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Author

Gordon, B.E.

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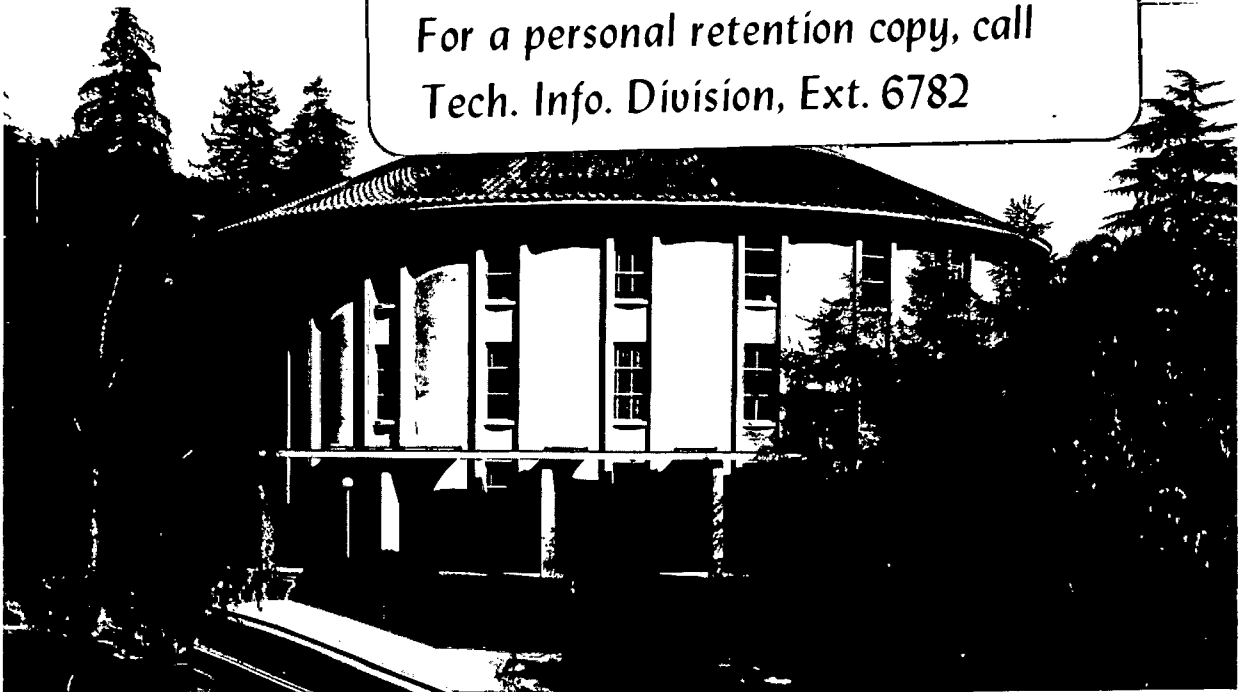
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THE GAS CHROMATOGRAPHIC SHIFT OF TRITIUM LABELED COMPOUNDS--AN APPLICATION
OF GAS LIQUID RADIOCHROMATOGRAPHY

B. E. Gordon, J. W. Otvos, W. R. Erwin, and R. M. Lemmon
Melvin Calvin Laboratory, Lawrence Berkeley Laboratory,
University of California, Berkeley, California 94720

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Division of the U.S. Department of Energy under Contract W-7405-ENG-48.

ABSTRACT. A small reduction in the gas-chromatographic retention times of a number of tritiated compounds compared to their unlabeled analogues has been observed. The shift appears to be proportional to the number of tritium atoms in a molecule.

Gas liquid radiochromatography (GLRC) has been in continuous use in this laboratory for some years in connection with carbon-14 ion beam impact studies (1,2) and tritium excitation labeling research (3,4). The equipment consists of a heated proportional flow counter (PFC) connected to the effluent of a conventional gas chromatograph. Radioactivity and mass are monitored with a two pen recorder. This system has been described in detail (5).

Because the PFC is downstream from the thermal conductivity bridge of the gas chromatograph, a radioactivity peak is displaced from the mass peak of the same compound by a distance proportional to the transit time between the two detectors. This displacement is the same for all radioactive compounds providing the carrier gas flow rate is constant. Thus, if the two pens of the strip chart recorder are adjusted with one labeled compound so that the radioactive and mass peaks coincide, they will do so for any labeled compound emerging from the chromatograph. For this purpose a toluene -¹⁴C standard is used and the upper pen (Hewlett Packard model 7100B recorder) is used as the radioactivity output while the lower pen provides the mass trace. The upper pen is at the end of a short length of 1/16 inch stiff stainless steel tubing and is bent slightly so that mass and radioactivity peaks from the standard coincide. With this slight modification the identification of emerging radioactive peaks is greatly facilitated through the use of unlabeled standards as markers. There is, however, a counting statistics limitation on the determination of the exact retention time of an emerging labeled compound. If the count rate is too low (i.e., <2000-3000 dpm) the peak is not sharply defined, leading to a problem when matching mass and radioactive peaks.

In most of our work, in particular in the excitation labeling studies, (3,4) samples have ample activity ($>10^4$ dpm).

The earlier work involving carbon -14 ion beam studies invariably showed excellent agreement between mass and radioactive peaks of various labelled compounds. When the tritium excitation labeling studies began, a persistent upscale shift in the radioactive traces of various tritiated compounds appeared. Reexamination of two labeled toluene standards (^{14}C and ^3H) also showed a very small shift for the latter. Fig. 1 shows the radiochromatogram of these two standards and the very slight shift of the radioactive peak for toluene- ^3H compared to its mass peak is evident. Replicate runs with the toluene- ^{14}C standard showed a standard deviation (1 σ) of the zero shift to be ± 2.5 seconds (~ 0.5 mm). The shift noted in Fig. 1 is real but close to the limit of detection.

Our excitation labeling studies involve the exposure of model compounds to a stream of carrier free excited tritium atoms and/or ions generated in a microwave discharge cell. A variety of reactions ensues among which may be exchange with hydrogens and addition to the parent compound. A cursory radiochromatographic examination of some of the labeled products also showed the tritium shift vis-a-vis the mass peak. On making this observation it became of interest to determine whether there was a correlation between the number of tritium atoms believed to be inserted into a molecule and the magnitude of the shift of the radioactive peak from the mass peak. An examination of the radiochromatograms of the labeled cyclohexyl analogs of benzoic acid and phenylalanine (Fig. 2a and b) showed this indeed to be the case, assuming 6 tritium atoms per molecule. The shifts were always toward earlier retention times. These preliminary findings prompted a re-examination of a number of gas liquid

radiochromatograms from earlier work. These also showed the same upscale shift but to varying degrees. The data also suggested that the magnitude of the shift depended upon the number of assumed tritium atoms per molecule. This is also evident in Fig. 3 where the reaction of benzene with the tritium plasma produced cyclohexene (4 tritium atoms) and cyclohexane (6 tritium atoms). The difference in the shift between the two compounds is small but significant. Therefore, all of the available chromatograms were carefully measured for the displacement between the radioactive and mass peaks to determine whether a correlation emerged between the magnitude of the shift and the putative tritium content of the labeled molecules. It should be mentioned that not all of the radiochromatograms were usable because sometimes either the mass or radioactivity peaks had been allowed to go offscale since, in the early phases of the work, there was no interest in the shift.

Table 1 documents the shift for those compounds for which such measurements could be made. The assumed tritium atoms/molecule is based on the use of pure T_2 to form the plasma and the assumption that during the labeling process, hydrogen atoms from the substrate are not involved in the reaction. Table 1 not only documents the shift, but suggests a proportionality between the number of tritium atoms/molecule and the degree of displacement. The near linearity of the shift (~ 3 sec/T/molecule) is probably fortuitous and considerably more work needs to be done to determine its quantitative nature. The reason why this shift is not usually observed in radiochromatograms of tritiated molecules is that, normally, carrier free labeling reagent is not used. Thus, tritium gas of one curie/mM has a ratio of T/H of about 0.02. This virtually ensures the entry of only one tritium per molecule for those that are

labeled, thus resulting in a shift very close to the noise level. Table 1 shows this minimum displacement for benzene, made from iodobenzene via a substitution reaction involving one tritium. This, incidentally, supports the suggestion of C.T. Peng (3) of the possibility of enhancing the substitution reaction via the use of iodinated analogues.

The displacements reported in Table 1 were observed with 1/4" packed columns having only modest resolution. There is the distinct possibility of markedly increasing the magnitude of the shifts by modest changes in the glc system such as using high resolution quartz capillary columns. It should be of great advantage in many labeling studies to be able to determine the number of tritium atoms/molecule. Such information combined with tritium nmr to locate the labeled positions, would make this and other labeling methods much more useful. Furthermore, substantial improvement in column resolution offers the possibility for increasing the specific activity of labeled compounds by separating, partially or completely, the earlier emerging tritiated peaks from the unlabeled molecules via trapping.

This effect of hydrogen isotopes on the retention times of compounds analyzed by gas chromatography has been reported in the literature. Thus, Wilzbach (6) noted an almost clean separation between cyclohexane and perdeuterocyclohexane, the latter emerging first. He found the same effect with tritiated cyclohexane and tritiated methylcyclohexane when compared with their unlabeled analogues. He even suggested that the magnitude of the shift could be used to determine the tritium atoms/molecule. Van Hook (7) reported shifts with deuterated compared to unlabeled ethanes and Gant (4) reported similar shifts for all the hydrogenated, deuterated and tritiated methanes. Those data were

expressed as a ratio of retention times and were all greater than one. For example, CH_4/CT_4 was 1.068. While this effect can be rationalized for hydrocarbons, where intermolecular interactions are weak and where isotope effects on volatility can be reasonably modeled, (9) it is somewhat surprising to observe it also for oxygenated compounds such as the silylated derivatives of carboxylic acids and even glucose, which was silylated by $-Si(CD_3)_3$ in all five hydroxyl positions (10). Evidently, hydrogen bonding effects are not overriding. Equally surprising, although not firmly established, is the apparent proportionality of the shift with the number of tritiums per molecule, independent of the molecular structure of the compounds analyzed.

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Table 1

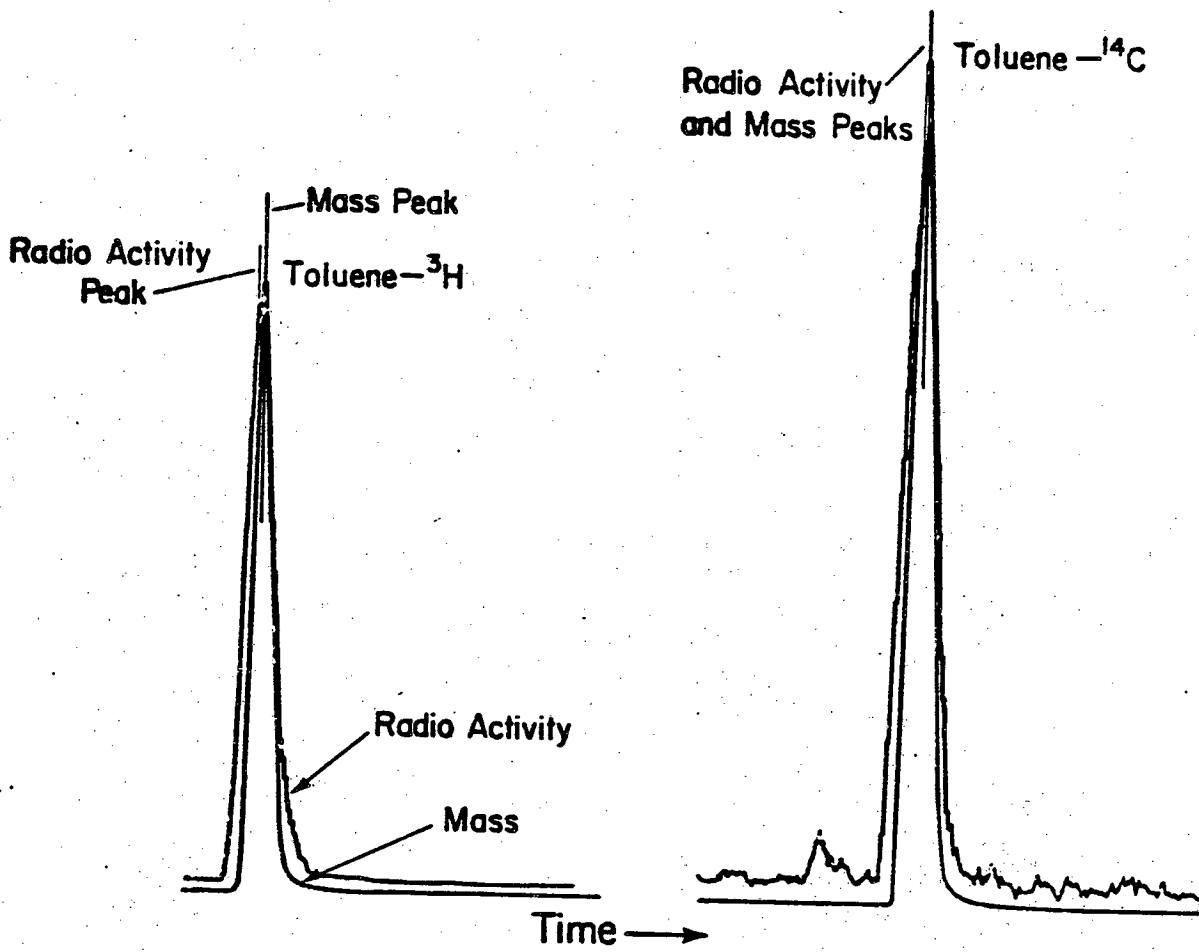
Retention Time Shift of Tritiated Molecules

<u>Parent Compound</u>	<u>Product</u>	<u>T (Putative)/Molecule</u>	<u>Shift, Seconds</u>
Benzoic Acid ¹⁾	Cyclohexane Carboxylic Acid	6	17.1
Phenyl Alanine ²⁾	Cyclohexyl Alanine	6	17.5, 20.8, 21.7
Benzene ³⁾	Cyclohexane	6	18.5, 18.5, 18.9
Benzene ⁴⁾	Cyclohexene	4	12.9
Cyclohexene ⁵⁾	Cyclohexane	2	6.5
Heptene-2	Heptane	2	10.6
Iodobenzene ⁶⁾	Benzene	1	3.2

1) Ovid-17, 178C isothermal

2) Ovid-17, 208C isothermal

3-6) Carbowax-20M programmed temp (50-250C)



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Fig. 1. GLRC's of toluene-³H and toluene-¹⁴C.

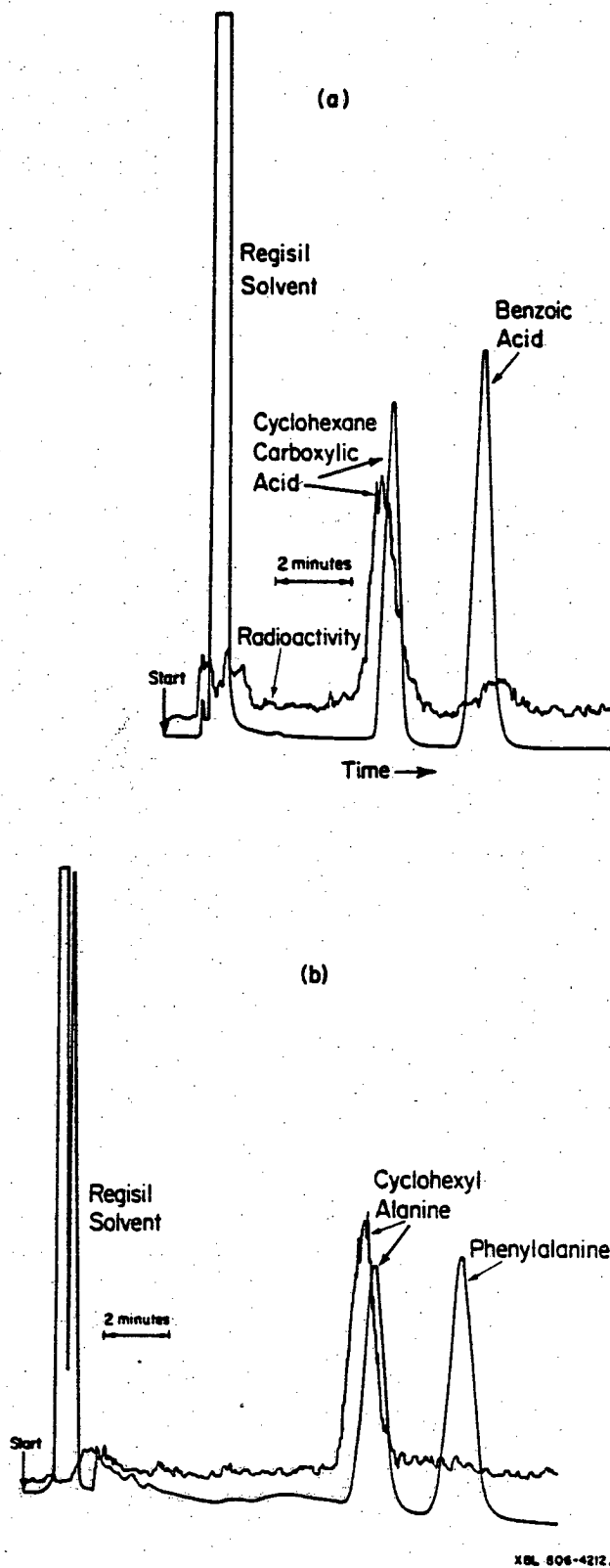


Fig. 2. GLRC's of cyclohexane carboxylic acid- $^3\text{H}_6$ (a) and cyclohexylalanine- $^3\text{H}_6$ (b).

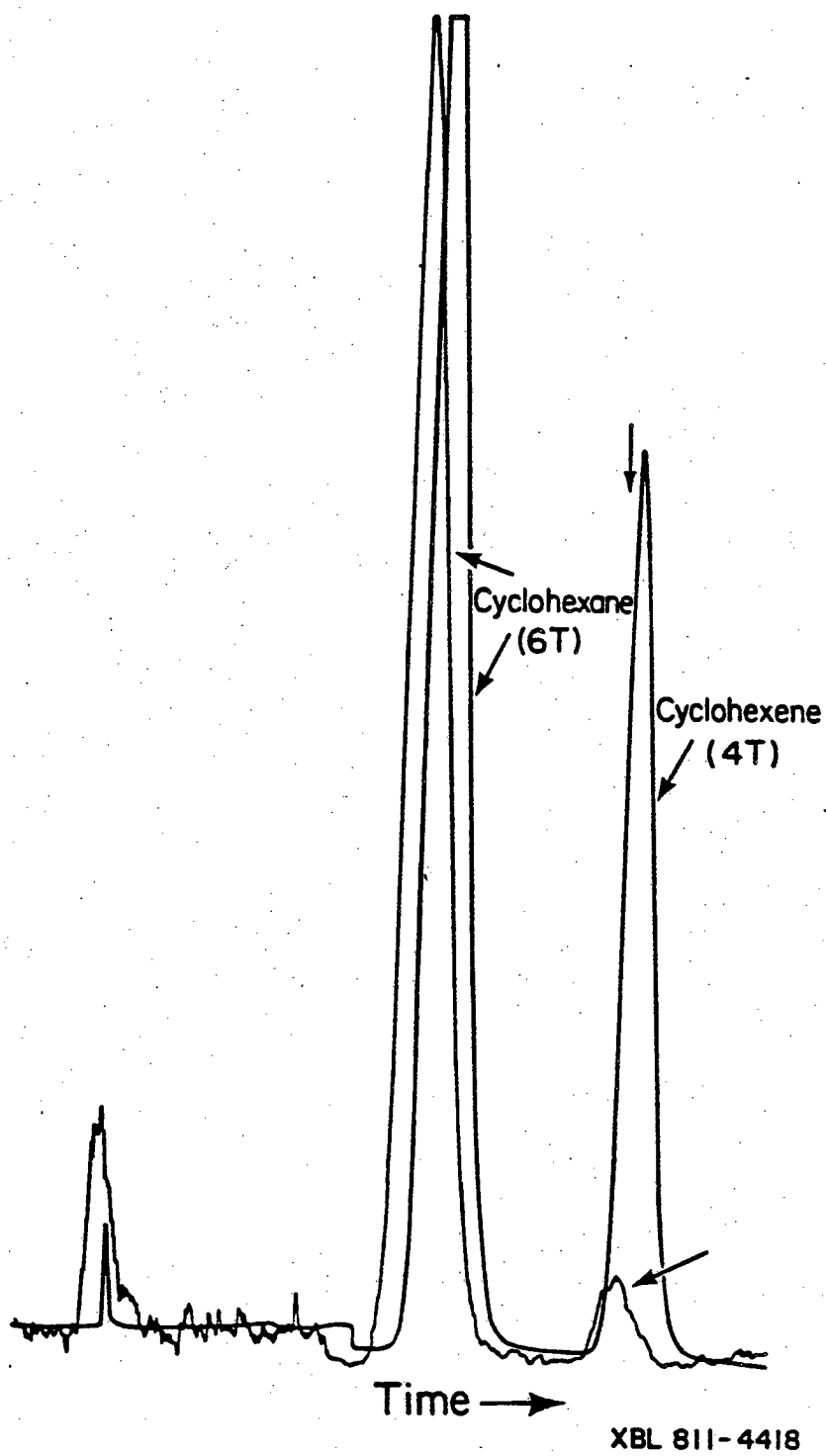


Fig. 3. Comparison of shifts of cyclohexene- $^3\text{H}_4$ and cyclohexane- $^3\text{H}_6$ (from benzene).

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