THE BASIC CONSTITUENTS OF THE VENOM OF SOME SOUTH AMERICAN TOADS

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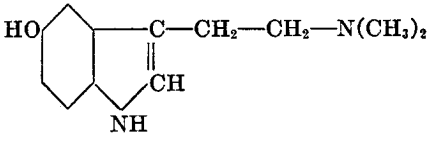
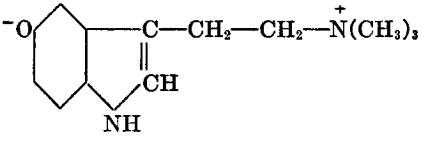
In the venom of the toads there is always present a group of substances designated the basic constituents (1). They are adrenalin and the indole derivatives, bufotenine (I), bufotenidine (II), dehydrobufotenine (III), and bufothionine (IV).



(I)



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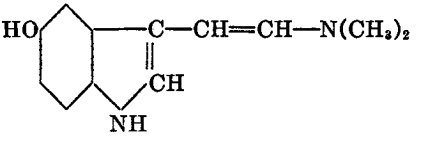

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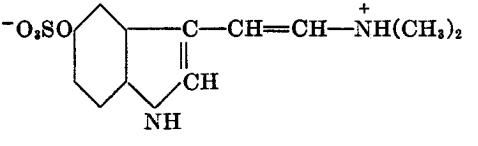

(IV)

In the work described in this paper these compounds have been investigated as components of the venom of some South American toads. New information is given on the venom of *Bufo chilensis*, *B. crucifer*, and *B. spinulosus* and some complementary data on *B. arenarum* and *B. paracnemis*.

EXPERIMENTAL

Methods—In some experiments dried venom was employed; in others the venom was expressed from the skins and dried *in vacuo*. The methods employed in each case are slightly different.

Dried Venom—The method described by Wieland, Hesse, and Huttel (2) was followed with some modifications. The dried venom is exhaustively

extracted with chloroform which dissolves the neutral constituents (bufagins). The residual venom is then dried and twice extracted with large amounts of cold methanol. The solution is evaporated at a low temperature, the residue is dissolved in a small amount of ethanol, and chloroform is added until no more precipitation takes place. The solution contains the toxic bufotoxins; the basic constituents are in the precipitate. This precipitate is well dried and dissolved in water. The faintly acid solution is filtered, brought to pH 2.5, and extracted with ether. (In all operations peroxide-free ether must be employed.) By evaporation of the ether, suberic acid is obtained from some venoms.

The residual water solution is now made alkaline with barium hydroxide and again extracted with ether, from which bufotenine is obtained as a yellow syrup. This syrup is dissolved in a small amount of hydrochloric acid and the solution divided into two parts. To one flavianic acid is added, and, by heating and then cooling, the bufotenine flavianate crystallizes as red needles, which can be purified by recrystallization from water, m.p. 131–133° (3). To the other solution picric acid is added; the resulting red bufotenine dipicrate, m.p. 174°, can be transformed into the yellow monopicrate, m.p. 180°, by boiling with benzene and recrystallizing from water (4). The bufotenine can be further characterized by transformation into bufotenidine hydroiodide, m.p. 215°, by treatment with methyl iodide (5).

The alkaline water solution is freed of barium by addition of dilute sulfuric acid and tested for bufotenidine by addition of flavianic acid. None of the samples contained this base. The solution is then concentrated at low temperature and about pH 7. From time to time, usually when the volume is reduced to one-half, a sample is taken, made slightly acid, and treated with picric acid. If dehydrobufotenine is present, a yellow picrate is obtained, which after recrystallization from absolute ethanol melts at 187°. From 50 per cent ethanol it melts indefinitely at 147–150°. Wieland and Wieland (4) point out that the low melting form is transformed into the high melting by boiling with mineral acids.

The dried venom residue which has been extracted with chloroform and with methanol can be further employed for the isolation of adrenalin by the method described by Deulofeu (6) after extraction with a 1 per cent water solution of acetic acid.

By this method all the above basic constituents of a toad's venom can readily be characterized. We have been unable to obtain bufothionine, which can be very easily isolated from dried toad skins.

Toad Skins—The well dried toad skins were extracted twice with large volumes of 70 to 80 per cent ethanol. The united extracts are concentrated at low temperature almost to dryness and the residue treated with

several portions of petroleum ether, until this solvent leaves no residue on evaporation.

The residue, insoluble in petroleum ether, is then treated with a small amount of absolute ethanol, when most of it dissolves, leaving insoluble inorganic salts and bufothionine. This insoluble residue is filtered and by crystallization from water, with the aid of charcoal if necessary, the bufothionine can be isolated as colorless prisms, m.p. 250° (darkening from about 240°), and further characterized by transformation into dehydrobufotenine hydrochloride (m.p. 244°) by boiling with 2 N hydrochloric acid.

The ethanolic solutions are then concentrated to a small volume and poured into a large amount of iced water. A precipitate, mainly bufagins, is removed; the filtrate containing the bases is concentrated at low temperature, filtered again if some precipitate appears, and brought to pH 2.5. From it suberic acid, bufotenine, dehydrobufotenine, and even bufotenidine, if present, can be isolated by the method described for the dried venom.

Adrenalin could not be isolated, even from skins of toads of which the dried venom contained it. It is apparently destroyed by the process.

In all cases mixed melting points with authentic preparations were determined.

Results

Bufo chilensis—This is the most common toad in Chile. From skin bufothionine, m.p. 252°, was isolated and characterized by transformation into dehydrobufotenine hydrochloride, m.p. 243°. From the alcoholic extract suberic acid melting at 143° was isolated. Further, bufotenine was characterized as its flavianate, m.p. 130–131°, and its red dipicrate, m.p. 172–173°, which on boiling with benzene passes into the yellow monopicrate, m.p. 179°. Dehydrobufotenine was isolated from the solution, after separation of the bufotenine, as the yellow picrate, m.p. 187°.

Bufo crucifer—This is a small toad from Brazil. Only 350 dried skins were available.

Bufothionine was characterized by its melting point (250°) and transformation into dehydrobufotenine hydrochloride (m.p. 240–241°). Bufotenine (picrate m.p. 173° and flavianate m.p. 130–131°) and dehydrobufotenine (picrate m.p. 187°) were found among the ethanol-soluble substances.

Bufo spinulosus—This is a common species of toad in Peru. Only bufothionine (m.p. 249°) and dehydrobufotenine (yellow picrate m.p. 187°) could be characterized. As the amount of material available was small (forty-three dried skins), the possibility that bufotenine or bufotenidine was present is not excluded.

Bufo paracnemis—This large toad from northern Argentina was studied by Deulofeu and Mendive (3) who isolated bufotenine and adrenalin from the dried venom. Working with the dried skin, we have been able to characterize bufothionine, m.p. 249°, which with boiling 2 N hydrochloric acid yielded dehydrobufotenine hydrochloride, m.p. 240–241°. Bufotenine was isolated as the red dipicrate, m.p. 174°, from which the base was regenerated and converted (by treatment with methyl iodide) into bufotenidine hydroiodide, m.p. 213–215°. Dehydrobufotenine (yellow picrate m.p. 187°) was also present, but adrenalin could not be characterized in the residual liquors.

Bufo arenarum—This is the predominant species in the central part of Argentina. In the dried venom bufotenine and dehydrobufotenine were found by Chen, Jensen, and Chen (7), adrenalin by Deulofeu (6), and bufothionine by Jensen (8). From the skins Wieland, Konz, and Mittasch (5) isolated bufothionine, bufotenine, and dehydrobufotenine. We have confirmed the presence of these bases in dried venom and dried skins with the exception that adrenalin could not be obtained from the skins.

DISCUSSION

The data here presented supplement the available information on another South American toad, *Bufo marinus*, in the secretion of which Abel and Macht (9) first characterized adrenalin. Chen and Chen (10) later demonstrated dehydrobufotenine and Deulofeu and Mendive (3) bufotenine. No bufothionine has been reported in the secretion, but as the secretions of *B. marinus* and *B. paracnemis* have practically the same chemical constituents, including the bufagins (3), we feel that bufothionine would be found if a search were made in the dried skins.

The venoms of those South American toads are characterized by the presence in its basic fraction of dehydrobufotenine, of its conjugated sulfuric acid derivative bufothionine, and of its reduction product bufotenine. The mechanisms of the interconversion of those three substances in the toad organism are unknown, but it is noteworthy that bufotenine possesses a pressor activity which is lacking in dehydrobufotenine and bufothionine. Bufotenidine, found in many species of toads from other regions, could not be detected in the toads which we examined. Its absence is especially certain in the cases of *Bufo paracnemis* and *B. arenarum*, of which we had large amounts at our disposal, and of *B. marinus*, a specimen of the dried venom of which was examined solely for bufotenidine.

SUMMARY

1. The basic constituents of the venom of the South American toads *Bufo chilensis*, *B. crucifer*, *B. spinulosus*, *B. paracnemis*, and *B. arenarum* have been investigated.

2. Bufothionine, bufotenine, and dehydrobufotenine are the most commonly present substances of this type. Adrenalin is always present in the dried venom. Bufotenidine has not been found in the dried secretions nor in the dried skins.

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