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A METHOD FOR SYNTHESIS OF ^{14}C-LABELLED STYRENE

Andrzej Majcherczyk, Annette Braun-Lüllemann, Aloys Hüttermann
Dep. of Technical Mycology, Institute of Forest Botany, University of Göttingen
Büsgenweg 2, 3400 Göttingen, FRG

SUMMARY

Side-chain and ring ^{14}C-labelled styrene were synthesised from labelled benzene and acetaldehyde via bromobenzene, Grignard-reagent and 1-phenylethanol. Uniformly ^{14}C-labelled styrene can be also prepared using this method. By-products were identified and the purity of products was determed by GC-MS.

Key Words: styrene-^{14}C, 1-phenylethanol-^{14}C, bromobenzene-^{14}C

INTRODUCTION

Styrene is an easily available, very common and cheap compound, produced since years on a large technical scale by a catalytic reaction of acetylene. Most styrene is used directly as a main substrate compound for different kinds of polymers. Additionally, because of its reactive double bond, styrene is a very useful compound for addition reactions and so, for the synthesis of various aromatic derivatives. Therefore, numerous procedures for a laboratory synthesis of aromatic derivatives starting from styrene can be found, but only few methods for synthesis of styrene itself (U-labelled styrene **ref. 1**, fluorinated styrenes **ref. 2**).

Recent years have seen a growing interest on a biological degradation of styrene or its polymerisation products. Studies on bioutilisation, degradation pathways and metabolism require the use of a ^{14}C-labelled styrene. Surprisingly, no labelled styrene was until now available and no small scale, laboratory methods for synthesis of styrene have been published in the last years. The necessity to start the synthesis from few available, labelled compounds, limited possible synthesis procedures. The objective of this work was to develope an easy, cheap and efficient method for the synthesis of total, ring and side-chain ^{14}C-labelled styrene.

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MATERIAL AND METHODS

All chemicals, if not stated otherwise, were obtained from Merck (Darmstadt, FRG). Bromobenzene was obtained from Aldrich (Steinheim, FRG). ^{14}C-U-labelled benzene (specific activity 4,48 GBq/mmol, 54,76 MBq/mg, radiochemical purity 99,2 %) was delivered by Amersham Buchler (Braunschweig, FRG), ^{14}C-U-labelled paraldehyde (specific activity 0,32 GBq/mmol, 37 MBq/ml, radiochemical purity 99 %) by NEN Research Products (Du Pont, Dreieich, FRG).

Styrene was synthesised according to the following reactions:

$$C_6H_6 + \tfrac{1}{2}Br_2 \xrightarrow{\text{(pyridine)}} C_6H_5Br \xrightarrow{Mg} C_6H_5MgBr + CH_3CHO \xrightarrow{(-5°C)} C_6H_5CHOHCH_3 \rightarrow \xrightarrow{KHSO_4} C_6H_5CHCH_2$$

Analysis of reaction products

Purity of reaction products and their structures were confirmed by analysis with GC-MS. Separation was achieved on a capillary column: 5 % Ph-Me-Silicone, 16 m x 0,2 mm, 0,33 µm film thickness; injection: 1 µl in spilt mode (1:50); gas: 1 ml/min He. Mass spectra were recorded in range of 45-450 m/z and compound identity confirmed by comparison with standard substances.

Synthesis of bromobenzene-^{14}C

Bromobenzene was synthesised according to the following reaction (**ref. 3**):

$$C_6H_6 + \tfrac{1}{2}Br_2 \xrightarrow{\text{(pyridine)}} C_5H_5Br$$

^{14}C-U-Labelled benzene (9,25 MBq), 25 g benzene and 0,25 g pyridine (dried over potassium hydroxide pellets) were placed in a 250 ml three-necked, round bottomed flask and equipped with a reflux condenser. A drying tube filled with KOH was connected at the top of the condenser and its outlet was fitted via PVC tubing with a HBr absorption device. The round bottomed flask was immersed in an ice bath, a thermometer and a separatory funnel containing 62,5 g bromine were placed to the other two joints of the flask.

Bromine dripped slowly into benzene under stirring in such a manner that the temperature did not exceed 30° C. After addition of all bromine, the mixture was kept for 1 h at 25-30° C in a water bath followed by heating to 65-70° C for another 45 min (or until the bromine has completely disappeared and no red bromine vapours were visible).

The reaction mixture was cooled, transferred to a separatory funnel and shaked successively with water and 10 % NaOH solution until washings remain alkali and the organic phase changed

colour from red-brown to yellow. Subsequently, excess of alkaline was removed from the organic phase by washing with water until neutral. The organic phase was separated finally from the water, transferred into a small round bottom flask and dried with $CaCl_2$ overnight. Solids were removed by filtration and the resulting crude bromobenzene (34 g) was used for further synthesis without purification. The composition of the reaction mixture and the yield of the reaction was calculated after GC-MS analysis. Purity of bromobenzene was 77 % and the yield calculated for the pure product 52 %.

Preparation of Grignard-Reagent

The reagent was prepared in a standard manner, using for 1 mol bromobenzene in 500 ml diethyl ether, 1 mol of magnesium turnings and 1 crystal of iodide as a catalyst. Ether and magnesium were placed in a three-necked, round bottomed flask (50 ml) equipped with a reflux condenser. The condenser was protected with a KOH filled drying tube and its temperature kept at 0° C with an external cooling device. A thermometer and a separatory funnel containing 34 g bromobenzene were attached to the round bottomed flask and ether mixed with magnesium by means of a magnetic stirrer. Bromobenzene was added carefully to keep the ether slowly boiling, if necessary the reaction mixture was cooled on ice. The beginning of the reaction (after about 2 min) was visible by a disappearance of the iodide colour, which later changed to an opalescent and finally a dark-brown colour. After addition of bromobenzene was completed, the mixture was boiled for about 30 min (about 40° C) until almost all the magnesium turnings disappeared. The Grignard-reagent was cooled to -10° C.

Synthesis of ring ^{14}C-labelled 1-phenylethanol

$$C_6H_5MgBr + CH_3CHO \longrightarrow C_6H_5CHOHCH_3$$

The reaction was carried out using the same apparatus as above. The separatory funnel was replaced with a new one, filled with 15 ml diethyl ether and 9,25 g acetaldehyde. Under mixing with a magnetic stirrer, the acetaldehyde solution was added very slowly (over 3 hours) to the Grignard-reagent to keep the temperature of the reaction below -5° C. At the end, the reaction mixture become milky and viscous, ice was added to the flask and products were transferred to a separatory funnel. Magnesium bromide was dissolved with an addition of 15 % sulphuric acid. The upper ether phase was separated, and the water phase washed four times with 50 ml portions of ether. The combined ether extracts were washed twice with water and evaporated on a rotatory evaporator. Confirmation of the reaction product and the purity of 1-phenylethanol were

determined by GC-MS. The yield of the synthesis, if bromobenzene from the first reaction was used, was calculated to be around 24 g and the purity of 1-phenylethanol determined to be approximately 85 %. The yield of the reaction, calculated for pure bromobenzene and 1-phenylethanol was 98 %.

Synthesis of chain ^{14}C-labelled 1-phenylethanol

The reaction was analogous to the coupling reaction above, except that ^{14}C-acetaldehyde was prepared directly by decomposition of paraldehyde. Paraldehyde (9,25 MBq) ^{14}C-labelled and 25 ml non-labelled) were placed together with 0,5 ml of concentrated sulphuric acid in a small two-necked round bottomed flask. A thermometer and a $CaCl_2$-filled drying tube were attached and the outlet of the drying tube connected by a Viton tube to a glass capillary. The end of capillary was inserted, instead of the separatory funnel from the previous apparatus, at the bottom of the round bottomed flask. Paraldehyde was heated to 37-40° C and acetaldehyde in gaseous form was led directly to the Grignard-reagent in such a manner that the reaction mixture was always below -5° C. The decomposition of paraldehyde was always kept below 40° C to prevent its simultaneous distillation with acetaldehyde (which take place at 42° C). The reaction was interrupted when about 70 % paraldehyde decomposed and continued after addition of another 25 ml of non-labelled compound. The reaction was stopped when about 80 % of paraldehyde had decomposed to prevent a possible explosion, and the products were extracted as previously described. The yield of the synthesis, calculated from the activity of product and substrate, was around 55 % (14,3 g crude product), and the purity determined to be 75 %. The yield calculated from active product was higher than calculated for total paraldehyde, due to double-step generation of acetaldehyde and addition of active compound in the first reaction step.

Synthesis of styrene from 1-phenylethanol

Styrene was obtained by a dehydration of ring or chain ^{14}C-labelled 1-phenylethanol under acidic conditions (**ref. 4**):

$$C_6H_5CHOHCH_3 \xrightarrow{KHSO_4} C_6H_5CHCH_2 + H_2O$$

A 250 ml three-necked round bottomed flask equipped with a separatory funnel and a thermometer was connected to a fractionating column. The end of the column was fitted with a double-necked distillation adapter with a thermometer and a condenser (cooled to 0° C) which was further coupled with a vacuum adapter and a three-necked round bottomed flask. The collecting flask was cooled in an ice bath and tightly closed. 1-Phenylethanol (26 g ring-labelled or

14,3 g chain-labelled) obtained from the previous reactions was added to the separatory funnel, Potassium hydrogen sulphate (6 g) and 1,2-dihydroxybenzene (0,03 g) were placed in the 250 ml flask, the apparatus evaporated to 150-170 Torr and the hydrogen sulphate heated to 190° C. 1-Phenylethanol was added slowly to keep pressure constant and the temperature permanently at 190° C. The resulting temperature at the top of the fractionating column was 110° C. After addition of the total 1-phenylethanol, the pressure was kept constant till the end of the reaction and finally reduced to 20 Torr until the distillation was complete. The yields of the reaction were determined after redestillation.

Redistillation of styrene

The distillation apparatus as above was also used for redistillation of styrene. The three-necked flask containing styrene from the previous steps was connected to the fractionating column and a glass capillary for vacuum distillation was attached. The condenser carried a two-limbed multiple receiver and vacuum adapter with small round bottomed flasks for uninterrupted collection of the fractions. The condenser was kept at 0° C with an external cooling device and the collecting flasks immersed in an ice bath. The distillation was carried out at 150-170 Torr. Styrene was collected after water, as the second fraction. The temperature was not allowed to rise over 60° C to prevent contamination with the remaining 1-phenylethanol. To obtain a better recovery, the pressure was reduced to 20 Torr at the end of the styrene distillation. The purity of styrene was determined by GC. The yield of dehydration, calculated for pure 1-phenylethanol and styrene, was approximatly 54 % (10 g crude product), the purity was 94,2 %, if 1-phenylethanol, obtained from ^{14}C-benzene of the upper reaction was used. Respectively, the yield was 40 % (4,5 g), and the purity 81,1 %, if 1-phenylethanol, obtained from ^{14}C-acetaldehyde was used.

RESULTS AND DISCUSSION

The methods used in the presented synthesis are very simple, easy to use and require only standard laboratory equipment and chemicals. The preparation can be organised in a such way to obtain differently labelled styrenes, using the same substrates and equipment. All steps of the synthesis were optimised if possible to use the same glass ware and minimise possible loses by sample transfer. Purification of intermediate products was in the presented methods not necessary and thus minimised any losses of material.

Studies on biodegradation of styrene, especially studies of metabolitsm and degradation pathways, are much simpler if different kinds of labelled styrene are available. Therefore,

the method of synthesis was adapted to meet these requirements, and results by combination of labelled and "cold" substrates in three types of ^{14}C-styrene are illustrated in Fig. 1. Because of our interest, side chain and ring labelled styrene were synthesised here, however, a synthesis of uniformly labelled styrene is possible using the same reactions.

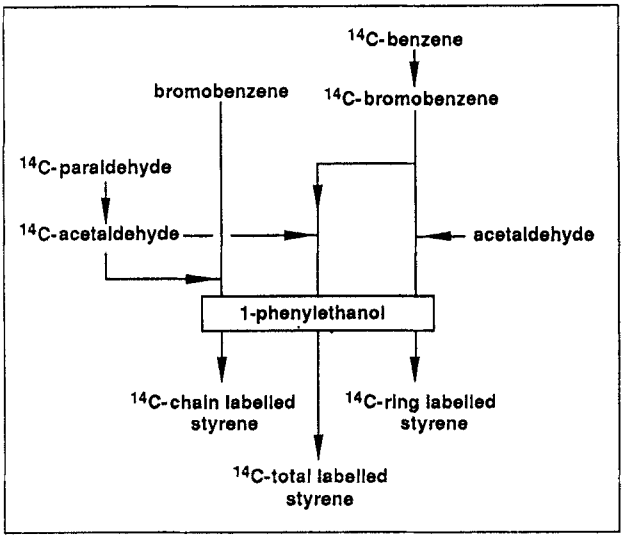

Fig. 1. Synthesis scheme of ^{14}C-labelled styrene

In our work, the yields of the reactions were higher than reported for standard procedures as the result of very carefully controlling of reaction temperature in all steps and very careful distillation of styrene. The ring labelled styrene was obtained with a purity of 94,2 %, a yield of total sythesis of 28 %, and a specific activity of 28490,412 Bq/mmol. The side chain labelled styrene was 81,1 % pure and the yield of total sythesis of 22 %. The resulting specific activity was 57784,566 Bq/mmol. The yield of the reaction was comparable to those obtained by Bachman and Lewis (2) for 4-fluorostyrene.

By-products of synthesis of ring-labelled styrene were ethylbenzene (0,8 %), bromobenzene (0,4 %), phenylethanol (2,7 %), dimethylbenzene (0,9 %) and biphenyl (1,0 %).

The synthesis of side-chain-labelled styrene lead to product contaminated with benzene (3,0 %), chlorobenzene (1,0 %), ethylbenzene (0,9 %), dimethylbenzene (0,9 %), chloromethylbenzene (0,7 %), phenol (5,4 %), phenylethanol (2,6 %) and biphenyl (4,4 %). Chlorobenzene, dimethylbenzene and chloromethylbenzene were contaminations of the utilised bromobenzene. Except small amounts of ethylbenzene and phenylethanol, all remaining by-products are not labelled and do not cause problems in degradation studies.

The synthesis of bromobenzene led to a mixture of benzene, mono- and dibromobenzene in a ratio of 18 : 77 : 5 % respectively. However, possible purification of monobromobenzene, important only for total synthesis (U-labelled styrene), is not economical and results in more losses than those of reaction of acetaldehyde with dibromobenzenes. A possible increase in the yield of the bromination step could be achieved by application of other drying techniques. However the preparation presented here was in our opinion a good compromise to simplify this procedure.

The next critical step in the synthesis was the dehydration of 1-phenylethanol. To obtain styrene in a high yield it was very important to keep the temperature as constant at 190° C as possible and to cool the condenser and collecting flask in order to prevent any losses of styrene under vacuum. The same precautions were necessary in the final distillation of styrene. The purity of synthesised styrene was checked by GC-MS (Fig 2).

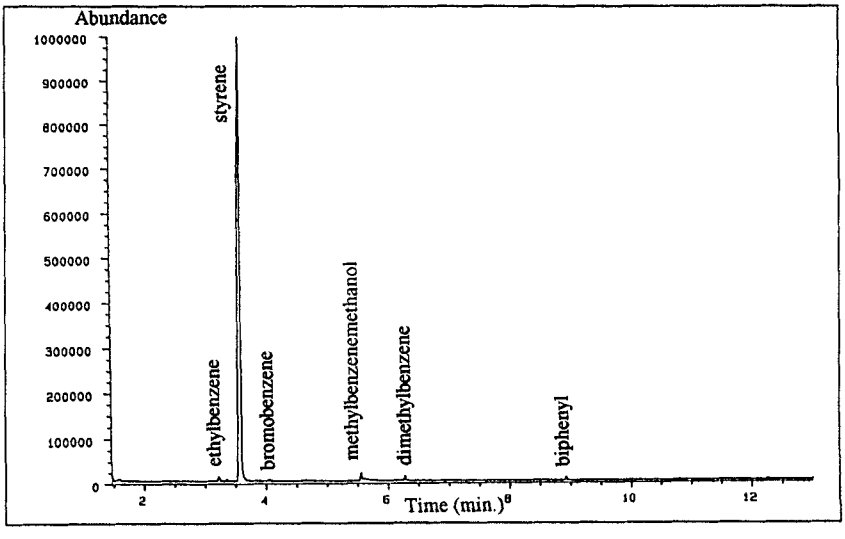

Fig. 2. Chromatogram of the synthesised ring-labelled styrene and by-products. Capillary column: 16 m x 0,2 mm, 5 % Phenylmethylsilicone - 32 μm film thickness; injection: 1 μl in spilt mode (1:50); gas: 1 ml/min He. Mass spectra were recorded in range of 45-450 m/z.

In our opinion, however, it is a good practice to start the reaction with cheap, unlabelled products and in all cases to check the quality and quantity of single steps by gas chromatography, and if possible to confirm the expected products by mass spectrometry.

The presented method allowed a preparation of styrene with a specific activity, sufficient for biological degradation experiments. It was simpler then a synthesis from acetylene-^{14}C, where total labelled styrene was obtained with 10 % yield (**ref. 1**). To produce styrene with a higher

purity grade, further destillation steps may be added. Dilution of the labelled compounds before the reactions, to obtain a labelled styrene with the required specific activity, instead of the synthesis of a high specific activity compound and subsequent dilution before use, allowed a simpler preparation. If necessary, it is of course possible to process all steps in a micro or submicro scale using undiluted compounds and special glass equipment.

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