[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

Studies on the Biosynthesis and Fragmentation of C^{14}-Labeled Cotton Cellulose and Seed Oil[1,2]

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A convenient method has been developed for determination of the label distribution within the anhydro-D-glucose units of C^{14}-labeled cellulose. The method is based on the periodate oxidation of cellulose followed by further oxidation of the resulting aldehyde functions with chlorous acid and hydrolysis of the product to glyoxylic and D-erythronic acids. The former compound was isolated as the 2,4-dinitrophenylhydrazone, which on decarboxylation provided the label distribution at C1 and C2. Complete and partial periodate oxidation of D-erythronic acid, isolated as the lactone, gave the label at C3, C4, C5 and C6. Application of this method to a sample of cotton cellulose-C^{14}, prepared from D-glucose-2-C^{14}, indicated that 58% of the label had remained at C2 and 25% was transferred to C5. These data and the distribution of label in the cotton seed oil indicate that such breakdown and resynthesis of D-glucose as occurs, takes place through the Embden–Meyerhof pathway.

Introduction of C^{14}-labeled D-glucose into the maturing cotton boll results in the formation of radioactive cotton cellulose[3,4] and cotton seed oil.[5] The introduction of D-glucose-1-C^{14} and D-glucose-6-C^{14}, respectively, into 21 day old maturing cotton bolls,[4] provided samples of radioactive cellulose with about 65% of the label at the original terminal position of the D-glucose units and 22% at the other terminal position. This indicated that despite a previous report,[3] at least a part of the radioactive cotton had been formed from the scission products of the C^{14}-labeled sugars. The exchange of label between the terminal positions apparently resulted from breakdown of the sugar through the Embden–Meyerhof pathway and the reversible isomerization of dihydroxyacetone-1-C^{14} 1-phosphate and D-glycerose-3-C^{14} 3-phosphate. Further evidence for the breakdown of C^{14}-labeled sugars to three carbon fragments was provided by the investigation of the radioactive cotton seed oil produced from D-glucose-6-C^{14}. This was converted to radioactive fatty acids and glycerol, the latter containing most of its activity at the terminal positions.

The present work is concerned with the confirmation of the above conclusions, through further experiments with D-glucose-2-C^{14}, and with the development of a convenient method for determination of the label distribution within the anhydro-D-glucose units of cellulose-C^{14}.

The procedures previously employed for the determination of C^{14} distribution in cellulose[6–9] require preliminary hydrolysis of the radioactive material and further manipulation of the resulting D-glucose and its derivatives. The inconvenient stage involving the isolation of pure D-glucose in small quantities has been eliminated by the direct oxidation of cellulose with sodium periodate[10–12] which, under controlled conditions, results principally in the cleavage of the C2–C3 bond. Further oxidation of the resulting aldehyde groups at C2 and C3 to carboxylic acid functions and the hydrolysis of the polymer provide glyoxylic acid and D-erythronic acid which can be used for determination of the C^{14}-label at the various positions of the anhydro-D-glucose unit.

The primary attack by periodate ion on cellulose (I) occurs at positions two and three, with consumption of one mole of periodate per anhydro-D-glucose unit. The uptake, however, does not cease at this value but continues because of over-oxidation at a rate dependent on the conditions employed.[10,13] Various conditions were therefore investigated in order to find a suitably rapid condition affording little over-oxidation and a maximum recovery of oxycellulose. The most satisfactory results were obtained from the employment of unbuffered 0.25 *M* solutions of sodium periodate. With an excess of this reagent (4 moles per anhydro-D-glucose unit of cellulose), the primary oxidation reaction appeared to be essentially completed after four days and the consumption of the oxidant within this period was in the range of 1.00–1.03 moles. Thus, repeated quantitative recovery of oxycellulose (II)[11] was achieved.

The next stage in the above process is the oxidation of the aldehyde groups in oxycellulose to carboxylic acid functions. Jayme and Maris[10] have studied this reaction, using bromine as the oxidant. After the hydrolysis of the product they obtained D-erythronic acid, as its barium and brucine salts, and a 40–50% yield of glyoxal, as the bis-(phenylhydrazone). Thus, it appeared that only the aldehyde group at the original C3 position had been oxidized by bromine. This difference in activity of the two aldehyde functions is consistent with the formulation of Mester.[11] The use of chlorous acid as an alternative reagent for this reaction has been studied by Davidson and Nevell[14] with partially (up to 27%) periodate-

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(6) E. J. Bourne and H. Weigel, *Chem. & Ind. (London)*, 132 (1954).

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(13) F. S. H. Head, *J. Textile Inst.*, **44**, T209 (1953).

(14) G. F. Davidson and T. P. Nevell, *ibid.*, **46**, T407 (1955).

oxidized celluloses. Recently the above reagent has been used for the quantitative oxidation of fully periodate-oxidized starches.[15] The conditions employed in this reaction (a 4 molar excess of *M* sodium chlorite solution in 0.5 *M* acetic acid) were applied to the oxidation of periodate oxycellulose (II) and the product III was isolated as the barium salt in good yield. Subsequent hydrolysis of III provided glyoxylic acid (IV) and D-erythronic acid (V). Working with one gram quantities of cotton cellulose, these products were conveniently isolated as the 2,4-dinitrophenylhydrazone and the 1,4-lactone in yields of 63 and 42%, respectively.

Decarboxylation of glyoxylic acid 2,4-dinitrophenylhydrazone[16] readily provided carbon dioxide from position two of the anhydro-D-glucose unit and the C^{14}-label associated with position one was calculated by difference.

The fragmentation of D-erythronic acid was somewhat more complicated. Complete periodate oxidation of sodium D-erythronate, according to the method described by Eisenberg[17] for potassium D-gluconate, provided carbon dioxide from C1, formic acid from C2 and C3 and formaldehyde from C4 of the tetronic acid. Thus, distribution of label at C1, C2, C3, (C4 + C5) and C6 of the anhydro-D-glucose unit was determined. In order to distinguish between C4 and C5, several attempts were made to find a suitable method for the isolation of the C2 or C3 of the erythronic acid. These attempts included the conversion of D-erythronic acid to the benzimidazole derivative[18,19] and Ruff degradation[20,21] of calcium D-erythronate to D-glycerose followed by oxidation to D-glyceric acid. Periodate oxidation of the benzimidazole derivative and D-glyceric acid was expected to provide formic acid from only C3 of the tetronic acid. Unfortunately, these reactions were not successful with one millimole quantities. Finally, the selective periodate oxidation of sodium D-erythronate was examined. It is known that, at low temperatures, periodate oxidation of glycolic acid[22] and glyoxylic acid[23] proceeds very slowly. Accordingly, sodium D-erythronate was oxidized partially with two moles of sodium periodate and the resulting glyoxylic acid from C1 and C2, and formaldehyde from C4 of the tetronic acid, were isolated as the 2,4-dinitrophenylhydrazone derivatives in reasonable yields. Decarboxylation of the former product provided carbon dioxide from C1 and the activity at C2 was calculated by difference. Thus, as shown in Fig. 1, it was possible to radioassay the (C1 + C2), (C3 + C4), (C3 + C4 + C5 + C6) fragments as well as the C2, C3 and C6 of the anhydro-D-glucose unit directly and thereby to calculate the radioactivities of C1, C4 and C5 by difference.

It should be noted that theoretically this scheme only applies to the anhydro-D-glucose units of the polysaccharide and does not include the D-glucose moieties at the terminal units. However, in view of the large molecular weight of cellulose and the overwhelming proportion of β-D-glucopyranosyl-(1→4) units, there is very little, if any, practical limitation involved on this account.

This method was tested with a sample of radioactive cellulose predominantly labeled at C6. All the samples were counted as barium carbonate at infinite thickness. These included the cellulose, the glyoxylic acid 2,4-dinitrophenylhydrazones from C1–C2 and C3–C4 and the D-erythrono-1,4-lactone, which were oxidized to carbon dioxide by the Van Slyke and Folch reagent at atmospheric pressure.[5,24] The distribution of the label at the terminal and intermediate positions of the above radioactive cellulose had been previously determined[25] by an alternative method.[4] The good agreement between these data and the more comprehensive results obtained by the new method is shown in Table I.

TABLE I

DISTRIBUTION OF LABEL WITHIN THE ANHYDRO-D-GLUCOSE UNITS OF CELLULOSE-6-C^{14}

Position	New data: μc./carbon atom	New data: Activity, %	Previous data,[25] activity, %
1	17.43[a]	21.2	21.7
2	2.82[b]	3.4	12.0
3	2.67[b,c] (2.87)[b,d]	3.2	
4	1.35[a]	1.6	
5	3.84[a]	4.7	
6	54.24[b,c] (54.71)[b,e]	65.9	66.2

[a] Calculated by difference. [b] These values were measured directly. [c] Derived from the complete periodate oxidation of sodium D-erythronate. [d] Obtained from the decarboxylation of glyoxylic acid 2,4-dinitrophenylhydrazone. [e] Derived from the oxidation of formaldehyde 2,4-dinitrophenylhydrazone.

The reliability of this method also was confirmed by the close agreement between the activity of the anhydro-D-glucose unit and the total activity of its fragments shown in Table II.

TABLE II

THE RADIOACTIVITY OF COTTON CELLULOSE-6-C^{14} AND ITS FRAGMENTS

Fragment	Activity, μc./mole
Total of C3 to C6	62.10
D-Erythronic acid (V)	62.24
Glyoxylic acid (IV)	20.25
Total of V + IV	82.49
Anhydro-D-glucose unit	83.66
Total to C1 to C6	82.35

A sample of C^{14}-labeled cellulose prepared in 9.3% radiochemical yield by the introduction of D-glucose-2-C^{14} into maturing cotton bolls,[26] as described before,[4] provided the data given in

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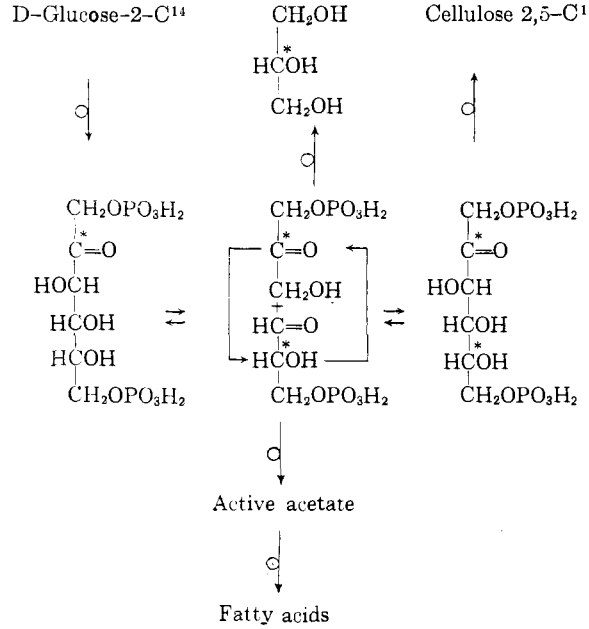
TABLE III

RADIOACTIVITY OF THE FRAGMENTS OF C^{14}-LABELED COTTON CELLULOSE PREPARED FROM D-GLUCOSE-2-C^{14}

Fragment	Activity μc./mole	Activity %
C1	35.5	4.6
C2	452.5	58.4
C3	52.9 (53.2)[a]	6.8
C4	21.9	2.7
C5	192.5	24.8
C6	20.8	2.7
Total	775.3	100.0
Glyoxylic acid	488.0	62.9
D-Erythronic acid	281.9	36.4
Total	769.9	99.3
Anhydro-D-glucose unit	760.2	98.0

[a] Obtained from decarboxylation of glyoxylic acid 2,4-dinitrophenylhydrazone.

Table III. The location of 58.4% of the label at C2 and 24.8% at C5 is consistent with the distribution pattern noted for the radioactive celluloses prepared[4] from D-glucose-1-C^{14} and -6-C^{14}. This confirms the contention that any breakdown and resynthesis of D-glucose occurring in the cotton boll takes place through the Embden–Meyerhof pathway and that the principal carbon exchanges result from the reversible isomerization of dihydroxyacetone 1-phosphate to D-glycerose 3-phosphate by the enzyme triose isomerase,[27] as shown below



The introduction of D-glucose-2-C^{14} into the maturing cotton bolls also resulted in the formation of radioactive cotton seed oil in 3% radiochemical yield which was isolated as described before.[5] This was converted to the fatty acids and glycerol. The distribution of the label within the glycerol molecule was ascertained through oxidation with sodium periodate[5] which provides formaldehyde from the terminal positions and formic acid from C2. The radioactivities of the cotton seed oil and its components are given in Table IV.

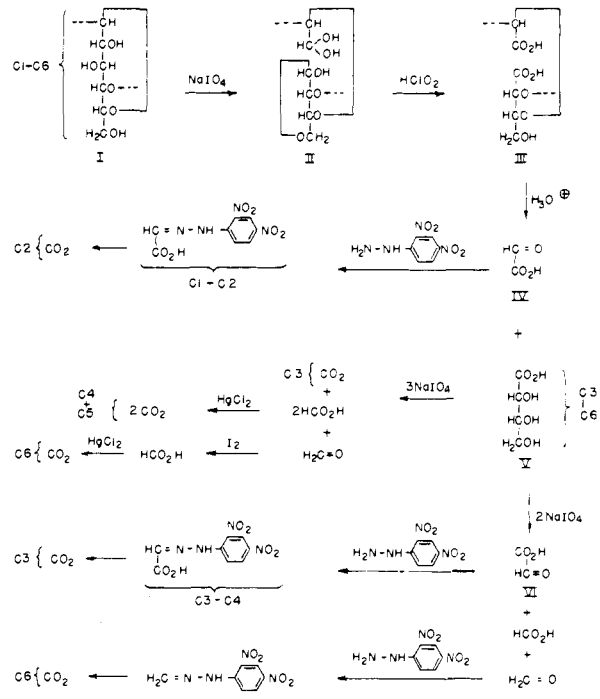


Fig. 1.—Degradation of cellulose for radioassay.

TABLE IV

RADIOACTIVITY OF THE COTTON SEED OIL PREPARED FROM D-GLUCOSE-2-C^{14}

Radioactive moiety	Activity, μc./carbon atom
Cotton seed oil	39.2
Fatty acids	43.4
Glycerol	
C1 + C3	21.9
C2	82.2

The conversion of D-glucose-6-C^{14} to the seed oil in the maturing cotton boll and its general implications has been discussed before.[5] It will be sufficient here to note that the product obtained from D-glucose-2-C^{14} follows the expected pattern. The glycerol derived from the former experiment contained most of its label at the equivalent terminal positions; whereas the glycerol isolated in the present work was predominantly labeled at C2. Thus, it is apparent that glycerol is derived from the triose moieties of D-glucose, presumably through the reduction with diphosphopyridine nucleotide (reduced form).[28] The conversion of C^{14}-labeled sucrose to specific fatty acids in the soybean has been determined by Simmons and Quackenbush.[29]

It remains to be shown whether the process of cellulose formation in the cotton boll is independent of the breakdown and resynthesis of the D-glucose moieties and proceeds by the direct polymerization

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of D-glucose phosphate as shown by the studies with *Acetobacter xylinum*.[30,31] Thus, distribution of the label in cotton cellulose may merely reflect the preceding reactions of the hexose and the pathways of its resynthesis. It is interesting to note that the distribution pattern of C^{14}-labeled celluloses produced by *Acetobacter xylinum*[7,31-33] is totally different from that of radioactive cotton celluloses. The former pattern has been shown to result from the operation of the pentose cycle,[34] whereas the distribution pattern of radioactive cotton celluloses, as noted before, is consistent with the Embden–Meyerhof pathway.

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Experimental

Periodate Oxidation of Cellulose.—In preliminary experiments, it was found that with 0.1 *M* solutions of unbuffered sodium metaperiodate, oxidation was very slow, and 8–10 days were required for completion of the primary oxidation. With 0.25 *M* periodate solution, however, the primary oxidation was completed after 4 days with uptakes, measured by a standard procedure,[35,36] in the range of 1.00–1.03 moles of periodate per anhydro-D-glucose unit. Subsequent oxidations were therefore carried out under these conditions with determination of the final uptakes only.

A homogeneous[26] sample (1 g., dry weight) of cotton cellulose was suspended in water, dispersed in the Waring blender and collected by filtration on a fritted-disc funnel. The damp cellulose was dispersed in 100 ml. of 0.25 *M* sodium metaperiodate solution and stored in the dark at room temperature (25–30°). Periodically, the reaction mixture was shaken vigorously in order to redisperse the cellulose, which, as oxidation proceeded, lost its fibrous nature and settled to the bottom of the flask. After 4 days, the insoluble periodate–oxycellulose was collected by filtration on a glass-fiber paper. Filtration on fritted discs was avoided due to the considerable losses of oxycellulose which occurred by dissolution when the discs became partially blocked by small fragments of oxycellulose. The oxycellulose was thoroughly washed with distilled water and then dried by washing with absolute ethanol, acetone and ether and by desiccation over phosphorus pentoxide; yield 0.97–1.01 g.

Oxidation of the Periodate Oxycellulose. (a) With *M* Sodium Chlorite in 0.5 *M* Acetic Acid.[15]—Oxycellulose (1 g.) was suspended in 50 ml. of an aqueous solution containing 4.52 g. of sodium chlorite and 1.5 g. of glacial acetic acid, and the mixture was stored at room temperature in a lightly stoppered flask. There was an immediate evolution of chlorine dioxide accompanied by a change in the color of the solution to a deep brown. After 1 hr., most of the oxycellulose had dissolved and after 3 hr., the remaining chlorine dioxide was removed by aeration at the water pump. The resulting pale yellow solution was treated with 20 ml. of a solution containing 10 g. of barium hydroxide octahydrate, and the heavy, white precipitate which formed was collected by centrifugation. It then was washed in a centrifuge bottle with water, 80% ethanol and absolute ethanol, collected by filtration, washed with ether and dried at 100° to give a white amorphous product; yield 1.82–1.87 g. (91–93%). The barium content of this product indicated it to have 76% of the theoretical carboxylic acid content.

(b) With 0.4 *M* Sodium Chlorite in 2 *M* Acetic Acid.[14]—Application of 0.4 *M* sodium chlorite (chlorite–aldehyde molar ratio of 3–4:1) in 2 *M* acetic acid as the oxidizing agent in the above reaction provided the barium salt in yields of only 42–54%.

Hydrolysis of Barium Carboxy-oxycellulose.—The barium salt (1.85 g.) was dissolved in 15 ml. of 2 *N* hydrochloric acid and the barium ion was precipitated by addition of the required amount of sulfuric acid. After removal of the barium sulfate by centrifugation, the solution and washings (2 *N* hydrochloric acid) were combined (*ca.* 30 ml.) and heated at 100° for 5–6 hr. The hydrolyzate was decolorized with activated carbon and treated with 90 ml. of a warm, freshly prepared, 1% solution of 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid. After storing overnight, the yellow precipitate was collected by filtration, washed with 10 ml. of water and dissolved in 50 ml. of 3.4% sodium bicarbonate solution.[16] The insoluble material was removed by filtration and washed with 20 ml. of aqueous sodium bicarbonate solution. The combined filtrate and washings were extracted with three 30-ml. portions of ethyl acetate and then acidified with 2 *N* hydrochloric acid. After storing for 1 hr., the yellow precipitate of glyoxylic acid 2,4-dinitrophenylhydrazone was collected by filtration, washed with 10 ml. of water and dried under reduced pressure over phosphorus pentoxide; yield 0.96–0.99 g. (61–63%, based on cellulose), m.p. 191–193°.

The combined filtrate and washings from the initial hydrazone precipitation were decolorized with activated carbon and neutralized (*p*H 5–6) with 40 g. of silver carbonate. The solution (250 ml.) was then passed through a column (80 × 28 mm.) of Amberlite IR-120 (H^+) resin and the acidic effluent and washings evaporated to dryness under reduced pressure. The residual gum was heated at 70–80° for 2 hr. under reduced pressure and, on cooling, crystallized in the form of long, waxy needles. The crystalline mass was extracted several times with warm ethyl acetate until most of the solid had dissolved. The combined extracts (250 ml.) were filtered and concentrated by means of an air stream. It is essential to avoid heating during this process since, with heating, the lactone tends to be deposited as a gum, causing a considerable decrease in the final yield. The crystalline mass obtained on concentration of the extracts was dissolved in 10–15 ml. of hot ethyl acetate, and this solution, after decantation from a small amount of gum and cooling at room temperature, and then at 0°, deposited colorless needles of D-erythrono-1,4-lactone; yield 0.232 g., m.p. 101–102°. Concentration of the mother liquor furnished an additional 0.079 g. of lactone, with the above melting point; over-all yield 0.311 g. (42% based on cellulose).

Periodate Oxidation of Sodium D-Erythronate.—A solution containing 118 mg. of D-erythrono-1,4-lactone (1 mmole) in 2.5 ml. of water was treated with 2.5 ml. of 0.4 *N* sodium hydroxide solution (1 mmole) and 20 ml. of 0.5 *M* sodium phosphate buffer at *p*H 5.8 (34.5 g. of sodium dihydrogen phosphate in 400 ml. of water and 100 ml. of 0.05 *N* sodium hydroxide). The system was swept for 5 min. with (carbon dioxide)-free nitrogen and then treated with a solution of 856 mg. of sodium metaperiodate (44 mmoles) in 10 ml. of water. The oxidation was continued for 1 hr. and the carbon dioxide, formic acid and formaldehyde produced were recovered as barium carbonate, according to the method described by Eisenberg,[17] in average yields of 98, 80 and 94%, respectively. These were radioassayed to determine the C^{14} distribution at C3 (C4 + C5) and C6.

Partial Periodate Oxidation of Sodium D-Erythronate.—A solution of 118 mg. of D-erythrono-1,4-lactone (1 mmole) in 2.5 ml. of water was treated with 2.5 ml. of 0.4 *N* sodium hydroxide solution and 5 ml. of 0.2 *M* acetate buffer at *p*H 4.6. The cooled (0–5°) solution was treated with a similarly cooled solution of 428 mg. of sodium metaperiodate (2 mmoles) in 10 ml. of water and stored at 0–5°. After 10 min., when tests with alkaline potassium iodide showed a negligible periodate ion content, a solution containing 250 mg. of barium chloride dihydrate in 5 ml. of water was added and the precipitated barium salts were removed by filtration. The filtrate was treated with 6 ml. of concentrated hydrochloric acid followed by 50 ml. of a warm, 1% solution of 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid. After 1 hr., the yellow precipitate was collected by filtration, washed with 10 ml. of water and dissolved in 25 ml. of 3.4% sodium bicarbonate solution. The insoluble residue of yellow formaldehyde 2,4-dinitrophenylhydrazone was collected by filtration, washed with 15 ml. of aqueous

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(34) Z. Gromet, M. Schramm and S. Hestrin, *Biochem. J.*, **67**, 679 (1957).

(35) P. Fleury and J. Lange, *J. pharm. chim.*, [8] **17**, 107 (1933).

(36) E. L. Jackson, "Organic Reactions," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1944, p. 341.

bicarbonate solution and 25 ml. of water and crystallized from hot absolute ethanol as yellow needles; yield 103 mg. (49%), m.p. 164–165° with preliminary softening at 162°.

The sodium bicarbonate extract and washings were extracted with two 25-ml. portions of ethyl acetate and then acidified with 2 *N* hydrochloric acid. After 1 hr., the precipitated yellow glyoxylic acid 2,4-dinitrophenylhydrazone was collected by filtration; yield 130 mg. (51%), m.p. 191–193°.

Similar yields of both products were obtained when the oxidation was carried out under unbuffered conditions.

Determination of C^{14} Distribution in Glyoxylic Acid 2,4-Dinitrophenylhydrazone.[16]—A portion of the hydrazone (254 mg., 1 mmole) was placed in a 10-ml. distillation flask and the system swept through with a current of (carbon dioxide)-free nitrogen. The flask was immersed in an oil-bath at 190°, and the temperature was raised rapidly to 205–210° and held at this level for 15–20 min. while the carbon dioxide released by decarboxylation was swept out by the nitrogen and trapped as barium carbonate[17]; recovery 93–97%. With the glyoxylic acid 2,4-dinitrophenylhydrazone derived from C1 and C2 of the anhydro-D-glucose units, this barium carbonate gave a direct measure of the specific activity of C2. Another portion (30 mg.) of the same sample of hydrazone was oxidized completely with the Van Slyke–Folch reagent[5,24]; the carbon dioxide was trapped as barium carbonate and radioassayed for the C^{14} content at C1 and C2. The specific activity of C1 then was determined by difference. Similarly, with the hydrazone derived from C3 and C4 of the anhydro-D-glucose units, the C^{14} activity at C4 was calculated by difference. Due to the limited amount of the hydrazone from C3-C4 available for decarboxylation, it was preferred, in these calculations, to use the value for the C3 activity obtained from the carbon dioxide liberated during the complete periodate oxidation of sodium D-erythronate.

Total Activity Measurements.—In addition to the two samples of glyoxylic acid, 2,4-dinitrophenylhydrazone, 20–30 mg. samples of the original cellulose, D-erythrono-1,4-lactone and formaldehyde 2,4-dinitrophenylhydrazone were similarly completely oxidized by the Van Slyke–Folch reagent. Radioassay of the recovered barium carbonate samples gave the total activities at C1-C6 and C3-C6 and a second value for the C6 activity, respectively.

Preparation of Benzimidazole Derivatives of D-Erythronic Acid.—A solution of calcium D-erythronate in 0.6 ml. of water, prepared by heating 152.7 mg. (1.29 mmoles) of D-erythrono-1,4-lactone with an excess of calcium carbonate, was treated for preparation of the benzimidazole derivative by the general method of Moore and Link[18]; yield of crude crystalline product 33–43 mg. (12–16%), m.p. 164–166°. This yield of the derivative was insufficient to permit subsequent periodate oxidation and isolation of the resulting fragments for radioassay.

Attempted Conversion of D-Erythrono-1,4-lactone to Calcium D-Glycerate Using Ruff Degradation and Bromine Oxidation.—A solution of calcium D-erythronate in 6 ml. of water, prepared by heating 236 mg. (2 mmoles) of D-erythrono-1,4-lactone with an excess of calcium carbonate, was treated with 0.3-ml. portions of 30% hydrogen peroxide solution as described by Isbell and associates.[21] The resulting weakly reducing solution (16 ml.) then was oxidized with 0.2 ml. of bromine, but attempts at the isolation of calcium D-glycerate were unsuccessful.

Isolation and Degradation of C^{14}-Labeled Cotton Seed Oil.—Seeds from four matured cotton bolls, which had been treated[26] with 150 μc. of D-glucose-2-C^{14}, were separated from the radioactive cellulose and ground in benzene suspension using a Waring blender. This product (7 g.) was then treated for extraction of the oil, as described previously[5]; yield of yellow cotton seed oil 1.12 g.

A sample of the oil (1.0 g.) was saponified and hydrolyzed[5] to yield 0.88 g. of fatty acids and 62 mg. of crude glycerol.

Oxidation of Cotton Seed Oil and its Components to Carbon Dioxide.—Samples of the oil (16.7 mg.) and fatty acids (15.8 mg.) were completely oxidized by the Van Slyke–Folch reagent to yield 224.3 and 205.5 mg. of barium carbonate, respectively. The glycerol (62 mg.), after dilution with an equal weight of unlabeled glycerol, was dissolved in 5 ml. of water and 15 ml. of 0.5 *M* sodium phosphate buffer (*p*H 5.8) and oxidized with a solution of 856 mg. of sodium metaperiodate in 10 ml. of water. After 1 hr., the formaldehyde and formic acid produced were recovered as barium carbonate, as described by Eisenberg,[17] in yields of 438.2 mg. (82%) and 197.4 mg (74%), respectively.

Counting Methods.—All samples were counted as barium carbonate at infinite thickness using a mica window Geiger tube,[37] connected to a decade scaler[38] and compared with a standard sample.[39] The samples were counted long enough to reduce the random counting error to ±2%.

(37) Thyrode 1B67/VG-IOA, Victoreen Instrument Co., Cleveland 3, Ohio.

(38) Potter Instrument Co., Inc., Flushing, N. Y.

(39) Tracerlab, Inc., Boston 10, Mass.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

The Controlled Thermal Decomposition of Cellulose Nitrate. V. C^{14}-Tracer Experiments[1,2]

BY F. SHAFIZADEH, M. L. WOLFROM AND P. MCWAIN

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Investigation of the products formed from the controlled ignition of cellulose-C^{14} nitrate, containing 58% of the label at C2 and 25% at C5 of the anhydro-D-glucose units, has indicated that a major fraction of glyoxal is derived from C2 and one of the adjacent carbon atoms and that C2 and C5 provide some of the carbon dioxide and formic acid fragments and very little of the formaldehyde. The principal initial pattern for the thermal decomposition of cellulose nitrate is thus established.

Reactions of cellulose nitrate have been the subject of extensive investigations.[3] In this Laboratory we have been concerned with the controlled thermal decomposition of cellulose nitrate and the course of the complex reactions therein involved. The first stage in this investigation was the identification and analysis of the products which are formed under different conditions as controlled by the pressure of the ignition chamber and the degree of nitration of the materials. Thus, it was shown that, under a variety of conditions, the carbon-containing volatile fragments consist mainly of carbon dioxide, carbon monoxide, formic acid, formaldehyde, glyoxal and hydrogen cyanide.[4]

(1) This work was carried out under contract (DA-33-019-ORD-2042; technical supervising agency, Ballistic Research Laboratories, Aberdeen Proving Ground, Md.) between the Office of Ordnance Research of the U. S. Army Ordnance Corps and the Ohio State University Research Foundation (project 679).

(2) Previous communication in this series: F. Shafizadeh and M. L. Wolfrom, THIS JOURNAL, **80**, 1675 (1958).

(3) J. Barsha in "Cellulose and Cellulose Derivatives; High Polymers," E. Ott, H. M. Spurlin and Mildred W. Grafflin, eds., Interscience Publishers, Inc., New York, N. Y., 2nd edition, 1954, Vol. V, Part II, p. 751.

(4) M. L. Wolfrom, J. H. Frazer, L. P. Kuhn, E. F. Dickey, S. M. Olin, R. S. Bower, G. G. Maher, J. D. Murdock, A. Chaney and Eloise Carpenter, THIS JOURNAL, **78**, 4695 (1956).