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DE NOVO SYNTHESIS AND DECOMPOSITION OF VERATRYL ALCOHOL BY A LIGNIN-DEGRADING BASIDIOMYCETE

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In studies of the metabolism of lignin-related aromatics by the ligninolytic basidiomycete *Phanerochaete chrysosporium* (strain ME-446), a compound was consistently found (TLC) in chloroform extracts of cultures. The substance, identified as veratryl alcohol (**1**), was first suspected to be a non-metabolizable degradation product of the aromatics studied, which included various guaiacyl- and veratryl-type compounds. Veratryl alcohol itself, in fact, was included in the first experiments. Further investigation with cultures containing ^{14}C-glucose as growth substrate revealed that **1** is synthesized *de novo* from glucose. Although the culture medium contained in addition to glucose 0.01 M phthalate or aconitate (buffers), and 0.6 mM L-asparagine (nutrient nitrogen), glucose was the sole source of veratryl alcohol carbon.

Introduction of the purified biosynthetic ^{14}C-veratryl alcohol into fresh cultures resulted in 40% decomposition to $^{14}CO_2$ in 20 days, showing that the fungus not only makes the compound, it also degrades it.

Russell *et al.* [1] found veratryl alcohol and veratraldehyde in cultures of a ligninolytic fungus (*Polystictus versicolor*), but considered them to be degradation products of the lignin-related aromatics or wood meal present in the cultures. Reports of synthesis or decomposition of veratryl alcohol by microorganisms were not found.

EXPERIMENTAL

Culture conditions. Cultures were grown under conditions optimized for the metabolism of lignin [2]. Each 125-ml culture flask contained, in a total of 10 ml basal medium [2]: 0.6 mM NH_4NO_3 and 0.6 mM L-asparagine as nitrogen sources, and 56 mM D-glucose. The medium was buffered at pH 4.5 with 0.01 M Na phthalate or 0.01 M Na aconitate. Flasks were seeded with $2 \cdot 10^6$ conidia from 3-week-old malt agar slants. Incubation was at 39°, without agitation, in intermittent light and under an atmosphere of essentially 100% O_2 [2].

Isolation and identification of veratryl alcohol (**1**). Veratryl alcohol was detected in the cultures after 5–6 days, and was present in a concentration of *ca* 30 μg/ml after 12 days. It was extracted from the cultures with $CHCl_3$ and purified by PLC [Si gel; C_6H_6–dioxane–HOAc (210:25:4)]. The product was identified as **1** by TLC on Si gel, using the above solvent (R_f 0.29) and also C_6H_6–EtOAc (3:1) (R_f 0.20), and C_6H_6–dioxane–HOAc (90:25:4) (R_f 0.67). **1** appeared as a purple spot on spraying with conc H_2SO_4–formalin (9:1) followed by brief heating at 140°. The identity of the product with veratryl alcohol was corroborated by its conversion to veratraldehyde (TLC) on oxidation with DDQ [3] and by PMR spectroscopy. PMR (270 MHz, $CDCl_3$): δ 1.6 (1 H, *s*, OH), 3.88 (3 H, *s*, OCH_3), 3.90 (3 H, *s*, OCH_3), 4.63 (2 H, *s*, CH_2), 6.84 (1 H, *d*, J = 8 Hz, *ortho* coupling), 6.90 (1 H, *dd*, J = 1.6 Hz, *meta* coupling, and 8 Hz, *ortho* coupling), 6.94 (1 H, *d*, J = 1.6 Hz, *meta* coupling) (aromatic protons).

Veratryl alcohol (**1**) *from glucose-*^{14}C. **1** was isolated from cultures grown as above with glucose-[U-^{14}C] ($1.38 \cdot 10^6$ dpm/mmol). It comprised 60% of the radiolabeled material in $CHCl_3$ extracts of 12-day cultures. Following isolation by PLC as above, its concentration was determined by UV (λ_{max}^{EtOH} nm (log ε): 279 (3.43), [4]), and its radioactivity determined in a modified Bray's scintillation fluid [5]. The specific radioactivity of the product was determined to be $2.17 \cdot 10^6$ dpm/mmol.

Decomposition of veratryl-^{14}*C alcohol.* Veratryl-^{14}C alcohol, isolated as above, was added in DMF to duplicate 1-day cultures (500 μg **1** in 25 μl DMF/culture). The cultures were grown as above (phthalate buffer). Evolved $^{14}CO_2$ was flushed from the flasks at 1–3 day intervals [2]. $^{14}CO_2$ was detected in 4-day cultures and the total trapped from the two cultures after 20 days amounted to 39 and 41% of the added radiolabel.

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