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# Antifungal and larvicidal cordiaquinones from the roots of *Cordia curassavica*

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**Abstract**

In addition to the known cordiaquinones A and B, two novel meroterpenoid naphthoquinones, named cordiaquinones J and K, have been isolated from the roots of *Cordia curassavica*. Their structures were elucidated by spectrometric methods including EI, D/CI mass spectrometry, $^{1}H$, $^{13}C$ and 2D-NMR experiments. The four naphthoquinones demonstrated antifungal activities against *Cladosporium cucumerinum*, *Candida albicans* and toxic properties against larvae of the yellow fever-transmitting mosquito *Aedes aegypti*. © 2000 Elsevier Science Ltd. All rights reserved.

*Keywords:* *Cordia curassavica*; Boraginaceae; Naphthoquinones; Cordiaquinones; Antifungal activity; Larvicidal activity

## 1. Introduction

*Cordia curassavica* Roemer and Schultes (Boraginaceae) is a 2 to 4 m high shrub widely spread in Central America and in the Caribbean Islands. In Panama, this plant is frequently encountered in dry areas below 2000 m (D'Arcy, 1987). The leaves of *C. curassavica* are used as a decoction for colds, flu, pneumonia or cough in Trinidad (Morton, 1981) and for colds, flu, cough, headache and parasitic diseases in Nicaragua (Barett, 1998). However, the roots are not reported to be part of any traditional medicine.

In our continuing search for new bioactive products from Panamanian plants, we previously described antifungal and larvicidal compounds isolated from the roots of *C. linnaei* Stearn (Ioset, Marston, Gupta & Hostettmann, 1998). Following this work, several other Panamanian species of the genus *Cordia* were investigated for their biological properties. Among them, the dichloromethane extract of the roots of *C. curassavica* was found to give marked activities against the phytopathogenic fungus *Cladosporium cucumerinum* (Homans & Fuchs, 1970), the yeast *Candida albicans* (Rahalison, Hamburger, Monod, Frenk & Hostettmann, 1991) and the larvae of the yellow fever-transmitting mosquito *Aedes aegypti* (Cepleanu, 1993). As no chemical or biological studies have previously been reported on this plant, the investigation of the roots of *C. curassavica* was undertaken.

## 2. Results and discussion

An analysis by HPLC coupled with diode array detection (DAD) of the crude dichloromethane extract showed the presence of four major compounds. According to their UV spectra, these compounds seemed to be naphthoquinone derivatives (Thomson, 1971) and cordiaquinone B (**1**) was identified as one of the constituents of the extract by HPLC-UV/DAD comparison with an authentic sample.

The extract was separated by column chromatog-

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raphy on silica gel. Compound **2** was obtained after further fractionation on Sephadex LH-20 and final purification on a silica gel column, while isolation of compounds **3** and **4** required fractionation on a diol column using low pressure liquid chromatography.

The structures of compounds **2**–**4** were determined by $^{1}$H-, $^{13}$C-NMR spectroscopy combined with EI and D/CI mass spectrometry.

Similar patterns were observed for the signals of the protons on the $sp^2$ hybridised carbons in the $^{1}$H-NMR spectra of compounds **2**–**4**: the signals of two protons appeared as a singlet at about $\delta$ 7.00 (H-2, H-3) and the signals of three protons arranged in a AMX system were seen between $\delta$ 7.50 and $\delta$ 8.00. These signals are characteristic of a 6-substituted 1,4-naphthoquinone (Ioset et al., 1998). The analysis of the $^{13}$C-NMR spectra of compounds **2**–**4** confirmed the presence of this substitution pattern on the naphthoquinone moiety and indicated that the non-naphthoquinone part of these molecules included eleven carbon atoms.

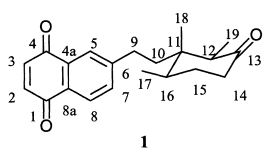
In the EI-MS of **2** a peak at $m/z$ 326 $[M]^{+}\cdot$ and in the D/CI-MS a peak at $m/z$ 344 $[M + NH_4]^{+}$) were in agreement with a molecular formula of $C_{21}H_{26}O_3$. After subtraction of the naphthoquinone unit, a partial elemental composition of $C_{11}H_{21}O$ could be calculated for the substituent whose structure was deduced from the combined analysis of the COSY, HSQC and HMBC spectra to allow the identification of cordiaquinone A. This compound was previously isolated from the roots of *C. corymbosa* G. Don. (Bieber, Messana, Lins, Da Silva Filho, Chiappeta & De Mello, 1990). A comparison of the UV, MS, $^{1}$H- and $^{13}$C-NMR data obtained experimentally for compound **2** showed they were in perfect agreement with those of cordiaquinone A.

A molecular formula of $C_{21}H_{24}O_3$ was suggested for compound **3** by the $^{13}$C-NMR spectrum and the presence of peaks at $m/z$ 324 $[M]^{+}\cdot$ in the EI-MS and at $m/z$ 342 $[M + NH_4]^{+}$ in the D/CI-MS. These MS analyses predicted the existence of two degrees of unsaturation in the substituent. These could be explained by the cyclisation of the non-naphthoquinone moiety and the presence on this cycle of a oxygen bridge linking the carbon atoms with signals at $\delta$ 86.4 (C-13) and $\delta$ 86.9 (C-11) in the $^{13}$C-NMR spectrum. The chemical shifts of these signals were indeed consistent with those found in the literature for such a structure (Marco, Sanz, Yuste, Carda & Jakupovic, 1991). A careful study of the HSQC, HMBC and COSY spectra allowed the identification of partial structures $CH_2$–$CH_2$–CH ($\delta$ 36.8, 30.3 and 56.2; C-9, C-10 and C-11) and CHO–$CH_2$–$CH_2$ ($\delta$ 86.4, 26.3 and 39.7; C-13, C-14 and C-15) that confirmed the position of oxygen attachment to the ring. Long distance correlations between carbon signals at $\delta$ 126.3, 150.7, 134.7 (C-5, C-6, C-7) and protons resonances centered at $\delta$ 2.80 (H-9a,b) showed that the first of these two partial structures was connected to the naphthoquinone part of the molecule. Other long distance correlations were observed between the carbon signals at 56.2, 46.0, 86.4 (C-11, C-12, C-13) and the signals at $\delta$ 1.12 (3H, *s*, $CH_3$-18) and $\delta$ 1.08 (3H, *s*, $CH_3$-19). Such results indicated that both methyl groups should be attached to C-12. These data led to the structure identification of compound **3**, which was found to be, to our knowledge, a new natural product named cordiaquinone J. A boat conformation was established for the cyclohexane ring because of the presence of the oxygen bridge.

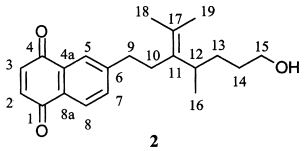
A molecular formula of $C_{21}H_{22}O_3$ was calculated for compound **4** after observation of ions at $m/z$ 322 $[M]^{+}\cdot$ in EI-MS and $m/z$ 340 $[M + NH_4]^{+}$ in D/CI-MS. The identification of two methyl, five methylene and one methine groups on the $C_{11}H_{17}O$ substituent was enabled by the study of the DEPT spectrum, the three last carbons being consequently quaternary carbons ($C_Q$). Among them, a ketone moiety was identified at $\delta$ 213.6 (C-13) and a methylene group was characterised by the presence of $^{1}$H-NMR signals at $\delta$ 4.95 (1H, *s*, H-17a) and $\delta$ 5.14 (1H, *s*, H-17b) connected with a $^{13}$C-NMR resonance at $\delta$ 113.9 (C-17) in the HSQC spectrum. Finally, the structure elucidation of the substituent was achieved by a combination of COSY, HSQC and HMBC spectra that gave evidence for $CH_2{=}C_Q$–$CH_2$–$CH_2$ (113.9, 146.2, 31.1 and 38.1; C-17, C-16, C-15 and C-14) and $CH_2$–$CH_2$–CH ($\delta$ 34.6, 29.8 and 56.6; C-9, C-10 and C-11) sequences. The latter chain was proved to be linked to the naphthoquine part of the compound by observation of long distance heteronuclear correlations between carbon C-9 ($\delta$ 34.6) and protons H-5 (1H, $\delta$ 7.84, *d*, $J$ = 1.9 Hz) and H-7 (1H, $\delta$ 7.71, *dd*, $J$ = 8.0, 1.9 Hz). Methyl groups at $\delta$ 0.99 and 1.18 ($CH_3$-18 and 19) were found to be attached to C-12 from HMBC correlations between these groups and $^{13}$C-NMR signals $\delta$ 56.6 and 38.0 (C-11 and C-12). Finally, the ketone function was established at the C-13 position because of HMBC correlations noted between this carbonyl group and $CH_3$-18 ($\delta$ 0.99), $CH_3$-19 ($\delta$ 1.18), H-14b ($\delta$ 2.24), H-11 ($\delta$ 2.33), H-15b ($\delta$ 2.60) H-14a and H-15a ($\delta$ 2.66). The structure of the non-naphthoquinone part of the molecule was confirmed by comparison of its spectroscopic data with those of nassauvirevolutin B, a coumarin derivative isolated from *Nassauvia revoluta* D. Don (Asteraceae) (Bittner, Jakupovic, Bohlmann & Silva, 1988). For steric reasons, the H-11 proton should occupy an axial position on the cyclohexanone ring while the cumbersome naphthoquinone group is in the equatorial position. To our knowledge, compound **4** has not previously been reported in literature. It was thus called cordiaquinone K.

The compounds isolated from the roots of *Cordia curassavica* were found to be equally active against

*Cladosporium cucumerinum* and *Candida albicans* in both bioautographic and dilution assays (Rahalison, Hamburger, Monod, Frenk & Hostettmann, 1994) and were almost equipotent to nystatin, a commercially available antifungal agent. Dilution tests were also performed on *Aedes aegypti* (Cepleanu, 1993) and showed similar larvicidal effects for all four cordiaquinones. Their activities were a little lower than the reference compound, plumbagin. The results of the three bioassays are reported in Table 1.



1



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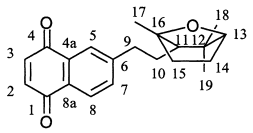

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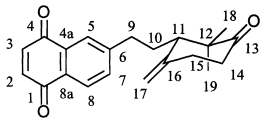

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## 3. Experimental

### 3.1. General

$^1$H and $^{13}$C NMR: Varian Unity Inova NMR instrument, Palo Alto, CA, USA. $^1$H- and $^{13}$C-NMR spectra were recorded in acetone-$d_6$ at 500.00 and 125 MHz, respectively. TMS: int. standard. UV: Varian DMS 100S UV-VIS spectrophotometer. UV spectra were recorded in MeOH. $[\alpha]_D$: Perkin-Elmer-241 polarimeter. TLC: silica gel 60 $F_{254}$ Al sheets (Merck) using petrol ether–EtOAc 1:1 and diol HPTLC plates (Merck) with petrol ether-acetone 9:1. CC: silica gel (63–200 μm; 750 × 65 mm i.d. and 63–200 μm; 400 × 20 mm i.d., Merck), Sephadex LH-20 (600 × 40 mm i.d., Pharmacia). LPLC: Lobar Lichroprep diol (40–63 μm; 270 × 25 mm i.d.; Merck). EI-MS and D/CI-MS: Finnigan MAT TSQ-700 triple stage quadrupole instrument. The identification of cordiaquinone B in the extract and the control of the purity of the isolated compounds were performed by HPLC/UV/DAD with a Nova-Pak RP-18 column (4 μm; 250 × 3.9 mm i.d.; Waters) using an MeOH–$H_2O$ gradient (35:65 → 100:0) in 30 min. The detection was performed at 210 and 254 nm.

### 3.2. Plant material

Roots of *Cordia curassavica* were collected in March 1997 at La Mesa, Altos de Campana National Park, Panama. A voucher is deposited at the National Herbarium of Panama (FLORPAN 2779) and at the Institut de Pharmacognosie et Phytochimie, Lausannne, Switzerland (No. 97030).

### 3.3. Extraction and isolation

Air-dried powdered roots of *Cordia curassavica* (668 g) were extracted at room temperature with dichloromethane to afford 16.9 g of extract. The dichloromethane extract was first fractionated by column chromatography on silica gel with a petrol ether–EtOAc gradient (4:1 → 0:1) giving frs 1–20. Fr. 16 was separated on Sephadex LH-20 with $CHCl_3$–MeOH (1:1). Further purification on a silica gel column using petrol ether-EtOAc (1:1) yielded 38 mg of compound **2**. Compound **3** (76 mg) and **4** (15 mg) were obtained after separation of fr. 7 on a diol LPLC system using petrol ether–acetone (9:1) as the mobile phase.

### 3.4. Biological assays

#### 3.4.1. Sample preparation for bioautographic assays

Geometric dilutions were obtained from freshly prepared stock solutions of isolated and reference compounds at a concentration of 1 mg/ml in an appropriate solvent. 10 μl of these solutions were applied on the TLC plates using graduated capillaries.

Table 1
Antifungal and larvicidal activities for compounds **1**–**4**

| Compound | *Cladosporium cucumerinum*[a] | *Cladosporium cucumerinum*[b] | *Candida albicans*[a] | *Candida albicans*[b] | *Aedes aegypti*[c] |
|---|---|---|---|---|---|
| **1** | 0.5 | 3 | 0.5 | 3 | 25 |
| **2** | 1 | 3 | 0.5 | 3 | 12.5 |
| **3** | 0.5 | 1.5 | 0.5 | 3 | 25 |
| **4** | 0.5 | 3 | 0.5 | 3 | 12.5 |
| Nystatin | 0.2 | 1 | 0.1 | 1 | – |
| Plumbagin | – | – | – | – | 6.25 |

[a] Minimal amount (μg) of compound to inhibit growth on a silica gel TLC plate.
[b] Minimal inhibition concentration MIC (μg/ml) of compound in a dilution assay.
[c] Minimal concentration (μg/ml) of compound required to kill all the larvae after 24 h.

*3.4.2. Bioautographic assays*

Direct bioautography with *C. cucumerinum*: after application of the samples on a silica gel 60 $F_{254}$ Al sheet (Merck), the TLC plates was developed in petrol ether–EtOAc 1:1 solvent system and thoroughly dried for complete removal of solvents. The plate was then sprayed with a suspension of *C. cucumerinum* in a nutritive medium and incubated for 2–3 days in polystyrene boxes with a moist atmosphere. Clear inhibition zones appeared against a dark grey background. Nystatin (Sigma) was used as reference compound.

Agar overlay bioautography with *C. albicans*: after application of the samples on a silica gel 60 $F_{254}$ glass plate (Merck), this was developed using a petrol ether–EtOAc 1:1 solvent system and thoroughly dried for complete removal of solvents. An inoculum of yeast ($\approx 10^7$ cells/ml) in molten malt agar (Biokar Diagnostics) was distributed over the plates. The medium solidified as a thin layer ($\approx 1$ mm) and the plates were then incubated overnight at 30°C in polystyrene boxes with a moist atmosphere. Inhibition zones were visible after spraying with an aqueous solution of methylthiazolyltetrazolium bromide (MTT) (2.5 mg/ml). Active compounds appeared as clear spots against a purple background. Nystatin (Sigma) was used as reference compound.

*3.4.3. Dilution assays*

Geometric dilutions of the isolated and reference compounds were freshly prepared in DMSO from stock solutions at 3 mg/ml (in DMSO). For *Candida albicans*, aliquots of these dilutions were added to malt agar medium (Biokar Diagnostics) inoculated with *C. albicans* ($10^5$ cells/ml) and distributed in hermetically closed 96-well plates. The final concentration of DMSO in the assay did not exceed 2%. Incubation was at 30°C for 24 h. In the case of *Cladosporium cucumerinum*, aliquots of the dilutions were added to Sabouraud agar medium (Biokar Diagnostics) which was distributed in 24-well plates. A suspension of *C. cucumerinum* in distilled water was spread over the agar. Well plates were closed hermetically and incubated at 30°C for 24 h. Control experiments without test compounds were carried out for verification of fungal growth. All samples were measured in duplicate. Nystatin (Sigma) was used as reference compound.

*3.4.4. Larvicidal assays*

Geometric dilutions of the isolated and reference compounds were freshly prepared from stock solutions at 10 mg/ml in DMSO. Aliquots of these dilutions were added to a graduated tube containing approximately 20 instar II larvae of *Aedes aegypti* in tap water and the final volume was adjusted to 10 ml. The tubes were incubated in darkness at 26–28°C for 24 h. Larvae lethality was observed under lab light. All samples were measured in duplicate. Plumbagin (Roth) was used as reference compound.

*3.5. Cordiaquinone A (**2**)*

$C_{21}H_{26}O_3$. P.M. 326. Dark yellow gum. UV: $\lambda_{max}^{MeOH}$(log $\varepsilon$): 251 (4.73), 257 (sh, 4.63), 342 (3.96). $[\alpha]_D$ +13° (acetone; *c*0.2). $^1$H-NMR: see Table 2. $^{13}$C-NMR: see Table 3. TSP-MS *m*/*z* (rel. int.): 344 $[M + NH_4]^+$ (22), 327 $[M + H]^+$ (100), 309 (14), 119 (54). EI-MS *m*/*z* (rel. int.): 326 $[M]^+\cdot$ (91), 284 (10), 267 (22), 242 (28), 212 (20), 186 (100), 172 (91), 143 (22), 115 (26), 95 (33), 85 (97), 69 (56). D/CI-MS *m*/*z* (rel. int.): 344 $[M + NH_4]^+$ (100), 342 (50), 272 (28).

*3.6. Cordiaquinone J (**3**)*

$C_{21}H_{24}O_3$. P.M. 324. Dark yellow gum. UV: $\lambda_{max}^{MeOH}$(log $\varepsilon$): 250 (4.55), 257 (sh, 4.50), 342 (3.74). $[\alpha]_D$ −37° (acetone; *c*0.2). $^1$H-NMR: see Table 1. $^{13}$C-NMR: see Table 2. TSP-MS *m*/*z* (rel. int.): 342 $[M + NH_4]^+$ (10), 325 $[M + H]^+$ (22), 307 (35), 279 (84), 175 (8), 159 (18), 130 (20), 119 (100), 101 (100). EI-MS *m*/*z* (rel. int.): 324 $[M]^+\cdot$ (36), 172 (48), 171 (96), 154 (52), 143 (97), 182 (28), 115 (100), 95 (22), 89 (37), 69 (21), 67 (32), 55 (32). D/CI-MS *m*/*z* (rel. int.): 342 $[M + NH_4]^+$ (100). HR-ESI-MS (positive mode) *m*/*z*: 325.1798 ($C_{21}H_{25}O_3$ $[M + H]^+$, requires 325.1798).

Table 2
$^1$H-NMR data for compounds **2**–**4** (acetone-$d_6$)

| H | **2** | **3** | **4** |
|---|---|---|---|
| 2 | 7.01 *s* | 7.00 *s* | 7.01 *s* |
| 3 | 7.01 *s* | 7.00 *s* | 7.01 *s* |
| 5 | 7.88 *d* (1, 9) | 7.85 *d* (1.3) | 7.84 *d* (1.9) |
| 7 | 7.76 *dd* (7.8; 2.0) | 7.72 *dd* (8.0; 1.8) | 7.71 *dd* (8.0, 1.9) |
| 8 | 7.96 *d* (7.8) | 7.95 *d* (7.8) | 7.95 *d* (7.8) |
| 9 | 2.85 *m* | 2.80 *m* | 2.74 *m* |
| | | | 2.60 *m* |
| 10 | 2.31 *m* | 1.63 *m* | 1.94 *m* |
| | | 1.72 *m* | 1.50 *m* |
| 11 | – | 1.37 *m* | 2.33 *dd* (12.0, 3.7) |
| 12 | 2.81 *m* | – | – |
| 13 | 1.47 *m* | 3.67 *d* (5.4) | – |
| 14 | 1.47 *m* | 1.93 *m* | 2.67 *m* |
| | | 1.59 *m* | 2.24 *m* |
| 15 | 3.53 *m* | 1.55 *m* | 2.67 *m* |
| | | 1.37 *m* | 2.57 *m* |
| 16 | 1.05 *d* (6.8) | – | – |
| 17 | – | 1.32 *s* | 5.14 *s* |
| | | | 4.95 *s* |
| 18 | 1.79 *s* | 1.12 *s* | 1.18 *s* |
| 19 | 1.72 *s* | 1.08 *s* | 0.99 *s* |

Table 3
$^{13}$C-NMR data for compounds **2**–**4** (acetone-$d_6$)

| C | 2 | 3 | 4 |
|---|---|---|---|
| 1 | 185.5[a] | 185.7[a] | 185.7[a] |
| 2 | 139.3[b] | 139.5[b] | 139.5[b] |
| 3 | 139.1[b] | 139.3[b] | 139.3[b] |
| 4 | 185.1[a] | 185.3[a] | 185.3[e] |
| 4a | 132.7[c] | 132.9[c] | 132.9[c] |
| 5 | 126.0 | 126.3 | 126.5 |
| 6 | 150.5 | 150.7 | 150.2 |
| 7 | 134.5 | 134.7 | 134.9 |
| 8 | 127.0 | 127.1 | 127.0 |
| 8a | 130.7[c] | 130.9[c] | 130.9[c] |
| 9 | 37.0 | 36.8 | 34.6 |
| 10 | 30.2 | 30.3 | 29.9 |
| 11 | 135.7 | 56.2 | 56.6 |
| 12 | 36.3 | 46.0 | 49.4 |
| 13 | 32.4[d] | 86.4 | 213.6 |
| 14 | 31.9[d] | 26.3[d] | 38.0 |
| 15 | 62.5 | 39.7 | 31.1 |
| 16 | 19.9[e] | 86.9 | 146.1 |
| 17 | 126.6 | 19.2 | 113.9 |
| 18 | 21.1 | 26.2 | 27.4 |
| 19 | 20.1[e] | 23.6 | 21.6 |

[a–e] Attributions can be interchanged.

### 3.7. Cordiaquinone K (*4*)

$C_{21}H_{22}O_3$. P.M. 322. Dark yellow gum. UV: $\lambda_{max}^{MeOH}$(log $\varepsilon$): 250 (4.67), 257 (sh, 4.61), 345 (3.91). $[\alpha]_D$ −46.4° (acetone; *c*0.37). $^1$H-NMR: see Table 1. $^{13}$C-NMR: see Table 2. TSP-MS *m*/*z* (rel. int.): 340 $[M + NH_4]^+$ (68), 119 (100). EI-MS *m*/*z* (rel. int.): 322 $[M]^+\cdot$ (100), 186 (48), 173 (34), 172 (46), 171 (44), 143 (50), 138 (42), 128 (29), 115 (73), 96 (58), 89 (32), 81 (35), 79 (36), 67 (44). D/CI-MS *m*/*z* (rel. int.): 340 $[M + NH_4]^+$ (100). HR-ESI-MS (positive mode) *m*/*z*: 323.1640 ($C_{21}H_{23}O_3$ $[M + H]^+$, requires 323.1641).

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