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A Chemoenzymatic Synthesis of 9(11)-Secosteroids using an Enzyme Extract of the Marine Gorgonian *Pseudopterogorgia americana.*

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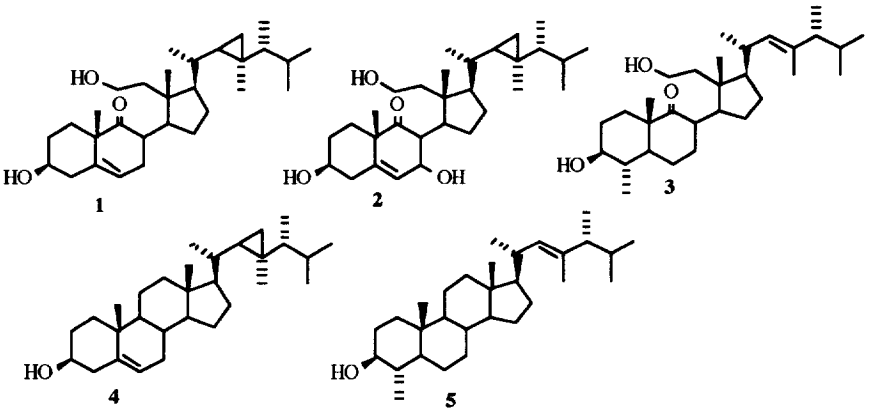
Abstract. A fortified enzyme preparation of the gorgonian *Pseudopterogorgia americana* has been developed which efficiently transforms a variety of sterols to their 9(11)-secosteroid derivative in high yield. NAD, NADP and glutamate dehydrogenase are key additives in this enzymatic conversion. In addition to naturally occurring metabolites, novel secosteroids have been prepared.

Marine invertebrates have proven to be a prolific source of novel steroids, many of which exhibit novel biological activity.[1] The gorgonian *Pseudopterogorgia* sp. produces three secosteroids, 9(11)-secogorgosterol (**1**), 7-hydroxy-9(11)-secogorgosterol (**2**), and 9(11)-secodinosterol (**3**), which exhibit inhibitory activity against protein kinase C, and potent anti-proliferative and anti-inflammatory activity.[2] Further, Fenical and co-workers[3] have demonstrated that these secosteroids afford an efficient fish-feeding deterrence for the source gorgonian. Secosteroids **1**, **2**, and **3** appear to be produced by a common enzymatic process, as in addition to the common functionalities in the C ring, the side chains are those of the biosynthetically related[4] sterols dinosterol (**4**) and gorgosterol (**5**). These latter monohydroxy sterols are the predominant sterols of *Pseudopterogorgia* spp. as well as numerous other dinoflagellate-bearing marine invertebrates.[1,5] We recently confirmed the identity of the biosynthetic

precursor to 9(11)-secogorgosterol (**1**), the most abundant secosteroid in *Pseudopterogorgia* sp., as being gorgosterol (**5**).[3]

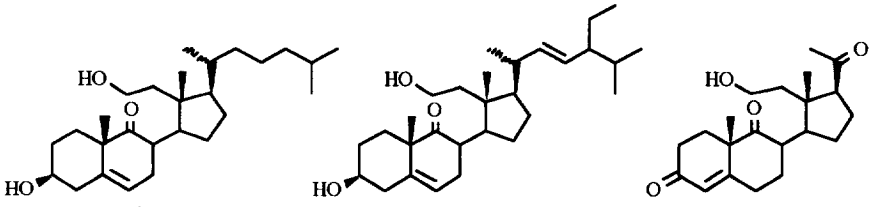
Herein, we report our preliminary studies directed at evaluating the capability of an enzyme extract of *Pseudopterogorgia americana* as a synthetic tool in 9(11)-secosteroid production. Since the gorgonian produces three 9(11)-secosteroids with different nuclei and side chains, we reasoned that the enzymes involved would be relatively non-selective and thus might provide a general synthetic route to 9(11)-secosteroids.



An acetone powder of *Pseudopterogorgia americana* was generated from a crude cell-free extract of a freshly collected gorgonian. The crude enzyme extract was prepared by homogenizing the gorgonian in a phosphate buffer (pH 7.7 with EDTA and dithiothreitol) and liquid nitrogen in a Waring blender. The crude homogenate was centrifuged (5,000 x g) to remove insoluble debris. The resulting supernatant was subsequently centrifuged at 18,000 x g for 4 hours, followed by successive filtration of the supernatant through 0.45 and 0.2 μm filters. The filtrate was slowly added to acetone at -78°C producing a voluminous white precipitate. Careful filtration and washing with cold acetone afforded a white solid. The yield of protein precipitate varied somewhat between individuals; typically this process generated 0.5 g acetone powder from 25 g of gorgonian.

The transformation of gorgosterol to its 9(11)-seco derivative was achieved by incubating the sterol (5 mg) and the acetone powder (100 mg) in a phosphate buffer at pH 7.7 with NAD (2 mg), NADP (2 mg) and L-glutamate dehydrogenase (0.5 mg) at 30°C for 48 hr. Purification of the steroids by preparative TLC yielded secogorgosterol in quantitative yield. 9(11)-Secogorgosterol (**1**) was identified by comparison of its ^{1}H-NMR with that of an authentic sample. As our initial determination

of substrate specificity, we examined the transformation of cholesterol, stigmasterol and progesterone, none of which are present in the gorgonian. All sterols were transformed to their 9(11)-seco derivatives (**6**, **7**, **8**) in modest to high yields. To our knowledge this represents the first chemoenzymatic preparation of a natural product using the enzymatic machinery of a marine invertebrate. Further, in addition to generating the secosteroids present in the gorgonian, the enzymatic process is applicable to the production of novel 9(11)-secosteroids.



6 78% **7** 18% **8** 24%

To determine which cofactors were required for this oxidative process, portions of an acetone powder were incubated with cholesterol under various conditions (see Table). Clearly, both NAD and NADP are required for optimal production of secosteroid. Glutamate dehydrogenase is employed to regenerate the oxidized form of the coenzymes during the incubation. The incubation time was determined from an incubation of cholesterol under the conditions described above, with aliquots being removed at various time intervals. Secosteroid production was found to reach a maximum at 48 hr. and appeared to decline in longer incubations.

Table. Effects of co-factors on secosteroid production.

Trial	NAD (mg)	NADP (mg)	% Yield of 9(11)-secocholesterol
1	2	0	50
2	0	2	18
3	2	2	97

We are currently investigating the full scope of this process and have initiated efforts to purify the enzymes responsible for secosteroid production. The conversion of progesterone, cholesterol and stigmasterol to their 9(11)-secosteroid derivatives suggests that this system is applicable to the production of a wide range of novel secosteroids.

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