TABLE V

CONSTANTS OF AMINO ACID ANILIDE HYDROGEN CITRATES

Amino acid anilide hydrogen citrate	M.p., °C. uncor.	Nitrogen, % Calcd.	Found
Alanine	87, foaming 128°	7.87	7.7
Valine	87, foaming 118°	7.29	7.3
Leucine	157–158, foaming 162°	7.04	7.0, 7.1

anilide hydrogen citrate were weighed into screw-cap vials. A 5.0-ml. volume of the appropriate buffer was then pipetted into each vial. After standing with occasional shaking, the *p*H was readjusted to the desired value. Then were added 16.0 mg. of enzyme (Nutritional Biochemicals papain lot no. 3781, or Merck ficin, or 5.0 mg. of Armour lot no. 10705 crystallized chymotrypsin–magnesium sulfate) plus 13.0 mg. of L-cysteine hydrochloride with each of the first two enzymes. Incubation proceeded at 40 ± 1° for 3 days (5 days for Table IV).

TABLE VI

CONSTANTS OF BENZOYLAMINO ACID ANILIDES

Anilide	M.p., °C.	Nitrogen, % Calcd.	Found	$[\alpha]^{25}D$
Bz-phenylalaninanilide	220–222	8.15	7.9, 7.9	+24.8 ± 1.0[a]
Bz-phenylalanylglycinanilide	244–245	10.47	10.3	+ 6.5 ± 1.0[b]
Bz-phenylalanylglycylglycinanilide[f]	247–249[h]	12.22	11.8, 11.8	+34.9 ± 1.5[c]
Bz-phenylalanylalaninanilide	243–244	10.15	9.8, 9.8	−17.4 ± 1.0[d]
Bz-tryptophananilide	199–200	10.96	11.0, 11.1	+43.4 ± 1.0[a]
Bz-tryptophylglycinanilide	200–201			+22.6 ± 1.5[e]
Monohydrate[g]	125–140	12.22	11.8, 11.8	

Solvent for rotations consisted of 1 part of 95% ethanol to 1 part of chloroform, by volume. [a] $c = 0.50$, [b] $c = 2.47$, [c] $c = 0.33$, [d] $c = 5.68$, [e] $c = 0.76$. [f] Calcd.: phenylalanine, 32.1; glycine, 24.9. Found: phenylalanine, 34.5; glycine, 24.7, by microbiological assay with *Lactobacillus brevis* and medium of S. W. Fox, T. L. Hurst and K. F. Itschner, THIS JOURNAL, **73**, 3573 (1951). [g] H_2O lost at 125° *in vacuo* for 2 hr. Calcd.: H_2O, 3.9. Found: H_2O, 3.5. [h] A m.p. of 236–240° was reported for material which was believed to be isomerically impure; O. K. Behrens and M. Bergmann, *J. Biol. Chem.*, 129, 597 (1939).

solution was concentrated under reduced pressure, the residual gum treated with 300 ml. of boiling ethyl acetate, and this liquid discarded. The residue was dried in a desiccator and stirred under hexane until solid; yield 22 g. For analysis, a sample was dried to constant weight in a pistol.

The hydrogen citrates of valinanilide and of leucinanilide were prepared similarly; their constants are presented in Table V. The leucinanilide hydrogen citrate obtained by recrystallization from amyl acetate was definitely microcrystalline, being composed of prisms. The other two citrates appeared as jagged chunks microscopically; their crystallinity was doubtful.

Products.—The constants of the new anilides produced are given in Table VI. Benzoylphenylalaninanilide has been described.[5] All melting points were determined on a Fisher–Johns block.

Enzyme Experiments.—1.00 mmole of the benzoyl-DL-amino acid and 1.00 mmole of the glycinanilide or alanin-

Crude anilides were recrystallized from aqueous ethanol to constant values. The modified biuret test (Table II) consisted of a standard biuret run in aqueous ethanol (1:2) as a solvent.

Acknowledgments.—The help of Mr. A. J. McMillan in synthesizing amino acid anilides and in analyzing products for nitrogen content is appreciated. Jacquetta Strifert Halverson first prepared the benzoyltryptophananilide. Appreciation is expressed to Dr. Randolph T. Major of Merck and Co., Inc., for the ficin and to Dr. Otto K. Behrens of Eli Lilly and Co. for his generosity in revealing unpublished notebook records of his own experiments on anilide synthesis.

AMES, IOWA

[CONTRIBUTION FROM THE STATE UNIVERSITY OF NEW YORK COLLEGE OF FORESTRY]

Preliminary Separations of Maple Hydrol Lignin

BY MÄRTA GRANATH AND CONRAD SCHUERCH

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A method has been developed for the separation of a dimer-rich fraction from hydrol lignin. Material higher in molecular weight than dimers is largely removed by solvent precipitation and monomers are then separated by countercurrent distribution. The extent of separation has been established by molecular weight determinations, distribution coefficients of monomers, paper chromatography and the isolation of the most important monomer.

The monomeric units isolated from various degradations have been important evidence of the aromatic structure of lignin. As yet there is little experimental evidence to indicate how these units are combined in the original polymer, for little progress has been made in the isolation of larger molecular fragments. Gustavsson and co-workers[1] have studied dimer-rich fractions obtained by the chromatography of spruce and birch ethanol lignin, and Pearl and Dickey[2] have separated and identified several crystalline dimeric compounds from the alkaline oxidation of lignosulfonic acids. Because of the complexity of lignin degradations, it seems profitable to study the dimeric fractions of several isolated lignins for comparison.

The present investigation concerns the separation of a dimer-rich fraction from hydrol lignin.[3] Hydrol lignin has the advantage of being more

(1) C. Gustafsson, K. Sarkanen, S. Kahila and E. Niskasaari, *Paper and Timber*, **33**, 74 (1951).

(2) I. A. Pearl and E. E. Dickey, THIS JOURNAL, **74**, 614 (1952).

(3) C. P. Brewer, L. M. Cooke and H. Hibbert, *ibid.*, **70**, 57 (1948).

stable to acid, alkali and oxidation than most lignins, but has the disadvantage that many competing reactions may be involved in its preparation, among them hydrogenation, hydrogenolysis, hydrolysis and ethanolysis. Although the removal of water from the reaction system should increase the lignin solubility and simplify the degradative reactions, little lignin was found to dissolve in the absence of water. This is explicable if acid-catalyzed solvolysis and not hydrogenolysis is responsible for the dissolution of the lignin[4] with water liberating the wood acids which serve as the necessary source of hydrogen ion. Reactions between hydrogen and the dissolved fragments presumably follow to yield the final product which has many similarities to a stabilized ethanol lignin.[4,5]

Hydrol lignin was prepared by necessity in the usual way and carried through the following three step separation: First a concentrated chloroform solution of hydrol lignin[6] from 450 g. of sugar maple wood was precipitated into ether as previously[5] to give an ether-insoluble and an ether-soluble fraction. Secondly, the ether solution was separated by centrifugation and concentrated *in vacuo* to give a residual chloroform solution. This was shaken with 1 *N* aqueous alkali (about three times) until extraction appeared essentially complete. The alkaline solutions were combined, acidified and extracted into butanol-1. By evaporation and weighing of a small aliquot, the yield of ether-soluble alkali-soluble hydrol lignin was found to be 20 g. Finally the concentration of butanol solution was adjusted to 2% and this solution was extracted in a countercurrent fashion against a half molal solution of commercial trisodium phosphate dodecahydrate. Nine separatory funnels, numbered zero to eight, were used in series as described by Craig,[6] and subsequent to partitioning nine fractions were obtained by acidification and re-extraction into the butanol layers. Ultraviolet absorption spectra indicated that there were pronounced concentrations of the lignin fragments in funnels zero and six and a definite minimum in funnels two and three. The thick grease obtained by evaporating combined butanol solutions zero and one contained primarily dimeric material free of 3-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanol (monomer I) and 3-(4-hydroxy-3-methoxyphenyl)-1-propanol (monomer II) but still contained any 1-(4-hydroxy-3,5-dimethoxyphenyl)-propane (monomer III) which was present. In our experience and according to published data,[3] monomer III is a very minor component of hydrol lignin. No other monomers have been reported. Monomers I and II were found in funnels five to eight. The above procedure therefore constitutes a separation of a dimer-rich fraction from hydrol lignin probably suitable for ultimate separation. Evidence for this claim follows.

Precipitation of Hydrol Lignin.—The most soluble fraction of ether-insoluble hydrol lignin has been shown to have a molecular weight of around 700 and less soluble fractions higher molecular weights.[5] The ether-soluble material has been shown to contain distillable monomers and a non-distillable resinous fraction.[3,5] The latter was presumed to be an intermediate dimer-rich fraction, and the present research undertaken to separate it without the use of the high temperatures required in distillation.

The Alkaline Extraction of Ether-soluble Hydrol Lignin.—In order to remove any material containing hydrogenated aromatic rings the ether-soluble fraction was extracted into aqueous alkali as described above. This simple alkaline extraction was later found not to remove all the syringyl derivatives from chloroform solution, for some 3-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanol (monomer I) remained in the "neutral" fraction. A "neutral" fraction from the alkaline hydrogenation of maple wood was also found to contain (4-hydroxy-3,5-dimethoxyphenyl)-ethane and a similar result has been reported by Pepper and co-workers.[7] Presumably extraction with alcoholic alkali is required in order to separate phenols of this type quantitatively from neutral material.[5,8]

The Effect of Monomer Concentration and Buffer *p*H on Monomer Partition Coefficients.—With a Beckman spectrophotometer, model DU, the ultraviolet absorption spectra of monomers I, II and III were first determined in butanol and the wave lengths of maximum absorption found to be 270 mμ for I, 265 mμ for II and 260 mμ for III. The optical densities (at these wave lengths) of solutions of the monomers were found to be linear functions of concentration over the usable range, *i.e.*, up to 100 mg. per liter.

A suitable solvent system for distribution of the phenols was found to be butanol and sodium phosphate solutions. Monomer I (56.2 mg.) was dissolved in 5 ml. of butanol and shaken with 5 ml. of phosphate buffer of *p*H 12.0. The butanol layer had, after separation and dilution 250 times, an optical density of 0.174 which from the relationship density:concentration for the monomer I, gave a total of 37.5 mg. in the butanol and 18.7 mg. in the buffer. The partition coefficient (amount in the butanol, the upper layer, divided by the amount in the buffer, the lower layer) was 2.00. The partition coefficients for monomer II (concn. 18.6 mg./ml.) and III (concn. 10.4 mg./ml.) determined in the same way were 1.82 and 12.3, respectively.

Since monomer I was present in largest amount, the effect of its concentration on its partition coefficient was investigated similarly in order to find at how high a concentration a predictable partitioning could be carried out. Using only an eight-transfer extraction this requirement is not rigorous[9] and the partition coefficient appeared sufficiently constant for our purposes at least to a concentration of 30 mg. per ml. (Table I).

TABLE I

RELATION OF MONOMER I CONCENTRATION TO PARTITION COEFFICIENT[a]

Concn., mg./ml. butanol	Partition coefficient	Concn., mg./ml. butanol	Partition coefficient
1.16	1.21	10.0	1.63
3.0	1.40	15.0	1.58
6.0	1.38	30.0	1.64

[a] Monomer I partitioned between butanol and 0.5 molal phosphate buffer at *p*H 11.90.

The effect of *p*H on the partition coefficient of monomer I was determined for the butanol–phosphate buffer system, using a single concentration of monomer and a constant phosphate concentration. Phosphate a: *p*H 11.4, 0.25 molal disodium phosphate and 0.25 molal trisodium phosphate. Phosphate b: *p*H 12.0, 0.15 molal disodium phosphate and 0.35 molal trisodium phosphate. Phosphate c: *p*H 12.5, 0.5 molal trisodium phosphate (C.P.). The relation *p*H:log partition coefficient was found to be linear at constant phosphate concentration with a slope close to −0.4 rather than −1.0 observed for other systems by Golumbic and co-workers.[8] This difference is probably attributable to the fact that butanol is an ionizing solvent whereas their organic phase was not. Measurements of *p*H were made with the Beckman model H *p*H meter employing a type B-1190E glass electrode.

Countercurrent Extraction.—From the measured *p*H of a 0.5 molal solution of technical trisodium phosphate (*p*H *ca.* 13) and the relation of the partition coefficients of the monomers to *p*H, the partition coefficients of monomers I and II were calculated to be about 0.5 under the conditions of the large scale extraction. Therefore, according to the calculations of Craig,[6] only negligible quantities of monomers I and II should be present in separatory funnels zero and one after the following eight-transfer countercurrent extraction. These compounds should be found chiefly in separatory funnels five to seven, and monomer III in separatory funnel zero.

In general Craig's method[6] was followed. A 2% solution of the lignin sample in butanol was placed in the first of nine 2-liter separatory funnels, numbered zero through eight;

(4) A similar conclusion is suggested by J. M. Pepper and H. Hibbert, THIS JOURNAL, **70**, 67 (1948).

(5) C. Schuerch, *ibid.*, **72**, 3838 (1950).

(6) L. C. Craig, C. Golumbic, H. Mighton and R. Titus, *J. Biol. Chem.*, **161**, 321 (1945).

(7) J. M. Pepper, C. J. Brounstein and D. A. Shearer, THIS JOURNAL, **73**, 3316 (1951).

(8) C. Golumbic, M. Orchin and S. Weller, *ibid.*, **71**, 2624 (1949).

(9) L. C. Craig, *Anal. Chem.*, **23**, 41 (1951).

in the other eight butanol, shaken with water, was placed. An equal portion of buffer (0.5 molal trisodium phosphate, commercial hydrate) was added to the first funnel. After shaking and equilibration, the buffer was transferred to the next funnel. This was followed by successive extractions and transfers until all funnels contained the two layers.[5] The fractions were isolated by acidification, separation and drying of the butanol, and concentration. The presence of the monomers in the expected places was established by the experiments which follow.

Paper Chromatography and Isolation of Monomer I.—Single drops of unconcentrated butanol solutions zero through eight from the countercurrent extraction were placed on individual strips of Whatman filter paper no. 1 (3.5 cm. by 40 cm.) together with drops of solution of the known monomers I, II and III and developed 4 to 6 hours with moist benzene:ligroin (2:1 by volume). Blue to green spots appeared upon spraying the dried, developed chromatograms with a 2% aqueous solution of phosphomolybdic acid and then exposing the moist strips to ammonia vapor.[10] With moist benzene:ligroin as developer monomers I and II were found in funnels five to eight, and with moist ligroin as developer monomer III was found exclusively in funnel zero.

Monomer I was also isolated in crystalline form from the vacuum distillation of combined butanol solutions five through seven.

Molecular Weight of Combined Fractions Zero and One.—The number average molecular weight of the thick grease from combined fractions zero and one was determined by an ebullioscopic method using a modified Menzies–Wright apparatus.[11] Five determinations with methyl ethyl ketone as solvent gave values from 460–484, a result close to, but perhaps slightly higher than, that expected of dimeric material. These two fractions therefore constitute a dimer-rich portion of hydrol lignin suitable for ultimate separation.

Source of Monomers for Physical Data. Monomer I. 3-(4-Hydroxy-3,5-dimethoxyphenyl)-1-propanol was obtained from hydrol lignin according to Hibbert's method.[3]

Monomer II. 3-(4-Hydroxy-3-methoxyphenyl)-1-propanol.—The ethyl ester of 4-acetylferulic acid (13.2 g.)[12] was reduced with low pressure hydrogen in 150 ml. of acetic acid with 0.1 g. of platinum oxide as catalyst. The catalyst was filtered off and the solution poured into ice. White crystals appeared which after distillation and crystallization from ether had a m.p. 45–46.5°. The saturated ester (6 g.) was dissolved in 200 ml. of anhydrous ether and reduced with 5 g. of lithium aluminum hydride.[13] The reduction yielded 3 g. of a colorless, slightly cloudy oil of $n^{25}D$ 1.5543 in agreement with previous workers.[3]

Monomer III. 1-(4-Hydroxy-3,5-dimethoxyphenyl)-propane.—Pyrogallol 1,3-dimethyl ether gave with propionyl chloride the propionate,[14] which through a Fries reaction[15] and Clemmensen reduction[16] yielded monomer III.

(10) R. F. Riley, THIS JOURNAL, **72**, 5782 (1950).

(11) H. Morawetz, *J. Polymer Sci.*, **6**, 117 (1951).

(12) L. S. Fosdick and A. C. Starke, THIS JOURNAL, **62**, 3352 (1940).

(13) C. F. H. Allen and J. R. Byers, *ibid.*, **71**, 2683 (1949).

(14) M. F. Hunter, A. B. Cramer and H. Hibbert, *ibid.*, **61**, 516 (1939).

(15) C. E. Coulthard, J. Marshall and F. L. Pyman, *J. Chem. Soc.*, 280 (1930).

(16) The Clemmensen Reduction in "Organic Reactions," John Wiley and Sons, Inc., New York, N. Y., 1942, Method III, p. 167.

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Constitution of Planteose[1]

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Planteose is shown to be 6-(α-D-galactopyranosyl)-β-D-fructofuranosyl-α-D-glucopyranoside. Hydrolysis by almond emulsin gives galactose and sucrose. Mild acid hyrolysis gives glucose and a ketose disaccharide, planteobiose, 6-(α-D-galactopyranosyl)-D-fructose (syn., melibiulose) which on reduction gives melibiitol (6-α-D-galactopyranosyl D-glucitol) and epimelibiitol (1-α-D-galactopyranosyl D-mannitol).

In 1943, Wattiez and Hans reported[2] the isolation from seeds of *Plantago major* and *P. ovata* of a new crystalline non-reducing trisaccharide, planteose. This compound of glucose, fructose and galactose was easily distinguishable from raffinose by its crystal habit, optical rotation, $[\alpha]D + 125.5°$, its melting point, 124°, its water of crystallization, 6.73% (corresponding to 2 H_2O), and by its optical rotation change during acid hydrolysis (ratio of final to initial rotation, 0.775). But of most importance was the fact that planteose was not attacked by yeast invertase, whereas raffinose is easily cleaved. Hydrolysis by emulsin was reported to be slow, but eventually led to complete breakdown to the component monosaccharides. A search of the literature has not revealed any work on planteose subsequent to the original publication of Wattiez and Hans.

A preliminary examination of planteose indicated that the sugar was indeed distinct from raffinose and that it had the characteristics described by Wattiez and Hans. Planteose was found to have very nearly the same papergram mobility as its isomer, raffinose. Partial acid hydrolysis produced glucose and a reducing disaccharide, planteobiose, which responded to the phloroglucinol-HCl papergram spray for fructose, and which was not oxidized by bromine water or alkaline iodine solution. The ease of the first step in acid hydrolysis pointed to the presence in planteose of a sucrose-type linkage. The attachment of the galactose unit to the fructose unit seemed probable both from the failure of invertase to act on planteose as well as from the chemical and chromatographic evidence that planteobiose was a galactosyl fructose. Observation that planteobiose had the same papergram mobility as the ketose disaccharide component of the melibiose pyridine isomerization mixture suggested that planteobiose was probably identical with melibiulose, with carbon 6 of the fructose unit as the point of attachment of the galactosyl unit. From the high optical rotation of planteose, one would expect the galactose to be present in the α-galactopyranosyl form. These considerations led to the formula for planteose given below.

(1) Journal Paper No. J-2122 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 1116. Presented before the Sugar Division of the American Chemical Society, Atlantic City, N. J., Sept., 1952.

(2) N. Wattiez and M. Hans, *Bull. acad. roy. med. Belg.*, **8**, 386 (1943); *C. A.*, **39**, 4849 (1945).