Studies on the Decomposition and Reactions of Urea. Part III. Reactions of Urea with Amines and Amino-acids.

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The reactions of urea with amines and amino-acids, specially in aqueous solution, are of particular interest in connection with the much controverted constitution of "ureastibamine," obtained by the action of excess of urea on *p*-stibanilic acid in warm aqueous solution (Brahmachari, *J. Indian Med. Res.*, 1922, **10**, 492). Since it is a complex mixture, the constituents of which cannot be easily isolated and purified partly due to their gelatinous character, the results simply based on their analytical data are likely to be misleading. Other similar aromatic amino-acids, which are not unstable like *p*-stibanilic acid and the reaction products of which can be easily obtained in pure and crystalline states, have, therefore, been selected for comparing their actions on urea under similar conditions. In the case of aniline (or the hydrochloride), the reactions have been carried usually with an increasing proportion of urea, while Baeyer (*Annalen*, 1864, **131**, 252), Girard (*Ber.*, 1873, **6**, 444), Davis and Underwood (*J. Amer. Chem. Soc.*, 1922, **44**, 2595), Weith (*Ber.*, 1876, **9**, 821), Fleisher (*ibid.*, 1876, **3**, 998), Davis and Blanchard (*J. Amer. Chem. Soc.*, 1923, **45**, 1816) have carried the reactions with an increasing amount of the base (aniline). Different results have, therefore, been obtained with respect to the relative yields of the substituted ureas formed.

Experimental.

Aniline (or hydrochloride) and urea.—The reaction products (phenyl and diphenylurea) are washed with water (20-30 c.c.) and separated by means of 50 % alcohol.

p-*Aminobenzoic acid and urea.*—A mixture of *p*-aminobenzoic acid (5 g.), urea (8 g.) and water (20 c.c.) was heated in a small flask on a water-bath for 2 hours. The amino-acid gradually dissolved and after about an hour a gelatinous precipitate separated out. The mixture was next diluted with water (20 c.c.) and filtered. The precipitate, after being thoroughly washed with water, was extracted with hot alcohol and filtered. The filtrate on evaporation yielded a white powder (2·2 g.) not melting below 275° and on analysis it was found to be carbamido-*p*-aminobenzoic acid. (Found: N, 15·23. $C_8H_8O_3N_2$ requires N, 15·55 per cent).

The precipitate, insoluble in alcohol, was dissolved in alkali and reprecipitated with acid and the precipitate was washed with alcohol. The residue (about 0·3 g.) was found to be carbo-di-*p*-aminobenzoic acid. (Found: N, 9·1. $C_{15}H_{12}O_5N_2$ requires N, 9·3 per cent).

The filtrate from the above precipitates, on acidification with dilute hydrochloric acid, yielded carbamido-*p*-aminobenzoic acid (1 g.) not melting below 270°. Evidently this was present in the solution as the ammonium salt, since the filtrate gave strong smell of ammonia on adding dilute alkali in the cold. The filtrate from the second precipitate was concentrated and then neutralised with dilute alkali and cooled, when a crystalline precipitate of *p*-aminobenzoic acid (0·8g.), m. p. 116°, was obtained. It must also have been present in the solution as the ammonium salt.

The results obtained with various amines, amino-acids, etc. are shown in the annexed tables.

Reactions of Amines and Aminoacids with Urea.

Reactant.	Condition of experiment.	Products formed.	M. p. and mixed m. p.	Remarks.
Aniline	Aniline (3·8 g.) + urea (4 g., 2 mol.) heated at 150° for about an hour	Phenylurea Diphenylurea	147° 235°	Total yield, 2·6g. Phenylurea, 2·2 g. (40%) Diphenylurea, 0·3 g. (7%)
Aniline hydrochloride	(*a*) Aniline hydrochloride (1·1 g.) + urea (4 g., 2 mol.) + water (20 c.c.) heated at 95-100° for 2½ hours	Phenylurea (0·7 g. 39%). Diphenylurea (0·1 g., 7%)	147° 235°	Filtrate from the precipitates yielded only a trace of diphenyl urea on refluxing for ½-1 hour.
	(*b*) Aniline hydrochloride (2·6g.) + urea (1·2g.) heated at 150° for 1 hour	Diphenylurea Phenylurea	235° 147°	Total yield, 70% Diphenylurea. 1·2 g. (59%) Phenylurea, 5 g. (18·2%)
	(*c*) Aniline hydrochloride (2 g.) + urea (4 g, 2 mol.), heated at 150° for 1 hour	Do	147°	Total yield, 1·3 g. (60%) Phenylurea, 0·9 g. (43%) Diphenylurea, 0·3 g. (18%)
8-Aminoquinoline dihydrochloride	Heated with equivalent quantities of urea in aqueous solution or in the dry state (15°)	Carbamido-8-aminoquinoline	206°	No corresponding diquinolyl derivative formed
6-Aminocoumarin hydrochloride	Heated under the above conditions	Carbamido-6-aminocoumarin	245° (*cf.* Dey and Seshadri, *J. Indian Chem. Soc.*, 1931, 8, 298)	No disubstituted urea derivative formed

Reactant.	Conditions of experiment.	Products formed.	M-p. and mixed m-p.	Remarks.
o-Nitroaniline	Heated with excess of urea at 160° or 180°	No action	.....	Similar results obtained with *p*-nitroaniline, 4-methoxy-2-nitroniline
p-Aminobenzoic acid	(*a*) Acid (5 g.), urea (8 g., 4 mol.) water (20 c.c.) heated on water-bath for 2 hrs. at about 95°	1. Carbamido-*p*-amino-benzoic acid (2·2 g.)	Not melting below 260°	
		2. Do (1 g.) (as ammonium salt)	Do	Carbo-di-*p*-amino benzoic acid formed only to a small extent (less than 0·3 g.)
		3. *p*-Aminobenzoic acid (0·8 g.) (as ammonium salt)	186°	
	(*b*) The above reaction carried at 75-80° for 2 hours	Carbamido-*p*-amino-benzoic acid (ammonium salt)	Not melting below 260°	Yield, 2-3% The bulk of the amino acid remained unreacted.
	(*c*) Acid (1·3 g.) urea (0·6 g.) and water (5 c. c.), heated on water-bath for 2 hrs.	Carbamido-*p*-amino-benzoic acid (0·8 g.)	Do	Filtrate contained ammonium salt of the amino-acid.
m-Aminobenzoic acid.	(*a*) Acid (5 g.), urea (6·7 g.) and water (15 c.c.), heated on water-bath for 2-2½ hrs.	1. Carbamido derivative (2·4 g.)	269°	
		2. Do (1·2 g.) (ammonium salt)	269°	
		3. *m*-Aminobenzoic acid	173°	

Reactant.	Condition of experiment	Products formed.	M.p. and mixed m.p.	Remarks.
m-Aminobenzoic acid	(*b*) Acid (1·5 g.), urea (1·5 g.) water (6·7 c.c.) heated as above	1. Carbamido derivative (0·8 g.).	169°	
		2. Do. (0·15 g.) (ammonium salt)	169°	
o-Aminobenzoic acid	Acid (5 g.), urea (8 g.), water (10-12 c.c.), heated on water-bath at 95°-100° for 2-2½ hours	1. Carbamido anthranilic acid (ammonium salt)	153°	
		2. Ammonium anthranilate		The ammonium salt was precipitated with absolute alcohol. No carbamido derivative formed
p-Arsanilic acid	Acid (5 g.), urea (6 g.), water (10 c.c.) heated on water-bath for 3 hours	Ammonium.*p* ilate		

Discussion.

In the reaction of *p*-aminobenzoic acid with excess of urea, the main products formed are (1) carbamido-*p*-aminobenzoic acid, (2) ammonium salt of the same and (3) ammonium-*p*-aminobenzoate. A disubstituted urea derivative is not formed to any marked extent (also compare the reaction of aniline hydrochloride in presence of excess of urea). Since ureastibamine is formed from *p*-stibanilic acid and urea under similar conditions, it is unlikely that it should contain any appreciable quantity of *sym*-diphenylurea-4 : 4-distibinic acid (Gray, *Proc. Roy. Soc.*, 1931, **B 108**,54). The reaction with arsanilic acid, to which *p*-stibanilic acid is very much allied, shows on the other hand, that no carbamido derivative is formed at all but simply the ammonium salt. It may be further noted that urea reacts very little with amines and amino-acids in aqueous solution at 75-80°. The following observations would throw further light on the constitution of the drug :

(*i*) An aqueous solution of ureastibamine is precipitated by an electrolyte, *e.g.*, NaCl or KI.

(*ii*) It gives diazo reaction. The presence of antimonic acid and other insoluble substances somewhat interferes with this test.

(*iii*) A concentrated aqueous solution of ureastibamine (2 g.) is heated with 5 % caustic soda solution at 70-75° for about 3 hours. An insoluble product (0·4 g.), consisting mainly of antimony oxide, gradually settles down. The filtrate, on acidification with dilute acetic acid, yields a voluminous precipitate (1·2 g.), which readily dissolves in dilute mineral acids (thus showing the absence of substituted urea derivatives). The above crude acid is purified by means of concentrated hydrochloric acid in the usual way and the pure stibanilic acid thus obtained is dried in *vacuo* over sulphuric acid. (Found : N, 5·0; Sb, 47·0. $C_6H_8O_3NSb$ requires N, 5·3; Sb, 46·1 per cent). Carbamido-*p*-stibanilic acid does not yield *p*-stibanilic acid when heated with dilute alkali as above. Hence the formation of *p*-stibanilic acid in the above experiment from the decomposition of the corresponding carbamido derivative (Bramhachari and Das, *J. Indian Med. Res.*, 1924, **12**, 423; Niyogi, *J. Indian Chem. Soc.*, 1928, **5**, 753), if formed at all, is precluded. Probably *p*-aminophenylstibinic acid exists in a polymerised form in which the amino group is involved or may be that the latter group is in a loose state of combination with antimonic acid.

Summing all these facts, it appears that *p*-stibanilic acid is the principal constituent of the drug and as in *Neostibosan* it should be mainly responsible for its therapeutic value. It is present partly as the ammonium salt and partly in the free state (*cf.* observation *i*) together with some unhydrolysed acetyl-*p*-stibanilic acid; the latter two colloidal substances are easily peptised in presence of antimonic acid and ammonia.

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