dures have given a series of peptides. All the data obtained can be rationalized by a unique sequence which forms a ring containing ten amino acids.

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Preparation of *p*-[(2-Amino-6-hydroxy-8-purinemethyl)-amino]-benzoic Acid[1]

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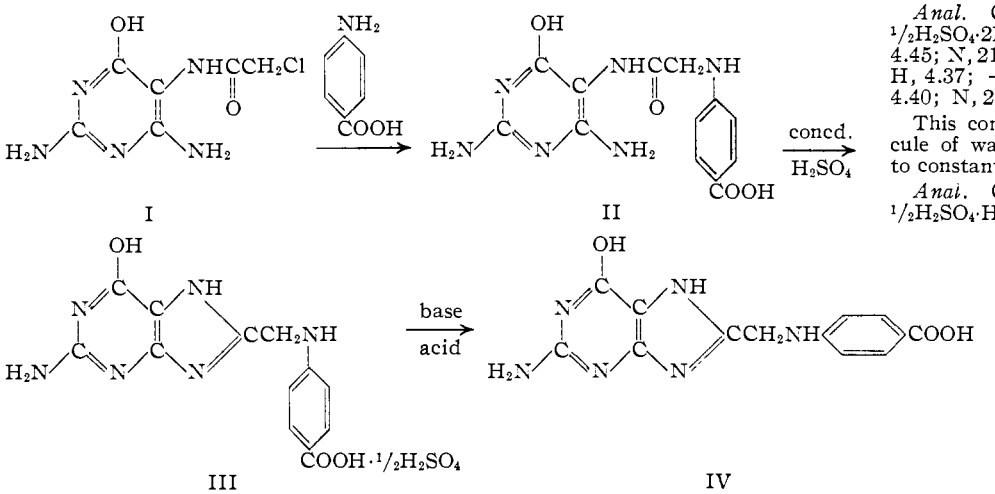
Pteroic acid, *p*-[(2-amino-4-hydroxy-6-pteridylmethyl)-amino]-benzoic acid, is a part of folic acid and is itself active for *S. faecalis R*[2]. Since simple modifications of the purines that occur in nucleic acids produce compounds with striking physiological properties, such as, for example, 6-mercaptopurine and azaguanine, it seemed to us to be interesting to prepare an analog of pteroic acid in which the pyrazine moiety is replaced by an imidazole ring: *p*-[(2-amino-6-hydroxy-8-purinemethyl)-amino]-benzoic acid (IV).

IV was therefore prepared according to the following scheme:



Experimental[3]

p-[(2,4-Diamino-6-hydroxy-5-pyrimidyl)-carbamylmethyl-amino]-benzoic Acid (II).—A solution of 4.50 g. (0.019 mole) of 2,4-diamino-6-hydroxy-5-chloroacetamidopyrimidine[4] (I) and 5.75 g. (0.042 mole) of *p*-aminobenzoic acid in 240 ml. of boiling water was refluxed over a small flame for 6 hours. During this time, a brown precipitate slowly formed. This was separated by filtration, washed with some water and dissolved in 1 *N* sodium hydroxide. The solution was treated with charcoal and then filtered. The product was reprecipitated by adding to the filtrate 1 *N* hydrochloric acid to a *p*H of about 7. The precipitate was collected by filtration and washed with water. Weight of this crude product was 3.2 g. (51.6%). After a second solution by alkali and precipitation by acid, the precipitate was ready for analysis after drying at 90–95° for 19 hours. The compound began to discolor at about 260° and did not melt up to 360°. The analysis showed that it was a hemihydrate, which was confirmed by subsequent reactions and analysis. By heating the hemi-hydrate at 180° for 15 hours to constant weight, the loss was 2.34%; calculated, 2.75%.

Anal. Calcd. for $C_{13}H_{14}N_6O_4 \cdot {}^1/_2H_2O$: C, 47.70; H, 4.62; H_2O, 2.75. Found: C, 47.07; H, 4.44; H_2O, 2.34.

This compound formed an unstable hydrochloride of undetermined structure when it was boiled with 4 or 6 *N* hydrochloric acid and then cooled in refrigerator overnight. Shining white crystals were obtained which, on washing with water, immediately hydrolyzed to the original compound II. It also formed an unstable sulfate of undetermined structure when it was dissolved in concentrated sulfuric acid at room temperature, diluted to about 6 *N* strength in cold water and allowed to stand overnight. White crystals were obtained, which on washing with water slowly hydrolyzed to II.

p-[(2-Amino-6-hydroxy-8-purinemethyl)-amino]-benzoic Acid (IV).—Compound II (3.27 g., 0.01 mole) was dissolved in 33 ml. of concentrated sulfuric acid and heated on a steam-bath for 2 hours. The acid solution was then filtered through asbestos fiber and the filtrate run into 750 ml. of water, which was cooled in an ice-bath. After standing in the refrigerator overnight, orange colored needles were obtained which did not hydrolyze to II on washing with water. A yield of 1.53 g. (39.7%) was obtained. After two more recrystallizations from concentrated sulfuric acid and water, it began to decompose at about 225° but did not melt by 290°. Analysis showed that this compound was a sulfate containing two molecules of water of crystallization (III).

Anal. Calcd. for $C_{13}H_{12}N_6O_3 \cdot {}^1/_2H_2SO_4 \cdot 2H_2O$: C, 40.51; H, 4.45; N, 21.81. Found: C, 40.52; H, 4.37; ———. C, 40.61; H, 4.40; N, 21.30.

This compound lost one molecule of water by drying at 110° to constant weight.

Anal. Calcd. for $C_{13}H_{12}N_6O_3 \cdot {}^1/_2H_2SO_4 \cdot H_2O$: C, 42.50; H, 4.12. Found: C, 42.07; H, 4.16.

When III was boiled with dimethylformamide and the solution run into water, the ring opened and a compound identical with II was obtained. This also was confirmed by analysis.

Anal. Calcd. for $C_{13}H_{14}N_6O_4 \cdot {}^1/_2H_2O$: C, 47.70; H, 4.62. Found: C, 47.89; H, 4.71.

When III was treated under milder conditions—dissolved in 0.5 *N* cold sodium hydroxide and precipitated by 0.5 *N* cold hydrochloric acid—a different compound, *p*-[(2-amino-6-hydroxy-8-purinemethyl)-amino]-benzoic acid (IV), was obtained. It decomposed at 315–317°. Although it had the same total composition as II, analyses proved the presence of one and a half molecules of water of crystallization, indicating that ring formation had occurred to give a purine derivative.

Anal. Calcd. for $C_{13}H_{12}N_6O_3 \cdot 1{}^1/_2H_2O$: C, 47.70; H, 4.62; N, 25.68; H_2O, 8.26. Found: C, 47.20; H, 4.61; N, 25.38; H_2O, 8.54.

The loss of water was determined by heating the compound at 180° to constant weight.

Other methods of ring closure to prepare IV directly from II were tried but without success. II was heated[5] at various temperatures under a vacuum of 15 mm. pressure. At 260–265°, decomposition occurred with the formation of a little brownish, greasy sublimate.

When II was boiled with 20% hydrochloric acid,[6] hydroly-

(1) Taken from a part of the thesis submitted by Chao-Shing Cheng to the Temple University Graduate Council in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(2) R. B. Angier, *et al.*, *Science*, **102**, 227 (1945); **103**, 667 (1946).

(3) All melting points are uncorrected. Analyses were performed by Micro-Tech. Laboratories, Skokie, Ill., and Huffman Microanalytical Laboratories, Wheatridge, Colo.

(4) G. H. Hitchings and G. B. Elion, THIS JOURNAL, **71**, 467 (1949).

(5) A. Bendich, J. F. Tinker and G. B. Brown, THIS JOURNAL, **70**, 3111 (1948).

(6) E. Fischer, *Ber.*, **30**, 560 (1897).

sis of the compound at the amide linkage to 2,4,5-triamino-6-hydroxypyrimidine occurred.

Also, heating II with oxalic acid[7] failed to bring about the desired ring closure.

When II was boiled with formamide,[8] analysis for carbon and hydrogen of the material isolated showed that it was not the desired product but might be guanine; however, a subsequent analysis for nitrogen proved that it was not the latter compound.

Anal. Calcd. for $C_5H_5N_5O$: C, 39.73; H, 3.33; N, 46.36. Found: C, 39.26; H, 3.02; N, 40.32, 40.28.

(7) E. Fischer and L. Ach, *ibid.*, **28**, 247 (1895).

(8) R. K. Robins, K. J. Dille, C. H. Willits and B. E. Christensen, THIS JOURNAL, **75**, 263 (1953).

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The Synthesis of β,β′-Dithiolisobutyric Acid

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Several years ago[1] a sulfur-containing polymeric material was obtained from asparagus juice which gave β,β′-dithiolisobutyric acid on reduction by sodium in liquid ammonia. Recent interest in dithiol acids following the characterization of α-lipoic acid[2] (6-thioctic acid) have made it desirable to have a readily available source of this lower analog.

β,β′-Diiodoisobutyric acid, the starting material for the syntheses attempted was found to be more accessible from the hydroiodination of α-iodomethylacrylic acid[3] than by reduction and displacement of α-hydroxy-β,β′-dichloroisobutyric acid previously used.[4]

Reaction of β,β′-diiodoisobutyric acid with thiourea gave the diisothiouronium salt in excellent yield. However, all efforts to hydrolyze this derivative to the desired β,β′-dithiolisobutyric acid failed. The reaction of sodium β,β′-diiodoisobutyrate with sodium benzyl mercaptide gave a good yield of α-benzylmercaptomethylacrylic acid and apparently no β,β′-dibenzylmercaptoisobutyric acid. A successful synthesis of the β,β′-dibenzylmercapto acid was achieved by a method paralleling that recently published by Reed and Niu[5] for α-lipoic acid. β,β′-Diiodoisobutyric acid was converted to its methyl ester with diazomethane. This in turn gave good yields of methyl β,β′-dibenzylmercaptoisobutyrate on reaction in ethyl acetate solution with sodium benzylmercaptide. The saponification of the methyl ester could be effected by shaking with alcoholic potassium hydroxide at room temperature. Attempts to speed the hydrolysis by warming resulted in β-elimination and the formation of α-benzylmercaptomethylacrylic acid. The free β,β′-dibenzylmercaptoisobutyric acid did not crystallize and was characterized as its disulfone. Reduction of the dibenzylmercapto acid by sodium in liquid ammonia was always attended by the formation of a polymeric gum in addition to the expected β,β′-dithiolisobutyric acid.

(1) E. F. Jansen, *J. Biol. Chem.*, **176**, 657 (1948).

(2) See O. A. Bessey, H. J. Lowe and L. L. Salomon, *Ann. Rev. Biochem.*, **22**, 545 (1953).

(3) K. N. Welch, *J. Chem. Soc.*, 257 (1930).

(4) J. W. E. Glattfeld and J. M. Schneider, THIS JOURNAL, **60**, 415 (1938).

(5) L. J. Reed and C. Niu, *ibid.*, **77**, 416 (1955).

Experimental

β,β′-Diiodoisobutyric Acid.—Ethyl bis-(hydroxymethyl)-malonate[3,6] was refluxed with hydriodic acid to give α-iodomethylacrylic acid.[3] An intimately ground mixture of 87.5 g. (0.46 mole) of α-iodomethylacrylic acid and 109 g. of potassium iodide was made into a paste with 196 g. of 95% phosphoric acid and heated for six hours on a steam-bath.[7] The reaction mixture was poured into ice-water, triturated to break up lumps and the product was collected on a filter. After drying over phosphorus pentoxide it was recrystallized from ethyl acetate–petroleum ether; m.p. 128–129°, yield 81 g. (52%). A mixture melting point with an authentic specimen prepared from dichloroacetone[4] showed no lowering.

Methyl β,β′-Diiodoisobutyrate.—An excess of ethereal diazomethane was added to 81 g. of the solid acid described above. The solution was dried over anhydrous magnesium sulfate, filtered and the ether removed *in vacuo*. This material was used for analysis; yield 77 g. (92%), m.p. 20.5–21°.

Anal. Calcd. for $C_5H_8I_2O_2$: C, 16.97; H, 2.28. Found: C, 17.4; H, 2.41.

Methyl β,β′-Dibenzylmercaptoisobutyrate.—A solution of 9.67 g. of sodium in 100 ml. of methanol was treated with 52.1 g. (0.42 mole) of benzyl mercaptan and evaporated to dryness *in vacuo*. A solution of 77 g. (0.21 mole) of methyl β,β′-diiodoisobutyrate in 100 ml. of ethyl acetate was then added and the mixture was refluxed for 1 hour and then poured into ice-water. The organic layer was separated, washed with cold 0.1 *N* sodium hydroxide solution, with water and then dried over magnesium sulfate. The solvent was removed *in vacuo* and the residue was recrystallized from methanol; yield 55.1 g. (73%), m.p. 44°.

Anal. Calcd. for $C_{19}H_{22}O_2S_2$: C, 65.86; H, 6.40. Found: C, 65.6; H, 6.49.

Hydrolysis of Methyl β,β′-Dibenzylmercaptoisobutyrate.—A mixture of 34.6 g. (0.1 mole) of methyl β,β′-dibenzylmercaptoisobutyrate in 120 ml. of 0.83 *N* potassium hydroxide in 90% methanol was shaken for 5 hours at room temperature. At that time another 20 ml. of the 0.83 *N* base was added and shaking continued for another hour. After being stored in the refrigerator overnight, the reaction mixture was acidified with concd. hydrochloric acid, filtered and concentrated *in vacuo*. The residue was dissolved in ethyl acetate, washed with water and again the solvent was removed *in vacuo*. The free β,β′-dibenzylmercaptoisobutyric acid remained as a non-crystalline gum. It was characterized by oxidation of its acetic acid solution with 30% hydrogen peroxide to *β,β′-dibenzylsulfonoisobutyric acid* which was recrystallized from dilute acetic acid; m.p. 181–182°.

Anal. Calcd. for $C_{18}H_{20}O_6S_2$: C, 54.5; H, 5.09. Found: C, 54.8; H, 5.33.

If the hydrolysis were carried out by warming, considerable quantities of the elimination product, **α-benzylmercaptomethylacrylic acid**, would separate in crystalline form. After recrystallization from nitromethane it melted at 93° dec.

Anal. Calcd. for $C_{11}H_{12}O_2S$: C, 63.43; H, 5.81. Found: C, 63.0; H, 5.90.

β,β′-Dithiolisobutyric Acid.—A solution of 11.5 g. (0.034 mole) of non-crystalline gummy β,β′-dibenzylmercaptoisobutyric acid in 20 ml. of toluene was added to 200 ml. of liquid ammonia. Small pieces of sodium were then added until a permanent blue color resulted. The excess sodium was destroyed with a little ammonium chloride, and the ammonia was allowed to evaporate in a nitrogen stream. The residue was acidified with concd. hydrochloric acid and extracted with ethyl acetate. The ethyl acetate solution was washed with water, dried and concentrated. The resulting gum was extracted with two 1-l. portions of warm petroleum ether (b.p. 90–100°) and these extracts were chilled to −30° for several days. The petroleum ether was decanted from the impure crystals and used to reëxtract the reduction product. There remained, insoluble in petroleum

(6) H. Gault and A. Roesch, *Bull. soc. chim.*, [5] **4**, 1411 (1937).

(7) H. Stone and H. Schlecter, *Org. Syntheses*, **30**, 33 (1950).