

Preparation of D-Arabinose-1-C¹⁴ and D-Ribose-1-C¹⁴¹

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By application of the cyanohydrin synthesis to D-erythrose, D-arabinose-1-C¹⁴ and D-ribose-1-C¹⁴ have been prepared in overall radiochemical yields of 30 and 8.5 percent, respectively. General acid catalysts in the cyanohydrin reaction appear to favor formation of the arabonic epimer. The epimeric acids resulting from the reaction of labeled cyanide and D-erythrose, and subsequent hydrolysis, were separated as crystalline potassium D-arabonate-1-C¹⁴ and cadmium D-ribonate-1-C¹⁴, respectively. The salts were converted to the corresponding lactones, and these were reduced to the sugars by use of sodium amalgam in the presence of sodium acid oxalate.

1. Introduction

As part of a program to make position-labeled sugars available for research workers in other laboratories, methods have been developed at the National Bureau of Standards for the preparation of D-glucose-1-C¹⁴, D-mannose-1-C¹⁴, D-mannitol-1-C¹⁴, D-fructose-1,6-C¹⁴, lactose-1-C¹⁴, and D-arabinose-5-C¹⁴ [1, 2, 3, 4, 5, 6].² The present report gives methods for the preparation of D-arabinose-1-C¹⁴ and D-ribose-1-C¹⁴. D-Arabinose-1-C¹⁴ was required as an intermediate in the preparation of D-glucose-2-C¹⁴, and both D-arabinose-1-C¹⁴ and D-ribose-1-C¹⁴ were needed for studies of the transformation of pentoses in biological systems. Rappoport and Hassid [7] obtained L-arabinose-1-C¹⁴ by application of the Sowden-Fischer nitromethane synthesis [8] to L-erythrose. The L-arabinose-1-C¹⁴ was separated in 3-percent yield by partition chromatography.

In light of prior work at the Bureau, it seemed desirable to attempt the synthesis of both D-arabinose-1-C¹⁴ and D-ribose-1-C¹⁴ by the cyanohydrin method, beginning with D-erythrose. It was found that in the reaction of cyanide with D-erythrose the presence of a general acid catalyst, such as bicarbonate or ammonium ion, favors formation of the arabonic epimer. The epimeric products of reaction were separated by the following steps: (1) Hydrolysis of the nitriles with aqueous sodium carbonate, (2) passage of the solution over a cation exchange resin to give the free acids, (3) neutralization of the acids with potassium hydroxide, and separation of labeled D-arabonic acid as crystalline potassium D-arabonate, (4) conversion of the potassium salts in the mother liquor to cadmium salts by passage of the solution over a cation exchange resin, and neutralization of the acid with cadmium hydroxide, and (5) separation of the labeled D-ribonic acid in the form of crystalline cadmium D-ribonate.

This procedure gave labeled potassium D-arabonate and cadmium D-ribonate in radiochemical yields of 50 and 23 percent, respectively. The potassium

D-arabonate-1-C¹⁴ was converted to D-arabono- γ -lactone-1-C¹⁴, and this was reduced with sodium amalgam in the presence of sodium acid oxalate.³ A 56-percent yield of the crystalline D-arabinose-1-C¹⁴ was separated without carrier; by use of carrier, the radiochemical yield was increased to 60 percent. As potassium D-arabonate-1-C¹⁴ was produced in 50-percent yield, the over-all radiochemical yield of D-arabinose-1-C¹⁴ was 30 percent, based on the cyanide originally used.

The cadmium D-ribonate-1-C¹⁴, prepared from the mother liquors of the potassium D-arabonate-1-C¹⁴, was converted to D-ribono- γ -lactone-1-C¹⁴, which was brought to crystallization by nucleation.⁴ The lactone was reduced with sodium amalgam and gave D-ribose-1-C¹⁴ in 37-percent yield. Inasmuch as 23 percent of the activity of the cyanide had been obtained as cadmium D-ribonate-1-C¹⁴, the over-all radiochemical yield of D-ribose-1-C¹⁴ was 8.5 percent. Work still in progress will undoubtedly raise the yields of both cadmium D-ribonate and D-ribose-1-C¹⁴.

2. Experimental Procedures

2.1 Preparation of D-Erythrose

For the preparation of sirupy D-erythrose, Sowden's method of periodate oxidation of a 4,6-substituted glucose [9] was applied to 4,6-ethylidene-D-glucose essentially as described by Rappoport and Hassid [7], but with certain convenient modifications. A mixture of 5.64 g of 4,6-ethylidene-D-glucose, mp 180° to 181° C [10] and 4.6 g of sodium bicarbonate was dissolved in an ice-cold solution containing 11.7 g of sodium metaperiodate in 150 ml of water. The solution was kept in an ice bath for a few minutes, and finally at room temperature for 2.5 hr; it was then freeze-dried. The fluffy residue was extracted with a total of 150 ml of hot ethyl acetate in 3 portions. The extract was filtered through a bed of decolorizing

³ In previous work at the Bureau, an equimolecular mixture of sodium oxalate and oxalic acid was used as an internal neutralizing agent. Crystalline sodium acid oxalate is a more convenient reagent, and is readily prepared, although apparently not commercially available. Use of the acid salt has resulted in somewhat higher yields of the sugar, possibly through closer pH control.

⁴ We are indebted to N. K. Richtmyer, of the National Institutes of Health, for seed crystals of D-ribono- γ -lactone.

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² Figures in brackets indicate the literature references at the end of this paper.

carbon and diatomaceous earth, and the solvent was removed by distillation under reduced pressure. The residue, amorphous 2,4-ethylidene-D-erythrose, was hydrolyzed by refluxing for 1 hr with 60 ml of 0.1-N sulfuric acid; the hydrolyzate was cooled, and deionized by passage through a column of mixed cation⁵ and anion⁶ exchange resins. The resulting solution was concentrated under reduced pressure to about 50 ml, and was then freeze-dried. The colorless sirup weighed 2.91 g and had a specific rotation, $[\alpha]_D^{20}$, of -17.3° . The cyanide-combining power of the sirup was about 90 percent of the theoretical, and consequently it was assumed that the residue contained 90 percent of D-erythrose. It was used without further purification for the cyanohydrin synthesis described in the following sections.

2.2. Effect of General Acid Catalysts in the Cyanohydrin Synthesis on the Yield of the D-Arabonic Epimer

Several cyanohydrin syntheses employing C¹⁴-labeled cyanide and sirupy D-erythrose were carried out on a semimicro scale under the conditions described below. After hydrolysis of the cyanohydrins, each mixture was treated with a large excess of non-radioactive potassium D-arabonate. From the radioactivity of the recrystallized potassium salt, the proportion of D-arabonic acid was calculated.

The mixtures, two of which are listed in table 1 as experiments 1 and 2, were frozen, and the tubes were sealed and allowed to stand at room temperature for 72 hr. They were then opened and heated on a water bath at 80° C for 7 hr in the presence of a stream of air. A few drops of water were added from time to time, but at the end of the hydrolysis period the solutions were allowed to evaporate to dryness. The residue in each tube was dissolved in 5 ml of water, and 500 mg of potassium D-arabonate was added. The mixture was warmed slightly to dissolve the salt, and the solution was filtered with the aid of a small amount of decolorizing carbon, and treated with 3 volumes of ethanol. Potassium D-arabonate crystallized freely from all preparations. After storage for 18 hr in a refrigerator, the mother liquor was removed from the crystals, which were washed in place with cold, aqueous ethanol (1:4). The samples were recrystallized once from water and ethanol, dried in a vacuum desiccator, and assayed. Experiments 1 and 2 reported in table 1 indicate that ammonium carbonate favors production of D-arabonic nitrile. A large-scale preparation reported in section 2.3, and also listed as experiment 3 in table 1, shows that sodium bicarbonate likewise favors production of the arabonic epimer. The results are in accord with the prior observation that general acid catalysts alter the proportion of the epimeric nitriles [3, 5].

⁵ Amberlite IR-100, analytical grade, Resinous Products Division of Rohm & Haas Co., Philadelphia, Pa.

⁶ Duolite A-4, Chemical Process Co., Redwood City, Calif.

TABLE 1. Effect of a general acid catalyst on the yield of the arabonic epimer obtained by the addition of cyanide to D-erythrose

Experiment	Reaction mixture ^a	Activity found in carrier potassium D-arabonate ^b	Arabonic epimer formed
	<i>Millimoles</i>		
1	{ <ul style="list-style-type: none"> • 0.0267 D-erythrose..... .0134 Na₂CO₃..... • 0.267 NaCN..... 	d 9.90	% 37.1
2	{ <ul style="list-style-type: none"> • 0.267 D-erythrose..... .134 (NH₄)₂CO₃..... • 0.267 NaCN..... 	d 14.80	55.6
3	{ <ul style="list-style-type: none"> • 4.0 D-erythrose..... 10.0 NaHCO₃..... • 3.1 NaCN..... 	f 6,950	49.6

^a Total volume was 1 ml in experiments 1 and 2, and 20 ml in experiment 3.

^b Activities determined by means of a vibrating-reed electrometer after a modified Van Slyke-Folch wet oxidation procedure (see [11]).

^c Assuming 90-percent purity of the erythrose sirup.

^d Calculated for entire 500 mg of carrier added.

^e Contained 26.7 μ c of carbon 14.

^f Radioactivity recovered in several crops.

^g Contained 14 mc of carbon 14.

2.3. Preparation of Potassium D-Arabonate-1-C¹⁴ from D-Erythrose by the Cyanohydrin Synthesis in the Presence of Sodium Bicarbonate

Ten milliliters of an aqueous solution containing 3.1 millimoles of sodium cyanide (14.0 mc of carbon 14) and 5 millimoles of sodium hydroxide was frozen on the sides of a small glass-stoppered tube. A small lump of solid carbon dioxide was added, and then a solution containing 5 millimoles of sodium bicarbonate and approximately 4 millimoles of D-erythrose in 10 ml of water. The loosely stoppered flask was placed in an ice bath, shaken until the contents had dissolved, and then kept in the bath for 1 day, and at room temperature for 5 days. The nitriles were hydrolyzed by heating on a steam bath for 7 hr, and the resulting ammonia was removed by evaporation of the solution to dryness in a stream of air. The residue was taken up in water and passed over a column containing 10 ml of cation exchange resin⁷ to remove sodium ions. The column was washed until the activity of the effluent was negligible, and the effluent was concentrated under reduced pressure to about 25 ml and neutralized with potassium hydroxide. Two grams of nonradioactive potassium D-arabonate was dissolved in the sirup and evaporation was continued to a volume of about 4 ml; the solution was then saturated with methanol. After 18 hr, a crop of potassium D-arabonate-1-C¹⁴ was obtained, which, when recrystallized and dried, weighed 2.23 g and had an activity of 5,980 μ c. By the further addition of 2.5 g of carrier potassium D-arabonate in 3 portions, 970 μ c of activity was obtained. Hence the total radiochemical yield of potassium D-arabonate-1-C¹⁴ was 6,950 μ c, or 50 percent of the activity of the original cyanide. The residual sirup was used for the preparation of cadmium D-ribonate-1-C¹⁴ (see sec. 2.6).

⁷ Amberlite IR-120, analytical grade, Resinous Products Division of Rohm & Haas Co., Philadelphia, Pa.

2.4. Preparation of Potassium D-Arabinose-1-C¹⁴ from D-Erythrose by the Cyanohydrin Synthesis in the Presence of Ammonium Carbonate

In another preparation of D-arabinose-1-C¹⁴, the ammonium carbonate method was employed. Five milliliters of a solution containing 0.7 millimole of sodium hydroxide and about 0.7 millimole of sodium cyanide with 5.4 mc of carbon 14 was frozen on the walls of a small glass-stoppered tube. Five milliliters of approximately 1 M ammonium carbonate was then frozen in the tube, and finally 1 ml of a solution containing 1 millimole of D-erythrose⁸ was added. The mixture was allowed to thaw in an ice bath and was then kept at room temperature for 3 days. It was treated essentially in the manner described in section 2.3, except that a crop of potassium D-arabonate-1-C¹⁴ (52 mg, containing 2,030 μC)⁹ was separated without the addition of carrier. The total radiochemical yield (2,446 μC , or 45 percent) was slightly less than that of the former preparation. However, as greater mechanical loss was incurred in this preparation because of the higher level of activity, it is believed that the two methods are equally satisfactory in the synthesis of potassium D-arabonate-1-C¹⁴.

2.5. Preparation of D-Arabono- γ -Lactone-1-C¹⁴ and Reduction to D-Arabinose-1-C¹⁴

An aqueous solution containing 446 mg (2.19 millimoles) of potassium D-arabonate with 1,190 μC of activity was passed through a column of cation exchange resin (see footnote 7). The column was thoroughly washed, and the effluent was evaporated at 60° C substantially to dryness. The residue, dissolved in a few milliliters of methanol, was transferred to a reduction tube such as that described previously [3]. Briefly, it consists of a test tube 2.8 by 20 cm fitted with a stirrer reaching to the bottom of the tube and with a sidearm for the introduction of the amalgam. The solution in the tube was concentrated in an air stream and seeded with D-arabono- γ -lactone.¹⁰ The tube was finally stored in a desiccator, and the contents was moistened from time to time with methanol. When lactonization was complete, as indicated by the disappearance of any sirupy phase (1 or 2 weeks), the material was reduced with sodium amalgam by the procedure described later.

The lactonization described above required considerable time. In order to avoid this delay, the following rapid procedure was developed: Two millimoles of potassium D-arabonate-1-C¹⁴ was passed

⁸ Because of uncertainty in the concentration of the cyanide solution, the erythrose was in larger excess than would ordinarily be added.

⁹ Except for the analyses in table 1, all assays of carbon 14 were made in formamide solution [12]. Details of the analysis of this crop of potassium D-arabonate-1-C¹⁴ are illustrative: The material was dissolved in water and the volume was adjusted to 10 ml. A 50- λ aliquot was diluted to 1 ml with water in a micromixing pipette. A 20- λ aliquot of this was diluted to 1 ml with formamide, and the solution gave 74.9 counts per second in a 2- π proportional counter in which 1 count per second is equivalent to 0.00271 μC .

¹⁰ The free acid may crystallize from the freshly prepared sirup at this point. Conversion of the acid to the lactone at room temperature requires at least a week.

through a column of cation-exchange resin, and the effluent was concentrated under reduced pressure to a sirup, which was transferred with a few drops of water to a reduction tube. Five milliliters of glacial acetic acid was added, and the tube was placed in a boiling-water bath and heated in a stream of air for 5 hr. After 1 hr of this period, 1 ml of water and 5 ml of acetic acid were added. At three 1-hr intervals thereafter, the material was treated with 5 ml of acetic acid only. At the end of the heating period, the pale-yellow residue was dissolved in 3 or 4 drops of methanol, seeded with D-arabono- γ -lactone, and placed in a desiccator over calcium chloride. It crystallized well within 24 hr.

When samples of nonradioactive D-arabonic acid were subjected to this procedure, the optical rotations, $[\alpha]_D^{20}$, 24 and 72 hr after the sirups were seeded with the crystalline lactone were, respectively, +66.0° and +67.6° (based on the weight of the lactone). These values correspond approximately to conversion of 91 and 94 percent, respectively, of the D-arabonic acid to the lactone.

Prior to the preparation of the labeled sugar, a series of sodium amalgam reductions was carried out on the nonradioactive lactone in order to determine optimum conditions. Sodium acid oxalate was employed as a neutralizing agent [6], and after removal of the salts in the manner described below, the reduction product was analyzed for reducing sugars by the modified Scales method [13]. It was found that a maximum yield of sugar (85 percent) is obtained with 4.6 g of 5-percent sodium amalgam per millimole of lactone.

In a typical reduction of the labeled lactone to the sugar, the reduction tube, containing 6.4 g of crystalline sodium acid oxalate and the lactone prepared from 446 mg of potassium D-arabonate-1-C¹⁴, having 1,190 μC of activity, was placed in an ice bath. After the addition of 20 ml of ice water, the stirrer was adjusted to produce vigorous stirring at the bottom of the tube, and 9.2 g of 5-percent sodium amalgam pellets¹¹ was added through the side arm. Stirring was continued for 3 hr, at the end of which time the mercury was removed, and the aqueous mixture was diluted with 5 volumes of methanol. The crystalline salts were separated, washed with methanol, and discarded, after it was ascertained that they were inactive. Phenolphthalein indicator was added to the mother liquor, previously cooled in ice water, and then aqueous sodium hydroxide until a faint but permanent pink color was obtained. The solution was concentrated under reduced pressure to about 10 ml, and treated with 5 volumes of methanol; the precipitated salts were separated, washed with methanol, tested for radioactivity, and discarded. The solution was concentrated to remove the alcohol, and passed over a column containing 25 ml of mixed anion and cation exchange resins (see footnotes 5 and 6); the resins were washed until the activity of the washings was negligible. The effluent was con-

¹¹ The amalgam was prepared in small pellets by pouring it in molten condition through a heated aluminum thimble, having a small hole in the bottom, into a 2-ft "shot tower" of mineral oil. The pellets were stored under mineral oil, and just before use, were blotted dry, weighed, and rinsed with benzene.

concentrated under reduced pressure and at a temperature less than 40° C to about 10 ml; at this stage the solution was filtered into a round-bottomed flask through a microfilter containing a small bed of decolorizing carbon,¹² and the combined solution and washings were freeze-dried in a 50-ml flask. The residue in the flask was dissolved in a few drops of methanol, and 2-propanol was added to incipient turbidity. Crystallization began immediately after nucleation. At the end of 2 days, the mother liquor was separated, and the crystals were washed in place with a few drops of a methanol-2-propanol mixture (2:1). For recrystallization, the crude material, dissolved in a few drops of water, was transferred to a standard-taper test tube, and the water was removed under reduced pressure. The thick sirup was dissolved in a few drops of methanol, and 2-propanol was cautiously added, until saturation was approached. The crystals that formed in 24 hr weighed 185 mg and contained 672 μc of activity. With the aid of 100 mg of nonradioactive D-arabinose as carrier, an additional 39 μc of activity was obtained from the mother liquor. The yield of D-arabinose-1-C¹⁴ (711 μc) from potassium D-arabonate-1-C¹⁴ was thus 60 percent.¹³

2.6. Separation of Cadmium D-Ribonate-1-C¹⁴

The mother liquor from the preparation of potassium D-arabonate-1-C¹⁴, described in section 2.3, was evaporated to remove the alcohol present. The residue was dissolved in water and passed over a column of cation exchange resin (see footnote 7). The effluent, containing 6,900 μc of activity, was then neutralized with cadmium hydroxide to the end point of phenolphthalein. After the addition of 1 g of nonradioactive cadmium D-ribonate, the solution was filtered with the aid of decolorizing carbon, and the filtrate was evaporated under reduced pressure to a thin sirup. Nucleation of the sirup with crystalline cadmium D-ribonate and careful addition of aqueous methanol resulted in a voluminous crystallization. The crystals were separated by filtration, and recrystallized by dissolving in 4 ml of hot water and adding successively 5 ml of methanol and water (1:1) and 2 ml of methanol and water (2:1). The hot solution was then saturated with methanol and allowed to cool slowly. The first crop of recrystallized cadmium D-ribonate-1-C¹⁴ weighed 678.6 mg and had an activity of 1,796 μc . After successive additions of two 1-g quantities of nonradioactive cadmium D-ribonate as carrier, and recrystallization of the resulting crops of salt, an additional quantity of cadmium D-ribonate-1-C¹⁴ containing 1,416 μc was recovered. The total yield of cadmium D-ribonate-1-C¹⁴ was thus 3,212 μc , corresponding to 23 percent of the C¹⁴-labeled cyanide used.

¹² This step removes a trace of oil that may be introduced with the sodium amalgam.

¹³ In other preparations, radiochemical yields up to 70 percent have been obtained.

2.7. Preparation of D-Ribono- γ -lactone-1-C¹⁴ and Reduction to D-Ribose-1-C¹⁴

A solution containing 679 mg of cadmium D-ribonate-1-C¹⁴, with an activity of 1,800 μc , was passed over a column of cation exchange resin (see footnote 7), and the effluent was concentrated under reduced pressure to a sirup. The sirup was transferred by use of water to three reduction tubes and evaporated to dryness by heating on a steam bath under a stream of dry air. The residue in each tube was dissolved in a few drops of water and the solution, after the addition of 5 ml of methyl cellosolve, was evaporated again. This process was repeated 4 times over a period of 2 days. Each residue was then dissolved in 2 ml of methyl cellosolve, and the solution was seeded with crystalline D-ribono- γ -lactone and stored in a desiccator over calcium chloride. From time to time the residues were moistened with methyl cellosolve. Crystallization occurred slowly, but ultimately all sirup disappeared, and the residues appeared to be entirely crystalline.

A series of reductions on nonradioactive D-ribono- γ -lactone showed that a maximum yield of sugar (70 percent) was obtained when 9.2 g of 5-percent amalgam and excess sodium acid oxalate were employed for the reduction of 1 millimole of lactone. Each of the three samples of lactone mentioned above was dissolved in 20 ml of ice water and reduced by use of the above quantities of amalgam and oxalate. The product from the three reductions was combined, the mercury was separated, and the mixture was diluted with 5 volumes of methanol. The resulting crystalline salts were separated by filtration, washed with methanol, and discarded after a test showed negligible radioactivity. The filtrate was concentrated under reduced pressure to a volume of about 15 ml and diluted with 75 ml of methanol; the sodium salts that separated were removed by filtration and washed with methanol. As a test showed that this second crop of salts likewise contained no appreciable radioactivity, it was discarded. The alcoholic filtrate was concentrated to about 10 ml, neutralized with aqueous sodium hydroxide in the presence of phenolphthalein until a permanent pink color was obtained, and immediately deionized by passage over a column of mixed cation and anion exchange resins (see footnotes 5 and 6). The effluent and wash liquor were combined and concentrated under reduced pressure to a volume of 6 ml; 750 mg of carrier D-ribose was dissolved in the sirup, and the solution was filtered through 1 ml of a mixture of decolorizing carbon and diatomaceous earth. The filtered solution was freeze-dried, and the residue was dissolved in 0.5 ml of ethanol. A mixture of ethanol and isoamyl alcohol was added to the point of incipient turbidity, and the solution was seeded with D-ribose and stored in a refrigerator. When crystal growth seemed complete (5 days), the mother liquor was removed with a capillary pipette, and the crystals were carefully washed, in place, with ethanol and dried over calcium

chloride. The crude product (768 mg) had an activity of 667 μc , or 37 percent of the activity of the cadmium D-ribonate-1- C^{14} used.

For recrystallization, the product was dissolved in a few drops of water, a drop of acetic acid was added, and the solution was filtered through decolorizing carbon. The filtrate was concentrated under reduced pressure to a thick sirup. The residue was dissolved in ethanol, and the solution was concentrated again under reduced pressure. The residue was dissolved in 0.5 ml of ethanol, and after addition of isoamyl alcohol to incipient turbidity, the solution was seeded and stored, first at room temperature and finally in a refrigerator. After 5 days, the product was separated, washed in place with a few drops of ethanol, and dried. The recrystallized D-ribose-1- C^{14} (540 mg) contained 465 μc of carbon 14. By use of carriers on the mother liquor from the recrystallization, nearly all of the radioactivity of the crude D-ribose-1- C^{14} was reclaimed.

3. References

- [1] H. S. Isbell, J. V. Karabinos, H. L. Frush, N. B. Holt, A. Schwebel, and T. T. Galkowski, *J. Research NBS* **48**, 163 (1952) RP2301.
- [2] H. S. Isbell, and J. V. Karabinos, *J. Research NBS* **48**, 2334 (1952) RP2335.
- [3] H. L. Frush and H. S. Isbell, *J. Research NBS* **50**, 133 (1953) RP2400.
- [4] H. L. Frush and H. S. Isbell, U. S. Atomic Energy Commission Report No. NBS-2308 (1953); *J. Research NBS* **51**, 167 (1953) RP2446.
- [5] H. S. Isbell, U. S. Patent 2606918, August 1952.
- [6] H. S. Isbell, U. S. Patent 2632005, March 1953.
- [7] D. A. Rappoport and W. Z. Hassid, *J. Am. Chem. Soc.* **73**, 5524 (1951).
- [8] J. C. Sowden and H. O. L. Fischer, *J. Am. Chem. Soc.* **69**, 1963 (1947).
- [9] J. C. Sowden, *J. Am. Chem. Soc.* **72**, 808 (1950).
- [10] R. C. Hockett, D. V. Collins, and A. Scattergood, *J. Am. Chem. Soc.* **73**, 599 (1951).
- [11] M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert, and P. F. Yankwich, *Isotopic carbon*, p. 92 (J. Wiley & Sons, Inc., New York, N. Y., 1949).
- [12] A. Schwebel, H. S. Isbell, and J. V. Karabinos, *Science* **113**, 465 (1951).
- [13] H. S. Isbell, W. W. Pigman, and H. L. Frush, *J. Research NBS* **24**, 241 (1940) RP1282.

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