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Radiolysis of binary mixtures. Part VIII *
Tritium Labelling by Direct Radiation Synthesis. The methanol-benzene system

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SUMMARY

A novel synthesis of tritiated compounds is reported using the direct irradiation of an aromatic-aliphatic mixture, one of the components of which is labelled with tritium. The irradiation of the methanol-tritiated benzene system has been used to illustrate the principle of the technique. The gamma irradiation of methanolic solutions of tritiated benzene yields the following tritiated scavenging products, some of which are specifically labelled under certain conditions : anisole; 1,3 — *and* 1,4-*cyclohexadienes;* 2,5-*phenylcyclohexadiene;* 1,3- *and* 1,4-*cyclohexadiene-methanols. The potential of the method as a general tritium labelling tool is discussed and compared with conventional procedures. Although the absolute yields of the labelled products from direct irradiation are small for relatively large total radiation doses (megarads), scavenging by non-labelled parent material can be used to give large chemical yields if required. The predominant advantage of the present method is that compounds labelled in specific positions with tritium can be obtained in a one-step chemical process whereas this synthesis may be difficult by other conventional labelling methods.*

INTRODUCTION

Two general one-step tritium labelling methods are currently in use, firstly, the Wilzbach gas irradiation procedure (1) which involves exposure of an organic compound to tritium gas and, secondly, heterogenous exchange with tritium oxide catalysed by the active group VIII transition metals (2).

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The latter method possesses significant advantages when compared with the Wilzbach technique (3, 4) and has consequently been used more extensively in general labelling work.

In some instances, isotope specificity in labelling is required and since the above two general methods do not usually produce specific isotope orientation, tedious chemical synthetic methods are the only alternative. There is thus a need for direct one-step methods which yield molecules tritiated in given positions.

It is the purpose of the present paper to describe a novel tritium labelling technique using radiation synthesis, which may produce useful specificity under certain conditions.

PRINCIPLE OF RADIATION SYNTHESIS

If two compounds (A) and (B) are exposed to ionizing radiation, a number of cross-products ($A\text{-}B_{\text{Prod}}$) are formed from the fragments of the parent compounds. If either A or B is labelled with tritium prior to irradiation, then the cross-products from the radiolysis will contain tritium in specific parts of the molecule. An ideal system for the present labelling method is one where A is an aliphatic molecule and B is aromatic. Ideally, benzene is a suitable aromatic since this compound can be pre-labelled with tritium to high specific activities by catalytic methods (2). Aliphatic molecules are suitable second components since these fragment readily during radiolysis. The fragments thus formed are then scavenged by the more radiation-stable benzene. In radiation chemistry, this process is termed the "protection effect" of aromatic compounds on the radiolytic degradation of aliphatics. Mechanisms proposed (5) to account for the protection effect (and therefore mechanisms applicable to the present radiation synthesis technique) may be broadly classified into two groups : (i) a physical interaction which includes charge or energy transfer and electron capture and (ii) a chemical process which is essentially free radical scavenging by the aromatic additive.

The possibility of radiation synthesis as a labelling tool arose during recent investigations (5-9) of the relative contribution of the above two mechanisms to the protection effect observed during the radiolysis of methanol-benzene. Firstly, a number of scavenging products including 1,4-cyclohexadiene-methanol; 1,3- and 1,4-cyclohexadienes; 2,5-phenylcyclohexadiene and anisole have been identified and separated by gas chromatography. Secondly, a study of isotope effects (9) in this system using benzene-d_6 involved the isolation of the above scavenging products, each of which was deuterated, in some cases, selectively.

Although the yields of the scavenging products from the direct irradiation are small in terms of a macro effect, typical G* values ranging from

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* G value = number of molecules formed per 100 electron volts of energy absorbed per gram of solution.

0.05 to 0.30, the technique described above constitutes an attractive method for specifically labelling molecules if tritium is used instead of deuterium. Being radio-active, tritium has a much greater sensitivity in detection than deuterium. Isotope dilution can thus be used to obtain high chemical yields of tritiated compounds.

Although the present work constitutes the first reported general method for the radiation synthesis of tritium labelled molecules, Wolf and co-workers (10) have shown how radiation synthesis can be used to prepare propanol-2,3-C^{14} from recoil C^{14} interactions involving ammonia-methanol solutions. In addition these workers (10) have radiolyzed ethylene-C^{14} methanol solutions in which selective radiation synthesis is brought about by ethylene scavenging of $\cdot CH_2OH$ radicals. To illustrate the present technique, deuterium will be used as a tracer for tritium, since low voltage mass spectrometry (11) and gas chromatography can be utilized to analyse for isotope on small quantities of material without extensive chemical degradation experiments which would be needed for tritum. The deuterium results then serve to establish the method as a preparative labelling tool for tritium. The system discussed will be cobalt-60 gamma irradiated methanol-benzene.

EXPERIMENTAL

Reagents used were commercially available but were rigidly purified, as shown below, in order to give maximum yields of scavenging products. Previous studies (6, 8) have shown that trace impurities in starting materials can markedly alter the course of the radiolysis and lead to a reduction of scavenging product yield.

Methanol

Methanol was purified using a modification of the technique of Baxendale and Mellows (6) as follows. B. D. H. methanol was distilled once from a 2 m fractionating column and the middle 80 % dried over sodium metal and redistilled under anhydrous conditions. The middle 80 % was refluxed with 2,4-dinitrophenylhydrazine-sylphuric acid, using the above column, methanol being withdrawn from the still head as required.

Benzene-d_6

Benzene-d_6 was prepared by the catalytic exchange methods previously described (2, 12, 13). Benzene (10 ml), heavy water (99.5 %, 20 ml) and Adams catalyst ($PtO_2.2H_2O$, 0.1 gm) were placed in a glass ampoule, frozen in a dry ice-acetone bath, degassed, sealed and heated to 120° C for 72 hours. The ampoule was then opened, and the aqueous layer removed and fresh D_2O (20 ml) added and the procedure repeated. Four successive exchanges were required to yield benzene-d_6 containing 98 atom percent deuterium. The benzene-d_6 was then dried with sodium sulphate, distilled, further dried with sodium and finally distilled from sodium.

Irradiations

Irradiations were carried out in a 2,000 curie cobalt-60 source to total doses of 6×10^{20} ev/ml at dose rates of approximately 3×10^{17} ev/ml/min. Fricke dosimeters were included with each irradiation. Samples for irradiation containing benzene or benzene-d_6 in methanol were made up volumetrically in pyrex irradiation vessels, outgassed by three successive freeze-thaw cycles and sealed off at $<10^{-3}$ mm Hg.

Analysis of Products

Anisole, cyclohexadiene-methanol and phenyl-cyclohexadiene were determined by gas chromatography (Perkin Elmer 800) using a 12 ft. 1.5 % silicone gum SE.30 on chromosorb column operated at various temperatures. The cyclohexadiene isomers were determined on the same instrument using a 5 % β, β′ oxydipropionitrile column operated at 60° C. Deuterium analysis on starting materials and products (where applicable) were performed under ow voltage conditions on a Metropolitan Vickers MS.2G instrument.

RESULTS

The effect of benzene concentration on the yields of anisole, cyclohexadiene-methanol and phenylcyclohexadiene are shown in Figure 1. It is observed that the first two compounds reach maximum G values at approximately 20 % by volume of benzene in methanol, whereas the last product reaches a maximum at 60-70 % benzene. In Fig. 2, it is clear that 1,4-cyclohexadiene reaches a maximum at approximately 40 % benzene although a plateau is observed over the range 25 % to 60 % by volume of benzene. However the 1,3-isomer of cyclohexadiene clearly has a different benzene concentration dependence from the 1,4-isomer (Fig. 2) a maximum being reached at approximately 60 % by volume of benzene. Fig. 3 shows the effect of benzene concentration on the ratio G(1,4-cyclohexadiene) / G(1,3-cyclohexadiene) and clearly illustrates that at low initial benzene concentrations the yield of the 1,4-isomer greatly predominates.

Replacement of benzene by fully deuterated benzene results in the observation of reverse isotope effects (9) (Table) for 1,4-cyclohexadiene, cyclohexadiene-methanol and phenylcyclohexadiene if the benzene mole fraction is less than 0.8. A constant positive isotope effect however is observed for anisole. The results thus show that the yields of cyclohexadiene, cyclohexadiene-methanol and phenylcyclohexadiene are greater in benzene-d_6 solutions than in benzene solutions. Thus by extrapolation to tritiated benzene the yields of scavenging products should be even more favourable. Hence for the remainder of the discussion, benzene-t_6 will be used to illustrate the label-

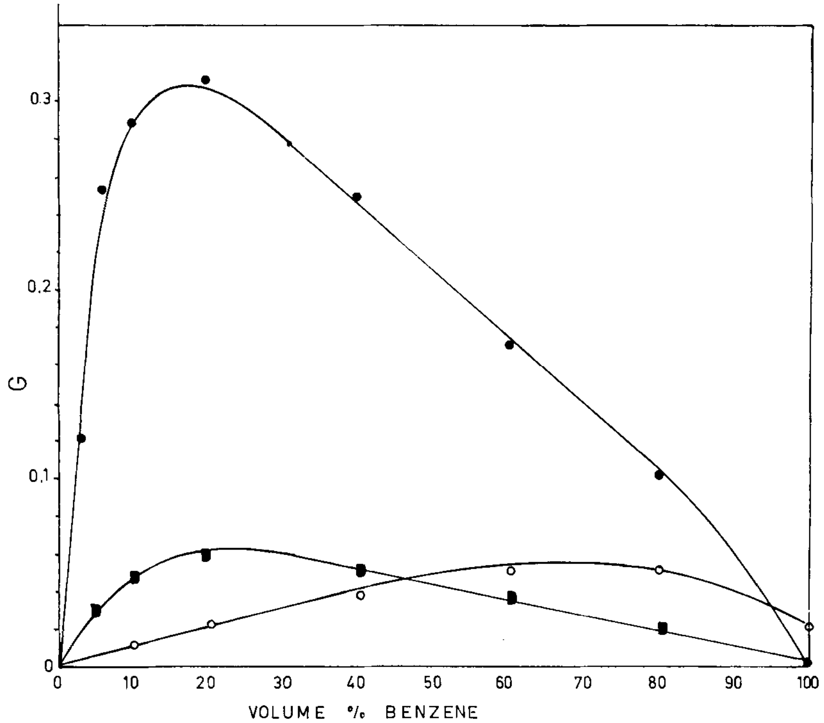

FIG. 1. Effect of benzene concentration on yields of scavenging products. Dose, 6×10^{20} ev./ml; dose-rate, 3×10^7 ev/ml/min.

— ● — ● — cyclohexadiene-methanol
— ■ — ■ — anisole
— ○ — ○ — phenylcyclohexadiene

ling properties of isotopic benzene in this reaction. Difficulties arise when the specificity of labelling is to be considered. While at lower benzene concentrations, a labelled species (reactions 1 and 2, using cyclohexadiene-methanol as a typical example) containing six tritons per molecule* will predominate, at high benzene concentrations (80 %) there is a high probability that a species containing seven tritons* per molecule will be formed according to reaction 3. The reason for the change in isotope orientation with concentration is that at low benzene concentrations, methanol will be in large excess and there is a greater probability of hydrogen atom addition in step 2 of equation 3. At high tritiated benzene concentrations, hydrogen for this step comes from the benzene and is therefore likely to be a triton.

Thus the technique gives generally labelled molecules which may contain specificity under certain irradiation conditions.

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* For illustration purposes, the theoretically possible maximum replacement of hydrogen atoms by tritons has been assumed.

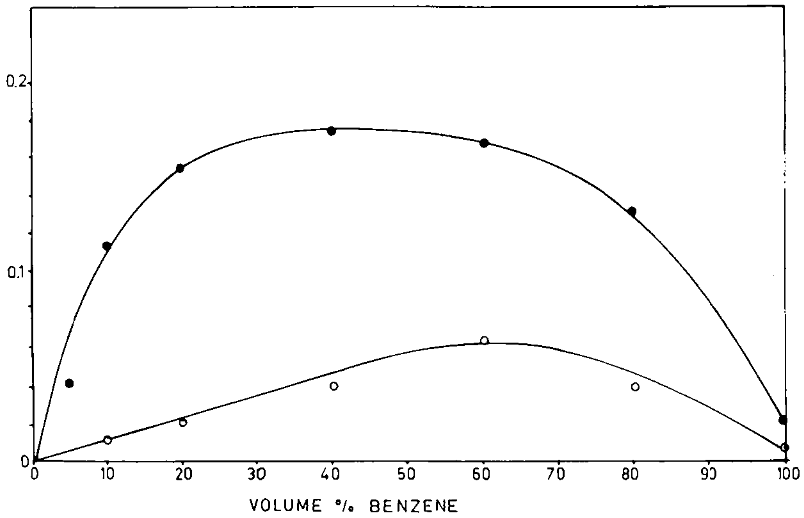


FIG. 2. Effect of benzene concentration on yields of cyclohexadienes. Dose and dose-rate as in figure 1.

— ● — ● — 1,4-cyclohexadiene
— ○ — ○ · — 1,3-cyclohexadiene

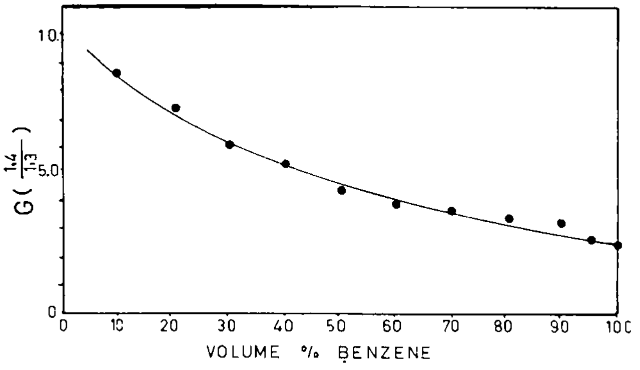
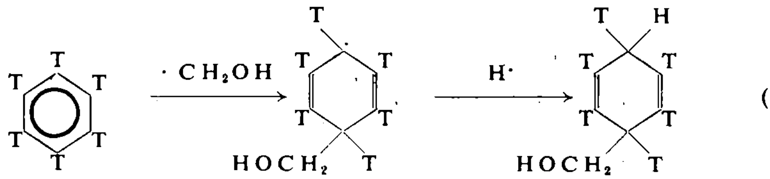


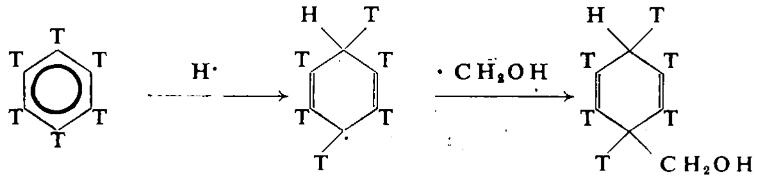
FIG. 3. Effect of benzene concentration on the ratio
G(1,4-cyclohexadiene)/G(1,3-cyclohexadiene)
Dose and dose-rate as in figure 1.

$$CH_3OH \xrightarrow{\rightsquigarrow} \cdot OCH_3 + \cdot CH_2OH + H^{\cdot} \qquad \text{(a)}$$

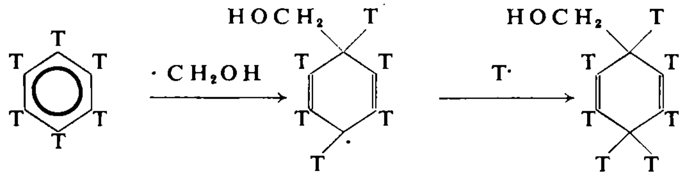


(b)

(1)



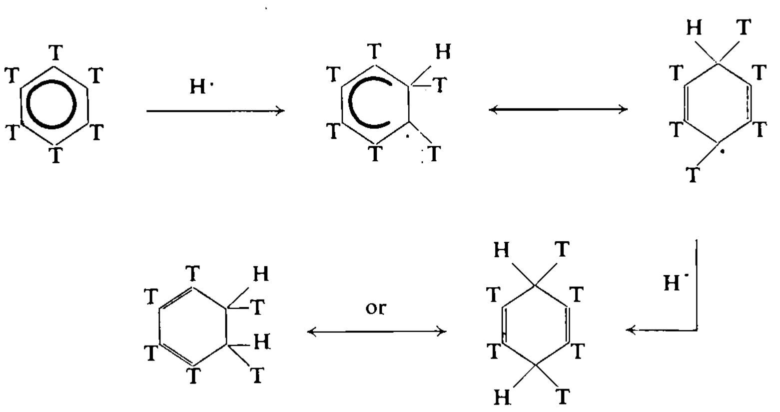
(2)



(3)

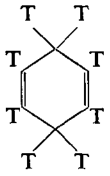
Applications of the Technique to Tritiated Cyclohexadienes and Phenylcyclohexadienes.

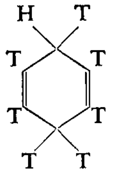
Recent data (8) indicate that the mechanism of formation of the 1,3- and 1,4-cyclohexadienes in the radiolysis of methanol-benzene solutions occurs via the formation of the cyclohexadienyl radical (equation 4).

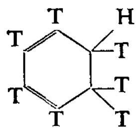


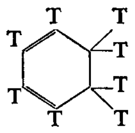
(4)

At low initial benzene concentrations the products shown in equation 4 are formed. However, at high initial benzene concentrations, significant concentrations of tritium would be present to yield species I to IV.

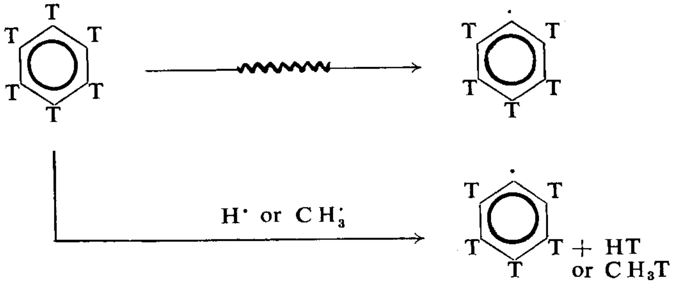

(I)


(II)


(III)

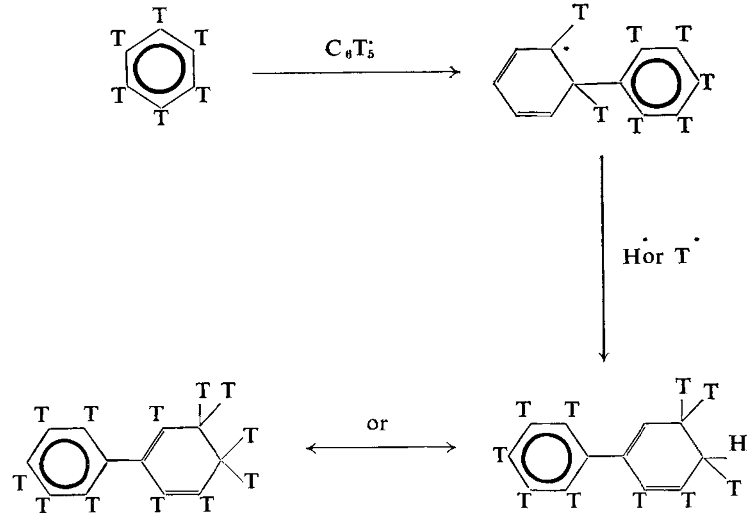

(IV)

In an analogous manner the phenylcyclohexadienes are formed through the phenylcyclohexadienyl radical (equations 5-7).



(5)

(6)



(7)

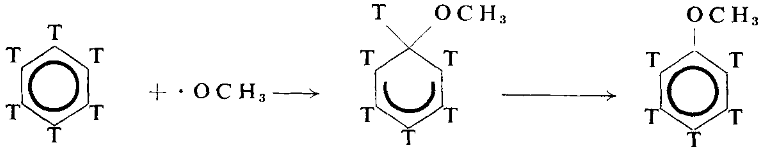
Application of the Technique to the Synthesis of Tritiated Anisole.

The results (Table), show that in contrast to the other scavenging products listed, anisole has a positive isotope effect associated with its formation over the whole benzene concentration range. This result suggests that anisole is formed by either an energetic substitution reaction (equation 8) or from the interaction of methoxy radicals with triplet benzene.

TABLE. Isotope Effects in the Radiolysis of Methanol-Benzene Solutions

Benzene (mole fraction)	Phenyl-2,5-cyclohexadiene	1,4-cyclohexadiene	Cyclohexadiene methanol	Anisole
1.00	2.25	2.30	—	—
0.90	1.16	1.10	0.70	1.50
0.81	1.02	0.95	0.69	1.40
0.65	0.94	0.90	0.70	1.23
0.41	0.91	0.90	0.75	1.31
0.23	0.90	0.95	0.70	1.50
0.10	0.89	0.90	0.82	1.43
0.05	0.87	0.85	0.80	1.60

Under these conditions anisole specifically labelled in the ring positions is obtained.



(8)

Comparison of Radiation Synthesis with other Labelling Methods.

When applicable, it is always recommended that the two generally accepted labelling methods previously discussed (i.e. Wilzbach (1) and catalytic exchange (2)) be used for tritiation. However in the Wilzbach gas exposure procedure, compounds such as the cyclohexadienes, phenylcyclohexadienes and cyclohexadiene-methanol would be expected to undergo predominant saturation in preference to substitution since this occurs with cyclohexene (84 % addition, 16 % substitution) and related compounds (14). In this situation, the main difficulty is radiochemical purification of the substituted product in the presence of a large excess of much higher specific activity addition compounds as impurities. In radiation synthesis the main scavenging products would be of the same order of specific activity and thus problems with radiochemical purification would be minimized.

In catalytic labelling with all Group VIII transition metals either by self-activation (15) or conventional exchange (2), extensive disproportionation occurs with the cyclohexenes and cyclohexadienes and in practice it is difficult to stop the reaction before this disproportionation reaction has reached completion, despite early work with deuterium oxide and cyclohexene on platinum which suggests that prior aging of the cyclohexene poisons disproportionation (16).

Even using a relatively new method recently reported involving addition-elimination exchange with TCl in the presence of tritium oxide, (17, 18), 1,3-cyclohexadiene exchanges readily after 48 hours at 130° C, but is accompanied by extensive disproportionation and polymerisation. At 110° C, where the competing reactions are minimized, exchange is very slow. With 1,4-cyclohexadiene, the TCl method even at 130° C is slow.

The remaining possible alternative is chemical synthesis by partial reduction of the parent aromatic compound. This again is difficult since labilization of isotope may occur and also a large variety of secondary products may be formed. Radiation synthesis under these conditions is an attractive alternative possibility for compounds of this chemical complexity.

With respect to anisole, Wilzbach labelling would give impurities which were highly radioactive. Catalytic exchange with tritium oxide is the most attractive in this instance, however, even on active platinum some exchange (although low) in the methyl group would be expected. Radiation synthesis should however, give specific ring labelling and is therefore an advantage with this compound.

In conclusion, it can be said that the radiation synthesis method is attractive for a wide range of aromatic-aliphatic mixtures. The present benzene-methanol system has been used for illustration. The critical feature of the feasibility of the technique would appear to involve a binary system where a relatively small number of predominant radiolysis products are formed rather than a large number of products in small yield. In terms of tritium labelling, the procedure is potentially useful for those compounds which undergo secondary reactions when tritiated either by the Wilzbach or catalytic exchange methods.

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