CONCLUSIONS.

1. It is necessary to amplify our first conclusions by the statement that there seems to be conclusive evidence that there are at least two color reacting constituents in Cascara Sagrada.

2. One color reacting constituent is practically insoluble in water and gives a deep cherry-red color with ammonia. The other color reacting constituent is soluble in water and gives an orange-brown color reaction with ammonia.

3. Both coloring constituents are soluble in ether.

4. It seems likely that the variation in the U. S. P. tests may in part be due to a difference in solubility of these two constituents.

5. It may be possible to derive a quantitative color test if these are the only two coloring constituents.

6. The bitter principle is soluble in water, accompanies or is the brown color reacting constituent and accompanies or is part of the therapeutically active principle, probably an anthraquinone.

7. The non-bitter principle is insoluble in water and is therapeutically active.

8. The bitter principle acts more slowly and produces marked griping, while the non-bitter principle acts more rapidly and with less griping.

Sample (1) was obtained from the Pharmacognosy Laboratory of the University of Illinois, College of Pharmacy, and was identified as Cascara microscopically by Prof. E. N. Gathercoal.

Sample(II) was obtained from the Pharmacy laboratory of the University of Illinois, College of Pharmacy.

Samples (III) and (IV) were obtained from a local drug store.

BIBLIOGRAPHY.

"Culbreth's Materia Medica and Pharmacology," 1917 Ed., p. 389.
Jowett, JOUR. A. PH. A., 1904, p. 288.
Kraemer, *A. J. P.*, 1912, p. 385.
L. Kofler, *Oesterr. Apoth. Ver.;* through *Chem. Abstr.*, 1919.

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THE STABILITY OF OFFICIAL PEPSIN PREPARATIONS.

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A. E. Taylor has stated[1] that "..... in general purine bodies tend to accelerate tryptic digestion." In 1922 Traut[2] observed that liquid medicinal pepsin preparations containing very small amounts of caffeine (a purine body) seemingly maintained their peptic activity more readily than without caffeine. It became of interest to determine whether other purine bodies, readily obtainable commercially and inexpensive could be used for this purpose in quantities so small as to be practically nontoxic. Purine derivatives available include the following: caffeine,

[1] *University of California Publications of Pathology*, I, p. 251 (1903–1907).

[2] November JOUR. A. PH. A., p. 686 (1922)—E. J. Traut and H. W. Vahlteich.

caffeine citrated, theobromine, theobromine and sodium salicylate, and uric acid. Xanthine, hypoxanthine, purine, guanine, adenine, allantoin, uracil, thymine, and cytosine are excluded because they are prohibitively expensive or entirely unobtainable.

It was also intended to compare the keeping qualities of pepsin preparations made according to the official directions except that the hydrochloric acid used is omitted, first, with such preparations containing purine derivatives, and second, with the official preparations containing both acid and purine derivatives. The preparations selected were the N. F. IV Glycerite of Pepsin and Elixir of Pepsin with the varying quantities of acid and purine bodies indicated in the table.

All of the preparations were made up from the same containerful of granular pepsin and from single lots of all other ingredients so as to eliminate any possible variables due to differences in materials or enzyme. It has been our experience that different dry pepsins deteriorate at different rates, a factor which it was important to eliminate in this work. Hence to account for such a possible deterioration of the dry pepsin used, a portion of it was retained for a determination of its proteolytic power both at the time the preparations were made and again after the two years. This particular specimen of granular pepsin showed no change in activity over the two-year period, eliminating the necessity for subtracting such a loss in activity of the liquid preparations.

To avoid a possibility of loss of enzyme by adsorption (admittedly a remote one) no talc or other clarifying medium was used in filtering the elixirs. The caffeine, theobromine, and theobromine and sodium salicylate, were dissolved in each case in 2 to 5 cc. of hot distilled water, this solution being added to the unfinished product before it was brought to volume. In making up the glycerites enough granular pepsin solution in water was made up for all of them and then aliquot portions pipetted off for each individual glycerite. The object of this was to assure that absolutely identical portions of enzyme were used in each glycerite. In making up the uric acid glycerite preparations the desired quantity of uric acid powder was dissolved in 40 to 50 cc. of glycerin with the aid of heat. For the uric acid elixir preparations the uric acid was dissolved in water with the cautious dropwise addition of 0.2*N* NaOH using neutral red as indicator to assure keeping the solution slightly on the acid side of p_H 7.0 so as to avoid decomposition of the purine group.

The preparations were stored in 250-cc. glass-stoppered bottles in 150- to 250-cc. quantities. They were kept on a shelf at a warm (in summer) south wall where the temperature from October to May was about 30° C. during the day and very likely was below 10° C. at night and when the school building was not occupied. They therefor encountered a twice daily change in temperature of 20° C. or more during most of the year.

The granular pepsin used had an activity of 1:3300 by the U. S. P. IX method.[1] It was used in exactly the quantities given in the N. F. IV for the elixir and glycerite without taking its higher activity (than 1:3000) into consideration. The preparations studied, with their original activities, activities after two years, per cent deterioration, and the p_H of each are tabulated as follows:

[1] As a matter of interest determinations were on the granular pepsin and the preparations when made up by Traut's modification of the milk method and an average of 10 to 12 determinations was found to check the U. S. P. determination in every case.

Pepsin preparation.	Proteolytic activity. Nov. 1923.	Proteolytic activity. Nov. 1925.	Deterioration in per cent.	p_H Nov. 1925.
Glycerite, N. F. IV	1:3300	1:3000	9	2.9
Glycerite, 1/4 HCl	1:3300	1:3100	6	2.9
Glycerite, no HCl	1:3300	1:3100	6	3.0
Glycerite, no HCl, 0.017 Gm. caffeine per 100 cc.	1:3300	1:3300	none	3.0
Glycerite, no HCl, 0.034 Gm. caffeine per 100 cc.	1:3300	1:3300	none	3.1
Glycerite, N. F. IV, 0.017 Gm. caffeine per 100 cc.	1:3300	1:3100	6	2.9
Glycerite, no HCl, 0.017 Gm. theobromine per 100 cc.	1:3300	1:3300	none	3.0
Glycerite, no HCl, 0.034 Gm. theobromine per 100 cc.	1:3300	1:3300	none	3.0
Glycerite, N. F. IV, 0.017 Gm. theobromine per 100 cc.	1:3300	1:3100	6	2.9
Glycerite, no HCl, 0.010 Gm. uric acid per 100 cc.	1:3300	1:3300	none	3.0
Glycerite, no HCl, 0.020 Gm. uric acid per 100 cc.	1:3300	1:3300	none	3.0
Glycerite, N. F. IV, 0.020 Gm. uric acid per 100 cc.	1:3300	1:3200	3	2.9
Elixir, N. F. IV	1:3300	Below 1:100	100	1.5
Elixir, 1/4 HCl	1:3300	1:1800	45	2.6
Elixir, no HCl	1:3300	1:2100	36	3.6
Elixir, no HCl, 0.017 Gm. caffeine per 100 cc.	1:3300	1:2000	40	3.6
Elixir, no HCl, 0.034 Gm. caffeine per 100 cc.	1:3300	1:2000	40	3.6
Elixir, N. F. IV, 0.017 Gm. caffeine per 100 cc.	1:3300	1:300	91	1.6
Elixir, no HCl, 0.017 Gm. theobromine per 100 cc.	1:3300	1:1800	45	3.6
Elixir, no HCl, 0.034 Gm. theobromine per 100 cc.	1:3300	1:1800	45	3.6
Elixir, N. F. IV, 0.017 Gm. theobromine per 100 cc.	1:3300	1:200	94	1.6
Elixir, no HCl, 0.010 Gm. uric acid per 100 cc.	1:3300	1:1700	49	3.5
Elixir, no HCl, 0.020 Gm. uric acid per 100 cc.	1:3300	1:1900	42	3.6
Elixir, no HCl, 0.040 Gm. theobromine and sodium salicylate per 100 cc.	1:3300	1:1800	45	3.6
Elixir, no HCl, 0.08 Gm. theobromine and sodium salicylate per 100 cc.	1:3300	1:1900	42	3.6

"1/4 HCl" means that the preparation was made up to contain 1/4 the HCl specified in the N. F. IV.

"no HCl" means that the preparation was made up to contain no added HCl whatever.

It seems reasonable to conclude from the above data that

(1) The Glycerite of Pepsin N. F. IV keeps admirably well.

(2) The Elixir of Pepsin N. F. IV lost its entire potency in two years or less (the preparations were not assayed during the two-year period).

(3) (*a*) The purine derivatives used as "preservatives" have no decided value for the purpose, and (*b*) the elimination of the HCl in the preparation of the elixirs is just as valuable a means of preserving their proteolytic activity as is the addition of the purine derivatives.

It is also interesting to observe that the p_H of the Elixirs of Pepsin, N. F. IV, which lost practically their entire activity is close to the optimum p_H for peptic

digestions. This suggests that the enzyme digests itself or its carrier and so loses its activity.

I wish to acknowledge the kindness of Mr. H. T. Graber of the Digestive Ferments Co., Detroit, Mich., for furnishing the granular pepsin used in this work.

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A SUGGESTED CHANGE IN THE OFFICIAL METHOD OF ASSAY OF CRESOL IN LIQUOR CRESOLIS COMPOSITUS.

BY EDWARD L. GRIFFIN.*

The Pharmacopœia of the United States, tenth decennial revision, provides under **Liquor Cresolis Compositus,** page 210, a method for the assay of cresol. In this method the cresol is determined as the increase in volume of the aqueous layer, which results when the kerosene and cresol mixture, obtained by distilling 50 cc. of liquor cresolis compositus with kerosene after the addition of sodium bicarbonate, is shaken with 15 per cent sodium hydroxide solution. The cresol is separated from this solution by treatment with hydrochloric acid and washed with a saturated solution of sodium chloride. Five grams of freshly ignited potassium carbonate is then added to the washed cresol, after which the mixture is shaken gently at frequent intervals during three hours and allowed to stand over night. The cresol, decanted from the potassium carbonate, must meet the U. S. P. distillation requirements for cresol. In short, the pharmacopœial requirements for this product are twofold: (1) The proportion of cresol present in 50 cc. must be such as to produce a 23- to 26-cc. volume increase in the sodium hydroxide solution in which it is dissolved. (2) The liberated cresols, when dry, must come within the definite distillation requirements; 90 per cent should distil between 195° and 205° C. as described.

This method of assay was applied to several samples of liquor cresolis compositus of known composition. In all cases the increase in volume of the sodium hydroxide solution, due to cresols, was within the prescribed limits. When the cresol was separated, washed, treated with 5 grams of freshly ignited potassium carbonate, and decanted as directed, the resulting product had become viscous, almost syrupy, and on distillation left a rather large residue. When ashed the residue showed the presence of potassium. When the distillation residue was treated with hydrochloric acid very little effervescence resulted, indicating that little or no carbonate was present. This suggested that the potassium carbonate had reacted to some extent with the cresol to form potassium cresylate. More detailed experiments were, therefore, undertaken.

Liquor cresolis compositus was made from a cresol of which 98 per cent came over between 195° and 205° C. when distilled according to Method II of the Pharmacopœia. This solution was then assayed according to the Pharmacopœia method. The dried cresol was decanted through cotton and no suspended potassium carbonate was carried with it. The residue after distillation at 205° was washed out of the flask with alcohol into a weighed platinum dish, evaporated on

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