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ENT-KAURENE AND *ENT*-BEYERENE DITERPENOIDS AND OTHER CONSTITUENTS OF *THECACORIS BATESII*

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ABSTRACT. Two novel diterpenoids, thecacorins A (**1**) and B (**2**), were isolated from *Thecacoris batesii* and their structures were established as *ent*-3β,20-epoxy-16-kaurene-3α,12β-diol and *ent*-15-beyerene-2β,3β-diol, respectively, on the basis of extensive spectroscopic analysis, especially, 1D NMR spectra, in conjunction with 2D experiments, COSY, NOESY, HMQC and HMBC.

KEY WORDS: Diterpenoids, Thecacorin A, Thecacorin B, *Ent*-3β,20-epoxy-16-kaurene-3α,12β-diol, *Ent*-15-beyerene-2β,3β-diol, *Thecacoris batesii*

INTRODUCTION

The genus *Thecacoris* (Euphorbiaceae) consists of about 19 species among which 10 occur in Africa and Madagascar; four of them are found in Cameroon [1, 2]. The literature has very scanty information on the genus. The leaf decoction of *T. batesii* is used as purgative and anti-rheumatic remedies in the medicinal plant therapy in Cameroon [3]. As part of our continuing program to study the chemical constituents of Cameroonian medicinal plants [4], we have examined the extracts of the bark of *T. batesii*. In this paper we describe the isolation and characterization of two diterpenoids **1** and **2** for which the trivial names thecacorin A and B, respectively, are proposed. Compound **2** is here reported for the first time from natural source while it was reported earlier as reduction product of the corresponding α-ketol (3-hydroxy-2-one) [5]. In addition the known compounds excoecarin D (**2b**), β-sitosterol and its β-*D*-glucopyranoside were also isolated. To the best of our knowledge, no previous phytochemical or pharmacological studies have been reported on *T. batesii*.

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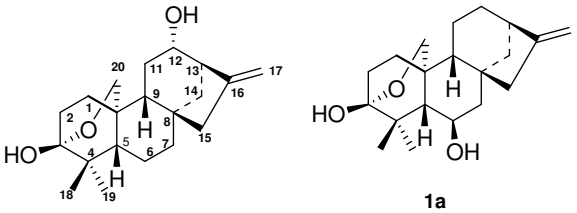
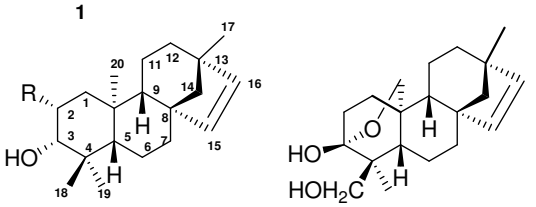


1 **1a**



2 R = OH
2a R = H **2b**

RESULTS AND DISCUSSION

Compound **1** was isolated as white needles with positive optical rotation ($[\alpha]^{25}_{D}$ + 9° (c 0.8, $CHCl_3$). The molecular formula was determined to be $C_{20}H_{30}O_3$ from HREIMS measurements. The IR spectrum showed a broad absorption band for hydroxyl groups at 3477 and 3417 cm^{-1}. Absorption bands at 1638 and 885 cm^{-1} due to a terminal double bond were also observed. The ^{1}H NMR spectrum of **1** (Table 1) showed signals for two tertiary methyl groups (δ 1.00, 1.04), two exocyclic methylene protons (δ 4.79, 4.86), one oxymethine proton (δ 3.77), one oxymethylene group (δ 4.33, 4.30) and one presumed allylic proton (δ 2.63). The combined analysis of the ^{13}C NMR and DEPT spectra of **1** (Table 1) revealed 20 carbon signals including five quaternary carbons among which one is dioxygenated (δ 98.3), nine methylene carbons one of which is exocyclic (δ 105.7), four methine carbons among which one is attached to a hydroxyl group (δ 71.5) and two methyl carbons. The NMR spectral data of compound **1** were similar to those reported for agallochin F [6] (**1a**) suggesting that compound **1** is a kaurene-type diterpenoid. The oxymethine carbon signal at δ 71.5 was assigned to the secondary hydroxyl carbon, C-12 from ^{1}H-^{1}H COSY and HMBC experiments. Thus the oxymethine proton signal (H-12, δ 3.77) showed COSY correlations with the allylic (H-13) and methylene (H_2-11) protons. The oxymethine proton also indicated correlations in the HMBC (Table 1) with C-9, C-13 and C-14 further confirming its position on C-12. The signal for H-12 appears as a pseudo triplet with a near zero coupling constant from which the configuration of the hydroxyl group at C-12 was determined as axial. Additional HMBC correlations (Table 1) were observed between C-3 (δ 98.3) and H_3-18, H_3-19, H_2-20 and H_2-2; C-20 (δ 69.2) and H-5, H_2-1 and H-9 and C-6 (δ 21.9) and H-8 and H_2-7. The relative stereochemistry of **1** was deduced from NOESY experiments. Thus, signals for H-5 and H-9 showed NOESY correlations clearly indicating the *cis*- relationship between the corresponding protons. The *cis*-relationship between H_2-20 and H_2-14 was also confirmed from the NOESY spectrum. On the basis of these data, thecacorin A (**1**) was deduced to be *ent*-3β,20-epoxy-16-kaurene-3α,12β-diol. To the best of our knowledge, compound **1** is reported here for the first time.

Table 1. ^{1}H (600 MHz), ^{13}C (150 MHz) spectroscopic data and important HMBC ^{2}J, ^{3}J-correlations for thecacorin A (**1**) in $CDCl_3$. Multiplicities and coupling constant in Hz are given in parentheses.

C / H	δ_H	δ_C	^{2}J, ^{3}J-correlated carbons ($^{1}H \rightarrow {}^{13}C$)
1a	2.16 (*m*)	35.3	C-2, C-3, C-5, C-10, C-20
1b	1.11 (*m*)	35.3	C-2, C-3, C-5, C-9, C-10, C-20
2a	2.19 (*m*)	30.0	C-1, C-3, C-10
2b	1.24 (*m*)	30.0	C-4, C-10
3	----	98.3	----
4	----	40.6	----
5	1.10 (*m*)	51.2	C-3, C-9, C-10, C-19, C-20
6a	1.58 (*m*)	21.9	C-5, C-7, C-8, C-10
6b	1.56 (*m*)	21.9	C-5, C-7, C-8, C-10
7a	1.53 (*m*)	39.4	C-5, C-6, C-8, C-14
7b	1.44 (*m*)	39.4	C-5, C-6, C-8, C-14
8	----	43.2	----
9	1.28 (*brd*, 9.7)	49.9	C-8, C-10, C-11, C-12, C-14, C-15, C-20
10	----	36.5	----
11a	1.86 (*ddd*, 5.0, 9.7, 15.6)	26.1	C-9, C-10, C-12
11b	1.65 (*brd*, 15.6)	26.1	C-8, C-9, C-10, C-12, C-13
12	3.77 (pseudo *t*[*])	71.5	C-9, C-13, C-14
13	2.63 (pseudo *t*[*])	51.8	C-8, C-11, C-12, C-15, C-16, C-17
14a	1.98 (*brd*, 12.0)	33.2	C-8, C-9, C-12, C-13, C-16
14b	1.03 (*dd*, 5.4, 12.0)	33.2	C-8, C-12, C-13
15	2.12 (*brs*)	48.3	C-7, C-8, C-9, C-13, C-14, C-16, C-17
16	----	152.2	----
17a	4.86 (*brs*)	105.7	C-13, C-15, C-16
17b	4.79 (*brs*)	105.7	C-13, C-15
18	1.04 (*s*)	27.7	C-3, C-4, C-5, C-19
19	1.00 (*s*)	18.6	C-3, C-4, C-5, C-18
20a	4.33 (*brd*, 10.2)	69.2	C-1, C-3, C-9, C-10
20b	4.30 (*brd*, 10.2)	69.2	C-1, C-3, C-9, C-10

[*]Apparent multiplicity *t* = *ddd*.

Compound **2** was isolated as an amorphous, white powder with an optical rotation of $[\alpha]^{25}_{D}$ = +11° (c 1.2, $CHCl_3$). The molecular formula of **2** was established to be $C_{20}H_{32}O_2$ from HREIMS. Infrared absorption bands at 3465 and 3410 provided evidence of hydroxyl groups while the IR absorption at 1670 cm^{-1} is attributable to a double bond. The ^{1}H NMR (Table 2) clearly indicated signals assignable to four tertiary methyl groups (δ 1.01, 1.02, 1.03 and 1.04), one 1,2-disubstituted olefinic double bond (δ 5.72, 5.48 *d* each, *J* = 5.4 Hz) and two oxymethines (δ 4.10, 3.21). The ^{13}C NMR and DEPT spectra of **2** (Table 2) showed 20 carbon signals which were analysed as four quaternary, six methines two of which are oxygenated (δ 71.6 and 78.9), six methylenes and four methyls. The similarity of the NMR data of **2** with those reported for stachenol {(**2a**) [5, 7]} strongly suggested that compound **2** is a beyerene type diterpenoid. The COSY spectrum showed correlation between the two oxymethine protons, one of these (at δ 4.10) also correlated with methylene protons at 2.09 and 1.20. These signals were consistent with a four-proton spin-system belonging to the C-1 to C-3 protons for ring A of stachenol (**2a**) with an additional OH-group at C-2. COSY correlations also define two additional spin systems, one consisting of H-5, H-6 and H-7 and another set of correlations which places H-9, H-11 and H-12 in one spin system. The relative stereochemistry of **2** was assigned based on NMR experiments. The ^{1}H NMR spectrum (Table 2) of compound **2** showed a coupling constant (*J* = 3.0 Hz) between the two oxymethine protons H-2 and H-3 indicating their *cis*-geometry. The *trans*-fusion of rings A/B was determined from NOESY experiment in

which H-5 showed correlations with H-9, H-1β and the oxymethine proton H-3. NOESY experiment also showed correlations between the methyl protons, H_3-20 and the olefinic proton H-15. The structure of thecacorin B (**2**) could thus be derived as *ent*-15-beyerene-2β,3β-diol. Compound **2** was reported earlier as reduction product of the corresponding α-ketol (3-hydroxy-2-one) [5]. The optical rotation, ($[\alpha]^{25}_D = +11^o$), is very close to the reported value ($[\alpha]_D = +12^o$). The ^{1}H and ^{13}C NMR spectroscopic data for compound **2** are reported here for the first time.

Table 2. ^{1}H (600 MHz), ^{13}C (150 MHz) spectroscopic data and important HMBC ^{2}J, ^{3}J- correlations for thecacorin B (**2**) in $CDCl_3$. Multiplicities and coupling constant in Hz are given in parentheses.

C / H	δ_H	δ_C	^2J, ^3J-correlated carbons ($^1H \rightarrow {}^{13}C$)
1β	2.08 (*dd*, 3.0, 14.4)	43.5	C-2, C-3, C-5, C-10, C-20
1α	1.20 (*dd*, 3.6, 14.4)	43.5	C-9, C-10, C-20
2	4.10 (q**, 3.0)	71.6	C-1, C-3, C-4, C-10
3	3.21 (*d*, 3.0)	78.9	C-4, C-18, C-19
4	----	38.6	----
5	0.93 (*dd*, 2.4, 11.4)	55.6	C-1, C-4, C-6, C-7, C-18, C-19
6a	1.60 (*m*)	20.0	C-5, C-7, C-8, C-10
6b	1.59 (*m*)	20.0	C-5, C-7, C-8, C-10
7a	1.70 (*dt*, 13.2, 3.0)	37.6	C-6, C-8, C-9, C-15
7b	1.37(*m*)	37.6	C-5, C-6, C-8, C-9, C-14
8	----	49.2	----
9	0.92 (*dd*, 6.6, 11.4)	54.1	C-1, C-5, C-8, C-10, C-11, C-12, C-14, C-15, C-20
10	----	36.9	----
11a	1.36 (*m*)	20.7	C-8, C-9, C-12, C-13
11b	1.58 (*m*)	20.7	C-8, C-9
12a	1.35 (*m)*	33.5	C-9, C-14
12b	1.25(*ddd*,6.0,12.0, 12.6)	33.5	C-9, C-11, C-13, C-14, C-17
13	----	44.1	----
14a	1.48 (*dd*, 2.4, 9.6)	61.6	C-7, C-8, C-9, C-12, C-13, C-17
14b	1.03*	61.6	C-8, C-9, C-15, C-17
15	5.72 (*d*, 5.4)	135.5	C-8, C-13, C-14, C-16
16	5.48 (*d*, 5.4)	136.8	C-8, C-13, C-14, C-15, C-17
17	1.01 (*s*)	25.3	C-13, C-14, C-16
18	1.03 (*s*)	30.4	C-3, C-4, C-5
19	1.02 (*s*)	17.9	C-3, C-4, C-5
20	1.04 (*s*)	16.8	C-1, C-5, C-9, C-10

* The signal is hidden under the methyl resonances and the value was read from HMQC spectrum.
** Apparent multiplicity is *q* = *ddd*.

Compound **2b** was isolated as colourless needles from hexane-EtOAC. The NMR data generated suggests that **2b** is excoecarin D isolated from *Excoecaria agallocha* and published as a normal beyerane derivative [8]. Excoecarin D was reported along with *ent*-beyerane and *ent*-kaurene terpenoids. The absolute configurations for the co-occuring *ent*-isomers were established from CD measurements. In the same paper it was reported that the relative stereochemistry of excoecarin D suggested from NMR data was confirmed by X-ray analysis. However, it is not clear how excoecarin D was taken as normal beyerane. Its co-occurrence with *ent*-diterpenoids rather suggests that biogenetically excoecarin D is an *ent*-beyerane derivative as shown in **2b**.

The absolute configurations of **1** and **2** were not established. They were taken to be *ent*-enantiomers in view of their co-occurrence with **2b** which is presumed to be an *ent*-beyerene.

EXPERIMENTAL

General. Melting points were measured with a Stuart Scientific Melting-point SMP1 apparatus and are uncorrected. Optical rotations were recorded using a JASCO P-1030 digital polarimeter. IR spectra were recorded using a Perkin Elmer System 2000 FT spectrometer with KBr disks. ^{1}H NMR (300 and 600 MHz) and ^{13}C NMR (75 and 150 MHZ) spectra were recorded on Bruker Avance instruments in $CDCl_3$ and MeOD with residual solvent peaks as internal references. COSY, NOESY, HMQC and HMBC experiments were performed with gradient enhancements. MS were obtained on a JEOL JMS-300 mass spectrometer. For column chromatography, Si gel (Merck) was used.

Plant material. Stem bark of *T. batesii* was collected in December 2002, from Kribi, in Littoral Province of Cameroon and the plant was identified by Mr V. Nana of the National Herbarium of Yaounde where a voucher specimen (18246/Sfcam) is deposited.

Extraction and isolation. The sun-dried and crushed stem bark of *T. batesii* (3 kg) was soaked in a mixture of methylene chloride-methanol (1:1) and pure methanol for 48 and 24 h, respectively, at room temp. The combined extracts were evaporated to give brown syrup (97 g). Part of this (90 g) was adsorbed onto silica gel and subjected to vacuum liquid chromatography, eluting with binary solvent systems hexane-EtOAc gradient, EtOAc and MeOH, consecutively. A total of five fractions A-E each *ca* 250 mL was obtained. Fractions A (38 g), B (3 g), C (3 g), D (2 g) and E (25 g) were eluted with n-hexane-EtOAc (3:1), (1:1), (1:3), EtOAc 100 % and MeOH 100 %, respectively. Repeated CC on fr. A using mixture of n-hexane-EtOAc gradient and for some cases preparative TLC in $CHCl_3$-MeOH (95:5) yielded β-sitosterol (45 mg), thecacorin B (**2**, 16 mg), thecacorin A (**1**, 20 mg) and excoecarin D (**2b**, 22 mg). The combined Frs. B and C (6 g) provided an amorphous powder which was later crystallized from methanol to give 3-*O*-β-D-glucopyranosylsitosterol (75 mg). Fr. D and E were combined (27 g), pre-adsorbed on to 25 g silica gel and put on 150 g of silica gel column in $CHCl_3$. Elution was carried out by introducing a methanol gradient, but 3-*O*-β-*D*-glucopyranosyl-sitosterol (25 mg) was the only non-intractable substance that was characterized from the 10 % methanol-$CHCl_3$ eluate.

*Ent-3β,20-epoxy-16-kaurene-3α,12β-diol (thecacorin A) (**1**).* White needles from *n*-hexane-EtOAc, m.p. 118-119 °C; $[\alpha]^{25}_{D}$ + 9° (c 0.8, $CHCl_3$); IR (KBr): ν_{max} 3477, 3417, 1638, 1463, 1325, 1135, 1110, 885 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz): Table 2; ^{13}C NMR ($CDCl_3$, 150 MHz), Table 2; APCI MS m/z (rel. int.): 319 $[M+H]^+$(5), 301 $[M + H - H_2O]^+$ (38), 283 $[M + H - 2H_2O]^+$ (15); HREIMS *m/z* 318.2165 calcd. for $C_{20}H_{30}O_3$: 318.2153.

*Ent-15-beyerene-2β,3β-diol (thecacorin B) (**2**).* White powder $[\alpha]^{25}_{D}$ +11° (c 1.2, $CHCl_3$); IR (KBr): ν_{max} 3465, 3410, 1670, 1455, 1380, 1310,1185, 1112, 1020 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz): Table 1; ^{13}C NMR ($CDCl_3$, 150 MHz), Table 1; APCI MS m/z (rel. int.): 305 $[M+H]^+$ (3), 287 $[M + H - H_2O]^+$ (75), 269 $[M + H - 2H_2O]^+$ (100); HREIMS *m/z* 304.2453, calcd. for $C_{20}H_{32}O_2$: 304.2335.

*Ent-3β,20-epoxy-15-beyerene-3α,18-diol (excoecarin D) (**2b**).* Colorless needles from *n*-hexane-EtOAc; physical and spectroscopic data were in agreement with the literature values [8].

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