Colorimetric Determination of 1-(4′-Nitrophenyl)-2-aminopropane-1,3-diol with 2,4,6-Trinitrobenzenesulfonic Acid in the Presence of Chloramphenicol

P. G. PIETTA ˣ, D. AGNELLINI, and M. PACE

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Abstract □ A colorimetric method based on the interaction between the chloramphenicol degradation product 1-(4′-nitrophenyl)-2-aminopropane-1,3-diol and the 2,4,6-trinitrobenzenesulfonic acid reagent was developed. Analytical solutions were reacted with the reagent at pH 9.1 for 20 min at room temperature, and the resulting color was measured at 340 nm. A linear relationship between absorbance and concentration occurred within the 5–25-μg/ml range under the conditions studied. Replicate analyses were in good agreement. An average recovery of 99.4 ± 0.4% was obtained for the synthetic mixtures.

Keyphrases □ Chloramphenicol degradation products—1-(4′-nitrophenyl)-2-aminopropane-1,3-diol, colorimetric analysis, in commercial chloramphenicol solutions □ 1-(4′-Nitrophenyl)-2-aminopropane-1,3-diol—colorimetric analysis, in commercial chloramphenicol solutions □ Colorimetry—analysis, chloramphenicol degradation products

Chloramphenicol solutions undergo hydrolysis (1–3), and one major degradation product is 1-(4′-nitrophenyl)-2-aminopropane-1,3-diol (I) (Scheme I). Since I has no antibiotic activity and is regarded as a contaminant in commercial drug preparations, its determination is important.

BACKGROUND

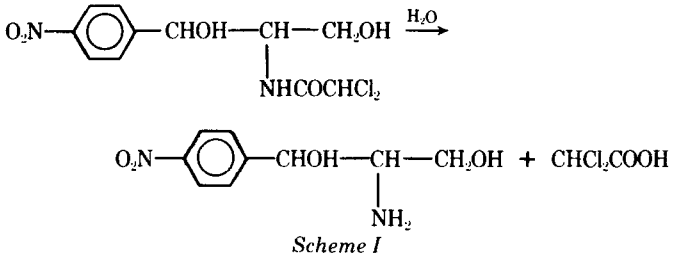
The interaction of I with 2,4,6-trinitrobenzenesulfonic acid reagent to give highly colored solutions led to the development of a new colorimetric method for I determination. This reagent previously was shown to be suitable for the colorimetric analysis of primary amino groups, amino acids, peptides, and proteins (4–7).

Existing analytical procedures for I include visible and UV spectrophotometry, polarography, and high-pressure liquid chromatography (HPLC) (6–9).

The standard colorimetric methods are based on the interaction of I with 1,2-naphthoquinone-4-sulfonate (10) and 1,4-naphthoquinone (11), but other chloramphenicol solution components (*e.g.*, chloramphenicol and acetate ions) can interfere with the reagents. Moreover, these procedures are not simple and involve the preparation of multireagent solutions.

This paper presents a new colorimetric method for the determination of microgram quantities of I in chloramphenicol solutions. At room temperature and pH 9.1, the procedure is not subject to chloramphenicol interference. This method has been applied successfully to the analysis of I in chloramphenicol dosage forms.

The synthesis and physicochemical properties of 2,4,6-trinitrophenyl-1-(4′-nitrophenyl)-2-aminopropane-1,3-diol (II) also are reported.



Scheme I

EXPERIMENTAL

Apparatus—Spectra and absorbance measurements were obtained by spectrophotometers[1–3] using matched quartz cells with a 1-cm optical path.

Reagents and Chemicals—2,4,6-Trinitrobenzenesulfonic acid was the chromogenic reagent. It was recrystallized before use according to a literature method (7). The 2,4,6-trinitrobenzenesulfonic acid reagent solution was prepared immediately prior to use by weighing accurately 100–500 mg of trinitrobenzenesulfonic acid and adding 0.1 ml of deionized water for each 100 mg of reagent.

Compound I was prepared quantitatively by acidic hydrolysis of chloramphenicol (12). The pKb value of the amino group was determined by acid–base titration to be 8.12.

Compound II was prepared by dissolving I (1 mmole), 2,4,6-trinitrobenzenesulfonic acid (200 mg), and sodium bicarbonate (200 mg) in water (10 ml); the solution became orange almost immediately, and the intensity increased over time. After 1 hr at room temperature, the solution was acidified to pH 2 and extracted with ethyl acetate. After washing with 1% $NaHCO_3$ and water, the solution was dried over sodium sulfate, the ethyl acetate was evaporated under vacuum, and the residue was crystallized from anhydrous ether–petroleum ether (275 mg, 65% yield, mp 85–88°).

TLC showed the product to be homogeneous in benzene–ethanol (9:1) and in *n*-butanol–acetic acid–water (4:1:1); IR (in mineral oil): 3360, 1620, 1590, 1570, 1515, and 1345 cm^{-1}; NMR: 3.6–3.8 (m, 1), 4.05 (d, 2), 7.45–7.7 (d, 2), 8.1–8.3 (d, 2), and 8.9–9.1 (d, 2). UV results are given in Fig. 1; log ε: 4.19 at 340 nm in 95% ethanol.

Anal.—Calc. for $C_{15}H_{13}N_5O_{10}$ (mol. wt. 423.20): C, 42.56; H, 3.09; N, 16.54. Found: C, 42.13; H, 3.12; N, 16.48.

The product was soluble in ethanol, methanol, ethyl acetate, and ether and was insoluble in carbon tetrachloride and chloroform.

Procedure—A sample of I was added to 2 ml of 0.1 *M* $Na_2B_4O_7$, and the volume was made up to 5.0 ml. Then 40 μl of trinitrobenzenesulfonic acid solution was added, and the solution was mixed rapidly.

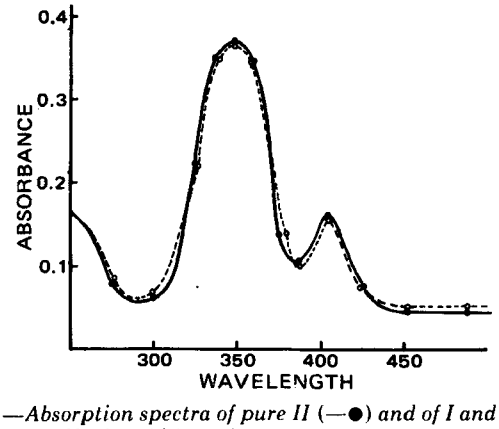


Figure 1—*Absorption spectra of pure II (—●) and of I and 2,4,6-trinitrobenzenesulfonic acid (– – ○).*

[1] Beckman Acculab 3.
[2] Beckman Acta III.
[3] Perkin-Elmer R 12, 60 MHz.

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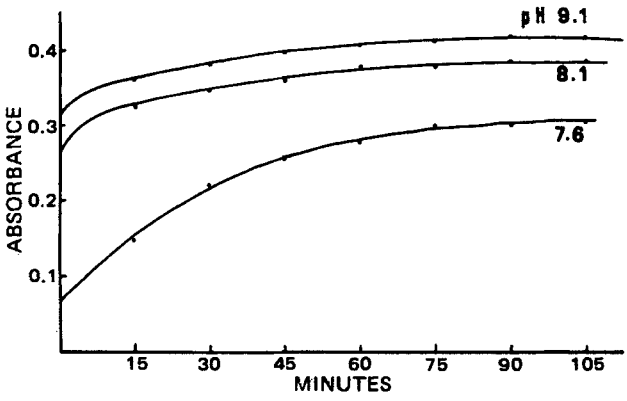

Figure 2—*Effect of pH on trinitrophenylation of I with 2,4,6-trinitrobenzenesulfonic acid and of coupling time on the final solution absorbance.*

After 20 min, the reaction was stopped by adding 5.0 ml of phosphate buffer (pH 4.5), and the absorbance at 340 nm was determined against a blank.

RESULTS AND DISCUSSION

When a I solution in 0.1 *M* $Na_2B_4O_7$ was added with 2,4,6-trinitrobenzenesulfonic acid, the solution became orange; the intensity increased during the initial 90 min at room temperature. A blank solution containing no amino compounds was pale yellow during this time. Upon acidification, the sample changed to a stable yellow while the blank became almost colorless. The UV–visible spectra of the acidified sample showed an absorption maximum at 340 nm (Fig. 1). No differences were found with pure synthetic II. This result indicated that the color was due exclusively to trinitrophenylation of the amino group.

The interaction between 2,4,6-trinitrobenzenesulfonic acid and I was affected by the pH and the reagent quantity. The higher the pH, the greater was the I reaction velocity with the reagent. However, in a strong alkaline solution, some decomposition of 2,4,6-trinitrobenzenesulfonic acid and II occurred. The optimum pH range for the trinitrophenylation was 8.1–9.1 at room temperature and fitted the amino group titration curve (pKb = 8.12, Fig. 2).

The reaction course was followed by the color development at 340 nm. Absorbance values increased through 120 min at room temperature and then stabilized. However, after 20 min at room temperature, the reaction was suitable for reproducible readings. Because the decomposition products absorbed less at acidic than at alkaline pH, the trinitrophenylation was stopped after 20 min by acidification with pH 4.5 phosphate buffer. The colors were stable for at least 2 hr, and the reference blank was unchanged under these conditions.

The color intensity also was affected by the 2,4,6-trinitrobenzenesulfonic acid concentration. Higher absorbances were obtained using up to a threefold reagent excess. Reagent quantities beyond this excess did not effectively increase the absorbance readings for I.

Standard curves, prepared by plotting absorbance *versus* I concentrations, showed a linear relationship up to 25 μg of I/ml.

The analysis is a semimicro procedure, and the sensitivity is in the range of 5 μg of I/ml. Reagent preparation is simple and rapid since 2,4,6-trinitrobenzenesulfonic acid is available commercially and is purified easily. The procedure requires ~20 min. The method is specific for I in the presence of chloramphenicol and normally employs components of chloramphenicol solutions (*e.g.*, *p*-oxybenzoates, sodium acetate, sodium borate, sodium chloride, and sodium phosphate).

A placebo was prepared containing chloramphenicol and some of the previously mentioned components; several weights of the components, each approximating the sample weight of commercial solutions, were spiked with a known amount of I and assayed. The average recovery was 99.4 ± 0.4%. Multiple analyses were performed on different commercial samples. Replicate analyses were in good agreement, and the samples assayed up to 35% of I.

Compound I in commercial chloramphenicol preparations is analyzed satisfactorily and rapidly by the described method.

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