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Labeled Substrates. I. The Synthesis of DL-2-Deuteriolactic Acid¹

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DL-2-Deuteriolactic acid has been prepared with incorporation of deuterium in approximately 75 atom per cent excess by concurrent hydrolysis and decarboxylation of bromomethylmalonic acid in refluxing deuterium oxide or by prior decarboxylation of the deuterium exchanged solid bromo di-acid to 2-deuterio-2-bromopropionic acid followed by hydrolysis with zinc carbonate.

In studies on the coupling of the oxidation of substrates to reductive biosynthesis, DL-2-deuteriolactic acid was utilized to give deuterium labeled phospho-pyridine nucleotides in the intact rat, and the utilization of the labeled nucleotides in glycogen and lipid synthesis has been reported.^{2a,b} The present report describes the synthesis of DL-2-deuteriolactic acid.

Deuterated lactic acid has been prepared from optically active lactic acid by exchange with deuterium oxide in the presence of a platinum catalyst at 120–130° for 45 hr.³ Incorporation occurs to the extent of 6% in both the α and β positions and the optical data show that the exchange proceeds with inversion of configuration.

For purposes of investigating reductive biosynthesis, since one would expect that any isotope in the methyl group would be incorporated into glycogen and lipids by the normal glycolytic reactions of lactic acid, it is necessary that the isotope be preponderantly in the 2-position. This has been accomplished by the preparation of DL-2-deuteriolactic acid with incorporation of deuterium up to 75 atom per cent by concurrent hydrolysis and decarboxylation of bromomethylmalonic acid in boiling deuterium oxide. The deuteriolactic acid was isolated as the zinc salt, with the labile deuterium removed by successive treatments with water and concentration to dryness.

An alternative procedure employed was the replacement of the acidic hydrogens of the bromomethylmalonic acid with deuterium by several exchanges with deuterium oxide, reisolation of the solid bromo di-acid and decarboxylation by heating to 2-deuterio-2-bromopropionic acid. Reaction of the bromopropionic acid, which contained over 70 atom per cent of deuterium, with zinc carbonate served to give the lactic acid as the zinc salt without loss of deuterium.

In these transformations, impure samples of bromomethylmalonic acid were used since it was

not found possible to isolate the pure bromo di-acid even after repeated recrystallizations from various solvents. Analysis of the zinc salt for lactic acid by the method of Barker and Sommerson⁴ indicated that the synthetic preparations had a lactic acid content of 50% or less than the expected value. That these low results are due to an isotope effect in the analytical method was shown by running two concurrent reactions with bromomethylmalonic acid: one in deuterium oxide and the other in water. The synthetic, unlabeled zinc lactate prepared in this fashion gave lactic acid values of 105% of the standard. The analytical method is based on color formation between *p*-hydroxydiphenyl and acetaldehyde, formed by reaction of concentrated sulfuric acid and lactic acid in a given time interval. If the formation of acetaldehyde occurs by removal of the α -hydrogen from lactic acid followed by decarboxylation and this hydrogen abstraction is the rate-determining step, then the observed isotope effect is easily understood.

The purity of the lactic acid was verified by analysis for zinc and water of hydration of the zinc hydrate and by the titration of the effluent on passing an aqueous solution of the zinc salt through an ion exchange resin. In all cases values near theoretical were obtained. Further proof of the identity of the deuterated compound was obtained from qualitative paper chromatography on filter paper. In the system, phenol, water, and formic acid, the lactic acid standard had an R_f of 88.4 and the synthetic deuterated lactic acid had an R_f of 88.2.

The position of the isotope was established both enzymatically⁵ and chemically. At least 90% of the deuterium in the labeled lactic acid is present in the 2-position. This was shown by observing

(4) S. B. Barker and W. H. Sommerson, *J. Biol. Chem.*, **138**, 535 (1941).

(5) Unpublished observations carried out in collaboration with Dr. Harold Strecker. The value of 90% of the theoretical amount of deuterium is probably the limit of accuracy of the experiment. It involves the colorimetric determination of glutamic acid, dilution with carrier glutamic acid, and two deuterium analyses. Furthermore, since the determination involves two enzymatic equilibria, any difference in the isotope effect of the enzymes involved would be reflected in the deuterium content of the glutamic acid.

(1) This investigation was supported in part by research funds from the U. S. Public Health Service and the Sugar Research Foundation.

(2) (a) H. D. Hoberman, *J. Biol. Chem.*, **232**, 9 (1958).
(b) H. D. Hoberman, *J. Biol. Chem.*, **233**, 1045 (1958).

(3) J. Bell, T. Hill, K. A. MacDonald, R. I. Reed, and A. MacDonald, *J. Chem. Soc.*, 3454 (1953).

that oxidation of the deuteriolactate by crystalline lactic dehydrogenase in the presence of α -ketoglutaric acid, ammonia, diphospho-pyridine nucleotide and highly purified glutamic dehydrogenase yields glutamic acid containing deuterium in a concentration equivalent to 90% of the calculated value.

The amount of deuterium in the methyl group was determined by oxidation of lithium lactate with a sulfuric acid-sodium dichromate mixture and isolation of the resulting acetic acid as the benzyl isothiuronium salt. Correction for washout of the isotope during the reaction by oxidation of unlabelled lithium lactate in a deuterated sulfuric acid-sodium dichromate mixture shows that the β - position contains less than 0.5% of the total isotope in the molecule.

EXPERIMENTAL⁶

*Bromomethylmalonic acid*⁷ was obtained by reaction of methylmalonic acid in ether with bromine until no further uptake of the halogen was observed. In the several preparations made, less than the expected equimolar quantity of bromine was absorbed. The crystalline solid obtained in each case represented less than 50% of the theoretical amount. In each preparation the solid obtained sublimed at about 135°; however the m.p. (actually a decomposition point with carbon dioxide evolution) varied from preparation to preparation. The various m.p. obtained are: 176–190°, 176–184°, 185–195°, 163–172°, and 142–145°. Repeated recrystallization from various organic solvents did not effect much change in the melting points so the impure solid was used in the subsequent reactions.

Lactic acid from bromomethylmalonic acid. A. DL-2-deuteriolactic acid. Bromomethylmalonic acid (4.5 g.) was dissolved in 5 ml. of deuterium oxide by heating to 50°. The solvent was removed *in vacuo* and the semicrystalline solid obtained refluxed in 5 ml. of deuterium oxide for 30 hr. At the end of this time 50 ml. of water and excess zinc carbonate were added to the reaction mixture and refluxing continued for 6 hr. The solution was filtered hot to remove the unreacted zinc carbonate, and the filtrate concentrated to dryness. The solid obtained was redissolved in water and concentrated to dryness several times to remove labile deuterium. To remove zinc bromide, it was then extracted with hot absolute ethanol until the extract gave a negative halide test. The residue was dissolved in the minimum amount of hot water and the zinc DL-2-deuteriolactate trihydrate precipitated by addition of absolute ethanol. Filtration, washing successively with ethanol, then with ether, and air drying overnight gave 2.45 g. of the zinc salt. Analysis of the zinc hydrate for lactic acid gave values of 30–50% of the standard.

B. Unlabelled lactic acid was obtained as the zinc hydrate by following the above procedure exactly with the substitution of hydrogen oxide for deuterium oxide. The solid

obtained weighed 2.70 g. Analysis of the zinc hydrate for lactic acid gave values of 105% of the standard.

Analysis of zinc-DL-2-deuteriolactate trihydrate. 298.3 mg. were eluted through Amberlite IR-120H until eluate was no longer acidic. Calcd. meq. of base: 2.00 meq.; found: 1.96 meq. 297 mg. were dried to constant weight. Calcd. H₂O: 18.1; found: 17.5. Zinc analysis: Calcd.: 22.0; found: 22.6.

Paper strip chromatography performed with the system described by Stark *et al.*⁸ using Whatman Filter paper #40 and developing with bromocresol green gave an *R_f* of 88.4 for standard lactic acid and an *R_f* of 88.2 for the synthetic DL-2-deuteriolactic acid.

The anhydrous zinc salt contained 44.2 atom per cent excess of deuterium.

2-Deuterio-2-bromopropionic acid. Bromomethylmalonic acid (72.1 g., 0.37 mol.) was treated with deuterium oxide (20 ml., 1.0 mol.) and the mixture warmed on a steam bath. The solvent was then removed *in vacuo* at 40–50°. After the process was repeated two more times, the sludge obtained was dried over phosphorus pentoxide for several days. The light brown, crystalline solid obtained was heated in an oil bath at 170°, at which temperature decarboxylation proceeded rapidly. After heating for 7 hr. the evolution of carbon dioxide virtually ceased. The liquid obtained was distilled *in vacuo* and a fraction boiling between 70–80° at 15 mm. of Hg was collected. This was carefully refractionated and a fraction boiling at 101–103° at 20 mm. of Hg was collected. It weighed 26.8 g., representing a yield of 45.2%. Mass spectrographic analysis showed that the compound had 70.8 atom per cent excess of deuterium.

DL-2-deuteriolactic acid from 2-deuterio-2-bromopropionic acid. To 25.8 g. (0.167 mol.) of DL-2-deuterio-2-bromopropionic acid in 150 ml. of water was added excess zinc carbonate and the mixture refluxed for 6 hr. The mixture was filtered to remove the unreacted zinc carbonate and the filtrate concentrated to dryness *in vacuo*. Extraction of the solid with absolute ethanol until the extract gave a negative halide test removed the zinc bromide. The residue was dissolved in water and concentrated to a small volume; crystallization was effected by the addition of ethanol. The crystals were filtered off, washed first with ethanol, then with ether, and air-dried overnight. A yield of 20.8 g. (87.5%) of the zinc hydrate containing 71.1 atom per cent excess of deuterium was obtained.

Degradation of DL-2-deuteriolactic acid. A. *Chemical oxidation.* To 500 mg. of labelled lithium lactate (containing 77.0 atom per cent D) was added 1.5 ml. of conc. sulphuric acid in 3.75 ml. of water and 1.78 g. of Na₂Cr₂O₇ in 3 ml. of water and the solution heated for 19 hr. on a steam bath. At the end of this time 200 ml. of water were added and the solution distilled until 125 ml. of distillate were collected. This was neutralized with standard sodium hydroxide. Reaction with *S*-benzylisothiuronium chloride gave the desired derivative, m.p. 144°. Admixture with authentic *S*-benzylisothiuronium acetate gave no depression of the melting point. Deuterium analysis gave 0.046 atom per cent excess for the salt or 0.21 atom per cent excess in the methyl group.

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(6) Deuterium analyses were performed by a modification of the method of J. Graff and D. Rittenberg, *Anal. Chem.*, **24**, 878 (1952). The mass spectrometer used for these analyses (Consolidated Electroynamics Inc., model 21-401) was purchased with funds made available by a grant from the National Heart Institute (No. AH-950), National Institutes of Health, Public Health Service.

(7) The literature values for this compound vary from 118–119° reported by H. Byk, *J. prakt. chem.*, (2) **1**, 19 (1870) to 165–170° by R. Meyer and P. Bock, *Ann.*, **347**, 105 (1906).

(8) S. B. Stark, A. E. Goodban, and H. S. Owens, *Anal. Chem.*, **23**, 413 (1951).