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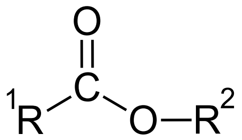
Molecular Spectroscopy
525 Verona Rd, Madison, WI 53711
(608) 276-6100
www.thermoscientific.com

picoSpin™ 45: The Fisher Esterification Reaction Synthesis of Isopentyl Acetate (Banana oil)

Dean Antic, Ph.D., Thermo Fisher Scientific, Boulder, CO, USA

1. Introduction

Esters are a class of compounds found widely in nature. Low molecular weight esters tend to have characteristic flavors and pleasant odors that are most often associated with essential oils, even though essential oils are a complex mixture.



The ^{1}R and ^{2}R group side chains can be linear or branch-chain aliphatic or aromatic groups and they can be the same or dissimilar groups.

The ester functional group can be synthesized by many methods. The simplest approach is Fisher esterification. By this method, esters are produced by refluxing a carboxylic acid and an alcohol in the presence of a concentrated acid catalyst. To exploit Le Chatelier's principle and shift the position of the equilibrium to the right we add an excessive of one of the reactants to the reaction mixture. Naturally, we choose the least expensive reactant, which, in this case, is the carboxylic acid. The reaction mechanism involves initial protonation of the carboxyl group, nucleophilic attack by the hydroxyl, proton transfer, and loss of water followed by loss of the catalyzing acid to produce the ester. The process is thermodynamically controlled yielding the most stable ester product. Typically, only primary and secondary alcohols are used in the Fisher method since tertiary alcohols are prone to elimination. In this lab, you will perform a Fisher Esterification to synthesize isopentyl acetate from isopentyl alcohol and acetic acid.

2. Purpose

The purpose of this experiment is to synthesize isopentyl acetate (3-methylbutyl acetate) via an esterification reaction between acetic acid and isopentyl alcohol (3-methylbutanol), using concentrated sulfuric acid as a catalyst. The product will be washed, distilled, then characterized using the Thermo Scientific™ picoSpin™ 45 NMR spectrometer.

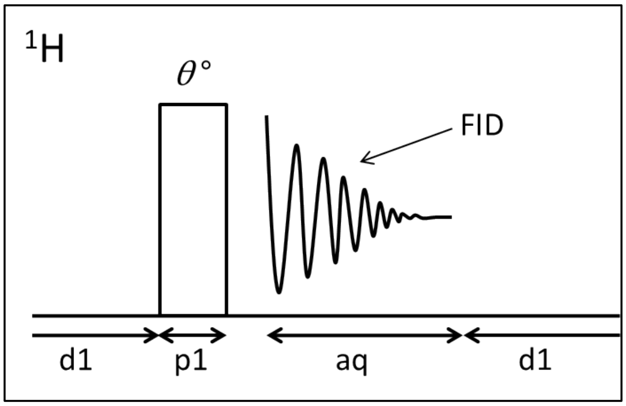
3. Literature

Adapted from: Gokel, H. D.; Durst, G. W. *Experimental Organic Chemistry;* McGraw-Hill, New York, 1980; pp 344-348.

CRC Handbook of Chemistry and Physics, 70th ed.; Weast, R.C., Ed.; CRC Press: Boca Raton, FL, 1990.

4. Pulse Sequence

In this experiment, we use a standard 90° single pulse experiment. The recycle delay time (d1) is adjusted to maximize signal intensity prior to signal averaging the next FID.



Sequence: $d1–[\theta°–aq–d1]_{ns}$
$\theta°$: Pulse rotation angle (flip angle)
FID: Free induction decay
d1: Recycle delay (µs) for spin-lattice relaxation
p1: R.F. transmitter pulse length (µs)
aq: Acquisition time (ms)
ns: # of scans (individual FIDs)

5. Procedures and Analysis

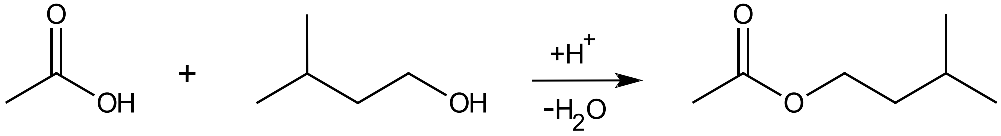
Time requirements: 3-3.5 hrs
Difficulty: Moderate
Sample: acetic acid, isopentyl alcohol, isopentyl acetate
Equipment/materials:

- Thermo Scientific™ picoSpin™ 45
- NMR processing software (Mnova)
- Ring stand
- Sand bath (or electric mantle)
- Boiling chips
- Separatory funnel
- Thermometer
- Clamps (flask or Keck)
- Simple distillation apparatus
  - 100 mL round bottom flask
  - 25 mL Erlenmeyer flask
  - Condenser
  - Three-way adapter
  - Vacuum adapter
- Thermometer adapter
- Iron ring
- Ice bath
- Syringe filter (optional filter)
- Syringe port
- Port Plug
- Tubing
- Reflux distillation apparatus
  - 50 mL round bottom flask
  - Condenser
  - Drying tube
- picoSpin accessory kit
  - 1 mL polypropylene syringes
  - 22 gauge blunt-tip dispensing needles
  - Drain tube assembly
- Inlet filter

Physical data:

Substance	FW (g/mol)	Quantity	MP (°C)	BP (°C)	Density (g/mL)
acetic acid (anhydr.)	60.05	25 mL	118		1.049
isopentyl acetate	130.19	product		142	0.876
isopentyl alcohol	88.15	20 mL		130	0.809
conc. H_2SO_4	98.08	5 mL			1.841
5% $NaHCO_3$	84.01	250 mL			1.0018
sat. NaCl		10 mL			
Na_2SO_4 (anhydr.)	142.04				

Reaction:

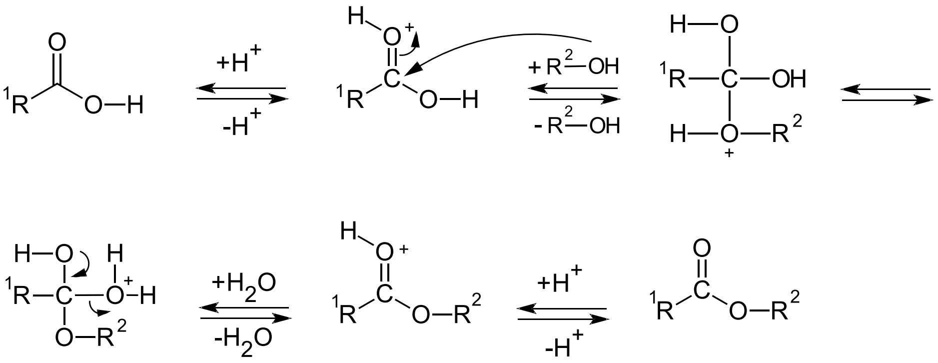


Acetic Acid

Isopentyl alcohol
(2-methylbutanol)

Isopentyl acetate
(2-methylbutyl acetate)

Mechanism:



Safety Precautions

CAUTION Eye protection should be worn at all times while using this instrument. Sulfuric acid is a strong dehydrating agent; wear protective gloves when pouring concentrated sulfuric acid. Acetic acid can burn the skin. Pour acetic acid in a fume hood. Avoid contact with skin and clothes.

⚠ **CAUTION** Avoid shock hazard. Each wall outlet used must be equipped with a 3-prong grounded outlet. The ground must be a noncurrent-carrying wire connected to earth ground at the main distribution box.

Experimental Procedure

- To a 100 mL round bottom flask add 25 mL (0.420 mol) glacial acetic acid **(**Caution: Hood) followed by 20 mL (0.185 mL) isopentyl alcohol (3-methyl-1-butanol). Swirl the flask to mix the layers.
- To the solution add (carefully, gloves) 5 mL concentrated sulfuric acid. Swirl the flask while sulfuric acid is added (heat generated).
- Add several boiling chips to the flask then place a reflux condenser with lightly greased joints on the flask as shown in Figure 1
- Bring the solution to boil using a sand bath, electric mantel or flame and reflux for 1 hr.

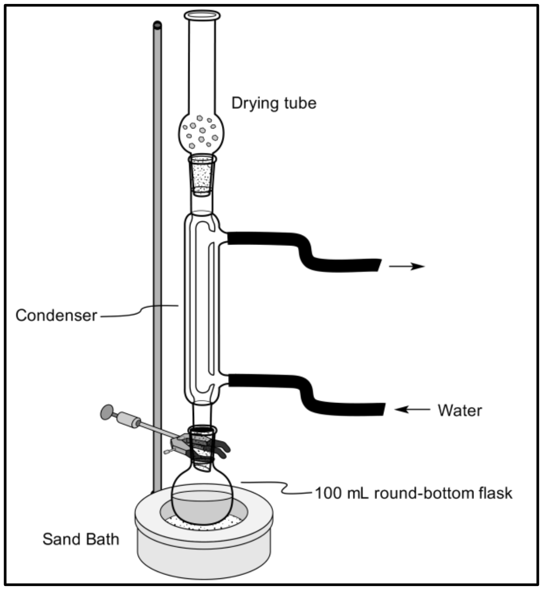


Figure 1. Reflux apparatus with exclusion of moisture

- After reflux is completed, allow the solution to cool to room temperature.
- Transfer the entire solution to a separatory funnel and add 50 mL distilled water. Swirl the solution, allow the layers to separate and remove the lower aqueous layer.
- Add another 25 mL portion of distilled water, shake the flask, and separate and remove the lower aqueous layer.
- Extract the organic layer with three 25 mL portions of 5% aqueous sodium bicarbonate solution to remove excess acetic acid (*Note*: Be careful, as carbon dioxide is given off during extraction.)
- Test the last extract and if the aqueous phase is not basic (pH paper), extract the organic layer with two more 25 mL portions of sodium bicarbonate solution ($NaHCO_3$).

- After removal of the acetic acid, wash the organic layer with two 5 mL portions of saturated salt solution.
- Transfer the organic layer to a 50 mL Erlenmeyer flask and dry over granular anhydrous sodium sulfate (Na_2SO_4) or magnesium sulfate ($MgSO_4$).
- After drying, the liquid should be clear; decant the organic layer into a 50 mL round bottom flask.
- Assemble a simple distillation apparatus as shown in Figure 2. Add several boiling chips and distill using a sand bath, electric mantel or flame. Cool the receiver flask in an ice bath.
- Collect the fraction that distills between 135°C and 143°C. The clear colorless product has an intense odor of bananas; it should be obtained in 80-90% yield.

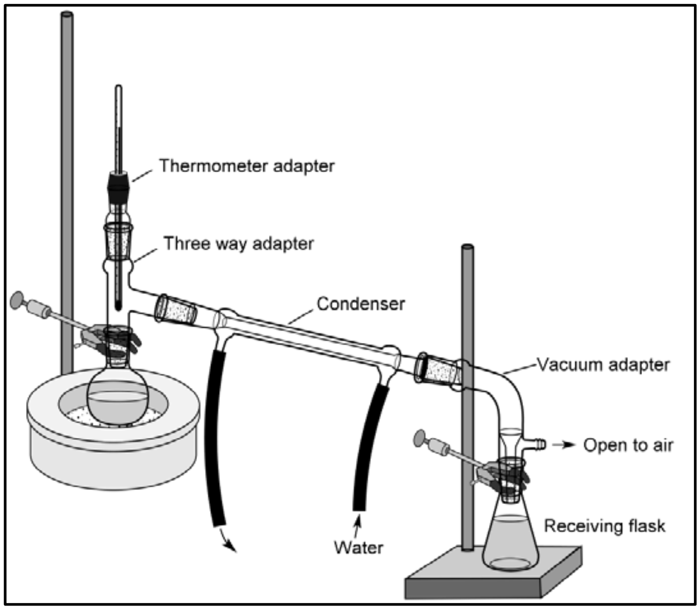


Figure 2 Simple distillation apparatus with sand bath

Analysis

Required:

Acquire ^{1}H NMR spectra of:

- Acetic acid (reactant)
- 3-Methyl butanol (reactant)
- 3-Methylbutyl acetate (distilled product)

Optional:

Acquire ^{1}H NMR spectra of:

- Initial reaction mixture (prior to reflux)
- Reaction mixture after reflux
- After H_2O wash and $NaHCO_3$ wash
- After $NaHCO_3$ wash, salt extraction and drying

NMR Sample preparation

Using a new, disposable 1mL polypropylene syringe fitted with a 1½"-22 gauge blunt-tip needle draw about 0.5 mL of glacial acetic acid (**Caution: Hood)** and transfer it to a 0.5 or 1 dram vial.

If available, to this sample, using a new, disposable 1mL polypropylene syringe fitted with a 1½"-22 gauge blunt-tip needle, rapidly draw out and add 6-10 droplets of tetramethylsilane (TMS). *NOTE:* TMS will begin to boil immediately after insertion of the syringe needle. Thus, sample transfer must be rapid.

Repeat this procedure for preparing samples of 3-methylbutanol and 3-methylbutyl acetate for NMR analysis. *NOTE:* Some of the *Optional* samples are aqueous and therefore TMS should not be added.

Instrumental procedure

The general procedure for sample analysis using a picoSpin NMR spectrometer is as follows:

Shim
- Ensure the NMR spectrometer is shimmed and ready to accept samples.

Pre-sample preparation
- Displace the shim fluid from the picoSpin capillary cartridge with air.
- Flush the cartridge with 0.1 mL of chloroform, and then displace the solvent with an air push. A small signal in your sample spectrum may appear at 7.24 ppm due to residual $CHCl_3$, it can be used to shift reference the spectrum.
- Set up the *onePulse* script according to parameters listed in the Pulse Script table.

Injection
- Using a 1 mL disposable polypropylene syringe fitted with a 1.5” long, 22 gauge blunt-tip needle, withdraw a 0.2 mL aliquot of sample.
- Inject about half the sample. Ensure all air bubbles have been displaced from the cartridge by examining the drain tube.
- Seal both the inlet and outlet ports with PEEK plugs.

Acquire
- Execute the onePulse script according to the values in the table of parameters provided

- Once the onePulse script has finished, prepare the cartridge for the next user by displacing the sample from the cartridge according to the following protocol: air, solvent, air.

Pulse Script: onePulse

Parameter	Value
tx frequency (tx)	proton Larmor frequency (MHz)
scans (ns)	16 or 25
pulse length (p1)	Instrument specific 90° pulse length
acquisition time (aq)	750 ms
rx recovery delay (r1)	500 µs
T1 recycle delay (d1)	10 s
bandwidth (bw)	4 kHz
post-filter atten. (pfa)	10 (11)[a]
phase correction (ph)	0 degrees (or any value)
exp. filter (LB)	0 Hz
max plot points	400
max time to plot	250 ms
min freq. to plot	-200 Hz
max freq. to plot	+1000 Hz
zero filling (zf)	8192
align-avg. data	✓
live plot	✓
JCAMP avg.	✓
JCAMP ind.	Unchecked

[a] Choose the instrument's default pfa values

6. Processing

Download the experimental JCAMP spectra files and open them by importing into Mnova. The free induction decay (FID) will undergo automatic Fourier transformation and a spectrum will be displayed.

To each spectrum, apply the following processing steps using the given settings:

Function	Value
Zero-filling (zf) & Linear Predict (LP)	16 k
Forward predict (FP)	From aq → 16 k
Backward predict (BP)	From -2 → 0
Phase Correction (PH)	PH0: Manually adjust
	PH1: 0
Apodization	

Exponential (LB)	0.6 Hz
First Point	0.5
Shift reference (CS)	Manually reference
Peak Picking (pp)	Manually Select Peaks
Integration (I)	Automatic Selection
Multiplet Analysis (J)	-

- Import each data file into the same workspace in Mnova. Manually apply Ph0 phase correction to each spectrum.
- Manually shift reference each spectrum using Mnova's TMS tool. Assign the TMS signal (0 ppm) or $CHCl_3$ signal (7.24 ppm), whichever is present.
- Identify and assign each signal in the spectra.
- Save the Mnova document, print each spectrum and paste into your lab notebook.

7. Results

Acetic acid (Figure 3) contains a carboxylic acid. Proton (^1H) NMR spectra of neat carboxylic acids are identified by a characteristic downfield (high frequency) chemical shift of the acid proton. The acidic nature of carboxylic acid protons make them strongly deshielded, with signals typically appearing between 11-12 ppm. Acidic protons also experience intramolecular hydrogen bonding and exchange rapidly, and rapidly exchanging protons tend to result in narrow signals. Adding a drop of D_2O (heavy water) to the sample causes the peak to disappear; this is evidence for the presence of a carboxylic acid, but then labile protons from alcohols, amines, thiols, phenols and enols will also exhibit this exchange behavior. Similarly, acidic protons experience intermolecular exchange with labile protons from other compounds, such as water, causing the signal to broaden and shift upfield (low frequency), closer to the labile proton chemical shift.

A characteristic feature of the ^1H NMR spectrum of 3-methylbutanol (Figure 4) is the presence of a coupled alcohol triplet appearing near 5 ppm. One reason a triplet structure is observed is that alcohol protons undergo dynamic exchange at various rates. Fast exchange leads to hydroxyl protons that do not couple with neighboring protons, resulting in a singlet. However, slow exchange allows time for coupling, such as that observed in 3-methylbutanol where the hydroxyl group proton couples with two methylene protons on the neighboring C_4 carbon, resulting in a triplet. Steric hindrance and intramolecular bonding can also interfere with dynamic exchange, allowing for coupling to adjacent protons. The C_4 protons (3.5 ppm) in turn couples with the alcohol and two methylene protons at position C_3, generating a quartet signal instead of a triplet.

As the reaction proceeds and 3-methylbutyl acetate is produced (Figure 5), two distinctive spectral features will appear in its NMR spectrum. The alcohol signal of 3-methylbutanol will disappear and the C_4 proton quartet will change into a triplet due to the loss of an adjacent alcohol proton; it will also shift downfield by 0.5 ppm, from 3.5 ppm to 4.0 ppm. The downfield shift of C_4 protons arises from increased deshielding of these protons as the adjacent alcohol group is transformed into the more electron-withdrawing ester functional group. Furthermore, evidence of the formation of product is seen in the presence of a singlet group appearing at just below 2 ppm; this signal belongs to the methyl ester group (C_1) of the carboxyl group (Figure 3 and Figure 5). The remaining signal groups due to protons within the isobutyl group ($C_{1\text{-}3,5}$ in 3-methylbutanol; $C_{5\text{-}7,9}$ in 3-methylbutyl acetate) are largely unchanged upon esterification of 3-methylbutanol.

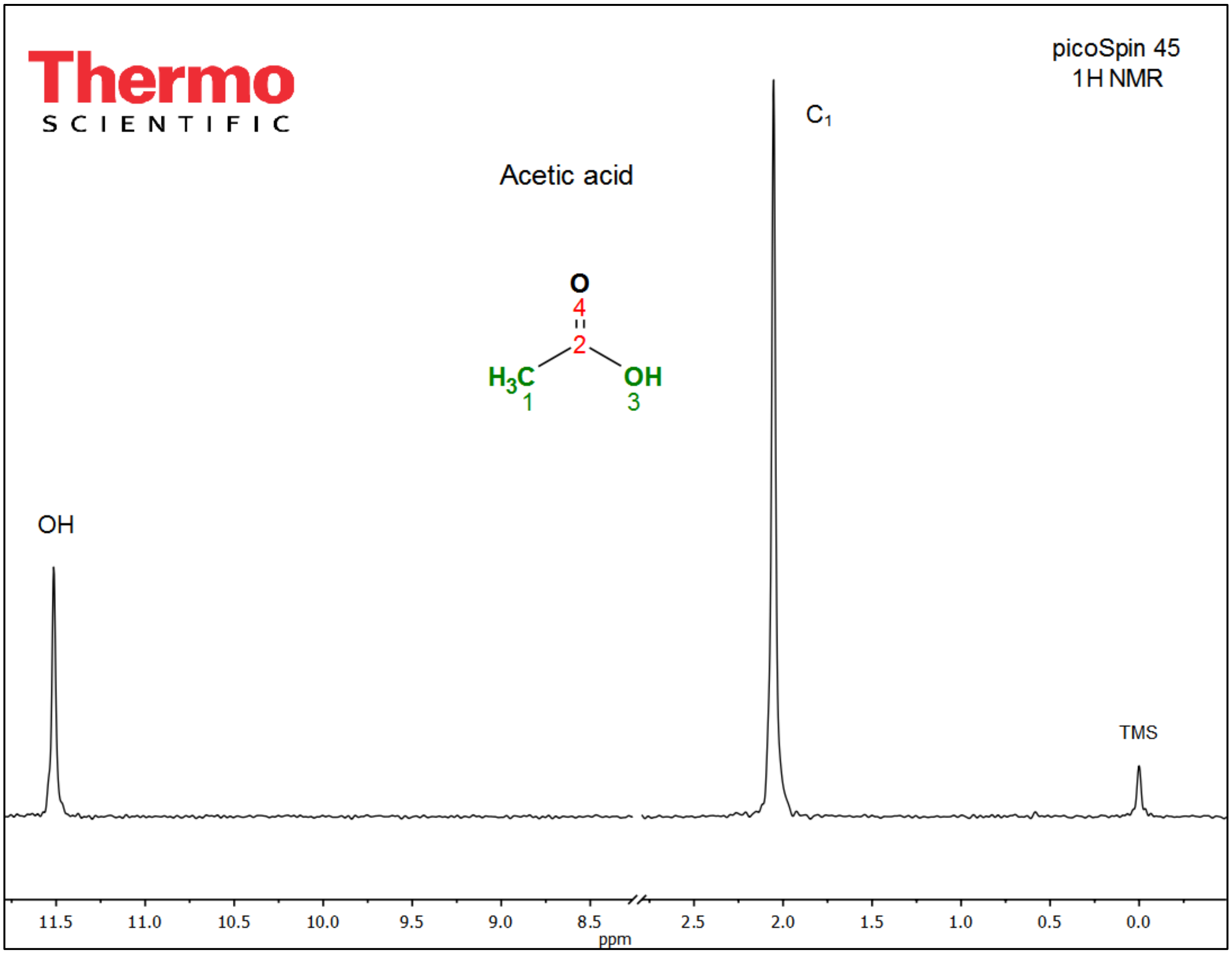


Figure 3 Full ^{1}H NMR (45 MHz) spectrum of acetic acid (anhdyr.; neat)

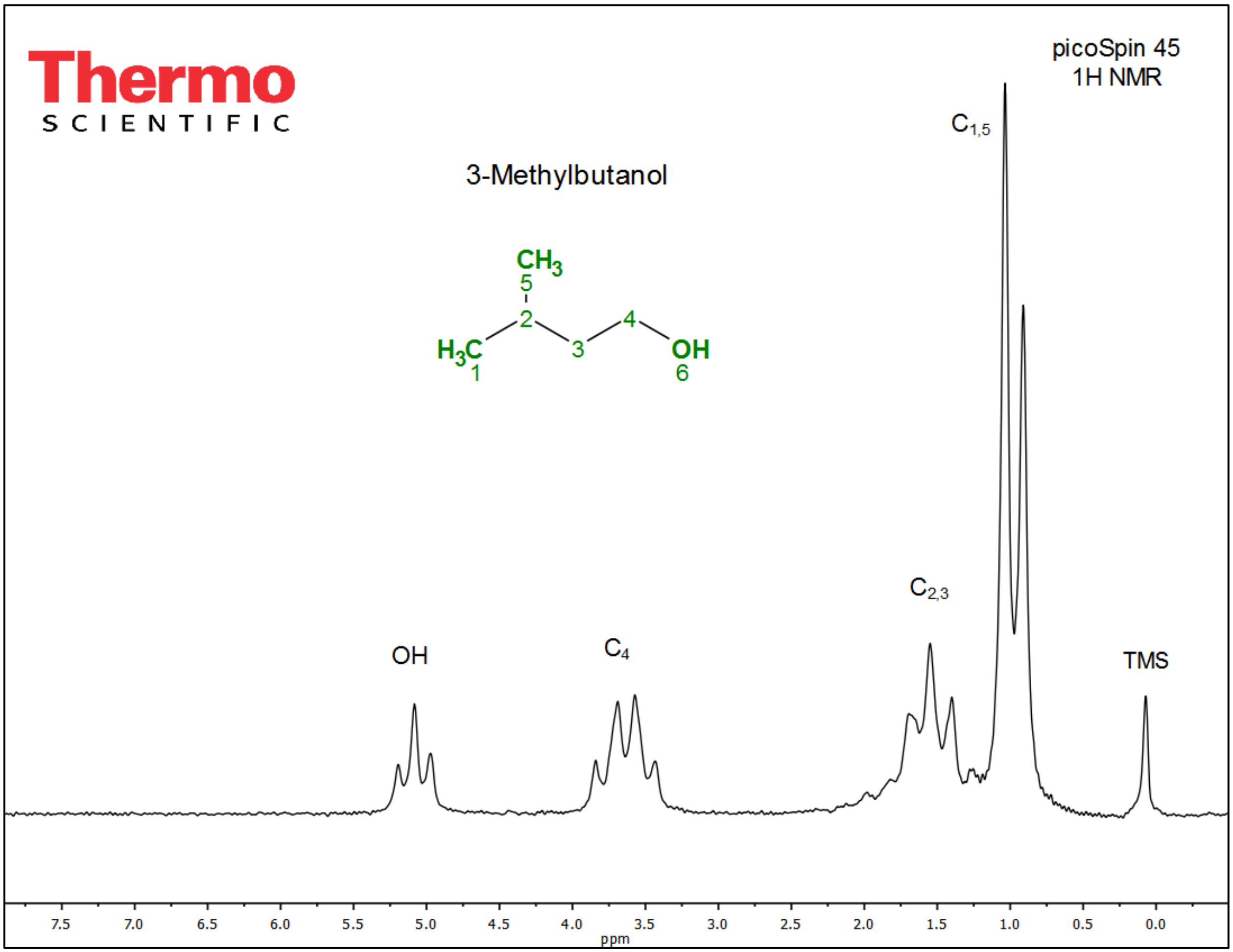


Figure 4 Full ^{1}H NMR (45 MHz) spectrum of 3-methylbutanol (neat).

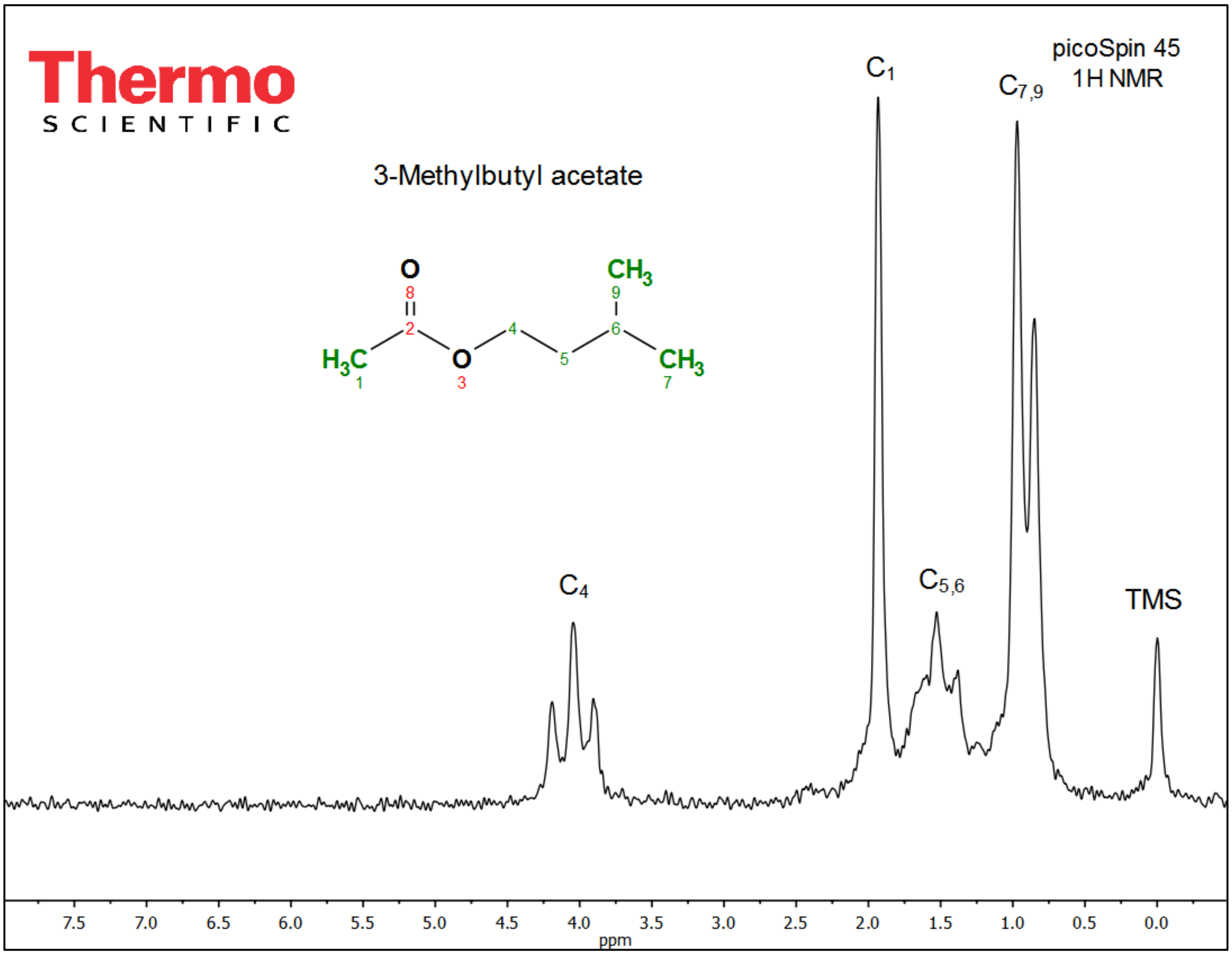


Figure 5 Full ^{1}H NMR (45 MHz) spectrum of 3-methylbutyl acetate (neat).

8. Comments

picoSpin 45 ^{1}H NMR proton spectra of neat acetic acid, 3-methylbutanol and 3-methylbutyl acetate are show in Figure 3-5. Chemical shifts and related NMR data are available in Table 1. Chemical shifts are referenced relative to TMS. Spectra are acquired from neat samples of reactants and product, and aliquots drawn from reaction mixtures. With the picoSpin 45 NMR spectrometer, it is not necessary to dilute samples prior to injection. However, 3-methylbutanol is somewhat viscous resulting in broadened signals, more so than 3-methylbutyl acetate, and dilution to 50% in $CDCl_3$ can improve signal resolution. Using a labile deuterated NMR solvent will result in the hydroxyl (OH) protons to exchange and its signal will diminish or disappear from the spectrum. Likewise, coupling to the methylene (C_4) proton will also be affected.

Presented in Figure 6- 8 are stacked ^{1}H NMR spectra acquired from neat reactants, isolated product, and spectra acquired from the reaction mixture during various stages of the experiment. These spectra are instructive in that they demonstrate the need for proper 'work up' of the reaction mixture prior to product distillation and isolation. Moreover, it is easy to visualize the changes in ^{1}H NMR spectra as reactants are converted to products. Figure 6 compares reactants and products, while Figure 7 includes the initial reaction mixture prior to addition of the acid catalyst (H_2SO_4). We see a hydroxyl signal appearing near 9.3 ppm, the apparent change of the methylene quartet to a triplet (~3.5 ppm), the appearance of a second methyl ester 'singlet' below 2.0 ppm and the broadening of all signals. The chemical shift of the OH group reflects the rapid exchange between the carboxylic acid proton and the alcohol, coalescing into one signal. Its position is dictated by the relative mole fraction of each component (acid and alcohol); that is, its chemical shift is linearly dependent on the mole fraction of the two labile protons under exchange. This is a well-known phenomenon to occur with mixtures of alcohols.

Looking at Figure 8 we see in the 'after reflux' spectrum (4th spectrum from the bottom) distinct and resolved singlet resonances due to the each type of carboxyl methyl group; one at 2.05 ppm from acetic acid and a second one at 1.93 ppm arising from the methyl ester product. The methylene ester (CH_2-O) appears shifted downfield to 4.0 ppm and is a clear triplet structure. In addition, due to the presence of mineral acid (H_2SO_4) the coalesced hydroxyl/carboxylic acid signal, appearing at 9.3 ppm in the initial reaction mixture, is further broaden and now centered on 9.7 ppm. The additional downfield shift arises from an increase of H^+ ions to the mixture.

After a water wash and neutralization with sodium bicarbonate ($NaHCO_3$), the merged labile hydroxyl/carboxylic acid proton signal (9.7 ppm) and the carboxylic acid signal (2.05 ppm) disappear (3rd spectrum from top). The methylene ester (CH_2-O) shows better resolution; also, a residual water signal is present. An additional $NaHCO_3$ wash, followed by salt extraction and drying of Na_2SO_4 (or $MgSO_4$) cleans up the product spectrum prior to distillation (2nd spectrum from top). The top two spectra in Figure 8 establish that even prior to distillation, the product 3-methylbutyl acetate is well isolated, and the 'before and after' distillation spectra appear nearly identical.

Table 1. ^{1}H NMR Spectral Data

Figure	Compound	Signal Group	Chemical Shift (ppm)	Nuclides	Multiplicity
3	acetic Acid	TMS	0	12 H	singlet
		HO-C(=O)CH_3	2.05	3 H	singlet
		HO-C(=O)CH_3	11.51	1 H	singlet
4	3-methylbutanol	TMS	0		singlet
		-CH-$(CH_3)_2$	0.90	6 H	doublet
		-CH_2-CH_2-CH-	1.47	2 H	triplet
		-CH-$(CH_3)_2$		1 H	multiplet

		$-CH_2-CH-(CH_3)_2$	3.56	2 H	quartet
		$HO-CH_2CH_2$	4.99	1 H	triplet
5	3-methylbutyl acetate	TMS	0	12 H	singlet
		$-CH-(CH_3)_2$	0.91	6 H	doublet
		$-CH_2-CH_2-CH-$	1.53	2 H	triplet
		$-CH_2-CH-(CH_3)_2$		1 H	multiplet
		CH_3COO-	1.93	3 H	singlet
		$O=CO-CH_2-CH_2$	4.05	2 H	triplet

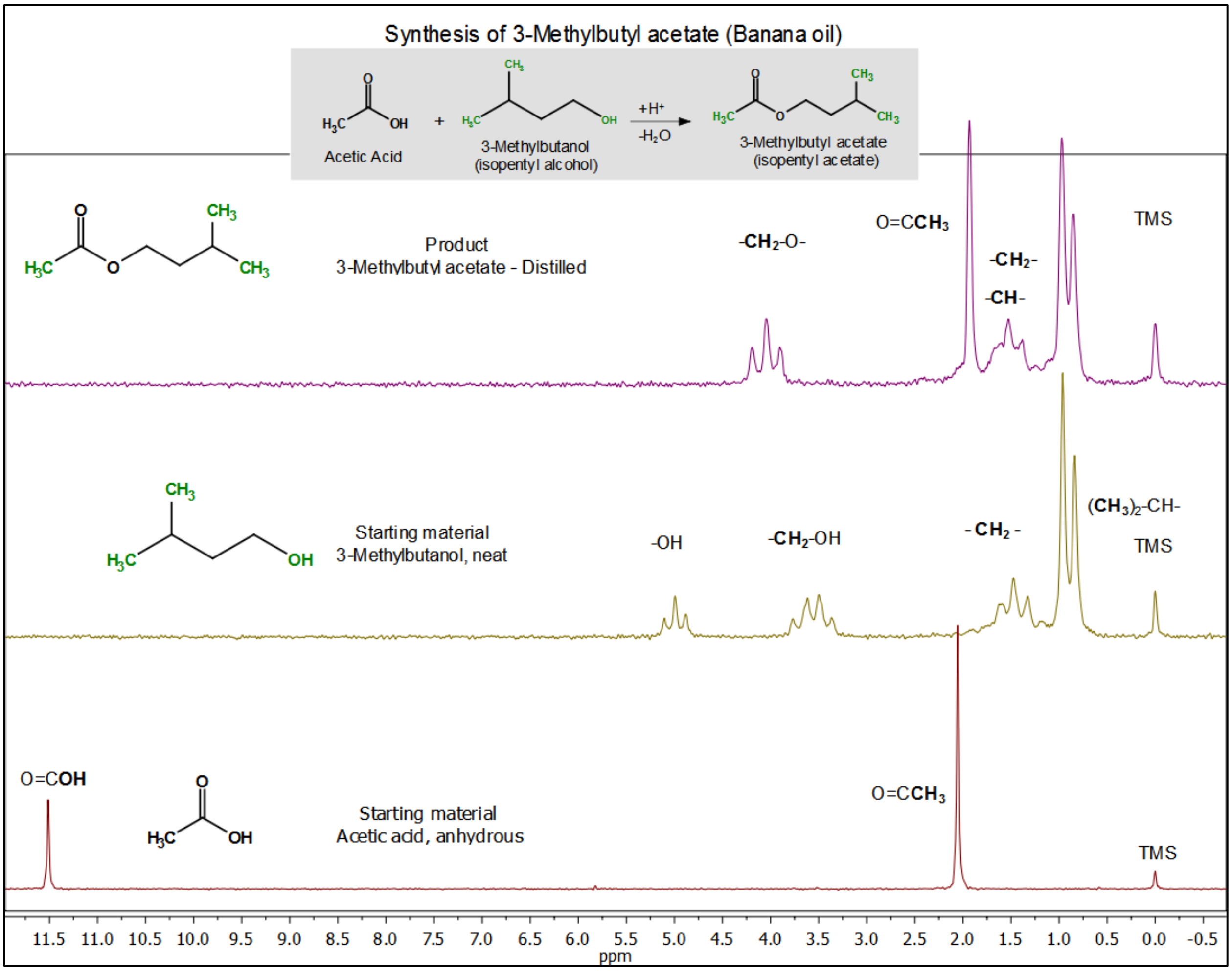


Figure 6 Full, stacked and labeled ^1H NMR (45 MHz) spectra of acetic acid and 3-methylbutanol (reactants), and 3-methylbutyl acetate (product)

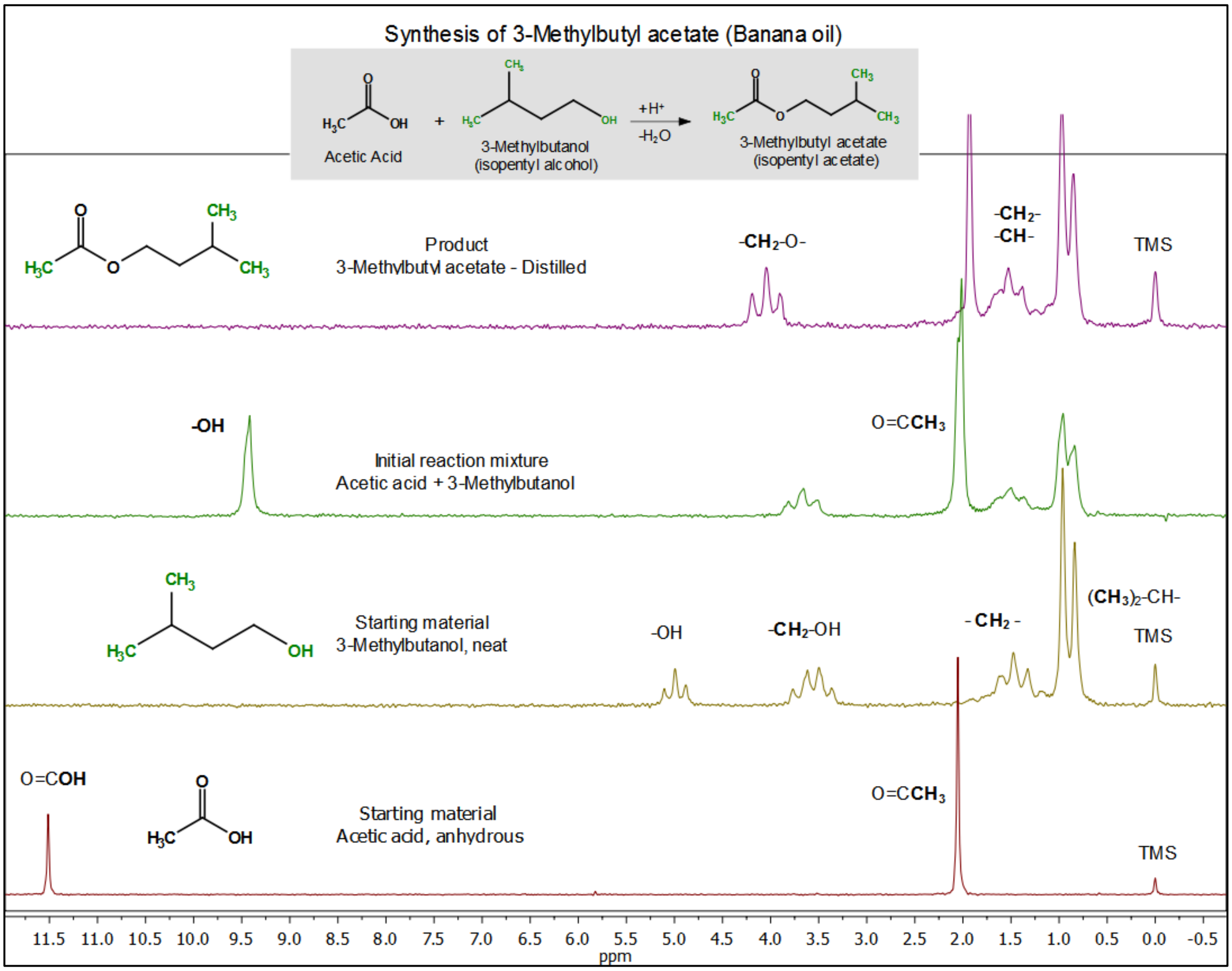


Figure 7 Full, stacked and labeled ^{1}H NMR (45 MHz) spectra of acetic acid and 3-methylbutanol (reactants), 3-methylbutyl acetate (product), and the initial reaction mixture prior to reflux.

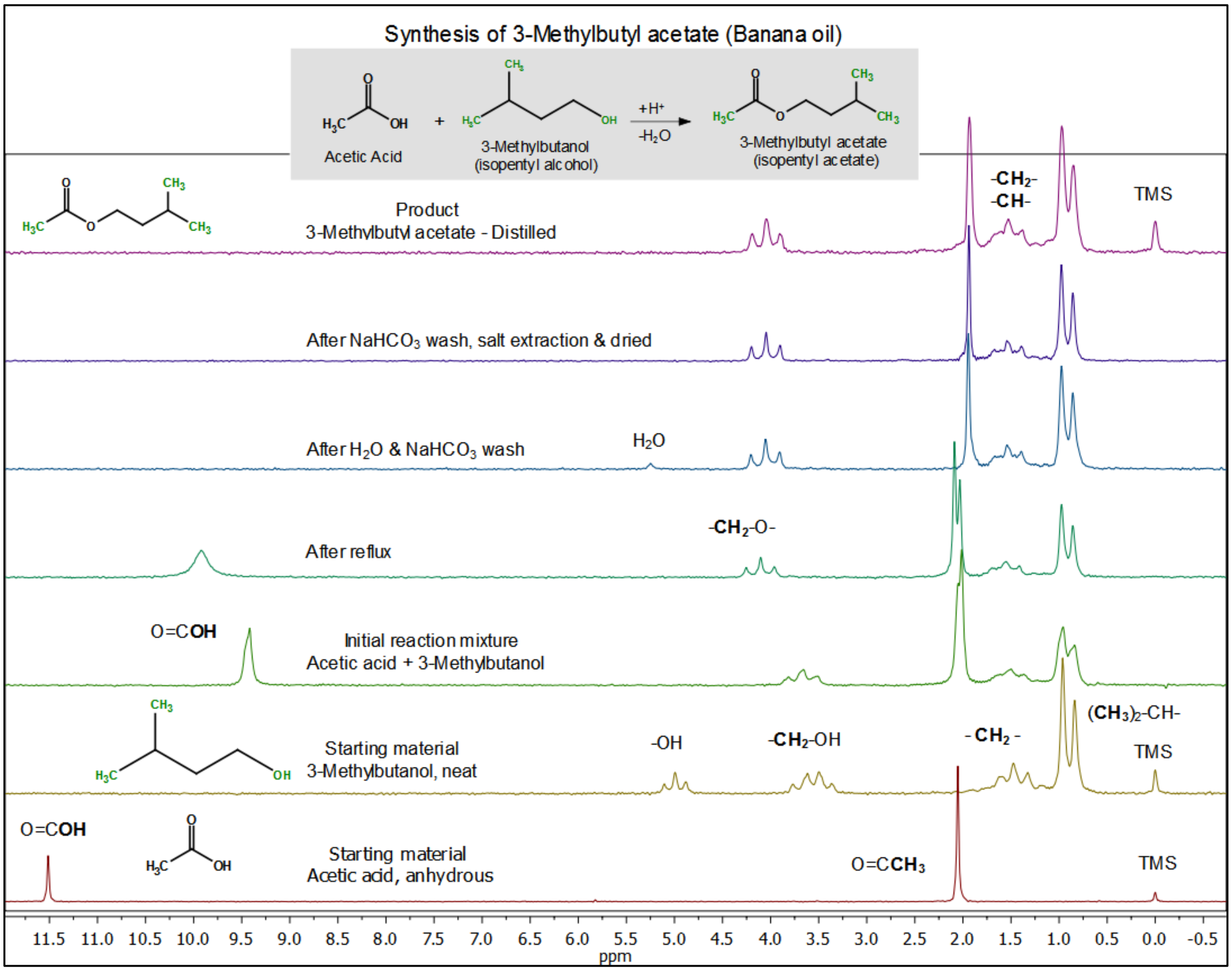


Figure 8 Full, stacked and labeled ^{1}H NMR (45 MHz) spectra of acetic acid and 3-methylbutanol (reactants), 3-methylbutyl acetate (product), and spectra acquired after different times during the experiment.

9. Own Observations

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