15 ml. of glacial acetic acid was cooled in an ice-bath and dry hydrogen chloride was passed through the solution for ten minutes. The intense red solid which separated was filtered by suction, and recrystallized from 70% ethanol saturated with hydrogen chloride. Addition of a 7.5% ferric chloride solution in glacial acetic acid to 3'-methoxy-4'-hydroxyflavylium chloride in the same solvent gave 3'-methoxy-4'-hydroxyflavylium ferrichloride, glistening crystals from glacial acetic acid. *Anal.* Calcd. for $C_{16}H_{13}O_3$-Cl·$FeCl_3$: Fe, 12.4. Found: Fe, 12.6.

3'-Methoxy-4'-hydroxyflavanone.—To a solution of 5 g. of 3'-methoxy-2',4-dihydroxychalcone in 150 ml. of ethanol, heated on the steam-bath, a 3% hydrochloric acid solution was added slowly until a permanent turbidity appeared. The solution was refluxed for 24 hours and cooled, causing the separation of pale yellow crystals. These were filtered, decolorized with charcoal, and recrystallized several times from absolute ethanol, and finally from dry ethyl acetate to give 3'-methoxy-4'-hydroxyflavanone; white crystals, m.p. 171–172°. *Anal.* Calcd. for $C_{16}H_{14}O_4$: C, 71.1; H, 5.2; MeO, 11.5. Found: C, 71.2; H, 5.3; MeO, 11.8.

3'-Methoxy-4,4'-dihydroxyflavan.—3'-Methoxy-4'-hydroxyflavanone (20 g.) and 5 g. of aluminum isopropoxide were covered with 150 ml. of absolute isopropyl alcohol in a round-bottom flask fitted with a short reflux condenser to which was attached another small condenser set for distillation. The solution was refluxed on the steam-bath with no water passing through the reflux condenser. The rate of distillation was 5 drops per minute. A positive test for acetone in the distillate given by 2,4-dinitrophenylhydrazine reagent showed that the reduction of the carbonyl group to the secondary alcoholic group was proceeding. The distillation was continued for about 30 hours, until the distillate, after refluxing for five minutes with water passing through the reflux condenser, gave a negative test for acetone. Most of the isopropyl alcohol was then removed under reduced pressure, and the cooled residue was hydrolyzed with cold dilute hydrochloric acid solution. The yellow crystalline product which separated was filtered, dried, and leached with hot petroleum ether (b.p. 60–110°), giving a white residue. Repeated recrystallizations from dilute ethanol gave 3'-methoxy-4,4'-dihydroxyflavan monohydrate; white needles, m.p. 160–161°. This m.p. was obtained when the temperature of the melting point bath was raised very gradually but, if the rate of heating was fast, the compound melted at a much lower temperature. Analytical values show that the compound retains water of crystallization unless dried under special conditions. *Anal.* Calcd. for $C_{16}H_{16}O_4 \cdot H_2O$: C, 66.2; H, 6.2; MeO, 10.7. Found after drying the compound over phosphorus pentoxide for three hours at 60° and 2 mm. pressure: C, 67.1; H, 6.3; MeO, 11.1. Calcd. for $C_{16}H_{16}O_4$: C, 70.6; H, 5.9. Found after drying the compound for eight hours over phosphorus pentoxide at 11° and 1 mm. pressure: C, 70.6; H, 6.0.

The diacetyl derivative of the above compound was prepared by adding 2 ml. of acetic anhydride to a solution of 0.1 g. of 3'-methoxy-4,4'-dihydroxyflavan in 5 ml. of anhydrous pyridine and keeping the solution overnight at room temperature. On dilution with 50 ml. of ice-water, crystals separated which washed with dilute hydrochloric acid and water and recrystallized from ethanol, gave 3'-methoxy-4,4'-diacetoxyflavan, white crystals, m.p. 121.5–123°. *Anal.* Calcd. for $C_{20}H_{20}O_6$: MeO, 8.9. Found: MeO, 8.7.

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Investigations on Lignin and Lignification. X. The Isolation and Characterization of the Native Lignin from *Kiri* Wood

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Previous work from this Laboratory[1,2] has established that the native lignin isolated from some woods by the method of Brauns[3] is representative of all the lignin present therein. Furthermore, our investigations have revealed that lignins differ not only from species to species, but also within the same species.[4] As an extension to our studies, we have selected to study the lignin from the Japanese tree, *Paulownia tomentosa*, otherwise known as "kiri" wood. To date, nothing has been reported in the literature on the nature of this lignin. Kiri native lignin was isolated in 0.2% yield with ethyl alcohol at room temperature. This lignin was obtained as a white amorphous powder, and the results of its characterization are recorded in Table I.

TABLE I

CHEMICAL COMPOSITION OF KIRI NATIVE LIGNIN

C, %	60.1
H, %	6.2
OCH_3, %	16.6
Acetate, OCH_3, %	13.5
Phenylhydrazone, OCH_3, %	15.2

Kiri native lignin was found to be soluble in methanol, ethanol, dioxane, 1% sodium hydroxide, pyridine, acetone and glacial acetic acid, but was insoluble in petroleum ether, benzene, ether and water. It reduced Fehling solution and gave positive color tests with phloroglucinol–hydrochloric acid and with the phenol reagent.

In Fig. 1 is presented the infrared absorption spectrum of kiri native lignin. It can be readily seen that it exhibits a marked similarity to the infrared spectra of other native lignins isolated in this Laboratory.[1–3,5]

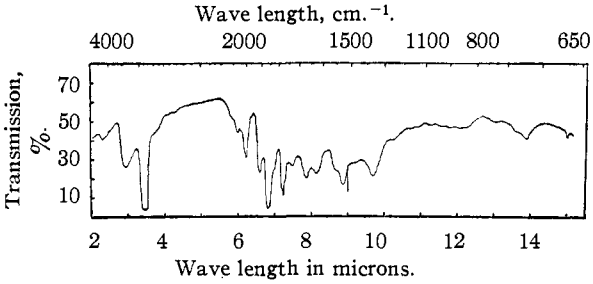

Fig. 1.—Spectrum of kiri native lignin.

Its ultraviolet absorption spectrum is shown in Fig. 2. It is similar to the absorption curves of other native lignins in that it gives an absorption peak at 282 mμ. However, the plateau from 295 to 320 mμ is quite unique.

Finally, kiri native lignin was compared with kiri lignins isolated with the aid of 10% alkali and 72% sulfuric acid. The data of this comparison are listed in Table II.

TABLE II

COMPARISON OF THE NATIVE AND CHEMICAL LIGNINS FROM KIRI WOOD

Lignin	C, %	H, %	OCH_3, %
Native	60.1	6.2	16.6
72% H_2SO_4	61.2	5.6	18.0
10% NaOH	60.9	5.7	17.4

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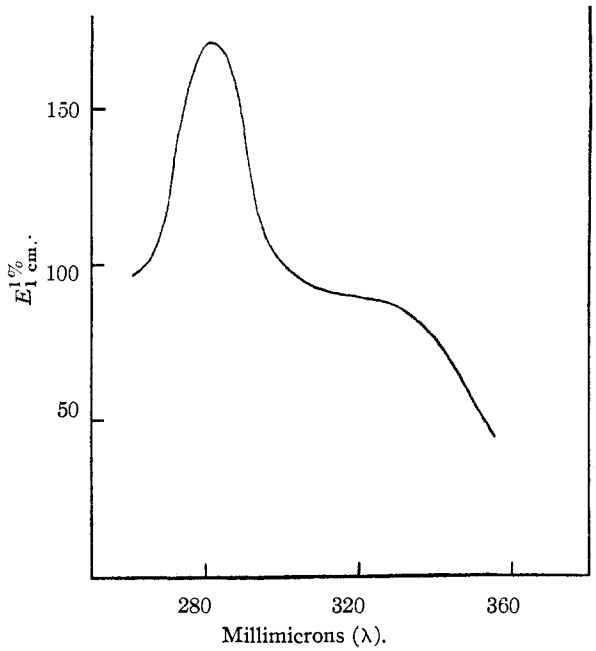

Fig. 2.—Spectrum of kiri native lignin.

It is readily observed that the methoxyl content of each of the chemical lignins is higher than that of the native lignin fraction. This discrepancy is due to either a change in the lignin complex on extraction with strong chemical reagents,[6] or to the fact that the total lignin present in kiri wood is inhomogeneous. Since kiri wood gave a strong positive Mäule color test,[7] which is specific for the presence of syringyl groups,[8] whereas the native lignin fraction gave only a fading cerise color, it appears that the latter explanation is more nearly correct. A similar situation was encountered in the study of the native lignins from oak and birch.

Experimental

Isolation of Native Lignin.—Air-dried kiri wood was stripped of its bark, ground to 40 mesh, and extracted with water and with ether. It was then extracted with ethyl alcohol until it no longer gave the phloroglucinol–hydrochloric acid color test. The alcohol was removed at reduced pressure. The reddish-brown residue was again washed with water and ether and dried *in vacuo*. It was then dissolved in dioxane, centrifuged, filtered and precipitated into an excess of ice-cold distilled water. The precipitate was dried, redissolved in dioxane to make a 10% solution and precipitated into ether. The above procedure was repeated until a constant methoxyl value was obtained.

The acetate and phenylhydrazone derivatives were prepared by the usual methods.[9]

Isolation of Chemically Prepared Kiri Lignins.—Extractive-free kiri wood was used in all cases; *i.e.*, the ground material was extracted in a Soxhlet apparatus first with 1:2 alcohol–benzene solution, and then with water.

Sulfuric acid lignin was isolated by the standard method.[9] The lignin content of the wood was found to be 22%, on a moisture-free basis. Alkali lignin[10] was also isolated by a standard method.

Ultraviolet Absorption Spectrum.—A solution of the lignin sample was prepared for spectroscopic analysis by dissolving 1 to 2 mg. of the sample in 50 ml. of the solvent (90 parts of purified dioxane to 10 parts of distilled water). A Beckman quartz spectrophotometer was used for the determination of the absorption curve.

Infrared Absorption Spectrum.—A Perkin–Elmer double beam recording infrared spectrophotometer was employed in this study.

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Preparation and Properties of Serum and Plasma Proteins. XXXIII. Specific Interactions of Prothrombin and Other Proteins with Barium Sulfate

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The effectiveness of barium sulfate in rendering plasma incoagulable, first observed by Bordet and Delange in 1912,[2] renders convenient the purification of prothrombin and serum prothrombin conversion accelerator and its plasma precursor. Alexander has demonstrated[3] that citrate brings about resolution of these proteins from barium sulfate, a finding which explains why prothrombin is not removed from citrated plasma by barium sulfate.

Calcium-free plasma, obtained by passage of blood over an ion exchange resin, yields to barium sulfate a fraction comprising 1% or less of the plasma proteins. This fraction contained, in addition to prothrombin and the precursor of serum prothrombin conversion accelerator, certain other proteins, apparently not involved in the coagulation mechanism. Sodium acetate, sodium chloride, glycine and glucose had no effect on the interaction; oxalate, which did not inhibit the interaction of prothrombin with barium sulfate, diminished the amount of inert protein removed.[4]

In our studies on the purification of prothrombin, we have investigated the effect of a number of substituted carboxylic acids on the interaction of these proteins, particularly of prothrombin, with barium sulfate.

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