TRITIATION OF THE NITROXIDE SPIN LABELED FATTY ACID 14-PROXYLSTEARIC ACID

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SUMMARY

The nitroxide spin labeled fatty acid 14-proxylstearic acid (1) was tritium labeled (likely at the 2-position) by a platinum black catalyzed exchange reaction in tritiated hot aqueous potassium hydroxide. The recovery of pure tritiated 1 was 56%, with a specific activity of 260 mCi/mM.

KEY WORDS: nitroxide, 14-proxylstearic acid, spin label, tritium

DISCUSSION

Proxyl[1,2] nitroxide spin labeled fatty acids are versatile molecules useful both as spin labels for studying biological systems[3] and as intermediates for the chemical synthesis of other spin labeled molecules such as phospholipids.[4] For use in quantitative studies involving the covalent attachment of these spin labels to proteins it was necessary to prepare a proxyl nitroxide fatty acid which was radiolabeled with tritium. Herein, we

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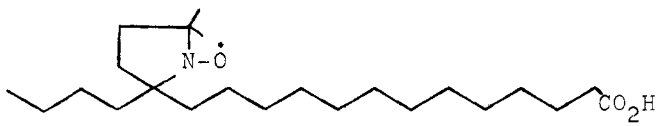
describe the direct incorporation of tritium into 14-proxylstearic acid (1)[5] by means of an exchange reaction catalyzed by platinum black in tritiated hot aqueous potassium hydroxide. Acid 1 was recovered from the labeling procedure in 56% yield and had a specific activity of 260 mCi/mM. Since the labeling procedure is closely analogous to that used by Atkinson et al.[6] for introduction of deuterium in the 2-position of aliphatic carboxylic acids, it is highly likely that the tritium atoms introduced into acid 1 are also at the 2-position.



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EXPERIMENTAL SECTION

<u>[2-^{3}H]-14-Proxylstearic Acid (1)</u>. In order to establish the stability of 14-proxylstearic acid[5] under the labeling conditions, a 15-mg sample of 1 was treated with 16 mg of platinum black and 0.45 ml of aqueous 1.0 N potassium hydroxide. This mixture was heated with stirring under N_2 at 100°C for 8 hr, then cooled. The catalyst was removed by filtration through celite. The filtrate was acidified to pH 4 by addition of 1.0 N hydrochloric acid and then extracted with three 5-ml portions of chloroform. The combined organic layers were concentrated in vacuo, affording

12 mg (75%) of crude **1**.

The actual tritium labeling experiment was done by Amersham Corp. (TR.1 procedure) on a 100-mg sample of **1**, with quantities of the other reactants scaled to the above experiment. After removal of labile tritium the sample was returned as the potassium salt dissolved in 25 ml of ethanol (total activity = 340 mCi). A 12-ml aliquot was concentrated to dryness (63 mg), dissolved in chloroform and applied to a 1000 micron Analtech 20 x 20 silica gel GF preparative thin layer chromatography plate. Elution with ether/hexanes (1:1) containing 1% acetic acid, isolation of the yellow band at R_F 0.6, and elution of this band gave 27 mg (56%) of pure **1** (acid form) with a specific activity of 260 mCi/mM.

ACKNOWLEDGMENT

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REFERENCES AND NOTES

1. "Proxyl" is a convenient abbreviation for a 2,2-disubstituted 5,5-dimethylpyrrolidine-N-oxyl nitroxide spin label.

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