528. *Synthesis of* 1 : 3-*Dihydroxy*[2-^{14}C]*acetone.*

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The synthesis of dihydroxyacetone described by Piloty (*Ber.*, 1897, **30**, 3161) has been improved and applied to the synthesis of dihydroxyacetone labelled with radioactive carbon. A new synthesis of dihydroxyacetone by acid decomposition of the sodium salt of 2-nitropropane-1 : 3-diol has been demonstrated in tracer experiments. This provides a rapid synthesis of labelled dihydroxyacetone when some dilution of radioactivity can be tolerated.

A synthesis of dihydroxyacetone labelled with ^{14}C was desired for investigations of carbohydrate metabolism. Since [^{14}C]formaldehyde (Arnstein, *Nature*, 1949, **164**, 361) and nitro-[^{14}C]methane (Sowden, *J. Biol. Chem.*, 1949, **180**, 55) had previously been prepared, the following synthesis of dihydroxyacetone (Piloty and Ruff, *Ber.*, 1897, **30**, 1656; Piloty, *ibid.*, p. 3161) appeared to be most useful :

$$3CH_2O + CH_3{\cdot}NO_2 \xrightarrow{\text{Alkali}} \underset{\text{(I.)}}{(HO{\cdot}CH_2)_3C{\cdot}NO_2} \xrightarrow{\text{Na–Hg}} \underset{\text{(II.)}}{(HO{\cdot}CH_2)_3C{\cdot}NH{\cdot}OH}$$

$$\xrightarrow{HgO} \underset{\text{(III.)}}{(HO{\cdot}CH_2)_2C{:}N{\cdot}OH} \xrightarrow{Br_2\text{–}H_2O} \underset{\text{(IV.)}}{(HO{\cdot}CH_2)_2CO}$$

With use of $^{14}CH_2O$ and $^{12}CH_3{\cdot}NO_2$ the product would be 1 : 3-dihydroxy[1 : 3-$^{14}C_2$]acetone, $(HO{\cdot}{}^{14}CH_2)_2CO$; with $^{14}CH_3{\cdot}NO_2$ and $^{12}CH_2O$, the 2-labelled compound would result. Further, by use of $^{14}CH_2O$ and $^{13}CH_3{\cdot}NO_2$, double labelling could be achieved.

Nitromethane may be condensed with aqueous formaldehyde solution, or with paraformaldehyde in organic solvents in presence of alkaline catalysts. The melting point of the product, trishydroxymethylnitromethane (I), has been variously reported as 144° to 158—162° (Henry, *Compt. rend.*, 1895, **121**, 210; Böeseken, *Rec. Trav. chim.*, 1915, **34**, 110; Schmidt and Wilkendorf, *Ber.*, 1919, **52**, 392; Kleinfeller, *ibid.*, 1929, **62**, 1584; Gorski and Makarov,

ibid., 1934, **67**, *B*, 996; de Maury, *Bull. Soc. chim.*, 1944, **11**, 281; Cason and Prout, *J. Amer. Chem. Soc.*, 1949, **71**, 1218). Using either aqueous or organic solutions we frequently obtained material melting as low as 130° which nevertheless gave good analytical results. The melting point was unchanged on crystallisation from ethyl acetate–chloroform, but recrystallisation from nitromethane (Cason and Prout, *loc. cit.*) gave white needles, m. p. 162°. It seemed likely that the variable melting point was caused by contamination with traces of the alkaline catalyst, especially since Gorski and Makarov (*loc. cit.*) had shown that alkali causes reversible decomposition. We found that if traces of inorganic material were removed by chromatography on alumina or treatment with ion-exchange resins, the melting point was raised to 176°.

Although Piloty (*loc. cit.*) and later Hersant and Linnell (*Quart. J. Pharm.*, 1931, **4**, 76) reduced (I) to the corresponding hydroxylamine (II) by sodium amalgam, we could not duplicate their yields (70 and 55%, respectively) (cf. Cason and Prout, *loc. cit.*, who obtained a yield of only 32·5%). In a search for a more reliable method, catalytic hydrogenation was investigated. In the presence of palladium–barium sulphate in methanol, hydrogen was rapidly taken up, but only trishydroxymethylmethylamine could be isolated. In aqueous solution, with the same catalyst, and in presence of oxalic acid as a trapping agent (Wilkendorf and Trénel, *Ber.*, 1923, **56**, 619; Schmidt, Ascherl, and Mayer, *ibid.*, 1925, **58**, 2430), hydrogen absorption was very slow; precise control was impossible and the product was a mixture containing mainly trishydroxymethylmethylamine oxalate.

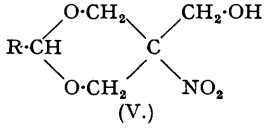
The use of aluminium amalgam in aqueous solution gave the hydroxylamine (II) in yields of up to 20%. An improved yield (65%) was obtained when (I), dissolved in tetrahydrofuran, was added to a stirred suspension of aluminium amalgam in ether.

Oxidation of (II) was carried out best in methanol. The oxime crystallised rather slowly and was always accompanied with a little oily material, which could not be removed by solvents; conversion into, and recovery from, the insoluble copper compound gave pure dihydroxyacetone oxime (III) in 65% yield. However, the crude oxime could be satisfactorily decomposed with bromine, so that this purification was normally omitted.

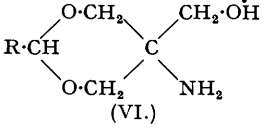
Owing to the solubility of dihydroxyacetone in many organic solvents, its isolation, particularly on the small scale used in this work, was attended with some loss. In most cases, the crystalline material was washed out with *n*-butanol; washings deposited further crops on storage. The yield of crystalline dihydroxyacetone obtained from the hydroxylamine (II) was 27%; the overall yield from nitromethane was 14·2% (cf. Hersant and Linnell, *loc. cit.*, 18·2% by Piloty's method).

The whole series of reactions was carried out with $^{14}CH_3 \cdot NO_2$; after as much dihydroxyacetone as possible had crystallised, the residual mother-liquor was evaporated and treated with inactive " carrier " dihydroxyacetone. A further quantity of less active material was thus obtained, and the overall radiochemical yield from nitromethane was 21·8%.

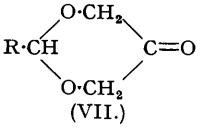
Several alternative routes to dihydroxyacetone or glycerol (which can be converted into dihydroxyacetone) were also investigated. 2-Nitropropane-1 : 3-diol, readily obtainable from trishydroxymethylnitromethane, yields 2-aminopropane-1 : 3-diol on reduction (Schmidt and Wilkendorf, *loc. cit.*). The amine hydrochloride reacted smoothly with sodium or barium nitrite at a slightly acid pH, 80% of the theoretical volume of nitrogen being evolved in 2—3 hours. The crude product was an oil giving a strongly positive acraldehyde test when heated with sodium hydrogen sulphate. However, no significant amount of glycerol or its benzoate could be isolated. In general, deamination of amino-ketones or -alcohols appears to give poor yields and mixed products (*e.g.*, Chiari, *Monatsh.*, 1898, **19**, 578; Pictet and Barbier, *Helv. Chim. Acta*, 1921, **4**, 925; Kalischer, *Ber.*, 1895, **28**, 1521; Neuberg and Kansky, *Biochem. Z.*, 1909, **20**, 460) and this approach was therefore abandoned.



(V.)



(VI.)



(VII.)

Periodate oxidation of the acetal (VI) of trishydroxymethylmethylamine to a cyclic acetal (VII) of dihydroxyacetone was considered possible, and such a protected compound might be more readily isolated. Trishydroxymethylnitromethane was converted into the acetal (V; R = Pr^n) by reaction with *n*-butaldehyde (Scattergood and MacLean, *J. Amer. Chem.*

Soc., 1949, **71**, 4153) and was slowly reduced by hydrogen in presence of Adams's catalyst. The product (VI; R = Pr^n) was characterised as the crystalline benzoate, m. p. 113—115°; reaction with *p*-nitrobenzoyl chloride afforded only an unidentified, halogen-containing product, m. p. 71—72°. When an aqueous solution of the amine (VI; R = Pr^n) reacted with periodic acid, large quantities of iodine were liberated, but the crude product gave only a poor yield of heterogeneous 2 : 4-dinitrophenylhydrazones.

A second successful synthesis of dihydroxyacetone, involving only three stages from nitromethane, was found in the action of strong acid on the sodium salt of 2-nitropropane-1 : 3-diol (VIII), which salt was readily obtained by the action of sodium methoxide on (I) :

$$\underset{\text{(I.)}}{(HO\cdot CH_2)_3C\cdot NO_2} \xrightarrow{NaOMe} \underset{\text{(VIII.)}}{(HO\cdot CH_2)_2C{:}NO_2^-Na^+} \xrightarrow{H_2SO_4} \underset{\text{(IV.)}}{(HO\cdot CH_2)_2CO}$$

That strong mineral acid might decompose the sodium salt in this way was suggested by the fact that to obtain 2-nitropropane-1 : 3-diol it was best to acidify (VIII) with salicylic acid in boiling ether (Den Otter, *Rec. Trav. chim.*, 1938, **57**, 13). Further, the synthesis was analogous to the conversion of nitro-deoxy-sugars (IX; R = H) into the corresponding aldoses (XI; R = H), and of compounds (IX; R = CH_2·OH) into ketoses (XI; R = CH_2·OH) (Sowden and Fischer, *J. Amer. Chem. Soc.*, 1944, **66**, 1312; 1945, **67**, 1713; 1946, **68**, 1511; 1947, **69**, 1048, 1963; Sowden, *ibid.*, 1950, **72**, 3325) :

$$\underset{\text{(IX.)}}{\begin{matrix}R\cdot CH\cdot NO_2\\ [CH\cdot OH]_n\\ CH_2\cdot OH\end{matrix}} \longrightarrow \underset{\text{(X.)}}{\begin{matrix}R\cdot C{=}NO_2^-Na^+\\ [CH\cdot OH]_n\\ CH_2\cdot OH\end{matrix}} \longrightarrow \underset{\text{(XI.)}}{\begin{matrix}R\cdot C{=}O\\ [CH\cdot OH]_n\\ CH_2\cdot OH\end{matrix}}$$

In many preliminary experiments, an aqueous solution of (VIII) was added to concentrated sulphuric acid under conditions used by Sowden (*loc. cit.*) in the synthesis of [1-^{14}C]glucose from (IX; R = H, *n* = 4). Removal of the excess of sulphuric acid by an aqueous suspension of calcium carbonate gave a solution which strongly reduced Fehling's solution in the cold. After treatment with ion-exchange resins and evaporation to dryness *in vacuo*, such solutions yielded viscous oils, which partly crystallised on seeding and prolonged storage. That dihydroxyacetone was, in fact, formed under these conditions was demonstrated as follows. $^{14}CH_3\cdot NO_2$ and formaldehyde were condensed and treated with sodium methoxide without purification of the intermediate (I). The radioactive sodium salt (VIII) was decomposed with acid; after de-ionisation, carrier dihydroxyacetone was added and the recovered oil crystallised on storage. By washing with alcohol 60% of the quantity of carrier added was recovered. Its radioactivity was unchanged on recrystallisation, and various derivatives were prepared and shown to have the same specific radioactivity. From the radioactivity of the isolated dihydroxyacetone, it was calculated that about 30% of the sodium salt had been converted into dihydroxyacetone. Where some dilution with carrier material can be tolerated, this method provides a rapid and convenient synthesis of labelled dihydroxyacetone. It is most convenient to carry out the initial condensation of $^{14}CH_3\cdot NO_2$ with paraformaldehyde in methanol; this solution of (I) is added to sodium methoxide in methanol, whereupon the sodium salt (VIII) is precipitated in nearly quantitative yield.

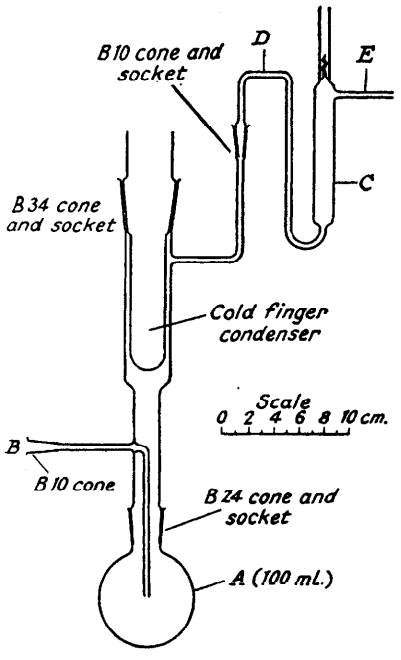
Experimental.

(M. p.s are uncorrected. Microanalyses by Drs. Weiler and Strauss, Oxford.)

Radioactivity Determinations.—The measurements were carried out on " infinite-thickness " samples, *i.e.*, 25 mg. or more of material/sq. cm., mounted on 1-sq.-cm. " Polythene " discs, as described by Popják (*Biochem. J.*, 1950, **46**, 560). Samples were counted with a helium-filled bell-shaped Geiger–Müller counter which had a thin mica window. The " background " of the instrument was 8—10 counts/min. and a sample which contained 10^{-3} μc. of ^{14}C/mg. of substance gave approx. 1100 counts/min. when counted as described above.

Preparation of Dihydroxyacetone by Piloty's Method.—(i) *Nitro*[^{14}C]*methane* (cf. Sowden, *loc. cit.*). $^{14}CH_3\cdot I$ ($26\cdot1 \times 10^{-3}$ μc./mg.; obtained from the Radiochemical Centre, Amersham) (6·1 g.) was distilled, with the aid of a vacuum manifold, into the reaction vessel (see figure). Flask *A* contained a mixture of silver nitrite (12·0 g.) suspended in non-radioactive nitromethane (13·4 g.) and was frozen in liquid air. The vessel was disconnected from the vacuum manifold and stoppered at *B*, and the contents of *A* were allowed to come to room temperature, with the cold finger containing solid carbon

dioxide in carbon tetrachloride. After an initial exothermic reaction, the contents were warmed at 85—90° for 1¼ hours. The solid carbon dioxide mixture was then replaced by an ice–salt freezing mixture, and the methyl nitrite also produced was blown out in a stream of dry air (introduced at *B*) and condensed in the break-seal tube (*C*) which was surrounded with liquid air. After being sealed at *D* and *E*, this tube was removed, and the flask and condenser were again connected to the vacuum manifold. The nitromethane was then distilled in vacuum in a closed circuit into a weighed flask cooled in liquid air. The yield was 14·64 g.



To determine the radiochemical yield, ω-nitro[ω-^{14}C]styrene was prepared. To a solution of the above nitromethane (0·6 g.) and redistilled benzaldehyde (1·06 g.) in methanol (2 ml.), cooled in ice and stirred, was added slowly sodium hydroxide (0·42 g.) in ice-water (1·5 ml.). At this point more methanol (3 ml.) was added to thin the mixture. Water (8 ml.) was added after a few minutes, and the solution added dropwise to concentrated hydrochloric acid (2 ml.) and water (3 ml.). The yellow oil which formed was separated by decantation of the aqueous supernatant liquor; it was washed with a little water, and crystallised by addition of ethanol. The pale yellow crystals, recrystallised from ethanol, had m. p. 56°. Specific radioactivity was 3·29 × 10^{-3} μc./mg. Therefore, the specific radioactivity of nitromethane = 8·03 × 10^{-3} μc./mg., and hence the radiochemical yield of $^{14}CH_3{\cdot}NO_2$ from $^{14}CH_3I$ = 73·6%.

(ii) *Trishydroxymethylnitromethane* (I). (a) *Preparation from* $^{14}CH_3{\cdot}NO_2$. Nitro[^{14}C]methane (4·67 g.; 8·03 × 10^{-3} μc./mg.), formaldehyde solution (40%; 17·50 g.), and anhydrous potassium carbonate (50 mg.) were mixed; after a short lag period the temperature rose to 50°, whereupon the flask was immersed in cold water to moderate the reaction. After 5 minutes, water (50 ml.) was added and the solution passed in succession through columns of Amberlite IR-100 (in the hydrogen cycle, 4·5 cm.² × 40 cm.) and Deacidite E (4·5 cm.² × 40 cm.). The columns were washed with water, a total of 2 l. of eluate being collected. This solution was evaporated in vacuum (bath-temp., <60°) and the crystalline residue dissolved in warm ethyl acetate (100 ml.). This solution was evaporated in vacuum, and the white crystalline residue washed out with chloroform, to yield 8·35 g., m. p. 162—165° (a second crop, 1·25 g., was obtained on concentrating the chloroform washings; total yield, 83·5%). The specific radioactivity was 3·06 × 10^{-3} μc./mg. A portion was recrystallised from nitromethane, forming long white needles, m. p. 176°, specific radioactivity 3·04 × 10^{-3} μc./mg.

(b) *Purification of material of low m. p. by chromatography.* Trishydroxymethylnitromethane, when prepared according to the method of Schmidt and Wilkendorf, or of Henry (directions of Hersant and Linnell), usually had m. p. 129—131° (Found: C, 32·15; H, 5·1; N, 9·5. Calc. for $C_4H_9O_5N$: C, 31·8; H, 6·0; N, 9·3%). A solution of this material (1·0 g.) in methanol (5 ml.) was passed through a column of alumina (Hopkin and Williams; 2 cm.² × 10 cm.); methanol was used as eluant, and 5 ml. fractions were collected. The pure compound (0·81 g.; m. p. 169—172°) was eluted in the second fraction.

(c) *Purification by ion-exchange resins.* Trishydroxymethylnitromethane (m. p. 129—131°; 51 g.) in water (500 ml.) was passed through columns of Amberlite IR-100 (5 cm.² × 50 cm.) and Deacidite E (5 cm.² × 50 cm.); washing was carried out with water, a total of 3 l. being collected. After evaporation to dryness *in vacuo* (bath-temp., <60°) the residue was dissolved in warm ethyl acetate (150 ml.). The warm solution was dried (Na_2SO_4), filtered, and treated with chloroform until crystallisation took place. The white crystals obtained in two crops had m. p. 167—169°, the yield being 44 g.

(iii) *Trishydroxymethyl[^{14}C]methylhydroxylamine* (II). Trishydroxymethyl[^{14}C]nitromethane (1·0044 g.; specific activity, 3·06 × 10^{-3} μc./mg. plus 1·9325 g. of inactive material; calculated specific activity, 1·05 × 10^{-3} μc./mg.) in tetrahydrofuran (25 ml.) was added to aluminium amalgam (prepared as described by Vogel, *J.*, 1927, 594, from 1·2 g. of aluminium foil) under dry ether (75 ml.). Water (2 ml.) was added to the stirred mixture, which was warmed to boiling. The bath was removed, and the ether continued to reflux during the next 15 minutes. More water (2 ml.) was added and, after further warming, the mixture was stirred at room temperature for 45 minutes. The alumina precipitate was filtered off and washed with fifteen 40-ml. portions of water. The centrifuged washings were combined and evaporated to dryness *in vacuo* (bath-temp., <55°). The crystalline residue was washed out with dry ether containing a little ethanol, to yield 1·675 g. (63·5%) of a white crystalline powder, m. p. 136—142°, specific activity, 1·10 × 10^{-3} μc./mg.

(iv) *Dihydroxy[2-^{14}C]acetone oxime* (III). Trishydroxymethyl[^{14}C]methylhydroxylamine (1·0 g.; specific activity, 1·10 × 10^{-3} μc./mg.), suspended in ethanol (20 ml.), was warmed at 65°; yellow mercuric oxide, freshly prepared from mercuric chloride (10 g.) and suspended in ethanol to form a thick paste, was added, with stirring, in portions. Initially a rapid blackening took place, and when the mixture had a pronounced yellow colour addition of mercuric oxide was stopped. The mixture was stirred for 2 hours at 60—65° and then filtered, and the residue washed with ethanol. The filtrate and washings, evaporated *in vacuo* (bath-temp., 40°), yielded an oil, which crystallised slowly. (This residue initially had a pronounced odour of formaldehyde.) A test portion did not reduce Fehling's solution in the cold. In later experiments, methanol was found to be a better solvent for this oxidation.

(v) *Dihydroxy*[2-^{14}C]*acetone* (IV). The above oxime, in water (8·0 ml.), was stirred during dropwise addition of bromine (0·5 ml.). Initially, the bromine was decolorised slowly, and there was a slow rise of temperature to 29°. After 10 minutes all the bromine had been added and an excess was present; the mixture was stirred for a further 1½ hours. At this time the solution was colourless. Hydrobromic acid was neutralised by addition of excess of lead carbonate, and the insoluble lead salts were filtered off. The filtrate and aqueous washings were passed in succession through columns of Amberlite IR-100 (H) (2 cm.^{2} × 25 cm.) and of Deacidite E (2 cm.^{2} × 30 cm.); the columns were washed with water, a total eluate of 2 l. being collected. After evaporation to dryness *in vacuo* (bath-temp., <40°) a yellow oil remained. It was dissolved in ethanol (5 ml.) and treated with ether (50 ml.). A white flocculent material was precipitated and soon coagulated to an oil. The turbid supernatant solution was decanted through a filter, and the filtrate evaporated to dryness *in vacuo* (bath-temp., <35°). A straw-coloured oil remained which was re-treated with ethanol (3 ml.) and ether (30 ml.). A little charcoal was added to the turbid solution, which was then filtered and evaporated to an oil as before. This oil crystallised quickly on seeding; after being kept overnight in a vacuum-desiccator with phosphoric oxide it was mixed with *n*-butanol (3 ml.) and filtered, and the residue washed with a little *n*-butanol–ether (1 : 2). There were obtained 105 mg. of a white crystalline powder, m. p. 76—78° (micro-block; rapid heating), specific activity 1·62 × 10^{-3} μc./mg. On storage, the butanol filtrate deposited further crops: 40 mg., of specific activity 1·53 × 10^{-3} μc./mg.; 32 mg., of specific activity 1·47 × 10^{-3} μc./mg. The total yield was 177 mg., a chemical yield from (II) of 27·2%. The butanol filtrate was dissolved in water (10 ml.), and inactive dihydroxyacetone added as carrier (1·0 g.). The solution was evaporated to dryness *in vacuo* (bath-temp., <35°) to yield an oil, which slowly crystallised. On treatment with *n*-butanol, 900 mg. of dihydroxyacetone were recovered, having specific activity 0·190 × 10^{-3} μc./mg. Hence, the total recovery of radioactivity = 449·5 × 10^{-3} μc., the radiochemical yield being 41·1%. The overall radiochemical yield of dihydroxyacetone from nitromethane = 21·8%.

Preparation of Dihydroxyacetone from the Sodium Salt of 2-*Nitro*[2-^{14}C]*propane*-1 : 3-*diol* (VIII).—(i) *Sodium salt* (VIII). $^{14}CH_3{\cdot}NO_2$ (1·1102 g., specific activity 8·03 × 10^{-3} μc./mg.; plus inactive material, 0·6078 g.; calculated specific activity, 5·19 × 10^{-3} μc./mg.) was mixed with formaldehyde solution (40%; 6·450 g.) and potassium carbonate (50 mg.). A vigorous reaction took place after a few minutes' shaking, and at 50° the flask was cooled with water. When the mixture had cooled to room temperature the solution was evaporated to dryness *in vacuo* (bath-temp., <50°). The crystalline residue was dissolved in methanol (50 ml.) and added to a solution of sodium (1 g.) in methanol (50 ml.). A white precipitate formed almost at once; it was filtered off after 1 hour at 0° to yield material [4·773 g. (91%)] containing 2 molecules of methanol of crystallisation.

(ii) *Dihydroxy*[2-^{14}C]*acetone*. The above sodium salt (4·773 g.) in water (35 ml.) was added slowly with stirring to a mixture of concentrated sulphuric acid (3 ml.) and water (5 ml.) cooled in an ice-bath (average internal temperature during addition, 5—10°). As each portion was added a blue-green colour developed and there was some gas evolution. When addition was complete (15 minutes) the solution was stirred for 10 minutes at room temperature. It was then added to a stirred suspension of calcium carbonate (10 g.) in water (50 ml.); when neutralisation was complete, the mixture was filtered and the solid washed with water (the filtrate reduced Fehling's solution in the cold). Inactive dihydroxyacetone (1·99 g.) was added to the filtrate and washings, which were then passed in succession through columns of Amberlite IR-100 (H) (5 cm.^{2} × 50 cm.) and of Deacidite E (5 cm.^{2} × 50 cm.). The columns were washed with water, a total of 2 l. being collected; this eluate was evaporated to dryness *in vacuo* (bath-temp., <40°). A brownish oil remained, which was dissolved in ethanol (20 ml.) and treated with ether (150 ml.). A flocculent precipitate formed; charcoal was added, and, after filtration, the clear solution was evaporated *in vacuo* (bath-temp., 30°). A pale yellow oil was obtained and was treated again with ethanol, ether, and charcoal. Removal of solvent as before gave a pale oil; this was seeded and kept in a vacuum-desiccator for 3 days. The crystalline product was filtered off after addition of *n*-butanol (5 ml.), to yield 1·025 g. of white crystals, m. p. 85—89° (slow heating in an oil-bath), specific activity, 0·877 × 10^{-3} μc./mg. The radiochemical yield from nitromethane = 10·1%.

In a second experiment, trishydroxymethylnitro[^{14}C]methane (3·0 g.; specific activity, 1·17 × 10^{-3} μc./mg.) in methanol (30 ml.) was treated with sodium (0·67 g.) in methanol (30 ml.). The sodium salt was filtered off and dissolved in ice-water (50 ml.), and this solution added to concentrated sulphuric acid (3 ml.) and water (4 ml.) in an ice-bath. The solution was worked up as described previously, but carrier dihydroxyacetone (2·03 g.) in water (10 ml.) was added only after the ethanol–ether precipitation. The dihydroxyacetone, isolated as usual (yield, 1·11 g.), had m. p. 85—88° (slow heating in oil-bath), specific activity, 0·340 × 10^{-3} μc./mg. A second crop (0·100 g.), of specific activity 0·342 × 10^{-3} μc./mg., was obtained from the alcoholic filtrate (ice-cold ethanol was used as filtering agent in this experiment). The charcoal used in treating the ethanol–ether solutions was suspended in water (20 ml.) containing inactive dihydroxyacetone (1·0 g.). Recovery of the material gave 0·70 g. of dihydroxyacetone, specific activity 0·077 × 10^{-3} μc./mg. [radiochemical yield from (I) = 13·3%].

For recrystallisation, 200 mg. of the first crop were dissolved in warm absolute alcohol (7·5 ml.), filtered, and treated with ether (25 ml.). The white crystals separating on cooling (24 mg.) had specific activity 0·342 × 10^{-3} μc./mg.

In order to establish the purity of dihydroxyacetone prepared in this way, three derivatives were prepared, by standard methods, from the material obtained as the first crop. The observed specific activities of these derivatives, and the calculated specific activities (based on the value 0·340 × 10^{-3} μc./mg. for dihydroxyacetone) are shown in the Table. The compounds had substantially the recorded m. p.s.

5-*Hydroxymethyl*-5-*nitro*-2-n-*propyl*-1 : 3-*dioxan* (V; R = Pr^n).—Trishydroxymethylnitromethane (10·5 g.), concentrated hydrochloric acid (5·6 ml.), water (6·7 ml.) and *n*-butaldehyde (5 g.) were shaken vigorously at room temperature for 40 hours. The solid, which had separated, and the supernatant

Observed and calculated specific radioactivities (10^{-3} μc./mg.) *of derivatives of dihydroxy*[2-^{14}C]*acetone.*

	Observed	Calc.[e]
Phenylosazone	0·112,[a] 0·112 [b]	0·114
2 : 4-Dinitrophenylhydrazone	0·115	0·113
Methylglyoxal disemicarbazone	0·159,[c] 0·165 [d]	0·164

[a] Original material. [b] Recrystallised. [c] First crop. [d] Second crop.
[e] Based on the value $0·340 \times 10^{-3}$ μc./mg. for dihydroxyacetone.

solution were then extracted with ether, and the residue remaining on evaporation of the ether was recrystallised from *n*-hexane, yielding the acetal (5·3 g.), m. p. 99—101°.

5-*Amino*-5-*hydroxymethyl*-2-n-*propyl*-1 : 3-*dioxan* (VI; R = Pr^n).—A solution of the above compound (5·3 g.) in methanol (65 ml.) was hydrogenated at room temperature and atmospheric pressure in the presence of Adams's catalyst (150 mg.). The uptake of hydrogen was approx. 30 ml./hour but after 3 days the theoretical volume (1660 ml.) had been absorbed. The catalyst was removed by filtration, and the solvent evaporated *in vacuo*. The residual oil (4·46 g., 98%) was characterised as the crystalline *benzoate*, prepared by reaction with benzoyl chloride in pyridine. After sublimation in a high vacuum this had m. p. 113—115° (Found: C, 64·3; H, 7·95; N, 5·2. $C_{15}H_{21}O_4N$ requires C, 64·5; H, 7·6; N, 5·0%). Similar reaction of the amine with *p*-nitrobenzoyl chloride in pyridine afforded only an unidentified, halogen-containing substance, m. p. 71—72° (after sublimation *in vacuo*) (Found: C, 49·5, 49·2; H, 3·6, 3·3; N, 8·0; Cl, 18·6%).

A solution of the acetal (V; R = Pr^n) (5·7 g., 0·0278 mole) and oxalic acid (1·76 g., 0·014 mole) in methanol (50 ml.)–water (35 ml.) was hydrogenated with 5% palladium–barium sulphate. The uptake of hydrogen was 15 ml./hour; Adams's catalyst (300 mg.) was therefore added, and the hydrogenation continued at 90 atm. for 5 hours. The solution was filtered, and the filtrate concentrated to about 50 ml., whereupon crystalline starting material separated (2·7 g.) (m. p. and mixed m. p. 98—100°). The filtrate was extracted with ether and the aqueous layer was evaporated to dryness. The residue was crystallised from ethanol by addition of ether, giving the *hydrogen oxalate* (0·75 g.), m. p. 176—177°, of (VI; R = Pr^n) (Found: C, 45·0; H, 7·2; N, 5·6, 5·0. $C_{10}H_{19}O_7N$ requires C, 45·3; H, 7·2; N, 5·3%).

2-*Nitropropane*-1 : 3-*diol* (cf. Den Otter, *loc. cit.*).—Finely powdered sodium salt of 2-nitropropane-1 : 3-diol (salt prepared as previously described, 45·0 g.), suspended in dry ether (1 l.), was added to a boiling solution of salicylic acid (30 g.) in dry ether (800 ml.). Enough dry ether to bring the volume to 2·5 l. was then added and the solution was refluxed for 90 minutes. The solution was filtered and evaporated to dryness *in vacuo*, yielding an oil which crystallised after about 30 minutes. The product was recrystallised from 270 ml. of ethyl acetate–chloroform (1 : 1·7). The yield was 16 g., and the m. p. 55—57°.

2-*Aminopropane*-1 : 3-*diol* (Schmidt and Wilkendorf, *loc. cit.*).—A solution of 2-nitropropane-1 : 3-diol (13·5 g.) and oxalic acid dihydrate (7·0 g.) in water (75 ml.) was hydrogenated in presence of 5% palladium–barium sulphate. When hydrogen uptake was complete, the solution was filtered and the filtrate concentrated *in vacuo*. Addition of acetone yielded 2-aminopropane-1 : 3-diol oxalate (12 g.), m. p. 200°.

Reaction of 2-*Aminopropane*-1 : 3-*diol with Nitrous Acid.*—(*a*) 2-Aminopropane-1 : 3-diol oxalate (2·7 g., 0·01 mole) in water (20 ml.) was treated with barium chloride (2·1 g., 0·005 mole) in water (10 ml.). The precipitated barium oxalate was removed by centrifugation and to the supernatant solution of aminopropanediol hydrochloride was added a solution of sodium nitrite (3·0 g.) in water (10 ml.). There was a vigorous evolution of nitrogen (300 ml. in 3 hours). The solution was filtered and evaporated to dryness *in vacuo* (bath-temp., <50°). The residue was extracted with dry methanol, and the solution was filtered and evaporated to dryness. This process was repeated twice more with methanol and once with methanol–ether (1 : 1). The final extract was evaporated and dried *in vacuo*, the yield being 1·46 g. This product gave a positive acraldehyde test. Repetition of this preparation on a larger scale gave a product which was more viscous than glycerol and could not be distilled *in vacuo* without extensive decomposition. Reaction of this material with benzoyl chloride in pyridine, a procedure which gives glycerol tribenzoate, m. p. 75°, in 75% yield, failed to give a solid benzoate.

(*b*) A solution of barium nitrite–barium chloride, prepared by shaking silver nitrite (1·9 g.) with a solution of barium chloride (dihydrate) (3·05 g., 0·0125 mole) for 10 minutes and filtration to remove silver chloride, was added, with cooling, to the aminopropanediol oxalate (1·68 g., 0·0125 mole) in water (25 ml.). The barium oxalate was removed by centrifugation and washed once with water. The combined supernatant solution began to evolve nitrogen; it was allowed to warm slowly. After 2 hours at room temperature it was heated for 10 minutes at 100°. The solution was then kept at room temperature for 16 hours, and evaporated *in vacuo* at 40°. The residue was extracted with methanol; the methanol was evaporated and the residue extracted with methanol–ether (1 : 1). This solution was filtered and evaporated. The oil was dried in a vacuum-desiccator. No glycerol tribenzoate was obtained by reaction of the product with benzoyl chloride in pyridine.

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