Colorimetric Method for Determination of 7-Aminocephalosporanic Acid (7-ACA) and Related Compounds

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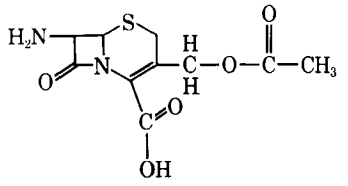
A reproducible colorimetric method is described for the determination of 7-aminocephalosporanic acid (7-ACA) and related compounds.

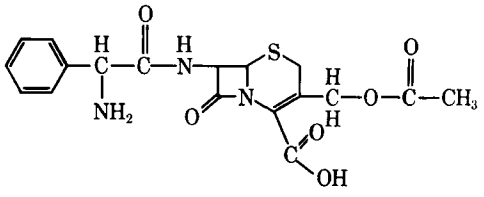
A NUMBER OF ACYL derivatives of 7-aminocephalosporanic acid (7-ACA) (I) and their antibacterial activities have been described (1–4). The D-phenylglycine derivative of 7-ACA, known generically as cephaloglycin[1] (II), has a broad spectrum of antibacterial activity. To determine the extent of acylation in the production of cephaloglycin, an assay was needed for small concentrations of 7-ACA. Methodology for the assay of 6-aminopenicillanic acid (6-APA) (III) in penicillin derivatives was investigated; however, the methods encountered had two basic drawbacks: (*a*) specificity (separation techniques utilized) and (*b*) sensitivity.

This paper describes a chemical method which permits the direct determination of small amounts of 7-ACA (0.2–1.5%) in cephaloglycin. At lower concentrations of 7-ACA (0.04%), a 25% error may be incurred. Optimum conditions were established for the rapid development of the 7-ACA-ninhydrin chromophore. Essentially no color formation was obtained with either cephaloglycin or phenylglycine under the same conditions.

7-Aminodesacetoxycephalosporanic acid (7-ADCA) (IV) and 6-aminopenicillanic acid (6-APA), both having an α-amino group adjacent to a β-lactam ring in common with 7-ACA, were found to respond in a similar manner. Likewise, 7-ADCA could be determined directly in small amounts (0.4–1.5%) in cephalexin[2] (V). At lower concentrations of 7-ADCA (0.1%), a 25% error may be incurred.



I



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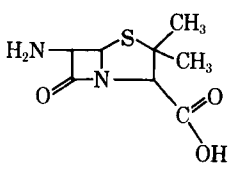

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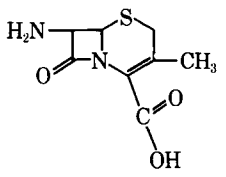

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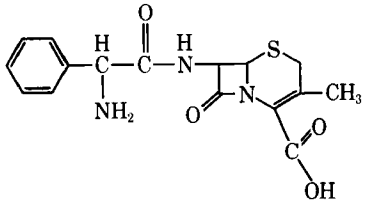

V

EXPERIMENTAL

Apparatus and Reagents—A spectrophotometer (Beckman model DU) with 1-cm. glass cells was used in this laboratory, but any suitable spectrophotometer may be used. (*a*) Buffer solution—2.0% w/v citric acid ($H_3C_6H_5O_7 \cdot H_2O$) in 0.8 *M* sodium hydroxide; (*b*) formic acid—C.P., 98–100%; (*c*) ninhydrin solution—5.0% w/v ninhydrin (certified triketohydrindene hydrate, Fisher Scientific Co.) in methylcellosolve (peroxide-free).

Recommended Procedure—*Method I—Determination of 7-ACA*—Accurately weigh approximately 7 mg. (±0.01 mg.) of sample and 7-ACA reference standard, and quantitatively transfer each to a 100-ml. volumetric flask. Dissolve the contents in 5.0 ml. of formic acid, dilute to volume with distilled H_2O, and mix thoroughly. Prepare a reference blank solution in a 100-ml. volumetric flask by diluting 5.0 ml. of formic acid to volume with distilled H_2O and mixing well. Add by transfer pipet to a test tube in the following order: 2.0 ml. of each prepared solution, 1.0 ml. of buffer solution, and 0.50 ml. of ninhydrin solution. Mix thoroughly, and after a 9-min. waiting period, determine the absorbance of each solution at 407 mμ on a suitable spectrophotometer, using 1.0-cm. glass cells and the reference blank solution in the reference cell.

Calculations:

$$\frac{\text{abs.}_{407}\ \text{sample} \times \text{mg. standard}}{\text{abs.}_{407}\ \text{standard} \times \text{mg. sample}} \times 100 = \%\ \text{7-ACA}$$

Method II—7-ACA in Cephaloglycin—Accurately weigh approximately 100 mg. (±0.1 mg.) of cephaloglycin sample, and quantitatively transfer to a 10-ml. volumetric flask. Dissolve the contents in 0.50 ml. of formic acid, dilute to volume with distilled H_2O, and mix thoroughly. Prepare a reference standard stock solution by dissolving 70 mg. of 7-ACA reference standard in 50 ml. of formic acid

Received June 12, 1968, from the Analytical Development-Physical Department, Eli Lilly and Co., Indianapolis, IN 46206

Accepted for publication August 19, 1968.

The author wishes to express his indebtedness to Dr. E. M. Van Heyningen and Dr. L. D. Hatfield for providing the compounds under investigation, and also to Mr. T. E. Cole for the amino acid analyzer assays provided.

[1] Cephaloglycin is the generic name given to 7-(D-α-aminophenylacetamido) cephalosporanic acid.

[2] Cephalexin is the generic name given to 7-(D-α-aminophenylacetamido) desacetoxy cephalosporanic acid.

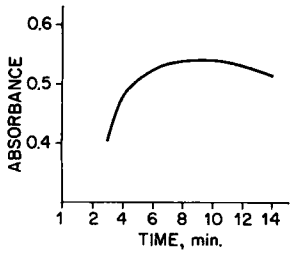

Fig. 1—Rate of color formation of 7-ACA chromophore.

and mixing well. Add 0.50 ml. of this stock solution to a 10-ml. volumetric flask, dilute to volume with distilled H_2O, and mix thoroughly. Prepare a reference blank solution in a 10-ml. volumetric flask by diluting 0.50 ml. of formic acid to volume with distilled H_2O and mixing well. Continue the assay as outlined in Method I, and calculate accordingly.

DISCUSSION

Standard Curve—A plot of the absorbance *versus* concentration was found to be linear and to pass through the origin, obeying Beer's law from 4–40 mcg./ml.

Stability of the Color—The color formation increases uniformly and rapidly, reaching a maximum in 9 min., then decreasing (Fig. 1). The yellow chromophore described exhibits a peak at 407 mμ; a secondary peak is also present at 570 mμ (Fig. 2).

Influence of Reagents—The color intensity was found to increase with a corresponding increase in pH, reaching a maximum at pH 4.5, then decreasing. An increase in pH not only influenced the rate of color development but also shifted the maximum absorbance peak to a higher wavelength (415 mμ). The intensity of the secondary peak (570 mμ) decreased with a pH increase. A pH 3 buffered system was selected, since cephaloglycin was found to be somewhat reactive at a higher pH. Both of the final concentrations of ninhydrin and methylcellosolve were found to influence the color formation. Optimum color formation was obtained when the final concentration of methylcellosolve (containing 50 mg./ml. of ninhydrin) was set at 14.3% v/v. Temperature fluctuations of ±3°, as well as light, did not affect the reaction rate. The effect of the 7-ACA standing in formic acid was found to be insignificant (0–3 min.).

Interferences—Several compounds were assayed by this procedure to determine the extent of color formation, as shown in Table I.

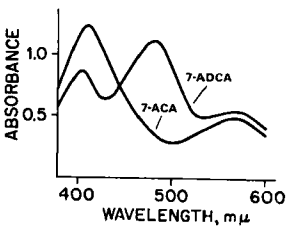

Fig. 2—Cary recording spectrophotometric curves of chromophores.

TABLE I—ABSORPTIVITY (a) AT 407 mμ

Compd.	a
7-ACA	15.3
7-ADCA	11.0
6-APA	5.4
Cephaloglycin	0.0018
Cephalexin	0.0035
Phenylglycine	0.33
NH_4Cl	0.16

Reproducibility—The following standard deviations were obtained on the methods outlined in Methods I and II of the *Procedure:* Method I—$2s = \pm 3.7\%$, $n = 6$; Method II—$2s = \pm 4.2\%$, $n = 6$.

A sample of cephaloglycin was assayed for 7-ACA by the colorimetric method, as well as by the amino acid analyzer. Agreement was obtained as follows: amino acid analyzer—1.35%; colorimetric method —1.42%.

7-ADCA and 6-APA—Essentially the same procedure was found to produce characteristic chromophores with 6-APA and 7-ADCA (Fig. 2); however, the rates of formation differed. In addition, the 7-ADCA color curve exhibited a third peak at 480 mμ which proved to be more suited to the direct determination of 7-ADCA in cephalexin. Maximum color development for the 7-ADCA chromophore was obtained in 18 min. at 407 mμ and in 50 min. at 480 mμ. The 6-APA chromophore reached maximum color development in 45 min. at 407 mμ.

The absorptivity for Compound 7-ADCA was 12.2 at 407 mμ and 11.5 at 480 mμ; for Compound cephalexin $a = 0.0065$ at 407 mμ and $a = 0.0035$ at 480 mμ; for Compound 6-APA $a = 9.55$ at 407 mμ.

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Keyphrases

7-Aminocephalosporanic acid (7-ACA), related compounds—analysis
Cephaloglycin—7-ACA determination
Ninhydrin—color reagent
Colorimetric analysis—spectrophotometer