

THE DIETARY ORIGIN OF EPIDIOXYSTEROIDS IN *ACTINIA EQUINA*. A CARBON-14 INCORPORATION EXPERIMENT

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ABSTRACT.—From *Actinia equina* sterol epidioxides **1–9** have been isolated. No epidioxides were found in the related species *Actinia fragacea*, *Aitapsia mutabilis*, and *Anemonia sulcata*. Compounds **1–9** from *Ac. equina* are structurally related to the steroids of its favorite prey *Mytilus edulis*. A carbon-14 incorporation experiment indicates that *Ac. equina* transforms exogenous sterols into epidioxides, supporting a dietary origin for those metabolites.

In the past few years sterol endoperoxides (epidioxysterols) have been frequently isolated from marine sources. The results known to date show that these compounds are present in certain sponges, tunicates, corals, sea anemones, and sea hares (1) but have never been isolated from seaweeds until a recent investigation on a halotolerant alga (2).

In addition to work on the isolation of new endoperoxides and their distribution in different phyla, some authors have also raised the question of the biological role of these compounds, principally trying to ascertain their origin and metabolism. In this sense, it has been suggested (a) that endoperoxides have a dietary origin and (b) that they are not a metabolic dead end but the precursors of keto and $\Delta^{5,7}$ -unsaturated steroids (3). Unfortunately, only circumstantial evidence has been presented so far to prove those transformations in the animal, and in fact there is a lack of structural correspondence between the epidioxysterols and the other sterols present in a given organism. The above-mentioned metabolic transformations of epidioxides are postulated on the basis of the feasibility of certain reactions that proceed well in vitro (4) and on the coexistence in the same animal of epidioxides with those end products (5).

The present paper provides for the first time unambiguous information on

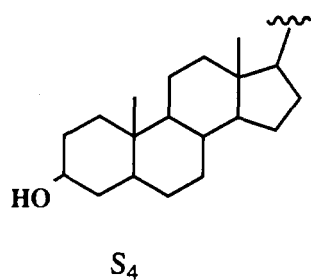
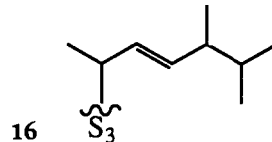
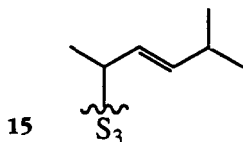
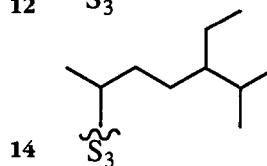
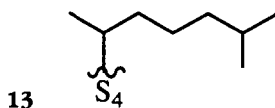
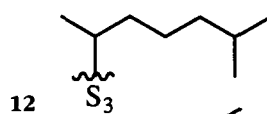
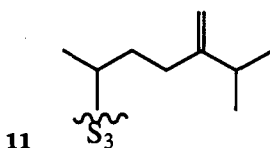
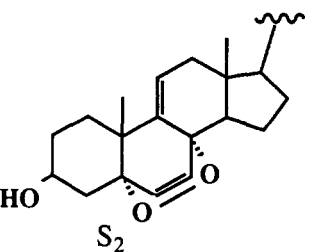
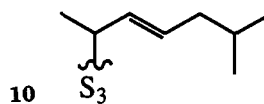
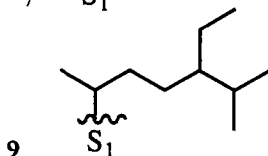
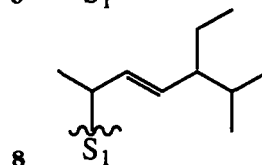
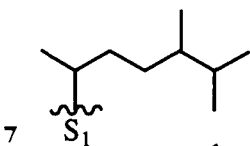
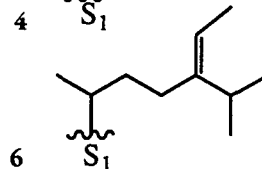
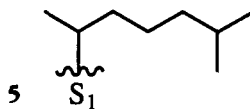
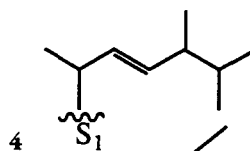
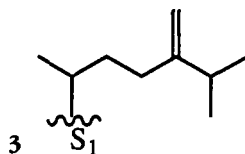
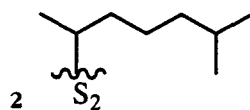
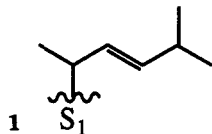
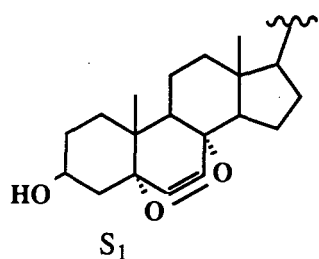
the dietary origin of these epidioxides as a result of a ^{14}C incorporation experiment on *Actinia equina* L. (Actiniidae). The distribution of epidioxides within the other cnidaria (*Actinia fragacea*, *Aitapsia mutabilis*, and *Anemonia sulcata*) is also studied.

RESULTS AND DISCUSSION

Extraction and chromatographic separation as described in the Experimental section led to the isolation of endoperoxides **1–9** from *Ac. equina*. Compounds **1–9** are already known as natural products, having previously been isolated from other marine sources (3). They were identified by comparison of their nmr and ms data with those of the literature and with authentic samples. In contrast, the other cnidaria studied (*Ac. fragacea*, *Ai. mutabilis*, and *An. sulcata*) were shown not to contain epidioxides.

We have also examined the steroid fraction of *Ac. equina*. Hplc isolation and spectroscopic data revealed the presence of cholestanol [**13**], cholesterol [**12**], 24-methylenecholesterol [**11**], *trans*-22-cholesterol [**10**], and β -sitosterol [**14**]. Some differences are observed between this composition and that found recently by Milkova *et al.* (6) for *Ac. equina* collected in the Black Sea.

A comparative analysis of the steroids and epidioxides isolated from our *Ac.*



equina showed no special structural coincidence; actually only the side chains of **2**, **3**, and **9** were present in both types of compounds. This fact, already observed in other cases (3), together with the absence of epidioxides in the other cnidaria examined and the different habitat in which these animals live, points to an exogenous origin for those metabolites.

Consequently, we turned our atten-

tion to mussels (*Mytilus edulis*), which constitute the basic food of the *Ac. equina* inhabiting our coast. The purpose was to look for a relationship between the steroids of *M. edulis* and the epidioxides of *Ac. equina*. The sterols of *M. edulis* were identified as **10**, **11**, **12**, **14**, **15**, and **16**. A comparison of their side chains with those of **1–9** shows an almost complete coincidence. In fact,

five of the six steroid side chains from the prey are represented in the epidioxides.

This finding is particularly interesting because no epidioxides were found in *M. edulis* and it is known that *Ac. equina* is not able to biosynthesize sterols *de novo* at least from simple precursors (7).

In order to pursue this point further, we decided to investigate the ability of *Ac. equina* to convert *in vivo* exogenous steroids into epidioxides. To achieve this goal we injected 4- $[^{14}\text{C}]$ -cholesterol into *Ac. equina* specimens maintained in an aquarium. After 2 days, the sterol and epidioxide fractions were isolated by chromatography. The major part of the label remained in the steroid fraction (74.5%) but the epidioxide mixture also showed radioactivity. In order to ensure that this activity was not due to the presence of possible high-specific-activity contaminants, we isolated by hplc of this fraction pure 5 α ,8 α -epidioxcholest-6-en-3 β -ol [5], which was shown to contain an activity of 1.3×10^4 dpm/mg, thus proving the *in vivo* transformation of steroids to epidioxides and supporting a dietary origin for those metabolites.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.— ^1H nmr (250 MHz) was recorded in a Bruker WM-250 and hplc separations on a Whatman Partisil ODS and μ Bondapak C_{18} columns were performed using a Waters Associates Model 590 chromatograph, equipped with a uv detector operated at 254 nm and R401 differential refractometer. 4- $[^{14}\text{C}]$ -Cholesterol (50 μCi) was supplied by DuPont (NEC-018), and the radioactivity was measured on a Beckman LS liquid scintillation system. Countings were performed with a 90% efficiency and stopped when a 1.4% (95% confidence) counting statistical error was attained. Hplc separations and nmr and ms were performed as previously described (1). All the compounds isolated were identified by their spectroscopic data and comparison with authentic samples.

BIOLOGICAL MATERIAL.—*Ac. equina* (red form), *Ac. fragacea*, *An. sulcata*, and *Ai. mutabilis* were collected at several places along the coast of Galicia (northwestern Spain) between March and May 1984. Specimens of *M. edulis* were collected in the same area. All specimens were identified by Dr. Victoriano Urgorri Carrasco of the Zoology

Department, Faculty of Biology, University of Santiago de Compostela, Spain. Voucher specimens were deposited in the collection of the Zoology Department, Faculty of Biology, University of Santiago de Compostela, Spain.

EXTRACTION AND ISOLATION.—Fresh biological material was extracted three times with MeOH and once with Et_2O . The extracts were concentrated *in vacuo* and submitted to cc on Si gel. Elution with C_6H_6 - Et_2O (95:5) produced a mixture of sterols, and elution with C_6H_6 - Et_2O (70:30) gave a mixture of epidioxides. Steroids and epidioxides were further isolated by reversed-phase hplc as described below.

From 6.5 kg of fresh *Ac. equina* (red form) we obtained 270 mg of a mixture of epidioxides and 5.5 g of sterols. Hplc of the epidioxides, eluting with H_2O -MeOH (7.5:92.5), gave, in order of elution, 40 mg of 1, 13 mg of 2, 5 mg of 3, 48 mg of 4, 60 mg of 5, 27 mg of 6, 4 mg of 7, 4 mg of 8, and 54 mg of 9.

From the steroidal fraction, the major component isolated pure from the column was identified as cholesterol [12] (4.6 g, 79%); the rest were isolated by reversed-phase hplc of the mixture, eluting with H_2O -MeOH (4:96) and giving, in order of elution, 10 (380 mg, 7%), 11 (320 mg, 6%), 12, 13 (58 mg, 1%), and 14 (385 mg, 7%).

From 3.5 kg of fresh *M. edulis*, using the same extraction and separation procedure as above, the following steroids were isolated: 10 (700 mg, 10%), 11 (2100 mg, 30%), 12 (2800 mg, 40%), 14 (70 mg, 1%), 15 (280 mg, 4%), and 16 (1050, 15%).

An identical isolation and separation procedure was followed in the study of *Ac. fragacea* (4.3 kg), *Ai. mutabilis* (110 g), *An. sulcata* (6.7 kg), and *M. edulis* (3.5 kg), which were shown not to contain epidioxides.

FEEDING EXPERIMENTS.—4- $[^{14}\text{C}]$ -Cholesterol (50 μCi , 0.368 mg) emulsified in Tween 60 in 50 μl of H_2O was injected into 20 specimens of *Ac. equina* (about 2 μl per animal), and the organisms were maintained in an aquarium for 48 h. Extraction as previously described yielded 623 mg of a dry residue (5.5×10^7 dpm, 74.5% of the original label). That mixture was submitted to preparative tlc [Si gel, MeOH- CH_2Cl_2 (5:95)], which afforded 120 mg of sterols (4.1×10^7 dpm, 74.5% of the recovered activity) and a fraction containing the sterol epidioxides that was further purified by hplc [MeOH- H_2O (86:14)]. This separation yielded 5 mg of pure 5 α ,8 α -epidioxcholest-6-en-3 β -ol [5], which showed an activity of 6.2×10^4 dpm (1.3×10^4 dpm/mg). Further verification of the identity of the epidioxides was carried out with an analytical μ Bondapak C_{18} column using MeOH- H_2O (90:10) as the mobile phase.

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