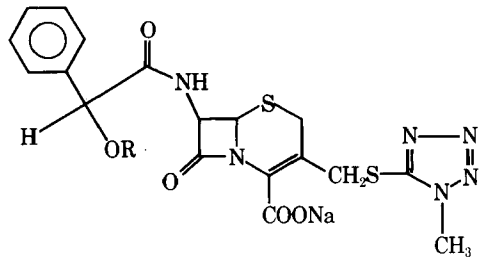
Conversion of Cefamandole Nafate to Cefamandole Sodium

JOSEPH M. INDELICATO ˣ, WILLIAM L. WILHAM, and BENITO J. CERIMELE

Abstract □ The rate of hydrolysis of the formyl moiety of cefamandole nafate was determined as a function of pH, temperature, and concentration of added sodium carbonate or tromethamine. The reaction rate was sensitive to hydroxide ion in the pH 5.5–8.0 range with half-life values of hours to minutes. Hydrolysis was rapid upon the addition of sodium carbonate or tromethamine. Chirality in the 7-D-mandelamido side chain was unaffected by hydrolysis.

Keyphrases □ Cefamandole nafate—hydrolysis of formyl moiety, effect of pH, temperature, and carbonate or tromethamine concentration □ Hydrolysis—cefamandole nafate formyl moiety, effect of pH, temperature, and carbonate or tromethamine concentration □ Antibacterial agents—cefamandole nafate, hydrolysis of formyl moiety, effect of pH, temperature, and carbonate or tromethamine concentration

The antibiotic cefamandole (I*a*), 7-D-mandelamido-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic acid, sodium salt, is active *in vitro* against a wide variety of Gram-positive and Gram-negative bacteria (1, 2). Attempts to prepare cefamandole sodium in crystalline form suitable for commercial use with respect to purity and long-term stability were unsuccessful. Since cefamandole nafate (I*b*), the formyl ester of cefamandole sodium, has the desired pharmaceutical characteristics, it is used in the clinical formulation of the antibiotic. Cefamandole nafate is indistinguishable from cefamandole microbiologically in routine clinical assay procedures (3) and is rapidly converted to cefamandole *in vivo* (4).



I*a*: R = H

I*b*: R = CH(=O)

During the development of the cefamandole nafate dosage form, it was found necessary to add sodium carbonate or tromethamine powder to the vial[1] to prevent turbidity when the drug is reconstituted with intravenous fluids. Turbidity is due to the precipitation of cefamandole nafate and cefamandole free acids when the pH of the solution slowly drops as a result of aqueous ester hydrolysis.

The purpose in this study was to determine the rate of hydrolysis of the formyl moiety as a function of pH, temperature, and the concentration of added sodium carbonate or tromethamine.

EXPERIMENTAL

Materials—D-6-Formylmandelamidopenicillanic acid (II), D- and L-cefamandole nafate[2], and D-formylmandelyl chloride[2] were used as supplied. The pH-stat[3] was calibrated with pH 7.00 buffer[4]. Sodium hydroxide solutions[4] (0.1 or 0.02 *N*) were employed in the constant pH titrations. All other chemicals were reagent grade and were used as received.

Kinetic Methods—The hydrolysis rates were followed by constant pH titration. The formic acid produced in the hydrolysis was titrated automatically with a pH-stat[3] fitted with a combination electrode[5]. Rate determinations were conducted at 25.0, 37.0, 45.0, and 55.0 ± 0.1°. Constant ionic strength (0.102 *N* ± 0.003) was established by prior addition of potassium chloride.

The formyl ester concentrations varied between 0.001 and 0.005 *M*. Carbon dioxide was excluded from the system with an argon blanket. Because methyl formate is volatile, a closed titration vessel (without argon) was used to measure its hydrolysis rate. From the recorded volume of standard sodium hydroxide solution consumed as a function of time, plots of log [ester] *versus* time were constructed. From these plots the pesudo-first-order rate constants were calculated. The fast reactions were followed for two half-lives, while the slow reactions were followed to 25% completion. In all cases, straight-line plots were obtained when one assumed that 1 mole of formic acid was produced for each mole of ester hydrolyzed.

The formation of any cephalosporic acids from the hydrolysis of the cephalosporin β-lactams would interfere in the constant pH titration of the formic acid produced by the ester hydrolysis. Such an interference is insignificant since the rate of base consumption due to β-lactam hydrolysis of cefamandole is ≤1/100 the rate of base consumption due to ester hydrolysis of cefamandole nafate. The rate of β-lactam hydrolysis of cefamandole nafate is assumed to be similar to that for cefamandole, because previous studies showed that side-chain alterations have little effect upon β-lactam reactivity (5).

Ester hydrolysis as the result of the addition of sodium carbonate or amines was also determined by NMR[6] at 25 ± 1°. Cefamandole nafate, 100 mg, and 18.6 mg (0.90 molar equivalent), 12.4 mg (0.60 molar equivalent), or 5.7 mg (0.275 molar equivalent) of sodium carbonate were dissolved in 1 ml of deuterium oxide immediately prior to insertion into the probe. Percent hydrolysis was calculated from the integrated peak intensities. The disappearance of the formyl proton at δ 8.2 and the appearance of the formate anion proton at δ 8.5 were monitored as a function of time. [These changes could also be monitored when water was substituted for deuterium oxide since the water peak (δ 4.8) does not interfere with the measurement.] The disappearance of the α-phenylmethine proton of cefamandole nafate at δ 6.2 and the appearance of the α-phenylmethine proton of cefamandole at δ 5.3 also could be followed, but monitoring this region was inconvenient since the δ 5.3 peak is located between the β-lactam peaks.

Similarly, cefamandole nafate and 1.0 molar equivalent of tromethamine or ethanolamine were dissolved in the appropriate volume of deuterium oxide to obtain an approximately 10% solution of the nafate. In these cases, it was necessary to monitor the disappearance

[1] Vials equal to 1 g of cefamandole (free acid) contain 1.11 g of cefamandole nafate and 0.063 g (0.28 mole equivalent) of sodium carbonate.

[2] Received from Dr. J. M. Greene, Lilly Research Laboratories. Synthesis of these antibiotics will be reported by Dr. Greene at a later date.

[3] Radiometer (TTT2), Copenhagen, Denmark.

[4] Fisher Scientific Co., Fair Lawn, N.J.

[5] A. H. Thomas (4049-L15), Philadelphia, Pa.

[6] A-60 or HA-100 spectrometers, Varian Associates, Palo Alto, Calif.

Table I—Apparent Pseudo-First-Order Rate Constants of Formate Ester Hydrolysis of Cefamandole Nafate

pH	Temperature	Number of Observations	$k \times 10^5$ sec^{-1} Mean	SE
5.5	37°	4	1.65	0.06
5.5	45°	4	3.99	0.32
5.5	55°	4	14.18	0.52
6.0	37°	4	3.54	0.20
6.0	45°	4	9.03	0.92
6.0	55°	4	30.70	3.18
6.5	25°	4	2.11	0.07
6.5	37°	4	8.24	0.25
6.5	45°	4	18.60	1.92
6.5	55°	4	52.25	3.89
7.0	25°	5	4.91	0.63
7.0	37°	4	19.40	0.69
7.0	45°	4	33.85	5.70
7.0	55°	4	133.25	10.37
7.4	25°	5	9.39	1.37
7.4	37°	5	40.40	2.53
7.4	45°	4	66.95	7.97
8.0	25°	8	29.00	1.83
8.0	37°	5	119.82	6.44
8.0	45°	4	174.88	8.16

of the α-phenylmethine proton of cefamandole nafate and the appearance of the α-phenylmethine proton of cefamandole. Peaks from *N*-formyl tromethamine made monitoring of the formyl proton impossible.

Retention of Chirality in 7-Mandelamido Side Chain of Cefamandole—D-Cefamandole nafate (0.513 g, 1 mmole) and sodium carbonate (0.106 g, 1 mmole) were dissolved in 25 ml of water and allowed to react for 30 min. At that time, the solution was layered with ethyl acetate, and the pH of the aqueous layer was adjusted to 2 with 1 *N* HCl. The ethyl acetate layer was collected and dried with anhydrous sodium sulfate, and the solvent was removed *in vacuo*. The 100-MHz NMR spectrum of the resulting foam was obtained in pyridine-d_5.

The pertinent features of the D-cefamandole free acid spectrum were δ 4.08 (d, $J_{H\text{-}H}$ = 5.0 Hz, C-6 proton), 4.46 (s, α-phenylmethine), and 8.41 (d, $J_{H\text{-}H}$ = 9.6 Hz, NH). The pertinent features of L-cefamandole free acid, obtained from L-cefamandole nafate by a procedure identical to the described one, were δ 3.96 (d, $J_{H\text{-}H}$ = 5.0 Hz, C-6 proton), 4.40 (s, α-phenylmethine), and 8.30 (d, $J_{H\text{-}H}$ = 9.6 Hz, NH). Each of these NMR spectra was free of the other isomer (>95% pure, the limit of this instrumental method).

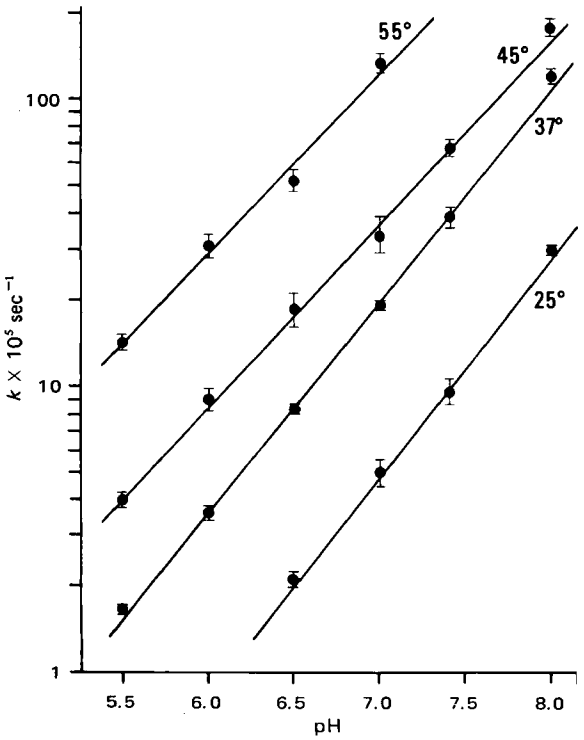


Figure 1—*The pH–rate profile of formate ester hydrolysis of cefamandole nafate. Coefficients of determination are 0.953 (25°), 0.995 (37°), 0.974 (45°), and 0.963 (55°).*

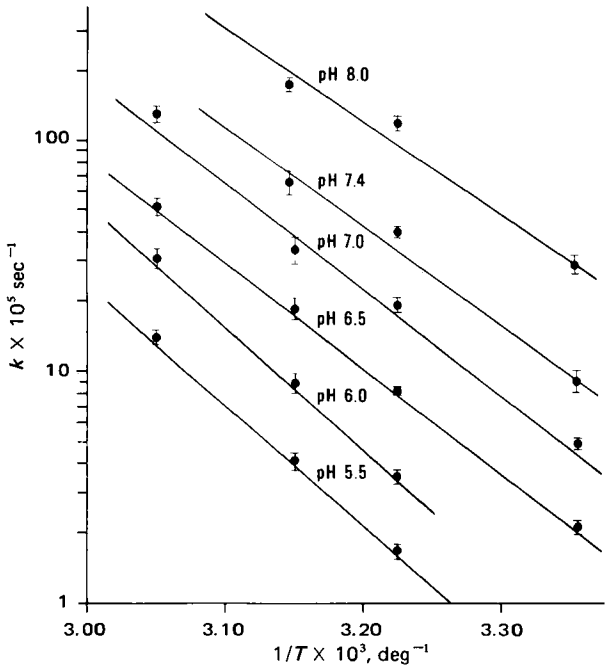


Figure 2—*Arrhenius plots of formate ester hydrolysis of cefamandole nafate. Key (E_a in kcal/mole): pH 5.5, 24.3; pH 6.0, 24.2; pH 6.5, 20.7; pH 7.0, 20.8; pH 7.4, 19.3; and pH 8.0, 18.0.*

***N*-Formyl Tromethamine**—Tromethamine (12.1 g, 0.1 mole) was allowed to react with methyl formate (6 g, 0.1 mole) in 120 ml of water at 25° for 24 hr, and the reaction mixture was lyophilized. The resulting solids were triturated with several 500-ml portions of hot acetone. Crystals were collected from the acetone solutions after cooling. Recrystallization from acetone yielded 4 g of *N*-formyl tromethamine, mp 104°; NMR (D_2O): 74% *trans*-configuration δ 3.27 (s, 6, CH_2OH) and 8.04 [s, 1, HC(=O)], 26% *cis*-configuration δ 3.23 (s, 6, CH_2OH) and 8.15 [s, 1, HC(=O)]; mass spectrum: M^+ 150; IR (mull): 1650 (amide) cm^{-1}.

Anal.—Calc. for $C_5H_{11}NO_4$: C, 40.27; H, 7.43; N, 9.39. Found: C, 40.17; H, 7.21; N, 9.52.

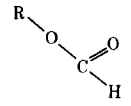
D-Isopropylmandelamide, Formate Ester—D-Formylmandelyl chloride (3.72 g, 20 mmoles), was dissolved in 50 ml of dichloromethane. Sodium bicarbonate, 4 g, was added to this solution, and the resulting suspension was cooled to 0°. Isopropylamine (1.18 g, 20 mmoles) dissolved in 25 ml of dichloromethane, was added dropwise to this suspension. The mixture was allowed to warm to room temperature, and the sodium bicarbonate was filtered after 2 hr. Dichloromethane was removed *in vacuo*, and the resulting solids recrystallized from hexane to yield 2.4 g (55%), mp 90°; NMR ($CDCl_3$): δ 1.18 (d, $J_{H\text{-}H}$ = 7 Hz, 6, CH_3CH), 4.40 [m, J = 7 Hz, 1, $(CH_3)_2CH$], 6.16 (s, 1, α-phenylmethine), 7.42 (m, 5, aromatic), and 8.16 [s, 1, $HC(=O)OR$]; IR (CCl_4): 1729 (ester), 1673, and 3420 (amide) cm^{-1}; mass spectrum: M^+ 221.

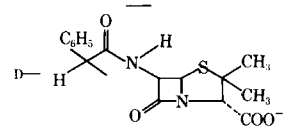
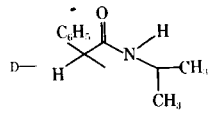
Anal.—Calc. for $C_{12}H_{15}NO_3$: C, 65.14; H, 6.83; N, 6.33. Found: C, 65.07; H, 6.76; N, 6.23.

RESULTS AND DISCUSSION

At a constant pH between 5.5 and 8.0, the formyl moiety of cefamandole nafate hydrolyzes by an apparent pseudo-first-order process. The effects of pH and temperature on the rate of hydrolysis are shown in Table I. The effect of the hydroxide ion in the pH 5.5–8.0 range is large, with the half-life at 37° decreasing from ~290 to ~7 min. The pH–rate profile for the hydrolysis at 25, 37, 45, and 55° (Fig. 1) is linear with coefficients of determination of 0.953 or better. The Arrhenius activation energy increases (Fig. 2) with a decrease in pH because nucleophilic attack at the formate carbonyl by a neutral water molecule is more difficult than nucleophilic attack by a negatively charged hydroxide ion.

Table II—Apparent Pseudo-First-Order Rate Constants of Formate Hydrolysis (pH 8.0, 25°) of Related Formate Esters



Compound	R	Number of Observations	$k \times 10^5$ sec^{-1} Mean	$k \times 10^5$ sec^{-1} SE	k_{obs} (D-Cefamandole) / k_{obs} (R—O—COH)
D-Cefamandole	—	8	29.0	1.83	—
L-Cefamandole	—	3	24.0	0.90	1.2
II		3	15.0	2.14	1.9
III		3	19.3	2.31	1.5
IV	CH_3	3	4.48	0.05	6.5

The rate of hydrolysis of cefamandole nafate (pH 8 and 25°) is 6.5 times greater than that of methyl formate (Table II). To determine if the increase in reactivity is the result of the effect of the mandelic moiety or of some interaction of it with the β-lactam-containing portion of the cephalosporin, several formylmandelic acid derivatives were prepared and their rates of hydrolysis were determined. It is apparent from the relative rates of formate hydrolysis (Table II) of the β-lactam-containing molecules, D- and L-cefamandole nafates, of II and of the non-β-lactam, III, that the increase in reactivity over that observed for methyl formate is merely the substituent effect of the mandelic moiety.

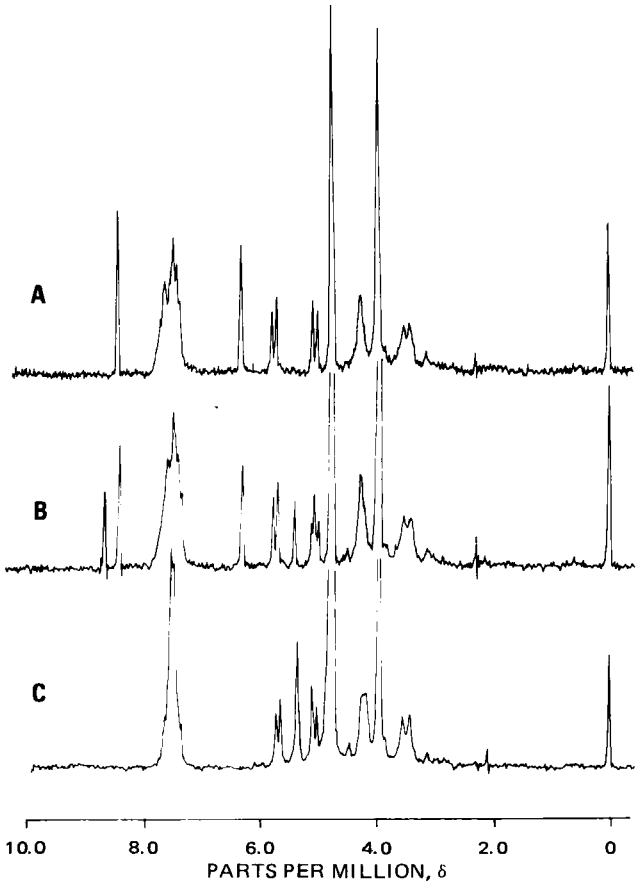


Figure 3—*NMR spectra (D_2O). Key: A, cefamandole nafate; B, cefamandole nafate hydrolyzed to ~28% cefamandole by the addition of 0.28 molar equivalent of sodium carbonate; and C, cefamandole.*

The hydrolysis of the formylmandelic derivatives may involve bond breakage at two possible sites, cleavage at the acyl—oxygen bond or cleavage at the alkyl—oxygen bond. Most esters hydrolyze by an acyl—oxygen cleavage, but alkyl—oxygen cleavage may occur in the hydrolysis of esters that yield stable carbonium ions, such as benzhydryl esters (6). In the case of cefamandole nafate, acyl—oxygen bond cleavage would result in the retention of chirality in the mandelic side chain; alkyl—oxygen cleavage would result first in a benzylic cation intermediate which, upon reaction with water, would yield a mixture of D- and L-cefamandoles. Because L-cefamandole possesses significantly lower microbiological activity than D-cefamandole[7], it is fortunate that D-cefamandole nafate hydrolyzes by acyl—oxygen cleavage to D-cefamandole. The D- and L-cefamandoles may be differentiated by their 100-MHz NMR spectra in pyridine-d_5[8].

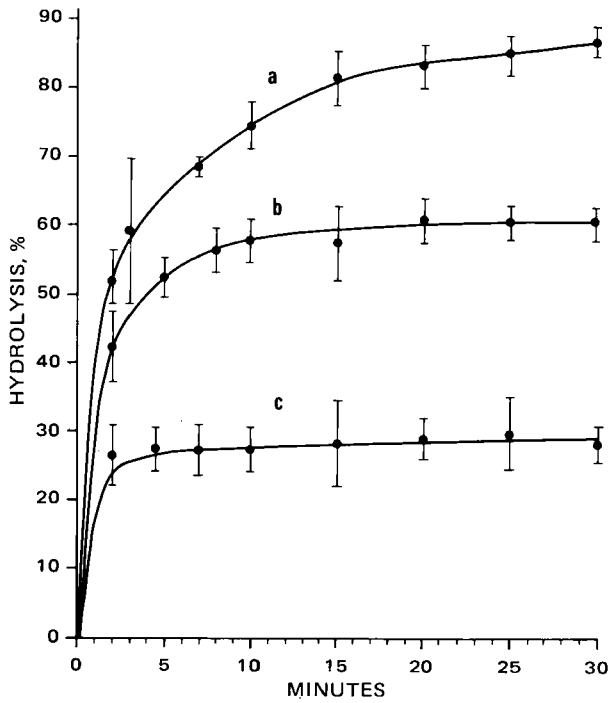
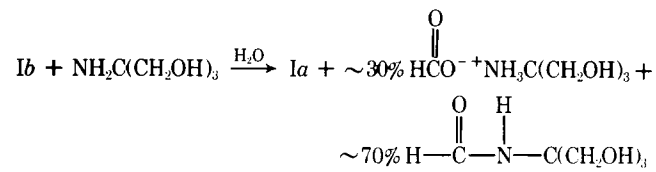


Figure 4—*Effect of 0.90 (a), 0.60 (b), and 0.28 (c) mole equivalent of sodium carbonate on the percent hydrolysis of cefamandole nafate to cefamandole at 25 ± 1°. Without the addition of sodium carbonate, no detectable hydrolysis was observed in 30 min and only ~10% hydrolysis was observed in 24 hr.*

[7] D. A. Preston, Lilly Research Laboratories, personal communication.
[8] G. W. Huffman, Lilly Research Laboratories, personal communication.



Scheme I

The rate of hydrolysis of cefamandole nafate to cefamandole upon the addition of sodium carbonate may be studied conveniently by NMR. The NMR spectrum of cefamandole nafate (Fig. 3A) differs from that of cefamandole (Fig. 3C) in the position of the α-phenylmethine proton peaks, at δ 6.2 and 5.3, respectively, and in the presence of a formyl proton peak at δ 8.2 in the ester. In cefamandole, the formyl proton is absent but occurs as a formate anion peak, δ 8.5 (Fig. 3B). By determining either the ratio of the cefamandole nafate methine peak (δ 6.2) to the cefamandole methine peak (δ 5.3) or, more conveniently, the ratio of the formyl peak (δ 8.2) to the formate anion peak (δ 8.5), one may calculate the percent hydrolysis at a given time. Figure 3B represents ~28% hydrolysis upon the addition of 0.28 mole of sodium carbonate.

Figure 4 shows the percent conversion of cefamandole nafate to cefamandole by 0.28, 0.60, and 0.90 molar equivalents of sodium carbonate at 25 ± 1°. The error indicated represents the 95% confidence interval determined from five experiments.

The aqueous hydrolysis of cefamandole nafate with tromethamine (Scheme I) and ethanolamine was also examined by NMR. In each case, upon the addition of 1.0 molar equivalent of amine, hydrolysis was complete within the time necessary to obtain the NMR spectrum. The NMR spectrum of the products of the tromethamine reaction indicated two new kinds of formyl peaks at δ 8.04 and 8.15 in addition to ~30% formate anion at δ 8.5. These new formyl peaks were identified as the formyl proton of *N*-formyl tromethamine in the *trans*- and *cis*-configurations of the amide bond (7). An authentic sample of *N*-formyl tromethamine was prepared by the reaction of methyl formate and tromethamine.

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[x] To whom inquiries should be directed.

Quantitative Determination of Theophylline in Pharmaceutical Dosage Forms by Differential Spectrophotometry

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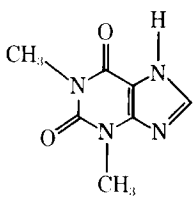
Abstract □ Theophylline was determined with good precision in tablets and capsules by differential spectrophotometry. Xanthines such as caffeine and theobromine did not interfere providing the total xanthine concentration was kept below 100 μg/ml. At a higher total xanthine concentration, nonlinearity occurred, presumably due to complex formation. This interference could be minimized by proper selection of the analytical wavelength.

Keyphrases □ Theophylline—analysis, differential spectrophotometry, pharmaceutical formulations □ Spectrophotometry, differential—analysis, theophylline in pharmaceutical formulations □ Aminophylline tablets and capsules—theophylline content analyzed by differential spectrophotometry

An argentimetric titration is described in the USP for the analysis of aminophylline and tablets containing aminophylline. The reactive component in the analysis is the theophylline (I) portion of aminophylline. The silver salt titration was compared with a spectrophotometric method, and some difficulties of the official method were discussed, particularly the effect of materials obscuring the end-point or interfering with filtration prior to titration (1). In addition, compounds with a structure similar to theophylline, such as theobromine (II), were reported (2) to interfere with the official method of analysis.

The direct spectrophotometric method, measuring the absorption peak in either acid (1) or base (3), is convenient and precise. However, it suffers from interference from other substances, such as theobromine and caffeine (III), absorbing in the same spectral region. Differential spectrophotometry offers the convenience and most of the precision of spectrophotometric



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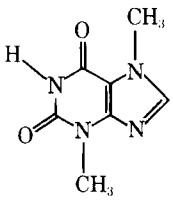

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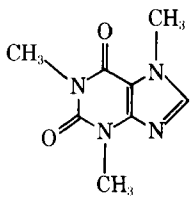

III