# Chemical Constituents of *Croton oligandrum* (Euphorbiaceae)

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A new clerodane diterpene derivative named crotonoligaketone was obtained from the stem bark of *Croton oligandrum* along with eight known compounds including crotonadiol, imbricatadiol, crotonzambefuran B, 7-acetoxytrachiloban-18-oic acid, 3-*O*-acetylaleuritolic acid, lupeol, $\beta$-sitosterol, and stigmasterol. The structures of the isolated compounds were established on the basis of their spectral data and by comparison with those reported in the literature.

*Key words:* Crotonoligaketone, Euphorbiaceae, *Croton oligandrum*

## Introduction

The genus *Croton* belongs to the subfamily Crotonoideae of the family Euphorbiaceae, one of the largest families of higher plants, the species of which are frequently monoecious. The genus *Croton* contains about 1300 species of trees, shrubs, and herbs and is found in the tropical and subtropical regions of the Northern and Southern Hemispheres. A number of the *Croton* species are known for their medicinal qualities, especially in Africa, Asia, and South America (Hutchinson and Dalziel, 1958; Salatino *et al.*, 2007). Plants of the genus *Croton* are commonly used for the treatment of non-communicable diseases, such as diabetes and cancers, and other ailments, such as digestive problems, dysentery, wounds, fevers, constipation, diarrhea, intestinal worms, malaria, pain ulcers, inflammation rheumatism, and other illnesses (Irvine, 1966; Salatino *et al.*, 2007).

*Croton oligandrum* Pierre ex Hutch. is a tropical tree of 9 – 15 m height (Baker and Wright, 1913). In Cameroon, the powder of the stem bark of *Croton oligandrum* is usually used to treat stomach disorders and splenomegaly, decoctions of the stem bark are used to treat pneumonia (Jiofack *et al.*, 2009); in Gabon, the same decoctions are used by pygmies to treat anemia (Betti *et al.*, 2013).

*Croton* chemistry is rather diverse; clerodane diterpenes, an extremely diverse group of terpenoids with more than 800 known compounds, seem to be one of the prevalent classes of terpenoids in this genus (Maciel *et al.*, 2003). To the best of our knowledge, there is very little phytochemical information on *Croton oligandrum*. Agnaniet *et al.* (2005) have only reported the composition of the essential oil obtained by hydrodistillation from the stem bark. The fact that this plant has hitherto not been studied in more detail prompted us to undertake an in-depth investigation of this plant in order to identify secondary metabolites which could be responsible for the observed biological activities. In the course of this study, a new clerodane diterpene derivative was isolated, along with eight known compounds, and identified by spectroscopic methods. We herein report on the structure elucidation of this new diterpene.

## Results and Discussion

The crude methylene chloride/methanol (1:1, v/v) extract of *Croton oligandrum* stem bark was subjected

to repeated silica gel column chromatography yielding nine compounds including one new metabolite.

Crotonoligaketone (**1**) was obtained as a white amorphous powder from the *n*-hexane/ethyl acetate (9.3:0.7 v/v) mixture. Its molecular formula $C_{23}H_{26}O_8$ was determined based on the NMR data in conjunction with HR-EI-MS which revealed the molecular ion peak at $m/z$ 430.1660 (calcd. 430.1628). Its IR spectrum showed strong absorption bands for a typical conjugated ketone at 1663.54 $cm^{-1}$ and esters at 1721.15 $cm^{-1}$, 1742.36 $cm^{-1}$, and 1748.12 $cm^{-1}$. The $^1H$ and $^{13}C$ NMR spectra, in conjunction with DEPT and HSQC (Table I), of **1** revealed the existence of a $\beta$-substituted furan ring [$\delta_H/\delta_C$ 6.35 (brs)/110.6 ppm; 7.31 (brs)/138.8 ppm; 7.42 (brs)/143.2 ppm; and $\delta_C$ 123.7 ppm (s)] (Ngadjui *et al.*, 2002), two vinyl methine groups at $\delta_H/\delta_C$ 6.61 (s)/128.8 ppm and 7.06 (s)/131.9 ppm, a conjugated ketone carbonyl group at $\delta_C$ 186.4 ppm, and three carboxy ester groups; the value of the chemical shifts of the carbon atom of these ester groups at $\delta_C$ 165.5, 169.5, and 172.7 ppm suggested that the first one is conjugated. In addition, four methyl groups occur in the molecule and were identified from their chemical shift and coupling patterns as a secondary group [$\delta_H/\delta_C$ 1.40 (d)/14.8 ppm] and three methyl ester groups [$\delta_H/\delta_C$ 3.87 (s)/53.0 ppm; 3.63 (s)/52.1 ppm; 3.64 (s)/52.8 ppm]. The spectral data of **1** were very similar to those of crotonzambefuran B (**4**), isolated from the same plant and previously isolated for the first time from *C. zambesicus* (Ngadjui *et al.*, 2002). Comparison of the NMR spectral data of compounds **1** and **4** showed almost the same chemical shifts, except for the signals of the protons of ring A. Careful examination of the spectra suggested that in the structure of **1** there is an additional conjugated carbon-oxygen double bond. This was confirmed by: (i) the molecular formula of **1** with an addtional oxygen atom but minus two hydrogen atoms; (ii) the NMR spectra with two vinyl methine groups in **1** instead of four in **4**; two quaternary carbon atoms at $\delta_C$ 150.1 and 157.5 ppm which can be assigned to the carbon atoms of two cross-$\alpha,\beta$-unsaturated carbonyl systems. From the foregoing data, crotonoligaketone (**1**) was identified as 15,16-epoxy-2-oxo-1(10),3,13(16)14-clerodatetraen-18,19,20-trioic acid trimethylester (Fig. 1). The relative configurations at C8 and C9 were investigated in a NOESY experiment. Correlation was observed between the methine proton at $\delta_H$ 1.64 ppm (H8) and the methylene protons at $\delta_H$ 2.25 and 2.35 ppm (H11). These data are consistent with the relative configuration of the groups at C8 and C9 as shown in **1**.

Table I. $^1H$ (300 MHz) and $^{13}C$ (75 MHz) NMR spectral data and HMBC correlations of compound **1** in $CDCl_3$.

| Position | $^1H$ NMR ($J$ in Hz) | $^{13}C$ NMR | HMBC (H-C) |
|---|---|---|---|
| 1 | 6.61 (1H, s) | 128.8 (d) | C5; C9 |
| 2 | – | 186.4 (s) | – |
| 3 | 7.06 (1H, s) | 131.9 (d) | C5; C18 |
| 4 | – | 157.5 (s) | – |
| 5 | – | 52.3 (s) | – |
| 6a | 2.88 (m) | 34.7 (t) | – |
| 6b | 2.75 (m) | 34.7 (t) | – |
| 7a | 1.66 (m) | 27.4 (t) | – |
| 7b | 1.66 (m) | 27.4 (t) | – |
| 8 | 1.64 (m) | 39.3 (d) | – |
| 9 | – | 56.1 (s) | – |
| 10 | – | 150.1 (s) | – |
| 11a | 2.35 (m) | 35.0 (t) | – |
| 11b | 2.25 (m) | 35.0 (t) | – |
| 12a | 2.54 (m) | 19.5 (t) | C11 |
| 12b | 2.47 (m) | 19.5 (t) | C11 |
| 13 | – | 123.7 (s) | – |
| 14 | 6.35 (brs) | 110.6 (d) | C13; C15; C16 |
| 15 | 7.42 (brs) | 143.2 (d) | C13 |
| 16 | 7.31 (brs) | 138.8 (d) | C14 |
| 17 | 1.40 (3H, d, 6.6) | 14.8 (q) | C8; C17 |
| 18 | – | 165.5 (s) | – |
| 19 | – | 169.5 (s) | – |
| 20 | – | 172.7 (s) | – |
| OMe-18 | 3.87 (s) | 53.0 (q) | C18 |
| OMe-19 | 3.63 (s) | 52.1 (q) | C19 |
| OMe-20 | 3.64 (s) | 52.8 (q) | C20 |

The carbon and proton resonances of **1**, as shown in Table I, were assigned using $^{13}C$ NMR data from DEPT, HMQC, HMBC, and COSY experiments.

In addition, eight known compounds were isolated (Fig. 1): crotonadiol (**2**) (Ngadjui *et al.*, 1999), imbricatadiol (**3**) (Schmeda-Hirschmann *et al.*, 2005), crotonzambefuran B (**4**) (Ngadjui *et al.*, 2002), 7-acetoxytrachiloban-18-oic acid **5** (Ngadjui *et al.*, 2002), 3-*O*-acetylaleuritolic acid (**6**) (McLean *et al.*, 1987), lupeol **7** (Reynolds *et al.*, 1985), $\beta$-sitosterol (**8**) (Lawrence and Zito, 1976), and stigmasterol (**9**) (Lawrence and Zito, 1976). The structures were identified by comparison with published data.

## Conclusion

The present study of *Croton oligandrum* resulted in the isolation of two pentacyclic triterpenes, lupeol (**7**) and 3-*O*-acetylaleuritolic acid (**6**), two sterols, $\beta$-sitosterol (**8**) and stigmasterol (**9**), and five diterpenes including one trachylobane, 7-acetoxytrachiloban-18-

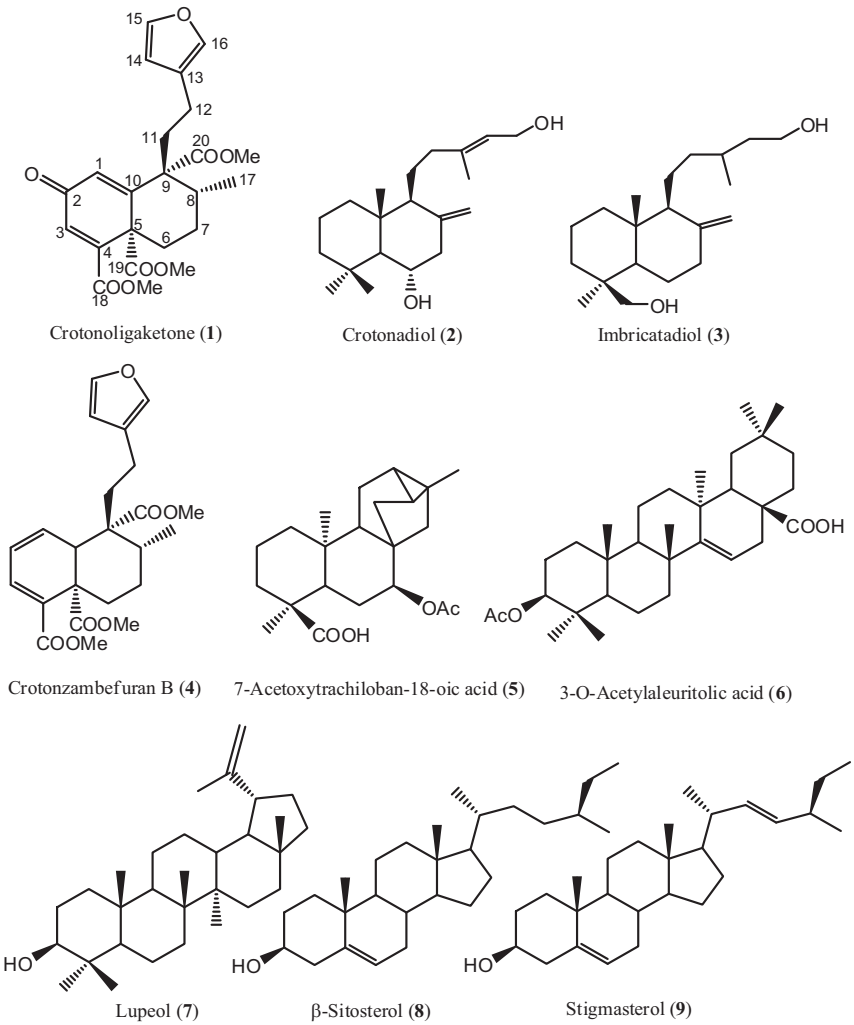


Fig. 1. Chemical structures of the compounds isolated from the stem bark of *Croton oligandrum*.

oic acid (**5**), two labdanes, crotonadiol (**2**) and imbricatadiol (**3**), and two clerodanes, crotonoligaketone (**1**) and crotonzambefuran B (**4**). Compounds **1**–**9** have been isolated from *C. oligandrum* for the first time, but compounds **2**, **4**, **5**, **7**, and **8** have previously been isolated from *C. zambesicus* (Ngadjui *et al.*, 2002). The cytotoxic effects of natural and semisynthetic labdanes from *C. oblongifolius* have been reported by Sommit *et al.* (2003). Several labdanes and clerodane diterpenes or their derivatives have been shown to possess gastroprotective activity (Schmeda-Hirschmann *et al.*, 2005). These activities and the presence of these compounds in *C. oligandrum* could explain why this plant is used to treat stomach disorders.

## Experimental

### *Instrumentation*

NMR spectra were recorded on Bruker (Billerica, MA, USA) DMX Avance 300 MHz and 600 MHz instruments equipped with an auto-tune probe and using the automation mode aided by the Bruker program Icon-NMR. $CDCl_3$ and $CD_3COCD_3$ were used as solvents and internal standards. HR-EI mass spectra were determined on a Waters (Milford, MA, USA) GCT-Premier spectrometer. IR spectra were recorded on a Shimadzu (Kyoto, Japan) FTIR-8700 Fourier transform infrared spectrometer with KBr disks. Melting

points were recorded using a Stuart Scientific (Redhill, Surrey, UK) melting point apparatus (SMP1) and are uncorrected. For column chromatography (CC), silica gel 60, particles size 0.04 – 0.063 mm (Merck, Modderfontein, South Africa), or Sephadex LH-20 (Sigma-Aldrich, Johannesburg, South Africa) were used. Analytical thin-layer chromatography (TLC) was performed using silica gel 60 PF254 + 366 (Merck) and silica gel 60-F254-precoated alumina sheets (Merck). Compounds on the plates were visualized under UV light (254 and 366 nm) and by spraying with vanillin/sulfuric acid.

*Plant material*

Stem bark and leaves of *Croton oligandrum* were collected in Nkol-nkoumou, Centre region, Cameroon in March 2013. The plant was identified by Mr. P. Mezili of the National Herbarium at Yaoundé, Cameroon, where a voucher specimen (No. 6687/SRF/Cam) has been deposited.

*Extraction and isolation*

The stem bark was dried and crushed. The obtained powder (3 kg) was extracted at room temperature with 5 L of methylene chloride/methanol (1:1, v/v) during 48 h followed by 3 L methanol for 24 h. The two extracts were separately concentrated under reduced pressure and combined on the basis of TLC, as the two extracts were found to contain almost the same compounds, to give 200 g of a brown extract, which was successively extracted with petroleum ether, EtOAc, and *n*-butanol. Evaporation of the solvents gave residues of 40, 130, and 15 g, respectively.

A portion of 110 g of the ethyl acetate extract was subjected to CC using silica gel 60 (0.04 – 0.063 mm, 120.0 g) and eluted with *n*-hexane/EtOAc and EtOAc/methanol gradients. Forty fractions of 300 mL each were collected and combined according to their TLC profiles monitoring in eight series, I to VIII. Crotonzambefuran B (**4**) (50 mg) and lupeol (**7**) (5 g) were directly filtered from fractions obtained with *n*-hexane/EtOAc (9:1) and (8.5:1.5), respectively. Series II [fractions obtained with *n*-hexane/EtOAc (9:1 – 8:2), 25 g] was subjected to CC on silica gel 60 (0.04 – 0.063 mm, 100 g), eluted with *n*-hexane/EtOAc (10:0 – 8:2). Fifty fractions of 100 mL each were obtained. $\beta$-Sitosterol (**8**) (10 mg), stigmasterol (**9**) (3 mg), 7-acetoxytrachiloban-18-oic acid (**5**) (30 mg), and 3-*O*-acetylaleuritolic acid (**6**) (100 mg) were obtained after filtration from the fractions eluted with *n*-hexane/EtOAc (9:1), (8.7:1.3), (8.4:1.6), and (8:2), respectively. Fractions eluted with *n*-hexane/EtOAc (9.3:0.7) were combined and filtered through Sephadex LH-20, eluted with $CHCl_3$/MeOH (7:3), to give crotonoligaketone (**1**) (30 mg). Series III [fractions obtained with *n*-hexane/EtOAc (7.5:2.5), 10.0 g)] was subjected to CC on silica gel 60 (0.04 – 0.063 mm, 30 g), eluted with $CHCl_3$/MeOH (10:0 – 8:2). Twenty fractions of 50 mL each were collected. Fractions obtained with $CHCl_3$/MeOH (9.5:0.5) and (9:1), respectively, were combined and filtered through Sephadex LH-20, eluted with $CHCl_3$/MeOH (7:3), to give imbricatadiol (**3**) (50 mg) and crotonadiol (**2**) (50 mg).

*Crotonoligaketone* (**1**): White amorphous powder. – $[\alpha]_D^{25} = -45.8°$ (*c* 0.1, MeOH). – UV (MeOH): $\lambda_{max}$ (log $\varepsilon$) = 207 (3.51), 249 nm (3.95). – IR (KBr): $\nu_{max}$ = 1748.12, 1742.36, 1721.15, 1663.54, 1634.33, 1251.74, 1220.95, 1215.85, 777.48 $cm^{-1}$. – NMR: see Table I. – EI-MS: $m/z$ (rel. int.) = 430.17 $[M]^+$ (7), 370.15 (38), 311.13 (62), 304.10 (88), 95.05 (91), 81.03 (100). – HR-EI-MS: $m/z$ = 430.1660 (calcd. for $C_{23}H_{26}O_8$, 430.1628).

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Agnaniet H., Akagah A., Mounzéo H., Menut C., and Bessière J.-M. (2005), Aromatic plants of tropical Central Africa. XLI. Volatile constituents of *Croton oligandrum* Pierre ex Hutch. growing in Gabon. J. Essent. Oil Res. **17**, 201 – 203.

Baker J. G. and Wright C. H. (1913), Flora of Tropical Africa, Vol. 6, Part 1. Royal Botanic Gardens, Kew (K), UK, p. 441.

Betti L. J., Yongo D. O., Mbomio O. D., Iponga M. D., and Ngoye A. (2013), An ethnobotanical and floristical study of medicinal plants among the Baka pygmies in the periphery of the Ipassa Biosphere Reserve, Gabon. Eur. J. Med. Plants **3**, 174 – 205.

Hutchinson L. J. and Dalziel J. M. (1958), Flora of West Tropical Africa, Revised by R. W. J. Keay, Vol. 1, Part 2, 2nd ed. White Press, London, UK, p. 221.

Irvine F. R. (1966), Woody Plants of Ghana. Oxford University Press, London, UK, p. 221.

Jiofack T., Ayissi I., Fokunang C., Nguedje N., and Kemeuze V. (2009), Ethnobotany and phytomedicine of the upper Nyongvalley forest in Cameroon. Afr. J. Pharm. Pharmacol. **3**, 144 – 150.

Lawrence J. A. and Zito S. W. (1976), Sterols and triterpenes from the fruits of *Artocapus altilis*. Phytochemistry **15**, 829 – 830.

Maciel M. A. M., Pinto A. C., and Kaiser C. R. (2003), NMR and structure review of some natural furoclerodanes. Magn. Reson. Chem. **41**, 278 – 282.

McLean S., Perpick-Dumont M., Reynolds W. F., Jacobs H., and Lachmansing S. S. (1987), Unambiguous structural and nuclear magnetic resonance spectral characterization of two triterpenoids of *Maprounea guianensis* by two-dimensional nuclear resonance spectroscopy. Can. J. Chem. **65**, 2519 – 2525.

Ngadjui B. T., Folefoc N. G., Keumedjio F., Dongo E., Sodengam B. L., and Connolly J. D. (1999), Crotonadiol, a labdane diterpenoid from the stem bark of *Croton zambesicus*. Phytochemistry **51**, 171 – 174.

Ngadjui B. T., Abegaz B. M., Keumedjo F., Folefoc N. G., and Kapche F. W. G. (2002), Diterpenoids from the stem bark of *Croton zambesicus*. Phytochemistry **60**, 345 – 349.

Reynolds W. F., Hughes D. W., Perpick-Dumount M., and Enriquez R. G. (1985), A pulse sequence for establishing carbon-carbon connectivities via indirect polarization transfer modulated by vicinal $^{1}$H-$^{1}$H coupling. J. Magn. Reson. **63**, 413.

Salatino A., Faria-Salatino M. L., and Negri G. (2007), Traditional uses, chemistry and pharmacology of *Croton* species (Euphorbiaceae). J. Braz. Chem. Soc. **18**, 11 – 33.

Schmeda-Hirschmann G., Astudillo L., Sepulveda B., Rodrıguez J. A., Theoduloz C., Yanez T., and Palenzuela J. A. (2005), Gastroprotective effect and cytotoxicity of natural and semisynthetic labdane diterpenes from *Araucaria araucana* resin. Z. Naturforsch. **60c**, 511 – 522.

Sommit D., Petsom A., Ishikawa T., and Roengsumran S. (2003), Cytotoxic activity of natural labdanes and their semi-synthetic modified derivatives from *Croton oblongifolius*. Planta Med. **69**, 167 – 170.