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Pharmaceutical Studies on 2-Aminoethanesulfonic Acid Derivatives. II.[1] 4-(Aminoethanesulfonylamino)antipyrine. (1). Stability Studies[2]

Shun-ichi Naito and Sachiko Bessho

Kyoto College of Pharmacy[3]

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Stability tests of 4-(aminoethanesulfonylamino)antipyrine in solution were made at different pH's, and it was observed that the chemical in aqueous solution was practically stable at room temperature through kinetical studies.

4-(Aminoethanesulfonylamino)antipyrine[4] (hereinafter abbreviated as taurinopyrine) was prepared in order to obtain a more potent and stable chemical than another antipyrine or aminoantipyrine derivatives with less side effects. Stability of taurinopyrine was mainly examined in the present series of work, comparing with aminopyrine.

Taurinopyrine is very stable to moisture, as its critical relative humidity (Fig. 1) is 98.9 %. Solubilities of taurinopyrine are as follows: 0.2% in benzene, 0.4% in acetone, 0.5 %in ethanol, 1% in methanol, 2% in water, and insoluble in ether, petroleum ether, benzine and ethyl acetate.

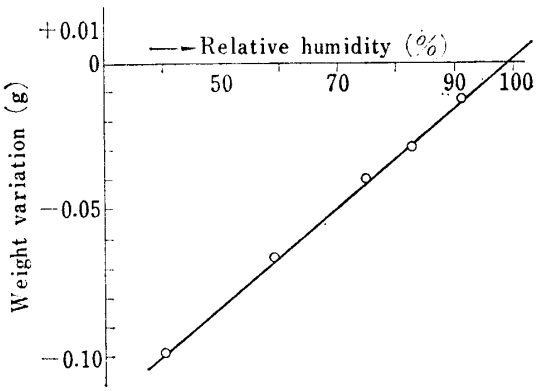

Fig. 1. Critical Relative Humidity[a] of Taurinopyrine at 37° after 4 Hours

a) Measured by the method described by R. Yamamoto and T. Takahashi (*Shionogi Kenkyusho Nempo*, **1954**, 455).

The thin-layer chromatography of the degradation products of taurinopyrine, heated in solution for 28 hrs on a boiling water bath, separated them into five spots when mixed solvents such as chloroform–*n*-butanol (22.5:1) were used. The five spots,[5] shown in Table I in detail, were detected in comparison with the thermal degradation products of aminopyrine and sulpyrine in solution. Less and no peculiar decomposition products of taurinopyrine were observed in the chromatography, as compared with another two antipyrine derivatives on the basis of *Rf* values.

In paper chromatography, many kinds of mixed solvent were used for successful separation of unchanged taurinopyrine from its degradation products, using 0.5% potassium ferricyanide solution and 1% ferric nitrate in 0.7 N nitric acid solution as a color developer. Unchanged taurinopyrine remained at the origin and its decomposition products were migrating, when a mixed solvent of chloroform–*n*-butanol (22.5:1) was used, whereas, unchanged amino-

1) Part I: S. Naito and K. Fukui, *Chem. Pharm. Bull.* (Tokyo), **18**, 820 (1970).
2) This constitutes Part VII of a series entitled "Studies on Stability and Stabilization of Pharmaceuticals" by S. Naito.
3) Location: *Misasagi, Yamashina, Higashiyama-ku, Kyoto.*
4) Synthetic method for this compound will be reported in due time as there are variety of methods. Pharmacological studies on this compound will be published in the following paper.
5) The five spots were also recognized by gas-chromatography and the results obtained would be discussed later. For example, glass column, SE-30 (15%), at 235°.

Table I. Thin-Layer Chromatography of the Thermal Degradation Products of Taurinopyrine, Aminopyrine and Sulpyrine in Aqueous Solution boiled in Water-Bath for 28 Hours

Spots No.	*Rf* values at 25°		
	Taurinopyrine	Aminopyrine	Sulpyrine
1	0[a]	0[b]	0[b]
2	0.1[b]	0.1[b]	0.1[b]
3	0.25[c]	0.25[c]	0.25[c]
4		0.3[b]	
5	0.4[b]		0.4[b]
6			0.53[b]
7		0.75[e]	0.78[f]
8	0.95[b,d]	0.95[b,d]	0.98[b,d]

a) Unchanged taurinopyrine.
b) color developer: 0.5% potassium ferricyanide solution and 1% ferric nitrate in 0.7N HNO_3 spot color: blue-bluish green
c) color developer: 1% ammonium phosphomolybdate solution spot color: gray
d) color developer: 5% *p*-dimethylaminobenzaldehyde in conc. HCl spot color: violet with taurinopyrine, yellowish violet with aminopyrine and sulpyrine
e) Unchanged aminopyrine.
f) Unchanged sulpyrine.
solvent: $CHCl_3$-*n*-BuOH (22.5:1)
absorbent: Kieselgel G (0.25 mm in thickness)

pyrine was separated from its degradation products, remaining at starting point, when a mixed solvent of ligroin, benzene and chloroform (18:9:1) was used. The substance at the starting point, *i.e.*, unchanged taurinopyrine or aminopyrine was extracted from the papers with water and re-examined through the thin-layer chromatography, using a mixed solvent of chloroform–*n*-butanol (22.5:1) for aminopyrine and taurinopyrine, and only unchanged aminopyrine or taurinopyrine was detected.

For the determination of taurinopyrine, several assay methods for aminopyrine and sulpyrine were investigated. Taurinopyrine is, however, negative to color reactions with *p*-dimethylaminocinnamaldehyde,[6] ferric chloride,[7] potassium naphtoquinone sulfonate[8] and indophenol.[9]

Table II. Absorbances in Color Variation after an Addition of 1% Ferric Nitrate in 0.7N HNO_3 for the Assay of Taurinopyrine

Time in minutes	mcg[b] of Taurinopyrine				
	blank	10.3	20.6	30.9	41.2
10	0	0	0	0	*a*)
15	0.005	0.060	0.112	0.198	0.365
20	0.011	0.080	0.168	0.335	0.490
30	0.020	0.115	0.229	0.360	0.490
35	0.012	0.138	0.289	0.420	0.550
40	0.011	0.125	0.265	0.399	0.528

a) Weak colorization was observed but not measured.
b) All the data are the mean values of three experiments under the same conditions.

6) K. Kato, *Yakuzaigaku*, **24**, 116 (1954).
7) P. Halfelfinger, B. Schmidli and H. Ritter, *Arch. Pharm.*, **297**, 641 (1964).
8) S. Ono and R. Onishi, *Yakugaku Zasshi*, **85**, 239 (1965).
9) S. Ono and R. Onishi, *Yakugaku Zasshi*, **85**, 245 (1965).

After separation from the thermal degradation products by paper chromatography, unchanged taurinopyrine or aminopyrine was assayed by the ferric nitrate method.[10] The final colored solution after an addition of ferric nitrate solution is unstable (Table II), and colorizing after thirty minutes was adopted for the assay.

From the assay results (Table III) given by the unchanged taurinopyrine or aminopyrine and prediction of the stabilities at high temperatures such as 40°, 50°, and 60±1°, the kinetics of degradation of taurinopyrine and aminopyrine could be interpreted on the baiss of the pseudo first order reactions. Several constants[11] concerning the predictions of stabilities of taurinopyrine and aminopyrine shown in Table III were derived from the results shown in Fig. 2. The predicted concentration after 2 years at 25° was 98.82% for taurinopyrine and 97.18% for aminopyrine, that is, the difference of the concentrations between the two drugs is little. Comparing with the rate constants at 25°, the degradation velocity of taurinopyrine may, however, be about half of aminopyrine. It is presumed that taurinopyrine in aqueous solution is more stable at room temperature than aminopyrine.

Table III. Tabulation of Slope (k_1),[a] Heat of Activation and Other Data derived from Arrhenius Plots of Pseudo First Order Rates for Thermal Degradation of Taurinopyrine and Aminopyrine in Aqueous Solutions

	Taurinopyrine	Aminopyrine
Rate constant(k_1) at 40°	9.9×10^{-4}	23.0×10^{-4}
Rate constant (k_1) at 50°	19.1×10^{-4}	54.5×10^{-4}
Rate constant (k_1) at 60°	38.5×10^{-4}	125.1×10^{-4}
Heat of activation, ΔHa in kcal/mole	14.1	17.5
P[b]	7	10
Predicted rate constant k_1,at 25°	3.2×10^{-4}	5.6×10^{-4}
Predicted concentration (%) after 10 months, at 25°	90.8	84.4
Assayed concentration (%) after 10 months, at room temperature	94.5	89.1

a) The rate constant k_1 is in reciprocal day. b) log(frequency factor)

Stability tests were also carried out at different pH's, in other words, 0.6 g of taurinopyrine was dissolved in 25 ml of water having pH 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0 prepared by an addition of 1N HCl, and pH 9.0 and 10.0±0.1 prepared by an addition of 1N NaOH, and the solutions were kept at 60° for 30 days. After storage, no significant changes of the pH and the content of taurinopyrine in many different pH solution, except slight brownish coloring

Table IV. Variation of pH's at Different Concentration of Taurinopyrine

Acid or alkali in 100 ml of water solution	Taurinopyrine added, (mg/ml)	pH
0.1 N HCl, 65.0 ml	24	6.10
	18	2.00
0.1 N HCl, 68.0 ml	24	5.15
	18	1.25
0.1N NaOH, 7.0 ml	24	8.95
	18	9.05
	25	8.79
None	20	8.75
	10	8.72

10) S. Naito, *Yakugaku Kenkyu*, **35**, 50, 136 (1963).
11) E.R. Garrett, *J. Am. Pharm. Assoc.*, **44**, 515 (1955); *idem*, *ibid.*, **45**, 171 (1956).
12) Measured by titration method. PH-meter, type M-4, Hitachi-Horiba (Tokyo) was used.

at pH 1.0, 2.0, 3.0, 9.0, and 10.0 were observed. Relationship between concentration of the chemical and pH was shown in Table IV for example.

In the Table, concentration of 18 mg/ml of taurinopyrine was used as a transient value for assuming when 24 mg/ml of taurinopyrine was decomposed at some degree at an accelerated temperature such as 60°. If some degradation of taurinopyrine was taken place, changes of pH of the solution must be observed at least at pH 6.10 or 5.15. Therefore, the fact that no significant changes of the pH of taurinopyrine in many different pH solution during 30 days at 60° were observed, also supports that no actual decompostion of taurinopyrine was happened in these experimental conditions as shown in Table V. In the case of an aqueous solution of taurinopyrine without any acid or alkali, no changes of pH through the kinetical study were comprehensible even after some decomposition of taurinopyrine, from the fact that almost same pH was observed at concentrations of 25, 20, and 10 mg/ml of taurinopyrine, as shown in Table IV.

Table V. Several Examples of Necessary Amount of Acid or Alkali for Adjusting pH and Concentrations of Taurinopyrine after Storage at 60°

pH	0.1N HCl added (in ml)	0.1N NaOH added (in ml)	Initial concentration (mg/20 ml)	Concentration after storage at 60° for 36 days (mg/20 ml)
4.0	14.4	0	500	500
5.0	13.6	0	500	510
6.0	12.9	0	500	480
10.0	0	1.6	500	440

The pH of 25 ml of the aqueous solution containing 0.6 g of taurinopyrine is 8.79, and the pK_b of the taurinopyrine is about 7.1.[12]

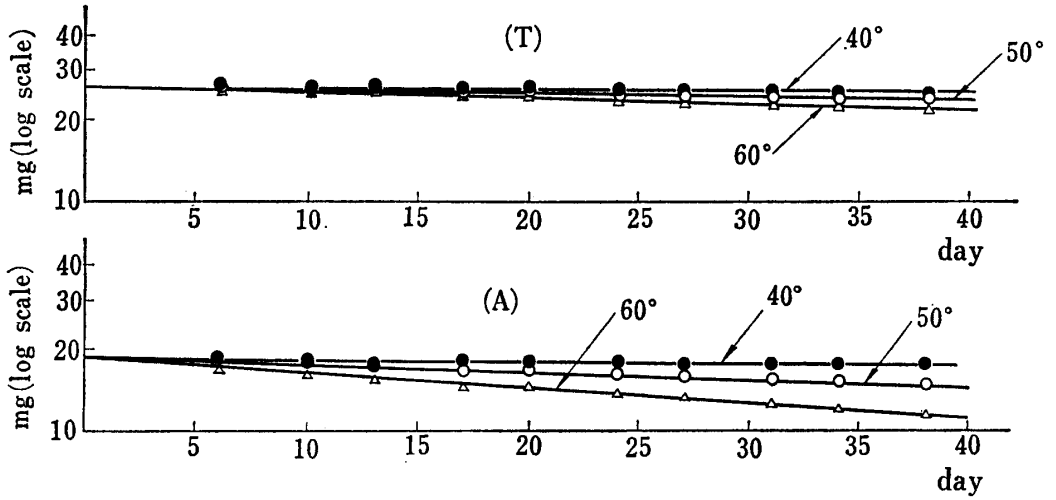

Fig. 2. Content of Taurinopyrine(T) or Aminopyrine(A) in Aqueous Solution

initial concentration: (T) 25.44 mg/ml at pH 8.8, (A) 18.20 mg/ml at pH 7.6. All the data are the mean values of three experiments under the same conditions.

Experimental

Sample——Taurinopyrine solution: 2.4 g of taurinopyrine (mp 185° (decomp.), recrystallized from water) was dissolved in 100 ml of water. Aminopyrine solution: 2.0 g of aminopyrine (mp 108°, recrystallized from ligroin) was dissolved in 100 ml of water. The solution is put into a colorless ampule (capacity, 1 ml). The samples were kept at 40°, 50°, and 60±1°,[13] and the active ingredient was assayed by sampling schedules.

13) Life-Tester, Model LT-6, Froint Sangyo Co., Ltd., Tokyo.

Determination of Taurinopyrine——Five microlitre of the sample solution was spotted on three filter paper, respectively, and chromatographed at 25° for 2 hr, using a mixture of $CHCl_3$–*n*-BuOH (22.5:1) as a developing solvent. Three chromatographed papers were cut into strips 1 cm above and below the original point and the strips were extracted with 4 ml of H_2O on a boiling water bath for 3 min, then 0.4 ml of supernatant fluid was put into a test tube. To this supernatant, water (0.6 ml) was added, and 0.6 ml of 0.5% potassium ferricyanide solution was mixed, and the solution was allowed to stand for 5 min. After that, 0.6 ml of 1% ferric nitrate in 0.7N HNO_3 was added to the solution which was allowed to stand for 35 min then 5 ml of water was added. The optical density of the above colored solution was determined at 720 mμ 5 min after the addition of water. The absorbances measured were corrected by deducting a blank value.

Determination of Aminopyrine——Five microlitre of the sample solution was dropped on three filter papers, and chromatographed at 25° for 2 hr, using a mixture of ligroin, $CHCl_3$ and benzene (18:1:9) as a solvent. The three chromatographed papers cut at one centimeter above and below the spotted line, were collected, then extracted with 4 ml of water in a centrifuge tube on a boiling water bath for 3 min, and 0.4 ml of the supernatant fluid was put into a test tube. Water (0.6 ml) was added, and 0.6 ml of 0.5% potassium ferricyanide solution was mixed and the solution was allowed to stand for 5 min. Then, 0.6 ml of 1% ferric nitrate in 0.7N HNO_3 was added to the solution which was allowed to stand for 5 min, then, 5 ml of water was added. The absorbance of the above colored solution was measured at 720 mμ 5 min after the addition of water. The optical density determined was corrected by deduction of a blank value.

Stability of Taurinopyrine Solution at Different pH's——In order to adjust pH, small amount of 0.1N HCl or 0.1N NaOH was added drop by drop to about 15 ml of the solution having 500 mg of taurinopyrine, and water was added to make the full volume of 20 ml, checking variation of pH by pH-meter. Several examples of necessary amount of acid or alkali for adjusting pH and concentrations of taurinopyrine after storage at 60° were shown in Table V.

In general, buffer solution is preferable for stability test at different pH's but HCl or NaOH was positively used for the test under consideration of making injectable solution.