STUDIES ON STEROIDS CCVIII. BUFADIENOLIDES FROM *BUFO ASPER*

KAZUTAKE SHIMADA, KAZUO OHISHI, and TOSHIO NAMBARA*

Pharmaceutical Institute, Tohoku University, Aobayama, Sendai 980, Japan

Iseli *et al.* disclosed the presence of bufogenins in *Bufo asper* Gravenhorst by paper chromatography (1), but isolation of these bufogenins and investigation on bufotoxins (2) from this toad venom have not been done. Here we report the isolation of five bufogenins (resibufogenin, cinobufagin, bufalin, bufotalin, marinobufagin) and three bufotoxins (resibufotoxin, bufalitoxin, vulgarobufotoxin) from the skin of this toad.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Kofler hot plate and are uncorrected. Ms and nmr spectra were recorded on a Hitachi M-52G spectrometer and a JEOL FX-100, respectively. Hplc was carried out on a Waters ALC/GPC 202 chromatograph equipped with a uv detector (280 nm) and a μBondapak C_{18} column (Waters Assoc., Milford, MA).

ANIMAL MATERIAL AND EXTRACTION.—Thirty-two Thai toads (*B. asper*) obtained from Vivarium Co. (Tokyo) were sacrificed by freezing in dry ice. The skins were immediately flayed off and extracted with EtOH at room temperature for a week.

ISOLATION AND IDENTIFICATION.—After removal of insoluble materials in the ethanolic extract by filtration through a layer of Celite, the filtrate was concentrated in vacuo below 50°, and partitioned with the hexane-H_2O and then with the EtOAc-H_2O systems. The EtOAc layer was concentrated in vacuo and the residue obtained was chromatographed on silica gel 60 (70-230 mesh) with *n*-hexane gradually enriched with EtOAc. Further purification by preparative tlc (silica gel HF_{254}) provided resibufogenin (40 mg), cinobufagin (2 mg), bufalin (6 mg), bufotalin (60 mg), and marinobufagin (1 mg). These bufogenins were identified by mp (3), ms (4), ^{1}H nmr (5), and finally by comparison with authentic samples.

The aqueous layer was percolated through a column of Amberlite XAD-2 resin (Rohm and Hass Co., Philadelphia, PA) to give bufotoxins. After thorough washing with distilled H_2O, the conjugated steroid fraction was eluted with stepwise-increasing concentrations of MeOH. Each eluate was subjected to column chromatography on silica gel employing EtOAc with stepwise-increasing concentrations of MeOH as eluent. Further purification was performed by hplc on a reversed phase column (6). Resibufotoxin (3 mg) (7), bufalitoxin (4 mg) (2), and vulgarobufotoxin (28 mg) (2) were unequivocally characterized by ^{1}H nmr, degradative means and finally by comparison with authentic samples. This is the first reported instance that the presence of bufotoxins in this toad venom has been clarified.

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