Photochemical Degradation of Diquat in Dilute Aqueous Solution and on Silica Gel

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In solution ^{14}C-ring-labeled diquat (6,7-dihydro-dipyrido[1,2-a:2',1'-c]-pyrazidiinium salt) was rapidly degraded by filtered radiation from a mercury vapor lamp and also by natural sunlight to give identical photochemical decomposition products. Chromatographic separation of the products formed at various stages during exposure indicated that as many as nine breakdown products were formed from diquat, though many of these were transient and underwent further degradation. Six decomposition products were isolated and identified and two pathways for the photochemical degradation of diquat are proposed. The minor route gives rise to very small amounts of fluorescent compounds with pyridone structures, while the major decomposition pathway leads to the already known TOPPS (1,2,3,4-tetrahydro-1-oxo-pyrido[1,2-a]-5-pyrazinium salt), together with picolinamide and picolinic acid. Under the action of sunlight, ^{14}C-ring- and ^{14}C-bridge-labeled diquat underwent photochemical breakdown, on silica gel surfaces, to give only TOPPS and picolinamide.

Diquat (6,7-dihydrodipyrido[1,2-a:2',1'-c]-pyrazi-diinium salt) as the dibromide (I) is the active constituent of Reglone (Plant Protection, Ltd.) which is widely used as a contact herbicide, as a desiccant, and for the control of water weeds. Available evidence (Funderburk and Lawrence, 1964; Smith, 1967) suggests that diquat is not metabolically degraded in certain plants, but undergoes rapid photochemical decomposition. Clearly the isolation and identification of any photochemical breakdown products derived from diquat, after spraying, would be greatly facilitated if this decomposition could be studied in a simpler system than on the surface of plants.

Funderburk et al. (1966) observed that two unidentified radioactive compounds were produced when ^{14}C-labeled diquat was irradiated in aqueous solutions using a mercury vapor lamp. Later work (Slade and Smith, 1967) showed that 1,2,3,4-tetrahydro-1-oxopyrido[1,2-a]-5-pyrazinium salt (II) (TOPPS) was formed together with several minor products when dilute aqueous solutions of diquat were exposed to filtered ultraviolet radiation.
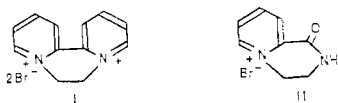
The work described here deals more fully with the photochemical breakdown of ^{14}C-ring- and ^{14}C-bridge-labeled diquat in dilute aqueous solutions and on silica gel. These studies were undertaken as the first part of a detailed investigation to determine the fate of diquat after foliar application.

METHODS AND MATERIALS

Chemicals. N,N'-Ethylene-[2,3,5,6,2',3',5',6',-[14]C$_4$] 2,2'-bipyridilium dibromide monohydrate (Dunn et al., 1966) and N,N'-ethylene-[7,8-[14]C$_2$] 2,2'-bipyridilium dibromide monohydrate (available from the Radio-chemical Centre, Amersham, England) were used. The specific activity of both materials was 2.5 mc. per mmole and their radiochemical purity was better than 99%.

Apparatus. The ultraviolet source consisted of a Hanovia Model 16 lamp fitted with a borosilicate filter 2 mm. thick, which effectively absorbed all radiation below 290 mμ. As there is very little radiation in sunlight with a wavelength below 300 mμ and none below 290 mμ (Luckiesh, 1930), it was assumed that the photochemical breakdown of diquat in solution, using this apparatus, would be similar to that occurring in sunlight.

Unless otherwise stated, the irradiations were conducted in a 250-ml. borosilicate glass beaker surrounded by aluminum foil to increase the photochemical efficiency of the radiation. The lamp and filter were centered over the beaker, with the filter just touching the rim of the beaker. Using this apparatus 50 or 100 ml. of diquat solutions, of the appropriate concentrations, could be irradiated. No oxygen, or air, was passed through the solutions and stirring was achieved using a magnetic stirrer.

In the case of diquat exposed to sunlight, the solution was poured into a borosilicate glass Petri dish (8-inch diameter) surrounded with aluminum foil and tightly covered with polythene sheeting 0.1 mm. thick. Polythene film of this thickness does not appreciably absorb radiation above 290 mμ. Exposure to sunlight was effected during May and June while the weather was sunny and relatively cloudless.

Ultraviolet Absorption Measurements. These were made on a Unicam S.P. 800 spectrophotometer using 1-cm. silica cells.

Radioactivity. The [14]C- in the various solutions was determined using a Packard Tri-Carb liquid scintillation counter (Model 574), by taking suitable aliquots, and adding 20 ml. of butyl-PBD-based scintillation fluid [1.6% 2-(4'-tert-butylphenyl)-5-(4"-biphenylyl)-1,3,4-oxdiazole, 17% naphthalene in dioxane].

Paper Chromatography. Unless otherwise stated, all chromatograms were run on Whatman No. 1 chromatography paper using a mixture of 1-butanol, acetic acid, and water (4:1:2) as developing solvent. Occasionally the paper was washed with distilled water for 24 hours prior to use.

Thin-Layer Chromatography. Silica gel plates 0.3 mm. thick (without fluorescent indicator) were used and developed using $6N$ hydrochloric acid.

RESULTS AND DISCUSSION

During the initial experiments concentrated solutions of unlabeled diquat (0.5 gram in 500 ml. of water) were irradiated for 80 hours with the filtered light, to enable larger quantities of the various breakdown products to be isolated for identification purposes. The undegraded diquat (I) and TOPPS (II) were removed from solution using a procedure (Morgan, 1966) in which 10 grams of Actibon C charcoal was stirred into the green fluorescent solution. All the organic material was adsorbed onto the charcoal, which was then filtered off and heated under reflux for 30 minutes with 250 ml. of 95% ethanol to remove only the less strongly adsorbed photoproducts. The residue obtained by evaporation of the ethanolic extract was taken up in a small volume of water and applied to the origin of a washed Whatman 3-mm. paper chromatogram.

After development the dried chromatogram was viewed under an ultraviolet lamp which indicated the presence of two compounds. One, having an R_f value of 0.62, exhibited intense blue fluorescence, while the other, with an R_f value of 0.80, showed ultraviolet absorption. Several minor fluorescent bands could be seen, but were present only in trace amounts.

The two major bands were cut out and eluted with distilled water. Evaporation of the extracts yielded approximately 5 mg. of the ultraviolet-absorbing compound and about 0.5 mg. of the fluorescent product. These two samples were rechromatographed separately on washed paper and then eluted with water. After removal of the water, the residues were dried for several hours using an oil vacuum pump, before being examined by mass spectroscopy.

Analysis by mass spectroscopy showed the material possessing the R_f value of 0.80 to have a molecular weight of 122, corresponding to a formula of $C_6H_6N_2O$. The breakdown pattern suggested the structure could be that of picolinamide (III), and this proved to be the case, as an authentic sample of picolinamide (III) gave an identical mass spectrum.
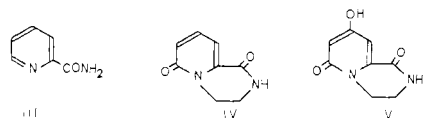
Mass spectra data for the fluorescent material were consistent with the presence of two products, the major constituent having a molecular weight of 164 and molecular formula $C_8H_8N_2O_2$, and a minor component (approximately 5% of the total fluorescent product) with a molecular weight of 180 corresponding to $C_8H_8N_2O_3$.

Although on washed paper chromatograms there appeared to be only one fluorescent band, on unwashed paper there were two fluorescent components with R_f values of 0.64 and 0.71, respectively. As these two degradation compounds showed strong fluorescence in both acidic and basic solutions (Bridges et al., 1966), and neither exhibited any black color with potassium iodoplatinate solution (Smith, 1960). it was inferred that they both possessed 2-pyridone structures. Consequently the product with the lower molecular weight was assigned the pyridone structure (IV), while the other compound, having an additional oxygen atom, was tentatively assumed to be the hydroxylated pyridone (V).

That picolinamide (III) should be formed from diquat (I) directly seemed unusual and it was considered likely that this compound (III) resulted from the photodecomposition of TOPPS (II). To test this hypothesis a solution containing 1 gram of TOPPS [as the chloride (Slade and Smith, 1967)] in 1 liter of water was irradiated for 24 hours. The solution was then extracted several times with benzene, and the combined extracts were evaporated to dryness. A white residue was obtained which could be recrystallized from a benzene and petroleum ether (boiling range 60° to 80°) mixture to give 3 mg. of white needles whose infrared spectrum (run as a liquid paraffin mull), melting point (106°), and mixed melting point (106°) were identical with those for authentic picolinamide (III).

This provided firm evidence that picolinamide could arise from the photochemical degradation of TOPPS (II). However, some of the amide (III) may be formed directly from diquat by fission of the ethylene bridge followed by oxidation of one of the pyridine rings.

The above experiments, which resulted in the isolation and identification of three further photodecomposition products of diquat, have given no information as to how many intermediates are involved. To resolve this problem 50-ml. portions of ^{14}C-ring- and ^{14}C-bridge-labeled diquat solutions containing 50 p.p.m. of ion were irradiated separately. Aliquots of 10 ml. were removed after 0, 4, 7, 24, and 36 hours, concentrated to small bulk, and applied to the origins of paper chromatograms, which were then developed and placed in contact with x-ray film for 3 weeks.

These results (Table I) indicate that when diquat solution is exposed to ultraviolet radiation for 36 hours only four decomposition compounds remain, and the predominant one contains none of the original bridge carbon atoms. The radioactive products with R_f values of 0.26 and 0.32 were so faint as to be hardly visible, indicating that they were present in negligible quantities. As picolinamide appears to be an important breakdown product of diquat, it was thought that the principal radioactive product (R_f 0.50) could be picolinic acid (VI) formed by hydrolysis of picolinamide (III) in the aqueous media. The other major radiocompound

Table I. Photochemical Decomposition of Diquat in Solution with Time

R_f Value of Radio Products	Hours										Structure
	0		4		7		24		36		
	Ring	Bridge	Ring	Bridge	Ring	Bridge	Ring	Bridge	Ring	Bridge	
0.20	S^a	S	S	S	S	S	W	W	—	—	Diquat(I)
0.26	—	—	—	—	—	—	W	M	W	W	Both ring and bridge
0.32	—	—	—	--	W	—	W	M	W	W	fragments
0.40	—	—	W	W	M	M	S	S	M	M	TOPPS(II)
0.44	—	—	W	W	W	W	—	—	—	—	Pyridone(VII)
0.48	—	—	—	W	—	W	—	W	—	—	Bridge fragments
0.50	—	—	W	—	W	—	S	—	S	—	Picolinic acid(VI)
0.64	—	—	—	—	M	—	W	W	—	--	Pyridones IV and V
0.74	—	—	—	—	W	—	M	W	—	—	
0.80	—	—	W	—	M	—	W	—	—	—	Picolinamide(III)

^{a} S, M, W, and — designate whether degree of film blackening on autoradiograms is strong, medium, weak, or nonexistent, and indicate the relative abundance of radioactive compounds present.

(R_f 0.40) was inferred to be TOPPS (II). The two eluted radioactive products were cochromatographed, both on paper and on thin layers of silica gel with authentic samples, thus confirming their identity. The picolinic acid was further characterized by cochromatography on paper using 2-propanol and concentrated ammonia (80 to 20) as eluent, and by co-electrophoresis.

Although as many as nine photodecomposition products may be present in solution at any one time (Table I), only TOPPS (II), picolinic acid (VI), and picolinamide (III) are present to any appreciable extent and the other components exist in only very minor amounts. The structures assigned to the various breakdown products are shown in the last column of Table I.

Very little degradation occurs during the first 4 hours of irradiation, but blackenings on the autoradiograms indicate that TOPPS (II), picolinamide (III), and picolinic acid (VI) may be formed in minute traces. Even after 4 hours an intensely fluorescent band (R_f 0.44) can be observed when the chromatogram is viewed under an ultraviolet lamp. The structure of this compound, on the basis of spectroscopic and electrophoretic data, is thought (Morgan, 1966) to be the monopyridone of diquat (VII). Recently this structural assignment has been confirmed by synthesis (Calderbank, 1968).
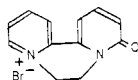
Table I also shows that initially picolinamide is the major decomposition product of diquat. This undergoes rapid hydrolysis, so that after 24 hours picolinic acid becomes the principal degradation product.

Information gained by irradiating ^{14}C-bridge-labeled diquat is misleading, since neither picolinamide nor picolinic acid shows on the autoradiograms and TOPPS appears as the only major breakdown product (Slade and Smith, 1967).

Little can be said concerning the minor photodecomposition entities with R_f values of 0.26 and 0.32, except that they appear to contain both ring and bridge carbon atoms derived from diquat. They may also possess relatively small carbon fragments, as their presence is not detected until later in the irradiation.

The nature of the material having the R_f value of 0.48 is uncertain and may possibly be due to fragments derived from the ethylene bridge.

As the two minor radioactive compounds (R_f 0.64 and 0.74) are formed from both ^{14}C-ring- and ^{14}C-bridge-labeled diquat and have R_f values identical to those of the pyridones (IV and V), it was considered that these radioactive bands may be due to the presence of the two pyridones. Subsequent elution of the products, followed by cochromatography on paper and silica gel with the fluorescent material previously obtained from the irradiation of nonradioactive diquat, confirmed this.

Slade and Smith (1967) reported that solutions of diquat are degraded completely to volatile fragments on prolonged irradiation. Since picolinic acid was the last identifiable end product in these experiments, an aqueous solution containing 50 p.p.m. of picolinic acid was irradiated to determine its rate of decomposition. The loss of picolinic acid from the solution was determined by the reduction in the ultraviolet absorption at 265 mμ. Breakdown was very rapid and after 16 hours no ultraviolet-absorbing material could be detected in solution.

Having studied the photodecomposition of ^{14}C-diquat in solution under artificial conditions, it became necessary to investigate the degradation under natural conditions, and also to obtain a measure of the relative amounts of the various degradation products formed. A solution containing 5 p.p.m. of ^{14}C-ring-labeled diquat was therefore exposed to sunlight as previously described. After various time intervals aliquots were removed and the diquat content was determined by measuring the ultraviolet absorption at 310 mμ. The radioactivity present in solution was also checked. These

Table II. Loss of Diquat and ^{14}C from Solution on Exposure to Sunlight

Time, Weeks	$E_{310}^{1\,Cm.}$	% Diquat Remaining	Activity in Solution, Counts/Sec./ Ml.	%^{14}C Remaining
0	0.54	100	3100	100
3	0.15	30	3080	99
4	0.10	20	2910	94
5	0.06	10	3050	98

Table III. Percentage Volatile Fragments Formed on Exposure of ^{14}C-Ring-Labeled Diquat in Solution to Sunlight

Time, Weeks	Nonvolatile ^{14}C in Solution, Counts/Sec./ 25 Ml.	% Volatiles
0	77,500	0
3	66,550	14
5	51,250	34

results (Table II) show that the breakdown of diquat is fairly rapid, 70% being degraded in 3 weeks. There is no observable loss of radioactivity from solution, indicating that any volatile fragments formed remain dissolved in the water.

To estimate these volatile fragments, 25-ml. portions were removed from the solution after 3 and 5 weeks' exposure and evaporated to dryness under reduced pressure at 40° C. The residues were taken up in 5.0 ml. of distilled water and the radioactivity in 1.0 ml. was determined. These results (Table III) show that after 5 weeks approximately one third of the original activity can be accounted for as volatile products.

After 3 and 5 weeks' treatment 20-ml. samples were taken from the solution, evaporated to small bulk, and applied to the origins of paper chromatograms which, after development, were placed in contact with x-ray film for 3 weeks. The resulting autoradiographs showed, in addition to diquat, the presence of seven radioproducts; however, only two predominated, the remaining five existing merely in trace amounts. The R_f values of these radioactive photocompounds (Table IV) correspond exactly to those observed when ^{14}C-ring-labeled diquat was irradiated with filtered ultraviolet light (Table I). For additional structural confirmation the bands corresponding to TOPPS (R_f 0.40), picolinic acid (R_f 0.50), and the pyridones (IV and V) (R_f 0.67 and 0.74) were eluted from the chromatograms with water and the evaporated residues successfully cochromatographed on paper and silica gel with the authentic materials. The component having an R_f value of 0.45 was judged to be the monopyridone of diquat (VII) on account of its distinctive blue fluorescence, and this has been shown to be correct (Calderbank, 1968). Therefore, the photodecomposition of diquat in aqueous solution by filtered ultraviolet radiation is essentially the

same as in sunlight, TOPPS (II) and picolinic acid (VI) being the major degradation products. In both instances the minor components appear to be identical.

The photochemical degradation of diquat on silica gel surfaces was investigated by applying 1 mg. each of the ^{14}C-ring- and ^{14}C-bridge-labeled herbicides in dilute solution (1 ml.) to separate areas of a silica gel chromatography plate. The plate was placed in a tray, covered with polythene sheeting (to exclude rain), and left out of doors for 6 weeks. After exposure the treated areas of the silica gel were carefully scraped off and extracted with water to remove all the radioactivity. These extracts were then applied to paper chromatograms from which autoradiograms were prepared. Two radioproducts with R_f values of 0.39 and 0.82 were obtained from the ^{14}C-ring-labeled diquat, while only one radiocompound (R_f 0.39) was produced from the ^{14}C-bridge-labeled herbicide. In both instances small amounts of the unchanged parent diquat were present. From the R_f values, and the fact that the compound with the greater movement contains no ethylene carbon atoms, it was deduced that the two products were TOPPS (II) and picolinamide (III). Cochromatography with known specimens on paper and silica gel surfaces confirmed this.

Photodecomposition of diquat on inert surfaces is thus simpler than in solution, and preliminary studies have indicated that small amounts of ^{14}C-TOPPS (less than 5% of the applied activity) are produced when plants treated with ^{14}C-diquat are exposed to sunlight.

Work in progress on the decomposition of the herbicide after its application to plant foliage will be reported later.

From the above studies two pathways (Figure 1) can be proposed for the photochemical decomposition of diquat in solution. The major pathway leads through TOPPS (II), picolinamide (III), to picolinic acid (VI) and volatile fragments, while the minor route gives rise to the two fluorescent pyridones (IV and V).

The importance of the pyridone (VII) during the breakdown of diquat is difficult to judge, as it is present

Table IV. Photochemical Decomposition of ^{14}C-Ring-Labeled Diquat in Solution by Sunlight

R_f of ^{14}C-Product	3 Weeks	5 Weeks
0.26	M^a	M
0.32	W	W
0.40	S	S
0.45	W	W
0.50	S	S
0.64	W	W
0.74	W	W

^{a} S, M, and W designate whether degree of film blackening on autoradiograms is strong, medium, or weak to indicate relative abundances of radioactive compounds.
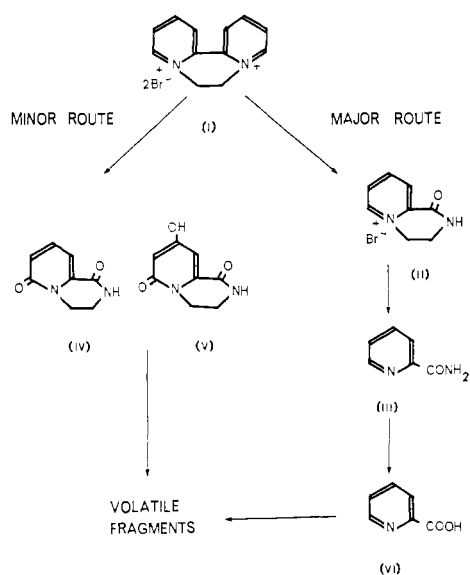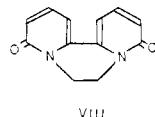

Figure 1. Proposed pathway for photochemical decomposition of diquat

in very small amounts, and detection is entirely due to its intense fluorescence. A highly fluorescent dipyridone (VIII) derived from diquat has also been synthesized (Farrington, 1966) but no evidence was obtained of its formation during the irradiation of diquat solutions.

VIII

The decomposition of diquat on silica gel surfaces gives rise to TOPPS and picolinamide as the only detectable products; in this instance hydrolysis to picolinic acid did not occur.

When diquat is used for the control of aquatic weeds, the chief factor resulting in loss of the herbicide is uptake by the weeds and adsorption to suspended silt and mud. This process is rapid and can account for removal of 90% of the applied diquat from the water within 24 hours (Yeo, 1967); thus loss of the chemical by the relatively slow process of photochemical decomposition will be slight.

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