F). The less soluble form melted at 159.7–160.3° when purified for analysis; the second isomer melted at 107.9–108.6°.

Anal. Calcd. for $C_{14}H_{16}O_2$: C, 77.7; H, 7.5. Found: (160° isomer) C, 77.5; H, 7.4; (108° isomer) C, 77.7; H, 7.4.

Reduced Esters, I.—Ethyl acetate distilled at 75–76° at 742 mm.; $n^{24}D$ 1.3718; *p*-bromophenacyl acetate,[15] m. p. 85.4–86.4°, no depression with an authentic sample.

Ethyl propionate distilled at 96–97° at 748 mm.; *p*-bromophenacyl propionate,[16] m. p. 61.8–62.8°, no depression with an authentic sample.

Ethyl isobutyrate distilled at 108–110° at 746 mm.; $n^{24}D$ 1.3889; *p*-bromophenacyl isobutyrate,[15] m. p. 75.6–76.4°, no depression.

β-Ketoesters, II.—Ethyl acetoacetate distilled at 87–89° at 24 mm.; $n^{24}D$ 1.4183; 2,4-dinitrophenylhydrazone,[17] m. p. 95.0–96.2°, no depression.

Ethyl α-propionylpropionate distilled at 192–193° at 752 mm., and gave with phenylhydrazine 4-methyl-3-ethyl-1-phenyl-5-pyrazolone,[18] m. p. 110.0–111.8°.

Ethyl α-isobutyrylisobutyrate[8] distilled at 202–203° at 750 mm., $n^{25}D$ 1.4252; semicarbazone,[19] m. p. 230–235°, uncor., dec.

(15) Judefind and Reid, THIS JOURNAL, **42**, 1043 (1920).
(16) Moses and Reid, *ibid.*, **54**, 2101 (1932).
(17) Strain, *ibid.*, **57**, 760 (1935).
(18) Schroeter, *Ber.*, **49**, 2719 (1916).
(19) Zeltner and Reformatsky, *J. Russ. Phys.-Chem. Soc.*, **38**, 105 (1906); *Chem. Centr.*, **77**, II, 316 (1906).

Summary

The reactions of 5,8-dimethyl-1-tetralone with zinc and ethyl bromoacetate, α-bromopropionate and α-bromoisobutyrate are described.

The corresponding reduced esters and β-keto-esters were isolated and identified as end-products of side-reactions involving zinc and the α-bromo-esters.

Mechanisms for the formation of these products are considered.

EVANSTON, ILLINOIS
COLUMBUS, OHIO RECEIVED JANUARY 16, 1948

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

Pyrimido[4,5-b]pyrazines (Pteridines[1]). III. Pteridinemono- and -dicarboxylic Acids[2]

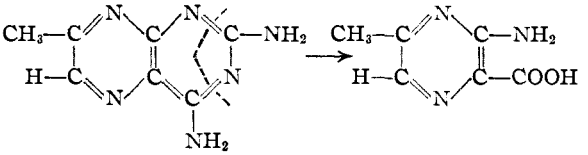
BY C. K. CAIN, M. F. MALLETTE[3] AND E. C. TAYLOR, JR.

Previous papers[4,5] from this Laboratory have reported the synthesis of a number of compounds containing the pteridine nucleus. These compounds have been for the most part those in which the pteridine nucleus has been substituted in the 2- and 4-positions by hydroxyl, amino or a combination of these two groups and in the 6- and 7-positions by hydrogen, alkyl, aryl or condensed ring groups. Because of the interesting biological properties of various pteridine derivatives, it seemed of interest to prepare derivatives in which one or two carboxyl groups occupy the 6- and 7-positions of the pteridine nucleus with the thought in mind that such substances might have different biological properties because of the presence of more reactive groups in these positions.

Table I lists the pteridinemono- and -dicarboxylic acids and their methyl esters which we have prepared together with significant data on their ultraviolet absorption spectra in solution. Three compounds previously reported by other investigators have been included for comparison.

The pteridinemono- and -dicarboxylic acids were prepared by oxidation of the corresponding methyl compounds with potassium permanganate according to standard procedures. Some of them proved extremely difficult to dry thoroughly for analysis as had been noted by Wittle, *et al.*,[6] in the case of Compound 3, Table I; accordingly, the acids were converted into the methyl esters by means of anhydrous methyl alcohol and dry hydrogen chloride. The methyl esters are crystalline compounds which may be purified by recrystallization and easily dried.

One of the compounds used for conversion to the acid by oxidation with potassium permanganate had been originally reported as 2,4-diamino-6-(or 7)-methylpteridine.[5] We have shown that the compound is the 7-methyl isomer by cleavage to 2-amino-6-methyl-3-pyrazinecarboxylic acid using the procedure of Weijlard, Tishler and

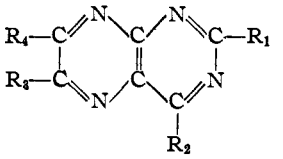


Erickson.[8] These authors showed by the cleavage reaction that the condensation of 2,4-dihydroxy-5,6-diaminopyrimidine with methylglyoxal results in the formation of 2,4-dihydroxy-7-methylpteridine. They were unable to detect the presence of the 6-methyl isomer in the product. Similarly, Mowat, *et al.*,[7] have shown that 2-amino-4-hydroxy-7-methylpteridine results from

(1) The common name "pteridine" is used to refer to pyrimido-[4,5-b]pyrazine.
(2) The work presented in this paper was undertaken in collaboration with the Office of Naval Research, Navy Department, Washington, D. C., and was aided by a grant to Cornell University by the Nutrition Foundation, Inc., New York City. It represents a part of a collaborative project on "Newer Members of the B Group of Vitamins."
(3) Present address: Department of Chemistry, University of Wyoming, Laramie, Wyoming.
(4) Cain, Mallette and Taylor, THIS JOURNAL, **68**, 1996 (1946).
(5) Mallette, Taylor and Cain, *ibid.*, **69**, 1814 (1947).
(6) Wittle, O'Dell, Vandenbelt and Pfiffner, *ibid.*, **69**, 1786 (1947).
(7) Mowat, *et al.*, *ibid.*, **70**, 14 (1948).
(8) Weijlard, Tishler and Erickson, *ibid.*, **67**, 802 (1945).

TABLE I

PTERIDINEMONO- AND -DICARBOXYLIC ACIDS AND METHYL ESTERS



Compound	Structure R_1	R_2	R_3	R_4	Ultraviolet absorption spectra[a] Maxima mu	Maxima log ε	Minima mu	Minima log ε
1	—OH	—OH	—COOH	COOH	265	4.24	235	3.89
					371	3.88	313	3.41
2	—OH	—OH	—$COOCH_3$	—$COOCH_3$	245	4.01	218	3.82
					267	4.02	253	3.99
					335	3.97	295	3.45
3[b]	—NH_2	—OH	—COOH	—COOH	258	4.34	235	4.00
					365	4.01	312	3.37
4[b]	—NH_2	—OH	—$COOCH_3$	—$COOCH_3$	240	4.03	233	3.01
					308	4.03	280	3.79
5	—NH_2	—NH_2	—COOH	—COOH	266	4.34	240	3.90
					370	3.97	315	3.21
6	—NH_2	—NH_2	—$COOCH_3$	—$COOCH_3$	263	4.21	233	3.76
					345	4.07	292	3.68
7	—OH	—OH	—H	—COOH	245	4.21	227	4.02
					360	3.90	294	3.18
8	—OH	—OH	—H	—$COOCH_3$	223	4.14	218	4.07
					325	4.07	270	2.86
9[c]	—NH_2	—OH	—H	—COOH	258	4.31	230	3.92
					372	3.87	304	3.03
10	—NH_2	—OH	—H	—$COOCH_3$	235	4.08	218	4.10
					330	3.94	290	3.30
11	—NH_2	—NH_2	—H	—COOH	258	4.26	230	3.96
					365	3.86	307	3.20
12	—NH_2	—NH_2	—H	—$COOCH_3$	330	3.94	275	3.51

[a] The acids were dissolved in 0.1 *N* sodium hydroxide and the esters in 0.1 *N* hydrochloric acid for absorption spectra measurements. See Experimental section. [b] Reference 6. [c] Reference 7.

the reaction of 2,4,5-triamino-6-hydroxypyrimidine with methylglyoxal diethyl acetal.

The carboxyl group in the 6-position of a 6,7-pteridinedicarboxylic acid is considerably less stable than a carboxyl group in the 7-position as shown by selective decarboxylation of 2,4-dihydroxy-6,7-pteridinedicarboxylic acid to 2,4-dihydroxy-7-pteridinecarboxylic acid by heating in quinoline solution. Purrmann[9] has reported that isoxanthopterin carboxylic acid (2-amino-4,7-dihydroxy-6-pteridinecarboxylic acid) readily loses carbon dioxide when heated to 260°. The isomeric xanthopterin carboxylic acid (2-amino-4,6-dihydroxy-7-pteridinecarboxylic acid) could not be decarboxylated.

The biological effects of these compounds have been studied for their action upon the growth of certain bacteria and also for their action on growth and hemoglobin formation in chicks by Daniel, Norris, Scott and Heuser of the School of Nutrition, Cornell University. These results are reported elsewhere.[10]

(9) Purrmann, *Ann.*, **548**, 284 (1941).

(10) Daniel, Norris, Scott and Heuser, *J. Biol. Chem.*, **169**, 689 (1947); **170**, 747 (1947); **178**, 123 (1948).

Experimental

The pteridinemono- and -dicarboxylic acids were prepared by oxidation of the corresponding methyl compounds with potassium permanganate. The methyl esters were prepared from the acids by means of anhydrous methyl alcohol and dry hydrogen chloride. The preparation of a typical acid and its methyl ester are described in some detail as an example of the method used throughout.

2,4-Dihydroxy-6,7-pteridinedicarboxylic Acid.—Dilute sodium hydroxide solution was added to a suspension of 4.3 g. (0.024 mole) of 2,4-dihydroxy-6,7-dimethylpteridine in 150 ml. of hot water until complete solution resulted. The solution was stirred mechanically and heated on the steam-bath while 15.0 g. (0.095 mole) of potassium permanganate was added portionwise over a period of three hours. The reaction mixture was cooled and filtered, the manganese dioxide twice extracted with hot dilute sodium hydroxide and the combined filtrates concentrated to a volume of 30 ml. by distillation under reduced pressure. Sufficient dilute hydrochloric acid was added to adjust the *p*H to 2, after which the solution was cooled to 0° and an equal volume of acetone was added. The resulting solid was collected by filtration and washed with cold water; yield, 3.8 g. (83%). The product was purified by dissolving in boiling 1 *N* hydrochloric acid and diluting with an equal volume of ethanol. The free acid crystallized as well-formed platelets exhibiting parallel extinction. Upon heating, the material started to darken at about 250° but showed no evidence of melting below

300°. The acid forms a yellow silver salt and gives a purple color when an aqueous solution is treated with aqueous ferrous sulfate.

2,4-Dihydroxy-6,7-pteridinedicarboxylic Acid, Dimethyl Ester.—To a solution of 10 g. of anhydrous hydrogen chloride in 50 ml. of absolute methanol was added 0.3 g. (0.0012 mole) of 2,4-dihydroxy-6,7-pteridinedicarboxylic acid and the mixture allowed to stand with occasional shaking for twenty-four hours. The mixture was then refluxed for five hours, followed by removal of the alcohol by distillation under reduced pressure. The residue was dissolved in water, the *p*H adjusted to 7 with dilute sodium hydroxide and the mixture cooled to yield 0.23 g. (69%) of light yellow plates. A sample was crystallized three times from water and dried at 140° *in vacuo* over phosphorus pentoxide for analysis. Upon heating, the crystals decomposed sharply at 246–247°.

Anal. Calcd. for $C_{10}H_8N_4O_6$: N, 20.00. Found: N, 20.10.

2-Amino-4-hydroxy-6,7-pteridinedicarboxylic Acid.—This acid was prepared by oxidation of 2-amino-4-hydroxy-6,7-dimethylpteridine[11] with potassium permanganate in the manner described by Wittle, *et al.*[6] The product was purified by dissolving in dilute sodium hydroxide and pouring the resulting solution into boiling dilute hydrochloric acid. The light yellow microcrystalline solid darkened slowly without melting on heating to 300° and gave a brown-green color test with ferrous sulfate.

2-Amino-4-hydroxy-6,7-pteridinedicarboxylic Acid, Dimethyl Ester.—This ester was prepared by the method of Wittle, *et al.*[6] *Anal.* Calcd. for $C_{10}H_9O_5N_5$: N, 25.08. Found: N, 25.15.

2,4-Diamino-6,7-pteridinedicarboxylic Acid.—The product resulting from the oxidation of 2,4-diamino-6,7-dimethylpteridine with potassium permanganate was purified by dissolving in hot dilute alkali and pouring into boiling dilute hydrochloric acid of such strength that the final solution was approximately *p*H 1. The light yellow microcrystalline solid darkened slowly without melting upon heating to 300°. An aqueous solution gave a brown color with aqueous ferrous sulfate.

2,4-Diamino-6,7-pteridinedicarboxylic Acid, Dimethyl Ester.—This ester was prepared in 67% yield from 0.8 g. of the acid by the method described above. Recrystallization from water gave long, rectangular yellow prisms exhibiting parallel extinction and darkening without melting upon heating to 300°. *Anal.* Calcd. for $C_{10}H_{10}O_4N_6$: C, 43.17; H, 3.62; N, 30.21. Found: C, 43.10; H, 3.32; N, 30.69.

2,4-Dihydroxy-7-pteridinecarboxylic Acid.—A 78% yield of this acid was obtained upon oxidation of 3.5 g. of 2,4-dihydroxy-7-methylpteridine with potassium permanganate. Recrystallization from 0.1 *N* hydrochloric acid gave small light yellow rods exhibiting parallel extinction, and decomposing at 270–275° when heated at a rate of 6° per minute. The product was soluble in hot water, formed a bright yellow silver salt and gave a purple color test with ferrous sulfate.

2,4-Dihydroxy-7-pteridinecarboxylic Acid, Methyl Ester.—A 48% yield was obtained by the esterification of 0.8 g. of the acid. The product was recrystallized either from methanol or from water to give a light red microcrystalline solid which darkened without melting upon heating to 300°. *Anal.* Calcd. for $C_8H_6O_4N_4$: N, 25.22. Found: N, 25.30.

2-Amino-4-hydroxy-7-pteridinecarboxylic Acid.—The preparation of this compound was carried out essentially as described by Mowat, *et al.*[7]

2-Amino-4-hydroxy-7-pteridinecarboxylic Acid, Methyl Ester.—Prepared in 83% yield from 0.7 g. of the corresponding acid, this ester was purified by dissolving in 5% sodium bicarbonate solution and pouring the resulting solution into boiling dilute hydrochloric acid. The light yellow, microcrystalline solid darkened without melting upon heating to 300°. *Anal.* Calcd. for $C_8H_7O_3N_5$: C, 43.44; H, 3.19; N, 31.67. Found: C, 43.39; H, 3.40; N, 31.23.

2,4-Diamino-7-pteridinecarboxylic Acid.—The oxidation of 1.6 g. of 2,4-diamino-7-methylpteridine with potassium permanganate resulted in a 75% yield of the acid. The product was purified by dissolving in dilute sodium hydroxide and pouring into boiling dilute hydrochloric acid. The light yellow microcrystalline solid darkened slowly without melting on heating to 300°, formed a yellow silver salt and gave a brown color with ferrous sulfate solution.

2,4-Diamino-7-pteridinecarboxylic Acid, Methyl Ester.—This ester resulted in 77% yield from 0.8 g. of the corresponding acid. The product formed fine light yellow needles showing parallel extinction when purified by recrystallization from water. *Anal.* Calcd. for $C_8H_8N_6O_2$: N, 38.17. Found: N, 38.10.

Proof of Structure of 2,4-Diamino-7-methylpteridine.—The product resulting from the condensation of 2,4,5,6-tetraminopyrimidine bisulfite and methylglyoxal[5] was shown to have the methyl group in the 7-position of the pteridine ring by cleavage to 2-amino-6-methyl-3-pyrazinecarboxylic acid by the method of Weijlard, Tishler and Erickson.[8] A mixture of 10 g. (0.057 mole) of 2,4-diamino-7-methylpteridine, 12 g. (0.30 mole) of sodium hydroxide and 100 ml. of water was heated in a stainless steel bomb at 180° for twenty hours. After cooling, the reaction mixture was acidified to *p*H 3 with concentrated hydrochloric acid and the resulting solid recrystallized from water. The melting point of this product, 211°, agreed well with that reported by Weijlard, *et al.* As a further check, decarboxylation of the acid resulted in a product which melted at 125° in good agreement with that reported by the aforementioned authors for 2-amino-6-methylpyrazine.

Proof of Structure of 2-Amino-4-hydroxy-7-methylpteridine.—The product resulting from the condensation of 2,5,6-triamino-4-hydroxypyrimidine bisulfite and methylglyoxal was shown to be identical with that reported by Mowat, *et al.*,[7] as resulting from the reaction of 2,5,6-triamino-4-hydroxypyrimidine and methylglyoxal diethyl acetal by a comparison of the absorption spectra and by a cleavage of 2-amino-6-methyl-3-pyrazinecarboxylic acid by the method described above.

Selective Decarboxylation of 2,4-Dihydroxypteridine-6,7-dicarboxylic Acid.—A solution of 0.05 g. of 2,4-dihydroxypteridine-6,7-dicarboxylic acid in 25 ml. of quinoline was refluxed for one hour. The grey solid obtained on cooling was dissolved in 5 ml. of 0.1 *N* hydrochloric acid and an equal volume of ethanol added. After cooling, the unchanged starting material was filtered off and the filtrate evaporated to a volume of 2 ml. The crystals which separated on cooling decomposed at 270–275°. This decomposition temperature as well as the ultraviolet absorption spectrum were in good agreement with those values found for 2,4-dihydroxypteridine-7-carboxylic acid prepared by the oxidation of 2,4-dihydroxy-7-methylpteridine.

Ultraviolet Absorption Spectra.—Because of the difficulty of obtaining analytical samples of the acids, their absorption spectra were determined on solutions prepared as follows: Approximately 1 mg. of an analytical sample of the ester was carefully weighed and dissolved in 100 ml. of 0.1 *N* sodium hydroxide solution. After the resulting solution had stood at room temperature for three days, the absorption spectrum was measured and checked twenty-four hours later. In no case was there a change in the spectrum upon checking and so it was assumed that saponification of the ester was complete.

The acids were too insoluble in 0.1 *N* hydrochloric acid for their absorption spectra to be measured. However, if sufficient hydrochloric acid was added to the above alkaline solutions to make the resulting solutions approximately 2 *N* acid, the sample remained in solution. Absorption spectra of these strongly acidic solutions resembled quite closely the spectra of the corresponding esters in 0.1 *N* hydrochloric acid.

(11) Sachs and Meyerheim, *Ber.*, **41**, 3957 (1908).

Acknowledgment.—The microanalyses were carried out by Miss Jane Wadhams.

Summary

The synthesis of several 6,7-pteridinedicarboxylic acids and 7-pteridinecarboxylic acids has been described. The ultraviolet absorption spectra of solutions of these compounds have been measured.

The reaction of 2,4,5,6-tetraminopyrimidine bisulfite with methylglyoxal has been shown to result in the formation of 2,4-diamino-7-methylpteridine by cleavage of the product to a known compound.

Selective decarboxylation of 2,4-dihydroxypteridine-6,7-dicarboxylic acid results in the loss of the carboxy group in the 6-position of the pteridine nucleus.

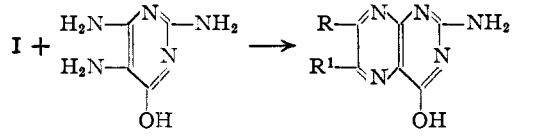
ITHACA, N. Y. RECEIVED APRIL 26, 1948

[CONTRIBUTION FROM LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID COMPANY]

Pteridine Chemistry. I

BY ROBERT B. ANGIER, COY W. WALLER, JAMES H. BOOTHE, JOHN H. MOWAT, JOSEPH SEMB, BRIAN L. HUTCHINGS, E. L. R. STOKSTAD AND Y. SUBBAROW

Despite unsatisfactory analytical data for their product, Karrer, *et al.*,[1] have reported the synthesis of 2-amino-4-hydroxy-7(or 6)-hydroxymethylpteridine by the reaction of *d,l*-glyceraldehyde and 2,4,5-triamino-6-hydroxypyrimidine (II) in a carbon dioxide atmosphere. However, considerable evidence collected previously in this Laboratory had led to the conclusion that the reaction of II with any three carbon compound (I) which might be expected to produce initially an hydroxy methyl- or halogenmethyldihydropteridine, actually gave primarily the fully aromatized methylpteridine with no substituent on the methyl group. This was particularly true when the reaction was carried out in a non-oxidizing atmosphere.



II III R = CH_3, R^1 = H
IV R = H, R^1 = CH_2COOH

For example, 2-amino-4-hydroxy-7-methylpteridine (III)[2] has been obtained pure from the reaction of II with such compounds as 1,3-dichloroacetone, 2,3-dichloropropional and α-bromotetronic acid[3] (lactone of 2-bromo-3-keto-4-hydroxybutanoic acid). The reaction with the latter compound was carried out in 6 *N* hydrochloric acid in which it might be expected to hydrolyze to form 1-bromo-3-hydroxyacetone. Preliminary work with *d,l*-glyceraldehyde had indicated that it also formed the same 7-methylpteridine (III).

To check our conclusions *d,l*-glyceraldehyde was treated with II under the conditions described by Karrer, *et al.*[1] The product was crystallized once in the manner described by the same authors and then further purified by two different methods. Each of these pure materials proved to be 2-amino-4-hydroxy-7-methylpteridine (III).

In a similar manner it has been found that ethyl 2,4-dibromo-3-ketobutanoate will react with II in a 2 *N* hydrochloric acid solution to give 2-amino-4-hydroxypteridine-6-acetic acid[4] rather than a bromo- or hydroxyacetic acid derivative. That the starting material was actually the 2,4-dibromo-derivative is evident since some of the same material was used to prepare the α-bromotetronic acid mentioned above.

The following mechanism is presented as a possible explanation of the products obtained in these particular reactions: the initial product of each reaction is an hydroxymethyl- or halogenmethyldihydropteridine, which, in the absence of a readily available oxidizing or dehydrogenating agent loses water or a hydrohalide. The resulting methylenepteridine then rearranges to form the fully aromatized methylpteridine.

Experimental

2-Amino-4-hydroxy-7-methylpteridine (III): A. 1,3-Dichloroacetone and 2,4,5-Triamino-6-hydroxypyrimidine (II).[5]—Dichloroacetone (1.4 g.), 3.7 g. of sodium acetate and 2.1 g. of the dihydrochloride of II were mixed and suspended in 35 cc. of water. A stream of carbon dioxide was passed through the mixture while heating under gentle reflux for one hour. After cooling, the resulting precipitate was filtered off, washed with water, methanol and ether and air-dried; yield 1.4 g. A portion of this product (0.30 g.) was dissolved in 10 cc. of a very dilute sodium hydroxide solution, decolorized with Norite, filtered, and 10 cc. of a 10 *N* sodium hydroxide solution added to the filtrate. Upon cooling, the sodium salt of the pterine crystallized out. This was filtered off and crystallized twice more in the same manner. The resulting sodium salt was dissolved in 8 cc. of water, filtered and acidified to *p*H 2.0 with hydrochloric acid. This was centrifuged, washed once with water, redissolved in 8 cc. of a very dilute sodium hydroxide solution and poured slowly into a well-stirred solution of hot dilute acetic acid. After cooling, the resulting crystalline material was filtered off, washed and dried; yield 0.05 g. The ultraviolet absorption spectra of this product were identical with those of 2-amino-4-hydroxy-7-methylpteridine.[2]

(1) Karrer, Schwyzer, Erden and Siegwert, *Helv. Chim. Acta*, **30**, 1034 (1947).

(2) (a) Prepared originally from methyl glyoxal and II; (b) Mowat, *et al.*, THIS JOURNAL, **70**, 14 (1948).

(3) Kumler, *ibid.*, **60**, 863 (1938).

(4) Prepared originally from methyl 3-keto-4,4-dimethoxybutanoate and II; Mowat, *et al.*, THIS JOURNAL, **70**, 14 (1948).

(5) Traube, *Ber.*, **33**, 1371 (1900).