Purification and Aid Equilibria of Glycinethymol Blue

Takashi YOSHINO*, Tadayoshi KUWANO**, Sadaaki MURAKAMI*
and Sigeru NAKANO***

(Received August 2, 1974)

Abstract

Glycinethymol Blue (GTB) was purified chromatographycally with cellulose and ion-exchange resin. Semi-Glycinethymol Blue was failed in purification because of its very insolubility in water. The acid formation constants of GTB have been investigated potentiometrically and spectrophotometrically. The formation constants of GTB are compared to those of the other Thymol Blue derivertives and the effects are discussed.

Introduction

Glycinethymol Blue (GTB), 3,3′-bis-(*N*-carboxymethylaminomethyl)-thymolsulphonphthalein, was first introduced by Körbl and Přibil as a usefull specific metal indicator for the chelatometric determination of copper in acid solution[1]. However, the acid formation constants of GTB have not been determined. So first of all it have been purified, and the acid formation constants have been measured by using potentiometric titration and the absorption measurements.

Experimental

The procedures and apparatus were those used previously[2].

Reagents

Stock solution of copper (II) *ion*, 1×10^{-2}M. Standardized titrimetrically with EDTA.

Buffers. Standard solutions were used[2].

Chromatography. Paper and cellulose chromatographic columns as described previously[3].

Synthesis of GTB

Glycine (2 g; 2.7×10^{-2} mole) with 37% formaldehyde (2 ml; 2.4×10^{-2} mole), Thymol Blue (4 g; 0.9×10^{-2} mole) and sodium hydroxide (1 g) were solved in glacial acetic acid. After reacting for 8 hours at 80°C, the solvent was distilled off under reduced pressure.

* Department of Chemical Technology
** Toyo Soda Manufacturing Co., Ltd.
*** Sumitomo Metal Mining Co., Ltd.

Separation and purification of GTB

The reaction mixture was developed on cellulose column using top layer solution of *n*-butanol which was saturated with 0.1% acetic acid. The three bands were appeared, Glycine (teiled orange band), probably mono-substituted product, Semi-Glycinethymol Blue (SGTB) (green) and GTB (yellow) respectively from the bottom of the column. GTB was completely separated repeating this procedure. The resulting crude GTB was converted into acid form with a cation-exchange column (Diaion SK–1).

The purity of the product was established by elemental analysis, potentiometric titration, paper chromatography and absorption spectrum. The product was the acid form of GTB. Found: C, 61.8%; H, 6.3%; N, 4.2%. Calculated for $C_{33}H_{40}O_9N_2S$: C, 61.86%; H, 6.29%; N, 4.37%.

Results

Reaction with copper (II)

GTB changed its colour from yellow to blue on reaction with copper (II) in the weakly acidic medium. Job's method showed it formes 1:1 and 2:1 (Cu:GTB) complexes.

Potentiometric titration

GTB was titrated with sodium hydroxide solution potentiometrically at pH from 3 to 11 (Fig. 1). The titration curve has the two well-defined inflections at $a=1$ and $a=2$ (where a=moles of base added per mole of GTB). In acidic side, one proton

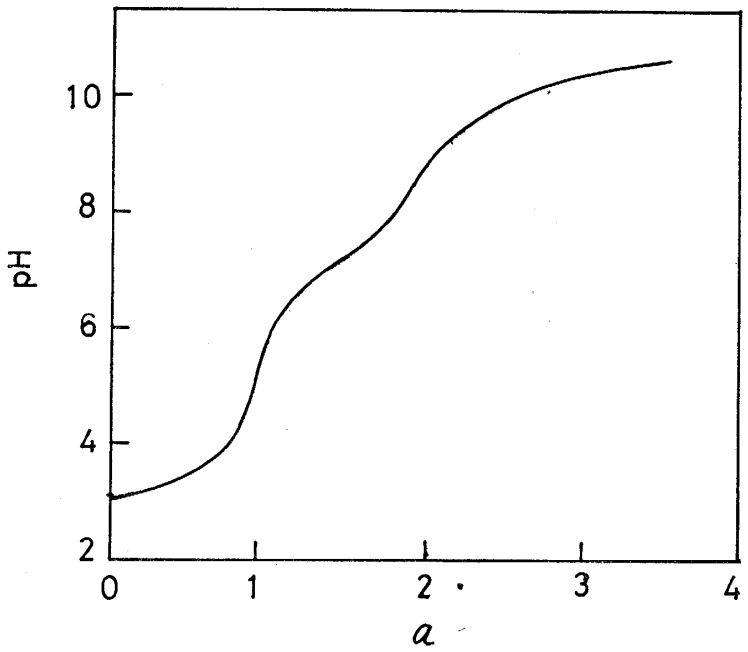


Fig. 1 Potentiometric titration curve of GTB at 25±0.1°C in 0.1 M KNO_3 solution.
GTB: 1.87×10^{-3} M.
a=moles of base added per mole of GTB.

dissociates. At higher pH three more protons dissociate, one between $a=2$ and $a=3$, the other two above $a=3$.

Absorption spectra

Absorption spectra of GTB at different pH values are shown inFig. 2. Between pH 2.5 and 5.5, the absorption curves are almost similar with the absorption maxima at 435 nm. The solution colour is yellow. Above pH 5.5, new bands appears near at 600 nm. With increase of pH, the absorbance fairly incraeses and this change is maximum at pH 8.7. The solution gives the colour to blue. With increase of pH from this value, the absorbance decreases and become minimum at pH 11.3 and again increases with increase of pH. During these two steps, the maximum absorbance is shifted very slightly to shorter wavelength and again to longer wavelength. The colour is changed from light to deep blue.

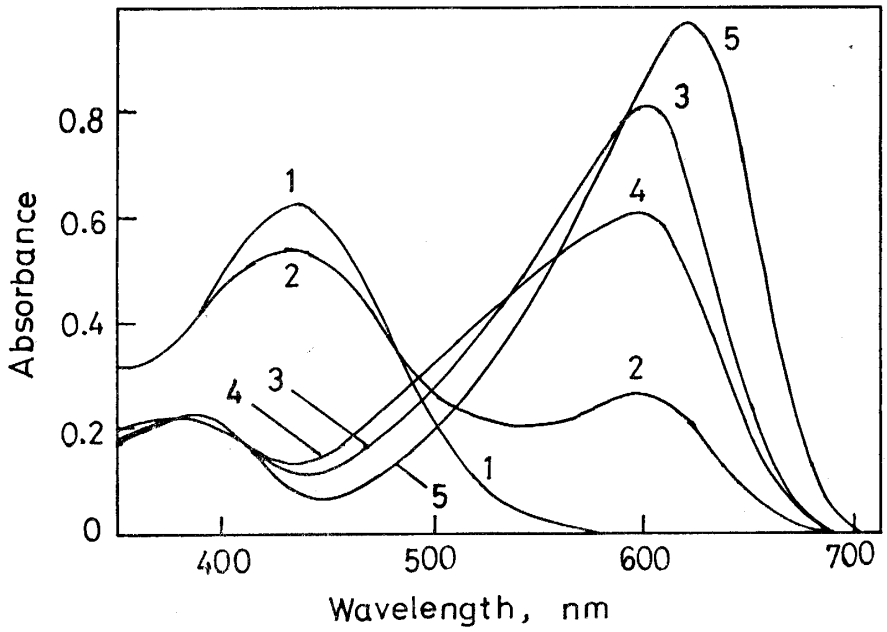


Fig. 2 Absorption spectra of GTB at different pH values.
GTB: 2.64×10^{-5} M.
pH: 1–3.65, 2–3.72, 3–8.71, 4–11.28, 5–12.80.

Discussion

Separation and purification of GTB and SGTB

Purification of the mono-substiutted product of Semi-Glycinethymol Blue (SGTB) was resulted in failure. The two points can be given as the reasons why SGTB is difficult to purify. These are teiling phenomenon of Thymol Blue (TB) band in cellulose column chromatograph and adsorption of SGTB on the ion-exchange resin. Figure 3 shows the paper chromatogram of the synthesis mixture. Band of TB is teiled and those of TB and SGTB overlap slightly each other. The separation fo SGTB from TB

needed many times of repeat of the cellulose column chromatography, resulting the decrease of the amount of the crude SGTB obtained. And addition to this, SGTB was adsorpted on the resin.

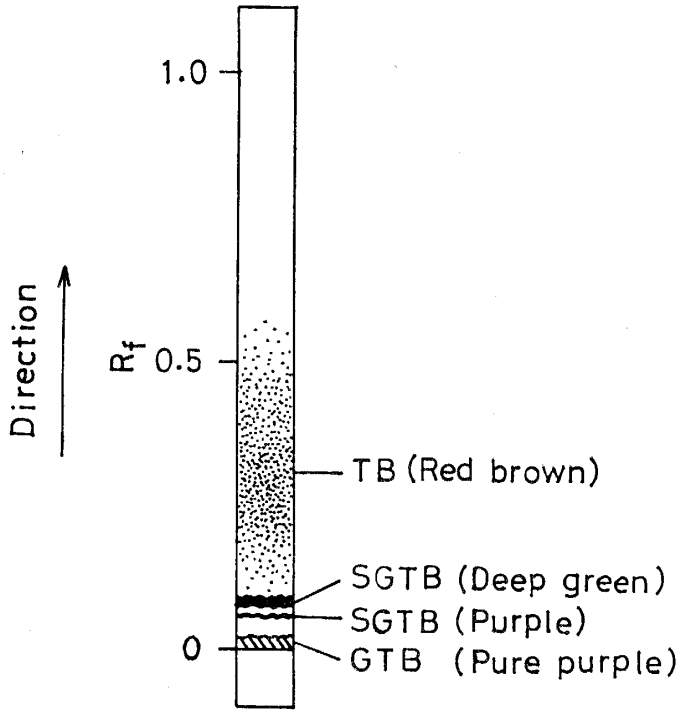


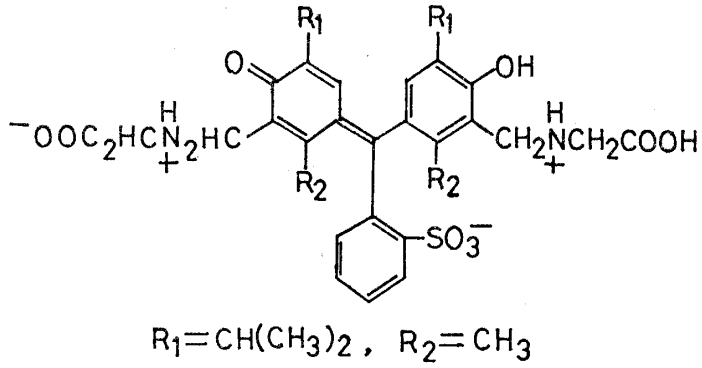
Fig. 3 Paper chromatogram of synthesis mixture of GTB.
Solvent: *n*-butanol satuarted with 0.1% acetic acid.
Developing time: 8 hours.

These behaviors of SGTB may be due to its very insolubility in water. SGTB may be mono-substituted product by N-methylglycine which has only one carboxylic group. Addition to this, TB has the substitutes both of isopropyl and methyl groups, on the other hand, Cresol Red (CR) has methyl and proton substitutes at the same positions of sulphonphthalein group. These result in the fact that the solubility order for these derivertives is SGTB<SGCR[4]<GTB<GCR[4]<SMTB[5]<SXO[3]<MTB[5]<XO[3], where SGCR (Semi-Glycinecresol Red) is obtained as the mono-substituted product when GCR (Glycynecresol Red) is prepared[4]. The each concentration of acetic acid in the developing solution increases in the latter system in the above order (0.1% for the SGTB–GTB, 1% for the SMTB–MTB[5] and 10% for the SXO–XO[3] respectively acetic acid saturated *n*-butanol). The increase of that caused the large trends both of teiling of the starting regents and adsorption of the product compounds. Separation of SGTB must await further investigations.

Equilibra of GTB

In acidic side, the absorption spectra are almost similar, although one proton may dissociate as seen from potentiometric data. In this pH range, two protons, one from

the sulphonic acid and the other from the carboxylic group, probably transfer to the nitrogens, and the zwitterion may be given by formula I. So, not large change of the spectra may suggest the dissociation from the carboxylic group. On alkaline medium, the equilibrium between the yellow to the blue GTB may exhibit the change of the electronic structure of the sulphonphthalein group, indicating the proton dissociation from the phenolic group. At higher pH, the behavior in the absorbance is quite similar to that of MTB[6]. Probably two protons attached to the nitrogens dissociate.



I

Formura I

Acid formation constants

Acid formation constants of GTB were calculated as previously, and listed in table 1. Those of TB derivertives, TB, SMTB[7], MTB[6] and GTB are listed in table 2, together with those of N-methylglycine (MG)[8] and N-methyliminodiacetic acid (MIDA)[9]. The former compounds have the general formula II, where X_1 or X_2 = H, $CH_2N(CH_2COOH)_2$ or CH_2NHCH_2COOH.

The introductions of the MIDA or MG groups into X_1 or both X_1 and X_2 cause the increase of the acidity of the phenolic group and that of the basicities both of the nitrogen and the carboxylic group of the substitute (when the ligand molecule has same groups more than one, the average value of the formation constants is compared). This

Table 1. Acid formation constants, wavelengths at absorption maxima and moler absorptivities of GTB

Ion	Wavelength	Absorptivity	Equilibrium constant
	λ_{max}nm	$\varepsilon \times 10^4$ l.mole^{-1}·cm^{-1}	log k
H_4L	435	1.65	2.87*
H_3L^-	435	1.65	6.98*
H_2L^{2-}	603	3.14	7.06
HL^{3-}	577	1.69	10.15
L^{4-}	615	4.11	11.67

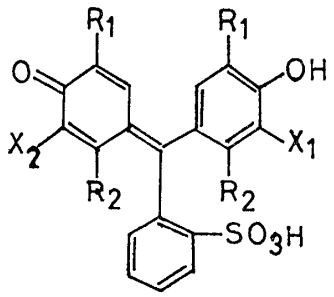
Data measured by means of pH titration at 25±0.1°C, μ=0.1 (KNO_3), the rest by means of spectrometry at the room temperature, μ=0.1 (KNO_3).

Table 2. Acid formation constants of Thymol Blue derivatives

log k	TB	SMTB	MTB	MG	MIDA
$\log k_{-COOH}$		2.0[a]	1.8[a]	2.24[a]	2.12[b]
		2.81[a]	2.0[a]		
		(2.4)*	3.04[a]		
			(2.3)*		
$\log k_{-OH}$	8.89**	7.60**	6.91**		
$\log k_{-\overset{+}{\underset{H}{N}}<}$		12.12**	11.14**	10.01[a]	9.65[b]
			12.94**		
			(12.04)*		

* Data respects the average value of each acid formation constant.

** Data measured by means of spectrometry at room temperature, $\mu=0.1$ (KNO_3), the rest determined by means of pH titration method. a: at 25°C, $\mu=0.1$; b: 20°C, $\mu=0.1$.



II

Formura II

show the MIDA and the MG groups react as electron acceptor. The sulphonphthalein group has three benzen rings and addition to this two of them can take quinoide structure. The π-electron cloud density may be distributed over the three benzen at the resonance. So, this group might act also as electron acceptor. However, MIDA or MG group may be more electrophilic than TB. And this trend is larger in MIDA than in MG. It is clear from that MIDA has two electrophilic carboxylic groups.

References

1) J. Körbl, E. Kraus and R. Přibl: Chem. Listy, **51**, 1809–1813 (1957)
2) T. Yoshino, S. Murakami, M. Kagawa and T. Araragi: Talanta, **21**, 79–85 (1974)
3) M. Murakami, T. Yoshino and S. Harasawa: Talanta, **14**, 1293–1307 (1967)
4) T. Yoshino, *et al.*: unpublished.
5) T. Yoshino, H. Imada, T. Kuwano and K. Iwasa: Talanta, **16**, 151–156 (1969)
6) T. Yoshino, H. Imada, S. Murakami and M. Kagawa: Talanta, **21**, 211–224 (1974)
7) T. Yoshino, S. Murakami and M. Kagawa: Talanta, **21**, 199–209 (1974)
8) F. Basolo and Y. T. Chen: J. Amer. Chem. Soc., **76**, 953–955 (1954)
9) G. Schwarzenbach, G. Anderegg, W. Schneider and H. Senn: Helv. Chim. Acta, **38**, 1147–1170 (1955)