 5% sodium hydroxide at room temperature, treated with 0.011 g of Norit, and filtered. The filtrate was acidified to pH 3.5 with 6N hydrochloric acid. The mixture was then heated to boiling and subsequently refrigerated. The yellow-green crystals were collected by filtration, washed with water and acetone, and dried *in vacuo* to yield 0.760 g (68% recovery) of **18**. A second recrystallization in the same manner yielded an analytically pure sample, mp >350°, tlc (2:1 1-butanol/5N aqueous acetic acid) 0.72, (2:1 1-propanol/1% aqueous ammonia) 0.70, (4:2:1 ethyl acetate/water/1-propanol) 0.73; uv (0.1N sodium hydroxide): λ max, nm (log ε), 214 (4.82), 232 (4.55), 272 (3.99), 362 (4.30); uv (0.1N hydrochloric acid): λ max, nm (log ε), 220 (4.40), 240 (sh), 308 (4.13), 362 (4.35), 366 (4.34); ir (potassium bromide): cm^{-1}, 3458 (w), 3321 (w), 3176 (w), 2770 (w), 1629 (s), 1564 (m), 1475 (m), 1330 (w), 1290 (w), 1180 (w), 1020 (w), 920 (w), 806 (w); ^{1}H-nmr (DMSO-d$_6$): δ 12.39 (bs, 1, N8-*H*), δ 8.4-8.3 (m, 2, C$_6$*H*$_5$), δ 7.4-7.3 (m, 3, C$_6$*H*$_5$), δ 7.26 (bs, 2, N*H*$_2$), δ 6.70 (bs, 2, N*H*$_2$).

Anal. Calcd. for C$_{12}$H$_{10}$N$_6$O: C, 56.69; H, 3.96; N, 33.05. Found: C, 56.48; H, 4.16; N, 32.87.

6-Phenylisoxanthopterin (2) from 7-Methoxy-6-phenyl-2,4-pteridinediamine (17).

In a Teflon beaker fitted with a special condenser, 0.250 g (0.932 mmole) of **17** was refluxed in 125 ml of 10% sodium hydroxide. The reaction's progress was monitored by tlc (4:2:1 ethyl acetate/water/1-propanol upper phase) by diluting and acidification of aliquots of the reaction mixture. After 168 hours of reflux, the hot reaction mixture was poured into 150 ml of boiling water, treated with 0.025 g of Norit, and filtered hot. The filtrate was acidified with 6N hydrochloric acid to pH 3.5-4.0 while hot. The mixture was boiled 10 minutes and refrigerated. The product was collected by filtration, washed with water and acetone, and dried *in vacuo* to yield 0.228 g (96%) of **2** as pale yellow crystals. The crude product was dissolved in 100 ml of 10% sodium hydroxide at room temperature and then filtered. The filtrate was heated to boiling and, while hot, acidified to pH 3.5 with 6N hydrochloric acid. The mixture was then cooled in the refrigerator. Filtration of the mixture, washing with water and acetone, and drying *in vacuo* yielded 0.216 g (95% recovery) of **2**. An analytical sample was prepared by similar alkali treatment followed by acidification three subsequent times, mp >300°, tlc (4:2:1 ethyl acetate/water/1-propanol) 0.44, (2:1 1-butanol/5N aqueous acetic acid) 0.68, (2:1 1-propanol/1% aqueous ammonia) 0.72; uv (0.1N sodium hydroxide): λ max, nm (log ε), 214 (4.79), 228 (4.58), 268 (3.99), 292 (sh), 362 (4.35); ir (potassium bromide): cm^{-1}, 3360 (w), 3157 (w), 1652 (s), 1596 (m), 1396 (w); ^{1}H-nmr (DMSO-d$_6$): δ 12 (vbs, 2, N3-*H*, N8-*H*), δ 8.3-8.1 (m, 2, C$_6$*H*$_5$), δ 7.5-7.3 (m, 3, C$_6$*H*$_5$), δ 7.2-7.1 (bs, 2, N*H*$_2$).

Anal. Calcd. for C$_{12}$H$_9$N$_5$O$_2$: C, 56.47; H, 3.55; N, 27.44. Found: C, 56.19; H, 3.44; N, 27.15.

2-Amino-3-ethoxycarbonyl-5-phenylpyrazine 1-Oxide (19).

A solution of 2.94 g (0.020 mole) of α-oxobenzeneacetaldehyde aldoxime (**14**) in 20 ml of 2-propanol was combined with 6.05 g (0.020 mole) of ethyl 2-amino-2-cyanoacetate p-toluenesulfonate salt (**9**), and the suspension was stirred 32.5 hours and then refrigerated overnight. Suction filtration and washing with a little ice cold 2-propanol yielded 3.48 g (67%) of crude product. Recrystallization from methanol yielded 2.70 g (78% recovery) of yellow needles. One additional recrystallization yielded an analytically pure sample, mp 145-146°, tlc (9:1 chloroform/methanol) 0.63, (2:1 1-propanol/1% aqueous ammonia) 0.72, (2:1 1-butanol/5N aqueous acetic acid) 0.69, (1:1 benzene/ethyl acetate) 0.32; uv (methanol): λ max, nm (log ε), 206 (4.24), 250 (sh), 280 (4.38); ir (potassium bromide): cm^{-1}, 3430 (m), 3385 (m), 3278 (m), 1696 (s), 1613 (s), 1552 (s), 1494 (m), 1402 (m), 1370 (m), 1354 (m), 1278 (m), 1238 (m), 1162 (s), 1108 (s), 1076 (m), 809 (s); ^{1}H-nmr (DMSO-d$_6$): δ 9.13 (s, 1, C6-*H*), δ 8.1-7.9 (m, 2, C$_6$*H*$_5$), δ 7.98 (bs, 2, N*H*$_2$), δ 7.6-7.4 (m, 3, C$_6$*H*$_5$), δ 4.43 (q, J = 6.7 Hz, 2, OC*H*$_2$CH$_3$), δ 1.38 (t, J = 6.7 Hz, 3, OCH$_2$C*H*$_3$); ^{13}C-nmr (DMSO-d$_6$): δ

165.40, δ 147.80, δ 139.93, δ 134.84, δ 130.42, δ 129.01, δ 128.78, δ 125.67, δ 124.48, δ 61.60, δ 14.17.

Anal. Calcd. for $C_{13}H_{13}N_3O_3$: C, 60.22; H, 5.05; N, 16.21. Found: C, 60.03; H, 4.87; N, 16.29.

Ethyl 3-Amino-5-chloro-6-phenyl-2-pyrazinecarboxylate (20).

In a preheated oil bath, 135 ml of dry N,N-dimethylformamide was heated to 80°, and 20.00 g (0.077 mole) of 2-amino-3-ethoxycarbonyl-5-phenylpyrazine 1-oxide (19) was added to the hot N,N-dimethylformamide. Dropwise addition of 25 ml of freshly distilled phosphorus oxychloride was initiated while the temperature of the reaction mixture was maintained between 80 and 90° with cooling from a cold water bath as required. After addition of the phosphorus oxychloride was completed, the mixture was stirred while the temperature was kept between 80 and 90° with a preheated oil bath. After 10 minutes of stirring, the reaction mixture was poured into 1300 ml of ice cold water. A brown precipitate formed immediately, but after a few minutes of stirring, the precipitate dissolved. After 15 minutes of stirring, a precipitate began to form again. The mixture was stirred several hours and then refrigerated overnight. After filtration and drying, the crude product was recrystallized from 750 ml of 100% ethanol to yield 15.66 g (74%) of crystals which were off-white in color. One additional recrystallization produced an analytically pure sample, mp 155-156°, tlc (9:1 chloroform/acetone) 0.66, (1:1 benzene/ethyl acetate) 0.72, (9:1 chloroform/methanol) 0.77; uv (methanol): λ max, nm (log ϵ) 210 (4.59), 223 (sh), 276 (4.37), 368 (3.95); ir (potassium bromide): cm^{-1}, 3440 (m), 3269 (m), 3196 (w), 3061 (w), 2983 (w), 1697 (s), 1619 (s), 1513 (m), 1457 (m), 1373 (m), 1316 (m), 1198 (m), 1150 (s), 969 (w); ^{1}H-nmr (DMSO-d_6): δ 7.8-7.3 (m, 7, NH_2/C_6H_5), δ 4.33 (q, J = 7.1 Hz, 2, OCH_2CH$_3$), δ 1.29 (t, J = 7.1 Hz, 3, OCH$_2$CH_3); ^{13}C-nmr (DMSO-d_6): δ 165.29, δ 154.13, δ 147.69, δ 138.58, δ 136.14, δ 129.07, δ 128.33, δ 127.99, δ 121.65, δ 61.20, δ 14.11.

Anal. Calcd. for $C_{13}H_{12}ClN_3O_2$: C, 56.22; H, 4.36; N, 15.13; Cl, 12.78; Found: C, 55.93; H, 4.45; N, 15.05; Cl, 12.98.

2-Amino-7-ethoxy-6-phenyl-4(3H)-pteridinone (21).

A 40 ml aliquot from a 0.2 M sodium ethoxide solution (formed by reacting 0.51 g (22.2 g-atoms) of sodium metal with 100 ml of super-dry ethanol) was added to 2.00 g of ethyl 3-amino-5-chloro-6-phenyl-2-pyrazinecarboxylate (20), and the mixture was refluxed 3 ½ hours. To this mixture, an ethanolic guanidine solution was added. (The ethanolic guanidine solution was formed by reacting 1.09 g (47.4 mmoles) sodium metal with 150 ml of anhydrous ethanol. When the sodium had reacted completely, 60 ml of this sodium ethoxide solution was combined with 1.65 g (17.2 mmoles) guanidine hydrochloride. Filtration removed the precipitated sodium chloride to yield an ethanolic guanidine solution.) After the mixture had refluxed 95 hours, the reaction mixture was acidified while hot by the addition of small portions of p-toluenesulfonic acid monohydrate until pH 3.5-4.0 was observed as indicated by pH paper (3.0-5.5). The reaction mixture was then refrigerated overnight. Filtration and washing first with water (50 ml) and then with acetone (10 ml) yielded 0.42 g (21%) of 21 after drying *in vacuo*. A 0.110 g sample of the crude product was recrystallized from 40 ml of N,N-dimethylformamide to yield 0.098 g (89% recovery) of pale yellow crystals. One additional recrystallization from the same solvent yielded an analytically pure sample, mp >350°, tlc (2:1 1-butanol/5N aqueous acetic acid) 0.73, (2:1 1-propanol/1% aqueous ammonia) 0.69, (4:2:1 ethyl acetate/water/1-propanol upper phase) 0.53; uv (0.1N sodium hydroxide): λ max, nm (log ϵ), 214 (4.74), 268 (4.03), 292 (sh), 362 (4.09); ir (potassium bromide): cm^{-1}, 3310 (w), 1685 (s), 1536 (m), 1413 (m), 1336 (w), 1241 (w), 1025 (w); ^{1}H-nmr (DMSO-d_6): δ 8.0-7.8 (m, 2, C_6H_5), δ 7.5-7.4 (m, 3, C_6H_5), δ 6.94 (bs, 2, NH_2), δ 4.48 (q, J = 7.12 Hz, 2, OCH_2CH$_3$), δ 1.39 (t, J = 7.12 Hz, 3, OCH$_2$CH_3).

Anal. Calcd. for $C_{14}H_{13}N_5O_2$: C, 59.36; H, 4.63; N, 24.72. Found: C, 59.25; H, 4.89; N, 24.61.

6-Phenylisoxanthopterin (2) from 2-Amino-7-ethoxy-6-phenyl-4(3H)-pteridinone (21).

A 10% sodium hydroxide solution (125 ml) was combined with 0.115 g (0.406 mmole) of 21 and the mixture was heated to boiling. After boiling 25 minutes, the mixture was filtered hot. The filtrate was acidified while hot to pH 3.5 with 6N hydrochloric acid and the mixture was refrigerated overnight. The pale yellow crystals were collected by filtration, washed with water and acetone, and dried *in vacuo* to yield 0.106 g (92%) of 2. These crystals were then redissolved in 100 ml of boiling 5% sodium hydroxide and stirred for 30 minutes. The solution was then treated with 0.011 g of Norit and filtered hot. The filtrate was acidified while heating to a boil with 6N hydrochloric acid to pH 3.5-4.0. After acidification, the mixture was allowed to cool to room temperature and filtered. Washing with water and acetone and drying *in vacuo* yielded 0.093 g (88% recovery) of purified product. Two additional recrystallizations yielded an analytically pure sample which was identical in all respects to the sample obtained by the full hydrolysis of 17.

Anal. Calcd. for $C_{12}H_9N_5O_2$: C, 56.47; H, 3.55; N, 27.44. Found: C, 56.18, H, 3.49; N, 27.27.

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