PAPER CHROMATOGRAPHIC STUDY OF JUTE HOLOCELLULOSE

by D. B. Das, P. K. Roy Chaudhuri *and* J. F. Wareham, *Group Laboratory, Messrs. Jardine Henderson, Ltd., Calcutta 35*

(Communicated by J. K. Chowdhury, F.N.I.)

(*Received May 28, 1954; approved for reading on August 5, 1955*)

Introduction

Jute contains about 10·5–14% lignin, 24–30% hemicellulose and 55–63% α-cellulose. From the carbondioxide values of jute, the total hemicellulose and the yield of furfural, its hemicellulose components have been subdivided into polyuronides, pentosans and hexosans. Little attempt has been made in the past to isolate and identify these hemicellulose components even by selective extraction and precipitation. The idea that polyuronide is polygalacturonic acid and pentosan is xylan, is based more on assumption than on experimental evidence.

With the development of partition chromatography the isolation and characterisation of the components of polysaccharides have become easier. The success of the preliminary partition chromatography (Sarkar, Mazumdar and Pal, 1950; Bose and Burma, 1952; Das, Roy Chaudhuri and Wareham, 1952) suggested that useful information might be obtained in applying the methods of paper chromatography (Consden, Gordon and Martin, 1944) as applied to sugars (Partridge, 1946) to a detailed study of sugar components in jute holocellulose and this communication describes the separation and identification of the different sugars present in jute holocellulose.

In selecting the acid for hydrolysing jute holocellulose, it was thought desirable to try a weak acid so as to prevent decomposition of sugar components as far as possible. In this connection the work of Spoehr (1947) on the use of formic acid as a hydrolysing agent for algenic acid seemed to be encouraging as he showed that uronic acid was not decarboxylated with boiling formic acid in contrast to boiling mineral acid solution. In addition the normal method of using mineral acids is rather cumbersome whereas in the formic acid method no neutralisation is required and therefore avoids the possible loss entailed through adsorption of the inorganic salts.

In view of the observations (Das, Mitra and Wareham, 1952, 1953, 1954) that some pentosans remain associated with the so-called α-cellulose, it is desirable to hydrolyse the holocellulose completely. The main difficulty encountered in this study is the drastic treatment required for complete hydrolysis of the holocellulose. The usual process of dissolving holocellulose is the 72% H_2SO_4 method of Norman and Jenkins (1933) which seemed to be too drastic for the detection of sugars which may be present only in traces. Hence it was thought desirable to try repeated treatments of formic acid, which being weak is unlikely to cause any serious decomposition of sugars. Another advantage of this procedure is to obtain several fractions and thus to study homogeneity of these fractions with respect to their composition. This procedure was also undertaken to establish the observation of Das, Mitra and Wareham (1952, 1953, 1954) that cellulosan is present in jute.

72% sulphuric acid has been used by Sarkar, Mazumdar and Pal (1950) for hydrolysing jute α-cellulose, 72% sulphuric acid and also hydrochloric acid of varying concentrations have been used here as hydrolysing agent for jute holocellulose in order to compare the results obtained by the mineral acids with those found by formic acid method.

METHODS AND MATERIALS

Jute.—The sample was white jute (*Corchorus Capsularis*) obtained from 24-Parganas District of West Bengal and was of good and soft quality. The whole fibre stem was purified by extraction with alcohol-benzene mixture (1 : 1) in a Soxhlet apparatus followed by washing in water. After drying in air, it was freed from adhering bark and specks by hand, cut into short length (1 cm.), any traces of grease absorbed in the process being afterwards removed by further extraction with alcohol-benzene mixture. The jute was then rinsed in distilled water, filtered and dried in air. This was then mixed thoroughly, and stored over 72% R.H. acid at 35°C. in a desiccator. The ash content of jute was determined by igniting samples of known dry weight (1 g.) till constant in weight. In order to characterise jute, the lignin content was determined by the method of Norman and Jenkins (1933), α-cellulose was estimated by treating the holocellulose obtained after chlorite treatment (Chattopadhyay and Sarkar, 1946) with 17·5% caustic soda solution according to A.C.S. method slightly modified by Sarkar, Chatterjee, Mazumdar and Pal (1948). The hemicellulose content was calculated by difference. Furfural was determined by the method of Hibbert *et al.* (1923) as modified by Chattopadhyay and Sarkar (1946). The formula given by Doreè (1947*a*) was used in the calculation. The carbondioxide value of the fibre was estimated by the method of Nanji, Paton and Link (cf. Doreè, 1947*b*). The results are given in Table 1.

TABLE 1

Chemical composition of jute

	(% on dry weight)
α-Cellulose	61·40
Hemicellulose	25·12
Lignin	11·78
Ash	0·81
Furfural	9·38
CO_2 value	1·26

A. Preparation of holocellulose.—

The holocellulose was prepared by sodium chlorite method (Taylor *et al.*, 1940) as used for jute by Chattopadhyay and Sarkar (1946), which involved the treatment of cut defatted jute with 0·7% sodium chlorite (liquor ratio 1: 50) for 2 hours at the boiling water bath at *p*H between 4·5 to 5 using acetic acid and sodium acetate buffer. It was then filtered, washed thoroughly with distilled water and then dried in air. The loss in weight recorded was 11·85% which approximately corresponds to the value of lignin content (11·78%). The lignin content of holocellulose was 0·33%.

B. Conditions of hydrolysis.—

Three methods of hydrolysis have been tried.

METHOD (i): *Sulphuric acid.*—Holocellulose was treated with 72% sulphuric acid (liquor ratio 1: 20) at temperature below 10°C. for 16 hours. The product was then diluted to a strength of 3% sulphuric acid, followed by heating in a water bath under reflux for 2 hours and filtering through a sintered crucible. The filtrate was then neutralised with barium carbonate, centrifuged and then passed through a charcoal filter. The clear solution was then evaporated under reduced pressure to a syrup when the distillate was treated for furfural yielding products with aniline reagent and phloroglucinol.

METHOD (ii): *Formic acid.*—0·5 g. jute holocellulose and 30 c.c. of 85% formic acid were taken in a test tube with ground joint (B24). The mixture was

thoroughly shaken to obtain as complete a suspension as possible and to avoid formation of lumps. The tube was then connected with an air condenser with glass joint, the upper end provided with a calcium chloride tube and the mixture boiled for 30 minutes in an oil bath maintained at 130–135°C. The tube was then cooled rapidly under a tap, the contents were filtered through a sintered crucible and washed with a few changes of water. The filtrate was termed as fraction 1 and the precipitate fraction 2.

Fraction 1.—The filtrate with its washings was then evaporated to dryness under reduced pressure in a water bath and the distillate tested for furfural yielding substances as usual. The residue was dissolved with a small quantity of water and again evaporated to dryness under reduced pressure. This was then refluxed with 30 c.c. of water in boiling water bath for 3 hours and again evaporated to dryness under reduced pressure. This process of dilution and distillation was repeated until the distillate was only faintly acidic to litmus. The residue was then taken with water and treated with activated charcoal and then filtered through a charcoal bed. The filtrate with washings was then found to be neutral which was subsequently evaporated under reduced pressure to a syrup.

Subfractions.—The residue (fraction 2 mentioned above) was estimated by drying at room temperature in a vacuum desiccator over P_2O_5 until constant in weight, further hydrolysed with 85% boiling formic acid (liquor ratio 1: 60) for 24 hours, and the filtrate termed as subfraction 2, and the residue again hydrolysed in the same way when the filtrate was denoted as subfraction 3. The process was repeated until subfraction 4, subfraction 5 and subfraction 6 were obtained. The residue left was termed as subfraction 7.

Subfractions 2, 3, 4, 5 and 6 were purified, decolourised and made to syrups in the same way as that of fraction 1, while for subfraction 7 sulphuric acid method of hydrolysis and purification, as explained above, was used.

Rate of hydrolysis.—In order to study the rate of hydrolysis of holocellulose and also to evaluate the percentage of these fractions and subfractions on the total holocellulose, 0·5 gm. holocellulose was hydrolysed with 30 c.c. of 95% boiling formic acid for the required length of time and at the end of this period, the contents were filtered through a tared sintered crucible, washed until free from acid. The filtrate was purified, decolourised and evaporated to dryness in the same way as before and then taken up with 1 c.c. water while the residue with its container (sintered crucible) was dried in an oven at 105°C. to constant weight. In all cases the first distillate was noted for furfural yielding substances as usual.

METHOD (iii): *Hydrochloric acid* of different concentrations (1*N*–6*N*) was tried for hydrolysing holocellulose, the procedure adopted being the same as that of formic acid, the duration of boiling in all cases was however 24 hours. The syrup was always slightly acidic.

C. *Chromatographic Techniques: Apparatus.*—

Round drums made up with galvanised sheets were used as chromatographic chambers while the solvent troughs were of glass. A vacuum desiccator top with grease was fitted on the chamber in order to make it air-tight. For inserting solvent during chromatography without disturbing the equilibrium a separating funnel was fitted on the lid of the desiccator, end of the former reaching to the solvent trough.

A conical flask with a fine glass jet connected with an electric air blower was found quite satisfactory for fine and uniform spray. Generally No. 1 Whatman filter paper was used whereas No. 3 occasionally, especially for isolation of sugars when larger quantity was desirable. *Goodbrand* air oven with arrangement for constant draught of pre-heated air (105°±2°C.) was used for drying and developing the chromatograms.

Solvents.—General solvents used are phenol saturated with water (9: 1) and *n*-butanol saturated with water (4: 1); the upper layers of the mixtures were only used as running liquids whereas the lower layers were placed at the bottom of the chambers.

The major advantage of selecting moist butanol and moist phenol as solvents is the fact that while with the former galactose runs slower than glucose and arabinose slower than xylose; these cases are reversed in the case of latter solvent and thus helping detection of these four sugars all of which are present in jute holocellulose. For certain specific purpose especially for separating galactose from glucose the upper layer of the mixture of ethyl acetate-water-pyridine (2: 2: 1) as suggested by Jermyn and Isherwood (1949) was used. The lower layer as usual was used at the bottom of the chamber.

Spray liquids.—In general a saturated water solution of aniline oxalate (Partridge, 1948*a*) was used. Ammoniacal silver nitrate solution was also tried occasionally but in view of the fact that the aniline oxalate develops a beautiful pink colour for pentose, yellow for rhamnose, brown to yellow for hexoses and a pinkish brown colour for uronic acid, aniline oxalate was preferred to silver nitrate.

General procedure.—As one dimensional chromatography has been used in all cases, it was sometimes essential to run the chromatograms as long as 72 hours in moist phenol and 128–168 hours in moist *n*-butanol. The latter procedure has been found to be quite suitable to separate glucose from galactose. In the case of ethyl acetate-water-pyridine mixture (Jermyn and Isherwood, 1949), it has been found that 32 hours are quite suitable for this purpose. 3 μl solution was used for each spot.

D. Identification of sugars.—

The colour developed by different sugars in the chromatogram after spraying with aniline oxalate together with their relative positions were the primary guides for their identification. But as there is the possibility of the presence of some of the sugars either only in traces or of similar R.F. value, both of which prevent separation, misleading information is likely to obtain by the normal chromatography alone. This is especially found to be true in the case of identification of galactose in the hydrolysate in presence of glucose, the former being present in jute holocellulose only in small proportion to that of the latter. For this purpose it is desirable to separate the individual sugars by paper chromatography followed by the characterisation by their physical properties (e.g. optical rotation) and compound formation. While this is being in progress and the results will be communicated later on, in the present investigations the sugars were separated by paper chromatography and provisionally identified in the following way.

No. 3 Whatman filter paper was generally used for this purpose. Consecutive spots of same dimension of the syrups of different fractions were spotted on the starting line of a 20 cm. wide paper (length 55 cm.) and the chromatogram was run in moist phenol for about 72 hours. It was then dried at 105°C. and a 2 cm. strip from side across the length was cut off and sprayed with aniline oxalate reagent and then the strips were developed by baking as usual. The different strips from the under-developed portion corresponding to the different bands of sugars were then cut off, across the width of the paper. These strips were then extracted separately with water and evaporated to a syrup as usual. The purity of different sugars obtained and their identifications were tested by further chromatography and whenever possible by specific colour tests as explained below:

(i) *By repeated chromatography.*—About a 7 cm. wide filter paper was spotted with three different spots, one with the test syrup, the next with the corresponding pure reference sugars and the last with a mixture of test solution and corresponding pure sugar. The chromatograph was then run in two different solvents for sufficient

length of time to effect separation of sugars of close R.F. values, if present. In most of the cases single non-diffused spot was observed indicating pure sugar but occasionally some mixtures of two sugars were also obtained. In the latter case the process of chromatographic separation (in these cases by No. 1 Whatman filter paper) as described above was repeated when pure sugars were obtained. This was especially experienced in separating and identifying galactose in presence of glucose.

From the rate of travel, the spot No. 2 was considered to be a di- or tri-saccharide. This isolated sugar when heated with naphthoresorcinol and hydrochloric acid and then extracted with ether, the extracted ether solution gave purple colour whereas the aqueous liquid was green indicating the presence of uronic acid and other non-extractable sugar. Spot No. 2 when further hydrolysed with 95% formic acid for 1 hour gave spots for methyl glucuronic acid and xylose only.

(ii) *By colour tests.*—Several colour reactions have been used to identify the sugars. These are naphthoresorcinol and hydrochloric acid test for Forsyth (1948); naphthoresorcinol and trichloracetic acid test of Partridge (1948*a*); aniline phthalate in butanol as suggested by Partridge (1949) and further extended by Hough, Jones and Wadman (1950); aniline oxalate in water (Partridge, 1948*b*) and lastly the usual basic lead acetate test. The test results of the different syrups with these coloured reagents are recorded in Table 5.

TABLE 2

Degree of hydrolysis and degradation of holocellulose by different acids

Acid used for hydrolysis	Concentration of the acid	Duration of hydrolysis (hrs.)	% Total holocellulose	Test for furfural on the first distillate (intensity of colour reaction)
Formic ..	95%	½	31·9	Faint
,, ..	,,	3	33·9	+
,, ..	,,	6	37·4	++
,, ..	,,	12	49·3	+++
,, ..	,,	24	73·0	++++
,, ..	,,	48	88·8	++++
,, ..	,,	72	96·0	++++
Hydrochloric ..	1*N*	24	38·0	++++
,, ..	2*N*	,,	62·0	++++
,, ..	4*N*	,,	84·4	++++
,, ..	6*N*	,,	85·0	++++
Sulphuric acid ..	72%	18	100·0 (approx.)	++++

Above results show that when the duration of hydrolysis with formic acid is ½ hour, decomposition of the pentoses and uronic acid is negligible or very little although about one-third of the total holocellulose is dissolved under this condition. Considering hemicellulose is likely to be attacked first than the α-cellulose, it should more or less represent the hemicellulose part of the holocellulose. As the jute holocellulose contains about 28·5% hemicellulose it seemed possible that the major part of hemicellulose, if not all, plus a small portion of α-cellulose are dissolved during this treatment.

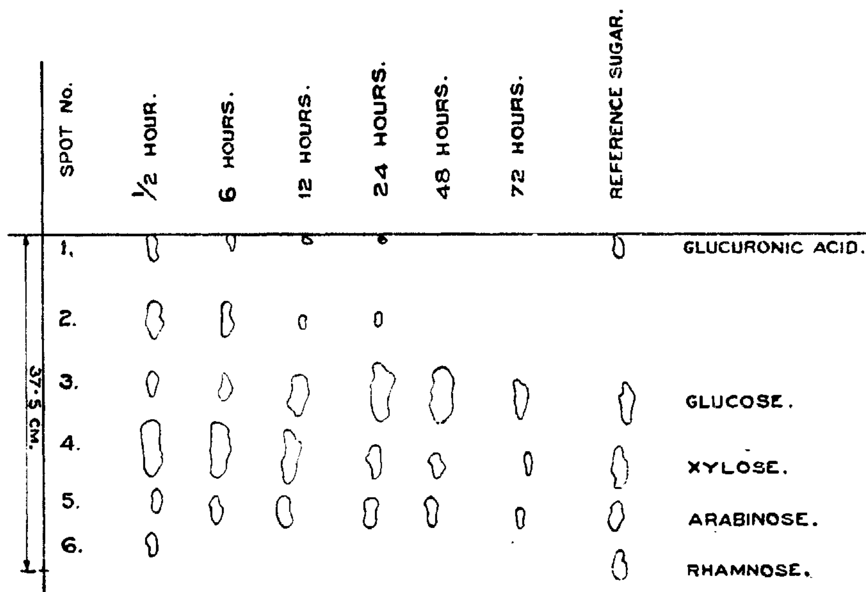

Fig. 1

Chromatogram in moist phenol. (72 hrs.) (Holocellulose hydrolysed by formic acid.)

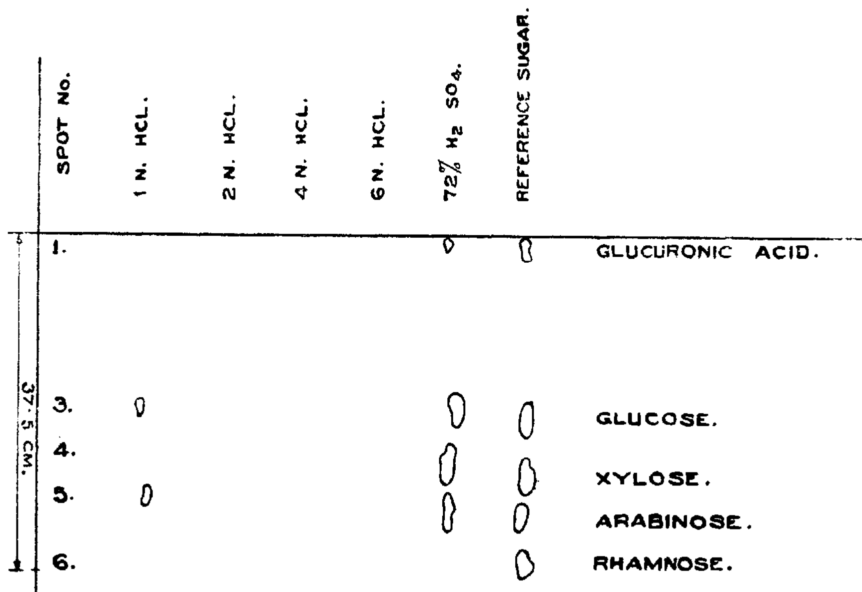

Fig. 2

Chromatogram in moist phenol. (72 hrs.) (Holocellulose hydrolysed by mineral acid.)

In the case of hydrolysis with hydrochloric acid, however, although the loss in weight with 1N acid is 38·0%, a value similar to that of formic acid when the duration is 6 hours, the high intensity of the furfural yield with the former method indicates that formic acid method is superior to hydrochloric acid method.

TABLE 3

Colour intensity of the spots shown in Figs. 1 and 2 after spraying with aniline oxalate solution

Ref. Sugar arranged in descending line	Glucuronic acid		Glucose and galactose	Xylose	Arabinose	Rhamnose
Spot No. (Figs. 1 and 2)	1	2	3	4	5	6
(1) Hydrolysate by formic acid; duration—						
(a) ½ hr. ..	+++	+++	+	++++	++	++
(b) 6 hrs. ..	++	+++	+	++++	+++	(+)
(c) 12 ,, ..	+	++	++	++++	+++	–
(d) 24 ,, ..	+	+(+)	+++	+++	+++	–
(e) 48 ,, ..	Faint	(+)	++++	+(+)	++	–
(f) 72 ,, ..	–	–	++++	+	+(+)	–
(2) Hydrolysed by HCl for 24 hrs.; strength of acid being—						
(a) 1N ..	–	–	+	–	+	–
(b) 2N ..	–	–	–	–	–	–
(c) 4N ..	–	–	–	–	–	–
(d) 6N ..	–	–	–	–	–	–
(3) Hydrolysed by 72% sulphuric acid ..	+	–	+++ (no galactose)	++	+	–
Reaction with aniline oxalate after spraying and roasting ..	Pinkish yellow	Yellowish pink	Yellow brown	Pink	Pink	Yellow

This is clearly evident from the results of chromatography that while six spots could be identified with the formic acid method, only two have been noticed with HCl acid method and four by 72% H_2SO_4 method. Galactose and rhamnose have been completely missed by both the mineral acids hydrolysis whereas the uronic acid could be found with H_2SO_4 hydrolysate only in a small quantity but none with HCl hydrolysate. Spot No. 2, while very strong in the formic acid hydrolysate, is absent in the mineral acid hydrolysate. All these, therefore, suggest in favour of using formic acid method of hydrolysis. To ensure less degradation and also for convenience it was, however, thought desirable to use more a diluted form of formic acid available in the market (85% Merck) and this has been used in all the subsequent experiments.

FIG. 3. *Distribution of holocellulose in different fractions*

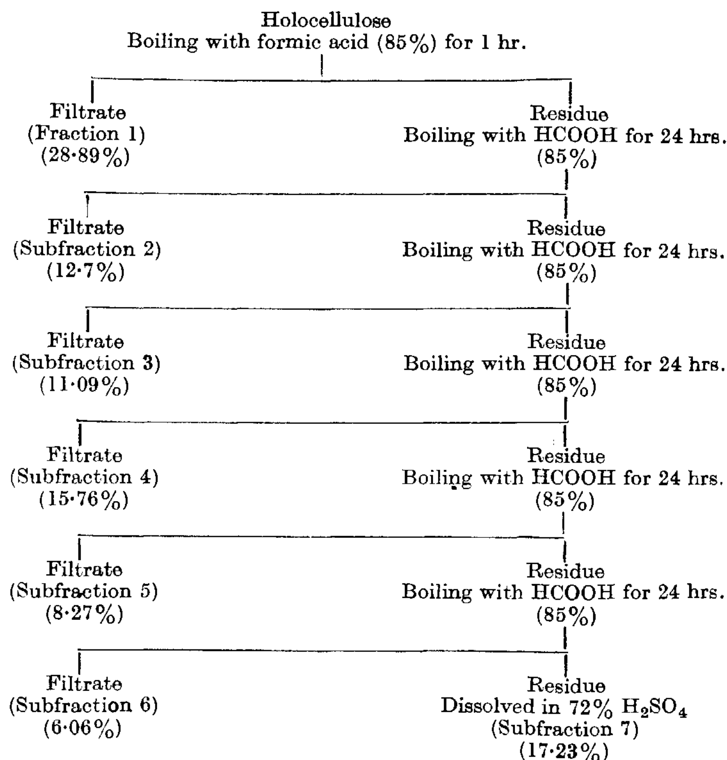

TABLE 4

Colour intensity of the spots shown in Fig. 4 after spraying with aniline oxalate

Ref. Sugar arranged in descending line	Glucuronic acid		Glucose and galactose	Xylose	Arabinose	Rhamnose
Spot No. (Fig. 4)	1	2	3	4	5	6
Fraction 1 ..	++++	+++	+	++++	++	+
Subfraction 2 ..	+	+	+++	++++	+++	−
,, 3 ..	−	−	+++	++	++	−
,, 4 ..	−	−	++++	+	+	−
,, 5 ..	−	−	++++	+	+	−
,, 6 ..	−	−	++++	(+)	+	−
,, 7 ..	−	−	++++	−	−	−
Reaction with aniline oxalate after spraying and roasting ..	Pinkish yellow	Yellowish pink	Yellow brown	Pink	Pink	Yellow

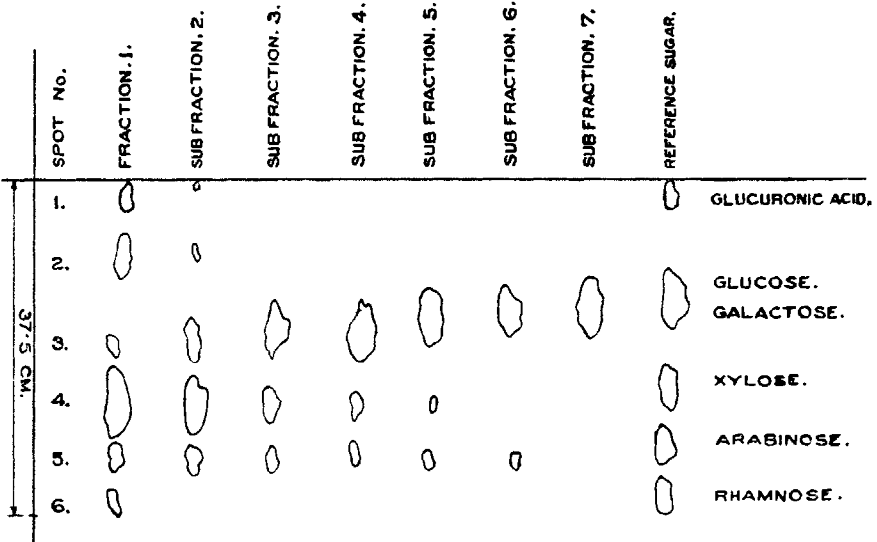

FIG. 4

Chromatogram of different fractions of jute holocellulose in moist phenol. (72 hrs.)

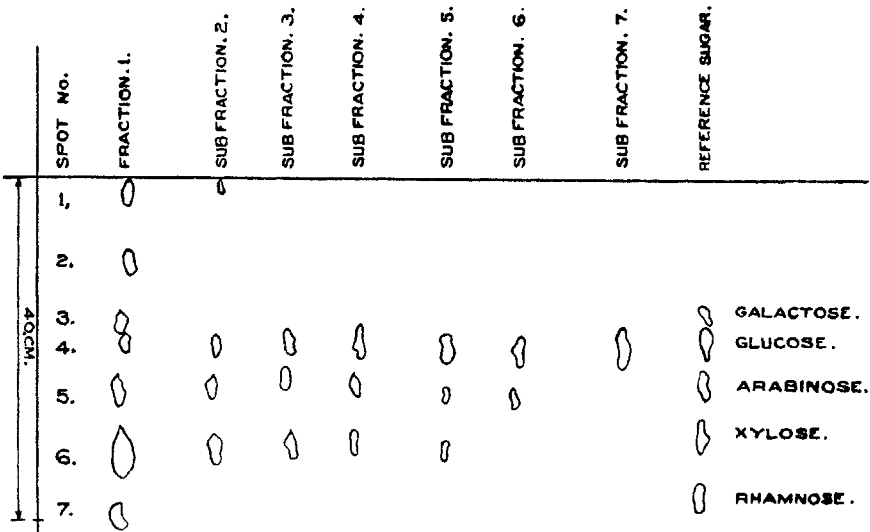

FIG. 5

Chromatogram of different fractions of jute holocellulose in moist *n*-butanol. (128 hrs.)

TABLE 5

Colour intensity of the spots shown in Fig. 5 after spraying with aniline oxalate

Ref. Sugars arranged in descending line	Glucuronic acid		Galactose	Glucose	Arabinose	Xylose	Rhamnose
Spot No. (Fig. 5) ..	1	2	3	4	5	6	7
Fraction 1	++++	+++	++	+	++	++++	+
Subfraction 2	+	(+)	—	+++	+++	+++	—
,, 3	—	—	—	+++	+++	++	—
,, 4		—	—	++++	++	+	—
,, 5	—	—	—	++++	+	+	—
,, 6	—		—	++++	+	—	—
,, 7	—	—	—	++++		—	—
Reaction with aniline oxalate after spraying and roasting	Pinkish yellow	Yellowish pink	Yellow to brown	Brown	Pink	Pink	Yellow

TABLE 6

Identification by colour tests

Colour reagents	SPOT NO. (from Tables 3 and 4)						
	in moist n-butanol (Table 4)						
	1	2	3	4	5	6	7
	in moist phenol (Table 3)						
	1	2	3	3	5	4	6
Aniline oxalate	Pinkish brown	Pinkish brown	Brown	Brown	Pink	Pink	Yellow
Heated with naphthoresorcinol and HCl	Violet blue extractable with ether	Green partly extractable with ether	Green not extractable with ether	Green not extractable with ether	Green not extractable with ether	Green not extractable with ether	Red not extractable with ether
Naphthoresorcinol and trichloracetic acid . .	Green	Green	Green	Green	Bluish green	Bluish green	Green
Aniline phthalate in n-butanol	Pink	Reddish brown	Yellowish brown	Yellowish brown	Red	Red	Yellowish brown
p-anisidine hydrochloride in n-butanol	Cherry red	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Heated with basic lead acetate	Light brown ppt.	Brown ppt.	Brick red ppt.	Brown ppt.	Brick red ppt.	Brown ppt.	No change in colour
Inference	Methyl glucuronic acid	Methyl glucuronic acid/xylose complex	Galactose	Glucose	Arabinose	Xylose	Rhamnose

Discussion

The results presented in Tables 2 and 3 and Figs. 1 and 2 show that under the conditions of our experiments hydrolysis by formic acid ensures less degradation of the different sugars present in jute holocellulose than in the case with either hydrochloric or sulphuric acid. Thus formic acid method has been found to be especially suitable in detecting rhamnose which is apparently present in jute holocellulose only in small quantities and also uronic acid.

Fig. 5 shows that glucose can be completely separated from galactose by means of moist *n*-butanol when the duration of chromatogram is as high as 128 hours. Using this solvent as well as that of Jermyn and Isherwood (1949) no galactose has been detected in the hydrolysate of jute holocellulose when the hydrolysis is carried out by 72% sulphuric acid method, whereas the same is found in fraction 1 (Fig. 5) obtained by the formic acid method. These observations together with the fact that formic acid can hydrolyse jute holocellulose, a part at least, to simple sugars suggest that this acid should preferably be used in hydrolysing different carbohydrates especially when identification of different sugar constituents and preparation of some oligosaccharides (Das, Mitra and Wareham, 1953) are aimed at. Practically all jute holocellulose (96%) is dissolved with formic acid when the duration of hydrolysis is 72 hours. In this respect the values recorded in Fig. 3, however, are much lower than those of the corresponding ones in Table 2. This is mainly due to the fact that in obtaining the values recorded in Fig. 3, the process of hydrolysis was not continuous as in the other case but was interrupted at different stages followed by washing and drying with the repeated formation of hard masses which are less prone to attack by the acid. The lower value is also due to the more dilute (85%) acid being used for obtaining the results recorded in Fig. 3 than that (95%) for the results of Table 2.

When however the duration of hydrolysis is high as is required for dissolving higher percentages of holocellulose, degradation of sugars occurs as is evident from the presence of furfural (Table 1). From Figs. 1 and 2 and Table 3 the absence of rhamnose, uronic acid and with only a trace of xylose is especially remarkable. Spot 2 is further hydrolysed and finally disappears when duration of hydrolysis is over 48 hours. Arabinose and glucose seem to be less prone to attack by formic acid. All these inferences support the recent study of Das, Mitra and Wareham (unpublished investigation) on the rate of decomposition of different sugars with formic acid obtained with Somogyi's (1945) reagent.

From the chromatographic results recorded in Figs. 4 and 5, Tables 4 and 5 and also the colour tests recorded in Table 6, methyl glucuronic acid, glucose, galactose, xylose, arabinose and rhamnose have been provisionally identified in jute holocellulose. This has been supported by separating these sugars and identifying them by further chromatography as explained previously. That the uronic acid component is gluco—and not galacto—has been concluded from its failure to give any mucic acid test but forms saccharic acid which was identified in the form of *k*-salt.

Spot No. 2 has been separated and identified as a compound of methyl glucuronic acid and xylose from the observation that on further hydrolysis of this compound with formic acid it splits up into these two sugars. This proves the existence of methyl uronic acid-xylose linkages in the jute hemicellulose chains.

The above results also show that the seven fractions or subfractions obtained by the formic acid method from the jute holocellulose differ in chemical composition. First two fractions represent most of the hemicellulose and only a small amount of glucose, coming presumably from α-cellulose. None of the other subfractions contain any uronic acid, oligosaccharide or rhamnose but all of them except the last one more or less contain glucose, arabinose and probably xylose thus supporting the view of Das, Mitra and Wareham (1952, 1953, 1954) that cellulosan exists in

jute. The absence of xylose or its presence only in traces in the last two fractions is due to the possibility of this sugar being seriously decomposed as the duration of hydrolysis is very great.

That arabinose is present in every fraction or subfraction of holocellulose and also that the rate of hydrolysis of arabinose in jute is slow supports the suggestion of Das, Mitra and Wareham (1953) that arabinose might be present in jute in pyranose form.

Summary

It has been shown that under the conditions of the experiments hydrolysis by formic acid causes much less destruction of jute holocellulose than is the case with mineral acids.

From the rate of hydrolysis of jute holocellulose it seems that the degree of decomposition of xylose, glucuronic acid and rhamnose by formic acid is quite marked whereas in the case of arabinose and glucose it is less pronounced especially in the latter.

The formic acid method has been found suitable for the detection of uronic acid and also rhamnose, the latter being present in jute only in a small amount.

Methyl glucuronic acid, galactose, glucose, xylose, arabinose and rhamnose have been chromatographically separated and provisionally identified from jute holocellulose.

A complex (spot No. 2) has been detected which indicates the presence of a linkage between methyl uronic acid and xylose.

It has been shown that galactose can easily be separated from glucose by using n-butanol as the solvent if the time of chromatograph is as high as 168 hours.

By fractional hydrolysis of jute holocellulose, seven fractions or subfractions were obtained. Some of these have been shown to differ in chemical composition.

The presence of pentose with glucose in almost all the subfractions supports the cellulosan theory.

It supports the suggestion that arabinose in jute, some of it at least, exists in pyranose form.

Acknowledgement

The authors record their thanks to the Directors, Messrs. Jardine Henderson, Ltd., for permission to publish this paper.

References

Bose, R. K., and Burma, D. P. (1952). Paper chromatography in the study of sugar constituents of jute hemicellulose. *Science and Culture*, **18**, 39-40.

Chattopadhyay, H., and Sarkar, P. B. (1946). A new method for the estimation of cellulose, with special reference to jute. *Proc. Nat. Inst. Sci. Ind.*, **12**, 23–46.

Consden, R., Gordon, A. H., and Martin, A. J. P. (1944). Qualitative analysis of proteins; a partition chromatographic method using paper. *Biochem. J.*, **38**, 224–232.

Das, D. B., Mitra, M. K., and Wareham, J. F. (1952). A study on the association of xylan with α-cellulose in jute by partition chromatography. *Science and Culture*, **18**, 249-250.

——— (1953). Association of glucose, arabinose and xylose in jute. *Nature*, **171**, 613-614.

——— (1954). Pentoses associated with jute α-cellulose. *Nature*, **174**, 228-229.

Das, D. B., Roy Chaudhuri, P. K., and Wareham, J. F. (1952). A note on the constitution of jute by paper chromatography. *Science and Culture*, **18**, 197.

Doreé, C. (1947*a*). Method of cellulose chemistry, London, Chapman & Hall, 377.

——— (1947*b*). *Ibid.*, 393.

Forsyth, W. G. C. (1948). Colour reagents for paper chromatography of sugar. *Nature*, **161**, 239-240.

Hibbert, H., Henderson, W. F., Johnsen, B., Mitscherling, W. O., and Wise, L. E. (1923). The preparation of standard cotton cellulose and the proposed method of analysis. *J. Indus. Eng. Chem.*, **15**, 748–751.

Hough, L., Jones, J. K. N., and Wadman, W. H. (1950). Quantitative analysis of mixtures of sugars by the method of partition chromatography. *J.C.S.*, **345**, 1702–1706.

Jermyn, M. A., and Isherwood, F. A. (1949). Improved separation of sugars on the paper partition chromatogram. *Biochem. J.*, **44**, 402–407.

Norman, A. G., and Jenkins, S. H. (1933). A new method for the determination of cellulose, based upon observation on the removal of lignin and other encrusting materials. *Biochem. J.*, **27**, 818–831.

Partridge, S. M. (1946). Application of paper partition chromatogram to the qualitative analysis of reducing sugars. *Nature*, **158**, 270-271.
——— (1948*a*). Filter paper partition chromatography of sugars. *Biochem. J.*, **42**, 238–250.
——— (1948*b*). Application to carbohydrate studies. *Biochem. J.*, **43**, Proc. XLVIII.
——— (1949). Aniline hydrogen phthalate as a spraying reagent for chromatography of sugars. *Nature*, **164**, 443.
Sarkar, P. B., Mazumdar, A. K., and Pal, K. B. (1950). Association of xylan with α-cellulose in jute. *Nature*, **165**, 897-898.
Sarkar, P. B., Chatterjee, H., Mazumdar, A. K., and Pal, K. B. (1948). The combination between lignin and polyuronic acid in jute fibre. *J. Textile Institute*, **39**, T1–T7.
Somogyi, M. (1945). A new reagent for determination of sugars. *J. Biol. Chem.*, **160**, 61–68.
Spoehr, H. A. (1947). The hydrolysis of alginic acid with formic acid. *Archives of Biochemistry*, **14**, 153.
Taylor, M. C., White, J. F., Vincent, G. P., and Cunningham, G. L. (1940). Sodium chlorite—Properties and reactions. *J. Indus. Eng. Chem.*, **32**, 899–903.

Issued December 23, 1955.