[CONTRIBUTION FROM NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

The Radiation-Induced Fluorescence and Fluorescence Spectra of Certain Quinoline Derivatives[1]

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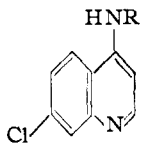
This investigation was stimulated by the discovery that certain of the useful antimalarial drugs of the 4-amino-7-chloroquinoline series, such as chloroquine (I, 4-(4-diethylamino-1-methylbutylamino)-7-chloroquinoline), could be determined quantitatively by a fluorometric technique involving preliminary exposure to ultraviolet irradiation.[5] Its aim was to establish the nature of the chemical change induced by the irradiation and, if possible, to establish the structure of the fluorophore produced. Although it has been demonstrated by analysis of ultraviolet absorption data that the development and subsequent diminution of fluorescence are due to major structural changes in the compounds, the work has unfortunately terminated before the ultimate objective of identification of the chemical transformations could be achieved. The purpose of the present paper is to report the results obtained in establishing some of the characteristics of these interesting transformations for I and two analogs, differing only in the structure of the amino alkyl group attached to the 4-amino nitrogen. It was



I. SN-7618[6], Chloroquine; R = $CH(CH_3)(CH_2)_3N(C_2H_5)_2$

II. SN-8137; R = $CH_2CH(OH)CH_2N(C_2H_5)_2$

III. SN-9584; R = $(CH_2)_3N(C_2H_5)_2$

IV. R = H

V. HNR = OH

surprising to find that, although the substituted 4-aminoquinolines (I, II, III) and the 4-hydroxyquinoline (V) all developed enhanced fluorescence under ultraviolet irradiation, the 4-aminoquinoline (IV) failed to exhibit this property.

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(5) Brodie, Udenfriend, Dill and Chenkin, *J. Biol. Chem.*, **168**, 319 (1947).

(6) The Survey Number, designated SN-, indicates the number assigned to a drug by the Survey of Antimalarial Drugs. Properties of these compounds are summarized in a recent monograph (Edwards Bros., Ann Arbor, Michigan, 1946).

Experimental

Borate buffer[5] was prepared by mixing five volumes of 0.6 *M* boric acid in 0.6 *M* potassium chloride with four volumes of 0.6 *M* sodium hydroxide; *p*H 9.5 (Beckmann *p*H meter).

Irradiation of SN-7618 (50 μM).—To 150 ml. of a 75 μM solution of SN-7618 in 0.01 *N* hydrochloric acid (38.7 mg./l. of the bis-dihydrogen phosphate salt of 4-(4-diethylamino-1-methylbutylamino)-7-chloroquinoline) was added thirty ml. of 0.05 *N* sodium hydroxide and 45 ml. of borate buffer. This solution (*p*H 9.5) was placed in a stoppered 150-ml. quartz flask and supported about 9 cm. below a Hanovia 500-watt ultraviolet lamp. Samples for determination of fluorescence and ultraviolet absorption were removed by pipet from the bottom of the flask without stirring.

Samples of SN-8137 (50 μM) and SN-9584 (25 μM) were similarly prepared and irradiated.

Fluorescence measurements were made on a Coleman model 12 photofluorometer. Samples were excited by a mercury arc passed through a B-1 filter (maximum transmittance at 365 mμ) and fluorescence was read through a PC-1 filter (completely absorbing below 425 mμ). Since the performance of the instrument is a delicate function of various environmental factors, even when operated through a constant-voltage transformer, it was continually recalibrated by setting to a scale reading of 40 with a solution of 500 γ/l. of quinine in 0.1 *N* sulfuric acid. Data on the development of fluorescence with time are summarized graphically in Fig. 1.

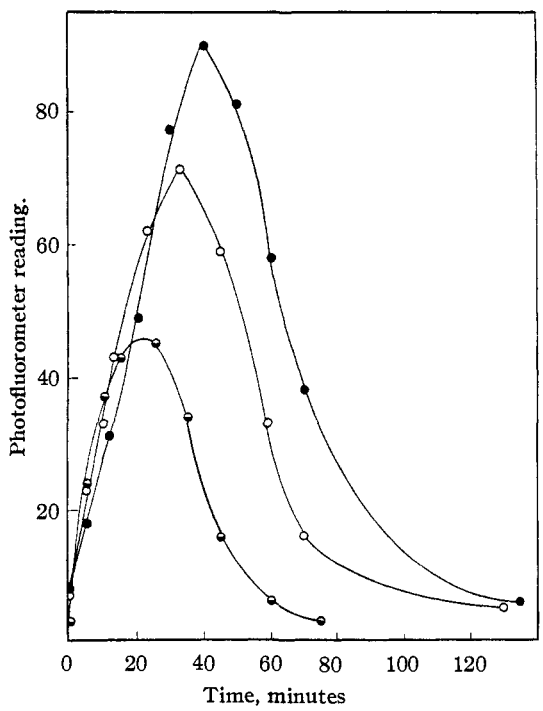


Fig. 1.—Enhancement of fluorescence by exposure to ultraviolet irradiation at *p*H 9.5: O, SN-7618, 50μM; ●, SN-8137, 50μM.; ⊖, SN-9584, 25μM.

Fluorescence spectra[7] were measured on solutions prepared by mixing five volumes of 750 mμ drug in water with one volume of 0.05 *N* sodium chloride and one-and-one-half volumes of borate buffer and irradiated in photofluorometer tubes 16 cm. from a Mazda 100-watt A-H4 ultraviolet lamp.[5] The tubes were withdrawn periodically and their fluorescence determined. Irradiation was stopped when the fluorescence had reached a maximum (*ca.* eight and one-half hours). Experiment showed that for these solutions there was no enhancement of fluorescence when 1% sodium sulfite was added before irradiation, in marked contrast to experiments at one-tenth this concentration of drug (see below).

The fluorescence spectra of irradiated and unirradiated drugs were photographed in a Steinheil GH spectrograph using three glass prisms. A low-pressure mercury arc lamp was used for the calibration lines while the fluorescence was excited by an H4 lamp emitting over 90% of its energy at 365 mμ. Eastman "40" photographic plates were used. Microdensitometer tracings of the negatives are presented graphically in Fig. 2. The density scale is logarithmic but the plates were not calibrated so that density comparisons are only approximate. The 404.7 mμ line is from the exciting light.

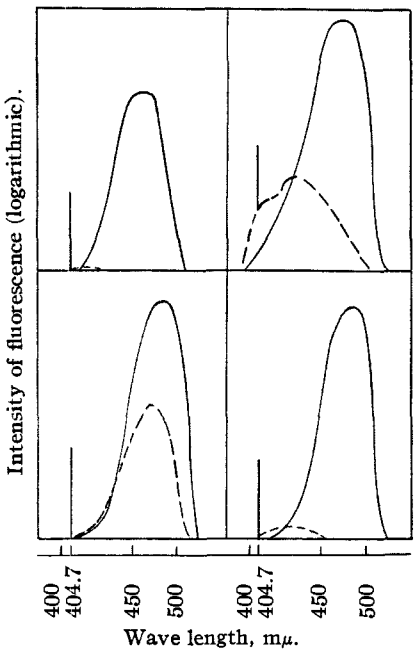


Fig. 2.—Fluorescence spectra before and after irradiation to maximum fluorescence at *p*H 9.5: ----, before; —, after; upper left, 7-chloro-4-quinolinol; upper right, SN-7618; lower left, SN-8137; lower right, SN-9584.

The maxima for the three drugs are almost identical (SN-7618, 470.5 mμ; SN-8137, 470.0 mμ; SN-9584, 472.0 mμ) and quite different from that for 7-chloro-4-quinolinol (460 mμ).

Incidental to these measurements, the fluorescent spectrum of quinine in 0.1 *N* sulfuric acid was found to have a very broad maximum between 445 and 469 mμ.

The fluorescence of the unirradiated drugs was also observed visually, SN-7618 showing violet fluorescence, SN-9584 a weaker violet and SN-8137 a blue-green.

Ultraviolet absorption data were obtained on a Beckmann quartz spectrophotometer (industrial model) using 1-cm. cells and a 0.40-mm. slit width. A blank consisting of ten parts of 0.01 *N* hydrochloric acid, two parts of 0.05 *N* sodium hydroxide and three parts of borate buffer gave a linear function of log I_0/I from 0.043 at 240 mμ to 0.012 at 310 mμ (I_0 = light transmitted through distilled water).

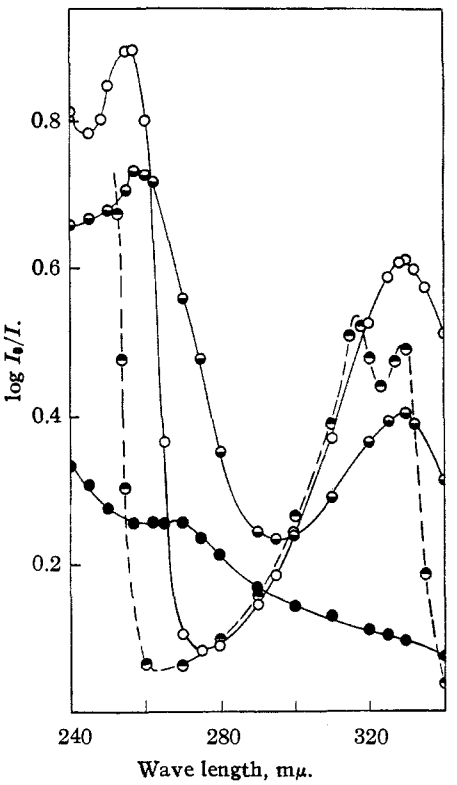


Fig. 3.—Ultraviolet absorption spectra for SN-7618, 7-chloro-4-quinolinol (V) and irradiated samples of SN-7618, *p*H 9.5, 50 μM; O, SN-7618; ◒, SN-7618 irradiated forty-five minutes; ●, SN-7618 irradiated 135 minutes; ◓, 7-chloro-4-quinolinol.

Aliquots of the samples used to determine the rate of development of fluorescence (data summarized in Fig. 1) were placed in a 1-cm. cell for determination of the change in ultraviolet absorption with length of ultraviolet irradiation. (It was shown that the exposure to ultraviolet light necessary to measure the absorption spectra was not of sufficient intensity or duration to cause any change in the samples.) Typical results are summarized in Figs. 3 and 4.

The most immediate and pronounced change in the spectra is an increase in absorption in each instance in the vicinity of 270 mμ. In order to present the character of the new absorption more clearly, the ten-minute irradiation spectra for SN-9584 has been corrected for the absorption of unchanged drug as follows. The largest decrease in absorption, occurring at 340 mμ, corresponded to a maximum of 93% of the undegraded drug (if none of the degradation products absorbed appreciably at this wave length, this would be a rough estimate of the maximum extent of degradation). In Fig. 5 we have sketched in, in addition to the observed absorption curve (heavy line), the calculated curve for 93% of the original SN-9584 (light line). The difference (dashed line) represents the minimum absorption for the fluorophore and its subsequent degradation products. Since, at ten minutes, there would be little subsequent degradation, this curve is probably our most reliable approximation of the absorption spectrum of the fluorophore in the region of 240 to 300 mμ. The characteristic maximum at about 270 mμ is also

(7) The authors are grateful to Dr. Scott Anderson, Anderson Consulting Laboratories, Champaign, Illinois, for supervision and assistance in the use of the Steinheil spectrograph.

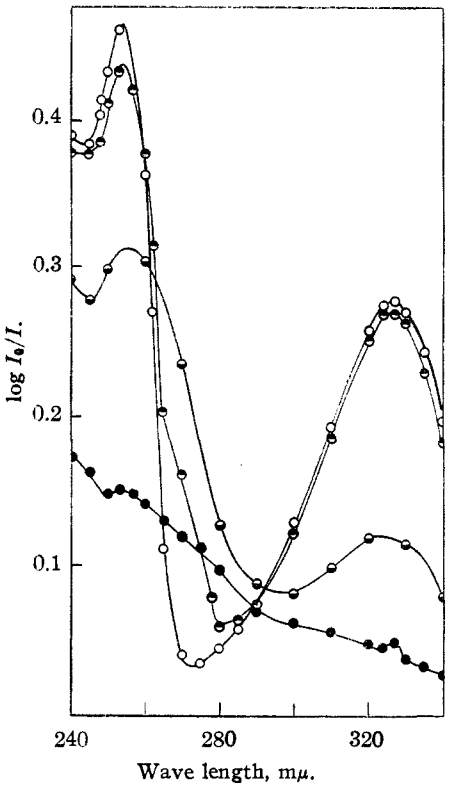


Fig. 4.—Ultraviolet absorption spectra for SN-9584 before and after exposure to irradiation, *p*H 9.5, 25μM: O, SN-9584; ◓, SN-9584 irradiated ten minutes; ◒, SN-9584 irradiated thirty-five minutes; ●, SN-9584 irradiated seventy-five minutes.

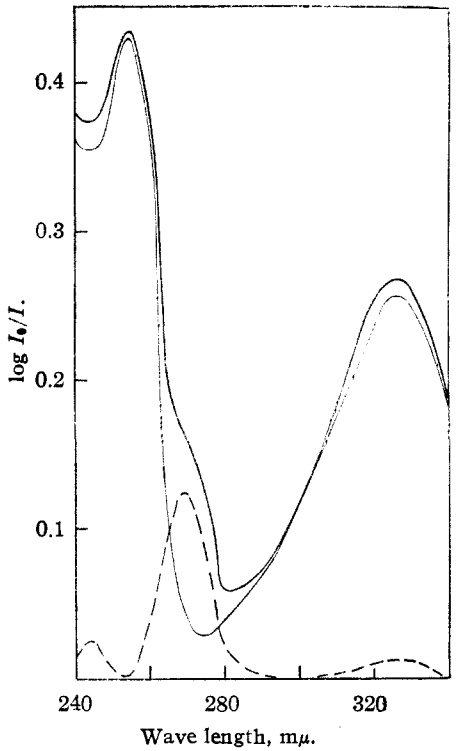


Fig. 5.—Ultraviolet absorption of product of irradiation from analysis of ultraviolet absorption of SN-9584 irradiated ten minutes: —, observed absorption (1); —, maximum contribution of unchanged SN-9584 (2); — — —, (1) minus (2).

indicated in curves similarly constructed from the data for longer irradiation of the various drugs (Fig. 6).

The Ultraviolet Absorption Spectrum of 7-Chloro-4-quinolinol.—Since the photolytic replacement by hydroxyl of the 4-alkylamino group of a quinoline did not seem unreasonable, we hoped to study the absorption spectra of irradiated solutions of 7-chloro-4-quinolinol. This scheme was not completed, although the spectrum was determined for a non-irradiated 50 μM solution of the compound in aqueous buffered media of *p*H 9.5 (Fig. 3). The twinned peak at 320–330 mμ resembles and approximates the position of that of the 7-chloro-4-alkylaminoquinolines. As indicated previously, the quinolinol does not fluoresce nor does it show the maximum at 270 mμ characteristic of the fluorophore.

It was found, however, that 7-chloro-4-hydroxyquinoline, but *not* 7-chloro-4-aminoquinoline, acquired fluorescence upon ultraviolet irradiation. The data for the former are presented graphically in Fig. 7.

Chromatographic Concentration of the Fluorophore from SN-8137.—Observations in our laboratory that the drugs under consideration are strongly absorbed from aqueous solution by Florosil led to attempts to use this absorbent in a chromatographic separation of unchanged drug from the fluorophore produced by irradiation. It was found that there was indeed a separation, the unchanged material being held in a narrow band near the top of the column, while the fluorophore occupied a less distinct region below. Such a column was colorless in daylight, but examination with a small ultraviolet lamp in darkness revealed the brilliant white fluorescence of unchanged drug and the pale blue of the fluorophore. The former was isolated and identified by sectioning of the column, elution with boiling ethanol and characterization as

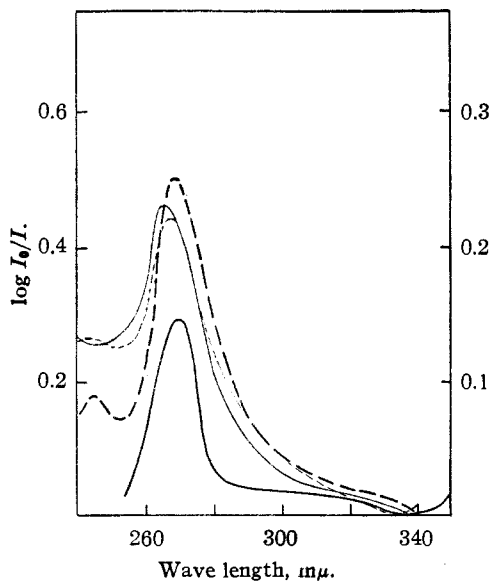


Fig. 6.—Approximate ultraviolet absorption of product of irradiation from analysis of ultraviolet absorption of irradiated SN-7618 (forty-five minutes, — — —), SN-8137 (sixty minutes, — -- —), SN-9584 (thirty-five minutes, —), SN-9584 (with sulfite, 230 minutes, —). (Use scale at right for thirty-five minutes SN-9584).

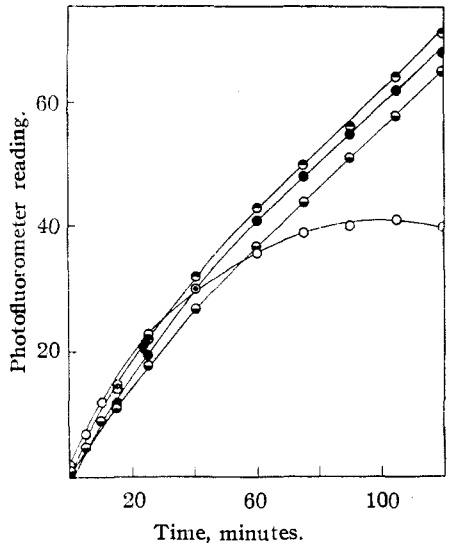


Fig. 7.—Enhancement of fluorescence of 7-chloro-4-quinolinol by exposure to ultraviolet irradiation at *p*H 9.5: O, 7-chloro-4-quinolinol, 50μM; ◓, 1% sodium sulfite added; ●, 1% hydroxylamine added; ◒, 1% cysteine added.

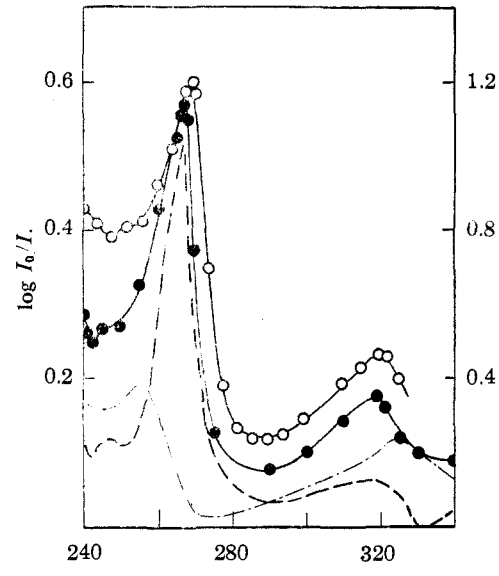


Fig. 8.—Ultraviolet absorption of concentrates of fluorophore from SN-8137; O, Eluate 1 (1) (scale at right); ●, Filtrate 7 (2); — - — - —, maximum contribution of SN-8137 to (2); ———, minimum contribution of fluorophore and further degradation products to (2).

the di-picrate. The fluorophore could be eluted with water or dilute alcohol. The progress of the elution was readily followed by ultraviolet examination of the column together with quantitative determinations of the fluorescence of the eluates with the photofluorometer. A 2.6 × 29-cm. column was prepared by pouring into it an aqueous slurry of 60/100 mesh commercial Florosil, then running several liters of water through in reverse at a rate which maintained the material in constant turmoil and washed out the finer dust, and finally allowing the packing to settle during a few hours to a constant depth. In one experiment, the irradiation of 1 g. (1.98 mM) of the drug in 3500 ml. of solution in a Pyrex flask for six-and-one-half hours under the Hanovia 500-watt ultraviolet lamp caused the fluorescence to rise from an initial value of 11 to a maximum of 66 (calibration: 500 γ/l. of quinine in 0.1 *N* sulfuric acid = 10). After standing four days (during which the fluorescence rose to 70), part of the solution (1400 ml., 0.79 mM) was filtered through the column of Florosil at *ca.* 500 ml./hr. Ultraviolet examination of the column showed that the unchanged drug was held near the top, while the fluorophore progressed slowly down the column and had just started to enter the filtrate with the last of the solution.

After unsuccessful extrusion attempts, the absorbent was dug out with a spatula; the lower 11 cm. which appeared to contain the fluorophore was boiled with 100 ml. of 95% ethanol, imparting to the latter a fluorescence of 99. (A 50-ml. test portion of ethanol after boiling with 5 g. of washed Florosil had a fluorescence of 2).

The ultraviolet absorption spectrum of the alcoholic solution of desorbed fluorophore above (which we shall call Eluate 1) was found to have maxima at 270 and 320 mμ, the former having about two and one-half times the intensity of the latter (Fig. 8).

Figure 8, together with some reasonable assumptions, provides the basis for further speculation upon the irradiation process. Let us suppose that the molecular extinction coefficient (ε) for the fluorophore (as indicated by data in Fig. 5) is roughly five to six times that of the drug. Then we should judge that the concentration of fluorophore in Eluate 1 approximated 20 μM. If we then assume proportionality of fluorophore concentration and fluorescence, it would seem that the 565 μM irradiated solution should have had, before chromatographing, a fluorescence of 2800 if it had been completely converted to the fluorophore. Its actual value of 70 suggests that the conversion was (70 − 11)/(2800 − 11) or about 2% complete.

In another experiment, the irradiation of 0.5 g. of the drug in 2 l. of solution (990 μM) for ten hours caused the fluorescence to rise from 8 to 56 (calibration as above). After standing two days (during which the fluorescence rose to 70) the solution was filtered through a 3.2 × 26-cm. column at *ca.* 200 ml./hr. The filtrates were collected in seven 250-ml. portions followed by one of 150 ml. It was evident that the fluorophore was not being held as strongly as in the previous experiment, since the fluorescence of the successive filtrates was 1, 3, 3, 10, 21, 37, 41 and 32, respectively. The ultraviolet absorption spectrum of Filtrate 7 is presented in Fig. 8.

The column was eluted with eight 100-ml. portions of 25% ethanol. Evaporation of eluates 2–6 to dryness under diminished pressure yielded distillates with no fluorescence. The residue was extracted with ethanol and attempts were made to isolate the fluorophore in the form of its picrate. A small quantity (0.02 g.) of material which decomposed slowly about 250° and melted with effervescence at 286–93° was obtained. Recrystallization of this material from ethanol and water afforded some long golden needles which began to decompose at 250°, melted with effervescence at 290–293°, and gave a positive Beilstein test for halogen. From micro-analysis of a 1.484-mg. sample, there was reported C, 45.24; H, 2.78.[8]

The Influence of Sulfite.—Other investigators[5] observed that the addition of various substances, especially cysteine, hydrazine, hydroxylamine and sulfite, before irradiation markedly retarded the radiation-induced degradation of the fluorophore. In this way, much more intensely-fluorescent solutions could be prepared. The influence of sulfite was checked by adding 1% of solid sodium sulfite to 25 μM SN-9584, prepared and irradiated precisely as before. The fluorescence data are summarized in Table I along with those for the same drug without sulfite. The latter are taken from Fig. 1 with reduction to the same scale of photofluorometer sensitivity.

(8) Analysis by Howard Clark.

TABLE I

DEVELOPMENT OF FLUORESCENCE OF SN-9584 ON IRRADIATION WITH AND WITHOUT SULFITE

Irradiation time, min.	1% Sulfite	Without sulfite
0	2	0.4
5	..	3.0
10	27	4.6
15	..	5.4
25	..	5.6
35	76	4.2
45	..	2.0
60	..	0.7
66	92	...
75	..	0.4
100	95	
140	93	
230	62	
300	12	
410	6	

It is evident that the presence of sulfite increased the optimum irradiation time from twenty-two to one-hundred minutes and the magnitude of the fluorescence by a factor of 17.

The ultraviolet absorption spectra of the irradiated samples were obtained in the manner previously described and are presented graphically in Fig. 9. The high absorption of the sodium sulfite solutions below 270 mμ is obvious from comparison with Fig. 4 and is in agreement with data from the literature.[9] Examination of Fig. 9 shows the degradation of the band centered at 327 mμ to be similar to that previously obtained without sulfite except that it increased a bit at first (thirty-five-minute irradiation).

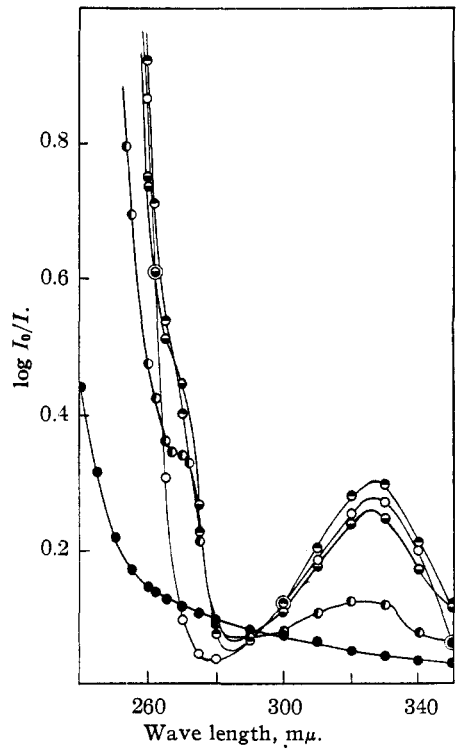


Fig. 9.—Absorption spectra of SN-9584 at *p*H 9.5 containing 1% sodium sulfite: ○, before irradiation; ◓, thirty-five minutes irradiation; ◒, 140 minutes irradiation; ◐, 230 minutes irradiation; ●, 410 minutes irradiation.

(9) Ghosh and Chakrabarti, *J. Indian Soc.*, **6**, 823 (1929).

Of significance also is the distinctive rise and fall during irradiation of the absorption at 270 mμ. If this absorption and the fluorescence are both plotted against the time of irradiation it will be seen that the maximum absorption corresponds nicely with the maximum fluorescence. This relationship holds true also for the previous three cases (see Figs. 3 and 4) and constitutes the second indication that the actual fluorophore possesses an absorption curve with a strong band at or near 270 mμ.

Absorption Spectra of Hydrazine, Hydroxylamine and Cysteine.—One suggestion for the efficacy of these reagents and sulfite in promoting development of fluorescence was their possible action as reducing agents.[10] In view of the observation on the marked absorption of sulfite below 260 mμ, it was considered of interest to check absorption spectra of other effective materials. The data, summarized in Fig. 10, indicates all begin to absorb strongly at or just below 260 mμ. This suggests (but certainly does not prove) that an internal screening against lower wave length radiation may be an explanation.

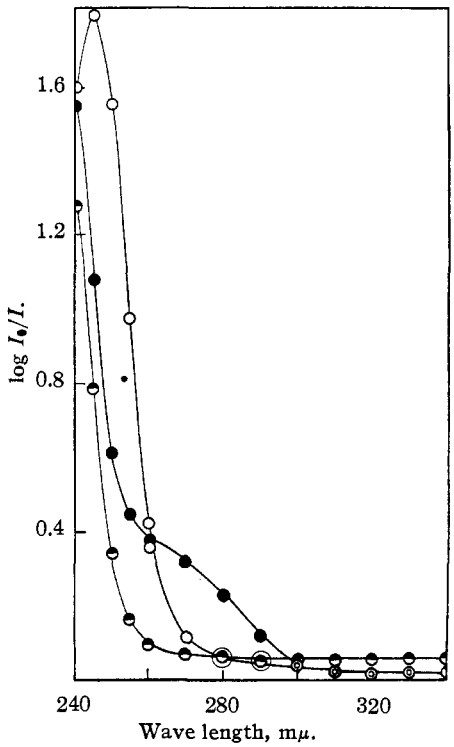


Fig. 10.—Absorption spectra for 1% aqueous solutions (*p*H 9.5) of hydrazine (○), hydroxylamine (◓) and cysteine (●).

An experiment was then undertaken in which two identical solutions of 25 μM SN-9584 in borate buffered media of *p*H 9.5 were irradiated side by side under the Hanovia lamp in flasks, one Pyrex and the other quartz, which were carefully matched for size and form. The light transmission of Pyrex drops to 30% at 300 mμ and is practically zero at 260, while quartz is highly transparent (92%) to as low as 260 mμ and only drops to 70% transmission at 240 mμ. The solution in quartz was thus subjected to the radiations of seven relatively strong mercury lines between 254 and 313 mμ from which the solution in Pyrex was reasonably protected. The data

(10) Bernard B. Brodie, private communication.

showed that the fluorescence of the solution contained in quartz attained a slightly greater magnitude (110%) than that in Pyrex, and reached its maximum in about 63% of the time required for the latter. It thus seems evident, from comparison with the sulfite experiment that screening alone cannot account for the marked effect of the fluorescence promoters.

Discussion

The changes in ultraviolet absorption which occur during irradiation of the various 4-amino-7-chloroquinoline drugs seem to demonstrate conclusively that both the development and subsequent diminution of fluorescence are accompanied by structural changes in the compounds. The parallel of the increase and subsequent decrease in absorption at 270 mμ with the increase and subsequent decrease in fluorescence must mean that the original drug undergoes a transformation to a fluorescent compound with an absorption maximum at 270 mμ. This compound is light sensitive and itself undergoes further transformation on continued exposure to ultraviolet irradiation.

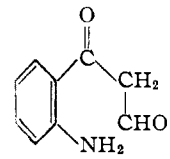
A careful examination and comparison of the ultraviolet absorption for the solutions of SN-9584 irradiated with and without sulfite (Figs. 4 and 9), reveals several interesting facts. In the first place, comparison of the last curves in each reveals them to be almost identical at wave lengths of 260 mμ and longer. It would thus seem that these two curves in this region correspond to rather complete degradation to the same product or products. The divergence below 260 mμ can be ascribed to increasing absorption by the sulfite.[9]

Comparison of the curves before irradiation (Figs. 4 and 9) reveals that the 327 mμ peak is identical but that sulfite has had a remarkable effect in increasing the intensity of the 260 mμ peak of the drug.

Careful examination of the intermediate curves reveals that the increase and subsequent decrease in the absorption at 270 mμ corresponds to the increase and subsequent decrease in fluorescence but that, whereas the magnitude of the increase in intensity of ultraviolet absorption corresponds quite closely, the magnitude of increase in fluorescence is seventeen-fold greater in the presence of sulfite. These observations, coupled with the fifteen-fold greater fluorescence of the longest-irradiated samples (see Table I), which seem, from their ultraviolet absorption, to correspond in extent of degradation, suggests that the sulfite may actually have not only a retarding influence on the irradiation process but may markedly promote the intensity of fluorescence of the compound so produced.

One other interesting comparison is the relative magnitude of absorption of the fluorophore in the 270 mμ and 320 mμ regions. The major peak at 270 mμ is unaffected but the peak at 320 mμ is relatively much more intense, especially in the early stages of irradiation. Since the relative absorption at 270 mμ and 320 mμ after 230 minutes of irradiation with bisulfite is nearly the same as for experiments without bisulfite, it seems possible that these two peaks *might* be due to similar but different irradiation products.

It is also a rather interesting coincidence that at least three of the reagents promoting fluorescence are reactive to a carbonyl group. This leads one to incline toward the possibility that the radiation-induced degradation of these quinoline derivatives might resemble the reported hydrolysis of pyridine to glutacondialdehyde when irradiated with light in the 254–266 mμ region.[11] No such studies on quinoline have been found in the literature nor has any reference been found to the ultraviolet absorption spectra of the type of product expected from such a transformation of 4-hydroxyquinoline (anthranoylacetaldehyde, VI).



VI

Summary

Measurements of both the fluorescence and the ultraviolet absorption spectra of irradiated solutions of three 4-amino-7-chloroquinoline drugs indicate the development and subsequent diminution of fluorescence to be accompanied by major structural changes in the compounds. It has been demonstrated that the fluorophore is an intermediate photolytic decomposition product which is rapidly and extensively further degraded.

Measurement of the fluorescence spectra of these drugs, as well as of 7-chloro-4-quinoline, before and after ultraviolet irradiation, is reported. The irradiated drugs give very similar fluorescence spectra, the maxima differing by about 100 mμ from that for the quinoline.

NOTRE DAME, INDIANA RECEIVED APRIL 5, 1948

(11) Freytag and Neudert, *J. prakt. Chem.*, **135**, 15 (1932); **136**, 193 (1933); **139**, 44 (1933).