Origin of the γ-Sitosterol present in Toad Venom

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SINCE the first isolation of a sterol, identified as γ-sitosterol, by Hüttel and Behringer[1] from the venom of the toads *Bufo vulgaris*, *Bufo vulgaris formosus*, and *Bufo arenarum*, several Papers have reported the presence of this sterol in the crude venom obtained from other toads.[2] γ-Sitosterol was found alone when the pure gland secretion was studied but mixed with cholesterol when the whole skin was extracted. It was recently reported[3] that γ-sitosterol is also present in the venom of *Bufo paracnemis* with which we are particularly concerned.

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It was always believed that the γ-sitosterol present in toad venom is derived from vegetable products present in the toad's diet,[4] but we now provide evidence that the γ-sitosterol encountered in toad poison is, at least in part, synthesized by the animal; as far as we know this is the first example of biosynthesis of γ-sitosterol in a vertebrate.

Several intact specimens of the toad *Bufo paracnemis* Lutz 1925 were injected subcutaneously with [2-^{14}C]-DL-mevalonic acid (DBED salt) ($7{\cdot}24 \times 10^9$ dose/min. per mmole), and two weeks after injection the venom from the parotid and tibial glands was collected by simple pressure. The crude solid venom was continuously extracted with chloroform–methanol (95 : 5) and then (80 : 20) and the combined extracts were chromatographed on alumina. From the first fractions γ-sitosterol was obtained and purified by sublimation and crystallization, m.p. 142—143°, $[\alpha]_D^{24} -39{\cdot}5°$ (*c* 1·1, chloroform); the product showed no absorption in the u.v. spectrum; the n.m.r. and i.r. spectra were in agreement with its structure. The γ-sitosterol had an activity of $3{\cdot}70 \times 10^5$ dose/min. per mmole, which represents a specific incorporation of 0·01% assuming only one isomer was utilized in the biosynthesis.

The value of incorporation, though somewhat low, is significant, and shows that mevalonic acid is used by the animal to synthesize the sterol, probably *via* squalene, in a way similar to the biosynthesis of sterols in the vegetable kingdom.[5] Although no pertinent data on the distribution of the label have yet been obtained, this experiment is considered to imply a pathway similar to the established scheme of plant steroid biosynthesis[6] without considering whether lanosterol or cycloartenol is the intermediate in the metabolism.[7] Further experimentation is necessary to prove that the two-carbon side chain present in γ-sitosterol is derived from methionine as in plants.[8] We thank the Consejo Nacional de Investigaciones Cíentificas y Técnicas (Buenos Aires) and FORGE (New York) for financial support.

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