Floral Resin of *Tovomitopsis saldanhae* (Guttiferae) and 7-*Epi*-nemorosone: Structural Revision

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Z. Naturforsch. **58 c**, 643–648 (2003); received January 27/April 2, 2003

The floral resin of *Tovomitopsis saldanhae* (Guttiferae) is composed of poliisoprenylated benzophenone and the major constituent is 7-*epi*-nemorosone which has now been revised.

Key words: Clusia, *Tovomitopsis*, Guttiferae, Resin

Introduction

The flowers of angiosperms offer various rewards for their pollinators, the most common being nectar and pollen. Floral resins are very uncommon rewards, known to occur up to now only in two angiosperm families and a few genera: Euphorbiaceae (species of the genus *Dalechampia* L., Armbruster, 1984) and Guttiferae (species of the genera *Clusia* L., *Chrysochlamys* Poeppig, and *Clusiella* Planch. & Triana; Bittrich and Amaral, 1997; Hammel, 1999). One has to be aware that the physical aspect of the reward (resin) cannot be taken as a clue of the chemical composition. Thus triterpenoids are the main constituents of the *Dalechampia* floral resin (Armbruster, 1984) and polyisoprenylated benzophenones are major components of *Clusia* floral resins (de Oliveira *et al.*, 1996, 1999; Porto *et al.*, 2000; Cuesta-Rubio *et al.*, 2001). Not all *Clusia* species offer a resinous reward, alternatively some offer pollen and others nectar. Thus the evolution of floral rewards within the genus prompted an investigation. We have looked for floral resinous rewards in other closely related genera. Recently we found a floral resin, secreted by the stamen filaments, in *Tovomitopsis saldanhae* Engl., a rare species from southeastern Brazil. *Tovomitopsis* is a small genus closely related to *Clusia*. Together with the genera *Chrysochlamys*, *Dystovomita* and *Tovomita* it forms the tribe Clusieae, one of the two major monophyletic groups of the subfamily Clusioideae (Gustafsson *et al.*, 2002). *Clusiella*, the other genus well known for producing flora resins, is only very distantly related to the Clusieae, belonging to the subfamily Kielmeyeroideae. In this case there is no doubt that its floral resins have evolved independently from *Clusia*. The presence of floral resin in *Tovomitopsis*, however, suggests two possibilities: either the resin evolved independently in both genera, or the flowers of the last common ancestor of *Clusia* and *Tovomitopsis* already produced floral resin and rewards like nectar and pollen evolved more recently in *Clusia*. If the first were true, the chemical composition of the floral resin in both genera might be different, if the latter is true, the chemical composition should be rather similar. To test these hypotheses, we proposed to analyze the floral resin of *Tovomitopsis*. *Tovomitopsis* is a genus very little investigated from a chemical point of view.

A review of previous investigations showed that an antibacterial vitamin E derivative was isolated from *Tovomitopsis psychotriifolia* Oerst., Planch. & Triana leaves (ethanolic extract) (Setzer *et al.*, 1995). This species from Costa Rica belongs probably rather into the genus *Chrysochlamys* (Hammel, 1999) and not into *Tovomitopsis*. The delimitation of both genera is controversial and needs further studies.

0939–5075/2003/0900–0643 $ 06.00

Results and Discussion

A flowering *Tomovitopsis saldanhae* specimen was located in the Atlantic rain forest in the north-eastern part of Minas Gerais, Brazil. The resin from the stamens of 5 flowers were collected with glass rods and then put into vials containing diethyl acetate. Solvent evaporation produced 70 mg of a resinous residue. Evaluation of the composition complexity and polarity by silica gel thin layer chromatography revealed that the mixture had one major component that was too polar to monitor by GC/MS. The crude mixture was therefore evaluated by ^{1}H NMR revealing signals that were characteristic of polyisoprenylated benzophenones possessing the bicyclo[3,3,1]nonanetrione moiety. Following the methodology previously established in our group (de Oliveira *et al.*, 1996), the crude resin was methylated with diazomethane and the resulting methylated mixture was submitted to preparative TLC. Isolation (18 mg) of the major component as its methyl derivative (~ 25 % of the mixture) and spectral analysis (^{1}H and ^{13}C NMR, MS, IR) revealed that this component was identical to one of 7-*epi*-nemorosone methyl derivatives (**1a**; Fig. 1), previously isolated from *Clusia nemorosa* (male flowers) (de Oliveira *et al.*, 1999), *C. insignis* (male) (Porto *et al.*, 2000) and *C. renggerioides*. This class of secondary metabolites (polyisoprenylated benzophenones) is characteristic of *Clusia* and now of *Tovomitopsis* floral resinous rewards. Its occurrence is relatively common, in fruits (Fuller *et al.*, 1999; Alves *et al.*, 1999; Henry *et al.*, 1999, 1995; Delle Monache *et al.*, 1991, 1988; Rama Rao *et al.*, 1978; Venkatswamy *et al.*, 1975; Krishnamurthy *et al.*, 1981; Santos *et al.*, 1998; Karanjgoakar *et al.*, 1973), latex (Lokvam *et al.*, 2000) and other plant parts (Gustafson *et al.*, 1992; Fuller *et al.*, 1999) of several Guttiferae genera (*Clusia*, *Garcinia*, *Symphonia*, *Rheedia* (= *Garcinia* s.lat.), *Vismia*, *Hypericum* and *Allanblackia*).

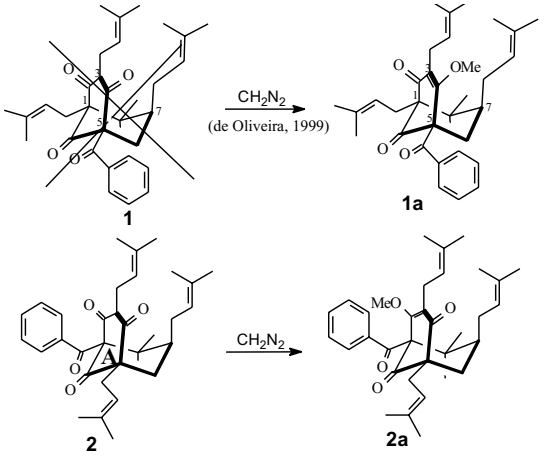


Fig. 1. 7-*epi*-nemorosone and 7-*epi*-nemorosone methyl derivative structural revisions.

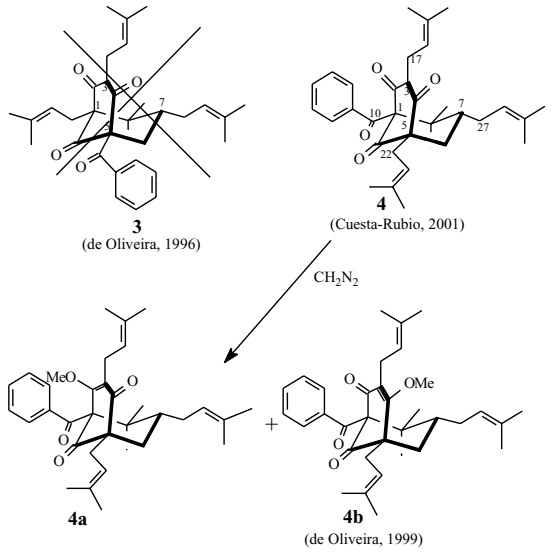
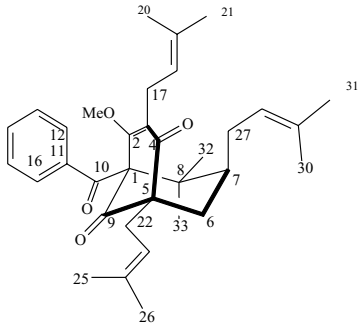


Fig. 2. Nemorosone methyl derivatives obtained with diazomethane, **4a** (revised by Cuesta-Rubio, 2001) and **4b** (de Oliveira, 1999, reconfirmed).

7-Epi-nemorosone structural revision

Revising the literature for the occurrence of polyisoprenylated benzophenones we became aware that structure **3** suggested for nemorosone by de Oliveira *et al.* (1996) was recently revised to structure **4** by Cuesta-Rubio (2001) (Fig. 2), who also suggested that an analogous structural misassignment might have occurred in the 7-*epi*-nemorosone methyl derivative (**1**) but due to the lack of an authentic sample their suggestion remained as a hypothesis. This prompted us to reinvestigate nemorosone and 7-*epi*-nemorosone methyl derivatives. Methylation of *Clusia rosea* flower resin with diazomethane allowed the isolation of the two nemorosone methyl derivatives I (**4a**) and II (**4b**), respectively (Fig. 2).



No.	C	H	No.	C	H
1	73.0		**18**	120.9	4.95
2	170.9		**19**	134.8	
3	122,1		**20**	17.9	1.64
4	193.5		**21**	25.7	1.66
5	63.4		**22**	30.1	2.50 e 2.60
6	41.9	2.12 e 2.18	**23**	119.5	5.05
7	48.5	1.44	**24**	133.1	
8	49.5		**25**	18.1	1.55
9	209.4		**26**	25.6	1.67
10	197.8		**27**	30.0	1.92 e 2.28
11	137.0		**28**	4.87	
12	128.6	7.61	**29**	132.6	
13	127.9	7.29	**30**	17.8	1.55
14	132.1	7.42	**31**	26.0	1.64
15	127.9	7.29	**32**	27.2	1.48
16	128.6	7.61	**33**	23.8	1.38
17	23.5	3.16 e 3.30	**34**	61.3	3.51

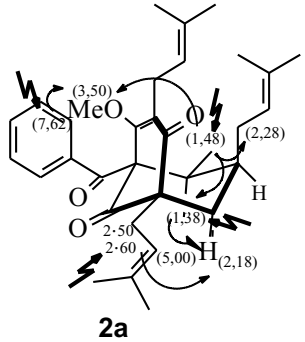
Fig. 3. ^{1}H and ^{13}C NMR chemical shift assignments for **2a**.

NMR reinvestigation of both **4a** and **4b** under Cuesta-Rubio's experimental conditions revealed that all their experimental observations (Cuesta-Rubio, 2001) could be reproduced on the methyl derivative **4a**, thus confirming that the revised and correct structure of nemorosone is **4**. Reinvestigation of *O*-methyl-nemorosone II (**4b**) (de Oliveira *et al.*, 1999) reproduced previous observations and the structure was reconfirmed. During these spectroscopic reinvestigations we realized that the misguided idea concerning nemorosone was mainly caused by enhancements arising from nOe experiments with unprecise excitations, at the time of our first isolation and identification. Thus the benzoyl moiety was erroneously situated on the carbon 5 when it should have been correctly linked to carbon 1. For the spectroscopic reinvestigations we had access to very selective excitations (INOVA 500, Varian with gradient and shaped pulses).

With these data in hands the spectroscopic reinvestigation of 7-*epi*-nemorosone was a must. As mentioned above the ^{1}H and ^{13}C NMR spectra of the *O*-methyl derivative of 7-*epi*-nemorosone isolated from *Tovomitopsis* were identical to the previously reported ones.

The main questions were not related to proton and carbon assignments which relied on C,H connectivities using 2D NMR and the HSQC and g-HMBC pulse sequences (Fig. 4), but about the substituents on carbon 1 and 5 and the relative configuration at C-7 which led us to focus on nOe experiments.

Using a non-degassed sample, NOESY 1D pulse sequence and setting the selective pulse onto one of the geminal methyl group (δ 1.48) provoked signal increments at the methyl group (δ 1.38, 1.17 %), *O*-methyl group (δ 3.51, 0.26 %), H-27 (δ 2.28, 2.54 %) thus linking the *O*-methyl enol ether moiety to the *gem*-dimethyl group. Setting the selective pulse onto the aromatic hydrogen (δ 7.61) provoked a 0.40 % enhancement of the methoxy group signal at δ 3.51 which set the benzoyl moiety on carbon 1 thus sharing with the methoxy and the *gem*-dimethyl groups the same hemisphere of **2a** (Fig. 1 and Fig. 4). Finally shaping the soft pulse in order to excite both hydrogens H-22 (δ 2.50 and δ 2.60) produced 2.57 % enhancement of the H-6 signal ressonating at δ 2.18. Other increments were observed but as mentioned above only the diagnostic ones were discussed. Based on the CH chemical shift (δ 48.5) (Porto *et al.*, 2000) the configuration at carbon 7 was never at stake but the conformation of this bicy-



2a

Fig. 4. Signal enhancements observed in NOESY 1D experiments with **2a**.

clo[3,3,1]nonendione moiety with an axial substituent at C-7 called for a better investigation.

2a *Ring A Conformation*: As 7-*epi* nemorosone **2** and its *O*-methyl derivative **2a** possess 7-isopentenyl substituents *cis* to the 1,3 dione and enone bridges their cumbersome structural spatial arrangements might be released by a change in ring **A** conformation going from chair to boat (Fig. 1). The flexibility of ring **A** and interconversion between chair and boat conformations was inferred from the temperature dependence of the chemical shifts, mainly H-27 which shifted from δ 2.28 (20 °C, CD_2Cl_2) to δ 2.18 (– 60 °C, CD_2Cl_2) suggesting a rapid conformational equilibrium. The predominance of the chair conformation can be suggested based on the nOe enhancement of the methoxy group (δ 3.51), Me equatorial (δ 1.48), and a very discrete increment of the axial methyl signal at δ 1.38 upon the excitation of H-27 (δ 2.28). According to the relative increment of the two angular methyl groups it can be suggested that the population ratio of chair/boat conformations at room temperature is over 10. The rational takes into account that similar increments would be expected in the boat conformation and no increment of the axial methyl group (pseudo axial to be more precise) is expected for the chair conformation. Finally the irradiation at δ 1.38 (Me_{ax} in the chair conformation **2a**), enhanced the amplitude of the double doublet at δ 2.18 (1H, dd, J = 13.6 and 6,9 Hz, H-6_{ax}) therefore assigned to H-$6_{ax.}$ The H-7/H-6_{ax} and H-7/H-6_{eq} coupling constants (6.9 and 1.5 Hz, respectively at 20 °C) are not fully consistent with the chair conformation for which similar coupling constants are predicted. These facts point toward a distorted chair conformation. The chair conformation was observed in X-ray diffraction experiments for analogous benzophenones (Rogers *et al.*, 1981; Santos *et al.*, 1998). Finally more NMR experiments are necessary to determine the exact ratio of boat and chair conformers present in solution and this is beyond the scope of the present paper.

Conclusions

The chemical composition of the floral resin of *Tovomitopsis saldanhae* is identical with that of certain *Clusia* species. This result seems to support the hypothesis that floral resin was already present in the last common ancestor of *Clusia* and *Tovomitopsis*. The fact that our preliminary investigations of the undoubtely independently evolved floral resin of *Clusiella* showed it to be chemically similar with that of clusias (and *Tovomitopsis*), but having also certain peculiarities, seems to fit in. Although being a plausible scenario, two arguments caution against too hasty conclusions: First, polyisoprenylated benzophenones occur in various tissues of several Guttiferae (see above). This means that such compounds had not to be "invented" independently more than once during evolution, as the plants could resort to these polyisoprenylated benzophenones for the production of floral resin. Or in other words, they were preadapted for using these compounds as floral reward. Secondly, the recent phylogenetic studies of *Clusia* and related genera based on DNA sequences (Gustafsson and Bittrich, in press) suggest an independent evolution of the floral resin in *Tovomitopsis* and *Clusia* to be slightly more parsimonious than a single origin. Lack of phylogenetic resolution and especially lack of data about the genus *Chrysochlamys*, however, preclude at present conclusive answers. We are also still ignorant about the chemical composition of floral resins in *Chrysochlamys tenuifolia Cuatr.*

Finally, the investigation of *Tovomitopsis saldanhae* floral resin allowed the structural revision of 7-*epi*-nemorosone.

Experimental

A voucher of *Tovomitopsis saldanhae* has been deposited at the Herbarium of the Biology Institute of UNICAMP by M. C. E. Amaral, V. Bittrich and L. Leoni under the number 2000/47 (UEC).

7-epi-O-Methyl nemorosone: 1-benzoyl-2-methoxy-8,8-dimethyl-3,5,7-tri(3-methyl-2-butenyl)-endo-bicyclo[3.3.1]non-2-ene-4,9-dione (**2a**)**.**

^{1}H NMR (300,07 MHz, $CDCl_3$/TMS): δ = 1.38 (3H, s, H-33), 1.44 (1H, overlap, H-7), 1.48 (3H, s H-32), 1.55 (6H, s, H-25 and H-30), 1.64 (6H, s, H-20 and H-31), 1.66 (3H, s, H-21), 1.67 (3H, s, H-26), 1.92 (1H, overlap, H-27), 2.12 (1H, dd, J = 13.6 and 1.5 Hz, H_{eq}-6), 2.18 (1H, dd, J = 13.6 and 6.9 Hz, H_{ax}-6), 2.28 (1H, m, H-27), 2.50 (1H, dd, J = 13.9 and 7.7 Hz, H-22), 2.60 (1H, dd, J = 13.9 and 7.0 Hz, H-22), 3.12 (1H, dd, J = 13.9 and

7.0 Hz, H-17), 3.30 (1H, dd, J = 15.7 and 6.0 Hz H-17), 3.50 (3H, s, OMe), 4.87 (1H, t, J = 6.4 Hz, H-28), 4.95 (1H, t, J = 5.5 Hz, H-18), 5.05 (1H, t, J = 7.3 Hz, H-23), 7.29 (2H, t, J = 8.1 Hz, H-13 and H-15), 7.43 (1H, tt, J = 7.3 and 1.5 Hz, H-14), 7.61 (2H, dd, J = 7.3 and 1.5 Hz, H-12 and H-16).

^{13}C NMR (75.45 MHz, $CDCl_3$/TMS): δ: 17.8 (C-30), 17.9 (C-20), 18.1 (C-25), 23.5 (C-17), 23.8 (C-33), 25.6 (C-26), 25.7 (C-21), 26.0 (C-31), 27.2 (C-32), 30.0 (C-27), 41.9 (C-6), 30.1 (C-22), 48.5 (C-7), 49.5 (C-8), 61.3 (OMe), 63.4 (C-5), 73.0 (C-1), 119.5 (C-23), 120.9 (C-18), 122.1 (C-3), 125.0 (C-28), 127.9 (C-13 and C-15), 128.6 (C-12 and C-16), 132.1 (C-14), 132.6 (C-29), 133.1 (C-24), 134.8 (C-19), 137.0 (C-11), 170.9 (C-2), 193.5 (C-4), 198.0 (C-10), 209.4 (C-9).

^{1}H NMR (499,88 MHz, $CDCl_3$/TMS) [observed signal enhancement in NOESY 1D]: δ = 1.38 (Me_{ax}) [1.48 (Me_{eq}); 2.18 (H-6); 7.62 (H-12 and H-16); 5.05 (H-23)], 1.44 (H-7) [2.18 (H_{ax}-6); 2.28 (H-27); 4.87 (H-28)], 1.48 (Me on C-8) [1.38 (Me); 2.28 (H-27); 3.51 (MeO); 4.87 (H-28); 4.94 (H-23)], 2.28 (H-27) [1.48 (Me on C-8); 1,92 (H-27); 3.51 (MeO); 4.87 (H-28)], 2.50 and 2.60 (H-22) [1.67 (Me-26); 2.18 (H_{ax}-6); 5.04 (H-23)], 3.30 (H-17) [3.16 (H-17); 3.51 (MeO); 4.94 (H-18)], 7.61 (H-12 and H-16) [3.51 (MeO); 4.95 (H-18); 5.05 (H-23); 7.29 (H-13 and H-15)].

Acknowledgements

The authors are indebted to FAPESP and CNPq for financial support. SMFM is thankful to CAPES and UFS for scholarship and the leave of absence respectively. The authors are particularly grateful to Mr. Lúcio Leoni (curator of The Herbarium in Carrancas, MG, Brazil) for his indispensable help in locating a plant of *Tovomitopsis saldanhae*.

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