

Note

Trifluoroacetic anhydride–sodium iodide as a reagent for the selective detection of nitroso compounds by thin-layer chromatography

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Nitroso compounds show great activity and considerable diversity of action^{1,2}, especially as carcinogens^{3,4}. For this reason their occurrence⁵, whether as synthetic derivatives^{2,6}, natural products² or accidental products in food processing⁷ or tobacco smoke², is of significant environmental concern. Consequently, there are numerous methods for the detection and determination of nitroso compounds^{2,8–10}. However, their selective determination in thin-layer chromatographic systems (TLC) is limited to a narrow range of detection reagents. Preussmann and co-workers^{11,12} described the silica gel TLC of wide variety of N-nitrosamines; detection was achieved using a 5:1 mixture of 1.5% of diphenylamine in ethanol and a 0.1% solution of palladium(II) chloride in 0.2% sodium chloride, and a solution of 0.5% sulphanic acid and 0.05% α -naphthylamine in 30% acetic acid (Griess reagent¹³), followed by UV irradiation. These reagents and also ninhydrin reagent (0.2–0.3%, ethanolic) were applied by Kroeller¹⁴ and Sen *et al.*¹⁵ for the detection and identification of nitrosamines in foodstuffs and tobacco smoke.

Yasuda and Nakashima¹⁶ accomplished the TLC detection of several aromatic nitroso compounds by heating the plates sprayed with acidic 2-naphthol solution [1% 2-naphthol in methanol–ethylene glycol–35% hydrochloric acid (10:8:2)]. Results for the TLC detection of nitrosamines with several common spray reagents were given in a comprehensive paper by Reio¹⁷.

In some procedures nitrosamines were derivatized to compounds exhibiting higher detectability (strong absorbance or chemiluminescence), prior to their chromatographic separation and subsequent determination. Thus, nitrosamines were pre-reduced to the corresponding hydrazines, followed by acylation with 4-nitroazobenzene-4'-carboxylic chloride¹⁸, or by coupling with anthranil^{19,20} or 5-nitrosalicylaldehyde²¹. Nitrosamines were also pre-reduced to amines and subsequently

dansylated^{22,23} or treated with N-(8-methoxy-5-quinolinesulphonyl)aziridine²⁴. Direct derivatization of nitrosamines was accomplished by treatment with 1-chloro-4-nitrobenzofuran²⁵ or 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole²⁶.

In previous papers we have reported procedures for the determination²⁷ and detection of sulphoxides and sulphimides²⁸ and nitroxide radicals and nitrones²⁹ using tfluoroacetic anhydride-sodium iodide (TFAA-I) reagent. We have found that this reagent also reacts with various nitroso compounds with simultaneous release of iodine. In this paper we present results for the application of the TFAA-I reagent to the detection of nitroso compounds in TLC.

EXPERIMENTAL

Materials

Acylating agents (TFAA, acetyl chloride, acetic anhydride), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, tetrachlorobenzoquinone (chloranil), cupferron, 1-nitroso-2-naphthol, N,N-dimethyl-4-nitrosoaniline, sulphanic acid and 2-naphthol were purchased from Aldrich (Milwaukee, WI, U.S.A.). Other nitroso compounds were prepared according to Ref. 30 and were all of the purity previously reported.

Reagents and solutions

The concentrations of nitroso compounds 1-5 (see Table II) were *ca.* $5 \cdot 10^{-2}$ - 10^{-3} M in anhydrous acetone. A 0.8 M solution of sodium iodide in anhydrous acetone and 0.8 M solution of TFAA in anhydrous acetone (prepared immediately before use) were used. Other acylating agents solutions were all 0.8 M in anhydrous acetone. Dragendorff reagent (Bi) was a 2% solution of potassium bismuth tetraiodide in 0.01 M hydrochloric acid. DDQ reagent was a 2% 2,3-dichloro-5,6-dicyano-1,4-benzoquinone solution in benzene. TCBQ reagent was a 2% tetrachlorobenzoquinone solution in benzene. Griess reagent (Gr R) was a solution of 0.5% sulphanic acid and 0.05% α -naphthylamine in 30% acetic acid. Other solutions were 2% iron(III) chloride (Fe) and 0.1% potassium permanganate (Mn).

Thin-layer chromatography

Precoated silica gel 60 F₂₅₄ aluminium sheets and precoated aluminium oxide 60 F₂₅₄ aluminium sheets (10 cm \times 5 cm), with a 0.2-mm thick layer (Merck, Darmstadt, F.R.G.), were used in all TLC experiments. The plates were spotted with an appropriate amount of compound (see tables), developed for a distance of 8 cm with acetone, air-dried and sprayed with sodium iodide solution and subsequently with TFAA solution. Nitroso compounds appeared almost immediately as brown spots on a white background, and were stable for more than 20 min.

RESULTS AND DISCUSSION

The results of the application of the various acylating agent-halide salt systems for the detection of nitroso compounds by TLC are presented in Table I. The most sensitive are TFAA-I and acetyl chloride-sodium iodide reagents. Subsequent modification of the reagent system by replacement of the halide salt or acetylating agent leads to differentiation of their detection selectivity. Thus, the use of the mild

TABLE I

COMPARISON OF VARIOUS ACYLATING AGENT-HALIDE SALT SYSTEMS FOR THE DETECTION OF NITROSO COMPOUNDS 1-3 AND 5 BY TLC

Silica gel plates. Solvent, acetone. -, Not detected; +/-, spot is detectable; +, distinct detection; ++, strong detection. Ac₂O = acetic anhydride; AcCl = acetyl chloride.

Compound applied			Ac ₂ O-NaI	AcCl-NaI	Ac ₂ O-LiBr	AcCl-LiBr	TFAA-I
No.	Structure ^a	Amount (μg/spot)					
1b	<chem>(i-Bu)2N-NO</chem>	1	-	-	-	-	-
		10	-	-	-	-	-
		50	-	+	-	-	+
1d	<chem>C1CCN(C1)NO</chem>	1	-	-	-	-	-
		10	-	+	-	-	+
		50	-	+	-	-	+
1f	<chem>CN(C)Nc1ccccc1NO</chem>	1	-	-	-	-	+
		10	-	+	-	+	++
		50	-	+	-	+	++
1g	<chem>Nc1ccccc1Nc2ccccc2NO</chem>	1	-	+/-	-	-	-
		10	+ ^b	+ ^c	+ ^c	+ ^c	+ ^b
		50	+ ^b	++ ^c	++ ^c	++ ^c	++ ^b
2	<chem>CN(C)Nc1ccccc1N(O)N</chem>	1	+	+	-	-	+/-
		10	++	+	-	-	+
		50	++	++	+	+/-	++
3	<chem>CN(C)N(S(=O)(=O)c1ccc(C)cc1)NO</chem>	1	+ ^d	+ ^d	-	-	+ ^d
		10	+ ^d	+ ^d	-	-	++ ^d
		50	+ ^d	+ ^d	-	-	++ ^d
5a	<chem>O=[N+]([O-])c1ccccc1</chem>	1	-	-	-	-	-
		10	-	-	-	-	-
		50	-	+/-	-	-	-
5b	<chem>Cc1ccc([N+](=O)[O-])cc1</chem>	1	-	+	-	-	+
		10	+/-	+	-	-	++
		50	+	++	-	+/-	++

^a i-Bu = Isobutyl; Me = methyl; Ph = phenyl.

^b Blue-brown spots after preheating to ca. 150°C, turning yellow after spraying with the reagent.

^c Blue-brown spots after preheating to ca. 150°C, turning blue-navy after spraying with LiBr, turning green after additional spraying with acetyl chloride.

^d Yellow spots after spraying with sodium iodide solution.

acetic anhydride-sodium iodide reagent allows the detection of N-nitroso-N,N-diphenylamine, N-nitroso-N-hydroxyphenylamine and N-nitrosamide 3, whereas the acetic anhydride-lithium bromide reagent detects only N-nitroso-N,N-diphenylamine. The more reactive systems based on TFAA and acetyl chloride detect a much wider range of nitroso compounds.

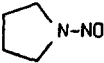
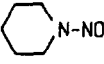
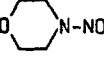
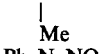

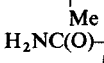
Results for the application of TFAA-I for the detection of nitroso compounds are summarized in Table II. It is evident that the detection limits of nitroso derivatives are strongly dependent on their structure. Thus, the detection limits of N-nitrosamines vary from 1 μg (per spot) for diaryl- and arylalkyl-N-nitrosamines (**1f** and **1g**) through 5–10 μg cyclic aliphatic N-nitrosamines (**1c–e**) to ca. 50 μg for acyclic aliphatic N-nitrosamines (**1a** and **1b**). N-Nitrosophenylhydroxylamine (**2**) (cupferron) and N-nitrosoamides **3** and **4** exhibit detection at ca. 1–5 μg .

Detection of C-nitroso compounds was even more influenced by structural factors. Thus, *p*-nitrosodimethylaniline (**5c**) and 1-nitroso-2-naphthol (**5d**) exhibit strong molecular absorbance themselves and they are detectable as yellow spots at levels lower than 0.5 μg in both the UV and visible light regions. Therefore, their reaction with TFAA-I cannot be applied to their detection.

Large differences in detection limits were observed for nitrosobenzene (**5a**) and

TABLE II

DETECTION LIMITS FOR NITROSO COMPOUNDS 1–5 WITH UV DETECTION (360 nm) AND USING TFAA-I AS THE DETECTION REAGENT

Compound applied		SiO_2			Al_2O_3		
No.	Structure ^a	Detection limit (μg)		R_F	Detection limit (μg)		R_F
		UV	TFAA-I		UV	TFAA-I	
1a	(i-Pr) ₂ N-NO	50	50	0.72	50	50	0.85
1b	(i-Bu) ₂ N-NO	50	50	0.72	50	50	0.78
1c	 N-NO	50	10	0.61	50	50	0.78
1d	 N-NO	50	10	0.52	50	50	0.80
1e	 N-NO	5	5	0.50	10	10	0.78
1f	Ph-N-NO	5	1	0.66	10	3	0.80
1g	 Ph-N-NO	3	1	0.58	10	1	0.85
2	Ph-N-NO	5	1	0.56	10	1	0.81
3	 $\text{O}^- \text{NH}_4^+$	50	1	0.67	50	1	0.81
4	 H ₂ NC(O)-N-NO	50	5	0.62	50	50	0.80
5a	Ph-NO	50	—	0.69	50	—	0.83
5b	<i>p</i> -Tol-NO	1	5	0.69	10	10	0.84

^a i-Pr = Isopropyl; i-Bu = isobutyl; Ph = phenyl; Me = methyl; Tos = tosyl; Tol = tolyl.

TABLE III

COMPARISON OF REAGENTS FOR THE DETECTION OF NITROSO COMPOUNDS BY TLC

Compound applied ^a		Detection reagent								
No.	Amount ($\mu\text{g}/\text{spot}$)	UV (360 nm)	I ₂	Bi	Fe	Mn ^b	TFAA-I	DDQ	TCBQ	Gr-R
1b	5	—	—	—	—	—	—	—	—	+ ^c
	25	+	+	—	—	—	—	—	—	+ ^c
1e	5	+/-	+	—	—	—	+	—	—	+ ^c
	25	+	+	—	—	—	+	—	—	+ ^c
1f	5	+/-	+/-	—	+ ^d	+/-	++	—	—	+ ^e
	25	+	+	—	+ ^d	+	++	—	—	+ ^e
1g	5	+	+	—	+ ^f	+	+	+ ^g	—	+ ^e
	25	+	+	—	+ ^f	+	+	+ ^g	—	+ ^e
2	5	—	+	—	+/- ^h	+/-	+	+ ⁱ	—	+/- ^c
	25	—	+	—	+ ^h	+	+	+ ⁱ	—	+ ^c
3	5	—	+	—	—	—	+	—	—	+ ^c
	25	+/-	+	+/- ^j	—	+/-	++	—	+/- ^k	+ ^c
5a	5	—	—	—	—	—	—	—	—	—
	25	+	—	—	—	+/-	—	—	—	—
5b	5	+	—	—	—	—	+	—	—	+ ^l
	25	+	—	—	—	+/-	++	—	—	+ ^l

^a For structures, see Table II.^b Grey-green spots.^c Pink-violet spots.^d Yellow-brown spots, turning green after preheating to *ca.* 150°C.^e Grey-green spots after UV irradiation.^f Navy blue spots, turning green after preheating to *ca.* 150°C.^g Grey-red spots.^h Orange spots.ⁱ Red-brown spots turning green after saturation with ammonia vapour.^j Orange spots after preheating to *ca.* 150°C.^k Lilac spots.^l Yellow-brown spots turning red brown.

p-nitrosotoluene (**5b**), apparently owing to the induction effect caused by the methyl substituent. Thus, whereas nitrosobenzene was undetectable even at a level of 50 μg on both silica gel and aluminium oxide plates, *p*-nitrosotoluene had detection limits of 5 μg (silica gel) to 10 μg (aluminium oxide).

A similar effect was observed with azoxybenzene derivatives. Thus, whereas azoxybenzene was not detected using TFAA-I even at a level of 50 μg per spot, its 2,2'-dimethoxy and 2,2'-dicarboxylic analogue were detectable at levels of 1 μg (acetyl chloride-sodium iodide) to 5 μg (TFAA-I) (silica gel plates).

Results are given in Table III for the evaluation of TFAA-I reagent in comparison with other spray reagents for the detection of representative nitroso compounds.

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