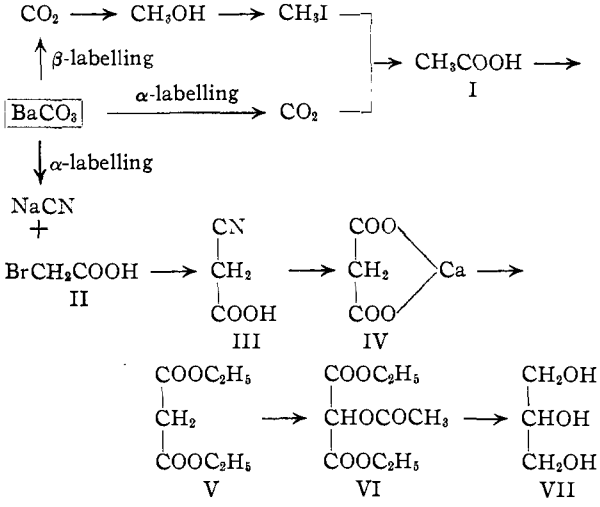
[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL CHEMISTRY AND THE BIOPHYSICAL LABORATORY, HARVARD MEDICAL SCHOOL]

A Synthesis of α- or β-C¹⁴-Labeled Glycerol

BY L. I. GIDEZ[1] AND M. L. KARNOVSKY

Radioglycerol, selectively labelled in the α-position (glycerol-1-(3)-C¹⁴) or the β-position (glycerol-2-C¹⁴) has been prepared from easily available radioactive starting materials, such as barium carbonate, acetic acid or sodium cyanide. The over-all yield starting from barium carbonate was of the order of 30%.

Glycerol has a unique relationship to both the lipids and the carbohydrates, and methods for making radioglycerol labelled in the α- or the β-position with C^{14} should be of particular interest to investigators studying carbohydrate and lipid metabolism and fermentation mechanisms. Such methods have recently been elaborated[2,3,4] and the scheme below describes the synthesis adopted in these laboratories and outlined previously.[2] Barium carbonate is shown as the ultimate starting material for both the α- and β-labelled products, but the synthesis may, of course, start at any convenient stage in the scheme.



α-Labelled glycerol is obtained from carboxyl-labelled acetic acid, or by the use of radiocyanide, and β-labelled glycerol results from the use of methyl-labelled acetic acid. These three starting materials are all commercially available, or may be synthesized by well-known methods[5,6,7] and their preparation will not be discussed. The intermediates of the above scheme (II – VI) were made by slight adaptations and modifications of established methods, and representative yields are given in the experimental section. The final step was the reduction of the tartronic acid ester (VI) with lithium aluminum hydride. The stoichiometry of such reactions has been worked out[8] and it has been shown that the reduction of an ester to an alcohol can be accomplished as follows

$$2RCOOR' + LiAlH_4 \longrightarrow LiAl(OR')_2(OCH_2R)_2$$

Thus, for each mole of the above triester, 1.5 moles of lithium aluminum hydride was required, and the products of the reaction were presumably lithium and aluminum alcoholates and glyceroxides, which were decomposed with water. Experience in this Laboratory has shown it to be necessary to remove dissolved salts from the resulting glycerol solution, especially if the product is to be distilled. This has been accomplished by passing the solution, containing glycerol and salts, through an ion-exchange column. The dilute ion-free solution of glycerol could then be concentrated by distillation of the water under reduced pressure, or by lyophilization.

The syntheses reported here were carried out on quantities that made addition of carrier unnecessary, with the object of more easily establishing the degree of "radio-purity" of the final product of the series of reactions. Our experiences lead us to believe that these operations may be carried out on smaller quantities, and carrier added where necessary, particularly in the final step. The over-all yields of radioglycerol were found to be about 30% from barium carbonate or acetic acid, or about 40% based on sodium cyanide.

The product was assayed by periodate oxidation and collection of the formaldehyde produced as the dimedone compound. This compound could be plated and counted, and represented the α-carbons of the glycerol molecule. The total carbon in the product was determined by wet combustion, and the carbon dioxide formed was counted as barium carbonate. The periodate assay and the combustion data revealed some carbon contamination derived, it is believed, from the ion-exchange column. Glycerol sample 1, which was passed through a column that had been subjected to a preliminary washing of exceptional thoroughness, proved to be free of this contamination. Distillation of the product, admixed with pure commercial glycerol, removed the extraneous material. Table I summarizes the results. A sample of formaldimedone obtained from α-labelled glycerol was recrystallized and counted. The same material was combusted, and counted as barium carbonate, and a factor obtained for the conversion of counts as formaldimedone to counts as barium carbonate. Under the conditions of counting in these laboratories, this factor is 1.04.

The localization of the radioactivity in the α- or β-carbons of the glycerol was established after oxidation with periodate. In the case of the α-labelled product, the formic acid obtained, representing the β-carbon, was counted after conversion to barium carbonate, and had a specific activity

(1) United States Atomic Energy Commission Predoctoral Fellow.
(2) M. L. Karnovsky and L. I. Gidez, *Fed. Proc.*, **10**, 205 (1951).
(3) A. P. Doerschuk, THIS JOURNAL, **73**, 821 (1951).
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(5) "Isotopic Carbon," John Wiley and Sons, Inc., New York, N. Y., 1949, pp. 172–179.
(6) J. A. McCarter, THIS JOURNAL, **73**, 483 (1951).
(7) Reference 5, pp. 193–195.
(8) R. F. Nystrom and W. G. Brown, THIS JOURNAL, **69**, 1197, 2548 (1947).

TABLE I

CHARACTERISTICS OF RADIO-GLYCEROL SAMPLES

Sample	Glycerol in product, %	Glycerol carbon, %	Total carbon, %	Glycerol C/Total C × 100	Activity, ct./min. mM. × 10^{-5} Formal dimedone	Activity, ct./min. mM. × 10^{-5} $BaCO_3$
α-Label 1	96.8	37.8	37.7	100.5	5.99	6.30
2	86.6	33.9	37.3	90.8	59.40	64.80
3[a]	97.5	38.2	37.2	103.0	9.62	10.85
β-Label 4	85.0	33.2	37.9	87.6	...	3.50
5[a]	95.5	37.4	38.1	98.1	...	1.03

[a] Samples 2 and 4 were diluted with pure glycerol and distilled *in vacuo* to give samples 3 and 5, respectively.

of between 0.4 and 2.5% of that of the whole molecule. In the case of the β-labelled glycerol, the formaldehyde representing the α-carbons was found to have a specific activity of 1.2–1.8% of that of the whole molecule. The reason for this apparent presence of radioactivity where it was not expected is not clear. It may be due either to radioactive contaminants of the glycerol or to inadequate selectivity of the degradation method.

As a check on the presence of radioactive contaminants, paper partition chromatograms were carried out according to the method of Hough[9] with several solvent systems. Ammoniacal silver nitrate was used to locate the components after development of the chromatogram. The synthetic radioglycerol behaved identically with pure glycerol, in that the R_f values were the same, and no other components were evident when the paper was scrutinized. Some strips were cut into uniform contiguous discs after development, and these discs were mounted on plates and counted. The results, as shown in Fig. 1, indicate the presence of two contaminants, one moving ahead of the glycerol zone and one behind it. Although the method is not quantitative, the indications are that these contaminants constitute not more than 5% of the total activity, and in the case of Sample 1 were scarcely detectable.

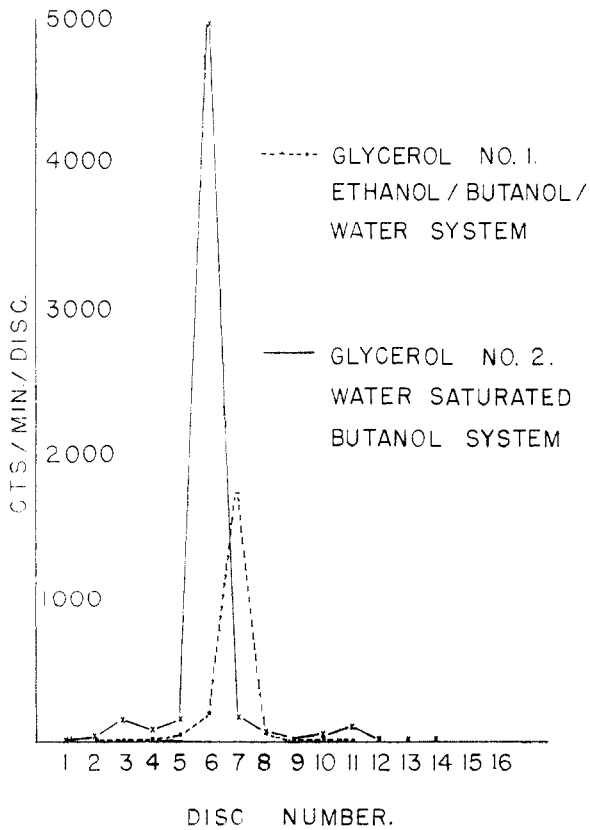

Fig. 1.—Paper-chromatographic examination of two radioglycerol samples. The paper was cut into contiguous discs, 20 mm. diameter, which were mounted and counted in a gas flow counter.

(9) L. Hough, *Nature*, **165**, 401 (1950).

Experimental

In these laboratories, quantities were chosen to yield about 6 g. of radioglycerol, without the addition of carrier at any intermediate stage.

Bromoacetic acid (II) was made by adapting the method of Natelson and Gottfried[10] so that 50% more bromine was used than in their directions, resulting in a greater conversion of acetic acid; yield 84%.

Calcium Malonate (IV).—The method of Weiner[11,12] was used, but with bromoacetate substituted for the chloro-compound for reasons of convenience; yield 81% (based on cyanide), 85% (based on bromoacetate).

Diethyl Malonate (V).—Free malonic acid was not isolated but the calcium salt was dehydrated *in vacuo* at 100° over P_2O_5, for 12 hours, and directly esterified with absolute alcohol and dry HCl gas[13,14]; yield 73%.

Diethyl Acetoxymalonate (VI).—Oxidation of diethyl malonate with lead tetraacetate was carried out according to the directions of Dimroth and Schweizer,[15] and the triester (diethyl acetoxymalonate; diethyl acetyltartronate) obtained after a smooth reaction; yield 83%.

Glycerol (VII).—Seven grams (0.2 mole) of lithium aluminum hydride was placed in a 500-ml. three-necked flask fitted with a reflux condenser and calcium chloride tube, a mercury sealed stirrer, and a dropping funnel whose stem was so bent as to deliver into the vortex of the stirred solution. Two hundred and fifty ml. anhydrous ether was added, and the mixture was refluxed for 1.5 hours. The turbid solution was chilled in an ice-bath, and 17.9 g. (0.082 mole) of diethyl acetoxymalonate in 200 ml. of dry ether added over a period of about one hour. The ice-bath was then removed, the mixture was allowed to come to room temperature, and finally was warmed (*ca.* 30–35°) on the water-bath for one hour. The solution was chilled again, and 200 ml. of water was added through the dropping funnel, at first very slowly and cautiously, to decompose the excess $LiAlH_4$, and the glyceroxides and alcoholates. The ethereal layer was separated and washed. Drying over sodium sulfate, and distillation indicated that no ether-soluble material remained. The washings and aqueous phase were combined and neutralized to *p*H 7 with H_2SO_4, filtered by suction, and the filtrate passed down an anion and cation exchange column.[16] The precipitate on the filter was washed free of radioactivity, and the washings put down the same column. The ion-free solution of glycerol was then concentrated by removal of water by lyophilization in an apparatus previously tested with inactive glycerol to ensure that carry-over was negligible. The final 20–30 ml. of water was removed *in vacuo* over P_2O_5. The product was of a very pale yellow color; yield 7.4 g. (86.6% pure, or 6.4 g. pure glycerol). This represents a yield of 85%, from diethyl acetoxymalonate. Portions of this material were admixed with pure glycerol and distilled at 140–145° (1–2 mm.) with a recovery of 90–95%, based on radioactivity and glycerol.

(10) S. Natelson and S. Gottfried, *Org. Syntheses*, **23**, 37 (1943).

(11) N. Weiner, "Org. Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 376.

(12) P. E. Yankwich, ref. 5, p. 191.

(13) E. Fischer, "Anleitung zur darstellung Organischer Präparate," 8 Aufl., 1908, p. 44.

(14) M. Conrad, *Ann.*, **204**, 126 (1880).

(15) O. Dimroth and R. Schweizer, *Ber.*, **56**, 1380 (1923).

(16) A mixture of Amberlite IR-120 and IR-4B or IR-400 is suitable.

Determination of Glycerol.—This was carried out by the method of Reeves,[17] in which the vicinal glycol is oxidized with periodic acid, and the formaldehyde from terminal carbinol groups collected and weighed as the dimedone complex.

Determination of Radioactivity.—All counting was carried out on stainless steel plates, in a gas flow counter, in the proportional range. The counter efficiency was about 45%.[18] Formaldimedone from the glycerol determination, particularly in the case of the α-labelled glycerol, was recrystallized twice from ethanol–water, centrifuged down, and plated from 30% alcohol. In order to obtain uniform plates, two or three drops of 95% ethanol were added to the material on the plate. The formaldehyde from the periodate oxidation was also distilled off, oxidized with $KMnO_4$, and counted as $BaCO_3$. The formic acid from the β-carbon of glycerol was oxidized with HgO to CO_2 and similarly counted.[19]

Combustion of Glycerol Samples.—These were combusted and their carbon content determined by the method of Van Slyke and Folch[20] with modifications in the reagents and apparatus.[21,22] The carbon dioxide was recovered and converted to $BaCO_3$ for counting.[23]

Paper Chromatograms.—These were carried out by the method of Hough.[9] The R_f values observed with several solvent systems were similar to those reported by that author. Up to 2 mg. of glycerol in solution was applied to each strip in a small spot by successive applications and evaporations with a hair-drier. The chromatograms were allowed to run for 18 hours.

Acknowledgments.—This research was supported in part by the United States Atomic Energy Commission. The authors wish to thank Mrs. Elizabeth Newton for assistance in the counting operations, and Dr. D. D. van Slyke for making available information on the combustion of radioactive samples.

(17) R. E. Reeves, THIS JOURNAL, **63**, 1476 (1941).
(18) C. V. Robinson, *Science*, **112**, 198 (1950).
(19) Y. J. Topper and A. B. Hastings, *J. Biol. Chem.*, **179**, 1255 (1949).
(20) D. D. Van Slyke and J. Folch, *ibid.*, **136**, 509 (1940).
(21) D. D. Van Slyke, J. Plazin and J. R. Weisiger, *ibid.*, **191**, 299 (1951).
(22) D. D. Van Slyke, R. Steele and J. Plazin, *ibid.*, **192**, 769 (1951).

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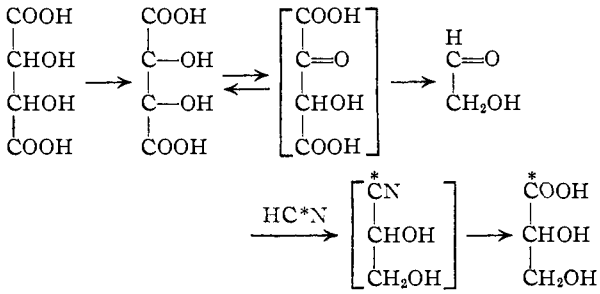
[CONTRIBUTION FROM THE DIVISION OF BIOCHEMISTRY, NOYES LABORATORY OF CHEMISTRY, UNIVERSITY OF ILLINOIS]

The Synthesis of Calcium D,L-Glycerate-1-C^{14} [1]

BY H. J. SALLACH[2]

In order to study some of the metabolic reactions of glyceric acid in the animal organism, D,L-glyceric acid-1-C^{14} has been prepared as its calcium salt by the addition of HC^{14}N to hydroxyacetaldehyde followed by the hydrolysis of the resulting nitrile.

Glyceric acid, labeled with C^{14} in the carboxyl carbon, has been synthesized as its calcium salt by the addition of radioactive hydrogen cyanide to hydroxyacetaldehyde. The resulting nitrile was hydrolyzed without isolation. The reactions involved are shown below. The radioactive carbons are marked with an asterisk.



Experimental

Hydroxyacetaldehyde was prepared according to the procedure of Fischer and Taube.[3] For this purpose tartaric acid was converted into dihydroxymaleic acid by treatment at −5° with 30% hydrogen peroxide.[4] The resulting acid was then decarboxylated by heating in pyridine.

Sodium cyanide,[5] 98.7 mg., having a total activity of 750 microcuries, was diluted with 452.9 mg. of 95% non-labeled sodium cyanide. Hydrogen cyanide (10.7 millimoles) was then generated from the sodium cyanide in a vacuum line by the addition of 10 ml. of concentrated sulfuric acid. The liberated gas was collected by means of a liquid nitrogen trap in a reaction flask containing a trace of sodium cyanide as a catalyst. A solution of 700 mg. of hydroxyacetaldehyde (11.6 millimoles) in 4 ml. of water, which had been prepared 24 hours earlier, was added to this reaction vessel. The addition reaction was allowed to proceed at 0° for 30 minutes and then for an additional 30 minutes at room temperature. At the end of this time, 4 ml. of concentrated hydrochloric acid was added, and the nitrile was hydrolyzed for 30 minutes. The solution was then neutralized with calcium carbonate, boiled for 10 minutes with a slight excess of the calcium carbonate and filtered. The calcium salt of glyceric acid was then precipitated by the addition of four to five volumes of ethanol. The product was recrystallized by dissolving in a minimum amount of hot water, decolorizing with charcoal, and reprecipitating with ethanol. The yield of calcium glycerate, based on the sodium cyanide used, was 33%.

Anal. Calcd. for $(C_3H_5O_4)_2Ca\cdot 2H_2O$[6]: Ca, 13.99; C, 25.18; H, 4.93. Found: Ca,[7] 13.92; C, 25.16; H, 4.87.

The calcium glycerate had an activity of 141.7 microcuries per millimole or 70.9 microcuries per millimole of free glyceric acid. The activity of the diluted sodium cyanide used in the synthesis was 70.1 microcuries per millimole.

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(1) Aided by grants from the United States Public Health Service and the Graduate College Research Fund of the University of Illinois.
(2) Department of Biochemistry, University of Colorado School of Medicine, Denver, Colorado.
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(4) H. O. L. Fischer and L. Feldmann, *ibid.*, **62B**, 854 (1929).
(5) Obtained from Tracerlab, Inc., Boston, Mass.
(6) P. F. Frankland and W. Frew, *Trans. J. Chem. Soc.*, **59**, 81 (1891).
(7) J. B. Niederl and V. Niederl, "Micromethods of Quantitative Organic Analysis," Second Ed., John Wiley and Sons, Inc., New York, N. Y., 1946, p. 62.