THE SYNTHESIS OF ^{14}C-TRANS-3-METHYL-2-HEXENOIC ACID LABELED IN VARIOUS POSITIONS*

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SUMMARY

The Emmons reaction was used for the synthesis of ^{14}C-trans-3-methyl-2-hexenoic acid. This compound is found in human sweat. 1-^{14}C-2-pentanone was prepared by the reaction at room temperature of ^{14}C-methylmagnesium iodide and butyronitrile.

INTRODUCTION.

Smith et al.[1], reported the presence of trans-3-methyl-2-hexenoic acid (TMHA) in the sweat of schizophrenic patients. Since information about the biological precursor of the acid and its metabolites seemed

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desirable, the syntheses of the labeled acid and a possible precursor-trans-3-methyl-2-hexenol, were undertaken. Should TMHA prove to be symptomatic of schizophrenia, the availability of labeled TMHA would be the basis for an "isotope-dilution" diagnostic test[2].

DISCUSSION.

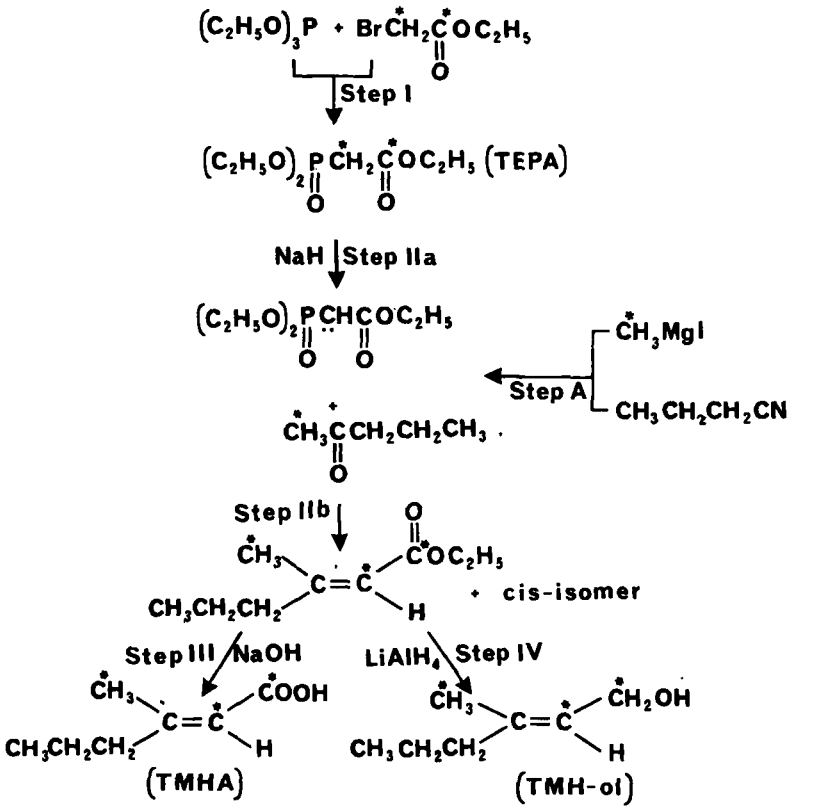
The Wadsworth and Emmons modification of the Wittig reaction[3] was chosen rather than the Reformatsky reaction[4, 5] since the overall yield of the desired compound is usually higher by this method. A second advantage is that only the cis and trans-2-enoic acids are formed while with the Reformatsky, considerable amounts of 3-hexenoic acids (cis and trans) are also formed. The synthetic method is outlined in Scheme I as is the synthesis of labeled 2-pentanone (Step A).

A number of runs using unlabeled ethyl and methyl bromoacetates in reaction with triethyl phosphate (STEP I) indicated that virtually quantitative yields could be obtained for triethylphosphonoacetate (TEPA) and diethylmethylphosphonoacetate (DEMPA). Four runs using labeled ethyl and methyl bromoacetates in the 2-position (3 runs) and the 1,2-positions (1 run) were carried out. These runs ranged from 1 to 13 mmoles of labeled acetate with total activities of 2-5 mCi's. The products from large runs were vacuum distilled giving 89% and 90% yields, while products of the smaller runs were used in the next step as such.

The syntheses of the esters of trans-3-methyl-2-hexenoic acid (TMHA) were carried out by preparing Wittig Reagent from either TEPA or DEMPA with sodium hydride in a dimethoxyethane solvent (STEP IIa). The 2-pentanone was then added (STEP IIb) at room temperature. The mixture was stirred for 24 hours before work-up. A 15/85 cis/trans mixture was obtained. The combined yields (of cis/trans isomers) ranged from 58 to 89%. The final run was different in that unlabeled TEPA was utilized and the label was introduced in the ester

by using 1-^{14}C-2-pentanone. This produced an ester in which the angular methyl group bore the label. The other esters synthesized carried the label in the 1-position and in one case, a dual label in the 1,2-positions.

SYNTHETIC SCHEME



* Indicates positions where label was introduced, though not all positions were simultaneously labeled.

The labeled 2-pentanone used in the previous step was not available commercially. One limitation in its preparation was the requirement that labeled methyl iodide be the source. It was used here mainly because of its ready availability and comparatively low cost. The best method was found to be the reaction of a nitrile and a Grignard[8] (STEP A) since it gave high yields under mild conditions and was free of side products. The yield for this reaction was about 65%. The radioactive run utilized 7 mmoles of ^{14}C-methyl iodide with a total activity of 20 mCi.

The esters of TMHA were hydrolyzed to the free acids by refluxing with 20% aqueous sodium hydroxide (STEP III). Since the ester is immiscible with the base, the reaction course could be determined by observing the disappearance of the upper phase. Complete hydrolysis took 5 hours. The free acid was then isolated by acidification with hydrochloric acid, extraction with ether and solvent stripping the dried ether extracts. Infrared and thin-layer chromatographic analysis confirmed the identity and purity of the TMHA thus prepared. Yields of acid in this step were 78-95%.

The reduction of the TMHA-esters (methyl and ethyl) to the unsaturated alcohols was carried out by room temperature reduction with lithium aluminum hydride (STEP IV)[9]. The suspension was stirred for 18 hours, hydrolyzed with mineral acid and extracted with ether. The ether extract contained the unsaturated alcohol which was free of cis-isomer. The alcohol was further purified by chromatography. The yield of alcohol was 95-99%.

In all of the syntheses, considerable amounts of the cis isomer were always obtained. The separation of isomers was always accomplished by preparative gas-liquid chromatography of the ester. The isomerically pure ester could then be hydrolyzed or reduced. No isomerization occurred during these steps.

EXPERIMENTAL.

^{14}C-Triethylphosphonoacetate. A 10 ml one-necked flask was charged with 2.03 g of 2-^{14}C-ethylbromoacetate (12 mmole, 2 mCi) and 2.16 g of triethylphosphite. The reactants were heated under reflux at 160°-165° C for 5 hours. The product was a pale yellow liquid. It was purified by distillation (140°-145°/9 mm) to give 2.41 g (89.5% yield) of the ester.

Ethyl 2-^{14}C-trans-3-methyl-2-hexenoate. A 100 ml reaction kettle was fitted with a thermometer, mechanical stirrer, dropping funnel and a reflux condenser with drying tube. The flask was charged with 25 ml of anhydrous dimethoxyethane and 463 mg of a 57% oil dispersion of sodium hydride (264 mg NaH, 11 mmole). The stirred dispersion was cooled to 0°-5° C and 2.4 g of ^{14}C-triethylphosphonoacetate (11 mmole, 1.78 mCi) in 3 ml of dimethoxyethane was added, drop-wise, so that the reaction temperature was kept below 6° C. After addition was complete the mixture was warmed to room temperature and stirred until hydrogen evolution had ceased. The resulting solution was cooled to 0°-5° C and 922 mg of 2-pentanone (10.7 mmole) was added drop-wise keeping the temperature below 6° C. After addition was complete the mixture was stirred at room temperature for 24 hours. The mixture was then hydrolyzed with water (20-30 ml) and extracted with three 25 ml portions of ether. The combined ether extracts were dried over anhydrous magnesium sulfate and solvent stripped using a stream of dry nitrogen gas. The product was purified by preparative scale gas-liquid chromatography. The product was a 15/85 cis/trans mixture.

1-^{14}C-2-pentanone. A three-necked flask was fitted with a thermometer, mechanical stirrer and a reflux condenser with drying tube. The flask was charged with 197 mg of magnesium turnings and 10 ml of anhydrous ether. The

dropping funnel was charged with a solution of 1.00 g of methyl iodide (7.04 mmole, 20 mCi) in 10 ml absolute ether. The methyl iodide was added slowly until Grignard formation was initiated and then at such a rate as to maintain gentle refluxing. After addition was complete an additional 100 mg of unlabeled methyl iodide was added to react with the residual magnesium. A solution of 486 mg of butyronitrile (7.04 mmole) in 10 ml of absolute ether was then added drop-wise over a ten-minute period[8]. The reaction was stirred overnight at room temperature.

The reaction mixture was then cooled and hydrolyzed with saturated aqueous sodium chloride (2-3 ml) followed by sufficient 10% hydrochloric acid to form two precipitate-free layers. The ether layer was then separated and the aqueous phase extracted with two, five ml portions of ether. The combined ether extracts were dried over anhydrous magnesium sulfate and distilled at 70° C, through a Vigreux column to remove the bulk of the ether. The residue, a dark liquid was purified by preparative-scale gas-liquid chromatography to give .415 g of pure 2-pentanone.

2-^{14}C-trans-3-methyl-2-hexenol[9]. A 100 ml three-necked flask was fitted with a thermometer, addition funnel and condenser with drying tube. To the flask was added 20 ml of absolute ether and 1.20 g of lithium aluminum hydride (31 mmole). The suspension was cooled to 0°-4° C and then 1.20 g of 2-^{14}C-trans-3-methyl-2-hexenoate (0.9 mmole, 5 mCi) in 8 ml of absolute ether was added drop-wise over a 10-minute period. The temperature was kept below 10° C. After addition, the mixture was warmed to room temperature and stirred for 24 hours. The mixture was then cooled to 0-5° C, hydrolyzed with water and sufficient 10% hydrochloric acid to give two precipitate free layers. The pH of the aqueous phase was checked to ensure that too much acid had not been used (final pH of 6). The ether layer was then separated and the aqueous phase extracted with two, 15 ml portions of ether.

The combined extracts were dried over anhydrous magnesium sulfate and solvent stripped by passing a stream of dry nitrogen through the solution. The yield was 70-72% with a B.P.$_{4mm}$=34° and $[n]_{24}^{D}$=1.4450.

Preparative chromatography was carried out using a Nester-Faust Co. (Wilmington, Delaware) apparatus with an electrostatic precipitator. The "Supelco" (Bellefonte, Pennsylvania) column was 10 ft. long, 1/2" ID annular, "bi-wall" stainless steel, packed with SE-30 on chromosorb-6W, 60/80 mesh. Helium gas was used at 30 lbs pressure. Nuclear magnetic resonance studies were made to establish structure and purity utilizing a Perkin-Elmer (Norwalk, Connecticut) R-12 Nuclear Magnetic Resonance Spectrometer using deuterated chloroform solutions.

The following compounds were synthesized by the methods described.

1. ethyl 2-^{14}C-trans-3-methyl-2-hexenoate
2. 2-^{14}C-3-methyl-2-hexenol-1
3. 2-^{14}C-3-methyl-2-hexenoic acid
4. methyl-2-^{14}C-trans-3-methyl-2-hexenoate
5. ethyl 1,2-^{14}C-trans-3-methyl-2-hexenoate
6. 1-^{14}C-2-pentanone
7. 1,2-^{14}C-trans-3-methyl-2-hexenoic acid
8. ethyl trans-^{14}C-3-methyl-2-hexenoate
9. trans-^{14}C-3-methyl-2-hexenoic acid

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