yield 2%. The product from hydrazinolysis of the α-L anomer failed to crystallize directly, but after acidic hydrolysis crystalline 2-amino-2-deoxy-L-lyxose hydrochloride was obtained; yield 30%, m.p. 155–164° dec.

Acknowledgment. The technical assistance of Miss Ramona Budd is gratefully acknowledged.

COLUMBUS 10, OHIO

[CONTRIBUTION FROM RAYONIER INC., OLYMPIC RESEARCH DIVISION]

The Ultraviolet Irradiation of Model Compounds Related to Cellulose[1]

ANDREW BEĆLIK AND J. KELVIN HAMILTON

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Cellobiose and cellopentaose, models for unmodified cellulose chains with terminal carbonyl groups, methyl β-cellobioside, model for an unmodified cellulose chain with a protected terminal carbonyl group, and cellobiitol and cellopentaitol, models for cellulose chains devoid of carbonyl groups, were irradiated with light of 2200–4000 Å, in the presence of air.

All five model compounds were fragmented, yielding comparable amounts of acidic compounds, of substances absorbing near 2600 Å, of monosaccharides, and of most of the predicted oligosaccharides of a lower degree of polymerization. These results were interpreted to show that ultraviolet light will, in the presence of air, initiate the fragmentation of oligosaccharide molecules and presumably also that of cellulose, whether carbonyl groups are present in the molecules or not.

The ultraviolet irradiation of cellulose is known to cause yellowing, reduction of the degree of polymerization, formation of carbonyl and carboxyl groups, and fragmentation of the molecules to a diversity of neutral and acidic non-volatile, volatile and gaseous products.[2–11] The following fragments have been identified: hydrogen,[9] carbon monoxide,[2,9] carbon dioxide,[2,3,9] D-glucose, and D-arabinose.[11] Also identified were di- and trisaccharides composed of D-glucose units and the corresponding compounds in which the terminal reducing glucose unit was replaced by D-arabinose.[11] Also observed but not identified were water-soluble acidic substances[11] and a water-soluble compound showing considerable ultraviolet absorption with a weak maximum near 2600 Å.[8,11]

Far ultraviolet radiation was shown to be considerably more effective in bringing about this degradation than light of the near ultraviolet.[5–7,11] Quantum yields of approximately 10^{-3} were calculated from the amount of carbon dioxide formed during degradation caused by radiation of 2537 Å.[5,9] Light of this wave length is commonly assumed to cause photolysis; the lesser degradation caused by light of the near ultraviolet is frequently referred to as photooxidation.[4,5,12]

The requirement for photolytic cleavage of a bond in a molecule is the absorption of light energy equal to, or greater than, the bond energy. For C—O and C—C bonds this would have to be light of a wave length less than 3400 Å. No absorption of light by the molecule is required for photooxidative degradation; the energy transfer occurs by collision of the molecule with some excited species created by action of light of the near ultraviolet upon such compounds of the environment as metallic impurities, peroxides, and dyes. Both kinds of degradation proceed, no doubt, by free radical mechanisms.

One of the unclarified aspects of the photodegradation of cellulose is the initiation of photolysis. A basic requirement would seem to be a chromophore absorbing below 3400 Å. Of these, only hydroxyl, carboxyl, and carbonyl groups occur commonly in cotton cellulose or wood celluloses. Hydroxyl and carboxyl groups would have to be ruled out, because they absorb below, or near 2000 Å—*i.e.* below the wave lengths which have been most frequently employed for the ultraviolet irradiation of cellulose. Potential carbonyl groups are present at C_1 in each terminal reducing D-glucose unit; carbonyl groups or potential carbonyl groups also occur as a result of oxidative damage at C_2, C_3, or C_6 of D-glucose units.[13] As carbonyl groups absorb near 2800 Å, they should theoretically be capable of initiating photolysis of the cellulose molecules, and are thus frequently held re-

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(1) (a) Contribution No. 56. (b) Presented at the 138th National Meeting of the American Chemical Society, New York, N. Y., September 1960.

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sponsible for the photodegradation of wood cellulose, presumably by analogy with the well established role of carbonyl groups in the heat degradation of wood cellulose.[14-16]

A modest amount of evidence points to the possibility of the acetal groups at C_1 of nonreducing D-glucose units acting as weak chromophores by absorbing near 2650 Å, and thus initiating photolysis of cellulose.[11,12,17]

One way to clarify the role of the carbonyl group in the photolysis of cellulose would be to compare the effect of ultraviolet light on samples of cellulose containing one or another kind of carbonyl group, or no carbonyl groups at all. As carbonyl derivatives of cellulose of known structure are relatively inaccessible, the more convenient approach of using model compounds was adopted. The required oligoglucoses of known structure could readily be isolated, and their structures modified by appropriate syntheses. The water solubility of such model compounds would make all degradation products readily accessible to analysis by chromatographic techniques. Published information on the ultraviolet irradiation of oligosaccharides is scarce[2,18,19]; carbon dioxide and carbon monoxide were the only identified degradation products.[2]

This paper deals with the effect of ultraviolet light on the following compounds: cellobiose and cellopentaose (models for cellulose molecules terminated by a reducing potential carbonyl group), methyl β-cellobioside (model for cellulose molecules having their reducing groups protected), and cellobiitol and cellopentaitol (models for cellulose molecules containing no carbonyl groups).

The products discovered in ultraviolet-irradiated wood cellulose indicated two modes of primary photolytic fission in the cellulose chains: that between C_1 of the D-glucose units and the attached glycosidic O atom, and that between C_1 and C_2 of the D-glucose units.[11] Both modes of fission occurred at the hypothetical "acetal chromophores." If the same "chromophores" initiate the same two modes of photolytic fission in the cellulose model compounds listed above, the following primary degradation products should be produced: D-glucose, D-arabinose, and 3-*O*-β-D-glucopyranosyl-D-arabinose from cellobiose; the same three sugars and also cellobiose, -triose, and -tetraose, as well as *O* - β - D - glucopyranosyl - (1→4) - *O* - β - D - glucopyranosyl-(1→3)-D-arabinose (or more simply: 3 - β - cellobiosyl - D - arabinose), 3 - β - cellotriosyl- and 3 - β - cellotetraosyl - D - arabinose from cellopentaose; D-glucose, D-arabinose, methyl β-D-glucopyranoside, cellobiose, and 3-β-glucopyranosyl-D-arabinose from methyl β-cellobioside; D-glucose, D-arabinose and D-glucitol from cellobiitol; and, finally, D-glucose, cellobiose, -triose, -tetraose, D-arabinose, 3-β-D-glucopyranosyl-, 3-β-cellobiosyl-, and 3-β-cellotriosyl-D-arabinose, as well as D-glucitol, cellobi-, cellotri- and cellotetraitol from cellopentaitol. The symbols of all these predicted primary degradation products are listed in Table I.

TABLE I

MONO- AND OLIGOSACCHARIDES PREDICTED AS PRIMARY PRODUCTS OF THE PHOTOLYSIS OF CELLULOSE MODEL COMPOUNDS

Ultraviolet-Irradiated Model Compounds	Primary Degradation Products[a]
Cellobiose	G; A, GA
Cellopentaose	G, G_2, G_3, G_4; A, GA, G_2A, G_3A, G_4A
Methyl β-cellobioside	G, G_2; A, GA; G—CH_3
Cellobiitol	G; A; S
Cellopentaitol	G, G_2, G_3, G_4; A, GA, G_2A, G_3A; S, GS, G_2S, G_3S

[a] Symbols used are: G = D-glucose, A = D-arabinose, G—CH_3 = methyl β-D-glucopyranoside, S = D-glucitol.

If, however, stronger chromophores—*e.g.*, carbonyl groups—or perhaps potential carbonyl groups are required for absorption of ultraviolet light and initiation of photolysis, then only the two reducing model compounds were expected to yield the fragments predicted in the above scheme. The main purpose of this investigation became, consequently, the verification of the occurrence of the predicted degradation products in the ultraviolet irradiated cellulose model compounds.

The results of ultraviolet irradiation were strikingly similar for all three groups of model compounds. The surface of all five compounds turned pale yellow, but the color appeared to reside in a thin layer which dissolved quickly upon contact with water, exposing layers of white and visually unchanged material. All but a few milligrams of each irradiated sample dissolved readily in water. The freshly prepared pale yellow solutions emitted a fairly strong, undefinable odor, which was indistinguishable from the odor emanating from wetted sheets of ultraviolet-irradiated wood cellulose.[11]

Solutions of the oligosaccharide alditols had a *p*H near 7.0, but the value for the other solutions was near 3.6. The titration values for the various samples (Table II) were quite comparable.

All solutions showed marked ultraviolet absorption, increasing toward shorter wave lengths. Each

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TABLE II

CHANGES IN SAMPLES OF CELLULOSE MODEL COMPOUNDS AFTER 80 HR. OF ULTRAVIOLET IRRADIATION

Observations after Irradiation[a]	Ultraviolet-Irradiated Model Compounds				
	Cellobiose	Cellopentaose	Methyl β-Cellobioside	Cellobiitol	Cellopentaitol
Weight loss, mg.	77	72	50	51	37
Water-insolubles, mg.	6	4	3.7	3.5	4.3
*p*H	3.5	3.6	3.6	7.4	7.0
Free acids, meq. × 100[b]	5.2	2.7	6.0	3.9	5.5
Total acids, meq. × 100[b]	10.1	7.6	13.6	7.9	10.8
Absorbance, A_{2600}[b]	1.46	.96	2.22	.65[c]	.96[c]
Specific rotation, $[\alpha]_D^{25°}$	+30.6	+9.8	—	−6.5	−4.0

[a] Per 100 cm.² irradiated surface. [b] Corrected for blank values. [c] Absorbance corrected to *p*H 3.6.

TABLE III

MONO- AND OLIGOSACCHARIDES DETECTED BY PAPER CHROMATOGRAPHY IN ULTRAVIOLET-IRRADIATED CELLULOSE MODEL COMPOUNDS BEFORE AND AFTER FRACTIONATION

Identified[a] Fragments in Ultraviolet-Irradiated Model Compounds	Cellobiose		Cellopentaose		Methyl β-Cellobioside		Cellobiitol		Cellopentaitol	
	Before Fract.	After Fract.	Before Fract.	After Fract.	Before Fract.	After Fract.	Before Fract.	After Fract.	Before Fract.	After Fract.
Cellotetraose	—	—	C / β	C / α	—	—	—	—	A,C,E / α,β	0[b]
3-β-Cellotriosyl-D-arabinose	—	—	—	C / α	—	—	—	—	A,C,E / α	0[b]
Cellotriose	—	—	A,C / β	C / α	—	—	—	—	A,C,E / α,β	0[b]
3-β-Cellobiosyl-D-arabinose	—	—	C / β	00[c]	—	—	—	—	A,C,E / α,β	0[b]
Cellobiose	—	—	A,C / β	A,B / α,β	A / β,ε	A,E / α,β	A,E[d] / α	000[e]	A,C,E / β	A,E / β,ζ
3-β-D-Glucopyranosyl-D-arabinose	—	A,B / α,β	—	A / β	A / ε	E / α	A,E[d] / α,β	E[d] / α	A,E / β	E / α,β
D-Glucose	A,B / β	A,B / α,β	—	00[c]	A / α,β	000[e]	A,B,E / α,β	A,E / α,β	A,C,E / α,β	A,E / α,β
D-Glucitol	—	—	—	—	—	—	A,B,E / β,ζ	A,E / β,ζ	A,C,E / β	E / β,ζ
D-Arabinose	A / β	A,B / α,β	—	A / β	A,E / β	A,E / α,β	A,E / α,β	A,E / α,β	C,E / β	E / α,β
Methyl β-D-Glucopyranoside	—	—	—	—	A / β	A / β	—	—	—	—

[a] The capital letters identify paper-chromatographic developers, the Greek letters spray reagents, with the aid of which identification was accomplished. The key for these letters is found in the Experimental part. The isolation of predicted degradation products which are listed in Table I, but do not appear in the first column of this table was not feasible because of inadequate chromatographic resolution. [b] Isolation was not attempted. [c] Isolation attempted, result negative. [d] These degradation products were not predicted. [e] Isolation not feasible because of inadequate chromatographic resolution.

curve had one weak maximum; at 2600 Å for solutions at *p*H 3.6, or at 2700 Å for those at *p*H 7.0. An increase in *p*H was also accompanied by an increase in absorbance—*e.g.*, from 0.96 at *p*H 3.5 to 1.45 at *p*H 7.0 in the case of cellopentaose. The specific rotation of the model compounds was reduced 1–3° by irradiation.

The infrared spectrum of irradiated cellobiose, determined in a potassium bromide pellet, was indistinguishable from that of the blank; the melting point of irradiated cellobiose was depressed 6°. These checks were not repeated with the other model compounds.

Several primary degradation products were detected and identified by paper chromatography. First, exploratory chromatograms showed the presence of various mono- and oligosaccharides in the irradiated model compounds. The small amounts of these products precluded the application of additional analytical techniques, and therefore every effort was made to check and recheck the initial paper chromatographic results by the use of a variety of developers and spray reagents. The systematic examination of all five irradiated model compounds revealed the presence of ten degradation products: eight reducing sugars, D-glucitol, and methyl β-D-glucopyranoside. Most of these ten compounds were detected in several model compounds, as shown in Table III. Mobilities of the model compounds and degradation products in one basic and two acidic paper chromatographic developers are listed in Table IV.

TABLE IV

RELATIVE MIGRATION RATES OF MONO- AND OLIGOSACCHARIDES

Sugar	$R_{Glucose}$ Values in Developer A[a]	Developer C[a]	Developer E[a]
Cellopentaose	0.00	0.00	0.00
Cellopentaitol	0.00	0.00	0.00
Cellotetraose	0.00	0.04	0.03
3-β-Cellotriosyl-D-arabinose	0.03	0.10	0.05
Cellotriose	0.06	0.21	0.07
3-β-Cellobiosyl-D-arabinose	0.12	0.33	0.12
Cellobiose	0.33	0.55	0.30
Cellobiitol	0.24	0.61	0.34
3-β-D-Glucopyranosyl-D-arabinose	0.52	0.70	0.50
Methyl β-Cellobioside	0.73	—	0.91
D-Glucose	1.00	1.00	1.00
D-Glucitol	0.90	1.17	1.19
D-Arabinose	1.51	1.24	1.47
Methyl β-D-Glucopyranoside	2.13	—	2.26

[a] The composition of the developers is indicated in the Experimental part.

The balance of all irradiated model compounds was fractionated by sheet paper chromatography. With the aid of appropriate reference compounds the fractions corresponding to most of the detected degradation products, and also the fractions corresponding to some of the predicted but as yet undetected degradation products were recovered. Unirradiated portions of the five model compounds were fractionated in exactly the same manner. Concentration and rechromatography of the fractions yielded these results: confirmation of the identity of most compounds detected prior to fractionation, with a few exceptions noted in Table III; detection of four additional degradation products; and an opportunity for a quantitative determination of the amounts of several degradation products.

The qualitative results are all listed in Table III. The identity of all but one of these degradation products rests upon comparison with an authentic specimen of the same compound, always chromatographed on the same strip of paper. The exception is the hitherto unknown compound 3-β-cellotriosyl-D-arabinose; it occurred as an impurity in the cellotetraose fraction isolated from irradiated cellopentaose, and was also detected in unfractionated irradiated cellopentaitol. The mixed oligosaccharides of D-glucose and D-arabinose can readily be differentiated from the oligoglucoses by the color they give with spray reagent α.[11,20] Most blank fractions contained little or none of the compounds detected in the corresponding fractions of irradiated model compounds.

(20) J. K. Hamilton and N. S. Thompson, *J. Am. Chem. Soc.*, 79, 6464 (1957).

The results of the quantitative paper chromatographic determination of the degradation products in most of the isolated fractions are listed in Table V. These data are corrected, where needed, for the blanks, which, with three exceptions, contained only negligible quantities of the corresponding compounds. The exceptions are indicated in footnotes to Table V. The quantitative data thus corroborated all but two of the earlier qualitative identifications. Cellobiose could, however, no longer be considered as an identified product of the photodegradation of methyl β-cellobioside, nor D-glucose as that of cellopentaitol.

Most of the predicted primary degradation products were shown to have actually been formed from the cellulose model compounds during ultraviolet irradiation. The detection of the remaining predicted primary degradation products was largely prevented by the limitations of paper chromatography. Furthermore, the three types of oligosaccharides used as model compounds, namely reducing sugar, glycoside, and alditol, yielded comparable amounts of the same degradation products. Two of the detected degradation products, cellobiose and 3-β-D-glucopyranosyl-D-arabinose, both arising from irradiated cellobiitol, were, however, not among those originally predicted.

Every detected degradation product arose apparently from initial cleavage of the model compound molecules in the vicinity of carbon atom No. 1 of the D-glucose units. Such cleavage must also have proceeded by the two modes of fission outlined in the introduction. A photolytic mechanism, initiated through absorption of light by the so-called acetal chromophores, would fit the data very well. This can not have been the only mechanism, since it could not account for the formation of cellobiose and 3-β-D-glucopyranosyl-D-arabinose from cellobiitol, as there is no acetal chromophore at C_1 of the D-glucitol unit of cellobiitol. These two degradation products may have arisen through photolysis initiated by light absorption at hydroxyl groups.[9,10]

The results of this investigation do not allow an accurate description of the pathway of the photodegradation of oligosaccharides. They do, however, clearly demonstrate one aspect of the problem: a strong chromophore, such as a carbonyl group, is not required for the photodegradation of oligosaccharides. By analogy, it is presumed that cellulose molecules completely devoid of carbonyl groups would also undergo photolytic degradation.

EXPERIMENTAL

All melting points are corrected; all evaporations were carried out under reduced pressure at 50°.

Cellulose model compounds. (1) *Cellobiose.* Commercial cellobiose, which contained a small amount of D-glucose, was recrystallized four times from 75% aqueous ethanol; m.p. 228–229° dec., $[\alpha]_D^{25}$ + 33.3° (*c* 2.0, in water). Paper

TABLE V

RESULTS OF QUANTITATIVE PAPER CHROMATOGRAPHIC ANALYSES ON THE DEGRADATION PRODUCTS ISOLATED FROM ULTRAVIOLET-IRRADIATED CELLULOSE MODEL COMPOUNDS

Product Formed Mmole × 100/100 Cm.[a]	Model Compounds Irradiated				
	Cellobiose	Cellopentaose	Methyl β-cellobioside	Cellobiitol	Cellopentaitol
Cellotetraose	—	1.88[a]	—	—	—
3-β-Cellotriosyl-D-arabinose	—	0.15[a]	—	—	—
Cellotriose	—	0.56[a]	—	—	—
Cellobiose	—	0.14[a]	—[b]	—	0.26
3-β-D-Glucopyranosyl-D-arabinose	0.18	—	0.04	0.33	0.04
D-Glucose	0.92[c]	—	—[d]	0.56	—[e]
D-Glucitol	—	—	—	1.00	0.50
D-Arabinose	0.12	—	0.09	0.14	0.05
Methyl β-D-glucopyranoside	—	—	1.72	—	—

[a] Determined indirectly; the appropriate fraction of the irradiated compound was hydrolyzed, and quantitative analysis was carried out on the monosaccharides in the hydrolyzates. [b] The quantity of cellobiose found in the blank was 84% of that found in irradiated methyl β-cellobioside. [c] The quantity of D-glucose found in the blank was only 20% of that found in irradiated cellobiose; the latter was corrected accordingly. [d] The D-glucose fraction could not be isolated because of overlapping with methyl β-cellobioside. [e] The quantity of D-glucose found in the blank was 73% of that found in irradiated cellopentaitol.

chromatography (developer B, spray reagent α) revealed only very slight traces of D-glucose in the purified product.

(2) *Cellopentaose.* This oligosaccharide and several of its homologs were prepared in this laboratory[21] as follows. Cotton linters was hydrolyzed for 6 hr. at 60° in 4% solution in 85% phosphoric acid. The hydrolyzate was fractionated on a 1:1 carbon-Celite column by gradient elution with aqueous ethanol. Cellopentaose emerged at an ethanol concentration of 30–35%, was then treated with carbon, and crystallized from water-methanol; white microcrystalline solid, m.p. 268.5° dec., $[\alpha]_D^{25}$ + 12.4° (*c* 0.75, in water); lit.,[22] m.p. 266–268° dec., $[\alpha]_D$ + 11° (*c*, 4.1, in water). A paper chromatographic check (as for cellobiose) revealed no impurities.

(3) *Methyl β-cellobioside.* This compound was prepared by a three-step synthesis from cellobiose octaacetate, using the procedure of Pacsu.[23] The final product was recrystallized three times from 95% ethanol; m.p. 191.5–192.5°, $[\alpha]_D^{25}$ − 19.4° (*c* 3.7, in water); lit.[23] m.p. 193°, $[\alpha]_D^{25}$ − 18.9°. A paper chromatographic check (developer A, spray reagent β) revealed only a small trace of cellobiose in the purified product.

(4) *Cellobiitol.* Cellobiose, purified as above, was reduced by sodium borohydride, using a slight modification of the method of Smith and co-workers.[24] The modification consisted in adding an excess of Amberlite IR-120(H) cation-exchange resin to the reaction mixture upon completion of the reduction, instead of acetic acid. The sodium-free filtrate was evaporated to a sirup, which was freed of borate by the methyl borate distillation method of Zill and co-workers.[25] Attempts at crystallizing the final product, a colorless glass, following the procedure of Wolfrom and Fields[26] and using cellobiitol seed crystals, were not successful. An attempt at crystallization *via* acetylation yielded a nonaacetate which also failed to crystallize. The colorless sirupy cellobiitol had the following rotation: $[\alpha]_D^{25}$ − 7.9° (*c* 0.94, in water); lit.,[26] $[\alpha]_D^{20-25}$ −7.8° (*c* 3–6, in water). No cellobiose, nor any other reducing sugar was detected in cellobiitol by paper chromatography, using developers A, B and E, and spray reagents α, β and γ.

(5) *Cellopentaitol.* Cellopentaose was reduced with sodium borohydride as described above, giving cellopentaitol, m.p. 284.5° dec., $[\alpha]_D^{25}$ −4.6° (*c* 3.3, in water), after recrystallization from aqueous ethanol.[26] Lit.,[26] m.p. 283–285° sl. dec., $[\alpha]_D^{20-25}$ −5.2° (*c* 3–6, in water). No cellopentaose, nor any other reducing sugar was detected in cellopentaitol by paper chromatography, using developers A, C and E, and spray reagent γ.

Reference compounds. Methyl β-D-glucopyranoside was synthesized from 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide[27] by the usual steps of methoxylation and deacetylation. Commercial samples of *D-glucose*, *D-arabinose*, and *D-glucitol* were used. *Cellotriose*, m.p. 205–207° dec., $[\alpha]_D$ +22.4° (*c* 3.1, in water); lit.,[22] m.p. 206–209° dec., $[\alpha]_D^{25}$ +21.6° (*c* 4, in water), was prepared in this laboratory[21] from partially hydrolyzed cotton linters by the method described above for cellopentaose. Cellotriose emerged from the column at an ethanol concentration of 13%, and was crystallized from water-methanol. *Cellotetraose*, m.p. 256–257° dec., $[\alpha]_D$ +16° (*c* 3.3, in water); lit.,[22] m.p. 252–253° dec., $[\alpha]_D$ +16.5° (*c* 3.4, in water), was also prepared by the above method.[21] This compound emerged from the column at an ethanol concentration of 20–22%, and was crystallized from water-methanol. *3-β-D-Glucopyranosyl-D-arabinose* and *3-β-cellobiosyl-D-arabinose* were synthesized as previously described.[11]

Irradiation. From previous experience with the ultraviolet degradation of wood cellulose,[11] it was assumed that the extent of degradation in oligosaccharides would depend on the area of the sample surface and the duration of ultraviolet irradiation, rather than on sample weight—*i.e.* depth of the irradiated layer. This assumption was upheld by the results of a few trial irradiations with crystalline cellobiose, which are given in Table VI, and consequently it was decided to conduct the comparative irradiation of the five cellulose model compounds with samples of equal surface area.

A duration of 80 hr. was chosen for irradiation after further trials with cellobiose, as an increase in the yield of primary products beyond that period appeared unlikely (Table VII).

The ultraviolet light source was a 360-watt, General Electric, UVIARC UA-3, mercury lamp (emission peaks at 2537, 2652, 3022, 3131, and 3654 Å). This lamp was mounted

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TABLE VI

CHANGES IN CELLOBIOSE SAMPLES OF VARIOUS WEIGHTS BUT EQUAL SURFACE AREAS AFTER 40 HR. OF ULTRAVIOLET

Cellobiose, Mg./100 Cm.²	Weight Loss, Mg./100 Cm.²	Free Acids[a] Meq. × 100/100 cm.²	Total Acids[a] Meq. × 100/100 cm.²	Absorbance,[a] A_{2600}/100 Cm.²
403	18.3	4.2	9.8	1.63
736	16.0	5.1	10.4	1.63
1170	13.0	5.1	11.1	1.47

[a] Corrected for blank values.

TABLE VII

CHANGES IN CELLOBIOSE SAMPLES OF EQUAL WEIGHT AND SURFACE AREA AFTER INCREASING DOSAGES OF ULTRAVIOLET

Ultraviolet, Hr.	Weight Loss, Mg./100 Cm.²	Free Acids[a] Meq. × 100/100 cm.²	Total Acids[a] Meq. × 100/100 cm.²	Absorbance,[a] A_{2600}/100 Cm.²
1	0.5	0.3	1.0	0.14
2	2.3	0.8	1.4	0.24
5	0.5	1.8	3.4	0.61
10	1.9	2.9	5.8	1.00
20	3.4	3.4	6.9	1.27
40	16.1	5.1	10.4	1.61
80	77.0	5.2	10.1	1.46

[a] Corrected for blank values.

vertically in the center of an adequately ventilated enclosure. Reasonably even deposits of the four crystalline model compounds, and a film of sirupy cellobiitol were prepared in tared Petri dishes of 9-cm. diameter by initiating the evaporation of their aqueous alcoholic solutions on the steam bath, and concluding it at room temperature. Sample clinging to the side-wall of the dishes was carefully removed, the deposits dried *in vacuo* over phosphorus pentoxide, and weighed. The samples of the disaccharides weighed approximately 450 mg., those of the pentasaccharides 250 mg. Uniformity of weight was not required, as the results were to be expressed and compared on the basis of sample surface area. The dishes were mounted vertically, without cover, with the deposit facing the lamp at a distance of 20 cm. The temperature of the sample surfaces was 50–52° during irradiation, and the air passing through the enclosure had a relative humidity of 60%. Duration of irradiation varied in exploratory runs, and was uniformly 80 hr. in the main runs.

Analysis of irradiated samples. After irradiation, the samples were redried, and dissolved in water at room temperature; the solutions were filtered, and adjusted to 25 ml. in volumetric flasks. Any water-insoluble material was dried and weighed. Blank solutions were prepared by dissolving corresponding quantities of the unirradiated model compounds in 25 ml. of water; these solutions were analyzed along with the solutions of the irradiated samples.

The *p*H, the optical rotation, and the ultraviolet absorption spectra were determined on suitable portions of these solutions; these portions were recovered for subsequent analyses.

Ten-milliliter aliquots were titrated with 0.05*N* sodium hydroxide, using phenolphthalein as indicator, to obtain the quantity of free acids formed. A threefold excess of 0.05*N* sodium hydroxide was immediately added to the titrated aliquot, the mixtures were allowed to stand for 30 min. at room temperature, and were then back-titrated with 0.05*N* hydrochloric acid.

The balance of each solution was concentrated and examined by paper chromatography. The following developers were used: (A) ethyl acetate–pyridine–water (8:2:1), (B) ethyl acetate–acetic acid–formic acid–water (18:3:1:4), and ethyl acetate–acetic acid–water in the following ratios: (C) 6:3:3, (D) 6:3:2, (E) 3:1:3 upper phase. All ratios were vol./vol. The following spray reagents were used: (α) *p*-anisidine–trichloroacetic acid–pyridine,[20] (β) silver nitrate–ammonia, (γ) bromophenol blue,[28] (δ) alkaline hydroxylamine followed by acidic ferric chloride,[29] a reagent for esters and lactones, (ϵ) periodate-permanganate,[30] and (ζ) *p*-anisidine hydrochloride in 1-butanol followed by potassium metaperiodate in aqueous acetone,[31] a reagent permitting detection and differentiation of reducing sugars and sugar alcohols on the same chromatogram.

Qualitative paper chromatograms were run on Whatman No. 1 paper, spotting 1–3 mg. of the irradiated sample, 1–3 mg. of the blank, and suitable quantities of reference compounds on the same strip. Several identical strips were spotted, developed by the descending method in developers A, B, C, and E, and sprayed with several of the above reagents.

Fractionation of irradiated samples and blanks was carried out by sheet paper chromatography. Whatman No. 3MM paper was used for cellobiose, cellopentaose and methyl β-cellobioside; the two alditols were fractionated on sheets of Whatman No. 3MM paper, to the upper edge of which were sewn wicks of Whatman No. 1 paper.[32] Descending chromatographic development was conducted with solvent B for cellobiose, D for cellopentaose, and E for the three other model compounds. The position of the reference compounds was located by spraying the marginal guide strips of the developed sheets with reagent α or β.

The fractionation of cellopentaose was carried out in two stages. After the first 24 hr. of development the outer pair of a double set of marginal guides was removed and sprayed. With their help the sheet was cut, parallel to the starting line, between the bands of the cellobiose and 3-β-cellobiosyl-D-arabinose fractions. The mono- and disaccharide fractions on the lower half of the sheet were ready for recovery. The development of the tri- and tetrasaccharides, located on the upper half, was resumed for an additional 78 hr. after attaching a fresh portion of Whatman No. 3MM paper to the cut edge by sewing. The other four model compounds were fractionated in the conventional manner.

Sections of the developed sheets matching the locations of the reference compounds in the guide strips were excised, and eluted with water. The water was evaporated and the residues of all eluates were extracted three times in succession with boiling ethanol of the following concentrations: for monosaccharides, 95%; for disaccharides, 90%; for trisaccharides, 85%; and for tetrasaccharides, 80%. The residues of the filtered and evaporated extracts were dried, weighed, and used for paper chromatography. The recovery of the blank fractions was accomplished in exactly the same manner.

The weights of the alcohol-soluble portions of all fractions, whether they derived from irradiated samples or from blanks, were quite similar, and of the order of 5–10 mg. Qualitative paper chromatographic checks, carried out on all fractions as above, revealed most of the material to be chromatographically immobile, presumably derived from the chromatographic paper. Quantitative analyses showed subsequently that the chromatographically mobile degradation-products

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represented only a small portion of the total weights of these fractions.

Quantitative determinations of most of the reducing sugars were carried out by a paper chromatographic-colorimetric method developed in this laboratory.[33] The cellobiose, -triose, and -tetraose fractions obtained from irradiated and blank cellopentaose samples were first hydrolyzed, using 4% aq. sulfuric acid at 100° for 6.5 hr., and the quantities of D-glucose in the processed hydrolyzates were determined by the above method.[33] The 3-β-D-glucopyranosyl-D-arabinose fractions were not large enough for this analysis, and no 3-β-cellobiosyl-D-arabinose was detected in the appropriate fractions. The hydrolyzate of the cellotetraose fraction from irradiated cellopentaose contained some D-arabinose, the quantity of which was also determined. This D-arabinose was assumed to be derived from 3-β-cellotriosyl-D-arabinose, another degradation product, which was present in the cellotetraose fraction as a contaminant.

The quantities of these oligosaccharides were then calculated, after correction for the blanks, from the quantities of the monosaccharides in the hydrolyzates. The quantity of 3-β-cellotriosyl-D-arabinose arrived at is almost certainly too low, some of that sugar having no doubt been located on the sheet immediately below the excised area of cellotetraose.

The quantities of methyl β-D-glucopyranoside and D-glucitol were finally estimated by visual comparison. The appropriate fractions from irradiated and blank methyl β-cellobioside, cellobiitol, and cellopentaitol, and solutions of authentic methyl β-D-glucopyranoside and D-glucitol in a suitable range of concentrations were, for this purpose, chromatographed side by side on the same strips of paper. Developer A was used for methyl β-D-glucopyranoside, developer E for D-glucitol, and spray reagent β for both.

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SHELTON, WASH.

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Lignin Model Compounds. Nitric Acid Oxidation of 4-Methylguaiacol[1]

IGOR SOBOLEV[1a]

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4-Methylguaiacol (I) reacts with dilute aqueous nitric acid giving rise to the following sequence of intermediates: I ⟶ 4-methyl-6-nitrosoguaiacol (II) ⟶ 4-methyl-6-nitroguaiacol (III) ⟶ 5-methyl-3-nitropyrocatechol (IV) ⟶ 2-hydroxy-5-methyl-3-nitro-1,4-benzoquinone (V). End products include nitrous oxide, nitrogen, nitric oxide, nitrogen dioxide, carbon monoxide, carbon dioxide, and oxalic acid. Nitrosonium (NO^+) ion mechanisms for the nitration of I and demethylation of III are proposed to account for the observed catalysis by nitrous acid. Similar reactions are believed to occur in lignin oxidations.

Oxidation with aqueous nitric acid is one of the oldest yet least understood reactions for degrading lignin. Only the simplest products have been identified so far: monomeric phenols and nitrophenols, oxalic, acetic and formic acids, carbon monoxide, and carbon dioxide. Reported reduction products from nitric acid include nitrogen, nitrous oxide, nitric oxide, nitrogen dioxide, and hydrogen cyanide. Some cleavage of aromatic methoxyl groups occurs also, and nitrous acid appears to be a required catalyst for the oxidation.[2] Oxidations of model compounds[3,4] have offered little new evidence concerning reaction mechanisms. In organic solvents, nitration of lignins involves some electrophilic displacement of aliphatic side chains by nitro groups.[5] Structures undergoing this displacement are chiefly 4-substituted guaiacyl end units containing benzyl alcohol and ether groups. To what extent displacement occurs in oxidations with aqueous nitric acid has not been established. The results of the present model compound study, applicable to portions of lignin, are believed to be of value primarily in indicating the role of nitrous acid in oxidations with nitric acid.

4-Methylguaiacol (I), a simplified lignin model with no side-chain oxygen, was oxidized with 2.6*N* "pure"[6] nitric acid at 70°. In a two-hour reaction, nearly all the compound was oxidized to oxalic acid, other water-soluble products, and to the gases listed in Table I. From the evidence presented

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(6) In this paper, "pure" nitric acid refers to solutions prepared from 70% reagent grade acid without addition of nitrous acid. "Nitrous acid" denotes all those chemical species determined as nitrous acid by the analytical method of Nelson, Kurtz, and Bray [*Anal. Chem.*, **26**, 1081 (1954)], *i.e.*, by colorimetric estimation of an azo dye formed in aqueous acetic acid.