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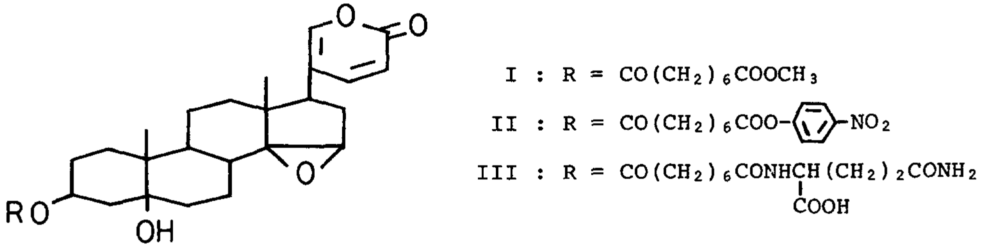
ISOLATION OF MARINOBUFAGIN 3-SUBEROYL-L-GLUTAMINE ESTER FROM THE SKIN OF BUFO AMERICANUS

Kazutake Shimada and Toshio Nambara*
Pharmaceutical Institute, Tohoku University, Aobayama, Sendai, Japan

Recent studies in this laboratory disclosed the occurrence of three new types of bufotoxins in which the succinoyl, adipoyl and pimeloyl groups are displaced for the suberoyl residue of the so-called bufotoxin, in the skin of Japanese toad, Bufo vulgaris formosus Boulenger[1]. In addition, the existence of bufogenin 3-sulfates and analogous conjugates of cardenolide named cardenobufotoxin in the toad was also demonstrated[1]. In this communication we wish to report the isolation of a novel conjugated bufadienolide which possesses L-glutamine instead of arginine in the hitherto known bufotoxin, from the skin of Bufo americanus.

Ten toads (Bufo americanus) obtained from Fisher Scientific Co. (Ithasca, Ill.) were sacrificed by freezing in dry ice, and the skins were immediately teared off and extracted with ethanol. The extract was chromatographed successively on silica gel using ethyl acetate/methanol and chloroform/methanol/water (80:20:2.5) as eluting solvents. Further purification by preparative thin-layer chromatography in chloroform/methanol/water (80:20:2.5) and subsequent gel chromatography on Sephadex LH-20 in methanol provided a new substance (III), 5 mg, mp 166-170° (decomp.), $[\alpha]_D^{20}$ + 15.1° (c=0.11 in MeOH/$CHCl_3$(1:1)), as colorless amorphous substance (from methanol/ether). This substance gave the negative ninhydrin and Sakaguchi tests and exhibited the n.m.r. signals at δ ($CD_3OD/CDCl_3$(1:1)): 0.80 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 3.55 (1H, s, 15α-H), 5.25 (1H, m, 3α-H), 6.30 (1H, d, J=10Hz, 23-H), 7.30 (1H, d, J=2Hz, 21-H), 7.85 (1H, q, J=10, 2Hz, 22-H). Being submitted to enzymatic hydrolysis with hog pancreas lipase (Sigma Chemical Co., St. Louis, Mo.) followed by methylation with diazomethane, III afforded marinobufagin 3-suberate methyl ester (I)[2], mp 105°, MS m/e: 570 (M^+), 189, 171, as colorless leaflets (from ether). Upon hydrolysis with 6 N hydrochloric acid glutamic acid was evidently characterized by amino acid analyzer. In order to confirm the amino acid moiety marinobufagin 3-suberoyl-D- and -L-glutamine and -glutamic acid esters were synthesized by the active ester method[3] starting from marinobufagin 3-suberate p-nitrophenyl ester (II). The marinobufagin 3-suberoyl-L-glutamine ester was readily differentiated from the D-isomer by i.r. spectra ($CHCl_3$) and proved to be identical with the natural product (III) in all respects.



I : R = $CO(CH_2)_6COOCH_3$

II : R = $CO(CH_2)_6COO{-}C_6H_4{-}NO_2$

III : R = $CO(CH_2)_6CONH\underset{\displaystyle COOH}{CH}(CH_2)_2CONH_2$

To the best of our knowledge this is the first recorded instance of the naturally occurring bufotoxin which possesses amino acid other than arginine. Further studies on the isolation and characterization of the new bufotoxin from the toad are being conducted in this laboratory and the details will be reported in the near future.

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