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SHORT COMMUNICATION

Inhibition of squalene synthase of rat liver by abietane diterpenes derivatives

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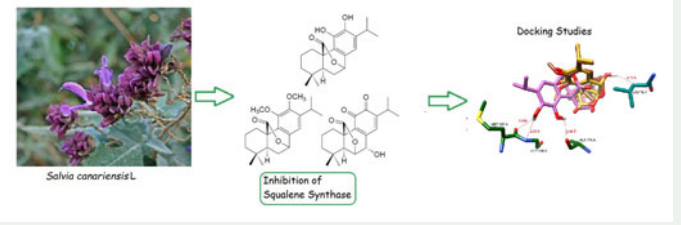
ABSTRACT

In the current study, chemical composition of cultivated *Salvia canariensis* L was determined. Carnosol was the main product isolated. We prepared more lipophilic derivatives from carnosol, and both isolated and semisynthetic abietane diterpenes were evaluated *in vitro* as inhibitors of squalene synthase. Among the compounds tested, carnosol was the most potent inhibitor (IC_{50} = 17.6 μM). These results highlight the great potential of this species for the production of new ingredients in nutritional supplements for the treatment of hyperlipidemia.



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Carnosol; Hyperlipidemia; Salvia; Cholesterol; Squalene synthase

1. Introduction

Hyperlipidemia is a well-established risk factor for the development of cardio and cerebrovascular diseases. For this reason, lipid lowering is one of the major approaches used for their primary prevention. A widely used strategy to decrease plasma cholesterol concentration is the use of HMG-CoA inhibitors such as statins. However, this enzyme plays a fundamental role in the biosynthesis of many other nonsteroidal molecules, so statins have potential adverse effects (Balaz and Wolfrum 2019). The inhibition of squalene synthase (SQS), an enzyme essential in the biosynthesis of the steroids, can prevent the cholesterol biosynthesis without

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disturbing the nonsterol pathway. Several SQS inhibitors have been developed such as zaragozic acid and quinuclidine (Kourounakis et al. 2010).

The genus *Salvia* consists of some 500 species, some of which have shown interesting biological activity (Jakovljević et al. 2019). In previous studies of the aerial part of *Salvia canariensis L.*, an endemism of the Canary Islands, carnosic acid **1** and carnosol **2** were isolated as the major phenolic diterpenes (Marrero et al. 2009), which have been reported to have broad biological activities like neuroprotective and hypolipidemic properties (González 2015; Alonso et al. 2019).

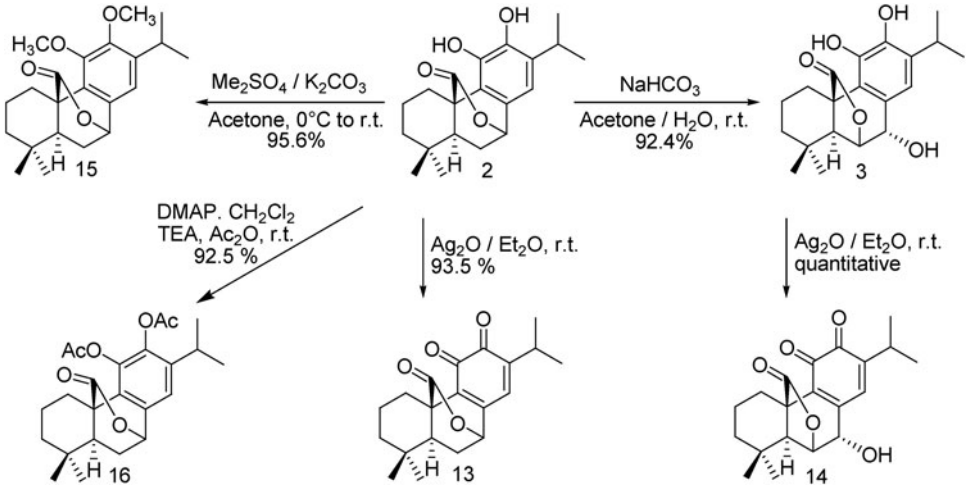
As a continuation of our work on the isolation and synthesis of diterpenes to identify bioactive components, and due to the great importance to discover potential SQS inhibitors, the purpose of this study was to provide the chemical profile of cultivated *S. canariensis*, obtain synthetic derivatives from the isolated products and evaluate their ability to inhibit SQS *in vitro*. These results make this plant to be considered as a crop that could have commercial importance as useful ingredient in nutritional supplements.

2. Results and discussion

The acetonic extract of cultivated *S. canariensis* was fractionated and purified following the protocol designed by Marrero et al. (2009). The analysis of the extract revealed carnosol **2** as the main product. The concentration of rosmanol **3** was also significant, with 7α-methoxyrosmanol **4**, 7α-ethoxyrosmanol **5** and galdosol **6** as minority constituents (Table S1). These compounds have been previously obtained from *S. canariensis* (Marrero et al. 2009). We also reported here, for the first time from this species, the isolation of rosmadial **7**, epirosmanol **8** and isorosmanol **9** previously found in *Rosmarinus officinalis* (Nakatani and Inatani 1984); isogaldosol **10** and demetilsalvicanol **11** previously isolated from *Salvia mellifera* (González et al. 1992); and 7β-methoxyrosmanol **12** identified previously in *Salvia dorrii* (Ahmed et al. 2006). On the other hand, in our study we could not detect carnosic acid **1**, probably due to the harvest time of the plant (Luis and Johnson, 2005). Although 16-acetoxycarnosol was isolated from *in vitro* culture of *S. canariensis* (Luis et al. 1992), we could not detect any abietane derivative oxidized at C-16.

All compounds were tested for SQS inhibitory activity at a concentration of 10 μM (Amin *et al,* 1992) (Table S1). Compounds **8**, **10**, **11** and **12** were not evaluated due to the small amount isolated. Most compounds exhibited weak inhibition activity of SQS, except carnosol **2** (IC_{50} = 17.6 μM). At this point, we decided to prepare some semisynthetic derivatives of carnosol **2**, where we change the hydroxyl groups for more lipophilic substituents (Scheme 1). The biological evaluation of these derivatives showed that 11,12-di-*O*-methylcarnosol **15** was weaker than carnosol **2**. However, the oxidation of rosmanol **3** to rosmaquinone **14**, improves the biological activity (Table S1).

To obtain more information about the mechanism as well as the mode of interactions of these phytochemicals with SQS, the three compounds with the best *in vitro* results were selected to perform a docking analysis (Table S2). Regularly, squalene inhibitors have been found interacting with Ala176, Met207, Gly208 and Leu76 residues and the ones nearby, mainly by Van der Waals and hydrophobic interactions, present in a large



Scheme 1. Semisynthesis of Carnosol derivatives.

hydrophobic pocket that the SQS receptor possess (Figure S3) (Ichikawa et al. 2012). The hydroxyl groups of carnosol and rosmaquinone may not interact with the R group of the residues, but with their skeletons. This would be the case for one of carnosol phenolic hydroxyl with the skeletal N of Gly208; a weak hydrogen or dipole interaction among these atoms could be possible, as their distance is 2.5 Å. This same hydroxyl has a 3.08 Å distance to the carbonyl of Met207. The second phenolic hydroxyl also has a 2.96 Å distance to the carbonyl of Ala176, so similar interactions can take part (Figure S4). The hydroxyl of rosmaquinone has Leu76 carbonyl at a distance of 4.35 Å. Thus, we can assume a weak dipole interaction from this group may be favouring rosmaquinone. These additional interactions, other than the hydrophobic ones, would partially explain why 11,12-dimethoxycarnosol (which have none of them) has the lowest docking score, even though it shares a very similar position in space with rosmaquinone. Even though carnosol and rosmaquinone have the best docking results, *in vitro* we see how carnosol has the lowest IC_{50} (17.6 μM), followed by 11,12-di-*O*-methylcarnosol (18.4 μM). Although this can look as a disagreement, we can observe that the docked poses give the correct insight for the *in silico-in vitro* relationship. The docked compounds are very close to each other in the cavities where is well known inhibitors bind (Ichikawa et al. 2012), but carnosol can be seen deeper in such cavity. Different studies for SQS inhibitors have made the observation that, the better the *in vitro* inhibition shown, the deeper such compound goes interacting in the hydrophobic cavity in the docking analysis (Kourounakis et al. 2010). In such way, the docking poses allows to explain why carnosol has a better interaction with the receptor by means of its spatial position.

3. Conclusions

We describe the majority diterpenes present in the extract of cultivated *S. canariensis*, which was confirmed to be a taxon strongly characterized by the presence of carnosol **2**. We isolated eleven abietane-type compounds. The present study portrays the first report of the isolation of six of these compounds from this species. We prepared

more lipophilic derivatives from carnosol, and both isolated and synthetic compounds were evaluated as SQS inhibitors. It was observed that carnosol **2**, rosmaquinone **14** and 11,12-di-*O*-methylcarnosol **15** showed *in vitro* SQS inhibitory effect. In order to obtain data about the interactions between these molecules and SQS, we performed *in silico* docking studies. To our knowledge, this is the first report of the inhibition of SQS by abietanes. Application of the findings from this docking study could led to the development of more potent SQS inhibitors aimed against atherosclerosis. We think that this species could be cultivated and used as a new useful ingredient in nutritional supplements with antioxidant and anti-atherosclerosis function.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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