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Short Communication

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Polyprenylated Benzophenones derivatives from *Cluisa minor* Fruits

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SUMMARY. *Clusia minor* fruits were investigated and three polyprenylated benzophenones were isolated using several chromatographic techniques. All the structures assigned (propolone D, hyperibone B and garcinielliptone I), including relative configuration, were elucidated by spectroscopic methods. These three benzophenones are reported for the first time in *Clusia genus*.

INTRODUCTION

Guttiferae (Clusiaceae) is a family of mainly tropical plants that comprises about 40 genera and 1200 species most of which are woody. The plants of the family are generally characterized by the presence of latex in most of their tissue [1]. Phytochemical investigations of *C. rosea* [2], *C. nemorosa* [3], and *C. grandiflora* [4] have revealed the presence of polyisoprenylated benzophenones in the fruits, roots, and leaves. A large number of polyisoprenylated benzophenone derivates with bicyclo-[3.3.1]-nonane-2, 4, 9-trione systems have been isolated from this family. This ring requires that the substituents at C-1 and C-5 be equatorial, whereas, prenyl group at C-7 has been observed both axial and equatorial [5]. In this investigation three polyprenylated benzophenones derivatives named propolone D, hyperibone B and garcinielliptone I were isolated from *Clusia minor* fruits.

MATERIAL & METHODS

General Experimental Procedures

Melting points were determined using a Baush & Lomb apparatus. Optical rotations were measured on a Perkin-Elmer 192 polarimeter equipped with a sodium lamp (589 nm) and a 10 cm microcell. ^{1}H NMR spectra were obtained with a Brucker DRX-600 spectrometer operating at 599.19 MHz and 150.858 MHz for ^{13}C, the UXNMR software package was used for NMR experiments in $CDCl_3$. ^{1}H-^{1}H DQF-COSY, ^{1}H-^{13}C HSQC, HMBC experiments were obtained using conventional pulse sequences. ESIMS was performed using a Finnigan LC-Q Advantage Max instrument (Termoquest, San Jose, CA) equipped with Xcalibur software. Exact masses were measured by a Q-Star Pulsar (Applied Biosystems) triple-quadrupole orthogonal time-of-flight instrument. HPLC separations were performed on a Waters 590 series pumping system equipped with Waters R401 refractive index detector and a Waters 10 µm µ-Bondapack C-18 column (300 x 7.8 mm). TLC analysis was performed with Macherey-Nagel precoated silica gel 60 F_{254} plates.

Plant Material

Clusia minor fresh fruits from were collected in the National Botanical Garden (Cuba) in July 2007. The plant was identified by Dr. Víctor Fuentes Fiallo and a voucher specimen (No. 482) was deposited in the Herbarium of the Fundamental Investigations of Tropical Plants Institute. The dried ethyl acetate extract was stored at environmental temperature in a dark place.

KEY WORDS: *Clusia minor,* Garcinielliptone I, Guttiferae, Hyperibone B, Polyprenylated benzophenones, Propolone D, Spectroscopic methods.

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Extraction and isolation

Fresh fruits (200 g) were extracted with ethyl acetate (x 3) for 7 days, and after filtration, the extract were concentrated under reduced pressure at 45 °C (57 g). A portion of this extract (2 g) was fractionated over Sephadex LH-20 column using methanol as solvent to furnish 3 fractions (1/1-1/3). Fraction 3/2 (1.3 g) on Vaccum-LC over silica gel eluting with 0-100% hexane-EtOAc mixture yielded 8 fractions (2/1-2/8). Fraction 2/3 (60.0 mg) was purified by RP-HPLC (μ-Bondapack C-18 column, MeOH-H_2O, 85:15, flow rate 2.5 mL/min) to give **1** (9.58 mg), **2** (4.13 mg) and **3** (6.70 mg).

RESULTS AND DISCUSSION

As a part of our study of plants of the Guttiferae family, we investigated the fruits of *Clusia minor*. Fresh fruits from *Clusia minor* were carefully collected and extracted with ethyl acetate. Part of the extract was fractionated on Sephadex LH-20 and silica gel and purified by RP-HPLC to give three polyprenylated benzophenone derivatives. These benzophenones (Fig. 1) were proposed as being propolone D (**1**), hyperibone B (**2**) and garcinielliptone I (**3**), previously isolated from *Cuban propolis* [7], *Hypericum scabrum* [6] and *Garcinia subelliptica* [8], respectively. Identifications were carried out on the basis of the evidence outlined below.

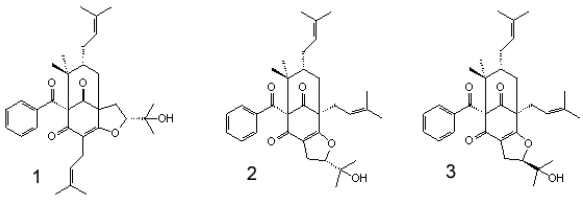

Figure 1. Polyprenilated benzophenones isolated from *Clusia minor*.

Propolone D (1): colourless oil; $[\alpha]_D$ + 48.5 ° (c 0.71, $CHCl_3$); ^1H and ^{13}C NMR data were consistent with those previously reported for hyperibone G [6] and propolone D [7]; ESIMS (positive mode) m/z 519 $[M+H]^+$. ESIMS/MS (positive mode) m/z 501 $[M+H-H_2O]^+$, 441 $[M+H-78]^+$, 397 $[M+H-122]^+$, 383 $[M+H-136]^+$, 327 $[M+H-192]^+$.

Hyperibone B (2): colourless oil; $[\alpha]_D$ – 42.2 ° (c 0.14, $CHCl_3$); ^1H and ^{13}C NMR data were consistent with those previously reported for hyperibone B [6]; ESIMS (positive mode) m/z 519 $[M+H]^+$. ESIMS/MS (positive mode) m/z 463 $[M+H-56]^+$, 451 $[M+H-68]^+$, 397 $[M+H-122]^+$, 359 $[M+H-160]^+$, 327 $[M+H-192]^+$.

Garcinielliptone I (3): colourless oil; $[\alpha]_D$ + 63.7 ° (c 0.37, $CHCl_3$); ^1H and ^{13}C NMR data were consistent with those previously reported for garcinielliptone I [8]; ESIMS (positive mode) m/z 519 $[M+H]^+$. ESIMS/MS (positive mode) m/z 463 $[M+H-56]^+$, 451 $[M+H-68]^+$, 397 $[M+H-122]^+$, 359 $[M+H-160]^+$, 327 $[M+H-192]^+$.

The molecular formula $C_{33}H_{42}O_5$ of compound 1 was deduced using MS, ^{13}C NMR and ^{13}C DEPT NMR analysis. The ESIMS spectrum showed an $[M+H]^+$ ion at *m/z* 519 demonstrated the molecular formula proposed. ^1H and ^{13}C NMR data (Table 1) suggested the presence of a bicyclo-[3.3.1]-nonane moiety, the skeleton most frequently encountered among polyprenylated benzophenone derivates isolated from Clusiaceae [2,9].

The evidence of de presence of three carbonyl groups was the presence of resonances corresponding to an unconjugated (δ 204.7) and two conjugated carbonyls (δ 193.2 and 193.5). ^{13}C chemical shifts at δ 78.6 (C-1), 193.2 (C-2), 115.9 (C-3), 172.3 (C-4), 60.1 (C-5), 39.2 (C-6), 42.7 (C-7), 48.0 (C-8) and 204.9 (C-9) supported the presence of the bicyclo-[3.3.1]-nonane moiety and indicating the presence of one methylene group (C-6), a methine group (C-7), and the analysis of the HMBC spectra allow us to assigned three quaternary sp^3 carbons (C-1, C-5 y C-8), a quaternary sp^2 carbon (C-3) and one unconjugated carbonyl group (C-9).

Analysis of ^1H RMN data exhibited only one set of signals, suggesting the absence of a tautomeric equilibrium. NMR data also permitted identification of the presence of three units C_5 and an unsubstituted benzoil moiety. The ^1H NMR spectrum exhibited signals for two vinylic proton (δ 5.03, δ 4.99), four vinylic methyl group (δ 1.58, 1.65, 1.65, 1.70) and four allylic protons (δ 1.77, 2.13, 3.06, 3.11), indicating the presence of two 3-methyl-2-butenyl groups in the molecule (C-17 to C-21 and C-27 to C-31) attached to C-3 and C-7, respectively. HMBC connectivities enabled differentiation between the isoprenoid groups at C-3 and C-7. Carbons C-2 (δ 193.2) and C-3 (δ 115.9) showed correlations to the C-17 protons indicating that this group is attached to C-3. Meanwhile carbons C-7 (δ 42.7) and C-8 (δ 48.0) showed correlations to the C-27 protons established that this isoprenoid is attached to C-7. The absence of further signals for sp_2 carbons suggested that the other remaining 3-methyl-2-butenyl group is modified. ^1H and ^{13}C NMR spectra indicated the presence of a 2-(2-hidroxypropyl)dihidrofuran ring , mainly for

Positions	1		2		3	
	δ ^{13}C	δ ^{1}H (J_{H-H} in Hz)	δ ^{13}C	δ ^{1}H (J_{H-H} in Hz)	δ ^{13}C	δ ^{1}H (J_{H-H} in Hz)
1	78.6	-	80.0	-	79.0	-
2	193.2	-		-	187.7	-
3	115.9	-	117.0	-	118.3	-
4	172.3	-	-	-	175.5	-
5	60.1	-	56.0	-	55.4	-
6ax	39.2	1.58 (overlapped)	39.3	1.96 d	39.7	1.50 t (12.7)
6eq		2.08 dd (13.2, 3.9)		1.98 d		2.00 dd (12.7, 3.9)
7	42.7	1.70 (overlapped)	43.7	1.59 (overlapped)	43.2	1.70 (overlapped)
8	48.0	-	48.0	-	47.6	-
9	204.9	-	204.2	-	206.5	-
10	193.5	-	193.0	-	193.1	-
11	136.9	-	135.0	-	136.6	-
12,16	128.1	7.50 d (7.4)	128.2	7.5 d (7.8)	128.0	7.61 d (7.4)
13,15	127.9	7.23 t (7.4)	127.9	7.26 m (7.8)	127.9	7.28 t (7.4)
14	132.0	7.37 t (7.4)	132.0	7.4 m (7.4)	132.1	7.41 t (7.4)
17	22.1	2.08 dd (13.2, 3.9)	27.4	2.93 dd (12.7, 10.4)	27.1	3.03 dd (14.9, 10.4)
		1.58 (overlapped)		2.98 dd (12.7, 8.0)		2.93 dd (14.9, 7.5)
18	120.6	5.03 m	92.4	4.80 t (10.4, 8.0)	93.0	4.83 dd (10.4, 7.5)
19	132.5	-	73.0	-	71.7	-
20	17.8	1.65 s	25.0	1.29 s	23.1	1.22 s
21	25.7	1.65 s	26.0	1.23 s	25.9	1.31 s
22a	30.3	2.70 dd (13.3, 10.4)	28.8	2.45 d (10.3, 7.8)	29.0	2.53 (2H) d
22b		1.86 dd (13.3, 6.0)		2.58 d (10.3, 6.6)		
23	90.2	4.63 dd (10.4, 6.0)	118.3	5.05 t	120.3	5.00 (overlapped)
24	71.1	-	136.0	-	134.7	-
25	23.9	1.23 s	18.0	1.65 s	18.1	1.69 s
26	26.6	1.39 s	26.0	1.68 s	25.9	1.71 s
27	26.7	2.13 m	26.7	2.2 dd	26.9	2.20 m
		1.77 (overlapped)		1.55 – 1.60(overlapped)		1.80 m
28	122.3	4.99 m	122.4	4.95 t	122.4	4.98 (overlapped)
29	133.6	-	134.0	-	133.4	-
30	17.9	1.58 s	17.7	1.55 s	17.9	1.58 s
31	25.9	1.70 s	25.7	1.66 s	25.9	1.66 s
32ax	16.4	1.20 s	15.8	1.10 s	15.8	1.13 s
33eq	23.1	1.39 s	23.7	1.38 s	23.7	1.40 s

Table 1. ^{1}H and ^{13}C NMR data for compounds **1**, **2** and **3** in $CDCl_3$. [a] Chemical shift values are in ppm from TMS, and values in Hz are presented in parentheses. All signals were assigned by DQF-COSY, HSQC, and HMBC experiments.

the unusual chemical shift of C-23 (δ 90.2), H-23 (δ 4.63) and C-24 (δ 71.1). In the HMBC spectrum cross-peaks between methylene protons at C-22 (δ 1.86 and 2.7) and C-5 (δ 60.1) and C-9 (δ 204.9) indicated that the dihydrofuran ring was formed between C-4 y C-5.

Assigment of the relative configuration at C-1, C-5, C-7 and C-22 was established by comparison with literature data [5,10,11]. The basic bicyclic ring system requires the phenyl ketone at C-1 and the bond between C-5 and C-22 to be in an equatorial orientation. Although $^{3}J_{H6ax-H7}$ was not observed, ^{13}C chemical shifts of the *gem*-methyl groups at C-8 permitted definition of an equatorial orientation of the 3-methyl-2-butenyl unit at C-7. The *gem*-methyl group at C-8 shows two ranges of ^{13}C chemical shifts: if the C-7 substituent is axial ranges will be δ 26-28 for Me-32ax and δ 22-25 for Me-33eq, while if the C-7 substituent is equatorial ranges will be δ 15-17 for Me-32ax and δ 22-24 for Me-33eq. The upfield shift of the C-32ax signal results from steric compression of a γ-gauche interaction between this carbon and the CH_2-27 of the equatorial isopentenyl group on C-7. On the other hand, the C-7 carbons are upfield shifted when their substituents are equatorial (δ 40-44). Taking account of these observations, in this case, the axial and equatorial C-8 methyl groups and the C-7 showed ^{13}C chemical shift in the range ob-

served for polyprenyl benzophenones with an equatorial isopentenyl group at C-7.

NMR data of this compound were identical with those reported for hyperibone G 6, althought some differences were observed with respect to optical rotation values, $[\alpha]_D$ =+ 48.5^0 for compound **1** and $[\alpha]_D$ = - 29.3 0 for hyperibone G, because of these compounds are probably enantiomers. Thus, the structure of compound **1** was consistent with propolone D, reported previously [7], and was assigned as shown in Figure 1.

Compounds **2** and **3** have the same molecular formula as compound **1** ($C_{33}H_{42}O_5$, determined by MS, ^{13}C NMR and ^{13}C DEPT NMR analysis) and their NMR data (Table 1) suggested the presence of a bicyclo-[3.3.1]-nonane derivative with dihydrofuran ring and an equatorial isopentenyl group at C-7 [5,10,11]. This ring was formed between C-4 and C-18 (HMBC correlations: H_2-17/C-3, C-4, C-18, C-19).

The NMR data of these compounds were very similar, but some differences in their ^{13}C NMR chemical shifts were observed for C-18 (δ 92.5 in **2** and δ 93.1 in **3**), for the carbons near C-18, and for the 1-methyl-1-hydroxyethyl side chain, consistent with these compounds being epimeric at C-18. Other differences were also observed in the chemical shifts of H_2-22 (δ 2.45 dd, 10.3, 7.8, and 2.60 dd, 10.3, 6.6 in **2**; δ 2.54, 2H, br d in **3**), C-23 (δ 118.3 in **2** and 120.3 in **3**) and C-24 (δ 135.5 in 2 and 134.7 in **3**). Theses differences are consistent with different orientations of the substituent at C-18 in these compounds and due to the steric effects between the C-18 1-methyl-1-hydroxyethyl and C-5 methylbut-2-enyl groups. 1D and 2D NMR data of **2** and **3** were identical to those reported previously for hiperibone B [6,7] and garcinielliptone I [7,8], respectively.

CONCLUSIONS

Several polyprenylated benzophenone derivatives have been isolated from plants of the family Guttiferae [1,2,5-7,9,11] but this is the first report about the presence of these three compounds (propolone D, hyperibone B and garcinielliptone I) in the *Clusia* genus. The results lead us to postulate that all polyprenylated benzophenones isolated, with the use of various chromatographics technics and spectroscopic methods, are derivatives of type A due to the presence of benzoyl group at C-1. The main differences observed in these compounds are respect to the optical rotation values suggesting the structures by comparison with literature. Although literatures reveals similar sign on optical rotation in compounds isolated from similar plants, this differences can depend of many factors such as temperature, solvent, equipment, purity of the compound.

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