version of this halide to its dibromide and of this dibromide to the ester are described. Molar refraction was calculated using the constants given by Kabachnik.[2]

Experimental

p-Ethylbenzenedichlorophosphine[3,4,5] was prepared by the reaction of *p*-ethylbenzene with phosphorus trichloride using aluminum chloride as catalyst. Excess phosphorus trichloride was removed by vacuum distillation prior to addition of phosphorus oxychloride to decompose the product–aluminum chloride complex. The phosphorus oxychloride–aluminum chloride complex was precipitated by addition of petroleum ether and separated by filtration.[6] The crude product obtained on evaporation of the solvent was used in the following reaction without purification. A study of yields from varying proportions of aluminum chloride, ethylbenzene and phosphorus trichloride gave a maximum yield of 70% of crude product with a molar ratio of 0.5/1/3, respectively, for preparations starting with 0.5 mole of ethylbenzene.

p-**Ethylbenzenedichlorophosphine dibromide** was prepared by addition of bromine to a carbon tetrachloride solution of the crude dichloride with cooling to dissipate the heat of reaction. The orange precipitate was not isolated but used directly in the esterification.

Dimethyl *p*-ethylbenzenephosphonate was prepared by addition of absolute methanol to the carbon tetrachloride suspension of the tetrahalide. Excess bromine was removed by washing with 10% sodium sulfite solution. The ester, which separates from the solution, was collected and fractionated under vacuum. The yield of ester boiling at 131–137° at 2 mm. was 70 g. from 53 g. of ethylbenzene or 66% of the theoretical amount. The yield based on the amount of dichloride used is 94% of the theoretical. The ester was refractionated to give a fraction boiling 133–135° at 2 mm. for analysis; $n^{25}D$ 1.5003; sp. gr. 1.141; d^{25}_0 1.141.

Anal. Calcd. for $C_{10}H_{15}O_3P$: P, 14.48; sapn. equiv., 107; molar refraction, 55.66. Found: P, 14.79; sapn. equiv., 106.8; molar refraction, 55.26.

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A Partial Criterion for the Comparison of Different Softwood Lignins

BY CONRAD SCHUERCH, JR.

A clear body of evidence has been developing which indicates that wood material is an interpenetrating system of hydrophilic and hydrophobic polymeric materials insoluble in neutral solvents. Although it is known that holocellulose contains several different hydrolyzable polysaccharides, there appear to be few research tools presently available which could establish the existence or non-existence of comparable heterogeneity in the hydrophobic or ligneous portion of wood.

Even in the case of hardwoods where the presence of two types of monomeric unit is proven and from which fractions of differing methoxyl content have frequently been isolated,[1] the degree of order in the substrate is difficult to assess because of uncertainty regarding the reliability of fractionation techniques and molecular weight determinations used up to the present. A single macromolecule with a single recurring pattern of guaiacyl and syringyl units would be expected to yield on random cleavage low molecular weight fractions of substantially different analyses, and these could give a false idea of the complexity of the system.

With softwood lignins, where the criteria of heterogeneity are still less obvious, it is commonly assumed that only polymer systems based on propylguaiacyl units are present and in consequence analyses are usually reported in terms of a hypothetical monomeric skeleton of nine carbon atoms. Although this hypothesis has been fruitful, a primary aim of structural studies should be to establish whether lignin is in fact a single polymeric material or several. To do so will require criteria which can establish whether or not isolated lignins are artifacts, that is, whether during each degradation process no new bonds are formed either by intermolecular polymerization or by intramolecular reaction.[2] Careful studies of molecular weight changes of fractionated lignins during chemical reaction might aid in establishing the absence of repolymerization but such studies have rarely been attempted. Perhaps the only evidence bearing on the second possibility (intramolecular reaction) is that obtained from comparative permanganate oxidations and hydrogenations of wood and isolated lignins.[1b,3,4,5] Other methods of comparison between derived lignins are highly desirable.

One simple method of evaluating softwood lignin preparations can be based on the fact that polymeric materials containing only propylguaiacyl units have an invariant ratio of per cent. methoxyl to per cent. skeletal carbon (C_{10}) equal to 31/120 or 0.258. So long as neither carbon nor methoxyl is lost or gained, the ratio remains the same—no matter what reactions the material has undergone, what number of entire propylguaiacyl units have been lost by scission, what fine structures are present, or what their degree of oxygenation or polymerization. It is therefore possible to compare this ratio in pure lignin preparations of the most divergent history or of widely different molecular weight. Fluctuations can be ascribed to the loss or gain of skeletal carbon or methoxyl in isolation *or to heterogeneity in the original starting material* but cannot be due to repolymerization or condensation reactions. In view of the frequency with which the latter are called upon to explain anomalous results, this ratio represents a useful limitation of uncertainty.

In Table I, there are listed the values of the methoxyl:carbon ratio for many isolated softwood lignins. It is apparent that these products are significantly different even according to this simple test of identity, and that all have lower ratios than

(1) C. Schuerch, THIS JOURNAL, **72**, 3838 (1950), (a) Table II and footnote 8; (b) footnote 19.

(2) The frequently applied criteria of color and solubility in a sulfite cook are tests of chemical change only and as such have utility in comparing "native" or supposedly unchanged lignins. They are clearly not applicable to derivatives of lignin which have been changed by the removal of reactive functional groups or by the introduction of elements of a hydrophobic solvent.

(3) D. Read and C. B. Purves, unpublished results indicate that cyclization occurs during some isolation methods.

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TABLE I

A COMPARISON OF THE RATIO METHOXYL TO SKELETAL CARBON[a] IN VARIOUS SOFTWOOD LIGNINS

No.	Substrate	Methoxyl, %	Skeletal carbon,[a] %	Ratio % OCH_3/% C
Theoretical values				
1	Any propylguaiacyl polymer	$(OCH_3)_1$	C_{10}	0.258
2	Probable pine lignin *in situ*[b]	16	67.5	.235
3	Probable spruce lignin *in situ*[b]	14	67.5	.207
4	Brauns native lignin formula[c]	$(OCH_3)_4$	C_{46}	.224
5	Klason lignin in wood[d]	13.5	65.6	.206
6	Klason lignin in wood[d]	12.0	60.7	.198
Alcohol-soluble lignins				
7	Klason reserve lignin[e]	12.5	60.2	.208
8	Brauns native lignin,[c] spruce	14.9	63.9	.233
Commercial type lignins				
9	Sulfonic acid from a fermented liquor[f]	9.15	46.68	.196
10	Barium sulfonates of different degrees of sulfonation: spruce A[g]	13.06	53.3	.245
11	spruce B[g]	12.73	51.2	.248
12	spruce C[g]	11.58	47.6	.243
13	spruce D[g]	9.75	40.2	.242
14	Sodium sulfonates from 85% A[h]	12.8	52.1	.246
15	Hemlock and 15% Fir B[h]	12.5	50.5	.248
16	Naphthylamine salt of β-sulfonic acid[d]	5.2	45.8[a]	.114
17	Alkali lignin A[i]	15.0	64.75	.232
18	Alkali lignin B[i]	14.0	65.02	.215
Complete lignin samples				
19	Spruce periodate[b]	12.2	61.4	.199
20	Hydrochloric acid lignin[j]	15.2	64.57	.235
Alcohol and other lignins				
21	Ethanol lignin[k]	10.42	57.6[a]	.182
22	Isobutanol lignin[l]	9.62	55.2[a]	.174
23	Amyl lignin[l]	7.89	51.9[a]	.152
24	Thioglycolic acid lignin[m]	11.7	47.0[a]	.249
25	Thioglycolic acid lignin[n]	(OCH_3) 0.92–0.95	C_{10}	.237–.245
26	Thioglycolic acid lignin[n]	(OCH_3) 0.84, 0.86	C_{10}	.217–.222
27	Cuproxam lignin[o]	16	63.6	.251

[a] Refers to total carbon minus carbon added by extractant. In no. 16) $61.2 - \frac{1.8 \times 120}{14} = 45.8$; in no. 21) $64.6 - \frac{24 \times 13.1}{45} = 57.6$; in no. 22) $66.02 - \frac{48 \times 16.4}{73} = 55.2$; in no. 23) $65.88 - \frac{60 \times 20.21}{87} = 51.9$; in no. 24) $54.6 - \frac{24 \times 10.1}{32} = 47.0$ [b] W. J. Wald, P. F. Ritchie and C. B. Purves, THIS JOURNAL, **69**, 1371 (1947). [c] F. E. Brauns, *ibid.*, **61**, 2120 (1939). [d] P. Klason, *Ber.*, **64**, 2733 (1931). These values are calculated on the basis of two different assumptions. [e] P. Klason, *ibid.*, **65**, 625 (1932). [f] K. Schwalbe and E. Prew, *Cellulosechemie*, **21**, 1 (1943). [g] H. Erdtman, B. O. Lindgren and T. Pettersson, *Acta Chem. Scand.*, **4**, 228 (1950). [h] Q. P. Peniston and J. L. McCarthy, THIS JOURNAL, **70**, 1324 (1948). [i] H. B. Marshall, F. Brauns and H. Hibbert, *Can. J. Research*, **13B**, 103 (1950). [j] E. Hägglund, *Cellulosechemie*, **4**, 73 (1923). [k] B. Holmberg and S. Runius, *Svensk Kemisk Tidskrift*, **37**, 189 (1925), quoted in ref. *l*. [l] E. Hägglund and H. Urban, *Cellulosechemie*, **8**, 69 (1927). [m] C. E. Ahlm and F. E. Brauns, THIS JOURNAL, **61**, 277 (1939). [n] B. Holmberg, *Papierfabrikant*, **36**, 218 (1938). [o] K. Freudenberg, H. Zocher and W. Dürr, *Ber.*, **62**, 1814 (1929).

that required of propylguaiacyl polymers. This is true of a complete sample—hydrochloric acid lignin (calculated to an ash and carbohydrate free basis) and also true of theoretical analyses for lignin *in situ* calculated by Klason, Brauns and Purves. Those values approaching that for a propylguaiacyl polymer most closely are apparently higher molecular weight lignin fractions (no. 10–15, 24, 25) and represent not all—though frequently a large amount—of the lignin in the wood. The lower molecular weight more soluble fractions from many methods of isolation have substantially lower methoxyl:carbon ratios. This is true of β-lignin sulfonic acids (no. 16), ether-soluble thioglycolic acid lignin (no. 26), and also native, reserve, and ethanol lignins, in which cases the insoluble higher molecular weight portions are not isolated.

Since many diverse methods give generally concordant results, it appears probable that the methoxyl:carbon ratio of lignin *in situ* is less than that required of pure propylguaiacyl polymers. The portion of protolignin which appears on chemical reaction as low molecular weight fragments is according to this criterion poorest in propylguaiacyl units. This interpretation seems not to explain all the observed variations in analyses and indeed may be quite incorrect[6]; but for the present, the assumption that lignin consists solely of one or

(6) The alternative explanation of a loss of methoxyl or gain in carbon during isolation cannot be ignored, but does not seem so probable, nor does it explain the theoretical values.

more propylguaiacyl polymers is certainly an oversimplification of the observed data.

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Experiments on the Mechanism of the Urea–Urease Reaction

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Although urease was the first enzyme to be isolated in a crystalline form,[1] in spite of the relative simplicity of its over-all reaction with urea and the detailed experiments of a number of workers, the reaction mechanism remains obscure. Now it has been shown elsewhere that the surface atoms of solid oxides possessing catalytic activity are very labile[2]: thus, using O^{18} as a tracer, it was found that aluminum oxide readily undergoes exchange of its surface oxygen with water vapor at room temperature, while many oxides suffer exchange with both water vapor and gaseous oxygen at elevated temperatures. In view of these results, and because urea possesses the grouping –CO–N– which is very common in protein, while so far as is known urease is wholly protein, we thought it worthwhile to examine whether the urea–urease reaction involves the chemical incorporation of all or part of the urea molecule into the enzyme, followed by the splitting out of the same structural elements from a neighboring part of the enzyme. Such a postulate can readily be tested by the use of isotopic urea: thus if $CO(N^{15}H_2)_2$ be used and either or both of the $-NH_2$ groups are incorporated into the enzyme, the first small amount of NH_3 evolved should contain less N^{15} than the main product, since it should be diluted with $N^{14}H_3$ from the enzyme: as the reaction proceeds the N^{15} content of the NH_3 evolved should approach that of the urea used. Similar remarks apply to the labeling of the urea with C^{13} or C^{14}. If an exchange reaction can be demonstrated, then after the reaction with isotopic urea the enzyme could, for example, be hydrolyzed and the various hydrolysis products examined for the presence of isotopic nitrogen or carbon. An experiment of this type should throw considerable light on the nature of the enzyme reaction and of the reactive centers in the enzyme.

A preliminary examination along these lines is reported here, using urea containing about 30 atom % excess of N^{15}. Considerable exchange of nitrogen has been found by us during the enzyme reaction, but some at least of this is due to exchange between the ammonia and the enzyme material.

Experimental

Preparation of Urease.—The enzyme was extracted from jackbean meal by the method of Sumner.[1] It was recrystallized from dilute acetone by addition of 0.5 *M* citrate buffer, *p*H 6.0.[3] The activity of the crystals, determined by the method of Van Slyke and Archibald,[4] was greater than 10^5 units per gram of dry enzyme.

Preparation of Urea.—Urea containing excess N^{15} was synthesized from ammonia and diphenyl carbonate by a modification of Henschel's[5] method.

Exchange Experiments.—In the first experiment 0.3 g. of the crystalline enzyme dissolved in distilled water was treated with 0.03 g. of urea. The middle fraction of the ammonia evolved was collected by aspiration in dilute hydrochloric acid.

In the second experiment, 0.3 g. of enzyme in 10 ml. of distilled water was treated with 0.006 g. of ammonia containing 30.5% N^{15}, passed by aspiration through the enzyme solution. The stream of air was continued until almost all of the ammonia had been collected in dilute hydrochloric acid. The enzyme reaction was then carried out on this pre-treated enzyme solution, using first 0.01 g. and then 0.02 g. of urea: almost complete recovery of ammonia being obtained from the first addition of urea before the second was added. The last runnings of the ammonia evolved from the second addition of urea were collected separately.

The ammonium chloride samples so obtained were converted to nitrogen, which was analyzed for N^{15} content by mass spectrometer.

	N^{15}, % Expt. no. 1	N^{15}, % Expt. no. 2
Original urea	29.1	30.8 ± 0.9
$N^{15}N_3$ before passage through enzyme	..	30.8 ± 0.9
$N^{15}H_3$ after passage through enzyme	..	23.8 ± 0.3
NH_3 evolved from 1st addition of urea	..	15.8 ± 5.0[a]
NH_3 evolved after 2nd addition of urea	20.0	22.7 ± 1.1
NH_3 evolved when reaction proceeds to completion	..	30.2 ± 1.3 30.0

[a] Sample contaminated with some air.

Discussion

The results reported do not allow us to decide whether or not our original suggestion as to the mechanism of the enzyme reaction is correct, but the exchange found with ammonia alone is of interest. The exchangeable ammonia in the enzyme can hardly be present as $COONH_4$ groups since the preparation involved much manipulation in buffer solutions containing large excesses of sodium and potassium salts. It is possible that the enzyme contains aldehyde residues which form imino groups with ammonia and that these groups take part in the enzyme eaction proper (*cf.*, the pyridoxal–pyridoxamine transformation.[6] Some support for the suggestion that aldehyde residues are an essential part of the reactive centers is found in the negative temperature coefficient of sulfite inhibition.[7] A reversible formation of imino groups as above would probably explain also the inhibition by ammonia of the rate of reaction between urea and urease.[8] Work along these and allied lines, and a repetition and extension of the experiments reported here, are in progress.

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