ate of the type of *p*-hydroxyphenylpyruvic acid. Suitable methoxylation of such an intermediate then produces the lignin building stones, polymerization of which results in lignin.

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[CONTRIBUTION NO. 336 FROM THE DEPARTMENT OF ORGANIC CHEMISTRY AND ENZYMOLOGY, FORDHAM UNIVERSITY]

Investigations on Lignins and Lignification. XX.[1a] The Biosynthesis of Methyl *p*-Methoxycinnamate from Specifically Labeled D-Glucose by *Lentinus lepideus*

BY H. SHIMAZONO,[1b] WALTER J. SCHUBERT AND F. F. NORD

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The fungus *Lentinus lepideus* was grown in media containing 1-C^{14}-D-glucose and 6-C^{14}-D-glucose. The activities of both were significantly incorporated into methyl *p*-methoxycinnamate, a normal metabolic product. The comparative distribution of activity in the ester from 6-C^{14}-D-glucose indicates that this compound may be synthesized *via* shikimic and prephenic acids. The distribution of the activity in the ester produced from 1-C^{14}-D-glucose, when compared with that from the 6-C^{14}-compound, shows that in the metabolism of glucose by this fungus, under these conditions, a pathway other than glycolysis is operative.

Introduction

The metabolism of the mold *Lentinus lepideus*, which is a member of the group of wood-destroying fungi, is of great interest in view of the enzyme system present, which is capable of decomposing the cellulose of wood. Several aromatic compounds are formed by wood-destroying fungi from the carbohydrates of wood, and some of these metabolic products are structurally very similar to the building units of lignin, which is also susceptible to attack by certain of these fungi. *Lentinus lepideus* is known to tolerate comparatively high concentrations of creosote. This mold is also reported to give rise to methyl *p*-methoxycinnamate, methyl cinnamate and methyl anisate, while growing on wood.[2] When this fungus is cultivated on a medium containing glucose, xylose, glycerol or ethanol as sole carbon source, methyl *p*-methoxycinnamate accumulates in each case under the same conditions.[3] The mechanism of formation of this ester from the above carbohydrates and alcohols is not yet known completely. Previous investigations of this Laboratory revealed[4] that acetate was not significantly incorporated into the ester. On the other hand, in the course of studies on the metabolism of amino acids, aromatization[5] of carbohydrates and the formation of phenolic acids have been reported. The present communication reveals that the ester formed by *Lentinus lepideus* is significantly derived from carbons 1 and 6 of glucose, and describes how the activities of these positions of glucose are distributed.

(1) (a) For paper XIX of this series see the preceding communication. (b) Postdoctorate fellow from the Forest Experiment Station, Dept. of Agriculture and Forestry, Tokyo.

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Experimental

Isolation of Methyl *p*-Methoxycinnamate.—*Lentinus lepideus* was grown for approximately 40 days at 22–25° in 500-ml. erlenmeyer flasks containing 200 ml. of a medium composed of: 1-C^{14} or 6-C^{14}-D-glucose, 2%; KH_2PO_4, 0.15%; Neopeptone (Difco-Bacto), 0.1%; $MgSO_4 \cdot 7H_2O$, 0.05%, and thiamine hydrochloride 2 mg./l. in tap water.

1-C^{14}-D-glucose was obtained from New England Nuclear Corporation, Boston, Mass., 6-C^{14}-D-glucose was synthesized from $KC^{14}N$, which was purchased from Tracerlab, Inc., Boston, Mass.

Upon termination of growth, the mycelium and culture medium were filtered, and the air-dried mycelium was extracted with ethanol in a Soxhlet apparatus for 8 hr. The ethanol extract was concentrated to dryness, and the residue was sublimed twice *in vacuo* at 75°. This was recrystallized from ethanol and water and gave a m.p. of 87–88°. The melting point showed no depression when mixed with an authentic sample.

Degradation of Methyl *p*-Methoxycinnamate and Determination of its Activity.—The scheme of degradation is shown in Fig. 1. One hundred mg. of methyl *p*-methoxycinnamate was saponified with methanolic alkali to *p*-methoxycinnamic acid, which was precipitated from water by adding hydrochloric acid; yield 70 mg., m.p. 172–173°. The activity of carbon-10 was calculated by subtracting the activity of this acid from that of the ester from which it was derived.

One hundred mg. of methyl *p*-methoxycinnamate was dissolved in 20 ml. of acetone, and about 250 mg. of finely powdered $KMnO_4$ was added in small portions with shaking and cooling. The solution was filtered and the precipitate washed with acetone. This precipitate was extracted with water on the steam-bath, and the clear filtrate was acidified with hydrochloric acid. The crude crystals were purified by precipitation from water by the addition of HCl solution; yield 50 mg. (65%), m.p. 184°. The m.p. after mixing with an authentic sample of anisic acid did not show a depression.

The presence of oxalic acid in this filtrate also was detected. It is believed that during the oxidation of the ester with $KMnO_4$ in acetone, oxalate is formed from the side chain of the methyl *p*-methoxycinnamate and is precipitated in acetone. Accordingly, oxalate is accumulated in the reaction mixture without further decomposition. The filtrate was concentrated *in vacuo* and the residue dissolved in *M*/4 potassium citrate buffer (*p*H 3.0). Hereupon, oxalic acid decarboxylase, prepared[6] from the mold *Collyvia veltipes*, was added, and the CO_2 evolved was absorbed in Ba$(OH)_2$ solution. Thirty-nine mg. of $BaCO_3$ was obtained. (25 mg. as oxalic acid; yield from ester 38%.) A wet

(6) H. Shimazono and O. Hayaishi, *J. Biol. Chem.*, **227**, 151 (1957); H. Shimazono, *J. Biochem., Japan*, **42**, 321 (1955).

combustion apparatus was used for this enzymic reaction. Doubling the specific activity of the resulting $BaCO_3$ gives the activity of positions 8 and 9 together.

Forty mg. of anisic acid was decarboxylated in quinoline with copper powder, and the CO_2 evolved was swept into barium hydroxide solution by means of nitrogen gas. Thirty-eight mg. of $BaCO_3$ was obtained. The activity of this carbonate gives that of carbon 7 of the ester.

One hundred mg. of anisic acid was heated with hydriodic acid (sp. gr. 1.70) at 155–165° in a Pregl apparatus, and the methyl iodide produced was absorbed into a 5% ethanolic solution of triethylamine. On standing overnight, about 70 mg. of white crystals of triethylmethylammonium iodide[7] was obtained upon evaporation of the solution. The activity of carbon 11 was obtained from the activity of this compound.

Upon cooling of this demethylated solution, 55 mg. of *p*-hydroxybenzoic acid was obtained, which after recrystallization from water gave a m.p. of 212°. Upon mixing with an authentic sample, the melting point did not show a depression.

The breakdown of the *p*-hydroxybenzoic acid was carried out essentially according to a previously reported method.[8] The activity of carbon 1 can be calculated from the difference between the activities of: (C-1,3,5) and (C-3,5). The sum of the activities of carbons 2 and 6 can be calculated from the activities of carbon 1 and carbons 1, 2, 6 (Fig. 1).

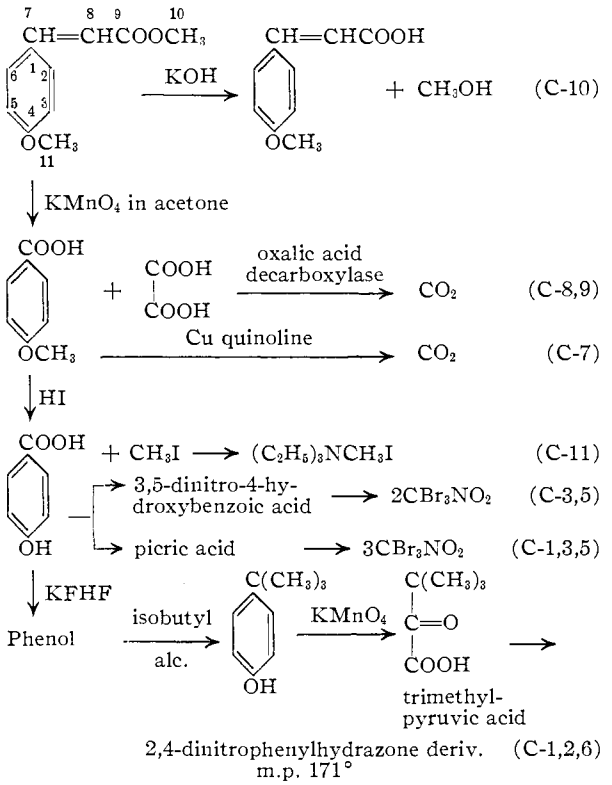

Fig. 1.

Results and Discussion

The activities of the compounds obtained by the degradation of methyl *p*-methoxycinnamate are shown in Tables I and II. It can be seen from these tables that the counts/min./mM. of the isolated ester derived from 1-C^{14}-D-glucose is almost the same as that of the glucose, while that of the ester from 6-C^{14}-D-glucose is more than twice as high as that of the glucose. Each compound obtained by the decomposition of the ester from 6-C^{14}-D-glucose is more active than the corresponding compounds from 1-C^{14}-D-glucose.

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(8) L. Reio and G. Ehrensvärd, *Arkiv Kemi*, **5**, 301 (1953).

TABLE I

ACTIVITY OF DEGRADATION PRODUCTS OF THE ESTER FORMED FROM 1-C^{14}-D-GLUCOSE

Compd. from 1-C^{14}-D-glucose	c./min./mM. compd. × 10^3
Glucose	109.6
Methyl *p*-methoxycinnamate	100.5
p-Methoxycinnamic acid	85.6
Anisic acid	78.2
p-Hydroxybenzoic acid	62.5
Oxalic acid	5.5
Anisic acid carboxyl carbon	14.2
Triethylmethylammonium iodide methyl carbon	13.3

TABLE II

ACTIVITY OF DEGRADATION PRODUCTS OF THE ESTER FORMED FROM 6-C^{14}-D-GLUCOSE

Compd. from 6-C^{14}-D-glucose	c./min./mM. compd. × 10^3
Glucose	75.7
Methyl *p*-methoxycinnamate	185.7
p-Methoxycinnamic acid	160.8
Anisic acid	149.1
p-Hydroxybenzoic acid	128.6
Oxalic acid	9.3
Anisic acid carboxyl carbon	32.7
Triethylmethylammonium iodide methyl carbon	23.5
Bromopicrin from picric acid	5.1
Bromopicrin from 3,5-dinitro-4-hydroxybenzoic acid	4.6
Carboxyl and keto carbon of trimethylpyruvic acid	42.4

TABLE III

DISTRIBUTIONS OF ACTIVITY IN METHYL *p*-METHOXYCINNAMATE FORMED FROM 1-C^{14}-D-GLUCOSE AND 6-C^{14}-D-GLUCOSE

Positions in methyl-*p*-methoxy-cinnamate C-No.	1-C^{14}-glucose c./min./mM. × 10^3	1-C^{14}-glucose % of ester	6-C^{14}-glucose c./min./mM. × 10^3	6-C^{14}-glucose % of ester
11	13.3	13.2	23.5	12.6
10[a]	14.9	14.5	24.9	13.4
8 + 9	5.5	5.4	9.3	5.0
7	14.2	14.1	32.7	17.6
1[a]	..	..	6.1	3.2
2 + 6[a]	..	..	72.7	39.1
3 + 5	..	..	9.2	4.9
4[a]				4.2

[a] Calculated value.

The comparative distributions of activity in the ester in the two experiments are shown in Table III. The percentage distributions in each carbon of the side chain are nearly identical, when comparing the esters obtained from 1-C^{14} and 6-C^{14}-D-glucose, but the absolute differences are entirely attributable to a uniformly greater dilution of C-1. In the ester produced from 6-C^{14}-D-glucose, significant activity was incorporated into carbons 7 and 2 or 6 of the phenylpropane moiety of the ester. These results are similar to those 5 obtained for tyrosine and shikimic acid biosyntheses from glucose.

Conclusion

Relating the results of the ester biosynthesis from 6-C^{14}-D-glucose to those of tyrosine metabolism, the possibility that methyl *p*-methoxycinnamate is synthesized by *Lentinus lepideus* from glucose *via* shikimic acid must be considered. However, in the ester biogenesis, the specific activity of carbon 1 underwent greater dilution, when compared with carbon 6. This probably is accounted for by an alternative oxidative decarboxylation of carbon 1 of glucose.

It also was observed that carbon 6 of glucose was markedly incorporated into the methoxyl carbon and the ester methyl carbon of the product. This result indicates that the methyl donor may not be a compound which could be derived from the citric acid cycle by this fungus. The unsymmetrical incorporation of carbons 1 and 6 of glucose into these positions gives further support of the occurrence in our organism of a pathway other than E.M.P. glycolysis. However, these considerations may be limited to the cultural conditions under which methyl *p*-methoxycinnamate is produced by *Lentinus lepideus*.

Acknowledgments.—The authors thank Dr. Wm. J. Robbins of the New York Botanical Gardens for the culture of *Lentinus lepideus* used in this study, which was supported by grants of the National Seience Foundation, the U.S. Public Health Service and the U.S. Atomic Energy Commission.

NEW YORK 58, NEW YORK

[CONTRIBUTION FROM THE CHEMISTRY DIVISION OF THE BRITISH COLUMBIA RESEARCH COUNCIL]

Phosphorylated Sugars. V.[1] Syntheses of Arabinofuranose and Arabinopyranose 1-Phosphates

BY R. S. WRIGHT AND H. G. KHORANA

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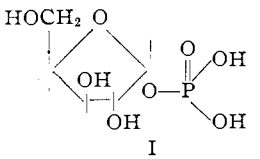
D- and L-arabinofuranose 1-phosphates have been synthesized by treatment of 2,3,5-tri-*O*-acylarabinofuranosyl bromides with triethylammonium dibenzyl phosphate, followed by hydrogenation and alkaline hydrolysis to remove, respectively, the benzyl and acyl groups. The products consisted largely of the α-anomers. The corresponding pyranose 1-phosphates were synthesized by analogous procedures using the appropriate tri-*O*-acylpyranosyl bromides. Methyl 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranoside was prepared as a crystalline substance in 50% yield by treatment of D-arabinose with methyl alcoholic hydrogen chloride followed by benzoylation and fractional crystallization of the products. Acetylation of D-arabinose in pyridine was shown to give mixtures of furanose and pyranose tetraacetates, with elevated temperatures favoring the formation of the furanose derivatives.

Since the first demonstration by Kalckar[2] of the enzymatic phosphorolysis of certain purine ribonucleosides, a number of investigations have dealt with nucleoside phosphorylases.[3] However, definitive information on important questions such as the mechanism of the action of such enzymes and their substrate specificities, especially with regard to sugar 1-phosphates, has largely been lacking. In recent papers from this Laboratory the syntheses of the anomeric D-ribofuranose 1-phosphates[4-6] were reported and from both chemical and enzymatic evidence it was established that the synthetic α-anomer[6] was identical with the ribose 1-phosphate obtained by the enzymatic phosphorolysis of ribonucleosides. It was thus clear that the ribonucleoside phosphorylases, at least those investigated,[4] brought about an inversion at the glycosidic center during the reaction that they catalyzed.[7] Further work directed to the question of substrate specificities of enzymes of this group required highly purified enzymes and some work along these lines will be reported elsewhere.[10] It also was necessary to make available synthetically some closely related sugar 1-phosphates for testing their suitability as substrates. The work reported in the present communication was therefore undertaken.

It was considered, on the basis of the results already obtained, that a possible substrate for the nucleoside phosphorylases should possess the furanose ring form and that the configuration of the phosphate group at C_1 be α. The two compounds that appeared of immediate interest were D-xylofuranose 1- (I) and D-arabinofuranose 1-phosphates (II). The synthesis of the latter was undertaken first for a number of reasons. Firstly, it differs



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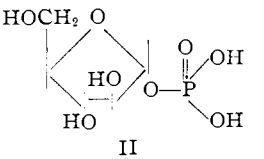

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(4) R. S. Wright and H. G. Khorana, THIS JOURNAL, **77**, 3423 (1955); **78**, 811 (1956).

(5) G. M. Tener and H. G. Khorana, *ibid.*, **79**, 437 (1957).

(6) G. M. Tener, R. S. Wright and H. G. Khorana, *ibid.*, **78**, 506 (1956); **79**, 441 (1957).

(7) It is worth noting that the recently discovered ribonucleotide pyrophosphorylases, which catalyze the reaction, purine or pyrimidine + 5 phosphoryl ribofuranose α-1-pyrophosphate[8,9] $\rightleftarrows$ ribonucleoside 5'-phosphate + pyrophosphate, also bring about an inversion at the glycosyl bond.

(8) A. Kornberg, I. Lieberman and E. S. Simms, *J. Biol. Chem.*, **215**, 389 (1955).

(9) C. N. Remy, W. T. Remy and J. M. Buchanan, *ibid.*, **217**, 885 (1955).

(10) W. E. Razzell and H. G. Khorana, *Biochim. Biophys. Acta*, in press.