are therefore much weaker bases than dialkylhydroxylamines (pK_a = 5.2[32]) or dialkylamino radicals (pK_a = 7.0 ± 0.5[33]), both of which must be protonated on nitrogen.[34] The absence of any epr signal at H_0 values between −3.5 and −7.5 is a line-broadening effect which results from a very rapid protonation and deprotonation of the radicals.

$$R_2NO\cdot + H^+ \underset{\text{fast}}{\overset{\text{fast}}{\rightleftharpoons}} R_2NOH\cdot^+$$

The absence of an epr signal from $-3.5 < H_0 < -7.5$ implies that the decrease in a^H_{NOH} for $\mathbf{1H^+}$ with increasing temperature (particularly in 1:1 (v/v) CF_3COOH–H_2SO_4 in which the proton hfc was not resolvable at 80°) cannot be due merely to the conversion of $\mathbf{1H^+}$ to **1**. That is, conversion to the unprotonated form, because of a decrease in H_0[9] or shift in the equilibrium constant, would require that the epr signal of $\mathbf{1H^+}$ first broaden and then disappear entirely before that due to **1** developed. However, the epr signal was present at all temperatures and the lines remained sharp as they coalesced. We therefore suggest that the geometry of $\mathbf{1H^+}$ at low temperatures differs from that at high temperatures.[24] The decreased interaction between the unpaired electron and the proton at the higher temperatures might, in principle, be due to (i) a more tetrahedral geometry at nitrogen, (ii) a shift of the proton into the nodal plane of the unpaired electron orbital, or (iii) a rapid inversion of the nitrogen atom ($k \sim 2 \times 10^7$ sec^{-1}). The first possibility seems unlikely since a^N remains unchanged or decreases slightly as the temperature is raised implying that the geometry of the nitrogen remains unchanged or that it becomes slightly more planar. Even if (ii) or (iii) provide the correct explanation for this phenomenon it is not obvious why $\mathbf{1H^+}$ behaves differently in concentrated sulfuric acid, nor is it clear why $\mathbf{2H^+}$ should behave differently from $\mathbf{1H^+}$ in both media. It is to be hoped that future studies of these radicals will answer some of the questions raised by the present work.

Acknowledgment. We are grateful to Professor Hoffman for a number of helpful comments and suggestions.

the ketone or alcohol group could have such a profound effect on pK. We therefore attempted to repeat the potentiometric titration on a very pure sample of 2,2,6,6-tetramethylpiperid-4-one N-oxyl.[30] There was no break in the titration curve from pH 8 to 2 and the nitroxide still gave a strong epr signal. It must be concluded that Rozantsev and Gintsberg titrated an impurity.

(28) H. Hogeveen, H. R. Gersmann, and A. P. Praat, *Recl. Trav. Chim. Pays-Bas*, **86**, 1063 (1967).

(29) E. G. Rozantsev and E. G. Gintsberg, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 571 (1965).

(30) Purified by slow sublimation at room temperature, mp 40° (lit.[31] mp 38°), 100% pure by glc on a 12-ft 10% silicone rubber column at 120°.

(31) R. Briere, H. Lemaire, and A. Rassat, *Bull. Soc. Chim. Fr.*, 3273 (1965).

(32) T. C. Bissot, R. W. Parry, and D. H. Campbell, *J. Amer. Chem. Soc.*, **79**, 796 (1957).

(33) R. W. Fessenden and P. Neta, *J. Phys. Chem.*, **76**, 2857 (1972).

(34) The pK_a for phenylhydroxylamine protonated on nitrogen is 2.0.[35] The estimated pK_a for protonation on oxygen is < -1.74.[35]

(35) A. Darchen and P. Boudeville, *Bull. Soc. Chim. Fr.*, 3809 (1971).

Pteridines. XXVIII. A New, General, and Unequivocal Pterin Synthesis[1]

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Contribution from the Department of Chemistry, Princeton University, Princeton, New Jersey 08540. *Received February 17, 1973*

Abstract: A versatile new synthetic route to pterins is described. Reaction of an α-ketoaldoxime or α-ketoketoxime with esters of α-aminocyanoacetic acid gives 2-amino-3-alkoxycarbonylpyrazine 1-oxides which are cyclized with guanidine to pterin 8-oxides. Deoxygenation of the pyrazine and pterin N-oxides, and the conversion of the latter to 7,8-dihydropterins, are described.

The classical synthetic route to pteridines involves condensation of a 4,5-diaminopyrimidine with an α,β-dicarbonyl compound, and it is a fair estimate that more than 90% of all known synthetic pteridines have been prepared by some modification of this condensation reaction.[3] Its usefulness and flexibility result from the wide diversity of substitution patterns possible with both components and the generally high yield with which the condensation proceeds. The use of α-ketoaldehydes in this so-called Isay pteridine synthesis[4] leads, however, to the preferential formation of 7- rather than 6-substituted isomers, while the use of other unsymmetrical α,β-dicarbonyl compounds necessarily gives products of ambiguous structure.[3,5] Thus,

(1) (a) Part XXVII: E. C. Taylor, S. F. Martin, Y. Maki, and G. P. Beardsley, *J. Org. Chem.*, **38**, 2238 (1973). (b) This work was supported in its initial stages by grants to Princeton University from the National Institutes of Health, Public Health Service (Grants No. CA-02551 and CA-12876), and from the U. S. Army Medical Research and Development Command (Contract No. DA-49-193-MD-2777). This is Contribution No. 1195 in the Army research program on malaria.

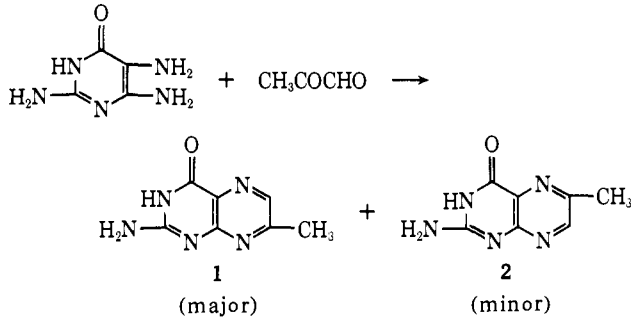
(2) Postdoctoral Fellow of the Swiss National Science Foundation.

(3) For general references on pteridine chemistry, see (a) "Pteridine Chemistry," W. Pfleiderer and E. C. Taylor, Ed., Pergamon Press, London, 1964; (b) R. C. Elderfield and A. C. Mehta, "Heterocyclic Compounds," Vol. 9, R. C. Elderfield, Ed., Wiley, New York, N. Y., 1967, pp 1–117; (c) W. Pfleiderer, *Angew. Chem., Int. Ed. Engl.*, **3**, 114 (1964).

(4) O. Isay, *Ber.*, **39**, 250 (1906).

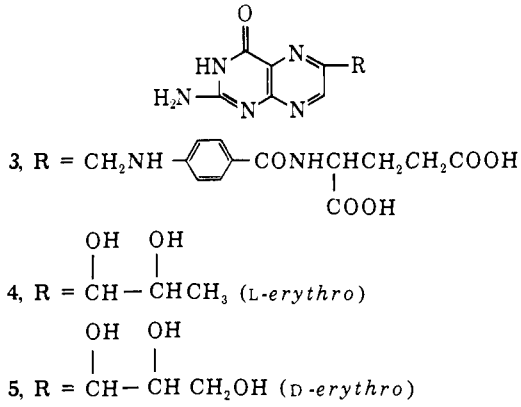
(5) See, for example, (a) R. F. W. Spickett and G. M. Timmis, *J. Chem Soc.*, 2887 (1954); (b) R. Tschesche and G. Sturm, *Chem. Ber.*, **98**, 851 (1965).

Scheme I



in the Isay-type synthesis (see Scheme I) of 7-methylpterin (**1**), the structure of the major product is determined by the preferred condensation of the more reactive of the two carbonyl groups (the aldehyde carbonyl) with the more nucleophilic of the ortho-situated amino groups (the 5-amino group), resulting in predominant formation of the 7-methyl isomer **1**.[3c] Depending upon the reaction conditions, this product may be contaminated with the isomeric 6-substituted derivative **2**, and removal of this isomeric "impurity" may be extremely difficult.

However, the majority of naturally occurring pteridines possess carbon substituents at position 6 rather than at position 7 (consider, for example, the structures of folic acid (**3**), biopterin (**4**), neopterin (**5**),



etc.) and the Isay reaction thus produces mainly the "wrong" isomer. Many attempts have been made to devise reaction conditions which would permit reversal of the normal direction of condensation so as to favor formation of the 6-substituted isomer (control of pH, addition of hydrazine, sodium bisulfite, or 2-mercaptoethanol,[6a] the use of α-hydroxy ketones rather than α,β-dicarbonyl compounds, etc.),[3] but it is now generally recognized that a mixture of isomers is probably inevitable and that separation may be either difficult or impossible.[6b]

Attempts to overcome this orientation problem by the design of alternate, unequivocal condensation reactions include the reaction of a 4-amino-5-nitrosopyrimidine with an active methylene compound bearing a nitrile group (the Timmis reaction),[9] the Boon and Leigh synthesis involving condensation of a 4-chloro-5-nitropyrimidine with an α-amino aldehyde or ketone, followed by reductive cyclization,[10] the condensation of a 4-amino-5-nitrosopyrimidine with phenacyl- or acetonylpyridinium salts,[11] and the use of pyrazine intermediates, which requires closure of the fused pyrimidine ring as the terminal step in the synthetic sequence.[12] This latter approach, however, has not enjoyed much use because of difficulties encountered in preparing suitably substituted pyrazine intermediates. In fact, some of the more versatile types of pyrazines suitable for subsequent conversion to pteridines (*i.e.*, 2-aminopyrazine-3-carboxylic acid derivatives) are actually best prepared by ring cleavage of preformed pteridines.[12c,d,13] Thus, none of these alternate syntheses has proven to be truly satisfactory; a general synthetic route to 6-substituted pteridines which is both unequivocal in the orientation of the 6 substituent, and versatile enough to allow introduction of multifunctional 6 substituents, has not until now been available.

We describe in this[14] and subsequent papers[15] the development of a new, general, and unequivocal synthesis of pteridines which solves this orientation problem. This new approach possesses the following special features: (a) it readily permits the unambiguous introduction at C-6 of a variety of side chains susceptible to chemical modification, and thus provides a simple entry into the pterin natural products possessing multifunctional C-6 substituents (such as biopterin, sepiapterin, neopterin, folic acid, etc.); (b) it permits variation in the nature of the pyrimidine substituents through a choice of the type of cyclization reaction employed in the terminal ring closure; (c) it produces initially a pteridine 8-oxide which can function as a useful intermediate for the selective introduction of substituents at position 7, and which upon reduction gives 7,8-dihydropteridines; (d) by suitable modification of the structure of the α-oximino carbonyl compound employed in the initial pyrazine cyclization, it provides as well an unequivocal procedure for the synthesis of the isomeric 7-substituted derivatives; and (e) because every pyrazine intermediate

(6) (a) C. B. Storm, R. Shiman, and S. Kaufman, *J. Org. Chem.*, **36**, 3925 (1971). These authors report the formation of pure 6-phenylpterin by the condensation of 2,4,5-triamino-6(1*H*)-pyrimidinone with phenylglyoxal at pH 4, but the yield was low (41%), and the insolubility of the product in this case facilitates isolation of the predominant isomer. (b) An excellent example of this intrinsic deficiency in the Isay-type pteridine synthesis is found in the recent work of Archer and Scrimgeour,[7] whose various oxidation studies on reduced derivatives of commercial 6-methylpterin were in part vitiated by the finding[8] that the latter "compound" was actually a previously unsuspected mixture of the 6 and 7 isomers.

(7) M. C. Archer and K. G. Scrimgeour, *Can. J. Biochem.*, **48**, 278 (1970).

(8) J. M. Whiteley, J. H. Drais, and F. M. Huennekens, *Arch. Biochem. Biophys.*, **133**, 436 (1969).

(9) For a bibliography of the Timmis reaction, see T. S. Osdene, ref 3a, pp 65–73.

(10) W. R. Boon and T. Leigh, *J. Chem. Soc.*, 1497 (1951).

(11) I. J. Pachter and P. E. Nemeth, *J. Org. Chem.*, **28**, 1197 (1963).

(12) Illustrative examples of this route to pteridines may be found in (a) S. Gabriel and A. Sonn, *Chem. Ber.*, **40**, 4850 (1907); (b) A. Albert, D. J. Brown, and G. W. H. Cheeseman, *J. Chem. Soc.*, 474 (1951); (c) E. C. Taylor, J. A. Carbon, and D. R. Hoff, *J. Amer. Chem. Soc.*, **75**, 1904 (1953); (d) E. C. Taylor, R. B. Garland, and C. F. Howell, *ibid.*, **78**, 210 (1956); (e) G. P. G. Dick and H. C. S. Wood, *J. Chem. Soc.*, 1379 (1955); (f) E. C. Taylor and W. W. Paudler, *Chem. Ind. (London)*, 1061 (1955); (g) W. B. Wright and J. M. Smith, *J. Amer. Chem. Soc.*, 77, 3927 (1955); (h) E. C. Taylor, O. Vogl, and P. K. Loeffler, *ibid.*, **81**, 2479 (1959); (i) F. Dalacker and G. Steiner, *Justus Liebigs Ann. Chem.*, **660**, 98 (1962).

(13) E. C. Taylor in "The Chemistry and Biology of Pteridines," a CIBA Symposium, G. E. W. Wolstenholme and M. P. Cameron, Ed., J. and A. Churchill, Ltd., London, 1954, pp 2–34.

(14) A preliminary communication on this work has appeared: E. C. Taylor and K. Lenard, *J. Amer. Chem. Soc.*, **90**, 2424 (1968).

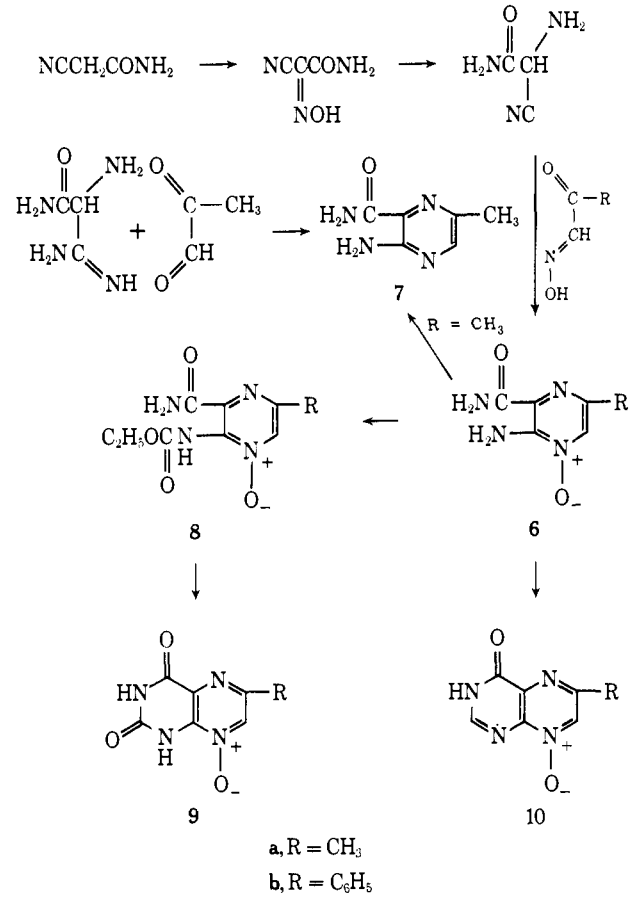
(15) (a) E. C. Taylor, K. L. Perlman, Y.-H. Kim, I. P. Sword, and P. A. Jacobi, *ibid.*, **95**, 6413 (1973); (b) E. C. Taylor and P. A. Jacobi, *ibid.*, **95**, 4455 (1973); (c) E. C. Taylor and R. F. Abdulla, *Tetrahedron Lett.*, 2093 (1973); (d) E. C. Taylor and T. Kobayashi, *J. Org. Chem.*, in press.

encountered prior to the pyrimidine ring annelation step is crystalline, the final pteridines are readily prepared in a high state of purity.

In the course of synthetic studies directed toward the preparation of aspergillic acid and derivatives, Sharp and Spring described[16] the reaction of alkyl α-aminonitriles (from aldehyde cyanohydrins and ammonia) with α-ketoaldoximes to give 3,5-disubstituted-2-aminopyrazine 1-oxides. It occurred to us that a proper choice of the 3 substituent in such 2-aminopyrazine 1-oxides should permit a subsequent ring closure to a pteridine 8-oxide. Thus, the condensation of α-aminocyanoacetamide[17] with oximinoacetone in glacial acetic acid solution gave 2-amino-3-carbamoyl-5-methylpyrazine 1-oxide (**6a**).[18] The structure of **6a** was confirmed by sodium dithionite reduction to 2-amino-3-carbamoyl-5-methylpyrazine (**7**), identical with an authentic sample prepared by the condensation of methyl glyoxal with aminomalonamidamidine.[20] Oximinoacetophenone reacted analogously with α-aminocyanoacetamide to give 2-amino-3-carbamoyl-5-phenylpyrazine 1-oxide (**6b**). The utility of these readily accessible pyrazine intermediates for pteridine synthesis was demonstrated by condensation with ethyl chloroformate to give the intermediate 2-ethoxycarbonylamino derivatives **8a** and **8b**, which were cyclized with sodium ethoxide to 6-methyllumazine 8-oxide (**9a**) and 6-phenyllumazine 8-oxide (**9b**), respectively. Similarly, cyclization of the pyrazines **6a** and **6b** with triethyl orthoformate in dimethylacetamide gave 6-methyl- and 6-phenyl-4(3*H*)-pteridinone 8-oxide (**10a** and **10b**, respectively). These reactions are summarized in Scheme II.

For the purposes of cyclization to pterins (2-amino-4(3*H*)-pteridinones), pyrazine *o*-aminoesters (*i.e.*, **11**) would be expected to be more reactive intermediates than the *o*-aminocarboxamides **6**. We have found that the condensation of ethyl α-aminocyanoacetate[21] with α-ketoaldoximes provides a simple and effective synthesis of these reactive and useful pyrazine intermediates. For example, condensation of ethyl α-aminocyanoacetate with oximinoacetone gave 2-amino-3-carbethoxy-5-methylpyrazine 1-oxide (**11a**), which was cyclized with guanidine in the presence of sodium methoxide to 6-methylpterin 8-oxide (**12a**). This latter compound could be reduced with aqueous sodium dithionite in almost quantitative yield to 6-methyl-7,8-dihydropterin (**13a**), identical with an authentic sample of **13a** prepared by the procedure of Boon and Leigh,[10] and also by the method of Pfleiderer and Zondler.[22] It is of particular interest that 6-methyl-7,8-dihydropterin (**13a**) has been shown to be a substrate for dihydrofolate reductase and has been used as a model for dihydrofolic acid in a number of enzymatic studies.[23] Potassium permanganate oxidation of **13a** proceeded quantitatively to give 6-methylpterin (**2** ≡ **14a**), identical with an authentic sample.[10]

Scheme II

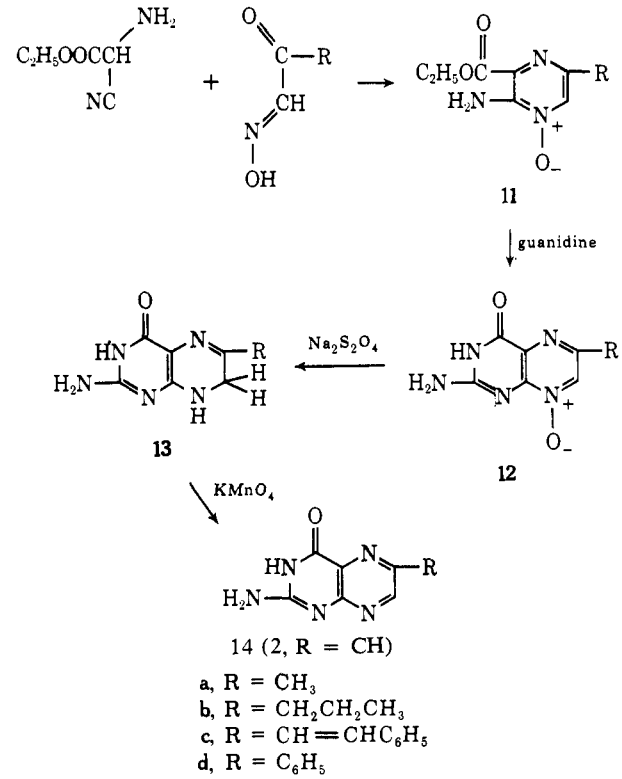


In analogous fashion, a number of 2-amino-3-carbethoxypyrazine 1-oxides (**11b–d**) were prepared by condensation of ethyl α-aminocyanoacetate with the appropriate α-ketoaldoximes (see Scheme III).[24]

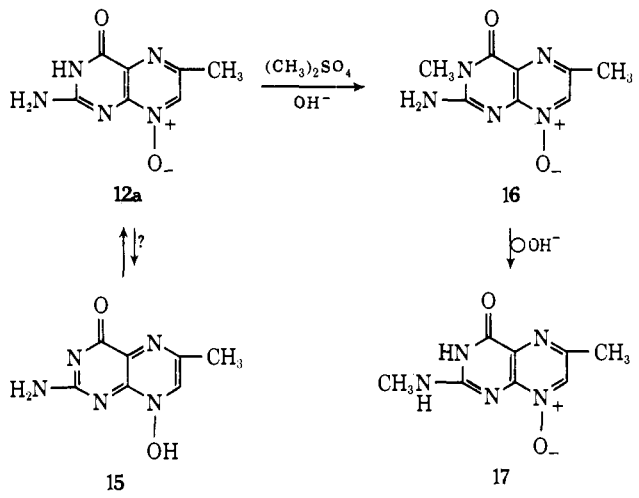
The possibility that the pterin 8-oxides **9**, **10**, and **12** might exist as the tautomeric 8-hydroxy derivatives (*i.e.*, **15**) appears to be excluded by comparison of the nmr spectra of the *N*-oxides with the spectra of the deoxygenated pteridines (for details see Experimental Section). It should be noted, however, that Pfleiderer and Hutzenlaub,[25] in connection with their preparation of 3-methyl-6-phenyllumazine 8-oxide, considered both tautomers as apparently probable, and we therefore record some additional experiments with **12a** which convincingly exclude the 8-hydroxy tautomer **15** as a major contributor (see Scheme IV). Thus, 6-methylpterin 8-oxide (**12a**) was methylated with dimethyl sulfate at pH 7–8 to give a monomethyl derivative which was shown conclusively to be the 3-methyl derivative **16** by subjecting it to a base-catalyzed Dimroth rearrangement[26] to give 2-methylamino-6-

(16) W. Sharp and F. S. Spring, *J. Chem. Soc.*, 932 (1951).
(17) A. H. Cook, I. Heilbron, and E. Smith, *ibid.*, 1440 (1949).
(18) Following completion of this work and publication of our preliminary communication (ref 14), it came to our attention that compound **6a** had been prepared independently by the same procedure during studies on the synthesis of new sulfanilamidopyrazines.[19]
(19) F. Chillemi and G. Palamidessi, *Farmaco, Ed. Sci.*, **18**, 566 (1963).
(20) O. Vogl and E. C. Taylor, *J. Amer. Chem. Soc.*, **81**, 2472 (1959).
(21) (a) A. H. Cook, I. Heilbron, and A. L. Levy, *J. Chem. Soc.*, 1594 (1947); (b) B. Ohta, *J. Pharm. Soc. Jap.*, **68**, 226 (1948); *Chem. Abstr.*, **48**, 4440g (1954).
(22) W. Pfleiderer and H. Zondler, *Chem. Ber.*, **99**, 3008 (1966).
(23) J. M. Whiteley and F. M. Huennekens, *Biochemistry*, **6**, 2620 (1967).
(24) We have recently found that benzyl α-aminocyanoacetate (as its methanesulfonic acid salt) also condenses readily with α-ketoaldoximes to give the corresponding 2-amino-3-carbobenzyloxypyrazine 1-oxides. Since these latter pyrazines are insoluble in water, this procedure has the advantage that products are readily isolated by pouring the reaction mixture into water. Reduction and/or cyclization then proceeded normally.
(25) W. Pfleiderer and W. Hutzenlaub, *Angew. Chem.*, 77, 1136 (1965).
(26) D. J. Brown in "Mechanisms of Molecular Migrations," Vol. 1, B. S. Thyagarajan, Ed., Wiley-Interscience, New York, N. Y., 1968, pp 209–245.

Scheme III



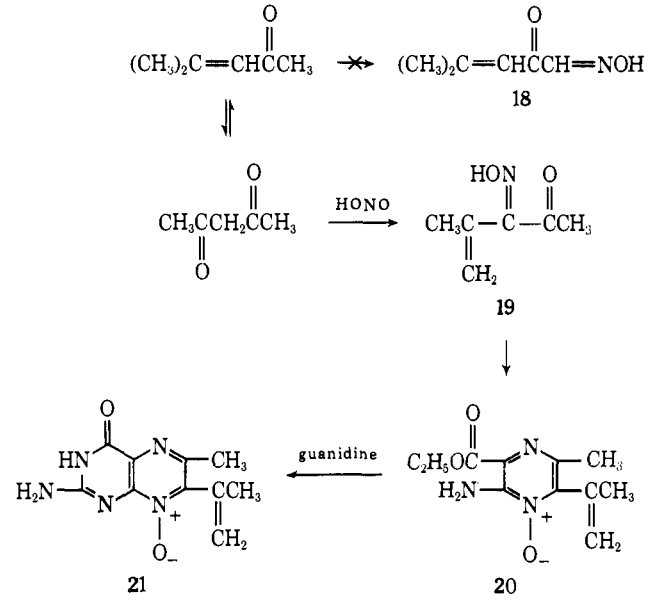
Scheme IV



methyl-4(3*H*)-pteridinone 8-oxide (**17**). This Dimroth rearrangement was conveniently followed by nmr spectroscopy; **16** exhibits two methyl signals at 2.36 (C_6–CH_3) and 3.30 ppm (N_3–CH_3). As the Dimroth rearrangement proceeds, the latter (*N*-methyl signal) is seen to move upfield to a terminal position of 2.83 ppm (the 2-methylamino grouping), while the 2.36 ppm signal remains unchanged. The percentage of Dimroth rearrangement at any given time is readily determined by integration. The uv spectra of **12a** and of **16**, both as neutral molecules and as cations, are essentially identical, as are their low pK_a values (the higher pK_a value for **16** could not be determined because of the intervening Dimroth rearrangement). Since the position of the C-7 proton signal in all of the pteridine 8-oxides examined remains essentially unchanged, and is the same as that exhibited by the C-7 proton signal in **12a**, we conclude that their structures are correctly represented as 8-oxides and not as 8-hydroxy derivatives (*i.e.*, **15**).

Many of the α-ketoaldoximes employed in the above reactions were prepared by simple nitrosation of the corresponding methyl ketones (*i.e.*, acetone, acetophenone). It should be noted, however, that direct oximation is ambiguous when the carbonyl group is flanked by unsymmetrical active methylene groupings. For example, nitrosation of mesityl oxide would be expected and has been claimed[27] to give the α-ketoaldoxime **18** (see Scheme V). We have now

Scheme V



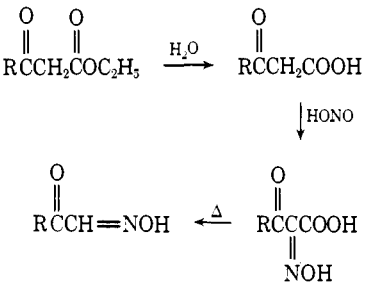
found, however, that this structural assignment is in error, and that the nitrosation product of mesityl oxide is in fact 2-methyl-3-oximinopent-1-en-4-one ("isonitromesityl oxide") (**19**).[28] The structure of **19** was confirmed not only by its definitive nmr spectrum (see Experimental Section) but by its cyclization with ethyl α-aminocyanoacetate to 2-amino-3-carbethoxy-5-methyl-6-isopropenylpyrazine 1-oxide (**20**). The structure of this latter compound was unambiguously confirmed by examination of its nmr spectrum (see Experimental Section). Condensation of **20** with guanidine in the presence of sodium methoxide then gave 6-methyl-7-isopropenylpterin 8-oxide (**21**).

Since analogous difficulties may be anticipated on nitrosation of other ketones,[29] we prefer the general synthetic route to α-ketoaldoximes outlined below in Scheme VI, which proceeds in high yield and is unambiguous. The β-keto esters which were not commercially available were prepared either by condensation of the appropriate methyl ketone with diethyl carbonate[30] or by reaction of alkyl Grignard reagents with ethyl cyanoacetate.[31]

Extensions of this new, general, and unequivocal approach to pteridines leading to syntheses, *inter alia*,

(27) L. Claisen and O. Manasse, *Ber.*, **22**, 526 (1889).
(28) Mesityl oxide is known to be in equilibrium with its prototropic isomer "isomesityl oxide:" F. H. Stross, J. M. Morgen, and H. de V. Finch, *J. Amer. Chem. Soc.*, **69**, 1627 (1947).
(29) See, for example, R. W. L. Clarke, A. Lapworth, and E. Wechsler, *J. Chem. Soc.*, **93**, 30 (1908).
(30) V. H. Wallingford, A. H. Homeyer, and D. M. Jones, *J. Amer. Chem. Soc.*, **63**, 2252 (1941).
(31) G. W. Anderson, I. F. Halverstadt, W. H. Miller, and R. O. Roblin, Jr., *ibid.*, **67**, 2197 (1945).

Scheme VI



of pteroic acid, 6-hydroxymethylpterin, folic acid, aminopterin, methotrexate, and the pteridine natural products possessing multifunctional C-6 substituents (*i.e.*, biopterin) are described in further papers in this series.

Experimental Section[32]

2-Amino-3-carbamoyl-5-methylpyrazine 1-Oxide (6a). To a solution of 3.00 g of α-aminocyanoacetamide[17] in 10 ml of glacial acetic acid was added 3.00 g of oximinoacetone in 10 ml of glacial acetic acid. An exothermic reaction ensued and some external cooling was necessary during the first 10 min to maintain the temperature at 20–25°. Yellow crystals started to separate after 10–15 min. The mixture was stirred at room temperature for 16 hr, and the yellow crystals were collected by filtration, washed with cold acetic acid followed by ether, and dried to give 3.00 g (62%) of 2-amino-3-carbamoyl-5-methylpyrazine 1-oxide, mp 218–219° (lit.[19] mp 235–236° from water). Recrystallization from ethanol or ethyl acetate gave bright yellow needles, but without change in the melting point. The compound gave a deep blue ferric chloride test in aqueous solution: nmr (CF_3COOH) δ 2.1 (3, s, C_5–CH_3), 7.83 (1, s, C_6–H); uv $\lambda_{max}^{C_2H_5OH}$ nm (log ε) 228 (4.05), 246 (4.24), 378 (3.88).

2-Amino-3-carbamoyl-5-methylpyrazine (7). To a solution of 0.30 g of 2-amino-3-carbamoyl-5-methylpyrazine 1-oxide in 7 ml of boiling water was added portionwise 3.50 g of sodium dithionite. The mixture was refluxed for 2 hr, the resulting suspension was cooled, and the precipitate was collected by filtration, washed with water, and dried to give 0.21 g (78%) of pale yellow crystals of 2-amino-3-carbamoyl-5-methylpyrazine, mp 203–204°. The material gave a negative $FeCl_3$ test and was identical in all respects with an authentic sample.[20]

2-Amino-3-carbamoyl-5-phenylpyrazine 1-Oxide (6b). To a suspension of 1.50 g of oximinoacetophenone in 10 ml of glacial acetic acid was added a solution of 1.00 g of α-aminocyanoacetamide in 5 ml of glacial acetic acid, and the resulting clear solution was stirred for 12 hr at room temperature. The resulting slurry of crystals was diluted with 50 ml of water and filtered, and the collected solid was washed well with water followed by ethanol and dried to give 0.75 g (32%) of 2-amino-3-carbamoyl-5-phenylpyrazine 1-oxide, mp 280–282°. Recrystallization from DMF gave long yellow needles with the same melting point: nmr (DMSO) δ 7.50 (3, m), 8.23 (2, m, C_3–C_6H_5), 9.16 (1, s, C_6–H); uv $\lambda_{max}^{C_2H_5OH}$ nm (log ε) 245 sh (4.08), 280 (4.38), 390 (3.81).

6-Methyllumazine 8-Oxide (9a). A mixture of 0.34 g of 2-amino-3-carbamoyl-5-methylpyrazine 1-oxide and 0.54 g of ethyl chloroformate was heated under reflux with stirring for 4 hr. Progress of the reaction could conveniently be followed by testing aliquots of the reaction mixture with ferric chloride; the starting material gives a strong positive (deep blue) test, while the 2-ethoxycarbonylamino derivative (*vide infra*) gives a negative test. The reaction mixture was then cooled and the white crystals were filtered off and dried to give 0.35 g (85%) of crude 2-ethoxycarbonylamino-3-carbamoyl-5-methylpyrazine 1-oxide (**8a**).

To a solution of 0.03 g of sodium dissolved in 10 ml of methanol was added 0.19 g of crude 2-ethoxycarbonylamino-3-carbamoyl-5-methylpyrazine 1-oxide, and the mixture was heated under reflux for 3 hr. The yellow sodium salt of the lumazine was collected by filtration and dissolved in water, and hydrochloric acid was added to pH 3–4. Filtration gave 0.15 g (86%) of white crystals of 6-methyllumazine 8-oxide: mp >320°; nmr (CF_3COOH) δ 2.25 (3, s, C_6–CH_3), 8.46 (1, s, C_7–H); uv $\lambda_{max}^{H_2O}$ nm (log ε) 248 sh (4.40), 268 (4.47), 290 sh (4.08), 385 (3.99).

6-Phenyllumazine 8-Oxide (9b). Reaction of 2-amino-3-carbamoyl-5-phenylpyrazine 1-oxide with ethyl chloroformate in xylene solution, as described above, gave 2-ethoxycarbonylamino-3-carbamoyl-5-phenylpyrazine 1-oxide (**8b**) (84% yield), which was cyclized to 6-phenyllumazine 8-oxide by heating with sodium methoxide in methanol: yield, 90%, mp >320°; nmr (CF_3COOH) δ 7.30 (3, m), 8.00 (2, m, C_6–C_6H_5), 8.93 (1, s, C_7–H); uv $\lambda_{max}^{H_2O}$ nm (log ε) 248 (3.88), 295 (4.14), 400 (3.36).

6-Methyl-4(3*H*)-pteridinone 8-Oxide (10a). A solution of 0.60 g of 2-amino-3-carbamoyl-5-methylpyrazine 1-oxide in 5 ml of triethyl orthoformate and 10 ml of DMF was heated at 140° (oil bath) with stirring for 16 hr. The precipitated solid was collected by filtration, washed well with water, ethanol, and ether, and dried to give 0.41 g (64%) of 6-methyl-4(3*H*)-pteridinone 8-oxide: mp >320°; nmr (CF_3COOH) δ 2.48 (3, s, C_6–CH_3), 8.66 (1, s), 8.80 (1, s, C_2–H and C_7–H); uv $\lambda_{max}^{H_2O}$ nm (log ε) 228 (4.05), 225 sh (4.05), 265 sh (4.13), 272 (4.13), 300 sh (3.86), 345 (3.86), 358 (3.86).

6-Phenyl-4(3*H*)-pteridinone 8-Oxide (10b). A solution of 0.30 g of 2-amino-3-carbamoyl-5-phenylpyrazine 1-oxide in a mixture of 5 ml of triethyl orthoformate and 10 ml of dimethylacetamide was heated under reflux with stirring for 12 hr. The resulting suspension of white crystals was cooled and filtered, and the collected solid was washed well with water followed by ethanol and dried to give 0.25 g (80%) of cream-colored crystals: mp >320°; nmr (CF_3COOH) δ 7.66 (3, m), 7.16 (2, m, C_6–C_6H_5), 8.45 (1, s, C_2–H), 9.10 (1, s, C_7–H); uv $\lambda_{max}^{H_2O}$ nm (log ε) 225 (4.23), 295 (4.44), 320 sh (4.16), 370 (3.89).

2-Amino-3-carbethoxy-5-methylpyrazine 1-Oxide (11a). A solution of 4.35 g of oximinoacetone and 15.00 g of ethyl α-aminocyanoacetate tosylate in 20 ml of absolute methanol was stirred for 24 hr at 35° (care should be exercised that the temperature does not rise higher than 35°). The clear brown reaction solution was then evaporated under reduced pressure and the residual brown oil triturated with 100 ml of water and then extracted with 3 × 300 ml of ethyl acetate, followed by 1 × 300 ml of chloroform. The combined extracts were dried over anhydrous sodium sulfate, and the solvents were evaporated under reduced pressure to give 4.85 g (49%) of pale yellow needles, mp 132–133.5°. An additional 0.80 g (total yield 5.65 g, 58%) of product was obtained by concentration of the above aqueous layer to 100 ml, followed by continuous extraction with methylene chloride, drying of the extracts (Na_2SO_4), evaporation, trituration of the brown oil with isopropyl ether, and recrystallization of the resulting yellow crystals from ether: nmr ($CDCl_3$) δ 1.40 (3, t), 4.5 (2, q, C_2H_5), 2.43 (3, s, C_5–CH_3), 8.13 (1, s, C_6–H), 7.3 (2, br, C_2–NH_2); uv $\lambda_{max}^{H_2O}$ nm (log ε) 226 (4.05), 246 (4.29), 378 (3.94).

2-Amino-3-carbethoxy-5-*n*-propylpyrazine 1-Oxide (11b). This compound was prepared in 52% yield from 15.00 g of ethyl α-aminocyanoacetate tosylate and 5.75 g of oximinomethyl-*n*-propyl ketone, as described above for the preparation of **11a**: yellow, silky needles, mp (from ether) 89–90°; nmr ($CDCl_3$) δ 1.41 (3, t), 4.41 (2, q, C_2H_5), 0.95 (3, t), 1.70 (2, m), 2.66 (2, t, C_3H_7), 7.96 (1, s, C_6–H); uv $\lambda_{max}^{C_2H_5OH}$ nm (log ε) 228 (4.08), 252 (4.30), 380 (3.91).

2-Amino-3-carbethoxy-5-styrylpyrazine 1-Oxide (11c). A solution of 18.00 g of ethyl α-aminocyanoacetate tosylate and 10.50 g of styryl oximinomethyl ketone in 60 ml of 2-propanol was stirred at room temperature for 16 hr. The yellow crystalline solid which separated from the dark solution was collected by filtration, washed well with 2-propanol, and dried to give 4.90 g (29%) of **11c**, mp 155–156° The analytical sample was prepared by recrystallization from ethanol without change in the melting point: nmr ($CDCl_3$) δ 1.45 (3, t), 4.50 (2, q, C_2H_5), 8.43 (1, s, C_6–H), 7.35 (5, m, C_6H_5), 6.96 (1, d), 8.26 (1, d, J = 17 Hz, CH=CH trans); uv $\lambda_{max}^{C_2H_5OH}$ nm (log ε) 315 (4.49), 402 (3.75).

2-Amino-3-carbethoxy-5-phenylpyrazine 1-Oxide (11d). A mixture of 15.00 g of ethyl α-aminocyanoacetate tosylate and 7.45 g of oximinoacetophenone in 30 ml of absolute methanol was stirred at 35° for 24 hr. A clear solution resulted after 5 min, a yellow crystalline solid started to separate after about 2 hr, and the reaction mixture practically solidified after 2.5 hr. Filtration gave 7.48 g (60%) of yellow crystals, mp 135–137°. The filtrate was concentrated under reduced pressure, and the residual brown oil was dis-

(32) Melting points are uncorrected and were determined on a Thomas-Hoover apparatus. Infrared data refer to Nujol mull spectra taken on a Perkin-Elmer 237B grating infrared spectrometer. Nmr data were obtained on a Varian A-60A instrument, using TMS as internal standard. Elemental analyses for all new compounds were in agreement with the assigned structures to within 0.3%. Results of these analyses were made available to the editor.

solved in 50 ml of ethyl acetate and transferred to a separatory funnel, and 100 ml of water and 250 ml of ethyl acetate were added. The aqueous layer was separated and extracted again with 2 × 300 ml of ethyl acetate and 1 × 300 ml of chloroform, the combined organic extracts were evaporated to dryness, and the residue was crystallized from 2-propanol or ethanol. This procedure gave an additional 1.78 g of product: total yield 9.26 g (74%); nmr ($CDCl_3$) δ 1.5 (3, t), 4.5 (2, q, C_2H_5), 7.9 (2, m), 7.43 (3, m, C_5–C_6H_5), 8.86 (1, s, C_6–H); uv $\lambda_{max}^{C_2H_5OH}$ nm (log ε) 245 sh (4.09), 278 (4.41), 392 (3.23).

2-Amino-3-carbethoxy-5-methyl-6-isopropenylpyrazine 1-Oxide (20). This compound was prepared in 51% yield from ethyl α-aminocyanoacetate and 2-methyl-3-oximinopent-1-en-4-one (**19**),[27] in acetic acid as solvent: mp (from ether) 121–122°; nmr ($CDCl_3$) δ 7.35 (2, br, C_2–NH_2), 1.43 (3, t), 4.53 (2, q, C_4H_5), 2.10 (3, t, J = ~2 Hz, CH_3C=), 2.50 (3, s, C_5—CH_3), 5.60 (1, m), 5.20 (1, m, =CH_2); uv $\lambda_{max}^{C_2H_5OH}$ nm (log ε) 233 (4.17), 253 (4.25), 287 sh (3.55), 378 (3.94).

6-Methylpterin 8-Oxide (12a). To a methanolic solution of guanidine (prepared by adding 5.60 g of guanidine hydrochloride to 240 ml of methanol containing 2.80 g of sodium, and removal of the precipitated sodium chloride) was added 8.00 g of 2-amino-3-carbethoxy-5-methylpyrazine 1-oxide. An orange precipitate gradually formed. The mixture was stirred at room temperature for 30 min, the methanol removed by evaporation under reduced pressure, and 150 ml of DMF added to the residue. The resulting solution was heated under reflux for 4 hr and cooled, and the yellow precipitate was collected by filtration and washed with ethanol. The crude product was dissolved in hot water and hydrochloric acid added to pH 3–4. Filtration then gave 5.50 g (70%) of pure 6-methylpterin 8-oxide: mp >320°; nmr (CF_3COOH) δ 2.36 (3, s, C_6–CH_3), 8.50 (1, s, C_7–H); uv $\lambda_{max}^{pH\ 4.82}$ nm (log ε) 270 (4.35), 290 sh (4.10), 378 (3.85); $\lambda_{max}^{pH\ 13}$ nm (log ε) 260 (4.54), 287 sh (3.92), 387 (3.94); $\lambda_{max}^{H_0=-1.85}$ nm (log ε) 257 (4.29), 287 sh (3.93), 352 (3.73).

The following pterin 8-oxides were prepared in the same manner from guanidine and the corresponding 2-amino-3-carbethoxy-5-substituted-pyrazine 1-oxides.

6-*n*-Propylpterin 8-Oxide (12b): 70% yield, mp >320°; nmr (CF_3COOH) δ 0.58 (3, t), 1.30 (2, m), 2.50 (2, m, C_3H_7), 8.16 (1, s, C_7–H); uv $\lambda_{max}^{0.1N\ NaOH}$ nm (log ε) 217 (4.01), 248 (3.90), 276 (4.31), 300 sh (3.99), 382 (3.70).

6-Styrylpterin 8-Oxide (12c): 71% yield, mp >320°; nmr (CF_3COOH) δ 6.7 (1, d), 7.3 (1, d, CH=CH); 7.1 (5, m, C_6H_5), 7.70 (1, s, C_7–H); uv $\lambda_{max}^{0.1N\ NaOH}$ nm (log ε) 245 (4.03), 280 sh (4.27), 320 (4.53), 415 (4.04).

6-Phenylpterin 8-Oxide (12d): 65% yield, mp >320°; nmr (CF_3COOH) δ 7.33 (3, m), 7.66 (2, m, C_6H_5), 9.00 (1, s, C_7–H); uv $\lambda_{max}^{0.1N\ NaOH}$ nm (log ε) 283 (4.73), 320 sh (4.29), 397 (4.15).

6-Methyl-7-isopropenylpterin 8-Oxide (21): 86% yield, mp >320°; nmr (CF_3COOH) δ 1.70 (3, t, J = ~2 Hz, CH_3C=), 2.33 (3, s, C_6–CH_3), 5.00 (1, d), 5.46 (1, d, CH_2=); uv $\lambda_{max}^{0.1N\ NaOH}$ nm (log ε) 262 (4.53), 290 sh (3.93), 385 (4.02).

6-Methyl-7,8-dihydropterin (13a). To a suspension of 0.10 g of 6-methylpterin 8-oxide in 10 ml of boiling water was added 1.00 g of sodium dithionite. The resulting solution was stirred at room temperature overnight and the colorless solid which had separated was collected by filtration, washed well with water followed by ethanol, and dried; yield, 0.08 g (90%), mp >320°. The uv, nmr, and ir spectra of this compound were identical with those of an authentic sample of 6-methyl-7,8-dihydropterin.[10,22] Its measured pK_a values [4.17 (±0.03) and 10.85 (±0.03)] were identical with those reported by Pfleiderer and Zondler[22] but differed slightly from those reported by Whiteley and Huennekens (3.2 and 10.6).[23] The reduction could also be effected (42% yield) with hydrogen and Raney nickel catalyst (room temperature, 50 psi of hydrogen).

6-Methylpterin (2 ≡ 14a). This compound was readily prepared in 94% yield by stirring a solution of 6-methyl-7,8-dihydropterin in 0.1 *M* potassium permanganate solution (made alkaline by addition of a small amount of 0.1 *N* sodium hydroxide) at room temperature for 10 min, bubbling in sulfur dioxide to dissolve the precipitated manganese dioxide. and filtering off the precipitated bright yellow, crystalline 6-methylpterin, identical with an authentic sample.[10]

6-Phenyl-7,8-dihydropterin (13d). A solution of 0.15 g of 6-phenylpterin 8-oxide in 7 ml of 0.5 *N* sodium hydroxide containing 0.55 g of sodium dithionite was heated under reflux for 15 min, cooled, and acidified to pH 1–2 with hydrochloric acid. The yellow solid which precipitated was collected by filtration and redissolved in 6 ml of hot 0.5 *N* sodium hydroxide, and the solution again acidified to give 0.14 g (88%) of 6-phenyl-7,8-dihydropterin hydrochloride, mp >320°.

Oxidation of this material with potassium permanganate, as described above, gave 6-phenylpterin (**14d**), identical with an authentic sample.[33]

3,6-Dimethylpterin 8-Oxide (16). Dimethyl sulfate (4 ml) was added slowly and with stirring over a period of 20 min to a solution of 1.00 g of 6-methylpterin 8-oxide in 80 ml of 0.1 *N* sodium hydroxide. The pH of the mixture was maintained at 7–8 by slow addition of 1 *N* sodium hydroxide (approximately 30 ml were necessary). The initially clear solution began to deposit yellow crystals after about 30 min. After 3 hr of stirring, the mixture was chilled to 0° and filtered, and the collected yellow solid was washed with ether, then with cold water, and dried: yield, 0.68 g (63%), mp >300°; nmr (CF_3COOH) δ 2.36 (3, s, C_6–CH_3), 3.30 (3, s, N_3–CH_3), 8.40 (1, s, C_7–H); uv $\lambda_{max}^{pH\ 3.93}$ nm (log ε) 270 (4.25), 292 sh (3.98), 382 (3.81); $\lambda_{max}^{H_0=-1.85}$ nm (log ε) 257 (4.19), 287 sh (3.99), 352 (3.62).

2-Methylamino-6-methyl-4(3*H*)-pteridinone 8-Oxide (17). A suspension of 0.20 g of 3,6-dimethylpterin 8-oxide in 4 ml of 2 *N* sodium hydroxide was boiled for 5 min. The pterin quickly dissolved to give a clear orange solution, from which the yellow sodium salt of the Dimroth rearrangement product rapidly separated. The suspension was cooled and filtered, and the collected sodium salt was dissolved in 5 ml of hot water. Acidification with 4 drops of concentrated hydrochloric acid (to pH 2–3) resulted in the precipitation of yellow crystals which were collected by filtration, washed with cold water followed by ethanol, and dried to give 0.18 g (90%) of 2-methylamino-6-methyl-4(3*H*)-pteridinone 8-oxide: mp >300°; nmr (CF_3COOH) δ 2.36 (3, s, C_6–CH_3), 2.83 (3, s, CH_3NH), 8.40 (1, s, C_7–H); uv $\lambda_{max}^{0.1N\ NaOH}$ nm (log ε) 265 (4.51), 295 sh (3.90), 400 (3.90).

(33) Prepared by the condensation of 2,6-diamino-5-(*p*-nitrophenylazo)-4(3*H*)-pyrimidinone with the morpholine enamine of phenylacetaldehyde; this work will be reported independently.