

## Radioactive Tracers—A New Tool in Organic Chemistry

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**R**ADIOACTIVE tracers are isotopes (often artificial) of elements, which emit characteristic radiations.<sup>1</sup> They became commercially available since 1946 as by-products of nuclear reactors. In America, radioactive isotopes of over sixty elements are now obtainable from the atomic pile.<sup>2</sup> At least nine more elements have radioisotopes which may be prepared in the cyclotron which was the only source of these isotopes before the advent of the atomic pile. Whereas in the cyclotron many hours of bombardment are required to produce a small amount of radioactive material, larger amounts of tracers became available through the use of an atomic pile.

Radioactive tracers are being widely used in industry and research. By the use of tracer elements in the study of chemical or biological reactions, vital information concerning the mechanism of plant and animal metabolism has been revealed. Besides their use in medicine and biology, radioactive isotopes promise help in the solution of many of the hitherto unsolved problems concerning surface phenomena, catalysis, free radicals, reaction kinetics and diffusion. A new light has been shed now on many problems in metallurgy, crystallography and high polymer science by the use of tracer elements.

To the organic chemist, radioisotopes have provided a new research tool whereby the course of complex reactions could be studied. The use of tracers in synthetic organic chemistry has led to the development of new micro-techniques and specialised vacuum techniques. The application of radioactive elements in organic chemistry is stressed in the present article.

### *Equipment in Radiochemistry*<sup>1</sup>:—

The radiation associated with radioactive elements is easily measured and gives a quantitative indication of the presence of such elements. If sufficiently sensitive measuring devices are used, it is possible to estimate the quantity of the active elements present, with an accuracy far beyond that which could be attained by the best of micro-balances. Thus it is possible to estimate amounts of elements as low as  $10^{-10}$  gm. to  $10^{-14}$  gm.

Radioactive elements emit radiations called alpha, beta and gamma rays which consist of doubly ionised helium, electron and photon streams respectively. Artificial isotopes almost exclusively radiate 'betas' and 'gammas.' These radiations have the common property of ionising the medium which they encounter and making it electrically conducting.

Studies in radiochemistry involve the use of a device known as the Geiger-Muller counter and the associated measuring gear. The Geiger counter has rightly been called the 'burette' of radiochemistry. A modern Geiger tube has often a central tungsten wire positively charged with a high voltage and a copper cylinder co-axial with the wire. The whole arrangement is encased in a glass sheath or made vacuum tight. The filling of the counter is often argon or alcohol vapour giving a total of 10 c.m. mercury pressure. When a radioactive substance is brought near this arrangement, a beta-particle might liberate an electron in the gas filling of the counter and this electron, as it accelerates towards the central anode wire, would

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liberate other electrons in its path by collision. There would thus be an avalanche of electrons depositing more or less simultaneously on the wire and depressing its positive potential until it is restored to its original value again.

Each beta-particle emitted by the radioactive substance would thus produce a "kick" in the positive potential which after amplification could be translated into mechanical action and counted on a revolution counter. Since the number of beta-particles emitted per second would be proportional to the amount of radioactive material present (all other factors being kept constant), we have, therefore, a means of titrating radioactivity. A detailed account of the equipment, methods of measurement and the applications of radioactive carbon in synthetic organic chemistry has been given by Calvin *et al.*<sup>3</sup>

#### Unit of Radioactivity<sup>1</sup>:—

The unit of radioactivity, called the "curie," is given by the number of particles emitted per second by one gram of pure radium. One "curie" corresponds to the emission of  $3.7 \times 10^{10}$  particles per second. In tracer work it is usual to express radioactivity in terms of "millicuries" or "microcuries," i.e.  $10^{-3}$  or  $10^{-6}$  curies, respectively.

#### Organic Syntheses:—

Organic chemistry being the chemistry of carbon compounds, it is not surprising that radioactive isotopes of carbon are by far the most widely used in the study of organic reactions, although radioactive isotopes of other elements, especially hydrogen (tritium), the halogens and phosphorous are also employed to a lesser extent.

Carbon has five isotopes of which  $C^{10}$ ,  $C^{11}$  and  $C^{14}$  are radioactive.  $C^{10}$  has a half-life of a few seconds and therefore

cannot be used.  $C^{11}$  has a half-life of 20 minutes and can be used in laboratories with cyclotron equipment.  $C^{14}$ , however, has a half-life of about 5,000 years and emits weak beta-rays. It is now obtainable from the atomic pile in a combined form such as,  $BaCO_3$  or  $KCN$  (asterisk denotes radioactive atom). Any compound labelled with  $C^{14}$  has thus to be synthesised from  $CO_2$  available from radioactive barium carbonate or potassium cyanide, and considerable efforts have been devoted towards this end.

Laboratory work involving the use of tracers must take into account the following:—

1. Health hazards: it is essential to conduct all work under a hood.
2. The need for high yields: this is desirable in order to conserve the valuable isotope.
3. The necessity for synthetic work to be carried out on a very small scale.
4. Isotope dilution that takes place due to exchange reactions.

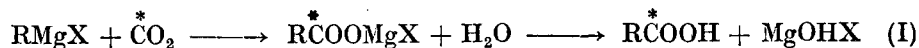
As far as possible the labelled atom should be introduced near the end of the sequence of reactions leading to the desired compound. The yields of the various reactions involved in the sequence must be carefully determined on the inactive chemicals so that some idea may be gained of the amount of labelled starting material needed in the final synthetic work.

The synthesis of carbon-labelled compounds usually consists of two stages:

- (a) Primary step of binding  $CO_2$  in one-carbon compounds suitable as starting materials for further syntheses.

(b) The next step is the formation of a carbon-carbon linkage.

Thus  $^*\text{CO}_2$  liberated from  $\text{Ba}^*\text{CO}_3$  is taken up in a Grignard reaction:

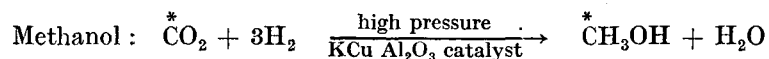
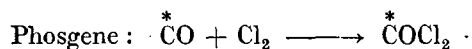


This reaction initially gave only moderate yields, but Weinhouse *et al.* have modified the method so that high yields of radioactive acids are now obtainable in the above reaction. Double decomposition of alkyl halides and  $\text{KCN}^*$  leads

finally to the active acids through the intermediate nitriles.

*One-carbon compounds*<sup>3</sup>:—

A few of the one-carbon compounds prepared are:



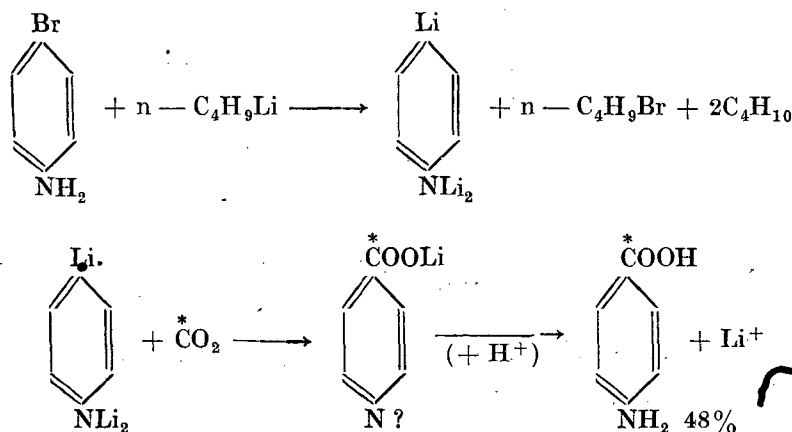
Similarly, other one-carbon compounds such as methyl iodide, formic acid, formaldehyde, etc., have also been prepared.

*Carboxylic acids*<sup>3</sup>:—

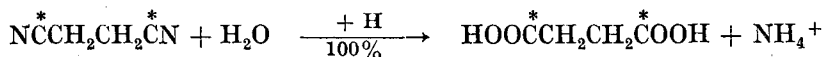
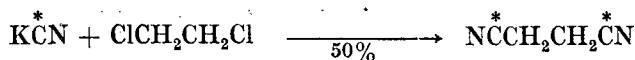
In the synthesis of functionally labelled carboxylic acids, their esters and amides, the Grignard reaction (I)

is widely used as indicated earlier. Thus, acetic acid  $l\text{-C}^{11}$  or  $\text{-C}^{14}$ , benzoic acid-carboxyl- $\text{C}^{14}$ , *l*-naphthoic acid-carboxyl- $\text{C}^{14}$ , palmitic acid- $l\text{-C}^{14}$  have been prepared by carbonation of the Grignard reagents from the corresponding bromo-compounds with labelled carbon dioxide.

*p*-Aminobenzoic acid-Carboxyl- $\text{C}^{14}$  has been prepared by the sequence:



The nitrile route has been used for the synthesis of succinic acid-1:4- $C^{14}$ , thus:

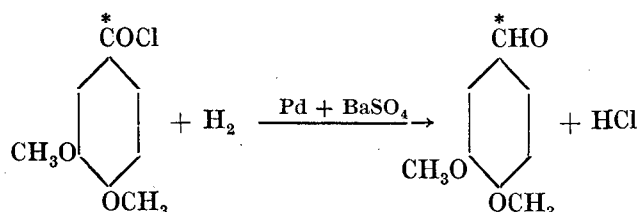
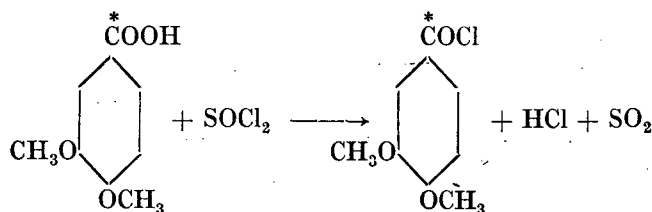


Non-functionally labelled acids and derivatives are prepared by the carbonylation of the Grignard reagents from labelled compounds. Thus, sodium

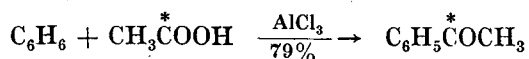
acetate- $\alpha$ - $C^{14}$ ,  $^*CH_3COONa$ , is prepared from  $^*CH_3I$ .

*Aldehydes and ketones*:—

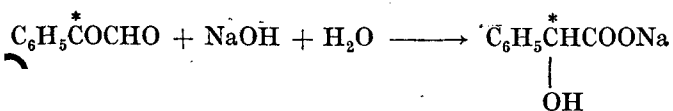
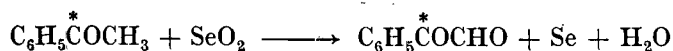
Functionally labelled veratraldehyde is prepared by the following route:



Acetophenone-carbonyl- $C^{14}$  has been prepared by two routes. One of them follows the malonic ester synthesis of keto-labelled benzoyl chloride while the other is by the Friedel-Craft route.



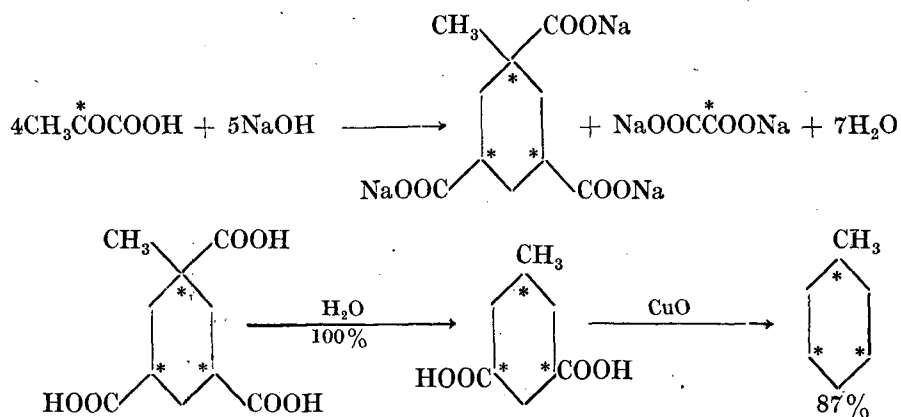
Among the alpha-hydroxyacids, mandelic acid- $\alpha$ - $C^{14}$  has been prepared by the following sequence of reactions:



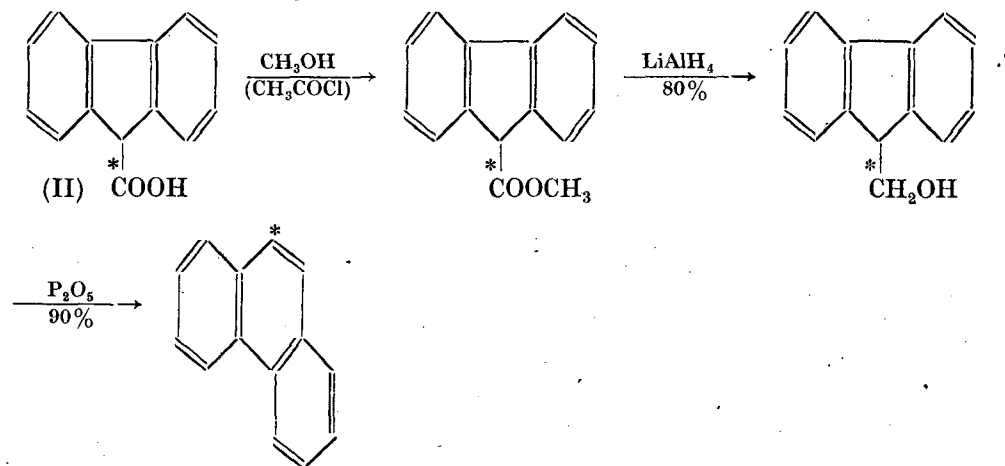
The syntheses of numerous amines, amino acids, alcohols, etc., have also been achieved. Labeled phenylalanine, tyrosine, and *dl*-tryptophan have been synthesised for biological studies.

*Aromatic hydrocarbons and carcinogens*<sup>3</sup>:—

Toluene-1:3:5- $C^{14}$  is obtained from pyruvic acid- $\alpha$ - $C^{14}$ .

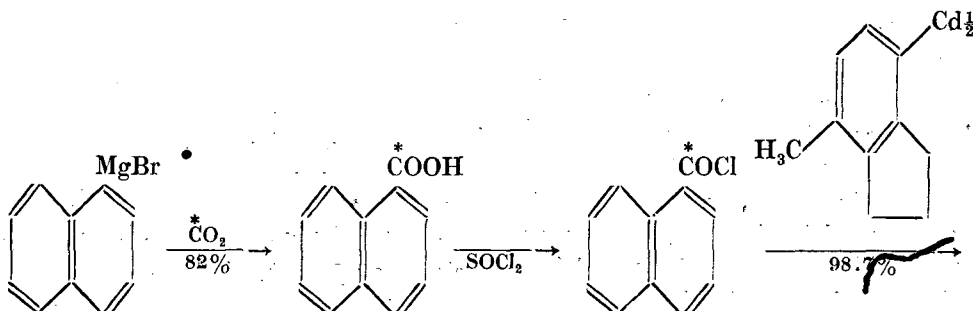


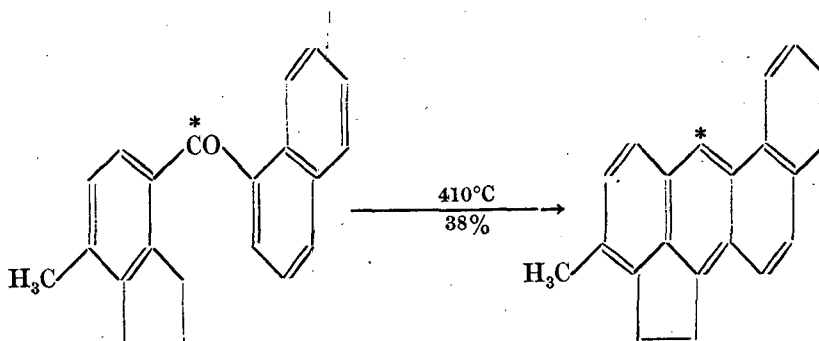
For carcinogenic studies, phenanthrene-9- $C^{14}$  has been prepared by the Wagner rearrangement of 9-fluorenyl-carbinol-10- $C^{14}$ .



(II) itself was obtained by the carbonation of the sodioderivative of fluorene. The synthesis of the highly active car-

cinogen, 20-methyl cholanthrene-11- $C^{14}$ , is accomplished by the Elb's synthesis as follows:—





Recently, steroids containing labelled carbon atoms have also been synthesised.

#### Study of Reaction Mechanisms:—

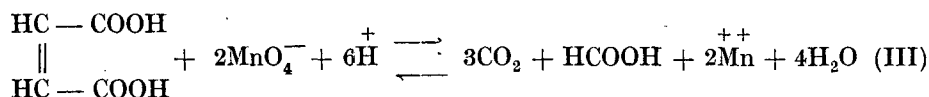
It is in the study of organic reaction mechanisms that radioactive isotopes, especially C<sup>14</sup>, have proved to be particularly useful. Recent work on such lines includes a study of the following:

- Synthesis and oxidation of fumaric acid<sup>4</sup>; the Willgerodt reaction<sup>5,6</sup>; mechanism of permanganate oxidation of

propane<sup>7</sup> and three-carbon acids; rearrangement studies on unsymmetrical alpha-diketones<sup>8</sup>, and the mechanism of the Fisher-Tropsch<sup>9</sup> synthesis of liquid fuels from coal.

#### Oxidation of fumaric acid<sup>4</sup>:—

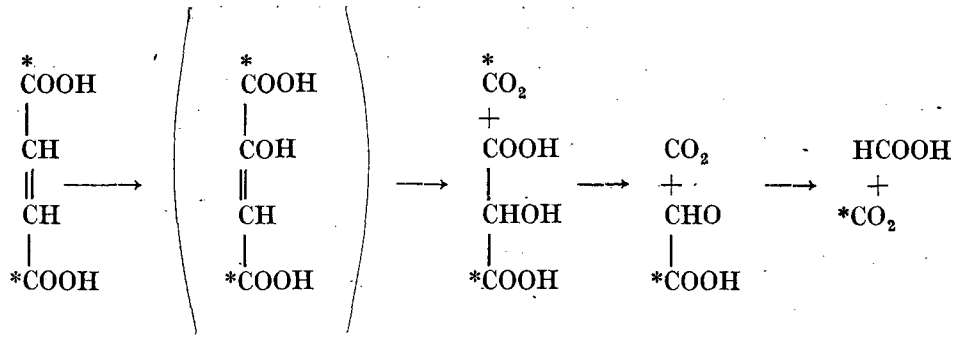
When fumaric acid is oxidised in 1.5N H<sub>2</sub>SO<sub>4</sub> by KMnO<sub>4</sub> at 35-50°, CO<sub>2</sub> and HCOOH are formed. The reaction can be represented quantitatively as follows:



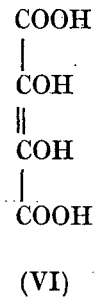
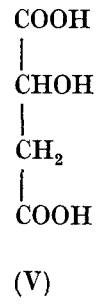
The formic acid formed could originate from either the methine or the carboxylic carbon atom or from both. Using radioactive C<sup>14</sup>, fumaric acid labelled in the two carboxyl groups was obtained. It was found that CO<sub>2</sub> formed on oxidation was highly radioactive, whereas the formic acid formed gave, on oxidation, carbon dioxide of only meagre activity. This experiment proves that formic acid formed on oxidation of fumaric acid arises from the methine carbon atoms. It may be mentioned here that the estimation of radioactivity is complicated by the exchange reactions that take place. Thus, CO<sub>2</sub> formed from ordinary carbon, as in the above case,

will not be completely free from radioactivity because of contamination by exchange reactions. However, the quantitative difference between the activity of the radioactive product and the weakly active side products being very large, the broad conclusions based on the more active samples are seldom erroneous.

Using radioactive hydrogen (tritium) it was also found that the hydrogen bonded to the methine carbon does not exchange with hydrogen ions during the oxidation. From the above results, the mechanism of the oxidation of fumaric acid may be briefly represented as follows:



The possible intermediates (IV) and (V) could be eliminated as they both yield 2 moles of HCOOH and 2 moles of CO<sub>2</sub>. In addition, (VI) could not be formed as the formic acid produced from it will not contain the original hydrogen from fumaric acid.



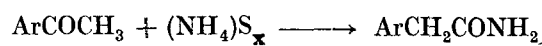
*Decarbonylation of alpha-ketoesters<sup>10</sup>:-*

The decarbonylation of α-keto esters is not only important in synthetic organic chemistry, but is also involved in biochemical reactions. In the pyrolysis of ethyl pyruvate-α-C<sup>14</sup>, CH<sub>3</sub>\*COCOOC<sub>2</sub>H<sub>5</sub>, between 110° and 130°, appreciable activity was not noticed in the carbon

monoxide formed. It is thus clear that the carbon monoxide originates from the carboxyl group of the α-keto esters and not from the keto group.

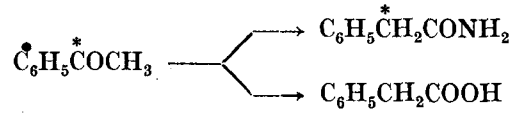
*The Willgerodt reaction<sup>7</sup>:-*

This reaction is used for the preparation of amides of arylacetic acids by heating methyl aryl ketones with aqueous yellow ammonium sulfide:



The mechanism of this interesting reaction was unknown till the use of radioactive tracers. Acetophenone-carbonyl-C<sup>14</sup> was sub-

jected to the Willgerodt reaction. The reaction mixture gave radioactive phenylacetamide and some inactive phenylacetic acid.



The formation of the amide in the Willgerodt reaction as shown above, clearly showed that it is produced by an oxidation-reduction mechanism along .

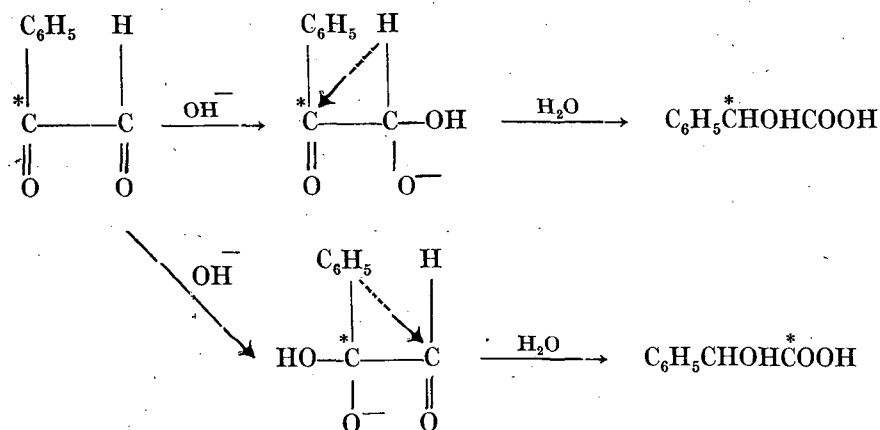
the alkyl chain and does not involve migration of carbon atoms. The inactive phenylacetic acid is probably formed by a different mechanism involving migration of a carbon atom.

*Rearrangement of unsymmetrical  $\alpha$ -diketones<sup>8</sup> :—*

The reaction of phenylglyoxal with aqueous alkali to form mandelic acid has been shown to follow the same second order kinetics as the benzil-benzilic acid rearrangement. Hence, -a

similar mechanism in the case of phenylglyoxal allows two possible modes of reaction: one, in which the aldehyde hydrogen shifts following hydroxyl-ion addition at the aldehyde group, and a second, in which a phenyl group migrates as the result of hydroxyl-ion addition at the keto group.

However, by labelling the carbonyl group, a distinction can be made since the two modes of reaction will lead to isotopically distinguishable products:—



Action of alkali on phenylglyoxal-carbonyl- $\text{C}^{14}$  was therefore studied and it was found that it was converted to mandelic acid without rearrangement of the carbon skeleton. The  $\text{CO}_2$  evolved by chromic acid oxidation of mandelic acid was found to be inactive.

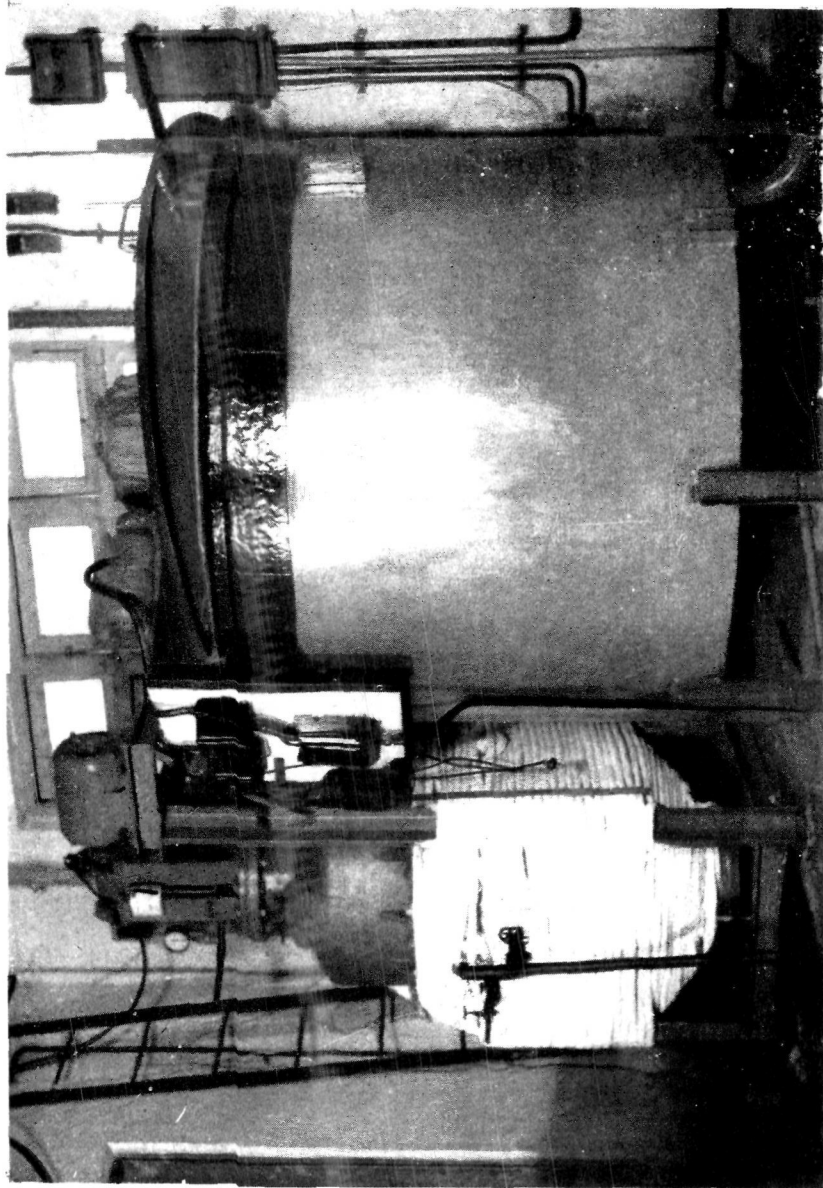
The above are only a few examples of the voluminous work being done in the study of reaction mechanisms. They serve to illustrate the versatility of this new weapon in the hands of the organic chemist.

*Radioactive isotopes as a Source of Radiation<sup>11</sup> :—*

An account of the use of radioactive tracers in organic chemistry would be incomplete without reference to their use as sources of radiation which may serve as initiators in organic reactions. If a suffi-

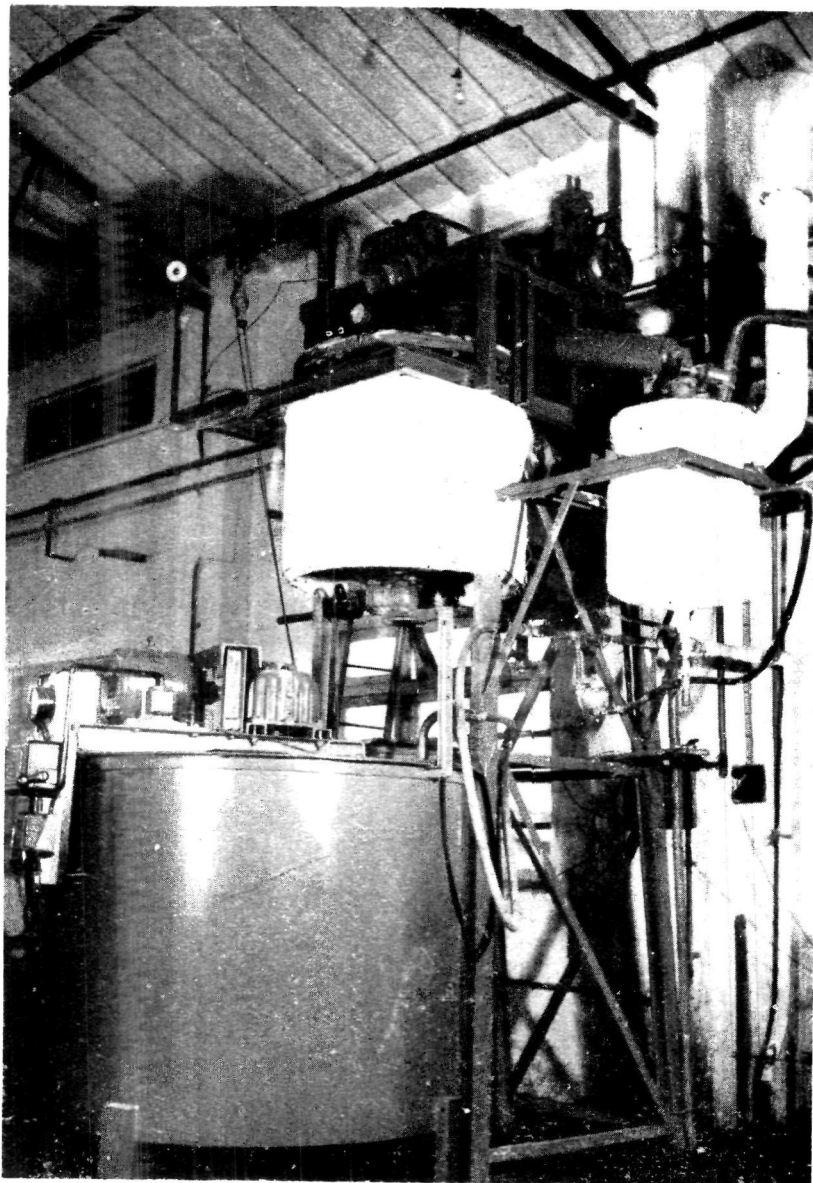
cient number of monochromatic neutrons of low energy could be made available, it is possible to measure chemical bond strengths accurately. Thus the initial appearance of a chemical reaction when a monochromatic neutron-beam is made to impinge on an organic compound, would be associated with the lowest bond strength in the molecule. For a hydrocarbon, it would be the weakest carbon-carbon linkage. Work along these lines is in progress in the U.S.A. The idea has great potentialities because, with a sufficient number of monochromatic neutrons, it might be possible to induce reactions which could be very accurately controlled. Likewise, by means of an intense radiation, it may also be possible to induce polymerisation of certain organic compounds, such as, e.g., allyl alcohol, allyl chloride and the like, which will not polymerise under normal conditions.





A REACTION SET-UP IN PILOT PLANT HOUSE No. 1,  
(Dyes and Intermediates Section)

Photo by: C. B. WADIKAR



A REACTION SET-UP IN PILOT PLANT HOUSE      No. 2.  
(*Dyes and Intermediates Section*)

*Photo by: C. B. WADIKAR*

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## Some Aspects of Research in Vat Dyes

B. SURYANARAYANA\*

**T**HE discovery of indanthrone by Bohn in 1901 opened up the important field of anthraquinone vat dyes. Since then dyes of this class<sup>1</sup> have been synthesised in large number and they now constitute the outstanding group of all dyes because of their excellent tinctorial and fastness properties. Among the synthetic dyes the anthraquinone vat dyes are the largest consumed next to the azo dyes and on value basis, they probably surpass the azo dyes even. Being particularly suitable for dyeing cotton and regenerated cellulose (which constitute about 70% of the textiles used)<sup>1</sup> they have assumed a great commercial importance in spite of their high cost.

*Some limitations of vat dyes and need for new vat dyes:—*

The general excellence in all respects, of the present range of vat dyes may give the impression that there is little scope for further improvement or extension of the range now available. A close examination, however, reveals that although the first member of the series was marketed more than fifty years ago, there are still no bright scarlet, bright red, greenish yellow and turquoise blue dyes among them, which possess fastness comparable to the best dyes of this

class<sup>2</sup>. Also, certain dyes, particularly yellows and oranges (e.g. Indanthrene Yellow GK and Indanthrene Golden Orange G) which are otherwise satisfactory have the drawback of tendering cellulose dyed with them, on prolonged exposure to light<sup>3</sup>. The need for the extension of the present range and the discovery of yellow and orange vat dyes which do not suffer from the tendering activity is therefore obvious.

Although the vat dyes owe their supremacy over other dyes to their high fastness, it should be realised that the conception of fastness is subject to continual change. New fastness requirements arise which were previously unknown. An example of this is provided by the fastness to new finishing processes<sup>2</sup> based on the synthetic resins such as anti-crease, anti-shrink, non-felting and flame-proof finishes. The range of dyes which suit these developments needs further extension.

The above instance is but one among the many new problems created for the dye chemist as a result of advances made in the field of textiles. Another example is the advent of synthetic fibres such as Nylon, Acrilon, Orlon, Dynel, Saran and Terylene. Viscose rayon being similar to natural cotton gave no

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