

Bioactive Steroidal Glycosides from the Starfish *Anasterias Minuta*

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Abstract: Cytotoxic fractions obtained by purification of the ethanolic extract of *Anasterias minuta* contain sulfated hexasaccharide glycosides. These compounds show antifungal activity against *Cladosporium cucumerinum*.

Introduction

Extracts and saponins isolated from starfish show a broad spectrum of biological effects: cytotoxic, hemolytic, antifungal and antiviral activities [1]. Although a high number of sulfated steroidal glycosides from starfish have been characterized in the last ten years [2], only a few studies concerning the biological activities of pure compounds have been reported. With the aim of correlating the antifungal activity of these compounds with their structures, we isolated and purified the glycoside fraction from the starfish *Anasterias minuta* and evaluated the antifungal activity of the pure saponins against *Cladosporium cucumerinum*.

Experimental

The organisms were extracted with ethanol and the aqueous extract was partitioned between water and cyclohexane. The aqueous phase was eluted through an Amberlite XAD-2 column, washed with water and the steroidal glycosides eluted with methanol. The methanol extract was purified by vacuum-dry column chromatography on silica gel C-18, using mixtures of methanol: water and methanol, and by Sephadex LH 60. Fractions containing the bioactive compounds were purified by reversed phase HPLC. The steroidal glycosides were characterized by $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, $^1\text{H-}^1\text{H COSY}$, HETCOR, FABMS and by enzymatic and acid hydrolysis.

Results and Discussion

Fractions obtained by purification of the ethanolic extract of the starfish *Anasterias minuta* by reversed phase C-18 chromatography were monitored with respect to their cytotoxic action against

Artemia salina [3]. The bioactive fractions contained sulfated steroidal glycosides and were further purified by Sephadex LH60 and HPLC. We were able to characterize three glycosides containing the same hexasaccharide chain but different steroidal aglycone structure. Acid hydrolysis of these glycosides and derivatization and analysis by glc of the monosaccharides showed the presence of quinovose, xylose, fucose and galactose in the ratio 2:1:1:2. Enzymatic hydrolysis with *Charonia lampas* glycosidase mixture rendered the corresponding triglycosides containing quinovose (1→2)-xylose (1→3)-quinovose attached at C-6 of the steroidal aglycone. The antifungal activity of the isolated glycosides was evaluated against *Cladosporium cucumerinum* [4] and correlated with the steroidal aglycone structure present in each glycoside.

References and Notes

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