

The specific activity approaches a constant value, which is estimated to $1\,500 \pm 500$ cpm/ μ g PAS. With this value and knowing the efficiency of the counter, the yield in the tritium labelling of PAS is calculated to 1.5 ± 0.5 mC/g PAS.

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Preparation of 2-Methyl[1- 14 C]-octadecanoic Acid

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2-Methyloctadecanoic acid has been prepared by several investigators, e. g. by Schneider and Spielman¹ from diethyl methylmalonate and hexadecyl iodide and by Morgan and Holmes² from methylmagnesiumiodide and 2-bromooctadecanoic acid.

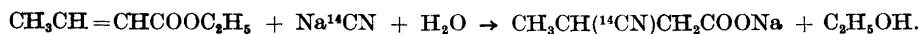
For the preparation of [carboxy- 14 C] 2-methyloctadecanoic acid a Kolbe electrolysis of hexadecanoic acid and sodium 3- 14 C]cyanobutyrate and subsequent hydrolysis of the cyano group seemed more suitable and was found to be convenient. The cyanobutyrate (previously prepared in a different way by Widequist³) was easily

obtained in comparatively good yield from ethyl crotonate and sodium 14 C]cyanide (cf. the preparation of methylsuccinic acid by Higginbotham and Lapworth⁴).

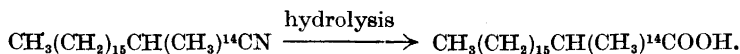
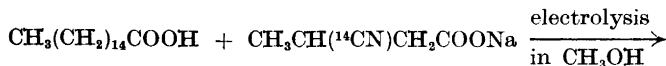
Experimental. Sodium 3- 14 C]cyanobutyrate (I). A solution of ethyl crotonate (0.91 g) in ethanol (3.6 ml) was mixed with 0.41 g of sodium 14 C]cyanide dissolved in 1.0 ml of water and the mixture refluxed on a water bath for 5 h. The solvents were removed by distillation and the residue extracted with boiling acetone. The insoluble fraction was filtered off. On cooling the filtrate, colourless crystals of I (0.72 g, 67 %) separated. They were dried at 0.4 mm Hg, 78°. (Found: C 44.0; H 4.7; N 10.1. Calc. for $C_5H_7NO_2Na$: C 44.4; H 4.5; N 10.4.)

2-Methyl[1- 14 C]octadecanoic acid (III). A solution of I (0.64 g of crude product) and hexadecanoic acid (7.0 g) in methanol (75 ml) was electrolysed between platinum electrodes as described by Stenhagen⁵. The precipitate (triacontane and a small amount of 2-methyloctadecanenitrile, II) was filtered off at room temperature and dissolved in boiling methanol. The solution was cooled to 20° and the precipitate again removed by filtration. The combined methanol solutions were evaporated and the residue extracted with ether, saturated with water. After removal of free acids from the ether solution by the ion exchanger Amberlite IRA 400, the ether was evaporated and the product hydrolysed by refluxing with ethanolic potassium hydroxide solution. Water was added to the hydrolysate, and hydrocarbons were removed by extraction with ligroin. Acidification of the alcohol-water layer and extraction of the 2-methyl[1- 14 C]octadecanoic acid with ether, yielded 0.65 g (46 %) of crude III. After recrystallization from methanol and ligroin, 0.36 g of pure acid, m.p. 54.5–55.0°, remained. Schneider and Spielman¹ give m.p. 54.5°, Cason, Wolfhagen, Tarpey and Adams⁶ 54.6–55.1° and Morgan and Holmes² 58°.

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I



II

III

The analyses were performed by Miss M. Westerdahl, Department of Analytical Chemistry, University of Lund.

The financial support of *Statens naturvetenskapliga forskningsråd* is gratefully acknowledged.

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Received January 7, 1957.

The Paramagnetic Resonance Absorption of a Semiquinone of FMN

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Recently Beinert¹ has presented strong evidence for the existence of a semiquinoid compound appearing as an intermediate in the oxidation-reduction reactions of flavinmononucleotide (FMN). In collaboration with Gutowsky and Rutledge he succeeded in obtaining a paramagnetic resonance signal characteristic of a free radical from FMN reduced by Zn in an acid 50% aqueous ethanol solution¹. They failed to obtain any signal with a pure aqueous solution*, because of the high dielectric losses in liquid water. It is, however, of the greatest importance to be able to investigate the paramagnetic resonance of this and similar substances as the postulated semiquinones of FAD, DPN and TPN when they are dissolved in water, the milieu of the enzymatic reactions in which they are supposed to take part^{2,3}.

By freezing the samples, which diminishes the dielectric losses greatly, and

* Personal communication.

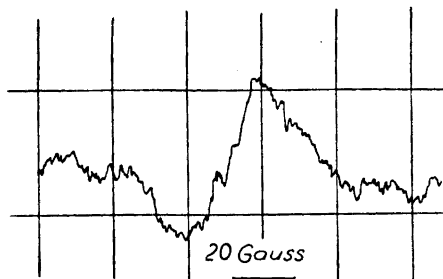


Fig. 1. Derivative of paramagnetic resonance absorption of 160 μ M FMN in 1 N HCl after reduction by solid Zn for 8 min and subsequent rapid cooling to about 80° K.

working at liquid N₂ temperatures, which improved the sensitivity, it has now been possible to record intense resonance signals from the semiquinone of FMN in dilute solutions. The FMN was dissolved in 1 N HCl and reduced by metallic Zn, until the color was brownish. A 0.20 ml sample was transferred to a small quartz tube, which immediately was dipped into liquid N₂. The solution rapidly solidified and was cooled down to 80° K. The sample was then transferred into the precooled high-Q cavity of the spectrometer⁴ operated at 9 400 Mc and the spectrum was recorded (Fig. 1). It did not change over a period of 30 min at liquid N₂ temperature. No signal could be obtained from FMN before adding Zn or after reoxidation in air, nor from a sample without FMN but reacted with Zn. The g-value of the resonance is practically identical with that of the free radical diphenylpicryl hydrazyl. No further signals could be seen in the region 2 600 to 3 900 gauss and no hyperfine structure has thus far been resolved.

Comparison of the surfaces under the absorption curve obtained by integration of Fig. 1 and that of a known amount of diphenylpicryl hydrazyl reveals that the concentration of free radicals is roughly half the initial concentration of FMN. This would appear to dispel any doubt that a semiquinone intermediate of FMN is really formed under the conditions described.

The technique used here will be of great value in cases when the intermediate is shortlived, since the low temperature will practically lock the reaction at a desired