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ISOLATION OF BUFALIN-3-SULFATE FROM THE SKIN OF *BUFO VULGARIS FORMOSUS* BOULENGER

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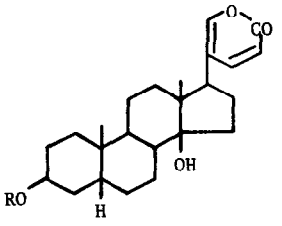
The occurrence of new type bufotoxins which contain other dicarboxylic acids than suberic acid, in the skin of *Bufo vulgaris formosus* Boulenger has previously been reported.[1] We now wish to communicate the separation of a novel conjugated bufadienolide, bufalin-3-sulfate, from the Japanese toad.

The ethanolic extract of the skin obtained from 600 toads was submitted to partition with the ether-water and ethyl acetate-water systems. The aqueous layer was percolated through a Amberlite XAD-2 resin employing 70% methanol as a eluant. The conjugated steroid fraction obtained was submitted to dry column chromatography on silica gel and eluted with ethyl acetate-methanol (2:1). The eluate was redissolved in chloroform-methanol-water (80:20:2.5) and chromatographed on silica gel impregnated with the aqueous phase. Subsequently repeated gel filtration on Sephadex LH-20 with use of methanol as a solvent afforded satisfactory separation of the desired substance. Trituration with acetone-methanol gave 5 mg of a new conjugate as colorless amorphous substance, mp 165.5-166.5°, $[\alpha]_D^{23}$ -33.1° (c=0.05, MeOH).

This compound resisted to usual acetylation, exhibited a positive test with barium-rhodizonate reagent for detection of sulfate ions[2] and infrared absorption bands at 1220, 1055 cm^{-1} due to a sulfate group. Inspection of n.m.r. spectrum showed signals at δ (1.3% solution in $CD_3OD/CDCl_3$(1:4)): 0.72 (3H, s, 18-CH_3), 0.98 (3H, s, 19-CH_3), 2.45 (1H, broad peak, 17α-H), 4.71 (1H, broad peak, 3α-H), 6.23 (1H, d, J=10Hz, 23-H), 7.23 (1H, d, J=2Hz, 21-H), 7.87 (1H, q, J=10, 2Hz, 22-H). Solvolysis in the usual manner[3] furnished bufalin (I) as an aglycone, whose structure was unequivocally characterized by thin-layer chromatography and mass spectrometry.

These evidences lent a support to assign the structure bufalin-3-sulfate and prompted

us to prepare the authentic sample from necessity of direct comparison. Condensation of bufalin with sulfuric acid was effected by treatment with N,N'-dicyclohexylcarbodiimide.[4] After neutralization with sodium hydroxide the product was purified by chromatography to provide the desired sodium bufalin-3-sulfate (II), mp 165-166.5°, $[\alpha]_D^{23}$ -35.7° (c=0.06, MeOH). The synthetic sample proved to be entirely identical with the natural product in every respect.



I : R = H

II : R = SO_3Na

To the best of our knowledges this is the first reported isolation of the bufadienolide sulfate from the natural source. It is to be noted that the cardiotonic as well as the hormonal steroids exist in the form of the sulfate in the animal kingdom. In view of the accumulating evidences for active participation of the steroidal conjugates, the physiological significance of the bufadienolide sulfate in the living animals is of particular interest. Further studies are being conducted in these laboratories and the details will be reported in the near future.

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