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ANTIFUNGAL AND LARVICIDAL MEROTERPENOID NAPHTHOQUINONES AND A NAPHTHOXIRENE FROM THE ROOTS OF *CORDIA LINNAEI*

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Key Word Index—*Cordia linnaei*; Boraginaceae; naphthoquinones; antifungal activity; larvicidal activity.

Abstract—Three new meroterpenoid naphthoquinones, the known cordiaquinone B and a new naphthoxirene have been isolated from the roots of *Cordia linnaei*. Their structures were established by spectrometric methods including EI, D/CI and FAB mass spectrometry, ^1H, ^{13}C and 2D NMR experiments. The naphthoquinones showed activity against *Cladosporium cucumerinum*, *Candida albicans* and the larvae of the yellow fever-transmitting mosquito *Aedes aegypti*, while the naphthoxirene derivative was found to be inactive in the same bioassays.

INTRODUCTION

Cordia linnaei Stearn is a shrub widely spread in Central and South America. In Costa Rica, a leaf decoction is used as a remedy for fevers and liver ailments [1]. Use of the roots in traditional medicine has not been reported.

In our systematic search for new natural antifungal and larvicidal compounds, many Panamanian plants have been screened [2]. The dichloromethane extract from the roots of *Cordia linnaei* was found to give interesting activities against the plant pathogenic fungus *Cladosporium cucumerinum* [3], the yeast *Candida albicans* [4] and the larvae of the yellow fever-transmitting mosquito *Aedes aegypti* [5]. As no chemical and biological studies have previously been reported on this plant, an investigation of the roots of *Cordia linnaei* was undertaken.

RESULTS AND DISCUSSION

The dichloromethane extract was first fractionated by column chromatography on silica gel. Further separations on silica gel afforded compounds **1** and **2**. Compound **3** required further purification on Sephadex LH-20, while **4** and **5** were obtained after Lobar fractionation on a diol column.

After HPLC coupled with diode array detection (DAD) of **1** to **5**, the UV spectra indicated these compounds to be naphthoquinone derivatives [6]. The structures of the compounds were determined by the use of ^1H, ^{13}C NMR spectroscopy, including 2D NMR experiments, EI and D/CI-MS. Relative configurations were obtained by NOE.

The ^1H NMR spectra of **1**, **2**, **4** and **5** exhibited the same signals for the aromatic region: two protons at δ 6.95 (H-2, H-3) and an AMX system (δ 8.00, *d*, $J = 7.8$ Hz, H-8; δ 7.89, *d*, $J = 1.7$ Hz, H-5; δ 7.57, *dd*, $J = 7.8$ Hz and 1.7 Hz, H-7). As a consequence, these four compounds all appeared to be 6-substituted naphthoquinones. The ^{13}C NMR spectra of the same compounds indicated the presence of substituents containing 11 carbon atoms in the aliphatic region of the spectra. In conjunction with the EI-MS of **2** (*m/z* 324 $[M]^+$), the unsaturation of the substituent could be explained by cyclisation. Evidence for the presence of a quaternary carbon (δ 43.6), 3 CH_3 (δ 7.7, 15.1 and 15.2), 4 CH_2 (δ 29.4, 30.8, 38.8 and 41.5) and 2 CH (δ 36.2 and 50.3) groups was provided by a DEPT experiment. The COSY spectrum and a HSQC experiment established the presence of the partial structures CH_2–CH_2 (δ 29.4 and 38.8, C-9 and C-10), CH_3-CH-CH_2-CH_2 (δ 15.1, 36.2, 30.8 and 41.5) and CH_3-CH (δ 7.7 and 50.3). The HSQC-TOCSY spectrum showed these latter two structural elements to belong to the same spin system, separated from the CH_2-CH_2 sequence by the quaternary carbon (δ 43.6). Linkage of the C-9 methylene group to the naphthoquinone

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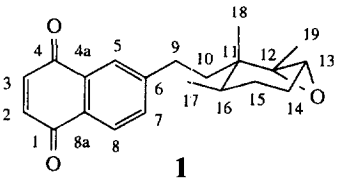

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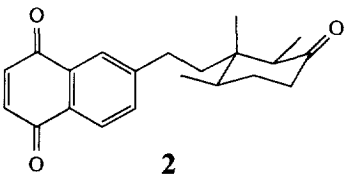

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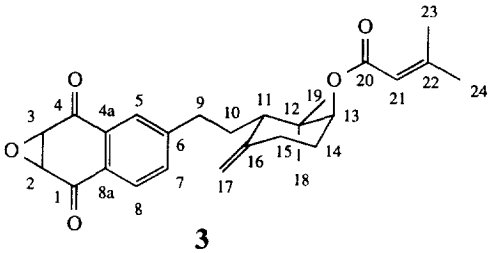

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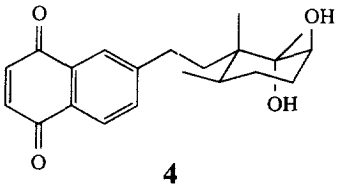

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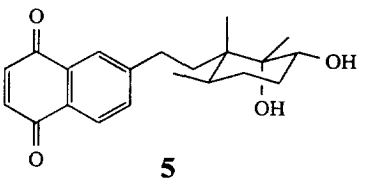

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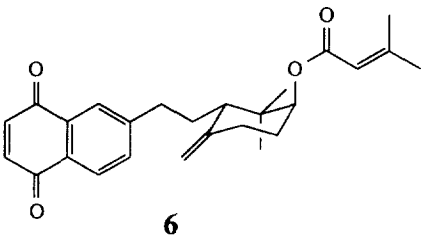

6

moiety was demonstrated by a HMBC experiment. The position of the ketone moiety (δ 213.1, C-13) and final confirmation of the carbon attributions were given by the HMBC experiment. Thus **2** was finally identified as cordiaquinone B, previously isolated by Bieber from *Cordia corymbosa* [7]. NOEs were obtained on methyl groups at δ 1.03 and 1.05 (CH_3-19 and CH_3-17) after irradiation of the methyl group at δ 0.63 (CH_3-18). This showed that the three methyl groups were all on the same side of the cyclohexane ring and that **2** had the same relative stereochemistry as found by Bieber from the biogenetic pathways [7] and confirmed by X-ray crystallography [8].

The ^{13}C NMR spectrum in $CDCl_3$ of compound **1** was quite similar to that of cordiaquinone B (**2**). However, the peak of the ketone carbon was absent and a new intense signal appeared at δ 62.4. This signal was shown to correspond to two distinctive carbons (δ 62.57 and 62.62, for C-H and a quaternary carbon, respectively) after measurement of ^{13}C and DEPT spectra in acetone-d_6. The molecular ion (*m/z* 324 $[M]^+$) in the EI-MS of **1** was identical to that of **2**, suggesting two unsaturations of the substituent at C-6. As all the ^{13}C NMR signals belonging to the terpenoid substituent were found between δ 15 and 65, cyclisation of the substituent and presence of an epoxide group, explained by the new signal at δ 62.4, were postulated. The epoxide group was confirmed by literature data [9] and the correlation (by HSQC) of the ^{13}C NMR signal at δ 62.4 with the broad singlet at δ 3.03 in the ^1H NMR spectrum [10]. An isolated CH_2-CH_2 sequence (δ 31.6 and 39.0, C-9 and C-10) linked to the naphthoquinone moiety and a CH_3-CH-CH_2-CH_2-CH chain (δ 15.9, 33.4, 24.1, 25.7 and 62.4) were identified after analysis of COSY and HSQC spectra. The structure of **1** was confirmed by HMBC experiments. The relative configuration was found from a series of NOE difference measurements. NOE between H-13 and CH_3-18 indicated the axial position of H-13. As a NOE was observed between CH_3-18 and the two methyl groups CH_3-17 and CH_3-19, these two latter substituents were in equatorial positions. To our knowledge, compound **1** (cordiaquinone E) is a new natural product.

The EI-MS of compounds **4** and **5** exhibited the same molecular ion $[M]^+$ at *m/z* 342 and a very similar fragmentation pattern. The Mr was confirmed by the presence of a pseudomolecular ion $[M+NH_4]^+$ at *m/z* 360 in the D/CI-MS. These results suggested the presence of two hydroxyl groups in the terpenoid moiety of **4** and **5**. In both cases, the presence of the first hydroxyl group was confirmed by a $[M-18]^+$ peak in the EI-MS. Signals for both carbon atoms substituted with hydroxyl groups were observed in the ^{13}C spectrum at δ 75.9 and 77.5 for **4** and at δ 73.6 and 78.5 for **5** (C-H and quaternary carbon, respectively) [11]. A cyclic side chain was required to explain the elemental composition of $C_{21}H_{26}O_4$, as in **1** and **2**. Identical CH_2-CH_2 and CH_3-CH-CH_2CH_2-CHOH sequences for both compounds **4** and **5** were obtained after analysis of COSY and HSQC spectra, indicating the two compounds to be isomers. Final structure elucidation of **4** and **5** was achieved from their HMBC spectra. Their relative configurations were determined using NOE experiments. For both compounds, a NOE resulting from the irradiation of CH_3-18 gave evidence for the equatorial position of CH_3-17 and CH_3-19. For **5**, a NOE between H-13 and CH_3-18 showed the axial substitution of H-13. This was confirmed by the large coupling constant of H-13 ($J = 11.8$ Hz) caused by trans-diaxial coupling with axial H-14. In the case of **4**, the absence of a NOE between H-13 and CH_3-18 indicated H-13 to be in an equatorial position. Small coupling constants of H-13 ($J < 6$ Hz) excluded any trans-diaxial relationship, which correlated with the results of the NOE experiment. Compound **4** (cordiaquinone F) and compound **5** (cordiaquinone G) are new natural compounds.

The ^1H NMR spectrum of **3** showed for the aromatic region an AMX coupling pattern similar to the one observed for the 6-substituted naphthoquinones. However, the singlet at δ 6.95 (H-2, H-3) was shifted to δ 4.00. The presence of an epoxide group at this location was in good agreement with ^1H NMR data for diosquinone [12]. Furthermore, the similarity of C-2 and C-3 ^{13}C shifts of **3** (δ 55.3) and of an epoxy derivative with similar structure, antiphenicol (δ 53.6 and 55.4) [13], agreed with the presence of an epoxide moiety. The EI-MS of **3** (*m/z* 422 $[M]^+$) was consistent with a molecular formula of $C_{26}H_{30}O_5$ and a structure with 5 more carbon atoms in the side chain than **1**, **2**, **4** and **5**. This difference could be explained by esterification (δ 166.1, quaternary carbon) of an isoprenylated unit with a cyclohexane hydroxyl. The allylic proton appeared at δ 5.64 (*s*, 1H) in the ^1H NMR spectrum and the two methyl groups at δ 1.91 (*s*, 3H) and 2.15 (*s*, 3H). The DEPT spectrum showed the presence of a methylene group (δ 109.8, CH_2) and of a CH linked to oxygen (δ 76.9). In addition, CH_2-CH_2-CH (δ 35.0, 27.4 and 52.0) linked to the aromatic ring and CH_2-CH_2-CH-O- (δ 30.7, 28.6 and 76.9) partial sequences were deduced from COSY and HSQC spectra. The positions of the methylene group and of the two other CH_3 moieties (δ 18.8 and 26.4) were decided from HMBC experiments. Accordingly, the structure of **3** (cordiaquinone H) was established. The small coupling constants of H-13 ($J < 4$ Hz) indicated an axial orientation for the ester group, whereas H-11 must be in an axial position for steric reasons. The identity of the naphthoquinone substituent was confirmed after comparison with NMR data for the terpenoid part of cordiaquinone C (**6**) [14]. The cordiaquinones A, B, C, D, meroterpenoid naphthoquinones, have been isolated from *Cordia corymbosa*, growing in Brazil [7].

Activities of the isolated compounds against *Cladosporium cucumerinum* and *Candida albicans* were evaluated by TLC bioautography. Quantification of the same bioactivities was achieved in an agar-dilution assay [15]. Larvicidal potency of the naphthoquinones against *Aedes aegypti* was investigated in a dilution test. The results of the three bioassays are reported in Table 3. Naphthoxirene **3** was inactive at the highest tested concentrations against the two microorganisms in both dilution assays, whereas the other naphthoquinones exhibited activities similar to those of the reference compound, nystatin, under the same conditions. Thus, the presence of the expoxide on the naphthoquinone ring is probably responsible for the lack of fungicidal activity of the naphthoxirene.

EXPERIMENTAL

General

Mp: uncorr. ^1H and ^{13}C NMR: $CDCl_3$ at 500.00 (^1H) and 125 (^{13}C) MHz, TMS as int. standard; UV: MeOH; TLC: silica gel 60 F_{254} Al sheets (Merck) and diol HPTLC plates (Merck); CC: silica gel (63–200 μm; 700 × 55 mm i.d., Merck), Sephadex LH-20 (400 × 13 mm i.d., Pharmacia). LPLC: Lobar Lichroprep diol (40–63 μm; 270 × 25 mm i.d., Merck); EI-MS, D/CI-MS and FAB-MS: Finnigan MAT TSQ-700 triple stage quadrupole instrument. Purity of the compounds was checked by HPLC with a Nova-Pak RP-18 column (4 μm; 150 × 3.9 mm i.d.; Waters) using an MeCN-H_2O gradient (30 : 70 → 100 : 0) in 30 min.

Plant material

Roots of *Cordia linnaei* were collected in October 1995 in Llano Carti, San Blas Islands, Panama. A voucher is deposited at the National Herbarium of Panama (FLORPAN 2263) and at the Institut de Pharmacognosie et Phytochimie, Lausanne, Switzerland (No. 94147).

Extraction and isolation

Air-dried powdered roots of *Cordia linnaei* (152 g) were extracted at room temp with CH_2Cl_2 to afford 2.34 g of extract.

The CH_2Cl_2 extract was first fractionated by CC on

Table 1. ^{1}H NMR data for compounds **1–5** ($CDCl_3$)

H	1	2	3	4	5
2	6.93 *s*	6.97 *s*	4.00 *bs*	6.92 *s*	6.92 *s*
3	6.93 *s*	6.97 *s*	4.00 *bs*	6.92 *s*	6.92 *s*
5	7.86 *d* (1.8)	7.89 *d* (1.9)	7.80 *d* (1.8)	7.86 *d* (1.9)	7.86 *d* (1.9)
7	7.54 *dd* (7.8; 1.7)	7.57 *dd* (7.8; 1.8)	7.57 *dd* (8.1; 1.8)	7.54 *dd* (8.0; 1.8)	7.54 *dd* (8.0; 1.8)
8	7.99 *d* (7.8)	8.02 *d* (7.8)	7.92 *d* (8.1)	7.97 *d* (7.8)	7.97 *d* (7.8)
9	2.85 *td* (12.7; 3.4)	2.80 *td* (13.1; 4.9)	2.85 *m*	2.96 *td* (13.2; 4.9)	3.15 *td* (13.2; 5.4)
	2.62 *td* (12.7; 3.4)	2.64 *td* (13.1; 4.9)	2.53 *m*	2.72 *td* (13.2; 4.9)	2.82 *td* (13.2; 4.9)
10	1.78 *m*	1.66 *m*	1.91 *m*	1.85 *m*	1.69 *m*
	1.66 *m*	1.65 *m*	1.88 *m*	1.70 *m*	1.67 *m*
11	—	—	1.79 *m*	—	—
12	—	2.60 *q* (6.8)	—	—	—
13	3.03 *bs*	—	4.71 *dd* (2.1; 3.6)	3.67 *dd* (5.9; 3.9)	3.72 *dd* (11.8; 4.9)
14	2.02 *m*	2.41 *m*	1.86 *m*	1.96 *m*	1.82 *m*
	1.72 *m*	2.41 *m*	1.63 *m*	1.57 *m*	1.38 *m*
15	1.42 *m*	1.93 *m*	2.35 *m*	1.55 *m*	1.48 *m*
	1.13 *m*	1.68 *m*	2.07 *m*	1.55 *m*	1.36 *m*
16	1.52 *m*	2.14 *m*	—	2.00 *m*	1.55 *m*
17	0.83 *d* (6.8)	1.01 *d* (6.4)	4.97 *bs*	0.98 *d* (6.9)	0.84 *d* (6.1)
			4.69 *bs*		
18	0.91 *s*	0.63 *s*	0.94 *s*	1.01 *s*	1.01 *s*
19	1.31 *s*	1.03 *d* (6.8)	0.82 *s*	1.34 *s*	1.28 *s*
21	—	—	5.64 *bs*	—	—
23	—	—	2.17 *d* (1.2)	—	—
24	—	—	1.89 *d* (1.2)	—	—

silica gel with a petrol-EtOAc gradient (4:1 → 0:1) giving frs 1–20. Compound **1** (118 mg) was obtained from fr. 7 by further separation on a silica gel column using $CHCl_3$-MeOH (19:1). Compound **2** (40 mg) was isolated after chromatography of fr. 12 on silica gel with $CHCl_3$-MeOH (49:1). Fr. 3 was subjected to CC on silica gel using $CHCl_3$-MeOH (49:1); further purification on Sephadex LH-20 with $CHCl_3$-MeOH (1:1) yielded 3 mg of **3**. Separation of fr. 16 by diol LPLC using petrol-EtOAc (1:1) gave **4** (37 mg) and **5** (9 mg).

6-[10-(11,12,16-trimethyl-12,13-epoxycyclohexyl) ethyl]-1,4-Naphthalenedione (Cordiaquinone E) (**1**)

Dark yellow oil. UV^{λ}_{max} nm (log ε): 250 (4.02), 258 (sh, 3.97), 340 (3.22); $[\alpha]_D$ −5.2° ($CHCl_3$; *c* 0.2); EI-MS *m/z* (rel. int.): 324 $[M]^+$ (17), 212 (21), 186 (40), 185 (14), 173 (23), 172 (26), 171 (22), 153 (100), 142 (24), 138 (12), 128 (10), 115 (11); ^{1}H and ^{13}C NMR: Tables 1 and 2.

6-[10-(11,12,16-trimethyl-13-oxocyclohexyl)ethyl]-1,4-Naphthalenedione (Cordiaquinone B) (**2**)

Yellow powder, mp 107–115°. UV^{λ}_{max} nm (log ε): 250 (4.29), 258 (sh, 4.23), 339 (3.48); $[\alpha]_D$ +3.9° ($CHCl_3$; *c* 0.6); EI-MS *m/z* (rel. int.): 324 $[M]^+$ (32), 187 (13), 186 (100), 173 (16), 172 (23), 171 (10), 139 (87), 115 (12), 111 (20), 97 (63), 84 (16), 83 (23), 69 (82), 67 (12); ^{1}H and ^{13}C NMR: Tables 1 and 2.

6-[10-(12,12-dimethyl-13-(22-methyl-21-butenoyl)-6-Methenyl-cyclohexyl)ethyl]-2,3-dihydro-2,3-epoxy-1,4-naphthalenedione (Cordiaquinone F) (**3**)

Yellow gum. UV^{λ}_{max} nm (log ε): 245 (4.06), 276 (3.32), 310 (sh, 3.10); $[\alpha]_D$ −12.9° ($CHCl_3$; *c* 0.2); EI-MS *m/z* (rel. int.): 422 $[M]^+$ (6), 323 (17), 235 (14), 135 (50), 123 (25), 122 (100), 121 (18), 107 (63), 93 (18), 91 (14), 85 (17), 84 (22), 83 (100), 81 (17), 79 (21), 77 (21); DC/I *m/z* (rel.int.): 440 $[M+NH_4]^+$ (100); ^{1}H and ^{13}C NMR: Tables 1 and 2.

6-[10-(11,12,16-trimethyl-12,13-dihydroxycyclohexyl)ethyl]-1,4-Naphthalenedione (Cordiaquinone G) (**4**)

Dark yellow oil. UV^{λ}_{max} nm (log ε): 251 (4.37), 258 (sh, 4.33), 345 (3.57); $[\alpha]_D$ +5.5° ($CHCl_3$; *c* 0.2); EI-MS *m/z* (rel. int.): 342 $[M]^+$ (24), 324 (6), 213 (15), 187 (16), 186 (100), 174 (24), 173 (72), 172 (34), 171 (10), 143 (9), 115 (7); DC/I *m/z* (rel. int.): 360 $[M+NH_4]^+$ (100), 342 $[M]^+$ (44), 243 (13); ^{1}H and ^{13}C NMR: Tables 1 and 2.

Table 2. ^{13}C NMR data for compounds **1–5** ($CDCl_3$)

C	1	2	3	4	5
1	185.2[a]	185.2[a]	191.0[a]	185.4[a]	185.5[a]
2	138.7[b]	138.7[b]	55.3[b]	138.7[b]	138.7[b]
3	138.4[b]	138.5[b]	55.3[b]	138.4[b]	138.5[b]
4	184.7[a]	184.7[a]	190.3[a]	184.9[a]	185.0[a]
4a	132.0[c]	132.0[c]	131.9[c]	131.9[c]	132.0[c]
5	126.8[d]	126.8[d]	126.8[d]	126.7[d]	126.8[d]
6	149.8	149.5	150.8	151.3	151.4
7	133.7	133.9	134.9	134.0	134.1
8	125.8[d]	125.9[d]	126.6[d]	126.0[d]	126.0[d]
8a	130.0[c]	130.0[c]	129.6[c]	129.7[c]	129.8[c]
9	31.6	29.4	35.0	32.2	32.6
10	39.0	38.8	27.4	38.7	40.6
11	38.8	43.6	52.0	43.0	43.6
12	62.4	50.3	39.1	77.5	78.5
13	62.4	213.1	76.9	75.9	73.6
14	25.7	41.5	28.6	28.4	30.6
15	24.1	30.8	30.7	25.7	29.1
16	33.4	36.2	146.2	33.1	37.9
17	15.9	15.1	109.8	16.6	16.5
18	17.9	15.2	26.4	17.4	12.2
19	20.8	7.7	18.8	21.8	15.8
20			166.1		
21			116.2		
22			156.7		
23			20.3		
24			27.4		

[a–d] Values in the same column with the same symbol may be interchanged

6-[10-(11,12,16-trimethyl-12,13-dihydroxycyclohexyl)ethyl]-1,4-Naphthalenedione (Cordiaquinone H) (**5**)

Dark yellow oil. $UV\lambda^{max}$ nm (log ε): 250 (4.37), 257 (sh, 4.33), 345 (3.55); $[\alpha]_D$ −4.8° ($CHCl_3$; *c* 0.3); EI-MS *m/z* (rel. int.): 342 $[M]^+$ (28), 324 (21), 296 (12), 225 (12), 223 (10), 213 (19), 212 (10), 187 (21), 186 (100), 185 (12), 174 (24), 173 (80), 172 (44), 143 (11); DC/I *m/z* (rel. int.): 360 $[M+NH_4]^+$ (100), 342 $[M]^+$ (21), 243 (6); ^1H and ^{13}C NMR: Tables 1 and 2.

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Table 3. Antifungal and larvicidal activities of isolated naphthoquinones

Compound	*Cladosporium cucumerinum*[a]	*Cladosporium cucumerinum*[b]	*Candida albicans*[a]	*Candida albicans*[b]	*Aedes aegypti*[c]
1	1	3	1	6	12.5
2	0.5	3	0.5	3	25
3	>50	>50	>50	>50	n.t.
4	0.5	1.5	1	6	50
5	1	3	1	6	25
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 an agar-dilution assay.
[c] Minimal concentration (ppm) of compound required to kill all the larvae after 24 hours
n.t. not tested

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