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 xanthoproteic 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.

AGRICULTURAL RESEARCH ADMINISTRATION
U. S. DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C. RECEIVED SEPTEMBER 28, 1943

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).

3,4-Methylenedioxy-α-amyl-cinnamylaldehyde was prepared analogously from heptanal (11.4 g.) and piperonal (15 g.). The fraction 150–160° (0.8 mm.) of the condensation product was purified *via* the semicarbazone as above. From butyl alcohol long needles, m. p. 155°, were obtained.

Anal. Calcd. for $C_{16}H_{21}O_3N_3$: N, 13.9. Found: N, 14.0. The pure aldehyde had b. p. 158–159° (0.9 mm.).

THE DANIEL SIEFF RESEARCH INSTITUTE
REHOVOTH, PALESTINE RECEIVED NOVEMBER 26, 1943

NEW COMPOUNDS

Some Aryl and Aralkyl Ureas

In connection with a study of the hypnotic properties of aryl ureas carried out by a pharmacological group in these Laboratories, a number of halogenated unsymmetrical aryl alkyl ureas have been prepared. Data on these and on some related unhalogenated ureas and symmetrically substituted ureas are presented in Table I.

Preparation of Secondary Amines.—The 3-chloro and bromo anisyl methyl and homo anisyl methyl amines (leading to ureas XVI–XIX) were prepared by halogenation of N-methyl anisyl and N-methyl homo anisyl amines in hydrochloric acid solution (XXIV in hydrobromic acid).

Whereas non-halogenated aromatic secondary amines are readily prepared by alkylation with alkyl halides, isolation of the secondary amine as the nitrosamine followed by reduction with stannous chloride, the alkyl bromides and iodides are unsuitable for reaction with halogenated aromatic amines,[1] the halogen being removed or displaced with resulting complications. By use of alkyl sulfates and alkyl toluene-sulfonates the desired alkyl groups can be introduced satisfactorily.

Ethylation was accomplished by stirring the primary amine with ethyl sulfate and water on the steam-bath until the layers had disappeared after which the conventional nitrosamine procedure was followed.

The secondary propyl and butyl amines corresponding to ureas IV and VI were obtained by warming 1 mol of alkyl toluene sulfonate with 2 mols of primary amine for three hours at 110–120°. The partially cooled melts were sludged with benzene and the bulk of the primary amines separated as salts of *p*-toluenesulfonic acid. Addition to the filtrate of alcoholic hydrogen chloride equivalent to about half of the remaining base precipitated virtually all of the remaining primary amines, after which the hydrochlorides of the secondary amines could be separated without trouble. In some runs sulfonamide formation was encountered.

Preparation of Ureas.—The symmetrically substituted ureas XX and XXI were prepared from ethyl isocyanate and the appropriate aromatic bases.

TABLE I

SECONDARY ARYL AND ARALKYL UREAS $R_1R_2NCONH_2$

	R_1	R_2	M. p., °C.[a]	Appearance	Cryst. solvent[b]	Empirical formula	Analyses, % Calcd. C	Calcd. H	Found C	Found H
I	C_6H_5	CH_2CH_2OH	110		Æ	$C_9H_{12}O_2N_2$	59.97	6.71	59.68	7.05
II[c]	2-Me-4-Cl-C_6H_3	C_2H_5	93	Stout prisms	Æ-H	$C_{10}H_{13}ON_2Cl$	56.46	6.16	56.59	6.19
III[d]	2-Me-4-Br-C_6H_3	C_2H_5	88.5–89	Rect. prisms	E-H	$C_{10}H_{13}ON_2Br$	46.70	5.10	47.00	5.35
IV	The same	*n*-C_3H_7	94.5–95.5	Prisms	H	$C_{11}H_{15}ON_2Br$	48.70	5.58	48.76	5.78
V[e]	2-Me-5-Cl-C_6H_3	C_2H_5	166–167	Felted needles	A-Aq	$C_{10}H_{13}ON_2Cl$	56.46	6.16	56.52	6.37
VI[f]	The same	*n*-C_4H_9	79.5–80	Prisms	H	$C_{12}H_{17}ON_2Cl$	59.86	7.12	59.84	7.44
VII[g]	4-Me-2-Br-C_6H_3	C_2H_5	116	Tiny prisms	E	$C_{10}H_{13}ON_2Br$	46.70	5.10	46.82	5.21
VIII[h]	2-Et-4-Br-C_6H_3	C_2H_5	95	Small stout prisms	E-H	$C_{11}H_{15}ON_2Br$	48.70	5.58	48.45	5.86
IX[i]	4-Et-C_6H_4	C_2H_5	122–124	Stout prisms	Æ-H	$C_{11}H_{16}ON_2$	68.70	8.39	68.99	8.62
X[j]	4-Et-2-Br-C_6H_3	C_2H_5	114	Stout prisms	E-H	$C_{11}H_{15}ON_2Br$	48.70	5.58	48.65	5.76
XI	2,4-$Me_2C_6H_3$	C_6H_5	73–74	Prisms	H	$C_{11}H_{16}ON_2$	68.70	8.39	68.75	8.50
XII	$C_6H_5CH_2$	CH_3	135	Needle prisms	Æ	$C_9H_{12}ON_2$	65.82	7.37	65.99	7.32
XIII	The same	*n*-C_4H_9	61–62	Needles	H	$C_{12}H_{18}ON_2$	69.84	8.80	69.80	8.72
XIV[k]	2-EtO-5-Br-C_6H_3	C_2H_5	124–124.5	Needles	Æ-H	$C_{11}H_{15}O_2N_2Br$	45.98	5.27	46.24	5.50
XV	4-MeO-C_6H_4-CH_2	CH_3	140–141	Flattened needles	A-Aq	$C_{10}H_{14}O_2N_2$	61.82	7.27	62.12	7.46
XVI	4-MeO-3-Cl-$C_6H_3CH_2$	CH_3	169–169.5	Leaflets	A	$C_{10}H_{13}O_2N_2Cl$	52.49	5.73	52.52	5.64
XVII	4-MeO-3-Br-$C_6H_3CH_2$	CH_3	178	Leaflets	A	$C_{10}H_{13}O_2N_2Br$	43.95	4.80	44.22	5.10
XVIII	4-MeO-3-Cl-$C_6H_3CH_2CH_2$	CH_3	117.5–118	Prisms	Æ	$C_{11}H_{15}O_2N_2Cl$	54.41	6.23	54.49	6.38
XIX	4-MeO-3-Br-$C_6H_3CH_2CH_2$	CH_3	116.5–117	Prisms	Æ-H	$C_{11}H_{15}O_2N_2Br$	45.97	5.27	46.03	5.30
	Symmetrically substituted ureas									
XX	2-Me-4-Br-$C_6H_3NHCONHEt$		230–232	Tiny felted needles	HAc	$C_{10}H_{13}ON_2Br$	46.70	5.10	47.00	5.45
XXI	2,4-Me_2-$C_6H_3NEtCONHEt$		76	Stout prisms	H	$C_{13}H_{20}ON_2$	70.86	9.16	70.62	9.19
XXII	2-Et-$C_6H_4NEtCON(COC_6H_5)_2$		128–129	Needles	B	$C_{28}H_{28}O_4N_2$	74.97 (N = 7.00)	6.04	75.00 (N = 6.95)	6.38
	Secondary Aralkylamine Hydrochlorides $R_1R_2NH\cdot HCl$									
XXIII	4-MeO-3-Cl-$C_6H_3CH_2$	CH_3	201–201.5	Needle prisms	A	$C_9H_{13}ONCl_2$	48.64	5.90	48.76	6.03
XXIV	4-MeO-3-Br-$C_6H_3CH_2$ (hydrobromide)	CH_3	202–203	Needles	A	$C_9H_{13}ONBr_2$	34.73	4.21	35.08	4.44
XXV	4-MeO-3-Cl-$C_6H_3CH_2CH_2$	CH_3	196	Felted needles	Æ	$C_{10}H_{15}ONCl_2$	50.84	6.41	51.00	6.50
XXVI	4-MeO-3-Br-$C_6H_3CH_2CH_2$	CH_3	215–216	Fine needle prisms	A	$C_{10}H_{15}ONClBr$	42.78	5.39	42.94	5.49

[a] All melting points corrected. [b] A = ethanol; Æ = ethyl acetate; E = ether; H = hexane; Aq = water; HAc = acetic acid; B = benzene. [c] B. p. (13 mm.) of secondary amine, 136°. [d] B. p. (0.25 mm.) of secondary amine, 96–99°. [e] B. p. (27 mm.) of secondary amine, 141°. [f] B. p. (1 mm.) of secondary amine, 125°. [g] B. p. (17 mm.) of secondary amine, 137°. [h] B. p. (3 mm.) of secondary amine, 135°. [i] B. p. (22 mm.) of secondary amine, 122–123°. [j] B. p. (3 mm.) of secondary amine, 107°. [k] B. p. (0.25 mm.) of secondary amine, 111°.

The secondary amines corresponding to the ureas I, XI–XIII and XV are known. The amines corresponding to ureas XVI–XIX were characterized as salts and data thereon are also included in Table I. For the other secondary bases boiling points are given. The derived ureas are themselves satisfactory as compounds of characterization.

(1) Baltzly and Buck, THIS JOURNAL, 63, 1757 (1941).