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Diterpenoids from the stem bark of *Croton zambesicus*

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Abstract

Three clerodane diterpenoids, crotozambefurans A, B and C were isolated from the stem bark of *Croton zambesicus* together with the known clerodane crotocorylifuran and two trachylobanes: 7β-acetoxytrachyloban-18-oic acid and trachyloban-7β, 18-diol. Betulinol, lupeol, sitosterol and its 3β-glucopyranosyl derivative were also obtained. The structures of crotozambefurans A, B and C were determined, respectively, as: 15,16-epoxy-1,3,13(16),14-clerodatetraen-20,12-olide-18,19-dioic acid dimethylester, 15,16-epoxy-1,3,13(16),14-clerodatetraen-18,19,20-trioic acid trimethylester and 15,16-epoxy-3,13(16),14-clerodatrien-19,1α:20,12-diolide-18-oic acid methylester, using spectroscopic analysis, especially, NMR spectra in conjunction with 2D experiments, COSY, HSQC, HMBC and TOCSY. © 2002 Published by Elsevier Science Ltd.

Keywords: *Croton zambesicus*; Euphorbiaceae; Stem bark; Isolation; Clerodane; Crotozambefurans A, B and C

1. Introduction

Croton zambesicus Müll. Arg. (Euphorbiaceae) has been used as traditional medicine for many applications such as fever, dysentery and convulsions (Irvine, 1966). It is widespread in Tropical West and Central Africa (Hutchinson and Dalziel, 1958). In our previous investigation (Ngadjui et al., 1999) we have reported two trachylobanes: 7β-acetoxytrachyloban-18-oic acid (**1**), trachyloban-7β, 18-diol (**2**), one labdane: crotonadiol (**3**) and one clerodane: crotocorylifuran (**4**). As a continuation of the investigation on the chemical constituents of *C. zambesicus*, we have harvested bulk quantities of the stem bark of this plant in order to isolate minor compounds and now we wish to discuss the results of our findings. In addition to the previously reported diterpenoids, (Ngadjui et al., 1999), we have isolated and characterized three new clerodane diterpenoids (**5**–**7**) for which the names crotozambefurans A, B and C, respectively, are proposed.

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2. Results and discussion

Repeated column chromatography of the organic extract of the stem bark of *C. zambesicus* yielded compounds **1**–**7** together with betulinol, lupeol, sitosterol and its glucopyranosyl derivative.

The molecular formula of crotozambefuran (**5**) was assigned as $C_{22}H_{24}O_7$ from NMR and EI–MS measurements. Its IR spectrum showed absorption bands typical for lactone (1763 cm^{-1}) and esters (1722 cm^{-1}). The ^1H and ^{13}C NMR signals (Table 1), of **5** revealed the existence of a β-substituted furan ring (δ_H 6.56 *t*, 7.60 *t*, 7.65 *brs* and δ_C108.2 *d*, 126.0 *s*, 140.1 *d*, 144.7 *d*) and three carbonyl ester groups; the value of the chemical shifts of these carbonyl ester groups at δ 166.9, 172.5 and 177.8 suggested that the former one is conjugated. In addition, three methyl groups were included in the molecule and they were identified from their chemical shifts and coupling patterns as a secondary group δ_H 1.13 and two methyl esters (δ_H 3.54 and 3.72). The third carbonyl group (δ 177.8) was deduced to to belong to a lactone moiety from HMBC data (Fig. 1). The spectral data of **5** were very similar to those of crotocorylifuran (**4**), which was isolated from the same plant (Ngadjui et al., 1999). Comparison of the NMR spectral

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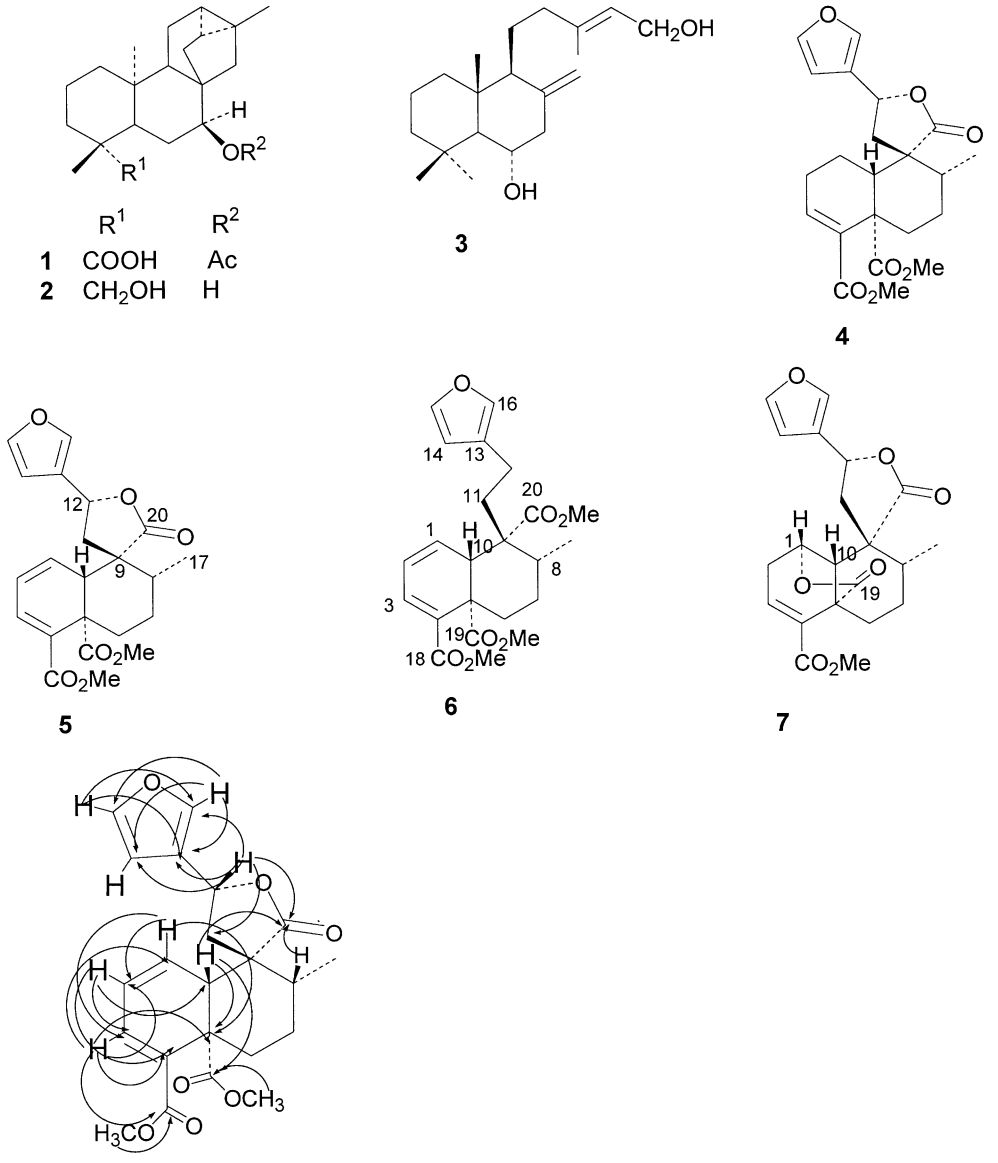

Fig. 1. Important HMBC correlations observed for compound **5**.

data of compounds **4** and **5** showed almost the same chemical shifts except for the signals of protons of ring A. Careful examination of the spectra suggested that in the structure of **5** there is an additional conjugated double bond. This was confirmed by: (i) the molecular weight of **5** which was less by two atomic mass units; (ii) the NMR which showed six vinyl proton signals instead of four observed in crotocorylifuran (**4**); (iii) the UV spectrum which showed a maximum absorption at λ_{max} 302 nm indicative of an extra conjugated bond on α,β-unsaturated carbonyl present in **4**. A λ_{max} value of 303 was obtained by empirical calculations for **5** as a substituted homoannular diene.

From the foregoing data, crotozambefuran A (**5**) was identified as 15,16-epoxy-1,3,13(16),14-clerodatetraen-20,12-olide-18,19-dioic acid dimethylester. The relative configuration at C-9 was investigated by use of NOE experiments. Irradiation of the H-12 proton signal at δ 5.60 led to a 4.1% enhancement of the H-10 signal (δ 3.02 *t*). Also irradiation of the latter signal enhanced H-11a, H-11b (5.1 and 7.2%, respectively). The other two axial proton signals H-8 (6.7%) and H-6 (δ 1.36 *br dt*, 6.6%) were also enhanced. The foregoing data are consistent with the relative configuration of the groups at C-8, C-9 and C-10, as shown in **5**.

Compound **6**, obtained as white needles from hexane–ethylacetate, was assigned the molecular formula as $C_{23}H_{28}O_7$ from HREIMS data. The ^1H and ^{13}C NMR spectra data (Table 1) of **6**, which showed a β-substituted furan ring, three carbonyl methyl ester groups, one secondary methyl group and a conjugated diene were similar to those of compound **5**. The ^{13}C NMR of **6** displayed three carbonyl ester groups at δ 167.4, 173.1 and 175.1. Furthermore the sp^3 oxymethine signal at δ_H

Table 1
NMR assignments of compounds **5** in CD_3OD and **6** in DMSO-d_6. Chemical shifts are given in ppm; multiplicities and coupling constant J (in parentheses) in Hz

	δ_C	δ_C	δ_C	δ_H	δ_H	δ_H
C/H	**5**	**6**	**7**	**5**	**6**	**7**
1	133.1 *d*	134.3 *d*	75.0 *d*	6.04 *dd* (3.2, 9.5)	6.62 *dd* (3.0, 9.7)	4.56 *brs*
2a	125.4 *d*	122.9 *d*	34.8 *t*	6.21 *ddd* (3.5, 5.3, 9.8)	6.12 *ddd* (3.2, 5.4, 9.6)	2.87 *dt* (2.7, 20.1)
2b	125.4 *d*	122.9 *d*	34.8 *t*	–	–	2.44 *m*
3	135.6 *d*	135.3 *d*	138.0 *d*	7.00 *d* (5.2)	6.99 *d* (5.4)	6.63 *brs*
4	136.1 *s*	134.8 *s*	137.1 *s*	–	–	–
5	47.5 *s*	47.1 *s*	45.4 *s*	–	–	–
6a	31.8 *t*	33.0 *t*	25.3 *t*	3.07 *dt* (3.2, 13.5)	2.90 *dt* (3.3, 13.4)	1.70 *m*
6b	31.8 *t*	33.0 *t*	25.3 *t*	1.36 *brdt* (3.4, 13.3)	1.44 *dt* (4.3, 13.4)	1.35 *m*
7a	28.2 *t*	28.8 *t*	26.7 *t*	2.06 *ddd* (2.6, 9.8, 13.6)	2.35 *m*	2.69 *brdd* (4.4, 17.0)
7b	28.2 *t*	28.8 *t*	26.7 *t*	1.56 *brdd* (3.6, 14.2)	1.55 *dq* (3.5, 14.0)	1.46 *brdd* (12.9, 17.2)
8	42.3 *d*	36.6 *d*	39.3 *d*	1.84 *m*	1.81 *m*	1.70 *m*
9	50.4 *s*	50.6 *s*	50.3 *s*	–	–	–
10	49.6 *d*	48.1 *d*	52.2 *d*	3.02 *t* (3.2)	2.96 *brt* (3.2)	2.23 *brs*
11a	41.4 *t*	33.9 *t*	42.3 *t*	2.65 *dd* (8.9, 14.3)	2.25 *m*	2.62 *dd* (7.7, 14.3)
11b	41.4 *t*	33.9 *t*	42.3 *t*	2.50 *dd* (8.2. 14.3)	2.05 *ddd* (3.4, 6.1, 13.0)	2.28 *dd* (9.7, 14.2)
12a	73.0 *d*	18.0 *t*	72.4 *d*	5.60 *t* (8.5)	2.30 *m*	5.45 *brt* (7.8)
12b	–	18.0 *t*	–	–	2.20 *m*	–
13	126.0 *s*	124.6 *s*	125.5 *s*	–	–	–
14	108.2 *d*	107.7 *d*	109.9 *d*	6.56 *brt* (0.8)	6.31 *t* (0.8)	6.70 *t* (0.7, 0.8)
15	144.7 *d*	143.2 *d*	145.3 *d*	7.60 *t* (1.6)	7.41 *t* (1.7)	7.73 *brt* (1.7)
16	140.1 *d*	139.0 *d*	141.9 *d*	7.65 *brs*	7.30 *brs*	7.87 *brs*
17	16.5 *q*	16.0 *q*	17.8 *q*	1.13 *d* (6.8)	1.15 *d* (6.9)	1.00 *d* (5.9)
18	166.9 *s*	167.4 *s*	165.6 *s*	–	–	–
19	172.5 *s*	173.1 *s*	175.4 *s*	–	–	–
20	177.8 *s*	175.1 *s*	176.0 *s*	–	–	–
OMe-18	51.2 *q*	51.0 *q*	52.6 *q*	3.72 *s*	3.72 *brs*	3.66 *s*
OMe-19	51.1 *q*	51.0 *q*	–	3.54 *s*	3.53 *brs*	–
OMe-20	–	50.6 *q*	–	–	3.60 *brs*	–

5.60 (*t*, $J=8.5$ Hz); δ_C 73.0 in **5** was replaced by a methylene group δ_H 2.30 and 2.20 (*m*, each), δ_C 18.0; the protons of this latter group showed cross peaks with the furan ring in the ^1H–^1H COSY and HMBC experiments. Thus the structure of crotozambefuran B (**6**) was determined as 15,16-epoxy-1,3,13(16),14-clerodatetraen-18,19,20-trioic acid trimethylester. The carbon resonances of **6** (Table 1) were assigned using DEPT, HSQC and HMBC experiments. The ^1H signals of **6** were generally well resolved and the coupling of these protons and the various sets of interacting protons were established by selective decoupling, TOCSY and ^1H–^1H COSY experiments leading to the confirmation of the proposed structure. The relative configuration at C-10 was deduced from NOE data as was done for **5**.

Crotozambefuran C (**7**) was isolated as white powder and its molecular formula was determined as $C_{21}H_{22}O_7$ from NMR and HREI–mass spectroscopy measurements. Its IR spectrum showed absorption bands for a conjugated carbonyl ester and lactone at ν_{max} 1719 and 1767 cm^{-1}, respectively. The ^{13}C NMR spectrum of **7** displayed 21 carbon signals assignable to two methyls, four methylenes, eight methines and seven quaternary carbons. The ^1H, ^{13}C NMR spectra and HMBC data were suggestive of one trisubstituted double bond (δ_H 6.63 *brs*, δ_C 138.0 *d*, 137.1 *s*), two lactones (δ 175.4, 176.0), one β-substituted furan ring (δ_H 6.70 *t*, 7.73 *t*, 7.87 *brs*; δ_C 109.9 *d*, 125.5 *s*, 141.9 *d*, 145.3 *d*), two sp^3 oxymethines (δ_H 4.56 *brs*, and 5.45 *brt* $J=7.9$ Hz; δ_C 75.0 and 72.4, respectively), one secondary methyl (δ_H 1.13) and one carbonyl methyl ester (δ_H 3.66; δ_C 165.6). The value of the chemical shift of the carbon of this later group is indicative of conjugated carbonyl.

Examination of the remaining carbon signals suggested that they constituted an octahydronaphthalene skeleton to which various substituents were connected. The positions of these substituents were deduced from a detailed analysis of the proton spectrum, ^1H selective decoupling and ^1H–^1H COSY experiments. The methyl group at δ_H 1.13 (*d*, H-17) displayed a cross peak with the proton at δ 1.70 (*m*, H-8): this proton was correlated to two other protons at δ 1.46 and 2.69 (*dd*, H-7, H-7′). In addition to their mutual coupling each of these two protons showed a cross peak with two protons at δ 1.70, 1.35 (*m*, H-6, H-6′). No further coupling to these protons was observed.

The sp^3 oxymethine proton signal at δ 5.45 (*t*, H-12) exhibited a cross peak with those of the two double-doublet protons at δ 2.62, 2.28 (H-11, H-11′) and this signal was also long-range coupled to that of the proton

on the furan ring. The chemical shift of H-12 (δ 5.45, *t*; 5.60 for **5**) suggested the same arrangement of furan and 5-membered ring lactone moieties as in **4** and **5**. The characteristic nature of the chemical shift and multiplicity of the H-12 signal for a proton attached to a carbon involved in the lactone group on one side and connected to a furan ring, on the other, has been described by Chen and Phillipson (1993). The signals of the vinyl proton at δ 6.63 (H-3) and the sp^3 oxymethine at δ 4.56 (H-1) showed a weak cross peak with the resonances of the protons at δ 2.44, 2.87 (H-2, H-2′); the H-1 signal also showed a weak coupling with H-10 (δ 2.23, *brs*). This should be due to the conformation shown in Fig. 2 where the two protons (H-1 and H-10) are orthogonal. The relative stereochemistry of the oxymethine in position 1 was then deduced to be α from the weak coupling of H-1 to H-10 (β-ax.). From the foregoing data, structure **7** was assigned to crotozambefuran C.

There is now a growing interest in the genus *Croton* and quite a number of recent reports including the present study clearly demonstrate the diversity of diterpenes with a variety of skeleton types (De Heluani et al., 2000; Kapingu et al., 2001), clerodanes and labdanes being by far the most common. From this and earlier study (Ngadjui et al. 1999) *C. zambesicus* may be considered as a rich source of clerodane and trachylobane diterpenes. Many of the diterpenoids possess cytotoxic (Roengsumran et al., 2001), anti-HIV-1 (Mekkawy et al., 2000) and anti-tumor properties (Grynberg et al., 1999).

3. Experimental

3.1. General

M.p.s uncorr.; UV: MeOH solution; IR: KBr disk; EI and HR MS: direct inlet 70 eV; ^{1}H and ^{13}C NMR (CD_3OD, DMSO-d_6) 600 or 300 MHz and 150 or 75 MHz, respectively, with the residual solvent peaks as internal references. HSQC, HMQC, TOCSY and HMBC experiments were performed with gradient enhancements.

3.2. Plant material

The stem bark of *C. zambesicus* was collected at Eloundem mountain, Yaounde, in the Central Province of Cameroon. Mr. P. Mezili of the National Herbarium in Yaounde identified the plant. Voucher specimen (8204/srfcam) is deposited at the National Herbarium, Yaounde, Cameroon.

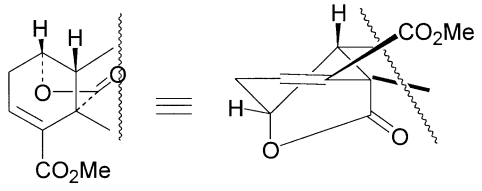

Fig. 2. Conformation observed in ring A for compound 7.

3.3. Extraction, isolation and characterization

The powdered, sun-dried stem bark of *C. zambesicus* (5 kg) was soaked in the mixture of CH_2Cl_2/MeOH (1:1) and pure MeOH for 24 and 2 h, respectively, at room temp. Concentration of the combined organic extract under red. press. yielded a greenish dark residue (195 g). Part of this extract (100 g) was subjected to column chromatography (silica gel 60, 300 g) and eluted with hexane followed by hexane–EtOAc gradient. Sitosterol (65 mg), lupeol (85 mg), crotocorylifuran (**4**, 22 mg) and betulinol (35 mg), were obtained from 5, 10, 15 and 20% EtOAc in hexane, respectively. Repeated column chromatography and PTLC on frs eluted with 25% ethyl acetate in hexane yielded: crotozambefurans A (**5**, 15 mg), B (**6**, 34 mg) and C (**7**, 20 mg). 7β-acetoxytrachyloban-18-oic acid (**1**, 28 mg), trachyloban-7β, 18-diol (**2**, 24 mg) and crotonadiol (**3**, 30 mg) together with sitosterol glucoside were eluted by hexane–EtOAc (1:1). Known compounds were identified from spectroscopic and physical data and comparison with published information and or with authentic specimens.

3.4. 15, 16-Epoxy-1,3,13(16),14-clerodatetraen-20,12-olide-18,19-dioic acid dimethylester, crotozambefuran A (*5*)

White solid, m.p. 143–146 °C; $[\alpha]_D^{25}$ −6.7° (MeOH, *c* 0.6); UV λ_{max}^{MeOH} nm (log ε): 207 (4.54), 302 (4.06); IR ν_{max}^{KBr}: 1763 (lactone), 1722 (ester), 1442, 1265 (C–O), 1154, 1081; EIMS *m*/*z* (rel. int.): 400 $[M]^+$ (20), 340 (42), 308 (100), 245 (24), 215 (38), 161 (84), 147 (52); CIMS *m*/*z* (rel. int.): 401 $[M+H]^+$ (100); ^{1}H NMR (600 MHz, CD_3OD), and ^{13}C NMR (150 MHz, CD_3OD): Table 1.

3.5. 15,16-Epoxy-1,3,13(16),14-clerodatetraen-18,19,20-trioic acid trimethylester, crotozambefuran B (*6*)

White needles in hexane–EtOAc, m.p. 108–109 °C; $[\alpha]_D^{25}$ −46.7° (MeOH, *c* 0.09); UV λ_{max}^{MeOH} nm (log ε): 209 (3.71), 298 (3.92); IR ν_{max}^{KBr} cm^{-1}: 1716 (C=O), 1571 (C=C), 1440, 1258 (C–O), 1208, 1100, 1020; EIMS *m*/*z* (rel. int.): 416 $[M]^+$ (5), 356 (15), 324 (100), 296 (40), 273 (15), 265 (20); HR–EIMS: found: *m*/*z* 416.4612, $[M]^+$ $C_{23}H_{28}O_7$ requires: 416.4672; ^{1}H NMR (300 MHz, CD_3OD) and ^{13}C NMR (75 MHz, CD_3OD): Table 1.

3.6. 15,16-Epoxy-3,13(16),14-clerodatrien-19,1α:20,12-diolide-18-oic acid methylester, crotozambefuran C (*7*)

White powder in hexane–EtOAc, m.p. 242 °C decomp.; $[\alpha]_D^{25}$ −25.0° (MeOH, *c* 0.11); UV λ_{max}^{MeOH} nm

(log ε): 209 (4.05); IR ν_{max}^{KBr} cm^{-1}: 1767 (lactone), 1719 (ester), 1624 (C=C), 1436, 1242 (C–O), 1163, 1120, 1027; EIMS m/z (rel. int.): 386 $[M]^+$ (20), 368 $[M-H_2O]^+$ (15), 355 $[M-OMe]^+$ (40), 339 (38), 310 (42), 254 (32), 248 (100), 216 (46), 201 (48); HR–EIMS: found: m/z 386.3915 $C_{21}H_{22}O_7$ requires: 386.3978; ^1H NMR (300 MHz, DMSO-d_6) and ^{13}C NMR (75 MHz, DMSO-d_6): Table 1.

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References

Chen, Y.C.Z.P., Phillipson, J.D., 1993. Clerodane diterpenoids from *Croton lechleri*. Phytochemistry 34, 265–268.

Heluani, De., Catalán, C.S., Hernández, C.A.N., Burgueño-Tapia, L.R., Joseph-Natan, E.P., 2000. Three new diterpenoids based on the novel sarcopetalane skeleton from *Croton sarcopetalus*. Journal of Natural Products 63, 222–225.

Grynberg, N.F., Echevarria, A., Lima, J.E., Pamplona, S.S.R., Pinto, A.C., Maciel, M.A.M., 1999. Anti-tumor activity of two 19-nor-clerodane diterpenes, *trans*-dehydrocrotonin and *trans*-crotonin, from *Croton cajucara*. Planta Medica 65, 687–689.

Hutchinson, L.J., Dalziel, J.M., 1958. Flora of West Tropical Africa, Revised by R.W.J. Keay. Vol. 1, Part 2, 2nd edn. White Press, London, p. 393.

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

Kapingu, M.C., Guillaume, D., Mbwambo, Z.H., Moshi, M.J., Uliso, F.C., Mahunnah, R.L.A., 2001. Diterpenoids from the roots of *Croton macrostachys*. Phytochemistry 54, 767–770.

Mekkawy, S.El., Meselly, M.R., Nakamura, N., Hattori, M., Kawahata, T., Otake, T., 2000. Anti-HIV-1 phorbol esters from the seeds of *Croton tiglium*. Phytochemistry 53, 457–464.

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

Roengsumran, S., Retson, A., Kuptiyanuwat, N., Vilaivan, T., Ngamrojnavanich, N., Chaichantipyuth, C., Phuthong, S., 2001. Cytotoxic labdane diterpenoids from *Croton oblongifolius*. Phytochemistry 56, 103–107.