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THE INSTABILITY OF STILBAMIDINE

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The recent simultaneous appearance of two publications, by Fulton and Goodwin (1946), and by Henry (1946), dealing with the photochemical instability of *cis*- and *trans*-stilbamidine in solution shows the existence of considerable divergence of opinion regarding the exact nature of the changes which occur. Barber, Slack, and Wien (1943) suggested that saturation of the ethylenic linkage of *trans*-stilbamidine on irradiation was due to addition of water at the double bond with carbinol formation. Henry (1946) showed that this suggestion was inadmissible, and his evidence was strongly in favour of dimerization with formation of a derivative of *cyclo*butane. This interpretation of the nature of the saturated irradiation product has recently been confirmed by Fulton and Dunitz (1947) by *x*-ray analysis and will therefore be assumed throughout the present communication.

Fulton and Goodwin (1946) were able to show the partial conversion of *cis*-stilbamidine into *trans*-stilbamidine on irradiation, and also deduced that *cis*-stilbamidine is not converted directly into the saturated product. On these points they and the present author are in complete agreement. On the other hand they were unable to find any evidence for the reverse *trans* $\rightarrow$*cis* change, whereas the present author found that partial conversion of *trans*-stilbamidine to *cis*-stilbamidine occurred over a wide range of concentrations and temperatures, equilibrium between the two forms being ultimately established under all conditions used. The value of the ratio dimer/*cis*-stilbamidine produced in the early stage of irradiation is determined by concentration and temperature; low temperature is favourable to dimer formation, which explains Fulton's (1943) finding that greater toxicity developed on irradiation at winter temperatures than at summer temperatures. Furthermore, no evidence was found for the "stabilization" of *trans*-stilbamidine in dilute solution which Fulton and Goodwin (1946) postulated in order to explain some of their results at low initial concentrations of *cis*-stilbamidine. At low concentrations, equilibrium between the *cis*- and *trans*-forms is rapidly established, and the equilibrium concentration of the *trans*-form is such that its rate of dimerization is very slow in comparison with its rate of reconversion to the *cis*-form, since the latter reaction is unimolecular whereas the former is bimolecular; this is doubtless the explanation of the apparent "stabilization" of the *trans*-stilbamidine. Their failure to obtain precipitation of *trans*-stilbamidine sulphate on irradiation of 0.05 per cent solutions of *cis*-stilbamidine sulphate is in agreement with the present author's finding that, at low concentrations, precipitation of *trans*-stilbamidine sulphate is not complete even in presence of a large excess of sodium sulphate, and consequently the normal bromometric method of analysis of mixtures of the two isomers fails to give reliable results in dilute solution. With the fluorescence-adsorption technique of Henry and Grindley (1942) no difficulty was experienced in demonstrating the complete reversibility of the *cis–trans* change at low concentrations, and it was in fact this property which was used to identify *cis*-stilbamidine as one of the irradiation products.

Fulton and Goodwin (1946) state that "It is very probable that the formation of the 'saturated' product by irradiating aqueous solutions of *trans*-stilbamidine is due to collision between activated *trans*- molecules. The high absorption coefficient of this compound indicates that a large proportion of the molecules present in an irradiated solution may be activated." In the first place, it is probably unusual for the mean life of a photochemically activated molecule to exceed 10^{-7} sec., so that there is little opportunity for any significant accumulation of activated molecules in the solution to occur. Secondly, if the saturated irradiation product arises as the result of carbinol formation it is difficult to see why collision of *two* activated molecules should be necessary. Even with dimer formation it is not necessary to make this assumption, as the energy of photochemical activation of a single molecule corresponding to the

wavelength of maximum absorption (329 mμ; 88,000 cals. per gram -mol.) is sufficient to bring about any ordinary chemical reaction which is likely to occur.

Hydrolysis of the amidine groups of stilbamidine was early found to be a dark reaction (Henry, 1945), and the nature of the first hydrolysis product, *trans*-4-amido-4′-amidinostilbene hydrochloride, was clearly stated. Recent examination of solutions of stilbamidine which had been stored for three years under various conditions shows that the factors upon which the rate of hydrolysis of the amidine groups primarily depends are the *p*H of the solution and the temperature. Exposure to light appears not to be of primary importance, but may influence the final results through conversion of the *trans*-stilbamidine to other compounds which may show different susceptibility to hydrolysis and produce hydrolysis products too soluble to be precipitated. A *p*H of 5 suppresses hydrolysis almost indefinitely. Unless the solutions used by Fulton and Goodwin (1946) were alkaline no significant degree of hydrolysis would be expected during their periods of insolation, particularly at the prevailing temperatures in Britain, and no crystallization would be expected even on prolonged exposure as substantial conversion of *trans*-stilbamidine to other products would have occurred in the early stages. Storage *in the dark*, at 40° C., of a one per cent solution of the hydrochloride at *p*H 7 will almost certainly produce a good crop of crystals of *trans*-4-amido-4′-amidinostilbene hydrochloride within three months. It may be added that experience here indicates that the method which they employed for detecting hydrolysis would be unsatisfactory for estimation of the extent of hydrolysis, as at low temperatures ammonia is difficult to aspirate completely, while elevation of the temperature is, under the necessary alkaline conditions —even with borax—liable to result in hydrolysis of the amidine groups. The only satisfactory method which has been found of estimating ammonium ion in presence of the amidine group was the formaldehyde method of Marcali and Rieman (1946), a separate "blank" being determined for each base encountered. The results of the long period storage tests are given in Table I. The drift of *p*H which occurred (through action on the glass, etc.) interferes to some extent with the deductions which can be made from the

TABLE I

HYDROLYSIS OF AMIDINE GROUPS IN SOLUTIONS STORED FOR 3 YEARS

Expt.	Compound	Conc. %	Conditions	*p*H Init.	*p*H Final	% Hydrol. to N_3 Cpd.	Remarks
A_1	Stilbamidine Hydrochloride	1.0	5° C., glass, dark	6.7	6.50	Nil	Some cryst. of N_4
A_2	Stilbamidine Hydrochloride	1.0	5° C., wax, dark	6.7	5.98	Nil	Some cryst. of N_4
B	Stilbamidine Hydrochloride	1.0	30–40° C., glass, dark	3.7	5.45	1.7	No visible N_3 cryst.
C	Stilbamidine Hydrochloride	1.0	30–40° C., glass, dark	4.5	5.92	5.6	Small tuft of N_3 cryst.
D_1	Stilbamidine Hydrochloride	1.0	30–40° C., glass, dark	6.7	6.75	35.5	Heavy crop of N_3 cryst.
D_2	Stilbamidine Hydrochloride	1.0	30–40° C., wax, dark	6.7	5.52	2.6	No visible N_3 cryst.
E_1	Stilbamidine Hydrochloride (Soln. init. insolated 1½ hrs.)	1.0	30–40° C., glass, dark	6.7	6.45	28.2	Some N_3 cryst.
E_2	Stilbamidine Hydrochloride (Soln. init. insolated 1½ hrs.)	1.0	30–40° C., wax, dark	6.7	6.05	5.3	No precipitation
F_1	Stilbamidine Hydrochloride	1.0	30–40° C., glass, diffused daylight	6.7	5.50	6.7	No precipitation
F_2	Stilbamidine Hydrochloride	1.0	30–40° C., wax, diffused daylight	6.7	5.65	6.3	No precipitation
G_1	Stilbamidine Isethionate	1.5	30–40° C., glass, dark	—	6.67	26.5	Some deposit of N_2 cpd.
G_2	Stilbamidine Isethionate	1.5	30–40° C., wax, dark	—	6.25	12.0	Some deposit of N_2 cpd.
H_1	Pentamidine Hydrochloride	1.0	30–40° C., glass, dark	7.2	5.65	4.8	No precipitation
H_2	Pentamidine Hydrochloride	1.0	30–40° C., wax, dark	7.2	5.15	1.3	No precipitation

NOTES.—1. The symbols N_4, N_3 and N_2 denote *trans*-stilbamidine and *trans*-4-amido-4′-amidinostilbene hydrochlorides and 4:4′-diamidostilbene respectively.

2. In Expts. E_1 and E_2 the total bromine absorption after the initial insolation was 49 per cent of the original bromine absorption.

results, but there is little doubt that *p*H and temperature are of primary importance in determining rate of hydrolysis and that it is not a surface action, as had previously been suggested (Henry, 1943).

Trans-4-amido-4′-amidinostilbene is more toxic than the parent compound. Its formation in the body—for which conditions of *p*H and temperature would be favourable—from stilbamidine adsorbed and retained for long periods may therefore in part account for the delayed toxic effects which have been observed. The occurrence of prolonged storage in the body is supported by recent examination of the urine of kala-azar patients some eighteen months after termination of their course of treatment with stilbamidine. Application of the fluorescence-adsorption technique of Henry and Grindley (1945), using 0.8 c.c. of urine, leaves little doubt that stilbamidine, or a closely related derivative, is still being excreted at a low level (0.005–0.03 mg. per 100 c.c.). In another patient, only 25 per cent of the stilbamidine isethionate (4,650 mg.) injected intravenously over a period of three months was excreted in the urine during the course of treatment, and 14 days after termination of the course the rate of excretion was steady at 0.1 mg. per 100 c.c.

Determination of the site or sites of storage of stilbamidine in the body is complicated by the difficulty of extracting the drug from adsorbing tissue by the usual organic solvents. It is possible that the answer may be provided by hydrolysing completely both tissue and adsorbed stilbamidine with conversion of the latter into the corresponding stilbene dicarboxylic acid, which can be detected and estimated by its fluorescence (cf. Henry, 1946), though with no great sensitivity. The point is important, in view of the recent use by Snapper (1947) of stilbamidine in the treatment of multiple myelomatosis. Pentamidine, which has been extensively used prophylactically against trypanosomiasis by van Hoof (1947) in the Congo, resembles stilbamidine in being strongly adsorbed by filter-paper, and is probably also stored in the body for long periods.

It seems highly probable that similar conditions of adsorption, storage, and slow release apply for the dimer after administration as appear to apply for stilbamidine. It has been shown (Henry, 1946) that the dimer is strongly adsorbed by filter-paper, and can readily be estimated by the fluorescence-adsorption technique because irradiation of a dry spot on filter-paper with short-wave (ca. 245 mμ.) ultraviolet light causes reversal of dimerization and production of *trans*-stilbamidine, which can then be estimated fluorimetrically. Two sheep were injected intravenously, one with 50 mg. of stilbamidine isethionate and the other with the same quantity of dimer isethionate, and the rate of excretion was followed for about 24 hours. The results of these tests are recorded in Table II,

TABLE II

EXCRETION OF *trans*-STILBAMIDINE AND DIMER IN THE URINE AFTER INTRAVENOUS INJECTION OF 50 MG. INTO SHEEP

Time of injections: 8.43 hours

Time	Urine vol., c.c.	Excreted in urine	
		mg./100 c.c.	mg.
trans-STILBAMIDINE			
10.25	65	2.0	1.3
12.30	50	5.5	2.8
14.15	50	5.0	2.5
15.20	20	3.5	0.7
16.15	48	1.3	0.7
08.50	42	0.7	0.3
09.50	50	0.8	0.4
11.55	60	0.9	0.5
Total excretion, mg.:			9.2
DIMER			
10.25	85	2.5	2.1
12.30	45	2.8	1.3
14.15	60	0.9	0.5
15.20	53	0.7	0.4
16.15	46	0.6	0.3
08.25	55	0.45	0.25
11.05	119	0.3	0.35
11.55	55	0.2	0.1
Total excretion, mg.:			5.3

and show that retention of the dimer in the body is closely similar to that of *trans*-stilbamidine. The estimation of the dimer was carried out by spotting-out the urine (neutralized to litmus with hydrochloric acid) on filter paper, washing radially, exposing the dry spots to short-wave ultraviolet radiation, and comparing the spots so produced with a series of standard spots which had been prepared in sheeps' urine and treated and developed in the same way.

The results of the *x*-ray examination by Fulton and Dunitz (1947) of the hydrocarbon obtained by complete hydrolysis of the saturated irradiation product and subsequent decarboxylation are of great interest. In the first place—and on the assumption that they first proved that carbinol formation was inadmissible (otherwise *cyclo*butane formation could have occurred during decarboxylation)—their results conclusively confirm the dimerization theory of the nature of this product, which had previously been deduced from kinetic

and other considerations. Secondly, the apparent rectangular, instead of square, shape of the *cyclo*butane nucleus of the molecule—confirmation of which will be awaited with much interest—has a very important bearing on the photochemical or thermal decomposition of the dimer. From considerations of bond-force constants and bond lengths (*cf.* Linnett, 1947) decomposition of the dimer would be expected to occur *across the longer sides of the rectangle.* According to the dimensions of the rectangle given by Fulton and Dunitz (1947) (there is an obvious printer's error in the published data) decomposition across the longer sides of the rectangle would involve some 9,000 cal./gram molecule *less* than decomposition across the shorter sides, and would result in formation of *trans*-stilbamidine and not *cis*-stilbamidine. There is already some evidence (Henry, 1946) that photochemical decomposition of the dimer produces *trans*-stilbamidine only, and not *cis*-stilbamidine or a mixture of the two isomers ; actual proof of this point is rendered difficult by the ready interconvertibility of the *cis*- and *trans*- forms on irradiation, and by the convertibility of *cis*-stilbamidine into the *trans*-isomer at high temperatures (Henry, 1945). The rectangular shape of the *cyclo*butane nucleus is likely to have some bearing upon the question of why *cis*-stilbamidine shows no tendency to undergo photochemical dimerization whereas *trans*-stilbamidine does so readily.

Summary

1. A number of points arising out of recent publications dealing with the photo-chemical changes which stilbamidine undergoes on irradiation are discussed.

2. Hydrolysis of the amidine groups has been shown to be dependent primarily upon the temperature and the *p*H of the solution. It is a dark reaction.

3. The dimer has been shown to be retained in the body after intravenous injection in the same way as is stilbamidine itself.

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