Microbiological Oxidation of Fusidic Acid

The tetracyclic triterpene derivative fusidic acid is of considerable interest because of its antibiotic activity[1] and because of the unusual *trans, syn, trans* arrangement of its A, B, and C rings[2]. We have initiated some experiments on the microbiological modification of the compound and have now isolated a crystalline product from a *Corynebacterium simplex* fermentation which has been identified as 3-oxofusidic acid.

The sodium salt of fusidic acid was added to a shaken *C. simplex* ATCC 6946 fermentation in yeast extract-peptone medium and incubated at 28 °C for 25 h. Product formation was followed by paper chromatography in the ZAFFARONI toluene-propylene glycol system[3].

The broth was adjusted to pH 3.3 and extracted with butyl acetate. The organic phase was treated with aqueous sodium hydroxide at pH 11, removing fusidic acid and leaving most of the 3-oxofusidic acid. The butyl acetate solution was washed with water, dried with sodium sulfate, and concentrated in vacuo. The resulting oil was taken up in ethyl alcohol, filtered free of an insoluble residue, transferred to water as a sodium salt, and extracted into methyl isobutyl ketone at pH 3.3. The extract was chromatographed on silicic acid by the procedures of HIRSCH and AHRENS[4]. The material eluted by 50% ether-hexane crystallized to give a 31% yield of 3-oxofusidic acid, m.p. 185–186 °C.

The structure of the isolated material was deduced from the following considerations. Elementary analysis (C, 72.06; H, 8.78), equivalent weight (518), pKa[1] (5.3), and UV-absorption (λ_{max}^{EtOH} 207 nm [ε 9,600]; $\lambda_{shoulder}^{EtOH}$ 213 nm [ε 9,100]) showed it to be not greatly altered from fusidic acid. The IR-spectrum (λ_{max}^{KBr} 5.75, 5.82, and 5.91 μ) indicated the presence of a new carbonyl group. If the absorption at 5.75 μ is assigned to the acetate carbonyl and the one at 5.91 μ to the acid carbonyl, as is usual, the absorption at 5.82 μ can be assigned to the carbonyl of a cyclic-6-membered ketone. Confirmation of this carbonyl group was obtained by treatment of a papergram of the product and fusidic acid with an acidified solution of 2,4-dinitrophenylhydrazine in ethyl alcohol. Fusidic acid gave no color, but the product gave a yellow color characteristic of saturated 3-ketones[5]. It was further found that the product gave a positive Cotton effect curve (RD in dioxane [c, 0.15], 24 °C: $[\alpha]_{400}$ + 21.2°, $[\alpha]_{310}$ + 590°, $[\alpha]_{285}$ + 217°, $[\alpha]_{275}$ + 360°), indicating a carbonyl chromophore; fusidic acid gave a plain positive dispersion curve (RD in dioxane [c, 0.19], 24 °C: $[\alpha]_{400}$ − 64°, $[\alpha]_{328}$ 0.00°, $[\alpha]_{270}$ + 960°).

The identification of the oxidation product as 3-oxofusidic acid was confirmed by direct comparison with an authentic sample, m.p. 193 °C, kindly furnished by Dr. W. O. GODTFREDSEN[6]. The two materials had identical chromatographic behavior (Rf 0.33–0.37; $R_{fusidic\ acid}$ 2.5), IR-spectra, and gave the same yellow color with 2,4-dinitrophenylhydrazine. In contrast, a sample of 11-oxofusidic acid, also furnished by Dr. GODTFREDSEN, gave an Rf of 0.26, a dissimilar IR-spectrum, and no color with 2,4-dinitrophenylhydrazine.

Zusammenfassung. *Corynebacterium simplex* oxydiert Fusidinsäure, ein Antibioticum aus *Fusidium coccineum*, zu 3-Oxofusidinsäure.

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Research Division, Wyeth Laboratories, Radnor (Pennsylvania, USA), May 2, 1966.

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[6] Dr. GODTFREDSEN has recently reported (W. O. GODTFREDSEN, W. VON DAEHNE, L. TYBRING, and S. VANGEDAL, J. Med. Chem. *9*, 15 [1966]) that 3-oxofusidic acid is formed in small amounts by the fusidic acid producing strain of *Fusidium coccineum*.

Formation of Red Pigment During Wood Decay Caused by White-Rot Fungi

Some wood-decaying fungi responsible for the white-rot of wood, cause it to get red in the first stage of the destroying process and later to get light. In the presented short communication the red pigment formation during the wood deterioration caused by the wood-rotting fungi mentioned is studied.

Spruce sawdust was used as the nutrient medium, moistened with a 3% water solution of peptone. Cultivation vessels of 1500 ml were filled with the sawdust, which was sterilized in streaming vapour and inoculated with 150 ml of mycelial pellets, grown on 3% malt extract, of the following species of fungi: *Trametes versicolor* (Fr.) Pilát and *Trametes gibbosa* (Pers.) Fr., i.e. representatives of white-rot fungi, and with *Fomes marginatus* (Fr.) Gill., a representative of brown-rot fungi. After 4 months' cultivation in the dark at a temperature of 25 °C, when the sawdust was completely covered with mycelium, the red pigment began to develop in cultivation vessels with white-rot fungi. Its properties and origin were the object of further studies. The above-mentioned pigment was soluble in benzene and tetrachlormethane. From this raw pigment the red pigment was later extracted according to HAYASHI[1] and the absorption spectrum measured on a recording spectrophotometer Unicam SP 700. The spectrum is characterized by 2 peaks in the regions of 333 nm and 480 nm, as can be seen in the Figure, curve 1a.

In order to elucidate the pigment formation, a whole series of further experiments were carried out. It is well

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known[2,3] that some polyphenolases isolated from roots of higher plants catalyse the oxidation of some amino acids in the presence of phenolic substances giving rise to the red pigment. Some conditions of the red pigment origin were also studied in microorganisms[4-6]. White-rot fungi excrete numerous oxidases into the medium[7-9]. This is why we have used the 3% malt extract as the medium on which the fungi were grown as a source of raw enzymes for the formation of a red pigment in vitro. 1 ml $M/100$ of catechol, 1 ml $M/5$ of glycine and 1 ml $M/50$ 1-hydroxyproline were added to 1 ml of dialysed cultivation medium in 1 ml $M/10$ phosphate buffer, pH 5.9. This mixture was incubated at a temperature of 39 °C for 2 h. During this time it showed the formation of a red pigment, the absorption spectrum of which is shown in the Figure, curve 1b. The pigment was formed only in the presence of a cultivation medium in which the fungi *Trametes versicolor* and *Trametes gibbosa* were cultivated. It was, however, not formed in the case of the fungus *Fomes marginatus*.

From the results it follows that the fungi producing polyphenolases catalysing the secondary oxidation of amino acids in the presence of phenols, are able to form the red pigment during the wood decay in the way described above. Fungi which do not produce polyphenolases of the type mentioned, do not form the red pigment.

Zusammenfassung. Holzzerstörende Pilze, die ins Medium Polyphenolasen ausscheiden, bilden bei der Zersetzung des Holzes, welches mit 3% Peptonlösung aufgesogen wurde, ein rotes Pigment. Die Pigmentbildung ist durch die sekundäre Oxydation von Aminosäuren und die katalysierende Rolle der Polyphenolasen verursacht.

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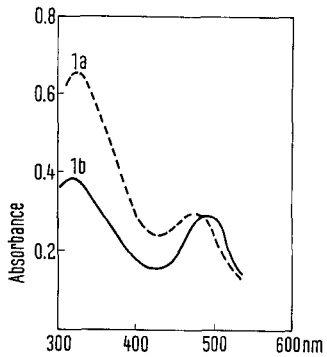

The absorption spectra of red colour solutions. Curve 1a: a red pigment isolated during wood decay by white-rot fungi. Curve 1b: a red pigment formed by the action of polyphenolase produced in the medium by white-rot fungi. Boiled enzymatic solution was used as blank.

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Postnatal Changes in Phosphatase and Non-Specific Esterase Activity in the Large Intestine of the Rat

During postnatal development of mammals there are changes in the histological structure of the gastrointestinal tract[1-5] and its enzyme pattern. This has been demonstrated histochemically[6-9] and biochemically[9-12]. The occurrence of enzymes in the large intestine during postnatal development has not received any attention at all, and only work on adult animals[13,14] has been performed. Since the large intestine may play a role in the absorption and metabolism of some substances[14], this has been studied during postnatal development of the rat in this paper.

Rats aged 1, 3, 7, 10, 15 and 20 days and adult animals were examined histochemically. Each age group contained 5 rats; sexes were represented about equally. Rats were decapitated 3 h after food deprivation. Excisions of the caecum and of the middle part of the oral and aboral half of the remainder of the large intestine were fixed in Baker's formol-calcium at 4 °C for 24 h, and alkaline and acid phosphatase were determined in paraffin sections together with AS- and α-esterase. ASD phosphate, ultrazol AS acetate, and α-naphthyl acetate were used as substrates; Fast red TR (Hoechst), hexatone basic fuchsin (Lachema) according to Barka, Fast blue RR (Hoechst) and Fast blue B (Lachema) were used as diazotates.

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