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ISOLATION OF A NEW TYPE BUFOTOXIN FROM SKIN OF BUFO VULGARIS FORMOSUS BOULENGER

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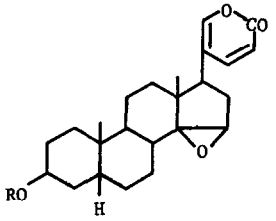
The structure of the so-called "bufotoxin" which was first isolated from the toad venoms by Wieland et al.,[1] has recently been elucidated to be the 3-suberoylarginine ester of bufogenin by degradative means.[2] In this communication we wish to report the isolation of a new type bufotoxin which possesses the succinoyl moiety instead of the suberoyl group in the hitherto known conjugated bufadienolides,[2,3] from the skin of Japanese toad Bufo vulgaris formosus Boulenger.

One thousand toads collected in the northeastern district of Japan were sacrificed by freezing in dry ice, and the skins were immediately teared off and extracted with ethanol. The extract was partitioned with ether-water and then with ethyl acetate-water. The aqueous layer was percolated through a column of Amberlite XAD-2 resin. After thorough washing with distilled water the conjugated steroid fraction was eluted with methanol. The eluate was redissolved in chloroform-methanol-water (80:20:2.5) and chromatographed on silica gel impregnated with the aqueous phase. Further purification by partition chromatography on silica gel followed by gel filtration on Sephadex LH-20 provided a new substance (III), mp 210.5-212°, $[\alpha]_D^{19}$ +22.8° (c=0.15, $CHCl_3$-CH_3OH (1:1)), as colorless prisms (from methanol).

This compound gave a positive result with the Sakaguchi's reagent and a negative ninhydrin test, and exhibited the n.m.r. signals at δ : 0.78 (3H, s, 18-CH_3), 1.01 (3H, s, 19-CH_3), 2.58 (4H, s, -$CO(CH_2)_2CO$-), 3.56 (1H, s, 15α-H), 5.03 (1H, broad peak, 3α-H), 6.20 (1H, d, J=9Hz, 23-H), 7.40 (1H, d, J=2.5Hz, 21-H), and 7.83 ppm (1H, q, J=9, 2.5Hz, 22-H). Upon hydrolysis with 6N hydrochloric acid arginine was evidently characterized by thin-layer

chromatography. In order to confirm the presence of a peptide bond involving the α-amino group of arginine, III was converted into the pyrimidine derivative (IV) by treatment with acetylacetone-potassium bicarbonate and then with diazomethane.[2,4] Unfortunately IV could not be obtained in the crystalline state, but its structure was unequivocally assignable on the basis of n.m.r. spectral data, δ : 0.78 (3H, s, 18-CH_3), 0.99 (3H, s, 19-CH_3), 2.26 (6H, s, pyrimidine-CH_3), 2.59 (4H, broad peak, -$CO(CH_2)_2CO$-), 3.50 (1H, s, 15α-H), 3.69 (3H, s, -$COOCH_3$), 5.05 (1H, broad peak, 3α-H), 6.15 (1H, d, J=9Hz, 23-H), 6.20 (1H, s, pyrimidine-H), 7.25 (1H, d, J=2.5Hz, 21-H), and 7.75 ppm (1H, q, J=9, 2.5Hz, 22-H).



I : R = H

II : R = $CO(CH_2)_2COOH$

III : R = $CO(CH_2)_2CONHCH(COOH)(CH_2)_3NHC(=NH)NH_2$

IV : R = $CO(CH_2)_2CONHCH(COOCH_3)(CH_2)_3NH$-(2,4-dimethylpyrimidine)

Being submitted to enzymatic hydrolysis with hog pancreas lipase, III afforded resibufogenin 3-hemisuccinate (II), mp 162-166.5°, $[\alpha]_D^{17}$ -11.4° (c=0.09, CH_3OH), as colorless prisms (from methanol-ether). The hydrolyzate proved to be identical in every respect with the synthetic sample which was readily obtained from resibufogenin (I) and succinic anhydride.

These evidences together led us to assign the structure 3-succinoylarginine ester of resibufogenin (III) to the new type bufotoxin. To the best of our knowledge this is the first recorded instance of the naturally occurring succinoylarginine ester of bufogenin. Further studies on the isolation of the new bufotoxin from the Japanese toads are being conducted in these laboratories and the details will be reported in the near future.

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