Polarography of Cephalosporin C Derivatives I: 3-(5-Methyl-1,3,4-thiadiazol-2-ylthiomethyl)-7-[2-(3-sydnone)-acetamido]-3-cephem-4-carboxylic Acid, Sodium Salt

D. A. HALL

Abstract □ 3-(5-Methyl-1,3,4-thiadiazol-2-ylthiomethyl)-7-[2-(3-sydnone)acetamido]-3-cephem-4-carboxylic acid, sodium salt was found to be polarographically reducible in acidic medium, producing two polarographic waves: the first a two-electron reduction, and the second a six-electron reduction. Both waves are diffusion controlled; however, only the first can be used to follow acid and base hydrolysis and β-lactamase degradation of the compound.

Keyphrases □ 3-(5-Methyl-1,3,4-thiadiazol-2-ylthiomethyl)-7-[2-(3-sydnone)acetamido]-3-cephem-4-carboxylic acid, sodium salt—polarographic reduction □ Cephalosporin C derivatives—polarographic reduction of 3-(5-methyl-1,3,4-thiadiazol-2-ylthiomethyl)-7-[2-(3-sydnone)acetamido]-3-cephem-4-carboxylic acid, sodium salt □ Polarography—reduction of 3-substituted cephalosporin C derivatives [3-(5-methyl-1,3,4-thiadiazol-2-ylthiomethyl)-7-[2-(3-sydnone)acetamido]-3-cephem-4-carboxylic acid, sodium salt]

The initial report of the polarographic reducibility of cephalosporins, specifically, cephalosporin C and two of its derivatives, cephalothin [7-(thiophene-2-acetamido)-cephalosporanic acid] and cephaloridine [7-(2-thienylacetamido)-3-(1-pyridylmethyl)-3-cephem-4-carboxylic acid betaine], was that of Jones *et al.* (1). The polarographic reduction wave of cephaloridine was used for quantitative analysis, while those of cephalothin and cephalosporin C were used for qualitative comparison.

This paper reports the d.c. polarographic reduction of 3-(5-methyl-1,3,4-thiadiazol-2-ylthiomethyl)-7-[2-(3-sydnone)acetamido]-3-cephem-4-carboxylic acid, sodium salt (I) and its analytical utilization for control and stability assays.

EXPERIMENTAL

Apparatus—Polarograms were recorded by an IR compensating automatic recording polarograph[1], employing a water-jacketed three-compartment polarographic cell (the indicating-electrode compartment was between the reference and counter-electrode compartments). The cell was maintained at 25.0 ± 0.2° and contained a saturated calomel reference electrode and a platinum foil auxiliary electrode. The compartments of the cell were separated by medium-porosity sintered-glass disks and 4% agar-saturated potassium chloride salt bridges. The dropping mercury electrode had an $m^{2/3}t^{1/6}$ value of 1.555 at open circuit in air-saturated 0.1 *M* potassium chloride solution, unless noted otherwise.

Coulometric runs were made using a mercury pool cathode in the same polarographic cell. Stirring was accomplished by means of a 600-r.p.m. synchronous rotator[2] and a one-turn screw-type impeller.

Procedure—Test solutions were prepared by weighing approximately 2.5 mg. of the compound of interest into a 25-ml. volumetric flask, adding the buffer, and diluting to the mark with deionized water; the pH of this solution was measured. Without delay, about 12 ml. of test solution was transferred to the polarographic cell, purged with argon[3] for about 10 min., and polarographed. The half-wave potential, $E_{1/2}$, and the diffusion current, i_d, were determined graphically using the maximum of the recorder trace.

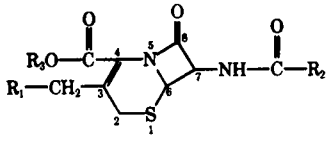
Buffer solutions were prepared from analytical reagent grade chemicals. Phosphate–citric acid–potassium chloride buffers were used at constant ionic strengths (2) of 0.4 and 0.6 *M* over the pH range of 2.3–6.0. A hydrochloric acid–potassium chloride buffer was used for pH 1.6 and was 0.1 *N* in hydrochloric acid. All buffer solutions were polarographically clean within the potential region of interest.

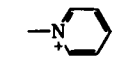
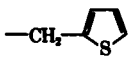
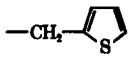
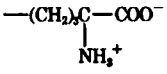
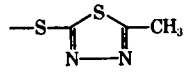
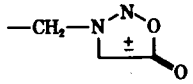
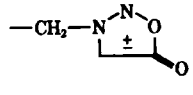
The total number of coulombs passed during coulometry was determined graphically. All coulometric runs were corrected for background current and were considered completed when the current was equivalent to the steady-state coulometric current of the background electrolyte.

RESULTS

Effect of pH—Compound I exhibits two polarographic waves (Fig. 1, solid lines), and both half-wave potentials are pH dependent. The results of the pH study at a concentration of 0.43 m*M* are shown in Table I. Waves I and II at this concentration show a pH dependence of $E_{1/2} = -0.45$–0.09 pH and $E_{1/2} = -0.76$–0.10 pH, respectively. As can be seen from Table I, the pH range in which both reduction waves are present is very narrow. A pH of 2.3 and an ionic strength of 0.6 *M* were selected for analytical purposes and were used throughout the rest of the study, unless noted otherwise.

Effects of Temperature and Mercury Height—Both waves I and II show a temperature dependence of +1.0% per degree. Waves I and II are dependent on the square root of the mercury height, corrected for back-pressure, within a relative standard deviation of ±1.6 and ±4.1%, respectively. These results indicate that both reduction waves are diffusion controlled.



	R_1	R_2	R_3
cephaloridine			zwitterion
cephalothin	$-OCOCH_3$		H^+
cephalosporin C	$-OCOCH_3$		H^+
I			Na^+
III	$-OCOCH_3$		Na^+

[1] Beckman Electroscan 30 Electroanalytical System, Beckman Instruments, Inc., Fullerton, Calif.

[2] E. H. Sargent & Co., Chicago, Ill.

[3] The Matheson Co., Inc., East Rutherford, N. J.

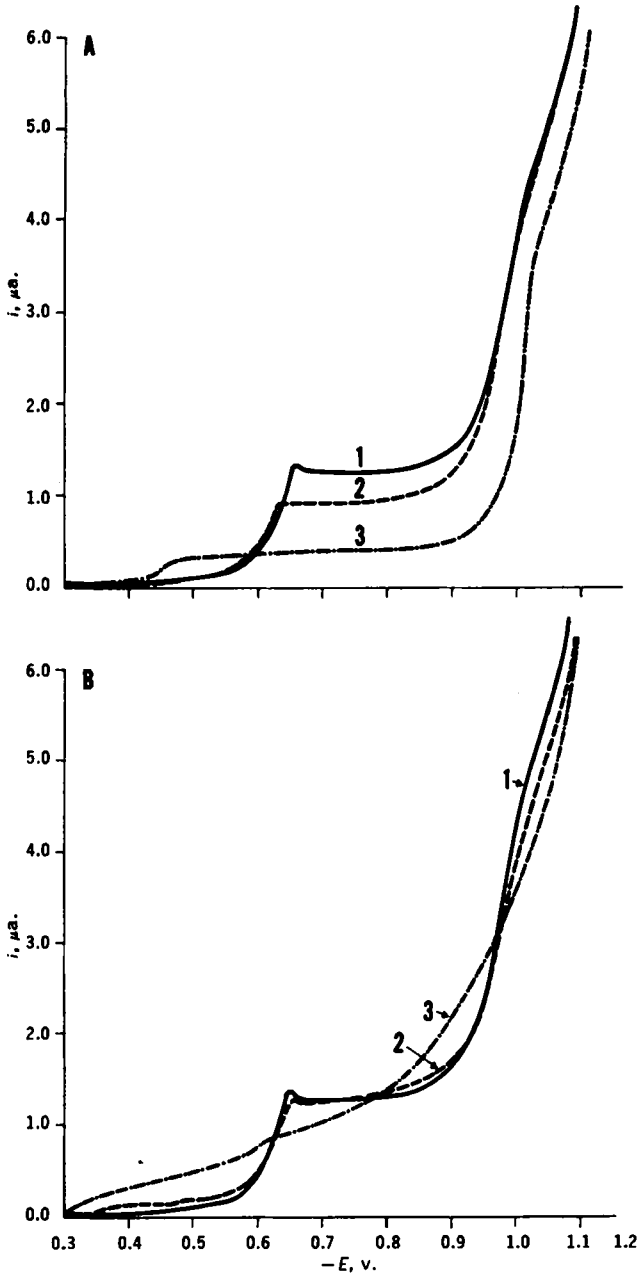


Figure 1—*(A) Polarography of acid hydrolysis sequence. Key: 1, initial; 2, 10 min. at 60°; and 3, 20 min. at 80°. (B) Polarography of base hydrolysis sequence. Key: 1, initial; 2, 0.1 meq. NaOH for 10 min. at room temperature; and 3, 0.1 meq. NaOH for 10 min. at 45°. All solutions were 0.22 m*M *in 1 at pH 2.0. The* $m^{2/3}t^{1/6}$ *of the dropping mercury electrode at open circuit in air-saturated 0.1* M *potassium chloride is 1.720.*

Concentration Dependence—The half-wave potentials of waves I and II are dependent on concentration, becoming more negative with increasing concentration (Table II). Wave I exhibits what appears to be a maximum of the first kind (3) that decreases more rapidly than the diffusion current with decreasing concentration and shows a negative dependence on surfactant[4] concentration; however, the addition of surfactant results in wave II merging with background. An increase in the ionic strength of the buffer from 0.4 to 0.8 *M* had no effect on the maximum. All data reported are in the absence of surfactant.

In the concentration range examined, *i.e.*, 0.07–0.43 m*M*, the diffusion currents of waves I and II are directly proportional to concentration (Table II). The dependences are i_{dI} = 4.41 μa./m*M* + 0.03 μa. and i_{dII} = 12.63 μa./m*M* − 0.15 μa., respectively. The *RSD* of waves I and II for four individually weighed 0.20 m*M*, 0.6 *M* ionic strength, pH 2.3 samples are ±2.6 and ±2.4%, respectively.

[4] Triton X-100.

Table I—Effect of pH on Polarographic Reduction of I

	Wave I			Wave II		
pH[a]	$-E_{1/2}$, v.	i_d, μa.	i/C, μa./m*M*	$-E_{1/2}$, v.	i_d, μa.	i/C, μa./m*M*
1.60	0.58	2.04	4.77	—	—	—
2.32	0.66	1.95	4.56	1.00	5.76	13.46
3.30	0.73	1.90	4.44	1.08	4.58	10.70
4.84[b]	—	—	—	1.26	4.52	10.56
5.98[c]	—	—	—	—	—	—

[a] Buffer systems described in *Procedure*. [b] Very poorly defined wave I. [c] Very poorly defined waves I and II.

Table II—Effect of Concentration on Polarographic Reduction of I at pH 2.3

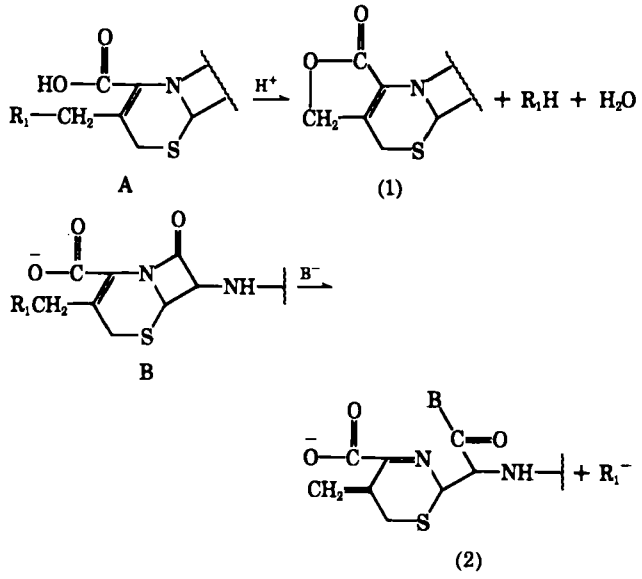
	Wave I			Wave II		
Concentration, m*M*	$-E_{1/2}$, v.	i_d, μa.	i/C, μa./m*M*	$-E_{1/2}$, v.	i_d, μa.	i/C, μa./m*M*
0.07	0.60	0.34	4.86	0.97	0.78	11.15
0.15	0.62	0.70	4.65	0.98	1.81	12.06
0.22	0.63	1.02	4.64	0.98	2.60	11.81
0.26	0.64	1.19	4.58	0.99	3.05	11.72
0.37	0.66	1.65	4.46	1.00	4.58	12.38
0.43	0.66	1.95	4.56	1.00	5.76	13.46

Acid Hydrolysis Stability—A 0.22 m*M*, 0.6 *M* ionic strength, pH 2.0 solution of I was subjected to three heating conditions. Results of two are shown in Fig. 1A. The polarograms show a decrease in wave I, essentially no effect on wave II, and the development of a reduction wave at −0.45 v. The third hydrolysis at 25° for 1 hr. did not detectably degrade I. A 1.95 m*M*, pH 2.3 solution of I was degraded at 80° for 15 min. and examined by an iodometric β-lactam assay and TLC. The iodometric assay, as compared to nondegraded I, indicated a 61% reduction in iodine uptake and a very large increase in the blank iodine uptake. The blank of the degraded material used approximately 1.7 times the iodine absorbed by the nondegraded sample. TLC in acetic acid–dioxane–acetone (1:2:8) on silica gel indicated a total loss of I, some material at the point of application, and two spots, both more mobile than I (iodine chamber development). The lead spot corresponded in R_f to the thiol-thiadiazole side chain of I. The less mobile spot corresponded to the lactone of I (Scheme IA) (1).

Base Hydrolysis Stability—Two 5.5-mg. samples of I were reacted with 0.1 meq. of base as 0.05 *N* NaOH under two different heating conditions. Polarograms of the resulting material at a concentration of 0.22 m*M* with respect to I and at a pH of 2.0 are shown in Fig. 1B. There is a decrease in both waves I and II and development of two ill-defined reduction waves at −0.35 and −0.48 v. A 2.5-mg. sample of I was degraded in 6 ml. of 0.17 *N* NaOH for 80 min. at room temperature. TLC, using the system previously described, revealed two new spots. The spot more mobile than I corresponded to the thiol-thiadiazole side chain of I. The second spot was less mobile than I and could be better described as a smear, most probably made up of material containing degraded β-lactam (see *Discussion*).

β-Lactamase Stability—Treatment of I with β-lactamase from *B. cereus* at pH 7 and 25° resulted in a 54% decrease in the diffusion current of wave I at pH 2.3 over 2 hr. The degradation produced a very small reduction wave at −0.36 v.

Polarography of 5-Methyl-1,3,4-thiadiazole-2-thiol (II)—Since II is indicated as a product of at least two different degradation paths of I and is also a probable intermediate in the synthesis of I (see *Discussion*), it seemed reasonable to try to develop a polarographic method to determine II. The compound is not reducible; therefore, it was decided to look into the possibility of an oxidation. The McIlvaine buffers used to determine I contain chloride ion, which results in an early anodic discharge at the dropping mercury electrode. However, II gives a well-defined anodic wave in pH 5.7, 0.7



Scheme I—Cephalosporin degradative pathways: A, acid hydrolysis; and B, base hydrolysis

M acetate buffer which is proportional to concentration within a relative standard deviation of ±6.0% (Table III). The half-wave potential is quite concentration dependent. The oxidation is assumed to involve the formation of an insoluble mercurous salt. This oxidation can be used as a supplemental analytical method to follow the degradation pathways of I which involve the loss of II and also as a control method for II in nondegraded I.

Coulometry—Coulometric reduction of I in pH 2.3 buffer on the limiting current plateau of wave I resulted in *n* values of 1.85 and 1.94. No anodic waves corresponding in $E_{1/2}$ to wave I were found in the electrolyzed solutions. Wave II was considered to be too close to background discharge for coulometric *n* value determination. However, comparison of diffusion currents for waves I and II indicates a five- or six-electron transfer for wave II.

Polarography of 3-Acetoxymethyl-7-[2-(3-sydnone)acetamido]-3-cephem-4-carboxylic Acid, Sodium Salt (III)—A 0.54 m*M* solution of III, in which an acetoxy group has been substituted for the thiol-thiadiazole group, in pH 2.3 McIlvaine buffer exhibits only one reduction wave at −1.01 v. with an *i/C* value of 14.3 μa./m*M*.

Polarography of 7-(Thiophene-2-acetamido)cephalosporanolactone (IV)—Compound IV, with a thiophene in place of a sydnone and an α,β-unsaturated lactone at the 3–4 position (Scheme IA) (1), showed no reduction waves in pH 2.3 McIlvaine buffer.

DISCUSSION

Cephaloridine, cephalothin, and cephalosporin C, as reported by Jones *et al.* (1), each exhibit one reduction wave. The polarographic $E_{1/2}$'s of the three compounds are pH dependent and concentration dependent, and the reduction wave of cephaloridine is diffusion controlled. Compound I exhibits two reduction waves, both showing the same characteristics with respect to pH and concentration as has been demonstrated. The $E_{1/2}$ concentration dependence of all four compounds is indicative of a polarographically irreversible reduction. Final solutions, completely electrolyzed with respect to the first reduction wave of I, showed no anodic wave with an $E_{1/2}$ corresponding to that of wave I, further proof of an irreversible reduction. Based on preliminary diffusion current data, Jones *et al.* (1) assumed that cephaloridine was reduced *via* a two-electron transfer. Coulometry, on the first reduction of I, confirms a net two-electron transfer for the compound and, based on *i/C* data, suggests a five- to six-electron transfer for the second reduction wave.

Jones *et al.* (1) made no attempt to assign the functionality in cephaloridine that undergoes reduction. Comparison of the reduction patterns of I and cephaloridine shows similar half-wave potentials for the second reduction of I and the reduction of cephaloridine. However, the former is a five- to six-electron transfer, and the latter is assumed to be a two-electron transfer. Sydnones, as reported by Zuman (4), are reduced in a single six-electron step under acidic conditions, which supports a hypothesis that wave II represents the reduction of the sydnone side chain of I, a functionality not present in cephaloridine.

Table III—Effect of Concentration on the $E_{1/2}$ and i_d of II

Concentration, m*M*	$-E_{1/2}$, v.	$-i_d$, μa.
0.30	0.09	0.76
0.60	0.11	1.67
0.91	0.12	2.64
1.21	0.12	3.52
1.51	0.13	4.41

The acid hydrolysis of I, as followed by TLC, results in the loss of II and the formation of the lactone of I. Polarographically, acid hydrolysis results in the loss of the first reduction wave and the retention of the second reduction wave. These two observations point to the first reduction of I as involving the side chain (II) at the 3-position. This conclusion is supported by the fact that III, which has an acetoxy group in the 3-position in place of the thiol-thiadiazole and the same acetamido grouping at the 7-position, exhibits only one reduction wave at −1.01 v., corresponding to the proposed sydnone reduction of I. Compound II is not reducible under similar conditions.

It was recently reported (5) that cephalosporin C, upon macroelectrolysis at a mercury pool electrode at pH 6.6, is converted to the deacetoxy compound, *i.e.*, the acetoxy group at the 3-position is reductively eliminated. It seems reasonable to assume, then, that the first reduction wave of I represents a similar elimination in which the thiol-thiadiazole group is expelled from the molecule. Conclusions from an extension of these arguments to the reduction waves of cephalosporin C and cephalothin, as reported by Jones *et al.* (1), are obvious. The reduction of cephaloridine, which probably also involves the 3-position side chain, may be complicated by its zwitterion character. Moreover, the first reduction wave of I is approximately 300 mv. more positive than the reduction wave of cephaloridine and approximately 600 mv. more positive than the reductions of cephalosporin C and cephalothin at the same pH. This indicates a substituent effect on the reduction caused by the functionality at the 3-position.

Cephalosporins, in general, easily undergo acid (6) and base (7) hydrolysis (Scheme I). Acid hydrolysis results in the loss of the R group at the 3-position and formation of an α,β-unsaturated lactone. Base hydrolysis opens the β-lactam moiety and simultaneously eliminates the R group at the 3-position. TLC has tentatively supported these degradation pathways for I. Enzymatic deactivation of cephalosporins is believed (7) to follow a reaction path similar to that of base hydrolysis, again with loss of the R group. As can be seen from Figs. 1A and 1B, the acid and base hydrolysis of I can be followed by polarography. The enzymatic degradation also results in loss of wave I of I. The key to this specificity is believed to be the loss of the R group at the 3-position. The identities of the new polarographic waves produced *via* the three degradation paths have not been established. The lactone functionality, from the polarography of IV, is not responsible for any of them. However, Structure 2 in Scheme IB could be polarographically reducible.

As mentioned under the polarography of II, II should also be a contaminant in nondegraded I. This conclusion is based on the reported (7) method of synthesis of compounds containing R groups at the 3-position other than —H or —$OCOCH_3$. The reaction is a nucleophilic displacement wherein an excess of free side chain is used to replace the naturally occurring —$OCOCH_3$ function of the fermentation product, cephalosporin C. The oxidation of II, therefore, should not only be useful in following the degradation of I, and presumably other cephalosporins with similar 3-position substituents, but should also be useful in examining newly synthesized cephalosporin for side-chain contaminant.

CONCLUSION

The polarographic reduction of I consists of two waves. The first is believed to be the two-electron reductive elimination of the 3-position substituent. The second is believed to be the six-electron reduction of the sydnone acetamido 7-position substituent. Both waves can be utilized as control methods for the analytical determination of I. Only the first wave, however, can be used to follow the

acid, base, and enzymatic degradations of I which involve the loss of the 3-position substituent.

When taking into consideration the work of Jones *et al.* (1), it is believed that the polarographic method should be generally applicable to all cephalosporins containing a leaving group at the 3-position. Those free leaving groups containing a thiol functionality should also be amenable to polarographic determination *via* oxidative mercurous salt formation.

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4-Anilidopiperidine Analgesics I: Synthesis and Analgesic Activity of Certain Ring-Methylated 1-Substituted 4-Propananilidopiperidines

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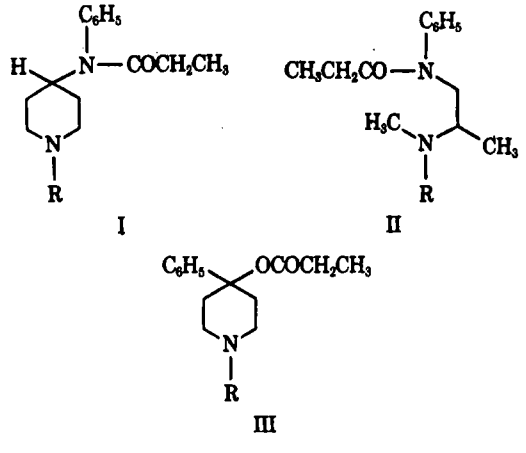
Abstract □ In view of the potency-enhancing effect of methyl substitution of the piperidine ring of the 4-phenylpiperidine analgesics and the alkylene chain of the acyclic basic anilide analgesics, the 1-methyl, 1-benzyl, and 1-phenylethyl derivatives of 2-methyl-, 3-methyl-, and 2,5-dimethyl-4-propananilidopiperidine were prepared. The analgesic activity of these compounds indicates that 3-methylation has the greatest effect in enhancing analgesic potency whereas 2-methyl and 2,5-dimethyl substitution is detrimental to analgesic activity.

Keyphrases □ 4-Anilidopiperidine analgesics—synthesis and activity of ring-methylated 1-substituted 4-propananilidopiperidines □ Analgesics, potential—synthesis of ring-methylated 1-substituted 4-propananilidopiperidines, structure–activity relationships □ Structure–activity relationships—4-anilidopiperidines and analgesic activity, effect of ring methylation

Fentanyl[1] (I, R = $C_6H_5CH_2CH_2$) is a potent narcotic analgesic which possesses a rapid onset and short duration of action (1). Its pharmacological profile is very similar to other morphinomimetic compounds, except that fentanyl is a considerably more potent narcotic analgesic (2). Structurally, fentanyl may be characterized as a 4-anilidopiperidine derivative. This class of synthetic narcotic analgesics exhibits structural features also found in the acyclic basic anilide analgesics (II) and the 4-phenylpiperidine analgesics (III).

In general, studies of structure–activity relationships in the 4-anilidopiperidine class (3–6) indicate that structural requirements for analgesic activity are similar to those established for both the acyclic basic anilides and the 4-phenylpiperidines. One aspect of the structure–activity relationships of the 4-anilidopiperidines that has not as yet been reported is the effect of piperidine ring methylation in this class. It is well established that methylation of the piperidine ring of the 4-phenylpiperidine analgesics (*e.g.*, the prodines) results in a significant increase in analgesic activity. In addition, introduction of a methyl substituent on the alkylene chain of the acyclic basic anilides provides for compounds of high analgesic activity. In this regard, however, it is important to note that the increase in analgesic activity in these analogs is highly dependent on the position of the methyl substituent relative to the basic nitrogen. In the 4-phenylpiperidines, 3-methylation and 2,3-, 2,5-, and 3,5-dimethylation result in high analgesic activities (7). Other methyl substitution patterns in this class generally reduce analgesic activity. In the acyclic basic anilide analgesics, methyl substitution α to the basic nitrogen affords the greatest enhancement of analgesic activity (3).



[1] Fentanyl citrate, Sublimaze, McNeil Laboratories, Inc.