spin coupling of 12–14 c.p.s. also agrees closely with that recently found for two non-equivalent methylene hydrogens in certain steroids.[10]

The group assignments of the n.m.r. lines from the yellow compound are summarized in Table I. It is evident from the above discussion of the n.m.r. data that the latter are in complete accord with the conclusion that the structure of the yellow compound formed when ellagic acid is benzylated is VI and not III.

## Experimental

**Acid Hydrolysis of the Yellow Compound.**—A solution of the yellow compound (60 mg.) in glacial acetic acid (2.0 ml.) was cooled and treated with 4 drops of concentrated sulfuric acid. After standing for 20 minutes a considerable quantity of colorless crystalline material had separated. Excess of water was added, the solid was collected and recrystallized from acetone–methanol. 3,3′,4-Tri-O-benzyl-5,5′-di-C-benzylellagic acid separated in colorless needles, m.p. 249°, which did not give a ferric chloride reaction.

*Anal.* Calcd. for $C_{49}H_{38}O_8$: C, 78.15; H, 4.82. Found: C, 78.2; H, 4.99.

The infrared spectrum was obtained on the solid material in the form of a KBr pressed disk using a Beckman IR-3 spectrophotometer[11] with NaCl optics.

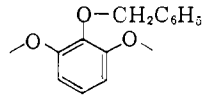
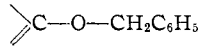
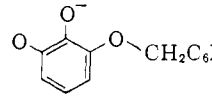
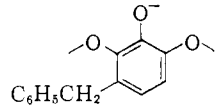
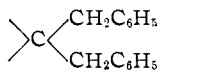
The high resolution n.m.r. spectrum was obtained at Varian Associates with their 60 megacycle spectrometer.[11]

(10) J. N. Shoolery and M. T. Rogers, THIS JOURNAL, **81**, in press (1959).

(11) Mention of manufacturers or of trade names of products or equipment does not imply that they are recommended by the Department of Agriculture over others not mentioned.

TABLE I

PROTON RESONANCE SHIFT AT 60 MEGACYCLES PER SECOND IN YELLOW COMPOUND FOR METHYLENE HYDROGENS

| Group | Shift, c.p.s. from benzene |
|---|---|
|  | 55 |
|  | 77 |
|  | 83 |
|  | 114 |
|  | 137, 149, 160, 172 |

The yellow compound was dissolved in deuterated chloroform to a concentration of about 0.2 *M*. The experimental procedure was the same as that described in part VIII.

**Acknowledgment.**—We wish to thank Edith Gong for obtaining the infrared spectral data, Mr. L. F. Johnson for obtaining the n.m.r. spectrum, and L. M. White for the elementary analyses.

PASADENA, CALIF.

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

# The Composition of Pyrodextrins. II. Thermal Polymerization of Levoglucosan[1]

BY M. L. WOLFROM, A. THOMPSON[2] AND R. B. WARD[2]

RECEIVED FEBRUARY 27, 1959

A fragmentation study of a polymer made by heating 1,6-anhydro-β-D-glucopyranose reveals that this anhydro moiety is capable of polymerizing when heated to produce the usual glucosidic linkages found in a pyrodextrin. Thus, in the dextrination of starch at high temperatures, the reaction may be a depolymerization destroying an α-D-(1 → 4)-linkage and forming a terminal anhydro group, followed by repolymerization to produce other glucosidic linkages.

It has been shown,[3] by isolative procedures, that during the production of pyrodextrins by roasting starch, without added acid catalyst, the normal α-D-(1 → 4)-linkages tend to disappear and α- and β-D-(1 → 6)-, and smaller quantities of β-D-(1 → 2)-linkages, are formed together with some 1,6-anhydro-β-D-glucopyranose end groups. This is in general agreement with the findings of other workers[4] employing other methods on pyrodextrins formed under acid catalysis. The fact that the reaction proceeds very rapidly at 200° and in the substantial absence of water or acid indicates that hydrolysis and reversion may not be significant factors under these conditions. It has been postulated that the initial step in the pyrolysis of cellulose,[5] is chain rupture with the formation of a 1,6-anhydro end group. Since an end product of the pyrolysis of starch and cellulose is 1,6-anhydro-β-D-glucopyranose, it is logical to assume such an intermediate step. The isolation of 1,6-anhydro-β-D-glucopyranose[3] from the hydrolyzate of a pyrodextrin formed at high temperature also lends support to this conception. We suggest that such an anhydro end group may also act as an intermediate in reforming the polymer and in producing other linkages in the molecule. The primary hydroxyl on carbon six of a nearby D-glucose unit may be the preferred point of reaction.

Pictet[6] obtained a polymer by heating 1,6-anhydro-β-D-glucopyranose at a temperature of 235–240°. We wish to report herein a fragmentation study of this polymer in which gentiobiose, isomaltose, maltose, cellobiose, sophorose and 1,6-anhydro-β-D-glucopyranose were isolated and

(1) Presented before the Chemistry Section of the Ohio Academy of Science at the Columbus, Ohio, Meeting, April 17–18, 1959.

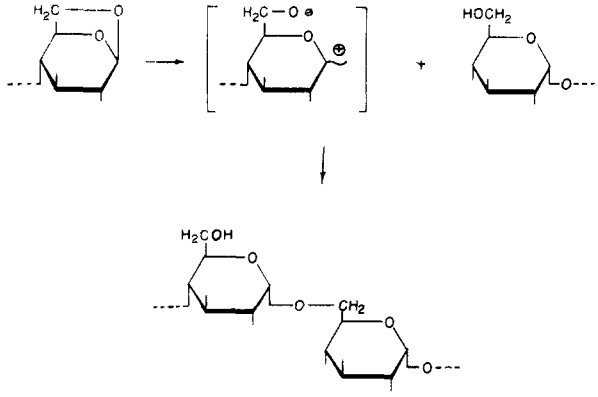
(2) Research Associate (A. T.) and Postdoctoral Fellow (R. B. W.) of the Corn Industries Research Foundation.

(3) A. Thompson and M. L. Wolfrom, THIS JOURNAL, **80**, 6618 (1958).

(4) G. M. Christensen and F. Smith, *ibid.*, **79**, 4492 (1957); J. D. Geerdes, Bertha A. Lewis and F. Smith, *ibid.*, **79**, 4209 (1957); Bernadine Brinhall, *Ind. Eng. Chem.*, **36**, 72 (1944).

(5) W. A. Parks, R. M. Esteve, Jr., M. H. Gollis, R. Guercia and A. Petrarca, *Abstracts Papers Am. Chem. Soc.*, **127**, 6E (1955).

(6) A. Pictet, *Helv. Chim. Acta*, **1**, 226 (1918).



identified as their crystalline acetates from the acid hydrolyzate of the polymer. This establishes the presence of α- and β-D-(1→6)-, α- and β-D-(1→4)-, β-D-(1→2)-linkages and 1,6-anhydro-β-D-glucose end groups in the polymer. The rather low proportion of (1→6)-linkages in the polymer is predictable since there are initially no primary hydroxyl groups in the reaction mixture. In the starch molecule, such an anhydro end group would probably react preferentially with a primary hydroxyl group giving rise to a preponderance of (1→6)-linkages. Thus, 1,6-anhydro-β-D-glucopyranose may undergo reaction with hydroxyl groups of other D-glucose molecules to form polymers having D-glucosidic linkages such as are found in the pyrodextrins. It would therefore appear probable that one path by which dextrins are formed by roa ting starch at a high temperature in the absence of acid, is the formation of anhydro end groups which in turn react with hydroxyl groups to form linkages different from those originally present.

## Experimental

**Thermal Polymerization of 1,6-Anhydro-β-D-glucopyranose.**—1,6-Anhydro-β-D-glucopyranose (100 g., m.p. 170°) was heated in an oil-bath in an open vessel at 235–240° for 15 min. The material, which foamed and became dark amber in color, was cooled, dissolved in a small volume of water and poured slowly with stirring into 2000 ml. of ethanol. The flocculent precipitate so formed was filtered, washed with ethanol and dried at 60° for several days. The material was then finely powdered and dried further at 60° under reduced pressure over phosphoric anhydride; yield 40 g.

**Acid Hydrolysis of Levoglucosan Polymer.**—A sample of the levoglucosan polymer (805 mg.) was dissolved in 250 ml. of 0.15 *N* sulfuric acid and heated in a boiling water-bath. Aliquots were removed at intervals and their copper reducing values determined (Fig. 1). During the hydrolysis a small amount of dark colored precipitate formed. An aliquot (80 ml.) was filtered and the residue dried; yield 8.5 mg. or 3.3% of the total weight of the initial polymer. The aliquots, after neutralization with barium carbonate, were filtered, concentrated and examined by paper chromatography using 1-butanol–pyridine–water (6:4:3 by vol.) or 1-butanol–ethanol–water (4:1:5 by vol., organic phase) and paper ionophoresis (0.2 *M* borate buffer, *p*H 10). The papers were sprayed with aniline hydrogen phthalate[7] or alkaline silver nitrate.[8] In the final hydrolyzate only D-glucose could be detected, but at intermediate times components behaving as 1,6-anhydro-β-D-glucose and two or more disaccharides were detectable.

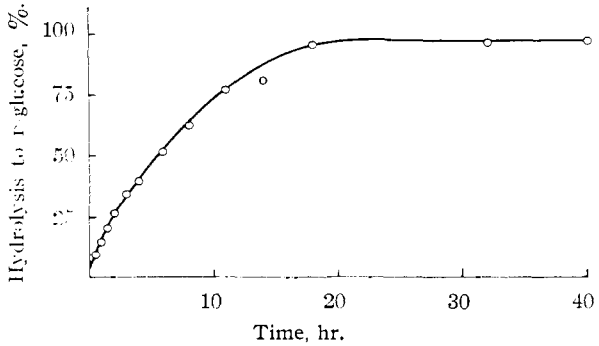



Fig. 1.—Acid hydrolysis of polymer formed by heating 1,6-anhydro-β D-glucopyranose (0.15 *N* $H_2SO_4$, 100°); ordinate determined by copper reducing value (Somogyi).

**Hydrolysis of 1,6-Anhydro-β-D-glucopyranose.**—A sample (0.251 g.) of the 1,6-anhydro-β-D-glucopyranose used for the preparation of the polymer was dissolved in 50 ml. of 0.15 *N* sulfuric acid and heated on a boiling water-bath for 16 hr. The reducing value indicated a 98% conversion to D-glucose.

**Hydrolysis and Fractionation of the Levoglucosan Polymer.**—The dried levoglucosan polymer (32.9 g.) was dissolved in 9 liters of 0.15 *N* sulfuric acid and heated with gentle stirring in a boiling water-bath. The hydrolysis was followed by copper reducing values and was stopped at 67% completion. The solution was neutralized with an excess of barium carbonate and concentrated under reduced pressure to 300 ml. This solution was placed on a column (800 × 80 mm., diam.) of Nuchar C Unground[9] which had been previously washed successively with dilute hydrochloric acid, water, dilute ammonium hydroxide, and then thoroughly with water until the effluent had a *p*H 7.5. The column was washed[10] with water (24 liters, fraction 1), 3% ethanol (12 liters, fraction 2), 5% ethanol (20 liters, fraction 3) and 10% ethanol (16 liters, fraction 4), the change in concentration being made when the elution of the fraction was complete as shown by its reaction to Benedict solution. Each fraction was evaporated under reduced pressure to a sirup and further dried by repeated solution in methanol and then evaporation to dryness. The yields were, respectively: 18.28, 0.56, 4.1 and 2.73 g.

Further analysis of fraction 1 by paper chromatography and electrophoresis indicated the presence of only D-glucose. The amorphous material (18.28 g.) was acetylated with 9 g. of anhydrous sodium acetate and 180 ml. of acetic anhydride by heating the mixture to the boiling point. After the vigorous reaction had subsided, the solution was again heated to boiling, cooled and poured into ice and water (2 liters) and stirred overnight. The suspension was then thrice extracted with chloroform. The combined extract was washed with water, sodium bicarbonate solution and water, dried with anhydrous sodium sulfate and evaporated to a sirup under reduced pressure, dissolved in ethanol and again evaporated to a sirup. The sirup was crystallized from ethanol; yield 20.7 g. Recrystallization from ethanol produced pure material, m.p. 130–131° unchanged on admixture with known β-D-glucopyranose pentaacetate, $[\alpha]^{20}D$ +3.9° (*c* 4, chloroform). After deacetylation only D-glucose could be detected by paper chromatography in the mother liquors.

Analysis of fraction 2 by paper chromatography indicated the presence of 1,6-anhydro-β-D-glucopyranose, a little D-glucose and a trace of disaccharides. The amorphous material (0.56 g.) was acetylated with sodium acetate (0.2 g.) and acetic anhydride (5 ml.) as described above; yield 390 mg. It was recrystallized from ethanol, m.p. 109–110° undepressed on admixture with 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-glucose, $[\alpha]^{20}D$ −62.5° (*c* 1, chloroform). The X-ray powder diffraction pattern was identical with that of 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-glucose.[11]

Analysis of fraction 3 by paper chromatography and paper electrophoresis indicated the presence of isomaltose or gentiobiose with a smaller amount of maltose or sophorose.

(7) S. M. Partridge, *Nature*, **164**, 443 (1949).

(8) W. E. Trevelyan, D. P. Procter and J. S. Harrison, *ibid.*, **166**, 444 (1950).

(9) A product of West Virginia Pulp and Paper Co., Chicago, Ill.

(10) R. L. Whistler and D. F. Durso, THIS JOURNAL, **72**, 677 (1950).

(11) A. Thompson, Kimiko Anno, M. L. Wolfrom and M. Inatome, *ibid.*, **76**, 1309 (1954).

The material (4.1 g.) was acetylated with 1.5 g. of sodium acetate and 33 ml. of acetic anhydride as described above to produce a sirup; yield 5.27 g. Crystallization from ethanol produced 290 mg. of crystalline material, m.p. 190–191° undepressed by authentic β-gentiobiose octaacetate. The X-ray powder diffraction pattern was identical with that of β-gentiobiose octaacetate. The mother liquors were evaporated to a sirup, dissolved in benzene and placed on a column (300 × 70 mm., diam.) of Magnesol–Celite[12] (5:1 by wt.). The column was developed with 3000 ml. of benzene–2-methyl-2-propanol (100:1 by vol.) and then extruded. The zones were located by streaking with indicator (1% potassium permanganate in 10% sodium hydroxide solution). Four zones 10–35 mm., 35–93 mm., 93–150 mm. and 150–175 mm. from the column top, were located and sectioned. Each zone was extracted with acetone, the solutions evaporated to sirups, and the material crystallized from ethanol.

The material from the top zone appeared to be a mixture of α-β-gentiobiose octaacetates and was not further investigated; yield 88 mg., m.p. 173°.

The material from the second zone from the column top exhibited an X-ray powder diffraction pattern identical to that of β-sophorose octaacetate; yield 123 mg., m.p. 186° undepressed on admixture with authentic β-sophorose octaacetate.

(12) W. H. McNeely, W. W. Binkley and M. L. Wolfrom, THIS JOURNAL, **67**, 527 (1945).

The material from the third zone from the column top appeared to be impure β-isomaltose octaacetate; yield 324 mg. It was rechromatographed on a Magnesol–Celite (5:1 by wt.) column (180 × 30 mm., diam.) and developed with 1100 ml. of benzene–2-methyl-2-propanol (100:1 by vol.). The material isolated from a zone 90–130 mm. from the column top crystallized and some crystals of β-sophorose octaacetate were separated mechanically; yield 10 mg.; m.p. 186°, the remaining material crystallized as β-isomaltose octaacetate; yield 75 mg., m.p. 146°. The melting point of each was undepressed upon admixture with authentic samples and exhibited X-ray powder diffraction patterns identical with those of known β-sophorose octaacetate and β-isomaltose octaacetate, respectively.

The material from the fourth zone from the column top produced crystals which exhibited an X-ray powder diffraction pattern identical with that of known β-maltose octaacetate; yield 92 mg., m.p. 157–158° undepressed by a known sample.

Paper chromatography of fraction 4 indicated the presence of a disaccharide and some trisaccharides. The amorphous material (2.73 g.) was acetylated with 1.4 g. of sodium acetate and 27 ml. of acetic anhydride as described above. The material which crystallized from ethanol exhibited an X-ray diffraction pattern identical with that of β-cellobiose octaacetate; yield 218 mg., m.p. 192° undepressed upon admixture with known β-cellobiose octaacetate.

COLUMBUS 10, OHIO

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[CONTRIBUTION FROM THE LABORATORY OF THE CHILDREN'S CANCER RESEARCH FOUNDATION]

# Infrared Spectra and the Structure of Polyriboadenylic Acid

BY R. S. MORGAN[1] AND E. R. BLOUT[2]

RECEIVED MARCH 18, 1959

Infrared spectra have been obtained from films of polyriboadenylic acid (poly A) prepared from acid and alkaline solutions. Such films rapidly exchange their OH and NH hydrogens upon exposure to $D_2O$. The infrared measurements have demonstrated the existence of two forms of poly A in the solid state. One of these forms, that obtained from acid solutions, may be oriented and then shows marked infrared dichroism. From the dichroic measurements certain conclusions may be made concerning the spatial arrangement of the purine, ribose and phosphate groups. These conclusions are compared with the proposed models for the acid form of poly A.

## Introduction

Polyriboadenylic acid (poly A) is the best known member of a class of enzymatically synthesized polymers, the polyribonucleotides, first prepared by Grunberg-Manago and Ochoa.[3] On the basis of fiber X-ray patterns, two models for the spatial structure of the polymer have been proposed.[4,5] Subsequent to the conception of these models, two new facts became known: first, that poly A possessed a titrable group with a $pK$ near 6 (identified as the N or $N_{10}$-amino group of the adenine moieties)[6] and second, that accompanying the protonation of this group, the polymer underwent a structural change in solution.[7] In the solid state, such a change was first observed by the present infrared study. X-Ray diffraction studies on solutions[8] and films[9] then established that the fiber pattern earlier known was due to the protonated polymer, which we will refer to here as "acid" poly A.

The present work was undertaken in the hope of adducing additional information concerning the spatial configurations of this polymer by means of infrared spectroscopy. This paper will present the spectra of oriented films of acid poly A obtained with polarized infrared radiation, the infrared spectra of the two forms (acid and alkaline) of the polymer in the solid state, and the spectra obtained from the corresponding deuterated polymers.

## Experimental

Films for infrared study were prepared by evaporating solutions of the polynucleotide of known $p$H onto AgCl disks. The films then were examined in a Perkin–Elmer model 21 double-beam spectrophotometer with a sodium chloride prism. The films were either exposed to room air or sealed (by means of a second AgCl window and a spacer) over a reservoir containing $D_2O$.[10] For dichroic measurements on acid poly A, orientation was achieved by wetting an acid film with 40% ethanol and stroking it until dry with a glass spatula. The resultant film was quite negatively birefringent, ($\Delta\eta$ −0.04). Although very weak negative birefringence could be produced in alkaline films by similar means, these films did not show definitive infrared dichroism. Oriented films were examined with a fixed AgCl polarizer in the sample beam only, the film being rotated so that the orientation axis was either parallel or perpendicular

(1) Huntington Laboratory, Massachusetts General Hospital, Boston 14, Mass.

(2) Chemical Research Laboratory, Polaroid Corporation, Cambridge 39, Mass.

(3) M. Grunberg-Manago and S. Ochoa, THIS JOURNAL, **77**, 3165 (1955).

(4) J. D. Watson, "The Chemical Basis of Heredity," Johns Hopkins Press, Baltimore, Md., 1957, p. 552.

(5) R. S. Morgan and R. S. Bear, *Science*, **127**, 80 (1958).

(6) R. F. Beers and R. F. Steiner, *Nature*, **179**, 1077 (1957).

(7) J. Fresco and P. Doty, THIS JOURNAL, **79**, 3928 (1957).

(8) J. Fresco, *J. Mol. Biol.* in press.

(9) R. S. Morgan and R. Byrne, submitted to *J. Mol. Biol.*

(10) H. Lenormant, A. Baudras and E. R. Blout, THIS JOURNAL, **80**, 6191 (1958).