Summary

It has been shown that the hypotensive properties of the roots and rhizomes of *Veratrum viride*, the American or Green Hellebore, are principally due to two hitherto undescribed crystalline alkaloids which have been named germidine and germitrine. Germidine has been identified as a mixed diester of the known alkamine germine, $C_{27}H_{43}O_8N$, with acetic acid and *l*-α-methylbutyric acid. Germitrine is a triester of germine containing in addition to the above acids one mole of *d*-methylethylglycolic acid.

Germitrine undergoes rapid methanolysis in 50% aqueous methanol at room temperature with the loss of the acetyl group and the formation of the diester alkaloid germerine (germine *l*-α-methylbutyrate *d*-methylethylglycolate), already known as a constituent of *Veratrum album*. The compound originally obtained from the triester fraction by crystallization from aqueous methanol and designated germitrine in our preliminary communication[13] was actually slightly impure germerine. Germitrine is also degraded to germerine by chromatography on acetic acid-washed alumina.

Germitrine is a powerful hypotensive agent. As little as half a microgram per kg. given intravenously elicits a marked fall in blood pressure in the anesthetized dog. The two diester alkaloids are somewhat less active.

Protoveratrine (*Veratrum album*), the corresponding triester of the alkamine protoverine, is likewise unstable in aqueous methanolic solution; in this case the volatile methanolysis products yielded on hydrolysis acetic acid as well as methylethylglycolic acid. Germine pentaacetate on similar treatment is partially degraded to a monoacetate.

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[CONTRIBUTION FROM THE LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID COMPANY]

2-Amino-4-hydroxy-6-pteridinecarboxaldehyde

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A pterincarboxaldehyde was first prepared from the natural fermentation *L. casei* factor by sulfurous acid cleavage by Hutchings, *et al.*[1] The fermentation *L. casei* factor has been synthesized and shown to be pteroyl-γ-glutamyl-γ-glutamylglutamic acid.[2] Therefore, the sulfurous acid method of cleavage should be applicable to the general class of pteroyl- compounds. Reported herein are two convenient methods of synthesizing 2-amino-4-hydroxy-6-pteridinecarboxaldehyde (I).

The first method of synthesis of I is the cleavage of pteroylglutamic acid with sodium sulfite solution acidified with excess acetic acid. This resulting solution reacted with a slight excess of iodine to precipitate I.

Petering, *et al.*,[3a] in a preliminary report indicated a pterincarboxaldehyde formation by oxidation of a polyhydroxyalkylpterin with lead tetraacetate. Forrest and Walker[4] by a similar oxidation of 6-tetrahydroxybutylpterin obtained a pterincarboxaldehyde characterized as I by the formation of a 2,4-dinitrophenylhydrazone and oxidation to 2-amino-4-hydroxy-6-pteridinecarboxylic acid (IV). Karrer, *et al.*,[5] have also reported a number of hydroxyalkylpterins.

Angier, *et al.*[6] have shown that the synthesis of substituted alkylpterins through a dihydropterin often results in methylpterins rather than the desired substituted methylpterins.

Weygand, *et al.*,[7] have confirmed the findings of Angier, *et al.*,[6] by the preparation of 2-amino-4-hydroxy-7-[D-*erythro*-2′,3′,4′-trihydroxybutyl]-pteridine from *p*-tolyl-D-isoglucosamine and 2,4,5-triamino-6-hydroxypyrimidine and subsequently periodic oxidation to 2-amino-4-hydroxypteridine-7-acetaldehyde.

Weygand, *et al.*,[7] also prepared I by periodic oxidation of 2-amino-4-hydroxy-6-[D-*arabo*-tetrahydroxybutyl]-pteridine. His product was characterized by analysis, oxidation to IV, preparation of a Schiff base with *p*-toluidine and methyl *p*-aminobenzoate and the preparation of pteroylglutamic acid.

The biological interest in the pteridine carboxaldehyde (I), such as the inhibition of xanthine oxidase by I as reported by Kalckar, *et al.*,[8] and Van Meter,[9] makes it desirable to have a preparative synthesis which will eliminate the possibility of isomer and pteridine acetaldehyde contaminants.

(1) Hutchings, *et al.*, THIS JOURNAL, **70**, 10 (1948); *Annals N. Y. Acad. Sci.*, XLVIII, 273 (1946).

(2) Boothe, *et al.*, *ibid.*, **70**, 1099 (1948); Mowat, *et al.*, *ibid.*, **70**, 1096 (1948); Boothe, *et al.*, *ibid.*, **71**, 2304 (1949); Mowat, *et al.*, *ibid.*, **71**, 2308 (1949); Semb, *et al.*, *ibid.*, **71**, 2310 (1949); Angier, *et al.*, *ibid.*, in press.

(3) (a) Petering and Weisblat, THIS JOURNAL, **69**, 2566 (1947); (b) Petering and Schmidt, *ibid.*, **71**, 3977 (1949).

(4) Forrest and Walker, *J. Chem. Soc.*, 83 (1949).

(5) (a) Karrer, *et al.*, *Helv. Chim. Acta*, **30**, 1031 (1947); (b) **31**, 777 (1948); (c) **32**, 423 (1949).

(6) Angier, *et al.*, THIS JOURNAL, **70**, 3029 (1948).

(7) Weygand, *et al.*, *Ber.*, **82**, 25 (1949).

(8) Kalckar, *et al.*, *J. Biol. Chem.*, **174**, 771 (1948).

(9) Van Meter and Oleson, *ibid.*, in press.

The second synthesis of I makes use of 2-amino-4-hydroxy-6-methylpteridine[10] III. The bromination of III in 48% hydrobromic acid by a modification of the method of Boothe, *et al.*,[10b] yielded 2-amino-4-hydroxy-6-dibromomethylpteridine (V). Purified V when hydrolyzed gave I identical with the product obtained from pteroylglutamic acid. The ultraviolet absorption spectra of I in 0.1 *N* sodium hydroxide as shown in Fig. 1 has a maximum at 275 mμ in addition to the maxima shown by the simple pteridines. The ultraviolet

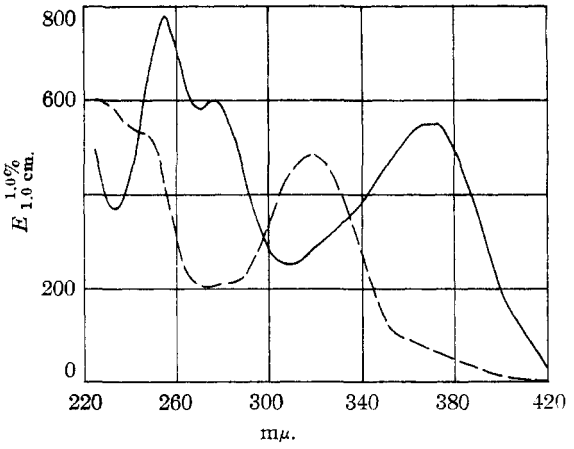


Fig. 1.—Ultraviolet absorption spectra of 2-amino-4-hydroxy-6-pteridinecarboxaldehyde: —, 0.1 *N* sodium hydroxide; - - -, 0.1 *N* hydrochloric acid.

absorption ratio in 0.1 *N* sodium hydroxide, obtained by dividing the density reading of the maximum at 255 mμ by the corresponding reading at 377 mμ, is much lower than that reported for other pteridines.[3b] This low ratio and added maximum were found most characteristic for the 6-pteridinecarboxaldehyde. The spectra for the phenylhydrazone (Fig. 2) and the oxime (Fig. 3) of I also differed from the usual pteridine spectra.

The report by Weygand, *et al.*,[7] stated that the 6-pteridinecarboxaldehyde (I) in 5 *N* sodium hydroxide gave the acid IV and 2-amino-4-hydroxy-6-hydroxymethylpteridine (VI) but no experimental data was given. Presented herein is a detailed experiment on the formation from I of the acid IV and the hydroxymethylpteridine VI and the separation of these products. Hutchings, *et al.*,[1] found that the methylpterin III and the acid IV were formed from the unoxidized solution of pteridinecarboxaldehyde resulting from the sulfurous acid cleavage of the fermentation *L. casei* factor. As postulated by Weygand, *et al.*,[7] and implied by Angier, *et al.*,[6] the pterin of Hutchings, *et al.*,[1] was possibly a dihydropterincarboxaldehyde which split out a molecule of water in the Cannizzaro type reaction to give the methylpterin III rather than the hydroxylmethylpterin VI.

The ultraviolet absorption data for the methylpterin III[10a] and the hydroxylmethylpterin VI (Fig. 4) are identical when calculated on a molec-

(10) (a) Mowat, *et al.*, THIS JOURNAL, **70**, 14 (1948); (b) Boothe, *et al.*, *ibid.*, **70**, 28 (1948); (c) Semb, U. S. Patent 2,477,426 (1949).

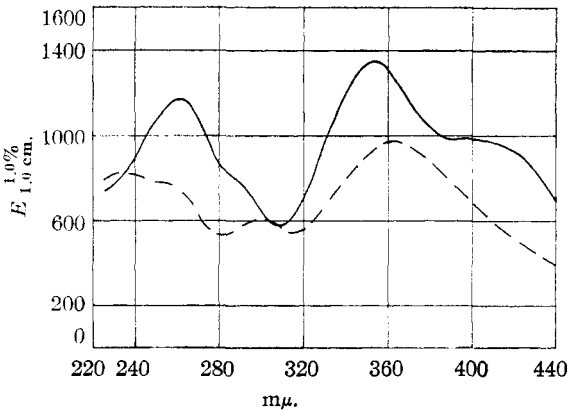


Fig. 2.—Ultraviolet absorption spectra of the phenylhydrazone of 2-amino-4-hydroxy-6-pteridinecarboxaldehyde: —, 0.1 *N* sodium hydroxide; - - -, 0.1 *N* hydrochloric acid.

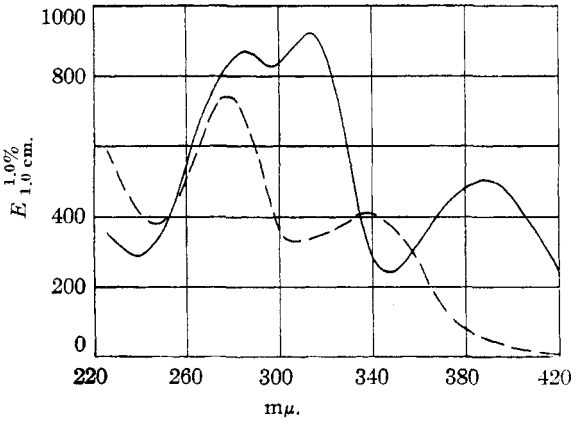


Fig. 3.—Ultraviolet absorption spectra of the oxime of 2-amino-4-hydroxy-6-pteridinecarboxaldehyde: —, 0.1 *N* sodium hydroxide; - - -, 0.1 *N* hydrochloric acid.

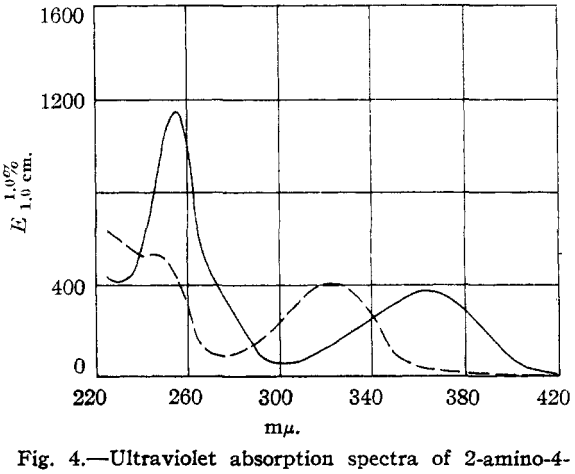


Fig. 4.—Ultraviolet absorption spectra of 2-amino-4-hydroxy-6-hydroxymethylpteridine: —, 0.1 *N* sodium hydroxide; - - -, 0.1 *N* hydrochloric acid.

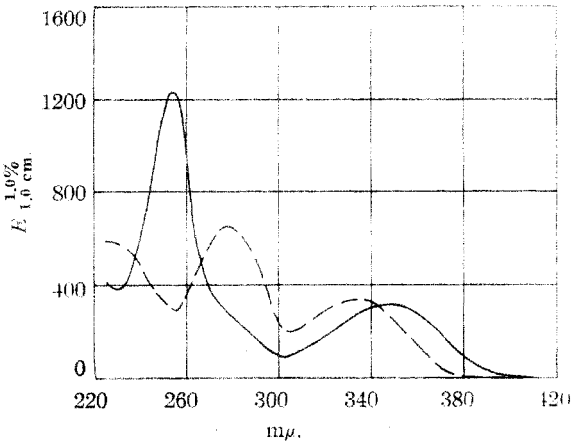


Fig. 5.—Ultraviolet absorption spectra of 2-acetylamino-4-hydroxy-6-methylpteridine: ——, 0.1 *N* ammonium hydroxide; - - -, 0.1 *N* hydrochloric acid.

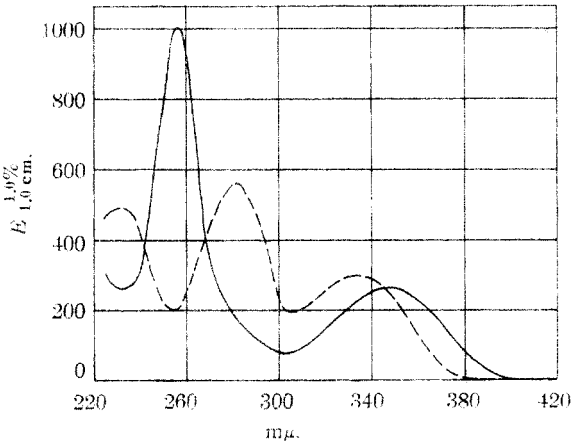
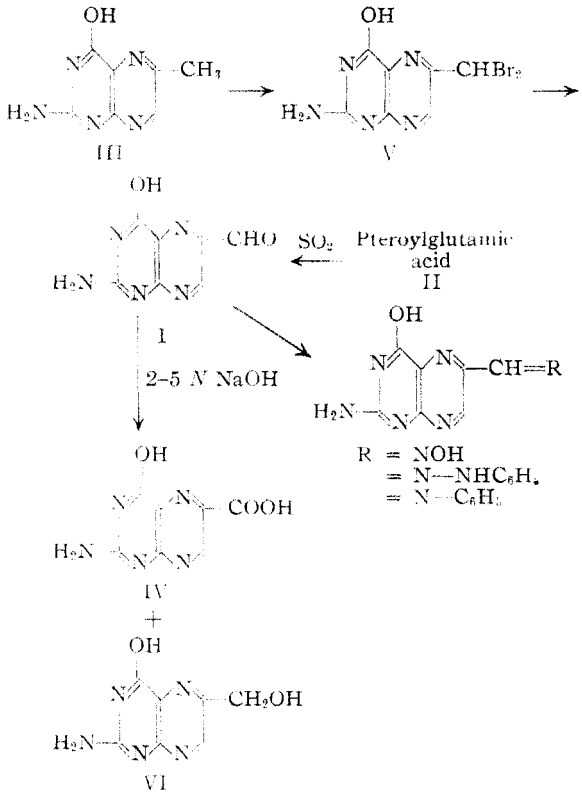


Fig. 6.—Ultraviolet absorption spectra of 2-acetylamino-4-hydroxy-6-acetoxymethylpteridine: ——, 0.1 *N* ammonium hydroxide; — — , 0.1 *N* hydrochloric acid.

ular basis. The spectra for the monoacetyl of III (Fig. 5) and the diacetyl of IV (Fig. 6) on a molecular basis are also identical. This data on the acetyl derivatives does not confirm the findings of Karrer, *et al.*,[5c] that acetylation of the hydroxylmethyl pterins result in a shift of 6–7 mμ in the maximum of the longer wave length. When the maximum density reading of the shorter wave length is divided by the maximum density reading of the longer wave length for the 0.1 *N* sodium hydroxide spectra a ratio of about 3.1 for the 6-alkylpterins and about 2.4 for the 7-alkylpterins[3b] is obtained. Inspection of the ultraviolet absorption data of Karrer, *et al.*,[5c] for the methylpterins and the hydroxymethylpterins and the calculation of the ratios for each from his data confirms the ratio of about 2.4 for the 7-substituted pterins but does not substantiate the 3.1 for the 6-isomers.

The melting points found for the monoacetyl of III and the diacetyl of VI were 315–320° (dec.) and 227–229° (dec.), respectively; however, substantially lower melting points of 272° (dec.) and 213° for these two compounds have been reported.[5c]



Experimental

2-Amino-4-hydroxy-6-pteridinecarboxaldehyde (I) from Pteroylglutamic Acid (II).—To a solution of 6 g. of pteroylglutamic acid dissolved in 900 cc. of water by adding a few drops of 10 *N* sodium hydroxide to affect solution was added 25 g. of sodium sulfite and 50 cc. of glacial acetic acid. The mixture was heated at 80–90° with stirring for thirteen hours. Cleavage of II was complete as shown by the chemical assay.[11] The reaction mixture was then clarified with charcoal, heated to boiling, diluted with a solution of iodine (15 g. of iodine and 30 g. of sodium iodide in 100 cc. of water) until a slight excess of iodine persisted for fifteen minutes. The product precipitated almost immediately, was collected after cooling to room temperature, washed and dried; wt. 1.8 g. It was recrystallized by dissolving in dilute sodium hydroxide solution, adding sodium sulfite and then acetic acid, clarifying with charcoal and reprecipitating by adding the iodine solution until a slight excess persisted. This product was identical in all respects to that obtained from hydrolysis of purified V; *i. e.*, ultraviolet absorption spectra, reactivity with aldehyde reagents and production of IV and VI in 2.5 *N* sodium hydroxide.

2-Amino-4-hydroxy-6-dibromomethylpteridine (V).—One hundred grams of 2-amino-4-hydroxy-6-methylpteridine III was added to a vigorously stirred solution of 310 g. of bromine in 3 l. of 48% hydrobromic acid. The mixture was stirred on a steam-bath for two and a half hours or until solution was complete. This solution was concentrated under water pump vacuum to one liter, cooled, clarified with charcoal, concentrated further under vacuum to 600 cc. where crystallization began. After cooling overnight the product was collected, washed with a small amount of acetic acid and then ether and dried; wt. 79 g. A second crop of 30 g. was obtained from the filtrate and

(11) Hutchings, *et al.*, *J. Biol. Chem.*, **168**, 705 (1947).

washed on concentrating and cooling. The product so isolated is the hydrobromide. Fifty grams of the above product was recrystallized from 500 cc. of 48% hydrobromic acid by dissolving at the boiling point, clarifying with charcoal and cooling overnight at 5°.

Anal. Calcd. for $C_7H_6ON_5Br_2 \cdot HBr$: C, 20.21; H, 1.45; N, 16.84; Br, 57.65. Found: C, 20.68; H, 1.79; N, 16.75; Br, 57.70.

The addition of V to cold water or alcohol resulted in the hydrolysis of the salt to the free base. This base invariably analyzed low (about 10%) for bromine indicating the formation of I.

2-Amino-4-hydroxy-6-pteridinecarboxyaldehyde (I).—A solution of 1 g. of purified V in 50 cc. of methyl cellosolve at 60–70° was added dropwise to 1500 cc. of boiling water. The mixture was stirred for twenty minutes. The product (I) was collected while the mixture was still boiling hot, washed and dried; yield 0.4 g.

Anal. Calcd. for $C_7H_5O_2N_5$: C, 43.98; H, 2.64; N, 36.64. Found: C, 43.45; H, 2.80; N, 36.97.

The Phenylhydrazone of I.—0.5 gram of purified V dissolved in 100 cc. of methyl cellosolve at 60–70° was added to a boiling solution of 3 g. of sodium acetate in 2500 cc. of water. Immediately to this mixture was added a solution of 0.75 g. of phenylhydrazine hydrochloride in 500 cc. of ethanol. After heating at boiling point and stirring for thirty minutes, the brick red precipitate was collected, washed and dried.

Anal. Calcd. for $C_{13}H_{11}ON_7$: C, 55.51; H, 3.94; N, 34.86. Found: C, 55.43; H, 4.61; N, 34.86 (cor. for 1.8% ash).

Oxime of I.—The oxime of the aldehyde (I) was prepared as described for the phenylhydrazone above substituting hydroxylamine hydrochloride (2 g.) for the phenylhydrazine hydrochloride.

Anal. Calcd. for $C_7H_6O_2N_6$: C, 40.78; H, 2.93; N, 40.77. Found: C, 40.89; H, 3.71; N, 40.84.

Anil of I.—The anil of I was prepared as described above for the phenylhydrazone using 2 cc. of aniline in place of the phenylhydrazine hydrochloride. The anil was not isolated until the reaction solution was cooled since it was found to be more soluble than the hydrazone or oxime.

Anal. Calcd. for $C_{13}H_{10}ON_6$: C, 58.64; H, 3.79; N, 31.56. Found: C, 57.72; H, 4.61; N, 31.53.

2-Acetylamino-4-hydroxy-6-dibromomethylpteridine.—A solution of 5 g. of V in 100 cc. of acetic anhydride was heated at refluxing temperature for thirty minutes. The resulting solution was clarified with charcoal and evaporated to dryness. The residue after recrystallization from acetic acid yielded 2 g. of purified product.

Anal. Calcd. for $C_9H_7N_5O_2Br_2 \cdot CH_3COOH$: C, 30.23; H, 2.54; N, 16.02; Br, 36.67. Found: C, 30.19; H, 2.79; N, 16.23; Br, 37.27.

Preparation of 2-Amino-4-hydroxy-6-hydroxymethylpteridine (VI) and 2-Amino-4-hydroxy-6-pteridinecarboxylic Acid (IV) from I.—A filtered solution of 10 g. of the pterin-6-carboxaldehyde (I) in 160 cc. of 2.5 *N* sodium hydroxide was cooled at 5° for five days. At the end of this period the fine needle-like crystalline precipitate was collected, washed with 2.5 *N* sodium hydroxide solution, alcohol and ether and dried: yield 6.2 g. of sodium 2-amino-4-hydroxypteridine-6-carboxylate. This compound was identical with the corresponding compound reported by Mowat, *et al.*[10a] The filtrate from VI on acidification to *p*H 7 with hydrochloric acid gave 5.5 g. of crude 2-amino-4-hydroxy-6-hydroxymethylpteridine. A sample was recrystallized twice from water for analysis.

Anal. Calcd. for $C_7H_7O_2N_5$: C, 43.52; H, 3.63; N, 36.26. Found: C, 43.3; H, 4.04; N, 36.4.

2-Acetylamino-4-hydroxy-6-acetoxymethylpteridine.—A mixture of 300 mg. of VI and 10 ml. of acetic anhydride was refluxed for two hours. The resulting solution was filtered and cooled. The needle-like crystalline product was collected, washed with ether and dried; yield 287 mg. It was recrystallized from water for analysis; m. p. 227–229° (dec.).

Anal. Calcd. for $C_{11}H_{11}O_4N_5$: C, 47.65; H, 4.00; N, 25.26. Found: C, 47.98; H, 4.68; N, 25.47.

2-Acetylamino-4-hydroxy-6-methylpteridine.—A mixture of 10 g. of 2-amino-4-hydroxymethylpteridine and a liter of acetic anhydride was refluxed for four hours. The solution was filtered while hot and cooled. The product was collected; wt. 6.3 g. For analysis a white crystalline compound resulted after several recrystallizations of the product from glacial acetic acid; m. p. 315–320° (dec.).

Anal. Calcd. for $C_9H_9N_5O_2$: C, 49.31; H, 4.14. Found: C, 49.52; H, 4.29.

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Summary

Two preparative methods of synthesis for 2-amino-4-hydroxy-6-pteridinecarboxaldehyde have been given; the first, by sulfite cleavage of pteroylglutamic acid, the second by dibromination of 2-amino-4-hydroxy-6-methylpteridine and subsequent hydrolysis.

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