Tigliane Diterpenoids from the Stem Bark of *Neoboutonia macrocalyx*

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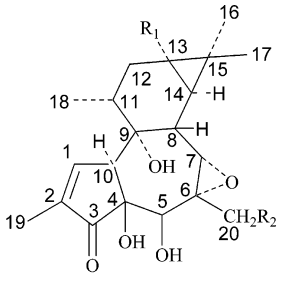
Neoboutonia macrocalyx is a plant used by traditional healers among the Meru community in Kenya. Chromatographic fractionation of the petroleum ether and dichloromethane extracts of this plant yielded one known (**1**) and three new tigliane-type diterpenoids (**2**−**4**). The chemical structures of the isolated compounds were established through spectroscopic data interpretation.

Neoboutonia macrocalyx Pax. (Euphorbiaceae), commonly known as ''Mutuntuki" (Kimeru), is found in central and eastern Kenya. The stem bark is used to treat headache and fever in traditional medicine. Extracts from the stem bark of this plant have shown antiplasmodial activity.[1] Several sterols and daphnane and tigliane diterpenoids have been isolated from other *Neoboutonia* species.[2,3] As part of a program on phytochemical investigations on Kenyan medicinal plants, we describe herein the isolation of a known compound, 6α,7α-epoxy-5*β*-hydroxy-12-deoxyphorbol-13-tetradecanoate (6α,7α-epoxy-4*β*,5*β*,9α,20-tetrahydroxy-13α-tetradecanoate-1-tiglien-3-one) (**1**),[4,5] and the structure elucidation of three new tigliane diterpenoid compounds (**2**−**4**) from the dichloromethane and petroleum ether extracts of the stem bark of *N. macrocalyx*.

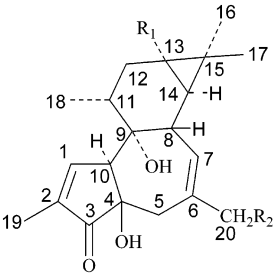
The structure of the known compound, 6α,7α-epoxy-5*β*-hydroxy-12-deoxyphorbol-13-tetradecanoate (**1**), was determined by comparison of generated spectroscopic data with values reported in the literature.[4,5] The spectroscopic data for this compound is included in Table 1 for comparison with other derivatives that were isolated together with **1**.

Comparison of the IR data for **1** and **2** revealed minor differences. Unlike **1**, which had one ester group (ν_{max} 1713 cm^{-1}), the IR spectrum of **2** revealed the presence of two ester groups (ν_{max} 1735, 1717 cm^{-1}). The ^{1}H NMR spectrum of **2** (Table 1) showed minor differences including the presence of six methyl groups at δ 0.89 (t, J = 6.3 Hz, Me-12″), 0.85 (t, J = 7.0 Hz, Me-16′), 0.93 (d, J = 6.7 Hz, H-18), 1.78 (s, H-19), 1.19 (s, H-16), and 1.09 (s, H-17) instead of five in **1**. Unlike in **1**, the two diastereotopic methylene protons attached to the oxygenated carbon (C-20) in **2** were observed distinctly at δ 4.80 (1H, d, J = 12.0 Hz, H-20a) and 3.82 (1H, d, J = 12.0 Hz, H-20b), suggesting esterification at this carbon. The region between δ 1.26 (br, m) and 1.60 (m) was more complicated in **2** due to an increase in the number of CH_2 groups in the ester groups (C-4′−C-15′, C-4″−C-12″). The presence of two signals at δ 2.30 (2H, t, J = 7.6 Hz, H-2′) and 2.30 (2H, t, J = 7.5 Hz, H-2″), superimposed on each other, confirmed the presence of two ester groups. The ^{1}H NMR data for the cyclopentyl and cyclohexyl rings of **2** were similar to those of **1**,[4,5] mellerin A,[2] and 12-deoxyphorbol-13-hexadecanoate,[6] while the data for the cycloheptyl ring were similar to those of montanin.[3] The rest of the ^{1}H NMR signals for **2** were similar to those of **1**. The ^{13}C NMR spectrum of **2** (Table 1) revealed minor differences from that of **1** and confirmed the presence of two ester groups (δ 175.3, C-1′; 173.4, C-1″). The presence of an ester group at C-20 was further



1 R_1 = $CH_3(CH_2)_{12}CO_2$, R_2 = OH

2 R_1 = $CH_3(CH_2)_{14}CO_2$, R_2 = $CH_3(CH_2)_{10}CO_2$



3 R_1 = $CH_3(CH_2)_{13}CO_2$, R_2 = OH

4 R_1 = $CH_3(CH_2)_{14}CO_2$, R_2 = $CH_3(CH_2)_{10}CO_2$

confirmed by the shift of the signal to δ 65.7 in **2** from δ 64.8 in **1**. The signals of the *gem*-dimethylcyclopropane skeleton were as reported for **1**[7,8] and other tigliane diterpenoids. The 17 carbon signals at δ 29.1−29.7 (C-4′−C-13′ and C-4″−C-10″) were assigned to the CH_2 groups in the two pendent ester chains. In addition to the two ester groups, the other part of the molecule contained 20 carbons and was proposed to be a tigliane-type diterpenoid skeleton on the basis of literature spectroscopic data.[2,8] COSY, HMBC, and HMQC spectra confirmed the existence of two side chain ester moieties in **2** (hexadecanoyl at C-13 and dodecanoyl at C-20). The connectivity of the methyl, methylene, methine, and quaternary carbons was determined on the basis of ^{1}H−^{13}C NMR long-range correlation signals in the HMBC spectrum. The EIMS fragments observed at m/z 239 and 185 were assigned to loss of a hexadecanoyl and a lauroyl ion fragment, respectively. The molecular formula, $C_{48}H_{80}O_9$, for **2** was deduced from the quasi-molecular ion adducts observed at m/z 829 $[M + C_2H_5]^+$ and 801 $[M + H]^+$ in the CIMS and confirmed by HREIMS (m/z 661.4830 for $C_{43}H_{65}O_5$). Consequently, the structure of **2** was confirmed as

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Table 1. NMR Data (500 MHz, $CDCl_3$) for Tigliane Diterpenoids **1**−**4**

	1		**2**		**3**		**4**	
position	δ_C, mult.	δ_H (*J* in Hz)	δ_C, mult.	δ_H (*J* in Hz)	δ_C, mult.	δ_H (*J* in Hz)	δ_C, mult.	δ_H (*J* in Hz)
1	163.9, CH	7.71, s	163.6, CH	7.69, s	161.2, CH	7.59, s	161.5, CH	7.61, s
2	134.2, qC		134.3, qC		132.8, qC		132.8, qC	
3	210.0, qC		209.8, qC		209.3, qC		209.1, qC	
4	72.6, qC		72.5, qC		73.8, qC		73.7, qC	
5a	71.6, CH	4.26, s	69.6, CH	4.28, s	38.7, CH_2	2.49, d (12.3)	39.0, CH_2	2.51, d (12.0)
5b		4.26, s		4.28, s		2.29, d (12.3)		2.38, d (12.0)
6	61.9, qC		60.7, qC		139.8, qC		135.0, qC	
7	65.7, CH	3.26, d (4.4)	65.7, CH	3.14, d (4.6)	130.3, CH	5.68, d (4.0)	133.8, CH	5.72, d (4.4)
8	36.3, CH	2.82, dd (4.4, 7.5)	36.2, CH	2.82, dd (4.6, 7.6)	39.2, CH	3.00, dd (4.0, 6.6)	39.5, CH	3.00, d (4.4, 6.6)
9	75.4, qC		75.3, qC		76.0, q*C*		75.9, qC	
10	49.7, CH	3.91 (s)	49.8, CH	3.90, s	55.8, CH	3.28, s	55.8, CH	3.29, s
11	38.2, CH	1.84 (m)	38.1, CH	1.84, m	36..3, CH	1.97, m	36.4, CH	1.96, m
12a	31.9, CH_2	2.06, dd (7.3, 15.3)	31.9, CH_2	2.03, dd (7.3, 15.5)	31.8, CH_2	2.06, dd (7.1, 14.7)	31.8, CH_2	2.06, dd (7.1, 14.8)
12b		1.56, dd (9.3, 15.3)		1.57, dd (9.0, 15.5)		1.55, dd (11.4, 14.7)		1.56, dd (11.3, 14.8)
13	64.1, qC		64.2, qC		63.4, qC		63.3, qC	
14	31.8, CH	1.13, d (7.5)	31.9, CH	1.12, d (7.6)	32.6, CH	0.83, d (6.6)	32.5, CH	0.81, d (6.6)
15	23.9, qC		24.0, qC		22.7, qC		22.7, qC	
16	22.8, CH_3	1.19, s	22.8, CH_3	1.19, s	23.2, CH_3	1.19, s	23.2, CH_3	1.19, s
17	15.7, CH_3	1.07, s	15.8, CH_3	1.09, s	15.3, CH_3	1.06, s	15.3, CH_3	1.07, s
18	19.0, CH_3	0.92, d (6.6)	19.0, CH_3	0.93, d (6.7)	18.5, CH_3	0.93, d (6.5)	18.5, CH_3	0.91, d (6.0)
19	9.7, CH_3	1.77, s	9.8, CH_3	1.78, s	10.1, CH_3	1.78, s	10.1, CH_3	1.78, s
20a	64.8, CH_2	3.83, s	65.7, CH_2	4.80, d (12.0)	68.3, CH_2	4.39, d (13.8)	69.5, CH_2	4.48, d (12.4)
20b		3.83, s		3.82, d (12.0)		4.27, d (13.8)		4.45, d (12.4)
1′	175.4, qC		175.3, qC		176.0, qC		175.9, qC	
2′	34.4, CH_2	2.30, t (7.5)	34.4, CH_2	2.30, t (7.6)	34.6, CH_2	2.30, t (7.5)	34.6, CH_2	2.30, t (7.5)
3′	24.8, CH_2	1.61, m	24.8, CH_2	1.61, m	24.8, CH_2	1.59, m	24.8, CH_2	1.61, m
4′	29.1, CH_2	1.26, m	29.1, CH_2	1.26, m	29.1, CH_2	1.26, m	29.1, CH_2	1.26, m
5′	29.2, CH_2	1.26, m	29.3, CH_2	1.26, m	29.2, CH_2	1.26, m	29.2, CH_2	1.26, m
6′	29.3, CH_2	1.26, m	29.4, CH_2	1.26, m	29.3, CH_2	1.26, m	29.2, CH_2	1.26, m
7′	29.4, CH_2	1.26, m	29.6, CH_2	1.26, m	29.3, CH_2	1.26, m	29.3, CH_2	1.26, m
8′	29.6, CH_2	1.26, m	29.6, CH_2	1.26, m	29.4, CH_2	1.26, m	29.4, CH_2	1.26, m
9′−11′	29.6, CH_2	1.26, m	29.6, CH_2	1.26, m	29.6, CH_2	1.26, m	29.6, CH_2	1.26, m
12′	32.0, CH_2	1.26, m	29.6, CH	1.26, m	29.6, CH	1.26, m	29.6, CH_2	1.26, m
13′	22.7, CH_2	1.26, m	29.6, CH_2	1.26, m	29.7, CH_2	1.26, m	29.6, CH_2	1.26, m
14′	14.1, CH_3	0.88, t (7.0)	32.0, CH_2	1.26, m	22.7, CH_2	1.26, m	31.9, CH_2	1.26, m
15′			22.7, CH_2	1.26, m	14.1, CH_3	0.89, t (5.6)	22.8, CH_2	1.26, m
16′			14.1, CH_3	0.85, t (7.0)			14.1, CH_3	0.87, t (7.0)
1″			173.4, qC				173.6, qC	
2″			34.2, CH_2	2.30, t (7.5)			34.3, CH_2	2.29, t (7.6)
3″			24.9, CH_2	1.61, m			24.9, CH_2	1.61, m
4″			29.1, CH_2	1.26, m			29.2, CH_2	1.26, m
5″			29.2, CH_2	1.26, m			29.2, CH_2	1.26, m
6″			29.3, CH_2	1.26, m			29.3, CH_2	1.26, m
7″			29.5, CH_2	1.26, m			29.4, CH_2	1.26, m
8″			29.6, CH_2	1.26, m			29.6, CH_2	1.26, m
9″			29.6, CH_2	1.26, m			29.6, CH_2	1.26, m
10″			29.7, CH_2	1.26, m			29.7, CH_2	1.26, m
11″			22.7, CH_2	1.26, m			22.7, CH_2	1.26, m
12″			14.1, CH_3	0.89, t (6.3)			14.1, CH_3	0.89, t (7.1)

6α,7α-epoxy-5*β*-hydroxy-12-deoxyphorbol-20-dodecanoate-13α-hexadecanoate (6α,7α-epoxy-4*β*,5*β*,9α-trihydroxy-13α-hexadecanoate-20-dodecanoate-1-tiglien-3-one).

The IR spectrum of compound **3** revealed minor differences from **2** including the presence of an isolated double bond (ν_{max} 1643 and 896 cm^{-1}) and an ester group (ν_{max} 1735 cm^{-1}). Unlike **2**, the ^1H NMR spectrum of **3** (Table 1) indicated five methyl groups at δ 0.89 (t, J = 5.6 Hz, H-15′), 0.93 (d, J = 6.5 Hz, H-18), 1.78 (s, H-19), 1.19 (s, H-16), and 1.06 (s, H-17) and was consistent with the presence of an ester group. The olefinic proton signal at δ 5.68 (1H, d, J = 5.0 Hz, H-7) confirmed the presence of a trisubstituted double bond in **3**. The presence of four diastereotopic protons at δ 2.49 (d, 2.3, H-5b), 2.29 (d, 12.3, H-5a), 4.39 (d, J = 13.8, H-20a), and 4.27 (d, J = 13.8, H-20b) suggested a 12-deoxyphorbol[5] skeleton, in which the C-20 hydroxyl group is free and an ester moiety is attached to C-13. Comparison of the ^1H NMR spectrum of **3** with the literature data revealed close similarity to the known compound 12-deoxyphorbol-13-hexadecanoate.[3,6] A comparison of the ^1H NMR data of **3** with those of 12-deoxyphorbol[8] and 13-*O*-acetyl-12-deoxyphorbol (prostatin)[9] revealed additional signals at δ 0.89 (3H, t, J = 5.6, H-15′), 1.26 (22H, br s, H-4′−H-14′), 1.59 (2H, m, H-3′), and 2.30 (2H, dd, J = 7.3, 7.5 Hz, 2′), suggesting that the compound is an ester of 12-deoxyphorbol and pentadecanoic acid. Comparison of the ^1H NMR data of **3** to those of other tigliane diterpenoids established the stereochemistry as 4*β*-OH, 8*β*-H, 9α-OH, 10α-H, 11α-CH_3, 13α-OR, and 14α-H.[6,8] The ^{13}C NMR spectrum of **3** (Table 1) revealed minor differences from that of **2**. The presence of two extra olefinic carbons (δ 139.8, s; 130.3, d) and absence of one ester group and three oxygenated carbons were noted, confirming a 12-deoxyphorbol ester skeleton. Correlations observed in the COSY, HMQC, and HMBC NMR spectra of **3** confirmed the presence of an ester group at C-13. The other part of the molecule contained 20 carbons. The molecular ion peak was observed at *m*/*z* 573 by CIMS and confirmed by HREIMS (*m*/*z* 494.3390 for $C_{32}H_{46}O_4$). The pentadecanoyl fragment ion was observed at *m*/*z* 225 in the EIMS, and the molecular formula was

deduced as $C_{35}H_{56}O_6$. Consequently, the structure of **3** was confirmed as 12-deoxyphorbol-13α-pentadecanoate (4β,9α,20-trihydroxy-13α-pentanoate-1,6-tigliadien-3-one).

The ^{1}H NMR spectrum of **4** (Table 1) revealed minor differences from that of **3**, including the presence of six methyl groups. The signals due to two diastereotopic allylic methylene protons attached to oxygenated carbon (C-20) in **4** were shifted slightly (δ 4.48, d, J = 12.4 Hz, H-20a and 4.45, d, J = 12.4 Hz, H-20b), suggesting substitution at this carbon. Comparison of the ^{1}H NMR spectrum of **4** with literature data for similar compounds revealed close similarity to 12-deoxyphorbol-13-hexadecanoate.[3,6] However, additional signals observed at δ 0.87 (3H, t, J = 7.0 Hz), 0.89 (3H, J = 7.1 Hz, H-12″), 1.26 (40H, br m, H-4′-H-15′ and H-4″−H-11″), 1.61 (4H, m, H-3′ and H-3″), 2.30 (2H, t, J = 7.5 Hz, H-2′), and 2.29 (2H, t, J = 7.6 Hz, H-2″) suggested that **4** is an ester of 12-deoxyphorbol-13-hexadecanoate with 4β-OH, 8β-H, 9α-OH, 10α-H, 11α-Me, 13α-OR, and 14α-H substituents.[2−8] The rest of the signals were similar to those of 12-deoxyphorbol-13-hexadecanoate.[3,6] The ^{13}C NMR spectrum of **4** (Table 1) revealed minor differences from 12-deoxyphorbol-13-hexadecanoate[3,6] and confirmed the presence of two ester groups (δ 175.9, 173.6). The shift in the signal due to the oxygenated methylene group (C-20) from δ 68.3 in **3** to 69.5 in **4** suggested substitution at that point. The presence of 12 extra ^{13}C NMR signals consisting of 10 methylenes, one methyl (δ 14.1), and one ester (δ 173.6) group confirmed the presence of two ester groups in **4**. The rest of the ^{13}C NMR data were similar to those of 12-deoxyphorbol-13-hexadecanoate.[3,6] The proton assignments of the suggested compound were further confirmed by COSY. The ester groups were assigned to C-13 and C-20 on the basis of COSY, HMQC, and HMBC NMR data. CIMS revealed three major peaks at *m*/*z* 495 (30%) 493 (25%), and 295 (100%). HREIMS gave a peak at *m*/*z* 662.5267 corresponding to a $C_{44}H_{70}O_4$ fragment ion. Since the molecular ion peak was not observed, it was not possible to determine unambiguously the size of the ester moiety at this stage. However, during this study, a similar tigliane diterpenoid, **2**, containing a 6α,7α-epoxide and a dodecanoyl ester at C-13 was isolated. Consequently, the second ester moiety was deduced as hexadecanoate ($CH_3(CH_2)_{14}CO_2$) and supported by the peaks at *m*/*z* 239 and 183 in the EIMS due to palmitoyl and lauroyl fragment ions, respectively. The structure of compound **4** was confirmed as 12-deoxyphorbol-13α-dodecanoate-20-hexadecanoate (4β,9α-dihydroxy-20-hexadecanoate-13α-dodecanoate-1,6-tigliadien-3-one), corresponding to $C_{48}H_{80}O_7$.

Tigliane diterpenoids have been isolated from many plants in Thymelaeaceae[10] and Euphorbiaceae.[2−9] They have interesting biological properties such as irritant, cytotoxic, carcinogenic, and antitumor activity.[9,10] Their presence in *N. macrocalyx* coupled with the reported cytotoxicity may be responsible for the observed antiplasmodial activity of the extracts, although this will have to be further investigated.

Experimental Section

General Experimental Procedures. $[\alpha]_D$ values were measured with a Perkin-Elmer 241 MC polarimeter. IR spectra were recorded on a Perkin-Elmer FT-IR 1600 spectrophotometer. UV spectra were recorded with a Beckman DU-6 spectrophotometer. All NMR spectra were recorded from a Bruker DRX-500 (^{1}H at 500 MHz; ^{13}C at 125 MHz) spectrometer as solutions in $CDCl_3$. ^{1}H−^{1}H COSY, HMQC, and HMBC NMR spectra were measured using standard Bruker pulse sequences. Chemical shifts are given on a δ (ppm) scale using residual $CHCl_3$ and $CDCl_3$ peaks at δ_H 7.26 and δ_C 77.0), respectively, as internal standards. CIMS were recorded from a Micro-Mass LCT spectrometer. HRIEMS were recorded from a Finnigan MAT TSQ 700 instrument. Column chromatography was performed on silica gel 60 (0.063−0.200 mm, 70−230 mesh ASTM, Merck) as the stationary phase. TLC and preparative TLC were performed using silica gel G/UV 254 (Macherey-Nagel).

Plant Material. The stem bark of *N. macrocalyx* was collected from Meru District in Kenya in October 2002. The plant was identified and authenticated by one of us (G.M.M.), and a voucher specimen (No. 008/04) has been deposited in the East African Herbarium.

Extraction and Isolation. After drying for 7 days under the shade, the plant sample was shred using an electric mill, with 1 kg of plant material extracted sequentially with 1000 mL of petroleum ether, dichloromethane, and methanol. Extraction with each solvent was repeated four times, and the extracts were evaporated in vacuo. The petroleum ether extract (3.0 g) was subjected to fractionation by column chromatography on silica gel (flow rate 2.0 mL/min), with a petroleum ether−ethyl acetate gradient (100:0−0:100), giving 142 × 10 mL portions, which were pooled on the basis of their R_f values and concentrated in vacuo to give eight fractions. The first four fractions were available in reasonable amounts and subjected to further purification by column chromatography. Multiple preparative TLC of the first fraction (179 mg) with *n*-hexane and ethyl acetate (3:1) afforded compound **4** (51.3 mg, R_f 0.70). Column chromatography of the fourth fraction on silica gel eluting with *n*-hexane−ethyl acetate (5:1) afforded a mixture, which was further purified by preparative TLC with *n*-hexane and ethyl acetate (3:1) to give compound **2** (17.7 mg, R_f 0.45). The CH_2Cl_2 extract (6.0 g) was subjected to vacuum-liquid chromatography to yield 12 portions, which were pooled to give five major fractions. The second fraction was available in reasonable amounts (905.5 mg) and was subjected to further column chromatography using petroleum ether−ethyl acetate gradient mixtures (100:0−0:100). Fractions 1−13 (36.2 mg) from the column chromatography were further purified by multiple preparative TLC, using chloroform−ethyl acetate (1:1 and 2:1) to yield compounds **1** (17.8 mg)[4,5] and **3** (9.3 mg, R_f 0.59), respectively.

6α,7α-Epoxy-4β,5β,9α-trihydroxy-13α-hexadecanoate-20-dodecanoate-1-tiglien-3-one (2): light orange oil; $[\alpha]^{25}_D$ +60.2 (*c* 2.3, Me_2CO); UV (CH_2Cl_2) λ_{max} (log ε) 252 (4.1) nm; IR (KBr) ν_{max} 3392, 3054, 2985, 2975, 2927, 2855, 1735, 1717, 1696, 1623, 1460, 1265, 1168, 740, 705 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; CIMS *m*/*z* 801.2 (100) $[M + H]^+$; EIMS *m*/*z* 662 (1) $[M - 2H_2O - HCO_2H - C_3H_6 - CH_3]^+$, 393 (1), 293 (5), 279 (1), 256 (2), 223 (2), 213 (3), 191 (4), 185 (6), 167 (19), 149 (82), 129 (11), 111 (19), 97 (25), 85 (31), 83 (39), 69 (70), 71 (78), 57 (95), 55 (100), 43 (85), 42 (80); HREIMS *m*/*z* 661.4830 (calcd for $C_{43}H_{65}O_5$, 661.4832); R_f 0.45 (3:1 *n*-C_6H_{14}−EtOAc).

4β,9α,20-Trihydroxy-13α-pentanoate-1,6-tigliadien-3-one (3): light yellow oil; $[\alpha]^{25}_D$ +41.3 (*c* 5.3, Me_2CO); UV (CH_2Cl_2) λ_{max} (log ε) 260 (3.82) nm; IR (KBr) ν_{max} 3400, 3053 2985, 2927, 2854, 1735, 1712, 1643, 1612, 1421, 1265, 1156, 896, 740, 704 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; CIMS *m*/*z* 573.0 (10) $[M + H]^+$; EIMS *m*/*z* 494 (1) $[M - 2H_2O - C_3H_6]^+$, 479 (1), 428 (1), 412 (5), 361 (4), 330 (2), 328 (2), 312 (4), 294 (4), 293 (3), 225 (3), 211 (10), 197 (6), 185 (30), 183 (8), 179 (7), 149 (9), 129 (10), 109 (11), 95 (11), 85 (16), 83 (50), 69 (47), 57 (98), 43 (100); HREIMS *m*/*z* 494.3390 (calcd for $C_{32}H_{46}O_4$, 494.3396); R_f 0.59 (1:1 $CHCl_3$−EtOAc).

4β,9α-Dihydroxy-20-hexadecanoate-13α-dodecanoate-1,6-tigliadien-3-one (4): light orange oil; $[\alpha]^{25}_D$ +50.6 (*c* 1, Me_2CO); UV (CH_2Cl_2) λ_{max} (log ε) 260 (3.92) nm; IR (KBr) ν_{max} 3379, 3053, 2985, 2927, 2855, 1735, 1717, 1707, 1652, 1612, 1421, 1265, 1172, 896, 740, 704 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; CIMS *m*/*z* 495.0 (30) $[M - 2H_2O - C_{16}H_{31}O + 2H]^+$, 493 (25) $[M - 2H_2O - C_{16}H_{31}O]^+$; EIMS *m*/*z* 663 (2), 662 (5) $[M - 2H_2O - CO - C_3H_6]^+$, 647 (3), 526 (1), 294 (2), 256 (3), 239 (1), 228 (4), 200 (11), 185 (20), 183 (2), 171 (18), 157 (30), 149 (12), 143 (19), 129 (71), 115 (25), 97 (24), 85 (50), 73 (94), 60 (100), 55 (100), 43 (87), 41 (91); HREIMS 662.5267 (calcd for $C_{44}H_{70}O_4$, 662.5274); R_f 0.70 (3:1 *n*-C_6H_{14}−EtOAc).

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Supporting Information Available: Table of 2D NMR data for the tigliane diterpenoids **2**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Kirira, P. G.; Rukunga, G. M.; Wanyonyi, A. W.; Muregi, F. M.; Gathirwa, J. W.; Muthaura, C. N.; Omar, S. A.; Tolo, F.; Mungai, G. M.; Ndiege, I. O. *J. Ethnopharmacol.* **2006**, *106*, 403−407.
(2) Zhao, W.; Wolfender, J.; Mavi, S.; Hostettmann, K. *Phytochemistry* **1998**, *48*, 1173−1177.
(3) Tchinda, A. T.; Tsopmo, A.; Tene, M.; Kamnaing, P.; Ngnokam, D.; Tane, P.; Ayafor, J. F.; Connolly, J. D.; Farrugia, L. J. *Phytochemistry* **2003**, *64*, 575−581.
(4) Zayed, S.; Hafez, A.; Adolf, W.; Hecker, E. *Experientia* **1977**, *33*, 1554−1555.
(5) Zayed, S.; Adolf, W.; Hecker, E. *Planta Med.* **1982**, *45*, 67−77.
(6) Ma, Q.; Liu, W.; Wu, X.; Zhou, T.; Qin, G. *Phytochemistry* **1997**, *44*, 663−666.
(7) Marco, J. A.; Sanz-Cervera, J. F.; Checa, J.; Palomares, E.; Fraga, B. M. *Phytochemistry* **1999**, *52*, 479−485.
(8) Gschwendt, M.; Hecker, E. *Tetrahedron Lett.* **1969**, *40*, 3509−3511.
(9) McCormick, I. R. N.; Nixon, P. E.; Waters, P. N. *Tetrahedron Lett.* **1976**, 1735−1736. Cashmore, A. R.; Seelye, R. N.; Cain, B. F.; Mack, H.; Schimidt, R.; Hecker, E. *Tetrahedron Lett.* **1976**, 1737−1739.
(10) Connolly, J. D.; Hill, R. A. *Dictionary of Terpenoids*; Chapman & Hall: London, 1991; Vol. 2.

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