LITERATURE CITED.

(1) Dohme and Englehardt, PROC. A. PH. A., 45 (1897), 193.
(2) Jowett, *Ibid.*, 52 (1904), 288.
(3) Beal and Tumminkatti, JOUR. A. PH. A., 14 (1925), 865; 15 (1926), 847.
(4) Daels, *Bull. acad. roy. méd. Belg.*, 29 (1919), 779; 30 (1920), 129.
(5) George D. Beal, JOUR. A. PH. A., 13 (1924), 215.
(6) J. H. Hoch, *Am. J. Pharm.*, 105 (1933), 64.
(7) Thorpe, Robinson and Miller, *J. Chem. Soc.*, 57 (1890), 38; 61 (1892), 1.
(8) George D. Beal and Ruth Okey, *J. Am. Chem. Soc.*, 39 (1917), 716.
(9) J. E. Driver and G. E. Trease, "The Chemistry of Crude Drugs" (1928), page 58 (Longmans, Green & Co., Ltd.).
(10) C. A. Browne, "Handbook of Sugar Analysis" (1912), page 374 (John Wiley & Sons, Inc., N. Y.).
(11) J. C. Munch, "Bioassays" (1931), page 778 (Williams and Wilkins, Baltimore).

STUDIES IN PERCOLATION.*

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DETANNATED TINCTURE OF CINCHONA.

Tinctura Cinchonæ Detannata was official in the N. F. 1886, 1896 and 1906. The tannins were removed from the tincture as the name implies. As early as 1866, Walker (1) briefly described a method by which he prepared a fluidextract of cinchona of a beautiful red color which held the cinchotannates in solution, by percolating with diluted alcohol. Meumann, in 1886 (2), discussed the method for preparing detannated tinctures by means of ferric hydroxide. His method could be applied to any drug but was outlined for cinchona. The ferric hydroxide magma was added to the bark and intimately mixed. The mixture was transferred to a percolator and percolation allowed to proceed.

A year later, Meumann published a second paper (3) in which he recommended 2 parts of ferrous sulphate to 3 parts of bark. Remington (4), in 1887, furnished a formula for the detannated tincture according to which the U. S. P. fluidextract was treated with ferric hydroxide. In 1888, Tiarks (5) reported on the method generally employed for preparing detannated fluidextracts. He mentions the use of albumin, gelatin, lime and ferric hydroxide. Keutmann (6), in 1903, described a method for detannating cinchona by percolating a mixture of the bark with calcium hydroxide after first forming a magma of the two with water and adding ammonium carbonate. It is doubtful whether he obtained a completely detannated tincture since he reports a cherry-red color for the preparation made in this manner, whereas a completely detannated tincture is yellowish in color. However, the red color may have been due to cinchona red (not to cinchotannic acid) which is reported to be *soluble* in ammonia water.

Experimental Part.—Cinchona bark, No. 20 powder was used. When assayed according to the U. S. P. X, it yielded 4.70%, 4.32% and 4.26% of total alkaloids in as many assays. The tannin content was determined by the hide powder method (7). Three determinations yielded 4.18%, 4.17% and 4.46%, respectively.

* Scientific Section, A. PH. A., Madison meeting, 1933.

The alkaloids of cinchona bark are supposed to be combined, in part at least, with cinchotannic acid. No therapeutic value, so far as alkaloidal action is concerned, seems to be attached to this peculiar combination (8). Inasmuch as cinchotannic acid, under certain conditions changes to cinchona red, an insoluble phlobaphene, the presence of the cinchotannic acid is supposed to be responsible for unsightly depositions in the tincture and other galenical preparations. Moreover, it has been reported that the cinchona red, as it is formed and deposited, occludes alkaloid. Hence, the formation of a deposit in the tincture is not only a cause of unsightliness, but may bring about a reduction in the alkaloidal content as well (9).

It seemed desirable, therefore, to remove the cinchotannic acid from such preparations in which this agent was not specially wanted. Instead of removing it from the tincture after the latter had been prepared, it seemed preferable to prevent its solution in the menstruum in the percolator. For this purpose calcium and ferric hydroxides might be regarded, *a priori*, as equally suitable. Moreover, either reagent would be expected to set free any alkaloids combined in the drug with cinchotannic acid, and thus possibly, aid in their solution.

DETANNATION WITH CALCIUM HYDROXIDE.

I. Enough CaO was used to yield 100 Gm. of $Ca(OH)_2$ when slaked with the theoretical amount of water. The slaked lime was mixed intimately with 100 Gm. of powdered cinchona bark, the mixture moistened with 150 cc. of alcohol and packed into a cylindrical percolator. Sufficient alcohol was added from time to time to yield 500 cc. of tincture. However, the percolate was collected in five fractions of 100 cc. each. These fractions were examined separately as was also a sixth fraction of 100 cc., *i. e.*, 100 cc. more than the formula for the preparation calls for. In the following table there will be found recorded the following data:

A. The length of time required for the percolation of each fraction.
B. The density of the percolate determined at 20° by means of a pycnometer.
C. The percentage of alcohol (by volume) ascertained by the U. S. P. X method.
D. The amount of extractive determined by the U. S. P. X method.
E. The alkaloidal content determined by the U. S. P. X assay method (U. S. P. X page 452).

	I.	II.	III.	IV.	V.	VI.
A.	10 hrs.	13 hrs.	15 2/3 hrs.	16 hrs.	15 1/2 hrs.	15 hrs.
B.	0.8359	0.8257	0.8280	0.8248	0.8195	0.8263
C.	75.60%	76.80%	81.48%	81.08%	81.08%	86.96%
D.	1.65%	0.77%	0.44%	0.14%	0.11%	0.15%
E.	1.02%[a]	0.45%[b]	0.28%[c]	0.17%[d]	0.13%[e]	0.18%[f]

[a] The average of 2 determinations, *viz.*, 1.07% and 0.97%.
[b] The average of 2 determinations, *viz.*, 0.45% and 0.45%.
[c] The average of 2 determinations, *viz.*, 0.30% and 0.26%.
[d] The average of 2 determinations, *viz.*, 0.15% and 0.18%.
[e] The average of 2 determinations, *viz.*, 0.14% and 0.11%.
[f] The average of 2 determinations, *viz.*, 0.20% and 0.16%.

The dregs were further extracted to completion with the same menstruum and the following determinations made:

Percentage extractive	1.96%
Percentage alkaloids	0.83%
Percentage tannin	0.11%

The resulting tincture was straw yellow in color. Upon standing for several months the color assumed a reddish tint. It gave no reaction with ferric chloride, hence may be assumed to have been free from tannin.

A. The period of percolation varied from 10 to 16 hours for each 100-cc. percolate.

B. With minor irregularities, the density of the five percolates constituting the tincture decreased with the extraction. In this respect the fractional percolates differ from those in which the bark was not mixed with calcium hydroxide (10). It may therefore be that, in the regular percolation, the cinchotannic acid is changed, in part at least, to an intermediate soluble product between it and the insoluble cinchona red which, on account of its greater density, causes a rise in density in the intermediate fractions.

C. Inasmuch as the alcohol used was 95 per cent by volume (not the official menstruum), it becomes apparent that the moisture of the air-dried drug was taken up, for the most part it would appear, by the strong alcohol reducing its ethanol content by about 20 per cent. From the table it also becomes apparent that the ethanol content of the percolate increased to about 81 per cent where it seemed to remain stationary so far as the official amount of percolate is concerned. But even the final percolate to approximate exhaustion, contained about 8 per cent less ethanol than the menstruum employed. It might be worth while to repeat the experiment in a dry atmosphere in order to ascertain whether the water taken up by the alcohol came from the mixture in the percolator (drug + $Ca[OH]_2$) or from the air.

D. The total extractive of the 500 cc. of tincture contained but 3.11 per cent of extractive, whereas percolate VI contained 0.15 per cent, making a total of 3.26 per cent (to this should be added the 1.96 per cent of the "exhaustive" extraction, making a total of 5.22 per cent) as compared with 20.27 per cent for the drug not treated with calcium hydroxide. Whereas the percentage of extractive decreases with each succeeding fraction of percolate, the percentages of extraction and the corresponding densities do not appear comparable. Thus for percolates II and III the corresponding figures are:

Density	0.8257	0.8280
Extractive	0.77%	0.44%

Although the density has increased slightly, the percentage of extractive has dropped materially. Again we observe some of the irregularities recorded in connection with the percolation of cinchona by itself with alcohol (11).

E. The alkaloidal content of the 500 cc. of tincture is only 2.05 per cent, percolate VI, supposed to represent more complete extraction, contained but 0.18 per cent making a total of 2.23 per cent. (Again there should be added 0.83 per cent making a total of 3.06 per cent.) The crude drug had assayed 4.42 per cent. These figures indicate a loss of more than 1 per cent. (It has been assumed that in the deposition of cinchona red from the tannin—containing tincture, alkaloid is occluded by the cinchona red formed. It may be possible that a like occlusion takes place in the percolators.) Roughly speaking the second fraction of percolate contained somewhat less than one-half as much alkaloid as the first fraction, the third fraction somewhat more than half as much as the second, the fourth somewhat more than one-half as much as the third. The fifth fraction contained not much less than the fourth, thus seeming to indicate that the last traces of alkaloid (12) still present were becoming more difficult to extract.

The $Ca(OH)_2$ must effect the solubility of substances other than the 4 + per cent of tannins, such as quinic acid, etc.

II. As in Experiment I, 100 Gm. of cinchona bark were mixed intimately with 100 Gm. of $Ca(OH)_2$ and the mixture percolated with 95 per cent alcohol until 500 cc. of tincture had been obtained. The product was of a light straw color which darkened but little upon standing. A slight whitish precipitate, presumably $CaCO_3$, resulted upon exposure to air. By way of comparison the corresponding

U. S. P. X tincture (however, 95 per cent alcohol was used) was prepared, also a third preparation using 67.5 per cent alcohol, which corresponds approximately to the menstruum of the U. S. P. X. In the last experiment the powdered cinchona bark was mixed with calcium hydroxide before it was packed in the percolator.

Some of the properties of these three tinctures are herewith tabulated:

	1. Tinct. Prepared with 95% Alc. and Lime.	2. U. S. P. Tinct.	3. Tinct. Prepared with 67.5% Alc. and Lime.
A. Time of percolation	79½ hrs.	85¾ hrs.	88½ hrs.
B. Sp. gr. 20°	0.8127	0.9302	0.9093
C. Percentage alcohol (vol.)	86.46%	68.30%	55.42%
D. Percentage of extractive	0.78% (13)	7.93%	2.10%
E. Percentage of alkaloid	0.44% (14)	1.19% (15)	0.925% (16)
F. Action upon litmus	Alkaline	Alkaline	Alkaline (17)
G. Test with $FeCl_3$	Negative	Positive	Negative

A. Whereas the periods of percolation varied as much as almost 10 hours it may be that this difference in time had no appreciable effect on the constants determined.

B. That the densities of 2 and 3, in the preparation of which a diluted alcohol had been employed, should be higher than that of 1 was to be expected. The density of 1, however, is lower than that of the corresponding tincture reported under Experiment I.

C. The ethanol content of tincture 1 is appreciably higher than that of the tincture reported under Experiment I. The ethanol content of tincture 2 is slightly higher than that of the menstruum employed, whereas that of tincture 3 is appreciably lower. It would appear, therefore, that even 67.5 per cent alcohol exerts a dehydrating effect on $Ca(OH)_2$.

Preparation of Quinic Acid.—Approximately 500 Gm. of the lime-treated dregs were extracted in a percolator with water until exhausted. The water extract was evaporated to dryness, the residue boiled with 95 per cent alcohol, and filtered. The alcohol removed material, which was of an amorphous character. The residue, insoluble in alcohol, was dissolved in warm water, decomposed with oxalic acid, the calcium oxalate filtered off and the quinic acid crystallized.

Fate of Tannic Acid in Drug.—Lime apparently removes the tannins from cinchona in the form of calcium tannate. Tannin determinations made on the dregs by means of the hide powder method, revealed little tannin. These determinations were made by first treating the bark with HCl so as to free the tannin.

Qualitative tests for tannins were made with ferric chloride and gelatin solution after first treating the bark with acid as stated above. No tests for tannins were apparent. The solutions were then carefully neutralized with ammonia and the tests with ferric chloride and gelatin repeated with the same results.

Further attempts were made to learn of the function of the lime in the detannation of the cinchona bark. After treating the dregs with HCl and filtering the aqueous solution, it was refluxed for 4 hours. For comparison a decoction of cinchona bark was filtered, the same quantity of HCl added and refluxed in the same manner. In the case of the bark, cinchona red was obtained and identified by precipitation as the barium compound, but in the case of the detannated dregs no cinchona red was obtained. The experiment in the latter case was repeated without treating the dregs with HCl and the same results were obtained.

PREPARATION OF TINCTURE OF CINCHONA U. S. P. X, AND DETANNATED TINCTURE OF CINCHONA.

III. A liter of each of the tinctures was prepared from red cinchona bark (18) which assayed 6.37 per cent and 6.82 per cent (19), respectively, of total alkaloids (20) in as many assays by the U. S. P. X method.

The following data were determined on each tincture employing the methods as outlined in the U. S. P. X.

	U. S. P. Tincture.	Detannated Tincture (21).
Sp. gr. 20°	0.9125	0.8928
Extractive	32.30%	2.03%
Alkaloids	1.15% (22)	1.01% (23)
Test with $FeCl_3$	Tannins present	No tannins present

BIBLIOGRAPHY.

(1) *Am. J. Pharm.*, 38 (1866), 411.
(2) *Pharm. Rund.*, 4 (1886), 197. (3) *Ibid.*, 4 (1886), 270.
(4) *Drugg. Circ.*, 3 (1887), 102.
(5) *Pharm. Rund.*, 6 (1888), 160.
(6) *Suedd. Apoth.-Ztg.* (1903); from *Pharm. Ztg.*, 48 (1903), 823.
(7) Villavecchia, "Applied Analytical Chemistry," 2 (1918), 338.
(8) "The properties and uses of cinchona correspond, proportionally in its strength with those of the following alkaloids (quinine, quinidine, cinchonine, cinchonidine). The cinchotannic acid being sufficient to impart astringency that is wanting in the alkaloids," Rusby, Bliss, Ballard, "Properties and Uses of Drugs," page 440.
(9) "While the precipitate consists largely of inert material, yet it carries down with it some of the active alkaloids and so weakens the preparation." Scoville, *Proc. Tercentenary of Cinchona* (1931), 214.
(10) JOUR. A. PH. A., 22 (1933), 641. (11) *Ibid.*
(12) Solubility of cinchona alkaloids at 25°.

1 Gm. quinine dissolves in 0.8 cc. ethyl alcohol.
1 Gm. quinidine dissolves in 32 cc. ethyl alcohol.
1 Gm. cinchonine dissolves in 120 cc. ethyl alcohol.
1 Gm. cinchonidine dissolves in 20 cc. ethyl alcohol.
(Merck's Index, 4th Edition.)

(13) The total extractive of the five fractions was 3.11 per cent. (See I.)
(14) Two assays gave the same results.
(15) No. 1—1.12%; No. 2—1.26%.
(16) No. 1—0.92%; No. 2—0.93%.
(17) This was more alkaline than 1.
(18) The tannin content of this bark was 2.3% and 2.5%, respectively, in as many assays, Villavecchia, "Applied Analytical Chemistry," 2 (1918), 338. The dregs showed a content of 0.31% and 0.42% tannins in as many assays.
(19) This corresponds to an average total alkaloidal content of 13.19 Gm. in the 200 Gm. of cinchona used in the percolation.
(20) Alkaloidal content of lime-treated dregs—0.37%. Alkaloidal content of U. S. P. X dregs—0.09%.
(21) This was prepared by the use of an excess of calcium hydroxide, mixed intimately with the bark and percolated. This tincture proved to be far more palatable than the U. S. P. X tincture.
(22) The average of three assays, *viz.:* 1.16%, 1.22% and 1.09%, respectively. The alkaloidal content of the liter of tincture is 11.50 Gm.
(23) The average of three assays, *viz.:* 1.02%, 0.99% and 1.03%, respectively. The total alkaloidal content of the liter of tincture is 10.10 Gm.