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# THE SECO-PSEUDOPTEROSINS, NEW ANTI-INFLAMMATORY DITERPENE-GLYCOSIDES FROM A CARIBBEAN GORGONIAN OCTOCORAL OF THE GENUS PSEUDOPTEROGORGIA

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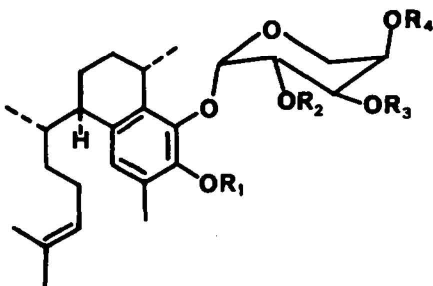
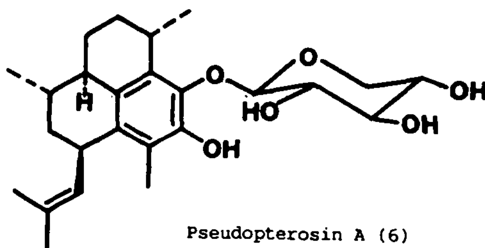
Abstract: A new class of diterpene-pentosides, the seco-pseudopterosins (1-4), has been isolated from a Caribbean sea whip of the genus Pseudopterogorgia. The new compounds are arabinose glycosides possessing aglycones of the serrulatane class, the compounds in the series are mono-acetate positional isomers, and they are related to the recently described pseudopterosins by bond cleavage at the C5 - C13 positions. The seco-pseudopterosins possess potent anti-inflammatory and analgesic activities equivalent to commercial anti-inflammatory drugs. The structures of these new compounds are suggested on the basis of comprehensive spectral analyses and upon chemical transformations.

As part of a program to explore the chemical defense adaptations of Caribbean octocorals, we have dedicated considerable effort to gorgonians of the complex genus Pseudopterogorgia.[1-5] Pseudopterogorgia species are among the most obvious invertebrates found on Caribbean reefs. Prior studies have shown this genus to be a rich source of cytotoxic compounds[2] and antiinflammatory agents,[4,5] and extracts of these animals produce significant feeding deterrence effects against carnivorous reef fishes.[6] As part of an expedition to the Florida Keys in 1980, our attention was drawn to a small, bushy Pseudopterogorgia species, extracts of which showed modest cytotoxicity and antimicrobial activity.[7] From this gorgonian, we have isolated four new bicyclic diterpenoid glycosides, the seco-pseudopterosins A-D (1-4). The seco-pseudopterosins are potent antiinflammatory and analgesic compounds related to pseudopterosin A, a metabolite from P. elisabethae.[4] The gorgonian collected in the Florida Keys was tentatively identified as P. kallos, a species securely assigned with some difficulty. In prior studies, collections securely identified as P. kallos were found to contain the kallolides, a series of "pseudopterane" diterpenoids.[5] We interpret our current findings to indicate that the new collection may represent a chemotaxonomically distinct Pseudopterogorgia species.[8]




Pseudopterosin A (6)

Seco-A (1) $R_1=R_2=R_3=R_4$ = H

Seco-B (2) $R_1=R_3=R_4$ = H, $R_2$ = Ac

Seco-C (3) $R_1=R_2=R_4$ = H, $R_3$ = Ac

Seco-D (4) $R_1=R_2=R_3$ = H, $R_4$ = Ac

(5) $R_1=R_2=R_3=R_4$ = Ac

(8) $R_1=CH_3$, $R_2=R_3=R_4$ = H

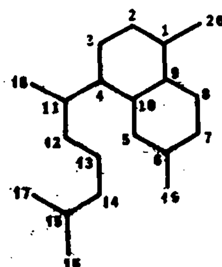


TABLE 1 - $^{1}H$ and $^{13}C$ NMR DATA FOR SECO-PSEUDOPTEROSINS A, C AND D[a,b]

| | Seco A | | Seco C | | Seco D | |
|---|---|---|---|---|---|---|
| C# | $^{1}H$ | $^{13}C$ | $^{1}H$ | $^{13}C$ | $^{1}H$ | $^{13}C$ |
| 1 | 3.10(1H,m) | 27.2 | 3.15(1H,m) | 27.0 | 3.14(1H,m) | 27.2 |
| 2 | 1.45(1H,m)<br>1.83(1H,m) | 28.1 | c | 27.9 | 1.82(1H,m)<br>1.49(1H,m) | 28.1 |
| 3 | c | 18.7 | c | 18.5 | 1.63(2H,m) | 18.9 |
| 4 | 2.60(1H,m) | 39.8 | 2.62(1H,m) | 39.5 | 2.61(1H,m) | 39.9 |
| 5 | 6.47(1H,s) | 121.2 | 6.48(1H,s) | 121.0 | 6.48(1H,s) | 121.1 |
| 6 | - | 127.8 | - | 127.7 | - | 127.7 |
| 7 | - | 146.5 | - | 146.4 | - | 146.7 |
| 8 | - | 137.0[e] | - | 137.0[e] | - | 137.1[e] |
| 9 | - | 129.2 | - | 129.1 | - | 129.3 |
| 10 | - | 142.1[e] | - | 141.7[e] | - | 142.0[e] |
| 11 | 1.95(1H,m) | 38.5 | 1.99(1H,m) | 38.5 | 1.92(1H,m) | 38.4 |
| 12 | c | 35.8 | c | 35.7 | 1.80(1H,m)<br>1.45(1H,m) | 35.8 |
| 13 | c | 26.3 | c | 26.2 | c | 26.4 |
| 14 | 5.14(1H,bt) | 125.0 | 5.15(1H,bt) | 124.8 | 5.15(1H,bt) | 125.0 |
| 15 | - | 131.1 | - | 131.1 | - | 131.1 |
| 16 | 1.69(3H,s)[e] | 25.6 | 1.70(3H,s)[e] | 25.7 | 1.70(3H,s)[e] | 25.7 |
| 17 | 1.61(3H,s)[e] | 17.7 | 1.61(3H,s)[e] | 17.7 | 1.61(3H,s)[e] | 17.7 |
| 18 | 0.71(3H,d,6.9) | 16.4 | 0.73(3H,d,6.9) | 16.4 | 0.72(3H,d,6.9) | 16.4 |
| 19 | 2.23(3H,s) | 21.1 | 2.18(3H,s) | 21.1 | 2.14(3H,s) | 21.1 |
| 20 | 1.16(3H,d,6.8) | 17.1 | 1.18(3H,d,6.8) | 17.3 | 1.19(3H,d,6.8) | 17.1 |
| 1' | 5.12(1H,d,2.7) | 104.1 | 5.18(1H,d,3.6) | 103.5 | 5.16(1H,d,3.4) | 103.4 |
| 2' | 4.14(1H,bd)[d] | 69.7[f] | 4.31(1H,dd,3.1,9.9) | 67.6 | 4.09(1H,dd,3.5,9.8) | 70.0 |
| 3' | 4.13(bd)[d] | 69.6[f] | 5.32(1H,dd,3.1,9.9) | 73.1 | 4.33(1H,dd,3.5,9.8) | 68.4 |
| 4' | 4.07(1H,bs) | 69.5[f] | 4.24(1H,bs) | 67.6 | 5.23(1H,bs)[d] | 71.6 |
| 5' | 3.83(1H,bd,12.3)[d]<br>4.33(1H,bd,12.3)[d] | 63.9 | 4.42(1H,bd,12.4)<br>3.85(1H,dd,12.4,2.4) | 63.5 | 3.89(1H,bd,13.1)<br>4.39(1H,dd,13.1,1.0) | 61.9 |
| OAc | | | 2.27(3H,s) | 20.9<br>171.5 | 2.26(3H,s) | 21.0<br>171.2 |
| OH | 8.30(1H,bs,exc.) | | 8.01(1H,bs,exc.) | | 8.17(1H,bs,exc.) | |

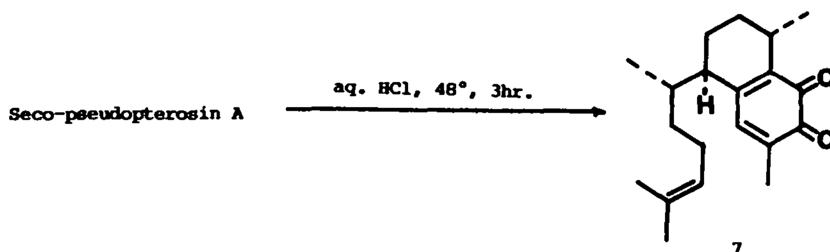
a. Proton NMR spectra were recorded at 360 MHz in $CDCl_3$ solution at 61°C. Assignments were aided by spin-decoupling experiments and J values shown are in Hz. Proton NMR values are in $\delta$ values with TMS as internal standard.
b. Carbon-13 NMR were recorded in $CDCl_3$ at 50 MHz. Multiplicities were obtained by single frequency off-resonance decoupling and they are not indicated above. Some assignments were made on the basis of evaluation of the "residual" coupling constants under off-resonance conditions.
c. Proton NMR resonances not assigned.
d. Signal broadening, even at higher temperatures, made measurement of J values impossible.
e,f. Signals within a vertical column may be reversed.

Specimens of Pseudopterogorgia sp. (collection #F-44) were collected by hand using SCUBA during an expedition of the research vessel CALANUS. The animals were stored frozen and within 2 months they were extracted with chloroform and ethyl acetate. The combined extracts were chromatographed over TLC grade silica gel and fractions containing polar compounds were further fractionated by silica HPLC. Purification yielded the seco-pseudopterosins A-D (1-4) as the major components of the extract. Seco-pseudopterosin A (Seco-A) was isolated as the major metabolite, while seco-pseudopterosins B-D were all found in lesser quantities. Seco-seudopterosin B, in particular, was isolated in such minor quantities that complete spectral data could not be obtained before decomposition.

Seco-pseudopterosin A,(1), analyzed for $C_{25}H_{38}O_6$ by high resolution mass spectrometry and by $^{13}C$ NMR (Table 1). The mass spectrum of this compound showed a fragment representing loss of $C_5H_8O_4$. This behavior, coupled with appropriate proton and carbon NMR bands, indicated that seco-pseudopterosin A contained a pentose-glycoside component, similar to that of a related series of glycosides, the pseudopterosins, typified by pseudopterosin A, (6).[4,9] Interpretation of the NMR spectral features of compound 1 indicated the presence of a bicyclic diterpenoid component containing a penta-substituted aromatic ring. We were particularly familiar with many of the spectral properties of similar carbon skeletons, since recent work with the pseudopterosins had just been completed.[9] As in the pseudopterosin series, the related isomers were easily recognized as mono acetate positional isomers by proton NMR. Indeed, acetylation of seco-pseudopterosins A-D, under standard conditions, yielded the same tetra-acetate 5.

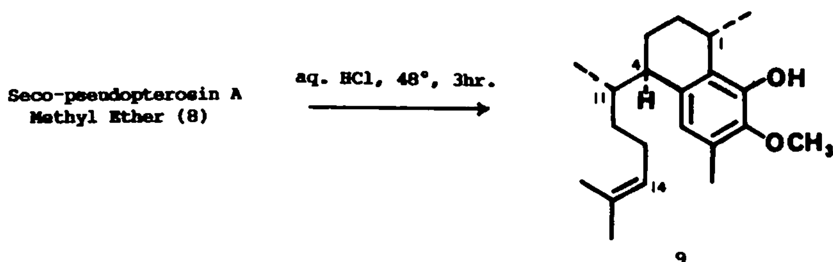
The complete structure determination of the seco-pseudopterosins was facilitated by interpretation of NMR data and by consideration of the results from several chemical transformations. Seco-A (1) possessed $^{13}C$ NMR features which were highly characteristic of its bicyclic skeleton. Six aromatic resonances were observed between 120 and 150 PPM, diagnostic of the catechol constellation. Unlike the $^{13}C$ NMR features of pseudopterosin A, the seco compound possessed an off-resonance doublet carbon at 121.2 PPM (assigned to C5), indicative that bond cleavage had occurred at the C5 - C13 positions.

In an attempt to examine the aglycone component of 1, the compound was hydrolyzed with mild HCl for 3 hours at 48°C. Hydrolysis yielded a single organic product, quinone 7, which could not be purified by chromatography without substantive loss on silica gel. The compound, obtained as a bright orange-red oil, analyzed for $C_{20}H_{30}O_2$ by mass spectrometry, and was recognized as an ortho-quinone by its highly-characteristic quinone spectral features (experimental). The production of an ortho-quinone confirmed that the aglycone of seco-A (1) contained the catechol functionality and no other oxygenation.




Seco-pseudopterosin A was next methylated with methyl iodide in acetone to obtain the mono methyl ether 8 in good yield. This ether was then utilized to determine the relative positions of hydroxyl, aromatic methyl, and glycoside linkages on the aromatic ring. Using proton NMR nuclear Overhauser enhancement Difference Spectroscopy (NOEDS) techniques, the methyl ether was readily positioned ortho to the aromatic methyl and glycoside functional groups. Irradiation of the methyl ether group (located at $\delta$ 3.79 in the NMR spectrum of 8) produced intense enhancement of the aromatic methyl resonance located at $\delta$ 2.33. Irradiation of the methyl group, in the reverse experiment, produced identical results. On the basis of this experiment and the production of 7 by hydrolysis, the methyl, methoxy, and glycoside components must be placed in sequential positions on the aromatic ring.

In order to further explore the structure of the aglycone, the methyl ether 8 was hydrolysed under mild acid conditions. Hydrolysis yielded a stable aglycone, 9, which was readily purified

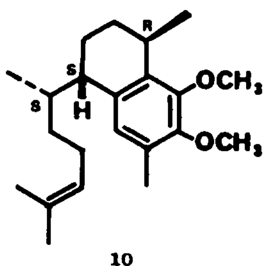



by HPLC. The stable aglycone 9 was an ideal derivative for continued NMR studies of the full structure of the aglycone. Using NOEDS methods, additional data was obtained to further define the structure of 9. Irradiation of the aromatic methyl group [ δ 2.22 (3H,s)] enhanced the lone aromatic proton [ δ 6.72(1H,s)] and the methoxy methyl group [ δ 3.78 (3H,s)]. In addition, a methine proton observed at δ 2.65 was strongly enhanced. These data confirmed our earlier findings with the glycoside, and also indicated that the aromatic methyl group was adjacent to the lone aromatic proton. Further, the enhancement of an unassigned methine proton suggested a point of substitution existed in close proximity to C5.

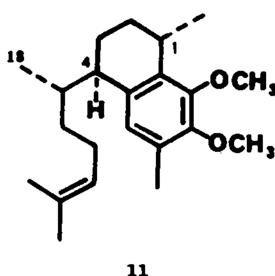
Extensive proton NMR decoupling experiments were also performed with the aglycone ether 9. Irradiation of the methine proton at C4 showed that another methine proton was positioned at C11. Irradiation of the C11 methine proton caused a high field methyl doublet to collapse to a singlet, thus allowing the positioning of a methyl group at this carbon. In a similar experiment, the methine proton at C1 was related to the presence of a secondary methyl group at this position. Another feature of the aglycone 9, which was also present in compound 1, was the terminal trisubstituted olefin. This group was readily confirmed as bearing two geminal methyl groups on the basis of $^{13}C$ NMR data, and upon the observance of significant allylic coupling of the methyl groups to the C14 olefin proton. The traditional terpenoid eight carbon side-chain in 9 (and hence in 1-4) was suggested based upon the favorable comparison of $^{13}C$ NMR data for this $C_8$ component with two model compounds, lanosterol[10] and ophibolin-C[11].

On the basis of the initial data obtained, the aglycone found in the seco-pseudopterosins was assigned to the known serrulatane [12] bicyclic ring system (also found in the compound biflorin[13]). The aglycone 8 was found to be similar to a dimethyl ether 10, reported as a synthetic derivative from the diterpenoid constitutents of Eremophila species. The diterpenoid 10 had been fully defined, including absolute stereochemistry, since it was produced from a compound described by X-ray crystallographic methods.[12] Methylation of aglycone 9 generated the aglycone 11, which was similar, but not identical, to the known 10. While most proton NMR resonances were highly comparable, the C18 methyl resonance in 11 was observed at δ 0.71 rather than at δ 0.96 as in 10. Other minor variations were observed in resonances due to protons in the side-chain. On the basis of this comparison, aglycone 11 was concluded to be diastereomeric at one or more centers.

Since there are 3 asymmetric centers in 10, it became important to pin-point the position(s) where these two isomers differed. Proton NMR analyses of compounds 1, 8, and 11 were performed with the specific goal of establishing the relative configurations of the alkyl




10

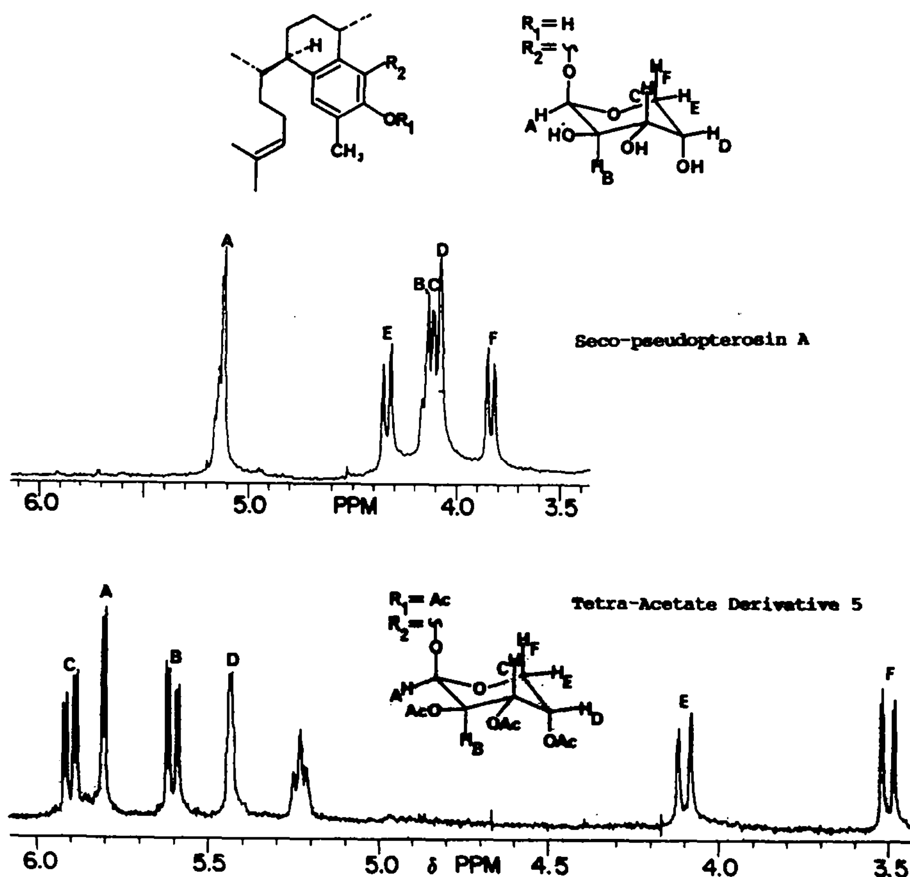



11

groups at C1 and C4. Since similar alkyl substituents are present in the pseudopterosin series, which was defined by X-ray methods, a comparison of relevant proton NMR data was performed. Using this approach and molecular models, the methyl group at C1 and the side-chain at C4 were illustrated to be trans. Coupling constant analysis illustrated that the seco-pseudopterosins possess identical conformations to that observed, by X-ray analysis, in pseudopterosin C. Diterpenoid **10** is known to possess the identical relative stereochemistry on the cyclohexane ring. Hence, aglycone **11** must be epimeric at C11. Because **10** has been shown to possess the C1R, C4S, C11S configuration, compound **11** must be C1R*, C4S*, C11R*. As indicated by the asterisks, the absolute stereochemistry cannot be determined by these comparisons. If, however, there is a consistent biosynthesis of this bicyclic series with the tricyclic pseudopterosins, the absolute stereochemistry is likely to be C1S, C4R, C11S.

Given that the structure of the aglycone component was secure, our attention was next focussed upon defining the sugar component. Proton NMR analyses of seco-A (**1**) and of the tetra-acetate **5** were performed, with particular attention paid to the mid-field region illustrating the sugar methine protons. Comparisons of **1** and **5**, and spin-decoupling experiments, allowed all protons to be assigned (see Figure 1). Of particular importance was the proton on the anomeric carbon ($H_A$), observed as the lowest field, non-aromatic nor olefinic, proton in seco-A (ca. 5.1). This proton, a doublet with J=2.7 Hz., was coupled to the adjacent proton at C2'. The proton at C2' was coupled to the methine proton at C3' by J=9 Hz. Since the C2' - C3' couplings were characteristic of axial-axial configurations, the C1' - C2' coupling constant of J=2.7 Hz must indicate that the proton at C1' is equatorial. From these data, the glycoside linkage in seco-pseudopterosin A was confirmed as alpha (axial). At 360 MHz, the remaining sugar methine protons were readily

**Figure 1 - $^1H$ NMR Interpretation of the Mid-Field Region of Seco-pseudopterosin A (1) and of the Tetra-Acetate Derivative 5**



resolved. Proton $H_D$, the C4' proton, was observed as a broadened multiplet with very small coupling constants. Because the magnitude of these couplings could not exceed 2-3 Hz in each case, the C4' proton must be equatorial. On the basis of these analyses, the sugar component of seco A was confirmed as alpha-arabinose. Unfortunately, attempts to isolate arabinose and to determine its optical rotation failed, presumably due to decomposition of the sugar under the strong acid conditions required for glycoside cleavage. Hence, the absolute stereochemistry of the arabinose component was not determined.

Interpretation of the proton NMR spectra of seco-pseudopterosins C and D (3,4) readily allowed the location of acetylation to be determined. In seco-pseudopterosin C, for example, the C3' sugar methine proton was shifted from $\delta$4.13 in 1 to $\delta$5.32. This low field shift strongly suggested that acetylation had taken place at the C3' position in 3. For seco-pseudopterosin D (4), analysis of the proton NMR data led to similar conclusions. In this case, the C4' methine proton was shifted from ca. $\delta$ 4.2 in 1 and 2 to $\delta$ 5.23, confirming the location of acetylation at C4'. NMR analyses of the sugar components were greatly aided by the classic papers produced on this topic in the late 1950's.[15-17]

The seco-pseudopterosins show antimicrobial activities against a wide variety of bacterial and fungal pathogens. Seco-pseudopterosin A, (1), for example, shows a minimum inhibitory concentration against Staphylococcus aureus of 8 micrograms per mL. More importantly, the seco-pseudopterosins are anti-inflammatory and analgesic agents with potencies near or exceeding those of several commercial drugs. Seco-pseudopterosin A, for example, at doses of ca. 50 micrograms per ear, shows 69% reduction of inflammation in the mouse ear edema assay. The related pseudopterosins are also potent anti-inflammatory and analgesic agents.[4] These two classes of novel bioactive natural products appear promising as lead structures in the design of new anti-inflammatory drugs.

## Experimental

General. Infrared spectra were recorded on a Perkin-Elmer model 137 spectrophotometer. Ultraviolet spectra were obtained in methanol using a Beckman Acta XIV spectrophotometer. Proton NMR spectra were recorded in deuteriochloroform at 360 MHz, using a spectrometer constructed from an Oxford magnet and Nicolet-1180E Fourier transform data system, at the UCSD NMR Facility. NOEDS experiments were performed essentially following the experiments outlined by Hall and Sanders[14]. Samples were prepared for NOEDS measurements by first degassing with Argon. Carbon-13 NMR were recorded in deuteriochloroform solution at 50 MHz using a Nicolet Wide-Bore spectrometer. Low-resolution mass spectra were obtained using a Hewlett-Packard Model 5930A mass spectrometer. High-resolution mass spectra were kindly provided by the Mass Spectrometry Resource Center, UC-Berkeley, under the direction of A. Burlingame. Optical rotations were measured using a Perkin-Elmer Model 141 polarimeter and 10 cm microcell. All solvents were dried and distilled from glass prior to use. Initial chromatographic separations were performed using a rapid elution method similar to "flash chromatography" but using vacuum techniques with TLC grade silica gel packed in a large scintered-glass filter funnel.

Collection, Extraction and Isolation of the Seco-pseudopterosins. Gorgonians tentatively identified as P. kallos (collection #F-44) were collected by hand using SCUBA in the Florida Keys near Cosgrove Shoal in June, 1980. The animals were immediately frozen, returned to the laboratory, and within 2 months extracted exhaustively with chloroform and then ethyl acetate. The extracts were combined and chromatographed over TLC grade silica gel using rapid elution methods. Fractions eluted with 60-85% ethyl acetate in isooctane were further purified by silica HPLC, using the same solvent mixtures, to obtain the seco-pseudopterosins A-D (1-4), as roughly 1.5% of dry weight gorgonian in total. Using normal phase methods, seco-pseudopterosins B, C and D eluted in advance of the more polar seco-pseudopterosin A. Only approximately 1 mg of seco-pseudopterosin B (2) was obtained from the initial chromatography. A poor quality proton NMR spectrum of this compound showed it to be the C2'-acetate isomer. However, before additional spectra could be obtained the compound decomposed.

Seco-pseudopterosin A (1). Seco-pseudopterosin A was isolated as a viscous oil which later solidified as an amorphous solid. Seco-pseudopterosin A showed the following spectral properties: $[\alpha]_D$ -118° (c 1.7, chloroform), UV (methanol) 224 nm ($\varepsilon$=10,000), 271 nm ($\varepsilon$=1380), 281 nm ($\varepsilon$=1490), after addition of 1 drop 5% KOH/methanol values shifted to 244, 281 and 291 nm, respectively. IR (chloroform) 3350, 3030, 2960, 2920, 1215 $cm^{-1}$; HRMS $M^+$ m/z (rel. intensity) 434.2683 (0.6) for $C_{25}H_{38}O_6$ (requires 434.2668), 302.2232 for $C_{20}H_{30}O_2$ (65), 218.1312 for $C_{14}H_{18}O_2$ (13) and 191.1069 for $C_{12}H_{15}O_2$ (100).

Seco-pseudopterosin C (3). Seco-pseudopterosin C was isolated as an amorphous solid which showed the following spectral features: $[\alpha]_D$ -89° (c 0.58, chloroform); UV (methanol) 223 nm ($\varepsilon$=7900), 271 nm ($\varepsilon$=2300), 281 nm ($\varepsilon$=2360), after addition of 1 drop 5% KOH/methanol values shifted to 248, 281 and 290 nm, respectively; IR (chloroform) 3400, 2960, 1740, 1460 $cm^{-1}$; HRMS m/z (rel. intensity) 302.2205 for $C_{20}H_{30}O_2$ (24, $M^+$ -$C_7H_{10}O_5$), 191.1074 for $C_{12}H_{15}O_2$ (100).

Seco-pseudopterosin D (4). Seco-pseudopterosin D was obtained as an amorphous solid which showed the following spectral properties: $[\alpha]_D$ -139° (c 0.6, chloroform); UV (methanol) 223 nm ($\epsilon$ =9900), 272 nm ($\epsilon$ =1890), 281 nm ($\epsilon$ =1860), after addition of 1 drop 5% KOH/methanol values shifted to 240, 282, and 292 nm, respectively; IR (chloroform) 3600, 3350 bd, 3020, 2960, 2920, 1735, 1490, 1450, 1410, 1370, 1320, 1210, 1075, 1000, and 780 $cm^{-1}$; HRMS $M^+$ m/z (rel. intensity) 476.2784 for $C_{27}H_{40}O_7$ (0.9), requires 476.2774, 302.2251 for $C_{20}H_{30}O_2$ (25), 300.2094 for $C_{20}H_{28}O_2$ (9), 285.1855 for $C_{19}H_{25}O_2$ (4.4), 229.1232 for $C_{15}H_{17}O_2$ (2.1) and 191.1070 for $C_{12}H_{15}O_2$ (100).

Acetylation of Seco-pseudopterosins A, C, and D. In multiple experiments, a slight excess of acetic anhydride was added to 20 mg samples of seco-pseudopterosins A, C, and D (1,3 and 4) in pyridine, cooled in ice. The solution was stirred at room temperature overnight, quenched with excess cold water and extracted with chloroform (3 X20 mL). The combined extracts were washed with 5% HCl, dist. water, and 5% sodium bicarbonate solution, then dried over anhyd. magnesium sulfate. Removal of solvent under reduced pressure yielded the tetra-acetate 5 in modest purity. Purification by silica HPLC yielded 5, from each isomer, as a viscous oil which showed the following spectral properties: $[\alpha]_D$ -108° (c 0.8, chloroform); UV (methanol) =228 nm ($\epsilon$ =11,000), 265 to 280 nm ($\epsilon$ =1200); IR (chloroform) 3030, 2960, 2920, 1745 $cm^{-1}$; LRMS $M^+$, m/z (rel. intensity) 602 (0.5) for $C_{33}H_{46}O_{10}$, 300 (0.2), 259 (35), 191 (9.3), base peak very low mass; Proton NMR (61°) $\delta$ 6.86 (1H,s), 5.62 (1H, dd, J=2.2, 11.9), 5.52 (1H, d, J=3.1), 5.42 (1H, bs), 5.28 (1H, dd, J=2.6, 10.2), 5.13 (1H, bt, J=5.9, 6.0), 4.32 (1H, bd, J=12.5), 3.78 (1H, dd, J=1.7, 13.1), 2.68 (1H, bs), 2.31, (3H, s), 2.29 (3H, s), 2.16 (3H, s), 2.04 (6H, s), 1.71 (3H, s), 1.62 (3H, s), 1.04 (3H, bs), 0.69 (3H, d, J=6.8).

Acid Hydrolysis of Seco-pseudopterosin A (1) Seco-pseudopterosin A (1.4 mg, 0.003 mmol), was combined with a solution consisting of 1 mL methanol and 1 mL 3 N HCl. The solution was sealed in a pyrex tube and warmed to 48°C for 3 hr. The tube was cooled, opened and diluted with water to 50 mL. The solution was extracted with chloroform (4 x 20 mL), and the combined chloroform extracts were washed with water and then 5% sodium bicarbonate. The solution was dried over anhyd. magnesium sulfate, filtered, and the solvent was removed under reduced pressure to yield ortho-quinone 7, as an orange-red oil (ca. 1 mg). The quinone showed the following spectral properties: UV (methanol) 224 nm ($\epsilon$ =6100), 281 nm ($\epsilon$ =1900), 420-460 nm ($\epsilon$ =500); LRMS $M^+$ m/z (rel. intensity) 302 (14) for $C_{20}H_{30}O_2$ ($M^+$+2), 218 (9.4), 173 (34), 158 (10); Proton NMR (360 MHz) $\delta$ 6.53 (1H, s), 5.16 (1H, bt), 4.14 (1H, m), 3.08 (1H, m), 2.64 (1H, m), 2.22 (3H, s), 1.71 (3H, s), 1.69 (3H, s), 1.21 (3H, d, J=6.8), 0.72 (3H, d, J=6.7).

Methylation of Seco-pseudopterosin A. A slight excess of methyl iodide was added to a stirredsolution of 1 (10.3 mg, 0.024 mmole) and excess anhyd. potassium carbonate in 30 mL acetone. The solution was refluxed for 7 hr and then diluted with excess water and extracted with chloroform (3 x 25 mL). The solvent extracts were combined, dried over anhyd. magnesium sulfate, and the solvent was removed under reduced pressure to yield the methyl ether derivative 6 (9.8 mg, 92%). Ether 6 showed the following spectral features: UV (methanol) 225 nm ($\epsilon$ =10,400), 269 ($\epsilon$ =1200), 275 nm ($\epsilon$ =1200); LRMS $M^+$ m/z (rel. intensity) 316 (7.1) for $C_{21}H_{32}O_2$ ($M^+$ $-C_5H_8O_4$); Proton NMR $\delta$ 6.81 (1H, s), 5.16 (1H, d, J=3.2), 5.14 (1H, bt), 4.73 (1H, bd, J=10.8), 4.27 (1H, bd, J=12.5), 4.14 (1H, bs), 4.13 (1H, dd, J=3.1, 9.5), 3.96 (1H, dd, J=3.2, 14.7), 3.87 (1H, m), 3.79 (3H, s), 3.02 (1H, m), 2.73 (1H, m), 2.64 (1H, s), 2.33 (3H, s), 1.71 (3H, s), 1.62, (3H, s), 1.16 (3H, d, J=6.9), 0.71 (3H, d, J=6.9). NOEDS Experiments: Irradiation of the aromatic proton at $\delta$ 6.81 enhanced the aromatic methyl at $\delta$ 2.33. Irradiation of the aromatic methyl at $\delta$ 2.33 enhanced the aromatic proton at $\delta$ 6.81 and also enhanced the methoxy methyl group at $\delta$ 3.79.

Hydrolysis of Seco-pseudopterosin A Methyl Ether (6). Seco-pseudopterosin A methyl ether (6) was hydrolyzed in a fashion identical to the hydrolysis of seco-A described above. From 7.9 mg compound 6 (0.018 mmole) was obtained 4.2 mg (75%) of the methyl ether aglycone 8. Aglycone 8 showed the following spectral features: UV (methanol) 225 nm ($\epsilon$ =9800), 275 nm ($\epsilon$ =1900), 282 nm ($\epsilon$ =2000), after addition of 1 drop 5% KOH/methanol values shifted to 244, 285 and 298 nm, respectively; LRMS $M^+$ m/z (rel. intensity) 316 (13) for $C_{21}H_{32}O_2$; Proton NMR $\delta$ 6.72 (1H, s), 5.51 (1H, bs, $D_2O$ exchg.), 5.15, (1H, bt), 3.78 (3H, s), 3.07 (1H, m), 2.65 (1H, bs), 2.22 (3H, s), 1.92 (2H, m), 1.76 (1H, m), 1.71 (3H, s), 1.63 (3H, s), 1.46 (1H, m), 1.44 (2H, m), 1.20 (3H, d, J=6.8), 0.71 (3H, d, J=6.9). NOEDS Experiments: Irradiation of the aromatic proton at $\delta$ 6.72 resulted in enhancement of the aromatic methyl at $\delta$ 2.22 and the C4 methine proton at $\delta$ 2.65. In the reverse experiment, irradiation of the methine proton (C4) at $\delta$ 2.65 enhanced the aromatic signal at $\delta$ 6.72. Irradiation of the aromatic methyl group at $\delta$ 2.22 resulted in enhancements of the aromatic proton at $\delta$ 6.72 and the methoxy methyl at $\delta$ 3.78.

Methylation of the Methyl Ether Aglycone 8. Methylation of the aglycone 8 was accomplished in a fashion identical to that described above for the methylation of seco-pseudopterosin A. The bis-methyl ether 10 was obtained in 85% yield starting with 12 mg of the aglycone 8. Aglycone 10 showed the following proton NMR spectral features: $\delta$ 6.72 (1H, s), 5.15 (1H, bt), 3.87 (3H, s), 3.80 (3H, s), 3.14 (1H, m), 2.65 (1H, m), 2.22 (3H, s), 1.72 (3H, s), 1.63 (3H, s), 1.16 (3H, d, J=6.8), 0.71 (3H, d, J=6.8).

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