Pergamon

PII: S0031-9422(97)00980-1

Phytochemistry, Vol. 49, No. 3, pp. 835–838, 1998
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Printed in Great Britain
0031–9422/98/$—see front matter

CYCLOARTANE TRITERPENES FROM *COMBRETUM QUADRANGULARE*

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(*Received in revised form* 7 *October* 1997)

Key Word Index—*Combretum quadrangulare*; Combretaceae; triterpenes; cycloartane derivatives.

Abstract—In addition to two known flavonoids, kumatakenin and isokaempferide, three new cycloartane triterpenes were isolated from the leaves of *Combretum quadrangulare*. Their structures were established by the means of 1D and 2D NMR techniques and physical data (MS, IR) as 1α,3β-dihydroxy-cycloart-24-ene-30-carboxylic acid, 1α,3β-dihydroxy-cycloart-24-ene-30-carboxylic acid methyl ester and (20ξ)-1α,3β,25-trihydroxy-cycloart-21-al-23-ene-30-carboxylic acid methyl ester. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Combretum quadrangulare Kurz is a shrub or tree, indigenous to southeast Asia, especially from Burma to Laos. The plant is commonly known as "Tram bâu" (Vietnam), "Kê 'khao" (Laos) or "Sâng kaê" (Cambodia) and the seeds are used in Vietnamese traditional medicine as a remedy against round- and tapeworm infections in humans [1]. Only the constituents of the flowers of this plant have been investigated previously [2]. In this paper we describe the isolation and structural elucidation of three new triterpenes with a 1,3-dihydroxy-cycloartane skeleton from the leaves of *C. quadrangulare*. Cycloartanes seem to be typical for the family of Combretaceae [3] and this genus in particular. Similar compounds, e.g. mollic acid, jessic acid and derivatives have already been isolated from other *Combretum* species such as *C. leprosum* [4], *C. elaegnoides* [5], *C. edwardsii* [6] and *C. molle* [7]. Two known 3-methoxy-flavone derivatives have been isolated also for the first time from *C. quadrangulare*.

RESULTS AND DISCUSSION

Extraction of the air dried leaves of *C. quadrangulare* with methylene dichloride, followed by repeated column chromatography (gel, silica gel and reversed phase chromatography) yielded compounds **1**–**3** and two known flavonoids 5,4′-dihydroxy-3,7-dimethoxyflavone (kumatakenin) and 5,7,4′-trihydroxy-3-methoxyflavone (isokaempferide).

Compound **1** showed a quasi-molecular ion in the ESI mass spectrum at *m/z* 485 $[M-H]^-$ corresponding to the molecular formula $C_{31}H_{50}O_4$. The IR spectrum indicated the presence of hydroxyl (3489 cm^{-1}), a cyclopropyl (2955 cm^{-1}), carbonyl (1706 cm^{-1}) and terminal methylene groups (887 cm^{-1}).

In the ^1H NMR spectrum of **1** (Table 1) two upfield shifted doublets at δ 0.57 and 0.86 (each $J = 4.1$ Hz) were assignable to a cyclopropyl methylene group (H_2-19) characteristic for cycloartanes. Additionally, a doublet at δ 4.87 ($J = 2.8$ Hz) was assignable to terminal methylene protons, while the signals at δ 3.94 and 5.58 indicated the presence of two carbons attached to oxygens. From the precise 2D NMR studies, the doublet of doublets at δ 5.58 ($J = 12.1$ and 3.6 Hz) and the singlet at δ 3.94 were ascribed to 3 α-axial and 1 β-equatorial protons. The high shift value of the H-3α signal can be explained by the solvent effect of pyridin-d_5.

The ^{13}C NMR spectrum of **1** showed the signals of 31 carbons (6 methyl, 11 methylene, 7 methine and 7 quarternary signals) and revealed the presence of a carbonyl function, a terminal methylene and two hydroxyl groups in this compound (Table 2). The shift values at δ 72.6 and 70.8 were assigned to C-1 and C-3, both substituted with a 1α and 3β hydroxyl group [5]. The signals at δ 106.6 and 156.7, characteristic for a terminal methylene group [8], were assigned to C-31 and C-24. The 2D NMR experiments (HSQC, HMBC and TOCSY) confirmed the 1,3-dihydroxy cycloartane structure of **1**, with a carboxylic group (C-28, δ

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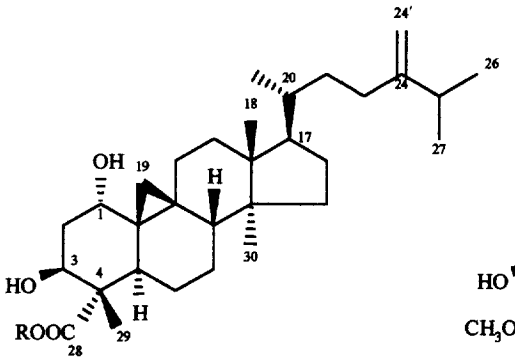
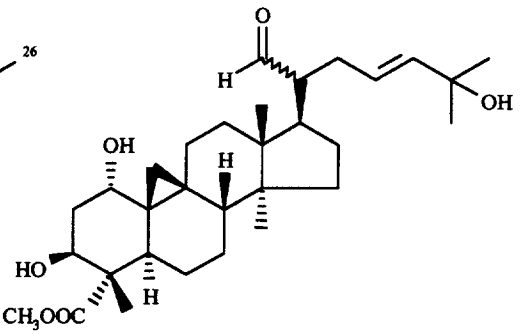


1 R = H

2 R = CH_3



3

180.6) at position C-4 (δ 55.7). The structure of the 1,5-dimethyl-hex-4-enyl side chain in position C-17 and the position of the methylene group at C-24 were also determined by the HMBC and TOCSY experiments, which showed long range correlations of the proton signals at δ 1.07 (H-26 and H-27) with the carbon signals at δ 156.7 (C-24), and 34.0 (C-25), the proton signals at δ 4.72 and 4.78 with the carbon signals at δ 34.0 (C-25) and 31.6 (C-23) and the proton signal at δ 0.93 (H-21) with the carbon resonances at δ 52.5 (C-17), 36.4 (C-20) and 35.3 (C-22). The stereochemistry of the cycloartane was in accordance to literature data [4–7]. Thus, compound **1** was a 1α,3β-dihydroxy-cycloart-24-ene-28-carboxylic acid.

The ^{1}H NMR and ^{13}C NMR data of **2** (Tables 1 and 2) closely coincided with those of **1**. The only

Table 1. ^{1}H NMR spectral data of compounds **1–3** (300 MHz, TMS as int. standard; **1** recorded in pyridine-d_5, **2** in methanol-d_4/$CDCl_3$ and **3** in methanol-d_4)

H	1	2	3
1	3.94 *s*	3.60 *s*	3.62 *t* (*J* = 2.8)
2	2.30 *m*	1.84 *m*	1.88 *q* (*J* = 2.8)
	2.53 *m*	1.92 *m*	1.94 *m*
3	5.58 *dd* (*J* = 12.1, 3.6)	4.60 *dd* (*J* = 11.7, 3.2)	4.62 *dd* (*J* = 11.6, 3.9)
5	3.46 *m*	2.63 *dd* (*J* = 11.2, 4.3)	2.60 *dd* (*J* = 12.1, 4.8)
6	1.29 *m*, 1.85 *m*	1.02 *m*, 1.18 *m*	1.08 *m*, 1.20 *m*
7	1.33 *m*	1.28 *m*, 1.32 *m*	1.28 *m*, 1.35 *m*
8	1.64 *m*	1.57 *m*	1.62 *m*
11	1.56 *m*, 2.75 *m*	1.28 *m*, 1.32 *m*	1.28 *m*, 1.35 *m*
12	2.77 *m*	1.36 *m*	1.48 *m*
15	1.25 *m*, 1.30 *m*	1.78 *m*	1.37 *m*, 1.65 *m*
16	1.32 *m*, 1.93 *m*	1.35 *m*	2.10 *m*
17	1.65 *m*	1.70 *m*	2.40 *m*
18	1.06 *s*	1.04 *s*	1.10 *s*
19	0.57 *d* (*J* = 4.1)	0.55 *d* (*J* = 4.1)	0.55 *d* (*J* = 4.0)
	0.86 *d* (*J* = 4.1)	0.76 *d* (*J* = 4.1)	0.77 *d* (*J* = 4.1)
20	1.46 *m*	1.47 *m*	2.44 *m*
21	0.93 *d* (*J* = 6.3)	0.97 *d* (*J* = 6.2)	9.55 *d* (*J* = 4.6)
22	1.21 *m*, 1.68 *m*	1.63 *m*	2.43 *m*
23	2.01 *m*, 2.33 *m*	1.74 *m*	5.63 *m*
24	—	—	5.71 *m*
24′	4.87 *d* (*J* = 2.8)	4.72 *s*, 4.78 *s*	3.79 *s*
25	2.27 *m*	2.29 *m*	—
26	1.07 *d* (*J* = 3.0)	1.08 *d* (*J* = 2.6)	1.34 *s*
27	1.07 *d* (*J* = 2.8)	1.08 *d* (*J* = 2.4)	1.32 *s*
29	1.72 *s*	1.17 *s*	1.18 *s*
30	1.02 *s*	1.04 *s*	1.10 *s*
CH_3COO-		3.76 *s*	

Table 2. ^{13}C NMR spectral data of compounds **1**–**3** (75 MHz, TMS as int. standard; **1** recorded in pyridine-d_5, **2** in methanol-d_4/$CDCl_3$ and **3** in methanol-d_4)

C	1	2	3
1	72.6	73.0	75.6
2	38.8	37.1	37.9
3	70.8	70.9	71.7
4	55.7	55.9	56.6
5	37.7	38.1	38.8
6	23.4	23.7	24.2
7	25.9	26.1	26.7
8	48.1	49.4	50.0
9	20.8	21.7	22.3
10	30.3	29.7	30.7
11	26.2	26.3	26.7
12	33.3	36.5	36.8
13	45.6	46.0	46.6
14	49.2	48.8	48.8
15	35.8	33.6	32.7
16	28.4	28.8	27.9
17	52.5	53.1	48.6
18	18.3	18.7	20.0
19	29.7	30.4	31.2
20	36.4	36.8	57.1
21	18.5	19.7	208.3
22	35.3	35.7	33.6
23	31.6	32.0	128.0
24	156.7	157.4	138.4
24′	106.6	106.6	52.9
25	34.0	34.7	82.6
26	22.0	22.2	25.3
27	22.1	22.3	25.0
28	180.6	178.9	179.4
29	9.8	8.9	9.4
30	19.8	18.7	20.0
CH_3COO		52.1	

differences were an additional proton singlet of δ 3.76 and a methyl carbon signal at δ 52.1 characteristic of a methyl ester moiety. In fact, the ESI mass spectrum of **2** (*m/z* 523 $[M+Na]^+$) showed a M_r of 500, 14 mass units higher than **1**. Thus, **2** was the corresponding methyl ester of **1**.

The high resolution ESI mass spectrometry of **3** showed a quasi-molecular peak at *m/z* 555.33 $[M+K]^+$, with an assignable molecular formula of $C_{31}H_{48}O_6$. The NMR experiments (see Tables 1 and 2) indicated that **3** had the same cycloartane structure as **2**, with 1α,3β-dihydroxy groups and a methyl ester function at C-4, but a structurally different side chain in position C-17. According to the NMR studies the side chain moiety of **3** had to consist of an aldehyde function (C-21, δ 9.55, δ 208.3), an OH-substituted quaternary carbon (C-25, δ 82.6), two geminal methyl groups (C-26 and C-27, δ 1.32 and 1.34, δ 25.0 and 25.3) and a double bond (C-23 and C-24, δ 5.63 and 5.71, δ 128.0 and 138.4). The structure of the 5-hydroxy-5-methyl-1-al-hex-3-enyl side chain was confirmed from the HMBC spectrum which showed cross peaks between the proton signals of H-17 (δ 2.40), H-21 (δ 9.55) and H-22 (δ 2.43) with the carbon signal of C-20 (δ 57.1). Furthermore, the H-24 signal (δ 5.71) exhibited long range couplings with the carbon signals of C-22 (δ 33.6), C-23 (δ 128.0), C-26 (δ 25.3) and C-27 (δ 25.0) (see Fig. 1). The configuration of C-20 is left undetermined. Thus, compound **3** is (20ξ)-1α,3β,25-trihydroxy-cycloart-21-al-23-ene-28-carboxylic acid.

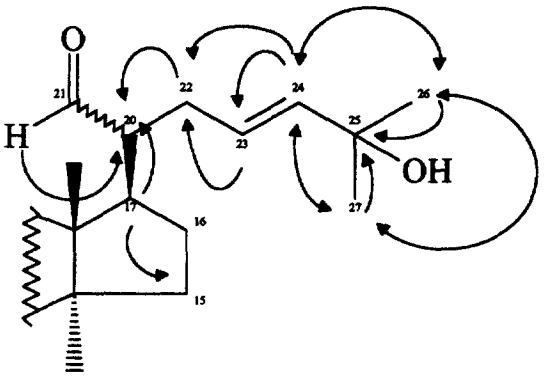


Fig. 1. ^1H-^{13}C long range correlations of the side chain moiety of **3**.

EXPERIMENTAL

General experimental procedures

IR: rolled on ZnSe (IFS 25, Bruker); ^1H (300 MHz) and ^{13}C NMR (75 MHz): AM-300, Bruker) in methanol-d_4, methanol-d_4/$CDCl_3$ = 1/1 or pyridine-d_5, using TMS as int. standard; MS: ESI mode on a Finnigan-Mat SSQ 7000.

Plant material

Fresh leaves of *Combretum quadrangulare* Kurz were collected in summer 1995 near the village Sa Dec, Dong Thap province in Vietnam. The identity of the species was confirmed by Prof. Vo van Chi, University of Medicine and Pharmacy, Ho Chi Minh City, Vietnam. A voucher specimen is deposited at the Institute of Pharmacognosy, University of Innsbruck, Austria.

Extraction and isolation

The air dried and pulverized leaves of *C. quadrangulare* (350 g) were extracted with CH_2Cl_2 in a Soxhlet apparatus for 3 days. Solvent was removed under vacuum to afford 28 g of residue. A part (6 g) of this extract was submitted to gel chromatography (Sephadex LH 20) with MeOH as mobile phase, to get three main fr (A–C). Fractions A and C, which contained the flavonoids, were purified by repeated gel chromatography and PTLC to yield 50 mg of

kumatakenin (from fraction A) and 9 mg of isokaempferide (from C).

Fraction B was subjected to CC (silica gel, 230–400 mesh) using *n*-hexane and EtOAc as eluents, and three subfractions (a–c) were collected. Fraction (a) (*n*-hexane–EtOAc, 9:1) was purified using LPLC (RP-18, MeOH–H_2O) to yield 18 mg of compound **2**. Pure **3** (25 mg) was obtained by repeated CC (silica gel, *n*-hexane–EtOAc) of fraction (b) (*n*-hexane–EtOAc, 1:1). Compound **1** (16 mg) was isolated from fraction (c) (EtOAc) by repeated LPLC (RP-18, MeCN–H_2O).

1α,3β-Dihydroxy-cycloart-24-ene-30-carboxylic acid (**1**). $C_{31}H_{50}O_4$; white, amorphons substance; mp 238–240°; IR ν_{max}^{ZnSe} cm^{-1} 3489, 2955, 1706, 1463, 1279, 887. ESI-MS 485 $[M-H]^-$. ^1H NMR and ^{13}C NMR (in pyridine-d_5) data are listed in Tables 1 and 2.

1α,3β-Dihydroxy-cycloart-24-ene-30-carboxylic acid methyl ester (**2**). $C_{32}H_{52}O_4$; white, amorphons substance; mp 190–194°; IR ν_{max}^{ZnSe} cm^{-1} 3459, 2952, 1703, 1464, 1263, 886. ESI-MS 523 $[M+Na]^+$. ^1H NMR and ^{13}C NMR (in methanol-d_4/$CDCl_3$ = 1/1) data are listed in Tables 1 and 2.

(20ξ)-1α,3β,25-Trihydroxy-cycloart-21-al-23-ene-30-carboxylic acid (**3**). $C_{31}H_{48}O_6$; white amorphons; mp 131–134°; IR ν_{max}^{ZnSe} cm^{-1} 3442, 2949, 1713, 1457, 1259; ESI-MS 555.33 $[M+K]^+$, 571.30 $[M+Na+MeOH]^+$ and 587.33 $[M+K+MeOH]^+$; ^1H NMR and ^{13}C NMR data (in methanol-d_4) are listed in Tables 1 and 2.

Kumatakenin. $C_{17}H_{14}O_6$; yellow needles; mp 248°; spectral data (^1H NMR, MS and IR) are in agreement with literature values [10].

Isokaempferide. $C_{16}H_{12}O_6$; yellow needles; mp 250–253°; spectral data (^1H NMR, MS and IR) are in agreement with literature values [11].

Acknowledgements—We thank Prof. Vo van Chi and Dr Tran Hung (University of Medicine and Pharmacy, Ho Chi Minh City, Vietnam) for their support in Vietnam, Dr S. Strum (Institute of Pharmacognosy, University of Innsbruck, Austria) and Dr K.-H. Ongania (Institute of Organic Chemistry, University of Innsbruck, Austria) for the MS measurement.

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