SYNTHESIS OF ^{14}C-LABELLED 5-HYDROXY-4-KETO-VALERIC ACID AND 4,5-DIOXO-VALERIC ACID

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This paper is dedicated to Prof. Dr. R. Appel on the occasion of his 60th birthday.

SUMMARY

5-Hydroxy-4-keto[4-^{14}C]valeric acid was prepared from 5-amino[4-^{14}C]levulinic acid. The synthesis of 5-hydroxy-4-keto[1-^{14}C]valeric acid (4) was achieved by Grignard reaction of 1-benzyloxy-4-bromo-2-butanone ethylene acetal (1) with $^{14}CO_2$ and subsequent removal of the protecting groups.
4,5-Dioxo[1- or 4-^{14}C]valeric acid was made by oxidation of the α-ketol group with cupric acetate.

Key words: 5-Hydroxy-4-keto[1-^{14}C]valeric acid, 5-hydroxy-4-keto[4-^{14}C]valeric acid, 4,5-dioxo[1-^{14}C]valeric acid, 4,5-dioxo[4-^{14}C]valeric acid

INTRODUCTION

In the course of our investigations on the biosynthesis of the antibiotic protoanemonin and its glucosidic precursors[1] in plants belonging to the family Ranunculaceae we needed specifically labelled 5-hydroxy-4-keto-valeric acid (HKV), 4,5-dioxo-valeric acid (DOVA) and HKV which was blocked at the hydroxyl function. The labelling pattern in protoanemonin biosynthesized from the radioactive tracers should provide evidence whether there was direct incorporation or not.

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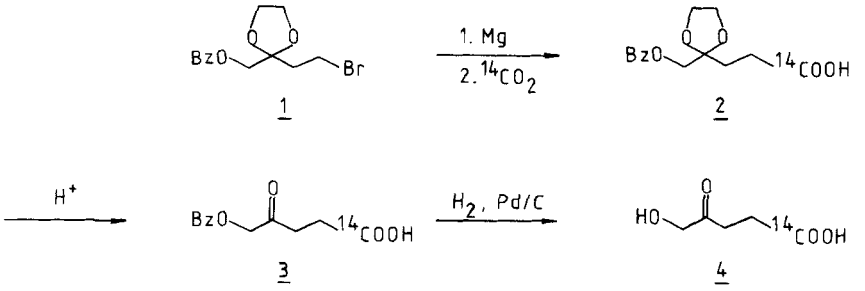
DISCUSSION

Deamination of 5-amino[4-^{14}C]levulinic acid hydrochloride (Amersham Buchler) by nitrous acid with the formation of 5-hydroxy-4-keto[4-^{14}C]valeric acid was accomplished with about 15% yield according to the method of Schlossberg et al.[2] Chromatography on Dowex columns mainly yielded labelled succinic acid.

Enzymatic synthesis of HKV labelled with ^{14}C at C-1, C-5 or C-1 and C-5 was achieved by the α-ketoglutarate:glyoxylate carboligase reaction[3]. Other methods for the preparation of HKV described in the literature were not suitable for labelling[4,5].

We therefore tried to synthesize [1-^{14}C]HKV by carboxylation of an appropriate Grignard reagent. Preliminary experiments showed, that 5-benzyloxy-levulinic acid which was prepared by treatment of 5-bromo-levulinic acid[6] with sodium benzylate was easily converted to HKV on catalytic hydrogenation. A bromide suitable for the Grignard reaction thus was 1-benzyloxy-4-bromo-2-butanone ethylene acetal (1):

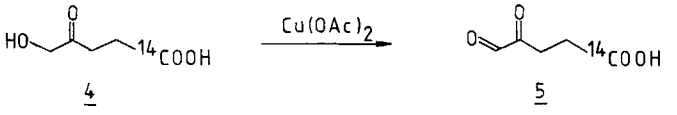


1 2 3 4

The preparation of compound 1 was accomplished by a five-step route starting with a mixed ester condensation between ethyl acetate and ethyl α-benzyloxyacetate to yield ethyl 4-benzyloxy-acetoacetate. Protection of the keto group with ethylene glycol and reduction of the ester function with lithium aluminium hydride gave 1-benzyloxy-4-hydroxy-2-butanone ethylene acetal, which was converted to the bromide 1 by the tosylate method[7]. Attempts to distil compound 1 were not successful, so that it was necessary to isolate the pure substance by preparative layer chromatography. The unlabelled carboxylic acids 2 and 3 from preliminary experiments could be crystallized. Analytical data for HKV were consistent with that obtained from an authentic sample prepared according to Lartillot et al.[6] Chromatographic

comparison of the p-bromo-phenacylesters also showed the identity of both unlabelled and [1-^{14}C]- or [4-^{14}C]HKV.

Oxidation of [1-^{14}C]HKV according to Zincke's method[8] with powdered cupric acetate under nitrogen yielded [1-^{14}C]DOVA (5), which was characterized as 4,5-dihydroxyimino[1-^{14}C]valeric acid[9]:



4 5

Non-radioactive 5 was also synthesized as described by Kissel and Heilmeyer[10] and had the same properties as the oxidation product.

EXPERIMENTAL

All physical data and elemental analyses were obtained from the unlabelled substances. The identity of the radioactive compounds was confirmed by chromatographic comparison with at least two solvent systems.

Melting points: microscope heating apparatus (Leitz). IR-spectra: Perkin Elmer spectrometer 157 G. ^{1}H-NMR-spectra: Varian EM 360 and Bruker WH 90. Elemental analysis: Perkin Elmer elemental analizer 240. Radiochromatogram scanning: TLC-scanner LB 2760 (Berthold) with amplifier high voltage unit BF 2301 and ratemeter integrator BF 2305 (Berthold). Radioactivity: liquid scintillation counter BF 8000 (Berthold) with HP 9815 A (Hewlett-Packard). Thin layer chromatography: TLC-foils (Schleicher & Schüll), visualization achieved by spraying with conc. H_2SO_4 (140°C). Preparative layer chromatography: silica gel 60 PF_{254} or 60 $PF_{254+366}$ (Merck) on 20x20 cm plates, detection by UV-light or radiochromatogram scanning, elution of silica gel with 500 ml dichloromethane, solvent system J_2 (see below) or methanol. Anion exchange chromatography: Dowex 1-X1 (Roth).

Solvent systems: A butanol/water/acetic acid (12:5:3), B benzene/dioxane/acetic acid (90:25:4), D cyclohexane/ethyl acetate

(85:15), G dichloromethane/acetone (100:1), J_1 chloroform/methanol/water (65:25:10) (lower phase), J_2 chloroform/methanol/water (65:35:10) (lower phase), K chloroform/acetone (8:2), P n-hexane/dichloromethane/i-propanol (90:5:5), C cyclohexane/ethyl acetate (2:3).

Barium[^{14}C]carbonate (20.9 mCi/mmol) and 5-amino[4-^{14}C]levulinic acid hydrochloride (58 mCi/mmol) were purchased from The Radiochemical Centre, Amersham Buchler.

5-Benzyloxy[1-^{14}C]levulinic acid ethylene acetal (2): 1.57 g (5.21 mmol) of 1-benzyloxy-4-bromo-2-butanone ethylene acetal (1) were dissolved in 17.5 ml absolute tetrahydrofuran and brought to reaction with 194.5 mg (8 mmol) magnesium turnings. The mixture was refluxed for ten minutes. Chromatographic analysis showed when the reaction was complete. In this case the more polar 1-benzyloxy-2-butanone ethylene acetal could be detected, R_F values (D): 0.46, (G): 0.46 (R_F values of bromide 1 (D): 0.55, (G): 0.77). The flask containing the Grignard reagent was connected to a vacuum line and cooled to -192°C. After evacuation $^{14}CO_2$ was generated by the dropwise addition of 10 ml conc. H_2SO_4 to 5 mCi (20.9 mCi/mmol) barium[^{14}C]carbonate and condensed into the reaction flask. After standing at room temperature for two hours, the reaction mixture was hydrolyzed with 10 ml saturated ammonium chloride solution and extracted exhaustively with ether. Chromatographic purification with system B yielded 1.41 mCi radiochemical pure 2, R_F values (P): 0.31, (J_1): 0.71, m.p. 62.5°C (n-hexane).

Anal. calcd. for $C_{14}H_{18}O_5$: C,63.15; H,6.81. Found: C,63.40; H,6.80.

IR (KBr): 2900, 1705, 1450, 1410, 1360, 1345, 1270, 1180, 1105, 1090, 1075, 1050, 950, 880, 750, 700, 675, 625 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ=2.27(m,4H,CH_2-2,3), 3.40(s,2H,CH_2-5), 3.96(s, 4H,acetal), 4.60(s,2H,benzyl-CH_2), 7.37(s,5H,aromat.), 10.1(s,1H,COOH).

5-Benzyloxy[1-^{14}C]levulinic acid (3): 1.41 mCi 2 were dissolved in a mixture consisting of 15 ml dioxane, 10 ml water and 0.5 ml conc. HCl and were refluxed for one hour. The acid was removed by evaporation with water at low temperature. The residue was dissolved in a little ethanol and chromatographed on silica gel with system P. 39.8 mg (0.87 mCi, 4.85 mCi/mmol) 3 were obtained, R_F values (P): 0.26, (J_1): 0.69, m.p. 75°C (CCl_4).

Anal. calcd. for $C_{12}H_{14}O_4$:C,64.84; H,6.30. Found:C,65.29; H,6.30.
IR (KBr): 2880, 1710, 1500, 1470, 1450, 1420, 1390, 1310, 1220, 1150, 1135, 1100, 1060, 1020, 920, 750, 740, 705, 645 cm^{-1}.
^{1}H-NMR ($CDCl_3$): δ=2.75(m,4H,CH_2-2,3), 4.15(s,2H,CH_2-5), 4.65(s,2H, benzyl-CH_2), 7.45(s,5H,aromat.), 10.9(s,1H,COOH).

5-Hydroxy-4-keto[1-^{14}C]valeric acid (4): To a solution of 0.8 mCi 3 in 4 ml ethyl acetate, 40 mg palladium/charcoal (10%) were added. The flask was connected to the hydrogenation apparatus. After stirring for 8 hours under hydrogen, chromatographic control showed, that 96% of the initial radioactivity was localized at the R_F value of the more polar HKV. Chromatographic separation (silica gel; system J_1) yielded 0.43 mCi pure [1-^{14}C]HKV. R_F values (J_1): 0.25, (A): 0.67, m.p. 100°C.
IR (KBr): 3430, 3380, 2930, 1725, 1690, 1450, 1430, 1405, 1315, 1225, 1210, 1125, 1090, 1010, 930, 740, 640 cm^{-1}.
^{1}H-NMR ([D_6]acetone): δ=2.70(m,4H,CH_2-2,3), 4.28(s,2H,CH_2-5), 5-7 (s,2H,OH and COOH).
A small part was reacted with p-bromophenacyl bromide to form the [1-^{14}C]HKV p-bromophenacyl ester, m.p. 102°C, R_F values (C): 0.35, (K): 0.42.

5-Hydroxy-4-keto[4-^{14}C]valeric acid[2]: 0.25 mCi 5-amino[4-^{14}C] levulinic acid hydrochloride (58 mCi/mmol) were diluted with 5.7 mg non-radioactive hydrochloride (Merck) and dissolved in 0.25 ml 1n H_2SO_4. After cooling to 0°C, a solution of 25 mg (0.36 mmol) sodium nitrite in 0.5 ml water was dropwise added. The solution was allowed to return to room temperature for 25 minutes and heated to 100°C for an additional five minutes. The mixture was transferred to a flask containing 10 ml water and adjusted with sodium hydroxide solution to pH 7.0. Purification was carried out by anion exchange chromatography on Dowex columns (formate form)[11]. The 0.038 mCi (15%) of radiochemical pure [4-^{14}C]HKV obtained were identical to HKV formed by the Grignard reaction and were also characterized as p-bromophenacyl ester.

4,5-Dioxo[1-^{14}C]valeric acid (5): 0.1 mCi [1-^{14}C]HKV were dissolved in 1 ml water. An excess of powdered cupric acetate (140 mg) was added and stirred continuously under nitrogen at room temperature for 3 days. Radiochromatogram scanning showed, that mainly the unpolar 4,5-dioxo-derivative 5 was formed (system A). Excess copper-II was precipitated by adding 10% oxalic acid solu-

tion. The salts were filtered off, and the filtrate was chromatographed on silica gel with system A. Elution with methanol yielded 0.058 mCi (58%) pure [1-^{14}C]DOVA, R_F values (J_1): 0.32, (A) 0.86. The analytical data were consistent with that obtained from a substance synthesized according to Kissel et al.[10] Compound 5 was characterized as 4,5-dihydroxyimino[1-^{14}C]valeric acid[9], R_F values (B): 0.46, (J_2): 0.61, m.p. 127°C. [4-^{14}C]DOVA was obtained by oxidation of [4-^{14}C]HKV in the same way. It is advisable to employ compound 5 immediately because decomposition occurs even at low temperatures.

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