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# Crassiflorone, a new naphthoquinone from *Diospyros crassiflora* (Hien)

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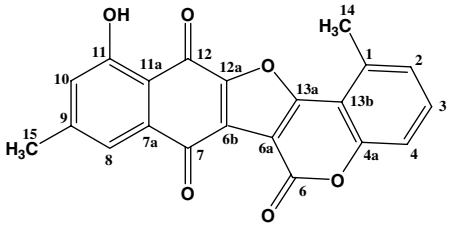
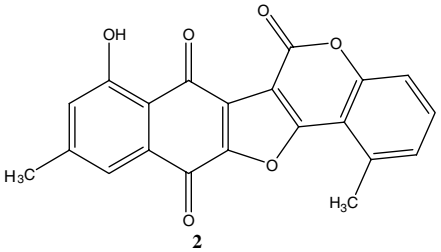
**Abstract**—A new naphthoquinone, 11-hydroxy-1,9-dimethyl-6*H*-naphtho[2′,3′:4,5]furo[3,2-*c*]chromene-6,7,12-trione, named crassiflorone, was isolated from the stem bark of *Diospyros crassiflora* together with the known compounds plumbagin, cyclocanaliculatin, gerberinol, lupeol, lupenone and betulinic acid. The structures of the compounds were established on the basis of 1D and 2D NMR spectroscopic data, as well as co-TLC with authentic samples. Some of the above compounds exhibited significant antimicrobial activity against bacteria and yeasts.


The genus *Diospyros* contains as many as 350 species, some of which are widely used for the treatment of many ailments.[1] Due to its use in folk medicine, the above taxon has attracted the attention of many scientists who have investigated the chemical constitution of many species and reported the presence of various classes of compounds including hydrocarbons, terpenes, naphthoquinones and coumarins.[1–4] In our systematic search for antimicrobial agents from the plant kingdom, we examined the chemical constitution of *Diospyros crassiflora* (a tree up to 25 m high and 80–120 cm width).[5] We report here a phytochemical investigation of the stem bark of *D. crassiflora* Hien collected from *Mount Fébé* in the Central Province of Cameroon in May 1998, as well as the antimicrobial activities of some of the above compounds against bacteria and yeasts. A voucher specimen (4924/SRFK) has been deposited at the Cameroon National Herbarium, Yaoundé.

The methylene chloride extract of the stem bark of *D. crassiflora* afforded, after repeated chromatography, seven compounds among which are, a new naphthoquinone derivative **1**, together with two naphthoquinone derivatives, plumbagin **3**[6] and cyclocanaliculatin **4**,[1] a coumarin, gerberinol **5**,[7] three triterpenes, lupeol, lupenone and betulinic acid.[1,8]




**1**




**2**

*Keywords*: *Diospyros crassiflora*; Ebenaceae; Crassiflorone; Bacteria; Yeasts.
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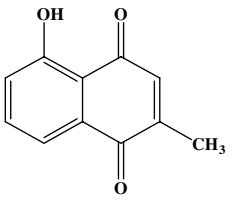


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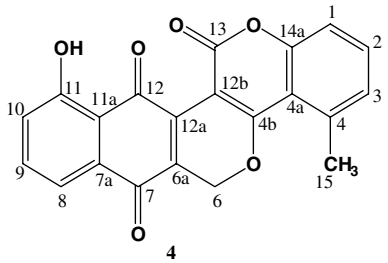


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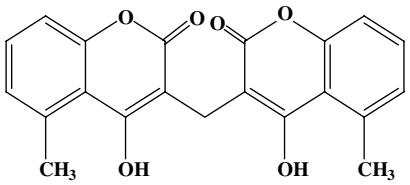


5

Crassiflorone **1** was obtained as the major component of the dichloromethane extract of the stem bark of *D. crassiflora*, and isolated by flash chromatography (solvent $CH_2Cl_2$–MeOH) using silica gel. It was soluble in dimethyl sulfoxide and crystallized from $CH_2Cl_2$–MeOH as orange crystals, mp 230–232 °C (uncorrected). The EI-MS suggested the molecular formula of crassiflorone to be $C_{21}H_{12}O_6$ (*m/z* 360.1 $[M]^+$, 99.6%) with 16° of unsaturation. The UV spectrum showed absorption maxima at $\lambda_{max}$ (MeOH) 283 and 341 nm (log $\varepsilon$ 2.45 and 2.53, respectively) typical of 5-methyl-4-oxycoumarin/2-oxychromenones,[3,9,10] and at 414 nm (log $\varepsilon$ 2.63) typical for the juglone derivatives.[3,10] The IR spectrum displayed a broad band at $\nu_{max}$ (KBr) 3421 $cm^{-1}$ (chelated OH), a band at 1597–1634 $cm^{-1}$ (aromatic ring and quinonoid carbonyl), and a further band at 1678 $cm^{-1}$ (coumarin carbonyl).[9,11] The $^1H$ and $^{13}C$ spectral data, by means of COSY, DEPT, HMQC, HMBC and NOESY experiments, allowed a full assignment of the NMR signals and led to structure **1**.

The analysis of the $^{13}C$ NMR spectral data of **1** (Table 1) through DEPT revealed 21 carbon atoms among which are two methyl groups ($\delta_C$ 21.5 and 23.2), five methine and 14 quaternary carbon atoms. Of the 14 quaternary carbon atoms, two were those of α,β-unsaturated ketones ($\delta_C$ 181.4 and 178.8), one was a chromenone carbonyl (α,β-unsaturated lactone) which resonated at $\delta_C$ 158.0 and four were linked to oxygen atoms in view of their deshielded chemical shifts at $\delta_C$ 153.9, 160.2, 156.2 and 165.5. Among the four oxygenated quaternary carbon atoms, one was linked to a hydroxyl group and thus accounted for the 12 hydrogen atoms present in compound **1**.

**Table 1.** $^{13}C$ NMR assignments of compounds **1** and **4** (125 MHz) in DMSO-$d_6$

| Atom no. | 1 | | 4 | |
|---|---|---|---|---|
| | $\delta_C$ (ppm) | DEPT | $\delta_C$ (ppm) | DEPT |
| 1 | 137.4 | C | 115.0 | CH |
| 2 | 127.5 | CH | 132.2 | CH |
| 3 | 132.1 | CH | 128.0 | CH |
| 4 | 115.2 | CH | 133.1 | C |
| 4a | 153.9 | C | 117.2 | C |
| 4b | — | — | 164.2 | C |
| 6 | 158.0 | C | 65.5 | $CH_2$ |
| 6a | 100.0 | C | 139.0 | C |
| 6b | 111.2 | C | — | — |
| 7 | 181.4 | C | 181.0 | C |
| 7a | 132.8 | C | 132.6 | C |
| 8 | 119.5 | CH | 119.0 | CH |
| 9 | 148.0 | C | 136.4 | CH |
| 10 | 122.8 | CH | 125.7 | CH |
| 11 | 160.2 | C | 161.8 | C |
| 11a | 113.4 | C | 113.7 | C |
| 12 | 178.8 | C | 180.6 | C |
| 12a | 156.2 | C | 139.8 | C |
| 12b | — | — | 105.0 | C |
| 13 | — | — | 154.5 | C |
| 13a | 165.5 | C | — | — |
| 13b | 114.5 | C | — | — |
| 14 | 23.2 | $CH_3$ | — | — |
| 14a | — | — | 152.1 | C |
| 15 | 21.5 | $CH_3$ | 20.8 | $CH_3$ |

High field $^1H$ NMR (DMSO-$d_6$, 500 MHz) signals assigned with the aid of $^1H$–$^1H$ COSY and NOESY (Fig. 1) experiments, showed one methyl group at $\delta_H$ 2.46 and two aromatic protons at $\delta_H$ 7.23 (br s, H-10) and 7.47 (br s, H-8), which were typical of a 7-methyljuglone unit without protons on C-2 and C-3,[10,12] and justified the presence of unit A (Fig. 2) in compound **1**. From the HMBC experiment (Table 2), the proton at $\delta_H$ 7.23 showed correlations with C-11 ($\delta_C$ 160.2), C-11a ($\delta_C$ 113.4) and C-15 ($\delta_C$ 21.5) (Table 2). Also, the proton at $\delta_H$ 7.47 showed correlations with C-7 ($\delta_C$ 181.4), C-7a ($\delta_C$ 132.8), C-10 ($\delta_C$ 122.8), C-11a and C-15. The signal at $\delta_H$ 11.91 (s, 1H) which exhibited HMBC correlations with C-10, C-11 and C-11a appeared to be that of a chelated hydroxyl group.

$^1H$–$^1H$ COSY and NOESY (Fig. 1) experiments, led to the assignment of the C-methyl group appearing at $\delta_H$ 2.61 to be that of a coumarin/chromenone system and

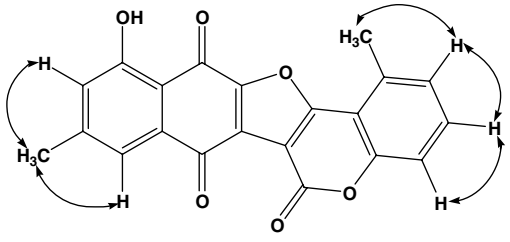


**Figure 1.** NOESY correlations of compound **1**.

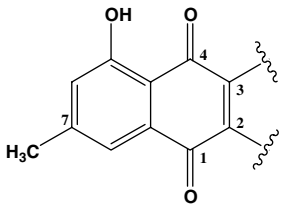


**Figure 2.** Unit A.

**Table 2.** $^{1}J$ (from HMQC), $^{2}J$ and $^{3}J$ correlations of crassiflorone (**1**)

| Proton $\delta_H$ (ppm) | Position | $^{1}J$-Correlated carbon | $^{2}J$- and $^{3}J$-correlated carbons |
|---|---|---|---|
| 11.91 | 11-OH | — | C-10, C-11, C-11a |
| 7.47 | 8 | 119.5 | C-7, C-7a, C-10, C-11a, C-15 |
| 7.45 | 3 | 132.1 | C-1, C-2, C-4, C-4a |
| 7.23 | 10 | 122.8 | C-8, C-11, C-11a, C-15 |
| 7.11 | 2 | 127.5 | C-1, C-3, C-4, C-13b, C-14 |
| 7.09 | 4 | 115.2 | C-2, C-3, C-4a, C-13b |
| 2.61 | 14 | 23.2 | C-1, C-2, C-13b |
| 2.46 | 15 | 21.5 | C-8, C-9, C-10 |

must therefore, be located on a benzene ring. The signals for the three remaining protons occurred at $\delta_H$ 7.09 (br d, $J = 8$ Hz, H-4), 7.11 (br d, $J = 8$ Hz, H-2) and 7.45 (t, $J = 8$ Hz, H-3), the above pattern being consistent only with a 1,2,3-trisubstituted benzene ring of an oxychromenone (5-methyl-4-oxycoumarin) and justified the presence of unit B (Fig. 3) in compound **1**. The signal at $\delta_H$ 7.45 appeared as a triplet due to coupling with H-2 and H-4, and the link to the carbon at $\delta_C$ 132.1 (C-3) was confirmed by 2D experiments. From the HMBC experiment, H-3 showed correlations with C-1 ($\delta_C$ 137.4), C-2 ($\delta_C$ 127.5), C-4 ($\delta_C$ 115.2) and C-4a ($\delta_C$ 153.9) (Table 2), while, H-2 showed correlations with C-1, C-3 ($\delta_C$ 132.1), C-4, C-13b ($\delta_C$ 114.5) and C-14 ($\delta_C$ 23.2).

The oxygen atom of unit B can be linked either to the C-2 or C-3 carbon of unit A. On this basis, the structure of crassiflorone could be either **1** or **2**; however, the quinonoid carbonyl resonance at C-7 ($\delta_C$ 181.4) of compound **1**, which is close in value to the coumarin carbonyl group as found for the quinone carbonyl C-12 ($\delta_C$ 180.6) of cyclocanaliculatin **4** (Table 1) and the HMBC correlation between H-8 and C-7 in structure **1** favour the C-3 linkage (leading to **1**). Also, the strong shielding effect on C-12 ($\delta_C$ 178.8) in compound **1** which normally appears at $\delta_C$ 189.7 (C-4) in a 7-methyljuglone (unit A with protons on C-2 and C-3)[13] and at $\delta_C$ 181.0 (C-7 with an hydroxylated methylene at the beta-position) in a cyclocanaliculatin (Table 2) can been seen as the result of the β-effect of the furan oxygen.[14] From the above data, **1** is considered to be the structure of crassiflorone. These data are also evidence for the linkage of unit B on C-2 and C-3 of unit A as shown in structure **1** in which the resulting furan ring constitutes the remaining degree of unsaturation. Crassiflorone gave fragmentations on EI-MS compatible with the suggested structure, notably the ion at $m/z$ 176.1 $[M+2H-186]^+$ (50%) attributed to a 5-methyl-4-coumarin fragment; ions at $m/z$ 134.1 $[M-226]^+$ (69%), 332.1 $[M-CO]^+$ (22%) and 106.1 $[M-254]^+$ (17%) were other important ions which are typical internal cleavages of quinonoid and coumarol rings.[7,15]

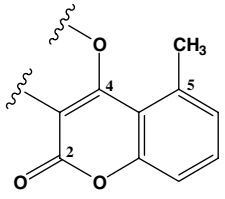


**Figure 3.** Unit B.

The antimicrobial activity of **1** and **5** was studied using the microdilution assay on a total of six microbial cultures belonging to four aerobic bacterial species (*Escherichia coli* LMP0101U, *Shigella dysenteriae* LMP0208U, *Staphylococcus aureus* LMP0206U, *Salmonella typhi* LMP0209U) and two *Candida* species (*Candida albicans* LMP0204U and *Candida krusei* LMP0311U). These strains were clinically isolated from the urogenital discharges of patients in the *Centre Pasteur du Cameroun* health institution and monitored in the Laboratory of Applied Microbiology and Molecular Pharmacology (LMP) of the University of Yaoundé I. The strains were activated at 37 °C for 24 h on nutrient agar (NA), Sabouraud Glucose Agar (SGA) (fungi).

The antimicrobial activity was evaluated on the basis of the minimal inhibition concentration (MIC). The inocula of microorganisms were prepared from 12 h broth culture and the suspensions were adjusted to 0.5 McFarland turbidity. The tested compounds were first dissolved in dimethyl sulfoxide 10% to the highest dilution (625 μg/mL), and serial twofold dilutions were made in a concentration range from 2.44 to 625 μg/mL in the 96-wells microplate containing nutrient broth. MIC values of the tested compounds against the above pathogens were determined according to the microdilution method.[16] Gentamycin (bacteria) and nystatin (yeasts) diluted in water were used as reference antibiotics. As shown in Table 3, compound **5** exhibited the strongest activity against *Shigella dysenteriae* and *Salmonella typhi* whilst compound **1** showed weak activities against the tested microbials (Table 3). The antimicrobial activity of compound **3** was described in our previous work on *D. canaliculata*.[3]

**Table 3.** In vitro antimicrobial activity of compounds **1** and **5**

| Tested microrganisms | 1[a] | 5[a] | GM/N[b] |
|---|---|---|---|
| Bacteria | | | |
| *Escherichia coli* | 19.53 | 19.53 | 10 |
| *Shigella dysenteriae* | 78.12 | 4.88 | 5 |
| *Salmonella typhi* | 19.53 | 4.88 | 10 |
| *Staphylococcus aureus* | 78.12 | 19.53 | 10 |
| Yeasts | | | |
| *Candida albicans* | 78.12 | 39.06 | 30 |
| *Candida krusei* | 78.12 | 39.06 | 30 |

[a] Results of the MIC recorded as the mean of triplicated experiments.
[b] GM: gentamycin; N: nystatin.

## Acknowledgements

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## References and notes

1. Mallavadhani, U. V.; Anita, K.; Panda; Rao, Y. R. *Phytochemistry* **1998**, *49*, 901–951.
2. Paknikar, S. K.; Pai Fondekar, K. P.; Kirtany, J. K.; Natori, S. *Phytochemistry* **1996**, *41*, 931–933.
3. Tangmouo, J. G.; Lontsi, D.; Ngounou, F. N.; Kuete, V.; Meli, A. L.; Manfouo, R. N.; Kamdem, H. W.; Tane, P.; Penlap, B. V.; Sondengam, B. L.; Connolly, J. D. *Bull. Chem. Soc. Ethiop.* **2005**, *19*, 81–88.
4. Arunendra, P.; Dinesh, K. K.; Rakesh, M. *Phytochemistry* **2004**, *65*, 2153–2158.
5. Letouzey, R.; Withe, F. *Flore du Cameroun, Ebénacées et Ericacées*. Muséum Nationale d'Histoire Naturelle, 16 rue Buffon Paris 5$^{e}$, 1970; Vol. 11, pp 57–63.
6. Tezuka, M.; Takahashi, C.; Kuroyanagi, M.; Satake, M.; Yoshihira, K.; Natori, S. *Phytochemistry* **1973**, *12*, 175–183.
7. Sengupta, P.; Sen, M.; Karuri, P.; Wenkert, E.; Halls, T. D. J. *J. Indian Chem. Soc.* **1985**, *LXII*, 916–919.
8. Muhamad, B. Z.; Jeffreys, J. A. D.; Waterman, P. G.; Zhong, S.-M. *Phytochemistry* **1984**, *23*, 1481–1484.
9. Okogun, J. I.; Enyenihi, V. U.; Ekong, D. E. U. *Tetrahedron* **1978**, *34*, 1221–1224.
10. Jeffreys, J. A. D.; Muhamad, B. Z.; Waterman, P. G.; Zhong, S. M. *Tetrahedron Lett.* **1983**, *24*, 1085–1088.
11. Gu, J. Q.; Graf, T. N.; Lee, D.; Chai, H. B.; Mi, Q.; Kardono, L. B. S.; Setyowati, F. M.; Ismail, R.; Riswan, S.; Farnswoeth, N. R.; Cordell, G. A.; Pezzuto, J. M.; Swanson, S. M.; Kroll, D. J.; Falkinham, J. O., III; Wall, M. E.; Wani, M. C.; Kinghorn, A. D.; Oberlies, N. H. *J. Nat. Prod.* **2004**, *67*, 1156–1161.
12. Terence, J. L.; Oliver, C. M. *J. Chem. Soc., Perkin Trans. 1* **1977**, 355–359.
13. Akella, V.; Sankaram, B.; Vaddu, V.; Marayana, R.; Madugula, M. *Phytochemistry* **1986**, *25*, 2867–2871.
14. Atta-Ur-Rahman *Nuclear Magnetic Resonance*; Springer: New York, Berlin, Heidelberg, Tokyo, 1986; pp 140–201.
15. Terence, J. L.; Oliver, C. M.; Douglas, S. *J. Chem. Soc., Perkin Trans. 1* **1976**, 2155–2161.
16. Zgoda, J. R.; Poter, J. R. *Pharm. Biol.* **2001**, *39*, 221–225.