

Application of the iodine–azide reagent for selective detection of thiophosphoryl compounds in thin-layer chromatography[☆]

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ABSTRACT

The application of the iodine–azide-induced reaction for the detection of thiophosphoryl compounds in thin-layer chromatographic systems is described. The detection limits of the derivatives tested were found to be at the nanomole level and depend on the nature of the P(S)_n function, particularly on the degree of polarity of the P–S bond and the number of sulphur atoms in the molecule. A comparison of the iodine–azide test reaction with other procedures used for thiophosphoryl compounds is presented. A procedure for the differentiation of thiophosphoryl compounds and sulphur compounds based on the combined molybdate procedure preceded by a preoxidation stage is demonstrated.

INTRODUCTION

Organophosphorus compounds, especially those containing P–S bonds, are of significant industrial interest [1,2]. Many of these compounds exhibit strong biological activity (*e.g.*, organophosphorus plant protection agents) and for this reason their occurrence is of significant environmental concern [3,4]. Analytical methods for thiophosphoryl compounds are numerous, most being based on chromatography-related techniques [4,5]. In this field,

thin-layer chromatography (TLC) combined with chemoselective detection has been considered as the method of choice, especially for non-volatile and thermally unstable organophosphorus derivatives [4–7]. Thus, phosphorothioates and phosphorodithioates were detected by TLC using silver nitrate solution alone [8] or in conjunction with chelating indicators (*e.g.*, bromocresol green) [8–11] and using copper(II) chloride solution and potassium hexacyanoferrate(III) solution [12] as subsequent spray reagents. Also, potassium iodoplatinate [4], palladium(II) chloride [4,13,14] and palladium(II) complexes with fluorescent indicators [*e.g.*, palladium(II)–calcein] [15] have been widely used for the detection of thiophosphoryl insecticides. Phosphi-

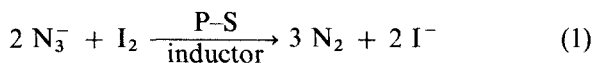
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nothioate complexes with metals were localized on TLC plates by means of a dithizone reagent [16,17].

The detection of thiophosphoryl compounds has also been achieved using 2,6-dibromobenzoquinone-4-chlorimine (DCQ) [18,19], an ammoniacal solution of 4-methyl umbelliferone preceded by bromine vapour treatment [19], fluorescein [20], ammonium molybdate reagent [12,21,22] and by potassium iodate solution [23] as spray reagents.

These compounds are visible in the UV region (254 nm) using fluorescent chromatoplates [24]. Phosphorothioates can be also monitored in eluates by means of conductivity or spectrophotometric detectors [25]. Several reports have described the use of the TLC-enzyme inhibition (TLC-EI) technique for the detection of a variety of organophosphorus compounds, including the P-S type [4,26-28].

However, most of the procedures cited above seem to employ general rather than specific detection reagents for thiophosphoryl compounds [4-7,12,21,22]. Considering this, the application of the iodine-azide reaction, selectively induced by the presence of sulphur-containing compounds, offers the opportunity for the differentiation of thiophosphoryl compounds from other organophosphorus derivatives. This reaction:



originally carried out by Raschig [29], has been explored extensively for decades in analytical chemistry [30-36], but there are only two reports on the chromatographic detection of phosphorothiono ester-based pesticides [32,36]. Recently we described the induction activity of P-S-containing compounds in the iodine-azide-induced reaction (eqn. 1) and its analytical applications [37]. As a continuation of this research, we present here results on the TLC detection of thiophosphoryl compounds using the iodine-azide spray reagent.

EXPERIMENTAL

Materials

All organophosphorus compounds were prepared according to ref. 38 and were all of the same purity as reported previously. Other reagents were prepared from chemicals purchases from Aldrich (Milwaukee, WI, USA).

Solutions and reagents

The concentrations of the compounds chromatographed were *ca.* $5 \cdot 10^{-2}$ – $1 \cdot 10^{-3}$ M in anhydrous dioxane [or in methanol (**5b**, **6b**) or benzene (**12**)].

A 1 M aqueous solution of sodium azide and a 1 M solution of iodine (in a 1 M aqueous solution of potassium iodide) were employed.

Dragendorff reagent (Bi) contained 0.11 g of bismuth(III) nitrate pentahydrate and 0.88 g of potassium iodide in 100 ml of 16% aqueous acetic acid. DDQ reagent was a 2% solution of 2,6-dibromo-*p*-benzoquinone-4-chlorimine in benzene. Fluorescein reagent (Flc) was a 0.25% solution of sodium fluorescein in dimethylformamide. Ninhydrin (Ninh) was a 0.1% solution of ninhydrin in ethanol. Copper(II) hexacyanoferrate(III) reagent (CFCN) was applied as a 2% aqueous solution of copper(II) chloride, followed by a 0.5% aqueous solution of potassium hexacyanoferrate(III). Palladium reagent (Pd) was a 0.5% solution of palladium(II) chloride in *ca.* 0.2 M hydrochloric acid. Silver reagent (Ag) was a 1% aqueous solution of silver nitrate. Iodate reagent (KIO₃) was applied as a mixture of a 5% aqueous solution of potassium iodate (25 ml) and a 1% aqueous starch solution (2 ml); the plates were pretreated with 20% hydrochloric acid.

Molybdate reagent (Mo) was prepared by dissolving 1 g of ammonium molybdate in 40 ml of water, followed by 3 ml of concentrated hydrochloric acid and 5 ml of 70% perchloric acid, and diluted while cooling with 100 ml of acetone. Tin(II) chloride reagent (Sn) was prepared by heating 1 g of tin(II) chloride dihydrate in 10 ml of concentrated hydrochloric acid until dissolved, followed by dilution with 40 ml of water and 50 ml of acetone. Perchloric acid was a 20% solution in ethanol, nitric acid (fuming) was a 90% solution, aqueous bromine solution was a saturated solution of bromine in water and hydrogen peroxide was a 10% aqueous solution.

Thin-layer chromatography

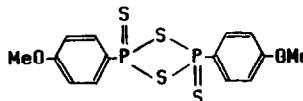
Precoated silica gel 60 F₂₅₄ aluminium sheets (10 cm × 5 cm) with a 0.2 mm thick layer (Merck, Darmstadt, Germany) were used for all TLC experiments. The plates were spotted with an appropriate amount of compound (deposition area *ca.* 0.2 cm²), developed for a distance of 8 cm with acetone, air

dried and sprayed with a freshly prepared 1:1 mixture of sodium azide and iodine solutions. Thiophosphoryl compounds appeared due to the

catalytic effect of the P-S bond as white spots on a yellow background, and were stable for more than 20 min.

TABLE I

DETECTION LIMITS OF THIOPHOSPHORYL COMPOUNDS WITH UV DETECTION (254 nm) AND USING IODINE (VAPOUR) AND THE IODINE-AZIDE SPRAY REAGENT

No.	Compound ^{a,b}	I ₂ ^c (μg)	UV (μg)	I ₂ -N ₃ ^d		R _F
				μg	nmol	
1a	(EtO) ₂ PSH	1	— ^e	5	32	0.72
1b	(EtO)(MenO)PSH	1	— ^e	5	19	0.75
2	(EtO) ₂ (EtS)P	1	— ^e	10	160	0.52
3	(EtS) ₃ P	3	30	30	140	0.63
4	Ph ₂ PSH	1	2	10	46	0.69
5a	(EtO) ₂ P(S)OH	1	— ^e	2	12	0.07
5b	(EtO) ₂ P(S)ONa	1	— ^e	2	10	0.08
6a	(EtO) ₂ P(S)SH	1	50	0.5	2.6	0.36
6b	(EtO) ₂ P(S)SNa	1	50	0.2	2.4	0.51
7	(EtO) ₂ P(S)SMe	10	50	— ^e		0.49
8	(EtO) ₃ PS	10	10	— ^e		0.72
9	(EtS) ₃ P	1	3	50	200	0.62
10	[(EtO) ₂ P(S)S] ₂	1	1	0.1	0.3	0.73
11	(EtO) ₂ P(S)OP(O)(OEt) ₂	0.3	— ^e	10	33	0.67
12		0.2	0.1	0.2	0.5	0.10; 0.50
13	Ph ₂ P(S)SH	0.2	1	0.1	0.4	0.74
14	[Ph ₂ P(S)S] ₂	0.2	0.2	0.1	0.2	0.71
15	Ph ₂ P(S)SMe	1	1	10	38	0.70
16	<i>t</i> -Bu(Ph)P(S)OH	0.2	10	10 ^e	47	0.22
17a	Me ₃ PS	0.3	— ^e	0.03	0.3	0.65
17b	Bu ₃ PS	0.3	— ^e	0.3	1.3	0.69
17c	Oct ₃ PS	0.3	— ^e	1	2.5	0.81
17d	Me ₂ PhPS	0.3	1	0.03	0.2	0.65
17e	MePh ₂ PS	0.3	1	0.3	1.3	0.65
17f	Ph ₃ PS	0.1	0.3	50 ^e		0.69
18	Ph ₂ (EtO)PS	0.3	0.3	50 ^e		0.71
19	(Me ₂ N) ₃ PS	1	1	10 ^e	51	0.68
20b	Ph(MeNH) ₂ PS	0.3	0.3	0.5	2.5	0.68
20c	Ph(PhNH) ₂ PS	0.1	3.0	10	31	0.69
20d	Ph(Me ₂ N) ₂ PS	0.3	0.3	— ^e		0.70
21a	Ph ₂ (NH ₂)PS	0.1	0.3	0.1	0.4	0.70
21b	Ph ₂ (MeNH)PS	0.3	0.3	0.5	2.2	0.69
21c	Ph ₂ (PhNH)PS			0.5	1.6	0.69
21d	Ph ₂ (Me ₂ N)PS	0.3	0.3	— ^e		0.70

^a Bu = *n*-Butyl; *t*-Bu = *tert.*-butyl; Et = ethyl; Me = methyl; Men = menthyl; Oct = *n*-octyl; Ph = phenyl; Naphth = 2-naphthyl.

^b Diethylphosphoric acid, diphenylphosphinic acid and triphenylphosphine oxide were undetectable using iodine-azide reagent.

^c Brown spots.

^d White spots.

^e Undetectable at 50 μg per spot.

Detection of thiophosphoryl compounds by the pre-oxidation-molybdate procedure

The chromatographic plates were treated with the oxidation agent (described in Table II), allowed to react for 10 min, sprayed with perchloric acid and finally heated in an oven to 180°C for 30 min. The plates were removed from the oven, cooled to room temperature, sprayed with the molybdate reagent and replaced in the oven for 5 min. After cooling, the plates were sprayed with tin(II) chloride solution.

Phosphates and phosphonates (resulting from the corresponding P-S compounds) appeared as blue spots on a buff background (the background can be bleached by placing the plate in a tank containing an atmosphere of ammonia vapour).

RESULTS AND DISCUSSION

The results of the application of the iodine-azide reagent for the detection of various types of thio-

TABLE II

DETECTION OF SULPHUR COMPOUNDS AND THIOPHOSPHORYL COMPOUNDS BY MEANS OF THE IODINE-AZIDE, THE MOLYBDATE AND THE PREOXIDATION-MOLYBDATE PROCEDURES

- = Not detected; +- = spot is detectable; + = distinct detection; ++ = strong detection.

Compound ^a	Amount (μg per spot)	Detection procedure (reagent)				
		I ₂ -N ₃	Mo ^b	(1) Br ₂ (aq.), (2) Mo	(1) HNO ₃ , (2) Mo	(1) H ₂ O ₂ , (2) Mo
HOCH ₂ CH ₂ SH	1	+	+	-	-	-
	10	++	++	-	-	-
HSC ₆ H ₄ CO ₂ H- <i>m</i>	1	+	-	-	-	-
	10	++	++	+ ^c	+ ^{-c}	+ ^{-c}
Naphth-SH	1	+	+	+ ^c	+ ^c	-
	10	++	++	+ ^c	+ ^c	+ ^c
S(CH ₂ CH ₂ OH) ₂	1	-	-	-	-	-
	10	-	-	-	-	-
PhSSPh	1	+	-	-	-	-
	10	+	-	+ ^c	+ ^c	+ ^c
PhNHC(=S)NH ₂	1	+	+	+ ^c	+ ^c	-
	10	++	++	+ ^c	+ ^c	+ ^c
DMSO	1	-	-	-	-	-
	10	-	-	-	-	-
(EtO) ₂ PSH	1	-	+	-	-	-
	10	+	++	+ ^b	+ ^b	+ ^b
(EtO) ₂ P(S)ONa	1	+-	+	+ ^{-b}	+ ^{-b}	+ ^{-b}
	10	+	++	+ ^b	+ ^b	+ ^b
(EtO) ₂ P(S)SNa	1	+	+	+ ^b	+ ^{-b}	+ ^{-b}
	10	++	++	+ ^b	+ ^b	+ ^b
[(EtO) ₂ P(S)S] ₂	1	+	+	+ ^b	+ ^b	+ ^b
	10	++	++	+ ^{+b}	+ ^{+b}	+ ^{+b}
<i>t</i> -Bu(Ph)P(S)OH	1	+	-	-	-	-
	10	+	+	+ ^b	+ ^b	+ ^b
PhP(S)(NHMe) ₂	1	+	-	-	-	-
	10	+	+	+ ^b	+ ^b	+ ^b
PhP(S)(NMe) ₂	1	-	-	-	-	-
	10	-	+	+ ^b	+ ^b	+ ^b
[Ph ₂ P(S)S] ₂	1	+	+	-	-	-
	10	++	+	+ ^b	+ ^c	+ ^c

^a Abbreviations as in Table I.

^b Blue spots.

^c Brown spots with or without molybdate spray.

phosphoryl compounds in comparison with their UV detection (254 nm) and using iodine are summarized in Table I.

It is evident that the detection limits of organo-phosphorus compounds, resulting from their own induction activity, are strongly dependent on their

TABLE III

COMPARISON OF REAGENTS FOR THE DETECTION OF THIOPHOSPHORYL COMPOUNDS BY TLC

- = Not detected; +- = spot is detectable; + = distinct detection; ++ = strong detection.

Compound	Amount (μg per spot)	Detection reagent								
		$\text{I}_2\text{-N}_3^a$	Pd^c	Ag^d	CFCN^g	Bi^h	KIO_3^i	DDQ^m	Ninh^n	Flc^q
$(\text{EtO})_2\text{PSH}$	3	+-	+	+	+-	-	-	+ ^{-k}	-	-
	30	+	+	++	+	+	+ ^j	+ ^k	+ ^o	+
Ph_2PSH	3	-	+	+	+	-	-	-	-	-
	30	+	+	+	+	+	+	+	+	+
$(\text{EtO})_2(\text{EtS})\text{P}$	3	-	+	+	-	-	-	-	-	-
	30	-	+	+	-	-	+	+ ^k	-	-
$(\text{EtS})_3\text{P}$	3	-	+	+	-	-	+	-	+ ^p	-
	30	-	+	+	-	+	+	-	+ ^p	+
$(\text{EtO})_3\text{PS}$	3	-	+	+ ^f	-	-	+ ^j	-	-	-
	30	-	+	+ ^f	-	-	+ ^j	-	-	-
$(\text{EtS})_3\text{PS}$	3	-	+	+	+	-	+	-	-	-
	30	+ ^b	++	+	+	+ ⁻	++	+ ⁻ⁱ	-	-
$(\text{EtO})_2\text{P(S)SH}$	3	+	+	-	+	-	-	+	+ ^{-o}	-
	30	+	+	+ ^f	+	-	+ ^j	+	+ ^o	-
$(\text{EtO})_2\text{P(S)SMe}$	3	-	+	+ ⁻	-	-	-	-	-	-
	30	-	+	+	+	-	+ ^j	-	-	-
$(\text{EtO})_2\text{P(S)OH}$	3	+	+	-	-	-	-	-	+ ^{-o}	-
	30	+	+	-	-	+	+ ^j	+	+ ^o	+
Ph_3PS	3	-	+	+ ^f	-	+ ⁻	+	+ ^l	-	-
	30	+ ^b	+	+ ^f	+ ^e	+	++	+ ^l	-	+ ⁻
Bu_3PS	3	+	+	+	+	+	+	+ ⁻	-	-
	30	+	++	+	++	+	+	+	-	+
$(\text{Me}_2\text{N})_3\text{PS}$	3	-	+	-	+	+	+	+ ⁻	-	-
	30	+ ^b	+	+ ^f	+	++	+	+	-	+
$\text{Ph}_2\text{P(S)SH}$	3	+	+	+	-	+	+	+	-	-
	30	++	+	+	-	+	+	+	-	+
<i>t</i> -Bu(Ph)P(S)OH	3	-	+	+	-	+	-	-	-	-
	30	+	+	+	-	+	+	-	-	+

^a White spots.

^b Brown spots.

^c Yellow-brown spots.

^d Dark yellow-brown spots.

^e After 0.5 h of exposure.

^f After 1 h of exposure.

^g Brown spots on grey-green background.

^h Orange spots.

ⁱ Brown spots.

^j Grey spots after saturation with ammonia vapour.

^k Navy-blue spots after saturation with ammonia vapour.

^l Lilac spots.

^m Green spots.

ⁿ Spots appeared after preheating to ca. 80°C.

^o Yellow spots.

^p Pink spots.

^q Lilac spots on yellow-green background.

structure and element contributions. Thus, organophosphorus compounds which do not contain a sulphur atom in the molecule exhibit no induction activity and are not detectable with the iodine–azide reagent (Table I). The activity of thiophosphoryl derivatives was found to be dependent on the nature of the $P(S)_n$ function. The results (Table I) reveal that the induction activity (resulting from this detection) of thiophosphoryl compounds is attributable more to the thiolate ($P-S^-$) function than to thio ester ($P-S-R$) or thiono ($P=S$) functions. Thus, the lowest detection limits are exhibited by phosphorodithioates **6a** and **6b**, the disulphide **9** (0.3–5 nmol) and alkyl and aralkyl phosphines sulphides (**17a–e**) (0.2–2.5 nmol). Triphenylphosphine sulphide (**17f**) and diphenylethoxyphosphine sulphide (**18**) exhibit high detection limits (>100 nmol), presumably owing to the influence of their electronegative substituents on polarization of the $P=S$ function. The detection of thioamides of organophosphorus acids was found to be dependent on the type of the amide moiety. Thus, primary (**21a**) and secondary thioamides (**20b**, **20c**, **21b**, **21c**) present show low to moderate detection limits (0.1–2.5 nmol) whereas tertiary thioamides (**20d** and **21d**) are inactive. Monothioacids **5a** and **16**, phosphorothioate **5b** and tetraethyl monothiopyrophosphate (**11**) exhibit moderate detection limits (10–30 nmol), whereas triphosphosphate **8**, dithiophosphate **7** and perthiophosphate **9** exhibit very poor detection limits (>100 nmol). A similar trend is observed for thiophosphoryl derivatives of trivalent phosphorus. Thus, dialkyl thiophosphites **1a** and **1b** [$P(S)H \rightleftharpoons P-SH$] exhibit low detection limits (5 nmol) whereas triethyl thiophosphite (**2**) and triethyl trithiophosphites (**3**) exhibit high detection limits (>100 nmol). Hence the iodine–azide reagent allows the micro-detection of aliphatic and mixed alkyl–aryl phosphine sulphides. The reagent also gives low detection limits of organophosphorus thioacids, their salts, partial esters and thioamides (primary and secondary) and disulphides, namely compounds with $P-S^-$ or $P-S-S-P$ functions. The other compounds tested, bearing $P=S$ or $P-S-R$ functions, exhibit a poor to moderate detection limits and the application of the iodine–azide reagent requires their prior hydrolytic activation to the $P-S^-$ function. The reagent shows no activity towards phosphoric acid and its partial or full esters, alkanephosphonic acids,

dialkylphosphinic acids and phosphine oxides.

It should be mentioned that the iodine–azide test reaction with thiophosphoryl derivatives is subject to interference from the presence of some sulphur compounds that do not contain phosphorus in the molecule. A similar interference problem also exists in the molybdate test, the most common test for phosphorus compounds [39,40]. Thus the application of the iodine–azide reagent or the molybdate reagent gives indiscriminately positive results for several thiophosphoryl and sulphur derivatives (Table II).

To overcome this lack of selectivity, we additionally developed a modified molybdate procedure based on the phosphoro–molybdate reaction but preceded by the oxidative elimination of potential sulphur reductants of molybdate (Table II). As a consequence, the combination of the iodine–azide test and the modified molybdate test permits the differentiation of thiophosphoryl and phosphoryl compounds and also phosphorus compounds from sulphur compounds.

The detection of various classes of thiophosphoryl compounds by means of the iodine–azide reagent and other representative spray reagents is presented in Table III.

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