New *ent*-Clerodane and Abietane Diterpenoids from the Roots of Kenyan *Croton megalocarpoides* Friis & M. G. Gilbert

DOI 10.1055/s-0042-108857
Planta Med

Publishing House and Copyright:
© 2016 by
Georg Thieme Verlag KG
Rüdigerstraße 14
70469 Stuttgart
ISSN 0032-0943

New *ent*-Clerodane and Abietane Diterpenoids from the Roots of Kenyan *Croton megalocarpoides* Friis & M. G. Gilbert*

Authors Beth Ndunda[1,2], Moses K. Langat[2,3], Dulcie A. Mulholland[2,3], Harry Eastman[2], Melissa R. Jacob[4], Shabana I. Khan[4,5], Larry A. Walker[4,5], Ilias Muhammad[4], Leonidah O. Kerubo[1], Jacob O. Midiwo[1]

Affiliations The affiliations are listed at the end of the article

Key words
- *Croton megalocarpoides*
- Euphorbiaceae
- abietane
- *ent*-clerodane
- *ent*-trachylobane
- diterpenoids
- electronic circular dichroism

Abstract

The roots of the endangered medicinal plant *Croton megalocarpoides* collected in Kenya were investigated and twenty-two compounds isolated. Among them were twelve new *ent*-clerodane (**1**–**12**) and a new abietane (**13**) diterpenoids, alongside the known crotocorylifuran (**4a**), two known abietane and four known *ent*-trachylobane diterpenoids, and the triterpenoids, lupeol and acetyl aleurotolic acid. The structures of the compounds were determined using NMR, HRMS and ECD. The isolated compounds were evaluated against a series of microorganisms (fungal and bacteria) and also against *Plasmodium falciparum*, however no activity was observed.

Supporting information available online at http://www.thieme-connect.de/products

Introduction

In the continued search for bioactive compounds from African *Croton* species, we investigated the phytochemistry of the endangered Kenyan *Croton megalocarpoides* Friis & M.G. Gilbert (Euphorbiaceae). *C. megalocarpoides* is a monoecious shrub or tree, growing up to eight meters tall in rocky places and is restricted to the semi-evergreen coastal bush lands or forests of East Kenya and South Somalia [1]. Taxonomically this species is related to *Croton mayumbensis* J.Léonard, *Croton mubango* Müll.Arg. and *Croton megalocarpus* Hutch., a plant that it is often confused with. All these species possess a grey scaly bark, their leaves are silvery beneath and produce *tri*-lobed fruits [2]. The *Croton* genus consists of over 1300 species of monoeceous and dioeceous trees, shrubs and herbs that are distributed worldwide with extreme diversity, especially in the West Indies, Southern Brazil, and Madagascar and are known for their ethno-medicinal value [3,4]. *C. megalocarpoides* is one of the fifteen *Croton* species that occur in Kenya and there are no reports of any ethno-medicinal usage for this species. However, the stem bark and the leaves of the related *C. mayumbensis* are used to treat microbial infections and human parasitic diseases [5] and the leaf sap of *C. megalocarpus* is used topically in East Africa to treat bleeding wounds, as an anthelmintic agent, and for the treatment of whooping cough [6].

Results and Discussion

In this study, twenty-two compounds were isolated from the roots of *C. megalocarpoides* including twelve new *ent*-clerodane diterpenoids (**1**–**12**), a new abietane diterpenoid (**13**), the known crotocorylifuran (**4a**) [7] (**Fig. 1**), the two known abietane diterpenoids, isolophanthin A [8] and abietic acid [9], four known *ent*-trachylobane diterpenoids, *ent*-3α,18-dihydroxytrachylobane [10], *ent*-trachyloban-18-ol [10], *ent*-trachyloban-18-oic acid [10,11], *ent*-3α-hydroxytrachyloban-18-al [12] and the triterpenoids, lupeol [13] and acetyl aleurotolic acid [14,15].

Compound **1** was isolated as a colourless oil and was determined to have a molecular formula of $C_{21}H_{28}O_5$ from HR-ESI-MS. The IR spectrum showed absorption bands for the carbonyl group of an ester at 1713 cm^{-1}, and at 3430 and 1695 cm^{-1} for a carboxylic acid group. The NMR spectra confirmed the presence of a furanoclerodane structure by resonances attribut-

received March 3, 2016
revised May 9, 2016
accepted May 10, 2016

Bibliography
DOI http://dx.doi.org/10.1055/s-0042-108857
Published online
Planta Med © Georg Thieme Verlag KG Stuttgart · New York · ISSN 0032-0943

Correspondence
Prof. Jacob O Midiwo
Department of Chemistry
University of Nairobi
Private Bag 30197–00100
Nairobi
Kenya
Phone: + 254 7 22 77 66 82
jmidiwo@uonbi.ac.ke

* Dedicated to Professor Dr. Dr. h.c. mult. Kurt Hostettmann in recognition of his outstanding contribution to natural product research.

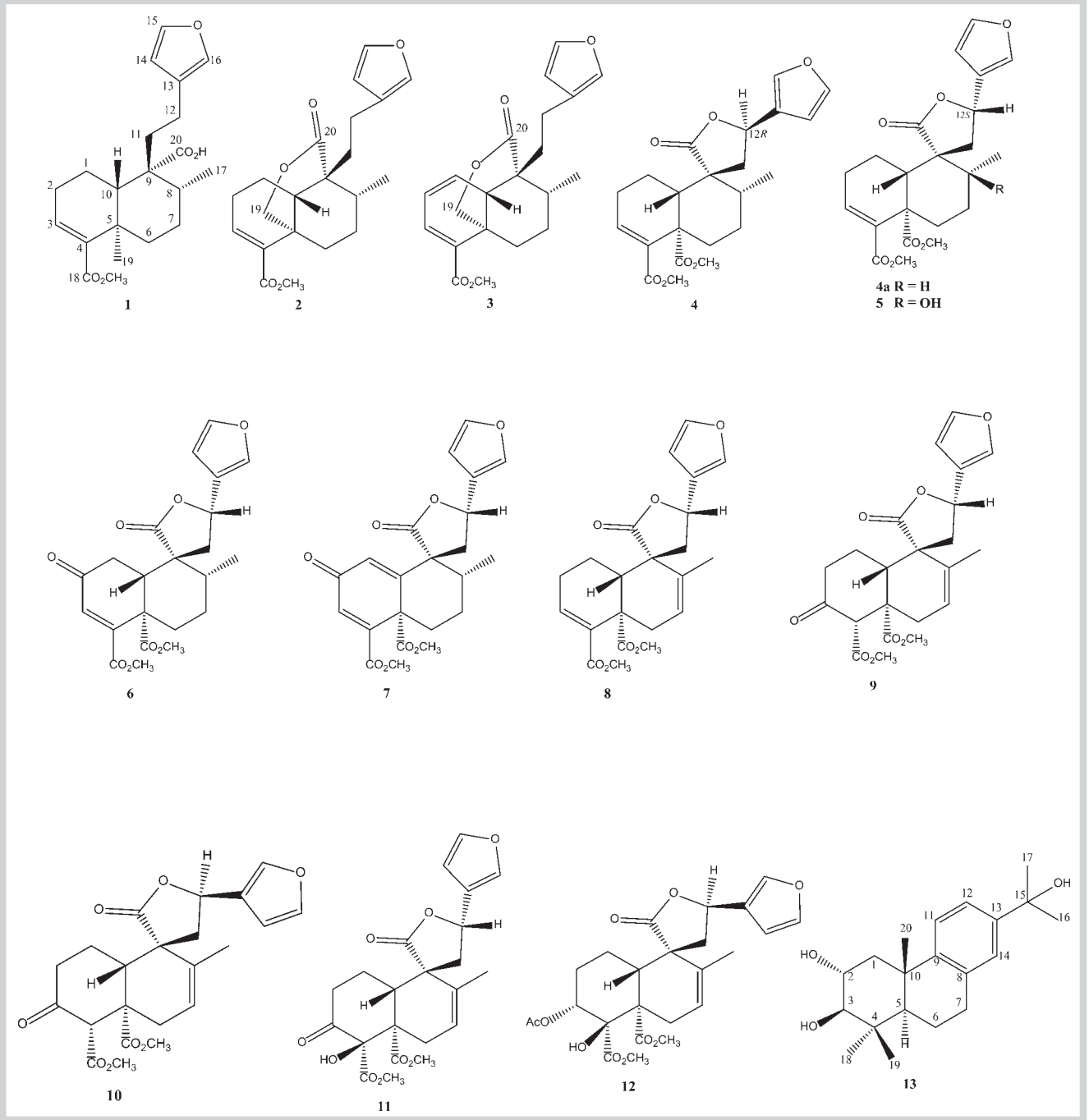

Fig. 1 Chemical structures for compounds isolated from *C. megalocarpoides*.

able to a β-substituted furan ring at δ_H 6.27 (br s, $W_{1/2}$ = 3.7 Hz, H-14), 7.23 (br s, $W_{1/2}$ = 3.4 Hz, H-16) and 7.35 (t, J = 1.6 Hz, H-15). The 3H-17 methyl group doublet (δ_H 1.15, d, J = 7.0 Hz) showed correlations in the HMBC spectrum with C-9 (δ_C 50.0) and C-8 (δ_C 37.3) resonances. Additional correlations were seen between the C-9 and H-10 (δ_H 1.62, m), H-8 (δ_H 1.61, m) and two H-7 (δ_H 2.15, δ_H 2.35) resonances, between the C-10 and 3H-19 (1.22, s) and H-8 resonances, between the C-20 carboxylic acid carbon (δ_C 182.9), H-10 and the two H-7 resonances, and between H-10 and C-4, whose chemical shift indicated the presence of a Δ^3-alkene (C-3, δ_C 137.6; C-4, 141.5). The corresponding H-3 alkene resonance (δ_H 6.67, dd J = 2.5, 5.0 Hz) showed correlations with the C-5 (δ_C 38.4) and C-18 (δ_C 167.9) carbonyl carbon resonances. A correlation between a methoxy group proton resonance (δ_H 3.69) and the C-18 resonance indicated the presence of a C-18 methyl ester.

The relative configuration of compound **1** was assigned using the NOESY experiment. Unfortunately the H-10 and H-8 resonances were too close to see a correlation, but correlations were seen between the 3H-17/H-8, H-8/H-11$_A$ (δ_H 2.35), H-11$_B$ (δ_H 1.20)/H-10 and, H-10/H-1β resonances, as expected for a clerodane diterpenoid (**Fig. 1 S**, Supporting Information). A specific rotation value of $[\alpha]_D$ −61.5° was measured for this compound indicating that compound **1** belonged to the *ent*-series as with the reported *ent*-

clerodane, laevinoid [16]. The negative Cotton effect at 240 nm obtained from electronic circular dichroism spectroscopy further supported this assignment. Compound **1,** 18-methoxycarbonyl-15,16-epoxy-*ent*-cleroda-3,13(16),14-trien-20-oic acid, was given the trivial name megalocarpoidolide A.

Compound **2** was found to be the 19,20-lactone derivative of compound **1** and was isolated as a white solid. HR-ESI-MS indicated a molecular formula of $C_{21}H_{26}O_5$ and the IR spectrum showed an absorption band at 1707 cm^{-1} for the carbonyl stretch of an ester group. The 3H-19 methyl group singlet present in compound **1** was replaced with an oxygenated methylene group (δ_H 4.40, dd, J = 12.0, 2.3 Hz; 4.82, d, J = 12.0 Hz, two H-19) in compound **2**, with a corresponding C-19 resonance at δ_C 75.7. The C-20 carboxylic acid group carbon resonance, present at δ_C 182.9 in compound **1,** had shifted upfield to δ_C 173.2 in compound **2**, and this, along with the molecular formula, indicated that a C-19, 20-lactone was present. The 2H-19 proton resonances showed a correlation in the HMBC spectrum with the C-20 carbonyl carbon resonance confirming this. The relative configuration for compound **2** was confirmed using the NOESY experiment, which showed correlations between one of the H-19's (δ_H 4.40, dd, J = 12.0, 2.3 Hz) and the H-1α (δ_H 1.37 m) resonance, the other H-19 (δ_H 4.82, d, J = 12.0 Hz) with H-6α (2.52 m) and H-7α (δ_H 1.57) resonances, between the 3H-17/2H-7 resonances, between the H-10/2H-11 resonances, and between the H-10/H-8 resonances (**Fig. 2 S**, Supporting Information). The ECD spectrum for this compound also gave a negative Cotton effect at 240 nm. This compound, 18-methoxycarbonyl-15,16-epoxy-*ent*-cleroda-3,13 (16),14-triene-,20,19-olide was given the trivial name, megalocarpoidolide B.

Compound **3**, megalocarpoidolide C, was the Δ^1 derivative of compound **2**, that was isolated as a white solid and assigned a molecular formula of $C_{21}H_{24}O_5$ from ESIMS, indicating an extra double bond compared to compound **2**. In addition to the characteristic resonances observed for compound **2**, two additional alkene carbon resonances were present at δ_C 125.7 (C-1) and δ_C 130.3 (C-2) with corresponding proton resonances partially overlapping at δ_H 6.20 and δ_H 6.22. These overlapped resonances showed coupling in the COSY spectrum with the H-10 (δ_H 2.75, br s, $W_{1/2}$ = 4.3 Hz) and H-3 (δ_H 6.83) resonances. These assignments were confirmed by the HMBC spectrum which showed correlations between H-1 and C-3 (δ_C 133.2), C-5 (δ_C 36.5), C-9 (δ_C 48.0), and C-10 (δ_C 43.9) and H-2 and C-3, C-4 and C-10 and between H-3 and C-1, C-2, C-5, C-18 (δ_C 167.4) and C-19 (δ_C 73.2). The relative configuration for compound **3** was the same as for compound **2** with correlations between one of the H-19's (δ_H 4.48, dd, J = 11.5, 1.0 Hz) with the H-6α (δ_H 2.79 m) and H-7α (δ_H 1.62) resonances, between the 3H-1 and 2 H-7 resonances, between the H-10 and 2H-11 resonances, and between the H-10 and H-8 resonances for the *ent*-clerodane diterpenoid, 18-methoxycarbonyl-15,16-epoxy-*ent*-cleroda-1,3,13(16),14-tetraen-20,19-olide, or megalocarpoidolide C.

Compound **4**, and **4 a** were both isolated as white solids, and were assigned the molecular formula of $C_{22}H_{26}O_7$ from HR-ESI-MS. The NMR spectra were very similar. Compound **4** displayed IR absorptions at 1715 and 1767 cm^{-1} for ester and lactone carbonyl stretches, respectively. Analysis of the 2D NMR spectra for compound **4** indicated that it was also a furanoclerodane diterpenoid with a β-substitued furan ring, but instead of a 20,19 lactone as in compounds **2** and **3**, a 20,12-lactone was present in compounds **4**–**12**. Three ester carbonyl carbons were present at δ_C 166.9, 173.3, and 176.5 and an oxymethine resonance was present at δ_C 71.9. As with the earlier compounds described, a methyl ester was placed at C-18 (δ_C 166.9, δ_H 3.68) in conjunction with a Δ^3 double bond (140.5, C-3; δ_C 137.2, C-4; δ_H 6.78, dd, J = 3.1; 6.9 Hz, H-3) and the C-19 methyl group was converted to a methyl ester (δ_C.173.3, δ_H 3.76). The ^{1}H NMR spectrum showed the presence of a secondary methyl group (δ_H 1.10, d, J = 6.8 Hz), ascribed to 3H-17, which was seen to be coupled to the H-8 (1.66 m) resonance. The HMBC spectrum showed correlations between the H-3 resonance and the C-18 carbonyl carbon and C-5 (δ_C 46.2) resonances. The C-5 resonance, in turn, showed correlations with the H-10 (δ_H 1.58 m) resonance which showed correlations with the C-19 carbonyl, (δ_C 173.3), C-9 (δ_C 51.4), C-11 (δ_C 42.5), and C-8 (δ_C 43.0) resonances. The C-9 resonance showed correlations with the 3H-17, H-10 and H-12 (δ_H 5.41, t, J = 8.4 Hz) resonances and the H-8, H-10, and H-12 resonances showed a correlation with the C-20 (δ_C 176.5) resonance, confirming the presence of a 20,12-lactone ring. The H-12 resonance also showed correlations with the C-14 and C-16 protons of the furan ring, confirming its assignment. For compounds **4**–**12** the NOESY spectrum was used to determine the configuration at C-12. A 3D model showed that for the 12*R* configuration a correlation would be seen between the H-12 and 3H-17 resonances, however if the 12*S* configuration was present, a correlation would be seen between the H-12 and the H-1α resonances. For compound **4** the 3H-17 resonance showed a strong correlation with the H-12 resonance, confirming the 12*R* configuration.

Compound **4 a** was found to be the C-12*S* epimer of compound **4** and was identified as the known compound crotocorylifuran. Crotocorylifuran was previously reported from the African species *Croton zambesicus* Müll.Arg. [7] and *Croton haumanianus* J. Léonard [17]. The NOESY spectrum for compound **4 a** showed a correlation between the H-1α resonance (δ_H 1.89) and the H-12 resonance (δ_H 5.43, t, J = 8.4 Hz), confirming the 12*S*-configuration. Thus compound **4** was a new C-12*R* epimer of the known crotocorylifuran **4 a** and was named 12-*epi*-crotocorylifuran.

Compound **5**, isolated as a white solid, was assigned a molecular formula of $C_{22}H_{26}O_8$ from HR-ESI-MS. The IR spectrum showed absorption bands that were similar to those of **4**. The ^{1}H and ^{13}C NMR spectroscopic data for compound **5** were similar to those of **4** and **4 a**, however, the 3H-17 doublet methyl proton resonance was replaced by a three proton singlet resonance (δ_H 1.26, s) in the ^{1}H NMR spectrum and an extra quaternary oxygenated carbon was present in the ^{13}C NMR spectrum (δ_C 72.7) which could be ascribed to C-8. Thus a hydroxyl group was placed at this position. The NOESY spectrum showed correlations similar to those observed for **4 a** where the H-12 oxymethine proton resonance (δ_H 5.39 t, J = 8.4 Hz) showed a correlation with the H-1α resonance (δ_H 1.84, m), enabling the configuration at C-12 to be determined as *S*. No correlation was seen between the H-10 and 3H-17 resonances, hence the 3H-17 group was assigned as α as in compounds **1**–**7** and the hydroxyl group was assigned as β. Compound **5**, 8β-hydroxycrotocorylifuran, has not been described previously.

Compound **6** was isolated as a white solid and assigned a molecular formula of $C_{22}H_{24}O_8$ from HR-ESI-MS. Compound **6** differed from compound **4 a** in having a ketone group at C-2, shown by a ketone carbonyl carbon resonance at δ_C 198.0 the ^{13}C NMR spectrum. The C-3 and C-4 resonances shifted from δ_C 140.3 and 136.5 in compound **4 a** to δ_C 131.8 and 153.2 in compound **6**. The ketone group was placed at C-2 due to correlations seen in the HMBC spectrum with the H-10 (δ_H 2.39, dd, J = 4.2, 17.0 Hz) and H-3 (δ_H 6.46, br s) proton resonances. The H-10 and the two

H-1 resonances showed a clear AMX coupling pattern (δ_H 2.72, dd, J = 4.2, 17.0 Hz, H-1α; δ_H 3.37 dd, J = 17.0, 17.0 Hz,). The NOESY spectrum showed a correlation between H-12 and the H-1α proton resonances, confirming the 12 *S*-configuration. Compound **6**, crotocorylifuran-2-one, has not been reported previously.

Compound **7** was isolated as a white solid and assigned a molecular formula of $C_{22}H_{22}O_8$ from HR-ESI-MS. The IR spectrum showed absorption bands at 1769, 1730, and 1663 cm^{-1} for carbonyl stretches of lactone, ester and ketone functional groups. The NMR spectra differed from those of compound **6** in that the H-10/2H-1 AMX system was no longer present, and instead of one alkene proton resonance, two were present (δ_H 6.76, br s, $W_{1/2}$ = 3.5 Hz; 6.86. br s, $W_{1/2}$ = 3.4 Hz) and two trisubstituted alkene double bonds were evident in the ^{13}C NMR spectrum (δ_C 128.0, 131.6, 150.7, 155.5). The double bonds were placed at $\Delta^{10,1}$ and Δ^3 as the two alkene resonances proton resonances both showed a correlation in the HMBC spectrum with a ketone carbonyl carbon resonance at δ_C 185.9, assigned as C-2, which had been shifted upfield from δ_C 198.0 in compound **6**. The shielded chemical shift of this ketone carbon resonance was attributed to the extra conjugation in ring A and has previously been noted in the clerodane diterpenoid, crotonoligaketone, isolated from the West African *Croton oligandrus* [18]. The ^1H and ^{13}C NMR chemical shifts for ring B, the lactone and furan ring for this compound, were similar to those of compound **6** and crotocorylifuran. The NOESY spectrum supported the 12*S* configuration as in compound **4 a** and **6**. This compound was a new crotocorylifuran derivative, 1,2-dehydro crotocorylifuran-2-one (megalocarpoidolide D).

Compound **8**, isolated as a white solid, was assigned a molecular formula of $C_{22}H_{24}O_7$ from HR-ESI-MS analysis. The IR spectrum showed absorption bands that were similar to those of crotocorylifuran. The structure differed from compound **4 a** in having a double bond at C-7. The ^1H NMR spectrum showed a vinyl methyl group proton resonance (δ_H 1.66, t, J = 1.1 Hz, 3H-17), which showed correlations in the HMBC spectrum with the alkene carbon resonances at δ_C 127.3 (C-7) and 130.6 (C-8) and a fully substituted carbon resonance at δ_C 53.0 (C-9). The 12*S* configuration was confirmed due to the observed correlations in the NOESY spectrum between the H-12 (δ_H 5.50, t, J=8.3 Hz) and the H-1α proton resonance (δ_H 1.78). The structure of compound **8** was thus determined as 7,8-dehydrocrotocorylifuran.

Compounds **9** and **10** were found to be C-12 epimers and their MS analysis indicated that both compounds had a molecular formula of $C_{22}H_{24}O_8$. The IR spectra were similar with carbonyl stretch bands at 1748 and 1720 cm^{-1}. The ^{13}C NMR spectrum of compound **9** was similar to that of compound **8** but showed no resonances for the Δ^3 double bond and showed a ketone carbon resonance at δ_C 200.9 (201.0 for compound **10**). This ketone resonance was assigned as C-3 and a proton singlet (δ_H 3.23) was assigned as H-4 with the corresponding C-4 resonance occurring in a downfield position at δ_C 67.0 (δ_C 67.2 for **10)**, due to adjacent ketone and ester groups. This assignment was supported by correlations seen in the HMBC spectrum between H-4 and the C-3 and C-18 (δ_C 168.0) carbonyl resonances and the coupling seen in the COSY spectrum between the H-10 (2.35)/two H-1 (2.45, 2.20), and two H-1/two H-2 (2.46, 2.76) resonances for compound **9**. The NOESY spectrum for compound **9** showed correlations between the H-12 oxygenated proton resonance (δ_H 5.47, t J = 8.6), and the H-1α resonance at (δ_H 2.20) confirmed the configuration at C-12 as *S*. The NOESY spectrum of **10** indicated a correlation between the H-12 (δ_H 5.51) and the H-1α resonance (δ_H 2.02) for compound **10**, indicating the 12*R* configuration. In both cases a strong correlation was seen between the H-4 and H-10 resonances, establishing H-4 as β and the methyl ester as α. Compound **9** was named as megalocarpoidolide E while compound **10** was named as megalocarpoidolide F.

Compound **11,** megalocarpoidolide G, isolated as a white solid, was found to be the 4β-hydroxy derivative of compound **9** and was assigned a molecular formula of $C_{22}H_{24}O_9$ from HR-ESI-MS. In addition to the esters (1747, 1719 cm^{-1}) and ketone (1658 cm^{-1}) absorption bands, an absorption band at 3353 cm^{-1}, characteristic of a hydroxyl group stretch, was seen in the IR spectrum. The ^1H and ^{13}C NMR chemical shifts followed patterns similar to those of compound **9** except for the presence of a fully substituted oxygenated carbon resonance at δ_C 82.7 and the absence of a C-4 methine carbon resonance, which occurred at δ_C 67.0 in compound **9**. A proton resonance at δ_H 4.20 (s) that did not correlate to any carbon resonance in the HSQC spectrum, was attributed to a tertiary hydroxyl group proton and showed correlations in the HMBC spectrum with carbon resonances at 201.9 (C-3), 171.3 (C-18), 82.7 (C-4), and 53.3 (C-5), hence a hydroxyl group was placed at C-4. The NOESY spectrum showed similar correlations to those observed for **9**, with correlations seen between the H-12 resonance (δ_H 5.47, t, J = 8.0 Hz) and the H-1α resonance (δ_H 2.14), indicating the 12*S*-configuration. The configuration of the C-4 hydroxy group was assigned as β due to correlations seen in the NOESY spectrum between the hydroxyl group proton resonance and the H-10 (δ_H 2.79, dd J = 4.8, 13.4 Hz) resonance.

Compound **12,** isolated as a white solid, was assigned a molecular formula of $C_{24}H_{28}O_{10}$ from its HR-ESI-MS. The ^1H NMR spectrum differed from that of compound **11** in lacking the C-3 keto group which was replaced by an acetate group (δ_H 2.01, s, 171.3). A proton resonance at δ_H 5.08 was assigned as H-3 and the HMBC spectrum showed correlations between H-3 and C-18 carbonyl carbon (δ_C 173.2) and the acetate carbonyl resonance. As with compounds **4** and **10**, the NOESY spectrum showed correlations between the H-12 oxygenated methine proton resonance (δ_H 5.45, t, J = 8.9 Hz) and the 3H-17 resonance, establishing the configuration at C-12 as *R*. The C-4 hydroxy group proton resonance (δ_H 3.82, s) showed correlations in the HMBC spectrum with the C-18, C-3 (δ_C 70.5), C-4 (δ_C 76.1), and C-5 (δ_C 52.1) resonances and correlations were seen in the NOESY spectrum between 4-OH/ and H-10 (δ_H 2.52) and, H-3β (δ_H 5.08, t, J = 2.6 Hz), and H-3β/ H-1β and H-1β/H-10, thus confirming both its configuration as β, and also the α orientation of the acetate group at C-3. Thus the structure was determined to be the 3α-acetoxy, 4β-hydroxy derivative of compound **11** or megalocarpoidolide H.

Compound **13** was obtained as a white solid and assigned the molecular formula of $C_{20}H_{30}O_3$ from HR-ESI-MS. This compound showed an IR absorption band at 3400 cm^{-1} for a hydroxyl group stretch. The presence of three aromatic proton resonances in the ^1H NMR spectrum (δ_H 7.23, 7.22 and 7.17) in conjunction with an isopropyloxy group indicated the presence of an abietane diterpenoid. Equivalent methyl group singlets (δ_H 1.58) were assigned to 3H-16 and 3H-17 of the isopropyloxy group, with C-15 (δ_C 72.5) showing correlations in the HMBC spectrum with the H-12 (δ_H 7.22) and H-14 (δ_H 7.17) resonances, and 3H-16/3H-17 resonance showing a correlation with the C-15 (δ_C 72.5) and C-13 resonances (δ_C 146.2). Two oxygenated methine resonances at δ_H 4.22 and 3.24, were seen to be coupled in the COSY spectrum and were assigned as H-2 and H-3 respectively. The corresponding C-3 resonance (δ_C 78.4) showed correlations with the H-5 (δ_H

1.42, dd, J = 2.5, 11.8 Hz), 3H-18 (δ_H 1.11, s) and 3H-19 (δ_H 1.08, s) resonances, the H-2 resonance showed a correlation with the C-4 (δ_C 38.5) resonance and the C-5 resonance showed correlations with the 3H-20 (δ_H 1.44, s), 3H-18 and 3H-19 resonances. The structure of this compound was determined to be a 2,3,15-trihydroxylated abietan-8,11,13-triene. The NOESY spectrum was used to assign the relative configuration of this compound. Correlations were observed in the NOESY spectrum between 3H-19/3H-20 and 3H-20/H-2 resonances establishing H-2 as *β*, and hence the hydroxyl group as *α* and between H-3/3H-18 and H-3/H-5 resonances, establishing H-3 as *α* and the 3-OH group as *β*. This compound was a novel diterpenoid, abieta-8,11,13-triene-2*α*,3*β*,15-triol, named isolophanthin E. Isolophanthin A (abieta-8,11,13-triene-3*β*,15-diol) and abietic acid were also isolated from this plant.

All isolated diterpenoids (**1**–**19, 4 a**) were tested for their antibacterial and antifungal activities against a panel of human pathogenic bacterial and fungal strains including *Candida albicans, Candida glabrata, Candida krusei, Aspergillus fumigatus, Cryptococcus neoformans, Staphylococcus aureus*, methicillin-resistant *S. aureus, Escherichia coli, Pseudomonas aeruginosa*, and *Mycobacterium intracellulare*. The antimalarial activity was also determined against chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum*. Cytotoxic activity was determined against VERO cells. All compounds were inactive at the highest tested concentration of 20 µg/mL (~ 50 µM) against bacteria and fungi and 4.76 µg/mL (~ 12 µM) against *P. falciparum* D6 and W2 strains. No cytotoxic activity was observed towards VERO cells up to 4.76 µg/mL (~ 12 µM).

C. megalocarpoides is a rich source of diterpenoids. Twenty-two compounds were isolated from the roots of *C. megalocarpoides* including twelve new *ent*-clerodane diterpenoids (**1**–**12**), a new abietane diterpenoid (**13**), the known crotocorylifuran (**4 a**), the two known abietane diterpenoids, isolophanthin A and abietic acid, four known *ent*-trachylobane diterpenoids, *ent*-3*α*,18-dihydroxytrachylobane, *ent*-trachyloban-18-ol, *ent*-trachyloban-18-oic acid, *ent*-3*α*-hydroxytrachyloban-18-al and the triterpenoids, lupeol and acetyl aleurotolic acid.

Materials and Methods

General experimental procedures

Optical rotations were measured on a JASCO P-2000 polarimeter at 21 °C and IR spectra recorded using a Perkin-Elmer (2000) spectrometer. 1D and 2D NMR spectra were recorded in $CDCl_3$ on a 500 MHz Bruker AVANCE NMR instrument at room temperature. Chemical shifts (δ) are expressed in ppm and referenced against the $CHCl_3$ resonances at 7.26 and 77.23 ppm for ^{1}H and ^{13}C-NMR respectively. The ESIMS were recorded on a Micromass Quattro Ultima LCMS with electrospray ionization and EIMS spectra were recorded on an Agilient 7890A instrument. Column chromatography was done using Merck silica gel 60 (0.063–0.200 mm) and Fluka Sephadex LH-20 as stationary phases. Analytical TLC was carried out using aluminium plates (0.25 mm) coated with silica gel (60 F254, Merck). Compounds were visualized under UV light at 254 or 365 nm, followed by spraying with 1% vanillin-H_2SO_4 spray reagent and heating.

Plant material

The roots of *C. megalocarpoides* were collected in July 2009 from the Kenyan Coastal region. The plant was identified at the University of Nairobi Herbarium in the School of Biological Studies and a voucher specimen, BN 2009/8, was deposited.

Extraction and isolation of compounds from *Croton megalocarpoides*

The root bark of *C. megalocarpoides* was air dried in the shade for 4 weeks after which it was ground into a fine powder. The fine powder (500 g) was sequentially extracted by cold percolation at room temperature with *n*-hexane, dichloromethane, and methanol (2 L solvent each, 24 h each). The extracts were concentrated using a rotary evaporator, combined and dried, yielding 9 g (1.8%) *n*-hexane, 47 g (9.4%) dichloromethane (DCM) and 16 g (3.2%) methanol extracts. From the DCM extract, 30 g of extract was adsorbed onto 30 g silica gel and subjected to CC on a silica gel column (300 g, 5 cm diameter × 35 cm long). Fractionation was done using a solvent gradient starting with *n*-hexane and gradually increasing the polarity using ethyl acetate. The volumes of the fractions collected were 75 mL. The process was monitored using analytical TLC plates. Purification of the fractions was done using a DCM/diethyl ether solvent system of varying ratios to afford two phytosterols, sitosterol (4.5 mg) and stigmasterol (4.1 mg) and two triterpenoids, acetylaleuritolic acid (12.9 mg) and lupeol (3.6 mg) in addition to the following compounds: megalocarpoidolide A (**1**; 84.4 mg), megalocarpoidolide B (**2**; 6.7 mg), megalocarpoidolide C (**3**; 16.7 mg), crotocorylifuran (**4 a**; 58.8 mg), 12-*epi*-crotocorylifuran (**4**; 13.4 mg), 8*β*-hydroxycrotocorylifuran (**5**; 3.5 mg); crotocorylifuran-2-one (**6**; 3.5 mg); megalocarpoidolide D (**7**; 16.8 mg), 7,8-dehydrocrotocorylifuran (**8**; 5.1 mg); megalocarpoidolide E (**9**; 14.3 mg), megalocarpoidolide F (**10**; 38.5 mg), megalocarpoidolide G (**11**; 23.3 mg), megalocarpoidolide H (**12**; 50.9 mg), isolophanthin E (**13**; 10.9 mg), isolophanthin A (6.1 mg), abietic acid (4.3 mg), 3*α*,18-dihydroxytrachylobane (13.0 mg), *ent*-trachyloban-18-ol (4.1 mg), *ent*-trachyloban-18-oic acid (3.8 mg), and *ent*-3*α*-hydroxytrachyloban-18-al (6.5 mg).

Megalocarpoidolide A (**1**): Colourless oil; $[\alpha]_D$ −61.5° ($CHCl_3$, *c* 0.00093); IR $\nu_{max}\cdot cm^{-1}$ (neat): 2923, 2853, 2400, 1713, 1695, 1251 cm^{-1}; CD Cotton effects: −2.80 (235 nm), +0.55 (210 nm), -0.95 (190 nm); ^{1}H NMR (500 MHz, $CDCl_3$) see **Table 2**;^{13}C NMR (500 MHz, $CDCl_3$) see **Table 1**. HR-ESI-MS *m/z* 359.18670 $[M-H]^+$ (calcd. for $C_{21}H_{27}O_5$, 359.18640).

Megalocarpoidolide B (**2**): White solid; $[\alpha]_D$ −57.6° ($CHCl_3$, *c* 0.00061); IR $\nu_{max}\cdot cm^{-1}$ (neat): 2927, 2867, 1707, 1200; CD Cotton effects: −0.81 (285 nm), -3.45 (238 nm), -2.91 (210 nm), +3.07 (187 nm); ^{1}H NMR (500 MHz, $CDCl_3$) see **Table 2**; ^{13}C NMR (500 MHz, $CDCl_3$) see **Table 1**. HR-ESI-MS *m/z* 359.18547 $[M+H]^+$ (calcd. for $C_{21}H_{27}O_5$, 359.18530).

Megalocarpoidolide C (**3**): White solid; $[\alpha]_D$ −51.7° ($CHCl_3$), *c* 0.0017); IR $\nu_{max}\cdot cm^{-1}$ (neat): 2927, 2851, 1707; ^{1}H NMR (500 MHz, $CDCl_3$) see **Table 2**; ^{13}C NMR (500 MHz, $CDCl_3$) see **Table 1**. ESIMS *m/z* 379.15174 for $[M+Na]^+$ (calcd. for $C_{21}H_{24}NaO_5$, 379.15160).

12-Epi-crotocorylifuran (**4**): White solid; $[\alpha]_D$ −50.4° ($CHCl_3$), *c* 0.0012); IR $\nu_{max}\cdot cm^{-1}$ (neat): 2923, 2851, 1767, 1715; ^{1}H NMR (500 MHz, $CDCl_3$) see **Table 2**; ^{13}C NMR (500 MHz, $CDCl_3$) see **Table 1**; HR-ESI-MS *m/z* 425.15648 for $[M+Na]^+$ (calcd. for $C_{22}H_{26}NaO_7$,425.15707).

Crotocorylifuran (**4 a**): White solid; $[\alpha]_D$ −10.8° ($CHCl_3$, *c* 0.00059); IR $\nu_{max}\cdot cm^{-1}$ (neat): 2924, 2851, 1763, 1715; ^{1}H NMR (500 MHz, $CDCl_3$) see **Table 2**; ^{13}C NMR (500 MHz, $CDCl_3$)

Table 1 ^{13}C NMR chemical shifts for compounds (**1–13, 4 a**) isolated from *C. megalocarpoides*.

No.	1	2	3	4	4 a	5	6	7	8	9	10	11	12	13
1	19.6	19.6	125.7	18.5	19.2	19.0	36.9	128.0	19.4	24.6	23.4	23.8	27.2	42.8
2	27.3	26.4	130.3	26.8	26.5	26.5	198.0	185.9	26.7	39.7	40.0	35.0	21.3	71.5
3	137.6	139.6	133.2	140.5	140.3	140.8	131.8	131.6	140.3	200.9	201.0	201.9	70.5	78.4
4	141.5	136.4	135.4	137.2	136.5	136.0	153.2	150.7	135.6	67.0	67.2	82.7	76.1	38.5
5	38.4	36.5	36.5	46.2	46.3	46.2	47.8	53.6	45.6	49.5	49.5	53.3	52.1	49.9
6	34.1	35.6	34.0	32.1	32.3	27.1	31.2	33.2	33.4	33.7	33.7	26.6	29.9	18.8
7	27.7	29.7	29.9	28.2	28.0	34.2	27.5	26.6	127.3	125.4	124.5	125.6	125.2	30.0
8	37.3	37.2	37.3	43.0	40.2	72.7	39.5	39.4	130.6	131.0	131.0	130.2	132.2	134.5
9	50.0	48.9	48.0	51.9	51.4	55.8	51.2	55.1	53.0	53.1	52.5	53.4	53.4	148.6
10	48.7	43.7	43.9	49.7	52.0	46.6	50.2	155.5	50.5	49.5	47.4	43.3	46.3	37.0
11	36.7	29.4	29.3	42.5	42.5	39.3	41.5	39.0	42.4	42.1	42.4	42.0	45.1	125.1
12	18.1	17.5	17.6	71.9	72.0	72.2	72.0	71.4	72.0	72.4	72.4	72.5	71.7	122.3
13	124.6	124.5	124.3	125.8	125.6	125.5	125.0	123.6	125.8	125.5	125.2	125.4	125.6	146.2
14	111.0	111.0	111.0	108.0	108.3	108.4	108.2	108.2	108.2	108.2	108.1	108.2	108.3	124.8
15	143.1	143.2	143.2	144.1	144.3	144.3	144.4	144.5	144.4	144.5	144.5	144.4	144.3	72.5
16	138.2	138.8	138.9	139.1	139.6	139.7	140.0	140.6	139.5	139.8	139.5	139.7	139.9	31.8
17	16.7	16.6	16.4	17.4	17.2	26.6	17.1	17.1	19.8	20.0	20.0	20.0	19.6	31.8
18	167.9	167.3	167.4	166.9	167.0	166.8	166.1	165.4	166.7	168.0	168.0	171.3	173.2	17.2
19	18.3	75.7	73.2	173.3	173.1	173.2	170.0	166.4	172.1	171.1	171.3	170.5	170.3	30.0
20	182.9	173.2	173.0	176.5	176.4	176.4	175.7	172.4	176.0	176.0	175.6	176.3	175.4	27.0
CO-Ac													171.3	
CH_3-Ac													21.3	
18-OCH_3	51.4	51.9	52.0	51.9	51.8	52.0	52.8	53.1	51.9	52.5	52.5	54.2	53.4	
19-OCH_3				51.6	51.8	51.8	52.8	53.3	52.4	52.5	52.5	52.3	53.7	

Table 2 ^{1}H NMR chemical shifts for compounds (**1–4, 4 a, 5, 6**) isolated from *C. megalocarpoides*.

No.	1	2	3	4	4 a	5	6
1α	1.80 m	1.37 m	6.20*	1.69 m	1.89 m	1.84 m	2.72 dd, 4.2, 17.0
1β	1.99 m	1.92 m		2.43 m	2.62 m	2.59 m	3.37 dd, 17.0, 17.0
2α	1.45 m	2.24 m	6.22*	2.26 m	2.37 m	2.42 m	–
2β	2.16 m	2.40 m		2.47 m	2.53 m	2.56 m	–
3	6.67 dd 2.5, 5.0	6.84 dd, 2.5, 5.0	6.83 m	6.78 dd, 3.1, 6.9	6.82 dd, 3.3, 4.2	6.86 dd 3.1, 4.2	6.46 br s $W_{1/2}$ = 3.3
4	–	–	–	–	–	–	–
5	–	–	–	–	–	–	–
6α	2.29 m	2.52 m	2.79 m	2.95 dt, 13.0, 3.5	2.91 dt, 13.3, 3.3	2.82 dt, 3.9, 13.3	2.85 br d, $W_{1/2}$ = 20.8
6β	1.97 m	1.33 m	1.60 m	1.08 dt 3.5, 13.0	1.09 dt 3.5, 13.3	1.46 dd 3.5, 13.3	1.29 m
7α	2.15 m	1.57 m	1.62 m	1.53 m	1.56 m	1.57 dt, 3.5, 14.4	1.62 m
7β	2.35 m	1.75 m	1.80 m	2.28 m	2.43 m	2.88 dd, 3.9, 14.4	2.44 m
8	1.61 m	1.91 m	1.94 m	1.66 m	1.57 m	–	1.62 m
9	–	–	–	–	–	–	–
10	1.62 m	1.80 m	2.75 br s, $W_{1/2}$ = 4.3	1.58 m	1.73 dd, 12.9, 2.6	2.24 dd, 2.4, 13.4	2.39 dd, 4.2, 17.0
11α	1.20 m	1.83 m	2.03 m	1.67 m	2.41 m	2.41 m	2.40 m
11β	2.35 m	2.44 m	2.53 m	2.43 m	2.41 m	2.70 dd 9.3, 14.9	2.40 m
12α	2.30 m	2.17 m	2.19 m	5.41 t, 8.4	5.43 t, 8.4	5.39 t, 8.4	5.41 t, 8.6
12β	2.32 m	2.35 m	2.34 dt, 4.9, 14.0	–	–	–	–
13	–	–	–	–	–	–	–
14	6.27 br s $W_{1/2}$ = 3.7	6.29 br s $W_{1/2}$ = 3.7	6.29 s	6.37 br s $W_{1/2}$ = 3.7	6.38 br s $W_{1/2}$ = 3.8	6.39 br s $W_{1/2}$ = 3.8	6.38 br s $W_{1/2}$ = 4.3
15	7.35 t, 1.6	7.36 t, 1.6	7.38 (*t*, 1.6)	7.43 m	7.43 m	7.46 t, 1.6	7.44 br s $W_{1/2}$ = 3.9
16	7.23 br s $W_{1/2}$ = 3.4	7.26 br s $W_{1/2}$ = 1.1	7.26 s	7.42 m	7.45 br s $W_{1/2}$ = 3.5	7.46 br s $W_{1/2}$ = 3.4	7.45 br s $W_{1/2}$ = 4.5
17	1.15 d, 7.0	0.96 d, 6.8	0.98 d, 6.6	1.10 d, 6.8	1.11 d, 6.5	1.26 s	1.04 d, 6.2
18	–	–	–	–	–	–	–
19α	1.22 s	4.40 dd, 2.3, 12.0	4.22 dd, 1.0, 11.5	–	–	–	–
19β		4.82 d, 12.0	4.48 dd, 1.0, 11.5	–	–	–	–
20	–	–		–	–	–	–
18-OCH_3	3.68 s	3.72 s	3.76 s	3.68 s	3.68 s	3.69 s	3.76 s
19-OCH_3	–	–		3.76 s	3.72 s	3.73 s	3.81 s
CH_3-Ac	–	–		–	–	–	–
20-CO_2H	11.5 br s	–		–	–	–	–

*Superimposed resonances

Table 3 ^{1}H NMR chemical shifts for compound (**7–13**) isolated from *C. megalocarpoides*.

No.	7	8	9	10	11	12	13
1α	6.76 br s $W_{1/2}$ = 3.5	1.78 m	2.20 m	2.02 m	2.14 m	1.86 m	2.72 dd, 3.1, 14.5
1β	–	2.37 m	2.45 m	2.41 m	2.35 m	2.22 m	1.72 dd, 3.4, 14.5
2α	–	2.41 m	2.76 m	2.37 m	2.59 m	1.69 m	4.22 m
2β	–	2.57 dt, 5.4, 19.0	2.46 m	2.74 m	3.00 m	2.64 m	–
3	6.86 br s $W_{1/2}$ = 3.4	6.98 dd, 2.0, 4.9	–	–	–	5.08 t = 2.6	3.24 br s $W_{1/2}$ = 8.7
4	–	–	3.23 s	3.18 s	–	–	–
4-OH	–	–	–	–	4.20 s	3.82 s	–
5	–	–	–	–	–	–	1.42 dd 2.5, 11.8
6α	3.12 dt, 13.5, 3.1	3.24 dd 6.6, 17.9	2.78 m	2.78 m	2.38 m	2.34 m	1.91 m
6β	1.43 dt, 4.0, 13.5	1.78 m	1.96 br d $W_{1/2}$ = 24.2 m	1.90 m	2.17 m	2.14 m	1.91 m
7α	1.71 m	5.82 dt, 1.2, 6.6	5.78 br d $W_{1/2}$ = 11.4	5.75 br d, $W_{1/2}$ = 11.4	5.77 br d, $W_{1/2}$ = 11.0	5.65 br d $W_{1/2}$ = 13.6	2.90 m
7β	2.78 dd, 5.4, 14.5	–	–	–	–	–	2.98 m
8	1.71 m	–	–	–	–	–	–
9	–	–	–	–	–	–	–
10	–	2.00 dd, 2.2, 12.7	2.35 m	2.15 m	2.79 dd, 4.8, 13.4	2.52 m	–
11α	2.76 m	2.34 m	2.51 m	2.77 m	2.51 m	2.40 m	7.23*
11β	2.64 dd, 10.9, 5.0	2.68 dd, 7.9, 14.1	2.74 m	2.35 dd 14.4, 9.0	2.72 dd, 8.5, 14.4	2.60 m	–
12	5.55 dd, 5.0, 10.9	5.50 t, 8.3	5.47 t, 8.6	5.51 t, 8.7	5.47 t, 8.4	5.45 t, 8.9	7.22*
14	6.43 br s $W_{1/2}$ = 4.0	6.39 dd 0.8, 1.8	6.39 br s $W_{1/2}$ = 4.3	6.40 br s $W_{1/2}$ = 3.8	6.40 br s $W_{1/2}$ = 3.9	6.39 br s $W_{1/2}$ = 4.3	7.17 br s $W_{1/2}$ = 3.4
15	7.46 br s $W_{1/2}$ = 4.3	7.44 t, 1.6	7.44 br s $W_{1/2}$ = 5.8	7.46 m	7.44 t, 1.6	7.43 br s $W_{1/2}$ = 4.4	–
16	7.55 br s $W_{1/2}$ = 3.6	7.45 br s $W_{1/2}$ = 3.3	7.46 br s $W_{1/2}$ = 3.9	7.47 m	7.46 br s $W_{1/2}$ = 3.5	7.46 br s $W_{1/2}$ = 4.0	1.56 s
17	1.18 d, 6.2	1.66 t 1.1	1.67 s	1.85 s	1.67 t, 1.2	1.66 s	1.56 s
18	–	–	–	–	–	–	1.11 s
19	–	–	–	–	–	–	1.08 s
20	–	–	–	–	–	–	1.44 s
18-OCH_3	3.83 s	3.70 s	3.73 s	3.75 s	3.86 s	3.70 s	–
19-OCH_3	3.63 s	3.71 s	3.74 s	3.75 s	3.71 s	3.72 s	–
Ac-CH_3	–	–	–	–	–	2.01 s	–

*Overlapped resonances

see **Table 1**; HR-ESI-MS *m/z* 425.15648 for $[M+Na]^+$ (calcd. for $C_{22}H_{26}NaO_7$, 425.15707).

8β-Hydroxy-crotocorylifuran (**5**): White solids; $[\alpha]_D$ −11.7° ($CHCl_3$), *c* 0.00032); IR $\nu_{max}\cdot cm^{-1}$ (neat): 2924, 2851, 1767, 1715; ^{1}H NMR (500 MHz, $CDCl_3$) see **Table 2**; ^{13}C NMR (500 MHz, $CDCl_3$) see **Table 1**; HR-ESI-MS *m/z* 441.15199 for $[M+Na]^+$ (calcd. for $C_{22}H_{26}NaO_8$, 441.15199).

Crotocorylifuran-2-one (**6**): White solid; $[\alpha]_D$ −9.2° ($CHCl_3$), *c* 0.00047); IR $\nu_{max}\cdot cm^{-1}$ (neat): 2923, 2851, 1710, 1685; ^{1}H NMR (500 MHz, $CDCl_3$) see **Table 2**; ^{13}C NMR (500 MHz, $CDCl_3$) see **Table 1**; HR-ESI-MS *m/z* 439.13631 for $[M+Na]^+$ (calcd. for $C_{22}H_{24}NaO_8$, 439.13634).

Megalocarpoidolide D (**7**): White solid; $[\alpha]_D$ −13.2° ($CHCl_3$, *c* 0.00034); IR $\nu_{max}\cdot cm^{-1}$ (neat): 2917, 1769, 1730, 1663, 1246, 1156; ^{1}H NMR (500 MHz, $CDCl_3$) see **Table 3**; ^{13}C NMR (500 MHz, $CDCl_3$) see **Table 1**; HR-ESI-MS *m/z* 437.12058 for $[M+Na]^+$ (calcd. for $C_{22}H_{22}NaO_8$, 437.12069).

7,8-Dehydrocrotocorylifuran (**8**): White solid; $[\alpha]_D$ −52.7° ($CHCl_3$), *c* 0.00091); IR $\nu_{max}\cdot cm^{-1}$ (neat): 2923, 2851, 1767, 1715; ^{1}H NMR (500 MHz, $CDCl_3$) see **Table 3**; ^{13}C NMR (500 MHz, $CDCl_3$) see **Table 1**. HR-ESI-MS *m/z* 423.14139 for $[M+Na]^+$ (calcd. for $C_{22}H_{24}NaO_7$, 423.14142).

Megalocarpoidolide E (**9**). White solid: $[\alpha]_D$ −24.2° ($CHCl_3$), *c* 0.00014); IR $\nu_{max}\cdot cm^{-1}$ (neat): 2917, 2864, 1748, 1720, 1174; ^{1}H NMR (500 MHz, $CDCl_3$) see **Table 3**; ^{13}C NMR (500 MHz, $CDCl_3$) see **Table 1**. HR-ESI-MS *m/z* 439.13591 for $[M+Na]^+$ (calcd. for $C_{22}H_{24}NaO_8$, 439.13634).

Megalocarpoidolide F (**10**): White solid; $[\alpha]_D$ −19.6° ($CHCl_3$, *c* 0.00029); IR $\nu_{max}\cdot cm^{-1}$ (neat): 2917, 2864, 1748, 1720; ^{1}H NMR (500 MHz, $CDCl_3$) see **Table 3**; ^{13}C NMR (500 MHz, $CDCl_3$) see **Table 1**. HR-ESI-MS (*m/z* 439.13591 for $[M+Na^+]$, calcd. for $C_{22}H_{24}NaO_8$, 439.13634).

Megalocarpoidolide G (**11**): White solids; $[\alpha]_D$ −7.3° ($CHCl_3$, *c* 0.00063); IR $\nu_{max}\cdot cm^{-1}$ (neat): 3353, 1747, 1719; ^{1}H NMR (500 MHz, $CDCl_3$) see **Table 3**; ^{13}C NMR (500 MHz, $CDCl_3$) see **Table 1**. ESIMS (*m/z* 455.13094 for $[M+Na^+]$, calcd. for $C_{22}H_{24}NaO_9$, 455.13125).

Megalocarpoidolide H (**12**): White solids; $[\alpha]_D$ −15.8° ($CHCl_3$, *c* 0.00048); IR $\nu_{max}\cdot cm^{-1}$ (neat): 2923, 2850, 1747, 1719; ^{1}H NMR (500 MHz, $CDCl_3$) see **Table 3**; ^{13}C NMR (500 MHz, $CDCl_3$) see **Table 1**. HR-ESI-MS (*m/z* 499.15716 for $[M+Na]^+$, calcd. for $C_{24}H_{28}NaO_{10}$, 499.15747).

Isolophanthin E (**13**): White solids; $[\alpha]_D$ +13.8° ($CHCl_3$, *c* 0.00043); IR $\nu_{max}\cdot cm^{-1}$ (neat): 3400; ^{1}H NMR (500 MHz, $CDCl_3$) see **Table 3**; ^{13}C NMR (500 MHz, $CDCl_3$) see **Table 1**. HR-ESI-MS (*m/z* 341.20886 for $[M+Na]^+$, calcd. for $C_{20}H_{30}NaO_3$, 341.20872).

Pharmacological assays

Antimicrobial activities: A modified CLSI (NCCLS 1998 and 2000) method as described by Samoylenko et al. (2009) [19], was followed for antimicrobial assays. Ciprofloxacin (ICN Biomedicals; ≥ 98% pure) for bacteria and amphotericin B (ICN Biomedicals; ≥ 98% pure) for fungi were used as positive controls. The tested

organisms were from the American Type Culture Collection (ATCC): *Candida albicans* (ATCC 90028), *Candida glabrata* (ATCC 90030), *Candida krusei* (ATCC 6258), *Aspergillus fumigatus* (ATCC 90906), *Cryptococcus neoformans* (ATCC 90113), *Staphylococcus aureus* (ATCC 29213), methicillin-resistant *S. aureus* (ATCC 33591), *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853) and *Mycobacterium intracellulare* (ATCC 23068).

Antiplasmodial activities: Antiplasmodial activities of the isolated compounds against two *P. falciparum* strains, D6 (chloroquine-sensitive) and W2 (chloroquine-resistant), were determined using the modified assay described by Trager and Jensen (1976) [20], also used by Makler, et al. [21] and Samoylenko, et al., (2009) [19]. DMSO was used as vehicle control and artemisinin (Sigma-Aldrich; purity ≥ 98%) and chloroquine (Sigma-Aldrich; purity ≥ 98% pure) were used as drug controls.

Cytotoxicity assay

The cell viability studies were done using monkey kidney fibroblasts (VERO) obtained from the American Type Culture Collection (ATCC). The assays were performed on 96-well microplates with the cells seeded at a density of 25000 cells/well and incubated for 24 h. Samples at different concentrations were added and plates were again incubated for 48 h. The number of viable cells was determined using Neutral Red according to a modified procedure of [22]. Doxorubicin (Sigma-Aldrich; ≥ 98–102% pure) was used as a positive control and DMSO as the negative control.

Supporting information

^{1}H, ^{13}C NMR and mass spectra of compounds **1**–**13** and **4a** and, DEPT, HSQC, COSY, NOESY and HMBC spectra for **1**, ECD spectra for **1** and **2** in addition to diagrams showing NOESY correlations for **1** and **2** are available as Supporting Information.

Acknowledgements

BN wishes to thank the University of Surrey, Guildford, UK, for providing laboratory space from where this work was done and also for NMR, IR and MS analysis. BN also thanks the University of Nairobi, International Foundation of Sciences (IFS), International Science Program (ISP-Sweden), Organization for Prevention of Chemical Weapons (OPCW), Natural Products Research Network for East and Central Africa (NAPRECA) for funds to support her PhD studies and supporting the research work that produced these results. The authors also wish to thank Ms. Marsha Wright (NCNPR, UM) for antimicrobial testing and Mr. John Trott for antimalarial and cytotoxicity assays and Mr. Colin Sparrow of University of Oxford for MS analysis. This work was supported by the NIH, NIAID, Division of AIDS, Grant No. AI 27094 (antifungal) and the USDA Agricultural Research Service Specific Cooperative Agreement No. 58–6408–1–603 (antibacterial).

Conflict of Interest

The authors declare that there is no conflict of interest.

Affiliations

[1] Department of Chemistry, University of Nairobi, Nairobi, Kenya
[2] Department of Chemistry, University of Surrey, Guildford, Surrey, United Kingdom
[3] School of Chemistry and Physics, University of KwaZulu-Natal, Durban, South Africa
[4] National Centre for Natural Products Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, MS, USA
[5] Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, MS, USA

References

1 *Beentje HJ.* Kenyan Trees, Shrubs and Lianas. Nairobi: Majestic Printing Works Ltd; 1994: 190–192
2 *Friis I, Gilbert MG. Croton megalocarpoides* sp. nov. An arborescent Euphorbiaceae from semi-evergreen vegetation in S. Somalia and E. Kenya. Nord J Bot 1984; 4: 327–331
3 *Caruzo MBR, Van Ee B, Cordeiro I, Berry PE, Riina R.* Molecular phylogenetics and character evolution of the "sacaca" clade: novel relationships of *Croton* section *Cleodora* (Euphorbiaceae). Mol Phylogenet Evol 2011; 60: 193–206
4 *Berry PE, Hipp AL, Wurdack KJ, Van Ee B, Riina R.* Molecular phylogenetics of the giant genus *Croton* and tribe Crotoneae (Euphorbiaceae sensu stricto) using ITS and *trnL-trnF* DNA sequence data. Am J Bot 2005; 92: 1520–1534
5 *Yamale SC, Koudou J, Samb A, Heitz A, Teulade JC.* Structural elucidation of a new furoclerodane from stem barks of *Croton mayumbensis* J. Leonard extracts. Int J Phys Sci 2009; 4: 96–100
6 *Kokwaro JO.* Medicinal Plants of East Africa, 3rd edition. Nairobi: University of Nairobi Press; 2009
7 *Ngadjui BT, Abegaz BM, Keumedjio F, Folefoc GN, Kapche GW.* Diterpenoids from the stem bark of *Croton zambesicus*. Phytochemistry 2002; 60: 345–349
8 *Yang LB, Li L, Huang SX, Pu JC, Zhao Y, Ma YB, Chen JJ, Leng CH, Tao ZM, Sun HD.* Anti-hepatitis B Virus and cytotoxic diterpenoids from *Isodon lophanthoides* var. *gerardianus*. Chem Pharm Bull (Tokyo) 2011; 59: 1102–1105
9 *Spessard GO, Matthews DR, Nelson MD, Rajtora TC, Fossum MJ, Giannini JL.* Phytoalexin-like activity of abietic acid and its derivatives. J Agric Food Chem 1995; 43: 1690–1694
10 *Kapingu MC, Guillaume D, Mbwambo ZH, Moshi MJ, Uliso FC, Mahunnah RL.* Diterpenoids from the roots of *Croton macrostachys*. Phytochemistry 2000; 54: 767–770
11 *Leong YW, Harrison LJ. Ent*-trachylobane diterpenoids from the liverwort *Mastigophora diclados*. Phytochemistry 1997; 45: 1457–1459
12 *Rayanil K, Limpanawisut S, Tuntiwachwuttikul P. Ent*-pimarane and *ent*-trachylobane diterpenoids from *Mitrephora alba* and their cytotoxicity against three human cancer cell lines. Phytochemistry 2013; 89: 125–130
13 *Sutomo S, Rianto S, Setyowati EP.* Isolation and identification of active compound of *n*-hexane fraction from Kasturi (*Mangifera casturi* Konsterm.) against antioxidant and immunomodulatory activity. J Biol Sci 2013; 13: 596–604
14 *Carpenter RC, Sotheeswaran S, Sultanbawa MUS, Ternai B.* ^{13}C NMR studies of some lupine and taraxerane triterpenes. Org Mag Resonance 1980; 14: 462–465
15 *Langat MK, Crouch NR, Pohjala L, Tammela P, Smith PJ, Mulholland DA. Ent*-kaure-19-oic acid derivatives from the stem bark of *Croton pseudopulchellus* Pax. Phytochem Lett 2012; 5: 414–418
16 *Wang G, Zhang H, Liu H, Yue J.* Laevinoids A and B: Two diterpenoids with an unprecedented backbone from *Croton laevigatus*. Org Lett 2013; 15: 4880–4883
17 *Tchissambou L, Chiarioni A, Riche C, Khoung H.* Crotocorylifuran and crotohaumanoxide, new diterpenes from *Croton haumanianus* J Leornard. Tetrahedron 1990; 46: 5199–5202
18 *Abega DF, Kapche DW, Ango PY, Mapitse R, Yeboah SO, Ngadjui BT.* Chemical constituents of *Croton oligandrum* (Euphorbiaceae). Z Naturforsch C 2014; 69: 181–185
19 *Samoylenko V, Jacob MR, Khan SI, Zhao J, Tekwani BL, Midiwo JO, Walker LA, Muhammad I.* Antimicrobial, antiparasitic and cytotoxic spermine alkaloids from *Albizia schimperiana*. Nat Prod Commun 2009; 4: 791–796
20 *Trager W, Jensen JB.* Human malaria parasites in continuous culture. Science 1976; 193: 673–675
21 *Makler MT, Ries JM, Williams JA, Bancroft JE, Piper RC, Gibbins BL, Hinrichs DJ.* Parasite lactate dehydrogenase as an assay for *Plasmodium falciparum* drug sensitivity. Am J Trop Med Hyg 1993; 48: 739–741
22 *Borenfreund E, Babich H, Martin-Alguacil N.* Rapid chemosensitivity assay with human normal and tumor cells *in vitro*. In Vitro Cell Dev Biol 990; 26: 1030–1034