Anal. Calcd. for $C_{14}H_{20}O_3N_2$: C, 63.61; H, 7.63. Found: C, 63.46; H, 7.68.

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p-[(2-Amino-4-hydroxy-6-pteridylmethyl)-*p'*-nitrobenzenesulfonylamino]-benzoic Acid and Intermediates

BY BARNEY J. MAGERLEIN AND DAVID I. WEISBLAT

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Recently the synthesis of pteroylglutamic acid was described in which the amino group of the *p*-aminobenzoylglutamic portion was protected by a tosyl group until after the formation of the pteridine nucleus.[1] This note describes the synthesis of crystalline *p*-[(2-amino-4-hydroxy-6-pteridylmethyl)-*p'*-nitrobenzenesulfonylamino]-benzoic acid (VII) and its glutamic acid analog VIIa (Chart I). The *p*-nitrobenzenesulfonyl derivatives are in most cases more readily crystallized than are the tosyl derivatives. This was noted particularly in the preparation of IVa and VII, both of which readily crystallized, although their tosyl counterparts failed to do so. The pteroic acid derivative VII showed 3–8% of folic acid-like activity when assayed with the test organism *S. faecalis* R. Removal of the *p*-nitrobenzenesulfonyl group of VII with 30% hydrogen bromide in acetic acid in the presence of phenol[2] gave a good yield of pteroic acid.

Acknowledgment.—The authors are indebted to Mr. L. Stubberfield and Mr. E. Stapert for the microbiological assays; to Dr. G. Pish and Mr. L. Scholten for the ultraviolet absorption data; to Mr. W. A. Struck and associates for the microanalyses; and to G. Staffen for technical assistance.

Experimental

Ethyl N-*p'*-Nitrobenzenesulfonyl-*p*-aminobenzoate (I).—One hundred grams of *p*-nitrobenzenesulfonyl chloride was added to 71 g. of ethyl *p*-aminobenzoate dissolved in 202 ml. of 2,4-lutidine at such a rate that the temperature did not exceed 80°. The solution was heated at 80° for 45 minutes and poured with stirring onto 600 ml. of ice and water. The crude product was collected by filtration, washed, and dried. It weighed 155 g. Recrystallization from 500 ml. of Cellosolve gave 103 g. (68.2% yield), m.p. 187–189°. After several more recrystallizations from Cellosolve and from ethanol it melted at 194–195°.

Anal. Calcd. for $C_{15}H_{14}N_2O_6S$: C, 51.42; H, 4.03; N, 8.00; S, 9.15. Found: C, 51.60, 51.72; H, 4.27, 4.35; N, 8.21, 8.20; S, 9.28, 9.52.

N-*p'*-Nitrobenzenesulfonyl-*p*-aminobenzoic Acid (II).—A solution of 3.5 g. of ethyl N-*p'*-nitrobenzenesulfonyl-*p*-aminobenzoate (I) in 30 ml. of 95% ethanol and 10 ml. of 10% sodium hydroxide was heated under reflux for 0.5 hour. The alcohol was distilled under vacuum. The residue was diluted with 50 ml. of water, acidified and filtered to give 2.84 g. (92.5% yield) of II, m.p. 249–256° dec. Two re-

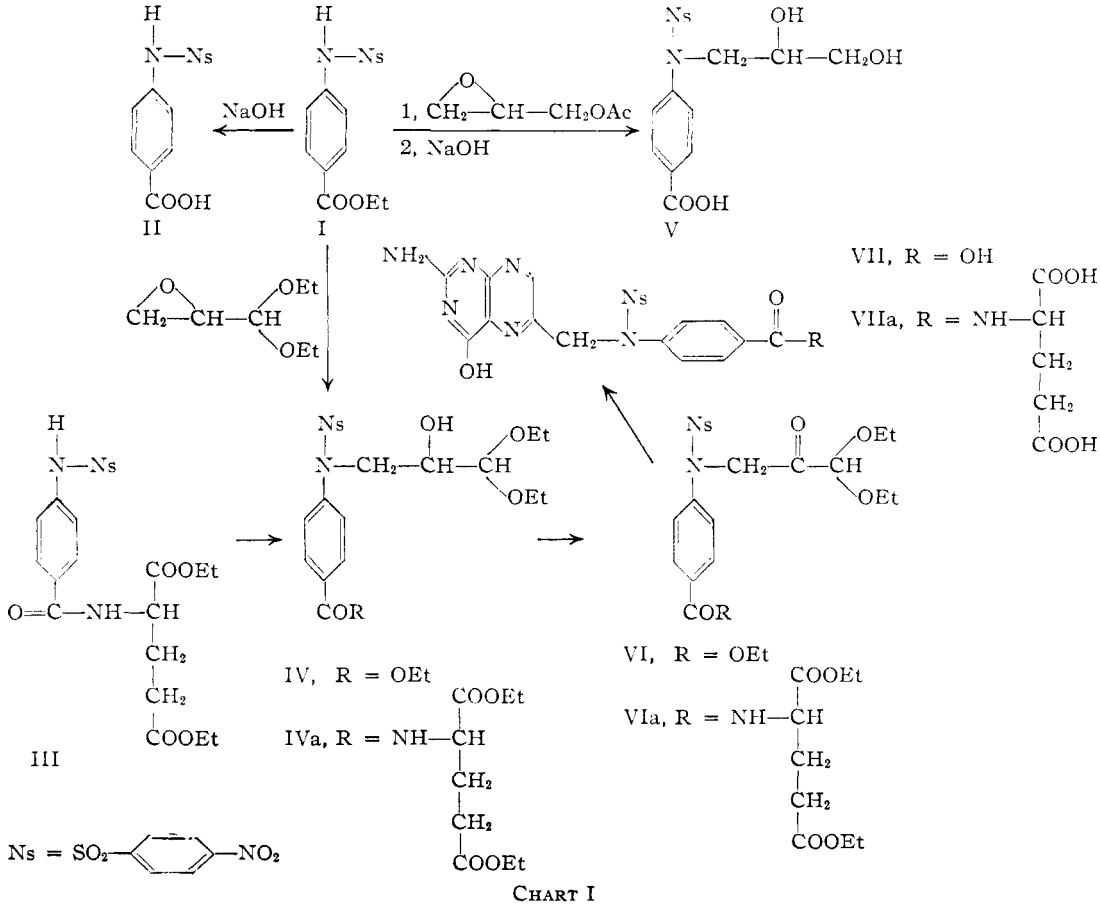

CHART I

(1) D. I. Weisblat, B. J. Magerlein, D. R. Myers, A. R. Hanze, E. I. Fairburn and S. T. Rolfson, THIS JOURNAL, **75**, 5893 (1953).

(2) D. I. Weisblat, B. J. Magerlein and D. R. Myers, *ibid.*, **75**, 3630 (1953).

crystallizations from acetic acid raised the melting point to 259–260° dec.

Anal. Calcd. for $C_{13}H_{10}N_2O_6S$: C, 48.44; H, 3.13; N, 8.69. Found: C, 48.27, 48.33; H, 3.19, 2.95; N, 8.50, 8.41.

Ethyl N-*p'*-Nitrobenzenesulfonyl-N-(3,3-diethoxy-2-hydroxypropyl)-*p*-aminobenzoate (IV).—A mixture of 12.0 g. of I, 5.8 g. of 2,3-oxidopropionaldehyde diethyl acetal and 3 drops of pyridine was heated at 135° for 2 hours. The dark brown melt was crystallized from 60 ml. of 2-propanol to give 15.9 g. (93.1% yield) of IV, m.p. 109–112°. Several recrystallizations from 2-propanol gave material melting 115–117°.

Anal. Calcd. for $C_{22}H_{28}N_2O_9S$: C, 53.22; H, 5.68; N, 5.64. Found: C, 53.33, 53.38; H, 5.60, 5.53; N, 5.73, 5.69.

Diethyl N-[N'-*p'*-Nitrobenzenesulfonyl-*p*-aminobenzoyl]-L-glutamate (III).—Diethyl *p*-aminobenzoyl-L-glutamate (3.22 g.) was dissolved in 6.3 ml. of pyridine and 2.22 g. of *p*-nitrobenzenesulfonyl chloride added. After the initial reaction the mixture was warmed on the steam-bath for one hour and then poured into 100 ml. of ice-water. The product collected by filtration, weighed 4.5 g. and melted at 154–161°. Several recrystallizations from dilute acetone raised the melting point to 165–167°.

Anal. Calcd. for $C_{22}H_{25}N_3O_9S$: C, 52.06; H, 4.96; N, 8.28. Found: C, 58.18; H, 4.97; N, 8.11.

Diethyl N-[N'-*p'*-Nitrobenzenesulfonyl-N'-(3,3-diethoxy-2-hydroxypropyl)-*p*-aminobenzoyl]-L-glutamate (IVa).—A mixture of 5.1 g. of III and 1.75 g. of 2,3-oxidopropionaldehyde diethyl acetal, and 3 drops of pyridine was fused at 140° for 2 hours. The dark melt was crystallized from 30 ml. of 2-propanol to give 5.3 g. (81.2% yield) of IVa which melted at 130–134°. Recrystallization from ethyl acetate gave white crystals, m.p. 136–138°.

Anal. Calcd. for $C_{29}H_{39}N_3O_{12}S$: C, 53.28; H, 6.01; N, 6.43. Found: C, 53.32, 53.68; H, 5.76, 5.84; N, 6.30, 6.21.

N-(*p'*-Nitrobenzenesulfonyl)-N-(2,3-dihydroxypropyl)-*p*-aminobenzoic Acid (V).—After fusion at 135° for 2 hours a mixture of 14.00 g. of I, 5.6 g. of glycidol acetate and 5 drops of pyridine was dissolved in 100 ml. of 80% ethanol and 40 ml. of 10% sodium hydroxide. This solution was heated under reflux for 1 hour, the alcohol distilled under vacuum, and the residue diluted with 100 ml. of H_2O. The cloudy solution was extracted with ethyl acetate and the extract discarded. Acidification of the aqueous fraction followed by extraction gave an acid fraction which when crystallized from 2-propanol weighed 6.8 g. (42.9% yield). It melted at 175–185°. After recrystallization from 2-propanol an analytical sample, m.p. 205–209°, was obtained.

Anal. Calcd. for $C_{16}H_{16}N_2O_8S$: C, 48.48; H, 4.07; N, 7.07. Found: C, 48.59, 48.74; H, 3.86, 4.10; N, 7.08, 7.21.

***p*-[(2-Amino-4-hydroxy-6-pteridylmethyl)-*p'*-nitrobenzenesulfonylamino]-benzoic Acid (VII).**—A heterogeneous mixture of 4.96 g. of hydroxyacetal IV, 5.26 g. of sodium dichromate, 35 ml. of chlorobenzene, 7 ml. of sulfuric acid and 23 ml. of water was stirred at 2–5° for 3 hours. The dark reaction mixture was diluted with ethyl acetate and washed with water and sodium bicarbonate solution. Evaporation of the solvent gave 4.9 g. of non-crystalline VI. This keto-acetal was not further purified but added to a mixture of 2.14 g. of 2,4,5-triamino-6-hydroxypyrimidine hydrochloride,[3] 1.7 g. of sodium acetate and 0.25 g. of potassium iodide in 50 ml. of acetic acid. This reaction mixture was stirred at 25° for one hour and heated under reflux for 2 hours. The solvent was distilled under vacuum. The residue was triturated twice with 20 ml. of 80% ethanol and then dried. It weighed 3.5 g.

One gram of crude pteridine was triturated with 30 ml. of concentrated hydrochloric acid. The acid solution was treated with 0.25 g. of Norite A and this diluted to 180 ml. with water. The precipitate was collected at the centrifuge and dried. It weighed 0.35 g. and showed the following ultraviolet maxima: $\lambda_{max}^{0.1\ N\ NaOH}$ 258 mμ, $E_{1\ cm}^{1\%}$ 541; 364 mμ, $E_{1\ cm}^{1\%}$ 135. A slurry of 0.15 g. of the partially purified VII and 0.80 g. of calcium hydroxide in 10 ml. of 0.1 *N* sodium hydroxide and 400 ml. of water was stirred at 25° for one hour, heated to 95° and filtered. The *p*H was adjusted to 3.0 and as the solution slowly cooled yellow needles of VII (0.07 g.) precipitated, $\lambda_{max}^{0.1\ N\ NaOH}$ 258 mμ, $E_{1\ cm}^{1\%}$ 700; 285 mμ, $E_{1\ cm}^{1\%}$ 311; 364 mμ, $E_{1\ cm}^{1\%}$ 180.

Anal. Calcd. for $C_{20}H_{15}N_7O_7S$: C, 48.29; H, 3.04; N, 19.71; S, 6.44. Found: C, 48.38, 48.34; H, 3.27, 3.43; N, 20.04; S, 6.57, 6.64.

N-[*p*-[(2-Amino-4-hydroxy-6-pteridylmethyl)-*p'*-nitrobenzenesulfonylamino]-benzoyl]-L-glutamic Acid (VIIa).—Diethyl N-[N'-*p*-nitrobenzenesulfonyl-N'-(3,3-diethoxy-2-hydroxypropyl)-*p*-aminobenzoyl]-L-glutamate was oxidized as described above in the preparation of VI to give crude crystalline VIa, m.p. 145–150° (75.5% yield). This product was not purified but condensed with 2,4,5-triamino-6-hydroxypyrimidine to give 28% of a crude pteridine fraction. This material was not further investigated.

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(3) W. Traube, *Ber.*, **33**, 1371 (1900).

Chloromycetin.[1] The Conversion of L-*threo*-*p*-Nitrophenylserinol to L-*erythro*-*p*-Nitrophenylserinol

BY GEORGE W. MOERSCH AND DAVID P. HYLANDER

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The recent publication by Logemann, *et al.*,[2] describing the production of L-*erythro*-*p*-nitrophenylserinol from the L-*threo*-form has prompted us to publish some of our own work in this direction. It is particularly interesting that while very similar methods were used, the reaction courses appear to be decidedly dissimilar.

Logemann, *et al.*, prepared L-*threo*-1-*p*-nitrophenyl-1-tosyloxy-2-dichloroacetamido-3-benzoyloxypropane by tosylation of the L-*threo*-diacylated *p*-nitrophenylserinol. Treatment of this tosyl derivative with potassium acetate in ethanol led to replacement by acetate with partial inversion or racemization at the C_1 center. By acid hydrolysis, an L-*erythro*-*p*-nitrophenylserinol, m.p. 112°, and an unknown, m.p. 154°, were found. The latter was suspected of being L-*threo*-*p*-nitrophenylserinol which melts 8 to 10° higher. The zero rotation reported is inapplicable to either substance.

Our own experience in converting *threo*-*p*-nitrophenylserinol to the *erythro*-form has been through mesylation of L-*threo*-1-*p*-nitrophenyl-2-benzamido-3-benzoyloxy-1-propanol.[3] On treatment of the 1-mesylate with potassium acetate in absolute ethanol potassium mesylate separates and from the solution is obtained a thick oil identifiable as the L-*erythro*-oxazoline by its infrared and by its subsequent reactions. Dilute acid readily opens the oxazoline and O → N shift in base gives an N,O-dibenzoyl derivative which can be hydrolyzed in base to L-*erythro*-N-benzoyl-*p*-nitrophenylserinol. Acid hydrolysis removes the amide to yield L(−)-*erythro*-*p*-nitrophenylserinol.

In order to obtain a crystalline *erythro*-oxazoline, the O-benzoyl was removed from L-*threo*-1-*p*-

(1) Chloromycetin is the registered trademark which Parke, Davis and Company has adopted for the antibiotic drug, chloramphenicol.

(2) W. Logemann, F. Lauria and E. Pella, *Gazz. chim. ital.*, **83**, 407 (1953).

(3) This substance has been reported by C. G. Alberti, *et al.*, *Chim. ind.*, **33**, 5 (1951). Our preparation will be reported in another connection.