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## occurrence of candicine( $\underline{p}$ -hydroxyphenylethyltrimethylammonium) in extracts of the skin of Leptodactylus pentadactylus pentadactylus\*

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CANDICINE has been known for more than 30 years to be a natural constituent of some plants, more precisely of some Argentinian Cactaceae 1-3. The compound has now been found to occur in a vertebrate tissue also.

The dry skin of five specimens of Leptodactylus pentadactylus pentadactylus, an Amazonium amphibian, was extracted twice with 70 per cent acetone. The combined extracts were concentrated under reduced pressure and the aqueous liquid shaken repeatedly with petroleum ether to remove fats. Then evaporation was continued and the syrupy residue taken up, by stirring, in 100 ml of 99 per cent ethanol. The liquid was then passed through a column of alkaline alumina. Elution was carried out with decreasing concentrations of ethanol, as described in detail in other papers. 4,5 The different eluates were suitably concentrated and then chromatographed on Whatman no. 1 paper.

Chromatograms of the 95 per cent ethanol eluates showed a spot which was indistinguishable from spots given by genuine synthetic candicine. In fact, candicine and the unknown spot gave identical color reactions with the Pauly reagent and with diazotized p-nitroaniline, but they also showed the same  $R_f$  value in four different solvent systems (0.55 in n-butanol - acetic acid - water (4:1:5), 0.07 in n-butanol - 25 per cent methylamine (8:3), 0.53 in methylethylketone - pyridine - 25 per cent methylamine - water (65:15:0.5:10), and 0.86 in KCl 20 per cent). Moreover, the unknown substance was eluted from the alumina column by the same ethanol concentration at which the elution of leptodactyline, the meta

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analogue of candicine occurred.

Candicine

The contemporaneous presence of leptodactyline in the 95 per cent ethanol eluates hindered the biological study of the unknown substance. Leptodactyline is in fact 5-20 times more active than candicine on the biological preparations usually employed. However, in spite of lack of biological evidence, the identification of the unknown substance found in the 95 per cent ethanol eluates seems to be beyond any reasonable doubt.

Leptodactyline

Thus, even in the animal kingdom p-tyramine may be N-methylated to the corresponding quaternary ammonium base.

By visual comparison of spots given by different known amounts of synthetic candicine and of spots produced by different amounts of the 95 per cent ethanol eluates it may be calculated that 1 g dry <u>Leptodactylus</u> skin contains 40-50 µg candicine.

In addition to candicine skin extracts of Lept. pentadactylus pentadactylus contain 1-2 µg of p-tyramine (the probable mother substance of candicine), 8 µg of leptodactyline, 130-150 µg of 5-hydroxytryptamine, 10-20 µg of histamine and different amounts of other unknown phenolic, indole and imidazole derivatives, including p-tyrosine, histidine and tryptophan.

It is possible that trace amounts of candicine are also present in extracts of the skin of Leptodactylus pentadactylus Dengleri (Costa Rica).

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