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QUANTITATIVE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF THE COMMERCIAL DYESTUFF G ACID AND GAMA ACID

KUEN-SHAN LEE* and TSAI-LUNG YEH
Union Industrial Research Laboratories, Industrial Technology Research Institute, Hsinchu 300 (Taiwan)
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SUMMARY

A convenient and accurate quantitative analytical method for the commercial dye products G acid, and Gama acid, has been established by ion-pair reversed-phase high-performance liquid chromatography. Good resolution among G acid, Schaeffer acid, R acid, Gama acid and amino-G acid was achieved. Appropriate selection of UV detection wavelength, 230 nm, has resulted in linear absorption responses over a wide concentration range, 0–8 μg. The application of the method to a study of the rate of ammonolysis of G acid to amino-G acid is described.

INTRODUCTION

The commercial product G acid (2-hydroxy-6,8-naphthalenedisulphonic acid, I) is an important dye intermediate currently manufactured from 2-naphthol by a two-stage sulphonation, first with concentrated sulphuric acid and then with 20% fuming sulphuric acid[1,2]. The sulphonation products are mainly G acid and the isomeric disulphonic acid, R acid (2-hydroxy-3,6-naphthalenedisulphonic acid, IV), residual monosulphonic acid, Schaeffer acid, (2-hydroxy-6-naphthalene sulphonic acid, III), and some trisulphonic acid. The separation of these products takes advantage of solubility differences between the alkali-metal salts of the sulphonic acids. G acid is first salted out as the dipotassium salt by potassium chloride, then R acid together with Schaeffer acid as their sodium salts by sodium chloride, while trisulphonic acid remains in the mother-liquor. Although the yield of G acid is 62% and that of R acid is only 13% with contamination by 2–3% Schaeffer acid, no thorough investigation of the reaction rate or optimum conditions has been reported. This is due to the fact that there is still no adequate quantitative analytical method for this purpose.

The stability of the colour of a synthetic dye depends exclusively on the purity of the intermediates used. Therefore, a fast and accurate qualitative and quantitative analytical method has to be established to detect any trace impurity such as R acid in G acid or Schaeffer acid in R acid, etc. A variety of qualitative methods have been reported including thin-layer chromatography[3], paper chromatography[4–11] and high-performance liquid chromatography (HPLC)[12–16]. The quantitative method remains the traditional titration method[17] which is not only time-consuming but also inac-

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curate, especially when interfering isomers are present which also react with the titrating reagent. For example, in this method the analysis of a mixture of G acid, R acid and Schaeffer acid requires three titrations: first, using the more reactive coupling reagent, diazotized *p*-nitroaniline solution, to determine the total amount of G acid, R acid and Schaeffer acid; secondly, titration with the less reactive coupling regent, diazotized *p*-toluidine or aniline to determine R acid and Schaeffer acid together; thirdly, addition of alcohol to precipitate R acid from Schaeffer acid or reaction with formaldehyde to elimininate Schaeffer acid, followed by titration with diazotized *p*-toluidine, to determine R acid.

Another commercial dye intermediate, Gama acid (2-amino-8-hydroxy-6-naphthalene sulphonic acid), can be prepared by first ammonolysis of G acid to amino-G acid and then by alkali fusion. The traditional titration method for Gama acid and amino-G acid in a mixture also suffers from similar difficulties in the titrations with diazotized *p*-nitroaniline and *p*-toluidine. Furthermore, amino-G acid and G acid have similar reactivities toward *p*-toluidine.

Since 1976, a few HPLC methods have been reported for naphthalenesulphonic acids[12–16], yet they are still only qualitative. This is due to inappropriate selection of column phase, mobile phase or UV detection wavelength and has resulted in poor resolution, long analysis times and lack of a linear response between UV absorption and sample concentration. The HPLC method reported in this paper, has excellent resolution, $R > 1.5$, among compounds I–V, which can be used for qualitative analysis, as well as quantitative analysis due to the linear relationship between absorption response and sample amount over the range 0–8 μg. Its application to the study of reaction rate and optimum reaction conditions is demonstrated in the ammonolysis of G acid.

EXPERIMENTAL, RESULTS AND DISCUSSION

UV absorption

In order to select an appropriate UV detection wavelength for HPLC analyssis of compounds I–V, a standard solution of each compound was prepared and UV spectra taken over the range 200–400 nm (Fig. 1). Using the Lambert–Beer law, $A = \varepsilon cb$ and $\varepsilon = aM$, the molar absorptivity, ε, and the absorptivity, a, of each compound at 230 nm and 254 nm were calculated and compared (Table I). (A = absorbance , c = concentration, b = path length and M = molecular weight of the solute).

Fig. 1 illustrates that at 254 nm only Gama acid and amino-G acid have significant absorption, while all the other compounds have weak absorptions. From Table I, it is seen that the absorptivity of Gama acid at 254 nm is almost ten times that of G acid. Thus the selection of 254 nm as the wavelength for HPLC UV detection in previous studies resulted in a non-linear response and was unsuitable for quantitative analysis. On the other hand, all compounds I–V have strong absorptions at 230 nm which is near their absorption maxima. Table I shows the narrow range of absorptivity, a_{230}, among the five compounds.

Chromatographic conditions

All HPLC analyses were carried out with a Shimadzu Model LC-3A equipped

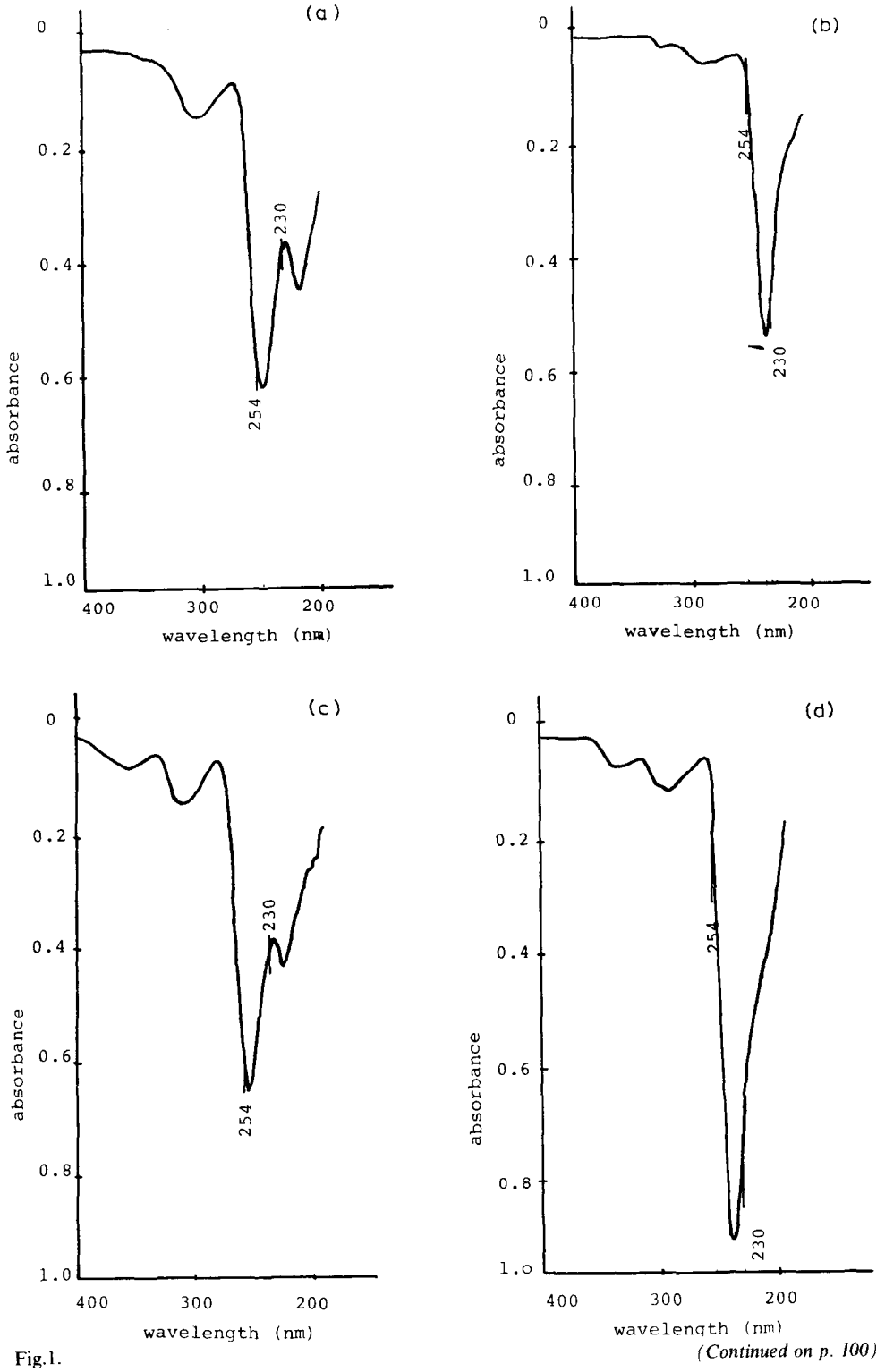

Fig.1.

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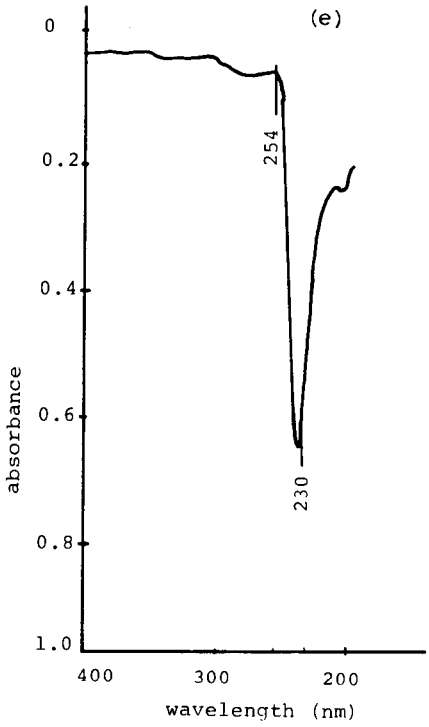

Fig. 1. UV absorption spectra of: (a) Gama acid, $2.0 \cdot 10^{-5}$ *M*; (b) Schaeffer acid, $9.0 \cdot 10^{-6}$ *M*; (c) amino-G acid, $2.0 \cdot 10^{-5}$ *M*; (d) G acid, $1.86 \cdot 10^{-5}$ *M*; (e) R acid, $8.6 \cdot 10^{-6}$ *M*.

with a CTO-2A column oven, SPD-2A variable wavelength UV detector and C-RIB data processor. UV spectra of compounds I–V were measured using a Shimadzu UV 200 double-beam spectrometer equipped with a Shimadzu U-125 MU recorder.

Reagents

Merck L.C. grade methanol and distilled water were used for the mobile phase. The ion pairing reagent (PICA) used in all analyses was tetrabutylammonium phosphate solution, 20 ml per bottle (Ajax Chemicals, Sydney, Australia) and was distilled from aqueous solution. Satisfactory results were also obtained with tetrabutylam-

TABLE I

MOLAR ABSORPTIVITIES AND ABSORPTIVITIES

	Acid				
	Gama	*Schaeffer*	*Amino-G*	*G*	*R*
MW	239	282	341	380	348
ε_{254}	$3.1 \cdot 10^4$	$4.4 \cdot 10^3$	$3.0 \cdot 10^4$	$5.4 \cdot 10^3$	$8.0 \cdot 10^3$
ε_{230}	$2.0 \cdot 10^4$	$5.8 \cdot 10^4$	$2.1 \cdot 10^4$	$4.1 \cdot 10^4$	$6.5 \cdot 10^4$
a_{254}	130	16	88	14	23
a_{230}	84	200	62	110	190

monium hydrogen sulphate solution (Waters, Milford, MA, U.S.A.). All other chemicals were reagent grade. The five solutes were in the following forms: G acid, dipotassium salt, 93%; R acid, disodium salt, 86%; Schaeffer acid, sodium salt dihydrate, 90%; Gama acid, free acid, 100%; amino-G acid, monopotassium salt, 100%.

The chromatogram shown in Fig. 2 was obtained under the following conditions (A): column, reversed phase, Hewlett-Packard 5-μm Hypersil ODS (25 cm × 4.6 mm I.D.); mobile phase, methanol–water (25:75 v/v) with 1% (v/v) AJAX PICA, flow-rate 2.0 ml/min; column temperature, 40°C; UV wavelength, 230 nm. The chromatogram shown in Fig. 3 was obtained under the following conditions (B): column, Merck 7-μm LiChrosorb RP-18 (25 cm × 4.6 mm I.D.); mobile phase, methanol–water (28:72) with 1% Waters PICA; flow-rate, 1.0 ml/min; column temperature, 25°C; UV wavelength, 230 nm. Both sets of conditions gave excellent resolution, $R > 1.5$, among the five compounds, but the elution sequence of Schaeffer acid and amino-G acid were exchanged under the different conditions.

Linearity

In order to study the linearity range for each compound, standard solutions were prepared by mixing reagent grade Gama acid (100%, 40.0 mg), amino-G acid (100%, 40.0 mg), Schaeffer acid (90%, 20.0 mg), G acid (93%, 20.0 mg) and R acid (86%, 20.0 mg) and completely dissolved in water by adding 0.1 *M* sodium hydroxide (2.0 ml). The volume was made up with water to 50 ml. Part of this solution was

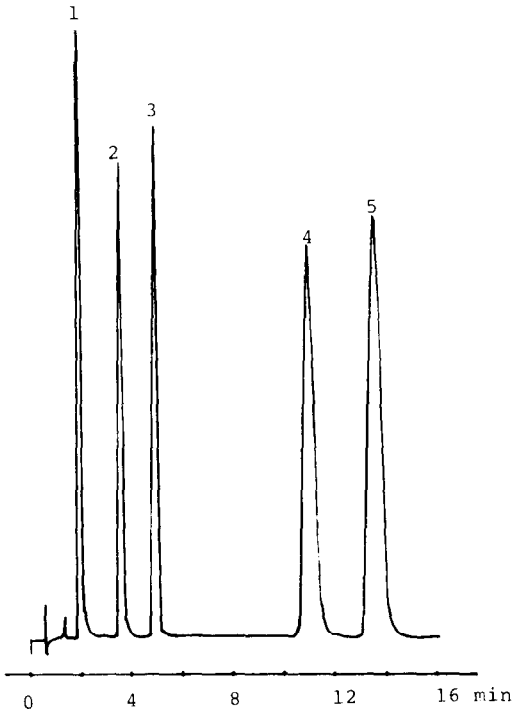

Fig. 2. Chromatogram of compounds I V using conditions A. Peaks: 1 = Gama acid; 2 = Schaeffer acid; 3 = amino-G acid; 4 = G acid; 5 = R acid.

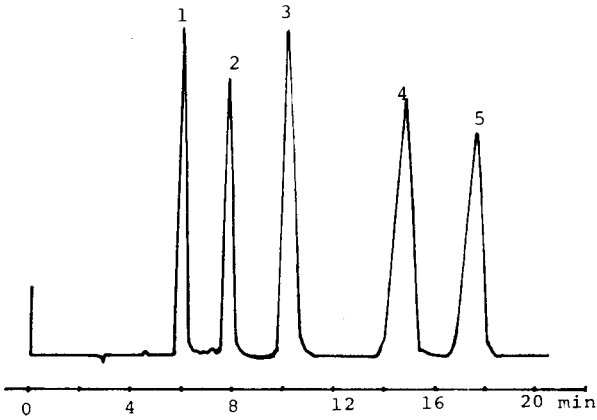

Fig. 3. Chromatogram of compounds I–V using conditions B. Peaks: 1 = Gama acid; 2 = amino-G acid; 3 = Schaeffer acid; 4 = G acid; 5 = R acid.

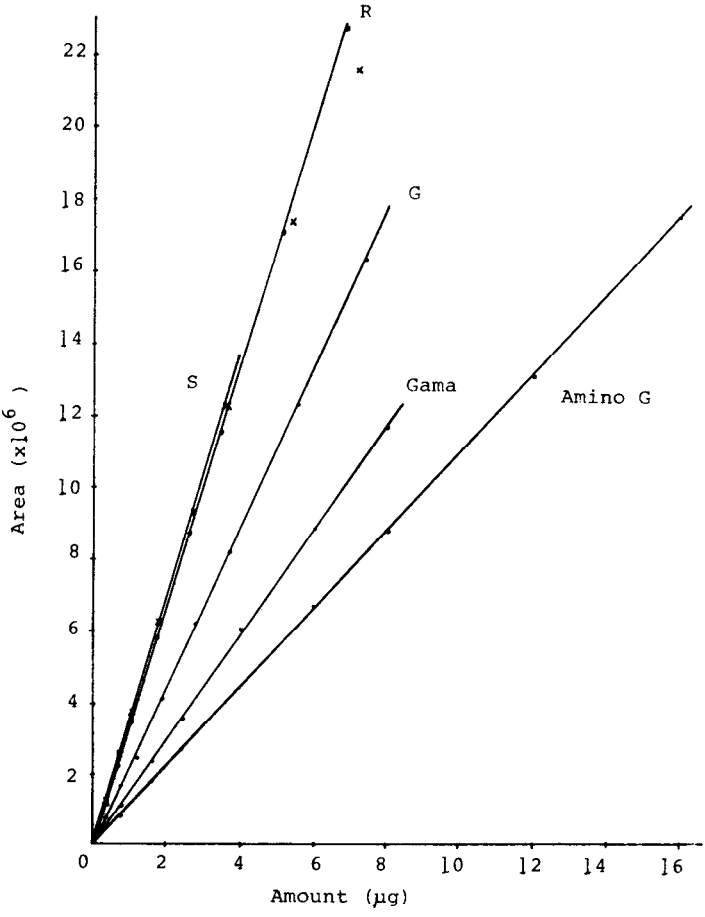

Fig. 4. Linearity of calibration curves for G acid, R acid, Gama acid, Schaeffer acid (S) and amino-G acid.

further diluted in water two-fold and five-fold. Then under chromatographic conditions A a 5–20-μl volume of the above mixture was injected and the HPLC results plotted as peak area *vs.* sample amount (Fig. 4).

It is seen that the five compounds have very good linear responses over a wide range of concentration and all the calibration curves pass through the origin. The relative average weight response factors among the five compounds were then calculated (Table II), and found to be in the increasing order; Schaeffer acid, R acid, G acid, Gama acid, amino-G acid, in accordance with the absorptivities at 230 nm in Table I.

Internal standard calibration

Since all the five compounds have linear responses over a wide concentration range, it is possible to establish an internal standard method for analysis of unknown samples.

G acid, R acid, and Schaeffer acid. A series of standard mixtures of the three acids were prepared containing a fixed amount of amino-G acid as an internal standard. The measured concentrations of each compound in the mixtures were chosen such that, with a fixed injection volume of 10 μl, each compound fell within its linear response range: G acid, 0.93–7.44 μg; R acid, 0.86–6.88 μg; Schaeffer acid, 0.18–

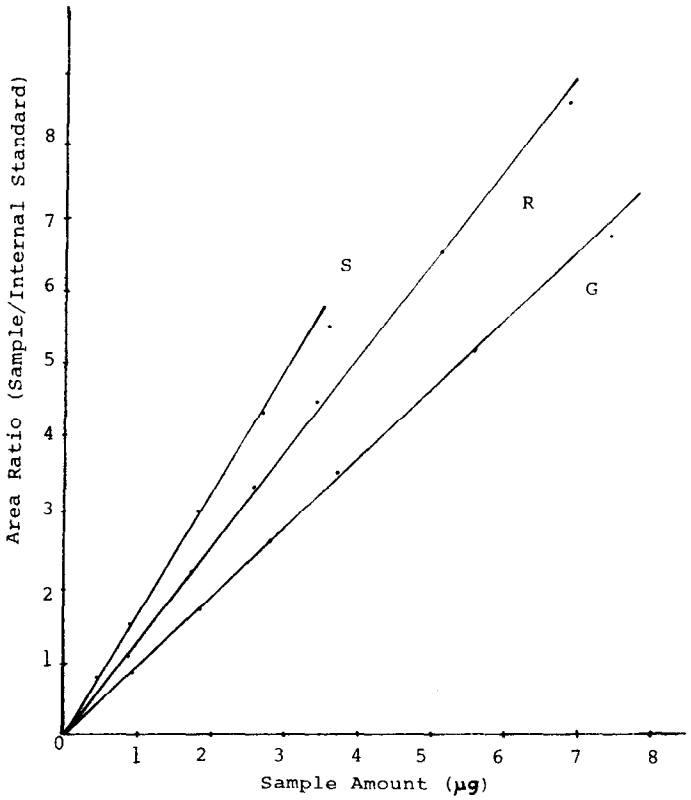

Fig. 5. Calibration curves for internal standard analysis of G acid, R acid and Schaeffer acid.

TABLE II

LINEARITY RANGES AND RESPONSE FACTORS

f = Sample wt./peak area.

	Acid				
	Gama	*Schaeffer*	*Amino-G*	*G*	*R*
Linearity (μg)	0–8.0	0–3.5	0–16.0	0–7.5	0–7.0
Wt. response factor, f	2.35	1	3.14	1.57	1.03

3.6 μg; the internal standard, amino-G acid was always 2.0 μg. The chromatographic results were plotted as the peak area ratio of the sample to amino-G acid *vs.* sample amount (Fig. 5).

It is seen that the relationships for the three acids are linear within the ranges in Table II, all three calibration curves passing through the origin.

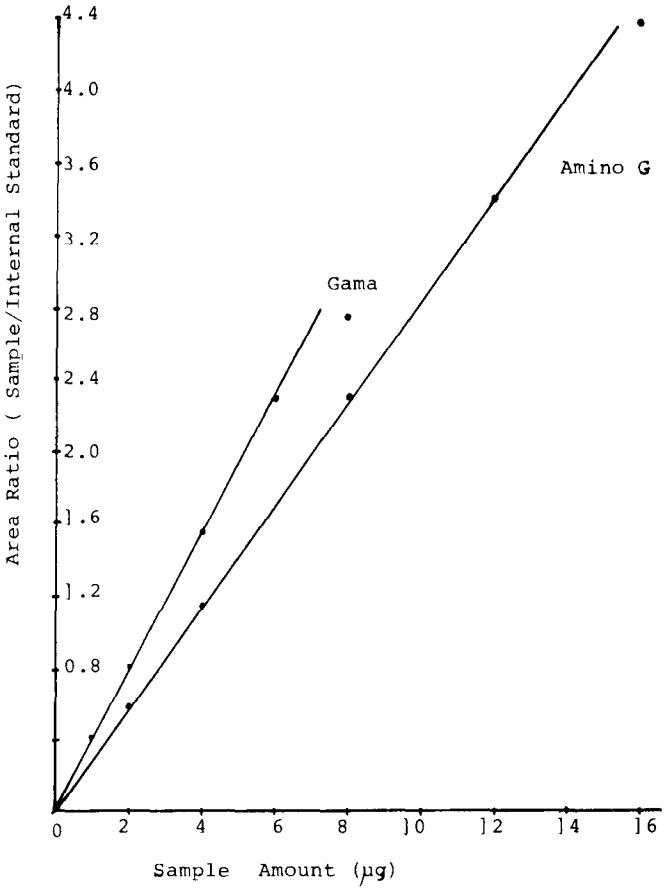

Fig. 6. Calibration curves for internal standard analysis of Gama acid and amino-G acid.

Gama acid and amino-G acid. A series of standard mixtures were prepared so that a fixed injection volume of 10 μl contained 1.0–8.0 μg Gama acid and 2.0–16 μg amino-G acid while the internal standard, Schaeffer acid, was always 0.9 μg. The results were plotted as the peak area ratio of sample to Schaeffer acid *vs.* sample amount (Fig. 6). Again, linear relationships for the two acids were obtained within the ranges in Table II, while each calibration curved passed through the origin.

Quantitative analysis of G acid and R acid mixture

In order to demonstrate the advantage of the quantitative HPLC method, it was compared with the traditional titration method.

HPLC internal standard method. A series of different sample solutions were made up (w/w) by mixing known amounts of G acid and R acid such that a fixed injection volume of 10 μl contained 2.0 μg amino-G acid as internal standard and the amounts of G acid and R acid fell in their linear response ranges (Table II). From the resulting peak area ratios of sample to amino-G acid, the detected amounts of G acid and R acid were then determined by interpolation using Fig. 5. The detected and actual values were then compared and percentage errors are listed in Table III. Except at very low concentrations, the error range of G acid is *ca.* 2% while for R acid it is *ca.* 1%. These narrow ranges illustrate the accuracy of the HPLC method.

Traditional titration method. As above, a series of known sample solutions were prepared and used for titration analysis. The total content of G acid and R acid was determined by titration with diazotized *p*-nitroaniline. Another sample was titrated with 0.1 *N* diazotized *p*-toluidine to determine R acid alone. Then the content of G acid was calculated by difference. The titration results and the actual values were then compared and the percentage errors were calculated (Table IV). It is seen that this method is inaccurate, the error fluctuating over a large range. The error for G acid is larger at lower concentrations, as high as 238%, while that for R acid is at 2–8%. This inaccuracy is due to the following factors; instability of the diazotized coupling reagent during titration in the daylight; critical influence of pH control on titration result and ambiguous determination of end-point.

Application of HPLC method to ammonolysis

The precursor of Gama acid —amino-G acid, can be prepared by ammono-

TABLE III

ANALYSIS OF G ACID AND R ACID BY HPLC INTERNAL STANDARD METHOD

Sample No.	*Actual values*				*Detected values*					
	G acid		*R acid*		*G acid*			*R acid*		
	Wt. (μg)	*% (w/w)*	*Wt. (μg)*	*% (w/w)*	*Wt. (μg)*	*% error*	*% (w/w)*	*Wt. (μg)*	*% error*	*% (w/w)*
1	3.55	95.4	0.172	4.6	3.59	1.1	95.4	0.172	0	4.6
2	2.80	76.5	0.860	23.5	2.86	2.1	76.7	0.869	1.0	23.3
3	1.87	52.1	1.72	47.9	1.91	2.1	52.3	1.74	1.2	47.7
4	0.933	26.7	2.58	73.3	0.951	1.9	26.9	2.59	0.4	73.1
5	0.187	5.4	3.27	94.6	0.196	4.8	5.6	3.27	0	94.4

TABLE IV

ANALYSIS OF G ACID AND R ACID BY TRADITIONAL TITRATION METHOD

Sample No.	*Actual value*				*Titration values*					
	G acid		*R acid*		*G acid*			*R acid*		
	Wt. (g)	*% (w/w)*	*Wt. (g)*	*% (w/w)*	*Wt. (g)*	*% error*	*% (w/w)*	*Wt. (g)*	*% error*	*% (w/w)*
1	0.674	95.4	0.0327	4.6	0.732	8.6	95.8	0.0317	3.1	4.2
2	0.532	76.5	0.163	23.5	0.537	0.9	76.7	0.163	0	23.3
3	0.355	52.1	0.326	47.9	0.409	15.2	56.3	0.318	2.5	43.7
4	0.177	26.7	0.489	73.3	0.268	51.4	36.5	0.467	4.5	63.5
5	0.0355	5.4	0.621	94.6	0.120	238	17.3	0.570	8.2	82.7

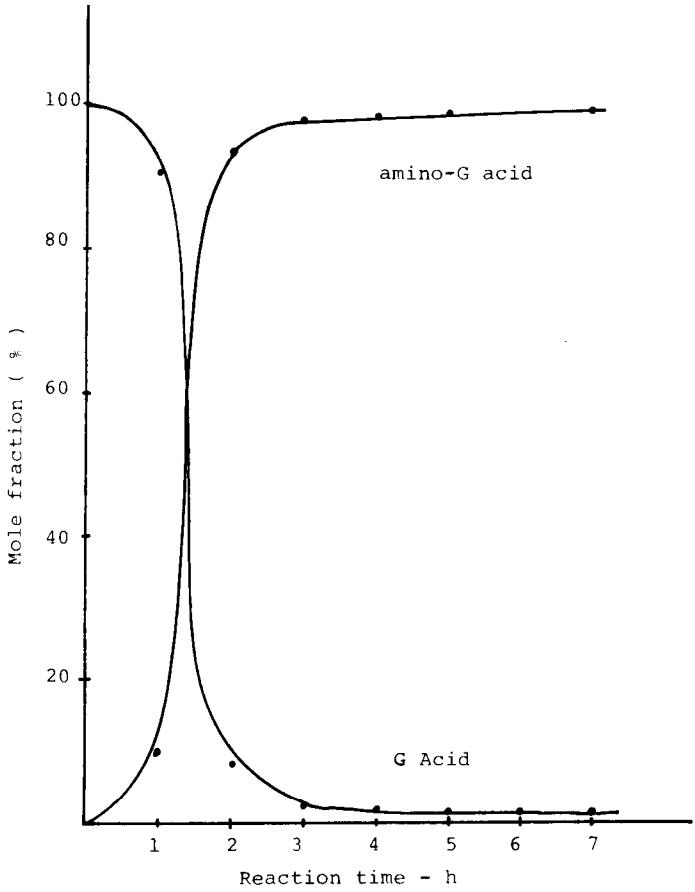

Fig. 7. Course of ammonolysis of G acid monitored by HPLC analysis.

lysis, the so-called Bucherer reaction of G acid. In order to investigate the optimum reaction conditions, the HPLC method was applied. The reaction was carried out by mixing 1.0 mol G acid, 1.2 mol ammonium sulphite (44%) and 4.0 mol aqueous ammonia water (28%) in a 2-l autoclave, followed by heating to 150°C for 7 h with a pressure drop from 150 to 100 p.s.i.g. At various times during the reaction, *ca.* 10-ml aliquots were removed and diluted 2000-fold for HPLC analysis. The result peak area ratios of G acid to amino-G acid were then converted into mole ratios by using the relative weight response factor, *f*, in Table II. The mole fractions (%) of G acid and amino G acid were calculated and plotted *vs.* reaction time to give conversion curves (Fig. 7). It is seen that the optimum conversion is reached after only 3 h under these conditions with *ca.* 98% conversion of starting G acid.

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