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Methyl esters of unsaturated fatty acids labeled with tritium in the methyl group

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Methyl esters of unsaturated acids have been labeled with tritium in the methyl group by a microtechnique which is a modification and refinement of the methylation procedure of Metcalf and Schmitz [1]. About 30 μl of 15% boron trifluoride methanol reagent [1] were placed in a reaction flask and frozen rapidly in liquid nitrogen (−196° C).

The flask was attached to a simple glass vacuum manifold and evacuated to 10^{-3} Torr. A glass reservoir flask containing 100 mC of tritium labeled methanol (80 mc/mM) was likewise frozen and evacuated simultaneously. The flasks were isolated from the vacuum pump and the methanol-^{3}H was vacuum distilled into the reaction flask. After the flask was closed the reagents were allowed to come to room temperature for equilibration. The flask was opened and the pressure was raised to atmospheric with N_2. Fatty acid (50 mg) was added and the solution was refluxed for 15 min. After being cooled, the

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solution was frozen in liquid nitrogen and the flasks were evacuated to 10^{-4} Torr. The unreacted methanol-^{3}H was recovered by vacuum distillation into the reservoir for future use. The tritium labeled methyl ester was washed from the reaction flask with ethyl ether and sodium bicarbonate solution was added. After ether extraction of the methyl esters, the resulting solution was dried over sodium sulfate and evaporated. The recovered sample was weighed and assayed for radioactivity by liquid scintillation counting. Chemical and radiochemical purity was determined as follows : Thin-layer chromatography was accomplished on an Eastman Chromagram plate impregnated with 10% alcoholic silver nitrate with benzene as the moving phase. All chromatograms were analyzed with a Packard Radiochromatogram scanner, model 7201. Further chemical and radiochemical purity determinations were accomplished by gas-liquid radiochromatography (GLRC) with the Cary-Loenco model 70 system [2].

Table 1 indicates the results of five experiments using this procedure. Four preparations were performed with the same batch of methanol-^{3}H which was recovered following each use. Decreasing specific activity indicates the successive dilution of the methanol-^{3}H. All the methyl ester preparations were shown to be both chemically and radiochemically pure by the techniques of thin layer and gas liquid chromatography described above. Limits of detection of impurities : Thin-layer scanning, 1.0×10^3 cpm, 1.0×10^{-2} mg; GLRC 2.2×10^4 cpm, 1.0 nanogram.

TABLE 1. ^{3}H-methyl ester preparations.

	Weight mg	Activity mC	Specific activity mc/mM
^{3}H-methyl 9-15 dienoate [a]	105.5	1.41	3.96
^{3}H-methyl linoleate	36.1	1.055	8.7
^{3}H-methyl linolenate	24.0	0.401	4.92
^{3}H-methyl linolenate	49.1	0.210	1.3
^{3}H-methyl linoleate	60.0	0.230	1.1

[a] Prepared using a separate batch of methanol-^{3}H.

The stability of these ^{3}H-methyl esters as regards release of tritium was compared to the stability of methyl esters labeled in the chain (prepared by Wilzbach tritiation procedures [3]), utilizing the techniques of microvapor-phase hydrogenation developed at this Laboratory [4]. Under these reaction conditions the chain-labeled methyl esters were found to release labile tritium while there was no tritium release with the ^{3}H-methyl esters nor was there ester interchange with unlabeled methyl esters. Thus ^{3}H-methyl labeling,

when applicable, may have distinct advantages over chain labeling with tritium.

Previously described methods of labeling fatty acid esters during esterification have utilized diazomethane-^{14}C [5, 6] and diazomethane after exchange with tritiated water [7]. In each of these methods the incorporation of radioactivity is quite small and the methods were designed as analysis tools rather than preparative procedures. The present method eliminates the use of hazardous diazomethane, yields compounds with usable specific activities and provides for efficient recovery and reuse of the radioactive methanol. By the technique of microesterification described herein, milligram amounts of valuable, labile, unusual or relatively unavailable fatty acids are readily reacted and labeled. The method is obviously also applicable to esterification with ^{14}C-labeled methanol.

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