soluble fraction (0.65 g., 9%) was recrystallized from alcohol and proved to be 2-phenylphenanthroxazole, m. p. 206–207°. The more soluble fraction (0.25 g., 2.7%) was 1-benzyl-2-phenylphenanthrimidazole; m. p. 241–241.5°. This compound has not been previously reported.

Anal. Calcd. for $C_{28}H_{20}N_2$: C, 87.46; H, 5.24; N, 7.27. Found: C, 87.69; H, 5.30; N, 7.12.

(b) **In Glacial Acetic Acid.**—The same quantities of quinone and amine were dissolved in 25 cc. of glacial acetic acid, refluxed for three hours and filtered while hot. The brown residue (3.1 g.) was digested with hot dioxane and subsequently with hot nitrobenzene. This left a green residue of phenanthroxazine; yield 2.2 g. (48%); m. p. > 360°.

Anal. Calcd. for $C_{28}H_{17}NO$: N, 3.66. Found: N, 3.46.

The original filtrate on cooling deposited 2-phenylphenanthroxazole which was recrystallized from alcohol; yield 1 g. (14%); m. p. 206–207°. Water was added to the filtrate from the oxazole, precipitating a gum. The latter was dissolved in a hot mixture of acetone and ether, from which 1-benzyl-2-phenylphenanthrimidazole separated on cooling. It was recrystallized from alcohol; yield 0.4 g. (4%); m. p. 241–241.5°.

Reaction of Phenanthraquinone with *n*-Butylamine in Toluene.—Although this reaction was carried out under various conditions, only intractable brown solids and gums were obtained.

Reaction of Phenanthraquinonimine with Benzylamine.—One gram (0.0048 mole) of phenanthraquinonimine and 0.53 cc. (0.0048 mole) of benzylamine were dissolved in 50 cc. of hot toluene and refluxed for three hours. The solution was steam distilled and the residue recrystallized from alcohol; yield of 2-phenylphenanthroxazole 0.83 g. (59%); m. p. 205–206°. No imidazole could be isolated.

Reaction of Phenanthraquinonimine with *n*-Butylamine.—Two grams (0.0097 mole) of phenanthraquinonimine and 0.96 cc. (0.0097 mole) of *n*-butylamine were dissolved in 35 cc. of hot toluene and refluxed for eight hours. After steam distillation, the residue was dissolved in a mixture of dry alcohol and benzene. A small amount (0.1 g.) of a red, amorphous solid remained. The solution was evaporated and the residue recrystallized from alcohol; yield of 2-propylphenanthroxazole 0.9 g. (35%); m. p. 85–86°. A mixed melting point determination with a sample prepared by Stein and Day[6] showed no depression.

Summary

1. It has been shown that primary amines which have two hydrogen atoms on the alpha-carbon atom react with retenequinone and phenanthraquinone (or their isosters, the quinonimines) to form the corresponding oxazoles.

2. The course of the reaction has been shown to consist of the following probable steps: (1) an aldol-type of condensation between the quinone and the amine; (2) a shift of hydrogen in the two adjacent triad systems from carbon to oxygen; (3) addition of OH across a —N=CH— linkage; and (4) oxidation of a dihydroöxazole (by a quinone) to an oxazole.

PHILADELPHIA, PA. RECEIVED APRIL 28, 1943

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, LABORATORIES OF THE MOUNT SINAI HOSPITAL, AND FROM THE SCIENTIFIC BUREAU OF BAUSCH AND LOMB OPTICAL CO.]

The Fluorescence of Vitamin A

BY HARRY SOBOTKA,[1] SUSAN KANN AND ERICH LOEWENSTEIN

The fading green fluorescence of vitamin A under ultraviolet irradiation has been used for its histochemical demonstration in slices of liver and other animal organs.[2] An attempt by one of us (E. L.) to utilize this fluorescence for the quantitative analysis of vitamin A solutions by a photoelectric fluorometer led to the observation that vitamin A preparations in alcoholic solution display upon irradiation an initial steep increase in fluorescence, followed by complete destruction of fluorescence during prolonged irradiation. In contrast to this, the fluorescence of vitamin A solutions in ether, chloroform, or benzene shows sometimes a small initial drop, but always assumes quickly a level of steady intensity which decreases but slowly.

The fluorescence of vitamin A in non-polar as well as in polar solvents is fairly proportional to its concentration over a range from 0.1–5.0 I.U./ml. under our experimental conditions. Although not quite as sensitive as the Carr–Price reaction, fluorescence may serve as a satisfactory basis for an analytical method. In concentrations below 0.1 I.U./ml. erratic results are often obtained. In polar solvents such as methanol, ethanol, or isobutanol fluorescence follows a curve such as "1" in Fig. 1. Symbatic curves are obtained for varying concentrations, the ordinates (intensity

(1) Supported by a Grant from Nutrition Foundation Inc. A report of the work was given at the Conference on vitamin research held at Gibson Island, Maryland, under the auspices of the A.A.A.S., July 20th, 1943.

(2) H. Popper, *Proc. Soc. Exptl. Biol. Med.*, **43**, 133 and 234 (1940); *Arch. Path.*, **31**, 766 (1941); H. Popper and R. Greenberg, *ibid.*, **32**, 11 (1941); R. Greenberg and H. Popper, *Am. J. Physiol.*, **134**, 114 (1941).

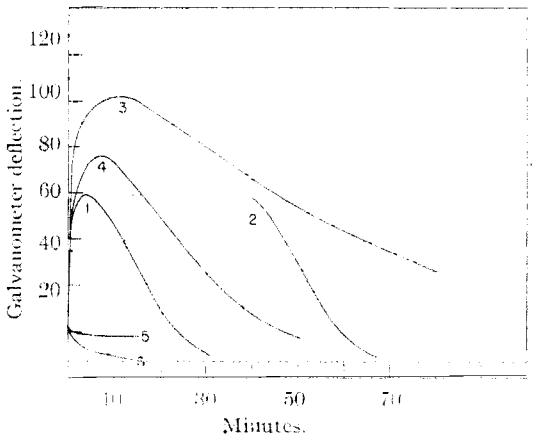

Fig. 1.—Fluorescence intensity of vitamin A solutions in ethanol: (1) vitamin A acetate 5 I.U./ml.; (2) the same, but illumination interrupted (dotted line); (3) same as 1 but flushed with carbon dioxide; (4) same with nitrogen; (5) same concentration of vitamin A acetate in benzene; (6) vitamin A alcohol 5 I.U./ml. in ethanol. The dotted line parallel to the base line indicates the upper limit of galvanometer deflection with the pure solvents. Galvanometer readings were taken every minute; the individual readings did not deviate more than one galvanometer division from the curves as plotted.

of fluorescence, given in ammeter readings) being proportional to the vitamin A concentration both for initial and for maximal fluorescence (Table I). The time elapsed, until maximum fluorescence is reached, is independent of the vitamin concentration, but is an inverse function of the intensity of the exciting radiation. The peak intensity of fluorescence, for a given initial intensity of fluorescence, is higher for high intensities of irradiation. The period for which the solution has been standing in the dark before irradiation, has no influence on these effects.

TABLE I

MAXIMUM GALVANOMETER DEFLECTION WITH ALCOHOLIC SOLUTIONS OF VARYING AMOUNTS OF VITAMIN A ACETATE

I.U./ml.	Deflection	I.U./ml.	Deflection
solvent	10	2.5	75
0.1	16	3.0	82
0.2	20	4.0	98
0.5	32	5.0	113
0.8	41	6.0	126
1.0	44	7.0	140
1.5	53	8.0	149
2.0	63	9.0	158

We further investigated whether the "secondary" reaction, involving decrease and ultimate disappearance of fluorescence, is of photochemical nature. When the light source was shut off, as soon as maximum fluorescence was reached, and irradiation was resumed after a dark period of, say, thirty-five minutes, the intensity of fluorescence was still at maximum and dropped, upon resumption of continuous irradiation, at a rate identical with that in the uninterrupted experiment (curve "2"). Intermittent irradiation, alternating with dark periods minute for minute, likewise showed complete standstill during the dark intervals. In view of the minute concentration of vitamin A in these observations, it was difficult to exclude oxygen below a stoichiometrically adequate limit. However, a slow current of small carbon dioxide or nitrogen bubbles passing through the vessel sufficed to influence the intensity curve; as shown in curve "3," a solution, flushed for fifteen minutes before, and throughout irradiation, showed a longer rise, leading to a higher maximum. The subsequent drop during continued irradiation proceeded one-fifth as fast with carbon dioxide-flushing, and less than one-half as fast with nitrogen-flushing (curve "4") compared to the rate without flushing.

The photoelectric fluorometer used consists of a mercury vapor lamp (85-watt mercury capillary arc); the beam of light passes first through an iris diaphragm, then through a filter which absorbs all visible light above 395 mμ (Corning no. 584). It then passes through a vertical slit, 13 × 32 mm., and hits the absorption cell (13 × 42 × 46 mm.) at a distance of 210 mm. from the light source. Adjacent to the broad side of the glass cell, a barrier-layer photoelectric cell is built into the lightproof housing of the absorption cell. Thus, the fluorescence, emanating from the fluid, hits the photoelectric cell at a right angle with the ultraviolet beam; it is filtered through Corning filter no. 038 which eliminates scattered ultraviolet rays. The photoelectric current generated in the cell deflects the mirror of a multiple-mirror galvanometer (sensitivity = 2.3×10^{-9} amp./mm.) the range of which may be altered by a suitable variable resistance.

If one removes the photoelectric cell, facing the absorption vessel, one may observe with the naked eye, looking perpendicular to the beam of ultraviolet light, that fluorescence increases at the side facing the source of light. About the time that maximal total fluorescence intensity would be recorded by the photoelectric cell, the vertical bright band begins to move gradually away from the light source and a non-fluorescent, dark band or halo appears in its wake. Under

certain conditions, a second band appears parallel to the first one in its original position; both bands now travel parallel and sometimes even a third one develops. These phenomena are presumably due to diffusion and convection and may be in the nature of Liesegang rings, caused by the opposite gradients of light intensity and oxygen concentration, under the assumption that dissolved oxygen is used up in the course of the "secondary" reaction. Since these phenomena were observed in a quiescent solution only, and since they may influence intensity measurements in a rather complicated manner, the effects of flushing with inert gases might have been caused by the resulting turbulence rather than by diminution of oxygen concentration; however, in control experiments with mechanical stirring instead of nitrogen bubbling, the fluorescence intensity took a course identical with that in the quiescent experiment.

The rise-and-fall type of curve was not only encountered in pure alcoholic solutions, but also in mixtures of ethanol or methanol with benzene up to definite proportions of admixture; above a percentage of 65–70 of benzene the fluorescence/time curve abruptly assumed the level type observed in pure benzene (curve "5").

All these observations were made with vitamin A concentrates (OLEUM PERCOMORPHUM Mead Johnson) which contain vitamin A in the form of its natural esters with higher fatty acids. They were reproduced in all essentials with a fresh sample of crystalline vitamin A acetate (Distillation Products Co.). Crystalline vitamin A alcohol, of which a fresh and an eight months old sample were tested, does not increase in fluorescence upon ultraviolet irradiation in alcoholic solution. The intensity of its initial fluorescence conforms with that of an equivalent concentration of its acetate, but it begins to drop immediately as illustrated by curve "6."

A vitamin A_2 concentrate, prepared from freshwater fish liver,[3] displayed the same features as the vitamin A concentrates used. This indicates that the difference between vitamins A_2 and A is vested in a part of the molecule that has no direct influence on fluorescence.

We are now studying the effect of these phenomena upon the Carr–Price reaction and upon the ultraviolet absorption with a view to elucidate the underlying mechanism. We are indebted to Mrs. W. Winternitz for valuable assistance.

Summary

The fluorescence of vitamin A esters in alcoholic solution first increases, then decreases under continued ultraviolet irradiation. Both processes are of photochemical nature, but the second one is impeded by flushing with inert gases. The phenomenon is shown by vitamin A_2 ester, but not by the free vitamin A alcohol.

(3) G. Wald, *J. Gen. Physiol.*, **22**, 391 (1939).

NEW YORK, N. Y.
ROCHESTER, N. Y. RECEIVED MAY 26, 1943

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, LABORATORIES OF THE MOUNT SINAI HOSPITAL]

Studies on Ionone. I. Cleavage of Ethyl Ionylidene Acetate[1]

BY HARRY SOBOTKA, EDITH BLOCH AND DAVID GLICK

β-Ionylidene acetaldehyde is an important intermediate for syntheses in the carotenoid group. The preparation of this substance by reduction of the corresponding ethyl ionylidene acetate has been claimed by Kuhn and Morris.[2] Neither Karrer[3] nor Krauze[4] found this method practicable or satisfactory; the latter claimed better yields by the reaction of β-ionone with the Grignard compound of bromoacetal, but they failed to characterize their product by physical constants or derivatives. Our own experiences with Kuhn's method, also with modifications using stannous chloride instead of chromous chloride and aniline instead of *o*-toluidine, and with chloro-acetal were likewise unsatisfactory.

In 1935 Davies, Heilbron, Jones and Lowe[5] had described the preparation of β-ionylidene acetaldehyde by the dry distillation of a mixture

(1) The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development, and The Mount Sinai Hospital.

(2) R. Kuhn and C. J. R. Morris, *Ber.*, **70**, 853 (1937); U. S. Patent 2,223,375 (April 22, 1941).

(3) P. Karrer and A. Ruegger, *Helv. chim. acta*, **23**, 284 (1940).

(4) M. V. Krauze and J. M. Slobodin, *J. Gen. Chem.* (Russian), **10**, 907 (1940).

(5) (a) W. H. Davies, I. M. Heilbron, W. E. Jones and A. Lowe, *J. Chem. Soc.*, 584 (1935); (b) I. M. Heilbron, W. E. Jones, A. Lowe and H. R. Wright, *ibid.*, 561 (1936).