

Synthesis and Properties of Paraquat (Methyl Viologen) and Other Herbicidal Alkyl Homologues

John H. Ross¹ and Robert I. Krieger*

A report of the physicochemical properties of 1,1'-dialkyl-4,4'-bipyridylium salts (viologens) is provided that is useful for residue analysis, biochemical research, and mechanistic studies of toxicity in plants and animals. This group of compounds is finding application as herbicides, anticoccidiostats, and other industrial uses. The solubility, redox potentials, ¹H NMR, partition coefficients, toxicity, synthesis, and radiosynthesis are summarized. Paraquat (methyl viologen) binds to glassware to the extent of 40% at 4 × 10⁻⁷ M. This observation requires consideration in the design of methods for paraquat residue determination and for experimental studies using low concentrations of viologens.

1,1'-Dialkyl-4,4'-bipyridylium salts (viologens) have found many applications as biochemical research tools (Michaelis and Hill, 1933; Peck and Gest, 1956; Guengerich et al., 1975) and are being developed commercially for such purposes as herbicides (Imperial Chemical Industries, Ltd., 1963, 1964, 1965a,b), electrochromic displays (van Dam and Ponjee, 1974; Belinko, 1976), electroplating metal brighteners (Passal, 1971), photographic light screens (Moore, 1976), film development accelerators (Willems, 1971), and an anticoccidiosis treatment for domestic fowl (Imperial Chemical Industries, Ltd., 1965c). Recently we have reported herbicidal activity toward duckweed (*Spirodela oligorrhiza*, Kurz) of paraquat and seven other alkyl homologues (Ross et al., 1979). Parallel studies using Sprague-Dawley rats revealed that the alkyl viologens and paraquat produced similar signs of toxicity (Ross and Krieger, 1979).

Reported syntheses of these compounds, though numerous, rarely include structural confirmation. The physical constants of these compounds were determined under similar conditions to allow more uniform comparison of the physicochemical effects of changing alkyl substituents on nitrogens of 4,4'-bipyridylium salts. Physicochemical constants of these compounds have been used to explain the herbicidal mode of action (Homer et al., 1960; Calderbank, 1968) and are important in developing residue quantitation methods. This article reports the synthesis and physical constants of a series of herbicidal 1,1'-dialkyl-4,4'-bipyridylium salts. Included also are procedures and results of the preparation of high specific activity ¹⁴CH₃-labeled substrate.

EXPERIMENTAL SECTION

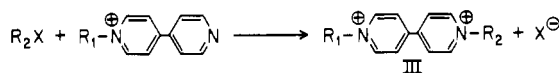
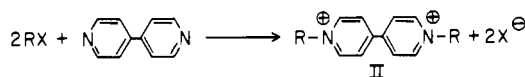
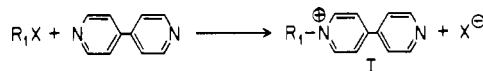
Materials. Alkyl halides and 4,4'-bipyridyl dihydrate were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI), and used without further purification. Benzyl viologen (chloride) was purchased from Gallard-Schlesinger Chemical Manufacturing Corp. (Carle Place, NY). Methyl viologen (paraquat, PQ) was purchased from Aldrich. Thin-layer chromatography (TLC) was conducted on precoated 250-μm silica gel G F-254 plates (Analtech Inc., Newark, DE) and was visualized by spraying with iodoplatinate (Waldi, 1965). Glass ampules (1-10 mL; Kimble Neutraglas) were purchased from VWR Scientific (San Francisco, CA).

Department of Environmental Toxicology, University of California, Davis, Davis, California 95616.

¹Present address: Agricultural Research Department, Stauffer Chemical, Mountain View, CA 94040.

General Procedures. Melting points were determined on a Thomas-Hoover apparatus (Arthur H. Thomas Co., Philadelphia, PA) and are uncorrected. ¹H NMR spectra were prepared by using a Varian EM 390 spectrometer (Varian Associates, Palo Alto, CA). Gas chromatographic analysis was performed on a Varian 2100 GLC (Varian Associates, Palo Alto, CA) using a 6-ft column packed with 12% SE-30 on Chromosorb W with a N₂ flow rate of 60 mL/min. Gas chromatography-mass spectrometry was done on a Finnigan Model 3200 at 70 eV (Finnigan Corp., Sunnyvale, CA). Elemental analyses were performed by C. F. Geiger (Ontario, CA). Ultraviolet spectra were determined on a Cary 14 UV-visible spectrophotometer. All compounds were dried at 110 °C for 2 h in vacuo prior to instrumental analysis. Liquid scintillation counting (LSC) was done with a Packard 2425 spectrometer (Packard Instruments, Downer's Grove, IL).

Radiosyntheses. Carbon-14 methyl-labeled paraquat (¹⁴CH₃PQ) was synthesized by using excess 4,4'-bipyridyl (2.8 mg, 18 μmol) previously recrystallized from water and vacuum desiccated (mp 113-114 °C) dissolved in nitrobenzene (0.05 mL) and placed into a 2-mL glass ampule. [¹⁴C]Methyl iodide (1.0 mCi at 55.7 mCi/mmol; California Bionuclear, Sun Valley, CA) was received in 0.75 mL of nitrobenzene and transferred to the ampule with three 0.2-mL nitrobenzene washes. The ampule was placed in a dry ice-acetone bath until the contents were frozen, and the neck was drawn out and sealed with a torch. Following gentle warming to room temperature, the ampule was checked for leaks by inversion in a beaker of warm water. The ampule was then heated in an oven for 23 h at 105 °C. After the ampule was cooled, the tip of the ampule was broken to allow addition of 6 μL (13.7 mg, 97 μmol) of methyl iodide (CH₃I) to the reaction mixture. The ampule was resealed and heated again for 24 h at 105 °C. Upon cooling to room temperature, the neck of the vial was scored and broken and the contents were removed by pipet with three 0.4-mL water rinses. The water phase was extracted with benzene (0.5 mL × 2) to remove unreacted CH₃I and dissolved nitrobenzene and transferred to a



10-mL volumetric flask containing water. Yield was 104% as determined by LSC of 10- μ L aliquots of the aqueous phase. The product cochromatographed by TLC (R_f 0.14) with authentic 1,1'-dimethyl-4,4'-bipyridylium ion (IIa) when developed in benzene-1-pentanol-methanol-1 N HCl (BPMH, 1.3:4:8:7). Radiopurity was 99% as determined by LSC of scraped active zones. Radioactivity was detected on TLC plates by autoradiography using Kodak X-Omat film. After catalytic hydrogenation (Soderquist and Crosby, 1972), the product cochromatographed as one peak by gas-liquid chromatography (GLC) with authentic 1,1'-dimethyl-4,4'-bipiperidine.

The specific activity of [^{14}C] PQ (83.4 mCi/mmol) was determined from counting a portion of the 10-mL stock solution by LSC and measuring the PQ concentration by colorimetry (Pack, 1967) at 600 nm. The specific activity of the catalytically hydrogenated product of this radiosynthesis (99.1 mCi/mmol) was determined by GLC of a portion of the hydrogenated product and LSC of other portions and compares favorably with the specific activity of [^{14}C] PQ determined colorimetrically.

1- ^{14}C Methyl-4-(4-pyridyl)pyridinium iodide ([^{14}C] PQ monoquat) was synthesized as described for [^{14}C] PQ starting with 2 mCi of [^{14}C] CH_3I (51 mCi/mmol) and 18.4 mg 4,4'-bipyridyl (118 μ mol), but additional nonlabeled CH_3I was not added and the reaction was terminated after 18 h. Preparative TLC of the aqueous extract of the reaction mixture in BPMH (1000- μ m silica gel G, Analtech) yielded a minor and a major radiolabeled band which corresponded to [^{14}C] PQ and [^{14}C] monoquat , respectively. A radiopurity determination of the isolated major product indicated that it was 97% pure and cochromatographed with authentic 1-(methylpyridyl)-4-pyridine iodide (Ia). Total recovery of radiolabel was 104%, of which 79% was recovered as [^{14}C] monoquat .

1- ^{14}C Methyl-1'-pentyl-4,4'-bipyridylium diiodide ([^{14}C] pentyl viologen) was synthesized by placing an ethanol solution of [^{14}C] monoquat (0.2 mCi) into a 2-mL glass ampule. Following evaporation of the solvent under a slow stream of nitrogen, 2 mL of pentyl iodide was added and the ampule was sealed and heated for 36 h at 105 $^\circ\text{C}$. The reaction mixture was extracted with 8.6 mL of H_2O , streaked on a preparative TLC plate, and developed in water-concentrated HCl-1-pentanol (100:15:2.5). The lower band (R_f 0.25) containing 51% of starting radiolabel was eluted from the plate in 97% radiopurity as determined by cochromatography with authentic methyl pentyl viologen (IIIb) in the solvent system just described. The upper band (R_f 0.36) consisted of starting material.

The radiolabeled compounds [^{14}C] monoquat and [^{14}C] pentyl viologen were difficult to elute from silica gel following isolation by preparative TLC. Elution required large volumes (200 mL) of 6 N HCl. The [^{14}C]-labeled bipyridyls streaked extensively on analytical TLC plates. Cochromatographic overlaying on the analytical plate (i.e., spotting cold standard followed by radiolabeled compound) did not reduce streaking. All the radiolabeled compounds including [^{14}C] PQ were premixed with cold standards before portions were spotted on analytical TLC plates for radiopurity determination. Even when cochromatographed by premixing, there was a small amount of elongation resulting in elliptical spots which decreased the apparent radiopurity.

Glass Binding of Paraquat. Modified Krebs-Ringer phosphate medium (KRP) as described by Smith et al. (1976) was used for PQ glass binding experiments. Calcium chloride was omitted from the medium because its addition resulted in a milky white precipitate. This medium contained inorganic constituents that are found in

human plasma or soil and water samples to be analyzed for PQ.

Varying amounts of unlabeled PQ were added to 3 mL of KRP containing a constant amount of [^{14}C] PQ (0.1 $\mu\text{Ci}/3\text{mL}$) in a 20-mL glass LSC vial. Following incubation of the medium for 1 h at 37 $^\circ\text{C}$ in a shaking water bath, 0.2 mL of the medium was withdrawn by plastic pipet and placed in a vial containing 1.0 mL of 1 N HCl. After addition of scintillation cocktail, the aliquots were counted by LSC and counting efficiency was determined by re-counting with [^{14}C]toluene internal standardization.

Syntheses. The following compounds prepared essentially by literature procedures are listed with the yields and physical constants observed. 1-Methylpyridinium iodide (Kosower, 1955): 36%; mp 117–118 $^\circ\text{C}$; ^1H NMR (D_2O) referenced to dioxane (4.0 ppm) on a 10-ppm scale, 9.03 (2 H, d), 8.78 (1 H, t), 8.28 (2 H, t), 4.60 (3 H, s). 1,1'-Dimethyl-4,4'-bipiperidine monohydrate (Soderquist and Crosby, 1972): 71%; mp 51–52 $^\circ\text{C}$; parent m/e 196.

1-Ethyl-4-(4-pyridyl)pyridinium Iodide (Ib). To a solution of 1.00 g (5.2 mmol) of 4,4'-bipyridyl dihydrate in 7 mL of nitrobenzene was added 0.42 mL (5.2 mmol) of ethyl iodide in a 10-mL glass ampule. The ampule was sealed with a torch and placed in an oven (105 $^\circ\text{C}$) for 18 h. On being cooled, the solidified reaction mixture was washed repeatedly with benzene to remove nitrobenzene odor and filtered. The crude product was dissolved in hot propanol-ethanol (4:1), and small red crystals formed. These crystals were discarded, and the mother liquor was evaporated to dryness and recrystallized from hot propanol-ethanol following Norit decolorization. The resulting orange crystals were rinsed with ice-cold propanol and dried in vacuo at 105 $^\circ\text{C}$.

1,1'-Diethyl-4,4'-bipyridylium Iodide (IIb). To 1.00 g (5.2 mmol) of 4,4'-bipyridyl dihydrate dissolved in 6 mL of nitrobenzene was added 1.03 mL (12.8 mmol) of ethyl iodide in a 10-mL ampule. The ampule was sealed and heated to 105 $^\circ\text{C}$ for 30 h. The solidified reaction mixture was extracted with benzene (6 \times 10 mL) and washed with propanol-ethanol (1:1). Recrystallization from absolute ethanol yielded orange-red needles.

1-Methyl-1'-propyl-4,4'-bipyridylium Iodide (IIIa). To 2 g (6.7 mmol) of Ia was added 5.0 mL (52 mmol) of propyl iodide in a 10-mL glass ampule. The ampule was sealed with a torch and heated to 105 $^\circ\text{C}$ for 18 h. The solidified bright red reaction mixture was extracted with water and the aqueous portion triturated with ethanol. The resulting crystals were recrystallized from 95% EtOH to yield small red plates.

Two compounds (IIa and IIIh) were anion exchanged. IIa was modified from an iodide to a chloride salt by placing a twofold excess of AgCl in a stirred flask containing an aqueous solution of IIa (iodide). AgI was removed by filtration and the filtrate triturated with acetone to obtain IIa chloride. IIIh was exchanged by dissolving it in water and adding a saturated solution of KI. Red crystals precipitated immediately and were collected by vacuum filtration and recrystallized from absolute ethanol.

Partition coefficients were determined by the method of Beroza et al. (1969) using water-saturated octanol partitioned against aqueous solutions [(2–3) $\times 10^{-5}$ M] of the bipyridylium salts. Concentration changes in the aqueous phase were determined by UV spectrophotometry at the absorption maxima of the respective compounds.

RESULTS AND DISCUSSION

$\text{S}_{\text{N}}2$ reactions of pyridine with alkyl halides have been claimed to be termolecular with the solvent concentration being one of the determinants of reaction kinetics (Norris

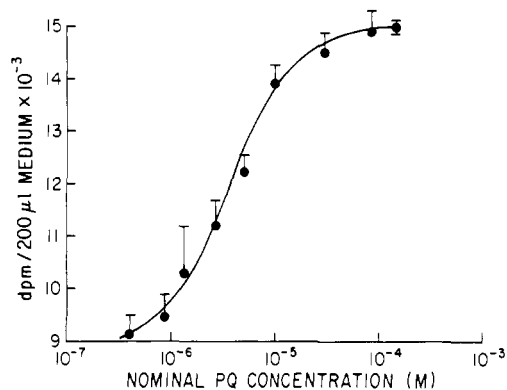


Figure 1. Debinding of [^{14}C] PQ bound to glass as a function of unlabeled PQ concentration. Varying amounts of unlabeled PQ were added to 3 mL of KRP containing a constant amount of [^{14}C] PQ ($0.1 \mu\text{Ci}/3 \text{ mL}$; $4.1 \times 10^{-7} \text{ M}$) in a 20-mL LSC vial. Following incubation of the solution for 1 h at 37°C in a shaking water bath, 0.2 mL of the solution was withdrawn by a plastic pipet and placed in a LSC vial containing 1.0 mL of 1 N HCl. After addition of scintillation cocktail the aliquots were counted and counting efficiency was determined by recounting with [^{14}C]toluene internal standardization. Each point represents the mean \pm SE of three different incubations.

and Prentiss, 1928; Swain and Eddy, 1948). Attempts to optimize reaction conditions were made by choosing a solvent with a boiling point in excess of the reaction temperature that would provide good solvation of the anionic leaving group and yet have relatively low water solubility and reactivity with the alkyl halide. Nitrobenzene was selected for these purposes. Whenever possible, the alkyl iodide was employed to favor high yields in this condensation (Shaw, 1961). These considerations were especially relevant in achieving high overall yields in radiosyntheses to provide maximal specific activities.

The effect of increased PQ concentrations on glass binding of a fixed quantity ($4.1 \times 10^{-7} \text{ M}$) of [^{14}C] PQ is shown in Figure 1. Glass binding of [^{14}C] PQ was 40% at the lowest nominal concentration and became negligible at PQ concentrations of $\geq 8 \times 10^{-5} \text{ M}$. PQ binding to glass was first observed when thoroughly washed scintillation vials which had previously contained [^{14}C] PQ before washing were filled with scintillation cocktail and counted for background. Background levels were 20 times normal. Binding studies of amino acid derivatives by powdered quartz have demonstrated that basic amino acids (e.g., lysine and arginine) are most tightly bound followed by neutral and finally acidic amino acids (Kavasmaneck and Bonner, 1977). PQ is bound very tightly by clay and silicic acid (Staiff et al., 1973). It is not surprising then that a doubly positively charged molecule such as PQ would be bound tenaciously by laboratory glassware. This observation has some interesting implications concerning determination of low levels of PQ in glass apparatus (e.g., residue studies and even PQ in marijuana smoke). At micromolar concentrations (0.1–1 ppm), substantial amounts of PQ will bind to glass unless one uses a cationic solute of high ionic strength (e.g., HCl or HClO_4) to displace it.

The name viologens was coined for disubstituted 4,4'-bipyridylium (bpy) salts because of the blue-violet color produced on one-electron reduction (Michaelis and Hill, 1933). The free radical formed reacts rapidly with oxygen ($k = 7.7 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$) to revert to the doubly charged parent compound (Farrington et al., 1973).

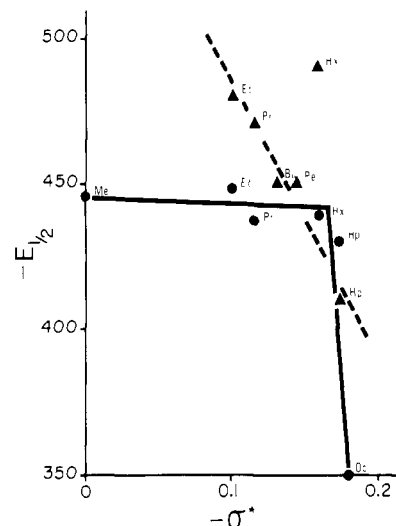
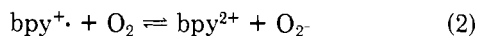
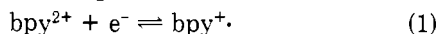


Figure 2. Dependence of redox potential ($E_{1/2}$ in millivolts) on alkyl inductive effect (σ^*) of side chains of viologens. $E_{1/2}$ determined in water (\bullet) from Homer et al. (1960) and Khaskin et al. (1972) was referenced to a normal hydrogen electrode. $E_{1/2}$ determined in acetonitrile (\blacktriangle) from van Dam and Ponjee (1974) was referenced to Ag/AgCl .

Addition of silver oxide to an aqueous viologen solution initially produces the quaternary ammonium hydroxide and the blue color indicative of free radical formation (Anderson, 1870; Weidel and Russo, 1882). In the presence of base, viologens decompose to 4,4'-bipyridyl with oxidation of the side chain to an aldehyde. The oxidation reaction is accompanied by the production of viologen free radicals and explains the nonobeyance of Beer's law by viologens in base (Corwin et al., 1968). All of the disubstituted bipyridylium compounds reported in the current study (IIa–i and IIIa–c) produced a blue-violet color when reduced by alkaline sodium dithionite solution while Ia and Ib did not.

It is the ability of the viologens to accept and release a single electron in biochemical systems that is thought to be the first steps in production of mammalian and plant toxicity (Bus et al., 1974; Calderbank, 1968). White (1970) demonstrated a correlation between redox potential and Taft's polar substituent inductive effect constant (σ^*) for short-chain electron-withdrawing nitrogen substituents. Figure 2 is a correlation of σ^* (Fellous et al., 1977) with redox potentials determined in acetonitrile (van Dam and Ponjee, 1974) and water (Khaskin et al., 1972; Homer et al., 1960). The correlation of redox potentials determined in water with σ^* appears anomalous because redox potential changes very little from methyl to hexyl. This may be due to precipitation of alkyl-4,4'-bipyridylium salts with chain lengths \geq pentyl following one-electron reduction in aqueous solution (van Dam and Ponjee, 1974). The lack of correlation of redox potentials determined in water with σ^* may be the result of the equilibrium between $\text{bpy}\cdot\text{X}$ (solid) and $\text{bpy}\cdot\text{X}$ (solution) where X is the anion and bpy^{2+} is the divalent cation. Thus, in aqueous solution the reduced long-chain bipyridyl ($\text{bpy}\cdot\text{X}$) is not in direct equilibrium with bpy^{2+} while in acetonitrile (eq 5) the partially dissociated bipyridylium salt (bpyX^+ ; van Dam, 1976) is in equilibrium with $\text{bpy}\cdot\text{X}$ which always stays in solution.

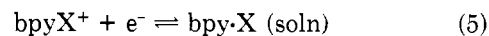
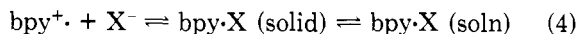
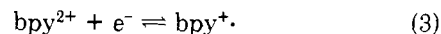


Table I. Properties of Some 4,4'-Bipyridylium Salts

compd no.	R ₁	R ₂	anion	yield, % ^a	recrystn solvent	color form	mp, °C ^b
Ia	methyl		I ⁻	80	1-PrOH-EtOH	orange needles	240-241
Ib	ethyl		I ⁻	47	1-PrOH-EtOH	orange leaflets	219-222
IIa	methyl	methyl	I ⁻	45	H ₂ O-EtOH	brt red plates	325
IIb	ethyl	ethyl	I ⁻	49	EtOH	orange needles	272-273 ^c
IIc	propyl	propyl	I ⁻	50	EtOH	brt red needles	259-260
IId	isopropyl	isopropyl	I ⁻	43	EtOH	brt yellow platelets	282-283
IIe	butyl	butyl	I ⁻	95	EtOH-5% H ₂ O	sm red plates	270-271
IIf	pentyl	pentyl	I ⁻	98	EtOH-1% H ₂ O	lg red plates	264-265
IIg	hexyl	hexyl	I ⁻	89	EtOH	lg gold-red plates	277-279
IIh	octyl	octyl	Br ⁻	94	1-PrOH-benzene	brt yellow plates	284-285 ^d
III	benzyl	benzyl	Cl ⁻			white needles	244-246
IIIa	methyl	propyl	I ⁻	62	5% H ₂ O-EtOH	sm red plates	250-251
IIIb	methyl	pentyl	I ⁻	66	5% H ₂ O-EtOH	sm red plates	251-252
IIIc	methyl	benzyl	Cl ⁻	20	EtOH-acetone	white plates	291-292

^a Following recrystallization. ^b All compounds decomposed at melting point. ^c Phillips and Mentha (1955) gave 273-274 °C. ^d Khaskin et al. (1972) gave 225 °C.

Table II. Nuclear Magnetic Resonance Chemical Shifts of Some 4,4'-Bipyridylium Salts

compd no.	proton chemical shifts, δ ^a (ppm)	
	ring	chain
Ia	9.03 (4 H, m), 8.58 (2 H, d), 8.10 (2 H, m)	4.65 (3 H, s)
Ib	9.21 (2 H, d), 9.00 (2 H, m), 8.62 (2 H, d), 8.13 (2 H, m)	4.95 (2 H, m), 1.92 (3 H, t)
IIa	9.35 (4 H, d), 8.80 (4 H, d)	4.70 (6 H, s)
IIb	9.40 (4 H, d), 8.81 (4 H, d)	5.02 (4 H, t), 1.99 (6 H, t)
IIc	9.36 (4 H, d), 8.80 (4 H, d)	5.05 (4 H, t), 2.48 (4 H, m), 1.34 (6 H, t)
IId	9.42 (4 H, d), 8.78 (4 H, d)	5.35 (2 H, m), 2.00 (12 H, d)
IIe	9.35 (4 H, d), 8.78 (4 H, d)	4.95 (4 H, t), 2.32 (4 H, m), 1.66 (4 H, m), 1.20 (6 H, m)
IIf ^b	9.67 (4 H, d), 9.05 (4 H, d)	5.12 (4 H, t), 2.47 (4 H, m), 1.92 (8 H, m), 1.30 (6 H, m)
IIg ^b	9.66 (4 H, d), 9.05 (4 H, d)	5.10 (4 H, t), 2.46 (4 H, m), 1.87 (12 H, m), 1.28 (6 H, m)
IIh ^b	9.67 (4 H, d), 9.06 (4 H, d)	5.12 (4 H, t), 2.48 (4 H, m), 1.72 (20 H, m), 1.25 (6 H, m)
III	9.37 (4 H, d), 8.74 (4 H, d)	7.75 (10 H, s), 6.12 (4 H, s)
IIIa	9.32 (4 H, m), 8.75 (4 H, m)	4.90 (2 H, m), 4.72 (3 H, s), 2.34 (2 H, m), 1.23 (3 H, t)
IIIb ^b	9.43 (4 H, m), 8.88 (4 H, d)	4.92 (2 H, m), 4.78 (3 H, s), 2.39 (2 H, m), 1.67 (4 H, m), 1.20 (3 H, m)
IIIc	9.37 (4 H, m), 8.78 (4 H, m)	7.80 (5 H, s), 6.17 (2 H, s), 4.73 (3 H, s)

^a Solvent was D₂O except where noted (footnote b) and on a 10-ppm sweep width where Me₄Si = 0 ppm; dioxane was used as an internal standard adjusted to 4.00 ppm. Final concentration of all compounds was (3-4) × 10⁻² M. ^b Solvent was CD₃OD.

The unusual range of colors of the crystalline compounds (Table I) first observed by Emmert and Stawitz (1923) is due to charge-transfer interactions of the anion with the bipyridylium molecule (Haque and Lilley, 1972; McFarlane and Williams, 1969). The least electronegative anion (I⁻) is more electron donating than Cl⁻ and results in a bathochromic shift in light absorption such that the iodides are yellow to red while chloride salts are white. This phenomenon was observed for IIh bromide (yellow crystals) that was exchanged with I⁻ to yield IIh iodide (red crystals). A similar observation was made for IIa iodide (red) and IIa chloride (white). In this context it is interesting to note that the melting point of these compounds does not change appreciably when the anion is changed: i.e., IIh (iodide) mp = 283-284 °C and IIh (bromide) mp = 284-285 °C; likewise IIa (iodide) mp = 325 °C and IIa (chloride) mp = 330 °C. Constants for the formation of charge-transfer complexes of IIa in aqueous solution are quite large ($k_{eq[Fe(CN)_6^{3-}]} = 52 \pm 5 M^{-1}$). Molar absorptivity of the complex is small ($\epsilon = 58-70 \text{ cm}^2/\text{mol}$; Nakahara and Wang, 1963) and results in the observation that the highly colored crystals form virtually colorless aqueous solutions at low concentration (Krumholtz, 1951). The color of the solutions is intensified in an organic solvent (e.g., MeOH), and this is probably due to only partial dissociation of the viologens in organic solvents (van Dam, 1976).

Previous NMR studies of IIa made assignments for ring proton chemical shifts based on the observation that one

of the two ring doublets (assigned 2, 6) was broader than the other ring doublet (assigned 3, 5) which "should be at low field due to the presence of an aromatic ring at position 4" (Haque et al., 1969). This assignment is exactly opposite of the known relative position of chemical shifts for 1-methylpyridinium iodide in which the 2, 6 protons are downfield of the 3, 5 protons (Reynolds and Priller, 1968). Analysis of 35 para-substituted pyridine compounds also confirms that the resonance of the 2, 6 protons is always further from Me₄Si than that of the 3, 5 protons including a bipyridyl with the rings conjugated by a vinyl group at the 4,4' position (Brugel, 1962). Further clouding the assignment of chemical shifts to ring protons is the purported equivalence of the 2 and 5 protons and the 3 and 6 protons of 4,4'-bipyridyl (Spotswood and Tanzer, 1967).

Assignment of chemical shifts to ring protons is facilitated in the current study because the effect of changing N substituents can be correlated with changes in ring proton resonance. Chemical shifts for the ring protons of the 1,1'-dialkyl-4,4'-bipyridylium salts (Table II) indicate that the resonance furthest downfield changes more than changing N-alkyl substituents than do the resonance of the other ring protons (IIa-IIe, Table II). Thus, the chemical shift furthest downfield changes from 9.35 to 9.42 ppm for IIa-IIe for protons 2 and 6 while the 3, 5 protons vary from 8.78 to 8.81 ppm for the same compounds. Change in resonance for the protons of the first alkyl carbon atom attached to the nitrogen varies even more

Table III. Properties of Some 4,4'-Bipyridylium Salts

compd no.	TLC, ^a <i>R_f</i>	UV _{max} , nm ($\epsilon \times 10^{-4}$) ^b	element analysis (C, H)		
			formula	calcd	found
Ia	0.25 ^c		C ₁₁ H ₁₁ N ₂ I	44.3, 3.7	40.9, 3.5 ^f
Ib	0.31 ^c		C ₁₂ H ₁₃ N ₂ I	46.2, 4.2	46.3, 4.1
IIa	0.13	254 (2.09) ^{d,e}	C ₁₂ H ₁₄ N ₂ Cl ₂	56.1, 5.5	55.9, 5.9 ^g
IIb	0.16	254 (1.33)	C ₁₄ H ₁₆ N ₂ I ₂	35.9, 3.9	35.5, 3.6
IIc	0.18	258 (1.72)	C ₁₆ H ₂₂ N ₂ I ₂	38.7, 4.5	38.8, 4.2
IId	0.16	257 (1.06)	C ₁₆ H ₂₂ N ₂ I ₂	38.7, 4.5	38.7, 4.2
IIE	0.27	258 (2.24)	C ₁₈ H ₂₆ N ₂ I ₂	41.2, 5.0	41.6, 5.2
IIf	0.46	258 (1.90)	C ₂₀ H ₃₀ N ₂ I ₂	43.5, 5.5	44.4, 5.9 ^h
IIg	0.78	259 (2.07)	C ₂₂ H ₃₄ N ₂ I ₂	45.5, 5.9	45.8, 6.1
IIh	0.32	258 (2.12)	C ₂₆ H ₄₂ N ₂ Br ₂	57.6, 7.8	57.3, 8.0
III	0.48	257 (2.10)	C ₂₄ H ₂₂ N ₂ Cl ₂		
IIIa	0.18	255 (2.28)	C ₁₄ H ₁₆ N ₂ I ₂	35.9, 3.9	36.0, 3.8
IIIb	0.24		C ₁₆ H ₂₂ N ₂ I ₂	38.7, 4.5	39.0, 4.8
IIIc	0.24		C ₁₈ H ₂₆ N ₂ Cl ₂	65.0, 5.5	64.7, 5.4 ^g

^a Solvent system, benzene-1-pentanol-methanol-1 N HCl (1.4:4:8:7). ^b Solvent H₂O with solute concentration of 2×10^{-5} M. ^c Streaked extensively in this system. ^d White (1970) gave 257 nm (2.36). ^e Downes (1967) gave 257 nm (2.02). ^f Did not meet theory, but compounds derived from it (IIIa-c) did. ^g Chloride salt obtained from AgCl exchange. ^h Compound exploded $\frac{3}{4}$ times; single smooth combustion did not meet theory.

than that for the α -ring protons (4.70–5.35 ppm for IIa–IIe) probably due to the alkyl inductive effect. Increasing electron induction by lengthening the alkyl chain could be expected to shield the ring protons α to the nitrogen and result in a decreased chemical shift for the α protons, but this is not seen. Additionally, the ability of the anion to donate charge to the ring can also effect the magnetic resonance of protons adjacent to the nitrogen (Haque et al., 1969; Reynolds and Priller, 1968) and this effect is dependent on the concentration of the compounds in solution.

For the above considerations, NMR for all compounds was conducted over a narrow concentration range. Unfortunately, the solubility of the viologens with alkyl chains \geq pentyl in D₂O is too low to get an adequate NMR spectrum so that MeOH-*d*₄ had to be used to dissolve these compounds. Conversely, the short-chain compounds (\leq butyl) are not very soluble in MeOH-*d*₄ so that one solvent could not be used for all NMR determinations. Since solvent character also affects the NMR spectrum of pyridinium compounds (Reynolds and Priller, 1968), a critical comparison of chemical shifts for the entire homologous series is prohibited.

The ultraviolet absorption maxima of the alkyl viologens shown in Table III (254–259 nm) are similar to those described for viologens with electron-withdrawing side chains (262–264 nm; White, 1970).

Increasing alkyl chain length on N-substituted pyridines results in increasing lipophilicity as measured by octanol–water partitions (Leo et al., 1971). The single largest change in partition coefficients for the 1-alkylpyridinium series occurs when the alkyl chain is lengthened from hexyl ($p = 0.0093$) to octyl ($p = 0.092$), yielding a ratio of octyl to hexyl of 9.9. Similar observations were made in the current work with 1,1'-dialkyl-4,4'-bipyridylium salts. The p values of IIh (0.19) and IIg (0.017) gave a ratio of octyl to hexyl of 11.2 which is comparable to the findings for the 1-alkylpyridinium compounds. The octanol–water partition coefficients of alkyl viologens with a chain \leq butyl were too small to measure.

Attempts to synthesize *tert*-butyl viologen, 2,2,2-trifluoroethyl viologen, and methoxymethyl viologen were unsuccessful. Yields of the addition product of 4,4'-bipyridyl and 2,2,2-trifluoroethyl iodide were $<5\%$ as estimated by TLC densitometry of the product. This yield could not be improved by increasing temperature to 200 °C in a sealed vial. Addition of water to the reaction

product of chloromethyl methyl ether and 4,4'-bipyridyl resulted in formation of a blue solution. The crystalline product isolated from anhydrous workup of the reaction mixture from another synthesis gave white needles which produced a violet color when dissolved in ethanol, but the color decreased on heating the solution. At 80 °C this solution was colorless, but on cooling it became violet again. This temperature-dependent color change was completely reversible and would occur repeatedly with the same solution. NMR analysis of a D₂O solution of the anhydrous product showed ring proton resonance at 9.32 (4 H, d), 8.70 (4 H, d), and 8.50 ppm (4 H, d) and alkyl proton resonance at 7.37 ppm (2 H, s) (relative to HOD, 4.52 ppm). The NMR spectrum and integrations are consistent with the structure methylene-1,4-bis(4-pyridyl)pyridinium ion. The reaction of *tert*-butyl chloride and 4,4'-bipyridyl exploded on repeated attempts when conducted in sealed ampules at 105 °C. The reaction mixture produced a violet solution in water. NMR analysis of the reaction mixture in Me₂SO-*d*₆ gave a typical ring resonance of disubstituted 4,4'-bipyridyl with a doublet at 4.00 ppm relative to Me₄Si. Over a 15-min period the doublet at 4.00 ppm coalesced to produce a singlet at 4.00 ppm. None of the products of the unsuccessful syntheses have been reported in the literature.

In related toxicological studies with selected viologens, pulmonary toxicity has been observed in rats (Ross and Krieger, 1979) and phytotoxicity has been observed in duckweed (Ross et al., 1979). Signs of acute toxicity in the rat included piloerection, dyspnea, lung hemorrhage, and adrenal enlargement. The benzyl homologue (IIIi) was at least 5 times as toxic to rats as paraquat. With respect to their herbicidal potency, however, none of the compounds were as toxic as paraquat. By use of the results of these studies as the basis for estimating selectivity, none of the viologens tested are any less toxic to mammals than plants compared to paraquat (Ross et al., 1979).

It is imperative that caution be exercised in handling alkyl viologens. All of the compounds tested were subsequently found to be toxic to rats (Ross and Krieger, 1979), and one compound (benzyl viologen) produces toxicity on a molar basis comparable to a lethal dose of sodium cyanide. During recrystallization of several viologens, a correlation between exposure and a bitter taste at the back of the tongue was noted. This suggests that these viologens are capable of dermal absorption which is not surprising considering the lethality of IIa following dermal exposure

(Christensen and Luginbyhl, 1975).

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Pesticide Residues in Imported Spices. A Survey for Chlorinated Hydrocarbons

James H. Sullivan

Pesticide analyses for residues of chlorinated hydrocarbons were performed on 28 spices from 25 producing countries. Several shipments were examined during several crop years. In all, 226 samples were analyzed. Low levels of DDT and BHC were detected consistently, the level being generally below 0.5 ppm. Residues of other chlorinated hydrocarbons such as Dieldrin, Endrin, and HCB were detected sporadically at a very low level. Except for oregano from Mexico, some of which is grown in an area where DDT is actively used, there is no relationship between pesticide residues and country of origin or individual spice. In view of the low level detected and the uniformity of detection, there does not appear to be any cause for concern.

The increased statutory and regulatory attention given to the use of pesticides and the subsequent reduction of residue tolerances has been of considerable interest to the membership of the American Spice Trade Association. As a group, it represents the major importers of spices which are grown throughout the world, especially in the developing nations where laws and practices are substantially different from ours.

Technical Services, McCormick & Company, Inc., Grocery Products Division, Baltimore, Maryland 21202.

Of particular interest are residues of the chlorinated hydrocarbons which continue to be used in some countries for a variety of reasons. In Mexico, for example, DDT is used for malaria control in the same region in which oregano is collected after harvest.

The significance of continued worldwide use of the chlorinated hydrocarbons was brought to our attention early in 1973 when several shipments of paprika imported from Spain were detained by the Food and Drug Administration (FDA) for having residues of benzene hexachloride (BHC) in excess of the tolerance of 1 ppm ap-