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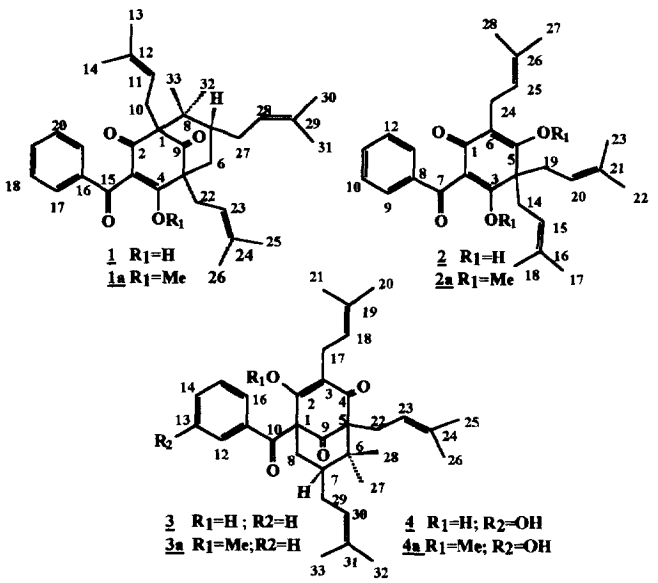
FLORAL RESINS OF *CLUSIA* SPP.: CHEMICAL COMPOSITION AND BIOLOGICAL FUNCTION

Cecília M. A. de Oliveira[2], André M. Porto[1], Volker Bittrich[3]
Ivo Vencato[4] and Anita J. Marsaioli*[1]

1-Instituto de Química , UNICAMP, CP 6154, Campinas 13081-970, SP, Brazil 2- Instituto de Química e Geociências-UFG, Goiás, Brazil. 3- Instituto de Biologia, UNICAMP, Campinas, SP Brazil, 4- UFSC, Florianopolis, SC, Brazil.

Abstract: The floral resins of five species of *Clusia* belonging to two taxonomic sections of the genus were investigated. These resins are used by pollinating bees for nest construction. The major components of these resins are polyisoprenylated benzophenones, a class of biologically active compounds. We found clusianone and three hitherto unknown compounds, grandone , nemorosone and hydroxy-nemorosone.

Guttiferae is a family of mainly tropical plants of ca. 40 genera and about 1200 species most of which are woody. The plants of the family are generally characterized by the presence of latex in most of their tissues.



The neotropical genus *Clusia* comprises ca. 250 spp. Several *Clusia* spp. offer floral resins as a reward for pollinating bees which use this material for nest construction.[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. Several compounds belonging to this class have shown HIV-inhibitory activity.[2] However, little is known about the chemical composition of *Clusia* floral resins and their role in the pollination process.[1,5] The floral resins of *C. grandiflora* (male), *C. rosea* (female), *C. insignis* (female), *C. nemorosa* (hermaphrodite) and *C. spiritu-sanctensis* (male) and *C. pernambucensis* (male)[6], cultivated at the "Fazenda Santa Elisa", Instituto Agronômico (IAC), Campinas, SP, Brazil, were carefully collected, scraping the viscous resins with small glass rods which were then dumped into vials containing organic solvents (diethyl ether or ethyl acetate). The fluid staminal oils were collected with capillaries or absorbed on small pieces of ultra pure filter paper which were dumped into vials containing organic solvent. For small flowers the task was better executed with the help of a dissecting microscope. Treatment of the resin with diazomethane allowed a better

separation of the major components, due to the greater stability of the methyl derivatives on silica gel. Clusianone 1, C_{33} H_{42} O_4, was the major component in *C. spiritu-santensis* (male) . Clusianone 1 was first isolated from the roots of *C. congestiflora* and was identified by X- ray diffraction analysis[7].

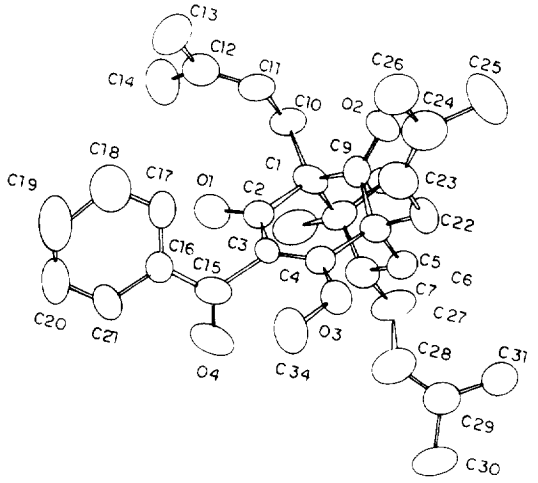
TABLE I: ^1H and ^{13}C chemical shifts of 2a, 3a and 4a, obtained by 1 D and 2D NMR spectroscopy (at 7 tesla and $CDCl_3$ solutions)

	2a		3a		4a	
C#	^{13}C	$^1H(\delta)$	^{13}C	$^1H(\delta)$	^{13}C	$^1H(\delta)$
1	188.0	-	65.1	-	65.2	-
2	123.4	-	169.9	-	170.0	-
3	169.6	-	123.2	-	123.0	-
4	53.8	-	193.1	-	193.0	-
5	170.7	-	74.4	-	74.1	-
6	118.0	-	47.8		47.9	-
7	196.5	-	42.5	1.66 (1H, overlap)	42.4	1.66(1H, overlap)
8	138.5	-	43.1	1.94 (1H, dd, J=3.4 and 12.6Hz eq.) 1.42(1H, t, J=12.6Hz, axial)	43.1	1.94 (1H, dd, J=3.4 and 12.6Hz eq); 1.42 (1H, t, J= 12.6 Hz,axial)
9	129.2	7.89 (1H, dd, J=7.4 and 1Hz)	207.9	-	207.9	-
10	128.6	7.42 (1H, t, J=7.4 Hz)	197.1	-	197.4	-
11	133.0	7.45 (1H, t, J=7.4 Hz)	137.0	-	138.3	-
12	128.6	7.42 (1H, t, J=7.4 Hz)	128.4	7,62(1H, dd, J=8 and 1Hz)	115.2	7.27 (1H, bs)
13	129.2	7.89 (1H, dd, J=7 and 1Hz)	127.9	7.32(1H, t, J=8Hz)	155.9	-
14	35.8	2.62 (2H, m)	132.1	7.44(1H, tt, J=8 and 1 Hz)	119.4	6.97 (1H, bd, J=7.7 Hz)
15	118.7	4.96 (1H, m)	127.9	7.32(1H, t, J=8Hz)	128.9	7.12 (1H, t, J=7.7Hz)
16	134.6	-	128.4	7,62(1H, dd, J=8 and 1Hz)	120.5	7.02 (1H, dd, J=7.7and 1 Hz)
17	25.9	1.65 (3H, s)	23.2	3.25(1H, dd, J=16 and 6.8Hz); 3.34(1H, dd, J=16 and 6.8Hz)	23.2	3.20(1H,dd,J=16 and 6.4 Hz); 3.35(1H, dd, J=16 and 6.4 Hz)
18	17.9	1.54 (3H, s)	121.5	5.00(1H, m)	121.4	5.00 (1H, m)
19	35.8	2.62 (2H, m)	134.4	-	134.5	-
20	118.7	4.96 (1H, m)	25.8	1.66(3H,s)	25.8	1.66 (3H, s)
21	134.6	-	18.0	1.66(3H, s)	17.9	1.66 (3H, s)
22	25.9	1.65 (3H, s)	29.5	2.50(1H, dd, J=13.7, 7.1Hz); 2.56 (1H, dd, J=13.7, 7.0Hz)	29.4	2.47(1H, dd ,J= 12.9 and 7 Hz); 2.57 (1H, dd, J=12.9 and 7 Hz)
23	17.9	1.54 (3H, s)	119.6	5.00(1H, m)	119.6	5.00 (1H, m)
24	22.7	3.14 (2H, d, J=6Hz)	133.0	-	133.1	-
25	122. 6	5.04 (1H, m)	25.6	1.66 (3H, s)	25.6	1.66 (3H, s)
26	131.6	-	18.1	1.66 (3H, s)	18.1	1.66 (3H, s)
27	25.6	1.58 (s)	24.4 eq.	1.34 (3H, s)	24.4 eq.	1.33 (3H, s)
28	17.9	1.57 (s)	16.1axial	1.19(3H,s)	16.1 axia	1.17 (3H,s)
29	-	-	27.6	2.10(1H,m); 1.66(1H, overlap)	27.6	2.09 (1H, m); 1.66 (1H, overlap)
30	-	-	122.5	4.97(1H,m)	122.4	4.98 (1H, m)
31	-	-	133.2	-	133.2	-
32	-	-	26.0	1.66(3H, s)	25.9	1.66 (3H, s)
33	-	-	17.8	1.55 (3H, s)	17.8	1.54 (3H,s)
OMe	61.6 59.5	3.92(3H, s) 3.57(3H, s)	61.6	3.45 (3H, s)	61.6	3.50(3H,s)
OH						6.12(1H, bs)

The absolute configuration was not assigned and no spectral data were provided. Clusianone 1 was more recently isolated from *C.sandinensis* and its spectral data were reported.[8] In our case we have obtained the methyl derivative 1a, by diazomethane treatment of the resin, which allowed its purification and full characterization.[9] X-ray diffraction analysis confirmed the proposed structure (ORTEP stereoview of 1a in figure 1). Analysis of ^1H,^{13}C and 2D NMR spectra, provided one bond (HETCOR) and multiple bond (COLOC) correlations allowing unambiguous assignments of all carbons[9] which were consistent with those reported for clusianone 1 (although we believe that some misassignments were reported previously due to the analysis performed on a keto-enolic mixture).[7] Grandone 2 was the second major component of *C. grandiflora* floral resin, (16%), it was isolated as its methyl derivative 2a. The analysis of the one and two dimensional ^1H

and ^{13}C NMR spectra, with homo and heteronuclear correlations at one and several bonds allowed the full assignment of its ^{1}H and ^{13}C signals [9] which are related with those of colupulone [10] and are depicted in table 1.

Figure 1- Stereoview of 1a obtained by X-ray diffraction analysis



Nemorosone, 3 , was the major component from floral resins of *C. rosea* (48%), *C. grandiflora* (69%), *C. insignis* (43%) and *C. nemorosa* (38%) (weight of isolated compound/ weight of crude methylated resin X 100; the quantification performed on the methyl derivative 3a, was also confirmed by GC of the methylated crude extract, DB-5 column). The main clues to the structure proposal of 3a were the spectral data of 1a and 2a. The proton NMR spectra of 1a and 3a were clearly distinct and the major difference was in the chemical shifts of two allylic protons at δ 3.20 and 3.35 in 3a were similar to those in 2a (δ 3.14) and were rather deshielded in relation to those in 1a at δ 2.41-2.58. The UV spectrum (λ_{max}=194 and 250 nm) of 1a indicated that the chromophores were also different from 3a (λ_{max}=194, 250 and 280 nm). The relative configuration of 3a was obtained by NOEDIF experiments and important increments were observed irradiating the methoxy group and observing positive increments at the allylic methylene at δ 3.20 and 3.35 (1.3%) , vinyl δ 5.00 (2.1%) and aromatic protons at δ 7.62(1.4 %). This information associated with the long range correlations, confirmed the structure of 3a. Compound 3 is a novel polyisoprenylated benzophenone. Hydroxy-nemorosone 4 was also isolated as its methyl derivative 4a, as the minor component of *C.nemorosa* floral resin. The main spectroscopic differences between 3a and 4a concerned the proton and carbon-13 NMR signals corresponding to the benzophenone moieties. Comparison of the carbon-13 NMR signals of 4a benzophenone portion with those of the 3-hydroxy-benzoic acid confirmed the proposed structure 4a. Figure 2 depicts the long range C,H correlations for 4a. Compound 4 is also a novel polyisoprenylated benzophenone. We have isolated but not yet fully characterized several other components of floral resins of 7 additional *Clusia spp.* which belong to the class of the polyisoprenylated benzophenone structurally related to those reported above. These results definitely establish the chemical composition of the major constituents of *Clusia* floral resins as polyisoprenylated benzophenones[5]. The fresh latex of the stem of *C. pernambucensis* (droplets of freshly cut stems) is composed of lanosterol and a polysaccharide, while the fluid floral oil (secreted by the stamens) of *C. grandiflora* is composed mainly of long chain diesters. Consequently they are both chemically distinct from the resins. Finally it is worth mentioning that the non methylated polyisoprenylated benzophenones are sensitive to acidic conditions (polymerize, decompose) while their methyl derivatives are not. It is therefore possible to understand

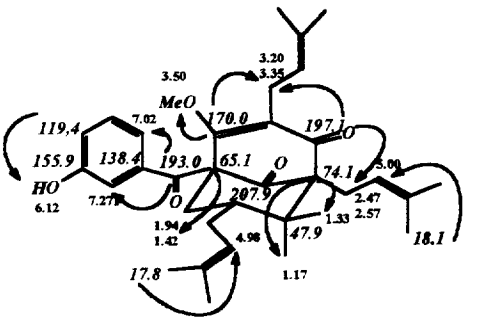


Figure 2: Long range C,H correlations for compound 4a (C-13 chemical shifts in italics)

why tropical bees find these resins so valuable for their nest construction.[1] The resins polymerize slowly during the nest construction and provide a waterproof protection for provisions and larvae with antiviral and probably antimicrobial activity. The major achievement of this work is to reveal the chemical composition of the pure floral resins (viscous) and floral oils (fluid) instead of whole plant organs.

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6. Voucher specimens have been deposited at the Universidade Estadual de Campinas (UEC) Herbarium by M.C.E. Amaral and V.Bittrich and V.Bittrich is responsible for the identifications. ***C. nemorosa*** G.Mey. hermaphrodite (# 95/150); ***C. nemorosa*** male (# 95/151); ***C. grandiflora*** , Spligt. male (# 95/152); ***C. grandiflora*** female (#95/153) ; ***C. rosea*** Jacq. female (# 95/154); ***C. spiritu-sanctensis*** G.Mariz & B.Weinberg , male, (# 95/185); ***Cl pernambucensis*** G.Mariz male (# 95/186).
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9. All compounds gave spectroscopic data in agreement with the assigned structures . The numbering system adopted in this paper is based on the IUPAC name given to the basic bicyclohexenone **Compound 1a (methyl clusianone, 3 - benzoyl - 4 - methoxy - 8,8 - dimethyl - 1,5,7 - tris(3-methyl - 2 -butenyl)-exo - bicyclo[3.3.1]non - 3 - ene - 2,9 - dione)**: M.p. 123-125 ^{0}C ; $[\alpha]_D$ +60.7 ^{0}(CHCl$_3$, c.1.4); IR film on NaCl ν (cm^{-1}) 2925(s), 1726(s), 1676(m) , 1640(s), 1597(s); MS molecular ion m/z 516 and major fragments at m/z 447 ($M^{+\cdot}$ - C_5H_9, 67%) , m/z 323 ($M^{+\cdot}$- C_5H_9 - C_9H_{16} 100%), m/z 105 ($C_7H_5O^+$, 47%). .Anal. Calcd for $C_{34}H_{44}O_4$: C, 79.03%; H, 8.58%; O, 12.39%. Found: C, 79.03 %; H, 8.48 %; O, 12.49%. ^{1}H NMR (300MHz, CDCl$_3$):δ 1.45(1H, t, J=13.5 Hz, H-6 axial), 2.08(1H, dd, J=13.5 and 4.4Hz,, H-6eq..), 1.90(1H, m, H-7), 2.45 (1H, dd, J=13.4 and 4.6 Hz, H-10), 2.58 (1H. dd, J=13.4 and 4.6 Hz, H-10), 4.81 (1H, m, H-11), 1.59 (6H, s, H-13, H-26), 1.54(1H, s, H-14), 7.82(2H, dd, J=7.6Hz and 1 Hz,H-17, H-21), 7.42(2H, bt, J=7.6 Hz, H-18, H-20), 7.55 (1H, tt, J=7.6 and 1 Hz ,H-19), 2.51(2H, overlap H-22), 5.09 (1H, m, H-23), 1.73 (3H, s, H-25), 2.18(1H, bd, J=14Hz, H-27), 1.73(1H, overlap, H-27), 5.09(1H, m, H-28), 1.70 (3H, s, H-30), 1.66(3H, s, H-31), 0.74(3H, s, H-32 ax), 1.05(3H, s, H-33 eq.), 3.62(3H,s, OMe) ; ^{13}C NMR (75.5 MHz, CDCl$_3$): δ 71.1(C-1), 195.5(C-2), 122.6(C-3), 172.9(C-4), 57.7(C-5), 40.2(C-6), 41.8(C-7), 47.0(C-8), 207.7(C-9), 24.6(C-10), 120.1(C-11), 134.2(C-12), 26.1(C-13), 18.1(C-14), 195.6(C-15), 137.9(C-16), 129.2(C-17, C-21), 128.6(C-18, C-20), 133.3(C-19), 30.7(C-22), 122.6(C-23), 133.1(C-24), 25.8(C-25), 18.0(C-26), 28.0(C-27), 120.1(C-28), 133.5(C-29), 25.9(C-30), 17.9(C-31), 15.9 (C-32), 22.4 (C-33), 60.7 (OMe) . **Compound 2a (methyl grandone, 2 - benzoyl - 3,5 -dimethoxy - 4,4,6 - tris(3-methyl - 2 -butenyl) - 2,5-cyclohexadien - 1 - one)**: oil ; IR film on NaCl: ν (cm^{-1}) 2966(w), 2925(m), 1674(m), 1652(s) , 1610 (s), 1449(m) ; EI HRMS molecular ion m/z 462.2770 calcd for. $C_{30}H_{38}O_4$, 462.27701 and major fragments 337.3(64%), 323.3(36%), 105.1 (85%), 41.0 (100%). ^{1}H NMR (300MHz, CDCl$_3$) see table I. ^{13}C NMR (75.5 MHz, CDCl$_3$) see table I. Anal. Calcd for $C_{30}H_{38}O_4$: C, 77.89%; H, 8.28 %; O, 13.83 %. Found: C, 77.68%; H, 8.20%; O, 14.12%. **Compound 3a (methyl nemorosone, 1-benzoyl - 2- methoxy - 6,6 - dimethyl - 3,5,7 - tris(3-methyl-2-butenyl)-exo - bicyclo[3.3.1]non - 2 - ene - 4,9 - dione)** : M.p. 88-91 ^{0}C ; $[\alpha_D]$ +150^0 (MeOH, c.0.8); IR film on NaCl: ν (cm^{-1}) 2966(m), 2916(m), 1720(s,), 1702(m) , 1654(s), 1604(s), 1241(m); EI HRMS molecular ion m/z 516.3239 calc for $C_{34}H_{44}O_4$ 516.32395 and major fragments at m/z 447.4 ($M^{+\cdot}$ - C_5H_9, 4 %) , m/z 298.4 (48%), m/z 87.1 (70.4%), m/z 74.1 (100%); ^{1}H NMR (300MHz, CDCl$_3$) see table I. ^{13}C NMR (75.5 MHz, CDCl$_3$) see table I. **Compound 4a (methyl-hydroxy- nemorosone, 1 - (3'-hydroxy-benzoyl) - 2 - methoxy - 6,6 - dimethyl - 3,5,7 - tris(3-methyl - 2 -butenyl)-exo - bicyclo[3.3.1]non - 2 - ene - 4,9 - dione)** M.p. 134-136 ^{0}C ; $[\alpha]_D$ = +142.8(MeOH, c.0.7); IR (KBr) ν (cm-1) 3365(s), 2966(m), 2913(m), 1721(s), 1705(s), 1654(s), 1608(s), 1239(m);HRMS molecular ion m/z 532,3188 calcd for $C_{34}H_{44}O_5$ 532.31887 and major fragments at m/z 463.5 ($M^{+\cdot}$ - C_5H_9, 80%) , m/z 339.3 ($M^{+\cdot}$- C_5H_9 - C_9H_{16}., 100%). ^{1}H NMR (300MHz, CDCl$_3$) see table I ;^{13}C NMR (75.5 MHz, CDCl$_3$) see table I
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