On the Mechanism of Pulp Bleaching. IV. Extracts from Unbleached Sulfite Pulp with Dimethyl Sulfoxide*

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As already reported, a part of the residual lignosulfonic acid in unbleached sulfite pulp can be dissolved by beating the pulp in water violently[1]. This lignin seems to have existed between fibrils. The chemical nature of this residual lignosulfonic acid was also investigated briefly. About 75~80% of the residual lignosulfonic acid remained, however, undissolved in the beaten pulp. It is therefore attempted, in the present communication, to isolate and investigate this residual lignosulfonic acid remaining in the beaten pulp.

Recently Björkman succeeded in extracting the so-called lignin-carbohydrate complex (LCC) with a solvent like acetic acid, dimethyl formamide or dimethyl sulfoxide, after having extracted the so-called milled wood lignin (MWL) from the finely milled wood powder with aqueous dioxane[2].

Lindgren[3] separated this LCC into a pure carbohydrate component consisting of carbohydrate and lignin chemically combined in a one to one ratio by using the technique of electrophoresis on glass fiber paper. Kawamura and Higuchi[4] isolated lignin preparation from beech wood by several different methods and recognized paper-chromatographically that a part of the lignin is chemically combined with xylose and xylobiose. In his investigation of the biosynthesis of lignin, Freudenberg obtained a compound consisting of both carbohydrate and coniferyl alcohol chemically combined[5]. A recent accumulation of experimental results seems to have confirmed almost definitely, the existence of a chemical combination of both the components in wood, although the mode of the combination remains to be investigated as yet.

It has, therefore, been attempted to dissolve the lignin, carbohydrate and lignin-carbohydrate complex, if any, from the unbleached sulfite pulp, which has already been beaten in water and extracted exhaustively with water, with dimethyl sulfoxide, to see if such materials can be extracted with this solvent and, if so, to investigate the chemical nature of the extracted materials.

Already extracted beaten pulp was repeatedly extracted further with dimethyl sulfoxide, and a grayish white powder was obtained. The yield was of the order of 10% of the pulp. The material contains about 6% methoxyl group, has ultraviolet absorption spectra characteristic of lignin, and gives a sugar mixture on hydrolyzation of the material. It is therefore evident that the materials contains both lignin and carbohydrate. In order to know whether the carbohydrate component and the lignin component exist independently or whether they are combined chemically in the material, the material was separated electrophoretically on a filter paper; it was found that there exists no chemical combination between the two components. The carbohydrate component and the lignin component were next separated by zone electrophoresis on cellulose powder, and both the components were investigated separately.

Preparation of the Dimethyl Sulfoxide (DMSO) Extract.—Unbleached sulfite paper pulp from spruce (*Picea jezoensis*, 1615 g.) was beaten in water until no more water-soluble residual lignosulfonic acid dissolved out, and then the beaten pulp was dried, crushed with a disk refiner, and respectively extracted with absolute dimethyl sulfoxide at room temperature (total amount of dimethyl sulfoxide: 50 l.). Figure 1 shows the ultraviolet absorption curves of DMSO extracts from spruce pulp. The dimethyl sulfoxide solution thus obtained was concentrated in vacuo and poured into alcohol, and the precipitate was dissolved in water and reprecipitated with alcohol. The yield of the water-soluble grayish-white "dimethyl sulfoxide extract" thus obtained was 18 g.

Dimethyl Sulfoxide Extract.—As no sugars are found in the extract by paper chromatography, the carbohydrate portion of the extract must be water soluble hemicellulose. The amount of hemicellulose in the extract and the component sugars were determined by hydrolyzing the extract (50 mg.) with 4% sulfuric acid (4 ml.) in an ampoule at 100°C for 8 hr.

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1) K. Sato and H. Mikawa, This Bulletin, **35**, 477 (1962).
2) A. Björkman, *Svensk Papperstidn.*, **60**, 243 (1957).
3) B. O. Lindgren, *Acta Chem. Scand.*, **12**, 447 (1958).
4) I. Kawamura and T. Higuchi, *J. Soc. Tex. Cell. Ind., Japan*, **7**, 269 (1951).
5) K. Freudenberg and G. Grion, *Chem. Ber.*, **92**, 1355 (1959).

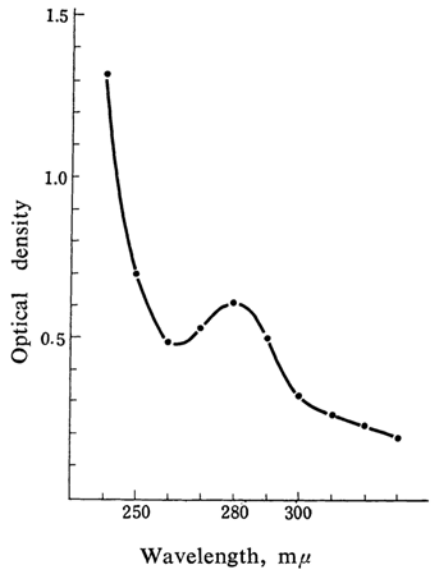

Fig. 1. Ultraviolet absorption curve of the "dimethyl sulfoxide extract" obtained by extracting the beaten unbleached sulfite pulp by dimethyl sulfoxide (52.0 mg. in 300 ml. water).

The total amount of the sugars was determined by Somogyi's method, and the component sugars, by paper chromatography[6]. The amount of the lignin in the extract was estimated from the ultraviolet absorption, the methoxyl content of lignin being assumed as 10.83% and the molar extinction at 280 mμ, as 2870 per CH_3O. The values obtained for the residual lignosulfonic acid in the previous paper[1] were used. The results are shown in Table I. It will be seen from the table that the carbohydrate component consists almost exclusively of xylose.

In order to know the nature of the lignosulfonic acid existing in the dimethyl sulfoxide extract, the extract was titrated conductometrically (Fig. 2). Assuming that all of the strong acid is attached to lignin as a sulfonic acid group, SO_3H/CH_3O was calculated as 0.32, which is considerably lower than that (0.5～0.6) of the ordinary lignosulfonic acid, and the presence of a weakly acidic group is not clear. To make clearer the nature of the residual lignosulfonic acid in the dimethyl sulfoxide extract, the isolation of it free from carbohydrate was attempted.

TABLE I. ANALYSES OF THE "DIMETHYL SULFOXIDE EXTRACT"

Lignin		35.1%	96.9%
carbohydrate		61.8%	
Component sugars	Xylose	94.6%	
	Arabinose* Mannose	1.6%	
	Glucose	2.4%	
	Galactose	1.4%	
CH_3O		5.33%	

* As arabinose shows the same R_f as that of mannose by paper chromatography, it was calculated as mannose.

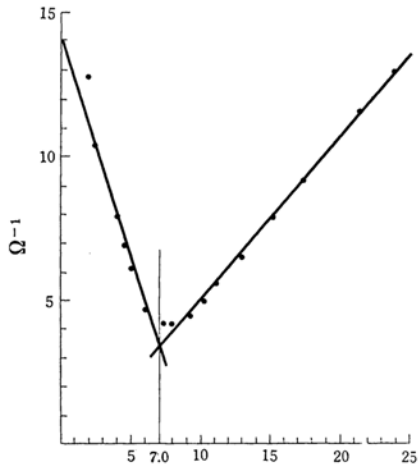

Fig. 2. Conductometric titration of the "dimethyl sulfoxide extract" obtained from the beaten pulp.

The simplest way to isolate the lignin in its sulfonated form, free from carbohydrate, is to sulfonate "the extract" in an acidic condition in order to hydrolyze the linkage between lignin and carbohydrate, if any, and to isolate the lignin in the form of lignosulfonic acid. The extract (4.73 g.) was dissolved in an ampoule in a cooking acid (100 ml. pH 4.2) prepared from 5% sodium hydroxide and sulfur dioxide and cooked at 135°C for 5 hr. The lignosulfonic acid was separated with 1-(*N*-piperidinoacetylamino)-naphthalene as usual. The analytical values of the lignosulfonic acid

TABLE II. ANALYSES OF THE DIMETHYL SULFOXIDE EXTRACT AND THE LIGNOSULFONIC ACID PREPARED FROM THE EXTRACT BY SULFONATION

	Dimethyl sulfoxide extract	Lignosulfonic acid prepared from the extract
CH_3O	5.33%	11.84%
S in SO_3H form	1.77%	4.54%
SO_3H/CH_3O	0.32	0.37
Phenolic OH/CH_3O	—	0.21～0.26
Type I OH/ Type II OH		1 : 4

6) H. Toda and T. Hamada, *J. Jap. Tech. Ass. Pulp. Paper Ind.*, **11**, 429 (1957).

thus prepared and that of the dimethyl sulfoxide extract are shown in Table II.

From the conductometric titration and the spectrum change during the titration[7], the ratio between the type I and the type II phenolic hydroxyl group was found to be ca. 1/4, which is a little lower than that between residual lignosulfonic acid dissolved by beating (1/3～1/4) and ordinary soft wood α-lignosulfonic acid (1/3).

On the Non-existence of the Linkage between Lignosulfonic Acid and Carbohydrate in the Dimethyl Sulfoxide Extract.—As it was thought to be highly possible that the lignin and carbohydrate in the dimethyl sulfoxide extract are chemically combined, the possibility of any linkage between the components was investigated by paper electrophoresis.

An aqueous solution of dimethyl sulfoxide extract was applied quantitatively (1.1 mg. in 0.05 ml.) on the cathodic side of a paper strip (Toyo Roshi Co., No. 50., 2×40 cm.) soaked with a 0.05 N sodium hydroxide solution and developed electrophoretically for 2.5～3 hr., the current density being maintained at 3 mA per paper strip. The paper strip was cut into pieces 2 cm. in length, and every piece was separately eluted with definite amount of water. The eluates were examined spectroscopically. As shown in Fig. 3, the absorption curves are characteristic of lignin. The lignin content of the eluates was therefore calculated from optical density at 280 mμ. For the carbohydrate assay, another paper strip developed similarly at the same time was eluted in just the same manner as in the case of lignin with 1 N sodium hydroxide. Carbohydrate was difficult to elute with water, but with sodium hydroxide it could be eluted very easily. Although the elution was repeated three times, the first elution was almost enough to extract all of the carbohydrate. The carbohydrate thus obtained was estimated as xylose by Somogyi's method.

The distribution of the lignin and the carbohydrate over the paper strip thus discovered is shown in Fig. 4. The values are corrected for the blank values due to the impurities coming from the paper, which were not very large. The total amount of the lignin and the carbohydrate thus recovered from the developed paper strip was nearly 100%, as is shown in Table III.

As is evident from the figure, carbohydrate and lignin migrate almost independently. As it does not seem very probable that some linkage between the components existed before the electrophoresis and that this linkage was ruptured during the electrophoresis, the existence of any chemical linkage may not be expected between lignin and carbohydrate in dimethyl sulfoxide extract, just as in the case

Table III.

Carbohydrate eluted	64.4%*
Lignin eluted	40.2%
Total	104.6%

* % based on the materials applied.

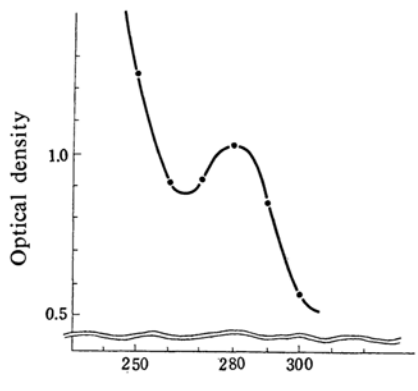

Fig. 3. Ultraviolet absorption curve of the solution of residual lignosulfonic acid obtained by developing electrophoretically the dimethyl sulfoxide extract on a paper strip and eluting the lignosulfonic acid from the strip.

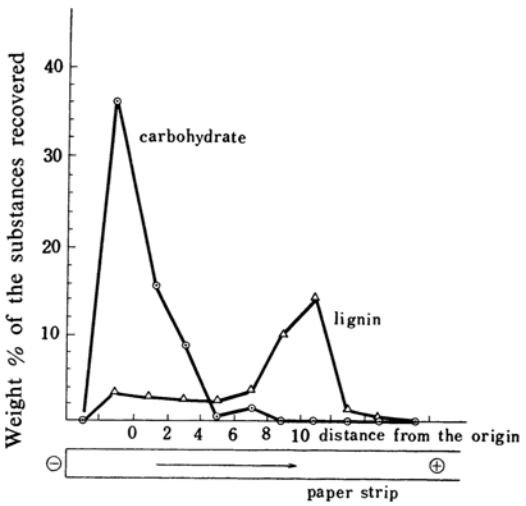

Fig. 4. Distribution of residual lignosulfonic acid and carbohydrate component of dimethyl sulfoxide extract after the development by electrophoresis on a paper strip. The ordinate means the weight percent of the substances obtained by eluting pieces of paper strip cut into 2 cm. length against the total amount of lignin or carbohydrate in the material applied to the origin.

7) H. Mikawa, K. Sato, C. Takasaki and K. Ebisawa This Bulletin, **28**, 653 (1955).

of the residual lignosulfonic acid isolated by beating.

Lignosulfonic Acid in Dimethyl Sulfoxide Extract Separated by Zone Electrophoresis.— In order to investigate the lignin in the extract in detail, the isolation of it free from carbohydrate was attempted. As it was found that electrophoresis is suited for this purpose, zone electrophoresis was applied to the extract. Cellulose powder (Schleicher u. Schüll), which had been exhaustively extracted with 0.05 N sodium hydroxide until the washings no longer showed any absorption at 280 mμ, was used together with 0.05 N sodium hydroxide as the carrier. Five hundred milligrams of DMSO extract was dissolved in a small portion of water, mixed with warm dilute agar-agar solution, and poured into a hole made in the slurry of cellulose powder at the origin of the cathodic side. After the agar-agar solution solidified, the extract was developed with a constant current density of 80 mA per 10 cm^2. This procedure was repeated six times, and then all the carbohydrate-free lignin portions were mixed and eluted with water, and the lignin was separated as barium salt using 1-(*N*-piperidinoacetylamino)-naphthalene as usual. The total yield was 0.557 g. The ultraviolet absorption curve of the lignosulfonic acid thus obtained is characteristic of lignin; the analytical values are listed in Table IV. It may be seen that the degree of sulfonation is low and that the ratio of type II phenolic hydroxyl group to the total phenolic hydroxyl groups is high. This lignosulfonic acid prepared by zone electrophoresis is otherwise not very different from the lignosulfonic acid reported previously, which had been obtained by a drastic beating of pulp.

TABLE IV. ANALYSES OF THE BARIUM SALT OF THE RESIDUAL LIGNOSULFONIC ACID EXTRACTED FROM BEATEN UNBLEACHED SULFITE PULP BY DIMETHYL SULFOXIDE AND PURIFIED BY ELECTROPHORESIS

CH_3O	11.01%
SO_3H/CH_3O	0.34
Total phenolic OH/CH_3O	0.17~0.21
Type I phenolic OH/CH_3O	0.04
Type II phenolic OH/CH_3O	0.13~0.17
Type I OH/type II OH	1 : 3~4
$COOH/CH_3O$	0.15~0.16
Diffussion coefficient of McCarthy's method[8]	9.25 cm^2/day

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8) V. F. Felicetta, A. E. Markham, Q. P. Peniston and J. L. McCarthy, *J. Am. Chem. Soc.*, **71**, 2879 (1951).