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Two polyisoprenylated benzophenones from the floral resins of three *Clusia* species

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Abstract

From the floral resins of *Clusia nemorosa* male, *C. nemorosa* hermaphrodite, *Clusia rosea* female, *Clusia grandiflora* male, *C. grandiflora* female, *Clusia insignis* male (all section *Chlamydoclusia*). *C. renggerioides* male (with pistillodium), *C. renggerioides* male (without pistillodium), *C. renggerioides* female (all section *Cordylandra*) belonging to the family Clusiaceae, we have isolated two novel polyisoprenylated benzophenones, nemorosone II and 6-*epi*-nemorosone, and a xanthone. The latter is the first member of this class of compounds ever isolated from the genus *Clusia*. HPLC allowed the quantification of these and other methylated benzophenones in the methylated resins revealing that indeed these are the major constituents of *Clusia* floral resins. Oleic, stearic and some unusual long chain esters and acids are the main constituents of the stamen oils.

Keywords: Floral resins; Stamen oils; *Clusia nemorosa* male, *C. nemorosa* hermaphrodite, *C. rosea* female, *C. grandiflora* female, *C. grandiflora* male, *C. insignis* male, *C. renggerioides* male (with pistillodium), *C. renggerioides* male (without pistillodium), *C. renggerioides* female; Clusiaceae; Section *Chlamydoclusia*, section *Cordylandra*; Novel polyisoprenylated benzophenones; Nemorosone II; 7-*Epi*-nemorosone; Xanthone; HPLC quantification

1. Introduction

The neotropical genus *Clusia* L. (Clusiaceae or Guttiferae) comprises about 250 species distributed from southern Florida to southern Brazil. The plants are woody and evergreen with often rather coriaceous and more or less carnose leaves. They occur as shrubs, small to medium-sized trees, hemi-epiphytes, and rarely lianas. As the great majority *Clusia* species are dioecious, some agent is necessary to guarantee that pollen from male flowers reaches the stigma of the female flowers (pollination). The resin, attracting female bees in search of nest construction material, serves as a reward for the bees, which by collecting floral resin on different plants, transport pollen to the stigmas. Some *Clusia* flowers not only secrete resin but additionally oils of different functions. In some species of the section *Chlamydoclusia* (e.g. *C. nemorosa* G. Mey. and *C. grandiflora* Splitg. and related species), the oil is secreted in droplets on the anther tips of the male flowers and acts as an accessory pollenkitt, i.e. it makes the dry, powdery pollen stickier so that it adheres better on the bees' body (Bittrich & Amaral, 1996a,b, 1997; Roubik, 1989).

The first investigation of *Clusia* floral resins (Oliveira, Porto, Bittrich, & Marsaioli, 1996) led to the isolation of four polyisoprenylated benzophenones, clusianone (**1**), grandone (**2**), nemorosone (**3**) and hidroxy-nemorosone (**4**), as their *O*-methyl derivatives (Fig. 1). Conscious that a thorough investigation was important we have now studied the floral resins and oils of *C. nemorosa* male, *C. nemorosa* hermaphrodite, *C. rosea* female, *C. grandiflora* male, *C. grandiflora* female, *C. insignis* male all belonging to the section *Chlamydoclusia* and *C. renggerioides* male (with pistillodium), *C. renggerioides* male (without pistillodium), *C. renggerioides* female belonging to the section

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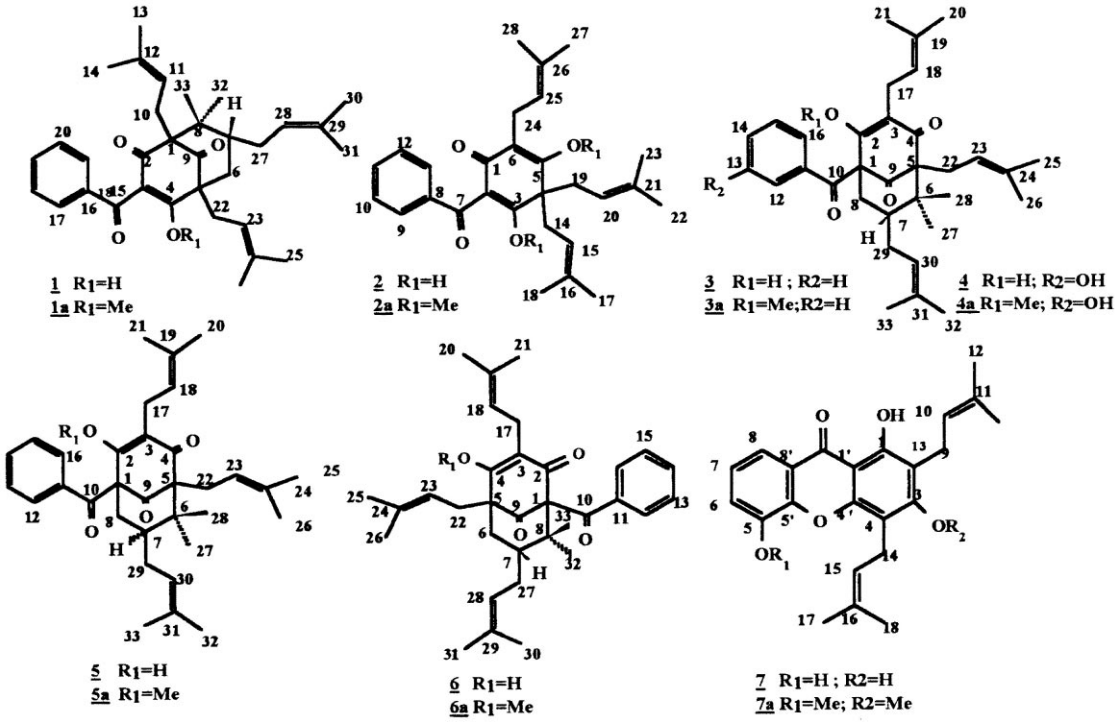

Fig. 1.

Cordylandra. In this paper, we report the isolation and characterization of two novel polyisoprenylated benzophenones possessing the bicyclo[3.3.1]nonenetrione moiety named 7-*epi*-nemorosone (**5**) and nemorosone II (**6**) along with the known xanthone (**7**). Our study was also extended to the stamen oils which we know now to be mainly constituted by fatty acids.

2. Results and discussions

Following the procedure described in our previous paper (Oliveira et al., 1996) the floral resins were collected by scraping them off the flowers with glass rods or spatulas. They were then dissolved in diethyl ether or ethyl acetate and treated with CH_2N_2. A combination of CC and TLC of the methylated resins gave two new polyisoprenylated benzophenones, (**5a**) and (**6a**), along with the xanthone (**7a**).

The molecular formula of (**5a**) was determined as $C_{34}H_{44}O_4$ *m/z* 516.3239 by HR mass spectrometry. The UV, MS, 1D and 2D NMR spectra (^1H, ^{13}C, $^1H \times {}^1H$, $^1H \times {}^{13}C$ one bond and multiple bonds) of (**5a**) were similar to those reported for nemorosone (**3a**) (Oliveira et al., 1996). The main $^1H \times H$ and $^{13}C \times {}^1H$ long range correlations are depicted in Fig. 2. Comparison of the ^1H NMR and ^{13}C NMR signals of (**5a**) and (**3a**) revealed some chemical shift differences [$\Delta\delta$((**5a**–**3a**))] mainly related to the bicyclo[3.3.1]nonenedione moiety ($\Delta\delta_{H\text{-}7} = -0.28$, $\Delta\delta_{H\text{-}8} = -0.48$ and 0.18, $\Delta\delta_{H\text{-}27} = 0.14$, $\Delta\delta_{H\text{-}28} = 0.19$, $\Delta\delta_{C\text{-}5} = -1.4$, $\Delta\delta_{C\text{-}6} = 1.7$, $\Delta\delta_{C\text{-}7} = 6.1$, $\Delta\delta_{C\text{-}8} = -1.2$, $\Delta\delta_{C\text{-}27} = 2.9$, $\Delta\delta_{C\text{-}28} = 7.8$). We suspected than epimerization of chiral C-7 center might be responsible for these effects but a Dreiding molecular model of 7-*epi*-nemorosone (**5a,**) revealed that the isopentenyl group (assuming the axial position) would produce a highly unstable molecule from the conformational point of view due to the two 1,3-diaxial interactions between the isopentenyl group and C-2 and C-4. It thus seemed likely that the conformation of ring B had changed from chair (compound (**3a**)) to boat (compound (**5a**)) along with the configuration of C-7 from R* (compound (**3a**)) to S* (compound (**5a**)). However the increments observed in the NOE difference spectra (Fig. 2) were not conclusive and we found that H-7 resonates at δ 1.38, almost 0.3 ppm more shielded than H-7 of compound (**3a**), and the carbonyl anisotropic shielding can be evoked to explain this effect when ring B adopts the boat conformation depicted in Fig. 2.

Additionally we used long range C, H correlations in the 2D NMR and NOE difference spectra (Fig. 2) to assign the two methylated enolic forms (**5a**) and (**5b**) (Fig. 3). In the ^1H NMR of the crude fractions of (**5a**) a small amount of (**5b**) was visualized but not isolated. (**5b**) was again detected in the HPLC analyses as a minor compound with an elution time similar to (**5a**) (shoulder).

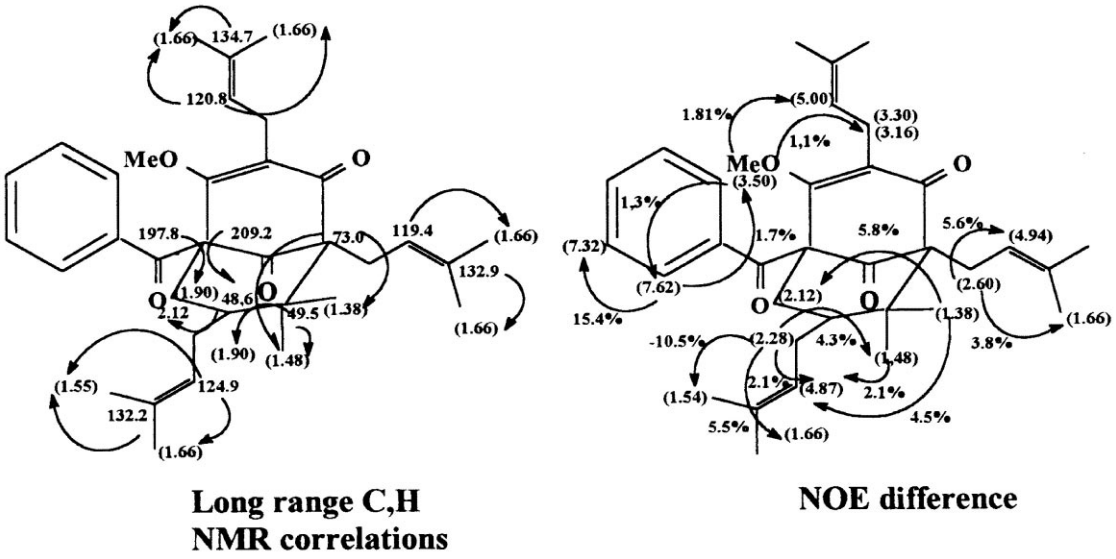

Fig. 2.

Compound (**6a**), (*O*-methyl nemorosone II) was isolated from the methylated floral resins of *Clusia rosea* and *C. renggerioides* male (with pistillodium) and its molecular formula was determined as $C_{34}H_{44}O_4$ at *m/z* 516.3239 by high resolution mass spectrometry. The UV spectrum revealed that chromophores belonging to the benzophenone (280 nm) and 1,3 diketone in the methyl enolic form moieties (250 nm) were present as in the case of compounds (**3a**), (**5a**), and (**6a**). The MS fragmentation pattern was compatible with the benzophenones (**3a**) and (**5a**), but the 1D and 2D NMR spectra (^1H, ^{13}C, $^1H \times {}^1H$, $^1H \times {}^{13}C$) and one bond and multiple bond (Table 1 and Fig. 4) comparison between (**3a**) and (**6a**) revealed that the main structural changes were to be found in the bicyclo[3.3.1]nonenedione moiety. Furthermore a significant modification was observed in the carbon chemical shifts at the bridgehead atoms. The bridgehead carbon alpha to the gem-dimethyl group in this position was confirmed by long correlations between H and C in the 2D NMR spectrum (Fig. 4) deshielding of the C-5 and concomitant shielding of C-1 in compound (**6a**) in relation to the corresponding carbons in (**3a**) was attributed to the exchange of functional groups.

Thus the benzophenone moiety was placed on the bridgehead carbon next to the gem-dimethyl group and the isopentenyl group at the bridgehead carbon next to the methylene of ring B. The IUPAC numbering system has been changed and C-1 is now placed, in compound (**6a**), next to the gem-dimethyl group and this has to be taken into account in Table 1. It is noteworthy that in compound (**6a**) the methoxy group is more deshielded (δ 4.00) than in (**3a**) and (**5a**) (δ around 3.45 and 3.50 respectively). The same is true

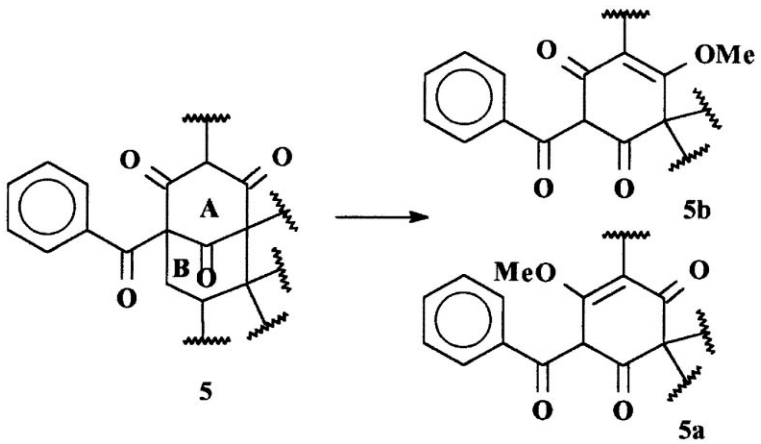

Fig. 3. Possible isomeric methyl enol ethers of **5** and **6** formed on methylation with diazomethane.

Table 1
^{1}H and ^{13}C chemical shifts of **5a** and **6a**, obtained by 1D and 2D NMR spectroscopy

	6a		**5a**	
C#	^{13}C	$^{1}H(\delta)$	^{13}C	$^{1}H(\delta)$
1	79.2	–	63.4	–
2	193.1	–	170.1	–
3	126.8	–	122.0	–
4	173.1	–	193.3	–
5	59.8	–	73.0	–
6	40.7	2.03 (1H, dd, J = 13.2 and 3.6 eq Hz.) 1.50 (1H, t, $J_{ax\text{-}ax} = J_{vic}$ = 13.2 Hz)	49.5	
7	43.7	1.66 (1H, overlap)	48.6	1.38 (1H, overlap)
8	48.8	–	41.9	2.12 (1H, m) 1.90 (1H, m)
9	207.3	–	209.2	–
10	194.0	–	197.8	–
11	136.2	–	136.8	–
12	128.5	7.45 (1H, dd, J = 9.6 and 1.6 Hz)	128.5	7.62 (1H, dd, J = 8 and 1 Hz)
13	127.7	7.23 (1H, t, J = 7.4 Hz)	127.8	7.32 (1H, t, J = 8 Hz)
14	131.9	7.38 (1H, tt, J = 7.8 and 1 Hz)	132.0	7.44 (1H, tt, J = 8 and 1 Hz)
15	127.7	7.23 (1H, t, J = 7.4 Hz)	127.8	7.32 (1H, t, J = 8 Hz)
16	128.5	7.45 (1H, dd, J = 9.6 and 1.6 Hz)	128.5	7.62 (1H, dd, J = 8 and 1 Hz)
17	23.3	3.15 (1H, dd, J = 15 and 7.2 Hz); 3.25 (1H, dd, J = 15 and 7.2 Hz)	23.6	3.30 (1H, dd, J = 16 and 6.8 Hz);3.16 (1H, dd, J = 16 and 6.8 Hz)
18	121.3	5.05 (1H, m)	120.8	5.00 (1H, tt,J = 6 Hz and 1 Hz)
19	134.4	–	134.7	–
20	26.0	1.66 (3H, s)	25.8	1.66 (3H, s)
21	17.9	1.66 (3H, s)	17.9	1.66 (3H, s)
22	29.7	2.45 (1H, dd, J = 14. and 6.9 Hz) 2.60 (1H, dd, J = 14. and 6.9 Hz)	30.2	2.50 (1H, dd, J = 21 and 7.8 Hz); 2.60 (1H, dd, J = 21 and 7.8 Hz)
23	119.5	5.05 (1H, m)	119.4	4.94 (1H, tt, J = 6 Hz and 1 Hz)
24	133.5	–	132.9	–
25	25.6	1.65 (3H, s)	25.6	1.66 (3H, s)
26	18.1	1.68 (3H, s)	18.2	1.66 (3H, s)
27	23.4	2.20 (1H, m); 1.70 (1H, m)	27.2 eq.	1.48 (3H, s)
28	122.6	5.05 (1H, m)	23.9 axial	1.38 (3H, s)
29	133.5		30.0	2.28 (1H, m); 1.90 (1H, overlap)
30	25.8	1.65 (3H, s)	124.9	4.87 (1H, tt, J = 6 Hz and 1 Hz)
31	17.9	1.69 (3H, s)	132.2	–
32	23.4 eq.	1.35 (3H, s)	26.0	1.66 (H, s)
33	15.7 axial	1.13 (3H, s)	17.8	1.55 (3H, s)
OMe	62.4	4.00 (3H, s)	61.4	3.50 (3H, s)

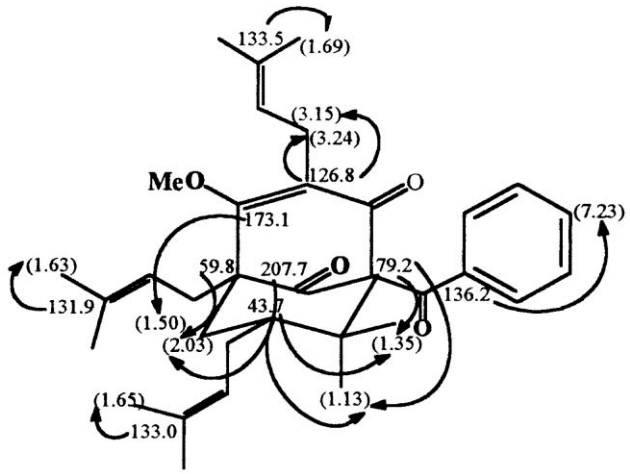

Fig. 4. Long range C, H correlations observed in 2D NMR spectrum of **6a**.

for the chemical shifts of the ring B methylene hydrogens compound (**3a**) (δ 1.94, H-8) corresponding hydrogens in compound (**6a**) (δ 2.03, H-6). The shielding effect of the benzophenone anisotropy on the methoxy group and methylene hydrogens in compound (**3a**) is absent in compound (**6a**) which shifts their resonating frequencies to higher values.

The full assignments of all ^{1}H and ^{13}C NMR signals of the two novel benzophenones as their *O*-methyl derivatives 7-*epi*-nemorosone (**5a**) and nemorosone II (**6a**) are in shown Table 1.

Due to the structural similarities of all compounds isolated from *Clusia* floral resins, the possibility existed that they might be artifacts arising from a common intermediate cyclizing under the chemical and chromatographic manipulation. We have therefore developed a chromatographic method that would rapidly detect and quantify the benzophenones in the resins. The methylated resins where analyzed by RP-HPLC (NOVAPAK C-18), which by means of calibration curves (Johnson & Stevenson, 1978) allowed the quantification of the isolated standards (**5a**), (**6a**) and (**7a**) as well as of the previously isolated benzophenones (**2a, 3a**) and (**4a**) (Oliveira et al., 1996).

The results are depicted in Table 2. They show that silica column chromatography could not have led to artifacts as the percentages distribution data obtained from the HPLC study were close to those obtained for the purified compounds. In addition to check each resin has its own individual composition. It is clear that the polyisoprenylated benzophenones are major components of the floral resins of *Clusia*. Compound (**7**), isolated as its methyl derivative (**7a**) from floral resins of *C. nemorosa* (hermaphrodite) was identified as a xanthone (Bennet & Huang Lee, 1989) previously obtained from a *Garcinia* species.

Finally, we investigated by GC/MS the composition of the stamen oils and observed that fatty acids like oleic and stearic acid are the main components. GC/MS allowed a further insight into the composition of the floral resins and showed that fatty acids were also present in the floral resins.

Based on the six main compounds isolated and detected by HPLC analysis of the floral resins of five *Clusia* species, we can conclude that the benzophenones present in the floral resins are certainly less oxidized than those isolated from fruits and leaves. Polyisoprenylated benzophenones show antimicrobial activity (Gustafson et al., 1992) and this fact raises further questions about the bioactivity of the natural form of these.

3. Experimental

3.1. General

Melting points were determined with a Kofler hot plate set up in a microscope Thermopan model (C.

Table 2
Quantification of the benzophenones **2a**–**6a** and of the xanthone **7a** of five floral *Clusia* resins

Resins	2a	3a	4a	5a	6a	7a
C. nemorosa male[a]	–	1.8	–	7.6	–	–
C. nemorosa hermaphrodite[a]	–	28.5	12.0	–	–	6.4
C.grandiflora male[a]	6.0	15.0	–	–	–	–
C. rosea female[a]	–	3.0	–	–	33.0	–
C. grandiflora female[a]	1.0	70.0	–	–	–	–
C. insignis male[b]	–	–	–	27.4	–	–
C. renggerioides with pistillodium male[b]	–	–	–	43.5	10.5	–
C. renggerioides with pistillodium male[b]	–	–	–	4.9	–	–
C. renggerioides without pistillodium male[b]	–	–	–	17.0	–	–
C. renggerioides female[b]	–	–	–	24.0	–	–

[a]Floral resins collected at IAC (Instituto Agronômico de Campinas).
[b]Floral resins collected at the Amazonas.

Reichert Optische Werke AG). 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) or Bruker AC 300P (300.1 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) or a Bruker AC 300P (75.5 MHz) spectrometer. $CDCl_3$ (77.0 ppm) was used as internal standard. Methyl, methylene, methine and carbon non-bonded to hydrogen were discriminated using DEPT-135° and DEPT-90° spectra (distortionless enhancement by polarization transfer). 2D NMR: standard H,H correlation and H, X correlation pulse sequences. GC/MS: HP-5890/5970 system equipped with either a J&W Scientific DB-5 fused silica capillary column (25 m × 0.2 mm × 0.33 μm). The 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).

3.2. HPLC

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 (about 10 mg of compound in 10 ml of acetonitrile). The concn. of compounds (**2a**–**7a**) was determined by comparing the area under each peak to standard curves generated by injection of standards **2a**–**7a** of known concentration. The detector response was linear over the concn range used.

3.3. Plant material

The floral resins of plants, cultivated at the 'Fazenda Santa Elisa', Instituto Agronômico de Campinas (IAC), Campinas, SP, Brasil, were collected by scraping off the viscous resins with small glass rods which were then dumped into vials containing Et_2O or EtOAc. 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* Splitg. male (95/152); *C. grandiflora* female (95/153); *C. rosea* Jacq. female (95/154), *C. renggerioides* male (without pistillodium) (91/28a) *C. renggerioides* female (91/28b) *C. insignis* male (91/26).

3.4. Isolation of products

3.4.1. Isolation of **5a**

Fresh resin (1.8 g) of *C. nemorosa* male resin was treated with CH_2N_2 in Et_2O (50 ml). The reaction mixture was kept at room temperature (in a safety hood) for the slow evaporation of the residual CH_2N_2 and the remaining solvent (free of CH_2N_2) was removed at reduced pressure. The residue was chromatographed on a silica gel column eluted with hexane–Et_2O (0 → 100%). Fractions from hexane–Et_2O (95:5) were combined to give 400 mg of (**5a**).

3.4.2. Isolation of **6a**

Fresh resin (0.8 g) of *C. rosea* female flowers was treated following the above mentioned procedure and successive column chromatographies eluted with hexane and increasing amounts of ethyl acetate (0–100%) produced (**6a**) (20 mg).

7-*epi*-*O*-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]. Oil, $[\alpha]_D^{20} = +140.7$ ($CHCl_3$, c. 3.1) IR: ν_{max}^{film} cm^{-1} 3446, 1716, 1657, 1608, 1384. EI HRMS 70 eV, *m*/*z* (rel. int.) 516.3239 [M]$^+$, 447 [M–C_5H_9, 72]$^+$, 323 (100), 281 (76), 105 (62,), 69 (100).

O-methyl nemorosone II: [1-benzoyl-2-methoxy-8,8-dimethyl-3,5,7-tris (3-methyl-2-butenyl)-exo-bicyclo [3.3.1] non-2-ene-4,9-dione]. Oil, $[\alpha]_D^{20} = +48.6$ ($CHCl_3$, c. 1.4). IR: ν_{max}^{film} cm^{-1} 3446, 1716, 1657, 1608, 1384. EI HRMS 70 eV *m*/*z* (rel. int) 516.3239 [M]$^+$, 447 [M–C_5H_9, 28]$^+$, 323 (45), 142 (29), 105 (24), 69 (100).

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