Notes

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Pharmaceutical Studies on 2-Aminoethanesulfonic Acid Derivatives. I. Aminoethanesulfonylphenetidine. (1). Stability Studies[1]

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Aminoethanesulfonylphenetidine[3] (hereinafter abbreviated as taurinophenetidine) was prepared in order to obtain a more potent chemical than *p*-acetamidophenol with less side effects. Stability of taurinophenetidine was mainly examined in the present series of work.

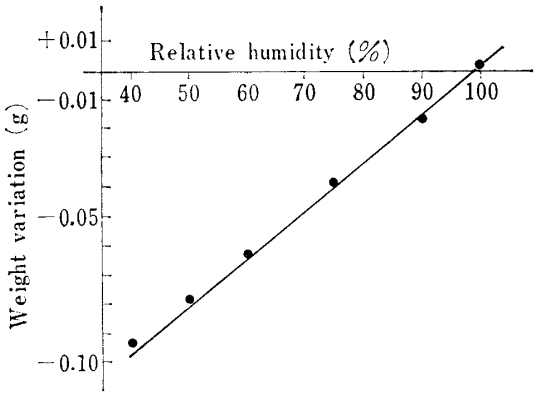
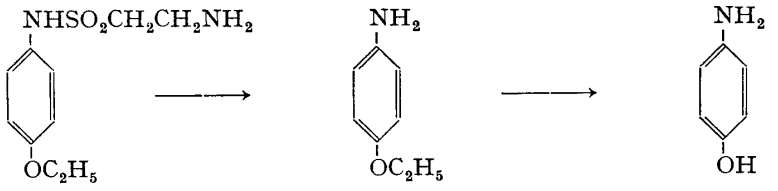


Fig. 1. Critical Relative Humidity[a] of Taurinophenetidine after Storage at 37° for 4 Hr

a) measured by the method reported by R. Yamamoto, T. Takahashi (*Shionogi Kenkyusho Nempo*, 455 (1954))

Taurinophenetidine is very stable to moisture, as its critical relative humidity (Fig. 1) is 99.2%. Solubilities of taurinophenetidine are as follows: 0.6% in ethyl acetate, 2% acetone, 0.7% in ethanol, 0.5% in chloroform and water, 0.3% in ligroine, 0.2% in benzene; soluble in methanol, acetic acid, and pyridine, and insoluble in ether, petroleum ether, and petroleum benzine.

The thin-layer chromatography of the degradation products of taurinophenetidine, heated in solution for 80 hr on a boiling water bath, separated them into 4 spots, when mixed solvents such as benzene and acetone (2:1) were used. The *Rf* values of the four spots, shown in Table I were coincided with those of admixture with the corresponded authentic compound, and mixed micro-melting point determination of the ethanolic extracts of each spot from thin-layer chromatograms showed no depression with the corresponded authentic compound, except oily *p*-phenetidine. As a result, the thermal degradation process of taurinophenetidine might be shown as following:



taurinophenetidine

In paper chromatography, many kinds of mixed solvent were used for successful separation of unchanged taurinophenetidine from its degradation products. Unchanged taurino-

1) This constitutes Part VI of a series entitled "Studies on Stability and Stabilization of Pharmaceuticals" by S. Naito.
2) Location: *Misasagi, Yamashina, Higashiyama-ku, Kyoto.*
3) Synthetic method for this compound will be reported in due time as there are varieties of methods. Pharmacological studies on this compound will be published in the following paper.

Table I. Thin-Layer Chromatography of the Thermal Degradation Products of Taurinophenetidine in Aqueous Solution heated in Water Bath for 80 Hour

Spot No.	*Rf* values at 25°				
	Taurine	*p*-Aminophenol	*p*-Phenetidine	Taurinophenetidine	Taurinophenetidine heated in solution
1	0				0
2				0.17	0.17
3		0.37			0.37
4			0.67		0.67

solvent: benzene and acetone (2:1)
adsorbent: Kieselgel G
color developer: 0.5% potassium ferricyanide solution and 1% ferric nitrate in 0.7N HNO_3
spot color: blue

phenetidine remained at the origin and its thermal degradation products moved, when a mixed solvent of acetone and hexane (1:15) was used. The color developer used was 0.5% potassium ferricyanide solution and 1% ferric nitrate in 0.7N nitric acid. The substance at the origin, *i.e.*, taurinophenetidine, was extracted from the paper with water and re-examined by a thin-layer chromatography, using a mixed solvent of benzene and acetone (2:1). No spot of thermal degradation products was found and only the unchanged taurinophenetidine was detected.

For the determination of taurinophenetidine, several assay methods for acetophenetidine were investigated.

Taurinophenetidine is, however, insensitive to color reactions with copper chloride,[4] sodium nitroprusside,[5] or 2,4-dinitrofluorobenzene.[6] After separation from the thermal degradation products by paper chromatography, unchanged taurinophenetidine was assayed by Methyl Orange solution.[7] The final colored solution is very stable at least for 2 hr. From the assay results (Fig. 2) for the unchanged taurinophenetidine and prediction of stabilities at high temperature such as 40°, 50°, and 60°, the kinetics of the degradation of taurinophenetidine could be interpreted as the pseudo-first-order reactions. Several constants[8] for the prediction of stabilities of taurinophenetidine, shown in Table II, were derived and the predicted concentration after 2 years at 25° was 56.2%.

On the other hand, no recognizable changes in the content of taurinophenetidine were found through paper chromatography after 67 days by storage at 40°, 50°, and 60° in a powder

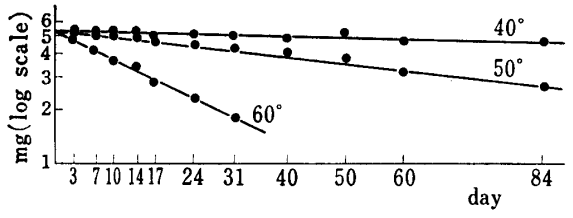


Fig. 2. Content of Taurinophenetidine in Aqueous Solution at pH 8.4

initial concentration: 5.25 mg/ml
All the data are the mean values of three experiments under the same conditions.

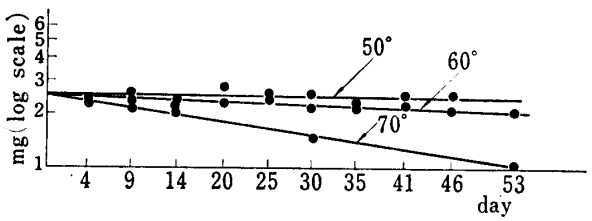


Fig. 3. Content of Taurinophenetidine in HCl Solution of pH 6.1

initial concentration: 2.5 mg/ml
All the data are the mean values of three experiments under the same conditions.

4) H.M. Hershenson and D.N. Hume, *Anal. Chem.*, **29**, 16 (1957).
5) M. Aoki and Y. Iwayama, *Yakugaku Kenkyu*, **23**, 273 (1957).
6) E. Nelson, I. O'Reilly, and T. Chulski, *Clin. Chim. Acta*, **5**, 774 (1960).
7) R.E. Keller and W.C. Ellenbogen, *J. Pharmacol.*, **106**, 77 (1952).
8) E.R. Garrett, *J. Am. Pharm. Assoc.*, **44**, 515 (1955); **45**, 171 (1956).

state. This fact shows that taurinophenetidine is remarkedly more stable in a powder form than in solution.

Stability test were also carried out at different pH's, such as pH 4.1, 5.2, and 6.1 prepared by an addition of 1N HCl, and pH 9.7 prepared by an addition of 1N NaOH. In the case of taurinophenetidine solution of pH 4.1 and 5.2, no decomposition was observed at 40°, 50°, and 60° for 53 days. Taurinophenetidine in solution of pH 9.7 was not so stable that no kinetic consideration was made, but in the case of the solution at 6.1, the kinetics of the degradation of taurinophenetidine could be interpreted as the pseudo-first-order reaction, as shown in Fig. 3 and in Table II.

Table II. 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 Taurinophenetidine in Aqueous Solution and HCl Solution of pH 6.1

	Aqueous solution	Solution of pH 6.1
Rate constant (k_1) at 40°	1.5×10^{-3}	
Rate constant (k_1) at 50°	7.5×10^{-3}	9.9×10^{-4}
Rate constant (k_1) at 60°	34.9×10^{-3}	4.0×10^{-3}
Rate constant (k_1) at 70°		16.6×10^{-3}
Heat of activation, ΔHa in kcal/mole	32.6	29.6
P[b]	21	17
Predicted rate constant k_1 at 25°	7.9×10^{-4}	1.7×10^{-5}
Predicted concentration(%) after 2 years, at 25°	56.2	98.8

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

Predicted concentration of taurinophenetidine after 2 years at 25° and at pH 6.1 was 98.8%. As mentioned above, taurinophenetidine solution is unstable in alkaline medium but the stability increases with increasing acidity of the medium. After storage, no significant changes in the pH were observed for all of the stability tests except at pH 9.7. Relationship between concentration of the chemical and pH was shown in Table III for example.

Table III. Variation of pH's at Different Concentration of Taurinophenetidine

Acid or alkali in 10.0 ml of water solution	Taurinophenetidine added, (mg/ml)	pH
0.01N HCl 7.8 ml	2.5	6.12
	1.0	5.95
0.01N HCl 9.8 ml	2.5	4.05
	1.0	2.30
0.01N NaOH 5.5 ml	2.5	9.92
	1.0	11.20
None	5.2	8.35
	2.0	8.32
	1.0	8.30

In the Table, concentration of 1.0 mg/ml of taurinophenetidine was used as a transient value for assuming when 2.5 mg/ml of taurinophenetidine was decomposed at some degree at an accelerated high temperature. If some degradation of taurinophenetidine was taken place, changes of pH of the solution must be observed at least at pH 4.0. Therefore, the fact that no significant change of the pH of taurinophenetidine in solution having pH 4.1 during

53 days at accelerated high temperature was observed as shown in Table IV, also supports that no actual decomposition of taurinophenetidine was happened in the experimental condition. In the case of an aqueous solution of taurinophenetidine without any acid or alkali or at pH 6.1, no changes of pH through the kinetical studies were comprehensible even after some decomposition of taurinophenetidine, from the fact that almost same pH was observed at different concentrations of taurinophenetidine, as shown in Table III.

Table IV. Necessary Amount of Acid or Alkali for Adjusting pH and Concentrations of Taurinophenetidine after Storage at 60°

pH	0.1N HCl added (approx. in ml)	0.1N NaOH added (approx. in ml)	Initial concentration (mg/20 ml)	Concentration after storage at 60° (mg/20 ml)
4.1	2.0	0	50	50 (after 53 days)
5.2	1.8	0	50	51 (after 53 days)
6.1	1.6	0	50	39 (after 53 days)
9.7	0	1.0	50	43 (after 24 days)

The pH of 100 ml of the aqueous solution containing 0.5 g of taurinophenetidine is 8.40 and the pK_b of taurinophenetidine is about 6.7.

Experimental

Sample——Taurinophenetidine (mp 149—153°, recrystallized from AcOEt) was dissolved in water. The solution was sealed in colorless ampules (capacity, 1 ml) and kept at 40°, 50°, 60°, or 70±1°,[9] and the active ingredient was assayed by sampling schedules.

Determination of pH's——Thirty millilitre of the solution having 75 mg of taurinophenetidine at different pH's in each ampule was kept at 40°, 50°, and 60±1° for 53 days. The pH of each solution after storage was determined by pH-meter.[10]

Determination of Taurinophenetidine——Sample solution of taurinophenetidine was spotted on a filter paper and chromatographed at 25°, using a mixture of acetone and hexane (1:15) as a developing solvent. The filter paper was cut into strips 1 cm above and below the original point and each strip was extracted with 4 ml of H_2O in a centrifuge tube on a boiling water bath for 3 min, and 3 ml of the supernatant fluid (containing 10—40 μg of taurinophenetidine) was placed in a test tube with a glass stopper. To this supernatant, 1 ml of the filtrate from an equivolume mixture of freshly prepared 0.5% Methyl Orange solution and 2.5% boric acid solution was added, and the mixture was allowed to stand for 5 min. Seven milliliters of AcOEt was added and the test tube was shaken vertically vigorously 20 times. After centrifuging for 5 min., the optical density of the AcOEt layer was determined at 410 mμ.

The absorbances measured were corrected by subtracting a blank value. Calibration curve was made by paper chromatography of a known concentration of taurinophenetidine solution.

Stability of Taurinophenetidine 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 50 mg of taurinophenetidine, and water was added to make the full volume of 20 ml, checking variation of pH by pH-meter. The pH of 20 ml of aqueous solution having 50 mg of taurinophenetidine was 8.4. Necessary amount of acid or alkali for adjusting pH and concentrations of taurinophenetidine after storage at 60° were shown in Table IV.

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.

9) Life-Tester, Model LT-6, Froint Sangyo Co., Ltd., Tokyo.
10) pH-Meter, Type M-4, Hitachi-Horiba, Tokyo.