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Recoil Labeling and Selective Radiation Labeling A Novel Synthesis of Propanol-2,3-$^{14}C_1$ *

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SUMMARY

*A recoil labeling method for the production of propanol-*2,3-$^{14}C_1$ *and other simple alcohols is described. In addition propanol-*2,3-^{14}C *and* 2*-methyl propanol-*3-^{14}C *were prepared by the irradiation of methanol solutions of ethylene-*^{14}C *and propene-*1-^{14}C*, respectively, in "scavenger" concentration. This latter approach called selective-radiation labeling is explored as a method for the preparation of specifically labeled compounds by "non-synthetic" methods. The advantages of selective radiation labeling are :* 1. *specificity of label;* 2. *ready control of desired specific activity;* 3. *efficient use of the labeling isotope in an appropriate precursor;* 4. *ease of purification and isolation of products;* 5. *minimum labor. Any source of penetrating radiation can be used in selective radiation labeling.*

RECOIL LABELING.

The recoil labeling of organic compounds has been discussed in a review by Wolf (1). The mechanistic aspects of the reactions of energetic carbon atoms has also been reviewed (2, 3). One of the problems involved in labeling compounds with carbon-14 is the need for an adequate nitrogen source since the nuclear reaction, $^{14}N(n, p)^{14}C$, is used. Usually the compound to be labeled is mixed with a nitrogen source, e.g. in the labeling of benzene, a

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solution in 2-methylpyrazine is used. For polar compounds ammonia suggested itself as a good nitrogen source. The hot-atom chemistry of carbon-14 in pure ammonia from the mechanistic point of view has been discussed by Cacace and Wolf (4). Most polar organic compounds will dissolve reasonable amounts of ammonia. Another advantage to the use of ammonia is that a second organic compound (e.g. the benzene-2-methylpyrazine system) is not present to add to the spectrum of labeled organic compounds produced. Its only disadvantage is that the partial pressure of ammonia can be rather high in some systems, necessitating the irradiation of a vessel in which the internal pressure may be above atmospheric.

A mixture of methanol and ammonia was irradiated as a model for the use of ammonia. The labeling process is outlined by equations 1 and 2.

$$NH_3 + \text{neutron} \rightarrow [^{14}C]\,\#\,{}^{*} + 3H\cdot + \text{proton} \tag{1}$$

$$[^{14}C]\,\# + CH_3OH \rightarrow \text{labeled organic compounds} \tag{2}$$

The simple labeled alcohols produced in this system are listed in Table 1 with their corresponding radiochemical yields. The yields are expressed as the amount of carbon-14 in the particular compounds divided by the total carbon-14 produced during the irradiation. The alcohols, except for the parent, were isolated by using carriers. The yields of ethanol and methanol are what one might expect for these products (cf. refs. 1 and 2), but the yield of the three carbon compound, n-propanol, is exceptionally high. In systems investigated up to that date the "substrate plus two carbons" yields had never been more than 0.1 % at best. The ethanol is probably produced in a hot insertion reaction by excited methylene-$^{14}C_1$(2, 3), equations 3 and 4.

TABLE 1. Product alcohols in recoil labeling of methanol.

Carbon-14 labelled alcohol	Radiochemical yield
Methanol	1.04 %
Ethanol	5.72 %
Propanol	2.63 %
2-Propanol	0.23 %
Butanol	0.16 %
Pentanol	0.13 %

$$[^{14} : \dot{C}\cdot]\,\#^{**} + CH_3OH \rightarrow [^{14} : CH_2]^{\ddagger}\ {}^{***} + \text{molecular fragment} \tag{3}$$

$$[: {}^{14}CH_2]^{\ddagger} + CH_3OH \rightarrow [^{14}CH_3\text{-}CH_2OH]^{\ddagger} \rightarrow {}^{14}CH_3\text{-}CH_2OH \tag{4}$$

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[*] # denotes excess kinetic energy.

[**] # denotes excess kinetic energy.

[***] ‡ denotes an atom or molecular fragment which is excited.

This mechanism requires specific labeling in the two position. This hypothesis is borne out by the degradative data, Fig. 1. Activity in the one position can arise from rearrangement of the excited intermediate (equation 4) or via alternate mechanisms of formation (2).

CH_3 —	— CH_2OH
95.1 %	4.9 %

FIG. 1. Activity distribution in ethanol

The propanol degradation proved to be most interesting. Fig. 2. The fact that 97.9 % of the activity appeared in the two and three carbons strongly suggested the reaction of a recoil-produced two carbon species with the organic substrate or with a moiety produced by radiolysis of this substrate *. The further fact that the labeling in the two and three positions was approximately equal indicated that the intermediate might be symmetrical. In recoil labeling, a particular product is frequently formed by more than one mechanism; hence an observation of completely equivalent labeling would have been unlikely. The two most probable mechanisms are outlined in equations 5 through 9.

CH_3 —	— CH_2 —	— CH_2OH
42.1 %	55.8 %	2.1 %

FIG. 2. Activity distribution in propanol

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* It must be remembered that the number of carbon-14 labeled species produced is very small relative to the total number of molecules present in the system. For example, a typical labeling run will produce carbon-14 containing species at concentrations between 10^{-4} to 10^{-6} mol fraction. The chances of direct radiolysis of labeled intermediates are very small under these reaction conditions.

$$\text{occur concomitantly}\left[\begin{array}{ll}[^{14}:\dot{C}\cdot]^{\#} + CH_3OH \rightarrow \rightarrow {}^{14}CH_2 = CH_2 \text{ or } [^{14}CH_2 = CH_2]^{\ddagger} & (5)\\ CH_3OH \quad \rightsquigarrow \cdot CH_2OH + H\cdot & (6)\end{array}\right.$$

$$^{14}CH_2 = {}^{(14)}CH_2 + H^{\cdot} \rightarrow {}^{14}CH_3\text{-}{}^{(14)}CH_2\cdot \tag{7}$$

$$^{14}CH_3\text{-}{}^{(14)}CH_2\cdot + \cdot CH_2OH \rightarrow {}^{14}CH_3\text{-}{}^{(14)}CH_2\text{-}CH_2OH \tag{8}$$

or

$$[^{14}CH_2 = CH_2]^{\ddagger} + CH_3OH \rightarrow \rightarrow {}^{14}CH_3\text{-}{}^{(14)}CH_2\text{-}CH_2OH \tag{9}$$

Our results do not rule out the reactions outlined in equations 10 and 11, but a consideration of the relative rates would favor H· addition as a first step.

$$^{14}CH_2 = {}^{(14)}CH_2 + \cdot CH_2OH \rightarrow {}^{14}CH_2\text{-}{}^{(14)}CH_2\text{-}CH_2OH \tag{10}$$

$$\cdot{}^{14}CH_2\text{-}{}^{(14)}CH_2\text{-}CH_2OH + CH_3OH \rightarrow {}^{14}CH_3\text{-}{}^{(14)}CH_2\text{-}CH_2OH + \cdot CH_2OH \tag{11}$$

The radiolysis of methanol has been studied by numerous people (5-7) and the production of the $\cdot CH_2OH$ radical is one of the most prominent reactions having a G value between 5 and 7.

This work would indicate that ammonia as a nitrogen source in recoil labeling is useful. The labeling achieved is of course subject to the usual strictures connected with recoil labeling. The level of activity in the product depends on the integrated neutron dose and the amount of carrier added in order to isolate the desired product. In principle small amounts of material at very high specific activities can be obtained but isolation techniques in most instances are complex. Preparation of a radiochemically pure material also has its hazards (1).

The recoil labeling study implied that the radiolysis of a mixture of methanol and labeled ethylene might result in the formation of labeled propanol. The main radiation damage to these systems is occasioned by the fast neutrons and gamma flux. The energy released in the nuclear transformations in the methanol-ammonia system contributes to only a very small fraction of the total radiolysis in the system.

SELECTIVE RADIATION LABELING.

Several samples of methanol-ethylene-$^{14}C_1$ mixtures were irradiated under conditions identical with those used in the recoil work. In each case about 10 ml (at NTP) of high specific activity ethylene-$^{14}C_1$ was dissolved in 20-25 grams of methanol. The mol fraction of ethylene-$^{14}C_1$ was thus about 7×10^{-4}. It can be seen from the data in Table 2 that the radiation labeling

TABLE 2. Labeled alcohols formed in the selective radiation labeling preparation of Propanol-2, 3-$^{14}C_1$.

Product alcohol	Yield (based on ethylene-$^{14}C_1$)
Methanol	0.57 %
Ethanol	0.38 %
2-Propanol	0.12 %
Propanol	41.5 %
Butanol	0.38 %

involved a very efficient use of the ethylene-$^{14}C_1$. Yields as high as 85 % are possible by appropriate choice of concentrations and reaction conditions. The distribution of activity found in the two and three position of propanol is given in Fig. 3. The two positions are essentially equivalent in activity.

CH_3 —	— CH_2 —	— CH_2OH
49.6 %	50.4 %	< 0.02 %

FIG. 3. Activity distribution in propanol prepared by radiation synthesis.

In order to further test the general applicability of the method and to provide evidence for the mechanism of formation of the labeled alcohols, mixtures of propene-1-$^{14}C_1$ and methanol were radiolyzed in a manner similar to that used for the ethylene-$^{14}C_1$-methanol mixture. A typical run produced the products listed in Table 3. Activity distribution found in the 2-methylpropanol-3-^{14}C is given in Fig. 4. The activity distribution in the 1-butanol showed about 90 % of the activity to be in the 2-position. This deviation from the expected 100 % in the 2-position may have been due in part to the method of degradation.

As pointed out in the section on recoil labeling, consideration of relative rates and other factors a mechanism involving hydrogen atom addition as a first step followed by radical recombination as the product forming step on the assumption that recoil formed ethylene-^{14}C is an intermediate. In the case of the direct radiolysis, the known intermediate ethylene is acting as a scavenger and can scavenge either hydrogen atoms or hydroxy methyl radi-

TABLE 3. Labeled alcohols formed in selective radiation labeling preparation 2-methyl-1-propanol-3-^{14}C

Product alcohol	Yield (based on Propene-1-$^{13}C_1$)
Methanol	0.06 %
Ethanol	0.04 %
Propanol	0.37 %
2-Propanol	0.31 %
2-Methylpropanol	70.03 %
1-Butanol	3.86 %

cals. The labeling experiments with propene-1-^{14}C would seem to favor scavenging of hydrogen atoms first. It is well known that in radical addition reactions of olefins the most stable radical is first formed and this is usually followed by an abstraction or radical recombination reaction. Thus the formation of the 2-propyl radical is preferred over the formation of the 1-propyl radical. The 2-methyl-1-propanol-3-^{14}C to 1-butanol-2-$^{14}C_1$ ratio of $> 18/1$ would support such product controlling reactions as outlined in equations 12-15.

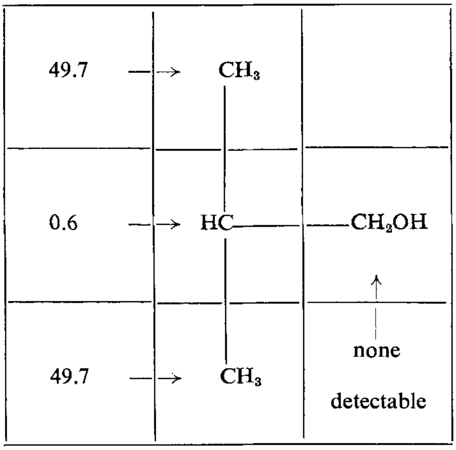
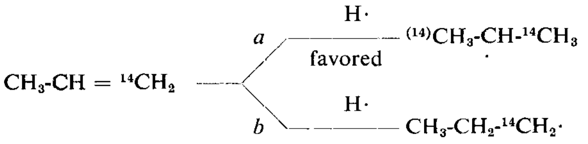
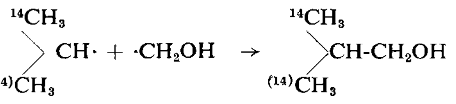


FIG. 4. Activity distribution in 2-methylpropanol.

$$\text{Mixture} \rightsquigarrow \text{H}\cdot + \cdot\text{CH}_2\text{OH} \qquad (12)$$



(13)



$$\left({}^{14}CH_3\right)\left({}^{(14)}CH_3\right)CH\cdot + \cdot CH_2OH \rightarrow \left({}^{14}CH_3\right)\left({}^{(14)}CH_3\right)CH\text{-}CH_2OH \qquad (14)$$

$$CH_3\text{-}CH_2\text{-}{}^{14}CH_2\cdot + \cdot CH_2OH \rightarrow CH_3\text{-}CH_2\text{-}{}^{14}CH_2\text{-}CH_2OH \qquad (15)$$

If the hydroxy methyl radical added first, followed by recombination or possibly hydrogen atom abstraction, the most stable radical intermediate $[CH_3\text{-}CH_2\text{-}{}^{14}\underset{\cdot}{C}H\text{-}CH_2OH]$ would lead to a favored formation of labeled 1-butanol which is not in accordance with the facts. Further work specifically designed to delineate the mechanism would be necessary to substantiate reaction sequences given by equations 12-15.

DISCUSSION OF SELECTIVE RADIATION SYNTHESIS.

In a selective radiation synthesis one is not dealing with a random or partially selective introduction of a radioactive element into a compound. Selective radiation synthesis as applied here involves the use of a labeled precursor as a scavenger for radicals (and conceivably ions in an appropriately chosen system) to produce a specifically labeled compound. The advantages of such a method are high yield (efficient use of labeled precursor), specific activities tailored to the requirements of the user and ease in isolation of the product. Thus selective radiation labeling is akin to ordinary synthetic methods.

Radiation as a means to bring about synthesis was outlined in a report by Able (8). Much has been written about the radiation chemistry of organic compounds and about attempts to use radiation as a means of bringing about useful compound formation. These studies concern themselves almost exclusively with chain reactions or reactions with high G values and are outside the scope of this paper. Some radiation chemistry studies afford examples of selective radiation labeling even though the intent of the work was basically studies in radiation chemistry. Holroyd and Klein (9) in using methyl-^{14}C iodide as a source of methyl radicals produced carbon-14 labeled compounds in what is a blend of selective and non-selective processes. Further examples of this type of work can be found in the papers of Cher *et al* (10) and Eberhardt *et al.* (11).

Examples of selective radiation labeling are few in number. In addition to the work reported in this paper there is an elegant description of a selective radiation labeling method for tritium compounds reported by Ekstrom and Garnett (12) and there is a report by Schwarz (13) on the preparation of trichloromethyl-$^{14}C_1$-cyclopentane and 1-chloro-2-trichloromethyl-$^{14}C_1$-cyclopentane. The yields reported were 5 % and 7.5 %, respectively, obtained from irradiation of equimolar mixtures of cylopentene and carbon tetrachloride-^{14}C with ^{60}Co gamma rays.

The carbon-14 examples are exemplary of the two approaches to selective radiation labeling. In the example cited in this paper the scavenger is labeled and the bulk solvent serves as a source of reactant radicals for the scavenger. This approach insures effective use of the labeled precursor. In the example of Schwarz an equimolar mixture of labeled and unlabeled material is used, again producing a desired specifically labeled product but with concomitant inefficient use of the labeled material. These approaches are summarized in Fig. 5.

1. Labeled material in low (scavenger) concentrations. Solvent is source of reactive fragments.

Labeled Material + Solvent
⇣
Radiolysis
↓
Specifically Labeled Product

Efficient use of Labeled Precursor

2. Labeled material in moderate to high concentrations (5 % to > 50 % of reaction mixture). Solvent is source of reactive fragments or serves as scavenger.

Labeled Material + Solvent
⇣
↓

Specifically Labeled Product

Inefficient use of Labeled Precursor

FIG. 5. Methods of Selective Radiation Synthesis.

CONCLUSION.

A recoil synthesis of propanol-2,3-$^{14}C_1$ has been compared with a selective radiation synthesis of propanol-2,3-$^{14}C_1$. A selective radiation synthesis of 2-methyl-propanol-3-^{14}C has also been described. No comparison is made with conventional synthetic methods but it is clear that the preparation of these two specifically labeled alcohols by the selective radiation method can compete most effectively and in all respects with any purely synthetic method designed to prepare these same compounds. The application of this method and its addition to the general methods for preparing labeled compounds has hardly begun. "Non-synthetic" (14) methods with the possible exception of Wilzbach labeling have not seen widespread use. The preparation of specifically labeled compounds has been the almost exclusive province of the purely synthetic approach. A knowledge of radiation chemistry coupled with a knowledge of the chemical properties of organic systems should allow the addition of SELECTIVE RADIATION LABELING, as a tool, to the methods the organic chemist has for the *facile* preparation of specifically labeled compounds. Its scope is not limited to carbon-14, deuterium or tritium, or to relatively simple organic compounds, nor is this method a panacea. It should nevertheless prove to be an useful method in the future.

EXPERIMENTAL.

1. *Preparation, Packaging and Irradiation of Sample for Recoil Labeling.* — Degassed C.P. grade methanol, 10.9098 grams, and ammonia, 1.4835 grams, were sealed under vacuum in a quartz vessel. The vessel was wrapped in quartz wool and placed in an aluminium cylinder preparatory to irradiation. The sample received 72 hours of irradiation in the Brookhaven reactor. The neutron flux was 3.9×10^{12} neutrons/cm^2 sec. The approximate absorbed dose was 7×10^7 rads (0.5 eV/molecule). The specific activity of this sample after irradiation was 3.73 mμc/mgC.

2. *Addition of Carriers.* — The carrier mixture consisted of 7.8378 grams of ethanol, 5.4755 grams of 2-propanol, 8.0200 grams of propanol, 6.5902 grams of butanol and 8.1302 grams of pentanol. The irradiated sample, 10.4 grams, was added to the carrier mixture and the mixture fractionated using a 40 plate spinning band column.

3. *Purification of Lower Boiling Alcohols.* — Methanol, ethanol, and 2-propanol were purified by preparative glpc. A dinonyl phthalate column (vide infra under Purification of Alcohols, Radiation Labeling) was used to separate the alcohols. A column temperature of 80° C was used. Repurification of each alcohol was carried out on a fresh column. The center cuts were collected and assayed.

CH_3OH *Anal.*, Found : Spec. Act., 0.036 mμc/mgC; C_2H_5OH *Anal.*, Found : Spec. Act., 0.189 mμc/mgC; i-C_3H_7OH *Anal.*, Found : Spec. Act., 0.0093 mμc/mgC.

4. *Purification of Higher Boiling Alcohols.* — The propanol, butanol and pentanol fractions were chromatographed as above with the substitution of 25 % by weight of UCON as the stationary liquid phase in the glpc column. The center cuts were collected and assayed.

C_3H_7OH *Anal.*, Found : Spec. Act., 0.073 mμc/mgC; C_4H_9OH *Anal.*, Found : Spec. Act., 0.005 mμc/mgC; $C_5H_{11}OH$ *Anal.*, Found : Spec. Act., 0.0033 mμc/mgC.

5. *Preparation and Degradation of Acetic-$^{14}C_1$ Acid.* — The center-cut ethanol-$^{14}C_1$, 0.5038 grams, was mixed with 10 ml of water and 1.1 ml of conc. sulfuric acid. The mixture was cooled (—15° C) and stirred. Potassium permanganate, 2.11 grams, was added portion-wise over a period of one hour. The precipitate, MnO_2, was dissolved by passing a stream of SO_2 into the

reaction mixture. The acidic aqueous solution was extracted with ether. The ether extract was washed with aqueous thallous hydroxide solution. The water was evaporated and the remaining solid extracted with absolute ethanol. The thallous acetate -$^{14}C_1$ was isolated and recrystallized from absolute ethanol.

Anal., Found : Spec. Act., 0.187 mμc/mgC.

The thallous acetate-$^{14}C_1$ was decarboxylated ⁽¹⁶⁾ and the methylamine (as a derivative) assayed.

Anal., Found : CO_2, Spec. Act., 0.0184 mμc/mgC; $C_8H_{10}N_2S$ *Anal.*, Found : N′-phenyl-N′-methyl thiourea, Spec. Act., 0.0445 mμc/mgC; correcting for inactive carbons, the methylamine specific activity was 0.356 mμc/mgC.

6. *Preparation and Degradation of Propanoic-$^{14}C_1$ Acid.* — The propanol-$^{14}C_1$ was oxidized and converted to thallous propanoate-$^{14}C_1$ as described previously (vide supra). The thallous propanoate-$^{14}C_1$ obtained was recrystallized from absolute alcohol.

Anal., Found : Spec. Act., 0.067 mμc/mgC.

The degradation was carried out as previously described for propanoic-$^{14}C_1$ acid. The assays of the decarboxylation, oxidation and derivatization follow.

Anal., Found : CO_2, carbon #1, Spec. Act., 0.0044 mμc/mgC; CO_2 carbon #2, 0.0115 mμc/mgC; thallium acetate, 0.098 mμc/mgC; N′-phenyl-N′-methyl thiourea, 0.0109; correcting for inactive carbon, the methylamine activity was 0.087 mμc/mgC.

7. *Preparation, Packaging and Irradiation of Sample for Radiation Labeling. Ethylene-^{14}C-Methanol.* — C. P. grade methanol was fractionated, dried over $CaSO_4$ and then flushed with a stream of argon. Approximately 30 ml of this methanol was placed in a pear-shaped flask. The flask was evacuated and the methanol outgassed. Ethylene-$^{14}C_1$ * was condensed into a quartz vessel on the same vacuum line. The methanol was then distilled into the quartz vessel. The sample was cycled through the liquid and solid stages several times to afford mixing. The vessel was brought to atmospheric pressure with helium and a sample taken for assay.

The vessel was placed on the vacuum line, the contents frozen, the vessel evacuated and then sealed. The sample weight was 24.4039 grams. The quartz vessel was wrapped with quartz wool and placed in an aluminium (2-S) cylinder preparatory to irradiation in the Brookhaven reactor. It was irradiated for one day at a flux of 9×10^{12} neutrons/cm² sec. This corresponds to an absorbed dose of approximately 7×10^7 rads (0.5 eV/molecule).

8. *Addition of Carriers and Preliminary Separation.* — A carrier mixture consisting of 2.0316 grams of ethanol, 2.0717 grams of 2-propanol, 3.0479 grams of propanol and 2.0859 grams of butanol was placed in an especially

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* Prepared by Dr. D. R. Christman, Brookhaven National Laboratory.

made vessel for mixing the carriers with the irradiated methanol. 75λ of bromine was added to the carrier to react with the ethylene-$^{14}C_1$ not consumed in the radiolysis.

The methanol mixture in the quartz vessel was frozen, the vessel opened, and the open end placed in the carrier solution. As the methanol mixture melted it ran directly into the carrier solution thus insuring complete reaction between bromine and unreacted ethylene-$^{14}C_1$. An aliquot was removed for analysis. The total activity contained in this mixture was 0.0623 mc (Spec. Act., 4.26 mμc/mgC). A preliminary fractionation using a three-eighths inch I.D. by ten inch long glass helice packed column was carried out. Three fractions were taken having the boiling point ranges : 45-65° C, 65-97° C, and 97-130° C.

9. *Purification of Alcohols.* — The alcohols were purified by means of preparative glpc. A half-inch I.D. by eleven foot column was packed with Johns-Manville, acid-washed 30-70 mesh firebrick. Twenty-five percent by weight of brick, dinonyl phthalate constituted the stationary liquid phase. The column was operated at 75° C.

A. — *Lower Boiling Alcohols.*

The 65-97° C fraction was separated on the column, each component individually trapped, and then each was again passed through a freshly prepared column. Center cuts of each alcohol were collected and analyzed.

CH_3OH *Anal.*, Found : Spec. Act. 0.0386 mμc/mgC; C_2H_5OH *Anal.*, Found : Spec. Act. 0.223 mμc/mgC; i-C_3H_7OH *Anal.*, Found : Spec. Act. 0.0595 mμc/mgC; n-C_3H_7OH *Anal.*, Found : Spec. Act. 14.34 mμc/mgC.

B. — *Higher Boiling Alcohols.*

The 97-130° C fraction plus the pot residue was redistilled. The distillate was diluted with 500 λ of carrier ethylene bromide. This mixture was separated on the dinonyl phthalate column, each fraction repurified on a fresh column, and center cuts of each collected and analyzed.

$C_2H_2Br_2$ *Anal.*, Found : Spec. Act., 1.25 mμc/mgC; C_4H_9OH *Anal.*, Found : Spec. Act., 0.306 mμc/mgC.

10. *Oxidation of Propanol-$^{14}C_1$ to Propanoic Acid-$^{14}C_1$.* — The purified propanol, 1.5 g, dissolved in 30 ml of H_2O and 3.3 ml of conc. H_2SO_4, was oxidized by adding 6.3 grams of $KMnO_4$ to the cooled (−15° C) solution over a period of one hour. The excess permanganate was reduced with 30 % H_2O_2 solution. The precipitate was removed and washed several times with hot H_2O. The filtrate plus the wash solutions were steam distilled. The distillate was titrated with thallous hydroxide solution (15).

The thallous propanoate-$^{14}C_1$ obtained was recrystallized several times from absolute ethanol.

The thallous propanoate-$^{14}C_1$ (14.20 mμc/mgC) was diluted with a mixture of inactive acetic, propanoic and butanoic acids. The mixture was dissolved in water and sufficient H_2SO_4 was added to hydrolyse the thallous propanoate-$^{14}C_1$. This solution was extracted with ether, the ether dried over Mg_2SO_4, and the bulk ether removed by fractional distillation. The residue was purified by separation on a glpc column (1/2″ I.D. × 11′ column, 30-70 mesh Johns-Manville acid-washed firebrick, liquid phase 25 %, by weight of brick, Dow-Corning (D.C.) 710 silicone oil). Stearic acid was not added to prevent unsymmetrical tailing and overloading because of creeping of the stearic acid. The peaks were nevertheless well separated. The column was operated at 90° C. A center cut of the propanoic acid-$^{14}C_1$ was converted to thallous propanoate-$^{14}C_1$ and again diluted with inactive thallous propanoate. This thallous propanoate-$^{14}C_1$ was purified by recrystallization from absolute ethanol.

Anal., Found : Spec. Act., 0.851 mμc/mgC.

11. *Degradation of Propanoic*-$^{14}C_1$ *Acid.* — The degradation scheme used was a modification of the method of Phares (16) based on the Schmidt reaction. The CO_2 from the decarboxylation of the propanoic-$^{14}C_1$ acid was collected and assayed. *Anal.*, Found : CO_2, Spec. Act. 0.001 mμc/mgC.

The ethylamine was oxidized to acetic acid. The acetic acid was converted to thallous acetate. The CO_2 from the decarboxylation of the acetic-$^{14}C_1$ acid was collected and assayed.

Anal., Found : CO_2, Spec. Act. 1.28 mμc/mgC.

The methylamine resulting from the Schmidt reaction on acetic acid was derivatized and assayed as N′-phenyl-N′-methyl thiourea.

Anal., Found : 0.157 mμc/mgC; (Methylamine spec. act.; corr. for inactive carbons in derivative was 1.26 mμc/mgC).

12. *Propene*-1-^{14}*C-Methanol Irradiation.* — The general procedure previously described was followed. Propene-$^{14}C_1$ (New England Nuclear Corp.) (~ 2.1 mg and 0.1 mc) was dissolved in 25.5856 g of methanol. The sample was irradiated for 6 hours at a flux of 9×10^{12} n/cm²/sec (~2×10^7 rads).

13. *Addition of Carriers and Preliminary Separation.* — A carrier mixture consisting of 3.1270 g n-butanol, 3.1423 g of isobutanol, 1.6157 g of n-propanol, 1.5109 g of isopropanol, 1.5360 g of ethanol and 150 λ bromine was added to the irradiated methanol. The total activity contained in this mixture was 0.116 mc (Spec. Act. 7.14 mc/mgC). A preliminary fractionation using the same fractionating column previously cited was carried out. Four fractions were collected having boiling point ranges of : 54°-64° C, 64°-72° C, 72°-99° C, and 99°-120° C.

14. *Purification of Alcohols.* — The alcohols were purified by means of preparative glpc.

A. — *Lower Boiling Alcohols.*

The lower boiling fraction, 54°-99° C, was separated as previously described. Each component was individually trapped, and rechromatographed on a freshly prepared column. A center cut of each was collected and analyzed.

CH_3OH *Anal.*, Found : Spec. Act. 0.0069 mμc/mgC; C_2H_5OH *Anal.*, Found : Spec. Act. 0.0512 mμc/mgC; i-C_3H_7OH *Anal.*, Found : Spec. Act. 0.400 mμc/mgC (Spec. Act. corrected for inactive carbon and based on 3.-5-dinitrobenzoate derivative); n-C_3H_7OH *Anal.*, Found : Spec. Act. 0.450 mμc/mgC (Spec. Act. corrected for inactive carbon and based on 3-5-dinitrobenzoate derivative).

B. — *Higher Boilding Alcohols and Halide Fraction.*

The 99°-120° C fraction and the 500 λ of the dibromopropane obtained were purified using the packing described previously. Center cuts of the alcohols and the dibromide were collected and analyzed.

i-C_4H_9OH *Anal.*, Found : Spec. Act. 40.09 mμc/mgC; n-C_4H_9OH *Anal.*, Found : Spec. Act. 2.22 mμc/mgC; $C_3H_6Br_2$ *Anal.*, Found : Spec. Act. 0.428 mμc/mgC.

15. *Dilution and Oxidation of 2-Methylpropanol-^{14}C to 2-Methylpropanoic-^{14}C Acid.* — Purified 2-methylpropanol-^{14}C (1.6043 g) was diluted with 37.9885 g of inactive 2-methylpropanol. The specific activity of the diluted mixture was 1.64 mμc/mgC. The 2-methylpropanol-^{14}C was oxidized to 2-methylpropanoic-^{14}C acid with $KMnO_4$ and Na_2CO_3 in H_2O (17). The 2-methylpropanoic-^{14}C acid was distilled. The free acid and the thallium salt were analyzed.

i-$C_4H_8O_2$ *Anal.*, Found : Spec. Act. 1.73 mμc/mgC; i-$C_4H_7O_2Tl$ *Anal.*, Found : Spec. Act. 1.73 mμc/mgC.

16. *Degradation of Thallium-2-Methylpropanoate-^{14}C.* — The modified method of Phares (16) was employed in the degradation. The CO_2 from the decarboxylation of thallium-2-methylpropanoate-^{14}C was collected and assayed.

Anal., Found : CO_2, Spec. Act. 0.0003 mμc/mgC.

The other product, 2-aminopropane-^{14}C was isolated, derivatized and assayed as the N'-phenyl-N-i-propyl thiourea.

$C_{10}H_{14}N_2S$ *Anal.*, Found : 0.725 mμc/mgC (corr. for inactive carbon, the 2-aminopropane-^{14}C Spec. Act. was 2.42 mμc/mgC).

17. *Degradation of 2-Methylpropanoic-^{14}C Acid.* — The bulk of the 2-methylpropanoic-^{14}C acid was converted to the silver salt.

$C_4H_7O_2Ag$ *Anal.*, Found : Spec. Act. 1.77 mμc/mgC.

The silver salt of 2-methylpropanoic-^{14}C acid was decarboxylated in the conventional manner using the Hunsdiecker reaction (18). The 2-bromopropane-^{14}C resulting from this reaction was isolated and hydrolyzed to 2-propanol-^{14}C. A KI-I_2 solution was added directly to the hydrolysis mixture until no more iodoform precipitated. The iodoform was collected and washed many times with H_2O, dried and sublimed.

CHI_3 *Anal.*, Found : Spec. Act. 3.22 mμc/mgC.

The filtrate and combined washings were evaporated to a minimum volume, cooled and acidified with H_2SO_4. Solid $Na_2S_2O_3$ was added until the iodine color disappeared. The solution was steam distilled and the distillate was neutralized with 0.5 N TlOH solution to the phenolphthalein end point. The solution was evaporated and the thallous acetate-^{14}C was purified in the usual manner.

$C_2H_3O_2Tl$ *Anal.*, Found : Spec. Act. 1.72 mμc/mgC.

The thallous acetate-^{14}C was decarboxylated. The CO_2 and the methyl-^{14}C-amine (as a derivative) were assayed.

CO_2 *Anal.*, Found : Spec. Act. 0.041 mμc/mgC; $C_8H_{10}N_2S$ *Anal.*, Found : Spec. Act. 0.435 mμc/mgC (Spec. Act. in methyl-^{14}C-amine corr. for inactive carbons in derivative was 3.56 mμc/mgC).

It should be noted that the method of degradation yields values for the methyl groups in the original acid as iodoform-^{14}C (from the 2-propanol-^{14}C) and as a methyl-^{14}C-amine derivative (from the decarboxylation of the acetic-^{14}C-acid resulting from the iodoform reaction on 2-propanol-^{14}C). The activity in these two methyl groups must a priori be the same. The actual values (3.22 mμc/mgC for the CHI_3 and 3.56 mμc/mgC for the CH_3NH_2) differ by about 10 %. This is within the precision one can expect from a chemical degradation of this type at these activity levels. An average of these two values is used to give the figure of 49.7 %/methyl group reported in the text.

18. *Preparation and Degradation of Butanoic-^{14}C-Acid.* — Butanol-^{14}C ((1.7595 g) was diluted with 10.6301 g of inactive butanol. The Spec. Act. of the diluted mixture was 0.321 mμc/mgC. The butanol-^{14}C was oxidized to butanoic-^{14}C acid by the same method as described under the oxidation of 2-methylpropanol-^{14}C. The butanoic-^{14}C acid was converted to the thallium salt.

$C_4H_7O_2Tl$ *Anal.*, Found : Spec. Act. 0.298 mμc/mgC.

Degradations were carried out as previously described. The results follow.

Anal., Found : CO_2, carbon #1, Spec. Act. 0.016; *Anal.*, Found : $C_3H_7O_2Tl$, carbons #2, 3 and 4, Spec. Act. 0.341; *Anal.*, Found : CO_2 carbon #2, Spec. Act. 0.879; *Anal.*, Found : $(CH_3CH_2NH_3)_2PtCl_6$ or $(C_2H_5NH_3)_2PtCl_6$, carbons #3 and 4, Spec. Act. 0.0448.

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