ence of pyridine and methoxalyl or ethoxalyl chloride, or in the presence of pyridine and acetic anhydride. A pyrroline-carboxylic acid was not obtained under the same conditions from N-ethoxalyl-β-amino-β-phenylpropionic acid.

3. The pyrroline-carboxylic acids were esterified with diazomethane and diazoethane. The products so obtained were identical with pyrroline derivatives prepared by the reaction of diazomethane and diazoethane upon the methyl and ethyl esters of 1,5-diphenyl-2,3-pyrrolidinedione-4-carboxylic acid, and the methyl ester of 1-phenyl-2,3-pyrrolidinedione-4-carboxylic acid.

4. A mechanism has been proposed for the ring closure reaction which relates it to the Perkin reaction and to the Claisen condensation.

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[CONTRIBUTION FROM THE BAKER LABORATORY OF CHEMISTRY AT CORNELL UNIVERSITY]

Pteridines. V. Deamination Studies on Certain Aminopteridines

BY E. C. TAYLOR, JR.,[1] AND C. K. CAIN

A previous paper[2] has described the different behavior toward acid hydrolysis of amino groups in the 2- and 4- positions of certain pyrimidine derivatives. There is also a difference in the reactivity of amino groups in the 2- and 6-positions of the purine nucleus, most strikingly demonstrated by the selective action of nitrous acid. Fischer[3] observed that the amino group of 2-amino-6-hydroxypurine (guanine) can be replaced by a hydroxyl group upon treatment with hot dilute (6 *N*) sulfuric acid and sodium nitrite but that 6-amino-2-hydroxypurine (isoguanine) is unaffected under the same conditions.[4] The latter has also been noted by other workers.[5,6] Hydrolysis of the amino group of isoguanine was effected, however, by boiling with 6 *N* hydrochloric acid for forty-five hours,[6,7] conditions which are also effective in hydrolyzing the amino group of guanine.[8]

A corresponding difference in the reactivity toward nitrous acid of the amino groups in the 2- and 4-positions of certain substituted pteridines was also observed. The amino group of 2-amino-4,6,7-trihydroxypteridine (leucopterin) may be replaced by a hydroxyl group upon treatment with strong (29 *N*) sulfuric acid and sodium nitrite,[9,10] whereas 4-amino-2,6,7-trihydroxypteridine (isoleucopterin) is not affected under the same conditions.[11] Likewise, a 2-amino-4-hydroxypteridine derivative (rhizopterin) is deaminated by the action of nitrous acid on a solution of the compound in a mixture of hydrochloric and acetic acids.[12] The substituents on the pyrazine portion of the pteridine nucleus seem to have a profound influence on the reactivity of the entire molecule, for 2-amino-4,6-dihydroxypteridine (xanthopterin) is deaminated normally under the conditions used above for rhizopterin,[12] although the molecule is completely disrupted by the action of nitrosylsulfuric acid.[13]

The purpose of the present investigation was to ascertain whether this behavior is confined to pterins of the xanthopterin–leucopterin series or is a general phenomenon and to find if possible some correlation between chemical reactivity and structure.

Studies on several variously substituted pteridines have shown that this difference in reactivity of amino groups in the 2- and 4-positions is not restricted to the above examples. An amino group in the 2-position of certain pterins may be replaced by a hydroxyl group by the action of nitrous acid. For example, 2-amino-4-hydroxypteridine and 2-amino-4-hydroxy-6,7-dimethylpteridine are smoothly converted to the corresponding 2,4-dihydroxypteridines by the addition of sodium nitrite solution to a solution of the pterin in boiling 7 *N* sulfuric acid. However, 2-amino-4-hydroxy-6,7-diphenylpteridine is unaltered not only under these conditions but even when a sulfuric acid solution is heated with nitrosylsulfuric acid. An amino group in the 4-position has been shown to be resistant to the action of nitrous acid; for example, 4-amino-2-hydroxypteridine and 4-amino-2-hydroxy-6,7-diphenylpteridine are unaffected under any of the above conditions. Contrary to expectation, it has been found that several 2,4-diaminopteridines are unaltered by nitrous acid regardless of the substitution on the pyrazine portion of the nucleus.

We have found that although an amino group in the 4-position of the pteridine nucleus is resistant to the action of nitrous acid, it may be removed readily by hydrolysis with dilute mineral acid under even milder conditions than required for the analogous hydrolysis in the purine series mentioned above. 2,4-Diaminopteridine, 2,4-diamino-6,7-dimethylpteridine and 2,4-diamino-

(1) U. S. Rubber Company Fellow in Chemistry, 1948-1949.
(2) Taylor and Cain, THIS JOURNAL, **71**, 2282 (1949).
(3) Fischer, *Ann.*, **215**, 253 (1882).
(4) Fischer, *Ber.*, **30**, 2226 (1897).
(5) Cherbuliez and Bernhard, *Helv. Chim. Acta*, **15**, 464 (1932).
(6) Purrmann, *Ann.*, **544**, 182 (1940).
(7) Spies, THIS JOURNAL, **61**, 350 (1939).
(8) Fischer, *Ber.*, **43**, 805 (1910).
(9) Wieland, Metzger, Schöpf and Bulow, *Ann.*, **507**, 226 (1933).
(10) Wieland and Purrmann, *ibid.*, **544**, 163 (1940).
(11) Wieland and Liebig, *ibid.*, **555**, 146 (1944).
(12) Wolf, Anderson, Kaczka, Harris, Arth, Southwick, Mozingo and Folkers, THIS JOURNAL, **69**, 2753 (1947).
(13) Schöpf and Kottler, *Ann.*, **539**, 128 (1939).

TABLE I
HYDROLYSIS CONDITIONS

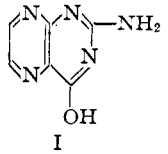
Pteridine	Concn. of HCl, *N*	Time, hr.	Temp., °C.	Product	Yield, %
2,4-Diaminopteridine	6	0.5	Reflux	2-Amino-4-hydroxypteridine	56
	6	30	Reflux	2,4-Dihydroxypteridine	63
2,4-Diamino-6,7-dimethylpteridine	6	0.5	Reflux	2-Amino-4-hydroxy-6,7-dimethylpteridine	93
	6	30	Reflux	2,4-Dihydroxy-6,7-dimethylpteridine	55
2,4-Diamino-6,7-diphenylpteridine	6	4	Reflux	2-Amino-4-hydroxy-6,7-diphenylpteridine	94.5
	6	30	Reflux	2-Amino-4-hydroxy-6,7-diphenylpteridine	92
	6	8	180°	2-Amino-4-hydroxy-6,7-diphenylpteridine	90
	(60% ethylene glycol 40% HCl)	20	Reflux	2,4-Dihydroxy-6,7-diphenylpteridine	12
				2-Amino-4-hydroxy-6,7-diphenylpteridine	80
4-Amino-2-hydroxypteridine	6	0.33	Reflux	2,4-Dihydroxypteridine	89.5
4-Amino-2-hydroxy-6,7-diphenylpteridine	12	1.5	160°	2,4-Dihydroxy-6,7-diphenylpteridine	98

6,7-diphenylpteridine are all converted in good yields to the corresponding 2-amino-4-hydroxypteridines by boiling for a short time with 6 *N* hydrochloric acid. Hydrolysis of the 4-amino group does not depend upon the presence of the 2-amino group, for both 4-amino-2-hydroxypteridine and 4-amino-2-hydroxy-6,7-diphenylpteridine are hydrolyzed readily to the corresponding 2,4-dihydroxypteridines.

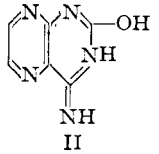
Upon long boiling with 6 *N* hydrochloric acid, an amino group in the 2-position may also be hydrolyzed. The reaction proceeds with great difficulty in the case of 2-amino-4-hydroxy-6,7-diphenylpteridine, perhaps because of the extreme insolubility of this compound in aqueous hydrochloric acid solution. Although unaffected by extended treatment with hot concentrated hydrochloric acid, partial hydrolysis does take place when the compound is refluxed for twenty hours in solution in a mixture of 60% ethylene glycol and 40% concentrated hydrochloric acid.

A summary of hydrolysis conditions and results is shown in Table I.

On the basis of the reaction of aminopteridines with nitrous acid and with mineral acid, it would appear that the compounds behave as though they exist in a single tautomeric form in acid solution, namely, one involving an amino group in the 2-position which should be removable with nitrous acid but stable to mild hydrolysis, and an imino group in the 4-position which should be stable toward nitrous acid but sensitive to hydrolysis by mineral acid. For example, 2-amino-4-hydroxypteridine and 4-amino-2-hydroxypteridine might be represented by formulas I and II, respectively.



I



II

Recent absorption spectra studies of some aminopurines[14] have suggested that these compounds are best represented by formulas showing an amino group in the 2-position and an imino group in the 6-position. The evidence is not conclusive, however, because much more strenuous conditions are necessary for the hydrolysis of the 6-amino group[6,7] than are necessary for the analogous reaction with the pteridines. In addition, there are simple purines where the 6-amino group is completely inert toward concentrated hydrochloric acid.[4]

The following consideration might also have some bearing on the difference in ease of hydrolysis of amino groups in the 2- and in the 4-positions of a pterin: An amino group in the 2-position is a part of a guanidine structure while an amino group in the 4-position is a part of an amidine structure. Since it is recognized that guanidine is much more stable to hydrolysis in acid solution than is an amidine, it might be expected that a 2-amino group would require more strenuous conditions for acid hydrolysis than a 4-amino group.

The failure of the 2,4-diaminopteridines to react with nitrous acid might be related to the explanation suggested for the decrease in the ease of diazotization of aniline when electron-attracting groups are introduced. For example, there is a progressive decrease in ease of diazotization in the series: aniline, 4-nitroaniline, 2,4-dinitroaniline, 2,4,6-trinitroaniline.[15] In 2-amino-4-hydroxypteridine, both nitrogen atoms of the pyrimidine ring are capable of acquiring a positive charge in acid solution, thus acting as effective electron attracting groups and causing diazotization to occur with difficulty. The presence of an additional salt-forming group in the 4-position, as in 2,4-diaminopteridine, might be the factor responsible for the failure of the compound to react with nitrous acid.

The 4-amino-2-hydroxypteridines utilized in these hydrolysis experiments have not been described previously; their synthesis and absorption spectra are therefore reported here. An unexpected difficulty was encountered in the synthesis of these compounds from 4,5,6-triamino-2-hy-

(14) Cavalieri, Bendich, Tinker and Brown, THIS JOURNAL, **70**, 3875 (1948).

(15) Saunders, "The Aromatic Diazo-Compounds," Longmans, Green and Company, New York, N. Y., 1936, pp. 3-16.

droxypyrimidine and the appropriate α,β-dicarbonyl compound. Using acid conditions similar to those developed for the synthesis of some 2,4-diaminopteridines[16] only the corresponding 2,4-dihydroxypteridines could be isolated, demonstrating ready hydrolysis of the 4-amino group. In order to obtain the desired 4-amino-2-hydroxypteridines, it was necessary to effect the condensation in neutral or slightly alkaline solution. The formation of the 2,4-dihydroxypteridines could not have resulted from conversion of the 4,5,6-triamino-2-hydroxypyrimidine to 5,6-diamino-2,4-dihydroxypyrimidine followed by condensation with the dicarbonyl compound, for it has been shown that the former pyrimidine is stable to acid hydrolysis.[2]

Experimental

Hydrolysis of Aminopteridines.—The hydrolysis experiments were carried out by heating 1.0 g. of the desired aminopteridine in hydrochloric acid under the conditions specified in Table I, removing the acid by evaporation to dryness under reduced pressure and purifying the solid residue as described previously.[17] Sulfuric acid of comparable strength gave the same results.

The identity of the product was established whenever possible by melting point determinations and in every case by a comparison of its absorption spectrum with that of an authentic sample. The latter was considered the preferable method of identification of the product because of the marked differences in absorption spectra exhibited by the various pterins.

Diazotization of 2-Amino-4-hydroxypteridine.—To a solution of 1.0 g. (0.00614 mole) of 2-amino-4-hydroxypteridine in 100 ml. of boiling 20% sulfuric acid was added slowly and with shaking a solution of 0.9 g. (0.013 mole) of sodium nitrite in 10 ml. of water. After standing at room temperature for five minutes, the reaction mixture was heated to boiling, allowed to stand for one hour and cooled to 0°. The orange solid was purified by dissolving in hot dilute ammonium hydroxide, treating with Norit and neutralizing with acetic acid to give 0.495 g. (49.2%) of 2,4-dihydroxypteridine. The identity of the product was established by mixed melting point and comparison of of the absorption spectrum with an authentic sample.

Diazotization of 2-Amino-4-hydroxy-6,7-dimethylpteridine.—The diazotization of this compound was carried out as described above to give a 43.8% yield of 2,4-dihydroxy-6,7-dimethylpteridine which was identified by mixed melting point and comparison of the absorption spectrum with an authentic sample.

Attempted Diazotization of 2-Amino-4-hydroxy-6,7-diphenylpteridine (A).—A sample of 0.05 g. of 2-amino-4-hydroxy-6,7-diphenylpteridine was added to 150 ml. of boiling 7 *N* sulfuric acid. The resulting clear yellow solution was treated at once with a solution of 1.0 g. of sodium nitrite in 20 ml. of water and allowed to stand without further heating. Bright yellow crystals in the form of small flat prisms exhibiting parallel extinction separated which were identified as the sulfate of the unreacted starting material by a positive qualitative test for sulfur and by an absorption spectrum determination, m. p. 300–303° (dec., uncor.). The free pteridine was obtained by dissolving the salt in dilute sodium hydroxide and precipitating with acetic acid. (B) A sample of 0.50 g. (0.00159 mole) of 2-amino-4-hydroxy-6,7-diphenylpteridine dissolved in 3 ml. of concentrated sulfuric acid was treated with a solution of nitrosylsulfuric acid prepared by adding 0.25 g. (0.00363 mole) of sodium nitrite to 4 ml. of cold concentrated sulfuric acid. The reaction mixture was heated to 50° for one hour, cooled, poured into ice and the solid filtered off and washed thoroughly with water. After suspension in hot dilute ammonium hydroxide and refiltering, washing and drying, 0.46 g. (88%) of yellow solid was obtained. It did not melt below 360° and its absorption spectrum identified it as the starting material.

Similar attempts to diazotize 2,4-diaminopteridine, 2,4-diamino-6,7-dimethylpteridine, 2,4-diamino-6,7-diphenylpteridine, 4-amino-2-hydroxypteridine and 4-amino-2-hydroxy-6,7-diphenylpteridine were also unsuccessful. It should be noted that no hydrolysis takes place when these pterins are dissolved in boiling 7 *N* sulfuric acid and allowed to stand without further heating. However, hydrolysis of a 4-amino group may be effected by refluxing a solution of the proper pterin in 7 *N* sulfuric acid for thirty minutes.

4-Amino-2-hydroxypteridine.—To a solution of 2.0 g. (0.0084 mole) of 4,5,6-triamino-2-hydroxypyrimidine sulfate[18] in 50 ml. of water adjusted to *p*H 5 with dilute sodium hydroxide was added 3.0 g. (0.0113 mole) of glyoxal bisulfite. The reaction mixture was heated to boiling, the *p*H adjusted to 9 and boiled for fifteen minutes. After neutralization with dilute hydrochloric acid, cooling and filtering, the light tan solid was washed with water followed by acetone and dried *in vacuo;* yield, 1.10 g. (80%). The product was purified by dissolving in hot 0.5 *N* sodium hydroxide, treating with Norit and acidifying the hot filtrate with acetic acid. A final recrystallization from 0.5 *N* acetic acid gave small rectangular prisms exhibiting parallel extinction. Essentially no change in appearance was noted upon heating to 360°.

Anal. Calcd. for $C_6H_5N_5O$: C, 44.2; H, 3.1; N, 42.9. Found: C, 44.5; H, 2.9; N, 43.3.

The absorption spectra of this compound are given in Table II.

4-Amino-2-hydroxy-6,7-diphenylpteridine.—A suspension of 1.0 g. (0.0039 mole) of 4,5,6-triamino-2-hydroxypyrimidine sulfate in 35 ml. of water was heated to boiling and the *p*H adjusted to 7 with dilute sodium hydroxide. A solution of 1.3 g. (0.0062 mole) of benzil in a mixture of 35 ml. of ethyl methyl ketone and 35 ml. of ethanol was added and the reaction mixture refluxed for four hours. The hot solution was filtered and the filtrate neutralized with acetic acid. After cooling to 0°, the light yellow crystalline solid which separated was collected by filtration and washed with warm ethyl methyl ketone followed by acetone; yield, 0.89 g. (73%). The product was recrystallized from 50% aqueous dimethylformamide to give flat prisms exhibiting oblique extinction and decomposing at 320–325° (uncor.).

Anal. Calcd. for $C_{18}H_{13}N_5O$: C, 68.6; H, 4.2; N, 22.2. Found: C, 68.5; H, 3.8; N, 22.3.

The absorption spectra of this compound are given in Table II.

TABLE II

	Maxima		Minima	
	mμ	Log ε	mμ	Log ε
4-Amino-2-hydroxypteridine	255[a]	4.32	232	3.95
	373[a]	3.85	310	3.04
	236[b]	4.11	222	4.01
	335[b]	3.93	274	2.98
4-Amino-2-hydroxy-6,7-diphenylpteridine	275[a]	4.38	248	4.22
	396[a]	4.12	335	3.39
	280[b]	4.19	257	4.09
	377[b]	4.18	325	3.57

[a] In 0.1 *N* sodium hydroxide. [b] In 0.1 *N* hydrochloric acid.

Summary

Several aminopteridines have been investigated as to their behavior toward nitrous acid and toward mineral acid. It has been found that, in

(16) Mallette, Taylor and Cain, THIS JOURNAL, **69**, 1814 (1947).

(17) Cain, Mallette and Taylor, *ibid.*, **68**, 1996 (1946).

(18) Bendich, Tinker and Brown, *ibid.*, **70**, 3109 (1948).

general, a 4-aminopteridine may be readily hydrolyzed by mineral acid but is resistant to the action of nitrous acid. A 2-aminopteridine requires considerably more strenuous treatment for hydrolysis to take place but reacts readily with nitrous acid to form the corresponding hydroxy compound unless an amino group is also present in the 4-position. Several explanations for this behavior are suggested.

The synthesis and absorption spectra of two new pterins, 4-amino-2-hydroxypteridine and 4-amino-2-hydroxy-6,7-diphenylpteridine, are reported.

ITHACA, N. Y. RECEIVED FEBRUARY 21, 1949

[CONTRIBUTION FROM THE RESEARCH LABORATORIES, MERCK & CO., INC., RAHWAY, N. J.]

A New Method of Resolution of DL-Threonine[1]

BY A. J. ZAMBITO, W. L. PERETZ AND E. E. HOWE

In 1937 West and Carter[2] reported a synthesis of DL-threonine in which one of the intermediates is N-formyl-O-methyl-DL-threonine. This synthesis not only represents the first practical method for the preparation of the racemic amino acid, but also affords a satisfactory method for the preparation of its optically active isomers. These same investigators found that this intermediate could be resolved with brucine and that D- and L-threonine subsequently resulted upon hydrolysis of the optically active derivatives.

Recently a new and improved synthesis of DL-threonine has been developed.[3] However, since neither N-formyl-O-methyl-DL-threonine, nor any other such suitable derivative which might readily lend itself to resolution is an intermediate in this synthesis, it became desirable to investigate other methods which might lead to the direct resolution of DL-threonine. Since threonine is not readily converted to N-formyl-O-methyl-DL-threonine, Fischer's[4] method for the resolution of DL-serine was first studied. In this method N-*p*-nitrobenzoyl-D-serine is precipitated as the quinine salt from an alcoholic solution. After decomposing the more soluble quinine salt of the L-derivative with alkali, the L-isomer is precipitated with brucine. When the method was applied to DL-threonine, however, it was found to be generally unsatisfactory.

We wish to report a simple and direct method of resolving DL-threonine in which only brucine is used, and in which, in contrast to most resolutions of this type, the physiologically active L-form separates first. The separation of the optically active isomers is based upon the difference in solubility of the brucine salts of the N-*p*-nitrobenzoyl derivatives of D- and L-threonine in methanol. In an extended study of the solubility of these derivatives under varying conditions, an interesting phenomenon was observed. Whereas the brucine salt of N-*p*-nitrobenzoyl-L-threonine is an almost colorless, highly insoluble compound, the brucine salt of the D-isomer exists in two forms. When first prepared in methanol a soluble, low-melting, deep orange-colored salt is obtained which slowly changes to an insoluble, high-melting, light yellow product. Under the proper conditions of time and temperature this change is kept at a minimum thus affording an excellent separation of the more insoluble L-isomer.

(1) Throughout this paper the nomenclature is used in the amino acid sense. For the sake of brevity however the subscript s has been deleted: Vickery, *J. Biol. Chem.*, **169**, 237 (1947).

(2) West and Carter, *J. Biol. Chem.*, **119**, 109 (1937).

(3) Pfister, Robinson, Shabica and Tishler, THIS JOURNAL, **70**, 2297-2298 (1948); Attenburrow, Elliot and Penny, *J. Chem. Soc.*, 310 (1948).

(4) Fischer and Jacobs, *Ber.*, **39**, 2942 (1906).

Experimental

N-*p*-Nitrobenzoyl-DL-threonine.—To a solution of 32 g. (0.269 mole) of DL-threonine in 1200 cc. of water and 270 cc. of normal sodium hydroxide at 0°, was added with vigorous agitation over a period of one hour, a total of 50 g. (0.269 mole) of *p*-nitrobenzoyl chloride (freshly distilled, m. p. 72–74°) in equal portions at three-minute intervals. Simultaneously, over this period of time, 135 cc. of 2 *N* sodium hydroxide solution were added dropwise. The solution was stirred for an additional twenty minutes and then acidified to congo red with 40 cc. of concentrated hydrochloric acid. After cooling in an ice-bath for one hour, the crude *p*-nitrobenzoyl-DL-threonine was filtered, and without drying was extracted with 300 cc. of boiling water. The insoluble portion, *p*-nitrobenzoic acid, weighed 4.1 g. The product began to crystallize from the hot solution immediately, and after chilling at 0–5° for one hour was filtered, washed with two 75-cc. portions of ice-cold water and dried at 60°; m. p. 159–162°. For further purification, the 56.5 g. of crude threonine derivative was extracted twice with hot ether to remove additional quantities of *p*-nitrobenzoic acid. The yield of pure *p*-nitrobenzoyl-DL-threonine was 50.7 g. (70.5%); m. p. 166–167°.

Brucine Salt of *p*-Nitrobenzoyl-DL-threonine.—To a warm solution (50–55°) of 64 g. (0.149 mole) of brucine Merck dissolved in 160 cc. of methanol was added 40 g. (0.149 mole) of *p*-nitrobenzoyl-DL-threonine, and the mixture was heated until solution was complete. With rapid stirring and scratching, the solution was cooled in an ice-bath, whereupon crystallization took place within a short time. The cooling and stirring were continued for a total of five minutes, during which time a heavy, yellowish precipitate separated out, and the temperature dropped to 25°. The flask was reheated to 50° with stirring, and then cooled to 25° over a five-minute period as in the previous case. The brucine salt of *p*-nitrobenzoyl-L-threonine was filtered, and the small amount of product adhering to the flask was transferred with the mother liquors. After washing the cake thoroughly in a mortar with 60 cc. of cold methanol, the slurry was transferred back to the funnel and washed, again using approximately 60 cc. of methanol for this operation. As much of the wash liquor as possible was removed by suction, after which the product was finally washed with two 50-cc. portions of ether, and