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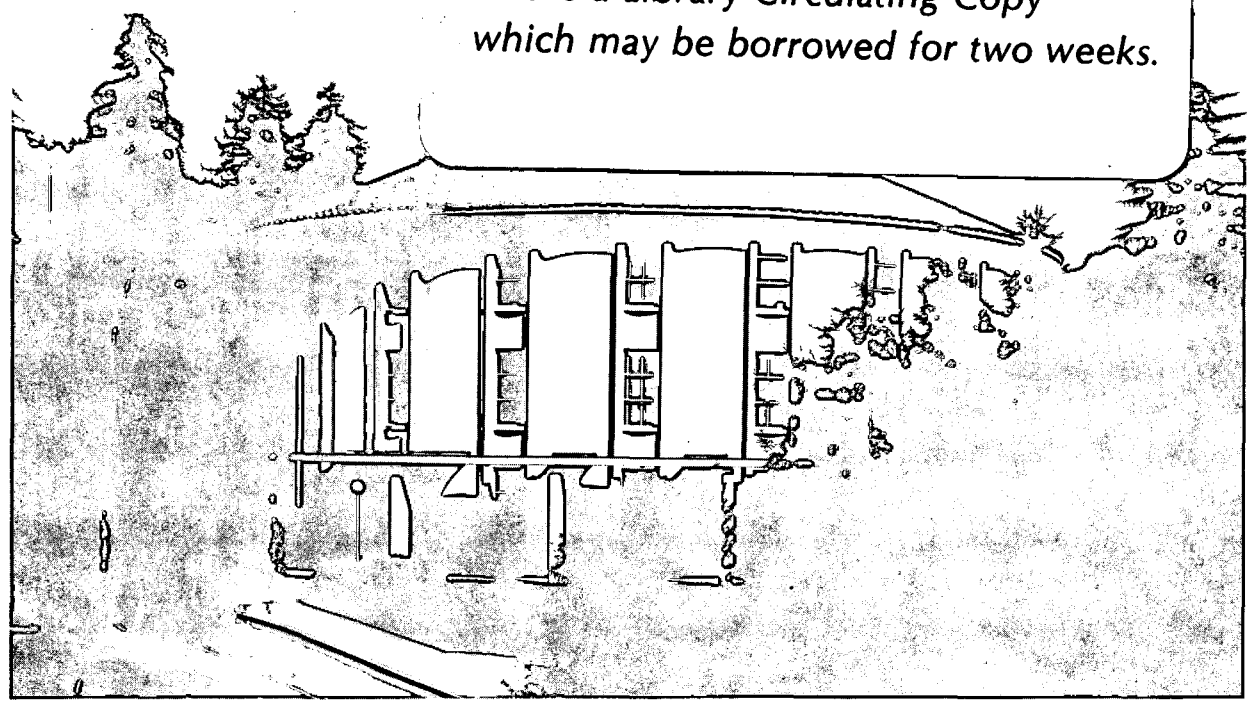
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H. Morimoto, P.G. Williams, and M. Saljoughian

June 1988

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TRITIUM LABELING BY THERMALLY GENERATED TRITONS.

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SUMMARY

The predominant effect of thermal atom irradiation on solid molecules is saturation of their aromatic functions. Only low levels of tritium exchange is observed for aliphatic solids. In contrast, liquids whose frozen surface can be rendered somewhat mobile at appropriate temperatures exhibit more exchange than addition. The rank order of effectiveness of several metals in promoting exchange/addition appears similar to the rank order for heterogeneous catalytic hydrogenation.

INTRODUCTION

Thermal tritons produced on the surface of a hot tungsten wire have been reported to label a range of molecules (refs. 1, 2). In particular, biomolecules have been labelled at high specific activity and are reported to retain full biological activity. However, few details were provided regarding their chemical and radiochemical identity, and purity.

We have selected benzene and xylene as model aromatic compounds for the thermal triton labelling studies under a variety of conditions.

METHODS

Materials

Substrates of spectroscopic purity were purchased from various suppliers and used without further purification. Metal wire of 0.25 mm diameter and > 99% purity was purchased from Alfa Products. Wire temperatures were calibrated by use of an optical pyrometer. Tritium gas was purchased from Oak Ridge National Laboratory containing 97.9% tritium.

Reaction Procedures

The reaction vessel was a 90 mL cylinder containing 2 stainless steel electrodes bridged by a 2 cm long metal filament. Pressure was monitored with a MKS pressure gauge. One millimeter of tritium gas pressure represented 308 mCi. For liquid substrates, 300 mL was placed in the bottom of the reaction vessel, frozen under liquid nitrogen, and flushed 3 times with helium gas. The sample underwent 2 cycles of freeze-thaw degas. Between cycles, the wire was heated to luminescence under high vacuum to remove any surface contamination. The cooling bath was exchanged for a bath of selected temperature, and 10 minutes was allowed for temperature equilibration. After reaction, the reaction vessel was kept at -196°C for 3 min to allow condensation of products before the sample was removed for analysis.

Gas Chromatography

A small aliquot of the sample was diluted and counted for radioactivity in a Packard 1500 TriCarb Liquid Scintillation counter. Another aliquot of 200,000 dpm was coinjected with cold standards for analysis by radio GLC, using a Varian 3700 with Carbowax 20M packed column. Temperature was controlled at 80°C for 6 minutes, then programmed to rise $10^{\circ}\text{C}/\text{min}$ to 225°C . Radioactivity was detected by a proportional flow counting tube (ref. 3) at an overall efficiency of 8%.

NMR Spectroscopy

An aliquot of the reaction products was diluted with perdeuterobenzene to a volume of 200 mL and placed in teflon tubes (Wilmad) then into a 5 mm glass NMR tube. Spectroscopy was carried out on an IBM AF 300 spectrometer (^3H at 320 MHz).

RESULTS

Gas chromatographic analysis of products from irradiation of benzene kept at -196° (FIG 1A), using a tungsten wire at 1000°C showed predominantly saturation, with 93% of the label in cyclohexane. There were small amounts of cyclohexene and higher boiling polymers, but labelled benzene was detected in only trace amounts. Tritium NMR spectroscopy of this reaction mixture (FIG 2) clearly shows tritium only in the region for saturated hydrocarbons. Assuming this labelling was a surface phenomenon, benzene was exposed to the thermal tritons for 3 one minute intervals, with each period separated by thawing of the substrate to expose a new surface to the tritons (FIG 3A). This increased the yield of

labelled benzene to 5%, cyclohexene to 50% and reduced cyclohexane to 28%. Since a frozen matrix always presents the same surface, in a subsequent experiment the benzene temperature was held at -77°C , and the radio GC analysis (FIG 3B) showed labelled benzene levels increased to greater than 50% of the total radioactivity, cyclohexane reduced to 15%, and visible levels of 1,4 cyclohexadiene and cyclohexene. This data is again supported by t NMR data.

Using the optimal conditions of 1 mm tritium pressure and 4 cm distance between the tungsten wire and substrate, tritiated benzene levels were increased to 60% (FIG 4A). Substituting platinum wire for tungsten further increased tritium exchange to 70% of the total radioactivity (FIG 4B) with only small amounts of tritiated cyclohexene formed. In contrast, using m-xylene as a substrate, platinum was ineffective in exchange labelling and tungsten wire showed good exchange (FIG 5). Tritium NMR of the reaction mixture showed equal incorporation into the 2 position, and into the 4,6 positions. Only a 5% trace of the tritium was present in the 5 position. The other aromatic resonances are probably due to the labelled o-xylene and p-xylene present as contaminants in the starting material.

A comparison of the effectiveness of various metals in promoting thermal tritium exchange into m-xylene is presented in TABLE 1. It is of note that the order is similar to that observed with metal catalyzed exchange, i.e., nickel gives exchange and platinum gives mainly saturation.

Initial interest in the use of thermal atoms as a labelling technique was stimulated by reports of its usefulness in introducing tritium into biological material with retention of biological activity. In our experiments, dipeptide solids were adsorbed onto GF/B filters and exposed to the thermal tritons produced by tungsten wire. The reaction products from phenylalanyl leucine were analyzed by t NMR (FIG 7). Resonances attributed to a saturated cyclohexyl group were observed but there was only very slight evidence for exchange into the phenyl group. The NMR spectrum (FIG 8) of reaction products from alanyl glycine irradiation also show only some resonances consistent with tritons in the methyl and alpha carbon of the alanyl residue.

In summary, the GC and t NMR spectroscopic data show that the major effect of thermal atom irradiation on solid aromatic molecules is saturation. Even under conditions where the surface of the substrate is mobile and some exchange is observed the resultant mixture contains highly labelled but saturated products.

ACKNOWLEDGEMENTS:

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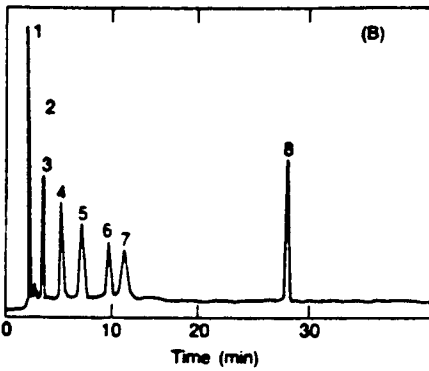
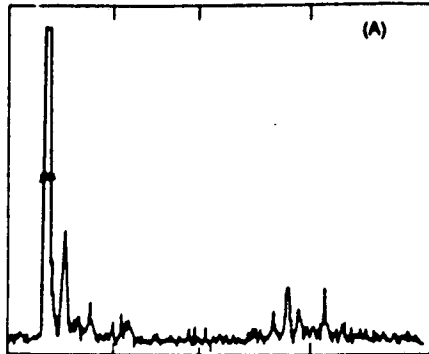
Fig. 1

Gas Chromatography
Varian 3700
Column: 30 ft
80° C for 15 minutes
10° C/min to 225° C

A Retention
Benzene
-150° C
1 mm diam.

B Mass markers

- 1 benzene
- 2 chloroform
- 3 cyclohexane
- 4 cyclopentane
- 5 1, 3 cyclohexadiene
- 6 1, 4 cyclohexadiene
- 7 benzene
- 8 bicyclohexyl

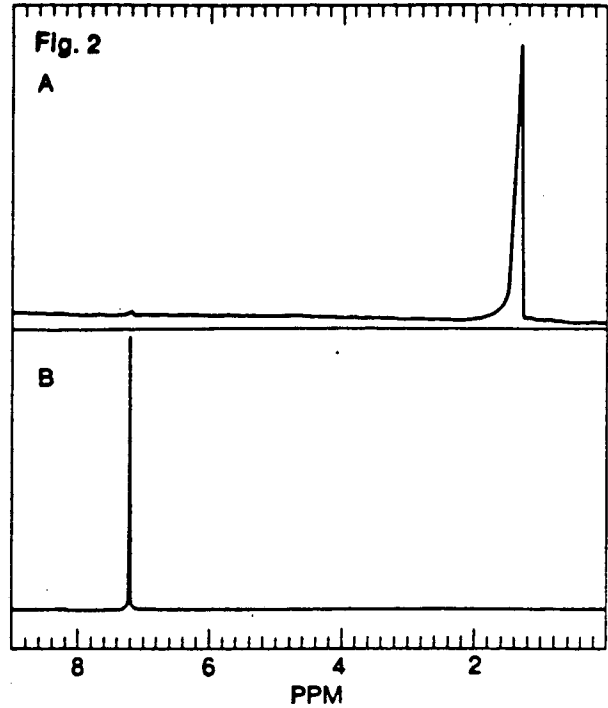


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Fig. 2

A

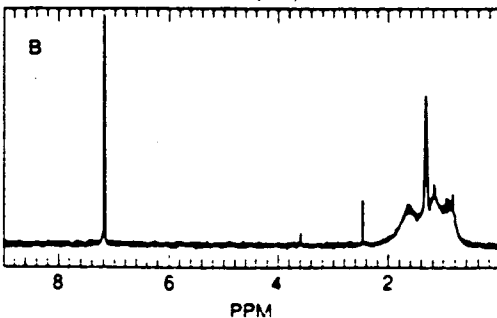
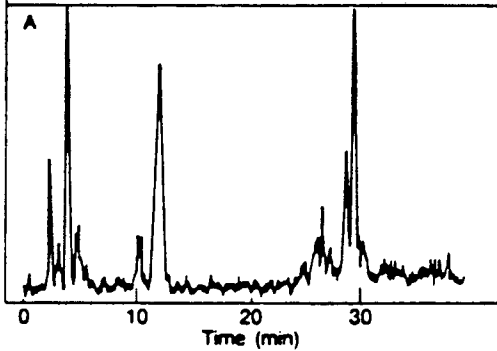
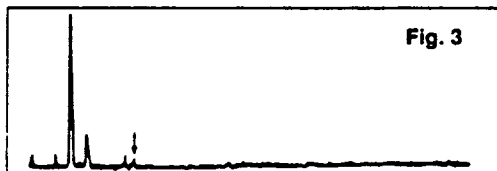
B



XBL 887-8475

Benzene
-77° C
1 mm
tritium

Fig. 3

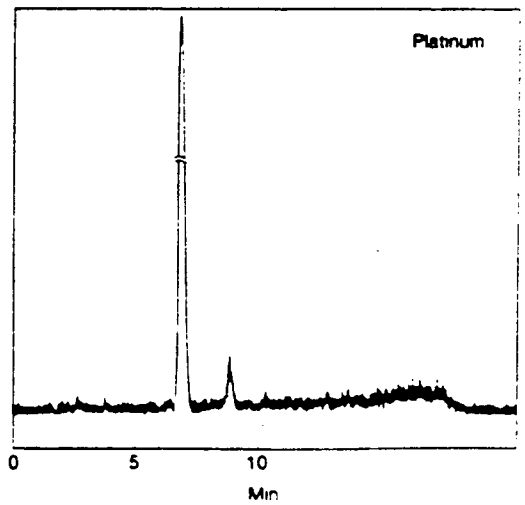
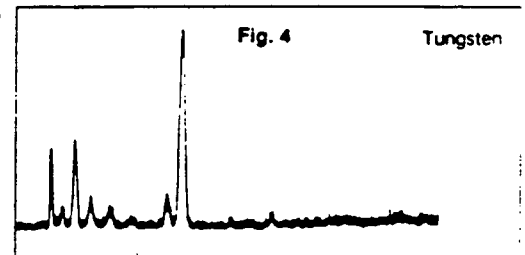


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Benzene
-77° C

Fig. 4

Tungsten



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Fig. 6

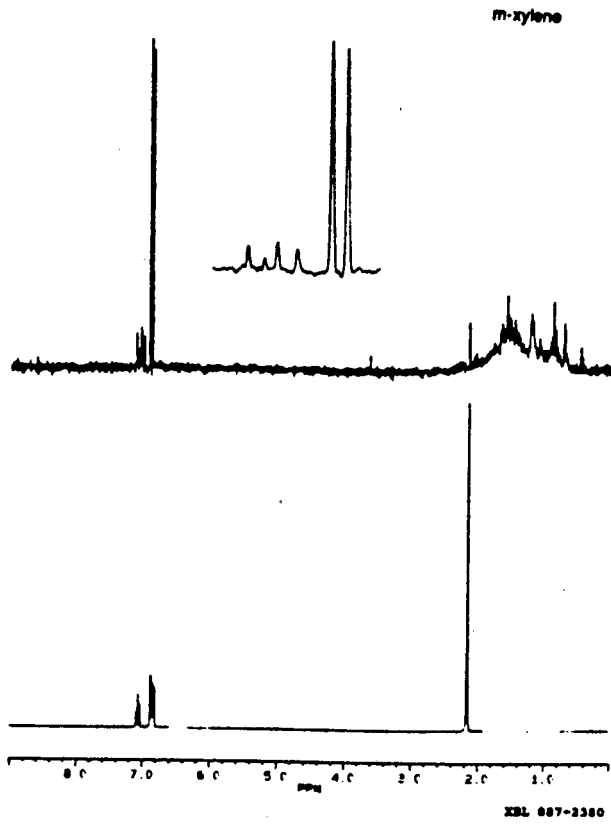
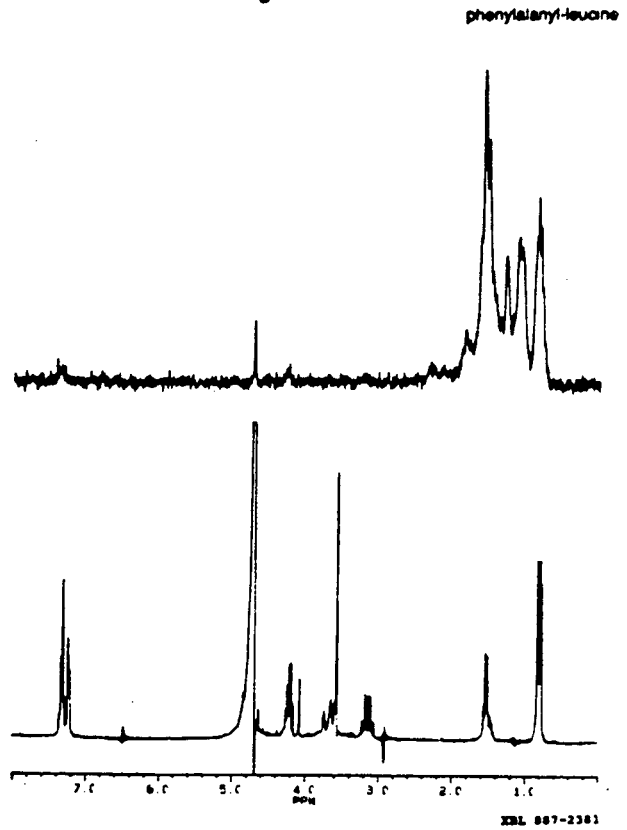
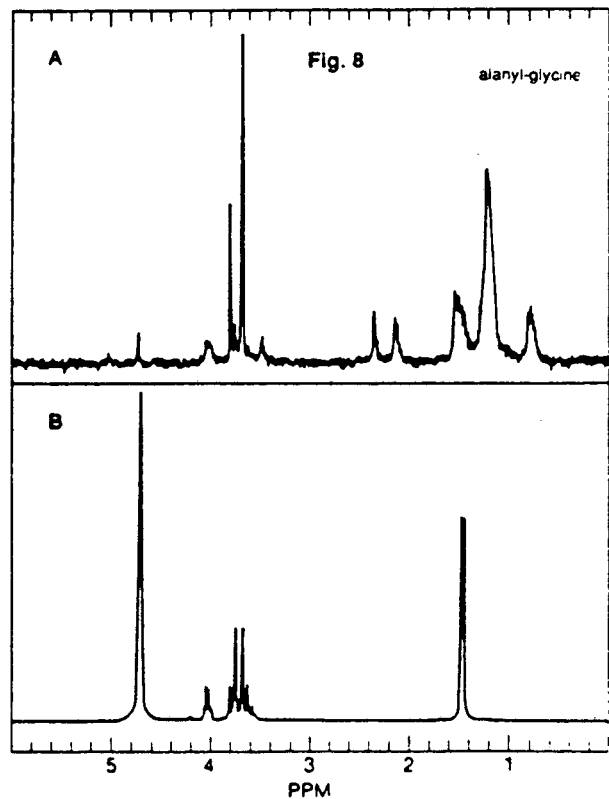


Fig. 7



TRITIATED M-XYLENE FORMED

METAL	% INCORPORATION	mCi
PALLADIUM	55	2.3
NICKEL	55	1.0
RHODIUM	45	0.1
IRIDIUM	45	2.2
TUNGSTEN	46	25.0
TITANIUM	32	5.0
PLATINUM	<5	0.5



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