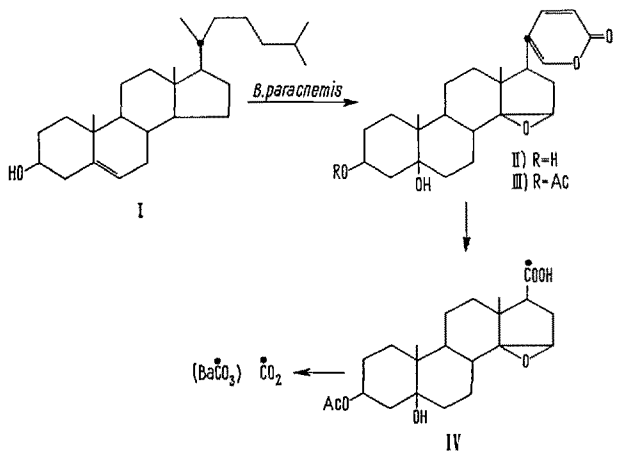
Biosynthesis of the Bufadienolide Marinobufagin in Toads *Bufo paracnemis* from Cholesterol-20-^{14}C

We have previously shown that pregnenolone-20-^{14}C resulted a very poor precursor of bufadienolides from animal origin[1]. As a consequence of this result, it was postulated that the biosynthetic precursor of animal bufadienolides should be cholesterol itself, in disagreement with the biosynthetic pathway to vegetal bufadienolides which are directly derived from pregnenolone[1,2] plus a still unknown 3-carbon unit precursor.

To test this hypothesis, several intact *B. paracnemis* specimens were inoculated s.c. with synthetic[3] cholesterol-20-^{14}C (I). The venom from the parotid and tibial glands was collected 78 and 107 days after injection, and marinobufagin (II) was isolated and purified by known methods[4]. In both cases compound II was found to be radioactive; activity values (see Table) show the slow metabolism of the animal, since the best incorporation resulted from the longer period of time. Immediately after collection of the venom, the animals were sacrificed, and cholesterol from liver and gall bladder was isolated and purified by known procedures[5]. Activity values of cholesterol are shown in the Table.



Precursor	Cholesterol-20-^{14}C (1.93×10^7 dpm/mM)	
Product	Specific activity dpm/mM	Specific incorporation (%)
Cholesterol	1.93×10^4 [a]	0.10
(from liver)	5.00×10^4 [b]	0.25
Marinobufagin	0.64×10^4 [a]	0.03
acetate (III)	1.96×10^4 [b]	0.10
	1.41×10^4 [c]	–
Etianic acid (IV)	1.33×10^4	–
$BaCO_3$	1.46×10^4	–

[a] 78 days collection. [b] 107 days collection. [c] Combined from both collections.

Marinobufagin (II) from both collections was combined and purified further as the acetate. Marinobufagin acetate (III) was oxidized to the etianic acid IV as described by SCHRÖTER et al.[6]. Compound IV was submitted to the Schmidt degradation reaction, and the evolved carbon dioxide was collected as barium carbonate, which had essentially the same specific activity of compound III.

The tabulated values clearly indicate that cholesterol-20-^{14}C is an excellent precursor of marinobufagin, and that this compound is solely labelled at C-20. Hence, the present results strongly support the hypothesis that the α-pyrone ring of animal bufadienolides is directly derived from the side-chain of cholesterol[7].

Zusammenfassung. Der biogenetische Ursprung des Marinobufagins in Kröten *Bufo paracnemis* wurde durch Injektion von Cholesterol-20-^{14}C nachgewiesen. Durch Abbau wurde festgestellt, dass die Gesamtaktivität des Produktes im C-20 des Marinobufagins enthalten ist.

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A. M. PORTO and E. G. GROS, Experientia *26*, 11 (1970). – After publication of our paper we were kindly informed by Prof. R. TSCHESCHE (University of Bonn) that he had arrived to the same conclusion.

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[7] We thank the Consejo Nacional de Investigaciones Científicas y Técnicas for financial support.

Sur les intermédiaires de la désalkylation en C_{24} du β-sitostérol par le criquet *Locusta migratoria* L.

Les insectes phytophages sont incapables de synthétiser le cholestérol et pourtant celui-ci leur est absolument nécessaire[1,2]. Ils peuvent l'obtenir par dégradation de certains phytostérols comme le β-sitostérol à défaut de le trouver dans leur nourriture[1,2]. SVOBODA[3,4] a montré que le lépidoptère *Manduca sexta* transforme le β-sitostérol en cholestérol par l'intermédiaire du desmostérol. WIENTJENS[2] à la suite de ses travaux sur la Blatte *Blatella germanica* a émis l'hypothèse que cette transformation devait se faire suivant un processus inverse de celui qui existe chez les végétaux supérieurs pour la synthèse des stérols en C_{29}; l'éthylidène-24 cholestérol (fucostérol) et le méthylène-24 cholestérol seraient des intermédiaires. Dans le but de vérifier cette hypothèse, nous avons utilisé un

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