Aureomycin-HCl (γ/cc.)	14	24	44
Mean value of absorbance	0.185	0.289	0.528
Calculated concentration from regression line (γ/cc.)	9.75	22.1	47.5
Deviation from known amount (%)	30.4	7.90	7.95
Concn. obtained from calibration curve (γ/cc.)	13.7	22.5	44.4
Deviation from known amount (%)	2.14	6.3	0.9

TABLE VII.

Multiple value	1	2	3
Absorbance	0.463	0.461	0.461

TABLE VIII.

Time of heating (min.)	3	5	10
Absorbance	0.409	0.463	0.449
〃	0.401	0.460	0.451

The authors wish to express their thanks to Prof. M. Ishidate of the Pharmaceutical Institute, University of Tokyo, for his valuable advices and instruction. Thanks are also due Dr. Y. Suzuki of the Ophthalmic Department, Chiba University Hospital, for the use of the spectrophotometer.

Summary

New colorimetric assay for Aureomycin with Th(IV) is described. A yellow complex has a maximum at 405 mμ at pH 4.0. It converts to an orange yellow complex on heating with dilute hydrochloric acid. These two color reactions conform to the Beer's law and can be applied to the colorimetric determination of Aureomycin.

(Received January 3, 1955)

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31. **Takeichi Sakaguchi** : Metal Chelate Compounds with Tetracycline Derivatives. III.* Colorimetric Determination of Aureomycin with Boric Acid.

(*Pharmaceutical Institute, University of Chiba***)

Current interest in Aureomycin and related compounds has increased the need for accurate means of assay. There are a few chemical methods available for the quantitative estimation of these antibiotics. Production of color by constituent functional groups of the tetracycline is the basis for the method presented here.

A solution (10 cc.) of concentrated sulfuric acid (sp. gr., 1.84) was added to a solution of Aureomycin Hydrochloride (about 5 cc.) to produce a somewhat stable orange red color (absorption maximum about 455 mμ), which turned brown.[1]

Addition of 1 cc. of 1% boric acid to a few cc. aliquot of Aureomycin Hydrochloride solution and made up to 5 cc. with concentrated sulfuric acid, produced a red violet color (absorption maximum 515~530 mμ) which was stable over a longer period (Fig. 1).

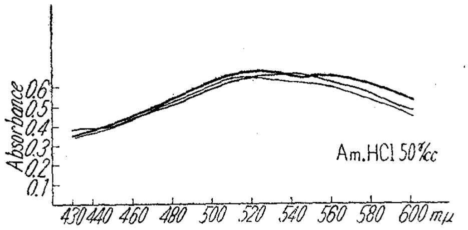

Fig. 1. Aureomycin-H_3BO_3-conc. H_2SO_4

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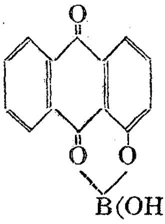
* Part II : This Bulletin, **3**, 166 (1955).

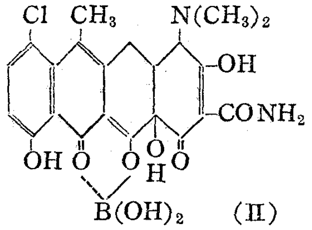
** Inohana-cho, Chiba (坂口武一).

[1] C. G. Van Arkel, A. M. Weshoff : Pharm. Weekblad, **86**, 389(1951)(C. A., **46**, 5276).

It is known that 1-hydroxyanthraquinone when treated with boric acid and concentrated sulfuric acid produces an intense color²⁾ (I), hence the following structure for colored chelate compound (II) of Aureomycin may be suggested.

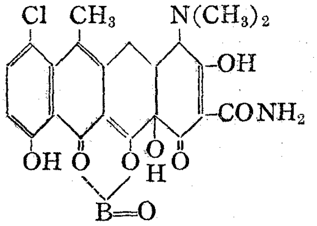


(I)



(II)

or



In the structural formula (II) Aureomycin is converted to Anhydroaureomycin which chelates with boric acid. This is evident because Anhydroaureomycin is obtained spectrophotometrically (445 mμ) by dilution of this boric acid complex with water. In the course of the reaction it was noted that the concentration of sulfuric acid over 85% was desirable to develop appropriate coloration. Consequently the use of a boric-sulfuric acid mixture, illustrated in Procedure II, was considered convenient. Further experiments indicated that Procedure II was more satisfactory than the above-mentioned method.

Reagents—Unless otherwise indicated, all reagents are C. P. or reagent grade. Boric acid solution (1%) was prepared by dissolving 1.0 g. of boric acid in 100 cc. of water. The specific gravity of conc. H_2SO_4 was 1.84. It is preferable to use the same commercial product in the series of the same experiments. Apparatus used was a Hitachi Spectrophotometer, Model EPB-V.

Standard Aureomycin Solution—A standard solution was prepared as previously described.* It contained 50 mg. of Aureomycin Hydrochloride in 100 cc. of water.

Procedure I—To a solution of 0.5~2.5 cc. of 50 mg% Aureomycin Hydrochloride in a 25-cc. test tube was added 1 cc. of 1% boric acid solution and it was made up exactly to 5 cc. with water. To this solution was added 10 cc. of conc. H_2SO_4 while cooling with ice water and then heated in a water bath at 80° for 5 minutes. The resulting solution was transferred into a volumetric flask (25 cc.) and brought up to the mark by the addition of conc. H_2SO_4.

The absorbance of the solution was measured at 515 mμ against a blank (distd. water). The solution contained 10~50 p.p.m. of Aureomycin Hydrochloride.

Standard Curve and Regression Line—From the results given in Table I, a regression line and a standard curve (Fig. 2) were established. The equation of the line was $y=0.0124x+0.028$, where x represents micrograms of Aureomycin Hydrochloride and y is the absorbance. The correlation coefficient was +0.9914. Standard deviation of absorbances and deviation of calculated results from experimental data are shown in Table II.

Effect of Sulfuric Acid Concentration—The absorbance at 515 mμ was found to be about the same for various concentrations of H_2SO_4, to which were added 4.5 and 6 cc. of water. The sample solution for colorimetry was prepared by adding conc. H_2SO_4 to the test solution which contained boric acid and made up to 25 cc. The results obtained are listed in Table III and it was found that the use of 5 cc. of water is preferable.

Color Formed Only by Sulfuric Acid—The absorption curves obtained by varying the concentration of H_2SO_4 from 100% to 60% are given in Fig. 3. It was found that the absorption band using

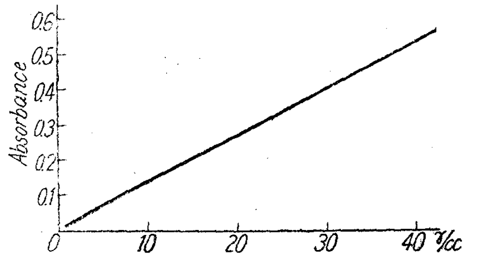


Fig. 2. Method of Boric-Sulfuric Acid (Procedure I)

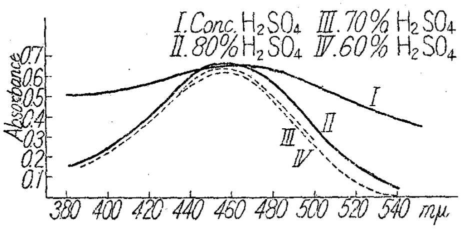


Fig. 3. Absorption Curves Produced by Various Concentration of Sulfuric Acid

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2) F. Feigl, U. P. Krumholz: Microchemie, Pregl-Festschrift, 77 (1929).

60~80% H_2SO_4 was rather narrow, while the band using conc. H_2SO_4 was wider. The absorption maxima of these curves were always at around 455 mμ indicating about equal absorbance.

Procedure II—Boric-sulfuric acid mixture: Ten g. of boric acid was dissolved in 100 cc. of conc. H_2SO_4.

To 0.5 to 2.5 cc. aliquot of the test solution was added 2 cc. of boric-sulfuric acid mixture and a red color developed. Conc. H_2SO_4 was then added up to 20 cc. and heated on a water bath at $80\pm3°$ for 5 mins. After cooling to room temperature it was made up to 25 cc. with H_2SO_4 and after 1~2 hrs. the absorption was measured at 530 mμ. Results are given in Table IV and absorption curves are illustrated in Fig. 5. The standard curve is shown in Fig. 6. It was found that procedure II was superior to the former.

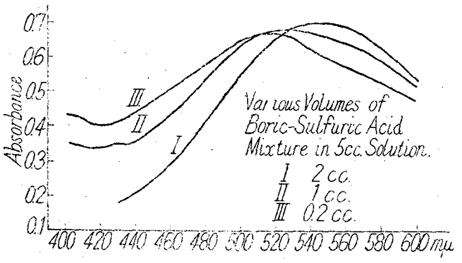

Fig. 4.

Variation of Boric Acid Contents in AM-H_2SO_4-conc. H_2SO_4 (Procedure II)

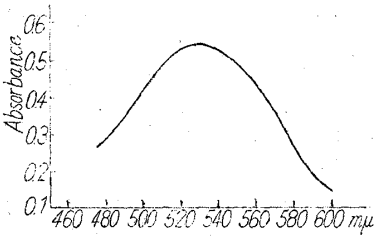

Fig. 5. AM-H_3BO_2-conc. H_2SO_4 (Procedure II) (AM-HCl 24.5 γ/cc.)

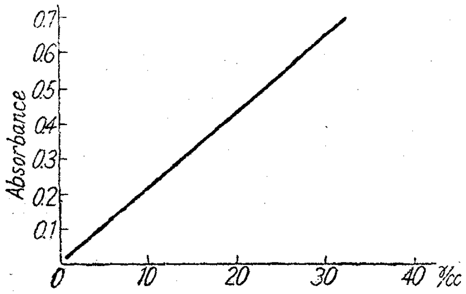

Fig. 6. Calibration Curve. Method of Boric-Sulfuric Acid Mixture (Procedure II)

Separation from Interferences—Five cc. of Aureomycin hydrochloride solution saturated with Na_2SO_4 was extracted 3 times with 2 cc. portions of butanol at pH 3~5. After the extraction, butanol extracts were combined and washed with water saturated with butanol and Na_2SO_4. The butanol layer was transferred to an evaporating dish and evaporated nearly to dryness on a boiling water bath.

The residue was rinsed with one or two drops of butanol and 1 cc. of boric-sulfuric acid mixture was added with thorough stirring. Five cc. of conc. H_2SO_4 was added to it as previously described, the resulting solution was transferred into a volumetric flask or a test tube (10 cc.), and brought up to the mark by the addition of conc. H_2SO_4. Absorbance measurement was made at 530 mμ against distd. water as a blank.

TABLE I. Absorbance vs. Concentration of Aureomycin Hydrochloride

Concn. of AM-HCl (γ/cc.)	10	20	30	40	50
Absorbance	0.135	0.280	0.390	0.540	0.640
〃	0.135	0.800	0.400	0.517	0.658
〃	0.145	0.290	0.408	0.537	0.630
Mean value	0.138	0.290	0.399	0.529	0.643

TABLE II. Deviation in Determination of Aureomycin Hydrochloride

AM-HCl (γ/cc.)	Absorbance				
14	0.199	0.181	0.190	0.170	0.186
34	0.456	0.468	0.456	0.445	0.442

AM-HCl (γ/cc.)	14	34
Absorbance, mean value	0.182_2	0.452_2
Standard deviation	0.011	0.010
Results from calibration curve	13.8 γ/cc.	33.6 γ/cc.
Deviation from calcd. value, %	1.43	1.18

Variation of boric acid contents in Aureomycin-boric-conc. sulfuric acid mixture is shown in Fig.

4. The use of 2 cc. of boric-sulfuric acid mixture in 25 cc. solution is preferable.

TABLE III. Effect of Water Content on Color Development
(cc. of water in 25 cc. of conc. H_2SO_4)

cc. of water	4	5	6
Absorbance at 515 mμ	0.274	0.285	0.280

TABLE IV. (according to Procedure II)

AM-HCl (γ/cc.)	4.2	6.1	12.2	18.4	24.5	30.6
Absorbance at 530 mμ	0.093	0.124	0.265	0.388	0.539	0.663
〃	0.093	0.121	0.260	0.388	0.539	0.663
〃	0.096	0.135	0.261	0.389	0.538	0.635
Absorbance at 525 mμ	0.094	0.122	0.258	0.384	0.535	0.658
〃	0.092	0.120	0.262	0.369	0.535	0.600
〃	0.097	0.135	0.255	0.382	0.535	0.626

The author wishes to acknowledge the helpful advices and suggestions of Prof. M. Ishidate of Pharmaceutical Institute, University of Tokyo. Analysis for the procedure I was carried out by K. Taguchi, and procedure II by A. Hanaki.

Summary

A sensitive colorimetric procedure using boric-sulfuric acid mixture for the determination of microgram quantities of Aureomycin Hydrochloride has been established. Working curves conform very closely to the Beer's law. The colored system was stable in the concentration of over 85% of sulfuric acid.

(Received February 3, 1955)

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32. **Eiji Ochiai und Hiroshi Yamanaka**: Polarisation der heterozyklischen Ringe mit aromatischem Charakter. CVIII. Eine neue Einführung der Nitril-Gruppe auf dem Pyrimidinkern.

(Pharmaz. Institut d. Mediz. Fakultät d. Universität Tokyo)*

Vor kurzem haben Ochiai und Suzuki[2] gezeigt, dass das 4-Chlorderivat des Pyridins, seines Methylhomologes bzw. Chinolins beim Erhitzen mit Natriumsulfit im wässerigen Medium in das entsprechende 4-Natriumsulfonat und das letztere beim Erhitzen mit Kaliumcyanid in das entsprechende 4-Cyanoderivat je mit befriedigender Ausbeute übergehen. Bei Anwendung dieser Methode auf dem 2- bzw. 6-Chlorderivat der Pyrimidin-Reihe haben wir nun eine neue Methode zur Darstellung ihrer 2- bzw. 6-Cyanoderivate entwickelt.

Eine Suspension von 2-Chlorderivat des Pyrimidins oder seines 4-Methylderivates bzw. 2,6-Dimethyl-4-chlorpyrimidins auf 10%iger wässerigen Natriumsulfit-Lösung wurde nämlich unter Rückfluss bis zur klaren Lösung erhitzt. Das beim Erkalten ausgeschiedene Natriumsulfonat wurde mit Alkalicyanid innig zerrieben und im Metallbade unter vermindertem Druck der Wasserstrahlpumpe erhitzt. Das hierbei übergegangene Destillat, welches das entsprechende Nitril vorstellt, wurde durch Umkristallisieren gereinigt. Die Resultate der Versuche sind in der folgenden Tabelle zusammengefasst.

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* Hongo, Tokyo (落合英二, 山中 宏).

[1] CVII. Mitteilung: Ochiai, Yokokawa: J. Pharm. Soc. Japan, **75**, 213 (1954).

[2] Dieses Bulletin, **2**, 247(1954).