

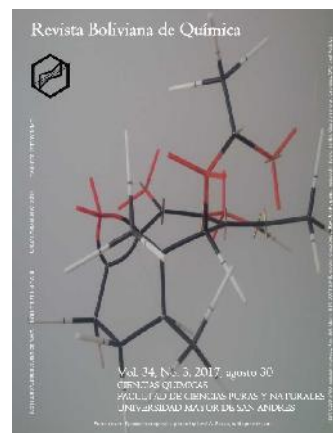


**SYNTHESIS OF THE NEW 4'-  
HYDROXY-3'-(3-METHYL-2-  
BUTENYL)CHALCONE  
BY MICROWAVE-ASSISTED  
CONDENSATION OF 4-HYDROXY-3-  
(3'-METHYL-2'-BUTENYL)  
ACETOPHENONE ISOLATED FROM  
SENECIO GRAVEOLENS AND  
BENZALDEHYDE**

**SÍNTESIS DE LA NUEVA 4'-HIDROXI-  
3'-(3-METIL-2-BUTENIL)CHALCONA  
POR CONDESACIÓN ASISTIDA POR  
MICROONDAS DE 4-HIDROXI-3-(3'-  
METIL-2'-BUTENIL) ACETOFENONA  
AISLADA A PARTIR DE SENECIO  
GRAVEOLENS Y BENZALDEHIDO**

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Gabriela Ibieta Jiménez<sup>1</sup>, José A. Bravo<sup>2</sup>, Yovana Quispe Coro<sup>3</sup>, Oscar Solís<sup>3</sup>, José L. Vila<sup>1,\*</sup>

<sup>1</sup>Natural Product Laboratory, Green Chemistry, Chemical Sciences Department, School of Pure and Natural Science FCPN, Universidad Mayor de San Andrés UMSA, P.O. Box 303, Calle Andrés Bello s/n, Ciudad Universitaria Cota Cota, phone +59122772269, La Paz, Bolivia, jvila@umsa.bo, www.umsa.bo

<sup>2</sup>Natural Product Laboratory, Phytochemistry, Chemical Sciences Department., School of Pure and Natural Science FCPN, Universidad Mayor de San Andrés UMSA, P.O. Box 303, Calle Andrés Bello s/n, Ciudad Universitaria Cota Cota, phone +59122792238, La Paz, Bolivia, jabravo@umsa.bo, www.umsa.bo

<sup>3</sup>Carrera de Química, Facultad de Ciencias Puras FCP, Universidad Autónoma Tomás Frías UATF, P.O. Box 36, Avenida del Maestro s/n, phones +591226227300, +591226223280, fax +591226226663, Potosí, Bolivia, quimicauatf@hotmail.com, quimica@uatf.edu.bo, www.uatf.edu.bo

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## ABSTRACT

In this article we report the synthesis of a new chalcone, 4'-hydroxy-3'-(3-methyl-2-butenyl)chalcone **2**, from the organic condensation assisted by microwave irradiation of benzaldehyde and 4-hydroxy-3-(3-methyl-2-butenyl)acetophenone **1**, the latter isolated from *Senecio graveolens*. Compound **2**, being of synthetic origin has not been so far isolated from natural sources. Structures were established by modern NMR techniques.

\*Corresponding author: [joselu62@hotmail.com](mailto:joselu62@hotmail.com)

## RESUMEN



En el presente artículo reportamos la síntesis de una nueva chalcona, 4'-hidroxi-3'-(3-metil-2-butenil)chalcona **2**, vía condensación orgánica asistida por irradiación de microondas de benzaldehído y 4-hidroxi-3-(3-metil-2-butenil)acetofenona **1**, aislada de la especie vegetal *Senecio graveolens*. El compuesto **2**, que es de origen sintético, no se ha aislado hasta ahora de fuentes naturales. Las estructuras fueron establecidas por técnicas modernas de RMN.

## INTRODUCTION

The species *Senecio graveolens*, Wedd. Asteraceae (quechua vernacular name: Chachacoma) is commonly used as traditional medicine remedy in the western highlands region of Bolivia, specially the Potosi department [1]. Medical indications of the fresh leaves aqueous hot infusion include stomachache, digestive relief like intestinal ache and flatulence; also the fresh leaves aqueous decoction is indicated for the treatment of anomalies of the menstrual cycle like white vaginal discharge and ovaries' disorders (emmenagogue) [1]. A specimen collected in La Paz Department was deposited at the Muséum d'Histoire Naturelle de Paris [2]. Previous research on the species afforded essential oil components: isovaleraldehyde, -pinene, -phellandrene, -terpinene, *p*-cymene, sabinene, -terpinene, 1-methyl-4-isopropenylbenzene, terpinolene, terpinen-4-ol, piperitenone, -eudesmol and -eudesmol; the antibacterial indexes of the essential oil over *Micrococcus luteus* and oxacillin-sensitive and oxacillin-resistant *Staphylococcus aureus*, and the antifungal activity over clinically isolated *Candida albicans* was established by these authors [3]. Another report on essential oils content mentions the characterization of sabinene, (+)-4-carene,  $\tau$ -terpinene,  $\beta$ -myrcene, 4-terpinenol and pulegone; the corresponding biological activity assays showed a strong activity against Gram (+) *Staphylococcus aureus* ATCC 29923 and a moderate activity against Gram (-) *Escherichia coli* [4]. Also, dihydroeuparin, 4-hydroxy-3-(isopenten-2-yl)acetophenone, 3-hydroxy-2,2-dimethyl-6-acetylchromane, 5-acetylsalicylaldehyde and 4-hydroxy-3(3'-hydroxyisopentyl)acetophenone were found in the aerial parts of the plant [5]. 4-hydroxy-3-(3-methyl-2-butenyl)acetophenone **1**, was found to be the major component of the alcoholic extract of the whole plant, and was assayed separately and as part of the alcoholic extract against breast cancer cell lines ZR-75-1, MCF-7 and MDA-MB-231, and non-tumorigenic MCF-10F cells: the alcoholic extract showed anticancer activity displaying a specific cytotoxic effect on cancer cells but compound **1** alone, didn't [6]. The plant is used in the highlands of Chile [6], Bolivia [7] and Peru and Argentina [8] against altitude hypoxia. A metabolomic altitudinal profiling study was done on samples collected between 4116 to 4611 masl in the XVth region of Chile; 2-hydroxy-5-(3-methylbut-2-enyl)acetophenone (**3**) besides compound **1**, 4-hydroxy-3-(3-methyl-2-butenyl)acetophenone were isolated and identified in the aerial parts of the plant [8]. The results showed differences in the chemical composition of *Senecio graveolens* growing at different altitudes and cytotoxic activity on MCF-7 cells (IC<sub>50</sub> 139  $\mu$ M) but no antibacterial properties for compound **3** [8]. The specific Gram-positive antibacterial activity of compound **1** isolated from *Senecio graveolens*, was established for methicillin-resistant strains of *Staphylococcus aureus* and *Mycobacterium smegmatis*, the compound was inactive against Gram-negative bacteria [9]. The permeabilizing effect of compound **1** on bacteria's membrane to avoid cell division was proposed by the authors [9]. *Senecio graveolens* was also the source of dihydroeuparin which was employed to afford synthetic oxime-derivatives of dihydroeuparin [10]. The aqueous 70% ethanol extract of leaves of *Senecio graveolens* collected in Bolivia's highlands at its lowest concentration produced a 12 mm inhibition halo in Petri dish on strains of *Staphylococcus aureus* [11]. Scopoletin was isolated from *Senecio nutans* and the phytochemical extract evaluated with respect to its antioxidant properties by ABTS and FRAP methods [12].

The condensation reported in the present article uses 4-Hydroxy-3-(3-methyl-2-butenyl)acetophenone (**1**) and benzaldehyde as precursors. 4-Hydroxy-3-(3-methyl-2-butenyl)acetophenone was previously isolated from *Senecio graveolens* and its antifungal properties were evaluated and defined regarding the mode of action, particularly against *Candida albicans* (strain ATCC 10231) [13,14]. Compound **1** is present as well in other plants like *Smilax sonchifolia* (Yacon) for instance [15]. Compound **1**, determined in *Helichrysum* species, was found to be active against Gram negative *Escherichia coli* and fungi like: *Penicillium sp.*, *Cladosporium herbarum* and *Phytophthora capsicii*; it was found inactive against *Aspergillus* species at a MIC of 100  $\mu$ g/mL [16], its full structure was established by conventional spectroscopic methods and by X-ray analyses [17]. Compound **1**, which is the major component of *Helichrysum italicum* was assayed and qualified as an inhibitor of leukotriene B<sub>4</sub> production at 100  $\mu$ M with a 95% inhibition (IC<sub>50</sub> 24  $\mu$ M) [18-20].

In this article we described the isolation of compound **1** from the aerial parts of *Senecio graveolens* whose chemical structure was established by us by the using of modern NMR techniques and the analysis of the corresponding spectra. Compound **1** was used in the organic condensation (*green chem* conditions) with benzaldehyde to afford the new: 4'-hydroxy-3'-(3-methyl-2-butenyl)chalcone **2**. As known, *green chem* conditions imply a number of advantages regarding classical synthetic methods, namely, cheap irradiation equipment, low temperatures, short irradiation times, short reaction times, access to energy control, potential better yields, eventual

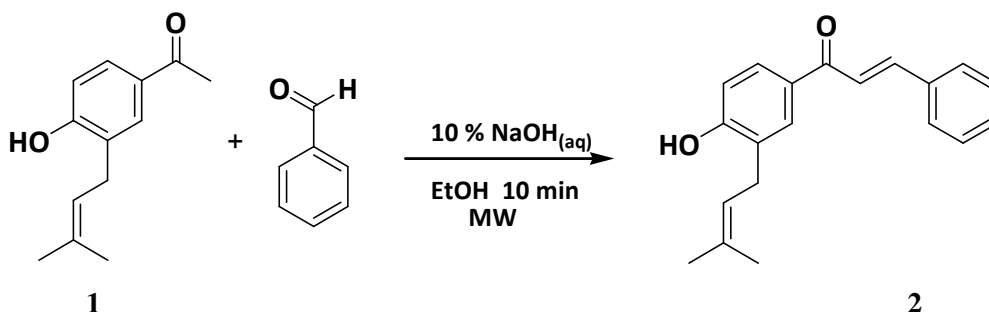


stereochemical specificity, environmental friendly solvents, etc. The present article is part of the further work we intend to develop in the field of MAOS (Microwave Assisted Organic Synthesis) [21].

## RESULTS AND DISCUSSION

### Microwave-assisted condensation synthesis of compound 2

The condensation reaction of **1** with benzaldehyde employed an alkaline medium (10% aqueous NaOH + 2 mL EtOH). After 10 minutes of irradiation, 98.8% aqueous AcOH<sub>glacial</sub> was added to the reaction mixture which collapsed into two phases subsequently separated in a separation funnel. The organic layer was extracted against water many times. Once evaporated the solvent of the organic layer a yellowish solid was submitted to an open LC column to afford the new 4'-hydroxy-3'-(3-methyl-2-butenyl)chalcone **2** whose structure was elucidated by NMR. Green synthesis of chalcones obey to the Claisen-Schmidt condensation which is easily attained with good yields depending on the nature of the reagents and desired products [21-23]. See Figure 1.



**Figure 1.** Microwave-assisted condensation of 4-Hydroxy-3-(3'-methyl-2'-butenyl)acetophenone (**1**) and benzaldehyde to afford 4'-hydroxy-3'-(3-methyl-2-butenyl)chalcone (**2**)

### Structural elucidation of 4-Hydroxy-3-(3'-methyl-2'-butenyl)acetophenone (**1**) isolated from the aerial parts of *Senecio graveolens*

Figure 2 shows the <sup>1</sup>H NMR full spectrum of **1**. Five spectral zones are distinguishable (all ciphers in ppm): 7.9-7.7 aromatics, alkenic, 7.0-6.8 aromatics, alkenic, 5.5-5.3 alkenic, 3.5-3.3, allyl, benzyl, 2.6-2.2, acetyl, and 1.8-1.7, allylic methyls. Assignments can be done *a priori* (see Figure 2). The integral value of the singlet of the two isolated and chemically equivalent methyl groups of isoprenyl is 5.86 for 6 protons δ 1.79 (*s*, 6H, CH<sub>3</sub>-C-3', H<sub>3</sub>C-4'). The next signal toward downfield is a singlet integrated as 2.94 for 3 acetyl protons δ 2.58 (*s*, 3H, CH<sub>3</sub>CO). Next peak integrated as 1.94 for 2 protons is the doublet for the allylic methylene equivalent protons of the isoprenyl group δ 3.40 (*d*, 2H, *J* 7.1 Hz, H-1'). Toward downfield we find the olefinic proton triplet of the isoprenyl group whose integral value is 1.00 for one proton δ 5.34 (*t*, 1H, *J* 6.6 Hz, H-2'). By rounding ciphers we find that protons 2' and 1' share the same coupling constant, namely *J* 7 Hz. We have assigned 12 protons so far, namely all protons of the benzene ring substituents (acetyl and isoprenyl, the hydroxyl proton is not visible in the proton spectrum). The signals of the three aromatic protons appear at down field: a doublet integrating as 1.22 or one proton δ 7.76 (*d*, 1H, *J* 8.2 Hz, H-5), and the doublet that integrates as 1.98 for two protons; first proton at δ 7.77 (*d*, 1H, *J* 9.5 Hz, H-6) and the second proton at δ 7.79 (*brs*, 1H, *J* indistinguishable, H-2). Obviously, the aromatic protons *J*-ortho coupling H-5/H-6 is defined by their proximate *J* values (8 and 9 Hz, respectively). The *J*-meta coupling between H-6 and H-2 is indistinguishable and it probably is less than 1 Hz. The full structural determination done *a posteriori* was achieved by the employ of 2DNMR experiments. The COSY experiment confirmed the <sup>1</sup>H NMR spectrum assignments (Figure 2). To the proton spectrum assignment (Figure 2) followed the carbon spectrum assignment (Figure 3) by means of the use of the proton assignments and the HSQC spectrum. These permitted the assignment of all hydrogenated carbons. For the assignment of the quaternaries we used the HMBC spectrum. The assignments written for the quaternaries in the carbon spectrum of Figure 3, namely C-3, C-1, C-3', C-4 and C=O, were deduced from correlations unequivocally established in the HMBC spectrum. The HMBC guided us thus to the establishment of the benzene ring correct substitution pattern as shown in Figure 2 or 4-Hydroxy-3-(3'-methyl-2'-butenyl)acetophenone (**1**). Figure 4 shows the H-C remote (<sup>2,3</sup>J<sub>H→C</sub>) correlations established from the HMBC spectrum.

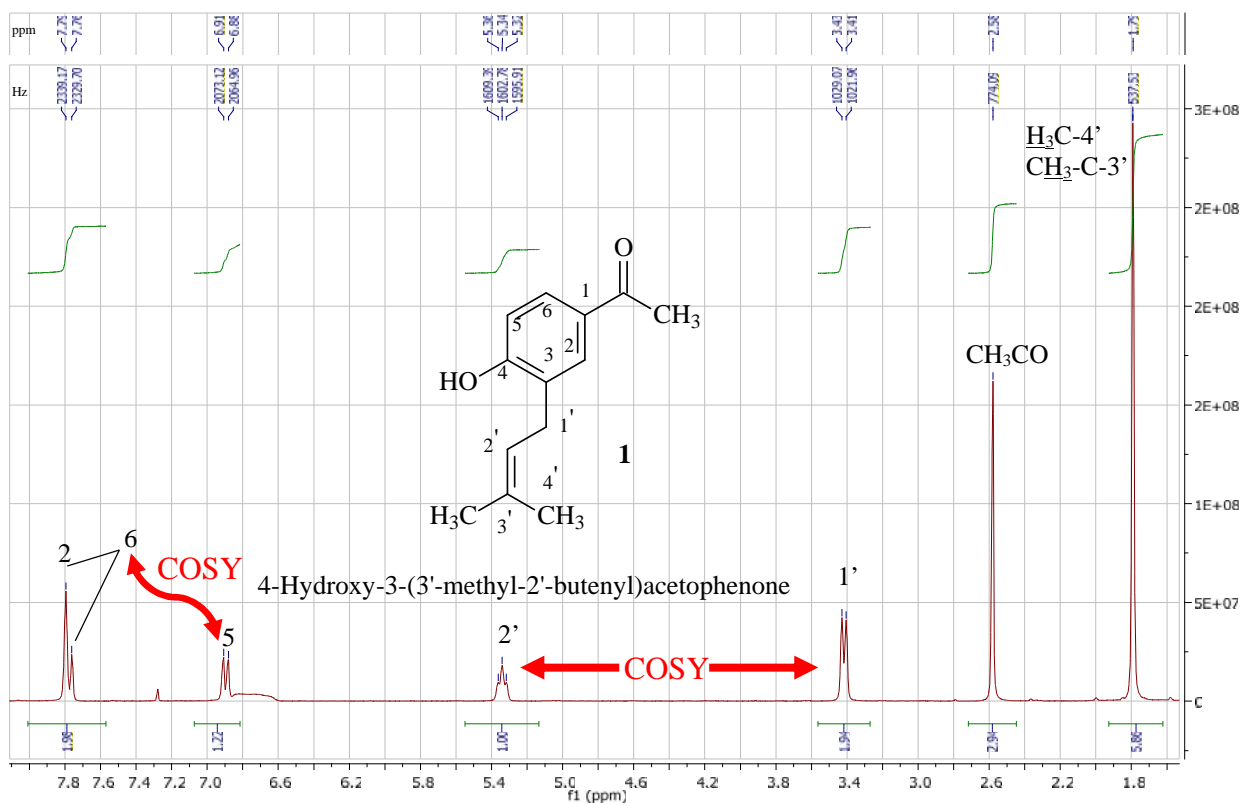


Figure 2. The  $^1\text{H}$  NMR spectrum of 4-Hydroxy-3-(3'-methyl-2'-butenyl)acetophenone (**1**), 300 MHz,  $\text{CDCl}_3$ ,  $\mu\text{H}$  from TMS

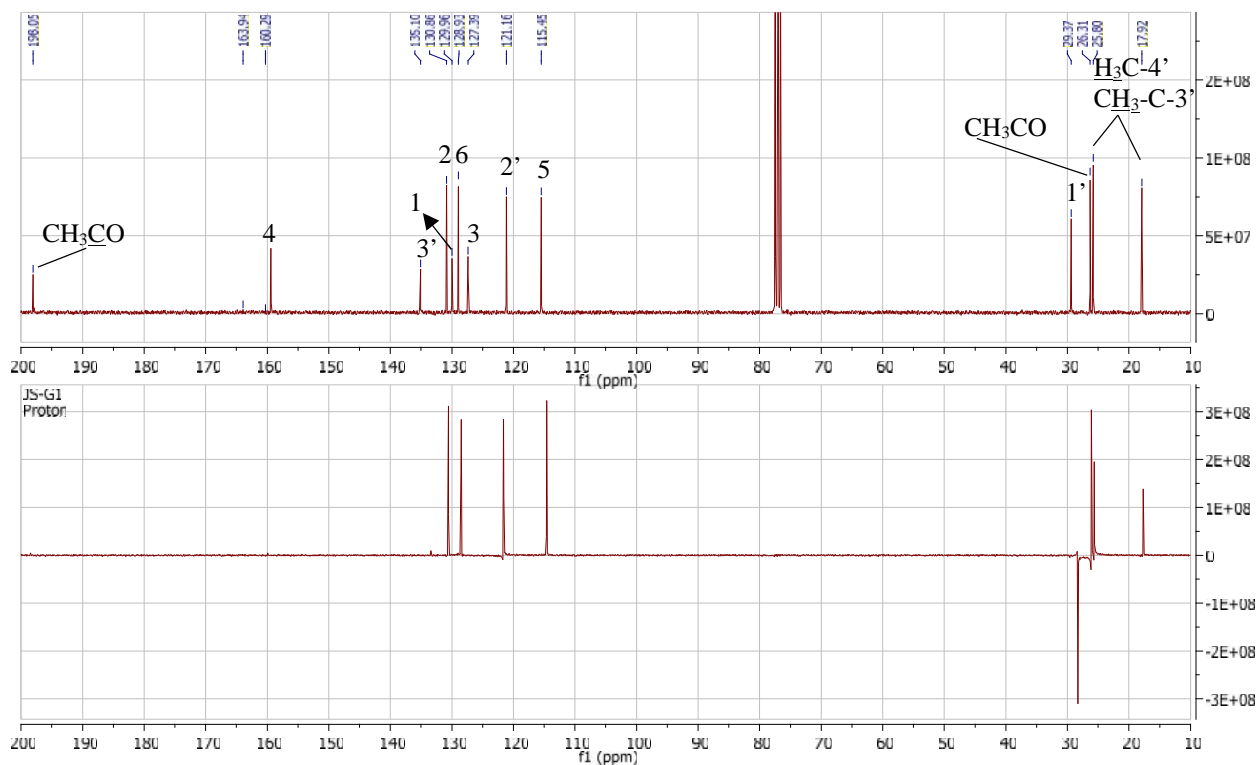
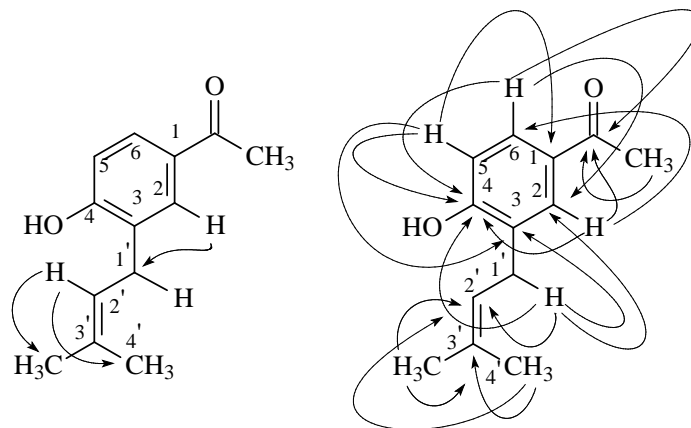


Figure 3. The  $^{13}\text{C}$  NMR spectrum and DEPT 135 experiment of 4-Hydroxy-3-(3'-methyl-2'-butenyl)acetophenone (**1**), 75 MHz,  $\text{CDCl}_3$ ,  $\mu\text{C}$  from TMS, assignments derived from the proton and HSQC assignments, complemented by HMBC correlations



Table 1 shows the NMR data for compound **1**. These data are congruent with those published for compound **1** in the literature [9,24].



**Figure 4.** H-C remote ( $^2J$ ,  $^3J$ ) correlations established from the HMBC spectrum of 4-Hydroxy-3-(3'-methyl-2'-butenyl)acetophenone (**1**)

**Table 1.** NMR data of compound **1**, HMBC correlations

Compound <b>1</b>		$\delta$ C ppm	$\delta$ C ppm	$\delta$ H ppm (J Hz)	HMBC H $^{2,3}$ J
Atom		<b>1</b>	Ref. [9]	<b>1</b>	<b>1</b>
<i>p</i> -Hydroxyacetophenone moiety					
1	C	129.96	130.2		
2	CH	130.86	130.8	7.79 brs	<u>COCH<sub>3</sub></u> , 6, 4
3	C	127.39	127.0		
4	C	159.41	159.1		
5	CH	115.45	115.5	7.76 d (8.2)	1, 3, 4
6	CH	128.93	128.9	7.77 d (9.5)	<u>COCH<sub>3</sub></u> , 2, 4
<u>COCH<sub>3</sub></u>	C	198.05	197.5		
<u>COCH<sub>3</sub></u>	CH <sub>3</sub>	26.31	25.8	2.58 s	<u>COCH<sub>3</sub></u>
<i>Isoprenyl moiety</i>					
1'	CH <sub>2</sub>	29.37	29.6	3.40 d (7.1)	2, 3, 4, 2'
2'	CH	121.16	121.0	5.34 t (6.6)	4', <u>CH<sub>3</sub>-C-3'</u>
3'	C	135.10	135.5		
4'	CH <sub>3</sub>	26.37*	26.3	1.79 s	2', 3'
CH <sub>3</sub> -C-3'	CH <sub>3</sub>	17.92*	17.9	1.79 s	2', 3'

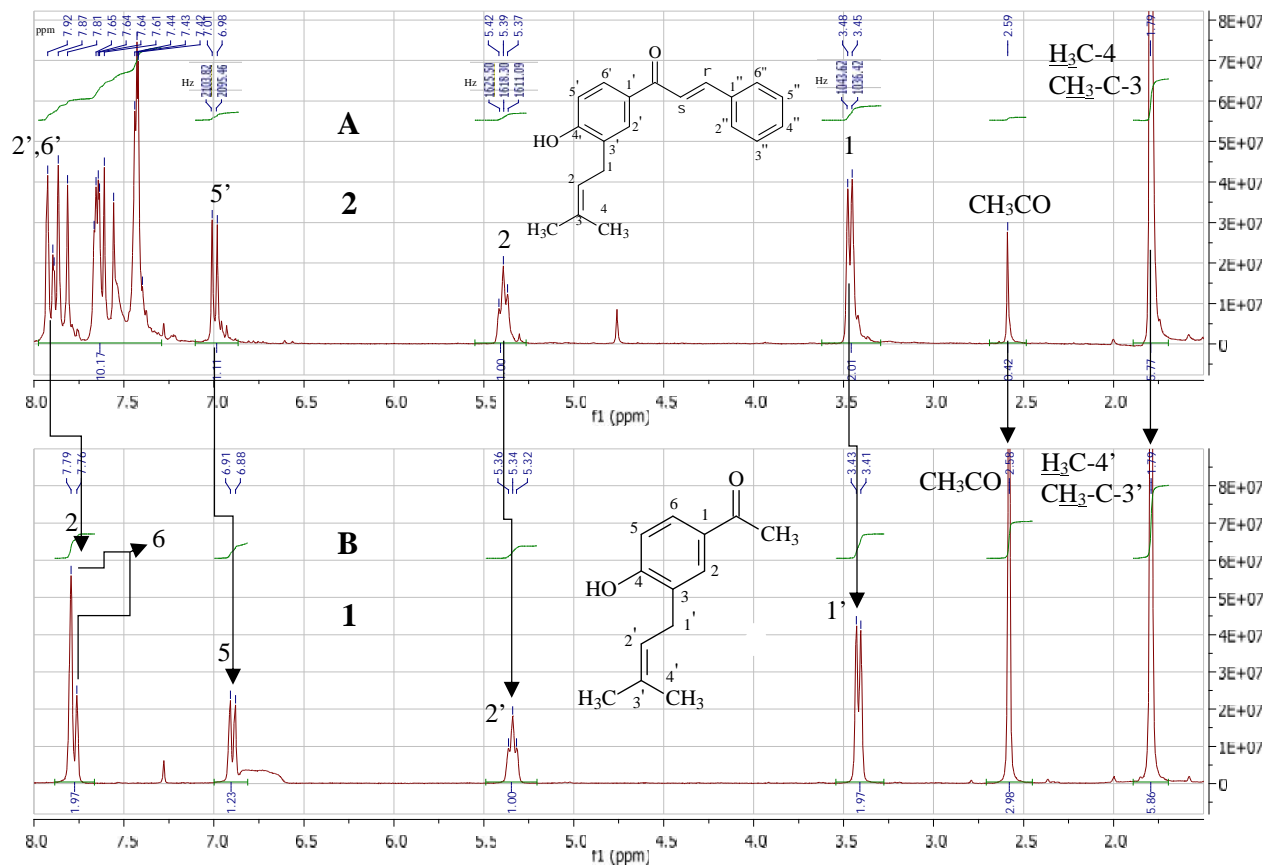
\*interchangeable values

#### Structural elucidation of 4'-hydroxy-3'-(3-methyl-2-butenyl)chalcone (**2**)

Figure 5 A and B is a comparative view of the  $^1\text{H}$ NMR spectra of compounds **2** and **1** respectively. This picture provides the spectral tool for the direct assignment of the *p*-hydroxyacetophenone and isoprenyl moieties of compound **2** thanks to the direct comparison of signals in both spectra (see partially assigned proton spectrum of **2** [Figure 5 A]). This means that protons H-5', H-2', H-1, CH<sub>3</sub>CO, H<sub>3</sub>C-4 and CH<sub>3</sub>-C-3 in Fig. 5 A can be directly assigned by simple comparison with the corresponding signals in the proton spectrum of **1** (Fig. 5 B). Moreover, from Figure 5 A it's deducible that the signal cluster in the range  $\delta$  8.00-7.25, includes the aromatic protons H-2' and H-6' of compound **2** (homologable to the aromatic protons H-2 and H-6 in compound **1**, Fig. 5 B). In this sense, it's possible *a priori*, on the basis of the similar shape of both signals, to assign the last three stripes at the left extreme of spectrum in Fig. 5 A to protons H-6' ( $\delta$  7.92, 7.90 and 7.89) and H-2' ( $\delta$  7.92) in **2**. Let us signal at this point that the assignment of H-6' ( $\delta$  7.90 and 7.89) and H-2' ( $\delta$  7.92) in **2** (Fig. 5 A) can be backed because there is a progressive shift toward low field of the proton signals of **2** (Fig. 5 A) with respect to those of **1** (Fig. 5 B) starting at



$\delta$  2.59 as follows:  $\delta$  2.58 (1)  $\rightarrow$   $\delta$  2.59 (2),  $\delta$  3.41 (1)  $\rightarrow$   $\delta$  3.45 (2),  $\delta$  3.43 (1)  $\rightarrow$   $\delta$  3.48 (2),  $\delta$  5.32 (1)  $\rightarrow$   $\delta$  5.37 (2),  $\delta$  5.34 (1)  $\rightarrow$   $\delta$  5.39 (2),  $\delta$  5.36 (1)  $\rightarrow$   $\delta$  5.42 (2),  $\delta$  6.88 (1)  $\rightarrow$   $\delta$  6.98 (2),  $\delta$  6.91 (1)  $\rightarrow$   $\delta$  7.01 (2),  $\delta$  7.76 (1)  $\rightarrow$   $\delta$  7.89 and 7.90 (2), and  $\delta$  7.79 (1)  $\rightarrow$   $\delta$  7.92 (2). Let us mention that the signal at  $\delta$  2.59 (2), is in reality the acetyl function of the acetophenone **1**, which remained in the mixture unreacted, or as a pollutant of the final product, the chalcone **2**; Figure 5 (2) proves this hypothesis since the integral value at  $\delta$  2.59 (2) is only half one proton with respect to the  $\delta$  2.58 (1) whose integral corresponds to 3 protons. The rest of integrals in **2** are congruent with the structure. Signals between  $\delta$  7.87 and  $\delta$  7.40 (Figure 6), should include the resonances for the aromatic protons 1''-6'' and the r,s-ethylenic system (see the expansion of this zone in Figure 6).



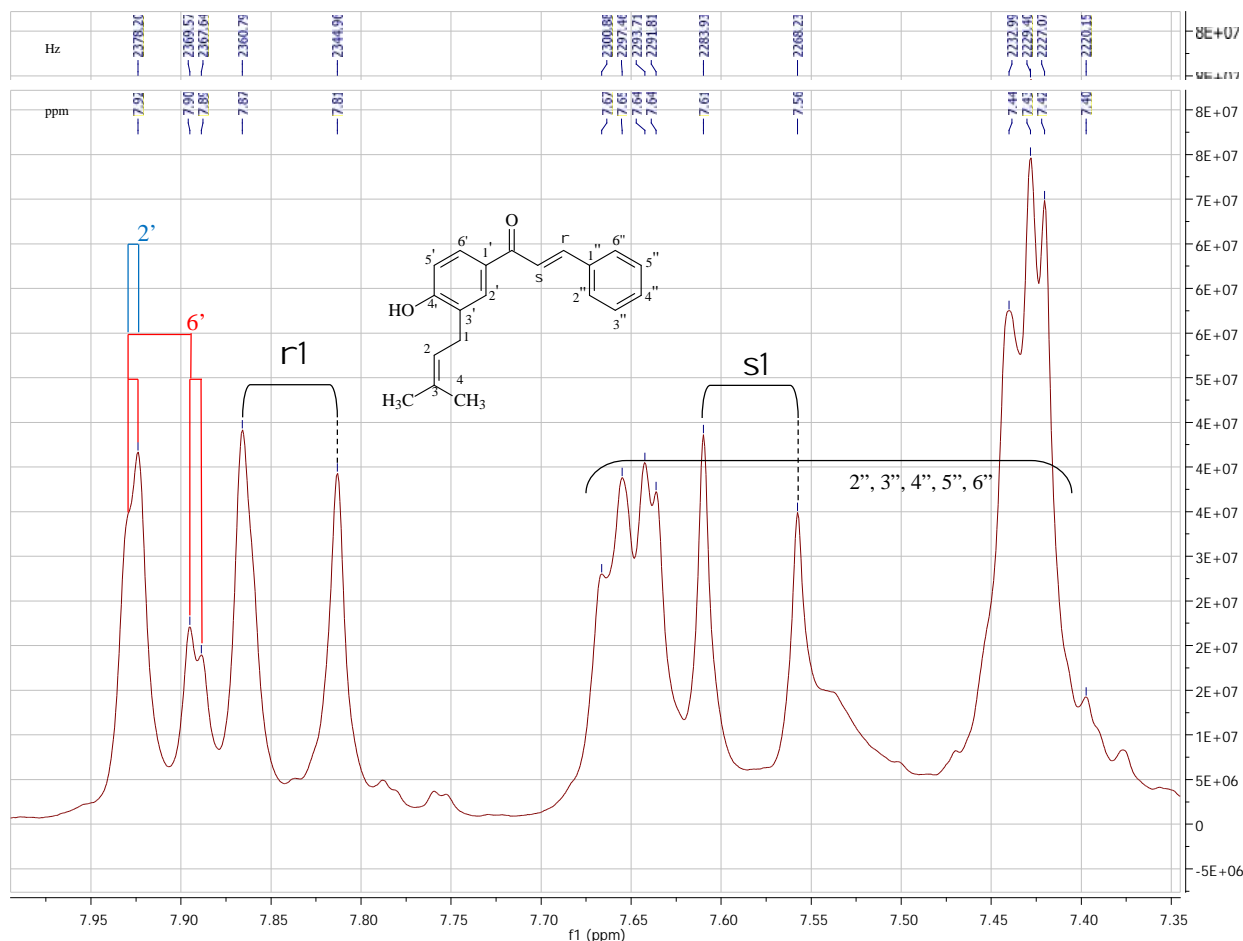
**Figure 5.** Comparative view of  $^1\text{H}$  NMR spectra of A: 4'-hydroxy-3'-(3-methyl-2-butenyl)chalcone (**2**) and B: 4-Hydroxy-3-(3'-methyl-2'-butenyl)acetophenone (**1**), 300 MHz,  $\text{CDCl}_3$ ,  $\text{U}_M$  from TMS

The expansion (Fig. 6) allows now to establish the *meta* coupling for H-6'  $\rightarrow$  H-2' in chalcone **2** by the measuring of  $J$  in Figure 6. The measured values are:  $^3J_{6' \rightarrow 5'} = 10.56$  Hz and  $^4J_{6' \rightarrow 2'} = 1.93$  Hz, both values are between the acceptable ranges for vicinal and long range proton couplings respectively in aromatic rings. For the characterization of the ethylenic system and the monosubstituted benzene ring of compound **2** it resulted very useful the employ of a bibliographic reference [21] and references therein, where a similar compound (benzylideneacetophenone or *trans*-chalcone) was structurally described, from where we extracted the  $^1\text{H}$  NMR spectrum (Figure 7, excerpt authorized by Bolivian Journal of Chemistry), punctually, the expansion of the aromatic and ethylenic systems. Figure 7 here, corresponds to Figure 2 in reference [21]. The ethylenic system appears in Fig. 7 as proton **B** with stripes at  $\delta$  7.87 and 7.82, and proton **C** with stripes at  $\delta$  7.59 and 7.53. Their respective equivalences in Fig 6 (Compound **2**) are: proton **s** with stripes  $\delta$  7.87 and 7.81, and proton **r**, stripes  $\delta$  7.61 and 7.56. The coupling constant  $^3J_{r \rightarrow s} = 15.8$  Hz is congruent with a "*trans*" coupling. The five aromatic protons of the vinyl-phenyl moiety of the *trans*-chalcone [21] (signaled as protons **D** in Fig. 7) are described there mostly by the four stripes:  $\delta$  7.69, 7.68, 7.67 and 7.66 and the three stripes:  $\delta$  7.46, 7.45 and 7.44. The corresponding signals in Fig. 6 (compound **2**) are: the four stripes  $\delta$  7.67, 7.65, 7.64 and 7.64, and the three stripes:  $\delta$  7.44, 7.43 and 7.42. These aromatic signals are not analyzable with regard to their coupling constants.





The last correlations between Fig. 6 and Fig. 7 are the remotely coupled aromatic protons 6' and 2' in compound **2** with stripes  $\delta$  7.92, 7.90 and 7.89, whose corresponding stripes in Fig. 7 are  $\delta$  8.07, 8.06, 8.04 and 8.04 designated as protons A. The difference between the chemical shifts of protons A (Fig. 7) and protons 6' and 2' (Fig. 6) like for instance  $8.06_{\text{Fig.7}} - 7.90_{\text{Fig.6}} = 0.16$  ppm lies on the structural difference between them, namely the isoprenyl substituent in compound **2** (Fig. 6), absent in the *trans*-chalcone. This  $^1\text{H}$  NMR comparative analysis proves by itself the identity of compound **2** as 4'-hydroxy-3'-(3-methyl-2-butenyl)chalcone. See Table 3 for the NMR data of **2**.



**Figure 6.** Expansion of the aromatic-ethylenic zone of the  $^1\text{H}$  NMR spectrum of 4'-hydroxy-3'-(3-methyl-2-butenyl)chalcone (**2**)

We have performed 2D NMR experiments with compound **2**. The COSY experiment clearly shows correlation peaks for vicinal ( $^3J$ ), and some remote couplings ( $^4J$ ). In the isoprenyl moiety:  $\text{H-2}^{3J_{\text{HH}}} \rightarrow \text{H-1}$  and  $\text{H-2}^{3J_{\text{HH}}} \rightarrow \text{H}_3\text{C-4}$ ,  $\text{H-2}^{3J_{\text{HH}}} \rightarrow \text{H}_3\text{C-3}$ . In the *p*-hydroxyacetophenone moiety:  $\text{H-6}^{3J_{\text{HH}}} \rightarrow \text{H-5}'$  and  $\text{H-6}^{4J_{\text{HH}}} \rightarrow \text{H-2}'$ . In the vinylphenyl moiety:  $\text{H-}\alpha^{3J_{\text{HH}}} \rightarrow \text{H-s}$ . The COSY expansion (Figure 8) shows a discrepancy with respect to the proton analysis based on Fig. 5, 6 and 7 regarding the coupling between H'6' and H-2'. The 2D experiment shows with this respect a pair of symmetrically disposed crosspeaks connecting H-5' with H-6', the latter being described in the horizontal proton projection of COSY with stripes at  $\delta$  7.90, 7.89 and 7.87 instead of  $\delta$  7.92, 7.90 and 7.89 as assigned in Figure 6. However, the comparative analysis of the  $^1\text{H}$  NMR spectra of compound **2** and *trans*-chalcone [21] affirms the results deduced from the 1D analysis purely. Figure 9 is a comparative view (and its expansions) of the  $^{13}\text{C}$  NMR spectra of **2** and **1**. This comparison allowed the assignment of the corresponding carbon signals in **2** (see Table 3), complemented by the employ of the HSQC spectrum (see Table 3). Figure 9 shows that all carbons corresponding to the moiety derived from the precursor 4-hydroxy-3-(3'-methyl-2'-butenyl)acetophenone (**1**) currently condensed with benzaldehyde under the form of compound **2**, can be directly assigned in **2** by examination of both spectra (comp. **1** and **2**). The assignment was done, indeed, by the close similarity of their chemical shift values (see Table 3). In Fig. 9 A' there remain 6 signals not assigned yet. From these, two correspond to alkenic carbons (r and s) and four to aromatic signals for the six aromatic carbons of the mono substituted benzene ring of compound **2**, namely:

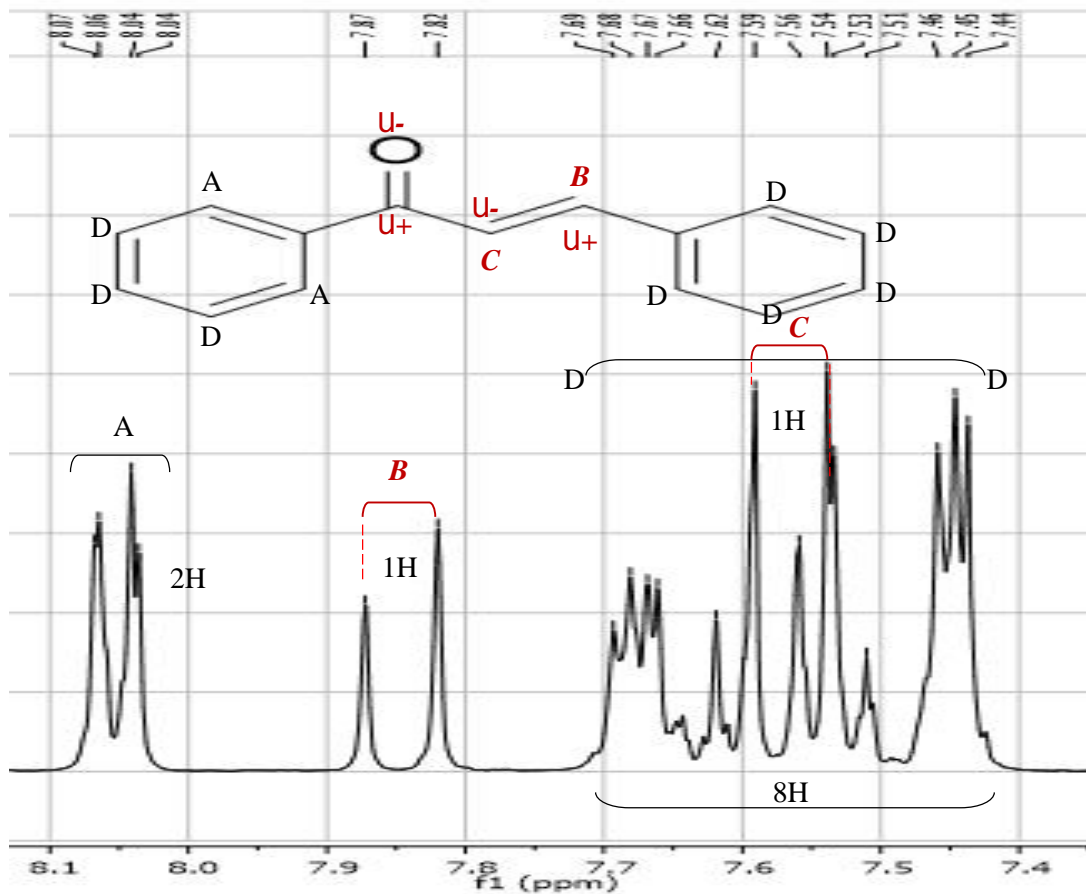


Figure 7. Excerpt from ref. [21] corresponding to: “Figure 2.  $^1\text{H}$  NMR spectrum of **1**, 300 MHz,  $\text{CDCl}_3$ ,  $\delta_1$  from TMS. Assignment by comparison with spectrum from the literature [7]”. With permission of Revista Boliviana de Química.

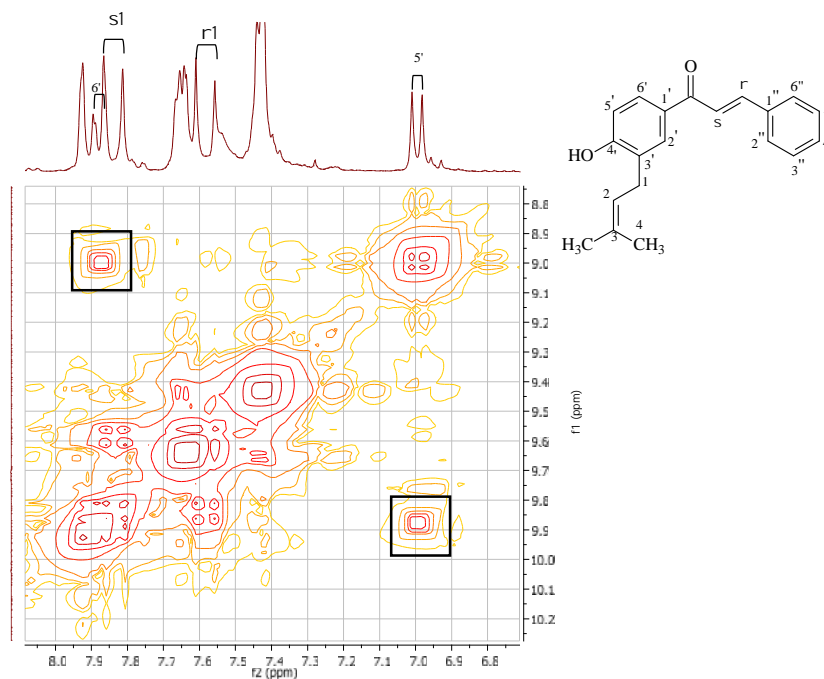


Figure 8. Expansion of the aromatic-ethylenic zones, COSY experiment of compound **2**



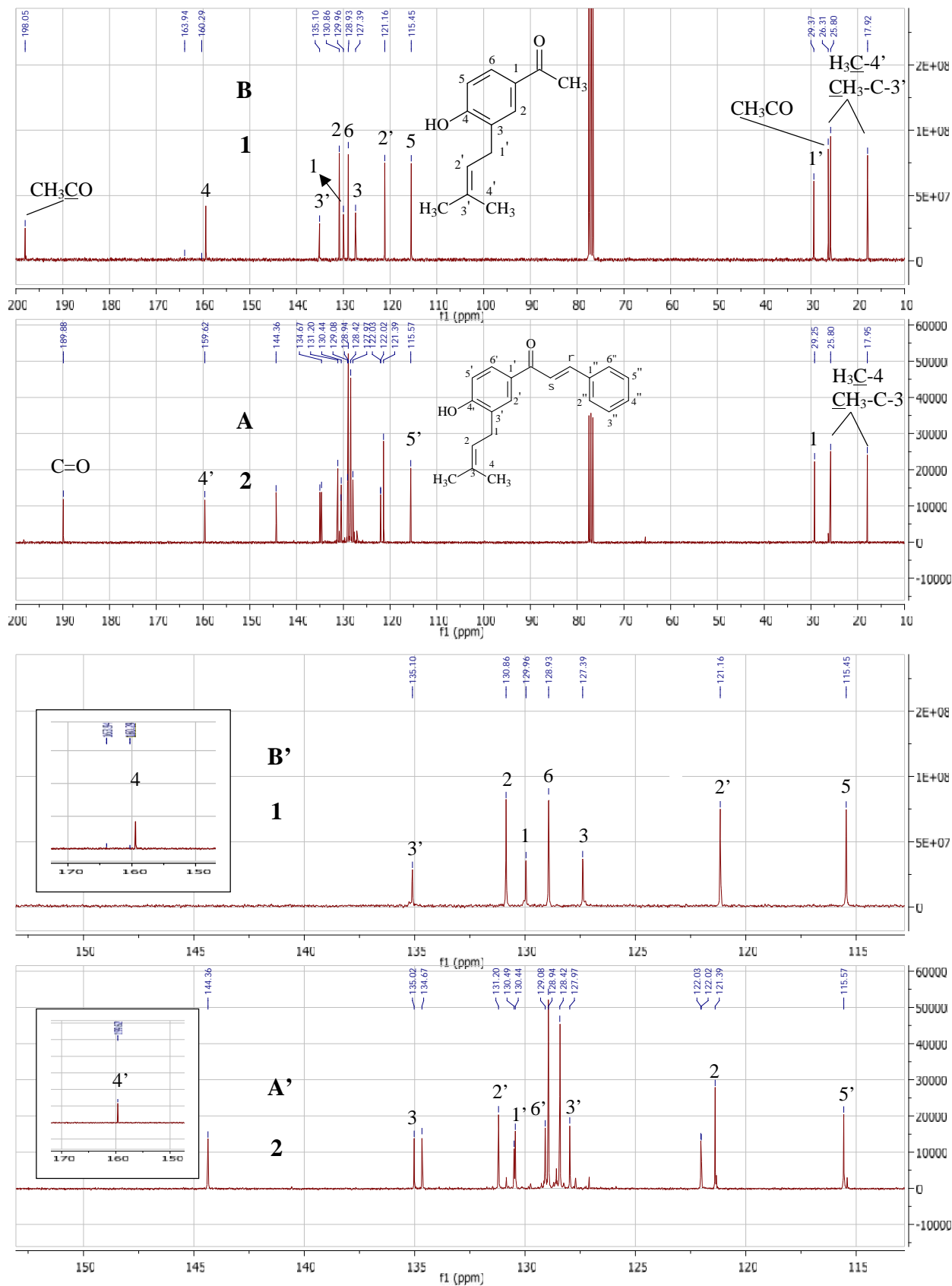


Figure 9. Comparative view of <sup>13</sup>C NMR spectra of A: compound 2 and B: compound 1, 75 MHz, CDCl<sub>3</sub>,  $\delta_c$  from TMS



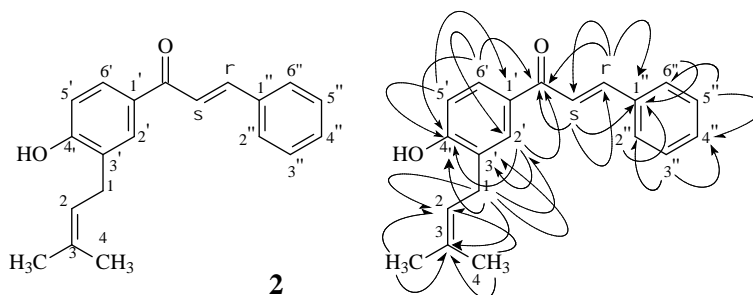
C-1'', C-2'',6'', C-3'',5'', C-4''. Table 2 shows the assignment of these six signals in compound **2** on the basis of calculations of chemical shifts from table C120 [25] (for the four aromatic signals), table C90 [26] (for the alkene  $\alpha,\beta$ ), and the chemical shift values of *trans*-chalcone [21]. This analysis is the completion of the full assignment of the  $^{13}\text{C}$  NMR spectrum of **2**.

**Table 2.** Assignment of  $^{13}\text{C}$  of the vinylphenyl moiety of compound **2**

$\text{ul}[\text{ppm}]$ (Fig. 9 A')	$\text{ul}[\text{ppm}]$ (calculated Ref. [25,26])	$\text{ul}[\text{ppm}]$ <i>trans</i> -chalcone Ref. [21]	Assignment Compound <b>2</b>
144.36	135.8 (C-r) <sup>[26]*</sup>	144.9 (C-r)	CH-r1
134.67	137.3 (C-1'') <sup>[25]</sup>	134.9 (C-1)	C-1''
130.49	127.7 (C-4'') <sup>[25]</sup>	130.6 (C-4)	CH-4''
128.94	126.2 (C-2'',C-6'') <sup>[25]</sup>	128.5 (C-2,C-6)	CH-2'',CH-6''
128.42	128.4 (C-3'',C-5'') <sup>[25]</sup>	128.5 (C-3,C-5)	CH-3'',CH-5''
122.03	112.3 (C-s) <sup>[26]*</sup>	122.1 (C-s)	CH-s1

\*Using the formula:  $\text{Ph-C}^{\text{H}}=\text{C}^{\text{H}}\text{H}_2$

Table 3 shows the HSQC correlations of compound **2**. This spectrum allowed to corroborate the assignment of H-6' as described by stripes at  $\delta$  7.90, 7.89 and 7.87 in Fig. 6, as suggested by the COSY spectrum (Fig. 8).



**Figure 10.** H-C remote ( $^2\text{J}$ ,  $^3\text{J}$ ) correlations established from the HMBC chart of 4'-hydroxy-3'-(3-methyl-2-butenyl)chalcone (**2**)

**Table 3.** NMR data of compound **2**, from Fig. 9A, Fig. 9A', Fig. 8, Fig. 6, Fig. 5, Table 2, and HMBC and HSQC spectra

Compound <b>2</b>		$\delta$ C ppm	$\delta$ C ppm	HSQC	$\delta$ H ppm (J Hz)	HMBC $\text{H}^{2,3\text{J}}$	$\underline{\text{C}}$
Atom		<b>2</b>	<b>1</b>	$\text{H}^1\text{J}$ $\text{C}$	<b>2</b>	<b>2</b>	
<i>p</i> -Hydroxyacetophenone moiety							
1'	C	130.44	129.96	-	-	-	-
2'	CH	131.20	130.86	→	7.93 d (1.9)	C=O, 4', 3'	-
3'	C	127.97	127.39	-	-	-	-
4'	C	159.62	159.41	-	-	-	-
5'	CH	115.57	115.45	→	6.99 d (8.4)	1', 4'	-
6'	CH	129.08	128.93	→	7.77 d (9.5)	C=O, 4', 2'	-
C=O	C	189.88	-	-	-	-	-
<i>Isoprenyl moiety</i>							
1	CH <sub>2</sub>	29.25	29.37	→	3.47 d (7.2)	2', 3', 3, 2	-
2	CH	121.39	121.16	→	5.39 t (7.2)	1, 4, CH <sub>3</sub> -C-3	-
3	C	135.02	135.10	-	-	-	-
4	CH <sub>3</sub>	25.80*	26.37	→	1.79 s	3, 2	-
CH <sub>3</sub> -C-3	CH <sub>3</sub>	17.95*	17.92	→	1.79 s	3, 2	-
<i>Vinylphenyl moiety</i>							
r1	CH	144.90	-	→	7.84 d (15.8)	C=O, 1'', s	-
s1	CH	122.03	-	→	7.54 d (15.7)	C=O, 1'', r	-
1''	C	134.67	-	-	-	-	-
2''	CH	128.94	-	→	7.43 m	3'', 1''	-
3''	CH	128.42	-	→	7.66 m	2'', 4''	-
4''	CH	130.49	-	→	7.40 m	3'', 5''	-
5''	CH	128.42	-	→	7.66 m	6'', 4''	-
6''	CH	128.94	-	→	7.43 m	5'', 1''	-

\*interchangeable values



The HMBC showed remote heteronuclear correlations for compound **2**. Table 3 shows the most important remote H-C couplings, presented graphically in Figure 10. From this information it's possible for instance, to unambiguously establish the links between the *p*-hydroxyacetophenone moiety and the isoprenyl moiety through the coupling between H-1 and C-3' and C-2'. The *p*-hydroxyacetophenone moiety can be linked with the vinylphenyl moiety by the coupling of H-r and H-s with C=O of *p*-hydroxyacetophenone. Figure 11 is the  $^{13}\text{C}$  NMR spectrum of compound **2** fully assigned. The NMR-based structural proposal for **2** was confirmed by the electrospray ionization mass spectra of **2** which show a M peak of  $m/z$  294 (M+H), corresponding to the molecular formula  $\text{C}_{20}\text{H}_{20}\text{O}_2$  for a molecular mass of 292 Da (see Figure 12). The molecular peak corresponds to  $m/z$  294. According to Dr. M. Bascopé, author of the spectra, the molecular and simultaneously base peak  $m/z$  294 was registered as  $m/z$  293.57. This value was rounded up to 294 by the computer. If we round down, 293.57 becomes 293 which is  $292+1$  or M+H. The spectra show two adducts of confirmation:  $m/z$  316 (M+H+Na) and  $m/z$  332 (M+H+K) and the dimer ( $2\text{M}+\text{H}+\text{Na}$ ) at  $m/z$  608.

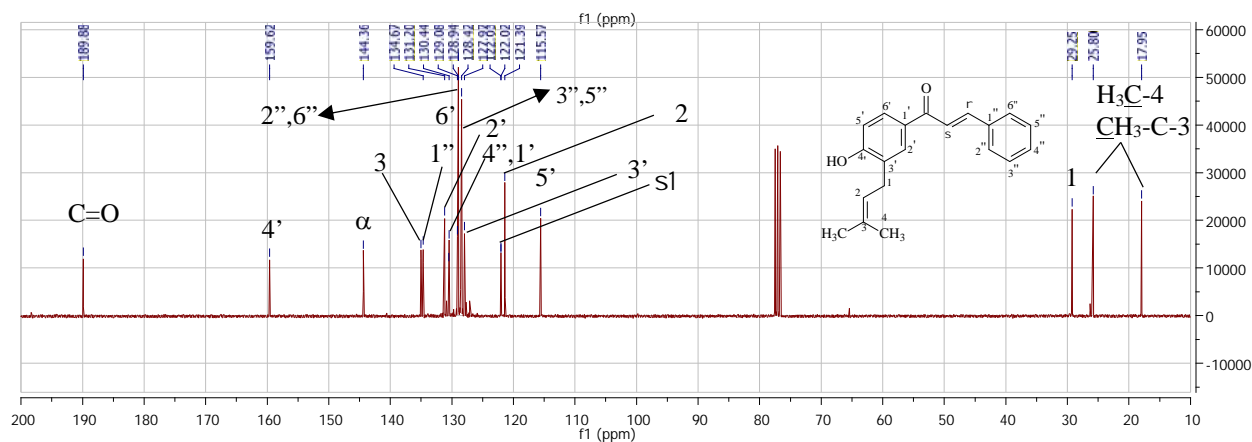


Figure 11.  $^{13}\text{C}$  NMR spectrum of compound **2**, fully assigned, 75 MHz,  $\text{CDCl}_3$ ,  $\text{U}_H$  from TMS

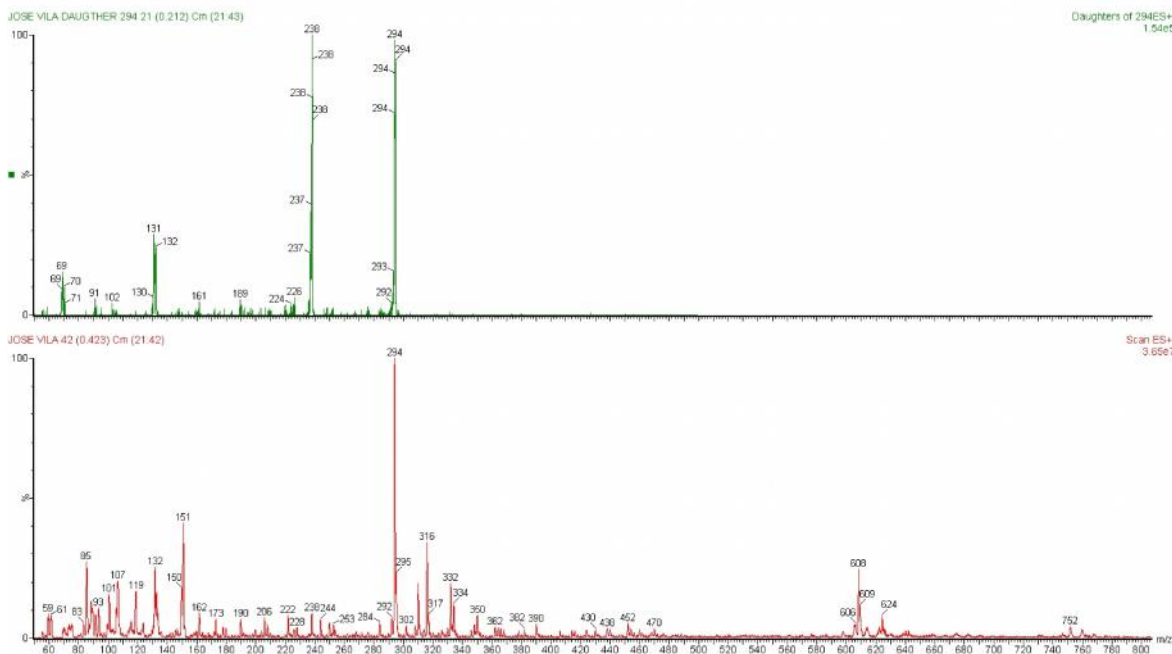
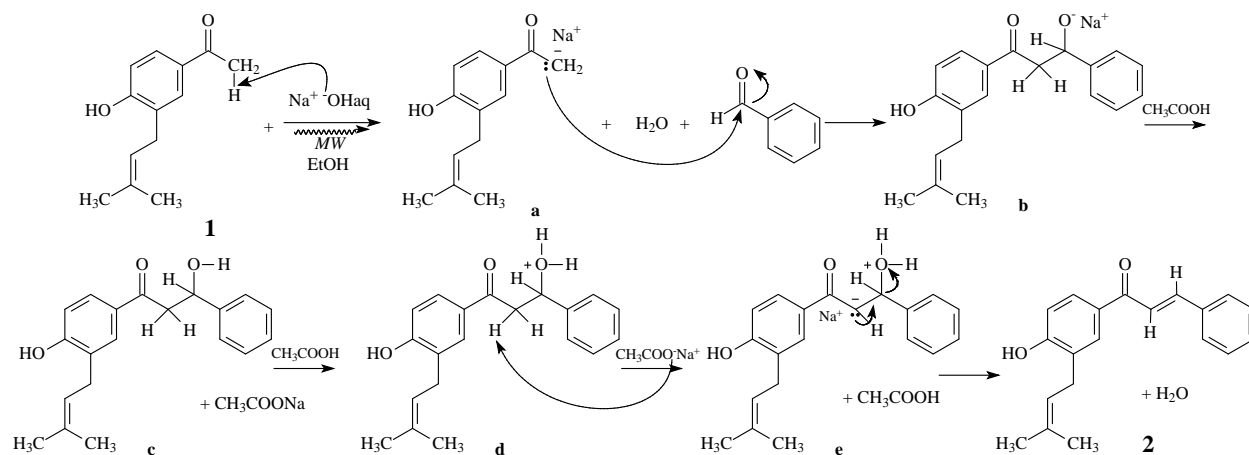


Figure 12. Electrospray ionization MS/MS spectra of compound **2**



### Mechanistic theoretical proposal for the condensation of compound 1 and benzaldehyde to afford compound 2

Some examples of theoretical mechanistic proposals for microwave-assisted synthesis are available in the literature *e.g.* [27]. Also, some mechanistic proposals were done by us for some classical synthetic methods [28 and references therein]. There isn't much difference from one method to the other in the compartment of molecules as precursors or intermediates. When a solvent is employed, an ionic mechanism is usually involved, this can back an ionic mechanism for a microwave-assisted reaction in an aqueous media like the one under current study here. Besides, the examples mentioned [27], are precisely ionic mechanisms. In this sense, the reaction depicted in Fig. 1, which used an aqueous basic solution can be interpreted mechanistically in an ionic path. Figure 13 shows the hypothetical reaction pathway followed by compound 1 and benzaldehyde when submitted to alkaline aqueous solution and MW irradiation.



**Figure 13.** Hypothetical mechanistic proposal for the synthesis of 4'-hydroxy-3'-(3-methyl-2-butenyl)chalcone (2) via microwave-assisted condensation of 4-Hydroxy-3-(3'-methyl-2'-butenyl)acetophenone (1) and benzaldehyde

The strong base NaOH attacks the acidic proton in  $\alpha$  of carbonyl of the *p*-hydroxyacetophenone moiety of compound 1 generating thus the strong base and nucleophilic:  $\text{Ar}(\text{C}=\text{O})\text{CH}_2^-$  (a). The carbonyl of benzaldehyde suffers the nucleophilic attack of intermediate a to form the adduct b. This sodium alkoxide (b) interchanges the weak acid  $\text{Na}^+$  for the stronger  $\text{H}^+$  (c) forming thus the secondary alcohol c and sodium acetate. The alcohol c is protonated by acetic acid to form the protonated alcohol d which by action of acetate over the hydrogen in  $\alpha$  of carbonyl produces the alkene 2 by dehydration (e) of the protonated alcohol d. Ethanol was added to the initial mixture as dissolvent for compound 1. The reaction is stereospecific since no traces of the isomer *cis*-chalcone was found.

## CONCLUSIONS

A new chalcone (2) has been synthesized as the condensation of a natural product (1) and a synthetic product (benzaldehyde) under green chemistry conditions and with a good yield. Chalcones possess a wide variety of biological activity applications. It's not pretended here to evoke a complete references list with a review sense, but instead only mentioning some examples like the anti-inflammatory, analgesic and antipyretic activity [29], or the antibacterial, antifungal and insecticide or as well antimutagenic activity of chalcones [30]. This suggests that *via* micro-wave assisted condensation, it is imaginable a series of biological assays [31] on any particular target to evaluate new molecules of synthetic or hemi-synthetic origin with a structure-activity study character.

## EXPERIMENTAL

### General



All equipment belongs to Department of Chemical Sciences UMSA; NMR spectrometer: Bruker DRX300, (300 MHz, 75 MHz), TMS used as internal standard. MestReNova software was used to manipulate NMR spectra. Microwave device: DAEWOO DC electronics, model KOC-1B0K, power: 170 W. IR spectrometer: Perkin-Elmer Spectrum BX FT-IR. UV spectrometer: BioTek,  $\mu$ Quant model. Melting point apparatus: Fisatom 430 D. Other equipment: BÜCHI rotavapor R-200 and heating bath B-490 plus diaphragm vacuum pump ILMVAC. ESI-MS/MS analyses: ESI-MS/MS was performed on a Waters Quattro Micro instrument operating in the positive-ion mode (Electrospray). Data acquisition was carried out with MassLynx 4.1 software with the following settings: capillary voltage, 2500 V; cone voltage, 30 V; extractor, 2 V; RF, 0.0 V; source temperature, 120 °C; desolvation temperature, 350 °C; cone gas flow, 50 L/h; desolvation gas flow, 350 L/h; LM 1 resolution, 15; HM 1 resolution, 15; ion energy 1, 0.2; MS/MS mode entrance, 15; MSMS collision energy, 30 eV (Gal-6S-P) and 30 eV (Gal-6S-IS); MS/MS mode exit, 15; LM 2 resolution, 15.0; HM 2 resolution, 15.0; ion energy 2, 2.0; Multiplier, 650; collision cell pressure, < 10<sup>-4</sup> mbar; collision gas, argon. Sample was introduced by direct infusion 10  $\mu$ L/min flow rate; spectrometer belongs to Centro de Investigaciones Químicas S.R.L (Responsible: Dr. Marcelo Bascopé). Synthesis monitoring (**2**) and isolation (**1**) by Silica gel 60 F254 TLC plates by Merck. Revealing agent: H<sub>2</sub>SO<sub>4</sub> 30 % aqueous solution plus heating. Chemicals: Benzaldehyde (p.a.), hexane (p.a.) and glacial acetic acid (p.a.) from Sigma-Aldrich. Methylene chloride and ethyl acetate (distilled). Ethanol and NaOH commercial.

#### Plant material

The species *Senecio graveolens* (Asteraceae) was collected in the municipal county of Tinquipaya, Tomás Frías province, Department of Potosí; GPS coordinates: 19°13'6" SW (southwest) y 65°49'35" W (west), a 3,800 m.a.s.l. in September 2016. Characteristic climate: cold (2 °C to 12 °C). The plant was taxonomically identified at the National Herbarium of Bolivia (LPB). A specimen voucher is deposited at LPB under the code YQC-1.

#### Isolation of compound 1

The leaves of the vegetal sample were dried at room temperature and were ground to afford a final weight of 15000g (15kg). This mass was entirely treated with a two-step process of solid-liquid extraction with 30 L of ethanol (96°) in 5 containers of 10L each for 14 days each step. Filtration and solvent evaporation (56°C) of the first ethanol extraction was done at reduced pressure until reaching 10% of the original volume of EtOH solution (3 L). No dry extract was obtained. No precipitate like crystals were noticed during the concentration of the first ethanol extract. The first extract was kept indefinitely in refrigerator at 4°C for further treatment. No weighing was done with the vegetal material after the first extraction and corresponding drying. Thus, the same original mass of vegetal material (after the first extraction) was extracted with EtOH 96° for the second time under the same conditions as the first one. During concentration (56°C) at reduced pressure orange-like colored crystals appeared quantitatively. In this later process the solvent volume was reduced approximately until the 10% of its original amount. Crystals were filtered from the solution (4.9 g, 0.03% of original sample). Silica-gel TLC assays were run in EtOAc/EtOH 1:1 at prudential stages of the process showing the presence of a major spot corresponding to compound **1** as the major constituent of the EtOH-2 extract. 4.9 g of **1** were submitted to re-crystallization for 24 hrs in cold EtOH (0°C) twice. After each 24 hrs crystals were filtered and washed with cold EtOH (0°C) in a Büchner funnel. The TLC assay (EtOAc/EtOH 1:1) over the re-crystallized **1** showed still impurity. A semi-micro silica gel (0.98 g) liquid chromatography in a Pasteur pipette was performed with **1**, EtOAc was used to dissolve the sample before separation in column. The first 24 fractions (5 mL each) extracted from column were eluted with of EtOAc giving rise to an orange-like colored pure compound according to TLC assay (EtOAc) corresponding to compound **1**. This was recrystallized in CH<sub>2</sub>Cl<sub>2</sub>. Other 23 fractions (5 mL each) were extracted from column by solvent EtOH giving rise to a second black compound according to TLC analysis (EtOAc/EtOH 1:1).

#### Microwave-assisted condensation synthesis of compound 2

NaOH (10% [0.2 g/2mL]) with EtOH 95% (2mL) and compound **1** (crystals, 488 mg) were stirred in heating plate at 35 °C in a 50 mL flask. Once the crystals of **1** dissolved, benzaldehyde (2.5 mL) was added to the reaction mixture. Mixture was irradiated under microwave conditions (170 Watts) four times during 10 min each with cooling intervals of 10 s. Water (25 mL) and AcOH (2.0 mL) were added to the reacting mixture. Two phases were formed after agitation and they were separated in a separation funnel. The organic layer was washed with distilled water thrice and separated in layers each time. The organic layer was put at 4°C in freezer overnight. A solid precipitate appeared in the bottom of the flask. Once separated by filtration it was washed with hexane and dichloromethane in a



silica gel Pasteur pipette. Yellow crystals were obtained that weighed 525 mg. The reaction yield was calculated as 71%.

#### Physicochemical data of compounds 1 and 2

##### Compound 1: 4-Hydroxy-3-(3'-methyl-2'-butenyl)acetophenone

MP: 94-95°C uncorrected; NMR; see Table 1.

##### Compound 2: 4'-hydroxy-3'-(3-methyl-2-butenyl)chalcone

MP: 106-107 °C uncorrected; NMR see Table 3; FTIR (solid):  $\nu_{\max}$  [ $\text{cm}^{-1}$ ] 827 (ArC-H  $\delta$  oop), 977 (ArC-H  $\delta$  ip,  $\delta$  oop), 1131 (ArC-H  $\delta$  ip, C-O st), [1250, 1283, 1334 (C-O st)], 1564 (C=C st), 1648 (C=O st), [3243, 3463, 3629 (O-H st)]; UV (EtOH<sub>50°C</sub>)  $\lambda_{\max}$  (log  $\nu$ ) nm: 263.0 (3.62) nm; ESI-MS/MS  $m/z$  69, 131, 238, 294 [(M+H)<sup>+</sup>];  $m/z$  85, 151, 294 [(M+H)<sup>+</sup>], 316 [(M+H+Na)<sup>+</sup>], 332 [(M+H+K)<sup>+</sup>], 608 [(2M+H+Na)<sup>+</sup>].

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