Anal. Calcd. for $C_{11}H_{10}N_4O$: C, 61.7; H, 4.7. *Found:* C, 61.4; H, 4.5.

2-Amino-5-phenylpyrazine-3-carboxylic Acid.—A mixture of 3.1 g. of 2-amino-5-phenylpyrazine-3-carboxamide in 200 ml. of 1 *N* sodium hydroxide was heated under reflux for 8 hours and the resulting clear solution adjusted to *p*H 3 with concentrated hydrochloric acid to yield 2.19 g. (70%) of yellow needles, m.p. 196° dec. Recrystallization from aqueous methanol did not change the melting point. The reported melting point for 2-amino-6-phenylpyrazine-3-carboxylic acid is 225° dec.[17]

Anal. Calcd. for $C_{11}H_9N_3O_2$: C, 61.4; H, 4.2. Found: C, 61.4; H, 4.5.

2-Hydroxy-5-phenylpyrazine-3-carboxylic Acid.—A solution of 0.511 g. of 2-amino-5-phenylpyrazine-3-carboxylic acid in 15 ml. of cold concentrated sulfuric acid was treated with a solution of 0.25 g. of sodium nitrite in 5 ml. of cold concentrated sulfuric acid. The resulting deep red solution was held at 0° for four hours, at room temperature for four hours, and then poured into ice. The frothy suspension of yellow solid was stirred at room temperature overnight and filtered, and the collected solid recrystallized first from water and then from ethanol to give 0.455 g. (88.5%) of long light yellow needles, m.p. 210° dec. The material exhibited a bright green fluorescence in dilute aqueous solution. A mixture melting point with an authentic sample of 2-hydroxy-5-phenylpyrazine-3-carboxylic acid, m.p. 203° dec. (reported[17,18] m.p. 200° dec.) was 208–209° dec.; infrared spectra of the two samples were identical. A mixture melting point with an authentic sample of 2-hydroxy-6-phenylpyrazine-3-carboxylic acid, m.p. 224° dec. (reported[17] m.p. 208–209° dec.; 217° dec.[18]) was 196–199° dec.

2-Amino-5,6-dimethylpyrazine-3-carboxamide (A).—A solution of 28.5 g. of aminomalonamidamidine dihydrochloride in 300 ml. of water at 10° was added slowly, with external cooling, to a solution of 13 g. of biacetyl in 60 ml. of ethanol. When the exothermic reaction had subsided, 30 ml. of concentrated ammonium hydroxide was added slowly, with concomitant separation of a heavy, bright lemon-yellow solid. The reaction mixture was stirred for several hours, cooled to 0° and filtered to yield 23.0 g. (92%) of a mixture of A and B, m.p. 255–260° dec. Ten grams of this material was placed in a soxhlet cup and extracted with absolute ethanol for five days. Evaporation of the ethanol extracts yielded 7.47 g. of a yellow solid which consisted predominately of 2-amino-5,6-dimethylpyrazine-3-carboxamide (A) but which contained a small amount of B. Vacuum sublimation readily separated these compounds and yielded A as a light yellow, crystalline sublimate, m.p. 255°.

Anal. Calcd. for $C_7H_{10}N_4O$: C, 50.6; H, 6.1. Found: C, 50.7; H, 6.3.

Higher Melting Isomer B.—The solid residue remaining in the soxhlet cup above was extracted for 10 minutes with 50 ml. of boiling 50% aqueous dimethylformamide. The cooled extract yielded 1.13 g. of a light yellow solid which proved to be a mixture of A and B by examination of its infrared spectrum. The residue from the above extraction was then recrystallized from 300 ml. of boiling 50% aqueous dimethylformamide to give 1.19 g. of pure B. This compound decomposes slowly above 280°; decomposition is complete between 320–330°. Since it does not have a characteristic melting or decomposition point, its purity was determined by solubility measurements in boiling 50% aqueous dimethylformamide (0.5 g. in 100 ml.) and by examination of its infrared spectrum. The sample was judged pure when it was shown that the above obtained 1.19 g. was completely extracted by eight successive 30-ml. portions of boiling 50% aqueous dimethylformamide, each portion yielding 0.15 g. ±0.02 g. of product with identical infrared absorption spectra; $\lambda_{\max}^{0.1N\ HCl}$ 244, 377 mμ; log ϵ 4.01, 4.06.

Anal. Calcd. for $C_7H_{10}N_4O$: C, 50.6; H, 6.1. Found: C, 50.3; H, 6.0.

2-Amino-5,6-dimethylpyrazine-3-carboxylic Acid.—A mixture of 5.8 g. of 2-amino-5,6-dimethylpyrazine-3-carboxamide (A) (the material obtained directly from the ethanol extraction described above may be used) and 40 ml. of 3 *N* sodium hydroxide was heated under reflux for 1.5 hours, chilled and the clear solution acidified to *p*H 3 with hydrochloric acid to yield 4.7 g. (81%) of cream-colored crystals, m.p. 208° dec. Recrystallization from water did not raise the melting point. This compound is reported[5] to melt with decomposition at 209–210°.

PRINCETON, N. J.

[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY, PRINCETON UNIVERSITY, AND THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

Pteridines. XIX. A Synthesis of 8-Substituted Pteridine-6-carboxylic Acids

BY EDWARD C. TAYLOR[1a] AND HARVEY M. LOUX[1b]

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A new route to pteridine-6-carboxylic acids is described in which a 4,5-diaminopyrimidine is treated with alloxan in dilute alkaline solution. The condensation proceeds *via* the intermediate formation of a *spiro* pteridine (XV) which undergoes subsequent cleavage with alkali. Utilization of 2,5-diamino-4-hydroxy-6-substituted aminopyrimidines leads to 2-amino-4-hydroxy-7-keto-8-substituted-7,8-dihydropteridine-6-carboxylic acids, and by this procedure both the 8-(D-1-sorbityl) and 8-(D-1-ribityl) derivatives are prepared. It is shown that initial condensation of the pyrimidine and alloxan in acidic solution leads *via* a deep purple anil (similar to I) to a 9-substituted pyrimido(5,4-g)pteridine (XXX), which cleaves under the reaction conditions to the 7-keto-8-substituted-7,8-dihydropteridine-6-carboxylic acid. Reduction under Clemmensen conditions then yields the corresponding 2-amino-4-hydroxy-8-substituted-7,8-dihydropteridine-6-carboxylic acid.

Discussion

Although the existence of pteridine glycosides in nature has never been demonstrated, the possibility that pteridines might be carried through their metabolic pathways with a sugar attached remains an intriguing possibility. The demonstrated *in vitro*[2–4] and *in vivo*[5] conversion of purines into pteridines implies that appropriate conversions of nucleosides would provide pteridine glycosides, at least as initial products; a mechanism for solubilizing the extremely insoluble pteridines would be found; and the close relationship between purines and pteridines, and between the pteridines and the flavins (such as riboflavin) would be strengthened. Striking support for the latter relationship is found in the recent isolation from *Eremothecium ashbyii* of the 8-ribityl derivative of dimethyllumazine (2,4-dihydroxy-6,7-dimethylpteridine) and its implication as an intermediate in the biosynthesis of riboflavin,[6,7] and in the isolation of the 8-ribityl deriva-

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[1] (a) Frick Chemical Laboratory, Princeton University, Princeton, N. J.; (b) National Science Foundation Pre-doctoral Fellow.

[2] A. Albert, *Biochem. J.*, **57**, x (1954).

[3] A. Albert, *ibid.*, **65**, 124 (1957).

[4] A. Albert in "The Chemistry and Biology of Purines," ed. by G. E. W. Wolstenholme and C. M. O'Connor, J. and A. Churchill Ltd., London, 1957, p. 97.

[5] I. Ziegler-Gunder, H. Simon and A. Wacker, *Z. Naturf.*, **11b**, 82 (1956).

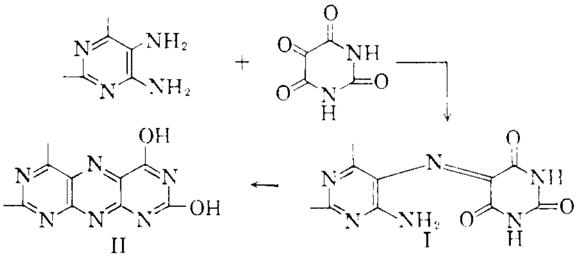
[6] T. Masuda, *Pharm. Bull.*, **5**, 28 (1957).

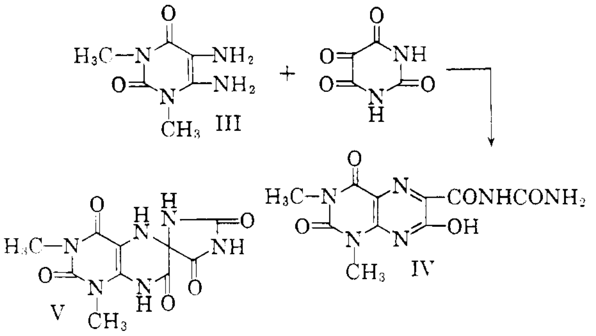
[7] T. Masuda, *ibid.*, **5**, 136 (1957).

tive of 2,4,7-trihydroxy-6-methylpteridine from the same source.[8] These considerations make very probable the existence of other 8-substituted pteridines in nature and stimulated us to examine possible synthetic routes for their preparation.

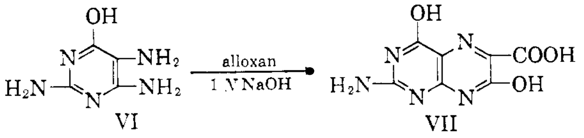
A few simple 8-alkyl pteridines have been reported previously and were prepared by methods involving the condensation of a suitable 4-substituted amino-5-aminopyrimidine with ethyl oxalate,[9] benzoin,[9,10,13] chloroacetyl chloride,[9] oxalic acid,[9] ethyl oxomalonate,[11] ethyl glyoxylate ethyl hemiacetal[12,13] or a 1,2-diketone.[12,13] 2,8-Dihydro-4-hydroxy-2-imino-8-methyl-6,7-diphenylpteridine has been made by an alternative approach involving the fusion of guanidine carbonate with 1,2-dihydro-3-methoxycarbonyl-1-methyl-2-oxo-5,6-diphenylpyrazine.[14] We wish to describe our own efforts in this direction which have utilized a new method of ring closure of diaminopyrimidines to pteridines[15] and have permitted the synthesis of pteridines substituted with sugar derivatives in the 8-position.

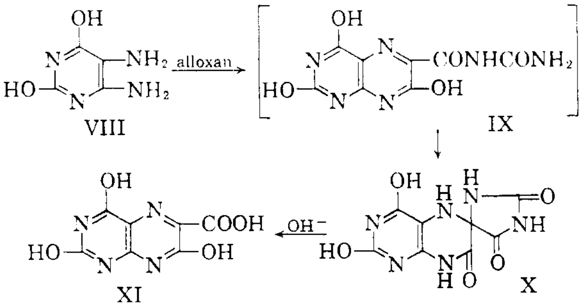
The reaction of 4,5-diaminopyrimidines with alloxan in acid solution has been shown to yield an intermediate deep purple anil (I) which, upon subsequent treatment with alkali, or upon prolonged heating in acid, cyclizes to a pyrimido(5,4-g)pteridine (II).[16] In neutral solution, however, a different reaction course is followed and pteridines are usually formed. The reaction of 1,3-dimethyl-5,6-diamino-2,4(1H,3H)pyrimidinedione (III) with alloxan was reported to yield 1,3-dimethyl-7-hydroxy-2,4(1H,3H)-pteridinedione-6-carboxyureide (IV),[17] although no evidence was cited to exclude the isomeric 7-carboxyureide structure. However, on the basis of arguments to be presented later in support of structure X, it appears more probable that the product of this reaction was the spiro compound V. Dimethylalloxan and III have been shown to give N-methyl 1,3-dimethyl-6-hydroxy-2,4-(1H,3H)-pteridinedione-7-carboxamide, the orientation of the product being determined by an ion-pair intermediate.[18,19] The reaction of dimethylalloxan and III in dilute acid, followed by boiling the reaction product in dilute alkali yields N-methyl-1,3-dimethyl-7-methylamino-2,4(1H,3H)-pteridinedione-6-carboxamide,[16,18] although the product was originally claimed to be a pyrimidopteridine.[20]





We have now found that the reaction of 4,5-diaminopyrimidines with alloxan in alkaline solution provides a convenient and efficient route to pteridine-6-carboxylic acids. The method may be illustrated by the reaction of 2,4,5-triamino-6-hydroxypyrimidine (VI) with alloxan in aqueous 1 *N* sodium hydroxide to give isoxanthopterincarboxylic acid (VII) in 97% yield. The identity of the product was confirmed by comparison with an authentic sample prepared from VI and diethyl oxomalonate.[21] The reaction of 2,4-dihydroxy-5,6-diaminopyrimidine (VIII) with alloxan under similar conditions gave desaminoisoxanthopterincarboxylic acid (2,4,7-trihydroxypteridine-6-carboxylic acid) (XI) in 55% yield. When these reactants were allowed to stand in dilute sodium hydroxide for one hour, a product was isolated for which the spiro structure X is suggested. This compound is isomeric with the 6-carboxyureide IX, but its ultraviolet absorption spectrum resembles that of a pyrimidine much more than a pteridine; the marked hypsochromic shift in the maximum of X as compared with XI cannot be reconciled with a carboxyureide structure, whose spectrum should be similar to that given by XI. The formation of a spiro compound from IX in alkaline solution par-





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(9) H. S. Forrest, R. Hull, H. J. Rodda and A. R. Todd, *J. Chem. Soc.*, 3 (1951).

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(11) G. B. Elion and G. H. Hitchings, *ibid.*, **75**, 4311 (1953).

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(13) W. E. Fidler and H. C. S. Wood, *ibid.*, 3980 (1957).

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(17) F. Sachs and G. Meyerheim, *Ber.*, **41**, 3957 (1908).

(18) H. Bredereck and W. Pfleiderer, *ibid.*, **87**, 1268 (1954).

(19) W. Pfleiderer, *ibid.*, **88**, 1625 (1955).

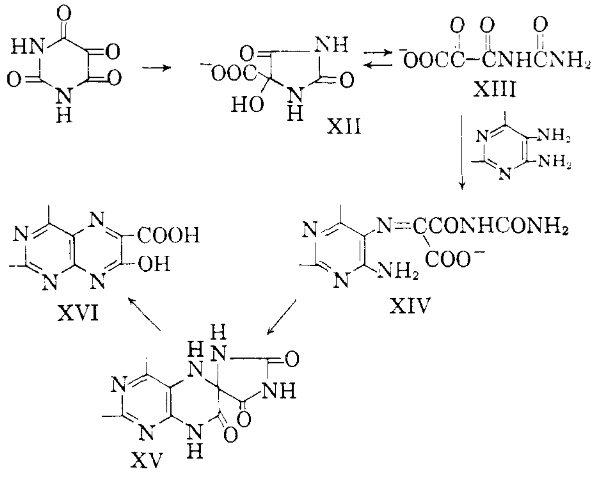
(20) O. DeGarmo, U. S. Patent 2,561,324; *C. A.*, **46**, 1595 (1952).

(21) R. Purrmann, *Ann.*, **548**, 284 (1941).

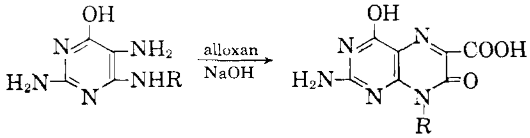
allels the recently reported conversion of 3-hydroxyquinoxaline-2-carboxyureides[22] and 3-hydroxy-1,4,5-triazanaphthalene-2-carboxyureides[23] to isomeric spiro derivatives, and thus may be consistent with the known propensity of the pteridine ring to undergo addition reactions across the pyrazine carbon–nitrogen double bonds.[24]

Compound X is evidently an intermediate in the conversion of VIII to XI, since it can be converted to XI in high yield under conditions which sufficed for the conversion from VIII. It is certain that 2,4,5,7-tetrahydroxypyrimido(5,4-g)pteridine, the product of the reaction of VIII with alloxan in acid solution,[16] is not an intermediate in the above conversion, since it is only partially converted to XI when subjected to identical reaction conditions.

It seems probable, in view of the above considerations, that alloxan undergoes an initial alkaline cleavage to alloxanic acid (XII), which exists in equilibrium in alkaline solution with the open chain monoureide of oxomalonic acid (XIII). Reaction of the keto group of XIII with the 5-amino group of the 4,5-diaminopyrimidine[25,26] would then yield XIV, which by cyclization to the spiro compound XV and finally hydrolysis, leads to the pteridine-6-carboxylic acid (XVI). This mechanism is supported by Pfleiderer's observation[19] that the sodium salt of the monomethyl amide of oxomalonic acid reacts with 1,3-dimethyl-5,6-diamino-2,4(1H,3H)-pyrimidinedione (III) to give the N-methyl amide of 1,3-dimethyl-7-hydroxy-2,4(1H,3H)-pteridinedione-6-carboxylic acid, and by our observation that the potassium salt of alloxanic acid can replace alloxan in the above syntheses.

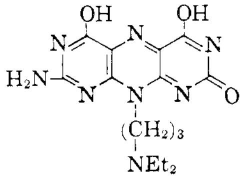


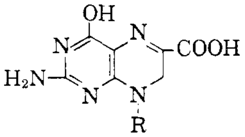
This new method for the synthesis of pteridine-6-carboxylic acids was then employed for the preparation of some 8-substituted derivatives of 2-amino-4-hydroxypteridines. The requisite 2,5-diamino-4-hydroxy-6-substituted aminopyrimidines (XVII–XXI) were prepared by reaction of 2-amino-4-hydroxy-5-phenylazo-6-chloropyrimidine[27] with ethylamine, 3-diethylaminopropylamine, 2,3-dihydroxypropylamine, D-glucamine and D-ribamine, respectively, followed by reduction with zinc dust and sulfuric acid. Because of the difficulties encountered in the isolation and purification of these unstable diaminopyrimidines, direct conversion to the corresponding pteridine-6-carboxylic acids (XXII–XXVI) was carried out by reaction with alloxan in alkaline solution.



XVII, R = -Et
XVIII, R = $-(CH_2)_3N(Et)_2$
XIX, R = $-CH_2CHOHCH_2OH$
XX, R = $-CH_2(CHOH)_4CH_2OH$
XXI, R = $-CH_2(CHOH)_3CH_2OH$

XXII, R = -Et
XXIII, R = $-(CH_2)_3N(Et)_2$
XXIV, R = $-CH_2CHOHCH_2OH$
XXV, R = $-CH_2(CHOH)_4CH_2OH$
XXVI, R = $-CH_2(CHOH)_3CH_2OH$





XXVII, R = $-CH_2CHOHCH_2OH$
XXVIII, R = $-CH_2(CHOH)_4CH_2OH$
XXIX, R = $-CH_2(CHOH)_3CH_2OH$

This conversion proceeded by alternate pathways depending on a seemingly minor experimental detail. If sufficient alkali was first added to the acidic reduction solution so that the solution was approximately 0.5 *N* in sodium hydroxide, and the alloxan was then added, the reaction followed the course previously outlined (XII → XVI). However, if alloxan was added directly to the reduction mixture, a deep violet color due to the anil (corresponding to I) appeared, which disappeared upon addition of alkali. In both cases, heating the reaction mixtures overnight brought about complete conversion to the pteridine-6-carboxylic acids. Confirmation of the assumption that the latter reaction conditions involved the intermediate formation of a pyrimido(5,4-g)pteridine, which then underwent cleavage, was obtained by the conversion of 2,5-diamino-4-hydroxy-6-(3-diethylaminopropyl)-aminopyrimidine(XVIII) to 2-keto-4,5-dihydroxy-7-amino-9-(3-diethylaminopropyl)-2,9-dihydropyrimido(5,4-g)pteridine (XXX) by heating with alloxan in the absence of alkali. Treatment of XXX with 0.5 *N* sodium hydroxide overnight resulted in complete conversion to XXIII. It has already been pointed out that 2,4,5,7-tetrahydroxypyrimido(5,4-g)pteridine does not undergo complete hydrolysis to a pteridine even under more vigorous conditions, and it thus appears that these 9-substituted pyrimido(5,4-g)pteridines are considerably more labile toward alkaline cleavage than the 9-

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(22) J. W. Clark-Lewis, *J. Chem. Soc.*, 422 (1957).

(23) J. W. Clark-Lewis and M. J. Thompson, *ibid.*, 430 (1957).

(24) A. Albert in "Current Trends in Heterocyclic Chemistry," ed. by A. Albert, G. M. Badger and C. W. Shoppee, Butterworths Scientific Publications, London, 1958, p. 20.

(25) W. Wilson, *J. Chem. Soc.*, 1157 (1948).

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

unsubstituted derivatives. This conclusion is in agreement with previous observations on the isoalloxazines; riboflavin, on treatment with dilute alkali, is hydrolyzed to a quinoxalone carboxylic acid, and lumiflavin behaves similarly.[28] By contrast, alloxazine itself is degraded only slowly by alkali.[29]

Conditions for the reduction of the lactam grouping in these 2-amino-4-hydroxy-7-keto-8-substituted-7,8-dihydropteridine-6-carboxylic acids were then examined, using the 8-(2,3-dihydroxypropyl) derivative XXIV as a model. Lithium aluminum hydride reduction was precluded because of the insolubility of this compound in suitable nonaqueous solvents, and all attempts to convert XXIV to its ethyl ester failed. Reaction with acetic anhydride yielded a diacetate which was not, however, appreciably more soluble than the parent compound. Reduction to 2-amino-4-hydroxy-8-(2,3-dihydroxypropyl)-7,8-dihydropteridine-6-carboxylic acid (XXVII) was finally effected under Clemmensen conditions. Application of these conditions to XXV and XXVI yielded the 8-sorbityl (XXVIII) and 8-ribityl (XXIX) derivatives of 2-amino-4-hydroxy-7,8-dihydropteridine-6-carboxylic acid, respectively. It is of interest that XXIX possesses the structure initially considered by Forrest and Mitchell[30] for the yellow Drosophila pigment, although further work indicated that the compound might better be represented as an 8-lactyl derivative.

Experimental[31]

2-Amino-4,7-dihydroxypteridine-6-carboxylic Acid (Isoxanthopterincarboxylic Acid (VII). Method A.—To a solution of 5.0 g. of 2,4,5-triamino-6-hydroxypyrimidine dihydrochloride in 150 ml. of 1 *N* sodium hydroxide was added a solution of 4.0 g. of alloxan monohydrate[32] in 50 ml. of water. The resulting yellow solution was heated under reflux for two days with mechanical stirring. The reaction mixture was then cooled and filtered to give 6.5 g. of a light tan sodium salt which was digested in 0.5 *N* hydrochloric acid for 3 hours on a steam-bath. Filtration of this acidic suspension yielded a light yellow microcrystalline solid which was washed thoroughly with water followed by acetone and dried; crude yield 5.06 g. (97%), m.p. >360°. The product was purified for analysis by dissolving in 0.1 *N* sodium hydroxide and reprecipitating with 6 *N* hydrochloric acid and was dried *in vacuo* at 156° for 18 hours over phosphorus pentoxide; $\lambda_{\max}^{0.1\ N\ \mathrm{NaOH}}$ 224, 259, 282 (shoulder), 347 mμ; log ϵ 4.57, 4.00, 3.53, 4.17.

Anal. Calcd. for $C_7H_5N_5O_4$: C, 37.7; H, 2.3; N, 31.4. Found: C, 37.6; H, 2.5; N, 31.1.

Method B.—To a solution of 4.0 g. of 2,4,5-triamino-6-hydroxypyrimidine dihydrochloride in 200 ml. of 1 *N* sodium hydroxide was added a solution of 6.0 g. of ethyl oxomalonate in 90 ml. of water, and the mixture was heated on a steam-bath for 14 hours. After the cooled reaction mixture had stood at room temperature for 2 days, it was acidified with 6 *N* hydrochloric acid, and the light yellow microcrystalline solid which separated was collected by filtration, washed well with water followed by acetone and dried; crude yield, 3.55 g. (85%), m.p. >360°.

Both samples of isoxanthopterincarboxylic acid were identical with an authentic sample prepared by the method of Purrmann,[21] as determined by comparison of ultraviolet absorption spectra and by paper chromatography.

Spiro Compound X.—Addition of 6.0 g. of alloxan monohydrate to a solution of 10 g. of 2,4-dihydroxy-5,6-diaminopyrimidine sulfate in 200 ml. of 1 *N* sodium hydroxide resulted in the immediate separation of an orange solid. The mixture was allowed to stand at room temperature for 1 hour and then filtered. The collected solid was dissolved in cold 0.1 *N* sodium hydroxide, a small amount of undissolved red solid removed by filtration and the filtrate acidified with glacial acetic acid to yield 6.24 g. (48%) of a microcrystalline solid, m.p. >360° (with darkening above 300°); $\lambda_{\max}^{1.0\ N\ \mathrm{NaOH}}$ 220.5, 278, 310 (shoulder) mμ; log ϵ 4.23. 4.01, 3.76.

Anal. Calcd. for $C_8H_6N_6O_5$: C, 36.1; H, 2.3; N, 31.6. Found: C, 36.2; H, 2.5; N, 31.6.

The small amount of red solid obtained above was dissolved in 1 *N* sodium hydroxide by warming on a steam-bath for 1 hour. Acidification of the resulting solution then yielded 0.49 g. of 2,4,7-trihydroxypteridine-6-carboxylic acid, identified by comparison with a known sample of this material (see below). The red solid was thus most probably the sodium salt of an intermediate hydrolysis product of X.

2,4,7-Trihydroxypteridine-6-carboxylic Acid (XI). Method A.—To a solution of 10 g. of 2,4-dihydroxy-5,6-diaminopyrimidine sulfate in 300 ml. of 1 *N* sodium hydroxide was added a solution of 6.0 g. of alloxan monohydrate in 75 ml. of water, and the resulting mixture was heated under reflux with mechanical stirring for 45 hours. Ammonia was continuously evolved and a light-colored solid started to separate from the reaction mixture after 3 hours. The cooled mixture was filtered and the crude sodium salt (9.52 g.) suspended in 200 ml. of 0.5 *N* hydrochloric acid. This mixture was heated on a steam-bath for 3 hours, cooled and filtered to give a white microcrystalline solid; yield 6.0 g. (55%), m.p. >360°. The product was purified by dissolving in 0.1 *N* sodium hydroxide and reprecipitating by the addition of 6 *N* hydrochloric acid; $\lambda_{\max}^{0.1\ N\ \mathrm{NaOH}}$ 227, 280, 341 mμ; log ϵ 4.43, 3.90, 4.17.

Anal. Calcd. for $C_7H_4N_4O_5$: C, 37.5; H, 1.6; N, 25.0. Found: C, 37.2; H, 1.7; N, 25.3.

Method B.—A solution of 5.0 g. of the spiro compound X in 150 ml. of 1 *N* sodium hydroxide was heated under reflux for 44 hours and then acidified with glacial acetic acid. Cooling caused the separation of 2.15 g. (51%) of 2,4,7-trihydroxypteridine-6-carboxylic acid, identical with a sample prepared by method A above.

Method C.—A solution of the dipotassium salt of alloxanic acid was prepared by adding 20 ml. of 40% aqueous potassium hydroxide to a solution of 10 g. of alloxan monohydrate in 20 ml. of water. After one hour it was added to a suspension of 12.5 g. of 2,4-dihydroxy-5,6-diaminopyrimidine sulfate in 300 ml. of water. The resulting mixture was heated under reflux overnight, filtered to remove a small amount of undissolved solid and the filtrate acidified with glacial acetic acid. The solid which separated was collected by filtration, washed with water followed by acetone and dried to give 7.5 g. (55%) of 2,4,7-trihydroxypteridine-6-carboxylic acid, identical with the products obtained by methods A and B above. Examination of the ultraviolet absorption spectrum of the filtrate (adjusted to 0.1 *N* sodium hydroxide) revealed the presence of a small amount of X.

2-Amino-4-hydroxy-5-phenylazo-6-ethylaminopyrimidine (XVII).—To a solution of 12 g. of 2-amino-4-hydroxy-5-phenylazo-6-chloropyrimidine[27] in 135 ml. of dimethylformamide was added 30 ml. of 30% aqueous ethylamine, and the mixture was heated to 90° and then allowed to stand for 2 hours. Dilution with 500 ml. of water caused the separation of a yellow-brown solid, which was collected by filtration, washed and dried; yield 7.0 g. (57%). The product was obtained in the form of orange microcrystals, m.p. 284° dec., by recrystallization from absolute ethanol; $\lambda_{\max}^{0.1\ N\ \mathrm{NaOH}}$ 248, 252(shoulder), 310(shoulder), 385, 413-(shoulder) mμ; log ϵ 4.26, 4.25, 3.62, 4.37, 4.27.

Anal. Calcd. for $C_{12}H_{14}N_6O$: C, 55.8; H, 5.5; N, 32.5. Found: C, 55.6; H, 5.4; N, 32.4.

2-Amino-4-hydroxy-5-phenylazo-6-(3-diethylaminopropyl)-aminopyrimidine (XVIII).—To a partial solution of 10 g. of 2-amino-4-hydroxy-5-phenylazo-6-chloropyrimidine in 100 ml. of dioxane was added 9.2 g. of 3-diethylamino-

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[28] T. Wagner-Jauregg in "The Vitamins," ed. by W. H. Sebrell, Jr., and R. S. Harris, Academic Press, Inc., New York, N. Y., Vol. III, 1954, p. 301.

[29] O. Kühling, *Ber.*, **24**, 2363 (1891).

[30] H. S. Forrest and H. K. Mitchell, THIS JOURNAL, **76**, 5658 (1954).

[31] All melting points are uncorrected. We are indebted for the microanalyses to Dr. Joseph F. Alicino, Metuchen, N. J.

[32] *Org. Syntheses*, **32**, 6 (1952).

propylamine, and the resulting mixture was allowed to stand at room temperature with occasional shaking for 20 hours. The solid which had separated out was collected by filtration and recrystallized from dioxane to give 6.55 g. (48%) of light yellow crystals, m.p. 201–203° dec.; $\lambda_{\max}^{0.1\ N\ \mathrm{NaOH}}$ 250, 313(shoulder), 384, 417(shoulder) mμ; log ε 4.26, 3.43, 4.34, 4.23.

Anal. Calcd. for $C_{17}H_{25}N_7O$: C, 59.5; H, 7.3; N, 28.6. Found: C, 59.4; H, 7.3; N, 28.4.

2-Amino-4-hydroxy-5-phenylazo-6-(2,3-dihydroxypropyl)-aminopyrimidine (XIX).—A mixture of 5.0 g. of 2-amino-4-hydroxy-5-phenylazo-6-chloropyrimidine, 3.0 g. of α-glycerolamine, 30 ml. of absolute ethanol and 20 ml. of dioxane was allowed to stand overnight at room temperature and then filtered to give 4.62 g. of an orange microcrystalline solid. The filtrate deposited an additional 0.39 g. of product after standing for one week; total yield 5.01 g. (82%). The product was purified by recrystallization from dilute ammonium hydroxide; m.p. 254° dec.; $\lambda_{\max}^{0.1\ N\ \mathrm{NaOH}}$ 250.5, 310(shoulder), 384, 412(shoulder) mμ; log ε 4.24, 3.36, 4.36, 4.26.

Anal. Calcd. for $C_{13}H_{16}N_6O_3$: C, 51.2; H, 5.5; N, 27.6. Found: C, 51.0; H, 5.5; N, 27.2.

2-Amino-4-hydroxy-5-phenylazo-6-D-glucaminopyrimidine (XX).—A mixture of 6.0 g. of D-glucamine, 8.4 g. of 2-amino-4-hydroxy-5-phenylazo-6-chloropyrimidine, 70 ml. of water and 100 ml. of dioxane was allowed to stand for 48 hours with occasional shaking. The mixture was diluted with 600 ml. of acetone and the precipitated solid collected by filtration; yield 6.67 g. (50%). Recrystallization from dilute ammonium hydroxide yielded the product in the form of a microcrystalline yellow-orange solid, m.p. 190° dec.; $\lambda_{\max}^{0.1\ N\ \mathrm{NaOH}}$ 250.5, 310(shoulder), 381, 412(shoulder) mμ; log ε 4.16, 3.29, 4.30, 4.16.

Anal. Calcd. for $C_{16}H_{22}N_6O_6$: C, 48.7; H, 5.6; N, 21.3. Found: C, 48.6; H, 5.8; N, 21.0.

2-Amino-4-hydroxy-5-phenylazo-6-D-ribaminopyrimidine (XXI) was prepared in 43% yield by a similar procedure from 5.61 g. of D-ribamine, 9.3 g. of 2-amino-4-hydroxy-5-phenylazo-6-chloropyrimidine, 100 ml. of water and 120 ml. of dioxane. Recrystallization of the crude product from dilute ammonium hydroxide yielded yellow-orange microcrystals, m.p. 165° dec.; $\lambda_{\max}^{0.1\ N\ \mathrm{NaOH}}$ 250.5, 310-(shoulder), 384, 410(shoulder) mμ; log ε 4.29, 3.68, 4.40, 4.30.

Anal. Calcd. for $C_{15}H_{20}N_6O_5$: C, 49.4; H, 5.5; N, 23.1. Found: C, 49.0; H, 5.5; N, 22.8.

2-Amino-4-hydroxy-7-keto-8-ethyl-7,8-dihydropteridine-6-carboxylic Acid (XXII). Method A.—A solution of 0.5 g. of crude 2,5-diamino-4-hydroxy-6-ethylaminopyrimidine[9] and 0.4 g. of alloxan monohydrate in 14 ml. of 1 *N* sodium hydroxide was heated under reflux for 16 hours and then acidified with hydrochloric acid. The precipitated light yellow solid was collected by filtration, washed with water and dried; yield 0.35 g. (67%), m.p. >360°. The material was purified by dissolution in 0.1 *N* sodium hydroxide followed by reprecipitation with hydrochloric acid; $\lambda_{\max}^{0.1\ N\ \mathrm{NaOH}}$ 261.5, 284(shoulder), 367 mμ; log ε 4.10, 3.67, 4.22.

Anal. Calcd. for $C_9H_9N_5O_4 \cdot H_2O$: C, 40.2; H, 4.1; N, 26.0; Found: C, 40.1; H, 4.0; N, 26.1.

When the material was purified by dissolution in dilute ammonium hydroxide followed by neutralization with hydrochloric acid, the ammonium salt of 2-amino-4-hydroxy-7-keto-8-ethyl-7,8-dihydropteridine-6-carboxylic acid separated.

Anal. Calcd. for $C_9H_{12}N_6O_4$: C, 40.3; H, 4.5; N, 31.3. Found: C, 40.2; H, 4.6; N, 31.3.

Method B.—To a suspension of 4 g. of 2-amino-4-hydroxy-5-phenylazo-6-ethylaminopyrimidine in 100 ml. of hot water was added 6.6 g. of zinc dust followed immediately by 6.4 ml. of 10 *N* sulfuric acid, and the mixture was heated under reflux for 2 hours. The reduced pyrimidine was only partially in solution at this time. The reaction slurry was decanted from the residual zinc, washed with two 10-ml. portions of hot water, and the washings added to the slurry. To this was added 2.8 g. of alloxan monohydrate followed immediately by 40 ml. of 4 *N* sodium hydroxide, and the mixture was heated under reflux for 15 hours. The reaction mixture was filtered from the zinc salts and the filtrate acidified with hydrochloric acid to give 2.68 g. (64%) of microcrystalline 2-amino-4-hydroxy-7-keto-8-ethyl-7,8-dihydropteridine-6-carboxylic acid, identical with the product obtained by method A above.

2-Keto-4,5-dihydroxy-7-amino-9-(3-diethylaminopropyl)-2,9-dihydropyrimido(5,4-g)pteridine (XXX).—To a suspension of 6.0 g. of 2-amino-4-hydroxy-5-phenylazo-6-(3-diethylaminopropyl)-aminopyrimidine in 150 ml. of boiling water was added 13.2 g. of zinc dust and 9.6 ml. of 10 *N* sulfuric acid. Boiling was continued for 10–15 minutes until the orange color of the solution had been discharged. The solution was decanted from the zinc, which was washed with two 10-ml. portions of hot water, and to the combined solutions was added 4.2 g. of alloxan monohydrate. A deep purple color formed immediately on mixing, followed by the separation of a yellow solid. The reaction mixture was then heated under reflux for 2 hours to give a clear, light yellow solution. Cooling caused the separation of a yellow solid which was collected by filtration, washed with water and dried; yield 6.13 g. (93%). Recrystallization from dimethylformamide yielded bright yellow microcrystals which did not melt below 360°; $\lambda_{\max}^{0.1\ N\ \mathrm{NaOH}}$ 242.5, 274, 438 mμ; log ε 4.27, 3.91, 4.30.

Anal. Calcd. for $C_{15}H_{20}N_8O_3 \cdot H_2O$: C, 47.6; H, 5.9; N, 29.6. Found: C, 47.2; H, 6.4; N, 30.0.

2-Amino-4-hydroxy-7-keto-8-(3-diethylaminopropyl)-7,8-dihydropteridine-6-carboxylic Acid (XXIII). Method A.—The reduction of 2-amino-4-hydroxy-5-phenylazo-6-(3-diethylaminopropyl)-aminopyrimidine was carried out as described above. Following the addition of alloxan monohydrate to the reduction mixture, 60 ml. of 4 *N* sodium hydroxide was added, and the resulting yellow solution was heated under reflux for 19 hours. The precipitated zinc salts were removed by filtration and the filtrate acidified with acetic acid. The finely-divided yellow solid which separated was collected by centrifugation, washed with water followed by acetone, and dried to yield 4.35 g. (74%) of crude product, m.p. 222–232° dec. Purification by dissolution in dilute ammonium hydroxide followed by reprecipitation with acetic acid raised the melting point to 262° dec. Aqueous solutions of the product exhibited a strong blue fluorescence; $\lambda_{\max}^{0.1\ N\ \mathrm{NaOH}}$ 263, 285(shoulder), 368 mμ; log ε 4.09, 3.52, 4.23.

Anal. Calcd. for $C_{14}H_{20}N_6O_4$: C, 50.0; H, 6.0; N, 25.0. Found: C, 49.9; H, 6.3; N, 24.7.

Method B.—A solution of 4.0 g. of 2-keto-4,5-dihydroxy-7-amino-9-(3-diethylaminopropyl)-2,9-dihydropyrimido-(5,4-g)pteridine in 150 ml. of 0.5 *N* sodium hydroxide was heated under reflux overnight, acidified with acetic acid, cooled and centrifuged to yield 1.85 g. (52%) of 2-amino-4-hydroxy-7-keto-8-(3-diethylaminopropyl)-7,8-dihydropteridine-6-carboxylic acid as pale yellow microcrystals. Comparison of ultraviolet absorption spectra showed the products prepared by methods A and B to be identical.

Method C.—The procedure outlined above in method A was repeated except that the sodium hydroxide solution was added to the reduction mixture before rather than after the addition of the alloxan monohydrate. The yield was 2.85 g. (49%) of a yellow microcrystalline solid which was shown by comparison of ultraviolet absorption spectra to be identical with the products obtained by methods A and B above.

2-Amino-4-hydroxy-7-keto-8-(2,3-dihydroxypropyl)-7,8-dihydropteridine-6-carboxylic Acid (XXIV).—2-Amino-4-hydroxy-5-phenylazo-6-(2,3-dihydroxypropyl)-aminopyrimidine (24 g.) was reduced with 52.8 g. of zinc dust and 38.4 ml. of 10 *N* sulfuric acid in 500 ml. of water as described above in method A. To the reduction mixture was added 16.8 g. of alloxan monohydrate followed by 240 ml. of 4 *N* sodium hydroxide, and the reaction mixture was worked up as previously described to yield 18.5 g. (79%) of crude product, m.p. 296° dec. Recrystallization from dilute ammonium hydroxide yielded pale yellow microcrystals, m.p. 306–310° dec. Aqueous solutions of this material exhibited a strong blue fluorescence; $\lambda_{\max}^{0.1\ N\ \mathrm{NaOH}}$ 263, 285-(shoulder), 396 mμ; log ε 4.10, 3.52, 4.25.

Anal. Calcd. for $C_{10}H_{11}N_5O_6$: C, 40.4; H, 3.7; N, 23.6. Found: C, 40.2; H, 3.8; N, 23.5.

Diacetyl Derivative of 2-Amino-4-hydroxy-7-keto-8-(2,3-dihydroxypropyl)-7,8-dihydropteridine-6-carboxylic Acid.—A suspension of 4.0 g. of 2-amino-4-hydroxy-7-keto-8-(2,3-dihydroxypropyl)-7,8-dihydropteridine-6-carboxylic acid in 30 ml. of acetic anhydride containing 4 drops of concentrated sulfuric acid was heated under reflux with occasional stirring for 3 hours. The resulting clear solution was evaporated to dryness under reduced pressure and the residue digested with water and then filtered to yield 3.5 g. (68%) of the crude acetylated product. Recrystallization from 95% ethanol yielded pale yellow crystals, m.p. 144–146° dec.; $\lambda_{\max}^{0.1\,N\ \mathrm{NaOH}}$ 220.5, 261.5, 363 mμ; log ε 4.34, 4.06, 4.17.

Anal. Calcd. for $C_{14}H_{15}N_5O_8$: C, 44.1; H, 4.0; N, 18.4. Found: C, 44.1; H, 4.2; N, 18.1.

2-Amino-4-hydroxy-7-keto-8-(D-1-sorbityl)-7,8-dihydropteridine-6-carboxylic acid (XXV) was prepared in 63% yield from 2-amino-4-hydroxy-5-phenylazo-6-D-glucaminopyrimidine by the procedure described in method A above. The crude product was recrystallized from water containing a few drops of glacial acetic acid to give a pale yellow microcrystalline solid, m.p. 343° dec. Aqueous solutions of this material exhibited a strong blue fluorescence; $\lambda_{\max}^{0.1\,N\ \mathrm{NaOH}}$ 263.5, 369 mμ; log ε 3.91, 4.04.

Anal. Calcd. for $C_{13}H_{17}N_5O_9$: C, 40.3; H, 4.4; N, 18.1. Found: C, 40.3; H, 4.6; N, 18.0.

2-Amino-4-hydroxy-7-keto-8-(D-1-ribityl)-7,8-dihydropteridine-6-carboxylic acid (XXVI) was prepared in 35% yield from 2-amino-4-hydroxy-5-phenylazo-6-D-ribaminopyrimidine by the procedure described above. The crude product was recrystallized from dilute acetic acid to yield a pale yellow microcrystalline solid, m.p. 345° dec. Aqueous solutions of this material exhibited a strong blue fluorescence; $\lambda_{\max}^{0.1\,N\ \mathrm{NaOH}}$ 263.5, 369 mμ; log ε 4.08, 4.22.

Anal. Calcd. for $C_{12}H_{15}N_5O_8$: C, 40.3; H, 4.2; N, 19.6. Found: C, 39.9; H, 4.2; N, 19.4.

2-Amino-4-hydroxy-8-(2,3-dihydroxypropyl)-7,8-dihydropteridine-6-carboxylic Acid (XXVII).—Zinc amalgam was prepared by shaking manually a mixture consisting of 10 g. of mossy zinc, 0.75 g. of mercuric chloride, 0.5 ml. of concentrated hydrochloric acid and 12 ml. of water. After 5 minutes, the liquid was decanted and the amalgam covered with 7.5 ml. of water and 10 ml. of concentrated hydrochloric acid. At once 4.0 g. of 2-amino-4-hydroxy-7-keto-8-(2,3-dihydroxypropyl)-7,8-dihydropteridine-6-carboxylic acid was added and the reaction mixture was heated under reflux for 20 minutes. The resulting yellow-green solution was cooled, adjusted to *p*H 9 with ammonium hydroxide and cooled at 5–10° for 2 days. Filtration yielded 2.62 g. (65%) of the ammonium salt of 2-amino-4-hydroxy-8-(2,3-dihydroxypropyl)-7,8-dihydropteridine-6-carboxylic acid. The product was purified by dissolution in dilute ammonium hydroxide followed by reprecipitation with glacial acetic acid, and finally by recrystallization from dilute acetic acid. It was obtained as a cream-colored microcrystalline solid, m.p. 332° dec. Aqueous solutions of this material exhibited a strong blue fluorescence; $\lambda_{\max}^{0.1\,N\ \mathrm{NaOH}}$ 260, 342 mμ; log ε 3.99, 4.15.

Anal. Calcd. for $C_{10}H_{13}N_5O_5$: C, 42.4; H, 4.6; N, 24.7. Found: C, 42.3; H, 4.6: N, 24.9.

2-Amino-4-hydroxy-8-(D-1-sorbityl)-7,8-dihydropteridine-6-carboxylic acid (XXVIII) was prepared in 53% yield from 2-amino-4-hydroxy-7-keto-8-(D-1-sorbityl)-7,8-dihydropteridine-6-carboxylic acid by the method described above. It was obtained in the form of a pale yellow microcrystalline solid, m.p. 346° dec. upon recrystallization of the crude product from very dilute acetic acid; $\lambda_{\max}^{0.1\,N\ \mathrm{NaOH}}$ 260, 356 mμ; log ε 4.01, 4.07.

Anal. Calcd. for $C_{13}H_{19}N_5O_8$: C, 41.8; H, 5.1; N, 18.8. Found: C, 41.6; H, 5.4; N, 18.9.

2-Amino-4-hydroxy-8-(D-1-ribityl)-7,8-dihydropteridine-6-carboxylic acid (XXIX) was prepared in 22% yield from 2-amino-4-hydroxy-7-keto-8-(D-1-ribityl)-7,8-dihydropteridine-6-carboxylic acid by the method described above. It was obtained in the form of a pale yellow microcrystalline solid, m.p. 354° dec. upon recrystallization from very dilute acetic acid. The identity of the product was established by comparison of its ultraviolet absorption spectrum with that given by the 8-(D-1-sorbityl) derivative above; $\lambda_{\max}^{0.1\,N\ \mathrm{NaOH}}$ 259, 353 mμ; log ε 4.04, 4.13.

PRINCETON, N. J.

[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY, PRINCETON UNIVERSITY]

Pteridines XX. 3-Amino-4(3*H*)pteridinone[1,2]

BY EDWARD C. TAYLOR, O. VOGL AND PAULA K. LOEFFLER

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3-Amino-4(3*H*)-pteridinone (VI) has been prepared by cyclization with ethyl orthoformate and acetic anhydride of the isopropylidene (II) and benzylidene (III) derivatives of 2-aminopyrazine-3-carboxyhydrazide (I), followed by very mild acid cleavage of the protecting groups. Because of the great ease with which VI may be hydrolyzed both by acid and by base to regenerate I, it would appear that pteridine intermediates of this type are unsuited for the preparation of pteridine "pseudo" glycosides.

There is an increasing body of evidence which indicates that purine[3–7] and pyrimidine[8–11] antimetabolites may be more effective as ribosides or ribotides than as the free bases, and that, indeed, prior ribosidation or ribotidation[12,13] *in vivo* may be a prerequisite first step in their biological utilization. Although pteridine glycosides have neither been prepared nor isolated from nature, their possible presence in biological systems is strongly suggested by a number of considerations which have been summarized in an accompanying paper.[14] As a part of a program directed toward the synthesis of pteridine glycosides, it was thought that "pseudo" glycosides in which the sugar grouping was attached through a substituent amino group rather than directly to the ring might be of interest as potential antifolic acid compounds. We report in this paper the preparation and properties of a

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(1) For the previous paper in this series, see O. Vogl and E. C. Taylor, THIS JOURNAL, **81**, 2472 (1959).

(2) This investigation was supported by a grant (C-2551) to Princeton University from the National Cancer Institute of the National Institutes of Health, Public Health Service.

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