THE CHEMICAL IDENTITY OF CERTAIN BASIC CONSTITUENTS PRESENT IN THE SECRETIONS OF VARIOUS SPECIES OF TOADS

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In a previous paper (1) we described the isolation of certain basic compounds, as flavianates, from the secretions of different species of toads and pointed out that some of these substances form series of identical compounds. We also reported that the secretions of certain species of toads contain basic constituents which possess a pharmacological action similar to that of epinephrine,[1] and we expressed the opinion that these substances are derivatives of tryptamine. The subsequent work of Wieland and his associates (6) proved that this inference was correct. These workers established the chemical structures of these active principles by synthesis and showed that they could be divided into two closely related chemical compounds: (*a*) bufotenine, 5-hydroxy-3-β-dimethylaminoethylindole, and (*b*) bufotenidine, the betaine of bufotenine.

In this paper we wish to report our findings concerning the more definite characterization of some of the flavianates previously described. For this purpose these compounds were converted into other salts which would allow a more precise identification.

The physiologically active flavianates obtained from ch'an su and the secretions of *Bufo bufo gargarizans*, *Bufo fowleri*, and *Bufo formosus* yielded picrates and hydriodide salts identical with the corresponding salts of bufotenidine. These salts showed similar melting points and gave no depression in melting points when mixed. The chemical analysis of each of the various salts also indicates that they are identical (Tables I and II).

[1] This base has been found to be present in ch'an su (2) and in the secretions of *Bufo marinus* (3), *Bufo arenarum* (4, 5), and *Bufo regularis* (4).

The flavianates obtained by us from the secretions of *Bufo bufo bufo* (*Bufo vulgaris*) and *Bufo viridis viridis* that were found to be similar to the above flavianates in physiological action gave picrates that seem to be identical with the picrate of bufotenine, as described by Wieland and coworkers (6). The chemical anal-

TABLE I

Bufotenidine Picrate

Calculated for $C_{13}H_{18}ON_2 \cdot C_6H_3O_7N_3$. C 51.01, H 4.70, N 15.67.

Source	M.p.	Analysis		
		C	H	N
	°C.			
Ch'an su	198	50.72	4.84	15.33
Bufo bufo gargarizans	198	50.88	4.94	15.46
Bufo fowleri	198	51.12	5.01	15.39
Bufo formosus	197	50.82	4.95	15.60

The salt crystallized from ethyl alcohol in red needles. Mixed melting points showed no depression.

TABLE II

Bufotenidine Hydriodide

Calculated for $C_{13}H_{18}ON_2 \cdot HI$. C 45.09, H 5.50, N 8.08.

Source	M.p.	Analysis		
		C	H	N
	°C.			
Ch'an su	209	44.92	5.65	7.92
Bufo bufo gargarizans	209	45.21	5.72	7.98
Bufo fowleri	209	44.88	5.69	8.00
Bufo formosus	209	45.02	5.22	8.12

The salt crystallized from methyl alcohol in prisms. Mixed melting points showed no depression.

yses also support the assumption of the identity of these salts with that of bufotenine (Table III).

Along with these physiologically active principles, a certain other basic constituent has been obtained as a flavianate (melting at 265–270°) from ch'an su and the secretions of *Bufo marinus* and *Bufo arenarum*; this compound, however, is physiologically inactive. It is identical with the product obtained on hydrolysis

of bufothionine, a sulfur-containing substance present in the secretions of certain toads (4, 7) (Table IV). This observation agrees with the view of Wieland and coworkers (6) in regard to the chemical identity of these basic constituents.

TABLE III

Bufotenine Picrate

Calculated for $C_{12}H_{16}ON_2 \cdot C_6H_3O_7N_3$. C 50.00, H 4.39, N 16.20.

Source	M.p.	Analysis		
		C	H	N
	°C.			
Bufo bufo bufo (Bufo vulgaris).....	177	49.84	4.61	15.94
Bufo viridis viridis.....	178	49.85	4.51	15.91

The salt crystallized from methyl alcohol-ether in red needles. Mixed melting points showed no depression.

TABLE IV

Picrate of Base, $C_{12}H_{14}ON_2$

Calculated for $C_{12}H_{14}ON_2 \cdot C_6H_3O_7N_3$. C 50.11, H 3.95, N 16.24.

Source	M.p.	Analysis		
		C	H	N
	°C.			
Ch'an su.....	183–184	50.15	4.12	15.95
Bufo marinus.....	183–184	49.98	4.22	16.01
Bufo arenarum.....	183–184	49.85	4.08	15.89
Bufothionine.....	183–184	50.02	4.18	15.92

The picrate crystallized from water in long yellow needles that contained 1 molecule of water of crystallization, which is given off at 100° in a vacuum. The analytical data given in the table are those found for the anhydrous salt. Mixed melting points of the various picrates showed no depression.

At present we have no definite information in respect to the exact chemical structure of this base, $C_{12}H_{14}ON_2$. We have found that its molecule contains one methylimide group; methoxyl groups are not present. On the basis of certain color reactions, we believe that, like bufotenine and bufotenidine, it is a derivative of indole.

EXPERIMENTAL

The different flavianates were converted into the various salts following the method of Wieland and his coworkers (6). Most of the analyses reported in this paper were carried out by Dr. Ing. A. Schoeller, Berlin-Schmargendorf. The analytical data given represent the average value of two or more determinations.

The flavianate of the base $C_{12}H_{14}ON_2$, melting at 265–266°, isolated from the secretion of *Bufo marinus* was converted into the free base and certain salts, which were analyzed. The free base was obtained on addition of ammonia to an aqueous solution of the acetate. It crystallized from alcohol in rhombohedrons which begin to darken at 200° and melt at 240° with decomposition; the crystals gradually darken when left in the open air (oxidation).

Analysis—$C_{12}H_{14}ON_2$. Calculated. C 71.29, H 6.93, N 13.86
Found. " 71.04, " 7.12, " 13.62

The following salts of the base $C_{12}H_{14}ON_2$ from *Bufo marinus* were prepared in the usual manner and found to have properties similar to those described by Wieland and coworkers (7) for the base obtained on hydrolysis of bufothionine.

The hydrochloride crystallized from alcohol-ether in needles, which begin to decompose at 212°, melt at 240°, and lose 1 molecule of water when dried *in vacuo* at 90°.

Analysis—$C_{12}H_{14}ON_2 \cdot HCl$.
Calculated. C 60.37, H 6.29, N 11.74, Cl 14.88, NCH_3 12.16
Found. " 59.88, " 6.18, " 11.51, " 14.56, " 12.10

The acid sulfate crystallized from alcohol-ether in rosette-like crystals, melting at 205°.

Analysis—$C_{12}H_{14}ON_2 \cdot H_2SO_4$. Calculated, N 9.33; found, N 8.98

The acetate crystallized from alcohol-ether in prisms, which begin to decompose at 210° and melt at 215°.

Analysis—
$C_{12}H_{14}ON_2 \cdot CH_3COOH$. Calculated, NCH_3 11.10; found, NCH_3 12.15

The hydriodide crystallized from alcohol-ether in rhomb-shaped crystals, which begin to darken at 220° and melt at 238°.

Analysis—

$C_{12}H_{14}ON_2 \cdot HI$.	Calculated.	C 43.64, H 4.55, N 8.49, NCH_3 8.79
	Found.	" 43.42, " 4.50, " 7.95, " 9.22

Hydrolysis of Bufothionine—35 mg. of bufothionine obtained from the secretion of *Bufo arenarum* (4) were boiled for 5 minutes with 1 cc. of 2 N HCl. The solution was evaporated *in vacuo* and converted into the picrate. The picrate crystallized from water in long needles, which showed a melting point of 183–184° and which showed no depression in melting point when mixed with the picrate of the base $C_{12}H_{14}ON_2$ from *Bufo marinus*.

SUMMARY

Bufotenidine has been shown to be present in ch'an su and in the secretions of *Bufo bufo gargarizans*, *Bufo fowleri*, and *Bufo formosus*. Bufotenine has been obtained from the secretions of *Bufo bufo bufo* (*Bufo vulgaris*) and *Bufo viridis viridis*. A basic constituent having the composition $C_{12}H_{14}ON_2$ was found in ch'an su and in the secretions of *Bufo marinus* and *Bufo arenarum*. This substance is identical with the compound that one obtains on hydrolysis of bufothionine, a sulfur-containing constituent present in the secretion of certain species of toads.

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