Derivatives of Khellinonequinone and their Aflatoxigenic Activity

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Khellinonequinone. Aflatoxigenic Activity

Nitrations of khellinone lead to the formation of a small amount of 3-nitrokhellinone and 5-acytetyl-6-hydroxybenzofuran-4,7-dione (khellinonequinone) as a main product. The latter compound reacts with primary amines to give the corresponding imino compounds. Reaction of khellinone with o-phenylenediamine involves condensation followed by cyclisation. While on the other hand treating with phenyl hydrazines gives the phenyl hydrazone. The pyrazolobenzofuran derivative was obtained by the action of hydrazine hydrate on khellinonequinone. Finally the reaction with malononitrile leads to the formation of the ylidene derivative. Two quinone derivatives showed a weak effect on mycelial growth and aflatoxin formation.

Introduction

The interesting biological activity of many benzofuran derivatives especially visnaginone and khellinone has recently attracted the attention of chemists. Anrep et al. [1] observed that khellinone (1) possesses coronary vasodilator action. Moreover some khellinone derivatives possess hypotensive, vasodilating and spasmyolytic activities, accordingly they have a wide use in formulation of medicaments for therapy of cerebral arteriopathies [2]. In addition some derivatives of khellinone show antibacterial activity as well as antiparasitic properties [3]. The reaction of quinones has been studies by several authors and was found to produce a mono [4, 5] or diamino derivative [6—8].

Therefore it became of interest to synthesize some new derivatives of khellinonequinone in order to test their effect on mycelial growth and as antiaflatoxigenic agents.

Results and Discussion

Khellinonequinone was prepared by nitration of khellinone (1) to give 5-acytetyl-6-hydroxybenzofuran-4,7-dione (2) as a main product together with a small amount of the 3-nitrokhellinone derivative (3). The IR spectrum of 2 reveals absorptions at 1685 and 1665 cm−1 (C=O) and at 3400–3500 cm−1 (−OH group) respectively. The 1H NMR spectrum of 3 reveals a −OH group at δ 11.45, the furan proton at C2 at δ 8.55, (s), 6 protons of 2 OCH3 groups appeared at δ 4.15 and 3.95 (s). Finally the COCH3 protons appeared with DMSO peak.

2 reacts with amines: aniline, p-toluidine, 2-amino-pyrazine and p-anisidine to give the corresponding imino compounds (4—7). The IR spectrum of 6 reveals absorption at 1690 cm−1 (C=O), a C=N at 1630 cm−1 and OH at 3380 cm−1. The 1H NMR spectrum of 4 shows signals at δ 2.65 (3H, CH3, s); at δ 6.9 and 7.75 (1H each, furan protons C2 and C3, d) and at δ 7.1—7.75 (5H aromatic protons, m).

The reaction of 2 with o-phenylenediamine involves condensation with the acetyl group followed by cyclization with the quinone carbonyl to form 8. The IR spectrum of 8 reveals a band at 1680 cm−1 (C=O), a C=N peak at 1630 cm−1 and OH peak at 3400–3450 cm−1. The 1H NMR spectrum of 8 shows signals at δ 7.25—8.35 (5H, the furan proton at C2 and 4 aromatic protons, m), at δ 7.15 (1H, for the furan proton at C3, d) and at δ 2.9 (3H, N=C−CH3, s).

When 2 was treated with phenylhydrazine, 2,4-dinitrophenylhydrazine and phenylsulfonylhydrazine, the hydrazones (9—11) were obtained. The IR spectrum of 10 shows bands at 3420—3450 cm−1 (OH), at 3100 cm−1 (NH), at 1680 and 1660 cm−1 (C=O group) at 1620 cm−1 (C=N) and at 1555 and 1335 cm−1 (C=NO2). The 1H NMR spectrum of 11 shows signals at δ 2.63 (3H, CH3, s), at δ 6.99 and 8.27 (1H each, furan protons at C3 and C2, d) at δ 7.68—7.88 (5 aromatic protons, m) and at δ 13.55 (1H, OH, s).

The reaction of 2 with hydrazine hydrate (2 moles) leads to the formation of the corresponding pyrazolobenzofuran derivative (12). The IR spectrum of 12 shows the absence of C=O groups and the
The reaction of 2 with malononitrile leads to the formation of the ylidene derivative (13). The IR spectrum of 13 reveals a $\gamma\text{C}=\text{O}$ at 1655 cm$^{-1}$, C=C=N group (2220 cm$^{-1}$) and an OH (3380–3480 cm$^{-1}$).

**Experimental**

All m.ps. are uncorrected. The IR spectra were recorded (KBr) on a Pye Unicam SP-1000 Spectrophotometer. $^1$H NMR spectra were obtained in CDCl$_3$ (DMSO) with a Bruker Wm 300 spectrometer with SiMe$_4$ as internal standard. Mass spectra were recorded on MS 30 and MS 9 (AEL) 70 eV. Microanalytical data were performed by the Microanalytical Laboratory at the National Research Centre, Cairo-Dokki. The compounds were analyzed for C, H, N. The reported analyses are correct within ±0.4% of the theoretical values.

5-Acetyl-6-hydroxybenzofuran-4,7-dione (2) and 3-nitrokellinone (3)

Dissolve 2 g of kollinone (1) in 5 ml of acetone while warming, then add 3 ml of conc. nitric acid dropwise while stirring. Pour on ice and filter, crystallize from benzene as golden yellow crystals of 2, m.p. 158–160 °C, yield ca. 85%, gives a red colouration with ferric chloride solution.

**Analysis for C$_{10}$H$_6$O$_5$ (206)**

Calcd  C 58.25 H 2.91,

Found  C 58.18 H 3.16.

Table. The effect of different concentration of kollinonequinone and its derivatives on mycelial growth and aflatoxin formation by *A. flavus* No. 182.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Conc. [Mm/1]</th>
<th>Final pH</th>
<th>Dry wt. of mycelium [g/50 ml]</th>
<th>Aflatoxin in culture filtrate in mycelium</th>
<th>Total aflatoxin [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>B  G  Total</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>5.0</td>
<td>2.05</td>
<td>287.4 47.3 334.7</td>
<td>1837.5 149.3 1986.8 100.0</td>
</tr>
<tr>
<td>2</td>
<td>0.1</td>
<td>4.7</td>
<td>1.52</td>
<td>98.6 40.6 139.2</td>
<td>129.7 116.2 245.9 16.6</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>4.7</td>
<td>1.25</td>
<td>62.3 34.6 96.9</td>
<td>107.1 87.0 194.1 12.5</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>5.0</td>
<td>0.80</td>
<td>59.1 22.3 81.4</td>
<td>76.1 41.8 117.9 8.6</td>
</tr>
<tr>
<td>10</td>
<td>0.1</td>
<td>5.0</td>
<td>1.09</td>
<td>99.7 30.8 130.5</td>
<td>195.1 90.3 285.4 17.9</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>5.0</td>
<td>0.94</td>
<td>70.7 24.3 95.0</td>
<td>123.4 59.7 183.1 12.0</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>4.7</td>
<td>0.83</td>
<td>52.4 19.8 72.2</td>
<td>74.1 43.5 117.6 8.0</td>
</tr>
</tbody>
</table>

*Initial pH = 4.2; * aflatoxin expressed as % of control.
Crystallization of the mother liquor from methanol gave red needles of 3, m.p. 180—182 °C, yield ca. 5%. Its alcoholic solution gave a green colour with ferric chloride solution.

**Analysis for C_{12}H_{11}O_{2}N (281) M^+ at m/e 281**
Calcd C 51.25 H 3.91 N 4.98.
Found C 51.02 H 3.74 N 4.69.

**Preparation of imino derivatives by the action of khellinonequinone (5-acetyl-6-hydroxybenzofuran-4,7-dione) (2) with amines**

**General procedure**

Dissolve 0.001 mole of 2 in absolute ethanol (20 ml), then add 0.002 mole of the appropriate amine while stirring for 2 h. Filter the solid obtained, wash with very dilute hydrochloric acid and crystallize from the suitable solvent.

4 was crystallized from cyclohexane as yellow crystals, m.p. 169—171 °C, yield 64%.

**Analysis for C_{12}H_{11}O_{2}N (281)**
Calcd C 68.33 H 3.91 N 4.99.
Found C 68.12 H 4.06 N 4.67.

5 was obtained in ca. 67% yields as dark yellow crystals from a mixture of benzene with cyclohexane, m.p. 200—202 °C.

**Analysis for C_{12}H_{11}O_{2}N (295)**
Calcd C 69.15 H 4.41 N 4.75.
Found C 69.50 H 4.37 N 5.16.

6 was prepared as yellow crystals from cyclohexane m.p. 150—152 °C, yield 75%.

**Analysis for C_{12}H_{11}O_{2}N (283)**
Calcd C 59.40 H 3.18 N 14.83.
Found C 58.89 H 3.22 N 14.42.

7 was obtained as yellowish crystals from benzene, m.p. 203—205 °C, yield ca. 71%.

**Analysis for C_{12}H_{11}O_{2}N (311)**
Calcd C 65.59 H 4.18 N 4.50.

8 was crystallized from cyclohexane and few drops of benzene as red crystals, m.p. 205—207 °C, yield ca. 63% its alcoholic solution gave a dark brown colour with ferric chloride solution.

**Analysis for C_{12}H_{11}O_{2}N (248)**
Calcd C 69.06 H 3.59 N 10.07.
Found C 68.75 H 3.47 N 10.02.

Reaction of khellinonequinone (2) with phenylhydrazines (9, 10, 11).

A mixture of 0.001 mole of 2 and 0.002 mole of the phenylhydrazine derivative in 20 ml of absolute ethanol is refluxed for $\frac{1}{2}$—1 h. Filter the solid so obtained and crystallize from the suitable solvent.

9 was obtained as faint brown crystals from ethanol m.p. 192.4 °C, yield ca. 66%.

**Analysis for C_{10}H_{12}O_{3}N (296)**
Calcd C 64.86 H 4.05 N 9.46.
Found C 64.45 H 3.91 N 9.17.

10 was crystallized from tetrahydrofuran as red crystals m.p. 258—259 °C, yield ca. 62%.

**Analysis for C_{10}H_{10}O_{4}N_{4} (286)**
Calcd C 49.74 H 2.59 N 14.51.
Found C 49.89 H 2.85 N 14.27.

11 was prepared as brown needles from methanol m.p. 193—195 °C, yield ca. 60%.

**Analysis for C_{10}H_{11}O_{4}N (359)**
Calcd C 53.50 H 3.06 N 10.88 S 8.9.
Found C 53.38 H 3.41 N 7.61 S 8.6.

Reaction of khellinonequinone (2) with hydrazine hydrate (12).

Add 0.3 ml of hydrazine hydrate to a solution of 1 g of 2 in absolute ethanol (50 ml). The reaction mixture is refluxed for 30 min then left to cool and filtration. The brown crystals of 12 are crystallized from ethanol, m.p. above 300 °C, yield 51%, its alcoholic solution gives a red colouration with ferric chloride solution.

**Analysis for C_{10}H_{10}O_{4}N_{3} (216)**
Calcd C 55.55 H 3.70 N 25.93.
Found C 55.49 H 3.55 N 25.46.

**Preparation of the ylidene derivative (13)**

A mixture of 2 g of 2 and 1.3 g of malononitrile in 50 ml of absolute ethanol was well stirred for 1 h. The red solid of 13 so obtained was filtered and crystallized from benzene as wine-red crystals, m.p. 300 °C, yield ca. 48%.

**Analysis for C_{10}H_{12}O_{2}N_{2} (254)**
Calcd C 56.93 H 2.19 N 10.22.
Found C 57.20 H 2.40 N 10.15.

**Biology:** microorganism *Aspergillus flavus* No. 182, a potent aflatoxin producer [9]. Culture medium: this was used according to Bullerman [10]. Cultivation: transfers were made from subcultures on Dox's agar slants to potato-dextrose-agar (PDA) plates for 7 d at 28 °C. Liquid cultures were grown in 250 ml Erlenmeyer flasks each containing 50 ml medium. Three concentrations of each of the two compounds (0.1, 1.0 and 0.5 mM/l) were added to the flasks. Unsupplemented flasks were taken as control. Flasks were then sterilized at 1.5 PSI for
15 min. Two discs each, 1 cm diameter were cut from 7 d old culture plates and used for incubating each flask. After incubation for 6 d at 25 °C. The culture medium from each flask was filtered off. Growth final pH and aflatoxin content were detected.

Mycelial dry weight determined according to Swaminathan, Koehler [11]. Preparation of corn steep liquor according to the method in [12].

Aflatoxin analysis

The aflatoxins in mycelia and culture filtrates were separately extracted using the methods of Saito et al. [13] and Bullerman [10], respectively. Aflatoxins were separated by TLC and the content estimated spectrophotometrically [14].

Results

Compound 2 at the 3 levels used (0.1, 1.0 and 5.0 mM), reduced mycelial growth to 74.1%, 60.9% and 39.0% respectively while effect of compound 10 was much more pronounced as it decreased mycelial growth to 53.1%, 45.8% and 40.1% respectively. The inhibitory effects of the two compounds on aflatoxin biosynthesis was more or less similar. Thus aflatoxin release was inhibited by the first compound to 16.6%, 12.5% and 8.6%, and the second compound to 17.9%, 12.0% and 8.6% of that of the control at the three levels used.

Discussion

The two compounds may be regarded as weak anti-aflatoxigenics or as plant fungicides preventing growth of Aspergillus.