Asperaldin, a New Aldose Reductase Inhibitor from *Aspergillus niger* CFR-1046

I. Fermentation, Isolation and Characterization

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Aldose reductase (EC 1.1.1.21) catalyzes the conversion of glucose to sorbitol and promotes the accumulation of sorbitol in various tissues under the condition of hyperglycemia such as diabetes mellitus. The accumulated intracellular sorbitol causes development of diabetic complications such as cataracts, neuropathy, retinopathy and nephropathy\(^1\). Inhibitors of aldose reductase have been shown to reverse these biochemical changes and have been proven effective in delaying and even preventing several diabetic pathologies. Thus, aldose reductase has become an attractive pharmacological target for the treatment of diabetic complications. It has been reported that inhibitors of aldose reductase reduce the tissue sorbitol content in diabetic animals and are useful as therapeutic agents for diabetic complications\(^2\).

In our screening programme on bioactive molecules through the fermentation route\(^3\)\(^-\)\(^7\), we screened 15 different strains of *Aspergillus* sp. for potential inhibitors against rat lens aldose reductase (RLAR). We found that, *Aspergillus niger* CFR-1046 produced a compound, termed by us as asperaldin, which exhibits potent inhibitory activity against RLAR. The present paper describes the fermentation, isolation, physico-chemical properties and biological activities of asperaldin.

The organism used in this study *Aspergillus niger* CFR 1046 was obtained from CFTRI culture collection. Submerged fermentation was carried out in a potato dextrose (soluble starch 0.4% and glucose 2%) medium. A portion of the mature agar slant was inoculated into 100 ml of potato dextrose broth in a 500 ml Erlenmeyer flask and incubated at 30°C on a rotary shaker at 250 rpm for 8 days. The fermented broth (3 liters) was treated with ethyl acetate (2 liters) for two hours followed by cheesecloth filtration to remove the biomass; the organic extract was separated and dried over anhydrous sodium sulfate and concentrated in vacuo to yield a crude solid (0.42 g). The residue was subjected to silica gel column chromatography. Elution of the crude extract was carried out using hexane, chloroform, ethyl acetate and methanol in different combinations. Two bed volumes were taken as a fraction. The fractions were analyzed by qualitative TLC (benzene-methanol, 3:2) and enzyme assay according to the method described by HAYMAN & KINOSHITA 1965. The active compound was eluted as a deep yellow band in the chloroform-ethylacetate (9:1) and the solvent was evaporated in vacuo to obtain 25 mg of amorphous red powder. The compound was redissolved in ethyl acetate and washed with 5% sodium bicarbonate solution. The carbonate washings were collected, acidified and reextracted into ethyl acetate to give 15 mg of red compound which was further purified by preparative TLC using benzene-methanol-acetic acid (3:2:0.1) as the mobile phase.

Asperaldin is an amorphous red powder. It is soluble in methanol, ethyl acetate, dimethyl sulfoxide, diethyl ether, sodium bicarbonate, sodium carbonate and sodium hydroxide, slightly soluble in chloroform and hexane, but insoluble in water. The Physico-chemical properties are summarized in Table 1. The El-MS spectra of the compound showed the molecular ions at m/z 205, based on the mass spectra the molecular formula of compound was established as C\(_{16}\)H\(_{18}\)O\(_{5}\).

The compound exhibits UV absorption at 232 nm, 265 nm and 310 nm at pH 3.8. However at pH 9.0, while the \(\lambda_{\text{max}}\) at 232 nm showed a hypochromic shift to 226 nm both the 265 nm peak and 310 nm peak showed a bathochromic shift to 286 nm and 355 nm respectively (Figure 1). This indicates that there are ionizable phenolic groups on aromatic rings. The broad IR absorption at 3437 cm\(^{-1}\) indicates –OH stretching frequencies. The peak at 2995 cm\(^{-1}\), 2912 cm\(^{-1}\) indicate aromatic C–H stretching. A broad ketonic absorption band at 1660 cm\(^{-1}\) indicate probably hydrogen bonded keto group. The structure was further confirmed by 2DHMQCT and GC-EIMS. The corresponding nuclear magnetic resonance signals are; \(^1\)H NMR (500 MHz, DMSO-d\(_6\)) \(\delta\) 0.8 (16 CH\(_3\)), 1.0 (11 CH\(_2\)), 1.2 (12, 13, 14 CH\(_2\)), 1.5 (15 CH\(_2\)), 6.0 (H-7), 6.5 (H-3), 6.95 (H-5). \(^13\)C NMR (500 MHz, DMSO-d\(_6\)) \(\delta\) 29.9 (16...
CH₂), 52.2 (11 OCH₂), 39.9 to 40.9 (-CH₂), 111.1 (H-7), 108.9 (H-3), 108.2 (H-5), 190.83a (4-CO), 181.7a (1-CO), 164.4a (6-CAr-OH), 163.6a (2-CAr-OH), 160.9a (8-CAr-O) [Carbon-13 assignments are interchangeable]. 1H NMR (500 MHz, methanol-d₄) δ 0.95 (16-CH₃), 1.31 to 1.37 (11-, 12-, 13-, 14-CH₂), 1.67 (15-CH₂), 6.55 (6-OH), 7.06 (7-H), 7.65 (3-H), 7.74 (5-H).

An aliphatic group corresponding to six carbons was observed at 0.8 (CH₃) and 1.0 to 1.5 (5-CH₂ groups) ppm. One of the –CH₂ (1.0 ppm) might have come from –OCH₂ group of hexyl moiety being subjected to ring current effect of the aromatic group present. This was confirmed by recording the 1H NMR spectrum in a second solvent namely d₄-methanol, where the signal at 1.0 ppm was not detected. It might have probably merged with the –CH₂ envelope at 1.31 to 1.67 ppm. Three separate single signals were seen at 6.0, 6.5 and 6.95 ppm, indicating aromatic protons. EI-MS analysis showed a parent peak at 205 (M⁺) and other fragment peaks like 178 (M⁺ – CO), 150 (178 – CO), 108 and 81 clearly indicating the aromatic group of the molecule. However a parent ion peak was distinctly missing. Based on EI-MS and 2DHMQCT analysis the proposed structure is shown in Figure 2 and chemically

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Table 1. Physico-chemical properties of asperaldin.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>red amorphous powder</td>
</tr>
<tr>
<td>Melting Point</td>
<td>decomposes at 170 - 175 °C</td>
</tr>
<tr>
<td>UV Spectrum</td>
<td></td>
</tr>
<tr>
<td>( \lambda_{max} ) nm (ε) at pH 3.8</td>
<td>232 (8,800), 265 (12,700), 310 (5,900)</td>
</tr>
<tr>
<td>( \lambda_{max} ) nm (ε) at pH 9.0</td>
<td>226 (21,600), 286 (15,400), 355 (5,800)</td>
</tr>
<tr>
<td>IR</td>
<td>3437, 2995, 2912 and 1660 cm⁻¹</td>
</tr>
<tr>
<td>Molecular formula</td>
<td>C₁₆H₁₈O₃</td>
</tr>
<tr>
<td>EI-MS m/z</td>
<td>205 (290 – 85) [GC-EIMS]</td>
</tr>
<tr>
<td>HPLC (Rt)</td>
<td>4.5 min (column: RP-C18, 25 X 4.6mm, mobile phase: methanol, 0.5ml/min, 265 nm)</td>
</tr>
<tr>
<td>GC (Rt)</td>
<td>21.4 min (column: HP-5, Conditions: 50°C, 2 min, 10°C/min; 220°C, 2min, 15°C/min; 270°C, 20min.)</td>
</tr>
<tr>
<td>TLC (Rf)</td>
<td>0.5 (normal phase silica gel plate, benzene-methanol-acetic acid (3:2:0.1) as mobile phase.)</td>
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</tbody>
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Fig. 1. UV Spectrum of asperaldin at pH 3.8 (— —) and at pH 9.0 (— —).
named as 2,6-dihydroxy-8-hexyl-oxy-1,4-naphthaquinone. The isolated compound exhibited a dose-dependent aldose reductase inhibition at an IC$_{50}$ of 27 µM (Figure 3). Due to the novel inhibitory nature and new structure, the compound is henceforth designated as asperaldin. Asperaldin compares better with reported RLAR inhibitors such as YUA001, produced by Corynebacterium sp. YUA-25, which has an IC$_{50}$ of 1.8 mM$^{9,10}$, and is moderate when compared to thiazocin A, produced by Actinosynnema sp. c-304, which has an IC$_{50}$ of 0.45 µM$^{11}$. Other RLAR inhibitors, salfredins$^{12}$, thermorubins and 2-hydroxy phenyl acetic acid$^{13}$ have also been reported. In contrast, chemically synthesized tolrestat, alrestatin and sorbinil have IC$_{50}$ of 0.035 µM, 2.7 µM and 1.5 µM, respectively$^{14}$.

Naphthaquinones have a versatile range of biological properties, including antifungal and antibacterial activities$^{15-19}$. Asperaldin was also tested for inhibition activity against commercial soybean 15-lipoxygenase (LOX-1)$^{20}$, commercial porcine pancreas lipase (PPL)$^{21}$, crude rat brain acetylcholinesterase (AChE)$^{22}$ and crude rat lens aldose reductase$^{8}$. Interestingly, asperaldin up to 300 µM did not show any inhibition against LOX-1, PPL and AChE. Also, asperaldin revealed no antimicrobial property up to a concentration of 200 µg by disk plate method against Bacillus subtilis, B. pumilis, E. coli, Pseudomonas aeruginosa, Penicillium notatum, Aspergillus niger, Saccharomyces cerevisiae and Candida utilis.

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