Tritium Labelling and Tritium N.M.R. - Part I Alpha-labelled Stearic, Palmitic, Myristic and Lauric Acids.

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Summary

Stearic, palmitic, myristic and lauric acids have been labelled with tritium in the alpha carbon position, and the position of the label confirmed using tritium N.M.R. spectrometry. The α-^{3}H acids were prepared using tritiated water with sulphuric acid or sodium hydroxide as catalysts. Specifically labelled compounds of high specific activity and radiochemical purity were obtained.

Introduction

Information from isotope tracer experiments on fatty acid metabolism has been limited by the ubiquity of long chain fatty acids, their relatively simple carbon framework and their rapid biological turnover *in vivo* and *in vitro*. The key problem of interpretation arises from the interchange of carbon fragments and of tritium labels during the rapid metabolism of these molecules. A major problem arises from the fact that conventional tritium labelling methods using hydrogenation catalysts lead to imprecise positioning of the isotope tracer in the fatty acid molecule.

A knowledge of the exact position of the isotope in the starting material is crucial to the study of fatty acid metabolism. Introduction of tritium or

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deuterium into the alpha position of fatty acids using either acid[1] or base[4,5,6,7] catalysed exchange, has been reported.

Van Heyningen et al., (1938)[1] reported introduction of stably bound deuterium to the alpha position of palmitic acid, using sulphuric acid as catalyst. Their assignment of the label to the alpha position was based on the results of degradative reactions. Atkinson et al., (1968)[6] used deuterium to label propionic and hexanoic acids in the α-position using KOH as catalyst. They determined the specificity of the position using proton N.M.R.

In the present work, tritium labelling of stearic, palmitic, myristic and lauric acids in the alpha-position was achieved using a modification of Van Heyningen's method, the position of the label in the molecule being established by tritium N.M.R. Stearic acid was also labelled in the α-position using NaOH as catalyst. This exchange using the sodium salt is considered to be a simpler labelling method since it has a shorter purification procedure and unwanted labelling of the labile acidic hydrogen is avoided.

Experimental

Preparation of High Specific Activity Tritiated Water - Raney-Nickel catalyst (1.80 g) was dried and degassed in a glass bulb on a vacuum line, for three hours at 300°C. Tritium gas was transferred onto the activated catalyst, following which water (0.2 ml) as vapour was transferred onto the catalyst and the bulb sealed. After mechanical turning of the mixture for 6 hours at 25°C, the tritiated water was removed from the catalyst by vapour transfer. This had a specific activity of ca. 16 Ci/ml. 5 Ci/ml tritiated water from the Radiochemical Centre, Amersham was also used.

Preparation of α-^{3}H-Fatty Acids

Sulphuric Acid method - Concentrated H_2SO_4 (2.00 g) was dripped onto the A.R. grade fatty acid (1.0 g) in a pyrex ampoule, followed by tritiated water (0.2 ml). The sealed ampoule was mechanically rotated for 64 hours at 100°C.

The purification of the tritiation reaction product was effected by ether extraction from water solution followed by removal of labile tritium from the carboxyl position via precipitation of the potassium salts and reacidification of this salt followed by further ether extraction. This procedure was repeated several times to constant specific activity.

The purified stearic acid was analysed by radio-gas-liquid chromatography of the methyl ester,[2] while the palmitic, myristic and lauric acids were analysed on a high pressure liquid chromatograph on a Waters' Fatty Acid Column fitted with both mass and radioactivity detectors. Specific activities are in Table 1.

Table 1

Specific Activities of α Tritiated Acids

	Activity T_2O Ci/ml	Specific Activity found (mCi/ml)	Theoret. Max.
Lauric acid	5 Ci/ml	9	84
Myristic acid	5	10	84
Palmitic acid	5	4	84
Stearic acid	5	32	84
Alkali Stearic acid	5	140	240
High Act. Stearic acid	16	1,110	1,330

Specific activities of the purified products were determined by liquid scintillation counting (channels ratio method) in a Packard "Tricarb" Scintillation Spectrometer using a toluene solution of POP and POPOP as scintillant.

After further purification by thin layer chromatography,[3] the specific activity was redetermined.

Positional analysis for the tritium label was carried out using tritium nuclear magnetic resonance spectroscopy in deuterochloroform as previously described.[8]

Sodium Hydroxide method - 50 µl of 5 Ci/ml tritiated water was added to 0.1 g sodium stearate, sealed in a glass ampoule, and heated at 160°C for 16 hours. The tritiated water was removed under vacuo and the sodium stearate dissolved in 10 mls water. Stearic acid was then extracted into ether after acidification with sulphuric acid. The product was then analysed using a High Pressure Liquid Chromatograph and tritium N.M.R.

Results

Thin layer chromatography of the free stearic acid and gas liquid chromatography of the methyl ester failed to reveal any impurities at levels above 2%. Radiochemical purity was established by radio-gas liquid chromatography when the only activity peak observed in addition to that of methyl stearate was a shoulder of height 10% of the parent due to stearic acid. Other radioactive impurities were estimated to be present at less than 2% of the parent activity. The purity of the other acids was established on a high pressure liquid chromatograph, which showed no detectable degradation products in either mass or activity detectors.

The tritium N.M.R. spectrum of the tritiated stearic acid (Fig. 1) shows only one peak. This peak has a chemical shift in good agreement with that predicted from the observed chemical shift for the α proton signal seen in the proton spectrum. Chemical shifts for protons are measured from

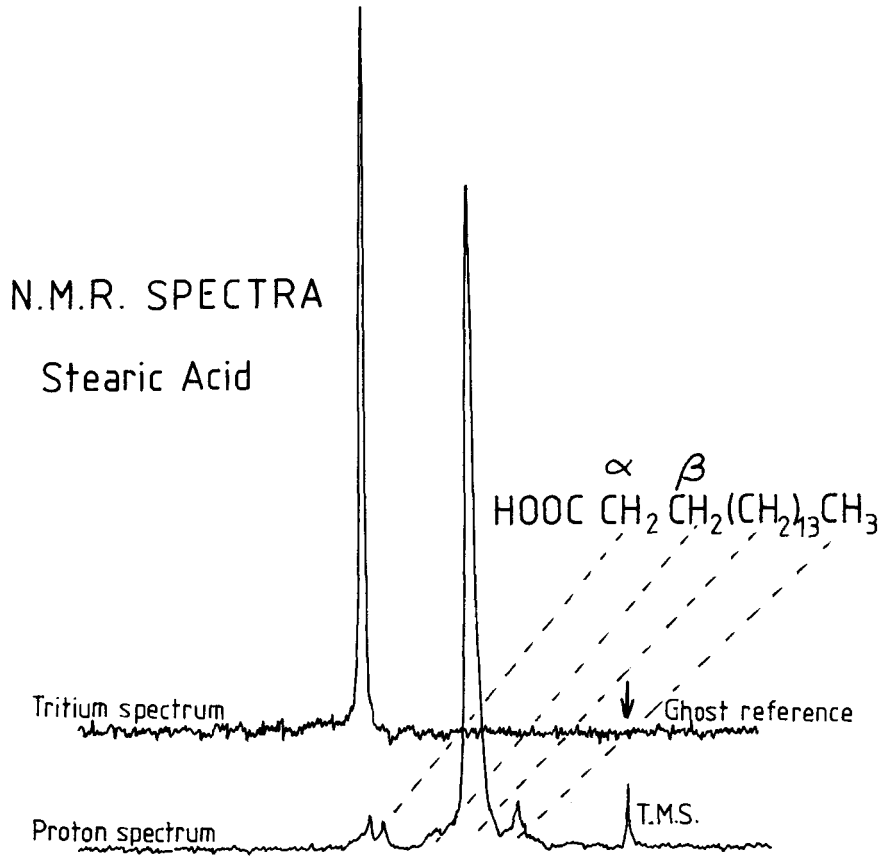

Figure 1.

the T.M.S. signal whereas the triton shifts are measured from the calculated position of the "ghost reference" signal[9] for the same standard. The slight shift of the tritium peak relative to the corresponding proton peak, in the display shown, arises because of a limitation of the plotter : both ^3H and ^1H scales are coincident in Hz whereas ideally they should be coincident in p.p.m.

Similar spectra were obtained for the other acids and results are shown in Table 2.

Table 2

Chemical Shifts for α Protons (δ^1H) and α Tritons (δ^3H) in Fatty Acids

Acid	$\delta(^1H)$ p.p.m.	$\delta(^3H)$ p.p.m.
[α-^{3}H]Stearic (alkali catalyst)	2.09	2.09
[α-^{3}H]Stearic (H_2SO_4 catalyst)	2.09	2.08
[α-^{3}H]Myristic	2.10	2.07
[α-^{3}H]Lauric	2.12	2.11

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