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Short Communication

# Application of trifluoroacetic anhydride–sodium iodide reagent for selective detection in thin-layer chromatography IV. Thin-layer chromatographic differentiation of nitrones, nitroxide radicals and nitrosoamines in mixtures

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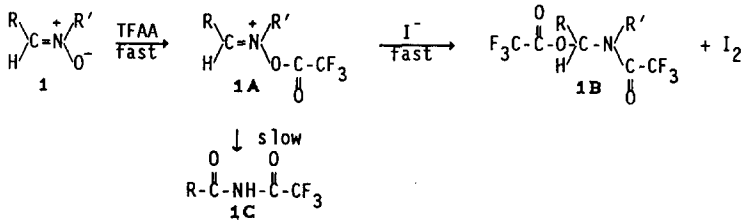
**Abstract**

Trifluoroacetic anhydride–sodium iodide mixture (TFAA-I) reacts with nitroxide radicals, nitrones and nitrosoamines with release of iodine. Methods for the differentiation of these groups of nitrogen derivatives containing the N–O moiety using TLC systems with TFAA-I detection reagent are described.

## 1. Introduction

In previous papers we have described the analytical application of trifluoroacetic anhydride–sodium iodide (TFAA-I) reagent for the TLC determination of nitrones (**1**) [1–3], nitroxide radicals (**2**) [1,2] and nitrosamines (**3**) [1,4]. TFAA-I reagent has been found to react very fast with these compounds with quantitative formation of iodine, according to Eqs. 1–3, and this feature constituted the basis of their detection, determination and micro-determination.

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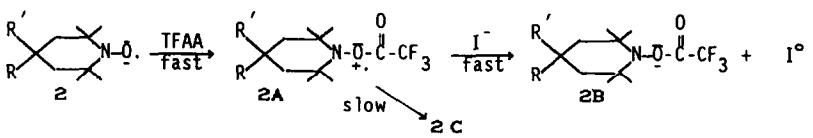


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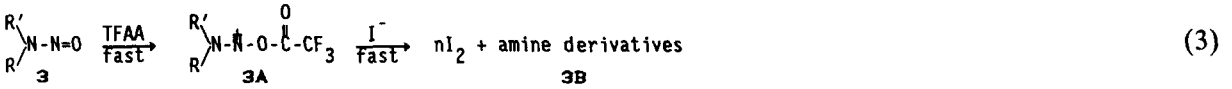


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In this paper we present our findings on the selective group determination of these compounds when they are present in mixtures.

## 2. Experimental

### 2.1. Materials

All reagents and solvents were purchased from Aldrich (Milwaukee, WI, USA) if is not stated otherwise. N-Phenyl-$\alpha$-phenyl nitrone (**1a**) and N-nitrosomethylphenylamine (**3a**) were prepared according to Refs. 5 and 6, respectively.

### 2.2. Solutions

N-Phenyl-$\alpha$-phenyl nitrone (**1a**), 4-oxo-2,2,6,6-tetramethyl-1-piperidinyloxy free radical (4-oxo-TEMPO; **2a**), N-nitroso-methylphenylamine (**3a**) solutions containing 2 mg of the compound in 1 ml of methanol (or acetic acid) were prepared. Other solutions used were a 0.5 *M* solution of sodium iodide in anhydrous acetone, a 1 *M* solution of trifluoroacetic anhydride in anhydrous acetone and a 1.5 *M* solution of ethanethiol in methanol.

### 2.3. Thin-layer chromatography

*Procedure I. TFAA-I detection*

Precoated silica gel 60 $F_{254}$ aluminium sheets and (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 amounts of compound (*ca.* 5 $\mu$g), developed for a distance of 8 cm with appropriate solvent [methanol or chloroform–acetone (4:1)], air dried and sprayed with sodium iodide solution, again air dried and subsequently sprayed with TFAA solution. Nitrones (**1**), nitroxide radicals (**2**) and nitrosoamines (**3**) appeared almost immediately as brown spots on a white background, and were stable for more than 20 min.

*Procedure II. EtSH–TFAA-I detection*

To 0.5 ml of a solution of **1**, **2** or **3** in methanol, 0.5 ml of a 1.5 *M* solution of ethanethiol in methanol was added. The mixture was allowed to stand for 3 h, then analysed according to procedure I.

*Procedure III. Ascorbic acid–TFAA-I detection*

To 1 ml of a solution of **1**, **2** or **3** in methanol, ascorbic acid (5 mg) was added and the mixture was stirred for 10 min and then analysed as according to procedure I.

*Procedure IV. Hydrogenation–TFAA-I detection*

To 1 ml of a solution of **1**, **2** or **3** in acetic acid–formic acid (9:1), palladium (10% Pd–C) (5 mg) was added. This mixture was stirred for 30 min and analysed according to procedure I.

*Procedure V. Silane–TFAA-I detection*

To 1 ml of a solution of **1**, **2** or **3** in acetic acid, triethylsilane ($Et_3SiH$) (3 drops) and trifluoroacetic acid (TFA) (3 drops) were added. The mixture was stirred for 20 min and analysed according to procedure I.

*Procedure VI. TFAA–TFAA-I detection*

Procedure I was followed but the final detection with TFAA-I was preceded by spraying the plate with TFAA solution (three times in 3-min periods). The plates were then sprayed with NaI and TFAA solutions.

*Procedure VII. Acidic treatment*

The plates were spotted with an appropriate amount (*ca.* 5 $\mu$g of each component) of compound **1**, **2** or **3**, developed (see procedure I), air dried and exposed to HCl vapour for 5 min (tank) or sprayed with TFA.

*Procedure VIII. TFAA–(TFAA-I)–NaI detection*

Two plates were spotted with a mixture of nitrone (**1a**), nitroxide radical (**2a**) and nitrosoamine (**3a**) (5 $\mu$g of each component), developed according to procedure I and sprayed

with TFAA solution (three times in 3-min periods). One of these plates was treated with TFAA-I reagent (see procedure I) and the other was sprayed with NaI solution.

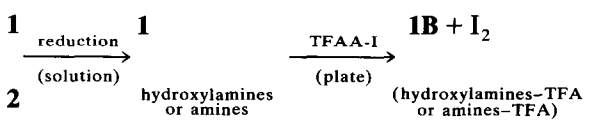
*Procedure IX. Ascorbic acid–(TFAA-I)–HCl–NaI detection*

A solution of a mixture of nitrone (**1a**), nitroxide radical (**2a**) and nitrosoamine (**3a**) was treated with ascorbic acid according to procedure III. Two plates were spotted with an appropriate amount of this solution (to deposit *ca.* 5 μg of each component) and developed according to procedure I. One of these plates was treated with TFAA-I reagent (see procedure I). The other plate was exposed to HCl vapours (tank) for 5 min (or sprayed with TFA), air dried and sprayed with NaI solution.

## 3. Results and discussion

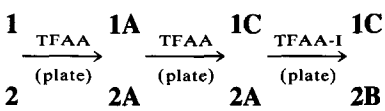
In order to determine selectively each of the N-oxy derivatives **1**, **2** and **3** in the mixture, some additional treatments are required that would allow their gradual selection, prior to the final detection with TFAA-I. Thus, nitrones (**1**) and nitroxide radicals (**2**) exhibit different redox properties; whereas nitrones are reduction-resistant compounds, nitroxide radicals are reduced easily various reagents, *e.g.*, hydroiodic acid [7], ascorbic acid [8], ethanethiol [9] and rhodizonic acid and its analogue [10]. The corresponding amines or hydroxylamines formed in this reaction are totally inactive towards TFAA-I.

We established analytical conditions for the selective reduction of nitrones in the presence of nitroxide radicals. Thus, the combination of the selective pre-reduction of nitroxide radicals into hydroxylamines or amines (by means of ascorbic acid, thiols or formate–palladium hydrogenation), with detection by means of the TFAA-I reagent of the reduction-resistant nitrones, allows the selective TLC differentiation of these two classes of N-oxy compounds:




Another possibility for the TLC differentiation of nitrones and nitroxide radicals is to use of the reverse mode of the TFAA-I procedure (TFAA is used in first stage, followed by addition of NaI). Thus, the corresponding N-O-trifluoroacetyl derivatives **1A** and **2A**, formed during the treatment of **1** and **2** with TFAA, rearrange to **1C** or **2C**, respectively, which are not able to oxidize iodide anion [1,3].

As these reactions proceed at different rates (reaction **1**→**1C** is much faster than reaction **2**→**2C**), the application of the reverse mode of TFAA-I detection permits the differentiation of these two types of N-oxy compounds according to:




The instability of nitrosoamines (**3**) in the presence of acid provides an opportunity for the elimination of these derivatives from their mixtures with nitrones and/or nitroxide radicals (all these N-O-derivatives are positive in TFAA-I detection). The subsequent decomposition of nitrosoamines in HCl vapour, followed by TFAA-I detection of the residual component, allows the selective detection of nitrones and/or nitroxide radicals in the two-component mixtures with nitrosoamines (**1** + **2** and **1** + **3**). Such TLC plates (after TFAA-I detection) revealed only the spots of the nitrones and/or nitroxide radicals, respectively.

We also attempted the selective detection of three-component mixtures of **1**, **2** and **3**:

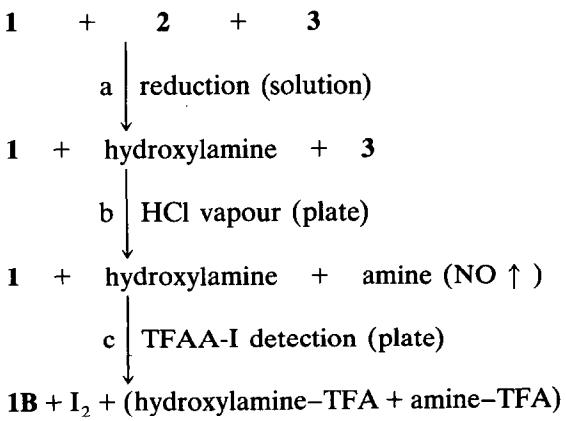
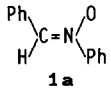
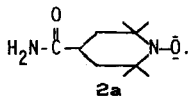
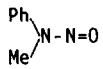


Table 1
Comparison of procedures for the selective detection of nitrones (**1a**), nitroxide free radicals (**2a**) and nitrosoamines (**3a**) by TLC

| Compound applied[a] | Procedure[b] | | | | | | |
|---|---|---|---|---|---|---|---|
| | I<br>TFAA-I[c] | II<br>EtSH[d]<br>TFAA-I[c] | III<br>Ascor.[d]<br>TFAA-I[c] | IV<br>H-Pd[d]<br>TFAA-I[c] | V<br>SiH-TFA[d]<br>TFAA-I[c] | VI<br>TFAA[c]<br>TFAA-I[c] | VII<br>HCl[c,e] |
|  **1a** | ++ | ++ | ++ | ++ | ++ | – | – |
|  **2a** | ++ | ++ | – | – | – | ++ | – |
|  **3a** | ++ | –/+ | ++ | – | –/+ | ++ | ++[f] |

Abbreviations: Me = methyl; Ph = phenyl; Ascor. = ascorbic acid; SiH = triethylsilane; TFA = trifluoroacetic acid; TFAA = trifluoroacetic anhydride. ++ = Strong detection; + = distinct detection; –/+ = spot is detectable; – = not detected.
[a] The amounts of **1**, **2** or **3** spotted were *ca.* 5 μg per spot (2–3 mm in diameter).
[b] Brown spots.
[c] The reaction was carried out on the TLC plate.
[d] The reaction was carried out in solution.
[e] HCl vapour.
[f] Brown spot turning green and subsequently blue on air exposure.

Table 2
Comparison of procedures for the TLC analysis of mixtures of nitrones (**1a**), nitroxide radicals (**2a**) and nitrosoamines (**3a**)

| Applied mixture of **1**, **2** and **3**[a] | TLC | | VIII | | IX | | |
|---|---|---|---|---|---|---|---|
| | TFAA-I[b] | $R_F$[c] | TFAA[d] (TFAA-I[b,e]) | NaI[d] | Ascor.[f] (TFAA-I[b,e]) | HCl[d] | NaI[d] |
| **1a** | ++ | 0.57 | –<br>(–)[e] | – | –<br>(++)[e] | – | –/+[b] |
| **2a** | ++ | 0.13 | –<br>(++)[e] | ++ | –<br>(–)[e] | –<br>– | –<br>– |
| **3a** | ++ | 0.65 | ++[g] | | –<br>(++)[e] | ++[g] | – |

Abbreviations and symbols as in Table 1.
[a] The mixture contained *ca.* 5 μg of each components.
[b] Brown spots on a white background.
[c] Silica plates. Solvent, chloroform–acetone (4:1).
[d] The reaction was carried out on the TLC plate.
[e] Detection with TFAA-I on the second plate.
[f] The reaction was carried out in solution.
[g] Brown spot turning green, and subsequently blue on air exposure.

The subsequent treatment of the three-component mixture with (a) ascorbic acid (in the starting solution) and (b) HCl vapour (on the TLC plate) allows the gradual elimination of nitroxide radicals (reduced to inactive hydroxylamines in stage a) and nitrosoamines (decomposed into amines in stage b), prior to the final detection of nitrones with TFAA-I reagent (stage c).

Comparison of this plate with the corresponding plates obtained (1) by instant TFAA-I detection of the starting mixture of **1**, **2** and **3** and (2) by HCl treatment followed by TFAA-I detection, permits the full TLC differentiation of all the N-oxy components in these mixtures.

The results of the differential detection of various compounds containing a semi-polar X–O bond with the TFAA-I as the final detection reagent are given in Tables 1 and 2.

## 4. References

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