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Crystalline Principles From Ch'an Su, the Dried Venom of the Chinese Toad.*

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In the river and lake regions of China there lives a large toad which secretes a whitish, slimy venom in some superficial organs located behind the eyes. These structures may be similar to the "parotid glands" and other smaller glands of the skin of the tropical toad, *Bufo agua*, the secretions of which were studied by Abel and Macht.[1] When the venom is collected by irritating the animal and is allowed to dry spontaneously, it is known as Ch'an Su.[2] This has been used empirically in Chinese medicine for hundreds of years. It has been employed in the treatment of canker sores, sinusitis, and many local inflammatory conditions, in the relief of toothache, and in the arrest of hemorrhages from the gums.[3] When administered internally in the form of a compound pill, it is said to be able to break colds.

Ch'an Su has been a subject of study by Japanese investigators[4, 5, 6, 7] under the name of "Senso" during the last few years. Their chemical results, however, do not agree, and their published pharmacological data can therefore only be accepted with reservation.

During 1923-25, one of us (Chen) made a preliminary study of several Chinese drugs in China. Ch'an Su was one of them. The potent action of its alcoholic and aqueous extracts on the cardiovascular system was then observed. Owing to the extensive work on ephedrine, a thorough investigation was postponed until in 1928. With the assistance of the Council on Pharmacy and Chem-

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1 Abel, J. J., and Macht, D. I., *J. Pharm. Exp. Ther.*, 1911-12, iii, 319.

2 Ch'an means the toad, and Su the venom. They are spelt according to the Mandarin pronunciation. See Y. W. Lee's "A New Chinese-English Dictionary," Shanghai, the Commercial Press, Ltd., 1918, 566 and 657.

3 Li, S. C., *Pentsao Kang Mu*, 1596 A. D., Chap. 42.

4 Hayashi, H., *Deut. Med. Wchnschr.*, 1911, xxxvii, 624.

5 Shimizu, S., *J. Pharm. Exp. Ther.*, 1916, viii, 347.

6 Kodama, K., *Acta Scholae Med. Univ. Imp. Kioto*, 1920, iii, 299; 1921-22, iv, 201 and 355.

7 Kotake, M., *Liebigs Ann. Chem.*, 1928, cdlxv, 1.

istry, American Medical Association, a supply of 1729 gm. of Ch'an Su was purchased from Tung Jen T'ang, Peiping, one of the oldest drugstores in China.[8] In the present study, Jensen was responsible for the chemical side and Chen for the pharmacological and pharmacognostical side. Four crystalline bodies have been separated so far. They can be characterized as follows.

1. *Cholesterol,* M. P. 146° C. (uncorrected), gives all the known color reactions for cholesterol. Spectroscopic examination showed an ergosterol content of 2 parts per thousand. The product, after irradiation, is now being subjected to biological tests for antirachitic elements.

2. *Bufagin,* M. P. 217° C. (uncorrected), is easily soluble in alcohol and chloroform, soluble in ether, and gives typical color reactions with chloroform plus concentrated sulphuric acid, and with acetic anhydride plus concentrated sulphuric acid. Abel was the first to name and isolate bufagin in crystalline form from *B. agua.*[1] The bufagin from Ch'an Su has a toxic action on the heart. When the frog's heart was perfused by the method of Howell and Cooke,[9] recently elaborated and extensively used by Sollmann and Barlow,[10] a concentration of 1:100,000 caused a brief acceleration followed by a rapid decrease in rate and amplitude. Relaxation became more and more incomplete until finally the ventricle stopped in systole. More dilute solutions depressed the contractions and rate. Auricular fibrillation and incomplete block were observed with a concentration of 1:10,000, and occasional extrasystoles with that of 1:20,000. A dilution of 1:10,000,000 had no effect. The M. S. D. (Minimal Systolic Dose) in frogs as determined by the one hour method was found to be 0.000045 gm. per gm. of body weight. In pithed cats, 1-1.5 mg. of bufagin proved fatal, due to the cardiac collapse. Sublethal doses had a prolonged pressor action.

3. *A Nitrogen-Containing Compound,* M. P. 200° C. (uncorrected), is soluble in chloroform and alcohol but insoluble in ether, produces color reactions with acetic anhydride plus concentrated sulphuric acid, and gives a positive Sakaguchi's test[11] probably due to the presence of arginine in the molecule. This is an entirely new principle isolated from Ch'an Su and possibly corresponds to the "bufotoxin" which Wieland[12] separated from the European toad,

[8] Chen, K. K., *Ann. Med. Hist.,* 1925, vii, 103.

[9] Howell, W. H., and Cooke, E., *J. Phys.,* 1893, xiv, 198.

[10] Sollmann, T., and Barlow, O. S., *J. Pharm. Exp. Ther.,* 1926, xxix, 233.

[11] Sakaguchi, S., *J. Biochem. Japan,* 1925, v, 25.

[12] Wieland, H., and Alles, R., *Ber. deut. chem. Gesell.,* 1922, lv, 1789.

Bufo vulgaris. The substance is more toxic than bufagin. Solutions of 1:1,000,000 and higher concentrations produced in the frog's heart a primary acceleration soon followed by a decrease in rate and amplitude with the occurrence of ventricular extrasystoles, auriculo-ventricular block as shown by the 2:1 rhythm, arrhythmia, ventricular intermittence and systolic standstill, while the auricles continued their bradycardia until final arrest was brought about. Concentrations of 1:2,000,000 to 1:10,000,000 caused some depression of the contractions and the rate. The M. S. D. in frogs was determined to be 0.000005 gm. per gm. of body weight. In pithed cats of ordinary size, 0.5-1.0 mg. caused death. Smaller quantities also raised the blood pressure, but not so much as bufagin.

4. *Epinephrine.* M. P. 212° C. (uncorrected), gives the typical color reactions with ferric chloride. This is the first time that this principle also has been obtained in crystalline form from Ch'an Su. Its presence makes the traditional belief justifiable that Ch'an Su stops hemorrhages, and shrinks the nasal mucosa to drain the infected sinuses. Abel' discovered epinephrine in the secretions of *Bufo agua* some 18 years ago. The epinephrine prepared from Ch'an Su reproduced all the known physiological responses in the animal body. When assayed biologically in pithed cats, it was proved to have exactly the same pressor activity as a commercial specimen.

Detailed accounts of this work will appear elsewhere.

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Influence of Cathode Rays on Leucocytes of Rabbits.

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It is well known that leucocytes are destroyed to a considerable extent if a large portion of an animal is exposed to about an erythema dose of roentgen rays which penetrate into the tissues under the skin. Bucky[1] has stated that very soft roentgen rays, "grenz rays," have a different effect on the leucocytes. According to him, a considerable portion of the leucocytes disappear from the circulating blood immediately after a small area of the skin has been exposed to

[1] Bucky, G., *Am. J. Roentg.*, 1927, xvii, 645.