Biochemistry of the Wood-rotting Fungi

4. METABOLIC PRODUCTS OF *TRAMETES SUAVEOLENS* (LINN.) FR.

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In an examination of the volatile metabolic products of the odoriferous fungus *Lentinus lepideus* Fr., Birkinshaw & Findlay (1940) identified the methyl esters of *p*-methoxycinnamic, and cinnamic acids. Another acid, anisic acid, was also present as an ester but the nature of the alcohol moiety was left undetermined, although it was considered that the ester was probably the methyl ester.

Another wood-rotting fungus which possesses a characteristic odour is *Trametes suaveolens* (Linn.) Fr. This fungus is frequently responsible for decay in willow trees. The odour of the natural fruit body is recorded by Ramsbottom (1923) as 'strong of anise', that of the mycelium in culture is described by Badcock (1939) as 'strong, almost anisaldehyde'. The identification of the volatile products of this fungus forms the subject of the present communication.

The fungus was grown on a liquid malt extract medium, and the volatile products in both the mycelium and the culture fluid were examined. The main product from both sources was methyl anisate (methyl *p*-methoxybenzoate). The odour of methyl anisate recalls that of *T. suaveolens* in culture, and this product is no doubt partly responsible for the smell produced by the fungus. The occurrence of anisic acid as its methyl ester in the products of *T. suaveolens* strengthens the probability that it is also combined in this form in the products of *Lentinus lepideus*. In addition to methyl anisate small amounts of anisaldehyde (*p*-methoxybenzaldehyde) and of free anisic acid (*p*-methoxybenzoic acid) were detected. Anisaldehyde is a constituent of many ethereal oils derived from higher plants, particularly of those containing anethole, from which it is produced by atmospheric oxidation. It occurs in oil of anise which *Trametes suaveolens* resembles in odour. The presence of small amounts of free anisic acid is to be expected, since anisaldehyde is readily oxidized to anisic acid when exposed to the air. The absence of appreciable amounts of cinnamic acid derivatives is of interest, since these were the major products isolated from *Lentinus lepideus*.

EXPERIMENTAL

Organism. The culture of *Trametes suaveolens* (Linn.) Fr. used was isolated from a sporophore of the fungus found growing on a willow tree near Oxford.

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Method of culture. The fungus was grown on a 5% aqueous solution of commercial malt extract distributed in 95 one-litre conical flasks each containing 350 ml. of the medium. The flasks were sterilized and inoculated with small portions of mycelium of *T. suaveolens* derived from malt-agar slopes; they were then incubated at 24°. The cultures were harvested after 34 days.

EXAMINATION OF METABOLIC PRODUCTS

Mycelium

This was separated from the culture fluid by filtration and subjected to steam distillation. About 5 l. of distillate were collected. It contained crystals (plates) which were increased in amount by chilling. These were collected and dried; 0.4 g., m.p. 48–48.5°. They were shown by analysis and by their failure to depress the m.p. of an authentic specimen of m.p. 48.5–49° to be methyl anisate. (Found: C, 65.2; H, 6.07; MeO, 37.1. Calc. for $C_9H_{10}O_2$: C, 65.04; H, 6.06; 2MeO, 37.35%.)

The filtrate from the steam distillate was extracted with ether. After removal of the ether the residue (0.86 g.) was partly crystalline. The crystals, freed from adherent oil by pressing on porous tile, weighed 0.3 g. and had m.p. 47–48°. They consisted of slightly impure methyl anisate.

Culture filtrate

The filtrate was extracted with 6 l. of ether. The extract (8.0 g.) was an oil containing crystals. After pressing on porous tile, the crystals weighed 1.47 g. and had m.p. 40–43°. These also proved to be impure methyl anisate.

The tile was ground up, together with that containing residues from the steam distillate of the mycelium, and extracted with ether. The total oil (6.81 g.), containing some crystals, was distilled *in vacuo*. The main fraction (Fraction I), 3.73 g., b.p. 123–125°/5 mm., was at first a colourless oil but later it crystallized. A small second fraction (Fraction II) was obtained of wt. 0.64 g., b.p. 140–175°/about 1 mm. There was an undistilled residue (2.01 g.) which showed signs of incipient decomposition, and was not further examined.

Treatment of distillates from ether extract of culture filtrate

(a) *Fraction I* (3.73 g., b.p. 123–125°/5 mm.). This distillate was at first an oil, but on touching the surface with a glass rod it set to a mass of crystals, with some oil. It was pressed on porous tile and gave 1.9 g. of crystals of m.p. 46.5–47.5°, consisting of impure methyl anisate. The oil (1.7 g.) was recovered from the porous tile by ether extraction and subjected to alkaline hydrolysis.

For this purpose, 0.53 g. was heated with aqueous *N*-NaOH (20 ml.) for 1 hr. under reflux. The solution required 17.20 ml. of *N*-HCl for neutralization. The neutral solution was ex-

tracted with ether, giving 0.10 g. of oil, which was dissolved in 4 ml. of ethanol and treated with 2:4-dinitrophenylhydrazine in HCl (Brady & Elmslie, 1926). A red precipitate, 0.13 g., m.p. 244–246°, was obtained. The m.p. was raised to 251–252° on recrystallization from ethyl acetate—brilliant red plates. A further amount of the dinitrophenylhydrazone was prepared directly from the oily portion of the distillate without hydrolysis. This was shown by analysis and comparison (mixed m.p.) with an authentic sample of m.p. 252° to be the 2:4-dinitrophenylhydrazone of anisaldehyde. (Found: C, 53.27; H, 3.86; N, 17.5; MeO, 9.9. Calc. for $C_{11}H_{12}N_4O_5$: C, 53.16; H, 3.82; N, 17.71; MeO, 9.8%.)

The ether-extracted neutral solution from the hydrolysis on acidification yielded crystals (0.31 g.) of m.p. 176–181°, consisting of impure anisic acid. Thus Fraction I of the distillate consisted mainly of methyl anisate together with a small amount of anisaldehyde.

(b) *Fraction II.* 0.64 g., b.p. 140–175°/c. 1 mm. This was a yellow oil containing a little crystalline material. As it was acidic it was dissolved in ether and washed with saturated aqueous $NaHCO_3$.

(c) *Acids from Fraction II.* On acidification of the $NaHCO_3$ solution, acids (0.05 g.) were precipitated. They were subjected to fractional sublimation in high vacuum, and gave two main fractions: (1) 12 mg., m.p. 102–104°; (2) 17 mg., m.p. 166–176°, after softening above 120°. Sublimates (1) was still a mixture which could not be satisfactorily separated into its components. Sublimates (2) on recrystallization from benzene gave 8 mg. of an acid of m.p. 180–181°, which was shown by comparison (mixed m.p.) with an authentic sample to consist of anisic acid.

(d) *Neutral portion of Fraction II.* The ether solution which had been washed with $NaHCO_3$ contained 0.47 g. of

oil which slowly developed crystals. A portion (0.19 g.) was subjected to alkaline hydrolysis by boiling with 5 ml. of $N-KOH$ in methanol for 1 hr. The crystalline K salt which formed on cooling was dissolved by addition of water (20 ml.), and the liquid after extraction with ether to remove unsaponifiable matter was acidified with HCl. The crystals (needles, 0.09 g.) which separated had m.p. 160–165°, raised to 180–182° on recrystallization from benzene. This was again anisic acid (mixed m.p., no depression).

Fraction II therefore contained an ester of anisic acid—doubtless methyl anisate—and a small amount of free anisic acid together with traces of other unidentified acids.

Neither Fraction I nor Fraction II showed any marked uptake of bromine when dissolved in CS_2 , which indicated the absence of appreciable amounts of cinnamic acid or derivatives.

SUMMARY

The volatile metabolic products of the fungus *Trametes suaveolens* (Linn.) Fr. grown on aqueous malt extract were examined. Methyl anisate was identified as the major product; anisaldehyde was also detected, but is present in much smaller amount. These two products are probably the chief contributors to the odour of the fungus in culture. A little free anisic acid was also detected; this may arise by atmospheric oxidation of the anisaldehyde.

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The Riboflavin Content of Food Served in Royal Air Force Messes

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As our knowledge of the role of riboflavin as an essential factor in human nutrition increases, the need for satisfactory estimation of the riboflavin content of food becomes more urgent. The microbiological method has been employed in many laboratories and has given reproducible results. However, if this method is to be accepted as reliable, it must give values in agreement with those obtained with other methods and in particular with those obtained with a reliable biological method. During the past two years food material prepared by a

method described by Macrae, Henry & Kon (1943) has become available. Since this consisted of a complex mixture of foodstuffs it appeared to be suitable for comparative tests from which the reliability of the microbiological method of estimation of riboflavin in foodstuffs might be ascertained. Any substances which might possibly interfere with such tests were likely to be present in a mixture of this kind. This comparative study allowed a determination of the daily riboflavin intake of R.A.F. personnel.