

## SCIENTIFIC SECTION

### A CONTRIBUTION TO THE CHEMISTRY, PHARMACOLOGY AND THERAPEUTICS OF AGAVE SALMIANA.\*

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#### INTRODUCTION.

About 25 years ago, by the initiative of a few leading physicians, the government established a scientific institution which was named "Instituto Médico Nacional." The object of this institution was to study the large number of native drugs and determine their therapeutic value, to classify the unknown specimens botanically, and to isolate their active principles and study them chemically. Most of the results of the work carried out by this institution are recorded in an interesting publication called "Anales del Instituto Médico Nacional" (1), of which only one edition was printed.

The following historical data were taken from this publication with the aid of Dr. E. Novoa, who offered his assistance at the library of the "Dirección de Estudios Biológicos de la Secretaría de Agricultura y Fomento," which has taken the place of the extinct Instituto Médico Nacional.

#### HISTORICAL.

"People in rural communities have found this plant (*Agave Salmiana* or Maguey) to be a panacea. It is claimed that there is no disease that will not yield immediately to treatment with the various applications of the products of this plant. The juice of its leaves is used for rubbing purposes in various conditions. The ancient Indians knew of the medicinal properties of this plant and used it in the treatment of different diseases. Country people to this date continue to use it in a similar manner in prescriptions or home remedies such as the following:

"To cure blows and contusions in the chest, use a beverage composed of about two liters of juice from the leaf of the plant, sugar and raisins. The juice is first evaporated on the open fire and then the other ingredients are added. The patient is instructed to keep a moderate diet."

For internal tumors the following formula was recommended:

"About 1 liter of the juice from roasted leaves together with some raisins, licorice, olate and cuautecomate wood, and four maguey thorns are disintegrated and mixed together. The mixture is concentrated on the open fire. The resulting concoction is given to the patient in small cup doses, one every twelve hours. The roasted leaf is also applied externally over the lesion to help healing."

"For gonorrhoea it is recommended to drink every day for about two weeks, half a liter of aguamiel (*Savia Agaves*) boiled previously."

"The gum or resin formed at the lower part of the stem or leaf is said to be a good toothache remedy, placing a small piece of the material over the affected tooth and keeping it there for some time."

The efficacy of such remedies is a matter of wide popular speculation. There are other applications so absurd that they are not mentioned here.

"In the year of 1790, a curandero (a practical healer using herbs to cure disease, also called hierbero) from Pátzcuaro arrived in the city of Mexico. His name was Nicolas de Viana and his

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nickname "El Beato" (The Bigot). He addressed the Tribunal of the Protomedicate, stating that he possessed the secret of an infallible cure for venereal diseases, which secret he had obtained from an Indian woman who had used it from time immemorial."

"The Royal Tribunal of the Protomedicate, knowing already of Viana's achievements, suggested that new experiments be carried out under the direction of an able physician. The well-known Dr. Jove was appointed to carry out the work. Viana was allowed to experiment at the hospitals, where it is said he cured completely over 100 patients with venereal disease. The cures were effected in 30 days.

"Doctors Jove, Guiral and Rada became so impressed with the results observed that they declared Viana's remedy to be a blessing to humanity. Further clinical experimentation was carried out by these physicians when Dr. Nuñez, Archbishop of Mexico, became interested in this work and commissioned Dr. Jove to perfect Viana's method and apply it at the other hospitals in a more scientific manner."

"While these experiments were carried out Dr. Balmis arrived at Vera Cruz and heard about them. After having been appointed Chief Surgeon he was requested to investigate this new method of curing venereal disease."

"Viana used three formulas in his method. One of them was a decoction with sudorific properties made of Savia agaves, agave roots, snake meat and castilian rose. Another formula was an infusion of several roots (Sasafrás, Saucó, Zarza), lemon and copal gums. These two were alternated with vegetable laxatives and enemas."

"Dr. Balmis introduced some rational modifications in these formulas and in their method of administration, and is said to have obtained very satisfactory results. Among the patients treated were found men, women, children of both sexes; old men, pregnant women, all classified as incurable even if using massive doses of mercury. The total number of cases successfully treated was about 350, out of which Dr. Jove treated more than 24 personally."

"Later on, Archbishop Haro became thoroughly convinced of the efficacy of this method (by that time Begonia had also been added to the various mixtures used) and wished that Spain should also be benefited by Viana's discovery. Dr. Balmis was appointed to carry this agave therapy to Spain, and he sailed in 1792 with a large supply of agave material and begonia."

"In July 1792 Dr. Balmis began to use his new therapy at the Hospital of Madrid. At the Hospital of San Juan de Dios 12 patients with gonorrhoea were chosen. Four were completely cured, others improved and two of the patients, in which the disease was too far advanced, died."

"At the Hospital de la Pasión seventeen new patients were observed, all women; some were cured, others improved and none died."

"At the Hospital General another twenty-five observations were carried. Of these cases twenty were cured."

This literature goes on giving detailed case reports to illustrate the action of the agent used by oral administration.

#### CHEMISTRY OF AGAVE SALMIANA.

There are several classes of agave of which *Agave Salmiana* or *Agave Americana* is the one dealt with in this paper. The common name in Spanish is "Maguety manso" ("Metl" in the Mexican language) (2). According to Segura, it was called "Teometl," "Tlacametl," "Centemetl." It grows in the valley of Mexico. The kind most sought is that grown in the prairies of Apam.

Lenoble found in the leaves of *Agave Americana* an acrid volatile oil, a gum-resin, chlorophyl, cellulose, acid calcium malate and other salts.

Prof. Palacios (3) in 1895 made a qualitative analysis of the juice of the plant and found a density of 1.022-1.024 in the liquid. It has an acid reaction and gives a copious flocculent precipitate upon addition of alcohol in excess. NaOH, Ca(OH)<sub>2</sub>, H<sub>2</sub>SO<sub>4</sub>, ammonium oxalate and barium nitrate all produce precipitates when added to this juice.

The juice obtained by expression was found to be very unstable, undergoing an acid fermentation unless sterilized or preserved with chloroform. The qualitative examination of the solution revealed the presence of saponins, reducing sugars, a soluble calcium salt, phosphorus, a small amount of protein and other common constituents of ordinary vegetable extractives.

Since the object of this investigation was mainly to find out whether products of this plant actually had any therapeutic value, our efforts were directed towards preparing a solution suitable for hypodermic administration. The first tests showed that the crude liquid was highly toxic to animals. The saponins in the solution were found to be hemolytic, and there was also a highly irritant substance contained in the stems; after several trials, by fractional precipitations using a large excess of alcohol which was later distilled off, the solution was concentrated to its original volume and thus freed from toxic and inert constituents. From this solution practically free from impurities two crystalline substances could be isolated. One was obtained by partial evaporation of the liquid in an acid medium, while the other was obtained by first neutralizing the solution and then adding an excess of alcohol. The first precipitate (from acid solution) is a light brown powder, partially soluble in water and forming a colloidal solution. The precipitate from the alkaline solution has a lavender color while moist, and dark gray in the dried form, being practically insoluble in water and the ordinary solvents. The acid precipitate contains about 15% calcium; the ash contains about 62% calcium. The ash from this same substance amounts to 24%. The phosphorus content of the acid powder is about 0.50% (cal. as  $P_2O_5$ ) and that of the lavender colored powder (alkaline) about 2%.

Solubility and hydrolysis tests showed that the substance was not a nucleo-protein. However, tests for mineral phosphorus were negative with magnesia mixture and uranium acetate. Ferric chloride gives no precipitate in the cold, but on warming, precipitation is observed.

The liquid much used by the natives as a drink called "Pulque" is a product of the agave plant. It is a white opalescent viscid liquid with a sour-sweet taste with a density of 1.025-1.046. This liquid, before it is manipulated or "doctored" to become pulque, in its fresh native state is called officially "Savis Agaves" in the new Mexican pharmacopœia. Rio de la Loza, Boussignault (2) and other authors have analyzed it with the following results:

According to Rio de la Loza its composition is:

Sugar.....	9.55%	K, Na, Ca, Mg, Cl, $SO_4$ , $PO_4$ , $SiO_2$ ...	0.726%
Gum and sol. albumen.....	0.540%	Water, resins, albuminoids and undetermined.....	9.180%

According to Boussignault its composition is:

Levulose.....	2.645%	Albumen.....	1.013%
Sucrose.....	6.171%	Ammonia.....	0.006%
Malic acid.....	0.353%	Mineral substances.....	0.621%
Gums.....	0.545%	Water.....	8.650%

"Savia Agaves" is alleged to have anti-scorbutic and anti-gonorrhœic properties. There is a proprietary preparation on the market called "Agmel" based on this substance, and its manufacturers claim that it has therapeutic properties.

## PHARMACOLOGY.

A solution containing about 1.5% soluble calcium salt and 0.01% phosphorus (as  $P_2O_6$ ) was prepared from the isolated principle and used hypodermically on rabbits in the first experiments. It was found that no appreciable effect was obtained even in doses of 2 cc. per Kg. wt.

The animals were kept under close observation during a week. In view of this apparent lack of toxicity it was decided to prepare a more concentrated solution, and one having the following approximate composition was prepared:

Soluble calcium (calc. as CaO).....	0.689%
Phosphorus (calc. as $P_2O_6$ ).....	0.067%
Acidity (in cc. <i>N</i> /10 NaOH per 100).....	90.00 %
Dissolved in normal saline solution.	

The amount of CaO in terms of calcium lactate would be about 4% against 1.5% of the solutions first used in the tests. The solution was equally well tolerated by the animals. There were no appreciable changes in the blood counts nor any other undesirable effects.

The author and several members of his staff were given 1- and 2-cc. injections of the concentrated solution without experiencing any toxic effects. The only disadvantage was that this stronger solution proved to be too painful and this was corrected by the addition of 1% stovaine. Although it was found that the painfulness was due to the acidity of the solution, the excess acidity could not be neutralized without causing the substance to precipitate. If applied hypodermically in a precipitated condition indurations were formed and the absorption was very slow.

## THERAPEUTICS.

It is impossible to state here the many uses which the maguey plant has been given as a medicinal agent. The solution described above was originally prepared in order to investigate the utilization of a soluble calcium salt, in its native condition. For the purpose Dr. Carl Nielsen's collaboration was requested but, unfortunately, other work prevented him from taking up the investigation. We wish to express our thanks to Dr. Albert Schneider for having carried out some tests with the original 1.5% solution.

The difficulty in securing white mice of known breed for nutrition experiments was an obstacle for the study of calcium fixation or retention using this agave solution. The work was temporarily postponed, and at that time, suggestions were offered to investigate the alleged anti-syphilitic properties of agave.

Dr. Miguel Cordero (4), Chief of the Division of Pharmacy and Chemistry of the Health Department, had at one time carried out some experiments in collaboration with Dr. Fernando Zárraga at the Hospital Morelos. These investigators had observed in a few cases the healing of secondary syphilitic ulcers with the oral administration of an Agave extractive. I found out accidentally that at a certain second-hand book shop there was an old treatise dealing with the curative properties of Agave extractives. The title of the book, edited in 1767 is, freely translated:

"Demonstration of the efficacy and virtues, newly discovered, of the roots of two plants from New Spain, species Agave and Begonia for the treatment of ve-

neral and scrofulous vices and other diseases that resist treatment with mercury and other known remedies. By Dr. Francisco Xavier Balmis, Surgeon Counselor to the Royal Armies, member of the Royal Academy of Medicine of Madrid, appointed by H. M. to verify the investigation carried out at Madrid concerning the curative properties of these plants."

This book is dedicated to the Duke de la Alcudia who apparently financed the investigation, and its object was to prove, against the skepticism of another physician, Dr. Piñera, that the Agave and Begonia treatment was a boon to humanity. Upon carefully examining this literature one can see that the basis of such treatment was to determine copious perspiration and to apply rather vigorous catharsis which, judged from modern therapy, strikes one as a rather veterinary-like procedure.

When it was decided to investigate the properties of the isolated principle, it was planned to ignore the use of sudorifics and cathartics in order to determine the action of the hypodermic solution alone. Dr. Demetrio Lopez, to whom I resorted, among the other physicians, for suggestions, was of the opinion that any beneficial effects as observed by Dr. Balmis had been probably caused by the sudorific action and by catharsis for, in the the absence of arsenic, bismuth or mercury, hardly any specific action could be expected from such a treatment.

The collaboration of several specialists on venereal diseases was secured and Dr. G. González Aréchaga (5), of the Health Department initiated the investigation, ably seconded by the students E. Novoa, who later carried out the animal experimentation with the glucosides of *Castela Nicholsoni*, and E. Sanchez Torres.

Ten cases of secondary syphilis were selected and the case reports have been published elsewhere ("Cronica Medica Mexicana," XXVII, No. 4, 158-168 (April 1928)). Seven patients improved and their ulcers healed completely leaving only a slight pigmentation, two showed little improvement, and one did not improve at all. Later on, Dr. Fernando Lopez selected ten other patients and the results were negative in all the cases treated. About 30 cases in all were investigated by different observers of which about one-half responded to treatment favorably and the other half failed to respond at all. The following conclusions could be derived from the clinical study:

1.—Among the 30 cases observed, primary, secondary and tertiary syphilitic subjects were treated.

2.—Cases with secondary ulcers responded best to the treatment.

3.—In tertiary syphilis astounding successes and failures were observed.

4.—Cases resistant to clinical improvement with specific agents responded surprisingly well to Agave substance, other similar cases did not improve at all.

5.—The effect on the Wassermann reaction was somewhat uncertain, and in some cases no relation could be established between clinical improvement and the modified Wassermann R.

6.—The following conditions were observed:

- (a) Marked clinical improvement and modification of W. R.
- (b) Marked clinical improvement and slight modification of W. R.
- (c) No clinical improvement and marked modification of W. R.
- (d) No clinical improvement and slight modification of W. R.
- (e) No clinical improvement and no modification of W. R.

Under (a) may be added: Marked clinical improvement and no modification of W. R.

7.—It was rather disappointing to observe that two patients with apparently the same type of lesions would respond differently to the same treatment: one would respond as though under specific treatment while the other showed absolutely no signs of improvement.

8.—The fact that Agave substance is capable of influencing, without any appreciable reaction, the involution of syphilitic ulcers in some cases and even determine an unmistakable clinical improvement in tertiary cases, suggests further study.

9.—The fact that in certain cases a modification of the W. R. is observed also warrants further investigation.

10.—Agave substance is applicable in cases of intolerance to specific treatment with about 40% or 50% probabilities of clinical improvement although no assurance of specific action may be had.

11.—One case in which an overdose of Agave substance was given by mistake (about 12 cc. of concentrated solution equivalent to 36 cc. of the original one and one-half per cent) slept during 24 hours. Somnolence induced by the administration of therapeutic doses of Agave substance was frequently observed. The larger doses of Agave substance are apt to cause slight fever but not true allergic reactions were observed. The substance may be considered practically non-toxic even for small children, in large doses.

Dr. Fernando Lopez did not present a written report of his 10 cases where no favorable results were observed. In his work, the maximum amount of Agave solution given was only 5 cc. of the dilute solution whereas, later, doses as large as 10 cc. of concentrated solution (equivalent to 30 cc. of the dilute solution) were given daily by other collaborators. However, good results were not necessarily obtained with the larger doses. Just why some patients respond to this treatment and others do not is not known.

At the time of writing this report there are some collaborators who are still investigating the problem and report consistently good results in hereditary syphilis.

#### DISCUSSION.

Whatever conclusions may be derived from the above data, they should be taken only from the clinical point of view, for there is no conclusive evidence of specific action. The solution used does not contain arsenic, bismuth or mercury, or any other metal of recognized anti-luetic properties. The precipitated substance contains as an impurity an undetermined amount of saponin (saponins are abundant in the Agave juice or exudate) although this impurity loses its hemolytic properties in the process of preparation and purification.

Calcium salts, soluble or insoluble, have never been found to possess any trypanocidal action so that there is no ground to suppose that this form of calcium is responsible for the effects observed.

If we consult the pharmacology of saponins (sapo-toxins and sapo-glucosides) (7) and (11) we see that these substances tend to alter protoplasmic surface tension and have a special affinity for cholesterol. The saponin contained in the solution used, in contrast with other similars, was observed to have the following properties:

(a)—It has no hemolytic properties *in vivo*. Many patients have taken considerable amounts of this solution by hypodermic administration over periods of from one to forty-eight weeks without any signs of hemolytic action. No hemolysis occurs *in vitro*.

(b)—It does not cause irritation to mucous membranes.

(c)—It does not possess the peculiar acrid taste and does not have sialagogue action. No symptoms of nausea, vomiting or diarrhea have been observed with its use.

(d)—Its hypodermic administration causes only a slight local reaction.

(e)—Systemic action: No appreciable effect on the nervous system has been observed unless the somnolence reported in some cases is due to it. The administration of even small quantities of other saponins to the animal may prove fatal, causing convulsions and paralysis of the respiratory center. No such effects have been observed by the substance under discussion. However, the original saponin occurring in the plant juice does possess the toxic properties of ordinary saponins.

Has the alteration of plasma surface tension any relation to the therapeutic effects observed? No experiments have been carried out along that line to determine if this sapogenin may cause effects on surface tension similar to those observed by sodium ricinoleate. It has been shown (6) that the cultivation of *Tubercle Bacilli* in a medium containing small amount of saponin does not inhibit their growth but it does affect appreciably their acid-resistant properties and morphology. It has also been shown that non-specific agents (8, 9, 10) like tuberculin may modify favorably the involution of a syphilitic ulcer but such an effect is usually brought about at the expense of some focal reaction of some intensity and in the case of Agave substance the patient improves unaware of any general or focal reaction. This, however, would be no proof that there is no physico-chemical or biological change at the site of the lesion if such be the mechanism of action of the substance under investigation.

#### CONCLUSIONS.

1.—A crystalline substance from the juice of *Agave Salmiana* has been isolated and used parenterally in the human being to investigate its alleged anti-syphilitic properties.

2.—In agreement with the data in ancient and recent literature on the subject some therapeutic properties have been observed without the use of cathartics or sudorifics.

3.—The results observed do not prove conclusively that such a substance may be depended upon as a specific treatment. Although some of the cases treated have been observed to improve clinically, eliminating as far as possible all chances of spontaneous involution of the lesions, others have failed to show any improvement under exactly the same treatment.

4.—The effect on the Wassermann reaction is not consistent and may be considered doubtful.

5.—Experimentation over a large number of cases under perfectly controlled conditions would be the only way to arrive at definite conclusions.

#### REFERENCES.

- (1) "Anales del Inst. Med. Nac.—Library of the Dir. de Est. Biol., Dept. of Agriculture," Mexico City.
- (2) Mexican Pharmacopœia (latest Edition of the Pharmaceutical Association).
- (3) *La Farmacia*, IV, No. 7 (July 15, 1895), Mexico.
- (4) M. Cordero, "Las Saponinas del Maguey" Archives of the Experimental Laboratory of the Department of Industry, Commerce and Labor, Mexico City.

(5) Bulletin No. 2 of the Department of Pharmacology, Central Chemical Laboratory, 1927-1928 (Dic. 20).—"Clinical Observations on the Parenteral Administration of Normal Agave Solution," by G. G. Colin, G. González Aréchaga.

(6) F. Wyss, *Compt. rend.*, 177 (1923), 719-720.

(7) Solmann, "Pharmacology."

(8) Kolmer, "Infection, Immunity and Specific Therapy," Saunders, Phila.

(9) Greenbaum and Wright, "Protein Therapy in Syphilis," *Arch. Dermat. and Syph.*, Chicago, 12 (1923), 773-920.

(10) R. D. Herrold, "Protein as an Adjunct Treatment of Resistant Syphilis," *Arch. Am. Med. Assoc. Bulletin*, No. 6 (Feb. 1926), 413-414.

(11) A. W. Van de Haar, "Galacturonic Saponins and Salts," *Ber.*, 55 (1923), 3041-3069.

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## NOTES UPON INDIAN EPHEDRAS.

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With the occurrence of several species of *Ephedra* in Western Tibet and Northern India it was thought that here lay a further possible supply of crude material for the production of ephedrine. However, seeing that not all species of *ephedra* contain ephedrine, and that in others the chief alkaloid present is the isomer pseudoephedrine it was thought to be of importance to make a chemical analysis of the Indian *Ephedras*. Books (1), (2) upon Indian materia medica list two *ephedras*, *E. vulgaris* and *E. pachyclada*, under the Persian name "Huma," and other names which are not familiar. It is considered to be the Soma of the Vedas. A personal visit to the Forestry Department at Dehra Dun revealed the fact that in Northern India there are three common species of *ephedra* and several varieties of the same. *Ephedra intermedia*, *E. Gerardiana*, and *E. foliata*, the last mentioned growing on the plains.

### ANALYSES.

With the samples kindly supplied we have undertaken in our Peking laboratories identical examinations for the alkaloids to those done upon Chinese *Ephredas* and published from time to time. The sample of *Ephedra intermedia* was sufficiently large to allow of our doing numerous analyses upon the various parts of the stem, the dry brittle stems loose in the package giving slightly higher values. The methods used were as recently published (3), number 3 by direct alkalization being found most suitable for regular assay work. This was checked with further analyses by modified methods. The percentage of alkaloid present was calculated from the acid titration values, the titrated solutions being subsequently tested by the "Biuret Test" (4) for the relative percentages of ephedrine and pseudoephedrine. The identity of these two isomers in the case of *Ephedra intermedia* was conclusively confirmed by the preparing of their hydrochloride salts with their characteristic melting points and optical rotation. Having but small samples of *Ephedra Gerardiana* the identity of the alkaloids depended on the Biuret reaction, which is quite reliable and final for practical purposes. The results are shown in Table I.