SHORT COMMUNICATION

Mechanism of the Ninhydrin Reaction on a Filter Paper

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It is known that color shades of reaction products of amino acids with ninhydrin on a paperchromatogram are different from one amino acid to another and also from one solvent to another used for development in a paperchromatography[1,2]. However, Ruhemann's purple (I, diketohydrindylidene diketohydrindamine) is known as the final product on the

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1) R. Consden et al., *Biochem. J.*, **38**, 224 (1944).

2) C. E. Dent, ibid., **43**, 169 (1948).

ninhydrin reaction of usual α-amino acid in aqueous solution[3]. These facts indicates that side reactions must occur on a paper besides a main reaction which gives I.

In a previous publication[4], it was reported that various by-products on the reaction of 11 α-amino acids with ninhydrin on a paper were separated from I by a paperchromatography using methanol-water (1 : 1, v/v). Therefore, different color shades of products on the reaction of various amino acids with ninhydrin on paper seem to be resulted from differences of quality and quantity of the by-products.

In the present communication, a blue pigment derived from phenylalanine by the side reaction is described. The pigment was easily separated by paperchromatography and extracted from the paper with methanol. The extract showed the maximum absorption at 608~610 mμ and decomposed and faded by heating with an excess of ninhydrin. By the addition of acetic acid or diluted hydrochloric acid into methanolic solution of the pigment, it turned to violet color showing its absorption spectrum not identical with that of I, but regained its original blue color by further addition of pyridine or ammonia.

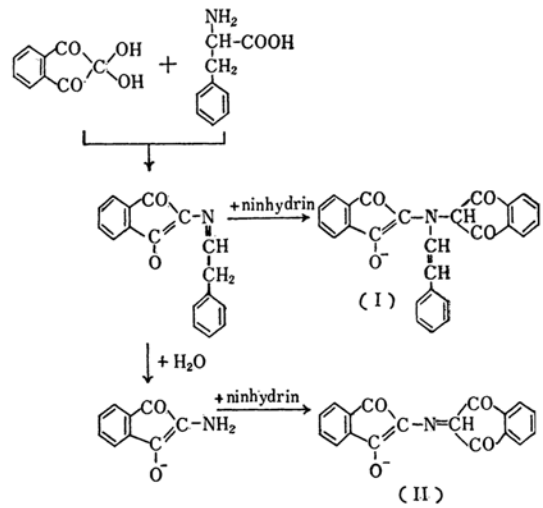
In an aqueous solution, no blue pigment was produced from phenylalanine by the ninhydrin reaction in spite of the presence of a piece of filter paper or air-bubbling into the solution.

When phenylalanine was heated with ninhydrin in absolute ethanol, black-green crystals, methanolic solution of which showed the same absorption spectrum and R_f value as those of the above blue pigment, were obtained. Namely a suspension of 1 g. of L-phenylalanine and 4 g. of ninhydrin in 50 ml. of absolute ethanol was heated at 60~70°C until carbon dioxide was no longer evolved. After the solution was cooled, the black-green crystals were filtered off (480 mg.) and washed with 50% methanol. They were recrystallized form benzene. M. p. (decomp.) 227~232°C. Found: C, 76.91; H, 3.81; N, 2.75. Calcd. for $C_{26}H_{17}O_4N$ (II): C, 76.65; H, 4.17; N, 3.43%.

When uniformly labeled ^{14}C-phenylalanine was used, 71% of the expected radioactivity was found in the blue pigment II. Consequently II must contain major parts of the skeleton of phenylalanine.

Comparing the infrared spectrum of I with that of II, both substances showed the absorption at 1670 cm^{-1}(carbonyl), 1640 and 1592cm^{-1}(benzene nucleus), and 1443 cm^{-1} (〉C=C〈$_{O-}$) but 1477 cm^{-1}(–N=C〈) was observed only in I. Characteristic absorption for II were at 1522, 1397 and 986 cm^{-1}(–CH=CH–), and 1351 and 1296 cm^{-1}(〉N–CH〈).

Therefore the reaction of ninhydrin with phenylalanine on a paper seems to proceed through the following two routes.



The present reaction mechanism of phenylalanine on a paper may be extended to those of other amino acids.

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3) D. J. McCaldin, *Chem. Revs.*, **60**, 39 (1960).
4) N. Tominaga, *Seikagaku*, **33**, 829 (1961).