OCCURRENCE OF SAPONINS GIVING RISE TO ASTERONE AND ASTEROGENOL IN VARIOUS SPECIES OF STARFISH

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Abstract—1. Pregnane steroids were detected in the acid hydrolysates of crude asterosaponins from starfish from the orders Spinulosida, Valvatida, and Forcipulatida.
2. Every species contained asterone (1) (in widely varying amounts).
3. Asterogenol (3) was detected in some species, but there was no obvious taxonomic pattern to its distribution.

INTRODUCTION

The saponins of starfishes, known as “asterosaponins”, have been the subject of considerable chemical, biological, and pharmacological interest. They are invariably found as complex mixtures. Each asterosaponin is composed of an oligosaccharide chain (or chains) and (usually) a sulphate moiety attached to a steroid aglycone. Classically, the aglycone has been liberated from the oligosaccharide and the sulphate by hydrolysis with mineral acid, and, from the hydrolysates, the pregnane asterone 1 has been the most commonly reported steroid (Burnell and ApSimon, 1983).

Kitagawa et al. (1978) demonstrated that the asterone obtained on hydrolysis of asterosaponins from Acanthaster plancis is actually derived by retro-aldolization of the genuine aglycone, thornasterol A 2. Since 1978, thornasterol A has been identified in many intact asterosaponins (see Burnell and ApSimon, 1983, and Burnell et al., 1985, for references). However, in 1980, we isolated a trihydroxypregnane, asterogenol 3, from the hydrolysates of asterosaponin mixtures from species of the genus Asterias (ApSimon et al., 1980). Subsequently, we were able to follow its seasonal variation in Asterias vulgaris (Burnell et al., 1985). Clearly, this C21 steroid cannot be derived by retro-aldolization of thornasterol A. Thus, asterone and asterogenol must arise from different asterosaponins.

To date most of the work on asterosaponins has used species from the order Forcipulatida. In the course of our investigations on Asterias vulgaris, we were able to obtain specimens of other species, which included members of the orders Spinulosida, Valvatida, and Forcipulatida. Herein we disclose the results of acid hydrolysies of asterosaponin mixtures from these organisms.

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MATERIALS AND METHODS

Samples were obtained in October from fishermen and by divers from sites in, or near, the Bay of Fundy (Burnell et al., 1982), except for Leptasterias polaris, which was collected in July by diving at a site near Rimouski, Quebec (see Table 1). The animals were kept on ice prior to freezing and storage at −30°C. The specimens were thawed, blended, and lyophilized to constant weight. The dry powder was extracted with chloroform (except benzene then dichloromethane for Leptasterias polaris) for 72 hr in a Soxhlet apparatus to remove fats. The remaining pulp was re-extracted with methanol for 72 hr. Following rotary evaporation of the methanol, the residue was dissolved in 0.05 N HCl (60 ml) and heated at reflux for 12 hr (Gilgan et al., 1976). The mixture was extracted with chloroform (100 ml then 2 x 25 ml), and the combined chloroform extracts were washed with distilled water until neutral (usually 3 x 100 ml), then dried over Na2SO4 and evaporated in vacuo to yield the nonpolar hydrolysate fraction. This was subjected to gas chromatographic analysis (4.3 m x 2 mm i.d. glass column packed with 3% OV-101 on Chromosorb W (HP), 80/100 mesh from Chromatographic Specialties, Brockville, Ontario, in a Perkin–Elmer 990 gas chromatograph equipped with a flame-ionization detector; oven temperature: 265°C, argon flow: ca. 20 ml/min). Five peaks were identified by comparison with synthetic
Data pertaining to the collection, water content, lipid extraction, and the yield of the nonpolar hydrolysate for each species are summarized in Table 1. Crossaster (Solaster) papposus had the highest lipid levels, and its methanolic extract also yielded the largest amount of chloroform-soluble hydrolysate. Hippasteria phrygiana had the lowest levels of both lipid and nonpolar hydrolysate. The yield of this nonpolar fraction was a rough indicator of the amount of asterosaponin-derived steroid we detected by gas chromatography. With genuine samples in samples in hand, we could identify and then quantify three pregnane steroids in the gas chromatograms. These were asterone 1, asterogenol 3, and the 17-epimer of asterone, iso-asterone 4. The latter compound is known to arise from asterone during hydrolysis (Burnell et al., 1985), so the asterone levels shown in Table 2 are actually the sums of the asterone and the iso-asterone levels.

As can be seen in Table 2, from the asterosaponins of every species we detected asterone as a product of lipid and nonpolar hydrolysate.

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Asterone and asterogenol in starfish

1985). Table 2 shows no clear-cut relationship between taxonomy and the occurrence of asterogenol. Asterosaponins have been implicated in the chemorecognition of starfish by potential prey organisms, notably molluscs (Mackie et al., 1968; Mackie, 1970), which respond with various, sometimes spectacular, avoidance reactions (Feder, 1967). Many of these molluscs are most sensitive to starfish species which prey on them locally (inter alia Feder and Christiansen, 1966; Thomas and Gruffydd, 1971; Margolin, 1975; Phillips, 1977). Considering the ubiquity of asterone-producing saponins in starfish, it appears that either these molluscs can differentiate between the various carbohydrate chains of the asterosapogenins, or that the minor aglycones (e.g. asterogenol) play an important rôle in species recognition.

Mackie et al. (1977) examined the levels of two C\textsubscript{27} steroids (marthasterone and dihydromarthasterone) in the acid hydrolysates of crude saponins from British starfish specimens. 

*Crossaster papposus* was the only species in which neither of these steroids was detected. This is an interesting contrast to our finding of relatively large amounts of C\textsubscript{27} steroids in this highly predatory species.

All the species in our study are fairly soft-bodied, with the exception of *Hippasteria phrygiana*, which is a hard species with thick spines. Asterosaponins undoubtedly benefit starfish as potent chemical defense agents (Burnell and ApSimon, 1983), so one might guess that *Hippasteria phrygiana* would be the least needy of a chemical defense system. Indeed, in this species we found only a small amount of asterone; similarly Mackie et al. (1977) have reported relatively low levels of the C\textsubscript{27} steroids in this species.

In starfish the major components of the free sterol and steryl ester fractions are almost invariably \Delta\textsuperscript{7} sterols (Goad, 1978). Starfish also contain significant amounts of steryl sulphates, but this steryl fraction is comprised mainly of cholesterol 5, a \Delta\textsuperscript{7} sterol, with a minor amount of 5x-cholest-7-en-3\beta-ol 6 (Goodfellow and Goad, 1973). This was the case for the peaks corresponding to cholesterol and steroid 6 in the gas chromatograms. However, at present we do not know whether our methanolic extract would contain the steryl sulphates. Hence, attributing the "cholesterol" levels in Table 2 to steryl sulphate content is conjectural, and it may be coincidental that the "cholesterol" levels in this fraction were always greater than the "5x-cholest-7-en-3\beta-ol" levels.

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REFERENCES


