PERGAMON

Phytochemistry 55 (2000) 755–768

PHYTOCHEMISTRY

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Polyisoprenylated benzophenones from *Clusia* floral resins☆

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Received 11 January 2000; received in revised form 1 May 2000

Abstract

From the floral resins of various *Clusia* species, seven polyisoprenylated benzophenones were isolated. HPLC allowed their quantification in all resins, revealing a distribution of benzophenone derivatives distinct from each other. In some species the staminal oils were collected and oleic, stearic and palmitic acids were the main constituents. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Clusia burchellii* male; *Clusia fluminensis* male; *Clusia hilariana* (red) male; *Clusia hilariana* (white) male; *Clusia insignis* male; *Clusia lanceolata* male; *Clusia panapanari* female; *Clusia paralicola* male; *Clusia pernambucensis* male; *Clusia scrobiculata* hermaphrodite; *Clusia spiritu-sanctensis* female; *Clusia spiritu-sanctensis* male; *Clusia weddelliana* male; Guttiferae; Polyisoprenylated benzophenones

1. Introduction

Herbivory and pollination are among the most studied plant/animal interactions. These are based on biomolecules playing a significant role in the communication of living organisms. Angiosperms use floral volatiles to attract their pollinators which are rewarded with pollen, nectar, nutritive oils or resins among others, although sometimes there are even no rewards at all. Resin is a rare reward limited to a few tropical genera like *Clusia*, a genus with about 250 species, whose flowers produce floral resins in many species. The viscous liquid is collected by bees and used as a nest construction material. Investigation of the chemistry of the floral resins revealed that they are composed of almost pure polyisoprenylated benzophenones (Oliveira et al., 1996, 1999). The attractive effect of *Clusia* flowers offering resin on some social bees acting as pollinators was indeed observed in field experiments and the volatile composition of 16 different species of *Clusia* flowers was recently concluded (Nogueira et al., 2000). The results indicated a correlation between the chemical composition, the taxonomic sections and the pollinators.

Flowers from the section Chlamydoclusia (*C. nemorosa* G. Mey. and *C. insignis* Mart. and related species; Oliveira et al., 1999), secrete floral resins and separately a staminal oil (in male flowers), which serves as an accessory pollen-kit. This peculiarity is not observed in flowers belonging to other sections. In all species some staminal or staminodial oils are jointly secreted with the resins thus reducing resin viscosity. In the section Cordylandra (and some species of section Phloianthera like *C. microstemon* Planch. & Triana) the floral resins, staminal oils and pollen (male flowers) are mixed together. In the male flowers of some species of the section Cordylandra (*C. renggerioides, C. spiritu-sanctensis, C. fluminensis, C. paralicola, C. pernambucensis*), the pollen is mixed with staminal oil inside a ball-like resin drop on the tip of the anther (Bittrich and Amaral, 1997).

The morphological differences between flowers belonging to these sections raises questions about the chemical composition and the chemical evidence of their role(s) in bee nest construction. This is addressed in the present investigation involving 11 floral resins of *Clusia* species belonging to the sections: Chlamydoclusia, Cordylandra, Phloianthera and Polythecandra.

☆ The authors dedicate this paper to Professor Otto R. Gottlieb to celebrate his 80th birthday.

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PII: S0031-9422(00)00292-2

2. Results and discussions

Fresh floral resins from various *Clusia* species were collected and treated with diazomethane, as previously described (Oliveira et al., 1996, 1999). While methylation of the resin allows the use of silica column chromatography for purification without decomposition of its components, it has the disadvantage of producing more than one derivative due to the keto-enol equilibrium. Figs. 1 and 2 depict all of the possible methylation products from polyisoprenylated benzophenones, possessing type **I** (R1, R2 and R3 are isoprenyl residues) and type **II** (R1, R2, R3 and R4 are three isoprenyl and one benzoyl residue) general structures. Compounds **Id** and **Ie** are only possible when the benzoyl unit is not at C-1 of structure **I**, and compound **IIc** is only possible when the benzoyl unit is at C-3 of structure **II**. Compounds **Ic**, **Id**, **Ie** and **IIc** have never been isolated or detected among the methylated products. The numbering system adopted is used for simplifying the discussion, and for comparison of the different classes of polyisoprenylated benzophenones; it does not attempt to follow IUPAC rules. Nevertheless, Section 4 employs IUPAC nomenclature for each compound. On the other hand, the assignments of the hydrogen and carbon chemical shifts use the numbering system used in the discussion. So far we have only isolated the methyl derivatives possessing the general structures **Ia**, **Ib**, **IIa**, **IIb**.

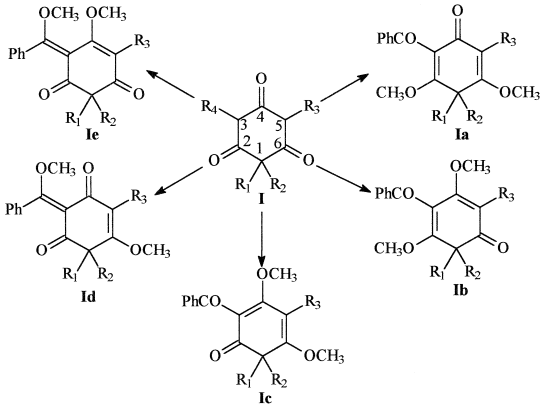

Fig. 1. Type **I** benzophenones and possible isomeric methyl enol ethers formed upon methylation with diazomethane.

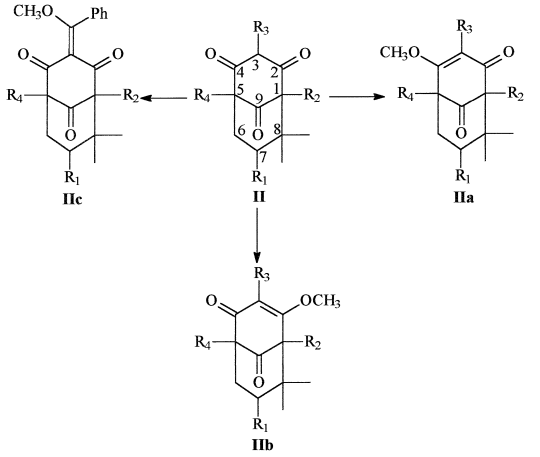

Fig. 2. Type **II** benzophenones and possible isomeric methyl enol ethers formed upon methylation with diazomethane.

The crude methylated resin of *C. weddelliana* was submitted to a series of silica gel columns, silica gel with 5% silver nitrate, thin layer chromatographies, with these resulting in isolation of methyl clusianone (**1a**, Fig. 3, Oliveira et al., 1996), **3b** and **4a** (Fig. 4). The UV spectrum of **3b** (λ_{max} 254 and 350 nm) is different from that previously reported for dimethyl grandone (**2a**, Fig. 3, λ_{max} 254 and 290 nm, Oliveira et al., 1996). The bathochromic shift of the $n\rightarrow\pi^*$ transition in the UV spectrum of **3b**, in relation to that of **2a**, is consistent with a dienone chromophore going from cross-conjugation to extended conjugation (**3b**). The presence of two methoxy groups (δ_H 3.54 and 3.46), and the absence of signals corresponding to two methyl groups at δ_H 0.70–1.50 in the ^{1}H NMR spectra of **3b** (Fig. 4), provided the first evidence of benzophenone derivatives possessing the general structure **I** (Fig.1). Six singlets 1.56 (3H), 1.60 (3H), 1.61 (3H), 1.65 (3H), 1.67 (6H) and 1.72 (3H) ppm, corresponding to seven methyl groups attached to double bonds, were assigned to C-13 (17.6 ppm), C-21 (17.7 ppm), C-16 (17.5 ppm), C-33 (17.8 ppm), C-32 and C-12 (25.4 and 25.5 ppm, both showing correlation to the hydrogen signal at 1.67 ppm) and C-20 (25.8 ppm), respectively, based on their C,H and H,H correlations in the 2D NMR spectra (HETCOR and COSY). The multiple signals corresponding to 5H in the region of 1.80 and 2.20 ppm were assigned to hydrogens 7, 7′, 8, 9 and 9′ based on their one bond C,H correlations to δ_c 42.9 (CH_2), 44.9 (CH) and 32.6 (CH_2), respectively. Signals at δ_H 2.50 (*dd*) and 2.58 (*dd*), each corresponding to 1H, were assigned to H-17 and H-17′, a methylene group which correlates with the C-17 signal at 42.9 ppm. The multiplet at δ_H 3.02 (2H) was taken as indicative of a methylene group between two double bonds and is characteristic of an isopentenyl residue located at C-5. The multiple signals at δ_H 4.97 and 5.08, integrating for three hydrogens, were assigned to the isopentenyl vinyl hydrogens, and the signals at δ_H 4.66 and 4.71 which had a one bond C,H correlation (HETCOR)

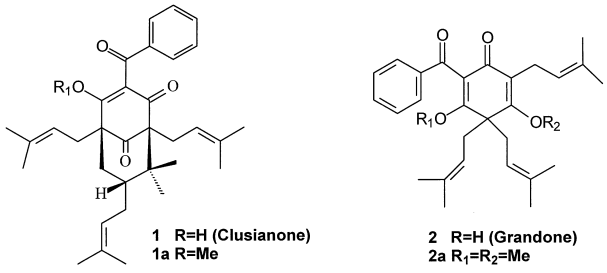

Fig. 3. Clusianone and Grandone and major methyl derivatives.

with the CH_2 at δ_C 111.9 (Table 1) were assigned to terminal double bond hydrogens. Signals at δ_H 7.47 (2H, *t*), 7.57 (1H, *tt*) and 7.95 (2H, *dd*) correlating with δ_C 128.9, 133.5, 129.3, respectively, (one bond) were consistent with the presence of a nonsubstituted benzoyl moiety. Analysis of the H,H–COSY spectrum provided spectral evidence for the above assignments and some of them will be discussed. Correlation of the allylic hydrogen H-29 (δ_H 3.02) with the methyl hydrogens (δ_H 1.65, H-33) and of the vinyl hydrogen H-30 (δ_H 5.08) with H-32 (δ_H 1.67) confirmed the above assignments. Similar correlations were observed between 4.97 (H-10) and 1.67 (H-12), 2.58 (H-17) and 4.97 (H-18), 1.72 (H-20). The absence of a carbonyl group at δ_C 205–209 in the ^{13}C NMR spectrum (characteristic of benzophenones derivatives possessing structure **II**) further characterized **3b** as a type **I** benzophenone. The full assignment of the carbon signals (Table 1) was obtained by using one and multiple bond 2D NMR C,H correlations (HETCOR and COLOC). The comparison of the carbon chemical

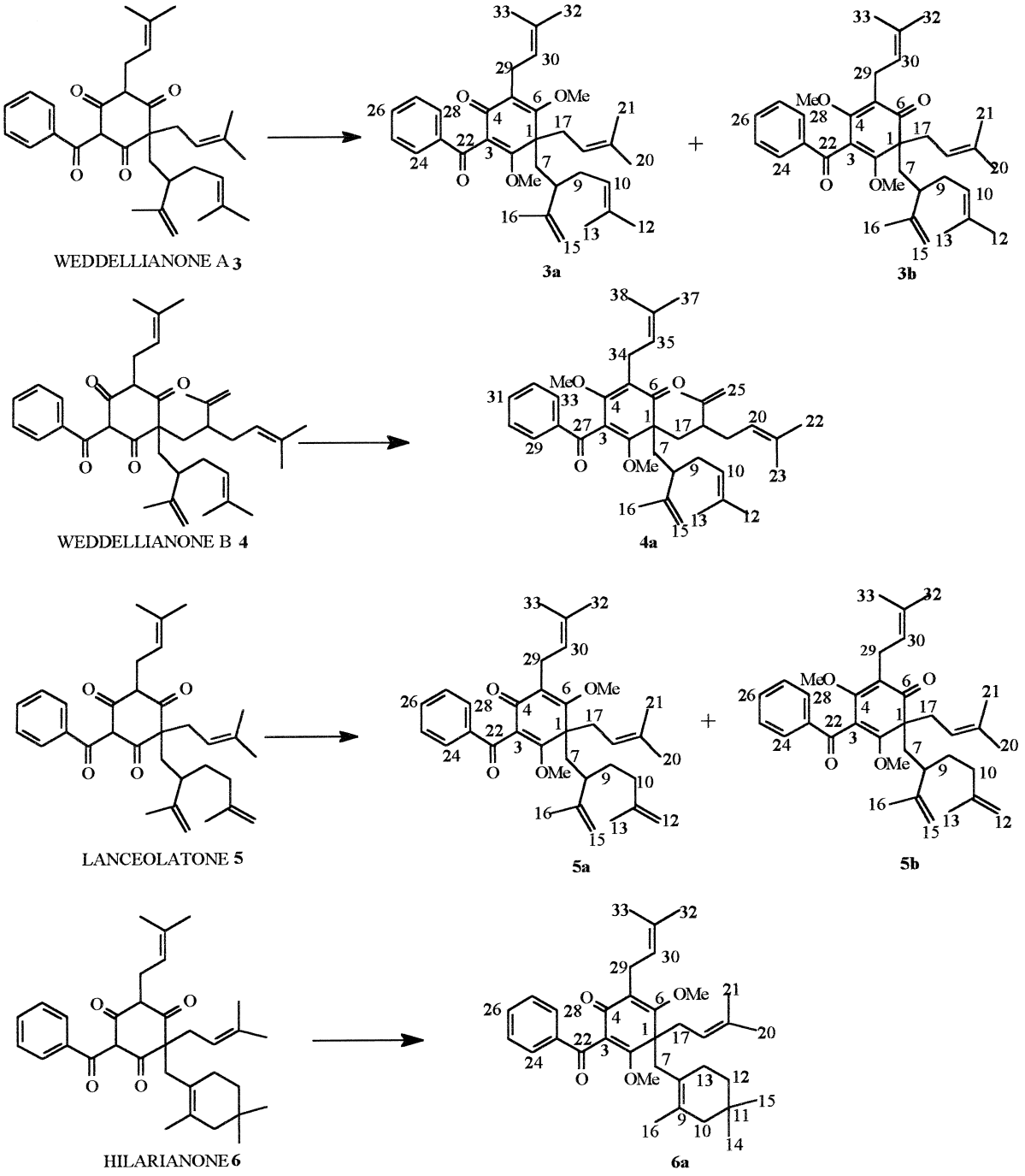

Fig. 4. Type **I** polyisoprenylated benzophenones isolated from *Clusia* floral resin.

shifts of **3b** with those of grandone showed that one of the substituents at carbon-1 was different in **3b**, while in **2a** both substituents were isopentenyl residues. Comparison of the carbon chemical shift of carbons 7 to 16 of **3b** with alkyl residues present in Guttiferones reported by Gustafson et al. (1992) revealed that a 10 carbon residue was attached to C-1. The high resolution mass spectrum showed a fragment at *m/z* 407.2132 which corresponds to the loss of a $C_{10}H_{17}$ radical from the molecular ion (absent). This compound was named weddellianone A (**3**).

Compound **4a** (Fig. 4) had a UV spectrum equal to that of **3b** (λ_{max} 254 and 350 nm) therefore possessing a similar chromophore. The high resolution mass spectrum depicted a molecular ion at *m/z* 598.3850 consistent with the molecular formula $C_{40}H_{54}O_4$, and by the addition of $C_{10}H_{17}$ (137 daltons) to the molecular mass of **3b**. The ^{1}H NMR spectrum was similar to that of **3b** except that signals corresponding to H-17, 17′ were missing. The ^{13}C NMR spectrum of **4a** had all sp^2 carbon chemical shifts rather similar to those of **3b**, but the signals at 118.6 and 134.9 ppm corresponding to C-18 and C-19 of **3b** were missing. On the one hand, signals at δ_c 122.7 (C-10), 132.0 (C-11), 147.9 (C-14) and 111.1 (C-15) ppm corresponded to two carbons each and had almost identical chemical shifts than those corresponding to the C-10, C-16 moiety of **3b** (Table 1). Therefore we proposed structure **4a** with two identical residues located at C-1. Finally long range C,H correlations in 2D NMR spectrum (COLOC) between carbon C-6 (δ_C 201.0 ppm)

Table 1
^{13}C shifts of compounds obtained by 1D and 2D NMR spectroscopy. The adopted numbering system is depicted in Figs. 4 and 5

C#	3a	3b	4a	5a	5b	6a	7a	8a	9a	9b
1	53.3	58.0	57.2	53.3	58.0	53.9	65.8	73.0	79.3	71.9
2	169.9	166.5	166.2	169.8	166.5	169.9	173.0	170.7	189.0	166.9
3	122.7	119.7	111.8	122.9	119.7	117.4	123.3	122.1	114.1	112.7
4	188.4	169.6	169.2	188.2	169.7	187.8	196.1	197.8	170.2	191.7
5	122.7	119.7	119.2	122.9	119.7	121.5	62.2	63.3	57.6	65.3
6	170.1	201.6	201.0	169.9	201.7	170.7	45.3	41.9	40.1	42.3
7	39.9	42.9	46.9	40.5	43.1	41.1	41.3	48.1	43.8	43.0
8	44.2	44.9	44.3	43.7	44.5	129.2	47.1	49.5	48.5	48.1
9	33.5	32.6	32.7	32.6	31.7	124.7	208.8	208.9	206.5	206.4
10	122.9	123.0	122.7	35.5	35.3	28.4	24.5	193.2	193.1	192.9
11	132.2	131.9	132.0	145.9	146.2	36.2	120.8	136.8	136.3	136.8
12	25.6	25.5	25.8	109.6	109.6	28.9	133.8	128.5	128.3	128.4
13	17.8	17.6	18.6	22.6	22.4	46.7	25.5	127.9	127.9	128.0
14	146.8	147.5	147.9	146.1	146.9	28.0	17.8	132.1	132.0	132.2
15	112.6	111.9	111.1	113.5	112.5	28.5	195.7	127.9	127.9	128.0
16	18.3	17.5	18.1	17.9	17.6	19.8	138.3	128.5	128.3	128.4
17	37.6	42.9	46.9	37.7	43.0	37.3	129.6	23.5	115.5	114.4
18	118.8	118.6	44.3	118.7	118.5	118.9	128.7	120.7	123.9	123.5
19	135.0	134.9	32.7	135.0	135.0	134.6	133.6	134.8	81.8	83.2
20	25.9	25.8	122.7	26.0	25.8	26.0	128.7	26.0	28.4	28.4
21	17.7	17.7	132.0	17.8	17.2	17.9	129.6	18.0	29.8	28.2
22	196.9	195.8	25.8	196.6	195.8	196.5	35.7	30.2	28.5	29.3
23	138.9	139.2	18.6	138.8	139.1	138.6	43.1	119.3	119.1	119.6
24	129.4	129.3	147.9	129.3	129.3	129.3	32.2	133.1	133.4	133.3
25	128.7	128.9	111.1	128.7	128.9	128.6	123.3	25.7	25.7	25.8
26	133.1	133.5	18.1	133.0	133.5	132.9	133.5	18.2	17.9	17.8
27	128.7	128.9	195.1	128.7	128.9	128.6	25.8	29.8	26.7	27.6
28	129.4	129.3	139.0	129.3	129.3	129.3	17.9	129.6	122.6	122.3
29	22.6	22.1	129.2	22.7	22.1	22.9	148.7	131.1	134.4	134.4
30	122.8	122.4	128.7	122.6	122.3	122.6	113.0	69.9	26.0	26.0
31	131.9	131.9	133.4	131.8	131.9	131.3	17.4	14.1	18.2	18.1
32	25.3	25.4	128.7	25.4	25.4	25.7	28.4	23.9	15.8ax	16.2ax
33	17.7	17.8	129.2	17.5	17.5	18.0	122.7	27.2	23.5eq	24.4eq
34	–	–	22.6	–	–	–	131.7	–	–	–
35	–	–	122.7	–	–	–	25.6	–	–	–
36	–	–	131.9	–	–	–	17.7	–	–	–
37	–	–	25.6	–	–	–	15.8ax	–	–	–
38	–	–	17.9	–	–	–	24.1eq	–	–	–
OMe	58.8	59.1	59.2	58.9	59.1	59.2	–	–	–	–
OMe	61.6	61.4	61.4	61.7	61.4	61.4	60.3	61.4	–	–
OAc	–	–	–	–	–	–	–	170.9	–	–
								21.0		

and hydrogens H-7 and H-17 (δ_H 2.03 ppm) and H-34 (δ_H 3.06 ppm) confirmed the proposed structure **4a**. The natural product from which **4a** was derived were named weddellianone B (**4**). Table 1 has the full assignment of all carbon signals. Curiously **4a** is optically inactive, while all other benzophenones are dextrorotatory. Therefore, based on the information above, we suggest that it is a meso compound, possessing the two stereogenic centers (C-8 and C-18) of opposite absolute configuration linked to C-1, a pseudoasymmetric center. Quantitative HPLC analysis of the crude methylated resin of *C. weddelliana* based on the calibration curve obtained with the standards **3b** and **4a** revealed that these compounds were present to 11.5% and 7% in the *C. weddelliana* floral resin (Table 2). The analysis also revealed that **3a** was present to about 8.5%, and as both **3a** and **3b** arise from the methylation reaction of **3** it was concluded that **3** is responsible for 20% of the chemical constituents of the floral resin of *C. weddelliana*.

Applying the above mentioned methodology to *C. lanceolata* floral resin, we isolated **3a, 5a** and **5b** (Fig. 4). Compound **3a** has a UV (λ_{max} 254 and 290 nm) spectrum identical to that of **2a** thus possessing the general structure **Ia**. Final structure **3a** was proposed by comparing its spectral data with those of **2a**, **3b** and **4a**. The ^{1}H NMR spectral features of **3a** and **3b** were similar, but in **3a**, the chemical shift differences between the two methoxy groups were larger than in **3b** and **4a** ($\Delta\delta_H$ **2a** = 0.35, **3a** = 0.48, **3b** = 0.08, **4a** = 0.03). Similarly, the $\Delta\delta_H$ of the two hydrogens belonging to the terminal bond H-15 and H-15′ is larger in **3a** (0.12 ppm) than in **3b** (0.05 ppm) and **4a** (0.05 ppm). Comparison of the carbon chemical shifts of **2a** and **3a** were almost identical but for the residue C7-C16 of **3a** which was similar to those of **3b**. The HRMS of **3a** and **3b** had similar fragmentation patterns. Structure **3b** was proposed, therefore **3a** and **3b** are isomeric methyl derivatives of the natural product, weddellianone A (**3**).

Compound **5a** had a UV spectrum identical to **3a**, and a similar ^{1}H and NMR spectrum except for a missing vinyl hydrogen on a trisubstituted double bond (4.91 ppm), and two extra signals at 4.67 and 4.69 ppm assigned to an additional terminal double bond. The ^{13}C NMR spectrum of **5a** and **3a** were similar except for the presence of two CH_2 functionalities in **5a** (35.5 and 109.6 ppm), and the absence of a CH_3 and of a vinyl CH. Full assignment of the carbon and hydrogen chemical shifts allowed the proposal of structure **5a** as a dextro-rotatory polyisoprenylated benzophenone which is derived from the novel natural product **5** named lance-olatone. As depicted in Fig. 1, additional isomeric methyl derivatives were expected and indeed compound **5b** possessing the general structure **Ib** (Fig. 1) was also isolated, and spectral differences between **5a** and **5b** were analogous to those observed between **3a** and **3b** previously discussed.

Quantitation (w/w) of **3a**, **3b**, **5a** and **5b** in the methylated *C. lanceolata* floral resin revealed that **3** and **5** are responsible for 31% of the weight of the total floral resin (Table 2).

From the methylated *C. hilariana* floral resin, we isolated the methyl derivatives **6a**, **10a** and **11a** (Fig. 5). Compounds **10a** and **11a** have already been described as floral resin components of *C. grandiflora* and *C. rosea* (Oliveira et al., 1999). The UV spectrum of **6a**, was identical to those of **2a**, **3a** and **5a**, therefore the general structure **Ia** was assumed. ^{1}H,^{1}H correlations were

Table 2
RP-HPLC quantification of the benzophenone methyl derivatives of derivatized *Clusia* floral resins

Floral resins	Section	General structure [Compound (HPLC quantification %)][c]
C. burchellii male	Cordylandra	**I** [**3a/5a** (5.0), **3b/5b** (17.2)]; **II** [**7a** (2.7), **1a**[b] (54.4)]
C. fluminensis male	Cordylandra	**I** [**3a/5a** (2.7), **3b/5b** (4.0)]; **II** [**7a** (10.0), **1a**[b] (37.0)]
C. grandiflora female	Chlamydoclusia[a]	**I** (1.0), **II** (70.0)
C. grandiflora male	Chlamydoclusia[a]	**I** (6.0), **II** (15.0)
C. hilariana (red) male	Phloianthera	**I** [**3a/5a** (10.3), **3b/5b** (1.0)]
C. insignis male	Chlamydoclusia[a]	**II** (27.4)
C. lanceolata male	Phloianthera	**I** [**3a/5a** (10.7), **5b** (20.4)]; **II** [**1a**[b] (5.8)]
C. nemorosa hermaphrodite	Chlamydoclusia[a]	**II** (40.5)
C. nemorosa male	Chlamydoclusia[a]	**II** (7.2)
C. pana-panari female	Cordylandra	**I** [**3a/5a** (1.0), **4a** (2.4)]; **II** [**7a** (6.9), **1a**[b] (63.7)]
C. paralicola male	Cordylandra	**I** [**3a/5a** (0.5), **3b/5b** (6.3), **4a** (4.9)]; **II** [**1a**[b] (74.6)]
C. pernambucensis male	Cordylandra	**I** [**3a/5a** (2.7), **3b/5b** (4.0)]; **II** [**7a** (10.0), **1a**[b] (37.0)]
C. rosea female	Chlamydoclusia[a]	**II** (36.0)
C. spiritu-sanctensis female	Cordylandra	**II** [**7a** (1.0), **1a**[b] (78.6)]
C. spiritu-sanctensis male	Cordylandra	**II** [**7a** (16.9), **1a**[b] (76.8)]
C. weddelliana male	Cordylandra	**I** [**3a** (8.5), **3b** (11.5), **4a** (7.0)]; **II** [**7a** (6.5), **1a**[b] (56.5)]
Bees nest	–	**II** [**1a**[b] (25.4)]

[a] Oliveira et al. (1999).
[b] Oliveira et al. (1996).
[c] **I** and **II** refers to general structures depicted in Figs. 1 and 2.

applied to the analysis of **6a** which showed unexpected spectral features like two shielded methyl groups (δ_H 0.85 and 0.82 ppm, usually characteristic of compounds possessing structure **II**) and two methoxy groups (δ_H 3.56 and 3.96 characteristic of the methyl derivatives of compounds possessing structure **Ia**).

One (HSQC) and multiple bond (HMBC) $^{13}C,^{1}H$ correlations between the hydrogens signals at δ_H 3.05, 3.17 (H-29, H-29′) and 2.59, 2.71 (H-17, H-17′) and carbon signals at δ_C 187.8 (C-4) and 53.9 (C-1), respectively, were evidence of an isopentenyl residue at C-5. Further correlations were observed between δ_H 2.77 (H-7) and δ_C 53.9 (C-1) and 129.2 (C-8) linking the cyclohexenyl moiety (from C-8 to C-16) to C-1. An intramolecular cyclization of the "ene" type of precursors like **3** or **4** can explain the origin of the third ring in **6a**.

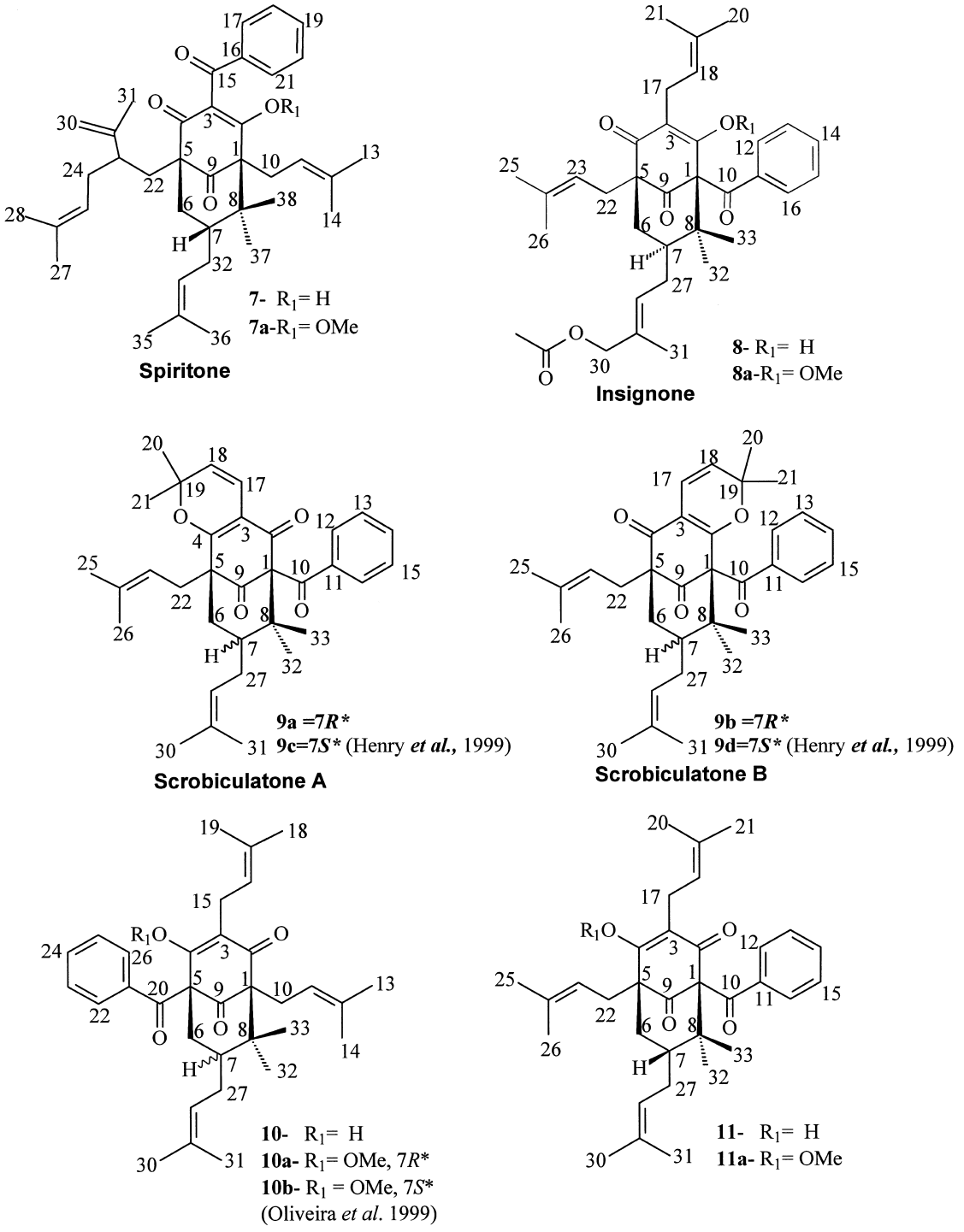

Fig. 5. Type **II** polyisoprenylated benzophenones isolated from *Clusia* floral resins.

From the methylated crude *C. spiritu-sanctensis* floral resin, we isolated **1a** and **7a** (Fig. 5). The UV (λ_{max} 254 nm) spectrum of **7a** was characteristic of type **IIa** or **IIb** methylated benzophenones (R_3 = benzoyl) and was similar to that of Clusianone (**1a**). The HRMS had a molecular ion at *m/z* 584.3866 corresponding to the molecular formula $C_{39}H_{52}O_4$ and a fragment at *m/z* 447.2623 correponding to the loss of a $C_{10}H_{17}$ ion from the molecular ion, indicating the presence of a C_{10} isoprenyl residue. Analysis of the ^{1}H NMR spectrum revealed the presence of two angular methyl groups (0.79 and 1.14 ppm) and one methoxy group (3.59 ppm), typical of type **II** benzophenone methyl derivatives. Signals at δ_H 4.55 (CH_2), 4.93 (CH) and 5.00 (2×CH) revealed the presence of five vinyl hydrogens. The AA′XX′Y spin system (7.44–7.90 ppm) revealed the presence of the unsubstituted benzoyl residue. Analysis of the ^{13}C NMR and H,C (HETCOR) and H,H (COSY) correlations confirmed the presence of a terminal double bond (113.0 ppm CH_2), of the 7*R** or *exo* relative configuration (CH δ_H 1.96, δ_C 41.3). Most signals had almost identical chemical shifts to those clusianone except for 10 carbons which had chemical shifts almost identical to the C_{10} residue of weddellianone (**3a** or **3b**). The relative position of all residues was obtained via differential NOE experiments and irradiation of the CH_3 (C-38) equatorial-like methyl group (though linked to a bicyclononanone moiety, this term is used in analogy to cyclohexane substituents) gave significant enhancement of the signals at δ_H 3.59 (OMe), 2.52 (H-10), 2.10 (H-32) tethering these hydrogens and consequently their carbons to the Me-38 neighborhood. Therefore structure **7a** was proposed, this being the methyl derivative of the novel natural product **7**, named spiritone. Quantitative HPLC experiments based on a calibration curve constructed with standard **7a** revealed that at least 1% (w/w) of the crude methylated resin is composed of **7a**.

Methylation of *C. insignis* floral resin followed by several separation methods, produced type **II** benzophenone **8a** and **10b**. The UV spectrum of **8a** (λ_{max} 245 and 278 nm) was characteristic of type **IIa** or **IIb** methylated benzophenones (R_3 = isoprenyl). The HRMS had a molecular ion at *m/z* 574.3298 corresponding to the molecular formula $C_{36}H_{46}O_6$ and a fragment at *m/z* 505.2523 corresponding to the loss of a C_5H_9 ion from the molecular ion, a clue of the presence of a C_5 isoprenyl residue. Analysis of the ^{1}H NMR spectrum revealed the presence of two angular methyl groups (δ_H 1.39 and 1.49), a methoxy group (δ_H 3.50 ppm), and a AA′XX′Y spin system of an unsubstituted benzoyl moiety (δ_H 7.29–7.60) characteristic of type **II** benzophenones. The simultaneous comparison of the ^{1}H and ^{13}C NMR spectral data revealed the presence of a CH_3COOCH_2 (δ_H 2.07, 4.42 and δ_C 21.0, 69.9, 170.0), and the absence of a vinyl methyl group, considering that three isoprenyl groups were expected. Homonuclear and heteronuclear correlations observed in the 2D NMR spectra (gCOSY, HSQC and gHMBC) were of major importance to assign the relative positions of one benzoyl, two C_5H_9 and one $C_5H_8OOCCH_3$ residues. The methylene attached to the acetoxy group (δ_C 69.9) had H,C long distance heteronuclear correlations with the vinyl methyl at δ_H 1.59 and a vinyl hydrogen at δ_H 5.20. The latter had a H,H homonuclear correlation with a CH_2 2.05 and 2.34, H-27, 27′). Finally the signal at δ_H 1.49 (H-7) has homonuclear correlations with the signals at δ_H 2.05 (H-27′ and H-6), 2.19 (H-6′) and 2.34 (H-27). It was therefore concluded that the acetoxy bearing isoprenyl group was tethered to C-7 and furthermore the chemical shifts of CH-7 (δ_H 1.49, δ_C 48.1) are consistent with a 7*S** or *endo* configuration. Finally the three bond C,H correlations (gHMBC) between the carbonyl group at δ_H 197.8 (C-4) and δ_H 3.30 (H-17), 3.14 (H-17′), 2.50 (H-22), 2.19 (H-6) and 2.05 (H-6′) established the final structure of **8a**, which is derived from the natural product **8** insignone.

C. scrobiculata floral resin is physically and chemically unusual. First it is black in contrast with the yellowish or orange colored resins of the other species, second it was not altered by methylation. Consequently the resin's components were separated without diazomethane treatment. Silica column chromatography and preparative TLC with 5% $AgNO_3$ were unsuccessful for the isolation of the **9a** and **9b** due to the rapid equilibration between the two isomeric forms. They were thus analyzed as a mixture. The UV spectrum showed absorption bands at λ_{max} 250 and 324 nm which were slightly different from benzophenones type **I** and **II**. The HRMS showed a molecular ion at *m/z* 500.2926 corresponding to a molecular formula $C_{33}H_{40}O_4$. Analysis of the ^{1}H NMR spectrum revealed the presence of eight methyl groups which were more shielded (0.60, 1.14, 1.21, 1.35, 1.39, 1.40 (2Me), 1.42) than those attached to double bonds. Two sets of doublets (J = 10.2 Hz) at 5.21, 5.39, 6.45 and 6.48 were assigned to a 2,2-dimethylpyran moiety by analogy with the data reported for a compound with similar characteristics isolated from the fruits of *C. plukenetii* by Henry et al. (1999). The *R** relative configuration at C-7 was established based on the discussions above (δ_C 43.8 for **9a** and 43.0 for **9b**; δ_H 1.70 for **9a** and **9b**). A comparison of our data with those in the above mentioned literature revealed that the compound isolated by Henry et al. (1999) is the 7*S** diastereomer (δ_C 48.5 for **9c** and 48.2 for **9d**; δ_H 1.50 for **9c** and 1.52 for **9d**).

It is worth mentioning that from the Cuban propolis, Rubio et al. (1999) have isolated a polyisoprenylated compound related to **9a** with a dihydro 2,2-dimethylpyran moiety, which showed antimicrobial and fungicidal activities.

Table 2 depicts the quantification by RP-HPLC of the methyl derivatives of the **3**, **4**, **5**, **7** and clusianone **1** in crude *Clusia* floral resins treated with diazomethane (*C. burchellii* male, *C. fluminensis* male, *C. hilariana* (red) male, *C. lanceolata* male, *C. panapanari* female, *C. paralicola* male, *C. pernambucensis* male, *C. spiritu-sanctensis* female, *C. spiritu-sanctensis* male, *C. weddelliana* male). The calibration curves were constructed with the above mentioned standards (Johnson and Stevenson, 1978) following a pre-established protocol (Oliveira et al., 1999). These data complement our previous observations that polyisoprenylated benzophenones are major components of *Clusia* floral resins and these additional data allowed conclusions concerning the chemistry of the floral resins in different *Clusia* sections. In the section Chlamydoclusia, the floral resins have type **II** benzophenones as major constituents and type **I** as minor components. The isoprenyl substituents in type **I** or **II** benzophenones are mainly composed of five carbons. In section Cordylandra, there is a predominance of compounds type **II** and among them clusianone (**1**) (ca 30–70%) is the major component, compounds type **I** are present in 3–20%. Section Phloianthera floral resins have compounds type **I** and **II** in almost equal amounts with at least one of the isoprenyl substituents possessing 10 carbon atoms and clusianone (**1**) is either absent or present in less than 10%. The section Polythecandra floral resins seem to resemble those of the section Chlamydoclusia (absence of clusianone (**1**) and with short isoprenyl substituents); however, only compounds possessing the general structure **II** were isolated and possess an additional ring, a pyran unit.

Approaching the question of the bees' nest construction, we located a *Trigona spinipes* bees' nest close to various *Clusia* plants, the floral resin of which the bees were observed to collect. Part of the nest was collected and extracted with chloroform. The crude nest extract was methylated and analyzed by GC/MS and by RP-HPLC (diode array). The GC/MS analysis revealed the presence of triterpenes, methyl derivatives of free fatty acids and methyl polyisoprenylated benzophenones (*m*/*z* at 69, 105, 283, 323 and 339). However, GC/MS is not appropriate for the analysis of methyl polyisoprenylated benzophenones due to their high molecular weight and also some undergo decomposition (Table 3). Methyl clusianone (**1a**) was identified and confirmed as one of

Table 3
CG/MS analysis of methylated resins and methylated stamen oils[a]

Species of *Clusia*	Chemical composition
C. burchellii[1,5]	Hexadecanoic methyl ester (K.I. 1927), octadecanoic methyl ester (K.I. 2124), octadecenoic methyl ester, 4-methyl benzenesulfonic methyl ester
C. grandiflora[a,1]	9-Hexadecanoic methyl ester, octadecanoic methyl ester, 5,8,11,14-icosatetrataenoic methyl ester
C. grandiflora[b,1]	9-Hexadecanoic methyl ester, octadecanoic methyl ester, 5,8,11,14-icosatetrataenoic methyl ester
C. hilariana[b](red)[1,5]	Benzoic methyl ester, tetradecanoic methyl ester (K.I. 1724), hexadecanoic methyl ester, 16-methyl heptadecanoic methyl ester, eicosanoic methyl ester, 9-octadecanoic methyl ester (K.I. 2104), octadecanoic methyl ester
C. lanceolata[b,1,5]	Benzoic methyl ester, tetradecanoic methyl ester, hexadecanoic methyl ester, 16-methyl heptadecanoic methyl ester, eicosanoic methyl ester, 9-octadecanoic methyl ester, octadecanoic methyl ester
C. nemorosa[c,1]	9-Hexadecanoic methyl ester, 9,12-octadecadienoic methyl ester
C. renggerioides[b,1,2,3]	Hexadecanoic methyl ester, 9-hexadecanoic methyl ester (K.I. 1753), 9-octadecanoic methyl ester
*C. renggerioides**[,b,4,5]	Hexadecanoic methyl ester, hexadecanoic methyl ester, 9-octadecanoic methyl ester
*C. renggerioides***[,b,4,5]	Hexadecanoic methyl ester, hexadecanoic methyl ester
C. rosea[a,1]	Octadecanoic methyl ester
C. spiritu-sanctensis[b,1,2]	Hexadecanoic methyl ester, octadecanoic methyl ester, 9-octadecanoic methyl ester
C. spiritu-sanctensis[b,1,5]	Benzoic methyl ester, 1,4-dimethoxy benzene, 4-methyl benzenesulfonic methyl ester, tetradecanoic methyl ester, hexadecanoic methyl ester, 9-hexadecanoic methyl ester, 9,12-octadecadienoic methyl ester, 9-octadecanoic methyl ester, octadecanoic methyl ester, pentacosane, hexacosane, eicosane
C. weddelliana[b,1,5]	Acid benzoic methyl ester, hexadecanoic methyl ester, octadecanoic methyl ester, 9-octadecenoic methyl ester
Trigona nest extract (methylated)	Octadecanoic methyl ester, triterpenes, polyisoprenylated benzophenones methyl derivatives

[a] Though detected as methyl ester derivatives the free fatty acids were first observed by TLC and by GC/MS, derivatization was a means to facilitate the GC/MS analysis [1]collected in Fazenda Santa Elisa (Instituto Agronômico de Campinas), Campinas, SP/Brazil; [2]collected capillary glass tubes; [3]collected with small filter paper; [4]collected in Amazonas/Brazil; [5]resins and oils staminal mixtured; *with pistillodium; **without pistillodium, a = female, b = male, c = hermaphrodite, K. I. = Kovats Index on DB-5 (Adams, 1995).

Table 4
Bioautography of floral resins *Clusia* and bees nest

Species of *Clusia*	*A. niger*	*B. subtilis*	*C. albicans*	*E. coli*	*R. equi*	*S. aureus*
C. grandiflora female	×	+	×	×	×	+
C. grandiflora male	×	+	+	×	×	+
C. insignis male	×	+	×	×	×	+
C. lanceolata male	–	+	+	×	×	+
C. nemorosa hermafrodite	×	+	×	×	×	+
C. renggerioides male	–	+	×	–	–	+
C. spiritu-sanctensis male	×	+	×	–	–	+
C. spiritu-sanctensis male[a]	×	–	×	–	–	–
C. weddelliana male	–	+	×	–	×	+
Clusianone methylated	–	–	×	–	–	–
Bees nest	–	+	+	×	–	+

[a] Methylated; (×) = not tested; (+) positive; (–) negative.

the major benzophenones present in the nest crude extract by RP-HPLC analysis and by co-injection with an authentic standard. We thus prove that floral resins are indeed part of the nest of these Trigona bees (Table 2).

Our survey for bioactivity of all methylated and non methylated resins and of the bees' nest extract using bioautography (Betina, 1973) revealed that the non-methylated polyisoprenylated benzophenones are largely responsible for the antimicrobial activity of the pure floral resins and of the nest extract. The micro-organisms used in the bioautography tests were: *Aspergillus niger* CCT 1435, *Bacillus subtilis* CCT 0089, *Candida albicans* CCT 0776, *Escherichia coli* CCT 5050, *Rhodococcus equi* CCT 0541 and *Staphylococcus aureus* CCT 4295. The results are shown in Table 4. Using the agar diffusion bioassay, Lokvam and Braddock (1999) have concluded that the female resin of *C. grandiflora* is more active than the male resin. Our data complement this observation by assigning the activity to polyisoprenylated benzophenones which are major components of the floral resins of *C. grandiflora* female (70% of type **II** and 1% type **I** polyisoprenylated benzophenones) and *C. grandiflora* male (15% of type **II** and 6% type **I** polyisoprenylated benzophenones) (Oliveira et al., 1999), as well as *C. spiritu-sanctensis* female (78.6% of **1** (Oliveira et al., 1996) and 16.9% for **7**) and *C. spiritu-sanctensis* male (76.8% of **1** and 1.0% of **7**, Table 2). One should be aware that quantification was performed on the methyl floral resins and with methylated isolated standards and that minor methyl derivatives were not taken into consideration. We further conclude that the more pronounced anti-microbial activity of the female resins is due to concentration differences in male and female resins (of equal species) of the same active components. The latter was visualized in the bioautography tests. It is also easy to understand that the female resins (e.g. *C. grandiflora* (Oliveira et al., 1999) and *C. spiritu-sanctensis* possessing such a high concentration of polyisoprenylated benzophenones, once removed from the flower, crystallize after 30 min. This phenomenon was first observed by Lokvam and Braddock (1999) for the female resin of *C. grandiflora*.

TLC and GC/MS analysis of recently collected pure staminal oil revealed the presence of free fatty acids and methylation was the derivatization of our choice. Therefore GC/MS analysis [*C. burchellii* male, *C. grandiflora* female, *C. grandiflora* male, *C. hilariana* (red) male, *C. lanceolata* male, *C. nemorosa* hermaphrodite, *C. renggerioides* male with pistillodium (collected in Central Amazonia/Brazil and Fazenda Santa Elisa/IAC-Campinas-SP/Brazil), *C. renggerioides* male without pistillodium (collected in Central Amazonia/Brazil), *C. rosea, C. spiritu-sanctensis* female, *C. weddelliana* male] of the methylated resins and methylated staminal oils (which were collected with small filter paper or capillary glass tubes, Table 3) confirmed the presence of fatty acids, detected as methyl esters, and some other components. The simultaneous analysis of the polyisoprenylated benzophenones is not feasible due to decomposition under the analytical conditions.

3. Conclusions

We have concluded the following about the *Clusia* floral resins and staminal oils: female and male floral resin chemistry does not diverge in the chemical structure of major components like fatty acids and polyisoprenylated benzophenones. Differences arise from minor components and from the ratio among major components, i.e. the chemical identity is maintained while the ratio may change from the male to female resins of the same species. From a resin chemistry point of view, it is up to now, possible to identify the section to which a species by scrutinizing whether the polyisoprenylated benzophenones present in the floral resins belongs to type **I** or **II**.

The staminal oil (secreted separately from the resin) is typical for sect. Chlamydoclusia, but is chemically different from those oils mixed with the floral resins. The oils mixed with the resin and that are responsible for making the resin less viscous are not characteristic for a specific section. Finally, we are aware that quantification of the fatty acids associated with the quantification of the benzophenones could solve the problem of the physical differences between male and female floral resins but this analysis is not available for the time being.

4. Experimental

4.1. General

FT-IR spectra were recorded with a Perkin–Elmer 298 spectrophotometer. ^{1}H NMR spectra were recorded with a Varian GEMINI 300 (300.1 MHz, Varian), Bruker AC 300P (300.1 MHz) or Varian INOVA (500 MHz) spectrometers. $CDCl_3$ was used as the solvent, with Me_4Si (TMS) as internal standard. ^{13}C NMR spectra were obtained with a Varian GEMINI 300 (75.5 MHz), a Bruker AC 300P (75.5 MHz) or a Varian INOVA 500 (125 MHz) spectrometers. $CDCl_3$ (77.0 ppm) was used as internal standard. Methyl, methylene, methine and carbon nonbonded to hydrogen were discriminated using DEPT-135° and DEPT-90° spectra (Distortionless Enhancement by Polarization Transfer); 2D NMR spectroscopy was performed with standard H,H correlation and H,C correlation pulse sequences (either HETCOR or HSQC for one bond correlation and long range HETCOR and HMBC for long range correlations) available in the spectrometers. The splitting of spin systems with second order perturbations is reported as J^* values. Optical rotation values were measured with a Polamat A polarimeter in a 0.1 dm cuvette. GC/MS analyses were carried out using a HP-5890/5970 system equipped with J.&W. Scientific DB-5 fused silica capillary column (25 m×0.2 mm×0.33 μm). Retention indexes were obtained by co-injecting the oil, and the standards with a C_{11}–C_{30} normal hydrocarbon mixture and applying the appropriate equation (Roubik, 1989). HRMS were carried out using a Micromass VG Auto Spec. spectrometer operating at 70 eV.

4.2. HPLC analyses and quantification

HPLC analyses were carried out using a HP system, SERIES II 1090 and UV diode array detector working at 254 nm, equipped with a NOVAPAK C-18 (Waters) column (3.9×150 mm, 4 μm, 60 Å). The best solvent system for the separation of the standards was an elution gradient from acetonitrile:water (60:40) to acetonitrile during 60 min (1 ml/min) at 40°C and 5 min of pure acetonitrile before restarting the cycle. The samples were filtered through a Millipore (MILLEX SR) filter (0.5 μm). Samples of 10 μl were injected, from about 10 mg of compound in 10 ml of acetonitrile. The concentration of compounds in the methylated floral resins was determined by comparing the area under each peak relative to standard curves generated by injection of standards **3a**, **3b**, **4a**, **4b**, **5a**, **7a**, **1a** (Oliveira et al., 1996) of known concentration. The detector response was linear in the operating concentration range.

4.3. Plant material

Most of the plants were cultivated at the "Fazenda Santa Elisa", Instituto Agronômico de Campinas (IAC), Campinas, SP, Brazil, and the floral resins were collected by scraping the viscous resins with small glass rods which were then placed into vials containing organic solvents (Et_2O or EtOAc). Voucher specimens were deposited at the Universidade Estadual de Campinas (UEC) Herbarium with voucher numbers M.C.E. Amaral & V. Bittrich; V. Bittrich is responsible for identifications. *C. burchellii* male (#97/3 masc.), *C. fluminensis* male (#97/249 masc.), *C. grandiflora* female (#95/153 fem.), *C. grandiflora* male (#95/152 masc.), *C. hilariana* (red) male (#97/248 masc.), *C. insignis* female, *C. insignis* male, *C. lanceolata* male (#96/27 masc.), *C. nemorosa* hermaphrodite (#95/150 herm.), *C. nemorosa* male (#95/151 masc.), *C. panapanari* female (#95/156 fem.), *C. paralicola* male (#97/5 masc.), *C. pernambucensis* male (#95/186 masc.), *C. renggerioides* male (#97/1 masc.), *C. renggerioides* male (without pistillodium) (#91/28a masc.), *C. rosea* female (#95/154 fem.), *C. scrobiculata* (Ribeiro, J.E.L.S. #1838, hermaphrodite), *C. spiritu-sanctensis* female (#95/185a fem.), *C. spiritu-sanctensis* male (#95/185 masc.), *C. weddelliana* male (#97/4 masc.).

4.3.1. General isolation procedure

Fresh resins of *C. hilariana* (white) male [137 mg, **6a** (8.0%)], *C. insignis* male [229 mg, **8a** (17%)], *C. lanceolata* male [300 mg, **3a** (3.9%); **5b** (3.9%); **5a** (4.2%)], *C. scrobiculata* [53 mg, **9a** and **9b** (51%), the methylation step was excluded for this particular resin], *C. spiritu-sanctensis* male [837 mg, **1a** (67.6%); **7b** (1.0%)], *C. weddelliana* male [360 mg, **1a** (34.8%), **5b** (4.6%), **6a** (7.0%)] were treated with diazomethane in Et_2O (excess). Reaction mixtures were kept at room temperature (in a safety hood) for the slow evaporation of residual diazomethane and the remaining solvent (free of diazomethane) was removed at reduced pressure. Residues were subjected to silica gel column chromatography, eluted with hexane–EtOAc or a hexane–Et_2O mixture, with increasing amounts of EtOAc (0–100%). Combined fractions were further purified by silica gel/silver nitrate (5%) preparative TLC eluted with benzene:EtOAc (5%).

*4.4. Dimethyl weddellianone A: 2-benzoyl-3,5-dimethoxy-4,6-bis(3-methylbut-2-enyl)-4-[5-methyl-2-(1-methylvinyl) hexa-4-enyl]cyclohexa-2,5-dien-1-one (**3a**)*

Yellow oil, IR (film NaCl) ν_{max} cm^{-1}: 2978, 2884, 2940, 1722, 1672, 1449, 1375, UV (MeCN) λ_{max} nm: 254 and 290, HRMS *m/z* (rel. int.): $C_{35}H_{46}O_4$, 530.3384 (M^{+}•-absent), found 407.2132 [M^{+}•$-C_9H_{15}=C_{26}H_{31}O_4$ requires 407.2214]$^{+}$ (4), 355.1554 (33), 353.1408 [M^{+}•-C_9H_{15}-C_4H_6]$^{+}$ (22), 339.1595 [M^{+}•-$C_{10}H_{16}$-C_4H_7]$^{+}$ (49), 337.1436 [M^{+}•-$C_{10}H_{17}$-C_4H_7-H]$^{+}$ (37), 335.1302 (20.5), 323.1286 [M^{+}•-C_9H_{15}-C_5H_9-CH_3]$^{+}$ (7), 313.1090 (21), 295.0987 (36), 105.0358 [C_7H_5O]$^{+}$ (100), 91.0563 [C_7H_7]$^{+}$ (22), 77.0393 [C_6H_5]$^{+}$ (34), 69.0702 [C_5H_9]$^{+}$ (28), ^{1}H NMR (300 MHz, $CDCl_3$/TMS): δ 1.58 (3H, *s*, H-21), 1.60 (3H, *s*, H-13), 1.62 (3H, *s*, H-16), 1.64 (6H, *s*, H-32 and H-33), 1.69 (3H, *s*, H-12), 1.76 (3H, *s*, H-20), 1.98–2.00 (3H, *bs*, H-8 and H-9), 2.12 (2H, *m*, H-7), 2.49 (1H, *dd, J* = 15.0 and 5.1 Hz, H-17), 2.69 (1H, *dd, J* = 15.0 and 9.0 Hz, H-17), 3.12 (1H, *dd, J* = 15.0 and 6.2 Hz, H-29), 3.23 (1H, *dd, J* = 15.0 and 6.2 Hz, H-29), 3.50 (3H, *s*, OMe), 3.98 (3H, *s*, OMe), 4.67 (1H, *bs*, H-15), 4.79 (1H, *bs*, H-15), 5.00 (2H, *m*, H-10 and H-18), 5.10 (1H, *bt, J* = 6.0 Hz, H-29), 7.41 (2H, *t, J* = 7.3 Hz, H-25 and H-27), 7.51 (1H, *tt, J* = 7.3 and 1.5 Hz, H-26), 7.89 (2H, *dd, J* = 7.3 and 1.5 Hz, H-24 and H-28). The assignments follow the numbering system adopted in Fig. 4.

*4.5. Dimethyl weddellianone A: 4-benzoyl-3,5-dimethoxy-2,6-bis(3-methylbut-2-enyl)-6-[5-methyl-2-(1-methylvinyl) hexa-4-enyl]cyclohexa-2,4-dien-1-one (**3b**)*

Yellow oil, IR (film NaCl) ν_{max} cm^{-1}: 2967, 2925, 2886, 1722, 1671, 1639, 1560, 1449, 1275, UV (MeCN) λ_{max} nm: 254 and 350, HRMS *m/z* (rel. int.): $C_{35}H_{46}O_4$, 530.3384 (M^{+}•-absent), found 407.2198 [(M^{+}•-C_9H_{15} = $C_{26}H_{31}O_4$ requires 407.2214]$^{+}$ (5.4), 355.1555 (35), 353.1434 (100), 339.1628 [M^{+}•-$C_{10}H_{16}$-C_4H_7]$^{+}$ (17), 337.1453 [M^{+}•-$C_{10}H_{17}$-C_4H_7-H]$^{+}$ (9), 335.1281 (24), 295.1004 (47), 283.0984 (31), 149.0275 (50), 105.0355 [C_7H_5O]$^{+}$ (92.5), 91.0554 [C_7H_7]$^{+}$ (26), 77.0386 [C_6H_5]$^{+}$ (47), 69.0691 [C_5H_9]$^{+}$ (43), 57.0697 (42), 55.0537 [C_4H_7]$^{+}$ (30), ^{1}H NMR (300 MHz, $CDCl_3$/TMS): δ 1.56 (3H, *s*, H-13), 1.60 (3H, *s*, H-21), 1.61 (3H, *s*, H-16), 1.65 (3H, *s*, H-33), 1.67 (6H, *s*, H-12 and H-32), 1.72 (3H, *s*, H-20), 1.90 (2H, *m*, H-9), 2.01 (1H, *m*, H-8), 2.06 (1H, *m*, H-7), 2.15 (1H, *m*, H-7), 2.50 (1H, *dd, J* = 15.0 and 7.5 Hz, H-17), 2.58 (1H, *dd, J* = 15.0 and 9.0 Hz, H-17), 3.02 (2H, *m*, H-29), 3.46 (3H, *s*, OMe), 3.54 (3H, *s*, OMe), 4.66 (1H, *m*, H-15), 4.71 (1H, *m*, H-15), 4.97 (2H, *m*, H-10 and H-18), 5.08 (1H, *m*, H-30), 7.47 (2H, *t, J* = 7.7 Hz, H-25 and H-27), 7.57 (1H, *tt, J* = 7.3 and 1.5 Hz, H-26), 7.95 (2H, *dd, J* = 8.0 and 1.5 Hz, H-24 and H-28). The assignments follow the numbering system adopted in Fig. 4.

*4.6. Dimethyl weddellianone B: (*meso*)-4-benzoyl-3,5-dimethoxy-2-(3-methylbut-2-enyl)-6,6-bis[5-methyl-2-(1-methylvinyl)hexa-4-enyl]cyclohexa-2,4-dien-1-one (**4a**)*

Yellow oil, $[\alpha]_D^{20} = 0$ (*meso*), IR (film NaCl) ν_{max} cm^{-1}: 2967, 2927, 2844, 1667, 1641, 1560, 1449, 1376, 1276, 1212, 1092, 733, UV (MeCN) λ_{max} nm: 254 and 350, HRMS *m/z* (rel. int.): $C_{40}H_{54}O_4$, found 598.3850 M^{+}• (4) (requires 598.4008), 462.2664 [M^{+}•-$C_{10}H_{16}$]$^{+}$ (13), 461.2611 (M^{+}•-$C_{10}H_{15}$) (14.4), 339.1600 [M^{+}•-$C_{10}H_{16}$-C_9H_{15}]$^{+}$ (100), 340.1634 (23), 327.1434(32), 283.0986 (37), 105.0384 (44), 91.0576(13), 77.0409 (11), 69.0730 [C_5H_9]$^{+}$ (32), ^{1}H NMR (300 MHz, $CDCl_3$/TMS): δ 1.55 (6H, *s*, H-13, H-23) 1.62 (6H, *s*, H-16 and H-24), 1.64 (3H, *s*, H-38), 1.69 (9H, *s*, H-12, H-22 and H-37), 1.91 (4H, *bs*, H-9 and H-19), 2.03 (6H, *bs*, H-7, H-8, H-17 and H-18), 3.06 (2H, *d, J* = 6.5 Hz, H-34), 3.50 (3H, *s*, OMe), 3.53 (3H, *s*, OMe), 4.66 (2H, *s*, H-15 and H-25), 4.71 (2H, *s*, H-5 and H-25), 4.96 (2H, *t, J* = 6.5 Hz, H-10 and H-20), 5.13 (1H, *bt, J* = 6.5 Hz, H-35), 7.45 (2H, *t, J* = 7.8 Hz, H-30 and H-32), 7.56 (1H, *t, J* = 7.3 Hz, H-31), 7.94 (2H, *dd, J* = 7.4 and 1.2 Hz, H-29 and H-33). The assignments follow the numbering system adopted in Fig. 4.

*4.7. Dimethyl lanceolatone: 2-benzoyl-3,5-dimethoxy-4,6-bis(3-methylbut-2-enyl)-4-[5-methyl-2-(1-methylvinyl) hexa-5-enyl]cyclohexa-2,5-dien-1-one (**5a**)*

Yellow oil, $[\alpha]_D^{20}$ + 45.5° ($CHCl_3$, c. 1.03), IR (film NaCl) ν_{max} cm^{-1}: 3070, 2967, 2935, 2884, 1673, 1646, 1598, 1440, 1364, 737, UV (MeCN) λ_{max} nm: 245 and 290, HRMS *m/z* (rel. int.): $C_{35}H_{46}O_4$, 530.3384 (M^{+}•-absent), found 407.2132 [(M^{+}•-$C_9H_{15}=C_{26}H_{31}O_4$ requires 407.2214]$^{+}$ (4), 355.1573 (48), 353.1443 [M^{+}•-C_9H_{15}-C_4H_6]$^{+}$ (54), 339.1596 [M^{+}•-C_9H_{15}-C_5H_9-CH_3]$^{+}$ (89), 337.1423 [M^{+}•-$C_{10}H_{17}$-C_4H_8]$^{+}$ (30), 335.1302 (20.5), 323.1302 [M^{+}•-C_9H_{15}-C_5H_9-CH_3]$^{+}$ (3.5), 313.1065 (24), 295.0970 (54), 105.0290 [C_7H_5O]$^{+}$ (100), 91.0478 [C_7H_7]$^{+}$ (28), 81.0628 (42), 77.0393 [C_6H_5]$^{+}$ (33), 69.0561 [C_5H_9]$^{+}$ (95), 57.0642 (42), 55.0478 [C_4H_7]$^{+}$ (70), ^{1}H NMR (300 MHz, $CDCl_3$/TMS): δ 1.40 (2H, *q, J* = 7.0 Hz, H-9), 1.60 (3H, *s*, H-21), 1.61 (3H, *s*, H-16), 1.64 (3H, *s*, H-33), 1.66 (3H, *s*, H-32), 1.69 (3H, *s*, H-13), 1.77 (3H, *s*, H-20), 1.83-1.87 (2H, *m*, H-10), 1.98 (1H, *m*, H-8), 2.16 (2H, *m*, H-7), 2.48 (1H, *dd, J* = 15.0 and 7.5 Hz, H-17), 2.62 (1H, *dd, J* = 15.0 and 7.5 Hz, H-17), 3.10 (1H, *dd, J* = 15.0 and 6.0 Hz, H-29), 3.22 (1H, *dd, J* = 15.0 and 6.0 Hz, H-29), 3.51 (3H, *s*, OMe), 3.97 (3H, *s*, OMe), 4.64 (1H, *bs*, H-12 or H-15), 4.67 (1H, *bs*, H-15 or H-12), 4.69 (1H, *bs*, H-12 or H-15), 4.74 (1H, *bs*, H-12 or H-15), 4.91 (1H, *bt, J* = 6.0 Hz, H-18), 5.10 (1H, *t, J* = 6.0 Hz, H-30), 7.41 (2H, *t, J* = 8.0 Hz, H-25 and H-27), 7.51 (1H, *tt, J* = 7.3 and 1.5 Hz, H-26), 7.88 (2H, *dd, J* = 7.3 and 1.5 Hz, H-24 and H-28). The assignments follow the numbering system adopted in Fig. 4.

*4.8. Dimethyl lanceolatone: 4-benzoyl-3,5-dimethoxy-2,6-bis(3-methylbut-2-enyl)-6-[5-methyl-2-(1-methylvinyl) hexa-5-enyl]cyclohexa-2,4-dien-1-one (**5b**)*

Yellow oil, $[\alpha]_D^{20}+146.6^\circ$ ($CHCl_3$, c. 0.58), IR (film NaCl) ν_{max} cm^{-1}: 2968, 2927, 2856, 1672, 1644, 1560, 1449, 1275, 1089, UV (MeCN) λ_{max} nm: 254 and 350, EIMS 70 eV, *m/z* (%): 530 ($M^{+\bullet}$), 339(100), 283(98), 105(21), 91 (15), 77(12), ^1H NMR (300 MHz, $CDCl_3$/TMS): δ 1.34 (2H, *m*, H-9), 1.56 (3H, *s*, H-21), 1.59 (6H, *s*, H-16 and H-33), 1.62 (3H, *s*, H-13), 1.68 (3H, *s*, H-32), 1.73 (3H, *s*, H-20), 1.82 (2H, *m*, H-10), 1.98 (1H, *m*, H-8), 2.10 (2H, *m*, H-7), 2.52 (1H, *dd, J* = 13.2 and 8.8 Hz, H-17), 2.60 (1H, *dd, J* = 13.2 and 8.8 Hz, H-17), 3.02 (2H, *m*, H-29), 3.46 (3H, *s*, OMe), 3.54 (3H, *s*, OMe), 4.62 (1H, *s*, H-15 or H-12), 4.66 (1H, *s*, H-12 or H-15), 4.69 (1H, *bs*, H-15 or H-12), 4.74 (1H, *bs*, H-12 or H-15), 5.00 (1H, *t, J* = 7.5 Hz, H-18), 5.06 (1H, *t, J* = 6.6 Hz, H-30), 7.48 (2H, *t, J* = 8.0 Hz, H-25 and H-27), 7.60 (1H, *tt, J* = 7.3,1.5 Hz, H-26), 7.96 (2H, *dd, J* = 7.3 and 1.5 Hz, H-24 and H-28). The assignments follow the numbering system adopted in Fig. 4.

*4.9. Dimethyl hilarianone: 2-benzoyl-3,5-dimethoxy-4,6-bis(3-methylbut-2-enyl)-4-methylalfa[1-(2,4,4-trimethyl-cyclohex-1-ene)]-cyclohexa-2,5-dien-1-one (**6a**)*

Yellow oil, $[\alpha]_D^{20}=+58.8^\circ$ ($CHCl_3$, c. 0.93), IR (film NaCl) ν_{max} cm^{-1}: 2949, 2923, 2861, 1674, 1650, 1610, 1450, 1364, 1276, 1225, 1100, 986, 690, UV ($CHCl_3$) λ_{max} nm: 288 and 250, HRMS *m/z* (rel. int.): $C_{35}H_{46}O_4$, found 530.3396 $M^{+\bullet}$ (3.2) (requires 530.3384), 462.2783 $[M^{+\bullet}\text{-}C_5H_8]^+$ (8.5), 394.2177 $[M^{+\bullet}\text{-}C_{10}H_{16}]^+$ (73), 339.1612 $[M^{+\bullet}\text{-}C_{10}H_{16}\text{-}C_4H_7]^+$ (58), 337.1465 $[M^{+\bullet}\text{-}C_{10}\text{-}H_{17}\text{-}C_4H_7\text{-}H]^+$ (37), 323.1316 $[M^{+\bullet}\text{-}C_9H_{15}\text{-}C_5H_9\text{-}CH_3]^+$ (38), 295.0982 (16), 137.1354 $[C_{10}H_{17}]^+$ (8), 121.1026 (19), 105.0360 $[C_7H_5O]^+$ (100), 91.0546 $[C_7H_7]^+$ (16), 77.0395 $[C_6H_5]^+$ (26), ^1H NMR (500 MHz, $CDCl_3$/TMS): δ 0.82 (3H, *s*, H-14), 0.85 (3H, *s*, H-15), 1.26 (2H, *m*, H-12), 1.60 (3H, *bs*, H-16), 1.63 (6H, *s*, H-21, H-33), 1.64 (3H, *s*, H-32), 1.64 (1H, overlap, H-10), 1.76 (3H, *s*, H-20), 1.76 (1H, overlap, H-10), 1.95 (2H, *m*, H-13), 2.59 (1H, *dd, J* = 14.0 and 5.0 Hz, H-17), 2.71 (1H, *dd, J* = 14.0 and 10.0 Hz, H-17), 2.77 (2H, *s*, H-7), 3.05 (1H, *dd, J* = 16.0 and 6.0 Hz, H-29), 3.17 (1H, *dd, J* = 16.0 and 6.0 Hz, H-29), 3.56 (3H, *s*, OMe), 3.96 (3H, *s*, OMe), 4.99 (1H, *m*, H-18), 5.02 (1H, *m*, H-29), 7.41 (2H, *t, J* = 7.7 Hz, H-25 and H-27), 7.50 (1H, *tt, J* = 7.5 Hz and 1.4 Hz, H-26), 7.88 (2H, *dd, J* = 7.5 and 1.4 Hz, H-24 and H-28). The assignments follow the numbering system adopted in Fig. 4.

*4.10. Methyl spiritone: 3-benzoyl-2-methoxy-8,8-dimethyl-1,7–bis(3-methylbut-2-enyl)-5-[5-methyl-2-(1-methylvinyl) hexa-4-enyl]-exo-bicyclo[3.3.1]non-2-ene-4,9-dione (**7a**)*

Yellow oil, IR (film NaCl) ν_{max} cm^{-1}: 2967, 2921, 2886, 1673, 1643, 1590, 1448, 1374, 1274, 737, UV (MeCN) λ_{max} nm: 254, HRMS *m/z* (rel. int.): $C_{39}H_{52}O_4$, found 584.3866 $M^{+\bullet}$ (62) (requires 584.3852), 447.2623 $[M^{+\bullet}\text{-}C_{10}H_{17}]^+$ (15), 323.1431 $[M^{+\bullet}\text{-}C_{10}H_{17}\text{-}C_5H_9\text{-}C_4H_7]^+$ (12), 256.2565 (33), 149.0392 (31), 137.1432 $[C_{10}H_{17}]^+$ (22), 129.1065 (16), 121.1160 (20), 111.1269 (25), 105.0544 $[C_7H_5O]^+$ (53), 69 $[C_5H_9]^+$ (85), 98.1063 (15), 95.0990 (38), 91.0671 $[C_7H_7]^+$ (17), 83.0884 (39), 81.0817 (60), 71.0968 (27), 69.0813 $[C_5H_9]^+$ (100), ^1H NMR (300 MHz, $CDCl_3$/TMS): δ 0.79 (3H, *s*, H-37$_{ax}$), 1.14 (3H, *s*, H-38$_{eq}$), 1.28 (1H, H-6), 1.36 (3H, *s*, H-31), 1.57 (1H, overlap, H-32), 1.57 (3H, *s*, H-28), 1.59 (6H, *s*, H-14 and H-36), 1.63 (1H, *m*, H-22), 1.67 (3H, *s*, H-35), 1.69 (6H, *s*, H-13 and H-27), 1.90-1.80 (1H, *m*, H-6), 1.98 (1H, *m*, H-23), 2.00 (2H, *m*, H-24), 2.00-1.80 (1H, *m*, H-22), 2.04–1.96 (1H, *m*, H-7), 2.10 (1H, *m*, H-32), 2.52 (1H, *dd, J* = 14.0 and 8.1 Hz, H-10), 2.74 (1H, *m*, H-10), 3.59 (3H, *s*, OMe), 4.55 (2H, *s*, H-30), 4.93 (1H, *m*, H-11), 5.00 (2H, *m*, H-25 and H-33), 7.44 (1H, *t, J* = 7.7 Hz, H-19), 7.57 (2H, *tt, J* = 7.3 and 1.5 Hz, H-18 and H-20), 7.90 (2H, *dd, J* = 7.0 and 1.5 Hz, H-17 and H-21). The assignments follow the numbering system adopted in Fig. 5.

*4.11. Methyl insigninone: 1-benzoyl-2-methoxy-8,8-dimethyl-3,5-bis(3-methylbut-2-enyl)-7-[(3-methyl-4-acetoxy-but-2-enyl)]endo-bicyclo[3.3.1]non-2-ene-4,9-dione (**8a**)*

Yellow oil, $[\alpha]_D^{20}=+92.7^\circ$ ($CHCl_3$, c. 1.56), UV (MeCN) λ_{max} nm: 245 and 278, HRMS *m/z* (rel. int.): $C_{36}H_{46}O_6$, found 574.32980 $M^{+\bullet}$ (11) (requires 574.3282), 505.2523 $[M^{+\bullet}\text{-}C_5H_9]^+$ (26), 469.3065 (4), 431.2180 (16), 363.1670 (18), 325.1521 (24), 323.1351 (72), 281.0898 (10) 269.0906 (16), 149.0314 (4), 105.0421 $[C_7H_5O]^+$ (100), 69.0754 $[C_5H_9]^+$ (29), 91.0610 $[C_7H_7]^+$ (6), 83.0884 (39), 81.0756 (3), 77.0442 (21), 69.0754 $[C_5H_9]^+$ (29), ^1H NMR (500 MHz, $CDCl_3$/TMS): δ 1.39 (3H, *s*, H-32), 1.49 (1H, overlap, H-7), 1.49 (3H, *s*, H-33), 1.59 (3H, *s*, H-31), 1.65 (3H, *s*, H-21), 1.67 (6H, *s*, H-26 and H-20), 1.68 (3H, *s*, H-25), 2.05 (2H, *m*, H-6 and H-27), 2.07 (3H, *s*, Me-Ac), 2.19 (1H, *dd, J* = 15.9 and 5.5 Hz, H-6), 2.34 (1H, *bd, J* = 14 Hz, H-27), 2.50 (1H, *dd, J* = 16.0 and 8.0 Hz, H-22), 2.60 (1H, *dd, J* = 16.0 and 8.0 Hz, H-22), 3.14 (1H, *dd, J* = 16.0 and 8.0 Hz, H-17), 3.30 (1H, *dd, J* = 16.0 and 8.0 Hz, H-17), 3.50 (3H, *s*, OMe), 4.42 (2H, *s*, H-30), 4.94 (1H, *t, J* = 1.2 Hz, H-19), 5.03 (1H, *t, J* = 1.2 Hz, H-23), 5.20 (1H, *t, J* = 7.3 Hz, H-28), 7.29 (2H, *t, J* = 8.0 Hz, H-13 and H-15), 7.43 (1H, *tt, J* = 8.0 Hz and 1.2 Hz, H-14), 7.60 (2H, *dd, J* = 8.0 and 1.2 Hz, H-12 and H-16). The assignments follow the numbering system adopted in Fig. 5.

*4.12. Scrobiculatone A: 1-benzoyl-8,8-dimethyl-5,7-bis(3-methylbut-2-enyl)-3,4-(2,2-dimethylpyran[5,6:3,4]exo-bicyclo[3.3.1]non-3-ene-2,9-dione (**9a**)*

Yellow oil, $[\alpha]_D^{20}=+44.7^\circ$ ($CHCl_3$, c. 0.19), IR (film KBr) ν_{max} cm^{-1}: 2974, 2927, 2856, 1722, 1698, 1644,

1586, 1447, 1414, 1337, 1308, 1266, 738, UV (MeCN) λ_{max} nm: 250 and 324, HRMS *m/z* (rel. int.): $C_{33}H_{40}O_4$, found 500.2926 $M^{+\bullet}$ (65) (requires 500.2916), 485.2693 $[M^{+\bullet}\text{-}CH_3]^+$ (16), 433.2393 $[M^{+\bullet}\text{-}C_5H_7]^+$ (12), 432.2312 $[M^{+\bullet}\text{-}C_5H_8]^+$ (39), 431.2237 $[M^{+\bullet}\text{-}C_5H_9]^+$ (8), 418.2137 $[M^{+\bullet}\text{-}C_5H_6O]+$ (33), 417.2119 $[M^{+\bullet}\text{-}C_5H_6O-H]^+$ (100), 364.1666 $[M^{+\bullet}\text{-}C_{10}H_{16}]^+$ (20), 363.1610 $[M^{+\bullet}\text{-}C_{10}H_{17}]^+$ (58), 309.1158 (91), 308.1065 (26), 293.0852 (26), 105.0397 $[C_5H_7O]^+$ (71), 83.9575 $[C_5H_7O]^+$ (10), 77.0420 $[C_6H_5]^+$ (25), 69.0728 $[C_5H_9]^+$ (44), 55.0737 (12), ^{1}H NMR (500 MHz, $CDCl_3$/TMS): δ 1.14 (3H, *s*, H-32$_{ax}$), 1.35 (3H, *s*, H-33$_{eq}$), 1.39 (3H, *s*, H-20), 1.40 (3H, *s*, H-21), 1.56 (7H, *s*, H-6, H-26 and H-31), 1.66 (3H, *s* broad, H-30),1.67 (3H, *bs*, H-25), 1.70 (2H, *m*, H-7 and H-27), 2.00 (1H, *d, J* = 1.37 Hz, H-6), 2.18 (1H, *m*, H-27), 2.56 (2H, *m*, H-22), 5.01 (1H, *m*, H-28), 5.03 (1H, *m*, H-23), 5.39 (1H, *d, J* = 9.9 Hz, H-18), 6.45 (1H, *d, J* = 9.9 Hz, H-17), 7.27 (2H, *m*, H-13 and H-15), 7.32 (1H, *tt, J* = 8.5 Hz and 1.4 Hz, H-14), 7.50 (2H, *dd, J* = 8.5 and 1.1 Hz, H-12 and H-16). The assignments follow the numbering system adopted in Fig. 5.

4.13. Scrobiculatone B: 1-benzoyl-8,8-dimethyl-5,7-bis (3-methylbut-2-enyl)-2,3-(2,2-dimethylpyran[5,6:3,2]exo-bicyclo[3.3.1]non-2-ene-2,9-dione (**9b**)

Yellow oil, $[\alpha]_D^{20}$ + 44.7° ($CHCl_3$, c. 0.19), IR (film KBr) ν_{max} cm^{-1}: 2974, 2927, 2856, 1722, 1698, 1644, 1586, 1447, 1414, 1337, 1308, 1266, 738, UV (MeCN) λ_{max} nm: 250 and 324, HRMS *m/z* (rel. int.): $C_{33}H_{40}O_4$, found 500.2926 $M^{+\bullet}$ (65) (requires 500.2916), 501.2977 $[M^{+\bullet}+H]$ (16), 485.2693 $[M^{+\bullet}\text{-}CH_3]^+$ (16), 433.2393 $[M^{+\bullet}\text{-}C_5H_7]^+$ (12), 432.2312 $[M^{+\bullet}\text{-}C_5H_8]^+$ (39), 431.2237 $[M^{+\bullet}\text{-}C_5H_9]^+$ (8), 418.2137 $[M^{+\bullet}\text{-}C_5H_6O]^+$ (33), 417.2119 $[M^{+\bullet}\text{-}C_5H_6O-H]^+$ (100), 364.1666 $[M^{+\bullet}\text{-}C_{10}H_{16}]+$ (20), 363.1610 $[M^{+\bullet}\text{-}C_{10}H_{17}]+$ (58), 309.1158 (91), 308.1065 (26), 293.0852 (26), 105.0397 $[C_5H_7O]^+$ (71), 83.9575 $[C_5H_7O]^+$ (10), 77.0420 $[C_6H_5]^+$ (25), 69.0728 $[C_5H_9]^+$ (44), 55.0737 (12), ^{1}H NMR (500 MHz, $CDCl_3$/TMS): δ 0.60 (3H, *s*, H-21), 1.21 (3H, *s*, H-32$_{ax}$), 1.40 (3H, *s*, H-33$_{eq}$), 1.42 (3H, *s*, H-20), 1.48 (1H, *m*, H-6), 1.54 (3H, *s*, H-26), 1.57 (3H, *bs*, H-31), 1.68 (3H, *bs*, H-30), 1.70 (3H, *bs*, H-25), 1.70 (2H, overlap, H-7 and H-27), 1.98 (1H, *m*, H-6), 2.10 (1H, *m*, H-27), 2.48 (2H, *m*, H-22), 5.01 (1H, *m*, H-28), 5.04 (1H, *m*, H-23), 5.21 (1H, *d, J* = 10.2 Hz, H-18), 6.48 (1H, *d, J* = 10.2 Hz, H-17), 7.27 (2H, *m*, H-13 and H-15), 7.43 (1H, *tt, J* = 8.5 Hz and 1.1 Hz, H-14), 7.62 (2H, *dd, J* = 8.5 and 1.1 Hz, H-12 and H-16). The assignments follow the numbering system adopted in Fig. 5.

4.14. Microbiological screening

The antimicrobial action of the floral resins *C. grandiflora* female, *C. grandiflora* male, *C. insignis* male, *C. lanceolata* male, *C. nemorosa* hermaphrodite, *C. renggerioides* male with pistillodium, *C. spiritu-sanctensis* male (methylated and no methylated), *C. weddelliana* male, clusianone and bees nest against selected bacteria and fungi was evaluated by the bioautography method (Betina, 1973) using 10^6 cells per ml in each case. Spots of the resins (100 μg l^{-1}) and chloramphenicol (10 μg l^{-1}, for antibacterial activity) or nystatin (10 μg l^{-1} for antifungal activity) were applied to 6 cm×6 cm TLC plates (Merck Silica gel 60 F_{254}) eluted with hexane: EtOAc (9:1), two identical TLC plates were made for each test. One plate was developed with an anisaldehyde sulfuric acid solution followed by heating and the second TLC plate was placed in a 60 mm diameter Petri dish covered with Nutrient Broth (NB-DIFCO, for bacteria) and Malt Extract (ME-DIFCO, for fungi) and inoculated with the test bacteria or fungi. Incubation time was 24 h at 37°C for bacteria and 48 h at 28°C for fungi. After incubation the plates were observed for inhibition zones. The inhibition halo was judged by comparison with that of the commercial nystatin or chloramphenicol. Screening was performed against the following microorganisms *Aspergillus niger* CCT 1435, *Bacillus subtilis* CCT 0089, *Candida albicans* CCT 0776, *Escherichia coli* CCT 5050, *Rhodococcus equi* CCT 0541 and *Staphylococcus aureus* CCT 4295 from the "André Tosello", Culture Collection Tropical (CCT), Campinas/SP/Brasil.

Acknowledgements

The authors thank FAPESP (Fundação de Apoio à Pesquisa do Estado de São Paulo) for support as well as by A. L. M. Porto for a CNPq scholarship. S. M. F. Machado is grateful to DQ/UF Sergipe for her leave of absence and to CAPES/PICD, C. M. A. de Oliveira is thankful to DQ/UF Goiás for her leave of absence and to CAPES/PICD, and V. Bittrich is indebted to FAPESP for a fellowship. The I.A.C. (Instituto Agronômico de Campinas, SP/Brazil) allowed us to use the cultivated plants on Fazenda St Elisa, and this is gratefully acknowledged.

References

Adams, R.P., 1995. Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Allured, Carol Stream, IL.

Betina, V., 1973. Bioautography in paper and thin-layer chromatography and its scope in the antibiotic field. Journal of Chromatography 78, 41–51.

Bittrich, V., Amaral, M.C.E., 1997. Floral biology of some *Clusia* species from Central Amazonia. Kew Bulletin 53, 617–635.

Gustafson, K.R., Blunt, J.W., Munro, M.H.G., Fuller, R.W., McKee, T.C., Cardellina, II, J.H., McMahon, J.B., Cragg, G.M., Boyd, M.R., 1992. The guttiferones, HIV-inhibitory benzophenones from *Symphonia glubulifera, Garcinia livingstonei, Garcinia ovalifolia* and *Clusia rosea*. Tetrahedron 48, 10093–10102.

Henry, G.E., Jacobs, H., Carrington, C.M.S., McLean, S., Reynolds, W.F., 1999. Prenylated benzophenone derivatives from Caribbean *Clusia* species (Guttiferae). Plukenetiones B-G and Xerophenone A. Tetrahedron 55, 1581–1596.

Johnson, E.L., Stevenson, R., 1978. Basic Liquid Chromatography. Varian, Palo Alto, CA.

Lokvam, J., Braddock, J.F., 1999. Anti-bacterial function in the sexually dimorphic pollinator rewards of *Clusia grandiflora* (Clusiaceae). Oecologia 119, 534–540.

Nogueira, P.C., Bittrich, V., Shepherd, G.J., Lopes, A.V., Marsaioli, A.J., 2000. The ecological taxonomic importance of flower volatiles of *Clusia* species (Guttiferae). Phytochemistry (in press)

Oliveira, C.M.A., Porto, A.L.M., Bittrich, V., Vencato, I., Marsaioli, A.J., 1996. Floral resins of Clusia spp: chemical composition and biological function. Tetrahedron Letters 37, 6427–6430.

Oliveira, C.M.A., Porto, A.L.M., Bittrich, V., Marsaioli, A.J., 1999. Two polyisoprenylated benzophenones from the floral resins of three *Clusia* species. Phytochemistry 50, 1073–1079.

Roubik, D.N., 1989. Ecology and natural history of tropical bees. Cambridge University Press.

Rubio, O.C., Cuellar, A.C., Rojas, N., Castro, H.V., Rastrelli, L., Aquino, R., 1999. A polyisoprenylated benzophenone from Cuban propolis. Journal Natural Products 62, 1013–1015.