137. *Studies on Degraded Esparto Cellulose.*

By G. O. Aspinall and W. B. Fordyce.

Degraded esparto cellulose capable of peptisation into a colloidal solution in water has been prepared. Methylation end-group assay showed that the degraded cellulose had an average chain length of 77 ± 3 glucose residues, and a similar value was obtained from periodate oxidation. The chemical accessibility of esparto cellulose, before and after degradation, has been investigated by acid hydrolysis and water sorption methods.

The heterogeneous degradation of cellulose by hot dilute (*ca.* 10%) mineral acid causes severe depolymerisation usually accompanied by destruction of the characteristic fibrous structure. After a rapid initial decrease, the degree of polymerisation tends to reach a limiting value of the order of 50—200 with the formation of fragments relatively resistant to further attack under the conditions of hydrolysis. These resistant fragments, whose exact size depends on the source and previous treatment of the original cellulose,[1] are considered to arise from preferential hydrolysis of the amorphous regions of the cellulose fibre and to represent the more highly ordered or crystalline regions. Indeed, methods for the determination of the degree of crystallinity of celluloses are based on the relative ease of accessibility to chemical attack of the amorphous regions.[2] Rånby [3] has shown that some of these resistant fragments (or cellulose micelles) will peptise to form aqueous sols in the pH range 3·5—9·5 and in the absence of electrolytes. Electron-microscopic measurements showed that the dimensions of the micelles (*ca.* 500 × 80 Å) were similar to those of the ordered regions or crystallites of the long filamentary fibrils obtained by ultrasonic treatment of cellulose fibres; [3,4] the lengths of the micelles corresponded to the values for the degrees of polymerisation of the derived cellulose nitrates measured viscometrically. These cellulose micelles, however, had not been examined by chemical methods, and this paper describes the results of an investigation of such a degraded cellulose prepared from esparto grass (*Stipa tenacissima* L.).

Esparto cellulose, obtained by exhaustive extraction of the delignified grass with dilute alkali to remove hemicelluloses, was hydrolysed with hot dilute sulphuric acid, and cellulose sols were obtained by repeated washing of the resulting hydrocellulose with water. Peptisation occurred after the fourth or fifth washing, and the colloidal particles, which showed the hydrophobic properties described by Rånby,[3] coagulated in the presence of a small amount of added electrolyte. The degraded esparto cellulose was methylated with rigorous exclusion of oxygen [5] and the quantity of tetra-*O*-methyl-D-glucose isolated from the hydrolysis of the methylated cellulose corresponded to one terminal non-reducing group per 77 ± 3 glucose residues. Estimation of the formic acid produced on periodate oxidation [6] indicated an average chain length of 69—72, a figure in reasonable agreement with the value obtained from the methylation studies.

It is interesting that chromatographic evidence was obtained for the presence of small quantities of xylose in the hydrolysate from the degraded cellulose and of small quantities of 2 : 3 : 4-tri-*O*-methyl and 2-*O*-methyl xyloses in the hydrolysate from the methylated cellulose. These observations provide further evidence for the extremely close association between cellulose and hemicelluloses in the plant.[7] It would be premature, however, to speculate whether this association is purely physical or whether chemical linkages exist between the two components.

Determinations of chemical accessibility, by two methods, were carried out on three cellulose samples : (1) the original esparto cellulose, (2) the " colloidal " degraded esparto cellulose (referred to as degraded esparto cellulose), and (3) the residual insoluble degraded

[1] Jörgensen, *Acta Chem. Scand.*, 1950, **4**, 185.
[2] Nickerson, *Adv. Carbohydrate Chem.*, 1950, **5**, 103.
[3] Rånby, *Discuss. Faraday Soc.*, 1951, No. **11**, 158; *TAPPI*, 1952, **35**, 53.
[4] Rånby and Ribi, *Experientia*, 1950, **6**, 12.
[5] McGilvray, *J.*, 1953, 2577.
[6] Halsall, Hirst, and Jones, *J.*, 1947, 1399, 1427.
[7] Adams and Bishop, *Nature*, 1953, **172**, 28.

esparto cellulose which did not undergo peptisation during the preparation of the "colloidal" cellulose (hereinafter called esparto hydrocellulose). In the first method, Nickerson's hydrolytic oxidation procedure [8] was followed, and the carbon dioxide liberated when each cellulose was digested in a boiling solution of ferric chloride and hydrochloric acid was measured gravimetrically. The carbon dioxide evolved from an equivalent quantity of glucose was measured concurrently. The results were calculated by Nickerson's formula [8] on the assumption that the rapid initial hydrolysis was due to attack in the easily accessible or amorphous regions, whilst the subsequent slow hydrolysis was due to attack in the more highly ordered regions, which are penetrated by chemical reagents only with difficulty. Fig. 1 shows the evolution of carbon dioxide from glucose and from the three cellulose samples, and Fig. 2 shows the percentage of each cellulose hydrolysed plotted against time. The sharp change in rate of hydrolysis of the celluloses over the first 2 hr. is brought out more clearly when the rate is plotted against time (Fig. 3). The rapid initial hydrolysis is complete after 2—3 hr. and from the extrapolation of the percentage hydrolysis–time curves to zero time, values for the chemical accessibility of the celluloses were obtained. The values quoted were obtained from the 2—4 hr. period, and although the shapes of the curves do not permit an exact extrapolation, the relation between the three celluloses is indicated, namely, that the degraded cellulose is slightly less, and the hydrocellulose more, accessible than the original esparto cellulose.

Cellulose	Accessibility (%)	Cellulose (%) hydrolysed after 8 hr.
Esparto cellulose	8·4	21
Esparto hydrocellulose	8·5	34
Degraded esparto cellulose	8·2	19

In the second method, the sorption of water vapour by the cellulose samples was measured and Fig. 4 shows a typical plot of moisture regain against relative humidity. The uptake of water for any cellulose is a function of the accessibility, and calculations from the characteristic sigmoid-shaped isotherms were made by using the theoretical sorption isotherm of Hailwood and Horrobin.[9] Again the results show the degraded esparto cellulose to be less accessible than the original esparto cellulose, whilst the hydrocellulose was slightly more accessible. Although both series of experiments suggest that the degraded "colloidal" cellulose is slightly less accessible than the parent esparto cellulose, as would be expected if hydrolysis of the esparto cellulose preferentially removes the less ordered or amorphous regions, the similarity between the two samples is a more striking feature of the results. It is interesting that Hermans [10] has shown, using the *X*-ray

Cellulose	Regain at 50% relative humidity	Accessibility (%)
Esparto cellulose	6·22	35
Esparto hydrocellulose	6·14	36
Degraded esparto cellulose	5·78	31

diffraction method of Hermans and Weidinger,[11] that colloidal cellulose micelles prepared by Rånby's method [3] had the same degree of crystallinity as the wood pulp from which they were derived. On the other hand, the esparto hydrocellulose was less highly ordered and was attacked 2·5 times more rapidly than the "colloidal" cellulose during the later stages of the hydrolytic oxidation (Fig. 3). As the hydrophobic character of the cellulose sols may be attributed to the high lateral order and high degree of internal hydrogen bonding, the process of peptisation appears to favour the separation of the highly ordered particles from the less highly ordered hydrocellulose.

The results of this investigation show that the cellulose sols from the hydrolysis of esparto cellulose contain particles of the same order of magnitude as those isolated by Rånby [3] from a number of sources. The average chain length of the degraded esparto cellulose (77 $\pm$ 3) is intermediate between those found by Rånby for the degraded cellulose

[8] Nickerson, *Ind. Eng. Chem. Anal.*, 1941, **13**, 423.
[9] Hailwood and Horrobin, *Trans. Faraday Soc.*, 1946, **42**, *B*, 84.
[10] Hermans, *Makromol. Chem.*, 1951, **6**, 25.
[11] Hermans and Weidinger, *J. Polymer Sci.*, 1949, **4**, 135.

from wood pulps and from the corresponding mercerised wood celluloses. Although these resistant colloidal particles arise from the preferential hydrolysis of the amorphous regions of tle original cellulose, it is unlikely that they can be identified with the crystalline regions. On the one hand, it is not known how far the crystallite size may decrease during

FIG. 1. *Evolution of carbon dioxide from (I) glucose, (II) esparto hydrocellulose, (III) esparto cellulose, and (IV) degraded esparto cellulose in boiling* $FeCl_3$–HCl.

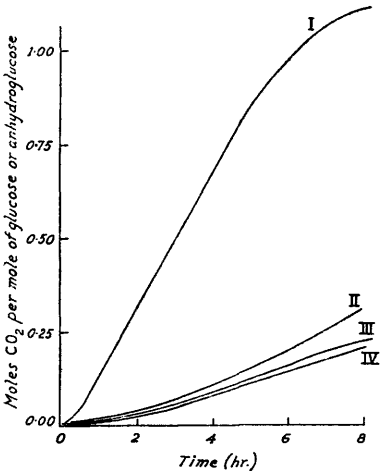


FIG. 2. *Hydrolysis–time curves for (I) esparto hydrocellulose, (II) esparto cellulose, and (III) degraded esparto cellulose.*

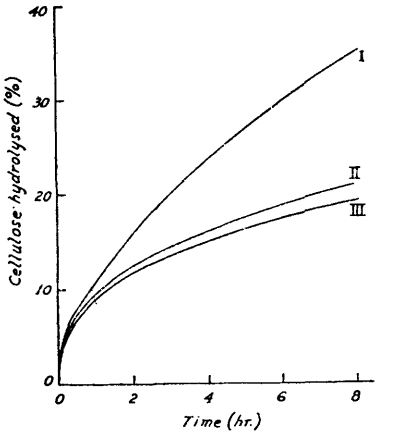


FIG. 3. *Rates of hydrolysis in* $FeCl_3$–HCl *of (I) esparto hydrocellulose, (II) esparto cellulose, and (III) degraded esparto cellulose.*

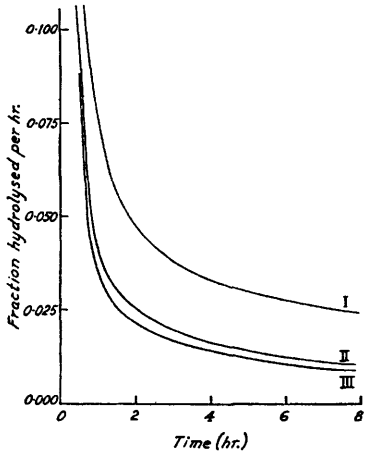
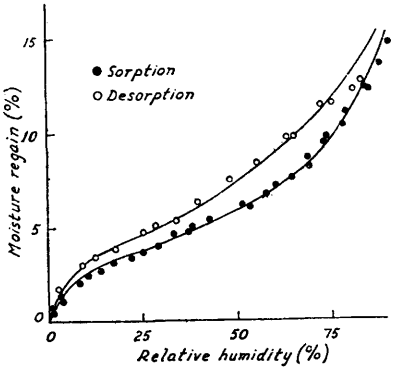


FIG. 4. *Typical water-vapour sorption–desorption isotherm for degraded esparto cellulose.*



the hydrolysis. On the other hand, there is evidence that as chain scission occurs a rapid crystallisation of the less ordered regions takes place causing an increase in size of the crystallites.[12–14] The fact that the isolated colloidal particles show little increase in degree of crystallinity (or correspondingly little decrease in accessibility) suggests that crystallite size, determining as it does the proportion of accessible glucose residues in the

[12] Hermans and Weidinger, *J. Polymer Sci.*, 1949, **4**, 317.
[13] Brenner, Frilette, and Mark, *J. Amer. Chem. Soc.*, 1948, **70**, 877.
[14] Howsmon, *Textile Res. J.*, 1949, **19**, 152.

surface layers of the cellulose particles, may be a more important factor in accessibility determinations than the geometric order prevailing throughout the interior of the crystallite.

Experimental

The following solvents (v/v) were used to separate the sugars and their derivatives : (*A*) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3; top layer), (*B*) butan-1-ol–ethanol–water (4 : 1 : 5; top layer), and (*C*) benzene–ethanol–water (169 : 49 : 15; top layer).

Preparation of Degraded Esparto Cellulose.—Esparto holocellulose was prepared from the grass as described by Chanda, Hirst, Jones, and Percival.[15] The holocellulose (100 g.) was extracted first with cold 4% sodium hydroxide solution (2 l.) for 24 hr. and then hot 6% sodium hydroxide solution (2 l.) for 24 hr. under a steam pressure of 40—45 lb. per sq. in., washed with water until free from alkali, and dried with ethanol to give esparto cellulose (70 g.).

Cellulose sols were prepared in a manner similar to that described by Rånby.[3] Esparto cellulose (30 g.) was heated at 100° with 10% sulphuric acid (300 c.c.) for 1—8 hr. The degraded cellulose was separated at the centrifuge and washed repeatedly with water (batches of 800 c.c.). Peptisation occurred at the 4th or 5th washing (pH 4—5) and continued until about the 10th washing. The cellulose sols were separated from fibrous material by centrifugation at 1500 r.p.m. and the colloidal cellulose was coagulated by the addition of sodium chloride (0·5 g. per l.). The light flocculent precipitate was separated at the centrifuge, washed twice with water (200 c.c.) to remove the electrolyte, and freeze-dried. The degraded cellulose was obtained in an average concentration of 2 g. per l. Chromatography of the hydrolysate (Monier-Williams) showed the presence of glucose and a trace of xylose.

Methylation of Degraded Esparto Cellulose.—Degraded esparto cellulose (11 g.) was methylated fifteen times with methyl sulphate and sodium hydroxide, rigorous precautions being used to exclude atmospheric oxygen.[5] The product was methylated twice more with methyl iodide and silver oxide, and the methylated cellulose was fractionated in hot chloroform–light petroleum (b. p. 60—65°) mixtures. Two main fractions, 1 (0·85 g.; OMe, 43·4%) and 2 (1·95 g.; OMe, 42·9%), were obtained and these were combined for subsequent work. Chromatography of a sample hydrolysate showed the presence of tetra-, tri-, and di-*O*-methylglucoses.

Hydrolysis of Methylated Cellulose and Separation of Methylated Sugars.—Methylated degraded esparto cellulose (2·6 g.) was heated with methanolic 3% hydrogen chloride (35 c.c.) in a sealed tube at 96° for 24 hr. and the resulting syrup was heated on the water-bath with 0·5N-hydrochloric acid (160 c.c.) for 16 hr. After neutralisation with silver carbonate and deionisation with Amberlite resins IR-100 and IR-4B, the solution was concentrated to a syrup (2·5 g.) which crystallised. The hydrolysate was fractionated on cellulose [16] (100 × 3 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3), saturated with water, as eluant to give four fractions.

Fraction 1. The syrup (43 mg.) travelled on the chromatogram at the same rate as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose but hypoiodite oxidation indicated only 61% of aldose. After further hydrolysis of the syrup with 0·5N-hydrochloric acid (25 c.c.) on the water-bath for 8 hr., the product was separated on filter sheets to give fractions 1*a* (25 mg.) and 1*b* (10 mg.). Fraction 1*a* crystallised and had m. p. and mixed m. p. (with 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose) 89—94° {$[\alpha]_D^{18}$ +100° ⟶ +80° (*c*, 0·09 in H_2O)}. The derived 2 : 3 : 4 : 6-tetra-*O*-methyl-*N*-phenyl-D-glucosylamine had m. p. and mixed m. p. 125—130°. Fraction 1*b* travelled on the chromatogram at the same rate as 2 : 3 : 6-tri-*O*-methyl-D-glucose and was not examined further. The corrected weight (29 mg.) of tetra-*O*-methyl-D-glucose corresponded to an average chain length of 77 ± 3 glucose residues.

Fraction 2. The chromatographically pure syrup (1·88 g.) crystallised readily and after recrystallisation from dry ether had m. p. and mixed m. p. (with authentic 2 : 3 : 6-tri-*O*-methyl-D-glucose) 108—113°, $[\alpha]_D^{18}$ +85° ⟶ +63° (*c*, 0·25 in H_2O), $[\alpha]_D^{18}$ +70° ⟶ −38° (*c*, 0·58 in methanolic 1% hydrogen chloride); OMe, 42·4%.

Fraction 3. The syrup (35 mg.) which did not crystallise had $[\alpha]_D^{18}$ +95° ⟶ +60° (*c*, 0·3 in H_2O) and travelled on the chromatogram at the same rate 2 : 3-di-*O*-methyl-D-glucose. Periodate oxidation [17] gave 0·86 mole of formaldehyde per mole of dimethyl sugar, estimated as the formaldehyde–dimedone compound.

Fraction 4. The syrup (194 mg.) which did not crystallise had $[\alpha]_D^{18}$ +72° ⟶ +55° (*c*, 0·15

[15] Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1289.
[16] Hough, Jones, and Wadman, *J.*, 1949, 2511.
[17] Bell, *J.*, 1948, 992.

in H_2O) and $[\alpha]_D^{18}$ $+73° \longrightarrow -50°$ (*c*, 0·26 in methanolic 1% hydrogen chloride). The syrup was converted into the methyl glycosides, and the product was oxidised with sodium metaperiodate solution.[17] The consumption of periodate corresponded to the presence of 66% of 2 : 6-di-*O*-methyl-D-glucose in the fraction. After destruction of excess of periodate, the chloroform-soluble extract was hydrolysed and chromatography showed the presence of a single sugar corresponding to 3 : 6-di-*O*-methyl-D-glucose.

In addition to the major components described, chromatographic evidence was obtained for the presence of traces of 2 : 3 : 4-tri-*O*-methyl- and 2-*O*-methyl-xylose (R_G 0·97 and 0·05 in solvent *C*).

Periodate Oxidation of Degraded Esparto Cellulose.—Oxidation of the cellulose (100 mg. batches) with potassium metaperiodate solution by the method of Halsall, Hirst, and Jones [6] yielded a practically constant amount of formic acid after 49 hr., corresponding to 1 mole per 23—24 glucose residues. The production of 1 mol. of formic acid per non-reducing end-group and 2 mols. per reducing end-group being assumed, this value corresponds to an average chain length of 69—72. A sample of degraded cellulose, prepared in a similar manner from Whatman No. 1 filter paper, yielded, on periodate oxidation, formic acid corresponding to an average chain length of 91—94.

Determination of Chemical Accessibility by Nickerson's Method.—The apparatus used was essentially that described by Conrad and Scroggie,[18] in which the carbon dioxide evolved was determined gravimetrically in absorption tubes packed with " Sofnolite " absorbent (15 g.) and magnesium perchlorate (10 g.). To ensure greater uniformity in the conditions under which cellulose and glucose were subjected to the oxidative hydrolysis the two reaction flasks were heated in the same bath at 132° ± 0·5°, and equal flow-rates of the air currents passing through the parallel reaction vessels were maintained.

Cellulose samples (1·5 g.) were heated in a boiling ferric chloride–hydrochloric acid mixture (150 c.c.; 0·6M; 2·4N), the absorption tubes being weighed periodically. The carbon dioxide evolved from samples of glucose (1·3 g.) treated under the same conditions in the parallel reaction flask was determined concurrently.

By means of Nickerson's formula [8] the experimental results (shown in Fig. 1 as carbon dioxide–time data) were converted into percentage hydrolysis–time curves (Fig. 2), from which the accessibilities were obtained by extrapolation to zero time through the 2—4 hr. period.

Determination of Accessibility by Water Sorption Measurements.—Water-vapour sorption and desorption measurements were made with a simple type of McBain–Baker sorption balance.[19] Cellulose samples under investigation were placed in a glass bucket suspended from a glass spring (sensitivity 14·42 cm./g.). The sorption chamber was connected to a high-vacuum pump through a manometer and a drying-tube (magnesium perchlorate), and a bulb containing water as the source of vapour was directly connected to the sorption chamber.

Zero readings were taken after complete evacuation of the system and after the source of water vapour had been completely degassed. The cellulose sample (*ca.* 0·15 g.) was then placed in the bucket and the system was evacuated (*ca.* 36 hr.) until the cellulose was thoroughly dry (as indicated by minimum extension of the spring). Small quantities of water vapour were then admitted, and after equilibrium had been established, the vapour pressure was measured directly by the change in the manometer levels and the moisture regain was calculated from the extension of the spring. The process was repeated until the maximum equilibrium pressure was reached. Desorption measurements were made by following the changes in water-vapour pressure and moisture regain during the gradual evacuation of the system. A typical plot of sorption–desorption data is given in Fig. 4 where moisture regain is plotted against relative humidity. Calculations of the accessibilities of the cellulose samples were made by means of the theoretical isotherm of Hailwood and Horrobin.[9] The best curves were obtained from the experimental data by the application of the method of least squares and Fig. 4 shows the close agreement between observed and calculated values for moisture regain when plotted against relative humidity in the case of the degraded esparto cellulose.

The authors thank Professor E. L. Hirst, F.R.S., for his interest and advice, Messrs. Alex. Cowan and Sons Ltd. for financial assistance, including the award of a scholarship to one of them (W. B. F.), and the Distillers Company Ltd. for a grant.

DEPARTMENT OF CHEMISTRY, UNIVERSITY OF EDINBURGH. [*Received, October 3rd,* 1955.]

[18] Conrad and Scroggie, *Ind. Eng. Chem.*, 1945, **37**, 592.
[19] McBain, " Sorption of Gases by Solids," London, 1932.