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.

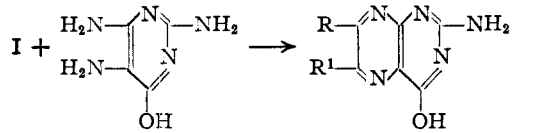
ITHACA, N. Y. RECEIVED APRIL 26, 1948

[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).

For purposes of analyses these pterins were all dried over phosphorus pentoxide for two hours at 100° and about 0.1 mm. pressure.

Anal. Calcd. for $C_7H_7ON_5$: C, 47.46; H, 3.96; N, 39.55. Found: C, 47.3; H, 4.52; N, 39.5 (cor. for 0.55% ash).

B. 2,3-Dichloropropional and II.—One gram of the triamine (II) dissolved in 50 cc. of 0.5 *N* hydrochloric acid was mixed with a solution of 0.9 g. of 2,3-dichloropropional in 50 cc. of ethanol. After ninety minutes at room temperature the solution was adjusted to *p*H 4.0. The precipitated product was collected, washed with ethanol and crystallized in the usual way from a 5 *N* sodium hydroxide solution. The crystalline sodium salt was filtered off, dissolved in water, treated with Norite A, precipitated from solution with acetic acid and crystallized again from a 5 *N* sodium hydroxide solution. The crystalline material was collected and converted to the free acid as described above; yield 0.40 g. The ultraviolet absorption spectra of this product were identical with those of 2-amino-4-hydroxy-7-methylpteridine.[2]

Anal. Calcd. for $C_7H_7ON_5$: C, 47.46; H, 3.96. Found: C, 47.52; H, 4.32.

2,3-Dibromopropional and II also react under identical conditions to give the same 7-methylpteridine (III).[6]

C. α-Bromotetronic Acid[3] and II.—A mixture of 0.50 g. of the triamine (II) and 0.70 g. of α-bromotetronic acid was dissolved in 12 cc. of 6 *N* hydrochloric acid and heated on the steam-bath for forty-five minutes. Carbon dioxide was given off, probably due to hydrolysis and decarboxylation of the α-bromotetronic acid. A small amount of solid was filtered off and the solution neutralized to *p*H 3.0. The product was collected by centrifuging, washed once with water, crystallized twice from 5 *N* sodium hydroxide in the usual manner and finally converted to the free acid; yield 0.125 g. For purposes of analysis this was crystallized once more as a sodium salt and converted again to the free acid. The ultraviolet absorption spectra were identical with those of (III).

Anal. Calcd. for $C_7H_7ON_5$: C, 47.46; H, 3.96. Found: C, 46.96; H, 4.42.

This compound as well as the product from B was oxidized in an alkaline permanganate solution to produce 2-amino-4-hydroxypteridine-7-carboxylic acid.[2b]

D. *d,l*-Glyceraldehyde and 2,4,5-Triamino-6-hydroxypyrimidine (II).—This reaction was run in the manner described by Karrer, *et al.*[1] A mixture of 1.4 g. of II, 25 cc. of water and 1 cc. of glacial acetic acid was ylaced in a flask and a stream of carbon dioxide passed through it. A solution of 0.9 g. of *d,l*-glyceraldehyde in 5 cc. of water was added and the mixture refluxed gently for two hours. After cooling well the resulting product was filtered off, washed and dried; yield 1.7 g. The ultraviolet absorption spectra of this crude compound and the ultraviolet absorption spectra of the oxidation product of a similarly prepared product both indicated that this material was almost entirely a 7-substituted pteridine.[2b]

A portion of the crude material (1.6 g.) was suspended in 100 cc. of water, brought to boiling and 50% sulfuric acid added until the solid had dissolved. The solution was treated with Norite, cooled and the crystalline product filtered off, washed and dried; yield 0.80 g.[1] This material was further purified by two methods.

One fraction (0.30 g.) was crystallized three times from a 5 *N* sodium hydroxide solution and converted to the free acid in the manner described in the first experiment above; yield 0.090 g. This is the same yield obtained in a similar purification of good 2-amino-4-hydroxy-7-methylpteridine obtained from methyl glyoxal.[2]

Anal. Calcd. for $C_7H_7ON_5$: C, 47.46; H, 3.96; N, 39.55. Found: C, 47.56; H, 3.92; N, 39.70.

Since it was considered possible that the strong alkali was in some manner decomposing the hydroxymethylpteridine to form a methylpteridine, another fraction (0.40 g.) of the once crystallized material was crystallized three more times from dilute sulfuric acid as described above; crystallized as clusters of needles; yield 0.15 g.

Anal. Found: C, 47.42; H, 4.32; N, 39.27.

Both of these purified products had ultraviolet absorption spectra which were identical with those of 2-amino-4-hydroxy-7-methylpteridine.[2,7]

2-Amino-4-hydroxypteridine-6-acetic Acid (IV).—The ethyl 2,4-dibromo-3-ketobutanoate prepared from 100 g. of ethyl acetoacetate[8] was dissolved in 600 cc. of ethanol and added to a solution of 60.0 g. of II in 800 cc. of 2 *N* hydrochloric acid. The solution was heated on the steam-bath for forty-five minutes, cooled and neutralized to *p*H 3.0 with 10 *N* sodium hydroxide. The resulting dark sludge was centrifuged and the supernatant liquid poured off. The residue was dissolved by adding 0.5 *N* sodium hydroxide to a total volume of one liter. To this was then added 750 cc. of a 10 *N* sodium hydroxide solution and the mixture was cooled overnight. The precipitate which had formed was filtered off, dissolved in 300 cc. of hot water, treated with Norite, filtered and 200 cc. of a 10 *N* sodium hydroxide solution added to the filtrate. After cooling, the product was collected and this purification was repeated. The resulting crystalline sodium salt was dissolved in 250 cc. of hot water, treated with Norite, filtered and then added to a hot solution of 35 cc. of acetic acid in 300 cc. of water. The product was filtered off, washed and dried; yield of yellow powder, 18.3 g.

For purposes of analysis 5.0 g. of this product was recrystallized once from a 2 *N* sodium hydroxide solution and three times from *N* sodium hydroxide solutions. The crystalline sodium salt was converted to the free acid by dissolving in water and acidifying to about *p*H 2.0 with hydrochloric acid; yield 2.1 g. of light cream colored product.

Anal. Calcd. for $C_8H_7O_3N_5$: C, 43.44; H, 3.17; N, 31.67. Found: C, 43.26; H, 3.31; N, 31.90.

The ultraviolet absorption spectra of this compound were identical with those of 2-amino-4-hydroxypteridine-6-acetic acid.[4]

Discussion

The foregoing experimental data prove that a large share of the product from the reaction of II and any of the three-carbon compounds described is 2-amino-4-hydroxy-7-methylpteridine. However, it is quite possible that the crude reaction products may contain small amounts of a halogen- or hydroxymethylpteridine. These could be formed due to small amounts of residual oxygen which might still be present even in the solution swept with nitrogen or carbon dioxide and which would oxidize small amounts of the intermediate dihydropteridine before it could split out hydrogen halide or water. This would explain the results described by Karrer and Schwyzer in a recent publication.[8] They treated the triamine (II) with dihydroxyacetone under conditions similar to those described for glyceraldehyde. The resulting pteridine was treated with *p*-aminobenzoylglutamic acid in a formic acid solution to obtain a product which contained some pteroylglutamic acid as shown by a biological assay. No yields were given but apparently only a small amount of impure product was obtained.

(6) Waller, *et al.*, THIS JOURNAL, **70**, 19 (1948).

(7) We have found that many of the various 2-amino-4-hydroxypteridines prepared in this Laboratory have required rather careful and extensive purifications before they became satisfactory analytical samples. Insufficiently purified products almost invariably gave low carbon and nitrogen values.

(8) Karrer and Schwyzer, *Helv. Chim. Acta*, **31**, 777 (1948).

Acknowledgment.—The authors wish to thank Mr. Louis Brancone and staff who performed the microanalyses and Anna de Grunigen who carried out the spectra determinations.

Summary

Descriptions are given for the synthesis of 2-amino-4-hydroxy-7-methylpteridine (III) from 2,4,5-triamino-6-hydroxypyrimidine (II) and each of the following: 1,3-dichloroacetone, 2,3-dichloropropional, α-bromotetronic acid and *d,l*-glyceraldehyde.

The synthesis of 2-amino-4-hydroxypteridine-6-acetic acid from 2,4,5-triamino-6-hydroxypyrimidine and ethyl 2,4-dibromo-3-ketobutanoate is described and a mechanism for these reactions is suggested.

PEARL RIVER, NEW YORK RECEIVED APRIL 1, 1948

[CONTRIBUTION FROM THE FOREST PRODUCTS LABORATORY,[1] FOREST SERVICE, U. S. DEPARTMENT OF AGRICULTURE]

A Flavonone from Douglas-Fir Heartwood[2]

BY JOHN C. PEW

A new compound has been found at the U. S. Forest Products Laboratory in Douglas-fir heartwood. This compound may be responsible for the resistance to sulfite pulping shown by the heartwood of this species. Erdtman[3] found a 3,5-dihydroxystilbene and its monomethyl ether in pine heartwood and showed these substances caused sulfite-pulping retardation. Douglas-fir heartwood, when subjected to Erdtman's method of separation, did not yield stilbene derivatives. Staining reactions, however, showed the presence of phenolic substances, and the crystalline compound isolated gave similar reactions.

The compound, accompanied by considerable extraneous matter, is extracted from the wood by methanol, ethanol, acetone or ethanol–benzene mixtures. Ether does not extract it, but dissolves it after extraction. Separation of the pure substance from the other extractives is somewhat difficult. The compound crystallizes as a hydrate from water in long, fine, colorless needles that melt with decomposition at 240–242° (dec.).[4] It is optically active, giving a value of $[\alpha]^{20}D + 46°$ (*c*, 4 in equal volumes of acetone and water) and $[\alpha]^{20}D + 13°$ (*c*, 4 in absolute alcohol). With ferric chloride it gave emerald-green to black colorations in aqueous solution, and with magnesium and hydrochloric acid it gave an intense purple-red color in alcoholic solution. This latter reaction suggested the structure of a flavanone. Atmospheric oxidation on a steam-bath of a 2 *N* sulfuric acid solution of the compound gave good yields of quercetin. Reduction of an alcoholic solution with zinc dust and hydrochloric acid gave the flavanone eriodictyol. Heating the compound with strong hydrochloric acid produced a compound with no optical activity. From these properties and the analysis the compound was formulated as 3,3′,4′,5,7 pentahydroxyflavanone.[5]

(1) Maintained at Madison, Wis., in coöperation with the University of Wisconsin.

(2) Presented before the American Chemical Society, New York, N. Y., Sept. 15–19, 1947.

(3) H. Erdtman, *Ann.*, **539**, 116 (1939).

(4) All melting points were corrected.

(5) Erdtman (private communication) suggests the compound be called "taxifolin."

The 3-hydroxyflavanones (or flavanolones)[6] belong to a class found in recent years in plants. The first example, designated alpinone,[7] and shown to be the 3,5-dihydroxy-7-methoxy-2-methylflavanone, was discovered in 1936 in a Japanese drug prepared from *Alpinia japonica*. Subsequently, three other 3-hydroxyflavanones were reported to occur naturally: fustin[8] (3,3′,4′,7-tetrahydroxyflavanone) from the heartwood of the *Rhus* sp.; ampeloptin[9] (3,3′,4′,5,5′,7-hexahydroxyflavanone) from *Ampelopsis meliaefolia* Kudo, a plant used as a drug and condiment in China; and pinobanksin[10] (3,5,7-trihydroxyflavanone) from the heartwood of *Pinus banksiana* and other *Pinus* sp. The presence of the 3-hydroxyflavanones in the heartwood of two species suggested the possibility of their occurrence in other woods. A South American wood, coigue (*Nothofagus dombeyi* Blume), the heartwood of which showed resistance to sulfite pulping, yielded naringenin and the corresponding 3-hydroxy compound, both of which gave cherry-red reduction colors with magnesium and hydrochloric acid. These two flavanones were also isolated from the heartwood of black cherry (*Prunus serotina* Ehrh). The 3-hydroxyflavanones were found to have a color reaction that appears to be characteristic. Their alcoholic solutions give deep anthocyanidin-like colors when treated with granulated zinc and hydrochloric acid, while the corresponding flavanones without the 3-hydroxy group and the flavanols, under similar treatment, remain colorless or give only weak pink to lavender tints. Both classes of flavanones readily give deep colors with magnesium and hydrochloric acid. If the filtrate from suspensions of ground wood (or other plant material) in methyl alcohol, after standing in contact a day or two, are not colored when acidified with hydrochloric acid but develop distinct colors on the subsequent addition of granulated zinc, the

(6) H. Erdtman, *Svensk Kem. Tid.*, **56**, 8 (1944).

(7) Y. Kimura and M. Hoshi, *Proc. Imp. Acad., Tokyo*, **12**, 285 (1936).

(8) T. Oyamada, *Ann.*, **538**, 44 (1939).

(9) M. Kotake and T. Kubota, *Ann.*, **544**, 253 (1940).

(10) H. Erdtman, *Svensk. Kem. Tidskr.*, **56**, 8 (1944).