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ISOLATION OF GAMABUFOTALIN 3-PIMELOYLARGININE ESTER FROM THE SKIN OF JAPANESE TOAD[1]

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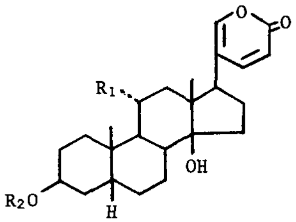
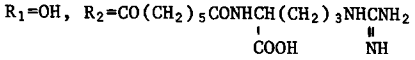
In recent years two novel types of bufotoxins in which the succinoyl and adipoyl groups are substituted for the suberoyl residue of the hitherto known bufotoxin,[2,3] have been separated from the skin of Japanese toad.[4,5,6] We now wish to report the isolation and characterization of two new bufotoxins, one of which possesses the pimelate as a dicarboxylic acid moiety, from the skin of Bufo vulgaris formosus Boulenger.

The ethanolic extract of the skin obtained from 1,800 toads was chromatographed repeatedly in the manner as previously reported[3,4] to give a new bufotoxin (I), mp 189-192° (decomp.), $[\alpha]_D^{18}$ -3.1° (c=0.16 in MeOH), as a colorless amorphous substance (from MeOH-acetone). It showed a positive result with Sakaguchi's reagent and a negative test with ninhydrin, and exhibited the n.m.r. signals at δ 0.75 (3H, s, 18-CH_3), 1.08 (3H, s, 19-CH_3), 3.70 (1H, m, 11β-H), 5.05 (1H, m, 3α-H), 6.25 (1H, d, J=10Hz, 23-H), 7.45 (1H, d, J=2Hz, 21-H), and 7.95 ppm (1H, q, J=10,2Hz, 22-H). Hydrolytic cleavage with 6N hydrochloric acid furnished arginine which was characterized by thin-layer chromatography. On enzymatic hydrolysis with a hog pancreas lipase preparation (Sigma Chemical Co., St. Louis), followed by methylation with diazomethane compound I afforded the methyl ester of gamabufotalin 3-hemipimelate (II), mp 193-195°, $[\alpha]_D^{18}$ -10.0° (c=0.05 in $CHCl_3$), ms m/e 175, 157, as colorless leaflets (from Et_2O). Subsequent acetylation with acetic anhydride and pyridine in the usual manner yielded the 11-monoacetate (III). Unfortunately this acetylated product could not be obtained in the crystalline state, but the direct comparison with an authentic specimen prepared from gamabufotalin in two steps showed the identity of two samples in every respect. These results lent a support to assign the structure gamabufotalin 3-pimeloylarginine ester to this new type bufotoxin. It is to be noted that this is the first recorded instance of the naturally occurring pimeloylarginine ester of bufogenin.

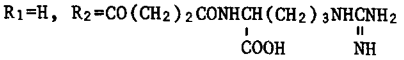
Another new bufotoxin (IV), mp $240-241^{\circ}$, $[\alpha]_{D}^{23}$ $-12\ 5^{\circ}$ (c=0 08 in 80% MeOH), was similarly separated as colorless needles (from aq MeOH) The presence of a peptide bond involving the α-amino group of arginine was confirmed by color tests and hydrolytic cleavage with hydrochloric acid Enzymatic hydrolysis and subsequent methylation with diazomethane in the similar fashion provided the methyl ester of bufalin 3-hemisuccinate (V), mp $165-166^{\circ}$, $[\alpha]_{D}^{14\ 5}$ $-14\ 9^{\circ}$ (c=0 13 in $CHCl_3$), as colorless needles (from Et_2O) It is evident from these data that this bufotoxin should be assigned to bufalin 3-succinoylarginine ester



I ·

$R_1=OH,\ R_2=CO(CH_2)_5CONH\underset{\underset{COOH}{|}}{CH}(CH_2)_3NH\underset{\underset{NH}{\|}}{C}NH_2$

II $R_1=OH,\ R_2=CO(CH_2)_5COOCH_3$

III : $R_1=OAc,\ R_2=CO(CH_2)_5COOCH_3$

IV ·

$R_1=H,\ R_2=CO(CH_2)_2CONH\underset{\underset{COOH}{|}}{CH}(CH_2)_3NH\underset{\underset{NH}{\|}}{C}NH_2$

V : $R_1=H,\ R_2=CO(CH_2)_2COOCH_3$

Further studies on the isolation of the new bufotoxin from the Japanese toad are being conducted in these laboratories and the details will be reported in the near future

REFERENCES AND FOOTNOTES

1 All the new compounds gave satisfactory elemental analyses.

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