O,O'-Dibenzoyl derivative of 4-hydroxyaminoquinoline 1-oxide underwent a similar pyrrolysis to give an enhanced CIDNP spectrum of benzene protons.

Pyrrolysis of O-acylhydroxylamines, induced by homolytic fission of N-O bond, is being examined in detail with a help of CIDNP method in our laboratory.

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A New Method for the Colorimetric Determination of Acrinol Base by Solvent Extraction with Tetrabromophenolphthalein Ethyl Ester

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In general, acid dye[2,3] is known to react with amine or quarternary ammonium salt to form a colored compound. Bromothymol blue,[4] bromocresol green[5] or bromophenol blue[6] has been used for the conventional determination of thiamine, quartenary ammonium compound or quinine.

Tetrabromophenolphthalein ethyl ester (TBPE) is well known as a pH indicator. Various amines and organic cations were found to be extracted with TBPE into 1,2-dichloroethane. The colors of the extracted species can be classified into the following three categories. (1) Blue species which is extracted by the presence of acrinol base, sparteine or thiamine. (2) Red-violet species which is developed by the presence of N,N-dimethylpiperazine, eserine or triethanolamine. (3) Yellow species which is the same color as reagent blank even in the presence of aniline, 3-aminoquinoline, pyridine, N,N-dimethylformamide, EDTA, or NTA.

The discussion of this paper deals with a spectrophotometric determination of acrinol base by solvent extraction with TBPE. The proposed method has a better reproducibility and a higher sensitivity. The titrimetric method[7] has been used for the determination of acrinol which is widely used as disinfectant. 3-Methyl-2-benzothiazolinone hydrazone-ferric chloride[8] was described as a spectrophotometric reagent for acrinol.

Experimental

Apparatus and Reagents——1) Apparatus: Shimadzu Model QR-50 spectrophotometer with 10 mm cuvettes. Iwaki Model KM shaker with a time switch. Toa Denpa Model HM-5 pH meter.

2) Reagents: All the chemicals were of reagent grade. All aqueous solutions were prepared by the use of deionized water.

a) Tetrabromophenolphthalein Ethyl Ester (TBPE) Solution: Weighed amounts of tetrabromophenolphthalein ethyl ester potassium salt (mol.wt. 700.1) were dissolved in ethyl alcohol.

1) Location: *a) Koyama-cho, Tottori-shi*; *b) Higashisenda-cho, Hiroshima-shi.*
2) M.E. Auerbach, *Anal. Chem.*, **15**, 492 (1943).
3) P.B. Marshall and E.W. Rogero, *Biochem. J.*, **39**, 258 (1945).
4) V.D. Gupta and DE. Cadwallader, *J. Pharm. Sci.*, **57**, 112 (1968).
5) H.M.N.H. Irving and J.J. Markham, *Anal. Chim. Acta*, **39**, 7 (1967).
6) M. Tatsuzawa, S. Nakayama and A. Okawara, *Bunseki Kagaku* (Japan Analyst), **19**, 761 (1970).
7) "The Japanese Pharmacopoeia VII-1, Part II," Nankodo, 1965, p. 1.
8) K. Kato, M. Umeda and S. Tsubota, *Yakugaku Zasshi*, **83**, 1180 (1963).

b) Standard Acrinol Base Solution: The standard solution of acrinol base was prepared by dissolving acrinol (6,9-diamino-2-ethoxyacridine monolactate monohydrate) in water; it was then standardized according to the titrimetric method[7] with thiosulfate. The working standard solutions were obtained by diluting this solution to the concentrations required for the experiments.

c) Buffer Solution: The borate–phosphate buffer was prepared by adding 3N sulfuric acid or 3 N sodium hydroxide solution to the 0.3 M potassium dihydrogen phosphate solution contained 0.1 M sodium borate.

Recommended Procedure——Pipette 2—8 ml of acrinol solution (2×10^{-5} M), 2 ml of TBPE solution (2×10^{-3} M) and 5 ml of borate–phosphate buffer solution (pH 9) into a separating funnel. Dilute the mixture to 25 ml with water and shake the solution for 2 min with 10 ml of 1,2-dichloroethane. After separation of the two layers, run off the extract into a glass tube through a filter paper to remove droplets of water. Measure the absorbance of the extract at 610 mμ using a reagent blank as a reference.

Result and Discussion

Absorption Spectra

Fig. 1 shows the visible absorption spectra of acrinol extracts with TBPE. It can be seen that the presence of a small amount of acrinol in aqueous solution leads to a considerable increase in the extraction. The absorbance maximum of the extract is at 610 mμ. This blue color in the organic phase may be attributed to the ion-pair formation between the TBPE anion and acrinol cation.

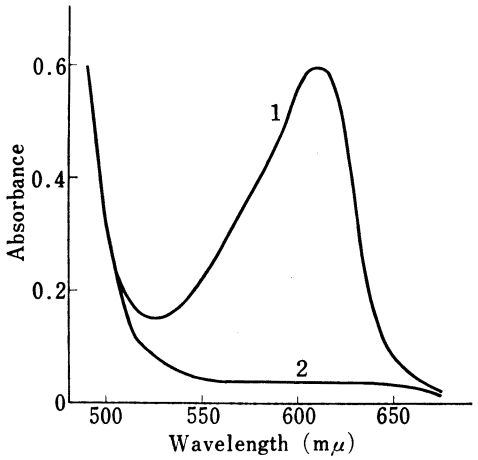

Fig. 1. Absorption Spectra
1: extract with 4.0×10^{-6}M acrinol
2: reagent blank
reference: water

Effect of pH

The effect of pH on the extraction was studied by extracting the acrinol base from a series of aqueous solutions buffered at various pH values. As is shown in Table I, the absorbance of the extract was almost constant when the pH of the aqueous phase lay within the range 8.5—10.0.

An extreme pH dependence was observed for the determination of quinine[6] or thiamine[4] with bromophenol blue or bromothymol blue. Similarly, the constant absorbance in any pH range could not be obtained, when bromophenol blue or bromo-

Table I. Effect of pH

pH of aqueous phase	Absorbance of extract[a]	Absorbance of reagent blank
7.0	0.417	0.045
8.1	0.585	0.047
8.5	0.596	0.045
8.9	0.597	0.044
9.4	0.595	0.047
9.7	0.600	0.047
10.0	0.593	0.044
10.4	0.569	0.048
11.0	0.448	0.044

a) extract with 4.0×10^{-6}M acrinol
wavelength: 610 mμ, reference: water

cresol green was used as an extractant for acrinol base.

Effect of Reagent Concentration

The influence of TBPE concentration on the extraction is illustrated in Table II. It is apparent that the concentration of TBPE should be maintained at more than 20-fold molar excess over acrinol to obtain a constant extraction. Excess amounts (2—10 ml) of the borate–phosphate buffer solution used in the recommended procedure had no influence on the absorbance of the extract. When the addition of the buffer solution was less than 2 ml, a good separation of the two layers was not observed.

Table II. Effect of TBPE Concentration

TBPE[a] (added)	mole ratio $\frac{\text{TBPE}}{\text{acrinol}}$	Absorbance (extract[b])	Absorbance (reagent blank)
0.1 ml	2	0.230	0.030
0.2 ml	4	0.405	0.041
0.5 ml	10	0.528	0.045
1.0 ml	20	0.575	0.045
2.0 ml	40	0.595	0.047
2.5 ml	50	0.602	0.047

a) 2×10^{-3} M solution, *b*) extract with 4.0×10^{-6} M acrinol
wavelength: 610 mμ, reference: water

Solvent for Extraction

The behavior of various solvents in the extraction was studied. Solvents were found to be classified into the following categories.

a) Those with which the presence of acrinol leads to a considerable increase in the extraction of blue TBPE: *e.g.* 1,2-dichloroethane, chloroform.

b) Those which do not extract the blue TBPE even in the presence of acrinol: *e.g.* carbon tetrachloride, cyclohexane, *n*-hexane, monochlorobenzene, toluene.

c) Those with which the blue TBPE is extractable even without acrinol: *e.g.* butyl acetate, ether, ethyl acetate, isoamyl alcohol, nitrobenzene, nitromethane, methyl isobutyl ketone.

1,2-Dichloroethane was found to be most suitable for the extraction of a TBPE-acrinol system.

Other Variables

Full color development was observed by 1 min shaking. Continued shaking up to 5 min produced no further change in absorbance.

The color intensity of dichloroethane extracts remains constant for 1 hr.

Normal room temperature fluctuations (16—24°) were without measurable effect in absorbance.

Calibration Curve and Precision

Fig. 2 shows the calibration curve for acrinol base by the recommended procedure described above. A linear relationship was observed between the absorbance of the extract and the concentration of acrinol base (1.6×10^{-6}—6.4×10^{-6} M) in the aqueous solution.

The reproducibility of the proposed method was estimated from the results of ten sample solutions, each with a final acrinol concentration of 4.0×10^{-6} M. The mean absorbance was 0.552, with a standard deviation of 0.006 absorbance unit.

Compositon of the Colored Species

The composition of the extracted species was determined to be 1:1 by the continuous variation method for acrinol and TBPE. The total concentration of acrinol plus TBPE was 1.6×10^{-5} M. The continuous variations plots at 610 mμ, 590 mμ and 630 mμ have a maximum at 0.5 mole fraction of TBPE. The extracted species with TBPE, therefore, can be suggested as $[\text{Acrinol}]^+\cdot[\text{TBPE}]^-$.

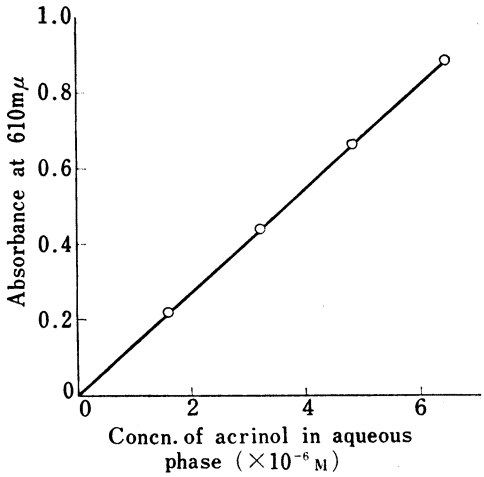

Fig. 2. Calibration Curve
reference: reagent blank

Effect of Foreign Substances

Table III shows the effect of foreign substances on the determination of acrinol. Chloride, glucose, lactose, borate, urea, and starch, which are apt to exist in pharmaceutical preparation with acrinol, do not interfere with the determination.

Analysis of Practical Samples

To test the validity of the proposed method commercial samples as medicine or disinfectant obtained from a drugstore were analyzed according to the recommended procedure and the titrimetric method[7] for comparison. The results obtained are summarized in Table IV.

Table III. Effect of Foreign Substances on the Determination of Acrinol Base

Substance added	Mole ratio (acrinol=1)	Recovery of acrinol (%)[a]
Ammonium sulfate	20000	100
Calcium chloride	20000	99
Sodium chloride	20000	100
Sodium nitrate	20000	101
Potassium bromide	20000	101
Zinc sulfate	20000	99
Antipyrine	20	107
Benzyl alcohol	20000	100
Boric acid[b]	10000	100
Benzethonium	0.1	115
o-Cresol	20000	98
Ethyl alcohol	20000	101
Glucose	20000	99
Lactose	20000	100
Nicotinamide	50	106
Phenacetine	5	105
Phenol	20000	97
Sodium acetate	20000	100
Sodium citrate	20000	101
Sdium salicylate	20000	100
Urea	20000	100
Vitamin B_1	5	108
Vitamin C	20000	98
Starch	(0.5%)	100

a) acrinol: 4.0×10^{-6}M as acrinol lactate
b) neutralized with NaOH solution

TABLE IV. Analysis of Practical Samples

Sample	Acrinol cation found	
	Titrimetric method	Proposed method
Disinfectant solution	2.71×10^{-3}M	2.80×10^{-3}M
Tablet	17.5 mg/g	17.9 mg/g
Ointment	0.698 mg/g	0.708 mg/g

a) Disinfectant Solution——Dilute a sample with water and treat the solution in the same manner as the recommended procedure.

b) Tablet——Dissolve a sample in dilute sulfuric acid. Filter the solution with glass filter and dilute the filtrate with water. Treat the solution in the same manner as the recommended procedure.

c) Ointment——Take a sample in a separating funnel and add 15 ml of ether. Extract the contents three times with 30 ml portions of 0.04 N sulfuric acid. Put together the whole extracts and dilute with water. Treat the solution in the same manner as the recommended procedure.