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ISOLATION OF BUFOTALIN 3-SUBEROYL-HISTIDINE AND -3-METHYLHISTIDINE ESTERS FROM THE SKIN OF BUFO MELANOSTICTUS SCHNEIDER

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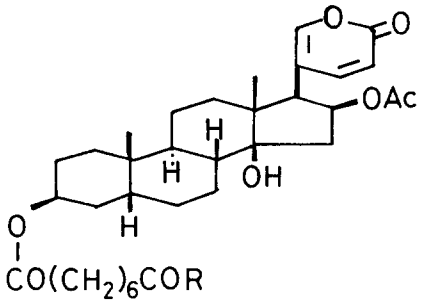
ABSTRACT: Two novel bufotoxins, bufotalin 3-suberoyl-histidine and -3-methylhistidine esters, were isolated from the skin of the Formosan toad, Bufo melanostictus Schneider.

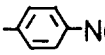
The structure of the so-called "bufotoxin" has been considered as bufogenin 3-suberoylarginine ester. Recent studies in this laboratory, however, disclosed the occurrence of three new types of bufotoxins in which the succinoyl, adipoyl and pimeloyl groups are displaced for the suberoyl residue of "bufotoxin", in the Japanese toad, Bufo vulgaris formosus Boulenger[1] and the tropical toad, Bufo marinus (L.) Schneider.[2] The existence of bufogenin 3-sulfates and analogous conjugates of cardenolide named cardenobufotoxin was also demonstrated.[1] In addition, we isolated a new bufotoxin having glutamine instead of arginine as an amino acid component from the skin of the North American toad, Bufo americanus.[3] In this communication we wish to report the isolation of two novel bufotoxins which possess histidine and 3-methylhistidine as an amino acid component from the skin of the Formosan toad, Bufo melanostictus Schneider.

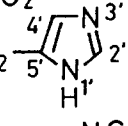
Eighty toads (Bufo melanostictus Schneider) obtained from VIVARIUM Co. (Tokyo, Japan) were sacrificed by freezing in dry ice, and the skins were immediately flayed and extracted with ethanol. The extract was purified in the manner previously described.[1] Further purification by high-performance liquid chromatography (μ BondapakC$_{18}$ column, methanol/0.3% $(NH_4)_2CO_3$(5:4)) provided new compound III (3 mg) as colorless amorphous substance (from methanol/ether), mp 193-193.5°(decomp.), $[\alpha]_D^{20}$ -8.1°(c=0.09 in methanol) and compound IV (3 mg) as colorless amorphous substance (from acetone), mp 170-174°(decomp.), $[\alpha]_D^{18}$ +5.6°(c=0.07 in methanol). These substances gave the negative ninhydrin and Sakaguchi tests and exhibited the following ^{1}H-nmr signals (in $CD_3OD/CDCl_3$). Compound III δ: 0.78 (3H, s, 18-CH_3), 0.98 (3H, s, 19-CH_3), 1.85 (3H, s, $OCOCH_3$), 5.06 (1H, m, 3α-H), 5.50 (1H, m, 16α-H), 6.18 (1H, d, J=10 Hz, 23-H), 7.00 (1H, br s, 4'-H), 7.30 (1H, d, J=3 Hz, 21-H), 8.16 (2H, m, 22-H and 2'-H); compound IV δ: 0.78 (3H, s, 18-CH_3), 0.98 (3H, s, 19-CH_3), 1.86 (3H, s, $OCOCH_3$), 3.65 (3H, s, N-CH_3), 5.06 (1H, m, 3α-H), 5.50 (1H, m, 16α-H), 6.18 (1H, d, J=10 Hz, 23-H), 7.00 (1H, br s, 4'-H), 7.32

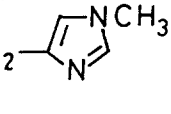
(1H, d, J=3 Hz, 21-H), 8.00 (1H, br s, 2'-H), 8.20 (1H, dd, J=10, 3 Hz, 22-H). Being subjected to enzymic hydrolysis with hog pancreas lipase (Sigma Chemicals Co., St. Louis, Mo.) followed by methylation with diazomethane,[1] both compound III and IV afforded bufotalin 3-hemisuberate methyl ester (I) as colorless needles (from ether/hexane), mp 171-173°, MS m/z: 614 (M^+), 189, 171.[1] Hydrolysis of these two with 6 N hydrochloric acid gave an amino acid component, which was assumed to be histidine, 1-methylhistidine, or 3-methylhistidine from the result obtained by amino acid analyzer. In order to confirm the amino acid moiety bufotalin 3-suberoyl-histidine, -1-methylhistidine and -3-methylhistidine esters were synthesized by the active ester method[1] through bufotalin 3-hemisuberate p-nitrophenyl ester (II). These conjugates were evidently differentiated from each other by thin-layer chromatography and high-performance liquid chromatography. Compounds III and IV thus proved to be identical with bufotalin 3-suberoyl-histidine and -3-methylhistidine esters in all respects, respectively.



I : R=OCH_3

II : R=O-  -NO_2

III : R=$NHCHCH_2$- 
COOH

IV: R=$NHCHCH_2$- 
COOH

To the best of our knowledge this is the first reported instance of the naturally occurring bufotoxins which possess histidine and 3-methylhistidine as an amino acid component. Further studies on the isolation and characterization of new bufotoxins from the toad are being conducted in this laboratory, and the details will be reported in the near future.

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