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A New Fluorinated Tyrosinase Inhibitor from a Chemically Engineered Essential Oil

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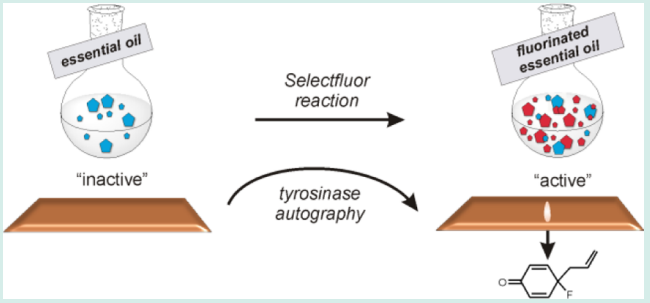
Supporting Information

ABSTRACT: The generation of fluorinated essential oils as a source of bioactive compounds is described. Most of the components of the natural mixtures were altered, leading to the discovery of a new fluorinated tyrosinase inhibitor.



KEYWORDS: *diversification, essential oil, fluorination, tyrosinase, bioactive compound*

Natural products are biologically validated starting points for the development of new drugs. They are the outcome of a long evolutionary process that has resulted in a unique assortment of skeletons with high affinity for biomolecules.[1]

Essential oils (EOs) are natural multicomponent systems[2] composed of low-molecular weight lipophilic compounds derived from different biosynthetic pathways.[3] In general, their production by plants is diversity oriented with the generation of complex mixtures of compounds that have the potential to regulate plant−insect and plant−mammal interactions. This bioactive volatilome is now emerging as a novel potential source of interesting lead structures for drug discovery.[3]

Several approaches have been proposed to increase the diversity of natural product mixtures, such us combinatorial biosynthesis[4] and related techniques.[5] Chemically engineered extracts (CEEs) represent alternative sources of molecules for the search of new bioactive compounds based on natural skeletons.[6−9] In this strategy, natural mixtures are chemically altered through reactions directed toward the incorporation of molecular fragments or elements that are relevant for bioactivity and rarely found in secondary metabolites.[10]

Fluorine is one such element, the incorporation of which into a molecule can modulate physicochemical properties such as pK_a, lipophilicity, hydrogen bonding, and electrostatic interactions, as well as metabolic stability (oxidative metabolism, hydrolytic metabolism, in vivo racemization).[11] The strategic use of fluorine substitution in drug design has led to the production of some of the key drugs available on the market.[12,13] The average proportion of fluorine in drugs is significantly higher than in natural products.[14] Natural organofluorines represent less than 1% of naturally occurring organohalogens.[15]

Considering that (a) small molecule natural products have had a significant impact on drug discovery, (b) 20−25% of drugs in the pharmaceutical pipeline contain at least one fluorine atom,[11] and (c) organofluorine compounds are virtually absent as natural products,[16] it becomes interesting to evaluate the effect of fluorination on the biological properties of natural mixtures of small molecules such as EOs (Figure 1).

For EO diversification, we used Selectfluor as a highly reactive fluorinating reagent that is safe, nontoxic, stable, and easy to handle.[17−20] Selectfluor can introduce fluorine atoms into molecules by reaction with double bonds, aromatic rings, and through the transformation of carbon−hydrogen bonds to

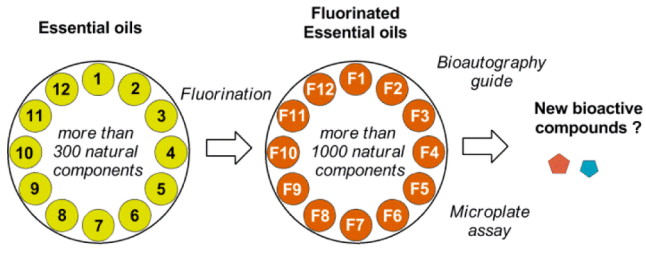


Figure 1. Diversification of essential oil mixtures by fluorination to generate libraries of biologically active compounds.

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carbon−fluorine bonds at saturated secondary and tertiary carbon sites.[21] According to the Dictionary of Natural Products,[22] 78% of the essential oil constituents include at least one nonaromatic carbon−carbon double bond in their structures, 22% contain at least one aromatic group, and 84% contain the types of C−H bonds that may be reactive. In addition, Selecfluor has been applied to the fluorination of other functional groups[23] that are present in essential oil components, such as enols (6.8%) and alkynes (2.5%). The fluorination of essential oils has not been reported to date.

The success of the CEEs approach lies in the power of numbers: to increase the chances of generating a bioactive compound, it is important to produce many compounds that incorporate the desired chemical feature or element. A series of 12 essential oils was fluorinated by reaction with Selectfluor in refluxing acetonitrile, and changes in chemical composition and bioactivity were evaluated. Incorporation of fluorine into the EO constituents was confirmed by ^{19}F NMR analysis with new peaks appearing between −97 and −197 ppm as expected for aromatic fluorine, α-fluoroketones, and α-fluoroenones.

The impact of the reaction on the chemical composition of the mixtures was evaluated by GC-MS, showing that most of the EO components were transformed by the reaction, expanding the chemical diversity of the mixtures. At least 60% of the peaks observed in the chromatograms of EOs disappeared after the reaction, and at least 88% of the peaks present in the gas chromatograms of the resulting fluorinated essential oils (FEOs) were absent in the chromatogram of the precursor EO (Figure 2a). The average number of major

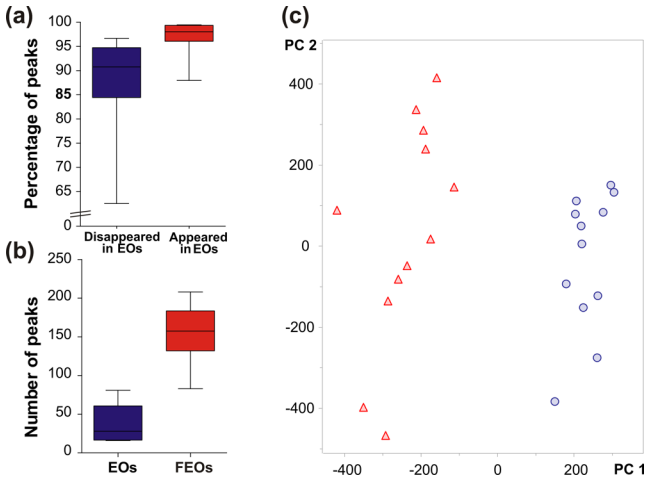


Figure 2. Box and whiskers plot for (a) percentage of peaks that disappeared from the chromatograms because of the reaction (blue) and that appeared in the chromatograms after the reaction (red) and (b) number of peaks from EOs (blue) and FEOs (red) detected in the GC-MS chromatograms. (c) Score plot of PCA of GC-MS data for EOs (blue circles) and FEOs (red triangles).

compounds detected in the mixtures increased from 37 to 155 due to the fluorination reaction (Figure 2b). This suggests that, on average, four products were generated from each natural precursor.

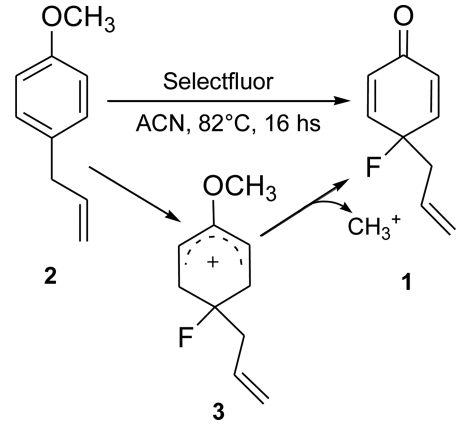
Changes in the composition of the mixtures were also evident from GC-MS coupled to principal component analysis (PCA). The score plot showed discrimination between two groups by PC1 and PC2: one corresponding to the FEOs (Figure 2, red triangles) and the other corresponding to the EOs (Figure 2c, blue circles). Similarly, PCA of the ^{1}H NMR spectra of the 24 mixtures showed discrimination between two groups by PC1, PC2, and PC3 (Figure S1 in the Supporting Information).

The effect of the reaction on the biomolecular properties of the mixtures was evaluated by comparing the tyrosinase inhibitory properties of the fluorinated and nonfluorinated mixtures. The discovery of tyrosinase inhibitors is attractive due to their potential applications in cosmetic, medicinal, and agricultural industries. This enzyme catalyzes the production of melanin and other pigments by oxidation of L-tyrosine.[24] Various dermatological disorders such as melasma, age spots, and sites of actinic damage, arise from an excessive level of epidermal pigmentation.[25] Additionally, the browning observed in vegetables and fruits after harvest is associated with tyrosinase activity, which produces a less attractive appearance and loss of nutritional quality.[26]

The tyrosinase inhibition properties of the mixtures were surveyed by TLC bioautography,[27] a technique particularly well-suited for the analysis of mixtures.[28] This methodology allows the evaluation of inhibitory properties of a sample developed on a TLC plate that is covered with a gel that contains enzyme and substrate. When applied to tyrosinase, fluorination was observed to enhance the inhibitory properties of two mixtures, the FEOs from *Ocimum basilicum* L., Laminaceae (FOB) and *Artemisia dracunculus* L., Asteraceae (FAD), which showed intense inhibition spots that were absent in the nonfluorinated EOs. A follow-up microplate assay[29] showed that the IC_{50} values for *O. basilicum* oil decreased from 278.4 ± 1.38 to 174.4 ± 1.45 μg/mL after fluorination. Similar results were obtained for *A. dracunculus*, in which the IC_{50} decreased from 232.2 ± 1.19 to 125.5 ± 1.56 μg/mL.

The main bioactive compound in both mixtures was identified as 4-allyl-4-fluorocyclohexa-2,5-dienone (**1**, Scheme 1). The identity of this compound was established by NMR

Scheme 1. Proposed Synthesis of 4-Allyl-4-fluorocyclohexa-2,5-dienone (1) from Methyl Chavicol (2) with Selectfluor through a *p*-Fluoro Cation (3)



(^{1}H, ^{19}F, and ^{13}C NMR), IR, and HRMS analyses. This fluorinated derivative could have been formed from the inactive natural component methyl chavicol (**2**, Scheme 1) that is present in both *O. basilicum* and *A. dracunculus* EOs.[30] This was confirmed by fluorination of pure **2** using the same reaction protocol previously employed for the EOs. The TLC bioactivity profile of the reaction showed the generation of the same

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bioactivity spot that was previously detected in *O. basilicum* and *A. dracunculus* FEOs.

It is interesting to note that compound **1** was a minor constituent of both bioactive fluorinated mixtures. Although comprising only 0.6 and 0.5% of the total peak areas of the chromatograms of *O. basilicum* and *A. dracunculus* FEOs (Figure S2), this active component was easily identified in the bioautography assay. Compound **1** could result from an addition−elimination process initiated with the generation of a *p*-fluoro cation (**3**, Scheme 1) from **2.** Release of an alkyl cation from the phenolic oxygen would thus result in the formation of the observed 4-allyl-4-fluorocyclohexa-2,5-dienone (Scheme 1).[31]

The inhibitory potency of fluorinated compound **1** (IC_{50} 59.14 ± 1.15 μM) was found to be similar to that of the known tyrosinase inhibitor kojic acid (IC_{50} = 42.16 ± 1.04 μM). Under these experimental conditions, the natural precursor of **1**, methyl chavicol (**2**), was inactive (IC_{50} > 1000 μM).

In summary, the generation of chemically engineered extracts through fluorination is described for the first time. Chemical diversification of a series of EOs led to the transformation of most of the components of the starting mixtures, producing fluorinated mixtures of expanded diversity (4-fold increase in the number of compounds). Fluorination increased tyrosinase inhibition in two mixtures. The use of a straightforward bioautographic assay enabled the identification of a minor fluorinated compound with similar inhibitory properties to kojic acid, generated in the mixture from a natural inactive precursor.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscombsci.6b00004.

Detailed experimental procedures and spectroscopic data (PDF)

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Author Contributions

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Notes

The authors declare no competing financial interest.

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