in the Folin method. Any autoclaved urine sample should yield a lower creatinine level when compared with the nonautoclaved standard than when compared with the autoclaved standard.

One ml. of a 24-hr. specimen of urine was mixed with 20 ml. of a saturated picric acid and autoclaved for 20 minutes. The solution was then cooled, and 1.5 ml. of a 10 per cent NaOH solution was added. The mixture was allowed to stand 10 minutes, after which it was diluted to volume. The creatinine standards were dissolved in dilute HCl. One creatinine standard was autoclaved with picric acid; the other was not. All solutions were compared and read in a photoelectric colorimeter. A few samples of urine and creatinine standards were autoclaved 40 minutes. The results of the total creatinine found in the various urines are given in Table 2.

TABLE 2

TOTAL CREATININE DETERMINATIONS BY METHOD OF ALBANESE

Urine sample	Calculated from autoclaved (20 min.)		Calculated from nonautoclaved			
	Creatinine standard (mg./ml.)	Creatinine zinc chloride standard (mg./ml.)	Creatinine standard (mg./ml.)	Deviation (%)	Creatinine zinc chloride standard (mg./ml.)	Deviation (%)
(Autoclaved 20 min.)						
H 83	1.36	1.36	1.37	+0.7	1.33	−2.2
C 51	1.00	0.99	1.00	0.0	1.02	+3.0
K 13	0.88	0.88	0.89	+1.1	0.88	0.0
C 64	1.27	1.26	1.28	+0.8	1.33	+5.5
A 2	0.54	0.54	0.55	+1.8	0.55	+1.8
A 7	1.75	1.74	1.76	+0.6	1.77	+1.7
A 1	1.23	1.23	1.20	−2.4	1.20	−2.4
B 2	1.91	1.93	1.88	−1.5	1.87	−3.1
B 3	0.92	0.93	0.91	−1.1	0.91	−2.1
B 9	1.80	1.81	1.76	−2.2	1.76	−2.8
B 10	1.47	1.48	1.52	+3.4	1.52	+2.7
K 88	1.56	1.56	1.60	+2.5	1.60	+2.5
(Autoclaved 40 min.)	Standards autoclaved 40 min.					
A 1	1.17	1.17	1.18	+0.9	1.18	+0.9
B 2	1.83	1.83	1.85	+1.1	1.85	+1.1
B 3	0.89	0.89	0.90	+1.1	0.90	+1.1
8	1.31	1.31	1.30	−0.8	1.30	−0.8

The deviation in results obtained with a nonautoclaved creatinine standard as compared with an autoclaved standard varied from −2.4 to +3.4 per cent, and more than 50 per cent of the determinations were higher when compared with the nonautoclaved standards than when compared with the autoclaved standards.

Summary: Creatinuria was found in all 42 diabetic clinic subjects of both sexes taken at random.

Total creatinine determined in the urines of diabetics by the Albanese modification showed a variable deviation (in magnitude and direction) when compared with total creatinine obtained by the Folin method.

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3. FOLIN, O. *J. biol. Chem.*, 1914, **17**, 469.
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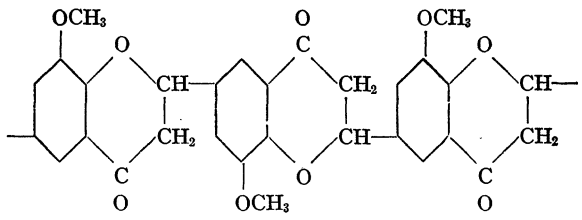
Interpretation of Lignin: The Synthesis of Gymnosperm Lignin

ALFRED RUSSELL

Northern Regional Research Laboratory,[1]
Peoria, Illinois

The existence of at least two varieties of lignin appears to be definitely established. One is associated with gymnosperms and gives, as significant fission products, only derivatives of catechol monomethyl ether (guaiacol). The other is associated with angiosperms and gives, as significant cleavage products, the same derivatives of catechol monomethyl ether and also some derivatives of pyrogallol-1,3-dimethyl ether. The possible occurrence of varieties of lignin giving other fission products is not excluded.

Examination of the analytical evidence accumulated during the past 70 years, excludes the improbable elaborate formulas previously proposed and leads to the conclusion that the lignin from gymnosperms is a polymeric 8-methoxy-dihydrobenzopyrone having the structure:



(I)

The lignin from angiosperms is likely constituted in a similar way but having pyrogallol-1,3-dimethyl ether nuclei terminally or otherwise attached or introduced. Such nuclei obviously could not replace directly the catechol monomethyl ether nuclei in (I) unless the migration of a methyl group occurs during degradation.

The material (I) is a cyclicized condensation (aldolization, loss of water—Claisen condensation) polymer of 2-hydroxy-3-methoxy-5-formylacetophenone (III) and is at once available by Fries rearrangement of vanillin monoacetate (II) through the steps (II) (III) (IV) (I).

This synthesis has been accomplished and the amorphous synthetic product, although somewhat darker in color, is qualitatively indistinguishable from a specimen of the lignin from gymnosperms. It has to give the same fission products, and its solubility characteristics and general behavior are the same. Moreover, absorption curves for the synthetic and the natural product are in agreement, and, so far as such comparisons are valid for amorphous materials, quantitative analytical measurements of the synthetic material and its derivatives are in harmony with calculated values and with the reported values for the lignin from gymnosperms.

For comparison purposes an exactly similar synthesis was carried out starting with p-acetoxybenzaldehyde. The product is analogous to that obtained from vanillin monoacetate.

It is to be noted that, in the above synthetic work, use has

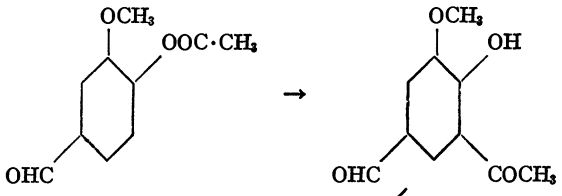
[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

been made of a new type of polymerization reaction—the polymerization of a monomeric bifunctional ketoaldehyde. In these cases the reaction amounts to a condensation polymerization, but it could, in other cases, be an addition process.

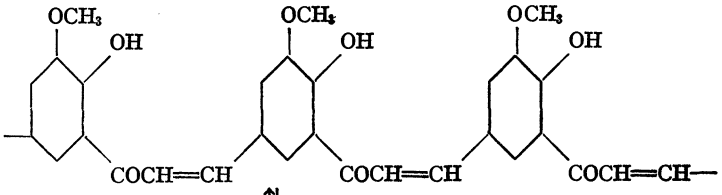
If water solubility were achieved by having enough phenolic hydroxyl groups present, materials built on the polydihydrobenzopyrone model would likely have tanning properties. It is conceivable that natural phlobatannins have just such a structure.

A detailed report of this work will appear elsewhere.

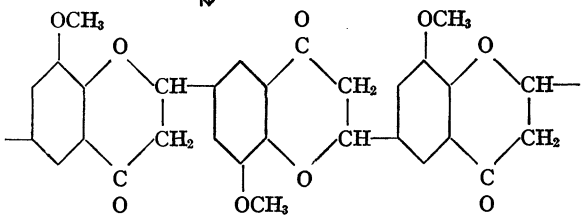
(II) → (III)



(IV)



(I)



IN THE LABORATORY

Ortho-Hydroxyphenylacetic Acid From an Amorphous Penicillin

HENRY FISCHBACH, T. E. EBLE, and JOSEPH LEVINE

Federal Security Agency, Food and Drug Administration, Washington, D. C.

Recently Welch, Randall, and Price (*6*) have directed the interest of antibiotic investigators toward the significance of some impurities in certain batches of amorphous commercial penicillin. By means of a biological assay technique (*7*) which they developed these investigators were able to determine the presence of a nonpenicillin component which enhanced the activity of crystalline penicillin. Hobby, Burkhardt, Hyman, and Levert (*4*) have also demonstrated the presence of an enhancement factor in certain lots of impure penicillin.

This laboratory undertook the task of isolating and identifying the constituents in a batch of the amorphous commercial penicillin in which Welch, *et al.* had found the enhancement factor.

Since the material described by Welch, *et al.* as containing the enhancement factor was shown to be acidic by these authors, and in view of our previous results in the application of partition chromatography to the resolution of the penicillins (*2, 3*), this technique was applied to the present problem. An investigation of numerous buffer and solvent systems finally resulted in the use of the subsequent conditions as the method of choice.

The crude penicillin was extracted four times at room temperature from an aqueous pH 2 buffer solution into ether, and the combined ethereal phases were evaporated to dryness, thus destroying any penicillin present. The residue was taken up in chloroform and added to a prepared chromatographic column in which silicic acid was the adsorbent and a 20 per cent potassium phosphate buffer of pH 3.6 the immobile solvent. The precautions mentioned in an earlier report (*2*) were followed in the preparation of the column. The chromatographic fractions subsequently referred to include the colorless as well as the colored zones on the column. With chloroform as the initial mobile solvent, 10 zones were eluted from