of *cis*-3-(2-thienyl)acrylic acid (1.54 g, 0.01 mole) in acetic anhydride (5.1 g, 0.05 mole) dropwise at 10—15°. After the addition, the mixture was stirred further for 1 hr at the same temperature. Precipitating crystals were filtered, washed with water, and recrystallized from ethyl acetate, mp 255—256°, yellow prisms (0.5 g, 2.5%). This compound was found to be **4c** by a mixed melting point determination with an authentic sample as well as spectral data.

The acidic filtrate from which **4c** was removed by filtration was poured into ice-cold water, extracted with ethyl acetate, washed with water, and dried. On evaporation of the solvent yellow crystalline residue was obtained, which was purified by chromatography analogously as above. Evaporation of the eluate *in vacuo* gave yellow prisms of **3c** (0.8 g, 40%). A mixed melting point determination of the above product with an authentic sample of **3b** did not show a depression.

Nitration of *trans*-3-(2-Thienyl)acrylic Acid (4a)——The nitration of **4a** was carried out in a similar manner as described above. The yellow crystals were recrystallized from ethyl acetate to obtain the analytical sample of **4a**, mp 255—256° (1.4 g, 72%).

Photochemical Isomerization of *cis*-(5-Substituted 2-thienyl)**acrylic Acids** (3a—c).——A general method of photochemical isomerization was shown as follows. A solution of the *cis*-(5-substituted 2-thienyl)acrylic acid (0.05 mole) in 50 ml of ethanol was irradiated for 6—8 hr with UV lamp (Manasuru Light 3650 Å, Manasuru Kagaku Co.) at room temperature. Yields, melting points of *trans* isomers obtained are as follows: **4a**, 0.5 g (64.9%), mp 146—147°; **4b**, 0.75 g (75%), mp 209—210; **4c**, 0.85% g (71%), mp 255—256°.

The Quantitative Determination of *cis/trans* Ratio of the Substituted Acrylic Acids——The quantitative determinations of the *cis/trans* ratios were carried out by gas chromatography (Shimazu model GC-5, 2% QF-1 on chromoscorb W, 2 m × ϕ3 mm stainless column, column temp. 140°, He 50 ml/min). A mixture of *cis* and *trans* isomers of each thienylacrylic acid was esterified by diazomethane in ether and subjected to the GC-analysis. No thermal isomerization was found to occurr under the above experiment.

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Stability of *p*-Hydroxybenzoic Acid Esters in an Acidic Medium[1]

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p-Hydroxybenzoic acid esters (parabens) have been generally used in pharmaceutical solution as preservatives.

Their stability has been mostly studied in a neutral[3,4] or alkaline solution,[3-7] and they have been considered as stable in an acidic medium.[7]

Presently a study was made of the stability of parabens in an acidic medium.

Experimental

Materials——Methyl and propyl parabens were used following recrystallization from an ethanol-water mixture. *p*-Hydroxybenzoic acid, a hydrolysate, used as standard in the quantitative determination of hydrolysate was recrystallized from an ethanol-water mixture.

Method——Methyl or propyl paraben was dissolved in 0.1 N, 0.01 N, 0.001 N, or 0.0001 N HCl solution to make a concentration of 5×10^{-4}M. Those solutions initially showed pH 1, 2, 3, and 4 respectively. Changes of pH before and after experiment were not observed except for a 0.0001 N HCl solution, of which pH changed to pH 4.3—4.5 at 40°, 60°, and 78° and to pH 5.0 at 100° after experiment.

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[1] This study was supported in part by a grant of the Ministry of Public Welfare, Japan.
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Each solution was filled in a ten-ml white ampule and the ampules were sealed. The ampules were stored at 40°, 60°, 78°, and 100° and the hydrolysate produced was chronologically determined.

The Analytical Method——Two ampules were used for each determination. Dilution was made with NaOH aqueous solution to make final concentration of NaOH above 0.01 N. The optical density of the diluted solution was measured at 303 nm and the residual ratio of paraben (C_p/C) was calculated with Eq. 1.

$$C_p/C = \frac{(A/C)-\varepsilon_a}{\Delta\varepsilon} \qquad \text{Eq. 1}$$

where A is the optical density of the test solution, C the initial concentration of paraben, ε_a and ε_p the molar absorption coefficient of *p*-hydroxybenzoic acid and paraben respectively at a certain wave length and $\Delta\varepsilon$ the difference of ε_p and ε_a. It is to be desired that the wave length which is to be used in determining $\Delta\varepsilon$ in Eq. 1 be a wave length showing the maximum difference in the spectra of paraben and *p*-hydroxybenzoic acid. Presently the wave length of 303 nm was adopted (Fig. 1). ε_p and ε_a in 0.01 N NaOH solution at 303 nm are the followings: $\varepsilon_{\text{methyl paraben}}=1.96\times10^4$, $\varepsilon_{\text{propyl paraben}}=2.06\times10^4$, $\varepsilon_a=3.40\times10^3$.

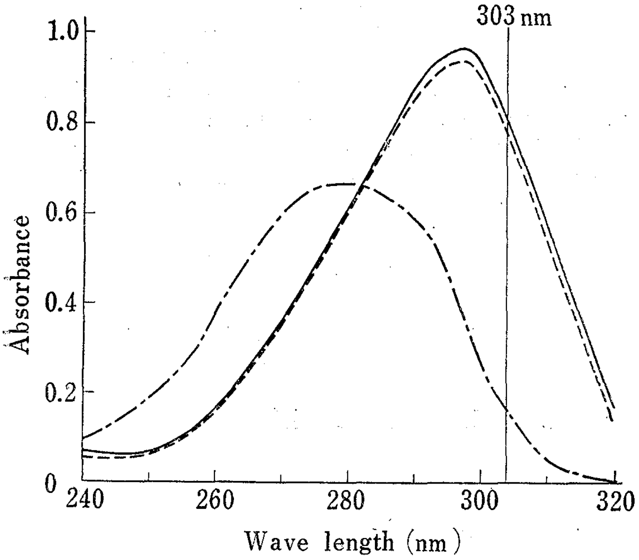

Fig. 1. Absorption Spectra of Compounds (4×10^{-5}M) in 0.01N NaOH

—·—·—: *p*-hydroxybenzoic acid; ———: propyl paraben; -------: methyl paraben

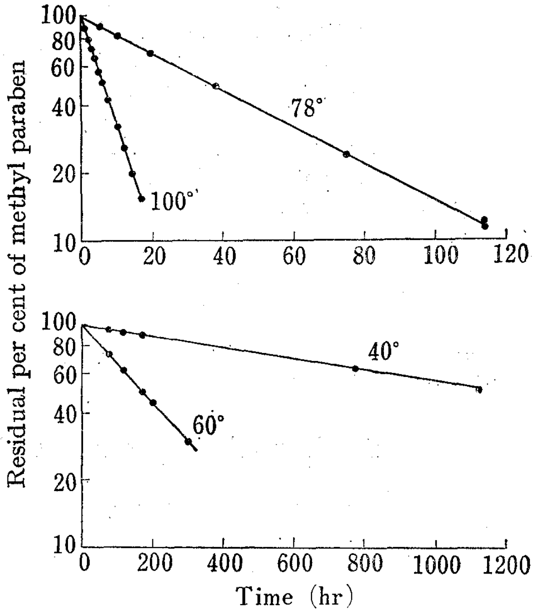

Fig. 2. First-order Graphs of the Thermal Degradation of Methyl Paraben in 0.1N HCl Solution

Table I. The Rate Constant and the Half-life of Hydrolysis of Methyl Paraben

Concn. of HCl (N)	Temp. (°C)	Rate Const., $k\pm\sigma^{a)}$ (hr^{-1})	Half-life, $t_{1/2}\pm\sigma^{a)}$ (day)
0.1	100	$(1.1207\pm0.0052)\times10^{-1}$	0.257 ± 0.001
	78	$(1.887\pm0.014)\times10^{-2}$	1.531 ± 0.011
	60	$(3.998\pm0.034)\times10^{-3}$	7.224 ± 0.062
	40	$(5.809\pm0.048)\times10^{-4}$	$49.71\pm0.41^{b)}$
0.01	100	$(1.052\pm0.066)\times10^{-2}$	2.74 ± 0.17
	78	$(1.911\pm0.066)\times10^{-3}$	15.11 ± 0.52
	60	$(3.90\pm0.16)\times10^{-4}$	74.1 ± 3.0
	40	$(6.76\pm0.75)\times10^{-5}$	$427\pm47^{b)}$
0.001	100	$(6.23\pm1.18)\times10^{-4}$	46.4 ± 8.8
	78	$(2.55\pm0.17)\times10^{-4}$	113.2 ± 7.7
	60	$(5.60\pm0.58)\times10^{-5}$	515 ± 54
	40	$(8.07\pm4.25)\times10^{-5}$	$360\pm190^{b)}$
0.0001	100	$(3.33\pm0.45)\times10^{-4}$	$87\pm12^{b)}$
	78	$(9.56\pm2.16)\times10^{-5}$	$302\pm68^{b)}$
	60	$(1.31\pm0.24)\times10^{-4}$	$220\pm40^{b)}$
	40	$(0.9\pm2.1)\times10^{-5}$	$3100\pm6900^{b)}$

a) σ indicates the standard error which was calculated with the weighted least squares method.

b) These data were not adopted in calculating the energy of activation and the frequency factor.

Result and Discussion

A logarithmic plotting of the residual ratio of methyl paraben against time at pH 1 showed a linear line at each temperature (Fig. 2). A similar result was obtained in each pH. These findings indicate that methyl paraben was apparently hydrolyzed following the first order kinetics. Propyl paraben was likewise hydrolyzed. The rate constant and the half-life of hydrolysis were summarized in Table I and II.

TABLE II. The Rate Constant and The Half-life of Hydrolysis of Propyl Paraben

Concn. of HCl (N)	Temp. (°C)	Rate Const., $k\pm\sigma$[a] (hr^{-1})	Half-life, $t_{1/2}\pm\sigma$[a] (day)
0.1	100	$(1.0273\pm0.0070)\times10^{-1}$	0.281 ± 0.001
	78	$(1.969\pm0.052)\times10^{-2}$	1.467 ± 0.038
	60	$(4.254\pm0.031)\times10^{-3}$	6.789 ± 0.050
	40	$(5.953\pm0.093)\times10^{-4}$	48.48 ± 0.76[b]
0.01	100	$(1.067\pm0.022)\times10^{-2}$	2.708 ± 0.056
	78	$(1.850\pm0.039)\times10^{-3}$	15.61 ± 0.33
	60	$(4.03\pm0.13)\times10^{-4}$	71.6 ± 2.3
	40	$(6.4\pm1.0)\times10^{-5}$	452 ± 71[b]
0.001	100	$(6.92\pm0.49)\times10^{-4}$	41.7 ± 3.0
	78	$(2.16\pm0.28)\times10^{-4}$	134 ± 17
	60	$(5.8\pm1.4)\times10^{-5}$	500 ± 120
	40	$(4.0\pm1.1)\times10^{-5}$	730 ± 210[b]
0.0001	100	$(1.83\pm0.23)\times10^{-4}$	158 ± 20[b]
	78	$(7.3\pm1.9)\times10^{-5}$	390 ± 100[b]
	60	$(9.4\pm5.4)\times10^{-6}$	3100 ± 1800[b]
	40	$(1.14\pm0.71)\times10^{-5}$	2500 ± 1600[b]

[b] σ indicates the standard error which was calculated with the weighted least squares method.
[a] These data were not adopted in calculating the energy of activation and the frequency factor.

Effect of temperature on the rate constant of hydrolysis was studied with the Arrhenius equation (Fig. 3), and the energy of activation and the frequency factor were calculated (Table III). In those calculations, the data at pH 4 were omitted because the pH of test solution was subjected to change during experiment.

The rate constant of hydrolysis at 25° was predicted with the rate constant at 100° and the energy of activation of 20.42±0.18 kcal/mole for methyl paraben and 19.79±0.13 kcal/mole for propyl paraben (Table IV). A pH-profile of the predicted rate constant of hydrolysis of

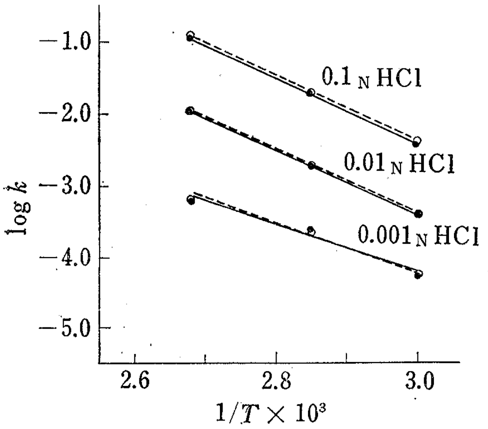

Fig. 3. Arrhenius Plots for Methyl and Propyl Parabens in Aqueous Hydrochloride Solution

—●—: methyl paraben; ···○···: propyl paraben

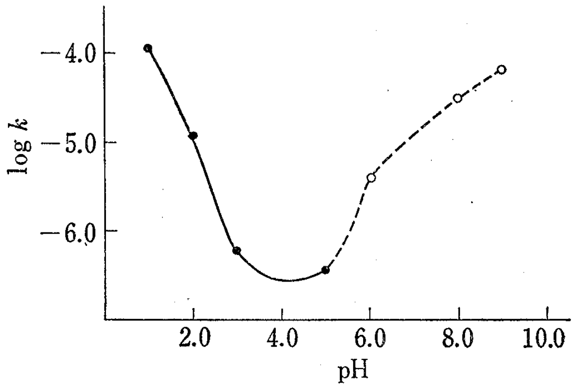

Fig. 4. Predicted log k—pH Profile of Methyl Paraben at 25°

The Values at pH 6, 8 and 9 were quoted from Ref. 3.

methyl paraben is shown in Fig. 4, where the values at pH 6, 8 and 9 were quoted from Ref. 3. It was found that methyl paraben was most stable in the neighbour of pH 4.

Sometimes some pharmaceutical preparations require sterilization in an autoclave as a manufacturing process. Hydrolysis of parabens in a steam sterilization under pressure, 121.5°, 20 min, was predicted (Table V). A considerable hydrolysis was predicted at pH 1, but at other pH parabens were stable.

Table III. The Energy of Activation and Frequency Factor of Hydrolysis of Parabens

	Concn. of HCl (N)	$E_a \pm \sigma^{a)}$ (kcal/mole)	$A \pm \sigma^{a)}$ (hr^{-1})
Methyl paraben	0.1	20.42±0.18	$(1.01\pm0.26)\times10^{11}$
	0.01	20.02±0.43	$(5.5\ \pm3.4\ )\times10^{9}$
	0.001	15.2 ±3.5	$(0.6\ \pm3.3\ )\times10^{6}$
Propyl paraben	0.1	19.79±0.13	$(4.03\pm0.73)\times10^{10}$
	0.01	20.28±0.26	$(8.0\ \pm3.0\ )\times10^{9}$
	0.001	12.7 ±1.5	$(1.9\ \pm4.0\ )\times10^{4}$

a) σ indicates the standard error which was calculated with the weighted least squares method.

Table IV. Predicted Rate Constant and Half-life of Hydrolysis of Parabens at 25°

	Concn. of HCl (N)	Rate Const., $k_{25}\pm\sigma^{a)}$ (hr^{-1})	Half-life, $t_{1/2}\pm\sigma^{a)}$ (day)
Methyl paraben	0.1	$(1.086\pm0.005)\times10^{-4}$	266± 13
	0.01	$(1.16\ \pm0.12\ )\times10^{-5}$	2490± 260
	0.001	$(6.1\ \pm1.5\ )\times10^{-7}$	47000±12000
	0.0001	$(3.27\ \pm0.64\ )\times10^{-7}$	88000±17000
Propyl paraben	0.1	$(1.255\pm0.042)\times10^{-4}$	230± 7.6
	0.01	$(1.083\pm0.081)\times10^{-5}$	2670± 200
	0.001	$(8.41\ \pm0.96\ )\times10^{-7}$	34300± 3900
	0.0001	$(2.23\ \pm0.37\ )\times10^{-7}$	130000±22000

a) σ indicates the standard error which was calculated with the weighted least squares method.

Table V. Predicted Rate Constant of Hydrolysis of Parabens under Steam Sterilization (121.5°, 20 min)

	Concn. of HCl (N)	Rate Const., $k_{121.5}\pm\sigma^{a)}$ (hr^{-1})	Predicted residual % after sterilization
Methyl paraben	0.1	$(4.96\pm0.16)\times10^{-1}$	84.77±0.46
	0.01	$(4.49\pm0.37)\times10^{-2}$	98.51±0.12
	0.001	$(2.79\pm0.57)\times10^{-3}$	99.91±0.02
	0.0001	$(1.49\pm0.22)\times10^{-3}$	99.95±0.01
Propyl paraben	0.1	$(4.42\pm0.10)\times10^{-1}$	86.30±0.30
	0.01	$(4.67\pm0.19)\times10^{-2}$	98.46±0.06
	0.001	$(2.96\pm0.24)\times10^{-3}$	99.90±0.01
	0.0001	$(7.8\ \pm1.1\ )\times10^{-4}$	99.97±0.004

a) σ indicates the standard error which was calculated with the weighted least squares method.