Preparation of Selectively Isotopically Labelled β-Hydroxypropionic Acid

Arie L. Gutman *,† **and Vered Ribon**
Department of Chemistry, Technion, Israel Institute of Technology, Haifa, Israel

Two efficient three-step one-pot procedures for the synthesis of any combination of selectively isotopically labelled β-hydroxypropionic acids from relatively inexpensive labelled sodium acetate and gaseous formaldehyde or carbon dioxide have been developed. [1-^{14}C]-, [2-^{14}C]-, [2-^{14}C,2-^{3}H]-, [2-^{13}C]-, and [2-^{13}C,2-^{2}H$_2$]-β-hydroxypropionates have been prepared. The question of selective reduction of the intermediate malonate half esters is discussed and the general procedures are described.

In the course of our studies on the biosynthesis of the biologically important clavulanic acid[1] we had to carry out some incorporation experiments with selectively isotopically labelled β-hydroxypropionate. Because of the interest prompted by its virucidal activity, two syntheses of [1-^{11}C]- and [1-^{14}C]-β-hydroxypropionic acid from 2-bromoethanol and ^{11}C or ^{14}C labelled KCN were reported some time ago.[2,3] More recently, Arigoni *et al.*[4] prepared the C-2 stereospecifically deuteriated compound using a mixture of chemical and enzymatic methods. As the reported syntheses of this compound in labelled form were too specific and therefore unsatisfactory for our needs, we developed a general method, which allows the synthesis of any combination of selectively ^{14}C-, ^{13}C-, ^{3}H-, ^{2}H-labelled β-hydroxypropionates from readily available labelled sodium acetate and/or carbon dioxide.

Two approaches, both proceeding through the intermediacy of the lithium enolate ion (**1**), were considered (Scheme 1). Sodium acetate with an excess of triethyl phosphate under reflux afforded in quantitative yield ethyl acetate,[5] which was separated from the high-boiling reagent by direct vacuum transfer into a frozen flask containing lithium di-isopropylamide (LDA) in THF. Warming up to −78 °C afforded the lithium enolate (**1**). Quenching of the latter with gaseous formaldehyde[6] gave upon acidic work-up ethyl β-hydroxypropionate (**2**), which was purified by bulb-to-bulb distillation. The required sodium salt (**3**) was obtained in 90% yield by basic hydrolysis of (**2**). In spite of the very high yields in the formation of ethyl acetate and in the hydrolysis step, the overall yield of (**3**) from sodium acetate *via* that approach was irreproducible, over a range of 20—40%. The second approach involved quenching of the lithium enolate (**1**) with CO_2, followed by acidic work-up to give monoethyl malonate (**4a**). The latter was converted into its lithium salt (**4b**) and reduced by refluxing with $LiBH_4$ in THF, which is known to reduce esters to alcohols while being totally inactive towards carboxylate anions.[7] One should note that $LiBH_4$ reduction of ethyl lithium malonate (**4b**) required unusually long reaction times. Thus, while the reduction of benzyl lithium malonate was complete within the usual 5 h period, the reduction of (**4b**) under the same reaction conditions required 72 h. This is probably due to the very slight solubility of the latter in THF owing to its hydrophilic character. The work-up involved addition of water, evaporation of the THF, and continuous extraction of the aqueous solution brought to pH 7 with $CHCl_3$ in order to remove most of the boric acid. Acidification of the β-hydroxypropionate containing aqueous solution and its continuous extraction with ether ensured the transfer of the β-hydroxypropionic acid into the organic layer. The colourless crystalline residue after evaporation of ether was essentially pure poly-β-hydroxypropionic acid (**5**), which could be hydrolysed by refluxing it with aqueous sodium hydroxide.[3] Elution of the basic solution through a sulphonic acid ion-exchange resin gave a dilute solution of β-hydroxypropionic acid, which was neutralised by NaOH and freeze-dried to give the sodium salt (**6**).

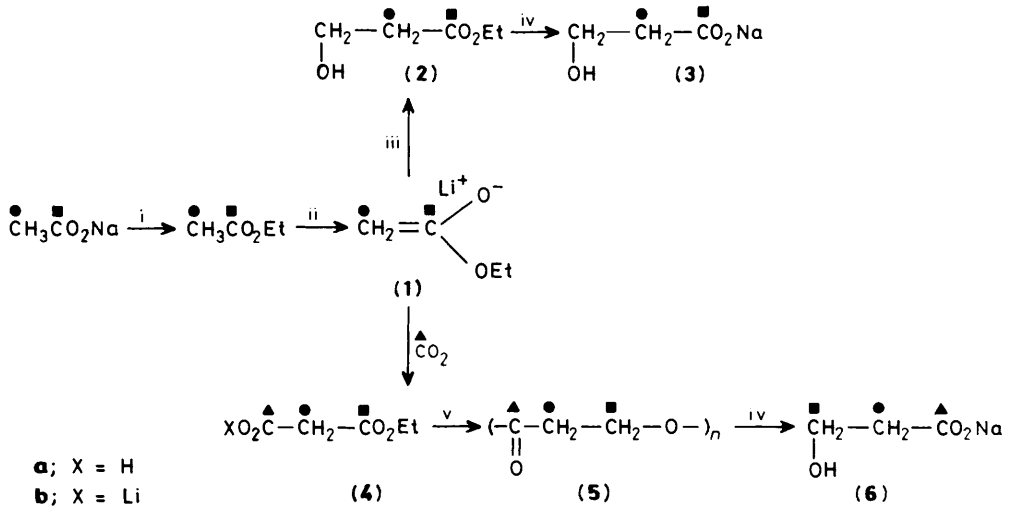
We were able to simplify considerably the method and improve yields by making use of the fact that quenching of the lithium enolate (**1**) with CO_2 directly gives the lithium salt (**4b**) which is used for reduction. The remaining di-isopropylamine and THF were evaporated and the colourless solid residue could then be treated with $LiBH_4$ solution. This improvement avoids the malonate isolation step and thus makes the method a one-pot procedure. The overall chemical and radiochemical yields of (**6**) from sodium acetate rose to 60—70%.

When C-1 labelled β-hydroxypropionate is required and the label is introduced from CO_2, the reaction could be carried out with any ester of acetic acid. Thus, we obtained [1-^{14}C]-benzyl malonate (**8**) by adding the *in situ* generated benzyl lithium enolate (**7**) to the ampoule containing radioactive CO_2, frozen by cooling with liquid air. Gradual warming of the ampoule to −78 °C was followed by bubbling in radioinactive CO_2 (as carrier) and acidic work-up. The radiochemical yield of (**8**) based on CO_2 was 80% (Scheme 2). As mentioned above the lithium salt of (**8**) could be easily converted into β-hydroxypropionate.

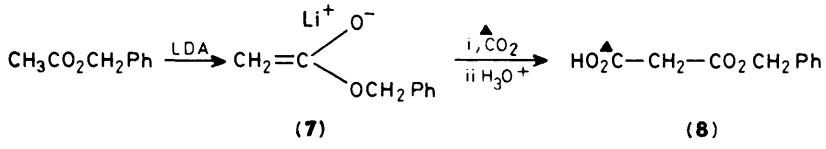
Although several procedures for selective reduction of acids in the presence of esters have been reported, they could not be applied to the reduction of the carboxylic end of malonate half esters. Treatment of (**4a**) or (**8**) with diborane under conditions generally accepted for selective reduction of acids[8,9] resulted in a complex mixture which, according to the n.m.r. spectrum, contained only traces of the desired β-hydroxypropionate esters. Another procedure,[10] which involves the *in situ* preparation of mixed carbonic carboxylic acid anhydrides and their reduction with sodium borohydride in aqueous THF to the corresponding alcohols, proved to be totally unsuitable for half esters of malonic acid. It appeared that mixed anhydrides formed from malonic acid half esters and chloroformates with triethylamine in THF are unstable and undergo spontaneous decarboxylation even at subzero temperatures (Scheme 3). When the labile malonate hydrogens were replaced by methyl groups, as in α,α-dimethyl monomethyl malonate (**9**), the mixed anhydride (**10**) was stable and it was possible to reduce it with $NaBH_4$ under usual conditions to give corresponding α,α-dimethyl methyl β-hydroxypropionate (**11**) (Scheme 3). The mechanistic reason for the ready decarboxylation of mixed anhydrides of malonic acid half esters and the synthetic utility of this finding have been described recently.[11]

In conclusion, from the two approaches to labelled β-hydroxypropionic acid outlined in Scheme 1, the formaldehyde route is apparently more direct and it could therefore be used for the synthesis of C-1 and/or C-2 labelled β-hydroxypropionates

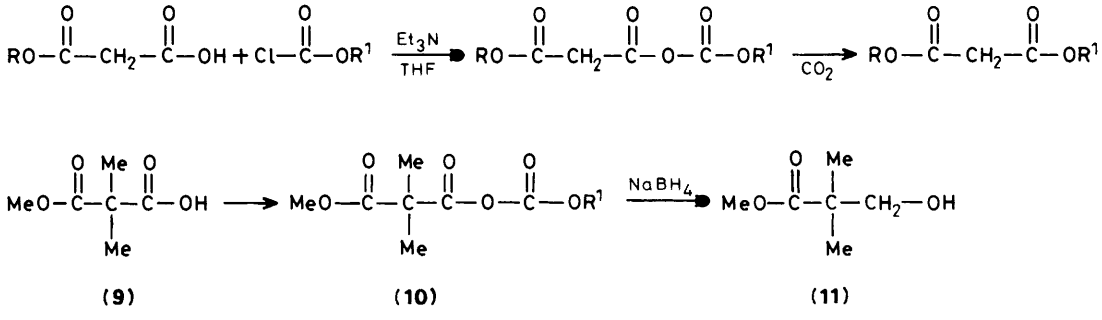
† Fellow of the Bat Sheva de Rothschild Foundation, 1984—1986.



Scheme 1. *Reagents:* i, Triethyl phosphate; ii, lithium di-isopropylamide (LDA); iii, gaseous formaldehyde; iv, aqueous NaOH; v, $LiBH_4$. ■, ●, ▲—Possibilities for ^{14}C and/or ^{13}C labelling



Scheme 2.



Scheme 3.

from appropriately labelled acetate. However, there are a number of considerations which would favour the somewhat longer malonate approach. Firstly, dry gaseous formaldehyde is generated by the thermal depolymerisation of paraformaldehyde at 150 °C and flushed with nitrogen into the reaction flask containing the lithium enolate reaction mixture at −78 °C. Even when a short-path wide inlet tube was used, the drastic (over 200 °C) difference in temperatures in the two flasks caused repolymerisation of some of the formaldehyde, which appeared as a crust on the surface of the reaction mixture. Because of this, and of some possible protic impurities originating from paraformaldehyde,[12] the chemical yields of the reaction were inherently low. Secondly, since labelled formaldehyde is not readily available this route could not be used for the synthesis of C-3 labelled β-hydroxypropionate. Finally, the malonate intermediate has a great potential for introducing deuterium and/or tritium labelling. The C-2 malonate hydrogens are readily exchanged simply by keeping the compound in D_2O at neutral pH.[13] If C-3 deuteriated or tritiated β-hydroxypropionate is required the reduction should be carried out with LiB^2H_4 or LiB^3H_4.[14]

Experimental

Materials and General Techniques.—[2-^{14}C]-,[2-^{3}H]-Sodium acetate and [^{14}C]carbon dioxide were purchased from Amersham International p.l.c. [2-^{13}C]Sodium acetate (99 atom% ^{13}C) and deuterium oxide (99.8 atom% D) were purchased from Aldrich Chemical Co. Radioactive counting was carried out with a Packard liquid scintillation counter, model 526, using [^{3}H]hexadecane and [^{14}C]hexadecane as internal standards. ^{1}H N.m.r. spectra were taken at 60 MHz on a Varian T-60 instrument and ^{13}C n.m.r. spectra were taken working at 100.58 MHz on a Bruker AM 400 WB instrument. Chemical shifts are reported in p.p.m. downfield from internal tetramethylsilane. Melting points (Pyrex capillary) are

uncorrected. Dry tetrahydrofuran (THF) was obtained by distillation from potassium.

Representative Procedure for the 'One-pot' Preparation of β-Hydroxypropionate (**6**) via *the Malonate*.—Lithium di-isopropylamide (LDA) was prepared by slow addition of a 1.43M-solution of butyl-lithium in hexane (8.4 ml, 12 mmol) to a cooled solution of di-isopropylamine (1.73 ml, 12 mmol) in dry THF (20 ml) in a three-necked flask with stirring under argon. The reaction mixture was stirred for 20 min at 4 °C, transferred under argon into a one-necked flask and solidified by cooling in liquid air. The frozen flask, still under argon, was connected through an inverted U-tube equipped with a vacuum tap to the frozen flask containing the ethyl acetate reaction mixture. [Labelled ethyl acetate was obtained by refluxing labelled sodium acetate (1.00 g, 12 mmol) with triethyl phosphate (5 ml; b.p. 215 °C) for 5 h]. Argon was pumped out and the evacuated system was closed *in vacuo* (0.2 mmHg). Gentle warming of the ethyl acetate reaction mixture caused the vacuum transfer of the volatile ethyl acetate into the frozen LDA-containing flask. The system was opened to argon and allowed to warm to −78 °C in order to generate the lithium enolate (**1**). After 1 h at that temperature the flask was plugged with a septum cap and dry carbon dioxide was gently bubbled through a needle into the solution at −78 °C for a period of 30 min. The reaction mixture was allowed to warm to 0 °C and evaporated to dryness under reduced pressure to leave crude ethyl lithium malonate (**4b**) as a yellowish crystalline material (1.1 g, 66% yield from sodium acetate). This was treated with dry THF (30 ml) and a 1.18M-solution of $LiBH_4$ in THF (20 ml, 23.6 mmol) and refluxed under argon for 72 h. The reaction mixture was cooled, water (20 ml) was added, and THF was removed on a rotary evaporator. The aqueous solution was brought to pH 7 and extracted for 48 h with chloroform in order to remove most of the boric acid. It was then acidified to pH 2.5 and extracted with ether for 48 h. Evaporation of the ether gave the white, partly crystalline, poly-β-hydroxypropionic acid (**5**); δ(D_2O) 2.5 (t, 2 H, CH_2CH_2CO, *J* 6 Hz) and 3.7 (t, 2 H, OCH_2CH_2, *J* 6 Hz).

The foregoing polymer (**5**) was dissolved in a 0.25M-solution of NaOH (20 ml) and refluxed for 5 h. The cooled liquid was poured onto a 10 mm diameter ion-exchange column packed with approximately 20 ml of (wet) Dowex 50W X8 in the acid form (200—400 mesh). The flow from the bottom of the column was regulated with a stopcock at 8—10 drops per minute. The column was washed with three 10-ml portions of distilled water and the last effluent collected had the pH of distilled water. The combined effluents, containing β-hydroxypropionic acid, were neutralized to pH 7.5 with 0.1M-NaOH and freeze-dried, leaving white crystalline sodium β-hydroxypropionate (**6**) (0.81 g, 60% overall yield from sodium acetate); δ(D_2O) 2.5 (t, 2 H, CH_2CH_2CO, *J* 6 Hz) and 3.8 (t, 2 H, OCH_2CH_2, *J* 6 Hz). Crystallization from ethanol afforded an analytical sample, m.p. 143—144 °C (lit.,[15] m.p. 143 °C).

Sodium [2-^{14}C,2-^{3}H]-β-*Hydroxypropionate*.—This compound was prepared from sodium[2-^{14}C,2-^{3}H]acetate (0.5 g, 1 mCi of ^{14}C, *ca.* 7 mCi of ^{3}H) in 70% overall chemical and radiochemical yield, as described for the unlabelled compound; m.p. 143—144 °C; the n.m.r. spectrum was identical with that of the unlabelled compound.

Sodium [2-^{13}C]-β-*Hydroxypropionate*.—This compound was prepared from sodium[2-^{13}C]acetate (1 g, 99 atom% ^{13}C) in 60% overall yield, as described for the unlabelled compound; m.p. 143—144 °C; $\delta_H(D_2O)$ 2.3 (2 H, $CH_2{}^{13}CH_2CO$, t, *J* 6 Hz, d, $^1J_{CH}$ 130 Hz) and 3.7 (2 H, $OCH_2{}^{13}CH_2$, t, *J* 6 Hz, d, $^2J_{CH}$ 3.5 Hz); $\delta_C(D_2O)$ (unlabelled compound) 160.8 (s, C-1), 40.1 (s, C-3), and 20.2 (s, C-2); ^{13}C enrichment 160.8 (d, *J* 40 Hz, C-1), 40.1 (d, *J* 40 Hz, C-3), and 20.2 (s, 99% enrichment, C-2).

Sodium [2-^{13}C,2-$^{2}H_2$]-β-*Hydroxypropionate*.—This compound was prepared from sodium[2-^{13}C]acetate (1 g, 99 atom% ^{13}C) in 65% overall yield. Deuterium was introduced at the malonate stage by dissolving the dry crude lithium[2-^{13}C]malonate (1.1 g), obtained as described for the unlabelled compound, in deuterium oxide (10 ml; 99.8 atom% D) at pH 8 and leaving it in the solution overnight. Freeze-drying was followed by $LiBH_4$ reduction and the usual work-up and isolation procedures. The product sodium [2-^{13}C,2-$^{2}H_2$]-β-hydroxypropionate had the following n.m.r. spectrum: $\delta_H(D_2O)$ 2.3 (0.4 H, $CH_2{}^{13}CH_2CO$, t, *J* 6 Hz, d, $^1J_{CH}$ 130 Hz) and 3.7 (2 H, $OCH_2{}^{13}CH_2$, br s); δ_C 160.8 (d, *J* 40 Hz, C-1), 40.1 (d, *J* 40 Hz, C-3), 20.2 (s, C-2, $^{13}CH_2$), and 19.9 (t, 18 Hz, C-2, $^{13}CD_2$).

Representative Procedure for the Preparation of β-*Hydroxypropionate* via *the Formaldehyde Approach: Sodium* [2-^{14}C,2-^{3}H]-β-*Hydroxypropionate* (**3**).—The LDA solution was prepared as described above from a 1.43M-solution of butyl-lithium in hexane (17.5 ml, 25 mmol) and di-isopropylamine (3.60 ml, 25 mmol) in dry THF (35 ml). Labelled ethyl acetate, obtained from sodium[2-^{14}C,2-^{3}H]acetate (2.05 g, 25 mmol, 250 μCi of ^{14}C, $^{3}H/^{14}C = 13.00 \pm 0.10$) and triethyl phosphate (9 ml), was added into the frozen LDA-containing flask by vacuum transfer, as described above. The system was opened to argon and the solution in the three-necked flask was allowed to warm up to −78 °C in order to generate the lithium enolate (**1**). After 1 h at that temperature a wide glass tube (12 mm in diameter), terminating *ca.* 1 cm above the surface of the reaction solution, was fitted into the middle neck of the flask. The other end of the glass tube was connected to a 100-ml round-bottomed two-necked flask containing paraformaldehyde (3 g) previously dried for 2 days in a desiccator over phosphorus pentaoxide. The flask was heated in an oil-bath at 180—200 °C, and the formaldehyde vapour produced by depolymerization was carried into the lithium enolate reaction solution by a slow stream of argon over a period of 30 min. (A white crust of repolymerized formaldehyde was always formed on the cold surface of the reaction mixture several minutes after beginning of the transfer.) After the addition of formaldehyde, the reaction mixture was allowed to warm up to *ca.* −10 °C and 1M-HCl (50 ml) was added. The organic layer was separated and the acidic aqueous layer was extracted with diethyl ether (3 × 50 ml). The combined organic extracts were washed with saturated $NaHCO_3$ (30 ml) and water (30 ml), dried (Na_2SO_4), and the solvent evaporated to afford a yellowish oil. Bulb-to-bulb distillation gave a colourless fraction of pure ethyl [2-^{14}C,2-^{3}H]-β-hydroxypropionate (**2**), b.p. 110 °C/23 mmHg (lit.,[16] 81 °C/13 mm); δ($CDCl_3$) 4.2 (q, 2 H, OCH_2CH_3, *J* 6 Hz), 3.8 (t, 2 H, OCH_2CH_2, *J* 5 Hz), 3.3 (br s, 1 H, OH, disappears with D_2O), 2.6 (t, 2 H, CH_2CH_2CO, *J* 5 Hz), and 1.2 (t, 3 H, CH_2CH_3, *J* 6 Hz); 0.88 g, chemical yield 30%; *ca.* 75 μCi ^{14}C, radiochemical yield 30%; $^{3}H/^{14}C = 11.90 \pm 0.10$. The observed decrease in the $^{3}H/^{14}C$ ratio from 13.0 in acetate to 11.9 in β-hydroxypropionate requires the reasonable tritium isotope effect of $k_H/k_T = 5.4$ in the generation of the lithium enolate (**1**). The foregoing ester (**2**) was hydrolysed with aqueous NaOH into the sodium β-hydroxypropionate (**3**) as described for the hydrolysis of the polymer (**5**).

Sodium [1-^{14}C]-β-*Hydroxypropionate* (**6**) *from* [^{14}C]*Carbon Dioxide and Benzyl Acetate*.—The LDA solution was prepared as described above from a 1.63M-solution of butyl-lithium in hexane (16.3 ml, 10 mmol), di-isopropylamine (1.45 ml, 10 mmol) in dry THF (20 ml). Benzyl acetate (1.5 g, 10 mmol) was added dropwise to the LDA solution at −78 °C and the reaction mixture was stirred at that temperature under argon for 1 h. The reaction solution (6 ml) containing *ca.* 2 mmol of the lithium enolate (**7**) was added under argon into the ampoule containing [^{14}C]carbon dioxide (1 mCi, 0.02 mmol), frozen in

liquid air. The contents of the ampoule were allowed to warm up to −78 °C and transferred back into the reaction flask. Dry radioinactive carbon dioxide was bubbled through the reaction mixture for 30 min, which was then allowed to warm up to 0 °C; 1M-HCl solution (40 ml) was then added. The organic layer was extracted with diethyl ether (3 × 30 ml) and the combined organic extracts were washed with saturated $NaHCO_3$ (30 ml) and water (30 ml), dried (Na_2SO_4), and evaporated to leave monobenzyl[1-^{14}C]malonate (**8**) as a colourless oil which solidified with time (1.00 g, 50% chemical yield; 500 μCi, 50% radiochemical yield): $\delta_H(CDCl_3)$ 10.2 (br s, 1 H, acidic), 7.1 (s, 5 H, Ph), 4.8 (s, 2 H, CH_2Ph), and 3.2 (s, 2 H, $OCCH_2CO$). The foregoing compound (**8**) was dissolved in water (20 ml) and the solution was neutralized (phenolphthalein) with lithium hydroxide and then evaporated under reduced pressure. The dry lithium salt was reduced with $LiBH_4$ in THF as described for the reduction of lithium ethyl malonate, except that in the case of lithium benzyl malonate a 5 h period under reflux was sufficient. Sodium [1-^{14}C]-β-hydroxypropionate was obtained in 90% chemical and radiochemical yield from (**8**).

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