Available online at www.sciencedirect.com
SCIENCE DIRECT®

Phytochemistry 65 (2004) 3083–3087

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Two labdane diterpenoids and a *seco*-tetranortriterpenoid from *Turreanthus africanus*

Pierre Tane [a,*], Mac Thomas Akam [b], Apollinaire Tsopmo [a], Chi P. Ndi [a], Olov Sterner [c]

[a] *Department of Chemistry, Faculty of Science, University of Dschang, P.O. Box 67, Dschang, Cameroon*
[b] *Department of Chemistry, Faculty of Science, University of Buea, P.O. Box 63, Buea, Cameroon*
[c] *Department of Organic and Bioorganic Chemistry, Lund University, P.O. Box 124, SE 221 00 Lund, Sweden*

Received 18 May 2004; received in revised form 8 October 2004

Abstract

Examination of the methylene chloride soluble portion of the acetone extract of the seeds of *Turreanthus africanus* yielded two labdane diterpenoids 12,15-epoxylabda-8(17),12,14-trien-16-al (**1**) and 16-acetoxy-12(*R*),15-epoxy-15β-hydroxylabda-8(17),13(16)-diene (**2**) and a limonoid, 17-*epi* 12-dehydroxyheudebolin (**3**). Structures elucidation was based on the analysis of spectroscopic data.

Keywords: *Turreanthus africanus*; Meliaceae; Labdane; Limonoid

1. Introduction

Species of the Meliaceae family are well-known for the production of terpenoid compounds including limonoids (Kimbu et al., 1984; Ayafor et al., 1994). The species *Turreanthus africanus* (Welw. ex. C.D.C.) Pellegr. is a tree distributed in tropical zone of Africa from Sierra Leone to the Congo and Angola. It is usually found in dense stands in rain forests along side lakes and streams (Kline, 1979; Kaiser, 1990). In Cameroon, the stem bark is used in the treatment of intestinal worms. In the course of our search for new antiparasitic agents from Cameroonian medicinal plants (Tchuendem et al., 1999; Bickii et al., 2000), the seeds of *T. africanus* were investigated. Fractionation and purification of the extract led to the isolation of two novel labdane diterpenoids **1** and **2**, and one limonoid **3**.

2. Results and discussion

A sample of the air-dried seeds of *T. africanus* was extracted by maceration in acetone. Concentration yielded an extract, which was defatted with hexane followed by extraction with methylene chloride. Fractionation of the CH_2Cl_2 extract was achieved by column chromatography over silica gel. The new diterpenoids **1** and **2** together with the novel limonoid **3** were obtained.

Compound **1** was obtained as orange oil. Its EIMS gave a molecular ion peak at *m*/*z* 300 and the molecular formula $C_{20}H_{28}O_2$ was deduced from this together with analysis of the NMR and HMQC spectra. The ^{1}H NMR spectrum (Table 1) of **1** displayed proton signals for an aldehyde function at δ 10.02, three methyl groups all appearing as singlet around 1 ppm, two olefinic protons signals at δ 6.66 and 7.26 each doublet ($J = 2.0$). Two more signals at δ 4.59 and 4.78 were characteristic of the exomethylene moiety at C-8 found in labdane diterpenoids (Kimbu et al., 1987; Itokawa et al., 1980). The ^{13}C NMR spectrum (Table 1) of **1** analyzed with the

---

[*] Corresponding author. Tel.: +237 3451735/7619546; fax: +237 3451202.
E-mail address: ptane@yahoo.com (P. Tane).

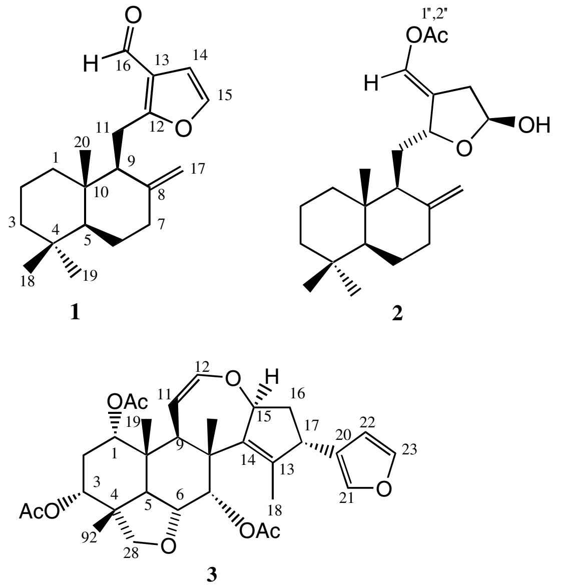
0031-9422/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2004.10.007

Table 1
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data (δ, multiplicity, *J*) for **1** and **2** in $CDCl_3$ with the solvent signals (7.26 and 77.0) as reference

Position	**1**		**2**	
	δ_C	δ_H	δ_C	δ_H
1	39.1	1.19/1.74 *m*	39.1	1.02 *dt* (3.6, 12.6) 1.67 *m*
2	19.3	1.53/1.60 *m*	19.3	1.47/1.56 *m*
3	42.0	1.23/1.43 *m*	42.0	1.20 *dt* (4.1, 13.4) 1.40 *m*
4	33.6	–	33.6	–
5	55.4	1.21 *m*	55.5	1.15 *dd* (2.5, 12.6)
6	24.1	1.36/1.76 *m*	24.3	1.34/1.75 *m*
7	37.8	2.02 *dt* (4.6, 13.0) 2.36 *ddd* (2.4, 4.1, 13.0)	38.2	2.03 *dt* (4.7, 13.0) 2.41 *ddd* (2.4, 4.0, 11.8)
8	148.0	–	148.3	–
9	54.6	2.44 *dd* (3.8, 10.3)	52.6	1.78 *m*
10	39.7	–	39.6	–
11	23.0	3.08 *dd* (3.8, 15.7) 3.15 *dd* (10.3, 15.7)	30.4	1.61/1.80 *m*
12	165.8	–	77.3	4.73 *m*
13	122.1	–	125.3	–
14	108.4	6.66 *d* (2.0)	35.7	2.74 *br d* (16.3) 2.68 *dd* (4.6, 16.1)
15	141.5	7.26 *d* (2.0)	97.0	5.60 *d* (4.6)
16	184.9	10.02 *s*	128.6	7.12 *d* (1.4)
17	107.4	4.59/4.78 *s*	107.1	4.65/4.87 *s*
18	21.7	0.84 *s*	21.7	0.80 *s*
19	33.6	0.91 *s*	33.5	0.89 *s*
20	14.3	0.81 *s*	14.4	0.70 *s*
1′			167.9	–
2′			20.7	2.17 *s*

aid of the HMQC spectrum revealed 20 carbon atoms including 3 methyles, 7 methylenes, 5 methines, and 5 quaternary carbons. The exomethylene was characterized on this spectrum by signals at 107.4 (*t*) and 148.3 (*s*). A detailed analysis of the ^{1}H–^{1}H COSY spectrum in conjunction with the HMQC spectrum established the presence of several spin systems, namely a $–CH_2–CH_2–CH_2–$, a $–CH–CH_2CH_2–$, a $–CH–CH_2–$, and a –CH–CH–. All the above data as well as those from the HMBC spectrum led to the completed structure elucidation of compound **1**. In the HMBC spectrum, pertinent correlations were observed between the aldehyde proton 16-H (δ 10.02) and C-12, C-13 and C-14, between proton 14-H and C-13, C-14, C-15 and C-16. Further correlation were observed between 9-H and C-1, C-5, C-8, C-11, C-12, C-17 and C-20 as well as well as between 5-H and C-3, C-9, C-18, C-19 and C-20.

The configuration of compound **1** was suggested by correlations observed in a NOSEY spectrum (Fig. 1). Correlations were observed from 1-Hα to 5-H, 9-H and 11-Hβ, from 5-H to 3-Hα, 7-Hα, 9-H and 19-H_3 as well as from 1-Hβ to 6-Hβ, 11-Hα and 18-H_3. On the basis of all spectroscopic evidences **1** was the novel labdane diterpenoid 12,15-epoxylabda-8(17),12,14-trien-16-al.



1 **2**

3

Fig. 1. Structure of isolated compounds.

Compound **2** was obtained as a yellowish oil, $[\alpha]_D = +35.0°$. Its IR spectrum showed absorption bands at υ_{max} 3404, 1701 and 1684 cm^{-1} representing hydroxyl, carbonyl and olefinic functionalities, respectively. The molecular formula $C_{22}H_{34}O_4$ attributed to **2** was deduced from microanalysis of EIMS and NMR data including the HMQC spectrum. The ^{1}H and ^{13}C NMR spectra (Table 1) of the decaline system in **2** showed close similarity with those of the same system in compound **1**. Differences between the two compounds were the absence of the aldehyde signal as well as the furan aromatic signals in both ^{1}H and ^{13}C NMR spectra of **2**. Analysis of ^{1}H NMR spectrum of compound **2** displayed apart of signals due to the decaline system, protons at δ 7.12 (*d*), 5.60 (*d*), 4.73 (*m*), 2.74 (*d*), and 2.68 (*dd*). The ^{13}C NMR spectrum in association with the HMQC spectrum showed an hemiacetal carbon at δ 97.0, an actetate function at δ 20.7 (*q*) and 167.9 (*s*), and an oximethine carbon at δ 77.3. Analysis of the HMBC spectrum (summarized in Fig. 2) showed how various parts fix together. pertinent correlations were observed on this spectrum between the hemiacetal proton 15-H and C-12, C-13 and C-14, between the olefinic proton 16-H (7.12) and C-1′, C-12, C-13 and C-14 as well as between 11-H_2 and C-8, C-9, C-10, C-12 and C-13. The compound was then characterized as the novel 16-acetoxy-12(*R*),15-epoxy-15β-hydroxylabda-8(17),13(16)-diene (**2**).

The stereochemistry of **2** was achieved by analysis of its NOESY spectrum (Fig. 2). Correlations were observed for example from 11-Hα to 1-Hβ and 20-H_3, from 12-H to 9-H, 11-Hβ and 14-Hβ as well as from 16-H to 9-H and 11-Hβ. The configurations at C-5, C-9, and C-10 were determined by observed correlations similar to those found for compound **1**. However, upon

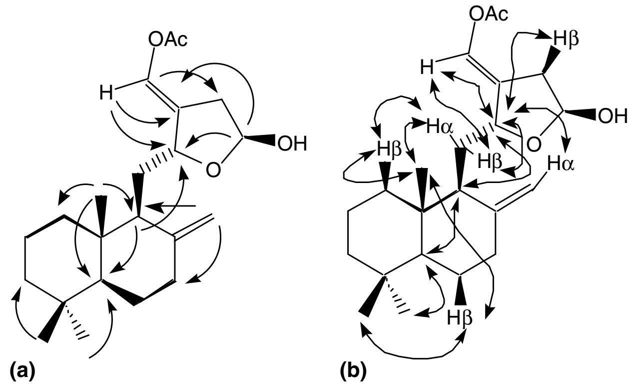


Fig. 2. Pertinent correlations observed with compound **2**: (a) HMBC correlations (H to C); (b) NOESY correlations.

standing at room temperature for days in chloroform, **2** slowly isomerized to a C-15 epimeric mixture (ca. 2:1).

Compound **3** was obtained from an hexane–EtOAc mixture as white crystals, m.p. 122–124 °C. Its IR spectrum displayed large absorption bands at υ_{max} 1732 and 1647 cm^{-1} for carbonyl and double bonds functionalities. The EIMS revealed fragment ion peaks at *m/z* 508, 448 and 388 produced by sequential elimination of three acetic acid units from the molecular ion peak at *m/z* 568. The molecular formula $C_{32}H_{40}O_9$ was then deduced from those observations as well as from NMR data. The ^1H NMR spectrum (Table 2) of **3** showed characteristic chemical shifts for acetyl groups at δ 2.02 (6H, s) and 2.13 (3H, *s*), three angular methyl groups at δ 1.03, 1.17, and 1.45 as well as a methyl linked to a sp^2 carbon at δ 1.78. The furan moiety was shown by proton signals at δ 6.31 (*d*, $J = 1.6$), 7.24 (*s*), and 7.31 (*d*, $J = 1.6$) while signals due to seven proton attached to oxygenated carbons were observed between δ 3.41–5.76. The ^{13}C NMR spectrum (Table 2) of **3** confirmed the presence of three acetate units with the shifts at δ 169.3, 169.5 and 169.7 for carbonyls and δ 20.6, 20.6 and 21.0 for methyls. Five oxygenated carbons were characterized by shifts between 71.3 and 85.1 while six olefinic carbons appeared at δ 96.7, 127.5, 139.0, 141.4, 142.7 and 144.2. The ^1H–^1H COSY spectrum showed how the proton sequences were and the HMBC correlations how the various parts were linked together. In that HMBC spectrum, major correlations were observed between 2-H_2 and C-1, C-2, C-4 and C-10, between 3-H and C-1, C-4, C-5, C-28 and C-29, between 9-H and C-1, C-5, C-7, C-11, C-12, C-19 and C-30. Further correlations were observed from 15-H to C-12, C-13, C-14 and C-17 as well from 17-H to C-13, C-15, C-20, C-21 and C-22. On the basis of the above spectroscopic data as well as by comparison with the data obtained for related compound heudebolin (Adesid and Okorie, 1973) and nimbilin (Ara et al., 1989), the compound was characterized as 17-*epi* 12-dehydroxyheudebolin (**3**).

Table 2
^1H (500 MHz) and ^{13}C (125 MHz) NMR data (δ, multiplicity, *J*) for **3** in $CDCl_3$ with the solvent signals (7.26 and 77.0) as reference

Position	δ_C	δ_H
1	71.3	4.79 *t* (2.8)
2	27.4	2.15 *dt* (2.4, 16.6) 2.25 *dt* (3.2, 16.6)
3	71.4	4.94 *t* (2.8)
4	42.0	–
5	40.3	2.59 *d* (12.2)
6	72.0	4.11 *dd* (3.0, 12.2)
7	73.8	5.76 *d* (3.0)
8	49.4	–
9	40.2	4.01 *dd* (1.8, 4.4)
10	39.0	–
11	96.9	4.03 *dd* (4.4, 7.3)
12	144.2	6.06 *dd* (1.8, 7.3)
13	143.0	–
14	141.4	–
15	85.1	5.55 *d* (7.2)
16	37.7	1.75 *d* (14.5) 2.37 *ddd* (7.2, 9.2, 14.5)
17	46.9	3.41 *d* (9.1)
18	15.8	1.78 *s*
19	15.7	1.03 *s*
20	127.5	–
21	139.0	7.24 *s*
22	110.1	6.31 *d* (1.6)
23	142.7	7.31 *d* (1.6)
28	77.8	3.49/3.56 *d* (7.5)
29	18.9	1.17 *s*
30	19.8	1.45 *s*
1-Ac	20.7/169.5	2.02 *s*
3-Ac	21.0/169.7	2.13 *s*
7-Ac	20.6/169.3	2.02 *s*

The stereochemistry of **3** was made by correlations observed in a NOESY experiment as well as by coupling constants from the proton spectrum. The coupling constants obtained for 5-H (*d*, $J = 12.2$), 6-H (*dd*, $J = 3.0$, 12.2), and 7-H (*d*, $J = 3.0$) led the determination of configuration at those positions. On the NOSEY spectrum correlations observed between 1-H and 3-H, 19-H_3 and 29-H_3, between 6-H and 19-H_3, 29-H_3 and 30-H_3 as well as those between 9-H and 5-H and 15-H allowed the complete determination of all stereocenters in compound **3**.

3. Experimental

3.1. General experimental procedures

Melting points were recorded with a Reichter microscope and are uncorrected. The optical rotations were measured with a Perkin–Elmer 341 polarimeter at 20 °C while the IR spectra were recorded with a FT-IR Shimazu Hyper 1.51 spectrophotometer. ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded in $CDCl_3$ using a Bruker ARX500 spectrometer with an inverse multinuclear 5 mm probe head equipped with a shielded gradient coil. The chemical shifts (δ) are reported in parts per million relative to chloroform (7.26 ppm for $C\mathit{H}Cl_3$ and 77.0 ppm for $\mathit{C}DCl_3$) while the coupling constants (J) are given in Hertz. COSY, HMQC and HMBC experiments were recorded with gradient enhancements using sine shaped gradient pulses. For 2D heteronuclear correlation spectroscopy the refocusing delays were optimized for $^1J_{CH} = 145$ Hz and $^nJ_{CH} = 10$ Hz. The raw data were transformed and the spectra evaluated with the standard Bruker UXNMR. EIMS and HREIMS spectra (direct inlet at 70 eV) were recorded with a JEOL SX102 spectrometer. Column chromatography was run on Merck silica gel 60. TLC analyses were carried out on silica gel GF_{254} precoated plates with detection accomplished by spraying with 15% H_2SO_4 followed by heating at 100 °C.

3.2. Plant material

The seeds of *T. africanus* was collected from neighbourhood of Yaounde, Cameroon, in December 1999. Authentication was done at the National Herbarium, Yaounde. The voucher specimens (UD 456) is deposited at the herbarium of the Botany Department, University of Dschang.

3.3. Extraction and isolation

The air-dried powdered seeds of *T. africanus* (2.1 kg) was extracted by percolation at room temperature with methylene chloride (3×7 l). Vacuum concentration by rotatory evaporation yielded 60 g of extract. Part of this extract (40 g) was subjected to column chromatography on silica gel eluting with a gradient system of hexane:EtOAc. Eighty one fractions of 250 ml were collected and were combined on the basis of their TLC profiles to four main fractions A–D. Fraction A (8 g, eluted with pure hexane) contained mostly fats and was not further investigated. Fraction B (1.5 g, eluted with hexane:EtOAc 95:5) was purified on a silica gel column and a chromatotron (elution with hexane:EtOAc 98:2) to give compound **1** (22 mg). Fraction C (5.2 g, eluted with hexane:EtOAc 90:10) was subjected to another silica gel column chromatography (elution with hexane:EtOAc 80:20) followed by gel permeation on Sephadex LH-20 (hexane:CH_2Cl_2 1:1) to afford compound **2** (150 mg). Finally, fraction D (1.2 g, eluted with hexane:EtOAc, 80:20) gave compound **3** (100 mg) after a silica gel column purification eluting with pure methylene chloride.

3.3.1. 12,15-Epoxylabda-8(17),12,14-trien-16-al (**1**)

Orange oil, $[\alpha]_D^{20}$ $+50.5°$ ($CHCl_3$, *c* 0.42); IR υ_{max} $CHCl_3$ cm^{-1}: 1715, 1684, 1621, 1415, 1370, 1118; ^1H NMR and ^{13}C NMR see Table 1; EIMS (probe) 70 eV, *m/z* (rel. int.) 300 $[M]^+$ (100), 285 (17), 271 (2), 267 (3), 215 (2), 191 (5), 176 (6), 164 (5), 137 (20), 110 (4), 95 (5), 81 (5), 57 (5); HREIMS 300.2092 (M^+, $C_{20}H_{28}O_2$ requires 300.2089).

3.3.2. 16-Acetoxy-12(R),15-epoxy-15β-hydroxylabda-8(17),13(16)-diene (**2**)

Yellowish oil, $[\alpha]_D^{20}$ $+35.0°$ ($CHCl_3$; *c* 1.4); IR υ_{max} $CHCl_3$ cm^{-1}: 3404 (OH), 1701, 1684, 1415, 1369, 1217, 1101; ^1H NMR and ^{13}C NMR see Table 1; EIMS (probe) 70 eV, *m/z* (rel. int.): 362 $[M]^+$ (0.4), 344 $[M - H_2O]^+$ (1), 319 $[M - Ac]^+$ (2), 302 $[M - AcOH]^+$ (11), 284 (14), 269 (10), 235 (6), 170 (23), 157 (26), 137 (48), 115 (100), 95 (27), 69 (31), 43 (53); HREIMS 362.2463 (M^+, $C_{22}H_{34}O_4$ requires 362.2457).

3.3.3. 17-Epi 12-dehydroxyheudebolin (**3**)

White crystals (hexane–EtOAc), m.p. 122–124 °C, $[\alpha]_D^{20}$ $-190.1°$ ($CHCl_3$, *c* 1.3); IR υ_{max} $CHCl_3$ cm^{-1}: 2943, 1732, 1647, 1436, 1371, 1215, 1128, 1059, 1024; ^1H NMR and ^{13}C NMR see Table 2; EIMS (probe) 70 eV, *m/z* (rel. int.): 568 $[M]^+$ (64), 525 $[M - Ac]^+$ (8), 508 $[M - AcOH]^+$ (100), 493 (15), 449 (33), 448 $[M - 2AcOH]^+$ (27), 419 (18), 388 $[M - 3AcOH]^+$ (17), 373 (31), 359 (60), 357 (23), 329 (22), 267 (19), 245 (19), 203 (50), 173 (35), 147 (44), 119 (22), 43 (100); HREIMS 568.2665 (M^+, $C_{32}H_{40}O_9$ requires 568.2672).

Acknowledgement

We gratefully acknowledge financial support from the International Science Program (ISP), Grant No. CAM:02, Uppsala University, Sweden.

References

Adesid, G.A., Okorie, D.A., 1973. Heudebolin, new limonoid from *Trichilia heudelotii*. Phytochemistry 12, 3007–3008.

Ara, I., Siddiqui, B.S., Faizi, S., Siddiqui, S., 1989. Two new terpenoids from root bark of *Azadirachta indica*. J. Nat. Prod. 52, 1209–1213.

Ayafor, J.F., Kimbu, S.F., Ngadjui, B.T., Akam, T.M., Dongo, E., Sondengam, B.L., Connolly, J.D., Rycroft, D.S., 1994. Limonoids from *Carapa grandiflora* (Meliaceae). Tetrahedron 50, 9343–9354.

Bickii, J., Njifutie, N., Ayafor, J.F., Basco, L.K., Ringwald, P., 2000. In vitro antimalarial activity of limonoids from *Khaya grandifoliola* C.D.C. (Meliaceae). J. Ethnopharm. 69, 27–33.

Itokawa, H., Morita, M., Mihashi, S., 1980. Labdane and bisnorlabdane type diterpenes from *Alpinia speciosa* K. Schum. Chem. Pharm. Bull. 28, 3452–3454.

Kaiser, J., 1990. Wood of the month – avodire: the light African wood with hidden strength. Wood of the Month Annual, Supplement to Wood and Wood Products. p. 22A.

Kimbu, S.F., Ayafor, J.F., Sondengam, B.L., Tsamo, E., Connolly, J.D., Rycroft, D.S., 1984. Carapolides D, E and F, novel tetranortriterpenoids from the seeds of *Carapa grandiflora* (Meliaceae). Tetrahedron Lett. 25, 1617–1620.

Kimbu, S.F., Ngadjui, B., Sondengam, B.L., Njimi, T.K., Connolly, J.D., Fakunle, C.O., 1987. A new labdane diterpenoid from the seeds of *Aframomum daniellii*. J. Nat. Prod. 50, 230–231.

Kline, M., 1979. *Turreanthus africanus* – Avodire. In: Flynn, J.H. Jr.Jr. (Ed.), A Guide to Useful Woods of The World. King Philip Publishing Co., Portland, Maine, p. 354.

Tchuendem, M.H.K., Mbah, J.A., Tsopmo, A., Ayafor, J.F., Sterner, O., Okunjic, C.C., Iwu, M.M., Schuster, B.M., 1999. Antiplasmodial sesquiterpenoids from the African *Renealmia cincinnata*. Phytochemistry 52, 1095–1099.