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Short Note

6-(1,3-Dihydroxy-3-phenylpropylidene)-5-hydroxy-2,2,4-trimethylcyclohex-4-ene-1,3-dione

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Abstract: A novel compound involved in the aroma of the fruit *Campomanesia lineatifolia* was isolated; the structure was determined by spectroscopic methods, mainly 1D and 2D NMR.

Keywords: champanone; chalcone; structure; biogenetic analysis

Introduction

Champanones A, B and C are compounds isolated from the fruit of *Campomanesia lineatifolia* R. & P. (Myrtaceae) [1,2]. These compounds are characterized by the presence of several methyl groups in the A ring of a flavonoid or chalcone. Here, we report the structure of the new champanone D on the basis of NMR, mainly HMBC experiment; in addition, the substitution pattern can explain the biosynthesis of the other compounds.

Results and Discussion

Champanone D, **2** was isolated as a yellow powder and its structure was assigned as follows. NMR spectra displayed the presence of three methyl groups due to the singlets at δ 1.43, 1.46 and 1.89 (3H each one); in addition, a dt (2H) was detected at δ 3.02 for a methylene group methylene, and a d

(J = 5.1 Hz) at δ 5.36 (1H). Finally, two more singlets were observed at δ 7.47 (5H, phenyl group) and at δ 13.84 (chelated hydroxyl group). COSY ^{1}H-^{1}H indicates a coupling between methylene at δ 3.02 with the singlet at δ 5.36; both signals correspond to a methylene and a methine group according to HMQC experiment.

Additionally ^{13}C JMOD experiment revealed the presence of three methyl groups at δ 7.94, 23.11 and 25.53, which according to HMQC experiment correlates with methyl groups located at δ 1.89, 1.46 and 1.43, respectively, then, the last two signals were assigned to *gem*-dimethyl group. Other detectable signals were a methylene group at δ 38.26, and three methine carbon atoms at δ 76.12, 125.85 and 128.97 coupling to the intense singlet at δ 7.47 in ^{1}H-NMR. Also six quaternary carbon atoms were displayed in ^{13}C JMOD at δ 52.40, 101.48, 107.35, 138.13, 161.32 and 182.91, as well as two carbonyl signals at δ 201.79 and 198.11. The existence of an oxygenated methine at δ 76.12 indicates the presence of an alcohol. A formula $C_{18}H_{20}O_5$ can be assigned to compound **2** based on these data.

When the spectroscopic properties of compound **2** were compared to those reported for other compounds isolated from *Campomanesia lineatifolia*, a high similarity was appreciated specifically with champanone B, since both possess three methyl groups (including a gem-dimethyl group), two carbonyl groups and a side phenyl ring (Figure 1).

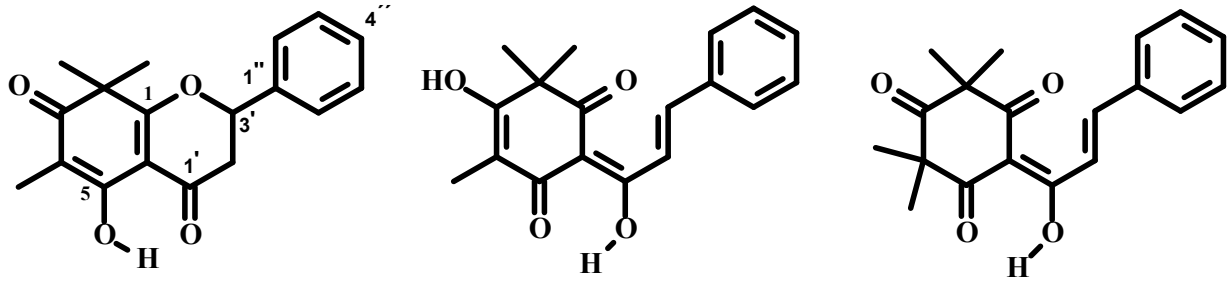

Figure 1. Structure of champanones from *Campomanesia lineatifolia.*

Nevertheless, the possibility that both compound have the same structure was excluded since ^{1}H and ^{13}C-NMR of champanone B were significantly dissimilar to those obtained in this work; in addition, HMBC experiment showed several anomalies. Thus, a carbonyl group at δ 198.11 ppm displayed long-range correlation with the methyl group and the gem-dimethyl group. However, the other carbonyl group at δ 201.79 only coupled to gem-dimethyl, which means coupling correlations involving five bonds (Figure 2, left). To meet all requirements of an HMB experiment, the new compound **2** should have several positions interchanged in relation to champanone B. If methyl and carbonyl groups were relocated as displayed in Figure 2 (right), all observed J^3 and J^2 long-range correlations would be correct.

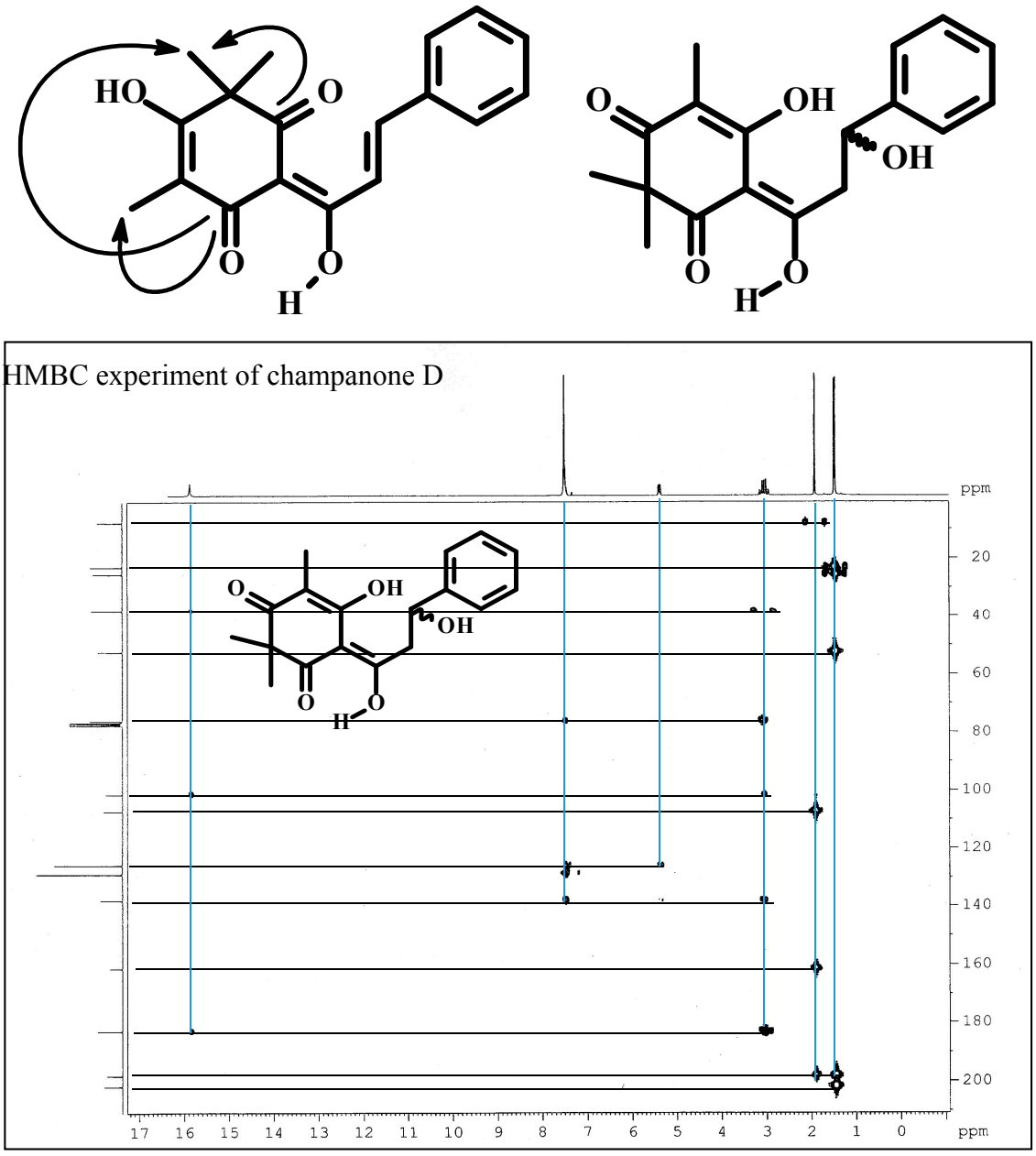

Figure 2. Improbable (left) and correct (right) structures of champanone D and HMBC ^{1}H-^{13}C.

Aside from champanone D, champanone C, whose chemical shifts are shown in Table 1, was also isolated.

The structure of champanone D could explain the biosynthesis of several types of compounds, since retro-enolization of the C-5 carbonyl group leads to two type of molecules. One of them with a gem-dimethyl at C-4 or free rotation around C-4, C-5 bond produces a molecule with a C-2-gem-dimethyl group, alike champanone C (Figure 3).

Phloroglucinol derivatives have mainly been reported in the species of the Myrtaceae and Hypericaceae families [3,4] and exhibit several biological activities including degenerative diseases and antibiotics, especially antivirus [5].

Table 1. ^{1}H and ^{13}C-NMR ($CDCl_3$) of several champanones, including champanone D **2**.

No.	Champanone C 1		Champanone C [1]		Champanone D 2		Champanone B [1]	
	δ^{1}H	**δ^{13}C**	**δ^{1}H**	**δ^{13}C**	**δ^{1}H**	**δ^{13}C**	**δ^{1}H**	**δ^{13}C**
1		186.4		186.0		161.32		197.4
2		48.84		48.4		52.40		48.2
2-Me	1.43 *s*	25.23	1.41 *s*	24.8	1.41 *s*	23.11	1.45 *s*	24.6
2-Me'	1.46 *s*	25.18	1.45 *s*	24.7	1.43 *s*	25.53	1.45 *s*	24.6
3		197.05		196.3		201.79		172.1
4		106.04		105.6		107.35		104.5
4-Me	1.82 *s*	7.08	1.80 *s*	6.6	1.87 *s*	7.94	1.92 *s*	6.7
5		164.50		164.0		182.91		191.0
6		103.53		103.1		101.48		105.8
1'		194.97		194.5		198.11		186.8
2'	2.85 *dd*	42.04	2.88 *dd*	41.6	3.00 *m*	38.26	7.92 *d*	123.3
	3.12 *dd*		3.10 *dd*					
3'	5.60 *dd*	81.62	5.58 *dd*	81.2	5.35 *d*	76.12	8.30 *d*	144.5
1"		126.45		129.6		138.13		135.3
2" y 6"	7.44 *m*	129.56	7.41 *m*	126.0	7.45 *s*	128.97	7.66 *m*	128.8
3" y 5"	7.48 *m*	130.03	7.47 *m*	129.1	7.45 *s*	128.97	7.39 *m*	129.0
4"	7.48 *m*		7.47 *m*	129.6	7.45 *s*	128.97	7.39 *m*	130.5
-OH	11.65 *s*		11.61 *s*		15.86 *s*		19.18 *s*	

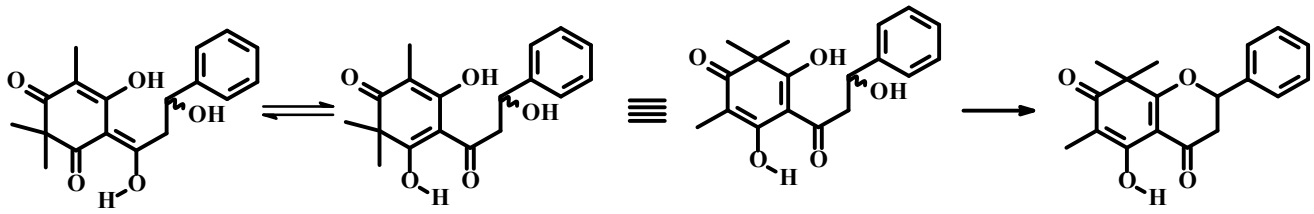

Figure 3. Proposed biosynthesis from Champanone D to Champanone C.

Experimental Section

^{1}H and ^{13}C-NMR spectra were recorded on a Bruker AMX ((Karlsruhe, Germany) operating at 300 and 75.0 MHz respectively, chemical shifts (δ) were reported in ppm and coupling constants (*J*) in Hz, $CDCl_3$ was used as solvent and internal standard. Infrared spectra were measured in KBr with a Thermonicolet Avatar 330 (Madison, WI, USA), mass spectra was recorded using a TQD triple quadrupole mass spectrometer with an orthogonal electrospray ionization source Z-spray (Waters, Milford, MA, USA) was used. Cone gas as well as desolvation gas was dry nitrogen. The cone gas and the desolvation gas flows were optimized at approximately 60 L/h and 1100 L/h, respectively. Other parameters optimized were: capillary voltage, 4.2 kV in positive ionization mode; lens voltage 0.3 V; source temperature, 105 °C and desolvation temperature, 400 °C. Dwell times of 0.01 s/transition were selected.

Silica gel 60 (200–300 mesh) (Merck, Darmstadt, Germany) and Sephadex LH-20 (Sigma, St. Louis, MO, USA) were used for all separations, while silica gel 60 F_{254} (Merck) was used for analytical thin

layer chromatography (TLC). The compounds were detected under UV light (254, 366 nm) and by spraying with H_2SO_4 (10%) followed by heating.

Dry seeds of *Campomanesia lineatifolia* (50 g) obtained in the local market were milled and then extracted by percolation (0.5 L) with ethanol. After evaporation, the residue (3.5 g) was dissolved in 100 mL of a mixture methanol/water (3:1 v/v), extracted with hexane (3 × 100 mL) and EtOAc (3 × 100 mL); the last extract yield a yellow powder (450 mg).

The compounds were purified by flash chromatography in a medium pressure chromatographic system, using a Biotage® SNAP Cartridge, KP-SIL, packed with 50 g of silica (50 μm), and run with Hex:EtOAc 95:5 (v/v) until EtOAc100%, flux rate 10 mL/min. Then, seventy fractions of 50 mL each were collected and monitored by tlc in silica gel chromatoplates 60 F_{254}, eluted with Hex:EtOAc (7:3, v/v), revealed with AcOH-H_2SO_4 spray and heated at 100 °C. Fractions with similar composition were mixed together to obtain only 7 chromatographic fractions. Fraction 3 (75 mg) was purified by repeated column chromatography until compound **1** (10 mg) was obtained, while from fraction 5 (65 mg) compound **2** was obtained (35 mg).

Champanone C, *5-hydroxy-6,8,8-trimethyl-2-phenyl-2H-chromene-4,7(3H,8H)-dione* (**1**), was isolated as yellow needles, m.p. = 152.3 °C–153.2 °C. MS (TQD EI+): *m*/*z* 321.27 (40) $[M+Na]^+$, 299.22 (70) $[M+H]^+$, 227.29 (100).

Champanone D, 6-(1,3-dihydroxy-3-phenylpropylidene)-5-hydroxy-2,2,4-trimethylcyclohex-4-ene-1,3-dione (**2**). Was isolated as amorphous yellow powder, m.p. = 139.5 °C–141.5 °C, FT-IR ν^{KBr}_{max} = 3045, 2989, 2930, 1734, 1645, 1630, 1440, 965. MS (TQD EI^+): *m*/*z* 339.25 (25) $[M+Na]^+$, 316.43 (70) $[M+H]^+$, 284.54 (100).

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Author Contributions

JFG and EC isolation and purification of compounds besides data analysis; WQ, data analysis and nmr support; FE study conception, structural analysis and manuscript writing.

Conflicts of Interest

The authors declare no conflict of interest.

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