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]

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

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

"sparing action" on the product of the blocked reaction. Previous evidence[3] involving pantothenate in the oxidation of pyruvate can also be explained on the basis of pantothenate mediating *cis*-aconitate synthesis.

That the above effect directly involves pantothenic acid was demonstrated by the reversing effect of both pantothenic and α-ketoglutaric acids on a pantothenic acid antagonist, N-α,γ-dihydroxy - β,β - dimethylvaleryl - β - aminobutyric acid, for *E. coli*. Further, the pantothenic acid requirement of *Proteus morganii* in a medium of inorganic salts, glucose, nicotinamide and cystine was appreciably decreased by α-ketoglutaric acid.

With *Lactobacillus arabinosus*, an oleic acid source ("Tween 80") or sodium glycocholate increased the antibacterial index from approximately 3,000 to 30,000 for the competitive inhibition of N-pantoyl-*n*-butylamine[4] of pantothenic acid functioning. Both substances added simultaneously did not enhance the effect. Since this organism presumably requires acetate for synthesis of sterols and fatty acids, this inhibitor appears to prevent the conversion of acetate to an intermediate common to both sterol and oleic acid synthesis.

The reported involvement of pantothenic acid in the conversion of glycine to threonine,[5] the demonstration by Lipmann, *et al.*,[6] of the presence of pantothenic acid in the coenzyme for acetylation of sulfanilamide and choline, and the results of the above *inhibition analyses* tend to indicate that many of the enzymatic reactions in which pantothenic acid functions involve the hypothetical "active" acetyl radical.

(3) Dorkman, *et al.*, *J. Biol. Chem.*, **144**, 393 (1942).
(4) Snell and Shive, *ibid.*, **160**, 287 (1945).
(5) Rossi and Cennamo, *Chem. Abstr.*, **40**, 6543 (1946).
(6) Lipmann, *et al.*, *J. Biol. Chem.*, **167**, 869 (1947).

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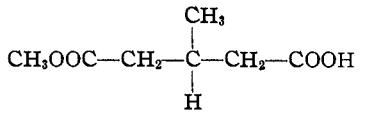
RECEIVED SEPTEMBER 19, 1947

REARRANGEMENT IN THE PREPARATION OF ESTER ACID CHLORIDES

Sir:

Cason[1] has recently drawn attention to the fact that during the preparation of the ester acid chlorides of the isomeric half esters of dibasic acids such as α-ethyl-α-butylglutaric acid by means of thionyl chloride rearrangement may occur, the derivatives obtained from the ester acid chlorides being mixtures derived from both isomers.

The writer has studied the conditions under which this type of rearrangement occurs when preparing the ester acid chlorides of the two enantiomorphs[2] of methyl hydrogen β-methylglutarate (I)



(I)

Rearrangement leads in this case to racemization and can be detected simply by pouring the ester acid chloride into water and measuring the rotation of the recovered half ester. In this way it has been found that no rearrangement takes place when the ester acid chloride is prepared by the action of oxalyl chloride in benzene solution. In case of thionyl chloride the occurrence and extent of rearrangement depend on the purity of the reagent and on the reaction temperature. If pure thionyl chloride (Kahlbaum "reinst, wasserhell") is used no rearrangement occurs if the reaction takes place at 30° and excess reagent is removed under reduced pressure on a water-bath kept at 50°. Use of less pure thionyl chloride (Kahlbaum, "purum") leads under the conditions just described to rearrangement, the extent of which increases if the reaction temperature is raised. (The ester acid chlorides have not been distilled.)

It is well known that anhydrides may be formed during the action of thionyl chlorides on acids and that the yield of acid chloride is lower if impure thionyl chloride is used or if the reaction temperature is too high.[3] With dibasic acids of the succinic and glutaric acid series thionyl chloride gives the cyclic anhydrides only. It appears likely that the rearrangement observed in the preparation of the ester acid chlorides occurs via the anhydrides.

That rearrangement may be avoided during the preparation of the ester acid chloride of (I) is evident from the fact that lengthening of the chain of the dextrorotatory enantiomorph of (I) by the Arndt–Eistert synthesis has given (+)-β-methyladipic acid identical with that derived from natural products.[2] Furthermore, the two enantiomorphs of (I) have been used as starting material for the synthesis of $d(+)$- and $l(-)$-3-methyltetracosanoic acids. The intermediate ester acid chloride was in case of one enantiomorph prepared by means of oxalyl chloride and in case of the other by means of pure thionyl chloride. The enantiomorphic long chain β-methyl substituted acids both melted sharply at 65.5° (cor.), and showed numerically equal optical rotations, $[M]^{25}D$ 13.2° (chloroform, *c*, 5.78). Mixed in equal proportions the acids gave a racemic compound melting sharply at 68.6° (cor.).

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RECEIVED SEPTEMBER 11, 1947

(1) J. Cason, THIS JOURNAL, **69**, 1548 (1947).
(2) S. Ställberg-Stenhagen, *Arkiv Kemi, Min., Geol.*, **25A**, No. 10 (1947).
(3) "Organic Syntheses," Coll. Vol. I, 2nd ed., p. 147.