not only readily discerns the presence of two closely related epimers but also locates the epimeric center.

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Occurrence of Novel Type Bufotoxin in Japanese Toad

The bufotoxin, isolated first from the toad venoms by Wieland, *et al.*,[1] was definitely characterized to be the 3-suberoylarginine ester of bufogenin by degradative[2,3] and synthetic means.[4,5] Recently a novel bufotoxin in which the succinoyl residue is substituted for the suberoyl group of the hitherto known conjugated bufadienolide, has been separated from the skin of Japanese toad.[6] We now wish to report the occurrence of an additional new type bufotoxin possessing adipic acid as a dicarboxylic acid moiety in the parotid glands of *Bufo vulgaris formosus* Boulenger.

One thousand toads collected in the northeastern district of Japan were freezed in dry ice, and the parotid glands were removed and extracted with cold ethanol. The extract was submitted to dry column chromatography on silica gel and eluted with ethyl acetate–methanol (1: 1). The eluate was redissolved in chloroform–methanol–water (80: 20: 2.5) and chromatographed on silica gel impregnated with the aqueous phase. Repeated purification by partition chromatography furnished a bufotoxin fraction which exhibited a single spot on the thin-layer chromatogram. Being subjected to enzymatic hydrolysis with the hog pancreas lipase preparation (Sigma Chemical Co., St. Louis), followed by methylation with diazomethane, this fraction gave a new substance (Ib), mp 185—187°, $[\alpha]_D^{14}$ +13.6° (c= 0.11 in $CHCl_3$), as colorless prisms (from ether) together with the known methyl ester of cinobufotalin 3-hemisuberate, mp 132.5—134.5°.[7]

The spectral inspection of Ib afforded the following data: NMR ($CDCl_3$ solution) δ: 0.80 (3H, s, 18-CH_3), 0.98 (3H, s, 19-CH_3), 1.84 (3H, s, 16β-$OCOCH_3$), 2.30 (8H, m, -$(CH_2)_4$-),

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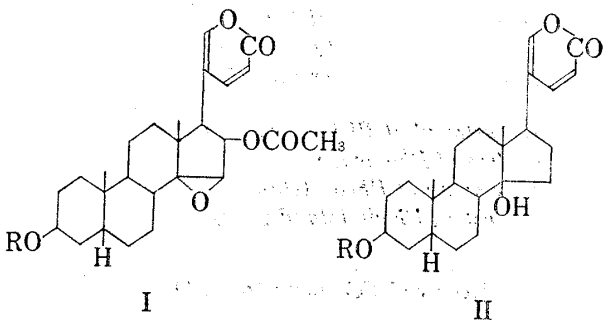
2.75 (1H, d, J=9 Hz, 17α-H), 3.60 (4H, s, $-COOCH_3$, 15α-H), 5.04 (1H, m, 3α-H), 5.40 (1H, d, J=9 Hz, 16α-H), 6.12 (1H, d, J=9 Hz, 23-H), 7.15 (1H, d, J=2 Hz, 21-H), 7.82 (1H, q, J=9, 2 Hz, 22-H); Mass Spectrum *m/e*: 584 (M^+), 161 (M^+-423), 143 (M^+-441). These evidences lent a support to assign the structure methyl ester of cinobufagin 3-hemiadipate to Ib and prompted us to prepare the authentic sample for direct comparison. Condensation of methyl hemiadipate with cinobufagin (Ia) was effected by treatment with N,N′-dicyclohexylcarbodiimide in the usual manner. The identity of natural and synthetic samples was unequivocally established by usual criteria.



a : R=H
b : $R=CO(CH_2)_4COOCH_3$

Chart 1

On partition chromatography described above further elution provided the second bufotoxin fraction. Enzymatic hydrolysis and subsequent methylation in the similar manner afforded the second new substance (IIb), mp 166—168°, $[\alpha]_D^{16}$ −3.1° (c=0.16 in $CHCl_3$), as colorless needles (from ether), besides the known methyl ester of arenobufagin 3-hemisuberate, mp 119—119.5°.[7]

Compound IIb showed the spectral characteristics as follows: NMR ($CDCl_3$ solution) δ: 0.68 (3H, s, 18-CH_3), 0.92 (3H, s, 19-CH_3), 2.30 (8H, m, $-(CH_2)_4-$), 3.58 (3H, s, $-COOCH_3$), 5.00 (1H, m, 3α-H), 6.15 (1H, d, J=10 Hz, 23-H), 7.18 (1H, d, J=2 Hz, 21-H), 7.75 (1H, q, J=10, 2 Hz, 22-H); Mass Spectrum *m/e*: 161 (M^+-367), 143 (M^+-385). These data led us to assign the structure methyl ester of bufalin 3-hemiadipate to IIb. The synthetic sample, similarly prepared from bufalin (IIa) and methyl hemiadipate by the N,N′-dicyclohexylcarbodiimide method, has proved to be entirely identical with the natural product.

It is evident from these results that adipic acid would consist of the genuine bufotoxin as a dicarboxylic acid component. Although the nature of amino acid linked to the hemiadipate still remains unclear, these new bufotoxins may possibly be the 3-adipoylarginine esters of bufogenin as judged from their chromatographic behaviors and the coexistence of the suberoyl and succinoyl homologs. To the best of our knowledge these are the first recorded instances of the naturally occurring hemiadipate of bufogenin. It is of particular interest that the dicarboxylic acids of even carbon number are in general capable of conjugating with bufogenin in the living animals.

Studies on the isolation of these new bufotoxins and elucidation of their complete structures are being conducted in these laboratories and the details will be reported in the near future.

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