PERGAMON

Phytochemistry 51 (1999) 171–174

PHYTOCHEMISTRY

Crotonadiol, a labdane diterpenoid from the stem bark of *Croton zambesicus*

Bonaventure T. Ngadjui[a,*], Gabriel G. Folefoc[a], Felix Keumedjio[a], Etienne Dongo[a], Beiban L. Sondengam[a], Joseph D. Connolly[b]

[a]*Department of Organic Chemistry, University of Yaoundé-1, P.O. Box 812, Yaoundé, Cameroon*
[b]*Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, UK*

Received 24 September 1998

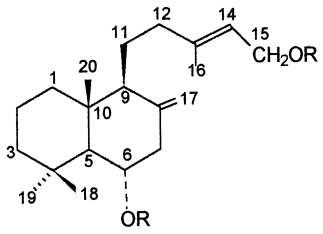
Abstract

A new labdane diterpenoid, crotonadiol, was isolated from an extract of 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. Lupeol, β-sitosterol and its 3-β-glucopyranosyl derivative were also isolated. The structure of crotonadiol was determined as 8(17),13-labdadiene-6α,15-diol, by spectral analysis. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Croton zambesicus; Euphorbiaceae; Stem bark; Labdane; Clerodane and trachylobane diterpenoids; Crotonadiol

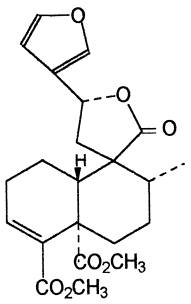
1. Introduction

Croton zambesicus Muell. Arg. (Syn. *C. amabilis* Muell. Arg.) is a shrub or small tree reaching 10 m in height and widespread in tropical west and Central Africa (Hutchinson & Dalziel, 1958). The stems are used for house posts in parts of West Africa. The roots are used as an aperient. The leaf decoction is used as a wash for fevers in Sierra Leone and Nigeria and internally for dysentery and convulsions (Irvine, 1961). Alkaloids (Shamma, Shine, & Dudock, 1967; Stuart & Byfield, 1971) and diterpenoids (Burke, Chan, Keith, Blount, & Manchand, 1981; Craveiro, Afianio, & Edilberto, 1982) have mainly been recorded from this genus. No previous phytochemical and pharmacological studies have been reported on *C. zambesicus*. As part of our continuing studies on Cameroonian plants of medicinal interest, we have examined the extract of the stem bark of *C. zambesicus*. This paper reports the isolation and structural elucidation of a new labdane diterpenoid, crotonadiol (**1**), together with the previously known clerodane, crotocorylifuran (**2**) (Burke et al., 1976; Burke et al., 1981; Tchissambou, Chiaroni, Riche, & Khuong-Huu, 1990), the trachylobanes, 7β-acetoxytrachyloban-18-oic acid (**3**) (Hasan, Healey, & Waterman, 1982) and trachyloban-7β, 18-diol (**4**) (Gonzalez, Breton, Fraga, & Luis, 1971;

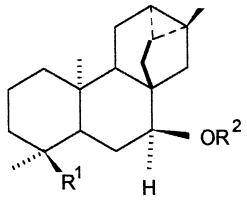


1 R = H

1a R = Ac



2



	R^1	R^2
3	COOH	Ac
4	CH_2OH	H

* Corresponding author. Fax: +23-53-88; e-mail: bngadjui@uycdc.uninet.cm.

PII: S0031-9422(98)00706-7

Gonzalez, Fraga, Hernandez, & Luis, 1973), the pentacyclic triterpenoid, lupeol (Ayafor, Ngadjui, Sondengam, & Tsamo, 1984) and sitosterol and its glucoside.

2. Results and discussion

Crotonadiol (**1**) was isolated from a chloroform–methanol (1:1) extract of dried powdered stem bark, as described in Section 3. Its molecular formula was determined as $C_{20}H_{34}O_2$ from NMR and EI-mass spectroscopy measurements. Crotonadiol showed IR absorption bands for hydroxy groups at ν_{max} 3500–3400 cm^{-1} and an exomethylene group at ν_{max} 1630, 890 cm^{-1}. The NMR spectra of **1** showed typical characteristics of a labdan-8(17), 13-diene skeleton [δ_H 4.61 and 4.90 (1H each, br s, 2H-17), 5.38 (1H, t, $J=6.9$ Hz, H-14), δ_C 108.2 (t, C-17), 145.5 (s, C-8), 123.2 (d, C-14), 140.3(s, C-13)]. The ^1H NMR spectrum of crotonadiol also indicated the presence of four tertiary methyl groups at 0.72, 1.02, 1.19 and 1.72 (3H each, s). The *E*-geometry of the side chain was established by the chemical shift of the methyl carbon at C-13 (δ_c 16.3). The chemical shift (δ_{Ca} 4.15) of the methylene group at C-15 which was coupled to the proton at C-14 (δ 5.38, t, $J=6.9$ Hz) indicated that this methylene group was hydroxylated. Furthermore the ^1H NMR spectrum of crotonadiol displayed a broad triplet doublet signal [δ 3.83 (1H, $J=4.9$, 10.7 Hz)] due to an oxymethine which was coupled to both a proton at 1.14 (d, $J=10.7$ Hz) and methylene protons at 2.04 (1H, br t, $J=12.0$ Hz) and 2.68 (1H, dd, $J=4.9$, 12.1 Hz). Therefore, this second hydroxy group should be attached to C-6 or C-11. However, the latter alternative position for the hydroxy group was discarded from HMBC and NOESY since the chemical shift (δ_{Ca} 1.7) of the proton at C-9 was observed at a similar position to those in a similar compound (Iwagawa, Yaguchi, Hase, Okubo, & Kim, 1992). The *trans*-relationship between the protons at C-5 and C-6 was deduced from the *diaxial* coupling constant ($J=10.7$ Hz). Thus crotonadiol was identified as labda-8(17), 13*E*-dien-6α, 15-diol. This structure was confirmed by both the ^{13}C NMR spectrum and the EI mass spectrum. The EI mass spectrum showed fragments at *m/z* 288, 273 and 255 corresponding to $[M\text{-}H_2O]^+$, $[M\text{-}H_2O\text{–}Me]^+$ $[M\text{-}2H_2O\text{–}Me]^+$, respectively. The ^{13}C NMR (Tables 1, 2 and 3) was fully assigned using DEPT spectra and by comparison of measured values with those reported for similar compound (Iwagawa et al., 1992). Crotonadiol (**1**) is reported here for the first time while its 6,15-di-*O*-glucopyranoside (gomojoside H) has been isolated from *Viburnum suspensum* (Caprifoliaceae) (Iwagawa et al., 1992).

Table 1
^{13}C NMR spectral data of **1** and **1a** in $CDCl_3$ at 90 MHz

C	1	1a
1	39.3 (t)	39.1 (t)
2	19.1 (t)	19.0 (t)
3	43.7 (t)	44.2 (t)
4	33.9 (s)	33.5 (s)
5	60.5 (d)	57.5 (d)
6	71.7 (d)	73.2 (d)
7	49.2 (t)	43.5 (t)
8	145.5 (s)	144.2 (s)
9	55.5 (d)	55.2 (d)
10	39.4 (s)	39.6 (s)
11	22.1 (t)	21.9 (t)
12	38.4 (t)	38.3 (t)
13	140.3 (s)	142.7 (s)
14	123.2 (d)	118.2 (d)
15	59.4 (t)	61.3 (t)
16	16.3 (q)	16.5 (q)
17	108.2 (t)	109.3 (t)
18	36.6 (q)	36.1 (q)
19	22.4 (q)	22.4 (q)
20	16.1 (q)	16.0 (q)
CO*Me*		21.1 (q)
CO*Me*		21.8 (q)
*CO*Me		170.1 (s)
*CO*Me		171.1(s)

3. Experimental

3.1. General

M.p.'s uncorr; UV-visible: MeOH solution; IR: KBr disk or $CHCl_3$ solution; EIMS direct inlet 70 eV. ^1H NMR (360 MHz) and ^{13}C NMR (90 MHz) recorded at room temp., residual solvent peaks as internal reference. HMBC, HMQC and NOESY experiments were performed with gradient enhancements.

3.2. Plant material

The stem bark of *Croton zambesicus* was collected at Eloundem mountain, Yaoundé, in the Central Province of Cameroon. A voucher specimen (No. 8204/SRFCAM) for the collection is deposited at the National Herbarium, Yaounde, Cameroon.

3.3. Extraction, isolation and characterization

The air-dried powdered plant material (2 kg) was macerated in CH_2Cl_2–MeOH (1:1). Removal of the solvent under red. pres. yielded a dark brown extract (60 g). Part (50 g) of this extract was subjected to repeated chromatographic fractionations on a silica gel column eluted with hexane followed by a hexane–EtOAc gradient. Sitosterol (40 mg), lupeol (35 mg) and crotocorylifuran (2; 15 mg) were obtained from frs eluted by hexane–EtOAc 19:1, 9:1 and 17:3, respectively; 7β-acetoxytrachyloban-18-oic acid (**3**; 20 mg), trachyloban-7β, 18-diol (**4**; 15 mg), crotonadiol (**1**; 20 mg) together with sitosterol glucoside (150 mg), were obtained from frs eluted by hexane–EtOAc (1:1). Known compounds were identified by comparison (m.p., ^1H, ^{13}C NMR) with

Table 2
^{1}J (from HMQC) ^{2}J and ^{3}J gradient HMBC correlations for compound **1**

Proton	Position	^{1}J correlated carbon	^{2}J, ^{3}J correlated carbons
5.38	14	123.2	16.3 (C-16)
4.90	17	108.2	49.2 (C-7); 55.5 (C-9)
4.61	17	108.2	49.2 (C-7); 55.5 (C-9)
4.15	15	59.4	123.2 (C-14); 140.3 (C-13)
3.83	6	71.7	
2.68	7	49.2	55.5 (C-9); 60.5 (C-5); 71.7 (C-6); 108.2 (c-17); 145.5 (C-8)
2.04	7	49.2	71.7 (C-6); 108.2 (C-17); 145.5 (C-8)
1.83	1	39.3	16.1 (C-20)
1.72	Me-16	16.3	38.4 (C-12); 123.2 (C-14); 140.3 (C-13)
1.64	9	55.5	16.3 (C-16); 39.4 (C-10); 22.1 (C-11); 38.4 (C-12)
1.53	2	19.1	43.7 (C-3); 33.9 (C-4)
1.48	2	19.1	33.9 (C-4)
1.19	Me-18	36.6	22.4 (C-19); 33.9 (C4); 43.7 (C-3); 60.5 (C-5)
1.14	5	60.5	22.4 (C-19); 33.9 (C4); 36.6 (C-18)
1.02	Me-19	22.4	33.9 (C4); 43.7 (C-3); 60.5 (C-5); 36.6 (C-18)
0.72	Me-20	16.1	39.3 (C-1); 39.4 (C-10); 55.5 (C-9); 60.5 (C-5)

Table 3
Volume integrated NOESY correlations observed for H-14, 2H-17, 2H-15, H-6β, H-7eq, H-7ax, 2H-12, H-5, Me-16, Me-18, Me-19 and Me-20 in crotonadiol (**1**)

From	to
H-5	H-9 (3.9) H-7a (1.5)
H-6β	H-7eq (1.6); Me-20 (1.5); Me-19 (2.2)
H-7ax	H-7eq (5.4); H-5 (0.7) H-9 (0.7)
H-7eq	H-7ax (6.2); H-6β (1.7), H-17a (2.1)
H-12a	H-12b (4.0)
H-12b	H-12a (3.7); H-9 (1.4); H-14 (0.9)
H-14	2H-15 (2.7)
H-15a	H-14 (2.0); Me-16 (2.0)
H-15b	H-14 (2.0); Me-16 (2.0)
H-17a	H-7eq (4.0); H-17b (13.0)
H-17b	H-17a (4.0); H-12 (1.3); H-11 (3.3)
Me-16	H-1 (3.3)
Me-18	Me-19 (3.1); H-3ax (1.2)
Me-19	Me-20 (7.1); H-6β (5.2); Me-18 (2.8); H-3eq (1.3); H-2ax (1-4)
Me-20	Me-19 (6.8); H-2ax (1.8); H-1eq (0.8)

The volme of integration (%) is given in parentheses.

authentic samples or published information. Sitosterol glucoside (60 mg), which was insoluble in the usual organic solvents, was acetylated using boiling Ac_2O (6 ml) for 2 h. The reaction mixture was evaporated in a petri dish to leave a residue which was chromatographed by CC (hexane–EtOAc, 3:2) to give white platelets of tetraacetate of sitosterol-3-β-D-glucopyranoside (55 mg, 80%) m.p. 166°C.

3.4. Crotonadiol (*1*)

Colourless oil; $[\alpha]_D^{25}$ −28°C ($CHCl_3$, *c* 0.12), UV λ_{max}^{MeOH} nm (log ε): 210 (4.10); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500–3400 (OH), 1630, 1530, 1420, 1200, 1100, 930, 890, 860, EIMS *m/z* (rel.int.): 306 ([M]$^+$, 10), 288 ([M-H_2O]$^+$, 25), 273 ([M-H_2O–Me]$^+$, 40), 255 ([M-Me–2H_2O]$^+$, 35), 243 (15), 203 (18), 190 (20), 187 (17), 153 (35), 109 (65), 69 (100); ^{1}H NMR (360 MHz, $CDCl_3$): δ 0.72 (3H, s, 3H-20), 1.02 (3H, s, 3H-19), 1.14 (1H, d, J=10.7 Hz, H-5), 1.19 (3H, s, 3H-18), 1.48 (1H, m, H-2a), 1.53 (1H, m, H-2b), 1.64 (1H, br d, J=11.8, H-9), 1.72 (3H, s, 3H-16), 1.83 (1H, m, H-1), 1.92 (1H, br dt, J=8.0, 14.0 Hz, H-12a), 2.04 (1H, br t, J=12.0 Hz, H_α-7), 2.17 (1H, m, H-12b), 2.68 (1H, dd, J=4.9, 12.1 Hz, H_β-7), 3.83 (1H, br dt, J=4.9, 10.7 Hz, H-6), 4.15 (2H, d, J=6.9 Hz, 2H-15), 4.61 (1H, br s, H-17a), 4.90 (1H, br s, H-17b) and 5.38 (1H, br t, J=6.9 Hz, H-14). ^{13}C NMR (90 MHz, $CDCl_3$): Table 1.

3.5. Acetylation of crotonadiol (*1*)

Compound **1** (12 mg) in CH_2Cl_2 (2 ml) was treated with Ac_2O (2 ml) in the presence of a catalytic amount of DMAP for 2 h. The reaction was monitored by TLC. The reaction mixture was mixed with celite (3 g) and evaporated into dryness in vacuo and the powder obtained was introduced on to a silica gel column and eluted with hexane–EtOAc (7:3) to give the diacetate **1a** (10 mg, 70%): colourless oil; UV λ_{max}^{MeOH} nm (log ε): 211 (3.80); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1700 (C=O), 1640 (C=C), 1580, 1500, 1450, 1400, 1100, 880. EIMS *m/z* (rel. int.): 390 ([M]$^+$, 15). ^{1}H NMR (360 MHz, $CDCl_3$): δ 0.74 (3H, s, 3H-20), 0.87 (3H, s, 3H-19) 1.01 (3H, s, 3H-18), 1.39 (1H, d, J=11.2 Hz H-5), 1.69 (3H, s, 3H-16), 1.98 (1H, br dt, J=7.8, 13.7 Hz, H-12a); 2.03 (3H, s, CH_3CO), 2.05 (3.14, s, CH_3CO), 2.20 (1H, m, H-12b), 2.69 (1H, dd, J=5.0, 12.1 Hz, H_β-7), 4.58 (2H, br d, J=6.7 Hz 2H-15), 4.62 (1H, br s, H-17a), 4.93 (1H, br s, H-17b), 5.03 (1H, dt, J=5.0, 10.2 Hz H-6), 5.30 (1H, br t, J=6.7 Hz H-14), ^{13}C NMR (90 MHz, $CDCl_3$): Table 1.

Acknowledgements

BTN acknowledges the Royal Society of London for a three-month grant to the Department of Chemistry of the University of Glasgow and the IFS (Stockholm).

References

Hutchinson, L. J. & Dalziel J. M. (1958). Revised by R.W.J. Keay (Ed.), *Flora of West Tropical Africa* (2nd ed., Vol. 1, Part 2, p. 393). London: White Frias Press.

Irvine, F.R. (1961). *Woody plants of Ghana* (p. 221). London: Oxford University Press.

Stuart, L., & Byfield, D. Y. (1971). *Phytochemistry*, *10*, 460.

Shamma, M., Shine, R. J., & Dudock, B. S. (1967). *Tetrahedron*, *23*, 2887.

Craveiro, A., Afianio, S., & Edilberto, R. (1982). *Phytochemistry*, *21*, 2571.

Burke, A. B., Chan, W. R., Keith, P. D., Blount, J. F., & Manchand, P. S. (1981). *J. Chem. Soc. Perkin Trans.*, *1*(10), 2666.

Tchissambou, L., Chiaroni, A., Riche, L., & Khuong-Huu, F. (1990). *Tetrahedron*, *46*, 5199.

Burke, B. A., Chan, W. R., Prince, E. C., Manchand, P. S., Eickman, N., & Clardy, J. (1976). *Tetrahedron*, *1881*, 32.

Hasan, C. M., Healey, T. M., & Waterman, P. G. (1982). *Phytochemistry*, *21*, 177.

Gonzalez, A. G., Fraga, B. M., Hernandez, M. G., & Luis, J. G. (1973). *Phytochemistry*, *12*, 1113.

Gonzalez, A. G., Breton, J. L., Fraga, B. M., Luis, J. G. (1971). *Tetrahedron Letters*, 3097.

Ayafor, J. F., Ngadjui, B. T., Sondengam, B. L., & Tsamo, E. (1984). *Planta Medica*, *50*, 205.

Iwagawa, T., Yaguchi, S., Hase, T., Okubo, T., & Kim, M. (1992). *Phytochemistry*, *31*, 1311.