Photoreactions of Riboflavin in the Presence of 2,4-Dichlorophenoxyacetic Acid (2,4-D)

Aerobic photolysis of aqueous buffered solutions of 2,4-dichlorophenoxyacetic acid (2,4-D) and riboflavin (RF) was studied. A 100-fold excess of 2,4-D with respect to RF concentration was used in every case. Spectroscopic and TLC analysis of reaction products showed that RF decomposes in the presence of 2,4-D. No detectable decomposition of 2,4-D was observed, even after 22 h of photolysis. Lumichrome, the principal photoproduct of RF in these conditions, was inhibited by 2,4-D. The rate of RF photodecomposition and lumichrome inhibition increased with 2,4-D concentration. The implication of these results on the possibility of a photodegradation of the herbicide via a sensitization by RF is discussed.

The riboflavin (RF) sensitized photodecomposition of 2,4-D has been postulated as an important route for the degradation of this herbicide (Crosby and Li, 1969) and related compounds (Crosby and Wong, 1973). Due to the potential importance of this reaction in the control of the fate and permanence of these herbicides in the environment we have undertaken a study of the mechanistic aspects of the interaction of excited riboflavin and 2,4-D. The aim of this preliminary report is to show that under visible irradiation, the presence of 2,4-D accelerates the decomposition of RF.

MATERIALS AND METHODS

Riboflavin (6,7-dimethyl-9-(D-1'-ribityl)isoalloxazine) from Sigma Chemical Co. was recrystallized twice from 2 N acetic acid and was then extracted with chloroform until no impurity could be detected by TLC. 2,4-Dichlorophenoxyacetic acid (2,4-D), technical grade, a gift from Atanor Chemical Co. (Argentine), was purified by recrystallization from ethanol–water and then from benzene. Lumichrome was prepared by prolonged irradiation of RF in methanol–water (50%) (Smith and Metzler, 1963) and it was then recrystallized from methanol. All solutions were prepared in a Britton–Robinson buffer (pH 6). Redistilled water was used. Silica gel G for thin-layer chromatography was obtained from E. Merck and thin-layer plates were prepared as previously reported (Smith and Metzler, 1963). The solvent system used was butanol–ethanol–water (7:2:1).

Irradiations were performed in a "merry-go-round" apparatus immersed in a thermostated bath (25 °C), with a tungsten visible lamp. Light was filtered and collimated with a glass lens, which removed all wavelengths below 340 nm. All spectral measurements were made on a Cary 17 spectrophotometer. Samples were prepared and handled in a dark room equipped with red photographic safelights.

RESULTS AND DISCUSSION

Buffered solutions 10^{-5} M in RF and 10^{-3} M in 2,4-D were photolyzed for 22 h. Under our conditions the only absorbing species was RF. The spectroscopic features of these solutions are shown in Figures 1 and 2. The absorption characteristics of RF in the region 500 to 300 nm are identical before irradiation either in the presence of 2,4-D or not (Figure 1). After photolysis, solutions containing only RF gave the typical spectrum of lumichrome, the well-known principal product of aerobic photolysis of the flavin in acid or neutral medium (Owen and O'Boyle, 1971). Solutions containing 2,4-D showed, according to the disappearance of the 445-nm band, that RF had been decomposed to the same extent, but lumichrome formation is nearly completely inhibited. Figure 2 shows the spectra of these solutions in the region 300–200 nm before and after irradiation, where the typical spectrum of 2,4-D can be recognized (Crosby and Li, 1969). The photolyzed solution shows a greater absorbance at the maxima, probably due to the background which can be seen as a long-wavelength tail. This background could arise from decomposition products of RF. The effect of changing concentrations of 2,4-D on the aerobic photolysis of RF was also studied. By spectroscopic and TLC analysis of reaction products at different stages of photolysis, we found the correlations shown in Tables I and II. The greater the concentration of 2,4-D the faster the rate of RF decomposition. There is also a noticeable effect of 2,4-D concentration on the yield of lumichrome after prolonged irradiation (Table II). An inhibition of lumichrome formation was observed. Such inhibition increased with 2,4-D concentration. These results suggest a different pathway for the disappearance of RF in the presence of 2,4-D. The excited flavin or a reactive intermediate generated by it is reacting with 2,4-D in a nonregenerative way. This reaction is even faster than the known photodecomposition of RF to lumichrome.

Table I. 2,4-D Effect on RF Photodecomposition

[2,4-D], M × 10^3	[RF],[a] M × 10^5
0	3.38
2.04	3.06
4.08	2.40
8.16	1.96

[a] Remaining RF after 3 h irradiation at room temperature. $[RF]_0$ (initial) = 4.5 × 10^{-5} M.

Table II. 2,4-D Effect on Lumichrome Formation

[2,4-D], M × 10^3	[Lu],[a] M × 10^5
0	4.4
2.04	3.8
4.08	2.9
8.16	2.2

[a] Lumichrome concentration after 17 h irradiation at room temperature. $[RF]_0$ (initial) = 4.5 × 10^{-5} M.

In a photosensitized reaction, the excitation energy may be directly transferred from the sensitizer to the acceptor transferring it to a reactive excited state, or it may be utilized in some reaction between the sensitizer, or an excited intermediate generated by it, and the acceptor. In every case, the sensitizer is regenerated in a secondary process. This regeneration is an important requirement assuming that a photosensitized reaction is a possible route for the degradation of the herbicide.

Of the two sensitization mechanisms the direct energy transfer from RF to 2,4-D may be disregarded since the excited states of RF are lower than those of the same multiplicity of 2,4-D (Moye and Winefordner, 1965; Song et al., 1972).

However, when irradiated with visible light, RF can sensitize the photooxidation of a large variety of substrates by a mechanism of the second type, in which the energy

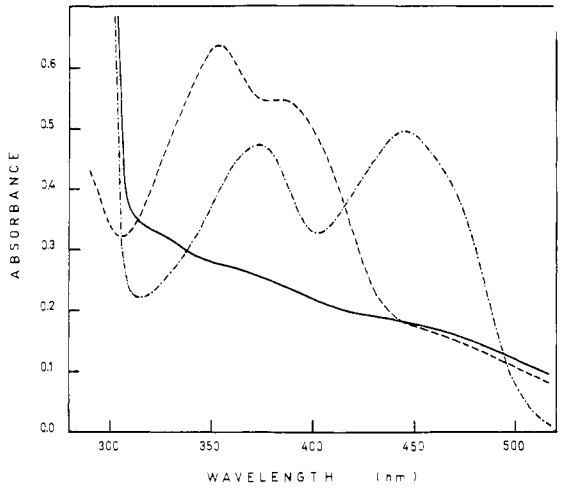

Figure 1. Absorption spectra (500-300 nm) of RF-2,4-D solutions (pH 5.8): (—) 2,4-D, 6×10^{-3} M, and RF, 6×10^{-5} M, after 22 h of irradiation; (- · -) 2,4-D, 6×10^{-3} M, and RF, 6×10^{-5} M, nonirradiated; (- - -) RF, 6×10^{-5} M, after 22 h of irradiation.

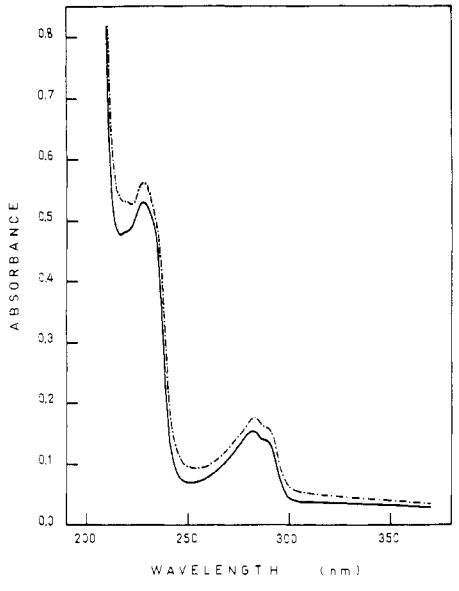

Figure 2. Absorption spectra (300-200 nm) of RF-2,4-D solutions (pH 5.8): (—) 2,4-D, 6×10^{-5} M, and RF, 6×10^{-7} M nonirradiated; (- · -) 2,4-D, 6×10^{-5} M, and RF, 6×10^{-7} M, after 22 h of irradiation. These solutions correspond to those in Figure 1 diluted 100 times before taking the spectra.

is not directly transferred to the acceptor (Taylor and Radda, 1971). The details of these reactions are generally unknown. The RF excited triplet state is the most probable reactive species that could react either directly with the substrate (2,4-D in its ground state) by hydrogen atom abstraction (eq 1a,b), or with O_2, resulting in an excited singlet state which could oxidize the substrate (eq 2a,b) where ^3RF is the excited triplet and RFH_2 the reduced form of RF. $^1O_2$ is the excited singlet of O_2 and S is the substrate. In both mechanisms, RF is regenerated to its ground state. Nevertheless, our results indicate that RF is not regenerated when irradiated in the presence of 2,4-D and although RF is completely destroyed, no appreciable disappearance of 2,4-D is detected.

$$^3RF + S \rightarrow RFH_2 + \text{oxidized products} \quad (1a)$$

$$RFH_2 + O_2 \rightarrow RF + H_2O_2 \quad (1b)$$

$$^3RF + O_2 \rightarrow RF + {}^1O_2 \quad (2a)$$

$$^1O_2 + S \rightarrow \text{oxidized products} \quad (2b)$$

It has been reported that the presence of RF caused a drastic increase in the rate of photodecomposition of the herbicide 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) (Crosby and Wong, 1973). However, the RF concentration was greater than that of the herbicide and no search for RF decomposition was carried out.

Our results do not explicity imply that 2,4-D is not being consumed. There is a great possibility that, in the experimental conditions used, 100-fold in excess of 2,4-D, a small disappearance of the herbicide, namely less than 1%, would not be detectable. It is then conceivable that working with an excess of RF, the reaction leading to a greater consumption of RF could also affect the 2,4-D concentration, which is in agreement with findings of other workers.

In conclusion, when RF is irradiated with visible light in the presence of 2,4-D it is not regenerated. It is even consumed at a greater extent than alone, so it could hardly act as a sensitizer. Further work is needed in order to obtain a better understanding of the photochemical reactions of RF in the presence of 2,4-D and their importance in the mechanism of degradation of the herbicide.

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