

THE CONSTITUTION OF PHOTOSTILBAMIDINE, THE IRRADIATION PRODUCT OF STILBAMIDINE

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Stilbamidine (4 : 4'-diamidinostilbene β -hydroxyethanesulphonate) has proved an active therapeutic agent in a number of protozoal diseases. During the treatment of Gambian sleeping sickness, Bowesman (1940) noted that solutions which had been made up for more than six hours were more toxic than those freshly prepared. Kirk (1942) suggested that the poisonous effects noted by him during the treatment of kala-azar in the Sudan were due to the use of old solutions of stilbamidine which had become toxic. Fulton and Yorke (1942) investigated the matter experimentally and showed that the increased toxicity was the result of photochemical change in the stilbamidine molecule. Similar changes were observed by Fulton (1943) in other diamidines with an unsaturated linkage. The saturation of the ethylene linkage under these conditions was indicated by the spectrographic observations of Goodwin (1943). Barber, Slack, and Wien (1943) came to the conclusion, without rigorous proof, that the irradiation product from stilbamidine was 4 : 4'-diamidinophenylbenzylcarbinol formed by the addition of water to the ethylenic bond. Henry (1943) suggested, as an alternative to carbinol formation, a number of other changes which may occur in solutions of stilbamidine exposed to light including dimerization, partial or complete hydrolysis of the amidine groups, and formation of *cis*-stilbamidine. The two latter suggestions could not be confirmed by Fulton and Goodwin (1946). In a later paper the same author (Henry, 1946) stated that aqueous solutions of *trans*-4 : 4'-diamidinostilbene undergo two concurrent changes—dimerization and *cis-trans* isomerism—when irradiated in tropical sunlight. The proof of the dimeric nature of the saturated compound formed was arrived at from the kinetics of the photochemical reaction. The nature of this saturated compound has now been investigated. Ciamician and Silber (1902) described the formation of a dimer from stilbene when

exposed for two and a half years to sunlight in benzene solution. Their work was confirmed by Stobbe (1914) who showed that the dimer could be isolated after a few days' exposure to sunlight. We have obtained the dimer described by them by irradiating stilbene in benzene with the light of a mercury vapour lamp and have used it as a reference compound to prove that the saturated product obtained from stilbamidine by irradiation is 1 : 2 : 3 : 4-tetra-(4'-amidinophenyl)-cyclobutane, which we have, for simplicity, called photostilbamidine.

Our thanks are due to May and Baker, Limited, for the gift of 50 g. stilbamidine with which these experiments were conducted.

EXPERIMENTAL

In the preparation of photostilbamidine, *trans*-4 : 4'-diamidinostilbene β -hydroxyethanesulphonate (50 g.) in 1 per cent (w/v) solution in distilled water was exposed in 500 c.c. volumes in pyrex conical flasks to direct sunlight. When freshly prepared, the solutions, before irradiation, gave rise immediately to a bulky precipitate on mixing with an equal volume of dilute sulphuric acid. After exposure of the solutions for 2 days, precipitation with sulphuric acid no longer occurred at once. At the end of one week's exposure to sunlight an equal volume of 2*N*-sulphuric acid was added to the contents of the flasks and on standing a crystalline precipitate separated. The yield of this first fraction after drying in air was 33 g. The solution was concentrated to 1,800 c.c. under reduced pressure at a temperature below 50° C., and on standing a further crop of crystals (1.9 g.) was obtained. The solution was partly neutralized with anhydrous sodium carbonate, and mixed with 600 c.c. of a saturated solution of sodium picrate; a bulky precipitate containing sulphate ions was formed which was collected, air dried, and triturated with 16 per cent aqueous hydrochloric acid. The free picric acid was collected and washed with hydrochloric acid. The combined filtrates were extracted with ether, and heated with norite to remove all traces of picric acid. A further

2.0 g. of photostilbamidine as sulphate was obtained on concentration of the solution. The absorption spectra of the various crystalline fractions and solutions were obtained during the course of the preparation. Apart from the presence of small amounts of unchanged stilbamidine, amounting to approximately 5 per cent of the original, the only other substance detected was photostilbamidine. The latter, as sulphate, was purified for analysis by recrystallization from water in which it was sparingly soluble; it crystallized in short rods with terminal branching, and melted between 280 and 290°. Purified *photostilbamidine sulphate* in solution showed only the faintest fluorescence in ultra-violet light in contrast to the marked fluorescence of solutions of stilbamidine. The air-dried product was analysed (Found: C, 44.5; H, 6.2; N, 12.6; H₂O, 15.7. C₃₂H₃₂N₈, 2H₂SO₄, 8H₂O requires C, 44.2; H, 6.0; N, 12.9; H₂O, 16.6%). Photostilbamidine sulphate dissolved in water and treated with the theoretical amount of barium chloride gave a very soluble crystalline dihydrochloride. When the solution of the latter was mixed with ammonium nitrate in excess, a crystalline *nitrate* was deposited. Recrystallization from water gave compact crystals, m.p. 288° (decomposition). (Found in air-dried solid: C, 46.6; H, 5.3; N, 20.1; H₂O, 5.7. C₃₂H₃₂N₈, 4HNO₃, 3H₂O requires C, 46.0; H, 5.1; N, 20.1; H₂O, 6.5%.)

Hydrolysis of stilbamidine and photostilbamidine sulphate

Photostilbamidine sulphate (1.3 g.) was mixed with potassium hydroxide (5 g.), water (5 c.c.), and absolute alcohol (45 c.c.), and boiled for 22 hours with the addition of water (15 c.c.) at the start of each day to dissolve some of the salts present. An equal volume of water was then added to the mixture and the alcohol distilled off. After removal of a small amount of insoluble material, the filtrate was acidified and yielded a gelatinous precipitate which became granular when heated on the water bath. It was collected, thoroughly washed with water, and redissolved in sodium bicarbonate solution; the solution was filtered from inorganic material and acidified. The product was again dissolved in aqueous sodium bicarbonate and reprecipitated. The yields of acid in two experiments each from 6.7 g. of starting material were 3.80 g. and 3.85 g., after drying at 90°. The product was nitrogen-free and required the theoretical amount of base for neutralization. On crystallization from glacial acetic acid, a small amount of insoluble material, possibly stilbenedicarboxylic acid, was removed. After three recrystallizations, 0.39 g. of the product required 35 c.c. of boiling glacial acetic acid for solution and gave 0.28 g. of a colourless product. Two forms of crystal were present, a needle-shaped variety being preponderant with some hexagonal plates. The *tetra-acid* was also soluble in ethyl alcohol, acetone and ethyl acetate and did not melt below 310°. (Found in solid dried at 90°: C, 69.4; H, 5.1; loss at 110°, 3.1. C₃₂H₂₄O₈, H₂O requires C, 69.3;

H, 4.7; H₂O, 3.2%.) The *methyl ester* was prepared by the action of an excess of diazomethane in ether on 0.1 g. of purified acid in 10 c.c. of methyl alcohol; after leaving overnight a clear solution was obtained, and when the solvent was removed an oil separated which soon solidified to a colourless solid. It crystallized from methyl alcohol in very small plates; m.p. 166°. (Found in air-dried material: C, 72.9; H, 5.4. C₃₃H₃₂O₈ requires C, 73.0; H, 5.4%.)

The hydrolysis of stilbamidine was found to be a much more difficult process. When 4:4'-diamidinostilbene dihydrochloride (2.5 g.), potassium hydroxide (10 g.) in water (10 c.c.) and absolute alcohol (90 c.c.) were refluxed for 20 hours in the dark, only 130 mg. of an acid were obtained by the procedure described above. In another experiment the same material (2.5 g.), sodium hydroxide (5 g.) in water (10 c.c.) and ethylene glycol (40 c.c.) were refluxed for 9 hours. The insoluble material present was removed and the filtrate concentrated under reduced pressure; the sodium salt obtained was collected, redissolved in water, and by the usual procedure yielded 0.54 g. acid, which was nitrogen-free, was insoluble in all common organic solvents and could not be crystallized. The ammonium salt, however, was very characteristic and crystallized readily in sheaves of leaflets. The solid first collected after alkaline hydrolysis weighed 1.57 g. and contained nitrogen. Hydrolysis of this material was effected by refluxing with 60 per cent sulphuric acid (25 c.c.) for 7 hours and then for a further 7 hours after addition of an equal volume of sulphuric acid of the same strength. On cooling the mixture was poured into water, the solid collected, and after purification by solution in sodium bicarbonate solution the yield of air-dried material obtained on acidification was 0.9 g. and had similar properties to the product of alkaline hydrolysis.

Esterification could not be satisfactorily effected by refluxing the acid (0.5 g.) with methyl alcohol (20 c.c.) and concentrated sulphuric acid (2 c.c.) on the water bath for 4 days. The desired product was, however, obtained by allowing suspensions of the acid, from both alkaline and acid hydrolysis, in methyl alcohol and ether to react with diazomethane. The bulk of the reaction product was very sparingly soluble and separated as small rhomboidal plates; a further small amount was obtained by concentration of the mother liquors. This methyl ester was very sparingly soluble in boiling methyl alcohol but crystallized well from dioxane in glistening fasciated laths; the addition of methyl or isopropyl alcohol to this solvent favoured quantitative separation. The ester from both products had m.p. 234°. Meyer and Hofmann (1917) gave the m.p. of dimethyl *pp'*-stilbene-dicarboxylate as 226–227° and Hager *et al.* (1944) recorded the m.p. as 227–228°.

Decarboxylation of 1:2:3:4-tetra-(p-carboxy-phenyl)-cyclobutane. Isolation of distilbene, isodistilbene and stilbene

The tetracarboxylic acid (0.5 g.) was mixed with quinoline (10 c.c.) and copper bronze (0.5 g.) and the

mixture boiled for 7 hours on a sand-bath. Evolution of carbon dioxide was slow at first but soon increased as the acid dissolved. On cooling, the mixture was poured into excess of 2*N*-hydrochloric acid and extraction of the product was effected by several portions of ether. The combined ether extracts were washed in turn with *N*-hydrochloric acid, sodium bicarbonate solution, and finally water. On removal of the solvent, 0.30 g. of a crystalline material was obtained. It was recrystallized from ether and separated as a mixture of fine prisms and colourless plates. The experiment was repeated with 1.5 g. acid, yielding 1.0 g. of product, which when refluxed with ether did not all go readily into solution. The less soluble portion was collected separately and crystallized from this solvent in the form of fine prisms, m.p. 163°. (Found: C, 93.3; H, 6.7. Calc. for $C_{28}H_{24}$: C, 93.3; H, 6.7%. M.W. in camphor, 292. Calc., 360.) The properties of this hydrocarbon agree with those described for distilbene by Ciamician and Silber and it must be 1:2:3:4-tetraphenylcyclobutane. The more soluble portion separated on concentration of the first mother liquors, as colourless plates. They were shown by m.p. and mixed m.p. with a pure specimen to be stilbene. The yield of stilbene was approximately 1/5 that of the other product. When decarboxylation under the same conditions was continued for 10 hours with 3.0 g. acid obtained from carefully purified photostilbamidine sulphate the product weighing 2.15 g. was highly coloured and contained a small amount of tarry material. It was also much more soluble in ether, and from this solvent large crystals of stilbene separated first. The only other product obtained was that melting at 163°. It seemed that some decomposition had occurred during this longer decarboxylation and stilbene was present in relatively greater quantity than in the preceding experiment. A final decarboxylation with similarly purified material (1.0 g.) lasting 4½ hours yielded 0.68 g. of product. By fractional crystallization from ether a large crop of the product m.p. 163° was obtained. The next fraction yielded hexagonal plates which when crystallized from ether or methyl alcohol melted at 149° in a total yield of approximately 70 mg. The only other product obtained was a few milligrams of stilbene. It appeared, therefore, that the yield of stilbene increased with increase of the reaction time, and the hydrocarbon m.p. 149° could not be isolated when the reaction was continued for more than than 4½ hours. The new hydrocarbon could be crystallized from ether or methyl alcohol and separated from the latter solvent in small tablets. As it is isomeric with distilbene, the name *isodistilbene* is proposed for it. (Found: C, 92.7; H, 6.8. $C_{28}H_{24}$ requires C, 93.3; H, 6.7. M.W. in camphor, 307. Calc., 360.)

Stilbene-4:4'-dicarboxylic acid on decarboxylation in the presence of quinoline and copper bronze for 4½ hours gave a theoretical yield of hydrocarbon. Careful fractionation from ether and absorption on alumina failed to reveal any other hydrocarbon than

stilbene. When stilbene and the hydrocarbon m.p. 163° were respectively heated with quinoline and copper under the same conditions, each substance was recovered unchanged from the reaction mixture.

Fractionation of the acid product from photostilbamidine

In order to throw light on the presence of stilbene obtained along with the hydrocarbon m.p. 163° in all the decarboxylation experiments, the photostilbamidine used in the preparation of the acid was examined for purity. In water it showed fluorescence similar to that of a weak solution of stilbamidine and spectrophotometric determinations indicated that about 5 per cent of this substance was present. Accordingly 14.4 g. of the original photostilbamidine was recrystallized from water and 0.62 g. of very sparingly soluble material with all the properties of stilbamidine sulphate, including characteristic absorption spectrum, was collected. Further light on the homogeneity of the original photostilbamidine was sought by an examination of the acid obtained from it. This acid was subjected to a fractionation process depending on the different acidities of the acids (cf. King and Ware, 1941). For this purpose 3.62 g. of acid were accurately neutralized with 27 c.c. of approximately *N*-sodium hydroxide in a total volume of about 150 c.c. of aqueous solution. The solution was then saturated with ether and a layer of 50 c.c. of the same solvent added. 2.7 c.c. of *N*-hydrochloric acid was then added slowly with shaking. Part of the precipitated acid in this first fraction remained undissolved by the ether. Ten further similar volumes of hydrochloric acid were added in turn, and the liberated acid dissolved in the freshly added ether layer in each case on shaking. After removal of solvent the weights of acid in grams obtained from each fraction in turn were as follows: 0.05 (insoluble) plus 0.06 (soluble); 0.17; 0.33; 0.40; 0.50; 0.45; 0.44; 0.44; 0.44; 0.15. All the fractions, in methyl alcohol, were methylated by means of diazo-methane. From each, the methyl ester already described of m.p. 166° was obtained. Only the insoluble part of the first fraction yielded the methyl ester of stilbene-dicarboxylic acid, m.p. 234°. The amount of this acid present was therefore probably less than 2 per cent and this amount failed to explain the presence of stilbene in the relatively large amounts found after decarboxylation.

Irradiation of Stilbene

In contrast to the rapidity and completeness with which photochemical change occurs in stilbamidine solutions, the reaction in stilbene solutions occurs relatively slowly and incompletely. The formation of a distilbene m.p. 163° was described by Ciamician and Silber (1902) who exposed stilbene, in benzene solution, for 2½ years to sunlight. The same product was obtained by Stobbe (1914) after 3 days' exposure, but the percentage yield obtained was not indicated

by any of these authors. We exposed 1 g. of stilbene in 10 c.c. of pure benzene to sunlight (not intense) for six weeks in a sealed tube but failed to isolate any of the dimerization product. The same solution was further exposed in a quartz cell 1 cm. deep to light of wavelength 250 $m\mu$ for 4 hours again without success. On further irradiation with the light from a mercury vapour lamp for 14 hours, the solvent was removed *in vacuo* and the crystalline residue redissolved in ether which was then concentrated. Fine prisms weighing 60 mg., readily distinguishable from the plates characteristic of stilbene, were the first to separate and a further smaller crop of prisms was obtained. On purification from ether the prisms melted at 163°, and from their properties, including stability to permanganate, non-absorption of bromine and unchanged melting point on admixture, appeared to be the same substance as that described by the above authors. There was no change in melting point on admixture with the decarboxylation product m.p. 163° described above. The substance produced by the irradiation of stilbene and the decarboxylation product were shown to be identical by means of x-ray crystallographic analysis (Fulton and Dunitz, 1947) and by comparison of their absorption spectra (Good-

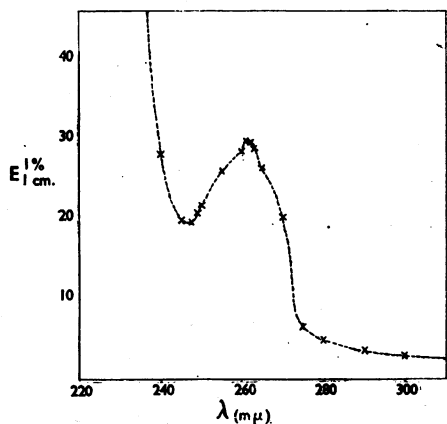


FIG. 1.—Absorption spectrum of 1:2:3:4-tetraphenylcyclobutane in cyclohexane.

win, 1947) in cyclohexane (Fig. 1). Theoretically the position of the absorption band and the intensity measured as $E_1^{1\%}$ should be very similar to those for toluene, and this was found to be the case.

		max.	$E_1^{1\%}$ 1 cm.
Compound	m.p. 163°	261 $m\mu$	29.9
Toluene	...	262 $m\mu$	32.7

When a stilbene solution in benzene was irradiated by the light of a mercury vapour lamp for 30 hours there was obtained besides unchanged stilbene and the hydrocarbon m.p. 163° a substance in yield of only a few milligrams, which crystallized in colour-

less needles from ether and had m.p. 213°. Analysis indicated that it contained oxygen. Because of the extremely small amount available its nature could not be determined. In another experiment in which 1.35 g. stilbene in benzene was exposed under the same conditions the yield of hydrocarbon m.p. 163° was 240 mg.

DISCUSSION

On exposure of stilbamidine solutions to sunlight the only product obtained, apart from small amounts of unchanged material, was photostilbamidine, isolated as sulphate. We now have clear chemical evidence that photostilbamidine is a dimerization product of stilbamidine. Analyses of photostilbamidine sulphate and nitrate show that the elements of water are not involved in the change of stilbamidine into photostilbamidine. When photostilbamidine was hydrolysed an acid was obtained different from stilbene-*p*:*p'*-dicarboxylic acid but of the same fundamental composition. It gave a methyl ester, m.p. 166° different from the methyl ester of stilbene-*p*:*p'*-dicarboxylate which has m.p. 234°. On decarboxylation of the acid from photostilbamidine, a hydrocarbon, $C_{28}H_{24}$, m.p. 163° was obtained identical with distilbene first isolated by Ciamician and Silber from the irradiation of benzene solutions of stilbene. A second isomeric hydrocarbon isodistilbene m.p. 149° was found in one decarboxylation experiment.

If during the irradiation of *trans*-stilbamidine the activated molecule retains its *trans*-structure, combination of two activated *trans*-molecules can give rise to two isomeric products which on hydrolysis and decarboxylation should yield the two tetraphenylcyclobutanes A and B.

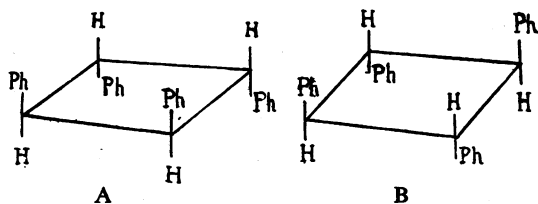


FIG. 2.

If however the activated molecule passed through or into a *cis*-form owing to free rotation, combinations involving *cis*-forms would give rise in addition to two other isomeric hydrocarbons.

It has been shown (Fulton and Dunitz, 1947) that the distilbene, m.p. 163° is represented by A with a centre of symmetry. The spatial configuration of the isomeride, isodistilbene, m.p. 149° has not yet been determined, but the probability is that

it has structure B. Since photostilbamidine in all experiments gave the hydrocarbon A, usually as the major component, the structure of photostilbamidine is that shown in A with four amidine groups in the *p*-positions of the phenyl groups.

Since isodistilbene was isolated on one occasion it is possible that photostilbamidine sulphate contains a component corresponding to structure B. The acid obtained by hydrolysis of photostilbamidine, on crystallization from acetic acid, showed inhomogeneity of crystal form and on decarboxylation under milder conditions gave two isomeric hydrocarbons apart from stilbene. On the other hand, careful fractionation of the acidic product obtained on hydrolysis of photostilbamidine failed to show the presence of any isomeric tetracarboxylic acid since all the fractions gave the same methyl ester m.p. 166°. The most likely explanation of this is that the isomeric tetracarboxylic acid which gives rise to the hydrocarbon, m.p. 149° must have been eliminated in the numerous purification stages involved in the preparation of the tetra-acid from photostilbamidine before subjection to decarboxylation.

The formation of stilbene in the decarboxylations of the tetra-acid is definitely not due to the presence of stilbene-dicarboxylic acid as an impurity. Stilbene arises from very specially purified photostilbamidine and its proportion increases with the duration of the decarboxylation process.

SUMMARY

Irradiation of a solution of *trans*-stilbamidine in sunlight gave a substance which on hydrolysis to the corresponding acid, followed by decarboxyla-

tion, yielded a hydrocarbon m.p. 163° identical with that obtained by irradiation of stilbene. The hydrocarbon previously described as di-stilbene has now been shown by x-ray crystallographic analysis to be 1 : 2 : 3 : 4-tetraphenyl-cyclobutane, containing a centre of symmetry. A second isomeric hydrocarbon m.p. 149° was also obtained in the same series of reactions, but its molecular configuration has not yet been determined. The isolation of these two hydrocarbons is in agreement with the view that stilbamidine undergoes dimerization on irradiation of its solutions.

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