Artigo

CALDENSINIC ACID, A BENZOIC ACID DERIVATIVE AND OTHERS COMPOUNDS FROM *Piper carniconnectivum*

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A benzoic acid derivative – caldensinic acid; *E*-phytyl hexadecanoate; β-sitosterol and stigmasterol mixture and phaeophytin a were isolated from the aerial parts of *Piper carniconnectivum*. The structures of these compounds were established unambiguously by IR, MS, 1D and 2D NMR analysis.

Keywords: 3,4-dihydroxy-5-(11'-carboxyl-3',7',15'-trimethylhexadeca-2'*E*, 6'*E*, 10'*E*, 14-tetraenyl) benzoic acid; *E*-phytyl hexadecanoate; phaeophytin a.

INTRODUCTION

During our search on the chemistry of Brazilian North-Northeast Piperaceae species we have isolated some amides,[1] flavonoids,[2] aristolactams,[3] propenylphenols,[4] terpenes,[4] phenylalkanoids[4] and benzoic acid derivative.[4] In this paper, we report the isolation of 3,4-dihydroxy-5-(11'-carboxyl-3',7',15'-trimethylhexadeca-2'*E*, 6'*E*, 10'*E*, 14-tetraenyl) benzoic acid; *E*-phytyl hexadecanoate, phaeophytin a, β-sitosterol and stigmasterol mixture from whole aerial parts of *Piper carniconnectivum* C. DC. (Piperaceae), known by the vernacular name "pimenta-longa", species native to the Amazon region, North of Brazil.[5] A flavonoid (galangin), a phenone (2-methoxy-4,5-methylenedioxypropiophenone), a coumarin (xanthyletin), three natural cyclopentenedione derivatives and four flavonoids: 5-hydroxy-7-methoxy-6-methylflavanone, 5-hydroxy-7-methoxy-8-methylflavanone, 5-hydroxy-7-methoxy-6,8-dimethylflavanone and 2'-hydroxy-4',6'-dimethoxy-3',5'-dimethylchalcone have recently been reported from this plant.[6]

RESULTS AND DISCUSSION

The mass spectrum of the compound **1** (Figure 1) showed a molecular ion peak at *m/z* 455,12 (M-H)-corresponding to $C_{27}H_{36}O_6$ and its IR spectrum showed a carbonyl absorption (1686 cm^{-1}) as well as a broad hydroxyl absorption (3438 cm^{-1}) typical of a carboxylic acid. The aromatic part of the ^1H NMR spectrum exhibited only two signals: δ_H 8.23, (d, *J* = 1.7 Hz, 1H) and δ_H 8.19 (d, *J* = 1.7 Hz, 1H) with chemical shifts and splitting pattern consistent with protons in *meta* position and suggested the presence of H-2 and H-6 of a 3,4,5-substituted benzoic acid unit. Furthermore, the ^1H NMR spectrum revealed the presence of a benzylic methylene resonance at δ_H 3.85 (d, *J* = 7.2 Hz, 2H), three signals: δ_H 5.81 (t, *J* = 7.2 Hz, 1H), δ_H 5.34 (brq, *J* = 5.5 Hz, 2H) and δ_H 7.21 (t, *J* = 7.3 Hz, 1H) to four olefin protons, four signals: δ_H 2.67 (brt, *J* = 7.7 Hz, 2H), δ_H 2.44-2.40 (m, 4H), δ_H 2.20 (m, 4H), δH 2.15 (m, 2H) to six methylene groups and three resonances [δ_H 1.84 (s, 3H), δ_H 1.69 (s, 3H) and δ_H 1.65 (s, 6H)] to four methyl substituents.

The ^1H NMR data associated with the presence of six signals to methylene carbons, four to methyl carbons, six to methyne carbons and ten to quaternary carbons in the APT spectrum (Table 1), beyond of the cross peaks in the HMBC spectrum, for the signal at δ_C 170.7 (carbonyl carbon) with δ_H 7.21 (olefin hydrogen 10') and δ_H 2.67 (methylene hydrogen 12'), provided evidence for the presence of a 3',7',15'-trimethylhexadeca 2',6',10',14'-tetraenyl -11'-carboxyl group in the benzoyl unit. The cross peaks for the signals at δ_C 29.5 (C-1') and δ_C 8.19 (H-2) in the HMBC spectrum supported the evidence for the presence of the hexadecane group at C-3, *orto* to the methyne proton (δ_H 8.19) and suggested the signal at δ_H 8.23 to H-6 and, consequently, two hydroxyl groups to C-4 and C-5 in benzoyl unit. The geometry of the double bonds at C-2', C-6' and C-10' were suggested by the ^1H x ^1H - NOESY spectrum, in which H-2' (δ_H 5.81) showed cross peak with H-4' (δ_H 2.15) confirming *E* configuration of the double bond at C-2'; the lack of correlation at H-6' (δ_H 5.34) with Me-18' (δ_H 1.65) and the presence of cross peaks between Me-18' (δ_H 1.65) with H-9' (δ_H 2.44-2.40) and H-5' (δ_H 2.20) supported *E* geometry at C-6'; finally the H-10' (δ_H 7.21) only showed cross peak with H-9' (δ_H 2.44-2.40) and H-8' (δ_H 2.20) suggesting *E* geometry at C-10' [7] (Figure 2). All the assignment of carbons and protons were inferred by interpretation of the spectral data: ^1H (1D and 2D: ^1H x ^1H-COSY, ^1H x ^1H-NOESY) and ^1H x ^{13}C-COSY. $^nJ_{CH}$ (n=1, HMQC; n=2 and 3, HMBC) Table 1. All these data, summarily described in the Table 1, compared with the literature,[7] supported the evidence of: 3,4-dihydroxy-5-(11'-carboxyl-3',7',15'-trimethylhexadeca-2'*E*, 6'*E*, 10'*E*, 14-tetraenyl) benzoic acid to **1**, previously isolated only from *Piper caldense*.

On the basis of the above spectroscopic data and comparison with the literature,[8] **2** was assigned as *E*-phytyl hexadecanoate related in the family Piperaceae for the first time.

The ^1H NMR spectrum of **3** (Figure 1) showed the presence of three sp^2 methyl singlets at δ_H 3.17, 3.37 and 3.66, a vinyl group at δ_H 7.96 (m, 1H), δ_H 6.27 (m, 1H), δ_H 6.14 (m, 1H) and three olefin hydrogens at δ_H 9.46 (s, 1H), δ_H 9.31 (s, 1H) and δ_H 8.53 (s, 1H) which are related to the hydrogens 5, 10 and 20 of the porphyrin skeleton of phaeophytin.[9-11] This data and the presence of an envelope of signals in the aliphatic region of the ^1H NMR spectrum of **3** and the resonance found to C-17[3] (δ_C 173.0) suggested the presence of a phytyl ester in the molecule. All assignments were based on 2D NMR spectra (HMBC, HMQC, ^1H x ^1H – COSY and NOESY) besides comparison with literature data[12] permitting to identify **3** as phaeophytin a, known to be a major degradation product *in natura* of chlorophyll *a*.[13] from Anthocerotaceae,[15] Poaceae,[16] Plagiochilaceae,[9] Dichapetalaceae,[12] Malvaceae,[14] Fabaceae[17] and Rubiaceae.[18] The spectral data and comparison with literature data[19] permitted to identify the compound **4** as a mixture of the steroids β-sitosterol (**4A)** and stigmasterol (**4B).**

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Table 1. ^{1}H (500MHz) and ^{13}C (125MHz) NMR spectral data of **1**, including heteronuclear 2D shift-correlated obtained by ^{1}H-^{13}C-COSY-$^{n}J_{CH}$ (n=1, HMQC; n=2 and 3, HMBC) experiments, in pyridine-d5, as solvent. δ (ppm) and *J* (Hz)

	^{1}H-^{13}C-COSY-$^{1}J_{CH}$		^{1}H-^{13}C-COSY-$^{n}J_{CH}$	
	δ_C	δ_H	$^{2}J_{CH}$	$^{3}J_{CH}$
C				
1	123.3			
3	129.4		2H-1’	
4	150.3			H-2, H-6, 2H-1’
5	146.6		H-6	
7	169.9			H-2, H-6
3’	136.4		2H-1’, 2H-4’, Me-17’	2H-5’
7’	134.8		2H-8’, Me-18’	2H-5’, 2H-9’
11’	133.8		H-10’, 2H-12’	2H-13’, 2H-9’
15’	132.3		Me-16’, Me-20’	2H-13’
19’	170.7			H-10’, 2H-12’
CH				
2	124.1	8.19 (d, *J*=1,7 Hz, 1H)		H-6
6	115.9	8.23 (d, *J*=1,7 Hz, 1H)		H-2
2’	123.8	5.81 (t, *J*=7,2Hz, 1H)	2H-1’	2H-4’, Me-17’
6’	125.8	5.34 (brq, *J*=5,5 Hz, 2H)	2H-5’	2H-4’, 2H-8’, Me-18’
10’	142.4	7.21 (t, *J*=7,3 Hz, 1H)	2H-9’	2H-8’, 2H-12’
14’	125.1	5.34 (brq, *J*=5,5 Hz, 2H)	2H-13’	2H-12’, Me-16’, Me-20’
CH_2				
1’	29.5	3.85 (d, *J*=7,2Hz, 2H)	H-2’	H-2
4’	40.4	2.15 (m, 2H)	2H-5’	H-2’, CH-17’
5’	27.4	2.20 (m, 4H)	2H-4’, H-6’	
8’	39.4	2.20 (m, 4H)	2H-9’	H-6’, H-10’, CH-18’
9’	28.8	2.44-2.40 (m, 4H)	2H-12’	
12’	28.1	2.67 (brt, *J*=7,7 Hz, 2H)	2H-13’	H-10’
13’	28.1	2.44-2.40 (m, 4H)	2H-8’	
CH_3				
16’	26.2	1.69 (s, 3H)		CH-20’
17’	16.7	1.84 (s, 3H)		H-2’, 2H-4’
18’	16.4	1.65 (s, 6H)		H-6’, 2H-8’
20’	18.1	1.65 (s, 6H)		CH-16’

^{1}H-^{1}H-COSY spectrum was also used in these assignments

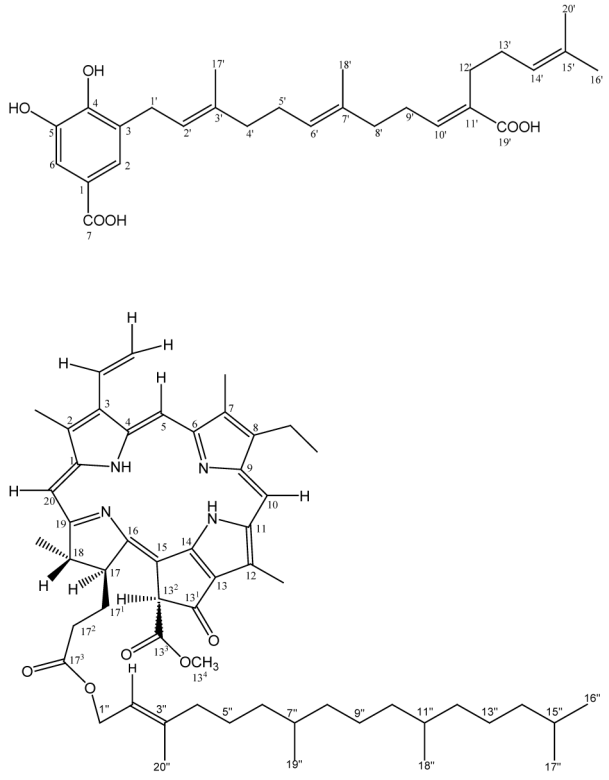

Figure 1. *Compounds isolated of Piper carniconnectivum C.DC (**1**) and (**3**)*

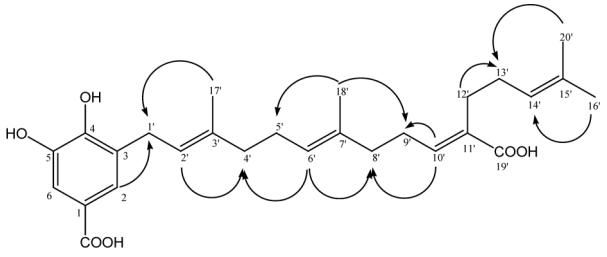

Figure 2. *NOE correlations of **1***

EXPERIMENTAL

General experimental procedures

IR spectra were obtained on a Perkin-Elmer FT-IR 1750 spectrophotometer in KBr disks and $[M-H]^-$ was obtained on HPLC-MS-MS analysis. A Shimadzu HPLC system coupled to a Quattro LC mass spectrometer (Watter, Manchester-UK) equipped with a Z-spray ESI source and operated in negative mode was used. Chromatographic separation was achieved on Shimadzu Shimpack C-18 column (4,6 x250 mm x 5 µm). Mobile phase was 0,1% aqueous formic acid and acetonitrile (15:85 v/v). The flow rate was set 1mL/min. and 20 µL of sample was injected into the column at 40 °C. The electrospray source was operated in the negative mode. The capillary potential was set a 2.5 kV, desolvation temperature 250 °C. Source temperature 110 °C, desolvation gas flow 250 L/h. Mass spectra was acquired in full scan mode (*m/z* 100-800 uma). `H NMR, ^{13}C NMR and 2DNMR spectra were recorded on Bruker 500 and Mercury-Varian 200 spectrometers in pyridine-d5 and $CDCl_3$ at 27 °C. Chromatography column (CC) was carried out on Sephadex LH-20 (Sigma) and silica gel 60 for analytical TLC (PF PF_{254}, Art. 7749, Merck).

Extraction and isolation

The plant was collected in the Garden of Emilio Goeldi Museum in Belém-PA, where a voucher specimen is deposited under the number MSP-009. The powdered material of *Piper carniconnectivum* (1300 g) was exhaustively extracted with 95% EtOH (4x2.0L) and provided 105 g of crude extract (8,0%). The extract was concentrated under red. Pres. and partitioned with hexane, $CHCl_3$, EtOAc. The hexane layer (12,5 g) was concentrated and chromatographed on a silica gel column, using hexane, hexane/$CHCl_3$, $CHCl_3$ and $CHCl_3$/MeOH as eluents resulting in 101 fractions (250 mL). Fractions were monitored by TLC and similar fractions were combined. The fraction 36-40, eluted in hexane/$CHCl_3$ (1:1), was chromatographed on silica gel column with gradient mixtures of hexane/$CHCl_3$ yielding 9 fractions (20 mL). Fraction 3, eluted in hexane afforded **2** (12 mg). The fraction 55-59, eluted in $CHCl_3$/hexane (7:3), subjected on silica gel column, using as eluents hexane, $CHCl_3$, EtOAc and MeOH in gradient mixture, provided 18 fractions (50 mL), which were reduced to 7 groups after TLC. Group 3 eluted in $CHCl_3$/hexane (7:3), after

successive preparative TLC with $CHCl_3$/hexane (8:2) afforded **4** (30 mg) and group 5 eluted in $CHCl_3$, submitted to preparative TLC with $CHCl_3$-MeOH (99:1), provided **3** (26 mg).

The $CHCl_3$ layer (11,0 g) was concentrated and chromatographed on Sephadex LH-20 colunm, eluted with MeOH afforded 152 fractions (10 mL) and similar fractions were combined after TLC. Fraction 9-12 was subjected to a new Sephadex LH-20 column eluted with MeOH:$CHCl_3$ (1:1) yield 23 fractions (25 mL). Fraction 6 (15.0 mg) afforded **1**.

The structures of **1**, **2**, **3** and **4** were elucidated as 3,4-dihydroxy-5-(11'-carboxyl-3',7',15'-trimethylhexadeca-2'*E*, 6'*E*, 10'*E*, 14-tetraenyl) benzoic acid; *E*-phytyl hexadecanoate, phaeophytin a and a β-sitosterol and stigmasterol mixture, respectively by interpretation of the spectral data (1D and 2D NMR, IV and MS).

*3,4-Dihydroxy-5-(11'-carboxyl-3',7',15'-trimethylhexadeca-2'E, 6'E, 10'E, 14'-tetraenyl) benzoic acid (***1***)*

Brown gummy solid. λ max. (KBr, cm^{-1}): 3438, 2921, 2855, 2560, 1686, 1599, 1548, 1513, 1443, 1382, 1300, 1215, 989, 893, 774. MS 455,12 $[M-H]P^-$. ^1H and ^{13}C NMR spectral data (Table 1).

^1H and ^{13}C NMR and IR spectral data of *E-phytyl hexadecanoate* (**2**), *Phaeophytin a* (**3**) and β-*sitosterol and stigmasterol* (**4**) were compared with literature data.[8-19]

SUPPLEMENTARY MATERIAL

Available in http://quimicanova.sbq.org.br, in PDF file, with free access.

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