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ABSTRACT

A review of the techniques and procedures associated with the use of tracer elements in organic chemistry is presented. Techniques for the manipulation and the synthesis into organic compounds of the isotopes of carbon, hydrogen, nitrogen, oxygen, sulfur and the halogens are outlined.

For publication in book, "Organic Techniques," edited by R.V.V. Nicholls, to be published by Reinhold Publishing Company.

(*) A portion of the work described in this paper was sponsored by the U.S. Atomic Energy Commission.

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Among the newer tools available to the organic chemist today are radioactive isotopes and abnormal concentrations of rare stable isotopes of the elements which he uses. These isotopes have made possible new approaches to many fundamental problems, such as mechanism studies; they have offered numerous new analytical methods, and in the field of biological chemistry they have been the key agent in many new studies, particularly of the type where the path and fate of labeled atoms, molecules, and even bacteria and viruses are to be determined. In industrial chemistry these special isotopes can be used both in research studies and in plant process control and development. As yet their applications have only begun - much of the work since the war has been directed toward the development of techniques and equipment such as preparing or isolating the desired isotopes, development of analytical tools such as mass spectrometers, Geiger-Mueller counters and other radioactive assay equipment, special methods for handling radioactive material and the synthesis of needed compounds and intermediates containing the radioactive and stable isotopes.

In this chapter we shall present a fundamental review of information for those isotopes that are commonly available for studies in organic chemistry and then discuss methods of synthesis and purification of labeled compounds. It is not felt possible to adequately cover the elements other than carbon and those elements which form carbon-element bonds, excluding metallo-organic and chelate compounds. This limitation is necessary because ionic and chelate compounds of almost every element in the periodic table can be prepared; to cover all of these radioactive matals would require a complete book in itself. Compounds such as sulfates and phosphates will also not be considered, but the radiochemistry of sulfur in covalent bonds will be reviewed.

We have also made no attempt to describe in detail a number of correlated techniques of primary importance to workers dealing with isotopic compounds. Three of the more important of these are the theory of measurement of radioactive materials (Geiger counters, proportional counters and ionization chambers), measurement of stable isotope concentrations and methods for the application of isotopes for the solution of specific types of problems. Excellent general and specific discussions of these subjects are to be found in current literature (1 through 21).

Isotopes Available: -

Although there are a number of isotopes, either stable or radioactive, of any element in the periodic table, only a limited number of these have practical applications for tracer experiments. Thus we find listed in the table of isotopes (22) five isotopes of carbon with masses of 10,11,12,13 and 14. Of these, C^{10} has so short a half-life, 20 seconds, that its use is experimentally impractical in most tracer experiments. Carbon 12 is the abundant natural occurring isotope, and Carbon 11, with a half-life of 20.5 minutes, depends upon the immediate availability of a cyclotron to prepare the radioactive material as needed and is useful only for special types of short-term experiments. This leaves C^{13} and C^{14} as important isotopes for tracer studies with this element.

Carbon. As previously mentioned, Carbon 13, which is the naturally occurring, less abundant isotope, and Carbon 14, which is a long-lived (5700 years) beta emitter, are the two important isotopes of this element for tracer work. Of these two isotopes, C^{14} is more important and is used more extensively; this is due to several factors: a) assay of C^{14} is faster and less expensive than that of C^{13} (the latter requires an accurate and expensive mass spectrograph which can measure only a small number of samples per day. Carbon 14 can be measured in simple and relatively inexpensive counting apparatuses which handle a

fairly large number of samples per day); b) the dilution which can be measured in C^{14} tracer studies is much larger by several factors of 10 than is possible with C^{13}; and c) equally important, the dilution possible per unit of isotope cost for C^{14} is greater than that for C^{13}.

The factors that initially slowed down the extensive use of C^{14} are rapidly being overcome. These include availability of the isotope in forms other than barium carbonate, health hazard considerations (see later discussion) lack of adequate analytical tools to measure the very soft beta emission, and need for development work in synthesis problems.

Hydrogen. Two isotopes of hydrogen are available for tracer studies, deuterium and tritium (H^2 and H^3). Deuterium, which is the less common stable isotope of hydrogen, can be readily assayed with a mass spectrometer as hydrogen gas, or by sensitive physical measurements on water, such as the density (23) or refractive index (23,24). Mass spectrograph measurements of deuterium as water are not practical because of water absorption on analytical equipment.

Tritium, which is a very soft beta emitter, can be effectively analyzed only as a gas either in a counter tube or in an ionization chamber (25,26,27). It can be detected in gas flow counters such as the Nucleometer (28) or "Q" gas counter (29, 30, 31), but self-absorption of the sample makes this a very inefficient method. In contrast to these difficulties, however, the isotope is available in large quantities at a very reasonable cost by nuclear synthesis in the atomic pile. This availability of tritium makes it possible to consider large scale tracer studies with the isotope. Thus one could easily study process problems in a small chemical factory or a large pilot plant with a curie of tritium (cost, $100).

Before isotopes of carbon were readily available for tracer experiments, deuterium was extensively used to mark carbon atoms. The possibility of hydrogen

exchange under the conditions of the experiment, and the need to prove the absence of such an effect, together with the cost of analysis, has reduced such use of tritium and deuterium to those cases where synthetic difficulties, health hazards or cost problems contraindicate the use of carbon isotopes.

Careful consideration must always be given to the possibilities of large isotope effects in the use and assay of deuterium and tritium. Even the passage of such a gas mixture through a tube or orifice in a mass spectrometer for assay can produce changes in isotope concentrations, and large effects can be found in biological systems.

Nitrogen and Oxygen. Nitrogen and oxygen are distinguished in organic tracer chemistry by having no useable radioactive isotopes; only concentrated samples of stable rare isotopes are available to the experimenter, and the mass spectrograph is needed for analytical measurements. Nitrogen 15 and oxygen 18 are the isotopes commonly used in experimental work. Although there is another stable isotope of oxygen, O^{17}, its natural concentration is very low and it has not yet been offered for sale.

Sulfur. Only one isotope of sulfur is commercially available to the organic chemist for tracer experiments, namely sulfur 35. The radiation of this isotope is very comparable to carbon 14 (0.169 mev β particle compared with a 0.156 mev β from carbon 14), so that counting equipment for solid samples of one isotope may be used for the other (32).

Although C^{14} may be readily cleaned from the surface of laboratory equipment such as beakers, sulfur is much more difficult to remove, so that cross contamination can be a more serious problem in work with this isotope.

Halogens. The radioactive isotopes of the halides that are commercially available for tracer studies are F^{18}, Cl^{36}, Br^{82} and I^{131}. No stable isotopes in abnormal concentrations are available.

Fluorine 18, a fairly hard β-emitter with a half-life of 112 minutes, is usually prepared using a cyclotron. This has seriously limited tracer studies

on fluorides. Chlorine, bromine and iodine each possesses a number of pile-produced radioactive isotopes which could be used for tracer work, but those listed as commercially available are convenient to use from the standpoint of long half-life and energetic radiation for easy counting and may be prepared by neutron bombardment in atomic piles in a pure form.

In Table I data for the commonly used isotopes in organic chemistry are listed. Natural concentrations, radiation energies and half-lives are given. More extensive information on the isotopes can be found in standard tables of isotopes (22,33).

Source of Isotopes. Concentrations of the four stable rare isotopes, C^{13}, D, N^{15}, and O^{18} may be purchased from several firms; these are listed in Table II. Most of the radioactive isotopes are available to research workers through the Isotopes Division of the U.S. Atomic Energy Commission, the Eldorado Mining and Refining (1944) Limited, Canada, or the Atomic Energy Research Establishment, England. Useful compounds synthesized from these isotopes can be purchased, either from the prime supplier or from a number of manufacturing and research companies. These are listed in Table III.

Health Hazards of Radioactive Isotopes: -

The irradiation hazards of the radioactive isotopes that have been discussed can be divided into two classes on the basis of the type and energy of radiation, half life of the isotope, and rate of elimination and localization of the element in the body. They are (1) those of negligible to slight hazard, which include C^{11}, F^{18}, S^{35}, Cl^{36}, T and C^{14} and (2) those of more severe hazard, which include I^{131} and Br^{82}. Any of these materials can be classified as a severe hazare from a radiation point of view if enough radioactivity is handled, and alternately, neither I^{131} or Br^{82} is particularly dangerous if used in small quantities (37, 38,39).

Table I

Commonly Used Isotopes in Organic Chemistry

Element	Natural Concentration	Radiation (Mev) Beta	Gamma	Half-life
C^{11}	--	0.95	no γ	20.5 min.
C^{13}	1.1%	Stable	--	--
C^{14}	12 dis./min./ g. Carbon	0.156	no γ	5700 yr.
D	0.0156%	Stable	--	--
T		0.0185	no γ	12.1 yr.
O^{18}	0.204%	Stable	--	--
N^{15}	0.38%	Stable	--	--
S^{35}	--	0.169	no γ	87.1 days
F^{18}	--	B^+ 0.95 (20%) 0.6 (80%)	no γ	112 min.
Cl^{36}	--	0.64	no γ	2×10^6 yr.
Br^{82}	--	0.465	0.547 0.737 1.35	34 hr.
I^{131}	--	0.314 0.500 .368 .283 .250 (6%)	0.638	8.0 days

Table II

Source of Isotopes (34, 35, 36)

Isotope	Source	Production Form	From whom obtained
C^{11}	Cyclotron bombardment	CO	Private arrangement with cyclotron facilities
C^{13}	Exchange reaction	KCN	Eastman Kodak Co., Rochester, New York (a)
C^{14}	Atomic pile bombardment	$BaCO_3$ Na_2CO_3	(a), (b). (c)
D	Electrolysis of water	D_2O	Stuart Oxygen Co., San Francisco, California
T	Atomic pile bombardment	T_2	(b), (c).
N^{15}	Exchange reaction	NH_4NO_3	Eastman Kodak Co., Rochester, New York
O^{18}	Electrolysis of water	D_2O, H_2O	Stuart Oxygen Co., San Francisco, California
S^{35}	Atomic pile bombardment	S, H_2SO_4	(a), (b), (c).
F^{18}	Cyclotron bombardment	variable	Private arrangement with cyclotron facilities
Cl^{36}	Atomic pile bombardment	KCl	(b), (c).
Br^{82}	Atomic pile bombardment	KBr NH_4Br	(b), (c). (a)
I^{131}	Atomic pile bombardment	NaI	(a), (b), (c).

(a) Atomic Energy Research Establishment, Harwell, Berks., England

(b) Isotopes Division, U. S. Atomic Energy Commission, Oak Ridge, Tenn., USA

(c) Eldorado Mining and Refining (1944) Limited, P.O. Box 379, Ottawa, Canada

Table III

Organizations Offering
Special Labeled Compounds for Sale

Isotope	Forms available	Organization
C^{11}	No special forms	
C^{13}	KCN, CH_3I, $BaCO_3$	Eastman Kodak Co., Rochester, New York.
C^{14}	A variety of common and special compounds. Write for further information.	Isotopes Division, U.S.A.E.C., Oak Ridge, Tenn. AERE, Harwell, Berks., England. Tracerlab, Inc., Boston, Mass. Texas Research Corp., Renner, Texas. Southern Research Inst., Birmingham, Ala. Radioactive Products, Inc., Detroit, Mich. U.S. Testing Labs., Inc., Hoboken, New Jersey. Nuclear Inst. and Chem. Co., Chicago, Ill.
D	D_2	Stuart Oxygen Co., San Francisco, Cal.
T	No special forms	
N^{15}	HNO_3 KNO_3, potassium phthalamide	Eastman Kodak Co., Rochester, New York.
O^{18}	No special compounds	
S^{35}	A variety of compounds. Write for further information.	Isotopes Division, A.E.C., Oak Ridge, Tenn. Tracerlab, Inc., Boston, Mass. Texas Research Corp., Renner, Texas. Abbott Laboratories, North Chicago, Ill. A.E.R.E., Harwell, Berks, England.
F^{18}	No special forms	
Cl^{36}	No special forms	
Br^{82}	Br_2, inorganic bromides and alkyl bromides	A.E.R.E., Harwell, Berks., England.
I^{131}	A variety of compounds. Write for further information	A.E.R.E., Harwell, Berks., England. Abbott Laboratories, North Chicago, Ill. Texas Research Corp., Renner, Texas. Tracerlab, Inc., Boston, Mass.

I^{131} and Br^{82}. Iodine owes its particular hazard to the ability of the thyroid to localize this element. Since this isotope possesses several fairly energetic γ rays as well as several β particles, considerable radiation can be delivered to the thyroid in a short time. In a normal adult about 20 and 25% of a dose of NaI^{131} will be concentrated in the thyroid and approximately 1/8 μc of I^{131} in the gland will produce 0.1 rep/day. The use of 50 to 100 μcuries of I^{131} in the laboratory certainly does not present any special problem from this point of view since the probability of injesting the entire quantity is small. Kamen has calculated that 200 μcuries of I^{131} is a safe maximum total dosage figure (7).

Bromine 82 is the only other isotope discussed in this chapter that is a γ emitter. Although it does not have the localizing action of iodine, the short half life often requires the manipulation of large quantities of the isotope, and under such conditions radiation to the hands and body should be carefully monitored.

C^{11} and F^{18}. Carbon 11 does not present a serious long-term radiation problem because of its 20 minute half life. The same consideration applies to F^{18} which has a half life of 112 min. With both of these isotopes, however, adequate shielding for the hands is required in working up large quantities of activity during the early time stages of a given experiment; glass of lucite shields will usually serve to stop the fairly energetic β particles.

Cl^{36}. Although Cl^{36} has a long half life and is a strong β emitter, the turnover time of this element in the body is fairly rapid and there is small probability of localization. In one measurement on a normal adult a 15 day half life for elimination has been observed (40).

Carbon 14. The fate of C^{14} in the body is very much a function of the type of compound in which it is ingested and, to a lesser extent, of the mode of ingestion. Thus far there has been only a limited amount of information on this subject

for either animals or humans, but this is rapidly being corrected. In general, if the radioactivity is in the form of simple organic molecules capable of being metabolized to CO_2 in the body then, following ingestion of the material, the radioactivity will be found widely distributed in all tissues and to the greatest extent will be excreted as carbon dioxide in the breath (41 through 47).

The types of compounds which have been shown to be rapidly handled in this manner are aliphatic fatty acids, amino acids, aliphatic alcohols, sugars and many other compounds normally found in the body. The most complete study on any one such compound has been done with glycine-2-C^{14} in mice and man (43, 46, 48). In the study on mice, which was made for six weeks, it was shown that 80 to 90% of the injected activity was excreted in this period of time. No selective holdup of activity in the bone or soft tissue was found within this time and the maximum half time for elimination of activity from the tissues was 10 to 12 days.

The studies on humans have shown a similar pattern for the period of six weeks, but longer studies with more complete measurements have introduced several extensions. When glycine-2-C^{14} is administered intravenously to humans, about 30% of the activity is eliminated as $C^{14}O_2$ with a half time of 3 hours, another 30% is eliminated with a half time of 30 hours and 30% more is exhaled with a half time of 10 to 12 days. (43). A total of about 85% of the injected dose is excreted in this manner in the breath. The urinary excretion of carbon-14, which constitutes some 10% of the injected dose was represented by three half times of 6 hours, 2 1/2 days and 50 days (48).

The remaining activity in the tissue, 2 to 5%, slowly decreases with a half time of approximately 50 days, but there was evidence for a yet slower component of elimination of C^{14}.

The studies of Skipper, et.al., (42,49) on the hazards of the use of C^{14} based on work with sodium bicarbonate given by intraperitoneal injections have shown that after 24 hours only 1.37% of the injected dose is left in the tissues, and that this figure decreases slowly thereafter (1 wk., 0.62%; 4 wks., 0.13%; and 12 wks., 0.12%). From this data the authors calculated that 50 mc. of C^{14} injected as $NaHC^{14}O_3$ would be required to produce 0.1 rep. exposure in a 70 kilogram man, assuming uniform distribution and comparable metabolism of C^{14} in man and mouse, except for the weight factor.

Longer studies on the bones of these mice showed long-term components of incorporated carbon 14, probably similar to those observed for glycine. The nature of the bone distribution has been discussed by Skipper and also by Bloom (50).

General correlation of data for the long-term excretion of many different compounds is now needed. Until these are available, each compound is a problem in itself with regard to dosage. Brues and Buchanan's tentative maximum retained dose of 30 μcuries C^{14} for man is still a reasonable figure on which to base safety considerations. (51).

Compounds which are so abnormal to the biological system that they cannot be broken up or enter into the metabolic cycle of the body are either retained in some reservoir such as the spleen or liver for long turnover times or are eliminated, usually via the urine or feces, and most probably with the bile for the latter case. In this elimination process the molecule may be conjugated, hydroxylated or otherwise modified to reduce toxicity and increase solubility.

Common examples of such compounds are the drugs. Thus, stilbamidine-amidine-C^{14}_2 (52), methadon-1-C^{14} (53), dibenzanthracene-9-C^{14} (54), morphine-N-methyl-C^{14} (55) and codeine-N-methyl-C^{14} (55) have all shown a small or negligible oxidation

to $C^{14}O_2$ in the animal body and have been eliminated mostly by the urine and feces. In certain diseases stilbamidine may be retained in the liver for long periods of time (52).

Tritium. Tritium oxide, when taken into the body, is rapidly equilibrated first with the water of the plasma and then with the total body water. This activity then slowly decreases as the body water is eliminated and replaced. The half time for the turnover of this body water is 9 to 14 days in man (56) and it can be decreased to as little as 2 1/2 days by augmenting the normal water intake. When HTO is inhaled, almost all of the inspired activity is taken into the body fluids, but inhalation of HT results in only about 0.05% of the activity appearing in the body (56).

In a recent study of retention of tritium in mice when administered as the oxide, a biological half life of 1.1 days for the body water was measured and a curve for tissue-bound tritium determined which was resolved into two components of 9 and 90 days half time. These two components represent comparable binding capacity and constitute about 17% of the activity present in the body water (57).

Tritium presents little radiation hazard because of the low energy of its β particle and its uniform body distribution. Kamen (7) has calculated that 14 millicuries of tritium would be required to produce a total dose of 1.0 rep in a 70 kg. man.

Sulfur 35. This radioactive isotope does not present an appreciable radiation hazard. The radiation is very soft and the half life only 87 days. The body absorption and turnover is in great part a function of the mode of injection. Orally injested sulfur and sulfate is not used much in body metabolic synthesis (58). Sulfur 35 is usually manipulated similarly to C^{14}.

Bremstrahlen. An additional radiation factor needs to be considered when handling large quantities of β emitters of low energies such as C^{14}, S^{35} or F^{18}. Although these β particles will not penetrate substantial container walls such as a glass vessel, the soft X-rays or "bremstrahlen" produced by β bombardment of the salts and vessel walls can produce a local radiation field. This radiation becomes appreciable at the 50 to 100 millicurie level for C^{14} and if larger quantities of radioactivity are handled for long periods of time, radiation dosage to the hands should be checked.

Equipment for Radioactive Work (59 through 66): -

Most organic tracer chemistry can be accomplished with a minimum amount of specialized laboratory facilities. Any clean, well-ventilated room is satisfactory. A good hood is necessary for radioactive work. Even with tracer level work with materials that are presumably not harmful if ingested, it is desirable to eliminate intake of radioactivity if this can be done.

The problems of cross contamination require that the radioactive laboratory be kept clean, and that, insofar as possible, the walls, floor, benches and hood be constructed of non-porous materials that are easily decontaminated. It is usually necessary to keep very high level and low level work separated to eliminate this cross contamination.

Radioactivity Determinations: -

There are a number of methods of varying sensitivity and precision for radioactive assay in tracer work. The choice of the method will depend on 1) the nature of radiation of the isotopes to be used in the studies, 2) desired sensitivity, 3) needed durability or ruggedness, 4) desired precision and 5) cost. Further special consideration such as directional counters for *in vivo* work must also be considered.

Geiger-Muller Counters. The most common method of radioactive assay is by means of the Geiger-Muller tube and a scaler. For organic chemical work, G. M. tubes may be conveniently divided into four classes, 1) thick-walled tubes for use with γ emitters, such as I^{131} and Br^{82}, 2) thick-end window tubes (greater than 3 mg./cm.^{2} window) for use with hard β emitters, as C^{11}, F^{18} and Cl^{36}, 3) thin-end window tubes for use with soft β emitters as C^{14} and S^{35} and 4) Geiger tubes in which the sample is placed in the tube itself for use with soft β emitters (flow-type counters).

There is a natural division of this last type of G. M. Tube into those units used for solid samples and those used for gaseous samples. A number of models of the flow-type gas counter for solid samples are commercially available (60) and are of particular interest in low level assay. The lack of window absorption in this counter makes it useful for C^{14} and S^{35} and it may even be used for detection of tritium, although the self absorption of the solid sample is very large. Because of the quenching action of small amounts of air that are sometimes difficult to eliminate from the tube and sample, this unit is somewhat less precise for routine work than the first three types of Geiger-Muller tubes.

Geiger-Muller counting of gaseous samples of low energy radiations can be made much more sensitive than is possible with solid samples (a). This is due to two reasons: 1) the self absorption of the sample is almost completely eliminated and 2) much greater sample sizes can commonly be used. This technique is generally used for low-level carbon 14 and for tritium assay (67).

(a) The exception to this generalization is found in the excellent low-level counting of carbon 14 as elementary carbon done by E. C. Anderson, J. R. Arnold and W. F. Libby, Rev. Sci. Instr., 22, 225 (1951).

Proportional Counters. Proportional counters designed to count solid samples in a flow-type windowless tube offer an excellent assay instrument for C^{14} and S^{35}. The sensitivity of such an instrument is the same as a comparable Geiger counter and the gas (usually methane) is not as sensitive to small amounts of air. Thus, considerably greater precision is routinely possible. A commercial example of such a unit is the Nucleometer (70).

Proportional counting of gaseous samples is comparable in sensitivity to the gaseous G. M. counting just mentioned or to the ionization chamber method described below (68, 69). Although a single commercial instrument is not available at present for this work, component parts such as the power supply, linear amplifier and scaler can be purchased and assembled.

Scintillation Counters. A recent development in the technique of radioactive measurement is the scintillation counter, which makes use of a photomultiplier tube and a phosphor which is sensitive to radiation (71, 72, 73). The instrument is very useful for directional counting. For counting gamma rays it is much more efficient than the G. M. tube.

Ionization Chambers. (20, 21, 74). Ionization chambers are used in a wide variety of counting levels in radiochemistry, but their greatest use in organic chemistry is for low-level activity and for precision assay of soft radiation samples. Thus this method of radioactivity measurement is used with tritium and carbon 14; the sample can be conveniently introduced into the chamber as gas, C_2H_2, H_2 or CH_4 for tritium or CO_2 for carbon 14. A Lindeman electrometer or a sensitive direct current amplifier can be used for current measurements for medium activity work or a vibrating reed electrometer for low level work. The latter unit is preferable in both cases, however, since it can be connected to an automatic recorder.

The sensitivity of the ionization chamber and the gas counter are comparable for C^{14} (about 0.01 dis./min./mg. barium carbonate) and both offer special advantages in certain types of industrial and biological research. While it is possible using G. M. tubes and solid samples to study biological reactions in small animals, such as mice and rats with one to 50 μcuries of C^{14}, the amount of radioactivity required for larger animals, such as man, is too great, both from the cost and radiation viewpoint. Thus, if 1 μcurie would serve for an experiment in a 20 gram mouse, 3 millicuries would be needed in a 60 kg. human. For such experiments ionization chambers or gas counters are almost a necessity; these instruments are 10^2 to 10^3 times more sensitive than solid counters.

In development work on a pilot plant scale or with a small industrial unit, the use of such sensitive measurements make studies possible with a reasonable financial outlay for radioactive materials and a negligible contamination problem. Since the ionization chamber, the vibrating reed electrometer and a good recording potentioneter can assay carbon 14 to 1% precision, this method is particularly useful in mechanism, rate and isotope effect studies.

Isotopic Synthesis: -

Preliminary Considerations. A number of special considerations should be made before a synthesis of a labeled organic compound is begun. They include such factors as the desired specific activity of the final product, amount of activity available for the synthesis, and the effect of radioactive decay in work with short half life isotopes.

The desired specific activity in terms of radioactivity per unit of weight will depend on the use to be made of the organic compound. Thus, if one desires

Br^{82} labeled dibromobenzene to study a reaction mechanism, the specific activity of the dibromobenzene prepared should be low enough so that the samples can be easily counted without special dilution and yet high enough so that any radioactivity incorporated into products of side reactions could be measured to the needed accuracy. Actual activities needed depends on the isotopes, the experiment and the counting equipment.

If the synthesized radioactive compound is to be used in a biological experiment in which extensive dilution with inactive material occurs, then quite high specific activity compounds are necessary. Thus, 1 μcurie of C^{14} per milligram organic compound has been found in this laboratory to be a convenient minimum specific activity for carbon metabolic studies with amino acids, fatty acids and metabolic intermediates. With such an activity level sufficient radioactivity can be given in one dose for in vivo and in vitro work and yet with small enough mass of material to avoid drastic flooding of the biological system. For drugs much higher specific activities may be necessary if the prescribed dose is small, as it is, for example, in stilbestrol.

From the specific activity required for the experiment and the amount of activity available for the synthesis the scale of the reaction may be calculated. Thus, if one has 10 millicuries of C^{14} (value $360) and desires to prepare sodium acetate-1-C^{14} with a specific activity of 5 μcuries/mg., the reaction would have to be done on a 24.4 mmole scale. Alternately, if one were studying a reaction mechanism and desired the sodium acetate to have a specific activity of 10^4 dis./min./mg., the preparation could be carried out on a 22 gram scale using but 0.1 millicurie of C^{14} (value $3.60) (b). With

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(b) 1 mc. = 2.2×10^9 dis./min.

$$\text{Scale of reaction} = \frac{\text{Total activity}}{\text{Desired sp. act.}} = \frac{0.1 \times 2.2 \times 10^9}{10{,}000} = 22{,}000 \text{ mg.}$$

$$= 22 \text{ grams}$$

other isotopes similar calculations can be made. If the half life is short, the decay losses during the preparation and the experiment must be considered so that the final specific activity is high enough to give needed counting precision.

For stable isotopes a comparable calculation of the required specific activity must be made using the precision of the spectrometer, the accuracy needed in the results, and the dilution expected in the experiment. In general much less dilution is possible with stable isotopes for a given amount of material than is possible with the radioactive materials. However, for oxygen and nitrogen tracer experiments there are no radioactive substitutes and in some biological experiments the lack of radiation hazard of the stable isotopes makes their use mandatory.

Carbon 11 and fluorine 18 require very rapid synthetic procedure. Since these two isotopes are usually made by cyclotron bombardment, this also means that research with them is only conveniently carried out in the immediate environs of a nuclear particle accelerator.

Standard small-scale organic reactions are usually adequate for C^{14}, C^{13}, N^{15}, O^{18} and D with such modification of existing procedure as is necessary. The value of the material calls for procedures giving a good yield and attention must be given to the nature of the starting material. As indicated previously, syntheses with radioactive isotopes will vary from large scale to micro reaction, depending on the specific activity of the final product and the amount of labeled material available.

Since it is often necessary to make radiochemical syntheses on a high yield and small scale, special considerations must be given to the adaptability of a preparation to such work, to the detection and elimination of radioactive

impurities from small amounts of product and to methods available for handling the compounds. If there is any question as to the exact position of label, degradation of the product may be necessary.

Choice of Synthesis Method. One of the best methods for the production of radiochemically pure labeled compounds is to develop or adopt a synthetic method which will either produce a pure compound directly or give a mixture of materials from which the desired product can be easily separated in a pure form. Thus, it may seem practical to prepare alanine-3-C^{14} ($C^*H_3CHNH_2CO_2H$) by the condensation of C^*H_3I with sodium acetamidomalonic ester followed by hydrolysis and decarboxylation of the intermediate thus formed. Actually it is found that this method will lead to a product containing small amounts of labeled sarcosine (N-methylglycine) (75). The separation of these two isomers is not easy on a small scale in good yields. Therefore, the preparation of this labeled alanine from labeled methyl iodide by condensation with alkali phthalimidomalinic ester is a more satisfactory method. Alternately, one can prepare propionic-3-C^{14} acid and by bromination and amination of the acid make alanine (75) labeled with carbon 14.

Chromatography. Adsorption and partition chromatography have found an important place in radiochemical preparations both as an analytical technique and as a purification procedure. The techniques are easily adapted to small scale work and are capable of producing a very pure product with negligible loss. (76, 77). Recent advances in the preparation and use of ion exchange resins and of paper chromatography has further extended the range and applicability of these methods to radiochemistry (78).

Some typical examples of the use of chromatography in radioorganic chemistry are as follows: The separation and purification of amino acid mixtures together

with various inorganic salts in the synthesis of amino acids by the use of ion exchange resins (75, 79, 80); the separation of plant acids on silica gel in photosynthesis studies (81); the purification and isolation of labeled cholesterol and other steroids on alumina (82); the purification of iodine 131 labeled thyroxine by one and two dimensional paper chromatography (83); and the separation of labeled glucose and fructose on hydrated magnesium acid silicate by elution with 95% ethanol (84).

One and two dimensional paper chromatography combined with radioautographs of the paper is one of the most powerful analytical tools available for the investigations of unknown mixtures of radiochemicals. Thus, in photosynthesis studies with radioactive carbon dioxide, two dimensional paper chromatography has been extensively used to identify the compounds formed (85, 86, 87). Figure 1. Paper chromatography and radioautographs is one of the best ways to check a non-volatile radioactive synthetic product to make sure it does not contain any other labeled compounds (75, 79, 88).

Vacuum system manipulation of volatile compounds. When small amounts of volatile organic compounds must be transferred from one container to another, extensive surface adsorption and volatilization losses may occur by the use of conventional handling methods. In such instances, a distillation transfer in a simple vacuum line can often give excellent results (12, 89, 90). Thus one can easily handle a few hundredths of a ml. to 1 ml. of such compounds as methyl iodide, ethanol or acetic acid with very small losses. Under these conditions radiation hazards due to possible inhalation of the material is also minimized, since the compound is never exposed to the air (12). See Figure 2.

Alternate procedures for the handling of volatile radioactive compounds include the use of a sweep system with a carrier inert gas (such as nitrogen or helium) and the use of an organic solvent to reduce the partial pressure of the radioactive compounds to the point where losses become negligible. The first of these systems is important where liquid air is not available, and labeled carbon dioxide must be transferred, as in a Grignard reaction.

Isotopic dilution. A convenient method for the quantitative analysis of a mixture of material for one component is by isotope dilution. This technique is particularly useful if it is very difficult or impossible to quantitatively isolate the desired component. This procedure may be used either to measure the amount of a radioactive compound in an unknown mixture, or by use of a labeled diluent, to determine the quantity of the normal material in the mixture (91).

The experimental procedure is very simple for radioactive materials. When the unknown mixture is not labeled, a known weight of the given component containing a known specific radioactivity is added to the mixture. This compound is then isolated pure from the mixture in sufficient quantity for analytical purposes. The decrease in the original specific activity of the component is used to determine the amount of the compound in the mixture.

If an unknown amount of a radioactive compound is present in a mixture containing no other labeled materials it can be similarly analyzed. A known quantity (weight) of the mixture containing a known amount of radioactivity is added to a known weight of the compound (inactive). By suitable purification a small amount of the pure compound is isolated. From the specific activity of this material the true specific activity of the radioactive compound (and the percent thereof in the mixture) can be calculated.

The simple relationships which may be formulated for radioactive isotopes are usually not satisfactory when stable isotopes are used. Here, the natural concentration of the isotope in the unlabeled material is often appreciable. In such instances, more elaborate equations are necessary to solve for the amount of desired material in the original unknown mixture. Equations for the calculation are given by a number of authors (92, 93, 94).

Partition. For certain special types of purification and analysis, partition of compounds between immiscible solvents or counter-current extraction offers some very useful possibilities (95, 96). Hand manipulation of separatory funnels or counter-current extraction units may be used in such work (97, 98, 99). The method is particularly useful for isolation and identification of small quantities of material and for analysis of mixtures of complex compounds from biological sources.

Degradation. The degradation of an organic compound to determine the positions of isotopic labeling is an important procedure in many tracer studies. In the synthesis of radioactive compounds it is used to confirm the synthetic procedure if there is any question on the nature of the reaction, and it is, of course, of prime importance in any mechanism studies.

The techniques used in such degradation studies are quite varied but usually consist of some type of selective oxidation or decarboxylation reaction. A number of typical degradation procedures are listed below as examples:

a) Degradation of acetic acid by decarboxylation of barium acetate to CO_2 (carbon 1) and acetone, followed by alkaline potassium triiodide oxidation of the acetone to give iodoform (carbon 2). (100, 101)

b) Degradation of lactic or propionic acid by chromic acid oxidation to CO_2 (carbon 1) and acetic acid, which can then be degraded as described above (102,103).

c) Degradation of pyruvic acid by oxidation to CO_2 (carbon 1) with ceric sulfate (104, 105).

c) Degradation of sugars in general and glucose in particular by (1) fermentation oxidation to lactic acid followed by degradation of the lactic acid as described above (103); (2) fractional oxidation of the sugar and various derivatives with lead tetraacetate (106); (3) fractional periodate oxidation (107).

e) Degradation of propylene by oxidation with permanganate or ozone to CO_2 (carbon 1) and acetic acid, which was further degraded as described above (108).

f) Degradation of benzoic acid by decarboxylation with sodium azide in concentrated sulfuric acid-chloroform solution (Schmidt reaction) (109).

g) Degradation of ethyl alcohol by sulfuric-chromic acid oxidation to acetic acid, followed by degradation of the acetic acid as described above (110).

h) Degradation of succinic acid by the Curtius reaction to give CO_2 (carbons 1 and 4) and ethylenediamine (carbons 2 and 3) (111).

i) Degradation of labeled cholesterol using oxidation and pyrolysis of derivatives (112).

REFERENCES

1) "Manual of Radioactivity," by G. Hevesy and F. A. Paneth, Oxford University Press (1938).

2) "Nuclear Radiation Physics," by R. Lapp and H. L. Andrews, Prentice-Hall, Inc., New York, N. Y. (1948).

3) "Elementary Nuclear Theory," by H. A. Bethe, John Wiley and Sons, Inc., New York, N. Y. (1947).

4) "An Introduction to Nuclear Physics," by S. Flueege, Interscience Publishers, Inc., New York, N. Y. (1946).

5) "Applied Nuclear Physics," by E. C. Pollard and W. L. Davidson, John Wiley and Sons, Inc., New York, N. Y., Second Edition, (1950).

6) "Mass Spectra and Isotopes," Second Edition, by F. W. Aston, Longmans, Green, Inc., New York, N. Y., (1942).

7) "Radioactive Tracers in Biology," by M. D. Kamen, Second Edition, Academic Press, Inc., New York, N. Y. (1951).

8) "Isotopic Tracers and Nuclear Radiation with Application to Biology and Medicine," by W. E. Siri, McGraw Book Co., New York, N. Y. (1949).

9) "Introduction to Radiochemistry," by J. Friedlander and J. W. Kennedy, John Wiley and Sons, Inc., New York, N. Y. (1949).

10) "Radioactive Indicators," by George Hevesy, Interscience Publishers, Inc., New York, N. Y. (1948).

11) "Radioactivity Applied to Chemistry," edited by A. C. Wahl and N. A. Bonner, John Wiley and Sons, Inc., June, 1951.

12) "Isotopic Carbon" by M. Calvin, C. Heidelberger, J. Reid, B. Tolbert and P. Yankwich, John Wiley and Sons, Inc., New York, N. Y. (1948).

13) "Radioisotopes in Pharmaceutical and Medical Studies," by D. L. Tabern, J. D. Taylor and G. I. Gleason. Nucleonics, 7, No. 5, 3-23; 7, No. 6, 40-55 (1950); and 8, No. 1, 60-77 (1951).

14) "A Manual of Artificial Radioisotope Therapy," by P. F. Hahn, Academic Press, New York, N. Y. (1951).

15) "Isotopic Tracer Techniques," by H. R. V. Arnstein and R. Bentley, Quarterly Reviews (Chem. Soc. London), 4, No. 2, 172 (1950).

16) "Biological Synthesis of Radioisotope Labeled Compounds," by N. H. Woodruff and E. E. Fowler. Nucleonics, 7, No. 2, 26 (1950).

17) "Chemical Syntheses of Radioisotope Labeled Compounds," by C. E. Crompton and N. H. Woodruff. Nucleonics, 7, No. 3, 49-64; 7, No. 4, 44-61 (1950).

18) "Measurements of Radioactivity." National Bureau of Standards Circular No. 476, U.S. Department of Commerce, Washington, D. C.

19) "Electron and Nuclear Counters," by S. A. Korff, D. Van Nostrand Company, New York, New York (1946).

20) "Ionization Chambers and Counters," by B. B. Rossi and H. H. Staub, McGraw-Hill Book Company, New York, N. Y. (1949).

21) "Ionization Chambers and Counters," by D. H. Wilkinson, Cambridge University Press (1959).

22) "Table of Isotopes," by G. T. Seaborg and I. Perlman, Rev. Mod. Phys., 20, 585-667 (1948).

23) A. S. Keston, D. Rittenberg and R. Schoenheimer, J. Biol. Chem., 122, 227 (1937).

24) D. Rittenberg and R. Schoenheimer, J. Biol. Chem., 111, 169 (1935).

25) M. B. Allen and S. Ruben, J. Am. Chem. Soc., 64, 984 (1942).

26) D. F. White, I. G. Campbell and P. R. Payne, Nature, 166, 628 (1950).

27) J. F. Black and H. S. Taylor, J. Chem. Phys., 11, 395 (1943).

28) A proportional counter, Radiation Counter Laboratories, Chicago, Ill.

29) A geiger counter, Nuclear Instrument and Chemical Corp., Chicago, Ill.

30) I. Gray, S. Ikeda, A. A. Benson and D. Kritchevsky, Rev. Sci. Inst., 21, 1022 (1950).

31) M. L. Eidinoff and J. E. Knoll, Science, 112, 250 (1950).

32) G. K. Schweitzer and J. T. Garrett, J. Phys. and Coll. Chem., 55, 195 (1951).

33) "Nuclear Data," National Bureau of Standards Circular No. 499, Sept. 1, 1950, Washington, D. C.

34) "Pile Produced Isotopes," available from Eldorado Mining and Refining (1944) Limited, P.O. Box 379, Ottawa, Canada.

35) "Radioactive Materials and Stable Isotopes," Isotope Division, Atomic Energy Research Establishment, Harwell, Berkshire, England.

36) "Isotopes, Catalogue and Price List," Isotopes Division, U.S. AEC, Oak Ridge, Tennessee.

37) "Safe Handling of Radioisotopes," Handbook No. 42, National Bureau of Standards, U.S. Dept. of Commerce, Washington, D. C. (1949).

38) "General Rules and Procedure Concerning Radioactive Hazares," U.S. AEC Isotopes Division Circular B1, Oak Ridge, Tenn.

39) J. Schubert, Nucleonics, 8, No. 2, 13-28; No. 3, 66-78; and No. 4, 59-67 (1951).

40) S. Threefoot, T. Ray, G. Burch and J. Cronvich, J. Lab. and Clin. Med., 36, 998 (1950).

41) J. C. Reid and H. B. Jones, J. Biol. Chem., 174, 427 (1948).

42) H. E. Skipper, L. White and C. E. Bryan, J. Biol. Chem., 180, 1187 (1949).

43) N. I. Berlin, B. Tolbert and J. Lawrence, J. Clin. Invest., 30, 73 (1951).

44) J. Govaerts, Science, 111, 467 (1950).

45) J. Schubert and W. D. Armstrong, J. Biol. Chem., 177, 521 (1949).

46) G. Nardi, Science, 111, 362 (1950).

47) R. Gould, F. Sinex, I. Rosenberg, A. Solomon and A. Hastings, J. Biol. Chem., 177, 295 (1949).

48) N. I. Berlin, B. M. Tolbert, H. C. Lee, and C. Lotz, Abstract, Am. Fed. for Clin. Res. Meeting, Chicago, Ill., Nov. 1, 1951.

49) H. E. Skipper, M. J. Bell and J. B. Chapman, Cancer Research, 10, 362 (1950).

H. E. Skipper, C. Nolan and L. Simpson, J. Biol. Chem., 189, 159 (1951)

50) W. Bloom, Anatomical Record, 103, 425 (1949).

W. Bloom, H. J. Curtis, and F. McLean, Science, 105, 485 (1947).

51) A. M. Brues and D. L. Buchanan, Cold Spring Harbor Symposia, Quant. Biol., 13, 52 (1948).

52) J. C. Weaver, B. Tolbert and B. Krueckel, Proc. Soc. Ex. Biol. and Med., 75, 741 (1950).

53) H. Elliot, F. Chang, I. Abdou and H. Anderson, J. Pharm. and Exp. Therap., 95, 494 (1949).

54) C. Heidelberger and H. B. Jones, Cancer, 1, 252 (1948).

55) H. Elliot. Private Communication.

56) "The Body Absorption, Distribution and Excretion of Tritium in Man and Animals," by E. A. Pinson and E. C. Anderson, U.S. AECU Document 937 (1950).

57) "Retention by Mice of Tritium Administered as the Oxide," R. C. Thompson, Hanford Engineering Works Report No. HW-21494, Hanford, Wash., June 19 (1951).

58) H. Tarver and C. Schmidt, J. Biol. Chem., 130, 67 (1937).

59) "Design of Laboratories for Safe Use of Radioisotopes," by D. R. Ward, Isotopics, 1, No. 2, 10 (1951), U.S. AEC, Isotopes Div., Oak Ridge, Tenn.

60) 1950-1951 Buyers Guide, Nucleonics, 7, No. 5, D1 (1950).

"Equipment for Radioactive Laboratories," Nucleonics, 7, No. 5, R1 (1950).

61) Symposium on Radiochemistry Laboratories; Articles by P. C. Tompkins, H. A. Levy, W. P. Norris, J. W. Swartout, N. B. Garden and C. N. Rice, Ind. and Eng. Chem., 41, 228 (1949).

62) G. G. Manov, Design of Small Scale Radiochemical Laboratory, U.S. AEC Report TID-388, March 1951.

63) P. C. Tompkins, O. Bizzell and C. D. Watson, Ind. and Eng. Chem., 42, 1469 (1950).

64) S. E. Eaton and R. J. Bowen, Nucleonics, 8, No. 5, 27 (1951).

65) O. M. Bizzell, Isotopics, 1, No. 1, 21 (1951), U.S. AEC, Isotopics Division.

66) "Design of Auxiliary Equipment and Services for a Radiochemical Laboratory," by C. H. Guest and L. G. Cook, National Research Council of Canada, Atomic Energy Project Report CRHR-386 (1948).

67) W. W. Miller, Science, 105, 123 (1947); S. C. Brown and W. W. Miller, Rev. Sci. Inst., 18, 496 (1947); and M. L. Eidinoff, Anal. Chem., 22, 529 (1950).

68) W. Bernstein and R. Ballentine, Rev. Sci. Inst., 21, 158 (1950).

69) D. D. Van Slyke, R. Steele and J. Plazin, J. Biol. Chem., 192, 769 (1951).

70) Radiation Counter Laboratories, Chicago, Ill.

71) F. H. Marshal and J. W. Coltman, Phys. Rev., 72, 528 (1947).

72) P. R. Bell, Phys. Rev., 73, 1405 (1948).

73) J. W. Coltman, Proced. of the I.R.E., 37, No. 6, 671 (1949) (A review).

74) C. D. Janey and B. J. Moyer, Rev. Sci. Inst., 19, 667 (1948).

75) "Synthesis of Several C^{14}-Labeled DL-Alanines," by R. Ostwald, P. Adams and B. Tolbert, Univ. of Calif. Radiation Lab. Report No. 962, unclassified, Oct. 13, 1950.

76) H. H. Strain, Anal. Chem., 22, 41 (1950).

77) H. H. Strain, Anal. Chem., 23, 25 (1951).

78) D. L. Clegg, Anal. Chem., 22, 48 (1950).

79) "An Improved Synthesis of Glycine-1-C^{14} and Glycine-2-C^{14} from C^{14} labeled Acetic Acid," by B. M. Tolbert, D. M. Hughes and R. Ostwald, Univ. of Calif. Rad. Lab., Report No. 705, May 16, (1950).

80) C. S. Cleaver and H. G. Cassidy, J. Am. Chem. Soc., 72, 1147 (1950).

81) R. E. Stutz and R. H. Burris, Plant Physiology, 26, 226 (1951).

82) D. Kritchevsky. Private Communication.

83) R. M. Lemmon, W. Tarpy and K. G. Scott, J. Am. Chem. Soc., 72, 758 (1950).

84) "The Biosynthesis of Carbon 14 labeled compounds. I. The chromatographic separation of Glucose and Fructose," by G. R. Noggle and R. A. Bolomey, Oak Ridge National Labs., Report No. 787, Unclassified, Aug. 17 (1950).

85) M. Calvin and A. A. Benson, Science, 109, 140 (1949).

86) A. A. Benson, J. A. Bassham, M. Calvin, T. Goodale, V. Haas and W. Stepka, J. Am. Chem. Soc., 72, 1710 (1950).

87) M. Calvin, J. Chem. Ed., 26, 639 (1949).

88) W. Tarpey, H. Hauptmann, B. Tolbert and H. Rapoport, J. Am. Chem. Soc., 72, 5126 (1950).

89) J. D. Cox, H. S. Turner and R. J. Warne, J. Am. Chem. Soc., 72, 3157 (1950).

90) B. M. Tolbert, J. Am. Chem. Soc., 69, 1529 (1947).

91) R. Abrams, Arch. Biochem., 30, 44 (1951).

92) M. Calvin, et.al., "Isotopic Carbon," John Wiley and Sons, Inc., New York, New York (1949), pp. 278 to 282.

93) F. C. Henriques, Jr. and Charles Margnetti, Ind. Eng. Chem., Anal. Ed., 18, 420 (1946).

94) H. Gest, M. D. Kamen and J. M. Reiner, Arch. Biochem., 12, 273 (1947).

95) J. D. Gregory and L. C. Craig, Annals New York Acad. Science, 53, 1051 (1951).

96) L. C. Craig and D. Craig, Techniques of Organic Chem. III, A. Weissberger, Editor, Interscience, New York (1950).

97) L. C. Craig, J. Biol. Chem., 155, 519 (1944).

98) M. W. Kies and P. L. Davis, J. Biol. Chem., 189, 637 (1951).

99) W. O. Ney, Jr., and H. L. Lochte, Ind. Eng. Chem., 33, 825 (1941).

100) H. A. Barker and M. Kamen, Proc. Natl. Acad. Sci., U.S., 31, 219 (1945).

101) S. Aronoff, V. Haas and B. A. Fries, Science, 110, 476 (1949).

102) P. Nahinsky and S. Ruben, J. Am. Chem. Soc., 63, 2275 (1941).

103) S. Aronoff, H. A. Barker and M. Calvin, J. Biol. Chem., 169, 459 (1947).

104) M. Utter, F. Lipmann and C. Werkman, J. Biol. Chem., 158, 521 (1945).

105) H. Wood, B. Vennesland and E. Evans, J. Biol. Chem., 159, 153 (1945).

106) S. Abraham, J. Am. Chem. Soc., 72, 4050 (1950).

107) H. Wood, N. Lifson and V. Lorber, J. Biol. Chem., 159, 475 (1945).

108) B. A. Fries and M. Calvin, J. Am. Chem. Soc., 70, 2235 (1948).

109) W. Doering, T. Taylor and E. Schoenewaldt, J. Am. Chem. Soc., 70, 445 (1948).

110) G. R. Bartlett and H. N. Barnet, Quart. J. of Studies on Alcohol, 10, 381 (1949).

111) A. A. Benson and J. A. Bassham, J. Am. Chem. Soc., 70, 3939 (1948).

112) H. N. Little and K. Bloch, J. Biol. Chem., 183, 33 (1950).

CARBON

Much of the chemistry involved in the synthesis of compounds containing isotopic carbon is, essentially, the chemistry of one and two carbon compounds. Once the proper intermediates are available, their incorporation into more complex organic compounds is limited only by the ingenuity of the investigator.

Inasmuch as many of the one and two carbon intermediates are gases or volatile liquids and since small quantities of material are generally used in efforts to obtain the highest possible specific activity, vacuum techniques and flow systems play an important role in many of these syntheses. Because of the low pressures used, relatively unstable compounds may be distilled and the materials contained within a closed system for the duration of the experiment.

The form in which the particular carbon isotope is available determines the initial steps in its utilization. Isotopic carbon is from prime suppliers as carbon monoxide-C^{11}, sodium cyanide-C^{13} and barium carbonate-C^{14}. Carbon-14 is also available in the form of a variety of organic compounds.

One carbon compounds:-

As has already been pointed out, the preparation of one carbon intermediates in good yields and in a high state of purity holds the key to success of many synthetic operations involving isotopic carbon. Of the one carbon compounds, carbon dioxide, sodium or potassium cyanide and methyl iodide are the most widely used. Formaldehyde and diazomethane have also found application in various syntheses.

A satisfactory method for the preparation of potassium cyanide, isotopically labeled, from barium carbonate involves reduction of carbon dioxide with potassium and ammonia:

$$C^{*}O_2 + 4K + NH_3 \xrightarrow{\Delta} KC^{*}N + KH + 2KOH$$

This method has been used to prepare potassium cyanide-C^{11} (1) and potassium cyanide-C^{14} (2) from the corresponding barium carbonate in yields of 90-96%. Recently an improved method for the preparation of the required potassium mirror has been reported (3).

Hydrogen Cyanide[2]. - Eight-tenths of a gram of clean potassium was wiped with a dry towel and dropped to the bottom of a nitrogen filled Pyrex tube 10 by 400 mm. A constriction was then quickly made about 4 cm. from the top and the tube was evacuated to 0.01 mm. Hg through flexible rubber tubing. Before melting the potassium, the whole tube was brushed with a hot flame to remove as much residual mineral oil as possible. The potassium was then melted and the still warm tube was shaken horizontally to distribute the potassium throughout its length. When cool, the tube was clamped in a horizontal position and individual spots of the lower surface were heated with a tiny flame while the other surfaces were cooled with a wet cloth. With practice, a mirror covering 95% of the inner surface is obtained.

The carbon dioxide from 8.50 mg. of $BaC^{14}O_3$ (ca. 3-6%; ca. 85 μc) and 179 mg. $BaCO_3$, and 2.0 mmole of NH_3 were condensed in the reaction tube with liquid nitrogen. The tube was sealed off at the constriction and placed in a steel bomb tube.

By keeping the nichrome-wound furnace at red heat, the steel bomb tube and the pyrex tube inside it could be brought to 620° C. in eight minutes (determined in "dry runs" with a Chromel-Alumnel thermocouple) and then held at that temperature

by reducing the input voltage. After twelve minutes more, the steel tube was removed, cooled, and opened. The Pyrex tube was chilled in liquid nitrogen and the (generally) small residual pressure released by heating with a hot flame.

The small amount of unreacted metal and the hydrides were decomposed with ethyl alcohol and water. The suspension was transferred to a generating flask on the vacuum line. The alcohol and dissolved gases were removed in vacuo, formic acid was added and the hydrogen cyanide was distilled into a trap along with about 1 cc. of water. (With inactive CO_2, the yield here is 90-96%, average d92%.)

The preparation of sodium cyanide-C^{14} by sodium azide reduction of radioactive barium carbonate has been carried out in 80% yield (4):

$$NaN_3 + BaC^*O_3 \xrightarrow{\Delta} NaC^*N + N_2 + BaO + O_2$$

Abrams (5) has prepared radioacyanide in average yields of 60-70% by the following method:

$$C^*O_2 + 2Mg \longrightarrow 2C^* + 2MgO$$

$$C^* + NH_3 \xrightarrow{1000^\circ} HC^*N + H_2$$

Another preparation of radioactive cyanide involves the decomposition of triphenylacetonitrile-1-C^{14} with sodium as shown below (6):

$$(C_6H_5)_3CNa \xrightarrow{CO_2} (C_6H_5)_3C\text{-}C^*OOH \longrightarrow (C_6H_5)_3C\text{-}C^*ONH_2 \longrightarrow (C_6H_5)_3C\text{-}C^*N$$

$$\xrightarrow[EtOH]{Na} (C_6H_5)_3CH + NaC^*N$$

McCarter (7) has recently reported a synthesis of cyanide-C^{14} in 90% yield by heating a mixture of potassium carbonate-C^{14} and zinc dust in a stream of ammonia:

Preparation of C^{14}-Cyanide from C^{14}-Carbonate[7]. - Powdered anhydrous C^{14}-potassium carbonate (0.001 mole) was thoroughly mixed with approximately 1 g. of zinc dust (Reagent grade) and the mixture was transferred to a porcelain combustion boat (Coors Size 2). The boat was then placed in a Vycor combustion tube (750 mm. in length and 19 mm. inside diameter) containing 3-4 g. of iron wire (0.01 inch diameter "for standardizing") in the form of a loose ball occupying the mid-portion of the tube. The boat was pushed into the tube until it touched the iron wire.

The end of the tube nearest the iron wire was connected to an apparatus for the preparation of dry ammonia gas. The other end was attached to a glass tube dipping beneath the surface of water in a test-tube in order to serve as an indicator of the rate of flow of the gas. Ammonia was prepared by warming concd. ammonium hydroxide and dried by passing through two towers of calcium oxide and one of sodium hydroxide pellets. A stream of ammonia was allowed to flow through the tube and when the air in the apparatus had been displaced the central portion of the combustion tube (that part occupied by the boat and iron wire) was heated electrically at 650° for 4 hours. During this time the flow of gas was maintained at a rapid rate but not so rapid as to force water out of the test-tube. The flow of ammonia was continued while the tube was allowed to cool to room temperature.

The boat and its contents were then transferred to an apparatus for the distillation of hydrogen cyanide. The boat was placed in a suitable erlenmeyer flask connected to a water-cooled condenser and 25 ml. of water was added. The tip of the condenser dipped below the surface of an excess (20%) of the theoretical amount of 1 N sodium or potassium hydroxide. The contents of the flask was then acidified by the addition of 2 N sulfuric acid and was heated gently until 15 to 20 ml. of distillate had been collected. The alkaline cyanide solution was then evaporated to dryness in vacuo or used directly.

Analysis by the argentimetric method of the distillate obtained in several experiments showed yields of 88-93% of theory with an average yield of 90%. The specific activity of C^{14}-labeled cyanide prepared by this procedure was unchanged from that of potassium carbonate used as the starting material. C^{14}-labeled potassium carbonate was readily obtained by passing $C^{14}O_2$ into a slight excess of 4 N potassium hydroxide and evaporating the solution to dryness. The yield of cyanide was decreased by heating the reaction mixture below 630° or over 670°. It was found that the conversion could be accomplished in higher yields in an iron tube but the product thus obtained was less pure than that obtained using the procedure described in this communication.

Labeled barium cyanide has been prepared by reduction of isotopic barium carbonate with ammonia at 850° (8).

Labeled methanol has been prepared in good yields by the reduction of carbon dioxide over a potassium oxide-copper oxide-alumina catalyst (9):

> Direct Reduction of Carbon Dioxide with Hydrogen[9]. - The carbon dioxide is generated in an evacuated system from barium carbonate by the action of concentrated sulfuric acid dropped from a pressure-equalizing funnel. The dioxide is condensed in a liquid-nitrogen trap, and the air which had been entrapped in the barium carbonate is pumped off. The amount of carbon dioxide used in a reduction may be checked by measurement of its pressure in a system of known volume.
>
> The carbon dioxide is reduced in a small hydrogenation bomb with a free volume of 300 cc. The bomb containing the catalyst is warmed with a flame and evacuated to a pressure of 30 microns. The bomb is then half immersed in liquid nitrogen, and the carbon dioxide is distilled in. The final pressure in the system of such a distillation into an iron bomb cannot usually be reduced below about 40 to 50 microns.
>
> After introduction of the carbon dioxide, the bomb is closed, removed from the line, and warmed to room temperature, and hydrogen is added to 4,000 psi. (Caution: Do not add hydrogen until the bomb is warm, for steel is brittle when very cold.) A booster pump is needed to add the hydrogen at sufficient pressure. Care must be taken when the hydrogen is forced into the bomb that the pressure of hydrogen on the supply side is always higher than the pressure in the bomb; this eliminates any possibility of carbon dioxide flowing

back into the storage tanks or booster pump. A high-pressure gauge should be placed on the pump side of the system as well as on the reaction vessel.

The reduction of the carbon dioxide may proceed with an initial pressure of 3,500 psi (room temperature), but this is very close to the point where reduction is incomplete. An initial pressure of 4,000 psi (final pressure, 7,000 psi) is preferable. The bomb is heated six hours at 285° C. The products of the reduction and the remaining carbon dioxide are caught in a combination spiral and sintered-glass disk trap cooled in liquid nitrogen; the hydrogen is discharged from the bomb through the spiral system at a rate of 1-2 l/min. The remaining products and the contents of the sintered-glass trap are distilled from the warmed bomb into the larger trap on the line.

The carbon dioxide that is not reduced is separated from the water-methanol mixture by distillation of the product through a spiral trap cooled in a Dry Ice bath. The remaining carbon dioxide is usually 3-4% of the initial gas.

The catalyst has been found to be slowly poisoned; it is unreliable when used for more than two reductions and, preferably, the catalyst should be used only once. The efficiency of any given batch of catalyst should be tested with an inactive run before use with radioactive material. The yield is found to be about 85% to 90% based on carbon dioxide. The product contains appreciable quantities of water, which cannot be separated from the methanol.

Methanol-C^{11} (9) and methanol-C^{14} (11) have been prepared by the reduction of methyl formate labeled with the respective isotope over copper chromite. In this procedure, only the formic acid moiety of the ester is labeled, thus causing a dilution in the final product.

Direct reduction of isotopic carbon dioxide with lithium aluminum hydride (12) has also been used to prepare labeled methanol:

$$4\overset{*}{C}O_2 + 3LiAlH_4 \longrightarrow LiAl(O\overset{*}{C}H_3)_4 + 2LiAlO_2$$

$$LiAlH_4 + 4ROH \longrightarrow LiAl(OR)_4 + 4H_2$$

$$LiAl(O\overset{*}{C}H_3)_4 + 4ROH \longrightarrow LiAl(OR)_4 + C^*H_3OH$$

ROH = n-butyl carbitol

The reaction as outlined above was carried out in diethyl carbitol with n-butyl carbitol serving as the decomposing alcohol. In lithium aluminum hydride reductions it has been noted that fission of the solvent ether may take place during the course of the reaction which, in cases as micro syntheses, may introduce impurities which are hard to separate from the desired product. Butyl carbitol was used in an effort to avoid low boiling contaminants and to afford a solvent from which the lower boiling methanol could be easily separated. In a study of this reaction it was observed that using tetrahydrofurfuroxy tetrahydropyrane as the solvent for the reducing agent and tetrahydrofurfuryl alcohol for alcholysis, methanol was obtained in 90% yield with only water and formaldehyde as impurities (13). Further work (14) has shown that with diethylene glycol diethyl ether as the solvent for the lithium aluminum hydride and benzyl alcohol, diethylene glycol monobutyl ether or ethylene glycol monophenyl ether for alcoholysis yields of isotopic methanol of 68-77% were obtained (based on conversion to methyl iodide). When tetrahydropyran was used as solvent for lithium aluminum hydride and alcholysis was carried out with diethylene glycol monobutyl ether, the yield was 66%. It is

adviseable to be aware of the possibility of fission products of the solvent whenever this reaction is carried out.

Conversion of methanol to methyl iodide can easily be carried out by heating with hydrogen iodide (10,11) or with phosphorus and iodine (9). In both cases cited, conversion of carbon dioxide to methyl iodide via methanol can be carried out uninterruptedly. A typical example follows:

Conversion of Methanol to Methyl Iodide[9]. - Into a Carius tube sealed to an 8-mm stopcock, 10 grams of iodine are introduced through a long-stemmed funnel. The tube is chilled in Dry Ice or liquid air (to prevent reaction of iodine and phosphorus), and 2 grams of red phosphorus and 3 ml. of water added. The water reduces the pressure in the tube (during the following reaction) since the hydrogen iodide formed dissolves in it; the water does not interfere with the conversion of the methanol to methyl iodide.

The tube is evacuated, and the methanol or methanol-water mixture is distilled in. The tube is removed from the line, and the stopcock is clamped on. A water jacket is added to the upper half of the tube, and the reaction mixture is warmed carefully (if necessary a cold bath is used to control the initial reaction). The reaction mixture is refluxed for 1 hour on the steam bath; then the tube is transferred to the vacuum line and the methyl iodide, together with part of the water, hydrogen iodide, and phosphine, is distilled, with pumping, into a trap and then distilled into a reaction vessel (about 100-cc volume) containing 10 ml. of water. This vessel is removed from

the line, warmed to room temperature, and shaken vigorously about 1 minute. It is then reconnected to the line, and the methyl iodide along with some water is distilled into a reaction tube containing 4 to 5 grams of phosphorus pentoxide. This tube is removed from the line, warmed to room temperature, and shaken intermittently for half an hour. The phosphorus pentoxide reacts with most of the phosphine and dries the methyl iodide; enough should be used to leave some dry powder after the methyl iodide is distilled. The methyl iodide is transferred to a storage vessel in vacuo. The yield is about 95% based on methanol.

Labeled diazomethane has been prepared from methylamine-C^{13} (15). The details follow:

Preparation of Methylamine Sulphate[15]. - ^{13}C-Methylphthalimide (3·19 g.) from phthalimide and labeled methyl iodide was heated under reflux with constant-boiling hydrochloric acid (7 ml., 100% excess) for 4 hours, the crystals deposited in the condenser being washed down with more acid from time to time. The reaction mixture was cooled to 0°, made alkaline with sodium hydroxide, and distilled in a Kjeldahl ammonia-distillation apparatus; the distillate was collected under 1·038N-sulphuric acid (19·00 ml.). 0·18 Ml. of 1·00N-methylamine solution was required to neutralise the excess of acid, so that 19·6 millimoles (100%) of methylamine had distilled. ^{13}C-Methylamine sulphate was obtained by evaporating the solution to dryness.

To a 3·55M-solution of methylamine sulphate (5·00 c.c., 17·75 millimoles) was added 10N-potassium hydroxide solution (3·55 cc.). Mesityl oxide 4·5 cc.) was run dropwise into the

magnetically stirred solution at 0°, then the reaction mixture was allowed to warm to room temperature during 2 hours. The solution was cooled to 0° again, and acetic acid (4.5 cc.) added dropwise followed by a solution of sodium nitrite (5 g. in 7 cc. of water). Finally, the nitroso-compound was extracted (continuously) with ether from the reaction product, and diazomethane generated from the extract as described above; 20.9 millimoles of diazomethane (59%) were thereby obtained. In two experiments on the 5-millimoles scale, the yield was 40%.

Isotopic formic acid has been prepared by the reduction of potassium bicarbonate-C^{14} (11), by hydrolysis of sodium cyanide-C^{14} (16), reduction of carbon dioxide-C^{14} with lithium borohydride (17) and oxidation of formaldehyde with "superoxol" (18). A typical experiment is detailed here:

Potassium Bicarbonate[11]. - 151.7 mg. of barium carbonate, containing approximately 0.94 millicurie of C^{14}, were placed in the reaction chamber connected to a dropping funnel and, via an inlet tube, to a trap. The separatory funnel contained 10 ml. of 40 per cent perchloric acid. Inside the trap was placed a glass test-tube, 18x180 mm., the side of which carried a small vent-hole 60 to 70 mm. from the bottom. In this tube, which was used later as the glass liner for the hydrogenation bomb, was placed 1.0 ml. of 0.832 N potassium hydroxide solution. The trap and contents were cooled in a dry ice-cellosolve mixture, and the apparatus was flushed out with a stream of carbon dioxide-free nitrogen gas. The alkali trap was then cooled in

liquid nitrogen, and the perchloric acid solution was added dropwise to the barium carbonate, with a slow stream of nitrogen passing through the apparatus. After 45 minutes the stop-cock connecting the the trap and separatory funnel was closed, the trap was evacuated by means of an oil pump to 1 or 2 mm. pressure, and the second stop-cock was closed. The evacuated trap was allowed to stand at room temperature for 24 hours.

The contents of the trap were refrozen by means of a dry ice-cellosolve mixture, and a small amount of carbon dioxide gas remaining in the trap was flushed out by means of a stream of nitrogen gas, and collected in a scrubber containing barium hydroxide. To the frozen solution in the trap were added 2 to 3 ml. of an aqueous suspension containing 50 to 100 mg. of freshly prepared palladium black catalyst, and this mixture was allowed to freeze as a layer above the carbonate-bicarbonate solution. The test-tube containing the frozen mixture was removed from the trap and sealed off at the top. The liquid was allowed to melt, a drop of phenophthalein solution was added through the side vent-hole, and 0.1 N sulfuric acid was added until the solution was only faintly pink.

Potassium Formate. - The tube containing the potassium bicarbonate and palladium black catalyst was placed in an Aminco high pressure hydrogenation bomb. The apparatus was filled with hydrogen at tank pressure (approximately 100 atmospheres) and heated at 70° with shaking for 24 hours. The hydrogenation mixture was then filtered to remove the catalyst. To this solution were added 258 mg. of non-isotopic potassium formate, and

the solution was evaporated to a semisolid mass in a stream of hot, dry air. The material was dried further in vacuo over phosphorus pentoxide and powdered by means of a glass rod.

Methyl Formate. - To the dry, powdered potassium formate was added 0.6 ml. of freshly distilled methyl sulfate in such a manner as to wet the ground glass joint of the flask. The reaction flask was heated slowly over a period of 2 hours to a temperature of 185°, by means of an oil bath, with a slow stream of nitrogen passing through the apparatus. The methyl formate was condensed in trap, which was cooled in a dry ice-cellosolve mixture. The uncondensed gases were passed through a barium hydroxide scrubber. The volume of methyl formate collected in the bottom of the trap was approximately 0.2 ml.

Methyl formate may be prepared by treating labeled potassium formate with methyl sulfate (11) or by heating sodium formate, methanol and sulfuric acid in a microdistillation apparatus (10).

Labeled formaldehyde has been prepared in 50-60% yields by partial oxidation of methanol over copper oxide (19,20), in 65-70% yield using a molybdenum-vanadium catalyst (21) and in 75-80% yield with a molybdenum-iron catalyst (22). A typical experiment is detailed:

Formaldehyde-C^{14} 19. - The catalyst for this oxidation is prepared by covering a copper screen with a heavy layer of freshly precipitated copper hydroxide. The hydroxide is precipitated from a cupric nitrate solution by the addition of dilute ammonium hydroxide; the precipitate is washed with water several times. The copper screen is then rolled, put into a quartz

tube (11 mm o.d., 25 cm long), dried, and reduced with hydrogen at 400° to 500° C. The methanol, which contains the water produced by the reduction of carbon dioxide with hydrogen, is carried over the catalyst bed (heated at 600° C) with a slow stream of air. To achieve the correct proportions of air and alcohol, the water-methanol mixture is heated in a small bubbler to about 70° C and the air is bubbled through it. The reduction products are caught in another small bubbler containing 1 ml of water. Unabsorbed gases are passed through a copper oxide furnace, and the carbon dioxide produced is absorbed in sodium hydroxide. The yield is found to be 50 to 60% based on methanol or 45 to 55% based on carbon dioxide.

A synthesis of formaldehyde-C^{14} from methyl-C^{14}-acetate follows this scheme (23):

$$CH_3CO_2C^{14}H_3 \xrightarrow{Cl_2} CH_3CO_2C^{14}H_2Cl \xrightarrow{H_2O} C^{14}H_2O$$

Urea-C^{14} has been prepared from barium carbonate via barium carbide and barium cyanide (24). Labeled urea has also been obtained by direct reaction of isotopic carbon dioxide and ammonia at high pressures (25) and by reaction of phosgene and ammonia (25).

Reduction of carbon dioxide-C^{11} with zinc has yielded carbon monoxide-C^{11} (26) and reduction of calcium carbonate-C^{13} with zinc has yielded carbon monoxide-C^{13} (27). Labeled carbon monoxide can also be obtained by exchange between carbon monoxide and labeled carbon dioxide (28). Treatment of isotopic carbon monoxide with chlorine yields labeled phosgene (26).

Other interesting one carbon compounds which have recently been reported are cyanamide-C^{14}, prepared by reduction of barium carbonate with ammonia in the presence of sodium azide (29), nitromethane from methyl iodide (30,31) and

methane (32) prepared by reduction of carbon dioxide over a nickel-thoria catalyst. Chlorination of this product yields carbon tetrachloride-C^{14} (32).

Details of many syntheses of one carbon compounds may be found in Calvin's "Isotopic Carbon". (33)

Establishment of a Carbon-Carbon Bond:-

The most common and most readily available one carbon intermediates are carbon dioxide and cyanide and incorporation of a carbon isotope into any larger molecule must, of necessity, center about methods for formation of carbon-carbon bonds involving these intermediates. Thus, carbonation of a Grignard reagent with isotopic carbon dioxide or treatment of a suitable halide with labeled cyanide are the most widely used steps in work involving radiocarbon. The number of carboxyl labeled acids which have been prepared is too great to be detailed here. Excellent expositions concerning the details of the carbonation of Grignard reagents have been published (34,35). A synthesis of acetic acid-1-C^{14}, which has been used to prepare carboxyl labeled acids through C-2 in yields of better than 90% (36), is detailed below:

Acetic Acid-1-C^{14} [35]. - The apparatus used for the synthesis is shown in Figure 3. The essential features are: a manifold H, attached to a high-vacuum system and carrying outlets for attaching the reaction flask K, the carbon dioxide generator F and G, a manometer C, and an inlet B for nitrogen. The carbonation flask K is fitted with an induction stirrer and is pear-shaped in order to permit freezing the Grignard solution without danger of cracking the flask. The carbon dioxide generator consists of an Erlenmeyer flask G for barium carbonate and a compensated funnel F for sulfuric acid. An Erlenmeyer flask is used in order to have the

barium carbonate in as shallow a pile as possible, to facilitate contact with the sulfuric acid. If the charge of barium carbonate is large enough to fill the flask to more than one-fifth of its total volume, a larger flask is substituted. In this case, a round-bottom flask is used since large Erlenmeyer flasks may collapse when evacuated. A drying tube E is placed between the generator and the manifold. The manometer C is the simple open mercury type.

The flask G is charged with 3.535 grams (17.9 mmoles) of barium radiocarbonate, the funnel F with 25 ml. of concentrated sulfuric acid (5-8 ml. of acid per gram of barium carbonate), and the carbon dioxide generator is assembled and attached to the maniforld. The system is evacuated to a pressure of about 0.1 micron, stopcock A is closed, and the system is tested for leaks. Then pure nitrogen is admitted through B until atmospheric pressure is reached; excess nitrogen passes out through the manometer. The plug M is removed, and 40 ml. (25.6 mmoles) of a 0.64 M solution of methylmagnesium iodide is placed in flask K by means of a pipet previously flushed with nitrogen and operated by a hypodermic syringe. The Grignard solution is diluted with 25 ml. of dry ether, and plug M is quickly replaced. Stopcock B is closed, and a bath of nitrogen is placed about K. Stopcock A is slightly opened for a moment to remove nitrogen and prevent an appreciable amount of it from being trapped in the solidifying solution; the stopcock is closed when the ether commences to boil. When the contents of the flask have been frozen solid, A is opened

again, and the system is evacuated to a pressure of 0.1 micron. Then A is closed, and the liquid nitrogen is replaced by a bath at -20°. When the contents of the flask have come to thermal equilibrium with the bath, the stirrer is started and carbonation is effected by dropping sulfuric acid onto the barium carbonate. The acid is added cautiously at first, so that the initial surge of gas does not carry out particles of barium carbonate, then as rapidly as possible without allowing the pressure to exceed 50 cm. When the initial evolution has subsided, flask G is warmed with a small flame to dissolve most of the barium sulfate and thereby facilitate reaction of remaining carbonate; in addition, the heating serves to expel the last traces of gas from the acid. The amnometer reading becomes constant in 5 to 15 minutes.

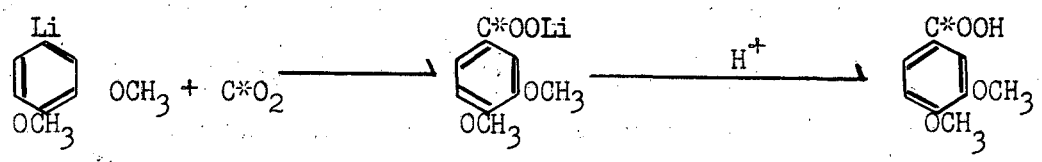
Since the presence of ether vapor interferes with the diffusion of carbon dioxide when the pressure of the carbon dioxide has fallen to a value near that of the ether, the flask K is again immersed in liquid nitrogen to draw any remaining carbon dioxide into K, the stopcock at H is closed, the flask is again warmed to -20°, and stirring is continued for 10 minutes. Nitrogen is then admitted to the system, and 10 ml. of sulfuric acid is added to hydrolyze the reaction product. A 30% excess of silver sulfate is added to K, which is still immersed in the cooling bath, and the mixture is stirred 5 minutes. The flask K is then removed from the manifold and fitted with a fractionating head, and the ether is removed by distillation.

The acetic acid is obtained by steam distillation, and the distillate is neutralized with standardized sodium hydroxide. The yield, determined from the amount of alkali required, is 89-94%. After filtration to remove a small amount of sediment which may be present, evaporation of the solution gives pure sodium acetate, whose weight agrees closely with that calculated from the titration value.

Details for preparation of a number of other carbcxyl l abeled acids are available (37,38).

In connection with the preparation of Grignard reagents, Glascock and Arrol (39) have reported the preparation of ethyl magnesium iodide in 55% yields by allowing gaseous ethyl iodide to react with a magnesium mirror. This technique may be a help in certain small scale vacuum line work.

Carbonyl labeled acids have also been prepared by several other methods. Carbonation of the corresponding lithium alkyls with isotopic carbon dioxide has yielded carboxyl labeled nicotinic (40), p-aminobenzoic (40) and veratric acids (41):



Veratric Acid (3,4-Dimethoxybenzoic Acid-Carboxyl-C^{14}) [41].-

A solution of n-butyllithium is prepared by refluxing overnight, in an atmosphere of purified nitrogen, a mixture of 2.5 grams of lithium, cut into small pieces, and 10 grams of n-butyl chloride, dissolved in 20 ml. of pentane. Then 80 ml. of pentane is added, and the solution is filtered anaerobically. The clear, colorless filtrate is found to be 0.632 M in butyllithium by adding excess

standardized acid to an aliquot and titrating the excess acid with standardized base.

The vacuum system is evacuated and filled with nitrogen as usual. Then 20.0 ml. (12.6 mmoles) of bytyllithium solution is pipetted into the carbonation flask, and the pentane is distilled in vacuum until about 1 ml. remains. The carbonation flask is chilled, and about 20 ml. of dry ether is distilled in from a flask attached to the manifold. The stirrer is run for a moment to mix the butyllithium and ether, then stopped. The solution is frozen with liquid nitrogen, and 30 ml. more ether is distilled in. This too is frozen. Purified nitrogen is admitted to the system, and 4.08 grams (18.8 mmoles) of 4-bromoveratrole, dissolved in a little ether, is added. When this has frozen, the system is evacuated and the manifold closed off from the pump.

The liquid nitrogen is replaced by isopropanol chilled to -60°, and as soon as the contents of the carbonation flask have become sufficiently fluid the stirrer is started. A precipitation of pure white veratryllithium commences in a few minutes, and stirring is continued for 15 minutes to permit the reaction to become complete. Carbonation is effected at -60° as rapidly as possible (7 minutes) with carbon dioxide from 1.25 grams (6.35 mmoles) of radioactive barium carbonate. Nitrogen is admitted to the system, and 10 ml. of concentrated hydrochloric acid is added to the reaction mixture. The appearance of a deep red color is reported. The cooling bath is removed, and the contents of the flask are stirred as they warm up. The acid is extracted from the reaction mixture with ether, then taken into sodium hydroxide. The water solution is warmed to remove ether,

treated with charcoal, filtered, and acidified. The reported yield of veratric acid is 1.03 grams (89.6% of theory based on barium carbonate); m.p., 176-178 uncor.; neutralization equivalent 184 (calculated, 182). No halide can be detected in the product by sodium fusion.

The preparation of 9-fluorenecarboxylic acid-carboxyl-C^{14} by carbonation of the appropriate sodium derivative has been described (42), and the Arndt-Eistert synthesis has been used to prepare pehnylacetic-1-C^{13} acid from carboxyl labeled benzoic acid (43).

Treatment of a halide with isotopic cyanide followed by hydrolysis of the resulting nitrile has also been used for the synthesis of carboxyl labeled acids. The synthesis of malonic acid-1-C^{14} is detailed below (44).

Malonic Acid-1-C^{14} [44]. - Ten grams of chloroacetic acid is dissolved in 15 ml. of water in a 100-ml. round-bottomed flask. The solution is warmed to 50°, neutralized with sodium carbonate (6 grams), and cooled to room temperature. To the slightly alkaline distillate from a cyanide preparation is added enough inactive sodium cyanide to make a total of 6 grams of this salt; the final volume of cyanide solution should be about 15 ml. The cyanide and chloroacetate solutions are mixed rapidly and heated on the steam bath for 40 minutes.

The solution is cooled, and to it is added slowly 5 grams of solid sodium hydroxide; when all the solid is dissolved the flask is warmed slowly on the steam bath and so heated for 1 1/2 hours. The last traces of ammonia are removed by bubbling steam through the hot solution for 10 minutes. A solution of 8 grams of anhydrous calcium chloride in 30 ml. of water is warmed to 50° and added slowly to the hot sodium malonate

solution. The precipitate of calcium malonate becomes crystalline on standing 15-20 hours. The calcium malonate is filtered and washed with several 10-ml. portions of ice-cold water, and sucked dry.

The dried calcium malonate is placed in a 200 ml. beaker, surrounded by an ice bath, with 15-20 ml. of reagent-grade ether. The two are mixed to a paste which is treated with 1 ml. of 12 N hydrochloric acid for each gram of dry salt; the acid is added dropwise at first. The solution is transferred to a continuous extractor, and ether extraction is carried out until no more malonic acid is obtained; 12 hours should be allowed for this step.

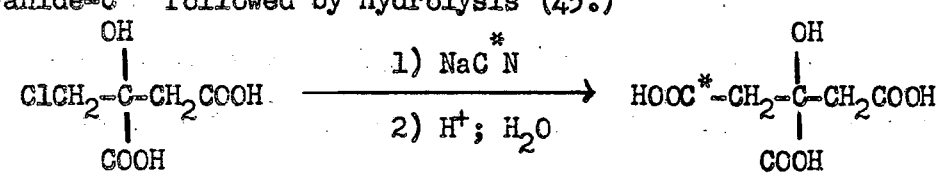
The product obtained by concentration of the ether solution is recrystallized from ether-petroleum ether mixture, if a very pure product is desired. Yield (crude product) 8.4 grams, 76% based on chloroacetic acid, m.p. 132° or higher.

Analysis (recrystallized product). Calculated for $C_3H_4O_4$:

C, 34.62; H, 3.87

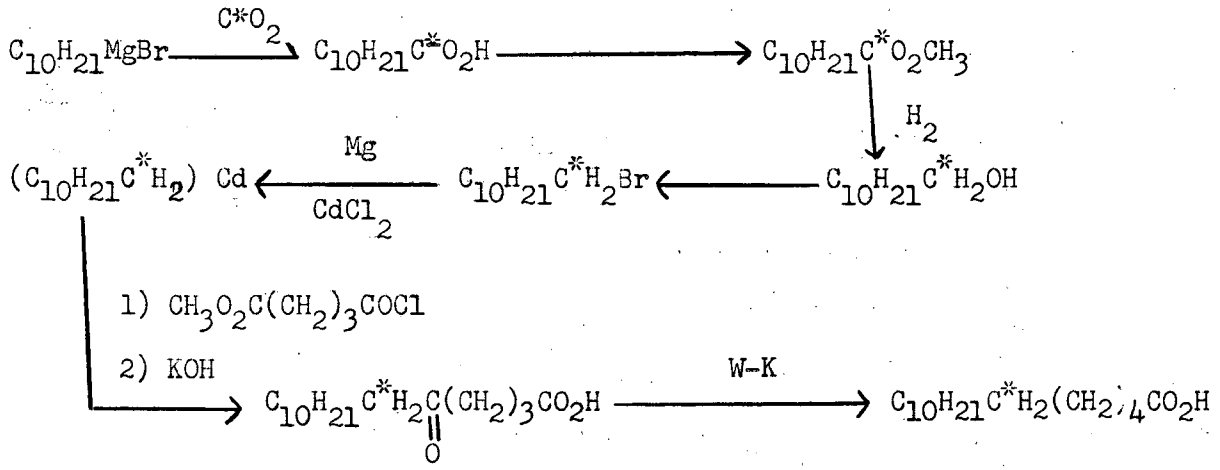
Found: C, 34.68; H, 3.82

Treatment of optically active chlorides with isotopic cyanide followed by hydrolysis is a good method for the preparation of optically active, labeled acids. A symmetrically labeled citric acid has been prepared in optically pure form by treatment of an isomer of α-chloro-β-carboxy-βhydroxybutyric acid with sodium cyanide-C^{14} followed by hydrolysis (45.)



Myristic acid-1-C^{14} has been prepared by refluxing potsssium cyanide-C^{14} with excess tridecylbromide in ethanol for three days and hydrolysis of the product (38).

The synthesis of labeled aliphatic compounds in which the label is at some position other than the functional groups involves conversion of one or another of the functionally labeled intermediates to the desired compound. The synthesis of labeled long chain aliphatic acids with the label at some position in the chain has been carried out in several ways. Crandall and Gurin (46) prepared n-octanoic acid-2-C^{14} by reduction of ethyl hexanoate-1-C^{14} with lithium aluminum hydride, conversion of the alcohol to the bromide and condensation with sodium ethylmalonate. Saponification and decarboxylation gave the desired acid. Treatment of an acid chloride with a dialkyl cadmium and reduction of the resulting ketome may also be used, with either of the condensing portions being labeled. Palmitic acid-6-C^{14} (47) and octanoic acid-7-C^{14} (48,49) have been prepared in this way. The reaction sequence for palmitic acid-6-C^{14} follows:



Of the several ways available to carry out any of these syntheses, it is often best to investigate each in an effort to find a process which gives the optimum yield. Thus propanol-1-C^{14} has been prepared by reduction of propionic acid 1-C^{14} with $LiAlH_4$ (50), by hydrogenation of propyl propionate over copper chromite (51) and

by high-pressure reduction of propionic acid (52). A detailed synthesis for propyl bromide via propyl alcohol from propionic acid is given below:

Propanol-1-C^{14} [52]. - Sodium propionate-1-C^{14} (1.44 g., 15.0 mmoles, 5.5×10^4 dis./min./mg.) was dissolved in 5 ml. water and treated with 15 ml. wet packed Dowax 50 cation exchange resin (250-500 mesh) to convert the salt to the free acid. The mixture was thoroughly stirred, filtered, and the filtrate plus washings added to a wet Cd-Ni hydroxide slurry prepared as follows: In a 50 ml. cetnrifuge tube 1.70 g. cadmium chloride·2-1/2 H_2O (7.43 mmoles) and 0.24 g. nickel nitrate hexahydrate (0.83 mmoles) were dissolved in 10 ml. water. Excess sodium hydroxide was added and the precipitate collected by centrifugation and washed 3-4 times with water.

The resulting solution of Cd-Ni propionate was evaporated to dryness on a steam bath using an air stream. (Note: In a test reaction, acidification of the Cd-Ni propionate followed by steam distillation of the acid showed that the conversion of sodium propionate to the dry Cd-Ni salt was quantitative.) The dry mixture was transferred to a 115 ml. stainless steel hydrogenation bomb containing 1.5 g. copper chromite catalyst. The bomb was maintained at a pressure of 50 microns for 4-8 hours to remove final traces of water. It was observed that the presence of moisture at this point markedly reduced yields.

The bomb was pressured to 3500 psi with electrolytic hydrogen and heated at 240°C. with shaking for nine hours. When cooled, the bomb was transferred to a vacuum line and the hydrogen released through a liquid nitrogen-cooled spiral trap containing a sintered glass disk. After evacuation, the bomb was held at

reduced pressure for five hours while warmed to approximately 80°C. with an infra red lamp and the distillate collected in the cold trap. The product consisted of 1.14 g. of a solution of 74% propanol in water (94% yield). The sample was oxidized to carbon dioxide and converted to barium carbonate for radioactivity measurements. Calculated specific activity, 8.9×10^3 dis./min./mg. $BaCO_3$; found, 8.5×10^3 dis./min./mg. $BaCO_3$.

Propyl-1-C^{14}Bromide.- The propanol-water mixture from the reduction was converted to the bromide using 4 ml. phosphorus tribromide, washed with water and dried over phosphorus pentoxide. The yield was 1.26 g. or 73% from propanol.

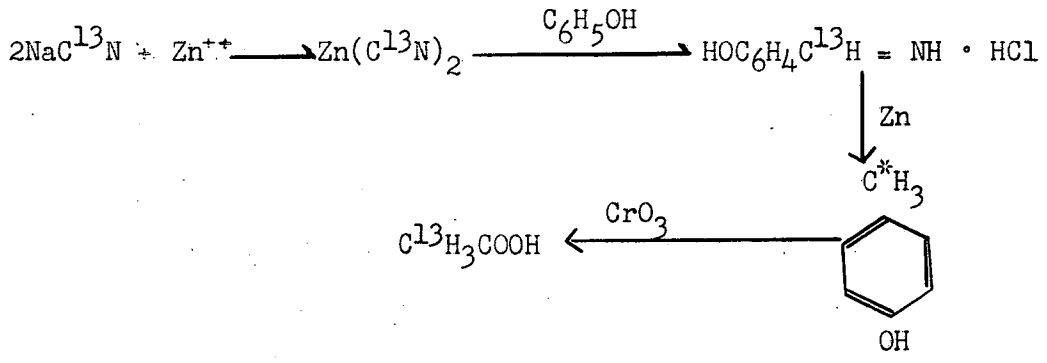
Propyl-1-C^{14} Iodide. - A propanol-water mixture prepared as described above was treated with 10 g. iodine, 0.62 g. red phosphorus and 3 ml. water. The propyl iodide was washed with water and dried over phosphorus pentoxide. The yield was 86% from the alcohol.

Among the non-functionally labeled compounds, methyl labeled acetic acid is a very important intermediate in various other syntheses. Carbonation of the Grignard reagent formed from methyl iodide-C^{14} (53) is a good direct method:

The Preparation of Sodium Acetate Labeled with Radioactive Carbon in the Methyl Group. [53]. - A 150 ml. conical reaction flask containing 50 ml. of dry ether and 0.5 gm. of magnesium turnings, was chilled with liquid nitrogen and 1 ml. (2.28 gm.) of methyl iodide distilled in. The reaction vessel was closed off and the ether refluxed 1 hour. The reaction flask was then cooled to -20° and carbon dioxide, that had been dried by passing through a spiral immersed in dry ice-acetone and freed of oxygen

and nitrogen by condensing with liquid air and evacuating at low pressures, was added until a pressure of about 30 cm. was maintained in the system. Stirring was then continued for 10 minutes. The reaction vessel was removed from the line and opened in the hood and the cold (-20° to -50°) Grignard complex decomposed with 15 ml. of 6 N sulfuric acid. After decomposition, an additional 35 ml. of water were added. 5 gm. of silver sulfate were added to precipitate the iodide present. The ether was distilled off and the acetic acid was steam-distilled from the reaction mixture with about 300 ml. of water. This distillate was exactly neutralized with 1 N sodium hydroxide solution with a glass electrode, evaporated to a small volume, filtered, evaporated to dryness, and dried in vacuo at 10^{-3} mm. pressure. The yield of white anhydrous sodium acetate was 70 to 75 per cent, the titration and weighings agreeing within a few tenths per cent.

a recent synthesis of methyl labeled acetic acid from methanol which does not involve preparation of methyl iodide has been reported (54). This method involves the formation of acetonitrile-2-C^{14} by treatment of methyl-C^{14}-hydrogen sulfate with potassium cyanide and proceeds in 87% yield. Anker (55) has prepared sodium acetate-2-C^{13} from labeled sodium cyanide by an ingenious method involving preparation of p-hydroxybenzaldimine hydrochloride:



Other compounds which may serve as useful intermediates include acetoacetic acid which has been prepared labeled in the 1, the 3 and the 1 and 3 positions with carbon-13 (56). The synthetic routes are outlined below:

(1) $2CH_3C^*O_2Et \xrightarrow{(C_6H_5)_3CNa} CH_3C^{*}{*}CH_2\text{-}C^*O_2Et$ (with $=O$)

(2) $CH_3C^*O_2CH_3 + Mg + BrCH_2CO_2Et \longrightarrow CH_3C^*CH_2CO_2Et$ (with $=O$)

(3) $CH_3CO_2CH_3 + Mg + BrCH_2C^*O_2Et \longrightarrow CH_3CCH_2C^*O_2Et$ (with $=O$)

Syntheses of malonic acid-2-C^{14} by treatment of bromoacetic-2-C^{14} acid with cyanide followed by hydrolysis and by decarbonylation of axalacetic-2-C^{14} acid have been reported (57). A recent synthesis of labeled malonic acid, which proceeds in 84% yield has been reported (58).

Pyruvic acid has been prepared labeled in the 1,2 or 2,3 positions with carbon-14 (59).

$CH_3CHO \xrightarrow{HC^*N} CH_3CHOHC^*O_2H$ (A)

$HC^*C^*H \longrightarrow C^*H_3C^*HO \xrightarrow{HCN} C^*H_3C^*HOHCOOH$ (B)

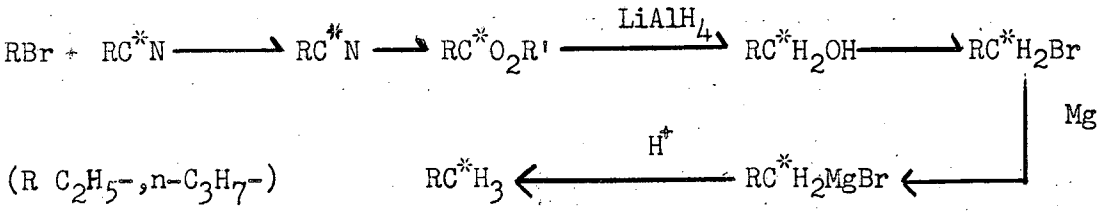
(A) or (B) $\longrightarrow CH_3CHOHCO_2Bu \xrightarrow{KMnO_4} CH_3CCO_2Bu$ (with $=O$) $\xrightarrow{NaOH} CH_3COCO_2Na$

$CH_3C^*O_2H + PBr_3 \longrightarrow CH_3C^*OBr \xrightarrow{CuCN} CH_3C^*OCN \longrightarrow CH_3C^*\text{-}COOH$ (with $=O$)

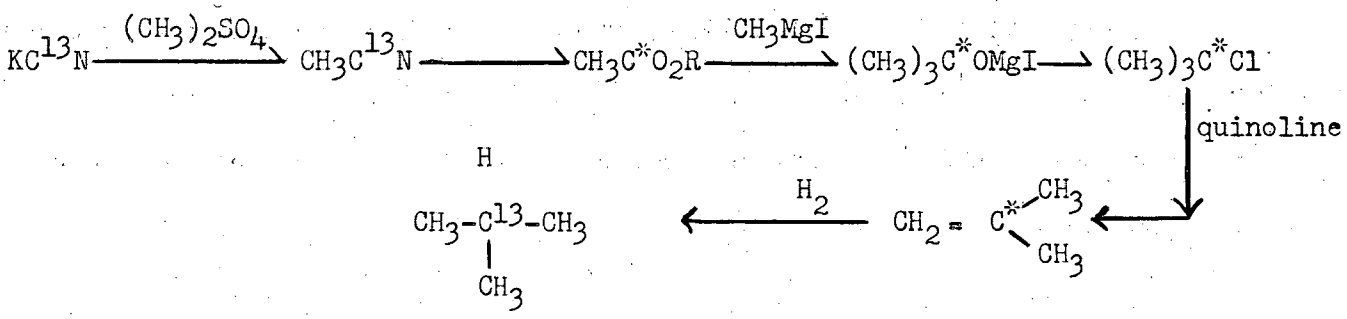
Alkanes and Alkenes: -

Using known methods, it has been possible to synthesize a variety of alkanes labeled with isotopic carbon (60). Methane-C^{13} was prepared by treatment of labeled methylmagnesium iodide with water. Propane-1-C^{13}, n-butane-1-C^{13} and

n-butane-1-C^{14} were prepared by the following reaction scheme:

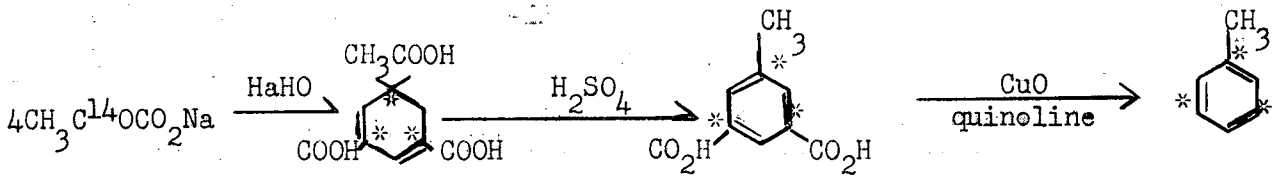


Butane-1-C^{14} was also prepared by hydrogenation of the labeled butene obtained upon treating allyl bromide with isotopic methylmagnesium iodide. Isobutane-1-C^{13} and isobutane-1-C^{14} were prepared by treating acetone with the appropriate labeled Grignard reagent and conversion of the addition product to labeled t-butyl iodide which, upon heating in the presence of hydrogen iodide, yielded isobutene. Hydrogenation gave the desired labeled isobutane. Isobutane-2-C^{13} was prepared in the following way:



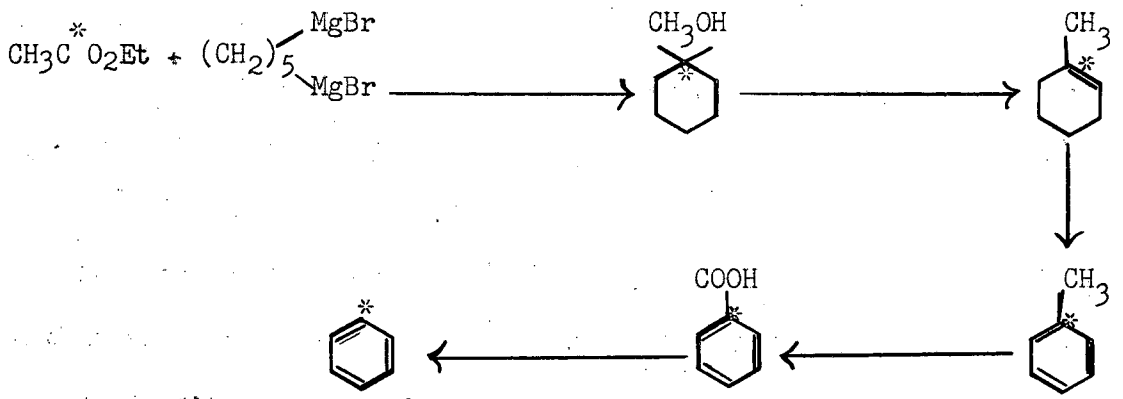
Among the unsaturated compounds, acetylene-C^{11} (1) and acetylene-C^{14} (61,62) have been prepared from barium carbide. Hydrogenation of acetylene-C^{14} to ethylene-C^{14} has been reported (63). Propene-1-C^{14} has been prepared by the pyrolysis of n-propyl-1-C^{14}-trimethylammonium hydroxide (64). This was found to be the most satisfactory method, most of the other methods tried resulted in a proton shift. 1-Butene-4-C^{14} has been prepared by treating allyl bromide with methylmagnesium-C^{14}-iodide (65).

Few syntheses of ring labeled aromatic compounds have been reported and, as yet no really satisfactory methods are available. Toluene-1,3,5-C^{14} has been prepared from sodium pyruvate-2-C^{14} (66). Uvitic acid is one of the intermediates:



Aromatic Compounds: -

The synthesis of toluene, benzoic acid and benzene, all ring labeled, has been reported by a reaction scheme which is outlined and detailed below (67):



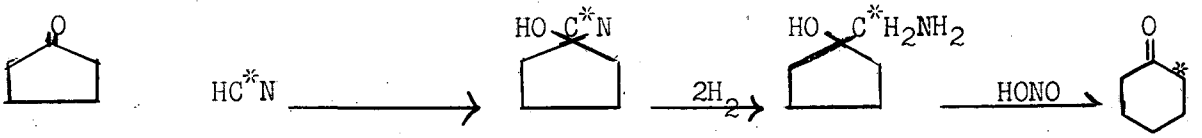
A Synthesis of Benzene, Toluene, and Benzoic Acid Labeled in the Ring with Isotopic Carbon [67]. - The synthesis of carboxyl-labeled ethyl acetate was accomplished by carbonation of methyl magnesium iodide with isotopic carbon dioxide, followed by reaction of the sodium acetate with diethyl sulfate in a modivication of the procedure of Sakami, Evans, and Gurin. Preparation of 1-methylcyclohexanol-1 labeled on carbon 1 was achieved by reaction of carboxyl-labeled ethyl acetate with the Grignard reagent from pentamethylene dibromide according to the procedure of Grignard and Vignon. Dehydration of the carbinol with iodine as described by Mosher afforded 1-methylcyclohexene, which was converted to toluene by vapor-phase dehydrogenation over platinized asbestos. The benzoic acid, prepared by oxidation of toluene with potassium permanganate, underwent smooth decarboxylation on treatment with

copper oxide and quinoline. The overall yields from barium carbonate varied from 35 to 50% for toluene, from 3 to 40% for benzoic acid, and from 25 to 40% for benzene.

Cyclization of 1-heptene-1-C^{14} over copper chromite yields labeled toluene, with a isotopic carbon atom appearing in the ring or in the methyl group (68). Condensation of acetone-2-C^{14} to mesitylene-1,3,5-C^{14} has been effected (69).

The syntheses of several polynuclear hydrocarbons have been carried out, but in all cases via cyclization of a labeled carboxylic acid. The carcinogens 1,2,5,6-dibenzanthracene-9-C^{14} (70) and 20 methylcholanthrene-11-C^{14} (71) have been prepared by known methods starting from naphthoic acid-2-C^{14} and naphthoic acid-1-C^{14}, respectively. Wagner rearrangement of 9-fluorenylcarbinol-10-C^{14} has yielded phenanthrene-9-C^{14} (42); the carbinol was prepared from the ester by reduction with lithium aluminum hydride. Fluorene-9-C^{14} (72) has been prepared from o-phenylbenzoic acid-1-C^{14}. The reported synthesis of -tetralone-1-C^{14} (38) opens the way to synthesis of ring labeled naphthalene derivatives.

Synthesis of cyclohexanone-2-C^{14} starting with cyclopentanone and labeled hydrogen cyanide and using the Tiffeneau rearrangement in one step has been reported (73). The reaction scheme is this:



Amino Acids: -

The synthesis of carbon labeled amino acids may be carried out according to general methods available in the literature.

The simplest synthesis of an α-amino acid involves bromination of a carboxylic acid followed by amination:

$$CH_2COOH \xrightarrow{Br_2} R\text{-}\underset{Br}{CH}COOH \xrightarrow{NH_3} R\text{-}\underset{NH_2}{CH}\text{-}COOH$$

This method is especially useful in isotopic syntheses since it permits labeling anywhere in the molecule. Leucine-1-C^{14} and leucine-2-C^{14} have been prepared in this manner (74). A detailed synthesis of glycine-2-C^{14} is described (75):

> Chloroacetic Acid [75].- A platinum boat charged with 1.4081 gm. of carboxyl-labeled anhydrous sodium acetate was placed in a horizontal glass tube and dried *in vacuo* at 3×10^{-2} mm. of Hg for 24 hours. The tube was then connected to a train of three traps, each of which was cooled with an isopropyl alcohol-dry ice mixture; the last trap was protected with a calcium chloride tube. Gaseous hydrogen chloride, dried with concentrated sulfuric acid, drierite, and anhydrous aluminum chloride, was passed slowly through the train. The tube was gradually heated and the liberated acetic acid distilled into the traps. When the reaction was complete, the three traps were connected to the vacuum line and the contents were distilled into a small reaction vessel (Figure 4), which was cooled with liquid nitrogen. The product contained 10 to 15 per cent water and a considerable amount of gaseous hydrogen chloride. The reaction vessel, protected by a low temperature condenser cooled with isopropyl alcohol-dry ice and fitted with a calcium chloride tube, was warmed to room temperature. 0.65 gm. of acetic anhydride was added and the mixture was refluxed for 1/2 hour to remove the water. A mixture of 0.02 gm. of iodine, 0.04 gm. of phosphorus, and 0.08 gm. of phosphorus pentachloride was added, and dry chlorine was passed through the system at reflux temperature for 2 1/2 hours (see Figure 4).

After the chlorination was completed, all of the material in the condenser and in the gas inlet tube was distilled in vacuo back into the reaction vessel, which was cooled in liquid nitrogen. The chloroacetic acid was then purified by fractional sublimation in vacuo onto a cold finger condenser filled with powdered dry ice.

The yield of pure product was 1.52 gm., m.p. 60°, which is 67 per cent based on anhydrous sodium acetate.

Glycine.- A mixture of 3.2 gm. of powdered ammonium carbonate, 10 ml. of concentrated ammonia, and 4 ml. of water was heated in a small three-neck flask, which was fitted through ground glass joints to a pressure-equalized dropping funnel, a Liebig condenser and a thermometer. After the salt had dissolved, 1.014 gm. of carboxyl-labeled chloroacetic acid in 3 ml. of water were added dropwise through the dropping funnel at such a rate that the temperature of the solution did not rise above 60°. The mixture was held at 60° for 6 hours and was then allowed to stand for 12 hours at room temperature. The solution was then concentrated until its temperature reached 112°. The distillate showed only very slight radioactivity. The yellowish solution was cooled to 70°, and 15 ml. of absolute methanol were added slowly with agitation. The mixture was cooled in a refrigerator for 1 hour. The precipitate was filtered and washed with methanol and ether.

The yield of pure white crystals, which showed no trace of chloride ion, was 0.54 gm. or 70 per cent (m.p. 225°, with decomposition). C 32.02, H 6.78: calculated, C 32.02, H 6.78. Upon concentration, the mother liquor gave 0.08 gm. of glycine which increased the yield to 0.62 gm. or 79 per cent, based on chloroacetic acid.

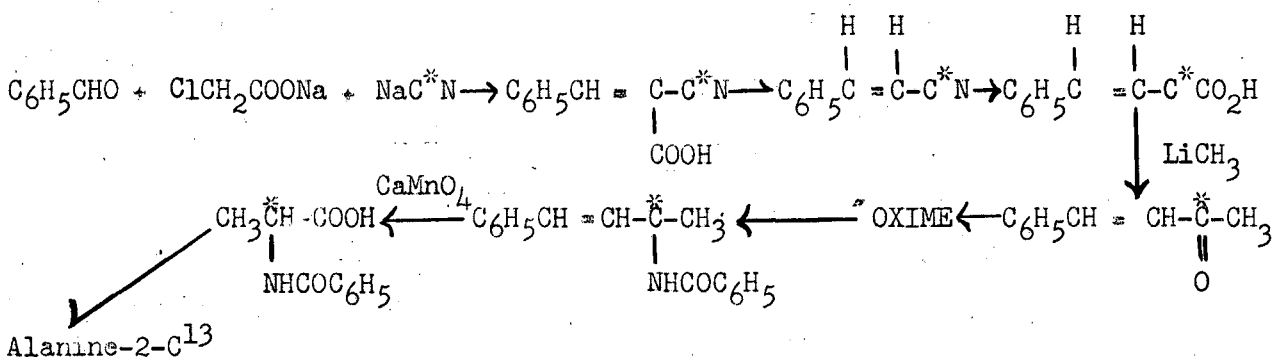
The chloroacetic acid used had a total activity of 299 microcuries. The glycine had a specific activity of 6.3×10^4 counts per minute per mg. and a total activity of 299 microcuries. From distillates and mother liquors, 50.4 microcuries were recovered.

The familiar sequence of the Strecker synthesis:

$$RCHO + HCN \longrightarrow R\text{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}\text{-}CN \longrightarrow R\text{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}\text{-}COOH$$

has been applied to isotopic syntheses. Carboxyl labeled leucine (38) and alanine (2,76) have been prepared by this method.

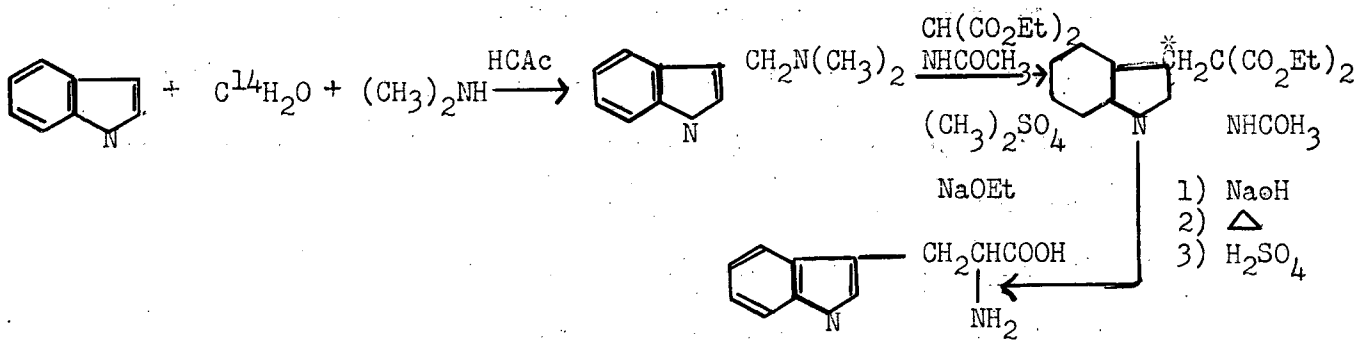
Alanine-2-C^{13} has been prepared from hydrogen cyanide-C^{13} using the reaction sequence outlined below (77):



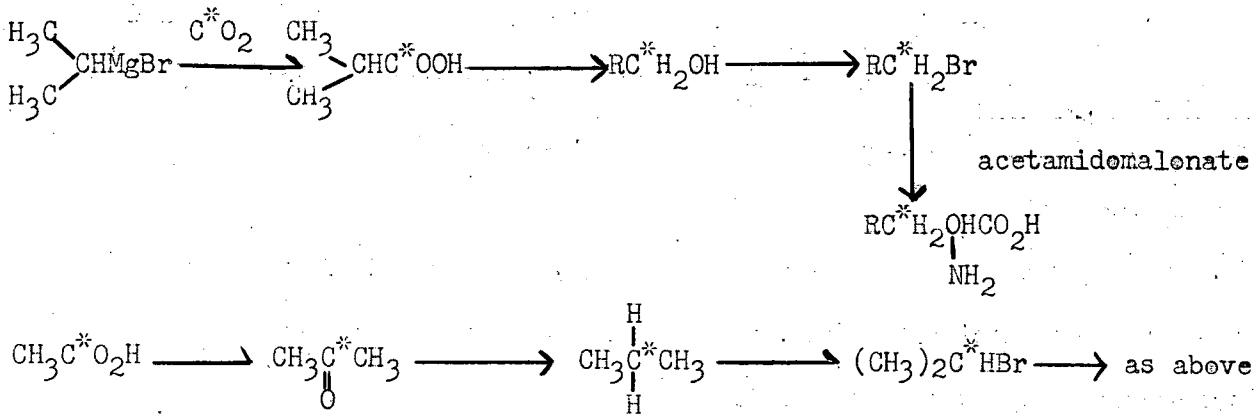
The same scheme has been applied to the synthesis of alanine-2-C^{14} (78). Anker (79) has prepared alanine-2-C^{14} in near quantitative yield by hydrogenation of the phenylhydrazone of pyruvic-2-C^{14} acid.

The acetamidomalonic ester type of synthesis has lent itself to the preparation of many amino acids labeled at carbon atoms other than the carboxy. Variations of this method included acylation of the amino group by groups other than acetyl and use of derivatives of the equally reactive cyanoacetic acid. It is possible, of course, to label either of the condensing portions.

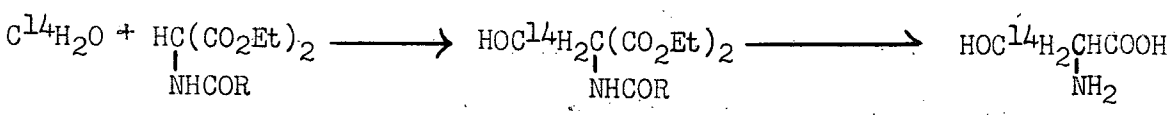
Using this method, tryptophane-3-C^{14} has been synthesized by the condensation of gramine (3-indolyl-methyl-C^{14}-dimethylamine) with ethyl acetamidomalonate (80):



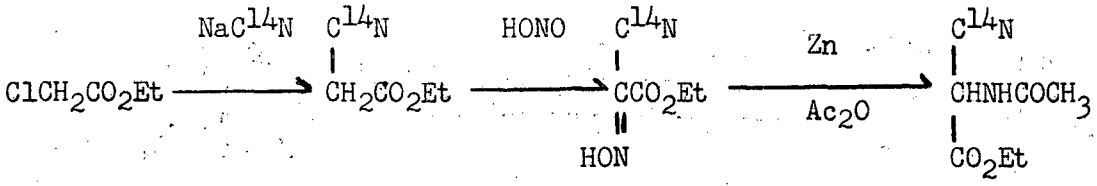
Tryptophane-2-C^{14} has been prepared by condensation of gramine with ethyl acetamidocyanoacetate-2-C^{14} (38). Lysine, ornithine and tyrosine, all labeled at C-2, have been prepared from labeled ethyl acetamidocyanoacetate (81). Condensation of acetamidomalonic ester and ethyl bromoacetate-2-C^{14} has been used to prepare aspartic acid-3-C^{14} (38). Leucine-3-C^{14} and leucine-4-C^{14} have been prepared via the scheme shown below (82): (Let R equal $(CH_3)_2CH$-)



Serine-3-C^{14} has been prepared by the condensation of formaldehyde-C^{14} with ethyl p-nitrobenzamidomalonate (83) and ethyl acetamidomalonate (21).



Histidine-1-C^{14} has been prepared by the condensation of 4-chloromethyl-imidazole with ethyl acetamidocyano-C^{14}-acetate (84). The synthesis of the acetamidocyanoacetate is outlined:

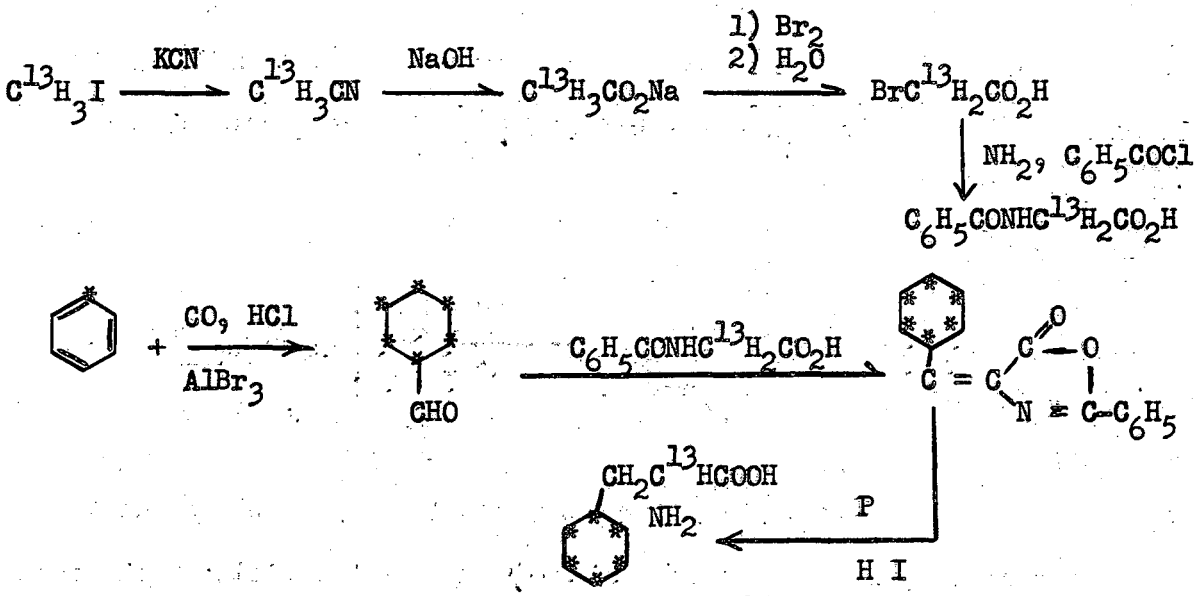


Methionine labeled with C^{13} and S^{34} has been prepared (85) by a method which centers about the condensation of labeled benzyl-β-chloroethylsulfide with ethyl sodiophthaliminomalonate. Phenylalanine labeled in the 1,3 and 5 positions of the ring has been prepared from pyruvic acid-2-C^{14}. This compound was condensed to give toluene which was converted to benzyl bromide-1,3,5-C^{14}. Condensation of the labeled benzyl bromide with acetamidomalonic ester gave ring labeled phenylalanine (86).

The well-known Gabriel phthalimide synthesis has been used to prepare glycine-1-C^{13} (53) from isotopic potassium cyanide.

Another useful route to labeled amino acids involves the azlactone synthesis. Phanylalanine-1,2-C^{14} (86,87) has been prepared from glycine-1,2-C^{14} using this method.

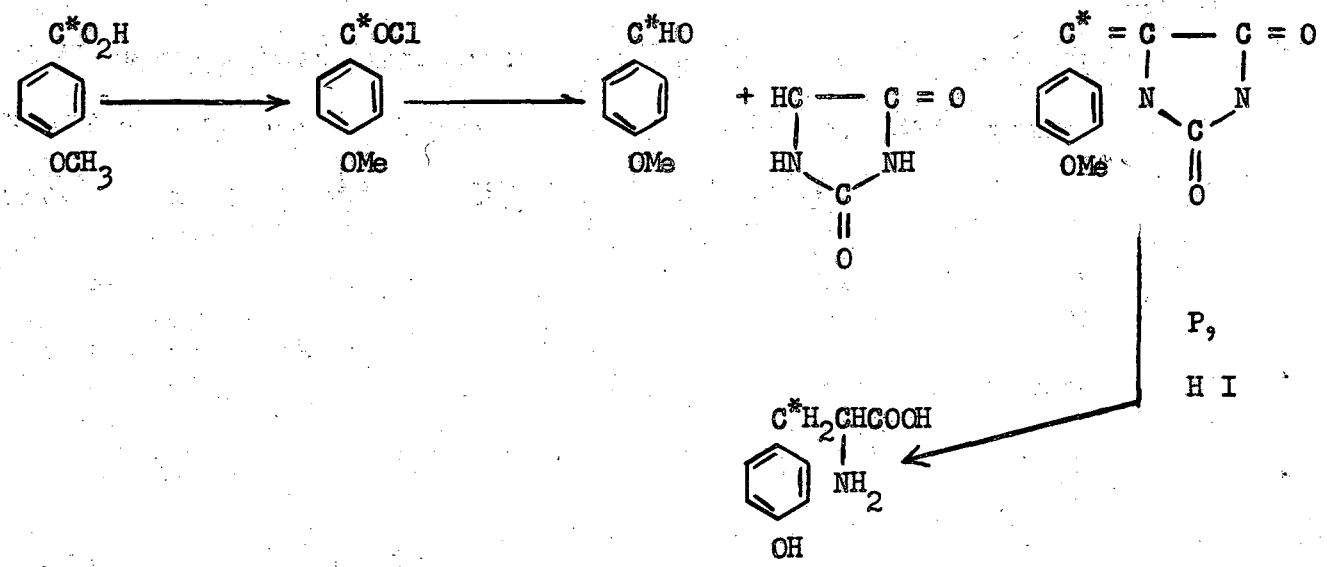
Phenylalanine labeled with carbon-14 in the ring and with carbon-13 in the alpha carbon has been prepared using the azlactone synthesis (88). A summary of the steps involved is given here:



Several other amino acid syntheses are worth noting. Reductive animation of alpha ketoglutaric acid-1,2-C^{14} (from condensation of diethyl oxalate-1,2-C^{14} and diethyl succinate) yielded glutamic acid-1,2-C^{14} (89).

Treatment of ketoornithine with isotopic sodium thiocyanate gives thiohistidine which, upon oxidation with ferric sulfate, yields labeled histidine [2-(4-imidazolyl-2-C^{14})-alanine] (90).

The hydantoin synthesis has been used to prepare tyrosine-3-C^{14} (91,92), tyrosine-1-C^{14} (93), valine-2-C^{13} (94) and carboxyl labeled tyrptophane (95). The synthesis of tyrosine-2-C^{14} is detailed below:



DL-Tyrosine-ρ-C^{14} 92. - Carbonyl-labeled p-anisaldehyde is prepared from 0.64 gram of labeled p-anisic acidby the Rosenmund Method. On the basis of the known yield in this synthesis, the quantity of aldehyde can be calculated to be 0.42 gram (3.08 mmoles). To the aldehyde, contained in a 14 by 100 mm heavy wall Pyrex ignition tube, is added 0.55 gram (5.5 mmoles) of hydantoin, 0.7 ml of dry diethylamine, and 1.2 ml of dry pyridine. The tube is sealed and heated 72 hours in steam. It is opened, and the solvent is removed in vacuum at room temperature; then pumping is continued 1.5 hours at 100°. The residue is stirred with three 2-ml portions of hot water, each portion being removed by a filter stick before the addition of the next. The yellow residue of anisalhydantoin weighted 0.60 gram after drying at 90°. This is a yield of 93% based on anisaldehyde and is in agreement with values around 90% found in pilot experiments with pure anisaldehyde.

To the anisalhydantoin, still contained in the ignition tube, is added 0.19 gram of red phosphorus and 2.4 ml of freshly distilled hydroidic acid (d, 1.7). A cold finger, made from a 5 ml conical centrifuge tube, is hung in the mouth of the test tube, and the mixture is refluxed 5 hours; 0.63 gram of iodine is then added, and the mixture is refluxed 5.5 hours longer.

The solution is filtered into a 30-ml flask and evaporated to dryness under vacuum at room temperature. To remove as much hydriodic acid as possible, the residue is dissolved in 5 ml of water and the evaporation is repeated; the residue is then dissolved in 5 ml of water. A yellow turbidity is present, which

increases with dilution. Water is added until no further increase in turbidity occurs (25 ml), and the solution is clarified by centrifugation. The solution is then adjusted to pH 5 with concentrated ammonia, and a slight precipitate which appears is removed by filtration. The filtrate is evaporated in vacuum, and the residue is heated 15 minutes at 100°. The residue is washed with water onto a filter, where it is well washed with water, followed by alcohol. The yield is 177 mg (36% based on anisalhydantoin). Reported analysis of a specimen prepared in this way (starting with inactive barium carbonate):

Analysis. Calculated for $C_9H_{11}NO_3$: C, 59.66; H, 6.2; N, 7.74.

Found: C, 58.15; H, 6.01; N, 7.76
58.24; 6.15 7.66

An interesting synthesis of glycine is the following (2):

$$2HCHO + KC^*N + NH_4Cl \longrightarrow H_2C = NCH_2C^*N \xrightarrow[EtOH]{H_2SO_4} H_2N - CH_2 - C^*N$$

$$H_2N - CH_2 - C^*N \xrightarrow{Ba(OH)_2} (H_2N \cdot CH_2C^*O_2)_2Ba$$

$$(H_2N \cdot CH_2C^*O_2)_2Ba \xrightarrow{H_2SO_4} H_2NCH_2C^*O_2H$$

Two groups of investigators (96,97) have used the following reaction scheme for the synthesis of lysine-e-C^{14}:

$$KC^{14}N + Cl(CH_2)_3Br \longrightarrow Cl(CH_2)_3C^{14}N \xrightarrow[Na]{CH(CO_2Et)_2} NC^* - (CH_2)_3 - CH(CO_2Et)_2$$

$$NC^* - (CH_2)_3 - CH(CO_2Et)_2 \xrightarrow{EtONO} NC^* * (CH_2)_3 - C(=NOH) - CO_2Et$$

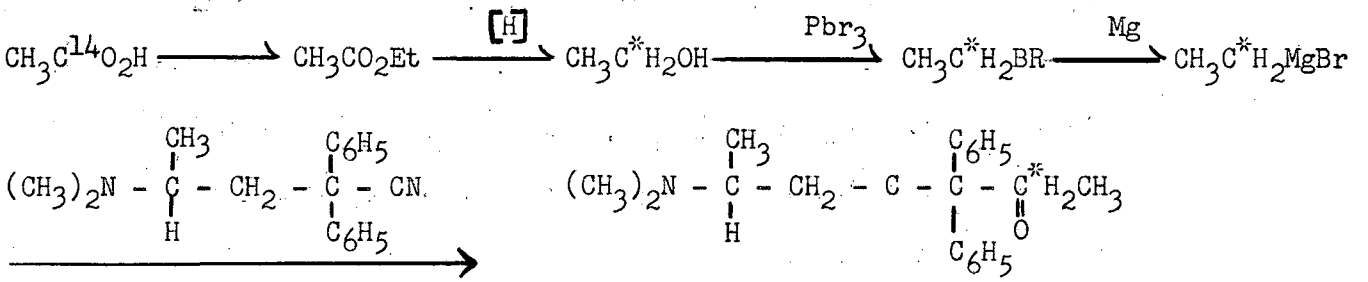
$$NC^* * (CH_2)_3 - C(=NOH) - CO_2Et \xrightarrow{H_2} H_2N - C^*H_2(CH_2)_3 - CH(NH_2)COOH$$

Methionine-methyl-C^{14} has been prepared by methylation of S-benzylhomocysteine with methyl iodide-C^{14} (11).

Other Compounds:-

Other compounds of biological interest have recently been prepared labeled with isotopic carbon.

In the field of drugs, labeled codeine (98), demerol (99) and methadon (100) have been prepared. Codeine was labeled with carbon-14 in the 3-methoxy group by treatment of pure morphine-N-oxide with methyl iodide-C^{14} in the presence of sodium. Demerol was prepared by reductive alkylation of 4-phenyl-4-carbethoxypiperidine with labeled formaldehyde and formic acid. The flow sheet for the methadon synthesis is given here:



Syntheses of phenobarbital-2-C^{14} (101) and chloretone (1,1,1-trichloro-2-methyl-C^{14}-2-propanol) (38) have also been reported.

Two syntheses of labeled choline are known. Treatment of β-dimethylaminoethanol with methyl iodide-C^{14} gave choline iodide (102), which was readily converted to labeled choline chloride and acetyl choline chloride. In another series of experiments (103), labeled acetylene was converted to ethylene and that to ethylene bromohydrin. Treatment of the bromohydrin with trimethylamine gave choline bromide, which was easily acetylated to acetylcholine bromide.

In the field of purines, adenine labeled with isotopic carbon in the 4 and 6 positions has been prepared (104). The synthesis involves condensation of phenylazomalononitrile, labeled in the cyano group, with formamidine hydrochloride.

Ethyl Formimino Ether Hydrochloride [104]. - A four-necked flask was equipped with a mercury-sealed stirrer, a sintered glass gas-dispersion tube, a calcium chloride drying tube, and an inlet

tube for the introduction of hydrogen cyanide. The flask was surrounded by a bath (alcohol-Dry Ice) kept at -10 to -20°. A mixture of one pound of commercial anhydrous ether and 225 cc. (3.86 moles) of absolute ethyl alcohol was placed in the flask. The stirrer was set in motion and the generation of hydrogen cyanide from 4 moles of sodium cyanide was begun. When approximately 3.5 moles of the hydrogen cyanide was collected, a vigorous stream of anhydrous hydrogen chloride was passed in and continued for ten minutes after the first appearance of turbidity (total time, thirty to forty-five minutes). The mixture was then allowed to warm overnight, without stirring, during which time the bath temperature reached 10°. The bath was cooled to -20° and the solvent was removed through the gas dispersion tube by suction. The residual solid was washed with two one-half pound portions of ether, and the ether removed as before. The white crystalline product (285 g., 90% based on hydrogen cyandie, in an experi- where it was measured accurately) was dried beiefly in air and stored in a tightly stoppered bottle. The compound slowly loses weight on exposure to air.

Anal. Calcd. for $C_3H_7ON \cdot HCL$: Cl, 32.4
Found: Cl, 38.9

Formamidine Hydrochloride. - Ethyl formimino ether hydrochloride (17.0 g., 0.18 mole) and 35 cc. of commercial absolute ethanol were placed in a dry Carius tube equipped with an inlet tube reaching an inch below the surface of the ethanol. The inlet tube was arranged so as to be easily detached. The tube was immersed in a Dry Ice-bath and connected to an ammonia generator. The generator consisted of a flask provided with a condenser

and an inlet tube for the introduction of air. A drying tower filled with pellets of potassium hydroxide was placed on top of the condenser. Ammonium nitrate (8.8 g., 0.11 mole containing ca. 25 atom per cent. excess N^{15} in the ammonium group) which had been dried over calcium chloride was dissolved in 5-10 c.c. of water and decomposed carefully by the addition of 11 cc. of 12 N potassium hydroxide. A brisk stream of air was drawn through the apparatus into an acid trap containing 5% aqueous boric acid and methyl violet indicator. Fifteen minutes after the addition of the potassium hydroxide, a water-bath was placed around the generating flask, the rate of air-sweep was decreased somewhat and the bath was warmed gradually to boiling in twenty minutes. After the bath had boiled for fifteen minutes, it was removed and the solution was boiled over a direct flame for fifteen minutes. Little or no ammonia remained in the generator.

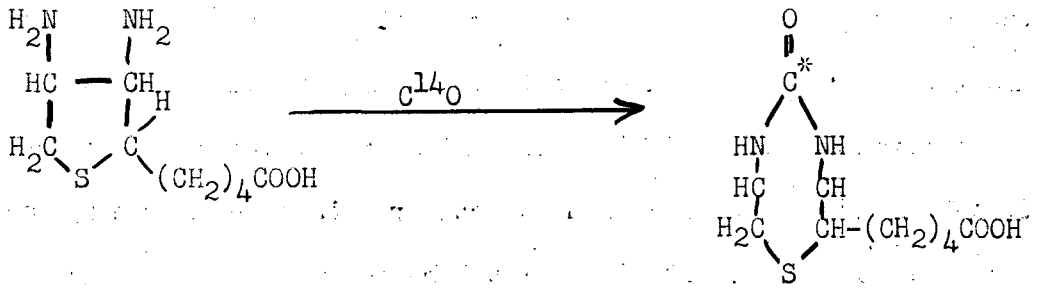
The bomb tube, with its inlet tube detached and included, was sealed and heated for two hours at 100°, during which time the tube was shaken occasionally. The tube was cooled, opened and any excess ammonia was collected in the aqueous boric acid trap by drawing a stream of dry air through the inlet tube for thirty minutes while the tube was surrounded by a bath at 55-60°. Usually with this ratio of reactants no excess ammonia was collected. Ammonia which was not recoverable varied from 0 to 8%. The hot ethanolic solution was filtered (the residue is 99% pure ammonium chloride), the alcohol was evaporated by means of a

stream of dry air, and the residual formamidine hydrochloride was dried in vacuo over phosphorus pentoxide and sulfuric acid.

anal. Calcd. for $CH_4N_2 \cdot HCl$: Cl, 44.1
Found: Cl, 43.8
(The analysis was carried out on material containing no excess N^{15}.)

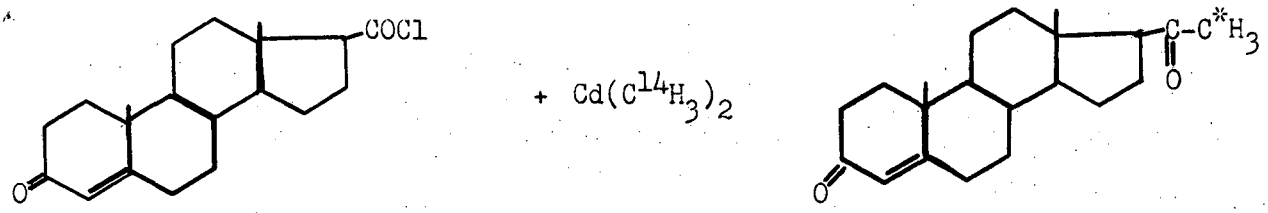
Treatment of 4,6-diamino-5-fromamido-C^{14} pyrimidine hydrochloride with 4-formylmorpholine has yielded adenine-8-C^{14} (105).

In the carbohydrate field, Sowden (30,31) has succeeded in preparing D-glucose-1-C^{14} and D-mannose-1-C^{14} by condensation of labeled nitromethane with D-abrinose and conversion of the resulting nitroalcohols to the corresponding hexoses. Treatment of L-xylosone with hydrogen cyanide-C^{14} has yielded L-ascorbic acid-1-C^{14} (106). Biotin (ureido-C^{14}) (107) has been prepared in the following manner:



Several syntheses of labeled steroids have recently been reported. Chain labeling of steroids can be more readily accomplished than can ring labeling, since intermediates are more readily prepared and, in general, fewer steps are required to complete the synthesis.

Progesterone-21-C^{14} has been synthesized by alkylation with dimethyl-C^{14} cadmium of 3-keto-Δ^4-etiocholenoyl chloride (108) and 3-acetoxy-Δ^5-etiocholenoyl chloride (109). In the latter case the resulting Δ^5-proenene-3-ol-20-one-21-C^{14} acetate was converted to progesterone by an Oppenauer oxidation. The general method:

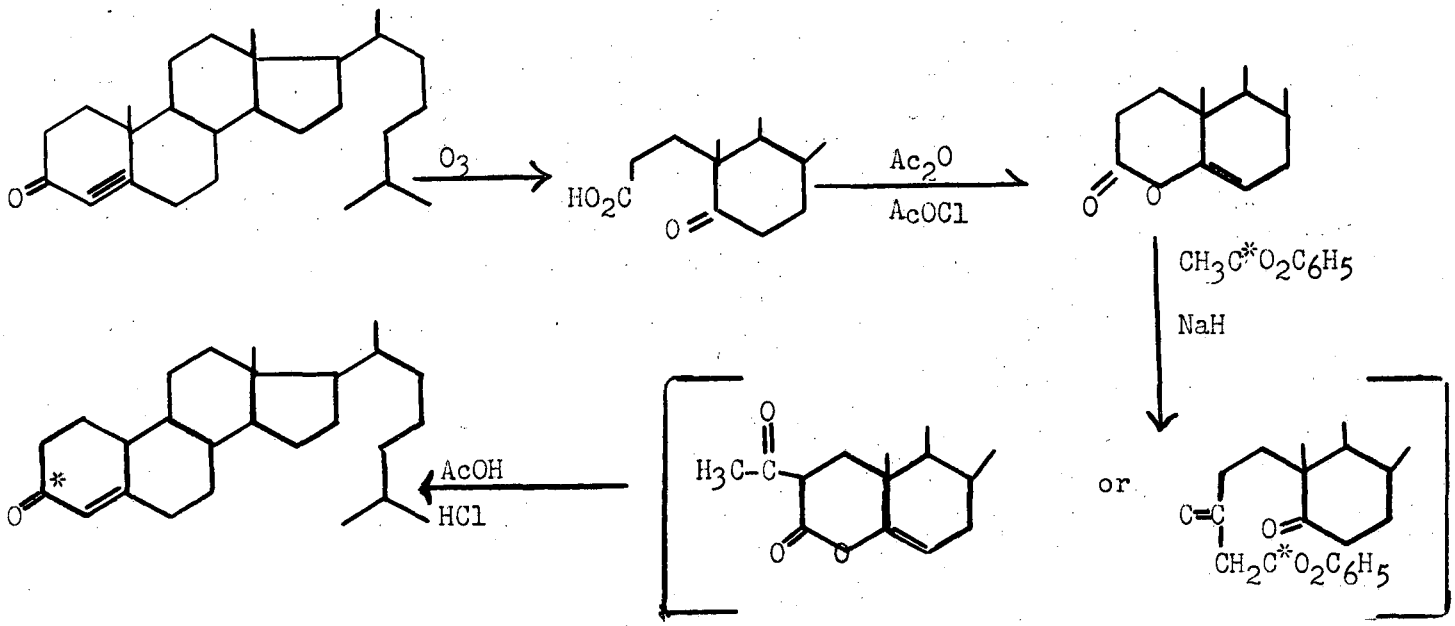


Treatment of androstene-3,17-dione-3-enol with labeled methylmagnesium iodide has been used to prepare 17-methyl-C^{14}-testosterone in good yield (109).

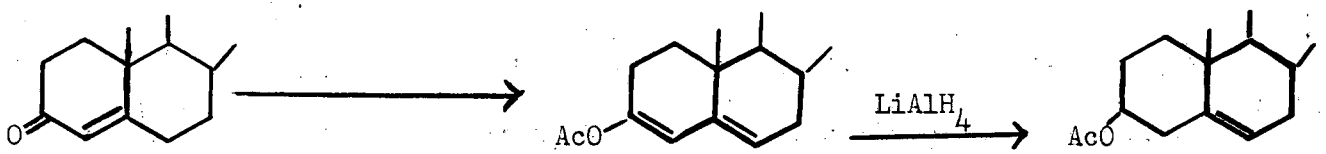
Recent syntheses of chloresterol by treatment of 25-ketonorcholesterol with methylmagnesium iodide followed by dehydration and hydrogenation (110,111) can serve as a model for the preparation of C-26 labeled cholesterol. The synthesis of cholesterol-26-C^{14} by this method, using radioactive methylmagnesium iodide, has been achieved (112).

Synthesis of ring labeled steroids presents a different problem. Total synthesis of steroid has been accomplished in a few cases (113,114,115,116) and in yields which preclude the use of labeled intermediates. However, opening of a ring with the loss of a carbon atom and replacing the lost atom with a carbon isotope offers an attractive way for ring labeling of steroids.

The synthesis of cholestenone-3-C^{14} and cholestenone-4-C^{14} has been achieved by Turner (117,118). The reaction scheme follows:



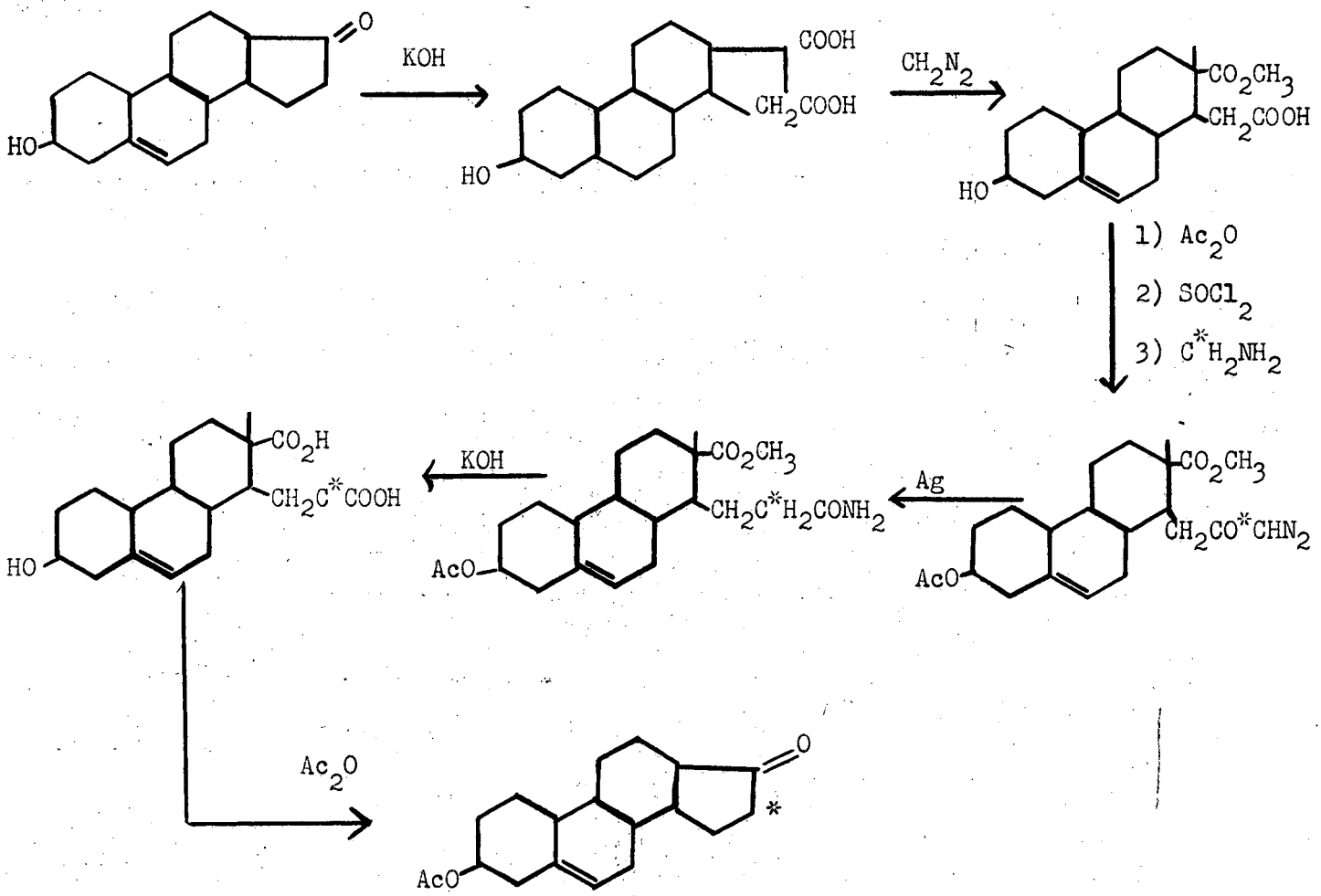
Treatment of the enol acetate of cholestenone with lithium aluminum hydride gives cholesterol in good yield (119). Ring labeled cholesterol has been prepared from ring labeled cholestenone in this manner (120).



Turner has succeeded in labeling the A ring of testosterone in the same manner (118,121). Recently, the synthesis of progesterone and desoxycorticosterone labeled in either the 3 or 4 position (122) has been reported. The method employed involves ozonization of 3-keto-Δ^4-etiocholenic acid to open ring A and give a keto acid similar to that obtained by Turner (117). The methyl ester of this acid is then treated with labeled ethyl bromoacetate in a Reformatsky reaction. Simultaneously, intermediate products undergo cyclization and dehydration to give the starting unsaturated ketone. The side chains are added by known methods.

Treatment of the lactone derived from the ring A keto acid with labeled methylmagnesium iodide gives a 4-labeled steroid in good yields and is an easier reaction to carry out than is the condensation with labeled phenyl acetate used by Turner. This method has been applied to the synthesis of cholestenone-4-C^{14} and testosterone-4-C^{14} (123). A study of the various methods available for incorporation of isotopic carbon into ring A of cholestenone has shown (124) that the yields from the phenyl acetate condensation, the Reformatsky reaction and the Grignard reaction were 45,30 and 70% respectively (calculated from ring A keto acid).

Dehydroisoandrosterone-16-C^{13} acetate (125) has been prepared in the following manner:



Biosynthetic methods: -

Many compounds which cannot be efficiently synthesized in the laboratory can be prepared conveniently by biosynthesis involving plants, animals or microorganisms.

The advantages of this method are obvious, since it puts a much larger variety of complicated labeled compounds within the reach of the investigator. The limitations of this method lie mainly in the fact that it may be wasteful of precious labeled intermediates. This is especially true of animal feeding experiments, where the labeled compound being fed may be metabolically utilized in a variety of ways. It is not usually possible to label any specific position by this method, which may prove a drawback in some experiments. The yields of the desired radioactive products may often be small and the isolation methods long and laborious.

It is not proposed to enumerate all the labeled matabolic products which can be formed from the feeding of radioactive substrates. Many of these have been described in a recent review (126).

Labeled glucose or fructose can be prepared from carbon dioxide-C^{14} using Turkish tobacco leaves (127). Labeled sucrose has been obtained by photosynthesis using the leaves of Canna indica (127) and by an ingenious enzymic synthesis (128) which can yield sucrose labeled in either the glucose or fructose portion:

$$\text{fructose-}C^{14} + \text{sucrose} \xrightarrow{\text{enzyme}} \text{glucose-1-fructoside-}C^{14}$$

$$\text{glucose-}C^{14}\text{-1-phosphate} + \text{fructose} \xrightarrow{\text{enzyme}} \text{glucose-}C^{14}\text{-1-fructoside}$$

Sugars labeled with carbon-14 have also been synthesized photosynthetically by barley seedlings and carbon dioxide-C^{14} (129).

The biosynthesis of starch-C^{13} by bean leaves using isotopic carbon dioxide has been discussed (130).

Labeled digitoxin and nicotine have also been biosynthesized by plants growing in an atmosphere of radioactive carbon dioxide (131,132).

Biosynthesis by microorganisms can yield various labeled fatty acis. Thus, clostridium acidi-urici incorporates carbon 14 into acetic acid during the fermentation of uric acid in an atmosphere of carbon dioxide-C^{14} (133). About half of the original radioactivity is utilized. Clostridium thermoaceticum utilizes 80% of carbon dioxide-C^{14} for acetic acid synthesis (134) and B. rettgeri converts isotopic carbon dioxide into acetic and butyric acids (135). Fermentation of ethanol by clostridium kluyveri results in incorporation of activity into butyric and hexanoic acids (136). The experimental details for several syntheses involving the use of microorganisms have been described in "Isotopic Carbon" (137).

Biosynthesis utilizing animals may either be carried out with the intact animal or with tissue of organ homogenates or slices. In general, the latter method is the more satisfactory.

Liver homogenates incubated with glycine-1-C^{14} yield labeled glutathione (138) and the transformation of labeled acetate to amino acids in the intact rat has been observed (139). The metabolic conversion of many labeled compounds, in intact animals and by tissue slices has been studied extensively, but in most cases the products are isolated only in enough quantity to identify. Often, if the biosynthesized material is of sufficiently high specific activity it may be diluted with inactive material and be used in further experiments. The degradation studies done on labeled cholesterol which was synthesized from acetate-C^{14} by liver slices is a case in point. (140).

The biosynthesis of uric acid labeled with carbon-14 has been studied using the pigeon as the experimental animal and with several radioactive substrates (141).

Using labeled amino acids (glycine-1-C^{14} and alanine-1-C^{14}) radioactive silk has been prepared biosynthetically (142) and labeled bufagin (143) has been obtained from toads fed algae which had been exposed to isotopic carbon dioxide.

REFERENCES

(1) R. D. Cramer and G. B. Kistiakowsky, J. Biol. Chem. 137, 547 (1941)

(2) R. B. Loftfield, Nucleonics 1, No. 3, 54 (1947)

(3) J. A. Box, Experientia 7, 258 (1951)

(4) A. W. Adamson, J. Am. Chem. Soc., 69, 2564 (1947)

(5) R. Abrams, Ibid., 71, 3835 (1949)

(6) R. Belleau and R. D. H. Heard, Ibid., 72, 4268 (1950)

(7) J. A. McCarter, Ibid., 73, 483 (1951)

(8) S. H. Zbarsky and J. Fischer, Can. J. Res. B27, 81 (1949)

(9) B. M. Tolbert, J. Am. Chem. Soc., 69, 1529 (1947)

(10) D. Harman, T. D. Stewart and S. Ruben, Ibid., 64, 2293 (1942).

(11) D. Melville, J. Rachele and E. Keller, J. Biol. Chem. 169, 419 (1947)

(12) R. F. Nystrom, W. H. Yanko and W. G. Brown, J. Am. Chem. Soc., 70, 411 (1948).

(13) J. D. Cox and R. J. Warne, Nature 165, 563 (1950)

(14) J. D. Cox, H. S. Turner and R. J. Warne, J. Chem. Soc., 1950, 3167

(15) J. D. Cox and R. J. Warne, Ibid., 1951, 1896

(16) S. Gurin, Symposium on Uses of Isotopes in Biological Research, University of Chicago, March 1947.

(17) J. G. Burr, W. G. Brown and H. E. Heller, J. Am. Chem. Soc., 72, 2560 (1950).

(18) A. Murray and A. R. Ronzio, AECU 834.

(19) M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich "Isotopic Carbon," John Wiley and Sons, Inc., New York, 1949, p. 166.

(20) C. Heidelberger, J. Biol. Chem., 179, 130 (1949)

(21) D. Elwyn and D. B. Sprinson, Ibid., 184, 465 (1950)

(22) H. R. V. Arnstein, Nature 164, 361 (1949)

(23) A. R. Jones and W. J. Skraba, Science 110, 332 (1949)

(24) A. Murray and A. R. Ronzio, J. Am. Chem. Soc., 71, 2245 (1949).

(25) M. Calvin, et.al., "Isotopic Carbon," p. 157

(26) J. L. Huston and T. H. Norris, J. Am. Chem. Soc., 70, 1968 (1948)

(27) S. Weinhouse, Ibid., 70, 442 (1948)

(28) J. T. Kummer, Ibid., 69, 2239 (1948)

(29) S. V. Schuching and F. W. Barnes, Ibid., 72, 3817 (1950)

(30) J. C. Sowden, Science 109, 229 (1949)

(31) J. C. Sowden, J. Biol. Chem., 180, 55 (1949)

(32) W. H. Beamer, J. Am. Chem. Soc., 70, 3900 (1948)

(33) M. Calvin, et.al., "Isotopic Carbon," pp. 151-171.

(34) W. G. Dauben, J. C. Reid and P. E. Yankwich, Anal. Chem., 19, 828 (1947)

(35) M. Calvin, et.al., "Isotopic Carbon," p. 178

(36) B. M. Tolbert, P. T. Adams and R. M. Noller, unpublished data.

(37) M. Calvin, et.al., "Isotopic Carbon," p. 180 ff.

(38) C. E. Crompton and N. H. Woodruff, Nucleonics 7, No. 3, 49 (1950)

(39) R. Glascock and W. J. Arrol, Nature 163, 61 (1949)

(40) A. Murray, W. W. Foreman and W. Langham, J. Am. Chem. Soc., 70, 1037 (1948)

(41) M. Calvin et.al., "Isotopic Carbon," p. 183

(42) C. J. Collins, J. Am. Chem. Soc., 70, 2418 (1948)

(43) C. Huggett, R. T. Arnold and T. I. Taylor, Ibid., 64, 3043 (1942)

(44) M. Calvin, et.al., "Isotopic Carbon," p. 191

(45) P. E. Wilcos, C. Heidelberger and V. R. Potter, J. Am. Chem. Soc., 72, 5019 (1950)

(46) D. I. Crandall and S. Gurin, J. Biol. Chem., 181, 829 (1949)

(47) W. G. Dauben, J. Am. Chem. Soc., 70, 1376 (1948)

(48) D. I. Crandall, R. Brady and S. Gurin, J. Biol. Chem., 181, 845 (1949)

(49) V. Lorber, M. Cook and J. Meyer, Ibid., 181, 475 (1949)

(50) R. F. Nystrom, W. H. Yanko and B. G. Brown, J. Am. Chem. Soc., 70, 441 (1948)

(51) B. A. Fries and M. Calvin, J. Am. Chem. Soc., 70, 2235 (1948)

(52) P. T. Adams, R. E. Selff and B. M. Tolbert, "Hydrogenation of Fatty Acids to Alcohols," University of California Radiation Laboratory Report #1300 (1951)

(53) B. M. Tolbert, J. Biol. Chem., 173, 205 (1948)

(54) D. N. Hess, J. Am. Chem. Soc., 73, 4038 (1951)

(55) H. S. Anker, J. Biol. Chem., 166, 219, (1946)

(56) W. Sakami, W. E. Evans and S. Gurin, J. Am. Chem. Soc., 69, 1110 (1947)

(57) G. A. Ropp, Ibid., 72, 4459 (1950)

(58) E. M. Gal and A. T. Shulgin, J. Am. Chem. Soc., 73, 2938 (1951)

(59) M. Calvin, et.al., "Isotopic Carbon," p. 209

(60) C. D. Wagner, D. P. Stevenson and J. W. Otvos, J. Am. Chem. Soc., 72, 5786 (1950)

(61) W. J. Arrol and R. Glascock, Nature 159, 810 (1947)

(62) W. J. Arrol and R. Glascock, J. Chem. Soc., 1948, 1534.

(63) W. J. Arrol and R. Glascock, Nature 161, 932 (1948)

(64) B. A. Fries and M. Calvin, J. Am. Chem. Soc., 70, 2235 (1948)

(65) R. B. Rieger and R. W. Blue, J. Org. Chem. 14, 505 (1949)

(66) D. M. Hughes and J. C. Reid, Ibid., 14, 516 (1949)

(67) M. Fields, M. A. Leaffer and J. Rohan, Science 109, 35 (1949)

(68) R. E. Powell and B. M. Tolbert, unpublished data.

(69) A. V. Grosse and S. Weinhouse, Science 104, 402 (1946)

(70) C. Heidelberger, P. Brewer and W. G. Dauben, J. Am. Chem. Soc., 69, 1389 (1947)

(71) W. G. Dauben, J. Org. Chem., 13, 313 (1948)

(72) F. E. Ray and C. R. Geiser, Science 109, 200 (1949)

(73) R. T. Arnold, UCRL 451

(74) H. Hauptmann, private communication.

(75) R. Ostwald, J. Biol Chem., 173, 207 (1948)

(76) S. Gurin and D. W. Wilson, Fed. Proc. 1, 114 (1942)

(77) D. Shemin, J. Biol. Chem., 162, 297 (1946)

(78) J. Baddiley, G. Ehrensvard and H. Nilson, Ibid., 178, 399 (1949)

(79) H. S. Anker, Ibid., 176, 1333 (1948)

(80) C. Heidelberger, Ibid.,179, 139 (1949)

(81) M. Fields, D. E. Walz and S. Rothchild, J. Am. Chem. Soc., 73, 1000 (1951)

(82) M. J. Coon and S. Gurin, J. Biol. Chem., 180, 1159 (1949)

(83) H. R. V. Arnstein, Nature 164, 361 (1949)

(84) L. P. Bouthillier and A. D'Iorio, Rev. Can. Biol., 9, 382 (1950)

(85) G. W. Kilmer and V. duVigneaud, J. Biol. Chem., 154, 247 (1943)

(86) B. Schepartz and S. Gurin, Ibid., 180, 663 (1949)

(87) S. Gurin and A. M. Delluva, Ibid., 170, 545 (1947)

(88) A. B. Lerner, Ibid., 181, 281 (1949)

(89) F. Kogl, J. Halberstadt and T. Barendregt, Rec. trav. chim., 67, 387 (1949)

(90) H. Barsook, C. L. Deasy, A. J. Haagen-Smit, G. Keighley and P. H. Lowy, Ibid, 176, 1383 (1948)

(91) J. C. Reid, Science 105, 208 (1947)

(92) J. C. Reid and H. B. Jones, J. Biol. Chem., 174, 427 (1948)

(93) R. B. Loftfield, J. Am. Chem. Soc., 72, 2499 (1950)

(94) J. Anatol, Compt. rend. 230, 1471 (1950)

(95) H. W. Bond, J. Biol. Chem., 175, 531 (1948)

(96) H. Barsook, C. L. Deasy, A. J. Haagen-Smit, G. Keighley and P. H. Lowy, Ibid., 176, 1383 (1948)

(97) P. Olynyk, D. B. Camp, A. M. Griffith, S. Woislowski and R. W. Helkamp, J. Org. Chem., 13, 465, (1948)

(98) F. N. H. Chang, J. F. Oneto, P. P. T. Sah, B. M. Tolbert and H. Rapoport, Ibid., 15, 634 (1950)

(99) W. Tarpey, H. Hauptmann, B. M. Tolbert and H. Rapoport, J. Am. Chem. Soc., 72, 5126 (1950)

(100) B. M. Tolbert, F. Christenson, F. N. H. Chang and P. P. T. Sah, J. Org. Chem. 14, 525 (1949)

(101) A. Murray and A. R. Ronzio, AECU 86.

(102) F. Christenson and B. M. Tolbert, UCRL 396.

(103) D. E. Walz, M. Fields and J. A. Gibbs, J. Am. Chem. Soc., 73, 2968 (1951).

(104) L. F. Cavalieri, J. F. Tinker and A. Bendich, Ibid., 71, 533 (1949).

(105) V. M. Clark and H. M. Kalckar, J. Chem. Soc., 1950, 1029.

(106) J. L. Burns and C. G. King, Science 111, 257 (1950)

(107) D. B. Melville, J. G. Pierce and C. W. H. Partridge, J. Biol. Chem., 180, 299 (1949).

(108) B. Riegel and F. S. Prout, J. Org. Chem., 13, 933 (1948).

(109) H. B. MacPhillamy and C. R. Scholz, J. Biol. Chem., 178, 37 (1949).

(110) A. X. Ryer, W. H. Gebert and N. H. Murrill, J. Am. Chem. Soc., 72, 4247 (1950).

(111) W. G. Dauben and H. L. Bradlow, Ibid., 72, 4248 (1950).

(112) W. G. Dauben and H. L. Bradlow, private communication.

(113) W. E. Bachmann, W. Cole and A. L. Wilds, J. Am. Chem. Soc., 61, 974 (1939).

(114) W. S. Johnson, J. W. Petersen and C. D. Gutsche, Ibid., 67, 2274 (1945).

(115) G. Anner and K. Meischer, Experientia 4, 25 (1948).

(116) R. B. Woodward F. Sonderheimer, D. Taub, K. Heusler and W. M. McLamore, J. Am. Chem. Soc. 73, 2043 (1951).

(117) R. B. Turner, Ibid., 69, 726 (1947).

(118) R. B. Turner, Ibid., 72, 579 (1950).

(119) W. G. Dauben and J. F. Eastham, Ibid., 72, 2305 (1950).

(120) W. G. Dauben and J. F. Eastham, private communication.

(121) R. B. Turner, Science 106, 248 (1947).

(122) R. D. H. Heard and P. Ziegler, J. Am. Chem. Soc., 72, 4328 (1950)

(123) G. Fujimoto, Ibid., 73, 1856 (1951).

(124) R. D. H. Heard and P. Ziegler, Ibid., 73, 4036 (1951).

(125) E. B. Hershberg, E. Schwenk and E. Stahl, Arch. Biochem. 19, 300 (1948).

(126) N. H. Woodruff and E. E. Fowler, Nucleonics 7, No. 2, 26 (1950).

(127) E. W. Putman, W. Z. Hassid, G. Krotkov and H. A. Barker, J. Biol. Chem., 173, 785 (1948).

(128) N. Wolochol, E. W. Putman, M. Duedoroff, W. Z. Hassid and H. A. Barker, Ibid., 180, 1237 (1949).

(129) S. Aranoff, H. A. Barker and M. Calvin, Ibid., 169, 459 (1947).

(130) L. G. Livingston and G. Medes, J. Gen. Physiol. 31, 75 (1947).

(131) E. M. K. Geiling and T. E. Kimura, Fed. Proc. 8, 308, (1949).

(132) E. M. K. Geiling, F. E. Kelsey, B. J. McIntosh and A. Ganz, Science 108, 558, (1948)

(133) H. A. Barker, S. Ruben and J. V. Beck, Proc. Nat. Acad. Sci., 26, 477 (1940).

(134) H. A. Barker and M. D.Kamen, Ibid., 31, 219, (1945).

(135) H. A. Barker, M. D. Kamen and V. Haas, Ibid., 31, 355 (1945).

(136) H. A. Barker, M. D. Kamen and B. T. Bornstein, Ibid., 31, 373 (1945).

(137) M. Calvin, et.al., "Isotopic Carbon," p. 272 ff.

(138) K. Block, J. Biol. Chem., 179, 1245 (1949).

(139) D. M. Greenbert and T. Winnick, Arch. Biochem. 21, 166 (1949).

(140) H. N. Little and K. Bloch, J. Biol. Chem., 183, 33 (1950).

(141) J. L. Karlsson and H. A. Barker, Ibid., 177, 597 (1949).

(142) P. C. Zamecnik, R. B. Loftfield, M. L. Stephenson and C. M. Williams, Science 109, 624 (1949).

(143) J. Doull, K. P. DuBois and E. M. K. Geiling, Fed. Proc. 8, 286 (1949).

HYDROGEN

There are three general ways in which a hydrogen label may be introduced into a compound: direct addition to an unsaturated linkage, replacement of hydrogen atoms already present or replacement of other atoms by heavy hydrogen. Syntheses involving all these methods will be discussed. Most of the literature covering the use of heavy hydrogen describes uses of deutarium because, until recently, this isotope alone was available; however, it must be understood that deuterium and tritium are interchangeable.

Hydrogenation of Double Bonds with Isotopic Hydrogen:-

There are many examples of the hydrogenation of couble bonds using heavy hydrogen. Schoenheimer and Rittenberg partially hydrogenated linseed oil in the presence of palladium (1); the iodine number of the oil was reduced from 170 to 79.5 and 5.74 atom percent of deuterium introduced. The syntheses of several deuterium labeled acids, prepared by hydrogenation, has been described by Bloch and Rittenberg (2,3). The preparation of methyl d_2-11,12-lithocholate by hydrogenation of methyl 3α-acetoxy-Δ^{11}-cholenate with deuterium gas is described here (4):

> Methyl d_2-11,12-Lithocholate[4].- Methyl 3α-acetoxy-Δ^{11}-cholenate was reduced with 95 per cent deuterium gas in 10 gm. portions by use of 2 gm. of $PtO_2 \cdot H_2O$ in 125 ml. of acetic acid-d. The solution was decanted from the catalyst and filtered quickly; the same catalyst was used to reduce about 150 gm. of product. The acetic acid-d was distilled in a closed system under diminished pressure and was used repeatedly. The accumulation of atmospheric water in repeated transfers, filtrations, etc., and the use of 95 per cent deuterium gas account for the incorporation of less than the calculated amount of deuterium. When rigid precautions are taken

to climinate these dilutions, the incorporation of the isotope is usually slightly in excess of theory. The methyl acetoxy-d_2-11, 12-lithochloate was saponified with N NaOH under a reflux for 1 hour, and the acid was esterified with methanol in the presence of H_2SO_4 at room temperature. The product contained 4.03 atoms per cent excess deuterium (1.69 atoms of deuterium per mole).

Tritium labeled hexesterol has been obtained by the hydrogenation of 2,3-di-(4-hydroxyphenyl)-2,4-hexadiene (diemestrol) with tritium gas over palladium (5).

The reduction of an unsaturated acetal with heavy hydrogen provides a way of obtaining a labeled aldehyde, which may be utilized in subsequent reactions. Leucine-β,γ-d and valine-β,γ-d have been prepared from isopentenal diethyl acetal and isobutenal diethyl acetal respectively by the series of reactions outlined below (6):

$$(CH_3)_2C = C(OEt)_2 \xrightarrow{D_2} (CH_3)_2\text{-}CH^*\text{-}CH^*(\text{-}OEt)_2 \xrightarrow[H^+]{H_2O}$$

$$(CH_3)_2\text{-}CH^*\text{-}CH_2^*\text{-}CHO \xrightarrow[NH_4Cl]{KCN} (CH_3)_2\text{-}CH^*\text{-}CH_2^*\text{-}\underset{NH_2}{CH}\text{-}COOH$$

Reductive amination of keto acids, using deuterium, has been used to prepare glutamic acid-α,β-d (7) and deuterioalanine (2).

It has recently been shown (8) that in hydrogenations with isotopic hydrogen, the equilibrium

$$H_2 + AcOT \rightleftharpoons HT + AcOH$$

plays a very important part. The acetic acid medium acts as an isotopic buffer, the carboxyl isotopic concentration of which determines, to a large extent, the isotopic ratio of the hydrogen atoms being incorporated into the compound being hydrogenated. The reaction studied was the hydrogenation of methyl 3α-acetoxy-Δ^{11}-cholenate, using deuterium and tritium. In this connection, it is interesting to note that in the

reductive amination of α-ketoglutaric acid (7), the resulting glutamic acid contained a much greater amount of deuterium when deuterium gas was used than when deuterium oxide was used.

Tritium containing methanol has been prepared by the hydrogenolysis of methyl formate over a copper oxide-chromium oxide catalyst (9). The tritium labeled methanol was converted to labeled methyl iodide by treatment with hydrogen iodide.

Replacement of Hydrogen Atoms by Isotopic Hydrogen:-

The hydrogen exchange reaction is the most popular method for the introduction of heavy hydrogen into an organic molecule. The advantage of this reaction lies in the fact that many complex compounds may be readily labeled without resort to a scheme of total synthesis.

Aromatic compounds will exchange ring hydrogens when treated with aluminum chloride and deuterium chloride (10). When phenol is treated with anhydrous deuterium chloride at slightly elevated temperatures exchange takes place in the 2,4 and 6 positions in the ring as well as in the hydroxyl group (11). Addition of heavy water to the thionyl chloride gives deuterium chloride readily.

In the presence of 80-90% deuterium sulfate many substances will readily undergo hydrogen exchange. This method has been used to prepare deuterobenzene (12), deuterophenylalanine (13), deuteroalanine (14,15) and deuteroleucine (14,15). Tritium labeled phenylalanine (16) has also been prepared in this way. Heavy sulfuric acid is made by treating sulfur trioxide with deuterium (12) or tritium oxide (16). Several fatty acids have been labeled using concentrated deuterium sulfate (17). One such preparation is described here:

Exchange of Deuterium between Palmitic Acid and D_2SO_4 [17].-

Concentrated D_2SO_4, 100 percent with respect to sulfuric acid, and containing 95 atom per cent of deuterium was prepared by

dissolving 1 mole of SO_3 in 1 mole of 95 per cent D_2O. 1 gm. of palmitic acid and 1.85 gm. of the 100 per cent D_2SO_4 were weighed into a test tube, and 0.186 cc. of 95 per cent D_2O added to bring the sulfuric acid concentration to 90 per cent. The neck of the tube was drawn to a long capillary and the tube placed in an oven at 98-100° for 50 hours. The tube was opened and the dark amber contents precipitated with water, and extracted with ether. The ethereal solution was extracted with alcoholic KOH, and the alkaline solution acidified with dilute H_2SO_4 and extracted again with ether. By this process the labile deuterium of the carboxyl group was completely replaced by ordinary hydrogen. After removal of the well dried ether the acid was recrystallized from aqueous acetone, yielding 913 mg. of palmitic acid, m.p. 62.5°, containing 4.32 atom per cent of deuterium. Assuming that D and H atoms have the same affinity for the carbon atoms, this corresponds to an exchange of 1.38 H atoms per molecule.

Similar tests on 1 gm. quantities of palmitic acid with 95 and 98 per cent D_2SO_4 gave 764 and 593 mg. of acid containing 5.04 and 4.62 atom per cent of deuterium respectively (corresponding to 1.79 and 1.48 atoms of deuterium per molecule). An experiment with 90 per cent D_2SO_4 at 130° yielded 933 mg. of palmitic acid containing 4.6 atom per cent of deuterium (1.47 atoms of deuterium per molecule).

The isotopic hydrogen enters the fatty acids in the alpha position under these conditions. Milder conditions, such as use of dilute acid with heavy water, also give exchange. Labeled glycine (15), proline (15), cystine (15,18), tyrosine (15), glutamic acid (15) arginine (18) and lysine (18) have all been p repared by this method.

Enolizable hydrogen atoms of ketones can be replaced by treatment with alkali and heavy water. In this way Δ^4-cholestenone was prepared by refluxing a solution of Δ^5-cholestenone in alcoholic sodium hydroxide containing D_2O (19). The cholestenone thus obtained contained 4.75 atom per cent excess deuterium. Recrystallization of this compound from a medium containing a minute amount of base yielded a pure product which contained only 0.19 atom per cent excess deuterium. The foregoing demonstrates the need of equilibration of hydrogen labeled compounds prior to use. It is generally accepted that the heavy hydrogen remaining after reflux of a labeled compound with base may be regarded as being stably bound.

Heating of a compound with heavy water and a suitable catalyst will give extensive exchange in many cases. Thus, fatty acids treated with alkaline D_2O and platinum at 130° will take up considerable deuterium. Labaled isocaproic acid has been obtained in this manner (20). Deuterobenzene has been prepared by passing the vapors of benzene and deuterium oxide over a nickel catalyst (21).

Deuterium labeled cholesterol has been prepared by heating cholesterol with heavy water, acetic acid and active platinum at 125° for four days (22). Selenium was found to give slow exchange and platinum alone, none.

Deutero cholesterol [22]. - One and one quarter gm. of platinum oxide suspended in a mixture of 40 ml. of deuterio acetic acid (containing 60 atom per cent D) and 13 ml. of 99 per cent D_2O were reduced with ordinary hydrogen; the excess hydrogen was replaced by nitrogen and 12.5 gm. of cholesterol were added to the mixture. The reaction flask was evacuated, sealed and shaken for three days in an oven at 127°. The solvent was distilled off *in vacuo*; the residue, which contained appreciable amounts of cholesteryl acetate, was taken up in ether, filtered, evaporated, and treated with 400 ml. of 95 per cent

ethanol containing 8 gm. of potassium hydroxide for 4 days at room temperature. The alcoholic solution was chilled, the crystals were filtered off, and a second crop was obtained by concentrating the filtrate. A third crop was secured from the mother liquor by dilution with water and extraction with ether. The combined crude sterol fractions (7.5 gm.), after several recrystallizations from acetone, yielded 4.9 gm. of cholesterol, m.p. 148° corrected, $[\alpha]_D^{26} = -39°$ (2 per cent in chloroform). The deuterium concentration was 4.16 atom per cent in excess.

Using the identical technique with tritium oxide, tritium labeled cholesterol has been prepared (23).

In a recent study of the deuteration of steroids (24) it was found that although saturated ketosteroids took up appreciable amounts of the isotope, the uptake increased with more keto groups per molecule and with increasing unsaturation. Hydroxysteroids underwent dehydration and hydrogenolysis, but these effects could be reduced by acylation prior to exchange. Varying temperature, catalyst and substrate in the deuteration of ^{4}-androsteno-3,17-dione showed that little exchange took place below 100°, most in dilute solution. The label in deuterated cholesterol was found to be 46% in the vicinity of the ^{5}-3 hydroxyl system and 54% in the isopropyl group of the side chain.

A process for the improved preparation of deuterated compounds has recently been reported (25). This process is based upon careful rectification of the mixtures resulting from exchange reactions. Preparation of CH_3OD and CD_3COOD is described, as is the necessary equipment.

Replacement of other atoms with isotopic hydrogen:-

The introduction of isotopic hydrogen into a compound by replacement of another element has been used in some cases to obtain labeled compounds. Deuterobenzene has

been prepared by the decarboxylation of calcium mellitate in the presence of $Ca(OD)_2$ (26):

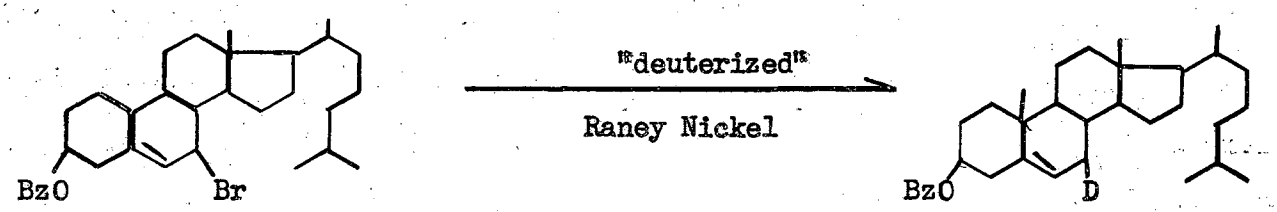
$$CaO + D_2O \longrightarrow Ca(OD)_2$$

$$C_6(CO_2)_6Ca_3 + 3Ca(OD)_2 \xrightarrow{\Delta} C_6D_6 + 6CaCO_3$$

Various labeled alkanes, among them methane-d, propane-1-d, n-butane-1-d and isobutane-2-d (27,28) and toluene-d (29) have been prepared by treating the appropriate Grignard reagents with either deuterium oxide or deuterium sulfate.

Decomposition of an aromatic diazonium salt using hypophosphorus acid in the presence of deuterium oxide (30) has been used to introduce deuterium into the benzene nucleus. Benzene-d and m-deuteronitrobenzene have been prepared in this manner.

Elegant methods for introducing one or two atoms of isotopic hydrogen into a single, known position in the steroid nucleus have been reported recently (31). In one case, 7-bromocholesteryl benzoate was treated with "deuterized" Raney nickel to give cholesteryl-7-d benzoate. Treatment of the bromide with deuterium and 5% palladium on charcoal also yielded cholesteryl-7-d benzoate.



Reduction of 7-ketocholesterol acetate ethylene mercaptol with "deuterized" Raney nickel permits introduction of two atoms of deuterium into the 7 position.

"Deuterized" Raney Nickel [31].- The Raney nickel catalyst (W-4) was prepared by the method of Pavlic and Adkins [Pavlic and Akkins, J. Am. Chem. Soc., 68, 1471 (1946).] and was found to contain about 165 cc. of hydrogen per cc. of catalyst (equivalent to about 0.7 g. of dry nickel). To 100 cc. of Raney nickel catalyst (under ethanol) was added 800 cc. of methylcyclohexane. The alcohol and any water present were removed by azeotropic distillation with methylcyclohexane at 70° (bath temperature) at 20 cm. To the dry catalyst was added 13.5 cc. of deuterium oxide (99.9 per cent.) and 1 liter of dry methylcyclohexane. The mixture was shaken well and warmed for fifteen minutes. The equilibrated deuterium oxide was removed by azeotropic distillation with methylcyclohexane. This equilibration was repeated five times with volumes of 13.5, 12.5 12, 12 and 11.5 cc. of deuterium oxide. The water from the last equilibration contained 88.5% deuterium oxide. Dry methylcyclohexane was added at each equilibration to replace the amount distilled. After the equilibration was complete, the "deuterized" Raney nickel was shaken with deuterium gas (95%).

7-d-Cholesterol from 7-bromocholesterol benzoate with "Deuterized" Raney Nickel as Catalyst. - To a suspension of 20 cc. of "deuterized" Raney nickel was added 2.00 g. of 7-bromocholesterol benzoate in 250 cc. of dry ethyl acetate. The mixture was shaken with deuterium (95%) under atmospheric pressure for one hour. The catalyst was removed by decantation and filtration with the aid of Celite. Because a considerable amount of the product remained adsorbed on the nickel, it was necessary to wash the catalyst several times with ethyl acetate to insure a quantitative recovery. The solvent was evaporated, and

the residue was hydrolyzed with 50 cc. of benzene, 125 cc. of methanol and a solution of 5 g. of potassium hydroxide in 5 cc of water. After refluxing one hour the solvents were removed in vacuo, water was added and the residue extracted with ether. The ether solution was washed with water, dried over sodium sulfate and evaporated to dryness. The semi-crystalline residue, 1.68 g., was chromatographed on 30 g. of alumina. The column was developed with ligroin (b.p. 30°), benzene: ligroin (1:1), benzene and mixtures of ether and benzene, and mixtures of methanol and ether. From the benzene and benzene-ether (3:1) eluates was obtained 960 mg. of crude 7-d-cholesterol, which upon rechrystallization from acetone gave 592 mg. of pure 7-d-cholesterol, m.p. 148-149°; $[\alpha]_D^{25}$ -40.5° ± 2° (11.36 mg. in 2.00 ml. of chloroform); the m.p. of the admixture with undeuterated cholesterol was not depressed. From the mother liquor was obtained 250 mg. of 7-d-cholesterol with m.p. 147.5-148.5°, giving a total of 844 mg. (63%) of 7-d-cholesterol. Infrared analysis of these samples revealed the presence of the characteristic absorption at 2100 $cm.^{-1}$ associated with the C-D bond. Quantitative analysis with the mass spectrometer indicated that the 7-d-cholesterol contained 1.55 atom per cent. excess deuterium, corresponding to 0.71 atom deuterium per molecule.

Other Methods:-

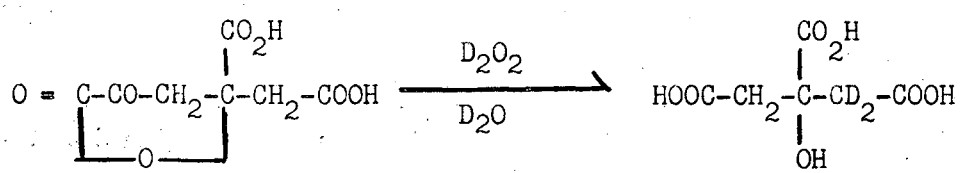
Another method of preparing labeled compounds involved synthesis with labeled intermediates. The intermediates may be labeled by any one of the methods described above. The decarboxylation of labeled malonic acid, prepared by exchange with heavy water, yields methyl-labeled acetic acid (32) which can be used for further synthetic

work. Methionine, labeled in the methyl group, has been prepared by the following reaction sequence (33):

$$CO \xrightarrow{D_2} CH_3^*OH^* \xrightarrow[HI]{P} CH_3^*I \xrightarrow[Na,NH_3\ (liq.)]{\text{homocysteine}} CH_3^*SCH_2CH_2\underset{NH_2}{\underset{|}{C}}HCOOH$$

Deuteroacetylene, prepared by treating calcium carbide with deuterium oxide, has been polymerized to give deuterobenzene (34); many other syntheses with this useful intermediate suggest themselves. Deuteroacetylene has been converted to deuteroacetaldehyde (2).

An interesting synthesis of deuterocitric acid involving the oxidation of oxalcitromalic lactone with deuterium peroxide in the presence of deuterium oxide has been reported (35):



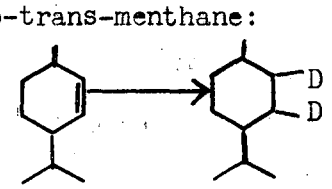
Deuterium Peroxide (35):-

A solution of eight gm. of phosphorus pentoxide in 6 cc. of deuterium oxide was heated for a short time, then cooled in an ice-salt mixture. Sodium peroxide was added, with stirring, to a pH of 7.6 and the solution acidified with 2 cc. of phosphoric acid-d_3. Vacuum distillation yielded 3.8 cc. of 29% deuterium peroxide.

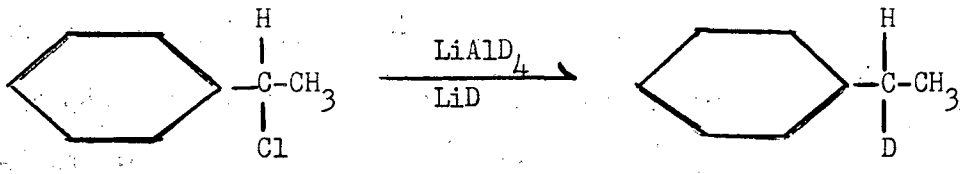
Optical Activity due to Isotopic Hydrogen:-

In connection with the introduction of hydrogen isotopes into various molecules, it is intersting to note that deuterated compounds may be optically active where their hydrogen analogs are not.

Alexander and Pinkus (36) reduced active trans 2-menthene with deuterium and obtained active 2,3-dideutero-trans-menthane:



An even more striking example is the preparation of optically active α-deuteroethylbenzene (37). The reduction of (-)-α-chloroethylbenzene ($[\alpha]_D^{25}$ -49.2°) with lithium aluminum deuteride-lithium deuteride in tetrahydrofuran gave an 80% yield or (-)-α-deuteroethylbenzene (α_D^{25} -0.30°).



Friedel-Crafts acetylation yielded optically active p-acetyl-α-deuteroethylbenzene which gave an optically active oxime. The activity of the oxime was not affected by several recrystallizations, and upon hydrolysis, the active ketone was regenerated.

Recent reports (38) of the preparation of tritium labeled lithium aluminum hydride and lithium hydride makes these reagents available for similar experiments.

Biosynthetic Preparations:-

The biosynthetic preparation of labeled compounds for further utilization finds little application in the case of heavy hydrogen. The great dilution of hydrogen atoms in the body, plus the possibilities for exchange militates against the use of biosynthesis as a preparative method. Many deuterium or tritium labeled compounds have been isolated from animal tissues after feeding of heavy water or a labeled precursor. Deuterium oxide feeding has yielded labeled cholesterol in rats (39) and similar results have been shown with rabbits using tritium (40). The feeding of deuterium oxide to rats or mice has resulted in the labeling of a host of body components. Among those isolated have been: glycine (41), aspartic acid (41), glutamic acid (41,42) proline (41), arginine (41), cystine (14,43), leucine (41) and tyrosine (41,42,43). Compounds other than amino acids have been: azaleic (42), heptoic (42), palargonic (42), stearic (44) and palmitic (44) acids and glycogen (42,45,46).

Feeding of labeled precursors other than water has given β-hydroxybutyric acid from deuterobutyric acid (47), glutamic acid and ornithine from deuteroproline (48),

tyrosine from deuterophenylalanine (13) and cholesterol which has been obtained after feeding deuteroleucine (20), deuterolsovaleric acid (20), deuteroethanol (49), deuterobutyric (49), deuteroalanine (49), deuterovaleric acid (49) and deuteromyristic acid (40).

Using deuteroacetate as a substrate, labeled succinic and citric acids have been obtained as yeast metabolites (50).

REFERENCES

(1) R. Schoenheimer and D. Rittenberg, J. Biol. Chem. 111, 175 (1935).

(2) K. Bloch and D. Rittenberg, Ibid., 155, 243 (1944).

(3) K. Block and D. Rittenberg, Ibid., 145, 629 (1942).

(4) B. A. Koechlin, T. H. Kritchevsky and T. F. Gallagher, Ibid., 184, 393 (1950).

(5) D. L. Williams and A. R. Ronzio, J. Am. Chem. Soc., 72, 5758 (1950).

(6) C. R. Kinney and R. Adams, Ibid., 59, 897 (1937).

(7) D. Rittenbert, S. Ratner and H. D. Hoberman, Ibid., 62, 2249 (1940).

(8) M. L. Eidinoff, J. E. Knoll, D. K. Fukushima and T. F. Gallagher, Abstracts 118th meeting of the American Chemical Society, September 1950, p. 660.

(9) D. Harman, T. D. Stewart and S. Ruben, J. Am. Chem. Soc., 64, 2293 (1942).

(10) A. Klit and A. Langseth, Z. physik. Chem. 176A, 65 (1936).

(11) H. Hart, J. Am. Chem. Soc., 72, 2900 (1950).

(12) C. K. Ingold, C. G. Raisin and C. L. Wilson, J. Chem. Soc., 1936, 915.

(13) A. R. Moss and R. Schoenheimer, J. Biol. Chem., 135, 415 (1940).

(14) R. Schoenheimer, D. Rittenberg and A. S. Keston, J. Am. Chem. Soc., 59, 1765 (1937).

(15) D. Rittenbert, A. S. Keston, R. Schoenheimer and G. L. Foster, J. Biol. Chem. 125, 1 (1938)

(16) S. Gurin and A. M. Delluva, Ibid., 170, 545 (1947).

(17) W. E. vanHeyningen and D. Rittenberg, Ibid., 125, 495 (1938).

(18) J. Stekol and W. H. Hamill, Ibid., 120, 531 (1937) .

(19) M. Anchel and R. Schoenheimer, Ibid., 125, 23 (1938).

(20) K. Bloch, Ibid., 155, 255 (1944).

(21) P. I. Bowman, W. S. Benedict and H. S. Taylor, J. Am. Chem. Soc., 57, 960 (1935).

(22) K. Bloch and D. Rittenberg, J. Biol. Chem. 149, 505 (1943).

(23) D. Kritchevsky, M. W. Biggs and N. K. Freeman, UCRL #644.

(24) D. K. Fukshima and T. F. Gallagher, Fed. Proc. 9, 174 (1950).

(25) K. Clusius and H. Knopf, Z. Naturforsch. 2b, 169, (1947).

(26) H. Erlenmeyer and H. Lobek, Helv. Chim. Acta 18, 1464 (1935).

(27) C. D. Wagner and D. P. Stephenson, J. Am. Chem. Soc., 72, 5785 (1950).

(28) C. D. Hurd and J. Azorlosa, Ibid., 73, 33 (1951).

(29) C. D. Hurd and J. Azorlosa, Ibid., 73, 37 (1951).

(30) E. R. Alexander and R. E. Burge, Ibid., 72,3100 (1950).

(31) D. K. Fukushima, S. Lieberman and B. Praetz, Ibid., 72, 5205 (1950).

(32) J. C. Halford and C. L. Anderson, Ibid, 58, 736 (1936).

(33) V. du Vigneaud, M. Cohn, J. P. Chandler, J. R. Schenk and S. Simonds, J. Biol. Chem., 140, 625 (1941).

(34) C. R. Clemo and A. M. McQuillen, J. Chem. Soc., 1935, 853.

(35) C. Martius and C. Schorre, Ann. 570, 140 (1950).

(36) E. R. Alexander and A. G. Pinkus, J. Am. Chem. Soc., 71, 1786 (1949).

(37) E. E. Eliel, Ibid., 71, 3970 (1949).

(38) K. E. Wilzbach and L. Kaplan, Ibid., 72, 5795 (1950) .

(39) D. Rittenberg and R. Schoenheimer, J. Biol. Chem. 121, 235 (1937).

(40) M. W. Biggs and D. Kritchevsky, Circulation 4, 34 (1951).

(41) G. L. Foster, D. Rittenberg and R. Schoenheimer, J. Biol Chem., 125, 13 (1938).

(42) G. E. Boxer and D. Stetten, Ibid., 153, 607 (1944).

(43) J. A. Stekol and W. H. Hamill, Proc. Soc. Exptl. Biol. Med. 35, 591 (1937).

(44) K. Bernhard and R. Schoenheimer, J. Biol. Chem. 133, 713 (1940).

(45) D. Stetten and G. E. Boxer, Ibid., 155, 231 (1944).

(46) G. E. Boxer and D. Stetten, Ibid., 155, 237 (1944).

(47) M. G. Morehouse, Ibid., 129, 769 (1939).

(48) M. R. Stetten and R. Schoenheimer, Ibid., 153, 113 (1944).

(49) S. Ratner, D. Rittenberg and R. Schoenheimer, Ibid., 135, 357 (1940).

(50) R. Sonderhoff and H. Thomas, Naturwiss. 24, 570 (1936).

OXYGEN

Incorporation of O^{18} into an organic molecule is generally achieved by exchange between an oxygen-containing compound and H_2O^{18}. Catalysts may be required and, in general, alcoholic or phenolic oxygen does not exchange.

The following table, taken from a review by Bentley (1) indicates the extent of exchange undergone by a variety of compounds under various conditions.

Compound	T °C	t (Hrs.)	Catalyst	Extent
Methanol	100	7		None
	25	24	0.1 N HCl	None
	25	48	0.1 N NaOH	None
Glycerol	100	6		None
Phenol	25	44		None
Acetic acid	100	48	HCl	Slight
	100	48	KOH	Complete
	100	48		None
Chloroacetic acid	25	24		Partial
Trichloroacetic acid	25	42		Complete
n-Butyric acid	100	24		None
Fumaric acid	25	44		None
	100	45		Partial
Maleic acid	100	45		Complete
Succinic acid	130	5		Complete
Benzoic acid	100	4	0.1 N HCl	Complete
	100	4		Complete
Citric acid	25	24		None
	75	24		Complete
	25	24	H_2SO_4	Partial
Phthalic acid	100	20		Slight
Terephthalic acid	100	20		Slight
Amyl acetate	25	44		None
Acetaldehyde	25	24		Complete

(Continued)

Compound	T °C	t (Hrs.)	Catalyst	Extent
Acetone	100	24	0.002 N HCl	Partial
	25	1	0.005 N NaOH	Complete
	25	1		Complete
Acetamide	25	71		None
Benzamide	100	23		None
Benzaldehyde	110	2		Complete
Urea	25	54		None
Glucose	100	48		
Fructose				Slight
Galactose				1 atom
Xylose	100	20		exchanges
Arabinose				
Alloxan hydrate	25	430		87% exchange
Diphenylmethyl carbinol	95	10.5		None
	95	22	KOH	None
Trimethyl carbinol	95	20		None
	95	2	H_2SO_4	None
Trianisyl carbinol	95	43		None
	95	24	H_2SO_4	Complete
Glycine	100	24		None
Glycine.HCl (pH 1.9)	100	24		Complete
Tyrosine	100	40		None
Diketopiperamine	100	48		None
Glycylglycine	100	24		None

Most organic work involving O^{18} has been in the field of reaction mechanisms. Hoberts and Urey have studied the kinetics of exchange between benzoic acid and water (2), the benzilic acid rearrangement (3) and the mechanism of ester hydrolysis, esterification and oxygen exchange (4), all with H_2O^{18}. Hydrolysis of γ-butylolactone in the presence of water containing heavy oxygen has yielded γ-hydroxybutyric acid labeled in the carboxyl group with O^{18} (5).

In the biological field, isotopic oxygen has been used as a tracer in photosynthesis (6) where it was found that the oxygen involved comes from the water rather than the carbon dioxide. The photoreduction reaction in photosynthesis has been recently studied with H_2O^{18} (7).

The fate of the sulfate-O^{18} group in the rabbit has been studied (8), the sulfate radical being chosen because it does not undergo exchange with water, whereas phosphate does.

REFERENCES

(1) R. Bentley, Nucleonics 2, No. 2, 18 (1948).

(2) I. Roberts and H. C. Urey, J. Am. Chem. Soc. 61, 2580 (1939).

(3) I. Roberts and H. C. Urey, Ibid., 60, 880 (1938).

(4) I. Roberts and H. C. Urey, Ibid., 61, 2584 (1939).

(5) F. A. Long and L. Friedman, Ibid., 72, 3692 (1950).

(6) S. Ruben, M. Randall, M. Kamen and J. L. Hyde, Ibid., 63, 877 (1941).

(7) G. D. Dorough and M. Calvin, Ibid., 73, 2362 (1951).

(8) A. H. W. Aten and G. Hevesey, Nature 142, 952 (1938).

NITROGEN

The introduction of heavy nitrogen into organic compounds involves the formation of a carbon-nitrogen linkage and use is made of the many well-known procedures for forming this bond. In almost every case, compounds containing isotopic nitrogen have been prepared for biological studies and most of the syntheses carried out with this isotope involve the preparation of amino acids, purines or intermediates leading towards one or the other class of compounds.

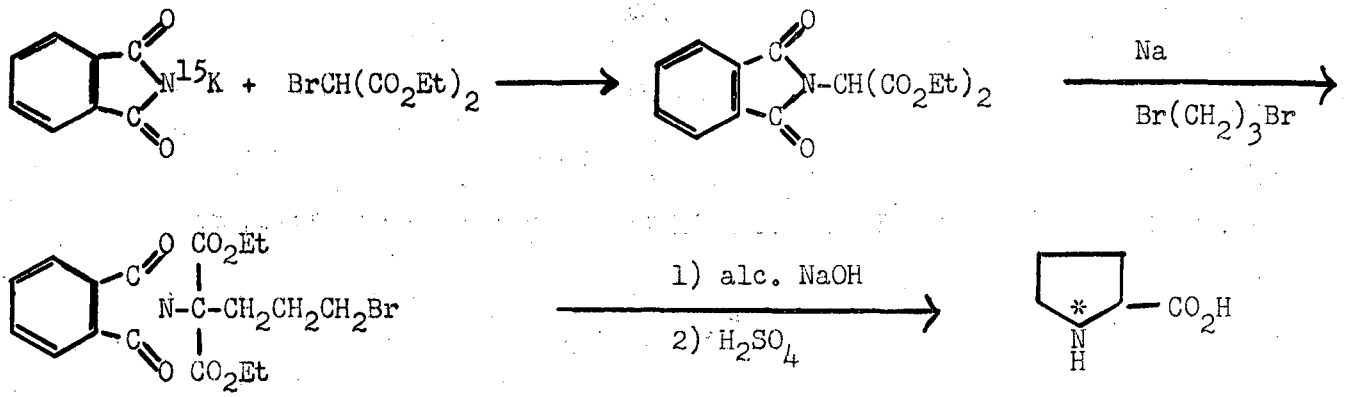
Amino Acids:-

A larte number of amino acids have been synthesized via the reductive amination (Knoop-Oesterlin) reaction. Schoenheimer and Ratner (1) made a careful study of this reaction and found that conditions including a 50% alcoholic solution, a palladium catalyst and one equivalent of ammonia for each keto group and one for each carboxyl group resulted in optimum yields. The non-amino nitrogen could be quantitatively recovered as $N^{15}H_3$ by distillation. Using this method, alanine, phenylalanine, tyrosine, norleucine, aspartic acid and glutamic acid were prepared (1).

The Knoop-Oesterlin reduction has also been used for the synthesis of γ-phenyl-α-amino-N^{15}-butyric acid (2).

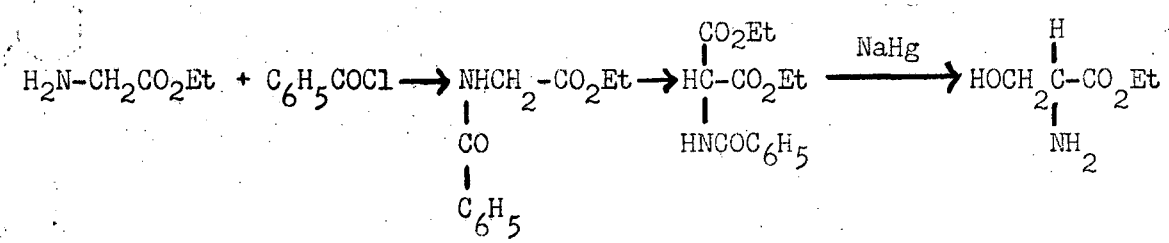
The Gabriel phthalimide synthesis has been used for the preparation of glycine-N^{15} (1,3,4). A study of this reaction (1) indicates that use of a copper oxide catalyst allows for reduction of the reaction temperature and that one step hydrolysis of the intermediate phthalimino ester with acetic and hydrochloric acis (5) increases the yields. A method for preparation of phthalimide from isotopic ammonia and phthalic acid has been reported (1).

A modified Gabriel phthalimide aynthesis, employing diethyl sodium phthalimido-malonate has been used for the preparation of proline-N^{15}: (3)

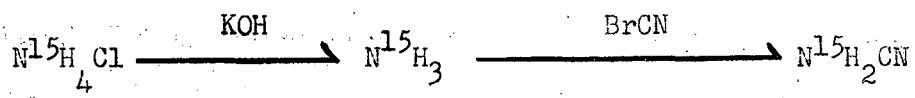


This synthetic scheme has also been applied to the preparation of serene-N^{15} (6).

Labeled serine has also been prepared by benzoylation of glycine-N^{15} followed by condensation with ethyl formate and reduction with sodium amalgam (3):

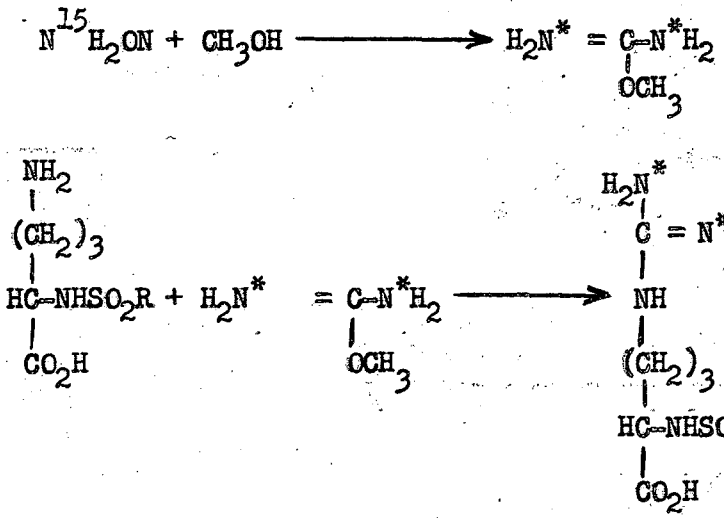


Creatinine-N^{15} has been made by condensation of cyanamide-N^{15} with sarcosine-N^{15} (7). The cyanamide is prepared from ammonia and cyanogen bromide:

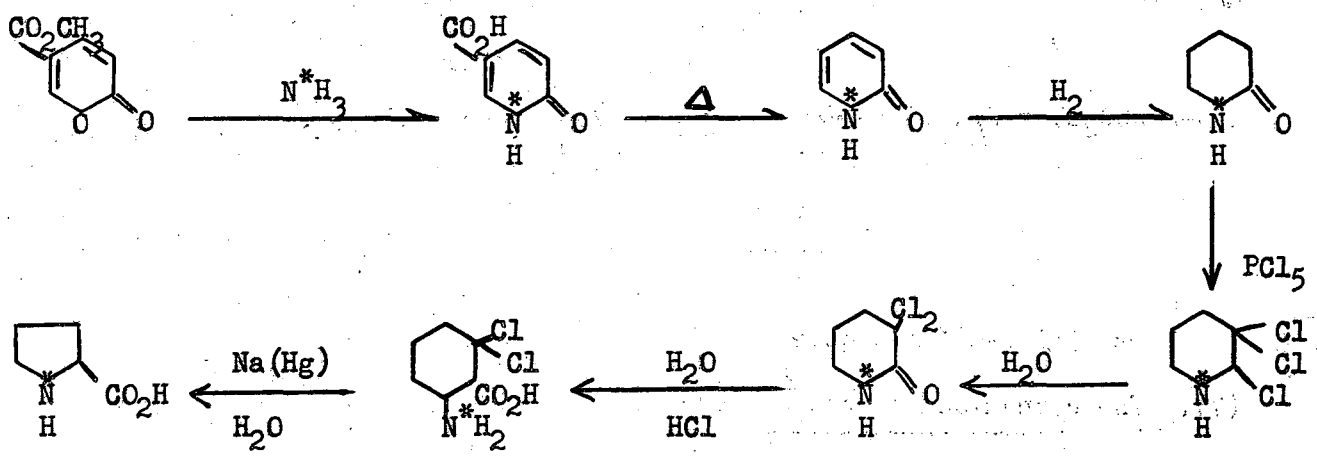


Modification of the Strecker synthesis using sodium cyanide in the presence of isotopic ammonium chloride has yielded alanine-N^{15} (3).

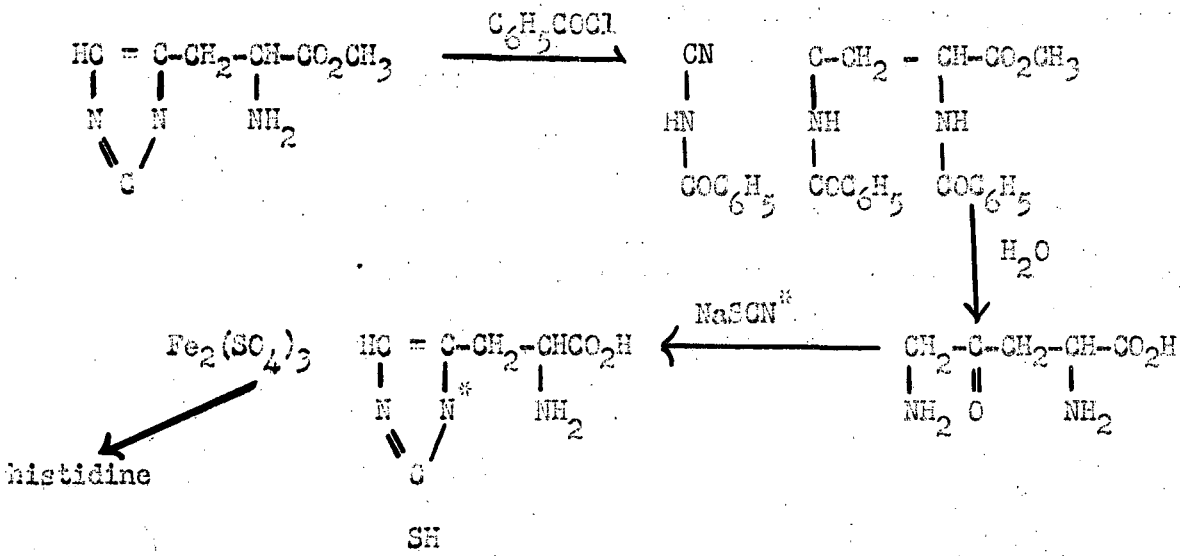
A novel synthesis of arginine-N^{15} has been worked out using the steps outlined below:



An elegant synthesis of proline and several pyridine derivatives, featuring the replacement of a ring oxygen with N^{15} has been reported (8). The starting materials are methyl coumalate and isotopic ammonia. The steps are:



Tesar and Rittenberg (9) have made use of the technique of partial breakdown of a cyclic compound, followed by introduction of an isotopic atom in place of one lost, in the synthesis of histidine:



The labeled sodium thiocyanate was prepared from isotopic ammonium nitrate in good yield:

$$3NH_4NO_3 + CS_2 + 2Fe(OH)_2 \longrightarrow 3NaSCN^{15} + 2\ FeS + S + 3NaNO_3 + 12\ H_2O$$

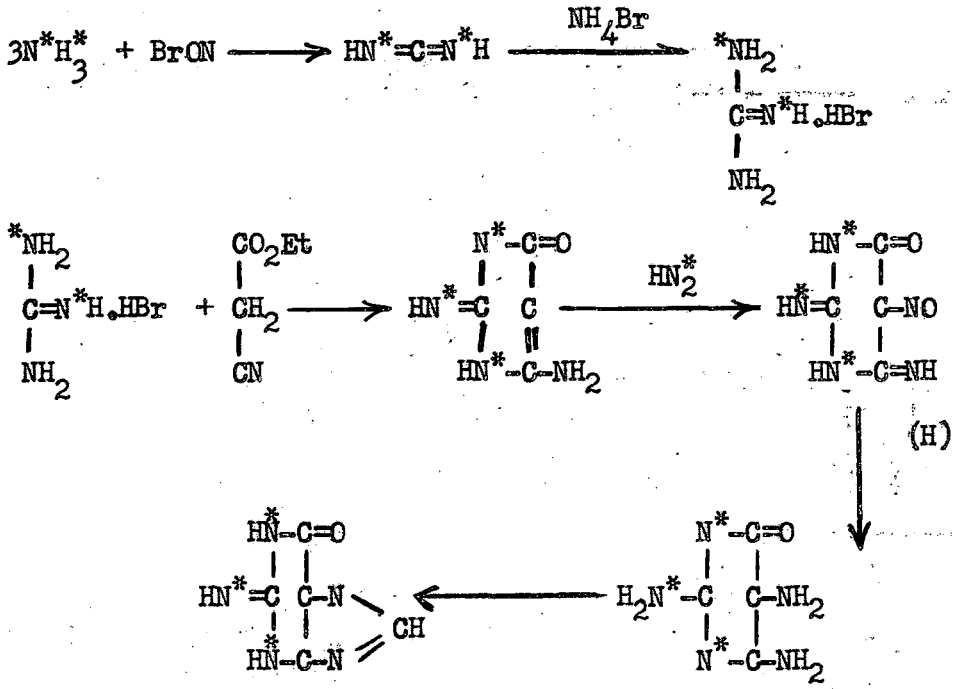
In a synthesis of 2-phenylindole, the Hofmann reaction involving alkaline hypobromite was used to prepare aniline-N^{15} from benzamide-N^{15} (10).

Purines:-

Of the purines which have been synthesized labeled in one or more nitrogen atoms there are: thymine-1,3-N^{15}, uracil-1,3-N^{15}, xanthine-1,3-N^{15}, and guanine-1,2,3-N^{15}, all prepared by Plentl and Schoenheimer (11). The synthesis of several of these involves the use of labeled thiourea:

$$H_2N^*CN + H_2S \xrightarrow[Se_2S_5]{H^+} H_2N^*\text{-}C(=S)\text{-}N^*H_2$$

The synthesis of guanine-1,3,5-N^{15} follows this flow-sheet?



The synthesis of the isotopic guanidine is detailed herein (11):

Guanidine Hydrobromide[11].- 4.77 gm. (3 moles) of isotopic ammonium chloride, 10.1 atom per cent N^{15} excess, were decomposed, special care being taken to include moisture. The ammonia was carried into a dry ice-cooled bomb containing 20 cc. of absolute ethyl alcohol. A gas inlet tube, especially constructed for this purpose, so as to fit the bomb tube was used. The small amount of ammonia that escaped was caught in a trap containing dilute sulfuric acid. When the absorption of ammonia in the alcohol was complete, the bomb tube was allowed to warm up to 0° and kept at this temperature in ice. A solution of 3.18 gm. of freshly prepared cyanogen bromide in 5 cc. of absolute ethyl alcohol was added, and the tube sealed and heated to 105-110° for 96 hours.

The length of time necessary for this reaction was determined by running a series of experiments with non-isotopic ammonia. The tubes were heated to 110° for various lengths of time and the contents tested with ammoniacal silver nitrate for the presence of cyanamide. At the end of 96 hours the test for cyanide was consistently negative.

The tube was cooled in ice, opened, cut off as straight as possible, and the open end fire-polished. The gas inlet tube was inserted, and the excess ammonia blown off by a slow stream of excess ammonia (10.1 atom per cent N^{15} excess) could thus be collected. The clear alcoholic solution was slowly evaporated to dryness and the residue dried in vacuo at room temperature. 4.02 gm. of guanidine hydrobromide were cooled. Although this product was not analytically pure, it was found to be quite satisfactory for the following condensation.

Other N^{15} labeled purines include isoguanine (12), uric acid (13), adenine (14) and orotic acid (15). All have been synthesized using standard methods and labeled intermediates. For the adenine synthesis, labeled formamide was prepared in this manner:

$$HCN + C_2H_5OH \xrightarrow{HCl} HC(=NH.HCl)(OC_2H_5) \xrightarrow{N^{15}H_3} HC(=N^{15}H.HCl)(N^{15}H_2)$$

4,6-Diamino-5-phenylazopyrimidine [14].- In a one-liter erlenmeyer flask was placed 29 cc. (29.6 g., 0.318 mole) of aniline and 85 cc. (1.02 moles) of concentrated hydrochloric acid. Ice was added to the mixture to maintain a temperature of 0 to 5°. Sodium nitrite (35.5 g., 0.50 mole, assay 97%) in 50 cc. of water was added in

small portions, while stirring. After ten minutes, 51 g. (0.63 mole) of anhydrous sodium acetate in 100 cc. of water was added, followed by 33 g. (0.50 mole) of malononitrile in 25 cc. of ethanol. After thirty minutes the phenylazomalonitrile was collected by filtration, washed with cold water and dried in air. The material may be recrystallized from benzene (5 cc. per g.) although the crude material could be used for the following reaction. The yield of recrystallized material was 48-52 g., 55-60%. A three-necked flask was provided with a Hershberg stirrer, a reflux condenser (protected with a drying tube) and two separatory funnels (one on top of the other). Formamidine hydrochloride prepared from 0.11 mole of ammonia was placed in the flask. To this was added 20 g. (0.118 mole) of phenylazomalononitrile in 30 cc of n-butanol (dried by distillation). In the bottom separatory funnel (connected to a drying tube) was placed 2.9 g. (0.13 mole) of sodium. Dry n-butanol (100-150 cc.) from the upper funnel was added to the sodium in small portions. The sodium butoxide solution was added to the reaction mixture in several portions, and the mixture refluxed gently for four hours. The mixture was cooled to 5° and the solid collected by filtration. The cake was washed alternately with water and alcohol and dried for a short time at 110°; yield, 15-18 g., 70-80%. This material was used in the next step without further purification, since the compound was difficult to recrystallize. When carefully dried ethanol was used as a solvent for the reaction the yield was frequently as low as 30%.

4,5,6-Triaminopyrimidine Sulfate. - 4,6-Diamino-5-phenylazopyrimidine (4.0 g., 0.0186 mole) was added to a boiling solution of 40 cc. of water

and 6 cc of ethyl cellosolve containing 4 g. of zinc dust. The mixture was boiled for 60-90 seconds while stirring. Sulfuric acid (6 cc. of 18 N) was added to the hot solution as rapidly as possible and the mixture decolorized with norite and filtered immediately. A lower yield resulted when the material was left in contact with acid. Upon cooling 2.4-3.4 g. (55-78%) of precipitate was collected. The 4,5,6-triaminopyrimidine sulfate thus obtained was used for the next step. The material could be recrystallized from 2 N sulfuric acid (20 cc. per g.) with an 85% recovery.

Anal. Calcd. for $C_4H_7N_5 \cdot H_2SO_4 \cdot H_2O$: N, 28.8

Found N, 28.7

Adenine Sulfate. - (a) 4,5,6-Triaminopyrimidine sulfate (0.78 g.) and 9 cc. of anhydrous formamide containing 0.3 cc. of 98% formic acid was heated in a bomb tube at 160-165° for two and one-half hours. The contents of the tube were chilled and the insoluble portion united with the residue on concentration of the filtrate in vacuo at ca. 150°. The residues were recrystallized from ca. 12 cc. of 2 N H_2SO_4; yield, 0.62 g. (95%).

(b) 4,5,6-Triaminopyrimidine sulfate (5.0 g., 0.0207 mole) was dissolved in 25 cc. of anhydrous fromic acid while warming the mixture gently. The solution was evaporated to dryness by warming gently in a stream of air. The 4,6-diamino-5-formylaminopyrimidine was transferred to a Carius tube and 20 cc. of formamide added. The tube was sealed and heated at 170° for two and one-half hours. The contents of the tube were cooled and the mixture filtered. The filtrate was evaporated to dryness and the two solid residues were recrystallized from 2 N sulfuric acid; yield, 3.1-3.5 g. (77-87%).

The yield was lowered when the 4,5,6-triaminopyrimidine sulfate was boiled with formic acid or when the heating period in the formamide was greater than two and one-half hours.

Anal. Calcd. for $(C_5H_5N_5)_2 \cdot H_2SO_4 \cdot H_2O$: N, 36.3; S, 8.28.

Found N, 36.3; S, 8.50.

Atom per cent. excess N^{15}, 4.82. Counter-current distribution revealed that the adenine sulfate was 98-100% homogenous with respect to ultraviolet absorbing impurities.

Exchange between N^{15} of organic compounds and other nitrogen has been attempted under mile conditions (16). It was found that the nitrogen of various amino acids, hippuric acids and the guanidino group of arginins did not exchange with the nitrogen of other compounds in aqueous solutions at 100°. The nitrogen of urea may be exchangable, but at a very slow rate even at 100°.

Biosynthetic Preparations:-

The biosynthesis of labeled nucleic acids by yeast has recently been reported (17). Using yeast of the Saccharomyces cerevisiae strain and labeled ammonium sulfate as the substrate, nucleic acids were isolated. Adanine picrate, guanine sulfate and silver pyrimidines, all labeled with N^{15}, were obtained from the nucleic acids.

REFERENCES

(1) R. Schoenheimer and S. Ratner, J. Biol. Chem. 127, 301 (1939).

(2) R. Schoenheimer, D. Rittenberg and A. S. Keston, J. Am. Chem. Soc. 59, 1765 (1937).

(3) D. Shemin, J. Biol. Chem. 162, 297 (1946).

(4) K. Bloch and R. Schoenheimer, Ibid. 138, 167 (1941).

(5) S. Ratner and H. T. Clarke, J. Am. Chem. Soc. 59, 200 (1937).

(6) D. Stotten, J. Biol. Chem. 144, 501 (1942).

(7) K. Bloch, R. Schoenheimer and D. Rittenberg, Ibid., 138, 155 (1941).

(8) M. R. Stotten and R. Schoenheimer, Ibid., 153, 113 (1944).

(9) C. Tesar and D. Rittenberg, Ibid., 170, 35 (1947).

(10) C. F. H. Allen and C. V. Wilson, J. Am. Chem. Soc. 65, 611 (1943).

(11) A. A. Plentl and R. Schoenheimer, J. Biol. Chem. 153, 203 (1944).

(12) A. Bendich, J. F. Tinker and G. B. Brown, J. Am. Chem. Soc. 70, 3109 (1948).

(13) L. F. Cavalieri, V. E. Blair and G. B. Brown, Ibid., 70, 1240 (1948).

(14) L. F. Cavalieri, J. F. Tinker and A. Bendich, Ibid., 71, 533 (1949).

(15) S. Bergstrom, H. Arvidson, E. Hammersten, N. A. Eliasson, P. Reichard and H. V. Ubisch, J. Biol. Chem. 177, 495 (1949).

(16) A. S. Keston, D. Rittenberg and R. Schoenheimer, Ibid., 127, 315 (1939).

(17) F. J. DiCarlo, A. J. Schultz, P. M. Roll and G. B. Brown, Ibid., 180, 329 (1949).

SULFUR

Radioactive sulfur may be used as either the S^{34} or S^{35} isotope; more work has been done with the latter. The isotopic material is generally available as the sulfate and for use in organic work conversion to sulfide or sulfur is necessary. An excellent procedure for conversion of barium sulfate to sulfur is detailed below (1):

Preparation of Barium Sulfate Containing Radiosulfur [1].-

Dried barium sulfate* (116 mg.) was spread in a thin layer on a platinum boat and placed in a Vycor tube. The air was expelled by a stream of hydrogen and the Vycor tube was heated at 900-1000° for two hours and then allowed to cool, a slow stream of hydrogen being maintained throughout the reduction. The issuing gases were bubbled through an absorption train consisting of 6 cc. of 0.5 N sodium hydroxide in a small test-tube and 1 cc. in a second tube.

The boat containing the barium sulfide* was placed along with 5 mg. of zinc dust in a 125-cc. ₹ 24/40 Erlenmeyer flask equipped with a separatory funnel and a delivery tube. The delivery tube was attached to the sodium hydroxide absorption train used with the reduction of the barium sulfate* and the apparatus was swept with oxygen-free nitrogen. Twenty cubic centimeters of 6 N phosphoric acid, which had been boiled with about 5 mg. of zinc dust to expel air and cooled somewhat, was placed in the separatory funnel. This acid was dropped onto the barium sulfide* at such a rate as to produce a slow evolution of hydrogen sulfide*, which

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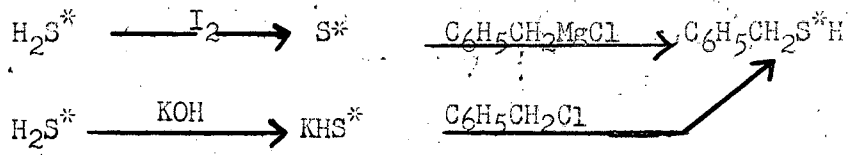
(*) An asterisk is used to indicate the presence of radiosulfur in a compound.

was absorbed in the sodium hydroxide scrubbers. When all the acid had been added, the reaction mixture was warmed slowly to its boiling point, allowed to cool in a stream of nitrogen and swept with nitrogen for one hour.

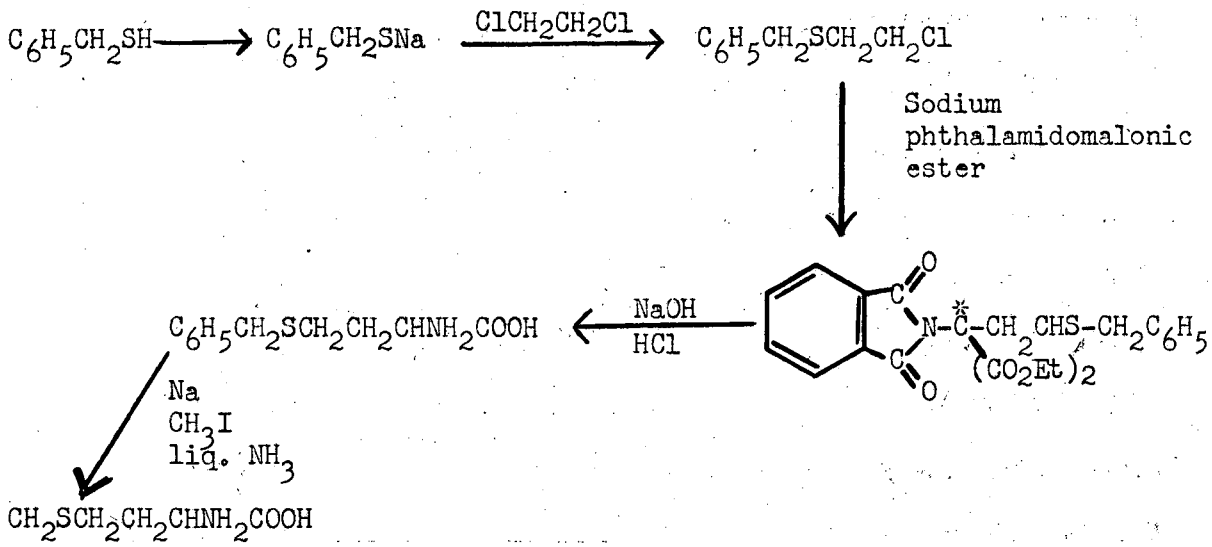
Fifteen cubic centimeters of 0.1 N iodine in potassium iodide and 1 cc. of concentrated hydrochloric acid were placed in a 50-cc. centrifuge cone. The 6 cc. of sodium sulfide* solution from the first scrubber was introduced at the bottom of the solution by means of a long slender pipet. The 1 cc. of solution from the second scrubber was used to wash the first and the washings were added to the iodine solution. The scrubbers were washed further with small portions of water until a nitroprusside or lead acetate test for the sulfhydryl group on the washings was negative. The portion of the transfer pipet coated with sulfur* was then broken off and placed in the iodine solution. After fifteen minutes the excess iodine was destroyed with a few drops of a freshly prepared solution of stannous chloride in 5 N hydrochloric acid. When the resulting suspension was allowed to stand overnight, the free sulfur* coagulated and was then collected by centrifugation. The precipitate was washed with water by centrifugation and decantation.

Amino Acids: -

The sulfur containing amino acids have all been prepared and in each synthesis radioactive benzyl mercaptan has been employed. This intermediate may be prepared in either of two ways. (2,3,4,5).



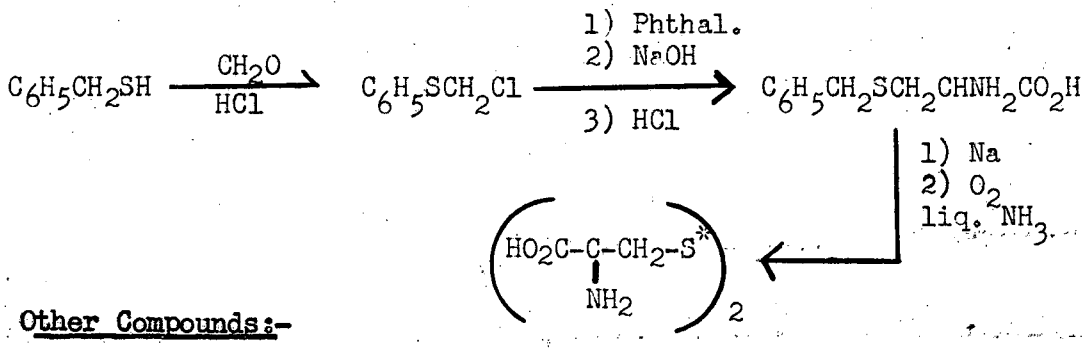
Methionine-S^{35} has been prepared via the phthalimidomalonic ester synthesis (3,5)



Using ethylene dichloride labeled with C^{13}, Gilmer and du Vigneaud (2) have synthesized methionine containing both C^{13} and S^{34}.

In an improved method of synthesis for methionine and cystine, the labeled benzyl mercaptan is condensed with ethyl γ-chloro-α-benzamidobutyrate (4) and with ethyl β-chloro-α-benzamido propionate (6,7) respectively. This yields benzylhomocystein, $C_6H_5CH_2SCH_2CH_2CHNH_2CO_2H$, in the first case and benzylcysteine, $C_6H_5CH_2SCH_2CHNH_2CO_2H$, in the second. The benzyl group is then removed with sodium in liquid ammonia followed by methylation or oxidation, as is required.

Cystine has also been prepared using the phthalimidomalonic ester synthesis (5):



Other Compounds:-

Mustards labeled with S^{35} have also been prepared. The synthesis of β,β'-dichlorodiethylsulfide-S^{35} has been carried out in the following manner: (8)

$$N_2S + \underset{\diagdown O \diagup}{CH_2\text{-}CH_2} \longrightarrow HOCH_2CH_2\text{-}C^*\text{-}CH_2OH_2OH \xrightarrow[CHCl_3]{SOCl_2} (ClCH_2CH_2)_2S^*$$

In another synthesis, the condensation of an active mercaptan with ethylene chlorohydrin was used (1):

$$R\text{-}S^*H + ClCH_2CH_2OH \xrightarrow{NaOH} R\text{-}S^*\text{-}CH_2CH_2OH \xrightarrow{HCl} R\text{-}S\text{-}CH_2CH_2Cl$$

$$(R = C_6H_5CH_2\text{-},\ C_4H_9\text{-})$$

The synthesis of labeled sulfanilamide has been achieved (9). In this preparation, acetanilide was sulfonated with $H_2S^{35}O_4$ and the acetyl sulfanilic acid converted to N-acetylsulfanilyl chloride with phosphorus pentachloride, and to N-acetylsulfanilamide with ammonia. Hydrolysis yielded the desired product. The preparation of the labeled sulfuric acid is described:

> Preparation of Labeled Sulfuric Acid [9]. - In a 100 ml., 3 necked flask, fitted with a dropping funnel, a gas inlet tube and a reflux condenser, was placed 7 mM of FeS and 1 millicurie of S^{35} as Na_2S. The condenser was fitted with 2 bubblers in each of which was placed 5 ml. of conc. HNO_3. A slow stream of N_2 was passed through the system, and 20 ml. 2.5 M HCl was slowly added through the dropping funnel to release H_2S. Finally, the solution was boiled for ½ hr. The H_2S which was swept into the bubblers was thus oxidized to a mixture of sulfate and sulfur.
>
> When all of the H_2S had been generated, the contents of the bubblers were transferred to a 125 cc. Erlenmeyer flask containing 7 mM of finely divided sulfur, with the aid of two 5 ml. portions of conc. HCl. The aqua regia mixture oxidized the sulfur to sulfate.

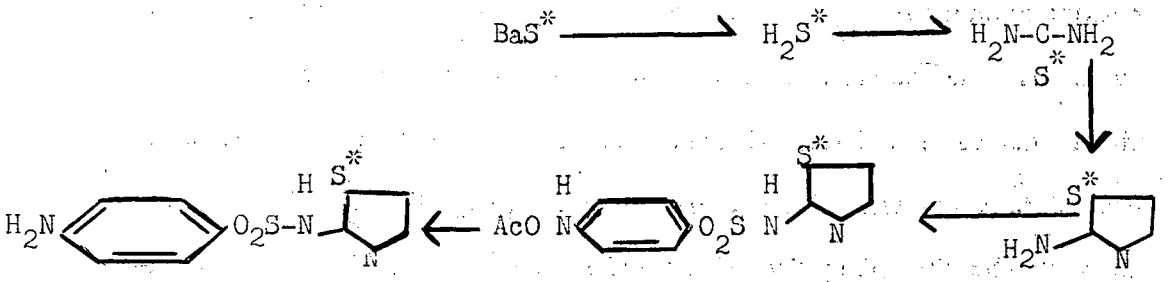
The H_2SO_4 was recovered by adding the acid mixtures, a drop at a time, to a 50 ml. centrifuge cone suspended in a stirred oil bath maintained at 160-170° C. The Erlenmeyer flask was washed out thoroughly with portions of HCl and water. Heating of the concentrated H_2SO_4 was continued for 15 min. after boiling had ceased, to insure removal of traces of nitric acid.

Among other compounds labeled with S^{35} which have recently been prepared is tetraethylthiouram disulfide (10), prepared by the following reaction sequence:

$$S^* + CS_2 \rightleftharpoons CS_2S^{*=} \rightleftharpoons S^{=} + CS_2^*$$

$$CS_2^* \quad (C_2H_5)_2NH \xrightarrow{KOH} KS^*C(=S)N(Et)Et \xrightarrow[\text{tetrathionate}]{\text{Sodium}} (Et)_2NC(=S)S-S-C(=S)N(Et)_2$$

Use of 2-(p-aminophenyl)-thiazole-S^{35} has been reported (11) but no preparative scheme is available. The synthesis of sulfathiazole in which the ring sulfur is labeled has been carried out according to the flow sheet shown below (12):



Thiourea-S^{35} has been synthesized in the manner described below (13):

Preparation of H_2S^{35} [13]. - The sulfur isotope, S^{35}, as obtained from the Atomic Energy Commission, is in the form of barium sulfide in 0.05 N barium hydroxide solution. The quantitative precipitation of the sulfur was accomplished by first transferring the solution into a 40-ml. centrifuge cone, the tip of

which had been drawn down to a diameter of about 7 mm. and a length of about 30 mm., then adding an excess of a solution which was 0.05 N in hydrochloric chloride and 0.1 N in cadmium chlorides. The quantity of reagent should be sufficient to neutralize all the barium hydroxide and give an acidic reaction to the mixture. The sulfur was thus precipitated as cadmium sulfide. The precipitate was separated by centrifuging and decanting the clear solution. After washing with water several times and centrifuging, the precipitate was dried while still in the cone.

The quantitative conversion of cadmium sulfide to hydrogen sulfide without the introduction of other gases was accomplished by breaking off the tip of the cone containing the cadmium sulfide into a 200 ml. flask into which had been introduced 20 g. of metaphosphoric acid. Connecting the flask to a vacuum system, evacuating to a pressure of 0.005 mm., then heating the acid to boiling, smoothly freed the sulfur as H_2S. As quickly as generated, the H_2S was removed from the system, while still under vacuum, by freezing out the gas with liquid nitrogen into a round-bottomed flask located at the end of a $CaCl_2P_2O_5$ purifying train. This receiver flask was equipped with a micro stopcock so that the flask and contents could be weighed. Cadmium sulfide, 71.9 mg., containing 8.2% of CdS^{35}, by this procedure gave 17.8 mg. of hydrogen sulfide (105%).

Thiourea Labeled with S^{35}.- The standard syntheses of thiourea using hydrogen sulfide utilize an excess of this gas. Since in this instance the hydrogen sulfide is the limiting factor, the procedure was modified in such a manner as to utilize completely all of the hydrogen sulfide containing the racioactive isotope.

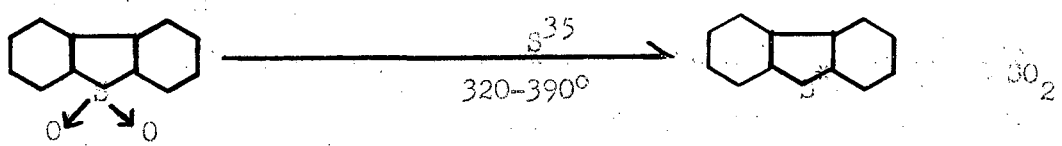
The flask containing the metaphosphoric acid was removed from the system and replaced by a 10-ml. flat-bottomed reaction flask containing a magnetic stirrer, 0.8 ml. of distilled water and 25 mg. of cyanamide (freshly prepared), and one small drop of concentrated ammonium hdyroxide. The contents of the flask were frozen with liquid nitrogen, the vacuum was re-established and the H_2S was transferred into it by warming the flask containing the frozen gas. The reaction vessel was closed off from the system and was allowed to warm to room temperature.

The mixture was then warmed to 40° and was stirred continuously for 24 hours with a magnetic stirrer. The solution was colored a faint yellow, which disappeared after 6 hours, followed by the appearance of a faint cloudiness. At the end of this period the contents of the flask were transferred into a vacuum sublimator using alcohol. All solvent was removed by evaporation and the thiourea was sublimed in a vacuum (0.02-0.03 mm.) at a temperature of 70-90°. A higher temperature causes the sublimation of impurities which lowered the melting point of the thiourea.

Since films of oils or waxes materially riasie the temperature of sublimation, it was found advantageous to remove all

oil films by washing the residues first with petroleum ether, then removing the ether and dissolving the thiourea in ethanol. The solvent was then removed by evaporation and the residue was sublimed in a vacuum. The isolation of thiourea by this method is quantitative. The thiourea obtained, 35 mg. (92.2% of the theoretical yield) melted at 171-173°. The compound possessed an activity of 0.535 mc./mg.

Preparation of dibenzothiophene-S^{35} by exchange between dibenzothiophene-5-dioxide and S^{35} has recently been accomplished (14).



Biosynthetic Preparations:-

Biosynthetic preparation of glutathione-S^{35} from yeast (Saccharomyces cerevisaie, Frohburg type) utilizing a substrate containing radioactive barium sulfate has been reported (15). Penicillin-S^{35} from P. chrysogenium (Q176 strain) using S^{35} in the substrate and from P. notatum (17) using radioactive sodium sulfate has been achieved. A specific activity of 0.05 mc/I.U. was obtained in the former cases. It was noted that a concentration of 50 mc. of S^{35} per liter did not affect the growth of the mold.

REFERENCES

(1) J. L. Wood, J. R. Rachele, C. M. Stevens, F. H. Carpenter and V. du Vigneaud, J. Am. Chem. Soc., 70, 2547 (1948).

(2) G. W. Gilmer and V. du Vigneaud, J. Biol. Chem. 154, 247 (1944).

(3) H. Tarver and C. L. A. Schmidt, Ibid., 130, 67 (1939).

(4) H. Tarver and C. L. A. Schmidt, Ibid., 146, 69 (1942).

(5) A. M. Seligman, A. M. Rutenberg and H. Banks, J. Clin. Invest. 22, 275 (1943).

(6) J. B. Melchior and H. Tarver, Arch. Biochem. 12, 301 (1947).

(7) J. L. Wood and L. V. Middlesworth, J. Biol. Chem., 179, 529 (1949).

(8) J. C. Boursnell, C. E. Francis and A. Wormall, Biochem. J. 40, 743 (1946).

(9) I. M. Klotz and J. B. Melchior, Arch. Biochem. 21, 35 (1949).

(10) L. Eldjarn, Acta Chem. Scand. 3, 644 (1949).

(11) H. Erlenmeyer, H. Meyer, H. Noll and E. Sorkin, Helv. Chim. Acta, 32, 1209 (1949).

(12) H. Noll, J. Beng, E. Sorkin and H. Erlenmeyer, Ibid., 34, 340 (1941).

(13) C. W. Bills and A. R. Ronzio, J. Am. Chem. Soc., 72, 5510 (1950).

(14) R. B. Brown, S. Kirkwood, L. Marion. S. Naldrett, R. K. Brown and R. B. Sandin, Ibid., 73, 465 (1951).

(15) R. G. Franklin, Science 89, 295 (1939).

(16) D. Rowley, J. Miller, S. Rowland and E. Lester-Smith, Nature 161, 1009 (1948).

(17) S. F. Howell, J. D. Thayer and L. W. Labaw, Science 107, 299 (1948).

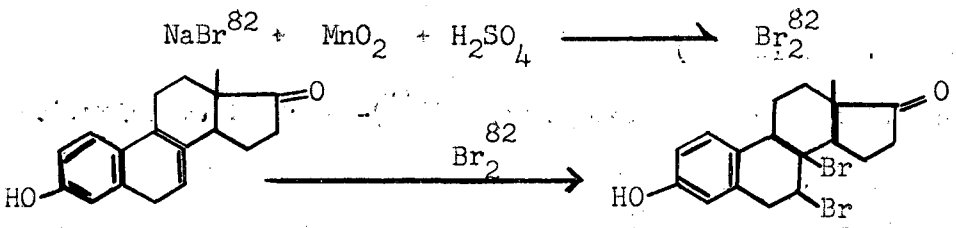
HALOGENS

Bromine:-

Bromine is generally obtained as the bromide, but if necessary can easily be oxidized to bromine and used as such directly. Its short half-life (34 hours) precludes any long range experiments and necessitates rapid manipulation.

Howarth (1) treated KBr^{82}. This gas was passed into a solution of hydrogen peroxide and procaine $H_2NC_6H_4CO_2CH_2CH_2N(C_2H_5)_2$ to give dibromoprocaine. The entire operation, starting from potassium bromide-Br^{82}, required ninety minutes.

The bromination of equilin to yield 7,8-dibromoestrone has been carried out as follows (2):

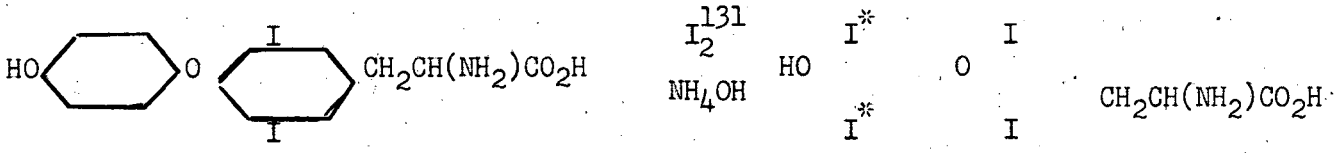


Bromination with bromine^{82} of several unsaturated acids has been accomplished (3) and triphenylethylene has been brominated to triphenylbromoethylene (4). The bromination of β-(6-methoxy-2-naphthyl)-β-ethyl-α,α-dimethylpropionic acid has also been reported (5).

Iodine:-

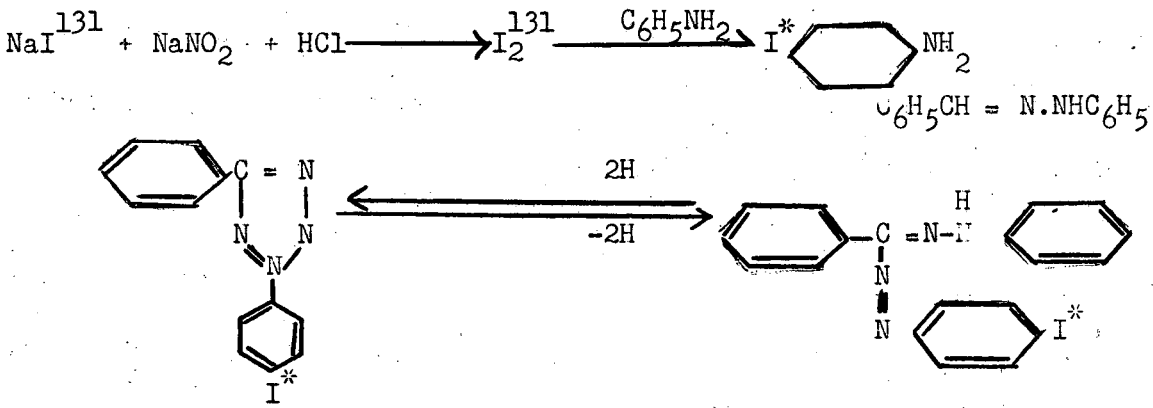
The organic chemistry of iodine^{131} is very similar to that of bromine^{82}. Isotopic iodine is obtained as the iodide and may either be used as such or oxidized to iodine. Several instances of incorporation of isotopic iodine into an aromatic nucleus by means of the Sandmeyer reaction utilizing potassium iodide^{131} will be noted later.

Direct iodination has been used in several cases. A solution of radioactive iodide was oxidized with potassium iodate and the resulting iodine^{131} used to iodinate α-estradiol (6). The mono- and diiodo^{131} estradiol derivatives were obtained. Preparation of mono- and diiodo^{131} thyroxine has also been accomplished (7,8).



Iodination of fluorescein with iodine^{131} chloride has yielded a diiodo compound which is assumed to be the 4,5-diiodo derivative (9), and treatment of casein with iodine^{131} has produced iodocasein (10).

The synthesis of 2,5-diphenyl-3-p-iodophenyl (I^{131})-tetrazolium chloride (11) has been carried out as follows:



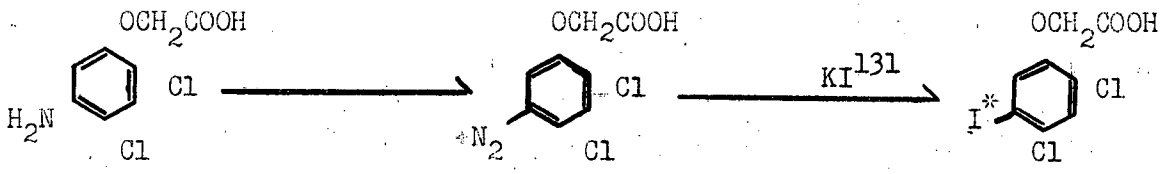
Several preparations have been reported in which the Sandmeyer reaction is used to prepare iodine^{131} labeled aromatic compounds. The synthesis of p-iodo^{131} phenyl sulfonic acid from p-diazobenzonesulfonic acid and potassium iodide^{131} (12) is an excellent example of this type of reaction and is detailed herein:

Isotopic Pipsyl Chloride[12] :- To the radioactive iodine, in solution, in the form of iodide ion, is added sufficient potassium iodide to make a total of 25 mg. The solution is adjusted to a pH above 7 and evaporated to a volume of less than 0.5 ml. The sample is transferred quantitatively, with the aid of several small portions of water, to a Pyrex test-tube and the volume is reduced to about 0.2 ml. A few small crystals of sodium sulfite are added. After cooling, an equal volume of concentrated hydrochloric acid is added followed by 25 mg. of diazobenzenesulfonic acid. After the initial evolution of nitrogen the sample is warmed to complete the reaction. The tube is cooled again and another 15 mg. each of diazobenzenesulfonic acid and potassium iodide are added and allowed to react as before. The reaction mixture is then saturated with sodium chloride and cooled, whereupon the sodium salt of p-iodophenylsulfonic acid crystallizes out. The crystals are centrifuged down and washed with bring. The combined mother liquor and washings are warmed and about 40 mg. of non-isotopic sodium iodophenylsulfonate, in solution in a small volume of water, is added. Upon cooling, the added sodium salt crystallizes out carrying down residual isotopic analog. This is also centrifuged down and washed with brine and the procedure repeated. The separate batches of sodium salt are dried and then each is dissolved in about one ml. of phosphorus exychloride containing a substantial excess of phosphorus pentachloride. The batches are pooled and the mixture is heated gently to ensure reaction. The reaction mixture is transferred to a separatory funnel containing ice-water and about 50 ml. of benzene. Small

portions of benzene are used to facilitate the transfer from the test tubes to the funnel. At this point, approximately 200 mg. of pure non-isotopic pipsyl chloride is added. The benzene layer is washed several times with cold water to remove excess phosphorus halides. The rate of hydrolysis of pipsyl chloride is negligible, under these conditions, as is indicated by the absence of any appreciable amounts of radioactivity in the wash water. The benzene solution is dried over anhydrous sodium sulfate and the drying agent is washed with benzene until it is substantially free of radioactivity.

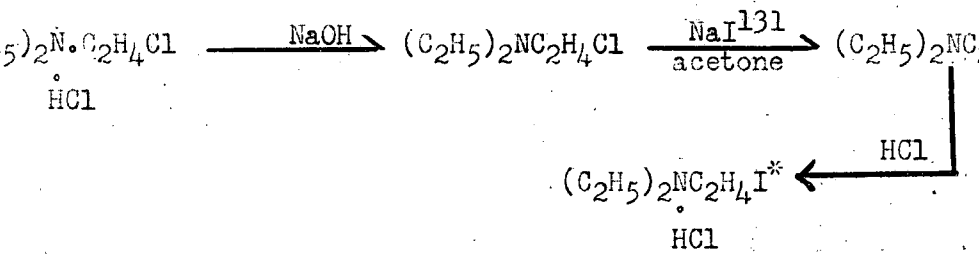
The combined benzene solution is evaporated to a small volume and transferred to a cold finger micro-distillation apparatus. The benzene is evaporated in a stream of air and the pipsyl chloride is distilled onto the cold finger at 150° at a few mm. of mercury pressure. The distilled material is washed into a vessel with a minimum of benzene. At this point more non-isotopic pipsyl chloride may be added to make a desired specific activity. The pipsyl chloride must be completely dissolved and thoroughly mixed to ensure isotopic homogeneity. Recrystallization may be achieved by adding petroleum ether to the benzene and concentrating the solution. The small amount of residual solvent is then drawn off with a capillary pipet and the crystals are dried at 70° for about one hour. The reagent has been successfully used even when the last recrystallization was omitted.

The use of the Sandmeyer reaction to prepare 2,4-dichloro-5-iodo^{131}-phenoxyacetic acid



has been noted (13,14). Other compounds prepared via this reaction include 2-iodo^{131}-3-nitrobenzoic acid (15) and trypan blue-iodine^{131} (3,3'-dimethylbiphenyl-4,4'-bis (2-azo-1-hydroxy-8-iodo-3,6-nephthalene disulfonic acid) (16,17).

A few compounds containing radioactive iodine have been prepared by exchange between bound iodine atoms and iodine^{131} in solution. The preparation of diethyl--iodo^{131} ethylamine hydrochloride (18) involved the following steps:



Labeled thyroxine has been prepared by direct exchange between thyroxine and radioactive iodide at pH 5 (8,19). The N-iodo-I^{131}-acetyl derivatives of several amino acids have been prepared by treating the corresponding N-bromo- or N-chloroacetyl derivative with a solution of sodium iodide-I^{131} in acetone (20).

Chlorine and Fluorine:-

The literature covering the use of the radioactive isotopes of chlorine and fluorine contains no record of the preparation of organic compounds labeled with isotopes of either of these elements.

REFERENCES

(1) F. Howarth, Nature 161, 857 (1948).

(2) G. H. Twombly, L. McClintoch and M. Engelman, Am. J. Obst. Gynecol. 56, 260 (1948).

(3) N. P. Buu-Hoi, M. Berger, P. Daudel, R. Daudel, S. May and C. Miguet, Helv. Chim. Acta, 29, 1334 (1946).

(4) E. Paterson, C. W. Gilbert, U. M. Gallagher and J. A. Hendry, Nature, 163, 801 (1949).

(5) P. Süe, J. Jacques, A. A. Horeau and R. Courrier, Compt. Rend., 227, 8 (1948).

(6) S. Albert, R. D. H. Heard, C. P. Leblond and I. Saffran, J. Biol. Chem., 177, 247 (1949).

(7) A. Horeau and P. Süe, Bull. Soc. Chim. Biol., 27, 483 (1945).

(8) R. M. Lemmon, W. Tarpey and K. G. Scott, J. Am. Chem. Soc., 72, 758 (1950).

(9) G. Boyack, G. E. Moore and D. F. Clausen, Nucleonics 3, No. 4, 62 (1948).

(10) C. F. Hamilton, M. H. Power and A. Albert, J. Biol. Chem., 178, 213 (1949).

(11) A. M. Seligman, R. Gofstein and A. M. Rutenberg, Cancer Res., 9, 366 (1949).

(12) A. S. Keston, S. Udenfriend and R. K. Cannan, J. Am. Chem. Soc., 71, 249 (1949).

(13) H. R. Mahler, R. J. Speer and A. Roberts, Science, 110, 562 (1949).

(14) J. W. Wood, W. C. Wolfe, H. M. Doukas, L. W. Klipp, T. D. Fontaine and J. W. Mitchell, J. Org. Chem., 14, 901 (1949).

(15) J. W. Mitchell, J. W. Wood, W. C. Wolfe and G. W. Irving, Science, 106, 395 (1947).

(16) H. S. Bloch and F. E. Ray, J. Nat. Cancer Inct., 7, 61 (1946).

(17) C. D. Stevens, A. Lee, P. H. Stewart, P. M. Quinlin and P. R. Gilson, Cancer Res., 9, 139 (1949).

(18) A. M. Seligman, A. M. Rutenberg and O. M. Friedman, J. Nat. Cancer Inst., 9, 261 (1949).

(19) E. Frieden, M. B. Lipsett and R. J. Winzler, Science, 107, 353 (1948).

(20) O. M. Friedman and A. M. Rutenberg, J. Am. Chem. Soc., 72, 3285 (1950).

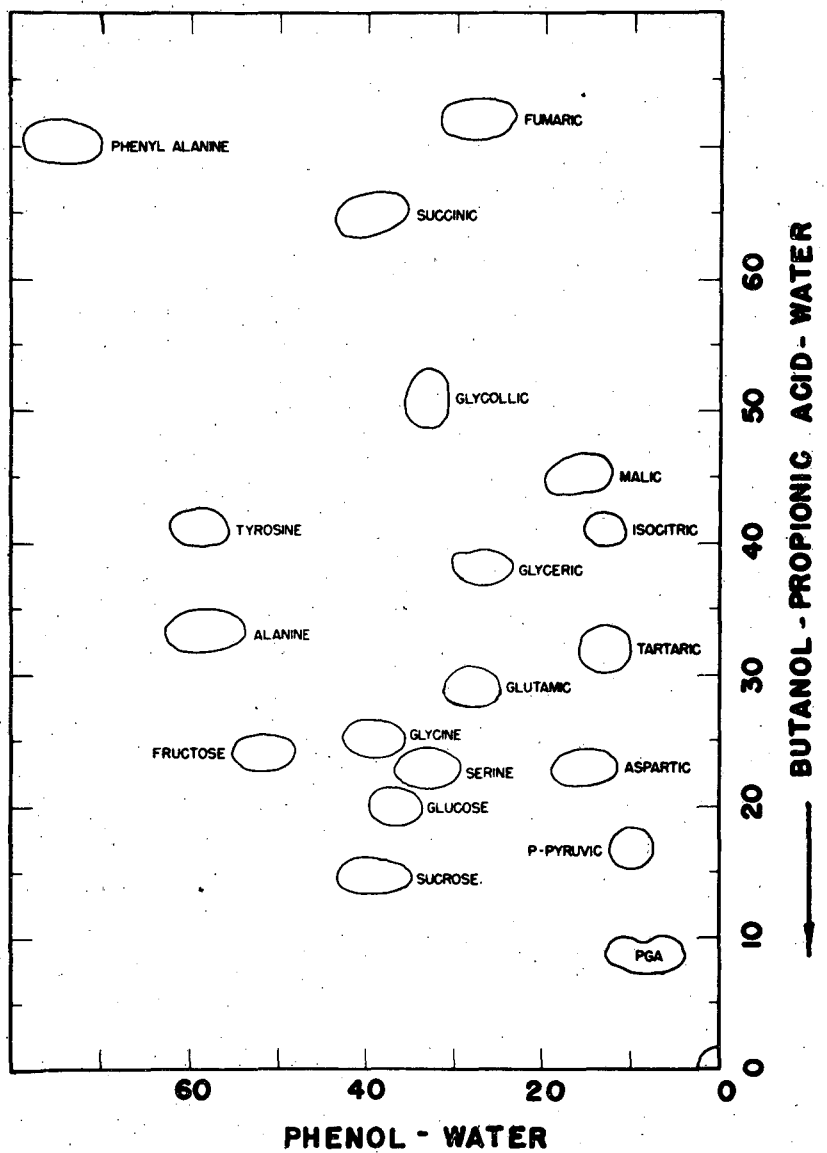


Fig. 1 - Distribution of a number of compounds found in photosynthesis studies of algae on two-dimensional paper chromatograph. The origin is in the lower right-hand corner. PGA is phosphoglyceric acid; P-pyruvic is phosphopyrivic acid. (A.A. Benson, et. al., Am. Chem. Soc., 72, 1710 (1950).

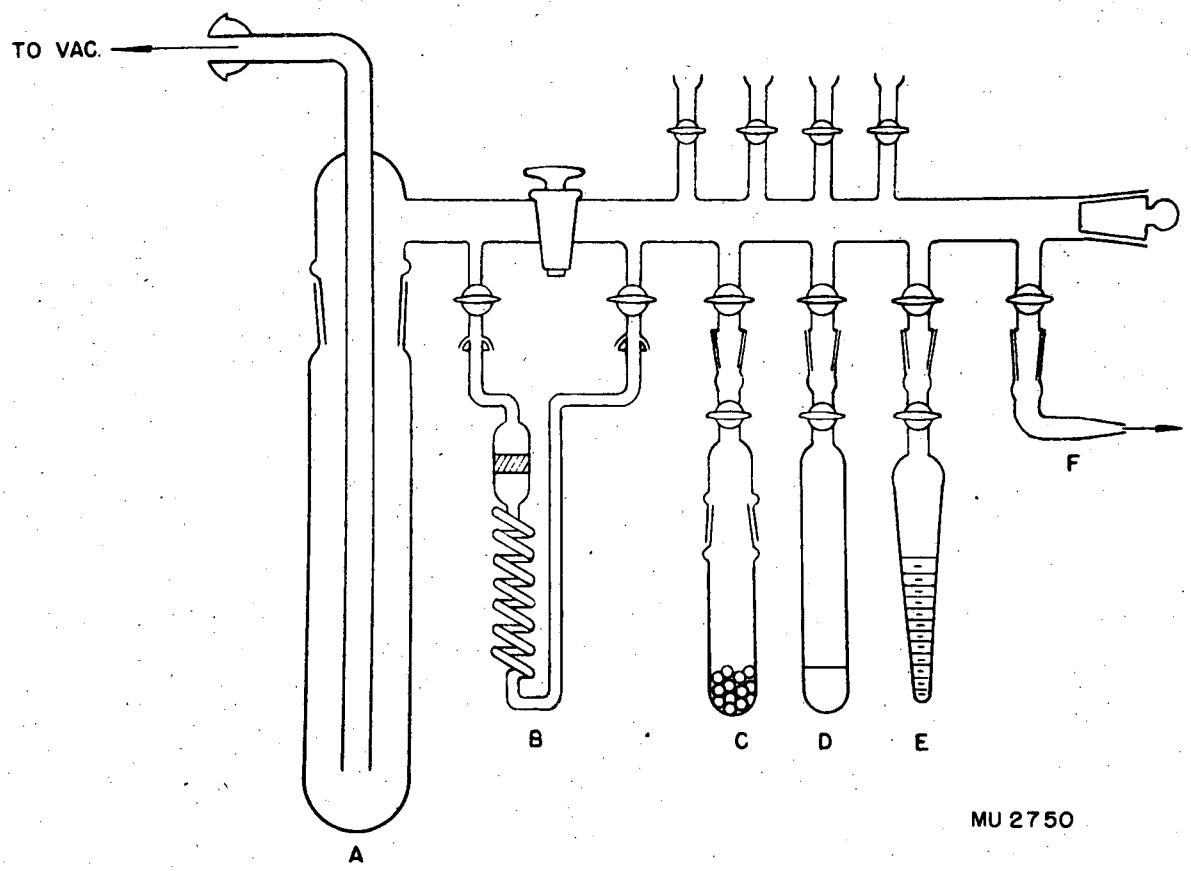


Fig 2 - A general utility vacuum line equipped with treatment vessels: a) trap; b) sprial-sintered glass trap used to separate organic vapors from a non-condensible gas such as H_2; c) vessel partially filled with glass beads and P_2O_5 used to dry aliphatic halides; d) washing vessel containing dilute alkali used to remove acid gases from volatile halides; e) graduated storage vessel. The upper outlets on the line are for attachment of manometers and vacuum gauges. (B. Tolbert, P. Christenson, F. Chang and P. Sah, J. Org. Chem., 14, 525 (1949).

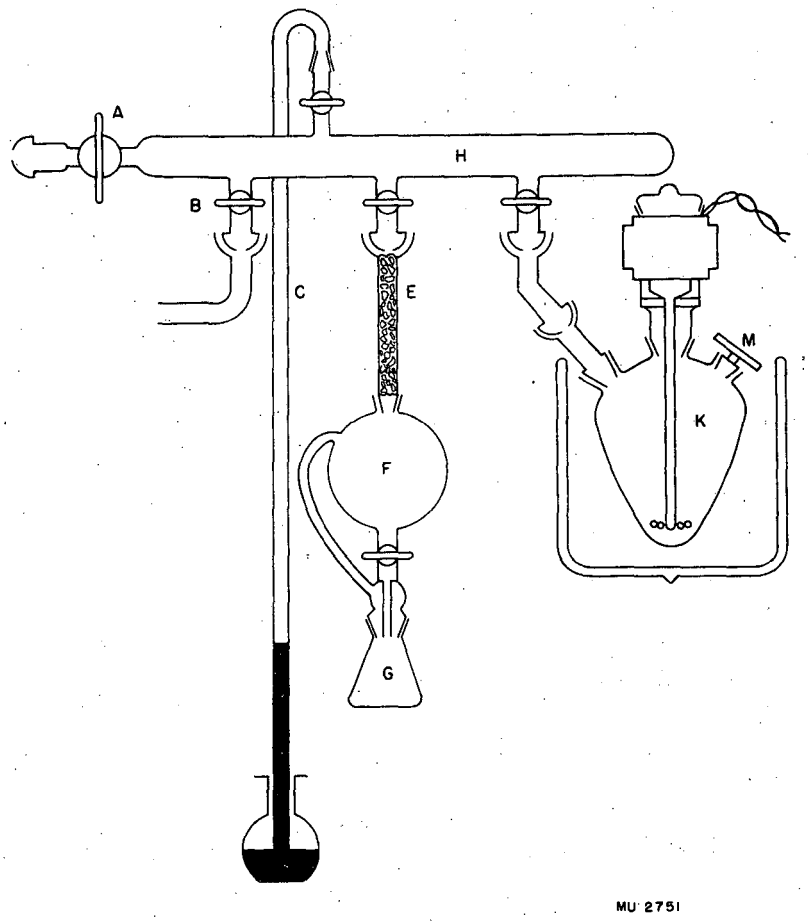


Fig. 3 - Grignard carbonation apparatus

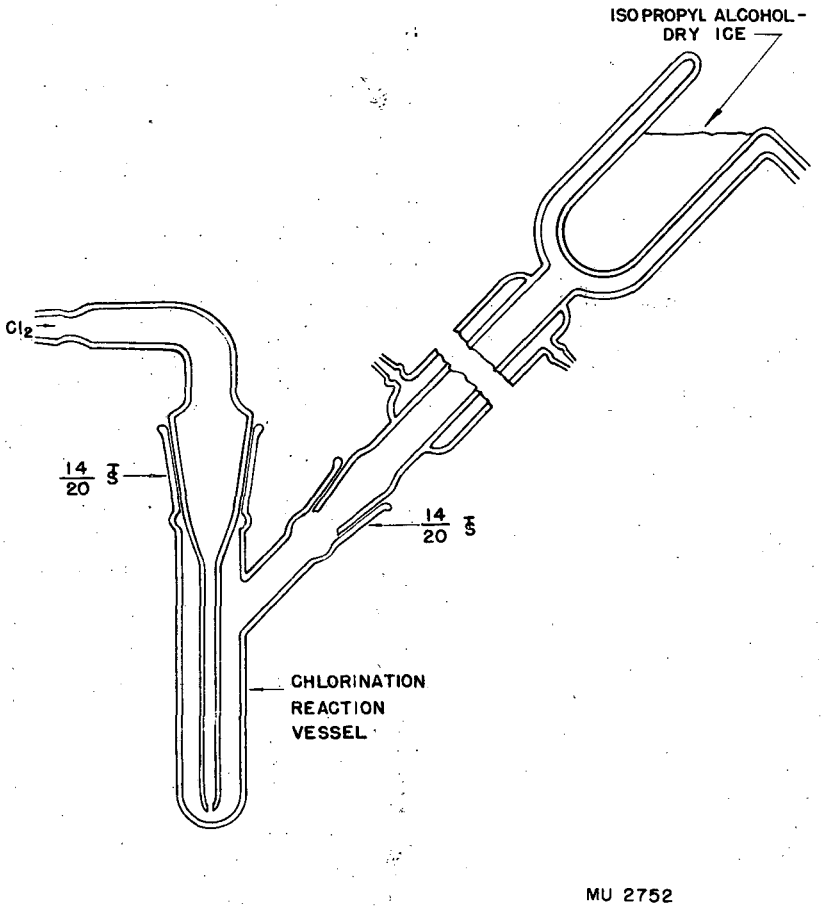


Fig 4 - Apparatus for synthesis of chloroacetic acid.