NOTES

On an Unstable Antifungal Metabolite from *Trichoderma koningii*

Isolation and Structure Elucidation of a New Cyclopentenone Derivative (3-Dimethylamino-5-hydroxy-5-vinyl-2-cyclopenten-1-one)

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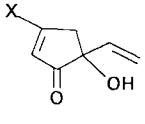
In the course of our search for new antifungal antibiotic from fungi we had isolated a fungal strain Y-87,2100 which produced a metabolite active against *Candida albicans*. The bioactive compound was unstable, losing its activity during concentration of its solution and had to be converted to a stable molecule **2** for characterisation. In this paper we report the fermentation and isolation of **1** as well as conversion of **1** to **2** followed by structure elucidation of **2**.

The fungal strain Y-87,2100, isolated[1] from a piece of rotten wood sample collected in Mulund, Bombay, was identified as *Trichoderma koningii* Oudemans. The strain was maintained either on potato dextrose agar or SABOURAUD's dextrose agar. The strain has been deposited as reference with the German Collection of Microorganisms and Cell Cultures, Braunschweig, Federal Republic of Germany (DSM No. 6244).

The producing organism was inoculated into a 500 ml wide mouth Erlenmeyer flasks containing 100 ml of seed medium (soluble starch 1.5 g, soyabean meal 1.5 g, glucose 0.5 g, NaCl 0.5 g, $CaCO_3$ 0.2 g, yeast extract 0.2 g, corn steep liquor 0.1 g, adjusted to pH 6.5 before sterilization). The flasks were incubated on a rotary shaker at 220 rpm for 60 hours at 26°C (±1°C). The production medium (soluble starch 15 g, soyabean meal 15 g, glucose 5 g, NaCl 5 g, $CaCO_3$ 2 g, corn steep liquor 1 g, $ZnSO_4 \cdot 7H_2O$ 0.22 mg, $CaCl_2$ 0.55 mg, $MnCl_2 \cdot 4H_2O$ 0.5 mg, $FeSO_4 \cdot 7H_2O$ 0.5 mg, $CuSO_4 \cdot 5H_2O$ 0.16 mg in 1000 ml of demineralized water, adjusted to pH 6.5 prior to sterilization), was distributed in 200 ml amounts in 1000 ml Erlenmeyer flasks and inoculated with 1% (v/v) of the seed culture. The fermentation was carried out on a rotary shaker at 220 rpm for 66 hours at 26°C (±1°C). The progress of fermentation was monitored by testing the bioactivity against *Candida albicans*, *Escherichia coli* 9632, and *Penicillium digitatum* 135. The culture filtrate exihibited bioactivity against *Staphylococcus aureus* 209P, *E. coli* 9632, *C. albicans*, *Aspergillus niger*, *P. digitatum* 135, *Fusarium culmorum* 100, *Trichophyton mentagrophytes*, and *Cladosporium* species.



1 X = - OH ?
2 X = - NMe_2
3 X = - OCH_3

The ethyl acetate extract (34 litres) of the culture filtrate (34 litres) was concentrated under reduced pressure to 1.2 litres (complete removal of the solvent leads to inactivation). To this 10.8 litres of petroleum ether (60~80) was added immediately and loaded onto a silica gel column (200~300 mesh, 1000 g, 46 cm × 8 cm), packed in petroleum ether (60~80) - ethylacetate (9 : 1). The elution was carried out under pressure using petroleum ether (60~80) - ethylacetate (9 : 1) followed by petroleum ether (60~80) - ethylacetate (1 : 3). Fractions were monitored by bioactivity and TLC (silica gel, petroleum ether (60~80) - ethylacetate (1 : 1), detection (uv) 254 nm, Rf: 0.53). The fractions containing compound **1** were combined and preserved at −40°C. Compound **1** was converted into a stable derivative **2** as follows:

To 200 ml of the solution containing compound **1**, 125 microlitre of dimethyl amine solution (35~40% w/v) was added in 25 microlitre lots with stirring over a period of five hours. The reaction was monitored by TLC (silica gel, ethylacetate - n-propanol - water (5 : 3 : 1), detection (uv) 254 nm, Rf: 0.41). The compound **2** was purified by silica gel column chromatography. The column was eluted using ethylacetate (12 bed volumes) followed by methanol - ethyl acetate (1 : 9) mixture (8 bed volumes). On the basis of TLC, fractions were combined and concentrated under reduced pressure to get 31 mg of compound **2**.

Compound **2** is a white amorphous powder with mp 137~139°C and $[\alpha]^{20}$ +69.33 (*c* 0.195, water). CI/EI-MS gave a molecular weight of 167. The molecular formula was established as $C_9H_{13}NO_2$ based on HREI-MS (M^+ *m/z* 167.0952). Compound **2** was soluble in water, methanol, chloroform, dimethyl sulphoxide, sparingly soluble in solvents such as acetone, acetonitrile, ethyl acetate and insoluble in petroleum ether (60~80). The compound **2** gave UV absorption λ_{max} (MeOH) nm ($E^{1\%}_{1cm}$) 280 (30,000) and an IR spectrum γ_{max} (KBR) cm^{-1} 3280, 1665, 1565, 1435, 1410, 1340, 1230, 1190, 1120, 1070, 1015. The 270 MHz ^1H NMR spectrum

showed following signals: (solvent $CDCl_3$) δ 2.83 (2H, S), 3.03 (3H, S), 3.1 (3H, S), 5.01 (1H, S, D_2O exchangeable), 5.21 (1H, dd, $J=10.5$, 1.1 Hz), 5.44 (1H, dd, $J=17.1$, 1.1 Hz), 5.88 (1H, dd, $J=17.1$, 10.5 Hz). (Solvent CD_3OD) δ 2.74 (1H, d, $J=17.0$ Hz), 3.0 (1H, d, $J=17.0$ Hz), 3.05 (3H, S), 3.13 (3H, S), 4.98 (1H, S), 5.16 (1H, dd, $J=10.5$, 1.1 Hz), 5.38 (1H, dd, $J=17.1$, 1.1 Hz), 5.89 (1H, dd, $J=17.1$, 10.5 Hz). The ^1H NMR of **2** showed well resolved splitting pattern for the protons in CD_3OD which helped to identify the proton spin systems. The compound has a very weakly acidic proton (exchanging with $D_2O/CDCl_3$ but not with CD_3OD) which can come from conjugated vinylogus systems. ^{13}C NMR spectrum (100 MHz in $CDCl_3$) of the compound showed the following signals: δ 200.94 (C=O), 174.13 (=C). 139.70 (=CH), 113.99 ($=CH_2$), 96.11 (=CH), 78.70 (C), 42.20 (CH_2), 40.36 (CH_3), 39.34 (CH_3). The multiplicities were derived from DEPT experiments.

Structure Elucidation of 2

Interpretation of spectroscopic data indicated the presence of following structural elements in **2**:

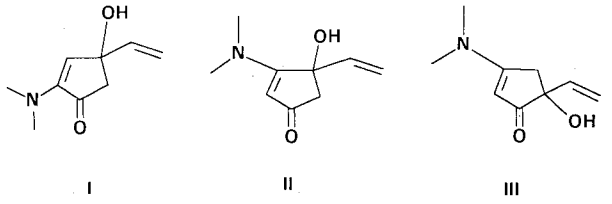
a) $Me_2N-C=C$ (HREI-MS (*m/z* 69.058) and ^1H, ^{13}C NMR)

b) $-CH=CH_2$ isolated spin system (^1H NMR)

c) $=CH$, CH_2 isolated spin system (^1H NMR)

d) C=O (keto), $-C-$ (^{13}C NMR)

Based on this interpretation following three structures were proposed.



I II III

The NOE experiments indicated the spatial proximity of CH and CH_2 groups (both singlets in ^1H NMR) to NMe_2 which fits best to structure III. Further ^{13}C NMR chemical shift calculation using Bremser data collection[2] gave the best agreement between the observed and calculated chemical shifts for structure III (199 (C=O), 173 (C=), 142 (–CH=), 113 ($=CH_2$), 99 (–CH=), 82 (–C–), 38 ($-CH_2$), 40 ($-CH_3$). $|\Delta\delta|$ n=2 ppm). The derivative **2** is then represented by structure III.

Based on this structure for compound **2** one can speculate that the NMe_2 group replaced a leaving group (X=OH?) present in the same position. It is known that ammonia/N-methyl aniline replaces the –OH group (leaving group) when it reacts with 1,2,4-cyclopentantrione.[3] A similar reaction might be taking place between Me_2NH and the bioactive metabolite. The structure of the compound may be represented by **1**.

Compound **2** did not exhibit any antibacterial or antifungal activity at 1 mg/ml concentration.

Isolation, structure elucidation and synthesis of a related inactive compound **3**, (5-hydroxy-3-methoxy-5-vinyl-2-cyclopenten-1-one) from *Trichoderma album* has been reported in the literature.[4]

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