SYNTHESIS OF [^{14}C]-LABELED N-tert-BUTYL- α -PHENYLNITRONE. A POTENTIAL SPIN-TRAPPING AGENT.

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Summary

A single step synthesis of the [^{14}C-ring]-N-tert-butyl- α -phenylnitrone (1) starting from the [^{14}C-ring] benzaldehyde is described. The product is obtained in high yield (90%) with a good level of purity.

Keywords: [^{14}C]-N-tert-butyl- α -phenylnitrone, spin-trapping.

INTRODUCTION

The lipophilic spin-trapping agent N-tert-butyl- α -phenyl nitrone (PBN) (1) significantly prevents the Adriamycin (ADR) cardiotoxicity in isolated rat heart (1). In fact its intracellular distribution on isolated myocardial preparation (2) suggests the protection of the specific sites where the generation of free radical pathogenic agents takes place (3,4). The interaction between the spin-trapping agent (PBN) and free radicals may provide an interesting way to limit the reaction cascade leading to cellular damage.

Among the different experimental techniques employed to study the spin-trapping of free radicals, electron paramagnetic resonance

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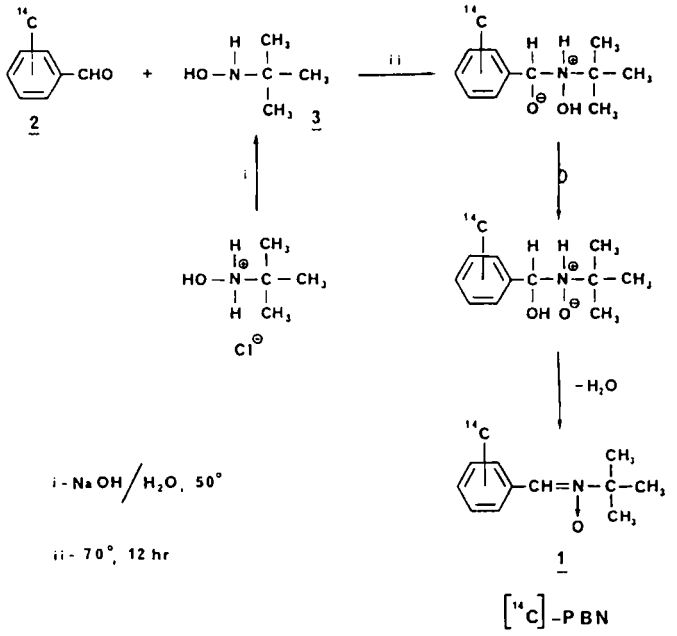
spectroscopy represents a valid tool to check the effectiveness of the employed spin-trapping agent (5).

However, with the aim to obtain a better comprehension of the PBN trapping mechanism, a more quantitative intracellular map of myocardial tissue is necessary. This goal can be attained using [^{14}C]-labeled-N-tert-butyl- α-phenylnitrone at sufficiently high level of specific activity so as to restrict the inherent PBN toxicity, taking into account also its low intracellular uptake.

In this paper we report a simple synthetic pathway (Scheme I) for [^{14}C]-PBN **1**, formed in a single pot from commercial [^{14}C]-benzaldehyde **2**.

Scheme I



2 3 1

[^{14}C]-PBN

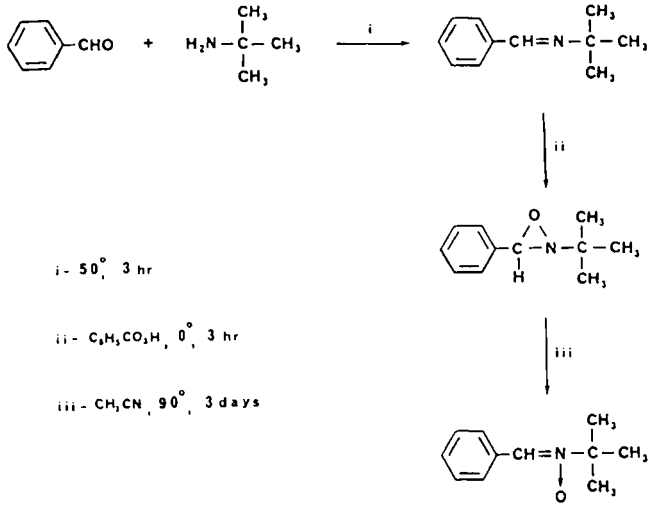
RESULTS AND DISCUSSION

First of all, as a general point, a synthetic procedure is valid for a radiochemical preparation if some important requirements are respected. In fact simple synthetic ways, which ensure

the personnel safety, good reaction yields, and guarantee an economic procedure should be always pursued (6-8).

Different reaction pathways can be proposed for the preparation of the N-tert-butyl- α-phenylnitrone. One of these is represented in Scheme II and concerns the initial preparation of the N-tert-butylbenzaldimine (**4**), its oxidation to 2-tert-butyl-3-phenyloxazirane (**5**) and at the end the ring opening to PBN (**1**) (9).

Scheme II



Some observations can be made regarding this Scheme. It is a complex pathway which requires three reaction steps; each one showing good yields (between 70%-80%) but with a low overall yield (about 35%). In particular, considering the high cost of the [^{14}C] benzaldehyde (**2**) used as starting compound, high activity of [^{14}C]-PBN can be prepared in economic way, only if the product can be obtained in very good yield.

Another difficulty involved in step ii), the preparation of 2-tert-butyl-3-phenyloxazirane (**5**), is its inclination toward fast hydrolysis producing the starting benzaldehyde (**2**) and N-tert-butyl-hydroxylamine (**3**) (9-12). This collateral behaviour

can be partially avoided employing particular reaction conditions, such as neutral condition and short purification time. Furthermore,in general,the oxaziranes are active oxygen compounds and show many reactions similar to those of organic peroxides (electron transfers or oxidative processes).

Another way of producing [^{14}C]-PBN (**1**), reported in Scheme I, was followed for this paper. Some important modifications, were made in this work to this well known method (9).

In contrast to the pathway of Scheme II, Scheme I is more simple and can be executed in a single pot, preparing preliminarly the free N-tert-butyl-hydroxylamine (**3**). The following nucleophilic attack on the carbonyl group, the subsequent proton migration and the final water elimination take place in sequential way in the same reaction vessel. The purification of the radioactive product has been performed in a different way with respect to the one reported in the literature (cf. ref. 9). In fact a more simple sublimation step of the crude product provides very high yield (about 90%) of [^{14}C]-PBN (**1**) with an elevated level of radiochemical purity (about 97%).

In conclusion, the modifications made in this study to the literature methods, furnished a more simple way to prepare [^{14}C]-PBN (**1**) in very high yield. In this paper, this method was applied , using sufficiently low level of total radioactivity, just like a test run, with the aim of proving the validity of the method. However if the preparation of [^{14}C]-PBN (**1**) should be performed again with higher level of radioactivity, the method described in this paper can be useful considering the high level of yield which it can provide.

EXPERIMENTAL

Material and Methods. [^{14}C]-labeled benzaldehyde was purchased by Sigma Chemical Co. (St. Louis, MO). N-tert-butyl hydroxylamine

hydrochloride and an inactive sample of N-tert-butyl-α-phenylnitrone were obtained by Aldrich Chem. Co. (Milwaukee, WI). The analysis of the starting compound and of the reaction products were carried using chromatographic methods. In particular, preparative gas liquid chromatography (s.s. E301 column long 3 m x 4 mm i.d. used at 70°C) was employed for the initial purification of [^{14}C]-labeled benzaldehyde. HPLC apparatus (Perkin Elmer LC 10) connected to a flow liquid scintillation monitor (Berthold LB 503) was performed for the identification of the filuted with isocratic mixture of acetonitrile: water = 50 :50 at the flow of 0.5 ml/min produced the best separative condition and allowed the identification of the product by comparison of the retention volume with that of an authentic sample of PBN.

A spectroscopic confirmation of the nature of the radioactive product [^{14}C]-PBN was obtained by I.R. analysis (Perkin Elmer Mod. 257), comparing the infrared signals with those of the commercial PBN and by ^{1}H N.M.R. analysis in CD_3COCD_3 solutions at 200.13 MHz on a Bruker AM 200 instrument.

Static determination of the radioactivity during the steps of the synthetic procedure were obtained by liquid scintillation counting on the Packard Mod. Tri-Carb 2260 XL spectrometer.

Preparation of [^{14}C]-N-tert-butyl-α-phenylnitrone (1).

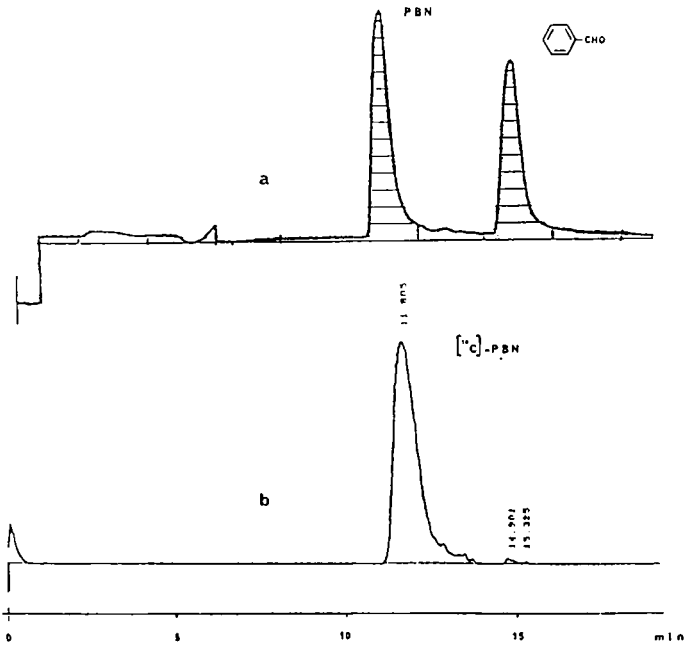
Commercial [^{14}C-ring]-benzaldehyde **2** (nominal activity about 740 kBeq and specific activity 233 MBeq/mmol) was diluted with 400 μl of inactive benzaldehyde used as carrier. This mixture was purified by g.l.c. method producing about 360 μl of [^{14}C]-radioactive benzaldehyde with specific activity: 163 Kbeq/mmol and total activity: 554 KBeq.

[^{14}C]-benzaldehyde (3.39 mmol) was carefully added drop by drop to a preformed mixture of N-tert-butyl-hydroxylamine-hydrochloride (450 mg. 3.58 mmol) and NaOH (180 mg, 4.5 mmol) in

1.4 ml of water, kept at 50° under magnetic stirring.

Phase separation was observed and this emulsion was left at 70° for 12 hours. The slurry mixture was cooled at r.t. and carefully transferred into a small glass apparatus where sublimation of the product took place. The internal pressure was slowly decreased, permitting the evaporation of the water and other volatile compounds avoiding any sudden boiling events involving the loss of the product. During this preliminary phase the temperature was kept between 30°-40°C. When a solid residue was observed on the bottom of the glass apparatus, the pressure was fixed at 10 mm Hg and the temperature was increased at 60°C (not more). White and long needles were slowly formed on the surface of the cold finger and the complete sublimation took about 2 days. About

FIGURE 1



a) HPLC analysis of standard PBN and benzaldehyde.

b) Radio-HPLC analysis of [^{14}C] PBN (**1**).

530 mg of [^{14}C] radioactive product was recovered m.p. 74-75° (yield: 89%) with total activity: 473 KBeq.

Infrared spectrum (in paraffin) confirmed all the main absorption frequencies (2900 cm^{-1}, 1580 cm^{-1}, 1460 cm^{-1}, 1380 cm^{-1}) characteristic of **1** including the finger print region. The spectrum was compared to the one of commercial PBN.

A more convincing confirmation of the structure came from NMR analysis of **1** carried out in deuterated acetone; δ(ppm): 1.61 (s, 9H, $C(CH_3)_3$), 7.45 (m, 3H, m and p aromatic protons), 7.85 (s, 1H, CH=N), 8.42 (m, 2H, ortho aromatic protons).

Radio-HPLC analysis (fig. 1) of the purified product showed the complete absence of the starting compound [^{14}C]-benzaldehyde and proved the chemical nature of the achieved product as [^{14}C-ring]-N-tert-butyl- α -phenylnitrone (**1**) with more of 97% of radiochemical purity. The specific activity of [^{14}C]-PBN was: 153 KBeq/mmol.

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