tions of *p*H, sodium ion concentration and temperature. However, reproducible results require the very careful control of all conditions and the re-equilibration of the column if any condition is altered. Thus, when Developer No. 5 is used and the column is warmed, re-equilibration for 4 days at 6° with 1000 ml. of developer is required.

This investigation has added three minor heme-containing components to the three that were detected by Allen, *et al.*,[7] in normal adult hemoglobin. Although Allen, *et al.*, failed to observe a component that moved more slowly than the main zone, such a component is apparent under the conditions employed here. Presumably, it is the same as the more slowly moving component observed by Morrison and Cook,[5] Huisman, *et al.*,[8] and Gutter, *et al.*[9] It may well be that undetected minor components are hidden in the main component or that the zones of some minor components may be heterogeneous. Zones A_{Ia} and A_{Ib} move through the column so rapidly even with Developer No. 4 that heterogeneity could well be hidden. The ratio of the heme to protein as derived from the ratio of the optical densities at 415 to 280 mμ is constant in all zones except zone A_{Ia+b}. A non-heme protein has been found to be eluted immediately ahead of A_{Ia}, and there are indications that either another non-heme protein is present in the region of A_{Ib} or that there are fewer hemes in this hemoglobin component. The point has not been pursued further.

The biosynthesis of hemoglobin does not seem to be influenced by the mass action effect of an excessively large amount of one of the constituents that goes to make it up. In the phenylketonuric individual, the presence of 30 to 40 times the normal amount of phenylalanine in the blood does not alter the nature of the hemoglobin components.

Although the present procedures are time consuming, they offer a sensitive method for the study of the hemoglobin components in hematological disorders.

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Isolation of a New Acidic Aromatic Amino Acid (*m*-Carboxy-α-phenylglycine) from Iris Bulb

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A new acidic aromatic amino acid, *m*-carboxy-α-phenylglycine was isolated from iris bulbs (*Iris tingitana var. Wedgewood*) by the use of ion-exchange resins and column chromatography. Its structure was determined from oxidation studies, elemental analysis, melting point and infrared absorption spectra. Synthetic *m*-carboxy-α-phenylglycine was prepared and found to be identical with the isolated material. *m*-Phthalic acid and *m*-phthalaldehydic acid were identified after oxidation of the amino acid, respectively, with alkaline permanganate and chloramine-T. The amount of this amino acid in the iris bulb was determined by quantitative paper chromatography with isolated material as the standard.

Introduction

Plant materials contain many amino acids which do not occur in proteins. In recent years, many new amino acids have been isolated from plants and characterized.[2] Storage organs have been particularly rich sources.

In an investigation of the non-protein nitrogen fraction of the iris bulb (*Iris tingitana var Wedgewood*), β-aminoisobutyric acid was isolated and identified.[3] In the isolation procedure, the acidic amino acids were removed by absorption on the salt form of an anion-exchange resin. Paper chromatography of this acidic fraction revealed several unknown compounds. One of them occupied the spot marked unknown No. 18 in Fig. 1 of our previous paper. This compound has been isolated and identified as *m*-carboxy-α-phenylglycine, an amino acid hitherto undescribed in the literature.

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(2) J. F. Thompson, S. I. Honda, G. E. Hunt, R. M. Krupka, C. J. Morris, L. E. Powell, O. O. Silberstein, Jr., G. H. N. Towers, R. M. Zacharius, *Botan. Rev.*, **25**, 1 (1959).

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Discussion

The acidic fraction from our previous isolation[2] was chromatographed on the acetate form of Dowex 1 and eluted with acetic acid. This process purified an unknown amino acid which was uncontaminated by any other ninhydrin active substance. After several recrystallizations from hot water, 400 mg. of a pure white crystalline material was obtained. Elemental analysis established an empirical formula of $C_9H_9O_4N$. The compound remained unchanged when heated with 6 *N* HCl for 24 hr. at 120°. Reaction with ninhydrin on paper produced a yellow color which gradually changed to the normal blue violet complex. When the ninhydrin reaction was performed in the test-tube under quantitative conditions, the color yield was 90% of theory.[4]

Migration of the isolated substance toward the anode in paper electrophoresis at *p*H 7 further confirmed the acidic nature of this compound. The rate of migration indicated no strongly acidic groups. With four oxygen atoms in the empirical formula, a dicarboxylic acid was likely.

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Elemental analysis also indicated an unsaturated compound, probably containing a benzene ring. Light absorption by an aqueous solution of the compound showed a maximum at 275 mμ, which also pointed to a benzene derivative. Tests with ferric chloride and with permanganate for phenolic groups or aliphatic double bonds were negative. Hydrogenation of the substance at room temperature with palladium-on-charcoal produced no change as judged by its R_F values in two solvent systems[5] as well as its color with ninhydrin. Reaction of the substance with the pyridoxal reagent[6] signified that it was an α-amino acid. The accumulated evidence indicated that the substance was a carboxy-α-phenylglycine. Oxidation of such a compound with alkaline permanganate should give one of the three possible phthalic acids, and reaction with chloramine T[7] should produce one of the three phthalaldehydic acids.

The product resulting from the oxidation with permanganate had an infrared spectrum which was identical with that of synthetic *m*-phthalic acid. Both the synthetic and the isolated material disappeared on heating above 310°. The melting points of the *p*-nitrobenzyl esters of the synthetic *m*-phthalic acid and the oxidized unknown were 207 and 209°, respectively. A mixture of the two compounds had a melting point of 208°.

Treatment of the unknown with chloramine-T resulted in a compound which gave a typical Fuchsin aldehyde test and which melted at 175°. Synthetic *m*-phthalaldehydic acid was prepared and also melted at 175°. The infrared spectra of the isolated and the synthetic aldehyde were identical. The phenylhydrazones of the synthetic material and the oxidized unknown had observed melting points of 183 and 184°, respectively.

m-Carboxy-α-phenylglycine was synthesized from *m*-phthalaldehydic acid by the Strecker synthesis.[8] The infrared spectra of the synthetic amino acid and a racemized sample of the isolated substance were identical. The synthetic material and the isolated amino acid were co-chromatographed in several solvent systems and were not separable.

Experimental

Isolation.—The procedure used in the isolation of β-aminoisobutyric acid consisted in desalting the protein-free 80% alcoholic extract from 45 kg. of homogenized iris bulbs on Dowex-50.[2] The desalted material was then put through a 7.5 × 50 cm. column of Dowex-1, X-4 (200 to 400 mesh) resin in the chloride form. After washing the column thoroughly with distilled water, the acidic amino acids were eluted with 1 *N* HCl. This acidic fraction was used as the starting material for the present isolation work. The 1 *N* HCl eluate was taken to dryness *in vacuo* at 35°, and the residue dissolved in a minimum of 0.5 *N* acetic acid. One-half of this solution was applied to a column (4.8 × 40 cm.) of Dowex-1, X4 (200 to 400 mesh) in the acetate form, and the acids were eluted with 0.5 *N* acetic acid at a rate of 2.6 ml. per min. After discarding the first 200 ml. of effluent, which contained no ninhydrin activity, collection of 13-ml. fractions was started. Paper chromatography revealed the unknown in fractions 153–180 uncontaminated by other ninhydrin reactive substances.

The second half of the acidic solution was fractionated in the same fashion and the fractions containing the unknown from the two batches were combined. After evaporation to dryness, 750 mg. of crude material was obtained and recrystallized four times from hot water to give a white crystalline product weighing 400 mg.

After being dried at 100° *in vacuo*, the crystalline material gave an elemental analysis of C, 54.4%, H, 4.90%, N, 7.00%, O, 33.7%, whereas the theoretical values for $C_9H_9O_4N$ are C, 55.4%, H, 4.63%, N, 7.18% and O, 32.8%. The compound decomposed slowly on heating at about 215°. The R_F value in phenol:water (8:3) was 0.40, and that in butanol: acetic acid:water (9:1:2.5) was 0.29.

Oxidation with $KMnO_4$.—A mixture of 135 mg. of unknown, 500 mg. of $KMnO_4$, 0.8 ml. of 2 *N* NaOH and 10 ml. of H_2O was refluxed for 90 min. The solution was cooled, acidified with H_2SO_4, and Na_2SO_3 was added until all color and MnO_2 were removed. After adding 30 ml. of H_2O the solution was refluxed for 15 min. On cooling the solution to room temperature, about 70 mg. of long white needle-like crystals was formed. The material which had been recrystallized from hot water disappeared slowly on heating above 310°. Comparison of this acid with the three synthetic phthalic acids (Eastman), which had been purified, showed that only the *m*-isomer (isophthalic acid) crystallizes out of hot water with the formation of long needle-like crystals. Synthetic *o*-phthalic acid melted at 195°, *m*-phthalic acid disappeared on heating above 310°, and *p*-phthalic acid similarly disappeared on heating above 300°.

The infrared spectra of the isolated oxidized compound and the synthetic isophthalic acid were identical, whereas the patterns of the *ortho* and *para* acids differed in many respects from the oxidized unknown.

The *p*-nitrobenzyl esters of the synthetic isophthalic acid and the isolated oxidized compound were made.[9] They showed essentially the same melting points 207 and 209°, respectively. The mixed m.p. was 208°.

Oxidation with Chloramine-T.—One hundred mg. of the amino acid was oxidized with chloramine-T according to the procedure of Dakin[7] and the resultant product was extracted from the acid solution with chloroform. The chloroform extract was evaporated to dryness and the residue dissolved in water. The phthalaldehydic acid was separated from *p*-toluenesulfonamide, a contaminant from the oxidation reaction, by passage through a column of 10 ml. of Dowex-1 in the acetate form. The phthalaldehydic acid was absorbed by the resin while the sulfonamide was completely washed through with water. The aldehyde was eluted with 2 *N* acetic acid and recrystallized from hot water to give 40 mg. of white needle-like crystals. It melted at 175.5° (uncorrected) as compared to 175° for a sample of the synthetic aldehyde. Both compounds gave typical Fuchsin tests for an aldehyde. The phenylhydrazones of the isolated and synthetic aldehydes were prepared and respective melting points of 182 and 183° were observed.

Comparison of Synthetic and Isolated Amino Acids.—About 25 mg. of the natural amino acid was heated at 120° in one ml. of 5 *N* NaOH for 6 hr. The NaOH was removed by passage through IRC-50, and the racemized acid was recrystallized from hot water. KBr discs of the synthetic material and the racemized sample were prepared and the infrared spectra obtained. The two spectral curves were identical.

The two amino acids were cochromatographed in several solvent systems[10] and did not separate. They both reacted similarly on development with ninhydrin on paper.

The amount of crude amino acid isolated corresponded to 7.5 mg./lb. of fresh iris bulbs or about 0.5% of the total ninhydrin activity. The *m*-carboxy-α-phenylglycine in a comparable batch of iris bulbs with use of isolated material for standard was determined by paper chromatography[9] to be 93 μg./g. fresh weight or 2.21% of the total amino nitrogen.[11] The residue remaining after extraction with 80% ethanol was hydrolyzed with acid and examined for the presence of *m*-carboxy-α-phenylglycine. There was no detectable amount present in this crude protein hydrolysate.

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