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*PRODUCTION OF ILLUDIN M AND OF A FOURTH CRYSTALLINE COMPOUND BY CLITOCYBE ILLUDENS**

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The isolation of three crystalline compounds from culture liquids of *Clitocybe illudens*, strain 72027-S, was recently reported.[1] Of the two antibacterial compounds, illudin M was obtained in relatively small amounts. A strain 14610-S has been found[2] which is more efficient under our conditions of culture than 72027-S for the production of illudin M. It produces larger amounts of this compound, and also produces relatively small amounts of illudin S. Isolation of illudin M consequently is simplified. A fourth compound, not antibacterial in our tests, has been isolated from culture liquids of this strain.

Clitocybe illudens (14610-S) was grown at 25° C. in 2800 ml. Fernbach flasks containing beechwood shavings and a corn-steep medium.[3] In about five weeks, at which time the mycelial mat completely covered the surface of the liquid, the activity against *Staph. aureus* was usually 32 dilution units[4] per ml. and against *Myco. smegma* 512 to 1024 dilution units per ml. Reflooding the mats with fresh corn-steep medium produced liquids with as high activity in about three weeks. Liquids tested three months after reflooding had retained their antibacterial activity; therefore, the mats were usually reflooded at convenient intervals of from 3 to 6 weeks. Mats kept for as long as 15 months continued to produce active culture liquids after being reflooded thirteen times.

The isolation procedure consisted of extraction of the culture liquid with chloroform (three extractions, 20, 10, and 10 per cent of the volume), and concentration of the chloroform extract *in vacuo*, to a heavy syrup. On standing, this deposited crystals which consisted of a mixture of illudin M and a new acidic compound. These were filtered off, washed with a solution of chloroform-hexane, and dried *in vacuo*. Separation of the two compounds was accomplished by sublimation, which left behind the acidic

compound, or by extraction of a solution of the compounds in ether with dilute bicarbonate solution, which left behind the illudin M. When obtained by the latter method, illudin M was best purified further by sublimation, which removed any traces of illudin S. (Most of the illudin S remained in the culture liquid after chloroform extraction.) If the extracted culture liquid was treated with Norit A according to the procedure previously described,[1] illudin S was obtained as crude crystals from the ethyl acetate extract of the concentrated aqueous acetone eluate. In five batches the yield varied from 0.04 to 0.17 g. per liter of culture liquid and averaged 0.08 g. per liter. The yield of sublimed illudin M was about 0.22 g. per liter, and represented 25 to 60 per cent of that present in the culture liquid as calculated from the activity of the latter. The ratio of the yields of illudin M to illudin S was: 1.29:1 for this strain as compared to 0.12:1 for strain 72027-S. The yield of the inactive acidic compound was about 0.05 g. per liter of culture liquid.

The acidic compound crystallized from 95 per cent ethanol in flat, prismatic rods. It melted at 216° (corr.). Analytical values agree with the molecular formula $C_{21}H_{22}O_7$, and with the presence of two acidic groups. Found:[5] C, 65.07; H, 5.88; N.E., 190: M.W., 365. Calc. for $C_{21}H_{22}O_7$ (386.30): C, 65.27; H, 5.74; N.E. 193; M.W., 386. The ultra-violet absorption spectrum showed maxima at 247 mμ (ϵ = 43,900) and at 330 mμ (ϵ = 2900) indicating the presence of an α,β-unsaturated carbonyl group.

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[1] These PROCEEDINGS, **36**, 300–305 (1950).

[2] We are indebted to Ross W. Davidson for this strain.

[3] The medium contained per liter, 1 g. KH_2PO_4, 0.5 g. KCl, 3 g. $NaNO_3$, 0.5 g. $MgSO_4 \cdot 7H_2O$, 40 g. dextrose and 5 g. Staley special nutrient 114 (corn-steep).

[4] Anchel, M., *J. Biol. Chem.*, **177**, 169–177 (footnote 1) 1948.

[5] Microanalyses were performed by the Huffman Microanalytical Laboratories, Denver, Colo.