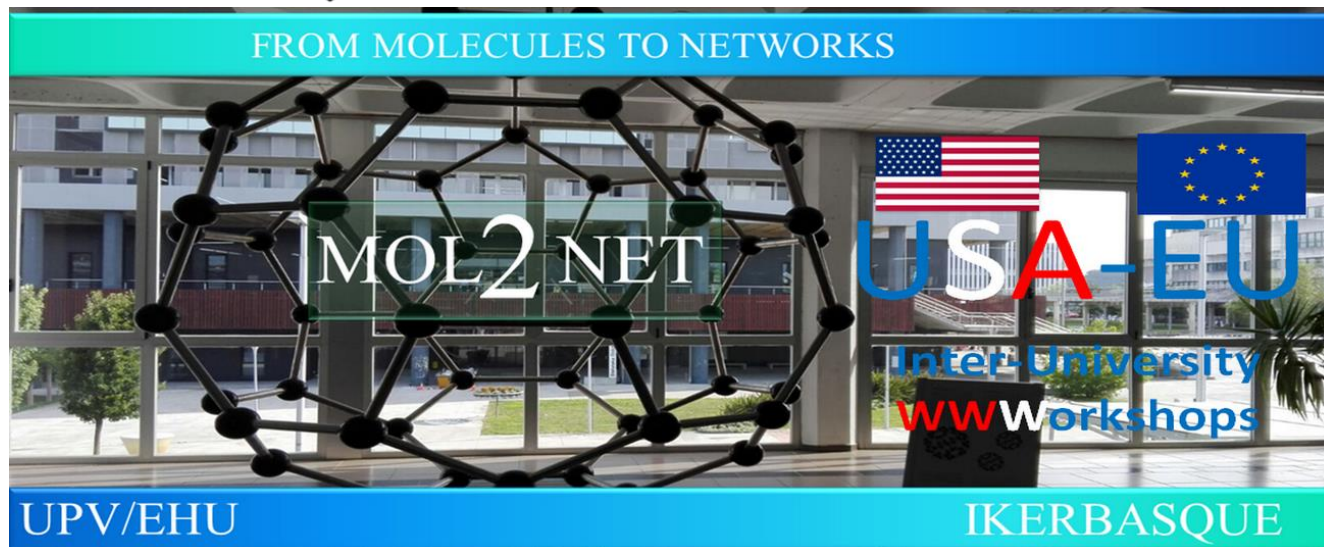




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Synthesis and structural elucidation of Isoliquiritigenin by Nuclear Magnetic Resonance

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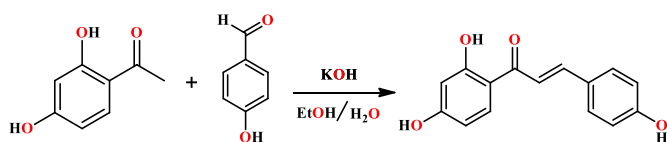
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Graphical Abstract



Abstract.

Chalcones, a class of natural compounds, characterized by having an α , β -unsaturated carbonyl linking two aromatic rings in their structure, are compounds of great biological relevance and wide variety of biological activities, being important research objects, which is why their obtainment synthetically have great relevance, being a more promising alternative than natural extractivism. Thus, this work aimed at the synthesis and structural elucidation of isoliquiritigenin, (2E)-1-(2,4-dihydroxyphenyl)-3-(4-hydroxyphenyl) prop-2-

	<p>en-1-one, using aldol condensation Claisen-Schmidt type in basic medium (KOH) of the acylated resorcin (1-(2,4-dihydroxyphenyl) ethan-1-one) with p-hydroxyaldehyde, being possible its identification by means of ^1H and ^{13}C NMR spectra.</p> <p>Keywords: Chalcones, Isoliquiritigenin, Claisen-Schmidt Condensation.</p>
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Introduction

Secondary metabolite extracts contain bioactive compounds with wide applicability in medicine. However, obtaining these compounds requires large amounts of plant material, since they have low yields and a great diversity of metabolic products. The synthesis of natural compounds becomes crucial, as it allows the obtainment of these compounds on a large scale and at low cost, allowing for more detailed analysis of bioassays both *in vitro* and *in vivo*, in addition to providing greater preservation of our flora (BERLINCK *et al.*, 2017).

Among the most therapeutically important natural compounds, chalcones are a class of molecules that are part of the biosynthesis of other secondary metabolites, such as flavonoids, categorized as one of the most important and diversified groups of polyphenols in the plant kingdom, as they have a vast diversity structural and a considerable pharmacological importance holding different biological activities (COUTINHO, 2009).

The two aromatic rings present in the molecule are responsible for allowing countless possibilities of substitution in chalcones, so this class can have its bioactivities modulated in different ways. In addition, its structural simplicity allows a wide variety of synthetic routes to be obtained (DIAZ-TELES *et al.*, 2016; NEUENFELDT *et al.*, 2015).

An example of a widely studied chalcone is isoliquiritigenin, known to have the most common pharmacological properties, such as anti-inflammatory (LIU, *et al.*, 2021), antimicrobial (GAUR, *et al.*, 2016), antioxidative (LIU, *et al.*, 2022) and anticancer (MAHAPATRA, *et al.*, 2020), both as more specific effects, such as antidiabetic WANG, *et al.*, 2020 and anti-angiogenic (MAHAPATRA, *et al.*, 2020). They are molecules that can be isolated from the roots and rhizomes of the genus *Glycyrrhiza*, in addition to the species *Dianthus chinensis*, *Astragalus membrabaceus*, used in Chinese folk medicine. (PENG *et al.*, 2015).

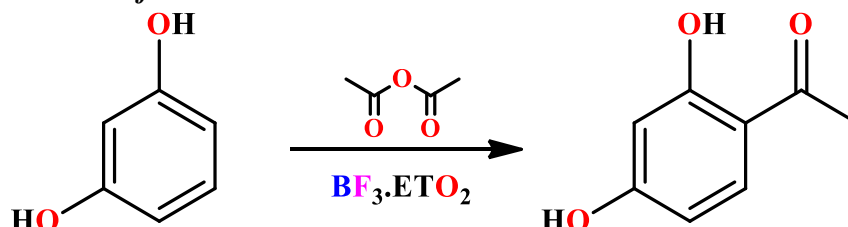
Because they have structural simplicity and present a vast plurality of biological activities, chalcones are one of the classes of molecules that has been gaining importance in the global research scenario (GOMES *et al.*, 2017; ZHUANG, *et al.*, 2017). Therefore, the present work had as objective the synthesis of isoliquiritigenin, through an acylation via Fries rearrangement followed by a Claisen-Schmidt condensation, with low cost and easy to acquire reagents.

Materials and Methods

Reaction I: preparation of chalcone precursor acetophenone

For the synthesis of 1-(2,4-dihydroxyphenyl) ethan-1-one (figure 1), 5g of benzene-1,3-diol were weighed and solubilized in 5.2 ml of acetic anhydride (1.1 eq). After total dissolution, 21.5 mL of boron trifluoride etherate ($\text{BF}_3 \cdot \text{EtO}_2$) were added, leaving the system under stirring for 15 minutes at a temperature of 60°C . After this time, the heating was stopped, just keeping the agitation until completing 24h. Purification was carried out by partitioning with water, ice and ethyl acetate, the organic phase was treated with anhydrous sodium sulphate, dried on a rotaevaporator and subjected to recrystallization from hexane, obtaining only the desired product. The reaction I procedure was based on the work by Wei *et al.* (2014).

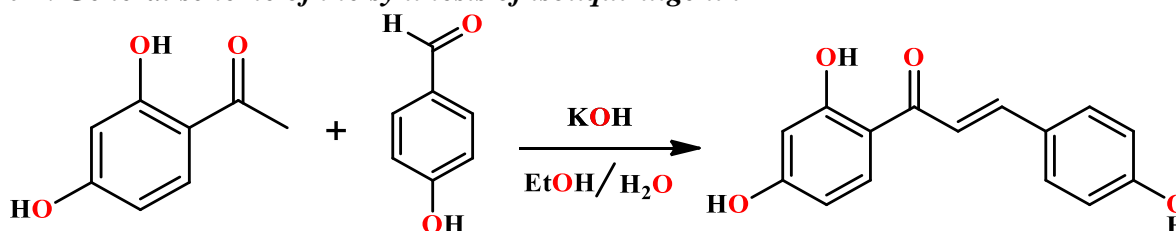
Figure 1: General scheme of reaction I



Reaction II: preparation of chalcone via Claisen Schmidt

In an erlemeyer flask, 905 mg (5.95 mmol) of the 1-(2,4-dihydroxyphenyl) ethan-1-one (synthesized in item 2.1) were weighed out and solubilized in ethanol, then 1.66 g were added (29.75 mmol) of potassium hydroxide and left under magnetic stirring. In another erlemeyer 867 mg (7.10 mmol) of 4-hydroxybenzaldehyde were weighed and solubilized in ethanol. The 4-hydroxybenzaldehyde solution was slowly added to the solution containing the acetophenone and potassium hydroxide, being left under magnetic stirring until completing 24 h. A change in color was observed, acquiring an orange hue with the presence of a precipitate, being monitored by Analytical Thin Layer Chromatography (TLC).

Figure 2: General scheme of the synthesis of isoliquiritigenin



After 24 h the pH was adjusted with HCl until obtaining a neutral pH 6~7, being purified by partitioning with ethyl acetate and distilled water. Subsequently, the organic phase was treated with anhydrous sodium sulfate and dried on a rotary evaporator, showing a yellowish solid.

The reaction was purified by Preparative Thin Layer Chromatography (CCDP), with elution system dichloromethane and methanol (5:5), resulting in a yellowish substance that was sent for spectroscopic analysis of Nuclear Magnetic Resonance.

Spectral Data and Physicochemical Characteristics of Isoliquiritigenin

(2E) -1-(2,4-dihydroxifenil) -3-(4-hidroxifenil) prop-2-en-1-ona:

Obtained in the form of an amorphous yellowish powder. ^1H RMN (500 MHz, CD_3OD) δ 7.95 (d, $J = 8.9$ Hz, 1H), 7.77 (d, $J = 15.3$ Hz, 1H), 7.59 (d, $J = 8.5$ Hz, 2H), 7.57 (d, $J = 15.3$ Hz, 1H) 6.84 (d, $J = 8.5$ Hz, 2H), 6.41 (dd, $J = 8.9, 2.3$ Hz, 1H), 6.29 (d, $J = 2.3$ Hz, 1H). ^{13}C RMN (125 MHz, CD_3OD) δ 193.51, 167.43, 166.29, 161.48, 145.64, 133.33, 131.79, 130.21, 127.83, 116.91, 114.72, 109.14, 103.83.

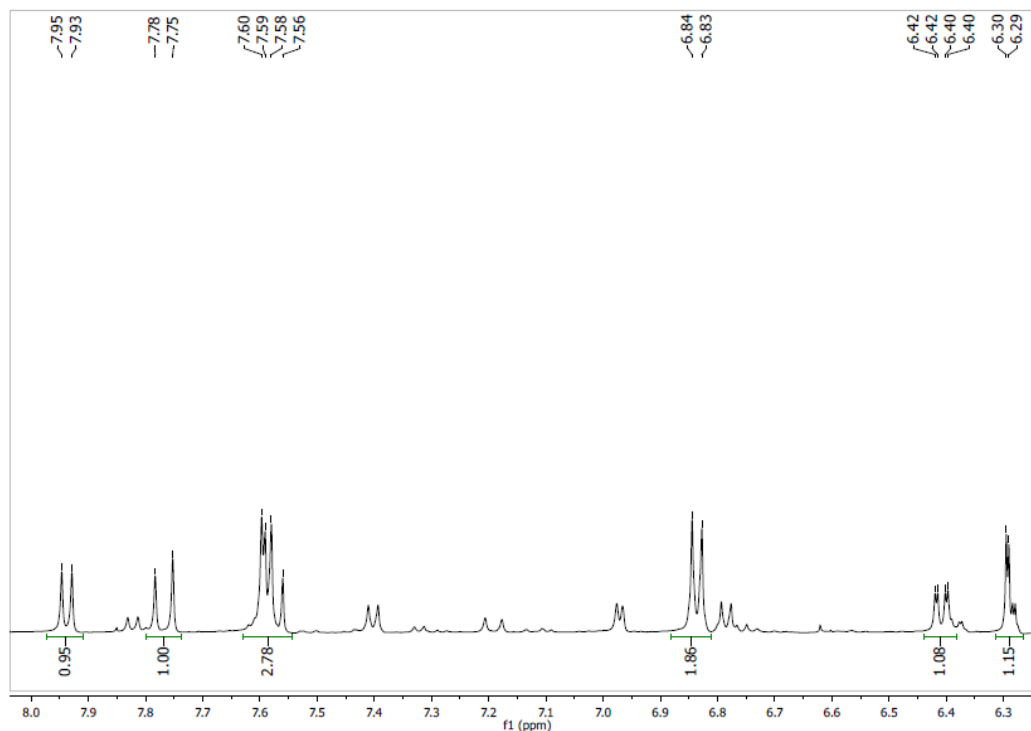
Results and Discussion

The synthesized compound was obtained as a yellowish amorphous solid, and was identified by ^1H and ^{13}C NMR spectroscopy at a frequency of 500 MHz and 125 MHz respectively, using deuterated methanol (CD_3OD) as solvent (Table 1).

Table 1: Comparison of the obtained ^1H and ^{13}C NMR spectral data with a literary model.

Posição	δ ^1H	δ ^{13}C	δ ^1H (Niu, et al., 2017)	δ ^{13}C (Niu, et al., 2017)
1	-	130.21	-	126.41
2, 6	7.59 (d, $J = 8.5$ Hz, 2H)	131.79	7.65-7.58 (m, 3H)	130.39
3, 5	6.84 (d, $J = 8.5$ Hz, 2H)	116.91	6.84 (d, $J = 8.7$ Hz, 2H)	115.49
4	-	161.48	-	160.12
1'	-	114.72	-	113.26
2'	-	167.43	-	166.07
3'	6.29 (d, $J = 2.3$ Hz, 1H)	103.83	6.28 (d, $J = 2.3$ Hz, 1H)	102.40
4'	-	166.29	-	165.00
5'	6.41 (dd, $J = 8.9, 2.3$ Hz, 1H)	109.14	6.41 (dd, $J = 8.9, 2.3$ Hz, 1H)	107.75
6'	7.95 (d, $J = 8.9$ Hz, 1H)	133.33	7.97 (d, $J = 8.9$ Hz, 1H)	131.94
α	7.57 (d, $J = 15.3$ Hz, 1H)	127.83	7.65-7.58 (m, 3H)	116.91
β	7.77 (d, $J = 15.3$ Hz, 1H)	145.64	7.79 (d, $J = 15.4$ Hz, 1H)	144.20
C=O	-	193.51	-	192.07

In the ^1H NMR spectrum (CD_3OD , 500 MHz) a richness of aromatic signals, characteristic of chalcones, can be observed (Figure 3). The presence of two doublets at δ_{H} 7.77 (d, $J = 15.3$ Hz, 1H) and δ_{H} 7.57 (d, $J = 15.3$ Hz, 1H) coupling together indicate the formation of the conjugated carbonyl system present in chalcones, together hence the presence of an ABX system at δ_{H} 6.29 (d, $J = 2.3$ Hz, 1H); δ_{H} 6.41 (dd, $J = 8.9, 2.3$ Hz, 1H) and δ_{H} 7.95 (d, $J = 8.9$ Hz, 1H), along with an AA'BB' system in δ_{H} 6.84 (d, $J = 8.5$ Hz, 2H) and δ_{H} 7.59 (d, $J = 8.5$ Hz, 2H), suggest the formation of isoliquiritigenin.

Figure 3: ^1H NMR spectrum of isoliquiritigenin expansion 6.0 to 8.0 ppm

The ^{13}C NMR spectrum (CD_3OD) showed compatible signals for 15 carbons, of which 9 are for methic type carbons and 6 for non-hydrogenated carbons (Figures 4 and 5). The presence of a conjugated ketone carbonyl at δ_{C} 193.51, corroborates the presence of the α,β -unsaturated system as seen in the hydrogen spectrum.

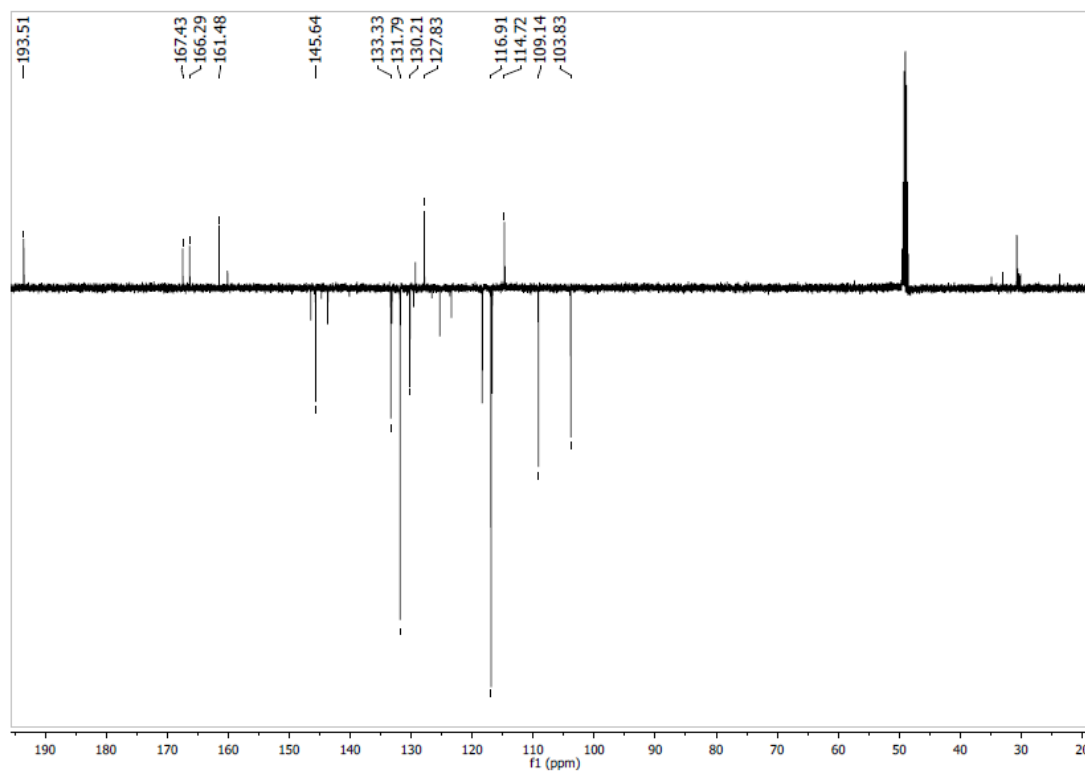
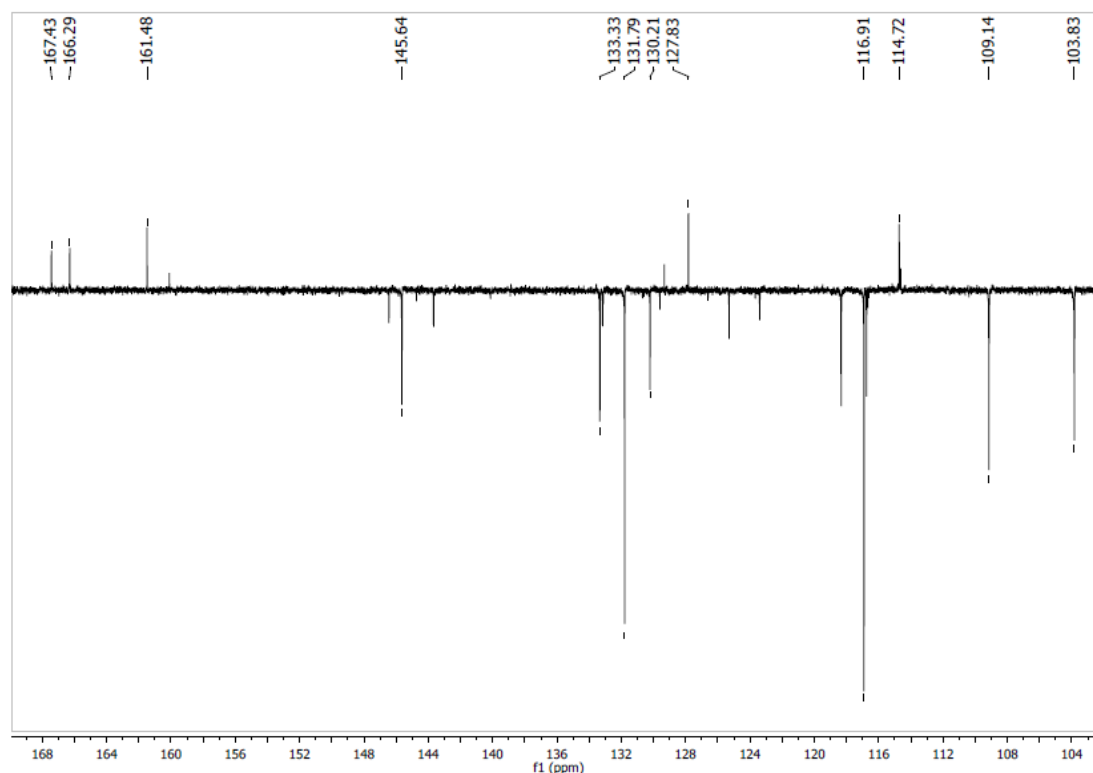
Figure 4: ^{13}C NMR spectrum of isoliquiritigenin.

Figure 5: ^{13}C spectrum of isoliquiritigen expansion in the 170 to 100 ppm region

Conclusions

In this work, it was possible to carry out the total synthesis of *(2E)-1-(2,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one* (isoliquiritigenin), a natural compound with low costs. Through methods known in the literature, such as acylation via Fries rearrangement and Claisen Schimidt condensation, and with cheap reagents such as *p*-hydroxybenzaldehyde and resorcin.

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