Journal of Labelled Compounds — October-December — Vol. VII, No. 4

Synthesis of Homovanillic Acid-2-^{14}C, Homoprotocatechuic Acid-2-^{14}C, 3', 4'-Dihydroxymandelic Acid-2-^{14}C and 1-(4 hydroxy-3-methoxyphenyl)-Ethylene Glycol-1-^{14}C.

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Received, March 11, 1971.

SUMMARY

Specifically labelled phenylacetic acid and mandelic acid derivatives, metabolites of L-Dopa, have been synthesized via *the intermediary labelled benzyl alcohols, which were prepared by reduction of the methyl esters of the appropriate benzoic acids. The benzyl alcohols have been converted to the corresponding phenylacetonitriles* via *the intermediate chlorides and to the cyanohydrins* via *the intermediate aldehydes in yields significantly higher than previously reported. Overall radiochemical yields of the title compounds were in the order of 25 % from $^{14}CO_2$ and specific activities ranged from 4-10 mC/mmole. The collated procedures have general applicability.*

INTRODUCTION AND DISCUSSION.

We have synthesized some substituted phenylacetic and mandelic acids, each ^{14}C labelled at the benzylic position of the side chain *(1-4)* [1]. Since interest in these compounds appears to be increasing, we sought to improve existing methods for their preparation as well as for analogous compounds.

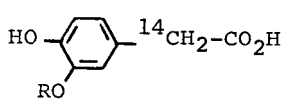
The required functional ^{14}C has been introduced into an appropriately substituted aromatic compound by a carbonation procedure [2, 3] or by the use of Cu^{14}CN [4], each resulting in a one carbon addition. We investigated the applicability of the essentially one step synthesis of α-keto acids [5], a two carbon addition, which, if successful, would allow either of the two carbons to be labelled. With these aromatic compounds, however, we found that the appropriate lithium derivative, formed *in situ* from the corresponding bromide, while yielding a carbonation product with CO_2, failed to react at a

favorable rate with tetramethylbutyl isonitrile. Accordingly, the carbonation procedure was adopted for this work. The acids resulting from this procedure have previously been reduced *via* their acid chloride derivatives [2] to the corresponding aldehydes which could possibly serve as intermediates for the present work. However, the alcoholic reduction products of these acids were readily obtained and provided easy access to the phenylacetic acids *(1, 2)* as well as the mandelic acid derivatives *(3, 4)*.

The carbonation product, 4-benzyloxy-3-methoxy-benzoic acid (*5*, Scheme I) was esterified *(6)* and reduced to the alcohol *(7)*. These reactions are essentially quantitative although, in practice, the alcohol was purified by column chromatography and obtained in overall yields of 80-85 %. This intermediate *(7)* was converted to the chloride *(8)* [6] which can yield the purified phenylacetonitrile. Purification was found to be unnecessary and hydrolysis of the crude nitrile yielded the acid *(9)* which was debenzylated [7] to provide pure homovanillic acid-2-^{14}C *(1)* in overall yield of 52 % from *7*. In addition, the alcohol *(7)* was oxidized [8] in high yield to the aldehyde *(10)*, which provided the intermediate necessary for the preparation of *3* and *4*.

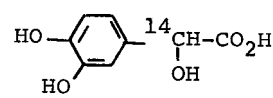
The cyanohydrin (*12*, Scheme II) has been reported as the acetate derivative [9] but we experienced considerable difficulty in obtaining a pure sample on a scale of 1-5 mmole and therefore thoroughly examined its preparation.

Cyanohydrins of oxygenated benzaldehydes are almost invariably prepared *via* the intermediate aldehyde-bisulfite adducts [10-12] and excellent yields have been obtained. The reported [9] synthesis of *12* does not involve the bisulfite adduct and the product is obtained only as the acetate derivative and in low yield. We investigated preparation of *12* with the bisulfite adduct of *10* but a variety of experimental conditions were uniformly unsuccessful, apparently due to the nonpolar solubility properties conferred by the benzyl group. Thin layer chromatographic (tlc) examination of the direct conversion of *10* to *12* (Scheme II) showed that product had been significantly formed after a short reaction period at room temperature, indeed, to a greater extent than



1 R = CH_3

2 R = H



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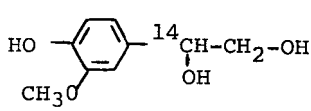

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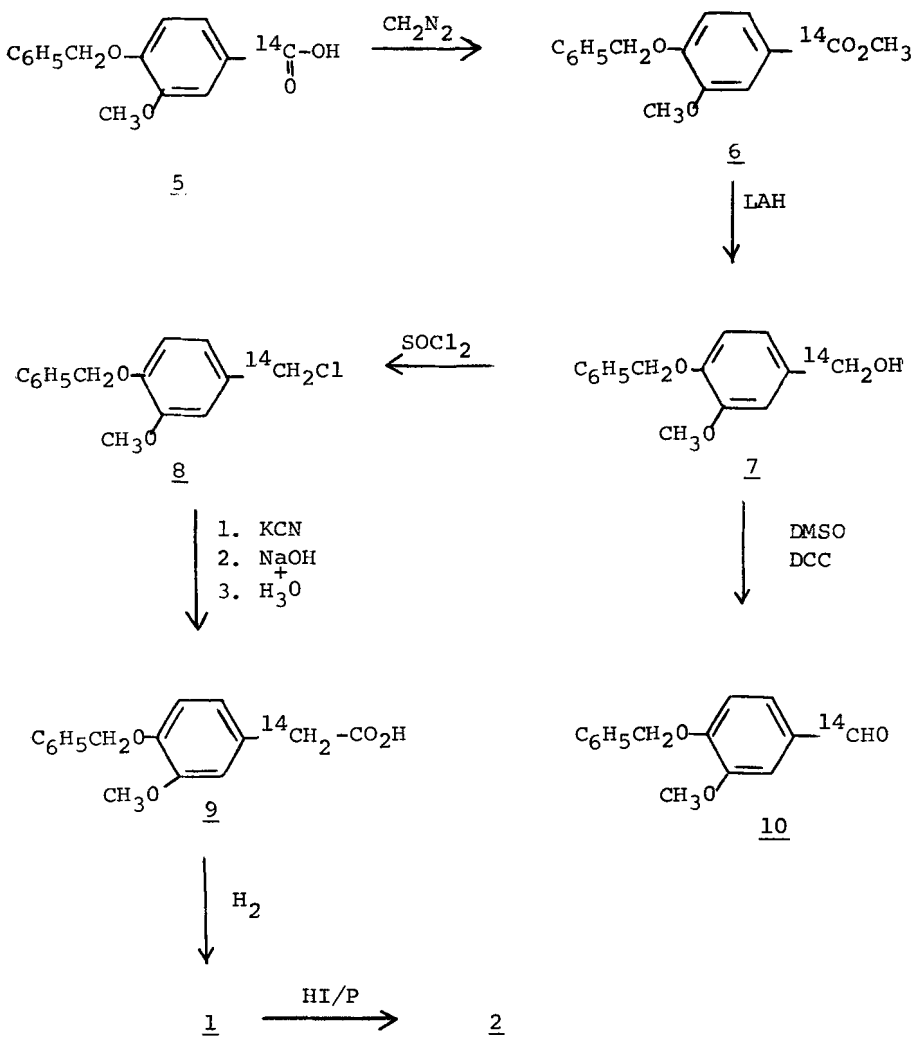
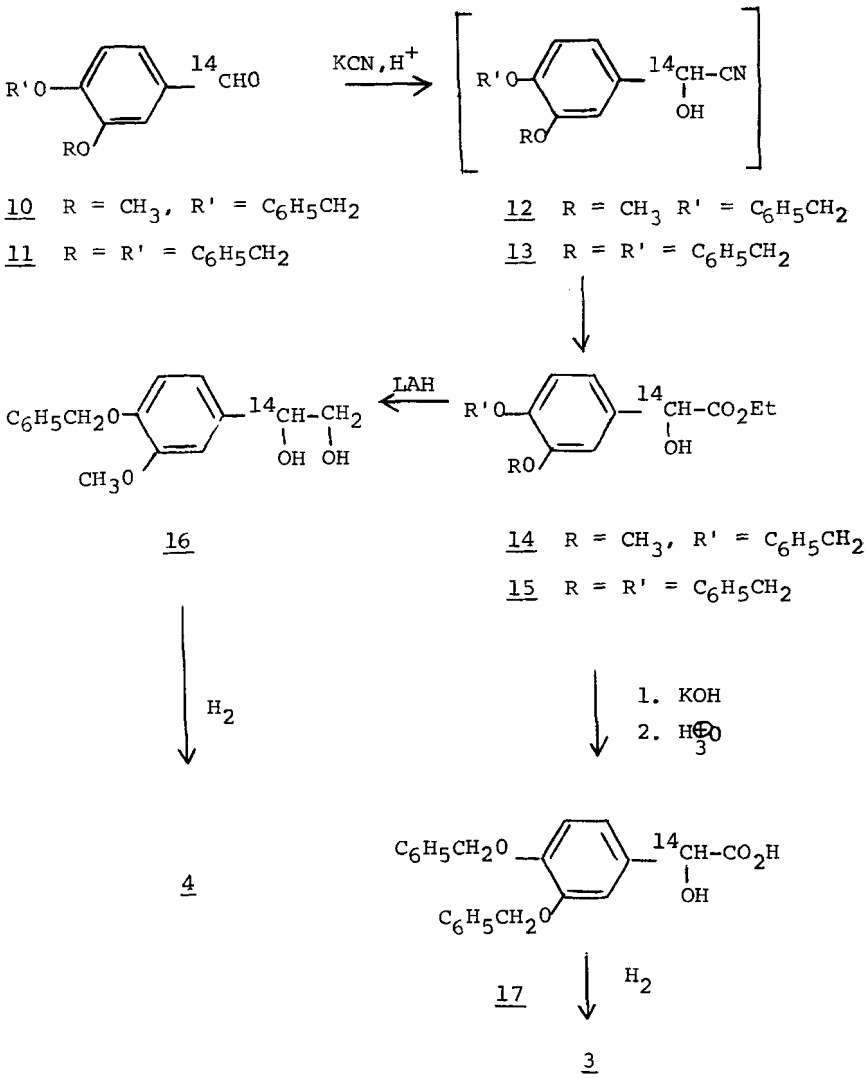


SCHEME I. Phenylacetic acids.

after 60 minutes in refluxing dioxane as described [9]. This was indicative of an unfavorable equilibrium causing reversal of the cyanohydrin to the aldehyde and HCN and an attempt to isolate the cyanohydrin *(12)* from the crude reaction mixture by silica gel chromatography was found to cause significantly more reversal of product to starting material. A two dimensional thin layer chromatography confirmed this instability characteristic of the cyanohydrin.

We were interested in *12* and *13* only as intermediates and therefore used tlc to determine the reaction conditions and stoichiometry required for maximum conversion of the aldehydes to the cyanohydrins. This conversion was found to approach 100 % and subsequent reactions were carried out immediately. Thus, by ethanolysis, *12* yielded the stable ester *(14)* which was reduced to the glycol *(16)* and hydrogenolysis provided the desired product *(4)*.



10 R = CH_3, R' = $C_6H_5CH_2$
11 R = R' = $C_6H_5CH_2$
12 R = CH_3 R' = $C_6H_5CH_2$
13 R = R' = $C_6H_5CH_2$
14 R = CH_3, R' = $C_6H_5CH_2$
15 R = R' = $C_6H_5CH_2$
16
17
4
3

SCHEME II. Mandelic Acid Derivatives.

These latter reactions were all carried out as modifications of the existing procedures and the final product *(4)* was obtained chromatographically pure in overall radiochemical yield of 57 % from the aldehyde *(10)* and 23 % overall from $^{14}CO_2$. This compares favorably with the reported [9] overall chemical yield of 12.5 % of *4* from nonlabelled benzyl vanillin.

By the same series of reactions, the analogous 3,4-dibenzyloxy-benzaldehyde-7-^{14}C *(11)* was prepared and converted *via* the cyanohydrin *(13)* to 3′, 4′-dihydroxymandelic acid-2-^{14}C *(3)* in comparable yield.

EXPERIMENTAL.

Radioactivity was measured by the liquid scintillation technique using a Packard Tricarb Model 2003 liquid scintillation spectrometer. All melting points are uncorrected.

Nonradioactive Starting Materials.

4-Bromoguaiacol. — Redistilled guaiacol (12.41 g, 0.10 mole, bp 202°/751 mm) was brominated in chloroform as described [15], yielding 61 % of fractionally distilled product, bp 54-57°/1μ.

4-Benzyloxy-3-methoxybromobenzene. — A 12.36 g (60.9 mmole) portion of 4-bromoguaiacol was treated with benzyl chloride as described [15]. The crude product obtained was recrystallized from ethanol resulting in a 60 % yield of product, 7.91 g, mp 60.5-61.0°.

5-Bromosalicylaldehyde. — Following the described procedure [16], redistilled salicylaldehyde, bp 96-96.5°/29 mm, was brominated in acetic acid in 91 % crude yield.

4-Bromocatechol. — The brominated salicylaldehyde was treated as described [17] yielding 31.2 % of product, mp 82.5-83.5°, recrystallized from carbon tetrachloride.

3,4-Dibenzyloxybromobenzene. — Benzylation of 4-bromocatechol was carried out as described [3]. After recrystallization from ethanol, the product was obtained in 57.8 % yield, mp 66-66.5°.

Radioactive Syntheses.

Benzylvanillic acid-7-^{14}C (5). — A 562 mg (2.0 mmole) portion of 4-benzyloxy-3-methoxybromobenzene was dissolved in ether, treated with 1.5 mmole of 1.5*N* butyllithium solution and carbonated with 1.0 mmole of $^{14}CO_2$ of specific activity 40 mC/mmole. A 71.7 % yield (183 mg) of the desired product was obtained by the described [2] workup procedure. The melting point of a nonradioactive sample prepared in the identical manner was 154-163°.

Benzylvanillic acid-7-^{14}C of approximately 10 mC/mmole was also obtained by this procedure as was 3,4-dibenzyloxybenzoic acid-7-^{14}C [3], prepared

from 3,4-dibenzyloxybromobenzene and $^{14}CO_2$ having specific activity again of 10 mC/mmole. In the latter instance, yields ranged up to 90 %.

Methyl benzylvanillate-7-^{14}C (6). — A 183 mg (0.717 mmole) portion of benzylvanillic acid-7-^{14}C was esterified with excess diazomethane in ether in essentially quantitative yield. A non-radioactive sample, mp 84.0-84.5° prepared in the identical manner, possessed a compatible NMR spectrum.

Anal. : Calcd for $C_{16}H_{16}O_4$: C, 70.57; H, 5.92; Found : C, 70.51; H, 6.12.

4-Benzyloxy-3-methoxybenzyl alcohol-7-^{14}C (7). — Methyl benzyl vanillate-7-^{14}C, 191 mg (0.7 mmole) was dissolved in 4 ml of tetrahydrofuran (THF) [freshly distilled from lithium aluminum hydride (LAH)] and added, over 15 minutes to a well stirred suspension of 75 mg (1.8 mmole) LAH in 30 ml THF. The mixture was stirred under nitrogen for 15 minutes, refluxed briefly and allowed to cool to room temperature. A 0.5 ml portion of ethyl acetate was added followed by 30 ml of ethyl ether, 0.5 ml water and 9 ml 1*N* H_2SO_4. The organic phase was washed with two 5 ml portions of water, then 5 ml of brine, dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo* to a residue of 177 mg. This was chromatographed on 25 g of silica gel (E. Merck #7734) with $CHCl_3$-ethyl acetate; 3 : 1. After a 50 ml forerun, 5 ml fractions were collected of which numbers 8-20 were combined and concentrated *in vacuo* to give 136 mg (0.555 mmole) of product. After admixture with 951 mg of unlabelled 4-benzyloxy-3-methoxybenzyl alcohol, specific activity was determined to be 4.20 mC/mmole. The total activity was 55.5 % of the initial $^{14}CO_2$.

By parallel reactions, material of specific activity of 10.2 mC/mmole was also obtained. In addition, 3,4-dibenzyloxybenzyl alcohol-7-^{14}C of a specific activity of 4.40 mC/mmole was obtained in exactly the same manner and in comparable yield.

4-Benzyloxy-3-methoxybenzyl chloride-7-^{14}C (8). — A suspension of 346 mg (1.42 mmole) of 4-benzyloxy-3-methoxybenzyl alcohol-7-^{14}C, 4.20 mC/mmole, in 4 ml of absolute toluene was chilled to 0°. With magnetic stirring and under N_2, 266 mg (1.85 mmole) of thionyl chloride was added in one portion. Stirring was continued at 0° for 3 hours after which time the homogeneous solution was evaporated *in vacuo* at 25° to a solid residue of 362 mg. By tlc (SiO_2, chloroform-ethyl acetate 3 : 1 elution), this residue showed a single spot at Rf 0.66 as compared with the starting material, Rf 0.32. The product may be sublimed at 60°/30 μ to yield material of mp 73.5-74.5°, lit. [6], 72-74°.

4'-Benzyloxy-3'-methoxyphenyl acetic acid-2-^{14}C (9). — The crude chloride obtained above (approx. 1.4 mmole) was dissolved in 14 ml of acetonitrile. Under N_2 and with magnetic stirring, 138 mg (2.13 mmole) of KCN and 0.1 ml of water were added. The resulting mixture was heated at reflux for 18 hr, cooled and evaporated *in vacuo* at room temperature to a thick residue which was extracted with three 15 ml portions of ether. These were combined, fil-

tered and evaporated to a thick residual oil which was treated with 6 ml of 10 % aqueous sodium hydroxide solution [7]. Under nitrogen, the mixture was magnetically stirred at reflux for 22 hours. After cooling, the solution was diluted with 6 ml of water and unreacted neutral materials were extracted by two 5 ml portions of ether. The aqueous phase was filtered and then acidified with 1.3 ml HCl (pH $<$ 2). The resulting mixture was chilled to 5° and allowed to stand for 4 hours with occasional stirring, then filtered and the precipitate washed with ice-water until chloride free. The product was dried at 25°/25 μ to a constant weight of 245 mg (0.90 mmole), 63.5 % from the benzyl alcohol *(7)*, mp 108-110°, lit. [13], 114-115°.

Homovanillic acid-2-^{14}C (1). — The entire sample obtained above was suspended in 15 ml of water and treated with 59 mg of 5 % Pd/C (20 % w/w). The mixture was hydrogenated at 25° and 1 atm until uptake ceased (6 hours). The mixture was heated to boiling, filtered and the precipitate thoroughly washed with boiling water. The filtrates were combined and concentrated *in vacuo* to a residual white solid (152.8 mg) which was sublimed at 110-115°/30 μ yielding 134.6 mg, 0.74 mM (52 % yield from 7), mp 140-142°, lit. [7, 14] 142-143°. The product was homogeneous by tlc (SiO_2, methanol, butanol, benzene, water, 4 : 3 : 2 : 1, elution, Rf 0.68) and had a specific activity of 22.47 μC/mg or 4.094 mC/mmole.

Homoprotocatechuic acid-2-^{14}C (2). — A sample of homovanillic acid-2-^{14}C, 127 mg (0.70 mmole), specific activity of 55 μC/mg and prepared by the above procedures, was added to a decolorized mixture of 62 mg (2.0 mg atom) red phosphorus and 1 ml 57 % HI solution. With magnetic stirring and under N_2, the mixture was treated as described [10] for preparation of this compound from homoveratric acid. The crude product obtained was purified by sublimation at 105°-110°/20 μ, yielding 82 mg (69 %), specific activity 59.54 μc/mg (10.01 mC/mmole), homogeneous by tlc (SiO_2, methanol, butanol, benzene and water, 4 : 3 : 2 : 1 elution, Rf 0.60).

Benzylvanillin-7-^{14}C (10). — Following the procedure of Pfitzner and Moffatt [8], 570 mg (2.32 mmole) of 4-benzyloxy-3-methoxybenzyl alcohol-7-^{14}C, specific activity of 4.2 mC/mmole, was dissolved in a mixture of 4.6 ml of dry benzene and 2.3 ml of dry dimethylsulfoxide. The solution was treated with 1.45 g of dicyclohexylcarbodiimide and 0.092 ml of 5M anhydrous phosphoric acid in DMSO, stoppered and stirred magnetically at room temperature for 18 hours. After the described [8] isolation procedure was carried out, the crude product was chromatographed on 125 g of silica gel (E. Merck # 7734) with 8 % ethyl acetate in benzene. Concentration of the appropriate fractions yielded 413 mg (1.7 mmole), 73.2 % of the desired product homogeneous by tlc. Yields ranged to 82 %.

In the same manner, 3,4-dibenzyloxybenzaldehyde-7-^{14}C *(11)* was prepared in similar yield (80 % from 0.42 mmole of 3,4-dibenzyloxy benzyl alcohol-7-^{14}C, specific activity of 4.40 mC/mmole.

DL-ethyl-4'-benzyloxy-3'-methoxymandelate-2-^{14}C (14). — Benzyl vanillin-7-^{14}C, 413 mg (1.7 mmole, 4.2 mC/mmole) and potassium cyanide (92 %), 1.7 g (25.3 mmole) were treated with 3.82 ml of dioxane and 0.94 ml water. After stirring at room temperature for 5 minutes, 0.57 ml (6.8 mmole) of hydrochloric acid was added. The mixture was stirred at room temperature an additional 30 minutes, diluted with 15 ml of ether and filtered. An additional 15 ml ether wash was added to the original filtrate which was then washed with two 5 ml portions of water, dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo* to a residual oil (467 mg), which showed by tlc analysis (SiO_2 chloroformethyl acetate 3 : 1 elution) a minimum of 90 % cyanohydrin, Rf 0.40 and the balance starting material at Rf 0.56. This mixture was dissolved in 5.1 ml of absolute ethanol and treated with 3.4 ml of HCl saturated ethanol at room temperature for 3.5 hours. The solvent was removed *in vacuo* at 30° and the residue treated with 25 ml of water. After stirring for 15 minutes, the resulting thick mixture was extracted with two 20 ml portions of ether, followed by two 20 ml portions of benzene. These were combined, dried, filtered and concentrated *in vacuo* to a residue of 468 mg which was chromatographed on 95 g of silica gel (E. Merck # 7734). The column was developed with chloroform-ethyl acetate, 3 : 1 and concentration of the appropriate fractions yielded 398 mg of the pure ester (1.26 mmole, 78 % yield from *10*). Non radioactive material prepared in exactly the same manner was of mp 120-121°, lit. [9], mp 120-123°.

By a similar process, 3,4 dibenzyloxybenzaldehyde-7-^{14}C, 215 mg (0.67 mmole, 4.40 mC/mmole) was treated with 10 mmole KCN and 2.7 mmole HCl. The resulting crude cyanohydrin (248 mg) was treated with 1.35 ml of HCl-saturated ethanol as described above. The resulting ester was not chromatographed.

DL-1-(4-benzyloxy-3-methoxyphenyl)-ethylene glycol-1-^{14}C (16). — A suspension of 115 mg (3.02 mmole) LAH in 15 ml of distilled THF was magnetically stirred under N_2 and heated at reflux for 10 minutes. The mixture was cooled to 35-40° and a solution of *14*, 398 mg (1.26 mmole) in 35 ml of THF was added over a period of 15 minutes maintaining the 35-40° temperature. After stirring at 40° for 15 minutes, the mixture was heated to reflux and held at that temperature for 10 more minutes. The flask was then cooled in an ice bath and decomposition effected by the successive addition of 1.2 ml water, then 0.6 ml H_2SO_4 in 12.2 ml of water. After the addition of 10 ml ether an aqueous phase separated which was extracted with two 20 ml portions of ether. These were combined with the organic phase and concentrated *in vacuo*. The residue was redissolved in ether, dried over magnesium sulfate, filtered and again concentrated *in vacuo* to a residual oil which crystallized on standing; 327 mg (1.19 mmole, 95 % yield). By tlc (SiO_2, ethyl acetate-ethanol, 95 : 5 elution), the product was homogeneous at Rf 0.31 and compared exactly with an authentic sample.

DL-1(4-hydroxy-3-methoxyphenyl)-ethylene glycol-1-^{14}C (4). — The crude glycol *(14)* obtained above was dissolved in 30 ml of absolute ethanol. After the addition of 164 mg 5 % Pd/C, the mixture was hydrogenated at 25° and 1 atmosphere for 30 minutes when uptake ceased. The mixture was filtered from the catalyst and the filtrate concentrated *in vacuo* to a residual oil which was chromatographed on 44 g of silica gel (E. Merck # 7734). The column was developed with 5 % ethanol in ethyl acetate. The appropriate 3 ml fractions were combined, filtered and concentrated *in vacuo* to an oil of constant weight of 210 mg. The product was homogeneous by tlc (SiO_2, ethyl acetate-ethanol 95 : 5 elution), Rf 0.27 and had specific activity of 22.8 μc/mg (4.2 mC/mmole). The radiochemical yield was 4.08 mc, 57 % from the aldehyde *(10)* and 23 % from $^{14}CO_2$.

DL-3',4'-dibenzyloxymandelic acid-2-^{14}C (17) [7]. — Crude ethyl 3', 4'-dibenzyloxymandelate-2-^{14}C, prepared from 3,4-dibenzyloxybenzaldehyde-7-^{14}C (0.67 mmole) as described above was suspended in 5 ml of water and treated with 145 mg of potassium hydroxide. The mixture was stirred and heated at reflux for 3 hours. The warm solution was extracted with two 15 ml portions of ethyl acetate which were combined and concentrated *in vacuo* to yield 117 mg of recoverable aldehyde *(7)*. The aqueous layer was then acidified to pH 1-2 with hydrochloric acid and the resulting mixture extracted with two 20 ml portions of ethyl acetate. These were combined, washed with two 5 ml portions of water, dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo* yielding 113 mg, 0.311 mmole (46 % from *11*).

DL-3',4'dihydroxymandelic acid-2-^{14}C (3). — The dibenzylmandelic acid-2-^{14}C was suspended in 10 ml of water and 23 mg of 5 % Pd/C were added. The mixture was hydrogenated at 1 atmosphere and at room temperature for 5 hours when uptake ceased. The catalyst was removed by filtration, thoroughly washing the precipitate with water, the product then isolated by lyophillizing the combined filtrates to provide 57 mg (0.31 mmole). By tlc (SiO_2, methanol, butanol, benzene, water, 4 : 3 : 2 : 1 elution), the product was homogeneous at Rf 0.50 and corresponded exactly to a non-radioactive sample of authentic material prepared in exactly the same manner, mp 136-137°, lit. [(11)], 145-146° after recrystallization from acetonitrile. Specific activity was 23.9 μc/mg (4.4 mC/mmole) and the radiochemical yield was 41 % from the aldehyde *(11)*.

ACKNOWLEDGMENT.

We thank Dr. T. Williams of our Physical Chemistry Department for n.m.r. spectra and Dr. F. Scheidl of our microanalytical laboratories for microanalyses.

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