

ECONOMICAL SYNTHESSES OF  $^3\text{H}$ - $^2\text{H}$  AND  $^{14}\text{C}$ -LABELED ETHYL ACETATES<sup>1</sup>

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Summary: Tritium and  $^{14}\text{C}$ -labeled isomers of ethyl acetate are excellent substrates for competitive dual-labeled radioactive isotopic measurements of  $\beta$ -deuterium and  $\beta$ -tritium isotope effects. Methods are given for preparation of the ethyl esters of acetic acid-2- $^3\text{H}$ , acetic acid-2- $^{14}\text{C}$ , and acetic acid-2,2- $^2\text{H}_2$ -2- $^3\text{H}$ , and of the acetic acid ester of ethanol-1- $^{14}\text{C}$  from the least costly labeled precursors in high purity (yield 70-80%) on the mmole scale.

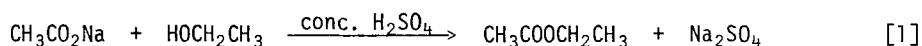
Keywords:  $\beta$ -tritium (deuterium) isotope effects, tritium-deuterium- $^{14}\text{C}$ -ethyl acetate, ketene- $^2\text{H}_2$ .

## INTRODUCTION

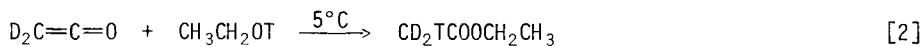
In investigations of transition state structures of acyl transfer processes,  $\beta$ -deuterium secondary isotope effects have been utilized as mechanistic tools quite broadly (2-6). The measurement of such effects by double-label competitive methods, as well as other investigations, is aided by the availability of variously labeled species of ethyl acetate. In this paper, we wish to report efficient and inexpensive approaches to ethyl acetate labeled with tritium, with tritium and deuterium in the  $\beta$ -position, and with  $^{14}\text{C}$ .

## DISCUSSION

Acetic acid-2- $^3\text{H}$  or -2- $^{14}\text{C}$  ethyl ester was prepared from the labeled sodium acetate and ethanol with concentrated sulfuric acid as catalyst (eq. 1).



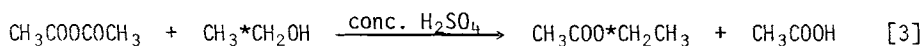
Acetic acid-2,2-<sup>2</sup>H<sub>2</sub>-2-<sup>3</sup>H ethyl ester was obtained from the reaction of ketene-<sup>2</sup>H<sub>2</sub> with ethanol-0-<sup>3</sup>H, according to eq. 2.



The reactants were obtained as follows: Ketene-<sup>2</sup>H<sub>2</sub> was prepared by the pyrolysis of acetone-<sup>2</sup>H<sub>6</sub> as described by Williams and Hurd (7), but slightly modified.

Ethanol-0-<sup>3</sup>H was obtained by the method of Gueive and Sparek (8).

Acetic acid ethyl-1-<sup>14</sup>C ester was obtained from the reaction of ethanol-1-<sup>14</sup>C and acetic anhydride with concentrated sulfuric acid as catalyst (eq. 3).



#### EXPERIMENTAL

Materials. The following radioactive materials were purchased: <sup>3</sup>H-CH<sub>3</sub>COONa 5 mCi, 13 Ci/mmol and 1-<sup>14</sup>C-CH<sub>3</sub>CH<sub>2</sub>OH 1 mCi, 0.05 mCi/mmol, Research Products International Corp., 2-<sup>14</sup>C-CH<sub>3</sub>COONa 1 mCi, 4.3 mCi/mmol, ICN Co., and <sup>3</sup>H-H<sub>2</sub>O 25 mCi, 0.45 mCi/mmol, New England Nuclear. Acetone-<sup>2</sup>H<sub>6</sub> (99.5% D) was supplied by Aldrich. All other materials were reagent grade. Instruments used for analytical measurements were: an Aerograph A-90-P<sub>3</sub> gas chromatograph supplied with a CARBOWAX 20 M (6' x 0.25") on Chromosorb W. 60/80 mesh support column, a Beckman IR-33 instrument, and a Beckman liquid scintillation counter LS-7500.

Acetic acid-2-<sup>3</sup>H or -2-<sup>14</sup>C-ethyl ester: The radioactive CH<sub>3</sub>COONa was dissolved in 5 mmol acetic acid or it was mixed with unlabeled CH<sub>3</sub>COONa to bring the quantity to 1 mmol and then dissolved in ~300 μL water. Twelve mmol absolute ethanol and 100 μL concentrated sulfuric acid were added and the mixture was refluxed for three hours. After the first fraction had been distilled out of the

reaction mixture, 5 mmol absolute ethanol was added to the residue and the procedure was repeated. About 3.5 mmol ester was obtained when 5 mmol acetic acid was reacted. A small amount of ethanol accompanying the ester was removed by decanting from granular  $\text{CaCl}_2$  and  $\text{K}_2\text{CO}_3$ .

The combined distillate contained 10-30% ethyl acetate and the remainder ethanol when ethanol was in large excess over  $\text{CH}_3\text{COONa}$ , as shown by gas chromatographic analysis. This mixture was separated on a basic alumina column (activity grade I, M. Woelm Eschwege, 1.2 x 110 cm) with ethyl acetate eluent. Two mL fractions were collected from the column and sampled for testing for radioactivity. The radioactive ethyl acetate was pooled from 16 mL eluent, and showed no ethanol by gas chromatographic analysis. The overall yield based on total radioactivity was larger than 70%.

Ketene- $^2\text{H}_2$ , Acetic acid-2,2- $^2\text{H}_2$ -2- $^3\text{H}$  ethyl ester. Ketene- $^2\text{H}_2$  was generated by pyrolysis of acetone- $^2\text{H}_6$  vapor over a 24 B & S Chromel a filament ( $\sim 3.5$  m) heated at 700-750°C. Unreacted acetone- $^2\text{H}_6$  was condensed in a side arm of the pyrolysis chamber and brought back to the boiling acetone flask through a siphon (see apparatus illustration in Ref. 7). The ketene- $^2\text{H}_2$  was passed through two dry ice-acetone traps to remove unreacted acetone- $^2\text{H}_6$  and other byproducts of the pyrolysis. The ketene- $^2\text{H}_2$  was then bubbled into ethanol-0- $^3\text{H}$  (prepared (8) by reaction of  $(\text{CH}_3)_2\text{Si}(\text{OCH}_2\text{CH}_3)_2$  with HOT, catalyzed by a small amount of 20% HCl, followed by distillation of the labeled ethanol directly away from the oily silicone product) at 5°C for three hours.

The conversion of ethanol was followed by withdrawing 0.5  $\mu\text{L}$  samples for gas chromatographic analysis. The reaction was terminated at the time when no peak for ethanol was observable. However, a small peak due to a byproduct, probably an acetate ester of dimethylsilanediol, also appeared (about 4% by gas-chromatographic area ratio). Column chromatographic purification of the product

was carried out as above and resulted in 18 mL of radioactive ethyl acetate. This was further distilled from anhydrous  $K_2CO_3$  and  $CaCl_2$  to remove the presumed silyl ester. The overall yield on radioactivity was 72%. Nearly complete (>95%) incorporation into ethyl acetate of the radioactivity initially present in ethanol can almost certainly be achieved by insuring exposure of all ethanol to ketene, through the use of efficient cold traps following the reaction vessel.

Acetic acid ethyl-1- $^{14}C$  ester. Twenty mmol of ethanol-1- $^{14}C$  was refluxed with 30 mmol acetic anhydride and 100  $\mu$ L conc. sulfuric acid for seven hours at 70°C. The product that was distilled out of the reaction vessel showed no trace of impurities that have O-H stretching vibrations in the IR. The ethyl acetate was then diluted ten fold (with cyclohexane), treated with a few drops of water to decompose excess acetic anhydride and further purified with anhydrous  $CaCl_2$  and  $K_2CO_3$  as described earlier. The overall yield, based on total radioactivity, was 78%.

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