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# A Color Reaction Given by Some $\alpha$-Amino Acids.

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A color reaction given by certain $\alpha$-amino acids was found during a study of the p-nitrobenzoylation of some of them. When a few milligrams of a reactive amino acid are dissolved in 2.0 cc of 5% sodium hydroxide in a test tube, cooled, and vigorously shaken for half a minute with 2.0 cc of a saturated solution of p-nitrobenzoyl chloride in carbon tetrachloride, no color develops. However, if 5 or 10 cc of n-butyl alcohol are now added and the tube is thoroughly agitated for a few seconds, a violet color appears in the butyl alcohol phase. The color persists for a few minutes and then gradually fades, becoming reddish and then colorless or pale yellow.

The violet color was given by *dl*-alanine, *dl*-valine, *dl*-norvaline, leucine, *dl*-norleucine, *dl*-phenylalanine, tyrosine, methionine, cystine, tryptophane, histidine, *dl*-lysine, *dl*-ornithine, $\alpha, \gamma$-diaminobutyric acid, arginine, and *dl*-citrulline. The test was negative on aspartic acid, glutamic acid, and hydroxyproline, and very faint on proline. It is presumed that the latter compound was not strictly pure. Glycine and *dl*-threonine gave dull orange colors, sarcosine a yellow, and glycine ethyl ester a pinkish brown. Negative results were obtained with every other substance tested, including the following: ammonia, $\beta$-alanine, urea, creatinine, adenine, uric acid, glycyl-*l*-leucine, ethyl allophanate, taurine, choline, brucine, $\alpha$-brom-$\epsilon$-benzoylaminocaproic acid, succinamide, m-aminobenzoic acid, and aniline.

When the volumes of reagents were those indicated above, the least amount of leucine which could be detected lay between 20 and 50 $\gamma$. The test was completely negative with the smaller quantity, but gave a good pink with the larger. The color produced was matched approximately by aqueous potassium permanganate.

Three different samples of p-nitrobenzoyl chloride gave similar results. One of the 3 samples was thrice recrystallized from carbon tetrachloride specially purified by successive thorough washings with mercury, concentrated sulfuric acid, sodium hydroxide solution, and water, followed by drying and distillation.[1] The recrystallized p-nitrobenzoyl chloride gave the color in undiminished intensity. The reagent may be dissolved in ether instead of

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[1] **Weissberger, A., and Proskauer, E.,** ***Organic Solvents,*** **Oxford, 1935, p. 156.**

in carbon tetrachloride with equally good color production when the solution is fresh, but after standing 2 weeks at room temperature the ethereal solution is no longer effective. Benzoyl chloride, m-nitrobenzoyl chloride, 3,5-dinitrobenzoyl chloride, p-brombenzoyl chloride, m-chlorbenzoyl chloride, and benzenesulfonyl chloride could not be substituted for p-nitrobenzoyl chloride. Other alcohols may be used in place of n-butyl alcohol. Color was produced with methanol (synthetic), ethanol (two different samples), isopropanol (distilled over lime), and isoamyl alcohol, but not by ether, ethyl acetate, acetone, or phenol.

The mechanism of the color production is not obvious. Isolated p-nitrobenzoyl leucine gave a positive test only when additional p-nitrobenzoyl chloride was used. If the butyl alcohol is added without agitation the violet color is produced at the junction of the aqueous and alcoholic layers and diffuses up into the latter where it eventually fades. The violet butyl alcohol solution becomes colorless on the addition of acetic acid, but the color is restored if excess sodium hydroxide is again added. When the acid solution is allowed to stand for some minutes restoration of the color by alkali is not possible. Aldehydes such as benzaldehyde and formaldehyde partly or completely inhibit the reaction, depending upon the conditions of the reaction and the amounts added. On dilution of the alcohol phase by nitrobenzene the color changes to blue; dilution by carbon tetrachloride causes the color to become red. There appears to be no analogy between the reaction described here and that given by phenylalanine according to Kapeller-Adler,[2] since in the present instance hydroxylamine completely inhibits color production. On the other hand, the reaction of alkaline alcoholic solutions of o- and m-dinitrobenzenes on fructose[3] gives colors which are similar in general behavior to those given in the present instance, particularly with respect to the changes in hue induced by dilution with suitable solvents.

*Summary.* A color reaction characteristic of many $\alpha$-amino acids has been described.

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[2] Kapeller-Adler, R., *Biochem. Z.*, 1932, **252**, 185.

[3] Joachimoglu, G., *Biochem. Z.*, 1915, **70**, 93.