techniques and buffers previously described.[41,42] The apparent pK_a values were determined spectrally by procedures previously employed.[43,44]

Potentiometric Titrations.—Potentiometric titrations were carried out at 23° with a glass electrode standardized at pH 4.00. Carbonate-free 0.100 N NaOH was added to a nitrogen-stirred solution of the particular mercaptopurine in pre-boiled water and the pK_a values calculated at seven equidistant points on the titration curve and averaged; 6MP, 2-amino-6-mercaptopurine and 6-mercapto-9-β-D-ribofuranosylpurine were titrated at concentrations of 0.01 M. 2-Amino-6-mercapto-9-β-D-ribofuranosylpurine was titrated at 0.0025 M.

For spectrophotometric and potentiometric studies, analytical samples of thioinosine (Ia) and thioguanosine (Ib) were used. 6-Mercaptopurine was purified as follows: A commercial sample of 6MP (0.15 g.) was dissolved in boiling pyridine (4.5 ml.) and cooled overnight in the refrigerator. The white prisms which formed were collected and washed with pyridine. The solid was triturated immediately with 4 ml. of water and the pH of the mixture adjusted to 5 with 1 N hydrochloric acid. The suspension was warmed to 50–60°, cooled, and the cream-colored solid collected and washed with 10 ml. of water. The recovery was 0.11 g. (dried at 15 mm. over NaOH). Paper chromatography in BuOH–AcOH–water (5:2:3) and in BuOH–HCOOH–water (85:0.2:14) using Schleicher and Schuell No. 597 paper showed only one spot. The pyridine mother liquor contained traces of a foreign spot.

2-Amino-6-mercaptopurine was recrystallized several times by methods previously described.[18] Paper chromatograms revealed the presence of a small impurity.

In spite of these purifications, it was impossible to establish sharp isosbestic points[45] in the spectra of 6MP and thioguanine, due probably to the slow decomposition with time (see Table I) already noted for these purines in dilute alkali. The relatively small errors in those two spectra do not alter the argument with regard to the allocation of ionization (between pH 4.9 to 9.6) to the dissociation of the 6-mercapto function; nor do they affect the accuracy of the spectrally-determined pK_a values (within the margin of error listed) in Table II.

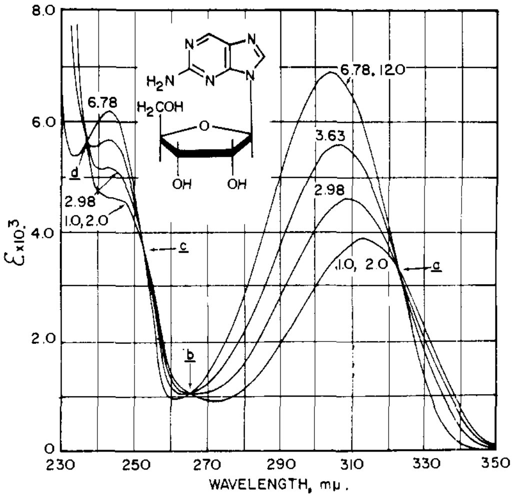

Fig. 5.

Key to Figures.—All the spectra listed were run in aqueous solutions at pH values indicated on the curves. The italicized letters refer to isosbestic points.

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(41) J. J. Fox, L. F. Cavalieri and N. Chang, THIS JOURNAL, **75**, 4315 (1953).

(42) J. J. Fox, N. Yung, J. Davoll and G. B. Brown, *ibid.*, **78**, 2117 (1956).

(43) D. Shugar and J. J. Fox, *Biochim. et Biophys. Acta*, **9**, 199 (1952).

(44) J. J. Fox and D. Shugar, *Bull. soc. chim. Belges*, **61**, 44 (1952).

(45) For a discussion of the significance of isosbestic points see J. J. Fox and D. Shugar, *Biochim. et Biophys. Acta*, **9**, 369 (1952). See also A. Bendich, in "The Nucleic Acids," Vol. I (Chargaff and Davidson, eds.), Academic Press, Inc., New York, N. Y., 1955, p. 81.

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

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

BY F. SHAFIZADEH AND M. L. WOLFROM

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Radioassay of the products formed from the controlled ignition of cellulose-C^{14} nitrates, predominantly labeled at C1 and C6 of the anhydro-D-glucose units, indicates that C1 gives mainly carbon dioxide and lesser amounts of formic acid and glyoxal (from C1 and C2) and that the major product from C6 is formaldehyde with lesser amounts of formic acid and carbon dioxide.

Several aspects of the controlled thermal decomposition of cellulose nitrate have been discussed in the previous papers of this series.[2-4] Thus, it has been noted that the controlled ignition of cellulose nitrate under reduced pressure provides a mixture of carbon-containing volatile fragments consisting mainly of carbon dioxide (and carbon monoxide), formic acid, formaldehyde and glyoxal. The quantitative analysis of these compounds and their relation to the chemical nature of the ignition process has been reported. In order to obtain further information concerning the ignition of cellulose nitrate, it was deemed pertinent to recover the ignition products, or derivatives thereof, and to correlate them with the various positions of the anhydro-D-glucose units of the cellulose nitrate, from which they originate, through the use of isotopic tracer techniques. Thus, the first step toward this goal was the preparation of specifically labeled cellulose-C^{14}. Several samples of these materials[5]

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(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). Preliminary communication: *Abstracts Papers Am. Chem. Soc.*, **132**, 16D (1957).

(2) Previous communication in this series: M. L. Wolfrom, A. Chaney and P. McWain, THIS JOURNAL, **80**, 946 (1958).

(3) 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).

(4) M. L. Wolfrom, J. H. Frazer, L. P. Kuhn, E. E. Dickey, S. M. Olin, D. O. Hoffman, R. S. Bower, A. Chaney, Eloise Carpenter and P. McWain, *ibid.*, **77**, 6573 (1955).

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

were obtained through the introduction of D-glucose-1-C^{14} and D-glucose-6-C^{14} into the maturing cotton-bolls according to a method first described by Greathouse.[6] However, contrary to our expectation, the labeling in these products (shown in Table I) was not completely specific.

TABLE I

DISTRIBUTION OF C^{14}-LABEL IN THE ANHYDRO-D-GLUCOSE UNITS OF RADIOACTIVE COTTON CELLULOSE

	% of total C^{14} in		
Sample, cellulose[a] from	C1	C2 to C5, inclusive	C6
D-Glucose-1-C^{14}	63.5	14.6	21.9
D-Glucose-6-C^{14}	21.7	12.0	66.2

[a] The distribution of the label in cellulose-6-C^{14} was ascertained by the close agreement of two independent experiments; these data represent the average results.

The next step was the efficient recovery and fractionation of the small amounts of the products in an uncontaminated form. For this purpose, the ignition system described before[3] was supplemented by a final trap, cooled with liquid nitrogen, for the condensation of carbon dioxide. Furthermore, the ignition system was modified so that it was possible to flush the ignition chamber with nitrogen without introducing carbon dioxide from ambient air into the condensing system.

The non-gaseous condensates were first treated with a solution of silver nitrate to precipitate any cyanide ions present, and the remaining solution was evaporated under freeze-drying conditions. The distillate contained formaldehyde and the volatile acids (mainly formic acid) and the residue contained glyoxal and other less volatile fragments. The distillate was neutralized and again evaporated in the same manner. This produced a solution containing formaldehyde which was recovered as the dimedone (5,5-dimethyl-1,3-cyclohexanedione) derivative and a residue containing sodium formate which was oxidized to carbon dioxide and recovered as barium carbonate.[7] Furthermore, the glyoxal in the original residue was recovered as glyoxal bis-(phenylhydrazone).[3]

The radioassay of the above products furnished the data given in Table II. These data concern the specific radioactivity of each product and its relation to the specific radioactivity of the original D-glucose units, without consideration of the product yields obtained. An accurate interpretation of the data is somewhat complicated by the lack of complete specificity in the labeling of the original tagged materials and by the fact that ignition may result in a complicated combination of primary and secondary reactions, before the products are cooled to the lower temperatures and condensed; it should be noted that the residual solvent (about 5% ethyl acetate) in the ignited film does not make any detectable contribution to the products considered here.[2] For simplicity in calculation, it has been assumed that the intervening carbon atoms (C2 to C5, inclusive) of the anhydro-D-glucose units are all equally radioactive and carry 3% of the activity in cellulose-6-C^{14} and 3.65% of that in cellulose-1-C^{14}. Mathematical calculations based on this approximation and the data given in Tables I and II result in the information, given in Table III, concerning the percentage of the fragments derived from C1 and C6, and the intervening carbons C2 to C5.

TABLE II

SPECIFIC RADIOACTIVITIES OF THE ORIGINAL CELLULOSE NITRATES AND THEIR IGNITION PRODUCTS

	Spec. act., μc. of C^{14} per g. atom of C		Comparative molecular activity, %		Ratio of sp. act. in prod. to "ave. C" in orig. D-glucose unit	
Substance	Cellulose-1-C^{14}	Cellulose-6-C^{14}	Cellulose-1-C^{14}	Cellulose-6-C^{14}	Cellulose-1-C^{14}	Cellulose-6-C^{14}
D-Glucose unit (original)[a]	4.63	16.65	100	100	1	1
Formaldehyde	6.17	46.22	22.19	46.22	1.33	2.76
Glyoxal	4.73	5.90	34.00	11.80	1.02	0.35
Formic acid	7.89	20.22	28.38	20.22	1.70	1.21
Carbon dioxide	7.89	14.86	28.38	14.96	1.70	0.89

[a] These values were obtained by the radioassay of the carbon dioxide resulting from the complete oxidation of the cellulose nitrate.

TABLE III

FRACTION OF EACH IGNITION PRODUCT WHICH ORIGINATES FROM THE VARIOUS CARBON ATOMS IN CELLULOSE NITRATE

Product	Cl, %	C2–C5, %	C6, %
Formaldehyde	11.2	23.6	65.2
Glyoxal	44.6	55.4	0 ± 4
Formic acid	36.3	47.2	16.5
Carbon dioxide	39.0	53.6	7.4

The quantitative analysis of the ignition products has been thoroughly investigated and described in detail.[2,3] Thus, the available data enable us to calculate the approximate mole fractions of C1 and C6 of the anhydro-D-glucose units that have been converted to each of the above products (Table IV).

TABLE IV

APPROXIMATE FRACTION OF C1 AND C6 OF THE ANHYDRO-D-GLUCOSE UNITS CONVERTED TO EACH OF THE PRODUCTS

Product	Yield in moles per mole[a] cellulose nitrate	Yield in moles per mole from C1	Yield in mole per mole from C2 to C5	Yield in mole per mole from C6
Carbon dioxide	1.1	0.43	0.59	0.08
Formic acid	0.35	.13	.16	.06
Glyoxal	.2	.09	.11	.00
Formaldehyde	.3	.03	.08	.19

[a] Based upon the anhydro-D-glucose unit; data of ref. 3.

These calculations indicate that the major products from C1 of the anhydro-D-glucose units are carbon dioxide and lesser amounts of formic acid and glyoxal, and the major product from the C6 moiety is formaldehyde with lesser amounts of carbon dioxide and formic acid. They also indicate that glyoxal is partly derived from the C1–C2 moiety and partly from other pairs of adjacent carbons within the C2 to C5 chain. Furthermore, the major bulk of formaldehyde has been traced to C6, although some appears to have been derived from other sources.

It should be noted that all the products shown below are not necessarily the original fragments of

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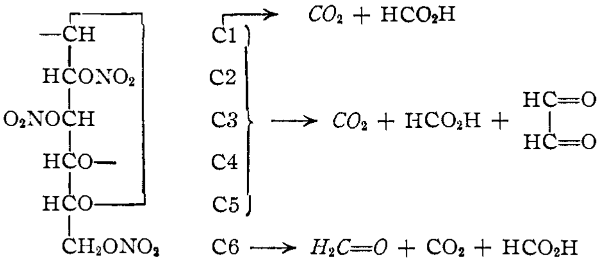
(6) G. A. Greathouse, *Science*, **117**, 553 (1953).

(7) F. Eisenberg, Jr., THIS JOURNAL, **76**, 5152 (1954); H. Fincke, *Biochem. Z.*, **51**, 253 (1913).

the anhydro-D-glucose units. In fact it would be more feasible to assume that some of these are derived from secondary reactions. The main products are in italics. Some aspects of the initial fragmentation reactions have been discussed by Mason and associates.[8] We hope to obtain further information concerning the fate of the intervening carbons, especially C2, through experiments with cellulose nitrate predominantly C^{14}-labeled at C2. In the above experiments, no attempt has been made to trace the origin of carbon monoxide which can be found among the ignition products.



The precipitate of silver cyanide isolated from the ignition products of both cellulose-1-C^{14} nitrate and cellulose-6-C^{14} nitrate displayed a considerable measure of radioactivity, indicating that the cyanide ions originate from the cellulose molecule and not from the solvent.

Acknowledgment.—We wish to express our gratitude to Dr. L. P. Kuhn for valuable suggestions and interest in this work. We are also pleased to acknowledge the assistance of Mr. P. McWain in ascertaining the distribution of the label in cellulose-6-C^{14}.

Experimental

Ignition of Cellulose Nitrate.—Purified radioactive cotton cellulose (4.5 g.) was converted, with mixed acids,[9] to cellulose nitrate containing 12.5% nitrogen. This was stabilized by the method of Reeves and Giddens,[10] dissolved in ethyl acetate and converted to a film. Since the material was highly polymeric, some difficulty was encountered in the latter process and it was necessary to gradually evaporate the highly viscous ethyl acetate solution in a crystallizing dish instead of utilizing the reported procedure.[4] The resulting film (7.5 g.) was ignited in small strips under a very gentle stream of nitrogen, at 75 mm. pressure, as described previously.[3] The ignition system, however, was supplemented by a final trap cooled with liquid nitrogen, to condense carbon dioxide, and was modified in a manner which made it possible to flush and evacuate the ignition chamber, after introduction of the cellulose nitrate strips, without contaminating the receivers with carbon dioxide from the air.

Isolation of the Ignition Products.—After ignition, the carbon dioxide condensed in the final trap was recovered as barium carbonate; yield 3.52 g. The solution of the other ignition products was immediately treated with 27 ml. of 1% aqueous silver nitrate and the resulting precipitate was filtered and washed; yield 147 mg.

The above filtrate was diluted to 150 ml. and evaporated under freeze-drying conditions. The residue was dissolved in 100 ml. of water and evaporated for a second time. The final residue contained glyoxal which was recovered as glyoxal bis-(phenylhydrazone) by repeated small scale chromatography over columns of silicic acid–Celite,[3] and purified by recrystallization from benzene; yield 406 mg.

The above distillates were combined and neutralized to *p*H 8 with 3 *N* sodium hydroxide and the neutral solution was again evaporated under freeze-drying conditions. The residue, containing sodium formate, was oxidized with mercuric chloride and the resulting carbon dioxide was recovered as barium carbonate; yield 1.685 g.

Finally, the distillate from the last evaporation was treated with 300 ml. of 0.4% aqueous dimedone (5,5-dimethyl-1,3-cyclohexanedione) reagent and the resulting precipitate of formaldehyde dimedone derivative (575 mg.) was recrystallized from 75% ethanol; yield 404 mg.

Counting Methods.—All samples were counted as solids at infinite thickness using a preflush flow counter[11] connected to a decade scaler,[12] and compared with a standard barium carbonate.[13] The samples were counted long enough to reduce the random counting error to ±2%. Samples of cellulose nitrate film and glyoxal bis-(phenylhydrazone) were oxidized with the Van Slyke–Folch[14] reagent and radioassayed as barium carbonate. The formaldehyde dimedone derivatives were counted as such and compared with a standard sample that was prepared from a solution of radioactive formaldehyde radioassayed as barium carbonate.[7] Thus, all of the samples counted were related to a single standard; this excluded the small precipitate of silver cyanide which was not quantitatively investigated.

Calculation Method.—The data recorded in Table III were derived from the results given in Tables I and II and from equations based on the assumption that the individual intervening carbons (C2 to C5) are all equally radioactive, and carry 3% of the activity in cellulose-6-C^{14} and 3.65% of that in cellulose-1-C^{14}. Thus, the total activity of a product (in per cent.) should have been partly derived from C1, partly from the carbons C2 to C5, and partly from C6, as shown in equations 1 to 3 for formaldehyde.

$$x + y + z = 1 \tag{1}$$

$$63.5x + 21.9y + 3.65z = 22.19 \tag{2}$$

$$21.7x + 66.2y + 3z = 46.22 \tag{3}$$

In equation 1, x is the fraction derived from C1, y is the fraction derived from C6 and z is the fraction derived from the other carbons. In (2), written for the experiment with cellulose-1-C^{14}, $63.5x$ and $21.9y$ are the activities of formaldehyde (in per cent.) derived from Cl and C6, respectively, and $3.65z$ is the remainder of the activity derived from C2 to C5. The same logic applied to (3) written for the experiment with cellulose-6-C^{14}.

Equations 4 to 6 apply to glyoxal which carries two adjacent carbon atoms of the original D-glucose unit.

$$x + y + z = 1 \tag{4}$$

$$(63.5 + 3.65)x + (21.9 + 3.65)y + (3.65 + 3.65)z = 2 \times 17.0 \tag{5}$$

$$(21.7 + 3)x + (66.2 + 3)y + (3 + 3)z = 2 \times 5.9 \tag{6}$$

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(8) G. Gelernter, L. C. Browning, S. R. Harris and C. M. Mason, *J. Phys. Chem.*, **60**, 1260 (1956).

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

(11) Radiation Counter Laboratories, Inc., Skokie, Ill.

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

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

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