[CONTRIBUTION FROM THE DEPARTMENT OF ORGANIC CHEMISTRY, MELLON INSTITUTE]

PTERIDINES. 6-SUBSTITUTED ISOXANTHOPTERINS AND THE 4-AMINO ANALOGS

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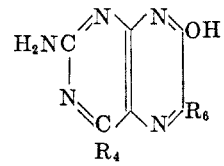
The pteridines most frequently discussed in the literature have a substituent in the 6-position (as xanthopterin and folic acid) and only hydrogen in the 7-position. However, the 7-hydroxypteridines derive interest from the fact that the 7-OH group is introduced during the *in vitro* degradation of folic acid by the action of enzymes and ultraviolet light (1). Further, the occurrence or derivation of 7-hydroxypteridines in biological material has been indicated by the finding of erythropterin in tubercle bacilli (2) and in the body pigment of insects (3).

The method used in the present work for the synthesis of 6-alkyl-7-hydroxypteridines was described by Purrmann (4). Condensation of 2,6-disubstituted 4,5-diaminopyrimidine bisulfite with α-keto esters in the presence of *N* acetic acid gave a very uniform series of derivatives. Whether these reactants give rise to 6-alkyl-7-hydroxypteridines (isoxanthopterins) or the isomeric 7-alkylxanthopterins apparently is in large measure influenced by the pH of the condensation medium (4, 5).

The series of derivatives is indicated below. Absorption spectra in the ultraviolet,[1] determined for solutions of these compounds, are very similar through the 4-hydroxy and 4-amino series. These values correspond closely to the absorption spectrum reported by Elion, *et al.* (5 b) for 6-methyl-isoxanthopterin and differ sharply from the 385 mμ maximum reported by these authors for 7-methylxanthopterin.



R_6 =	R_4 = OH	R_4 = NH_2
$CH_2COOC_2H_5$	R_4 = OH (I)	R_4 = NH_2 (VI)
CH_2COOH	R_4 = OH (II)	R_4 = NH_2 (VII)
CH_3	R_4 = OH (III)	R_4 = NH_2 (VIII)
$CH(COOC_2H_5)CH_2COOC_2H_5$	R_4 = OH (IV)	R_4 = NH_2 (IX)
C_6H_5	R_4 = OH (V)	R_4 = NH_2 (X)

It is interesting to note that the 6-acetic acid derivative (II) is stable enough to permit isolation of the free acid. Under comparable conditions of hydrolysis and neutralization, certain rather similar quinoxaline derivatives underwent spontaneous decarboxylation (6, 7).

[1] In some cases the Beckman, and at times, the Cary recording spectrophotometer was used. Curves were determined from 230 mμ to 400 mμ.

Solutions of the compounds described in the present paper are fluorescent and the compounds were readily characterized by the use of partition chromatography on filter paper as previously reported (8). With the use of paper chromatography, Korte (9) found evidence for the presence of 2-amino-4,7-dihydroxy-6-pteridineacetic acid in ichthyopterin.

Seven of these compounds were tested for their potential ability to increase the survival time of mice bearing transplanted mouse leukemia of lymphatic and myelogenous type by Menten and Green of Children's Hospital in Pittsburgh. Some of the compounds were also submitted to the National Cancer Institute for testing.

EXPERIMENTAL

Condensations of substituted 4,5-diaminopyrimidine bisulfite salts (10 a, b) with α-keto-esters were carried out by adding the reactants to a large volume of *N* acetic acid already heated to 90°, and then rapidly raising the temperature till the solution was refluxing. The procedure is described under the preparation of ethyl 2-amino-4,7-dihydroxy-6-pteridine-acetate (I).

Ethyl 2-amino-4,7-dihydroxy-6-pteridineacetate (I). Although Elion, *et al.* (5 b) utilized I as an intermediate in the synthesis of 6-methylisoxanthopterin (III) neither the ethyl ester nor the corresponding pteridineacetic acid (II) was isolated.

In the present study 1500 ml. of *N* acetic acid was preheated to about 90°; the addition of 40 g. of 2,4,5-triamino-6-hydroxypyrimidine bisulfite (0.22 mole) was followed immediately by the addition of 55 g. of the sodium salt of ethyl oxalacetate (0.26 mole). The mixture was shaken and rapidly heated during 6–8 minutes to a refluxing temperature of about 125°. The solids dissolved within 2–3 minutes and very soon a needle-like yellow precipitate began to separate. Sulfur dioxide fumes were noticeable. Refluxing was continued for 60–90 minutes. Yield 25 g. (42%).

Extraction of the crude orange-yellow product with hot alcohol left a tan solid, which could be recrystallized from pyridine or large volumes of alcohol. The ester could be dissolved by brief warming with two equivalents of 0.1 *N* sodium hydroxide and recovered on neutralization. On prolonged contact with excess alkali, hydrolysis occurred.

For analysis the ester was dried in a vacuum oven at 60°.[2] On subsequent drying at 140° and 2 mm. there was a loss of volatile material, corresponding to 0.5 mole-equivalent of water. A dried sample exposed to the atmosphere of the room regained weight equal to 0.5 mole-equivalent of water.

Anal. Calc'd for $C_{10}H_{11}N_5O_4 \cdot 0.5H_2O$: C, 43.80; H, 4.38; N, 25.55; H_2O, 3.3.
Found: C, 42.5; H, 4.39; N, 24.71; H_2O, 3.1.

Ultraviolet spectrum: Maxima (in 0.1 *N* NaOH), 253, 278, 340 mμ. Minima, 245, 292 mμ.

A small second crop from the original mother liquor showed maxima at 352 and 379 mμ when dissolved in 0.1 *N* sodium hydroxide.

2-Amino-4,7-dihydroxy-6-pteridineacetic acid (II). Hydrolysis of the ethyl ester was with 5 *N* sodium hydroxide at room temperature, or by heating with 0.5 *N* sodium hydroxide. In the presence of 100 ml. of 0.5 *N* sodium hydroxide the ester (5.3 g.) dissolved rapidly, was refluxed for 1 hour, and was filtered hot. Acidification in the presence of ice with *N* acetic acid (or to *p*H 2) gave a light brown powder. The spectrum resembles that of the corresponding 6-methyl derivative and not that of isoxanthopterin-6-carboxylic acid (5).

Anal. Calc'd for $C_8H_7N_5O_4 \cdot 0.75\ H_2O$: C, 38.32; H, 3.39; N, 27.94; H_2O, 5.4.
Found: C, 37.90; H, 3.42; N, 28.01; Loss on drying, 5.34.

Ultraviolet spectra: Maxima (in 0.1 *N* NaOH), <230, 245–255, 340 mμ. Minimum, 290 mμ.
Maxima (in 0.1 *N* HCl), <220, 288, 337 mμ. Minima, 256, 309 mμ.

[2] The purified compounds did not melt below 330° except as noted.

2-Amino-4,7-dihydroxy-6-methylpteridine (III). (*6-Methylisoxanthopterin*). Decarboxylation of the acetic acid substituent to give the 6-methylpteridine derivative was carried out by warming (85–100°) a suspension of the pteridine (II) with excess 2 *N* hydrochloric acid, or better, with excess *N* acetic acid. A rapid evolution of carbon dioxide caused vigorous foaming. This pteridine has been described by Elion, *et al.* (5 a, b).

The absorption maxima and E_M values of an analyzed sample indicate 6-methylisoxanthopterin. In contrast, 7-methylxanthopterin has a maximum at 385 mμ (5 b).

Ultraviolet spectra: Maxima (in 0.1 *N* NaOH), <230, 250–255, 272–276, 338 mμ. Minima, 245, 290 mμ.

Maxima (in 0.1 *N*H Cl), <230, 290, 337 mμ. Minima, 260, 305 mμ.

Diethyl 2-amino-4,7-dihydroxy-6-pteridinesuccinate (IV). Condensation of the potassium salt of diethyl oxalylsuccinate (11) with 2,4,5-triamino-6-hydroxypyrimidine bisulfite in *N* acetic acid gave the expected pteridine; yield 50%. For analysis 0.5 g. was refluxed with 11 ml. of hot pyridine; the hot solution was filtered and the mother liquor was concentrated under reduced pressure till solid began to separate. After the addition of a large volume of acetone, 0.42 g. of a flocculent white powder was collected; m.p. 299° with decomposition. The sample was dried in the vacuum oven at 110°.

Anal. Calc'd for $C_{14}H_{17}N_5O_6$: C, 47.86; H, 4.84; N, 19.94.

Found: C, 47.10; H, 4.65; N, 19.95.

Ultraviolet spectrum: Maxima (in 0.1 *N* NaOH), <230, 256, 275, 340 mμ. Minima, 245, 292 mμ.

Ethyl 2,4-diamino-7-hydroxy-6-pteridineacetate (VI). The condensation of 2,4,5,6-tetraminopyrimidine bisulfite (10) with the sodium salt of ethyl oxalacetate was carried out in the manner described under I. A faint orange color was removed from the crude product by washing with alcohol and a yellow solid was obtained in good yield, 72%. This ester had previously been utilized by Elion, *et al.* (5 b) as an intermediate in synthesizing the methyl derivative (VIII).

Anal. Calc'd for $C_{10}H_{12}N_6O_3$: C, 45.45; H, 4.54; N, 31.81.

Found: C, 45.06; H, 4.70; N, 31.27.

Ultraviolet spectrum: Maxima (in 0.1 *N* NaOH), <230, 250–255, inflection at 280, 342 mμ. Minimum, 291 mμ.

2,4-Diamino-6-methyl-7-hydroxypteridine (VIII). After hydrolysis of the ethyl ester (VI) with 0.5 *N* sodium hydroxide and neutralization with acetic acid in the presence of ice, a cream-colored solid which analyzed as the pteridineacetic acid (VII) was recovered. The nitrogen content of this material increased upon repeated solution in alkali and precipitation with acid.

Anal. Calc'd for $C_8H_8N_6O_3$(VII): C, 40.68; H, 3.39; N, 35.6.

Found: C, 40.77; H, 3.36; N, 36.02.

For ready preparation of the methyl derivative the ester (VI) was refluxed for 30 minutes with *N* sulfuric acid and the hot solution filtered from undissolved solid. The crude product was collected on cooling and was examined by partition chromatography on paper with the use of butanol-morpholine-water as a developing solvent (8). Three spots were observed corresponding to simultaneously developed samples of the known ester, acid, and methyl derivative. After twice dissolving the crude product in alkali and precipitating with excess hot *N* acetic acid, the product gave a single spot, which checked with the methyl derivative on the paper chromatogram. This purified product analyzed correctly and the curves in the ultraviolet for solutions in 0.1 *N* NaOH and 0.1 *N* HCl corresponded to those reported by Elion, *et al.* (5 b).

This pteridine (VIII) was also prepared by condensation of 2,4,5,6-tetraminopyrimidine bisulfite in *N* acetic acid with amyl pyruvate.

Diethyl 2,4-diamino-7-hydroxy-6-pteridinesuccinate (IX). Condensation of diethyl potassium oxalylsuccinate (11) with 2,4,5,6-tetraminopyrimidine bisulfite gave a 59% yield of the pteridine (IX); m.p. 322–324° with decomposition.

Anal. Calc'd for $C_{14}H_{18}N_6O_5$: C, 48.00; H, 5.14; N, 24.00.

Found: C, 47.94; H, 5.01; N, 24.14.

Ultraviolet spectrum: Maxima (0.1 *N* NaOH), <235, 252–254, 338 mμ. Minimum, 295 mμ.

6-Phenylisoxanthopterin (V). Ethyl benzoylformate (Eastman) was condensed with the pyrimidine in the usual manner; yield 40%.

Anal. Calc'd for $C_{12}H_9N_5O_2$: C, 56.47; H, 3.53; N, 27.45.
Found: C, 56.52; H, 3.66; N, 27.24.

Ultraviolet spectrum: Maxima (in 0.1 *N* NaOH), 226, 265, 360 mμ. Minima, 257, 309 mμ.

2,4-Diamino-6-phenyl-7-hydroxypteridine (X). This 4-amino analog of V was obtained in 76% yield from the condensation of 2,4,5,6-tetraminopyrimidine bisulfite with ethyl benzoylformate in *N* acetic acid.

Anal. Calc'd for $C_{12}H_{10}N_6O$: C, 56.69; H, 3.94; N, 33.07.
Found: C, 56.24; H, 4.18; N, 32.78.

Ultraviolet spectra: Maxima (in 0.1 *N* NaOH), <230, 268, 362 mμ. Minima, 255, 308 mμ.
Maxima (in 0.1 *N* HCl), <230, inflection at 240, 306, 362 mμ. Minima, 265, 325 mμ.

SUMMARY

6-Phenyl and 6-alkyl derivatives of 2-amino-4,7-dihydroxypteridine, as well as the 4-amino analogs, have been synthesized with the use of α-keto esters. Very similar curves were obtained for the ultraviolet absorption spectra of the various 6-alkylpteridines in alkaline solution. The isolation of 2-amino-4,7-dihydroxy-6-pteridineacetic acid is reported.

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