[CONTRIBUTION FROM THE RADIATION LABORATORY AND DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY]

Observations on the Radiation Decomposition of Some C^{14}-Labeled Compounds[1]

BY B. M. TOLBERT, P. T. ADAMS, EDWARD L. BENNETT, ANN M. HUGHES, MARTHA R. KIRK, R. M. LEMMON, R. M. NOLLER, R. OSTWALD AND MELVIN CALVIN

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Varying amounts of radiation decomposition during storage in the dry form have been found in the cases of C^{14}-labeled valine, norvaline, norleucine, choline chloride, calcium glycolate and cholesterol. These data indicate that investigators using labeled organic compounds should make frequent checks of the purity of these compounds in order to exclude the possibility of the presence of decomposition products.

The work described in this paper was initiated by a recent observation that considerable radiation decomposition had occurred to a sample of choline-methyl-C^{14} chloride which had been synthesized in these laboratories last spring. This observation, together with a similar one made by Dr. Sidney Weinhouse on samples of C^{14}-labeled calcium glycolate which were synthesized here four years ago, has illustrated the need for information on the rate of decomposition of C^{14}-labeled organic compounds. Since this group (Bio-Organic Group of the Radiation Laboratory) has carried out during the past six years the syntheses of several dozen labeled organic compounds, we have available a number of such compounds which could be examined for evidence of radiation decomposition. From these compounds we picked a list of twelve which had been exposed to large amounts of self-radiation since their syntheses and for which there are unequivocal data to show their high degree of purity at the time of synthesis. All of these compounds had been stored in dry, solid form in a dark cabinet at room temperature. The extent of the radiation decomposition was established by making paper chromatograms of each of the compounds and determining, by means of radioautographs of the chromatograms, if other radioactive constituents had appeared during storage. Similar paper chromatograms had been prepared at the times of synthesis and in all cases there had been no observable (or, at most, less than one per cent.) radioactive impurity. By comparing the old radioautographs with the ones recently prepared, we have measured the percentage of radioactive impurities which have appeared since the times of synthesis. In the case of choline, which had been stored in an evacuated sealed tube, we were able to determine the amount of a volatile radioactive product, namely, trimethylamine, which had formed during storage. None of the other compounds were stored in sealed tubes and any volatile decomposition products would not be detected unless they comprised a considerable fraction (at least 10%) of the radioactivity of the freshly prepared compound. The data which were obtained are summarized in Tables I and II.

The data of Tables I and II show that there is a general relationship between the amounts of radiation (R.e.p.) and the likelihood of finding some radiation decomposition; however, the choline, cholesterol and stilbamidine provide major exceptions.

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TABLE I

COMPOUNDS SHOWING MORE THAN ONE PER CENT. RADIATION DECOMPOSITION

Compound	Date prepared	Sp. act. (μc./mg.)	R.e.p.[a] × 10^{-6}	Non-vol. radioac. prods. obs. on paper chromatograms[b]	De-comp., %
Valine-4,4′-C_3^{14}·HCl	Nov. 1951	6.0	10	1	1–2
Norvaline-3-C^{14}·HCl	Jan. 1951	17.7	46	6	5
Norleucine-2-C^{14}	March 1952	17.4	20	4	2
Choline-methyl-C_1^{14} chloride	May 1952	13.0	11	0[c]	63
Calcium glycolate-1-C^{14}	Feb. 1949	5.8	30	1	13[d]
Calcium glycolate-2-C^{14}	Feb. 1949	4.0	21	1	22[d]
Cholesterol-4-C^{14}	Aug. 1951	6.5	7	0[e]	~40[e]

[a] These values were calculated: (R.e.p.) Roentgens equivalent physical = $kN\bar{E}$(R.e.p./e.v.)/wt. in g., where k = fraction of radiation absorbed (arbitrarily given the value of 1 in our calculations); N = total number of events since the time of synthesis; $\bar{E}$ = average energy of the radiation (= 50,000 e.v. for C^{14}); R.e.p./e.v. is taken as 1.9×10^{-14}. [b] These observations were made in February, 1953. [c] The main decomposition product of the methyl-labeled choline is the volatile trimethylamine. Further details are recorded in the experimental part of this paper. [d] These values were determined (private communication) by Dr. Sidney Weinhouse who found that radioactive formate was present in these percentages. The formic acid was steam distilled from the glycolic acid and its radioactivity measured as CO_2 after oxidation by mercuric ion. [e] Approximately 40% destruction (based on the amount no longer precipitable by digitonin) of the labeled cholesterol has been reported to us (private communication) by Professors W. G. Dauben and I. L. Chaikoff. These observations will be reported in detail in a separate publication.

TABLE II

COMPOUNDS SHOWING LESS THAN ONE PER CENT. RADIATION DECOMPOSITION

Compound	Date prepared	Sp. act. (μc./mg.)	R.e.p. × 10^{-6}
Glycine-2-C^{14}·HCl	Dec. 1951	4.9	6
Guanine hydrochloride-4-C^{14}	March 1950	2.6	9
8-Azaguanine-4-C^{14}	March 1950	3.8	13
Adenine sulfate-4,6-C_2^{14}	March 1950	2.9	10
8-Azaadenine-4,6-C_2^{14}	March 1950	4.4	15
Stilbamidine diisethionate-amidine-C_2^{14}	Feb. 1951	13.7	31
Thyroxine-α-C^{14}	May 1950	1.0	3
Succinic acid-2-C^{14}	Dec. 1949	3.9	14

Our data are too scanty to permit predictions as to the susceptibility of various organic groups to radiation damage—this we hope to do later with the accumulation of further information. For the present, however, it should be emphasized that users of C^{14}-labeled compounds would be well advised to check the purity of their compounds frequently,

particularly if the compound is of high specific activity and/or was prepared several years ago. For checking such purity, paper chromatography provides a very convenient tool, especially for finding non-volatile radioactive impurities. However, it may also be useful for volatile impurities if these compounds have either a basic or acidic function and can be kept in a non-volatile form on a paper chromatogram by using, respectively, an acidic or a basic solvent (for example, the chromatography of choline which is reported in this paper). There are several references in the literature[2-5] to the use of paper chromatography for determining the purity of radioactive preparations.

Experimental

The valine-4,4′-C_2^{14}, norvaline-3-C^{14}, and norleucine-2-C^{14} which were found to show radiation decomposition were all prepared *via* an acetamidomalonate synthesis using as starting materials, respectively, isopropyl-methyl-C_2^{14} iodide, propyl-1-C^{14} iodide, and butyl-1-C^{14} iodide. All of these preparations will be the subject of a forthcoming publication. The final purification of the labeled compounds was accomplished by elution from an ion-exchange column. Paper chromatography of the preparations (phenol–water in one direction, butanol–propionic acid–water in the other) showed that there was less than one per cent. of radioactive impurities present.

The preparation of the labeled choline has been reported earlier.[6] The material described in the present paper was prepared at a later date (May, 1952) using the same procedure. The later preparation included, in addition, the same paper chromatographic search for radiochemical impurities as was carried out on all the labeled compounds mentioned in this report. Again, these impurities were less than one per cent. of the preparation.

Since the radiation decomposition of the choline is so extensive (63%) we made some efforts to determine what the principal decomposition products were. Upon opening a sealed tube of the choline a pronounced odor of acetaldehyde was observed. The presence of acetaldehyde was established by the preparation directly, from the choline sample dissolved in water, of the 2,4-dinitrophenylhydrazone. This derivative, after recrystallization once from glacial acetic acid and twice from ethanol, was found to have a melting point of 146° (literature value[7] 147°). It was also found to be completely non-radioactive.

About two-thirds of the radioactivity of the original choline preparation was volatile—it was lost in the operation of making a plate for counting the choline chloride. That this volatile product is trimethylamine was demonstrated as follows: (1) Aqueous alkali was added to a freshly opened tube of the choline and the volatile products were collected in a connected trap which contained a saturated solution of picric acid in ethanol and which was cooled in a liquid nitrogen bath. The trap was then removed, closed off from the atmosphere, and warmed, with shaking, to about 60°. After the trap was cooled back to 0°, crystalline picrate appeared. The solvent was removed by filtration and the precipitate was dried. Its melting point after two recrystallizations from ethanol was 211° (literature value[8] 216°) and its activity was 1.67 mc./mmole (the activity of the original choline chloride was 1.82 mc./mmole; both values determined by direct plating). (2) When the labeled choline chloride from a freshly opened tube was placed in acid solution and an aliquot portion paper chromatographed (one-dimensionally) using a *n*-butanol–concd. HCl–water (4:1:1 by vol.) solvent, two radioactive spots were obtained. The faster moving spot (higher R_F value) contained 63% of the total activity and the slower moving spot (choline) contained 37% of the activity on the paper. The faster moving spot was cut out, eluted, and the material co-chromatographed with labeled trimethylamine hydrochloride. These materials proved to be chromatographically identical (one-dimensional paper chromatogram with the same acidic butanol solvent).

The syntheses of the labeled glycolic acids, and the criteria for their purity at the time of preparation, have been described in an earlier publication.[3] The same applies to the guanine, 8-azaguanine, adenine and 8-azaadenine,[9] to the glycine,[10] to the succinic acid[11] and to the stilbamidine.[12] The thyroxine synthesis (R. M. Lemmon, unpublished work) was carried out using glycine-2-C^{14} and following the original procedure of Harington.[13]

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