TABLE I

DATA ESTABLISHING THE IDENTITY OF "PHENYL-VOLEMOSAZONE" WITH D-MANNOHEPTOSE PHENYLOSAZONE

Substance	Melting point: Older literature	Melting point: Present measurements	Mixed melting point: With "phenyl-volemosazone"	Mixed melting point: With D-altroheptose phenylosazone	$[\alpha]^{20}D$ in methyl Cellosolve
D-Altroheptose phenylosazone	197°[a]	194–195°	183–185°	.....	−54.4° (no mutarotation during 120 hours)
"Phenyl-volemosazone"	196°[b]	194–195°	.....	183–185°	+55.4° → +17.0° (96 hours)
D-Mannoheptose phenylosazone	About 200°[c]	199–200°	194–195°	185–187°	+55.9° → +17.2° (96 hours[d])

[a] LaForge and Hudson, *J. Biol. Chem.*, **30**, 61 (1917). [b] Fischer (ref. 2). [c] Fischer and Passmore, *Ber.*, **23**, 2226 (1890). [d] The mutarotation was still proceeding after forty-eight hours but had reached completion at ninety-six hours. It is so slow that the initial rotation can be measured easily.

tion of volemitol excludes this sugar. In later years Ettel[4] has suggested that "volemose" is identical with the naturally occurring ketose sedoheptulose (D-altroheptulose), but this inference proves to be unsound.

While "phenyl-volemosazone" is the useful trivial name of a definite crystalline substance, the place of which in the system of carbohydrate formulas is now established, it appears to us that the name "volemose" is inherently so indefinite that it should be discarded or used only in a historical way. On the other hand, the name "volemulose," which Bertrand[5] gave to a sirupy ketoheptose product that he made by the biochemical oxidation of volemitol with the organism *Bacterium xylinum* (classified at the present time as (*Acetobacter xylinum*) and assumed to be a single sugar because of the known specificity of action of this organism, may indeed prove to be an early name of D-mannoheptulose or D-altroheptulose. A repetition of Bertrand's experiment, which we plan to undertake, is required before a decision can be made, since Bertrand's rule of specificity for this organism indicates that either or both of these ketoheptoses could be expected from volemitol. The melting point which he reports for "phenyl-volemulosazone" (205°) apparently excludes the possibility that it was a mixture of the phenylosazones from the two sugars (see Table I).

We thank Dr. Raymond M. Hann and Mr. John Sipes for supplying the volemitol, which they prepared from sedoheptulose by reduction with sodium amalgam.

Summary

Emil Fischer's directions for preparing from the naturally occurring volemitol the crystalline substance which he named "phenylvolemosazone" have been repeated and the crystalline product which we have obtained in about the yield that he reported proves to be D-mannoheptose phenylosazone.

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(4) Ettel, *Collection Czechoslov. Chem. Commun.*, **4**, 519 (1932); Tollens-Elsner, "Kurzes Handbuch der Kohlenhydrate," 1935, p. 405; Beilstein's "Handbuch," 4th ed., vol. 31, 1938, p. 363.

(5) Bertrand, *Compt. rend.*, **126**, 764 (1898); *Bull. soc. chim.*, [3] **19**, 348 (1898); *Ann. chim.*, [8] **3**, 209, 287 (1904).

[CONTRIBUTION FROM THE DIVISION OF INDUSTRIAL AND CELLULOSE CHEMISTRY, MCGILL UNIVERSITY, AND FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

The Elementary Composition of Lignin in Northern Pine and Black Spruce Woods, and of the Isolated Klason and Periodate Lignins[1]

BY W. J. WALD,[2] P. F. RITCHIE[3] AND C. B. PURVES

At the present time most investigators believe that lignins isolated from woods consist in great measure of oxygenated phenylpropane units condensed together in an unknown way to an undetermined extent. The very substantial evidence for this view has been adequately summarized by Freudenberg,[4] Hibbert,[5] Percival,[6] Phillips[7] and others. It is also agreed that chemical changes occur in lignin during its isolation from wood by acidulated alcohols, thioalcohols or phenols, by strong mineral acids or alkalies, by sulfite solutions or by many other reagents. Such changes are probably greatly increased when elevated temperatures are required for the isolation, as in technical wood pulping processes. The general

(1) Presented at the Atlantic City meeting of the American Chemical Society, April, 1946.

(2) The results with Northern Pine wood are from a thesis submitted by W. J. Wald to the Faculty of the Massachusetts Institute of Technology in partial fulfilment of the requirements for the degree of Doctor of Philosophy, 1940.

(3) The results with Black Spruce wood were obtained by P. F. Ritchie, Gottesman Foundation Scholar, in 1945, at McGill University.

(4) Freudenberg, *Ann. Rev. Biochem.*, **8**, 88 (1939).

(5) Hibbert, *ibid.*, **11**, 183 (1942); *Paper Trade J.*, **113**, 35 (July 24, 1941).

(6) Percival, *Ann. Repts. Chem. Soc.*, **39**, 142 (1942).

(7) Wise, "Wood Chemistry," Reinhold Publishing Corp., New York, N. Y., 1944, review chapters by M. Phillips, pp. 272–368.

impression obtained is that lignin during isolation may be in part converted to more complex, insoluble substances, in part cleaved to simpler, more soluble units, and may also become condensed with elements derived from the medium of extraction.

At this point opinions diverge. Some authorities, including Freudenberg,[8] consider that the changes are superficial and that the oxygenated phenylpropane complexes characteristic of isolated lignins exist preformed in the wood. The possibility that lignin is originally hydroaromatic in nature cannot yet be entirely overlooked, particularly since the aliphatic side chains of crystalline, phenolic lignans often present with lignin in woods readily undergo cyclization to tetrahydronaphthalene derivatives and hence to naphthalenes.[9] Schütz and Sarten,[10] after discussing the indecisive evidence available, conclude that lignin suffers deep-seated transformation during isolation and that no sharp line of chemical demarcation exists between the carbohydrate and non-carbohydrate components of wood. Hilpert and his collaborators[11] maintain the extreme position that isolated lignins are nothing more than the resinified debris of very reactive carbohydrates of unspecified type.

Since substantial differences usually exist between the carbon and hydrogen contents of aromatic, hydroaromatic and carbohydrate substances, definite knowledge of the composition of lignin *in situ* would tend to discriminate between these possibilities. Twenty years ago, Fuchs[12] attempted to gain this information from the difference between the average composition of entire woods and of the carbohydrate components of woods. The undeveloped state of wood analysis at that time forced him to assume that 71.5% of a wood consisted of hexosans and pentosans in a 2:1 ratio and also to assume reasonable analyses for the wood and for these components. The composition calculated for the "lignin" in the remaining 28.5% of the wood was then corrected by assuming that ash, protein, resin and wax had the average composition C, 50.0; H, 6.4% and amounted to 4.1%. Fuchs pointed out that the result found for "*genuines Lignin*," C, 63.1; H, 5.9%, was independent of its chemical nature or state of chemical combination. Although similar calculations have been made from time to time by others,[8] the underlying analytical data seem to have been assumed rather than specially determined with the greatest possible accuracy. The present article includes an attempt to remedy this deficiency and to avoid the assumptions that were inevitable at the earlier date.

(8) *E. g.*, Freudenberg, Lautsch and Piazolo, *Cellulosechem.*, **21**, 95 (1943).

(9) Haworth, *Ann. Repts. Chem. Soc.*, **33**, 270 (1936).

(10) Schütz and Sarten, *Cellulosechem.*, **22**, 1 (1944).

(11) *E. g.*, Hilpert and Hellwage, *Ber.*, **68**, 380 (1935).

(12) Fuchs, "Chemie des Lignins," J. Springer, Berlin, 1926, p. 74.

Resins, waxes and proteins were first eliminated from the wood meals by thorough extractions with neutral solvents. Advantage was then taken of the fact that holocellulose fractions prepared by the original method of Kurth and Ritter[13] account, with the Klason lignin, for substantially all of the wood. Direct knowledge of the holocellulose–lignin ratio and of the analyses of the wood and holocellulose then made it possible to calculate the composition of the wood lignin without further assumptions.

The solution of the problem of isolating lignin in a chemically unchanged form probably requires new methods that avoid high temperatures and employ neutral, chemically inert reagents. Brauns'[14] extraction of "native lignin" with 96% ethanol at room temperature met these requirements, but the failure of 97% of the lignin present to be so extracted suggests that "native lignin" is not identical with the bulk of the lignin in wood. Another approach involves the selective dissolution of the holocellulose by enzymes such as those present in the digestive juices of the Weinberg snail. The exploration of this route by Ploetz[15] suggests that much of the residual lignin is combined with carbohydrate, although incidental extractions with cupri-ethylenediamine solutions during the work increased the risk of chemical change. A third possible method originated in the discovery by Jackson and Hudson[16] that cellulose oxidized with cold periodic acid becomes soluble in hot water, whereas Freudenberg and his collaborators[17] briefly stated that lignin is apparently unchanged by this oxidant. However, a decrease from 16 to 10% in the methoxyl content of the lignin was observed. Pennington and Ritter,[18] who recently used periodic acid to free lignin sulfonic acid fractions from traces of carbohydrates, found that a methoxyl-containing fragment of the lignin was lost during the treatment.

Lignin has now been isolated by alternately oxidizing wood with aqueous sodium paraperiodate at *p*H 4 and 20° and extracting the residue with boiling water at *p*H 6.5 to 7. Although the method avoids changes in the lignin caused by exposure to unduly high temperatures, to acids and to alkalies, changes of an oxidative nature are obviously not to be excluded.

Experimental

Materials.—Coarse sawdust from carefully peeled logs of a fifty-year old Northern Pine and a one hundred and forty year old Black Spruce were separately and exhaustively extracted in large Soxhlets (no rubber connections) with hot alcohol–benzene (1:2) for approximately sixty hours. Four-hour extractions with hot 95% ethanol and with hot water followed, after which the wood

(13) Kurth and Ritter, THIS JOURNAL, **56**, 2720 (1934).

(14) Brauns, *ibid.*, **61**, 2120 (1939).

(15) Ploetz, *Ber.*, **73**, 57, 61, 74, 790 (1940).

(16) Jackson and Hudson, THIS JOURNAL, **59**, 2049 (1937); **60**, 989 (1938).

(17) Freudenberg, Sohns and Janson, *Ann.*, **518**, 62 (1935).

(18) Pennington and Ritter, THIS JOURNAL, **68**, 1391 (1946); **69**, 187 (1947).

meals were allowed to dry in the air. Tests for nitrogen were negative. The 60 to 100 mesh and the 60 to 80 mesh fractions, respectively, of the two wood meals were used throughout the work and were stored in tightly stoppered large glass bottles.

Analytical Methods.—Analyses for moisture, Klason lignin, ash and pentosan were carried out in duplicate or triplicate by standard U. S. Forest Products Laboratory methods[19] with the following minor deviations. Moisture was determined by heating 2-g. samples at 105° for eight hours, since three to five hour periods gave less reproducible results. The 72% sulfuric acid used in the lignin estimations was precooled to 0° before use in order to avoid appreciable heating of the sample before mixing was complete. The mixture was allowed to remain for sixteen hours at 10°, instead of for two hours at 20°, before being diluted with water as the next step in completing the estimation. This change gave minimum, more reproducible Klason lignin values. All of the Klason lignins were free from sulfur.

Holocellulose was prepared by three-to-four-minute chlorinations of the moist wood alternated by extractions with alcohol–pyridine (1:1) as described by Kurth and Ritter.[13] All-glass equipment was used. One or two per cent. of lignin was left in the holocellulose to minimize the risk of oxidation and a subsequent bleaching step was omitted for the same reason. No nitrogen could be detected in any of the holocellulose samples.

Methoxyl determinations were made by Vieböck and Schwappach's method,[20] in the case of spruce as modified by Peniston and Hibbert.[21] Combustion analyses were on the semi-micro scale and with air-dry samples. Moisture and ash determinations were simultaneously carried out on separate samples and the results were used to correct the combustion data to a moisture and ash free basis.

Oxidation of Wood with Periodate.—Sodium paraperiodate ($Na_3H_2IO_6$), about 35 g., was dissolved by shaking in about 1800 cc. of water acidulated with acetic acid. The acidity was then adjusted to *p*H 4.1, by adding glacial acetic acid. On the following day the solution, if necessary, was filtered through sintered glass. After adjustment with water to a volume of 2 liters, a 5-cc. aliquot of the filtrate was analyzed for periodate by the standard arsenite–iodine titration developed by Fleury and Lange.[22] Spruce wood meal weighing 101.9 g. (cor. for 7.46% moisture) was added and the temperature of the mixture was maintained at not more than 20° with the help of a cold water-bath. The mixture was slowly, continuously and mechanically stirred for sixty hours at 20°. When desired, the consumption of periodate with time was followed by withdrawing small aliquots and estimating the unused periodate they contained by the titration already described. The amount consumed was obtained by difference from the blank minus the amounts withdrawn in any previous aliquots.

After sixty hours of oxidation, the mixture was filtered through sintered glass and the wood residue washed with cold water until the washings contained no iodate, as shown by their negative reaction with potassium iodide and acid. The washed residue, whose dry weight was close to that of the original wood, was then boiled under reflux with 8 liters of distilled water to dissolve the oxidized portion of the carbohydrates. After filtration and washing with water, the wet residue was submitted to another oxidation–water extraction cycle. Drying was always through methanol and benzene but only small samples for analysis were dried until the completion of the final cycle. The results are summarized in Fig. 3.

Filtrates and washings rich in iodate and periodate were treated with sufficient strong caustic soda to precipitate the insoluble paraperiodate. The filtrate from this operation, containing the sodium iodate, was saturated at 80° with bromine,[23] or at 100° with chlorine[24] to reoxidize iodate to the paraperiodate. The facile recovery of the latter in 80 to 90% yield in a state of 95 to 100% purity made possible great economies in the use of this expensive salt.

Chemical Instability of Lignin at 105°.—The accepted method of drying wood or lignin for analytical purposes consists of heating the sample in air at 105° until the weight becomes approximately constant. A sample of spruce Klason lignin, heated in this way for six hours, decreased in weight by 8.21%, but when dried to constant weight at room temperature *in vacuo* over phosphorus pentoxide the decrease was only 7.63%. This decrease corresponded exactly to the water content as determined by the Karl Fischer method.[25] Heating at 105° therefore had removed about 0.6% of the dry substance of the Klason lignin. More prolonged periods of heating (Table I) caused an additional loss of 0.2 to 0.5% of the lignin substance. These losses in weight were accompanied by decreases of two to three per cent. in the carbon content of the dry lignin, as compared to the values obtained by allowing for the "moisture" content of unheated, air dried samples. Since heating air-dry spruce Klason lignin in a stream of nitrogen, carefully purified from traces of oxygen, produced similar results, the decrease in carbon content was tentatively attributed to a slight thermal decomposition rather than to an oxidation.[26] A similar change was exhibited by a

TABLE I

ANALYSES[a] OF WOOD AND LIGNIN AFTER HEATING AT 105°

	Hours at 105°	C, %	H, %	% Change in weight[b]
	In air			
Spruce wood	0[c]	51.2	6.2	(+7.49)
	24	48.8	6.1	−0.21
	48	48.5	6.3	− .40
Spruce Klason	0[c]	67.4	5.4	(+8.2)
lignin	24	64.1	6.1	−0.23
	48	63.8	6.1	− .28
Pine Klason	0[c]	67.4	5.9	....
lignin	10	64.2	6.2	....
		63.5	6.5	....
	In nitrogen			
Spruce Klason	0[c]	67.4	5.4	(+8.2)
lignin	24	64.1	6.1	−0.24
	48	64.7	6.1	− .50

[a] Corrected for ash. The duplicate combustions were all within 0.18% of the averages quoted for C and H. [b] Based on weight after drying for six hours at 105°. [c] On air-dry samples and analysis corrected to base weight after drying for six hours at 105°.

(19) "Methods Used at the Forest Products Laboratory for the Chemical Analysis of Pulps and Pulpwoods," Forest Products Laboratory, Madison, Wis., 1939.

(20) Vieböck and Schwappach, *Ber.*, **63**, 2818 (1930).

(21) Peniston and Hibbert, *Paper Trade J.*, **109**, No. 17, 46 (1939).

(22) Fleury and Lange, *J. pharm. chim.*, **17**, 107, 196 (1933).

(23) Lange and Paris, *J. pharm. chim.*, **21**, 403 (1935).

(24) Hill, THIS JOURNAL, **50**, 2678 (1928); *cf.* "Inorganic Syntheses," Vol. I, McGraw-Hill Book Co., New York, N. Y., 1929, p. 169.

(25) Mitchell, *Ind. Eng. Chem. Anal. Ed.*, **12**, 390 (1940).

(26) Moore, "Coal," John Wiley and Sons, New York, N. Y., 1940, pp. 32–36, discusses the evolution of small amounts of carbon dioxide and lower hydrocarbons from coals heated *in vacuo* at 100° after being degassed at room temperature.

sample of spruce wood meal which had been extracted, thoroughly and in succession, with alcohol–benzene, alcohol and hot water.

It is important to note that the conventional method of drying used in the present research underestimated the true dry weights of Klason lignin and wood by not more than a few tenths of one per cent. In consequence, the carbon and hydrogen percentages corrected for apparent "moisture content" were high only by smaller, variable amounts. The necessity of using unheated samples for analytical purposes is obvious.

Composition of Lignin in Wood.—In calculating the composition of "lignin removed" from those of the wood and the residual holocellulose, the ratio by weight existing between these two constituents of the wood must be determined as accurately as possible. The yield of holocellulose (Table II, column A) when subtracted from 100 gave the percentage of "lignin removed" as shown in the fifth column. This calculation assumed no error in the estimation of holocellulose and automatically corrected for the small amount of lignin deliberately left in the latter. Alternatively, the Klason lignin determinations on wood and holocellulose could be assumed to be nearly correct and their difference (column 6) gave a value for "lignin removed." When making this computation, it was necessary to express the amount of lignin in the holocellulose as a percentage of the wood (column B). The data in columns 5 and 6 are not identical and the probable error noted in the average of each pair corresponds to the amount by which the lignin-free holocellulose plus the Klason lignin failed to account for exactly 100% of the wood. Numerous other workers have obtained material balances of the same order of precision. Although this precision probably owed something to a chance cancellation of errors of unknown size,[27] consideration shows that precise knowledge of such errors would throw light upon the chemical nature of the "lignin removed," rather than alter its average composition. The largest divergence, occurring in analysis 1, was ± 0.3%, and the ratio of "lignin removed" to holocellulose was accordingly in doubt by ± 1.5%, or by ± 0.6% on the basis of the wood. In the other three cases, the ratio was presumably correct to ± 0.5% or to ± 0.2%.

TABLE II

RATIO OF HOLOCELLULOSE TO LIGNIN REMOVED[a]

Anal.	Holocellulose, % Yield (A)	Lignin,[b] % (B)	Klason Lignin, % (C)	Lignin removed, % (100-A)	(B-C)	Average
			Northern Pine wood			
1	72.3	1.3	28.4	27.7	27.1	27.4 ± 0.3
2	73.0	1.8	28.9	27.0	27.1	27.1 ± 0.1
			Black Spruce wood			
3	72.1	0.8	28.8	27.9	28.0	27.9 ± 0.1
4	72.6	1.3	28.8	27.4	27.5	27.4 ± 0.1

Error in ratio (Analysis 1), $\frac{27.4 \pm 0.3}{72.6 \pm 0.3}$ or ±0.6%

[a] All analyses corrected for moisture and ash. [b] As percentage of wood.

(27) Wise, Murphy and D'Addico, *Paper Trade J.*, **122**, No. 2, 35 (1946).

The first two lines of Table III summarize the carbon, hydrogen and methoxyl contents determined for the holocellulose and wood, all data being corrected for moisture and ash. Line 3 of the table expresses the analyses of the holocellulose (line 1) as fractions of the wood, and the carbon, hydrogen and methoxyl contents of the "lignin removed" were obtained by difference. The limits of divergence between the duplicate or triplicate analyses were carried forward additively in the calculations and created an uncertainty of ± 0.7% in the percentage of carbon in the lignin. Fortunately the calculated analyses were not very sensitive to slight changes in the holocellulose–lignin ratio and the additional possible error from this cause was only ± 0.25%. Although the extreme calculated error in this, the most unfavorable case, was ± 1% in the carbon, and also in the hydrogen and methoxyl analyses, the possible limits were smaller in the other three cases (Table IV) and the true values are probably not far from C, 67.5; H, 6%. The second analysis with pine wood was made with a 60 to 100 mesh fraction obtained by regrinding already sieved, coarser sawdust and was therefore not quite comparable to the first analysis. The variation in the methoxyl percentages may be attributed in part to this difference.

TABLE III

CALCULATION OF LIGNIN COMPOSITION[a]

(Analysis 1)

	Base weight	C	H	OCH_3
Holocellulose	100	44.9 ± 0.03	6.2 ± 0.2	1.2 ± 0.05
Wood	100	51.0 ± 0.1	6.2 ± 0.1	5.1 ± 0.1
Holocellulose[b]	72.6	32.6 ± 0.1[b]	4.5 ± 0.2[b]	0.9 ± 0.04[b]
Lignin removed	27.4	18.4 ± 0.2	1.7 ± 0.3	4.2 ± 0.2
Lignin	100	67.2 ± 0.7	6.1 ± 1.0	15.2 ± 0.6

Applying possible error of 0.6% in lignin–holocellulose ratio.

Lignin (cor.) C, 67.2 ± 1.0 H, 6.1 ± 1.0 OCH_3, 15.2 ± 0.75%

[a] All analyses corrected for moisture and ash. [b] Corrected to wood basis by factor 72.6/100.

"Native lignin" from the same pine wood (Table IV) had carbon and methoxyl values substantially below those calculated for the residual, ethanol-insoluble lignin, and the analyses by Brauns[14] suggest that the same remark is also true of "native lignin" from spruce. Harris[28] also considered on other grounds that "native lignin" was not identical with the bulk of the lignin in wood. Analyses tabulated by Phillips[7] for spruce and pine lignins obtained by various authors, using sulfuric, hydrochloric, hydrochloric–phosphoric acids, caustic soda or cupraammonium, also reveal carbon con-

(28) Harris, *Ind. Eng. Chem.*, **32**, 1049 (1940).

TABLE IV

THEORETICAL AND OBSERVED ANALYSES OF PINE AND SPRUCE LIGNINS

		C, %	H, %	OCH_3, %
Extreme calculated values				
Pine Anal.	1	66.2–68.2	5.1–7.1	14.4–16.0
Pine	2	67.6–69.3	5.0–6.7	16.4–17.9
Spruce	3	67.2–67.8	5.5–6.5	14.1–14.3
Spruce	4	67.4–68.0	5.0–6.1	13.6–14.0
Probable values				
Pine *in situ*		67.5	6	16(?)
Spruce *in situ*		67.5	6	14
Isolated lignins				
Native pine		60.7	6.8	12.9
Native spruce[a]		63.9	6.15	14.9
Pine Klason[b]		67.4	5.9	15.9
Spruce Klason[b]		67.4	5.4	15.4
Spruce Klason[b]		65.7	5.8	
Spruce periodate lignin		62.1	5.8	10.7
Theoretical lignin units				
Oxygenated phenyl propane resins[c]		61–70	6	15.8
Hydroaromatic derivatives		60–68	9	15.3
Dimethyl pentosans[d]		52.5	7.5	38.8

[a] Brauns, ref. 14 (drying conditions not stated). [b] Analyzed air-dry and corrected for moisture. [c] Cramer, Hunter and Hibbert, ref. 29. [d] Hilpert and Hellwage, ref. 11.

tents ranging between 62.4 and 64.0%. Freudenberg, Lautsch and Piazolo,[8] however, quote values as high as C, 67%, for certain lignins isolated from spruce and their results agree well with those both found and calculated for Klason lignin in Table IV. It is possible that the widespread practice of analyzing lignins after drying at elevated temperatures is responsible for the customary deficiency of 2 to 5% in carbon content. A review[29] of various "lignin building units" suggested from time to time in the literature shows that the majority require carbon contents of 53 to 65.7%, the exceptions being three proposed by Freudenberg,[17,30] with 67.2 to 67.7% carbon. Although the carbon content of a recent variant of the phenylpropane structures, 2-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone and its dismutation isomers[31] is only 61%, the substance readily yielded a resin with a carbon content of 70% (Table IV). Less drastic resinification might well leave the composition within the acceptable range.

Acceptable, too, are the compositions of the structures recently put forward by Jayme and Hanke[32] for spruce lignin *in situ*. These structures contain up to 68.2% of carbon and consist of hexosan units in which two or three of the hydrocarbon hydrogen atoms are replaced by guaiacyl residues. The introduction into theoretical lignin formulas of many hydroaromatic units appears to be inadmissible, because such units would raise the hydrogen content from the calculated value of 6% to the neighborhood of 9%. A glance at the composition of methylated pentosans eliminates them from consideration as possibilities for lignin *in situ*.[11] If less highly methylated carbohydrates are assumed in order to reduce the methoxyl content to the proper range, the gross discordance in carbon content is increased. Isolated spruce and pine Klason lignins had their customary methoxyl contents of 15.4 to 15.9%, but for unknown reasons the former exceeded the calculated value by about 1.4%. The observation could not be checked for pine lignins because the calculated methoxyl contents failed to agree closely among themselves.

Periodate Lignins.—The attempt to isolate lignin of the calculated composition, without using acids or alkalies, commenced with small-scale oxidations of the extracted pine and spruce woods, holocelluloses and Klason lignins with 1% aqueous sodium paraperiodate at 20°. The solutions were kept buffered to *p*H 4.1 throughout the experiments, since it was known[33] that secondary oxidations were at a minimum in that *p*H range.

Some of the results in the spruce series of experiments are summarized in Fig. 1, which omits the very similar rate plots obtained with pine. A fairly rapid initial reaction was noted in all three cases (plots II, III, IV) and was followed by a slower secondary consumption of periodate. Klason lignin, although oxidized to the smallest extent, absorbed the equivalent of one atom of oxygen for each Brauns–Hibbert[14,34] lignin building unit of molecular weight 840. The fortuitous

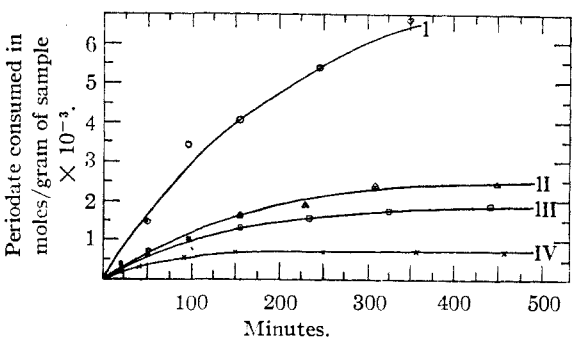

Fig. 1.—Moles of $Na_3H_2IO_6$ consumed at *p*H 4.1 and 20° per gram sample: plot I, spruce Klason lignin with 3% $Na_3H_2IO_6$; plot II, spruce holocellulose; plot III, extracted spruce wood; plot IV, spruce Klason lignin, all with 0.5% $Na_3H_2IO_6$.

(29) Wald, Ph.D., Thesis, Massachusetts Institute of Technology, 1940.

(30) Freudenberg, *Naturwissenschaften*, **27**, No. 14, 227 (1939); *Angew. Chem.*, **52**, 362 (1939).

(31) Cramer, Hunter and Hibbert, THIS JOURNAL, **61**, 509 (1939).

(32) Jayme and Hanke, *Cellulosechem.*, **21**, 127 (1943). The effect of the method of drying on the carbon content of lignin was briefly noted and the status of the lignin–carbohydrate complex theory was extensively discussed.

(33) Grangaard, Gladding and Purves, *Paper Trade J.*, **115**, No. 7, 41 (1942).

(34) Brauns and Hibbert, THIS JOURNAL, **55**, 4720 (1933).

nature of this agreement became apparent when the concentration of the periodate used for each gram of Klason lignin was increased (plot I), all other conditions being unchanged. Since an increase in the concentration of periodate also increased its consumption by wood and holocellulose, the amount of oxidation attained in any experiment probably depended on physical factors. Trial showed that holocellulose required repeated oxidations under the above conditions, alternated with hot water extractions, before it entirely dissolved, even although much periodate remained unused in each oxidation.

In order to limit further the secondary oxidations, whose nature was unknown, the action of 2.6% aqueous periodate was restricted to three hours and the oxidation–hot water extraction cycle was repeated seventeen times on the same wood sample. As the holocellulose was selectively removed as hot-water solubles, the percentage of Klason lignin in the undissolved residue steadily increased from the initial figure of 28.8 to 60.7%. The rate of increase was not greatly altered (Fig. 2) by grinding the residue almost to colloidal dimensions in a Waring blendor before the eighth and eleventh oxidations. The tedium of the

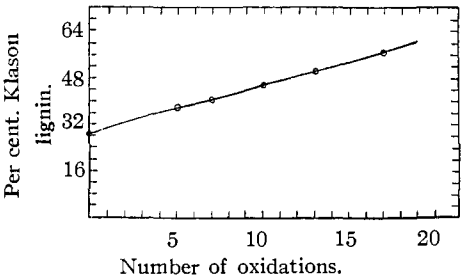

Fig. 2.—Increase in Klason lignin content of residue from spruce wood repeatedly oxidized by excess of 2.6% $Na_3H_2IO_6$ at *p*H 4.1 and 20° for three-hour periods.

oxidations was eventually lessened by increasing the time for each oxidation from three to sixty hours, as described in the Experimental Portion. Although the amount of periodate initially present was nearly constant in the successive oxidations (Fig. 3) the amount consumed by the

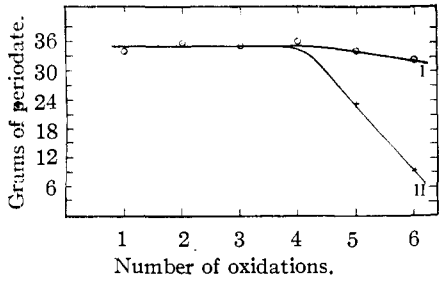

Fig. 3.—Dry spruce wood 101.9 g., oxidized for sixty hours six times with 2 liters of 1.75% $Na_3H_2IO_6$ (35 g.) at *p*H 4.1 and 20°: plot I, g. periodate added; plot II, g. periodate consumed.

wood residue decreased very sharply from 100% when the greater part of the holocellulose had been removed. The product obtained after six oxidation–hot water extraction cycles (sample X) contained 86% of Klason lignin and accounted for 80% of that initially present in the wood. The fate of the remaining 20% was uncertain, for extensive search in the washings and residual periodate liquors failed to locate it, and in one later experiment the recovery of Klason lignin was nearly quantitative. Periodate lignin (sample X) when re-oxidized five times under the same conditions very slowly consumed periodate and slowly decreased in weight by 15%. The product (sample Y) contained 96.6% of Klason lignin. A fresh sample of Klason lignin decreased in weight by 28% in a parallel series of oxidations.

Both samples of periodate lignin were quite free of iodine, were bright brown in appearance and upon microscopic examination were seen to retain much of the detailed morphological structure of the wood. They remained insoluble in methanol, ethanol, ether, benzene, chloroform and dioxane even when suspensions in these liquids were boiled for several hours. The addition of 2% of hydrogen chloride to the suspensions in hot dioxane or in methyl, ethyl, butyl and amyl alcohols, however, yielded yellow solutions and a dark brown resin that remained insoluble when boiled with a fresh amount of the acidified solvent. When submitted to the holocellulose estimation, periodate lignin displayed the usual yellow color during each chlorination, but became red-brown during each extraction with hot alcohol–pyridine. Seven chlorination–extraction cycles dissolved the sample completely and no trace of holocellulose was found. When distilled with 12% hydrochloric acid in a standard pentosan estimation, the distillate gave no pink color with aniline hydrochloride test paper and no precipitate formed until several hours after the addition of phloroglucinol. The periodate lignin probably contained no pentosans, although the acid distillation produced a small amount of formaldehyde, which was isolated as a slowly separating, alcohol-soluble phloroglucide, and as the crystalline dimethone derivative, m. p. 185–186°. Periodate lignin dissolved completely in a standard calcium bisulfite pulping liquor in six hours at 135°.

It is obvious from Table IV that the carbon and methoxyl contents of the periodate lignin X were definitely lower than the values calculated for the lignin *in situ*. Since the periodate products were obtained by the oxidation of wood at 20° and *p*H 4, alternated with water extractions at 100° and *p*H 6.5, any resinification of the lignin during the isolation was probably of minor importance. It is much more probable that periodate lignins are oxidized derivatives of the lignin as it exists in wood.

Dr. H. W. Johnston, formerly of the Pulp and Paper Research Institute of Canada, Professor E.

Hauser of the Massachusetts Institute of Technology, Drs. C. B. Stanwood and C. A. Turner, of the Great Northern Paper Company, all helped in the procurement and preparation of the wood meals. One of us (P. F. R.) wishes to express thanks to The D. S. and R. H. Gottesman Foundation of New York for a Scholarship and to the Canadian Pulp and Paper Association for a summer maintenance grant.

Summary

1. The composition of lignin as it exists in Northern Pine and Black Spruce wood was found to be about C, 67.5; H, 6%, by an indirect method that involved no assumptions concerning the chemical nature of the lignin. Methoxyl contents were calculated to be in the ranges 14.4 to 17.9% and 13.6 to 14.3%, respectively. The analyses were those of aromatic, rather than hydroaromatic or carbohydrate, substances.

2. Klason lignins isolated from the same woods had carbon and hydrogen analyses agreeing with the calculated values, provided the lignins were not dried by heating at 105°. When this precaution was neglected, the analyses were 2 to 5% low in carbon and resembled those published for most other isolated lignins. The methoxyl content of spruce, and perhaps of pine Klason lignin was about 1.5% higher than the calculated values. Most suggested structural formulas are too deficient in carbon content to represent lignin *in situ*.

3. Spruce or pine wood meal was alternately oxidized with aqueous sodium paraperiodate at *p*H 4 and 20° and boiled with water at *p*H 6.5 to dissolve the oxidized holocellulose. The residual, insoluble "periodate lignin" was only slowly attacked by the oxidant, contained no holocellulose and 86 to 96% of Klason lignin. Analysis showed that "periodate lignin" had undergone chemical change, presumably an oxidation rather than a resinification.

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[CONTRIBUTION FROM THE ABBOTT LABORATORIES]

Some Schiff Bases of Free Amino Acids[1]

BY FLOYD C. MCINTIRE

Schiff base formation appears to be almost unrecognized among the various reactions between amino acids and aldehydes or ketones.[2] Although the esters and salts of various amino acids have long since been reported to form Schiff bases with aromatic aldehydes,[3] there are very few Schiff bases of the free amino acids on record. Bergmann and Zervas[4] reported the preparation of some monoarylidine derivatives of the diamino acids, but there appear to be no unequivocal reports of the preparation of Schiff bases of the free monoamino acids. Attempts to prepare these from the Schiff bases of the monoamino acid esters or salts have resulted in decomposition. Dakin[5] obtained preparations which had the correct analyses for benzylidineglycine, -alanine and -leucine, but he considered these preparations to be polymers. They were not crystalline and they were prepared under rather drastic conditions with very low yields. Gulland and Mead[6] studied amino acid Schiff base formation as related to *p*H in aqueous-alcohol solutions. They concluded that the Schiff base formation takes place primarily above *p*H 7 and is a reversible reaction so that the isolation of a Schiff base of a monoamino acid would be highly improbable.

The purpose of this paper is to report the preparation of some Schiff bases of free amino acids, particularly of the monoamino acids. These compounds have been prepared in crystalline form and in good yield by the reaction of amino acids with *o*-hydroxy aromatic aldehydes under very mild conditions. The conclusion that these compounds are actually Schiff bases is based upon the following criteria: (1) elementary analyses check well with the theoretical values; (2) representative members have been hydrolyzed under very mild conditions to yield the corresponding amino acids in good yield; (3) representative members have been hydrogenated to yield the corresponding N-arylamino acids.

Experimental

Materials.—2-Hydroxy-1-naphthaldehyde was prepared by the method of Duff and Bills.[7] 2-Methoxy-1-naphthaldehyde was prepared by methylation of 2-hydroxy-1-naphthaldehyde with dimethyl sulfate. Other aldehydes were purchased from Eastman Kodak Company.

All of the monoamino acids and glutamic acid were used in the free amino acid form.

The basic amino acids were prepared for use as follows: Lysine monohydrochloride was dissolved in water and shaken overnight with an excess of silver oxide. Most of the silver was removed from solution by precipitation with

(1) Presented in part before the Division of Biological Chemistry at the 110th meeting of the American Chemical Society, Chicago, September 9–13, 1946.

(2) Clarke, "Amino Acids" in "Organic Chemistry, An Advanced Treatise," 2nd ed., edited by Gilman, *et al.*, John Wiley and Sons, New York, N. Y., 1943.

(3) Bergmann, Ensslin and Zervas, *Ber.*, **58**, 1034 (1925); Gerngross, *Biochem. Z.*, **108**, 84 (1920); Gerngross and Zühlke, *Ber.*, **57**, 1482 (1924).

(4) Bergmann and Zervas, *Z. physiol. Chem.*, **152**, 282 (1926); **172**, 277 (1927).

(5) Dakin, *J. Biol. Chem.*, **82**, 439 (1929); **84**, 675 (1929).

(6) Gulland and Mead, *J. Chem. Soc.*, 210 (1935).

(7) Duff and Bills, *J. Chem. Soc.*, 1307 (1934).