*Studies on the Absorptiometric Determination of Microamounts of Boron. V. The Necessity of Methyl or Ethyl Oxalate in the Color Reaction of Boric Acid with Curcumin**

By Masatoshi MIYAMOTO

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The color reaction of boric acid with curcumin, whereon the most sensitive method for absorptiometric determination of boron is based, is well-kown, but it has not yet been clearly revealed although when Schulemberger1) first studied the reaction in 1866. Spicer and Strickland2) have, however, contributed valuable information on the reaction product. The present paper deals with facts concerning the color reaction overlooked for a long time. The study has been initiated with the purpose of extracting an effective product from a curcumin-oxalic acid reagent solution sufficiently aged by refluxing;3) thus, it is hoped, a stable and reliable reagent for instant use can be obtained.

Results and Discussion

The Effects of Various Acids on Color Reaction.—It has been reported that the presence of oxalic,4) tartaric5) and salicylic6) acids intensify the color resulting from the reaction between

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1) M. E. Schulemberger, *Bull. Soc. Chim.*, [2], 5, 194 (1866).

2) G. S. Spicer and J. D. H. Strickland, *J. Chem. Soc.*, **1952**, 4644, 4650.

3) M. Miyamoto, *Japan Analyst* (*Bunseki Kagaku*), **11**, 635 (1962).

4) C. E. Cassal and H. Gerrans, *British Food Journal*, 210 (1902).

5) F. Michel, *Mikrochemie ver. Mikrochim. Acta*, **29**, 63 (1941); *Chem. Abstr.*, **35**, 5412 (1941).

6) C. H. Cribb and F. W. F. Arnaud, *Analyst*, **31**, 147 (1906).

TABLE I. EFFECT OF VARIOUS ACIDS ON COLOR DEVELOPMENT

Exp., No.	Curcumin solution*1		Net absorbancy at 550 mμ for 1 μg. boron in the presence of		
	Solvent	Acid added	None	Oxalic acid	Hydrochloric acid
1	Ethanol	—	—	0.126	0.021
2	Ethanol	Hydrochloric	0.098*2	0.311*3	—
3	Ethanol	Oxalic	0.404	—	0.400
4	Ethanol	Malonic	0.190	0.326	—
5	Ethanol	Tartaric	0.000	0.152	—
6	Ethanol	*o*-Phthalic	0.016	0.011	0.000
7	Ethanol	Salicylic	0.000	—	—
8	Acetone	—	—	—	0.000
9	Acetone	Oxalic	0.068	—	0.362
10	Diethyl ketone	Oxalic	0.124	—	0.120

*1 Refluxed for 4 hr.
*2 Analogous absorbancies were obtained for 0.5, 1.5 and 2.0 μg. of boron.
*3 Proportional to the amounts of boron.

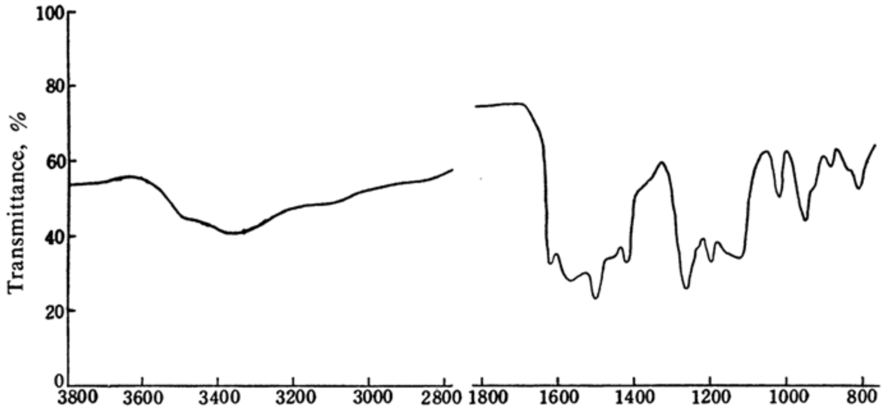

Fig. 1. Infrared absorption spectrum of curcumin.
Curcumin, 0.5 mg., and KBr, 200 mg.

curcumin and boric acid. The author's results in the 4th column of Table I show that the presence of oxalic acid is effective for the color reaction under the conditions stated in the experimental section below, whereas those of hydrochloric, malonic, tartaric, *o*-phthalic and salicylic acids are ineffective (cf. Nos. 2 to 7). The slight effect of malonic acid may possibly be attributed to small amounts of oxalic acid contaminating the malonic acid used (cf. "Effect of various esters" below). No correlation is observed between the effects and the first dissociation constants of the acids added to a curcumin solution. The effect of oxalic acid on the reaction is unique.

A comparison of the results shown in the 5th column, Nos. 1 and 2, shows that the presence of hydrochloric acid promotes the color reaction in the presence of oxalic acid. This may be attributed to the effect of hydrochloric acid (hydrogen ions) on the aging reaction of a reagent solution, whereby aging reaction proceeds during evaporation for the color reaction. However, the aging reaction does not proceed enough during the evaporation, as is revealed by a lower result of No. 2, 5th column, than that of No. 3, 4th column. In either cases, both oxalic and hydrochloric acids were present at the evaporation stage; a curcumin-oxalic acid solution aged fairly enough by 4 hours' refluxing[3] was used in the latter case. Tartaric and *o*-phthalic acids are not only ineffective but also interfere with the color reaction in the presence of oxalic acid, as is shown in the 5th column. This may be attributed to the formation of borate

TABLE II. INFRARED ABSORPTION BANDS OF CURCUMIN

Wave number, cm^{-1}		Assigned to
Author	Reported[9]	
3360	3400	-OH (Hydrogen bonded)
1625	1625	>C=O (Conjugated hydrogen bonded ring of β-diketone)
1570	1600 ?	-C=C- (Conjugated)
1510	1500	-C=C- (Ring)
1430	—	-OH (β-Diketone[10])
1265	—	-OH (Phenolic[11]) >C=O (Conjugated hydrogen bonded ring of β-diketone[10])
1200	—	-OH (Phenolic[11]), -C-O-C- (Aryl)
1125	—	?
1025	—	-C-O-C- (Aryl)
960	962	-C=C- (Trans), -OH (β-Diketone[10])

complexes* with the acids.

When acetone is used as the solvent in preparing a reagent solution, the reagent solution gives an intense color only in the presence of hydrochloric acid (cf. Nos. 8 and 9). A reagent solution prepared with diethyl ketone as the solvent gave a little coloration (cf. No. 10). However, because of insufficient data, no conclusion was possible except that the solution might not have a strong color-developing power, if, indeed, it has any.

The foregoing results indicate that the presence of oxalic acid is necessary in the color reaction between boric acid and curcumin when ethanol is used as the solvent in preparing a reagent solution and that the presence of both of hydrochloric and oxalic acids is required when acetone is used as the solvent. These facts suggest a close relation between the color reaction and the solvent of the reagent solution used.

The Aging Reaction of the Curcumin-Oxalic Acid Reagent.—*Absorption Spectrum.*—The aging phenomenon of a curcumin-oxalic acid reagent solution is pronounced in an ethanol medium. However, no interpretation of this phenomena has been made thus far. The structure[8,9] of curcumin reveals no possibility of a reaction whereby curcumin reacts with either oxalic acid or ethanol in the solution or is converted to a compound effective in the coloration of boric acid. The structure was confirmed by absorption spectroscopic studies. The infrared and near-ultraviolet absorption curves obtained by the author are shown in Figs. 1 and 2, respectively. The infrared absorption bands of a purified curcumin in the solid state may be assigned as shown in Table II. The absorption band, max 265 mμ and ε 12500, observed in an ethanol medium indicates the possible existence of the keto-enol ring structure[12] of curcumin in the medium (cf. curve

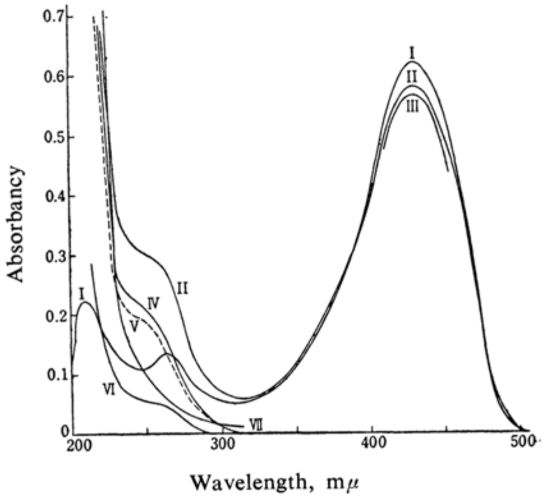

Fig. 2. Absorption spectra of ethanolic solutions of curcumin-oxalic acid reagent and related compounds.

I : Curcumin, 1.09×10^{-5} M
II : Curcumin-oxalic acid (refluxed for 6 hr.), 1.09×10^{-5} and 3.97×10^{-3} M, respectively
III : Curcumin-oxalic acid (left to stand for 29 days), the same concentrations as curve II
IV : Oxalic acid (refluxed for 6 hr.) or diethyl oxalate, 3.97×10^{-3} M
V : Difference between curves I and II
VI : Oxalic acid (fresh solution), 1.5×10^{-3} M
VII : Oxalic acid (aqueous solution), 1.5×10^{-3} M

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* The complex formation of boric acid with tartaric acid in an acid medium is suggested by the increasing solubilities[7] of boric acid in water with the increasing amounts of tartaric acid added.

7) W. F. Linke, "Solubilities of Inorganic and Metal-organic Compounds," Vol. I, D. Van Nostrand, New York (1958), p. 267.

8) V. Lampe, *Ber.*, **51**, 1347 (1918).

9) L. J. Bellamy, G. S. Spicer and J. D. H. Strickland, *J. Chem. Soc.*, **1952**, 4653.

10) S. Bratož, D. Hadži, and G. Rossmy, *Trans. Faraday Soc.*, **52**, 464 (1956).

11) K. Kuratani, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zassi*), **73**, 928 (1952).

12) D. S. Rasmussen, D. D. Tunnicliff and R. R. Brattain, *J. Am. Chem. Soc.*, **71**, 1068 (1949).

I, Fig. 2). Only a little decrease in the absorbancy of a curcumin-oxalic acid reagent solution at 430 mμ was observed after it had been refluxed for 6 hr., indicating a good stability³⁾ of the reagent solution (cf. curves I and II). A decrease of about 10% was also observed after allowing reagent solution to stand at room temperature for 29 days. However, no appreciable change in the shape of the absorption curve was observed in either case.

A comparison of curve I, obtained for a curcumin solution, with curve II obtained for an aged reagent solution leads to curve V, analogous to the absorption curve of the ethyl oxalate (curve IV) but different from that of oxalic acid (curve VII). Seemingly this implies that the aging reaction may simply be the esterification reaction of oxalic acid with the ethanol used as a solvent rather than a reaction with which curcumin is concerned. This expectation was substantiated by the facts that a fresh curcumin solution to which ethyl oxalate has been added develops the color with boric acid as much as a sufficiently aged reagent solution does and that this is also the case when a refluxed ethanolic solution of oxalic acid has been added instead of the ester. On the contrary, a very weak coloration takes place with a refluxed ethanolic curcumin solution to which a fresh ethanolic solution of oxalic acid has been added. The results obtained are shown in Tables III and IV.

TABLE III. COLOR DEVELOPMENT WITH A CURCUMIN SOLUTION MIXED WITH AN OXALIC ACID SOLUTION

Curcumin soln.	Oxalic acid soln.	Net absorbancy at 550 mμ for 1 μg. boron
Fresh	Fresh	0.090
Refluxed	Fresh	0.087
Refluxed	Refluxed	0.395
Fresh	Refluxed	0.399

TABLE IV. EFFECT OF VARIOUS ESTERS ON THE COLOR DEVELOPMENT

Oxalate ester	Solvent	Net absorbancy at 550 mμ for 1 μg boron
Dimethyl	Methanol	0.355
Diethyl	Methanol	0.392
Diethyl	Ethanol	0.405
Diethyl	Acetone	0.404
Di-*n*-butyl	Ethanol	0.003
Di-*n*-butyl	*n*-Butanol	0.002

The Effects of Various Esters.—As Table IV shows, a curcumin solution to which diethyl oxalate has been added develops the color with boric acid independently of the solvent used in preparing the reagent solution. Dimethyl oxalate acts as does diethyl oxalate. Dibutyl oxalate (presumably any higher ester of oxalic acid than propyl oxalate) and esters other than that of oxalic acid do not contribute to the color reaction at all. These facts indicate the unique and important role of dimethyl oxalates in the color reaction.

Acetone has been frequently used as a solvent in place of ethanol in preparing a curcumin-oxalic acid reagent solution. It is dimethyl or diethyl oxalate but not oxalic acid which enables the reagent solution to develop the color with boric acid. The ester should be formed from alcoholic impurities in any acetone used. This will be seen from the fact that the purer the acetone, the lower the intensity of the resulting color, as Table V shows. No difference in the shape of the absorption curve was observed between the colored product obtained from an acetonic curcumin-oxalic acid solution and that obtained from an ethanolic curcumin-diethyl oxalate solution (cf. curves II and III, Fig. 3). It has been

TABLE V. COLOR DEVELOPMENT WITH CURCUMIN AND OXALIC ACID DISSOLVED IN VARIOUS PURITY GRADES OF ACETONE

Acetone	Net absorbancy at 550 mμ for 1 μg. boron	
	Fresh soln.	Refluxed soln.
JIS 1st grade	0.200	—
JIS special grade	—	0.362
Absorption spectroscopic grade	0.138	0.203
Purified	0.017	0.104

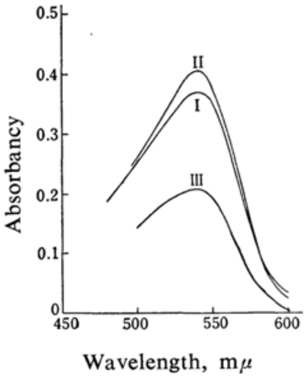

Fig. 3. Absorption spectra of ethanolic solutions of colored products.
Reagent solutions used are:
I: Curcumin-dimethyl oxalate as a methanolic solution
II: Curcumin-diethyl oxalate as an ethanolic solution
III: Curcumin-oxalic acid as an acetonic solution refluxed for 8 hr.

said[13] that commercial acetone some times contains methanol as an impurity. The author observed strong absorption bands at 206.5 and 214 mμ and around 1.9 μ which may be attributed to such impurities as water and alcohols present in commercial acetones.

With this view, the role of hydrochloric acid in the color reaction, whereby an acetonic reagent solution is able to develop a strong color with boric acid with the aid of hydrochloric acid (cf. Table I), may be understood as that of an acid catalyst in the esterification which occurs during evaporation for the color reaction. The presence of insufficient amounts of either of the esters in the reaction mixture may cause low and scattered results. For this reason,* the amounts of aqueous sample solutions assigned to the color reaction should be regulated[3] in order to retain ethyl oxalate against hydrolysis. The fact that an insufficiently aged reagent solution or a reagent solution containing relatively small amounts of oxalic acid results in low and scattered results[14] may also be attributed to insufficient amounts of ethyl oxalate formed in the reagent solutions. Seemingly, for the same reason, highly precise results may hardly be expected by using an acetonic reagent solution prepared with a commercially available acetone containing even relatively small amounts of alcoholic impurities, even if a working curve is renewed for a particular reagent solution prepared with a particular lot of acetone as the solvent.

The Composition of the Colored Product. — The composition of the colored product has been studied by Schulemberger[1] and by Clarke and Jackson.[15] Spicer and Strickland[2] have presented a structure for a red complex, rosocyanin,** formed in the presence of hydrochloric acid; they have found the boron curcumin ratio to be 1 to 2. They have also stated that another red complex, rubrocurcumin, having a 1 to 1 to 1 composition of boron, curcumin, and oxalic acid is formed when the reaction takes place in the presence of oxalic acid, and they have presented a structure for this complex. However, the foregoing discussion reveals that further studies of the colored products, especially of rubrocurcumin, are necessary.

No distinct difference in absorption maximum and shape was observed between absorption curves of ethanolic solutions of the colored product obtained in the presence of methyl and ethyl oxalate respectively (cf. curves I and II, Fig. 3). This might imply that the same product is obtained in the presence of either of the esters. The author leaves uncovered such problems as the role of the esters in the reaction mechanism and the composition of colored product, merely indicating that methyl or ethyl oxalate is necessary for the color reaction between curcumin and boric acid.

Experimental

The chemicals, such as curcumin, oxalic acid, ethanol and hydrochloric acid, the apparatus, the procedure of color development, and the method of measuring color intensity are the same as in the previous reports.[3,14,16] The other chemicals used were of JIS special or guaranteed grade. The methanol, acetone, and diethyl ketone used as solvents for preparing curcumin-oxalic acid solutions had been purified by distillation with a quartz apparatus after having been dried with anhydrous sodium carbonate. Variations will be indicated for particular cases.

The Effect of Various Acids on Color Development. — A boric acid solution containing 1.00 μg. of boron associated with either one of the other substances, 2.00 mg. (3.2 meq.) of oxalic acid, or 1 meq. of hydrochloric acid was subjected to the color reaction with a curcumin solution containing one of the various acids to be tested. The curcumin solution was prepared by dissolving 40.0 mg. of curcumin and 80.0 meq. of hydrochloric, oxalic, malonic, tartaric, *o*-phthalic or salicylic acid in 100 ml. of ethanol, acetone, or diethyl ketone. This mixture was refluxed for 4 hr. in order to promote an aging reaction such as might take place in the case of an ethanolic curcumin-oxalic acid solution. The refluxing was continued for 8 hr. in the case of an acetonic solution because of the low boiling point of acetone. The reaction product was extracted by ethanol, and the absorbancy was measured at 550 mμ. Blank tests gave absorbancies less than 0.033. The results are shown in Table I.

Absorption Spectra. — The infrared absorption spectrum of curcumin was obtained with a recording spectrophotometer, Hitachi Model EPI-2, a pellet consisting of 0.5 mg. of curcumin and 200 mg. of potassium bromide being used. The curcumin was purified by recrystallization[14] from ethanol until a constant melting point, 173°C, was obtained. The results are shown in Fig. 1. For obtaining the absorption spectra of the various solutions shown in Fig. 2, a manual spectrophotometer, Hitachi Model EPU-2, equipped with 1 cm. cells was used, the reference liquids being the same

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[13] T. Kuwata, "Yozai," Maruzen Co., Tokyo (1940), p. 258.

* The hydrolysis of the resultant colored product during evaporation for the reaction may not be a principal reason for regulating the volume of the aqueous sample solution to be taken. The coloration is observed at the end of the evaporation, and it proceeds further by baking.[3]

** Rosocyanin, so called by these workers, should be distinguished from the "rosocyanine" of the previous workers.[1,15]

[14] M. Miyamoto, *Japan Analyst* (*Bunseki Kagaku*), **12**, 115 (1963).

[15] L. Clarke and C. L. Jackson, *Am. Chem. J.*, **39**, 696 (1908).

[16] M. Miyamoto, *Japan Analyst* (*Bunseki Kagaku*), **12**, 120 (1963).

as the solvents of the sample solutions. Ethanolic solutions of curcumin-oxalic acid, curcumin, oxalic acid, and diethyl oxalate were diluted with ethanol to an appropriate concentration just before the measurements so as to give appropriate absorbancies, although their concentration was originally 40.0 mg./100 ml. and 0.400 M as to the curcumin and oxalic acid equivalent respectively. For obtaining the absorption spectra of reaction products extracted by ethanol, a recording spectrophotometer, Hitachi Model EPS-2, and 1 cm. celles were used, the reference liquid being ethanol. The results are shown in Fig. 3.

Color Development with a Curcumin and an Oxalic Acid Solutions Prepared Independently.—An ethanolic solution of curcumin, 80.0 mg./100 ml., and one of 0.800 M oxalic acid were prepared. Two milliliter portions of the solutions were mixed together just before use, and the color-developing power of the mixed solutions was tested by using 1.00 μg. of boron. The test was also carried out after the solutions had been independently subjected to refluxing for 2 hr. The results are shown in Table III.

The Effects of Various Esters.—A reagent solution containing 40.0 mg. of curcumin and 40.0 mmol. of the various esters to be tested was prepared by dissolving the respective substances in methanol, ethanol, acetone, or *n*-butanol. The color-developing power of the solution was tested by using 1.00 μg. of boron. The esters tested were dimethyl, diethyl, and di-*n*-butyl oxalates. The results are shown in Table IV. When *n*-butanol was the solvent, two layers were formed by adding the reagent solution to a boric acid solution, and the system was not dried by the evaporation at 55°C leaving the solvent because of its high boiling point. In this case, it was assumed that drying completed when the aqueous layer had disappeared. None of the color-developing power was observed when the oxalate esters were replaced by any of the following esters: the dimethyl and diethyl esters of acetic, malonic, and *o*-phthalic acids, and methyl and ethyl salicylates.

Color Development with Curcumin and Oxalic Acid Dissolved in Various Purity Grades of Acetone.—Curcumin-oxalic acid solutions were prepared by using various purity grades of acetone as a solvent. Immediately after the preparation, the color-developing power of the solutions was tested by using 1.00 μg. of boron, to which 1.00 meq. of hydrochloric acid had previously been added. The test was also carried out after the solutions had been refluxed for 8 hr. The results are shown in Table V. Of the various grades of acetone, a spectroscopic-grade aceton was that of Eastman Kodak, and a purified acetone was obtained by the following procedure: acetone, JIS first grade, was stirred in the presence of powdered potassium permanganate for 3 days, then double distilled after dehydration with anhydrous sodium carbonate.

Summary

The coloration of microamounts of boric acid with an ethanolic solution takes place in the presence of oxalic, but not in that of other acids. Near-ultraviolet, visible, and infrared absorption spectroscopic studies have revealed that the aging reaction of an ethanolic curcumin-oxalic acid reagent solution is simply the esterification of the oxalic acid with the solvent used. The resulting diethyl oxalate is necessary to the color reaction. The role of the ester has not been interpreted yet; however, it is so unique that it can be performed only by dimethyl oxalate, not by the other esters. The esterification of oxalic acid in an acetonic reagent solution may be attributed to the presence of alcoholic impurities present in aceton used. The composition of the colored reaction product, rubrocurcumin, presented by Spicer and Strickland[2] has not been substantiated yet in relation to the necessity of the esters to the color reaction.

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Research Laboratory
Tokai Electrode Mfg. Co., Ltd.
Fujisawa, Kanagawa-ken