

limits. Figures as high as 319 and 330 mg. % were found in 2 females which were spent. The blood sugar also showed no distinctive relationship.

The function of the high blood calcium in fish cannot be the formation of exceptionally strong bone. On the other hand, the eggs were found especially rich in calcium, which suggests that the calcium may be required for their elaboration. In connection with this study, attention should be called to the observation of Riddle¹ and others to the effect that the blood calcium increases markedly in birds at the period of ovulation. This has been attributed to the requirement incidental to the formation of the shell.

¹ Riddle, O., and Reinhart, W. H., *Am. J. Physiol.*, 1926, lxxvi, 660.

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Synthesis of Racemic 2, 5-Dihydroxy Phenyl Alanine (Gentic Alanine)

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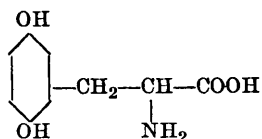
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In 1922, in cooperation with Dr. Casimir Funk, we attempted the synthesis of the gentisic alanine by the Sasaki¹ and Hirai^{2, 3} methods, namely condensation of dimethoxy gentisic aldehyde with glycine anhydride in presence of anhydrous sodium acetate and acetic anhydride, and subsequent splitting of the diketo piperazine compound by means of prolonged heating with strong hydriodic acid and red phosphorus.

We failed to obtain the desired compound at that time and, due to pressure of other work, temporarily dropped the problem. Early in 1927, we again resumed this work and succeeded in preparing a crystalline substance which gave all the reactions of the desired compound. A Kjeldahl nitrogen determination gave the correct analysis for nitrogen, but due to the small amount of material available, we were unable to do a complete combustion at that time and were therefore loathe to publish our results.

As K. Hirai⁴ has lately reported the synthesis of this amino acid, and as our compound appears to be identical with Hirai's in all respects, we feel justified in reporting our work.

Splitting of the 2,5-dimethoxy benzal glycine anhydride to racemic 2, 5-dihydroxy phenyl alanine.



1.1 gm. of 2,5-dimethoxy benzal glycine anhydride were mixed with 10 cc. hydriodic acid (s.g. 1.7) and 0.8 gm. red phosphorus and the mixture refluxed in an oil bath at 110° C. for 7 hours. The mixture, on cooling, was diluted with water, filtered and the filtrate acidified with glacial acetic acid. 20% lead acetate solution was added to complete precipitation. The precipitates of lead iodide and lead phosphate were filtered and to the clear colorless filtrate, ammonia was added until a precipitate was no longer formed. This casein-like precipitate which is the lead salt of the amino acid was filtered and washed well with water.

The lead salt was suspended in water and decomposed with hydrogen sulfide gas. The precipitate of lead sulfide was filtered, the excess hydrogen sulfide removed by a stream of CO₂ gas and the clear filtrate was evaporated to dryness in vacuum under CO₂ gas.

The crystalline residue was taken up in a small amount of water containing a trace of sulfur dioxide gas and the solution allowed to stand in a vacuum dessicator over sulfuric acid. Beautiful ten-sided prisms, probably rhombic, formed. These crystals weighed 0.23 gm. On recrystallization from water containing a trace of sulfur dioxide, 0.12 gm. of the decahedrons was obtained.

The compound melted at 204 to 205° C. (uncorrected), with decomposition. It was readily soluble in warm water, slightly soluble in cold water and insoluble in most organic solvents. Its aqueous solution gradually turned to a reddish brown on addition of ammonia. With a drop of ferric chloride solution, it gave a greyish-green color which gradually turned dark and finally gave a black precipitate on standing.

It reduced an ammoniacal silver nitrate solution very quickly in the cold. It also gave a positive ninhydrin reaction for presence of a free α-amino group.

Nitrogen analysis — Found —7.26%.

Calculated for C₉ H₁₁ O₄ —7.10%.

Although this amino acid has not been found in nature, it is of special significance from a biochemical standpoint, since its structure leads to the hypothesis that it may be a possible precursor of hemogentisic acid in alkaptonurics.

¹ Sasaki, T., *Ber. d. deutsch. chem. Gesellsch.*, 1921, liv, 163.

² Hirai, K., *Biochem. Zeit.*, 1921, cxiv, 67.

³ Hirai, K., *Biochem. Zeit.*, 1926, clxxvii, 449.

⁴ Hirai, K., *Biochem. Zeit.*, 1927, clxxxix, 88.