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Synthesis and Antibacterial Activity of Juglone Derivatives

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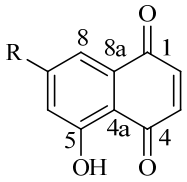
Abstract: Nitro and halogen substituted derivatives of the juglone naphthoquinone were synthesized and assayed for their antibacterial activity. 8-Nitrojuglone was obtained as the exclusive product from the direct nitration of juglone with nickel (II) nitrate and *p*-toluenesulphonic acid. In addition, a series of five 8-halojuglone derivatives were synthesized via a solvent-free Friedel-Crafts acylation reaction. One of the acylation reactions afforded an anthraquinone-type derivative as the minor product. The 8-nitrojuglone derivative displayed the most notable activity against *S. aureus*. However, all of the 8-halojuglone derivatives were found to be less active than juglone against the bacteria assayed.

Key words: Naphthoquinone, nitration, Friedel-Crafts acylation, anthraquinone, antibacterial activity.

1. Introduction

The juglone naphthoquinone (1) (Fig. 1) is synonymous with the well-known allelopathic nature of the black walnut tree (*Juglans nigra*) [1-2]. Furthermore, it displays significant antimicrobial [3], antitumor [4], antihypertensive [5] and enzyme inhibition activities [6]. The larvicidal and molluscicidal activities of juglone (1) along with some of its synthetic derivatives were recently evaluated. Some of the synthetic bromo derivatives were identified as remarkably potent larvicidal and molluscicidal agents [7].

7-methyljuglone (2) (Fig. 1), which is the methyl derivative of juglone (1), occurs naturally in the *Ebenaceae* family of plants [2, 8]. Juglone (1), 7-methyljuglone (2), and a series of synthetic halogen juglone derivatives were shown to exhibit noteworthy antitubercular activity [8]. In addition, synthetic amino derivatives of juglone are also another group of bioactive compounds with moderate lung tumour cytotoxicity [9]. However, thus far, no nitro substituted juglone derivative has ever been synthesised and biologically assayed. In light of these interesting accounts, it was proposed to synthesise and evaluate the antibacterial activity of a new nitro derivative together with some new and known halogen derivatives of juglone (1).



R = H (**1**)
R = Me (**2**)

Fig. 1 Juglone (1) and 7-methyljuglone (2).

2. Experiment

2.1 General Experimental Procedures

Melting points were determined using a Stuart Scientific SMP1 apparatus and are uncorrected. UV and IR spectra were recorded on a Perkin Elmer

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Lambda 25 UV/VIS and Perkin-Elmer System 2000 FT-IR spectrometer, respectively. ^{1}H NMR spectra were recorded on a Bruker Avance 400 spectrometer, while ^{13}C NMR spectra were recorded on a Bruker Avance 300 with $CDCl_3$ as the solvent and TMS as the internal standard. HRESIMS measurements were conducted on a Bruker micro TOFQ mass spectrometer. TLC (silica gel 60 F_{254}) was employed to check the purity of all the compounds. Silica gel 60 (0.040-0.063 mm) was used as the stationary phase for all column chromatography separations.

2.2 Synthesis of 5-Hydroxy-8-Nitro-1,4-Naphthalenedione (8-Nitrojuglone) (3)

Nickel(II) nitrate hexahydrate (1.00 g, 3.8 mmol) was added to a solution of juglone (0.35 g, 2.0 mmol) in acetone (30.0 mL). *p*-Toluenesulphonic acid (5.10×10^{-3} g, 0.3 mmol) was subsequently added and the reaction mixture was refluxed at 50 ^{o}C for 24 hours. Excess acetone was removed under vacuo to afford a dark reddish residue. The residue was partitioned between EtOAc and water. The EtOAc extracts were combined, dried ($MgSO_4$) and concentrated under vacuum. Purification of the crude product by silica gel column chromatography (EtOAc/*n*-hexane, 1:9), followed by recrystallization from chloroform afforded 3 as a red crystalline solid (51 mg, 11%). Mp 120-123 ^{o}C; UV (MeOH) λ_{max} (nm) (log ε): 205.11 (4.57), 229.75 (4.46), 402.90 (3.87); IR (KBr) ν_{max} (cm^{-1}): 3409 (O-H), 1671 and 1644 (C=O), 1603 (aromatic), 1523 (asymmetric NO_2), 1352 (symmetric NO_2); δ_H (400 MHz, $CDCl_3$, ppm): 12.31 (1H, s, OH), 7.68 (1H, d, $J_{6,7}$ = 9.0 Hz, H-7), 7.39 (1H, d, $J_{6,7}$ = 9.0 Hz, H-6), 7.04 (2H, s, H-2 and H-3); HRESIMS: m/z 218.0131 $[M-H]^-$ (calcd. for $C_{10}H_4NO_5$, 218.0089).

2.3 General Procedure for the Synthesis of 8-Halojuglone Derivatives (6a-6e)

A mixture of sodium chloride (8.00 g, 137.0 mmol) and aluminium chloride (40.00 g, 300.0 mmol) was heated until melting (180-185 ^{o}C). The corresponding 4-halophenol derivative (10.7 mmol) and maleic anhydride (4.00 g, 40.8 mmol) were subsequently added to the ionic melt and stirred for 2 minutes, after which it was rapidly poured into a mixture of hydrochloric acid (12 M) and ice. The mixture was then left to stand for 30 minutes and the resulting chocolate precipitate was filtered and dried overnight at room temperature.

The solid residue was then exhaustively extracted with *n*-hexane in a Soxhlet extractor at 70 ^{o}C. The *n*-hexane extracts were combined and concentrated under *vacuo*. Purification of the product was finally accomplished by either recrystallization from *n*-hexane or silica gel column chromatography ($CHCl_3$/*n*-hexane, 3:20).

2.3.1 8-Chloro-5-Hydroxy-1,4-Naphthalenedione (6a)

UV (EtOH) λ_{max} (nm) (log ε): 211.92 (4.53), 254.47 (4.18), 430.75 (3.66); IR (KBr) ν_{max} (cm^{-1}): 3413 (O-H), 3068 (C_{Ar}-H), 1664 and 1643 (C=O), 1602 (aromatic); δ_H (400 MHz, $CDCl_3$, ppm): 12.64 (1H, s, OH), 7.66 (1H, d, $J_{6,7}$ = 9.1 Hz, H-7), 7.26 (1H, d, $J_{6,7}$ = 9.1 Hz, H-6), 6.97 (2H, s, H-2 & H-3); δ_C (300 MHz, $CDCl_3$, ppm): 190.1 (C-4), 183.0 (C-1), 161.7 (C-5), 141.3, 140.7 (C-2 or C-3), 137.3 (C-7), 127.2 (C-8), 126.8 (C-8a), 125.8 (C-6), 116.4 (C-4a); HRESIMS: m/z 206.9846 $[M-H]^-$ (calcd. for $C_{10}H_4ClO_3$, 206.9849).

2.3.2 8-Chloro-5-Hydroxy-7-Methyl-1,4-Naphthalenedione (6b)

UV (EtOH) λmax (nm) (log ε): 216.79 (4.50), 255.71 (4.14), 432.50 (3.45); IR (KBr) ν_{max} (cm^{-1}): 3429 (O-H), 3058 (C_{Ar}-H), 2965 (C_{sp^3}-H) 1663 and 1646 (C=O), 1608 (aromatic); δ_H (400 MHz, $CDCl_3$, ppm): 12.58 (1H, s, OH), 7.22 (1H, s, H-6), 6.93 (2H, s, H-2 and H-3), 2.51 (3H, s, CH_3); δ_C (300 MHz, $CDCl_3$, ppm): 189.9 (C-4), 183.6 (C-1), 161.0 (C-5), 149.4 (C-7), 141.3, 137.0 (C-2 or C-3), 127.7 (C-8), 127.4 (C-8a), 126.3 (C-6), 115.0 (C-4a), 22.2 (CH_3); HRESIMS: m/z 221.0014 $[M-H]^-$ (calcd. for $C_{11}H_6ClO_3$, 221.0005).

2.3.3 8-Chloro-5-Hydroxy-7-Ethyl-1,4-Naphthalenedione (6c)

UV (EtOH) λ_{max} (nm) (log ε): 217.64 (4.58), 256.18 (4.18), 432.50 (3.72); IR (KBr) ν_{max} (cm^{-1}): 3424 (O-H), 3066 (C_{Ar}-H), 2975, ($C_{sp}{}^3$-H), 1668 and 1645 (C=O); 1605 (aromatic); δ_H (400 MHz, $CDCl_3$, ppm): 12.61 (1H, s, OH), 7.23 (1H, s, H-6), 6.93 (2H, s, H-2 & H-3), 2.91 (2H, q, $J_{9,10}$ = 7.5 Hz, $C\underline{H}_2CH_3$), 1.31 (3H, t, $J_{9.10}$ = 7.5 Hz, $CH_2C\underline{H}_3$); δ_C (300 MHz, $CDCl_3$, ppm): 189.9 (C-4), 183.6 (C-1), 161.2 (C-5), 154.5 (C-7), 141.3, 136.9 (C-2 or C-3), 127.5 (C-8), 127.1 (C-8a), 124.8 (C-6), 114.9 (C-4a), 28.1 ($\underline{C}H_2CH_3$), 13.6 ($CH_2\underline{C}H_3$); HRESIMS: m/z 235.0180 $[M-H]^-$ (calcd. for $C_{12}H_8ClO_3$, 235.0167).

2.3.4 8-Bromo-5-hydroxy-1,4-naphthalenedione (6d)

UV (EtOH) λ_{max} (nm) (log ε): 212.18 (4.55), 254.71 (4.21), 431.15 (3.75); IR (KBr) ν_{max}(cm^{-1}): 3432 (O-H), 3068 (C_{Ar}-H), 1663 and 1639 (C=O); 1602 (aromatic); δ_H (400 MHz, $CDCl_3$, ppm): 12.63 (1H, s, OH), 7.65 (1H, d, $J_{6,7}$ = 9.2 Hz, H-7), 7.25 (1H, d, $J_{6,7}$ = 9.2 Hz, H-6), 6.97 (2H, s, H-2 and H-3); δ_C (300 MHz, $CDCl_3$, ppm): 190.2 (C-4), 183.1 (C-1), 161.7 (C-5), 141.3, 140.8 (C-2 or C-3), 137.3 (C-7), 127.2 (C-8), 126.8 (C-8a), 125.9 (C-6), 116.3 (C-4a); HRESIMS: m/z 250.9342 $[M-H]^-$ (calcd. for $C_{10}H_4BrO_3$, 250.9349).

2.3.5 8-Bromo-5-Hydroxy-7-Methyl-1,4-Naphthalenedione (6e)

UV (EtOH) λ_{max} (nm) (log ε): 216.77 (4.57), 256.01 (4.13), 432.16 (3.65); IR (KBr) ν_{max}(cm^{-1}): 3436 (O-H), 3059 (C_{Ar}-H), 2926 ($C_{sp}{}^3$-H), 1664 and 1647 (C=O), 1606 (aromatic); δ_H (400 MHz, $CDCl_3$, ppm): 12.55 (1H, s, OH), 7.20 (1H, s, H-6), 6.91 (2H, s, H-2 & H-3), 2.49 (3H, s, CH_3); δ_C (300 MHz, $CDCl_3$, ppm): 189.9 (C-4), 183.6 (C-1), 160.9 (C-5), 149.4 (C-7), 141.2, 137.0 (C-2 or C-3), 127.6 (C-8), 127.3 (C-8a), 126.3 (C-6), 114.9 (C-4a), 22.3 (CH_3); HRESIMS: m/z 264.9466 $[M-H]^-$ (calcd. for $C_{11}H_6BrO_3$, 264.9500).

2.4 1,5,8-Trihydroxy-3-Methylanthracene-9,10- Dione (Helminthosporin) (7)

Mp: 228-230 ^oC (lit. 228.5-230.5 ^oC [15]); UV (MeOH) λ_{max} (nm) (log ε): 230.36 (4.54), 253.84 (4.18), 287.01 (3.86), 488.03 (3.98); IR (KBr) ν_{max}(cm^{-1}): 3419 (OH), 1635 and 1615 (C=O); δ_H (400 MHz, $CDCl_3$, ppm): 13.03 (1H, s, 5-OH), 12.34 (1H, s, 8-OH), 12.16 (1H, s, 1-OH), 7.71 (1H, s, H-4), 7.31 (2H, s, H-6 and H-7), 7.13 (1H, s, H-2), 2.50 (3H, s, CH_3); δ_C (300 MHz, $CDCl_3$, ppm): 191.0 (C-9), 187.0 (C-10), 163.2 (C-1), 158.6 (C-8), 158.0 (C-5), 149.5 (C-3), 133.6 (C-4a), 130.0 (C-6 and C-7), 125.0 (C-2), 120.8 (C-4), 113.2 (C-8a and C-10a), 22.8 (CH_3); HRESIMS: m/z 269.0457 $[M-H]^-$ (calcd. for $C_{15}H_9O_5$, 269.0455).

2.5 2-Chloro-1,5,8-Trihydroxy-4-Methylanthracene-9, 10-Dione (8)

Mp: 240-242 ^oC; UV (MeOH) λ_{max} (nm) (log ε): 233.79 (4.52), 257.14 (4.11), 294.64 (3.81), 501.74 (3.86); IR (KBr) ν_{max} (cm^{-1}): 3415 (O-H), 1637 and 1613 (C=O); δ_H (400 MHz, $CDCl_3$, ppm): 13.37 (1H, s, 5-OH), 13.28 and 12.18 (2 × 1H, 2s, 1-OH and 8-OH), 7.66 (1H, s, H-3), 7.38 and 7.31 (2 × 1H, 2d, $J_{6,7}$ = 9.4 Hz, $J_{6,7}$ = 9.4 Hz, H-6 and H-7), 2.79 (3H, s, CH_3); δ_C (300 MHz, $CDCl_3$, ppm): 191.5 (C-9), 188.4 (C-10), 158.5 (C-8), 158.0 (C-5), 135.7 (C-4), 131.2 (C-4a), 131.0 (C-3), 129.6 (C-2), 129.2 (C-6 and C-7), 117.8 (C-9a), 113.6 (C-8a), 112.5 (C-10a), 23.1 (CH_3); HRESIMS: m/z 303.0074 $[M-H]^-$ (calcd. for $C_{15}H_8ClO_5$, 303.0066).

2.6 Antibacterial Assay

The minimum inhibitory concentration (MIC) was determined by the broth micro-dilution method [20-21]. Bacterial suspensions were prepared in Mueller-Hinton broth and the turbidity was adjusted to that of a 0.5 McFarland solution. Gentamicin and tetracycline were used as positive controls, whereas DMSO was used as a negative control in the assay. Test compounds were dissolved in DMSO and diluted with water to prepare the working solution of 500 μg/mL concentration in not more than 10% DMSO. Serial two-fold dilutions of the compounds were made in the range of 250.0-3.9 μg/mL in 96 well tissue culture plates. The plates were then

inoculated with 100 μL of bacterial suspension, sealed and incubated for 24 h at 37 °C. 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) (50 μL, 0.2 mg/mL in sterile distilled water) was used to indicate the viability of the bacterial cells. A yellow colour was interpreted as no bacterial growth, whereas a purple colour was interpreted as bacterial growth. The MIC was determined as the lowest concentration of a compound which prevented a colour change from yellow to purple.

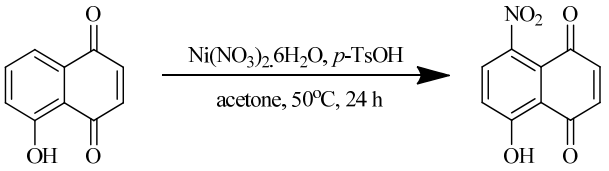
3. Results and Discussion

3.1 Synthesis

The new nitro derivative (3) was obtained from the direct nitration of juglone (1) with nickel (II) nitrate and catalytic p-toluenesulphonic acid (Scheme 1). The method was chosen because of its exclusive ortho-regioselectivity [10]. The IR spectrum of 3 revealed the presence of the nitro substituent as two moderately-intense absorption bands at 1523 cm^{-1} and 1352 cm^{-1}. Unexpectedly, the X-ray crystallographic data of 3 showed that the product is a para-nitro substituted juglone derivative (Fig. 2) [11]. This fascinating observation may be due to the formation of nickel (II)-juglone complexes which facilitate nitro substitution at the para position and not at the ortho position [12].

A series of five 8-halogen substituted juglone derivatives (6a-6e) was prepared from the solvent-free, Friedel-Crafts acylation reaction of the respective 4-halophenol derivatives (4a-4e) with maleic anhydride (5) (Scheme 2). Two of the derivatives, 6c and 6d, are new compounds, whereas the remaining derivatives are known compounds [8, 13-14].

The acylation reaction of 4b recorded the lowest reaction yield of 8% (Table 1). This is due to the formation of the known anthraquinone, helminthosporin (7) (Fig. 3) as the minor product (1%) [14-16]. This observation was also observed in the acylation reaction of 2-chloro-4-methylphenol, which afforded a mixture of polymeric products, with one of



(1) **(3)**

11%

Scheme 1 The direct nitration of juglone (1).

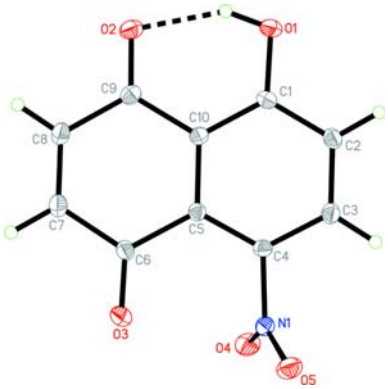
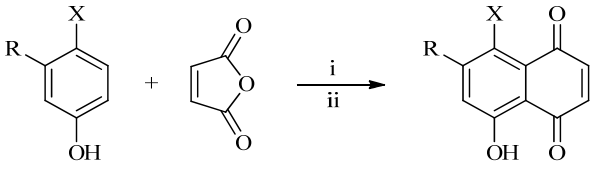


Fig. 2 The ORTEP plot of 8-nitojuglone (3).



(4a-e) **(5)** **(6a-e)**

(i) $AlCl_3$ (liq), NaCl (liq), 180-185°C, 2 mins. (ii) $H_3O^{\oplus}$

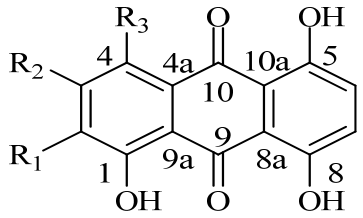
	X	R
(a)	Cl	H
(b)	Cl	Me
(c)	Cl	Et
(d)	Br	H
(e)	Br	Me

Scheme 2 The synthesis of 8-halojuglone derivatives (6a-6e).

Table 1 The melting point and reaction yield of the 8-halojuglone derivatives (6a-6e).

Derivative	X	R	Melting point (°C)	Yield[b] (%)
6a	Cl	H	202-204 (201-202)[a]	10
6b	Cl	Me	158-160 (160-161)[a]	8
6c	Cl	Et	108-111	14
6d	Br	H	206-209	24
6e	Br	Me	148-150 (154)[a]	10

[a]Literature melting point; [b]Isolated yields.



$R_1 = H, R_2 = Me, R_3 = H$ (**7**)
$R_1 = Cl, R_2 = H, R_3 = Me$ (**8**)

Fig. 3 Helminthosporin (7) and anthraquinone (8).

the minor products being a chloro substituted anthraquinone (8) (Fig. 3). However, no juglone derivative was obtained from the reaction mixture of 2-chloro-4-methylphenol and maleic anhydride (5). Therefore, the presence of a chloro and a methyl substituent on 4b activated it for further reaction with maleic anhydride to form the anthraquinone-type minor product. Reductive dechlorination finally afforded helminthosporin (7) [14].

Furthermore, the 4-halo substituent in the phenolic reactants (4a-e) is crucial for the regiochemical control of the Friedel-Crafts acylation reaction.

The UV spectra of 7 and 8 revealed four absorption bands which are characteristic of an anthraquinone [17]. In addition, the ^{1}H NMR spectra of 7 and 8 showed three intramolecular hydrogen-bonded hydroxyl resonances at δ_H 12.16-13.03 and 12.18-13.37, respectively [18].

3.2 Antibacterial Activity

Juglone (1), synthetic derivatives (3), (6a-6e) and the anthraquinone-type derivatives, (7) and (8) were subjected to antibacterial assay against four bacterial strains: *Bacillus cereus* (ATCC 10876), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Klebsiella pneumonia* (ATCC 13883). The results are summarized in Table 2. 8-Nitrojuglone (3) displayed the most notable activity against *S. aureus*. This indicates a possible relationship between the nitro substituent of 3 and its notable activity against *S. aureus* [19]. In addition, both of the anthraquinone-type derivatives, (7) and (8) displayed higher antibacterial activity than those of juglone (1).

Table 2 The MIC (mM) of juglone (1) and its synthetic derivatives against four bacterial strains.

Compound/ control antibiotic	Minimum inhibitory concentration, MIC (mM)			
	Gram-positive bacteria		Gram-negative bacteria	
	B. cereus	*S. aureus*	*E. coli*	*K. pneumoniae*
Juglone (1)	0.36	0.72	0.36	0.36
3	0.29	0.14	0.57	0.57
6a	0.60	0.60	0.60	0.60
6b	0.56	1.1	0.56	0.56
6c	0.53	1.1	0.53	0.53
6d	0.50	0.99	0.50	0.50
6e	0.47	0.94	0.47	0.47
7	0.23	0.46	0.23	0.23
8	0.21	0.41	0.21	0.21
Gentamicin	-	-	0.027	0.014
Tetracycline	0.016	<0.004	-	-

On the contrary, all of the 8-halojuglone derivatives (6a-6e) exhibited lower antibacterial activity than those of juglone (1).

4. Conclusion

8-Nitrojuglone (3) was obtained as the sole *para*-isomeric product from the direct nitration of juglone (1) with nickel (II) nitrate and *p*-toluenesulphonic acid. The 4-halo substituent in the phenolic reactant controls the regiochemistry of the Friedel-Crafts acylation reaction. 8-Nitrojuglone (3) is the most active derivative against *S. aureus*. Both of the anthraquinone-type derivatives, (7) and (8), are more active than juglone (1) against the bacteria assayed. However, none of the 8-halojuglone derivatives (6a-6e) are more active than juglone (1) against the bacteria assayed.

Acknowledgments

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