3-METHOXY-2,5-TOLUQUINONE FROM *ASPERGILLUS* SP. HPL Y-30,212

FERMENTATION, ISOLATION, CHARACTERIZATION AND BIOLOGICAL PROPERTIES

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3-Methoxy-2,5-toluquinone (**I**), described in the literature as a synthetic intermediate, was isolated for the first time from a natural source namely the fermentation broth of an *Aspergillus* strain. The compound showed moderate activity against various Gram-positive and Gram-negative bacteria.

In the course of our screening program to find novel antibiotics from fungi, a strain of *Aspergillus* sp. No. HPL Y-30,212 was isolated from the soil collected near Jodhpur, India. From this strain we report the isolation of 3-methoxy-2,5-toluquinone (**I**), hitherto described in literature only as a synthetic intermediate[1,2]. The present paper deals with fermentation, isolation, chemical characterization and biological properties of the antibiotic and is the first report on the isolation of the compound from a natural source.

Fermentation

A few loopfuls of the sporulated culture from a slant maintained on Sabouraud agar were transferred to a 250-ml conical flask with 50 ml liquid medium containing 0.5% glucose, 1.5% corn starch, 1.5% soybean meal, 0.1% corn steep liquor, 0.2% yeast extract, 0.5% NaCl and 0.2% $CaCO_3$ at pH 6.5. This was grown on a rotary shaker for 60 hours at 25°C and was used as a seed culture.

The media used for antibiotic production contained 1.5% soybean meal, 0.3% beef extract, 0.5% Casamino acids, 1% soluble starch, 1% maltose, 1% dextrin and 0.4% $CaCO_3$. The pH of the medium was adjusted to 6.5 prior to sterilization. A 500-ml flask containing 100 ml of the above media was inoculated with 1% of the seed culture and incubated for 72 hours at 28°C.

Determination of the activity was made by the disc diffusion assay using *Staphylococcus aureus* 209 P, *Escherichia coli* 9632 and *Candida albicans* as test organisms.

Isolation of the Antibiotic

The fermented broth (10 liters) was extracted with ethyl acetate (2×1 liter). After evaporation of the solvent, the crude residue (4 g) was chromatographed on a silica gel column. Elution with benzene gave the quinone as a yellow powder (8 mg). Recrystallization from ethyl acetate - petroleum ether gave an analytically pure sample.

Further elution of the silica gel column with benzene - ethyl acetate led to the isolation of two more antibiotics identified as penicillic acid (**V**, 32 mg) and neoaspergillic acid (**VI**, 130 mg).

Physico-chemical Properties

3-Methoxy-2,5-toluquinone was obtained as a yellow crystalline solid, mp 145~146°C, showing the

following properties. The compound is soluble in alcohol, acetone, chloroform and other common organic solvents. The UV spectrum in methanol showed maxima at 265 nm (ε 18,696) and 365 nm (1,216). The infrared spectrum revealed sharp peaks at 1686, 1658 and 1610 cm^{-1}. The NMR spectrum showed signals at δ 2.08 (3H, d, J=2.5 Hz, C_1-CH_3), 3.83 (3H, s, OCH_3), 5.86 (1H, d, J=2.5 Hz, C_4-H), 6.83 (1H, m, C_6-H).

The compound gave the following elemental analysis: C 63.02, H 5.12% while $C_8H_8O_3$ requires C 63.15, H 5.26%.

The mass spectrum indicated peaks for the molecular ion at M^+ 152, m/z 124 ($M^+ - C{=}O$), 109, 96 and 69.

Biological Properties

3-Methoxy-2,5-toluquinone exhibited rather moderate antibacterial and antifungal activity as shown in Table 1. The acute toxicity in mice (LD_{50}, i.p.) was found to be 7.4 mg/kg.

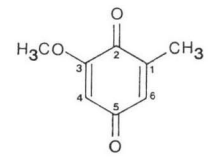
Results and Discussion

The antibiotic coprinin (**II**) (4-methoxy-2,5-toluquinone) is produced by several fungi including *Coprinus radius* and *Coprinus similis*[3]. The yellow crystalline antibiotic from fungus Y-30,212 while showing the same molecular weight (M^+ 152) and molecular formulae ($C_8H_8O_3$) as coprinin, clearly differed in its physico-chemical properties. In the NMR spectrum the olefinic proton at C_4 appeared as a doublet (J=2.5 Hz). The presence of this cross carbonyl-coupling with the olefinic proton at C_6 was considered as the major criterion in suggesting that antibiotic from Y-30,212 was perhaps isomeric to coprinin. This together with the fact that the mp (145~146°C) of antibiotic from Y-30,212 was considerably lower than reported for coprinin (mp 175°C) led us to assign the alternate structure (**I**).

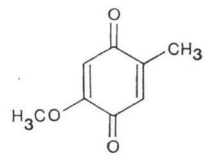
The above conclusion was confirmed by direct comparison (mp, mixed mp, IR and NMR) of the antibiotic with a sample synthesized unambiguously by the following route.

Table 1. Antimicrobial spectrum.

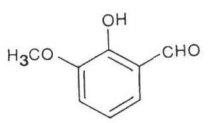
Test organisms	Minimum inhibitory concentration (MIC) (μg/ml)
	3-Methoxy-2, 5-toluquinone
Staphylococcus aureus 209 P	5
Staphylococcus aureus R 85	5
Bacillus subtilis	2
Bacillus cereus	5
Streptococcus faecalis	50
Escherichia coli TEM	50
Escherichia coli 9632	>100
Pseudomonas aeruginosa	>100
Proteus vulgaris	10
Enterobacter cloacae	>100
Klebsiella pneumoniae	50
Serratia marcescens	2
Salmonella typhimurium	50
Candida albicans	25



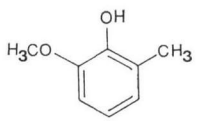
I



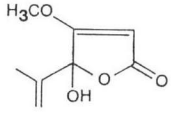
II



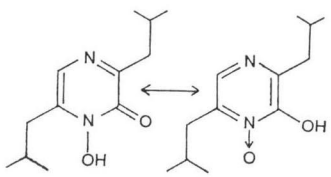
III



IV



V



VI

O-Vanillin (**III**) was reduced by modified HUANG-MINLON procedure[4] to afford 2-methyl-6-methoxyphenol (**IV**). This on oxidation with peracetic acid[5] gave 3-methoxy-2,5-toluquinone.

The strain also co-produces two more antibiotics identified as penicillic acid (**V**)[6] and neoaspergillic acid (**VI**)[7].

Acknowledgements

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