SYNTHESIS OF CARBON-13 LABELED 5-(DIETHYLPHOSPHONO)-2-PENTANONE ETHYLENE KETAL, A REAGENT FOR SYNTHESIS OF MULTI C-13 LABELED STEROIDS

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SUMMARY

Alkylation of ethyl acetoacetate-$^{13}C_4$ with ethylene oxide and ethylene oxide-$^{13}C_2$ afforded 2-acetylbutyrolactone-$^{13}C_4$ (4a) and the hexalabeled form (4b), respectively. Treatment of the lactone with an excess of 48% hydrobromic acid effected decarboxylation with bromide displacement to yield 5-bromo-2-pentanone labeled with 3 or 5 carbon-13 atoms (5). The bromo ketone was converted to the ethylene ketal with ethylene glycol and p-toluenesulfonic acid catalysis. Displacement of the bromide by sodio diethyl phosphite gave the required ethylene ketals of 5-(diethylphosphono)-2-pentanone (7). A convenient process for preparation of ethylene-$^{13}C_2$ oxide from acetic-$^{13}C_2$ acid is also described.

Key Words: 5-Diethylphosphono-2-pentanone, carbon-13, multi-labeled steroid

INTRODUCTION

The measurement of cortical steroids at low concentrations in biological media by mass spectrometric methods has caused a need for these complex steroids multilabeled with carbon-13. In order for the labels to be retained after subjection to potential metabolic action or mass spectrometric fragmentation it was essential to introduce the labeled atoms in the steroid skeleton rather than the susceptible 17-side chain. Henrick, et al[1] reported methods for construction of the A-ring of the steroid with the key step being the reaction of 5-(diethylphosphono)-2-pentanone ethylene ketal with an enol lactone such as 7. This procedure, a useful variation of the general method of Velluz and coworkers,[2] appeared to be well suited for introduction of multiple C-13 labels in the Ring A-B region of the steroid system. This manuscript de-

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scribes the synthesis of the phosphono ketal labeled at positions 1, 2, 3 (7a) and 1, 2, 3, 4, 5 (7b) with 99 atom percent ^{13}C.

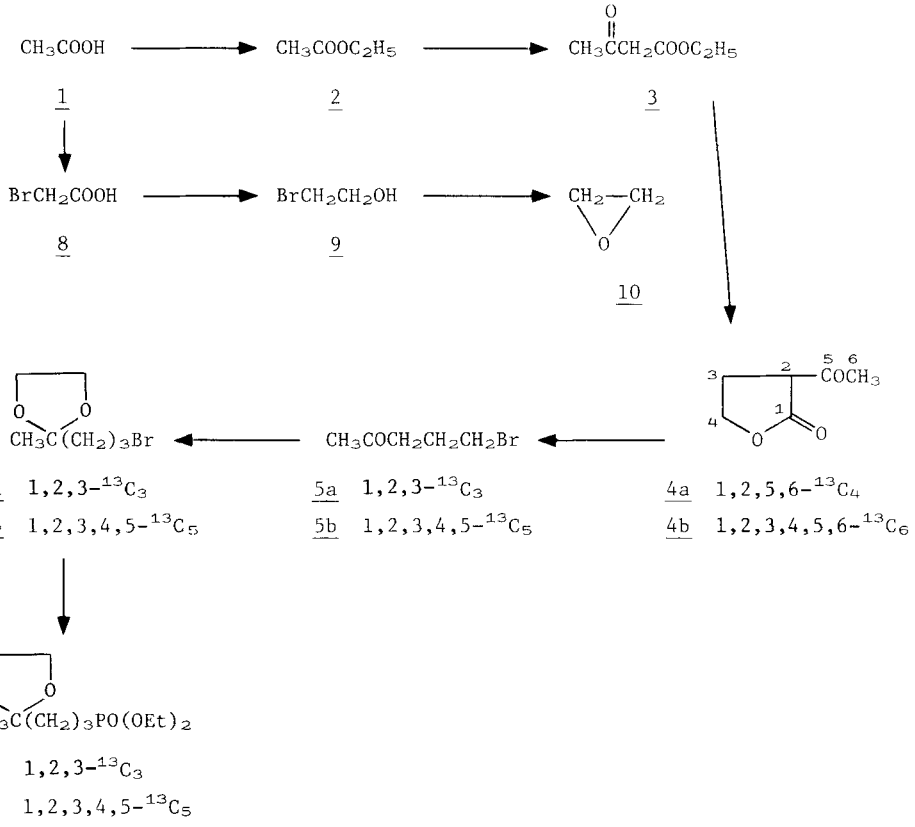
DISCUSSION

Starting from acetic-$^{13}C_2$ acid (1) we prepared ethyl acetate-$^{13}C_2$ (2) in 83% yield by heating with diethyl phosphite[3] at 140°, followed by fractional distillation of the acetate ester. Self-condensation of ethyl acetate as catalyzed by potassium hydride[4] afforded ethyl acetoacetate-$^{13}C_4$ (3) in yields of 57-70%. Although the 90% yield reported[4] was not realized, the method is far superior to the similar method employing sodium hydride. The tetra ^{13}C labeled acetoacetate (3) was alkylated with ethylene oxide in ethanolic sodium ethoxide[5] to afford butyrolactone-$^{13}C_4$ (4a) in 53% yield. The labels are at positions 2 and 3 of the ring and both carbons of the acetyl group. Treatment of the lactone with an excess of 48% hydrobromic acid at room temperature for 24 hours resulted in decarboxylation with insertion of bromide to yield 5-bromo-2-pentanone-1,2,3-$^{13}C_3$ (5a). This process, a modification of the method of Boon,[6] gave 5 in yields of 75-85%. When conducted at lower temperatures the reaction did not proceed to completion and with insufficient hydrogen bromide lower yields of a product apparently contaminated by hydroxy ketone were obtained.

The bromoketone (5a) was converted to its ethylene ketal (6a) by reflux with ethylene glycol and p-toluenesulfonic acid in benzene with water separation. Reaction of the bromo ketal (6a) with sodio diethyl phosphite in dimethylformamide at 90-100° afforded the phosphono ketal--1,2,3-$^{13}C_3$ (7a).[7]

Preparation of the phosphono ketal labeled at all five carbons (7b) required alkylation of ethyl acetoacetate-$^{13}C_4$ (3) with ethylene-$^{13}C_2$ oxide. The deuterated oxide has been prepared in 70% yield previously[8] by chlorohydration of ethylene and decomposition of the resultant ethylene chlorohydrin in alkali. However, we considered it to be more convenient, if not more economical, to use doubly labeled acetic acid as a starting material for the oxide. Acetic-$^{13}C_2$ acid was brominated to give bromoacetic-$^{13}C_2$ acid (8) in 90% yield. Reduction of the bromoacid with borane[9] in tetrahydrofuran at 0-5°

gave 2-bromoethanol-$^{13}C_2$ (9) in 79% yield. Ethylene$^{13}C_2$ oxide was obtained in 68% yield when 9 was heated to 130° and treated with 20% sodium hydroxide. Alkylation of ethyl acetoacetate-$^{13}C_4$ (3) with the labeled ethylene oxide afforded a 40% yield of acetylbutyrolactone-$^{13}C_6$ (4b). The lactone was converted to 5-(diethylphosphono)-2-pentanone-$^{13}C_5$ ethylene ketal (7b) in the manner described for the tris labeled material (7a).



EXPERIMENTAL

Ethyl Acetate-$^{13}C_2$ (2). Acetic-$^{13}C_2$ acid (1, 99 atom % ^{13}C) 70 g (1.13 mole) and 145.3 ml (1.13 mole) of diethyl phosphite were heated at 130-145° for 4 hours. The condenser was replaced by a distillation head and the product distilled to afford 101.2 g at b.p 77-80°. Redistillation through a short Vigreux head gave 89.6 g (83%) at b.p. 75-76°.

Ethyl Acetoacetate-$^{13}C_4$ (3). A 23% oil dispersion of potassium hydride (55 ml, 0.316 mole) was twice washed with cyclohexane to remove oil. The hydride was suspended in 295 ml of dry cyclohexane and warmed to 50° with stirring. Then a solution of 52 g (0.577 mole) of ethyl acetate-$^{13}C_2$ in 177 ml of cyclohexane was added dropwise over 1 hour with gradual elevation of the temperature to 80°. The mixture was stirred at reflux for 3 hours and about 3/4 of the solvent was removed by distillation. The mixture was cooled in ice and treated with 175 ml of ether and 142 ml of 2 N hydrochloric acid. The layers were separated and the aqueous portion was thrice extracted with 90-ml portions of ether. The combined ether extracts were washed with 25 ml of saturated sodium bicarbonate and dried over magnesium sulfate. The solvent was distilled through a Vigreux head, followed by distillation of the product at 48-50°/6 mm Hg. The IR spectrum was very similar to that of unlabeled ethyl acetoacetate. HNMR ($CDCl_3$) 1.27 (3H, t, CH_3 of C_2H_5), (3H, q centered at 2.30, J_1 = 130 Hz, J_2 = 6 Hz $^{13}CH_3{}^{13}CO$), (2H, doublet of triplets centered at 3.45, J_1 = 146 Hz, J_2 = 7 Hz, $^{13}CH_2$), 4.2 (2H, m, $-OCH_2-$).

Bromoacetic-$^{13}C_2$ Acid (8). A mixture of 14.8 g (0.239 mole) of acetic-$^{13}C_2$ acid and 1.26 g (6 mmole) of trifluoroacetic anhydride was heated to 100° for 5 minutes. Then 2 ml of bromine was added from the top of the condenser. A drying tube was attached and heating was continued for 10 minutes whereupon another 10 ml of bromine was added rapidly dropwise through the condenser. Two more additions of 4.5 and 3.5 ml of bromine were made at succeeding 40- and 80-minute intervals for a total of 20 ml (0.389 mole) of bromine. The total heating period was 2 hours, 40 minutes. Carbon tetrachloride was added and distilled off in successive portions until a colorless distillate was obtained. Distillation of the residue at 25 mm Hg in a 155-165° bath gave 29.0 g (86%) of crystalline product; HNMR ($CDCl_3$) doublets centered at 3.9, J_1 = 158 Hz, J_2 = 5 Hz.

2-Bromoethanol-$^{13}C_2$ (9). To a solution of 56 g (0.397 mole) of bromoacetic-^{13}C acid in 155 ml of dry tetrahydrofuran at 0-5° was added, dropwise over 30 minutes, 483 ml (0.483 mole) of 1 M borane in tetrahydrofuran. The mixture was stirred at 0-5° for 1.5 hours and at ambient tempera-

ture for another 2 hours. The mixture was again cooled to 0-5° and 243 ml of water was added slowly. Ether (200 ml) was added followed by 48.3 g of potassium carbonate with stirring until the solid had dissolved. The ether was separated and the aqueous portion extracted with another three 50-ml portions of ether. The ether extract was dried over magnesium sulfate and evaporated to leave 40 g (79%) of clear liquid, HNMR 2 sets of doublets of multiplets center at 3.6 and 3.9; I.R. 3.15 μ (OH).

Ethylene-$^{13}C_2$ Oxide (10). Bromoethanol-$^{13}C_2$ (9), 40 g (0.316 mole) was heated to 130° in a 3-neck flask fitted with a dropping funnel, distillation head and thermometer. Then 66 ml (0.33 mol) of 20% sodium hydroxide was added dropwise over 15 minutes. The ethylene oxide evolved was collected in a dry ice cooled graduated receiver giving a total of 11 ml (68%). Addition of more alkali to the reaction flask did not produce further product.

The material was dried over sodium hydroxide pellets at -78° for 1 hour and used in the next step.

2-Acetyl-$^{13}C_2$-butyrolactone-1,2-$^{13}C_2$ (4a). To 80 ml of a solution of sodium ethoxide in ethanol (from 4.6 g, 0.2 g-atom sodium) was added 26.8 g (0.2 mole) of ethyl acetoacetate-$^{13}C_4$ (3). The solution was chilled to 0° and a solution of 10 ml (0.2 mole) ethylene oxide in 15 ml of ethanol was added all at once. The flask was tightly stoppered and kept in the ice bath for 2 hours followed by 22 hours at room temperature. Ethanol was removed *in vacuo* and the residue was treated with 100 ml of ice cold 2 N hydrochloric acid. The mixture was extracted with one 75-ml portion and two 40-ml portions of dichloromethane. The extract was dried over magnesium sulfate and evaporated. The residue was distilled at reduced pressure through a Vigreux head to give 10.5 g of recovered starting material at 60-88°/3.5 mm Hg and 13.9 g (53%) of product at 88-100°/3.5 mm; I.R. 5.8 and 5.95 μ (C=O); ^{13}C NMR 29.29 (q, C-6 CH_3), 53.00 (m, C-2 CH), 172.73 (d, C-1 $C(=O)-O$), 200.16 (q, C-5, $C(=O)$).

2-Acetylbutyrolactone-$^{13}C_6$ (4b). (4b) was prepared in a similar manner using the ethylene-$^{13}C_2$ oxide (10) to afford 11.5 g (40%) of product with a recovery of 10.4 g (36%) of starting acetoacetate (3). I.R. 5.8 and 6.0 μ

(C=O), ^{13}CNMR 23.65 (q, C-3, CH_2), 29.29 (q, C-6, CH_3), 53.00 (m, C-2, CH), 67.10 (m, C-4, CH_2O), 172.73 (d, C-1, COO), 200.16 (q, C-5, C=O).

5-Bromo-2-pentanone-1,2,3-$^{13}C_3$ (5a). A solution of 17.1 g of 2-acetylbutyrolactone-$^{13}C_4$ (4a) in 140 ml of 48% hydrobromic acid was kept at ambient temperature for 21 hours. The mixture was diluted with 420 ml of water and extracted with 100 ml of dichloromethane. After three additional extractions with 50 ml portions of dichloromethane the organic extract was dried over magnesium sulfate. The solvent was distilled at atmospheric pressure through a Vigreux. Residual solvent was removed by brief application of vacuum (25 mm Hg) at room temperature to leave 16.2 g (74%); I.R. 6.0 μ (C=O); TLC (pentane-ether, 1:1) R_f 0.8, no starting material was present at R_f 0.3.

The penta labeled compound, 5-bromo-2-pentanone-$^{13}C_5$ (5b) was similarly obtained in 76% yield. I.R. 6.0 μ (C=O).

In unlabeled runs yields of 80-90% were observed. If a ratio of 4 ml of 48% hydrobromic acid to 1 g of 4 was used yields dropped to 60-65% and a peak at 9.5 μ appeared in the infrared spectrum. HNMR ($CDCl_3$) 2.2 (3H, s, CH_3), 2.2 (2H, m, CH_2), 2.7 (2H, t, CH_2C=O), 3.5 (2H, t, CH_2Br).

5-Bromo-2-pentanone-1,2,3-$^{13}C_3$ Ethylene Ketal (6a). A mixture of 16.2 g of the bromo ketone (5a), 6.0 ml of ethylene glycol, 241 mg of p-toluenesulfonic acid, and 170 ml of benzene was heated under reflux for 5.5 hours with water separation by a Dean-Stark trap. The solution was cooled to room temperature and added dropwise to 100 ml of 4% sodium bicarbonate at 0-5° with stirring. The benzene phase was separated and washed with 100 ml of water. After drying over magnesium sulfate the solvent was removed under reduced pressure through a Vigreux head followed by distillation of the product to afford 16.3 g (80%) at b.p. 60-66°/0.9 mm Hg; I.R. no C=O at 6.0 μ.

The $^{13}C_5$ labeled bromo ketal (6b) was prepared in a similar manner from 5b in 77% yield.

Unlabeled runs averaged 80% yield; I.R. no C=O at 5.95 μ; HNMR ($CDCl_3$). 1.3 (3H, s, CH_3), 1.9 (4H, t, CH_2CH_2), 3.5 (2H, t, CH_2Br), 4.0 (4H, s, ketal).

5-(Diethylphosphono)-2-pentanone-1,2,3-$^{13}C_3$ Ethylene Ketal (7a). To a solution of 13.5 g (97.8 mmole) of freshly distilled diethyl phosphite in 195

ml of dry ether was added 2.25 g (97.8 mg-atom) of sodium spheres. The mixture was stirred at reflux for 2.5 hours with consumption of the sodium. Dimethylformamide (154 ml) was added followed by a solution of 16.3 g (76.9 mmole) of the bromo ketal (6a). The mixture was heated at 65° to distill off most of the ether, then the temperature was raised to 95° and maintained there for 1 hour. The dimethylformamide was removed in vacuo causing precipitation of sodium bromide. The mixture was extracted thoroughly with ether with filtration to remove salts. Evaporation of ether and distillation of product afforded 16.1 g (83%) at b.p. 130-138°/1 mm Hg; ^{13}CNMR 23.61 (q, C-5, CH_3), 39.55 (octet, C-3, CH_2), 109.49 (t, C-2, O-C-O).

The 5-(diethylphosphono)-2-pentanone-$^{13}C_5$ ethylene ketal (7b) was similarly obtained in 86% yield from the bromo ketal (6b); ^{13}CNMR 16.86 (m, C-4, CH_2), 23.54 (m, C-5+C-1, CH_3+CH_2P), 28.36 (q, C-5, CH_2P), 39.50 (m, C-3, CH_2), 109.36 (m, C-2, O-C-O).

Unlabeled runs averaged 84%, b.p. 139-140°/1 mm. HNMR ($CDCl_3$) 1.3 (3H, s, CH_3 next to ketal), 1.3 (6H, t, CH_3 next to CH_2), 1.8 (6H, m, $CH_2CH_2CH_2$), 3.9 (4H, s, ketal), 4.1 (4H, m, CH_2 next to CH_3).

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