

NO-CARRIER-ADDED ^{11}C -LABELLING OF BENZENOID COMPOUNDS IN RING POSITIONS BY CONDENSATION OF NITRO- ^{11}C METHANE WITH PYRYLIUM SALTS

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SUMMARY

A new synthesis is described for three no-carrier-added nitro- ^{11}C benzenes **3a-c** by condensation of nitro- ^{11}C methane (**1**) with the appropriate pyrylium salts **2a-c** in the presence of a base such as *t*-BuOK in *t*-BuOH. For synthesizing 4-nitro- ^{11}C anisole (**3a**), tetrabutylammonium fluoride was successfully used as an auxiliary base. The best results were obtained in the synthesis of **3a**. The conversion of **1** with 4-methoxypyrylium perchlorate (**2a**) yielded **3a** of a radiochemical purity of up to 82 % and a mean specific radioactivity of 30 GBq/ μmol (0.8 Ci/ μmol) within 20 min. Related to **1**, the reproducible radiochemical yields of **3a** are in the range of $77\pm 5\%$ (decay-corrected). 2,6-Dimethyl-4-methoxy-nitro- ^{11}C benzene (**3b**) was prepared by reaction of **1** with 2,6-dimethyl-4-methoxypyrylium perchlorate (**2b**) in radiochemical yields of about 37 % (decay-corrected) within 10 min. 2-Nitro- ^{11}C mesitylene (**3c**) was obtained by condensation of **1** with 2,4,6-trimethylpyrylium tetrafluoroborate (**2c**) in radiochemical yields of about 29 % (decay-corrected) within 20 min. $^{13}\text{C}/^{11}\text{C}$ Co-labelling experiments were carried out in order to confirm the identity of **3a-c** and the position of the label.

Keywords: ^{11}C -ring labelling, nitro- ^{11}C methane, 4-nitro- ^{11}C anisole, 2,6-dimethyl-4-methoxy-nitro- ^{11}C benzene, 2-nitro- ^{11}C mesitylene

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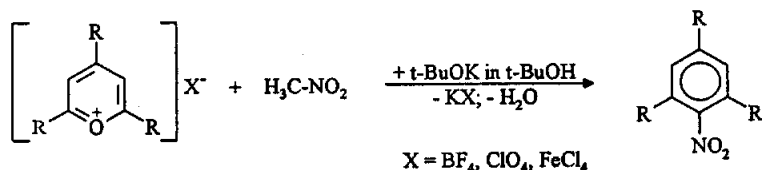
INTRODUCTION

Radiotracers labelled with ^{11}C ($t_{1/2} = 20.4$ min) are used in nuclear medical imaging with positron emission tomography (PET). In many cases such radiotracers undergo metabolic processes. Metabolisation may cause problems related to the fate of the label. Therefore we deal with possibilities to position the ^{11}C label in metabolically stable moieties such as aromatic rings.

In earlier papers we described the first n.c.a. labelling of various benzenoid compounds with ^{11}C in their rings by reaction of pentamethinium salts with nitro- ^{11}C methane as nitro- ^{11}C benzene [1], 3-nitro- ^{11}C anisole [2] and nitro- ^{11}C toluene derivatives [3]. These syntheses are based on the principle of synchronous six-electron cyclization of hexatriene systems into aromatics according to [4]. However, this reliable method has limitations due to the restricted availability of non-radioactive precursors [2, 3].

We therefore investigated another possibility of synthesizing substituted nitro- ^{11}C benzenes. This method consists in the condensation of pyrylium salts with nitro- ^{11}C methane. It has previously been presented and published in abstract form [5, 6].

The conversion of 2,4,6-trisubstituted pyrylium salts by nitromethane into many aromatic 2,4,6-trisubstituted nitrocompounds according to Scheme 1 has been known for a long time [7-10] but was not used for labelling with the carbon isotopes ^{11}C , ^{13}C or ^{14}C :

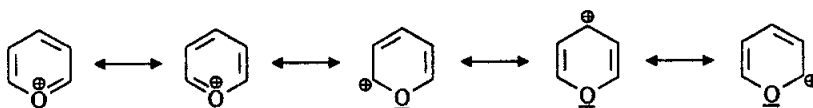


Scheme 1

Pyrylium salts are "key" compounds in organic chemistry [10-12]. They can be converted into various compounds, mostly by exchanging the oxygen atom.

As defined, pyrylium salts consist of a cyclic-conjugated oxonium cation with a π -electron sextet and an anion, such as perchlorate, tetrafluoroborate, tetrachloroferrate or chloride. The ground state of the pyrylium cation can be described by two

oxonium-Kekulé structures and three carbenium-pyran structures according to Scheme 2 [11, 12]:



Scheme 2

These resonance formulas express the two essential properties of the system: the aromaticity and the high susceptibility to nucleophilic attack in positions 2, 4 and 6.

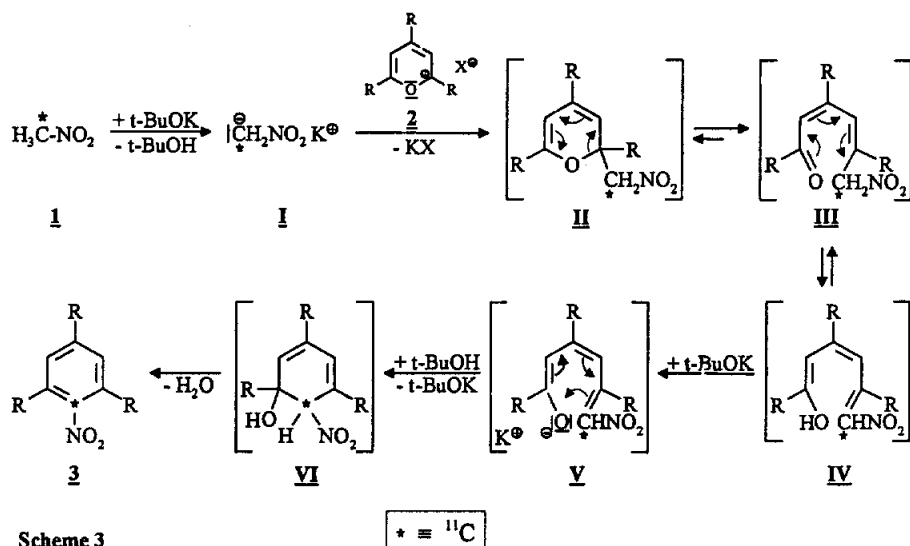
RESULTS AND DISCUSSION

Method of labelling

The labelling method is based on Scheme 3, as first described by Dimroth et al. [9]. The reaction comprises the opening of the heterocyclic ring of the pyrylium salt **2** by attack of the carbanion of nitro-[¹¹C]methane (**1**) to yield dienon **III**, followed by the intramolecular nitro-aldol condensation with formation of a substituted nitro-[¹¹C]benzene **3**.

The potassium salt of nitro-[¹¹C]methane (**I**) is formed by reaction of potassium tert-butyrate with nitro-[¹¹C]methane (**1**). The carbanion of **I** is added in 2- or 6-position to the carbenium cation of the pyrylium salt **2**, followed by electrocyclic ring opening of the 2Hi-pyran **II** with formation of a 6-nitro-[^{6-¹¹C]hexa-2,4-dien-1-ol **III** (thermal reaction control). Compound **III** is at tautomeric equilibrium with the 6-nitro-[^{6-¹¹C]hexa-1,3,5-trien-1-ol **IV**. Under the influence of a second equimolar amount of potassium tert-butyrate, the equilibrium is shifted to **IV** by formation of its potassium salt **V**. In the presence of tert-butanol the ring is closed. The 6-nitro-[^{6-¹¹C]cyclohexa-2,4-dien-1-ol **VI** formed is converted into the nitro-[¹¹C]benzene **3** by elimination of water.}}}

It can be assumed that the subsequent cyclization reaction from **V** via **VI** to **3** according to Scheme 3 is also based on the principle of the synchronous six-electron cyclization of hexatriene systems into aromatics according to [4]. This fact was not mentioned by Jutz and Wagner [4].



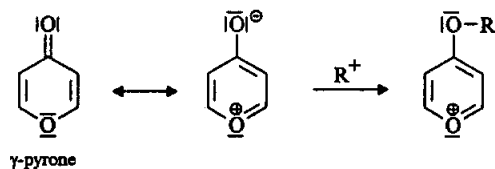
Scheme 3

Synthesis of the pyrylium salts

There are two synthetic principles of preparing pyrylium salts [12]:

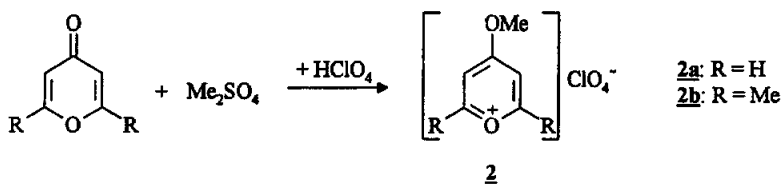
- Syntheses starting from an existing heterocyclic compound;
- Syntheses using ring closure reactions.

The electrophilic addition of alkylating agents to γ -pyrones is one method, starting from an existing heterocyclic compound. In this way many pyrylium salts can be synthesized. The possible electrophilic attack on the oxygen of the carbonyl group can be explained by the mesomeric structure of the γ -pyrone according to Scheme 4:



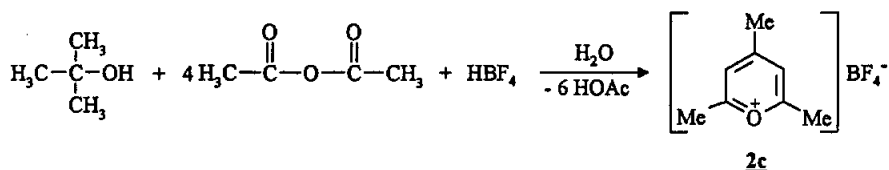
Scheme 4

In this way we synthesized the 4-methoxypyrylium perchlorate (**2a**) from γ -pyrone (4H-pyran-4-one), using dimethyl sulphate as methylation agent, followed by addition of perchloric acid (Scheme 5) [13]. The 2,6-dimethyl-4-methoxypyrylium perchlorate (**2b**) was synthesized from 2,6-dimethyl- γ -pyrone in the same manner according to Scheme 5 [14]:



Scheme 5

A third pyrylium compound, the 2,4,6-trimethylpyrylium tetrafluoroborate (**2c**), was synthesized by a ring closure reaction. This synthesis is based on the diacetylation of isobutene, which is formed *in situ* by dehydration of tert-butanol. In this case acetic anhydride, tert-butanol and tetrafluoroboric acid are converted according to Scheme 6 [15]:



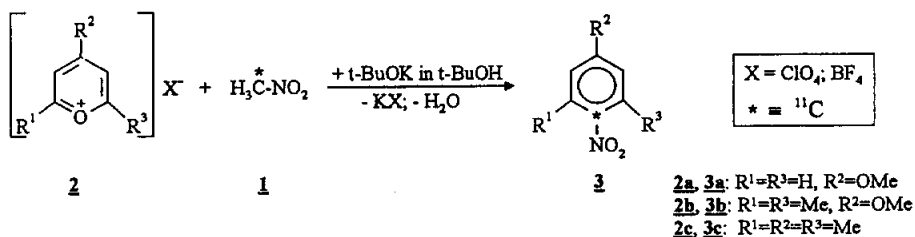
Scheme 6

Synthesis of the nitro-[1-¹¹C]benzenes

Initial attempts to synthesize 4-nitro-[4-¹¹C]anisole (**3a**) were made by heating a mixture of 8 mg 4-methoxypyrylium perchlorate (38 μmol of **2a**), 250 μl hexamethylphosphoric triamide (HMPT), 25 μl 1.6 M butyllithium in hexane (40 μmol) and nitro-[¹¹C]methane (**1**) at 170 °C for 10 min, but they did not work. These reaction conditions, which are similarly used for the synthesis of 3-nitro-[3-¹¹C]toluene and 4-nitro-[4-¹¹C]toluene [3] from their appropriate pentamethinium salts and **1**, are not suitable for the desired condensation. The condensation reaction requires a protic solvent such as tert-butanol. This necessity was already reflected in the reaction mechanism according to Scheme 3.

Potassium tert-butyrate in tert-butanol was therefore used for the following condensation experiments. The application of this solvent-base combination was described in most literature formulas, e.g. also for the syntheses of 2,6-dimethyl-4-methoxynitrobenzene [10] and 2-nitromesitylene [9].

The results of the ring closure experiments for preparing the nitro-[1-¹¹C]benzenes **3a-c** according to Scheme 7 are listed in Table 1.



Scheme 7

Preparation of 3a

After trapping **1** in a mixture of 250 μl tert-butanol, 38 μmol pyrylium salt **2a** and 76 μmol potassium tert-butyrate (molar ratio = 1:2) and heating this reaction mixture at 100 $^\circ\text{C}$ for 20 min, only 7.7 % **3a** was obtained (Expt. 1).

The reaction conditions for the synthesis of **3a** were therefore optimized, using tetrabutylammonium fluoride as an auxiliary base and HMPT as an additional solvent.

The order of the addition of bases for the trapping of **1** in a solvent/ pyrylium salt mixture was shown to be of importance. The yields of **3a** increased considerably (to about 58 %) when the following order was used: addition of 12 mg tetrabutylammonium fluoride trihydrate (38 μmol) to a mixture of 8 mg **2a** (38 μmol) in 250 μl HMPT, trapping of **1**, addition of 38 μl 1 M potassium tert-butyrate in tert-butanol, and heating of the reaction mixture at 120 $^\circ\text{C}$ for 20 min (Expt. 4). A further increase in the yields of **3a** was achieved by using a greater amount of tert-butanol (250 μl) (Expts. 5 and 6). Thus, starting from **1**, **3a** was prepared in a radiochemical purity of up to 82 % and with a mean specific radioactivity of 30 GBq/ μmol (0.8 Ci/ μmol). Related to **1**, the reproducible radiochemical yields of **3a** are in the range of 77 \pm 5 % (decay-corrected). A radiochromatogram of unpurified **3a** is shown in Fig. 1.

The successful preparation of 4-nitro-[4-¹¹C]anisole (**3a**) demonstrates the fact, that the known method according to Scheme 1 is not limited to the syntheses of 2,4,6-trisubstituted nitrobenzenes as described in [7-10]. This method is also suitable for preparing p-substituted nitrobenzenes.

Table 1: Percentage of the nitro-[¹¹C]benzenes **3a-c** in the reaction mixture after syntheses according to Scheme 7 under various reaction conditions. General conditions: trapping the nitro-[¹¹C]methane (**1**) in a mixture of a solvent and 38 μmol pyrylium salt **2a-c**, addition of a base before or after trapping **1**, reaction temperature: 120 °C

Expt.	Amounts of solvents [μl] and bases [μmol]				Reaction time [min]	Percentage [%] of 3 (decay-corrected)
	HMPT	t-BuOH	t-EuOK	Bu ₄ NF·3H ₂ O		
Synthesis of 3a						
1	-	250	76	-	20	7.7 ^{b)}
2	-	250	38	38	20	9.4
3	250	250 ^{a)}	38 ^{a)}	38 ^{a)}	20	7.7
4	250	38 ^{a),c)}	38 ^{a),c)}	38	20	57.7
5	250	250 ^{a)}	38 ^{a)}	38	20	71.4
6	250	250 ^{a)}	38 ^{a)}	38	20	81.9
Synthesis of 3b						
7	250	250 ^{a)}	38 ^{a)}	38	20	0
8	-	250	20 ^{a),c)}	-	10	37.0
Synthesis of 3c						
9	-	250	76	-	20	0 ^{b)}
10	250	250 ^{a)}	38 ^{a)}	38	20	0
11	-	250	38	38 ^{d)}	20	20.8 ^{e)}
12	-	250	38 ^{a),c)}	38	20	15.1 ^{f)}
13	-	250	76	38	20	12.8 ^{g)}
14	-	250	20 ^{a),c)} 40 ^{a),c)}	- -	10 20	17.1 29.0 ^{h)}
15	250	250 ^{a)}	38 ^{a),c)}	-	20	0
16	-	250	38 ^{a),c)}	-	20	15.6

^{a)} addition after trapping [¹¹C]CH₃NO₂ (**1**) in the reaction mixture

^{b)} reaction temperature: 100 °C

^{c)} addition as 1 M solution of t-BuOK in t-BuOH

^{d)} use of Bu₄NF on silica gel (1.1 mmol F/g resin)

^{e)} as well as 38.4 % of unconverted [¹¹C]CH₃NO₂ (**1**) and 32.2 % [¹¹C]CH₃ONO

^{f)} as well as 57.7 % of unconverted [¹¹C]CH₃NO₂ (**1**)

^{g)} as well as 57.3 % [¹¹C]CH₃ONO

^{h)} after a second addition of 20 μmol t-BuOK and further heating for 10 min

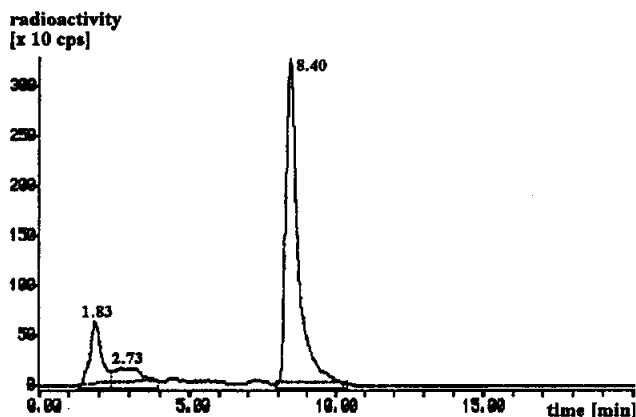


Fig. 1: Radiochromatogram obtained from the reaction mixture of the 4-nitro-[4-¹¹C]anisole (**3a**) synthesis
 1.83 min: [¹¹C]CH₃ONO; 11.6 %
 2.73 min: [¹¹C]CH₃NO₂ (**1**); 6.5 %
 8.40 min: 4-nitro-[4-¹¹C]anisole (**3a**); 81.9 %

Preparation of **3b** and **3c**

The syntheses of 2,6-dimethyl-4-methoxy-nitro-[1-¹¹C]benzene (**3b**) and 2-nitro-[2-¹¹C]mesitylene (**3c**) by reaction of their appropriate pyrylium salts **2b** or **2c** with **1** were not possible under the above-described optimized reaction conditions for the preparation of **3a** (Expts. 7 and 10). We are unable to interpret these results.

Other reaction conditions had therefore to be worked out. **3c** was obtained in yields of about 21 % (Expt. 11) as follows: **1** was trapped in a mixture of 250 µl tert-butanol, 8 mg of **2c** (38 µmol), 34.6 mg tetrabutylammonium fluoride on silica gel (1.1 mmol tetrabutylammonium fluoride/g carrier Δ 38 µmol tetrabutylammonium fluoride) and 38 µl 1 M potassium tert-butyrate in tert-butanol. This reaction mixture was heated at 120 °C for 20 min. **3c** decreased to about 15 % when pure tetrabutylammonium fluoride trihydrate (38 µmol) was used (Expt. 12).

Further optimization experiments for synthesis of **3b** and **3c** were carried out using potassium tert-butyrate (after trapping of **1** in tert-butanol) without the auxiliary base tetrabutylammonium fluoride. Starting from **1**, **3b** was obtained in shares of about 37 %, using **2b** and potassium tert-butyrate in a molar ratio of about 2:1 within 10 min reaction time (Expt. 8; see Fig. 2).

When **3c** was prepared in the same manner, it was only obtained in percentages of about 17 % (Expt. 14). A second addition of 20 µmol potassium tert-butyrate (i.e.

a molar ratio of **2c** and potassium tert-butyrate = 1:1) and further heating for 10 min produced 29 % **3c** (Expt. 14; see Fig. 3). By using **2c** and potassium tert-butyrate in a molar ratio of about 1:1 at the beginning of the reaction, only an astonishingly low percentage of \approx 16 % **3c** was synthesized after 20 min heating (Expt. 16). This conversion did not take place in a mixture of HMPT and tert-butanol (Expt. 15).

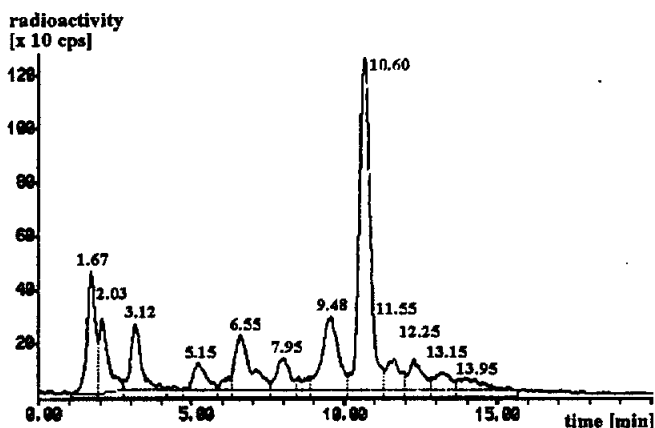


Fig. 2: Radiochromatogram obtained from the reaction mixture of the 2,6-dimethyl-4-methoxy-nitro-[1-¹¹C]benzene (**3b**) synthesis
 1.67 min: [¹¹C]CH₃ONO; 11.6 %
 3.12 min: [¹¹C]CH₃NO₂ (**1**); 6.5 %
 10.60 min: 2,6-dimethyl-4-methoxy-nitro-[1-¹¹C]benzene (**3b**); 37.0 %

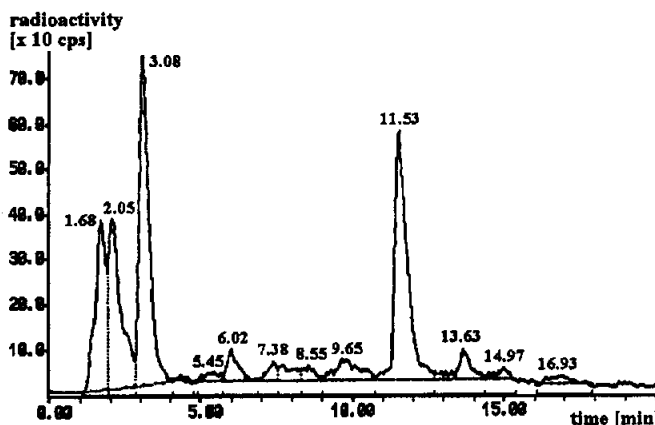


Fig. 3: Radiochromatogram obtained from the reaction mixture of the 2-nitro-[2-¹¹C]mesitylene (**3c**) synthesis
 1.68 min: [¹¹C]CH₃ONO; 11.5 %
 3.08 min: [¹¹C]CH₃NO₂ (**1**); 22.9 %
 11.53 min: nitro-[2-¹¹C]mesitylene (**3c**); 29.04 %

The radiochromatograms of unpurified **3b** and **3c** shown in Figs. 2 and 3 contain many peaks from unidentified products.

The high percentages of [^{11}C]methyl nitrite (see footnotes^{a,b}) of Table 1) resulted from the undesired isomerization reaction of **1** into [^{11}C]methyl nitrite under the influence of bases [2].

*Experiments for synthesizing **3b** and **3c** with alternative bases*

Radiochemical conversions of the pyrylium salts **2b** and **2c** with **1** were also carried out using triethylamine or sodium hydroxide in ethanol. The results of these experiments are not included in Table 1. After adding 76 μmol triethylamine to a mixture of 38 μmol **2b** and **1** in 250 μl ethanol and heating at 120 $^{\circ}\text{C}$ for 10 min no **3b** was obtained. Triethylamine in ethanol, which was used for the preparation of 2,4,6-triphenylnitrobenzene starting from 2,4,6-triphenylpyrylium tetrafluoroborate and nitromethane according to [16], proved to be too weak for such radiochemical conversions.

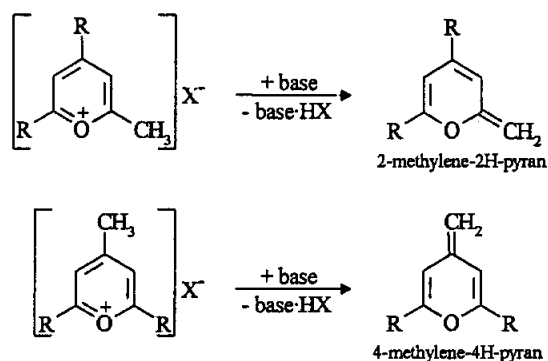
The radiochemical yields of the nitro-[^{11}C]benzenes **3b** and **3c** were low when using sodium hydroxide in ethanol. After adding a solution of 29 μmol sodium hydroxide in 40 μl ethanol to a mixture of 38 μmol **2b** and **1** in 250 μl ethanol and heating at 120 $^{\circ}\text{C}$ for 10 min, the share of **3b** was 14 %.

Similarly, **3c** was obtained in a 9 % share by the use of 38 μmol **2c** and **1** in 250 μl ethanol, addition of 75 μmol sodium hydroxide in 100 μl ethanol and heating this mixture at 120 $^{\circ}\text{C}$ for 20 min.

The condensation of 2,4,6-trimethylpyrylium perchlorate with nitromethane (19fold excess) into 2-nitromesitylene, using sodium hydroxide in ethanol, was described by Dimroth et al. [8]. But the quantitative ratios of the conversions with nitro-[^{11}C]methane are in inverse proportion to the corresponding nonradioactive preparations.

An explanation for the low yields of **3b** and **3c** in comparison with those of **3a** may be the formation of "anhydrobases" of the pyrylium salts. Such "anhydrobases" (2-methylene-2H-pyrans or 4-methylene-4H-pyrans) are formed by treatment of

pyrylium salts containing methyl (or benzyl) groups in the 2-, 4- or 6-position with bases [11] according to Scheme 8.



Scheme 8

This may reduce the precursor amount significantly and therefore limits the yields.

¹³C/¹¹C Co-labelling experiments were carried out in order to unequivocally affirm the identity of **3a-c** and their labelling positions. The positions of the ¹¹C label were confirmed by analyses of the ¹³C-NMR spectra of 4-nitro-[4-¹³C]anisole, 2,6-dimethyl-4-methoxy-nitro-[1-¹³C]benzene and 2-nitro-[2-¹³C]mesitylene which were synthesized by the same method used for **3a-c** through addition of nitro-[¹³C]-methane immediately after the trapping of nitro-[¹¹C]methane. The strong ¹³C signals of 4-nitro-[4-¹³C]anisole ($\delta = 141.5$ ppm), 2,6-dimethyl-4-methoxy-nitro-[1-¹³C]benzene ($\delta = 145.5$ ppm) and 2-nitro-[2-¹³C]mesitylene ($\delta = 149.7$ ppm) corresponded to the ¹³C signals of the authentic unlabelled compounds. MS analyses of 4-nitro-[4-¹³C]anisole ($m/z = 155$ (M+1)), 2,6-dimethyl-4-methoxy-nitro-[1-¹³C]benzene ($m/z = 183$ (M+1)) and 2-nitro-[2-¹³C]mesitylene ($m/z = 167$ (M+1)) indicated a molecular ion peak which differed from the corresponding molecular ion peak of the authentic unlabelled compounds by 1.

EXPERIMENTAL

Materials

γ -Pyrone (99 %), 2,6-dimethyl- γ -pyrone (99 %) and t-BuOH (99.3 %) were purchased from Aldrich. Dimethyl sulphate (for synthesis) and perchloric acid (p.a.,

70 %) were obtained from Laborchemie Apolda, Germany, acetic anhydride (p.a.) from Feinchemie Sebnitz, Germany, and tetrafluoroboric acid (purum, 40 %) from Chemiewerk Nunchritz/ BT Dohna, Germany. HMPT, *t*-BuOK and BuLi (1.6 M in hexane), all of synthesis quality, were purchased from Merck. Bu₄NF·3H₂O (purum) and Bu₄NF on silica gel (1.1 mmol F/ g carrier) were obtained from FLUKA. For co-labelling experiments nitro-[¹³C]methane (99 atom % ¹³C) was purchased from Aldrich.

Methyl iodide for synthesis (Merck), nitromethane 99 %, 4-nitroanisole 97 % (Aldrich), 2-nitromesitylene 99 % (ABCR GmbH & Co., Germany) and 2,6-dimethyl-4-methoxy-nitrobenzene (synthesized according to [10]) were used as reference substances.

Analysis

The ¹³C-NMR spectra were recorded on a Bruker MSL 300 NMR spectrometer at 75.475 MHz, the ¹H-NMR spectra on an AC P 200 (Bruker) at 200.13 MHz. The ¹³C-NMR spectra of the ¹³C-ring-labelled nitrobenzenes were recorded on a Varian INOVA 400 spectrometer at 100.6 MHz with CDCl₃ as internal standard.

Mass spectrometric analyses were carried out on a Micromass tandem quadrupole mass spectrometer (Quattro LC) operated in the MS mode. Mass spectral data were recorded in the positive ESI mode using a cone voltage of 20 V (for 2,6-dimethyl-4-methoxy-nitrobenzene) or 30 V (for 4-nitroanisole and 2-nitromesitylene). A solution of the nitrobenzene or their corresponding ¹³C-ring-labelled nitrobenzene in MeOH was infused at a flow rate of 5 μl/min.

To determine the extent of the reaction conversion, the radiochemical purity of the reaction products and the specific radioactivity of 4-nitro-[4-¹¹C]anisole, an HPLC system (JASCO) was used, including a pump, a Rheodyne injector with a 20 μl loop, a LiChrospher 100 RP-18 endcapped column (5 μm, 150 mm x 3.3 mm, Merck) and a UV detector coupled in series with a radioactivity detector FLO-ONE\Beta 150TR (Canberra Packard). The HPLC analyses (see radiochromatograms in Fig.1-3) were carried out at a flow rate of 0.5 ml/min with the following linear gradient of the eluents: 0 min - 70 % buffer/ 30 % MeCN; 10 min - 0 %

buffer/ 100 % MeCN; 20 min - 0 % buffer/ 100 % MeCN; buffer = phosphate buffer pH 7 (c[NaH₂PO₄] = 0.26 mM; c[Na₂HPO₄] = 0.51 mM).

A second HPLC system was used to confirm the identity of the radioactive products **3a** (6.44 min), **3b** (10.09 min) and **3c** (14.98 min). These analyses were done with a NUCLEOSIL 120 RP-18 column (5 μm, 125 mm x 4 mm, Macherey-Nagel) eluted isocratically with isopropanol/water (40/60) containing 0.1 M ammonium formate at a flow rate of 0.5 ml/min.

The specific radioactivity of 4-nitro-[4-¹¹C]anisole was calculated by means of a calibration curve using different concentrations of the nonradioactive 4-nitroanisole in relation to their UV adsorbance response.

Synthesis of the precursors

4-Methoxyppyrylium perchlorate (2a)

Compound **2a** was prepared according to [13] as follows. A mixture of γ-pyrone (2.5 g, 26 mmol) and dimethyl sulphate (3.5 g, 28 mmol) was heated at 60 °C for 1 h with a brownish colour appearing. Then a solution of perchloric acid (60 %, 5 ml, 46 mmol), acetic anhydride (15 ml) and diethyl ether (20 ml) was added at room temperature. After stirring for 30 minutes the crystalline solid separated, was filtered through a frit glass filter, washed with n-propanol and diethyl ether and dried. The pale crystals had m.p. 84-88 °C (lit. m.p. 76-77 °C [13]).

Yield: 2.0 g Δ 36.5 %.

¹³C-NMR: 75.475 MHz, CD₃OD, TMS Δ 0, δ in ppm

49.8 (4-OCH₃); 102.7 (CH, (C3) and (C5)); 113.6 (C, (C4)); 167.7 (CH, (C2) and (C6))

Analysis calcd. for C₆H₇ClO₆: C, 34.2; H, 3.3; Cl, 16.9.

Found: C, 33.8; H, 3.2; Cl, 16.2.

2,6-Dimethyl-4-methoxyppyrylium perchlorate (2b)

Compound **2b** was prepared according to [14] as follows. A mixture of 2,6-dimethyl-γ-pyrone (2.5 g, 20 mmol), dimethyl sulphate (4.0 g, 32 mmol) and methanol (0.25 ml) was heated at 50 °C for 1 h with a complete solution occurring and a

yellow colour appearing. Perchloric acid (70 %, 9.5 g, 66 mmol) was added while cooling the solution with ice water. After stirring for 2 h at 0 °C, the crystalline solid separated, was filtered through a frit glass filter, washed with acetone and diethyl ether and dried. In this way only 0.7 g colourless crystals were obtained. The yield of the pyrylium salt was increased by addition of diethyl ether to the filtrate, with grey crystals precipitating. The isolated crystals were purified by being dissolved in acetone and then precipitated with diethyl ether. The colourless crystals had m.p. 182-192 °C (lit. m.p. ~ 190 °C [14]).

Total yield: 2.1 g Δ 43.7 %.

¹H-NMR: 200.13 MHz, CD₃CN, TMS Δ 0, δ in ppm

2.73 (s, 6 H, 2-CH₃ and 6-CH₃); 4.24 (s, 3 H, 4-OCH₃); 7.32 (s, 2 H, H-C(3) and H-C(5))

¹³C-NMR: 75.475 MHz, CD₃CN, TMS Δ 0, δ in ppm

21.4 (2-CH₃ and 6-CH₃); 60.9 (4-OCH₃); 109.6 (CH, (C3) and (C5)); 179.3 (C, (C2) and (C6)); 180.5 (C, (C4))

Analysis calcd. for C₈H₁₁ClO₆: C, 40.2; H, 4.6; Cl, 14.9.

Found: C, 39.7; H, 4.7; Cl, 15.2.

2,4,6-Trimethylpyrylium tetrafluoroborate (2c)

Compound **2c** was prepared according to [15] as follows. Tetrafluoroboric acid (40 %, 7 ml, 39 mmol) was slowly added to a stirred mixture of acetic anhydride (50 ml, 530 mmol) and t-BuOH (4 ml, 42 mmol) at such a rate that the final temperature reached approximately 100 °C. The dark brown solution was allowed to cool down to 80 °C, and was then chilled to 5 °C in an ice bath. The separation of the salt began and was completed by addition of diethyl ether (100 ml). The crystalline solid was filtered through a frit glass filter, washed with diethyl ether (30 ml) and dried. The pale yellow crystals (3.2 g) were recrystallized from a mixture of ethanol and methanol (1 : 1, 60 ml) containing 3 drops of tetrafluoroboric acid. The colourless crystals had m.p. 207-213 °C (lit. m.p. 224-226 °C [15]).

Yield: 2.0 g Δ 24.4 %.

¹H-NMR: 200.13 MHz, CD₃CN, TMS Δ 0, δ in ppm

2.78 (s, 3 H, 4-CH₃); 2.93 (s, 6 H, 2-CH₃ and 6-CH₃); 7.85 (s, 2 H, H-C(3) and H-C(5))

Analysis calcd. for C₈H₁₁BF₄O: C, 45.8; H, 5.2.

Found: C, 45.7; H, 5.4.

Synthesis of 2,6-dimethyl-4-methoxy-nitrobenzene (as reference substance for 3b)

This compound was prepared according to [10] as follows. A suspension of t-BuOK (0.946 g, 8.4 mmol) in t-BuOH (15 ml) was slowly added to a solution of 2,6-dimethyl-4-methoxy-pyrylium perchlorate (1 g, 4.2 mmol) in nitromethane (10 ml, 185 mmol) with an orange colour appearing. The reaction mixture was refluxed for 45 min. The potassium perchlorate separated in the reddish brown reaction mixture was then filtered through a frit glass filter. The filtrate was evaporated and the residue recrystallized from a mixture of methanol and water (2 : 1, 15 ml). The pale needles had m.p. 47.6-50.6 °C (lit. m.p. 49.5-50.5 °C [10]).

Yield: 0.3 g Δ 39.5 %.

¹H-NMR: 200.13 MHz, CDCl₃, TMS Δ 0, δ in ppm

2.24 (s, 6 H, 2-CH₃ and 6-CH₃); 3.73 (s, 3 H, 4-OCH₃); 6.52 (s, 2 H, H-C(3) and H-C(5))

¹³C-NMR: 75.475 MHz, CDCl₃, TMS Δ 0, δ in ppm

18.4 (2-CH₃ and 6-CH₃); 55.5 (4-OCH₃); 113.8 (CH, (C3) and (C5)); 132.4 (C, (C2) and (C6)); 145.5 (C, (C1)); 160.0 (C, (C4))

Radiosyntheses

[¹¹C]Carbon dioxide

¹¹C was produced on the modified U-120 cyclotron at the Research Center Rossendorf Inc. by the ¹⁴N(p,α)¹¹C nuclear reaction giving [¹¹C]CO₂ [1].

[¹¹C]Methyl iodide

[¹¹C]Methyl iodide was prepared by the standard one-pot procedure [17] via reduction of [¹¹C]CO₂ with LiAlH₄, hydrolysis and treatment of the formed [¹¹C]methanol with hydroiodic acid.

Nitro-[¹¹C]methane (1)

The gas-solid reaction of [¹¹C]methyl iodide with silver nitrite according to [18] resulted in nitro-[¹¹C]methane (**1**). In this procedure the [¹¹C]CH₃I was driven by an N₂ stream (flow rate = 40 ml/min) through a heated glass column (i.d. 3 mm, length 4 cm, oven temp. 80 °C) containing AgNO₂ (0.4 g). The [¹¹C]CH₃NO₂ (**1**) was contaminated with 2–4 % [¹¹C]methyl nitrite.

4-Nitro-[4-¹¹C]anisole (3a)

The gaseous [¹¹C]CH₃NO₂ (**1**) thus produced was introduced into a cooled 2 ml vessel (10 °C) containing HMPT (250 μl), 4-methoxyppyrylium perchlorate (**2a**) (8 mg, 38 μmol) and Bu₄NF·3H₂O (12 mg, 38 μmol). After addition of a solution of t-BuOK (4.3 mg, 38 μmol) in t-BuOH (250 μl) the well-sealed vessel was heated at 120 °C for 20 min.

2,6-Dimethyl-4-methoxy-nitro-[1-¹¹C]benzene (3b)

The gaseous [¹¹C]CH₃NO₂ (**1**) was trapped in a 2 ml vessel containing t-BuOH (250 μl) and 2,6-dimethyl-4-methoxyppyrylium perchlorate (**2b**) (9 mg, 38 μmol). After addition of t-BuOK (1 M in t-BuOH, 20 μl, 20 μmol) the well-sealed vessel was heated at 120 °C for 10 min.

2-Nitro-[2-¹¹C]mesitylene (3c)

The gaseous [¹¹C]CH₃NO₂ (**1**) was trapped in a 2 ml vessel containing t-BuOH (250 μl) and 2,4,6-trimethylppyrylium tetrafluoroborate (**2c**) (8 mg, 38 μmol). After addition of t-BuOK (1 M in t-BuOH, 20 μl, 20 μmol) the well-sealed vessel was heated at 120 °C for 10 min followed by a second addition of t-BuOK (1 M in t-BuOH, 20 μl, 20 μmol) and further heating at 120 °C for 10 min.

¹¹C/¹³C Co-labelling experiments

4-Nitro-[4-¹¹C/¹³C]anisole

The gaseous [¹¹C]CH₃NO₂ (**1**) was trapped in a cooled 2 ml vessel (10 °C) containing HMPT (250 μl), 4-methoxyppyrylium perchlorate (**2a**) (8 mg, 38 μmol)

and $\text{Bu}_4\text{NF}\cdot 3\text{H}_2\text{O}$ (12 mg, 38 μmol). Nitro- ^{13}C methane (2 μl , 37 μmol) was added. After addition of $t\text{-BuOK}$ (1 M in $t\text{-BuOH}$, 38 μl , 38 μmol) and $t\text{-BuOH}$ (250 μl) the well-sealed vessel was heated at 120 °C for 30 min.

Using a syringe, the reaction mixture was diluted with water (10 ml) and passed through an activated RP-18 cartridge (Chromafix C18 ec, Macherey-Nagel). After washing the cartridge with water (10 ml), 4-nitro- $[4\text{-}^{11}\text{C}/^{13}\text{C}]$ anisole was eluted with acetonitrile (2 ml) and analysed by two different HPLC systems mentioned above.

After decay of the ^{11}C radioactivity, the solvent was evaporated. The purification of 4-nitro- $[4\text{-}^{13}\text{C}]$ anisole was made by flash chromatography using a silica gel column (i.d. 1 cm, length 4.3 cm) and a mixture of petrolether and ether (9 : 1). The separated fractions (each 1 ml) of 4-nitro- $[4\text{-}^{13}\text{C}]$ anisole were combined and evaporated. The purified solid product was dissolved in CDCl_3 (0.7 ml) and analysed by ^{13}C -NMR. A part of this CDCl_3 solution was evaporated for MS investigations in MeOH.

2,6-Dimethyl-4-methoxy-nitro- $[1\text{-}^{11}\text{C}/^{13}\text{C}]$ benzene

The gaseous $^{11}\text{C}\text{CH}_3\text{NO}_2$ (**1**) was trapped in a 2 ml vessel containing $t\text{-BuOH}$ (250 μl) and 2,6-dimethyl-4-methoxy-pyrylium perchlorate (**2b**) (9 mg, 38 μmol). Nitro- ^{13}C methane (2 μl , 37 μmol) was added. After addition of $t\text{-BuOK}$ (1 M in $t\text{-BuOH}$, 40 μl , 40 μmol) the well-sealed vessel was heated at 120 °C for 30 min. The reaction mixture was analysed by two different HPLC systems.

After decay of the ^{11}C radioactivity, the reaction mixture was evaporated. The purification of 2,6-dimethyl-4-methoxy-nitro- $[1\text{-}^{13}\text{C}]$ benzene was made by flash chromatography using a silica gel column (i.d. 1 cm, length 3.8 cm) and a mixture of petrolether and ether (9 : 1). The separated fractions (each 1 ml) of 2,6-dimethyl-4-methoxy-nitro- $[1\text{-}^{13}\text{C}]$ benzene were combined and evaporated. The purified solid product was dissolved in CDCl_3 (0.7 ml) and analysed by ^{13}C -NMR. A part of this CDCl_3 solution was evaporated for MS investigations in MeOH.

2-Nitro-[2-¹¹C/¹³C]mesitylene

The gaseous [¹¹C]CH₃NO₂ (**1**) was trapped in a 2 ml vessel containing t-BuOH (250 μl) and 2,4,6-trimethylpyrylium tetrafluoroborate (**2c**) (8 mg, 38 μmol). Nitro-[¹³C]methane (2 μl, 37 μmol) was added. After addition of t-BuOK (1 M in t-BuOH, 40 μl, 40 μmol) the well-sealed vessel was heated at 120 °C for 30 min. The reaction mixture was analysed by two different HPLC systems.

After decay of the ¹¹C radioactivity, the reaction mixture was evaporated. The purification of 2-nitro-[2-¹³C]mesitylene was made by flash chromatography using a silica gel column (i.d. 1 cm, length 2.5 cm) and petrolether. The separated fractions (each 1 ml) of 2-nitro-[2-¹³C]mesitylene were combined and evaporated. The purified solid product was dissolved in CDCl₃ (0.7 ml) and analysed by ¹³C-NMR. A part of this CDCl₃ solution was evaporated for MS investigations in MeOH.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. D. Scheller of the Dresden University of Technology and Dr. M. Scheunemann of our institute for recording the NMR spectra. The authors also thank Mrs. K. Fischer for recording the mass spectra and Dr. J. Römer for his helpful discussion of this work.

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