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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[5] L. M. REINEKE, Analyt. Chem. *28*, 1853 (1956).
[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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