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# Champanones, yellow pigments from the seeds of champa (*Campomanesia lineatifolia*)

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**Abstract**

Chemical investigation of the methanol extract of the seeds of *Campomanesia lineatifolia* Ruiz and Pav. (Myrtaceae) led to the isolation of two new β-triketone type compounds, named champanones A (**1**) and B (**2**), together with the known 2,3-dihydro-5-hydroxy-6,8,8-trimethyl-2-phenyl-4H-1-benzopyran-4,7(8H)-dione (champanone C) (**3**). The structures of **1** and **2** were determined to be 2,2,4,4-tetramethyl-6-(1-oxo-3-phenylprop-2-enyl) cyclohexane-1,3,5-trione (occurs as an enol form) and 2,2,4-trimethyl-6-(1-oxo-3-phenylprop-2-enyl)cyclohexane-1,3,5-trione (occurs as an enol form), respectively, by means of spectroscopic analysis. The three compounds showed mild antimicrobial activity.


*Keywords:* *Campomanesia lineatifolia*; Myrtaceae; Champanone; Champa; β-Triketones; Pigments

## 1. Introduction

Champa, (*Campomanesia lineatifolia* Ruiz and Pav.) (Myrtaceae), also known as palillo, guayaba de mono o guayaba de anselmo, is a fruit plant native to Amazonas region. However, currently this plant is widely distributed in Colombia, not only in the Amazonas but also in Antioquia, Boyacá, Cauca and Cundinamarca areas, where it is known for its edible fruits and for the pigments of its seeds, which are locally used in painting (Villachica, 1996). There are no studies reported on the chemical composition of this plant, and phytochemical investigation for *Campomanesia* species are very limited (Schmeda-Hirschmann, 1995; Limberger et al., 2001). As part of our research for bioactive compounds from Colombian plants (Duan et al., 2002; Nakano et al., 2004; Ramos et al., 2004; Nakagawa et al., 2004), we have investigated constituents of the seeds. The methanol extract of the seeds was found to contain yellow pigments. We report herein on the isolation and structure elucidation of the three pigments **1** and **2** and **3**, named as champanones A, B and C, respectively. In addition, antimicrobial activity of the three compounds is described.

## 2. Results and discussion

Champanone A (**1**) was isolated as yellow needles. The molecular formula of **1** was established as

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$C_{19}H_{20}O_4$ by HREIMS data. The IR spectrum showed a band at 1655 $cm^{-1}$ corresponding to a conjugated ketone and at 1555 $cm^{-1}$ for a hydrogen-bonded conjugated ketone. UV absorptions were found at 356, 240 and 204 nm confirming the presence of a conjugated system. The $^1H$ NMR spectrum of **1** showed signals for four singlet methyls ($\delta$ 1.41 (6H) and $\delta$ 1.46 (6H)), five-proton multiplets assignable to a phenyl group ($\delta$ 7.43 (3H) and 7.68 (2H)), a two-proton singlet ($\delta$ 8.02) and a one-proton singlet ($\delta$ 18.15) indicative of a very strong intramolecular hydrogen bond (Hellyer and Pinhey, 1966). The signal at $\delta$ 8.02 collapsed into AB doublet ($\delta$ 7.97 and 8.03, $J = 16.0$ Hz) when the $^1H$ NMR spectrum was recorded in $CDCl_3/CD_3OD$. The $^{13}C$ NMR of **1** exhibited signals due to the methyl groups ($\delta$ 23.9 and 24.1), eight quaternary carbons ($\delta$ 53.9, 57.1, 108.3, 146.8, 186.1, 197.6, 202.6 and 210.0), in addition to signals for a phenyl group ($\delta$ 129.0 (CH) × 2, 129.1 (CH) × 2, 131.2 (CH), 134.7 (C)). The HMQC spectrum correlated the singlet at $\delta$ 8.02 with the carbon signals at $\delta$ 121.0 and 146.8, confirming the presence of a *trans*-1,2-disubstituted olefin. The HMBC spectrum showed long-range correlations as summarized in Fig. 2 which showed that champanone A is a β-triketone type compound. It should be noted that the hydrogen-bonded enol-proton exhibited correlations with C-6, C-1′ and C-2′. Thus, it is clear that the C-1′ carbonyl presents as an enol form in $CDCl_3$ solution and the occurrence of other enol structures was ruled out. The fragmentation pattern in EI-MS, particularly *m*/*z* ions at 70 (dimethylketene) and 242 (M-dimethylketene), and 77, 103 and 131 (characteristics of the cinnamoyl group) further provided evidence for the structure of **1**. On the basis of these data Champanone A was determined to be the new β-triketone, 2,2,4,4-tetramethyl-6-(1-oxo-3-phenylprop-2(*E*)-enyl)cyclohexane-1,3, 5-trione (Fig. 1). This compound was stable in

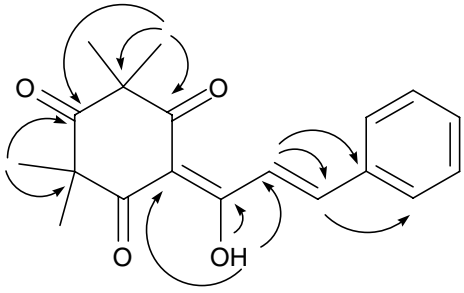


Fig. 2. Important long-range correlations observed for **1** in HMBC experiments.

$CDCl_3$ solution at least several days when stored at 4 °C. The complete assignments of $^1H$- and $^{13}C$ signals for **1** are shown in Table 1. Champanone A is a dehydro analogue of grandiflorone (**4**), isolated from steam-volatile oils of *Leptospermum* species (Hellyer and Pinhey, 1966; Porter and Wilkins, 1998; van Klink et al., 1999).

Champanone B (**2**) was isolated as yellow needles. The molecular formula of **2** was assigned as $C_{18}H_{18}O_4$ on the basis of HREIMS data. The EI-MS spectrum of **2** was similar to that of compound **1**, regarding to the presence of ions *m*/*z* 77, 103, and 131, indicating a cinnamoyl moiety, and of ions at *m*/*z* 242 (M-methylketene), 228 (M-dimethylketene) and 70 (dimethylketene) suggesting β-triketone structure. The IR spectrum showed a band at 1655 $cm^{-1}$ for conjugated carbonyl groups and at 1555 $cm^{-1}$ for a hydrogen-bonded conjugated ketone. The $^1H$ NMR spectrum of **2**, recorded immediately after dissolving in $CDCl_3$, showed signals for methyl singlets [$\delta$ 1.45 (two Me) and 1.93 (one Me)], AB doublet signals due to a *trans*-olefin ($\delta$ 8.30 and 7.92, $J = 16.1$ Hz), five-protons of a benzene ring, and two enol proton signals at $\delta$ 19.18 and 6.40. However, the spectrum became more complex within a few hours, suggesting that other tautomeric forms may be generated

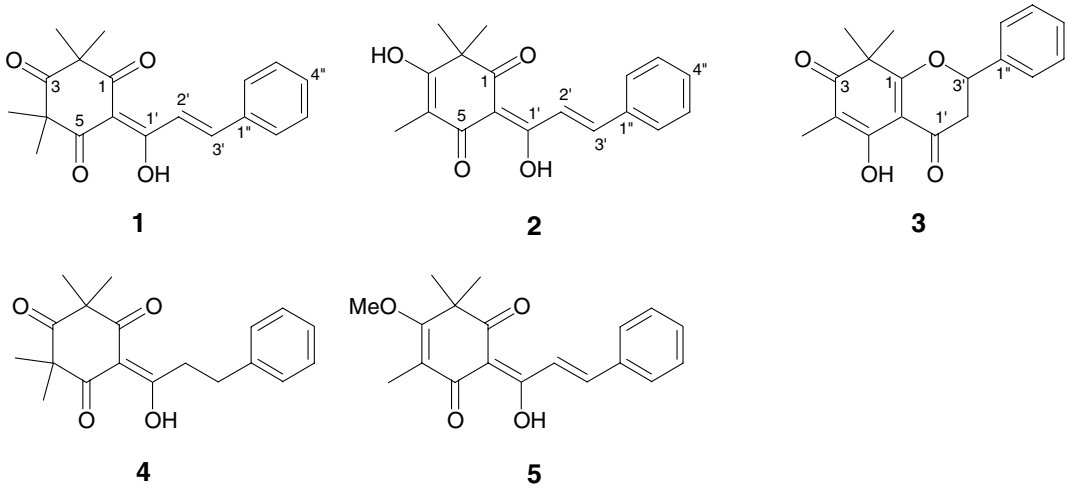


Fig. 1. Structures of compounds **1**–**5**.

Table 1
NMR data of compounds **1**–**3** ($CDCl_3$)

| No. | **1** | | **2** | | **2**[a] | | **3** | |
|---|---|---|---|---|---|---|---|---|
| | $\delta^1$H | $\delta^{13}$C | $\delta^1$H | $\delta^{13}$C | $\delta^1$H | $\delta^{13}$C | $\delta^1$H | $\delta^{13}$C |
| 1 | – | 197.6 | – | 197.4 | – | 196.8 | – | 186.0 |
| 2 | – | 57.1 | – | 48.2 | – | 48.5 | – | 48.4 |
| 2-Me | 1.41 *s* | 23.9 | 1.45 *s* | 24.6 | 1.35 *s* | 24.3 | 1.41 *s* | 24.8 |
| 2-Me′ | 1.41 s | 23.9 | 1.45 *s* | 24.6 | 1.35 *s* | 24.3 | 1.45 *s* | 24.7 |
| 3 | – | 210.0 | – | 172.1 | – | 175.9 | – | 196.3 |
| 4 | – | 53.9 | – | 104.5 | – | 103.5 | – | 105.6 |
| 4-Me | 1.46 *s* | 24.0 | 1.92 *s* | 6.7 | 1.82 *s* | 7.5 | 1.80 *s* | 6.6 |
| 4-Me′ | 1.46 *s* | 24.0 | – | – | – | – | – | – |
| 5 | – | 202.6 | – | 191.0 | – | 190.5 | – | 164.0 |
| 6 | – | 108.3 | – | 105.8 | – | 104.8 | – | 103.1 |
| 1′ | – | 186.1 | – | 186.8 | – | 185.7 | – | 194.5 |
| 2′ | 8.02 *s* | 121.0 | 7.92 *d*(16.1) | 123.3 | 7.83 *d*(15.5) | 123.5 | 2.88 *dd* (17.0, 3.8)<br>3.10 *dd* (17.0, 14.0) | 41.6 |
| 3′ | 8.02 *s* | 146.8 | 8.30 *d*(16.1) | 144.5 | 8.22 *d*(15.5) | 143.0 | 5.58 *dd* (14.0, 3.8) | 81.2 |
| 1″ | – | 134.7 | – | 135.3 | – | 134.9 | – | 129.6 |
| 2″, 6″ | 7.68 *m* | 129.0 | 7.66 *m* | 128.8 | 7.68 *m* | 128.4 | 7.41 *m* | 126.0 |
| 3″, 5″ | 7.43 *m* | 129.1 | 7.39 *m* | 129.0 | 7.46 *m* | 129.1 | 7.47 *m* | 129.1 |
| 4″ | 7.43 *m* | 131.2 | 7.39 *m* | 130.5 | 7.46 *m* | 130.6 | 7.47 *m* | 129.6 |
| enol OH | 18.15 *s* | | 19.18 *s* | | 19.34 *brs* | | 11.61 *s* | |
| enol OH | – | | 6.40 *s* | | Obscure | | – | |

Coupling constants (*J*) are given in Hz.
[a] NMR data in DMSO-$d_6$.

by equilibration since new enolic protons began to appear upon time at $\delta$ 18.71, 18.30 and 18.72. Analysis of the complex $^1$H NMR spectrum, particularly signals of down-field hydrogen-bonded protons and the AB doublets of olefinic protons indicated the formation of three compounds in addition to the original compound **2** (55% remained after one day). In spite of this unfavorable situation, the HMBC spectrum of **2** recorded within several hours after making a NMR sample exhibited cross peaks (Fig. 3) sufficient to assign the structure. On the basis of the whole data described above, the structure of **2** was established as 2,2,4-trimethyl-6-(1-oxo-3-phenyl-prop-(2*E*)-enyl)cyclohexane-1,3,5-trione (naming is for a carbonyl form) (Fig. 1). It is found that compound **2** exists exclusively in one of tautomeric structures, that is, C-3 and C-1′ bis-enol form in $CDCl_3$ solution (just after dissolution), since the enol proton at $\delta$ 19.18 (1′-O*H*) exhibited HMBC correlations with C-6 and C-1′. In contrast, the sample was much more stable in DMSO-$d_6$ than in $CDCl_3$. Single set of $^1$H/$^{13}$C signals (listed in Table 1) was obtained in DMSO-$d_6$ solution even after several days. The strongly hydrogen-bonded enolic proton (1′-O*H*) was observed at $\delta$ 19.34 as a very broad signal. In DMSO-$d_6$ solution, the molecule takes the same C-5, C-1′ bis-enol structure as found in $CDCl_3$, as judged from the similarity of the NMR spectra in both solvents. The crystalline sample stored at 4 °C was stable for several months. A methyl ether derivative (**5**) at C-3-OH of champanone B was recently isolated from *Desmos dumosus* (Annonaceae) and its X-ray structure was reported (Wu et al., 2002). The geometry of the C-6 and C-1′ double bond in **2** was tentatively assigned as *E* from analogy of the structure of **5**.

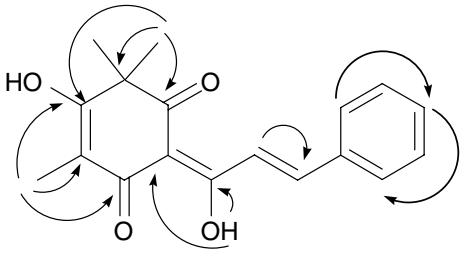


Fig. 3. Important long-range correlations observed for **2** in HMBC experiments.

Compound **3** was isolated as an amorphous yellow powder. The molecular formula of **3** was assigned as $C_{18}H_{18}O_4$ on the basis of HREIMS data. The $^1$H NMR spectrum showed signals for three singlet methyls ($\delta$ 1.41, 1.45 and 1.80), multiplet five-proton signals of a phenyl group ($\delta$ 7.39–7.49) and three protons ($\delta$ 2.88, 3.10, 5.58) assignable to a –CH(OR)–$CH_2$– moiety. Analysis of the HMQC and HMBC data established the structure of compound **3** as 2,3-dihydro-5-hydroxy-6,8,8-trimethyl-2-phenyl-4H-1-benzopyran-4,7(8H)-dione (naming for the particular enol from) (Fig. 1). The hydrogen-bonded enol-proton at $\delta$ 11.61 (5-O*H*) showed HMBC correlations with the expected three carbons (C-4, C-5 and C-6). Champanone C was stable in $CDCl_3$ solution at least a week. This compound has been recently reported from *Desmos* spp. (Annonaceae) without giving any trivial name (Wu et al., 2003). Thus, we named compound **3** as champanone C. The $[\alpha]_D$ value of champanone C was

~0°, although the configuration at C-3′ (C-2 of flavone numbering) was not further examined.

The three champanones displayed mild antimicrobial activity. Thus, compound **1** showed activity against *Micrococcus luteus*, MIC 30 μg/ml; *Staphylococcus aureus*, MIC 30 μg/ml; *Bacillus subtilis*, MIC 30 μg/ml; *Pseudomonas aeruginosa*, MIC 30 μg/ml; and *Streptococcus faecalis*, MIC 15 μg/ml. Compound **2** was active against *M. luteus*, MIC 30 μg/ml and compound **3** against *B. subtilis*, MIC 30 μg/ml; and *S. faecalis*, MIC 30 μg/ml. These results are in agreement with earlier work which described β-triketones as responsible for the antimicrobial activity of essential oils of *Eucalyptus* and *Leptospermum* species (Porter and Wilkins, 1998; Perry et al., 1997; Ghisalberti, 1996). Furthermore, it is also reported that Manuka oil, particularly a β-triketone rich chemotype, has activity against pathological bacteria, e.g., *Staphylococcus*, *Listeria*, *Enterococcus* and some fungi, e.g., *Trichophyton*, *Microsporum*, as well as anthelmintic and insecticidal activities (Douglas et al., 2001).

In conclusion, we have isolated two new yellow pigments, named champanones A and B, from the seeds of *C. lineatifolia* and established their structures. β-Triketone natural products were thought to be a relatively rare class of secondary metabolites, arising from multiple C-methylation of a $C_6$–$C_3$–$C_6$ type precursors. They are so far found only in Myrtaceae (mainly, *Eucalyptus* and *Leptospermum* genera) (van Klink et al., 1999; Ghisalberti, 1996), Annonaceae (*Uvaria, Desmos*) (Wu et al., 2002; Hufford et al., 1981) and Leguminosae (*Dalea*) (Dreyer et al., 1975) families. We recently isolated champanone A also from methanol extract of *Phyllanthus niruli* (Euphorbiaceae) (unpublished results). Systematic studies may reveal that β-triketone type compounds are more widely distributed in the plant kingdom.

## 3. Experimental

### 3.1. General

Melting points were determined on a Yazawa BY-1 hot-stage micro melting point apparatus and were uncorrected. Optical rotations were measured on JASCO DIP-360 polarimeter. IR spectra were recorded on a Perkin–Elmer FT-IR Paragon 500 spectrophotometer and UV on a Shimadzu UV-200 spectrometer. NMR spectra were measured in $CDCl_3$ on a Bruker DRX500 (500 MHz for $^1H$, 125 MHz for $^{13}C$) instrument using TMS as internal standard. When recorded in DMSO-$d_6$, $^1H$ chemical shifts were referenced to the residual proton of the solvent ($\delta_H$ 2.50) while $^{13}C$ chemical shifts were referenced to the solvent signal ($\delta_C$ 39.50). EIMS were recorded on a Shimadzu QP-5050 spectrometer with a direct inlet system at 70 eV, and FAB-MS and HREIMS on a JEOL JMS-AX505H.

### 3.2. Plant material

Fruits of champa (*Campomanesia lineatifolia*) were collected from Miraflores, Boyacá, Colombia, in July 2001 and identified by C. Garzón. A voucher specimen (BGF-220) has been deposited at the Instituto de Ciencias Naturales de la Universidad Nacional de Colombia, Bogotá, Colombia.

### 3.3. Extraction and isolation

The seeds (270 g dried weight) obtained from fruits of *C. lineatifolia* were milled and extracted with MeOH/acetone (1:1, v/v) two times at room temperature, during 48 h. Evaporation of the solvent under vacuum yielded 20.5 g of crude extract. This extract was subjected to silica gel flash column chromatography, using a discontinuous gradient of hexane, AcOEt and MeOH. Part of the MeOH eluate (400 mg) was subjected to silica gel column chromatography using a discontinuous gradient of hexane/AcOEt (10:1, 8:1, 6:1, 4:1, 2:1, 1:1) to give compound **1** (12 mg) in the fractions eluted with hexane/AcOEt 6:1. Compounds **2** and **3** were enriched in the fractions eluted with hexane/AcOEt 1:1 and 2:1, respectively, and subsequently separated by prep. TLC to give 41 mg of **2** and 14 mg of **3**.

### 3.4. Champanone A (*1*)

Yellow needles; m.p. 92–93 °C. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 356 (3.63), 240 (3.97), 204 (3.87); IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3030, 2980, 2940, 2870, 1720, 1655, 1620, 1549, 1410, 1040, 970; NMR data, see Table 1; EIMS *m/z* (%): 312 $[M]^+$ (100), 297 (6), 242 (7), 241 (11), 235 (11), 227 (8), 217 (8), 214 (10), 200 (8), 138 (20), 131 (65), 115 (8), 103 (33), 96 (98), 81 (37), 77 (13), 70 (11); HREIMS *m/z* 312.1332 (Calcd. for $C_{19}H_{20}O_4$, 312.1361).

### 3.5. Champanone B (*2*)

Yellow needles; m.p. 134–135 °C. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 376 (3.55), 310 (3.86), 229 (3.62); IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3560, 3090, 3010, 2980, 2940, 2860, 1720, 1655, 1621, 1580, 1520, 1470, 1430, 1230, 1170, 1030, 980, 940; NMR data, see Table 1; EIMS *m/z* (%): 298 $[M]^+$ (29), 283 (13), 281 (12), 255 (5), 242 (3), 228(5), 227 (11), 221 (22), 217 (29), 194 (13), 171 (9), 131 (100), 115 (16), 103 (51), 82 (15), 77 (35), 70 (11), 67 (30), 55 (9); HREIMS *m/z* 298.1175 (Calcd. for $C_{18}H_{18}O_4$, 298.1205).

### 3.6. Champanone C (*3*)

Yellow needles, m.p. 147–148 °C. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 375 (3.42), 316 (3.74), 230 (3.60); IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3560, 3433, 3090, 3010, 2980, 2927,

2365, 1720, 1672, 1623, 1580, 1562, 1470, 1411, 1287, 1257, 1230, 1176, 1033, 989, 940; NMR data, see Table 1; EIMS *m/z* (%): 298 $[M]^+$ (100), 283 (45), 255 (17), 242 (8), 228 (8), 227 (34), 207 (11), 194 (20), 193 (10), 166 (24), 151 (24), 138 (54), 131 (38), 123 (17), 115 (11), 110 (10), 105 (11), 104 (32), 103 (40), 96 (15), 91 (60), 83 (69), 77 (43), 70 (52), 55 (23); HREIMS *m/z* 298.1173 (Calcd. for $C_{18}H_{18}O_4$, 298.1205).

### 3.7. Antimicrobial assay

The antimicrobial testing of the pigments **1**–**3** was performed by the diffusion agar method (Acar, 1980), using *Micrococcus luteus* (UCMC 139) *Serratia marcescens* (UCMC140), *Salmonella enteriditis* (ATCC4931), *Bacillus subtilis* (ATCC21556), *Staphylococcus aureus* (ATCC65384), *Pseudomonas aeruginosa* (ATCC10145), *Escherichia coli* (ATCC8739), *Streptococcus faecalis* (UCMC145), and *Candida albicans* (ATCC10231) from stock cultures of the Departamento de Farmacia de la Universidad Nacional de Colombia. All assays were carried out in triplicate, seeded in Mueller Hinton agar for bacteria and yeast. Discs were impregnated with 30 μg of each pigment solubilized in DMSO-water and placed on the agar. Discs with streptomycine sulphate (30 μg) were also used for comparison. The cultures were incubated for 48 h at 35 °C for bacteria and 22 °C for *C. albicans*. Qualitative evaluation of the inhibition was performed by visual method, measuring the inhibition halos around the discs. Minimum inhibitory concentration (MIC) values were determined applying the broth dilution method (Amsterdam, 1996) only in the cases where the compounds showed inhibition halos of more than 3 mm.

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