



Figure 1. Thin-layer chromatogram of Abate standards (first 6 spots viewed from left to right) and samples (last 2 spots at far right) separated from a 4-to-1 mixture of surface water and settled sewage

ylazoaniline in 95% 2-propanol. (Caution! Bromine vapor is hazardous. Use a ventilated hood.) Areas of sharp red spots with R_f values of 0.11 ± 0.01 on a bright yellow background indicated the amount of Abate. Spot areas were outlined, transferred to tracing paper, and measured by superposition on millimeter graph paper.

RESULTS AND DISCUSSION

The per cent recovery of Abate from the samples was determined by averaging the spot areas from the duplicate aliquots and comparing this area to the curve derived from the six standards on the same plate. The typical spot area for 9 μg . of Abate applied in 15 μl . of acetone was 51 sq. mm., the area for 24 μg . was 172 sq. mm., and areas for microgram values between were directly proportional. Assuming no error in areas of six spots measured for standard spot area vs. microgram curves, the deviation of points from the straight line was never greater than 0.5 μg . A typical standard curve for 9 to 24 μg . of Abate applied in 15 μl . of acetone gave a straight line with a slope of 1.2 μg . per 10 sq. mm. Best results were obtained by using freshly prepared plates and allowing sufficient time for dissipation of bromine.

Figure 1 shows a typical chromatogram of Abate. The first six increasing spot areas represent Abate standards of 9, 12, 15, 18, 21, and 24 μg . spotted in 15 μl . of acetone, and the last two spots represent

duplicate 15- μl . aliquots of an Abate residue dissolved in 50 μl . of acetone. Referring to Figure 1, comparison of the last two spot areas with those of the standards led to an average Abate recovery of 68% from a 4-to-1 mixture of surface water and settled sewage spiked with 53 μg . per liter of Abate. Examination of Figure 1 clearly shows that interfering materials in the sewage mixture are either left behind or chromatographed away from Abate.

Table I presents recoveries of Abate from distilled water, surface water, and a 4-to-1 mixture of surface water and settled sewage. It also gives volumes of acetone used to dissolve dry residues prior to spotting duplicate 15- μl . portions onto the thin layers. An average recovery of 75% with an average deviation of 6% was obtained. A freshly prepared 4-to-1 mixture of surface water and settled sewage did not have a background value. Recoveries from distilled water or surface water appear to be the same as from a 4-to-1 mixture of surface water and settled sewage. Thus, impurities in either surface water or a 4-to-1 mixture of surface water and settled sewage do not appear to affect Abate recoveries appreciably.

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Nature of Compounds Uniformly Labeled with Radiocarbon in a Benzene Ring

Mass spectrometric measurements show that a majority of the labeled molecules present in ring-UL- ^{14}C benzoic acid ring-UL- ^{14}C diphenamid contain more than one radiocarbon atom per

molecule. The possible implications of these results for experimentalists using ring-labeled compounds are discussed.

It is now common practice in residue studies to employ compounds uniformly labeled with radiocarbon in a benzene ring. All these compounds are made ultimately from the same starting material, radiocarbon-labeled benzene. Benzene- ^{14}C is prepared commercially by procedures that involve the trimerization

of symmetrical two-carbon reaction intermediates, customarily carried out at very high specific activities. Unlabeled benzene is then added to reduce the specific activity to usable levels. From these considerations, it can be predicted that both radiobenzene itself and compounds prepared from it will contain radioactive

molecules containing more than one ^{14}C atom per molecule. As a continuation of studies on the mass spectra of ^{14}C -labeled compounds (Occolowitz, 1968) this question has been explored.

RESULTS

Two compounds, ring-labeled benzoic acid and ring-labeled diphenamid (*N,N*-dimethyldiphenylacetamide), were selected for study. The studies were carried out in a CEC 21-110 double-focusing instrument employing an electron multiplier detector. After establishment of background values with cold material the radioactive compound was introduced. Three scans were made and the results were averaged. The radiobenzoic acid had a specific activity of 20.6 μCi per mmole, as determined by liquid scintillation counting. The value obtained by mass spectrometry was 23.8 μCi per mmole, showing that all of the radiocarbon is accounted for in this study. The mass spectrometric data are summarized in Table I.

In terms of molecular population the most abundant species is that containing two ^{14}C atoms per molecule (33%). Twenty-nine per cent of the labeled molecules have but one ^{14}C atom, while 23% have three. Detectable amounts of labeled molecules containing six ^{14}C atoms were present. In terms of the distribution of the label, about 60% of the radioactivity is present in molecules containing either two or three ^{14}C atoms per molecule. Since the starting material of high specific activity for radiobenzene synthesis—for example, acetylene—will contain many molecules having both carbon atoms labeled, there is a greater than random possibility that adjacent ^{14}C atoms will occur in the benzene ring.

These studies were extended to include the herbicide diphenamid-UL-ring- ^{14}C . The specific activity by liquid scintillation was 5.3 μCi per mg., while 6.0 μCi per mg. was obtained by mass spectroscopy. The data obtained are summarized in Table II. The most abundant molecular species contains two ^{14}C atoms per molecule and accounts for 57% of the radioactivity. The method of synthesis ensures that all of the ^{14}C atoms in the multi-labeled molecules reside in the same benzene ring—i.e., it was prepared from radiodiphenylacetone nitrile prepared in turn through the reaction of benzaldehyde, radiobenzene, HCN, and BF_3 (Mills, 1948).

Table I. Molecular Composition of Ring-Labeled Benzoic Acid

No. of ^{14}C Atoms/Molecule	% of Total Molecules	% of Labeled Molecules	% of Total Radioactivity
0	83.59	0	0
1	4.80	29.2	12.8
2	5.44	33.2	29.2
3	3.76	22.9	30.2
4	1.80	11.0	19.3
5	0.51	3.1	6.8
6	0.10	0.6	1.6

Table II. Molecular Composition of Ring-Labeled Diphenamid

No. of ^{14}C Atoms/Molecule	% of Total Molecules	% of Labeled Molecules	% of Total Radioactivity
0	98.74	0	0
1	0.45	35	19
2	0.65	52	57
3	0.09	7	12
4	0.07	6	12

DISCUSSION

The predominance of multilabeled molecules in uniformly ring-labeled compounds has implications for the research worker using them. In tracer work with radio-carbon-labeled compounds, isotope effects are assumed to be small and are customarily ignored (Wang and Willis, 1965). However, in dealing with multilabeled molecules this assumption may not be entirely valid. Molecular species containing as many as six ^{14}C atoms may exhibit both primary and secondary isotope effects of sufficient magnitude to alter the quantitative results of an experiment. Isotopic effects could occur in both the organic chemical reactions employed in synthesis or in the biochemical reactions encountered in the use of the ring-labeled substrate. Because of the widening use of ring-labeled material in biochemical studies, it would be worthwhile to investigate the question of possible isotope effects.

When the ^{14}C atom in a labeled molecule containing one ^{14}C atom decays, the degradation products contain no further radioactivity and can be ignored. However, the decay of a ^{14}C atom in a multilabeled molecule will lead to radioactive decay products. Since labeled compounds are used in studies designed to quantitate residues in the 1-p.p.m. to 1-p.p.b. range, this point is important. Despite the relatively long half life of radiocarbon, storage times of one year or longer should result in the formation of impurities in the 1-p.p.m. range. If these residues were selectively concentrated in biological materials, erroneously high levels of radiocarbon would be detected. Thus, ring-labeled compounds used in residue studies should be repurified if they have been stored for an appreciable length of time.

The results reported here emphasize the desirability of developing a practical synthesis of ^{14}C -labeled benzene with only one ^{14}C atom per molecule.

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