A METHOD FOR THE PREPARATION OF TRITIUM LABELLED HYDROCARBONS OF HIGH SPECIFIC ACTIVITY.

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SUMMARY

Tritiated hydrocarbons may be prepared by Kolbe electrolysis of carboxylic acids which have been labelled by catalytic exchange in tritiated water.

A study of hydrocarbon utilization by micro-organisms required a labelled mixture of the n-alkanes, triacontane ($C_{30}H_{62}$), hexacosane ($C_{26}H_{54}$) and docosane ($C_{22}H_{46}$). The anticipated incorporations were expected to be very low and, as it was also likely that metabollic products would be extensively diluted, it was desirable to have starting material of very high specific activity.

Preparation of high molecular weight hydrocarbons labelled with carbon-14 presented a formidable problem and so, despite possible disadvantages associated with the kinetic isotope effect and non specificity, it was decided to label with tritium.

Two general methods for tritium labelling involve exchange reactions, either with tritium gas (1) or with tritiated water and a heterogeneous catalyst (2). Gas exchange has been widely used but suffers from the disadvantage that it causes extensive degradation with the subsequent difficulty of radiochemical purification. Evans (2) has described the extensive use of platinum catalysed exchange reactions in either tritiated water or acetic acid at temperatures of 100-130°C. Very high specific activities have been obtained and degradation reactions are often less important or at least do not produce impurities of higher specific activity than the desired product.

Two difficulties arise with respect to the C_{20} - C_{30} n-alkanes. Firstly, it is difficult to secure pure samples free from isomeric impurities and secondly they are immiscible with water. We believe that the technique described in this paper circumvents these difficulties and gives a general procedure for preparation of a wide variety of tritium labelled hydrocarbons. Two stages are involved. A suitable carboxylic acid or mixture of acids (as sodium salts) is first subjected to a platinum catalysed exchange with tritiated water at 110 - 120°C. Excess tritiated water and any labile tritium is removed and the acids are then subjected to Kolbe electrolysis (3,4) which involves coupling of two hydrocarbon radicals (reaction 1).

$$2\ R\ CO_2^- \xrightarrow{\text{electrolysis}} R - R + 2\ CO_2 + 2e \qquad (1)$$

If two different acids, in equimolar proportions are used, the products are usually three hydrocarbons in the statistically expected proportions (reaction 2).

$$4\ RCO_2^- + 4\ R'CO_2^- \longrightarrow R\text{-}R + 2R\text{-}R' + R'\text{-}R' + 8CO_2 + 8e \qquad (2)$$

This technique was used in the present case where an equimolar mixture of the sodium salts of palmitic and lauric acids were used to prepare the required mixture of triacontane, hexacosane and docosane. The exchange reaction with 40 Ci of tritiated water gave a product with a specific activity of 0.04 Ci/ m mole. This product was diluted fourfold with inactive acids and after electrolysis and subsequent purification, the mixed hydrocarbons had the expected specific activity of 0.02 Ci/m mole. Thus, the presence of impurities of high specific activity could be discounted.

The alternative technique of exchange with tritium gas has recently been reported for preparation of tritiated n-nonacosane (5). In this particular case, however, the hydrocarbon was available as an extract from broccoli and the tritiated hydrocarbon could be purified by repeated thin-layer chomatography.

EXPERIMENTAL

(a) Tritiation Procedure

The tendency of aqueous solutions of fatty acid sodium salts to foam when subjected to vacuum evaporation necessitated the use of a special reaction tube illustrated in fig. 1. The pear-shaped flask (A) and the anti-splash bulb (B) each had a volume of about 25ml. Platinum oxide (0.05g.) was introduced into (A), using ethanol (5 ml.) and was then reduced in a stream of pure hydrogen. Most of the supernatant ethanol was removed and mixed sodium palmitate

and sodium laurate (0.1g.) in water (0.5 ml.) added. The flask was attached to a vacuum manifold at (C) and carefully evacuated to remove all the water and ethanol. When the contents of the flask were completely dry, tritiated water (1.0ml., 40 Ci) was distilled in by cooling the flask in liquid nitrogen. The flask was then flame sealed at (D), warmed to room temperature and placed in a thermostatted oil bath at 110° for 24 hours. After completion of the

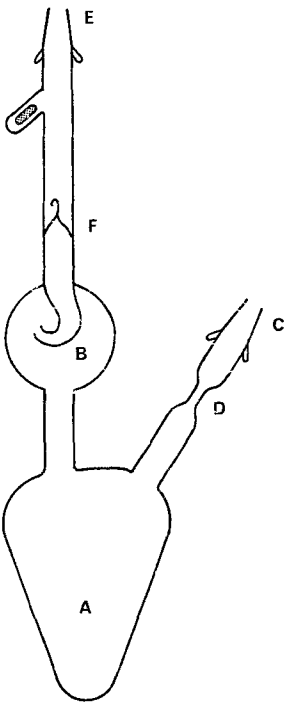

Fig. 1 Reaction tube for catalytic exchange in tritiated water.

exchange tritiation, the flask was reattached to the manifold at (E), the seal at (F) broken and all the tritiated water transferred back to its reservoir. Several portions of aqueous ethanol (~5 ml.) were then distilled into the flask (A) and subsequently removed to displace exchangeable tritium. The flask was then removed from the manifold and the labelled sodium salts dissolved in methanol and transferred to the electrolysis cell shown in fig. 2. Additional sodium palmitate/laurate mixture (0.3g.) was added and dilute sodium methoxide (≡ 0.2g. sodium metal) added to bring the total volume to 60 ml.

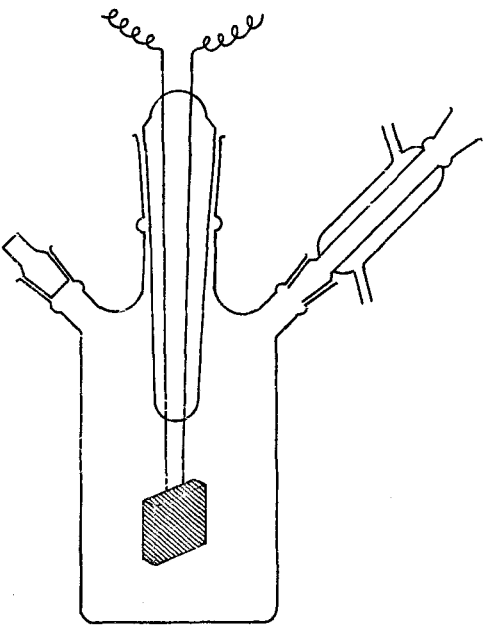

Fig. 2 Electrolysis cell. The platinum electrodes (3 cm. x 2 cm.) are mounted on a frame of pyrex glass rod.

The solution (1 ml) was further diluted with methanol (100 ml) and three portions of this solution (0.1 ml each) were then removed for tritium assay, and gave a mean value of 6.54×10^5 dpm which corresponded to a specific activity of 10.1 mCi/m mole or 40.4 mCi/m mole for the original undiluted acids.

Electrolysis of the methanolic solution at 100v and 0.9A was then allowed to proceed for 3 3/4 hours at room temperature (by cooling with iced water). The hydrocarbons were isolated by evaporation of the methanol, addition of aqueous acid and extraction with ether. The ether solution was dried ($MgSO_4$), filtered and evaporated. The hydrocarbons were purified by chromatography on a column of alumina using cyclohexane solvent. Gas chromatographic analysis of a micro portion gave three peaks only corresponding to triacontane, hexacosane and docosane in the expected proportions 1 : 2 : 1 by peak area. No impurities could be detected either by gas chromatography or by infrared spectral analysis. Labelled hydrocarbons (0.004g) in cyclohexane (30 ml.) were diluted further (1 ml. in 100 ml.) and portions (0.1 ml.) used for tritium assay to give a mean value of 1.78×10^4 dpm which corresponded to a specific activity of 22 Ci/m mole.

Chemical yields from the electrolysis were observed in many trial experiments and found to fluctuate considerably between 10 and 50%. This may be due to small scale operation but a systematic study was not undertaken. Experience with larger scale operations indicates that yields of around 50% are usual (3,4).

(b) Radiochemical Assay

Samples of solution to be assayed (0.1 ml.) were dissolved in a toluene solution of butyl-PBD (Ciba)(0.8%, w/v) (10 ml) in standard screw cap counting vials. The counter was a Philips Liquid Scintillation Analyser equipped with automatic external standardisation and automatic computation to give sample activity directly as absolute dpm.

(c) Gas Liquid Chromatography

A Pye 104 Gas Chromatograph was used. Samples were examined on 5' x 1/8" columns of either non-polar E-30 (10%), or polar PEG-20M (20%), stationary phases. The solid support in the former case was 100-120 mesh siliconised Diatomite 'C' and in the latter case 100-120 mesh J.J.'s 'C'. The carrier gas flow (nitrogen) was 60 ml/min at 46 psi and a flame ionisation detector was used at 300°C. Temperature programming was used with the non-polar column (120°C-300°C at 6°C/min.) and the polar column was maintained isothermally at 225°C.

(d) Infra Red Spectroscopy

A Perkin Elmer Infracord was used.

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