Notes

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Mikiko Kataoka, Takeshi Miyata, Keiko Yogi, and Yoshitoshi Kasé : Application of Ninhydrin-Sulfuric Acid Reagent to the Determination of Micro Amounts of 3-Dimethylamino-1,1-di(2-thienyl)but-1-ene (Ohton).

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In the previous papers,[1,2] a new method for the determination using ninhydrin-sulfuric acid reagent of 1-methyl-3-(di-thien-3-ylmethylene)piperidine (a synthetic antitussive, trade name "Asverin", AT-327) and its derivatives possessing thiophene nuclei have been reported. In the present paper, it was reported the determination, with the same reagent, of 3-dimethylamino-1,1-di(2′-thienyl)but-1-ene (trade name "Ohton"), a synthetic analgesic having narcotic properties and a similar chemical structure to AT-327.

Though some methods for the determination of Ohton have been reported, most of them are unspecific and insensitive. The reagents used for qualitative analysis[3~6] were as follows: concentrated H_2SO_4, Erdmann's, Mandelin's, Fröhde's, and Mecke's Marquis' reagents. For the determination of Ohton, the following methods were available: measurement of ultra-violet absorption[7] in aqueous solution (sensitivity, 1 μg./ml.), concentrated H_2SO_4[8] and methylorange[9] (sensitivity, more than 10 μg./ml.), and bromine iodide methods[5] (sensitivity, no good).

According to Feigl,[10] thiophene derivatives unsubstituted in the α-position condense with ninhydrin in concentrated sulfuric acid to yield colored quinoidal compounds. Ohton, possessing two thiophene nuclei, was colored by the same reagent and its absorption maximum appears at 540 mμ.[1]

The intensity of characteristic color developed by the above-mentioned reaction depends on the concentration of Ohton. The color developed after 24 hrs., at 25~30° exhibited an absorption maximum at 540 mμ. The absorbance at 540 mμ obeyed to Beer's Law over the range of 1 to 30 μg./ml. The recovery from human urine was 96.6 ± 3.8%.

Experimental

Reagent——10 mg. of ninhydrin (triketohydrinden hydrate) crystals was dissolved in 100 ml. of thiophene-free concentrated sulfuric acid. The reagent should be freshly prepared.

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1) Y. Kasé, T. Yuizono, M. Kataoka, T. Miyata: Kumamoto Pharm. Bull., **6**, 20 (1965).
2) T. Yuizono, Y. Kasé, A. Kawano, H. Okubo, M. Kataoka: J. Pharm. Pharmacol., **18**, 112 (1966).
3) H. Iwasaki: Kagaku to Sōsa, **8**, 49 (1955).
4) H. Nariyuki: *Ibid.*, **8**, 36 (1955).
5) B. Ikuta, T. Morikawa: *Ibid.*, **8**, 76 (1955).
6) S. Nishiyama: Eiseikagaku, **4**, 74 (1956).
7) Y. Inano: Nihon Hōigaku Zasshi, **9**, 1 (1955).
8) H. Kajita: Seikagaku, **27**, 456 (1955).
9) S. Nishiyama, H. Kozuka, R. Tanba: Eiseikagaku, **4**, 29 (1956).
10) F. Feigl: "Spot Tests in Organic Analysis," 6th ed., 473 (1960). Amsterdam, Elsevier Publishing Co.

Standard Solution of Ohton——114 mg. of 3-dimethylamino-1,1-di(2′-thienyl)but-1-ene hydrochloride (white crystals, m.p. 169~170°) was dissolved in 100.0 ml. of distilled water. It contains 1.0 mg. Ohton in 1 ml. The solution was diluted further if necessary.

Procedures——One ml. of 1% sodium carbonate solution and 20 ml. of ether were added to 10 ml. of Ohton solution (1.0, 2.0, 5.0, 10.0 and 30.0 μg./ml.) in a separating funnel. The mixture was shaken 200 times/min. and after 15 min. the ether layer was removed. The aqueous layer was extracted 5 times with each 20 ml. of ether. Ten ml. of *N*/500 sulfuric acid was added to the collected ether extracts and the mixture was shaken for 15 min. After ether layer was removed, 4 ml. of the ninhydrin reagent was added to 2.0 ml. of the aqueous solution under ice-cooling and stirring. The mixture allowed to stand at 25~30° for 24 hrs. in a dark place, and the absorbance at 540 mμ was measured with a Beckmann type spectrophotometer. As a blank, a mixture of one part of distilled water and two parts of ninhydrin reagent was used.

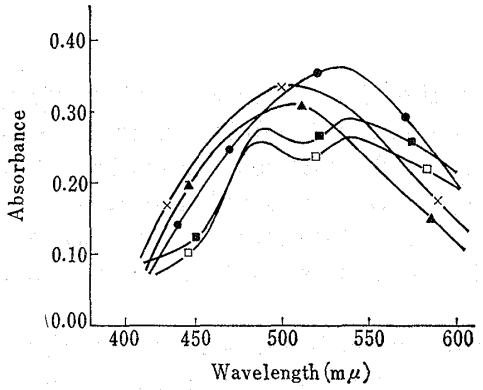

Fig. 1. Influence of Ratio of Ohton Solution and Ninhydrin Reagent on Absorption Maximum

10.0 μg./ml. of Ohton solution was used.
Absorption maximum after 24 hrs.
Temperature 25~30°.
×——× Ohton solution : Ninhydrin reagent=1:1
▲——▲ 1:1.5
●——● 1:2
■——■ 1:3
□——□ 1:4

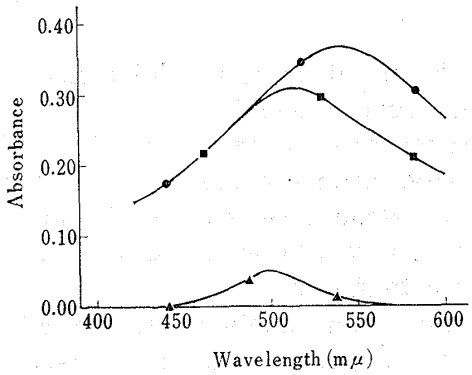

Fig. 2. Influence of Concentration of Sulfuric Acid on Absorption Maximum

10.0 μg./ml. of Ohton solution was used.
The ratio of mixing, Ohton solution : Ninhydrin reagent=1:2.
Absorption maximum after 24 hrs.
Temperature 25~30°.
●——● Concentrated Sulfuric acid (95%)
■——■ 70% Sulfuric acid
▲——▲ 50% Sulfuric acid

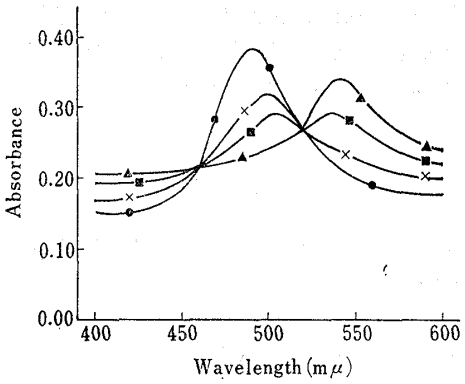

Fig. 3. Time Course of Absorption of Ohton

10.0 μg./ml. of Ohton solution was used.
The ratio of mixing, Ohton solution : Ninhydrin reagent=1:2.
Temperature 25~30°.
●——● 1 hr.
×——× 4 hr.
■——■ 8 hr.
▲——▲ 12~30 hr.

Specificity——As described in the previous paper,[1] Ohton showed the specific absorption (λ_{max}=540 mμ) which was quite different from those produced by other thiophen compounds.

Optimum Condition of Reaction——A) Ratio of mixing: To one part of Ohton solution (10 μg./ml.) were added 1.0, 1.5, 2.0, 3.0 and 4.0 parts of ninhydrin reagent. An absorption maximum after 24 hrs. appeared at 510 mμ and it shifted to 490 mμ after 30 hrs. in the ratio of 1:1, and the similar tendency was also seen in the ratio of 1:1.5. In the ratio of 1:2, an absorption maximum after 24 hrs. appeared at 540 mμ and it did not change within 30 hrs. In the cases of 1:3 and 1:4, the absorption maxima were not distinct over the range of 490 to 550 mμ. The ratio of 1:2 seemed the most suitable condition of mixing, at which the most intense absorption was observed (Fig. 1).

B) Concentration of sulfuric acid: To one part of Ohton solution containing 10 μg./ml. were added 2 parts of various concentrations of sulfuric acid (containing ninhydrin in 0.01%). An absorption maximum after 24 hrs. appeared at 500, 510 and 540 mμ with 50%, 70% and 95% sulfuric acid solution, respectively and the absorptivity was proportional to the concentration of sulfuric acid. Therefore, 95% was an optimum concentration for sulfuric acid (Fig. 2).

C) Optimum concentration of ninhydrin in the reagent: In order to confirm the optimum concentration of ninhydrin for the color reaction, ninhydrin reagents of 0.001, 0.005, 0.01, 0.02 or 0.05% in conc. sulfuric acid were mixed with Ohton solution (10 μg./ml.) in the ratio of 1:2. When the concentration of ninhydrin was 0.001% and 0.005%, absorption peak after 24 hrs. appeared at 490 mμ and 520 mμ, respectively. When the concentration of ninhydrin was more than 0.01%, absorption peak was always seen at 540 mμ and significant increase was not observed in the absorbance. Therefore, 0.01% of ninhydrin was the optimum concentration.

D) Optimum time for optical measurement: Since the absorptivity of the reaction product changes with time, the optimum time for measurement was investigated. In the mixing ratio 1:2, absorption maximum was observed at 490 mμ at first, but the intensity decreased gradually with time and a new absorption maximum appeared at 540 mμ concomitantly with decrease of the former, and the latter reached a maximum after 12 hrs. (Fig. 3). The absorbance of Ohton (10 μg./ml.) was measured at every one hour for 48 hrs. and it was found that it needed 12 hrs. to get to the maximum absorption and began to decrease after 30 hrs. Therefore, it may be concluded that measurement should be carried out at the 24 hrs. after mixing.

E) Temperature: The colored solution (ratio of mixing of 1:2), was kept below 20°, 25~30°, 31~35° and above 40°, and the changes of absorbances were followed up.

1) Below 20°, the color did not get to the maximum absorption after 24 hrs.

2) At 25~30° or 31~35°, the color reached to the maximum of absorption in 12 hrs. and it did not change even after 30 hrs.

3) Above 40°, the color reached the maximum after 4 hrs., but the intensity of absorbance was very low.

So, it is preferable to allow the solution to stand at 25~30°.

Calibration Curve——The absorbance at 540 mμ obtained under the optimum conditions described above obeyed to Beer's Law over the range of 1 to 30 μg./ml. of Ohton (Fig. 4).

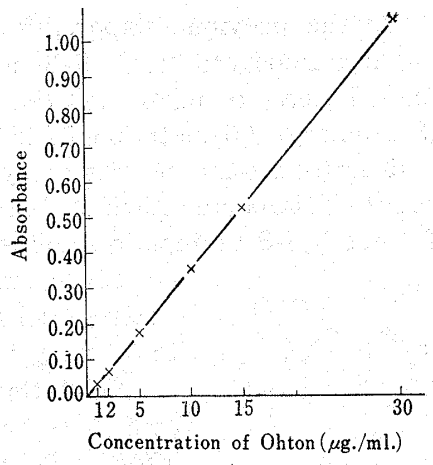

Fig. 4. Calibration Curve

Table I. Recovery of 3-Dimethylamino-1,1-di(2′-thienyl)but-1-ene Hydrochloride from Human Urine

Concentration μg./ml.	No. of Sample	Found μg./ml.	Found %
1.0	7	0.99±0.05	99.0±4.5
2.0	7	1.90±0.07	95.0±3.7
5.0	7	4.81±0.21	96.1±4.2
10.0	7	9.74±0.33	97.4±3.3
30.0	7	28.7 ±0.99	95.5±3.3
		Average	96.6±3.8

Recovery——One ml. of 10% sodium carbonate solution was added to 10 ml. of human urine which contained 1.0, 2.0, 5.0, 10.0 or 30.0 μg./ml. of the drug. Each alkaline urine sample was extracted with ether as described above. As a blank, urine which did not contain Ohton was used. The recovery was 96.6±3.8%, over the range of 1.0~30.0 μg./ml.