257. *The Photochemical Instability of* cis- *and* trans-4 : 4'-*Diamidinostilbene.*

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Aqueous solutions of *trans*-4 : 4'-diamidinostilbene [*trans*-(I)] on insolation undergo two concurrent changes—dimerisation and *cis–trans* isomerisation—the proportion in which the products of the two reactions are formed being dependent upon both concentration and temperature.   At all concentrations and temperatures the reactions proceed until equilibrium is established between the *cis*- and *trans*-forms, the equilibrium thereafter slowly drifting owing to conversion of the latter into the dimer.   The dimerisation of *trans*-(I) is only very slightly reversible in sunlight, but under the influence of short ultra-violet waves (*ca.* 2500 A.) reversal of dimerisation is rapid and substantial, equilibrium being ultimately established.   *Cis*-(I) has no tendency to

dimerise except indirectly *via* trans-(I). The yellow colour developing on irradiation of trans-(I) is attributed to a secondary reaction product of cis-(I), produced from trans-(I). The product of the dimerisation of trans-(I) is strongly adsorbed by filter-paper and can be estimated fluorimetrically. Solutions of trans-(I) fluoresce in both the blue and the green-yellow of the spectrum, the former preponderating at low, and the latter at intermediate, concentrations. The fluorescence exhibits marked concentration- and temperature-quenching.

INCREASED toxicity of solutions of trans-4 : 4′-diamidinostilbene (stilbamidine) [trans-(I)] on exposure to sunlight was first reported by Fulton and Yorke (*Ann. Trop. Med. Parasit.*, 1942, **36**, 134), and has since been confirmed by Barber, Slack, and Wien (*Nature*, 1943, **151**, 107), Fulton (*Ann. Trop. Med. Parasit.*, 1943, **37**, 48), and Kirk and Henry (*ibid.*, 1944, **38**, 99). The increase in toxicity is also accompanied by decrease in trypanocidal activity (Fulton, *loc. cit.*), pronounced modification of the absorption spectrum (Goodwin, *Ann. Trop. Med. Parasit.*, 1943, **37**, 59), and reduction in capacity for reacting with bromine and permanganate (Barber *et al.*, *loc. cit.*; Fulton, *loc. cit.*). The monomethyl derivative of trans-(I) was found to be affected by irradiation in a closely similar way, and 4 : 4′-diamidinotolane underwent a two-fold increase in toxicity and marked yellowing on irradiation. The 4 : 4′-diamidines of the dimethyl derivative of trans-(I) and of the saturated compounds 1 : 3-diphenoxypropane, 1 : 5-diphenoxypentane, and 1 : 2-diphenylethane showed no significant modification of toxicity, trypanocidal activity, or absorption spectrum on irradiation, though the first developed a strong yellow colour.

FIG. 1.
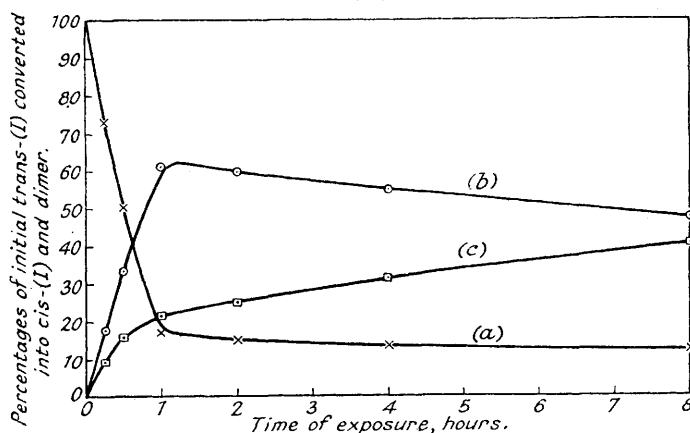
Insolation of 0·2% *soln. of* trans-(I) *hydrochloride. Solution* (47 c.c.) *contained in* "*Pyrex*" *flasks*, 50 c.c. *capacity. Temp.*, ca. 48°. trans-(I) = (a) ; cis-(I) = (b) ; dimer = (c).

From the effect upon the chemical and physical properties of trans-(I) it was clear that it was the ethylenic linkage which was affected by the irradiation, resulting in saturation of the compound. The reaction occurring was considered by Barber *et al.* (*loc. cit.*) to be the addition of water at the ethylenic linkage with formation of 4 : 4′-diamidinophenylbenzylcarbinol (II). This assumption appeared to explain all the known facts, including Fulton's (*loc. cit.*) finding that irradiation and subsequent desiccation yielded a product whose composition corresponded to addition of water to trans-(I), and strong supporting evidence was produced by them. Goodwin (*loc. cit.*) pointed out that, while the modification of the absorption spectrum was in conformity with this view, the observed results would equally well be explained by any process involving saturation of the ethylenic linkage. He also pointed out that the stilbene derivatives can obviously exhibit cis– trans-isomerism, but added : " . . . in the case of the parent substance the isomerides show different band intensities rather than any marked change in position. Although this type of isomerism probably plays a part in determining the photochemical reactivity of the compounds, the slight effect which may be shown in their spectra can, at this stage, be ignored ". Barber *et al.* (*loc. cit.*) considered cis–trans-isomerisation as a possibility, but decided against its occurrence.

The present author's preliminary experiments with freshly prepared 1% solutions of trans-(I) hydrochloride exposed to direct tropical sunlight confirmed the partial conversion into a very soluble bromine-absorbing compound capable of giving rise to a blue fluorescence, similar to that of the parent compound, on exposure to ultra-violet light, and it seemed very probable that this compound was cis-(I), which would be expected to be more soluble than the trans-isomer. At the same time examination of the saturated irradiation product appeared to throw doubt upon its being (II), and to indicate that it was a product of the dimerisation of (I). In consequence the view was put forward (Henry, *Nature*, 1943, **152**, 690) that the main reactions occurring on irradiation were dimerisation, giving 1 : 2 : 3 : 4-tetra-(4′-amidinophenyl)*cyclo*butane (III), and cis–trans-isomerisation (cf. also Kirk and Henry, *loc. cit.*). Shortly after the publication of these views Devine (*Ann. Trop. Med. Parasit.*, 1944, **38**, 35) put forward similar views without, however, giving fresh supporting evidence.

It was suggested (Henry, *loc. cit.*) that the primary photochemical reaction might be cis–trans-isomerisation followed by dimerisation of the resulting cis-isomer, as normally being the more reactive of the two forms. From the shapes, at all concentrations, of the experimental curves (cf. Figs. 1 and 2) in the immediate vicinity

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of the origin it is evident, however, that this is not the case, and that the two products arise independently, and directly, from the original *trans*-(I). In point of fact *cis*-(I) shows no tendency to dimerise except indirectly *via trans*-(I) (*vide infra*).

Trans-(I) has a strong blue fluorescence in dilute solution and when absorbed in filter-paper, and its fluorescent and adsorptive properties have been made the basis of an approximate method of estimation applicable to dilute solutions in water and in the biological fluids (Henry and Grindley, *Ann. Trop. Med. Parasit.*, 1942, **36**, 102; 1945, **39**, 1). It also absorbs bromine quantitatively, and the conditions necessary for its accurate bromometric estimation have been determined (Henry, *J.*, 1945, 870).

FIG. 2.
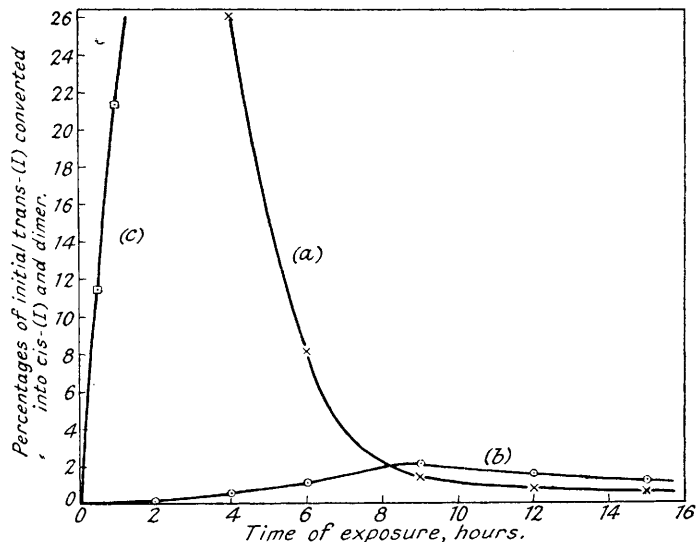
Insolation of 15% *soln. of* trans-(1) *isethionate. Solution* (30 *c.c.*) *contained in " Pyrex " flasks,* 50 *c.c. capacity. Temp.,* 45°. trans-(I) = (a); cis-(I) = (b); dimer = (c).
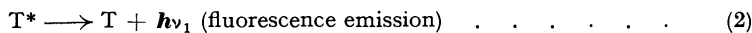
DISCUSSION.

Insolation of neutral solutions of *trans*-(I) gives rise to two principal irradiation products in proportions depending on both concentration and temperature. The principal irradiation product at low concentrations is unsaturated and develops fluorescence on exposure to light, and it and *trans*-(I) are freely interconvertible by irradiation in solution, *via* an equilibrium which, at constant temperature, is independent of concentration. There can therefore be no doubt that this irradiation product is *cis*-(I), and failure of previous workers to detect it is attributable to the conditions—fairly low concentration and rather elevated temperature—required for its formation in substantial proportion.

The principal insolation product at higher concentrations is saturated and non-fluorescent, and is presumably the same product as that which Barber *et al.* consider to be (II). Concurrent formation of (II) and *cis*-(I) from *trans*-(I) should produce the two compounds in ratio independent of concentration, since each would be in effect the result of a unimolecular reaction. That this ratio is definitely not independent of concentration is strong evidence against the validity of the theory that the saturated product is (II). Furthermore, if hydration at the ethylenic linkage can occur at all it is not easy to see why it does not do so at low concentrations. Anhydrous *trans*-(I) isethionate can be converted, by insolation of very concentrated solutions at low temperature, almost quantitatively into the anhydrous, saturated irradiation product *without change in weight*, so disproving carbinol formation and showing polymerisation or an intramolecular change involving saturation, for which there seems no reasonable mechanism. Since the kinetics of the photochemical reaction favour dimerisation as opposed to formation of a higher polymer, there is little doubt that the saturated product is (III), the dimer of *trans*-(I), and this is assumed henceforth. The salts of these compounds are highly ionised in aqueous solution, have low solubilities in ethanol, and the free bases are nearly insoluble in the usual organic solvents. Direct proof of dimerisation by molecular weight determinations therefore appeared too unpromising and was not attempted.

The *cis–trans*-change is freely reversible in direct sunlight, diffuse daylight, and the filtered light (3000—4000 A.) of the mercury lamp. Under these conditions of irradiation the dimerisation reaction shows signs of being only slightly reversible even at high concentrations, but under the influence of the unfiltered light of the mercury lamp reversal of dimerisation, both in solution and when adsorbed on filter-paper and dried, is rapid. There is evidence that reversal of dimerisation produces *trans*-(I) only, and that equilibrium is ultimately established between dimer and *trans*-(I) on the one hand and *trans*-(I) and *cis*-(I) on the other. Reversal of dimerisation is effected by light of frequency 2700—2400 A., which coincides with the absorption band of the

irradiation product found by Goodwin (*loc. cit.*).   Filtration of the light through a 1% solution of the dimer effectively removes the wave-lengths responsible for the reversal of dimerisation.

Since none of these reactions takes place to any detectable extent in the dark it is concluded that all of the compounds concerned are thermo-stable, and that, at room temperature at any rate, all of the reactions are photochemical only.   They may be summarised as follows, in which T, C, and D refer to *trans*-(I), *cis*-(I) and dimer respectively, and * to activated forms :

$$T + h\nu_1 \longrightarrow T^* \qquad\qquad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \tag{1}$$

$$T^* \longrightarrow T + h\nu_1 \text{ (fluorescence emission)} \quad . \quad . \quad . \quad . \quad . \quad . \tag{2}$$

$$T^* \longrightarrow C \qquad\qquad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \tag{3}$$

$$T^* + T \longrightarrow D \qquad\qquad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \tag{4}$$

$$C + h\nu_2 \longrightarrow C^* \longrightarrow T \qquad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \tag{5, 6}$$

$$D + h\nu_3 \longrightarrow D^* \longrightarrow 2T \qquad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \tag{7, 8}$$

Reaction (3) tends to reduce the fluorescence efficiency at all concentrations, and reaction (4) readily accounts for the marked concentration-quenching which *trans*-(I) exhibits.   Neither *cis*-(I) nor the dimer fluoresces in the visible spectrum, but each develops fluorescence on suitable irradiation due to partial conversion into *trans*-(I).

Owing to the almost complete irreversibility of dimerisation in sunlight *trans*-(I) continues to dimerise after equilibrium between *trans*-(I) and *cis*-(I) has been established.   In consequence a steady decrease in concentration of both forms occurs, but the ratio of the two remains constant at a value of 4—5, independent of the initial concentration over the range 1·0—0·02%.   On insolation of concentrated solutions of *trans*-(I) isethionate, values of the final equilibrium *cis*-(I)/*trans*-(I) ratio much lower than the above are obtained.   This is attributed to slight reversal of dimerisation, confirmed by prolonged insolation of spots of dimer solution on filter paper, maintaining the *trans*-(I) concentration at a higher value than corresponds to the true equilibrium value. Insolation of these solutions after dilution to 1% results in rapid readjustment of the *cis*-(I) and *trans*-(I) concentrations to give the *cis*/*trans* ratio characteristic of the diluted solutions.

Variation with temperature of the dimer/*cis*-(I) ratio for the early stages of insolation of solutions (0·2%) of *trans*-(I) hydrochloride shows that at low temperatures very little *cis*-(I) would be produced at this concentration, and that the dimer/*cis*-(I) temperature curve approximates to a rectangular hyperbola.   It is not known whether a similar relationship holds for other concentrations.   At constant initial concentration of *trans*-(I) the rate of conversion of *trans*-(I) into *cis*-(I) and dimer is, in the early stages of the reaction, approximately independent of the temperature, and depends only upon the amount of light received.   Each molecule of dimer produced, however, results from combination of one activated molecule of *trans*-(I) with one unactivated molecule [reaction (4)].   The total proportion of primarily activated *trans*-(I) molecules which are deactivated by conversion into *cis*-(I) and dimer is therefore represented by the sum of the *cis*-(I) and half of the dimer produced.   This sum increases (Table II) as the temperature rises, so that, on the assumption that the quantum efficiency of reaction (1) is independent of the temperature, there are fewer activated molecules remaining at the higher temperatures to revert to *trans*-(I) with fluorescence emission.   It follows from this that the fluorescence should diminish as the temperature is raised, a conclusion which is confirmed by experiment.   Temperature-quenching also occurs at very low concentrations, at which dimerisation is negligible, showing that the probability of activated *trans*-(I) molecules changing to *cis*-(I) increases considerably as the temperature rises, while the mean life of the activated molecules so far as fluorescence-emission is concerned probably remains substantially constant.

The fluorescence phenomena which *trans*-(I) exhibits are too complex for satisfactory analysis by the crude methods available.   The fluorescence band is broad, and stretches, apparently continuously, over roughly half the visible spectrum.   At low concentrations the visual effect, without filters, is essentially blue, but as the concentration increases the band moves further into the longer wave-lengths, the visual effect changing through green-blue and blue-green to green.   Viewed through appropriate filters, the maximum blue fluorescence occurs at about 0·1% (isethionate) and fades out completely at 10% ; the yellow-green fluorescence is maximum at about 2% and extends through, with decreasing intensity, to both the highest (38%) and the lowest (0·01%) concentration examined.   The existence of a maximum in the overall fluorescent effect is thus quite definite.   The general effects of heating are to displace the band towards the shorter wave-lengths, and to cause a substantial decrease in the total fluorescence.   Cooling produces the opposite effects.   The fluorescence phenomena are not affected by exclusion of oxygen, and the temperature effects are reversible.   At all concentrations greater than 0·1% the fluorescence, with vertical irradiation, is confined to the meniscus, showing absorption of the exciting radiation in a thin surface layer.   As concentration is reduced below 0·1% penetration into the body of the solution increases.

Examination through light filters of standard spots of *trans*-(I) on filter paper, as used in the fluorescence-adsorption estimation, shows the presence of green and yellow in the fluorescence spectrum irrespective of concentration even down to 0·05 mg. per 100 c.c.   Compound *trans*-(I) produced on dry filter-paper from *cis*-(I) or dimer by irradiation of dried spots of the solutions gives exactly the same effect, as do also dry spots of *trans*-4-amino-4'-amidinostilbene.

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The general trend of the dimer/*cis*-(I) ratio with concentration in the early stages of insolation of *trans*-(I) was apparent from the main insolation experiments. To obtain further data, with concentration the only variable factor, solutions (0·1; 0·2; 0·4%) of the hydrochloride were insolated for sufficient times to give 20% conversion. At these concentrations absorption of the light responsible for the photochemical action is virtually complete in a thin surface layer of the solution. In spite of this it was found that the times required for 23% conversion were 8, 12, and 17·5 mins. respectively, while the actual quantities of activated *trans*-(I) converted into *cis*-(I) and dimer [*cis*-(I) plus dimer/2] in equal times (12 mins.) were as 5 : 6 : 7, showing that the quantum efficiency of dimer plus *cis*-(I) production increases with concentration. This is doubtless associated with the observed concentration-quenching of the fluorescence.

The initial rates of formation of dimer and *cis*-(I) may be expressed by the equations

$$d[D]/dt = k'[T^*][T] \qquad \qquad \text{(a)}$$

and
$$d[C]/dt = k''[T^*] \qquad \qquad \text{(b)}$$

from which the ratio, R, of the initial rate of formation of dimer to that of *cis*-(I) is given by the equation

$$R = (d[D]/dt)/(d[C]/dt) = k'[T^*][T]/k''[T^*] = K[T] \qquad \text{(c)}$$

The value of the ratio R_2/R_1 for initial concentrations T_2 and T_1 (T_2 the higher; other conditions constant) is given by

$$R_2/R_1 = T_2/T_1 \qquad \qquad \text{(d)}$$

In practice, with the concentrations used (0·1—0·4%), it was not easy to determine the slopes of the tangents to the dimer and *cis*-(I) curves at the origin, and it was necessary to use experimental ratios determined at

FIG. 3.
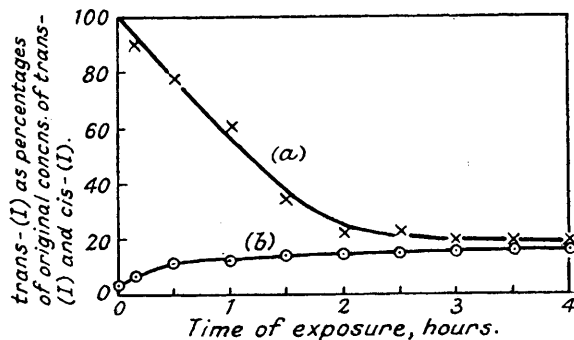
Exposure of 0·02% *solution of* trans-(I) *and* cis-(I) *to north-sky light.* *Temp.,* 36°.
(*a*) = trans-(I) *remaining in the solution of* trans-(I).
(*b*) = trans-(I) *produced in the solution of* cis-(I).

about 20% conversion of the *trans*-(I). The ratio, R', so determined, is lower than the true ratio, R, at the origin (cf. Fig. 1), and divergence increases as the initial concentration falls. The ratio R'_2/R'_1 was found to be greater than the ratio T_2/T_1 by a factor of 1·3—1·4. In view of the experimental difficulties the results obtained may be considered as offering strong support for the dimerisation theory.

The slow rate of dimerisation following the establishment of the equilibrium between *cis*-(I) and *trans*-(I) indicated that *cis*-(I) does not dimerise on insolation. On this assumption it was predicted that on insolation of a 1% solution of *cis*-(I) hydrochloride the *trans*-(I) concentration would rapidly rise to a maximum of about 18% of the original *cis*-(I) concentration and would then fall slowly so as to maintain the *cis*-(I)/*trans*-(I) ratio constant at 4—5. The rate of formation of dimer [from *trans*-(I)] would initially be *nil*, would rise to a maximum coinciding in time with the maximum of the *trans*-(I) curve, and would then decrease somewhat. The dimer–time curve would therefore show a point of inflexion. In spite of some interference from impurities in the *cis*-(I) hydrochloride used, the predicted features were reproduced almost exactly (cf. Fig. 4). Failure of the apparent *trans*-(I) to show a subsequent fall is attributed to interference by the secondary irradiation product responsible for the appearance of the yellow colour (q.v.). That the true concentration of *trans*-(I) does subsequently decrease follows from the existence of the point of inflexion in the dimer curve.

The photo-dimerisation mechanism of concentration-quenching may be of more frequent occurrence than is at present suspected. According to Weiss (*Trans. Faraday Soc.*, 1939, **35**, 37) all organic molecules which fluoresce strongly have fully conjugated bond systems. They are therefore potentially capable of dimerisation. If reversal of dimerisation is effected by radiation of similar frequency to that which produces dimerisation, and if equilibrium is largely in favour of the monomer, dimerisation might easily escape detection. The effect upon fluorescence of photo-dimerisation must be distinguished from that due to the formation of associated molecules in concentrated solution (*e.g.*, of many dyestuffs), which has already occurred before irradiation.

The proof that (III) has adsorptive powers comparable with those of *trans*-(I) is of importance as indicating a high degree of adsorption and retention in the body, as has been found in the case of *trans*-(I) (Henry and

Grindley, *loc. cit.*; Kirk and Henry, *loc. cit.*) and as indicating a possible cause of the delayed toxic effects produced by the dimer in human beings (Kirk and Henry, *loc. cit.*). Determination of the rate of excretion of (III) after intravenous injection will be investigated by the fluorescence-adsorption technique as soon as opportunity permits.

That *trans*-(I), both in solution and in the solid state, tends to develop a yellow colour on irradiation, the cause of which has not so far been explained, has been observed by previous workers (Fulton and Yorke, *loc. cit.*; Barber, *et al.*, *loc. cit.*; Kirk and Henry, *loc. cit.*). Kirk and Henry thought that it was due in part to the formation of *cis*-(I), but *cis*-(I) is colourless (Slack, private communication), and it has been observed that most of the acquired yellow colour is carried down along with *trans*-(I) sulphate. Solid *trans*-(I) hydrochloride and isethionate are both very slightly yellow, and concentrated solutions are strongly coloured. The natural yellow colour of a 1% solution of *trans*-(I) hydrochloride is weak, but on insolation the colour of the solution steadily increases and continues to do so after the main photochemical reaction is complete and the *cis*-(I)–*trans*-(I) equilibrium established. A concentrated (15%) solution of the isethionate is strongly coloured. On insolation the colour steadily diminishes until, at completion of the main photo-chemical change, it is only about 20% of the original colour, while 40% solutions become nearly colourless after full insolation. Crude

FIG. 4.
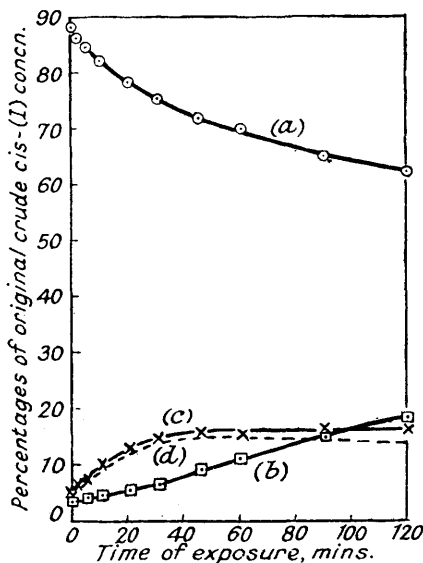
Insolation of 1% *solution of crude* cis-(I) *hydrochloride. Solutions* (23 c.c.) *contained in " Pyrex " flasks, 50 c.c. capacity. Temp.,* 45°.

(a) = cis-(I) *remaining.*	(c) = trans-(I) *produced (expt.).*
(b) = *dimer produced.*	(d) = trans-(I) *corrected for yellow irradiation product.*

cis-(I) hydrochloride prepared from insolated *trans*-(I) hydrochloride is invariably coloured. On insolation in solution (1%) a definite increase in the intensity of colour ensues, while insolation of the solid results in rapid yellowing. It appears, therefore, that there is a close connection between the yellowing on irradiation and the presence of *cis*-(I), suggesting that the yellow colour is due to a product of the further photochemical change of *cis*-(I). The colour is destroyed on bromination, from which it is concluded that the compound responsible for it is bromine-absorbing. Whether the absorption of bromine is due to addition at an ethylenic linkage or to substitution has not been ascertained.

The difference in behaviour on irradiation of *trans*-dimethyl-(I) as compared with that of *trans*-(I) and *trans*-monomethyl-(I) is of considerable interest. In absence of any chemical investigation of the photo-chemical instability of the mono- and the di-methyl derivatives it may be assumed that the former undergoes dimerisation and *cis–trans* isomerisation similar to *trans*-(I), while the latter is either substantially unchanged, or undergoes *cis–trans* isomerisation only. Fulton (*loc. cit.*) attributed the difference in behaviour to absence of a reactive hydrogen atom in the dimethyl derivative, while Henry (*Nature*, 1943, **152**, 690) suggested steric hindrance as being the cause. Whatever is the true explanation of the difference it is of interest to observe that the behaviour of these compounds on irradiation is closely similar to that of anthracene and its mono-*meso*-substituted derivatives on the one hand, which dimerise on irradiation, and its di-*meso*-substituted derivatives on the other hand, which are unaffected by irradiation (Bergmann, *Trans. Faraday Soc.*, 1939, **35**, 1052). Also of interest is the close parallel between the behaviour on insolation of *trans*-(I) and of the cinnamic acids, which likewise undergo *cis–trans* isomerisation and dimerisation on insolation, and reversal of dimerisation under the action of the short ultra-violet waves.

EXPERIMENTAL.

Main Insolation Experiments.—Direct tropical sunlight between 11·30 and 13·30 hours, during which period its intensity remains fairly constant, was used, and, where necessary, experiments could be carried over from day to day since the photochemical changes cease when irradiation is discontinued. The solutions, freshly prepared in artificial light, were contained in " Pyrex " flasks which were tied, lying flat, on a board suitably holed and fitted with a perpendicular rod which was kept always pointing direct into the sun. In this way normal incidence of the sunlight was maintained, and the board was kept gently shaken so that during any one experiment all solutions were subjected to similar conditions of exposure, and of mixing. Most solutions were warmed to 42—45° before exposure so as to maintain a nearly constant temperature throughout the insolation period.

After exposure the total bromine absorption was determined on one aliquot, giving *cis*-(I) plus *trans*-(I) and dimer by difference from 100%, and a second aliquot was warmed to 60°, excess of sodium sulphate solution added dropwise to precipitate *trans*-(I) as the sulphate, the solution cooled overnight, filtered through sintered glass, and the bromine absorption of an aliquot of the filtrate determined to give the *cis*-(I). The bromination technique previously described (Henry, *J.*, 1945, 870; *Analyst*, 1945, **70**, 259) was used, the almost complete precipitation of the *trans*-(I) and the presence of *cis*-(I) being confirmed by spotting-out the *cis*-(I) filtrate on filter-paper and examining it in ultra-violet light (cf. Henry, *J.*, 1945, 870). Simultaneous precipitation of much of the dimer sulphate assisted by tending to carry down the *trans*-(I) sulphate. The conditions used were not such as to precipitate any *cis*-(I) sulphate. The *trans*-(I) used was free from *cis*-(I), but a small correction to all results was necessary to allow for unprecipitated *trans*-(I) [blank *cis*-(I) estimation at zero time]. The results of these experiments are summarised in Table I, and parts are illustrated in Figs. 1 and 2.

TABLE I.

Expt.	Initial temp.	Initial concn. of *trans*-(I).	Dimer/*cis*-(I) ratio at early stage.	Final equilibrium *cis*-(I)/*trans*-(I) ratio.
I	45°	15% isethionate	400	1·8
II	45	2% hydrochloride	28	1·4 *
III	28 †	1% ,,	7·3	4·6
IV	48	0·2% ,,	0·5	4·0
V	28 †	0·2% ,,	0·9	4·6
VI	45	0·02% ,,	0·05	4·7 ‖
VII A ‡	42	0·1% ,,	—	5·0
VII B ‡	18	0·1% ,,	—	4·4

* Equilibrium had not been established.
† Temperature rose to *ca.* 45° in the later stages.
‡ These results show the comparatively small effect of temperature upon the final equilibrium (photochemical) *cis*-(I)/*trans*-(I) ratio.
‖ Obtained by bromometric analysis after concentration and fractionation.

Dependence of Dimer/Cis-(I) *Ratio on Temperature.*—Two solutions (0·2%) of *trans*-(I) hydrochloride were insolated for 15 minutes, one being maintained at 19° and the other at *ca.* 64° by running hot and cold water respectively over the flasks. There was occasional interruption of the insolation for very brief periods in the high temperature flask, allowing for which it may be assumed that, other conditions being constant, the total *trans*-(I) changed into *cis*-(I) and dimer is independent of the temperature. The results are summarised in Table II, which includes comparable results for average temperatures of 33° and 48° derived from Expts. IV and V respectively (Table I).

TABLE II.

Date	6.1.45	23.12.44	26.12.44	6.1.45
Approx. temperature	19°	33°	48°	64°
Cis-(I), C (%)	8·9	17·7	17·5	17·1
Dimer, D (%)	16·9	16·5	9·5	6·7
Trans-(I), T (%)	74·2	65·8	73·0	76·2
Cis-(I) plus dimer (%)	25·8	34·2	27·0	23·8
Ratio dimer/*cis*-(I)	1·90	0·93	0·54	0·39
C/(C + D)	0·34	0·52	0·65	0·72
D/(C + D)	0·66	0·48	0·35	0·28
(C + D/2)/(C + D)	0·67	0·76	0·83	0·86

Preparation of Crude Cis-(I) *Hydrochloride.*—A solution of *trans*-(I) hydrochloride (4 g. in 20 l.) was fully insolated at 45° and then evaporated, finally to dryness, in the dark at 35°. The residue was treated with water (5 c.c.) at 35° and filtered to remove undissolved *trans*-(I) hydrochloride, and the filtrate was allowed to evaporate to dryness. Bromometric analysis of crude *cis*-(I) hydrochloride so obtained (3·51 g.) showed it to contain, as hydrated hydrochlorides : *cis*-(I), 88·4; *trans*-(I), 4·5; dimer, 4·0; free H_2O, 3·0%. The recovered products of the insolation of the original *trans*-(I) hydrochloride were : *cis*-(I), 3·10 g.; *trans*-(I), 0·66 g.; dimer, 0·14 g.; giving 4·7 for the *cis*-(I)/*trans*-(I) equilibrium ratio for 0·02% solution, and 0·045 for the dimer/*cis*-(I) ratio.

Reversibility of Cis-(I)–Trans-(I) *Change.*—(i) Solutions (0·02%) of *cis*-(I) hydrochloride, prepared as above, and *trans*-(I) hydrochloride were exposed simultaneously under identical conditions (temp. 36°) to bright north-sky light, and the *trans*-(I) estimated in each solution at suitable intervals by the fluorescence-adsorption method. The results are given in Fig. 3, and show that the same equilibrium mixture arises from both *cis*-(I) and *trans*-(I). The *cis*-(I)/ *trans*-(I) equilibrium ratio given by this method was 4·4.

(ii) A solution of *cis*-(I) hydrochloride (0·5 g. in 2·5 l.), prepared as above, was fully insolated and the solution evaporated, finally to dryness, in the dark at 35°. The residue was treated with water (1 c.c.) at 35° to remove most of the *cis*-(I). The undissolved material, on fractionation, yielded 0·04 g. (8%) of crystals which were shown to be *trans*-(I) hydrochloride (composition, fluorescence and adsorption, behaviour on insolation, bromine absorption, and insolubility of sulphate).

Evidence for Polymerisation.—The saturated irradiation product can be isolated as the sulphate in reasonably pure condition by addition of sodium sulphate to the slightly acid solution and recrystallisation from hot, slightly acidified, water, the acidity being necessary to prevent hydrolysis of the amidino-groups. The purest product obtained gave : N, 12·9; SO_4, 22·2; loss at 110°, 13·3; loss at 200°, 15·8. Calc. for $C_{32}H_{32}N_8,2H_2SO_4,8H_2O$ (III) : N, 12·8; SO_4, 22·1; H_2O, 16·6. Calc. for $C_{16}H_{18}ON_4,H_2SO_4,3H_2O$ (II) : N, 12·8; SO_4, 22·1; H_2O, 12·4%. Decision between the

two formulæ depended, therefore, entirely upon the content of water of crystallisation. As the sulphate does not dehydrate readily, even on prolonged heating at 110°, it was obviously unsuitable for deciding between the two alternatives owing to the possibility of other changes occurring.

The oxalate of the irradiation product is comparatively insoluble, and dehydrates at room temperature over sulphuric acid. It is, however, difficult to purify, as it does not readily redissolve in hot water and will not crystallise from a slightly acid medium. Results obtained with oxalate of doubtful purity were intermediate between the requirements of (II) and (III); the conclusions were therefore indefinite, and efforts along these lines were discontinued.

The results of the kinetic experiments showed that, at the very high concentrations (40%) which can be obtained with *trans*-(I) isethionate, negligible proportions of *cis*-(I) and *trans*-(I) would remain in the solution fully insolated at low temperature, thus avoiding the need for purification. *Trans*-(I) isethionate readily dehydrates over sulphuric acid at 35° (Found for material so dried : N, 10·65; S, 12·15; bromine absorption, 309 mg./g. Calc. for $C_{16}H_{16}N_4,2HO\cdot C_2H_4\cdot SO_3H$: N, 10·85; S, 12·40%; bromine absorption, 310 mg./g.), suffers no further loss at 110° (24 hours) and only slight loss (0·05% per hour) at 200°. In spite of the rather low figures for N and S the salt, dried to constant weight over sulphuric acid at 35°, may safely be assumed to be anhydrous. The isethionate of the irradiation product is extremely soluble and does not crystallise; instead, on evaporation the solution finally sets to a glassy solid which, over sulphuric acid at 35°, dries out until the weight reverts to the original weight of anhydrous *trans*-(I) isethionate. This has been repeated several times with the same result, and disproves carbinol (II) formation and shows polymerisation of internal rearrangement. In one experiment *trans*-(I) isethionate (1·5893 g.) dried over sulphuric acid at 35° to 1·5612 g. Water (3 c.c.) was then added and the solution insolated for 8 hours at 10°. On evaporation (35°) and subsequent drying, in the dark, over sulphuric acid at 35° the weight became constant, after 13 days, at 1·5613 g. The dehydration curve was quite smooth. The final product, dissolved and made up to 100 c.c. for analysis (solution *A*), contained 0·45% of *trans*-(I) and 0·25% of *cis*-(I) giving a *cis*-(I)/*trans*-(I) ratio of 0·6, and 99·3% conversion of the original *trans*-(I) to dimer.

On insolation, or irradiation with filtered light from the mercury lamp, of solution *A* (equivalent to 1·1% dimer hydrochloride) the concentration of *trans*-(I) decreased from 4·5 to 1·7 mg./100 c.c. and that of *cis*-(I) increased from 2·5 to 5 mg./100 c.c., to give a *cis*-(I)/*trans*-(I) ratio of 3. Other similarly prepared solutions gave ratios of 4 and 5 after irradiation.

Reversal of Dimerisation.—(i) *Reversal in solution.* Solution *A* underwent no detectable change on irradiation (45 minutes) with the filtered (3000—4000 A.) light of the mercury lamp, other than the *trans*-(I)–*cis*-(I) readjustment described. On irradiation with the unfiltered light of the lamp there was a marked, and steady, increase in the concentration of both *trans*-(I) and *cis*-(I) in the solution, particularly evident on examining a spot of the solution on filter-paper, but less so on examining the fluorescence of the bulk solution on account of concentration-quenching. Using the fluorescence-adsorption method the concentration of *trans*-(I) increased steadily from 4 mg./100 c.c. at zero time to 66 mg./100 c.c. after 115 minutes, at which time equilibrium had still not been reached. Bromometric analysis of the solution at this stage showed that, of the original dimer, 6·8% had been converted to *trans*-(I) and 15·3% to *cis*-(I), giving a *cis*-(I)/*trans*-(I) ratio of 2·2. On repeating with a 0·1% solution (250 c.c.) of dimer, irradiation being continued for two hours after equilibrium as determined by the fluorescence-adsorption method had been reached, brommetric analysis of the solution gave : *cis*-(I), 65; *trans*-(I), 21; dimer, 14%; *cis*-(I)/*trans*-(I), 3·1; dimer/*cis*-(I), 0·22. The higher value of the *cis*-(I)/*trans*-(I) ratio found in this experiment suggests that reversal of dimerisation produces *trans*-(I) only, which is then partially converted into *cis*-(I); but conditions were too complex, and data too scanty, for a decision on this point to be possible at present. The dimer/*cis*-(I) ratio is what would be expected for the concentration used.

(ii) *Reversal on dry filter-paper.* A drop (Oreyer pipette) of dimer solution, spotted out on filter-paper and dried, shows, in filtered ultra-violet light, only the fluorescence due to the small quantities of *trans*-(I) and *cis*-(I) present. On insolation (1 hour) some development of fluorescence in the empty outer zone of the spot is detectable, but the effect is small. On very brief exposure to the unfiltered light of the mercury lamp and subsequent examination in the filtered light, a brilliant fluorescent spot is observed, the area of which is dependent upon the concentration of the dimer solution used. Maximum development of fluorescence occurs within 1 second. With a 1% solution of dimer the fluorescence which develops covers practically the whole area wetted by the drop, but as concentration is reduced the radius of the fluorescent area diminishes (showing strong adsorption of the dimer) until, at the low concentrations which would be encountered in the excretion of the injected compound, the fluorescent effect is an annulus exactly similar in appearance to that given by *trans*-(I) (cf. Henry and Grindley, *loc. cit.*). Spots of dimer solution (0·1—10 mg./100 c.c., as hydrochloride) on filter-paper showed no fluorescence below 0·5 mg./100 c.c., while the 10 mg./100 c.c. spot showed 0·1 mg./100 c.c. against *trans*-(I) standards. After brief exposure to the unfiltered light of the mercury lamp the fluorescence of the spots, on comparison with *trans*-(I) standards, gave 0·05—5·0 mg./100 c.c. of *trans*-(I). At each concentration the developed fluorescence was that of *trans*-(I) at half the concentration. By comparison, after development, of a spot of an unknown dimer solution in water or the biological fluids with a series of standard spots similarly prepared (washed if necessary) and developed, there is no doubt that satisfactory estimation of the unknown could be effected and, for example, rate of excretion after injection determined.

(iii) *Wave-band producing reversal of dimerisation.* The rate of development of the fluorescence of spots of dimer solution (50 mg./100 c.c.) on filter-paper on exposure to the full light of the mercury lamp passed through various filters (water, dimer solution, and solutions of lead acetate, contained in a silica capsule giving 1 cm. depth of liquid) was used for this purpose. The dimer spot was placed beneath the capsule and irradiated for known periods with the full light of the lamp, and its fluorescence was then compared with a similar spot fully developed. With water there was immediate and full development; with dimer solution (1%) no significant development after 60 seconds' exposure; with lead acetate (1 : 25; 2700 A.) approximately 50% development after 60 seconds exposure; with lead acetate (1 : 300; 2500 A.), full development after 30 seconds' exposure; with lead acetate (1 : 1500; 2400 A.), full development after 15 seconds' exposure. The maximum of the absorption band of an irradiated solution (1%) of *trans*-(I) was found by Goodwin (*loc. cit.*) to be at 2510 A.

Adsorptivities of Trans-(I), Cis-(I), *and Dimer.*—Using the technique of Henry and Grindley (*loc. cit.*) three spots of each solution (50 mg./100 c.c.) were prepared by transferring 0·1 c.c. to filter-paper. One spot of each solution was left unwashed, one of each washed radially with water (0·2 c.c.), and one of each washed radially with N/10-hydrochloric acid (0·2 c.c.). After drying the *cis*-(I) spots were developed in filtered ultra-violet light, and the dimer spots in unfiltered light of the mercury lamp. The fluorescences of corresponding spots of the compounds were then compared. The unwashed spots and those washed with water showed no significant differences in fluorescent areas, but washing with acid caused much more washing out of *cis*-(I) and dimer, which were similar, than of *trans*-(I). The adsorptivity of the dimer (filter-paper) is therefore rather less than that of *trans*-(I) and comparable with that of *cis*-(I).

*Variation of Dimer/*Cis-(I) *Ratio with Concentration.*—Solutions (200 cc.; 0·1; 0·2, 0·4%) of *trans*-(I) hydrochloride were insolated at 43°, for 9, 12, and 15 minutes respectively, and the solutions analysed brommetrically. The results are given in Table III, from which it is calculated on a proportionality basis that 23% total conversion of the original *trans*-(I) would have occurred in 8, 12, and 17·5 minutes respectively, and that in equal times (12 minutes), the actual quantities of activated *trans*-(I) converted into *cis*-(I) and dimer would have been as 5 : 6 : 7.

TABLE III.

Init. concn. of *trans*-(I)HCl, g./100 c.c.	0·1	0·2	0·4
Time of insolation, min.	9	12	15
Cis-(I) formed, % of original	20·8	14·0	7·1
Dimer formed, % of original	5·1	9·0	12·8
Total *trans*-(I) changed, %	25·9	23·0	19·9
Time for 23% change, mins..................................	8	12	17·5
Cis-(I) plus dimer/2, %....................................	23·4	18·5	13·5
Dimer/*cis*-(I) ratio, R'	0·245	0·64	1·80
R'0·2/R'0·1	2·6 (= 2 × 1·3)		
R'0·4/R'0·2		2·8 (= 2 × 1·4)	

Insolation of Cis-(I) *Hydrochloride Solution* (1%).—The experiment was carried out, with material prepared as described above, as for *trans*-(I) solutions, each flask (capacity 50 c.c.) containing 23 c.c. of the solution, heated to 45° immediately before insolation. After exposure the solutions were analysed bromometrically in the usual way. In carrying out the analysis of these solutions it was observed that there appeared to be some lag in the completion of the bromine-absorption, in consequence of which the period of contact with the bromine was increased, for all estimations, from the usual 2—3 minutes to 10 minutes. The lag is attributed to rather slow absorption of bromine by the yellow irradiation product, which was present in small quantity in the original *cis*-(I) preparation and further increased during insolation of the solution, and not to bromination of *cis*-(I) taking place more slowly than that of *trans*-(I), though this possibility has not been ruled out. That the yellow compound does absorb bromine is indicated by the fact that bromination of the yellow solution by addition of excess of freshly prepared bromine water (removing the excess by a current of air) destroys the yellow colour.

The results of the insolation of the *cis*-(I) hydrochloride solution are shown graphically in Fig. 4. Failure of the *trans*-(I) concentration to show a decrease in the later stages is attributed to the presence of the yellow compound which, since it absorbs bromine (under the conditions used) and is largely precipitated together with *trans*-(I) sulphate, appears in the analysis as *trans*-(I). The observed *trans*-(I) concentration is, in fact, the true *trans*-(I) plus most of the yellow compound, which latter increases with the time of insolation. The true *trans*-(I) concentration should therefore be as represented by the dotted curve.

Products of Hydrolysis of Cis-(I), Trans-(I), *and the Saturated Insolation Product.*—Complete removal of the nitrogen could be effected, though with difficulty, by prolonged refluxing in the dark with aqueous-ethanolic sodium hydroxide until evolution of ammonia ceased. After removal of the ethanol the acids could be precipitated as amorphous or gelatinous solids by acidification of the diluted aqueous solutions. The compounds *cis*-(I) and *trans*-(I) produce *cis*- and *trans*-stilbene-4 : 4'-dicarboxylic acids respectively on hydrolysis. Assuming no rupture of the *cyclo*butane ring during hydrolysis, (III) would give 1 : 2 : 3 : 4-tetra-(4'-carboxyphenyl)*cyclo*butane (V); while (II) would give 1 : 2-di-(4'-carboxyphenyl)ethan-1-ol (VI).

The acid produced as above from the saturated insolation product [containing only small quantities of *cis*-(I) and *trans*-(I)] was redissolved in slight excess of sodium hydroxide, the solution largely diluted, and the acid reprecipitated by rapid addition, with stirring, of excess of hydrochloric acid to the hot solution. The acid was filtered off, washed with cold water, and air-dried to constant weight. The product was readily and completely soluble in ethanol, was nitrogen- and ash-free and had no m. p. up to 310° (Found : equiv., 141·4). Over concentrated sulphuric acid at 30° it readily dehydrated to constant weight (24 hours; 4·55% loss), and the dehydrated material recovered nearly the whole of the loss in weight on exposure to the air for two days [equiv. calc. for dehydrated material, 135. (V) requires 134; (VI) requires 143]. The acid is therefore assumed to be (V).

Crude specimens of *cis*-(IV) and *trans*-(IV) were also prepared, as above, from the corresponding amidines. Equal quantities (0·02 g.) of these, and of (V), were dissolved in a small excess of sodium hydroxide, neutralised, and the solutions diluted to 20 c.c. (100 mg. of acid/100 c.c.). These solutions were spotted-out on filter-paper in the usual way and the dry spots examined in the filtered light of the mercury lamp. The spot prepared from *trans*-(IV) gave a strong fluorescence immediately, and there was no change on further irradiation (10 minutes). That prepared from *cis*-(IV) showed only a faint fluorescence at first, due to the presence of a little *trans*-(I), but during exposure fluorescence steadily developed over the whole area wetted by the drop, exactly as had previously been found for *cis*-(I). The spot prepared from (V) showed no initial fluorescence, and no fluorescence had developed after irradiation (10 minutes) with the filtered light. On irradiation of this spot with unfiltered light of the lamp fluorescence developed over the whole area of the spot until it was comparable in intensity with that of the *cis*-(IV) spot fully developed. Exposure for about 1 minute was required for maximum fluorescence to be reached, as compared with about one second in the case of a similar spot of (III). It is evident, therefore, that (V) is also unstable under the action of the short ultra-violet waves and reverts to *trans*-(IV).

The solutions of the sodium salts of *cis*-(IV), *trans*-(IV), and (V) were insolated (15 minutes), with shaking, and again spotted out. The spot from (V) showed no initial fluorescence and no fluorescence developed on exposure, showing that the compound is stable to sunlight. The spots prepared from *cis*-(IV) and *trans*-(IV) after insolation each had the same initial fluorescence, much less than that of uninsolated *trans*-(IV) solution, and on exposure extensive fluorescence due to *cis*-(IV) developed to the same extent in each. It is evident, therefore, that on insolation *cis*-(IV) and *trans*-(IV) undergo *cis-trans*-isomerisation until equilibrium between the two forms is established.

The fluorescence of the *trans*-(IV) spot was only 5—10% of that of a *trans*-(I) spot at the same equivalent concentration, showing the depressing effect on fluorescence of replacement of the amidino-groups by carboxyl groups.

The mercury lamp used throughout this work was provided with a white glass filter transmitting little light outside the range 3000—4000 A. (suppliers' description). For concentration- and temperature-quenching observations the solutions were contained in open test-tubes mounted so that all could be irradiated, heated (oven) or cooled simultaneously. To exclude oxygen the solutions were oven-heated (110°; 1 hour) and a layer of toluene was added while hot.