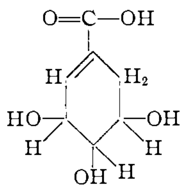
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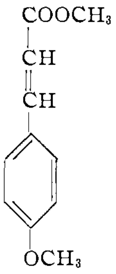
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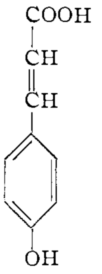
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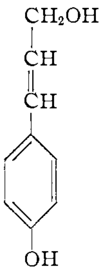
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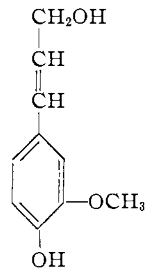
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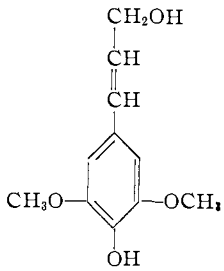

IV

Acknowledgments.—The author expresses his appreciation to the Conference Board for International Exchange for a travel award. His participation in the program of this Laboratory was made possible through grants of the U. S. Public Health Service, the National Science Foundation and the U. S. Atomic Energy Commission to Dr. F. F. Nord. He wishes to thank Dr. B. D. Davis for supplying a culture of A 170–143 S, for helpful discussions and his and Dr. E. S. Mingioli's advice in using this organism for the bioassay. The culture of Lelep was obtained through the courtesy of Dr. Wm. J. Robbins, Director of the New York Botanical Gardens. The 5-aldo-1,2-isopropylidene-D-xylofuranose, needed to prepare 6-C^{14}-D-glucose, was a generous gift from Dr. H. S. Isbell, Washington 25, D. C. Samples of shikimic acid were received from Dr. H. O. L. Fischer, Berkeley, Cal., and Dr. U. Weiss of the New York Botanical Gardens. The author also wishes to thank Drs. W. J. Schubert and D. D. Clarke of this laboratory for continued discussions and assistance, and B. Lopez-Ramos for carrying out the microbiological assays for shikimic acid.

NEW YORK 58, N. Y.

[CONTRIBUTION NO. 313 FROM THE DEPARTMENT OF ORGANIC CHEMISTRY AND ENZYMOLOGY, FORDHAM UNIVERSITY]

Investigations on Lignin and Lignification. XVII. Evidence for the Mediation of Shikimic Acid in the Biogenesis of Lignin Building Stones

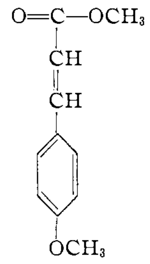
BY G. EBERHARDT AND WALTER J. SCHUBERT*

RECEIVED DECEMBER 21, 1955

A 2,6-C^{14}-shikimic acid was incorporated into a living sugar cane plant. The lignin isolated from this plant contained radioactivity. Vanillin, obtained by oxidative degradation of the lignin, shows a distribution of radioactivity which is comparable to the distribution of C^{14} in the originally incorporated shikimic acid. Thus, this acid may be considered as a precursor of the aromatic ring of lignin building stones.

In the preceding paper of this series,[1] details of the biogenesis of methyl *p*-methoxycinnamate (I) from glucose, a metabolic product of the fungus *Lentinus lepideus* (Lelep), were studied. The mechanism of formation of this compound was found to be related to that of the biogenesis of certain aromatic amino acids. Due to the structural similarity of compound I to the postulated building stones of lignin (II, III, IV),[2] these results point to a relationship with the biogenesis of lignin.

Shikimic acid (V) is regarded as a direct precursor of the aromatic ring[3] of the amino acids phenylalanine, tyrosine, tryptophan and *p*-aminobenzoic acid. Recent experiments[4] indicate that after the introduction of radioactively labeled shikimic acid into freshly cut wheat and maple plants, there was considerable activity in the lignin portions of these plants.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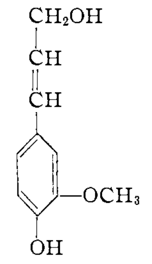
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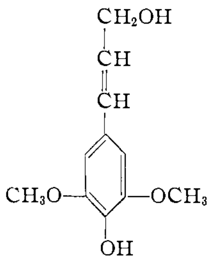
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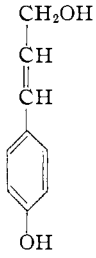
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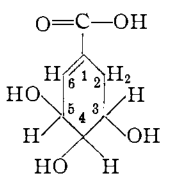
V

The experimental results to be reported here show that shikimic acid, without any rearrangement of the carbon atoms of its six-membered ring, is also to be considered as a precursor of the aromatic rings of the lignin building stones, and, accordingly, that this transformation parallels the

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* Proctor and Gamble Co. Fellow, 1953–1955.

(1) G. Eberhardt, THIS JOURNAL, **78**, 2832 (1956).

(2) F. F. Nord and J. C. Vitucci, *Arch. Biochem.*, **15**, 465 (1947).

(3) M. E. Rafelson, Jr., G. Ehrensvärd and L. Reio, *Exp. Cell Res., Suppl.*, **3**, 281 (1955). Compare also R. Robinson, "The Structural Relations of Natural Products," Clarendon Press, Oxford, 1955, p. 30.

(4) S. A. Brown and A. C. Neish, *Nature*, **175**, 688 (1955).

mechanism of formation of the aromatic amino acids.

The experiment involved the following steps: (a) Shikimic acid, specifically labeled with C^{14} in positions 2 and 6 of the ring, was prepared by microbiological means. (b) This acid was incorporated into a living lignin-producing plant. (c) The lignin of the plant was isolated, and its activity determined. (d) The lignin was oxidatively degraded to vanillin. (e) The distribution of C^{14} in the aromatic ring of the vanillin was determined by degradation of this compound.

Experimental

Specifically labeled shikimic acid was prepared by fermentation of 6-C^{14}-D-glucose by *E. coli* mutant 83-24.[5] Such shikimic acid contains 52% of its total activity in position 6 and 43% in position 2.[5] The activity of the compound amounted to about 20 μc. in 100 mg.

An aqueous solution of this acid was incorporated into the leaves of a growing, fully developed sugar cane plant (*Saccharum officinarum*) as follows. The youngest leaves of the plant were cut across with a razor blade about 5 cm. from the tips. The cut ends were dipped immediately into test-tubes containing 2 ml. of the aqueous solution of the radioactive material.

After several days of continued metabolism, the leaves were removed and the stem of the plant was cut, dried and pulverized, and the resulting powder continuously extracted with water. The extracted and dried plant material was then submitted to treatment with Schweizer reagent, and then to a modified alkaline-nitrobenzene oxidation,[6] and the resulting vanillin isolated. This compound was then purified by sublimation, and by recrystallization from petroleum ether.

The distribution of the radioactivity in the ring carbons of the vanillin was determined according to the degradations[7] shown in Scheme I.

The activities in positions C_2, C_5 and C_6 were determined by the above three series of degradations, which were performed on a millimolar scale. The following individual reactions were performed according to previously described procedures.

For C_2: Vanillin (1 m*M*) → vanillin acetate (85%) → 2-nitrovanillin,[8] m.p. 134° (47%) → bromopicrin[7] (77%) → $BaCO_3$,[9] 66.4 mg., total yield from vanillin to C_2: 28%.

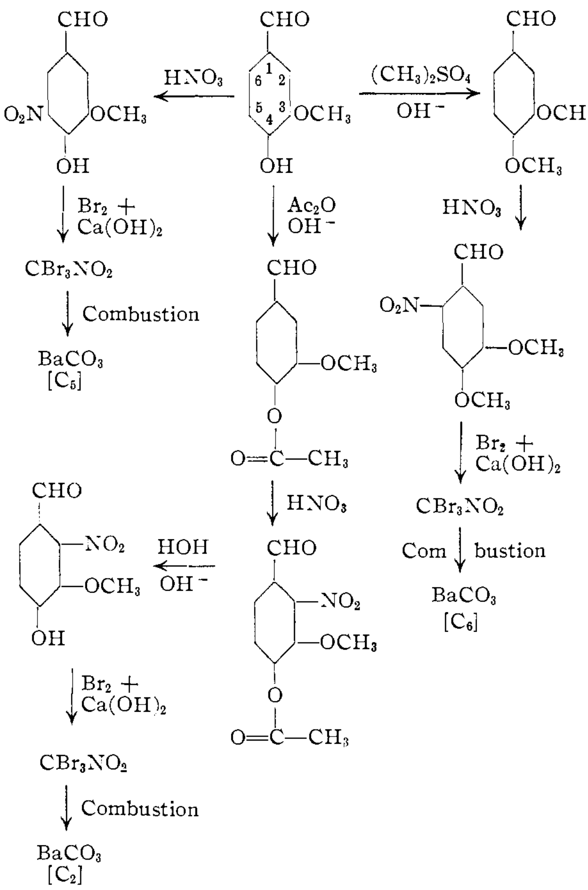
For C_5: Vanillin (1 m*M*) → 5-nitrovanillin[10] (68%), m.p. 174°, → bromopicrin (68%) → $BaCO_3$, 77.6 mg., total yield from vanillin to C_5: 39%.

For C_6: Vanillin (1 m*M*) → veratraldehyde[11] (95%) → 6-nitroveratraldehyde,[12] m.p. 132° (72%) → bromopicrin (41%) → $BaCO_3$, 46 mg., total yield from vanillin to C_6: 22%.

Determination of the Radioactivity.—All measurements are referred to an infinitely thick layer of $BaCO_3$. The counting was performed with the aid of an end window counter.

Results and Discussion

The results of the counting of the plant material (Table I) indicate that upon introduction of specifically C^{14}-labeled shikimic acid into the sugar cane, the active material was incorporated by the plant into non-water-extractable components of the stem. Counting of the isolated Klason lignin indicates that the radioactivity in its essential part is located in the lignin, as this component had a much greater specific activity than did the entire stem. Furthermore, the vanillin obtained by oxidative degradation of the lignin has a somewhat increased specific activity when compared to the lignin from which it was derived.



SCHEME I

TABLE I

DISTRIBUTION OF ACTIVITY IN THE PLANT MATERIAL

Plant material	Activity, cts./min.
Stem (ground and water-extracted)	6
Klason lignin (10% of weight of stem)	42
Vanillin	58

The vanillin molecule has eight carbon atoms, while a lignin building stone has "10.5" (*i.e.* coniferyl alcohol = 10, and sinapyl alcohol = 11). If the two oxidized C-atoms of the propyl side chain of the lignin building stones are inactive, then an increase in the specific activity from the Klason lignin (42 cts./min.) to vanillin could be calculated as 55 cts./min. In fact, the activity of the isolated vanillin amounted to 58 cts./min. Therefore, it may be concluded that the β- and γ-carbon atoms of the propyl side chain of the coniferyl alcohol contain no appreciable radioactivity.

The degradation of the vanillin (Table II) and counting of the resulting $BaCO_3$, as described above, shows the following distribution of activity in the aromatic ring of the vanillin: C_6 contains 44%, C_2 41%, and C_5 0% of the total activity of the vanillin. Thus, we find a distribution of activity

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(5) P. R. Srinivasan and D. B. Sprinson, personal communication.

(6) R. Creighton, J. McCarthy and H. Hibbert, THIS JOURNAL, **63**, 3049 (1941).

(7) J. Baddiley, G. Ehrensvärd, E. Klein, L. Reis and E. Saluste, *J. Biol. Chem.*, **183**, 777 (1950).

(8) I. Pisovschi, *Ber.*, **43**, 2137 (1910).

(9) D. D. Van Slyke and J. Folch, *J. Biol. Chem.*, **136**, 509 (1940).

(10) I. Pearl and D. Beyer, *J. Org. Chem.*, **16**, 219 (1951).

(11) K. Rosenmund, *Ber.*, **43**, 3415 (1910).

(12) E. B. Marr and M. T. Bogert, THIS JOURNAL, **57**, 1329 (1935).

in the aromatic ring of vanillin which agrees well with the original distribution of C^{14} in the six-membered ring of the incorporated shikimic acid, *i.e.*, the results give evidence that the cyclohexene ring of the acid was converted directly into the aromatic rings of lignin without randomization of the carbon atoms.

TABLE II

DISTRIBUTION OF ACTIVITY IN THE VANILLIN

	Activity, cts./min.	Percentage distribution of the total activity
Vanillin	58	100
C-6	204	44
C-2	190	41
C-5	0	0

Conclusions

After absorption of specifically C^{14}-labeled shikimic acid through the leaves of a sugar cane plant, it has been established that some of this compound was metabolized by the plant and was incorporated into a non-water extractable component of the stem of the plant. The analytical evidence indicates that the activity was incorporated to a great extent in the lignin. The degradation of the lignin, *via* vanillin, discloses that the activity, located in the aromatic ring, is comparable to the distribution of the activity in the incorporated shikimic acid.

From these results, it can be concluded that shikimic acid is an intermediate on the pathway from carbohydrates[13] formed by photosynthesis, to the aromatic rings of the lignin building stones.

Acknowledgments.—G. E. wishes to thank the Conference Board for International Exchange for a travel award and to express his appreciation to Drs. D. Sprinson and P. R. Srinivasan for discussions and experimental advice. His participation in the program of this Laboratory was made possible by grants of the U. S. Public Health Service, the National Science Foundation and the U. S. Atomic Energy Commission to Dr. F. F. Nord. The authors wish to thank Dr. Wm. J. Robbins for helpful discussions and his courtesy in arranging for obtaining the cane sugar plants which were grown under the supervision of Mr. L. Politi.

[13] F. F. Nord and J. C. Vitucci, *Nature*, **160**, 224 (1947); W. J. Schubert and F. F. Nord, THIS JOURNAL, **72**, 980 (1950).

NEW YORK 58, N. Y.

[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

Structure of Corn Hull Hemicellulose. I. Partial Hydrolysis and Identification of 2-*O*-(α-D-Glucopyranosyluronic Acid)-D-xylopyranose[1,2]

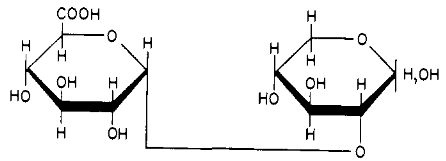
BY R. MONTGOMERY, F. SMITH AND H. C. SRIVASTAVA

RECEIVED DECEMBER 29, 1955

Graded hydrolysis of the acidic hemicellulose from the hulls of the kernels of maize (*Zea mays*) with dilute sulfuric acid yields arabinose, xylose, galactose and an aldobiouronic acid fraction. By treating the aldobiouronic acid fraction first with methanolic hydrogen chloride and then with acetic anhydride, two crystalline acetates of a methyl aldobiouronoside methyl ester have been produced. One of these acetates (II or B, m.p. 178–180°, $[\alpha]_D + 163°$ in chloroform) has been shown to be derived from 2-*O*-(α-D-glucopyranosyluronic acid)-D-xylopyranose. Methylation of II followed by reduction and remethylation gave methyl 2-*O*-(2,3,4,6-tetra-*O*-methyl-D-glucopyranosyl)-3,4-di-*O*-methyl-D-xylopyranoside (III), the structure of which has been proved by the fact that upon hydrolysis it yields 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-D-xylose.

Corn hulls which are the outer coverings of the kernels of maize (*Zea mays*) are obtained as a by-product in the manufacture of corn starch by the wet-milling process. The major component of the corn hull is a hemicellulose which can be extracted with dilute alkalies. Previous studies[3] have shown that it is composed of xylose (48%), arabinose (35%), galactose (7%) and uronic acid (10%).

Since the corn hulls represent a very cheap by-product and since the hemicellulose component of the hulls appears to show promise as an adhesive, thickener or stabilizer, a detailed study of the structure of the corn hull hemicellulose has been undertaken as part of a project[2] designed to establish in a general way the relationship between the structure and physical properties of polysaccharide gums. A preliminary report of the methylation studies on



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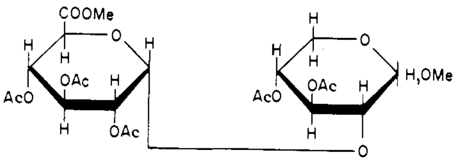

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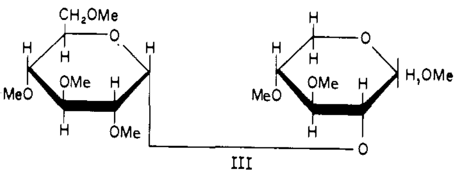

III
↓
2,3,4,6-Tetra-*O*-methyl-D-glucose
3,4-di-*O*-methyl-D-xylose

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(1) Paper No. 3481, Scientific Journal Series, Minnesota Agricultural Experiment Station, University of Minnesota.

(2) This research was done under contract with the U. S. Department of Agriculture and authorized by the Research and Marketing Act of 1946. The contract was supervised by the Northern Utilization Research Branch of the Agricultural Research Service.

(3) M. J. Wolf, Majel M. MacMasters, John A. Cannon, E. C. Rosewall and C. E. Rist, *Cereal Chem.*, **30**, 451 (1953).