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A New Depsidone from *Aspergillus nidulans*

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A compound has been isolated from the mycelium of *Aspergillus nidulans* grown on chloride free substrate. The proposed structure of this compound is that of tris-dechloronornidulin, the first nonchlorinated fungal depsidone. Tris-dechloronornidulin has been shown to be a direct precursor for the formation of nidulin. The presence of orsellinic acid in the broth has been demonstrated.

The production of secondary metabolites in *Aspergillus nidulans* (earlier considered as *Aspergillus ustus*) has been investigated by several authors. In 1945 Kurung [1] reported the presence in the fermentation broth of a principle active against *Mycobacterium tuberculosis* and *Mycobacterium sanae*. Hogeboom and Craig [2] in 1946 isolated two crystalline products each containing covalently bound chlorine. In a series of papers Dean *et al.*[3–5] reinvestigated the chlorine containing compounds produced by *A. nidulans*. Besides the two compounds found by Hogeboom and Craig, named nornidulin and dechloronidulin by Dean *et al.*, a third chlorinated compound was isolated that was given the name nidulin.

Dean *et al.* established the structure of nidulin from which followed the structures of nornidulin and dechloronornidulin (Fig. 1). The suggested structure of nidulin was confirmed by Beach and Richards.[6] Though depsidones are common lichen metabolites, nidulin and its two derivatives are the only depsidones isolated from fungi to date.

In all previous investigations the organism has been grown on Czapek-Dox medium with glucose as carbon source. The Czapek-Dox salt solution contains 0.05 % potassium chloride, the source of the chlorine introduced into the organic substances. By growing the organism on Czapek-Dox medium free of potassium chloride, we found that *A. nidulans* produces a series of chlorine free phenolic substances.

One of the products excreted into the culture medium was identified as orsellinic acid. The presence of orsellinic acid in *A. nidulans* is nor surprising since, in addition to being a frequently met metabolite in moulds, orsellinic acid should presumably be involved in the biosynthesis of nidulin and related

compounds. The identity of orsellinic acid was established from its UV-spectrum as well as by thin-layer and gas chromatography.

The major component in ether extracts of dry mycelia was isolated by recrystallizations from toluene. The compound had a melting point of 185–187°C. It was insoluble in water, aqueous sodium hydrogen carbonate, and 0.5 M sodium carbonate but soluble in acetone, ethanol, but less soluble in ether. The compound reacted with tetrazotized *o*-dianisidine giving a brown color but showed a negative ferric chloride reaction. Mass spectroscopy indicated a molecular weight of 326 and elemental analysis gave the empirical formula $C_{19}H_{18}O_5$.

On hydrogenation with platinum oxide catalyst, the compound consumed 0.85 mequiv. of hydrogen suggesting the presence of one unsaturated bond. By fusing the compound with potassium hydroxide one of the degradation products was identified as orcinol by thin-layer and gas chromatography.

When the compound was left overnight in 0.5 M sodium hydroxide under nitrogen, it was completely hydrolyzed giving rise to an acidic compound with a molecular weight of 344 (mass spectroscopy) suggesting the presence of a lactone bridge. The occurrence in the IR-spectrum of the original compound of a strong absorption band characteristic of a carbonyl group (1710 cm^{-1}) supports this suggestion.

NMR data (solvent deuterated acetone, internal standard TMS) give evidence for the presence of four C-methyl groups, two of which are presumably substituted on aromatic nuclei (δ 2.12 ppm, δ 2.37 ppm). One of the remaining methyl groups seems to be bound to a vinyl group (a doublet centered around 1.79 ppm, $J = 6$ cps) and the fourth methyl group gives rise to a singlet at δ 2.04 ppm. A vinyl proton appears as a doublet centered around 5.50 ppm and three protons show up as aromatic protons in the region 6.4–6.6 ppm. Thus all carbon bound protons of the compound are accounted for and nothing contradicts the placement of the fourth methyl group on a vinylic C-atom.

Treatment of the unknown compound with methyl iodide and potassium carbonate in acetone gave a product with a molecular weight of 354 and containing two *O*-methyl groups (δ 3.8 ppm).

The data presented support a close structural relationship of the unknown compound with nidulin, *i.e.* a depsidone derivative carrying two phenolic hydroxyl groups, two aromatic methyl groups, and an unsaturated side chain identical with that of nidulin. Fig. 1 shows a proposed structure for the new depsidone derivative that could be named tris-dechloronornidulin. It follows that the acid obtained by hydrolysis will be named tris-dechloronornidulinic acid.

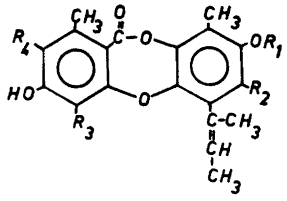

Fig. 1. $R_1 = CH_3$; $R_2 = R_3 = R_4 = Cl$, nidulin. $R_1 = H$; $R_2 = Cl$; $R_3 = H$; $R_4 = Cl$, dechloronornidulin. $R_1 = R_2 = R_3 = R_4 = H$, trisdechloronornidulin.

If the organism is grown on a chlorine-free medium until a good production of tris-dechloronornidulin is obtained, addition of potassium chloride causes a gradual disappearance of tris-dechloronornidulin and a simultaneous appearance of nidulin. Furthermore, ^{14}C-labelled tris-dechloronornidulin (biologically labelled from acetate-^{14}C) is incorporated to a high degree into nidulin when added to a nidulin producing culture. These biosynthetic experiments further confirm the proposed structure for the isolated depsidone derivative.

EXPERIMENTAL

Culture conditions. Aspergillus nidulans CMI 85,473 (NRRL 2006) was grown at 28°C for 30 days as a surface culture in 1 l Fernbach's flasks each containing 300 ml of substrate medium ($NaNO_3$ 2.0 g, KH_2PO_4 1.0 g, $MgSO_4.7H_2O$ 0.01 g, $FeSO_4.7H_2O$ 0.01 g, yeast extract 1.0 g, glucose 40 g, and distilled water to 1 l). In the biosynthetic experiments the organism was grown on a shake table (150 ml medium/500 ml conical flask) with 0.5 % KCl added to the original culture medium.

Isolation and identification of orsellinic acid. The culture filtrate was acidified with HCl and extracted with ether. The acidic material was reextracted into a fresh solution of aqueous $NaHCO_3$. After acidification, the acids were again taken up into ether. The ether solution contained an acid that was indistinguishable from authentic orsellinic acid on thin-layer chromatogram (silica gel, G, cyclohexane – dioxane – acetic acid, 45/18/1, R_F 0.26). The UV-spectrum and the gas chromatographic behaviour of the substance extracted from the thin-layer plate further confirmed its identity as orsellinic acid (Aerograph 600, Chromosorb W, 20 % SE).

Isolation of tris-dechloronornidulin. The dried mycelium from 11 Fernbach flasks was extracted with ether in a Soxhlet apparatus. The ether solution was then shaken with aqueous $NaHCO_3$, taken to dryness in a rotary evaporator and the residue repeatedly crystallized from toluene. The yield of pure product, m.p. 185 – 187°C, was 1.1 g (λ_{max} 261 nm; ε 12 300). (Found: C 70.04; H 5.50; O 24.36. Calc. C 70.00; H 5.52; O 24.50.)

O-*Methyl-trisdechloronidulin.* 200 mg of tris-dechloronornidulin in 20 ml of acetone were refluxed for 24 h with 1.2 g K_2CO_3 and 25 ml of methyl iodide. The reaction mixture was evaporated to dryness *in vacuo* and the residue treated with ether and water. A small volume of methanol – water (1/1) was added to the ether phase. The methoxy derivative crystallized as colorless plates after slow evaporation of the ether. Yield 345 mg (80 %), m.p. 136 – 139°C, (OCH_3 16.8; calc. 17.6).

Tris-dechloronornidulinic acid. A sample of tris-dechloronornidulin was dissolved in 0.5 M NaOH and was left overnight at room-temperature under nitrogen. The alkaline solution was acidified with HCl and extracted with ether. After treating the ether solution with aqueous $NaHCO_3$ the acidic compound was reextracted into ether after acidification of the aqueous solution. The residue obtained after evaporation of the ether was recrystallized from toluene yielding slightly yellow crystals, m.p. 191 – 193°C (decomp.); equiv. by titration 369.

Degradation of tris-dechloronornidulin. A small sample of tris-dechloronornidulin was fused with KOH for a few minutes. After dissolving in water the reaction mixture was acidified with HCl and then extracted with ether. The residue from the evaporated ether solution was sublimed *in vacuo* at 100°C. The sublimed substance showed UV-absorption as well as thin-layer and gas chromatographic properties that were identical with those of authentic orcinol.

Biosynthetic experiments. ^{14}C-Labelled tris-dechloronornidulin was prepared by adding sodium acetate-2-^{14}C to a producing culture. After two days' exposure to the radioactive substrate tris-dechloronornidulin-^{14}C was isolated as described.

The labelled sample was dissolved in a minimal volume of ethanol and then added to a nidulin producing culture. After two days nidulin and remaining tris-dechloronornidulin-^{14}C were isolated from the dried mycelium by extraction with ether and thin-layer chromatography (cyclohexane – dioxane – acetic acid, 45/18/1, nidulin R_F 0.58, tris-dechloronornidulin R_F 0.24). Scanning of the chromatogram in a radioscanner indi-

cated that about 50 % of the extracted radioactivity was localized in nidulin, with the remaining radioactivity mainly in the reisolated tris-dechloronornidulin.

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REFERENCES

1. Kurung, J. M. *Science* **102** (1945) 11.
2. Hogeboom, G. H. and Craig, L. C. *J. Biol. Chem.* **162** (1946) 363.
3. Dean, F. M., Roberts, J. C. and Robertson, A. *J. Chem. Soc.* **1954** 1432.
4. Dean, F. M., Erni, A. D. T. and Robertson, A. *J. Chem. Soc.* **1956** 3545.
5. Dean, F. M., Deorha, D. S., Erni, A. D. T., Hughes, D. W. and Roberts, J. C. *J. Chem. Soc.* **1960** 4829.
6. Beach, W. F. and Richards, J. H. *J. Org. Chem.* **28** (1963) 2746.

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