For cations, the points of ionic cloud point shift values *versus* either lyotropic numbers or standard free energies of formation in water are scattered and do not fall on a single curve. This lack of correlation is ascribed to the fact that all cations except sodium, potassium, ammonium, rubidium, and cesium form complexes with the ether groups of octoxynol 9. The cloud points of polyoxyethylated surfactants are thus affected by cations in two ways. First, all cations tend to become hydrated, binding water and altering its structure. Second, there is a specific interaction by complexation between the ether groups of the surfactants and most cations, which predominates over their dehydrating effect to various degrees, resulting in net salting in or positive Δ values (4, 5). The ionic cloud point shift values for the complexing cations are probably related to the stability constants of the complexes they form with the ether linkages of the polyoxyethylated surfactants and the standard free energy changes of complexation.

Anions do not form complexes with the ether groups. Therefore, their effect on the cloud points of nonionic surfactants depends entirely on their interaction with water, which is also the dominant factor in ranking the anions in the Hofmeister series.

The ionic cloud point shift values of Table IX refer to octoxynol 9. They are higher than the corresponding absolute Δ values for polyoxyethylated surfactants having practically the same average number of ethylene oxide units per molecule and the same HLB but lacking an aromatic ring. Calculating the ionic cloud point shift values with the aid of Eq. 2 from the data of Ref. 5, it was found that polyoxyl 10 cetyl ether and polyoxyl 10 stearyl ether had the lowest absolute Δ values. Those of polyoxyl 10 oleyl ether were somewhat higher, but still well below the corresponding values for octoxynol 9. As the polarity of the hydrocarbon moiety of the surfactants decreased, the ionic cloud point shift values at a given W also decreased. Since the HLB values of the four surfactants were the same within one unit, this observation points up the major shortcoming of the HLB system, namely, that it does not take into account the nature of the hydrophobic moiety of the surfactants (8, 38).

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Dissolution Profile Determination of a Multicomponent Product Using a Rapid Liquid Chromatographic Analysis

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Abstract □ A system was developed which is capable of monitoring the dissolution profiles of each of the active components in a two-component combination product. The UV spectra of the two drugs (metoprolol and hydrochlorothiazide) overlap considerably, making conventional UV analysis of either component unrealistic. By resolving the two drugs on a high-performance liquid chromatographic (HPLC) system both can be quantitated after separation. The analysis is sufficiently short to allow for the use of a six-station dissolution tester in tandem with an HPLC for determination of dissolution profiles. The HPLC system and equipment, can monitor six samples of this product at 5-min intervals in real analysis time.

Keyphrases □ Dissolution—multicomponent product, rapid HPLC, metoprolol, hydrochlorothiazide □ Metoprolol—dissolution profile, multicomponent product with hydrochlorothiazide, rapid HPLC □ Hydrochlorothiazide—dissolution profile, multicomponent product with metoprolol rapid HPLC.

The determination of the release rate of an active drug from pharmaceutical dosage forms has become a standard technique in pharmaceutical development, research, and quality control testing laboratories. This information is valuable to formulators in selecting an optimum solid dosage formulation. *In vivo–in vitro* correlations can often be established which are

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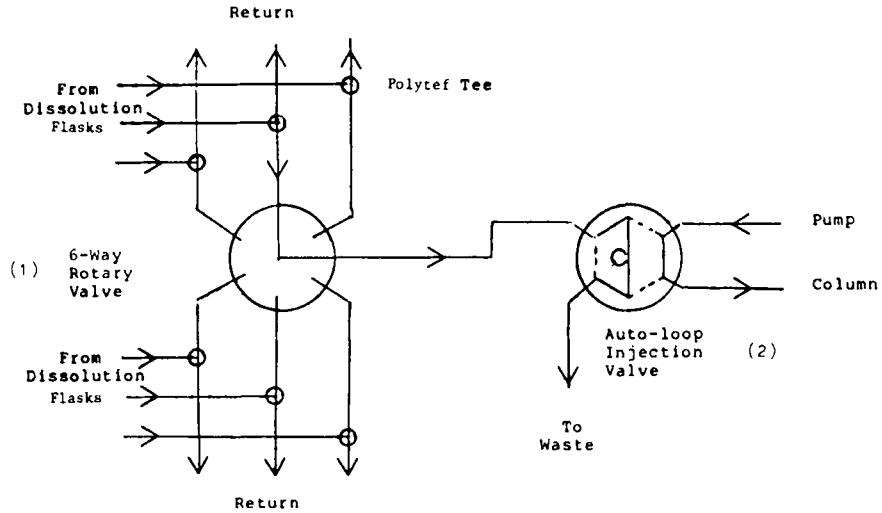

Figure 1—*Schematic diagram of the sampling and injection hardware. Key: (1) six-way rotary valve driven by a 60° stepping actuator; (2) loop injector of the liquid chromatograph.*

useful for predicting bioavailability, and at times it is possible to employ dissolution to satisfy the FDA requirements concerning the bioavailability of a product.

Many dissolution techniques have been proposed, and several are in general usage. The most frequently used techniques are the USP rotating basket and USP rotating paddle methods (1). Other methods that have been employed are the spin filter (2, 3), the rotating flask (4), and the USP disintegration basket (5). Typically, samples of the test solution are taken at one or more time points, and the concentrations of the active component(s) are determined. Various analytical techniques are employed for evaluation concentrations. Wet chemistry techniques can be used to prepare the sample for analysis, or the samples can be analyzed directly without preparation depending on the particular drug excipients and the relative concentration ranges. Several automated dissolution methods have been proposed (6–8). In addition, several commercial instruments are available which will sample, store, and prepare samples for UV analysis[1]. The development of high-performance liquid chromatographic (HPLC) techniques and equipment has simplified the analysis of aqueous samples. This method can be employed for analyzing samples taken from the dissolution medium using a fraction collector, or the solution can be sampled automatically (9).

This paper presents HPLC techniques and equipment which can be used in conjunction with most dissolution test methods. The system is capable of sequentially sampling six streams from the dissolution apparatus and subjecting these samples to chromatographic analysis in <5 min. In operation, all six streams from a dissolution test unit[2] are continuously pumped, through in-line filters, into a six-way rotary valve[3], which selects one stream to be diverted to the injection loop of a chromatograph, while the other streams are returned to the dissolution flasks. Kent *et al.* (10) used a similar valve to monitor the UV absorbance of six dissolution streams. This system has the advantage of performing an HPLC separation and analysis of several components of a multicomponent product during the dissolution experiment.

EXPERIMENTAL

Equipment—An HPLC[4] with programmable external events and a pneumatic loop valve injector was used. The position of the injection valve was controlled by a program in the chromatograph that used solenoids to control the pneumatic pressure to the valve. "Tee" fittings were placed in the pneumatic lines to control the action of a 60° stepping actuator. This actuator was used to rotate a six-way rotary valve[5]. As shown in Fig. 1, each of six streams of dissolution medium from a dissolution unit is pumped to the six-way valve, where one stream is selected to go to the injection valve and the rest are returned through polytef tee fittings to the dissolution unit. The stream going to the injection valve flows through the injection loop and then to waste (~2–3 mL of medium is lost in a 30-min analysis).

On program command (external event), the injection valve switches from the load position to the inject position, and the sample is washed onto the analytical column. At the same time, the pneumatic pressure switches the 60° stepping actuator to the return position. The next external events command causes the injector valve to return to the load position, and the stepping actuator is advanced simultaneously to select the next stream from the dissolution unit and send it through the injection loop. The injection loop is thus continuously flushed with new sample throughout the time required for the chromatographic separation.

Chromatographic Conditions—Since rapid turnaround is required for this application, the chromatography was optimized with a short (5-cm) column and a high flow rate. An analysis time of 30–45 s/sample was the targeted range.

The rapid HPLC method used a 50 × 2.5-mm column packed with nucleosil 5 SA. At ambient temperature, an isocratic mobile phase composed of 0.015 M $KMeSO_3$, 0.025 M tetramethylammonium chloride, and 50% acetonitrile was employed at a flow rate of 2.7 mL/min (~150 atm) with a 10-μL injection loop. The detection was at 212 nm, with 0.2 AUFS attenuation; resolution of the two components was achieved within 0.8 min. The samples in the dissolution experiment were started at 0.8-min intervals so that the timing of the analysis would be synchronized with the dissolution time. This process allowed six samples to be analyzed in sequence within 5 min.

Additionally, as an alternate system, a microprocessor[6] was used to control the sequence of steps and provide the timing in an automated HPLC system. All of the instrument conditions were kept constant, except for the pneumatic actuation and the HPLC pumping system. In this case, two solenoid valves[7] were used under the control of the microprocessor. The microprocessor has 16 I/O ports, which are extensions of a resident versatile interface adapter. Two of these ports were used to control the solenoid valves. The pneumatic pressure released by the solenoid valves was used to simultaneously power an automatic injector and 60° stepping actuator[8]. The timing and the sequence

[1] SASDRA; Technician Industrial Systems, Tarrytown, N.Y.; DISOGRAPH; Hanson Research Corp. Northridge, Calif.

[2] Hanson, multiple, six-station, variable-speed model 72R-QC; Hanson Research Corp. Northridge, Calif.

[3] Altex model 50-11, six-way Teflon rotary valves.

[4] Model 5020LC with external events and pneumatic loop valve injector; Varian Instrument Co.

[5] Rheodyne model K 50-03 60° stepping actuator with two model 50-11 six-way Teflon rotary valves.

[6] AIM 65; Rockwell International, Anaheim, Calif.

[7] Rheodyne Model 7163 solenoid valve.

[8] Rheodyne Model 7010 sample injector valve with Rheodyne Model 7001 RV pneumatic actuator.

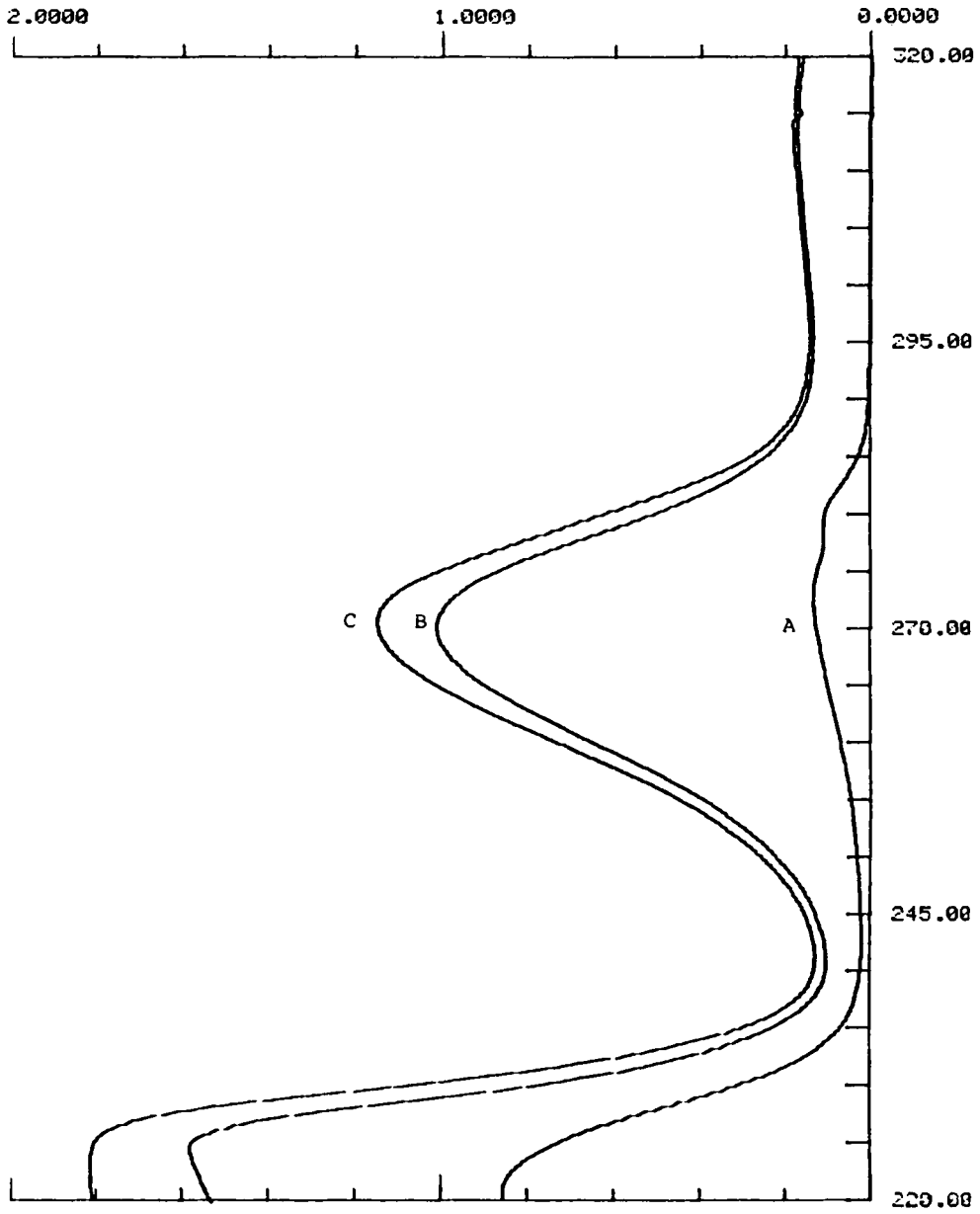

Figure 2—*UV spectra of metoprolol and hydrochorothiazide. Key: (A) metoprolol, 0.1 mg/mL; (B) hydrochlorothiazide, 0.025 mg/mL; (C) metoprolol-hydrochlorothiazide 0.1–0.025 mg/mL.*

of steps were programmed in BASIC. Driving the injector to the load or inject positions was done by sending the appropriate signals through the I/O port to the appropriate solenoid (one for inject position, one for load position). System delays were easily accomplished by sending the BASIC program into a loop for the appropriate period of time.

The advantages of the microprocessor-based system are that (*a*) it can be retrofitted to an existing HPLC pump and detector, (*b*) all of the hardware is commercially available and is relatively inexpensive, and (*c*) the programming on the microprocessor can be tailored to specific applications and is independent of another operating system (such as the timed event functions necessary on the chromatograph for this procedure).

Dissolution Apparatus and UV Analyses—The USP paddle method was used at 50 rpm employing 1 L of distilled water as the dissolution medium. The dissolution profiles were determined by continuously monitoring the absorbance of the media through a series of flow cells in a UV-visible spectrophotometer[9]. The media were circulated through polytef tubing with an in-line filter holder[10] using a thick prefilter[11]. A wavelength of 252 nm was selected, where the sum of the absorbance is due to the hydrochorothiazide and the absorbance due to the metoprolol will be in a convenient range for analysis.

[9] Zeiss Model DM-4, with multiple cell attachment; Karl Zeiss, New York, N.Y.
[10] Swinex, 47 mm; Millipore Corp., Bedford, Mass.
[11] Cat. No. AP25 040 00; Millipore Corp., Bedford, Mass.

RESULTS AND DISCUSSION

This system is applicable for either single-component products (which have low extinction coefficients or some inactive material in the formulation that interferes with normal analysis) or multicomponent products. The obvious advantage is that the release rate profiles of the individual components in multicomponent products can be determined. The system proposed here can analyze six separate dissolution samples within a 5-min turnaround time. This allows for the dissolution experiment and the HPLC analysis of the active components to be conducted simultaneously.

In developing an HPLC method for use in conjunction with a standard six-sample dissolution apparatus, analysis time is the prime consideration. For example, if the required dissolution profile allows for 5-min time intervals between groups of six samples for this apparatus, ~0.8 min is available for each chromatogram. For separations which cannot be performed this quickly, longer sampling intervals such as 10 or 15 min would be required.

The product, metoprolol-hydrochorothiazide, was prepared in three strengths: 50:25, 100:25, and 100:50 mg. Each of the strengths was analyzed for dissolution profiles and each compared using the two different analytical methods. All results are the mean of six tests.

The UV spectra of metoprolol and hydrochorothiazide are shown in Fig. 2. The spectra are seen to overlap at all points of the UV spectrum. It would nearly be impossible to develop an accurate multiple-wavelength analysis for this combination product. HPLC analysis would be an obvious method of choice in this case.

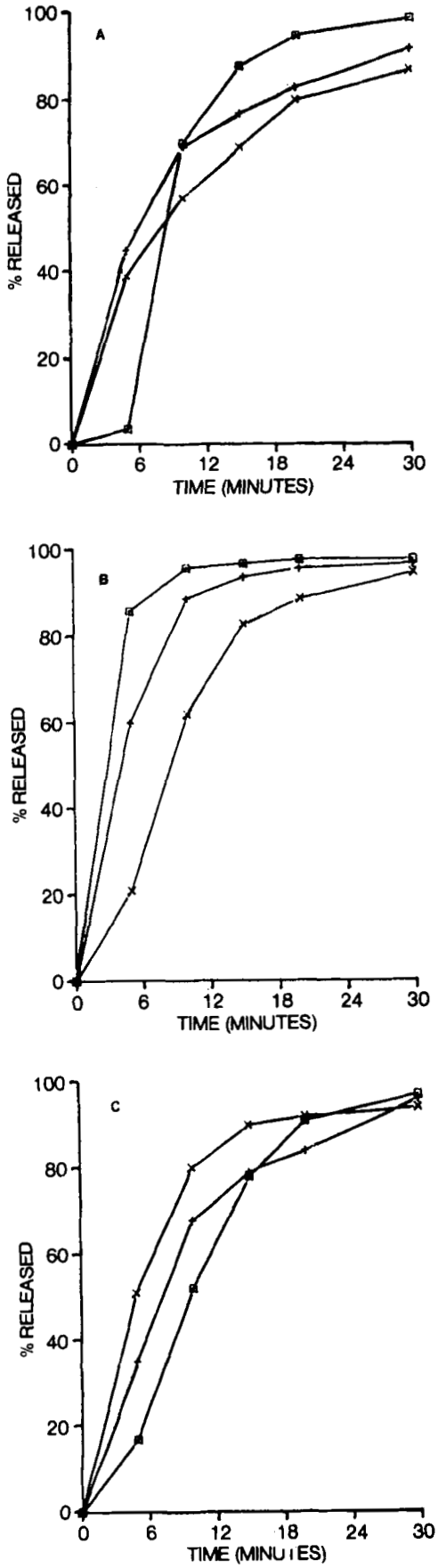

Figure 3—*Comparison of the dissolution rates of (A) 50:25-, (B) 100:25-, and (C) 100:50-mg combinations of metoprolol-hydrochlorothiazide. Key: (+) UV analysis (combination); (x) HPLC analysis of metoprolol; (□) HPLC analysis of hydrochlorothiazide.*

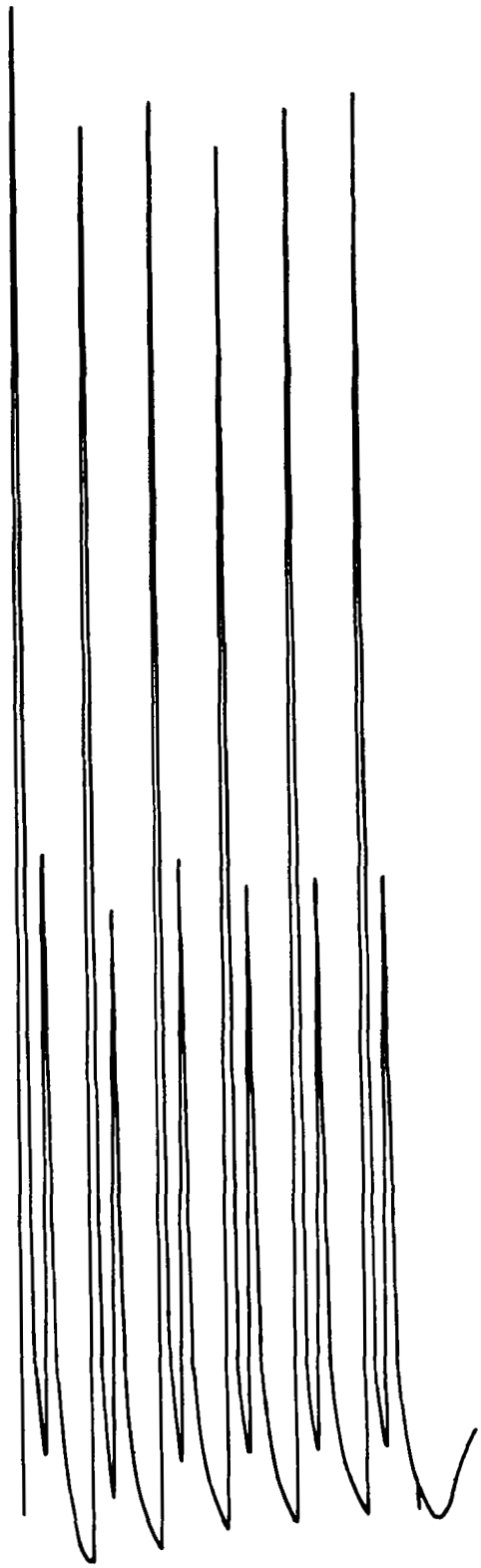

Figure 4—*Chromatogram showing six injections made in a 5-min period.*

The three strengths of metoprolol-hydrochlorothiazide were tested using this rapid HPLC method, and comparison data were generated using the UV analysis method (Fig. 3). It can be seen that the UV analysis yields a dissolution profile which is a composite of the individual release rates measured by the rapid HPLC method. For these sets of experiments, the analysis time for each batch of tablets required ~1.5 h using the rapid HPLC technique. For a conventional HPLC analysis technique, the time required to pull manual aliquots and analyze each sample is much greater. Before this technique was introduced into our laboratory, it required ~5 h to analyze a similar dissolution.

Table I—Peak Heights of Metoprolol–Hydrochlorothiazide Standard Solutions

	Concentration in Simulated Gastric Fluid, mg/L									
	10:5		20:10		60:30		80:40		100:50	
	MET[a]	HCT[b]	MET	HCT	MET	HCT	MET	HCT	MET	HCT
	6.3	4.2	17.1	8.4	58.7	24.8	75.0	32.0	98.0	41.0
	6.3	4.1	17.2	9.1	58.1	32.5	75.0	32.0	98.0	42.0
	6.3	4.2	17.0	8.6	56.5	23.7	74.2	31.2	98.0	41.0
	6.1	4.0	17.0	8.2	56.5	24.3	75.7	31.6	96.5	40.5
Mean	6.3	4.1	17.1	8.6	57.5	24.1	75.0	31.7	97.6	41.1
SD	0.1	0.1	0.1	0.4	1.1	0.6	0.6	0.4	0.8	0.6

[a] MET, metoprolol. [b] HCT, hydrochlorothiazide.

The advantage of a rapid screening technique for monitoring individual release rates of the active components of a multicomponent product is shown in Fig. 3. The composite release from the 100:25- and 100:50-mg formulations appear similar; however, the hydrochlorothiazide is the faster component in Fig. 3B (100:25 mg) and the slower component in Fig. 3C (100:50 mg).

A typical chromatogram is shown in Fig. 4, where six analyses were completed. Peak height measurements for a series of standards are listed in Table I. Linear regression analysis on the mean peak height values of metoprolol and hydrochlorothiazide *versus* concentration yielded correlation coefficients of 0.9996 and 0.9994, respectively.

The intent of these experiments was to demonstrate the utility of a novel approach to analyzing the dissolution rate profile of combination products. For this article, we selected an example of a dual-entity product which was manufactured in several strengths. The ease of operation and facility of this technique is demonstrated in the reduced time required for quantitative analysis of each of the two components of this combination product. The progress being made in HPLC techniques and equipment should allow this technique to be developed for other combination products and for single-entity products which have excipients that interfere in normal UV analyses.

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Some Effects of 1-(2,4-Dichlorophenyl)-4-dimethylaminomethyl-1-nonen-3-one Hydrochloride on *Escherichia coli* GK-19

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Received December 27, 1982, from the **Department of Applied Microbiology and Food Science, College of Agriculture*, ‡*Department of Microbiology, College of Medicine*, and §*College of Pharmacy, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0, Canada.* Accepted for publication April 26, 1983.

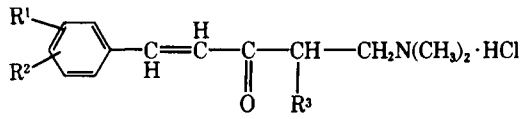
Abstract □ 1-(2,4-Dichlorophenyl)-4-dimethylaminomethyl-1-nonen-3-one hydrochloride (Id) was shown to inhibit the growth of *Escherichia coli* GK-19 at a concentration of 50 μg/mL in a medium of pH 6.5. Maximal antibacterial activity was found during the logarithmic growth phases rather than at the early stationary phase. Electron microscopy revealed that Id caused lysis, and inhibition of respiration and retardation of RNA and protein syntheses occurred in the bacteria with this compound at 50 μg/mL.

Keyphrases □ 1-(2,4-Dichlorophenyl)-4-dimethylaminomethyl-1-nonen-3-one hydrochloride—antibacterial activity in *Escherichia coli*, effects of concentration, temperature, and medium pH □ Antibacterial activity—1-(2,4-dichlorophenyl)-4-dimethylaminomethyl-1-nonen-3-one hydrochloride against *Escherichia coli*, effects of concentration, temperature, and medium pH

Previous work from these laboratories showed that a number of Mannich bases derived from conjugated styryl ketones had antibacterial properties (1–3). The aim of the present investigation was twofold. First, it was hoped to unravel some of the factors affecting the action of a representative Mannich base on bacterial growth, and second, to discern the site or sites of action of the compound toward the bacteria.

EXPERIMENTAL

Synthesis of Compounds—Compound Ia was prepared by the literature method (4); Ib, d–f were synthesized by a previously described procedure (5), as was Ic (6).



Ia: $R^1 = R^2 = R^3 = H$
Ib: $R^1 = R^2 = H$; $R^3 = (CH_2)_4CH_3$
Ic: R^1 = 3-OH; $R^2 = H$; $R^3 = (CH_2)_4CH_3$
Id: R^1 = 2-Cl; R^2 = 4-Cl; $R^3 = (CH_2)_4CH_3$
Ie: R^1 = 2-Cl; R^2 = 6-Cl; $R^3 = (CH_2)_4CH_3$
If: R^1 = 3-Cl; R^2 = 4-Cl; $R^3 = (CH_2)_4CH_3$

Measurement of Bacterial Growth—In this study, an isolate of *Escherichia coli* K-12 strain designated GK-19 (7) was employed. The bacteria were grown in a minimal salts medium (8) to which casamino acids (0.5% w/v) and thiamine (7.5 μg/mL) had been added. Adjustment of the pH was made using Sörensen's buffer solutions (9). The cultures were grown in Erlenmeyer flasks