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Colorimetric Determination of 1-(2-*o*-Chlorobenzoyl-4-chlorophenyl)-5-glycylaminomethyl-3-dimethylaminocarbonyl-1*H*-1,2,4-triazole Hydrochloride Dihydrate with 3,5-Dibromosalicylaldehyde

RIKIO IKENISHI,* TAKAYASU KITAGAWA, and EIZO HIRAI

Shionogi Research Laboratories, Shionogi & Co., Ltd., Fukushima-ku, Osaka 553, Japan

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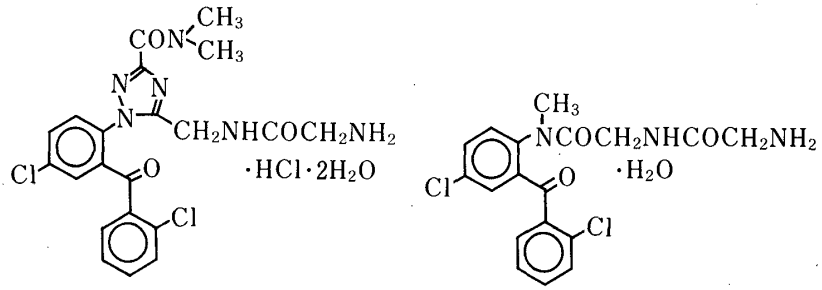
A colorimetric assay method was established for 1-(2-*o*-chlorobenzoyl-4-chlorophenyl)-5-glycylaminomethyl-3-dimethylaminocarbonyl-1*H*-1,2,4-triazole hydrochloride dihydrate (**1**), a sleep inducer, based on the reaction of **1** with 3,5-dibromosalicylaldehyde (DBSA) to give a red-colored substance in the presence of piperidine. Our previously established method employing DBSA was also applicable to **1**. Compound **1** was determined with good precision and specificity.

The general applicability of the assay method to compounds containing a glycinamide group was also examined.

Keywords——open-ring benzodiazepine; 1-(2-*o*-chlorobenzoyl-4-chlorophenyl)-5-glycylaminomethyl-3-dimethylaminocarbonyl-1*H*-1,2,4-triazole hydrochloride dihydrate; 3,5-dibromosalicylaldehyde; piperidine; colorimetry; sleep inducer; peptide

In a recent series of studies on ring-opened derivatives of 1,4-benzodiazepine,[1] 1-(2-*o*-chlorobenzoyl-4-chlorophenyl)-5-glycylaminomethyl-3-dimethylaminocarbonyl-1*H*-1,2,4-triazole hydrochloride dihydrate (**1**) was developed as a sleep inducer. The purpose of the present work was to establish an assay method for **1**. The titrimetry of salts such as the hydrochlorides of organic bases, though difficult by the usual technique, can be done effectively in the presence of mercuric acetate in acetic acid.[2] This titration method, however, suffers from the problem of subsequent disposal of the poisonous metal. In the previous paper,[3] a minor tranquilizer, 4-chloro-2-(*o*-chlorobenzoyl)-*N*-methyl-N^{α}-glycylglycinanilide (**2**), was assayed by a colorimetric method based on coloration with reagent 3,5-dibromosalicylaldehyde (DBSA) to check the quality of the drug in the raw material. Since it was



1 **2**

Chart 1

found later that the reaction of **2** with DBSA occurred at the glycinamide residue in the side chain to give a colored substance,[4] we anticipated that the assay method for **2** might be applicable to **1**. When **1** was treated according to the same procedure, the same coloration

was formed and thus **1** could be determined.

This paper describes the result of the analysis of **1** by the DBSA method and the applicability of the method to various peptide derivatives having a glycinamide group.

Experimental

Apparatus——A Shimadzu spectrophotometer, model UV-190, was used for absorbance measurement.

Reagents——DBSA–dimethyl sulfoxide (DMSO) Test Solution: Dissolve 1.91 g of DBSA (reagent grade, Tokyo Chemical Industry Co., Ltd.) in 100 ml of DMSO. Prepare the solution before use.

Piperidine–DMSO Test Solution: Dilute 5 ml of piperidine (special grade, Wako Pure Chemical Industries, Ltd.) with DMSO to made 100 ml.

Standard Solution: Accurately weigh about 250 mg of standard material of **1** into a 50-ml volumetric flask, then dissolve it in, and dilute to the mark with, DMSO. Pipet 1, 1.5, 2, 2.5, 3, 3.5, and 4 ml of the solution into seven 10-ml volumetric flasks, and dilute to the marks with DMSO.

The standard **1** was prepared by recrystallization from AcOEt–MeOH–H_2O (10 : 1 : 1).

Assay Procedure——Accurately weigh about 10 mg of the sample into a 10-ml volumetric flask, then dissolve it in, and dilute to the mark with, DMSO. Pipet 1 ml of the solution into a 10-ml volumetric flask, add 1 ml of DBSA–DMSO test solution, mix well, and immediately cool in an ice bath. Add 1 ml of piperidine-test solution, 7 ml of DMSO and then 0.5 g of anhydrous sodium sulfate. Stopper tightly and heat for 1.5 h at 70 °C. After cooling of the flask in a water bath, transfer exactly 1 ml of the reaction solution to a 10-ml volumetric flask and dilute to the mark with piperidine–DMSO test solution. Read the absorbance at 558 nm using a solution obtained in the same manner with 1 ml of DMSO as the blank.

Standard Curve: Proceed with 1 ml of a standard solution according to the assay procedure. Make a standard curve by plotting the absorbance *vs.* the concentration of the standard solution.

Measurement of the Absorption Spectra——A DMSO solution of the sample (1 mg/ml) was prepared and DBSA–DMSO test solution was added to 1 ml of the solution. The solution was treated according to the assay procedure. The absorption spectrum of the resulting solution was recorded.

Results and Discussion

Absorption Spectrum

The absorption spectrum of the colored substance developed by treating **1** according to the assay procedure was almost in accord with that observed for the reaction solution of **2**, as shown in Fig. 1.

Assay Condition

All the conditions which had been examined for the determination of **2**, *i.e.*, the effects of temperature, reagent concentration, *etc.*, were reexamined. The optimal conditions determined for **1** were completely in accord with those for **2**. Thus, the method established for **2** can be directly applied to **1** as such.

The yield of the color reaction was determined to be *ca.* 85% by assuming the conversion of **1** into the estimated colored substance.[4] In order to increase the yield, various base catalysts

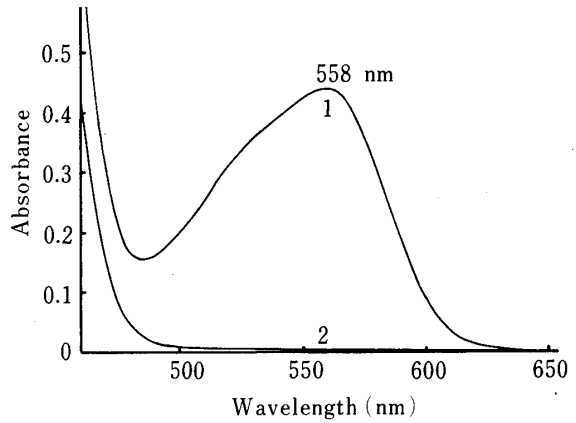


Fig. 1. Visible Absorption Spectra for Compound **1**

1, sample solution; 2, blank solution.

other than piperidine (anilines, alkylamines, *etc.*) were tested in the color reaction. No catalyst superior to piperidine was found.

Effect of Impurity in the Raw Material

The raw material may contain **3**, formed by ring closure, as an impurity. A mixed sample of **1** containing 10% impurity was assayed. No effect on the determination was observed.

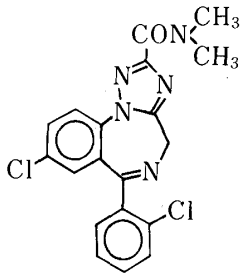


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Chart 2

TABLE I. Determination of Compound **1** in Raw Material Samples

Lot. No.	Run	Taken (mg)	Found (mg)	Content (%)
	1	10.39	10.30	99.1
52	2	10.39	10.48	100.9
	3	10.39	10.38	99.9
	1	10.21	10.28	100.7
53	2	10.21	10.28	100.7
	3	10.21	10.15	99.4
	1	12.74	12.93	101.5
54	2	11.16	11.30	101.3
	3	10.46	10.36	99.0
	1	9.88	10.01	101.3
55	2	9.92	9.96	100.4
	3	11.78	12.02	102.0
	1	10.98	11.15	101.5
56	2	10.86	11.08	102.0
	3	10.40	10.51	101.1
	1	10.38	10.41	100.3
57	2	12.34	12.29	99.6
	3	11.28	11.25	99.7
	1	11.44	11.60	101.4
58	2	10.46	10.46	100.0
	3	11.36	11.32	99.6
	1	11.68	11.79	100.9
59	2	10.20	10.21	100.1
	3	10.24	10.31	100.7
	1	10.22	10.04	98.2
60	2	10.88	10.95	100.6
	3	11.94	12.09	101.3
	1	10.56	10.66	100.9
61	2	12.20	12.46	102.1
	3	10.40	10.53	101.3

TABLE II. Color Development of Peptides with 3,5-Dibromosalicylaldehyde

Compound	λ_{max} (nm)
Glycylglycine	537
Glycylglycylglycine	548
Glycylglycylglycylglycine	546
Glycyl-L-alanine	535
Glycyl-D-alanine	536
Glycyl-L-leucine	540
Glycyl-L-tyrosine	540
[1-Glycine]-ACTH-(1—18)-octadecapeptide amide[a]	535
450192[b]	556
450088[c]	554

a) K. Inouye, M. Shin, I. Kikkawa, and H. Otsuka, *Annual Report of Shionogi Research Laboratory*, **20**, 2 (1970).
b) 2′,5-Dichloro-2-(3-glycylaminomethyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-benzophenone.
c) 4-Chloro-2-(*o*-chlorobenzoyl)-*N*-methyl-N^{α}-glycylglycinanilide.

Analytical Results for the Raw Material

According to the established method, **1** was assayed with a precision (coefficient of variation, less than 2%) comparable to that obtained with **2**. Table I shows the analytical results for the raw material samples (ten lots).

The results are consistent with the color reaction mechanism presented in the previous paper.[4]

Reaction of Compounds Having a Glycinamide Group with DBSA

Several other compounds having a glycinamide group reacted with DBSA under the assay conditions established for **1**. Color developed in all the reaction mixtures. The maximum wavelengths, as shown in Table II, were 535—556 nm, suggesting the formation of the same colored product as observed for **1**. The results indicate that the same assay method should also be applicable to these compounds.

References and Notes

1) K. Hirai, T. Ishiba, H. Sugimoto, T. Fujishita, Y. Tsukinoki, and K. Hirose, *J. Med. Chem.*, **24**, 20 (1981) and preceding papers in the series on "Benzophenone-Related Compounds."
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4) R. Ikenishi, T. Kitagawa, and E. Hirai, *Chem. Pharm. Bull.*, **32**, 609 (1984).