SYNTHESES AND ANTIBACTERIAL SCREENING OF JUGLONE DERIVATIVES

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SYNTHESES AND ANTIBACTERIAL SCREENING OF JUGLONE DERIVATIVES

by

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Daniel Teoh Chuan Tan

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LIST OF ABBREVIATIONS

Organic Chemistry and Reagents

AcOH = acetic acid

$B(OAc)_3$ = boron triacetate

DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene

Et_3N = triethylamine

EtOH = ethanol

i-PrOH = isopropanol

LAH = lithium aluminium hydride

LTMP= lithium tetramethylpiperidide

MeOH = methanol

NBS = *N*-bromosuccinimide

NMO = *N*-methylmorpholine-*N*-oxide

OAc = acetate

OEt = ethoxyl

OMe = methoxyl

$OSiMe_3$ = trimethylsilyloxy

PCC = pyridinium chlorochromate

$Pd(PPh_3)_4$ = tetrakis(triphenylphosphine)palladium(0)

p-TsOH = *p*-toluenesulphonic acid

$(Tf)_2O$ = trifluoromethanesulfonic anhydride

THF = tetrahydrofuran

TMSCl = trimethylsilyl chloride

TPAP = tetrapropylammonium perruthenate

rac = racemic

Spectroscopy

$CDCl_3$ = deuterated chloroform

COSY = Correlation Spectroscopy

DEPT = Distortionless Enhancement by Polarisation Transfer

GC-MS = Gas Chromatography-Mass Spectrometry

HRESIMS = High Resolution Electron Spray Ionisation Mass Spectrometry

IR = Infrared spectroscopy

J = coupling constant

NMR = Nuclear Magnetic Resonance spectroscopy

TMS = tetramethylsilane

UV = Ultraviolet spectroscopy

amu = atomic mass unit

d = doublet

dd = doublet of doublets

m = multiplet

ppm = parts per million

s = singlet

t = triplet

Microbiology

B. cereus = *Bacillus cereus*

E. coli = *Escherichia coli*

K. pneumoniae = *Klebsiella pneumoniae*

MHB = Mueller Hinton Broth

MIC = minimum inhibitory concentration

MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide

NA = nutrient agar

S. aureus = *Staphylococcus aureus*

Miscellaneous

TLC = thin layer chromatography

eq = equivalent(s)

h = hour(s)

id = internal diameter

liq = liquid

mins = minutes

rt = room temperature

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SINTESIS DAN PENSKRINAN ANTIBAKTERIA TERBITAN JUGLON

ABSTRAK

Sebatian semula jadi juglon pernah digunakan sebagai bahan permula di dalam sintesis pelbagai sebatian bioaktif kuinon seperti okromisinon. Di dalam kajian ini, terbitan juglon disintesiskan daripada dua jenis bahan permula, iaitu juglon dan bukan juglon. Prekursor okromisinon, 1,2,3,4-tetrahidro-8-hidroksi-3-metilbenz[*a*]antrasen-7,12-dion (**19**) disintesiskan daripada juglon melalui dua langkah tindak balas. Langkah yang pertama ialah tindak balas Diels-Alder, manakala langkah kedua ialah tindak balas pengaromatikan. Di samping itu, terbitan baru *para*-nitrojuglon (**51**) diperoleh sebagai hasil isomerik tunggal daripada tindak balas penitratan langsung juglon. Tindak balas berkenaan melibatkan pengunaan nikel(II) nitrat sebagai agen penitratan. Tindak balas pengasilan Friedel-Crafts di antara beberapa terbitan 4-halofenol dan maleik anhidrida turut dijalankan. Tindak balas pengasilan tersebut menghasilkan lima terbitan 8-halojuglon (**30**, **31**, **52**, **53** dan **54**). Dua daripada lima terbitan berkenaan (**52** dan **54**) merupakan terbitan baru. Salah satu daripada tindak balas pengasilan itu memberikan antrakuinon (**69**) sebagai hasil minor. Antrakuinon (**69**) kemudiannya dikenal pasti sebagai sebatian semula jadi helmintosporin. Kesemua terbitan sintetik juglon diuji terhadap dua jenis bakteria Gram positif dan dua jenis bakteria Gram negatif. 8-nitrojuglon (**51**) menunjukkan aktiviti signifikan terhadap bakteria Gram positif *S. Aureus* manakala, helmintosporin (**69**) menunjukkan aktiviti yang paling tinggi terhadap kedua-dua bakteria Gram negatif yang diuji. Namun begitu, kesemua terbitan 8-

halojuglon (**30**, **31**, **52**, **53** dan **54**) menunjukkan aktiviti antibakteria yang lebih rendah berbanding dengan juglon.

SYNTHESES AND ANTIBACTERIAL SCREENING OF JUGLONE DERIVATIVES

ABSTRACT

The juglone natural product has been used as the starting material in the syntheses of various bioactive quinones like ochromycinone. The present study involves the syntheses of juglone derivatives from both juglone and non-juglone starting materials. The ochromycinone precursor, 1,2,3,4-tetrahydro-8-hydroxy-3-methylbenz[*a*] anthracene-7,12-dione (**19**) was synthesised from juglone in two steps. The first step was the Diels-Alder reaction and the second step was the aromatisation reaction. In addition, a new *para*-nitrojuglone derivative (**51**) was obtained as the sole isomeric product from the direct nitration of juglone. The reaction involved the use of nickel(II) nitrate as the nitrating agent. The Friedel-Crafts acylation reactions between various 4-halophenol derivatives and maleic anhydride were also carried out. The acylation reactions yielded five 8-halojuglone derivatives (**30**, **31**, **52**, **53** and **54**), two of which (**52** and **54**) are new derivatives. One of the acylation reactions afforded an anthraquinone (**69**) as the minor product. The anthraquinone (**69**) was later identified as the known naturally-occurring helminthosporin. All of the synthetic juglone derivatives were assayed against two Gram-positive and two Gram-negative bacterial strains. 8-Nitrojuglone (**51**) exhibited significant activity against the Gram-positive *S. aureus* bacteria. Furthermore, helminthosporin (**69**) displayed the highest activities against both

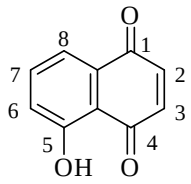
of the Gram-negative bacteria assayed. However, all of the 8-halojuglone derivatives (**30**, **31**, **52**, **53** and **54**) showed lower antibacterial activities than those of juglone.

CHAPTER 1

INTRODUCTION

1.1 A Brief Introduction to Juglone

The walnut tree has a unique way of flourishing in its natural environment. It inhibits the occurrence, growth and germination of certain competing plants like potato, tomato, apple and alfalfa by synthesising a chemical toxin (Rietveld, 1983). This observation was first noted by the Roman naturalist, Pliny the Elder (23-79 A.D.) in the first century AD (Thomson, 1971; Rietveld, 1983). The relationship between these inhibited plant species and the walnut tree is known as 'allelopathy'. The allelopathic property of walnut trees is found to be attributed to juglone (**1**) (Thomson, 1971; Harbone, 1998).



(**1**)

Juglone (**1**) is a bicyclic naphthoquinone which comprises a 1,4-benzoquinone ring and a phenol ring fused together. The IUPAC name of juglone (**1**) is 5-hydroxy-1,4-naphthalenedione. Juglone (**1**) has a molecular formula of $C_{10}H_6O_3$ and a molecular weight of 174.15 g mol^{-1}. It is a dark orange-brown solid which is sparingly soluble in water.

1.2 The General Uses of Juglone

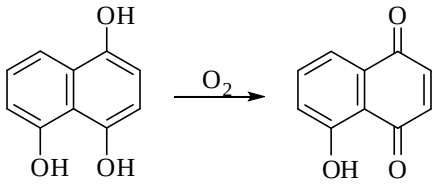
The intense orange-brown colour of juglone (**1**) has been used for centuries as a dye for wool and human hair. The use of juglone (**1**) as a natural dye has now been applied in cosmetic products like sunscreen oil and tanning agents (Soderquist, 1973; Levy, 2001; Bechtold and Mussak, 2009). In the dye industry, juglone (**1**) is known by its trade name as CI Natural Brown 7 (Bechtold and Mussak, 2009).

Native South Americans have been reported to immobilise fishes by throwing fresh walnut hulls into the water. The immobilised fishes will then rise up to the surface for collection (Thomson, 1971; Soderquist, 1973). This practice of fishing employs juglone (**1**) as a fish depressant (Westfall *et. al.*, 1961)

The use of juglone (**1**) as a traditional treatment for various skin infections have been in practice for many years by North Americans (Soderquist, 1973). The extract of freshly macerated black walnut hulls is applied topically for fungal infections like ringworm and viral infections like herpes (Clark *et. al.*, 1990; Inbaraj and Chignell, 2004).

1.3 The Natural Occurrence and Isolation of Juglone

Juglone (**1**) and its precursor, α-hydrojuglone (**2**) are mainly concentrated in the roots, bark, fruit hulls and shells; and to a smaller extent in the leaves of the walnut tree. However, Both of these chemical constituents are not found in the edible walnut. Upon exposure to air, the non-toxic α-hydrojuglone (**2**) is immediately oxidised to the toxic juglone (**1**) (Soderquist, 1973; Rietveld, 1982) (Scheme 1.1).

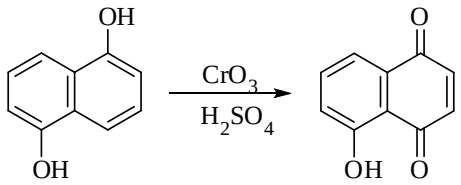


(**2**) (**1**)

Scheme 1.1: The air oxidation of α-hydrojuglone (**2**).

Juglone (**1**) is found predominantly in the black walnut tree (*Juglans nigra*) species (Thomson, 1971). According to Daugherty and co-workers, juglone (**1**) is only found exclusively in the Juglandaceae family (walnut family) of plants (Daugherty *et. al.*, 1995). However, Cai and co-workers later discovered that juglone (**1**) was also present in *Diospyros lycioides*, a plant belonging to the Ebenaceae family (Cai *et. al.*, 2000).

Vogel and Reischauer first isolated juglone (**1**) as a yellow crystalline from fresh walnut shells in 1856. They initially named the isolate 'nucin' (Soderquist, 1973). The structural confirmation of juglone (**1**) was later determined by synthesis by Bernthsen and Semper in 1887 (Daglish, 1950). In their study, Bernthsen and Semper employed the chromic acid oxidation of 1,5-dihydroxynaphthalene (**3**) for the synthesis of juglone (**1**) (Friedham, 1934; Thomson, 1948) (Scheme 1.2).



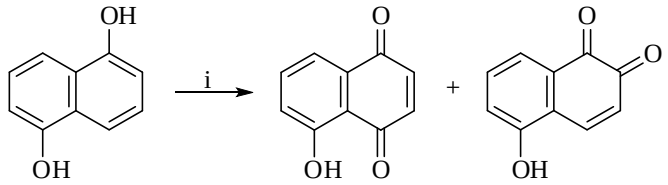
(**3**) (**1**)

Scheme 1.2: The oxidation of 1,5-dihydroxynaphthalene (**3**) by chromic acid oxidation, as carried out by Bernthsen and Semper, 1887.

Since then, the 1,5-dihydroxynaphthalene (**3**) oxidation pathway was employed as the only synthetic pathway in the synthesis of juglone (**1**) (Wakamatsu *et. al.*, 1984; Oelgemöller *et. al.*, 2006).

1.4 Reported Methods for the Synthesis of Juglone (1)

Wakamatsu and co-workers were the first synthetic chemists to employ a catalytically oxygenation reaction of 1,5-dihydroxynaphthalene (**3**) to afford juglone (**1**) (Scheme 1.3) (Wakamatsu *et. al.*, 1984).

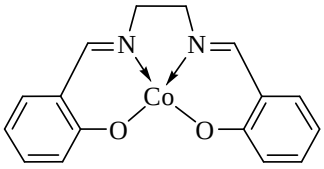


(**3**) (**1**) (**5**)

71% 14%

(i) O_2, salcomine (**4**) (10 mmol%), CH_3CN, rt, 30 mins

Scheme 1.3: The salcomine oxidation of 1,5-dihydroxynaphthalene (**3**), as documented by Wakamatsu and co-workers, 1984.

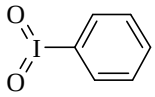


(**4**)

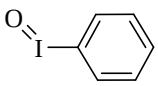
Salcomine (**4**) is a square planar cobalt(II) coordination complex. It functions as an oxygen carrier in the oxidation reaction by binding reversibly to molecular oxygen (Appleton, 1977). The salcomine-catalysed oxidation reaction only requires 10 mmol% of salcomine and a short reaction time (30 minutes). Moreover, it avoids the use of strong mineral acids like sulphuric acid and strong oxidising reagents like

chromic acid (Wakamatsu *et. al.*, 1984). However, the reaction is partially regioselective as a 1,2-quinone by-product (**5**) was also isolated from the reaction mixture (Scheme 1.3). Overall, the reaction furnished juglone (**1**) in 71% yield, while the 1,2-quinone by-product (**5**) was isolated in 14% yield.

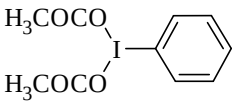
Barret and Daudon later utilised six hypervalent iodine compounds as oxidising agents in the oxidation of 1,5-dihydroxynaphthalene (**3**) to juglone (**1**) (Barret and Daudon, 1990). The iodine oxidants used in the study were iodylbenzene (**6**), iodosylbenzene (**7**), bis(acetoxy)iodobenzene (**8**), bis(trifluoroacetoxy)iodobenzene (**9**), bis(trifluoroacetoxy)iodopentafluorobenzene (**10**) and bis(trifluoroacetoxy)iodoperfluorohexane (**11**) (Figure 1.1).



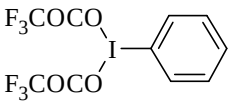
(**6**)



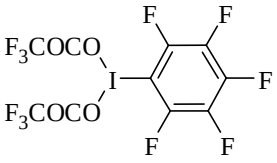
(**7**)



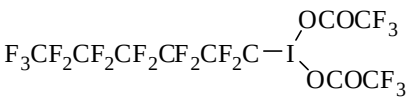
(**8**)



(**9**)



(**10**)



(**11**)

Figure 1.1: The hypervalent iodine compounds (**6**-**11**) which were used as oxidants in the work of Barret and Daudon, 1990.

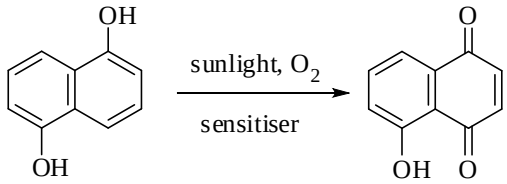
Iodine oxidant (**11**) gave an excellent reaction yield of 91% for the oxidation of **3** to juglone (**1**) (Entry 6, Table 1.1). However, approximately 3 equivalents of the oxidant (**11**) were required for the reaction. Moreover, the reaction time was much longer (1½ hours).

Table 1.1: The oxidation of **3** with various hypervalent iodine compounds (**6-11**) (Barret and Daudon, 1990).

Entry	Oxidizing agent (equivalent)*	Solvent	Reaction condition	Reaction time (h)	Yield (%)
1	(**6**) (0.51 equiv) and vanadyl acetylacetonate (0.04 equiv)	toluene	reflux	2	30
2	(**7**) (2.20 equiv)	CH_3CN: H_2O (2:1)	rt	-	47
3	(**8**) (2.20 equiv)	CH_3CN: H_2O (2:1)	0^oC	1	26
4	(**9**) (-)	-	-	-	58
5	(**10**) (-)	-	-	-	76
6	(**11**) (3.33 equiv)	CH_3CN: H_2O (2:1)	0^oC	1½	91

* The starting material (**3**) is taken as 1.00 equivalent.
"-"denotes not given.

In 2006, Oelgemöller and co-workers conducted the dye-sensitised solar photooxygenation reaction of 1,5-dihydroxynaphthalene (**3**) (Scheme 1.4) (Oelgemöller *et. al.*, 2006). They explored the used of various soluble and solid-supported dye sensitisers. The soluble dye sensitisers used were rose bengal (RB) and methylene blue (MB); while the solid-supported dye sensitisers used were rose bengal on Merrifield resin (RB_{MF}) and methylene blue on ion-exchange resin (MB_{IE}). A parabolic trough reactor equipped with aluminium mirrors was used to illuminate the reaction mixture with medium concentrated sunlight (Figure 1.2).



(**3**) (**1**)

Scheme 1.4: The dye sensitised solar photooxygenation of **3,** as reported by Oelgemöller and co-workers, 2006.

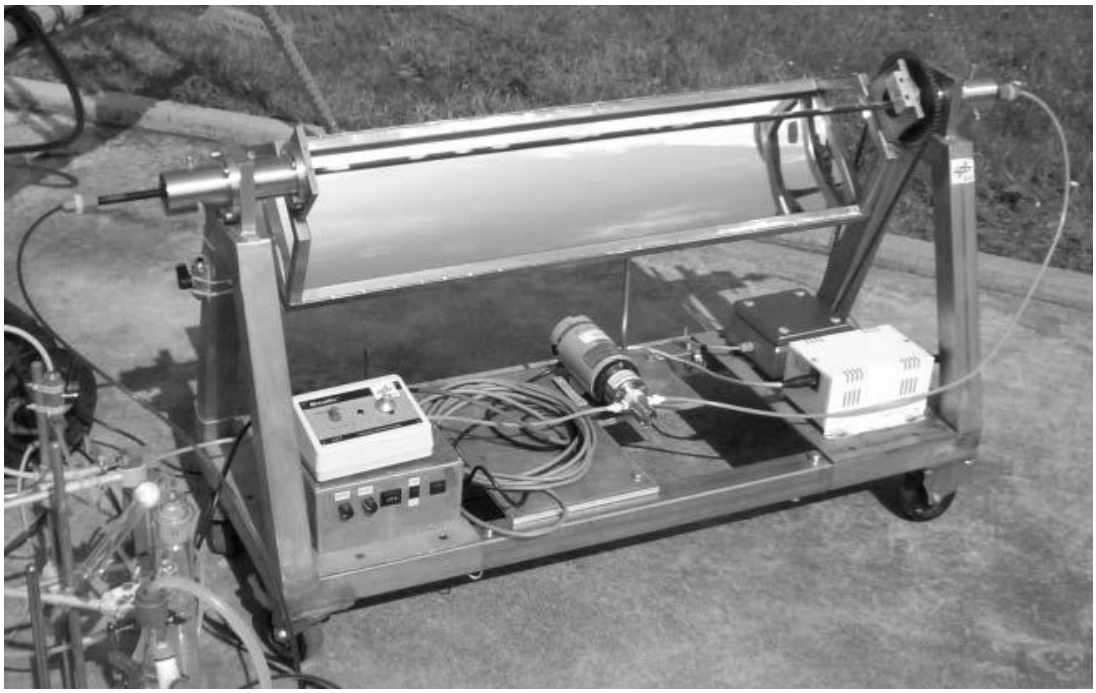


Figure 1.2: The parabolic trough solar reactor which was used by Oelgemöller and co-workers (Oelgemöller *et. al.*, 2006).

The highest reaction yield (79%) was achieved with rose bengal, RB as the dye sensitiser and acetone as the reaction solvent (Entry 6, Table 1.2).

Table 1.2: The dye sensitised solar photooxygenation of **3** with various soluble and solid-supported sensitisers (Oelgemöller *et. al.*, 2006).

	Entry 1	Entry 2	Entry 3	Entry 4	Entry 5	Entry 6
3 (g)	2.00	2.00	2.00	2.00	2.00	2.00
Sensitizer (g)	RB (0.10)	MB (0.05)	RB_{MF} (1.00)	RB_{MF} (0.50)	MB_{IE} (2.00)	RB (0.10)
Solvent (ml)	*i*-PrOH (250)	*i*-PrOH (250)	*i*-PrOH (250)	*i*-PrOH (250)	*i*-PrOH (250)	Acetone (250)
Illumination time (h)	4	4	4	4	4	4
Amount of photons recorded (mol)	2.0	2.1	1.3	1.7	2.0	2.1
Yield (%)	75	62	53	33	31	79

RB = rose bengal
MB = methylene blue
RB_{MF} = rose bengal on Merrifield resin
MB_{IE} = methylene blue on ion-exchange resin

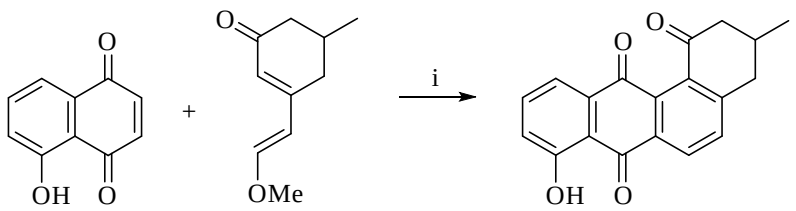
The dye sensitised photooxygenation reaction is a mild and environmentally benign method. It avoids the use of toxic solvents like acetonitrile and excessive chemicals. (Oelgemöller *et. al.*, 2006). Nevertheless, the reaction yield is considerably affected by weather and solar illumination conditions.

1.5 The Use of Juglone (1) and Its 2-Bromo Acetyl Derivative (14) as Starting Materials in Organic Synthesis

Juglone (**1**) plays a vital role in the syntheses of various naturally-occurring and biologically active quinones (Oelgemöller *et. al.*, 2006; Carreño and Urbano, 2005). Most of these quinones possess the juglone (**1**) core in their structural framework. Furthermore, the electron withdrawing carbonyl functionalities of the 1,4-benzoquinone moiety of juglone (**1**) also warrants it as a superior Diels-Alder dienophile (Sankararaman, 2005).

Ochromycinone (**12**) is an archetypal naturally-occurring quinone which possesses a juglone (**1**) core in its molecular structure. It is a member of the angucyclinone sub-class of antibiotics (Hasnah, 2005). Due to its bio-active significance, the total synthesis of ochromycinone (**12**) has attracted much attention from synthetic organic chemists (Krohn *et. al.*, 2004; Kaliappan and Ravikumar, 2007).

The Diels-Alder synthesis of racemic ochromycinone (**12**) was first successfully developed by Guingant and Barreto in 1987 (Guingant and Barreto, 1987). A stiochiometric equivalent of boron triacetate was employed to promote the Diels-Alder cycloaddition of juglone (**1**) and dienone (**13**) (Scheme 1.5).



(**1**) (**13**) rac-(**12**)

75%

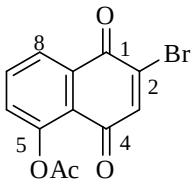
(i) $B(OAc)_3$ (1equivalent), CH_2Cl_2, 20°C

Scheme 1.5: The boron triacetate-promoted Diels-Alder synthesis of racemic ochromycinone (**12**) (Guingant and Barreto, 1987).

The Lewis acid-promoted Diels-Alder reaction of juglone (**1**) and dienone (**13**) afforded racemic ochromycinone (**12**) in 75% yield.

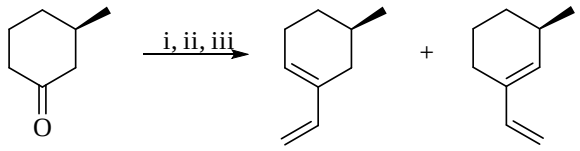
Guingant and Barreto's watershed achievement set the precedence for the syntheses of more complex and diverse angucyclinones via the Diels-Alder strategy (Hasnah,

2005; Carreño and Urbano, 2005). Juglone (**1**) and its 2-bromo acetyl derivative (**14**) have been used as the dienophilic reactants.



(**14**)

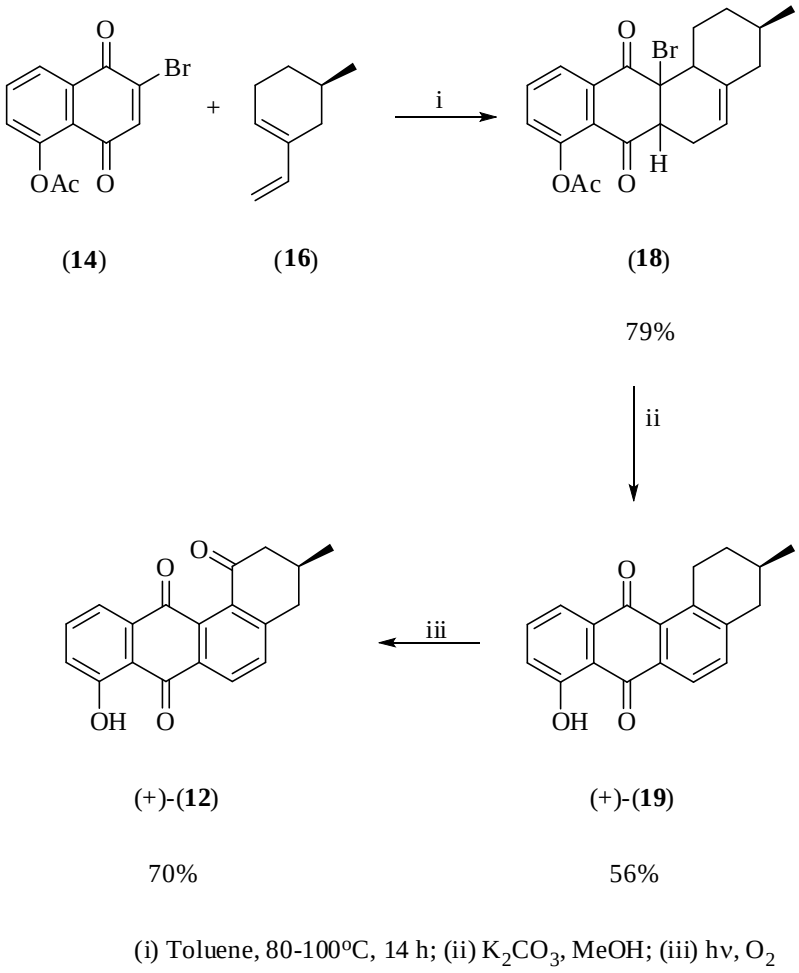
In 2004, Krohn and co-workers synthesised enantiomerically pure (+)-ochromycinone (**12**) from 2-bromo acetyl juglone (**14**) and chiral diene (**16**) (Scheme 1.7) (Krohn *et. al.*, 2004). They applied a non-Lewis acid promoted Diels-Alder cycloadditon for their asymmetric work. However, both of the reactants, **14** and **16** were refluxed at temperatures of 80-100^{o}C for 14 hours. The chiral diene (**16**) was prepared from commercially available (*R*)-(+)-3-methylcyclohexanone (**15**) in three synthetic steps (Scheme 1.6).



(**15**) (**16**) (**17**)

(i) LTMP, THF; (ii) $(Tf)_2O$, -78^{o}C; (iii) $Pd(PPh_3)_4$, $SnBu_3$

Scheme 1.6: The synthesis of chiral diene (**15**) (Krohn *et. al.*, 2004).



Scheme 1.7: The asymmetric Diels-Alder synthesis of (+)-ochromycinone (**12**), as reported by Krohn and co-workers, 2004.

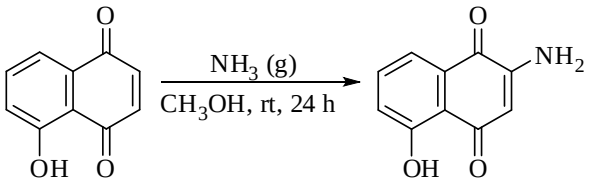
The Diels-Alder cycloadduct (**18**) was obtained in 79% yield from **14** and **16**. The ochromycinone precursor (**19**) was obtained in 56% yield from **18**. Enantio-pure ochromycinone (**12**) was obtained in an overall yield of 31% from **14** and **16**.

2-Bromo acetyl juglone (**14**) was the choice dienophile in the work of Krohn and co-workers due to the utilisation of the slightly labile chiral diene (**16**). The absence of an electron donating group on **16** warrants it a less reactive diene as compared to dienone (**13**) which was used in the work of Guingant and Barreto (Krohn *et. al.*, 2004). However, the bromine substituent of **14** is known to efficiently direct the

regioselectivity of the Diels-Alder reaction (Krohn *et. al.*, 2004; Carreño and Urbano, 2005).

Thus far, a non-Lewis acid promoted Diels-Alder synthesis of the ochromycinone precursor (**19**) involving juglone (**1**) has not been carried out at room temperature.

Apart from that, Arnone and co-workers reported a direct amination method involving juglone (**1**) as the reactant (Scheme 1.8) (Arnone *et. al.*, 2007).



(**1**) (**20**)

20%

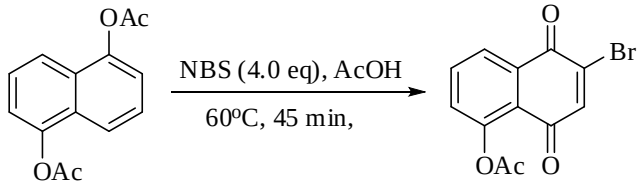
Scheme 1.8: The direct amination of juglone (**1**), as described by Arnone and co-workers, 2007.

The reaction afforded 2-aminojuglone (**20**) in a modest 20% yield. Arnone and co-workers proposed that the hydroxyl functional of juglone (**1**) controls the regioselectivity of the reaction (Arnone *et. al.*, 2007). Nevertheless, a direct nitration has never been attempted before on juglone (**1**).

1.6 The Syntheses of Juglone Derivatives from Non-juglone Starting Materials

The synthesis of the aforementioned 2-bromo acetyl juglone (**14**) (Section 1.5) is described by Heinzman and Grunwell (Scheme 1.9) (Heinzman and Grunwell,

1980). The single-step procedure employed 1,5-diacetoxynaphthalene (**21**) as the starting material and *N*-bromosuccinimide, NBS as the brominating reactant.



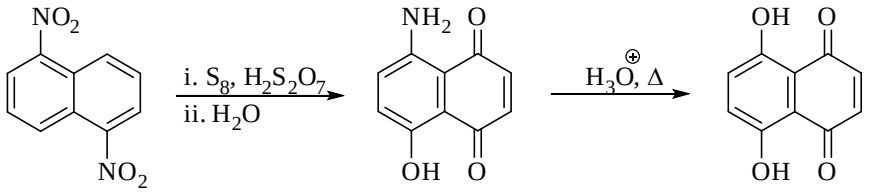
(**21**) (**14**)

>90%

Scheme 1.9: The synthesis of 2-bromo acetyl juglone (**14**), as documented by Heinzman and Grunwell, 1980.

The efficient, regioselective reaction furnished 2-bromo acetyl juglone (**14**) in a high yield of over 90%. Heinzman and Grunwell observed that the bromine atom selectively substitutes at the position which is '*ortho*' to the acetoxy substituent in the reactant.

8-Aminojuglone (**23**) is also synthetically prepared from a non-juglone starting material. It was first isolated as an intermediate from the synthetic pathway of 1,5-dinitronaphthalene (**22**) to naphthazarin (**24**) (Scheme 1.10) (Fariña *et. al.*, 1985).

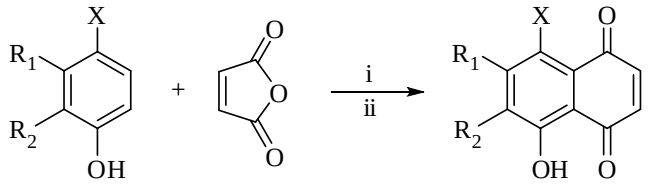


(**22**) (**23**) (**24**)

Scheme 1.10: The synthesis of naphthazarin (**24**) (Fariña *et. al.*, 1985).

Fariña and co-workers obtained 8-aminojuglone (**23**) in a moderate yield of 65% from **22**. Elemental sulphur was used to reduce the nitro groups while oleum, $H_2S_2O_7$ was used as the strong oxidising agent (Fariña *et. al.*, 1985).

In 2007, Mahapatra and co-workers synthesised and assayed 8-halo-5-hydroxy-1,4-naphthoquinone derivatives (**29**-**32**) for their anti-tubercular activities (Mahapatra *et. al.*, 2007). The 8-halojuglone derivatives (**29**-**32**) were synthesised from 4-halogen substituted phenols (**25**-**28**) and maleic anhydride (**33**) via a solvent-free, rapid Friedel-Crafts acylation reaction (Scheme 1.11). All of the 8-halojuglone derivatives (**29**-**32**) were obtained in moderate yields of 25-30% (Table 1.3).



(**25**-**28**) (**33**) (**29**-**32**)

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

	X	R_1	R_2		X	R_1	R_2
(**25**)	F	Me	H	(**29**)	F	Me	H
(**26**)	Cl	Me	H	(**30**)	Cl	Me	H
(**27**)	Br	Me	H	(**31**)	Br	Me	H
(**28**)	Cl	H	Me	(**32**)	Cl	H	Me

Scheme 1.11: The Friedel-Crafts acylation of various 4-halogen substituted phenols (**25**-**28**) with maleic anhydride (**33**), as reported by Mahapatra and co-workers, 2007.

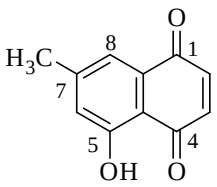
Table 1.3: The percentage yields of the 8-halo-5-hydroxy-1,4-naphthoquinone derivatives (**29**-**32**) as obtained by Mahapatra and co-workers, 2007.

Starting material	Product	Yield (%)
25	**29**	25
26	**30**	30
27	**31**	27
28	**32**	30

1.7 The Biological Activities of Juglone (1) and Some of Its Derivatives

As mentioned earlier (Section 1.2), the antimicrobial properties of juglone (**1**) has been well known and applied in North American folkloric medicine (Clark *et. al.*, 1990). Clark and co-workers evaluated the antifungal activity of juglone (**1**) with eight commercial antifungal agents: clotrimazole, triacetin, tolnaftate, griseofulvin, zinc undecylenate, selenium sulphide, liriodenine and liriodenine methiodide. The study showed that juglone (**1**) exhibits moderate antifungal activity, with an efficacy that is comparable with zinc undecylenate and selenium sulphide.

Cai and co-workers later identified Juglone (**1**) and its natural methyl derivative, 7-methyljuglone (**34**) as two of the six active compounds which are present in the methanolic extract of *Diospyros lycioides* (Cai *et. al.*, 2000). *Diospyros lycioides* is a Namibian plant which is commonly used for tooth cleaning. Cai and co-workers tested the isolated compounds against four oral bacterial strains; two of which were Gram-positive bacteria (*Streptococcus mutans* and *Streptococcus sanguis*) and the reamaining two were Gram-negative bacteria (*Porphyromonas gingivalis* and *Prevotella intermedia*). Their results showed that juglone (**1**) displayed the most significant antibacterial activity against all the oral bacteria tested.



(**34**)

Apart from that, Juglone (**1**) has also been reported to demonstrate various biological activities like anti-tumour, antihypertensive and enzyme inhibition activities (Martinez and Benito, 2005). Furthermore, juglone (**1**) and its synthetic derivatives have also been shown to display both larvicidal and molluscicidal activities (Ribeiro *et. al.*, 2009).

Bhargava and Westfall were one of the earliest researchers to report on the anti-tumour activity of juglone (**1**) (Bhargava and Westfall, 1968). They assayed extracts of black walnut (*Juglans nigra*) on transplanted mammary adenocarcinoma tumours in mice. The results showed that the juglone constituent of the extracts was able to depress the mammary adenocarcinoma tumour growth rate significantly.

In 1998, Sugie and co-workers reported the effect of juglone (**1**) on male rats which were affected with intestinal tumour (Sugie *et. al.*, 1998). The rats were injected with azoxymethane; a known carcinogen which induces colon carcinoma. Based on the results obtained, Sugie and co-workers suggested juglone (**1**) as a promising chemopreventive agent for human intestinal tumours.

Very recently, Aithal and co-workers discovered that juglone (**1**) implicates the cell death of melanoma tumour cells by both apoptosis (programmed cell death) and necrosis (premature cell death) processes (Aithal *et. al.*, 2009). They also noted that

juglone (**1**) exhibits potential cytotoxic (cell toxicity) and genotoxic (gene toxicity) effects on melanoma tumour cells.

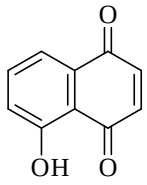
In addition, Neuhauscarlslie and co-workers observed that juglone (**1**) showed moderate antihypertensive activity (Neuhauscarlislie *et. al.*, 1997). An *in vitro* guinea pig papillary muscle model was employed by Neuhauscarlslie and co-workers for their study.

A detailed study on the interaction of juglone (**1**) with three commercial antihypertensive drugs; verapamil, nifedipine and diltiazem was later conducted by Bhosale and co-workers (Bhosale et. al., 1999). Bhosale and co-workers carried out their study on an isolated frog heart.

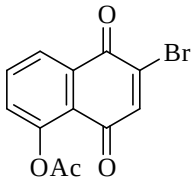
Ilina and co-workers reported that juglone (**1**) moderately inhibits the HIV-1 reverse transcriptase, RT enzyme (Ilina et. al., 2002; Martinez and Benito, 2005). The RT enzyme is now a major target for the search of new anti-HIV drugs (Ilina et. al., 2002).

Juglone (**1**) also demonstrates significant inhibition activity against three essential enzymes which are involved in the metabolic pathways of the *Helicobacter pylori* bacteria (Kong *et. al.*, 2008). The potent, multi-targeted inhibition of juglone (**1**) against these enzymes makes it a prospective lead compound for the development of peptic ulcer drugs.

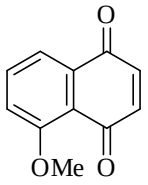
Ribeiro and co-workers recently assayed juglone (**1**) and 2-bromo acetyl juglone (**14**) together with twelve other known natural and synthetic naphthoquinones, (**35-46**) for their larvicidal and molluscicidal activities (Figure 1.4) (Ribeiro *et. al.*, 2009). The larvicidal activities were assayed against *Aedes aegypti* while the molluscicidal activities were assayed against the *Biomphalaria glabrata* snail species. They noted that naphthoquinones are generally more active as a molluscicide than as a larvicide. They also observed that juglone (**1**) and its synthetic derivatives **35**-**41** displayed noteworthy larvicidal and molluscicidal activities. The brominated juglone derivatives **14**, **36** and **39** were among the remarkable larvicides and molluscicides.



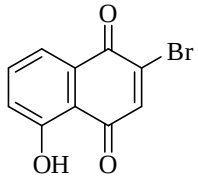
(**1**)



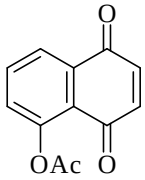
(**14**)



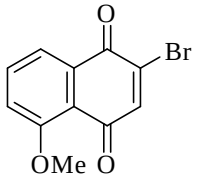
(**35**)



(**36**)



(**37**)



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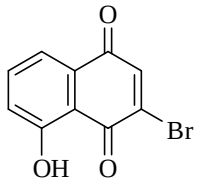

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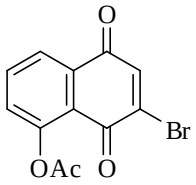
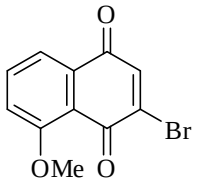
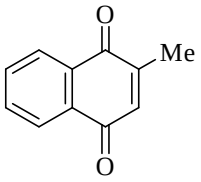


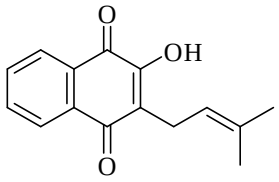
(**40**)



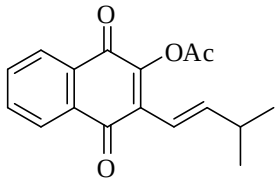
(**41**)



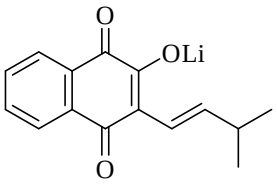
(**42**)



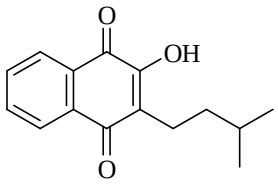
(**43**)



(**44**)



(**45**)



(**46**)

Figure 1.3: The natural and synthetic naphthoquinoines which were used in the larvicidal and molluscicidal studies of Ribeiro and co-workers, 2009.

1.8 Research Objectives

In view of all the reported literature hitherto, there still remains a pressing need to synthesise various juglone derivatives from both juglone (**1**) and non-juglone starting materials. These synthetic juglone derivatives can further be subjected to biological assays.

The present work consists of three main research objectives, notably:

I. To synthesise juglone derivatives from juglone (**1**) and non-juglone starting materials;
II. To characterise all synthetic compounds;
III. To evaluate the antibacterial activities of all synthetic compounds against Gram-positive and Gram-negative bacteria.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Chemicals and Materials

2.1.1 Chemicals

The following is a list of commercial chemicals and reagents used for the synthesis, and characterisation of all titled compounds. All solvents used were of analytical grade reagents. Unless otherwise stated, all solvents and reagents were used without further purification.

1. Acetone, AR grade (QRëC)
2. Aluminium chloride, anhydrous powder (Merck, Germany)
3. Benzene, chem. pure grade (Systerm, Malaysia)
4. Benzophenone, 99% (Acros Organics, Belgium)
5. 4-Bromo-3-methylphenol, 98% (Sigma-Aldrich, USA)
6. p-Bromophenol (TCI, Japan)
7. Calcium chloride, anhydrous granular (R & M Chemicals, UK)
8. Calcium hydride (Riedel-de Haën, Germany)
9. 4-Chloro-3-ethylphenol, 97% (Sigma-Aldrich, USA)
10. Chloroform, AR grade (QRëC)
11. Chloroform-d, for NMR, 99.8% D, stabilised with silver foil, 0.03 v/v % TMS (Acros Organics, Belgium)
12. 4-Chloro-3-methylphenol, for synthesis (Merck, Germany)
13. 4-Chlorophenol, for synthesis (Merck, Germany)
14. Cyclohexanone, extra pure (Merck, Germany)
15. 1,8-Diazabicyclo[5.4.0]undec-7-ene, ≥97.5% (Sigma-Aldrich, USA)

16. Dichloromethane, AR grade (QRëC)
17. Diethyl ether, AR grade (Lab-Scan, Thailand)
18. Ethanol, 99.7%, denatured, AR grade (QRëC)
19. Ethyl acetate, AR grade (Fischer Scientific, UK)
20. Hydrochloric acid, 37%, AR grade (QRëC)
21. Juglone (5-hydroxy-1,4-naphthoquinone), ≥95% (Fluka, Switzerland)
22. Lithium aluminium hydride, powder, for synthesis (Merck, Germany)
23. Magnesium sulphate, dried (BDH Chemicals, England)
24. Maleic anhydride, 99% (Acros Organics, Belgium)
25. 3-Methylcyclohexanone, 97% (Sigma-Aldrich, USA)
26. *N*-Methylmorpholine-*N*-oxide, 97% (Aldrich, USA)
27. Molecular sieves type 4A, powder (Fluka, Switzerland)
28. Nickel(II) nitrate hexahydrate, crystallised, ≥98% (Fluka, Switzerland)
29. Petroleum ether 60-80^{o}C, AR grade (Lab-Scan, Thailand)
30. Potassium bromide, spectrograde powder (International Crystal Labs, USA)
31. Sodium chloride, AR grade (Fisher Scientific, UK)
32. Sodium hydride, 60% suspension in paraffin oil, for synthesis (Merck, Germany)
33. Sodium lumps (Riedel-de Haën, Germany)
34. Tetrahydrofuran, AR grade (Lab-Scan, Thailand)
35. Tetrapropylammonium perruthenate, 97% (Aldrich, USA)
36. Toluene, AR grade (R&M Chemicals, UK)
37. *p*-Toluene Sulphonic acid, monohydrate (R&M Chemicals, UK)
38. Triethylamine, for synthesis (Merck, Germany)
39. Triethyl phosphonoacetate, ≥97% (Fluka, Switzerland)

40. Trimethylsilyl chloride, for synthesis (Merck, Germany)

41. Zinc chloride, AR grade (R&M Chemicals, UK)

2.1.2 Materials

The following is a list of materials used for the extraction and separation of all titled compounds.

1. Cellulose extraction thimbles, 32mm (internal diameter) x 100mm (Whatman Paper, England)
2. Silica gel 60, for column chromatography, (0.040-0.063 mm), (230-400 mesh ASTM) (Merck, Germany)
3. TLC silica gel 60 F254, aluminium sheets, 20cm x 20cm (Merck, Germany)

2.2 General Synthetic Experimental Methods

2.2.1 Reaction Monitoring

Thin Layer Chromatography (TLC) was employed to monitor the progress of all reactions conducted for more than 2 minutes. TLC monitoring was conducted at intervals of 30 minutes. The starting material of each reaction was spotted on the TLC plates for comparison (Landgrebe, 2005). Solvents systems used for developing the TLC plates were ethyl acetate: *n*-hexane (1:25), (1:19), (1:9), chloroform: *n*-hexane (1:9), (1:4) and toluene: *n*-hexane (1:1). The TLC plates were visualised under ultraviolet radiation at wavelengths of 254 and 365 nm by using a 230V, 8W UV Vilber Lourmat lamp.

2.2.2 Non-air-sensitive Reactions

All non-air-sensitive reactions were conducted at normal atmospheric pressure. All reaction flasks were affixed with a calcium chloride drying tube.

2.2.3 Air-sensitive Reactions

Unless otherwise stated, all air-sensitive reactions were conducted under a closed nitrogen atmosphere. Prior to reaction, all glassware were pre-dried overnight in an oven at 120^{o}C. The pre-dried glassware was assembled hot and left to cool under a steady stream of nitrogen (Furnis *et. al.*, 1989).

Reaction solvents and liquid reagents were pre-dried overnight and distilled under a steady flow of nitrogen. The freshly distilled solvents and reagents were then used immediately. Diethyl ether, tetrahydrofuran and toluene were dried and distilled over sodium wire and benzophenone. For the distillation of every 100.0 ml of the solvent, 1.00 g of sodium and 1.00 g of benzophenone were used (Furnis *et. al.*, 1989). Benzophenone functions as a colour indicator. A dark intense violet colour indicates that the solvent is dry. In addition, chloroform, dichloromethane, triethylamine and trimethylsilyl chloride were dried and distilled over granular calcium hydride. For the distillation of every 10.0 ml of the solvent or reagent, 0.05 g of calcium hydride was used.

All dry solvents and reagents were transferred into the reaction flask by using standard syringe techniques (Aldrich, 1997). This involved the use of glass syringes equipped with 12 inch long, 20 gauge pliable stainless steel needles. Rubber septa were used to facilitate the passage of the syringe needle into the reaction flask.

2.2.4 Column Chromatography Separation

Reaction mixtures (analytes) were separated by silica gel column chromatography. The ratio of silica gel to analyte used was approximately 20-25: 1 (Zubrick, 2001). All columns were packed wet under a positive pressure of nitrogen. *n*-Hexane was used in the packing of all columns.

Components of the analyte were separated and eluted by gradient or stepwise elution method. The polarity of the solvent mixture (eluent) was gradually increased with elution (Landgrebe, 2005). The selection of the eluent was based on TLC analyses. A constant nitrogen flow was used as a positive pressure to elute the column.

The fractions were separately collected in volumes of 15.0-20.0 ml. The composition of each fraction was analysed by TLC. Fractions with similar compositions were combined and concentrated under vacuum. The entire column chromatography process was repeated until a pure compound was obtained.

2.2.5 Recrystallisation

Single-solvent recrystallisation was also employed for the purification of all solid products. The recrystallisation solvent is one which completely dissolves the sample when hot but partially dissolves it at room temperature (Zubrick, 2001). Conical flasks were used for all recrystallisations.

The solid sample was first dissolved in the hot recrystallisation solvent. Next, the solution was filtered and the filtrate was then concentrated until 1/2-1/3 of its original volume (Landgrebe, 2005). The hot, concentrated solution was left to cool