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New Method for the Rapid Extraction of Natural Products: Efficient Isolation of Shikimic Acid from Star Anise

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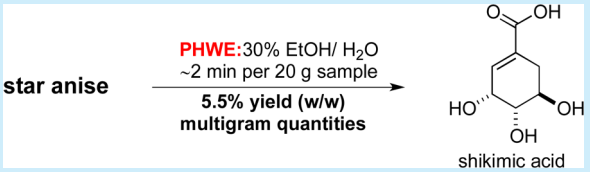
Supporting Information

ABSTRACT: A new, practical, rapid, and high-yielding process for the pressurized hot water extraction (PHWE) of multigram quantities of shikimic acid from star anise (*Illicium verum*) using an unmodified household espresso machine has been developed. This operationally simple and inexpensive method enables the efficient and straightforward isolation of shikimic acid and the facile preparation of a range of its synthetic derivatives.



Shikimic acid (**1**) is a key intermediate in the eponymous metabolic pathway used by plants and microbes to synthesize aromatic amino acids and other secondary metabolites (Figure 1).[1] Although it is of fundamental importance to these organisms, the shikimate pathway is not operative in mammals.[2] Consequently, this has led to the specific development of herbicides, antifungal, antibacterial, or antimalarial agents that operate by inhibiting enzymatic processes along this pathway.[3] Shikimic acid derivatives have also been shown to exhibit useful biological activity. Most notably, the well-known antiviral drug oseltamivir (**2**) (Tamiflu), which acts as a viral neuraminidase inhibitor, is used to treat seasonal influenza and has been deployed during H1N1 influenza outbreaks.[4] Furthermore, fluorinated shikimate analogues have been shown to inhibit *P. falciparum* and have been tested as antimalarial drugs.[5] In addition, shikimic acid-derived (−)-zeylenone (**3**) displays anticancer, antiviral and antibiotic behavior,[6] and triacetylshikimic acid exhibits anticoagulant and antithrombotic activity.[7]

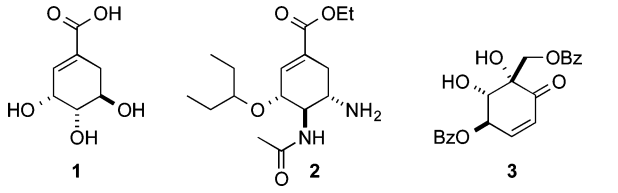


Figure 1. Shikimic acid (**1**), oseltamivir (**2**), and (−)-zeylenone (**3**).

Because of the significant biological properties of shikimic acid and its analogues, these molecules continue to be of interest to synthetic chemists.[8] Moreover, three contiguous stereocenters, an olefin, and a carboxylic acid group are present in this 6-membered carbocycle. As such, it represents a very flexible and useful starting point for organic synthesis and the preparation of biologically relevant and complex molecules.[4−7,8e,9] This is certainly evident in nature where shikimate-derived natural products constitute a class of interesting and challenging synthetic targets.[10]

Shikimic acid is found in many plant species, but is noted to be in particularly high abundance in Chinese star anise (*Illicium verum*).[8e] Dried star anise pods are inexpensive and are readily available in kilogram quantities.[11] The amount of shikimic acid present varies, but reports estimate an average of 3.3% by dry mass.[12] In 1996, Adams and co-workers reported the Soxhlet extraction of shikimic acid from star anise.[13] Some 9 years later, Payne and Edmonds improved this process.[14] This approach simply involved extracting ground star anise with ethanol in a Soxhlet extractor for 2 h. However, it still featured a protracted and rather cumbersome isolation procedure. Ultimately, by this approach, Payne and Edmonds were able to isolate 0.6−1.74 g (2.4−7.0% w/w) of pure shikimic acid.[14] This process has reportedly been adapted and employed to isolate shikimic acid from plants of the *Illicium* genus commercially in China.[15] Variations on the Soxhlet extraction of shikimic acid from star anise continue to be reported.[16] Other related methods that feature the extraction of shikimic acid by refluxing star anise in a range of solvents for extended periods of time (i.e., maceration) have also been disclosed.[17] An ionic liquid-based ultrasound-assisted extraction method has also recently been developed.[18]

Ongoing work in our laboratory is focused on developing novel processes for the rapid isolation of multigram quantities of natural products. Because of the above-mentioned challenges associated with its isolation, we identified shikimic acid as a suitable molecule for us to test the utility of our new method for low cost, rapid, pressurized hot water extraction (PHWE).[19] Herein, we report the use of an unmodified household espresso machine for the rapid PHWE of multigram quantities of shikimic acid from star anise. This represents, to our

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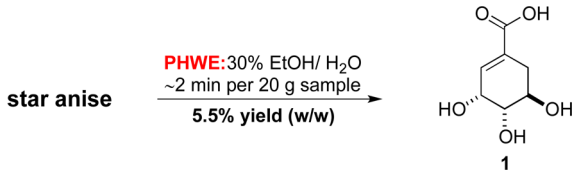
knowledge, the first example of the laboratory use of a simple espresso machine to facilitate the extraction of natural products (other than caffeine) from plant material. This new approach for the multigram isolation of shikimic acid from star anise avoids a number of problems associated with established methods.

PHWE has been previously undertaken on a custom-built system for analysis of shikimic acid content on samples of up to 0.5 g of dry plant material.[20] Although high extraction efficiency was reported, natural product **1** was not isolated, and the method was not repeated on a semipreparative scale. We sought to specifically utilize relatively cheap, unsophisticated, and commercially available equipment to achieve the extraction of multigram quantities of star anise.[21] Consequently, given that the pump in an espresso machine enables the continuous flow of water at temperatures up to 96 °C and at pressures of typically 9 bar, we believed that such a system would be suitable for our purposes.[22]

In our approach, ground star anise (20 g) was packed in the portafilter (sample compartment) of an espresso machine and extracted with a 30% ethanol/water solution (200 mL).[23,24] This was achieved very quickly (~2 min per 20 g sample) and repeated with another 20 g sample of star anise. The extracts were combined, silica gel (20 g) was added, and the suspension was evaporated to dryness. The ensuing solid was washed with dichloromethane and ethyl acetate before extraction with a 10% acetic acid/ethyl acetate solution. The solvent was evaporated, and the residue was washed with dichloromethane then dried to provide shikimic acid as an off-white solid (2.21 g, 5.5% w/w) that was sufficiently pure, as judged by ^{1}H and ^{13}C NMR (Scheme 1). Thus, this method does not require purification by

Scheme 1. Isolation of Shikimic Acid from Star anise

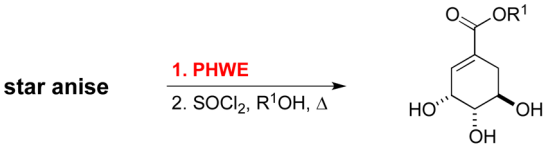


flash or ion-exchange chromatography. The isolation of shikimic acid is greatly simplified by this process primarily because the extraction of unwanted plant material and colored impurities appear to be minimized. We believe that this is a product of the very short residence times involved in the extraction process coupled with the low ethanol content of the solvent medium.

Synthetic work employing shikimic acid almost always involves protection of some or all of the functional groups.[25] For this reason, we sought to investigate a range of synthetic transformations using the evaporated aqueous extract directly. This approach also served to simplify product isolation. Initially, the esterification of the carboxylic acid group of shikimic acid was undertaken by heating the crude extract in methanolic or ethanolic HCl. Methyl and ethyl esters **4** and **5** were successfully isolated after purification by flash chromatography in 4.7% (2.03 g from 40 g of star anise) and 5.4% yields (2.49 g from 40 g of star anise), respectively (Scheme 2).

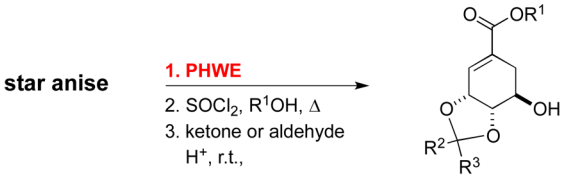
Next, the crude PHWE extract was refluxed in methanolic HCl (as before). However, ester **4** was not isolated but immediately reacted with 2,2-dimethoxypropane and Amberlyst to provide acetonide **6** (Scheme 3). By this approach, 4.76 g of

Scheme 2. Preparation of Methyl and Ethyl Shikimate



4 R^1 = Me: **4.7% yield (w/w)**
5 R^1 = Et: **5.4% yield (w/w)**

Scheme 3. Preparation of Shikimic Acid Derivatives



6 $R^1 = R^2 = R^3$ = Me: **4.0% yield (w/w)**
7 $R^1 = R^2 = R^3$ = Et: **4.2% yield (w/w)**
8 R^1 = Me, R^2 = Ph, R^3 = H: **3.5% yield (w/w)**

shikimic acid derivative **6** was isolated from 90 g of star anise (equivalent to 4.0% yield of shikimic acid w/w). It should be noted that the extraction of dry star anise, functionalization, and isolation of acetonide **6** required only 8 h from start to finish. By analogy, 13.15 g of 3-pentanone-derived *syn*-diol **7** was prepared from 200 g of star anise (equivalent to 4.2% yield of shikimic acid w/w). This effectively demonstrates that this sequence can be scaled up to give multigram quantities of valuable synthetic intermediates. Furthermore, compound **7** is an early stage intermediate en route to oseltamivir (**2**).[26] A similar sequence delivered 2.34 g of product **8** from 40 g of star anise as a mixture of diastereomers (equivalent to 3.5% yield of shikimic acid w/w).

In summary, we have developed a new PHWE method using an unmodified household espresso machine and applied it to successfully effect the rapid extraction of shikimic acid from star anise (~2 min per 20 g sample). This approach is uncomplicated, quick and efficient for the isolation of gram-scale quantities of natural product **1** and subsequent synthesis of its derivatives. It should be noted that our process significantly simplifies the isolation of the shikimic acid, and further chemical transformations can be performed directly on the crude extract. This novel strategy complements existing techniques employed for the extraction of natural products and represents a viable, cost-effective alternative to more complicated approaches. We also believe that this technique represents a useful tool that can streamline bioprospecting procedures. With this in mind, subsequent studies will explore the scope of this methodology, and investigations into the extraction and isolation of natural products from a range of other plants are currently underway. Our results will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and ^{1}H and ^{13}C NMR spectra for compounds **1** and **4**–**8**. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b00936.

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Notes

The authors declare no competing financial interest.

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(24) Ethanol is used as organic modifier in our PHWE method. We found that a 30% ethanol/ water mixture provided optimal results in the extraction of shikimic acid from star anise. The use of organic modifiers in PHWE is well-established (see ref 19).
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