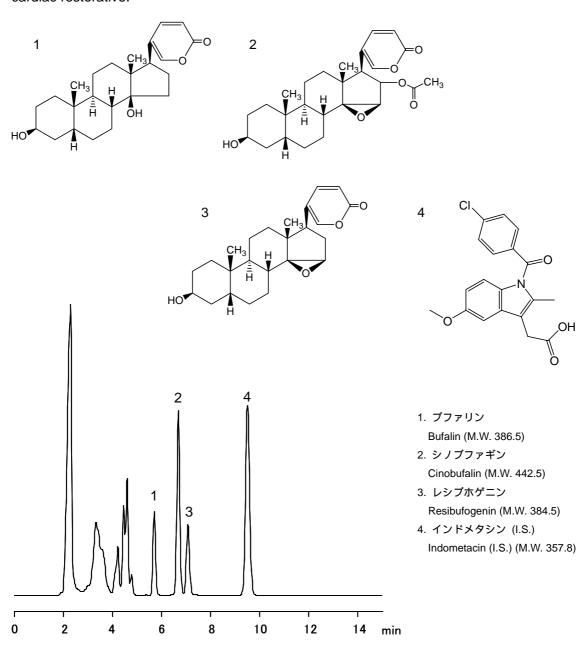
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Toad venom is one of the crude medicines listed in The Japanese Pharmacopoeia. It has long been used as cardiac restorative, antalgestic, or alexipharmic. Bufo steroid, its main component, does not accumulate in the body, and is receiving an attention as a highly safe cardiac restorative.



## [HPLC Conditions]

Column : CAPCELL PAK C<sub>18</sub> MGII S5 ; 4.6 mm i.d. x 150 mm

Mobile phase :  $CH_3CN / 0.1 \% H_3PO_4 = 60 / 40$ 

Flow rate : 0.5 mL/minTemperature :  $40 \,^{\circ}\text{C}$ Detection : UV 300 nm Inj. vol. :  $10 \,\mu\text{L}$ 

Pretreatment : Toad venom was cut into small pieces, and ground in a mortar with a

pestle. 0.5 g of the ground toad venom was dispersed in a mixed solvent (50 mL, water / methanol = 1 / 1). The dispersion was refluxed for 60 min. After cooling it down, the dispersion was filtered with filter paper. The residue was rinsed with the mixed solvent. The filtered liquid and the mixed solvent used for rinsing were added together and adjusted to 100 mL in a volumetric flask. The solution was further filtered with a 0.2- $\mu$ m filter. 10 mL of the filtered solution and 5 mL of the internal standard solution (indometacin, 250  $\mu$ g/mL in methanol) were added together, adjusted to 25 mL in a volumetric flask with

methanol, and introduced to HPLC.

 $\Re$  1  $\mu$ g/mL = 1 ppm

## [References]

1) The Japanese Pharmacopoeia, (14th~15th Edition) 第 14~15 改正, 日本薬局方