Researches by the Chromatography. IV.
On the Impurities of Cotton Cellulose and Wood Cellulose

By Akira HATANO and Hiroshi SOBUE

Introduction

In previous papers[1,2,3] we have reported some preliminary investigations carried out in order to determine qualitatively or quantitatively the hydrolyzate of some species of wood pulps by means of paper chromatography. From these investigations it was evident that glucose, xylose, mannose, arabinose, galactose and uronic acids of these sugars are the building units of the polysaccharides of the wood pulps investigated. Recently remarkable progress has been made

1) H. Sobue and A. Hatano, *J. Chem. Soc. Japan, Ind. Chem. Sect.*, **54**, 460 (1951).
2) H. Sobue and A. Hatano, ibid. **55**, 131 (1952).
3) H. Sobue, K. Matsuzaki, A. Hatano and Y. Arisawa, *J. Soc. Tex. Cell. Ind. Japan*, **8**, 79 (1952).

in the purification of wood pulps in both scientific and industrial fields, and high grade wood pulps are now being appreciated as the materials for tire cord rayon, acetate rayon, nitrocellulose, Bemberg etc. instead of cotton linters.

A number of research workers have studied the difference of the chemical components, morphological structures, fine structures, chain length distribution and reactivity or accessibility between these two different cellulosic materials[4]. The authors have studied their impurities and obtained some interesting results, which are expected to offer a number of unsolved problems. These will be described below.

Materials and Methods

Egyptian cotton linters (Gossypium cananilles), Japanese red pine (Pinus densiflora) and Japanese beech (Fagus crenata) were used as the materials. Their chemical composition is analysed by Tappi standard method and is tabulated in Table 1. The

Table 1

Chemical Components of Cotton Linters, Red Pine and Beech

	Cotton Linters	Red Pine	Beech
	%	%	%
Total Cellulose	99.0	54.7	56.8
Alpha Cellulose	96.8	38.3	41.5
Beta Cellulose	1.1	4.2	7.2
Ash	0.18	0.27	0.33
Resin	0.45	3.08	2.67
Lignin	—	28.0	23.6

raw cotton is extracted with alcohol-benzene (1 : 1) for six hours and boiled carefully with 1% sodium hydroxide for fifteen hours. The sample is washed with water and then with diluted acetic acid and washed again with water and dried in air. Thus, purified cotton is obtained. The material is infused in 69% sulfuric acid and kept for three days at 0~5°C, and this sulfuric acid solution is diluted into 2% sulfuric acid with water and hydrolysis is carried out under boiling. The hydrolyzate is neutralized with barium carbonate and concentrated by evaporation in a vacuum. The aliquot of the concentrated solutions is placed on one end of a filter-paper strip and developed with *n*-butanol-acetic acid-water (4 : 1 : 1) and partitioned into its component. Toyo filter paper No. 50 is used exclusively in these experiments. Standard sugars and amino acids are obtained from commercial sources. Strips are dried in air and some of them are sprayed with an acetone solution of ninhydrin and the amino acids are identified. Some of them are sprayed with a *n*-butanol solution of anilin-hydrogen-phthalate, and the monosaccharides and uronic acids are identified.

Experimental Results and Discussion

In the hydrolyzates of raw cotton linters, there are found arabinose and small amounts of galactose, galacturonic acid and glucuronic acid. It is not clear whether these uronic acids are the original components of linters or produced in the process of hydrolysis. According to the result of determination with a photoelectric photometer[1], arabinose is about 1.5% of glucose in the hydrolyzates. Pectin has been noticed in raw cotton linters and is said to be present in the fiber inner surface, primary wall or secondary wall with the components of wax. A long time ago, Ehrlich[5] et al. stated that pectin is composed of 4 moles of *dl*-galacturonic acid, 1 mole of *l*-arabinose and 1 mole of *d*-galactose. But the details on the relationship between cellulose and arabinose, galactose etc. will be discussed in the following paper.

The hydrolyzate of purified cotton linters contains almost no impurities besides the traces of arabinose and glucuronic acid. This fact shows that the prification of cotton linters is easy and is thought to be an important difference from the case of wood pulps[2,3].

The amino acids in the hydrolyzates of the samples which show positive ninhydrin reactions are cystine, aspartic acid, asparagine, arginine, glutamic acid, alanine and tryptophane. The *Rf* value of various amino acids are illustrated in Table 2.

Table 2

Rf Values of Amino Acids in Butanol-Acetic Acid-Water (4 : 1 : 1) mixed Solution at 25°C.

Amino Acids	*Rf* Value
Crystine	0.08
Arginine	0.14
Aspartic Acid	0.24
Glutamic Acid	0.31
Asparagine	0.37
Alanine	0.43
Tryptophane	0.60

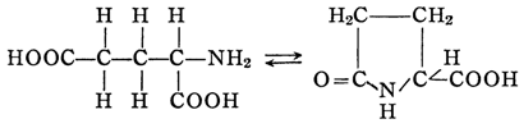
All of these amino acids are contained in any of the cotton linters, red pine and beech,

4) J.W. Bailey, *Ind. Eng. Chem.*, **30**, 40 (1938); A. Meller. *Paper Trade J.*, **124**, No. 9, 104 (1947); W. Klauditz, *Holz Roh-u Werkstoff*, **4**, 314 (1941); E.J. Lorand and E.A. Georgi, *J. Am. Chem. Soc.*, **59**, 1166 (1937); H. Haas, *Das Papier*, **2**, 397 (1948); R. Bartunek, *Cellulosechemie*, **22**, 56 (1944).

5) F. Ehrlich, *Z. Angew. Chem.*, **43**, 1072 (1930).

although in different amounts. The amino acids in the hydrolyzates are naturally assumed to have originated from the proteins made of polypeptide links which are composed of these amino acids. These proteins are assumed to be the rudiments of the protoplasmas of plant cells. The living cells stop their functions and their protoplasma is supposed to adheres in a thin layer to the inside of the secondary cell wall which is made of cellulose molecules. It is of extreme interest to this cover that the amino acids composing the proteins appear in the same kinds, but in not the same amounts, in any of the cotton linters, red pine and beech.

On the chromatogram colorized with ninhydrin, an elliptical spot is found in a fairly wide area in the region of around 0.3~0.4 *Rf* value. Glutamic acid which is a monoamino dicarboxylic acid shows an equilibrium reaction in its aqueous solution as shown in Fig. 1. The reaction is said to proceed readily to



Gultamic Acid — Pyrrolidoncarboxylic Acid

Fig. 1.—Equilibrium Reaction of Gultamic Acid

the right, when its aqueous solution is heated or is made acidic[6]. This iminized pyrrolidoncarboxylic acid is not colorized reddish violet with ninhydrin but is colorized yellow. On the other hand, the spraying with bromophenol-blue indicator, which is made up as a 0.1% solution in 95% ethanol, on this acid makes its differentiation easy by colorizing it yellow in the blue background. Pyrrolidoncarboxylic acid is synthesized from purified standard glutamic acid or glutamine and it is found that the *Rf* value of this synthesized pyrrolidoncarboxylic acid which is determined corresponds perfectly to that of the above mentioned spot. Therefore, it is presumed that a part of glutamic acid in cotton linters etc. is iminized in the process of hydrolysis and changes to pyrrolidoncarboxylic acid. And among the amino acid components, aspartic acid which is another monoaminodicarboxylic acid is iminized to some extent, but it is much more stable and unchangeable.

Approximate determination of the amino acids in each material is carried out by using the photoelectric photometer[1] and the planimeter[7], and it is recognized that the content of the protein in raw cotton linters, red pine and beech is about 0.2~0.3% respectively.

On the other hand, almost no amino acids are noticed in the purified linters. Hitherto, the crude protein content in plants has been determined by multiplying the nitrogen content with 100/16 i.e. 6.25, and it is thought that the author's value is a little smaller than the values of other workers. This is supposed to be due to the loss of protein on account of its change to fumine and besides this possibility, the presence of the nitrogen materials other than protein, such as particular alkaloids, nitrates, ammonia, acid amides etc. must be taken into consideration. The nitrogen contents of plants are fairly different according to the seasons of harvesting, the parts of wood, the growing places, the age of plants and the nitrogen contents of the soil.

In the hydrolyzates of red pine and beech sulfite rayon pulps, cystine, alanine and pyrrolidoncarboxylic acid are constantly found and also the hydrolyzates of viscose rayon and staple fibers made of sulfite rayon pulp constantly contain pyrrolidoncarboxylic acid. But in sulfate rayon pulps treated by alkali cooking, its content is very small. On the other hand, lignin separated from wood is also said to contain proteins[8]. The fact that the amino acids still remain intact in spite of the relatively drastic treatment such as cooking of wood or production process of viscose from rayon pulps, suggests the possibility that the proteins of wood are combined very firmly with the cellulosic materials of the cell wall. It also seems probable that the nitrogen components of wood pulps consume chlorine by producing chloramine in the process of bleaching, but these components are present in such minute amounts that their effects are thought to be almost negligible. However, it is also probable that the nitrogen in a minute amount behaves in a particular fashion in high grade wood pulps.

Summary

Egyptian cotton linters, its purified linters, Japanese red pine and beech were hydrolyzed. It has been shown by paper chromatography that the hydrolyzates contain certain monosaccharides, uronic acids and amino acids.

6) C. Okinaka, *Sexagint*, **1927**, 27.

7) R.B. Fisher, D.S. Parsons and G.A. Horrison, *Nature*, **161**, 764 (1948).

8) L. Polohelmo, *Biochem. Z.*, **165**, 463 (1925); **214**, 161 (1929); M. Phillips, *J. Assoc. Official agr. Chem.*, **15**, 118 (1932); **22**, 422 (1939); S. A. Waksman and K. R. Stevens, *Ind. Eng. Chem. Anal. Ed.*, **2**, 167 (1930).

Raw cotton linters have been found to contain, besides glucose, about 1.5% arabinose, and a small amount of glactose, glucuronic acid and galacturonic acid.

The amounts of the various amino acids contained in the hydrolyzates of raw cotton linters, red pine and beech are somewhat different from each other, but the kinds of the amino acids are the same and they are assumed to be cystine, aspartic acid, aspargine, arginine, glutamic acid, alanine and tryptophane.

Glutamic acid changes to pyrrolidoncarboxylic acid in the process of hydrolysis of the materials and shows special colorizations reactions with ninhydrin and bromo-phenol-blue indicator.

Purified cotton linters and sulfate rayon pulps treated by alkali cooking do not contain any amino acid, but sulfite rayon pulps contain cystine, alanine and pyrrolidoncarboxylic acid.

Faculty of Engineering,
University of Tokyo, Tokyo