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 C14 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[6,24]; the carbon dioxide was trapped as barium carbonate and radioassayed for the C14 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 C14 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 C14-Labeled Cotton Seed Oil.—Seeds from four matured cotton bolls, which had been treated[26] with 150 μc. of D-glucose-2-C14, 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 (pH 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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The Controlled Thermal Decomposition of Cellulose Nitrate. V. C14-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-C14 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

(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.

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]

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

In order to obtain further information regarding the nature of the reactions involved, it was deemed necessary to correlate these fragments with the various positions in the anhydro-D-glucose units of the cellulose nitrate, from which they originate. Investigations along these lines were carried out through two different methods. The first method involved the controlled ignition of cognate materials such as dextran nitrate, pectic acid nitrate and the nitrates of several hexitols. On the basis of the results thereby obtained, it was concluded that formaldehyde was derived from the primary nitrate groups.[4] The second method was based on tracing the carbon-containing fragments through the use of C^{14}-labeled cellulose nitrates. The C^{14}-labeled celluloses were prepared[5] according to the method of Greathouse[6] through the introduction of D-glucose-1-C^{14} and -6-C^{14} into the maturing cotton bolls. The resulting radioactive celluloses contained about 65% of the label at the original terminal position of the anhydro-D-glucose units and 22% at the other terminal position. The data obtained from the radioassay of the ignition products of cellulose-1-C^{14} and -6-C^{14} nitrates were interpreted through mathematical equations based on the assumption that the remainder of the label was equally distributed between the intervening positions (C2 to C5). It subsequently has been shown that this assumption is justified, since the deviations from the average[7] are well within the experimental error of ±2%. Thus, it was shown that 45% of glyoxal is derived from C1–C2 and the remainder from other pairs of adjacent carbons within the C2 to C5 chain. It also was shown that formic acid and carbon dioxide are derived 36 and 39%, respectively, from C1; 47 and 54% from C2 to C5; and 16 and 7% from C6. Furthermore, the origin of formaldehyde was traced as 11% from C1, 24% from C2 to C5 and 65% from C6. These results show that C6 constitutes the main origin of the formaldehyde, but that small amounts also arise from the other carbons. It also was shown that the cyanide ions originated from the cellulose molecule and not from the solvent.

The present work describes further experiments with cellulose-2-C^{14} which confirm the above data and provide further information concerning the fate of the intervening carbons, particularly C2. Cellulose-2-C^{14} was obtained by the introduction of highly active[8] D-glucose-2-C^{14} into maturing cotton bolls according to the method of Greathouse[6] as described before.[5] The product, obtained in a radiochemical yield of 9.3%, was purified, cut and homogenized. A portion of this homogeneous, radioactive cellulose was nitrated with mixed acids[9] and another portion was used for ascertaining the distribution of the label (Table I) within the anhydro-D-glucose units.[7] The radioactive cellulose nitrate was dissolved in ethyl acetate and

converted to a film. This was ignited under reduced pressure in an atmosphere of carbon dioxide-free nitrogen. The resulting ignition products were collected and fractionated as before.[2] The carbon dioxide and formic acid fragments were converted to barium carbonate directly. The glyoxal and formaldehyde fragments were isolated as the bis-(phenylhydrazone) and dimedone derivatives, respectively. These products were purified, and oxidized with the Van Slyke–Folch reagent[10] at atmospheric pressure[11] and the resulting carbon dioxide was converted to barium carbonate. Radioassay of the barium carbonate samples provided the specific radioactivity of the fragments given in Table II.

TABLE I

DISTRIBUTION OF THE LABEL[7] WITHIN THE ANHYDRO-D-GLUCOSE UNITS OF CELLULOSE-2-C^{14}

Fragment	Activity Specific, μc./mole	%
C1	35.5	4.6
C2	452.5	58.4
C3	52.9	6.8
C4	21.9	2.7
C5	192.5	24.8
C6	20.8	2.7
Anhydro-D-glucose unit	760.2	98.0

TABLE II

SPECIFIC ACTIVITY OF THE CELLULOSE NITRATE AND ITS IGNITION PRODUCTS

Radioactive moiety	Activity Specific, μc./mole	%[a]
Av. carbon atom of cellulose	129.2	16.6
Glyoxal	341.2	44.8
Carbon dioxide	115.1	15.1
Formic acid	96.5	12.6
Formaldehyde	31.1	4.1

[a] Total activity of C1 to C6 of the anhydro-D-glucose units is regarded as 100.

The high specific activity of glyoxal (Table II) indicates that a major fraction of this compound must have been derived from C2 and one of the adjacent carbon atoms. The previous results[2] indicated that 45% of glyoxal is derived from C1–C2. At the other extreme, the very low activity of formaldehyde indicates that it must originate principally from the low activity carbons (Table I): C6, C4, C1 and C3; a major contribution from C4, C1 and C3 is excluded on the basis of the previous results.[2] On the other hand, the difference between the specific activity of C6 (Table I) and that of formaldehyde (Table II) indicates that a small fraction of this compound must have been derived from the other carbons. Furthermore, the specific activities (Table II) of carbon dioxide and formic acid, being somewhat below the single carbon average, indicate that a less than average portion of these products must have been derived from the more radioactive carbons, C2 and C5.

These conclusions are completely consistent with our previous data. The net result of all these

(5) F. Shafizadeh and M. L. Wolfrom, THIS JOURNAL, **77**, 5182 (1955).

(6) G. A. Greathouse, Science, **117**, 553 (1953).

(7) M. L. Wolfrom, J. M. Webber and F. Shafizadeh, THIS JOURNAL, **81**, 1217 (1959).

(8) Obtained from Dr. H. S. Isbell, National Bureau of Standards, Washington, D. C., and Volk Radio-Chemical Co., Chicago 40, Ill.

(9) A. L. Olsen and J. W. Greene, "Laboratory Manual of Explosive Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1943, p. 25.

(10) D. D. Van Slyke and J. Folch, J. Biol. Chem., **136**, 509 (1940).

(11) F. Shafizadeh and M. L. Wolfrom, THIS JOURNAL, **78**, 2498 (1956).

investigations is that formaldehyde originates mainly from C6 and very little from C2. In contrast, glyoxal is derived mainly from C2 and one (largely C1) of its adjacent carbons, and very little from C6. The origins of carbon dioxide and formic acid are less clear cut. It appears that they are derived rather heavily from C1,[2] and somewhat less than average from the other positions (including C2 and C5). Thus, as in the periodate oxidation reactions, the oxidation state of the products is consistent with the oxidation state of the carbon atom from which they mainly originate. The demonstrated deviations from the principal pattern shown in Fig. 1, as well as the large differences in the yield of the individual fragments, can be attributed to competing fragmentation processes and to subsequent secondary reactions, such as the postulated example

$$HC=O \qquad HC=O$$
$$| \qquad\qquad |$$
$$HC=O \longrightarrow CO_2H \longrightarrow CO_2 + H_2C=O$$
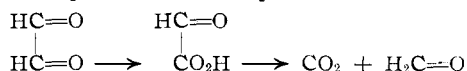
It is to be noted that since some over two moles (per monomer unit) of carbon monoxide is formed[12] from cellulose nitrate under these conditions, the formation of this product cannot be ascribed to any one carbon.
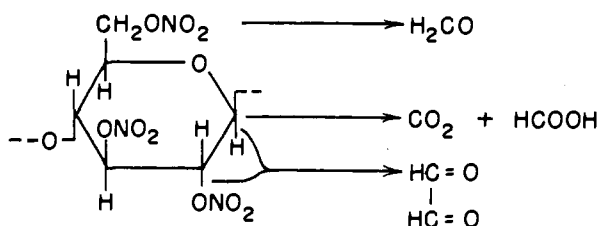

Fig. 1.—Principal initial pattern for the thermal decomposition of cellulose nitrate.

Experimental

Preparation of Cellulose-2-C[14].—Several cotton plants, *Gossypium hirsutum* var. *Paula*, were cultivated in 12-inch pots in a green house. The plants responded very well to regular fertilizing and the use of the large pots. A total of 150 μc. of highly active commercial[8] D-glucose-2-C[14] was introduced into four 21-day old cotton bolls as described before.[5] The resulting radioactive cotton cellulose was separated from the seeds, which were used for another experiment,[7] and purified[6]; yield 3 g. In order to prepare homogeneous samples the product was cut with scissors. The cut fibers were suspended in water, blended in a Waring blender

and filtered on a fritted glass Büchner funnel. The resulting pad was cut into small pieces (3 mm. diam.), suspended in water and blended. This process was repeated several times until the suspension of the small pieces of the fiber appeared to be quite homogeneous and there was no long fiber to adhere on a glass rod. The resulting pulp then was dried and divided into several samples used for the nitration and radioactivity determinations. The latter experiments indicated a radiochemical yield of 9.3%. Several preliminary experiments aimed at dissolving the highly polymeric cotton cellulose and recovery of intact material were unsuccessful.

Ignition of Cellulose-2-C[14] Nitrate.—A 1-g. sample of homogenized cellulose-2-C[14] was nitrated with a mixture of 22 ml. of 70% nitric acid, 3.4 ml. of water and 35.3 ml. of 95% sulfuric acid[9] and the product was stabilized by washing with 1% ammonia.[13] A parallel non-radioactive experiment indicated that the product contained 12.52% nitrogen, as determined by the du Pont nitrometer. The radioactive product was dissolved in 75 ml. of ethyl acetate and the solution was converted to a transparent film free of tension; yield 1.2 g. Thus, the previous difficulties in obtaining a clear film from the highly polymeric cotton cellulose nitrate were obviated by the pulping of cotton cellulose. The above film was cut into small strips and a 1-g. sample was ignited in an atmosphere of nitrogen, at 75 mm. pressure, as described before.[2]

Isolation of the Carbon Fragments.—The carbon dioxide resulting from the ignition of cellulose-2-C[14] nitrate (1 g.) was collected in a trap cooled with liquid nitrogen and converted to barium carbonate; yield 348 mg. The other products collected in the ignition system and two spiral traps, cooled with solid carbon dioxide and acetone, were dissolved in 100 ml. of water and the solution was treated with 4 ml. of 0.1 N silver nitrate. A small precipitate of silver cyanide was filtered and the filtrate was fractionated by evaporation under freeze-drying conditions, as described before.[2]

The glyoxal in the residue was recovered as the bis-(phenylhydrazone) and recrystallized from benzene; yield 72 mg., m.p. 168–170°. A sample of the product (17.1 mg.) was oxidized with the Van Slyke–Folch reagent[10] at atmospheric pressure,[11] and the resulting carbon dioxide was converted to barium carbonate; yield 199 mg.

The distillate was neutralized with 3 N sodium hydroxide and re-evaporated. The sodium formate in the residue was oxidized to carbon dioxide with mercuric chloride and recovered as barium carbonate; yield 366 mg.

The final distillate was treated with 300 ml. of 0.4% dimedone (5,5-dimethyl-1,3-cyclohexanedione) reagent, and the resulting crude precipitate of formaldehyde dimedone derivative (57 mg.) was recrystallized from aqueous ethanol; yield 45 mg., m.p. 189–190°. A sample of this material (20.9 mg.) was oxidized to carbon dioxide with the Van Slyke–Folch reagent, and the product was converted to barium carbonate; yield 246 mg.

Counting Methods.—The samples of barium carbonate were plated in 1-inch planchets and counted at infinite thickness with a mica window Geiger tube[14] connected to a decade scaler,[15] and the counts were compared with that of a standard sample.[16] The samples were counted long enough to provide a random counting error of less than ±2%. The results are presented in Table II.

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(12) M. L. Wolfrom, A. Chaney and P. McWain, THIS JOURNAL, **80**, 946 (1958).

(13) R. E. Reeves and J. E. Giddens, *Ind. Eng. Chem.*, **39**, 1303 (1947).

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

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

(16) Tracerlab, Inc., Boston, Mass.