

A Sensitive Test for Tertiary Amines Using α,γ -Anhydroaconitic Acid

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A sensitive reagent, derived from *trans*-aconitic acid and acetic anhydride has been studied for use in a colour test for tertiary amines. The reactive compound in the reagent has been identified by infrared spectroscopy as acetic α,γ -anhydroaconitic anhydride and confirmed by other experiments. Modified syntheses of α,γ -anhydroaconitic acid and β,γ -anhydroaconitic acid are reported. Preparation of the reagent and a procedure for its use are described. The limits of identification for tertiary amines are given.

This study was undertaken with the intention of finding a sensitive and simple method of detecting tertiary amines. A comparison of tests for tertiary amines using different aliphatic polycarboxylic acids¹ revealed that one using aconitic acid was the most favourable with regard to sensitivity. Furthermore, this reaction takes place at room temperatures, whether it is in bulk liquid solution or on absorbing materials such as paper, cellulose-powder or silica gel. Thus, the reagent is particularly useful as a spray reagent for chromatograms.

The reagent was made by mixing *trans*-aconitic acid and acetic anhydride in the ratio 2 to 3. After standing 1 - 3 days, depending on the temperature, the thick suspension became a light yellow-brown (sometimes greenish) homogeneous liquid. The liquid was diluted with acetic anhydride to a concentration corresponding to 0.5 % aconitic acid and in 12 h the sensitivity of the mixture achieved its maximum. Apparently the aconitic acid undergoes structural changes during standing. A syrup, (A), considered to be the active compound, was isolated by freeze-drying and this could be redissolved in acetic anhydride to give the original sensitive reagent.

β,γ -Anhydroaconitic acid was prepared using the method of Thorpe,² by warming *trans*-aconitic acid with acetic anhydride in chloroform. The β,γ -anhydride was recrystallized from benzene as needles or flakes with half a mole of benzene of crystallization. When dissolved in water the crystals gave *cis*-aconitic acid.^{3,4} The reaction product also contained α,γ -anhydroaconitic acid. This method has been modified and used for the preparation of α,γ -

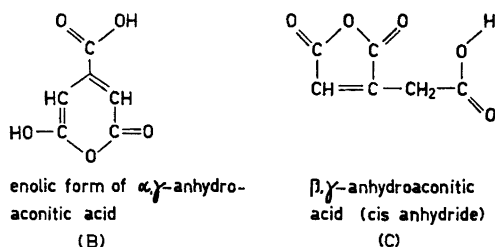


Fig. 1.

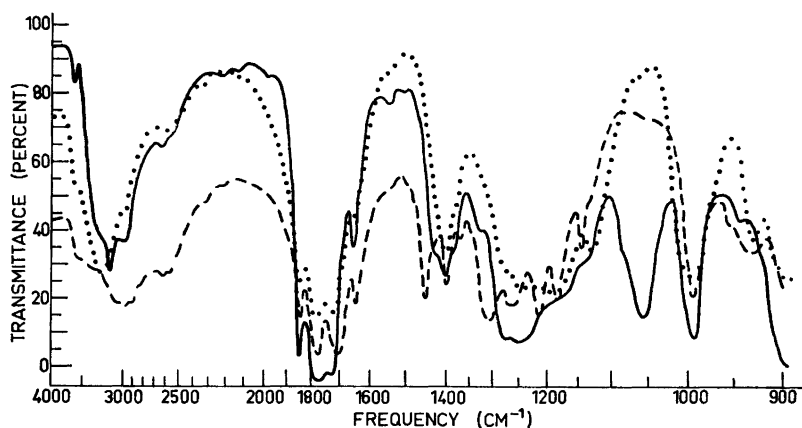


Fig. 2. Infrared spectra of, — the active compound in the reagent (film on NaCl prism), \cdots α,γ -anhydroaconitic acid (KBr phase), - - - β,γ -anhydroaconitic acid (KBr phase).

anhydroaconitic acid, which was recrystallized from ethyl acetate.⁵ At 140° the α,γ -anhydride rearranges to the β,γ -anhydride,² and a modified method for the preparation of β,γ -anhydroaconitic acid was worked out on the basis of this information.

Infrared spectroscopy studies of A, synthesised anhydroaconitic acids (B,C), and aconitic acids, together with a literature survey of anhydrides and carboxylic acids gave the following information.

Of the three infrared spectra in Fig. 2, the spectra of A and B bear the closest resemblance. A and B give absorption bands at 1665 cm^{-1} , typical of carboxylic acids with internal hydrogen bonding,⁶ whereas C gives a stronger absorption at 1645 cm^{-1} . C gives an absorption at 1465 cm^{-1} (CH_3 - and $-\text{CH}_2$ -⁶ deformation vibrations) but neither A nor B, which therefore exist in their enolic forms. The methyl group of acetic anhydride⁶ gives absorptions at 1430 cm^{-1} and 1375 cm^{-1} and shoulders in these areas in the spectra of A indicate a partial acetylation of the carboxyl group in α,γ -anhydroaconitic acid. This view is strengthened by the presence of the absorption at 1060 cm^{-1} , which is typical for open chain anhydrides.⁶

Bearing in mind the results of the infrared investigation a series of reagents prepared from aconitic acids and their anhydrides were compared with regard to sensitivity towards tertiary amines. 0.5 % α,γ -anhydroaconitic acid reagent was found to be the most sensitive and 0.1 μg of 4-benzyl-pyridine could be detected on paper. It was also found that at room temperature this reagent reached maximum sensitivity 10–15 min after mixing, thus indicating the time for acetylation of the carboxyl group in the anhydro acid.

As to the mechanism of the reaction resulting in the colouring compound, only preliminary investigations have been made, similar to those with the malonic acid reagent.¹ The reaction seems to commence with a base catalyzed condensation between one molecule of anhydroaconitic acid and one of acetic anhydride. However, the coloured compound is not aromatic in this case.

The colour obtained with this reagent was a clearer red than with any other reagent. The coloured solution was chromatographed on a thin-layer plate, coated with silica gel and developed in acetone/chloroform (30/70) and gave one red component with an R_F 0.25. This indicated that α,γ -anhydroaconitic acid reagent ought to be suitable for a quantitative colourimetric determination of small amounts of tertiary amines. Investigation of a suitable method is in progress.

EXPERIMENTAL

β,γ -Anhydroaconitic acid. A suspension of 1 part *trans*-aconitic acid in 7.5 parts of xylene (b.p. 139°) was heated to reflux and the water formed in the reaction removed as an azeotrope with xylene. The reaction time was about 2 h. The hot xylene solution of anhydride was filtered, the filtrate evaporated to dryness and the residue recrystallized from benzene to give an 85 % yield of the acid. M.p. 76–78°. (Lit.³ 78–78.5°).

α,γ -Anhydroaconitic acid. 17.4 g (0.1 mole) *trans*-aconitic acid was suspended in 50 ml methylene chloride and 11.7 g (0.115 mole) acetic anhydride added with stirring, and the temperature was allowed to rise slightly above room-temperature, before cooling, to make sure the reaction started. The reaction flask was kept in a refrigerator (0–5°) for 3 days, after which time white fluffy crystals were collected. Yield 11.5 g (74 %). M.p. 132–134°. (Lit.⁵ 135°). On one occasion compact, light yellow crystals of apparently the same quality were collected.

Reagent. A solution of 0.5 % α,γ -anhydroaconitic acid in acetic anhydride is ready for use after standing for 10 min. Usually the reagent can be stored in a polythene bottle for at least one month without loss of sensitivity.

Procedure. A drop of an acetic anhydride solution of the tertiary amine was mixed with one or two drops of the reagent solution and an intense violet colour was formed. Using the reagent as a spray on paper, a red or blue-red colour was obtained where tertiary amines were present.

Limits of detection. The lower limits on paper and silica gel are 5×10^{-4} $\mu\text{mole}/\text{cm}^2$ and 1×10^{-1} $\mu\text{mole}/\text{cm}^2$, respectively. Reagents made from β,γ -anhydroaconitic acid or *trans*-aconitic acid gave limits on paper of 1×10^{-2} $\mu\text{mole}/\text{cm}^2$ and 5×10^{-3} $\mu\text{mole}/\text{cm}^2$, respectively.

Remarks. All glass-ware used for this test had to be absolutely free from alkali as the colour reaction is base catalyzed.

trans-Aconitic acid (Fluka AG) of technical quality (90%) was used without further purification. Acetic anhydride of analytical grade was used in all syntheses and experiments.

When using the test for thiazoles on paper it was found that practically no colour appeared. In solution, however, the final colour does not differ much from that of other tertiary amines. This is probably due to a comparatively long reaction time for thiazoles. On paper, the acetic anhydride vapourizes or reacts with cellulose fibres so that the ideal

reaction medium does not exist for the time needed for reaction. This difference in obtaining colour in solution and on paper may eventually be used as a test for thiazoles.

When tertiary amines exist in the gaseous state it is possible for them to be absorbed onto papers impregnated with isostearic acid⁷ (Emery Industries Inc., U.S.A.). Papers dipped in a solution of 30 g acid in 100 ml solvent gave optimum results when the air containing small amounts of amine, was drawn through the paper. Isostearic acid was chosen because it is liquid at room temperature, resistant to oxidation and neutral to the reagent. Papers impregnated with isostearic acid are stable for a considerable time.

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