COMMUNICATIONS

Photolysis of 5-Amino-4-chloro-2-phenyl-3(2*H*)-pyridazinone

The photolysis of 5-amino-4-chloro-2-phenyl-3(2*H*)-pyridazinone (pyrazon) in both aqueous solution and on silica gel plates resulted mainly in the formation of polymeric material. The photolysis in sunlight was relatively rapid. Two of the products isolated from the reaction in aqueous solution were identified as 5-amino-4′-chloro-2,2′-diphenyl-4,5′-iminodi-3(2*H*)-pyridazinone and 2,5,7,10-tetrahydro-2,7-diphenylpyrazino[2,3-*d*:5,6-*d*′]dipyridazine-1,6-dione. The former was also formed after sunlight exposure of pyrazon absorbed on silica gel.

Pyrazon (5-amino-4-chloro-2-phenyl-3(2*H*)-pyridazinone, I) is a selective herbicide for the control of broadleaf weeds in sugar beets and table beets. We report here some results obtained during a study of the effects of natural and artificial radiation on this material.

EXPERIMENTAL

Starting Material. Pyrazon was obtained from BASF in analytical grade. Purity was established by thin-layer and gas chromatography.

Irradiation Conditions. A 1-l. aqueous solution of pyrazon (275 ppm) was irradiated with a 450-W Hanovia lamp through a Corex filter for 6 hr, while nitrogen was passed through the reaction mixture. The reaction mixture was cooled and the temperature of the reaction never exceeded 30 °C. Aqueous solutions of pyrazon of approximately the same concentration were exposed to sunlight in 1-l. borosilicate glass flasks for 6 hr in early November of 1970 and for 1 month between mid-April and mid-May of 1971. Control samples in actinic glass containers were also "exposed" for these periods. Nitrogen was not used for the sunlight exposures.

Sunlight exposure of pyrazon and two of its photoproducts, II and III (*vide infra*), on silica gel plates were made as follows: 2 μg of 10 mg/ml solutions of the materials were spotted on a 100 μ-thick silica gel G (according to Stahl) plate. The silica gel plate was then covered with a quartz plate (Corning glass filter CS 9-54). Controls were created by simply placing a tiny piece of opaque tape over the spots not to be exposed. These exposures were made for periods of 4 hr to 1 week.

Isolation of the Photoproducts. The irradiated solution was extracted twice with a total of 500 ml of ethyl acetate, and the combined extracts were dried over anhydrous sodium sulfate, filtered, and evaporated to dryness under reduced pressure. The residue was dissolved in 10 ml of acetonitrile and added to 2 g of silica gel (J. T. Baker 80–200 mesh), after which the solvent was evaporated on a flash evaporator. This residue was then added to the top of 30 g of silica gel in a 15 × 1-in. glass column. Elution with 130 ml of benzene–ethyl acetate (4:1) gave the yellow material, IV; elution with 570 ml of benzene–ethyl acetate (3:1) gave pyrazon; further elution with 300 ml of this solvent gave another yellow compound, III; elution with 220 ml of benzene–ethyl acetate (1:3) gave a white solid, II. Elution with 400 ml of ethyl acetate gave a very small amount of material (V) which we did not characterize, and elution with 200 ml of ethyl alcohol gave the major product(s), a brown solid (VI) which remained at the origin in the thin-layer system used.

Before recrystallization of the materials, a 166-mg extract from the 1-month sunlight exposure gave 5 mg of IV, 90 mg of pyrazon, 13 mg of III, 21 mg of II, *ca.* 1 mg of V, and 35 mg of VI. Recrystallization of II, III, and IV from acetonitrile resulted in material melting at 320, 312–15, and 285–87 °C, respectively.

Thin-Layer Chromatography. Eastman silica gel sheets with fluorescent indicator were used. Elution (10 cm of solvent front) with ethyl acetate–hexane (2:1) gave the following R_f values: I, 0.65; II, 0.20; III, 0.57; IV, 0.88; V, 0.05; and VI, 0.00.

Identification of Photoproducts. Products were characterized by mass and infrared spectroscopy and by elemental analysis. Compounds, II, III, and IV from both laboratory and natural irradiations were found to be identical by thin-layer chromatography and infrared spectroscopy.

Analysis. Elemental analyses of II and III and molecular weight determination of VI were performed by Schwarzkopf Microanalytic Laboratory, Woodside, N.Y.

RESULTS AND DISCUSSION

The mass spectra of II and III suggest strongly that these materials are 5-amino-4′-chloro-2,2′-diphenyl-4,5′-iminodi-3(2*H*)-pyridazinone and 2,5,7,10-tetrahydro-2,7-diphenylpyrazino[2,3-*d*:5,6-*d*′]dipyridazine-1,6-dione, respectively (Figure 1). The parent ion of the former appears at *m/e* 406 with an isotope distribution (Beynon, 1960) indicating the presence of only 1 chlorine atom. The base peak at *m/e* 371 arises by loss of the chlorine atom. Other prominent peaks occur at *m/e* 301 (loss of $C_6H_5N_2\cdot$), *m/e* 273 (loss of $C_6H_5N_2\cdot$ and CO), *m/e* 237 (loss of HCl from 273), *m/e* 105 ($C_6H_5N_2^+$), and *m/e* 77 ($C_6H_5^+$). The mass spectrum of III exhibits a parent/base peak at *m/e* 370 and prominent peaks at *m/e* 265 (loss of $C_6H_5N_2\cdot$), *m/e* 237 (loss of $C_6H_5N_2\cdot$ and CO), *m/e* 105, and *m/e* 77. The spectrum indicates that no chlorine is present. The spectrum of both materials also exhibits a peak at *m/e* 185. The latter may arise as shown in Figure 2.

The infrared spectra of both II and III show absorbance in the pyridazine region between 1414 and 1444 cm^{-1} (Rao, 1963) as well as absorbance due to secondary amine and amide. The elemental analyses for carbon, hydrogen, and nitrogen for both II and III agree with the calculated values.

The formation of II from I may be thought of as a substitution reaction, where the 5-amino-4-chloro-2-phenylpyridazin-3-onyl radical replaces a chlorine atom. (The reaction may also occur *via* nucleophilic substitution, but we have no experimental evidence to support either pathway.) A similar type of substitution may be considered in the conversion of II to III. Analogous reactions were observed in the photochemical formation of chlorinated anilinoazobenzenes from chloroanilines and chloroazobenzenes (Rosen *et al.*, 1970).

The infrared spectrum of IV exhibits no absorptions for pyridazine and amide. Its mass spectrum indicates that it is of high molecular weight with a mass of at least 541. Other ions appear at m/e 449 (?), 369 (base peak) 355, 342, 341, 325, 314, 313, 249, 194, 119, 93, 91, and 77. Absence of an ion at m/e 105 as well as absence of pyridazine and amide absorption in the infrared spectrum indicates that extensive degradation or rearrangement of the pyrazon molecule occurred. Because of the small amounts of material available, no more attempts to identify IV were made; nor is any work on the structure of this compound contemplated.

The major product of the solar irradiation of pyrazon in water was VI, a material whose average molecular weight (determined by osmometry in pyridine solution) was greater than 9000. The infrared spectrum of VI, in addition to exhibiting the large broad bands indicative of polymeric material, was similar to the spectrum of II. Although VI has not been well characterized, it is reasonable to suggest that it is formed by the same pathway as II. A molecule of II may undergo two reactions: (a) internal cyclization to form III; or (b) trimerization. The trimer may then react to form longer chains. The ultraviolet absorption spectrum of II exhibits a maximum at 292 nm (that of I is at 286 nm) in methanol, indicating that it is capable of absorbing solar energy directly.

The results obtained by exposing I, II, and III to sunlight when absorbed on silica gel are in agreement with the above suggestion. Within 4 hr, both II and material remaining at the origin after elution were formed from pyrazon, while no III was detected. Within the same exposure period, II was also converted to material which remained at the origin after elution. Again III was not detected, a result due to the concentration effect in the solid state. It is known that concentrated systems favor polymerization over cyclization. Exposure of III under these conditions resulted in no reaction. The "dark" controls of I and II also remained unchanged.

The photolysis of pyrazon by sunlight appears to be a relatively rapid reaction when the material is dissolved in water or when absorbed on silica gel. It should be remembered that the experiment in aqueous solution, where almost half the pyrazon was lost in 1 month, was performed in a borosilicate glass flask. This means that only about 10% of the light principally responsible for energizing the reaction was transmitted (Calvert and Pitts, 1966). In a previous experiment (early November 1970), when the total amount of solar energy below 3100 Å is approximately 1/6 of the total that reaches the earth's surface at this latitude (Forsythe and Christison, 1929) in April–May, trace amounts of III were detected within 6 hr.

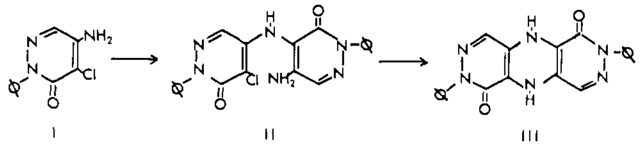

Figure 1. Structure of Compounds I, II, and III

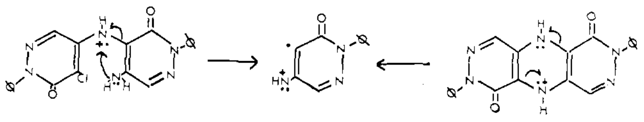

Figure 2. Possible pathways for formation of peak at m/e 185 from both II and III

ACKNOWLEDGMENT

The authors express their appreciation to Olwen Van Es, Rutgers University, and to Martin Schulman, Varian Aerograph, for mass spectra; to N. Drescher, Badische Anilin- & Soda Fabrik for supply of pyrazon; and to K. L. Loening, Chemical Abstracts Service, for nomenclature of II and III.

LITERATURE CITED

Beynon, J. H., "Mass Spectrometry and Its Application to Organic Chemistry," Elsevier, Amsterdam, 1960, p 298.
Calvert, J. G., Pitts, J. N., "Photochemistry," Wiley, New York, N.Y., 1966, p 748.
Forsythe, W. F., Christison, F., *Gen. Elec. Rev.* **32**, 664 (1929).
Rao, C. N. R., "Chemical Applications of Infrared Spectroscopy," Academic Press, New York, N.Y., 1963, p 325.
Rosen, J. D., Siewierski, M., Winnett, G., J. AGR. FOOD CHEM. **18**, 494 (1970).

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Received for review February 25, 1971. Accepted October 21, 1971. Presented in part at the Division of Pesticide Chemistry, 152nd meeting, ACS, Washington, September 1971. Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08903.

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Metabolism of Limonin and Limonoate by Microorganisms: The Conversion to Nonbitter Compounds

A survey was made of microorganisms capable of metabolizing limonin to nonbitter compounds. It was found that several species of bacteria obtained from soil grew well on a medium containing limonin or limonoate as a single carbon source. Two nonbitter metabolites from the cultural medium were isolated and identified as deoxylimonin and deoxylimonic acid.

Limonin (Figure 1) is a bitter principle of citrus juices (Arigoni *et al.*, 1960; Emerson, 1948; Higby, 1938; Kefford, 1959). The bitterness due to limonin develops gradually in juices after extraction from certain varieties of oranges, lemons, and grapefruit. The intact fruits do not normally contain limonin but rather a nonbitter precursor, limonoate A-ring lactone (Maier and Beverly, 1968; Maier and Margileth, 1969). This nonbitter precursor converts to limonin under acidic conditions (Maier and Beverly, 1968) and the conversion is also accelerated by the action of limonoate D-ring lactone hydrolase, which has been shown to be present in citrus fruit (Maier *et al.*, 1969). In the citrus industry this phenomenon is referred to as delayed bitterness (Joslyn and Pilnik, 1961; Kefford, 1959).

In dealing with this bitterness problem, two lines of research are underway at our laboratory. The first is that of acceler-