the amount of aldehyde bound by the protein will be largely influenced by the character of the side-chains of the various amino acids present in the protein, although the side-chains as such may not react directly with formaldehyde. It seems likely that the order of sequence of the amino acids of the peptide chain also would exert an influence on the amount of aldehyde binding. Further work is in progress in this laboratory to ascertain the effect of other substituted groups on the binding of formaldehyde.

Summary

1. The reactions between solutions of $l(+)$-aspartic and $l(+)$-glutamic acid containing two equivalents of sodium hydroxide and various amounts of formaldehyde have been followed by polariscopic and hydrogen-ion measurements.

2. Each of these amino acids reacts with formaldehyde, mole per mole, to form definite compounds. Each of these latter compounds combines with further amounts of formaldehyde when the aldehyde concentration is increased, giving unstable compounds that cannot be isolated.

3. The equilibrium constants of the above reactions have been calculated.

GENEVA, N. Y. RECEIVED FEBRUARY 10, 1943

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING AND THE LIGNIN AND CELLULOSE RESEARCH LABORATORIES, UNIVERSITY OF WASHINGTON]

Volatile Hydrogenation Derivatives of Lignin

BY A. BAILEY

A qualitative survey of the effects of hydrogenation of lignin involving the variables of solvent, catalyst and source of lignin was completed. This study indicated the most favorable point of attack for a quantitative study and served as a development and proving ground for apparatus able to effect the extremely difficult separation of compounds in a condition of purity sufficient to permit identification. This report covers the qualitative survey and the first portion of a quantitative study of the hydrogenation products of butanol lignin. The compounds thus far identified were separated by more exacting technique than that reported in any previous investigation. These compounds are largely new hydrogenation products and would not have been separated in the distilling columns used by other workers. As new products, they have constitutional interest.

Catalytic hydrogenation of Willstätter lignin was effected in 1925 by Fierz-David and Hannig.[1] Klason (sulfuric acid) lignin was hydrogenated in 1935 by Moldavskii and Vainshtein[2] and in 1938 by Harris, D'Ianni and Adkins.[3] Many research reports have been published in subsequent years. The hydrogenation reaction has high intrinsic interest and worth since yields are higher than by other reactions, the reaction is inherently one of stabilization rather than disintegration, and the products have been of great constitutional interest and value.

Eight compounds were identified in the present experiments in the fraction of the reaction mixture which boiled below butanol. The yields of these are shown in Table I.

TABLE I

HYDROGENATION PRODUCTS AND YIELDS

Compound	Yield, %
Water	17.9
2,3-Dimethylbutane	0.6
Methyl alcohol	0.2
Tetrahydrofurfuryl alcohol	1.2
Ethyl alcohol	0.3
2,2-Dimethylbutanol	1.0
Secondary butanol	1.2
Methylisopropylcarbinol	0.3

The yield of water was not reported by Harris, D'Ianni and Adkins but 7.5–8.7% was reported by Cooke, *et al*[4]. This value is undoubtedly too low since a Widmer column was used to effect the separation. Cooke did not report efficiency data but according to the data of Baker, Barkenbus and Roswell[5] the H. E. T. P. of a Widmer column

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(1) H. E. Fierz-David and M. Hannig, *Helv. Chim. Acta*, **8**, 900 (1925).

(2) B. L. Moldavskii and S. M. Vainshtein, *Khim. Tverdogo Topliva*, **6**, 656 (1935).

(3) E. E. Harris, J. D'Ianni and H. Adkins, THIS JOURNAL, **60**, 1467 (1938).

(4) L. M. Cooke, *et al.*, *ibid.*, **63**, 3056 (1941).

(5) R. H. Baker, C. Barkenbus and C. A. Roswell, *Ind. Eng. Chem., Anal. Ed.*, **12**, 468 (1940).

was 8.2 cm. which would limit the efficiency of Cooke's column to 6.1 theoretical plates. By present standards, this is extremely low. Calculations indicate that this column would require a boiling point separation of the order of 60° for quantitative separation of a two-component mixture. In addition, the authors noted other sources of loss of water. The yield of 18% of water reported here was obtained in columns packed with glass helices and of extremely high efficiency,[6] and is believed to represent very nearly the maximum yield of water in this particular reaction. The volatile components boiling below butanol were distilled off through a column having an efficiency equivalent to 40 theoretical plates and then distilled in cascade through another 40-plate column, recycled through the same 40-plate column, and then through a 60-plate column, reducing the volume of distilland in each distillation to increase efficiency.[7] This procedure made the separation easily quantitative.

A positive explanation of the low yield of methanol is not apparent. It is suspected that part of the methoxyl may be accounted for in the 2,3-dimethylbutane, the 2,2-dimethylbutanol, and the methylisopropylcarbinol. Aliphatic chains have been isolated previously in this Laboratory from the same lignin[8] although largely without the methyl side-chains. It is conceivable that the methyl side-chains arise from the splitting of the methoxyl and the subsequent wandering of the methyl radical. On the other hand, the as yet unidentified high boiling products may show the presence of methoxyl.

The ethanol reported here is probably derived from the hydrogenolysis of β-ethyl-α-methylacrolein,[8] since the ethyl radical is an uncommon constituent of lignin.

The presence of tetrahydrofurfuryl alcohol is of great interest since furfural has long been known in the past as a product of the destructive distillation of wood, although derived from pentosans rather than from lignin. This first and direct isolation of tetrahydrofurfuryl alcohol as a reaction product of lignin appears to be the first direct evidence of the existence of the furan ring in lignin as suggested by the model experiments which resulted in the synthesis of dehydrodiisoeugenol and Erdtmann's acid (*cf.* refs. 9, 10). It thus supports in that respect the hypothesis that lignin contains condensed rings of the coumaron and chroman type. Explanations of the presence of compounds of higher boiling point than butanol in the distillate are offered by the phenomena of azeotropism and codistillation.

The relatively large number of other compounds which were identified is a direct tribute to the efficiency of the fractionating columns used. No previous study of lignin hydrogenation products has employed high efficiency fractionation and none of the columns previously used could have detected the presence of these compounds in the above quantities. They therefore represent a difficult advance over previous studies. The compounds undoubtedly have structural significance which may be more evident when all of the hydrogenation products are identified. The large number of alcohols obtained apparently either occur as fairly stable ethers since they were not obtained by hydrolysis,[8] or else represent hydrogenolysis products. In either case, these compounds are decidedly not in harmony with the simple type of polymerization and ring conjugation hypothesized by some workers.[9,11] It seems likely that the ultimate structure will be quite complex, requiring much more evidence than is now available before a rational approach can be attempted.

The preliminary qualitative study made possible these generalizations.

1. The use of a copper chromite catalyst produced lower yields and a completely different series of products from butanol lignin than did Raney nickel.
2. Water as a solvent produced lower yields and different products than did butanol or dioxane as solvents.
3. Contrary to many reported experimental data, the solvent seemed to affect the kind of products formed instead of merely influencing the rate or extent of formation.
4. The source and chemical history of the lignin had a major influence on yield.
5. In general, change of solvent or catalyst tended to produce a different series of products.
6. Optimum yield was obtained from butanol

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[6] A. Bailey, *Ind. Eng. Chem., Anal. Ed.*, **13**, 487–491 (1941).

[7] All of these columns were generally similar in construction, operation, and testing to that described.[6] The first 40-plate column had a packed section 150 × 1.6 cm. i. d.; the intermediate 40-plate column 150 × 0 8 cm. i. d.; the 60-plate column was the one described.[6]

[8] A. Bailey, THIS JOURNAL, **64**, 22 (1942).

[9] K. Freudenberg, *Ann. Rev. Biochem.*, **8**, 81 (1939).

[10] K. Freudenberg, M. Meister and E. Flickinger, *Ber.*, **70**, 500 (1937).

[11] H. Hibbert, *Paper Trade J.*, **113**, T539 (July 24, 1941).

lignin, in anhydrous butanol as the solvent, over Raney nickel, at temperatures just below the critical temperature of butanol, and at as high a pressure as was consistent with reasonable safety.

The higher yields obtainable with nickel catalyst would appear to be a reflection of the partially aromatic structure of lignin since nickel has more or less specificity toward the benzene nucleus. This result is in contrast to the work of Harris, D'Ianni and Adkins.

Experimental

Preparation of Lignin.—Western hemlock (*Tsuga heterophylla* Sargent) chips from a commercial sulfite chipper were digested in equal volumes of *n*-butanol and water in a 25-liter stainless steel rotating autoclave at 160°. Repeated digestions with fresh solvent were carried out to ensure removal of all free[12] lignin. The solution of lignin in butanol was shaken with water until carbohydrates were removed. The absence of carbohydrates was proved by a negative α-naphthol test and an absence of phenylhydrazone and osazone formation. The water in the butanol was driven off by distilling. The distillate consisted of the binary azeotrope which changed to butanol as the water was removed. The boiling point of the vapor was 117° for several hours to effect the concentration to a thick sirup for storage. The dehydration aspects of this procedure have been more fully discussed elsewhere.[13]

Hydrogenation of Lignin.—The thick lignin sirup was heated on the steam-bath with stirring and addition of anhydrous butanol until homogeneous. Five large samples, for the determination of dry solids, were quickly poured into tared containers. After determining the dry solids, the lignin present in the five samples was calculated. Each of the samples was transferred separately to a one-liter bomb, 10 g. of Raney nickel added and the total volume made up to 700 ml. with anhydrous butanol. Hydrogen was pumped into the bomb to a pressure of about 3500 p. s. i. at room temperature. Heating to 260° required approximately one hour and increased the pressure to about 6500 p. s. i. The bomb itself was of the Adkins type,[14] was in fact made in the University of Wisconsin shops, and was mounted and rocked according to Adkins' specifications.[14] Heating and agitation were continued for several hours after pressure drop was noted; the total time was about twenty-five hours. In five hydrogenations about 410 g. of free butanol lignin, representing 80% of the total lignin in the wood, was treated. Each 28 g. of lignin absorbed one mole of hydrogen.

Isolation of Reaction Products.—Earlier isolations of hydrogenation products from other hydrogenations by conventional apparatus gave impure fractions which did not permit accurate analysis and necessitated the development of special apparatus for precise quantitative separation. Some of these have been described in separate publications.[6,15,16] The five reaction mixtures described above were hydrogenated under identical conditions and then united. The volatile products reported here were distilled off through a 40-plate column leaving the solvent and less volatile components behind. This distillate was then fractioned in cascade through a 40-plate column of low holdup, through another 40-plate column of very low holdup, and then through a 60-plate column of very low holdup, according to the technique already described.[6] Samples of 0.5 ml. were removed at a time from the final column, allowing the column to operate under total reflux (for at least an hour) until equilibrium was again established, when another 0.5 ml. sample was removed. Yields were determined by the weight of distillate and the elimination curve. All fractions were extremely pure; no contaminants were discovered in any of the identification procedures.

Identification of Products

(1) **Water.**—The butanol–water azeotrope was distilled in the columns mentioned with specially-designed, original heads,[6] which permitted removal of only the butanol-saturated water layer. This weighed layer was in turn distilled through the 60-plate column to determine the amount of water present by the elimination curve. Identity was established by boiling point, characteristic surface tension effects, mixture with an authentic sample, ability to form hydrates such as those formed with anhydrous copper sulfate, and the Scriba and the Biltz tests.

(2) **2,3-Dimethylbutane.**—Boiling point 58.0°; specific gravity, d^{20}_4 0.6620; refractive index n^{20}_D 1.3745; reactions—inert to all reagents.

(3) **Methanol.**—The 3,5-dinitrobenzoate melted at 108.1° and the *p*-nitrobenzoate at 95.8°.

(4) **Tetrahydrofurfuryl Alcohol.**—The *p*-nitrobenzoate melted at 46.2° and the 3,5-dinitrobenzoate at 83.0.°

(5) **Ethyl Alcohol.**—The *p*-nitrobenzoate melted at 56.0° and the 3,5-dinitrobenzoate at 93.0°.

(6) **2,2-Dimethylbutanol-4.**—The 3,5-dinitrobenzoate melted at 83.1°.

(7) ***s*-Butyl Alcohol.**—The *p*-nitrobenzoate melted at 25.2° and the 3,5-dinitrobenzoate at 75.0°.

(8) **Methylisopropylcarbinol.**—The α-naphthyl carbamate melted at 108.0° and the phenyl carbamate at 67.8°.

All of the above data are in close agreement with literature values.

The exigencies of war necessitated suspension of the latter part of these experiments involving the hydrogenation products boiling above butanol. Preliminary separations have been effected and mass quantities now await separation and identification. When and if conditions again permit, it is hoped to complete and communicate the final phase of this study.

Acknowledgment.—The author is indebted to the kindness of Mr. L. K. Henke, Mechanician at the University of Wisconsin, who constructed the hydrogenation bomb.

Summary

Eight low boiling hydrogenation products of lignin were separated and identified. Six of

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(12) A. Bailey, *Paper Trade J.*, **110**, TS 1–6 (Jan. 4, 1940).

(13) A. Bailey, *Ind. Eng. Chem.*, **34**, 483 (1942).

(14) H. Adkins, "Reactions of Hydrogen," Univ. of Wisconsin Press, Madison, Wis., 1937.

(15) A. Bailey, *Ind. Eng. Chem., Anal. Ed.*, **14**, 71 (1942).

(16) A Bailey, *ibid.*, **14**, 177 (1942).

these were new hydrogenation products of lignin. Data on optimum conditions of catalyst, solvent, and kind of lignin are recorded.

SEATTLE, WASHINGTON RECEIVED JANUARY 6, 1943

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY AND THE KEDZIE CHEMICAL LABORATORY, MICHIGAN STATE COLLEGE OF AGRICULTURE AND APPLIED SCIENCE]

The Isolation of β-Amyrin from the Leaves and Seeds of Alfalfa

BY L. CARROLL KING, CHARLES D. BALL, BYRON RIEGEL, CARL E. SCHWEITZER, PERRIN G. SMITH AND EDWIN W. MEYER

In the systematic investigation of the components of the unsaponifiable fractions from alfalfa leaf and seed oils, the occurrence of α-spinasterol and carnaubyl alcohol in the leaf meal oil,[1] and of α, β and δ-spinasterols in the seed oil[2] has been reported. In addition to these alcohols, β-amyrin has been isolated[3] from both sources. It was obtained from alfalfa leaf meal oil as the allophanate or as the acetate during the fractionation of that oil for vitamin K_1. It was also isolated from the unsaponifiable fraction of alfalfa seed oil as the acetate after removal of most of the sterols.

Hydrolysis of the acetate or allophanate gave the free alcohol, $C_{30}H_{50}O$. This was converted to the benzoate and to the *p*-nitrobenzoate. The identity of this substance from these alfalfa sources with β-amyrin is indicated by the agreement of the melting points and specific rotations of the alcohol and several of its derivatives, as recorded in Table I, with reported values. β-Amyrin and its derivatives from the two alfalfa sources were directly compared by melting points and mixed melting points.

TABLE I

Source	Elemi gum[a]		Alfalfa seeds		Alfalfa leaves	
Substance	M. p., °C.	$[\alpha]_D$	M. p., °C.	$[\alpha]_D$	M. p., °C.	$[\alpha]_D$
β-Amyrin	192–194	89.1	196–198	86.5	199–200	86.1
Acetate	238–239	82.0	238–239	79.8	238–240	85.1
Benzoate	231–232		233–234	100.1	232–233.4	98.4
p-Nitrobenzoate	257–258	93.2	257–258	95.6	257–258	

[a] J. L. Powers and W. E. Powers, *J. Am. Pharm. Assoc.*, 29, 175 (1940).

The authors from Northwestern University wish to express their appreciation to the Abbott Laboratories for their generous support of this work.

Experimental[4]

Isolation from Alfalfa Seeds.—From ground alfalfa seeds (Hardigan) was obtained a 11.8% yield of oil[2] which gave about 4% of unsaponifiable material. Fractional crystallization of 150 g. of this material from moist ether separated it roughly into three parts: a solid fraction melting above 120°, a second solid fraction melting below 120° and an oily residue. The higher melting fraction has been previously described.[2] The material melting below 120° (weight 4 g.) was fractionally crystallized from 95% ethanol. By this method more material melting above 120° was removed, leaving a fraction that melted over a range of 30–115°. This fraction was dissolved in 500 ml. of warm acetic anhydride and allowed to stand overnight. The solid material which separated was removed by filtration and dissolved in 200 ml. of boiling acetone. On cooling a flocculent precipitate formed which was removed. Further crystallization from acetone gave waxy material melting at 64–66°. It was insoluble in cold concd. sulfuric acid and appeared to be a hydrocarbon. The acetone filtrates were concentrated to 100 ml. and allowed to stand. Large rod-like and large flaky crystals slowly separated along with more of the flocculent precipitate. The mixture was filtered in such a way as to retain only the large rod-like and flaky crystals. These were then separated manually. The flaky material consisted mostly of β-spinasteryl acetate. The rod-like material was recrystallized from acetone to give 0.5 g. of beautiful crystals melting at 238–239°; $[\alpha]^{19}_D$ +79.8° (50.6 mg. made up to 2 ml. with chloroform, α^{19}_D +4.04°; *l*, 2 dm.). The crystals were identified as β-amyrin acetate.

Anal. Calcd. for $C_{32}H_{52}O_2$: C, 81.99; H, 11.18. Found: C, 81.83, 81.79, 81.97; H, 11.41, 11.05, 11.20.

β-Amyrin.—A portion of the above acetate was saponified with 5% alcoholic potassium hydroxide. The product was crystallized from 95% ethanol giving material that melted at 195–198°; $[\alpha]^{18}_D$ +86.5° (24.8 mg. made up to 2 ml. with chloroform, α^{18}_D +2.15°; *l*, 2 dm.).

Anal. Calcd. for $C_{30}H_{50}O$: C, 84.44; H, 11.81. Found: C, 84.55, 84.52; H, 11.41, 11.85.

This alcohol on heating with acetic anhydride gave in almost quantitative yield, a product identical with the original acetate; m. p. 234–238°.

β-Amyrin Benzoate.—A solution of 50 mg. of the above β-amyrin in 1 ml. of pyridine was treated with about 0.5 ml. of benzoyl chloride. The reaction mixture was heated for three hours on a steam-bath and the product recovered

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[1] B. Riegel, C. E. Schweitzer and P. G. Smith, *J. Biol. Chem.*, **129**, 495 (1939).

[2] L. C. King and C. D. Ball, THIS JOURNAL, **61**, 2910 (1939); **64**, 2488 (1942).

[3] The study of the constituents of alfalfa seeds was carried out at Michigan State College (L. C. K. and C. D. B.) and that on the leaves at Northwestern University. The discovery that β-amyrin occurs in both sources is due to Dr. King, who is now located at Northwestern University.—B. R.

[4] All melting points are corrected.