A CHEMICO-PHARMACEUTICAL STUDY OF CINCHONA

A thesis submitted to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

by

Justin Lawrence Powers

Date August 3 ...., 1935.

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The author wishes to express his appreciation to Professor Edward Kremers for suggesting this problem, and for his constant aid and advice while the work was in progress. Working under his direction during the past year has been a highly esteemed privilege, a great pleasure, and an inspiration.

To Professor W. O. Richtmann the author is deeply indebted for aid in obtaining early references relating to this problem, and for an introduction to certain literature of pharmacognosy and pharmacy with which he would not have otherwise become familiar.

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ERRATA

P. 3, line 9, for "will be" read "are".

P. 10, line 20, for "both" read "each".

P. 83, line 8, for "U. S. P." read "U. S. P. X".

P. 84, line 6, for "U. S. P." read "U. S. P. X".

P. 84, line 15, for "U. S. P." read "U. S. P. X".

P. 86, line 11, for "absorbtion" read "adsorption".

P. 87, line 5, for "indicated" read "given".

P. 118, line 3, for "Cinchona nova" read "Cinchona nova, Linne".

P. 128, line 8, for "Cinchona officinalis" read "Cinchona officinalis, Linne".

P. 129, line 15, for "liter" read "1 liter".

INTRODUCTION

A galenical and chemical study of cinchona was undertaken with the hope that it might result in a contribution to an understanding of the mechanism of the process of percolation. An investigation having the same aim was carried out by Milton Wruble[1] in this laboratory between 1930 and 1933, the results of which were published in 1933.[2] Wruble, working with cinchona, reported certain surprising anomalies which were observed during its percolation with alcohol. Cinchona was chosen by Wruble for his studies because the alkaloidal content of the drug and of its galenical preparations could be determined with a high degree of accuracy, and because of the problems presented in connection with the stability of the tincture and fluidextract of the drug. Inasmuch as the instability of these two preparations was known to be due, in part at least, to little understood changes of cinchotannic acid extracted along with the cinchona alkaloids by the usual methods, attention was given by Wruble to the preparation of a detannated tincture.[3]

For the first part of this investigation it was considered advisable to repeat Wruble's percolation experiments with a cinchona from a different source and having a different alkaloidal content. In this way his results, insofar as cinchona is concerned, could be verified, or if there were very wide deviations from his observations, these differences could be pointed out. As an aid to a more complete interpretation and explanation of the anomalies observed in the percolation of cinchona, it was thought that a chemical examination

of the constituents of cinchona, exclusive of the alkaloids, might prove helpful. Particularly was an examination of cinchotannic acid and cinchona red deemed advisable because of the role of these constituents in causing precipitation in the tincture and fluidextracts of cinchona for long periods of time after being prepared. This dissertation, which is a report of these studies, is accordingly for convenience divided into two main parts, a galenical and a chemical. In the galenical part will be included the results obtained in the investigations devoted for the most part to the percolation and extraction of cinchona. In the chemical part the results of a laboratory investigation of some of the minor constituents of cinchona are presented.

1) Dissertation, University of Wisconsin, (1933).
2) Jour. Am. Ph. Assn., 22, (1933), p. 643.
3) Ibid., 23, (1934), p. 208.

GALENICAL PART

Drug Used. The cinchona* used in this study was supplied through the courtesy of Parke, Davis and Company, Detroit, Michigan, from their regular stock, and coarsely ground by them to an approximately No. 20 powder, especially for this work.

Alkaloidal Content of Drug. The total alkaloids of cinchona were determined according to the official method.[1] The data used together with the results obtained are tabulated below.

Sample	Weight	Aliquot part used representing:	Wt. alkaloidal residue.	Per cent. alkaloids
1	5 grams	4 grams	0.3628 grams	9.07
2	5 grams	4 grams	0.3672 grams	9.18
3	5 grams	4 grams	0.3650 grams	9.13
			Average	9.13

The alkaloidal content of this drug is much higher than that found in cinchona purchased on the open market. In order to comply with the U. S. P. X requirements, cinchona must contain not less than 5 per cent of total alkaloids. The cinchona used by Wruble[2] in his percolation experiments, according to his assay, contained only 4.43 per cent of total alkaloids. It is thus seen that for comparative percolation studies a drug much richer in alkaloidal content than that used by Wruble was employed.

* The writer wishes to express his appreciation to Mr. F.O. Taylor, Chief Chemist of Parke, Davis and Company, who was instrumental in having a generous supply of cinchona furnished for this investigation.

Tannin Content of Drug. The percentage of tannin in the cinchona used in these studies was determined according to a method described by Villavecchia[3]. Three samples were analyzed for tannin content by this method, with the following results: 3.42 per cent, 3.65 per cent, and 3.72 per cent.

Moisture Content of Drug. The moisture content was determined by the xylene method[4]. Each of two 25 gram samples of powdered cinchona were boiled under a reflux condenser with 100 cc. of xylene. Each sample yielded 2.5 cc. of water which represented 10 per cent of moisture in the powdered drug.

Extraction of Powdered Cinchona with Successive Solvents. Two 50 gram samples of cinchona were extracted by means of a Soxhlet extraction apparatus until exhausted by each of the following solvents in the order named: petroleum ether, ether, chloroform, and alcohol. Between each extraction the drug was thoroughly dried. The solvent in each case was allowed to evaporate spontaneously at room temperature, but in no single instance did crystalline material separate. The dregs left after the extraction by alcohol were transferred to a beaker and extracted successively by heating with the following solvents in the order named: water, a 0.5 per cent aqueous solution of hydrochloric acid, and finally by a 0.5 per cent aqueous solution of sodium hydroxide. These solvents were evaporated at a temperature of 60°, the last two after neutralization. The residues after being dried to constant weight were weighed.

	Sample 1		Sample 2	
	Wt. Ext. (grams)	Per cent	Wt. Ext. (grams)	Per cent
Petroleum ether	0.4258	0.85	0.3920	0.79
Ether	1.3482	2.70	1.3452	2.69
Chloroform	1.0128	2.03	0.9088	1.82
Alcohol	8.2508	16.52	8.0616	16.13
Water	1.0840	2.17	1.0724	2.14
HCl 0.5 per cent	6.2200	12.44	6.1750	12.35
NaOH 0.5 per cent	5.7752	11.45	5.8454	11.68
Dregs	18.3500	36.70	18.9500	37.90
Moisture	5.0000	10.00	5.0000	10.00
Totals	47.4668	94.86	47.7504	95.50

The various extracts were examined superficially. The petroleum ether extract was green in color, of a fat-like nature, and possessed a characteristic aromatic odor. The ether extract upon thorough drying could be pulverized. It was found to consist of a mixture, the greater part of which was soluble in dilute hydrochloric acid. The addition of Mayer's reagent to hydrochloric acid solutions produced precipitates indicative of the presence of alkaloids. Both alkaloids and tannin were found in the chloroform, alcohol, water, and dilute hydrochloric acid extracts.

Percolation Experiments. It was mentioned in the introduction that Wruble's [27] experiments which had been designed to study the mechanism of the process of percolation had been repeated. Reasons were also given for the desirability of so doing. Wruble attacked the problem by attempting to learn something concerning the way in which the menstruum functions as it traverses downward through a column of drug contained in a percolator. The ideal way to have done this would have been to segment the drug contained in a percolator into a definite number of equal layers and draw off samples of the percolate at the bottom of each segment. This, however, could not be accomplished in a satisfactory manner. The technique finally developed by Wruble involved the use of a number of percolators, each representing an hypothetical segment of a large percolator. The quantity of drug in each percolator (hypothetical segment) was not the same, but each differed from the preceding one by a regular decrement. In this way it was possible to reserve a portion of the percolate from each fraction collected from all of the hypothetical segments, and still approximate in all essentials the conditions which should prevail in a large percolator.

Procedure. This experimental procedure used by the writer was adapted from the published description of the method used by Wruble. Five and one half kilograms of cinchona were divided into ten parts. The first portion weighed 1000 grams, the second weighed 900 grams, the third weighed 800 grams, and so on down to the tenth, which weighed 100

grams. The 1000 gram portion of drug was moistened with 750 cc. of 95 per cent alcohol, and placed in a covered container for six hours. It was then packed in a percolator, enough alcohol was then added to saturate the drug, the percolator was covered, and the drug was allowed to macerate during twenty four hours. Percolation, the rate of flow of the percolate carefully controlled, was then started. The menstruum used throughout for this first 1000 gram portion, until 10 liters of tincture had been collected, was 95 per cent alcohol. Each one of the other portions of drug was moistened with a proportionate quantity of alcohol, and treated in an identical manner during thirty hours before the percolation was started. Alcohol, however, was not used to percolate these portions, but instead, the tincture from the preceding percolator was employed for this purpose. Thus, from the percolator containing 1000 grams of cinchona, a first portion of 1 liter of tincture was drawn off, 100 cc. were reserved, and 900 cc. were used for the percolation of the 900 grams of drug which had been macerated with alcohol in the second percolator. In like manner, a first portion of 900 cc. of percolate was collected from the second percolator, of which 100 cc. were reserved, and 800 cc. were used for the percolation of 800 grams of alcohol saturated drug contained in a third percolator. This procedure was carried out successively with the other portions of drug, in such a way that from the tenth and final percolator, containing 100 grams of drug, only 100 cc. of percolate were collected in each

series. Ten series of extractions were carried out successively on the same drug. Thus, it is seen that from 5.5 kilograms of drug a total of 10 liters of tincture were collected in 100 cc. portions.

The 100 samples of tincture collected were, with the exception of the determination of the hydrogen ion concentration, subjected to the same examination as those which had been prepared by Wruble. The specific gravity of each was determined by means of a pycnometer at 20°. The extractive was determined according to the method of the U. S. P. X.[5] The alkaloidal content was determined according to the official method.[1] The results of these various determinations are tabulated for each series in Table No. 1. A comparison of the results of specific gravity, extractive, and alkaloidal content determinations reported by Wruble, and those obtained by the writer are presented in Table No. 2. Following the tabulation for each series, the same material is presented graphically in order to show to a better advantage not only the comparison of results, but also the irregularities obtained by both of us.

In Table No. 3 are presented averages for each series of specific gravity, extractive, and alkaloidal content determinations. This table is also followed by a graphical representation of the same data. It was thought that such a presentation would more accurately show the great similarity in results obtained with two different samples of cinchona, than the somewhat enlarged graphs accompanying Table No. 2. This proved to be true. In Table No. 4 are

presented averages for each percolator, and this table is also followed by graphs representing the same data.

Discussion. An examination of Table 2, in which are presented comparisons of results obtained by Wruble and by the writer in a series of percolation experiments with cinchona from different sources and of widely varying alkaloidal content, shows that while the results are not identical there is still a surprising agreement between them. The graphs accompanying the table also illustrate this point. Wruble's results and his discussion of them were available when this work was started, and as many as possible of his suggested sources of errors were eliminated. It is perhaps best to quote from Wruble's report and point out any differences in the procedure, conditions, or observations made by the writer.

Following the tabulation of his results, Wruble makes the following statement:

"From these many results it is to be noted that certain anomalies are present. In every series the maximum point is reached followed by a decrease. Such results are not readily explainable. No doubt, however, certain surface phenomena, such as adsorption, absorption and perhaps others of which we know little, are responsible in a great measure for this anomaly."

The anomalies noted by Wruble were also observed by the writer. It should be pointed out, however, that in at least one series a maximum point was not reached and followed by a decrease. In series No. 4 it will be seen that the last point reached was the high point. Inasmuch as in this particular series the same exception was met with by both Wruble and the writer, it seems worth pointing out, even though no

explanation can be offered.

Concerning the possible sources of error due to difficulties in maintaining a constant rate of flow of percolate, and the influence of varying intervening maceration periods, Wruble wrote as follows:

"Errors.- These experiments presented a number of difficulties in technique some of which were only realized during the procedure. It must be admitted that at their best they represent only an approach to ideal conditions. While as many of the variables in percolation were controlled as nearly as possible to be identical in each of the ten percolators, it was found quite impossible to maintain them at comparative rates of flow for any length of time. The rates would generally decrease after being adjusted, sometimes increase and in a number of instances stop flowing altogether.

Where the rate decreased it was adjusted to run faster so that the final volumes in all percolators would be collected at comparative rates and if it increased the percolator was allowed to run slower in like manner. If a percolator stopped and was not noticed soon thereafter (this did not occur frequently), the rate at which it was set was determined by the judgment developed in carrying out the rather large number of extractions.

Percolators were started at approximately the same time each morning and stopped at a fixed time every day. Experimentation was not interrupted at any other time but was carried out seven days a week. Here again, the ideal manner of conducting such extractions would have been to continue percolation from the very beginning without a single interruption. While we may assume that the intervening maceration periods, having been the same in each case would tend to equalize this error, it must not be forgotten that during these macerations numerous changes took place. (See Series IX and X)"

The writer used a glass tube of 4 mm. diameter as an outlet from the percolator. This was connected by means of a rubber tube to another glass tube with an external diameter of 3 mm. By means of a screw clamp on the rubber tube no difficulty was experienced in regulating the rate of flow at 10 drops per minute, and maintaining it at that rate. In fact, it was frequently found to be unnecessary to make

any adjustments during a period of 14 hours. Since the number of hours required by the writer to collect a given volume of tincture was invariably less than that required by Wruble, it seems possible that he might have used a delivery tube so small that it frequently became clogged. In the experiments carried out by the writer, the percolators were started at 8:00 A.M., and stopped at 10:00 P.M. each day of the week from the beginning until the end of the ten series. Regardless of efforts to overcome suggested sources of error, approximately the same irregularities were observed as those reported by Wruble. The reference to Series IX and X in the above quotation relates to the fact that a period of three months elapsed between the completion of the first eight series of experiments and those mentioned. This period of maceration did not apparently produce any very marked changes in the results obtained, as a comparison of the accompanying graphs following the tabulation of those series shows.

Wruble considered that fluctuations in temperature might have contributed to the production of the irregularities reported by him. Concerning this possibility he wrote as follows:

"Throughout the many months in which these experiments were conducted the changes in temperature must have affected the solubility of the constituents more or less. Some of the latter series were made in the warmer months of the year, the earlier in the fall months while those in between during the winter months. The room temperature during these various periods of the year must of necessity have varied somewhat.

In spite of the errors that have been enumerated it is believed that inasmuch as the large number of extractions made show strikingly the repetition that has already been pointed out, we can feel justified in believing that such an anomaly exists in the case of cinchona."

The experiments carried out by the writer were started late in October, 1933, and completed the latter part of April, 1934. During this period the temperature was maintained between 68° and 74° F. at all times. The results obtained indicate that the fluctuations of temperature within reasonable limits have very little influence on the extraction of cinchona, inasmuch as Wruble's experiments were doubtlessly subjected to wider variations than those of the writer.

From either Table III in which are shown averages for each series, or from Table IV in which are shown averages for each percolator, it was possible to compute readily the averages for the entire series. The results of such computations are tabulated below.

Average Specific Gravity		Average Per cent of Extractive		Average Per cent of Alkaloids	
Wruble	Powers	Wruble	Powers	Wruble	Powers
0.8366	0.8399	5.42	6.00	0.75	1.24

The above values indicate what the specific gravity, per cent of extractive, and per cent of alkaloids would be for a tincture prepared by combining the hundred samples of percolate which were reserved in 100 cc. portions. These values, when considered in connection with the alkaloidal content of the drugs used by Wruble and by the writer, may be used to arrive at some interesting data. Some pertinent values in this connection are given below. (See next page)

	Wt. of Drug.	Per cent of Alkaloids.	Wt. of available Alkaloids.
Wruble	5500 grams	4.43	242.65 grams
Powers	5500 grams	9.13	502.15 grams

It is now possible to calculate the weight of alkaloids extracted from the drug and contained in the 10 liters of tincture collected, and to determine what per cent of total alkaloids available in the drug this weight represents.

	Total wt. tr. collected.	Total wt. alkaloids in tr.	Per cent of available alkaloids extracted.
Wruble	8366 grams	62.75 grams	25.85
Powers	8399 grams	104.15 grams	20.74

The criterion of quality of the galenical preparations of cinchona such as the fluidextract and tincture is the alkaloidal content. In a series of experiments such as those which have been described, it might have been expected that the alcoholic tinctures prepared from a drug containing 9.13 per cent of total alkaloids would have shown a much higher alkaloidal content than those prepared from a drug containing only 4.43 per cent. It was shown, however, that while the ratio of alkaloidal content of the two drugs was approximately 1 : 2, the ratio of the average alkaloidal content of the corresponding tinctures was 75 : 125. It was also determined that alcohol extracted 25.85 per cent of the total alkaloids from the drug having the lower alkaloidal content, and 20.74 per cent from the drug containing the higher percentage of alkaloids.

The alkaloids of cinchona occur in part free, and in part combined with cinchotannic acid. The cinchotannates of the alkaloids are much less soluble in alcohol than are the free alakloids. This slight solubility of the cinchotannates of the alkaloids in alcohol presents an explanation for the comparatively small percentage of alkaloids extracted during these experiments. It is not possible, however, to find so ready an explanation for the fluctuations in alkaloidal content which were observed at different intervals in the menstruum as it passed through a column of the drug contained in a percolator. The suggestion was made by Wruble that this anomaly was due to surface phenomena such as adsorption and absorption. Cinchona contains, in addition to alkaloids, cinchotannic acid, cinchona red, quinic acid, and possibly uncombined quinovic acid, any one of which might, by the formation of insoluble combinations with the alkaloids, contribute to the irregularities observed during the percolation of cinchona. The fats and related constituents should not be ignored in attempting to formulate an explanation. Any explanations that might be offered are still open to proof. However, the results obtained by the treatment of cinchona with calcium hydroxide or milk of lime previous to extraction with alcohol indicate the plausibility of the assumption that the constituents mentioned do influence to a marked extent the efficiency of alcohol as a menstruum for the extraction of the alkaloids of the drug. This phase of the subject will be discussed in more detail in connection with the detannated

tincture and fluidextract.

Table I

Series I

Amt. of Drug Gms.	Rate Hours	Sp. Gr. 20°	Extractive Wt. of Sample	Extractive Wt. of Extractive	Extractive Percent	Alkaloidal Content Wt. of Sample	Alkaloidal Content Wt. of Alkaloid	Alkaloidal Content Percent
1000	52½	0.8583	8.583	0.8106	9.44	6.8684	0.1210	1.76
900	49	0.8598	8.598	0.9944	11.51	6.8784	0.1704	2.33
800	42 3/4	0.8549	8.549	0.8406	9.93	6.8392	0.1604	2.32
700	36	0.8698	8.698	0.9722	11.18	6.9584	0.1514	2.18
600	30½	0.8608	8.608	0.8742	10.15	6.8864	0.1516	2.20
500	25	0.8628	8.628	0.8901	10.20	6.9024	0.1462	2.12
400	21½	0.8629	8.629	0.9304	10.78	6.9032	0.1488	2.16
300	16 3/4	0.8589	8.589	0.8698	10.12	6.8712	0.1398	2.02
200	11½	0.8619	8.619	0.8750	10.15	6.8952	0.1406	2.04
100	6¼	0.8616	8.616	0.8708	1022	6.9828	0.1402	2.03

Series II

Amt. of Drug Gms.	Rate Hours	Sp. Gr. 20°	Extractive Wt. of Sample	Extractive Wt. of Extractive	Extractive Percent	Alkaloidal Content Wt. of Sample	Alkaloidal Content Wt. of Alkaloid	Alkaloidal Content Percent
1000	50½	0.8286	8.2860	0.2836	3.43	6.6288	0.0624	0.94
900	47	0.8371	8.3710	0.4846	5.78	6.6968	0.0824	1.23
800	42	0.8367	8.3670	0.4408	5.26	6.6936	0.0832	1.24
700	36 3/4	0.8408	8.4080	0.4962	5.90	6.7264	0.0822	1.22
600	30½	0.8410	8.4100	0.4974	5.90	6.7280	0.0812	1.21
500	26½	0.8448	8.4480	0.4900	5.80	6.7584	0.0832	1.23
400	22	0.8403	8.4030	0.4910	5.84	6.7224	0.0788	1.17
300	16½	0.8377	8.3770	0.4366	5.21	6.7016	0.0742	1.11
200	11 3/4	0.8338	8.3380	0.3956	4.75	6.6704	0.0732	1.08
100	5 3/4	0.8348	8.3480	0.3906	4.68	6.6784	0.0724	1.08

Series III

Amt. of Drug Gms.	Rate Hours	Sp. Gr. 20°	Extractive Wt. of Sample	Extractive Wt. of Extractive	Extractive Percent	Alkaloidal Content Wt. of Sample	Alkaloidal Content Wt. of Alkaloid	Alkaloidal Content Percent
1000	58¼	0.8232	8.2320	0.2160	2.62	6.5856	0.0450	0.68
900	49	0.8446	8.4460	0.5200	6.18	6.7568	0.0846	1.25
800	41½	0.8477	8.4770	0.4988	5.88	6.7816	0.0926	1.37
700	36 3/4	0.8450	8.4500	0.5832	6.90	6.7600	0.0904	1.34
600	30¼	0.8430	8.4300	0.5569	6.58	6.7440	0.0870	1.21
500	26	0.8448	8.4480	0.5846	6.92	6.7584	0.0892	1.32
400	22	0.8477	8.4770	0.5876	6.91	6.7816	0.0948	1.39
300	17	0.8416	8.4160	0.5426	6.45	6.7328	0.0792	1.08
200	12¼	0.8427	8.4270	0.6014	7.13	6.7416	0.0840	1.27
100	5½	0.8382	8.3820	0.5096	6.08	6.7056	0.0736	1.10

Series IV

Amt. of Drug Gms.	Rate Hours	Sp. Gr. 20°	Extractive Wt. of Sample	Extractive Wt. of Extractive	Extractive Percent	Alkaloidal Content Wt. of Sample	Alkaloidal Content Wt. of Alkaloid	Alkaloidal Content Percent
1000	49½	0.8207	8.2070	0.1674	2.04	6.5656	0.0370	0.56
900	47½	0.8367	8.3670	0.4396	5.25	6.6936	0.0696	1.04
800	40¼	0.8419	8.4190	0.4810	5.71	6.7352	0.0820	1.22
700	36¼	0.8384	8.3840	0.4888	5.82	6.7072	0.0792	1.18
600	31½	0.8400	8.4000	0.4928	5.85	6.7200	0.0810	1.21
500	26	0.8411	8.4110	0.5352	6.24	6.7288	0.0824	1.24
400	21¼	0.8424	8.4240	0.5602	6.63	6.7392	0.0864	1.25
300	16¼	0.8401	8.4010	0.5516	6.56	6.7208	0.0798	1.17
200	10 3/4	0.8425	8.4250	0.6024	7.15	6.7400	0.0906	1.34
100	6	0.8459	8.4590	0.6338	7.49	6.7672	0.0986	1.42

Series V

Amt. of Drug Gms.	Rate Hours	Sp. Gr. 20°	Extractive Wt. of Sample	Extractive Wt. of Extractive	Extractive Percent	Alkaloidal Content Wt. of Sample	Alkaloidal Content Wt. of Alkaloid	Alkaloidal Content Percent
1000	55½	0.8188	8.1880	0.1168	1.42	6.5504	0.0308	0.45
900	46½	0.8272	8.2720	0.3304	3.99	6.6176	0.0580	0.87
800	43	0.8423	8.4230	0.5492	6.52	6.7384	0.0754	1.12
700	36¾	0.8451	8.4510	0.5746	6.78	6.7608	0.0902	1.33
600	31½	0.8421	8.4210	0.6048	7.18	6.7368	0.0880	1.34
500	27	0.8429	8.4290	0.6488	7.69	6.7432	0.0928	1.37
400	22	0.8448	8.4480	0.6124	7.24	6.7584	0.0982	1.45
300	16½	0.8421	8.4210	0.5514	6.66	6.7368	0.0890	1.32
200	10¾	0.8417	8.4170	0.5708	6.78	6.7336	0.0932	1.37
100	5½	0.8420	8.4200	0.5778	6.86	6.7360	0.0924	1.37

Series VI

Amt. of Drug Gms.	Rate Hours	Sp. Gr. 20°	Extractive Wt. of Sample	Extractive Wt. of Extractive	Extractive Percent	Alkaloidal Content Wt. of Sample	Alkaloidal Content Wt. of Alkaloid	Alkaloidal Content Percent
1000	48½	0.8172	8.1720	0.1050	1.16	6.5376	0.0284	0.43
900	45 3/4	0.8250	8.2500	0.2460	2.97	6.6000	0.0550	0.83
800	41¼	0.8387	8.3870	0.4476	5.33	6.7096	0.0734	1.09
700	35½	0.8439	8.4390	0.5544	6.57	6.7512	0.0830	1.23
600	31¼	0.8408	8.4080	0.5376	6.39	6.7264	0.0830	1.27
500	26¼	0.8417	8.4170	0.5632	6.69	6.7336	0.0876	1.31
400	20 3/4	0.8434	8.4340	0.5602	6.64	6.7472	0.0954	1.41
300	15½	0.8444	8.4440	0.5684	6.73	6.7552	0.0978	1.43
200	11 3/4	0.8415	8.4150	0.5694	6.79	6.7320	0.0922	1.37
100	6 3/4	0.8427	8.4270	0.5796	6.86	6.7416	0.0960	1.42

Series VII

Amt. of Drug Gms.	Rate Hours	Sp. Gr. 20°	Extractive Wt. of Sample	Extractive Wt. of Extractive	Extractive Percent	Alkaloidal Content Wt. of Sample	Alkaloidal Content Wt. of Alkaloid	Alkaloidal Content Percent
1000	49¼	0.8191	8.1910	0.1144	1.27	6.5528	0.0306	0.45
900	45¼	0.8217	8.2170	0.1772	2.15	6.5396	0.0502	0.76
800	40	0.8329	8.3290	0.3098	3.72	6.6632	0.0560	0.84
700	35 3/4	0.8409	8.4090	0.4586	5.45	6.7272	0.0832	1.24
600	31¼	0.8421	8.4210	0.5702	6.77	6.7368	0.0862	1.26
500	24 3/4	0.8421	8.4210	0.5804	6.89	6.7368	0.0884	1.31
400	21½	0.8432	8.4320	0.5856	6.94	6.7456	0.0974	1.44
300	14 3/4	0.8441	8.4410	0.5844	6.92	6.7528	0.0890	1.31
200	12	0.8419	8.4190	0.5632	6.79	6.7352	0.0918	1.36
100	5¼	0.8420	8.4200	0.5652	6.71	6.7360	0.0934	1.39

Series VIII

Amt. of Drug Gms.	Rate Hours	Sp. Gr. 20°	Extractive Wt. of Sample	Extractive Wt. of Extractive	Extractive Percent	Alkaloidal Content Wt. of Sample	Alkaloidal Content Wt. of Alkaloid	Alkaloidal Content Percent
1000	48½	0.8168	8.1680	0.0846	1.05	6.5344	0.0286	0.43
900	50	0.8211	8.2110	0.1902	2.31	6.5688	0.0480	0.74
800	45	0.8264	8.2640	0.2744	3.32	6.6112	0.0488	0.71
700	35 3/4	0.8371	8.3710	0.4652	5.55	6.6968	0.0774	1.15
600	32	0.8449	8.4490	0.5646	6.68	6.7592	0.0902	1.33
500	25 3/4	0.8418	8.4180	0.5682	6.72	6.7344	0.0859	1.27
400	20½	0.8435	8.4350	0.5770	6.84	6.7480	0.0958	1.41
300	15 3/4	0.8441	8.4410	0.5740	6.80	6.7528	0.0908	1.34
200	10½	0.8436	8.4360	0.5648	6.69	6.7488	0.0932	1.38
100	6¼	0.8417	8.4170	0.5630	6.70	6.7336	0.0904	1.34

Series IX

Amt. of Drug Gms.	Rate Hours	Sp. Gr. 20°	Extractive Wt. of Sample	Extractive Wt. of Extractive	Extractive Percent	Alkaloidal Content Wt. of Sample	Alkaloidal Content Wt. of Alkaloid	Alkaloidal Content Percent
1000	50	0.8160	8.1600	0.0750	0.92	6.5280	0.0250	0.38
900	50½	0.8215	8.2150	0.1916	2.33	6.5720	0.0436	0.66
800	45½	0.8243	8.2430	0.2396	2.90	6.5944	0.0484	0.75
700	38	0.8331	8.3310	0.3892	4.67	6.6648	0.0682	1.02
600	32	0.8399	8.3990	0.4622	5.51	6.7192	0.0796	1.18
500	25½	0.8431	8.4310	0.6104	7.23	6.7448	0.0898	1.37
400	20 3/4	0.8427	8.4270	0.5882	6.99	6.7416	0.0916	1.36
300	17	0.8437	8.4370	0.5812	6.90	6.7496	0.0988	1.46
200	11	0.8440	8.4400	0.5756	6.82	6.7520	0.0984	1.47
100	5 3/4	0.8421	8.4210	0.5686	6.75	6.7368	0.0926	1.36

Series X

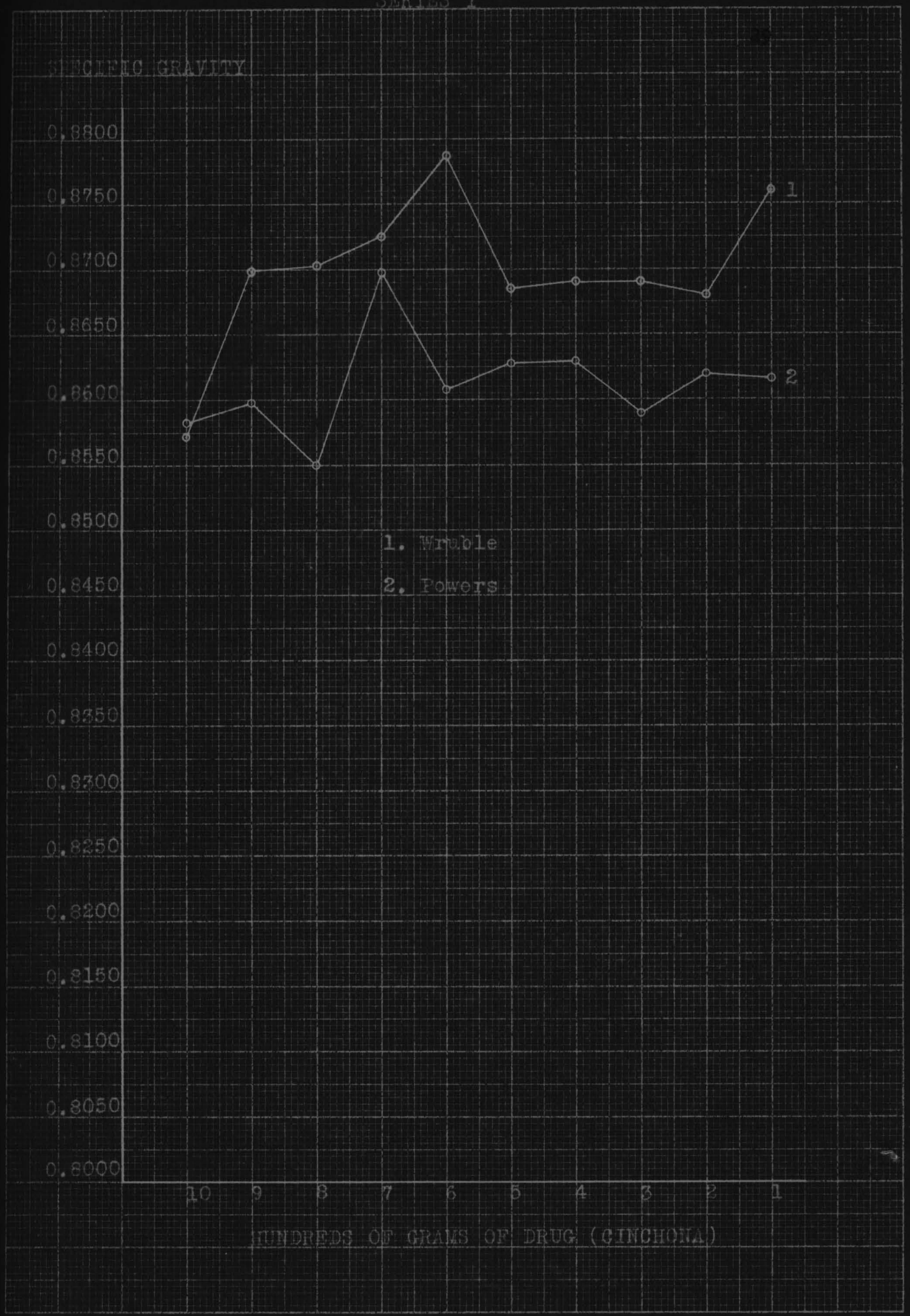
Amt. of Drug Gms.	Rate Hours	Sp. Gr. 20°	Extractive Wt. of Sample	Extractive Wt. of Extractive	Extractive Percent	Alkaloidal Content Wt. of Sample	Alkaloidal Content Wt. of Alkaloid	Alkaloidal Content Percent
1000	49	0.8177	8.1770	0.0618	0.76	6.5416	0.0186	0.28
900	45	0.8207	8.2070	0.1562	1.90	6.5656	0.0422	0.64
800	41½	0.8223	8.2230	0.2316	2.80	6.5784	0.0476	0.72
700	35½	0.8297	8.2970	0.3104	3.75	6.6376	0.0666	1.00
600	29 3/4	0.8357	8.3570	0.4316	5.28	6.6856	0.0734	1.09
500	24 3/4	0.8432	8.4320	0.5866	6.95	6.7456	0.0924	1.37
400	21	0.8407	8.4070	0.5616	6.68	6.7256	0.0854	1.27
300	15½	0.8432	8.4320	0.5822	6.90	6.7456	0.0914	1.35
200	9 3/4	0.8438	8.4380	0.5822	6.89	6.7504	0.0892	1.32
100	4½	0.8412	8.4120	0.5666	6.73	6.7306	0.0842	1.25

Table II

Series I

Amt. of Drug Gms.	Rate Hours		Sp. Gr. 20°		Percent of Extractive		Percent of Alkaloids	
	Wruble	Powers	Wruble	Powers	Wruble	Powers	Wruble	Powers
1000	78 3/4	52½	0.8572	0.8583	9.05	9.44	1.17	1.76
900	71¼	49	0.8699	0.8598	11.09	11.51	1.59	2.33
800	61½	42 3/4	0.8703	0.8549	12.50	9.93	1.58	2.32
700	52 3/4	36	0.8725	0.8698	13.00	11.18	1.63	2.18
600	48½	30½	0.8787	0.8608	14.50	10.15	1.63	2.20
500	41	25	0.8685	0.8628	13.20	10.20	1.54	2.12
400	34 3/4	21½	0.8691	0.8629	12.40	10.78	1.53	2.16
300	22½	16 3/4	0.8691	0.8589	12.65	10.12	1.41	2.02
200	13½	11¼	0.8681	0.8619	13.24	10.15	1.50	2.04
100	7 3/4	6¼	0.8761	0.8618	15.80	10.22	1.74	2.03

SERIES I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0.8600
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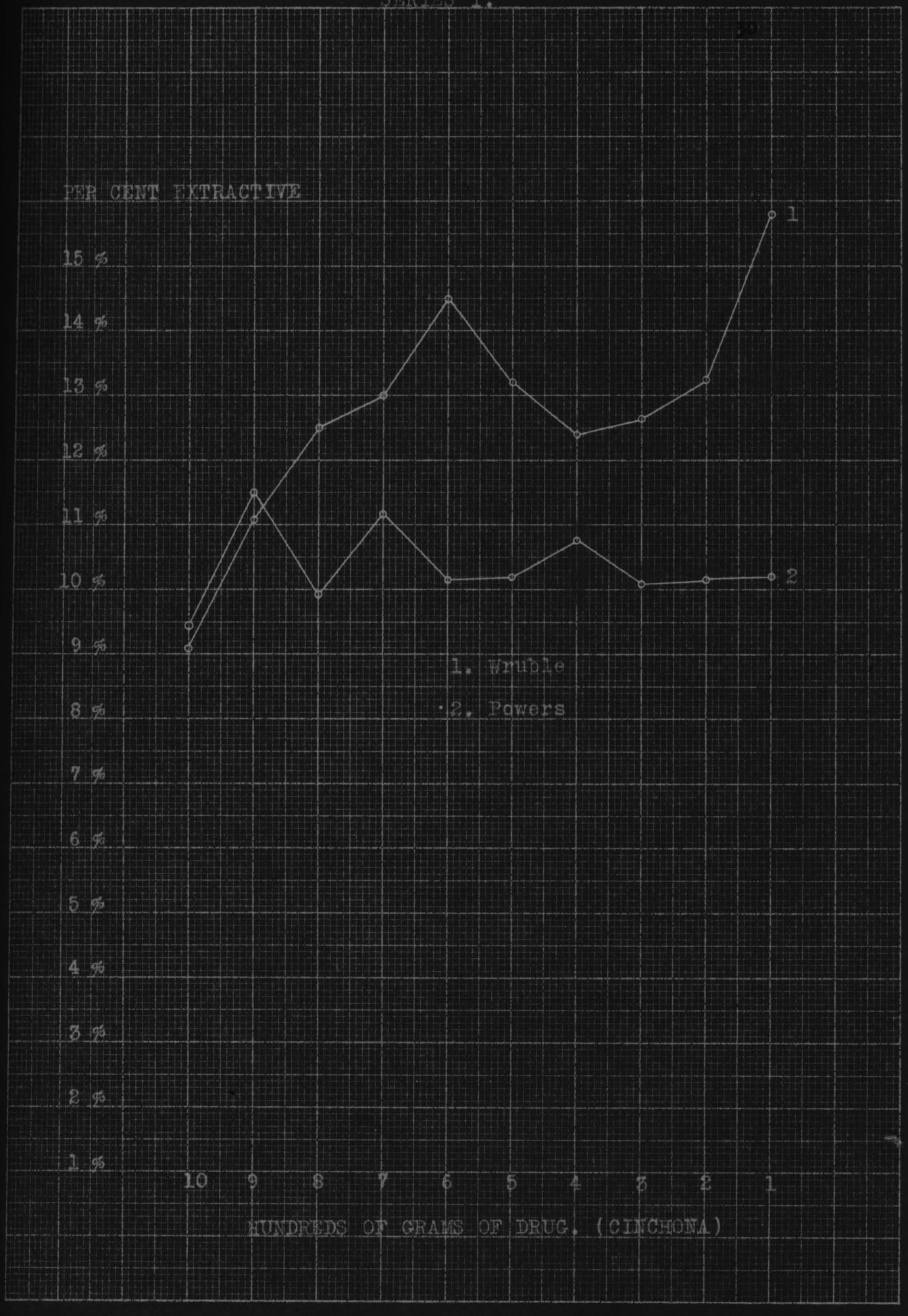
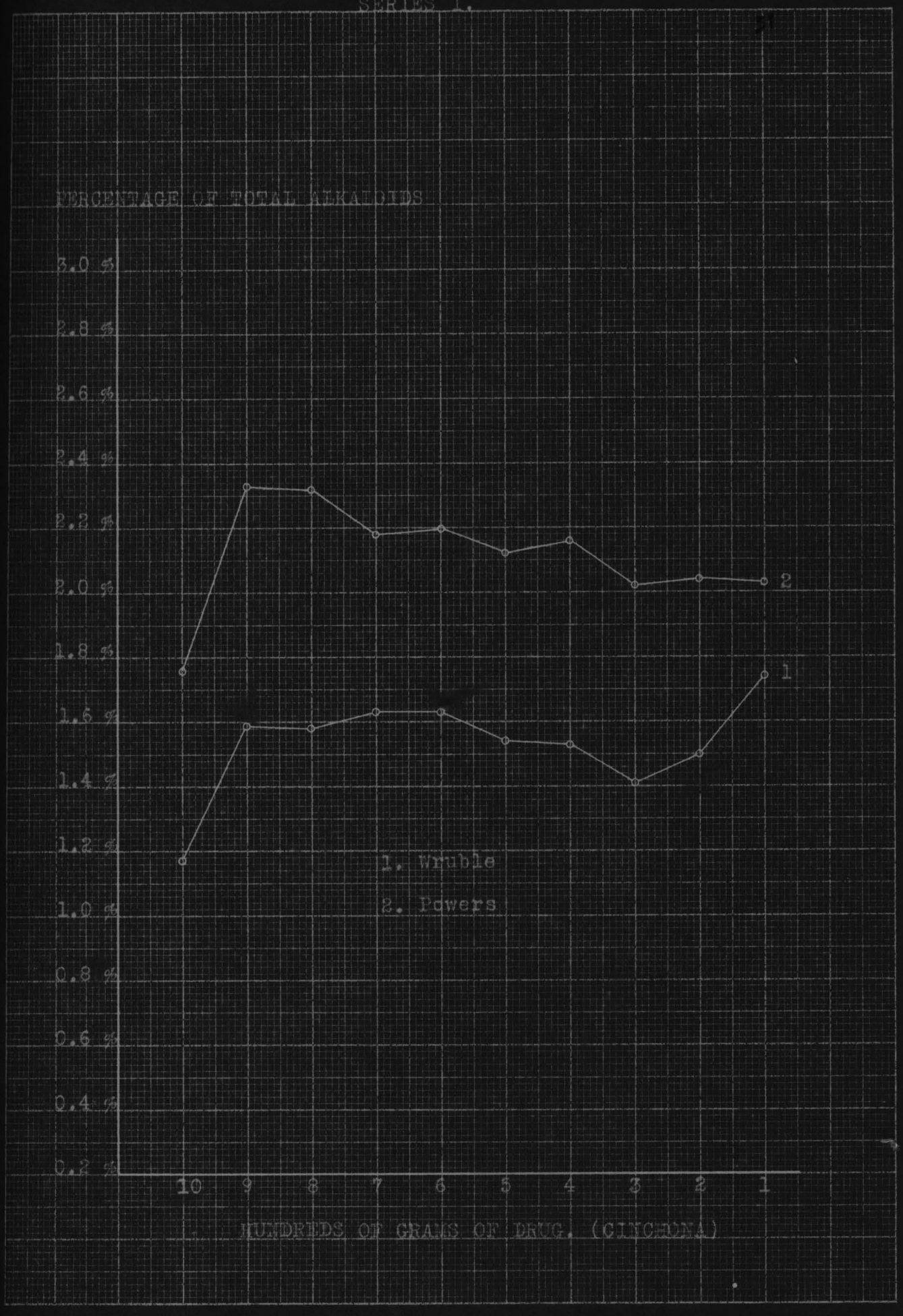
0.8500
0.8450
0.8400
0.8350
0.8300
0.8250
0.8200
0.8150
0.8100
0.8050
0.8000
1
2
1. Wrable
2. Powers
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAMS OF DRUG (CINCHONA)

SERIES I.

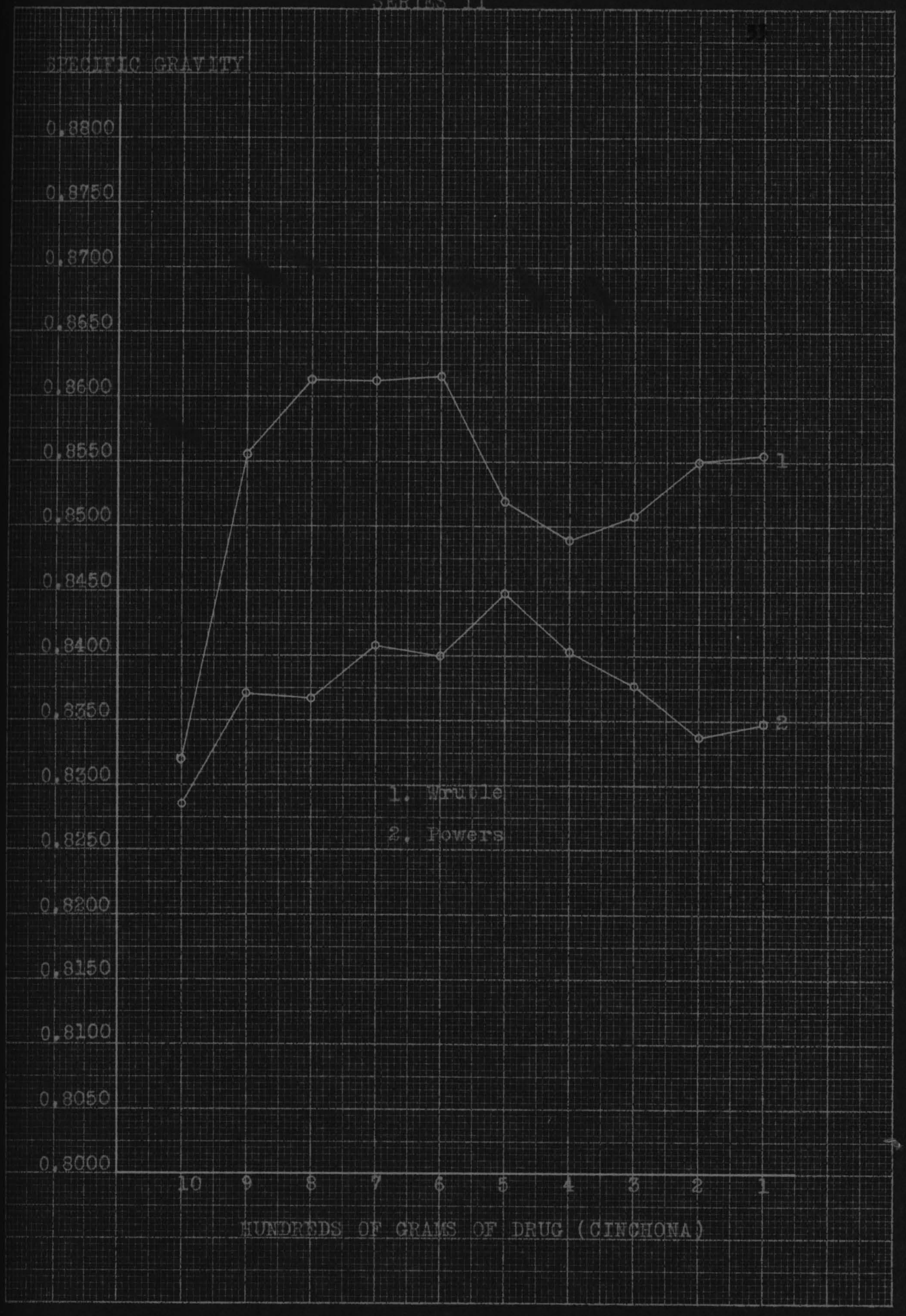

PER CENT EXTRACTIVE
15 %
14 %
13 %
12 %
11 %
10 %
9 %
8 %
7 %
6 %
5 %
4 %
3 %
2 %
1 %
1
2
1. Wruble
2. Powers
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAMS OF DRUG. (CINCHONA)

SERIES I.


PERCENTAGE OF TOTAL ALKALOIDS
3.0 %
2.8 %
2.6 %
2.4 %
2.2 %
2.0 %
1.8 %
1.6 %
1.4 %
1.2 %
1.0 %
0.8 %
0.6 %
0.4 %
0.2 %
2
1
1. Wruble
2. Powers
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAINS OF DRUG. (CINCHONA)

Series II

Amt. of Drug Gms.	Rate Hours		Sp. Gr. 20°		Percent of Extractive		Percent of Alkaloids	
	Wruble	Powers	Wruble	Powers	Wruble	Powers	Wruble	Powers
1000	77¼	50¼	0.8320	0.8286	3.72	3.43	0.60	0.94
900	72½	47	0.8555	0.8371	8.35	5.78	0.91	1.23
800	62 3/4	42	0.8613	0.8367	10.67	5.26	1.33	1.24
700	50½	36 3/4	0.8612	0.8408	10.06	5.90	1.24	1.22
600	47½	30¼	0.8616	0.8410	10.11	5.90	1.02	1.21
500	37 3/4	28¼	0.8519	0.8448	8.75	5.80	0.79	1.23
400	32¼	22	0.8489	0.8403	8.10	5.84	0.71	1.17
300	24	16½	0.8508	0.8377	8.75	5.21	0.93	1.11
200	10 3/4	11 3/4	0.8549	0.8338	9.40	4.75	0.82	1.08
100	6½	5 3/4	0.8555	0.8348	9.30	4.68	0.81	1.08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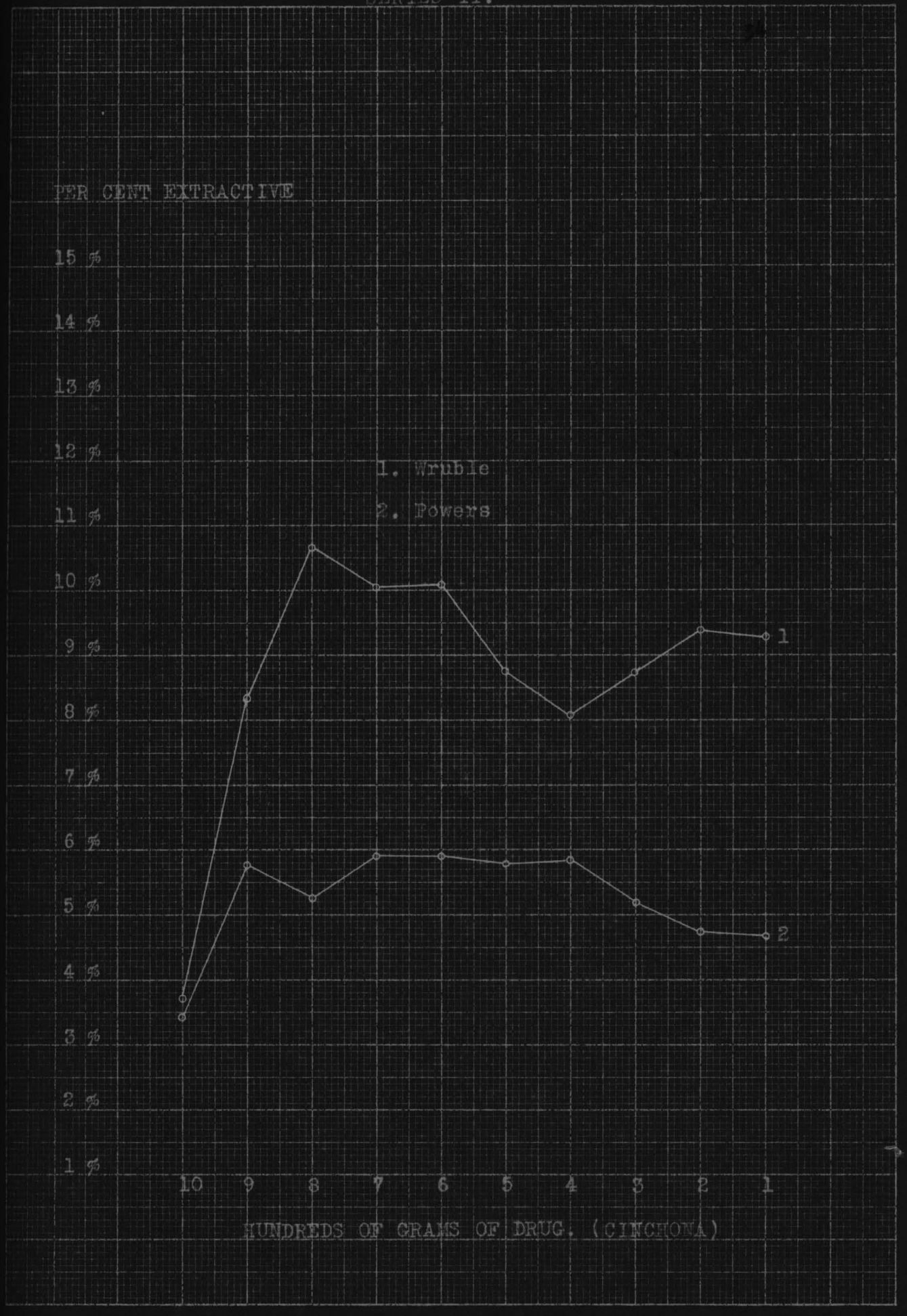
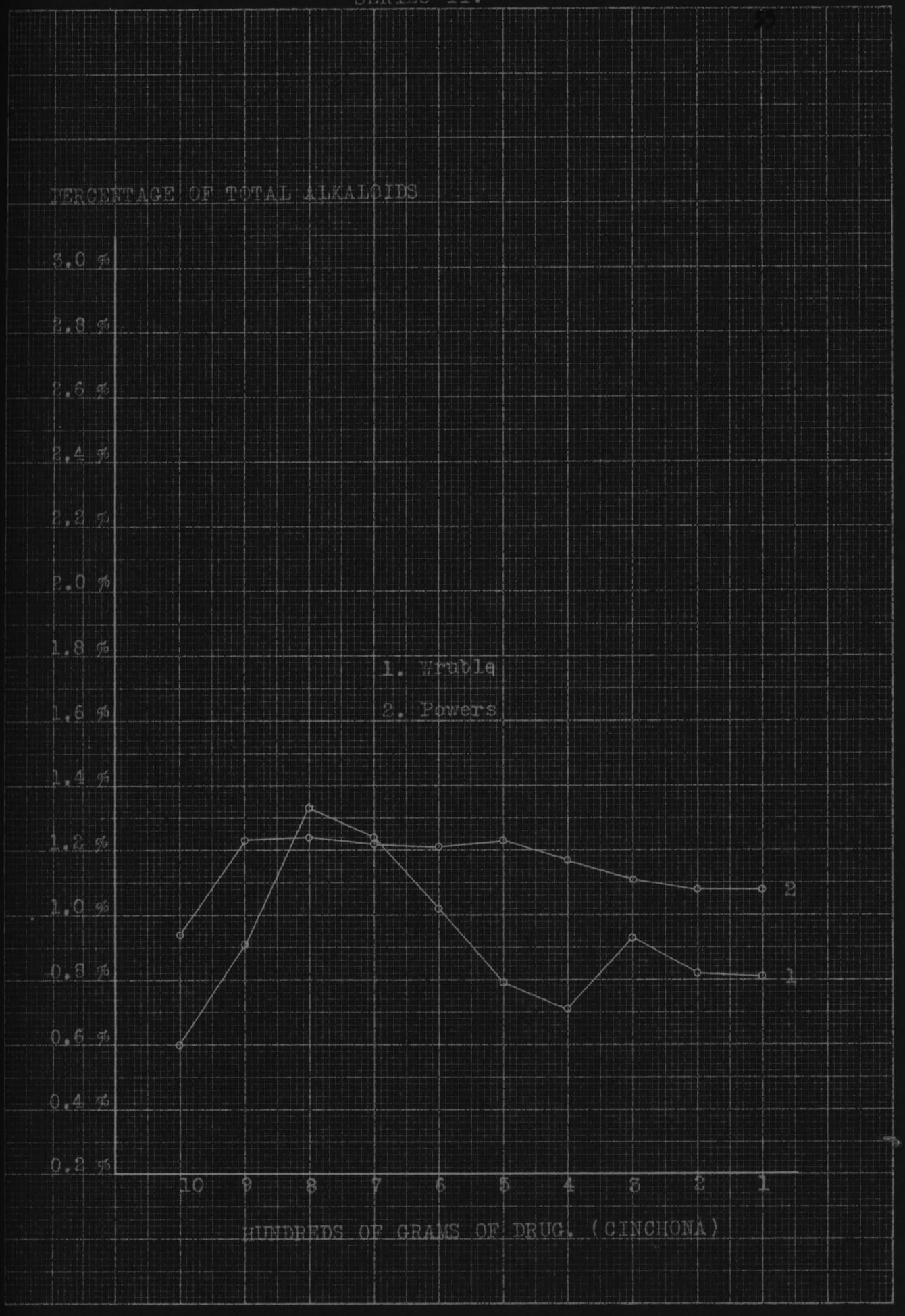
0.8600
0.8550
0.8500
0.8450
0.8400
0.8350
0.8300
0.8250
0.8200
0.8150
0.8100
0.8050
0.8000
1
2
1. Wruble
2. Powers
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAMS OF DRUG (CINCHONA)

SERIES II.


PER CENT EXTRACTIVE
15 %
14 %
13 %
12 %
11 %
10 %
9 %
8 %
7 %
6 %
5 %
4 %
3 %
2 %
1 %
1. Wruble
2. Powers
1
2
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAMS OF DRUG. (CINCHONA)

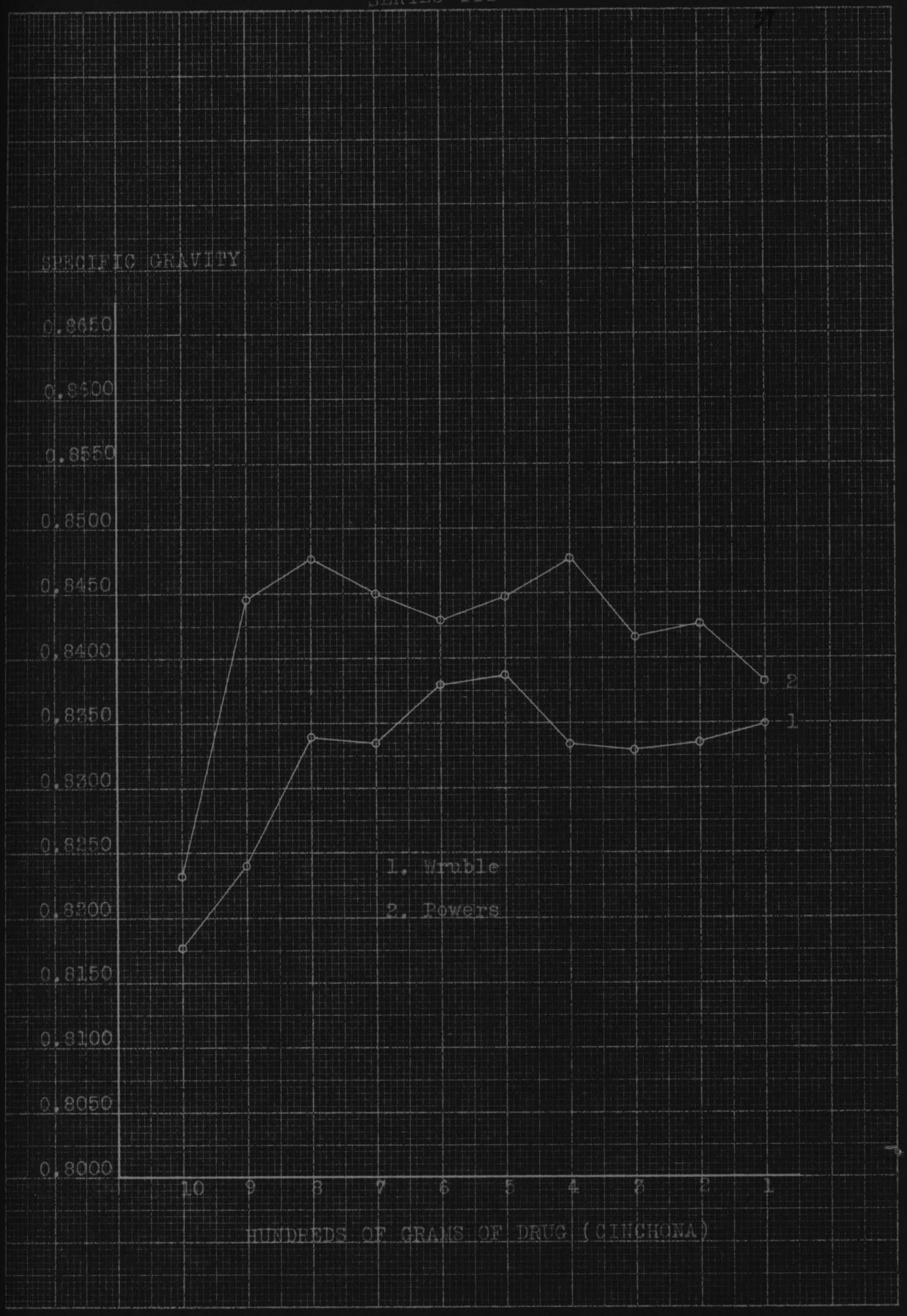
SERIES II.


PERCENTAGE OF TOTAL ALKALOIDS
3.0 %
2.8 %
2.6 %
2.4 %
2.2 %
2.0 %
1.8 %
1.6 %
1.4 %
1.2 %
1.0 %
0.8 %
0.6 %
0.4 %
0.2 %
1. Mrubla
2. Powers
2
1
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAMS OF DRUG. (CINCHONA)

Series III

Amt. of Drug Gms.	Rate Hours		Sp. Gr. 20°		Percent of Extractive		Percent of Alkaloids	
	Wruble	Powers	Wruble	Powers	Wruble	Powers	Wruble	Powers
1000	76¼	58½	0.8177	0.8232	2.05	2.62	0.39	0.68
900	70	49	0.8240	0.8446	3.10	6.18	0.62	1.25
800	58½	41½	0.8339	0.8477	5.23	5.98	0.91	1.37
700	51¼	36 3/4	0.8335	0.8450	5.88	6.90	0.99	1.34
600	42½	30¼	0.8379	0.8430	5.95	6.58	0.69	1.21
500	38½	28	0.8387	0.8448	6.12	6.92	0.78	1.32
400	30 3/4	23	0.8334	0.8477	5.12	6.91	0.94	1.29
300	22½	17	0.8329	0.8416	4.72	6.45	0.65	1.08
200	14½	12¼	0.8335	0.8427	5.20	7.13	0.68	1.27
100	8½	5½	0.8349	0.8382	6.87	6.08	0.76	1.10

SERIES III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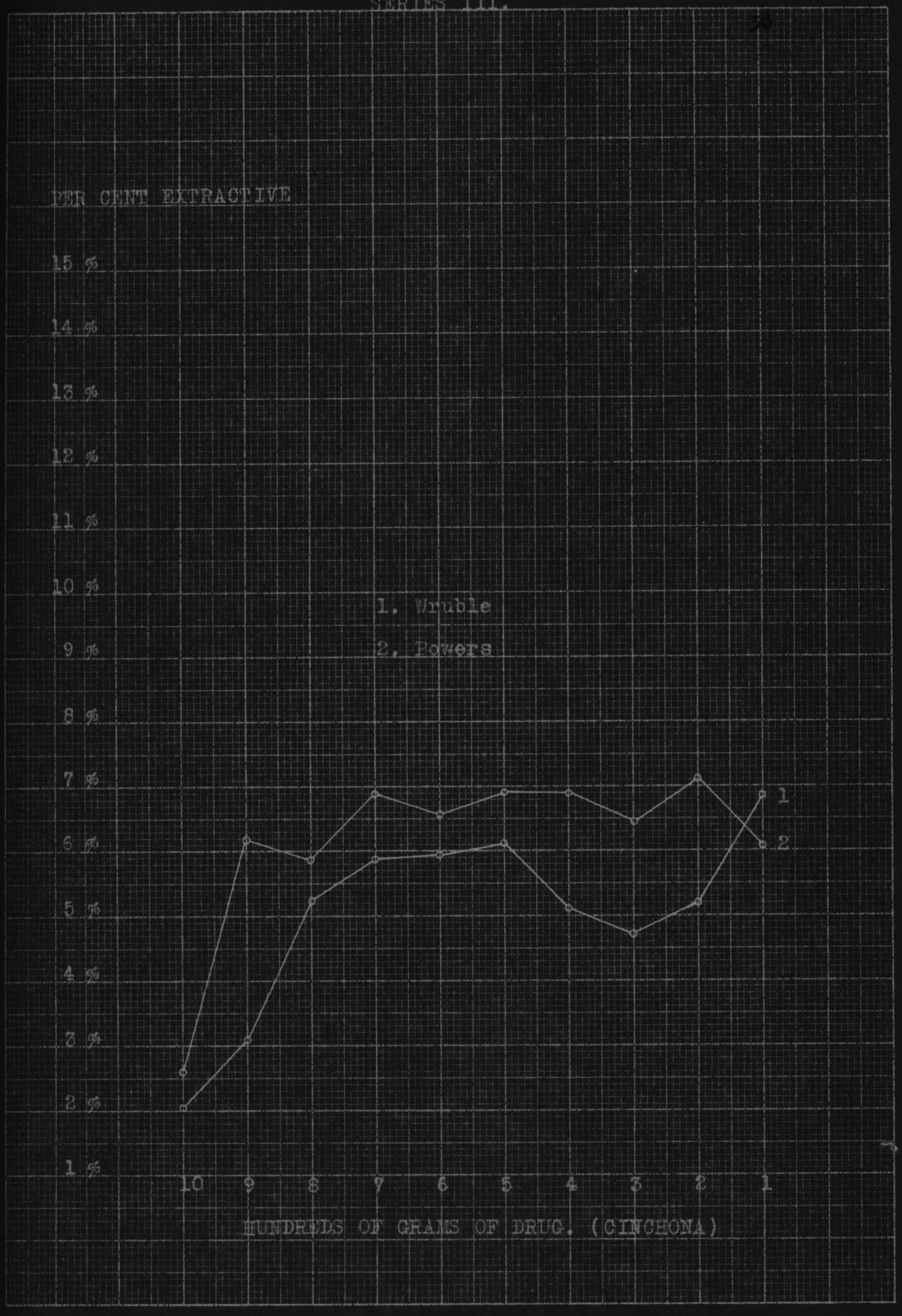
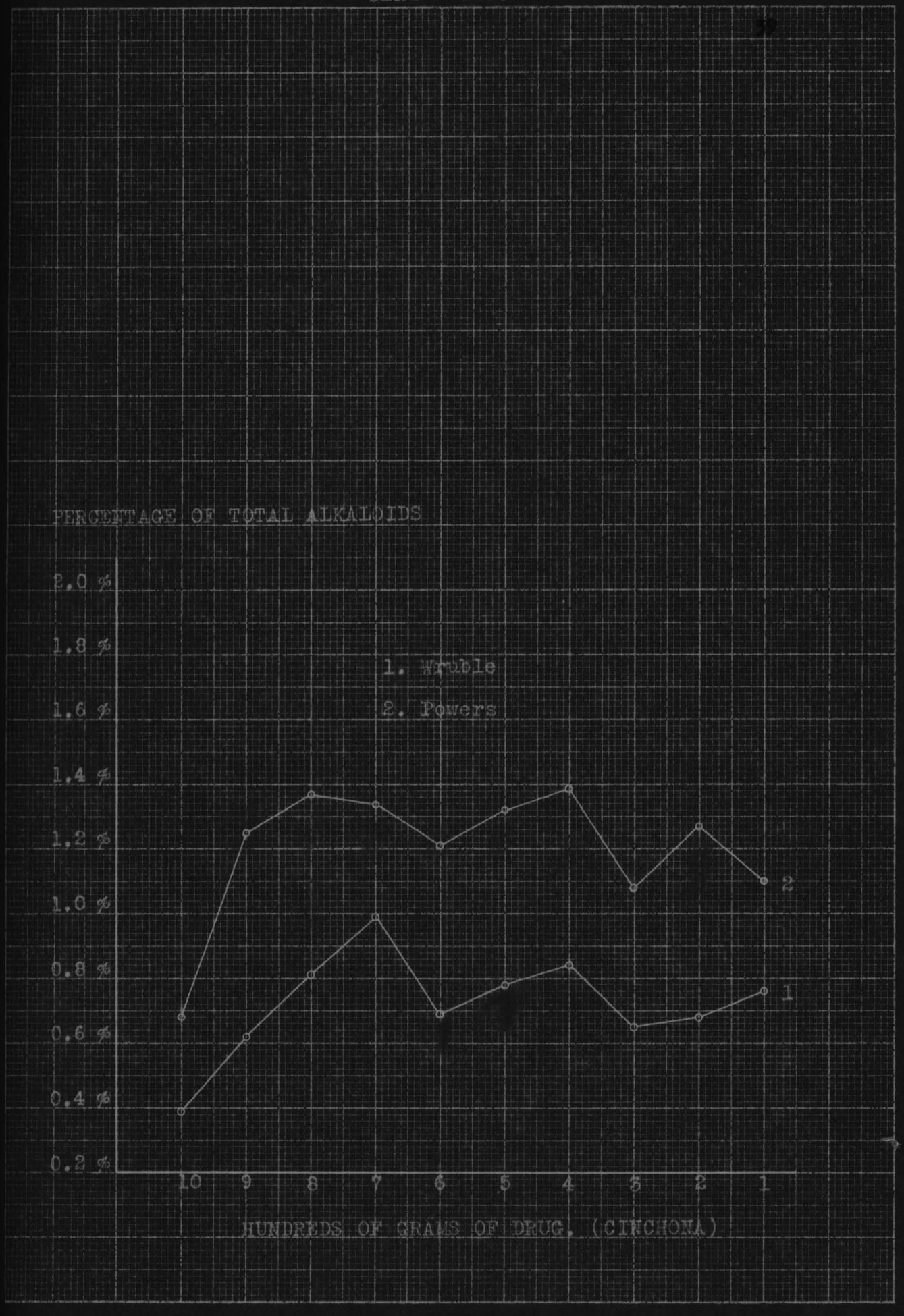
0.8550
0.8500
0.8450
0.8400
0.8350
0.8300
0.8250
0.8200
0.8150
0.8100
0.8050
0.8000
2
1
1. Wruble
2. Powers
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAINS OF DRUG (CINCHONA)

SERIES III.


PER CENT EXTRACTIVE
15 %
14 %
13 %
12 %
11 %
10 %
9 %
8 %
7 %
6 %
5 %
4 %
3 %
2 %
1 %
1. Wruble
2. Powers
1
2
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAMS OF DRUG. (CINCHONA)

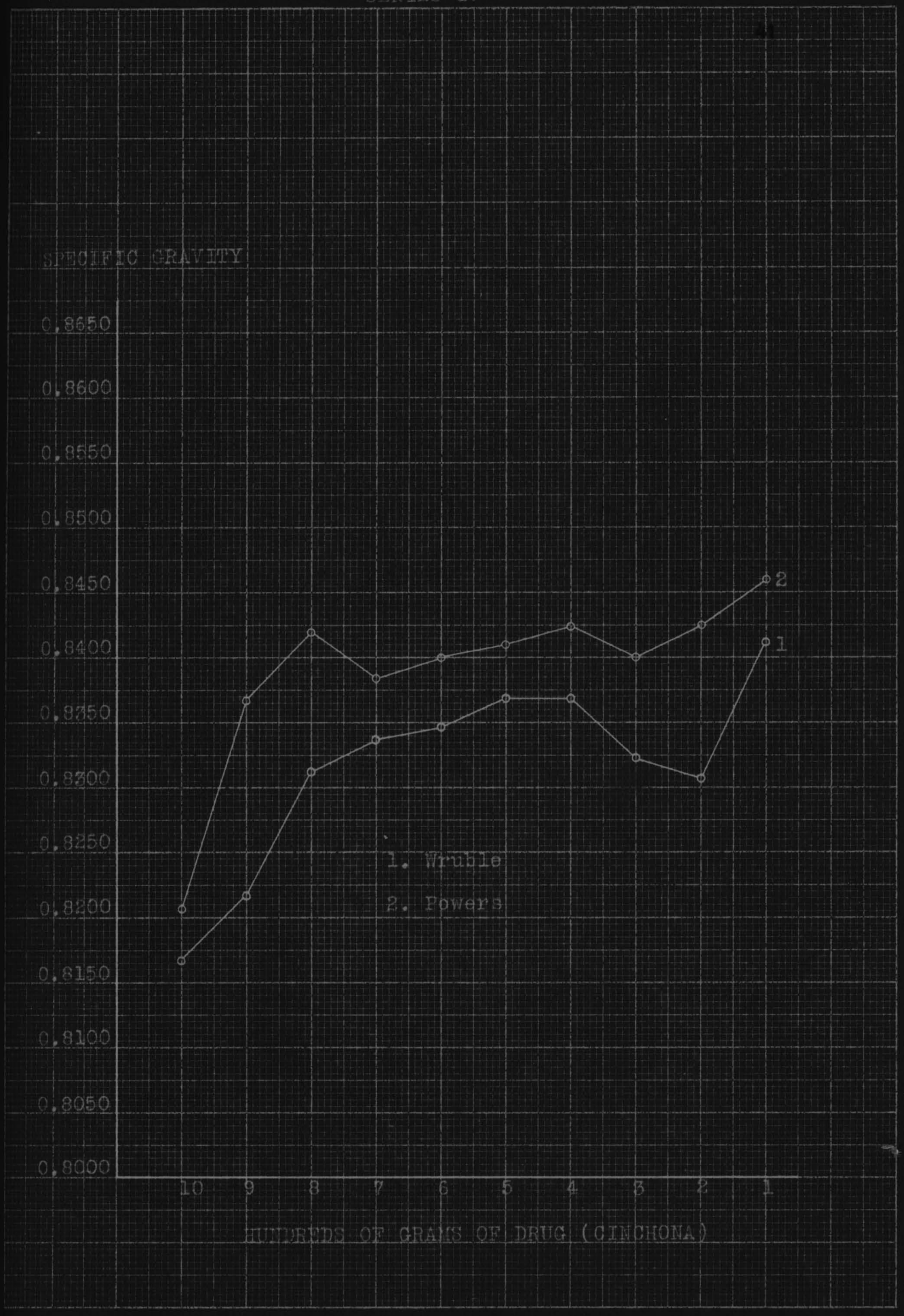
SERIES III.


PERCENTAGE OF TOTAL ALKALOIDS
2.0 %
1.8 %
1.6 %
1.4 %
1.2 %
1.0 %
0.8 %
0.6 %
0.4 %
0.2 %
1. Wrubble
2. Powers
2
1
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAMS OF DRUG. (CINCHONA)

Series IV

Amt. of Drug Gms.	Rate Hours		Sp. Gr. 20°		Percent of Extractive		Percent of Alkaloids	
	Wruble	Powers	Wruble	Powers	Wruble	Powers	Wruble	Powers
1000	58 3/4	49½	0.8167	0.8207	1.52	2.04	0.30	0.56
900	49½	47½	0.8217	0.8367	2.84	5.25	0.47	1.04
800	44½	40¼	0.8312	0.8419	4.46	5.71	0.69	1.22
700	40	38¼	0.8337	0.8384	5.10	5.82	0.65	1.18
600	37¼	31½	0.8347	0.8400	----	5.85	0.76	1.21
500	34¼	28	0.8368	0.8411	5.90	6.24	0.77	1.24
400	28½	21¼	0.8368	0.8424	5.70	6.63	0.79	1.25
300	23½	16¼	0.8323	0.8401	4.74	6.56	0.72	1.17
200	18 3/4	10 3/4	0.8307	0.8425	4.72	7.15	0.70	1.34
100	9	6	0.8412	0.8459	6.90	7.49	0.80	1.42

SERIES IV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0.8500
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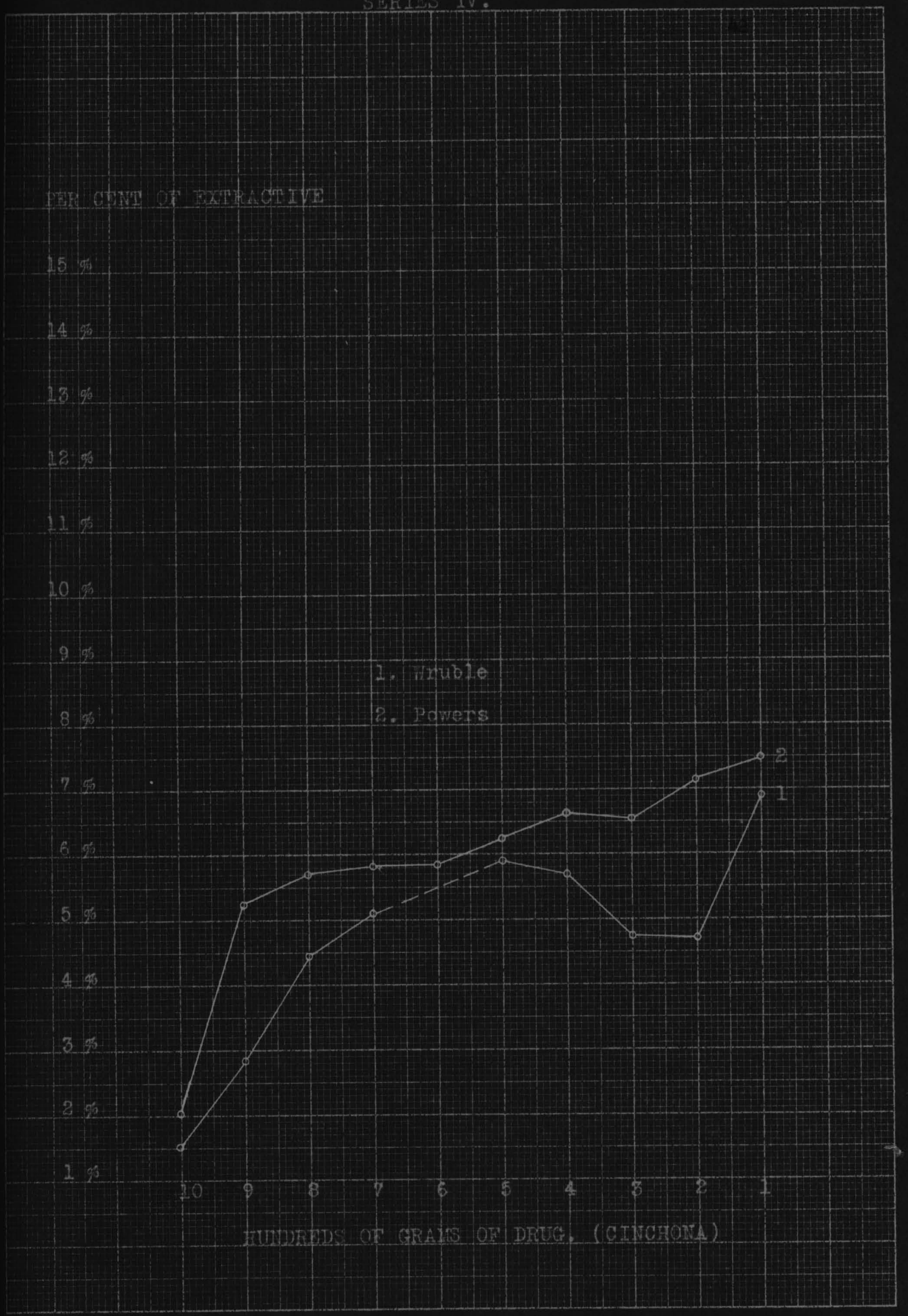
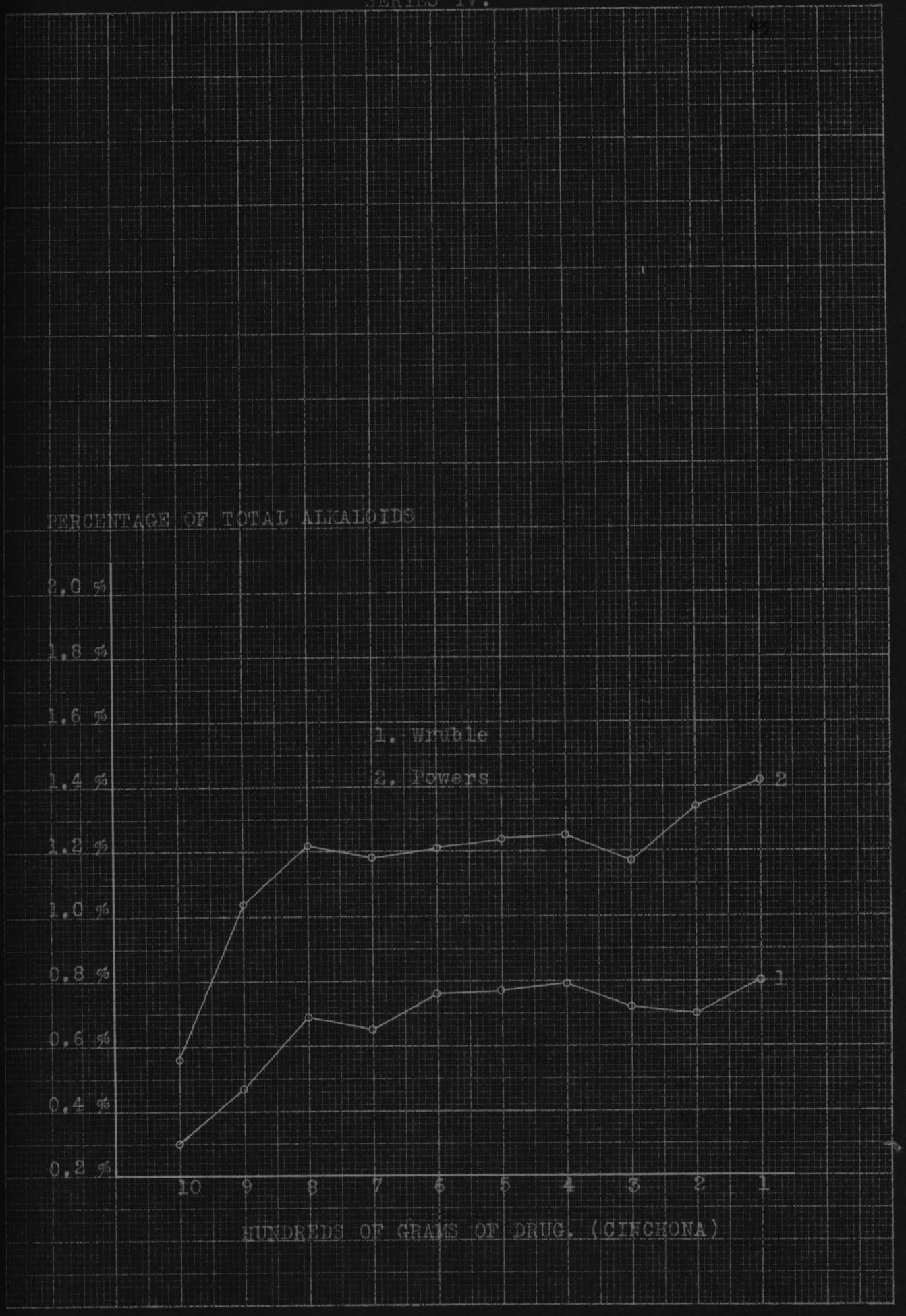
0.8400
0.8350
0.8300
0.8250
0.8200
0.8150
0.8100
0.8050
0.8000
2
1
1. Wruble
2. Powers
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAMS OF DRUG (CINCHONA)

SERIES IV.

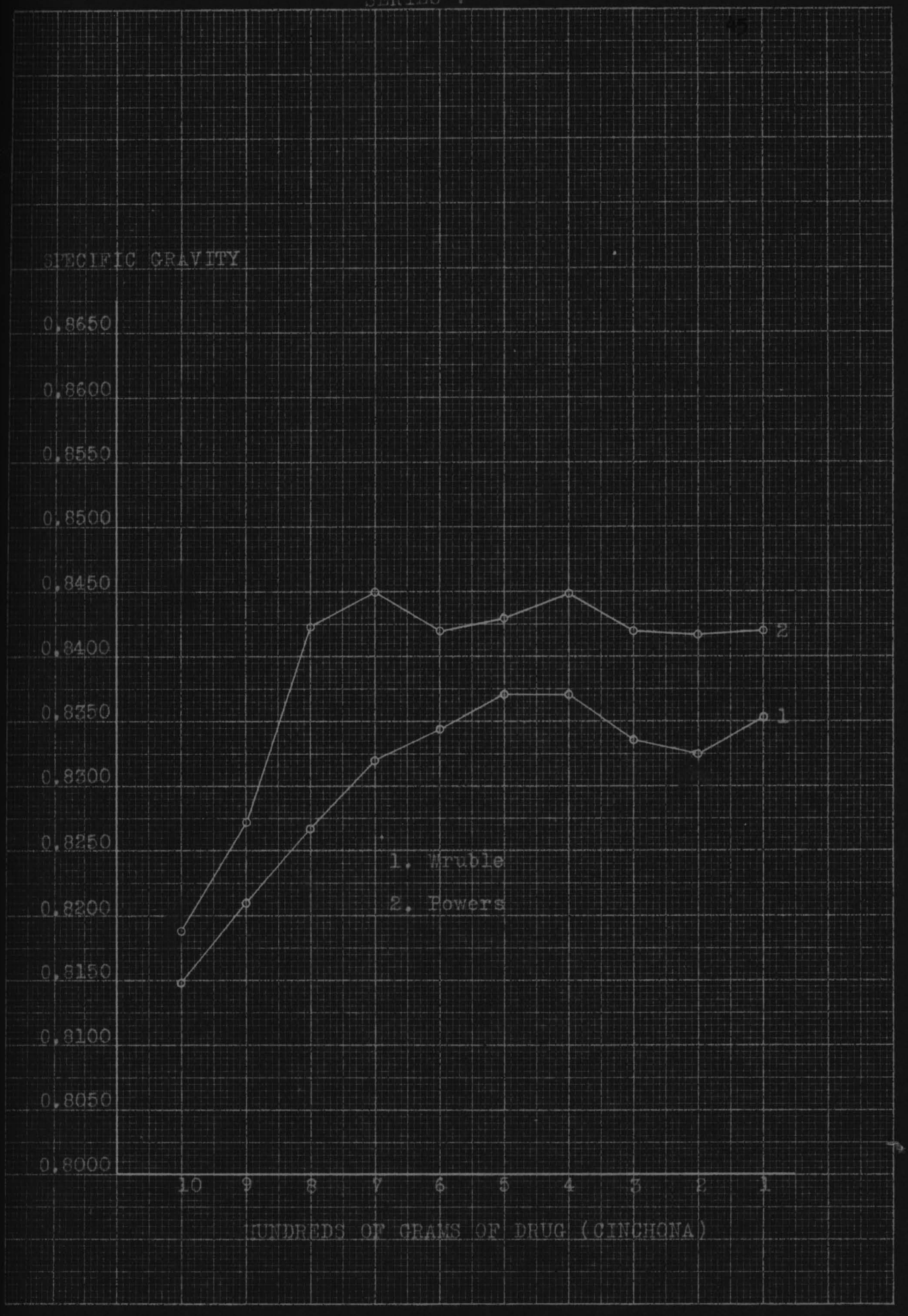

PER CENT OF EXTRACTIVE
15 %
14 %
13 %
12 %
11 %
10 %
9 %
8 %
7 %
6 %
5 %
4 %
3 %
2 %
1 %
1. Mruble
2. Powers
2
1
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAINS OF DRUG, (CINCHONA)

SERIES IV.


PERCENTAGE OF TOTAL ALKALOIDS
2.0 %
1.8 %
1.6 %
1.4 %
1.2 %
1.0 %
0.8 %
0.6 %
0.4 %
0.2 %
1. Wrubble
2. Powers
2
1
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAMS OF DRUG. (CINCHONA)

Series V

Amt. of Drug Gms.	Rate Hours Wruble	Rate Hours Powers	Sp. Gr. 20° Wruble	Sp. Gr. 20° Powers	Percent of Extractive Wruble	Percent of Extractive Powers	Percent of Alkaloids Wruble	Percent of Alkaloids Powers
1000	50½	55½	0.8148	0.8188	1.16	1.42	0.41	0.45
900	44 3/4	46½	0.8210	0.8272	2.01	3.99	0.47	0.87
800	41½	43	0.8267	0.8423	3.32	6.52	0.77	1.12
700	39 3/4	38 3/4	0.8320	0.8451	4.64	6.78	0.79	1.33
600	36½	31½	0.8344	0.8421	5.42	7.18	0.61	1.34
500	34½	27	0.8371	0.8429	5.88	7.69	0.61	1.37
400	26¼	22	0.8371	0.8448	6.15	7.24	0.97	1.45
300	22¼	16½	0.8336	0.8421	5.40	6.66	0.64	1.32
200	14 3/4	10 3/4	0.8325	0.8417	4.88	6.78	0.51	1.37
100	8½	5½	0.8353	0.8420	5.65	6.86	0.76	1.37


SERIES V
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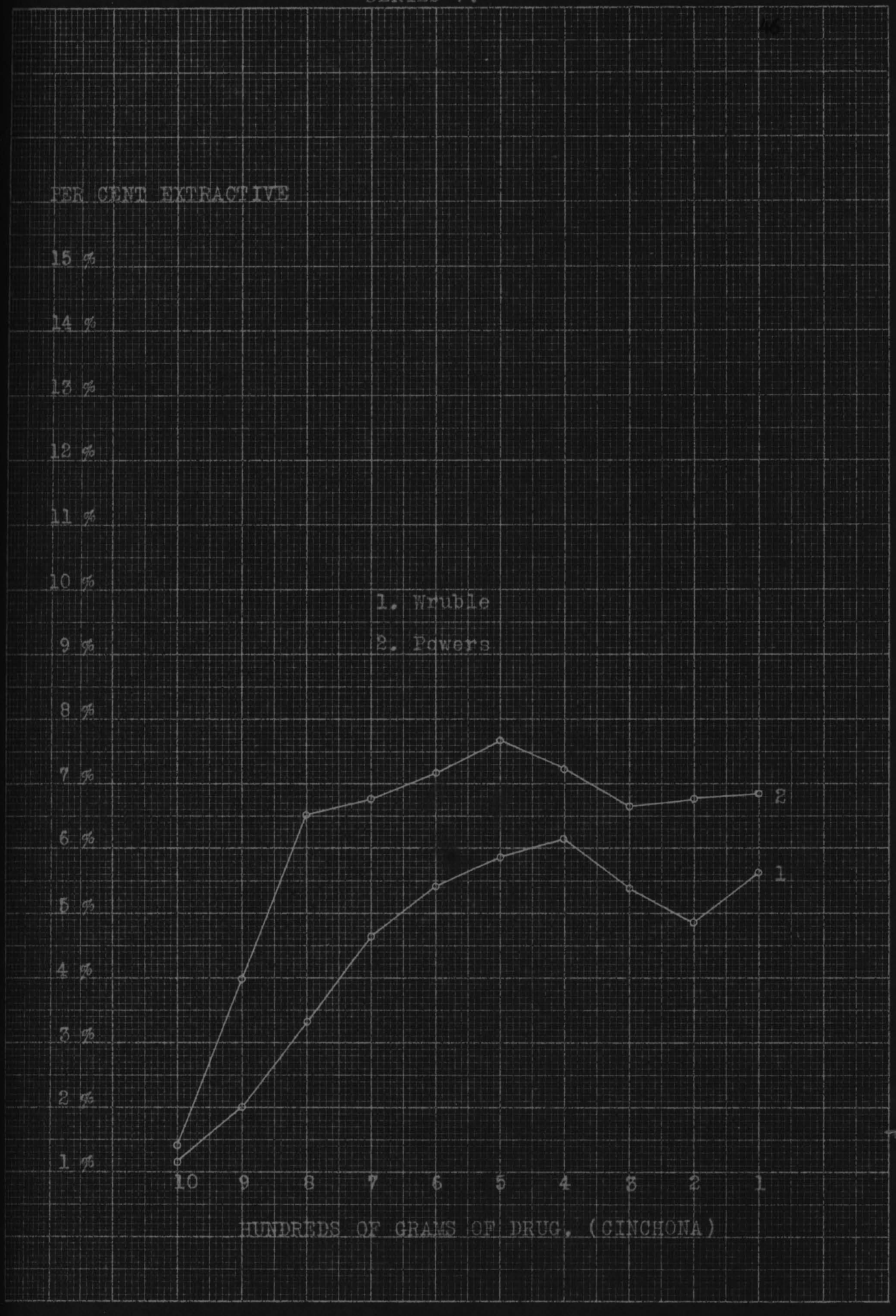
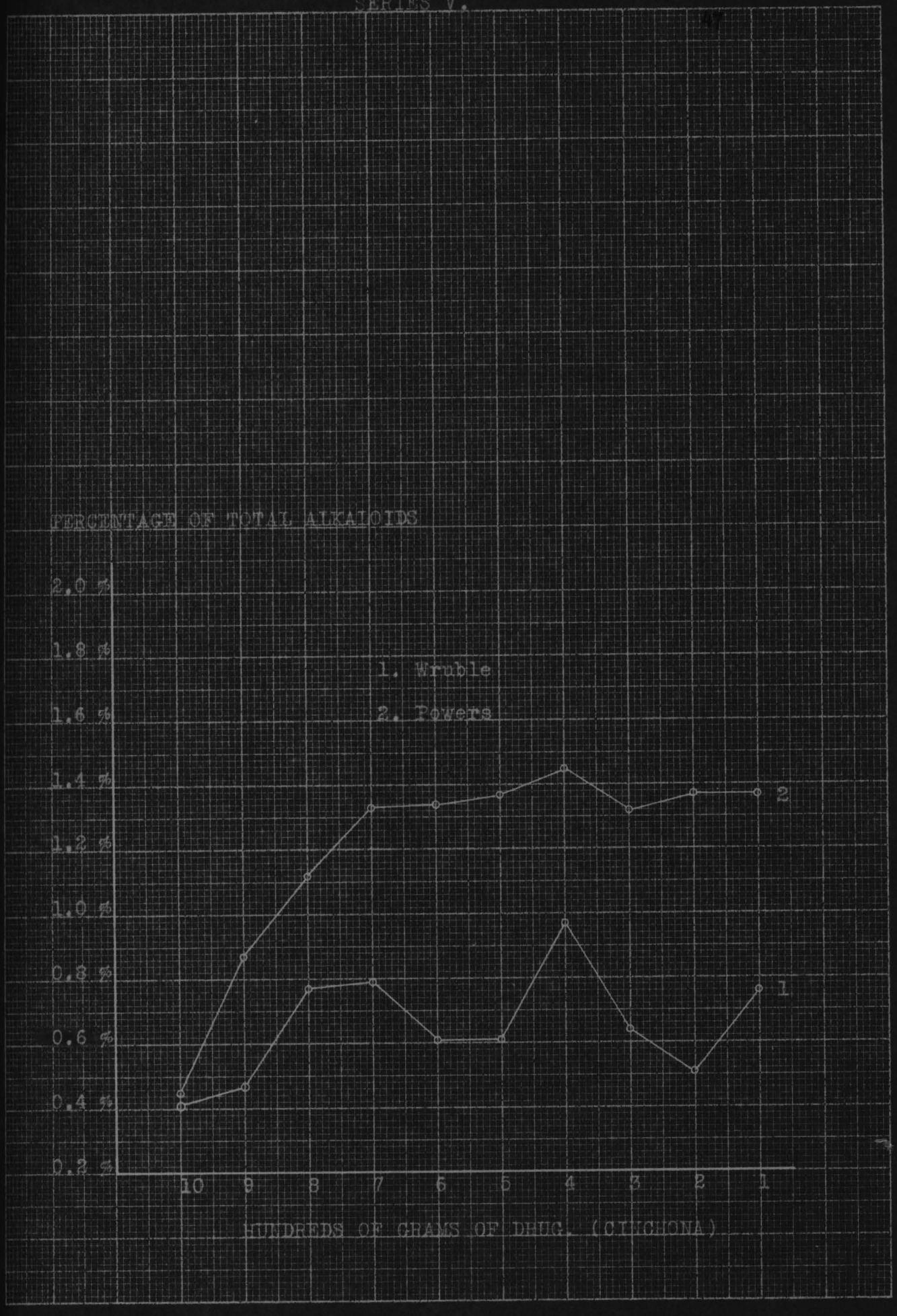
0.8450
0.8400
0.8350
0.8300
0.8250
0.8200
0.8150
0.8100
0.8050
0.8000
2
1
1. Mruble
2. Powers
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAMS OF DRUG (CINCHONA)

SERIES V.


PER CENT EXTRACTIVE
15 %
14 %
13 %
12 %
11 %
10 %
9 %
8 %
7 %
6 %
5 %
4 %
3 %
2 %
1 %
1. Wruble
2. Powers
2
1
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAMS OF DRUG, (CINCHONA)

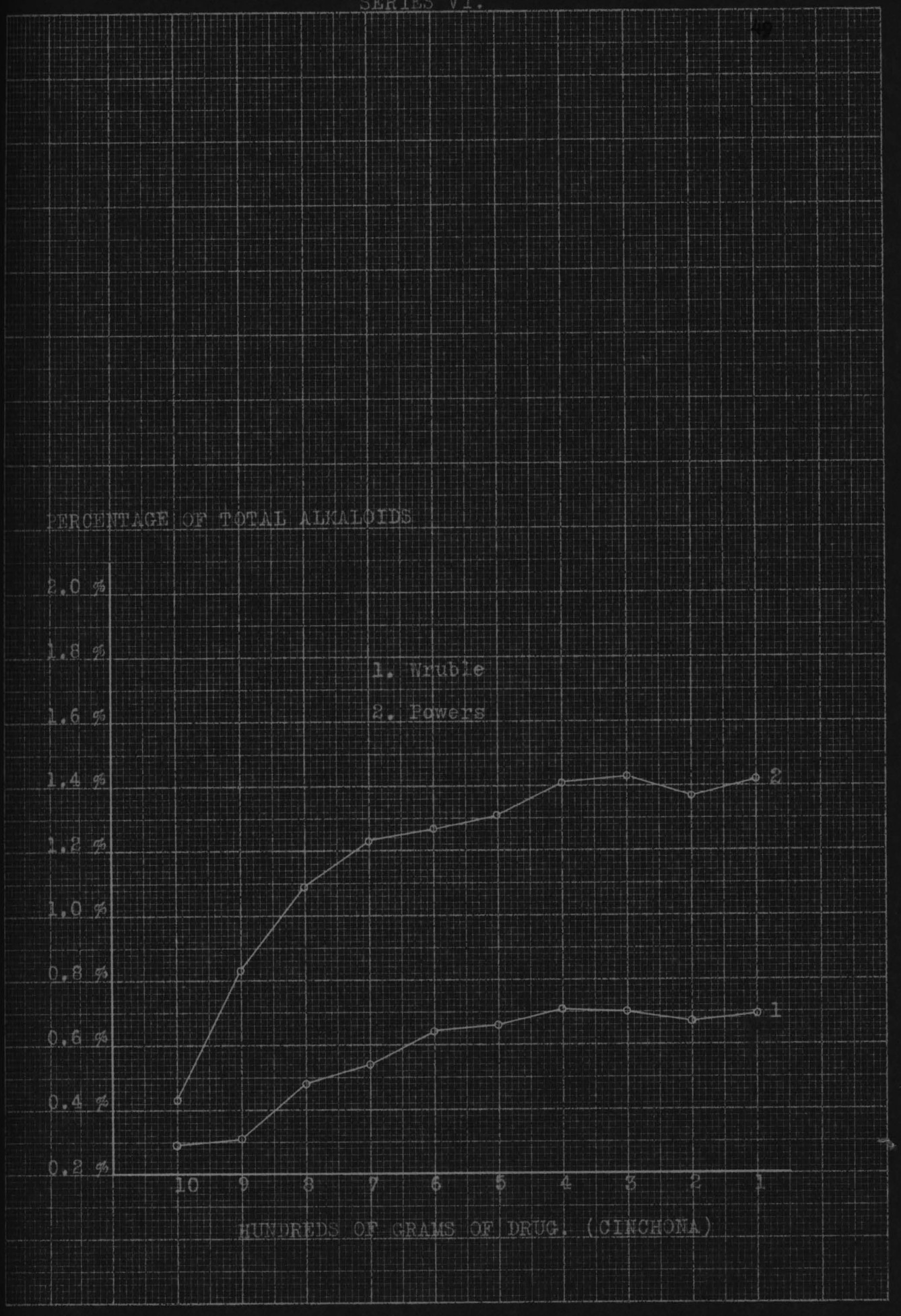
SERIES V.

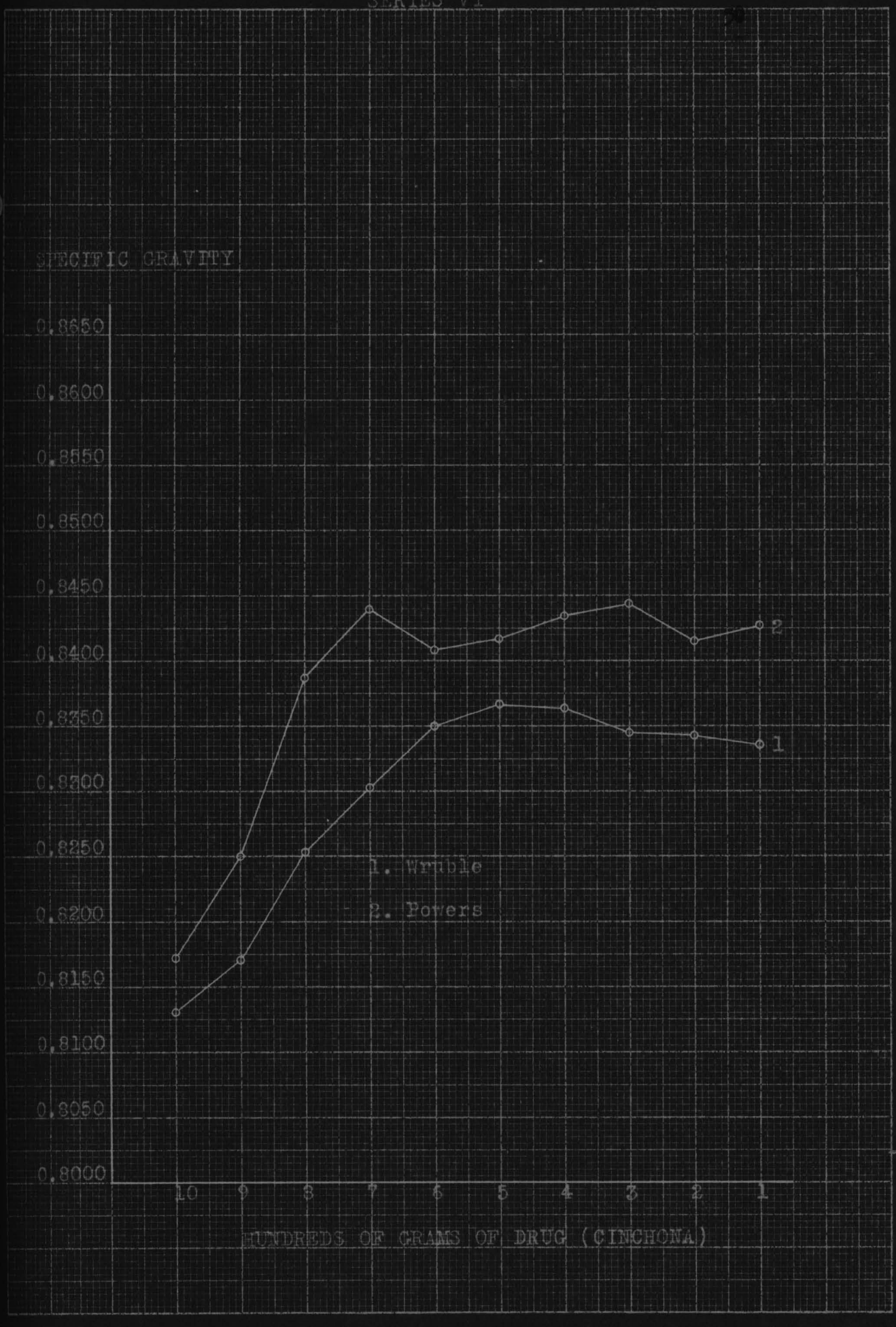

PERCENTAGE OF TOTAL ALKALOIDS
2.0 %
1.8 %
1.6 %
1.4 %
1.2 %
1.0 %
0.8 %
0.6 %
0.4 %
0.2 %
1. Wruble
2. Powers
2
1
10
9
8
7
6
5
4
3
2
1
HUNDREDS OF GRAMS OF DRUG. (CINCHONA)

Series VI

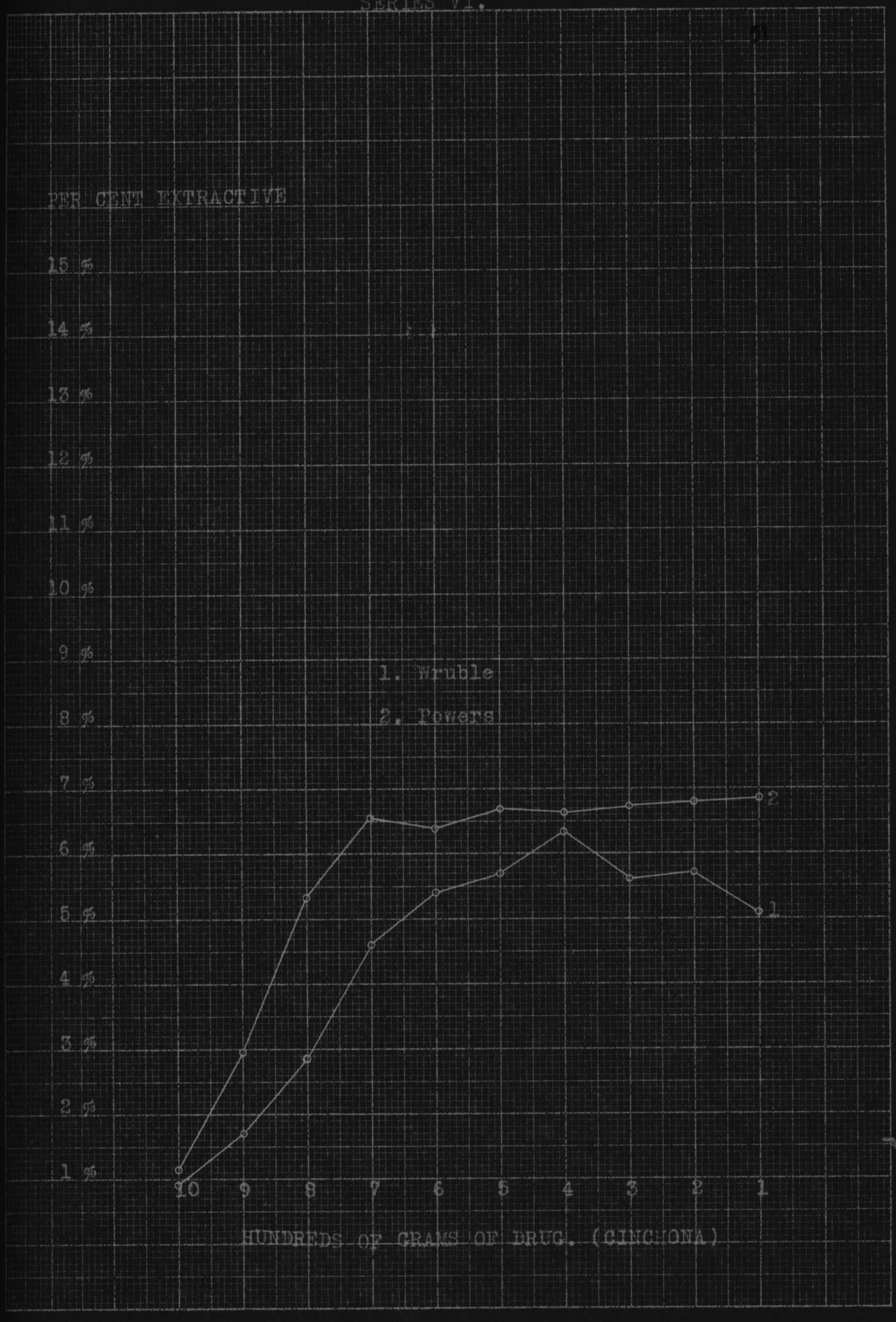
Amt. of Drug Gms.	Rate Hours		Sp. Gr. 20°		Percent of Extractive		Percent of Alkaloids	
	Wruble	Powers	Wruble	Powers	Wruble	Powers	Wruble	Powers
1000	53	48½	0.8131	0.8172	0.93	1.13	0.29	0.43
900	47½	45 3/4	0.8169	0.8250	1.70	2.97	0.31	0.83
800	44½	41¼	0.8253	0.8337	2.85	5.33	0.48	1.09
700	41	35½	0.8303	0.8439	4.80	6.57	0.54	1.23
600	38¼	31¼	0.8349	0.8408	5.42	6.39	0.64	1.27
500	32 3/4	26¼	0.8387	0.8417	5.70	6.89	0.66	1.31
400	27¼	20 3/4	0.8364	0.8434	6.35	6.84	0.71	1.41
300	23 3/4	15½	0.8345	0.8444	5.62	6.72	0.70	1.43
200	17 3/4	11 3/4	0.8343	0.8415	5.72	6.78	0.67	1.37
100	8 3/4	6 3/4	0.8336	0.8427	5.10	6.86	0.69	1.42

SERIES VI.


PERCENTAGE OF TOTAL ALKALOIDS
2.0 %
1.8 %
1.6 %
1.4 %
1.2 %
1.0 %
0.8 %
0.6 %
0.4 %
0.2 %
1. Wruble
2. Powers
2
1
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAMS OF DRUG. (CINCHONA)


SERIES VI
SPECIFIC GRAVITY
0.8650
0.8600
0.8550
0.8500
0.8450
0.8400
0.8350
0.8300
0.8250
0.8200
0.8150
0.8100
0.8050
0.8000
2
1
1. Wrmble
2. Powers
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAMS OF DRUG (CINCHONA)

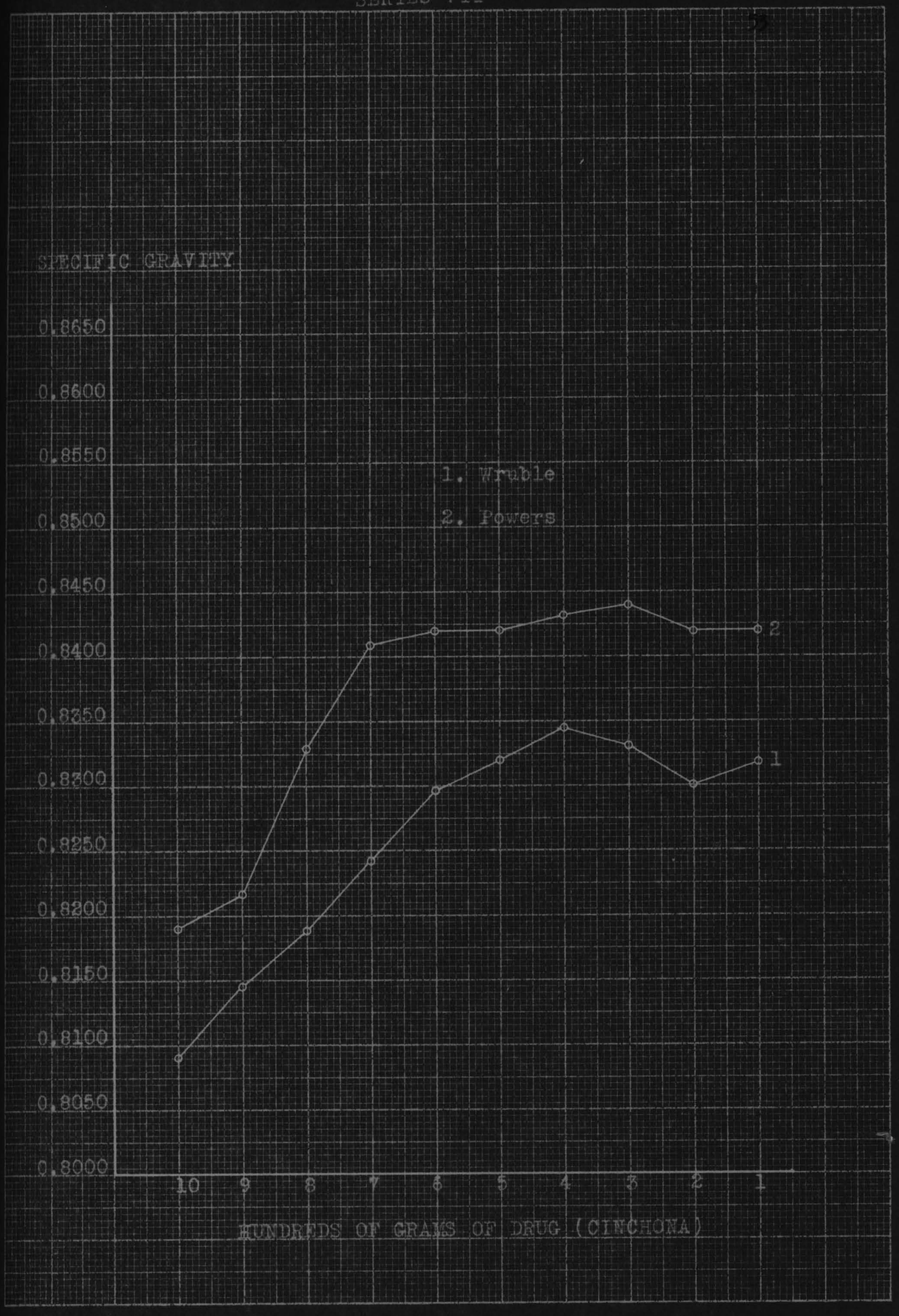
SERIES VI.


PER CENT EXTRACTIVE
15 %
14 %
13 %
12 %
11 %
10 %
9 %
8 %
7 %
6 %
5 %
4 %
3 %
2 %
1 %
1. Wruble
2. Powers
2
1
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAMS OF DRUG. (CINCHONA)

Series VII

Amt. of Drug Gms.	Rate Hours		Sp. Gr. 20°		Percent of Extractive		Percent of Alkaloids	
	Wruble	Powers	Wruble	Powers	Wruble	Powers	Wruble	Powers
1000	52½	49½	0.8096	0.8191	0.72	1.27	0.24	0.45
900	45½	45½	0.8145	0.8217	1.46	2.15	0.37	0.76
800	43	46	0.8188	0.8329	2.40	3.72	0.59	0.84
700	41 3/4	35 3/4	0.8242	0.8409	3.83	5.45	0.62	1.24
600	37¼	31¼	0.8296	0.8421	5.18	6.77	0.72	1.26
500	34¼	24 3/4	0.8320	0.8421	5.45	6.89	0.78	1.31
400	25 3/4	21½	0.8345	0.8432	6.25	6.94	0.81	1.44
300	22½	14 3/4	0.8336	0.8441	6.15	6.92	0.82	1.31
200	19¼	12	0.8298	0.8419	5.12	6.79	0.79	1.36
100	7 3/4	5¼	0.8318	0.8420	5.50	6.71	0.86	1.39

SERIES VII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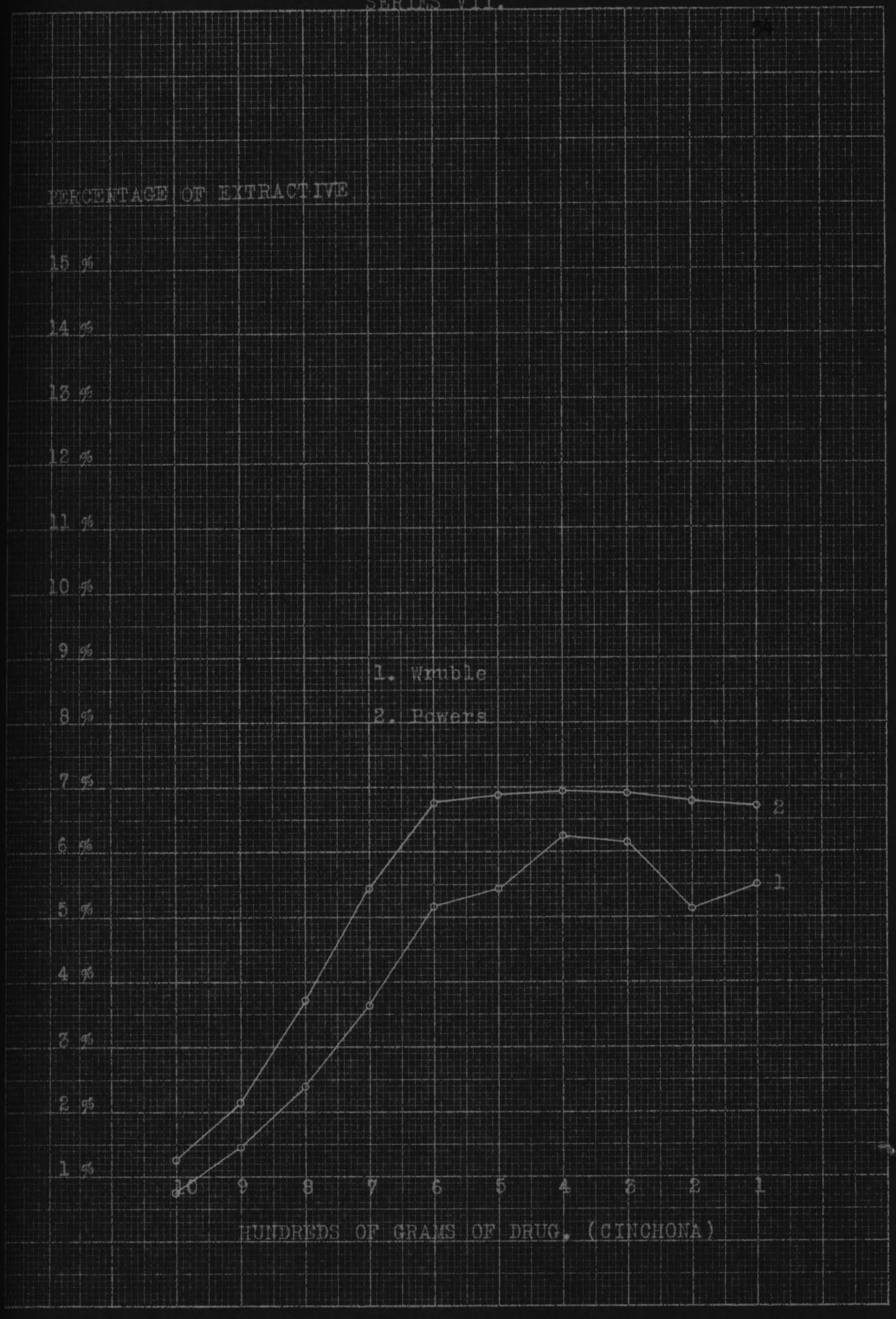
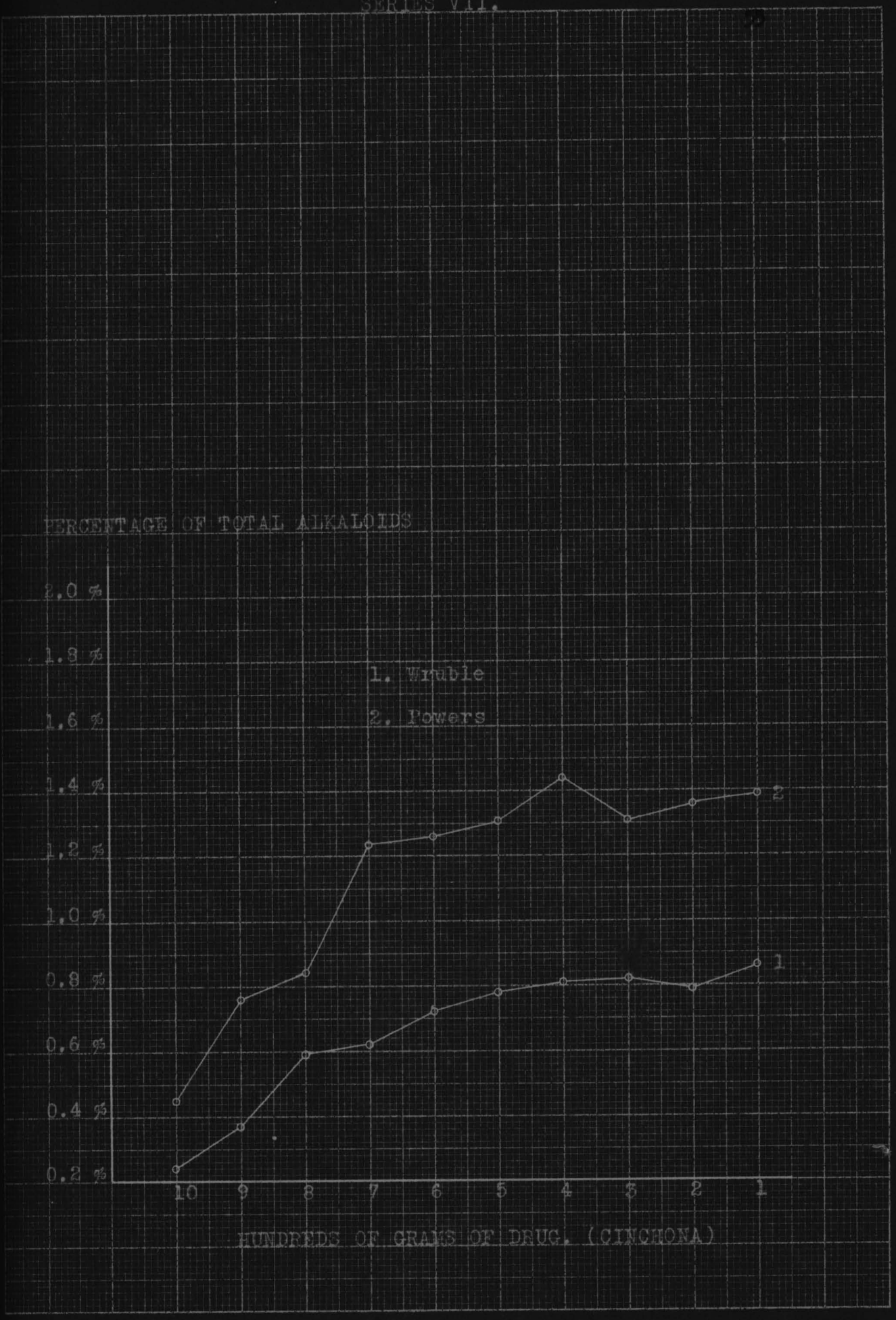
0.8500
0.8450
0.8400
0.8350
0.8300
0.8250
0.8200
0.8150
0.8100
0.8050
0.8000
1. Wruble
2. Powers
2
1
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAMS OF DRUG (CINCHONA)

SERIES VII.


PERCENTAGE OF EXTRACTIVE
15 %
14 %
13 %
12 %
11 %
10 %
9 %
8 %
7 %
6 %
5 %
4 %
3 %
2 %
1 %
1. Wmble
2. Powers
2
1
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAMS OF DRUG. (CINCHONA)

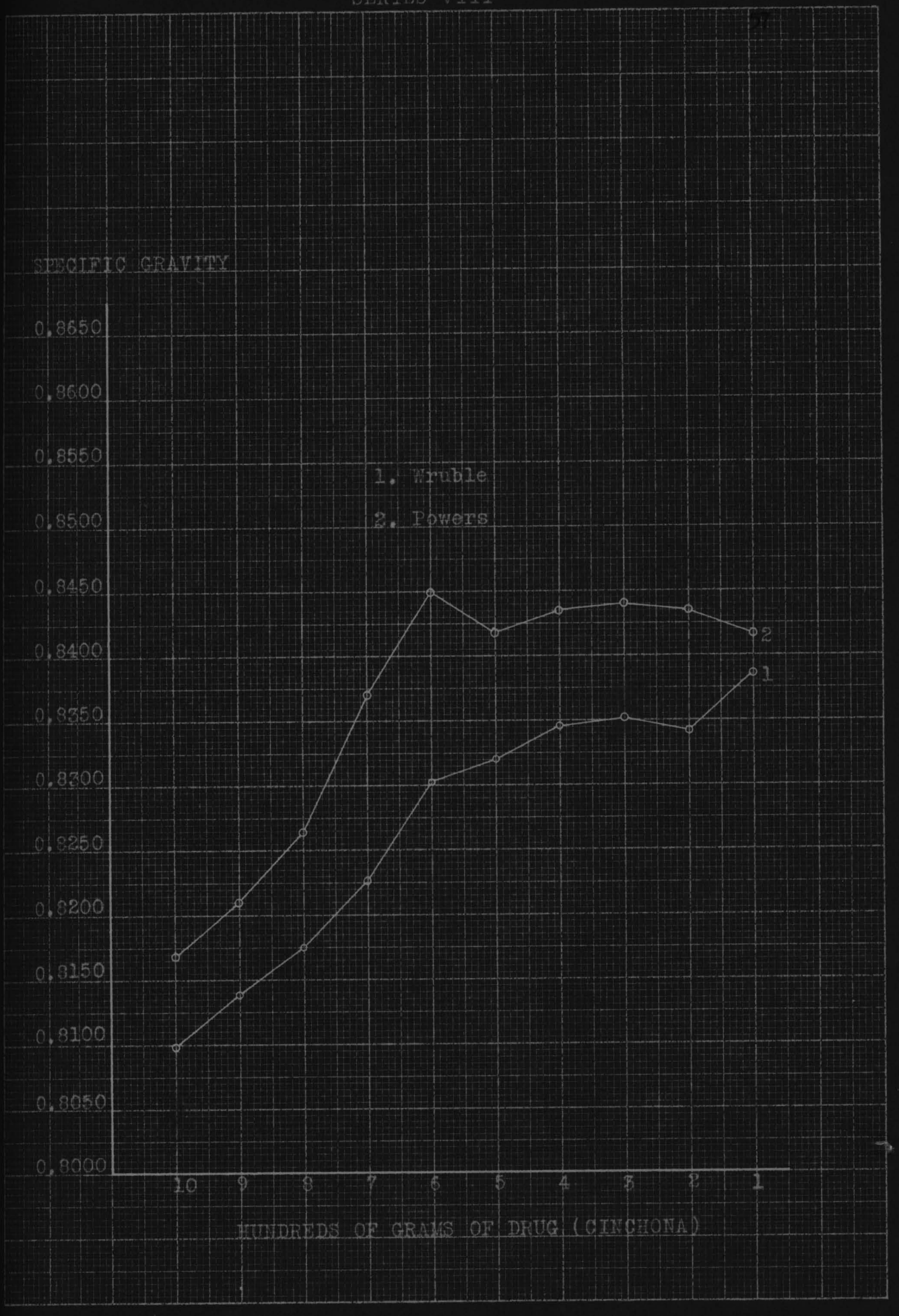
SERIES VII.


PERCENTAGE OF TOTAL ALKALOIDS
2.0 %
1.8 %
1.6 %
1.4 %
1.2 %
1.0 %
0.8 %
0.6 %
0.4 %
0.2 %
1. Wrubie
2. Powers
2
1
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAMS OF DRUG. (CINCHONA)

Series VIII

Amt. of Drug Gms.	Rate Hours		Sp. Gr. 20°		Percent of Extractive		Percent of Alkaloids	
	Wruble	Powers	Wruble	Powers	Wruble	Powers	Wruble	Powers
1000	50 3/4	48½	0.8098	0.8168	0.54	1.05	0.24	0.43
900	44½	50	0.8138	0.8211	1.42	3.31	0.32	0.74
800	41½	45	0.8175	0.8264	2.23	3.32	0.38	0.71
700	37½	35 3/4	0.8226	0.8371	3.33	5.55	0.60	1.15
600	34¼	32	0.8302	0.8449	5.00	6.68	0.68	1.33
500	30	25 3/4	0.8321	0.8418	5.80	6.72	0.74	1.27
400	27¼	20½	0.8346	0.8435	6.82	6.84	0.82	1.41
300	23½	15 3/4	0.8352	0.8441	6.70	6.80	0.67	1.34
200	18¼	10½	0.8342	0.8436	6.40	6.69	0.79	1.38
100	8½	6¼	0.8386	0.8417	7.00	6.70	0.83	1.34

SERIES VIII


SPECIFIC GRAVITY
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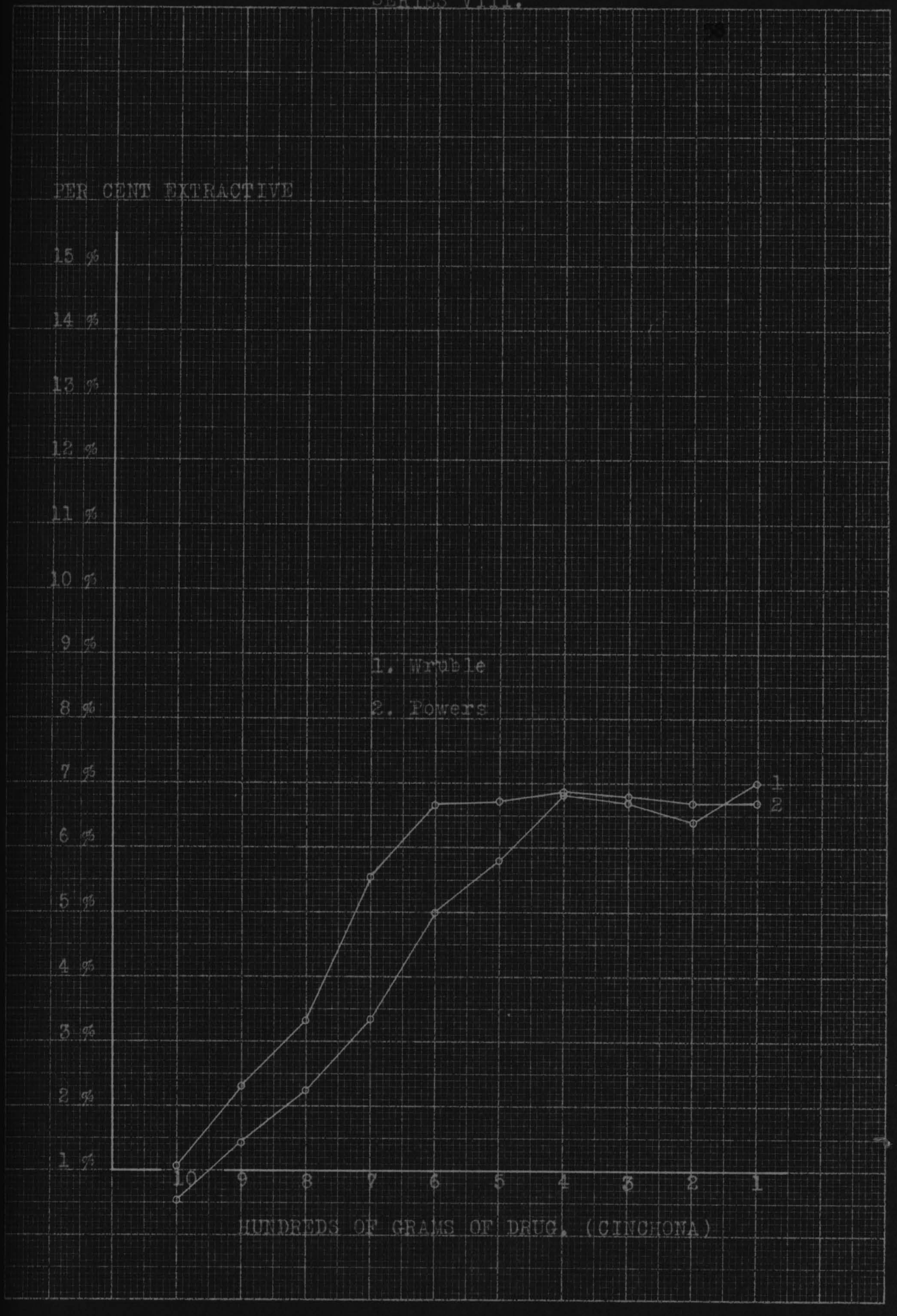
0.8550
0.8500
0.8450
0.8400
0.8350
0.8300
0.8250
0.8200
0.8150
0.8100
0.8050
0.8000
1. Wruble
2. Powers
2
1
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAMS OF DRUG (CINCHONA)

SERIES VIII.


PER CENT EXTRACTIVE
15 %
14 %
13 %
12 %
11 %
10 %
9 %
8 %
7 %
6 %
5 %
4 %
3 %
2 %
1 %
1. Wrable
2. Powers
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10
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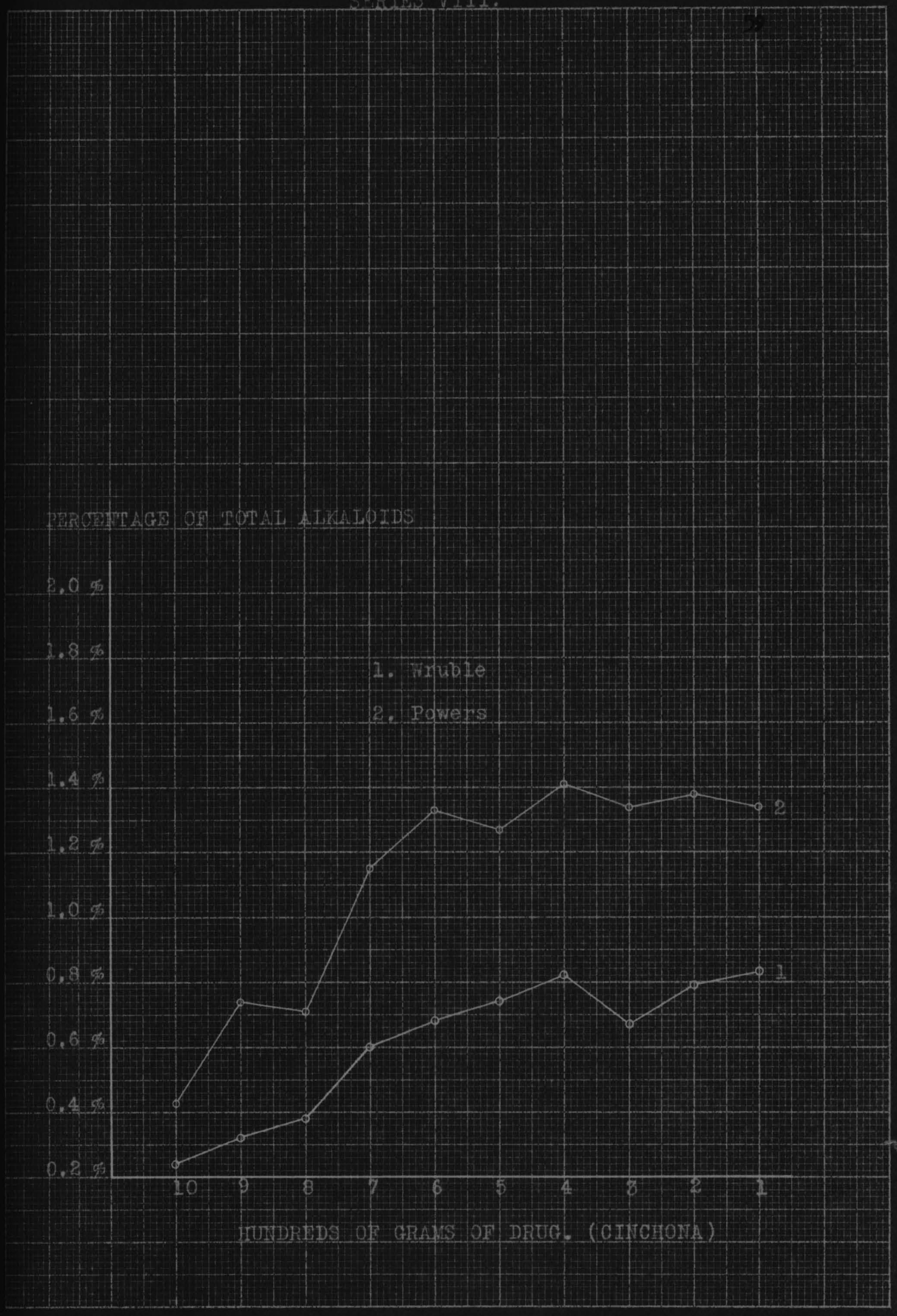
8
7
6
5
4
3
2
1
HUNDREDS OF GRAMS OF DRUG. (CINCHONA)

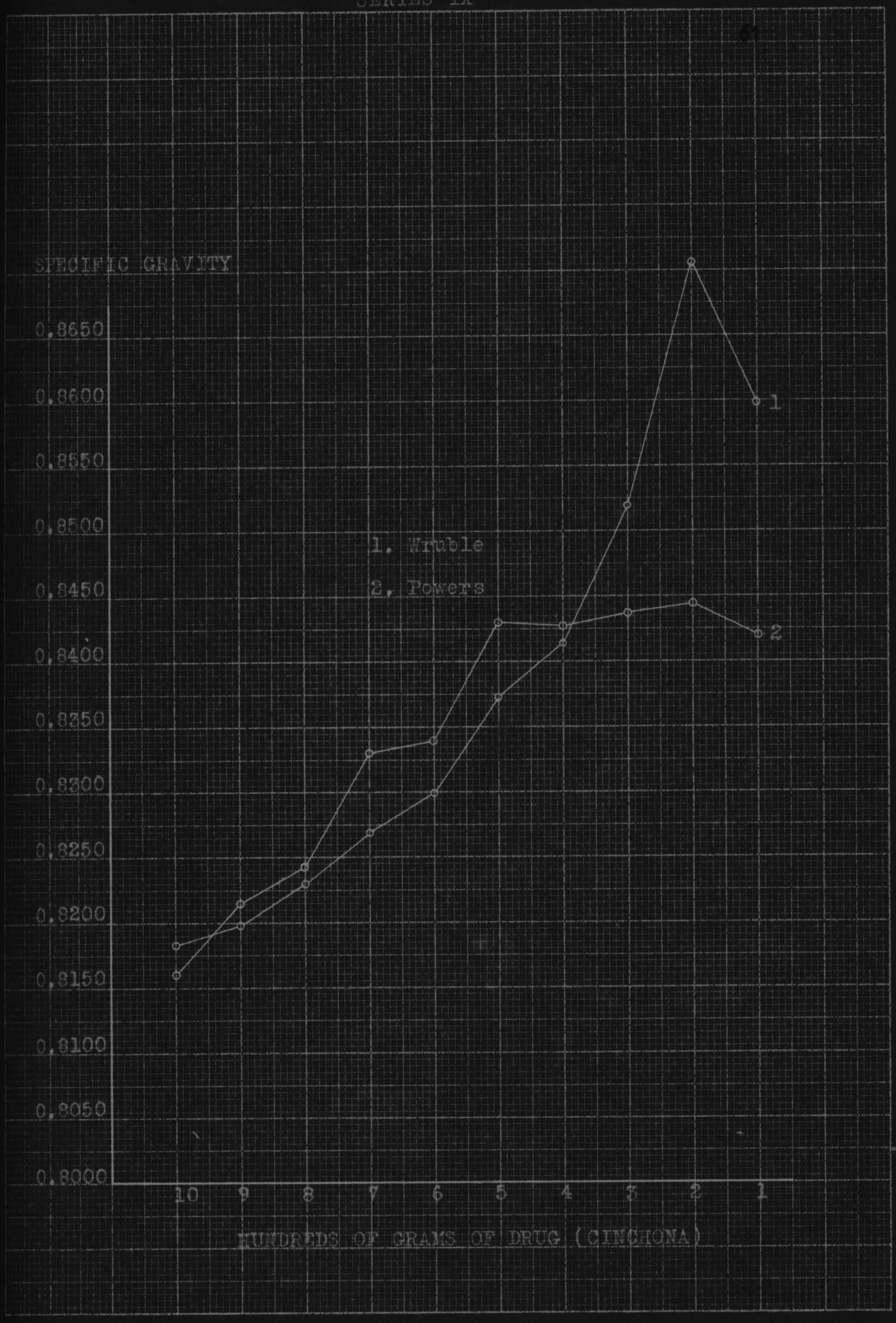
SERIES VIII.


PERCENTAGE OF TOTAL ALKALOIDS
2.0 %
1.8 %
1.6 %
1.4 %
1.2 %
1.0 %
0.8 %
0.6 %
0.4 %
0.2 %
1. Wruble
2. Powers
2
1
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAMS OF DRUG. (CINCHONA)

Series IX

Amt. of Drug Gms.	Rate Hours		Sp. Gr. 20°		Percent of Extractive		Percent of Alkaloids	
	Wruble	Powers	Wruble	Powers	Wruble	Powers	Wruble	Powers
1000	44½	50	0.8183	0.8160	0.47	0.92	0.24	0.38
900	41½	50¼	0.8198	0.8215	0.92	2.33	0.26	0.66
800	37¼	45½	0.8229	0.8243	1.63	2.90	0.39	0.75
700	34	36	0.8268	0.8331	2.53	4.67	0.59	1.02
600	32½	32	0.8298	0.8339	3.39	5.51	0.66	1.18
500	31¼	25¼	0.8373	0.8431	4.87	7.23	0.72	1.37
400	26 3/4	20 3/4	0.8414	0.8427	5.72	6.99	0.75	1.36
300	21½	17	0.8519	0.8437	8.65	6.90	0.90	1.46
200	11½	11	0.8706	0.8440	9.30	6.82	0.90	1.47
100	6	5 3/4	0.8598	0.8421	8.90	6.75	0.96	1.36

SERIES IX


SPECIFIC GRAVITY
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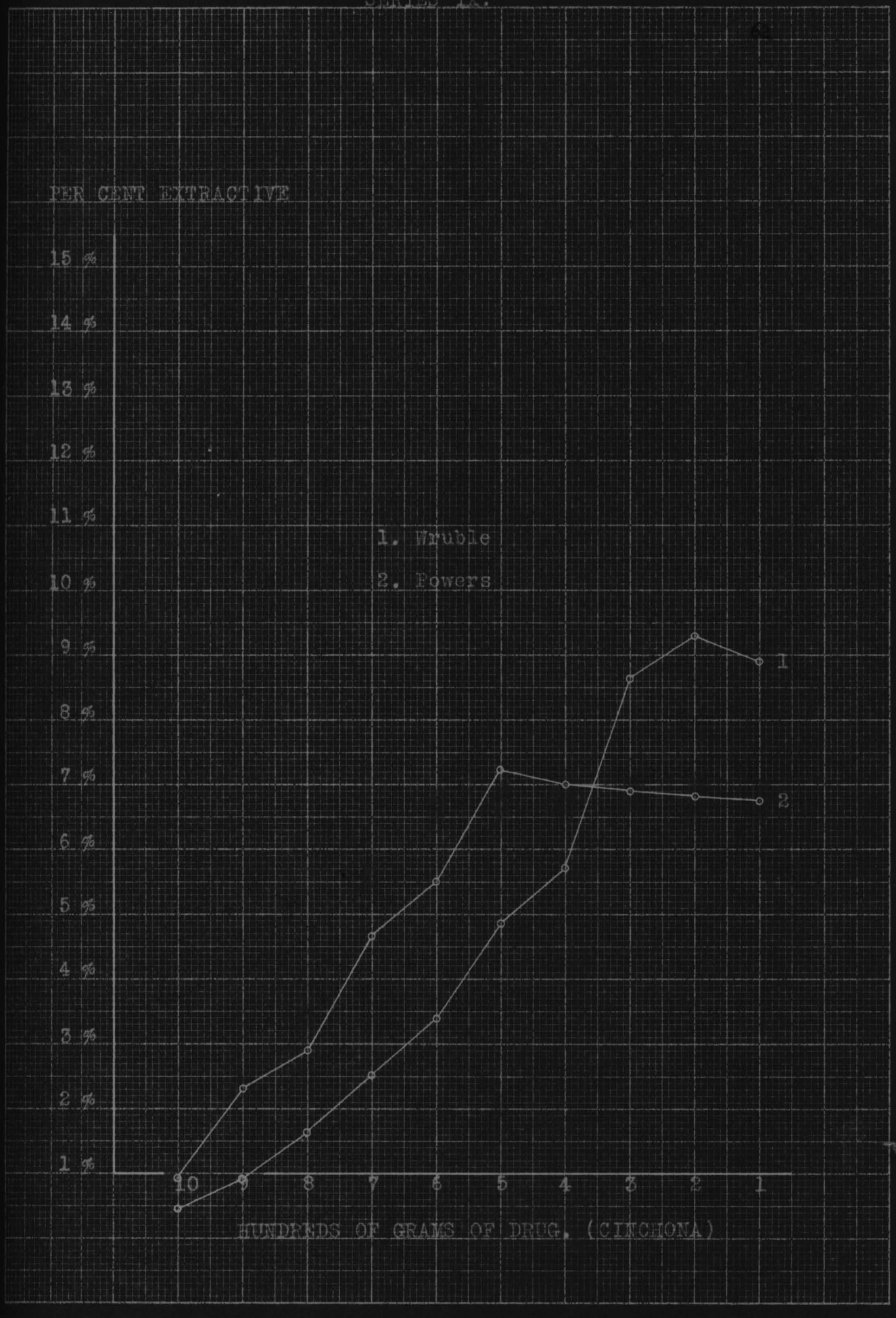
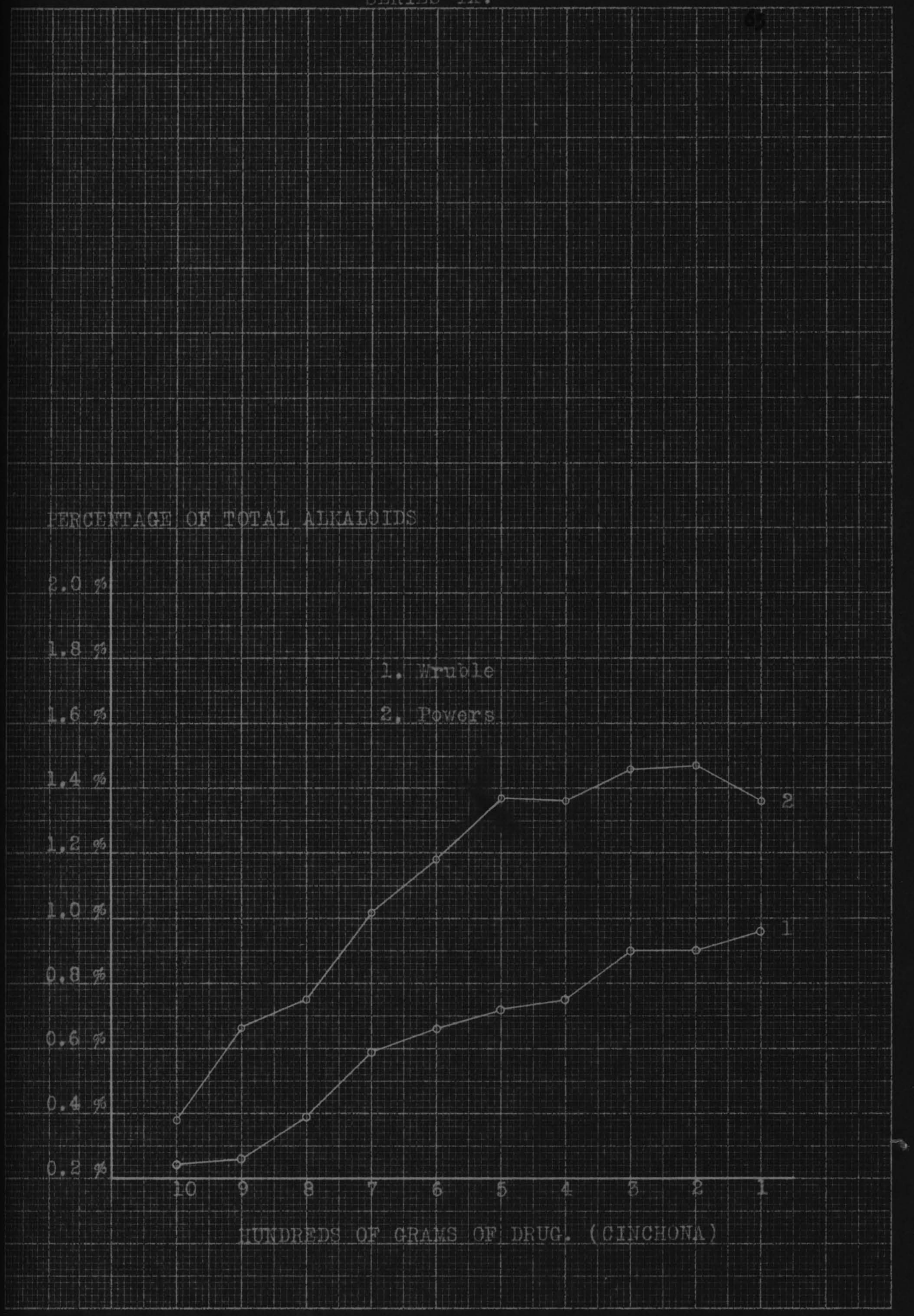
0.8550
0.8500
0.8450
0.8400
0.8350
0.8300
0.8250
0.8200
0.8150
0.8100
0.8050
0.8000
1. Wruble
2. Powers
1
2
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAMS OF DRUG (CINCHONA)

SERIES IX.


PER CENT EXTRACTIVE
15 %
14 %
13 %
12 %
11 %
10 %
9 %
8 %
7 %
6 %
5 %
4 %
3 %
2 %
1 %
1. Wruble
2. Powers
1
2
10
9
8
7
6
5
4
3
2
1
HUNDREDS OF GRAMS OF DRUG. (CINCHONA)

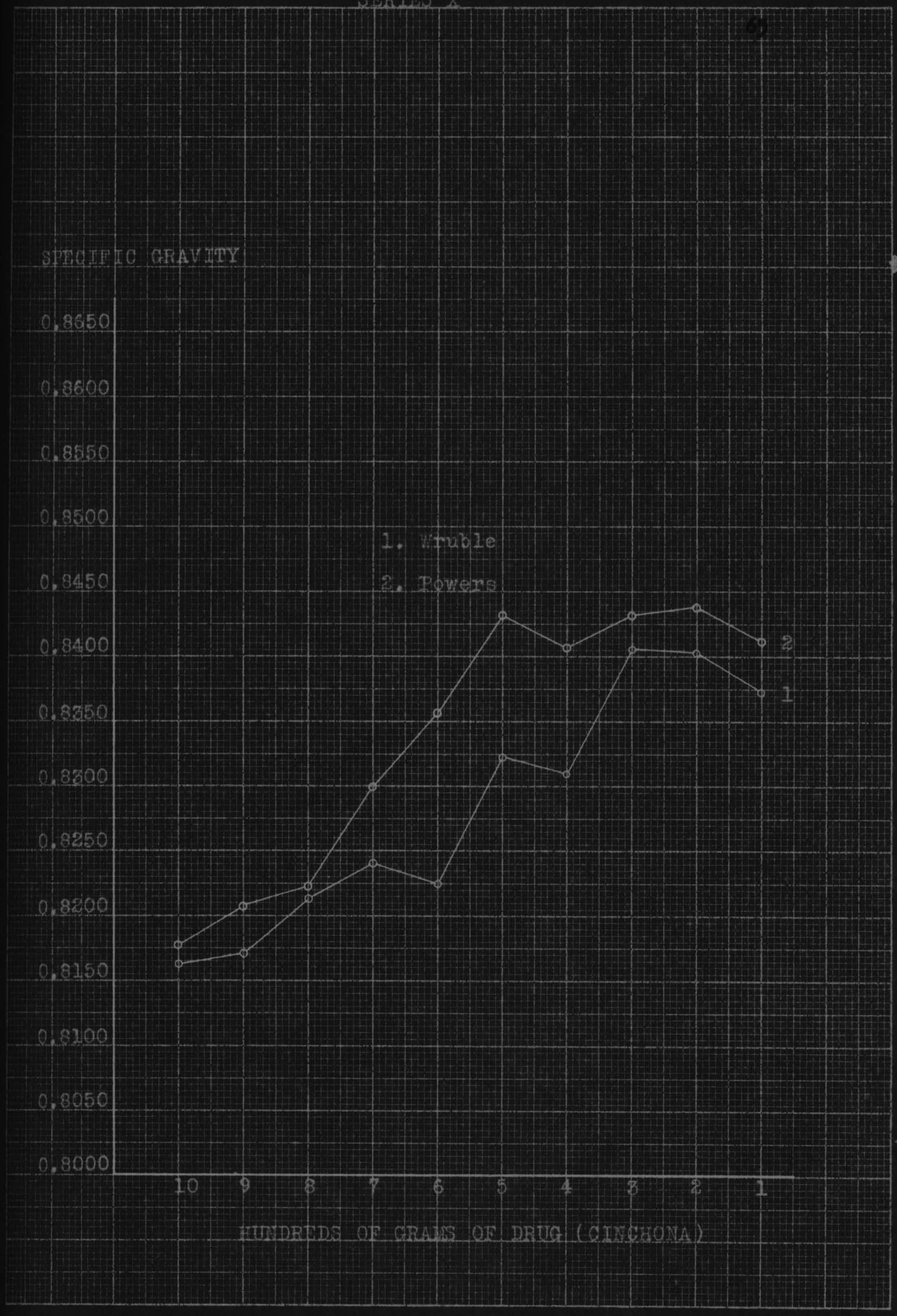
SERIES IX.


PERCENTAGE OF TOTAL ALKALOIDS
2.0 %
1.8 %
1.6 %
1.4 %
1.2 %
1.0 %
0.8 %
0.6 %
0.4 %
0.2 %
1. Wruble
2. Powers
2
1
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAMS OF DRUG. (CINCHONA)

Series X

Amt. of Drug Gms.	Rate Hours		Sp. Gr. 20°		Percent of Extractive		Percent of Alkaloids	
	Wruble	Powers	Wruble	Powers	Wruble	Powers	Wruble	Powers
1000	46½	49	0.8162	0.8177	0.46	0.76	0.18	0.28
900	40	45	0.8171	0.8207	0.90	1.90	0.28	0.64
800	37 3/4	41½	0.8213	0.8223	1.44	2.80	0.48	0.72
700	33	35½	0.8239	0.8297	2.23	3.75	0.50	1.00
600	31½	29 3/4	0.8224	0.8357	2.32	5.28	0.39	1.09
500	27 3/4	24 3/4	0.8323	0.8432	3.85	6.95	0.49	1.37
400	25½	21	0.8310	0.8407	4.26	6.68	0.66	1.27
300	20	15½	0.8406	0.8432	6.62	6.90	0.79	1.35
200	10 3/4	9 3/4	0.8403	0.8438	5.62	6.89	0.47	1.32
100	7½	4½	0.8373	0.8412	5.72	6.73	0.62	1.25

SERIES X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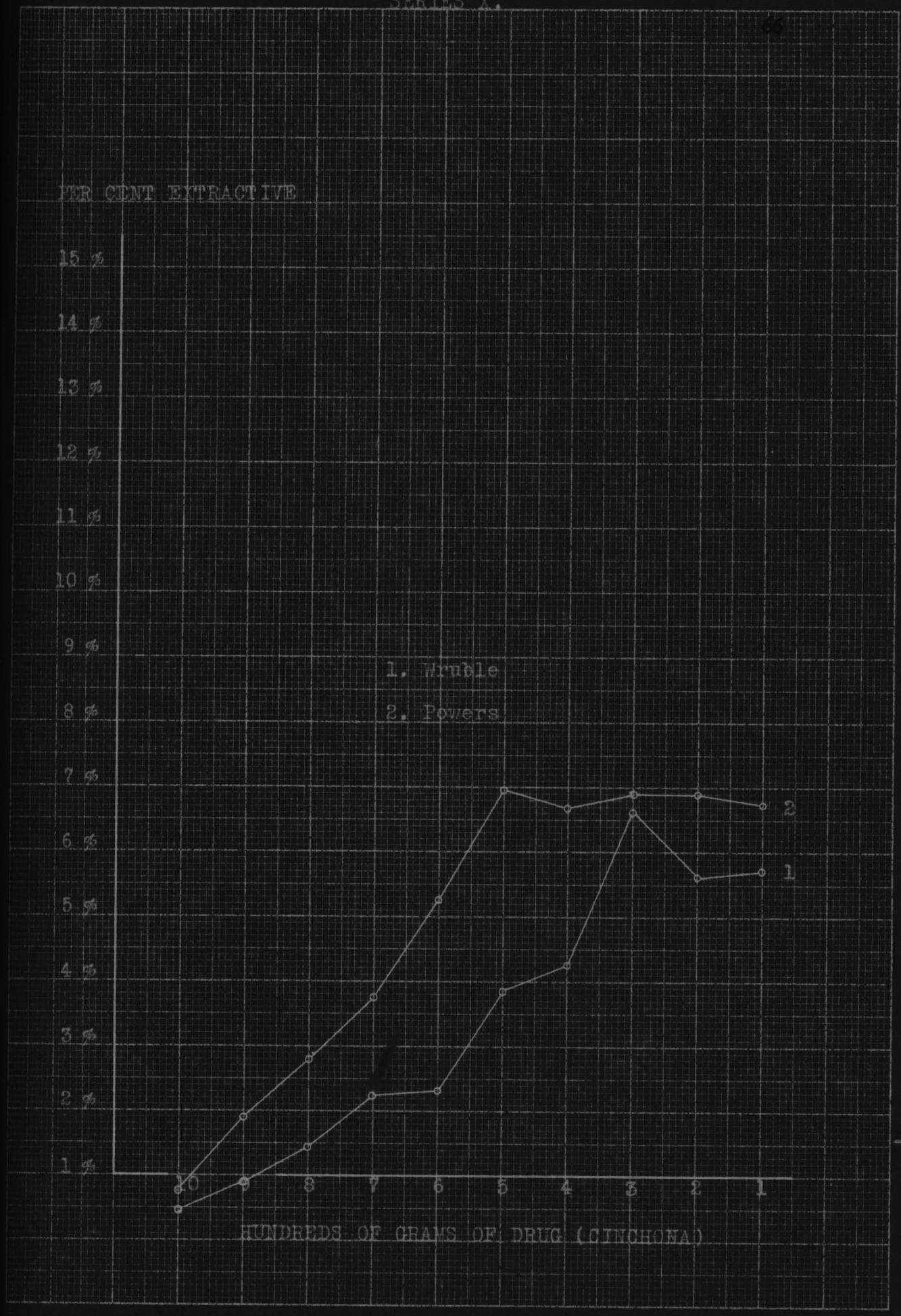
0.8500
0.8450
0.8400
0.8350
0.8300
0.8250
0.8200
0.8150
0.8100
0.8050
0.8000
1. Wruble
2. Powers
2
1
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAINS OF DRUG (CINCHONA)

SERIES X.


PER CENT EXTRACTIVE
15 %
14 %
13 %
12 %
11 %
10 %
9 %
8 %
7 %
6 %
5 %
4 %
3 %
2 %
1 %
1. Mrubble
2. Powers
2
1
10
9
8
7
6
5
4
3
2
1
HUNDREDS OF GRAMS OF DRUG (CINCHONA)

SERIES X.

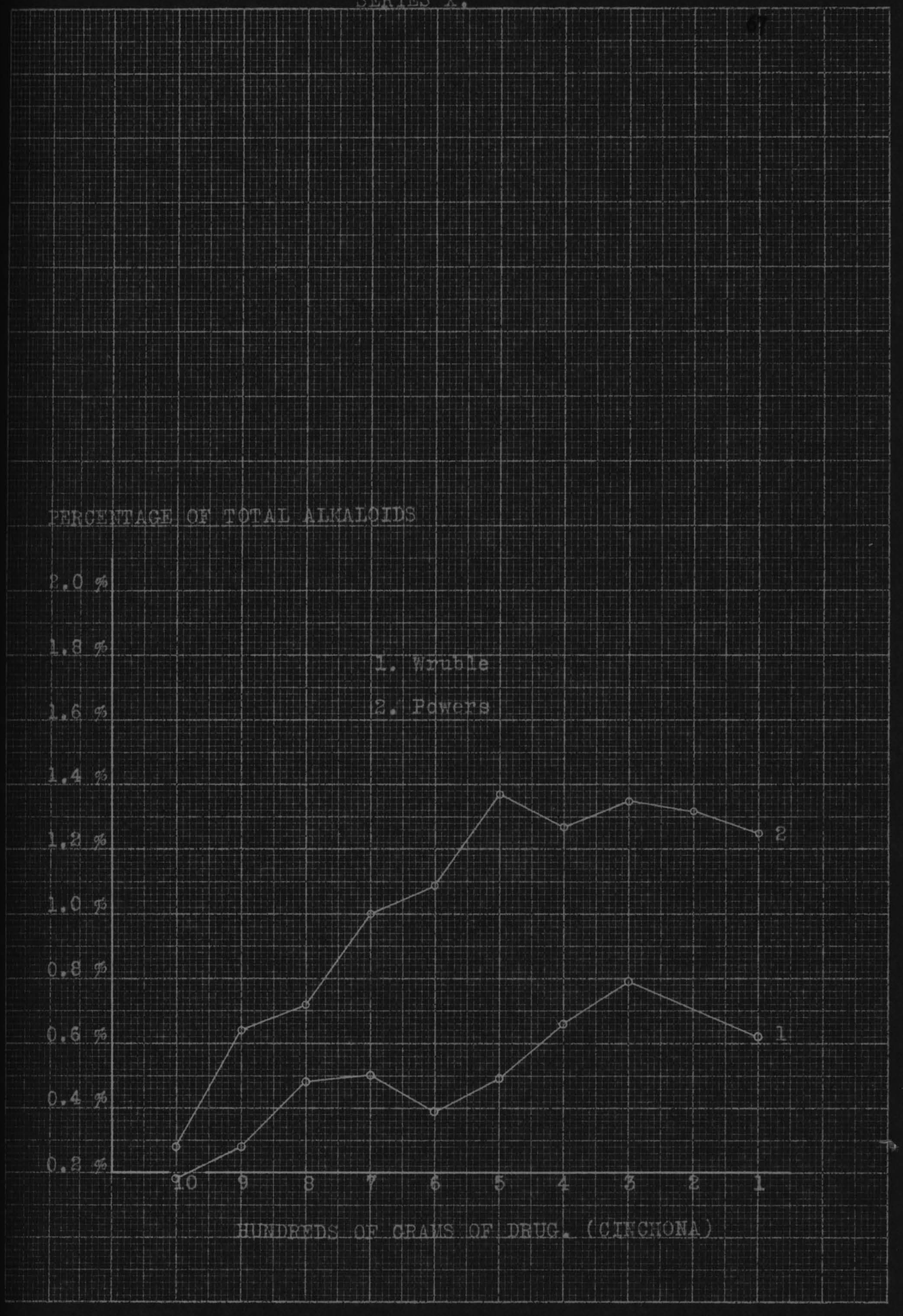
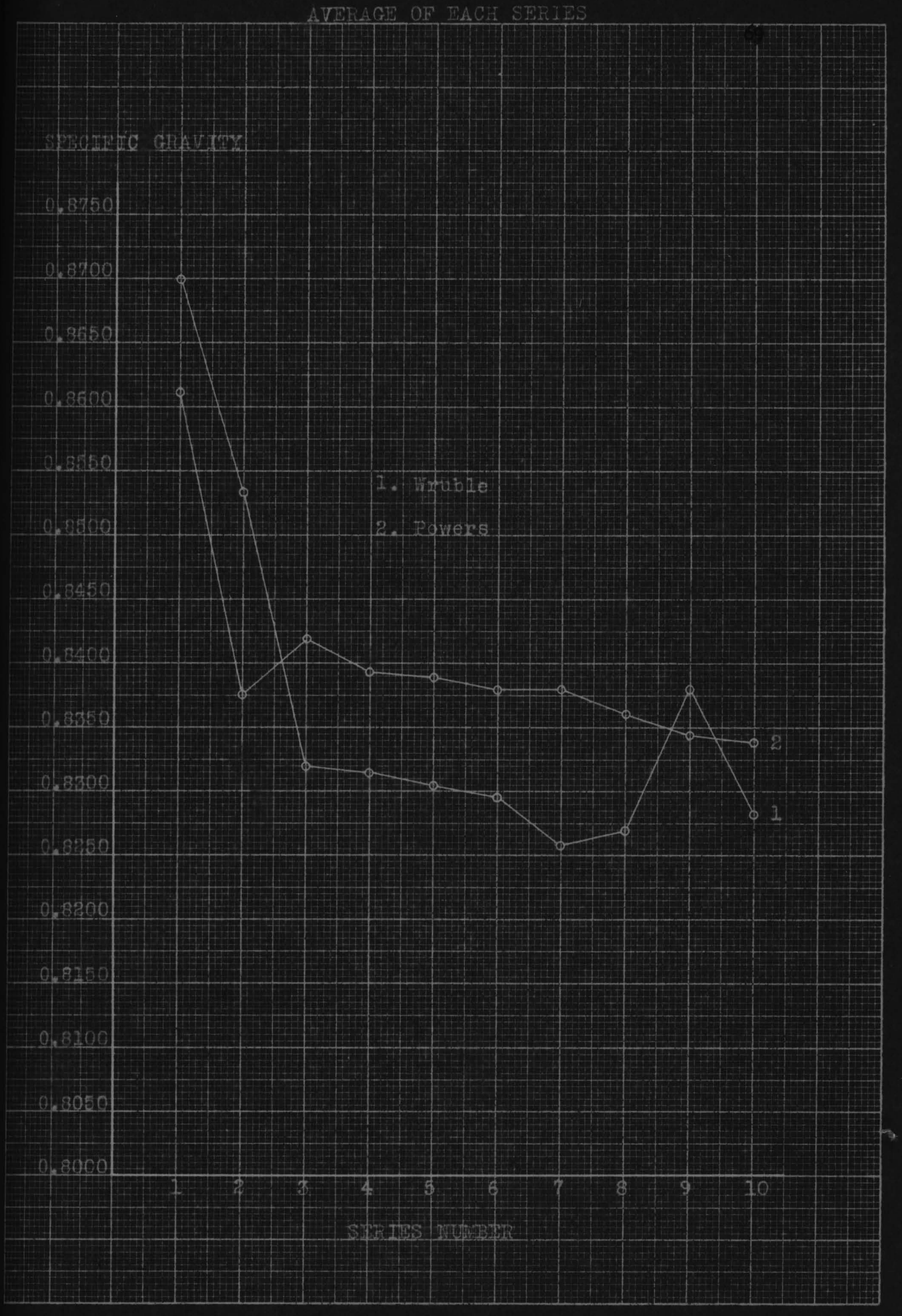

PERCENTAGE OF TOTAL ALKALOIDS
2.0 %
1.8 %
1.6 %
1.4 %
1.2 %
1.0 %
0.8 %
0.6 %
0.4 %
0.2 %
1. Wruble
2. Powers
2
1
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAMS OF DRUG. (CINCHONA)

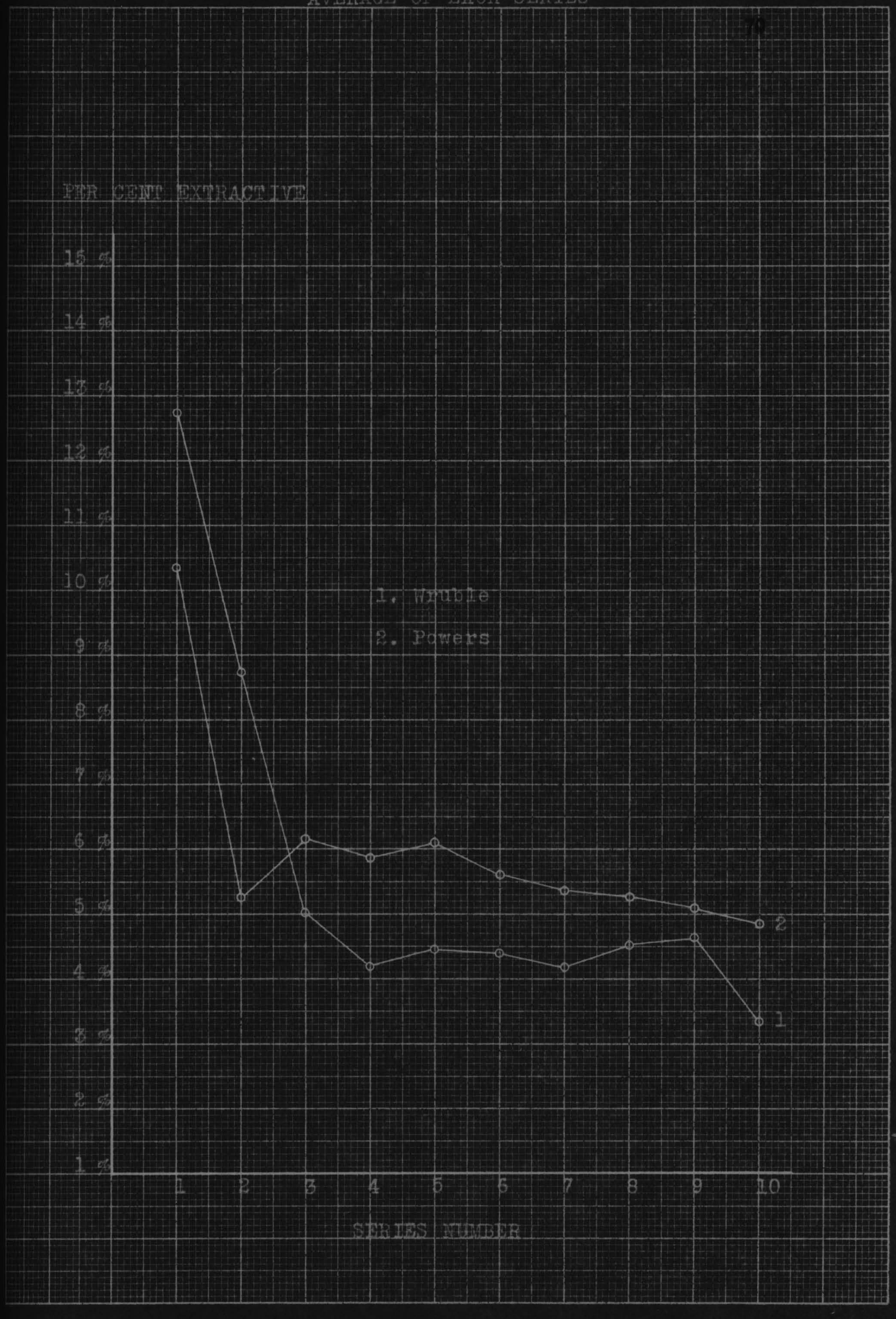
Table III

Average of each Series.

	Sp. Gr.		% Extractive		% Alkaloid	
Series	Wruble	Powers	W ruble	Powers	Wruble	Powers
I	0.8700	0.8612	12.74	10.37	1.53	2.12
II	0.8534	0.8376	8.72	5.26	0.92	1.15
III	0.8320	0.8419	5.02	6.17	0.72	1.20
IV	0.8316	0.8390	4.19	5.87	0.67	1.16
V	0.8305	0.8389	4.45	6.11	0.65	1.20
VI	0.8296	0.8379	4.40	5.61	0.57	1.18
VII	0.8258	0.8380	4.19	5.36	0.66	1.14
VIII	0.8269	0.8361	4.52	5.27	0.61	1.11
IX	0.8379	0.8344	4.64	5.10	0.64	1.10
X	0.8282	0.8338	3.34	4.86	0.49	1.03


AVERAGE OF EACH SERIES
SPECIFIC GRAVITY
0.8750
0.8700
0.8650
0.8600
0.8550
0.8500
0.8450
0.8400
0.8350
0.8300
0.8250
0.8200
0.8150
0.8100
0.8050
0.8000
1. Wruble
2. Powers
2
1
1 2 3 4 5 6 7 8 9 10
SERIES NUMBER

AVERAGE OF EACH SERIES



AVERAGE OF EACH SERIES

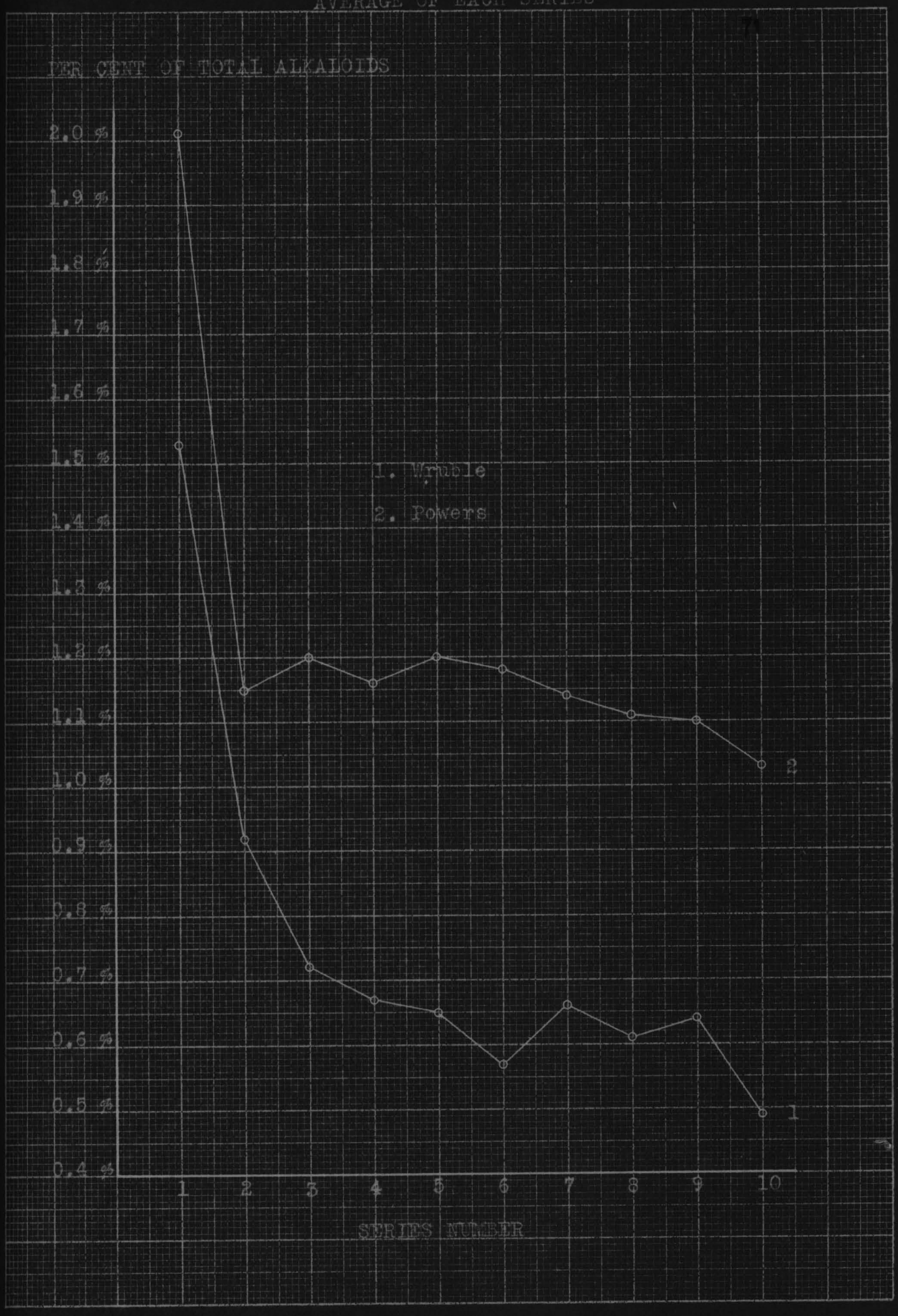
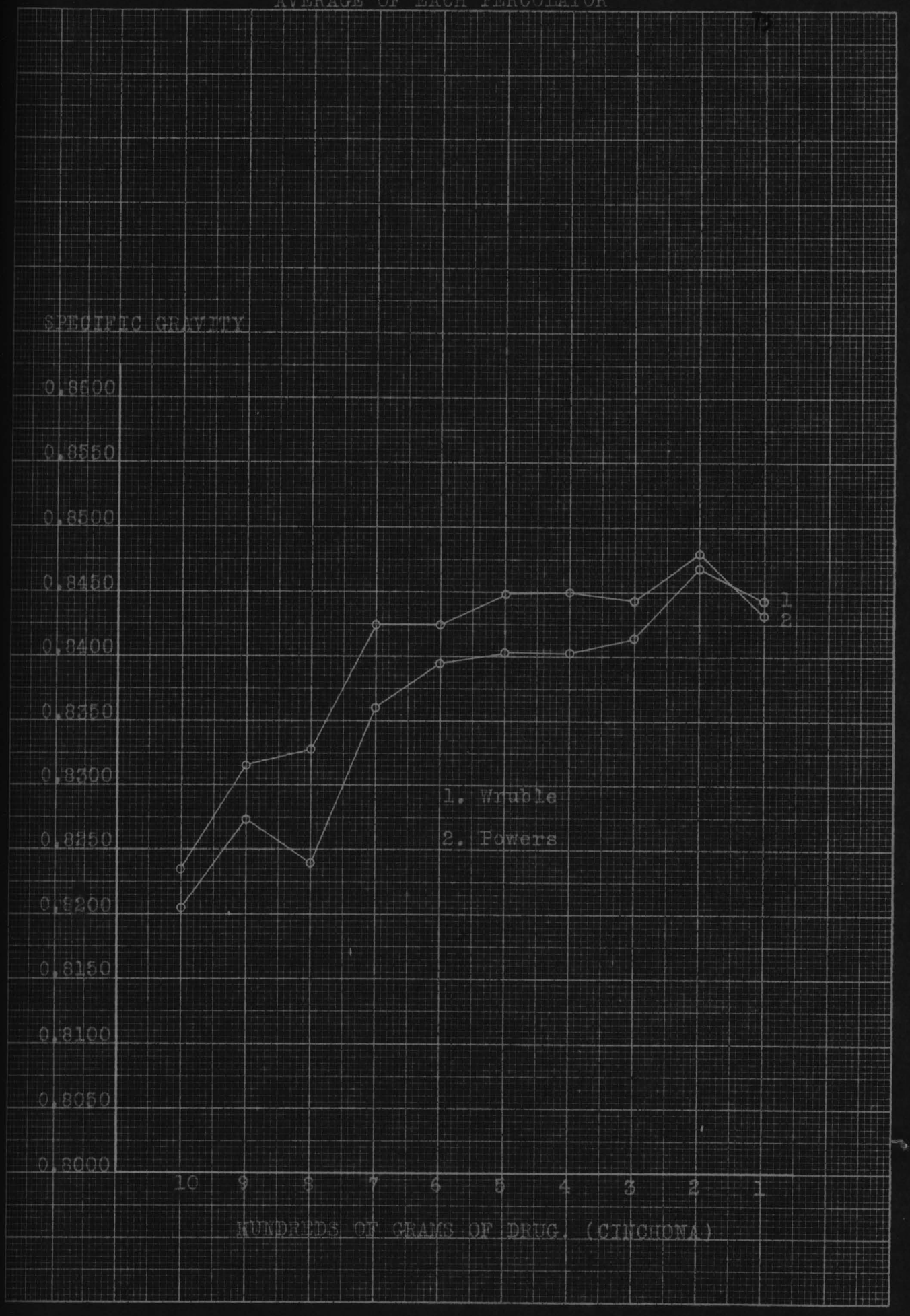


Table IV

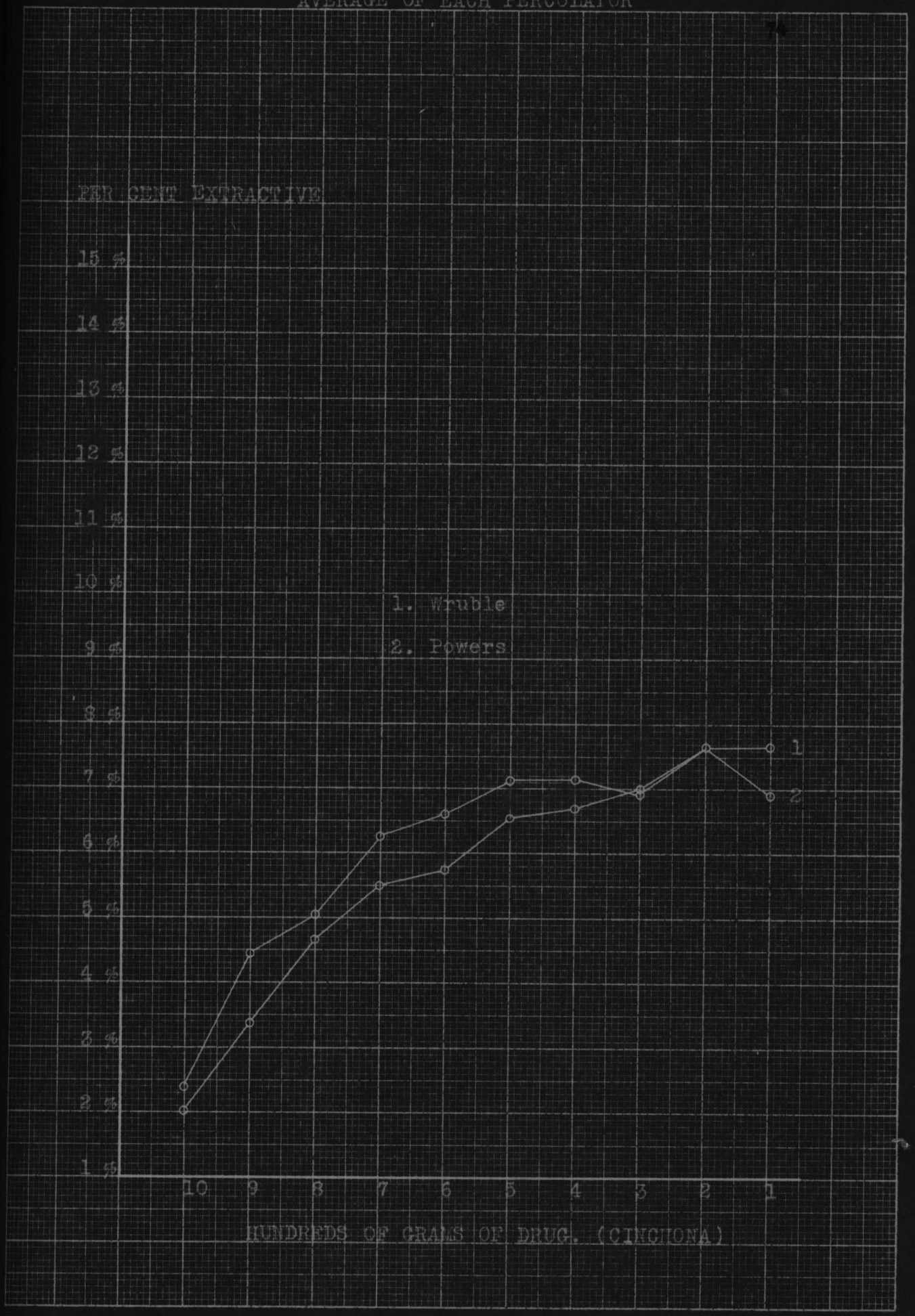
Average of each Percolator.

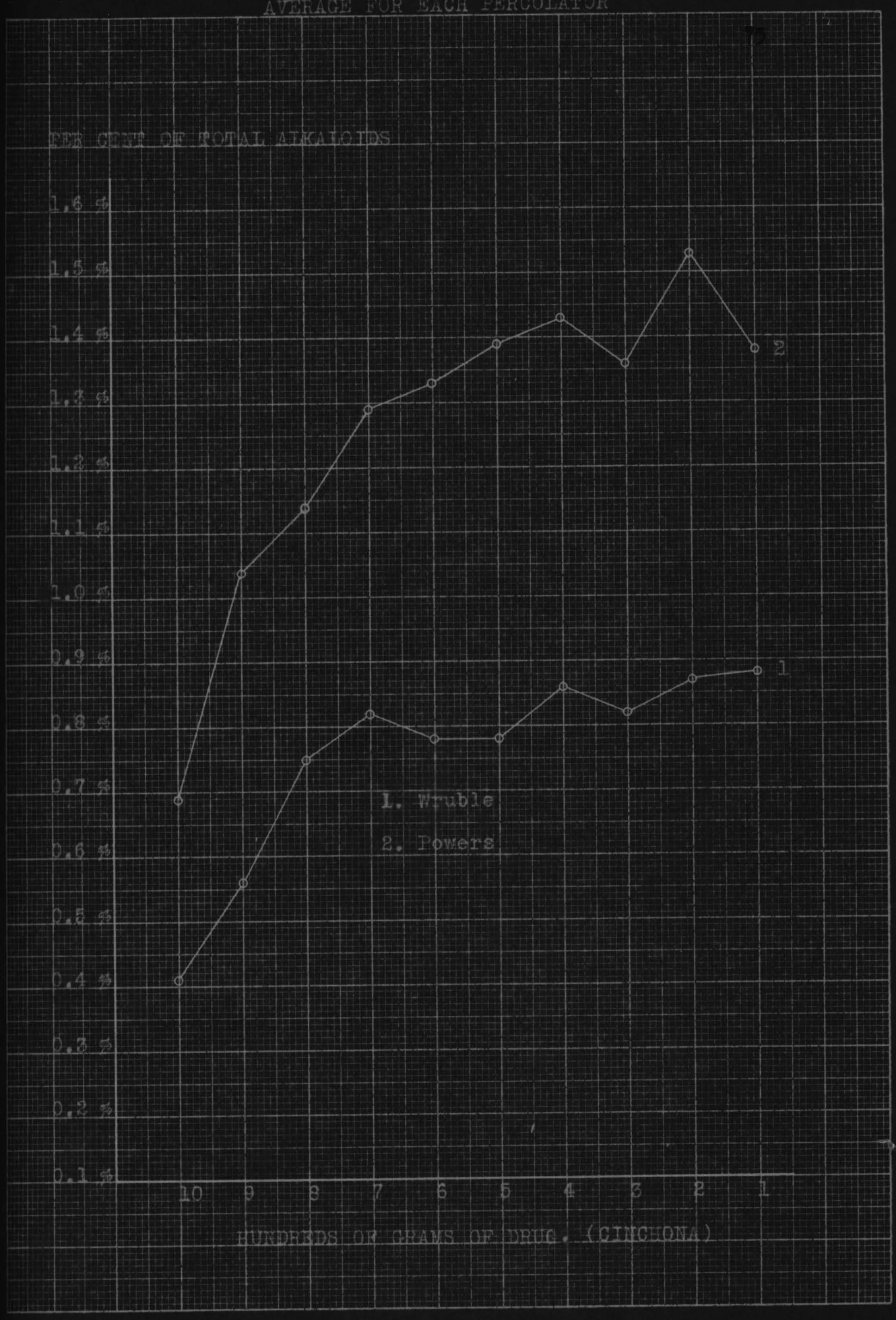
Percolator	Amt. of Drug Gms.	Sp. Gr. 20°		% Extractive		% Alkaloid	
		Wruble	Powers	Wruble	Powers	Wruble	Powers
I	1000	0.8205	0.8235	2.02	2.41	0.41	0.69
II	900	0.8274	0.8315	3.38	4.44	0.56	1.04
III	800	0.8239	0.8328	4.67	5.04	0.75	1.14
IV	700	0.8361	0.8424	5.50	6.26	0.82	1.29
V	600	0.8394	0.8424	5.73	6.61	0.78	1.33
VI	500	0.8403	0.8448	6.55	7.13	0.78	1.39
VII	400	0.8403	0.8451	6.69	7.15	0.86	1.43
VIII	300	0.8415	0.8440	7.00	6.93	0.82	1.36
IX	200	0.8468	0.8479	7.66	7.66	0.87	1.53
X	100	0.8444	0.8432	7.67	8.91	0.88	1.38

AVERAGE OF EACH PERCOLATOR



AVERAGE OF EACH PERCOLATOR




AVERAGE FOR EACH PERCOLATOR
PER CENT OF TOTAL ALKALOIDS
1.6 %
1.5 %
1.4 %
1.3 %
1.2 %
1.1 %
1.0 %
0.9 %
0.8 %
0.7 %
0.6 %
0.5 %
0.4 %
0.3 %
0.2 %
0.1 %
2
1
1. Wruble
2. Powers
10 9 8 7 6 5 4 3 2 1
HUNDREDS OF GRAMS OF DRUG. (CINCHONA)

Detannated Tincture of Cinchona. Wruble[6] prepared a completely detannated tincture of cinchona by mixing the powdered drug with an equal weight of calcium hydroxide, and then percolating the mixture with 95 per cent alcohol.

In order to determine the minimum proportion of calcium hydroxide to cinchona necessary for the formation of a detannated preparation, four tinctures were prepared with varying quantities of calcium hydroxide mixed with the drug previous to the percolation of it. For the purposes of comparison, a fifth tincture was prepared without previously mixing the drug with calcium hydroxide. For convenience in referring to them, these tinctures will be designated by numbers as follows:

	Wt. drug.	Wt. $Ca(OH)_2$	Vol. tincture collected.
Tincture No. 1	100 grams	100 grams	500 cc.
Tincture No. 2	100 grams	75 grams	500 cc.
Tincture No. 3	100 grams	50 grams	500 cc.
Tincture No. 4	100 grams	25 grams	500 cc.
Tincture No. 5	100 grams	00 grams	500 cc.

The calcium hydroxide used in the preparation of the first four tinctures was obtained by adding the calculated amount of water to a weight of calcium oxide necessary to convert it to the desired amount of calcium hydroxide. The cinchona was mixed with the calcium hydroxide, the mixture was moistened with sufficient alcohol to render it damp, and it was placed in a closed container for six hours. The moistened mixture was then transferred to a percolator, enough alcohol

was added to saturate it, and it was allowed to macerate during 24 hours. Percolation was then started and allowed to proceed at the rate of 20 drops per minute, until 500 cc. of percolate were collected. The same procedure was followed for Tincture No. 5, except that the drug was not mixed with calcium hydroxide.

The specific gravity of each tincture was determined at 25° by means of a pycnometer. The per cent of extractive was determined by allowing 10 cc. of each tincture, measured from a pipette, to evaporate spontaneously at room temperature, and then drying the residue to constant weight at 60°. From the specific gravity, the weight of each sample was determined and the per cent of extractive was calculated upon that basis. The per cent of alkaloids in each tincture was determined according to the method of the U. S. P. X., using 20 cc. samples. The weight of each sample was determined from the corresponding specific gravity, and the per cent by weight of alkaloids calculated therefrom. Tannin was determined by the same method as that used for the estimation of tannin content of the drug. The results of these determinations are tabulated below. (See next Page)

Tr. No.	Sp. Gr.	Per cent of Extractive	Per cent of Tannin	Per cent of Alkaloids
1.	0.8176	1.68	0.00	1.30
2.	0.8196	2.37	0.14	1.32
3.	0.8221	2.65	0.23	1.36
4.	0.8254	3.42	0.60	1.33
5.	0.8292	4.17	1.15	1.21

Tincture No. 1 was light yellow in color, and retained the same color after standing one year. There was no formation of a precipitate, except a small amount of crystalline material which separated at once. This was presumably calcium carbonate. Tinctures 2,3, and 4 were all of a cherry red color, the intensity increasing with the decrease in the quantity of calcium hydroxide used. After standing for a few weeks, a red deposit started to form which increased with time. After a year, a considerable quantity had separated. Tincture No. 5 was of a deep brownish-red color, and while a deposit of solid material started soon after its preparation, the quantity was less than in the three tinctures numbered 2,3, and 4. If any dependence can be placed upon the values obtained for tannin content of the various tinctures, it becomes apparent that calcium hydroxide, especially when used in quantities equal to the weight of the drug, prevents the extraction by alcohol of substances other than tannin. This may be illustrated by adding together the per cent of tannin and the per cent of alkaloid in each tincture, and then subtracting this sum from the per cent of extractive. The difference represents the per cent of extractive exclusive of tannin and alkaloid.

Tr. No.	Per cent of Extractive	Per cent Tannin plus Per cent Alkaloid	Difference (Per cent Extractive exclusive of Tannin and Alkaloid)
1.	1.68	1.30	0.38
2.	2.37	1.46	0.91
3.	2.65	1.59	1.06
4.	3.42	1.93	1.49
5.	4.17	2.36	1.71

From the above tabulation it is seen that as the ratio of calcium hydroxide to drug decreases, the per cent of extractive exclusive of tannins and alkaloids increases. It therefore appears that calcium hydroxide forms alcohol insoluble combinations not only with tannin, but also with other substances which otherwise would have been extracted.

The red color and the rapidity with which precipitates form in tinctures 2,3, and 4 indicates that calcium hydroxide, if used in sufficient quantities, prevents the extraction of cinchona red from the drug by alcohol. From the results obtained in the extraction with alcohol of cinchona which has been boiled with milk of lime it appears that in the presence of water a smaller amount of calcium hydroxide prevents the extraction of objectionable material than when water is absent. (See detannated fluidextract of cinchona.)

Fate of Cinchotannic Acid. Attempts were made to determine the fate of cinchotannic acid in a drug treated with calcium hydroxide. A sample of the dregs from tincture No. 1 was boiled with 1 per cent hydrochloric acid in an effort to

liberate the tannin from its calcium salt, the mixture was filtered, and the filtrate was tested for the presence of tannin by the addition of ferric chloride solution. No positive test could be obtained. This, however, might be expected since cinchotannic acid condenses readily in the presence of even low concentrations of mineral acids with the formation of cinchona red. Various organic acids were used in a manner similar to that mentioned for hydrochloric acid, but the same results were obtained in each instance. Carbon dioxide was passed into an aqueous suspension of the dregs for some time, and the mixture then filtered. The filtrate showed no tannin to be present. It appears that cinchotannic acid if regenerated from its calcium salt by acids is immediately decomposed.

Detannated Fluidextract of Cinchona. In the preparation of quinovin 1 kilogram of cinchona was boiled during several hours with 6 liters of milk of lime. The milk of lime was prepared by slaking 500 grams of lime and suspending the resulting calcium hydroxide in water. After filtering the mixture, the residue was air dried and then used for the preparation of the fluidextract.

The air dried dregs, representing 1 kilogram of drug, were moistened with 95 per cent alcohol and macerated for 6 hours in a closed container. The mixture was then packed in a percolator and sufficient alcohol was added to saturate it. The percolator was covered and the mixture was left to macerate for 48 hours. At the end of the maceration period perco-

lation was started, the first 850 cc. of percolate were reserved, and the process was continued until the drug was exhausted of alkaloids. Between 9 and 10 liters of percolate had to be collected in order to accomplish this. The percolate collected after the first 850 cc. had been reserved was concentrated to 150 cc. by distilling off the alcohol. Upon cooling the concentrated percolate, crystalline material separated. This was filtered off, dried, and found to weigh 29 grams. The dried material was identified as a mixture of alkaloids. It was found to dissolve readily in a 1 per cent aqueous solution of hydrochloric acid, and such a solution, upon the addition of alkaloidal reagents such as Mayer's, Wagner's, and Picric acid T. S., produced characteristic precipitates. These alkaloids were presumably a mixture of those of cinchona least soluble in alcohol, such as quinidine, cinchonine, and cinchonidine.

The filtrate from the alkaloidal material and the alkaloids was added to the 850 cc. of percolate originally reserved in order to bring the total volume of the fluidextract to 1000 cc. The fact that the alkaloids failed to dissolve was an indication that the fluidextract was saturated. The fluidextract was filtered from the alkaloids and portions of it were used for the determination of specific gravity, per cent extractive, and alkaloidal content. The results of these determinations are tabulated below. (See next page.)

Sp. Gr. at 25°	Per cent of Extractive	Alkaloidal content Per cent by weight	Alkaloidal content Grams per 100 cc.
0.8439	13.58	7.09	6.02

The U.S.P. X requires that fluidextract of cinchona shall contain not less than 4 grams and not more than 5 grams of the total alkaloids of cinchona in each 100 cc. It is seen that the detannated fluidextract exceeds the U.S.P. minimum requirement by more than 50 per cent. In order to make use of the alkaloids which had been separated from the percolate upon concentration, and at the same time dilute the detannated fluidextract so that it would comply with the U.S.P. requirements, the following procedure was used: One tenth, 2.9 grams, of the total alkaloids which had been obtained as stated above was dissolved in 20 cc. of a 5 per cent aqueous solution of hydrochloric acid. This solution was then diluted to 100 cc. with alcohol and mixed with an equal volume of the fluidextract. The 200 cc. of fluidextract thus obtained should contain 8.9 grams of the total alkaloids of cinchona, or 4.45 grams per 100 cc., which is within the limits of pharmacopoeial requirements. The same determinations were carried out with this preparation as previous to dilution, the results of which are tabulated below.

Sp. Gr. at 25°	Per cent of Extractive	Alkaloidal content Per cent by weight	Alkaloidal content Grams per 100 cc.
0.8440	10.33	5.39	4.55

The fluidextract prepared according to the method outlined above was of a clear brown color. It gave no test for tannin and the color precluded the presence of cinchona red. After standing for several weeks no precipitate formed. If there is any advantage to administering a preparation containing the total alkaloids of cinchona in the same proportion in which they occur in the drug, but without the disadvantage of the instability of the U.S.P. fluidextract, it would appear that this preparation might be useful.

For comparative purposes a fluidextract was prepared according to the U.S.P. directions. Five hundred grams of cinchona were moistened with a menstruum consisting of a mixture of 50 cc. of glycerin, 50 cc. of a 10 per cent aqueous solution of hydrochloric acid, and 400 cc. of alcohol, and macerated for 6 hours in a tightly closed container. The mixture was transferred to a percolator and enough of Menstruum I added to saturate the drug. After 48 hours, percolation was started and continued until the drug was exhausted, using the rest of Menstruum I and following that by a second menstruum consisting of a mixture of 4 volumes of alcohol and 1 volume of water. The first 425 cc. of percolate were reserved, the remainder was concentrated to 75 cc. and mixed with the reserved portion. An assay showed the presence of 8.94 grams of alkaloids per 100 cc. of fluidextract. A 100 cc. portion of this was diluted with an equal volume of Menstruum II (4 volumes of alcohol and 1 volume of water). The specific gravity, per cent of extractive, and alkaloidal content were determined, and the results

obtained are tabulated below.

Sp. Gr. at 25°	Per cent of Extractive	Alkaloidal content: Per cent by weight	Alkaloidal content: Grams per 100 cc.
0.9545	26.76	4.57	4.46

It is seen that the U.S.P. method for the preparation of the fluidextract is efficient insofar as extraction of the alkaloids is concerned. However, it extracts along with the alkaloids a large percentage of inert material which is positively disadvantageous in that it slowly precipitates and carries along with it varying proportions of the alkaloids, thereby weakening the activity of the fluidextract. From this standpoint the detannated fluidextract would be of some advantage, inasmuch as the percentage of extractive in addition to the alkaloids is much less than in the U.S.P. preparation.

Continuous Extraction of Cinchona with Alcohol. Ten kilograms of cinchona were extracted with alcohol by means of a Lloyd Continuous Extraction Apparatus during two weeks. The extract was concentrated within the apparatus to the consistence of a thick syrup. The volume of the concentrated extract was 3.75 liters, and its weight was 3.5 kilograms. A weighed portion of the concentrated extract was allowed to evaporate at room temperature, and was then dried to constant weight at 60°. The dried residue was found to correspond to 46.74 per cent of the weight of the alcoholic extract. The 3.5 kilograms of extract therefore represented approximately 1.64 kilograms of the dried extract, and this corresponded to 16.4 per cent of the weight of the drug.

As has already been mentioned, the drug used contained, according to the average of three assays, 9.13 per cent of alkaloids. The dregs from the extraction were air dried and assayed for alkaloidal content. The average of two assays showed that these still contained 5.81 per cent of the alkaloids. These results show that alcohol is not an efficient solvent for the extraction of cinchona alkaloids. That it was not an efficient solvent for other principles of cinchona was shown by the high percentage of extractive obtained by exhausting cinchona with the menstruum employed for the preparation of the U. S. P. X. fluidextract of the drug (see page 83). It was found that this menstruum is capable of extracting practically all of the alkaloids and from 30 to 40 per cent of additional extractive. In the manufacture of the fluidextract, however, this ability of the menstruum

to remove such a high percentage of extractive in addition to the alkaloids is a decided disadvantage. This excessive extractive, sometimes designated as "plant dirt" or "Ballaststoffe", is considered to be responsible for the precipitates which so frequently form in galenical preparations such as the fluidextract of cinchona [8]. In precipitating, it carries with it varying quantities of the active principles of the drug as was shown in the report of an examination of cinchona red (page 114). It is also entirely possible that the superfluous extractive of a galenical preparation may, due to absorbtion of the active principles, hinder to a great extent their absorbtion through the gastro-intestinal tract [9].

The 3.5 kilograms (3.75 liters) of the concentrated alcoholic extract from cinchona were extracted successively with the following solvents: petroleum ether, ether, chloroform, ethyl acetate, and acetone.

Extraction with Petroleum Ether. The concentrated alcoholic extract, in divided portions, was extracted by shaking it in a separatory funnel with petroleum ether. The extraction with each portion was continued until a sample of the petroleum ether upon evaporation left no residue. The petroleum ether was distilled off. The residue that remained was of a light green color and of about the consistence of petrolatum. It appeared to consist chiefly of fats and fat-like substances. The washings obtained by shaking a small portion of it with one per cent aqueous hydrochloric acid produced no precipitates upon the addition of alkaloidal

reagents. It therefore appeared that petroleum ether, as would be expected, was quite selective in its action. The weight of fats obtained by extraction with petroleum ether was 120 grams. The saponification number was determined for the fat. The results obtained are indicated below.

	Weight	Cc. 0.50 N. KOH	Saponification No.
Sample 1.	1.4898	6.35	119
Sample 2.	2.1272	9.15	123

Extraction with Ether. Attempts to follow the petroleum ether extraction with ether were unsuccessful. The ether removed the alcohol and left a semi-solid, plastic and sticky, unmanageable mass. In order to overcome this difficulty the concentrated alcoholic extract was mixed with about an equal bulk of purified oak sawdust (U. S. P. X., p. 455), the mixture was thoroughly dried and then powdered by means of a ball mill. The powdered material was next extracted with ether in a continuous extraction apparatus until the solvent appeared to remove no additional extractive. The ether war removed by distillation. The residue which remained was very similar in appearance to that from the petroleum extraction. The ether removed 35 grams of fat-like material. A portion of this when tested for alkaloids as described under the petroleum ether extract showed that some had been removed by the ether. The saponification number of the ether extract was then determined and the results of this determination are tabulated below.

	Weight.	Cc. 0.50 N. KOH.	Saponification No.
Sample 1.	1.3648	12.25	251
Sample 2.	1.1874	11.30	248

Because of the similarity between the petroleum ether and ether extracts the two were combined and saponified by means of alcoholic potassium hydroxide. A report of an examination of the fats will be found in the chemical part of this thesis in the chapter entitled "Cinchona Fats".

Extraction with Chloroform. The residue left after extraction with ether was followed in the continuous extraction apparatus by chloroform. The extraction was considered to be complete when 10 cc. of the chloroform solution upon evaporation left only a small residue. The chloroform was removed by distillation and left a nearly black, semi-solid, plastic mass which weighed 245 grams. A portion of the extract when dissolved in water produced a green color upon the addition of ferric chloride solution, thereby indicating the presence of tannin in the extract. When a portion of the extract was shaken with a 1 per cent aqueous solution of hydrochloric acid and filtered, the filtrate gave characteristic precipitates upon the addition of alkaloidal reagents such as Mayer's, Wagner's, and Picric Acid T. S., thus indicating that chloroform removed some of the alkaloids of cinchona. Various attempts were made to separate the chloroformic extract into crystalline components, but no satisfactory results were obtained.

Extraction with Ethyl Acetate. Because ethyl acetate is reputed to be somewhat selective in the extraction of certain types of tannins, it was used to follow chloroform. After several days of continuous extraction, however, it failed to remove more than a slight amount of material. Upon distillation of the solvent, only 7 grams of a yellow, amorphous, and very hygroscopic solid remained. It was not possible to characterize this, although qualitative tests indicated the absence of alkaloids and tannins.

Extraction with Acetone. The ethyl acetate was followed by acetone as solvent in the extraction of the concentrated alcoholic extract. The extraction was continued until no more material was dissolved by the solvent. The acetone was distilled off, and the residue which remained weighed 165 grams. It was nearly black in color and of a semi-solid consistence. Qualitative tests showed the presence of tannin and alkaloids in the extract. Attempts to separate the extract into crystalline components were unsatisfactory.

It was found that by this series of extractions that the only solvents at all selective in their action were petroleum ether and ether. The other solvents, chloroform, ethyl acetate, and acetone, did not extract at all satisfactorily the concentrated alcoholic extract. The sawdust mixture was extracted with alcohol until exhausted and then the alcohol was distilled off. The concentrated alcoholic extract weighed 2.75 kilograms. It was used as a source of tannin, of which it contained an appreciable quantity, for

hydrolysis and other experiments. Interest in other phases of the investigation prevented a return to a more intensive study of those extracts described above.

1). United States Pharmacopoeia, X, (1926), p. 109.

2). Jour. Am. Pharm. Assn., 22, (1933), p. 641.

3). Villavecchia, Applied Analytical Chemistry, 2, (1918), p. 338.

4). Circular 134, The Forest Service of the United States Department of Agriculture, (1908), pp. 1 - 7.

5). United States Pharmacopoeia, X, (1926), p. 466.

6). Jour. Am. Pharm. Assn., 23, (1934), p. 208.

7). United States Pharmacopoeia, X, (1926), p. 165.

8). Schweiz. Apoth.-Ztg., 67 (1929), p. 73.

9). Pharmacia, 14, (1934), pp. 284, 298.

CHEMICAL PART

CINCHOTANNIC ACID

CINCHOTANNIC ACID.

Cinchona bark, which was introduced into European medicine over three hundred years ago, during the early years of its medicinal use was believed to owe its curative properties to the presence of an astringent principle. It was compared with willow bark which was used as a febrifuge, and which likewise contains tannin.[1] The first references in the literature which related this astringent principle of cinchona bark to tannin appeared during the latter part of the eighteenth century. To Dolfuss[2] should probably go the credit for having first recorded the presence of tannin in this drug, which observation he announced in 1787. Four years later, and independently, Fourcroy[3] reported the results of his investigations of cinchona bark. He observed that infusions of cinchona bark blackened crystalline iron sulphate, and that the addition of lime water produced a precipitate. Iron metal rubbed with moistened cinchona bark was observed by Fourcroy to become black. He considered these results to be due to some principle similar to, but not identical with gallic acid. Shortly after Fourcroy's contribution was published, Berthollet[4] reported results of an investigation which were essentially the same as those of the former author. It is interesting to note, however, that Berthollet considered the astringent principle of cinchona bark to be a derivative of gallic acid. In this connection he stated: "En traitant le quinquina avec l'eau froide, il se dissout très peu de la poudre rougeâtre, que je regarde comme une

combinaison d'acide gallique, selon les preuves que je citerai après; c'est de ce principe duquel dépend la propriété du quinquina, de noircir les dissolutions de fer ..."

In 1810, Reuss[5] described in much detail the procedure and results obtained in an attempt to carry out a systematic separation of the constituents of cinchona bark. He prepared an alcoholic extract of the drug which was evaporated to dryness, and by means of different solvents and reagents separated into five parts which were designated by the author as follows:

(1) L'amer cinchonique

(2) Le rouge cinchonique

(3) Le cinchonate de Chaux

(4) Tannin

(5) Du muquex végétal insipide

The observation was made that an aqueous infusion prepared from an alcoholic extract contained more tannin than an infusion made directly from the drug. Such infusions were characterized by their effect on "red sulphate of iron", and the precipitating effects of lime water and solutions of glue upon them. Pfaff[6] in 1815 recognized the presence of tannin in aqueous infusions of cinchona bark by their effect on solutions of iron salts and solutions of glue.

In 1820 Pelletier and Caventou[7] announced the isolation of quinine and cinchonine, and in the same publication described a method for preparing the tannin of cinchona bark. They prepared an infusion of the drug with water slightly acidulated with hydrochloric acid, and precipitated this with

magnesium oxide. This precipitate was dissolved in diluted acetic acid as completely as possible. The solution was treated with lead acetate, the precipitate was collected, washed, suspended in water, and decomposed with hydrogen sulphide. Attention was called to the fact that this solution of tannin showed properties more comparable to the tannins of kino and catechu than to nutgall tannin.

Concerning the knowledge of the composition of cinchotannic acid in 1852, Schwarz[8] made the following statement: "Das Chinin und Cinchonin so wie die Chinasaeure sind ofters gegenstand von Untersuchungen gewesen, welche die Ausmittelung ihrer Zusammensetzung zum Zweke hatten. Ueber die Zusammensetzung der Chinagerbsaeure und des Chinaroth ist bis jetzt nichts bekannt." Schwarz subjected cinchotannic acid to a more thorough chemical examination than it had previously received. He described a new method for its isolation and purification, which involved the preparation of an aqueous decoction of the drug, the removal of cinchonared by precipitation with magnesium oxide, and then precipitation of the tannin with lead acetate. The lead salt thus obtained was suspended in water, and decomposed by hydrogen sulphide, the lead sulphide was removed by filtration, and the filtrate was evaporated to dryness in a desiccator over sulphuric acid. From the results of an elementary analysis the formula $C_{14}H_{16}O_9$ was assigned to cinchotannic acid. Schwarz also reported the following observations concerning the properties and reactions of this product: (a) cinchotannic acid boiled with hydrochloric acid is completely decom-

posed with the formation of beautiful red flakes which dissolve in alkaline liquids with a leek-green color, (b) the acid subjected to dry distillation produces a distinct odor of phenol, (c) an aqueous solution produces a precipitate when sulphuric acid is added to it, and (d) that an aqueous solution of it, if made alkaline, will on exposure to air rapidly absorb oxygen and change to cinchona red, during which time carbon dioxide is generated.

Rembold[9], in 1868, prepared cinchotannic acid according to the method described by Schwarz, and hydrolyzed it by boiling with dilute sulphuric acid. Cinchona red was formed as an insoluble precipitate which was filtered off. The filtrate was found to contain a sugar which gave reactions characteristic for dextrose. The cinchona red was fused with potassium hydroxide, and Rembold reported the isolation of protocatechuic acid from the melt. From the results of a combustion, the formula $C_{28}H_{22}O_{14}$ was deduced for cinchotannic acid.

The most recent and most intensive investigation on cinchotannic acid was that of Schütt[10], the results of which were published in 1900. Schütt prepared cinchotannic acid by extracting cinchona bark with ether and then with alcohol. The alcoholic extract was concentrated to a small volume, a and poured into a large quantity of water. The aqueous solutior was filtered from the water-insoluble material, the filtrate treated with lead acetate, and the lead salt of cinchotannic acid which precipitated was removed by filtration, washed until free of lead and acetic acid, and dried

on a porous plate. The dried lead salt was suspended in alcohol, and decomposed with hydrogen sulphide. The lead sulphide was removed, and the filtrate was freed from hydrogen sulphide by passing a current of carbon dioxide through the alcoholic solution. When the cinchotannic acid was wanted for use in his experiments, it was obtained by distilling the alcohol under reduced pressure in a current of carbon dioxide. Schütt was unable to obtain the material in a crystalline condition. However, he determined the carbon and hydrogen by combustion, and from the results of his analysis assigned the formula $C_{43}H_{50}O_{20}$ to cinchotannic acid. Hexaacetyl and hexabenzoyl derivatives were also prepared in an amorphous condition, and formulas were assigned to them after molecular weight determinations and combustions. Schütt hydrolyzed a sample of his product with diluted sulphuric acid, and reported that he obtained an osazone, presumably of glucose, from the solution left after removing the cinchona red formed in the reaction. However, he did not consider that this indicated that the tannin of cinchona is glucosidal in character. He states in this connection: "Von einem Glykosidcharackter der Chinagerbsaeure zu sprechen besteht keine Berechtigung, da die geringe Mengen von Dextrose, welche bei der Einwirkung von verdünnter Schwefelsaeure neben Kohlensaeure und Chinarot auf den Gerbstoff der Chinarinde entstehen, beweissen dürften, dass der Chinagerbstoff, wie überhaupt die Gerbstoffe in Pflanzenreiche kaum als eine selbständige, rein darstellbare atomgruppe bestehen können."

Nierenstein[11] reports that he has examined two authentic barks of different species of cinchona, which were found to contain no catechin-like substance. He states that any further work upon this tannin will have to be far more botanically precise than those which have been carried out, if it is to be of any value to plant chemistry.

The difficulty in preparing cinchotannic acid, and the ease with which it changes to the phlobaphene, make it appear that it will be among the last of the tannins to have any light thrown on its structure. Quite obviously the work which has been done on it really throws very little light upon its actual constitution. It is likely to remain in the list of unclassified tannins for some time to come.

Experimental.

Preparation of Cinchotannic Acid. Cinchotannic acid was prepared by the method used by Schdtt[10], and by modifications of it. The following described method was found to be the most satisfactory of any used, and was employed several times. One kilogram of cinchona bark was extracted by percolation with 95 per cent alcohol until the percolate was only slightly colored. In order to accomplish this, it was necessary to collect from five to seven liters of percolate. The alcohol was recovered by distillation, the percolate being concentrated to about 250 cc. The concentrated percolate was poured into two liters of cold water, and the water-insoluble material which separated was filtered off and used for the preparation of cinchona red. The filtrate was treated with an aqueous solution of lead acetate until no further precipitation occurred. The precipitate, consisting principally of the lead salt of cinchotannic acid, was collected on a filter, washed repeatedly with water until the washing gave no test for lead, and finally dried on a porous plate. The dried material was suspended in 300 cc. of 95 per cent alcohol, and hydrogen sulphide was passed into the suspension for several hours. The lead sulphide which precipitated was removed by filtration, and the filtrate containing cinchotannic acid was freed from hydrogen sulphide by passing carbon dioxide through the liquid. The alcohol was then removed by distillation under reduced pressure in an atmosphere of carbon dioxide. The The yield varied from 2 to 5 grams.

Properties of Cinchotannic Acid. Cinchotannic acid prepared by the above method was of a light yellow color, and occurred in the form of scales. It was very freely soluble in water, alcohol, and acetone. It was insoluble in ethyl acetate, ether, petroleum ether, and benzene. Very dilute aqueous solutions produced a green color with ferric salts. Precipitates were formed with solutions of calcium hydroxide, barium hydroxide, sodium hydroxide, and potassium hydroxide from which the acid could not be regenerated. Upon exposure to the air, cinchotannic acid rapidly became dark red in color, and insoluble in water. It was found best to preserve cinchotannic acid in an alcoholic solution in sealed containers, in which the air had been displaced by carbon dioxide.

Attempts to Hydrolyze Cinchotannic Acid. Many attempts were made to isolate recognizable products from alkali fusions, as well as from hydrolyzing the product with different strengths of solutions of alkalies. It was impossible to obtain satisfactory results, although many varied conditions were employed.

Attempt to Determine if Cinchotannic Acid Is Combined with a Sugar. Both Rembold[9] and Schdtt[10] reported dextrose as a product of the acid hydrolysis of cinchotannic acid. The latter author claimed to have prepared an osazone having a melting point of 202°, which he believed to be glucosazone. In order to determine if the cinchotannic acid prepared from cinchona used in these experiments yielded a sugar upon acid hydrolysis, the following

procedure was used: Two grams of cinchotannic acid were added to 25 cc of a 5 per cent solution of sulphuric acid in water. Almost immediately a red precipitate formed. The mixture was boiled during thirty minutes, and then filtered. The filtrate was treated with lead acetate to remove the sulphuric acid, and then the excess lead was removed by means of hydrogen sulphide. After filtering, the solution was boiled to remove the hydrogen sulphide, and divided into two parts. One portion was boiled with Fehling's solution, and set aside for some time. No reduction of the copper compound occurred. To the other portion was added 0.5 gram of phenylhydrazine hydrochloride, an equal weight of anhydrous sodium acetate, and the mixture was heated in a boiling water bath during two hours. No precipitate formed even after the mixture had stood 24 hours.

Pyrolysis of Cinchotannic Acid. One gram of cinchotannic acid was placed in a pyrex test tube, 2 cm. in diameter by 20 cm. in length, which was provided with an horizontal side tube of 5 mm. in diameter, placed about 5 cm. from the mouth. This was connected with an air condenser, the end of which was attached to a suction flask. The system was evacuated of air by means of a high vacuum oil pump which reduced the pressure to less than 1 mm. By means of an oil bath, heat was applied to the test tube containing the cinchotannic acid. When the temperature of the bath had risen to 245°, a small quantity of white, prismatic crystals collected in the upper part of the condenser. In the upper part of the test tube, yellowish white, needle shaped crystals

collected. The heat was continued until considerable charring took place in the lower part of the test tube, at which time the heat was removed, and after cooling, the test tube was disconnected from the rest of the system. The white crystals were scraped from the wall of the condenser, and the yellow crystals from the wall of the test tube. The melting point of the white crystals was found to be 102 - 103°, while that of the yellow crystals was found to be 170 - 180°. The charred residue in the test tube was extracted with a small amount of a 5 per cent aqueous solution of sodium hydroxide, the mixture was filtered, and the filtrate was acidified with hydrochloric acid. The acidified solution was extracted several times with ether, the ethereal extract was dried with anhydrous sodium sulphate, and the ether allowed to evaporate spontaneously. The residue which remained was nearly white, and appeared to be crystalline. The melting point was 195 - 200°, with decomposition. When mixed with an authentic sample of protocatechuic acid, the mixed melting point was 198°, with decomposition. The melting point of the protocatechuic acid alone was 198°, with decomposition.

The white crystals which collected in the condenser were freely soluble in water, and dilute aqueous solutions were colored green by the addition of ferric chloride, and turned to violet upon the addition of ammonia water. This reaction is characteristic of the ortho-dihydroxy compounds. The material was recrystallized from a mixture of high boiling petroleum ether and benzene, after which it melted sharply at 104°.

When mixed with an authentic sample of catechol, melting point 104°, there was no depression in the melting point. Large enough quantities of either product of pyrolysis could not be obtained to prepare derivatives.

The preceding pyrolysis experiments were repeated, using cinchotannic acid which was mixed with (a) an equal weight of calcium oxide, (b) an equal weight of barium hydroxide, and (c) an equal weight of sodium hydroxide. In each of the three instances, catechol was formed and collected in the condenser, but no protocatechuic acid could be isolated from the melt.

1). M. Nierenstein, The Natural Organic Tannins, London, (1934), p. 228.

2). Crell's Chemische Annalen, ii, (1787), p. 147; through Nierenstein, The Natural Organic Tannins, London, (1934), p. 227.

3). Annales de Chemie, 8, (1791), p. 113.

4). Annales de Chemie, 16, (1793), p. 172.

5). Reuss, Nouvelle analyse du principe febrifuge du quinquina, Moscow, (1810); Göttingische gelehrte Anzeigen, (1812), p. 601; Jour. Pharm., I, (1815), p. 488.

6). Jour. Pharm., I, (1815), p.556.

7). Ann. Chim. et Phys., 15, (1820), pp. 308 & 337.

8). Jour. prakt. Chem., 56, (1852), p. 76.

9). Jour. prakt. Chem., 103, (1868), p. 217.

10). B. Schütt, Zur chemischen Charakteristik der Bestandteile der Chinarinde, Dissertation, Hanover, (1900).

11). M. Nierenstein, The Natural Organic Tannins, London, (1934), p. 228.

CINCHONA RED

CINCHONA RED.

Cinchona Red, the phlobaphene which is characteristic of the barks of the various species of cinchona, was first described in 1810 by Reuss[1], when he reported the results of his investigations upon cinchona bark. He extracted the bark with alcohol, evaporated the resulting liquid to dryness, and separated the dried extract into five distinct parts. To the amorphous, red, water-insoluble portion he applied the name, rouge cinchonique. Ten years later Pelletier and Caventou,[2] in the same publication in which they announced the discovery of quinine, called attention to a rouge cinchonique soluble and a rouge cinchonique insoluble which they obtained during the course of their investigations. In 1828, Berzelius[3] repeated the experiments of Pelletier and Caventou relating to this particular phase of the work, and extended their observations. He concluded that the rouge cinchonique soluble of these investigators was a tannin similar to that from nutgalls. The rouge cinchonique insoluble he designated as unlösliches Chinarot. Berzelius also called attention to the readiness with which cinchotannic acid changes into cinchona red upon contact with air.

Some years later, Schwarz[4] prepared what he considered to be a pure form of cinchona red, by depriving cinchona bark of all the material soluble in boiling water, and then exhausting the dregs with ammonia water. The latter product was acidified with hydrochloric acid which precipitated the

cinchona red and quinovin. The precipitate was then boiled with milk of lime, which formed an insoluble combination with cinchona red, but a soluble salt of quinovin. The mixture was filtered, and the residue was decomposed with hydrochloric acid, washed with water until free of the acid and calcium chloride, and finally was dissolved in ammonia water, and again precipitated with hydrochloric acid. The cinchona red obtained in this way was not crystalline, but Schwarz determined the carbon and hydrogen content of his product by a combustion analysis, and from the results obtained, carbon, 53.6 - 54.4 per cent and hydrogen, 5.4 - 5.7 per cent, deduced the empirical formula $C_{12}H_{14}O_7$. He assumed that it was formed from cinchotannic acid, the formula of which he had also determined from the results of a combustion of amorphous material, according to the following equation: $C_{14}H_{16}O_9 + 3\,O = C_{12}H_{14}O_7 + 2\,CO_2 + H_2O$.

Guiraud-Boissenot[5], in 1854, reported the results of his examination of cinchona red. By distillation of the dry material, he claimed to have obtained pyrogallic acid, a carmine red substance, and an empyreumatic oil. As the result of a combustion analysis, he assigned to it the empirical formula, $C_{14}H_8O_6$. He added also that it contained traces of calcium and iron.

The next publication relating to cinchona red was that by Rembold[6]. This author reported that cinchona red obtained by the method described by Schwarz was identical with the product obtained from the spontaneous decomposition of cinchotannic acid upon exposure to the air. He reported that he

was able to isolate protocatechuic acid from the melt of the phlobaphene by fusion with potassium hydroxide. From the results of a combustion analysis, carbon, 57.5 per cent and hydrogen, 3.9 per cent, Rembold calculated for and assigned to cinchona red the formula, $C_{28}H_{22}O_{14}$.

The most recent work upon cinchona red is that of Schütt[7] who prepared it first by extracting cinchona bark with ether, and then with alcohol. The alcoholic extract was evaporated to a small volume, and poured into a large quantity of water. The water-insoluble portion which separated was filtered off, dried, and dissolved in alcohol. This solution was boiled for some time with activated charcoal, cooled, and filtered. The filtrate was poured into water, and the water-insoluble material which separated was considered by him to be cinchona red. This was separated from the liquid by filtration, dried at 105°C, and portions of it used for an elementary analysis. The results of his analyses showed that his product, which was amorphous, contained 65.05 per cent of carbon, and 6.46 per cent of hydrogen, from which he calculated the formula for cinchona red to be $C_{26}H_{29}O_9$.

The utter futility of attempting to determine the ultimate composition of amorphous organic compounds is strikingly shown in the very decided lack of agreement in the results obtained by various workers upon cinchona red. It is extremely doubtful that the investigators whose works have been referred to were working with identical substances. In the case of cinchona red prepared by the method described by Schütt, it has been possible to prove conclusively that the

product is contaminated with varying proportions of quinovin. It has also been possible to show that the phlobaphene or cinchona red which separates from galenical preparations of cinchona upon standing, contains appreciable quantities of alkaloids as well as quinovin.

Experimental.

Preparation of Cinchona Red. Fifty grams of the water-insoluble material, which had been obtained in the preparation of cinchotannic acid when the concentrated alcoholic extract from 1 kilogram of cinchona was poured into water, were air dried, and then dissolved in 500 cc. of alcohol. Ten grams of activated charcoal (Darco) were added to the alcoholic solution, and the mixture was boiled under a reflux condenser during 2 hours. The charcoal was removed by filtration, and the process was repeated. The clear alcoholic solution was poured into 4 liters of water, and the reddish brown precipitate which formed, considered by Schütt[7] to be cinchona red, was filtered off and dried.

Examination of Cinchona Red. Inasmuch as Schütt had prepared cinchona red from a drug which had been extracted with ether before being extracted with alcohol, it seemed advisable to extract the product, prepared as above, with a fat solvent. Accordingly, 25 grams of cinchona red were placed in an extraction thimble, and extracted in a Soxhlet extractor with petroleum ether. When completely extracted, the petroleum ether was distilled off, and the residue which remained was of an oily appearance similar to that of the petroleum ether extract of the drug. It weighed 1.47 grams, representing 6 per cent of the weight of cinchona red. Its saponification number was determined, and found to be 146.

The cinchona red thus purified was extracted with ether

in the Soxhlet extraction apparatus. An ether extract weighing 3.76 grams was obtained. This extract was washed with 0.5 per cent hydrochloric acid, and found to contain alkaloids. The weight after washing and drying was 2.1 grams. This material was dissolved in alcohol, water was added until it began to precipitate, and it was set aside for several days. Nearly white crystals formed which did not melt sharply. Additional recrystallizations did not remedy this condition. It was suspected that this material might be a phytosterolin. Accordingly it was subjected to acid hydrolysis according to the method used by Power and Salway[8] for this type of compound.

Hydrolysis of the Ether Extract of Cinchona Red. Two g grams of the purified material were dissolved in 100 cc. of hot amyl alcohol, and 40 cc. of an aqueous 15 per cent solution of hydrochloric acid were added, together with enough ethyl alcohol to make a homogenous liquid. The mixture was boiled under a reflux condenser during 2 hours. Definitely crystalline material separated, which was filtered off after cooling the mixture, washed with alcohol, and dried on a porous plate. The substance melted with decomposition at 315°C. The melting point, together with the insolubility of the product of hydrolysis in the amyl alcohol mixture, eliminated phytosterolin from consideration. The substance was found to possess acidic characteristics, but quinovic acid was not immediately suspected because the melting point obtained was higher than any reported for that acid.

In order to learn more concerning the acid, an elementary

analysis was made, with the following results:

	Wt. of Sample	Wt. of CO_2	Wt. of H_2O	Percent of: Carbon	Hydrogen
I.	0.0694	0.1894	0.0574	74.43	9.40
II.	0.0668	0.1822	0.568	74.26	9.49

From the above results, the formula, $C_{30}H_{46}O_5$, may be calculated. Molecular weights computed from the titration values are tabulated below:

	Wt. of Sample	Cc. N/10 KOH required for neutralization.	Molecular weight computed, assuming a monocarboxyllic acid
I.	0.1872	7.55	248
II.	0.1415	5.80	244
		Average	246

The molecular weight computed from the formula derived from the combustion is 486. If it is assumed that the compound is a dicarboxyllic acid, the calculated neutral equivalent would be 243, which is in close agreement with the results found. These data, when compared with those reported by Wieland[9], with the exception of the melting point for quinovic acid, made it appear that the ether extract, after having been freed from alkaloids, was quinovin, and the product obtained by its hydrolysis was quinovic acid. In order to determine defiritely if this supposition was true, an authentic sample of quinovin was prepared as described on page 122, and this was hydrolyzed in exactly the same way as the compound obtained from cinchona red. The melting point of the quinovic acid thus prepared was found to be 315°, and a mixture of

the two compounds also melted at this temperature. To characterize further the product of hydrolysis of the material extracted from cinchona red with ether as quinovic acid, the triacetyl and the monoacetyl derivatives were prepared in exactly the same manner as described on page ___ for the preparation of the corresponding derivatives made from authentic quinovic acid. The melting points were the same, and when the parallel derivatives were mixed, there were no depressions in the melting points of the mixtures.

Examination of Cinchona Red from Tincture of Cinchona. The tinctures, the preparation of which was described in the galenical part of this report, after standing in a dark place during ten months, had deposited a quantity of red flaky material, commonly referred to as cinchona red. This material was collected on a filter and dried. From about 8 liters of tincture, 12.5 grams of the dried material were obtained.

Qualitative Tests. One-tenth of a gram of the dried material obtained from the tinctures was boiled with 10 cc. of a 0.1 per cent aqueous solution of hydrochloric acid. The mixture was cooled and filtered, and the filtrate was divided into three parts. Each portion was tested for the presence of alkaloids by the addition of a few drops of Mayer's Reagent to the first, Wagner's Reagent to the second, and Picric Acid T.S. to the third portion. In each instance a precipitate was formed, indicating thr presence of alkaloids.

Quantitative Determination of Total Alkaloids. Two and one-half grams of cinchona red from the tinctures of cinchona were assayed for total alkaloids, according to the official method.[10] An aliquot part, representing 2 grams of the sample, was found to contain 0.2494 gram, or 12.47 per cent of total alkaloids.

Isolation of Quinovin. Five grams of cinchona red were digested with 25 cc. of milk of lime. The mixture was filtered, and the filtrate was acidified with hydrochloric acid. The gelatinous precipitate which formed was filtered off and dried. It was identified by hydrolyzing in the way previously described for quinovin. The product of hydrolysis, of which only a trace was obtained, was identified by its melting point, 315°, and its melting point, which remained unchanged, when mixed with quinovic acid.

Hydrolysis and Pyrolysis of Cinchona Red. Experiments identical with those described under cinchotannic acid were applied to cinchona red from the two sources described above. No products arising from such treatment could be isolated and identified.

1.) Reuss, Nouvelle analyse du principe febrifuge du quinquina, Moscow (1810);
Göttingishe gelehrte Anzeigen, p. 601, (1812);
Jour. Pharm., I. (1815), p. 488.

2.) Ann. Chim. et Phys., 15, (1820), pp. 308 &337.

3.) Jahres-Berichte uber die Fortschriftte der physischen Wissenschaften, VII., (1828), p. 253.

4.) Jour. prakt. Chem., 56, (1852), p. 76.

5.) Jour. Pharm., 25, (1854), p. 199.

6.) Jour. prakt. Chem., 103, (1868), p.217.

7.) B. Schütt, Zur chemischen Charakteristik der Bestandteile der Chinarinde, Hanover, Dissertation, Munich, (1900).

8.) Jour. Chem. Soc., 103, (1913), p. 399.

9.) Ann., 453, (1927), p. 83.

10.) U. S. P. X, p. 109.

QUINOVIN

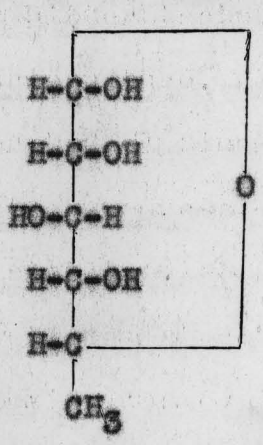
QUINOVIN.

Quinovin (Ger. Chinovin) has been known since 1821, when it was discovered by Pelletier and Caventou[1] in the bark of false cinchona, Cinchona nova, and was reported by them under the name of acide quinovique. Later investigators named this substance chinovabitter because of its intensively bitter taste, and a lack of understanding concerning its true nature. Hlasiwetz[2,3] in 1851 described a method for hydrolyzing quinovin with hydrochloric acid, and reported two products of hydrolysis, one amorphous and the other crystalline. He recognized the acidic properties of the crystalline material, and noted upon heating it that carbon dioxide was evolved, and a new acid was formed. Several years later Hlasiwetz[4] characterized the amorphous component obtained from the hydrolysis of quinovin as a sugar. Having thus established the glucosidal character of the acide quinovique of Pelletier and Caventou or chinovabitter of other authors, Hlasiwetz suggested that the name chinovin be applied to the glucoside, and that chinovasäure be restricted to the acid component. At the same time a method of preparation for the glycoside was described, and several salts of quinovic acid were reported.

In the interim between the discovery of quinovin and the work of Hlasiwetz upon it, as well as subsequently, a large number of publications appeared in which the presence of this principle was reported in nearly all of the different species of cinchona and in other members of the Rubiaceae

family.[5-15]

The sugar component of the glucoside quinovin was studied by E. Fischer and C. Liebermann[16]. They established the fact that it is a rhamnose type of sugar and gave it the name chinovose. Later work upon the sugar by Freudenberg and Raschig[17] established the structure given below, which indicates that it is d-Epi-rhamnose.



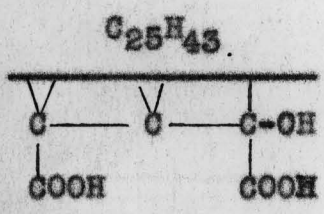
The phenylosazone of this sugar melts at 191°C.

C. Liebermann[18-19] described a method for the preparation of quinovin, reporting yields of from 7 to 12 grams from 12 kilogram quantities of cinchona. The following information concerning quinovic acid obtained by hydrolysis of quinovin was reported: (a) An elementary analysis showed 72.5 per cent of carbon and 9.25 per cent of hydrogen, from which the empirical formula, $C_{32}H_{46}O_6$, was deduced. (b) The acid heated to above 300°C. lost carbon dioxide, and a new acid, pyroquinovic acid, was formed. These results substantiated those of Hlasiwetz[3], which indicated that quinovic acid is not a monocarboxyllic acid.

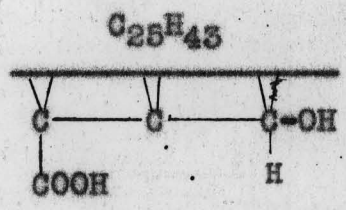
Comparatively recently, H. Wieland and his co-workers[20-23]

have subjected quinovic acid to a very thorough chemical examination in an attempt to determine its structure. The quinovic acid used was obtained by hydrolysis of quinovin with alcoholic hydrochloric acid. An elementary analysis indicated that the acid contained 74.2 per cent carbon and 9.53 per cent hydrogen, from which the empirical formula, $C_{30}H_{46}O_5$, was calculated. The melting point was recorded as 298°C. A triacetyl derivative having a melting point of 180° was prepared, which upon boiling with methyl alcohol produced a monacetyl derivative which melted at 284°C. Heated to 300°C. under reduced pressure, carbon dioxide was eliminated from quinovic acid, forming another acid, pyroquinovic.

The results obtained by Wieland led to the assignment of formula I to quinovic acid, and formula II to pyroquinovic acid.

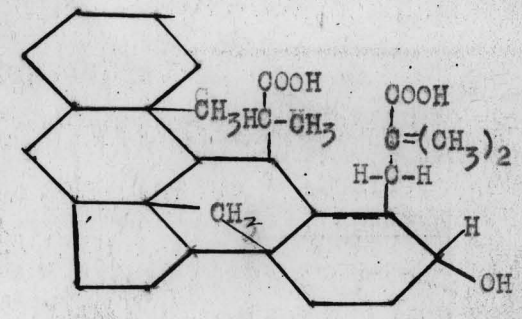


I.



II.

More recent investigations have led to the assignment of the formula given below as possibly representing the structure of quinovic acid.



Quinovin was prepared and hydrolyzed to form quinovic acid, and triacetyl and monacetyl derivatives of the latter were prepared for comparison with derivatives of the material obtained from cinchona red in order to establish beyond doubt its identity. An osazone was prepared from the sugar portion of the molecule for the same reason.

EXPERIMENTAL

Preparation of Quinovin. Quinovin was prepared by a modification of the method described by C. Liebermann[18] as follows: One kilogram of cinchona was boiled during four hours with 5 liters of milk of lime, which was prepared by slaking 500 grams of lime, and suspending the resulting calcium hydroxide in the water. The mixture was filtered while still warm, the filtrate was concentrated by evaporation on a sand bath to a volume of 500 cc., and again filtered. The solution of the calcium salt of quinovin thus obtained was treated with an excess of hydrochloric acid, and the precipitate which formed was filtered off, washed with water until free of acid, and redissolved in milk of lime with the aid of moderate heat. The mixture was filtered, and the quinovin again precipitated by the addition of hydrochloric acid. The yellowish-brown precipitate of crude quinovin was washed with water several times, and then dried. It was dissolved in alcohol, and the solution was decolorized by boiling with charcoal. After the removal of the charcoal by filtration, water was added to the filtrate until it became slightly cloudy, when it was set aside for several hours. Slightly yellow crystals separated, which became pure white after one recrystallization from diluted alcohol. The flake-like crystals of quinovin were dried on a porous plate. A yield of 1.7 grams of quinovin was obtained, which compares favorably with a yield of 12 grams from 12 kilograms of

cinchona reported by Liebermann.

Hydrolysis of Quinovin. One-half gram of quinovin was dissolved in 30 cc. of hot amyl alcohol, and 10 cc. of an aqueous 15 per cent solution of hydrochloric acid were added, together with enough ethyl alcohol to make a homogeneous solution. The mixture was heated in a flask under a reflux condenser during two hours. Upon cooling the mixture, crystals of quinovic acid separated, which were removed by filtration, washed with cold alcohol, and thoroughly dried over phosphorus pentoxide in a vacuum desiccator. Quinovic acid prepared in this way melted at 315°C. with decomposition. The melting point of quinovic acid reported by Wieland [20] is 298°C.

Acetylization of Quinovic Acid. One-half gram of quinovic acid was heated with 3 cc. of acetic anhydride under a reflux condenser during ten minutes. About half of the acetic anhydride was removed under diminished pressure. Upon cooling, crystals of triacetyl-quinovic acid separated, and were filtered off. These were dried on a porous plate, and then recrystallized from acetone. The crystals purified in this way were found to melt at 179 - 180°C. The melting point reported by Wieland is 180°C.

Monoacetyl-quinovic Acid. One-half gram of the triacetyl-quinovic acid was added to 15 cc. of 90 per cent methyl alcohol, and boiled during one hour. Upon cooling this liquid, crystals separated which were filtered off, and dried upon a porous plate. These melted at 280 - 282°C. After one recrystallization from acetic acid, the product melted with

decomposition at 285°C. Wieland reported a melting point for this compound of 284°C.

Preparation of Chinovose Osazone. The mixture of amyl alcohol, hydrochloric acid, and ethyl alcohol used in the hydrolysis of quinovin was distilled with steam until no amyl alcohol passed over in the distillate. The aqueous liquid which remained in the distilling flask was neutralized with sodium hydroxide, and evaporated to dryness. The residue was extracted with absolute alcohol, and the alcoholic extract was evaporated to dryness. The smear which remained was taken up in 20 cc. of water, one gram of phenyl hydrazine hydrochloride, and one gram of anhydrous sodium acetate were added, and the mixture was heated in a boiling water bath during three hours. A very small quantity of needle-shaped, yellow crystals formed, which were filtered off and dried on a porous plate. The melting point was 189 - 190°C. The melting point reported by Fischer[16] was 191°C.

1). Jour. Phar., (2) 8, (1821), p. 112.

2). Ann., 78, (1851), p. 247.

3). Ann., 79, (1851), p. 129.

4). Ann., 111, (1859), p. 182.

5). Winckler, Rep. Pharm., 49, (1834), p. 116.

6). Ibid., 51, (1835), p. 193.

7). Ibid., 81, (1842), pp. 42, 51, 332.

8). Ibid., 91, (1845), p. 314.

9). Winckler, Ber., 4, (1871), p. 206.

10). Buchner, Rep. Pharm., 53, (1835), p. 1.

11). Petersen, Ann., 17, (1835), p. 164.

12). Schnedermann, Ann., 45, (1845), p. 277.

13). Schwarz, Jour. pr. Chem., 56, (1852), p. 76.

14). Rochleder, Jour. pr. Chem., 102, (1867), p. 16.

15). References 5 - 14 inclusive according to Husemann-Hilger, II, p. 1405, (1884).

16). Ber., 26, (1893), p. 2415.

17). Ber., 62, (1929), p. 373.

18). Ber., 16, (1883), p. 926.

19). Ber., 17, (1884), p. 868.

20). Ann., 453, (1927), p. 83.

21). Ann., 479, (1930), p. 1930.

22). Ann., 488, (1931), p. 242.

23). Ann., 497, (1932), p. 140.

CINCHONA FATS

The so-called cinchona fats have received very little attention from plant chemists working upon the drug. This is doubtless due to the fact that the bark contains only small quantities of the fatty material, and that this has not been considered important from a medicinal standpoint. As early as 1855, attention was called to the presence of fat-like substances in cinchona bark by Lauber[1], Reichardt[1], and Reichel[2], but no effort was made to separate the material into its components, and to characterize them. At the worlds exposition in Paris in 1859, Koerner[3] exhibited under the name of <u>cinchocerotin</u>, a crystalline principle which he had isolated from cinchona bark, and which he considered to be a wax. This principle was examined some years later by Helms[4], who reported it to have a melting point of 130°C. and the empirical formula, $C_{27}H_{48}O_2$, as calculated from the results of an elementary analysis.

In 1883, C. Liebermann[5], while studying quinovin, reported the finding of an apparently indifferent substance which he was able to crystallize, and which melted sharply at 139°C. From an elementary analysis, the formula was calculated to be $C_{30}H_{48}O_2$. A year later, Liebermann[6] reported further upon the properties of this substance. He found that boiling with hydriodic acid yielded a hydrocarbon $C_{10}H_{16}$. He therefore proposed the name <u>oxyquinoterpene</u> for the original substance. Subsequently he subjected this oxyquinoterpene to a more careful chemical examination.[7] An acetyl derivative was prepared which melted at 124 - 126°C. The specific rotation of the original substance was retermined, and found

to be equal to -39.2°. Other physical and chemical properties caused Liebermann to believe that he was dealing with a substance closely related to cholesterin. He suggested that the name oxyquinoterpene be abandoned, and that the name cholestole be used in its place.

O. Hesse[8], working simultaneously with but independently of C. Liebermann, isolated a crystalline principle from the petroleum ether extract of Cinchona officinalis. Hesse, from the results of an elementary analysis, calculated the empirical formula to be $C_{20}H_{34}O$, recorded a melting point of 139° for the compound, and 124° for its acetate. In a chloroformic solution it was found to be laevogyrate, $[\alpha]_D$ = - 34.4°. Color reactions led him to believe that the compound with which he was working was closely related to cholesterin. He named this principle cinchol, and stated that he believed it to be identical with Liebermann's oxyquinoterpene. From China cuprea (false cinchona) he obtained a similar substance to which he gave the name cupreol. In a subsequent publication, Hesse[9] reported that he considered cinchol and cupreol to be isomeric compounds.

Schütt,[10] from the results of a study of an ether extract of cinchona bark, reported a phytosterol melting at 134 - 135°, which he considered to be identical with cinchol which had been previously reported by Hesse[8]. This phytosterol was insoluble in the liquid obtained by saponifying the entire ether extract. The solution of soaps was examined by the lead salt method. The fatty acids were precipitated

fractionally with barium acetate, and from the results of a determination of the barium in each of three fractions obtained, Schütt reported the presence of arachidic, stearic, and palmitic acids. These results have been confirmed by another method. The presence of glycerin was also reported by Schütt.

Examination of the Cinchona Fats. In the section of this report in which were described the continuous extraction of cinchona with alcohol, and the attempts to separate the concentrated extract resulting therefrom into more or less definite components by means of selective solvents, reference was made to 120 grams of petroleum ether extract and 35 grams of ether extract. These two extracts of fat-like materials were combined and saponified by heating them in a liter round bottom flask under a reflux condenser during 6 hours with a solution prepared by dissolving 25 grams of potassium hydroxide in 300 cc. of 70 per cent alcohol. After saponification was complete, the mixture was diluted with an equal volume of water, and the greater part of the alcohol was removed by distillation. The aqueous liquid left after the distillation of the alcohol was extracted with several successive portions of ether in order to remove the undissolved material and other ether soluble components. The combined ethereal extracts were dried by means of anhydrous sodium sulphate, and then the ether was distilled off. The residue which remained was of a semisolid consistence, yellow in color, and weighed 18 grams.

Characterization of Phytosterol. The 18 grams of undissolved material, which had been extracted from the soap solution by means of ether, were acetylated by heating during 1 hour with 35 cc. of acetic anhydride under a reflux condenser. The reaction mixture was diluted with water and warmed slightly to decompose the acetic anhydride, and then extracted with ether. The ethereal solution was washed in a separatory funnel with successive portions of aqueous 1 per cent sodium hydroxide solution which was followed with water. The ethereal solution was dried with anhydrous sodium sulphate after which the ether was removed by evaporation on a steam bath. Alcohol was added to the residue, which dissolved upon boiling. When the alcoholic solution was cooled, crystals separated which were filtered off by suction. The filtrate upon evaporation yielded additional crops of crystals. A portion of the acetate was saponified by treating it with N/2 alcoholic potassium hydroxide. The substance thus obtained was, together with the acetate, given to Dr. Ole Gisvold for further purification and characterization. Concerning these samples, Gisvold reports as follows:

"The crude sterol and sterol acetate which had a yellowish appearance, were combined and acetylated by refluxing them with acetic anhydride for one hour. The reaction mixture was diluted with water and warmed in order to decompose the acetic anhydride present. The mixture was shaken out with ether. The ethereal layer was removed and shaken with dilute sodium hydroxide and then with water. The greater portion of the ether was removed on the steam bath. Alcohol was added and the mixture upon cooling deposited a mass of crystals which were removed by suction and washed once with cold alcohol. Several more crops were obtained. All the crops were then combined and recrystallized from alcohol and ether. M.P. 133 - 133.5° Further recrystal-

lization failed to raise the melting point.

A portion of the acetate was saponified by boiling with alcoholic potassium hydroxide. The free sterol thus obtained after recrystallization from ether melted at 139 - 140°. Further recrystallization failed to raise the melting point.

Small portions of the acetate were recovered from the acetate mother liquor. The residue left was oily in nature, mixed with sterol acetate. No further attempt was made to recover the sterol acetate that was left."

The purified sterol when dissolved in chloroform, produced upon the addition of a few drops of concentrated sulphuric acid a deep red color (Hesse's reaction). When dissolved in acetic anhydride, the addition of sulphuric acid produced a blue coloration (liebermann's reaction). A 5 per cent, weight in volume, solution of the sterol in chloroform was prepared and examined for optical activity. It was found to be laevorotatory and to possess an observed optical rotation of - 1.75°. From the observed polariscopic reading in a 100 mm. tube at 25°C., the specific optical rotation, $[\alpha]_d^{25^\circ}$ = - 35°, was calculated.

From the above data it appears that cinchona contains sitosterol, the principal phytosterol found in the higher plants. The melting point reported for sitosterol varies between 137° and 141°. The specific optical rotation of it varies between - 33.9° and - 36.64° when observed at 18°C. The melting point of sitosterol acetate has been recorded as ranging between 125.6° and 137°. These constants are comparable to those obtained by the writer in the examination of the sterol from cinchona.

Separation of the Fatty Acids. The solution of soap, from which the phytosterol had been extracted with ether, was treated with an excess of sulphuric acid to liberate the free fatty acids. The liberated fatty acids were removed from the acidulated liquid by extraction with ether. Some difficulty was experienced in this extraction because of the separation of a layer between the ether and water layers which appeared to act as an emulsifying agent, and which would not dissolve completely in either phase. This finally was separated as sharply as possible and the emulsion-like layer obtained was centrifuged. By this treatment a small amount of a dark brown solid was obtained which was later purified and characterized as a phytosterolin, under which heading it will be discussed. The combined ethereal extracts were washed several times with water to remove sulphuric acid, and then dried by means of anhydrous sodium sulphate. The acidulated aqueous liquid, from which the fatty acids and the phytosterolin had been separated, was neutralized with potassium hydroxide and the solution concentrated by allowing it to evaporate spontaneously. The solution was later used for determining qualitatively the presence of glycerin.

Separation of the Liquid from the Solid Fatty Acids.

The ether was removed from the ethereal solution of the fatty acids by distillation in an atmosphere of carbon dioxide. The residue which remained was of a semisolid consistence, light green in color, and weighed 60 grams. For accomplishing the separation of the saturated from the unsaturated fatty acids, the lead-alcohol method of Twitchell[11] as modified by Schwarz[12] was used. The 60 grams of mixed fatty acids were dissolved in 200 cc. of boiling alcohol, and to the solution while still hot was added, with constant stirring, a nearly boiling solution consisting of 50 grams of lead acetate in 150 cc. of alcohol. The mixture was allowed to cool to room temperature and then placed in an ice box and kept at a temperature of between 10 and 15° during three days. It was then filtered, the residue washed with cold alcohol, and the washings added to the filtrate. After drying, the residue weighed 12.5 grams. This residue, consisting for the most part of the lead salts of the solid or saturated fatty acids, was further purified by dissolving it in 200 cc. of boiling alcohol, allowing the solution to cool to room temperature,and placing it in an ice box over night. The lead salts which separated from the alcoholic solution upon cooling were filtered off, dried, and found to weigh 12 grams. The filtrate was added to the combined first filtrate and washings mentioned above. The fatty acids were considered to be separated into two fractions, (A) solution of lead salts of the liquid unsaturated fatty acids in cold alcohol, and (B) lead salts of the solid saturated fatty acids, soluble

in hot, but insoluble in cold alcohol.

Liberation of the Liquid Unsaturated Fatty Acids.

Fraction A. The alcoholic solution of the lead salts of the unsaturated fatty acids was evaporated to one third its volume and cooled over night at between 10 and 15°. No separation occurred, which indicated the absence of lead salts of saturated fatty acids. Hydrogen sulphide was conducted into the alcoholic solution until a filtered sample showed no further precipitation of lead sulphide. The mixture was heated to boiling in order to coagulate the lead sulphide, and then filtered. The residue was thoroughly washed with warm alcohol to remove any adsorbed fatty acids and the washings added to the filtrate. Carbon dioxide was passed into the alcoholic solution until the odor of hydrogen sulphide was completely dissipated. The alcohol was removed by distillation, in an atmosphere of carbon dioxide, under reduced pressure. The residue, from which no attempt was made to remove the acetic acid, was of a brownish-yellow color, and of an oily appearance.

Bromination of the Liquid Unsaturated Fatty Acids. The mixture of liquid fatty acids which measured 30 cc. were dissolved in 300 cc. of acetic acid, and to this solution 600 cc. of ether were added. The mixture was cooled to 5°, and a solution of 1 part of bromine in 2 parts of acetic acid was added drop by drop until the bromine was present in excess. The solution was then placed in a refrigerator during 24 hours. No precipitate formed, which is an indica-

tion of the absence of linolenic acid inasmuch as the hexabromide of this acid is insoluble in ether. The solution was poured into 3 liters of water, the ether was allowed to evaporate spontaneously, and the precipitate which formed was filtered off. The precipitate was then dissolved in ether, and the solution formed was dried by means of anhydrous sodium sulphate. The ether was distilled off, and the residue was dissolved in boiling petroleum ether. The petroleum ether solution was cooled and crystals separated which were filtered off. The solution upon concentration deposited additional crystals which were filtered off and combined with the first crop. These were twice recrystallized from petroleum ether. The mother liquors were combined and examined for the presence of oleic acid.

Characterization of Linolic Acid Tetrabromide. The crystals obtained from the petroleum ether solution melted at 113 - 114°, a melting point in close agreement with the actual melting point of linolic acid tetrabromide, 114 - 115°. The bromine content was determined according to the Stepanow[13] method by decomposing the brominated fatty acid with metallic sodium in absolute alcohol. The halogen was then precipitated by the addition of N/10 silver nitrate solution, and the excess of the latter was determined by titration with N/10 potassium thiocyanate solution. The results are indicated below.

Per cent of bromine in linolic acid tetrabromide.

	Found	Calculated
Sample 1	53.14	
Sample 2	52.90	53.33

The melting point of the brominated compound, the bromine content, and the solubility of the compound indicates that linolic acid is one of the unsaturated fatty acids obtained from the saponified cinchona fats.

Liberation of Oleic Acid from the Dibromide. The filtrate from the linolic acid tetrabromide should consist of a petroleum ether solution of oleic acid dibromide. The petroleum ether was distilled off and the residue which remained, a thick oily appearing liquid, was dissolved in 150 cc. of 90 per cent alcohol. Ten grams of 20 mesh metallic zinc were added to the solution, and the mixture was heated under a reflux condenser during 6 hours. The clear liquid was decanted, the zinc was washed with several portions of alcohol, and the washings were added to the liquid which had been decanted. The alcohol was distilled off, the residue was poured into 500 cc. of water, and diluted sulphuric acid was added to decompose the zinc salt of the free fatty acid. The mixture was heated for 30 minutes on a water bath, cooled, and then shaken twice with ether in a separatory funnel. The ether was distilled off, and the residue was heated with N/2 alcoholic potassium hydroxide to saponify any ethyl ester of oleic acid that might have been formed. The alcohol was evaporated on a steam bath, the residue dissolved in water, and the solution acidified with sulphuric acid. The free fatty acid was then extracted with ether, the ethereal solution was dried with anhydrous sodium sulphate, and the ether distilled off. The oily residue which remained was

characterized as oleic acid.

Characterization of Oleic Acid. The dark red, oily residue above referred to was found to solidify at 3° which is about the congealing point for an authentic oleic acid. Two grams of the acid were neutralized with a solution of potassium hydroxide, and oxidized by means of potassium permanganate, according to the method of Hazura as outlined by Rosenthaler.[14] The white solid which separated was filtered off, dried on a porous plate, and recrystallized from alcohol. The melting point of these crystals was 136°, which is in close agreement with the melting point reported for dihydroxystearic acid. These characteristics of the acid liberated from the petroleum ether soluble brominated fatty acid should be sufficient to indicate oleic acid.

Liberation of the Saturated Fatty Acids. Fraction B. The mixture of lead salts of solid and saturated fatty acids previously referred to, was suspended in a 10 per cent aqueous solution of nitric acid, and warmed until decomposed. The liberated fatty acids were filtered off, washed several times with water, and dissolved in ether. The ethereal solution was dehydrated with anhydrous sodium sulphate, and the ether was distilled off, leaving a mixture of free solid and saturated fatty acids.

Separation of the Saturated Fatty Acids from One Another. Seven grams of the dried mixture of fatty acids were dissol-

ved in 140 cc. of boiling alcohol. The solution was cooled to 20°, and the precipitate which formed was filtered off, dried, and the melting point determined and found to be 72° - 74°. The filtrate was cooled to 0°, and a further precipitate formed, which after being filtered off and dried, melted at 68° - 70°. The filtrate was concentrated to 100 cc., and again cooled to 0°, when more solid material separated. This was filtered off and dried. The melting point of this fraction was found to be 65° -67°. The solution was further concentrated to 50 cc., and a fraction with the same melting point was obtained. The solution was allowed to evaporate spontaneously, and the residue left after being dried melted at 59° - 60°. It is seen that five fractions were obtained.

Fraction number	Melting point
1.	72° - 74°
2.	68° - 70°
3.	65° - 67°
4.	65° - 67°
5.	59° - 60°

Fractions 1 and 2 were combined and treated as previously described, as were also fractions 3 and 4. Fraction 5 was also dissolved in alcohol, and fractionally precipitated by concentration and cooling of the solution. Fractions having similar melting points were combined and treated as before. Ultimately the fatty acids were resolved into three fractions as tabulated below. (See next page)

Fraction number	Melting point
1.	75° - 76°
2.	67° - 68°
3.	61° - 62°

The first fraction was of a light yellowish-white color, the second was perfectly white, and the third was very slightly tinged with green. Neither fraction was crystalline.

Characterization of Fraction 1. It was noted that the melting point of this fraction was close to the melting points which have been reported for arachidic acid. In order to further characterize this acid, its molecular weight was determined by titration with N/10 alcoholic potassium hydroxide, using phenolphthalein as indicator. The results are given below.

Wt. sample	Cc. N/10 KOH used
0.5000 grams	15.99
Mol. wt. found.	**Mol. wt. calc. for arachidic acid**
310.90	312.32

Preparation of the Para-bromphenacyl Ester. Reid[15] and his co-workers recommend the preparation of p-bromphenacyl esters as a means of identifying fatty acids. The procedure used was as follows: The solution of the potassium salt of the fatty acids from the titration mentioned above was made definitely acid to phenolphthalein by the addition of a trace of the free fatty acid. To this solution was added the cal-

culated quantity (0.43 grams) of p-bromphenacylbromide, and the mixture was boiled during one hour. The solution was cooled, and the material which separated was filtered off, dried, and recrystallized from alcohol. Nearly white microscopic crystals were obtained which melted at 89° - 90°. Inasmuch as the p-bromphenacyl esters of arachidic and stearic acids melt within one degree of each other, a mixture was made of equal parts of the ester prepared as described above with an authentic ester of stearic acid. The mixed melting point was not sharp and was considerably depressed. The melting point reported by Reid for the p-bromphenacyl ester of arachidic acid was 90°.

From the melting point, the molecular weight, and the melting point of the p-bromphenacyl ester, it appears that Fraction 1 is composed of almost pure arachidic acid.

Characterization of Fraction 2. The melting point of this fraction is in close agreement with the melting point of pure stearic acid, 69°. The molecular weight was determined in exactly the same manner as described for the fraction characterized as arachidic acid. The results are given below.

Wt. sample	Cc. N/10 KOH used
0.5000 grams	17.7
Mol. wt. found	**Mol. wt. calculated for stearic acid**
282	284.29

The p-bromphenacyl ester of this acid was prepared in the same way as previously described. The melting point of the ester after recrystallization from alcohol was 90°, and there was no depression in the melting point when it was mixed with an authentic sample of the p-bromphenacyl ester of stearic ester.

The melting point, the molecular weight, and the p-bromphenacyl ester characterizes this fraction as stearic acid.

Characterization of Fraction 3. This fraction melts near to the generally accepted melting point of palmitic acid, 62.6°. Titration values from which the molecular weight was calculated are given below.

Wt. sample	Cc. of N/10 KOH used
0.5000 grams	19.35
Mol. wt. found	**Mol. wt. calculated for palmitic acid**
257.91	256.26

The p-bromphenacyl ester prepared as described above melted at 85°. The melting point reported by Reid was 86°.

The melting point, the molecular weight, and the derivative prepared of this fraction indicate that it is composed principally of palmitic acid.

Identification of a Phytosterolin. As was mentioned in the description of the separation of the fatty acids from the acidified soap solution by extraction with ether, a layer formed between the aqueous and the ethereal phase. This was drawn off and centrifuged to break up what appeared to be an

emulsion. A dark brown solid separated from the liquid, which was then decanted. The solid was dried and found to weigh 1.2 grams. It was soluble in boiling alcohol, and insoluble in cold alcohol. Its appearance could not be changed by dissolving it in hot alcohol, and allowing it to precipitate upon cooling. It was finally dissolved in boiling alcohol, a small amount of charcoal was added, and the mixture was boiled for some time, and then filtered while still hot. When the filtrate, which was nearly colorless, cooled, there was a separation of white crystals. These were filtered off, recrystallized from alcohol, and dried in a desiccator over phosphorous pentoxide. The dried crystals obtained in this way melted at 285°. Since this melting point was within the range of the melting points of a large number of phytosterolins* which had been isolated and characterized by Power [16], and inasmuch as the acetates of these are somewhat characteristic, an acetate was prepared by boiling a small portion of the material with acetic anhydride. When the mixture cooled, the acetate separated, was filtered off, and recrystallized from alcohol. This acetate melted at 165°, a melting point within the range of the various ones reported by Power.

The original substance gave a positive carbohydrate test

* This term was coined by F.B. Power to designate a class of compounds which upon acid hydrolysis yield a sugar and a phytosterol. For a complete list of individual phytosterolins, see a contribution by Matlack, Jour. A. Ph. A., XVIII, (1929), p. 28.

with alpha-naphthol and sulphuric acid, and after hydrolysis with hydrochloric acid it reduced Fehling's solution. These reactions, indicative of a phytosterolin, made it seem advisable to hydrolyze the substance. This was done according to the method used by Power.

Hydrolysis of the Phytosterâãäå. One half gram of the substance was dissolved in 30 cc. of hot amyl alcohol, 10 cc. of an aqueous 15 per cent solution of hydrochloric acid were added, together with enough alcohol to make a homogeneous solution. The mixture was heated in a flask under a reflux condenser during 2 hours. The amyl alcohol was distilled off with steam. Crystals remained with the water in the flask through which the steam had been passed. These were filtered off, and the filtrate was retained for the preparation of an osazone. The crystals were recrystallized from alcohol and then from ether. The melting point was 138° - 139°. The melting point of a mixture made from equal parts of this material with the phytosterol previously described was unchanged. An acetate was prepared which melted at 133°.

Preparation of an Osazone. The filtrate obtained after steam distilling the reaction mixture in the hydrolysis of the phytosterolin, and filtering off the phytosterol, was neutralized with sodium hydroxide. One gram of phenylhydrazine and 1 gram of anhydrous sodium acetate were added to the solution, and the mixture was heated in a boiling water bath during 2 hours. A very small quantity of yellow, needle shaped crystals separated which were dried on a porous

plate, and the melting point determined. This osazone melted at 200° - 202°. The quantity obtained was too small to re-crystallize, so the identity of the sugar portion cannot be stated.

Identification of Glycerol. The aqueous solution left after the liberation of the free fatty acids from the soap solution was neutralized with potassium hydroxide solution, and evaporated as far as possible on a steam bath. The residue remaining was extracted with a mixture of 3 parts of 95 per cent alcohol and 1 part of ether, and the solvents were evaporated. A yellow syrup remained which had a sweet taste, and was miscible with water and with alcohol in all proportions. A few drops of the syrup were heated with about 0.5 grams of potassium bisulphate. This produced the characteristic odor of acrolein.

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