THE SYNTHESIS OF ISOTOPICALLY LABELLED ORGANIC COMPOUNDS

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Introduction

Most tracer studies necessitate the synthesis of isotopically labelled materials and the last few years have seen important developments in this branch of radiochemistry. It therefore seems appropriate to survey the methods used for the synthesis of organic compounds labelled with isotopes of the more important elements, *viz.*, hydrogen, carbon, nitrogen, oxygen, sulphur, phosphorus, and the halogens. From time to time information on certain aspects of this subject has been summarized [1–9] but we shall lay special emphasis on recent developments so that this contribution may be regarded as an extension to the survey which formed part of Arnstein and Bentley's review.[1] Both chemical and biological methods of synthesis are covered and an account is given of methods of degradation used for the location of labelled atoms in organic compounds.

Information concerning the sterling-area availability of the isotopes and their compounds is provided in a catalogue published by the Isotopes Division of the Atomic Energy Research Establishment. This document, together with one entitled "An Introductory Manual on the Control of Health Hazards from Radioactive Materials",[10] provides much useful information concerning the health hazards and manipulation of these substances.

Isotopic Synthesis. General Considerations.—The selection of the tracer *element*, where any choice exists, is made on the basis of factors such as stability of labelling, ease of synthesis and assay, isotope half-life, concentration of isotope available, influence of radiation effects, etc. Similarly the choice of *isotope* (radioactive or stable) will depend on whether mass spectrometric assay is obligatory (see below), on the radioactive half-life, the dilution

[1] Arnstein and Bentley, *Quart. Reviews*, 1950, **4**, 172.
[2] Calvin, "Isotopic Carbon", Chapman & Hall, London, 1949.
[3] *Cold Spring Harbor Symp.*, Vol. 13.
[4] Crompton and Woodruff, *Nucleonics*, 1950, **7**, (3), 49; (4) 44.
[5] Grove and Catch, *Brit. Med. Bull.*, 1952, **8**, 234.
[6] Kamen, "Radioactive Tracers in Biology", 2nd Edn., Academic Press Inc., New York, 1951.
[7] Lawrence and Hamilton, "Advances in Biological and Medical Physics", Vol. 1 (1948); Vol. 2 (1951).
[8] Tabern, Taylor, and Gleason, *Nucleonics*, 1950, **7**, (5), 3; (6), 40; **8**, (1), 60.
[9] Woodruff and Fowler, *ibid.*, 1950, **7**, (2), 26.
[10] Available from The Director, The National Physical Laboratory, Teddington. Middlesex.

expected during the experiment and, in certain cases (notably with hydrogen isotopes), on differences arising from an isotope effect.[11]

Ideally, an isotopic synthesis should be simple and should combine a high recovery of isotope with low dilution by unlabelled material. A high yield is sought for reasons of economy and the isotope is therefore introduced at as late a stage in the synthesis as is possible, yields being improved by careful selection of reaction conditions, by the use of unorthodox methods and new reagents, and by refinements in experimental technique. The concentration of isotope in the product must be sufficient to permit assay at the end of the tracer experiment, and, further, the gross quantity of labelled compound must not be so large as to disturb the biological or other system under investigation. Hence, dilution during synthesis must be prevented or restricted to an extent determined by the isotope used, its half-life, its initial concentration, the nature of the system being investigated, and the sensitivity of the method of analysis.

In any particular synthesis the loss involved if low yields are obtained must be set against the extra effort required to secure higher yields. Similarly, if dilution is to be avoided or kept very small it will usually be necessary to work on a small scale (often below 10 millimoles) and to accept the attendant experimental difficulties.[12]

In the *manufacture* of labelled compounds it is customary to work both for high yield and low dilution [12] and this is made rather easier by the use of larger quantities of isotope than would be handled by individual research workers. In many cases, even for much biological work, a 10- or 100-fold dilution during synthesis is permissible. It is then advantageous to work on the ordinary small laboratory scale or, alternatively, to work initially on a smaller scale and to improve both yield and purity at difficult stages by judicious addition of pure carrier. This process of carrier dilution has been applied to the resolution of racemates [13] although it is not always wholly successful.[14] In certain cases a very high dilution and low yield can be accepted, particularly in chemical tracer investigations; thus Loftfield [15] converted $^{14}CO_2$ into 2-chloro[1 : 2-$^{14}C_1$]*cyclo*hexanone, for use in a study of the Faworskii reaction, *via* an 8-stage synthesis over which the dilution was approximately 30,000-fold. The overall yield of isotope from $^{14}CO_2$ to the end product of the degradation of the reaction product was approximately 0·003% and could have been as little as 0·2% of this value without effect on the accuracy of the results.

Detailed descriptions of experimental methods, *e.g.*, vacuum-manipulation of volatile compounds, are included in many of the papers cited in this Review; more comprehensive accounts have been given by Calvin [2] and Catch.[12]

[11] Ropp, *Nucleonics*, 1952, **10**, (10), 22.
[12] Catch, "Radio-isotope Techniques", Vol. II, H.M.S.O., London, 1952, p. 100.
[13] *E.g.*, Wood and Gutman, *J. Biol. Chem.*, 1949, **179**, 535.
[14] Arnstein, Hunter, Muir, and Neuberger, *J.*, 1952, 1329.
[15] "Use of Tracers in Organic Reaction Mechanism Studies", Brookhaven Conference Report, BNL–44(C–10), Jan. 1950, p. 59.

Purity of Labelled Compounds.—The usual criteria of purity of organic compounds may also be applied to labelled compounds but are often inadequate. Thus, as a result, for example, of carrier dilution during a synthesis a labelled compound that is pure by normal standards may contain a chemical trace of a radioactive impurity with a gross activity of a similar order to that in the principal compound. This situation arises from the very large dilution many radioactive isotopes may undergo before detection becomes impossible : it is much less important when stable isotopes are used.

Sensitive determination of chemical impurities is sometimes valuable,[16] but the most satisfactory tests of purity are those designed to demonstrate the extent to which the isotope is associated with the compound being studied.

Thus an isotopic compound must be purified not only to constant melting point, boiling point, refractive index, etc., but also to constant isotopic composition, and reliance should not be placed on one method of purification alone. If, for example, the purity of an acid is determined by Duclaux distillation, the acid and isotope content of the fractions should correspond within experimental error. Similarly the same values for partition coefficients should be obtained by chemical analysis and by isotope assay. Paper chromatography combined with autoradiography is especially useful in the detection of radioactive impurities.[17] Keston, Udenfriend, and Cannan [18] have developed an ingenious method of analysis for amino-acids in which the mixed acids and authentic pure specimens of each expected component are converted into *p*-iodophenylsulphonyl derivatives, one with the [^{131}I]- and the others with the [^{35}S]-compound. Representative samples are then mixed and chromatographed together and the distribution of radioactivity on the chromatogram is examined. The ratio of sulphur- to iodine-activity (and these are of widely differing character) is constant throughout each pure band and its value provides a critical assay for the acid under examination.

The positive absence of any specific impurity may be established by adding the non-radioactive impurity as carrier and re-separating on paper.

Finally, if the compound is subjected to degradation, the sum of the isotope contents of the fragments should agree with that of the whole compound determined directly.

Many of the conventional methods of purification (*e.g.*, precise fractional distillation) are quite unsuitable on the relatively small scale of most isotopic syntheses. Losses are involved in all purification procedures and it is therefore desirable to devise synthetic methods which give very pure products or products containing unexceptionable or easily removable impurities. Thus, if carbon dioxide is reduced by lithium aluminium hydride dissolved in diethylene glycol diethyl ether, the methanol product is contaminated with ethanol formed by scission of the solvent. Separation is impracticable

[16] Hughes, Williams, and Young, *J.*, 1951, 1279.
[17] *E.g.*, Putman and Hassid, *J. Biol. Chem.*, 1952, **196**, 749.
[18] Keston, Udenfriend, and Cannan, *J. Amer. Chem. Soc.*, 1949, **71**, 249.

but the use of an alternative solvent [19] gives methanol free from ethanol. Paper chromatography is applicable on the small preparative scale [17] and has been very widely used in the separation and purification of labelled substances found in biological tracer experiments.[20]

Multiple Labelling.—Some compounds behave as though labelled in more than one atom in the molecule, whether these atoms are of the same or different elements. In such compounds a distinction may be drawn between those that contain molecules bearing more than one labelled atom and those that do not.

Thus, if methyl iodide (containing say 5 atoms % ^{14}C) is converted into the Grignard reagent and then carboxylated with $^{14}CO_2$ (also containing 5 atoms % ^{14}C) some 0·25% of the molecules of acetic acid produced will (apart from any possible isotope effect) contain two ^{14}C atoms. This acetic acid could be distinguished, by mass-spectrometric assay of derived ethylene, from a mixture of $^{14}CH_3{\cdot}^{12}CO_2H$ and $^{12}CH_3{\cdot}^{14}CO_2H$ of the same overall isotopic composition at each carbon atom. This type of distinction is occasionally important,[21] but for most practical purposes the two samples behave in an identical manner and are quite indistinguishable by radioactive counting methods. At tracer concentrations the difference is beyond the sensitivity of the mass spectrometer. It is therefore satisfactory for most purposes to use mixtures of singly labelled compounds in place of "truly" multiply labelled compounds. The singly labelled compounds may be prepared separately in higher yield since conditions are more readily designed to give a high yield from only one reactant. Each compound is, however, diluted by the others. Effective multiplication of labelling also occurs when a labelled compound has the appropriate degree of symmetry or has been synthesised *via* a symmetrical intermediate.

Nomenclature.—The nomenclature used for isotopically labelled organic compounds in this Review is that proposed jointly by the Editorial Board of the Biochemical Society and the Editors to the Chemical Society and has already been applied in part in publications of those societies.[22] So far as it affects the compounds named in this Review it is as follows.*

The symbol for the isotope introduced is placed in square brackets directly attached to the front of the name, as in [^{14}C]urea.

When more than one position in a substance is labelled by means of the same isotope the number of labelled atoms is added as a right-hand subscript (cf. ordinary formulæ), as in [$^{14}C_2$]glycollic acid.

When isotopes of more than one element are introduced, their symbols are arranged in alphabetical order, including ^{2}H and ^{3}H for deuterium and tritium respectively.

The isotopic prefix precedes that part of the name to which it refers, as in 2-

[19] Cox, Turner, and Warne, *J.*, 1950, 3167.

[20] *E.g.*, Benson, Bassham, Calvin, Goodale, Haas, and Stepka, *J. Amer. Chem. Soc.*, 1950, **72**, 1710; Wmteringham, *Nucleonics*, 1952, **10**, (3), 52.

[21] Wood, *J. Biol. Chem.*, 1952, **194**, 905. [22] *J.*, 1951, 3516: 1952, 5061.

* The full scheme may be obtained from the Editor, The Chemical Society, with whose co-operation this account of it has been written.

acetamido-7-[^{131}I]iodofluorene, α-naphth[^{3}H]oic acid ($C_{10}H_7 \cdot CO_2{}^3H$), sodium [^{14}C]formate, 1-amino[^{14}C]methyl*cyclo*pentanol ($NH_2 \cdot {}^{14}CH_2 \cdot C_5H_8 \cdot OH$).

When not sufficiently distinguished by the foregoing means, the positions of isotopic labelling are indicated by arabic numerals, Greek letters, or prefixes (as appropriate), placed within the square brackets and before the symbol of the element concerned, to which they are attached by a hyphen ; examples are [1-$^{2}H_1$]ethanol($CH_3 \cdot CH^2H \cdot OH$), [1-^{14}C]aniline, [α-^{14}C]leucine, [*carboxy*-^{14}C]leucine, [*Me*-^{14}C]*iso*leucine, [6 : 7-$^{14}C_2$]xanthopterin, [$\alpha\beta$-$^{14}C_2$]-maleic anhydride, [1-^{14}C : 2-^{13}C]acetaldehyde, [$\beta\gamma$-$^{13}C_2$: ^{34}S]methionine, [β-^{14}C : $\alpha\beta$-$^{2}H_2$: ^{15}N]serine, 2 : 4-diamino[1 : 2 : 3-$^{15}N_3$]pyrimidine.

When the position of isotopic labelling is indeterminate, the possible positions are specified together with the number of atoms which are labelled, as in [*ar*-$^{14}C_1$]benzaldehyde (one ^{14}C in the benzene ring), [4 : 6-$^{14}C_1$]adenine (one ^{14}C, at position 4 or 6), D-[1 : 6-$^{14}C_1$]fructose (one ^{14}C, at position 1 or 6). (The device illustrated in the last two examples is an extension of the " editorial " proposals.)

Hydrogen

Hydrogen has two useful tracer isotopes, the stable deuterium (^{2}H or D) and the weak β-emitter tritium (^{3}H or T, $\tau_{\frac{1}{2}} \sim 12$ years). Both are available as water and, in general, syntheses are applicable to both isotopes.

Deuterium can be diluted some 10^3—10^4-fold with normal hydrogen before the accuracy of analysis is seriously reduced, but for tritium (~ 50 atoms % ^{3}H, ~ 1 c per ml.) the corresponding figure is of the order of 10^{10}.

However, the isotopes should be used with caution,[23] particularly in biological applications, first because many hydrogen atoms readily undergo exchange or replacement, and secondly because of the potentially large isotope effect.[11] The element received particular attention in Arnstein and Bentley's review,[1] an annual bibliography [24] is published, and there have been several other reviews.[6, 25] Treatment here will therefore be brief.

Deuterium.—Methods of assay [26] referred to by Arnstein and Bentley have been augmented by a spectrographic method based on the stretching frequency of the O–^{2}D bond.[27]

Almost all syntheses reported since the last Review fall into one or other of Arnstein and Bentley's three categories, namely, hydrogen exchange, hydrogen addition, or group replacement by hydrogen. Therefore, only syntheses showing particular points of chemical interest will be noted.

Hydrogen Exchange.—[1-^{2}H]Ethanol has been prepared from the product

[23] Verley, Rachele, du Vigneaud, Eidinoff, and Knoll, *J. Amer. Chem. Soc.*, 1952, **74**, 5941.

[24] " A Review of the Properties of Deuterium Compounds ", U.S. Dept. of Commerce, N.B.S. (1946 onwards).

[25] Kimball, " Bibliography of Heavy Hydrogen Compounds ", McGraw-Hill, New York, 1949 (to 1945).

[26] Kirshenbaum, " Physical Properties and Analysis of Heavy Water ", McGraw-Hill, New York, 1951.

[27] Trenner and Walker, Perkin-Elmer Instrument News, Fall, 1952.

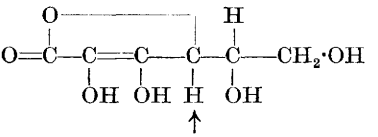
of a bromine degradation of the silver salt of an α-enriched propionic acid [28] and from diazoethane : [29]

$$Me{\cdot}CHN_2 \xrightarrow[\text{in } Et_2O]{^2H_2O} Me{\cdot}C^2HN_2 \xrightarrow{R{\cdot}CO_2{}^2H} Me{\cdot}C^2H_2{\cdot}O{\cdot}COR \longrightarrow Me{\cdot}C^2H_2{\cdot}OH$$

$$[R{\cdot}CO_2{}^2H = 3 : 5 : 1\text{-}(NO_2)_2C_6H_3{\cdot}CO_2{}^2H]$$

Partial chromic acid oxidation of the alcohol [28] showed further enrichment of the intermediate aldehyde $CH_3{\cdot}C^2HO$, the protium-alcohol being preferentially destroyed. The enriched propionic acid has also been electrolysed [30] to yield $CH_2{:}C^2H_2$.

An interesting application of a deuterated diazo-compound has been made by Leitch and his collaborators during extensive and careful studies of the synthetic chemistry of deuterium. Diazomethane, either prepared from enriched nitromethane or enriched directly, was polymerised in ethereal solution by copper powder to yield polydideuteromethylene [31] $[C^2H_2]_n$. Other papers in this series deal with the synthesis of various alkyl halides and polyhalides, deuteroformaldehyde, etc. Replacement of hydrogen in the biologically important ascorbic acid dissolved in heavy water was studied by Weigl.[32] Infra-red analysis suggested the lability of the hydrogen atom at C_4.



Addition Reactions.—Two methods have been used for the partial reduction of the acetylenic bond. By the use of the chromous chloride in ^2HCl Ronzio prepared [$^2H_4$]ethylene, which polymerised [33] rather more readily than the corresponding protium compound to yield a polydeuteroethylene. An alternative method, based on the use of certain deuterised Raney nickels, was established by Khan.[34] This procedure has been utilised for the syntheses of [1-^{2}H]acetaldehyde : [35]

$$Me{\cdot}C{:}C{\cdot}Me \longrightarrow Me{\cdot}C^2H{:}C^2H{\cdot}Me \longrightarrow Me{\cdot}C^2H{-}C^2H{\cdot}Me \longrightarrow$$

$$HO{\cdot}C^2HMe{-}C^2HMe{\cdot}OH \longrightarrow 2Me{\cdot}C^2HO$$

By low-temperature addition of ^2HCl to anethole, followed by a bimolecular dehalogenation with reduced iron powder, [2 : 5-$^2H_2$]hexœstrol

[28] Cornforth and Popják, *Nature*, 1949, **164**, 1053.
[29] Curran and Rittenberg, *J. Biol. Chem.*, 1951, **190**, 17.
[30] Kruis, *Naturwiss.*, 1948, **35**, 155.
[31] Leitch, Gagnon, and Cambron, *Canad. J. Res.*, 1950, **28**, *B*, 256.
[32] Weigl, *Analyt. Chem.*, 1952, **24**, 1483.
[33] Ronzio, U.S. At. Energy Comm. Rep., LA-14 78 ; cf. ref. 31.
[34] *J. Amer. Chem. Soc.*, 1952, **74**, 3018.
[35] Blacet and Brinton, *ibid.*, 1950, **72**, 4715.

having a slightly lower physiological activity than that of the protium compound has been prepared.[36]

The fundamental importance of the equilibrium:

$$H_2 + R{\cdot}CO_2H^* \rightleftharpoons HH^* + R{\cdot}CO_2H$$

in catalysed hydrogen-addition and -exchange reactions has recently been demonstrated.[37] The deuterated carboxyl group plays the part of an isotopic buffer.

Replacement Reactions.—The simple deuterated hydrocarbons are readily prepared by interaction of deuterium oxide with suitable carbides, but [$^2H_4$]allene [38] represents an unusual by-product from a synthesis of [$^2H_4$]-methylacetylene from Mg_2C_3. The acetylenic hydrogen readily undergoes exchange. Several workers have studied the preparation of [2-^{2}H]propan-2-ol ($Me_2C^2H{\cdot}OH$) and [2 : 2-$^2H_2$]propane by catalytic reduction of acetone.[39] It appears to be impossible to prevent partial exchange with the α-hydrogen atoms during preparation of the hydrocarbon in this manner but it is probable that an authentic propanol may be prepared by catalytic or lithium aluminium hydride reduction of the ketone. As is found with acetaldehyde, oxidation of the deutero-alcohol is relatively slow.[40] Schissler, Thompson, and Turkevich [41] have devised a method for introducing one, two, or three deuterium atoms at a single carbon atom through Zn–AcO^2H reduction of suitable halides.

Deuterochloroform has been prepared from trichloroacetophenone and from calcium trichloroacetate by the haloform reaction.[42]

In an ingenious synthesis of [1 : 1-$^2H_2$]allyl acetate,[43] the crystalline adduct of anthracene and acrylic ester was reduced with lithium aluminium deuteride and the product acetylated. The complex was readily decomposed by heat (see next page).

By hypophosphorous acid deamination of diazonium salts in ^2H_2O, Alexander and Burge [44] have introduced specific labels into aromatic compounds, but the efficiency of the reaction is poor since protium enters the nucleus preferentially.

In a study of optical activity due to the presence of deuterium, Alexander [45] employed the useful lithium aluminium deuteride reduction of a toluene-*p*-sulphonate to prepare *trans*-*p*-[3-^{2}H]menthane. The material had $[\alpha]_D^{25} = -0{\cdot}09° \pm 0{\cdot}01°$ while the corresponding protium compound was quite inactive.

[36] Lacassagne, Buu-Hoï, Chamorro, Xuong, and Hoán, *Compt. rend.*, 1950, **231**, 1384.

[37] Eidinoff, Knoll, Fukushima, and Gallagher, Abs. 118th Amer. Chem. Soc. Mtg., 1950, p. 66Q.

[38] Lord and Venkateswarlu, *J. Chem. Phys.*, 1949, **20**, 1237.

[39] Friedman and Turkevich, *J. Amer. Chem. Soc.*, 1952, **74**, 1669; Williams, Krieger, and Day, Abs. 122nd Amer. Chem. Soc. Mtg., 1950, 22M.

[40] Westheimer and Nicolaides, *J. Amer. Chem. Soc.*, 1949, **71**, 25.

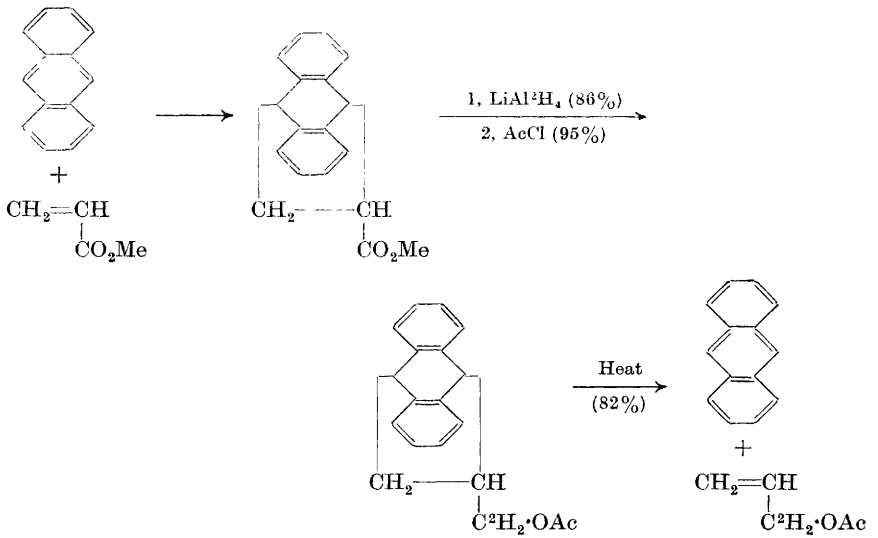
[41] U.S. At. Energy Comm. Rep., AECU-1387.

[42] Boyer, Bernstein, Brown, and Dibeler, *J. Amer. Chem. Soc.*, 1951, **73**, 770; Earing and Cloke, *ibid.*, p. 769.

[43] Bartlett and Tate, *ibid.*, 1953, **75**, 91.

[44] Alexander and Burge, *ibid.*, 1950, **72**, 3100.

[45] Alexander, *ibid.*, p. 3796.



Miscellaneous.—It is not possible to describe here the valuable work on the deuterium-labelling of steroids which has been carried out by Gallagher and others.[46] These compounds are frequently more readily accessible than the carbon-labelled substances and are therefore useful for biological studies, but it is necessary to ascertain the position and stability of the label as well as to devise methods for its introduction at specific positions.

There have also been occasional biosyntheses with deuterium, but this is not a valuable method owing to the existence of a rapidly changing hydrogen pool in the living cell.

Tritium.—Tritium has become available at very high specific activity as a result of pile synthesis [6] by the reaction $^{6}Li(n,\alpha)^{3}H$. It is a very soft β-emitter (max. 0·018 mev) and must therefore be analysed [47] by a gas counting tube, ionisation chamber, or scintillation counter,[48] preferably as hydrogen or as a hydrocarbon derived from water of combustion.

In general, synthetical methods follow those of deuterium but certain specific examples have been reported.

Intermediates.—Syntheses of lithium tritide and the useful lithium aluminium tritide have been announced.[49]

Exchange Reactions.—Tritiated methanol has been prepared, (i) by hydrolysis of the ester derived from diazomethane and α-naphth[^{3}H]oic acid,[23] and (ii) by a replacement reaction involving the catalytic reduction of methyl formate.[50] Other compounds prepared by exchange methods

[46] Gallagher, "Isotopes in Biochemistry", Churchill, London, 1951, p. 28; Nolin and Jones, *Canad. J. Chem.*, 1952, **30**, 727; Bell and Thomson, *J.*, 1952, 572.

[47] Glascock, *Biochem. J.*, 1952, **52**, 699.

[48] Farmer and Berstein, *Science*, 1953, **117**, 279; Hayes and Gould, *ibid.*, p. 480.

[49] Wilzbach and Kaplan, *J. Amer. Chem. Soc.*, 1950, **72**, 595.

[50] Harman, Stewart, and Ruben, *ibid.*, 1942, **64**, 2293.

include phenylalanine,[51] stearic acid,[52] benzene,[53] and a range of steroids[54] including cortisone.[55]

Addition Reactions.—Succinic acid [56] and hexœstrol [57] have been prepared by this method, the addition of tritium apparently being favoured over that of protium in the latter case.

Replacement Reactions.—[1-^{3}H]Ethanol has been prepared [49] by lithium aluminium tritide reduction of ethyl acetate, and styrene [58] by toluene-*p*-sulphonic acid dehydration of the hydrogenation product of acetophenone.

In an extensive review of aromatic substitution Melander [53] has prepared a series of tritiated aromatic compounds mostly *via* the appropriate Grignard compound.

Miscellaneous.—Tritiated stilbene [59] is self-luminous and may be used as a constant light source for the standardisation of photomultiplier tubes.

There have been biosyntheses of labelled nucleic acids.[60]

Carbon

Three isotopes of carbon[2] have been used in tracer studies. ^{11}C, a positron emitter ($\tau_{\frac{1}{2}} \sim 20$ minutes) is made in the cyclotron [6] by the reaction ^{10}B(d,n)^{11}C, but, although its energetic radiation makes detection very easy, it has been little used since the longer-lived ^{14}C became readily available in 1946. ^{13}C, the heavy stable isotope, is separated from normal carbon (containing $\sim 1{\cdot}1\%$ of ^{13}C) by fractional distillation of carbon monoxide [61] or by means of isotopic exchange reactions.[62] It is available as $Ba^{13}CO_3$ and $K^{13}CN$ and is usually analysed as carbon dioxide in the mass spectrometer.[63] ^{14}C, a weak β-emitter ($\sim 0{\cdot}15$ mev ; $\tau_{\frac{1}{2}} \sim 5600$ years), is made in the pile by the reaction ^{14}N(n,p)^{14}C. Depending on the choice of nitrogenous target material the ^{14}C may be obtained in a variety of compounds,[64, 65] but it is normally available as $Ba^{14}CO_3$ from which other compounds are prepared by chemical or biological synthesis.[66] ^{14}C is usually analysed by counting as 'carbon dioxide or barium carbonate,[67] but

[51] Gurin and Delluva, *J. Biol. Chem.*, 1947, **170,** 545.
[52] Rosenthal and Kritchevsky, Univ. California Radiation Lab. Rep., 1131.
[53] Melander, *Acta Chem. Scand.*, 1948, **3,** 95 ; *Arkiv Kemi*, 1950, **2,** 213.
[54] *E.g.*, Biggs and Kritchevsky, *Arch. Biochem.*, 1952, **36,** 430.
[55] Fukushima, Kritchevsky, Eidinoff, and Gallagher, *J. Amer. Chem. Soc.*, 1952, **74,** 487.
[56] Williams and Ronzio, U.S. At. Energy Comm. Rep., AECU-2126.
[57] *Idem, J. Amer. Chem. Soc.*, 1950, **72,** 5787.
[58] Berstein, Bennett, and Fields, *ibid.*, 1952, **74,** 5763.
[59] *Idem, Nucleonics*, 1953, **11,** (2), 64.
[60] Eidinoff, Riley, Knoll, and Marrian, *J. Biol. Chem.*, 1952, **199,** 511.
[61] London, "Mass Spectrometry", Institute of Petroleum, London, 1952, p. 141.
[62] Stewart, *Nucleonics*, 1947, **1,** (2), 18.
[63] Wilson (Ed.), "Preparation and Measurement of Isotopic Tracers", Edwards, Ann Arbor, Mich., 1948.
[64] Ref. 2, p. 6.
[65] Croatto, Giacomello, and Maddock, *Ric. sci.*, 1951, **21,** 1598.
[66] More than 80 ^{14}C-labelled compounds are available from the Radiochemical Centre, Amersham.
[67] Neville, *Atomics*, 1952, **3,** 309 ; Smith, *ibid.*, 1953, **4,** 29.

occasionally more complex compounds are counted directly, either as solids[68] or in solution.[69,70]

Chemical Syntheses.—Definite location of isotopic atoms can normally be achieved by chemical synthesis but, if there are symmetrical intermediates, (effective) multiple labelling may result. In some cases unexpected molecular rearrangements occur; thus when Loftfield[71] attempted to prepare [1-^{14}C]*cyclo*pentanecarboxylic acid by the sequence: [1-^{14}C]*cyclo*pentanol $\xrightarrow{PBr_3}$ *cyclo*pentyl bromide $\xrightarrow{NaCN}$ *cyclo*pentyl cyanide $\longrightarrow$ *cyclo*pentanecarboxylic acid, degradation showed $\sim$20% of the ^{14}C to be in the methylene-carbon atoms of the ring.

Syntheses with ^{13}C and ^{14}C are similar and will not be discussed separately in this Review. However, the most highly enriched ^{13}C normally available (65—75 atoms % of ^{13}C) can be diluted only $\sim 10^3$ times with normal carbon before the accuracy of analysis is reduced, while the corresponding figure for ^{14}C ($\sim$ 5 atoms % of ^{14}C; $\sim$ 3 millicuries per milliatom) is 10^6—10^8.

One-carbon Compounds and Simple Intermediates.—Some recent developments are outlined in Table 1.

Functionally Labelled Carboxylic Acids.—The carboxylation of organo-

[68] *E.g.*, Hogness, Roth, Leifer, and Langham, *J. Amer. Chem. Soc.*, 1948, **70**, 3840.
[69] Schwebel, Isbell, and Karabinos, *Science*, 1951, **113**, 465.
[70] Audric and Long, *Research*, 1952, **5**, 46.
[71] Loftfield, *J. Amer. Chem. Soc.*, 1951, **73**, 4707.
[72] Ref. 1, p. 180.
[73] Von Schuching and Barnes, *J. Amer. Chem. Soc.*, 1950, **72**, 3817.
[74] Williams and Ronzio, *ibid.*, 1952, **74**, 2407.
[75] Adamson, *ibid.*, 1947, **69**, 2564; Henneberry and Baker, *Canad. J. Res.*, 1950, **28**, *B*, 345; Maĭmind, Tokarev, and Shamyakin, *Doklady Akad. Nauk S.S.S.R.*, 1951, **81**, 195 (*Chem. Abs.*, 1952, **46**, 3889); Claus, Abs. 121st Amer. Chem. Soc. Mtg., 1952.
[76] McCarter, *J. Amer. Chem. Soc.*, 1951, **73**, 483.
[77] Abrams, *ibid.*, 1949, **71**, 3835.
[78] Spyker and Neish, *Canad. J. Chem.*, 1952, **30**, 461.
[79] Cox and Warne, *J.*, 1951, 1895.
[80] Heard, Jamieson, and Solomon, *J. Amer. Chem. Soc.*, 1951, **73**, 4985.
[81] Murray and Ronzio, *ibid.*, 1952, **74**, 2405.
[82] Weygand and Schaefer, *Chem. Ber.*, 1952, **85**, 310.
[83] Grant and Turner, *Nature*, 1950, **165**, 153.
[84] Burr, Brown, and Heller, *J. Amer. Chem. Soc.*, 1950, **72**, 2560.
[85] Wagner, Stevenson, and Otvos, *ibid.*, p. 5786.
[86] Adams, Selff, and Tolbert, *ibid.*, 1952, **74**, 2416.
[87] Arrol and Glascock, *J.*, 1948, 1534.
[88] Monat, Robbins, and Ronzio, U.S. At. Energy Comm. Rep., AECU-672.
[89] Arrol and Glascock, *J.*, 1949, S335.
[90] Kilmer and du Vigneaud, *J. Biol. Chem.*, 1944, **154**, 247.
[91] Kramer and Kistiakovsky, *ibid.*, 1941, **137**, 554.
[92] Cox and Warne, *J.*, 1951, 1893.
[93] Kögl, Halberstadt, and Barendregt, *Rec. Trav. chim.*, 1949, **68**, 387.
[94] Ostwald, *J. Biol. Chem.*, 1948, **173**, 207.
[95] Fields, Rothchild, and Leaffer, *J. Amer. Chem. Soc.*, 1952, **74**, 2435.
[96] Ropp, *ibid.*, 1950, **72**, 4459; Gal and Schulgin, *ibid.*, 1951, **73**, 2938.
[97] Bennett, *ibid.*, 1952, **74**, 2420.
[98] Fields, Walz, and Rothchild, *ibid.*, 1951, **73**, 1000.

TABLE 1. *One-carbon compounds and simple intermediates* [72]

Compound	Starting material, method, and yield	Ref.
$*CN{\cdot}NH_2$	$Ba*CO_3$ (heated with NH_3 and NaN_3) : 94%	73
$KN*CO$	$NH_2{\cdot}*CO{\cdot}NH_2$ (heated with K_2CO_3) : 70—80%	74
Na*CN or K*CN	$Ba*CO_3$ (heated with NaN_3) : 75—93%	75
	K_2*CO_3 (heated with Zn in NH_3) : 90%	76
	$*CO_2$ (*via* carbon) : 59—70%	77
	$H{\cdot}*CO_2Na$ (heated with $NaNH_2$) : > 85%	78
$*CH_3{\cdot}NH_2$	$*CH_3I$ (Gabriel reaction) : 98%	79
	Na*CN (catalytic reduction) : 85%	80
$*CH_2N_2$	$*CH_3{\cdot}NH_2$ (*via* nitrosomethylurea) : 57—68% (55% from $BaCO_3$)	79
$H{\cdot}*CHO$	$*CH_3{\cdot}OH$ (catalytic oxidation) : 77—81%	81
	$*CH_3{\cdot}OH$ (catalytic oxidation) : 86%	82
$H{\cdot}*CO_2Na$	K*CN (alkaline hydrolysis) : ~ 100%	83
	$*CO_2$ (reduction with $LiBH_4$) : 73%	84
$*CH_4$	$*CH_3I$ (Grignard reaction) : 86%	85
$*CH_3{\cdot}OH$	$*CO_2$ (reduction by $LiAlH_4$) : 89%	19
	$H{\cdot}*CO_2H$ (hydrogenation of Cd–Ni salt) : 85%	86
$*CH{:}*CH$	$*CO_2$ (reduction with Ba metal) : > 90%	87
	$Ba*CO_3$ (reduction with Ba metal) : 98%	88
$*CH_2{:}*CH_2$	$*C_2H_2$ (reduction with $TiCl_3$) : 96—98%	89
$*CH_2Cl{\cdot}CH_2Cl$	Na*CN (*via* $CH_3{\cdot}*CN$, $CH_3{\cdot}*CH_2{\cdot}NH_2$, and $*CH_2{:}CH_2$) : 56%	90
$*CH_3{\cdot}*CHO$	$*C_2H_2$ (catalytic addition of H_2O) : 75%	91
$*CH_2{\cdot}*CH_2{\cdot}O$	$*C_2H_4$ (*via* $HO{\cdot}*CH_2{\cdot}*CH_2Cl$) : 85—95%	92
$(*CO_2H)_2$	$H{\cdot}*CO_2Na$ (440°/0·01 mm.) : 90%	93
$Cl{\cdot}CH_2{\cdot}*CO_2H$	$CH_3{\cdot}*CO_2Na$ (Cl_2–PCl_5, P_4, I_2) : 67%	94
$Br{\cdot}CH_2{\cdot}CO_2H$	$CH_3{\cdot}CO_2Na$ (Br_2–$CH_3{\cdot}COCl$) : 79—84%	95
$CH_2(CO_2H)_2$, $CN{\cdot}CH_2{\cdot}CO_2H$, $CH_2(CN)_2$	Conventional syntheses from Na*CN or $*CH_3{\cdot}CO_2Na$	96, 97
$CN{\cdot}*CH(NHAc){\cdot}CO_2Et$	$CN{\cdot}*CH_2{\cdot}CO_2Et$ (nitrosation, catalytic reduction) : 76%	98
$*CN{\cdot}CH(N_2Ph){\cdot}CN$	Na*CN (*via* $*CN{\cdot}CH_2{\cdot}CO{\cdot}NH_2$) : 46—53%	97

metallic compounds, especially of Grignard reagents, gives excellent yields of carboxylic acids (up to 98%) and is probably the most useful first step in isotopic syntheses with carbon ; [99] the hydrolysis of nitriles, prepared from inorganic cyanides, is less important [99] and other methods, *e.g.*, reduction of keto-acids,[100] are relatively unimportant. Most simple derivatives can be satisfactorily prepared by standard methods. Volatile esters are conveniently made by the reaction of alkyl sulphates [101] or phosphates [102] with salts of carboxylic acids, while volatile acid chlorides are best prepared by the reaction of the acids and phthaloyl chloride.[103]

Non-functionally Labelled Carboxylic Acids.—These may be made by the above methods, starting with suitably labelled alkyl halides, etc. The

[99] Ref. 1, p. 181 ; Ref. 2, p. 172.

[100] *E.g.*, Jorgenson, Bassham, Calvin, and Tolbert, *J. Amer. Chem. Soc.*, 1952, **74**, 2418.

[101] Sakami, Evans, and Gurin, *ibid.*, 1947, **69**, 1110.

[102] Ropp, *ibid.*, 1950, **72**, 2229. [103] Cox and Turner, *J.*, 1950, 3176.

acetoacetic ester [104] and malonic ester [105] syntheses, and the reduction of keto-acids,[106] may be applied when appropriate.

Aldehydes and Ketones.—The Rosenmund reduction of acyl halides is the method of choice for the synthesis of isotopically labelled aldehydes,[107] although for benzaldehyde [108] the method of McFadyen and Stevens [109] gives rather lower but more consistent yields. Several aldehydes, *e.g.*, [1-^{14}C : 2-^{13}C]-acetaldehyde [110] and -benzaldehyde [111] have been prepared by oxidation of the corresponding alcohols.

Several ketones, including acetone,[112] [1-^{14}C]*cyclo*hexanone,[113] and [1-^{14}C]*cyclo*pentanone,[71] have been synthesised by the pyrolysis of salts of carboxylic acids. The Friedel–Crafts reaction has been applied to the small-scale synthesis of a number of functionally labelled aralkyl ketones (yields 71—89%),[114] including cyclic ketones.[115] The malonic [116] and acetoacetic ester [117] syntheses, reaction of acyl halides with cadmium alkyls,[116] and reaction of nitriles with Grignard reagents [117] have obviously a wide application. [2-^{14}C]*cyclo*Hexanone has been obtained in 25% yield by application of the Tiffeneau reaction to 1-amino[^{14}C]methyl*cyclo*pentanol.[118] Arnstein and Bentley [119] have synthesised 1 : 3-dihydroxy[2-^{14}C]acetone from nitro[^{14}C]-methane and formaldehyde.

Alcohols and Amines.—By far the most satisfactory route to labelled primary alcohols lies in the reduction of acids, acyl halides, and esters with lithium aluminium hydride,[120] yields exceeding 95% being easily obtained in small-scale preparations.[103, 121, 122] If high-pressure equipment is available, the hydrogenolysis of esters over copper chromite,[123] or of the cadmium–nickel salts of acids,[124] may be used. The alcohol chosen for esterification may be that formed in the reduction [123] or one of much higher or lower boiling point.[125] Secondary and tertiary alcohols have most frequently been obtained by the Grignard reaction.

[104] *E.g.*, Coon and Abrahamson, *J. Biol. Chem.*, 1952, **195,** 805.
[105] *E.g.*, Coon, Abrahamson, and Greene, *ibid.*, 1952, **199,** 75.
[106] Dauben, *J. Amer. Chem. Soc.*, 1948, **70,** 1376.
[107] Ref. 2, p. 197.
[108] Geissmann, Univ. California Radiation Lab. Rep. 1233.
[109] *J.*, 1936, 584.
[110] Ehrensväard, Reio, Saluste, and Stjernholm, *J. Biol. Chem.*, 1951, **189,** 93.
[111] Douglass, U.S. At. Energy Comm. Rep., ORNL-1206.
[112] *E.g.*, Aronoff, Haas, and Fries, *Science*, 1949, **110,** 476.
[113] Speer, Humphries, and Roberts, *J. Amer. Chem. Soc.*, 1952, **74,** 2443.
[114] Speer and Jeans, *ibid.*, p. 2443.
[115] *E.g.*, Collins, *ibid.*, 1951, **73,** 1038.
[116] Dauben, Reid, Yankwich, and Calvin, *ibid.*, 1950, **72,** 121.
[117] Cerwonka, Brown, and Anderson, *ibid.*, 1953, **75,** 28.
[118] Arnold, U.S. At. Energy Comm. Rep., AECU-575.
[119] *J.*, 1951, 2385.
[120] Brown, " Organic Reactions ", Vol. VI, Wiley, New York, 1951, p. 469.
[121] Pack and Tolbert, Univ. California Radiation Lab. Rep., 1957 (Sept. 1952).
[122] Turner and Warne, *J.*, 1953, 789.
[123] Tolbert, Christenson, Chang, and Sah, *J. Org. Chem.*, 1949, **14,** 525.
[124] Adams, Selff, and Tolbert, *J. Amer. Chem. Soc.*, 1952, **74,** 2416.
[125] Hauptman, Adams, and Tolbert, *ibid.*, p. 2423.

Several routes to labelled glycerol have been explored. [1-^{14}C]Glycerol has been prepared in 60% yield (from $Ba^{14}CO_3$) by the following series of reactions: [126]

$$Ba^{14}CO_3 \longrightarrow Na^{14}CN \xrightarrow{CH_2Ph\cdot O\cdot CH_2\cdot CHO} CH_2Ph\cdot O\cdot CH_2\cdot CH(OH)\cdot{}^{14}CN \xrightarrow[2,\ HCl\text{–}EtOH]{1,\ Ac_2O} CH_2Ph\cdot O\cdot CH_2\cdot CH(OAc)\cdot{}^{14}CO_2Et \xrightarrow{LiAlH_4} CH_2Ph\cdot O\cdot CH_2\cdot CH(OH)\cdot{}^{14}CH_2\cdot OH \xrightarrow[hydrogn.]{Cat.} HO\cdot CH_2\cdot CH(OH)\cdot{}^{14}CH_2\cdot OH$$

In a similar synthesis from glycollic aldehyde, glycerol has been obtained in about 13% yield (from $Na^{14}CN$).[127] Both [1-^{14}C]- and [2-^{14}C]-glycerol have been made from the appropriately labelled malonic ester in an overall yield of 30% (from $Ba^{14}CO_3$): [128]

$$CH_2(CO_2Et)_2 \xrightarrow{Pb(OAc)_4} AcO\cdot CH(CO_2Et)_2 \xrightarrow{LiAlH_4} HO\cdot CH(CH_2\cdot OH)_2$$

Schlenk, Lamp, and DeHaas [129] have devised a synthesis for glycerides, specifically labelled in the glycerol residue.

Many alcohols have been converted into *alkyl halides* by conventional methods.

A number of *amines* has been prepared by standard methods. Methylamine, ethylamine,[130] and aniline,[95] are obtained in good yield from the appropriate acids by the Schmidt reaction. Other primary amines have been made by reduction of oximes [131] and nitriles,[90] and by the Gabriel reaction.[79] Secondary and tertiary amines have been synthesised by alkylation methods.[132]

Olefins have been prepared by dehydration of alcohols [133] and by dehydrohalogenation of alkyl halides,[85] but the thermal decomposition of quaternary ammonium hydroxides gives a more certain location of the double bond (*e.g.*, the preparation of [1-^{14}C]prop-1-ene.[133] *Saturated hydrocarbons* are made by reduction of olefins,[85] or from alkyl halides by reduction (Zn–Cu couple [134] or lithium aluminium hydride [135]) or by reaction of the Grignard compound with water.[85] In a few cases ketones have been reduced by the Wolff–Kishner method, *e.g.*, in the preparation of [$^{14}C_1$]*cyclo*hexane.[113]

Hydroxy-acids.—Some α-hydroxy-acids are conveniently prepared by the cyanohydrin synthesis. Thus [*carboxy*-^{13}C]lactic acid is obtained in 94—96% yield from $Na^{13}CN$, and [$\alpha\beta$-$^{13}C_2$]lactic acid in 40% yield from

[126] *Chem. Eng. News*, 1952, **30**, 1872.
[127] Doerschuk, *J. Amer. Chem. Soc.*, 1951, **73**, 821.
[128] Gidez and Karnovsky, *ibid.*, 1952, **74**, 2413.
[129] Schlenk, Lamp, and DeHaas, *ibid.*, p. 2550.
[130] Phares, *Arch. Biochem.*, 1951, **33**, 173.
[131] Wilson, *J. Amer. Pharm. Assoc., Sci. Edn.*, 1950, **39**, 687.
[132] *E.g.*, Walz, Fields, and Gibbs, *J. Amer. Chem. Soc.*, 1951, **73**, 2968.
[133] Fries and Calvin, *ibid.*, 1948, **70**, 2235.
[134] Gordon and Heimel, *ibid.*, 1951, **73**, 2942.
[135] Phillips, Trevoy, Jaques, and Spinks, *Canad. J. Chem.*, 1952, **30**, 844.

$Ba^{13}CO_3$ (*via* acetylene and acetaldehyde [101]). Another method of wide application is the halogenation and subsequent hydrolysis of carboxylic acids; glycollic and lactic acids have been made on the small scale in excellent yields in this manner.[136] In a few cases it is advantageous to prepare and reduce a keto-acid, *e.g.*, malic acid from oxaloacetic ester.[100] Anderson and Rahman [137] have described a useful synthesis of [$^{14}C_2$]glycollic acid, in which potassium [^{14}C]carbonyl is prepared from [^{14}C]carbon monoxide in liquid ammonia, and then hydrolysed. A yield of 80% from $Ba^{14}CO_3$ is claimed. Mandelic acid has been made as follows:

$$Ph\cdot{}^{14}CO\cdot CH_3 \xrightarrow{SeO_2} Ph\cdot{}^{14}CO\cdot CHO \xrightarrow[H_2O]{NaOH-} Ph\cdot{}^{14}CH(OH)\cdot CO_2H \quad (75\%\ \text{yield})$$

It has been demonstrated by degradation that the carbon chain does not suffer rearrangement.[138] Asymmetrically labelled citric acid has been prepared by reaction of (—)-β-carboxy-γ-chlorobutyric acid with [^{14}C]-cyanide followed by hydrolysis.[139] It was converted enzymically into α-ketoglutaric acid labelled only in the γ-carboxyl group.[140]

TABLE 2. *Amino-acids*

Amino-acid and position of label	Reference and method
Glycine ; [*carboxy*-^{14}C]	141 ; *a*
DL-Alanine ; [*carboxy*-^{14}C] ; [α-^{14}C) ; [β-^{14}C]	142 ; *a*
D- and L-Serine ; [β-^{14}C : $\alpha\beta$-$^{2}H_3$: ^{15}N]	143 ; *b*(2)
DL-Aspartic acid ; [4-^{14}C]	144 ; *b*(2)
DL-Aspartic acid ; [3-^{13}C : 4-^{14}C]	110 ; *b*(2)
L-Threonine ; [γ-^{14}C : ^{15}N]	145 ; *b*(1)
L-Threonine ; [α-^{14}C]	146 ; *b*(1)
DL-Valine ; [α-^{14}C]	147 ; *c*
DL-Valine ; [β-^{13}C]	148 ; *d*
L-Valine ; [γ-^{13}C]	149 ; *a*
DL-Ornithine ; [α-^{14}C]	98 ; *b*(1)
D- and L-Glutamic acid ; [1 : 2-$^{14}C_2$]	93, 150 ; *c*
DL-Glutamic acid ; [5-^{14}C]	150 ; *b*(2)
L-Histidine ; [*carboxy*-^{14}C]	151 ; *c*
DL-Leucine ; [*carboxy*-^{14}C] ; [α-^{14}C]	125 ; *a*
L-Leucine ; [*carboxy*-^{14}C]	152 ; *d*
DL-*iso*Leucine ; [*Me*-^{14}C]	153 ; *d*
DL-Lysine ; [α-^{14}C]	98 ; *b*(1)
D- and L-Lysine ; [α-^{14}C]	14 ; *b*(1)
D- and L-Phenylalanine ; [α-^{13}C : *Ph*-$^{14}C_1$]	154 ; *c*
DL-Phenylalanine ; [*carboxy*-^{14}C]	155 ; *d*
DL-Tyrosine ; [*carboxy*-^{14}C]	156 ; *d*
DL-Di-iodotyrosine ; [*carboxy*-^{14}C]	156

Methods: *a*, *via* the α-halogeno-acid; *b*(1), synthesis using labelled acylamino-malonate, -cyanoacetate, or -acetoacetate; *b*(2), ditto, other reactant labelled; *c*, hippuric acid synthesis; *d*, Strecker or hydantoin synthesis; *e*, reductive amination of keto-acid.

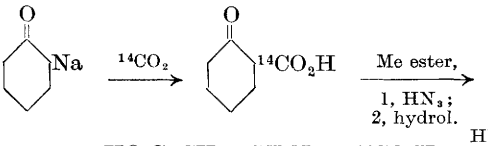
[136] Hughes, Ostwald, and Tolbert, *J. Amer. Chem. Soc.*, 1952, **74**, 2434.
[137] Anderson and Rahman, Brookhaven National Lab. Rep., 103.
[138] Brown and Neville, quoted in "Isotopic Carbon" (ref. 2), p. 214.
[139] Wilcox, Heidelberger, and Potter, *J. Amer. Chem. Soc.*, 1950, **72**, 5019.
[140] Ref. 1, pp. 191—194.

Amino-acids (see Table 2).—[α-^{13}C]Glycine has been prepared by an interesting method : [157]

$$K^{13}CN + CS(NHPh)_2 \xrightarrow{PbCO_3} N^{13}C{\cdot}C(NHPh){:}NPh \xrightarrow{LiAlH_4}$$

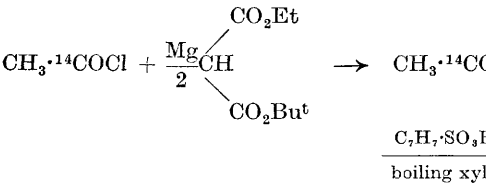
$$NH_2{\cdot}^{13}CH_2{\cdot}C(NHPh){:}NPh \xrightarrow{Hydrol.} NH_2{\cdot}^{13}CH_2{\cdot}CO_2H$$

The valuable method of partial degradation and resynthesis has been used to introduce ^{14}C at $C_{(2)}$ of the glyoxaline ring of L-histidine.[158] Arnstein *et al.*[14] have converted $^{14}CO_2$ into [*carboxy*-^{14}C]lysine in 12% yield :



$$HO_2C{\cdot}[CH_2]_4{\cdot}CH(NH_2){\cdot}^{14}CO_2H \xrightarrow{HN_3} NH_2{\cdot}[CH_2]_4{\cdot}CH(NH_2){\cdot}^{14}CO_2H$$

Keto-acids.—A number of labelled keto-acids has been prepared, usually by the application of standard methods (see Table 3). [β-^{14}C]Acetoacetic ester has been made as follows : [167]



[141] Bloch, *J. Biol. Chem.*, 1949, **179,** 1245.
[142] Ostwald, Adams, and Tolbert, *J. Amer. Chem. Soc.*, 1952, **74,** 2425.
[143] Elwyn and Sprinson, *J. Biol. Chem.*, 1950, **184,** 465.
[144] Wang, Winnick, and Hummel, *J. Amer. Chem. Soc.*, 1951, **73,** 2390.
[145] Meltzer and Sprinson, *J. Biol. Chem.*, 1952, **197,** 461.
[146] Krasna, Peyser, and Sprinson, *ibid.*, 1952, **198,** 421.
[147] Adams and Tolbert, *J. Amer. Chem. Soc.*, 1952, **74,** 6272.
[148] Anatol, *Compt. rend.*, 1950, **230,** 1471.
[149] Fones, Waalkes, and White, *Arch. Biochem.*, 1951, **32,** 89.
[150] Speer, Roberts, Maloney, and Mahler, *J. Amer. Chem. Soc.*, 1952, **74,** 2444.
[151] Borsook, Deasy, Haagen-Smit, Keighley, and Lowy, *J. Biol. Chem.*, 1952, **196,** 669. [152] *Idem, ibid.*, 1950, **184,** 529.
[153] Cerisia, Jenkins, and Degering, *J. Amer. Pharm. Assoc., Sci. Edn.*, 1951, **40,** 341.
[154] Lerner, *J. Biol. Chem.*, 1949, **181,** 281.
[155] Henneberry, Oliver, and Baker, *Canad. J. Chem.*, 1951, **29,** 229.
[156] Loftfield, *J. Amer. Chem. Soc.*, 1950, **72,** 2499.
[157] Ehrensvärd and Stjernholm, *Acta Chem. Scand.*, 1949, **3,** 971.
[158] Borsook, Deasy, Haagen-Smit, Keighley, and Lowy, *J. Biol. Chem.*, 1950. **187,** 839. [159] Anker, *ibid.*, 1948, **176,** 1337.
[160] Thomas, Wang, and Christensen, *J. Amer. Chem. Soc.*, 1951, **73,** 5914.
[161] Curran, *J. Biol. Chem.*, 1951, **191,** 775.
[162] Crandall, Brady, and Gurin, *ibid.*, 1949, **181,** 845.
[163] Dauben, *J. Amer. Chem. Soc.*, 1948, **70,** 1376.
[164] Weinman, Chaikoff, Dauben, Gee, and Entenman, *J. Biol. Chem.*, 1950, **184,** 735.
[165] Weinman, Chaikoff, Stevens, and Dauben, *ibid.*, 1951, **191,** 523.
[166] Heidelberger and Hurlbert, *J. Amer. Chem. Soc.*, 1950, **72,** 4704.
[167] Dauben and Bradlow, *ibid.*, 1952, **74,** 5204.

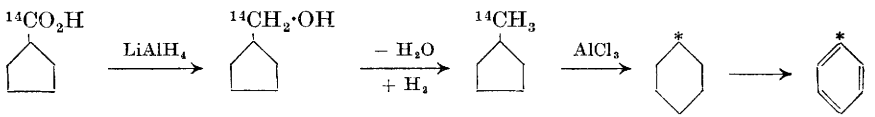
tert.-Butyl esters have also been used in the preparation of oxaloacetic acid, which may be obtained in 90% yield from the *tert.*-butyl ester by the above technique.[166]

TABLE 3. *Keto-acids*

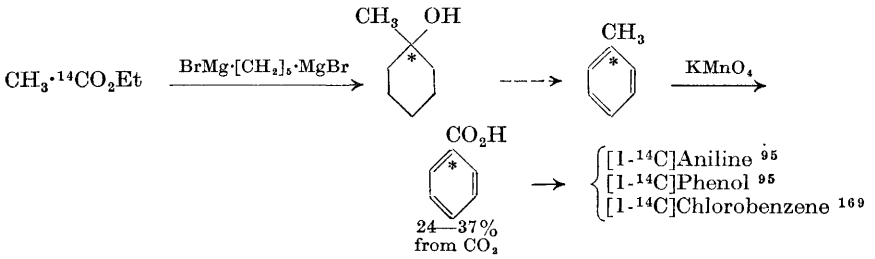
Keto-acid and position of label	Reference and method (see p. 421)
Pyruvic acid : [*carboxy*-^{13}C]	159, (*a*)
Pyruvic acid : [α-^{14}C]	160, (*a*)
Acetoacetic acid : [*carboxy*-^{13}C] ; [β-^{13}C] ; [*carboxy* : β-$^{13}C_2$]	101, (*b*)
Acetoacetic acid : [β-^{14}C]	161, (*c*)
$CH_3 \cdot {}^{14}CH_2 \cdot CO \cdot [CH_2]_4 \cdot CO_2Et$	162, (*d*)
$n\text{-}C_{10}H_{21} \cdot {}^{14}CH_2 \cdot CO \cdot [CH_2]_3 \cdot CO_2H$	163, (*d*)
$n\text{-}C_5H_{11} \cdot {}^{14}CH_2 \cdot CO \cdot [CH_2]_8 \cdot CO_2H$	164, (*d*)
$n\text{-}C_{12}H_{25} \cdot {}^{14}CH_2 \cdot CO \cdot [CH_2]_3 \cdot CO_2H$	165, (*d*)
$^{14}CO_2H \cdot CH_2 \cdot CO \cdot CO_2H$	144, 166, (*b*)
$CO_2Et \cdot {}^{14}CH_2 \cdot CO \cdot CO_2Et$	96, (*b*)
$^{14}CO_2H \cdot {}^{14}CO \cdot [CH_2]_2 \cdot CO_2H$	93, 150, (*b*)

Methods : *a*, *via* acetyl cyanide ; *b*, acetoacetic ester condensation ; *c*, *via* $CH_3 \cdot CO \cdot CH(^{14}CO \cdot CH_3) \cdot CO_2Et$; *d*, cadmium alkyl and $COCl \cdot [CH_2]_n \cdot CO_2R$.

Ring-labelled Aromatic Compounds.—Benzene derivatives have been prepared in which the isotope (*a*) is uniformly distributed within the ring, or (*b*) bears a definite orientation to a substituent. Compounds of the first kind, *e.g.*, [*ar*-$^{14}C_1$]benzaldehyde,[154] may be made from benzene itself, which has been synthesised directly by two methods. In the first of these,[113] [1-^{14}C]*cyclo*hexanone (*q.v.*) is reduced to *cyclo*hexane and dehydrogenated to [$^{14}C_1$]benzene (22% yield from $Ba^{14}CO_3$). The second [122] gives a 75% yield from $^{14}CO_2$:



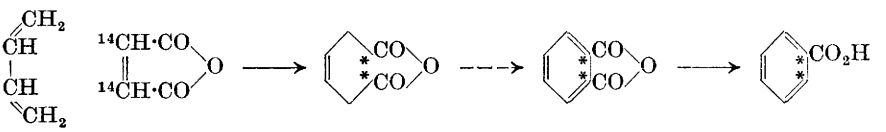
Several useful specifically labelled benzene derivatives have been prepared in rather low yields : [168]



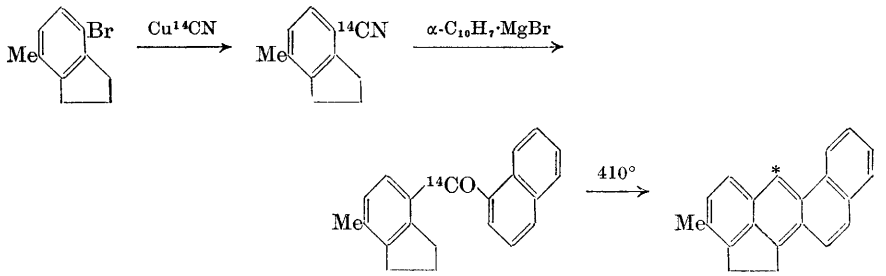
A potentially valuable synthesis of [1 : 2-$^{14}C_2$]benzoic acid *via* [αβ-$^{14}C_2$]-

[168] Fields, Leaffer, Rothchild, and Rohan, *J. Amer. Chem. Soc.*, 1952, **74**, 5498.
[169] Fields, Gibbs, and Walz, *Science*, 1950, **112**, 591.

maleic anhydride [170] has been outlined in a review article : [171]



Most polycyclic compounds have been made from [*carboxy*-^{14}C]-aroic acids by intramolecular acylation (*e.g.*, 2-methyl[4-^{14}C]naphtha-1 : 4-quinone [172] and [9-^{14}C]anthracene [173]), by application of the Wagner rearrangement (*e.g.*, 1 : 2-benz[3 : 4-$^{14}C_1$]anthracene [174] and [5 : 6-$^{14}C_1$]chrysene [175]), or by means of the Elbs reaction (*e.g.*, 20-methyl[11-^{14}C]cholanthrene [176]).



It has also been found possible to prepare naphthalene and α-naphthol containing ^{14}C by irradiation of quinoline oxalate in a nuclear reactor.[65]

Many ring-labelled *heterocyclic compounds* have been synthesised, usually by adaptations of standard methods ; purines and pyrimidines have received most attention. Some of these are listed in Table 4.

Steroids.[187]—Partial syntheses of several biologically important steroids

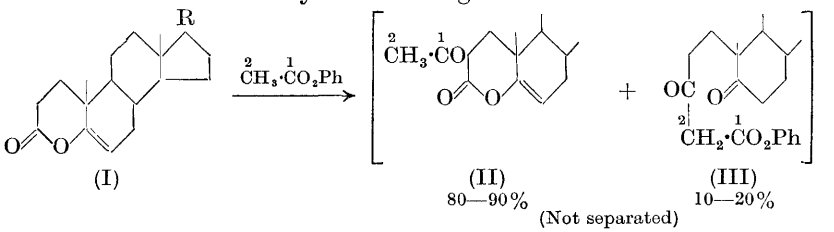
[170] Nystrom, Loo, and Leak, *J. Amer. Chem. Soc.*, 1952, **74**, 3434.
[171] Nystrom, Loo, Mann, and Allen, quoted in ref. 4.
[172] Liang-Li and Elliott, *J. Amer. Chem. Soc.*, 1952, **74**, 4089.
[173] Stevens and Holland, *Science*, 1950, **112**, 718.
[174] Collins, Burr, and Hess, *J. Amer. Chem. Soc.*, 1951, **73**, 5176.
[175] Toffel, Jones, and Collins, *ibid.*, 1953, **75**, 397.
[176] Martin and Baker, U.S. At. Energy Comm., File No. NP-3177.
[177] Bennett, *J. Amer. Chem. Soc.*, 1952, **74**, 2432.
[178] Mandel and Brown, *ibid.*, p. 2439.
[179] Abrams and Clark, *ibid.*, 1951, **73**, 4609.
[180] Weygand and Grossinsky, *Chem. Ber.*, 1951, **84**, 839.
[181] Bennett, Skipper, Mitchell, and Sugiura, *Cancer Res.*, 1950, **10**, 644.
[182] Bentley and Neuberger, *Biochem. J.*, 1952, **52**, 694.
[183] Miller, Gurin, and Wilson, *J. Amer. Chem. Soc.*, 1952, **74**, 2892.
[184] Anker and Boehne, *ibid.*, p. 2431.
[185] Weygand, Mann, and Simon, *Chem. Ber.*, 1952, **85**, 463.
[186] Williams and Ronzio, *J. Amer. Chem. Soc.*, 1952, **74**, 2409.
[187] Twombly, *Vitamins and Hormones*, 1951, **9**, 237.
[188] Turner, *J. Amer. Chem. Soc.*, 1950, **72**, 579.
[189] Fujimoto, *ibid.*, 1951, **73**, 1856 ; Heard and Ziegler, *ibid.*, p. 4036.

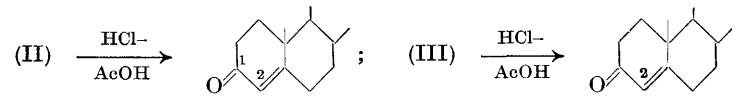
TABLE 4. *Heterocyclic compounds*

Compound	Ref.; yield from $BaCO_3$ (see p. 423)
[2-^{14}C]Uracil	177 (32—40%); 178 (60%[a])
[2-^{14}C]Thymine	177 (20—28%)
[6-^{14}C]Orotic acid	166 (38%)
[4 : 6-$^{14}C_1$]Adenine	97 (17%)
[8-^{14}C]Adenine	179 (55%[b])
8-Aza[4 : 6-$^{14}C_1$]adenine	97 (17%)
[2-^{14}C]Guanine	177 (40—50%)
[4-^{14}C]Guanine	97 (38%)
[8-^{14}C]Guanine	180 (70—75%)
8-Aza[2-^{14}C]guanine	181 (8%)
8-Aza[4-^{14}C]guanine	97 (43%)
2 : 6-Diamino[2-^{14}C]purine	177 (15—20%)
[6-^{14}C]Uric acid	182
4-Hydroxy[4 : *carboxy*-$^{14}C_1$]glyoxaline-5-carboxyamide	183
[6 : 7-$^{14}C_2$]Xanthopterin	184 (5%)
[2-^{14}C]Folic acid	185 (1·8%)
[9-^{14}C]Folic acid	82 (3·65%[c])
[^{14}C]Thiamine (vitamin B_1)	186

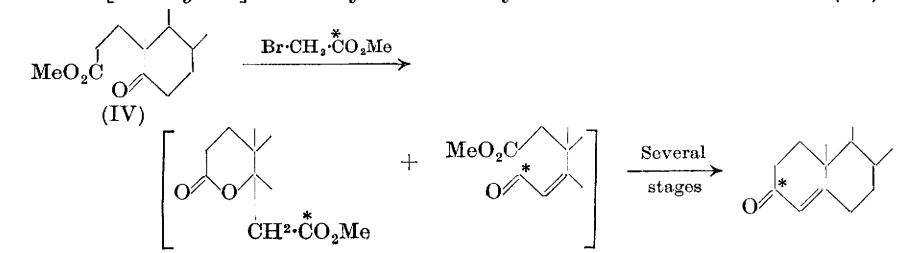
[a] From [^{14}C]urea. [b] From sodium [^{14}C]formate. [c] From [^{14}C]methanol.

have been devised. One synthesis of ring A-labelled steroids is as follows :[188]





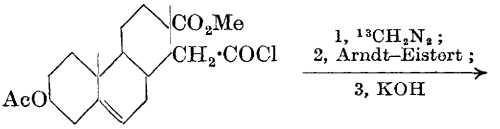
[3-^{14}C]- and [4-^{14}C]-Cholest-4-en-3-one (R = ·CHMe·$[CH_2]_3$·$CHMe_2$) and testosterone (R = OH) have been made in this way. Better yields are obtained by reaction of (I) with [^{14}C]methylmagnesium iodide, followed by cyclisation.[189] Alternatively, the isotope may be introduced in methyl bromo[*carboxy*-^{14}C]acetate by Reformatsky reaction with the keto-ester (IV) :

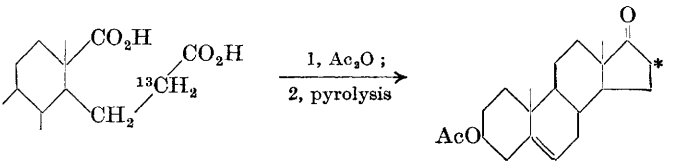


Cholestenone,[188] progesterone, and deoxycorticosterone acetate [190] have been prepared by this method.

Ring-labelled cholesterol may be made by reduction of cholestenone with sodium borohydride.[191]

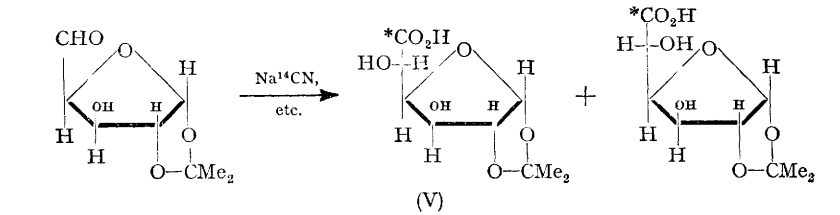
Herschberg *et al.*[192] have labelled dehydro*iso*androsterone acetate in ring D by the following procedure :

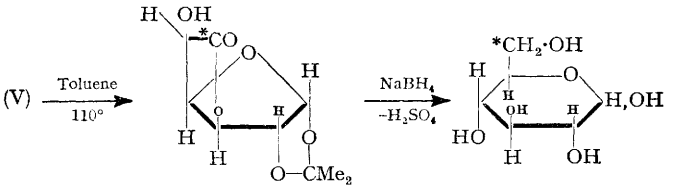




Steroids labelled in the side chain so far prepared include [26-^{14}C]-cholesterol [193] and [21-^{14}C]progesterone.[194]

Carbohydrates.—Carbohydrates labelled in the 1-position have been made by the Fischer–Kiliani method (*e.g.*, D-galactose,[195] D-glucose, and D-mannose [196]) and also by the nitromethane method of Sowden and Fischer [197] (*e.g.*, L-arabinose and L-ribose,[198] D-glucose and D-mannose [199]). Sowden [200] has prepared D-[6-^{14}C]glucose by the following method :





[190] Fujimoto, *J. Amer. Chem. Soc.*, 1950, **72**, 4328.
[191] *E.g.*, Dauben and Eastham, *ibid.*, 1951, **73**, 4463.
[192] Herschberg, Schwenk, and Stahl, *Arch. Biochem.*, 1948, **19**, 300.
[193] Ryer, Gebert, and Murill, *J. Amer. Chem. Soc.*, 1950, **72**, 4247.
[194] Riegel and Prout, *J. Org. Chem.*, 1948, **13**, 933.
[195] Topper and Stetten, *J. Biol. Chem.*, 1951, **193**, 149.
[196] Isbell, Karabinos, Frush, Holt, and Schwebel, *J. Res. Nat. Bur. Stand.*, 1952, **48**, 163.
[197] Sowden and Fischer, *J. Amer. Chem. Soc.*, 1947, **69**, 1963.
[198] Rappoport and Hassid, *ibid.*, 1951, **73**, 5524.
[199] Sowden, *J. Biol. Chem.*, 1949, **180**, 55.
[200] *Idem*, *J. Amer. Chem. Soc.*, 1952, **74**, 4377.

Both D- and L-[1-^{14}C]ascorbic acid have been synthesised by the osone method.[201]

Biological Syntheses.—Biosynthetic methods are particularly well suited to the preparation of natural products, many of which it is difficult or impossible to synthesise by chemical means. Wholly specific labelling is not easily achieved, but for many purposes is unnecessary. The syntheses frequently start from very simple intermediates, and optically active compounds are obtained in the natural configuration. Dilution of isotope is usually greater than in chemical syntheses, but may in favourable cases be kept small, or substantially avoided. Yields vary widely, *e.g.*, from up to 70% in the photosynthetic preparation of sucrose [202] to an estimated yield of less than 0·005% [203] in the preparation of digitoxin in *Digitalis purpurea*.[204]

Microbiological Methods.—These have been used extensively and frequently give high yields. Autotrophic bacteria grown on $^{14}CO_2$ invariably produce uniformly labelled compounds, but there are many partial syntheses brought about by micro-organisms in which control of labelling may be exercised.

The autotrophic bacterium *Thiobacillus thiooxidans* has been employed [205] in the production of bacterial protein and derived amino-acids from $^{14}CO_2$. About 15% of the ^{14}C was recovered as separated uniformly-labelled amino-acids; dilution was very slight. Better radiochemical yields have been obtained [206] by using *Rhodospirillum rubrum*, which assimilates equimolar quantities of carbonate and ethanol. The three-fold dilution of activity is relatively unimportant, but the non-uniform labelling to be expected is a more serious disadvantage.

Some syntheses by which specifically labelled compounds can be made are listed in Table 5.

Among more complex compounds prepared microbiologically may be mentioned streptomycin,[213] which has been isolated from cultures of *S. griseus* grown on uniformly labelled glucose.

Photosynthetic Methods.—Photosynthesis in whole plants is extremely inefficient for preparative purposes; nevertheless a number of drugs and complex natural products have of necessity been prepared in this way,

[201] Hamilton and Smith, *J. Amer. Chem. Soc.*, 1952, **74**, 5162; Burns and King, *Science*, 1950, **111**, 257.

[202] Scully, Stavely, Skok, Stanley, Dale, Craig, Hodge, Chorney, Watanabe, and Baldwin, *ibid.*, 1952, **116**, 87.

[203] Ref. 6, p. 272.

[204] Geiling, Kelsey, McIntosh, and Ganz, *Science*, 1948, **108**, 558.

[205] Frantz, Feigelman, Werner, and Smythe, *J. Biol. Chem.*, 1952, **195**, 423.

[206] Tarver, Tabachnik, Canellakis, Fraser, and Barker, *Arch. Biochem.*, 1952, **41**, 1.

[207] San Pietro, *J. Biol. Chem.*, 1952, **198**, 639.

[208] Ref. 2, p. 274.

[209] Ref. 2, p. 276.

[210] Isbell and Karabinos, *J. Res. Nat. Bur. Stand.*, 1952, **48**, 438.

[211] Foster, Carson, Anthony, Davis, Jefferson, and Long, *Proc. Nat. Acad. Sci.*, 1949, **35**, 663.

[212] Ajl and Kamen, *J. Amer. Chem. Soc.*, 1951, **73**, 2349.

[213] Karow, Peck, Rosenblum, and Woodbury, *ibid.*, 1952, **74**, 3056.

TABLE 5. *Microbiological syntheses*

Compound	Precursor and organism	Yield ; dilution	Ref.
[$\alpha\beta$-$^{14}C_1$: ^{15}N]Aspartic acid	[$\alpha\beta$-$^{14}C_1$]Fumaric acid ; *Escherichia coli*	47% ; small	207
n-[$\alpha\gamma$-$^{14}C_2$]Butyric acid (etc.)	[*Me*-^{14}C]Acetic acid (etc.) ; *B. rettgeri*	60—70% ; small	208
n-[3-^{14}C]Hexanoic acid (etc.)	*n*-[*carboxy*-^{14}C]Butyric acid (etc.) ; *Cl. Kluyveri*	80% ; ~25%	209
D-[1 : 6-$^{14}C_1$]Fructose	D-[1-^{14}C]Mannitol ; *Acetobacter suboxydans*	54% ; small	210
[*carboxy*-$^{14}C_2$]Fumaric acid	[1-^{14}C]Ethanol ; *Rhizopus nigricans*	40—60% ; ~40%	211
[$\alpha\beta$-$^{14}C_2$]Succinic acid	[*Me*-^{14}C]Acetic acid ; *Escherichia coli*	37% ; ~10×	212

including colchicine,[214] digitoxin,[204] morphine,[215] nicotine,[216] and pyrethrins.[217] Further, tobacco mosaic virus has been labelled [218] with ^{14}C by growing infected plants in $^{14}CO_2$. Photosynthesis in detached leaves, however, affords an efficient means for the preparation of some important carbohydrates. Using the leaves of *Canna indica*, Putman and Hassid [17] have obtained very pure glucose, fructose, and sucrose of high specific activity in an aggregate yield of $\sim 70\%$. Bean leaves appear to be the most suitable for the biosynthesis of starch ; [219] dilution is negligible and yields of 18—36% of purified starch have been obtained.[219, 220]

Animal Biosyntheses.—In favourable circumstances relatively high yields of particular substances may be obtained after the assimilation of suitably labelled precursors. Karlsson and Barker [221] have obtained recoveries of ^{14}C in uric acid excreted by pigeons injected with formate, [α-^{14}C]glycine and [*carboxy*-^{14}C]glycine of 40, 61, and 17% respectively. In the first case $> 98\%$ of the ^{14}C was found in $C_{(2)} + C_{(8)}$ of the uric acid, and in the last 87% was in $C_{(4)}$. Similarly, after feeding of [8-^{14}C : 1 : 3-$^{15}N_1$]adenine to rats, some 35% of isotope fed was isolated from the tissues in six separated ribonucleotides ; adenylic acids *a* and *b* together accounted for 70% of the recovered isotope.[222] In most cases, however, the bulk of the isotope is distributed generally in the animal, largely diluted with normal carbon. Thus feeding with glucose or lactate and simultaneously injecting [^{14}C]-bicarbonate into fasted rats has the result that the glycogen laid down in the liver may contain up to $\sim 2{\cdot}5\%$ of the ^{14}C administered ; the glucose

[214] Walaszek, Kelsey, and Geiling, *Science*, 1952, **116**, 225.
[215] McIntosh, Kelsey, and Geiling, *J. Amer. Pharm. Assoc., Sci. Edn.*, 1950, **39**, 512.
[216] Ganz, Kelsey, and Geiling, *Bot. Gaz.*, 1951, **113**, 195.
[217] Pellegrini, Miller, and Sharpless, *J. Econ. Entomol.*, 1952, **45**, 532.
[218] Schonfellinger and Broda, *Monatsh.*, 1952, **83**, 837.
[219] Livingston and Medes, *J. Gen. Physiol.*, 1947, **31**, 75.
[220] Gibbs, Dumrose, and Acher, U.S. At. Energy Comm. Rep., AECU-283.
[221] *J. Biol. Chem.*, 1949, **177**, 597.
[222] Marrian, Spicer, Balis, and Brown, *ibid.*, 1951, **189**, 533.

obtained by hydrolysis has ~ 97% of its ^{14}C content in $C_{(3)}$ and $C_{(4)}$.[223] Glutathione has been prepared from the liver of a rabbit previously injected with [^{14}C]bicarbonate.[224] The yield was about 0·1% and it was shown by enzymic degradation that ~ 53% of the activity was in the carboxyl group of the glutamic acid residue. Other substances synthesised in animals for biological studies include glucuronic acid,[225] plasma phospholipids,[226] squalene,[227] and bufagin.[228]

Syntheses in isolated animal tissues appear to have many advantages over the use of whole animals, but have been comparatively little used. Anfinson has prepared crystalline [^{14}C]ovalbumin [229] and [^{14}C]ribonuclease [230] by incubation of hen oviduct minces and bovine pancreas slices respectively with $^{14}CO_2$, and labelled hæmin has been prepared by incubation of duck blood with precursors such as acetate and glycine.[231] Brady and Gurin [232] have shown that labelled cholesterol may be obtained in up to 17% yield from [*Me*-^{14}C]acetate in rat-liver slices.

Enzymic Syntheses.—These have had a rather limited application in spite of their great specificity and efficiency. By the use of sucrose phosphorylase, sucrose labelled in the fructose or glucose residue has been prepared.[233] A similar procedure using maltose phosphorylase, which catalyses the reaction :

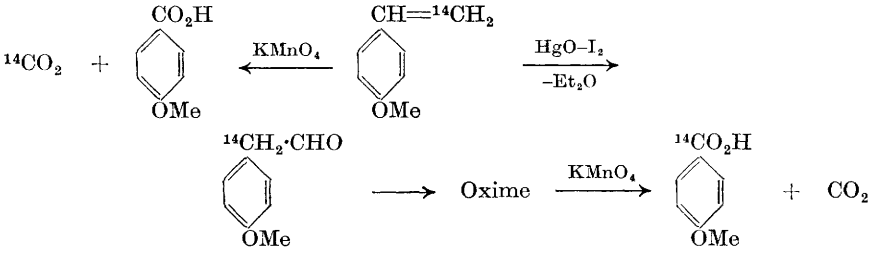
Maltose + Inorganic phosphate $\rightleftharpoons$ β-D-Glucose-1 phosphate + D-Glucose

has been used for preparation of maltose labelled in either the reducing or the non-reducing glucose residue.[234] The glucose-1 phosphate was itself prepared from labelled starch by use of potato phosphorylase.[233] Other compounds prepared enzymically include L-[*carboxy*-^{14}C]malic acid [235] and oxaloacetic acid.[236] Enzymic methods for the resolution of racemic amino-acids [237] are well suited for use with isotopically labelled compounds.[238]

Degradation of Labelled Compounds.—It is often necessary to locate the isotope in an organic compound, and degradation plays an important part in many biological and chemical tracer studies. The degradation must be designed so as to permit the isolation of specific fragments of the molecule in a form suitable for isotopic analysis. Most chemical syntheses lead to unambiguously labelled compounds, but in some cases unexpected molecular

[223] Shreeve, Feil, Lorber, and Wood, *J. Biol. Chem*, 1949, **177**, 679.
[224] Krimsky and Racker, *ibid.*, 1952, **198**, 721.
[225] Packham and Butler, *ibid.*, 1952, **194**, 349.
[226] Weinmann, Chaikoff, Entenman, and Dauben, *ibid.*, 1950, **187**, 643.
[227] Langdon and Bloch, *J. Amer. Chem. Soc.*, 1952, **74**, 1869.
[228] Doull, Dubois, and Geiling, *Arch. int. Pharmacodyn.*, 1951, **86**, 454.
[229] Anfinson and Steinberg, *J. Biol. Chem.*, 1951, **189**, 739.
[230] Anfinson, *ibid.*, 1950, **185**, 827.
[231] *E.g.*, Shemin and Wittenberg, *ibid.*, 1951, **192**, 315.
[232] Brady and Gurin, *ibid.*, 1951, **189**, 371.
[233] Wolochow, Putman, Doudoroff, Hassid, and Barker, *ibid.*, 1949, **180**, 1237.
[234] Fitting and Putman, *ibid.*, 1952, **199**, 573.
[235] Kaufman, Korkes, and del Campillo, *ibid.*, 1951, **192**, 301.
[236] Lorber, Utter, Rudney, and Cook, *ibid.*, 1950, **185**, 689.
[237] See, *e.g.*, Birnbaum, Levintow, Kingsley, and Greenstein, *ibid.*, 1952, **194**, 455.
[238] *E.g.*, Hassan and Greenberg, *Arch. Biochem.*, 1952, **39**, 129.

rearrangements occur, and these are revealed only on degradation. In biological syntheses degradation of the product is essential, except for those applications (*e.g.*, isotopic dilution analysis) where the molecule as a whole is to be traced. The methods used must themselves be proved on unequivocally labelled reference compounds, or confirmed by alternative methods. Leete *et al.*[239] converted biosynthetic *N*-methyltyramine by methylation and Hofmann degradation into *p*-methoxystyrene, which was then converted into *p*-anisic acid :



Direct oxidation revealed a molecular rearrangement which vitiated the original procedure.

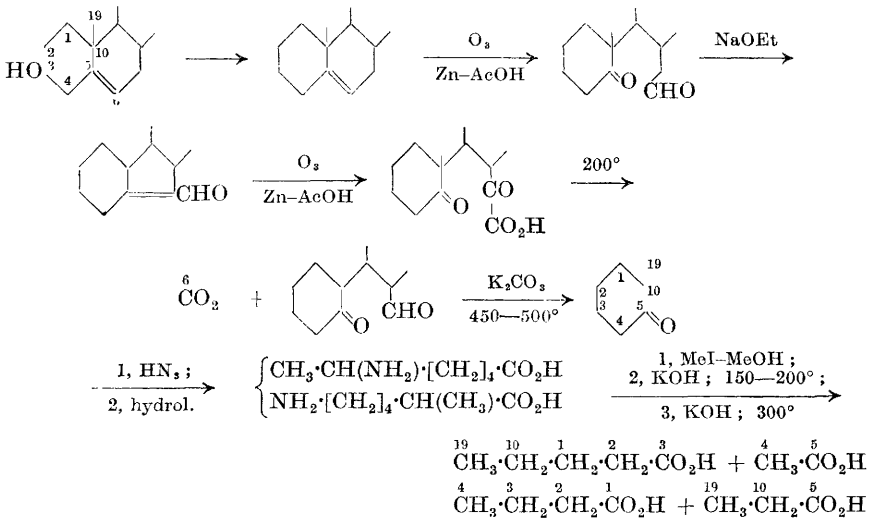
Differences in reaction rates caused by isotopic substitution (the isotope effect [11]) may interfere with degradation procedures. Two types of reactions may be distinguished. In those where the relevant labelled atom can be present at the end of the reaction only in a single compound, an isotope effect can in any case only be detected during the reaction, and is avoided by carrying the reaction to completion. On the other hand, when the labelled atom is distributed between two or more compounds, an isotope effect, if it occurs, cannot be avoided in this way, but appropriate corrections can be made. Reactions of the first type include decarboxylations, combustions to carbon dioxide (*e.g.*, acetic acid, urea, xanthhydrol ureide), and the absorption of carbon dioxide by alkali. Incomplete combustions will also give false results for more obvious reasons. The second type is exemplified by the iodoform reaction on [1-^{14}C]acetone. Intermediates in degradation procedures are commonly isolated and purified as derivatives. Isotope effects have been observed in the reaction of benzophenone with 2 : 4-dinitrophenylhydrazone,[240] and of formaldehyde with dimedone,[241] both reactions falling into the first category.

Some of the most common end products of degradation procedures are difficult to purify on the very small scale. Iodoform is a very important example, and here a specific oxidation procedure has been devised.[242]

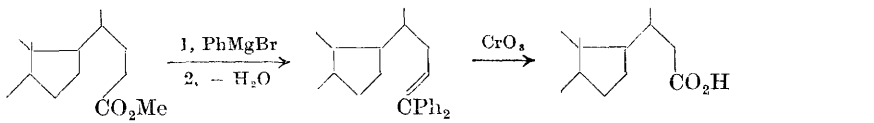
As an example of the scope of degradative methods, two recent partial degradations of biosynthetic cholesterol may be mentioned. Cornforth, Hunter, and Popják [243] have isolated all the carbon atoms of ring A,

[239] Leete, Kirkwood, and Marion, *Canad. J. Chem.*, 1952, **30**, 749.
[240] Brown and Holland, *ibid.*, p. 438.
[241] Downes, *Austral. J. Sci. Res.*, 1952, **5**, *A*, 521.
[242] Shreeve, Leaver, and Siegel, *J. Amer. Chem. Soc.*, 1952, **74**, 2404.
[243] Cornforth, Hunter, and Popják, *Biochem. J.*, 1953, **54**, 590.

$C_{(6)}$ and $C_{(19)}$:



The four acids were separated by partition chromatography and further degraded.[244] Wüersch, Huang, and Bloch [245] converted cholesterol into dihydrocholesteryl acetate, which was oxidised to 3β-hydroxy*allo*cholanic acid and acetone (derived from $C_{(25)}$, $C_{(26)}$, and $C_{(27)}$). Repeated application of the Barbier–Wieland degradation procedure to the acid permitted the isolation of all the carbon atoms of the side chain.



Nitrogen

There is only one isotope of nitrogen which is suitable for tracer studies. This is the stable ^{15}N, which is readily available as nitrate (and hence nitrite) or as phthalimide of about 60 atoms % excess of ^{15}N. It is usually prepared [62, 246] from ammonia enriched from the natural abundance of 0·38% in some suitable fractionating device :

$$^{15}NH_{3(gas)} + {}^{14}NH_{4(solution)}^{+} \rightleftharpoons {}^{14}NH_{3(gas)} + {}^{15}NH_{4(solution)}^{+}$$

Stimulated by the biological importance of compounds such as proteins, amino-acids, nucleic acids, etc., the essential synthetic chemistry of the isotope was largely established several years ago and was reported by

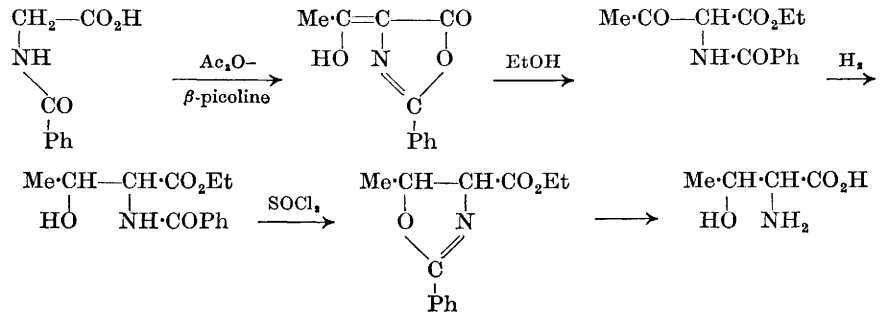
[244] Hunter and Popják, *Biochem. J.*, 1951, **50**, 163.
[245] Wüersch, Huang, and Bloch, *J. Biol. Chem.*, 1952, **195**, 439.
[246] Wilson, " Preparation and Measurement of Isotopic Tracers ", Edwards, Ann Arbor, Mich., 1946.

Arnstein and Bentley [1] and others.[7] The following notes supplement these surveys.

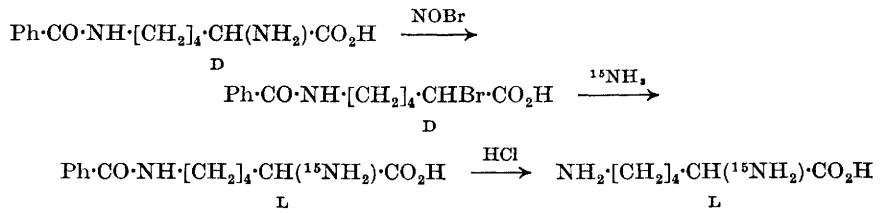
Intermediates.—Clusius,[247] by operating a thermal diffusion column with atomic nitrogen produced by an electric discharge, has obtained 99·8% $^{15}N_2$ and has converted this gas into nitrous and nitric acids. Hydroxylamine has been obtained [248] by electrolytic reduction of nitric acid.

The synthesis of urea from ammonia and diphenyl carbonate has given rise to explosions, and alternative procedures to avoid this phenomenon have been developed.[249]

Amino-acids.—β-Alanine [250] and anthranilic acid [251] (and hence tryptophan) have been prepared from labelled phthalimide, the former by condensation with acrylic acid, the latter by the Gabriel method which has also been utilised for the synthesis of [α-^{15}N]- and [δ-^{15}N]-ornithine in both L- and DL-forms.[252] Condensation of ornithine with labelled urea yielded citrulline, degradation establishing that ^{15}N was present only in the terminal group. Threonine and *allo*threonine have been prepared,[145, 253] both by direct amination of the corresponding α-bromo-β-methoxybutyric acid and after the following reactions :



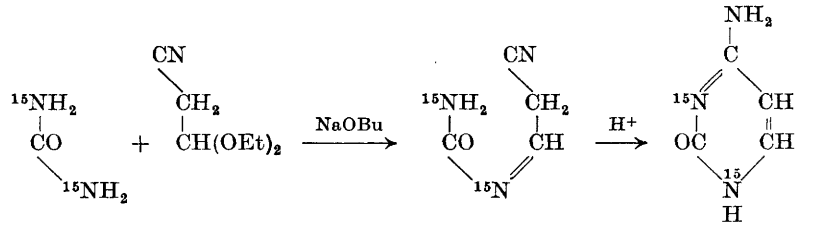
A synthesis of considerable potential value [14] has been applied to the preparation of L-[α-^{15}N]lysine.



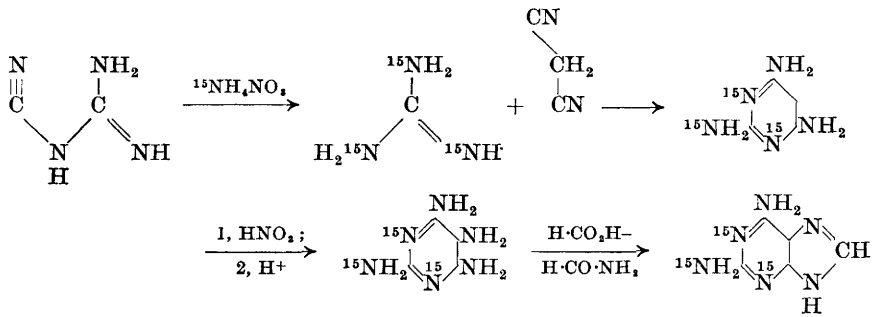
[247] Clusius, *Helv. Chim. Acta*, 1950, **33**, 2122, 2134 ; 1952, **35**, 1103.
[248] Farago and Roberson, Abs. Amer. Chem. Soc. 122nd Mtg., 1952, 41M.
[249] Buzard and Bishop, *J. Amer. Chem. Soc.*, 1952, **74**, 2925 ; Williams and Ronzio, *ibid.*, p. 2407.
[250] Graff and Hobermann, *J. Biol. Chem.*, 1950, **186**, 369.
[251] Partridge, Bonner, and Yanofsky, *ibid.*, 1952, **194**, 269.
[252] Stetten. *ibid.*, 1951, **189**, 499 ; Hirs and Rittenberg, *ibid.*, 1950, **186**, 429.
[253] Shulgin, Lien, Gal, and Greenberg, *J. Amer. Chem. Soc.*, 1952, **74**, 2427

DL-[^{15}N]Valine [254] and L-[^{15}N]glutamic acid [255] have been synthesised by the Knoop technique of reductive amination and there have been biosyntheses of aspartic acid [256] (from [2 : 3-$^{14}C_2$]fumaric acid and ammonia by *Escherichia coli*), glutamine [257] (from red beets), and tryptophan [251] (from a yeast supplied with [^{15}N]anthranilic acid), and a whole range of labelled amino-acids has been separated on the large scale by ion-exchange chromatography.[258]

Purines, Pyrimidines, etc.—The majority of recent synthetic work in this field has been concerned with ^{14}C, but improved syntheses of cytosine [259] and 2[^{15}N] : 4-diamino[1 : 3-$^{15}N_2$]pyrimidine [260] avoid the formation of undesirable by-products by utilising an alkoxide-catalysed condensation of isotopic urea with cyanoacetaldehyde diethyl acetal :



A synthesis of [1 : 3-$^{15}N_2$]uric acid [261] *via* 5-nitrobarbituric acid has been carried out and the oxidative degradation of this and other compounds of the group has been studied.[262] 2[^{15}N] : 6-Diamino[1 : 3-$^{15}N_2$]purine [263] has been prepared from labelled guanidine in excellent yield :



[254] Behrens *et al.*, *J. Biol. Chem.*, 1948, **175**, 765.
[255] Barker, Hughes, and Young, *J.*, 1951, 3047.
[256] Wu and Rittenberg, *J. Biol. Chem.*, 1949, **179**, 847.
[257] Hood, Lyman, and Tatum, *Arch. Biochem.*, 1951, **30**, 351.
[258] Åqvist, *Acta Chem. Scand.*, 1951, **5**, 1031.
[259] Bendich, Gettler, and Brown, *J. Biol. Chem.*, 1949, **177**, 565.
[260] Bendich, Geren, and Brown, *ibid.*, 1950, **185**, 435.
[261] Benedict, Forsham, and Stetten, *ibid.*, 1949, **181**, 183.
[262] Cavalieri and Brown, *J. Amer. Chem. Soc.*, 1948, **70**, 1242 ; Cavalieri, Tinker, and Brown, *ibid.*, 1949, **71**, 3973.
[263] Bendich, Furst, and Brown, *J. Biol. Chem.*, 1950, **185**, 423.

[1 : 3-$^{15}N_2$]Hypoxanthine [264] has been synthesised from thiourea by standard methods and by deamination of adenine. Deamination of guanine similarly yielded [1 : 3-$^{15}N_2$]xanthine.

Biosynthesis [265] of several members of this group of compounds has been carried out with bacteria,[266] yeasts,[267] and tissue,[222] and with the intact animal.[268]

Miscellaneous.—Studies of reaction mechanisms involving nitrogen have necessitated syntheses of several aromatic derivatives including phenyl-[β-^{15}N]hydrazine,[269] phenyl azide,[270] 3 : 5-dinitrobenzazide,[272] diazoaminobenzene,[271] and all three singly labelled *p*-dimethylaminoazobenzenes.[273]

Nembutal [274] and the carcinogen, 2-[^{15}N]acetamidofluorene [275] have been prepared.

Biosynthesis of several porphyrins following assimilation of singly and doubly labelled glycine have been carried out [276] and followed by elegant degradations to show the origin of the various atoms. Prodigiosin,[277] a tripyrrylmethane pigment of bacterial origin, has been similarly studied. Other biosyntheses include those of stercobilin [278] and nicotinamide [279] (from indole by a selected strain of *Neurospora*).

Oxygen

Since the radioactive isotopes of oxygen (^{14}O, ^{15}O, ^{19}O) have very short half-lives they are unsuited to tracer work and it has been necessary to utilise the stable ^{18}O and ^{17}O which occur in atmospheric oxygen to the extent of 0·20 and 0·04% respectively.[280] Arnstein and Bentley [1] discuss the problem of the mass-spectrometric assay of ^{18}O and refer to the latter author's review of the subject.[281] More recently Dole has also contributed a comprehensive review [282] which contains an extensive bibliography.

It is not practicable to separate ^{18}O by electrolysis of water, but processes of (1) fractional distillation of water, methanol,[283] or carbon monoxide,[61] (2) thermal diffusion, or (3) chemical exchange [284] have been

[264] Gettler, Roll, Tinker, and Brown, *J. Biol. Chem.*, 1949, **178,** 259.
[265] Ref. 46; Wilson, *op. cit.*, p. 152; Brown, *op. cit.*, p. 164.
[266] Reichart and Estborn, *J. Biol. Chem.*, 1952, **188,** 839.
[267] Di Carlo, Schultz, Roll, and Brown, *ibid.*, 1949, **180,** 329, 333.
[268] Bendich, Brown, Phillips, and Thiersch, *ibid.*, 1950, **183,** 267.
[269] Ref. 247, p. 2122.
[270] Clusius and Weisser, *Helv. Chim. Acta*, 1952, **35,** 1548. [271] *Idem, ibid.*, p. 1524.
[272] Bothner-By and Friedman, *J. Amer. Chem. Soc.*, 1951, **73,** 5391.
[273] Fones and White, *Arch. Biochem.*, 1949, **20,** 118.
[274] Van Dyke, Scudi, and Tabern, *J. Pharmacol.*, 1947, **90,** 364.
[275] Argus and Ray, *Cancer Res.*, 1951, **11,** 423.
[276] Ref. 46; Shemin and Wittenberg, *op. cit.*, p. 41.
[277] Hubbard and Rimington, *Biochem. J.*, 1951, **46,** 220.
[278] London, *J. Biol. Chem.*, 1950, **184,** 373.
[279] Bonner and Wasserman, *ibid.*, 1950, **185,** 69.
[280] Nier, *Phys. Review*, 1950, **77,** 789.
[281] Bentley, *Nucleonics*, 1948, **2,** (2), 18; cf. ref. 3.
[282] Dole, *Chem. Reviews*, 1952, **51,** 263.
[283] Dostrovsky, Hughes, and Llewellyn, *Bull. Res. Counc. Israel*, 1951, **1,** 133.
[284] Boyd and White, *Ind. Eng. Chem.*, 1952, **44,** 2202.

successfully employed. Clusius, by operating a series of six 14-m. diffusion units for many months, prepared 250 ml. of 99% $^{18}O_2$, but the isotope is usually supplied as $H_2{}^{18}O$ of much lower enrichment, from which oxygen may be generated [285] as required. It can be assayed [1, 281, 282] in carbon dioxide or oxygen by the mass spectrometer or by precise density determinations on derived water. The main field of application [281, 282] has been to the study of reaction mechanisms (*e.g.*, ester [286] and lactone [287] hydrolysis), but it is also useful for the assay of oxygen in organic compounds,[288] particularly in those substances such as fluoro-compounds which do not normally yield accurate results by established methods ; other applications have been to geochemistry [289] (*e.g.*, for the determination of paleo-temperatures), and to metabolic [290] and photosynthetic studies, although in a recent paper [291] the validity of certain fundamental premises in this work has been called in question. It is also of interest that the ^{18}O content of atmospheric oxygen is higher than would be expected if it were in equilibrium with the oxygen of natural waters.

Where possible, the isotope is incorporated into organic compounds by a process of exchange with $H_2{}^{18}O$. Tabulated data which have been presented by Bentley and by Dole indicate that carbonyl compounds generally show a ready exchange, particularly when this is catalysed by acid or alkali. The carboxylic acids too may undergo exchange although it is suppressed by the presence of alkali when the acids are in anionic form.

Labelled alcohols cannot be prepared by such methods unless powerful labilising groups are present in the molecule, and it is therefore necessary to employ the conventional methods of halide hydrolysis. Similarly, phenols must be prepared by fusion of the corresponding sulphonic acid with $Na^{18}OH$.

The oxygen of amides, peptides, urea, etc., is quite inert towards $H_2{}^{18}O$ as are those of many inorganic ions of biological significance such as phosphate, sulphate, and nitrate. These ions may be labelled by interaction of heavy water with the appropriate anhydride.

In his review, Dole lists a number of organic reactions which have been studied by ^{18}O tracer techniques and offer routes for the synthesis of labelled esters, amides, ethers, etc. More recently Bender [292] has prepared carbonyl-labelled esters *via* the corresponding imidates.

Phosphorus

The biological importance of phosphorus, coupled with a ready availability of the radioactive isotope, ^{32}P, stimulated a variety of early tracer

[285] Bentley, *Biochem. J.*, 1950, **45**, 591.
[286] Bunton, Comyns, and Wood, *Research*, 1951, **4**, 383.
[287] Long and Friedman, *J. Amer. Chem. Soc.*, 1950, **72**, 3692.
[288] *E.g.*, Kirschenbaum, Strong, and Grosse, *Analyt. Chem.*, 1952, **24**, 1361.
[289] Urey, *Science*, 1948, **108**, 489 ; Silverman, *Geochim. Cosmochim. Acta*, 1951, **2**, 26.
[290] Cohn, *J. Biol. Chem.*, 1949, **180**, 771.
[291] MacKenzie and Milner, *J. S. Afr. Chem. Inst.* 1952, **4**, (1), 79.
[292] Bender, *J. Amer. Chem. Soc.*, 1951, **73**, 1626.

applications which have been the subject of several excellent reviews.[293] A formidable number of labelled compounds has been isolated during the course of this work, but there have been relatively few deliberate syntheses.

^{32}P ($\tau_{\frac{1}{2}} = 14{\cdot}3$ days) is the longest-lived of the radioactive isotopes and decays with emission of a β-particle of maximum energy 1·7 mev. First produced by the $^{31}P(n,\gamma)^{32}P$ reaction when ordinary phosphorus was bombarded by slow neutrons, it can also be made more satisfactorily and at higher specific activity by a variety of other reactions using a radium–beryllium neutron source, a cyclotron, or an atomic pile, *viz.* : $^{31}P(d, p)^{32}P$; $^{34}S(d,\alpha)^{32}P$; $^{32}S(n,p)^{32}P$; $^{35}Cl(n,\alpha)^{32}P$. Methods for the extraction [294] of ^{32}P as phosphate at very high specific activity from pile-irradiated sulphur have been described : it is essential to ensure that the material is at a uniform oxidation level before use.[295] The energetic nature of the radiation may necessitate some care in manipulating appreciable quantities but it also simplifies analytical techniques for which either solid or liquid counting is suitable.[296]

The phosphate ion does not normally undergo exchange [297] with the phosphorus of organic compounds or with the other oxy-acids of phosphorus, and such compounds must therefore be synthesised by chemical or biological methods. Preparations of various useful intermediates, including the phosphorus oxy-acids and poly-acids,[298] phosphorus halides,[299] and phosphorus oxychloride have been reported. This last-named compound is especially valuable and may conveniently be made by Axelrod's modification of Lindberg's method in which phosphorus pentachloride is heated with radioactive phosphate.[300] The inevitable dilution of activity entailed in this method may be avoided by treating carbonyl chloride with ferric phosphate and fractionating the products.[301]

Chemical Syntheses.—Esters and similar derivatives of phosphoric acid prepared by standard methods (*e.g.*, H_3PO_4 or $POCl_3$ + alcohol, or Ag_3PO_4 + alkyl halide) account for a large number of the recorded chemical syntheses, *e.g.*, those of tributyl,[302] tri-*o*-tolyl,[303] *p*-nitrophenyl,[300] propanediol,[304], glycerol α- and β-,[299a, 305] glucose,[306] cholesteryl,[307] 2-aminoethyl,[299b] and di-(2-aminoethyl)[307] phosphate. Radioactive vitamin K substitute [307]

[293] Ref. 6, p. 279 ; Wood, *Atomics*, 1951, **2**, 217.
[294] Arrol, *Nucleonics*, 1953, **11**, (5), 26.
[295] Thomas and Nicholas, *Nature*, 1949, **163**, 719. [296] Ref. 6, p. 279.
[297] Gourlay, U.S. At. Energy Comm. Rep., AECU-1763.
[298] Hull, *J. Amer. Chem. Soc.*, 1941, **63**, 1269 ; Vogel and Podall, *ibid.*, 1950, **72**, 1420 ; Götte and Frimmer, *Angew. Chem.*, 1952, **65**, 53.
[299] (*a*) Chargaff, *J. Amer. Chem. Soc.*, 1938, **60**, 1700 ; (*b*) Chargaff and Keston, *J. Biol. Chem.*, 1940, **134**, 515.
[300] Axelrod, *ibid.*, 1948, **176**, 295.
[301] Gardiner and Kilby, *J.*, 1950, 1769.
[302] Baldwin and Higgins, *J. Amer. Chem. Soc.*, 1952, **74**, 2431.
[303] Hodge and Sterner, *J. Pharmacol.*, 1943, **79**, 225.
[304] Lampson and Lardy, *J. Biol. Chem.*, 1949, **181**, 697.
[305] Popják and Muir, *Biochem. J.*, 1950, **46**, 103.
[306] Ref. 304, p. 693.
[307] Morrison and Crowley, Univ. California Radiation Lab. Rep. 1759.

(2 methyl-1 : 4-naphthaquinol diphosphate) and phosphoryl choline [308] have also been synthesised.

Physiologically active compounds which have been investigated include the "nerve gas" D.F.P.[309] (di*iso*propyl phosphorofluoridate), the insecticides "OMPA" (octamethylpyrophosphoramide) or "Pestox III",[310] "Iso-pestox" (*NN'*-di*iso*propylphosphorodiamidic fluoride), tetra-alkyl pyrophosphorates,[311] and Parathion (diethyl *p*-nitrophenylphosphorothionate = diethyl *p*-nitrophenylmonothiophosphate).[312] The last compound is obtained in rather poor yield from $^{32}POCl_3$ or $^{32}PCl_5$:

$$H_3PO_4 \longrightarrow POCl_3 \xrightarrow[1000°]{C} PCl_3 \xrightarrow[150°]{S\text{–}AlCl_3} PSCl_3 \longrightarrow$$

$$(EtO)_2P(S)Cl \longrightarrow (EtO)_2P(S)\cdot O\cdot C_6H_4\cdot NO_2\text{-}p$$

$$P \longrightarrow PCl_5 \xrightarrow{P_2S_5} PSCl_3 \longrightarrow p\text{-}NO_2\cdot C_6H_4\cdot O\cdot P(S)Cl_2 \longrightarrow$$

$$p\text{-}NO_2\cdot C_6H_4\cdot O\cdot PS(OEt)_2$$

but Hein and McFarland observed [312] that by using pile-irradiated phosphorus trichloride doubly (P and S) labelled parathion of 94% purity and activity 2 μc/mg. was produced in 26% yield. The activity was due to the reactions $^{31}P(n, \gamma)^{32}P$ and $^{35}Cl(n,p)^{35}S$.

The biological behaviour of a number of phosphine oxides and phosphinic and phosphonic acids and their derivatives which were prepared from active phosphorus trichloride has been investigated by Morrison and Crowley.[307]

Biosyntheses.—It is impracticable to detail the very many phosphorus-containing compounds which have been isolated during the extensive biological experiments with radiophosphorus (for a summary see Kamen [6]), and there have been relatively few deliberate syntheses.

There have been enzymic syntheses of adenosine triphosphate,[313] phosphoglyceric acid,[314] phosphocreatine,[315] glucose-1 and -6 phosphate,[305, 315] and of the attendant phosphoglucomutase.[316]

Much work has been devoted to the isolation of phospholipids and nucleic acids from cell-free systems, from systems involving surviving tissue slices, and from intact organisms.[317] The labelling of proteins for immunological studies has been well reviewed by Wormall,[318] but there have also been numerous instances of phosphorus-containing proteins isolated from metabolising systems.

[308] Riley, *J. Amer. Chem. Soc.*, 1944, **66**, 512.
[309] Witten and Miller, *ibid.*, 1948, **70**, 3886; Saunders and Worthy, *J.*, 1950, 1320.
[310] Gardiner and Kilby, *Biochem. J.*, 1952, **51**, 78.
[311] Roan, Fernando, and Kearns, *J. Econ. Entomol.*, 1950, **43**, 319.
[312] Lockau, Ludicke, and Weygand, *Naturwiss.*, 1950, **38**, 350; Murray and Spinks, *Canad. J. Chem.*, 1952, **30**, 497; Hein and McFarland, *J. Amer. Chem. Soc.*, 1952, **74**, 1856.
[313] Kornberg and Price, *J. Biol. Chem.*, 1951, **191**, 535.
[314] Sutherland, Posternak, and Cori, *ibid.*, 1949, **181**, 153.
[315] Meyerhof and Green, *ibid.*, 1950, **183**, 377.
[316] Jagannathan and Luck, *ibid.*, 1949, **179**, 569.
[317] Ref. 6, p. 286; ref. 46, p. 152.
[318] Wormall, *Brit. Med. Bull.*, 1952, **8**, 224; Francis, Mulligan, and Wormall, *Nature*, 1951, **167**, 748.

Smith secured the incorporation of phosphorus into vitamin B_{12} by growing *Streptomyces griseus* on labelled media [319] although the yields were very poor. Other entities which have been labelled include flies,[320] bacteria,[321] bacteriophages,[322] viruses,[323] and blood cells.[324]

Sulphur

The stable isotopes of sulphur find limited application in tracer studies. Thode *et al.* report some interesting variations in the $^{32}S : {}^{34}S$ ratio for materials derived from various natural sources [325] and there has also been some application [90] to biochemical studies, but interest has centred largely on use of the more convenient radioactive isotope ^{35}S, which has been reviewed by Tarver [326] and others.[327, 328] The isotope ($\tau_{\frac{1}{2}} = 87{\cdot}1$ days) decays with emission of a low-energy (0·169 mev) β-ray similar to that of ^{14}C and is now usually prepared by pile irradiation of potassium chloride[327] : $^{35}Cl(n,p)^{35}S$. After oxidation, it can be extracted from the target material as sulphate possessing a very high specific activity, although for most purposes it is diluted with inactive carrier. The radiation hazard is slight and techniques suitable for ^{14}C are generally appropriate, although memory effects due to surface adsorption are far more pronounced with sulphur. It is usually determined with counters of thin-window or gas-flow type in samples of barium or benzidine sulphate, particular care being taken to ensure quantitative oxidation to the sulphate radical.[329]

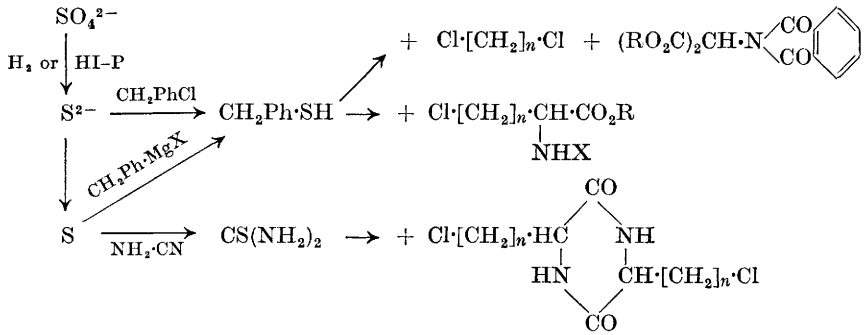
Chemical Syntheses.—A range of useful intermediates has been prepared, including sulphur,[330] sulphide,[331] the oxides and oxy-acids,[332] thiocyanate,[333] thionyl chloride,[334] carbon disulphide,[335] thiols,[330, 336] thiourea,[337] and

[319] Smith, *Biochem. J.*, 1952, **52,** 384, 387.
[320] Radcliff, Bushland, and Hopkins, *J. Econ. Entomol.*, 1952, **45,** 509.
[321] Harper and Morton, *J. Gen. Microbiol.*, 1952, **7,** 98.
[322] Kozloff and Putman, *J. Biol. Chem.*, 1950, **182,** 229, 243.
[323] Graham, *Canad. J. Res.*, 1950, **28,** *E*, 186.
[324] Reeve, *Brit. Med. Bull.*, 1952, **8,** 181.
[325] Szabo, Tudge, Macnamara, and Thode, *Science*, 1950, **111,** 464.
[326] Cf. ref. 7, Vol. 2, p. 281.
[327] Ref. 6, p. 300.
[328] Erichsen and Müller, *Angew. Chem.*, 1952, **64,** 580.
[329] Young, Edson, and McCarter, *Biochem. J.*, 1949, **44,** 179 ; Larson, Maas, Robinson, and Gordon, *Analyt. Chem.*, 1949, **21,** 1206 ; Rollinson and Creamer, Abs. Amer. Chem. Soc. 122nd Mtg., 1952, 18B.
[330] (*a*) Seligman, Rutenburg, and Banks, *J. Clin. Invest.*, 1943, **22,** 275 ; (*b*) Wood, Rachele, Stevens, Carpenter, and du Vigneaud, *J. Amer. Chem. Soc.*, 1948, **70,** 2547.
[331] Cooley, Yost, and McMillan, *ibid.*, 1939, **61,** 2970 ; Henriques and Margnetti, *Ind. Eng. Chem., Anal.*, 1946, **18,** 476.
[332] Berry and Peterson, *J. Amer. Chem. Soc.*, 1951, **73,** 5197 ; Huston, *ibid.*, p. 3049 ; Ames and Willard, *ibid.*, p. 164 ; Masters and Norris, *ibid.*, 1952, **74,** 2395.
[333] Wood and Kingsland, *J. Biol. Chem.*, 1950, **185,** 833 ; Eldjarn, *Acta Chem. Scand.*, 1953, **7,** 343.
[334] Johnson, Norris, and Huston, *J. Amer. Chem. Soc.*, 1951, **73,** 3052.
[335] Eldjarn, *Acta Chem. Scand.*, 1949, **3,** 644.
[336] (*a*) Wood and van Middlesworth, *J. Biol. Chem.*, 1949, **179,** 529 ; (*b*) Walling, *J. Amer. Chem. Soc.*, 1948, **70,** 2561.
[337] Bills and Ronzio, *ibid.*, 1950, **72,** 5510.

toluenesulphonyl chloride.[338] Keston *et al.* have synthesised[18] *p*-iodobenzenesulphonyl chloride for use in their elegant method for the analysis of amino-acid mixtures.

Amino-acids.—Major effort has been devoted to the sulphur-containing amino-acids, particularly cystine and methionine, for which a number of preparative methods have been devised. Most of these involve a preliminary synthesis of toluene-ω-thiol, by interaction of a benzyl halide with an alkali sulphide [330, 339] or, better, by interaction of sulphur with a benzylmagnesium halide.[330, 336a] Kilmer and du Vigneaud,[90] by condensation of sodium hydrogen sulphide with excess of [$^{13}C_2$]ethylene dichloride, followed by a phthalimidomalonate synthesis, sodium reduction in liquid ammonia, and finally *S*-methylation, prepared doubly labelled [$\beta\gamma$-$^{13}C_2$: ^{34}S]methionine in yields of 5% on the carbon and 11% on the sulphur.

However, in order to conserve isotopic material it is better to condense the sulphide with a suitable preformed carbon chain with,[326, 330, 339–342] or without,[336a] protection of the amino-acid end group. More recently, isotopic thiourea has proved a useful donor of the methylthio-group,[343] and Fry [344] has described two methods based on serine which are suitable for synthesis of cystine.



Optically active compounds have been prepared by an application of the isotope dilution technique,[13] or by biosyntheses with yeast [345] or bacteria.[346]

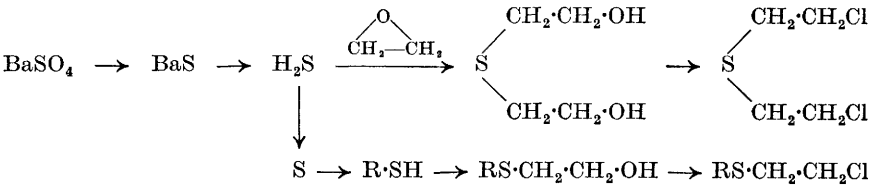
By direct neutron-irradiation of " cold " material, Ball, Solomon, and Cooper [347] succeeded in producing cystine of low specific activity, supporting their claim by degradation studies which established the sole site of radio-

[338] Ray and Soffer, *J. Org. Chem.*, 1950, **15**, 1037.
[339] Tarver and Schmidt, *J. Biol. Chem.*, 1939, **130**, 67.
[340] *Idem, ibid.*, 1942, **146**, 69.
[341] Wood and Gutmann, *ibid.*, 1949, **179**, 535.
[342] Melchior and Tarver, *Arch. Biochem.*, 1947, **12**, 301.
[343] Bloch, Abs. Amer. Chem. Soc. 118th Mtg., 1950, 22c.
[344] Fry, *J. Org. Chem.*, 1950, **15**, 438.
[345] Schlüssel, Maurer, Hock, and Hummel, *Biochem. Z.*, 1951, **322**, 226; Williams and Dawson, *Biochem. J.*, 1952, **52**, 314; Wood and Mills, *J. Amer. Chem. Soc.*, 1952, **74**, 2445.
[346] Cowie, Bolton, and Sands, *Arch. Biochem.*, 1952, **35**, 140.
[347] Ball, Solomon, and Cooper, *J. Biol. Chem.*, 1949, **177**, 81.

activity in the sulphur atom. Lipp and Weigel were, however, unsuccessful in parallel experiments.[348]

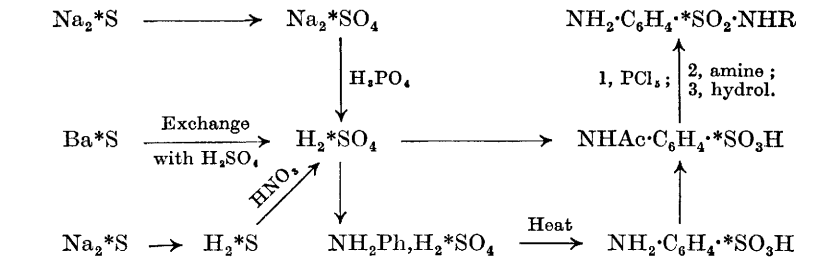
The phthalimidomalonate synthesis has also been applied to [^{35}S]cystathionine (2-amino-2-carboxyethyl 3′-amino-3′-carboxypropyl sulphide),[349] and Weiss and Stekol describe a diketopiperazine method, starting from homoserine, which is suitable for the preparation of any γ-alkylthio-α-aminobutyric acid, if necessary in optically active form (*e.g.*, cystathionine, homolanthionine, ethionine).[350] *iso*Cysteine,[351] taurine,[352] and cystamine [352] have all been synthesised by chemical means, and a microbiological preparation of glutathione has been reported.[353]

Drugs.—Preparation of a wide range of pharmacologically important compounds has been reported. Mustard gas,[354, 355] and the derived sulphoxide and sulphone,[355] together with several related compounds,[330b] were synthesised by several groups and now find some application in immunological studies.[318] Yields are excellent.



BAL (2 : 3-di[^{35}S]mercaptopropan-1-ol) has been prepared in moderate yield from the dibromopropanol.[356]

Radioactive compounds of the sulphonamide group which have been studied include sulphanilic acid,[357, 358] its di-iodo-derivative,[358] sulphanilamide,[359] sulphapyridine,[360] and sulphathiazole.[361]



[348] Lipp and Weigel, *Naturwiss.*, 1952, **39**, 189.
[349] Rachele, Reed, Kidwai, Ferger, and du Vigneaud, *J. Biol. Chem.*, 1950, **185**, 817. [350] Weiss and Stekol, *J. Amer. Chem. Soc.*, 1951, **73**, 2497.
[351] Dziewiatkowski and Wingo, *Proc. Soc. Exp. Biol.*, 1949, **70**, 448.
[352] Eldjarn, *Acta Chem. Scand.*, 1951, **5**, 677.
[353] Woodward, *J. Franklin Inst.*, 1951, **251**, 557.
[354] Axelrod and Hamilton, *Amer. J. Path.*, 1947, **23**, 389.
[355] Boursnell, Francis, and Wormall, *Biochem. J.*, 1946, **40**, 743.
[356] Young, *Science*, 1946, **103**, 439; Peters, Spray, Stocken, Collie, Grace, and Wheatley, *Biochem. J.*, 1947, **41**, 370.
[357] Ingraham, *J. Amer. Chem. Soc.*, 1952, **74**, 2433.
[358] Myers, *Cancer Res.*, 1950, **10**, 234.

Other physiologically active compounds which have been labelled include "antabuse" (tetraethylthiuram disulphide),[335] 2-toluene-*p*-sulphonamidofluorene,[362] insulin sulphate,[363] methionine sulphoximine,[364] penicillin,[365] pentothal (sodium 5-ethyl-5-1′-methylbutyl-2-[^{35}S]thiobarbiturate],[8] phenothiazine,[366] 2-*p*-aminophenylthiazole,[367] and the insecticide "Parathion" (diethyl *p*-nitrophenyl phosphorothionate).[368]

Miscellaneous.—Chemical syntheses of xanthates,[369] alkyl sulphates,[370] tetramethylthiuram disulphide,[371] and dibenzothiophen and its 3-acetamido-derivatives [372] have been carried out and attention has been drawn to a suitable synthesis for dithizone.[373]

Biosynthesis.—Mention has already been made of the sulphur-containing amino-acids and peptides which have been prepared by biosynthetic methods. There has also been a very considerable effort devoted to the synthesis of penicillin [374] with high specific activity. Various organisms, proteins, and viruses have been labelled in connection with biological studies.

Halogens

Fluorine.—The most suitable isotope for tracer work, ^{18}F, has a half-life of only 112 minutes and it must be made in a cyclotron or similar device. There have been few applications or syntheses.

Chlorine.—The isotopes, ^{34}Cl and ^{38}Cl, have rather short half-lives (about $\frac{1}{2}$ hour) and have therefore found very limited tracer application. More recently ^{36}Cl ($\tau_{\frac{1}{2}} \sim 10^6$ years, radiation β 0·73 mev) has become available and one or two syntheses have been recorded. The labelled γ-isomer of "benzene hexachloride" required for isotope dilution analysis of the commercial product has been reported.[375] Acetanilide has been chlorinated, hydrolysed, and treated with carbonyl chloride to yield *p*-[^{36}Cl]chlorophenyl *iso*cyanate from which δ-*p*-[^{36}Cl]chlorophenyl hydantoic acid was prepared by interaction with glycine.[376] Standard methods of zinc-catalysed

[359] Klotz and Melchior, *Arch. Biochem.*, 1949, **21**, 35; Fingl, Christian, and Edwards, *J. Amer. Pharm. Assoc.*, 1950, **39**, 693.
[360] Bray, Francis, Neale, and Thorpe, *Biochem. J.*, 1950, **46**, 267.
[361] Noll, Bang, Sorkin, and Erlenmeyer, *Helv. Chim. Acta*, 1951, **34**, 340.
[362] Ray and Argus, *Cancer Res.*, 1951, **11**, 274.
[363] Stadie, Haugaard, and Vaughan, *J. Biol. Chem.*, 1952, **199**, 729.
[364] Roth, Wase, and Reiner, *Science*, 1952, **115**, 236.
[365] du Vigneaud, Wood, and Wright, "The Chemistry of Penicillin", Princeton Univ. Press, p. 892.
[366] Lazarus and Rogers, *Nature*, 1950, **166**, 647.
[367] Noll, Sorkin, and Erlenmeyer, *Helv. Chim. Acta*, 1949, **32**, 609.
[368] Jensen and Pearce, *J. Amer. Chem. Soc.*, 1952, **74**, 3184.
[369] Gaudin and Carr, *Analyt. Chem.*, 1952, **24**, 887.
[370] Croes and Ruyssen, *Bull. Soc. Chim. biol.*, 1951, **33**, 1837.
[371] Craig, Davidson, Juve, and Geib, *J. Polymer Sci.*, 1951, **6**, 1.
[372] Brown, Kirkwood, Marion, Naldrett, Brown, and Sandin, *J. Amer. Chem. Soc.*, 1951, **73**, 465. [373] Irving and Bell, *Nature*, 1952, **169**, 756.
[374] Hawell, Thayer, and Labaw, *Science*, 1948, **107**, 299; Maas and Johnson, *J. Bact.*, 1949, **58**, 361; Smith and Hockenhull, *J. Appl. Chem.*, 1952, **2**, 287.
[375] Craig and Tryon, Abs. Amer. Chem. Soc. 122nd Mtg., 1950, 12L.
[376] Woeber, *J. Amer. Chem. Soc.*, 1952, **74**, 1354.

$H^{36}Cl$ esterification of *cis-* and *trans*-3-chloropropenol were utilised by Hatch, Morgan, and Tweedie for preparation of the 1 : 3-dichloropropenes : [377] there was no exchange of chlorine. A general method of synthesising labelled chlorides by exchange with aluminium chloride has been suggested.[378]

Bromine.—Several radioactive isotopes are known, of which ^{82}Br ($\tau_{\frac{1}{2}} \sim 34$ hours) is most suitable for tracer studies, despite an appreciable hazard presented by the decay process, which involves a cascade of 3 γ-rays associated with an initial β-disintegration.[379] The isotope, which is prepared by neutron-irradiation of a suitable bromide either organic or inorganic [$^{81}Br(n, \gamma)^{82}Br$], has found application in chemical, medical, pharmaceutical, immunological, and entomological studies, where the ease of counting with ordinary thick-walled Geiger–Müller tubes is an advantage.[380] The short half-life precludes any lengthy syntheses but it has been used for kinetic and preparative esterification studies,[381] and certain aromatic bromides have been prepared by direct exchange with the inorganic (lithium, aluminium) halides.[382]

Addition of elementary bromine, obtained by oxidation, to the appropriate unsaturated compounds has yielded 7 : 8-dibromœstrone [383] and a range of aliphatic dibromo-acids.[384] The isotope has also been substituted into the molecules of dyes required for tumour localisation (dibromotrypan-blue,[385] dibromo-Evans-blue). Several groups have synthesised the œstrogen bromotriphenylethylene.[386] Howarth has studied the action of procaine by studies with the labelled dibromo-analogue.[387]

Iodine.—The physiological significance of iodine in minute quantities soon stimulated biological studies with a radioactive isotope at a time when only ^{128}I ($\tau_{\frac{1}{2}} \sim 25$ min.) was available.[388] The development of the cyclotron and atomic pile made available ^{130}I ($\tau_{\frac{1}{2}} \sim 12\frac{1}{2}$ hours) and ^{131}I ($\tau_{\frac{1}{2}} \sim 8$ days), and today virtually all iodine tracer work is carried out with the last-named isotope.[389] This decays by one of two alternative routes which, however, both yield β-particles and a series of fairly energetic γ-rays. It is prepared by reactions, $^{130}Te(d,n)^{131}I$ or $^{130}Te(n,\gamma)^{131}Te \xrightarrow[(\tau_{\frac{1}{2}}\ 30\ hr.)]{-\beta} {}^{131}I$ or is separated from fission products and is available as iodine, iodide, or iodate, or in a range of useful intermediates and physiologically important compounds

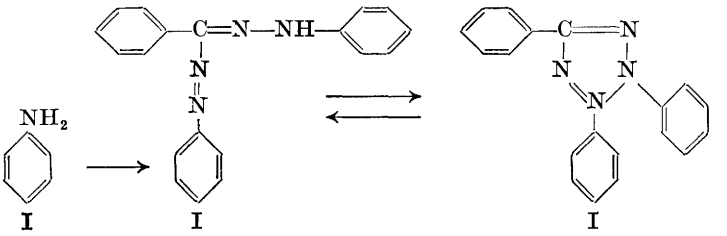
[377] Morgan, Hatch, and Tweedie, *J. Amer. Chem. Soc.*, 1952, **74**, 1826.
[378] Wallace and Willard, *ibid.*, 1950, **72**, 5275. [379] Ref. 6, p. 331.
[380] Winteringham, *Nature*, 1949, **164**, 183.
[381] *Idem*, *J.*, 1949, S 416 ; Baret and Pichat, *Bull. Soc. chim.*, 1950, **17**, 1294.
[382] Kieffer and Rumpf, *ibid.*, 1951, **18**, 584.
[383] Twombly, McClintock, and Engelman, *Amer. J. Obst. Gynaecol.*, 1948, **56**, 260.
[384] Buu-Hoï, Berger, Daudel, Daudel, May, and Miguet, *Helv. Chim. Acta*, 1946, **29**, 1334.
[385] Moore and Tobin, *J. Clin. Invest.*, 1943, **22**, 155.
[386] Paterson, Gilbert, Gallagher, and Hendry, *Nature*, 1949, **163**, 801 ; Twombly, Schoenewaldt, and Meisel, *Cancer Res.*, 1951, **11**, 780 ; Apelgut, Cheutin, Mors, and Berger, *Bull. Soc. chim.*, 1952, **19**, 533.
[387] Howarth, *Nature*, 1948, **161**, 857 ; 1949, **163**, 679.
[388] Ref. 3, p. 139. [389] Ref. 6, p. 336.

required for analytical, chemical, and medical studies. The separation [390] and assay [391] of the isotope have been reviewed.

Five major methods for the introduction of active iodine into organic compounds have been developed, *viz.*, esterification,[392] halogen exchange, direct iodination with iodine or iodine chloride, the Sandmeyer reaction, and biosynthesis.

Compounds prepared by exchange with inorganic halide include both aryl [393] and alkyl [394] halides, iodoacetamide, iodoacetamido-acids,[395] mustard-gas analogues,[396] and thyroxine.[397]

Elementary iodine prepared from iodide by oxidation with iodate, hydrogen peroxide, hypochlorite, or nitrous acid has been utilised in preparations of *p*-iodoaniline and hence iodotetrazolium salt,[398] chiniofon



(8-hydroxy-7[^{131}I]-iodo-quinoline-5-sulphonic acid),[399] iodinated œstradiols,[400] pheniodol,[401] and thyroxine [402] and its analogues,[397b, 402] and for labelling a wide range of products such as fats,[403] fibres,[404] polystyrene,[405] etc.

Wormall [318] has discussed the important subject of the labelling of proteins by methods which include iodination or reaction with *p*-iodophenyldiazonium chloride.

Elementary iodine has also been utilised for the preparation of iodotriphenylethylene from the corresponding Grignard compound,[406] and

[390] Arrol, *Nucl. Sci. Abs.*, 1952, **6**, 151.
[391] Bruner and Perkinson, *Nucleonics*, 1952, **10**, (10), 57.
[392] Ludes and Endler, *Chem. Abs.*, 1952, **46**, 9145.
[393] Kristjanson and Winkler, *Canad. J. Chem.*, 1951, **29**, 154.
[394] Heydring and Winkler, *ibid.*, p. 790.
[395] Friedman and Rutenburg, *J. Amer. Chem. Soc.*, 1950, **72**, 3285.
[396] Seligman, Friedman, and Rutenburg, *Cancer*, 1950, **3**, 336, 342.
[397] (*a*) Frieden, Lipsett and Winzler, *Science*, 1948, **107**, 353; (*b*) Lemmon, Tarpey, and Scott, *J. Amer. Chem. Soc.*, 1950, **72**, 758; (*c*) Taurog, Briggs and Chaikoff, *J. Biol. Chem.*, 1952, **194**, 655.
[398] Seligman, Gofstein, and Rutenburg, *Cancer Res.*, 1949, **9**, 366.
[399] Albright, Tabern, and Gordon, *Amer. J. Trop. Med.*, 1947, **27**, 533.
[400] Albert, Heard, Leblond, and Saffran, *J. Biol. Chem.*, 1949, **177**, 247.
[401] Free, Page, and Woollett, *Biochem. J.*, 1950, **48**, 490.
[402] Gross and Leblond, *J. Biol. Chem.*, 1950, **184**, 489; Michel, Roche, and Tata, *Bull. Soc. Chim. biol.*, 1952, **34**, 366, 466.
[403] Rutenburg, Seligman, and Fine, *J. Clin. Invest.*, 1949, **28**, 1105.
[404] Sankey, Mason, Allen, and Keating, *Pulp and Paper Mag., Canada*, **52**, 136.
[405] Tubis and Jacobs, *Nucleonics*, 1952, **10**, (9), 54.
[406] Morrison, Univ. California Radiation Lab. Rep., 1719.

iodination with ^{131}ICl has served for the preparation of di-iodofluorescein,[407] 3′ : 5′-[$^{131}I_2$]di-iodo-*A*-methopterin,[4] 3 : 5-[$^{131}I_2$]di-iodofolic acid,[4] [$^{131}I_4$]-tetraiodophenolphthalein,[4] and iodinated penicillins.[4] Inactive iodine chloride in conjunction with active iodide has been employed for the preparation of iodinated sulphanilamide and sulphapyridine,[408] and for the carcinogen 2-acetamido-7-[^{131}I]iodofluorene.[409]

The Sandmeyer reaction is convenient for the preparation of aromatic compounds and has been employed for the preparation of the analytically important *p*-iodobenzenesulphonyl chloride [18] and for 2-[^{131}I]iodo-3-nitrobenzoic acid,[410] iodinated dyes (Nile-blue,[411] trypan-blue [412]), a D.D.T. analogue,[413] iodo- and iodoso-benzene,[414] 2 : 4-dichloro-5-[^{131}I]iodophenoxyacetic acid,[415] etc.

Biosynthetic methods have frequently been employed during studies of thyroid metabolism [416] and have found some application in protein labelling.

One novel method for the preparation of the simpler iodides through a modified Szilard–Chalmers reaction has also been described.[417]

[407] Roe, Hayes, and Bruner, *J. Amer. Chem. Soc.*, 1951, **73**, 4483.
[408] Bloch and Ray, *J. Nat. Cancer Inst.*, 1946, **7**, 61.
[409] Weisburger, *J. Amer. Chem. Soc.*, 1950, **72**, 1758.
[410] Mitchell, Wood, Wolfe, and Irving, *Science*, 1947, **106**, 395.
[411] Sloviter, *J. Amer. Chem. Soc.*, 1949, **71**, 3360.
[412] Stevens, Lee, Stewart, Quinlin, and Gilson, *Cancer Res.*, 1949, **9**, 139.
[413] Jensen and Pearce, *J. Amer. Chem. Soc.*, 1952, **74**, 2436.
[414] Aten and Aten, *ibid.*, p. 2411.
[415] Wood, Wolfe, Doukas, Klipp, Fontaine, and Mitchell, *J. Org. Chem.*, 1949, **14**, 900.
[416] Gross and Leblond, *J. Biol. Chem.*, 1950, **184**, 489 ; Gross and Pitt-Rivers, *Biochem. J.*, 1953, **53**, 645.
[417] Glueckauf and Ray, *J.*, 1949, S 330.