

SCIENTIFIC SECTION

COMPARATIVE CHEMICAL EXAMINATION OF DIFFERENT BRANDS OF ACRIFLAVINE HYDROCHLORIDE (ACRIFLAVINE) AND ACRIFLAVINE BASE ("NEUTRAL" ACRIFLAVINE).*

BY GEORGE W. COLLINS, Ph.C., Sc.D. AND ARANKA STASIAK, M.D.

I. INTRODUCTION.

Acriflavine and the base, "Neutral" Acriflavine are two of the several anti-septics introduced during the World War. They are dye products of the acridine series, the latter, a substance first obtained from coal-tar. The preparation and properties of 3:6 diamino-10-methylacridinium chloride (acriflavine-base) were first described by Benda.¹

Acriflavine base was introduced by Ehrlich, who found it to possess some effectivity in trypanosome infection, hence he called it "Trypaflavine," under which name it is generally described in German literature. In England and the United States the hydrochloride of 3:6 diamino-10-methylacridinium chloride is the product more generally known as acriflavine, although the free base (which in this country is also called "neutral" acriflavine) is at times called by the same name,² hence the confusion in nomenclature.

The more recent use of acriflavine as a dye antiseptic is based on the pioneer work of Browning and co-workers.³

Subsequently much work has been done, a review of which previous to 1919 was published in the Reports of the Council on Pharmacy and Chemistry.⁴ From time to time since then statements have occurred that this or that product was better. There have been published statements, particularly by E. G. Davis, to the effect that the British and French acriflavines were better than the American make, although scientific, chemical or controlled clinical evidence was lacking. In view of this, it seemed advisable to examine chemically a few specimens of American, British and French products.

Standards for the products were described in "New and Nonofficial Remedies"—1925.⁵ These standards, with some additional ones, were first used to determine the relative purity of the products. The problem was first undertaken by one of us, a Fellow of the International Health Board of the Rockefeller Founda-

* From the American Medical Association Chemical Laboratory, Chicago.

¹ L. Benda, "Ueber das 3:6-diamino-acridin," *Ber. d. deutsch chem. Gesselsch.*, 45 (1912), 1787.

² Scientifically speaking, the substance commercially available under the name acriflavine should have been called acriflavine hydrochloride; this recommendation has been carried out in later editions of "New and Nonofficial Remedies."

³ C. H. Browning and W. Gilmour, "Bactericidal Action and Chemical Constitution with Special References to Basic Benzol Derivatives," *J. Path. Bact.* (July 1913), 144.

⁴ "Reports of the Council on Pharmacy and Chemistry" for 1919, page 38.

⁵ Generally abbreviated "N. N. R." A volume published annually by the Council on Pharmacy and Chemistry of the American Medical Association. It describes new remedies of merit which are not official in the United States Pharmacopoeia. The 1928 standards, as will be noted, have been radically revised as a result of the investigations.

tion; as time did not permit completion of the work, it was taken up in greater detail by the other. The report follows:

II. EXAMINATION OF "ACRIFLAVINE."

BY ARANKA STASIAK, M.D. (BUDAPEST) CHICAGO.¹

Original packages of the various brands of acriflavine (acriflavine hydrochloride) appearing on the open market in this country and abroad were purchased in 1926 from different sources.

The products examined in 1926 were:

1. Acriflavine—The Abbott Laboratories, Chicago.
2. Acriflavine—The Abbott Laboratories, Chicago.
3. Acriflavine—Boots Pure Drug Co., Ltd., Nottingham.
4. Acriflavine—(Heyl) National Aniline & Chemical Co., Inc., New York.
5. Acriflavine—(Heyl) National Aniline & Chemical Co., Inc., New York.
6. Gonacrine—Les Établissements Poulenc Frères, Paris.

The specimens of the National Aniline and Chemical Company, Inc., were labeled as acriflavine di-hydrochloride; those of the Abbott Laboratories as mono-hydrochloride, whereas, in the case of the other specimens, no statement was given regarding the hydrogen chloride content.

The color and appearance of the various products differed somewhat. Specimens 1 and 2 were a bright red, while the others were a reddish brown. Specimens 2 and 3 were odorless; 4 and 5 had a marked odor of acetic acid; 6 had an acrid irritating odor, as also had No. 1, though in the latter case this odor was less marked.

TABLE I.—ANALYSES OF VARIOUS BRANDS OF ACRIFLAVINE.

Brands.	Ash.	Water-insoluble matter.	Loss on drying at 100° C.	Chlorine content.	Nitrogen content.	Hydrogen-chloride content.
1 Abbott Laboratories, Chicago				22.06	12.67	11.32
	0.65 ¹	0.04	14.7	24.35 ²	13.97	12.48
2 Abbott Laboratories, Chicago	0.96	0.37	18.4	22.35	13.15	11.59
3 Boots Pure Drug Co., Ltd., Nottingham	0.02	0.03	17.2	22.31	12.61	12.20
				23.59	13.34	12.91
4 National Aniline & Chemical Co., Inc., New York	0.23	0.05	15.03	21.43	12.89	11.19
5 National Aniline & Chemical Co., Inc., New York	0.58	0.23	14.3	21.70	13.05	11.33
				23.17	13.93	12.09
6 Les Établissements Poulenc Frères, Paris	0.73	0.02	12.5	23.85	13.00	12.99
				24.41	13.33	13.31
Theoretical ³				23.95	14.19	12.31

¹ Figures are reported in terms of percentage.

² The figures reported in italics are calculated for the specimen dried over sulphuric acid by Collins (see the following article). The other brands were not determined, owing to the lack of material.

³ Figures are calculated on anhydrous basis.

¹ Fellow of the International Health Board of the Rockefeller Foundation. This work was done during the spring of 1926.

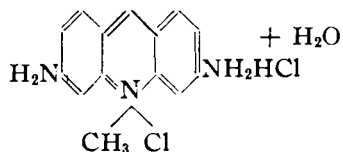
All products were found to comply with the qualitative tests described in "New and Nonofficial Remedies"—1925. Quantitative estimations made were: the loss on drying at 100° C., the water-insoluble matter, ash, chlorine, nitrogen and hydrogen chloride content.

The quantitative results are given in the foregoing table.

Particularly interesting were the p_H curves of the electrometric titrations. All curves (except the one of Specimen 2) were found to be characteristic, with only one sudden rise, indicating that the compounds appeared to be monohydrochlorides. The curve for Specimen 2 indicated a monohydrochloride, but several rises which were noted were probably due to other ingredients having a buffer action. A smooth curve, therefore, may be considered as a criterion of the purity of a product.

The chloride determinations indicated the correctness of the formula in "New and Nonofficial Remedies," namely, two chlorine atoms, one (as the electrometric titration shows) for hydrogen chloride, and the other attached to the acridine nitrogen (not as hydrochloride).

The formula which first appeared in New and Nonofficial Remedies was



It was later shown that there was no evidence of definite water of crystallization.

SUMMARY.

1. Comparative chemical analyses were made of different brands of acriflavine in 1926.
2. The results obtained show no marked difference in the purity of the different products, and do not justify the statement that the foreign product is superior to the domestic. All the products, as is to be expected, are pronouncedly acid in reaction and are in reality acriflavine hydrochloride.
3. The p_H curve of the electrometric titration may indicate the purity of the products.

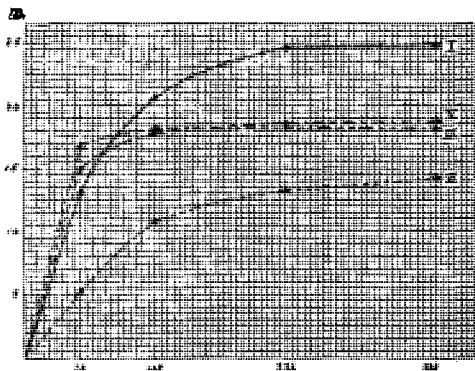
III. FURTHER OBSERVATIONS ON ACRIFLAVINE HYDROCHLORIDE.

BY GEORGE W. COLLINS, Sc.D.

It was suggested in the preceding paper¹ that the p_H curves of the electrometric titration might indicate the purity of the product; this led to further investigation on this compound. One of each of the various brands used in the foregoing paper was subjected to further analytic determinations, namely: the loss on drying at 100° C., and over sulphuric acid in a partial vacuum at various time intervals, the chlorine and hydrogen chloride content.

¹ Dr. Stasiak was unable to finish the work begun, as her time in this country was limited.

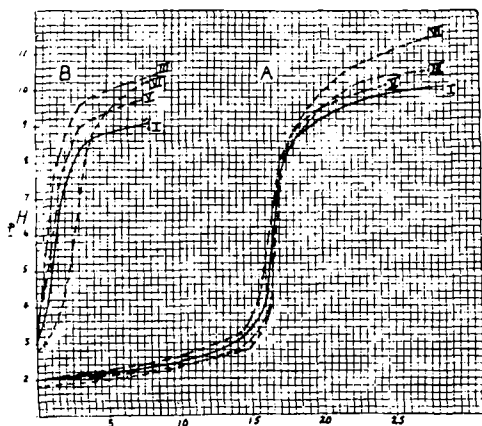
It was noted that on drying the various products at 100° C. there was a gradual loss in weight as the time element increased (see Chart I). On determining the



Time in hours.

Curve numbers agree with the brand numbers.
Chart I.—Rate of decomposition by heat at 100° C.

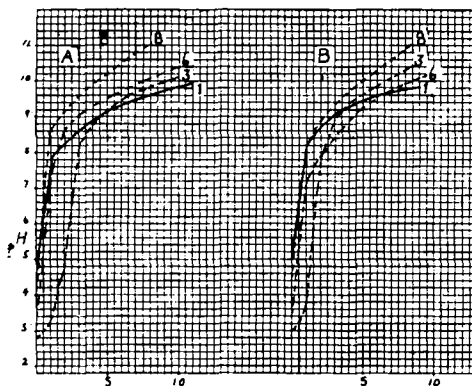
amount of chlorine¹ present in these dried specimens, it was found to be equivalent to only one chlorine atom, whereas originally there were two chlorine atoms (see Table I). On making an electrometric titration,² a rather abrupt smooth curve (see Chart II, Part B, Curves) was obtained, showing that it was quite evident that the hydrogen chloride had been driven off, as well as the water on heating at 100° C., and that the percentage of hydrogen chloride lost was concordant with that of the theoretical amount; in fact, only traces of hydrogen chloride remained and the product be-



Cubic centimeters of tenth-normal sodium hydroxide consumed by 0.5 Gm.

Curve numbers agree with the brand numbers.

Chart II.—Electrometric titration curves on: A.—Acriflavine hydrochloride previously dried over sulphuric acid. B.—Acriflavine hydrochloride after drying at 100° C. for 258 hours.



Cubic centimeters of tenth-normal sodium hydroxide consumed by 0.5 Gm.

Curve numbers agree with the brand numbers.

Chart III.—Electrometric titration curves on acriflavine base (neutral acriflavine): A.—Undried material (calculated for the dried). B.—Dried material.

¹ The chlorine content was determined by a modification of the Lehman Method [Lehman, "Ueber die Bestimmung des Arsens in Salvarsan und Neo-Salvarsan," *Apoth. Ztg.*, 27 (1912), 545] for the determination of arsenic in organic compounds. The modified method has been used for several years in this and other laboratories, although a similar modification quite recently was described by Salkin [Bernard Salkin, "Analysis of Organic Silver Compounds," *JOUR. A. PH. A.*, 16 (1927), 140]. An accurately weighed quantity (about 0.25 Gm.) of the specimen was transferred to a 250-cc. Erlenmeyer flask, and dissolved in 10 cc. of distilled water; one gram of silver nitrate, which had been previously dissolved in about 10 cc. of distilled water, was added; followed by the addition of about 10 cc. of sulphuric acid, finally about 2 Gm. of finely powdered

haved in other respects as acriflavine base (neutral acriflavine) rather than as acriflavine hydrochloride.

The specimens that were exposed over sulphuric acid in a desiccator with a partial vacuum, obtained constant weight in a shorter period of time than when dried at 100° C. The electrometric titrations made on these dried specimens produced curves (see Chart II, Part A, Curves), which were smooth and characterized by only one sudden rise (confirming the observations noted by Stasiak), indicating that all the products were monohydrochlorides and not a dihydrochloride, as stated on the label of one of the products. Furthermore, the various products produced curves (see Chart II, Part A, Curves) which practically coincide: showing that there was no difference in the products as to the hydrogen chloride content, in other words, they were all monohydrochlorides, and that the hydrogen chloride was not removed by this method of drying.

The results of the analytical determinations are recorded in Table II.

TABLE II.—COMPARATIVE DATA ON SPECIMENS OF VARIOUS BRANDS OF ACRIFLAVINE.

(A) Dried Over Sulphuric Acid. (B) Dried in an Electric Oven at 100° C.

Brands. ¹	Specimen dried over sulphuric acid.			Specimen dried at 100° C.		
	Loss on drying.	Hydrogen chloride content (calculated from electrometric titration).	Loss on drying.	Total chlorine content (including hydrogen chloride).	Hydrogen chloride content (calculated from electrometric titration).	Chlorine content ⁴ (calculated).
1 Abbott Laboratories, Chicago	9.34 ²	12.27	24.74	14.20	1.22	13.01
3 Boots Pure Drug Co., Ltd., Nottingham	5.45	12.34	18.04	13.37	0.39	12.99
5 National Aniline & Chemical Co., Inc., New York	6.34	11.85	18.54	13.91	1.15	12.78
6 Les Établissements Poulenc Frères, Paris	2.45	12.34	14.19	15.16	1.99	13.23
Theoretical		12.31 ³	11.97 ³

¹ Numbers agree with those in the preceding article.

² Figures are reported in terms of percentage.

³ Figures are based on the loss of one molecule of hydrogen chloride.

⁴ These figures were obtained by calculating the hydrogen chloride to chlorine and subtracting it from the total chlorine content.

REVISION OF STANDARDS.

Based on the foregoing data, rigorous standards for identity and purity were elaborated. These were submitted to the respective manufacturers for comment and then, with certain slight modifications suggested by the manufacturers, the following standards were adopted for inclusion in "New and Nonofficial Remedies:"

ACRIFLAVINE HYDROCHLORIDE.—Acriflavinae Hydrochloridum.—Acriflavine.—3:6 Diamino-10-methylacridinium chloride monohydrochloride.—2:8 Diamino-*N*-methylacridinium chloride hydrochloride.

potassium permanganate were added in several portions, carefully agitating after each respective addition; ultimately the flask and contents were allowed to digest on the steam-bath for at least thirty minutes. The solution was decolorized with a solution of hydrogen peroxide by the aid of heat, so as to remove all traces of the manganese oxides that were formed. The precipitate of silver chloride was collected on a Gooch crucible, washed with nitric acid and water, followed by alcohol and ether, and dried to constant weight at 105° C.

² The usual hydrogen electrode and calomel cell were used in these determinations.

Acriflavine hydrochloride is a brownish red, odorless, crystalline powder. It is soluble in about 3 parts of water and in alcohol; nearly insoluble in ether, chloroform, liquid petrolatum, fixed and volatile oils; the solutions are dark red in color and fluoresce on dilution; an aqueous solution (1 in 250) is distinctly acid when measured by means of the hydrogen electrode.

Add a few drops of hydrochloric acid to an aqueous solution of acriflavine hydrochloride which is just sufficiently diluted to be fluorescent: the fluorescence disappears, but partially reappears on further dilution with water; add 2 drops of sulphuric acid to about 1 cc. of an aqueous solution (1 in 250) and agitate the mixture: an orange-red crystalline precipitate is produced. An aqueous solution (1 in 5) when mixed with an equal volume of a saturated sodium bicarbonate solution produces a marked effervescence (*distinction from acriflavine base*). Dissolve 1 Gm., in 250 cc. water; to a 10-cc. portion add 1 cc. diluted hydrochloric acid; add 1 cc. barium chloride solution: no precipitation occurs (*distinction from proflavine*); to another portion of 10 cc. add 1 cc. formaldehyde solution: no precipitation occurs (*distinction from proflavine*).

When tested for arsenic according to the U. S. Pharmacopœia X, the product should meet requirements for the test for arsenic (page 428, Arsenic Test). Dry about 1 Gm., accurately weighed, to constant weight over sulphuric acid in a partial vacuum: the loss does not exceed 7 per cent. Incinerate about 1 Gm., accurately weighed, previously dried over sulphuric acid: the ash does not exceed 1 per cent. Dissolve about 1 Gm., accurately weighed, previously dried over sulphuric acid in 250 cc. of warm water; collect the insoluble matter, if any, in a weighed Gooch crucible; wash the insoluble matter with hot water, dry and weigh the residue: the insoluble matter does not exceed 0.5 per cent.

The nitrogen content determined according to the method described under acriflavine base corresponds to not less than 13.7 per cent, nor more than 14.3 per cent, when calculated to the dried substance. The chlorine content, determined according to the method described under acriflavine base corresponds to the percentage of nitrogen found multiplied by the factor 1.688 (N to Cl in $C_{14}H_{14}N_3 Cl.HCl$) plus or minus 1 per cent, when calculated to the dried substance. Transfer about 0.5 Gm., accurately weighed, to a 400-cc. beaker, and dissolve in 100 cc. of water: the hydrogen chloride content determined by titration with tenth normal sodium hydroxide solution using the hydrogen electrode and a calomel cell (normal potassium chloride solution) corresponds to not less than 12.0 per cent, nor more than 12.5 per cent, hydrogen chloride, when calculated to the dried substance.

CONCLUSIONS.

1. Trustworthy results could not be obtained when the compound was dried at 100° C.
2. The compound does not contain a molecule of water of crystallization as heretofore claimed, but the water is present in varying proportions.
3. The hydrogen chloride could be completely removed at 100° C., on prolonged exposure; and only one chlorine atom remained, namely: the one attached to the nitrogen, as was suggested by Stasiak in the preceding article.
4. The hydrogen chloride is not removed by prolonged exposure over sulphuric acid in a partial vacuum, and the products so dried, yielded smooth curves with only one sudden rise, which were concordant with theory; and there was present only one hydrogen chloride molecule and not two as some authors and firms have stated.
5. Furthermore, the p_H curves of the electrometric titration appears to be an excellent criterion in the determination of the identity and purity of the compound.
6. A more appropriate name for the product was proposed and adopted for N. N. R., *i. e.*, Acriflavine Hydrochloride in place of Acriflavine.
7. Standards for Acriflavine Hydrochloride have been prepared based on work herein reported.

IV. EXAMINATION OF ACRIFLAVINE BASE (SO-CALLED NEUTRAL ACRIFLAVINE).

BY GEORGE W. COLLINS, Sc.D.

In view of the previous work it was deemed advisable to make a comparative chemical examination of the various brands of Neutral Acriflavine (as it is erroneously known). Original packages of the several brands were purchased on the open market, except one specimen of the Abbott Laboratories' brand which was obtained from the manufacturer.

The brands examined were:

1. Neutral Acriflavine—The Abbott Laboratories, North Chicago.
2. Neutral Acriflavine—The Abbott Laboratories, North Chicago.
3. Neutral Acriflavine—Boots Pure Drug Co., Ltd., Nottingham.
4. Neutral Acriflavine—Boots Pure Drug Co., Ltd., Nottingham.
5. Neutral Acriflavine (Heyl)—National Aniline & Chemical Co., Inc., New York.
6. Neutral Acriflavine (Heyl)—National Aniline & Chemical Co., Inc., New York.
7. Neutral Acriflavine—National Aniline & Chemical Co., Inc., New York.
8. Gonacrine-chloromethylate de DIAMINOACRIDINE—Les Établissements Poulenc Frères, Paris.
9. Gonacrine-chloromethylate de DIAMINOACRIDINE—Les Établissements Poulenc Frères,¹ Paris.

The products of the Abbott Laboratories were labeled diamino-methyl-acridinium-chloride; two specimens of the National Aniline & Chemical Co., Inc., were designated as (Heyl) 3-6 Diamino-*N*-Methyl-Acridinium, and a third, 3-6-Diamino-10-Methyl-Acridinium Chloride; those of the Boots Pure Drug Co., Ltd., bore no statement as to their chemical composition other than the designation, neutral acriflavine, "A modified form of acriflavine without action upon metals and specially suitable for injection;" and those of Les Établissements Poulenc Frères were labelled Gonacrine-chloro-methylate de DIAMINO-ACRIDINE, which was identical with the label that was on their acriflavine (acriflavine hydrochloride).

TABLE III.—APPEARANCE OF THE VARIOUS BRANDS OF NEUTRAL ACRIFLAVINE EXAMINED.

Brand.	Color.	Appearance.
1 Abbott Laboratories	Red	Fine uniform powder
2 Abbott Laboratories	Red	Fine uniform powder
3 Boots Pure Drug Co., Ltd.	Light reddish brown	Coarse, heterogeneous powder, with particles of a purple cast
4 Boots Pure Drug Co., Ltd.	Light reddish brown	Coarse uniform powder
5 National Aniline & Chemical Co., Inc.	Light brown	Fine uniform powder
6 National Aniline & Chemical Co., Inc.	Purple	Coarse powder with black particles
7 National Aniline & Chemical Co., Inc.	Purple	Fine powder with black particles
8 Les Établissements Poulenc Frères	Tan	Fine uniform powder
9 Les Établissements Poulenc Frères	Tan	Fine uniform powder

¹ After this chemical investigation had been completed and the new standards proposed for N. N. R., had been accepted by the manufacturers whose products had been found acceptable, it was noted in one of the trade journals (*Oil, Paint and Drug Reporter*, April 23, 1928) that negotiations had been completed for the merger of the Usines des Rhône and the Établissements Poulenc Frères to be known as Société des Usines Chimiques Rhône-Poulenc.

The appearance of the various brands differed markedly; for comparisons see Table III. All the products were found to be odorless. The solubility of the various products (1 Gm. in 250 cc. of water) differed slightly; Specimens 1 and 2 were of a light red color, while the others were of a brownish red color; all the solutions contained some floating particles (suggestive of dirt); Specimens 6 and 7 appeared to contain the greater amount (see Table III). The reaction of the aqueous solutions appeared to be neutral to congo paper but acid when determined by the electrometric method. (For p_H values see later comments.)

The various products were found to comply with the qualitative tests as described in the 1926 edition of the New and Nonofficial Remedies, except that in a few instances the intensity of color, opalescence and turbidity varied somewhat, particularly in accordance with the depth of color of the product. Upon the addition of barium chloride solution to a uniformly acidified aqueous solution of each of the brands, Specimens 2, 3 and 4 produced no turbidity; Specimen 1 yielded a faint turbidity; all others were of the same intensity, a slight turbidity increasing on standing (probably indicative of the presence of sulphate as an impurity). The quantitative estimations made were: the water-insoluble matter, the loss on drying at 100° C., the "sulphated" ash, the chlorine and nitrogen contents and electrometric titrations on the undried and material dried to constant weight at 100° C.

The results of the quantitative determinations are tabulated in Table IV.

TABLE IV.—ANALYSES OF VARIOUS BRANDS OF NEUTRAL ACRIFLAVINE.

Brands.	Water-insoluble matter.	Loss on drying at 100° C.	"Sulphated" ash.	"Sulphated" ash (calculated as sodium chloride).	Chlorine content.	Nitrogen content.
1 Abbott Laboratories (North Chicago)	0.06 ¹	10.06	4.71	3.88	15.37	15.660
2 Abbott Laboratories (North Chicago)	0.06	10.17	4.73	3.89	15.33	15.4
3 Boots Pure Drug Co., Ltd. (Nottingham)	0.05	8.51	2.71	2.23	14.92	15.73
4 Boots Pure Drug Co., Ltd. (Nottingham)	0.06	8.91	11.28	9.28	18.88	14.68
5 National Aniline & Chemical Co., Inc. (New York)	0.06	8.68	9.56	7.87	17.81	15.18
6 National Aniline & Chemical Co., Inc. (New York)	0.21	8.67	3.37	2.78	15.18	15.68
7 National Aniline & Chemical Co., Inc. (New York)	0.15	8.76	4.09	3.36	15.38	15.82
8 Les Établissements Poulenc Frères (Paris)	0.08	5.66	4.90	4.03	15.02	14.81
9 Les Établissements Poulenc Frères (Paris)	0.04	6.15	4.60	3.80	15.14	14.97
Theoretical ³	10.00 ²	4.00 ²			13.67	16.18

¹ Figures are reported in terms of percentage.

² Figures in italics are limits described by New and Nonofficial Remedies, 1926.

³ Figures are calculated on the anhydrous basis.

From the results recorded in Table IV, it is particularly interesting to note the uniformity in moisture content of the products of each respective manufacturer; also that all specimens examined were within the limits required by N. N. R., 1926, except those of the Abbott Laboratories, which were just above

the limit, while the products of the Les Établissements Poulenc Frères were exceedingly low when compared with the others.

The "sulphated" ash of the various brands was found to be high, except one of the Boots Pure Drug Co., Ltd., and one of the National Aniline & Chemical Co., Inc., which fell within the limit prescribed by N. N. R., 1926. It might not be amiss to point out the pronounced difference in the "sulphated" ash content of the specimens of the products of the Boots Pure Drug Co., Ltd.,¹ which vary from 2.71 per cent to 11.28 per cent and those of the National Aniline & Chemical Co., Inc., ranging from 3.37 per cent to 9.56 per cent; this when calculated as sodium chloride, means that the consumer is paying a high price for table salt.

The p_H values for solutions of the products (0.5 Gm. in 100 cc. of distilled water, previously boiled to remove carbon dioxide) ranged between 2.8 and 4.8 (see Chart III, which contains representative curves of each of the various brands). As was expected, the curves of the electrometric titrations performed on the undried material and that dried at 100° C., in three brands were concordant, indicating that there was no appreciable titratable hydrogen chloride present. Specimen 3 (see Chart III, Curve 3), undried, showed a comparatively larger titratable acidity; however, this specimen when dried for 198 hours at 100° C. showed a marked diminution of this value, as would have been expected from the work reported on acriflavine hydrochloride. This is strongly presumptive evidence that the hydrogen chloride from the acriflavine hydrochloride had not been all removed in the preparation of this particular batch of acriflavine base. In further support of this contention, Table IV shows this specimen to have a comparative low "sulphated" ash. From the results correlated in Table IV and the curves obtained by electrometric titration (see Chart III), it is quite evident that a smooth abrupt curve might be considered as a criterion of the purity of the product.

Working with acriflavine base (neutral acriflavine) Burke and Rodier² have shown that various solvents yielded different p_H values. Hence, it appears evident that solutions of acriflavine base (so-called neutral acriflavine) as prepared and used in practice, might vary in p_H due to the various solvents used and in the impurities present; its germicidal action appearing weaker in an acid medium but increasing on an increase in alkalinity.³ Furthermore, it appears quite probable that the variation in p_H might account for some of the unfavorable results reported clinically.

OPTICAL, CRYSTALLINE EXAMINATION.

During the investigation one of the manufacturers had presented to the Council on Pharmacy and Chemistry a specimen of acriflavine base (Neutral

¹ It so happened that while this investigation was in progress the Boots Pure Drug Co., Ltd., submitted specimens of Acriflavine and Neutral Acriflavine to the Council on Pharmacy and Chemistry for inclusion in N. N. R. Their product, Neutral Acriflavine, bore the same control number as one of the specimens under scrutiny (see Table IV, No. 3), which was found to yield 2.84 per cent of "sulphated" ash, which agreed favorably with the one reported in the table. (See "Annual Reports of the Chemical Laboratory of the American Medical Association," Vol. 19-20 (1926-1927) for complete details.)

² Victor Burke and E. A. Rodier, "Preparation of Neutral Acriflavine Solutions for Intravenous Injection," *J. Lab. Clin. Med.*, 13 (1927), 231.

³ A. H. Eggerth, "The Bactericidal Action of Acridine Dyes and the Adjuvant Effect of Serum," *J. Infec. Dis.*, 38 (1926), 440.

Acriflavine) with which the firm was desirous of replacing their present market product. The product was claimed to have been manufactured by a new process, using animal charcoal in final purification. This particular product was found to contain 12.88 per cent chlorine, considerably less than the theoretical amount present in the compound and unlike in color being a bright, light red color, and differing from the other specimens in some of the qualitative tests. It was deemed advisable, therefore, to investigate its crystalline character to ascertain whether or not it really differed from the other brands under examination. The collaboration of Dr. Albert J. Walcott, professor of mineralogy and crystallography of Northwestern University, was obtained. Two different specimens of the various brands under examination and the new proposed product were sent to Dr. Walcott for crystallographic examination. Dr. Walcott reported as follows:

1. The acriflavine base of the samples submitted exists in a crystalline state and in a state which appears to be amorphous. The "Amorphous" acriflavine base is very finely divided and is in suspension or in solid solution in the crystalline particles. The crystalline part of acriflavine base is biaxial.
2. There is also a possibility that acriflavine base consists of two different substances, *viz.*, a crystalline substance and a very finely divided substance which appears to be amorphous, and which is in suspension or in solid solution in the crystalline substance.
3. The color of acriflavine base is due to the very finely divided part which appears to be amorphous. The "amorphous" acriflavine base of the different samples varies in manner of distribution and in degree of division. This may explain the reason for the difference in color.

REVISION OF STANDARDS.

Based on the foregoing data, rigorous standards for identity and purity were elaborated. These were submitted to the respective manufacturers for comment, and then with certain slight modifications suggested by the manufacturers, the following standards were adopted for inclusion in New and Nonofficial Remedies:

ACRIFLAVINE BASE.—Neutral Acriflavine.—The base 3:6-diamino-10-methylacridinium chloride.—2:8 diamino-*N*-methylacridinium chloride.

Acriflavine Base is a brownish red, odorless, granular powder. It is soluble in about 3 parts of water; incompletely soluble in alcohol; nearly insoluble in ether, chloroform and the fixed oils; the aqueous solution is brownish red in color, and fluoresces on dilution.

Add a few drops of hydrochloric acid to an aqueous solution of acriflavine base which is sufficiently diluted to be fluorescent: the fluorescence disappears, but partially reappears on further dilution with water. Add 2 drops of sulphuric acid to about 1 cc. of an aqueous solution (1 in 250) and agitate the mixture: an orange-red crystalline precipitate is produced. An aqueous solution (1 in 5) does not effervesce on the addition of an equal volume of a saturated solution of sodium bicarbonate (*distinction from acriflavine hydrochloride and proflavine*).

Dissolve 1 Gm. in 250 cc. water; to a 10-cc. portion add 1 cc. diluted hydrochloric acid, add 1 cc. barium chloride solution: no precipitation occurs (*distinction from proflavine*); to another portion of 10 cc. add 1 cc. formaldehyde solution: no precipitation occurs (*distinction from proflavine*).

When tested for arsenic according to the U. S. Pharmacopœia X, the product should meet requirements for arsenic (page 428, Arsenic Test). Dry about 1 Gm., accurately weighed, to constant weight at 100° C.; the loss does not exceed 7.0 per cent. Dissolve about 1 Gm., accurately weighed, previously dried at 100° C., in about 250 cc. of warm water, collect the insoluble matter, if any, in a weighed Gooch crucible, wash the insoluble matter with hot water, dry and weigh the

residue: the insoluble matter does not exceed 0.5 per cent. Heat with an excess of sulphuric acid at a low temperature about 1 Gm., accurately weighed, previously dried at 100° C.; the "sulphated" ash does not exceed 3.5 per cent., when calculated as sodium chloride. Transfer about 0.3 Gm., accurately weighed, to a 500-cc. Kjeldahl flask, and determine the nitrogen content according to the method described in "Medical War Manual No. 6, Laboratory Methods of the United States Army," page 221. (The sulphuric acid digestion should be carried on at least 20 hours.) The percentage of nitrogen corresponds to not less than 15.5 per cent, nor more than 16.3 per cent, when calculated to the dried substance.

Transfer about 0.25 Gm., accurately weighed to a 250-cc. Erlenmeyer flask, dissolve in 10 cc. of water, add about 0.5 Gm. of silver nitrate, previously dissolved in about 10 cc. of water, followed by 10 cc. sulphuric acid; finally add about 2 Gm. of powdered potassium permanganate in several portions; allow the flask and contents to digest on a steam-bath for at least 30 minutes; decolorize the solution by addition of hydrogen peroxide with the aid of heat, to remove traces of the manganese oxides formed; filter the precipitate of silver chloride on to a Gooch crucible, wash the precipitate with nitric acid and water, and dry to constant weight at 105° C.: the percentage of chloride corresponds to not less than the percentage of nitrogen found according to the preceding assay multiplied by the factor 0.844 (N to Cl in $C_{11}H_{14}N_3Cl$), when calculated to the dried substance, nor more than the percentage of nitrogen found times 0.844 plus 3.0 per cent, when calculated to the dried substance. Transfer about 0.5 Gm., accurately weighed, to a 400-cc. beaker, and dissolve in 100 cc. of water: the hydrogen-ion concentration determined by titration with tenth-normal sodium hydroxide solution, using the hydrogen electrode and a calomel cell (normal potassium chloride solution) corresponds to a p_H of not less than 3 nor more than 7.

SUMMARY.

1. Comparative chemical analyses were made of different brands of Neutral Acriflavine in 1926.

2. The appearance of the various products differed markedly.

3. The compound apparently does not contain a molecule of water of crystallization.

4. Two of the manufacturers' products vary considerably in their "sulphated" ash content, and none of the brands examined were within the limit as prescribed in "New and Nonofficial Remedies," 1926.

5. The p_H curve of the electrometric titration appears to indicate the purity of a product.

6. The results obtained indicate that the product of the American manufacturers is at par with those of the foreign, and do not substantiate the claims, "that the foreign is superior to the domestic."

7. The difference in color (in the dry state) appears to be due to the fineness of the powder and the presence or absence of a very small amount of impurity removable by animal charcoal.

8. A more appropriate name was proposed and adopted by N. N. R., for the product: *i. e.*, Acriflavine Base in place of Neutral Acriflavine.

9. A complete revision of the standards of Acriflavine Base (Neutral Acriflavine) of "New and Nonofficial Remedies" resulted from this investigation.

See your Railroad Agent for attractive rates and routes to Rapid City, South Dakota, the A. P. H. A. Convention City—Week of August 26th.