Aristophenones A and B. A New Tautomeric Pair of Polyisoprenylated Benzophenones from *Garcinia aristata*

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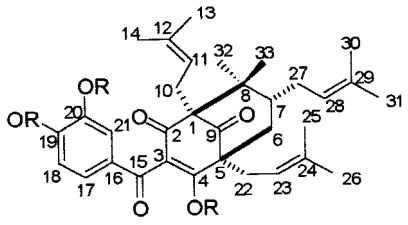
A tautomeric pair of new isoprenylated benzophenones, aristophenone A (**1a**) and B (**1b**), have been isolated from *Garcinia aristata* fruits. Their structures has been determined using high-field 2D NMR techniques.

Plants of the family Guttiferae, in particular those belonging to the genera *Clusia* and *Garcinia*, produce a series of oxidized and isoprenylated benzophenones.[1−5] These compounds are thought to be of mixed shikimate and acetate biosynthetic origin. In a previous paper we examined propolis collected in a small area, Nuevitas, in Cuba, and we described the isolation, structural elucidation, and antimicrobial and antifungal activities of a new polyisoprenylated benzophenone named propolone A.[6] Now we have investigated the fruits of *Garcinia aristata* (Griseb.) Borhidi (Guttiferae), an endemic plant of Cuba for which no chemical studies have been reported. Here we describe the isolation and structural elucidation of aristophenone, a new tautomeric pair of prenylated benzophenone derivatives (**1a**, **1b**) based on the bicyclo[3.3.1]nonane-2,4,9-trione system.

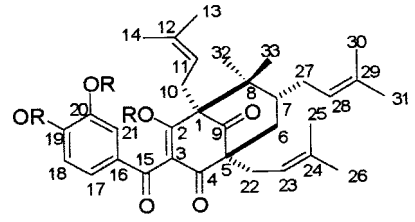
Aristophenone was obtained from the hexane extract of *Garcinia arista* by HPLC. The molecular formula $C_{33}H_{42}O_6$ was indicated using MS, ^{13}C NMR, and ^{13}C DEPT NMR analyses. The EIMS of aristophenone gave a molecular ion at *m*/*z* 534, and additional peaks at *m*/*z* 137 and 69 suggested the presence of a 3,4-dihydroxybenzoyl group ($C_7H_5O_3$) and isoprenyl substituents, respectively. UV aborptions at 280 and 228 nm revealed a chromophore with extended conjugation. The IR spectrum exhibited absorption bands for hydroxyl (3350 cm^{-1}) and carbonyl groups (1730, 1680, 1638 cm^{-1}).

Aristophenone was present in $CDCl_3$ solution as a tautomeric pair (**1a**, **1b**) in a ratio of 1:1, as evidenced by two sets of NMR signals, the consequence of the presence of the enolizable 1,3-diketone system. Analysis of the 1D and 2D NMR spectra in $CDCl_3$ with homo- and heteronuclear direct and long-range correlations allowed assignment of ^1H and ^{13}C NMR signals for aristophenone A (**1a**) and B (**1b**) as listed in Table 1. In the 600 MHz NMR spectra, six vinyl protons between δ 4.81 and 5.28 and 12 vinylic methyl groups in the region δ 1.46 to 1.82 indicated the presence of three isopent-2-enyl groups for each tautomer: a 3-methyl-2-butenyl group (from C-27 to C-31) linked to the methyne at C-7, as evidenced by a COSY experiment, and a second and third 3-methyl-2-butenyl group (from C-10 to C-14 and from C-22 to C-26) linked to the basic skeleton at C-1 and C-5 quaternary carbons, respectively, as evidenced by COSY and HMBC correlations. Four aliphatic methyl proton singlets between δ 1.23 and 1.37 were assigned to the two *gem*-dimethyl groups (C-32 and C-33), correlated by HMBC to each other and to the C-8 quaternary carbons. An aromatic AMX system was evident for both **1a** and **1b** from proton resonances at δ 6.64 (J = 8.3), 7.02 (J = 8.3, 2.2), and 7.05 (2.2) and at δ 6.64 (J = 8.3), 7.09 (J = 8.3, 2.2), and 7.08 (2.2), respectively. Characteristic ^{13}C NMR resonances, including those for substituted aromatic carbons at δ 128.1, 143.5, and 149.5 for **1a** and δ 128.1, 143.3, and 149.6 for **1b** and two conjugated carbonyl groups, respectively, at δ 194.8 and 194.5, were indicative of the presence of a 3,4-dihydroxybenzoyl group in the structure of the tautomeric pair aristophenone A (**1a**) and B (**1b**).

The bicyclo[3.3.1]nonane structures and the location of the functionalities for **1a** and **1b** were deduced from HMBC data and by comparison with the data of clusianone reported in the literature[1]. Aristophenone is closely related to clusianone, differing only in the 3,4-dihydroxybenzoyl group, of shikimate biogenesis similar to that of xanthochymol, and in the C-7 configuration.[7] This skeleton is the most frequently encountered among the bridged bicyclic prenylated benzophenone derivatives of the Guttiferae.



1a R=H
2 R=CH_3CO-



1b R=H
3 R=CH_3CO-

The tautomeric pair was acetylated to afford the regioisomeric pair aristophenone A acetate (**2**) and aristophenone B acetate (**3**). HMBC data enabled differentiation between the two isomers. For **2** there were cross-peaks between the enol carbon C-4 (168.2) and the protons at C-6

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Table 1. ^{1}H NMR and ^{13}C NMR Data for **1a** and **1b** in $CDCl_3$[a]

		1a		1b		
position	DEPT	δ_C	δ_H (J_{H-H} in Hz)	δ_C	δ_H (J_{H-H} in Hz)	HMBC[b]
1	C	69.4		66.2		
2	C	196.5		198.0		
3	C	115.9		115.6		
4	C	198.6		196.9		
5	C	58.8		63.0		
6	CH_2	38.6	2.25 eq dd (13, 3), 2.14 ax dd (13, 10.5)	40.1	2.17 eq dd (13, 3) 2.00 ax dd (13, 10.5)	4, 8, 9, 22, 27
7	CH	40.2	1.60 m	40.7	1.55 m	1, 5, 28, 32, 33
8	C	50.6		51.3		
9	C	208.3		208.6		
10	CH_2	26.2	2.76 dd (14, 8) 2.65 dd (14, 6)	25.3	2.80 dd (14, 8), 2.70 dd (14, 6)	2, 8, 9, 12
11	CH	120.3	5.12 dd (8, 6)	118.7	4.81 (8, 6)	1, 13, 14
12	C	135.0		135.0		
13	Me	25.3	1.73 s	25.6	1.71 s	11, 14
14	Me	17.7	1.58 s	17.5	1.62 s	11, 13
15*[c]	C	194.8		194.5		
16*	C	128.1		128.1		
17*	CH	125.2	7.02 dd (8.3, 2.2)	125.7	7.09 dd (8.3, 2.2)	15, 19, 21
18*	CH	114.8	6.64 d (8.3)	114.8	6.64 d (8.3)	16, 20
19*	C	149.5		149.6		
20*	C	143.5		143.3		
21*	CH	116.8	7.05 d (2.2)	116.8	7.08 d (2.2)	15, 17, 19
22	CH_2	30.5	2.66 dd (14, 8) 2.62 dd (14, 6)	31.4	2.63 dd (14, 8), 2.53 dd (14, 6)	4, 6, 9, 24
23	CH	119.7	5.28 dd (8, 6)	119.2	5.18 dd (8, 6)	5, 25, 26
24	C	134.9		134.9		
25	Me	18.0	1.59 s	17.9	1.68 s	23, 26
26	Me	26.1	1.75 s	25.7	1.65 s	23, 25
27	CH_2	28.5	1.96 ddd (14, 9, 9) 2.21 ddd (14, 7, 1)	28.6	2.07 m (2H)	6, 8, 29
28	CH	124.4	4.92 dd (9, 7)	124.3	4.95 dd (9, 7)	7, 30, 31
29	C	132.1		132.2		
30	Me	17.7	1.53 s	17.1	1.46 s	28, 31
31	Me	26.0	1.81 s	26.2	1.82 s	28, 30
32	Me	23.7	1.23 s	23.6	1.29 s	1, 7, 8, 33
33	Me	15.8	1.37 s	15.7	1.34 s	1, 7, 8, 32

[a] Chemical shift values are in ppm from TMS, and *J* values in Hz are presented in parentheses. Carbon multiplicities were determined using DEPT experiments. All signals were assigned by DQF-COSY, HSQC, and HMBC experiments. [b] Carbons that correlate with the proton resonance. [c] * No distinction can be made between the two tautomers.

and C-22, while the carbonyl group at C-2 (δ 196.5) showed correlations only to the C-10 protons. For **3** the carbonyl carbon C-4 (196.9) showed correlations to the C-6 and C-22 protons, while the enol carbon C-2 (169.1) showed correlations only to the C-10 protons.

The basic bicyclic ring system in **1a** and **1b** required that the isopentenyl chains at C-1 and C-5 be equatorial. The relative stereochemistry of the remaining chiral carbon at C-7 was determined by the coupling constant analysis, by NOE data obtained from a ROESY spectrum, and by comparison with reference compounds. A 10.5 Hz coupling between the methylene protons H-6ax and H-7 required these protons to be diaxial; thus the isopentenyl group at C-7 was equatorial. The H-6eq proton signal showed an NOE interaction with H-7 and with one of the methylene protons at C-27. NOE interactions between Me-33ax and one of the methylene protons at C-27 and H-6ax were also consistent with the structures **1a** and **1b**.

Examination of the reported values of vicinal coupling constants for a series of prenylated benzophenones gave two ranges. In the case of methyl nemorosone I and II and guttiferone B,[2,5,8] the ^3J (vicinal) coupling constant between H-6_{axial} and H-7 has a value of 10−12 Hz, with an *R*-configuration for C-7 (the 3-methyl-2-butenyl substituent occupies the equatorial position in the predominant chair conformation). In these compounds there exists a chair conformation for the B-ring of the bicyclo[3.3.1]nonane system, confirming the high value of the vicinal coupling constant. On the other hand, there are other examples where the coupling constant between H-6_{axial} and H-7 reaches a value of 7−7.5 Hz. That is the case for the compounds guttiferone A and F,[5,9] plukenetione E acetate, and plukenetione G.[10] All of them possess the *S*-configuration, and these ^3J values are not characteristic of the chair conformation of cyclohexane derivatives (J_{aa} = 10−12 Hz, $J_{ae} = J_{ee} = 2-5$ Hz), suggesting that both chair and twist-boat conformations exist in similar proportions and contribute to the vicinal coupling constant observed. This is due to the two 1,3-diaxial interactions between the isopentenyl group and C-2 and C-4 in chair conformation. To clarify this point, a semiempirical computational procedure for conformational search and energy minimization (AM1, MOPAC v6) was performed to determine the most probable conformation of B ring for the *R* and *S* stereoisomers. The results, showing high-energy differences (>5.5 kcal/mol) between the most stable chair and the minor conformers, assess the chair as the predominant conformation for stereoisomer *R*. However, the twist-boat and the chair conformers of the *S* stereoisomer have an energy difference < 0.7 kcal/mol, suggesting that both conformations are represented at the equilibrium.

Experimental Section

General Experimental Procedures. Melting points were determined using a Bausch & Lomb apparatus. Optical rotations were measured on a Perkin-Elmer 192 polarimeter

equipped with a sodium lamp (589 nm) and a 10 cm microcell. UV spectra were obtained with a Beckman DU 670 spectrophotometer and IR spectra with a Bruker IFS-48 spectophotometer. A Bruker DRX-600 spectrometer, operating at 599.19 MHz for ^{1}H and 150.858 for ^{13}C, using the UXNMR software package, was used for NMR experiments in $CDCl_3$. ^{1}H−^{1}H DQF-COSY (double quantum filtered COSY), ^{1}H−^{13}C HSQC, HMBC, and ROESY experiments were obtained using conventional pulse sequences.[6] The EIMS spectrum was obtained from a VG-PROSPEC mass spectrometer (70 eV). The FABMS spectrum was recorded in a glycerol matrix in the negative ion mode on a VG ZAB instrument (Xe atoms of enrgy of 2−6 kV). HPLC separations were performed on a Waters 590 series pumping system equipped with a Waters R401 refractive index detector and a Waters μ-Bondapak C18 column. A computational procedure for conformational search and energy minimization was performed using the AM1[11] method, implemented in the MOPAC v6 program.[12]

Plant Material. Fruits of *Garcinia aristata* (Griseb.) Borhidi were collected in the Jardín Botánico Nacional (Habana, Cuba) in June 1998 and identified by Dr. Victor Fuentes Fiallo. A voucher specimen (No. 700) is deposited in the Herbario del Instituto de Investigaciones Fundamentales de La Agricultura "Alejandro de Humboldt".

Extraction and Isolation. Fresh fruits (533 g) were extracted with *n*-hexane (× 2) for two weeks. A yellow solid was obtained after concentration under reduced pressure, and this was purified by HPLC (μ-Bondapack C-18 column, MeOH/H_2O 9:1, flow rate 2 mL/min). Successive crystallizations from hexane gave crystalline aristophenone (mixture of **1a** and **1b** in $CHCl_3$ solution).

Aristophenone A (1a) and B (1b): yellow cubes ($CHCl_3$); mp 82 °C; $[\alpha]_D^{25}$ +58° (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 280 (4.21) and 228 (4.34) nm; IR (KBr) ν_{max} 3350, 1730, 1715, 1644, 1220, 1151 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; EIMS *m*/*z* 534 (3) M^+, 466 (2), 315 (15), 177 (27), 137 (87), 69 (100); *anal*. C 74.38%, H 9.08%, calcd for $C_{33}H_{42}O_6$, C 74.71%, H 9.15%.

Aristophenone A acetate (2): colorless oil; $[\alpha]_D^{25}$ + 53° (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 250 (4.16) nm; IR (KBr) ν_{max} 1783, 1730, 1715, 1644 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.43 (1H, dd, J = 8.3, 2.2 Hz, H-17), 7.35 (1H, d, J = 2.2 Hz, H-21), 7.04 (1H, d, J = 8.3 Hz, H-18), 5.27 (1H, dd, J = 8, 6 Hz, H-23), 5.13 (1H, dd, J = 8, 6 Hz, H-11), 4.93 (1H, dd, J = 9, 7 Hz, H-28), 2.77 (1H, dd, J = 14, 8 Hz, H-10a), 2.66 (1H, dd, J = 14, 8 Hz, H-22a), 2.65 (1H, dd, J = 14, 6 Hz, H-10b), 2.62 (1H, dd, J = 14, 6 Hz, H-22b), 2.37 (3H, s, −$COCH_3$), 2.34 (3H, s, −$COCH_3$), 2.25 (1H, dd, J = 13, 3 Hz, H-6eq), 2.21 (1H, ddd, J = 14, 7, 1 Hz, H-27a), 2.14 (1H, dd, J = 13, 10.5 Hz, H-6ax), 2.06 (1H, s, −$COCH_3$), 1.97 (1H, ddd, J = 14, 9, 7 Hz, H-27b), 1.82 (3H, s, Me-31), 1.76 (3H, s, Me-25), 1.74 (3H, s, Me-13), 1.61 (3H, s, Me-14), 1.59 (1H, m, H-7ax), 1.58 (3H, s, Me-26), 1.54 (3H, s, Me-30), 1.38 (3H, s, Me-33), 1.24 (3H, s, Me-32); ^{13}C NMR ($CDCl_3$, 600 MHz) δ 208.7 (C-9), 196.6 (C-2), 192.6 (C-15), 169.3 (−*C*OCH_3), 168.6 (−*C*OCH_3), 168.2 (−*C*OCH_3), 157.6 (C-4), 143.4 (C-19), 139.6 (C-20), 135.2 (C-12), 135.0 (C-24), 133.4 (C-16), 132.1 (C-29), 130.3 (C-17), 124.4 (C-28), 123.0 (C-21), 120.4 (C-11), 120.2 (C-18), 119.8 (C-23), 118.8 (C-3), 69.3 (C-1), 62.6 (C-5), 50.8 (C-8), 40.3 (C-7), 38.4 (C-6), 31.6 (C-22), 28.7 (C-27), 26.2 (C-10), 26.1 (C-26), 25.9 (C-31), 25.4 (C-13), 23.9 (C-32), 20.8 (−CO*C*H_3), 20.5 (−CO*C*H_3), 20.1 (−CO*C*H_3), 18.1 (C-25), 17.8 (C-14), 17.6 (C-30), 15.8 (C-33); EIMS *m*/*z* 660 (58) M^+, 618 (16), 221 (4), 69 (100); *anal*. C 74.42%, H 8.11%, calcd for $C_{39}H_{48}O_9$, C 74.74%, H 8.26%.

Aristophenone B acetate (3): colorless oil; $[\alpha]_D^{25}$ + 54° (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 248 (4.25) nm; IR (KBr) ν_{max} 1778, 1726, 1712, 1642 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.46 (1H, dd, J = 8.3, 2.2 Hz, H-17), 7.36 (1H, d, J = 2.2 Hz, H-21), 7.06 (1H, d, J = 8.3 Hz, H-18), 5.18 (1H, dd, J = 8, 6 Hz, H-23), 4.95 (1H, dd, J = 9, 7 Hz, H-28), 4.82 (1H, dd, J = 8, 6 Hz, H-11), 2.90 (1H, dd, J = 14, 8 Hz, H-10a), 2.83 (1H, dd, J = 14, 6 Hz, H-10b), 2.63 (1H, dd, J = 14, 8 Hz, H-22a), 2.53 (1H, dd, J = 14, 6 Hz, H-22b), 2.28 (3H, s, −$COCH_3$), 2.24 (3H, s, −$COCH_3$), 2.18 (1H, dd, J = 13, 3 Hz, H-6eq), 2.21 (2H, m, H-27), 2.10 (1H, dd, J = 13, 10.5 Hz, H-6ax), 2.06 (1H, s, −$COCH_3$), 1.83 (3H, s, Me-31), 1.72 (3H, s, Me-25), 1.71 (3H, s, Me-13), 1.62 (3H, s, Me-14), 1.60 (3H, s, Me-26), 1.56 (1H, m, H-7ax), 1.46 (3H, s, Me-30), 1.34 (3H, s, Me-33), 1.27 (3H, s, Me-32); ^{13}C NMR ($CDCl_3$, 600 MHz) δ 209.0 (C-9), 196.8 (C-4), 193.2 (C-15), 169.0 (−*C*OCH_3), 168.7 (−*C*OCH_3), 168.1 (−*C*OCH_3), 158.3 (C-2), 143.5 (C-19), 140.1 (C-20), 135.1 (C-12), 135.0 (C-24), 133.4 (C-16), 132.3 (C-29), 130.2 (C-17), 124.9 (C-28), 123.2 (C-21), 120.8 (C-18), 120.1 (C-3), 119.2 (C-23), 118.8 (C-11), 72.3 (C-1), 63.2 (C-5), 51.6 (C-8), 40.6 (C-7), 40.1 (C-6), 31.5 (C-22), 28.8 (C-27), 26.6 (C-10), 26.1 (C-31), 25.8 (C-26), 25.5 (C-13), 23.7 (C-32), 20.5 (−CO*C*H_3), 20.1 (−CO*C*H_3), 19.8 (−CO*C*H_3), 17.9 (C-25), 17.8 (C-14), 17.6 (C-30), 15.7 (C-33); EIMS *m*/*z* 660 (54) M^+, 618 (11), 221 (4), 69 (100); *anal*. C 74.39%, H 8.15%, calcd for $C_{39}H_{48}O_9$, C 74.74%, H 8.26%.

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