

# Investigating Biogenetic Hypotheses of the *Securinega* Alkaloids: Enantioselective Total Syntheses of Secu'amamine E/ent-Virosine A and Bubbialine

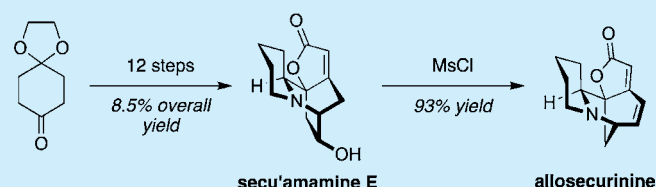
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## S Supporting Information

**ABSTRACT:** The synthesis of the *Securinega* alkaloid secu'amamine E (*ent*-virosine A) has been accomplished for the first time in 12 steps and 8.5% overall yield. In addition, bubbialine has been prepared and characterized. These two alkaloids and bubbialidine, all featuring an azabicyclo[2.2.2]octane core, were rearranged to their azabicyclo[3.2.1]octane congeners, a framework found in many *Securinega* alkaloids. These experiments suggest that azabicyclo[2.2.2]octane derivatives could serve as intermediates in the biosynthesis of the rearranged azabicyclo[3.2.1]octane products.



The *Securinega* alkaloids consist of a group of more than 60 known secondary metabolites isolated from plants of several genera of the Phyllanthaceae family, including *Securinega*, *Phyllanthus*, *Flueggea*, and others.<sup>1</sup> Some of these plants have been used in traditional folk medicines across Asia, Africa, and Amazonia, and subsequently, several compounds of the family were shown to exhibit antimalarial, antibacterial, antitumor, and central nerve system activities.<sup>1,2</sup> Among the *Securinega* alkaloids, securinine constitutes the most abundant and best-studied representative featuring the securinine skeleton (Scheme 1A), and the group includes a series of isomeric neosecurinine-type and two lower homologues of norsecurinine- and neonorsecurinine-type, respectively (see Scheme 1A for an overview). Structurally, these compounds can be divided into groups featuring either azabicyclo[2.2.2]- or azabicyclo[3.2.1]octane BC ring systems with a fused pyrrolidine or piperidine ring. Their interesting biological activities combined with the bridged tetracyclic core structure triggered numerous synthetic efforts toward *Securinega* alkaloids.<sup>1,2c,3,4</sup>

Secu'amamine E (**1**) was isolated along with two new congeners from *Securinega suffruticosa* var. *amamiensis* by Ohsaki et al. in 2009<sup>5</sup> and constitutes a member of the neosecurinine series. The enantiomer *ent*-**1** has also been isolated as virosine A from *Flueggea virosa*,<sup>6</sup> although this relationship was not revealed in the subsequent publication.<sup>5</sup> The lower homologue of **1**, bubbialidine (**2**), the synthesis of which we have reported recently,<sup>7</sup> belongs to the neonorsecurinine family and was isolated along with the isomeric bubbialine (**3**).<sup>8</sup> While allosecurinine (**4**) is a natural epimer of securinine, neither enantiomer of allonorsecurinine (**5**) has

been obtained from a natural source, in contrast to securinol A (**6**) and viroallosecurinine (**7**).<sup>9</sup>

The biosynthetic origin of both the A ring and the C/D system has been investigated over decades<sup>10</sup> and can be traced back to lysine and tyrosine catabolites, which merge to the postulated tricyclic intermediate **8** (Scheme 1B). The origin of the B/C bicyclic ring system, however, received much less attention and, therefore, remains an interesting subject for scientific investigation. The general biosynthetic pathway formulated in the 1970s suggested that intermediate **8** can either react via reduction and 1,6-addition to the azabicyclo[2.2.2]octane system to form e.g. **1** or via reduction and amination to the azabicyclo[3.2.1]octane system to directly afford **4**.<sup>10</sup> An alternative biogenetic hypothesis suggests that compounds featuring the azabicyclo[2.2.2]octane ring system **1** could be intermediates, of which the azabicyclo[3.2.1]octane compounds of type **4** would result through subsequent rearrangement involving an aziridinium ion such as **9**, which has already been postulated by Horii et al. in the 1960s.<sup>11</sup> This hypothesis has, to the best of our knowledge, first been suggested by Magnus et al. in 1993,<sup>12</sup> but later received little attention,<sup>4g</sup> and recent review articles do not mention this mechanistic possibility.

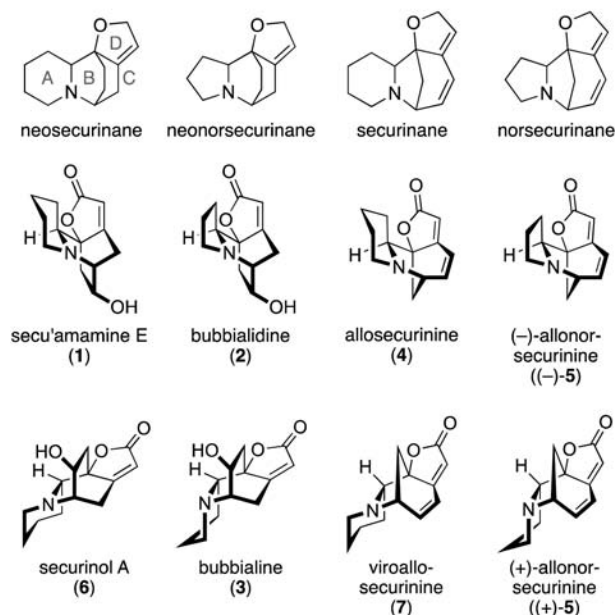
In this study, we provide experimental evidence for the second hypothesis by converting three different azabicyclo[2.2.2] natural products to the rearranged azabicyclo[3.2.1] congeners. Along these lines, the first total syntheses of secu'amamine E/*ent*-virosine A (**1**) and bubbialine (**3**) are reported.

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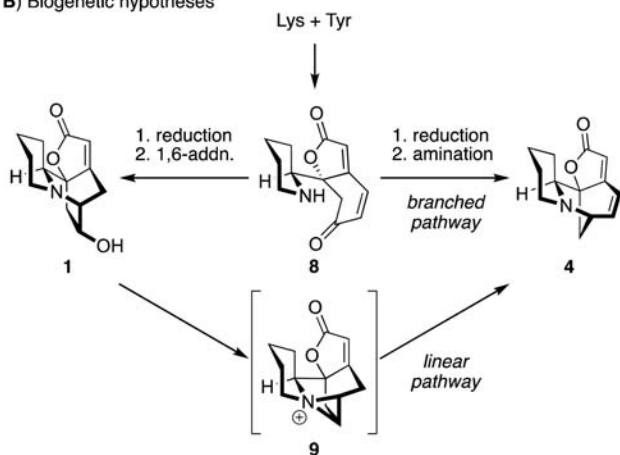
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Scheme 1. General Structures and Biogenetic Hypotheses of Securinega Alkaloids

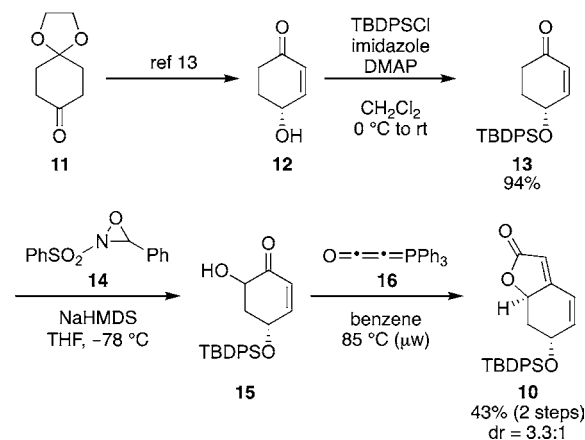
## A) Securinega alkaloid structures



## B) Biogenetic hypotheses



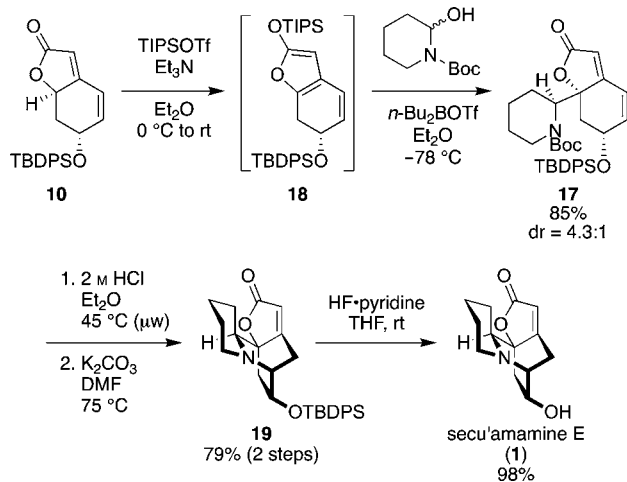
Analogous to our earlier work,<sup>7</sup> the key intermediate in our synthesis of secu'amamine E (**1**) is TBDPS-protected (+)-aquilegiolide (**10**), for which a shorter synthesis was developed (Scheme 2). Ketal **11** was transformed into enone **12** in four steps and 37% overall yield following procedures developed by Hayashi et al. during their total synthesis of (+)-panepophenanthrin.<sup>13</sup> To this goal, a proline-catalyzed stereoselective  $\alpha$ -addition of nitrosobenzene, a reduction of the ketone using K-selectride followed by reductive N–O cleavage, and finally an acid-catalyzed ketal hydrolysis with concomitant elimination of water led to the desired  $\gamma$ -hydroxy enone **12**. The hydroxyl function was protected with a TBDPS group to give fully protected enone **13** in 94% yield. For the subsequent  $\alpha$ -hydroxylation, we first investigated the use of Rubottom oxidation conditions.<sup>14</sup> While <sup>1</sup>H NMR analyses indicated clean formation of the corresponding silyl enol ethers using either TMSCl or TESCl and NaHMDS, the subsequent reaction with *m*CPBA led to decomposition of the substrate, and complex mixtures were obtained. We then evaluated Davis' oxaziridines as oxidants and treatment of the preformed sodium enolate with 3-phenyl-2-(phenylsulfonyl)oxaziridine (**14**)<sup>15</sup> at  $-78^\circ\text{C}$

Scheme 2. Synthesis of Bicyclic Key Intermediate **7**

yielded  $\alpha$ -hydroxy ketone **15**. In addition, clean formation of the product was also observed when a mixture of enone **13** and oxaziridine **14** in THF at  $-78^\circ\text{C}$  was reacted with NaHMDS. However, isolation and purification turned out to be difficult, as enone **15** was not stable during flash column chromatography. Moreover, *N*-benzylidenebenzenesulfonamide, the byproduct formed from oxaziridine **14**, displayed very similar retention properties on silica as the hydroxy ketone **15**. When instead (+)-(8,8-dichlorocamphorylsulfonyl)oxaziridine was used to generate a different byproduct, none of the desired product **15** was detected. The best results were obtained by washing the organic fraction with aqueous NaHSO<sub>3</sub>, therefore removing most of the imine and aldehyde contents, and followed by filtration with apolar solvent to remove insoluble benzenesulfonamide. To setup the butenolide D ring, we applied an efficient one-pot addition/Wittig reaction sequence using the Bestmann ylide ((triphenylphosphoranylidene)ketene, **16**).<sup>16</sup>  $\alpha$ -Hydroxy enone **15** was heated in benzene in the presence of ketene **16** to give the bicyclic key intermediate **10** in 43% yield over two steps as a mixture of diastereoisomers (3.3:1), which was inconsequential and the isomers were not separated. While lower temperatures only slowed the reaction, the addition of triethylamine did not have any impact on yield.

The A ring was introduced using a one-pot procedure reported by Busqué, de March et al. producing the desired product **17** in high yield and good selectivity (Scheme 3).<sup>17</sup>

Scheme 3. Enantioselective Synthesis of Secu'amamine E



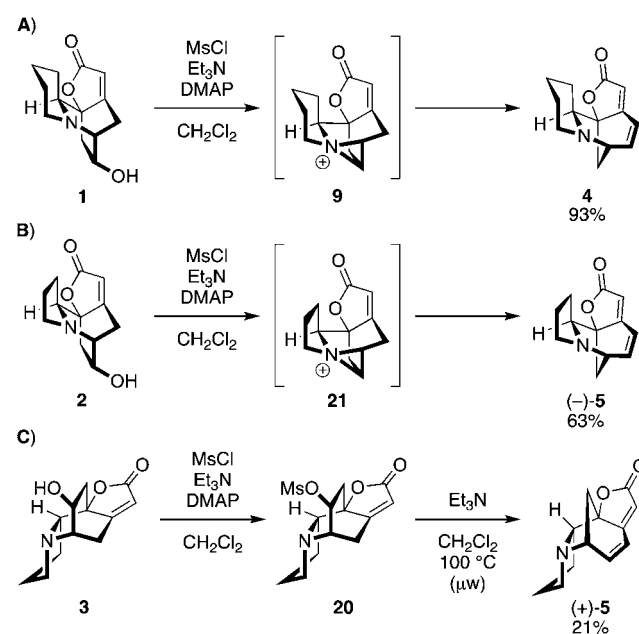
Since the reaction proceeds via silyl enol ether **18**, both diastereoisomers of intermediate **10** lead to the same product. The two diastereoisomers generated could be separated by silica column chromatography. The Boc protecting group was then removed with ethereal HCl solution at elevated temperature to give the desired hydrochloride in 97% yield. When the salt was heated in a suspension of dipotassium phosphate in DMF the desired tetracycle **19** was isolated in varying yields. Applying more basic potassium carbonate in this transformation afforded silyl ether **19** in a yield of 81%. Final deprotection of the hydroxyl function by treatment with excess HF·pyridine afforded secu'amamine E (**1**) in 98% yield. Thus, the neosecurinane alkaloid **1** was synthesized in 12 steps in an overall yield of 8.5%. All analytical data obtained from the synthetic material were identical with those reported for the authentic natural product. The optical rotation of  $-45.7^\circ$  ( $c = 0.10$ , MeOH) of the synthetic alkaloid **1** matched the value of  $-43.8^\circ$  ( $c = 0.08$ , MeOH) reported by Ohsaki et al.<sup>5</sup>

In order to profile the ecotoxicity of this alkaloid, we tested the synthetic material for toxicity against the freshwater crustacean *Thamnocephalus platyurus* (beavertail fairy shrimp).<sup>18</sup> In an acute toxicity assay, five concentrations of alkaloid **1** ranging from 1.23 to 100  $\mu\text{M}$  were incubated with 10 animals for 24 h, each concentration in three separate experiments. Each experiment was evaluated by visual inspection of the animals before and after incubation. However, no toxicity of alkaloid **1** could be observed in this concentration range. In addition to secu'amamine E (**1**), we have obtained bubbialidine (**2**) and bubbialine (**3**) following a previously published route.<sup>7</sup> In this context, bubbialine (**3**) was characterized for the first time during this work.

We then focused on evaluating the feasibility of the hypothesis outlined above, i.e. if the azabicyclo[2.2.2]octane ring system **1** constitutes an intermediate en route to azabicyclo[3.2.1]octane compounds of type **4**. To test the generality of the dehydrative rearrangement outlined above in Scheme 1B, we treated synthetic samples of secu'amamine E (**1**), bubbialidine (**2**), and bubbialine (**3**) with MsCl in the presence of DMAP and Et<sub>3</sub>N. Alkaloids **1** and **2** reacted within seconds at room temperature to give the rearranged alkaloids allosecurinine (**4**) and (–)-allonorsecurinine ((–)-**5**), respectively, in up to 93% yield (Scheme 4A and B). In contrast, bubbialine (**3**) reacted to give the corresponding mesylate **20** under these conditions, which could be isolated and purified by chromatography on silica gel (Scheme 4C). To effect the rearrangement of the sulfonate **20**, forcing conditions had to be applied (100 °C, microwave, 6 h) and (+)-allonorsecurinine ((+)-**5**) was isolated in low yield and moderate purity. These experimental observations can be explained by the mechanistic rationale outlined in Scheme 4. Formation of the aziridinium intermediate **9** or **21** is highly favored for alkaloids with an antiperiplanar configuration of the amino and hydroxy groups on the C ring, as the nitrogen is well aligned to donate electron density into the  $\sigma^*$  orbital of the exocyclic C–O bond. Thus, the structure allows for rapid extrusion of the mesylate via such a mechanism. However, when the groups are arranged in a synperiplanar configuration, such as for **3**, this orbital interaction is less feasible and the mesylate has to leave in an S<sub>N</sub>1 fashion.

As a consequence of these mechanistic observations, we speculate that neosecurinane- and neonorsecurinane-type alkaloids possessing a [2.2.2]-bicyclic core serve as direct biosynthetic intermediates of securinane- and norsecurinane-type alkaloids with an unsaturated [3.2.1] core. Accordingly, the

**Scheme 4.** Dehydrative Rearrangement of *Securinea* Alkaloids



biosynthesis of  $\alpha,\beta,\gamma,\delta$ -unsaturated *Securinea* alkaloids would be extended by an additional intermediate featuring an OH group and thus follow a linear pathway (Scheme 1B). Based on the hypothesis by Busqué, de March et al., intermediate **8** may also be formulated as an alcohol instead of the ketone.<sup>17</sup> Analogous to the transformation of **10** reported in Scheme 3, the tricyclic amine might derive naturally from the bicyclic lactones (+)-aquilegiolide and/or (+)-menisdaurilide via vinyllogous Mannich reaction and thus already contain the hydroxy group.

In summary, we have completed the first enantioselective total synthesis of secu'amamine E (**1**) in 12 steps and 8.5% overall yield. Key transformations are a butenolide formation using the Bestmann ylide (**16**), a vinyllogous Mannich reaction to introduce the A ring, and an intramolecular aza-Michael addition to form the B ring. Furthermore, three synthetic natural products (**1**, **2**, and **3**) featuring an azabicyclo[2.2.2]-octane core were rearranged to their azabicyclo[3.2.1]octane congeners supporting a linear biogenetic hypothesis for *Securinea* alkaloids. Thus, neo(nor)securinane-type alkaloids with an antiperiplanar configuration on the C ring might serve as natural precursors of the (nor)securinane-type alkaloids.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b03716.

Detailed experimental procedures, analytical data, and NMR spectra (PDF)

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## Notes

The authors declare no competing financial interest.

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