Biosynthesis of Animal and Plant Bufadienolides. Parallel Experiments with Pregn-5-en-3-ß-ol-20-one-20-^{14}C in *Scilla maritima* and *Bufo paracnemis*

It has been shown that pregnenolone plays a very important part in the biosynthesis of cardenolides and related compounds[1]. In experiments with *Helleborus atrorubens*, TSCHESCHE et al.[2] have found that pregnenolone-21-^{14}C was a good precursor of the bufadienolide hellebrin, but as far as we know no experimental work has been done on the biosynthesis of bufadienolides from animals using this steroid. Previous experiments on the biosynthesis of marinobufagin have shown[3] that cholesterol was a precursor of the bufadienolide in the toad *B. marinus*, but considering that the label was on carbon-4 and the general observation that the steroid nucleous is a relative stable moiety while the side chain is extensively cleaved during its metabolism, this result did not clarify the biosynthetic problem of the α-pyrone ring of marinobufagin.

Continuing with our work on the biosynthesis of toad venoms[4] several intact specimens of the toad *Bufo paracnemis* Lutz 1925 were injected s.c. with pregnenolone-20-^{14}C[5] and 33 and 80 days after injection the venom from the parotid and tibial glands was collected by simple pressure. Marinobufagin was isolated by chromatographic procedures already described[6].

At the same time, trying to find a correlation between bufadienolides from both origins, 2 bulbs of *Scilla maritima* (red squill) were inoculated with the same labelled steroid. The plants were harvested 14 days after injection and the bufadienolide scilliroside was isolated by known methods[7]. The results are shown in the Table.

Tracer experiments with pregnenolone-20-^{14}C in *Scilla maritima* and in *Bufo paracnemis*

	Precursor specific activity (dpm/mM)[a]	Bufadienolide isolated	Specific activity (dpm/mM)	Specific incorporation (%)
Scilla maritima	3.9×10^8	Scilliroside	2.2×10^6	0.56
Bufo paracnemis	7.3×10^8	Marinobufagin	6.5×10^2[b]	0.0001
			6.8×10^2[c]	0.0001

[a] Radioactivities were determined with a Packard Tri-Carb Model 3305 liquid scintillation spectrometer in the usual scintillation solutions. [b] 33 days' collection. [c] 80 days' collection.

The results clearly indicate that, as expected, pregnenolone was a good precursor of the plant bufadienolide scilliroside, but it was incorporated into the animal bufadienolide marinobufagin in such a value that could be neglected.

Accordingly, it is possible to postulate that, in the case of toads, cholesterol would be first converted into an intermediate with a bile acid structure[8] which could be subsequently transformed into the bufadienolide by unexceptional steps; the isolation from toad venom[9] of 7-α-hydroxy and 7-β-hydroxy-cholesterol, a critical intermediate in the biosynthesis of bile acids[10], would support this hypothesis although no 7-hydroxy-bufadienolide has been detected yet. In the case that cholesterol were metabolized to pregnenolone, this would not be used by the animal to synthesize bufadienolides[11].

Zusammenfassung. Pregnenolon-20-^{14}C wird von roten Meerzwiebeln als Vorstufe für herzwirksame Glykoside verwendet, dagegen kann *Bufo paracnemis* diese Produkte, subkutan appliziert, nicht in die üblichen Krötengifte überführen.

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Synthetic Substitute Enzymes: Part III Glutamic Acid Copolymers with Lysozyme-like Activity[1]

Significant lysozyme-like activity was reported by us for copolymers of Glu and Phe, and of Glu and γ-cholesteryl-L-glutamate[2]. These polymers were synthesized in view of the formulation of a mechanism for the β-(1-4)-glucosaminidase activity of lysozyme[3]. The most important feature of this mechanism, the protonation of the glycosidic oxygen atom at the point of fission of the polysaccharide chain by the unionized γ-carboxyl of Glu residue 35, and the stabilization of the carbonium ion formed after bond fission by the carboxylate ion of Asp residue 52, are made possible since these 2 amino-acid residues are located in hydrophobic and hydrophilic regions of the enzyme molecule respectively. Synthesis of Glu copolymers with Phe and γ-cholesteryl-L-glutamate were, therefore, carried out from the N-carboxyanhydrides of γ-benzyl-L-glutamate and the hydrophobic

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