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Note

Sequential thin-layer chromatography of paraquat and related compounds

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Paraquat (1,1'-dimethyl-4,4'-bipyridinium) is a non-selective contact herbicide that is highly toxic to man[1] and animals[2]. Its toxicity is due largely to its specific cytotoxic effect on mammalian lungs[2]. Analysis of paraquat by thin-layer chromatography presents a special problem. Chromatographic systems such as those used for choline and other quaternary amines were inadequate[3–5]. Modification of these systems resulted in the formation of multiple spots[5–8]. Cellulose thin-layer and paper in combination with the solvent system, benzene-*n*-pentyl alcohol–methanol–1 *N* HCl (1:1:2:1) were used to separate paraquat from diquat and from impurities present in a technical grade product[9], however the time required for this method was long (*i.e.* 1 h for the cellulose and silica plates, and 5 h for the paper). Recently, a sequential thin-layer chromatographic (STLC) method was developed to analyze the organophosphorus insecticide leptophos [O-(4-bromo-2,5-dichlorophenyl) O-methyl phenylphosphonothioate] and its metabolites[10].

This communication reports a rapid thin-layer chromatographic method which offers fast and convenient analyses for paraquat and its possible degradation products.

EXPERIMENTAL

Chemicals

Paraquat and its related compounds used in this study were provided by Chevron Chemical Co. (Richmond, Calif., U.S.A.) The following analytical grade compounds were investigated: paraquat dichloride [1,1'-dimethyl-4,4'-bipyridinium dichloride]; QINA [4-carboxyl-1-methylpyridinium chloride]; monopyridone [1,2-di-hydro-1,1'-dimethyl-4,4'-bipyridinium-2-one chloride]; monoquat [1-methyl-(4'-pyridyl) pyridinium methylsulfate]; dipyridone [1,2,1',2'-tetrahydro-1,1'-dimethyl-4,4'-bipyridyl-2,2'-dione]. ^{14}C-methyl paraquat [bis(N-methyl-^{14}C)-4,4'-bipyridinium], specific activity 30 mCi/mmol, was obtained from Amersham–Searle, Arlington Heights, Ill., U.S.A.).

Thin-layer plates

Gelman type SA, ITLC, silicic acid-impregnated glass-fiber sheets (Gelman Instruments Company, Ann Arbor, Mich.. U.S.A.), were used.

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Solvents

The solvents used were: (A) benzene–*n*-amyl alcohol–methanol–1 *N* HCl (1:1:2:1) and (B) acetonitrile–water–ammonia (40:9:1).

Procedure

Aliquots (10 μl) of acetone solutions (1 μg/ml) of paraquat and the related compounds were spotted on the glass-fiber sheet. Chromatograms were developed in a lined, pre-equilibrated tank, up to 3 cm with solvent A for 7 min, then for 30 min after developing to 16 cm with solvent B for 26 min. The respective regions were identified by visualization in an iodine chamber.

The radiochemical purity of ^{14}C-methyl paraquat was evaluated using the same sequential ITLC system. For the characterization of impurities, a mixture of paraquat and structurally-related compounds was added to the ^{14}C-methyl paraquat solution and the chromatographic pattern was subsequently determined. The standards were detected by their color in iodine vapor. The sheets were cut into 5 mm strips and placed in the scintillation medium. The latter was composed of 1 volume of Triton X-100 and 2 volumes of toluene containing 5 g of 2,5-diphenyloxazole (PPO) and 200 mg of 1,4-bis[2(5-phenyloxazolyl)-benzene] (POPOP) per liter. Radioactivity was determined using a Beckman model LS-100 liquid scintillation spectrometer.

RESULTS AND DISCUSSION

The herbicide paraquat causes severe pulmonary changes in man and animals, which may begin after most of the chemical is metabolized and excreted. Efforts to evaluate the effects of residues and any potential metabolites of paraquat require, in part, the development of a simple, accurate method, suitable for the separation and detection of paraquat and its degradation products in biological and non-biological systems.

The R_F values (average of 3 developments) of paraquat and related compounds in 2 single solvents and in a 2-solvent sequential system are listed in Table I. In a preliminary study no single solvent system was found capable of separating all these compounds. In solvent A all the compounds moved from the origin, but QINA and

TABLE I

R_F VALUES OF PARAQUAT AND RELATED COMPOUNDS ON ITLC SHEETS USING SINGLE AND SEQUENTIAL SOLVENT SYSTEMS*

Solvent A: benzene–*n*-amyl alcohol–methanol–1 *N* HCl (1:1:2:1). Solvent B: acetonitrile–water–ammonia (40:9:1). Sequential solvent system: solvent A for 3 cm, followed by solvent B for 30 min after developing to 16 cm.

*Compounds**	*Solvent A*	*Solvent B*	*Solvent A followed by Solvent B*
Paraquat	0.21	0	0.19
QINA	0.58	0.28	0.34
Monopyridone	0.53	0	0.49
Monoquat	0.42	0	0.54
Dipyridone	0.58	0.78	0.79

* Chemical names are listed under Experimental,

dipyridone had the same R_F value (0.58), with monopyridone moving too close to allow its separation from them (R_F: 0.53). Paraquat and monoquat, with R_F values of 0.21 and 0.42 respectively, were well separated, both from each other and from the other compounds. On the other hand, when solvent B was used, QINA and dipyridone moved and separated from the other three compounds, which did not move from the origin. The R_F values for QINA and dupyridone were 0.23 and 0.78 respectively.

By employing a 2-solvent sequential TLC system which developed the ITLC sheets first with solvent A (for 3 cm) followed by solvent B (for 30 min after it reached 16 cm), a good resolution of all compounds tested was obtained (Table I). The R_F values were as follows: paraquat 0.19, QINA 0.34, monopyridone 0.49, monoquat 0.54 and dipyridone 0.79.

The radiochemical purity of ^{14}C-methyl paraquat was evaluated using this system (STLC). This compound was found to be 97.20% pure (Fig. 1). The impurities were identified by STLC to be QINA (0.75%), monopyridone (0.80%) and a polar compound that did not move from the origin and accounted for 1.27% of the total radioactivity. This component might have been formic acid formed by N-oxidative demethylation of the N-methyl pyridine ring.

The method of analysis described in this communication is very useful for the separation and identification of paraquat-type compounds. It is convenient in

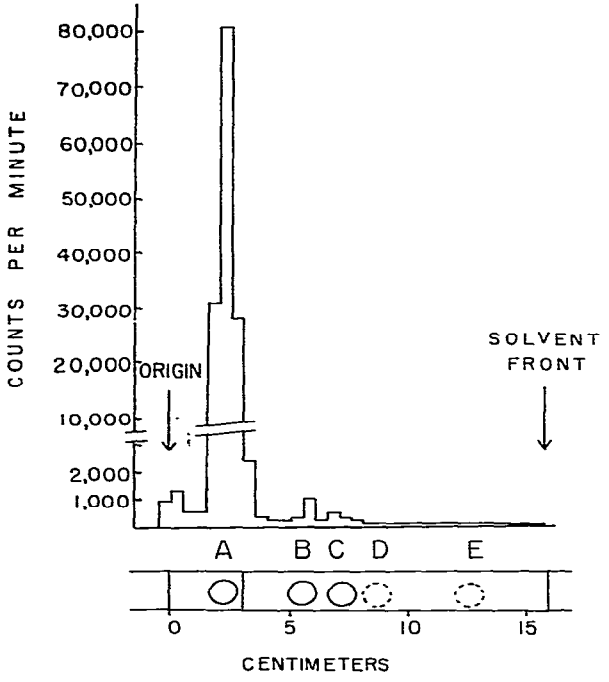

Fig. 1. Chromatogram and scan for ^{14}C-methyl paraquat, using Gelman type SA, ITLC silicic acid-impregnated glass-fiber sheets, following sequential elution with solvent A benzene–*n*-amyl alcohol–methanol–1 *N* HCl (1:1:2:1) for 3 cm, and solvent B acetonitrile–water–ammonia (40:9:1) for 30 min after developing to 16 cm. The letters A to E refer to the following compounds: A, paraquat: B, QINA; C, monopyridone; D, monoquat and E, dipyridone.

handling the chromatograms and is highly reproducible. It combines good resolution with rapid solvent development and offers an added convenience, enabling appropriate regions to be separated for quantitative determinations in a liquid-scintillation spectrometer. The ITLC strips could also be extracted with suitable solvents for the unequivocal indentification of each compound utilizing other techniques such as infrared spectroscopy and mass spectrometry.

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