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Liquidambar styraciflua: a renewable source of shikimic acid

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Dedicated to Professor Bradford P. Mundy on the occasion of his 70th birthday

Abstract

An isolation procedure is presented that yields 2.4–3.7% w/w pure shikimic acid from the seeds of *Liquidambar styraciflua* (Sweetgum). Shikimic acid, the starting material in the commercial synthesis of the antiviral agent oseltamivir and an important intermediate in the biosynthesis of aromatic amino acids in plants, was found by HPLC to be abundant in the granular, aborted seeds (6.5% w/w) while present only in small amounts in the developed, fertile seeds (0.14% w/w). This extraction technique makes *L. styraciflua*, which is found in 40 states of the continental US, a potential renewable source of this important natural product.

Keywords: *Liquidambar styraciflua*; Shikimic acid; Plant natural products

Shikimic acid (Fig. 1) was first isolated in 1885 by Eijkman from the fruit of the Japanese plant *Illicium religiosum* Sieb.[1] The elucidation of its structure nearly 50 years later,[2] and the discovery that shikimic acid (**1**) was found to play an important role in the biosynthesis of the three aromatic amino acids phenylalanine, tyrosine, and tryptophan[3] resulted in intensified research efforts toward the synthesis of the compound,[4] its isolation from other organisms,[5] the identification of its metabolites,[6] and its transformation into potential chemotherapeutics. This latter area of research has lead to the syntheses of various bioactive compounds from **1**,[7] including that of the neuraminidase inhibitor oseltamivir phosphate.[8] The drug, sold under the trade name Tamiflu, is currently the most promising treatment for avian flu, the H5N1 subtype of influenza A.[9] Concerns over an outbreak of the disease have elevated the need for **1**,[10] although the availability of newer syntheses[11] of Tamiflu have emerged which may alleviate the demand. Currently, however, the majority of the supply of **1** is met by extraction from the fruit of *Illicium verum* (Chinese star anise) and the fermentation process of Frost and co-workers.[12]

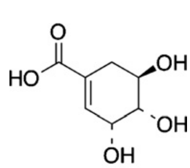

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Fig. 1. Structure of (−)-shikimic acid.

In 1961, Plouvier reported the presence of **1** in the leaves of *Liquidambar styraciflua*, more commonly known as the sweetgum tree.[13] Sweetgum exhibits a rapid growth rate of 0.6–0.9 m per year,[14] is very resilient, and is widely

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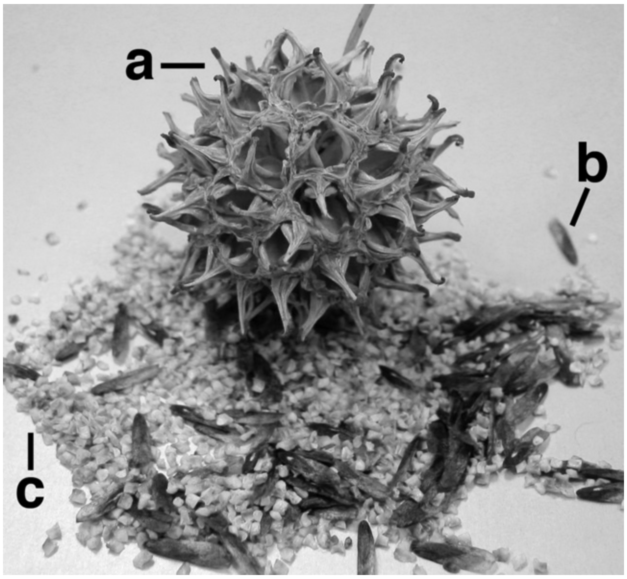

Fig. 2. Two types of seeds in the (a) fruit of *L. styraciflua*: (b) long, fertile seeds and (c) granular, aborted seeds.

cultivated outside its natural habitat both for its ornamental value and as a hardwood for lumber.[15] Mature trees reach 18–23 m in height, span 10–15 m in diameter,[16] and produce gumball-sized fruit (Fig. 2), which have not previously been reported to contain **1**.[5a,17] This is perhaps a consequence of the highly dense and intractable nature of the seed pods, which make the seeds difficult to separate from the fresh fruit. The fruit releases its seeds during autumn through pores 3–4 mm in diameter.[14] We observed that the fruit, which are harvested while green, undergoes dehydration to allow easy access to the seeds.[18] In this study, the average mass of one dried fruit was 4.2 g and yielded approximately 1.2 g of seed, representing roughly 27% seed by mass. Because **1** is commonly found in the tissues of many types of plants,[5] we sought to quantify the amount of **1** in *L. styraciflua* and develop a facile procedure for its extraction from this potentially renewable source.[19]

Fruit from the sweetgum was collected from the lower branches of trees in Claremont, CA from mid-October to late November.[20] Upon drying, either over a period of two weeks at room temperature or for 24 h in a warm oven, the pods open, allowing for the collection of the seeds within. Portions (approx. 35 g) of the seed material obtained from *L. styraciflua* were stirred overnight in 400 mL of deionized water at 65 °C. The extracts were subjected to three treatments with Norit activated charcoal, followed by filtration and concentration under reduced pressure until approximately 50 mL of liquid remained. After allowing the solution to cool, 75 mL of isopropyl alcohol was added to each solution to precipitate a white solid. This mixture consisted of **1** along with other extracted material, the whole of which decomposed if removed from suspension and exposed to air. The mixture was heated to boiling (to redissolve **1**) and passed through a preheated, medium porosity fritted filter. The filtrate was concentrated under reduced pressure to yield a clear, yellow syrup. The syrup was reconstituted with water and chromatographed on an Amberlite IRA-400 (acetate) column using the eluents water (20 mL) and 25% acetic acid (125 mL), respectively. The acetic acid fractions were concentrated under reduced pressure to obtain **1** (amorphous free acid) in an average yield of 3.23% (w/w from dry mass of seed).[21]

Dry seed pods of *L. styraciflua* yield a mixture of two types of seeds (Fig. 2), oblong, winged seeds that are capable of germinating, and granular seeds that have been aborted.[22] Although the isolation procedure described above utilized both seeds together, we sought to determine whether there was a predominance of **1** in either type of seed. Thus, the seeds were separated by hand, and quantification of **1** from the extracts was performed via HPLC analysis (see Supplementary data for HPLC traces),[23] revealing that the majority of **1** originates from the aborted seeds (6.5% w/w) rather than from the fertile seeds (0.14% w/w).[24] This difference is striking, given the large number of plant metabolites dependent on **1** via the shikimate pathway.[6] For example, in the leaves of higher plants, **1** was reported to inhibit phosphoenolpyruvate carboxylase, suggesting that the acid could act to regulate the amount of carbon available for the production of secondary compounds in plants.[25] Comparative studies of other flora with large aborted seed to fertile seed ratios are underway.

In summary, we report that the common sweetgum, through the seeds of its annual fruit, yields **1** in amounts comparable to that of *Illicium verum*. We feel this represents a significant and viable alternative source of **1**, given the enormous numbers of fruit obtainable per tree[26] coupled with recent advancements in sweetgum reproductive technology[27] that will assure its availability into the future.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.02.140.

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