solved in 50 ml. of 1% methanolic hydrogen chloride and allowed to stand at room temperature for one week. Periodically, aliquots were analyzed by chromatography and suitable indicators on solvent D. This technique showed the gradual disappearance of the aldotriouronic acid (R_x 0.35), the appearance and disappearance of the acidic methyl glycoside (R_x 0.46) and the complete formation of the non-reducing methyl ester, methyl glycoside (R_x 0.86).

The ester-glycoside (0.943 g.) was isolated by evaporation of excess solvent after the removal of chloride ion with silver carbonate. It was dissolved in a mixture of 10 ml. of anhydrous methanol and 20 ml. of methyl iodide and cooled to 0°. One gram each of silver oxide and Drierite were added, and the mixture, after remaining at 0° for 4 hr., was maintained at room temperature for an additional 18 hr. The mixture was then heated under reflux for 1 hr., and the product was isolated (0.941 g.). Another methylation, employing methanol as solvent, was accomplished under reflux for 8 hr. with fresh reagents. Five additional methylations with acetone as solvent had to be carried out before the product was soluble in methyl iodide only. Three additional Purdie methylations gave 1.1 g. of ether-soluble oil.

The methylated aldotriouronic acid, in 50 ml. of anhydrous ether, was added dropwise to 1.1 g. of lithium aluminum hydride in an equal volume of ether. The mixture was refluxed gently for 1 hr., allowed to stand overnight and refluxed for an additional 0.5 hr. The excess lithium aluminum hydride was then destroyed with a small amount of ethereal ethyl acetate.

The organic mixture was shaken with 500 ml. of water and the mixture was evaporated free of organic solvent. The aqueous suspension was filtered, the washings combined with the filtrate, and the clear, aqueous solution was neutralized by shaking with an excess of Amberlite IR 120(H) and Duolite A-4 ion-exchange resins. The cloudy sirup obtained after evaporation was triturated with ether to give, after evaporation, 0.773 g. of a clear, colorless sirup.

The partly methylated trisaccharide was methylated three times with Purdie reagents. The color developed in this reaction was removed from an aqueous solution with mixed acid–base resins. Evaporation gave a thick, colorless sirup weighing 0.63 g.

Anal. Calcd.: OCH_3, 48.9. Found: OCH_3, 46.6; $[\alpha]^{25}D$ +49° (*c* 0.64, water).

Hydrolysis of the Methylated Trisaccharide and Identification of Components.—The methylated trisaccharide (0.47 g.) was dissolved in 140 ml. of *N* sulfuric acid and heated under gentle reflux for 12 hr. The hydrolyzate was worked up using barium carbonate and mixed acid–base resins. Evaporation gave a light yellow sirup (0.437 g.) which, upon chromatography in solvents J, K and L, revealed three major spots corresponding in mobility and color reactions to authentic 2,3-di-O-methylxylose (R_g 0.48), 3,4-di-O-methylxylose (R_g 0.55) and 2,3,4,6-tetra-O-methylglucose (R_g 1.0) (solvent J). A very slight trace of a slow-moving spot (R_g 0.1) was also detected.

The sirupy mixture of methylated sugars were separated on solvent L. The chromatograms were run for 48 hr., and the tetramethylglucose which ran off the end of the paper was collected in shallow glass dishes in the bottom of the chromatographic jars. The tetramethylglucose was separated from a large amount of colored debris by chromatography for 6 hr. on solvent M. The sections of the paper corresponding to the sugars were cut out, extracted with water, filtered, deionized with mixed acid–base resin and triturated with chloroform. Evaporation gave 70 mg. of 2,3-di-O-methylxylose, 72 mg. of 3,4-di-O-methylxylose and 110 mg. of 2,3,4,6-tetra-O-methylglucose which crystallized spontaneously.

The crystalline tetramethylglucose was recrystallized from ether and petroleum ether. The first 20 mg. was collected for analysis and melted at 89–90° and showed $[\alpha]^{25}D$ +83° (*c* 1, water) constant value.

Twenty milligrams of 2,3-di-O-methylxylose was employed for the preparation of the characteristic anilide derivative.[34] The m.p. and mixed m.p. was 125–126°. Two crystalline structures exist for the anilide of 2,3-di-O-methylxylose melting at 125 and 145°, respectively.[35]

The lactone derivative of 3,4-di-O-methylxylose was prepared using 28 mg. of sirup by standard techniques.[36] The 3,4-di-O-methyl-D-xylono-δ-lactone melted at 65–66° and showed $[\alpha]^{25}D$ −19.4° (*c* 1.2, water).

Acknowledgment.—The authors wish to thank Dr. F. Smith, University of Minnesota, for authentic specimens of 2,3- and 3,4-di-O-methylxylose and Dr. R. L. Whistler of Purdue University for samples of authentic specimens of xylobiose, xylotriose and xylotetraose. The assistance of Dr. H. W. Kircher and Mr. R. G. Rogerson during the isolation of the hemicelluloses and also in carrying out electrophoresis experiments is gratefully acknowledged.

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[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

Reduction of the Products of Periodate Oxidation of Carbohydrates. V. The Constitution of Cellulose[1]

BY IRWIN J. GOLDSTEIN, J. K. HAMILTON, R. MONTGOMERY AND F. SMITH

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Cellulose has been subjected to prolonged oxidation with sodium periodate and with periodic acid. The polyaldehyde so formed was reduced with sodium borohydride to the corresponding polyalcohol. Hydrolysis of the latter gave erythritol, glycolic aldehyde and also small amounts of glycerol and glucose (0.1–0.2%). After retreatment of the cellulose polyalcohol with periodate the product still contained glucose residues. The structural significance of these findings is discussed.

In previous communications[2–4] a new method has been reported for the determination of the fine structure of polysaccharides. This general method based upon periodate oxidation, followed by reduction and hydrolysis, has been extended in order to gain further insight into the detailed structure of cellulose, the most abundant of all naturally occurring organic substances.

It is generally accepted that cellulose is a long chain polysaccharide in which the anhydroglucose residues are joined predominantly by 1,4-β-glycosidic bonds.[5,6] Most investigators assume

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that the cellulose found in nature, whether from wood, cotton, bacteria or animals, is entirely unbranched and differs only in chain length and other minor physical properties. However, the manner in which cellulose is isolated determines, to a large extent, certain of its physical properties. If cellulose, especially wood cellulose, is considered as a system rather than a distinct entity, then the hemicelluloses, which are intimately associated with the cellulose, must be included. It is also possible that native cellulose may contain, in addition to a small percentage of linkages other than the β-1,4 type, a very small number of anhydro units other than D-glucose (*e.g.*, D-xylose, D-mannose).

The general methods of end-group assay that have been applied to other polysaccharides such as starch and glycogen are not particularly applicable to the cellulose molecule. Complete methylation of all the available hydroxyl groups followed by hydrolysis and determination of the ratio of 2,3,4,6-tetra-*O*-methyl-D-glucose to 2,3,6-tri-*O*-methyl-D-glucose has given variable results as to the chain length, although all experiments show that the vast majority of glucose residues are joined through positions 1 and 4. The conditions of methylation determine the amount of 2,3,4,6-tetra-*O*-methyl-D-glucose; relatively large quantities are isolated in the presence of oxygen indicating short chains, whereas very little, if any, is obtained in a nitrogen atmosphere indicating extremely long chains.[5,7-9]

The method of periodate oxidation, in which the terminal glucose residues having adjacent α-, β-, γ-triol groups[10,11] give rise to titratable formic acid, is also said to be of questionable value when applied to cellulose.[12] Thus any over consumption of periodate that results in the production of formic acid alters the value for the apparent chain length.

The consumption of one mole of periodate per anhydroglucose residue[13] agrees with the results of methylation with respect to the mode of union of the anhydroglucose residues, and these results, together with the specific rotation of cellulose and its derivatives before and after hydrolysis, indicate that the polysaccharide is joined predominantly by 1,4-β-glucosidic bonds. It appears to be a linear polymer although the existence of branches in the cellulose molecule recently has been reported.[14]

Another widely used method for the calculation of the chain length of the cellulose molecule is by the determination of the intrinsic viscosity in cupriethylenediamine hydroxide or cuprammonium hydroxide and subsequent substitution of these values in the Staudinger equation.[15] Chemical methods have been devised recently for determining the reducing end in polysaccharides including cellulose by making use of the cyanohydrin reaction[16-18] and by transforming the reducing end to a glucitol residue.[19]

The prolonged periodate oxidation of cellulose, followed by the reduction and hydrolysis of the derived polyalcohol,[2] appeared to offer an alternative method for the study of the fine structure of this polysaccharide. Based upon graded acid hydrolysis, acetolysis, methylation or periodate oxidation (*i.e.*, periodate consumption and formic acid estimation), the proportion of linkages in the cellulose molecule other than 1,4 is very small and would not be detectable from the consumption of periodate. If, however, glucose units are joined through position 3 and 4 or 2 and 4 or any other combination of linkages such that no α,β-glycol groups are present, they would be unattacked by periodate and could be detected and estimated[2] by sensitive spectrophotometric methods.[20]

This paper is concerned with the determination and characterization of glucose after prolonged periodate oxidation of cellulose, followed by reduction and hydrolysis. The specimens of cellulose examined were filter paper (two sources), native Empire cotton and wood cellulose. "Over-oxidation" was considered advantageous and certain of the oxidations were carried out at a rather high concentration of sodium periodate or periodic acid for relatively long periods of time. Every effort was made to eliminate the possibility of incomplete oxidation of all adjacent hydroxyl groups. Such a situation may arise from intermolecular interactions which result in fixed "*trans*"-hydroxyl groups and subsequent resistance to periodate oxidation[21] or the possibility that the ring conformation of certain of the anhydroglucose residues is such that attack by periodate is hindered or prevented. However, the fact that glucose still survives when the derived polyalcohol itself is treated with periodate points to the presence of linkages other than 1,4 in cellulose. In nearly all cases the amount of glucose detected following oxidation, reduction and hydrolysis was in the range of 0.1 to 0.2% based upon the weight of cellulose. By following the above procedure, periodate stable glucose units have been detected in wood cellulose derived from Western Hemlock. This would indicate that the presence of anomalous glucose residues is not restricted to cellulose from cotton but that it is also present in the wood cellulose system. It is known from periodate studies that certain glucomannans associated with wood cellulose from Western Hemlock contain a very small number of linkages other than the 1,4-type,[22] hence the actual number of unattacked glucose residues arising from the so-called true cellulose (polyglucosan) will require further

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TABLE I

Expt.	Type of cellulose	Time, days	Temp., °C.	Cellulose, g.	Vol. reaction mixture, ml.	Periodate concn., *M*	Periodate consumption (moles per anhydroglucose unit)	Glucose after oxidation, %
I[a]	Whatman filter paper	64	2–4	5	245	0.2	1.0	0.2
II	Empire cotton	41	2–4 and 11 for last 20 days	17	500	.4	1.3	.1
III[b]	Empire cotton	85	2–4	42	1500	.4	1.4	.2
IV	Empire cotton	56	2–4	65	2000	.4	1.2	.2
V	Wood cellulose	22	22–24	17	600	.4	1.6	.1
VI	S and S filter paper	86	2–4	5.7	500	.1	1.0	..
VII[c]	S and S filter powder, polyalcohol from expt. VI	15	2–4	4.6	200	.1	2.5	.1

[a] The polyalcohol obtained in this experiment was reoxidized with sodium periodate (0.1 *M*) for 30 days at 2–4°; reduction with sodium borohydride followed by hydrolysis gave a hydrolyzate in which glucose was detected by paper chromatography. [b] Periodic acid was used and after 24 days the concentration was increased to 0.5 *M*. [c] The acid produced corresponded to approximately 1.7 moles of acid per anhydroglucose unit.

study. The amount of glucomannan associated with this wood cellulose was 1.3%, and as only a trace amount of the glucose from this polysaccharide is left unattacked by periodate, the major portion of the glucose that is stable to periodate arises from the cellulose. The possibility that an anomalously linked polyglucosan is intimately associated with the cotton cellulose used in this study also exists, although more recent experiments indicate that this possibility is unlikely.[23]

In the case of the Empire cotton, the amount of D-glucose found after carrying out the above scheme of periodate oxidation, reduction and hydrolysis corresponded to one glucose unit per 500 to 1000 glucose residues. The results also showed that the major products of hydrolysis of the cellulose polyalcohol were erythritol[24] and glycolic aldehyde which clearly arise from those glucose residues joined by 1,4-glucosidic bonds. In a separate experiment the glucose (0.13%) remaining after periodate oxidation was isolated in the crystalline form and characterized as the *p*-nitroaniline derivative. The erythritol was readily crystallized while the glycolic aldehyde was identified as the di-*p*-nitrophenylhydrazone of glyoxal.

The manner in which the periodate-immune glucose residues of cellulose from Empire cotton are joined to other units is now being investigated by methylation studies of the polyalcohol obtained from the cellulose by periodate oxidation followed by reduction.

Experimental

The celluloses employed in these experiments were from filter paper pulp (Whatman No. 1) and filter paper powder (S and S 289), raw Empire cotton and wood cellulose (sulfite) from Western Hemlock.

The Empire cotton was hand picked and not chemically treated in any way. After defatting by extraction with chloroform and with toluene, it had an average chain length greater than 3200 as determined by viscometric studies. Upon hydrolysis with cold 72% sulfuric acid in the usual way, it gave glucose and extremely small amounts of xylose (0.2%), arabinose (0.2%) and perhaps a trace of mannose as determined by chromatographic analysis. No other sugars or uronic acids were noted.

Periodate Oxidation of Cellulose.—Cellulose, dried to constant weight *in vacuo*, or of known moisture content, was dispersed in sodium periodate or periodic acid and the resulting suspension diluted to volume to give a final periodate ion or periodic acid concentration of 0.2, 0.4 or 0.5 *M*, respectively. While the reactions were carried out in most cases at 2–4°, in certain experiments temperatures of 11–12° and 21–22° were employed for part of the oxidation. At frequent intervals the reaction mixtures were thoroughly shaken and the periodate consumption determined in the usual manner[8,9] on an aliquot (5 ml.) removed from the reaction mixture and diluted to 25 ml.; 10 ml. of this 25 ml. was treated with excess 0.2 *N* sodium arsenite (10 ml.), sodium bicarbonate (1 g.) and water (25 ml.) in the presence of a few crystals of potassium iodide. The mixture was shaken and allowed to stand for 15 minutes and the excess arsenite back titrated with 0.01 *N* iodine using amylose as the indicator. A blank determination was carried out simultaneously.

As the oxidation proceeded the cellulose shrunk considerably. This was particularly noticeable in the case of the Empire cotton which filled the reaction liquid at the outset but finally formed only a layer on the bottom of the container. The material had no tensile strength.

The results of seven periodate oxidations are presented in Table I.

Isolation of the Cellulose Polyaldehyde.—The insoluble cellulose polyaldehyde (expt. II) was removed from the reaction mixture, washed thoroughly with distilled water and dialyzed against distilled water for several days. In certain instances the polyaldehydes were electrodialyzed to remove inorganic salts. The purified polyaldehyde was dried by solvent exchange using absolute ethanol, acetone, ethyl ether and by desiccation *in vacuo* over calcium chloride (yield 15 g.). The yield in nearly every case was greater than 90% and in certain cases was greater than 95%. The polyaldehyde was a white amorphous powder; it was insoluble in water but soluble in 10% aqueous potassium acetate and in an aqueous solution in which sodium borohydride had been allowed to decompose. Infrared analysis showed that the cellulose polyaldehyde did not contain any carbonyl groups.[25]

After purification by electrodialysis the polyaldehyde did not contain any acidic groups as indicated by titration with 0.05 *N* sodium hydroxide. On standing the polyaldehyde reacted slowly with the dilute alkali.

Anal. Calcd. for $C_6H_8O_5 \cdot {}^1/_2H_2O$: C, 42.6; H, 5.3. Calcd. for $C_6H_8O_5 \cdot H_2O$: C, 40.4; H, 5.6. Found (for sample (experiment VI) dried for 2 days *in vacuo* over solid sodium hydroxide and for 2 hr. *in vacuo* at 55°): C, 39.8; H, 5.7. Found (for material dried *in vacuo* over anhydrous calcium chloride): C, 43.0; H, 5.9.

Reduction of the Cellulose Polyaldehyde.—(a) The polyaldehyde (14 g. from expt. II) was suspended in water (200 ml.) and sodium borohydride (1.5 g. in 20 ml. of water) was added with stirring. Dissolution of the material occurred almost immediately. The alkaline solution was left for 4 hr. and an additional amount of sodium borohydride (1.5 g. in 20 ml. of water) was added and the reaction solution allowed to stand overnight. An additional 1.0 g. of

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sodium borohydride was added. After 1 hr. an acidified aliquot was found to be non-reducing to Fehling solution. The alkaline solution was neutralized with glacial acetic acid and concentrated under diminished pressure at 40–50° to a volume of 50 ml.

(b) An attempt was made to reduce a suspension of the cellulose polyaldehyde from experiment VI with hydrogen under pressure (900 lb./sq. in.) at 80–90° using a Raney nickel catalyst. After 6.5 hr. the product was recovered unchanged. In another experiment a sample (0.55 g.) of polyaldehyde from experiment I was suspended in water (75 ml.) and successfully reduced in 15 hr. at 60° and 750 lb./sq. in. pressure of hydrogen; the clear solution did not reduce Fehling solution and gave erythritol after hydrolysis with dilute sulfuric acid but not before. The success of the second catalytic hydrogenation may have been due to alkali left in the particular sample of Raney nickel catalyst.

Isolation of the Cellulose Polyalcohol.—A 5-ml. aliquot of the above solution from experiment II (a) was dialyzed against approximately 1 liter of distilled water. A Molisch test of the water outside the dialysis casing gave a bright green color, identical with that from the aqueous solution within the membrane, thus indicating that organic material was dialyzing out. The polyalcohol in the dialysis casing appeared to be slightly soluble, and upon concentration *in vacuo* to a small volume and centrifugation a white precipitate was isolated. It was found to be soluble in hot water or hot aqueous ethanol and upon cooling these solutions, long white threads (similar to retrograding amylose) appeared. These were removed by centrifugation and dried by solvent exchange using anhydrous methanol and ethyl ether; the cellulose polyalcohol showed $[\alpha]^{23}D$ −26° in water (*c* 1.0).

Anal. Calcd. for $C_6H_{12}O_6$: C, 43.9; H, 7.3. Found: C, 42.7; H, 7.3.

Hydrolysis of the Cellulose Polyalcohol.—To a 40-ml. aliquot (from Experiment III, one-half of the total volume from the reduction of 10 g. of cellulose polyaldehyde), 12 *N* hydrochloric acid (4 ml.) was added and the solution refluxed for 10 hr. The cooled hydrolyzate was neutralized and deionized by passage through a cation exchange column (Amberlite IR-120) and an anion exchange column (Duolite A4), respectively. The resulting neutral solution was concentrated under diminished pressure to a sirup. When large volumes of methanol were added and were removed under diminished pressure in order to remove the last traces of borate salts, the sirup was dissolved in a small volume of methanol and allowed to evaporate slowly, crystalline erythritol was obtained (0.93 g.), m.p. and mixed m.p. 120–122°.

Qualitative Chromatographic Analysis.—Paper partition chromatographic analysis of the hydrolyzate of the above polyalcohol (after removing 0.93 g. of crystalline erythritol) on Whatman No. 1 filter paper using 1-butanol:ethanol:water (4:1:5) as the irrigating solvent, showed the presence of erythritol and small amounts of glycerol and glucose. The origin of the glycerol is not yet known.

Quantitative Chromatographic Analysis.—The amount of glucose present in the hydrolyzates of the polyalcohols from the various experiments was determined spectrophotometrically at 490 mμ by the phenol–sulfuric acid method of Dubois, *et al.*,[20] after separation by paper chromatography using 1-butanol:ethanol:water (4:1:5) for 36 to 60 hr. (see Table I).

Reoxidation of Cellulose Polyalcohol.—A sample of the cellulose polyaldehyde obtained from experiment VI was reduced with sodium borohydride in the normal manner described above. The polyalcohol was treated with sodium periodate (0.12 *M*) at 2–4°. There was a continuous production of formic acid and consumption of periodate. The solution was periodically neutralized with dilute sodium hydroxide and the reaction finally stopped, by the addition of ethylene glycol, when the consumption of periodate was approximately 2.5 moles per mole of anhydroglucose of the original cellulose. The production of formic acid at this stage was about 1.7 moles of acid per mole anhydroglucose as indicated by the amount of *N* sodium hydroxide required to maintain neutrality of the reaction mixture. The resulting solution (after removal of excess periodate with barium chloride) was again reduced with sodium borohydride and the solution hydrolyzed with *N* sulfuric acid, neutralized and deionized by successive passage through cation (Amberlite IR-120) and anion (Duolite A4) exchange columns, respectively. The neutral solution was concentrated under diminished pressure to a sirup. The glucose, separated from other hydrolytic products by paper partition chromatography using 1-butanol:ethanol:water (4:1:5) as the irrigating solvent, corresponded in R_f to a glucose standard. Quantitative paper partition chromatography showed that 0.1% of the original cellulose was present as glucose.

Isolation of Glucose from Cellulose Polyaldehyde. Reduction of Cellulose Polyaldehyde with Sodium Borohydride.—To a suspension of cellulose polyaldehyde (72.5 g., prepared from Empire cotton as described above) in cold water (3 l.) was added an ice-cold solution (300 ml.) of sodium borohydride (28.2 g.). The reaction mixture which foamed considerably was stirred and allowed to stand overnight (12 hr.) in the cold room. At this point there was obtained a turbid solution with only small amounts of undissolved material. To ensure complete reduction, an additional portion of sodium borohydride (10 g. in 100 ml. of water) was then added and the mixture stirred for 2 hr.

The reaction mixture was neutralized with dilute hydrochloric acid and evaporated to dryness *in vacuo* at 40°. To the dried residue obtained above was added 2% methanolic hydrogen chloride (500 ml.). The reaction mixture was refluxed for 10 hr. and concentrated *in vacuo*, adding methanol to maintain a volume of about 250 ml. This operation served to remove most of the hydrogen chloride and the glycolic aldehyde dimethylacetal. The glycolic aldehyde in a portion of the distillate was characterized directly in the usual way as the di-*p*-nitrophenylhydrazone of glyoxal, m.p. and mixed m.p. 307°; lit.[26] m.p. 306–307°.

After filtering the sodium chloride from the methanolysis reaction mixture, the filtrate was concentrated *in vacuo* to dryness and dissolved in hot 50% ethanol. On standing, crystalline erythritol (15.5 g.) was deposited, m.p. and mixed m.p. 120°. From the mother liquor additional erythritol (13 g.) was obtained.

Acid Hydrolysis of the Methanolized Cellulose Polyalcohol.—The concentrated sirupy residue (approx. 25 g.) was dissolved in *N* sulfuric acid (150 ml.) and the solution refluxed for 10 hr. The solution was neutralized ($BaCO_3$), filtered and concentrated, whereupon additional erythritol (8 g.) crystallized. To ensure removal of all inorganic salts the residue was dissolved in water and deionized by passage first through a cation (Amberlite IR 120)[27] and then through an anion (Duolite A4)[28] exchange resin.

Paper chromatographic analysis of the solution showed the presence of only erythritol and glucose. Removal of most of the water *in vacuo* yielded a sirup from which more erythritol (2 g.) was deposited on standing at 5°. The mother liquors were concentrated *in vacuo* to remove water, ethanol being added from time to time to facilitate the process.

Treatment of the Residue with Benzaldehyde.—To a portion (5 g.) of the dry sirupy residue (15 g.) was added benzaldehyde (10 g.) and 50% sulfuric acid (5 ml.). The reaction mixture was shaken for several hours and then kept overnight. The dibenzylidene derivative of erythritol (2 g.) was filtered and recrystallized from ethanol, m.p. and mixed m.p. 199°; lit.[29] m.p. 201°. After adding water (20 ml.) to the filtrate, it was extracted three times with chloroform to remove the excess of the benzaldehyde and any residual di-*O*-benzylidene-erythritol.

Chromatographic Separation of D-Glucose and Erythritol.—The above residue containing glucose and erythritol was dissolved in 1-butanol:pyridine:water (6:4:3) and applied to the top of a cellulose column. The automatic fraction collector was adjusted to collect fractions (15 ml.) at 30-minute intervals. Samples from tubes 1–27, when spotted on paper and sprayed with Tollens ammoniacal silver nitrate reagent, stained deeply, and upon concentrating the combined fractions erythritol (1.45 g.) was obtained.

Tubes 29–35 contained the glucose component as revealed by Tollens spray. Concentration gave D-glucose (31 mg. corresponding to 0.13% of the weight of the original polyaldehyde) which was chromatographically identical with an authentic specimen on two solvent systems (pyridine:ethyl acetate:water, 1:2.5:3.5; and 1-butanol:acetic acid:water, 2:1:1). The addition of a few drops of 50% ethanol gave crystalline D-glucose, m.p. and mixed m.p. 149°, $[\alpha]^{25}D$ +51° equilibrium value in water (*c* 1). It was identified as

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(27) A product of the Rohm and Haas Co., Philadelphia, Pa.

(28) A product of the Chemical Process Co., Redwood City, Calif.

(29) E. Fischer, *Ber.*, **27**, 1535 (1894).

the crystalline N-*p*-nitrophenyl-D-glucosylamine, m.p. and mixed m.p. 185°, and $[\alpha]^{25}D$ −188° in pyridine (*c* 1); lit.[30] m.p. 184°, $[\alpha]D$ −192° (pyridine).

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ST. PAUL, MINNESOTA

[CONTRIBUTION FROM THE FISHERIES RESEARCH BOARD OF CANADA, CHEMISTRY SECTION OF THE TECHNOLOGICAL STATION]

Marine Sterols. IV. 24-Dehydrocholesterol: Isolation from a Barnacle and Synthesis by the Wittig Reaction

BY U. H. M. FAGERLUND AND D. R. IDLER

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A new sterol, 24-dehydrocholesterol, has been isolated from the barnacle *Balanus glandula*. It represents 34.2% of the total sterols of the barnacle. The major component (59.8%) is cholesterol. The new sterol also has been synthesized *via* the Wittig reaction and the synthetic product shown to be identical with the natural one. A previously reported sterol obtained by the dehydrohalogenation of 25-bromocholesterol and assigned the structure 25-dehydrocholesterol has been shown to be a mixture of 40% 25-dehydrocholesterol and 60% 24-dehydrocholesterol.

A previous investigation of the sterols of molluscs resulted in the identification of 24-methylenecholesterol as a major sterol component of several species of molluscs.[1,2] Its subsequent synthesis *via* the Wittig reaction has been reported.[3,4] Our study of the sterols of marine invertebrates has now been extended to a crustacean, the barnacle *Balanus glandula*. To the best of our knowledge cholesterol is the only sterol which has been isolated and characterized from crustaceans.[5-10] The infrared spectra of crude barnacle sterols demonstrated the absence of any significant quantity of 24-methylenecholesterol. Chromatography of the azoyl esters[11] of the crude sterol mixture resulted in three zones. The sterol of the fastest moving zone was identified as cholesterol and represents 59.8% of the total sterols. The middle zone which represents 6% of the total sterol will be the subject of a future report. The slowest moving zone (34.2%) when hydrolyzed was found to be contaminated with a small amount of $\Delta^{5,7}$-sterol which was removed with maleic anhydride. The purified sterol and its derivative had properties different from those of any known sterol. Hydrogenation of the sterol with Adams catalyst in glacial acetic acid produced cholestanol with the uptake of 2 moles of hydrogen and demonstrated that the original sterol was a readily reducible cholestadienol. The modified Liebermann–Burchard reaction[12] showed that the cholestadienol had a double bond in the 5-position and that the second double bond made no significant contribution to the reaction, thus eliminating most nuclear double bonds from consideration. Ozonolysis resulted in the isolation of acetone as its 2,4-dinitrophenylhydrazone in good (51%) yield and completed the identification of the sterol as 24-dehydrocholesterol. The synthesis of this sterol *via* the Wittig reaction from 3β-acetoxy-5-cholenaldehyde and triphenylphosphine-isopropylidene was carried out in a manner analogous to that previously reported for the synthesis of 24-methylenecholesterol and 25-dehydrocholesterol. The properties of the synthetic sterol and its derivatives were identical with those of the sterol from barnacle.

With respect to the comparative biochemistry of marine invertebrates the question of the significance of 24-dehydrocholesterol in barnacles must await a comprehensive study of the sterols of other crustaceans. However, by analogy with Mollusca the results of this report already appear of interest. In Mollusca, as we ascend the evolutionary scale, we find a high percentage of Δ^7-sterols in chiton,[13,14] large quantities of 24-methylenecholesterol in Pelecypoda[1,2] and almost entirely cholesterol in Gastropoda and Cephalopoda.[15] Descending the evolutionary scale in Crustacea we find that cholesterol is the principal if not the only sterol (other than small amounts of provitamin D) in Malacostraca (crabs, shrimps, etc.);[13] Cirripedia (barnacle) has been shown here to contain cholesterol as a major component but also 24-dehydrocholesterol in large amounts. It remains to see whether cholesterol either continues to decrease or is absent in Ostracoda, Copepoda and Branchiopoda. The day may not be too far distant when the sterols of a marine invertebrate will be predictable from their position in the evolutionary scale. From the bio-

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