A CHEMICAL STUDY OF CH 'AN SU, THE DRIED VENOM OF THE CHINESE TOAD, WITH SPECIAL REFERENCE TO THE ISOLATION OF EPINEPHRINE.*

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(Received for publication, March 19, 1929.)

A brief summary of our work on Ch 'an Su appeared recently in a preliminary communication (1). A historical account of the toad poison and a pharmacognostic study of Ch 'an Su by us have been published elsewhere (2).

The chemical investigation of the secretion of the toad was begun by Pelletier (3), and continued by Gratiolet and Cloëz (4), Fornara (5), Calmels (6), Phisalix and Bertrand (7), Bertrand (8), and Faust (9). The highly potent substances prepared by Phisalix and Bertrand, and by Faust were still amorphous products. Abel in association with Macht (10) was the first to isolate bufagin and epinephrine in crystalline form from the American tropical toad, *Bufo agua*. Wieland and his coworkers (11) later succeeded in obtaining crystalline bufotalin and bufotoxin from the European common toad, *Bufo vulgaris*. Apparently the European toad does not secrete epinephrine. Handovsky (12) separated from the same species a substance which he thinks is a pyrrole derivative and distinctly different from epinephrine. Novaro (13) claimed that the secretion of *Bufo marinus* contains epinephrine, but he did not isolate epinephrine in chemically pure form.

Ch 'an Su has been investigated under the name Senso by several Japanese workers during the last few years. Shimizu (14) isolated from Ch 'an Su cholesterol and bufagin, $C_{18}H_{24}O_4$, m.p. 209–210°, which he believed to be identical with Abel's bufagin,

* This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

without, however, giving any chemical proof of such identity. He also separated an amorphous substance which he termed bufotoxin and which did not contain nitrogen, and further indicated the probable presence of epinephrine by color reactions and biological tests, but was not able to isolate the latter in chemically pure form. Kodama (15) gave the formula $C_{27}H_{34}O_7$ for bufagin, m.p. 222–223°, and claimed to have succeeded in obtaining bufotoxin to which he ascribed the formula $C_8H_{10}O_2$, m.p. 203–204°, in crystalline form. Kotake (16), repeating the work, found the formula for bufagin to be $C_{29}H_{38}O_7$, m.p. 220–221°, and Kodama's bufotoxin to be a chlorine-containing compound, $C_{27}H_{35}O_5Cl$, m.p. 218–220°. One can see from the foregoing that the chemical results so far obtained differ widely.

Using the same procedure as has been applied by Abel and Macht in the isolation of epinephrine from *Bufo agua*, we were able to obtain this substance in crystalline form from Ch'an Su as will be seen from the experimental part of this paper, and thus prove the presence of epinephrine in the Chinese product.

Furthermore we have found evidence of the chemical identity of the bufagin which was isolated by us from Ch'an Su with Abel's bufagin. The melting points of both products, purified from the same solvent, were the same and showed no depression when the two substances were mixed. We also prepared acetyl derivatives of both products which had the same melting point and showed no depression in melting point when mixed. We realize only too well that the identity of these two compounds can be made certain only by preparing several derivatives and decomposition products and comparing them. But unfortunately we had only 100 mg. of Abel's bufagin at our disposal. As soon as an opportunity presents itself we hope to be able to settle this question definitely.

Our main aim in taking up the chemical investigation of Ch'an Su was however to see if it also contained a substance which was similar in its chemical composition to the bufotoxin isolated by Wieland and Alles (11) from the European toad, *Bufo vulgaris*. Wieland and Alles were able to show that the molecule of bufotoxin is made up of bufotalin and suberyl-arginine. We have been successful in obtaining from Ch'an Su a nitrogen-containing substance which seems to be closely related in its chemical com-

position to the bufotoxin of Wieland. Our product gives a positive Sakaguchi test (17), thus indicating the presence of arginine in the molecule. We have also isolated from Ch'an Su a fatty acid which seems to be suberic acid. Our finding in Ch'an Su of a compound which is apparently similar in its chemical composition to the bufotoxin of Wieland refutes the claim of the Japanese chemists, Shimizu (14) and Kodama (15), to have isolated bufotoxin from Ch'an Su, as their compounds do not contain nitrogen. As very thorough analytical work is required to establish the empiricial formulas of these compounds we shall report later in more detail about this phase of our work on toad poison. Here we shall describe only the isolation of epinephrine from Ch'an Su.

EXPERIMENTAL.

150 gm. of finely powdered Ch'an Su[1] were mixed thoroughly with 400 cc. of 1 per cent acetic acid and let stand for 2 days in the dark. There was formed such a colloidal emulsion, due to the presence of mucin in Ch'an Su, that it was impossible to effect a separation either by filtration or by centrifugation at high speed. After addition of 600 cc. of 96 per cent alcohol, a fairly good separation was obtained by centrifuging the solution at high speed for 15 minutes. The alcoholic water solution, which still had quite an amount of matter in colloidal suspension, was evaporated to about 300 cc. under diminished pressure in a carbon dioxide atmosphere. To the thick and slimy mass about 200 cc. of water were added and then enough basic lead acetate solution until a clear separation of solid matter and solvent was effected. The solution was filtered with suction and the precipitate washed well with 1 per cent acetic acid. The lead was removed from the filtrate with hydrogen sulfide and the filtrate from the lead sulfide evaporated to about 250 cc. under diminished pressure in a carbon dioxide atmosphere. After filtration the solution was shaken out three times with chloroform to remove organic matter like bufagin and other substances. The watery solution was then further evaporated to about 30 cc. with the same precautions.

[1] One will have to protect his nose from the inhalation of the powder, which is very irritating, causing sneezing and hypersecretion.

To the clear dark red solution concentrated ammonia was added until the reaction was strongly alkaline. Petroleum ether was added to the solution to prevent further oxidation and the mixture put in the ice box overnight. The precipitate was filtered off with suction, washed well with dilute ammonia water, then with alcohol and ether. About 350 mg. of a grayish looking crystalline powder were obtained from 150 gm. of Ch'an Su. This powder already possessed a high degree of purity as shown by the melting point and by biological comparison with other samples of epinephrine from *Bufo agua*, prepared by Abel. The product was purified by dissolving in acetic acid and again precipitating with ammonia. After two precipitations a very pure colorless product, showing the crystalline form of epinephrine, was obtained.

The product melted at 212° with decomposition; when mixed with epinephrine, obtained from *Bufo agua*, no depression in melting could be observed.

Analysis.—(Micro-Kjeldahl.)
3.663 mg. substance: 2.005 cc. 0.01 N HCl.
3.515 " " : 1.977 " 0.01 " "
$C_9H_{13}NO_3$. Calculated N. 7.65 per cent.
Found. " 7.66 and 7.86 per cent.

The specific rotation of our product was found to be

$$[\alpha]_D^{22} = -49.83°$$

This value is in close agreement with that reported for *l*-epinephrine by different investigators.

SUMMARY.

A chemical study of the constituents of the dried Chinese toad poison, Ch'an Su, was made. The blood pressure-raising principle of Ch'an Su was isolated in crystalline form and found to be epinephrine.

We wish to express our appreciation to Professor Abel for supplying us with a sample of epinephrine and bufagin, obtained by him from the toad, *Bufo agua*.

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CORRECTION.

The statement in the text that the mixed melting point of bufagin from Bufo agua and from Ch'an Su and that of their acetyl derivatives showed no depression is incorrect. The mixed melting point of the above named compound shows a marked depression. This suggests that the bufagin from Bufo agua is chemically different from the bufagin from Ch'an Su. In a later paper the relationship of these two compounds will be shown.