SYNTHESIS OF DETERIUM LABELED PLANT ETHYLENE PRECURSOR

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SUMMARY

Synthetic methods for the preparation of β -deuterium labeled 2-keto-4-methylbutyric acid were investigated. Vinyl chloride was first reacted with the ethyl oxalyl chloride moiety using aluminum chloride as condensing agent and the addition of methyl mercaptan followed. Deuterium labeling was achieved by using $NaBD_4$ reduction in pyridine.

Key Word : Plant Ethylene Precursor, 2-Keto-4-methylbutyric acid, Deuterium Labeling

INTRODUCTION

Ethylene has been recognized as an endogenously produced substance which promotes fruit ripening and regulates some aspects of plant growth. L-Methionine and some derivatives such as methional and 2-keto-4-methylthiobutyric acid (hereafter, KMBA) are oxidized enzymatically with production of ethylene (1,2,3). KMBA is a widely used oxygen-radical scavenger and has been used for the detection of hydroxy-like radical species in a variety of systems.

This paper describes a synthesis of β -deuterium labeled KMBA from ethyl oxalyl chloride. α -Deuterium labeled methionine was prepared by the treatment(4) of methionine with D_2O-aluminum sulfate, but the α -protons of methionine or KMBA are easily exchangeable. Therefore β -labeled KMBA is more useful in the study of biosynthesis of ethylene.

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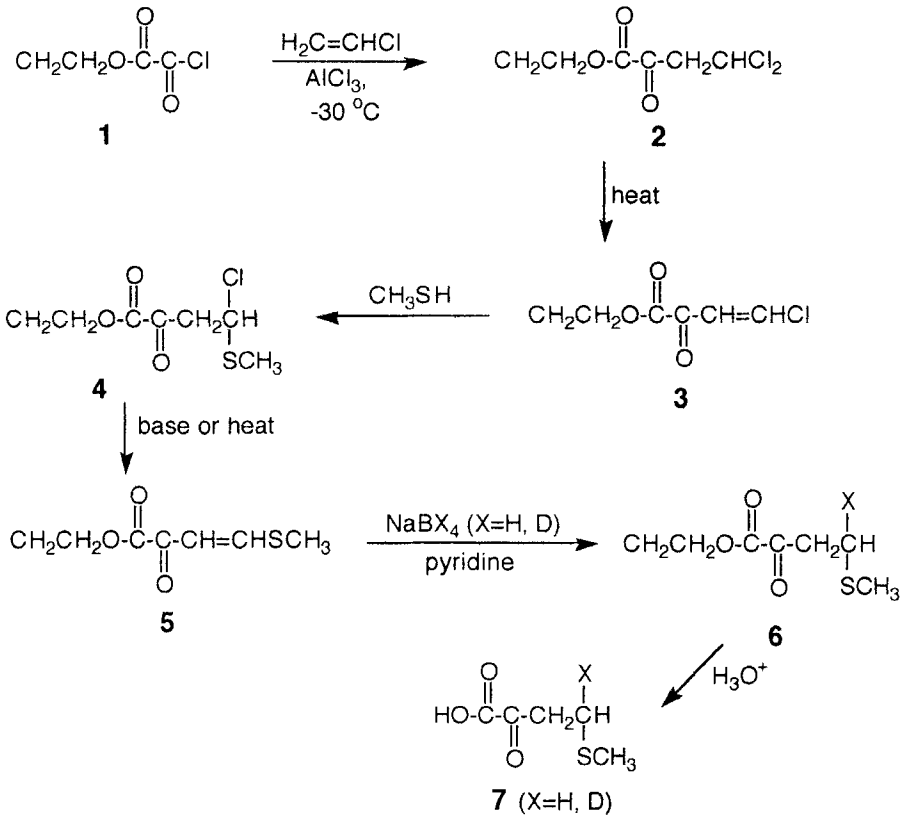
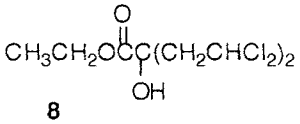
RESULT AND DISCUSSION

For the synthesis of labeled KMBA, our strategy was to construct the main frame of KMBA, then introduce label at C-3 and C-4 by the hydrogenation of a substrate with unsaturation between C-3 and C-4 such as vinyl ketones **3** or **5** in Scheme I. The addition(5) of methyl mercaptan could be performed before or after

Scheme I



hydrogenation. The preparation of the substrate **3** began by chlorovinylation of ethyl oxalyl chloride with vinyl chloride. Dichloro compound **2** was obtained using a modified procedure(6) for the synthesis of β -chlorovinyl ketone, that is, the reaction temperature must keep under -25 ℃ for 24 h. Purification of **2** was not attempted. After filtering off the crystalline bis dichloro substituted compound **8**, the filtrate was



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distilled under vacuum to give 35~45 % yield of yellow liquid **3** resulting from loss of HCl during distillation. The ^1H NMR spectrum of **3** indicated that **3** consisted of more than 95 % trans isomer.

Hydrogenation of **3** was attempted using several different catalyst such as PtO_2, 5 % Pd/charcoal, 5% $Pd/BaSO_4$, 5% Rh on alumina, and tris (triphenyl phosphine) chloro Rh[1](7) in different solvents. None of them were successful. Spectral data from all the reactions indicated a mixture of several species such as hydrogenolysis product, alcoholic product, and some unknowns, but none of **4**. After failing at hydrogenation of **3**, addition of methyl mercaptan was attempted. The addition proceeded smoothly without base in high yield (85%). The ^1H NMR spectrum showed that **7** consisted of 75 % trans and 25 % cis-isomer. But hydrogenation of **7** was also unsuccessful. Diimide reduction(8) was attempted with **7** but only unsaturated alcoholic products were obtained.

It has been reported that reduction of α , β -unsaturated ketones with $NaBH_4$ gives only the products of 1,4-addition rather than 1,2-addition in the presence of excess pyridine(9). Reduction of **5** was carried out in pyridine with $NaBH_4$. Exclusion of moisture was attempted but the formation of saturated alcohols indicates that a proton source is available to convert the metal hydride salt of the enolate to enol which could then be reduced, probably through the keto form. As soon as **5** was added to pyridine-$NaBH_4$ solution at room temperature, the solution turned red and the reduction was complete within 10 min. The crude product was subjected to chromatography to give about 30 % of **6** and 30% of saturated alcohol **8**. The ^1H NMR spectrum of **6** showed two triplets at 2.7 and 3.1 ppm for the α ,β -,methylene protons, a singlet at 2.1 ppm for thiomethyl protons, and a triplet at 1.3 and a quartet at 4.3 ppm for ethyl protons. About 4% of **5** accompanied these products and could not separated, but was removed by acid hydrolysis in the next step. The reduction yield could be raised by oxidizing the saturated alcohol **8** with a DMSO-acetic anhydride mixture(10).

Hydrolysis of **6** was carried out under both acid and base conditions(11). Under 1N HCl condition **6** was hydrolyzed slowly at 50 ℃ to give cleanly β -ketoacid **7** without any contamination. In the case of base hydrolysis the reaction was very fast but the product was contaminated with unsaturated keto acid **5**. The ^1H NMR

spectrum of **7** showed two triplets at 2.8 and 3.2 ppm for two α ,β -methylene protons and a singlet at 2.1 ppm for thiomethyl protons; which is consistent with the reported ^{1}H NMR data(12).

In order to introduce deuterium at the unsaturated double bond of **5**, $NaBD_4$ was used instead of $NaBH_4$ following the same procedure as described above. The ^{1}H NMR spectrum of β -labeled **6** showed a singlet at 2.1 ppm for the thiomethyl protons, and two multiplets at 2.8 ppm and 3.2 ppm with the intensity 1:2, indicating that 2.8 ppm multiplet represents one proton from the β -methylene and 3.2 ppm the two protons from the α -methylene group. Hydrolysis of deuterium labeled **6** was carried out under the same conditions as described above, that is, under 1 N HCl conditions and **6** hydrolyzed slowly at 50 ℃. The ^{1}H NMR spectrum of β -deuterium labeled **7** showed a singlet at 2.1 ppm for the thiomethyl protons, and two multiplets at 2.8 ppm and 3.2 ppm for the α ,β -methylene protons.

Hydrolysis of **5** was attempted before reducing with $NaBH_4$ as an alternative pathway. With 1N NaOH the reaction was complete within 5 min but the following reduction of unsaturated keto acid yielded a mixture of several components including **5,6,7** and unknowns which could not be separated.

EXPERIMENTAL

Ethyl oxalyl chloride and other reagents were obtain from Aldrich Chemical Co., and were used without further purification. THF and dioxane were dried by distillation from Na-benzophenone. Pyridine was dried by distillation from CaH_2 and kept over molecular sieves. Proton and carbon NMR spectra were recorded on a Brucker AMX-300. Chemical shifts are reported as values in parts per million relative to tetramethylsilane (0.0) as an internal standard.

Ethyl 4-Chloro-2-oxo-3-butenoate (3)

A mixture of 18.4 mL (0.16 mole) of ethyl oxalyl chloride and 22 g (0.16 mole) of aluminum chloride was dissolved in 90 mL of 1,2-dichloroethane at 0 ℃. The temperature of the mixture then brought to -30 ℃ using STIR-KOOL. Dry vinyl chloride was introduced into the solution through an inlet tube reaching to the bottom

of vessel. The vinyl chloride was generated by the decomposition of 42 mL of dichloroethane with 50 g of NaOH in 150 mL of 60% aqueous ethanol. The temperature of the generating flask was set at 40~42 ℃ so that the vinyl chloride produced was effectively used for the reaction. The reaction took 24 h for completion and then it was set aside for another 24 h at -30 ℃. The resultant dark brown solution was poured into a mixture of 200 g of ice and 100 mL of water. The mixture was allowed to stir for 30 min., then separated and the organic layer was washed with water, dried over sodium sulfate, filtered, and the solvents were evaporated. The oily residue was triturated with 70 mL of hexane and put in the refrigerator overnight. Filtration gave 6.5 g of white crystal of 3-ethoxycarbonyl-3-hydroxy-1,1,5,5-tetrachloropentane **8** (mp 108-110 ℃). The filtrate in 100 mL dichloroethane was treated with pH 7 buffer 400 mL for 1h, then separated and dried over sodium sulfate, and the solvent evaporated. The residue was distilled under vacuum to give 12.1 g (47%) of yellow liquid (bp 87 ℃ at 11 mmHg): ^{1}H NMR ($CDCl_3$) of **8.** δ 5.98 (pair of d, 2H, -$CHCl_2$), 4.30 (q, 2H, -OCH_3), 3.91 (br s, 1H, OH), 2.91 (m, 4H, -CH_2-), 1.40 (t, 3H, -CH_3); Anal. Calcd for $C_8H_{12}O_3Cl_4$: C, 32.24; H, 4.03; Cl, 47.62. Found: C, 32.48; H, 4.04; Cl, 47.43. ^{1}H NMR ($CDCl_3$) of 3: 7.70 and 7.14 (pair of d, 2H, -CH=CHCl, J= 18 Hz), 4.38 (q, 2H, -OCH_2-), 1.40 (t, 3H, -CH_3). For **3**: ^{13}C NMR ($CDCl_3$) 179.78 and 160.54 (two ester groups),142.66 and 160.54 (-CH=CHCl), 62.70 (-OCH_2-), 13.77 (-CH_3). Anal. Calcd for $C_6H_7O_3Cl$: C, 44.32; H, 4.34. Found: C, 44.55; H, 4.32.

Ethyl 4-Methylthio-2-oxo-3-butenoate (5)

A solution of **3** (4.0 g, 0.025 mole) in 20 mL THF was cooled to -75 ℃ and methyl mercaptan about 16 mL was introduced through a septum. The solution was allowed to warm to room temperature. Reaction continued until the solution became colorless (about 2 days). Excess methyl mercaptan was removed and the residue was treated with 30 mL of methylene chloride and 50 mL of pH 7 phosphate buffer for 1h. After separating the organic layer, it was dried, and the solvents evaporated. The residue was distilled under vacuum to collect 3.52 g (81%) of **5** as a yellow liquid (bp 97 ℃ at 5 mmHg): ^{1}H NMR ($CDCl_3$) 8.15, 7.56, 7.02, and 6.62 (four pair

of d, 75% trans 25% cis, 2H, -CH=CHS-), 4.32 (q, 2H, $-OCH_2-$), 2.48 and 2.42 (two s, 3H, $-SCH_3$, trans and cis), 1.36 (t, 3H, $-CH_3$). ^{13}C NMR ($CDCl_3$) 179.68, 177.89, 161.94, 161.04, 159.64, 153.85, 116.30, and 115.23 (two carbonyl ester and -CH=CHS-, trans and cis), 61.90 and 61.75 ($-OCH_2-$, trans and cis), 14.41 and 14.38 ($-SCH_3$ and $-CH_3$). Anal. Calcd for $C_7H_{10}O_3S$: C, 48.28; H, 5.75. Found: C, 48.34; H, 5.90.

Ethyl 4-Methylthio-2-oxobutyrate (6)

To a solution of 0.38 g (0.01 mole) $NaBH_4$ in 30 mL of pyridine, 0.25g (0.002 mole) of **5** was added over a period of 3 min in a nitrogen atmosphere. After 10 min the dark red solution was poured into a mixture of 35 mL of conc. HCl and 50 g of ice. The mixture was allowed to stand for 30 min and extracted several times with dichloromethane. The solvent was removed and residue was subjected to flash chromatography (eluant: $CHCl_3$). The product **6** so obtained was dried under vacuum to give 0.11g (31%) of pale yellow oil: ^1H NMR ($CDCl_3$) 4.28 (q, 2H, $-OCH_2-$), 3.10 and 2.73 (two t, 4H, $-CH_2CH_2S-$), 2.07 (s, 3H, $-SCH_3$), 1.31 (t, 3H, $-CH_3$). For the preparation of β -D labeled **6** the same synthetic procedure was followed except $BaBD_4$ rather $NaBH_4$. ^1H NMR of β -D labeled **6** ($CDCl_3$): 4.28 (q, 2H, $-OCH_2-$), 3.10 and 2.73 (two m, 4H, $-CH_2CHDS-$), 2.07 (s, 3H, $-SCH_3$), 1.31 (t, 3H, $-CH_3$).

4-Methylthio-2-oxobutyric Acid (7)

A solution 100 mg of **6** in 3 mL of 1N HCl was heated at 50~55 ℃ for 9 h. The reaction mixture which formed two layers at the initial stage of the reaction turned to a clear pale yellow after 2 h. Sodium chloride was added to the reaction mixture was then extracted with ether. The ethereal layer was washed with aqueous sodium bicarbonate to remove the acidic substances. The aqueous layer was washed with ether, acidified with HCl, salted out with sodium chloride, and extracted with ether. Evaporation of the ether and vacuum dry left **7** as a viscous oil (60 mg, 72 %): ^1H NMR (acetone-d_6) 3.21 and 2.79 (two t, 2H, $-CH_2CH_2S-$), 2.12 (s, 3H, $-SCH_3$). The same recipe was applied for the synthesis of β -D labeled **7**. ^1H NMR of β -D labeled **7** (acetone-d_6): 3.21 and 2.79 (two m, 4H, $-CH_2CHDS-$), 2.12 (s, 3H, $-SCH_3$).

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