

Note

Trifluoroacetic anhydride–sodium iodide as a reagent for the selective detection of nitrones and nitroxide radicals by thin-layer chromatography

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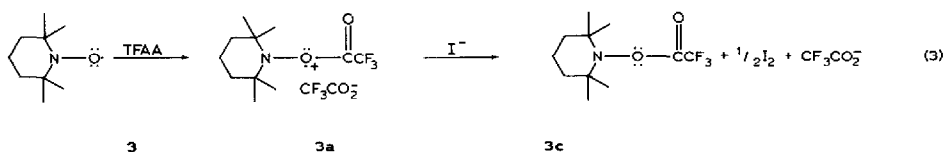
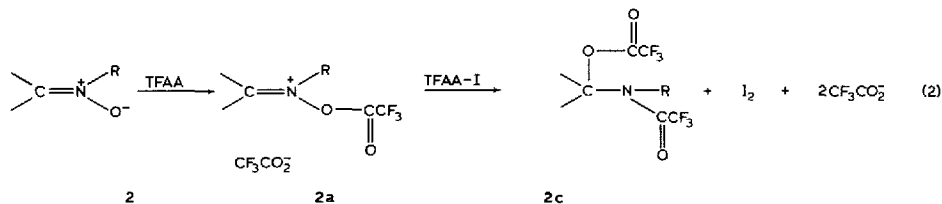
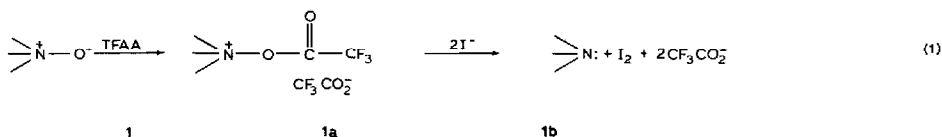
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The importance of N-oxo compounds is connected with their technological applicability and use as reagents in organic and bioorganic chemistry¹. In this class of nitrogen derivatives, special interest is focused on the properties and applications of nitroxide radicals and nitrones. Nitroxide radicals play a special role in spin labelling^{2,3}, whereas nitrones are important reagents in organic synthesis⁴ and also serve as excellent spin traps in electron paramagnetic resonance techniques^{5,6}. Hence the selective detection and determination of nitrones and nitroxide radicals are still important problems in nitrogen analytical chemistry^{7–9}.

Recently, we reported the application of trifluoroacetic anhydride–sodium iodide reagent (TFAA–I) for the microdeterminations of sulphoxides¹⁰, nitrones¹¹ and nitroxide radicals¹². We have also used this reagent for the selective detection of sulphoxides and sulphimides by thin-layer chromatography (TLC)¹³.

Here we report a selective procedure for the detection of nitrones and nitroxide radicals in the presence of amine N-oxides, based on the application of TFAA–I reagent. This reagent is able to convert amine N-oxides (**1**) into the corresponding amines (**1b**), nitrones (**2**) into the corresponding trifluoroacetamidoalkyl trifluoroacetate (**2c**) and nitroxide radicals (**3**) into the corresponding hydroxylamine trifluoroacetates (**3c**), with the simultaneous formation of elemental iodine, according to the equations¹² on the next page.

Because the rate of reaction of TFAA–I reagent and related systems with N-oxo compounds is influenced by their structures, there is the possibility of establishing conditions which allow the selective detection of nitrones and nitroxide radicals in the presence of aliphatic amine N-oxides.



EXPERIMENTAL

Materials

Trifluoroacetic anhydride (TFAA), trimethylamine N-oxide dihydrate, pyridine N-oxide and quinoline N-oxide hydrate were purchased from Aldrich (Milwaukee, WI, U.S.A.). Trimethylamine N-oxide was dehydrated according to ref. 14 and pyridine N-oxide and quinoline N-oxide were dehydrated by double vacuum distillation.

Nitroxides **3a-i** were purchased from Aldrich. Nitrones **2a-h** were prepared according to ref. 15 and were all of the same purity as reported previously.

Solutions

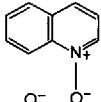
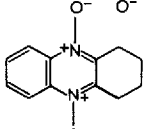
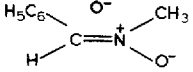
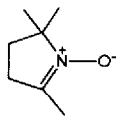
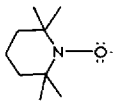
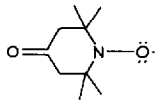
A 0.8 M solution of sodium iodide (NaI) in anhydrous acetone and a 0.8 M solution of TFAA in anhydrous acetone (prepared immediately before use) were used.

The concentrations of the compounds **1**, **2** and **3** chromatographed were *ca.* $5 \cdot 10^{-2}$ – 10^{-3} 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% solution of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone in benzene. Other solutions were iron(III) chloride (Fe), 2% aqueous; potassium permanganate (Mn), 0.1% aqueous; 4-dimethylaminobenzaldehyde (DAB), 5% methanolic; ninhydrin reagent (NH), 2% in butanol saturated with water; bromophenol blue (Ind), *ca.* 0.05% in ethanol; and reduced sodium nitroprusside (SN), for which a 1.2% aqueous solution of sodium nitroprusside (10 ml) was reduced with sodium borohydride to give a clear, deep-red solution, 0.8 ml of acetic acid (1 M) was added and, after 2 min, 5 ml of water were added.

TABLE I
COMPARISON OF VARIOUS ACYLATING AGENT-HALIDE SALT SYSTEMS FOR THE DETECTION OF AMINE OXIDES 1, NITRONES 2 AND NITROXIDE RADICALS 3 BY TLC

Silica gel plates. Solvents: acetone and methanol (2g). +/-, Spot is detectable; +, distinct detection; ++, strong detection.

Compound applied		Ac_2O-NaI	$AcCl-NaI$	$Ac_2O-LiBr$	$AcCl-LiBr^a$	TFAA-I	TFAA-LiBr ^a	
No.	Structure	$\mu g/spot$						
1c		1	-	-	-	-	-	-
		10	+	+	-	-	+	-
1d		1	-	+	-	-	+	-
		10	+/-	++	-	-	++	-
2a		1	-(+ ^b)	-(+ ^c)	-	-	+	-
		10	-(+ ^b)	-(+ ^c)	-	-	++	-
2g		1	-	-(+ ^b)	-	-	-	-
		10	+	-(+ ^b)	-	-	+	-
3a		1	-	+/-	-	-	+/-	-
		10	+/-	+	-	+	+	+
3b		1	-	+	-	-	+	-
		10	+/-	+	-	+/-	+	+

^a Red-brown spots.

^b After 30 min exposure.

^c A spot of iodine appeared after 10 min exposure.

Thin-layer chromatography

Pre-coated silica gel 60 F₂₅₄ aluminium sheets and pre-coated aluminium oxide 60 F₂₅₄ aluminium sheets (10 cm × 5 cm), with a 0.2-mm thick layer (Merck, Darmstadt, F.R.G.), were used for 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. Nitrones 2 and nitroxides 3 appeared almost immediately as brown spots on a white background, and were stable for more than 20 min.

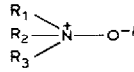
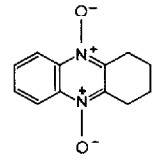
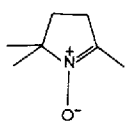
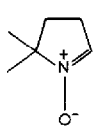
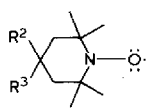
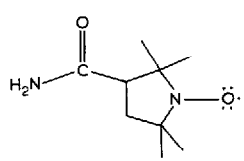
RESULTS AND DISCUSSION

The results of the application of various reagents, related to TFAA-I detection systems, for the detection N-oxo compounds by TLC are presented in Table I. These

results reveal that the most sensitive system for the detection of nitrones and nitroxide radicals is TFAA-I. Comparable properties were observed for acetyl chloride-sodium iodide reagent, which is slightly less sensitive for the detection of nitrones, however.

TABLE II

DETECTION LIMITS FOR AMINE OXIDES 1, NITRONES 2 AND NITROXIDE RADICALS 3 USING TFAA-I AS THE DETECTION REAGENT

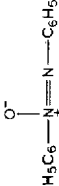
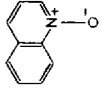
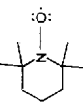
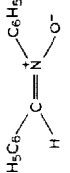
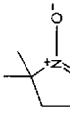
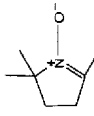
No.	Structure	Substituents			SiO_2		Al_2O_3			
		R^1	R^2	R^3	Detection limit		R_F^a	Detection limit		R_F^a
					μg	$nmol$		μg	$nmol$	
1d					5.0	23.3	0.52	10.0	46.6	0.68
										
2a		C_6H_5	H	CH_3	1.04	7.7	0.26	22.4	17.8	0.62
2b		C_6H_5	H	C_6H_5	1.0	5.0	0.70	2.3	11.7	0.72
2c		$m-ClC_6H_4$	H	C_6H_5	1.04	4.5	0.65	2.1	9.0	0.73
2d		$p-CH_3OC_6H_4$	H	C_6H_5	1.0	4.4	0.67	2.0	8.8	0.72
2e		$p-ClC_6H_4$	H	$tert.-C_4H_9$	5.7	22.3	0.61	11.4	44.5	0.79
2f		$p-O_2NC_6H_4$	H	$tert.-C_4H_9$	1.2	5.2	0.58	2.3	10.4	0.72
2g					5.0	31.0	0.05	7.5	45.5	0.81 ^c
					5.0	31.0	0.75 ^c			
2h					3.0	26.4	0.05	5.0	44.0	0.76 ^c
					3.0	26.4	0.75 ^c			
3a			H	H	1.1	7.1	0.66	2.2	14.2	0.68
3b				$-O-$	1.0	5.9	0.65	2.0	11.8	0.70
3c			H	OH	0.96	5.58	0.65	3.0	17.6	0.71
3d			CH_3	OH	1.08	5.8	0.71	2.2	12.0	0.78
3e			$tert.-C_4H_9$	OH	1.08	4.7	0.72	2.2	9.6	0.77
3f			$C_6H_5CH_2$	OH	1.1	4.27	0.73	2.2	8.5	0.80
3g			H	TosO	0.9	2.75	0.7	2.0	6.2	0.75
3h			H	NH_2	2.8	16.0	0.06	5.2	30.0	0.16
3i					1.04	5.6	0.51	2.08	11.2	0.59

^a Acetone.

^b Trimethylamine N-oxide (1a) did not give a positive test with TFAA-I even for applied amounts $\leq 50 \mu g/spot$, whereas pyridine N-oxide (1b) and quinoline N-oxide (1c) did for applied amounts $\geq 10 \mu g/spot$.

^c Methanol.

TABLE III
COMPARISON OF REAGENTS FOR THE DETECTION OF N-O COMPOUNDS BY TLC

Compound	Detection reagent												
	Structure	Amount applied ($\mu\text{g/spot}$)	UV	I_2	TFAA-I	Bi^{a}	Mn^{b}	Fe	NH	Ind	SN^{c}	DDQ	DAB ^d
 <chem>O=[N+]([O-])c1ccccc1</chem>	1	+/- ^e	-	-	-	-	-	-	-	-	-	-	-
	10	++ ^e	-	-	-	-	-	-	-	+/- ^f	-	-	+
	50	+++ ^e	+/-	-	-	-	+/-	-	-	+/- ^f	-	-	+
 <chem>O=C1N([O-])CCCC1c2ccccc2</chem>	1	+	-	-	-	-	-	-	-	-	-	-	-
	10	+	+	-	-	+	+	+	-	+/- ^g	-	-	-
	50	+	+	-	-	+	+	+	-	+ ^g	-	-	-
 <chem>O=C1N([O-])CCCCC1c2ccccc2</chem>	1	-	-	+/	-	-	+	-	-	-	+	-	-
	10	+/-	+	+	-	-	+	-	-	-	+	-	-
	50	+	+	+	-	-	+	-	-	-	+	-	-
 <chem>O=C1C=C([O-])N1c2ccccc2</chem>	1	+	+	+	+	-	-	-	-	-	-	-	-
	10	+	+	+	+	-	+	-	+ ^h	+/- ^g	-	-	+/-
	50	+	+	+	+	-	+	-	+ ^h	+/- ^g	-	+	+
 <chem>O=C1C=C([O-])N1c2ccccc2</chem>	1	+	+	+	-	-	-	-	+/- ^j	-	-	-	-
	10	+	+	+	-	-	+	-	+	+	-	-	-
	50	+	+	+	-	-	+	-	+	+	-	-	-
 <chem>O=C1C=C([O-])N1c2ccccc2</chem>	1	+	+	+	-	-	-	-	+/- ^k	-	-	-	-
	10	+	+	+	-	-	+	-	+ ^k	-	-	-	-
	+	+	+	+	-	-	+	-	+ ^k	-	-	-	-

^a Red spots.
^b Grey-green.
^c Blue spots on a grey-green background.
^d Yellow spots.
^e Brown spots after UV irradiation.
^f Grey-blue spots on a blue-violet background.
^g Grey-blue spots on a pink-blue background.
^h Grey spots after preheating to ca. 100°C.
ⁱ Red spots, turning to green after saturation with ammonia vapour.
^j Grey-blue spots after preheating to ca. 150-200°C.
^k Yellow-brown spots after preheating to ca. 150-200°C.

The replacement of sodium iodide with lithium bromide leads to the more selective detection of nitroxide radicals, but a decrease in sensitivity also occurs. These results clearly indicate the superiority of TFAA-I as a detection reagent for nitrones and nitroxide radicals by TLC.

Results for the application of TFAA-I for the detection of amine N-oxides **1**, nitrones **2** and nitroxide radicals **3** are summarized in Table II. It is evident that the detection limits of N-oxy compounds are strongly influenced by their structures. Thus, aliphatic amine N-oxides do not react at all with TFAA-I, heterocyclic amines N-oxides react slowly with high detection limits, the acyclic nitrones are detectable at 5–10 nmol and cyclic aliphatic nitrones (both aldo and keto nitrones) are detectable at ca. 30–40 nmol. The detection limits of nitroxide radicals vary from 5 to 10 nmol.

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

ACKNOWLEDGEMENT

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