the other hand, the methoxylated ester, methyl *p*-methoxycinnamate, is a known metabolic product of the wood-rotting fungus, *Lentinus lepideus*.[4]

Hence, since it seemed possible that substances might be formed by fungi which, while not chemically identical with the lignin of higher plants, at least have certain properties sufficiently characteristic to be considered as "lignin-like," it was of interest to determine the nature of this fraction of a fungus and to attempt to isolate this product by less drastic means, such as have recently been employed to isolate native lignin from white Scots pine wood.[5a,b]

The wood-rotting fungus, *Trametes pini*, was selected for this purpose, since it was reported[3] to produce 54.08% of "lignin."

Experimental

Fifty-ml. portions of the nutrient medium employed in previous experiments,[6] contained in 125-ml. erlenmeyer flasks, were sterilized by autoclaving for 20 min. at 15 lb. pressure. Each flask was inoculated with a 3-ml. spore-mycelial suspension of *Trametes pini* (obtained from Centraalbureau voor Schimmelcultures, Baarn, Holland), and incubated in the dark at 27–28°. After one month of growth, the mycelia were filtered off, dried and ground to 60 mesh in a mill.

The dried powder was analyzed for its "lignin" content by a standard method,[7] and was found to contain 23.7% of "sulfuric acid lignin." This "lignin" was subjected to analysis, with the results reported in Table I.

Since extraction of sawdust with ethyl alcohol in a Soxhlet apparatus has been demonstrated to be an efficient method of isolating an unaltered lignin from wood,[8] this procedure was also applied to the ground *Trametes pini* mats. Thus, the dried powder was extracted for 24 hours in a Soxhlet apparatus with 95% ethyl alcohol. The extract was concentrated by distillation, whereby a reddish-brown residue remained. This was dissolved in dioxane and precipitated by filtering into ether. It amounted to about 1% of the weight of the dry mats. The resulting powder was purified by repeated precipitations from dioxane into ether. The product was submitted to microanalysis, and these results are also recorded in Table I.

TABLE I

COMPOSITION OF LIGNIN-LIKE COMPONENT OF *Trametes pini*

	Prod. isolated with H_2SO_4, %	Prod. extracted with EtOH, %
C	54.12	55.21
H	4.33	5.78
OCH_3	1.55	12.76

Like native white Scots pine lignin,[5a,b] the product isolated from *Trametes pini* with ethyl alcohol reduces Fehling solution, is soluble in ethyl alcohol, methyl alcohol, dioxane, pyridine, dil. sodium hydroxide and glacial acetic acid, and insoluble in water, ether, benzene and petroleum ether. However, unlike native white Scots pine lignin, it does not give the characteristic color reactions with phloroglucinol or aromatic amine reagents.

Thus, the enzymatic formation from carbohydrate of a fungal product containing 12.66% methoxyl and resembling true lignin in many respects, has been demonstrated in the wood-rotting fungus, *Trametes pini*.

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(5) (a) W. J. Schubert and F. F. Nord, THIS JOURNAL, **72**, 977 (1950); (b) W. J. Schubert and F. F. Nord, *ibid.*, **72**, 3835 (1950).

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Heterocyclic Basic Compounds. XIII. 4-Aminocoumarin Derivatives

BY DAVID P. SPALDING,[1] HARRY S. MOSHER[2] AND FRANK C. WHITMORE

The preparation of 4-aminocoumarin derivatives was undertaken to supply samples for testing as antimalarial drugs. The starting point in these syntheses was 4-hydroxycoumarin obtained from methyl acetylsalicylate by the method of Stahmann.[3] Anschütz[4] successfully prepared 4-chlorocoumarin by the action of phosphorus pentachloride on 4-hydroxycoumarin, but he failed to report the yield. In the present work phosphorus oxychloride was found to be a superior reagent. 4-Chlorocoumarin was found to react vigorously with orpholine to produce 4-morpholinocoumarin in good yield. The reaction with 3-diethylaminomethyl-4-hydroxyaniline was not so vigorous, and resulted in the product 4-(4′-hydroxy - 3′ - diethylaminomethylanilino)-coumarin, an analog of the quinoline antimalarial Camoquine,[5] in much lower yield. Neither of these last two compounds showed any indication of antimalarial activity in the *P. gallinaceum* infection in chicks.[6]

Experimental

4-Chlorocoumarin.—4-Hydroxycoumarin (25 g.) was refluxed with 15 ml. of phosphorus oxychloride for two hours. After hydrolyzing the mixture in ice-water, the reaction product was separated and extracted with 500 ml. of ethanol and the purified product obtained by crystallization. The yield was 27.7%, 7.7 g., m. p. 89–91°. Anschütz[4] reports m. p. 91–92°.

4-Morpholinocoumarin.—A mixture of 6 g. of morpholine and 4.1 g. of 4-chlorocoumarin produced a deep red solution; a vigorous reaction immediately followed solution. On standing the reaction mixture set to solid. After thorough washing with cold water the product was crystallized from 50-ml. portions of ethanol to a constant melting point of 139–141°. The yield was 71% (3.8 g. light orange colored plates).

Anal. Calcd. for $C_{14}H_{13}O_3N$: N, 6.12. Found: N, 6.06.

4-(4′-Hydroxy-3′-diethylaminomethylanilino)-coumarin.—2-Diethylaminomethyl-4-acetylphenol[5] (11.8 g.) was hydrolyzed by heating two hours with 50 ml. of water and 50 ml. of concentrated hydrochloric acid. After

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(3) Stahmann, *et al.*, THIS JOURNAL, **65**, 2285 (1943).

(4) Anschütz, *Ann.*, **367**, 200 (1909).

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(6) We are greatly indebted to Dr. R. J. Porter of the University of Michigan for these results.

adjusting the *p*H to approximately 4 with 40% sodium hydroxide, 8.8 g. of 4-chlorocoumarin was added and reflux was continued for seventeen hours. The reaction mixture was made basic with concentrated ammonium hydroxide and extracted with 400 ml. of chloroform in three equal portions. After drying the chloroform solution over magnesium sulfate, the solution was concentrated to 250 ml. and dry ether was added until the solution became cloudy. On cooling 5.0 g. of brown solid separated which on recrystallization from 500 ml. of methanol gave 4.0 g. (23.6%) of light-green solid, m. p. 210–211°.

Anal. Calcd. for $C_{20}H_{22}O_3N_2$: N, 8.27. Found: N, 8.38.

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Modified Synthesis of Pteroylglutamic Acid

BY SHOJIRO UYEO, SATOSHI MIZUKAMI, TOKUO KUBOTA AND SHUZO TAKAGI

The use of trihaloacetones in the synthesis of pteroylglutamic acid from 2,4,5-triamino-6-hydroxypyrimidine (I) and *p*-aminobenzoylglutamic acid (II) has been described by Hultquist and Dreisbach[1] and by Weygand and Schmied–Kowarzik[2].

This method seems preferable to the standard method of Waller, *et al.*,[3] in which α,β-dihalopropionaldehyde is used in place of trihaloacetone. In the latter case dihydropteroylglutamic acid should result initially and this intermediate should be dehydrogenated automatically in the reaction medium or oxidized chemically with iodine,[4] sodium bichromate[5] or mercuric acetate[6] to the aromatic pteridine.

The details of the original procedures were not available to us, but the condensation of equivalent quantities of (I), (II) and 1,1,3-trichloroacetone in water at room temperature for several days while the *p*H was maintained at 4 by the addition of sodium bicarbonate solution gave crystalline pteroylglutamic acid of about 85% purity in a yield of only 1.4%. Under these conditions the solution and product darken, so the reaction was modified by adding sodium bisulfite in order to avoid the oxidation of (I) by trichloroacetone. This was found to have a pronounced beneficial effect, giving after purification a 37% yield of crystalline pteroylglutamic acid of about 80% purity. In a similar manner, the same product was obtained in a 15% yield when 1,1-dichloro-3-bromoacetone was substituted for trichloroacetone.

It seems that the sodium bisulfite may play a role not merely as an antioxidant, but also as a catalyst to effect the combination of the three components to give pteroylglutamic acid. Semb[7] found that 2-amino-4-hydroxy-6-methylpteridine, instead of the 7-methyl derivative, was the principal product when methylglyoxal was allowed to react with 2,4,5-triamino-6-hydroxypyrimidine in water solution at *p*H 7 in the presence of sodium sulfite. Seeger, *et al.*,[4] obtained chiefly 2,4-diamino-6-methylpteridine by using 2,4,5,6-tetraaminopyrimidine in place of the triamine in the same reaction. These results seem to be in accordance with ours, although our finding was done before the appearance of their papers.

Other reducing agents, such as sodium hydrosulfite or ascorbic acid did not show any appreciable influence on the yield.

Experimental

1,1-Dichloro-3-bromoacetone (III) and 1,1,3-Trichloroacetone (IV).—These two compounds were prepared according to the procedure of Cloez.[8] III was recrystallized from petroleum ether forming colorless needles, m. p. 28–30°.

Anal. Calcd. for $C_3H_3OCl_2Br$: C, 17.5; H, 1.5. Found: C, 17.6; H, 1.6.

IV distilled at 77–79° at 25 mm. and crystallized on cooling as colorless needles melting at 13.6–15.5°.

Anal. Calcd. for $C_3H_3OCl_3$: C, 22.3; H, 1.9. Found: C, 22.3; H, 1.9.

Both III and IV formed dihydrates when each compound was poured into the same amount of water. III dihydrate formed colorless plates, m. p. 53–55°.

Anal. Calcd. for $C_3H_3OCl_2Br\cdot 2H_2O$: C, 14.9; H, 2.9. Found: C, 14.9; H, 2.9.

IV dihydrate, m. p. 47–48°, was isomorphous with the trichloro compound and did not depress the melting point when admixed with III dihydrate.

Anal. Calcd. for $C_3H_3OCl_3\cdot 2H_2O$: C, 18.2; H, 3.6. Found: C, 18.4; H, 3.7.

Both dihydrates are easily soluble in water.

Pteroylglutamic Acid.—To a solution of 1.13 g. of 2,4,5-triamino-6-hydroxypyrimidine,[9] 2.13 g. of *p*-aminobenzoyl-L-glutamic acid[10] and 1.5 g. of sodium bisulfite in 150 cc. of water was added a solution of 1.3 g. of 1,1,3-trichloroacetone in 60 ml. of water. The acidity of the resulting mixture was adjusted and maintained at *p*H 4 throughout the reaction with frequent addition of sodium bicarbonate solution. After standing for ninety-five hours at room temperature the precipitation was complete. The product was then filtered off and washed with water, alcohol and ether. The crude material was purified twice according to the method of Waller, *et al.*,[3] by reprecipitation and 1.30 g. of yellowish-orange crystals were obtained. The purity by chemical assay[11] was about 80%. The substance was reprecipitated once more in a similar manner and the purity was raised to about 92%; yield 1.04 g. For analysis it was further reprecipitated thrice and dried at 140° *in vacuo* for five hours. The dried pale yellow crystals were very hygroscopic and on exposure to air they gained in weight quite rapidly, changing color to yellow. The analyses agreed with values calculated for partially rehydrated material.

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(9) Traube, *Ber.*, **33**, 1371 (1900).

(10) Van der Scheer and Landsteiner, *J. Immunology*, **29**, 373 (1935).

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