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NEMOROSONOL, A DERIVATIVE OF TRICYCLO-[4.3.1.0^{3,7}]-DECANE-7-HYDROXY-2,9-DIONE FROM *CLUSIA NEMOROSA**

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Key Word Index—*Clusia nemorosa*; Guttiferae; nemorosonol; polyisoprenylated benzophenone; 2D NMR.

Abstract—The structure and relative stereochemistry of nemorosonol, isolated from the fruits of *Clusia nemorosa*, has been determined by high field 2D ^{1}H(400 MHz) and ^{13}C(100 MHz) NMR spectroscopy. Nemorosonol represents a novel polyisoprenylated benzophenone with the acetate derived benzene ring modified to a tricyclo-[4.3.1.0^{3,7}]-decane skeleton.

INTRODUCTION

For some time, the genera *Garcinia*, *Clusia* and *Rheedia* (subfamily Clusioideae) have been known to be the source of a series of polyisoprenylated compounds [1–9]. Biogenetically, these molecules may be regarded as polyisoprenylated benzophenones in which the acetate derived benzene ring is modified to a bicyclo-[3.3.1]-nonane-2,4,9-trione moiety by intervention of prenyl groups. This is illustrated by the known representatives of this class of compounds, xanthochymol (**1**) [1, 2], isoxanthochymol [1], bronianone [3, 4] garcinol [5, 6] (=camboginol [5, 7]), isogarcinol [5, 6] (=cambogin [6, 7]); clusianone **2** [8] and kolanone [9]. The pertinent structures had been the subject of several revisions and were convincingly settled by means of X-ray crystallographic methods [2, 6, 8].

In this paper we describe the isolation and structural elucidation of nemorosonol (**3**) from *Clusia nemorosa*. Nemorosonol (**3**) is a new representative of this class of metabolites and is characterized by its unique tricyclo-[4.3.1.0^{3,7}]-decane-2,9-dione moiety.

RESULTS AND DISCUSSION

Nemorosonol (**3**) was obtained as optically active colourless cubes from the benzene extract of the fruits. Preliminary IR, MS and NMR spectral studies gave values strongly resembling those of other known members of this class of molecules. Thus, the mass spectrum (M^+ 502) afforded the elemental composition as $C_{33}H_{42}O_4$ and exhibited a base peak at *m/z* 105, arising from the diagnostic $[C_6H_5-CO]^+$ fragment ion [9]. The IR spectrum revealed the presence of a conjugated (ν_{max} 1620 cm^{-1}) and an unconjugated (ν_{max} 1725 cm^{-1}) carbonyl function in the molecule. The ^{1}H NMR spectrum (60 MHz, CCl_4) exhibited, besides the signals for an unsubstituted phenyl ring and the vinylic protons of three isoprenyl groups, a resonance (δ16) at a value typical for a hydroxyl proton in six-membered chelated ring [5]. In a similar manner to other members of this group of metabolites, nemorosonol, upon methylation, gave two isomeric enolethers as shown by the replacement of the enolic hydroxyl signal with a methyl resonance at either δ3.18 or 3.37 respectively. The mass spectra of both methyl derivatives exhibited the base peak at the same *m/z* value as found for the parent **3** while the IR and NMR spectra disclosed the presence of an additional, tertiary, hydroxyl function attached to the aliphatic part of the molecule and whose signals were obscured by exchange processes in the spectra of **3**.

The above findings distinguished nemorosonol from isomeric clusianone (**2**) and kolanone [8, 9], and were accomodated in partial structures **3a** and **3b** where, on the basis of the gross structure and the number of unsaturations (6), ring B of the new metabolite had to have a tricyclic arrangement of carbon atoms.

Information about the remaining structural details of nemorosonol were inferred from high-field ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR. The assignment of the resonances in terms of proton and carbon-13 chemical shifts, δ_H, δ_C, ^{13}C-multiplicities and interproton coupling constants, J_{HH}, were performed by means of standard one- and two-dimensional (1D, 2D) FT NMR techniques and the pertinent spectral data are collected in Table 1. First, proton 2D chemical shift correlation (COSY) [10] experiments were run to establish the presence of groupings with contiguous (uninterrupted by quaternary atoms) proton-proton connectivities *via* J_{HH} couplings. These were then followed by carbon-13 spectral editing (DEPT) [11] and carbon–proton heteronuclear chemical shift correlation experiments mediated by one-bond J_{CH} couplings [10]. Proton–proton coupling constants were obtained from 1D spectra.

Upon inspection of data related to proton-bearing carbon atoms it became immediately evident that, in

*Part 3 in the series 'Chemistry of *Clusia* genus'. For Part 2 see Delle Monache, F., Delle Monache, G., Cavalcanti, J. F. and Pinheiro, R. M. (1987) *Tetrahedron Letters* **28**, 563.

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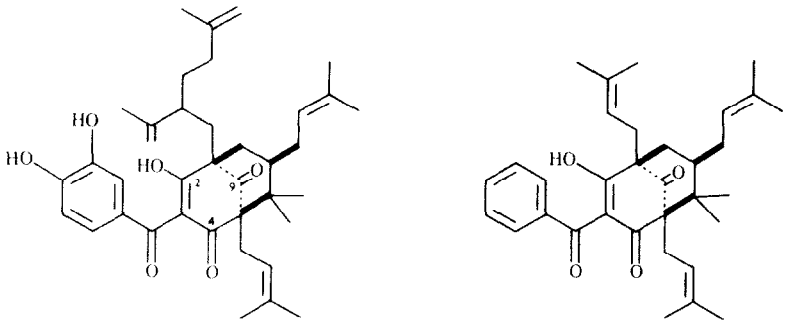
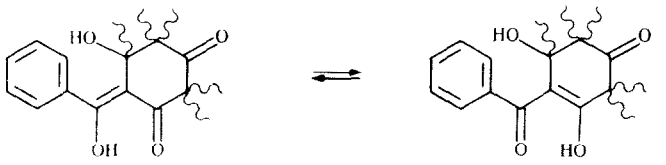
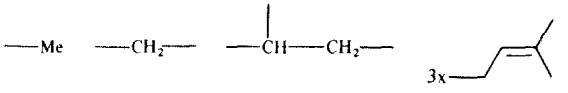


1 **2**



3a **3b**

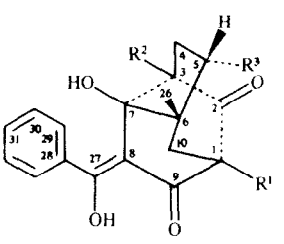


4a **4b** **4c** **4d**

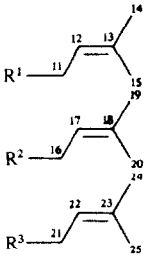
addition to the three isoprenyl groups (**4d**) detected in the preliminary proton spectra of **3**, partial structures **3a** and **3b** have to accomodate groups **4a**–**4c**. Since groups with just one free valence (**4a**, **4d**) may only act as substituents, this finding indicated that the three-ring system should include in its framework groups **4b**, **4c**, the six quaternary carbon atoms of ring B and a further quaternary C-atom giving its resonance in the aliphatic range, i.e. a total of 10 carbon atoms.

Assignment of the quaternary carbon resonances to individual sites of the molecule leading to the complete structure of the new metabolite was achieved by a series of heteronuclear carbon-proton 2D chemical shift correlation experiments mediated by multiple bond $^{n}J_{CH}$ (n = 2,3,4) spin–spin interactions [10]. The relevant carbon–proton connectivities revealed by these measurements are summarized in Table 2. Distinction between two-bond and longer range interactions was aided by a series of selective heteronuclear NOE difference experiments, giving major ^{13}C signal enhancements *via* two-bonds C-H dipolar interactions. As can be seen from the data in Table 2, multiple-bond carbon–proton connectivities gleaned by these experiments gave a direct definition of the tricyclo-[4.3.1.$0^{3,7}$]-decane-dione moiety complete with the positions of the individual substituent groups within the molecular framework.

Molecular models disclosed that, by its formation, the tricyclic system itself defines the relative configurations at the chiral centres C-1, C-3, C-6 and C-7. The relative stereochemistry at the remaining chiral carbon atom (C-5) was determined by homonuclear ^{1}H–^{1}H NOE experiments in which resonances due to H-4A, H-4B, H_3-26 were subjected in separate experiments to selective pre-irradiation. The resulting signal enhancements detected in the difference mode disclosed that the methyl group at C-6 and the isoprenyl substituent at C-5 assume a *trans* steric disposition with respect to the five-membered ring encompassing carbon atoms 3,4,5,6 and 7. The structure **3** of nemorosonol is now being studied by crystallography for determination of absolute configurations.



3



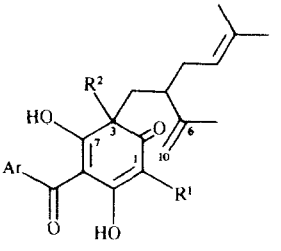


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Table 1. NMR spectral parameters*

Position	δ_C	δ_H	$^nJ_{i,j}$
1	63.61 (*s*)	—	—
2	209.42 (*s*)	—	—
3	62.81 (*s*)	—	—
4	40.36 (*t*)	2.05(A); 1.56(B)†	$^2J_{4A,4B} = -13.0$; $^3J_{4A,5} = 8$; $^3J_{4B,5} = 7$
5	48.66 (*d*)	1.57†	$^3J_{5,21A} = 7.6$; $^3J_{5,21B} = 7.6$
6	47.34 (*s*)	—	—
7	83.88 (*s*)	—	—
8	109.86 (*s*)	—	—
9	199.15 (*s*)	—	—
10	46.40 (*t*)	1.56(A)†; 1.48(B)	$^2J_{10A,10B} = -13.5$
11	25.73 (*t*)	2.92(A); 2.88(B)	$^2J_{11A,11B} = -15.3$; $^3J_{11A,12} = 7.5$; $^3J_{11B,12} = 7.5$; $^5J_{11A,14} = {}^5J_{11B,14} = {}^5J_{11A,15} = {}^5J_{11B,15} = 0.5$
12	120.20 (*d*)	5.59	$^4J_{12,14} = 1.5$; $^4J_{12,15} = 1.5$
13	133.46 (*s*)	—	—
14	26.15 (*q*)	1.71	
15	17.99 (*q*)	1.64	
16	29.88 (*t*)	2.61(A); 2.18(B)	$^2J_{16A,16B} = -15.3$; $^3J_{16A,17} = 7.5$; $^3J_{16B,17} = 7.5$; $^5J_{16A,19} = {}^5J_{16B,19} = {}^5J_{16A,20} = {}^5J_{16B,20} = 0.5$
17	120.38 (*d*)	5.15	$^4J_{17,19} = 1.5$; $^4J_{17,20} = 1.5$
18	133.09 (*s*)	—	—
19	26.07 (*q*)	1.54†	
20	17.74 (*q*)	1.40	
21	33.53 (*t*)	2.10(A);2.075(B)	$^2J_{21A,21B} = -15.0$; $^3J_{21A,22} = 7.5$; $^3J_{21B,22} = 7.5$; $^5J_{21A,24} = {}^5J_{21B,24} = {}^5J_{21A,25} = {}^5J_{21B,25} = 0.5$
22	123.81 (*d*)	4.95	$^4J_{22,24} = 1.5$; $^4J_{22,25} = 1.5$
23	131.52 (*s*)	—	—
24	25.83 (*q*)	1.63	
25	17.99 (*q*)	1.49	
26	19.12 (*q*)	0.90	
27	174.88 (*s*)	—	—
28	135.27 (*s*)	—	—
29	128.30 (*d*)	7.04†	$^3J_{29,30} = 7.5$
30	130.16 (*d*)	7.47†	$^3J_{30,31} = 7.8$
31	128.30 (*d*)	7.04†	$^3J_{31,32} = 7.8$
32	130.16 (*d*)	7.47†	$^3J_{32,33} = 7.5$
33	128.30 (*d*)	7.04†	
C-27–OH	—	16.00	
C-7–OH	—	2.00	

*In C_6D_6 at 25°. Chemical shifts (in ppm) relative to internal TMS. Mutual interproton couplings (in Hz) are given only once, at their first occurrence in the Table.

†Coupling constants for overlapping multiplets were obtained from spectra run in $CDCl_3$.

From a biosynthetic point of view, nemorosonol (**3**) and clusianone (**2**) or its bicyclic analogues, are closely related. The difference may consist in the number of cyclization steps of the presumed common precursor **5** [5]. A bicyclic system is generated by cyclization involving carbons 6-1, whilst a tricyclic skeleton may arise through the formation of two bonds between carbons C-6 (-7) and C-10 (-1) respectively.

EXPERIMENTAL

General. Low field NMR spectra were obtained on a Varian EM-360 instrument in CCl_4 soln. High-field ^1H (400 MHz) and ^{13}C (100 MHz) spectra were recorded on a Varian XLA-400 spectrometer in C_6D_6 and $CDCl_3$ solns at ambient temps. Of the two solvents, C_6D_6 was preferentially employed owing to the higher (~90%) predominance of the tautomeric form **3a** in this liquid. Standard pulse sequences were used to obtain the 2D correlation spectra. Proton–proton chemical shift correlation spectra were obtained in the absolute value (non-phased) mode acquiring the time-domain data into a 2048 × 512 data table which, after pseudo-echo filtering, was transformed into frequency-domain as a 2048 × 2048 data matrix. Heteronuclear 2D chemical shift correlation experiments were performed by acquiring the time-domain data in a 8192 × 512 data table and the transformation size was 8192 × 1024. One-bond ^1H–^{13}C chemical shift correlations spectra were run with broad-band ^1H-decoupling in the F1 dimension. Multiple bond ^1H–^{13}C chemical shift correlation spectra were obtained by the standard hetero-correlation sequence without ^1H decoupling in F1.

In these experiments, the relevant time-delay periods were systematically varied such as to obtain an efficient coherence transfer for a wide range of J_{CH} couplings. Selective heteronuclear NOE spectra were obtained in the difference mode, using

Table 2. Relevant multiple-bond ^{13}C-^{1}H connectivities in **3***

C	$^{n}J_{H}$					
1	$^{2}J_{10A}$;	$^{2}J_{10B}$;	$^{2}J_{11A}$;	$^{2}J_{11B}$		
2	$^{3}J_{10A}$;	$^{3}J_{10B}$;	$^{3}J_{11A}$;	$^{3}J_{11B}$;	$^{3}J_{16A}$;	$^{3}J_{16B}$
3	$^{2}J_{4A}$;	$^{2}J_{4B}$;	$^{2}J_{16A}$;	$^{2}J_{16B}$;	$^{3}J_{5}$	
6	$^{2}J_{5}$;	$^{2}J_{10A}$;	$^{2}J_{10B}$;	$^{2}J_{26}$		
7	$^{3}J_{4A}$;	$^{3}J_{4B}$;	$^{3}J_{16A}$;	$^{3}J_{16B}$;	$^{3}J_{26}$	
8	$^{3}J_{27OH}$;	$^{4}J_{26}$				
9	$^{3}J_{10A}$;	$^{3}J_{10B}$;	$^{3}J_{11A}$;	$^{3}J_{11B}$		
13	$^{3}J_{11}$;	$^{2}J_{12}$;	$^{2}J_{14}$;	$^{2}J_{15}$		
18	$^{3}J_{16}$;	$^{2}J_{17}$;	$^{2}J_{19}$;	$^{2}J_{20}$		
23	$^{3}J_{21}$;	$^{2}J_{22}$;	$^{2}J_{24}$;	$^{2}J_{25}$		
27	$^{2}J_{27OH}$;	$^{3}J_{29}$;	$^{3}J_{33}$			
28	$^{3}J_{27OH}$;	$^{2}J_{29}$;	$^{3}J_{30}$;	$^{3}J_{32}$;	$^{2}J_{33}$	

*Inferred from chemical shift coordinates (δ_{C_i},δ_{H_j}) of correlation peaks in 2D maps resulting from $^{n}(C_i,H_j)$ multiple-bond coherence tranfer. Correlations between C_ith carbon atom H_ith proton are represented by the pertinent coupling pathway, $^{n}J_{H_j}$.

carefully adjusted low-power ^{1}H pre-irradiation. Pre-irradiation times and power levels were set by running the ^{1}H spectra on the decoupled coil and using the coupler channel in the heteronuclear mode with low duty-cycle gating.

Homonuclear, ^{1}H–^{1}H, NOE experiments were performed in the difference mode using the frequency cycling technique for highly selective pre-irradiations [12]. Pre-irradiation times and irradiation power levels were carefully set before each experiment. In order to avoid signal overlapping in the spectral regions of interest, $CDCl_3$, C_6D_6 and their mixtures were alternatively used to prepare the samples.

Plant material. Fruits of *Clusia nemorosa* G. F. W. Meyer were collected near Maceio' (Alagoas, Brazil) and identified by Dr. G. Lopez Esteves (Meio Ambiente, Maceio'). Voucher specimens are deposited at the Herbarium of Centro Chimica dei Recettori under the cipher CN-84.

Extraction and fractionation. Roughly ground fresh fruits (950 g) were extracted with C_6H_6 (×2) to give a dark red residue (22 g) which on silica gel with C_6H_6–EtOAc afforded seven fractions; N1 (400 mg; C_6H_6–EtOAc, 19-1), N2 (2.5 g; 19-1), N3 (4 g; 19-1), N4 (6.6 g; 9-1), N5 (3.9 g; 9-1), N6 (1.1 g; 4-1) and N7 (2.7 g; 3-2). Further purification of N2 (silica gel; C_6H_6–hexane, 1:1) gave two chromatographically pure fractions, N2a (150 mg) and N2b (950 mg). Successive crystallization from MeOH and hexane of the latter gave crystalline nemorosonol.

Nemorosonol (**4**). Colourless cubes (mp 83–85° from hexane), which became yellow on exposure to light; $[\alpha]_D = +203$ (*c* 0.7). UV, λ_{max}^{MeOH} nm: 245 (log ε 4.05) and 338 (log ε 3.88); IR, $\nu_{max}^{CCl_4}$ cm^{-1}: 3550, 3510, 1725, 1615; ^{1}H NMR (60 MHz) δ: 15.30 (1H, *s*), 7.40 (5H, *s*), 5.30–4.70 (3H, *m*), 2.60–1.45 (*m*), 1.15 (3H, *s*); EIMS (probe) 70 eV, *m/z* (rel. int.): 502 $[M]^+$ (42), 487 (2), 484 (2), 474 (2), 456 (3), 433 $[M-C_5H_9]^+$ (28), 419 (2), 415 (2), 405 (7), 397 (4), 387 (2), 379 (6), 378 (5), 377 $[433-C_4H_8]^+$ (6), 366 (11), 311 $[433-C_9H_{14}]^+$ (100), 399 $[433-C_9H_{16}]^+$ (44), 298 (7), 295 (11), 255 (26), 241 (7), 233 (11), 229 (7), 191 (6), 177 (14), 163 (22), 123 (7), 121 (6), 105 $[C_6H_5-C\equiv O^+]$ (100), 77 (36), 69 (96). Methylation of nemorosonol (170 mg) with CH_2N_2 gave after purification on silica gel (C_6H_6–hexane, 2:1) the enol ethers **a** (70 mg) and **b** (70 mg). Enol ether **a**; oil $[\alpha]_D + 249$ (*c* 0.5). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3430, 1720 and 1615 cm^{-1}; ^{1}H NMR (60 MHz) δ: 7.95–7.75 (2H, *m*), 7.55–7.30 (3H, *m*), 5.05 (3H, *m*), 4.85 (1H, *s*, exchangeable with D_2O), 3.18 (3H, *s*), 2.55–1.45 (*m*), 1.05 (3H, *s*); ^{13}C NMR (25.2 MHz, $CDCl_3$) δ: 211.5 (CO), 195.2 (CO), 163.6 (=C–OR), 138.4 (C-1), 133.4, 133.3, 132.7 (3 × C=), 131.7 (C-31), 129.1 (*d*; C-29, C-33), 128.6 (*d*; C-30, C-32), 123.8, 120.3, 120 (*d*; × CH=), 116.8 (C=), 85.5 (C-OH), 62.8, 58.9, 48.6 (3 × >C–), 56.9 (*q* OMe), 48.4 (*d*, >C H), 48.2, 39.9, 33.0, 30.8, 25.8 (*t*; 5 × CH_2), 26.1, 26.1, 25.9, 20.1, 18.1, 18 and 17.7 (*q*; 7 × Me); EIMS (probe) 70 eV, *m/z* (rel. int.): 516 $[M]^+$ (8), 501 (1), 488 (1), 485 (1), 448 (20), 447 $[M-C_5H_9]^+$ (19), 433 (8), 419 (6), 411 (3), 401 (3), 393 (5), 391 $[447-C_4H_8]^+$ (14), 380 (7), 379 (5), 325 $[447-C_9H_{14}]^+$ (80), 323 $[447-C_9H_{16}]$ (35), 301 (3), 295 (5), 281 (7), 269 (50), 257 (5), 255 (7), 243 (20), 191 (8), 165 (13), 135 (7), 123 (5), 121 (5), 105 (100), 77 (20), 69 (70). Enol ether **b**; oil, $[\alpha]_D + 138$ (*c* 0.5). UV λ_{max}^{MeOH} nm: 294 (log ε 3.91); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3505, 1725, 1628; ^{1}H NMR (60 MHz), δ: 7.45–7.05 (5H, *m*), 5.30 (1H, *s*, exchangeable with D_2O), 5.0 (3H, *m*), 3.37 (3H, *s*), 2.55–1.45 (*m*), 1.18 (3H, *s*); ^{13}C NMR (25.2 MHz, $CDCl_3$) δ: 210 (CO), 192.4 (CO), 165 (MeO–C=), 132.9 (C-1), 132.7, 132.6 (3 × C=), 129.6 (*d*, C-31), 128.8 (*d*; C-29, C-33), 128.3 (*d*; C-30, C-32), 123.7, 120.6, 120 (*d*; 3 × CH=), 117.1 (C=), 85.6 (–C–OH), 65.6, 63.9, 48.4 (3 × –C–), 57.2 (*q*, OCH_3), 49.9 (*d*, CH), 45.1, 42.0, 33.4, 30.1, 25.3 (*t*; 5 × CH_2), 26.2, 26.0, 25.8, 18.8, 18.1, 18.0 and 17.9 (*q*, 7 × Me); EIMS (probe) 70 eV: 516 $[M]^+$ (3), 501 (3), 485 (3), 484 (6), 473 (3), 469 (5), 457 (3), 447 $[M-C_5H_9]^+$ (2), 419 (4), 415 (7), 325 $[447-C_9H_{14}]^+$ (5), 323 $[447-C_9H_{16}]^+$ (8), 311 (9), 309 (9), 297 (8), 295 (7), 281 (5), 269 (6), 265 (4), 255 (5), 247 (8), 241 (7), 161 (17), 135 (8), 129 (15), 123 (9), 121 (6), 105 (100), 77 (15), 69 (80). Both the enol ethers were recovered unchanged after acetylation (pyridine–Ac_2O) or methylation (Na_2CO_3–Me_2SO_4).

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