230. *The Bromination of 4 : 4′-Diamidino-αγ-diphenoxypropane and of 4 : 4′-Diamidino-stilbene.*

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Bromination of 4 : 4′-diamidino-αγ-diphenoxypropane (propamidine, I) in aqueous solution involves substitution in the benzene rings, whereas bromination of *trans*-4 : 4′-diamidinostilbene (stilbamidine, II) consists of rapid quantitative addition at the ethylenic linkage. Both (I) and (II), and their bromination products, can form complexes with free bromine and iodine. The bromination of *trans*-(II) is reversible while that of *cis*-(II) is irreversible. Compound *cis*-(II) has no fluorescence in the visible spectrum.

A study of the bromination of these compounds was undertaken primarily to devise a satisfactory procedure for studying the kinetics of the photochemical decomposition of *trans*-(II) in aqueous solution; preliminary experiments had indicated that bromination of an irradiated solution before and after precipitation of *trans*-(II) as the sulphate, which has a low solubility in water, would afford a ready means of following the progress of the reaction.

At 35° the bromination of (I) in dilute solution with a 50% excess of bromine proceeds smoothly with entry of two bromine atoms into the benzene rings. Under these conditions there is usually some separation of bright orange-yellow needles towards the end of the reaction. At 25° or with higher concentrations, this separation of crystals is much more pronounced. These crystals consist of a tetrabromo-complex of the hydrobromide of brominated (I) and, in solution, the additional bromine is readily available for oxidation purposes (*e.g.*, neutral potassium iodide). The tetrabromo-complex loses two atoms of bromine on being warmed, reverting to a dibromo-complex which is stable at 100° in absence of moisture; it readily loses its remaining active bromine at 200°. Both complexes have a strong odour of bromine and, on exposure to a moist atmosphere, the active bromine is ultimately lost leaving dibromopropamidine hydrobromide (III). The formation of the complex involves no liberation of acid, and is therefore simply an addition process.

There is evidence that all the amidines which have been encountered can form similar complexes with bromine and iodine. If the concentration of either (I) or free bromine is sufficiently high or the temperature sufficiently low, (I) produces an immediate orange-yellow precipitate of the bromo-complex of the unbrominated molecule which seriously interferes with the normal bromination process. This precipitate dissolves on being heated and, later, on allowing the solution to cool, a complex of (III) crystallises. On addition of excess of bromine in potassium bromide to a 1% solution of *trans*-(II) an immediate orange-yellow precipitate

is formed if the temperature is fairly low. Since this precipitate redissolves on shaking it is presumably a bromo-complex of unbrominated *trans*-(II).

Following the course of the bromination of (I) or the estimation of *trans*-(II) and the products of its photochemical decomposition necessitates addition of potassium iodide to react with the excess of bromine present in the reaction system. If concentrations and reaction temperature have been suitably chosen there is no solid bromo-complex present in the solution. On addition of the potassium iodide reddish-brown flocculent precipitates are formed unless the concentrations are very low. These precipitates gradually dissolve as the titration of the iodine with thiosulphate proceeds but, sometimes, they become crystalline or gummy; in either case they redissolve only with difficulty. After all free iodine in solution has been reduced further shaking results in return of the brown colour of the iodine. In these cases alternate shaking and titration of the iodine must be continued until there is no more solid in suspension before addition of starch and the completion of the titration. This process can be very troublesome, and for quantitative work, particularly when estimating *trans*-(II), conditions should be chosen so as to minimise the effect. Iodine in potassium iodide has no direct action on the ethylenic linkage of *trans*-(II), but a tetraiodo-complex rapidly precipitates.

There is another source of error in the estimation of *trans*-(II). Even when all traces of undissolved iodo-complex have disappeared and the titration has been carried to its end-point with starch and thiosulphate there is invariably a rapid and persistent return of the blue colour, due to slow liberation of bromine and reversion to the original *trans*-(II). In contrast a stable end-point is obtained with (I) in spite of the acidity developed during the bromination. In solution, in presence of potassium iodide, the liberated bromine is removed as fast as it is formed and debromination can proceed to a significant extent. The first end-point reached in the titration immediately after all traces of solid iodo-complex have disappeared must therefore be taken as the correct end-point. As the liberation of bromine is proceeding at the same time as the iodo-complex is dissolving the importance of using only a small excess for the bromination will be appreciated. The rate of debromination is reduced by lowering the temperature; this, however, aggravates the difficulties due to precipitation of the complex on account of its reduced solubility.

The reversibility of the bromination of *trans*-(II) has been confirmed by demonstrating (by application of the method of spotting-out on filter-paper, Henry and Grindley, *Ann. Trop. Med. Parasitol.*, **1942**, **36**, 102; **1945**, **39**, 1) that, after removal of excess of bromine by a current of air, *trans*-(II) slowly appears in the solution. The *trans*-(II) produced in presence of potassium iodide has been isolated *via* the tetraiodo-complex. The reverse reaction takes place in the dark; it appears to be uninfluenced by exposure to direct sunlight, except that any *trans*-(II) previously produced is partially converted into *cis*-(II). Compound *trans*-(II) may not be the only reaction product, and the possibility of condensation to form a *cyclo*butane derivative cannot be ignored. It is not yet known whether the dibromo-derivative *viz.*, αβ-dibromo-αβ-(4 : 4′-diamidinophenyl)-ethane (IV), is the racemic or the *meso* form.

It was considered that debromination of (IV), which would probably take place in presence of body fluid and tissue as readily as in presence of potassium iodide, might have important pharmacological use. Compound *trans*-(II) is highly trypanocidal *in vitro*, and has a strong curative action in the early stages of trypanosomiasis; it is, however, ineffective in the late stages of the disease owing to its inability to penetrate the blood-brain barrier (Lourie, *Trans. Faraday Soc.*, **1943**, **39**, 340). Compound (IV) is less strongly adsorbed by filter-paper than is *trans*-(II). If it is also less strongly adsorbed by body tissue it might be capable of penetrating the blood-brain barrier in significant amount, subsequently undergoing loss of bromine to give *trans*-(II) which should then be capable of exerting an action upon the parasites in the nervous system. Should this occur it would be closely parallel, in effect, to the behaviour of tryparsamide. The toxicity to mice of (IV) is, however, approximately twice as great, on a molecular basis, as that of *trans*-(II) which might preclude its successful use in the manner indicated.

The only specimens of *cis*-(II) which have been available are those recovered from irradiated solutions of *trans*-(II), and have not been entirely free from unchanged *trans*-(II). Brominated *cis*-(II) appears to show no tendency to revert to the parent compound, a small amount of the *trans*-compound which slowly made its appearance being attributed to debromination of a little *trans*-(II) present as an impurity in the original preparation. During bromometric estimations of *cis*-(II), produced by irradiation, no persistent return of the blue colour, similar to that found in the estimation of *trans*-(II), has been observed.

Recent experiments leave no doubt that *cis-trans* isomerisation is one of the photochemical changes which *trans*-(II) undergoes in solution, and that equilibrium is established between the two forms. There is also no reasonable doubt that *cis*-(II) has no fluorescence of its own in the visible spectrum, the fluorescence which rapidly appears in a solution of the *cis*-compound, on exposure to ultraviolet light (Barber, Slack, and Wien, *Nature*, **1943**, **151**, 107; R. Slack, Private Communication), being attributed to the *trans*-compound produced by the action of the light. This phenomenon is spectacularly evident on spotting-out a solution of *cis*-(II) on filter-paper, allowing it to dry and exposing it to ultra-violet light. A small amount of *trans*-(II) present in the solution of the *cis*-compound is readily detected by the central annulus of typical *trans*-(II) fluorescence which shows up immediately on exposure to ultra-violet light, and can be estimated by comparison with standard spots provided this is done at once, before the fluorescence resulting from the presence of the *cis*-compound begins to interfere. On first exposure the zone outside the fluorescent *trans*-(II) annulus is devoid of fluorescence, but with exposure fluorescence steadily develops in this area to an extent and intensity depending upon the concentration of *cis*-(II) in the solution, and can completely mask the original fluorescence due to the *trans*-(II).

The hydrochloride of *cis*-(II) has a very indefinite melting point; on melting it is partly converted into *trans*-(II).

Experimental.

Materials.—The materials available were the isethionate of (I) and the hydrochloride and isethionate of *trans*-(II). The isethionates can be converted to the hydrochlorides by addition of concentrated hydrochloric acid and recrystallisation from water. The hydrochloride of (I) had m. p. 281—283°, and *trans*-(II) hydrochloride had no m. p. up to 325° (Ashley *et al.*, *J.*, 1942, 103, gave 292° and 300° respectively). In both cases N and Cl′ contents were slightly low owing, probably, to slight hydrolysis having occurred. Solutions of *trans*-(II) have a definite yellow colour, uninfluenced by repeated crystallisation.

Hydrolysis of the amidine groups can occur in suitable circumstances in the case of *trans*-(II), but hydrolysis to a small extent would have little effect upon the course of the bromination. The first hydrolysis product of *trans*-(II), 4-amido-4′-amidinostilbene monohydrochloride dihydrate (Henry, *Nature*, 1943, **152**, 690), brominates quantitatively and also forms complexes with bromine and iodine. It deposits as well-formed needles from 1% solutions of *trans*-(II) hydrochloride left standing for some months, has m. p. 320° (Found : N, 12·5; Cl′, 10·4; Br absorption, 470 mg. per 1 g.; loss at 110°, 10·3. $C_{16}H_{15}ON_3,HCl,2H_2O$ requires N, 12·5; Cl′, 10·5; Br absorption, 473 mg. per 1 g.; H_2O, 10·7%), and forms a nitrate of low solubility, m. p. 291°. From the low solubility in water, the close similarity of its fluorescence and adsorption properties to those of *trans*-(II) (Henry and Grindley, *loc. cit.*) and the fact that it can be formed from the latter in the dark there can be no doubt that the compound examined was the *trans*-modification.

Bromination Apparatus and Procedure (see Henry, *Analyst*, 1945, **70**, 259).

Bromination Results.—(a) 4 : 4′-*Diamidino-αγ-diphenoxypropane.* At 36° in 0·005M solution with a 50% excess of bromine over that required for formation of the dibromo-derivative the reaction was virtually complete in 12—16 hours. After this stage a further slow bromine reduction above four atoms per mol. continued owing, probably, to further slow substitution occurring.

(b) trans-4 : 4′-*Diamidinostilbene.* Exposure of the solution to daylight prior to the addition of the bromine must be avoided as ultra-violet light produces changes which result in reduction of bromine-absorbing capacity. In addition, concentrations, temperature and amount of excess bromine must be such that there is no interference due to the formation of complexes or to the reversal of bromination. Under favourable conditions and with correct attention to the above points, the bromine absorption of *trans*-(II) hydrochloride dihydrate was invariably within 1% of theoretical (428 mg. per 1 g.). Duplicate estimations on 10 c.c. of a 1% solution were 427, 429 mg. per 1 g. The bromine absorption of the isethionate, dried at 110°, was 312 mg. per 1 g. (calc. : 310 mg.). With both hydrochloride and isethionate the same absorption was obtained after 30 minutes' contact with the bromine as after 3 minutes' contact, showing no detectable bromination of the benzene rings during the contact period.

Tetrabromo-complex of Dibromopropamidine Hydrobromide (V).—On addition of 0·05N-bromine in 2% potassium bromide (1200 c.c.) to (I)-isethionate (5 g.) in water (500 c.c.) an immediate, bulky, brownish-yellow precipitate of the bromo-complex of the hydrobromide of unbrominated (I) was produced, which could not be redissolved by shaking for three hours or by addition of more water (500 c.c.) and further shaking. The precipitate was dissolved by heating to 65—70°, the solution maintained hot for 6—8 hours to ensure completion of bromination and more bromine (1·5 c.c.) added to replace loss. On allowing to cool, a heavy crop of bright orange-yellow needles formed (yield of air-dry material, 88%) (Found : N, 5·6; active Br, 32·1. $C_{17}H_{18}O_2N_4Br_2,2HBr,4Br$ requires N, 5·9; active Br, 33·6%). It is probable that the *compound* is anhydrous, but this was difficult to establish owing to the difficulty of drying without loss of bromine. On exposure to a warm, humid atmosphere bromine was steadily lost until, after 7 days, all traces of bromo-complex had disappeared and a colourless, *anhydrous* residue of (III) remained (Found : N, 9·0; Br′, 24·9. $C_{17}H_{18}O_2N_4Br_2,2HBr$ requires N, 8·9; Br′, 25·7%).

On heating (V) at 110° in a small bulb provided with a long stem, to minimise access of air, bromine was expelled until the residue was of constant weight corresponding to the *dibromo-complex* (2 hours, 16·7% loss; 3 hours, 17·1%; 18 hours, 17·4%. Loss of 2Br from (V) requires 16·8%). Subsequent heating for 1½ hours at 150° resulted in a further loss of 0·2%, but at 200° rapid loss of bromine occurred. The product having constant weight obtained at 110° (64 hours) lost 19·5% on exposure to moist air at atmospheric temperature ($C_{17}H_{18}O_2N_4Br_2,2HBr,2Br$ requires 20·2% loss).

The *hydrochloride* of (III), produced by precipitation from solution with conc. hydrochloric acid and recrystallisation from acidified water, had m. p. 288° (Found : N, 9·7; Cl′, 13·2. $C_{17}H_{18}O_2N_4Br_2,2HCl$ requires N, 10·3; Cl′, 13·1%). After prolonged hydrolysis with alcoholic sodium hydroxide ionisable halogen was still 13·1%, showing no hydrolysis of bromine and that substitution was in the benzene rings.

In order to show that no liberation of acid accompanies formation of the bromo-complex, and in consequence that no substitution of hydrogen occurs (*e.g.*, the imino-hydrogen atoms) the hydrochloride of (III) was made the starting point so as to minimise the effect of acidity resulting from the normal bromination. The theoretical quantity of bromine in potassium bromide to produce (V) was added (yield 90%; active Br, 31%), the complex separated and the filtrate debrominated by a stream of air. The acidity was the same as in the blank.

Tetraiodo-complex of trans-(II) *Hydriodide.*—Dropwise addition with constant shaking of 0·1N-iodine in potassium iodide to dilute solutions of *trans*-(II) hydrochloride at 60° precipitated the *tetraiodo-complex* of *trans*-(II) hydriodide as dark-brown, microscopic needles, irrespective of the proportion of iodine to *trans*-(II) used. The theoretical proportion of iodine produced nearly quantitative precipitation of *trans*-(II) as the iodo-complex. The filtrates from the precipitates, after removal of any free iodine, were neutral. The separated complex dissolved only very slowly on shaking with potassium iodide solution, and direct estimation of active iodine in this way was not satisfactory. It was moderately stable at 110° (loss 1% per hour) but, at 200°, the active iodine was fairly rapidly expelled leaving a nearly colourless residue having constant weight (Found : Loss, 48·7. $C_{16}H_{16}N_4,2HI,4I$ requires 49·4%).

Reversibility of Bromination of trans-(II).—(*a*) The hydrochloride of *trans*-(II) (1% solution) was brominated, in artificial light, by addition of a slight excess of strong, freshly prepared bromine water, each drop of which produced a yellow precipitate which dissolved on stirring. The excess of bromine was quickly removed by passing a stream of air for five minutes, the solution was then spotted-out on filter-paper, again aerated for two hours and again spotted-out. Thereafter it was left to stand at 35° in the dark and was spotted-out at intervals.

(*b*) The hydrochloride of *cis*-(II) containing 2·5% of *trans*-(II) as determined by the spotting-out test, was brominated under the same conditions as in (*a*). Again, each drop of bromine water produced a yellow precipitate which dissolved on stirring. The excess of bromine was removed as above, and the solution spotted-out at similar intervals.

(*c*) The hydrochloride of *trans*-(II) (0·077 g.), brominated by the normal analytical procedure, absorbed bromine equivalent to 22·23 c.c. of 0·01854N-$Na_2S_2O_3$. At this stage, the solution (*ca.* 100 c.c.) contained, in addition to (IV), potassium bromide and iodide, sodium tetrathionate and starch. It was left to stand at 35°, the liberated iodine titrated with the same thiosulphate solution at frequent intervals and the decolorised solution spotted-out as above.

It was found, in the case of these spots, that a second zone of fluorescence developed rapidly in the dark outside

the *trans*-(II) annulus. This effect was not due to *cis*-(II), as *cis*-(II) gives spots which are stable in the dark for at least twelve hours and do not develop fluorescence until exposed to ultra-violet light. In order to avoid interference by this outer zone fluorescence in the comparison of the *trans*-(II) annulus with the standards it was necessary either to make the comparison immediately after the spot had dried, or to wash the spot radially immediately after preparation, by the technique developed by Henry and Grindley (*loc. cit.*). The latter procedure is preferable, as a stable preparation results. This effect was hardly detectable in Expts. (*a*) and (*b*) and did not interfere with the estimation of the *trans*-(II) produced.

(*d*) Brominated solutions of *trans*-(II) (*ca.* 1%), plus 10% potassium iodide solution, heated on a water bath, rapidly liberated free iodine, and, after about four hours, a considerable crop of crystals of the tetraiodo-complex of *trans*-(II) hydriodide had formed in the hot solution.

In Expt. (*a*) *trans*-(II) appeared in the solution at a constant rate of 2·0 mg. per 100 c.c. per day, while in Expt. (*b*) its rate of appearance was only 0·2 mg. per 100 c.c. per day. The results of Expt. (*c*) are given in the table. In no case was fluorescence due to *cis*-(II) detected.

Time.		Extra $Na_2S_2O_3$ required (c.c.).	Proportion of (IV) debrominated (%).	Concn. of *trans*-(II) (mg./100 c.c.).	
Hrs.	Mins.			(i) From fluorescence.	(ii) From extra $Na_2S_2O_3$ required.
—	5	0·15	0·7	0·5	0·5
—	35	1·15	5·1	3	3·7
1	40	2·70	12·1	—	8·9
2	5	3·25	14·6	10	10·6
5	—	5·50	24·7	17	18·0
5	25	5·75	25·7	20	18·8

Conversion of cis-(II) *to* trans-(II) *on melting.* With 0·0013 g. of *cis*-(II), containing 2·5% of *trans*-(II), in the m. p. tube, melting commenced at 239° but was not complete until 267°. The lower portion of the tube was then ground with water (2·6 c.c.) to give a solution containing the equivalent of 50 mg. per 100 c.c. of the original *cis*-(II) and the solution spotted-out on filter paper. The *trans*-(II) concentration was estimated, from the fluorescence, to be 15—20 mg. per 100 c.c., representing 30—40% of the original *cis*-(II). The typical fluorescence, due to the presence of *cis*-(II), rapidly developed in the outer zone of the spot during exposure to ultra-violet light.

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