Pteridines. XXXVII. A Total Synthesis of L-*erythro*-Biopterin and Some Related 6-(Polyhydroxyalkyl)pterins

Edward C. Taylor* and Peter A. Jacobi

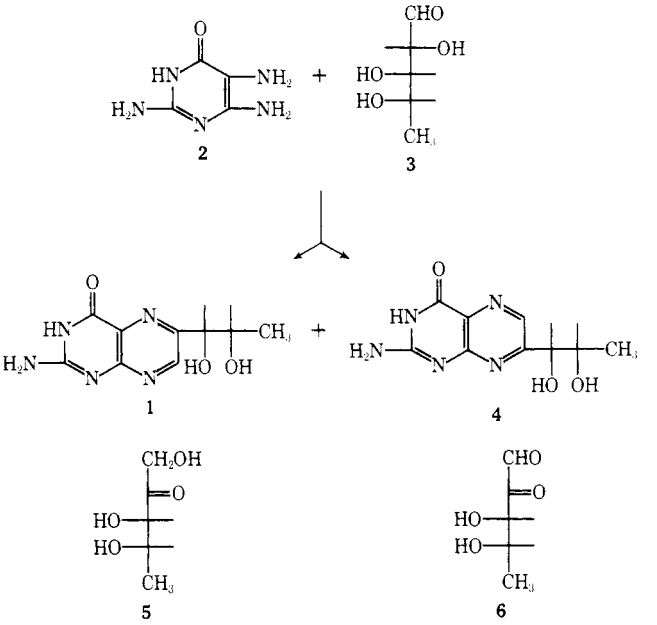
Contribution from the Department of Chemistry, Princeton University, Princeton, New Jersey 08540. *Received February 4, 1975*

Abstract: An unequivocal synthesis of L-*erythro*-biopterin [6-(L-*erythro*-1′,2′-dihydroxypropyl)pterin] (**1**) is described which comprises the following steps: (1) cupric acetate oxidation of 5-deoxy-L-arabinose (**3**) to its osone **6**, (2) transoximation with acetone oxime to the α-ketoaldoxime **7**, (3) condensation with benzyl α-aminocyanoacetate methanesulfate to give 2-amino-3-benzyloxycarbonyl-5-(L-*erythro*-1′,2′-dihydroxypropyl)pyrazine 1-oxide (**9b**), (4) cyclization with guanidine to biopterin 8-oxide (**10**), and (5) deoxygenation with sodium dithionite. The overall yield of **1** from **3** was 12%. In analogous fashion, 6-(D-*arabino*-tetrahydroxybutyl)pterin (**16a**) and 6-(D-*threo*-trihydroxypropyl)pterin (D-*threo*-neopterin) (**16b**) were prepared starting from D-glucose and D-xylose, respectively. A much improved synthesis of the bis-sulfone **22** from L-rhamnose is also described.

L-*erythro*-Biopterin (**1**) is one of the most ubiquitous of the naturally occurring pteridines. It is found widely distributed in microorganisms, insects, algae, amphibia, and mammals[3,4] and is the most abundant of the naturally occurring pterins found in human urine.[5] In its 5,6,7,8-tetrahydro form, it functions as an essential enzyme cofactor in a number of hydroxylation and oxygenase reactions, including the conversion of phenylalanine to tyrosine,[3] tyrosine to Dopa,[6,7] melanine synthesis,[8,9] and both tryptophan[10-13] and dihydroorotic acid[14] hydroxylation. Many other biological oxidation and/or dehydrogenation reactions apparently involve tetrahydropteridine cofactors which are probably biopterin or closely related derivatives; these include the 17-α-hydroxylation of progesterone, the biosynthesis of the prostaglandins,[15] the conversion of long-chain alkyl ethers of glycerol to fatty acids and glycerol, hydroxylation of cinnamic acid to *p*-coumaric acid, the introduction of unsaturation into the carotenes and fatty acids, sterol biosynthesis, and ω oxidation of long-chain saturated fatty acids. Furthermore, there is now indirect evidence to support the postulate that either tetrahydrobiopterin and/or closely related tetrahydropterins play a critical role in cellular electron transport, including photosynthesis.[3]

This widespread utilization of biopterin in normal metabolic processes has stimulated much work aimed at its synthesis since, despite its ubiquity in nature, biopterin is unusually difficult to obtain from natural sources. Most previous syntheses have utilized the classical "Isay synthesis" of the pteridine ring system, which involves the condensation of a suitable diaminopyrimidine (in this case, 2,4,5-triamino-6-hydroxypyrimidine, **2**) with an appropriate sugar intermediate. As a consequence of this inherently ambiguous cyclization process,[16] mixtures of both biopterin (**1**) and isobiopterin (**4**) (in which the sidechain occupies the 7 position in the pteridine ring) are obtained, and separation must then be effected by laborious and often destructive chromatographic techniques. For example, condensation of **2** with 5-deoxy-L-arabinose (**3**) gives a complex mixture of products from which biopterin (**1**) and isobiopterin (**4**) can be isolated in a ratio of 15:85 (see Scheme I).[17] This unfortunate isomer ratio is apparently unaffected by a wide variety of added reagents which, in simpler examples, often enhance the amount of the desired 6 isomer relative to the undesired 7 isomer.[17-20] Attempts to improve upon this classical approach include the condensation of **2** with the *d,l*-ketose **5** (which gives *d,l*-**1** in 12% yield as part of a complex mixture)[21] and the condensation of **2** with the osone **6**,

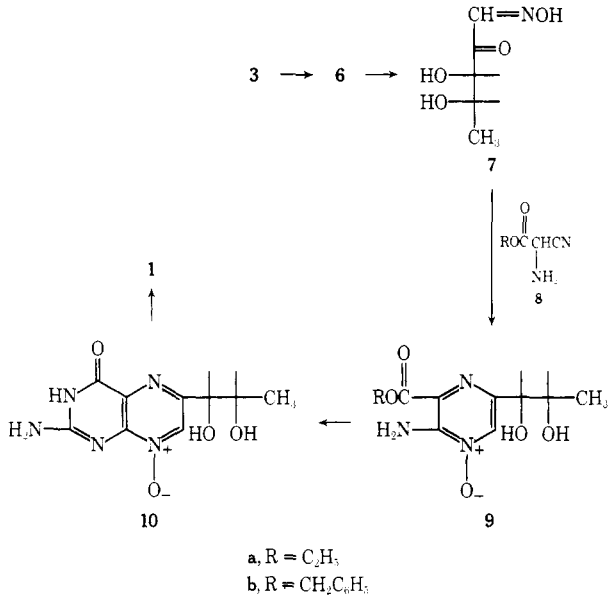
Scheme I



which gives **1** and **4** in an improved ratio of 8:2 (again separated by chromatography).[22] The only truly unequivocal synthesis of L-*erythro*-biopterin (**1**) which has been described involves the condensation of 2-amino-4-chloro-5-nitro-6-hydroxypyrimidine with 1-amino-1-deoxy-L-*erythro*-pentulose, followed by reductive cyclization and subsequent oxidation[23] (12% overall yield).

We describe in this paper a new synthesis of L-*erythro*-biopterin[24] (**1**) which utilizes our recently described new synthetic approach to 6-substituted pterins and pteridines.[16,25-28] This approach involves the condensation of an α-aminonitrile with an α-ketoaldoxime to give a 2-amino-3,5-disubstituted pyrazine 1-oxide, which is subsequently converted to a 6-substituted pteridine or pterin by an appropriate cyclization process followed by deoxygenation. Application of this concept to the synthesis of biopterin would involve the sequence of reactions depicted in Scheme II and the α-ketoaldoxime intermediate (**7**) derived from 5-deoxy-L-arabinose (**3**). Since this latter compound is itself a rare sugar, available only by degradation of naturally occurring L-rhamnose, we initiated our investigations of the feasibility of Scheme II by examination of some more readily accessible model compounds. We describe below our results with a

Scheme II

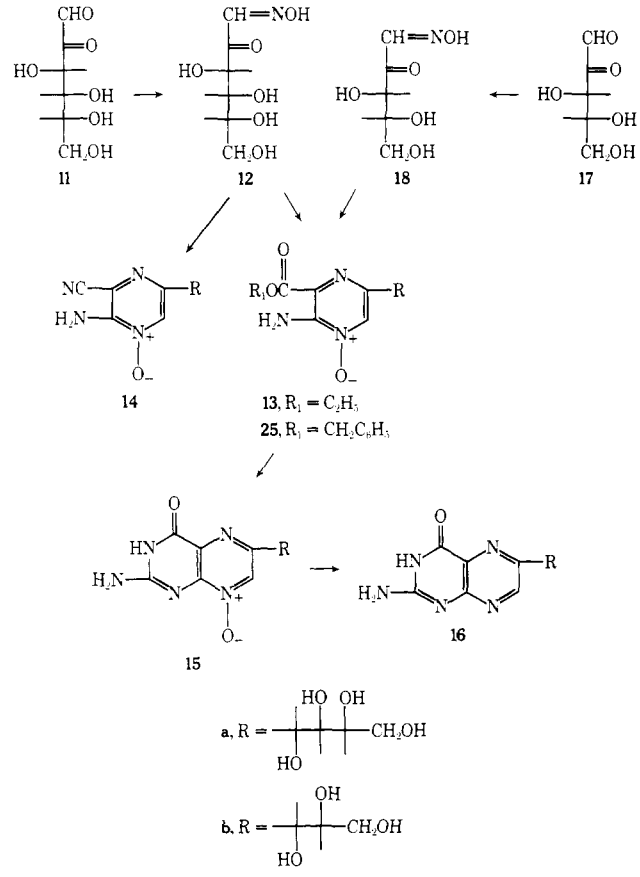


number of model systems, their eventual successful extension to the preparation of the α-keto aldoxime **7**, and its successful conversion to **1**.

We chose as our initial model sugar D-glucosone (**11**), available in relatively pure form by hydrolysis of its corresponding osazone.[29] Our first problem was the unambiguous and regioselective conversion of this α-keto aldehyde to the corresponding α-keto aldoxime **12** as a model for the projected conversion of **6** to **7**. There are apparently no reports in the literature describing the direct conversion of a glyoxal derivative to its monoaldoxime, although there are numerous references to the conversion of α-keto aldoximes (prepared by alternative procedures) to their corresponding dioximes. We were able to confirm these latter observations with phenylglyoxal, which could not, in our hands, be converted to phenylglyoxal 2-oxime with hydroxylamine; the only product isolated was phenylglyoxal dioxime. Clearly, an oximation reagent less reactive than hydroxylamine itself was required, and in the end this desired selectivity was achieved through the use of acetone oxime, a compound which has been used previously for the oximation of highly reactive aldehydes.[30] The success of this transoximation reaction is undoubtedly a reflection both of the extremely weak nucleophilicity of acetone oxime and its considerable steric bulk. Thus, even following heating at 50° over a period of 5 h and in the presence of a sixfold excess of acetone oxime, no trace of phenylglyoxal dioxime could be found (TLC); the only product formed was phenylglyoxal 2-oxime.

This technique was then successfully applied to D-glucosone (**11**) as follows. An aqueous solution of **11** was treated with a slight excess of acetone oxime at pH 4. Although there was no visible change in the reaction solution after 24 h of stirring at room temperature, solvent was removed, and the resulting bright yellow gum was dissolved in ethanol and treated with ethyl α-aminocyanoacetate (**8a**). The desired 2-amino-3-ethoxycarbonyl-5-(D-*arabino*-tetrahydroxybutyl)pyrazine 1-oxide (**13a**) separated in crystalline form. Similarly, treatment of the crude osone monoaldoxime **12** with aminomalononitrile gave the corresponding 2-amino-3-cyanopyrazine 1-oxide **14a**. Furthermore, it was found that the purity of **11** employed in these conversions had little effect on the eventual outcome of the above reaction course. Thus, D-glucosone prepared by cupric acetate oxidation of D-glucose and obtained in 40% yield as a mixture of at least eight compounds could be utilized directly in the above conversions with no significant lowering of yield of the crystalline pyrazine 1-oxide intermediate. This latter development was to have important consequences for our future work directed toward the synthesis of **1**, since it is extremely difficult to obtain the osones of sugars other than glucose by decomposition of their corresponding osazones. Finally, treatment of **13a** with guanidine in the presence of a large excess of sodium methoxide led directly to 6-(D-*arabino*-tetrahydroxybutyl)pterin 8-oxide (**15a**) whose constitution was established by periodate cleavage to pterin-6-carboxaldehyde 8-oxide, followed by reaction with hydroxylamine to give the known 6-oximinomethylpterin 8-oxide.[27] Sodium dithionite reduction of **15a** in hot pH 7 buffered aqueous solution gave 6-(D-*arabino*-tetrahydroxybutyl)pterin (**16a**).[31]

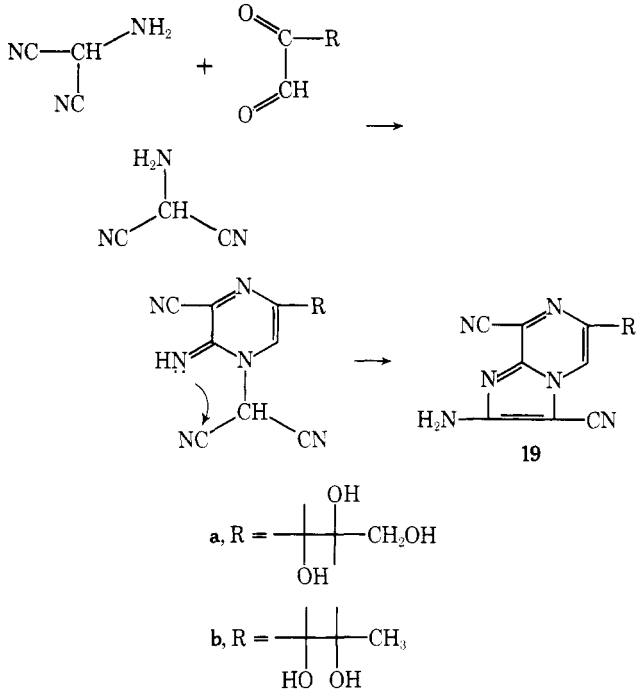
Scheme III



In order to establish the generality of these conversions, a second series of model experiments was examined. Thus, D-xylosone (**17**), available in 60% yield by cupric acetate oxidation of D-xylose,[32] was treated overnight with acetone oxime under conditions identical with those described above for the conversion of **11** to **12**. Again, following the removal of solvent, the residual gum was dissolved in ethanol and treated with ethyl α-aminocyanoacetate (**8a**). To our disappointment, there was no indication that the desired 2-amino-3-ethoxycarbonyl-5-(D-*threo*-trihydroxypropyl)pyrazine 1-oxide (**13b**) had been formed. The inescapable conclusion was that the transoximation reaction had failed on **17** despite the successful conversion under identical reaction conditions of **11** to **12**.

It was apparent that some independent means of assessing the progress of the transoximation reaction was desirable, and this fortuitously became available as a result of an incidental observation made in the course of some preliminary cyclization reactions attempted with **17**. We had made

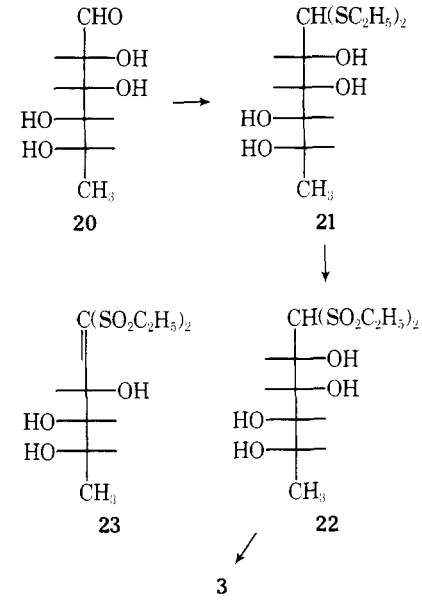
the intriguing observation that the osone **17** reacted with aminomalononitrile to give a highly fluorescent compound which, on the basis of analytical and spectroscopic evidence, appears to possess structure **19a.** Analogous derivatives are



obtained by reaction of aminomalononitrile with phenylglyoxal or with methylglyoxal.[33] The intense fluorescence of these imidazopyrazines provided the key for monitoring the conversion of the osone **17** to the corresponding α-keto aldoxime **18.** Thus, periodic aliquots of the mixture of the osone **17** with acetone oxime were withdrawn and tested with excess aminomalononitrile, and this procedure was continued until a reaction aliquot revealed none of the highly fluorescent **19a** (by TLC). At this point it was assumed that the transoximation process was complete. We were thus able to determine that the conversion of D-xylosone (**17**) to its corresponding monoaldoxime (**18**) could be successfully effected in a period of 3 h by heating at 50° in water at pH 7 in the presence of a threefold excess of acetone oxime. Excess reagent was then removed by extraction, and the desired pyrazine 1-oxide (**13b**) was then prepared by condensation with ethyl α-aminocyanoacetate (**8a**). Finally, cyclization of **13b** with guanidine in the presence of a large excess of sodium methoxide led directly to 6-(D-*threo*-trihydroxypropyl)pterin 8-oxide (**15b**) which, upon reduction with sodium dithionite, gave 6-(D-*threo*-trihydroxypropyl)pterin (D-*threo*-neopterin) (**16b**).[34] We were thus ready to attempt the extension of these studies on model systems to biopterin itself.

5-Deoxy-L-arabinose (**3**) is normally prepared by degradation of naturally occurring L-rhamnose (**20**), and although a variety of approaches have been explored to effect this degradation, by far the most convenient appeared to be that developed by Hough and Taylor.[35] This route involves the preparation of L-rhamnose diethylmercaptal (**21**), subsequent oxidation with aqueous perpropionic acid to give 1,1-diethylsulfonyl-L-manno-2,3,4,5-tetrahydroxyhexane (**22**), and final cleavage with dilute ammonium hydroxide to give the desired **3** (see Scheme IV). Unfortunately, however, a major by-product in the oxidation of **21** to **22** is the vinyl bis-sulfone **23,** which in turn is only very slowly cleaved to **3**. Normally, this mixture must therefore be separated prior to further conversions, and, as a result, pure 5-deoxy-L-arabinose has been available in only very limited

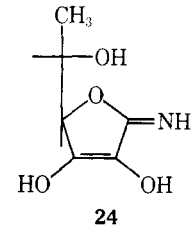
Scheme IV



quantities. In contrast, we have found that the oxidation of **21** to **22** can be effected in 97% yield, without contamination by the vinyl bis-sulfone **23,** through the use of *m*-chloroperbenzoic acid in anhydrous dioxane. 5-Deoxy-L-arabinose (**3**) was then obtained by treatment of **22** with dilute ammonium hydroxide at room temperature, followed by deionization with Amberlite IR-120 and IR-4B resins. Using this modified procedure, it was eventually possible to prepare 5-deoxy-L-arabinose routinely on a 30-g scale.

The next step projected for the synthesis of biopterin (see Scheme II) was the oxidation of **3** to 5-deoxy-L-arabinosone (**6**), and it was anticipated that this conversion could be effected with cupric acetate, as we had previously observed both with D-glucose and with D-xylose. Initial efforts in this direction were disappointing, however, and typically resulted in conversions of less than 10%.

The oxidation of carbohydrates to osones with cupric acetate is a complex reaction, and one for which even moderate success requires a subtle manipulation of a number of experimental variables. The major problem encountered in the proper adjustment of these variables is the estimation of the progress of the reaction, i.e., in the present instance, a determination of the amount of **6** present in the complex reaction mixture. The traditional method for carrying out this analysis has involved precipitation of the osone as its phenylosazone, but this turned out both to be time consuming and unreliable. A technique which was eventually found to be far superior involved the initial conversion of the osone to imino-5-deoxy-L-araboascorbic acid (**24**) by treatment of



24

the crude oxidation mixture with sodium cyanide and subsequent titration of the enediol functionality present in **24** with iodine. Each of these steps has been shown to proceed virtually instantaneously and in quantitative yield with other sugar osones,[36] and by suitable calculation the initial concentration of osone **6** could thus be easily determined. Using this procedure, which rapidly and consistently gave quantitative data reproducible to within ±2%, it was possi-

ble to carry out a large number of trial oxidations in sequence on **3**, and eventually conditions were found which allowed the preparation of **6** in yields comparable to those obtained with D-glucose.

The next step in the projected sequence leading to biopterin (see Scheme II) was the conversion of 5-deoxy-L-arabinosone (**6**) to the α-keto aldoxime **7**. The transoximation reaction with acetone oxime appeared to be proceeding successfully, as judged by the absence of the highly fluorescent imidazolopyrazine **19b** upon treatment of an aliquot of the reaction mixture with aminomalononitrile, but subsequent addition of ethyl α-aminocyanoacetate (**8a**) failed to give isolable amounts of 2-amino-3-ethoxycarbonyl-5-(L-*erythro*-1′,2′-dihydroxypropyl)pyrazine 1-oxide (**9a**). Although it appeared from TLC and spectral evidence that this latter pyrazine was indeed being formed, it could not be isolated from the reaction mixture. The usual technique of extraction of simple 2-amino-3-ethoxycarbonyl 5-substituted pyrazine 1-oxides from aqueous solution using organic solvents was not applicable in the present instance because of the very high solubility of **9a** in water.

A possible resolution of this dilemma appeared to lie in modifying the structure of **9** in such a manner as to either decrease its water solubility or render it more likely to crystallize from the organic solvents utilized in its preparation. There are, however, a limited number of such modifications which are compatible with the requirement that **9** function as a precursor for the eventual synthesis of biopterin. An obvious possibility was the use of esters other than ethyl α-aminocyanoacetate in the condensation reaction leading to **9**. In fact, a number of such esters were prepared and subjected to rigorous testing in model systems, and of these, benzyl α-aminocyanoacetate (**8b**) appeared to be the most promising. For example, condensation of **8b** with pyruvaldehyde 1-oxime led conveniently to 2-amino-3-benzyloxycarbonyl-5-methylpyrazine 1-oxide which was readily obtained simply by pouring the reaction mixture into water, from which the highly insoluble product separated in high yield. By contrast, the corresponding ethyl ester failed to separate from water and had to be isolated by extraction with organic solvents.[16] Similarly, D-xylosone monoaldoxime (**18**) reacted smoothly with **8b** to give crystalline 2-amino-3-benzyloxycarbonyl-5-(D-*threo*-trihydroxypropyl)pyrazine 1-oxide (**25b**) (see Scheme III); in contrast to the ethyl ester **13a**, the benzyl ester was conveniently purified from residual starting materials and inorganic impurities by washing with water. Subsequent guanidine cyclization proceeded normally to give **15b**, and thus this sequence of reactions utilizing the benzyl rather than the ethyl ester again proved much more convenient.

Returning to the synthesis of biopterin, it was gratifying to find that the use of benzyl α-aminocyanoacetate (**8b**) satisfactorily resolved the problem of isolation of the intermediate pyrazine 1-oxide **9**. Thus, condensation of **8b** with the monoaldoxime **7** proceeded smoothly to give **9b** as a crystalline intermediate.[37] Cyclization with guanidine in the presence of a large excess of sodium methoxide then gave 6-(L-*erythro*-1′,2′-dihydroxypropyl)pterin 8-oxide (biopterin 8-oxide) (**10**) which, on reduction with sodium dithionite in aqueous solution buffered to pH 7, gave L-*erythro*-biopterin (**1**), identical in all respects with the naturally occurring enzyme cofactor.

Experimental Section[38]

D-Glucosazone. The following procedure is modified from that of Richtmyer.[39] A solution of 60 g (0.33 mol) of anhydrous D-glucose and 130 ml (1.32 mol) of phenylhydrazine in 3 l. of water containing 80 ml of glacial acetic acid was heated at 80° with efficient stirring for a period of 3 h. During this time the desired osazone slowly separated as a pale-yellow, fluffy solid which, after cooling to room temperature, was collected and washed successively with 10% aqueous acetic acid, water, cold ethanol, and finally ether. Final purification was accomplished by crystallization from 20 parts of 50% aqueous pyridine to yield 60 g (50%) of D-glucosazone, mp 208–209° (lit.[39] mp 208–209°).

D-Glucosone (11). The following procedure is modified from that of Bayne.[29] A suspension of 20 g (0.056 mol) of D-glucosazone in 600 ml of absolute ethanol was mixed with 1.0 l. of water in a 3-l. round-bottomed, three-necked flask fitted with a sealed stirrer, condenser, and dropping funnel. Glacial acetic acid (12 ml) and freshly distilled benzaldehyde (32 ml, 0.32 mol) were added, and the mixture was refluxed vigorously with efficient stirring. After 2–3 h, all starting material had dissolved, and from this point reflux was continued an additional 1.5 h. The condenser was then reversed, and 600 ml of distillate was collected during a period of about 1 h, with the concurrent addition of 1.0 l. of water. After this time, the mixture was cooled overnight to complete the precipitation of benzaldehyde phenylhydrazone. The reaction mixture was then filtered with suction, and the solid residue was washed with two 200-ml portions of water. The combined filtrate and washings were concentrated to about 400 ml under reduced pressure (bath temperature 40°) and extracted with six 75-ml portions of ether, then warmed to 60° to remove dissolved ether, and stirred for 10 min with Darco G-60 activated carbon. Following the removal of carbon by suction filtration, the slightly yellow filtrate was evaporated under reduced pressure (bath temperature 40°) to a thick syrup. This syrup was dissolved in 10 ml of water, and 200 ml of hot absolute ethanol was added slowly with thorough mixing. After standing 10 min, any precipitate was removed by filtration, and the filtrate was concentrated and dried (by repeated dissolution and concentration from absolute methanol) to give 6.3 g (63%) of very pale yellow **11** as a thick syrup.

Phenylglyoxal 2-Oxime. A solution of 1.0 g (6.6 mmol) of phenylglyoxal monohydrate in 10 ml of water was adjusted to pH 4 and stirred at room temperature in the presence of 0.5 g (6.8 mmol) of acetone oxime. After stirring overnight, the colorless precipitate was filtered, washed with water, and dried to yield 0.9 g (91%) of colorless crystals, identical in all respects with an authentic sample.[40] No trace of phenylglyoxal dioxime could be detected in the crude reaction mixture (TLC), even following heating at 50° over a period of 5 h.

D-Glucosone Aldoxime (12). A solution of 5.0 g (0.028 mol) of D-glucosone (**11**) in 30 ml of water was adjusted to pH 4 with dilute ammonium hydroxide and stirred for 24 h at room temperature in the presence of 2.3 g (0.032 mol) of acetone oxime. After this period, the reaction mixture was extracted with three 20-ml portions of ether and evaporated under reduced pressure (bath temperature 40°) to complete dryness with repeated dissolution and concentration from absolute ethanol. The pale-yellow gum obtained was used in the following reaction without further purification.

2-Amino-3-cyano-5-(D-*arabino*-tetrahydroxybutyl)pyrazine 1-Oxide (14a). A solution of 5.0 g (0.026 mol) of D-glucosone aldoxime (**12**) in 50 ml of absolute ethanol was treated with 9.0 g (0.036 mol) of finely powdered aminomalononitrile tosylate, and the resulting suspension was stirred at room temperature. Solution (dark red) was generally complete within 1 h, and the precipitation of **14a** began shortly thereafter. After stirring a total of 36 h, the very thick suspension obtained was filtered and pressed dry as thoroughly as possible. The crude material was then washed with ice-cold ethanol and dried to yield 4.6 g (64% overall from **11**) of a pale beige solid. The analytical sample crystallized from ethanol in the form of slightly off-white clusters of needles: mp 235° dec; NMR Me_2SO-d_6 δ 8.28 (1, s, C_6-H), 7.69 (2, br s, NH_2), 5.22–4.42 (5, m, CH).

2-Amino-3-ethoxycarbonyl-5-(D-*arabino*-tetrahydroxybutyl)pyrazine 1-Oxide (13a). Method A. A solution of 5.0 g (0.026 mol) of D-glucosone aldoxime (**12**) in 50 ml of absolute ethanol was treated with 9.0 g (0.030 mol) of ethyl α-aminocyanoacetate (**8a**) tosylate, and the resulting suspension was stirred at room temperature for 36 h. During this period of time all starting materials slowly passed into solution (deep red), and the desired product separated as a pale yellow, flocculent precipitate. The material was collected, washed with ice-cold ethanol, and dried to yield 4.7 g of **13a** (55% overall from **11**), which after recrystallization from methanol melt-

ed at 203–205°. NMR (Me_2SO-d_6) δ 8.28 (1, s, C_6-H), 7.42 (2, br s, NH_2), 5.40–4.00 (5, m, CH), 4.37 (2, q), 1.31 (3, t, COOEt).

Method B. To a boiling solution prepared from 14.4 g (0.080 mol) of anhydrous D-glucose in 30 ml of water and 750 ml of methanol was added 60 g (0.30 mol) of cupric acetate hydrate, and the suspension was boiled an additional 10 min before cooling and filtering to remove cuprous oxide. Excess cupric acetate in the filtrate was precipitated with hydrogen sulfide, and the resulting thick suspension was filtered through Celite and washed with methanol. The combined filtrate and washings were concentrated under reduced pressure (bath temperature 40°) to a clear, bright-yellow syrup, from which acetic acid was removed by three concentrations from 200-ml portions of water. The amount of D-glucosone (**11**) present in this mixture was estimated at 6.0 g (40%) by treatment of a small aliquot with phenylhydrazine in dilute acetic acid, and recovery of the D-glucosazone thus produced. The crude syrup was then taken up in 30 ml of water and adjusted to pH 4 by the dropwise addition of concentrated ammonium hydroxide. Acetone oxime (12.6 g, 0.17 mol) was added, and the resulting solution was heated in an oil bath maintained at 40° for 24 h. After this period of time, the reaction mixture was clarified with a small amount of charcoal and then extracted with six 30-ml portions of ether to remove excess acetone oxime before concentrating in vacuo (bath temperature 40°) to a bright-yellow gum. This material was dried thoroughly first by repeated dissolution and concentration from absolute methanol and finally under high vacuum to remove any traces of water. The crude D-glucosone aldoxime (**12**) thus prepared was covered with 50 ml of absolute ethanol, to which was added 12.0 g (0.040 mol) of ethyl α-aminocyanoacetate (**8a**) tosylate. The resulting gummy suspension was warmed gently to dissolve most of the suspended materials, and it was then allowed to stir at room temperature for 36 h to give a thick precipitate which was collected by filtration, washed with ice-cold ethanol, and dried to give 5.1 g (51% overall based on **11**) of **13a**, identical in all respects with the material obtained following method A above.

6-(D-*arabino*-Tetrahydroxybutyl)pterin 8-Oxide (15a). To a solution of 0.33 g (1.09 mmol) of 2-amino-3-ethoxycarbonyl-5-(D-*arabino*-tetrahydroxybutyl)pyrazine 1-oxide (**13a**) in 4 ml of anhydrous DMF were added, in order, 0.22 g (2.30 mmol) of guanidine hydrochloride and 0.32 g (5.93 mmol) of freshly prepared sodium methoxide. The suspension was then heated overnight in an oil bath maintained between 70 and 75°, after which time any residual solid material was dissolved by the addition of 6 ml of water. The deep-orange solution was clarified to a bright yellow with a small amount of activated carbon, and the pH was adjusted to 3–4. After cooling overnight to complete precipitation, the bright-yellow microcrystalline solid was collected by filtration, washed with a small amount of ice-water, and dried to give 0.19 g (59%) of chromatographically homogeneous material (R_f 0.67 in 0.5% potassium carbonate). The analytical sample, crystallized from 20% aqueous acid, had mp >300°, with darkening above 250°. Uv λ_{max} (0.1 N NaOH) nm (log ε): 387 (3.95), 297 sh (3.90), 268 (4.53).

A sample of 0.07 g (0.23 mmol) of the material prepared as described above was added slowly to a solution of 0.17 g (0.74 mmol) of potassium periodate in 8 ml of 1 N sulfuric acid to give a bright-yellow solution. After stirring 30 min, the pH was adjusted to 5–6, and the resulting suspension was allowed to stand overnight. The granular precipitate (0.03 g) was then collected and treated with an excess of hydroxylamine in pyridine to give 6-oximinomethylpterin 8-oxide, identical in all respects with an authentic sample.[27]

6-(D-*arabino*-Tetrahydroxybutyl)pterin (16a). A suspension of 100 mg (0.33 mmol) of 6-(D-*arabino*-tetrahydroxybutyl)pterin 8-oxide (**15a**) in a buffered solution prepared from 24 ml of distilled water and 1 ml of Beckman concentrated pH 7 buffer was stirred under gentle reflux until all material had dissolved. Sodium dithionite (65.0 mg, 0.37 mmol) was then added to the resulting bright-yellow solution, and stirring and heating were continued for an additional 30 min. During this period of time, the color of the reaction mixture slowly clarified to a pale yellow with the concomitant separation of the desired product. After cooling several hours, the pale-yellow precipitate of **16a** was collected, washed with ice-water, and dried to give 62 mg (65%) of chromatographically homogeneous material (R_f 0.16 in 1% NH_3/1-propanol (1:2) (lit.[31] R_f 0.16)). The analytical sample crystallized from 20% aqueous acetic acid as a pale-yellow microcrystalline solid which decomposed slowly above 220°. Uv λ_{max} (0.1 N NaOH) nm (log ε): 255 (4.37), 365 (3.87).

D-Xylosone (17). A solution of 1.2 g (8.0 mmol) of D-xylose in 3 ml of water was diluted with 75 ml of methanol, and after heating with stirring to a vigorous boil, 6.0 g (30.1 mmol) of cupric acetate hydrate was added in one portion. The resulting suspension was boiled an additional 10 min before cooling and filtering through Celite to remove cuprous oxide. The mother liquors were then filtered through a 2.5-cm column packed with 14 cm of Dowex 50-WX4 prepared in the usual manner (followed by thorough washing with methanol). The column was eluted with an additional 200 ml of methanol, and the clear colorless eluate was concentrated at 40° (10 Torr) to a pale-yellow syrup, from which residual acetic acid was removed by concentrating from two 200-ml portions of water. In a blank experiment, the yield of D-xylosone was determined as follows. The syrupy osone from 1.2 g (8.0 mmol) of D-xylose was dissolved in 25 ml of water, and the solution was neutralized with 1 N NaOH. After passing nitrogen through the solution for 10 min, a threefold excess of sodium cyanide (1.2 g, 24.5 mmol) was added to react with the D-xylosone to form imino-D-ascorbic acid.[36] Nitrogen was bubbled through the solution for an additional 20 min, with stirring, and the solution was then acidified to congo red (pH 3–5) with 6 N HCl. Excess cyanide was removed by a stream of nitrogen, and the sample was titrated with a 0.1 N methanolic solution of iodine.

ml of 0.1 N I_2 required	43
theory	80
% osone = 54	

3,7-Dicyano-2-amino-5-(D-*threo*-1,2,3-trihydroxypropyl)imidazo[1,2-*a*]pyrazine (19a). A solution of 0.60 g (0.004 mol) of D-xylosone and 1.14 g (0.009 mol) of aminomalononitrile tosylate in 5 ml of ethanol was stirred at room temperature overnight. The yellow solid which had separated was collected by filtration, washed with ether, and recrystallized from ethanol to give 0.12 g (10%) of **19a:** mp 220–223° dec; uv λ_{max} (C_2H_5OH) nm (log ε) 257 (2.66), 390 (2.16); ir 2210 cm^{-1}.

2-Amino-3-ethoxycarbonyl-5-(D-*threo*-trihydroxypropyl)pyrazine 1-Oxide (13b). The syrupy osone from 1.2 g (8.0 mmol) of D-xylose was taken up in 5 ml of water, and the solution was adjusted to pH 7 by the dropwise addition of concentrated ammonium hydroxide. Acetone oxime (2.0 g, 27.4 mmol) was then added, and the resulting solution was stirred in an oil bath maintained at 50°. After a period of 3 h, the reaction mixture was clarified to a bright yellow with 20 mg of Darco G-60, diluted with an additional 15 ml of water, and extracted with three 10-ml portions of ether to remove excess acetone oxime. Concentration at 40° (10 Torr) then gave a heavy bright-orange gum which was dried under high vacuum for 2 h. The resulting material, consisting of crude D-xylosone aldoxime (**18**), was covered with 10 ml of absolute ethanol and warmed gently to effect partial solution. Ethyl α-aminocyanoacetate (**8a**) tosylate (1.4 g, 4.7 mmol) was added, and the mixture was stirred at room temperature for 24 h. During this period, all starting materials passed into solution, and the desired product separated. The reaction mixture was cooled for several hours, filtered, washed with ether, and dried to give 0.80 g of pale-yellow **13b,** contaminated with a small amount of ammonium tosylate. Crystallization of the crude material from ethanol gave 0.55 g (46% overall based on **17**) of **13b** as elongated fibers: mp 180–182°; NMR (Me_2SO-d_6) δ 8.30 (1, s, C_6-H); 7.47 (br s, NH_2); 5.30–3.40 (4, m, CH); 4.34 (2, q), 1.35 (3, t, COOEt).

6-(D-*threo*-Trihydroxypropyl)pterin 8-Oxide (15b). This material was prepared in 61% yield from 0.30 g (1.09 mmol) of 2-amino-3-ethoxycarbonyl-5-(D-*threo*-trihydroxypropyl)pyrazine 1-oxide (**13b**), 0.22 g (2.30 mmol) of guanidine hydrochloride, and 0.32 g (5.93 mmol) of sodium methoxide as described above for the analogous preparation of **15a;** R_f 0.68 in 0.5% potassium carbonate. The analytical sample, crystallized from 20% aqueous acetic acid, decomposed slowly over a range of 220–300°. Uv λ_{max} (0.1 N NaOH) nm (log ε): 387 (3.94), 294 sh (3.90), 265 (4.54).

A sample of the above material, when treated with hydrated periodic acid followed by an excess of hydroxylamine, gave 6-oximinomethylpterin 8-oxide as previously described (cf. **15a**).

6-(D-*threo*-Trihydroxypropyl)pterin (16b). This material was prepared in 59% yield from 100 mg (0.37 mmol) of 6-(D-*threo*-trihy-

droxypropyl)pterin 8-oxide (**15b**) and 70 mg (0.40 mmol) of sodium dithionite as described above for the preparation of **16a**; R_f 0.25 in 1% NH_3–1-propanol (1:2). The analytical sample crystallized from 20% aqueous acetic acid as a white microcrystalline solid which decomposed slowly above 230°. Uv λ_{max} (0.1 N NaOH) nm (log ϵ): 2.55 (4.38), 3.65 (3.88).

Benzyl α-Aminocyanoacetate (8b) Methanesulfonate. Heavy-duty aluminum foil (4.2 g of 1 cm^2 pieces) was amalgamated for 2 min with 100 ml of 2% mercuric chloride, and the resulting amalgam was washed twice with water, then with ethanol, tetrahydrofuran, and ether and was finally covered with 100 ml of tetrahydrofuran. A solution of 20 g (0.10 mol) of benzyl α-oximinocyanoacetate[41] in 120 ml of ether was added to the amalgam (in a 500-ml three-necked, round-bottomed flask equipped with addition funnel and efficient reflux condenser), and the mixture was stirred vigorously during the addition of 6 ml of water at a rate just sufficient to maintain reflux. After addition was complete (0.5 h), the resulting slurry of aluminum salts was cooled briefly, and the reaction contents were filtered through a pad of Celite (large funnel), the collected salts being washed thoroughly with 250-ml portions each of tetrahydrofuran and ether. The combined filtrate and washings were then treated with 12.0 g (0.12 mol) of methanesulfonic acid with vigorous stirring, and the nearly colorless solution obtained was diluted slowly to 1000 ml with ether to induce the crystallization of **8b**. The mixture was stirred an additional 30 min at room temperature and then chilled briefly before filtering, washing with ether, and drying to give 15.3 g (53.5%) of snow-white **8b** methanesulfonate, mp 157–160°. The melting point was raised to 164–166° by crystallization from ethanol–ether.

The properties of the hydrochloride salt of **8b** (mp 92–94°) were identical with those reported.[42] This method for the preparation of **8b** represents a marked improvement over the previously published procedure.[42]

2-Amino-3-benzyloxycarbonyl-5-methylpyrazine 1-Oxide. A solution of 0.50 g (5.75 mmol) of pyruvaldehyde 1-oxime and 0.85 g (2.97 mmol) of benzyl α-aminocyanoacetate (**8b**) methanesulfonate in 5 ml of anhydrous methanol was stirred in an oil bath maintained at 35° for 24 h. During this period, the desired product partially separated in the form of bright-yellow elongated needles. The reaction mixture was then poured into 50 ml of ice–water and allowed to stand for 1 h before filtering, washing with water, and drying, yield 0.62 g (81%). The analytical sample, mp 158–160°, was recrystallized from methanol: NMR ($CDCl_3$) δ 8.13 (1, s, C_6-H); 7.4 (2, br s, NH_2); 7.32 (5, s, –Ph); 5.33 (2, s, CH_2Ph); 2.43 (3, s, C_5–CH_3).

The above material, on cyclization with guanidine under previously described conditions,[16] gave 6-methylpterin 8-oxide, identical with an authentic sample prepared from 2-amino-3-ethoxycarbonyl-5-methylpyrazine 1-oxide.

2-Amino-3-benzyloxycarbonyl-5-(D-*threo*-trihydroxypropyl)-pyrazine 1-Oxide (25b). To a solution of crude D-xylosone aldoxime (**18**) in ethanol, prepared from 1.2 g (8.0 mmol) of D-xylose as described above (*cf.* **13b**), was added 1.4 g (4.9 mmol) of benzyl α-aminocyanoacetate (**8b**) methanesulfonate, and the mixture was stirred at room temperature for 24 h. During this period, all starting materials passed into solution, and the desired product separated. The reaction mixture was cooled for several hours, filtered, washed with ice-cold ethanol and water, and dried to give 0.68 g (46% overall from **17**) of ale-yellow **25b**. The analytical sample crystallized from ethanol in the form of white microcrystalline clusters of needles: mp 173–174°; NMR (Me_2SO-d_6) δ 8.30 (1, s, C_6-H), 7.48 (2, br s, NH_2), 7.32 (5, s, Ph), 5.33 (2, s, CH_2Ph); 5.3–3.4 (4, m, CH).

L-Rhamnose Diethylmercaptal (21). L-Rhamnose (**20**) hydrate (66 g, 0.36 mol) was added with vigorous stirring to an ice-cold suspension of 100 ml of ethanethiol in 67 ml of concentrated HCl. The product began to precipitate within a few minutes, and after 10 min the reaction mixture had completely solidified. The solid mass was broken up with a glass rod, filtered, and washed with ice-water to remove traces of colored material. The colorless product, after drying under high vacuum overnight, weighed 84 g (81%). Recrystallization from water gave elongated plates, mp 137–138° (lit.[43] mp 137–138°).

1,1-Diethylsulfonyl-L-*manno*-2,3,4,5-tetrahydroxyhexane (22). L-Rhamnose diethylmercaptal (**21**, 25 g, 0.092 mol) was dissolved in 250 ml of anhydrous dioxane with gentle warming. The resulting solution was cooled to 10° in an ice bath, and 80 g (0.46 mol) of 98% *m*-chloroperbenzoic acid was added in small portions, with the temperature maintained between 15 and 20°. A precipitate began to form after 20–30 min, but a clear solution was obtained after addition was complete (45 min). After 15 min, the solution was allowed to warm to room temperature and stirred an additional 3 h, during which time the product partially separated. The mixture was then diluted with 250 ml of ether and cooled briefly to 0°. After filtering, washing thoroughly with ether, and drying, the pure white product weighed 30 g (97%), mp 183–184° (lit.[35] mp 178–180°).

5-Deoxy-L-arabinose (3) Hydrate. Thirty grams (0.090 mol) of 1,1-diethylsulfonyl-L-*manno*-2,3,4,5-tetrahydroxyhexane (**22**) was dissolved in dilute ammonium hydroxide (250 ml, pH 9–10), and within a few minutes diethylsulfonylmethane (mp 101–102°) separated. The mixture, which slowly turned a very pale yellow, was stirred at room temperature for 16 h, diethylsulfonylmethane was filtered off, and the filtrate was deionized with Amberlite IR-120 and IR-4B resins. After continuous extraction with chloroform for 6 h to remove any residual diethylsulfonylmethane, the colorless aqueous solution was concentrated in vacuo (bath temperature 40°) to give a pale-yellow syrup of **3**·H_2O. After several concentrations from absolute methanol and finally drying under high vacuum, the syrup weighed 13 g (96%); phenylosazone mp 171–173° (lit.[35] mp 172–174°).

5-Deoxy-L-arabinosone (6). Ethanol (75 ml) was added to a solution of 1.2 g (7.9 mmol) of 5-deoxy-L-arabinose (**3**) hydrate in 6 ml of water. The solution was brought to a vigorous boil, and 12.0 g (60.1 mmol) of cupric acetate hydrate was added in one portion with efficient stirring. The resulting suspension was boiled for exactly 7 min and then chilled immediately by stirring in an ice bath. After 1 h, the precipitated cuprous oxide and excess cupric acetate were removed by filtration and washed thoroughly with methanol; the washings were added to the initial filtrate. The combined mother liquors were then filtered through a 2.5-cm column packed with 14 cm of Dowex-50WX4 prepared in the usual manner (followed by thorough washing with methanol). The column was eluted with an additional 200 ml of methanol, and the resulting clear, colorless solution was concentrated in vacuo (bath temperature 40°) to a pale-yellow syrup, from which residual acetic acid was removed by concentration from two 200-ml portions of water. The yield of **6** was determined as 40% by conversion to imino 5-deoxy-L-araboascorbic acid (**24**) and titration with 0.1 N iodine (cf. D-xylosone, **17**).

2-Amino-3-benzyloxycarbonyl-5-(L-*erythro*-1′,2′-dihydroxypropyl)pyrazine 1-Oxide (9b). The syrupy osone from 1.2 g (7.9 mmol) of 5-deoxy-L-arabinose (**6**) hydrate, prepared as described above, was taken up in 3 ml of water and adjusted to a pH of exactly 3.5 by the dropwise addition of 5% ammonium hydroxide. Acetone oxime (0.6 g, 8.2 mmol) was then added, and the resulting solution was stirred in an oil bath maintained at 50°. After a period of 6 h, the reaction mixture was diluted with an additional 15 ml of water and extracted with three 10-ml portions of ether to remove excess acetone oxime. Concentration at 40° (10 Torr) then gave a bright yellow gum which was dried under high vacuum for 2 h. This material, consisting of crude 5-deoxy-L-arabinosone aldoxime (**7**), was covered with 10 ml of absolute ethanol and warmed gently to effect solution. Benzyl α-aminocyanoacetate (**8b**) methane sulfonate (1.1 g, 3.8 mmol) was added, and the mixture was stirred at room temperature for 36 h. During this period of time, all starting materials passed into solution and a small amount of the desired product separated. The reaction mixture was diluted with 15 ml of ether and chilled overnight, and the precipitate was filtered, washed with ether and water, and dried to give 0.46 g (47% overall from **6**) of a light-beige solid. The analytical sample crystallized from ethanol as a pale-yellow microcrystalline solid: mp 165–166°; NMR (Me_2SO-d_6) δ 8.32 (1, s, C_6-H), 7.53 (2, br s, NH_2), 7.35 (5, s, Ph), 5.31 (2, s, CH_2), 4.53–3.22 (4, m, CHOH–CHOH), 0.91 (3, d, CH_3).

6-(L-*erythro*-1′,2′-Dihydroxypropyl)pterin 8-Oxide (10b). This compound, mp >300°, was prepared in 76% yield from 0.35 g (1.09 mmol) of 2-amino-3-benzyloxycarbonyl-5-(L-*erythro*-1′,2′-dihydroxypropyl)pyrazine 1-oxide (**9b**), 0.22 g (2.30 mmol) of guanidine hydrochloride, and 0.32 g (5.93 mmol) of sodium methoxide, as described above for the analogous preparation of **15a**; R_f 0.31 on Whatman no. 1 paper, 30-cm path, butanol–acetic acid–

water (50:15:35). It was analytically pure as obtained directly from the reaction mixture but could be recrystallized with little or no loss from 20% aqueous acetic acid. Uv λ_{max} (0.1 N NaOH) nm (log ϵ) 385 (3.95), 295 sh (3.90), 265 (4.54).

L-*erythro*-Biopterin (1). This compound was prepared in 83% yield by reduction of 6-(L-*erythro*-1′,2′-dihydroxypropyl)pterin 8-oxide (**10**) with sodium dithionite, as described above for the preparation of **16a**; R_f 0.60 in 1:1 1-propanol–water. Synthetic biopterin, crystallized from 20% aqueous acetic acid, was obtained as a pale-yellow microcrystalline solid and had the same chromatographic behavior in three different solvent systems as authentic samples of the natural product[23,44] and with a sample of synthetic L-*erythro*-biopterin prepared by the alternative unequivocal route.[23] Its mass spectrum was superimposable with the published spectrum.[45] Uv λ_{max} (0.1 N NaOH) nm (log ϵ) 254 (4.37), 365 (3.86).

The biological activity of this synthetic L-*erythro*-biopterin was determined as follows:[46] it was reduced in situ[47] and quantitated via spectrophotometric measurements.[47,48] The tetrahydrobiopterin (BH_4) was demonstrated to be considerably more potent than synthetic dimethyltetrahydropterin ($DMPH_4$) as a cofactor for tyrosine hydroxylase (TH). This was established for partially purified preparations of TH from bovine adrenal medulla, guinea pig adrenal, and guinea pig brain. In each case the marked increase in TH activity observed with BH_4 as compared with $DMPH_4$ was associated both with an increased V_{max} and a decreased K_m.[49] The superiority of BH_4 supports the suggestion that it is the naturally occurring cofactor for TH as has been established for liver phenylalanine hydroxylase by Kaufman.[50]

References and Notes

(1) For the previous paper in this series, see E. C. Taylor, R. F. Abdulla, K. Tanaka, and P. A. Jacobi, *J. Org. Chem.*, **40,** 2341 (1975).
(2) This work was supported by a grant (CA-12876) to Princeton University from the National Cancer Institute, National Institutes of Health, Public Health Service.
(3) For a discussion and references, see H. Rembold and W. L. Gyure, *Angew. Chem., Int. Ed. Engl.*, **11,** 1061 (1972).
(4) W. Pfleiderer, *Angew. Chem., Int. Ed. Engl.*, **3,** 114 (1964).
(5) T. Fukushima and T. Shiota, *J. Biol. Chem.*, **247,** 4549 (1972).
(6) T. Nagatsu, M. Levitt, and S. Udenfriend, *J. Biol. Chem.*, **239,** 2910 (1964).
(7) T. Lloyd and N. Weiner, *Mol. Pharmacol.*, **7,** 569 (1971).
(8) N. Kokolis and I. Ziegler, *Z. Naturforsch., Teil B,* **23,** 860 (1968).
(9) I. Ziegler, *Z. Naturforsch., Teil B,* **18,** 551 (1963).
(10) E. M. Gal, J. C. Armstrong, and B. Ginsberg, *J. Neurochem.*, **13,** 643 (1966).
(11) S. Hosoda and D. Glick, *J. Biol. Chem.*, **241,** 192 (1966).
(12) W. Lovenberg, E. Jequier, and A. Sjoerdsma, *Science,* **155,** 217 (1967).
(13) T. Noguchi, M. Nishino, and R. Kido, *Biochem. J.,* **131,** 375 (1973).
(14) G. W. Kidder and L. L. Nolan, *Biochem. Biophys. Res. Commun.*, **53,** 929 (1973).
(15) B. Samuelsson, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **31,** 1442 (1972).
(16) For a discussion of the limitations of this route to pteridines, see E. C. Taylor, K. L. Perlman, I. P. Sword, M. Sequin-Frey, and P. A. Jacobi, *J. Am. Chem. Soc.*, **95,** 6407 (1973).
(17) (a) E. L. Patterson, R. Milstrey, and E. L. R. Stokstad, *J. Am. Chem. Soc.*, **78,** 5868 (1956); (b) H. Rembold and H. Metzger, *Chem. Ber.*, **96,** 1395 (1963).
(18) R. Tschesche, B. Hess, I. Ziegler, and H. Machleidt, *Justus Liebigs Ann. Chem.*, **658,** 193 (1962).
(19) H. S. Forrest and J. Walker, *J. Chem. Soc.*, 2077 (1949).
(20) F. Weygand, A. Wacker, and V. Schmied-Kowarzik, *Chem. Ber.*, **82,** 25 (1949).
(21) M. Viscontini, R. Provenzale, and W. F. Frei, *Helv. Chim. Acta,* **55,** 570 (1972).
(22) J. Weinstock, U.S. Patent 3,505,329.
(23) K. J. M. Andrews, W. E. Barber, and B. P. Tong, *J. Chem. Soc., Chem. Commun.*, 120 (1968).
(24) Preliminary communication: E. C. Taylor and P. A. Jacobi, *J. Am. Chem. Soc.*, **96,** 6781 (1974).
(25) E. C. Taylor, K. L. Perlman, Y.-H. Kim, I. P. Sword, and P. A. Jacobi, *J. Am. Chem. Soc.*, **95,** 6413 (1973).
(26) E. C. Taylor and T. Kobayashi, *J. Org. Chem.*, **38,** 2817 (1973).
(27) E. C. Taylor and K. Lenard, *Justus Liebigs Ann. Chem.*, **726,** 100 (1969).
(28) E. C. Taylor and P. A. Jacobi, *J. Am. Chem. Soc.*, **95,** 4455 (1973).
(29) S. Bayne in "Methods in Carbohydrate Chemistry", Vol. II, R. L. Whistler and M. L. Wolfrom, Ed., Academic Press, New York, N.Y., 1963, p 421.
(30) H. L. Wehrmeister, U.S. Patent 3,124,613.
(31) (a) H. S. Forrest and J. Walker, *J. Chem. Soc.*, 79 (1949); (b) F. Weygand, H. Simon, K. D. Keil, and H. Millauer, *Chem. Ber.*, **97,** 1002 (1964).
(32) R. Weidenhagen, *Z. Wirtschaftsgruppe Zuckerind.*, **87,** 711 (1937).
(33) R. E. Drake, Jr., Senior Thesis, Princeton University, 1971.
(34) M. Viscontini and R. Provenzale, *Helv. Chim. Acta,* **51,** 1495 (1968).
(35) L. Hough and T. J. Taylor, *J. Chem. Soc.*, 3544 (1955).
(36) (a) L. L. Salomon, J. J. Burns, and C. G. King, *J. Am. Chem. Soc.*, **74,** 5161 (1952); (b) J. K. Hamilton and F. Smith, *ibid.*, **74,** 5162 (1952).
(37) It is conceivable, of course, that the transoximation reaction could have led to an α-oximino aldehyde rather than to an α-keto aldoxime; cyclization would then have given a 6-substituted pyrazine 1-oxide, and finally a 7- (rather than a 6-) substituted pteridine (i.e., isobiopterin). However, this remote but potentially very disturbing possibility could be definitely excluded, since the crystalline pyrazine 1-oxide intermediates were homogeneous by TLC (a technique which readily detects even traces of 6-substituted pyrazine 1-oxides in the presence of the 5-isomers). The penultimate pterin 8-oxides were also homogeneous by TLC.
(38) Satisfactory microanalytical data have been obtained for all new compounds reported. The Editors have been supplied with these data, which are available upon request.
(39) N. K. Richtmyer in "Methods in Carbohydrate Chemistry", Vol. II, R. L. Whistler and M. L. Wolfrom, Ed., Academic Press, New York, N.Y., 1963, p 127.
(40) Aldrich Chemical Co., Milwaukee, Wis.
(41) G. Shaw and D. V. Wilson, *J. Chem. Soc.*, 2937 (1962).
(42) H. T. Clarke, J. R. Johnson, and R. Robinson, "The Chemistry of Penicillin", Princeton University Press, Princeton, N.J., 1949, p 728.
(43) E. Zissis and N. K. Richtmyer, *J. Am. Chem. Soc.*, **74,** 4373 (1952).
(44) We are indebted to the Smith Kline Laboratories, Philadelphia, Pa., for supplying us with this material.
(45) Y. Iwanami and M. Akino, *Tetrahedron Lett.*, 3219 (1972).
(46) We are deeply indebted to Dr. Barbara Petrack of the Pharmaceuticals Division of Ciba-Geigy Corp. for this biological evaluation of our synthetic material.
(47) S. Kaufman, *J. Biol. Chem.*, **242,** 3934 (1967).
(48) T. Nagatsu, K. Mizutani, I. Nagatsu, S. Matsuura, and T. Sugimoto, *Biochem. Pharmacol.*, **21,** 1945 (1972).
(49) B. Petrack, V. Fetzer, and R. Altiere in "Frontiers in Catecholamine Research", E. Usdin and S. Snyder, Ed., Pergamon Press, New York, N.Y., 1973, pp 97–100.
(50) S. Kaufman, *Proc. Natl. Acad. Sci. U.S.A.*, **50,** 1085 (1963).