THE CONSTITUENTS OF THE ROOTS OF AMERICAN GINSENG.*

BY YING C. WONG.

Ginseng has been esteemed by the Chinese for several thousand years. The roots obtained from plants growing in China and Korea furnish one of the valuable and reliable medicines. The properties may be summarized in a general way as being alterative and tonic. The price of the best Chinese roots ranges from $480 to $800 a pound. Owing to the great demand for Chinese Ginseng, the native plants have become very much reduced as the wild roots are most highly esteemed; the commercial supplies are largely met by the drug obtained from cultivated plants. For almost a hundred years, American Ginseng has been exported to China, and at the present time more than a million pounds are shipped annually. The wild American roots are considered to be much more valuable than those collected from cultivated plants.

The name Ginseng or Schinseng is applied to the roots of *Panax quinquefolium* (American Ginseng) and *Panax Ginseng* (Chinese Ginseng), perennial herbs, belonging to the family of Araliaceae, the former growing in rich woods in the eastern United States and Canada, and the latter indigenous to the mountainous forests of eastern Asia and cultivated in northern China, Korea and Japan. Ginseng has not been subjected to any very careful scientific investigation, as all preliminary examinations regarding its constituents have shown that they are not of such a striking character as those of the alkaloidal drugs. The drug undoubtedly possesses some value in medicine, and it was considered desirable to investigate its constituents with the view of throwing some light on its value as a medicine.

As it is very difficult to obtain any large quantity of authentic specimens of wild roots and also due to the high price of the same, these investigations were made with commercial material which was apparently derived from cultivated plants.

American Ginseng appears to have been first chemically examined by Rafinesque,[1] who stated that he found in it a camphor-like body, on which depends the taste of the drug and to which he gave the name "panacine."

S. S. Garrigues[2] also examined the root, and he observed the presence of a substance which he called neither an alkaloid nor a glucoside, and he gave it the name "panaquilon." Garrigues assigned to this substance the formula $C_{12}H_{28}O_9$. He further showed that "panaquilon," when treated with strong acids, was converted into a white substance, with the escape of carbon dioxide and water. For this white substance, Garrigues proposed the name "panacon" ($C_{11}H_{19}O_4$).

* The writer desires to express his gratitude and thanks to Prof. Henry Kraemer for having suggested this investigation and assisted in obtaining the samples, and for his kind guidance throughout the course of the work.

The work was carried out at the University of Michigan in partial fulfillment of the requirements for the degree of Master of Science. (See p. 310 April JOURNAL, A. PH. A. '21, also p. 816, Vol. IX, JOURNAL A. PH. A.—Editor.)

[1] *Am. J. Pharm.*, 1854, 26, 510.

[2] *Ibid.*, 1854, 26, 511.

Davydow[1] investigated these principles discovered by Garrigues but added nothing new to our information concerning them.

EXPERIMENTAL.

CHEMICAL EXAMINATION.

The material employed for this part of the investigation consisted of the roots of the above described American Ginseng, which was perfectly authentic. These roots were from cultivated plants ranging from 4–6 years old. They were dry and were ground into powder. As a preliminary experiment, a small portion of 20 gramme of the ground material was tested for the presence of an alkaloid, but with a negative result.

In order to ascertain whether an enzyme were present, 100 Gm. of the ground material were macerated with water at the ordinary temperature for two days. To the expressed and filtered liquid about twice its volume of alcohol was added, when a gelatinous precipitate was produced. This was collected, washed with a little alcohol, and dried in a vacuum over sulphuric acid, when it amounted to about 0.2 Gm. It responded to the biuret reaction, and slowly hydrolyzed starch, thus proving the presence of an enzyme.

Another portion of 34 Gm. of the ground material was successively extracted in a Soxhlet apparatus with various solvents, when the following amounts of extracts, dried at 100° C., were obtained:

Petroleum ether extracted	0.1699 Gm.	equals	0.47%
Ether extracted	0.2891 Gm.	equals	0.85%
Chloroform extracted	0.0433 Gm.	equals	0.12%
Ethyl acetate extracted	0.4192 Gm.	equals	1.23%
Alcohol extracted	5.9464 Gm.	equals	17.49%
Total	6.8679 Gm.	equals	20.16%

For the purpose of a more detailed examination, a quantity of 2000 Gm. of the powdered material was extracted with hot 92% alcohol, when, after the removal of the greater portion of the solvent, 410 Gm. of a dark brown, viscid extract were obtained.

The whole of the above mentioned extract was mixed with cold water, and a yellowish brown emulsion was formed in which small globules of a dark brown oleoresin were suspended. In order to separate these globules, the whole liquid was shaken out three times with ether. The united ethereal liquid (A), which contained the oleoresin, was of a dark brown color, while the aqueous liquid (B) which contained the saponin and other substances was yellowish brown.

Examination of the Ethereal Liquid (A)

After the removal of the ether, a dark brown syrupy extract was obtained which amounted to 36 Gm. This was mixed with a little water and distilled with a current of steam. The distillate was extracted with ether, and the solvent removed, when a small amount of about 0.5 Gm. of a pale yellow essential oil was obtained. This had a pleasant odor, to which undoubtedly the odor of the drug is due. To a little of this oil, a drop of concentrated sulphuric acid was added, a yellowish

[1] *Phar. Zeitschs. Rusl.*, pp. 97, 113, 13, and *Am. J. Pharm.*, 1890, 338.

brown color was produced, which upon the application of gentle heat for a few minutes changed to pink. When another small portion was treated with aniline and hydrochloric acid, a blood red color was produced on standing, and so it yielded the color reaction for furfural.

After the above operation, there remained in the distilling flask a dark brown, resinous substance. The water was decanted and petroleum ether added to dissolve out the resinous substance. This mixture was transferred to a separatory funnel and washed with hot water. The petroleum ether layer was then separated and the solvent removed. The resinous mass obtained amounted to 18 Gm.

This dark brown resinous mass was dissolved in alcohol and heated under a reflux condenser with an alcoholic solution of potassium hydroxide for four hours. When saponification was completed, the alcohol was evaporated off, water added, and the alkaline mixture transferred to a separatory funnel and extracted several times with ether. The ethereal extracts were then united, washed with a small quantity of water, dried, and evaporated. A small amount of a crystalline substance was obtained, which separated out from absolute alcohol in plates, and when dried gave a melting point between 132–134°. A small amount of this substance was dissolved in chloroform and the solution shaken with an equal quantity of concentrated sulphuric acid, the chloroform layer on standing for a while was colored red and then purple. This substance, thus, reacted to the phytosterol color reaction, and it is seen to be a phytosterol, which was too small an amount for any further separation and investigation.

Examination of the Aqueous Liquid (B).

This aqueous liquid contained the saponin and a small amount of sugar. As stated above, the main point of this work was to isolate this saponin in its pure state and to study its nature. To isolate it, the aqueous liquid was treated with a saturated solution of lead subacetate by which it was precipitated as a lead salt. The precipitate was suspended in water and hydrogen sulphide passed through it. It was filtered and the filtrate exactly neutralized with potassium hydroxide and allowed to stand for a few hours after the addition of barium hydroxide. The barium compound of the saponin was collected and suspended in water and freed from barium by means of carbon dioxide. The solution was evaporated in a vacuum and the concentrated residue dissolved in methyl alcohol and finally precipitated by pouring the alcoholic solution into a large volume of ether (about six times that of the alcoholic solution). To purify it, this process was repeated three times; the final product obtained was of a slightly yellowish white color; it amounted to 36 Gm. after being dried. A determination of its ash content was made which amounted to 1.04%.

Properties of the Saponin: This saponin dissolves readily in water and warm glycerin. It is slightly soluble in 95% alcohol and in glacial acetic acid, but moderately in hot alcohol and methyl alcohol. It is insoluble or almost so in ether, chloroform, benzene, amyl alcohol and ethyl acetate. When dried, it is of light yellowish white color, and it imparts a yellowish color to its water, glycerin, and alcoholic solutions. In the dry and powdered state, it is amorphous. It melts at 170–172° C. It has a slightly bitter taste.

In water solution, this saponin produces decided foaming under agitation. Solutions as weak as 1 per 1000 exhibit this peculiarity. This frothing property can easily be shown by placing a small quantity of the saponin in a beaker, the latter put under the tap and water run down on it. It is non-toxic to animals or fish, and has no haemolytic action.

It is acid to litmus and phenolphthalein. Concentrated sulphuric acid dissolves it with first a reddish color, changing into violet, and later into more intense red. With a mixture of equal parts of alcohol and sulphuric acid in the presence of a drop of ferric chloride, it gives a bluish green color. When its solution is added to a permanganate solution, the latter is reduced. This saponin is precipitated by neutral as well as basic lead acetate, and also by barium hydroxide. Its water solution does not reduce Fehling's solution, but if it has first been heated with a dilute mineral acid, the Fehling solution is reduced. The heating with dilute mineral acids produces a crystalline substance sapogenin, and a pentose. It does not contain nitrogen and its empirical formula is $(C_5H_{12}O_5)X$.

Substance* 0.3288 Gm. gave 0.6120 CO_2 and 0.1871 H_2O C = 50.79; H = 6.26 percent.
0.3362 Gm. gave 0.6288 CO_2 and 0.1890 H_2O C = 50.83; H = 6.31 percent.
$(C_5H_{12}O_5)X$ requires C = 51.06; H = 6.38 percent.

*The saponin used in the combustion analysis was dried at 110°.

The Acetyl Derivative of the Saponin: During the process of investigation it was noticed that this saponin could be acetylated, yielding a definite product. This derivative was prepared by heating 2 Gm. of saponin with 2 Gm. of sodium acetate and 8 Gm. of acetic anhydride for one hour in a reflux condenser. The mixture was poured into water and the precipitated solid collected, washed and dried in a vacuum desiccator over sulphuric acid. The product thus obtained was a dark mass, which melted below 100° C. It was readily soluble in the ordinary organic solvents, but could not be crystallized. A combustion analysis showed the following results:

Substance dried at 110° 0.3848 Gm. gave 0.7616 CO_2 and 0.1886 H_2O C = 53.98; H = 5.51%
$(C_{17}H_{22}O_{10})X$ requires C = 53.76; H = 5.5%

A determination of the acetyl value of this acetylated product was made by saponifying 0.5 Gm. with alcoholic potash for half an hour, removing the alcohol by evaporation, acidifying with 1:10 sulphuric acid and distilling. The distillate was titrated with $\frac{N}{10}$ NaOH and 0.339 Gm. of acetic acid was found to be present which corresponds to 0.2929 Gm. CH_3CO or 58.58% of the acetylated saponin. This acetyl value together with the results of the combustion would indicate that the acetylation had taken place in the whole molecule of the saponin.

Hydrolysis of the Saponin.

Formation of a Sapogenin and a Pentose: It has already been mentioned that a substance insoluble in water results when the saponin in solution is heated with a dilute mineral acid. To obtain the substance, 5 Gm. of the saponin were dissolved in a little water and then 50 Cc. of a 10% aqueous solution of hydrogen chloride added, and the mixture heated on a water-bath for four hours, when a crystalline hydrolytic product separated out from the hot liquid. This product

was collected, carefully washed with water, and dried, the filtrate being set aside for the subsequent examination of the sugar.

This sapogenin was of a light brown color. It was insoluble in water, chloroform, ether or benzene, and slightly soluble in absolute alcohol, but more readily so in alcohol containing a little water. When it was dissolved in acetic anhydride, a little chloroform added, and then a few drops of concentrated sulphuric acid, a purplish red color was produced, which finally disappeared. When a solution of the substance in aqueous alcohol was agitated, a copious frothing was produced, but the permanency of the latter is not so great as in the case of the saponin itself. It melted at 188–191°. A combustion analysis showed the following results:

Substance dried at 110° 0.1614 Gm. gave 0.4114 CO_2; 0.1260 H_2O. C = 69.51; H = 8.73%
$(C_{21}H_{38}O_5)X$ requires C = 69.23; H = 8.78%

The filtrate obtained above was examined for sugars. It was concentrated on the water-bath and the concentrated liquid divided into three portions to be tested for the presence of glucose, pentose and galactose respectively, but the first and third resulted negatively.

In order to arrive at a quantitative relation between the saponin and the liberated pentose, 1 Gm. of the saponin was distilled with 12% hydrochloric acid as carried out in the provisional method of the A. O. A. C.[1]

This yielded 0.01662 Gm. of furfural phloroglucide. A duplicate experiment was performed on the same amount of saponin, which yielded 0.01760 Gm. of furfural phloroglucide. The average of these two results, that is 0.01711 Gm., is equivalent to 0.022689 Gm. of pentose or 2.2689 percent of the saponin taken.

A quantitative determination of sapogenin liberated from the saponin was also carried on two samples of the saponin, by the above method. The first one of 2 Gm. was heated for one hour which yielded 0.5604 Gm. of dry sapogenin or 28.02% of the saponin, and the second of 1.6 Gm. was heated for four hours, yielding 0.4826 Gm. of sapogenin or 30.16% of the saponin.

From these experiments, the yield of pentose and that of sapogenin were below those figures which were expected from theoretical considerations, and these discrepancies might have been caused by incomplete heating and hydrolysis.

Microscopical Examination.

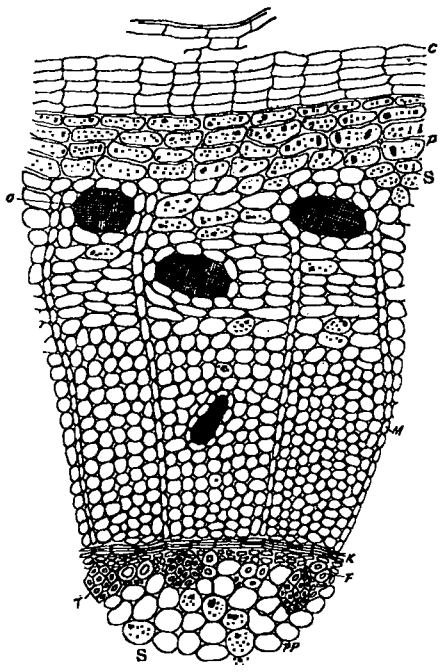
A number of papers on the microscopic structure of this root have been published, the most important of these being the one by Davydow.[2] In the description, he stated that the parenchyma cells near the rather thick cork contain clusters of crystals of calcium oxalate, while in the examination of this root, the writer failed to find any of these crystals present. The accompanying plate shows the general appearance of a cross section of the root.

The cross sections examined were obtained from both fresh and dry roots, the latter being soaked in dilute alcohol, and the following internal structures were observed:

CORK: The cork consists of four to six rows of tabular cells which are nearly free from starch the walls having large pores and reticular thickenings.

[1] U. S. Dept. of Agric., Bur. of Chem., *Bul.* 107 (rev.), p. 54.

[2] Rep., *Apoth. Ztg.*, 1890, 137.



Transverse section of American ginseng root. *C*—cork; *P*—cortical parenchyma; *M*—medullary rays; *K*—cambium; *F*—wood fibers; *T*—tracheae; *PP*—pith parenchyma; *O*—oleoresin secretion cavities; S—Starch grains.

CORTICAL PARENCHYMA: The outer layers of the cortical parenchyma cells are long in outline with rounded corners, while those bordering the cambium are smaller and round in outline. The parenchyma of the cortex contains numerous starch grains which exhibit one or two flattened surfaces; among the parenchyma cells occur large schizogenous ducts in which an oleoresin is secreted.

CAMBIUM: The cambium zone is marked by its dark brown color. The cells are rectangular, and they are usually not clearly seen because the walls are partially collapsed.

MEDULLARY RAYS: The medullar ray cells are tangentially elongated, and somewhat rectangular in outline.

TRACHAE: The vessels are angled in outline. They show either simple or bordered pores and recticular thickenings.

WOOD FIBERS: The wood fibers are thick-walled and highly angled.

PITH PARENCHYMA: The pith parenchyma cells are rounded in outline and as large as the cortical parenchyma cells, which also contain considerable starch. Saponin occurs in some of the parenchyma cells of both the cortex and pith, and its presence may be detected by the formation of a violet-red coloration with concentrated sulphuric acid.

GROUND OR POWDERED GINSENG: The color of powdered ginseng is yellowish white. It has a feeble odor and a sweet, slightly aromatic taste, somewhat analogous to that of licorice powder. Under the microscope, numerous starch grains are seen, which are mostly spheroidal, varying from 0.005 to 0.02 mm. in diameter; some of which exhibit one or two flattened surfaces; and in some a small cleft is visible. The vessels are mostly in broken fragments. The wood fibers are long, occurring in groups, singly and also in broken fragments, with thickened walls, and slightly angled. Large fragments of cork cells, parenchyma tissues, medullar rays and oleoresin secretion canals with yellowish color are also seen.

SUMMARY AND PHARMACOLOGICAL TESTS.

The material used for this investigation consisted of the roots of American Ginseng, *Panax quinquefolium.*

A preliminary test was performed for the presence of an alkaloid, but with a negative result. Another one showed the presence of a relatively small amount of an enzyme.

An extract of the material, made by hot 92% alcohol and after the removal of the solvent, was treated with a little water and the mixture extracted several times with ether. The ethereal portion showed a very small amount of a pale yellow essential oil, and a dark brown resinous substance, which reacted to the phytosterol reaction.

The aqueous liquid was subsequently examined and it was found to contain an appreciable amount of sugar, and a saponin which after purification was a

light yellowish white amorphous powder melting at 170–172°. It yielded an acetyl derivative which was a dark brown mass melting below 100°. On hydrolysis this saponin was resolved into a pentose and a sapogenin which was of a darker color than the original saponin melting at 188–191°.

It has been mentioned above that this saponin is not toxic to animals or fish and has no haemolytic action. This conclusion was indicated by the following experiments:

(A) 0.5 Gm. of the saponin was dissolved in 25 Cc. of physiological salt solution, thus making it 2% in strength. Ten test tubes each containing $^1/_2$ Cc. of a 2.5% three-times-washed sheep red corpuscles were prepared, and physiological salt solution was added to each to make the final volumes 3.5 Cc. Into these tubes were pipetted 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 Cc. respectively of the above prepared saponin solution. The tubes were then shaken and allowed to stand at room temperature. In 24 hours the corpuscles had settled down, leaving a colorless liquid at the top in all the tubes. The tubes were allowed to stand for one week, but no change was observed.

Another set of experiments was carried out as above except the tubes were kept in the incubator at 37° instead of at room temperature. The results were the same.

(B) A 10% solution of this saponin was prepared, 2 Cc. was injected to each of two rabbits and one guinea pig intravenously and another 2 Cc. to another guinea pig subcutaneously. All these animals were examined every day, which resulted in no symptoms of physiological activity.

(C) A 1–2000 solution of the saponin was prepared in a large glass jar and a gold fish weighing 28 Gm. put in. The jar was placed outside of a window and the fish fed with fish food. For two days, it was just as healthy as it was at the beginning. At the end of four days no change was shown, it was taken out and its weight noted, which showed neither increase nor decrease.

THE RELATION OF THE DISSOCIATION OF HYDROGEN TO ENZYMIC ACTIVITY.* PEPSIN STUDIES.

BY HOWARD T. GRABER.

Sörensen demonstrated that the measure of the reaction of hydrolysis by Pepsin, and also Catalase, is not dependent upon the titrable acidity but rather upon the "H" ion concentration.

He showed that the action of these enzymes has an optimum at a definite "H" ion concentration and that the presence of other ions exerts an influence which, while not measureable, should not be neglected. He pointed out that he could produce one and the same "H" ion concentrations by means of either of two methods:

1—By the use of what he termed regulators or buffers, or

2—By the dilution of the acid.

It was also shown that regardless of what buffer controls the acidity, and irrespective of the concentration of the other ions, the enzyme exerts the same activity if the concentration of the "H" ions is the same.

*Read before the Detroit Section of the American Pharmaceutical Association, May 13, 1921.