Color Reactions for Certain Amino Acids

BY HENRY TAUBER

We have observed that certain amino acids are converted to chromogens when heated. Alcoholic extracts containing these chromogens become more deeply colored when alkali is added, but on subsequent acidification become colorless or much lighter in color.

The procedure has been as follows. Ten mg. of the amino acid in a dry Pyrex test-tube is heated over a burner until the first color change occurs, which may be to yellow or brown depending on the amino acid. Overheating should be avoided since it destroys the chromogens. After cooling, 3 cc. of ethyl alcohol is added and the solution boiled for thirty seconds and the resulting solution divided equally among three small test-tubes. To the first is added 0.2 cc. of 0.1 *N* sodium hydroxide, to the second 0.2 cc. of 0.1 *N* sulfuric acid, and to the third 0.2 cc. of water.

Twenty-one biologically important amino acids have been found to behave as follows: three (*dl*-alanine, *dl*-valine and *dl*-iso-leucine) when heated sublime completely, leaving no pigment or residue. Twelve (*l*-cystine, *l*-cysteine, glycine, *l*-hydroxyproline, *dl*-methionine, glutamic acid, *dl*-aspartic acid, *dl*-serine, *l*-proline, *d*-arginine-monohydrochloride, *dl*-lysine, and *d*-lysine) change to yellow, brown or black decomposition products without chromogenic properties. Six (phenylalanine, *l*-tyrosine, *l*-leucine, *l*-histidine, *l*-tryptophan and *dl*-threonine) form chromogens which react as follows when subjected to the above tests: *l*-tyrosine, *l*-tryptophan and *dl*-threonine turn reddish brown on heating, exhibit a light brown or reddish color in alcoholic solution, become more deeply colored on the addition of alkali and turn a light brown or, in the case of *l*-tyrosine, a yellow color on acidification. *l*-Histidine-monohydrochloride becomes light brown on heating, light yellow in alcoholic solution, deep yellow on the addition of alkali and almost colorless on acidification. *dl*-β or *l*-β-phenylalanine and *l*-leucine on heating partially sublime, turn yellow and give a yellow or light yellow color in alcoholic solution. This color turns to a deep yellow on addition of alkali and becomes almost colorless on subsequent acidification. In the case of the β-phenylalanines the alkaline alcoholic solution exhibits a greenish-yellow fluorescence which is particulary strong in ultraviolet light, without the addition of alkali.

This fluorescent pigment from phenylalanine has been isolated in a more concentrated form as follows. One gram of phenylalanine is heated in a large Pyrex test-tube and stirred with a thermometer until the temperature of the material reaches 250°. After cooling, the crystalline reaction mixture is extracted four times with 15-cc. portions of acetone. The deep yellow, fluorescent solution is concentrated to 10 cc., filtered and evaporated to dryness in vacuum. Ninety-five mg. of reddish-brown hygroscopic material is obtained. Dilute alcoholic solutions of this pigment show intense fluorescence in ultraviolet light but not in daylight. On the addition of alkali, however, deep greenish-yellow fluorescence is shown in daylight. In acetone the fluorescence is much stronger than in alcoholic solution. The pigment gives a much more intense xantho-proteic reaction (deep orange-red) than phenylalanine. After acetone extraction of the pyrolyzed reaction mixture there is left 550 mg. of white material and some substance is lost owing to sublimation.

We are aware that these amino acids are not the only compounds that react in this manner. We believe, however, that these observations are interesting enough to warrant recording. Especially remarkable is the strongly fluorescent compound that forms on the pyrolysis of phenylalanine.

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Substituted α-Amyl-cinnamylaldehydes

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α-Amyl-cinnamylaldehyde is, because of its jasmin-like odor, manufactured industrially from heptanal and benzaldehyde.[1] It seemed possible that the same synthesis with substituted benzaldehydes, which by themselves exhibit pleasant odors, would give especially valuable products. This was, however, not the case. 4-Methoxy-, 3,4-methylene-dioxy- and 3,4-dimethoxy-α-amyl-cinnamylaldehydes have been synthesized and studied; the first of them has been described briefly before.[2] The substituted benzaldehydes proved less reactive than benzaldehyde itself in this condensation, as in other similar cases.[3] In all these experiments, a mixture of pyridine and piperidine proved a more convenient catalyst, than the commonly used potassium hydroxide. Vanillin did not react at all with oenanthal under these conditions.

Experimental

4-Methoxy-α-amyl-cinnamylaldehyde.—A mixture of heptanal (12 g.), anisaldehyde (14 g), pyridine (100 cc.) and piperidine (10 cc.) was heated on the water-bath for eight hours, poured out into ice-cold dilute sulfuric acid and extracted with ether. The product was twice distilled at 0.3 mm.; b. p. 145°; yield, 10 g.

Anal. Calcd. for $C_{15}H_{20}O_2$: C, 77.6; H, 8.6. Found: C, 77.6; H, 9.0.

The **semicarbazone** separated from a mixture of the aldehyde (1.5 g.), semicarbazide hydrochloride (0.75 g.) and potassium acetate (0.6 g.) in aqueous alcohol at room temperature. It formed needles, m. p. 143–145°, when crystallized from propyl alcohol. Calcd. for $O_{16}H_{23}O_2N_3$: N, 14.5. Found: N, 14.3.

3,4-Dimethoxy-α-amyl-cinnamylaldehyde.—Veratraldehyde (13 g.) and heptanal (9.5 g.) were condensed by heating for eight hours in presence of pyridine (50 cc.) and piperidine (5 cc.). The condensation product had b. p. 165° (0.6 mm.).

Anal. Calcd. for $C_{16}H_{22}O_3$: C, 73.3; H, 8.4. Found: C, 73.4; H, 8.7.

The semicarbazone, prepared as above, was recrystallized from propyl alcohol and formed needles, m. p. 175°.

Anal. Calcd. for $C_{17}H_{25}O_3N_3$: C, 64.0; H, 7.8; N, 12.7. Found: C, 64.4; H, 8.4; N, 13.0.

(1) Rutowski and Korolew, *J. prakt. Chem.*, [2] **119**, 272 (1928).
(2) I. G. Farbenindustrie A. G., French Patent 628,739 (1927).
(3) Molt, *Rec. trav. chim.*, **56**, 233 (1937).