Biochem. J. (1972) **128**, 1371–1372
Printed in Great Britain

The Intermediacy of 3-Oxo Steroids in the Conversion of Cholest-5-en-3β-ol into 5α-Cholestan-3β-ol by the Starfish *Asterias rubens* and *Porania pulvillus*

By A. G. SMITH, R. GOODFELLOW and L. J. GOAD
Department of Biochemistry, University of Liverpool, P.O. Box 147, *Liverpool L69 3BX, U.K.*

(*Received* 25 *May* 1972)

In a previous communication (Smith & Goad, 1971*a*) we reported that the starfish *Asterias rubens* (Phylum Echinodermata, Class Asteroidea) can convert injected cholest-5-en-3β-ol into 5α-cholestan-3β-ol, and that the latter compound can be further metabolized to give 5α-cholest-7-en-3β-ol, which is the predominant sterol of this animal (Smith & Goad, 1971*b*; Goad *et al.*, 1972). In mammalian tissues it is reported that the reduction of cholest-5-en-3β-ol to give 5α-cholestan-3β-ol proceeds through the intermediate production of cholest-4-en-3-one (Shefer *et al.*, 1964, 1965). The present communication shows that a route involving 3-oxo steroids is also operative in the production of 5α-cholestan-3β-ol by *A. rubens* and *Porania pulvillus*.

Materials and methods

[4-^{14}C]Cholest-5-en-3β-ol (50mCi/mmol) was purchased from The Radiochemical Centre (Amerhsam, Bucks., U.K.). [4-^{14}C]Cholest-4-en-3-one (7.7mCi/mmol) was synthesized from [4-^{14}C]cholest-5-en-3β-ol by an Oppenauer oxidation. [3α-^{3}H]Cholest-5-en-3β-ol (61.4mCi/mmol) was prepared by reduction of cholest-5-en-3-one with ^{3}H-labelled $NaBH_4$.

The labelled steroids were emulsified in 0.2ml of aq. 5% (v/v) Tween 80 and injected into the body cavity at the base of one 'leg' of the starfish. The animals were then maintained in aquaria held at about 10°C. At the end of an incubation the non-saponifiable lipid was isolated and the sterols were obtained by t.l.c. on silica gel developed with chloroform. 5α-Cholestan-3β-ol, 5α-cholest-7-en-3β-ol and cholest-5-en-3β-ol were separated by a combination of t.l.c. on silica gel impregnated with $AgNO_3$ and the formation, and t.l.c. on silica gel, of the sterol epoxides as described previously (Smith & Goad, 1971*a*).

Results and discussion

T.l.c. of the non-saponifiable lipid (1.67μCi) obtained from *A. rubens* 65h after injection of [4-^{14}C]cholest-4-en-3-one (5.0μCi) showed that the 3β-hydroxy sterols were the predominantly labelled compounds (Fig. 1), but radioactivity was also present in unchanged cholest-4-en-3-one and in material that co-chromatographed and also crystallized with 5α-cholestan-3-one. T.l.c. of the labelled 3β-hydroxy sterols on $AgNO_3$-impregnated silica gel revealed that all the radioactivity was associated with the band chromatographing with 5α-cholestan-3β-ol and 5α-cholest-7-en-3β-ol, which did not separate from each other. The possibility that [4-^{14}C]-cholest-4-en-3-one was reduced by *A. rubens* to give cholest-4-en-3β-ol, which also chromatographs with 5α-cholestan-3β-ol and 5α-cholest-7-en-3β-ol on the $AgNO_3$-impregnated silica-gel t.l.c. system employed, was investigated. A portion of the radioactive sterol (7.26×10^5 d.p.m.) was added to a mixture of unlabelled 5α-cholestan-3β-ol (17.8mg), 5α-cholest-7-en-3β-ol (51.5mg) and cholest-4-en-3β-ol (40.0mg), and the epoxides were formed by treatment with *m*-chloroperbenzoic acid. T.l.c. of the resulting mixture on silica gel demonstrated two radioactive bands. One (30%) corresponded to 7α,8α-epoxy-5α-cholestan-3β-ol, and the other (70%) ran with 5α-cholestan-3β-ol and 4β,5β-epoxycholestan-3β-ol, which co-chromatographed. 7α,8α-Epoxy-5α-cholestan-3β-ol (25.8mg; 6.0×10^4 d.p.m.) was isolated and purified by preparative t.l.c. and then recrystallized several times. The specific radioactivity remained constant (2337, 2248, 2353, 2274 and 2352d.p.m./mg) and thus

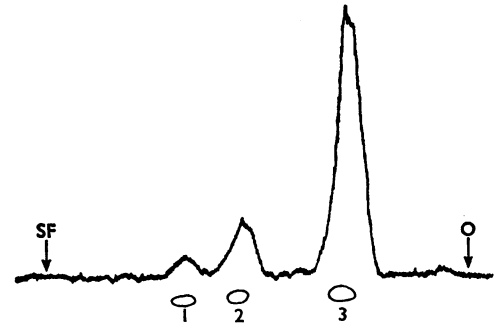

Fig. 1. *T.l.c. radioscan of the non-saponifiable lipids isolated from A. rubens after injection of* [4-^{14}C]-*cholest-4-en-3-one*

The silica-gel t.l.c. plate was developed with chloroform. O, Origin; SF, solvent front; 1, 5α-cholestan-3-one; 2, cholest-4-en-3-one; 3, 5α-cholestan-3β-ol and other 3β-hydroxy sterols.

established the conversion of [4-^{14}C]cholest-4-en-3-one into 5α-cholest-7-en-3β-ol. A portion of the radioactive fraction containing 5α-cholestan-3β-ol and 4β,5β-epoxycholestan-3β-ol (1.08×10^5 d.p.m.) was refluxed in ether with $LiAlH_4$, which converted the latter compound into cholestan-3β,5β-diol (Plattner *et al.*, 1948). T.l.c. showed that 97% of the recovered radioactivity was presented in 5α-cholestan-3β-ol (6.50×10^4 d.p.m.), and this was confirmed by addition of carrier (101 mg) and crystallization to constant specific radioactivity (609, 571, 574, 592 and 579 d.p.m./mg). Thus the major product of cholest-4-en-3-one reduction in *A. rubens* is 5α-cholestan-3β-ol, with negligible formation of cholest-4-en-3β-ol.

The obligatory intermediacy of a 3-oxo steroid in the conversion of cholest-5-en-3β-ol into 5α-cholestan-3β-ol was demonstrated by the injection of [4-^{14}C,3α-^{3}H]cholest-5-en-3β-ol (4.0 μCi of ^{14}C; ^{3}H/^{14}C ratio 1.87) into a specimen of *A. rubens*. The non-saponifiable lipid (0.41 μCi of ^{14}C) was extracted after 5 days and the isolated 5α-cholestan-3β-ol had a ^{3}H/^{14}C ratio of 0.13 after three recrystallizations. This extensive loss of the 3α-^{3}H therefore confirms that the formation of 5α-cholestan-3β-ol from cholest-5-en-3β-ol in *A. rubens* proceeds predominantly through a 3-oxo steroid intermediate. Chromic acid oxidation of the 5α-cholestan-3β-ol gave 5α-cholestan-3-one with a ^{3}H/^{14}C ratio of 0.03, showing that most of the ^{3}H was in the 3α-position. This retained ^{3}H could result from reintroduction during reduction of a 3-oxo steroid intermediate, as suggested in a similar study on 5α-cholestan-3β-ol production (Björkhem & Gustafsson, 1971). Alternatively it may indicate that a small proportion of the 5α-cholestan-3β-ol is produced by direct reduction of the Δ^5-bond of cholest-5-en-3β-ol without the intermediacy of a 3-oxo steroid (Rosenfeld & Gallagher, 1964). At present a decision between these two explanations is not possible. The unchanged [4-^{14}C,3α-^{3}H]cholest-5-en-3β-ol recovered from the above incubation had an increased ^{3}H/^{14}C ratio of 2.40, which remained constant after formation of the 5α,6α-epoxide and several recrystallizations. This increased ^{3}H/^{14}C ratio is in accord with the operation of a kinetic isotope effect that limits the rate of utilization of the 3α-^{3}H-labelled species of cholest-5-en-3β-ol during the dehydrogenase step involved in 3-oxo steroid formation. A similar isotope effect has been observed during the operation of the 3β-hydroxy steroid dehydrogenase in other biological systems (Björkhem 1969, Björkhem & Gustafsson, 1971).

The above studies were also extended to another starfish, *P. pulvillus*. The [4-^{14}C,3α-^{3}H]cholest-5-en-3β-ol administered had an initial ^{3}H/^{14}C ratio of 2.78. After 137h incubation the ^{3}H/^{14}C ratios for the recovered 5α,6α-epoxycholestan-3β-ol and 5α-cholestan-3β-ol were 4.08 and 0.12 respectively, demonstrating that a 3-oxo steroid is also an intermediate in stanol formation in this species.

We thank the Science Research Council for financial support and Professor T. W. Goodwin, F.R.S., for his interest and encouragement.

Björkhem, I. (1969) *Eur. J. Biochem.* **8**, 337
Björkhem, I. & Gustafsson, J. A. (1971) *Eur. J. Biochem.* **21**, 428
Goad, L. J., Rubinstein, I. & Smith, A. G. (1972) *Proc. Roy. Soc. Ser. B* **180**, 233
Plattner, A., Heusser, H. & Kulkarni, A. B. (1948) *Helv. Chim. Acta* **31**, 1885
Rosenfeld, R. S. & Gallagher, T. F. (1964) *Steroids* **4**, 515
Shefer, S., Milch, S. & Mosbach, E. H. (1964) *J. Biol. Chem.* **239**, 1731
Shefer, S., Milch, S. & Mosbach, E. H. (1965) *J. Lipid Res.* **6**, 33
Smith, A. G. & Goad, L. J. (1971*a*) *FEBS Lett.* **12**, 233
Smith, A. G. & Goad, L. J. (1971*b*) *Biochem. J.* **123**, 671