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ACKNOWLEDGMENTS

Supported in part by the Committee on Faculty Research, University of Mississippi, and the Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi.

The authors thank Westwood Pharmaceuticals, Buffalo, N.Y., and Rohm and Haas Research Laboratories, Spring House, Pa., for the antimicrobial screening data listed in Tables II and III, respectively. The authors also acknowledge the authentic samples of oxonantenine from Dr. J. A. Lamberton, CSIRO Chemical Laboratories, Melbourne, Australia; lysicamine from Dr. A. Cave, Universite De Paris-Sud, France; 2-*N*,*O*-dimethylliriodendronine from Dr. M. P. Cava, University of Pennsylvania, Philadelphia, Pa.; and atherosperminine picrate from Dr. I. R. C. Bick, University of Tasmania, Australia.

Formylation of Glucose by Cefamandole Nafate at Alkaline pH

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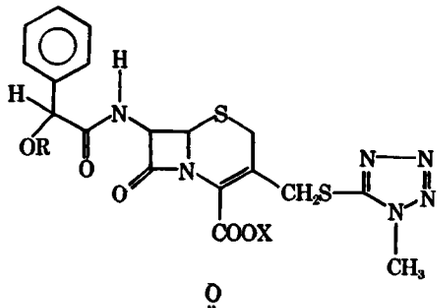
Received March 3, 1980, from *Lilly Research Laboratories, Division of Eli Lilly and Company, Indianapolis, IN 46285.* Accepted for publication April 25, 1980.

Abstract □ An increase in the hydrolysis rate of the formyl moiety of cefamandole nafate was observed when the commercial product was reconstituted in 5% dextrose in water relative to the rate when the formulation was dissolved in water for injection or in 0.9% saline. The increase in ester cleavage was the result of nucleophilic attack of glucose on the formyl ester moiety. This transesterification produced small amounts of D-glucose-6-formate and other D-glucose diformates. The formation of these products is of no clinical significance since the antibiotic potency and stability of cefamandole are unaffected and no toxicological differences were observed in animal studies or in clinical trials when formulated cefamandole nafate was administered with or without glucose.

Keyphrases □ Cefamandole nafate—hydrolysis in presence of glucose □ Glucose—effect on hydrolysis of cefamandole nafate □ Hydrolysis—cefamandole nafate, effect of glucose

Cefamandole nafate (I*a*) is the formyl ester of the sodium salt of cefamandole [7-D-mandelamido-3-[[(1-methyl - 1*H* - tetrazol - 5 - yl)thio]methyl] - 3 - cephem - 4 - carboxylic acid, I*b*]. Cefamandole nafate is converted rapidly *in vivo* (1) and *in vitro* (2) to cefamandole. Against Gram-positive organisms, cefamandole has microbiological activity comparable to that of cephalothin and cefazolin; against Gram-negative bacteria, cefamandole clearly is superior to these antibiotics both in its activity and its spectrum (3).



I*a*: R = CH, X = Na
I*b*: R = H, X = H

BACKGROUND

Cefamandole nafate, which may be administered either intramuscularly or intravenously, maintains microbiological potency in commercial intravenous fluids for >96 hr at 4°. However, an increase in the rate and extent of hydrolysis of the formyl moiety has been observed (Fig. 1) when formulated cefamandole nafate[1] is reconstituted in commercial 5% dextrose in water relative to the rate when the formulation is dissolved in water for injection or in 0.9% saline. This change in the hydrolysis rate of the formyl moiety is related to the glucose concentration (Fig. 2) and occurs only in the formulated product.

A similar two- to threefold increase in the hydrolysis rate of phenyl acetates in the presence of glucose was reported previously (4). This increase was presumed to be the result of nucleophilic attack on the carbonyl carbon by the hemiacetal alkoxide ion derived from glucose; however, neither glucose-1-acetate nor any other glucose ester was isolated.

Glucose and other carbohydrates have been implicated in the biological inactivation of penicillins at pH 7.4 and higher (5, 6). This inactivation was shown to be the result of nucleophilic attack on the β-lactam ring, resulting in the formation of penicilloyl sugar esters, which subsequently hydrolyze to penicilloic acids (7, 8).

The purposes of this study were to define the role of glucose in the

[1] Mandol, Eli Lilly and Co., Indianapolis, Ind. 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.

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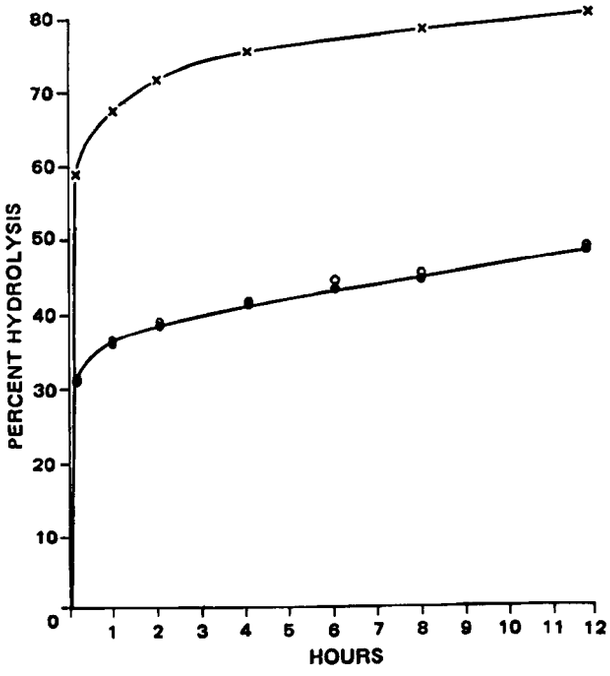


Figure 1—*Effect of water for injection (●), 0.9% saline (○), and 5% dextrose (×) on the percentage of hydrolysis of cefamandole nafate to cefamandole when formulated cefamandole nafate was reconstituted at 25°.*

hydrolysis of cefamandole nafate and to identify any products that could arise from this drug–diluent interaction.

EXPERIMENTAL

Materials—Cefamandole nafate formulated with 0.28 mole equivalent of sodium carbonate[1] and cefamandole sodium[2] were used as supplied. The intravenous fluids were obtained commercially[3]. All other chemicals were reagent grade and were used as received.

Rate of Cefamandole Nafate Hydrolysis—High-pressure liquid chromatography (HPLC) was used to determine the extent of hydrolysis of the formyl ester of formulated cefamandole nafate dissolved in various intravenous fluids. The chromatographic system consisted of an injector[4], a pump[5], a reversed-phase column[6], and a UV detector[7] set at 260 nm. The mobile phase was water–acetonitrile–acetic acid (85:13:2) at a flow rate of 2.0 ml/min.

Peak heights of the cefamandole and cefamandole nafate present in solution after reconstitution (concentration of 2 mg/ml) were measured and compared with standard curves.

Glucose solutions were prepared by dilution of a commercial 10% dextrose solution[3] with water for injection[2].

Preparation of D-Glucose Formates—α-D-Glucose (10 g, 5.55×10^{-2} *M*) was dissolved in 100 ml of 98% formic acid and stirred for 0.5 hr at room temperature. Formic acid was removed *in vacuo*. The resulting syrup was dissolved twice in 50 ml of water and lyophilized to remove residual formic acid. The crude preparation separated into three spots of R_f 0.21, 0.31, and 0.48 when it was chromatographed on paper [butanol–ethanol–water (4:1:1)] and visualized with aniline oxalate and heat. The R_f value for D-glucose is 0.21 when chromatographed with this system.

The prepared syrupy residue was dissolved in 20 ml of absolute ethanol to which 30 ml of water-saturated methyl ethyl ketone was added. It was placed on a 4 × 25-cm (~180 g) column[8], which had been rinsed exhaustively with water-saturated methyl ethyl ketone.

The column was developed with water-saturated methyl ethyl ketone using an automatic fraction collector to collect 10–15-ml samples. Drops of the collected fractions were spotted on paper, sprayed with aniline oxalate, and heated to show which tubes contained reducing sugars. On this basis, tubes 1–46 and 64–83 were discarded; tubes 47–58, 59–63, 84–93, 94–103, 104–113, and 114–147 were divided as indicated into six fractions. The solvent was removed *in vacuo*.

Isolation of D-Glucose Formates—*α- and β-D-Glucose-6-formates*—The residues obtained from tubes 104–113 and 114–147, both of R_f 0.31, were identified by their 360-MHz NMR[9] spectra as being a mixture of α- and β-D-glucose-6-formates; field desorption mass spectrum: M^{+1} 209; IR (D_2O): 1700 (ester) cm^{-1}.

Anal.—Calc. for $C_7H_{12}O_7$: C, 40.39; H, 5.81. Found: C, 40.10; H, 6.19.

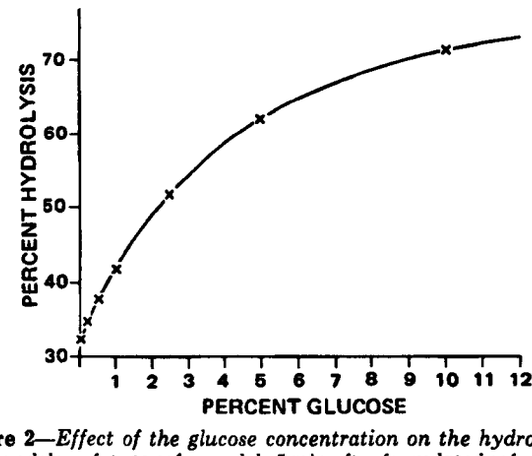


Figure 2—*Effect of the glucose concentration on the hydrolysis of cefamandole nafate to cefamandole 5 min after formulated cefamandole nafate was reconstituted.*

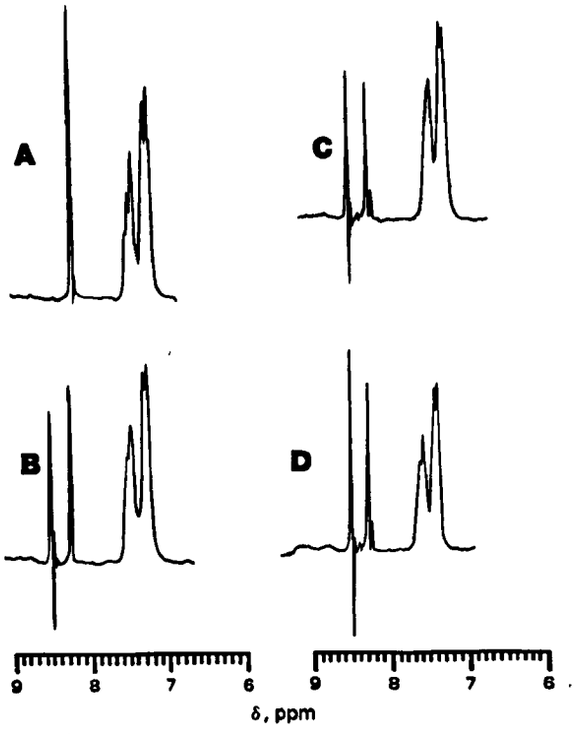
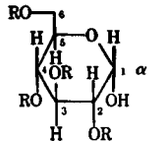
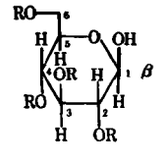


Figure 3—*NMR spectra in water. Key: A, cefamandole nafate; B, commercial cefamandole nafate hydrolyzed to ~28% cefamandole by the 0.28 mole equivalent of sodium carbonate present in the formulation; C, commercial cefamandole nafate dissolved in 5% dextrose; and D, commercial cefamandole nafate in water spiked with a crude mixture of D-glucose-6-formate, D-glucose-2,6-diformate, D-glucose-3,6-diformate, and D-glucose-4,6-diformate.*

[2] From production lots, Eli Lilly and Co., Indianapolis, Ind.
[3] Travenol Laboratories Inc., Deerfield, Ill.
[4] Model U6K, Waters Associates, Milford, Mass.
[5] Model 6000A, Waters Associates, Milford, Mass.
[6] Partisil 10 ODS, Whatman Inc., Clifton, N.J.
[7] Model LC-55, Perkin-Elmer, Oak Brook, Ill.
[8] Avicel, microcrystalline cellulose, FMC American Viscose Division, Newark, Del.
[9] WH-360, Brüker, Zürich, Switzerland.

Table I—360-MHz NMR Spectra of the Formylated Glucoses[a]

Proton	α-D-Glucose	α-D-Glucose-6-formate	α-D-Glucose-2,6-diformate	α-D-Glucose-3,6-diformate	α-D-Glucose-4,6-diformate
					
H_1	δ 5.21 (d) 4 Hz (H_1–H_2)	δ 5.19 (d) 4 Hz (H_1–H_2)	δ 5.40 (d) 4 Hz (H_1–H_2)	δ 5.28 (d) 4 Hz (H_1–H_2)	δ 5.27 (d) 4 Hz (H_1–H_2)
H_2	δ 3.54 (dd) 4 Hz (H_2–H_1), 9 Hz (H_2–H_3)	δ 3.54 (dd) 4 Hz (H_2–H_1), 9 Hz (H_2–H_3)	δ 4.83 (dd) 4 Hz (H_2–H_1), 9 Hz (H_2–H_3)	δ 3.80 (dd) 4 Hz (H_2–H_1), 10 Hz (H_2–H_3)	δ 3.70 (dd) 4 Hz (H_2–H_1), 9 Hz (H_2–H_3)
H_3	δ 3.72 (dd) 9 Hz (H_3–H_2), 9 Hz (H_3–H_4)	δ 3.69 (dd) 9 Hz (H_3–H_2), 10 Hz (H_3–H_4)	δ 3.96 (dd) 9 Hz (H_3–H_2), 9.5 Hz (H_3–H_4)	δ 5.13 (dd) 10 Hz (H_3–H_2), 9 Hz (H_3–H_4)	δ 3.97 (dd) 9 Hz (H_3–H_2), 10 Hz (H_3–H_4)
H_4	δ 3.42 (dd) 9 Hz (H_4–H_3), 9 Hz (H_4–H_5)	δ 3.44 (m) Seen only when H_5 was irradiated	δ 3.61 (dd) 9.5 Hz (H_4–H_5), 10 Hz (H_4+H_5)	δ 3.72 (dd) 9 Hz (H_4–H_3), 10 Hz (H_4–H_5)	δ 4.93 (dd) 10 Hz (H_4–H_3), 10 Hz (H_4–H_5)
H_5	δ 3.83 (m)	δ 4.04 (ddd) 10 Hz (H_5–H_4), 4 Hz (H_5–H_6'), 2 Hz (H_5–H_6'')	δ 4.12 (dd) 10 Hz (H_5–H_4), 4 Hz (H_5–H_6'), 2 Hz (H_5–H_6'')	δ 4.16 (dt) 10 Hz (H_5–H_4), 4 Hz (H_5–H_6)	δ 4.31 (dt) 10 Hz (H_5–H_4), 3 Hz (H_5–H_6)
H_6'	δ 3.76 (dd) 12 Hz (H_6'+H_6''), 6 Hz (H_6'+H_5)	δ 4.39 (dd) 10 Hz (H_6'+H_6''), 4 Hz (H_6'+H_5)	δ 4.44 (dd) 12 Hz (H_6'+H_6''), 4 Hz (H_6'+H_5)	δ 4.49 (broad s)	δ 4.36 (broad s, possibly *AB*)
H_6''	δ 3.84 (dd) 12 Hz (H_6''–H_6'), 2 Hz (H_6''–H_5)	δ 4.45 (dd) 10 Hz (H_6''–H_6'), 2 Hz (H_6''–H_5)	δ 4.50 (dd) 12 Hz (H_6''–H_6'), 2 Hz (H_6''–H_5)	δ 4.49 (broad s)	δ 4.36 (broad s, possibly *AB*)
6-Formyl	—	δ 8.16 (s)	δ 8.20 (s)	δ 8.19 (s)	δ 8.17 (s)
2-Formyl	—	—	δ 8.24 (s)	—	—
3-Formyl	—	—	—	δ 8.34 (s)	—
4-Formyl	—	—	—	—	δ 8.27 (s)

Proton	β-D-Glucose-6-formate	β-D-Glucose-2,6-diformate	β-D-Glucose-3,6-diformate	β-D-Glucose-4,6-diformate
				
H_1	δ 4.54 (d) 8 Hz (H_1–H_2)	δ 4.89 (d) 8 Hz (H_1–H_2)	δ 4.78 (d) 8 Hz (H_1–H_2)	δ 4.75 (d) 8 Hz (H_1–H_2)
H_2	δ 3.26 (dd) 8 Hz (H_2–H_1), 8 Hz (H_2–H_3)	δ 4.69 (dd) 8 Hz (H_2–H_1), 9 Hz (H_2–H_3)	δ 3.50 (dd) 8 Hz (H_2–H_1), 10 Hz (H_2–H_3)	δ 3.42 (dd) 8 Hz (H_2–H_1), 8 Hz (H_2–H_3)
H_3	δ 3.45 (m) Seen only when H_3 and H_5 were irradiated	δ 3.76 (dd) 9 Hz (H_3–H_2), 9 Hz (H_3–H_4)	δ 4.97 (dd) 10 Hz (H_3–H_2), 10 Hz (H_3–H_4)	δ 3.80 (dd) 8 Hz (H_3–H_2), 9 Hz (H_3–H_4)
H_4	δ 3.4 By analogy with diformates	δ 3.59 (dd) 9 Hz (H_4–H_3), 9 Hz (H_4–H_5)	δ 3.73 (dd) 10 Hz (H_4–H_3), 10 Hz (H_4–H_5)	δ 4.94 (dd) 9 Hz (H_4–H_3), 10 Hz (H_4–H_5)
H_5	δ 3.7 (m) Irradiation at δ 3.7 decouples H_6	δ 3.75 (ddd) 9 Hz (H_5–H_4), 5 Hz (H_5–H_6'), 2 Hz (H_5–H_6'')	δ 3.82 (ddd) 10 Hz (H_5–H_4), 4.5 Hz (H_5–H_6'), 2.5 Hz (H_5–H_6'')	δ 3.98–4.03 (m)
H_6'	δ 4.34 (dd) 12 Hz (H_6'–H_6''), 5 Hz (H_6'–H_5)	δ 4.39 (dd) 12 Hz (H_6'–H_6''), 5 Hz (H_6'–H_5)	δ 4.41 (dd) 12 Hz (H_6'–H_6''), 4.5 Hz (H_6'–H_5)	δ 4.34 (dd) 12 Hz (H_6'–H_6''), 6 Hz (H_6'–H_5)
H_6''	δ 4.50 (dd) 12 Hz (H_6''–H_6'), 4 Hz (H_6''–H_5)	δ 4.55 (dd) 12 Hz (H_6''–H_6'), 2 Hz (H_6''–H_5)	δ 4.53 (dd) 12 Hz (H_6''–H_6'), 2.5 Hz (H_6''–H_5)	δ 4.40 (dd) 12 Hz (H_6''–H_6'), 2 Hz (H_6''–H_5)
6-Formyl	δ 8.16 (s)	δ 8.20 (s)	δ 8.19 (s)	δ 8.17 (s)
2-Formyl	—	δ 8.29 (s)	—	—
3-Formyl	—	—	δ 8.34 (s)	—
4-Formyl	—	—	—	δ 8.27 (s)

[a] The solvent was deuterium oxide, and the external standard was 1,1,2,2-tetradeutero-2-trimethylsilylethanesulfonic acid sodium salt.

α-D-Glucose-2,6-diformate and β-D-Glucose-3,6-diformate—Semipreparative separation of the residue from tubes 47–58 (R_f 0.48) was obtained *via* HPLC. The chromatographic system consisted of an injector[10] with a 1-ml loop, a pump[5], a semipreparative reversed-phase column[11], and a UV detector[12] set at 220 nm at 0.1 aufs. The mobile phase was water at a flow rate of 4.0 ml/min. The sample size was 10 mg.

The fraction could be separated by this method into three peaks which

[10] Model 7120, Rheodyne, Berkeley, Calif.

[11] μBondapak C_{18} (10 μm), 30 cm × 7.8 mm i.d., Waters Associates, Milford, Mass.

[12] Technicon model 635 LC, Varian Associates, Palo Alto, Calif.

Table II—Estimated Quantities of Formylated Glucose Formed upon Reconstitution of 1-g Vial of Cefamandole Nafate with 5% Dextrose

Diluent	Volume, ml	Time after Reconstitution	Percent of Cefamandole Nafate Hydrolyzed to Cefamandole	Unreacted[a] Glucose, mg	6-Formyl Glucose[b], mg
Water	3	3–5 min	28	—	—
		30 min	29	—	—
5% Dextrose	3	3–5 min	47	~105	~45
		3 hr	52	~110	~40
	50	3–10 min	57	~2410	~90
	100	30 min	60	~4900	~100
		3 hr	88	~4890	~110
	3 (first step), then diluted to 100 (second step)	30 min	52	~4940	~60
		3.5 hr	64	~4910	~90

[a] Calculation based on the difference between glucose added and formylated products observed. [b] Estimated from NMR peaks assigned to the formyl ester proton at C-6 of glucose. Quantities reported are in terms of milligrams of glucose-6-formate as if it were the only formylated glucose produced.

represented α-D-glucose-2,6-diformate, β-D-glucose-3,6-diformate, and a mixture of β-D-glucose-2,6-diformate and α-D-glucose-3,6-diformate. The residue was recycled three times before collection of the peak fractions directly onto dry ice–acetone. The lyophilized fractions were characterized by 360-MHz NMR spectroscopy.

α-D-Glucose-4,6-diformate—The crystalline residue from tubes 59–63 (~700 mg) (R_f 0.48) was redissolved in methyl ethyl ketone and produced 300 mg of needles, mp 144–147°, upon standing. This product was identified by the 360-MHz NMR spectrum as α-D-glucose-4,6-diformate; field desorption mass spectrum: M^{+1} 237.

Anal.—Calc. for $C_8H_{12}O_8$: C, 40.68; H, 5.12. Found: C, 40.90; H, 4.85.

NMR Assay—The extent of hydrolysis of the formyl moiety of commercially formulated cefamandole nafate in water for injection and 5% dextrose was determined by 90-MHz NMR spectroscopy[13]. The NMR spectrum of the 5% dextrose solution contained additional peaks in the formate region not present when water was used for reconstitution. The peaks were attributed to a mixture of glucose formates formed in solution by transesterification with cefamandole nafate.

The extent of transesterification that resulted from reconstitution of formulated cefamandole nafate with varying volumes of 5% dextrose was determined by NMR spectroscopy at 25 ± 1°. Commercially available 1-g vials of cefamandole nafate were reconstituted with 3, 50, and 100 ml of 5% dextrose prior to insertion into the probe. The degree of transesterification was calculated from the integrated peak intensities. The disappearance of the nafate formyl proton at δ 8.32, the appearances of the various proposed glucose formyl protons at δ 8.26, 8.40, and 8.47, and the appearance of the formate anion proton at δ 8.50 were monitored as a function of time. The water peak (δ ~4.8) did not interfere with the measurement.

Product Identification: Comparison with Synthesized Glucose Formates—*NMR*[13] *Spectroscopy*—Authentic samples of D-glucose-6-formate and a mixture of D-glucose-6-formate and glucose diformates were added to commercial 1-g vials of cefamandole nafate reconstituted with 3 ml of water. In each case, an additional peak was observed in the formate region having the same chemical shift (δ 8.26) as the major new peak observed when formulated cefamandole nafate was reconstituted with 5% dextrose.

HPLC—The presence of glucose-6-formate and the glucose diformates in glucose solutions of formulated cefamandole nafate was verified by HPLC. The high-pressure liquid chromatograph[5] was equipped with a variable-wavelength UV detector[14] set at 213 nm and 0.32 aufs, an injector[4], and a reversed-phase column[15]. The mobile phase was water at a flow rate of 1 ml/min.

Commercial 1-g vials of formulated cefamandole nafate were reconstituted with either 6 ml of water for injection or with 6 ml of 5% dextrose. Peaks having retention times identical to the glucose formates were observed in the vials reconstituted with 5% dextrose. The height of these peaks increased when an authentic mixture of glucose-6-formate and the glucose diformates was added to this solution.

Kinetic Methods—The hydrolysis rates of D-glucose-6-formate and D-glucose-4,6-diformate were followed by titration at constant pH with 0.1 and 0.2 *N* NaOH (2). Constant ionic strength (0.1 *N*) was established by prior addition of potassium chloride. The formyl ester concentrations varied between 0.001 and 0.006 *M*.

The relative rates of hydrolysis at C-4 and C-6 were determined by NMR analysis. The formate region of D-glucose-4,6-diformate was scanned before and after the addition of 1.0 mole equivalent of sodium carbonate. The relative hydrolysis rates were calculated from changes in the heights of the C-4 and C-6 formyl proton peaks using:

$$\frac{\ln([A]/[A_0])}{\ln([B]/[B_0])} = \frac{k_A}{k_B} \qquad \text{(Eq. 1)}$$

The rates of hydrolysis at C-4 and C-6 were assumed to be independent and competing reactions.

RESULTS AND DISCUSSION

The hydrolysis of cefamandole nafate to cefamandole was examined by NMR and HPLC techniques. In Fig. 3A, the NMR resonance peak at δ 8.32 was assigned to the methine proton of the intact formyl ester of cefamandole nafate. The formyl moiety of cefamandole nafate hydrolyzed rapidly in solution due to the sodium carbonate present in the formulated vial. The resulting formate anion appeared as a resonance peak at δ 8.5 (Fig. 3B). However, when the formulated cefamandole nafate vial was reconstituted with 5% dextrose, additional resonance peaks appeared in the NMR spectrum (Fig. 3C). The appearance of these new peaks suggested the formation of formyl glucose esters.

Attempts to isolate the labile glucose esters from the aqueous cefamandole solution were unsuccessful. However, authentic glucose formates were prepared for NMR and chromatographic comparison. Separation and identification of the formylated products from the reaction of glucose and formic acid were complicated by the existence of α- and β-anomers. However, once separated, 360-MHz NMR spectroscopy allowed identification of the esters without derivatization or degradation studies (Table I).

The chemical shifts and coupling constants of α-D-glucose-6-formate, when compared with α-D-glucose, suggested that C-1 through C-4 were identical. However, the downfield chemical shifts of H-6′ and H-6″ of α-D-glucose at δ 3.76 and 3.84, respectively, to δ 4.39 and 4.45 in α-D-glucose-6-formate, plus the minor downfield chemical shift of H-5 in α-D-glucose-6-formate, were indicative of formylation of the C-6 hydroxyl group. The formyl ester proton appeared at δ 8.16.

The glucose diformates were identified by a comparison of their respective chemical shifts with α-D-glucose-6-formate. The downfield shift of H-2 from δ 3.54 in α-D-glucose-6-formate to δ 4.83 in α-D-glucose-2,6-diformate, the minor changes in the chemical shift of H-1 and H-3, and the absence of change in the chemical shift for H-5 and H-6 were indicative of formylation at both the C-2 and C-6 hydroxyl groups. The formyl ester protons appeared at δ 8.24 (C-2) and 8.20 (C-6).

Identification of α-D-glucose-3,6-diformate and α-D-glucose-4,6-diformate was possible by similar comparisons with α-D-glucose-6-formate. The major changes in chemical shifts occurred for protons on C-3 and C-4 for the respective diformates, with minor changes in the chemical shift for the protons on adjacent carbons.

Similar assignments were made for the β-anomers, which exist in solution in equilibrium with the α-anomers. The chemical shifts of the formyl ester protons were identical for the α- and β-forms of each compound, except for D-glucose-2,6-diformate, where the C-2 formyl ester

[13] EM-390, Varian Associates, Palo Alto, Calif.
[14] Model 970A, Tracor, Austin, Tex.
[15] μBondapak C_{18} (10 μm), 2.5 cm × 3.9 mm i.d., Waters Associates, Milford, Mass.

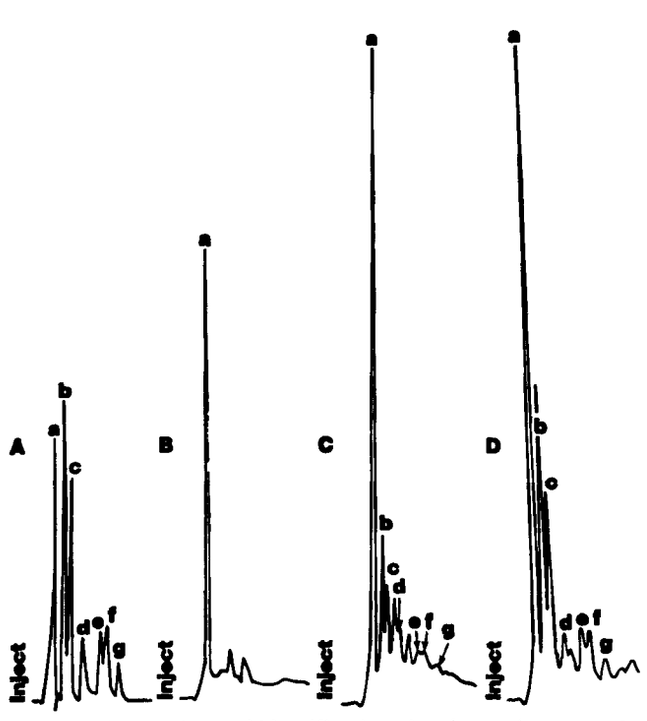


Figure 4—*Separation and identification of D-glucose formates when commercial cefamandole nafate was reconstituted with 5% dextrose. A 30-cm × 3.9-mm i.d. μBondapak C_{18} column was used. The solvent was water. The flow rate was 1 ml/min. The UV detector was operated at 213 nm at 0.32 aufs. Key: A, crude mixture of D-glucose formates; B, commercial cefamandole nafate reconstituted with 6 ml of water, where minor peaks represent unidentified trace impurities (0.03%); C, commercial cefamandole nafate reconstituted with 5% dextrose; and D, Sample C spiked with a crude mixture of D-glucose formates. Peak a was formate anion, peak b was α-D-glucose-6-formate, peak c was β-D-glucose-6-formate, peak d was a mixture of β-D-glucose-2,6-diformate and α-D-glucose-3,6-diformate, peak e was α-D-glucose-2,6-diformate, peak f was β-D-glucose-3,6-diformate, and peak g was a mixture of α- and β-glucose-4,6-diformate.*

protons differed in chemical shift by 0.05 ppm. This anomeric difference in chemical shift may be accounted for by intramolecular hydrogen bonding between the formyl ester carbonyl and the C-2 hydroxyl group of the α-anomer.

NMR spiking experiments with authentic glucose-6-formate and glucose diformates (Fig. 3D) clearly demonstrated the formation of 6-formylated glucose products when formulated cefamandole nafate was reconstituted with 5% dextrose. Because the chemical shifts for the 6-formyl ester proton of both glucose-6-formate and the various glucose diformates were indistinguishable, it was possible to identify the new product only as a 6-formyl glucose ester.

Table III—Apparent Pseudo-First-Order Rate Constants of Formate Ester Hydrolysis of D-Glucose-6-formate and Cefamandole Nafate

pH	Temperature	n	$k \times 10^5$ sec^{-1} Average	SE
		D-Glucose-6-formate		
6.0	25°	4	0.38	0.04
	37°	4	1.95	0.07
	45°	4	3.59	0.33
7.4	25°	3	8.37	0.62
	37°	5	27.7	3.60
	45°	4	37.8	6.50
		Cefamandole Nafate[a]		
6.0	37°	4	3.54	0.20
	45°	4	9.03	0.92
7.4	37°	5	40.40	2.53
	45°	4	66.95	7.97

[a] Taken from Ref. 2.

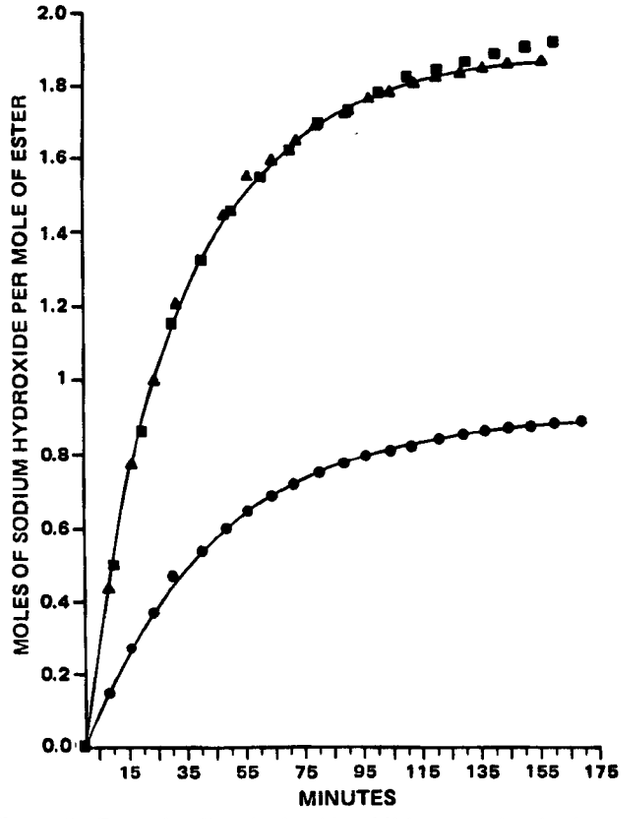


Figure 5—*Rate of sodium hydroxide addition to maintain the ester solution at a constant pH (7.4 at 37°) with a pH-stat. Key: ●, D-glucose-6-formate; ▲, D-glucose-4,6-diformate; and ■, theoretical curve for consumption of base by D-glucose-4,6-diformate, assuming the rate at C-4 is 2.7 times that at C-6 and the reactions are independent and competing.*

The quantity of formylated glucose formed when cefamandole nafate was reconstituted with 5% dextrose was dependent on the volume of diluent introduced (Table II). This conclusion was based on integration of the NMR peak assigned to the formyl ester proton at C-6 of all of the glucose formates. The values ranged from ~45 mg of formylated glucose at a diluent volume of 3 ml to ~100 mg at a diluent volume of 100 ml. With reconstitution in a stepwise fashion, first by adding 3 ml of 5% dextrose and then by diluting to 100 ml with 5% dextrose, only ~60 mg of formylated product was formed.

Less formylated product was produced in the stepwise addition of 5% dextrose because the extent of transesterification was dependent both on the amount of glucose available for reaction and on the pH of the reaction medium, transesterification being base catalyzed. Immediately upon reconstitution, the solution was alkaline due to the 0.28 mole equivalent of sodium carbonate present in the formulation. Within moments, the pH of the reconstituted vial dropped to ~7 as the formic acid produced upon hydrolysis of the formyl moiety was neutralized by the sodium carbonate. Therefore, the addition of glucose after the solution pH approached neutrality did not result in the formation of significantly more formylated glucose.

The quantities of formylated glucose in Table II were approximations in terms of milligrams of glucose-6-formate per 1-g vial of cefamandole nafate. However, HPLC spiking experiments with a mixture of authentic D-glucose-6-formate, D-glucose-2,6-diformate, D-glucose-3,6-diformate, and D-glucose-4,6-diformate clearly demonstrated the formation of these compounds when formulated cefamandole nafate was reconstituted with 5% dextrose (Fig. 4).

The chemical reactivity of the formyl moiety of D-glucose-6-formate was similar to the reactivity of the formyl moiety of cefamandole nafate (Table III). At physiological conditions (pH 7.4 and 37°), the respective half-lives were 29 and 42 min. D-Glucose-4,6-diformate was more reactive than D-glucose-6-formate (Fig. 5). While it was impossible to determine which ester of D-glucose-4,6-diformate was undergoing hydrolysis by titration, an NMR hydrolysis experiment involving introduction of 1.0 mole equivalent of sodium carbonate to D-glucose-4,6-diformate sug-

gested that the ester at C-4 hydrolyzed ~2.7 times faster than the C-6 ester. The rates of D-glucose-2,6- and D-glucose-3,6-diformate hydrolysis were not measured since sufficient quantities of material were not easily obtainable.

While the interaction between cefamandole nafate and glucose is of chemical interest, it is not clinically significant. The antibiotic potency and stability of cefamandole were unaffected by this reaction, and no toxicological differences were observed in animal studies[16] or in clinical trials when formulated cefamandole nafate was administered in the presence or absence of glucose. In mice, the intravenous LD_{50} for D-glucose-6-formate was estimated to be between 5000 and 8000 mg/kg.

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ACKNOWLEDGMENTS

The authors thank Mr. Jon Paschal for 360-MHz NMR data and interpretations and Dr. Al Dinner and Mr. Charles Novotny for preparative HPLC assistance.

Simultaneous Determination of Aspirin and Salicylic Acid in Bulk Aspirin and in Plain, Buffered, and Enteric-Coated Tablets by High-Pressure Liquid Chromatography with UV and Fluorescence Detectors

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Received December 10, 1979, from the *National Center for Drug Analysis, Food and Drug Administration, St. Louis, MO 63101.*
Accepted for publication April 17, 1980.

Abstract □ A quantitative high-pressure liquid chromatographic method that uses a reversed-phase column coupled to UV and fluorescence detectors was developed to determine aspirin and salicylic acid in bulk aspirin and in plain, buffered, and enteric-coated tablets. The aspirin was dissolved, filtered, and injected into the chromatograph. The UV absorbance of aspirin was determined at 254 nm, and the fluorescence of salicylic acid was measured at 425 nm. Excipients and impurities did not interfere. Recoveries of 100% were obtained for aspirin and salicylic acid from simulated tablet formulations. Results obtained by the USP XIX procedure and the proposed method were compared. The coefficient of variation for the aspirin analysis was 0.59%; for salicylic acid, it was 1.69%. The rate of hydrolysis of aspirin to salicylic acid in the solvents used was <0.05%/hr.

Keyphrases □ Aspirin—high-pressure liquid chromatographic determination □ Salicylic acid—high-pressure liquid chromatographic determination □ Analgesics—aspirin, high-pressure liquid chromatographic analysis □ High-pressure liquid chromatography—analysis, aspirin and salicylic acid

Several methods have been reported for the determination of aspirin and salicylic acid. These methods have employed GLC (1–5), high-pressure liquid chromatographic (HPLC) (4–6), UV (7), IR (8), titrimetric (9), fluorometric (10), and automated (11, 12) procedures. Spectrophotometric (13), fluorometric (14, 15), HPLC (5, 16), and automated (17) procedures have been used to determine salicylic acid in the presence of aspirin.

BACKGROUND

In 1978, a national survey of the quality of aspirin tablets on the market was conducted in these laboratories. Portions of the bulk drug substances used in the manufacture of the formulations sampled were collected whenever possible and analyzed with the finished products. The USP methodology (18) for these products was validated, and the literature was examined for alternative procedures with stability-indicating possibilities.

During this period, an HPLC method for the simultaneous determination of aspirin and salicylic acid was reported (6). After the problems associated with the Baum and Cantwell procedure (6) and the troublesome formulations and techniques associated with the USP XIX procedure (18) were considered, the possibility of simultaneously determining aspirin and salicylic acid was investigated in these laboratories.

The simultaneous determination of aspirin and salicylic acid developed in these laboratories is an extension of an HPLC–fluorescence procedure used to determine only the salicylic acid levels in aspirin formulations during the national survey. This earlier HPLC–fluorescence procedure (19) was based on the HPLC conditions of Ali (4) and the observation of the fluorescence characteristics of salicylic acid at pH 4.5 by Shane and Miele (14).

The procedure described in this report determined aspirin and salicylic acid in the formulations precisely and accurately. The manipulation of

0022-3549/80/1000-1188$01.00/0