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Identification of Amphilectosins as Key Intermediates in Pseudopterosin Biosynthesis

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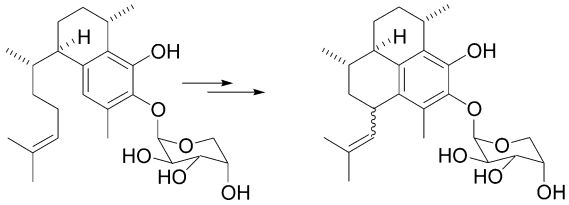
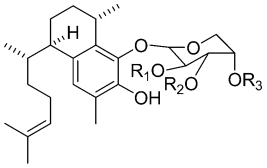


Amphilectosins A and B have been identified from the organic extract of *Pseudopterogorgia elisabethae* collected in the Florida Keys, along with seco-pseudopterosins and pseudopterosins. The structures of the amphilectosins, "C-12−C-13 dehydro seco-pseudopterosins", suggested that these metabolites provide the biosynthetic link between the seco-pseudopterosins (serrulatane diterpenes) and pseudopterosins (amphilectane diterpenes). This biosynthetic relationship was confirmed through various radiolabeling experiments. Incubation studies with the amphilectosins revealed the selective transformation of amphilectosin A to pseudopterosin Y and the transformation of amphilectosin B to pseudopterosin F, which suggests that the α/β stereochemistry for the isobutenyl group in the pseudopterosins arises from the selective ring closure of the *cis*- and *trans*-amphilectosins.

Introduction

The pseudopterosins (e.g., **1** and **2**, Scheme 1) represent a class of structurally diverse diterpene glycosides isolated from the marine octocoral *Pseudopterogorgia elisabethae*.[1] Collections of *P. elisabethae* from various geographic locations contain different members of the pseudopterosin family. Currently there are 26 known pseudopterosin derivatives (A−Z). All known pseudopterosin congeners contain an amphilectane skeleton and a glycoside linkage at either C-9 or C-10. Structural variations for this class of diterpenes are limited to the identity of the glycoside, the degree of its acetylation, and the stereochemistry for the isobutenyl group on C-1.

The structurally related seco-pseudopterosins A−D (**3**−**6**, Figure 1) belong to the serrulatane class of diterpenes and were initially isolated from *Pseudopterogorgia kallos* collected in the Florida Keys.[2] More recently, novel seco-pseudopterosins and pseudopterosins were reported to co-occur in *P. elisabethae* collected in the Florida Keys and Old Providence Island.[3,4]



3 $R_1 = R_2 = R_3 = H$
4 $R_1 = Ac$; $R_2 = R_3 = H$
5 $R_2 = Ac$; $R_1 = R_3 = H$
6 $R_3 = Ac$; $R_1 = R_2 = H$

FIGURE 1. Seco-pseudopterosins A−D (**3**−**6**).

The pseudopterosins and seco-pseudopterosins are antiinflammatory agents, which exhibit a novel spectrum of activity when compared to existing topical antiinflammatory agents. In animal models the pseudopterosins

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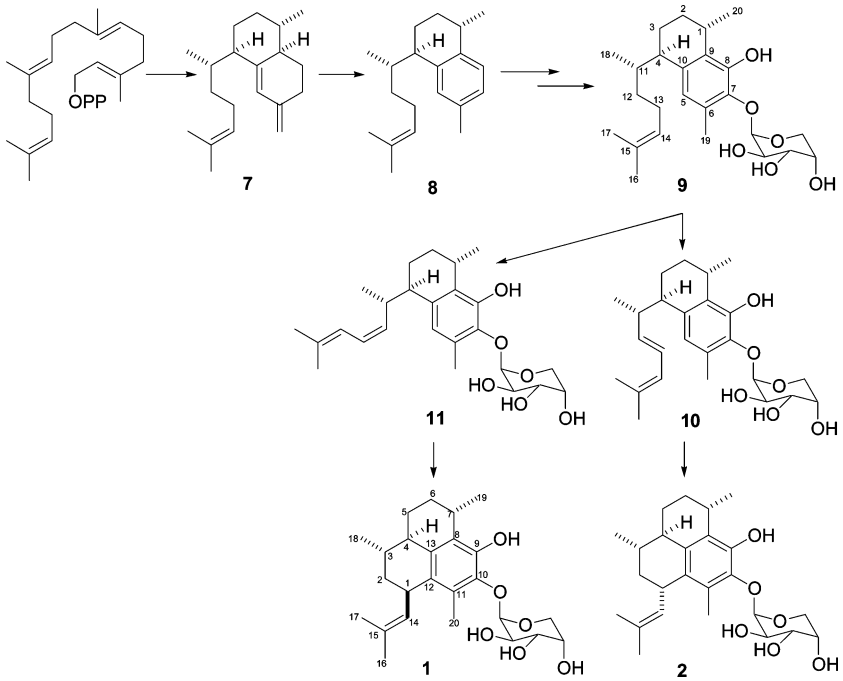
SCHEME 1. Involvement of Seco-Pseudopterosins and Amphilectosins in Pseudopterosin Biosynthesis



block edema produced by acute application of phorbol 12-myristate 13-acetate (PMA).[5] In harvested human polymorphonuclear granulocytes (PMNs), the pseudopterosins block calcium ionophore-induced degranulation and release of leukotriene B (LTB),[5] neutrophil myeloperoxidase, and lactoferrin.[6] Recent studies have indicated that the release of eicosanoids is blocked without interrupting biosynthesis.[3] Pseudopterosin A has been found, in cultured cells, to stabilize nuclear lamina in dividing sea urchin embryos[7] and decrease phagosome formation in *Tetrahymena* cultures activated with calcium or zymosan.[3,8] Additionally, pseudopterosins are currently used as cosmetic additives.[9]

We recently described in vitro and in vivo systems, which were developed as tools to elucidate the biosynthesis of the pseudopterosins. Previously we used our in vitro technique to confirm the intermediacy of elisabethatriene (**7**) and erogorgiaene (**8**) in pseudopterosin biosynthesis.[10−12] In a continuation of these metabolic studies, we have conducted a study to search for, and evaluate, the intermediacy of additional putative precursors in pseudopterosin biosynthesis. Specifically, our goal was to identify the mode of cyclization of serrulatanes to the pseudopterosins with the amphilectane ring system. Herein, we report the structures of three novel diterpene glycosides, seco-pseudopterosin J (**9**), amphilectosin A (**10**), and amphilectosin B (**11**) and report the intermediacy of these diterpene glycosides in pseudopterosin biosynthesis.

Results and Discussion

Samples of *P. elisabethae* were collected at a depth of 80 ft off Long Key, FL, and dried by lyophilization. Dried coral material was extracted, the solvent was removed under reduced pressure, and the crude organic material was subjected to solvent partitioning. Fractionation of the methylene chloride partition using gradient silica gel flash chromatography gave 11 fractions (F1−F11). Subjection of F11 to reversed phase C18 HPLC afforded pseudopterosin F (**1**), pseudopterosin Y (**2**), and three novel diterpene glycosides: seco-pseudopterosin J (**9**), amphilectosin A (**10**), and amphilectosin B (**11**). The known compounds (**1** and **2**) were identified by comparison of their spectral data with that previously reported.[4,14]

Seco-pseudopterosin J (**9**) was isolated as a colorless oil. A molecular formula of $C_{25}H_{38}O_6$ was established by HR ESI FT-ICR, which requires 7 degrees of unsaturation. The ^{1}H NMR spectrum of **9** was similar to that reported for seco-pseudopterosin A.[2] However, a NOESY correlation between H_3-19 and H-1′ suggested that the arabinose moiety resides on C-7. To confirm the position of the glycoside, **9** was methylated and the glycoside was hydrolyzed to afford seco-pseudopterosin J methyl ether

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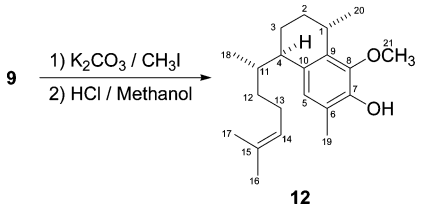
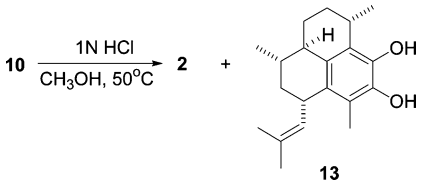
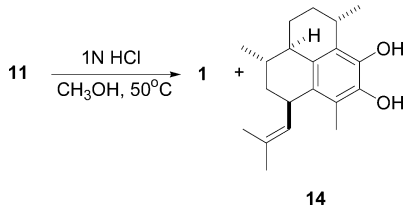
SCHEME 2. Synthesis of 12



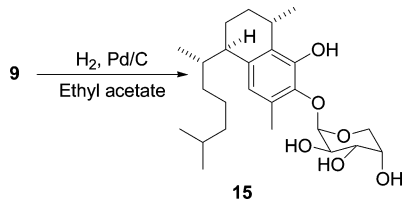
SCHEME 3. Synthesis of 2 and 13



SCHEME 4. Synthesis of 1 and 14



SCHEME 5. Synthesis of 15



aglycone (**12**, Scheme 2). The ^{1}H NMR spectrum for **12** was similar to that previously reported.[4] Furthermore, NOESY correlations H-1/H_3-21 and H_3-20/H_3-21, as well as those for H-5/H_3-19 and H_3-19/H-7 (OH), indicated that the methoxy group resides on the C-8.

Amphilectosin A (**10**) was purified as a colorless oil. The HR ESI FT-ICR of **10** showed a sodium adduct at 455.2404, suggesting a molecular formula of $C_{25}H_{36}O_6$, which requires 8 degrees of unsaturation. The ^{1}H NMR spectrum of **10** was similar to seco-pseudopterosin J (**9**) with the exception of the olefinic region, which showed the addition of two signals, suggesting the presence of an additional double bond. COSY correlation data showed that the C-14 olefinic proton (δ 5.80, J = 11 Hz) had cross-peaks with the C-13 olefinic proton (δ 6.13, J = 11, 15 Hz), the C-16 methyl (δ 1.71), and the C-17 methyl (δ 1.74) protons. The C-13 olefinic proton showed vicinal coupling with the C-12 olefinic proton (δ 5.59, J = 6, 15 Hz), which in turn showed COSY interactions with the C-11 methine (δ 2.62). The large coupling constant (J = 15 Hz) for the C-12 and C-13 olefinic protons indicates a trans relationship. The ^{1}H NMR spectrum of amphilectosin A (**10**) was also similar to the reported spectrum of helioporin G, which has the same diterpene skeleton as **10**, but possesses a methylene dioxy moiety at C-7−C-8.[13] Corey at el. previously synthesized a synthetic derivative of amphilectosin A, which, upon treatment with acid and heat, was cyclized stereospecifically to pseudopterosin Y aglycone (**13**).[15] To further confirm the structure of amphilectosin A (**10**), a small sample of this compound was transformed to pseudopterosin Y (**2**) and to aglycone **13** using analogous conditions (Scheme 3). The ^{1}H NMR spectra for **2** and **13** were identical to that reported in the literature.[4,15]

Amphilectosin B (**11**) was isolated as a colorless oil. The UV spectrum and HRMS of **11** were similar to that of amphilectosin A (**10**), indicating that these may be structurally related molecules. Furthermore, the COSY spectra of amphilectosin A (**10**) and amphilectosin B (**11**) revealed that these diterpene glycosides had the same connectivity. The only significant difference between the ^{1}H NMR spectra of amphilectosin A and amphilectosin B was the smaller coupling constant (J = 11.1 Hz) observed for the C-12 and C-13 olefinic protons of **11**. Similarities between the various spectral data for **10** and **11** and differences in the coupling constant data for the C-12 and C-13 olefinic bond suggest that **11** is the cis isomer of **10**. The ^{1}H NMR of the diterpene portion of **11** was also similar to that of helioporin F, which is the cis isomer of helioporin G. Interestingly, amphilectosin B was transformed to pseudopterosin F and its aglycone (**14**) when treated with acid and heat (Scheme 4), which is the opposite stereochemical outcome observed for the acid-catalyzed cyclization and hydrolysis of amphilectosin A (**10**).

Biosynthetic Experiments. The co-occurrence of pseudopterosins with seco-pseudopterosins in *P. elisabethae* suggested the possibility that the latter undergoes a ring closure reaction to give rise to the pseudopterosins. The isolation of the amphilectosins appears to provide a possible mechanism for this ring closure, a nucleophilic attack of the aromatic ring on the C-12−C-13 double bond. To evaluate the intermediacy of seco-pseudopterosins and amphilectosins in pseudopterosin biosynthesis, seco-pseudopterosin J (**9**), amphilectosin A (**10**), and amphilectosin B (**11**) were produced in radiolabeled form by incubating [1-^{3}H] GGPP (20 μCi) with a cell-free extract of *P. elisabethae*. To confirm radiochemical purity of these three metabolites, a portion of each was purified to constant specific activity by HPLC, derivatized, and re-purified to constant specific activity. Metabolites are deemed to be radiochemically pure when the specific activity of the purified derivative is the same as that of the isolated natural product. Thus, to evaluate the radiochemical purity of seco-pseudopterosin J (**9**) we needed to develop a method to derivatize this molecule. Specifically, we converted seco-pseudopterosin J to its dihydro analogue (**15**) using a palladium-catalyzed hydrogenation (Scheme 5). HRMS of analogue **15** gave a sodium adduct at 459.2395, which suggested the molecular formula $C_{25}H_{40}O_6Na$ and 6 degrees of unsaturation, thus confirming the hydrogenation. The ^{1}H NMR spectrum of analogue **15** was similar to that of seco-pseudopterosin J with the exception of the appearance of two methyl doublets (δ 0.69, J = 6.9 Hz; δ 1.17, J = 6.9 Hz) and the absence of an olefinic triplet. The ^{13}C NMR spectrum showed resonances consistent with aliphatic methyl groups (δ 23.2) and more importantly did not show resonances for olefinic carbons.

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After confirming the structure of analogue **15**, we then tested the radiochemical purity of seco-pseudopterosin J (**9**), amphilectosin A (**10**), and amphilectosin B (**11**) by derivatization of each compound and purification of the derivative to constant specific activity. Seco-pseudopterosin J (**9**) (1000 DPM, 3.8×10^6 DPM/mmol) was converted to its dihydro analogue (**15**) (Scheme 5), and this was shown to have a similar specific activity (860 DPM, 3.0×10^6 DPM/mmol). Amphilectosin A and amphilectosin B were cyclized and hydrolyzed to their respective pseudopterosin aglycones, and these were shown to have similar specific activities as their amphilectosin precursors. Amphilectosin A (7.4×10^4 DPM/mmol) was transformed to pseudopterosin Y aglycone (6.7×10^4 DPM/mmol), and amphilectosin B (6.1×10^4 DPM/mmol) was transformed to pseudopterosin F aglycone (5.3×10^4 DPM/mmol).

An initial goal of this work was to determine if seco-pseudopterosins are precursors to pseudopterosins. Thus, we incubated ^{3}H-seco-pseudopterosin J (9040 DPM, 3.8×10^6 DPM/mmol) with a cell-free extract and monitored for the production of ^{3}H-labeled pseudopterosins. Rigorous purification of pseudopterosin F and pseudopterosin Y from the cell-free extract using HPLC and subsequent scintillation counting of the pseudopterosins indicated the transformation of seco-pseudopterosin J (**9**) to pseudopterosin F (770 DPM, 4.2×10^5 DPM/mmol) and pseudopterosin Y (860 DPM, 5.4×10^5 DPM/mmol). To confirm that the recovered pseudopterosins were radiochemically pure, pseudopterosin F (**1**) and pseudopterosin Y (**2**) were hydrolyzed to their respective aglycone derivatives **13** and **14**, which were purified to constant specific activity, and shown to have specific activities similar to those of their "parent" pseudopterosins (**13**, 480 DPM, 4.9×10^5 DPM/mmol; **14**, 430 DPM, 4.2×10^5 DPM/mmol).

The enzymatic transformation of seco-pseudopterosin J (**9**) to the pseudopterosins supports our hypothesis that the amphilectane skeleton found in pseudopterosins arises from the serrulatane skeleton of the seco-pseudopterosins. This transformation also supports our hypothesis that the amphilectosins undergo a ring closure reaction to give rise to the pseudopterosins. To address the involvement of the amphilectosins in pseudopterosin biosynthesis we tested the transformation of seco-pseudopterosin J to amphilectosin A (**10**) and amphilectosin B (**11**) and the transformation of **10** and **11** to the pseudopterosins.

The transformation of seco-pseudopterosin J (**9**) to the amphilectosins was evaluated by incubating labeled seco-pseudopterosin J (37 155 DPM, 3.5×10^5 DPM/mmol) with a cell-free extract of *P. elisabethae*. Rigorous purification of the amphilectosins from the cell-free extract by repeated HPLC injections and subsequent scintillation counting indicated that both amphilectosins were radioactive. Radiochemical purity for the recovered amphilectosins (**10**, 1580 DPM, 6.3×10^4 DPM/mmol; **11**, 1150 DPM, 4.6×10^4 DPM/mmol) was addressed by chemical derivatization to their respective pseudopterosin aglycone derivatives, which after rigorous purification were shown to have specific activities similar to those of their amphilectosin precursors (**13**, 1500 DPM, 6.0×10^4 DPM/mmol; **14**, 1075 DPM, 4.3×10^4 DPM/mmol).

The intermediacy of amphilectosins in pseudopterosin biosynthesis was confirmed by examining the transformation of labeled amphilectosins to pseudopterosins using a cell-free extract. Amphilectosin A (25 410 DPM, 7.4×10^4 DPM/mmol) and amphilectosin B (20 130 DPM, 6.1×10^4 DPM/mmol) were incubated separately with cell-free extracts of *P. elisabethae* and monitored for the production of labeled pseudopterosins. Interestingly, amphilectosin A gave rise specifically to pseudopterosin Y (1589 DPM, 6.4×10^4 DPM/mmol), and amphilectosin B afforded only pseudopterosin F (1290 DPM, 5.4×10^4 DPM/mmol). Radiochemical purity for these transformations was addressed by the chemical transformation of each pseudopterosin to their respective catechol derivative **13** and **14**. After purification and scintillation counting, these derivatives were found to have specific activities similar to those of their amphilectosin precursors (**13**, 1460 DPM, 6.4×10^4 DPM/mmol; **14**, 1200 DPM, 5.0×10^4 DPM/mmol).

The selective biosynthetic conversion of amphilectosin A to pseudopterosin Y and amphilectosin B to pseudopterosin F suggests that this is the key enzymatic step in the conversion of seco-pseudopterosins to pseudopterosins. However, our observation of the stereospecific acid-catalyzed transformation of amphilectosins to the pseudopterosins suggested that this ring closure may not be an enzymatic process. To address this possibility, we performed a biosynthetic experiment with a boiled control. Thus, a cell-free extract was heated at 100 °C for 1 h prior to incubation with labeled amphilectosin A (25 880 DPM, 7.4×10^4 DPM/mmol). The recovered pseudopterosins were found to be unlabeled, thus indicating that this ring closure is under enzymatic control. The stereospecific acid-catalyzed ring closure is intriguing and is consistent with analogous observations made by Corey in his synthesis of the pseudopterosin aglycone.[15]

The transformation of seco-pseudopterosin J to the amphilectosins could proceed either via an oxidation/dehydration or directly from a dehydrogenation. The former mechanism requires the existence of a seco-pseudopterosin derivative with an alcohol at either C-12 or C-13. As a preliminary measure to determine whether such an intermediate is produced, we performed a radioactivity-guided isolation. Seco-pseudopterosin J (100 200 DPM, 2.6×10^6 DPM/mmol) was obtained from a *P. elisabethae* cell-free extract that was incubated with 20 μCi [1-^{3}H] GGPP. ^{3}H-Labeled **9** (99 200 DPM) was then incubated with a cell-free extract, and aliquots were taken every 15 min for 1 h. The aliquots were partitioned, and the methylene chloride partitions were subjected to normal-phase HPLC. A detailed analysis of the radioactive HPLC fractions of the first aliquot indicated that the only radioactive metabolites were amphilectosins A and B. Purification and analysis of the remaining aliquots indicated the presence of radioactivity in the amphilectosins and pseudopterosins. No other radioactive compounds were detected in any of the aliquots, other than recovered radioactivity in seco-pseudopterosin **9**. These data suggest that the seco-pseudopterosins are directly transformed to the amphilectosins with no intermediate step.

The above data provide the biosynthetic link between seco-pseudopterosins and pseudopterosins, members of the serrulatane and amphilectane diterpenes, respec-

tively. The isolation of the amphilectosins, together with the biosynthetic experiments, indicates that the seco-pseudopterosins are transformed to their C-12−C-13 dehydro analogues, thus facilitating the ring closure presumably by nucleophilic attack of the aromatic ring on the newly introduced double bond. It is also now clear that the formation of pseudopterosins with α- and *β*-isobutenyl groups is due to the selective ring closure of the *cis*- and *trans*-amphilectosins. The results of these biosynthetic experiments, together with our previously published findings, are summarized in Scheme 1.

Experimental Section

General Experimental Procedures. [1-^{3}H] Geranylgeranyl diphosphate (60 Ci/mmol) was purchased from a commercial supplier. ^{1}H NMR spectra were recorded in $CDCl_3$ at 400 MHz. Homonuclear COSY and 1-D proton NOESY were used to establish atom connectivites and spatial relationships. Silica gel (400−600 mesh grade) was used for flash chromatography experiments.

Coral Material. *P. elisabethae* was collected by SCUBA in the Florida Keys at a depth of 80 ft and allowed to dry in the sun or immediately flash frozen with liquid nitrogen. The sun-dried material was further dried by lyophilization, while the flash frozen coral material was stored at −80 °C.

Extraction and Isolation. Dried *P. elisabethae* (305 g) was extracted with ethyl acetate (2 × 1000 mL) and methylene chloride (2 × 1000 mL). The extracts were filtered, combined, and evaporated to dryness under reduced pressure, yielding a black gummy residue (120 g). The crude material was dissolved in methanol/water (9:1) (600 mL) and partitioned with hexanes (3 × 600 mL) to give a nonpolar extract (65 g). The ratio of the methanol/water layer was adjusted to 1:1, and the aqueous layer was partitioned with methylene chloride (3 × 1000 mL) to afford a yellow oil (3.8 g). The hexanes and methylene chloride partitions were dried over anhydrous sodium sulfate, filtered, and concentrated to dryness under reduced pressure. Silica gel and methylene chloride were added to the methylene chloride extract, and the sample was evaporated to dryness under reduced pressure.

The silica gel-bound residue was loaded on a silica gel flash column and eluted with a stepwise gradient of hexanes and ethyl acetate (100−0% hexanes) to afford 11 fractions. Fraction 11 was subjected to semipreparative RP-C18 HPLC using a gradient of acetonitrile/water (50−100%) as mobile phase to afford seco-pseudopterosin J (**9**, 6.2 mg), amphilectosin A (**10**, 10.5 mg), amphilectosin B (**11**, 4.7 mg), pseudopterosin F (**1**, 5.2 mg),[14] and pseudopterosin Y (**2**, 9.4 mg).[4]

Seco-pseudopterosin J (9). Colorless oil; Compound **9**: $[\alpha]^{20}_D$ −80° (*c* 0.0013, CH_2Cl_2); IR (CH_2Cl_2) 3370, 3020, 2990, 2920. ^{1}H NMR (400 mHz, $CDCl_3$) *δ* 6.48 (1 H, s), 5.13 (1 H, d), 5.12 (1 H, br t), 4.39 (1 H, d, 12.7 Hz), 4.12 (3H, m), 3.88 (1 H, d, 14.8 Hz), 3.13 (1 H, m), 2.62 (1 H, m), 2.28 (3 H, s), 2.05 (2 H, m), 2.00 (1 H, m), 1.81 (1H, m), 1.69 (3 H, s), 1.66 (2 H, m), 1.61 (3 H, s), 1.45 (2 H, m), 1.44 (1 H, m), 1.17 (3 H, d, 6.9 Hz), 0.69 (3 H, d, 6.9 Hz). ^{13}C NMR (100 mHz, $CDCl_3$) *δ* 146.5, 141.8, 137.0, 131.2, 129.1, 127.8, 124.8, 121.0, 103.3, 70.0, 69.6, 69.1, 63.6, 39.4, 38.5, 35.6, 27.8, 27.0, 26.2, 25.7, 20.9, 18.4, 17.7, 17.2, 16.4. HRMS *m/z* (ESI FT-ICR) found for $C_{25}H_{38}O_6Na$ 457.2251, observed 457.2249.

Amphilectosin A (10). Colorless oil; Compound **10**: $[\alpha]^{20}_D$ −85° (*c* 0.0038, CH_2Cl_2); IR (CH_2Cl_2) 3500, 3030, 2920 1710 cm^{-1}. ^{1}H NMR (400 mHz, $CDCl_3$) *δ* 6.47 (1 H, s), 6.13 (1 H, dd, $J_{1,2}$ = 10.8 Hz, $J_{1,3}$ = 15.1 Hz), 5.80 (1 H, d, 10.8 Hz), 5.58 (1 H, dd, $J_{1,2}$ = 6.2 Hz, $J_{1,3}$ = 15.2 Hz), 5.10 (1 H, d, 2.27 Hz), 4.34 (1 H, d, J = 12.4 Hz), 4.12 (3 H, m), 3.83 (1 H, d, J = 12.2 Hz), 3.10 (1 H, m), 2.63 (1 H, m), 2.20 (3 H, s), 2.10 (2 H, m), 1.85 (1 H, m), 1.80 (1 H, m), 1.74 (3 H, s), 1.71 (3 H, s), 1.65 (2 H, m), 1.43 (1 H, m), 1.13 (3 H, d, 6.8 Hz), 0.87 (3 H, d, 6.2 Hz). ^{13}C NMR (100 mHz, $CDCl_3$) *δ* 146.4, 142.0, 137.4, 135.8, 133.0, 128.9, 127.7, 125.5, 125.1, 121.8, 103.7, 69.5, 69.3, 63.7, 53.4, 42.5, 41.1, 27.1, 26.9, 25.9, 20.8, 19.2, 18.2, 17.0, 16.2. HRMS *m/z* (ESI FT-ICR) found for $C_{25}H_{36}O_6Na$ 455.2404, observed 455.2402.

Amphilectosin B (11). Colorless oil; Compound **11**: $[\alpha]^{20}_D$ −62° (*c* 0.0014, CH_2Cl_2); IR (CH_2Cl_2) 3500, 3010, 2990 1730 cm^{-1}. ^{1}H NMR (400 mHz, $CDCl_3$) *δ* 6.51 (1 H, s), 6.06 (1 H, d, 11.1 Hz), 5.90 (1 H, d, 11.7 Hz), 5.28 (1 H, dd, $J_{1,2}$ = 11.1 Hz, $J_{1,3}$ = 11.1 Hz), 5.12 (1 H, d, 3.4 Hz), 4.39 (1 H, d, 12.3 Hz), 4.12 (2 H, m), 4.07 (1H, m), 3.88 (1 H, d, 11.7 Hz), 3.14 (1 H, m), 2.97 (1 H, m), 2.60 (1 H, m), 2.26 (3 H, s), 2.00 (1 H, m), 1.86 (1 H, m), 1.74 (3 H, s), 1.70 (3 H, s), 1.46 (1 H, m), 1.17 (3 H, d, 6.8 Hz), 0.89 (3 H, d, 6.9 Hz). ^{13}C NMR (100 mHz, $CDCl_3$) *δ* 147.0, 141.5 135.9, 135.1, 134.7, 127.5, 123.3, 121.7, 120.3, 103.2, 70.1, 69.6, 69.0, 66.3, 63.6, 41.5, 37.4, 27.0, 26.9, 26.3, 21.1, 20.2, 18.2, 18.0, 17.1. HRMS *m/z* (ESI FT-ICR) found for $C_{25}H_{36}O_6Na$ 455.2404, observed 455.2408.

Conversion of Amphilectosin A (10) to Pseudopterosin Y (2). To 5 mg (11 *μ*mol) of **10** was added a 1 N HCl methanolic solution (2 mL). After being agitated at 50 °C for 3 h, the solution was cooled to 25 °C, and water was added (10 mL). The solution was extracted with methylene chloride (3 × 2 mL), the organic extracts were combined and dried over anhydrous sodium sulfate, and the solvent was allowed to evaporate under a stream of nitrogen. The residue was purified by semipreparative RP-C18 HPLC using acetonitrile/water (50−100%) as a mobile phase to afford **2** (4.4 mg, 91%). All spectral data collected were identical to that previously reported.[4]

Conversion of Amphilectosin B (11) to Pseudopterosin F (1). Conversion of **11** (2.3 mg, 5.3 *μ*mol) to **1** (2.0 mg, 87%) was performed as described above for the conversion of amphilectosin A to pseudopterosin Y. The ^{1}H NMR of **1** was identical to that described in the literature.[14]

Conversion of Amphilectosin A (10) to Pseudopterosin Y Aglycone (13). Conversion of **10** (4.3 mg, 9.9 *μ*mol) to **13** (2.7 mg, 91%) was performed as described above for the conversion of **10** to **2** except the reaction was allowed to proceed for 8 h. The ^{1}H NMR of **13** was identical to reported literature values.[15]

Conversion of Amphilectosin B (11) to Pseudopterosin F Aglycone (14). Conversion of **11** (3 mg, 6.9 *μ*mol) to **14** (1.8 mg, 88%) was performed as described for the preparation of **13**. The ^{1}H NMR of **14** was identical to reported literature values.[15]

Catalytic Hydrogenation of Seco-Pseudopterosin J (9) to 14,15-Dihydro-Seco-Pseudopterosin J (15). Compound **9** (3 mg, 6.9 *μ*mol) was dissolved in ethyl acetate (10 mL) and transferred to an Erlenmeyer vacuum flask containing 3% Pd−C and a magnetic stir bar. The reaction vessel was maintained under a positive pressure of hydrogen and vigorously stirred at 25 °C for 8 h. The resulting heterogeneous mixture was filtered through a column of Celite, and the solvent was allowed to evaporate under a stream of nitrogen. The organic residue was purified by semipreparative RP-C18 HPLC using acetonitrile/water (50−100%) as a mobile phase to afford **15** (2.3 mg, 75%) as a colorless oil. Compound **15**: $[\alpha]^{20}_D$ −57° (*c* 0.0015, CH_2Cl_2). IR (CH_2Cl_2) 3500, 3020, 2920 cm^{-1}. ^{1}H NMR (400 mHz, $CDCl_3$) *δ* 8.12 (1 H, s), 6.48 (1 H, s), 5.13 (1 H, d, 2.4 Hz), 4.08 (3 H, m), 4.39 (1 H, d, 12.0), 3.88 (1 H, dd, $J_{1,2}$ = 1.9 Hz, $J_{1,3}$ = 12.7), 3.28 (1 H, m), 3.13 (1 H, m), 2.62 (1 H, m), 2.28 (3 H, s), 1.17 (3 H, d, 6.9 Hz), 1.04 (3 H, d, 6.3 Hz), 0.98 (3 H, d, 6.5 Hz), 0.69 (3 H, d, 6.9 Hz). ^{13}C NMR (100 mHz, $CDCl_3$) *δ* 146.5, 141.8, 137.0, 129.1, 127.8, 121.0, 103.3, 70.0, 69.6, 69.1, 63.6, 39.9, 39.4, 38.5, 35.6, 28.2, 27.8, 27.0, 26.2, 23.2, 23.2, 20.9, 18.4, 17.2, 16.4. HRMS *m/z* (ESI FT-ICR) found for $C_{25}H_{40}O_6Na$ 459.2391, observed 459.2395.

Methyl Ether Aglycone Derivative of Seco-Pseudopterosin J (12). To a stirred solution of **9** (4.0 mg, 9.2 *μ*mol) in dry acetone (10 mL) was added an excess of anhydrous potassium carbonate and methyl iodide. After being refluxed for 8 h, the reaction mixture was allowed to cool to room

temperature, the acetone evaporated under a stream of nitrogen, water was added (5 mL), and the aqueous layer was extracted with methylene chloride (3 × 5 mL). The combined methylene chloride layers were dried over anhydrous sodium sulfate, filtered, and allowed to evaporate under a stream of nitrogen. To the crude organic material was added a 1 N HCl methanolic solution (2 mL). After being agitated at 50 °C for 8 h, the solution was cooled to 25 °C and water was added (10 mL). The solution was extracted with methylene chloride (3 × 10 mL), the organic extracts were combined and dried over anhydrous sodium sulfate, and the solvent was evaporated under a stream of nitrogen. The residue was purified by semipreparative RP-C18 HPLC using acetonitrile/water (50−100%) to afford **12** (2.6 mg, 90%). All spectral data collected were identical to that previously reported.[4]

Preparation of the Cell-Free Extract. Flash frozen *P. elisabethae* (150 g) was homogenized in a blender with liquid nitrogen and 300 mL of a 0.1 mM tris-HCl buffer (pH = 7.7) containing 3 mM EDTA and 0.035% β-mercaptoethanol. The homogenate was centrifuged at 9600*g* for 15 min, the pellet was discarded, and the supernatant was centrifuged at 39000*g* for 60 min. The resulting supernatant (cell-free extract) was stored in 40-mL aliquots at −80 °C.

Incubation of Cell-Free Extract with ^{3}H-GGPP and Purification of ^{3}H-labeled 9, 10, and 11. A 40-mL aliquot of cell-free extract was incubated with 20 μCi [1-^{3}H] GGPP (50−60 Ci/mmol) for 24 h at 29 °C. The incubation mixture was quenched and extracted with ethyl acetate (3 × 40 mL), the organic extracts were combined and dried over anhydrous sodium sulfate, filtered, and concentrated to dryness under reduced pressure. The organic reside was partitioned between hexanes and methanol/water (9:1) three times. The ratio of the methanol/water layer adjusted to 1:1, and the layer was partitioned with methylene chloride (3 × 30 mL). The methylene chloride partition was then subjected to gradient normal-phase HPLC using hexanes/ethyl acetate (60−100%) as mobile phase, and the most polar UV active fraction (λ = 283 nm) was collected and subjected to reversed-phase HPLC. Acetonitile/water (50−100%) was used as a mobile phase to afford seco-pseudopterosin J (**9**, 37 530 DPM, 3.5 × 10^5 DPM/mmol), amphilectosin A (**10**, 52 820 DPM, 7.4 × 10^4 DPM/mmol), and amphilectosin B (**11**, 20 130 DPM, 6.1 × 10^4 DPM/mmol).

Incubation of Cell-Free Extract with ^{3}H-Labeled 9, 10, and 11. Incubation of triturated **9**, **10**, and **11** was performed by transferring labeled metabolites into a 40-mL falcon tube using ethyl acetate, and the solvent was removed under a stream of nitrogen. To the falcon tube were added glycerol (4 mL), Tween 20 (0.05%), and 0.1 mM tris-HCl buffer (1 mL). The mixture was vortexed for 10 min, followed by the addition of 1 aliquot of cell-free extract (40 mL). The cell-free extract was incubated at 29 °C for 24 h and then quenched and extracted with ethyl acetate (3 × 40 mL). Target molecules were purified from the organic residue as described above.

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Supporting Information Available: Spectral data for seco-pseudopterosin J (**9**), amphilectosin A (**6**), amphilectosin B (**7**), and 14,15-dihydro-seco-pseudopterosin J (**15**). This material is available free of charge via the Internet at http://pubs.acs.org.

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