Since the yield of 6-tetrahydroxypterine by the above procedure was small, our attention was centered on the more direct synthesis of III by the condensation of I with osones (II). The reaction of I with II is rapid and yields III in good quantity. The conditions for obtaining the preferred isomer appear to be reversed from that described above—*i. e.*, I and II at *p*H 5–9 yield the 6-isomer, while the condensation of I-bisulfite and II in strongly acidic solution yields a mixture richer in the 7-isomer.

Although details of work on pure isomers will be published later it is deemed worthy to report the synthesis of the isomeric mixture of III and the preparation of the isomeric mixture of formylpterine (IV) from III by the method outlined.

D-Glucosone was heated with an equivalent amount of 2,4,5-triamino-6-hydroxypyrimidine bisulfite in 75% acetic acid at 75° for forty-five minutes. The mixture was cooled and the precipitate collected. The product was exhaustively extracted with hot alcohol and dried. Yield of III was 60%, $[\alpha]^{26}_{D}$ −70.9° (169.2 mg. per 100 ml. of *N* NaOH). Absorption spectrum in 0.1 *N* NaOH showed maxima at 252 mμ and 360–362 mμ with ε of 19,000 and 7940, respectively.

Anal. Calcd. for $C_{10}H_{13}N_5O_5$: C, 42.39; H, 4.62; N, 24.71. Found: C, 42.17; H, 4.92; N (Kjeldahl), 25.11.

III was oxidized with lead tetraacetate to IV, an isomeric mixture, obtained in 85% yield. IV contained ash which was hard to remove. It exhibited strong carbonyl activity forming oximes, hydrazones and Schiff bases readily. IV treated with a slight excess of barium permanganate gives V, identity of which was established by its ultraviolet absorption, titration curve and analysis.

Anal. Calcd. for $C_7H_5N_5O_2H_2O$: C, 40.2; N, 33.5. Found: C, 38.8; N (Kjeldahl), 31.5 (cor. for 4.90% ash).

THE UPJOHN COMPANY H. G. PETERING
KALAMAZOO, MICHIGAN D. I. WEISBLAT

RECEIVED AUGUST 18, 1947

ANTAGONIST FOR PTEROYLGLUTAMIC ACID

Sir:

We wish to report the synthesis of a potent pteroylglutamic acid antagonist, N-[4-{[(2,4-diamino-6-pteridyl)-methyl]-amino}-benzoyl]-glutamic acid. In the course of an investigation of analogs of pteroylglutamic acid, this compound was prepared from 2,4,5,6-tetraminopyrimidine sulfate,[1] 2,3-dibromopropionaldehyde, and *p*-aminobenzoylglutamic acid under the conditions described for the synthesis of pteroylglutamic acid.[2] Purification of the crude product was accomplished by a method very similar to that used for pteroylglutamic acid.[3]

The purified product was obtained crystalline as clusters of yellow needles, and in 0.1 *N* sodium hydroxide solution it shows ultraviolet absorption maxima at 260, 284 and 370 mμ, and minima at 239, 271 and 333 mμ. *Anal.* Calcd. for $C_{19}H_{20}O_5N_8 \cdot 2H_2O$: C, 47.9; H, 5.1; N, 23.5. Found: C, 47.3; H, 5.18; N, 23.4. Magnesium salt: Calcd. for $C_{19}H_{18}O_5N_8Mg \cdot 3H_2O$: C, 44.2; H, 4.7; N, 21.7; Mg, 4.7. Found: C, 44.6; H, 4.85; N, 21.4; Mg, 4.82. The biological properties have been examined by Dr. B. L. Hutchings and Dr. E. L. R. Stokstad of the Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York. The inhibition ratio for half-maximum inhibition of the growth of *Streptococcus faecalis R* is 1.9, 0.7 and 0.4 at concentrations of pteroylglutamic acid of 0.003, 0.005 and 0.01 microgram per 10 ml., respectively.

Details of the synthesis and properties of this and related compounds will be the subject of subsequent communications.

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RECEIVED SEPTEMBER 19, 1947

BIOSYNTHESES INVOLVING PANTOTHENIC ACID

Sir:

In *Escherichia coli* cysteic acid appears to prevent competitively the decarboxylation of aspartic acid to β-alanine which results in pantothenic acid becoming a limiting growth factor.[1] Under our testing conditions the rate of pantothenic acid synthesis is determined by the ratio of cysteic to aspartic acid, and exogenous substances allowing growth to occur at a lower rate of pantothenic acid synthesis produce an increased antibacterial index.[2]

Such an effect is obtained with citric, *cis*-aconitic or α-ketoglutaric acids. The antibacterial index over a thirty-fold range in aspartic acid concentrations was 300 in the medium containing these substances but only 30 in their absence. Oxalacetic and pyruvic acid were inactive alone, but a mixture of both necessitated a slight increase in the concentration of cysteic acid to obtain the same growth inhibition. Acetate alone possessed some activity. Pantoic acid was inactive. The apparent "sparing action" of *cis*-aconitic acid on the pantothenic acid requirement of *E. coli* is not equaled by its precursors; hence, it appears that pantothenic acid deficient cells are unable to convert effectively pyruvate and oxalacetate to *cis*-aconitate (or ketoglutarate). This datum explains the previously reported[1] enhanced activity of glutamic over aspartic acid in preventing the toxicity of cysteic acid. The transamination reaction produces both aspartic and α-ketoglutaric acids, the latter having a

(1) Traube, *Ber.*, **37**, 4545 (1904).
(2) Angier, *et al.*, *Science*, **103**, 667 (1946).
(3) Waller, *et al.*, THIS JOURNAL, **69**, in press (1947).

(1) Ravel and Shive, *J. Biol. Chem.*, **166**, 407 (1946).
(2) Molar ratio (analog to metabolite) just necessary for maximum inhibition of growth.