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Synthesis of Pteroylglutamic Acid. III

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Two methods of synthesizing pteroylglutamic acid have been described.[4] Another approach to the synthesis of this compound involves the reaction of N-(*p*-aminobenzoyl)-glutamic acid with a three carbon compound to form a stable intermediate which after isolation could be condensed with 2,4,5-triamino-6-hydroxypyrimidine to form the desired product.

The most satisfactory intermediate of this type was prepared from the three carbon compound, reductone (2,3-dihydroxyacrylaldehyde).[5] Euler and Martius[6] described the reaction of reductone with urea in a dilute mineral acid solution to form the two expected products, 2,3-dihydroxy-2-ene-propylideneurea and bis-(2,3-dihydroxy-2-ene-propylidene)-urea. O'Meara, *et al.*,[7] described the reaction of an impure reductone solution with *p*-aminobenzoic acid to obtain a crystalline compound but no analyses or physical data were given for the product.

pounds which might be used as intermediates in the preparation of various analogs of pteroylglutamic acid. These compounds are listed in Table I. As is evident from the formula these compounds could exist in several tautomeric forms. However, in writing the formula of the product the enediol structure of the starting compound has been retained since it is at least the initial product of the reaction. Irrespective of the tautomeric nature of the solid it was thought probable that in solution there would be present at least a small percentage of the keto-aldehyde tautomer. The fact that compounds (II) and (VI) were found to react readily with an alcoholic solution of phenylhydrazine to form the corresponding diphenylhydrazones indicated that this might be true. In contrast to these results attempts at treating the ester (VI) with 2,4,5-triamino-6-hydroxypyrimidine[8] in aqueous or methanolic solution gave little or no product. However, when hot ethylene gly-

TABLE I

p-(2,3-DIHYDROXY-2-ENE-PROPYLIDENEAMINO)-BENZOIC ACID AND DERIVATIVES HO HO | | HC=C—CH=N—C$_6$H$_4$—COR

R =	M. p., °C.[a]	Yield, %	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
—OH (I)	255–258	90	57.96	4.38	6.76	57.97	4.46	6.72
—OC_2H_5 (II)	189–190	95	61.27	5.57	5.96	61.71	5.34	6.19
—NH_2 (III)	>200 dec.	90	58.24	4.89	13.59	57.95	5.65	13.85
—$NHCH_2COOH$ (IV)	>210 dec.	94	54.54	4.58	10.61	53.72	4.18	10.11
—$NHCH_2COOC_2H_5$ (V)	196–198	75	57.51	5.52	9.59	56.93	5.35	9.56
—$NHCH(CH_2COOC_2H_5)COOC_2H_5$ (VI)	121–125	80	58.16	6.17	7.14	58.32	6.78	7.18
—$NHCH(CH_2CH_2COOH)COOH$[b] (VII)	195–200	40–65						

[a] Corrected for exposed stem of thermometer. [b] Obtained only in a crude state.

In this Laboratory reductone reacted with diethyl N-(*p*-aminobenzoyl)-glutamate in a dilute hydrochloric acid solution to produce diethyl N-{*p*-(2,3-dihydroxy-2-ene-propylideneamino)-benzoyl}-glutamate (VI). In a similar way a number of other derivatives of *p*-aminobenzoic acid were treated with reductone to form com-

col was used as a solvent the reaction proceeded satisfactorily and the product was obtained by diluting with water and filtering. As was true in the first synthesis[4a] this material was very impure and contained 10 to 30% pteroylglutamic acid (5 to 21% molal yield) as determined with *S. faecalis* R. The best yield was obtained either by using a one to three mole excess of 2,4,5-triamino-6-hydroxypyrimidine or by using a 0.5 mole excess of the pyrimidine and one equivalent of a basic condensing agent such as sodium acetate or disodium phosphate.

Compounds (I), (II) and (VII) have each been

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(4) (a) Waller, *et al.*, THIS JOURNAL, **70**, 19 (1948); (b) Hultquist, *et al.*, *ibid.*, **70**, 23 (1948).
(5) Euler and Martius, *Ann.*, **505**, 73 (1933).
(6) Euler and Martius, *C. A.*, **28**, 3382 (1934).
(7) O'Meara, McNally and Nelson, *Nature*, **154**, 796. (1944).
(8) Traube, *Ber.*, **33**, 1371 (1900).

reacted with 2,4,5-triamino-6-hydroxypyrimidine in the manner described above. The crude product obtained from VII was active in stimulating the growth of both *S. faecalis* R and *L. casei*. The products obtained from I and II were active in stimulating the growth of *S. faecalis* R but were only very slightly active for *L. casei*. The yield of the expected pteroic acid from I and II compared favorably with the yield of pteroylglutamic acid from VI. The reaction appears to be generally applicable to intermediates of the type described.

Experimental[9]

Ethyl N-(*p*-Aminobenzoyl)-glycinate.—N-(*p*-Aminobenzoyl)-glycine[10] (4.0 g.) was suspended in a solution of 50 cc. of absolute ethanol and 3 cc. of concentrated hydrochloric acid and refluxed for two hours. The solution was evaporated to a sirup which crystallized. This was dissolved in 60 cc. of water, filtered and then neutralized with a saturated sodium carbonate solution. The material oiled out but soon crystallized. This was recrystallized once from a water–ethanol solution.

For purposes of analysis this was recrystallized twice from benzene; white crystals, m. p. 93–95°.

Anal. Calcd. for $C_{11}H_{14}O_3N_2$: C, 59.45; H, 6.35; N, 12.61. Found: C, 59.83; H, 6.71; N, 12.78.

Diethyl N-{*p*-(2,3-Dihydroxy-2-ene-propylideneamino)-benzoyl}-glutamate (VI).—Reductone (5.0 g.) (0.057 mole) prepared by the method of Euler and Martius[5] was dissolved in 100 cc. of water and added to a mixture of 16 g. (0.05 mole) of diethyl N-(*p*-aminobenzoyl)-glutamate[4a] and 7 cc. of concentrated hydrochloric acid in a solution of 1600 cc. of water and 100 cc. of ethanol. This was stirred rapidly for two hours and then stood in the refrigerator for several hours. It was then filtered, washed with ether, and dried; yield 16.7 g. (85%). This was sufficiently pure for subsequent reactions but may have contained water of crystallization since it had the rather low melting range of about 65–73°.

For analyses this was purified by dissolving it in chloroform at room temperature and then adding petroleum ether. The product oiled out but crystallized after standing. This purification was repeated once; cream colored crystals, m. p. 121–125°.

General Procedure for the Preparation of *p*-(2,3-Dihydroxy-2-ene-propylideneamino)-benzoic Acid (I) and Its Derivatives.—Compounds (I), (II), (III), (IV), (V) and (VII) were prepared by methods analogous to that described above for compound (VI) except that no ethanol was used in the solvent. With the exception of the acid (VII) these products were all crystalline and pale yellow to light orange in color. The acids (I) and (IV) and the amide (III) were rather insoluble in most solvents but they analyzed satisfactorily without purification. The esters (II) and (V) were readily recrystallized from ethanol or chloroform.

The Diphenylhydrazones of the Esters (II) and (VI).—A. Ethyl *p*-(2,3-dihydroxy-2-ene-propylideneamino)-benzoate (II) (0.2 g.) and 0.2 cc. of phenylhydrazine were dissolved in 10 cc. of ethanol containing 2 drops of acetic acid and refluxed for twenty minutes. Upon cooling the product separated; yield 0.2 g. This was recrystallized once from benzene and once from chloroform; long, yellow thread-like needles; m. p. 203–205°.

Anal. Calcd. for $C_{24}H_{25}O_2N_5$: C, 69.36; H, 6.07; N, 16.86. Found: C, 69.84; H, 5.42; N, 17.21.

B. Diethyl N-{*p*-(2,3-dihydroxy-2-ene-propylideneamino)-benzoyl}-glutamate (VI) (0.5 g.) and 0.9 cc. of phenylhydrazine were dissolved in 10 cc. of ethanol containing 3 drops of acetic acid and refluxed for twenty minutes. Upon cooling the product separated; yield 0.42 g. This was recrystallized once from benzene and once from ethanol; m. p. 157–159°.

Anal. Calcd. for $C_{31}H_{36}O_5N_6$: C, 65.01; H, 6.34; N, 14.68. Found: C, 64.79; H, 5.95; N, 15.06.

Pteroylglutamic Acid.—Seven grams (0.018 mole) of diethyl N-{*p*-(2,3-dihydroxy-2-ene-propylideneaminobenzoyl}-glutamate (VI), 5.0 g. (0.036 mole) of 2,4,5-triamino-6-hydroxypyrimidine[8] and 200 cc. of ethylene glycol were placed in a 250-cc. flask and heated to 135° for thirty minutes with continuous stirring. The solution was cooled almost to room temperature and poured into 600 cc. of water. A little concentrated hydrochloric acid was added to bring it to *p*H 3–4. The mixture was then filtered, washed with water, methanol and ether and dried; yield of crude 5.5 g. (63%). This was shown to be 20% pteroylglutamic acid by bioassay with *S. faecalis* R. Real yield was 12.6%.

This material was purified by the method of Waller, *et al.*,[4a] to obtain a crystalline product. The ultraviolet and infrared spectra, crystallography and biological activity of this compound, the previously prepared synthetic pteroylglutamic acid,[4] and the naturally occurring liver *L. casei* factor were found to be identical.

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Summary

1. *p*-(2,3-Dihydroxy-2-ene-propylideneamino)-benzoic acid and six of its derivatives have been prepared by treating reductone (2,3-dihydroxyacrylaldehyde) with *p*-aminobenzoic acid or a derivative.

2. Pteroylglutamic acid has been prepared by condensing diethyl N-{*p*-(2,3-dihydroxy-2-ene-propylideneamino)-benzoyl}-glutamate with 2,4,5-triamino-6-hydroxypyrimidine.

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(9) All melting points are corrected.

(10) Purchased from the National Aniline Division of Allied Chemical and Dye Corporation.