Isolation of cytochalasine B from tomatoes contaminated with the fungus *Hormiscium sp.*

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A substance of molecular formula $C_{29}H_{37}NO_5$ was isolated from tomato paste made of a material contaminated with the fungus *Hormiscium sp.* and also from culture media of pure *Hormiscium sp.* Physical and chemical study of this substance and its derivatives evidenced its identity with the antibiotic cytochalasine B (phomin). The production of cytochalasine B by *Hormiscium sp.* has not been reported as yet.

A crystalline bitter principle of m.p. 218°C and $[\alpha]_D^{25}$ +84° (ethanol), isolated from some tomato pastes was shown by elemental analysis and high-resolution mass spectrometry to have molecular formula $C_{29}H_{37}NO_5$.

Its mass spectrum revealed peaks characteristic of a benzyl group at *m/e* 388 (M-91) and 91, in addition to 479 (M^+).

The p.m.r. spectrum showed signals at δ 0.65 and 0.90 (doublets, J = 6.5 cps) — two secondary methyl groups, a multiplet at δ 7.20 attributable to 5 protons of an aromatic ring and a singlet at δ 8.20 ascribable to a proton of the lactam grouping.

Bands in the infrared spectrum are indicative of a carbonyl group (1720 cm^{-1}), a double bond (1655 cm^{-1}), hydroxy and amido groupings (3400 cm^{-1}), and a monosubstituted benzene ring (705, 770 cm^{-1}).

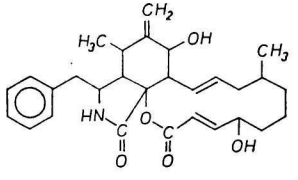
The ultraviolet spectrum displayed an intense maximum at 220 nm (log ε 4.65) and inflections due to an aromatic ring at 257, 265, and 269 nm.

Upon catalytic hydrogenation over a platinum catalyst in ethanol the bitter substance afforded tetrahydro and hexahydro derivatives; their molecular ion peaks appeared at *m/e* 483 and 485, respectively. The presence of peaks at *m/e* M-91 and 91 indicated that the reduction of benzene ring did not occur. Therefore it could be deduced that the substance under investigation contained three double bonds.

The bitter principle yielded, when acetylated, an amorphous diacetyl derivative. Its infrared spectrum consisted of bands with characteristic stretching vibrations of carbonyl groups in an ester and a lactam groupings at 1730—1760 cm^{-1} (broad), C—O grouping in an ester group at 1240 cm^{-1}, C=C double bonds at 1660 cm^{-1}, C—H bonds in an aromatic ring and of unsaturated C=C groups at 3033, 3070, and 3090 cm^{-1} and N—H bond at 3400 cm^{-1}.

The N—H grouping in the acetyl derivative was also seen in the p.m.r. spectrum at δ 6.12. Singlets at δ 1.93 and 2.1 are associated with protons of acetyl groups, whereas doublets centred at δ 1.0 and 0.80 (J = 6.5 cps) with secondary methyl groups.

Spectral data of the bitter principle and its derivatives indicated the identity with cytochalasine B (phomin) (*I*). Comparison with an authentic specimen proved this assumption to be correct.



I

The antibiotic phomin, having a cytostatic activity, was isolated from Phoma-species (strain S 298) by *Rothweiler* and *Tamm*, who also reported its structural formula [1, 2].

Cytochalasine B isolated from *Helminthosporium dermatioideum* is of the same structure [3, 4].

We found that cytochalasine B isolated from tomato paste is a secondary metabolite of the fungus *Hormiscium sp.* Pure cultures of this fungus were shown to contain the same substance as tomato pastes investigated [5, 6].

Tomatoes contaminated with this fungus are of bitter taste due to the presence of the secondary metabolite cytochalasine B.

Experimental

The melting point was determined on a Kofler micro hot stage. Samples for analysis were dried for 6 hrs over P_2O_5 at 0.01 torr.

Infrared spectra were measured with a UR-10 (Zeiss, Jena) spectrophotometer, ultraviolet spectra with a UV/ORD-5 JASCO spectrophotometer, mass spectra with an AEI-MS 902 spectrometer, and p.m.r. spectra with an HA 100 Varian apparatus in dimethyl sulfoxide, tetramethylsilane being the internal reference substance. Optical rotation was determined on a 143 A Bendix—Ericsson objective polarimeter.

Isolation of the bitter principle

Tomato paste (5 kg) was extracted under stirring with acetone and filtered. The filtrate was clarified with basic lead acetate and acetone was removed under diminished pressure. The residue was diluted with distilled water and extracted with light petroleum, which was not worked up. The aqueous solution was further extracted with chloroform, the organic layer dried with anhydrous sodium sulfate and evaporated to dryness. The residue (60 mg) was chromatographed over alumina—G Woelm neutr. in chloroform or chloroform—methanol 94 : 6 using preparative thin layer plates (30 × 20 cm).

Spots were visualized with concentrated sulfuric acid; the band containing the bitter substance of R_F 0.4 and 0.66, respectively turned pink with this reagent. The bitter

principle eluted with chloroform from the proper band amounted to 18 mg; after recrystallization from chloroform 13 mg, m.p. 217–218°C, $[\alpha]_D^{25}$ +84° (c = 0.1; ethanol). Phomin [2] m.p. 218°C, $[\alpha]_D^{25}$ +85°.

For $C_{29}H_{37}NO_5$ (479.6) calculated: 72.62% C, 7.77% H, 2.92% N; found: 72.30% C, 7.56% H, 2.76% N.

Peaks in the mass spectrum at m/e: 479 (M^+), 461, 443, 425, 396, 388, 370, 352, 334, and 91 (100%).

Saprophytic cultivation of the isolated fungus *Hormiscium sp.* on the malt agar and a similar isolation process afforded 3200 mg of phomin [6].

Acetylation of cytochalasine B

Cytochalasine B (50 mg) was dissolved in pyridine (2 ml) and acetic anhydride (1 ml) and allowed to stand overnight at room temperature. After 24 hrs the solvent was evaporated *in vacuo* from a steam bath and the foamy residue (58.5 mg) was purified by dissolving in chloroform and filtering through a little column of alumina Woelm (acidic).

Diacetyl phomin, amorphous, $[\alpha]_D^{25}$ +82.5° (c = 0.212; ethanol).

For $C_{33}H_{41}NO_7$ (563.7) calculated: 70.31% C, 7.33% H; found: 69.80% C, 7.35% H.

Hydrogenation of cytochalasine B

Cytochalasine B (53.6 mg) dissolved in ethanol (10 ml) was hydrogenated over Adams catalyst (20 mg) at room temperature. After 4 hrs the catalyst was filtered and the filtrate evaporated under diminished pressure. The residue consisted, according to thin-layer chromatography, of two products, which were separated by column chromatography over alumina (grade IV) with chloroform. The substance of R_F 0.54; $[\alpha]_D^{25}$ −50.3 ± 2° (c = 3.7; ethanol) was shown to be tetrahydro derivative. Peaks in the mass spectrum at m/e: 483 (M^+), 465, 447, 437, 432, 392, 374, 358, 356, 346, 338, 316, 190, 91 (100%). The second substance of R_F 0.39, hexahydro derivative revealed peaks in its mass spectrum at m/e: 485 (M^+), 467, 449, 434, 394, 376, 358 (100%), 348, 340, 330, 318, 190, 91.

References

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