Notes

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Colorimetric Determination and Detection of Tertiary Amines with *cis*-Aconitic Anhydride[1]

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cis-Aconitic anhydride reacts with aliphatic *tert*-amines in alcoholic solution in the presence of acetic anhydride to produce a yellow color, which can be applied to detection and determination of aliphatic *tert*-amines and alkaloids.

Limits of identification are about 1—3 μg/0.5 ml, while the calibration curves for the determination show linear correlations in the range of concentration 1—10 μg/ml (3-dimethylamino-1-propanol), 1.7—17 μg/ml (2-dimethylaminoethanol), 3—30 μg/ml (nicotine), 5—50 μg/ml (homatropine and cinchonine) or 15—90 μg/ml (reserpine and aconitine).

Methods for detection of tertiary amines by the reaction with *cis*-aconitic anhydride to give a red coloration have been reported by Okuma[3] and Groth, *et al.*[4] Their reports showed the validity of the methods with solid and liquid sample of amines, but no example with solutions was given. These methods were applied only to the qualitative detection of a few amines.

Several colorimetric determinations of tertiary amines with some dyes have been reported but these are not specific for *tert*-amine.[5,6] Schenk, *et al.*[7] have stated a method for the determination of aromatic *tert*-amines using tetracyanoethylene, without referring to aliphatic *tert*-amines.

We found that when aliphatic *tert*-amines in alcoholic solution react with *cis*-aconitic anhydride and acetic anhydride, the yellow color and yellowish green fluorescence appear. This color reaction is found to be widely usable for detection and determination of aliphatic *tert*-amines and alkaloids.

Experimental

Reagents——*cis*-Aconitic Anhydride: The reagent (Sigma Chemical Company) is dissolved in absolute ethanol to give a 0.01 M solution. The solution must to be prepared once a week.

Acetic Anhydride: Wako Pure Chemical Industries, special grade.

Sample Amines: Commercially available sample amines purified by distillation are dissolved in absolute ethanol at a concentration of 10 to 100 μg/ml for detection and 1 to 100 μg/ml for determination.

Apparatus——Absorption spectrum and optical density measurements were obtained with a Hitachi model 124 recording spectrophotometer and a Hitachi model 139 spectrophotometer.

Fluorescence spectrum was measured with a Hitachi model 204 spectrofluorometer.

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1) This work was presented at the 19th Annual Congress of Japan Society for Analytical Chemistry at Nagoya, Oct. 1970.
2) Location: *a) Mizuho-cho, Mizuho-ku, Nagoya; b) Yoshidashimoadachi-cho, Sakyo-ku, Kyoto.*
3) S. Okuma, *Yakugaku Zasshi*, **75**, 1124 (1955).
4) A.B. Groth and M.E. Dahlen, *Acta Chem. Scand.*, **21**, 291 (1967).
5) R.M. Silverstein, *Anal. Chem.*, **35**, 154 (1963).
6) H.M.N.H. Irving and J.J. Markham, *Anal. Chim. Acta*, **39**, 7 (1967).
7) G.H. Schenk, P. Warner, and W. Bazzelle, *Anal. Chem.*, **38**, 907 (1966).

Procedure 1——Detection: To a mixture of 0.1 ml of acetic anhydride and 0.2 ml of *tert*-amine solution in a micro test tube, 0.2 ml of *cis*-aconitic anhydride solution is added. The mixture is warmed in a water bath at 50° for 5 min. Yellow appears when *tert*-amines are present. It is desirable to compare the color with the reagent blank.

Procedure 2——Determination: Three ml of sample amine solution is added to a mixture of 0.2 ml of acetic anhydride and 0.5 ml of *cis*-aconitic anhydride solution in a 20 ml ground stoppered flask. After heating the mixture for 15 min in a water bath at 50° and allowing to stand for from 20 to 40 min below 15°, the absorbance is measured at 405 nm.

Results and Discussion

The best conditions for the determination at each stage of the procedure were investigated.

Time and Reagent

Fig. 1 shows the effect of the amount of acetic anhydride on the absorbance. As shown in Fig. 1, the best condition was 0.2 ml of acetic anhydride. The absorbance at 405 nm was not affected by the amount of *cis*-aconitic anhydride solution within the range from 0.4 ml to 0.8 ml.

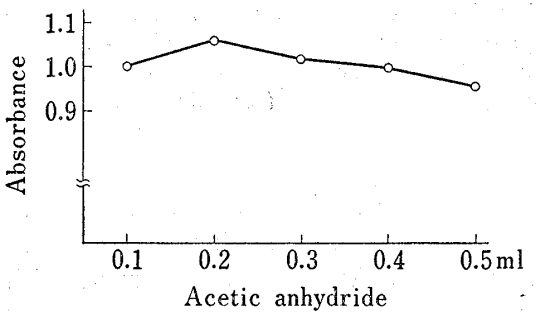

Fig. 1. Effect of the Amount of Acetic Anhydride on the Absorbance
(3-dimethylamino-1-propanol taken: 30 μg)

The effect of heating time on the absorbance is shown in Fig. 2, which indicates that the absorbance becomes constant between 14 and 16 min of heating time. Thus, 15 min heating for the quantitative procedure and 5 min heating for detection purposes are found to be sufficient.

Standing Time

With a reaction solution containing nicotine, the absorbance increased gradually for 35 min of standing time at room temperature after heating (Fig. 3). Thus, 40 min of standing time was taken for nicotine.

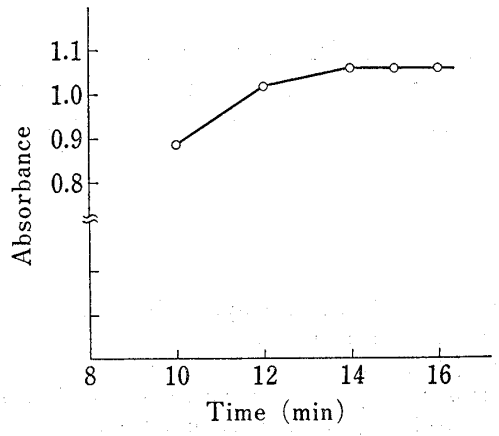

Fig. 2. Effect of Heating Time on the Absorbance
(3-dimethylamino-1-propanol taken: 30 μg)

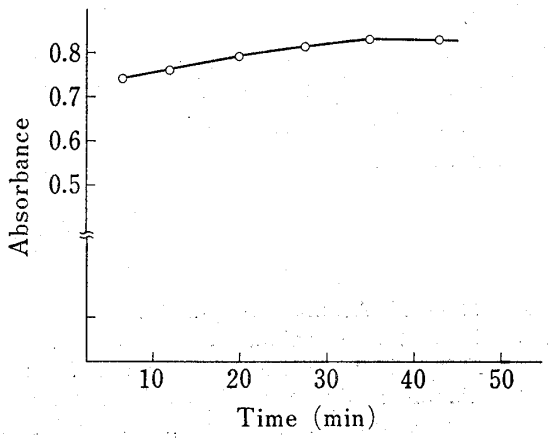

Fig. 3. Effect of Standing Time on the Absorbance
(nicotine taken: 90 μg)

Solvent

Methanol, ethanol, *n*-propanol and *n*-butanol are usable as solvent. Among them, ethanol is the most preferable one for color development. Nonpolar solvents, such as benzene, chloroform and carbon tetrachloride do not give sufficient coloration.

Absorption Curve

Fig. 4 shows the absorption spectrum of the reaction mixture of 3-dimethylamino-1-propanol and *cis*-aconitic anhydride. The maximum optical density was found at 405 nm. It emits also a yellowish green fluorescence as shown in Fig. 5. Excitation at 365 nm gives maximum emission at 492 nm. Since the fluorescence is somewhat unstable at room temperature, it is not applicable to the quantitative determination of amines.

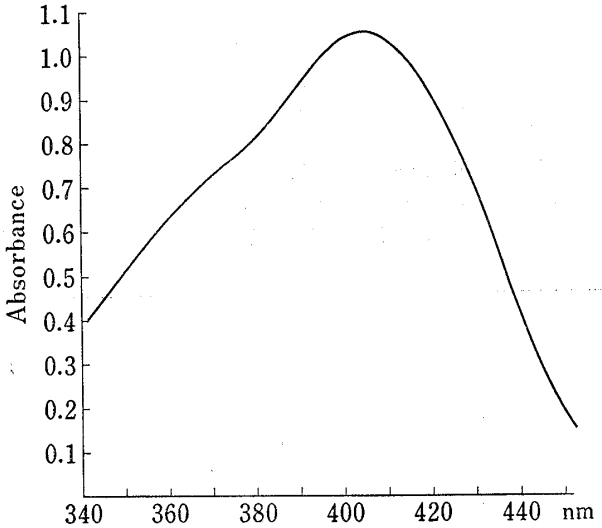

Fig. 4. Absorption Spectrum of the Reaction Product

(3-dimethylamino-1-propanol taken: 30 μg)

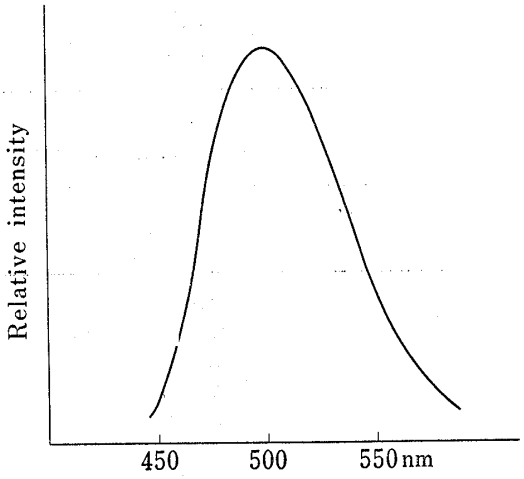

Fig. 5. Fluorescence Emission Spectrum of the Reaction Product

(2-dimethylaminoethanol taken: 51 μg excitation wavelength: 365 nm)

Determination

The calibration curves show good linear correlations in the range from 1 μg/ml to 17 μg/ml for aliphatic *tert*-amines (*e.g.* 3-dimethylamino-1-propanol: 1—10 μg/ml, 2-dimethylaminoethanol: 1.7—17 μg/ml) and 3 μg/ml to 90 μg/ml for alkaloids (*e.g.* nicotine: 3—30 μg/ml, homatropine and cinchonine: 5—50 μg/ml, reserpine and aconitine: 15—90 μg/ml).

In five runs, the coefficients of variation were less than 2.1% (3-dimethylamino-1-propanol and 2-dimethylaminoethanol), 2.4% (nicotine and homatropine) and 3.0% (reserpine and aconitine).

Detection

Tertiary amines are detectable as shown in Table I. It is found that limits of identification are 1—2 μg/0.5 ml for the aliphatic *tert*-amines and 1—3 μg/0.5 ml for the alkaloids.

This test was applied to some drugs containing *tert*-amines. When the drugs contain *tert*-amine salts such as hydrochloride or tartarate, the following pretreatment is necessary.

To 1 ml of an aqueous solution of the test sample containing about 10 mg of *tert*-amine salt, 2 ml of 10% sodium hydroxide solution is added and the amine is extracted from the solution twice with each 20 ml portion of ether. After the extract is washed three times with each 20 ml of distilled water, the solution is evaporated to dryness under reduced pressure. The residue dissolved in ethanol is submitted to the detection procedure.

Limits of identification of these drugs are shown in Table II.

Results of other amine compounds except aliphatic *tert*-amines are shown in Table III.

Both aromatic *tert*-amines and aliphatic *sec*-amines give the same color as aliphatic *tert*-amines. But as shown in Table III, these compounds showed poor sensitivity, 30—500 μg/0.5 ml. All the aromatic *sec*-amines examined (*i.e.* N-ethylaniline, N-butylaniline and diphenylamine) show no coloration.

Table I. Limits of Identification of Aliphatic Tertiary Amines

Compounds	Limit of identification (μg/0.5 ml)
3-Dimethylamino-1-propanol	1
2-Dimethylaminoethanol	2
N,N-Dimethylcyclohexylamine	2
Nicotine	1
Homatropine	3
Cinchonine	3
Aminopyrine	3

Table II. Limits of Identification of Medical Supplies

Compounds	Limit of identification (μg/0.5 ml)
Chlorpromazine	9
Levomepromazine	10
Perphenazine	10
Imipramine	8
Amitriptyline	8
Hydroxyzine	9
Diphenhydramine	7
Triplennamine	8
Chlorpheniramine	8
Diphenylpyraline	9
Promethazine	9

Table III. Limits of Identification of Aromatic Amines and Aliphatic *sec*-Amines

Compounds	Limit of identification (μg/0.5 ml)
N,N-Dimethyl-*m*-toluidine	30
N,N-Dimethyl-*p*-toluidine	110
N,N-Dimethylaniline	100
Pyridine	470
Piperidine	100
Diethanolamine	500

The present method gives no measurable color for aromatic and aliphatic *prim*-amines, for example, *p*-toluidine, *m*-toluidine, *n*-butylamine and *iso*-propylamine. Aminopyrine which contains a tertiary amino group gives the distinctive coloration but melubrine and aminoantipyrine give no color.