UDC 632.951 : 543.43

109. Takashi Mitsui, Hiroshi Ozaki, Etsuko Toda, and Hajime Sano : Insecticide Determination. II.*1 Colorimetric Determination of Dimethyl 1,2-Dibromo-2,2-dichloroethyl Phosphate (Dibrom).

(*Research Laboratory, Chugai Pharmaceutical Co., Ltd.**2)

Colorimetric determination of the insecticide, DDVP, was previously reported. This method of determination was found to be applicable to another insecticide, Dibrom, which undergoes reddish yellow coloration by reaction with acetone in the presence of alkali. Colorimetric determination of Dibrom by the use of this reaction was attempted, but the absorbance so varied according to the reaction temperature, time and pH, that various conditions of the reaction were examined. Good results were obtained from the following examinations.

Experimental and Results

1) **Relationship between Wave Length and Absorbance**——A solution of 100 mg. of Dibrom, accurately weighed, is dissolved in Me_2CO and made up exactly to 100 ml. To 10 ml. of this solution placed in a glass-stoppered test tube, 5 ml. of a solution of 2.0 g. of KOH dissolved in 100 ml. of dehyd. EtOH and filtered is added and the mixture is maintained at 25° for 2 hr. One milliliter of this solution is diluted with 15 ml. of distilled water or EtOH and absorbance of this solution is measured at each wave length.

As shown in Fig. 1, the absorbance became maximum at 370～375 mμ, and subsequent measurements were made at 370 mμ.

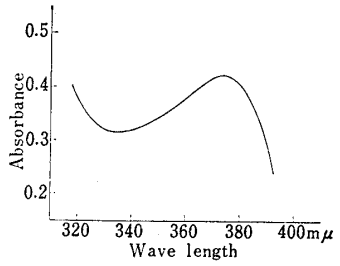

Fig. 1. Absorption Spectrum of Dibrom-Acetone Complex

2) **Relationship between Reaction Temperature and Time**——Two solutions of about 5 and 10 mg. of Dibrom accurately weighed, dissolved in Me_2CO are brought exactly to 10 ml. respectively, placed in glass-stoppered test tubes, and 5 ml. of the ethanolic KOH solution, the same as above, is added. These solutions are allowed to stand at 20, 25 or 30° to examine the effect of temperature on coloration. As shown in Fig. 2, the absorbance was not stabilized by standing at 20°, even after 180 min., and gave an upward curve. At 25°, the absorbance became stable after 100～140 min., both with 5 and 10 mg. of the sample. A similar upward curve was observed at 30°, same as at 25°, and the absorbance became stable in 100～140 min. Therefore, temperature is suitable at 25～30° but considering the use of Me_2CO for this reaction, lower temperature is desirable and subsequent experiments were carried out at 25～26°.

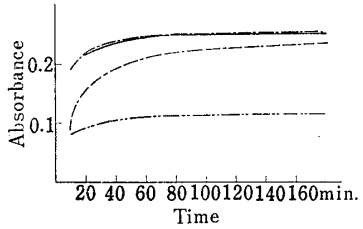

Fig. 2. Rate of Color Development

- - - - - - - - - - : 30°(10 mg.)
———————— : 25°(10 mg.)
— — — — — — — — : 20°(10 mg.)
— - — - — - — - — - : 25°(5 mg.)

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3) **Relationship between Absorbance and pH**——The sample was treated by the foregoing conditions, colored solution was allowed to stand at 25° for 2 hr., and 15 ml. of buffer solutions of various pH was added to 1 ml. of the colored solution. Results on the measurement of absorbance of these solutions at 370 mμ are shown in Fig. 3 which indicate that the absorbance greatly varies accordins to the pH of solution. This is especially marked in the range of pH 7.0~8.0, in which the absorbance varies greatly with slight difference in pH. There is little effect of pH on the absorbance in the range of pH 5.0~6.0 but the sensitivity is poor in relationship between the concentration and absorbance. In other words, the slope of the straight line plotted by taking the concentration on the abscissa and absorbance on the ordinate becomes very small.

When 15 ml. of dehyd. EtOH was used, a good sensitivity and reproducible results were obtained, and dehyd. EtOH was used for all subsequent experiments.

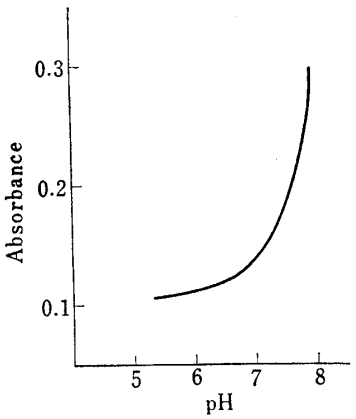

Fig. 3. Relationship between Absorbance and pH

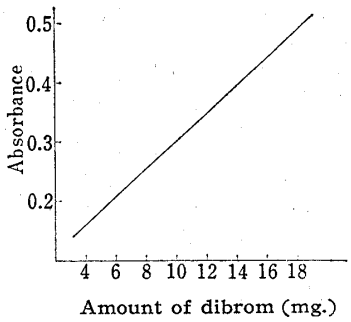

Fig. 4. Calibration Curve

4) **Calibration Curve**——Dibrom is purified by recrystallization, 200 mg. of this purified Dibrom is accurately weighed, and dissolved in Me_2CO to make exactly 100 ml. To each of the glass-stoppered test tubes, 2, 3, 4, 5, 6, 7, 8 or 9 ml. of this solution is placed and Me_2CO is added to make exactly 10 ml. To each of these solutions, 5 ml. of the above-mentioned ethanolic KOH solution is added, allowed to stand at 25° for 2 hr., and its 1 ml. is mixed with 15 ml. of dehyd. EtOH to measure the absorbance at 370 mμ. A solution not containing any sample of Dibrom is treated with the same reagents in the same manner to be used as the blank. The calibration is obtained by plotting absorbance on the ordinate and concentration of Dibrom (weight) on the abscissa, as shown in Fig. 4.

5) **Purification of Dibrom**——Fifteen milliliters of Dibrom is placed in a well-cooled flask and dissolved in a small amount of Et_2O as possible (ca. 45 ml.).

This solution is filtered, chilled hexane is added until white turbidity is produced, and the mixture is allowed to stand at below 0°. The crystals that separate out are collected by filtration and washed with a small quantity of hexane. Repetition of this procedure for 3~4 times gives Dibrom as white crystals. Purification was carried out in a freezing laboratory of below 5° and all the laboratory wares used were chilled to below 0°. About 3~5 mg. of purified crystals are obtained by a few repetitions of recrystallization from 50 ml. of crude Dibrom.

6) **Determination of Various Dibrom Samples and Comparison with the Infrared Absorption Spectral Method**——One hundred milligrams of various kinds of Dibrom samble is accurately weighed, dissolved in Me_2CO, and brought exactly to 100 ml. Ten milliliters of this solution is placed in a glass-stoppered test tube and this is treated by the foregoing procedure. The absorbance of the solution is measured and purity of each sample is calculated from the calibration curve. Determination was carried out on each of these samples and the one allowed to stand at 100° for 100 hr. These values were compared with the values obtained by the IR spectral method and the result is shown in Table I. The result of t-test of difference in corresponding values between these two methods showed good agreement, with $t=0.494<t(7, 0.05)=2.365$.

Table I. Comparision of Colorimetry and Infrared Absorpton Spectral Method

Sample	No.	Colorimetry (%)	IR method (%)
A	1	93.51	90.57
	2	81.66	84.24
B	1	100.38	96.44
	2	84.47	88.39

C	1	90.49	83.30
	2	71.66	73.17
D	1	85.32	84.43
	2	40.99	58.34
		$t=0.494$	

Each sample was allowed to stand at 100° for 100 hr.
No. 2 is the one allowed to stand at this condition.

7) Periodical Change of 5% Dibrom Emulsifiable Concentrate——Periodical change of Dibrom emulsifiable concentrate was examined, together with examination of *epi*-chlorohydrin, a stabilizer for Diazinon and was known to have stabilizing effect on other organophosphorus insecticides, as a stabilizer for Dibrom. For this purpose, two kinds of concentrate were prepared, A : containing 5% of *epi*-chlorohydrin and B : not containing it. The concentrates were maintained at 100, 70, 60 and 50°, and periodical change of the content of Dibrom was determined by the foregoing colorimetric method. Preliminary examinations were made to see whether the emulsifier, solvent, and *epi*-chlorohydrin interfered in this coloration or not. The determined values were slightly lower than with pure sample and the values were corrected accordingly (Table II). Results obtained from these examinations are shown in Fig. 5 in which the periodical change at 100° is not included but is given in Table III. The figures given in Tables III, V and the figures on the ordinate in Fig. 5 are the determination values (in %) when the value observed immediately after preparation of the concentrate is taken as 100%. As will be seen from these results, *epi*-chlorohydrin has no stabilizing effect on Dibrom or rather promotes decomposition. Further statistical treatment was made to presume the days required until 10% reduction at 30, 25 and 20°, and following data were obtained (Table IV). Experiments were further made to see whether these values (shown in Table IV) would agree with the values when the concentrates are maintained at room temperature (ca. 25°).

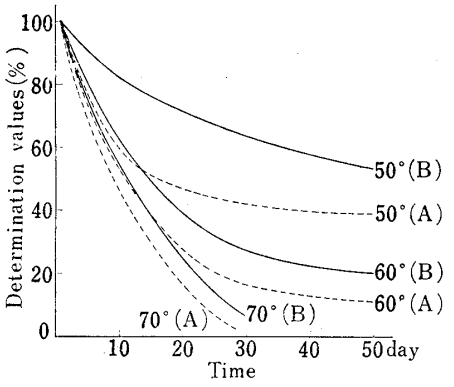

Fig. 5.
Thermal Degradation
of Dibrom in Concentrate

Table II. Effect of other Insecticides, Emulsifier and Solvent on Dibrom

	g. (ml.)/0.1 g. Dibrom	Recovery (%)
Lindane	0.5 g.	93.9
DDT	1.0 g.	101.5
Dieldrin	0.5 g.	120.6
Diazinon	0.5 g.	101.5
Malathion	1.0 g.	115.1
Emulsifier+Solvent	2.0 g.	97.4
Kerosene	10.0 ml.	98.7

Table V shows the periodical change at a room temperature.
From this result, the days required until 10% reduction at room temperature were 1.5 and 3~4 months in A and B respectively.
These values show good agreement with the estimates shown in Table IV.

Table III. Periodical Change of 5% Dibrom Concentrate at 100°

Period maintained (days)	A (%)	B (%)
1	26.6	30.9
2	0	0

Table IV. The Days Required until 10 % Reduction (days)

	30°C	25°C	20°C
A	30	45	75
B	60	100	180

Table V. Periodical Change of 5% Dibrom Concentrate at Room Temperature

Period maintained (months)	A (%)	B (%)
1	97.5	—
2	84.2	—
3	70.0	90.1
4	54.8	89.9
5	47.5	71.0

Relationship between the kind of emulsifier used and decomposition of Dibrom is presented in Table VI, in which the figures represent the determined values (in %) of periodical change, taking the content of Dibrom immediately after preparation of the concentrate as 100 %. The emulsifiers used in the present experiments were all pure products except sodium laurylsulfonate which was of 40 % purity. Therefore, it does not consist solely of anionic emulsifier.

No. 11 and 12 are commercial products of unknown composition for emulsification of Dibrom. These results showed that Dibrom is fairly stable when nonionic emulsifier alone is present but undergoes fairly rapid decomposition in the presence of anionic emulsifier alone. The decomposition rate does not seem to change with the change in molar amount of nonionic emulsifier in a range of 14~20 moles. The rate of decomposition seems rather to change by the mixing ratio of the nonionic and anionic emulsifiers.

Table VI. Stability of Dibrom in the Presence of Emulsifiers

	Emulsifier	Residual content of Dibrom After 2 days (%)	After 5 days (%)
1	Polyoxyethylene octylphenylether (6 mole)	86.4	68.0
2	Polyoxyethylene nonylphenylether (16 mole)	95.0	73.4
3	Polyoxyethylene sorbitanmonooleate (40 mole)	94.9	78.0
4	Calcium dodecylbenzenesulfonate	71.1	46.2
5	Sodim laurylsulfonate (40%)	93.5	76.5
6	Polyoxyethylene nonylphenylether (14.0 mole 50%) Calcium dodecylbenzenesulfonate (50%)	77.4	59.4
7	Polyoxyethylene nonylphenylether (16.0 mole 50%) Calcium dodecylbenzenesulfonate (50%)	74.8	56.5
8	Polyoxyethylene nonylphenylether (18.5 mole 50%) Calcium dodecylbenzenesulfonate (50%)	75.0	60.0
9	Polyoxyethylene nonylphenylether (20.0 mole 50%) Calcium dodecylbenzenesulfonate (50%)	74.5	61.7
10	Polyoxyethylene nonylphenylether (16.0 mole 55%) Calcium dodecylbenzenesulfonate (45%)	79.7	66.8
11	——	81.2	58.9
12	——	82.5	56.0

Temp. 70°C

Discussion

Reaction mechanism of the foregoing colorimetric method is being examined and still remains obscure. It is doubtful whether the value obtained by this determination shows the true purity of Dibrom or not, or whether impurities and decomposition products do or do not affect coloration. For this reason, the values were compared with that from the infrared absorption spectral method and the two values were found to agree well, as shown in Table I, indicating that this method can be used for determination. The present method takes rather a long time for determination but the procedure is simple and Dibrom in an oil and concentrate can be determined without extraction. However, the determined values tend to be smaller when these base mate-

rials are present (cf. Table II) but the method is applicable by slight correction.

The present method utilizes colorimetric determination of the insecticide DDVP but the color developed by Dibrom is more labile than that of DDVP. While the determination value of DDVP is hardly affected in the presence of other insecticides, the value of Dibrom is greatly affected by other insecticides, as shown in Table II, but no examination has yet been made on a method for separatory determination. Periodical change of the concentrate, and relationship between the kind of emulsifier and rate of decomposition of Dibrom are still in the stage of preliminary investigation. Further examinations are being continued.

The authors wish to express their thanks to Goro Tatsui, Director of Research Laboratory of Chugai Pharmaceutical Co., Ltd., for permission to publish this work. They are indebted to many coworkers in the Laboratory for their earnest assistances.

Summary

A colorimetric method for the determination of DDVP is modified to adapt for the determination of Dibrom, which based on the color complex formed between Dibrom and acetone in the presence of alkali. The values obtained from this method showed good agreement with the values from the infrared absorption spectral method. Periodical change of 5% Dibrom emulsifiable concentrate was studied at 100, 70, 60, and 50° by this colorimetric method, and statistical treatment was made to presume the days required until 10% reduction at 30, 25, and 20°. Experiments were further made to see whether these values would agree when the concentrates were maintained at room temperature. From this result, the days required until 10% reduction at room temperature were 1.5, and 3~4 months in the emulsifiable concentrate containing 5% of *epi*-chlorohydrin and the one not containing it, respectively. These values showed good agreement with the values obtained from the statistical treatment. *epi*-Chlorohydrin has no stabilizing effect on Dibrom or rather promotes decomposition. Dibrom in the emulsifiable concentrate is fairly stable when nonionic emulsifier alone is present, but undergoes fairly rapid decomposition in the presence of anionic emulsifier alone.

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110. Shigeru Yoshida : Infrared Spectra of Benzamide, its *p*-Substituted Derivatives, Pyridinecarboxylic Acid Amides and Pyrazinamide.*[1]

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Infrared absorption spectra of benzamide, *p*-substituted benzamides, picolinamide, nicotinamide, isonicotinamide and pyrazinamide will be dealt with in this paper. The infrared spectra of benzoic acid, its *p*-substituted benzoic acids, pyridinecarboxylic acids and pyrazinecarboxylic acid has been previously reported,[1)] indicating that benzoic acids form dimeric structure (I), which was observed usually in aliphatic carboxylic acids; and that pyridinecarboxylic acids and pyrazinecarboxylic acids with nitrogen atoms in their nuclei form an intramolecular hydrogen bond structure (II) between carboxylic acid and nitrogen atom in the nucleus, without forming dimeri c structure. Especially, a carboxylic acid which possesses a nitrogen atom at *ortho*-position such as picolincarboxylic acid and pyrazinecarboxylic acid, forms intermolecular hydrogen bond structure (III).