

## THE PREPARATION OF [ $^{14}\text{C}$ ] AND [ $^3\text{H}$ ] LABELLED BENZENE OXIDE

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### SUMMARY

Benzene oxide -[U- $^{14}\text{C}$ ] was prepared from benzene -[U- $^{14}\text{C}$ ] by modifications of methods described for the inactive compound.

Benzene oxide-[3,6- $^3\text{H}$ ] was prepared by decomposition of 3,6-bis-trimethylsilyl-1,4-cyclohexadiene with tritiated water, bromination of the 1,4-cyclohexadiene-[3,6- $^3\text{H}$ ] so obtained, epoxidation and dehydrobromination.

With the latter method benzene oxide-[3,6- $^3\text{H}$ ] can be prepared at a much lower cost and higher specific activity than benzene oxide-[U- $^{14}\text{C}$ ].

**Key Words:** Benzene oxide, Tritium, Carbon-14

### INTRODUCTION

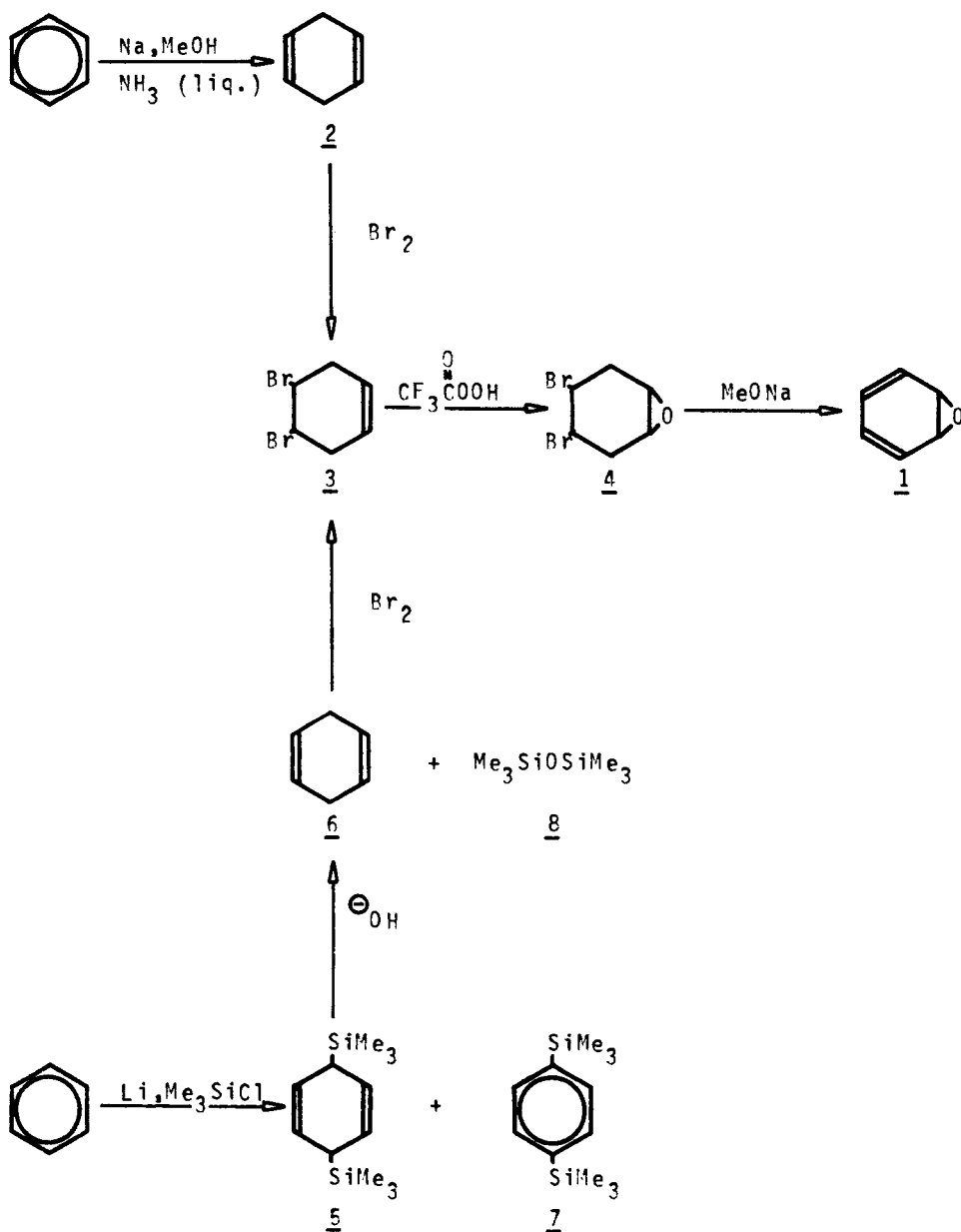
The microsomal enzyme epoxide hydratase is responsible for the hydration of epoxides produced from aromatic and olefinic compounds by the monooxygenases (1,2). Such epoxides are thought to be the reactive metabolites of polycyclic hydrocarbons (2).

Some evidence suggested that microsomal membranes contain at least two epoxide hydratases, one with a very broad substrate specificity and one more specific for benzene oxide (3).

Clarification of this point required a sensitive and rapid enzyme assay.

The development of a radiometric assay in our laboratory using radiolabelled benzene oxide as substrate fulfilled these requirements (4).

The routes used for the synthesis of [ $^{14}\text{C}$ ] and [ $^3\text{H}$ ] labelled benzene oxide (**1**) are shown in Scheme 1.

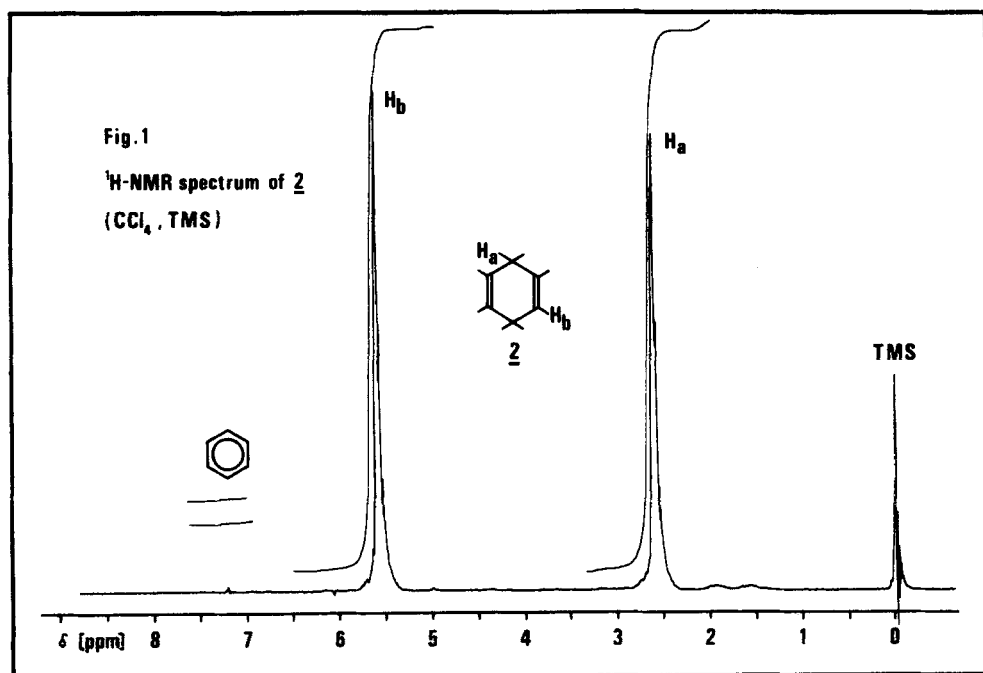


Scheme 1

## RESULTS AND DISCUSSION

Benzene oxide- $[\text{U-}^{14}\text{C}]$  1 was prepared according to the method first described by VOGEL et al. (6) starting with benzene- $[\text{U-}^{14}\text{C}]$ , which was reduced according to BIRCH (7) to 1,4-cyclohexadiene- $[\text{U-}^{14}\text{C}]$  2.

Using particular experimental conditions an almost complete conversion of benzene was achieved and 2 obtained of excellent purity (Fig. 1) and in good yield.



The bromination of 2 had to be carried out under anhydrous conditions otherwise the yield of 4,5-dibromocyclohexene- $[\text{U-}^{14}\text{C}]$  3 was reduced by formation of the tetrabromide (8).

4,5-dibromocyclohexene oxide- $[\text{U-}^{14}\text{C}]$  4 was prepared according to (9) in excellent yield and very good purity without recrystallization.

The preparation of benzene oxide- $[\text{U-}^{14}\text{C}]$  followed as described (6). The reaction time of 5 min recommended in (6) always led to

a very low yield (15 - 20 %) and 4 was recovered unchanged. The reaction time was therefore increased to 30 min when a yield of 60 % benzene oxide-[U- $^{14}\text{C}$ ] was obtained.

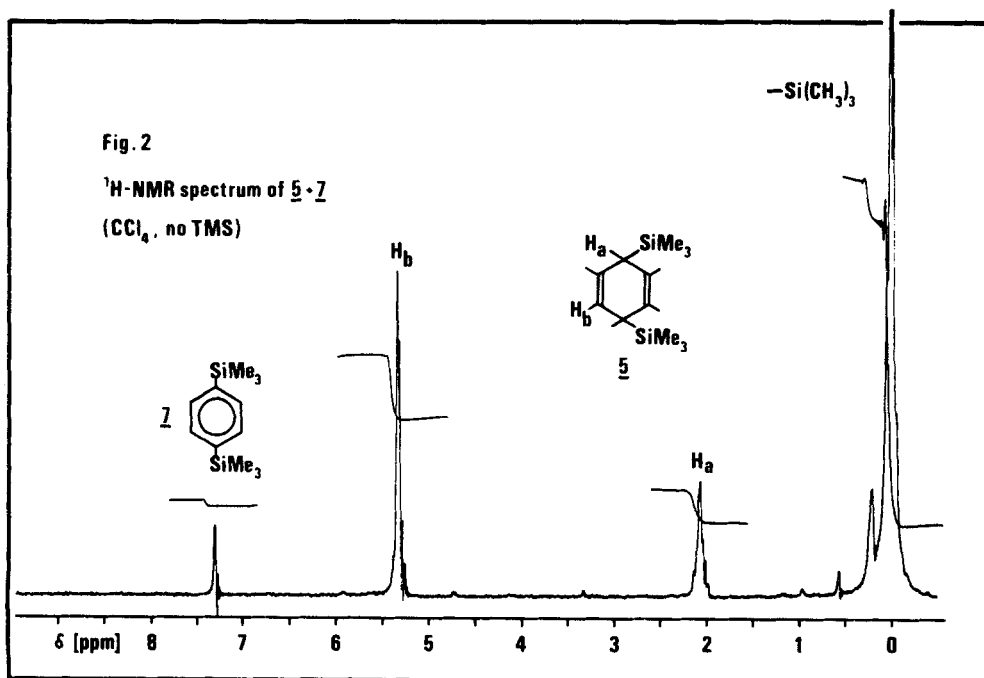
The overall yield of benzene oxide-[U- $^{14}\text{C}$ ] was 30 % and the specific activity starting with 1 mCi benzene-[U- $^{14}\text{C}$ ] was 7.4 mCi/mol.

The high cost of benzene-[U- $^{14}\text{C}$ ] and the requirement for a higher specific activity of benzene oxide forced us to find another method to prepare radioactive benzene oxide.

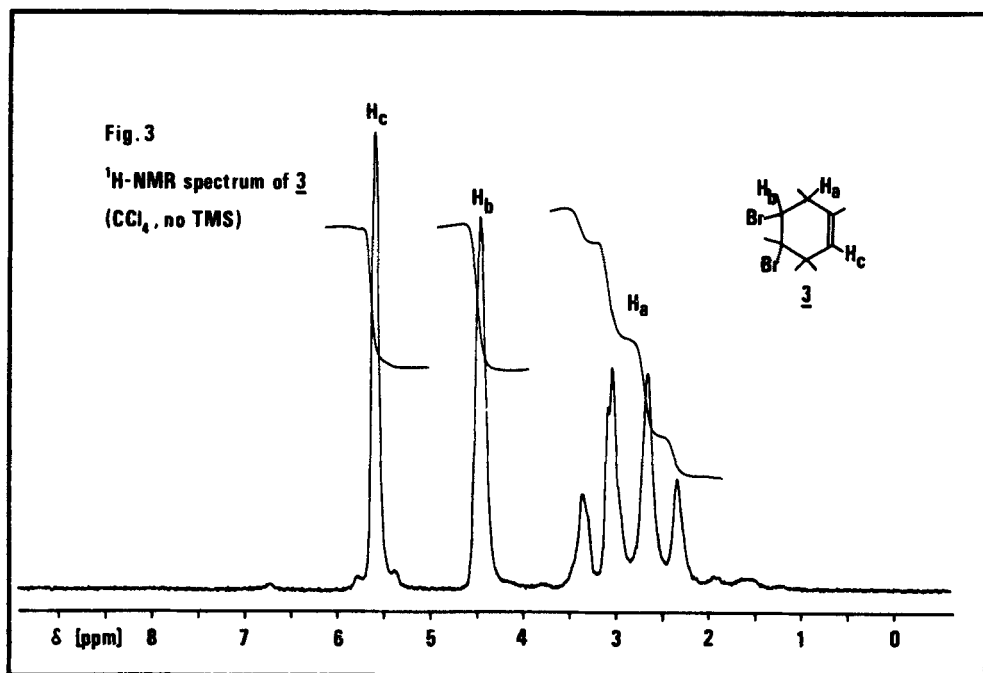
Since  $^3\text{H}$  may be introduced into a compound more easily than  $^{14}\text{C}$  we prepared [ $^3\text{H}$ ]labelled benzene oxide.

For this purpose the decomposition of 3,6-bis-trimethylsilyl-1,4-cyclohexadiene 5 (prepared according to DUNOGUES et al. (10)), with tritiated water was utilised.

The decomposition product consisted of a mixture of 1,4-cyclohexadiene-[3,6- $^3\text{H}$ ] 6, benzene-[1,4- $^3\text{H}$ ], originating from 1,4-bis-trimethylsilyl-benzene 7 formed during the silylation reaction (Fig. 2), and bis-trimethylsilylether 8.



Since the three compounds have similar boiling points separation of the mixture by simple distillation was not very efficient. The mixture was distilled only once, the amount of 6 determined by  $^1\text{H}$ -NMR and the mixture used for the bromination. Working up led to 3 without any trace of the bis-trimethylsilylether 8 (Fig. 3). The epoxidation and the dehydrobromination were conducted as described above.



The dehydrobromination of 4,5-dibromocyclohexene oxide- $[3,6\text{-}^3\text{H}]$  to benzene oxide- $[3,6\text{-}^3\text{H}]$  was accompanied by only a slight intramolecular isotopic effect.

The overall yield of benzene oxide- $[3,6\text{-}^3\text{H}]$  was 22 % and the specific activity starting with 25 mCi of tritiated water was 18.0 mCi/mol.

Although both the yield of benzene oxide- $[3,6\text{-}^3\text{H}]$  and the radioactive conversion are lower than in the case of benzene oxide- $[\text{U-}^{14}\text{C}]$  the synthesis of benzene oxide- $[3,6\text{-}^3\text{H}]$  as described offers several advantages:

1. It is easier to prepare 6 through decomposition of 5 than by the BIRCH reduction of benzene.
2. Tritiated water is cheap and readily available in high specific activities. Thus benzene oxide-[3,6-<sup>3</sup>H] can be prepared with a specific activity more than 100 times higher than in the case of benzene oxide-[U-<sup>14</sup>C] at the same cost.
3. The described silylation reaction (10) is not only applicable to benzene and alkyl benzenes but also to polycyclic aromatic hydrocarbons (11), therefore the decomposition of those silylated compounds with tritiated water can be used as a source of ditritio derivatives of polycyclic hydrocarbons.

#### EXPERIMENTAL

The <sup>1</sup>H-NMR spectra were recorded on a VARIAN EM 360 Spectrometer. Melting and boiling points are uncorrected.

##### 1,4-Cyclohexadiene-[U-<sup>14</sup>C] 2

A mixture of 10.15 g dry benzene containing 1 mCi of benzene-[U-<sup>14</sup>C] (The Radiochemical Centre, Amersham, England) and 12.43 g dry methanol was dropped with vigorous stirring in the course of 70 min at -65° C to the deep blue solution of 7.48 g sodium in 200 ml of anhydrous liquid ammonia. Stirring was continued for 16 h when discoloration to a gray suspension had occurred. The ammonia was then allowed to evaporate over a period of 12 h. The residue was decomposed at 0-5° C with 50 g ice. The organic phase was washed with 10 ml ice-water, 10 ml ice-cold 2 wt. % sulfuric acid, 10 ml ice-water again, and finally dried over magnesium sulfate. Distillation over sodium yielded 6.77 g (65 %) 1,4-cyclohexadiene-[U-<sup>14</sup>C], b.p. 89.0° C, with specific activity 7.6 mCi/mol. <sup>1</sup>H-NMR (Fig. 1) showed 2 containing 0.38 wt. % benzene.

3,6-Bis-trimethylsilyl-1,4-cyclohexadiene 5 was prepared from 11.90 g dry benzene, 3.20 g finely chopped lithium, 66.40 g

chlorotrimethylsilane and 40 ml dry THF according to (10).

The raw product was recrystallized from methanol yielding 21.73 g of white platelets (m.p. 50-52° C) consisting according to  $^1\text{H}$ -NMR (Fig. 2) of a mixture of 91.3 wt. % 5 and 8.7 wt. % 7. Yield of 5 : 58 %.

#### 1,4-Cyclohexadiene- $[\text{3,6-}^3\text{H}]$ 6

To a mixture of 6.90 g potassium hydroxide and 8 ml water containing 25 mCi of  $[\text{3H}]$ water (New England Nuclear Corp., Boston, USA) 18.60 g of the mixture of 5 and 7 in 45 ml of 2-ethoxyethanol was added at 0-7° C over 90 min with vigorous stirring. The resulting suspension was then stirred for 3 h at 5° C and 14 h at 24° C.

The volatile components of the reaction mixture were isolated by distillation under reduced pressure. The fraction b.p. 30-35° C/25 mm was washed twice with 20 ml ice water, dried over magnesium sulfate and distilled over a 26 cm VIGREUX column.

The fraction b.p. 89-81° C (17.0 g) was collected. It contained 30 wt. % 1,4-cyclohexadiene according to  $^1\text{H}$ -NMR and only traces of benzene. Yield of 1,4-cyclohexadiene- $[\text{3,6-}^3\text{H}]$  was 5.10 g (84 %) and the specific activity 33.1 mCi/mol.

#### 4,5-Dibromocyclohexene- $[\text{U-}^{14}\text{C}]/[\text{3,6-}^3\text{H}]$ 3

A mixture of 8.70 g dry bromine in 25 ml of dry chloroform was added with stirring at -70° C to a mixture of 4.36 g 1,4-cyclohexadiene- $[\text{U-}^{14}\text{C}]/[\text{3,6-}^3\text{H}]$  and 25 ml of dry chloroform in the course of 30 min.

The solvent was removed by distillation and the residue fractionated.

Yield of pure 4,5-dibromocyclohexene- $[\text{U-}^{14}\text{C}]/[\text{3,6-}^3\text{H}]$  was 10.18 g (78 %), b.p. 111° C/19 mm, m.p. 36-37° C.

4,5-Dibromocyclohexene oxide- $[\text{U-}^{14}\text{C}]/[\text{3,6-}^3\text{H}]$  4 was prepared ac-

cording to (9).

Starting with 10.08 g 4,5-dibromocyclohexene-[U- $^{14}\text{C}$ ]/[3,6- $^3\text{H}$ ] 10.43 g (97 %) 4,5-dibromocyclohexene oxide-[U- $^{14}\text{C}$ ]/[3,6- $^3\text{H}$ ] was obtained. This was pure enough (m.p. 67-68 $^{\circ}$  C, lit. (9) 65-68 $^{\circ}$  C) to use without recrystallization.

Benzene oxide-[U- $^{14}\text{C}$ ] 1 was prepared according to (6) with the exception that the reaction time of the dehydrobromination was 30 min.

Starting with 10.24 g 4,5-dibromocyclohexene oxide-[U- $^{14}\text{C}$ ] of specific activity 7.5 mCi/mol 2.26 g (60 %) of benzene oxide-[U- $^{14}\text{C}$ ] was obtained as an orange liquid, b.p. 28 $^{\circ}$  C/15 mm (lit. (6) 27 $^{\circ}$  C/14 mm), with a specific activity of 7.4 mCi/mol.

Benzene oxide-[3,6- $^3\text{H}$ ] 1 was prepared as described above. The specific activity of 4,5-dibromocyclohexene oxide-[3,6- $^3\text{H}$ ] was 32.7 mCi/mol and dropped to 18.0 mCi/mol in the case of benzene oxide-[3,6- $^3\text{H}$ ].

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