Progesterone Metabolism by the Echinoderms *Asterias rubens* and *Marthasterias glacialis*

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The echinoderms *Asterias rubens* and *Marthasterias glacialis* metabolize injected [4-¹⁴C]progesterone to give labelled 3 β -hydroxy-5 α -pregnan-20-one and 3 β ,6 α -dihydroxy-5 α -pregnan-20-one. These radioactive products are converted by the animals into conjugated forms that are soluble in aqueous methanol, and which have mobilities on t.l.c. similar to the asterosaponins.

Marine invertebrate animals contain a wide variety of sterols, some of unique structure. In the phylum Echinodermata, the starfish (Class Asteroidea) are proving to be of particular interest. These animals contain predominantly Δ^7 sterols, in contrast with most other classes of animal, which possess Δ^5 sterols (Goad *et al.*, 1972; Grossert, 1972). The starfish *Asterias rubens* produces 5 α -cholest-7-en-3 β -ol both by synthesis *de novo* (Smith & Goad, 1971a) and by metabolism of dietary cholesterol (Smith & Goad, 1971b). The utilization of cholesterol proceeds via a 3-oxo sterol to give first 5 α -cholestan-3 β -ol, which can then be converted into 5 α -cholest-7-en-3 β -ol (Smith *et al.*, 1972).

The Asteroidea also contain a group of novel compounds, the asterosaponins (Grossert, 1972), which

have toxic properties (Rio *et al.*, 1965) and apparently act as a spawning inhibitor in the starfish (Ikegami *et al.*, 1972b). Characterization of asterosaponin A has shown that the steroidal aglycone is a pregnane derivative, 3 β ,6 α -dihydroxy-5 α -pregn-9(11)-ene-20-one (I) (Ikegami *et al.*, 1972a; Sheikh *et al.*, 1972; Shimizu, 1972; ApSimon *et al.*, 1973). In the saponin there is a 3 β -sulphate group (Ikegami *et al.*, 1973) and a tetrasaccharide comprising two molecules each of quinovose and fucose attached by a glycosidic linkage with the 6 α -hydroxyl group (Ikegami *et al.*, 1972c). Other asterosaponins contain steroidal aglycones based on the cholestane skeleton (Turner & Smith, 1971; Sheikh *et al.*, 1972), but in all cases reported to date the 6 α -hydroxyl group and $\Delta^{9(11)}$ bond are present.

Since progesterone (II) has been reported (Ikegami *et al.*, 1971) to be present in the gonads of an asteroid, *Asterias amurensis*, we decided to examine the metabolism of progesterone by two species of starfish.

Specimens of *Asterias rubens* and *Marthasterias glacialis* were injected (in June 1972) with [$4\text{-}^{14}\text{C}$]progesterone ($5\mu\text{Ci}$; 61mCi/mmol) and the animals maintained for 86h in sea water at $10\text{--}12^\circ\text{C}$. The animals were then homogenized (Waring Blender) and extracted with chloroform-methanol (2:1, v/v), and the filtrate was separated into the chloroform and aqueous-methanol phases.

The chloroform extracts contained 7–14% of the original radioactivity administered to the animals and the two extracts were treated in an identical manner to identify the labelled products. The material was chromatographed on alumina (Brockmann grade III) eluted with mixtures containing increasing proportions of diethyl ether in light petroleum (b.p. $40\text{--}60^\circ\text{C}$) to give three fractions containing labelled compounds which chromatographed on t.l.c. (silica gel developed with ethyl acetate-chloroform, 7:13, v/v) with unchanged progesterone (II; R_F 0.46), 3β -hydroxy- 5α -pregnan-20-one (III; R_F 0.35) and a possible pregnanediol (R_F 0.22).

The labelled material co-chromatographing with 3β -hydroxy- 5α -pregnan-20-one (III) was purified by preparative t.l.c. and a portion subjected to analysis by radio-g.l.c. on 1% SE-30 as stationary phase. Only one radioactive peak was observed, which had a retention time corresponding exactly to the mass peak of the added carrier 3β -hydroxy- 5α -pregnan-20-one (III). Addition of 3β -hydroxy- 5α -pregnan-20-one (III) and crystallization gave a constant specific radioactivity. Also formation and crystallization to constant specific radioactivity of the derivative 3β -hydroxy- 5α -pregnan-20-one semicarbazone further confirmed the identity of the labelled compound.

In other marine invertebrates testosterone and 17α -hydroxyprogesterone are metabolites of progesterone (Lehoux & Sandor, 1970; Teshima, 1972). These compounds had R_F values similar to that of 3β -hydroxy- 5α -pregnan-20-one (III) on the t.l.c. system used above. Therefore these compounds were added to a portion of the radioactive material and the mixture was acetylated. T.l.c. (silica gel developed with ethyl acetate-chloroform, 1:9, v/v) showed that the radioactivity co-chromatographed with 3β -acetoxy- 5α -pregnan-20-one (R_F 0.68), but negligible radioactivity was associated with testosterone acetate (R_F 0.49) or 17α -hydroxyprogesterone (R_F 0.19). Elution of the 3β -acetoxy- 5α -pregnan-20-one and crystallization gave a constant specific radioactivity.

The t.l.c. system used to purify the 3β -hydroxy- 5α -pregnan-20-one would not be expected to separate this compound from possible mono-ene metabolites such as 3β -hydroxy- 5α -pregn-7-en-20-one or 3β -hydroxy- 5α -pregn-9(11)-en-20-one. Treatment of a

sample of the labelled material with *m*-chloroperbenzoic acid in chloroform to epoxidize any olefinic material gave only one radioactive product, which co-chromatographed with unchanged 3β -hydroxy- 5α -pregnan-20-one. No radioactivity was observed with a mobility corresponding to the more polar epoxides, which would be obtained from unsaturated compounds.

The most polar radioactive metabolite in the chloroform extract had an R_F on t.l.c. indicating it to be a diol. Admixture and crystallization with 5α -pregnane- 3β , 20α -diol and 5α -pregnane- 3β , 20β -diol resulted in a progressive fall in specific radioactivities, indicating non-identity of the labelled material with those compounds. Since the pregnane derivative found in the saponins has a 3β , 6α -diol structure, 3β , 6α -dihydroxy- 5α -pregnan-20-one (IV) was synthesized by treatment of 3β -acetoxy- 5α -pregn-5-en-20-ethylene ketal with diborane (Gurst *et al.*, 1973). On t.l.c. this compound (IV) had an R_F identical with that of the radioactive compound. Moreover when mixed with the labelled material both the diol and the derived diacetate crystallized to constant specific radioactivity. Treatment with *m*-chloroperbenzoic acid and t.l.c. indicated that no labelled unsaturated compounds were present.

The aqueous-methanol extract from the *A. rubens* experiment contained approx. 25% of the original administered radioactivity. T.l.c. on silica gel developed with either butan-1-ol-ethyl acetate-water (4:1:2, by vol.) or butan-1-ol-acetic acid-water (12:3:5, by vol.) showed that radioactivity was associated with material that had R_F values in the two systems (0.07 and 0.35 respectively) similar to those reported for the asterosaponins (Mackie *et al.*, 1968). Elution of the material of R_F 0.35 in the second t.l.c. system and hydrolysis with 2M-HCl gave the steroidal aglycones by extraction with chloroform. The aqueous phase was evaporated to dryness, treated with trimethylchlorosilane and hexamethyldisilazane in pyridine and the resulting mixture analysed by g.l.c. on 1% SE-30 with temperature programming at 2°C/min over the range $130\text{--}190^\circ\text{C}$. Mannitol was added as an internal standard and peaks were observed corresponding to the trimethylsilyl ethers of glucose and also of quinovose and fucose, which are constituents of the asterosaponins. Examination of the steroid aglycone fraction by t.l.c. showed radioactivity running with both 3β -hydroxy- 5α -pregnan-20-one (III) and 3β , 6α -dihydroxy- 5α -pregnan-20-one (IV). Elution, addition of the appropriate carrier and crystallization gave a constant specific radioactivity for both compounds.

Under the conditions used in the present experiments we observed no significant metabolism of progesterone (II) along the more usual routes to give, for example, 17α -hydroxyprogesterone or testosterone, which are metabolites reported in other marine

invertebrates (Teshima, 1972). The reduction of progesterone to 3 β -hydroxy-5 α -pregnan-20-one by the starfish is not altogether unexpected, since it has previously been demonstrated (Smith *et al.*, 1972) that cholest-4-en-3-one is reduced to give 5 α -cholestan-3 β -ol by *A. rubens*, thus establishing the presence of a steroidal Δ^4 -3-one reductase system in this animal.

The production of 3 β ,6 α -dihydroxy-5 α -pregnan-20-one (IV) by the starfish is of particular significance in relation to possible asterosaponin biosynthesis. The presence of the radioactive diol in a conjugated aqueous-methanol-soluble form, which had chromatographic properties similar to those of the asterosaponins, suggests that these compounds may be biosynthesized by the starfish. However, the labelled 3 β -hydroxy-5 α -pregnan-20-one recovered on acid hydrolysis of the aqueous-methanol fraction could not have been present in an asterosaponin, but it may perhaps have occurred as a 3 β -glycoside derivative. The exact nature of these labelled compounds in the aqueous-methanol extract remains to be elucidated, as does the manner of introduction of the $\Delta^9(11)$ bond into the steroidal aglycone of the asterosaponins.

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