N-Halo Derivatives VI: Microbiological and Chemical Evaluations of 3-Chloro-2-oxazolidinones

MASANORI KOSUGI *, JAMES J. KAMINSKI, SALLY H. SELK, IAN H. PITMAN ‡, NICOLAE BODOR ˣ, and TAKERU HIGUCHI ‡

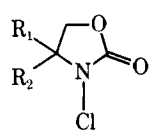
Abstract □ Comparative antimicrobial activity of 3-chloro-2-oxazolidinone (I), 3-chloro-4-methyl-2-oxazolidinone (II), 3-chloro-4,4-dimethyl-2-oxazolidinone (III), and *N*-chlorosuccinimide (IV) was evaluated in aqueous buffers in the absence and presence of 5% horse serum. All four compounds had similar bactericidal activity in the absence of horse serum, but I and III had superior activity relative to IV when serum was present. Compound III was considerably more stable with respect to loss of positive chlorine and bactericidal activity than I and II when stored in 0.1 *M* sodium dihydrogen phosphate buffered to pH 7.0 at 40°. Thus, III is potentially the most useful bactericidal agent of those evaluated. The chlorine potentials of I, II, and III, the rate constants for transfer of positive chlorine from I and III to morpholine in aqueous solutions, and the hydrolytic stabilities of I and III with respect to loss of positive chlorine were evaluated. These data, together with previously calculated data for IV, are used to rationalize the observed bactericidal activities.

Keyphrases □ Nitrogen–halogen compounds—3-chloro-2-oxazolidinones, antimicrobial activity screened, chlorine potentials evaluated □ 3-Chloro-2-oxazolidinones—antimicrobial activity screened, chlorine potentials evaluated □ Antimicrobial agents, potential—3-chloro-2-oxazolidinones screened □ Chlorine potentials—3-chloro-2-oxazolidinones evaluated □ Structure–activity relationships—3-chloro-2-oxazolidinones and antimicrobial activity

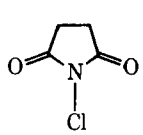
Recent studies on the chemical (1–4) and antimicrobial properties (4, 5) of *N*-chloro compounds led to the conclusion that 3-chloro-4,4-dimethyl-2-oxazolidinone (III) should be an extremely useful bactericidal agent (6). The minimum inhibitory concentration (MIC) of III against bacteria such as *Staphylococcus aureus, Escherichia coli, Salmonella typhimurium,* and *Bacillus subtilis* was approximately 1000 ppm, and III was an effective bactericidal agent in the presence of 5% horse serum, a potential denaturing agent (5). In addition, the absence from the molecule of hydrogen atoms adjacent to the nitrogen–chlorine bond (3) is expected to stabilize its solutions against loss of positive chlorine [*cf.*, the stability of its 3-bromo analog (2)], and the product of hydrolytic decomposition of its nonchlorinated analog would be the relatively nontoxic 2-amino-2-methyl-1-propanol (7).

The objectives of these studies were twofold: (*a*) to compare the antimicrobial activity of III with that of related compounds, 3-chloro-2-oxazolidinone (I), 3-chloro-4-methyl-2-oxazolidinone (II), and *N*-chlorosuccinimide (IV); and (*b*) to compare some chemical properties of these compounds such as the chlorine potential, rate of positive chlorine transfer to a nitrogen-containing acceptor, and stability of aqueous solutions with respect to loss of positive chlorine with their antimicrobial properties.

This comparative study was made to provide useful information for the design of new *N*-chloro antimicrobial agents.



I: $R_1 = R_2 = H$
II: $R_1 = CH_3$, $R_2 = H$
III: $R_1 = R_2 = CH_3$



IV

EXPERIMENTAL

Equipment—Spectrophotometric measurements were made on instruments[1] with thermostated cell compartments. All thermostated water baths were maintained at 25.0 ± 0.1°.

Materials—All reagents were of analytical grade unless otherwise stated. *N*-Chlorosuccinimide[2] was used without further purification. All water was double distilled using a Pyrex still. 2-Oxazolidinone and 4,4-dimethyl-2-oxazolidinone were prepared as described previously (8).

3-Chloro-2-oxazolidinone (I)—Chlorine was bubbled through 30 ml of an aqueous solution of 2-oxazolidinone (8.7 g, 0.1 mole) for 0.5 hr at 0°. A white solid, which precipitated, was extracted with dichloromethane. The dichloromethane extract was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The white solid that was obtained was sublimed (40°/0.2 mm Hg) and yielded 8.57 g (0.071 mole, 71%) of I, mp 59–61°; UV (H_2O): λ_{max} 248 nm (ϵ = 296 M^{-1} cm^{-1}); PMR ($CDCl_3$): δ 3.6–5.0 (*AA'BB'*, 4H) ppm.

Anal.—Calc. for $C_3H_4ClNO_2$: C, 29.65; H, 3.32; Cl, 29.2; N, 11.53. Found: C, 29.95; H, 3.65; Cl, 28.7; N, 11.77.

3-Chloro-4-methyl-2-oxazolidinone (II)—This compound was prepared from 4-methyl-2-oxazolidinone in essentially the same way as I was prepared from 2-oxazolidinone, resulting in a liquid, bp 85–90°/1 mm Hg; IR (neat): 3000, 1780, 1390, 1195, and 1040 cm^{-1};

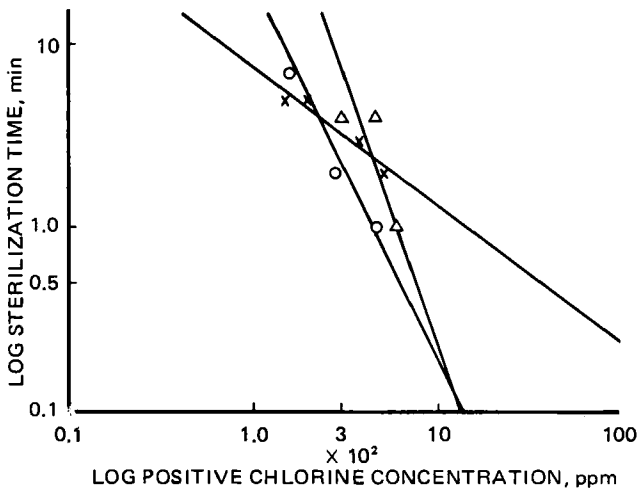


Figure 1—*Plot of the sterilization time for a suspension of* Staph. aureus *against the concentration of I* (○), *II* (△), *and III* (×) *expressed as parts per million of positive chlorine. Solutions were buffered to pH 7.0 with sodium dihydrogen phosphate.*

[1] Cary 14, 15, and 16 spectrophotometers, Varian Instruments, Inc.; and Durrum-Gibson stopped-flow spectrophotometer, Durrum Instrument Corp.

[2] Aldrich Chemical Co.

Table I—Effect of Horse Serum on the Contact Bactericidal Efficiency of I, III, and IV[a]

Compound	Positive Chlorine, ppm	Horse Serum, %	Sterilization Time[b], min					
			Staph. epidermidis	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*Staph. aureus*	*B. bronchiseptica*
I	298	0	0.5[c]	0.5	0.5	0.5	1	0.5
	298	5	1	0.5	0.5	0.5	3	0.5
III	311	0	2	0.5	1	0.5	2	0.5
IV	311	5	3	0.5	1	0.5	3	0.5
	301	0	0.5	0.5	0.5	0.5	0.5	0.5
	301	5	7	3	8	9	>10	5

[a] pH 4.6 in 0.1 *M* sodium acetate. [b] Time intervals screened were 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 min. [c] Due to limitations in the time scale of the antimicrobial experiment, sterilization time intervals of 0.5 min may not be a true indication of the actual contact time required to sterilize the microorganisms tested. Sterilization of the organisms during this time interval should be more correctly interpreted as less than or equal to 0.5 min.

PMR ($CDCl_3$): δ 4.5 (m, 1H), 4.0 (m, 2H), and 1.4 (d, 3H, J = 6 Hz) ppm.

Anal.—Calc. for $C_4H_6ClNO_2$: C, 35.44; H, 4.46; Cl, 26.2; N, 10.33. Found: C, 35.64; H, 4.59; Cl, 26.5; N, 10.14.

3-Chloro-4,4-dimethyl-2-oxazolidinone (III)—This compound was prepared from 4,4-dimethyl-2-oxazolidinone, using the method described for I and II. Sublimation of the reaction product at 60°/0.25 mm Hg yielded a white solid, mp 71–72.5°; UV (H_2O): λ_{max} 248 nm (ϵ = 274 M^{-1} cm^{-1}); PMR ($CDCl_3$): δ 4.23 (s, 2H) and 1.40 (s, 6H) ppm.

Anal.—Calc. for $C_5H_8ClNO_2$: C, 40.15; H, 5.39; Cl, 23.7; N, 9.37. Found: C, 40.36; H, 5.36; Cl, 23.4; N, 9.35.

Chlorine Potentials—Chlorine potentials were calculated from the measured equilibrium constants for the reactions of I, II, or III with succinimide to yield the appropriate 2-oxazolidinone and IV or from those for the reverse reactions. The calculation method was described previously (9). In a typical experiment, a volume of a freshly prepared solution of the *N*-chloro compound in water was mixed with an equal volume of a solution of the appropriate nonchlorinated compound in 0.1 *M* phosphate buffer. The ionic strength of the reaction solution was 1.0 *M*. The reaction solution was equilibrated at 25°, and then its UV absorbance was measured. The method of calculating equilibrium constants from absorbance measurements was essentially the same as that described previously (10).

Rates of Chlorine Transfer—A volume of a freshly prepared solution of I or III (10^{-3}–10^{-4} *M*) in water was mixed with an equal volume of an aqueous solution of morpholine (10^{-1}–10^{-2} *M*). The pH value of the latter solution was adjusted with perchloric acid, and the ionic strength was maintained at 2.0 *M* with sodium perchlorate. Pseudo-first-order rate constants for the chlorine transfer were calculated from UV absorbance changes accompanying the reaction.

Table II—Stability of Aqueous Solutions[a] of I–III at 40° with Respect to Loss of Positive Chlorine and Bactericidal Activity

Compound	Positive Chlorine[b], Initial, ppm	Sterilization[c] Time, min	Positive Chlorine[d], Final, ppm	Sterilization[c] Time, min
I	465	1	30	>30
II	610	1	4	>30
III	534	2	527	2

[a] The solutions were buffered to pH 7.0 using 0.1 *M* sodium dihydrogen phosphate. [b] Concentration expressed as parts per million of positive chlorine at the time of preparation of the sterilizing solutions. [c] Time for sterilization of a suspension of *Staph. aureus*. Time intervals screened were 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, and 30 min. [d] Concentration of positive chlorine remaining in the sterilizing solution after storage for 14 days at 40°.

Table III—Chlorine Potentials, pK_{cp} Values, of I–IV at 25°

Compound	pK_{cp}
I	7.01[a]
II	7.41[a]
III	8.01[a]
IV	7.71[b]

[a] The ionic strength of 1.0 *M* was adjusted using sodium perchlorate. [b] From Ref. 9.

Stability of I and III in Aqueous Solutions—Solutions of I and III in 0.1 *M* sodium acetate solutions buffered at pH 4.6, in 0.1 *M* sodium dihydrogen phosphate solutions buffered at pH 7.0, and in sodium borate solutions buffered at pH 9.3 were stored at 40°. The stability of the solutions with respect to loss of positive chlorine was determined from results of iodometric assays for positive chlorine.

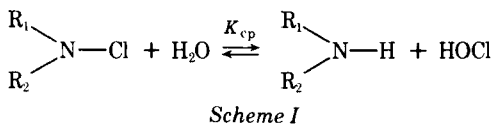
Antimicrobial Activity of I–IV—The organisms and methods were the same as those described previously (5).

RESULTS AND DISCUSSION

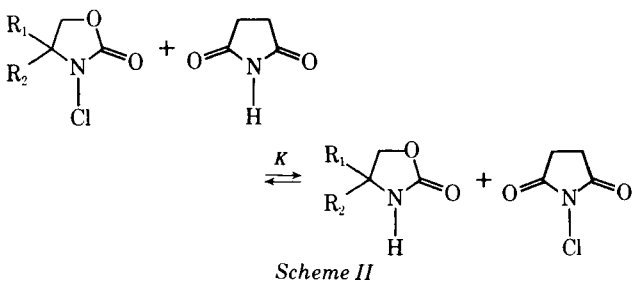
A bactericidal efficiency test was conducted to determine the contact time required to sterilize a bacterial suspension by particular concentrations of the test substances. *Staphylococcus aureus* (ATCC 6538) was the bacterium used, and the test solutions were buffered to pH 7.0 with 0.1 *M* sodium dihydrogen phosphate. The concentration of positive chlorine and, hence, the concentration of *N*-chloro compound in the solutions were determined by iodometric assay. Each compound had similar bactericidal activities as judged by this test[3] (Fig. 1).

The second type of test involved comparison of the time required to sterilize suspensions of various organisms in aqueous buffers and in the same buffers containing 5% horse serum (Table I). The data demonstrate that IV was much less active in the solution containing the serum (*i.e.*, in the presence of extraneous organic material including proteins) than in the pure buffer, whereas I and III were active bactericidal agents in the absence and presence of the horse serum denaturant.

The third test was designed to evaluate the length of time that aqueous solutions of I–III remain bactericidal. Such solutions were prepared in 0.1 *M* sodium dihydrogen phosphate buffered to pH 7.0 and divided into two portions. One portion was assayed for positive chlorine content and then subjected to the bactericidal efficiency test using *Staph. aureus*. The other portion was stored at 40° for 14 days when it was assayed for positive chlorine content and subjected to the bactericidal efficiency test. The solutions of I and II lost most of their



Scheme I



Scheme II

[3] The nonchlorinated oxazolidinone analogs of I (1823 ppm), II (1554 ppm), and III (1404 ppm) did not sterilize the bacterial suspension in 30 min. Therefore, the bactericidal activity of the 3-chloro derivatives is much greater than that of the parent compounds.

Table IV—Equilibrium Constants[a] for Exchange of Positive Chlorine between an *N*-Chloro Compound and a Nitrogen-Containing Receptor

Reaction	pH	*K*
IV + 2-oxazolidinone	6.59	0.2
I + succinimide	6.76	5.0
IV + 4-methyl-2-oxazolidinone	6.85	0.5
IV + 4,4-dimethyl-2-oxazolidinone	6.83	2.0
III + succinimide	6.68	0.5

[a] The ionic strength of 1.0 *M* was adjusted using sodium perchlorate. The analytical wavelength was 270 nm.

Table V—Second-Order Rate Constants for Transfer of Positive Chlorine from I, III, and IV to Morpholine at 25° and Ionic Strength 1.0 *M*

Compound	pH	k_{obs}, M^{-1} sec^{-1}	k, M^{-1} sec^{-1}[a]
I	6.78	1.0	
	7.19	3.2	112 ± 13
	7.72	9.2	
III	6.76	0.24	
	7.25	0.82	
	7.33	0.78	24 ± 3
	7.70	2.2	
	7.73	1.7	
IV			6667[b]

[a] Values of k are independent of pH and refer to the reaction between the morpholine neutral molecule and the neutral *N*-chloro compound:

$$k_{obs}\left[1 + \frac{[H^+]}{K_{a,\text{morpholine}}}\right] = k$$

The pKa of morpholine was taken to be 8.77 (15). [b] From Ref. 9.

positive chlorine content during 14 days; consequently, the bactericidal activity of the solutions was reduced (Table II). The solution of III retained both its original positive chlorine content and its bactericidal activity during 14 days at 40°.

The second phase of this study was concerned with comparing the bactericidal properties of I–IV with some of their chemical properties. The chlorine potential, pK_{cp} value of an *N*-chloro compound has been defined (9) as $-\log K_{cp}$, where K_{cp} is the equilibrium constant for the reaction shown in Scheme I.

The chlorine potential values (Table III) were calculated from the equilibrium constants described in Table IV, which are for positive chlorine exchange between I, II, or III and succinimide or for the reverse reactions (Scheme II).

The methods used for converting the latter equilibrium constants into chlorine potential, pK_{cp}, values were described previously (9). The chlorine potential is thus a measure of the thermodynamic stability of an *N*-chloro compound relative to its nonchlorinated analog. An *N*-chloro compound spontaneously transfers its chlorine to another nitrogen-containing molecule if the chlorine potential of the product is larger than that of the reactant. Hence, the fact that I–IV have similar chlorine potentials (within the range of 7.51 ± 0.50) indicates that they would have similar "thermodynamic tendencies" to transfer their positive chlorine to receptor molecules.

These receptor molecules may be constituents of the solvent (*i.e.*, to yield hypochlorous acid in water), of extraneous organic material (*e.g.*, proteins and other denaturing agents), or of bacteria. The reaction of the *N*-chloro molecule or of hypochlorous acid with molecules that are constituents of bacteria has been suggested (5, 11) to be involved in the bactericidal action of *N*-chloro compounds. The similarity of the chlorine potentials of these molecules thus seems to be consistent with the observation that they have similar bactericidal activity in aqueous suspensions of bacteria that are not contaminated with a denaturing agent such as horse serum.

Earlier (12), it was suggested that pK_{cp} values of *N*-chlorinated imides and amides were related to the acid dissociation constants of the imides or amides by a rather complex relationship. The use of this relationship leads to the prediction that the pK_{cp} values of I, II, and III would be 8.0, 8.5, and 8.4, respectively. The fact that the actual values were slightly smaller than the predicted values is probably due to the different electronic character of the nitrogen atom in the oxazolidinones as compared to that in the amides and imides discussed previously (12). However, it is apparent that the linear free energy relationship described previously (12) does provide a useful means of obtaining an estimate of the pK_{cp} value of an *N*-chloro compound.

The mechanism of chlorine transfer from *N*-chloro compounds to other organic molecules (*e.g.*, proteins or bacterial constituents) may involve hydrolysis of the molecules to hypochlorous acid, their chlorination by hypochlorous acid, and direct transfer of the chlorine to the receptor (13). It was argued previously (14) that the second mechanism was likely to be relatively important because the rate of chlorine exchange between molecules such as IV and nitrogen-containing receptors was much faster than the rate of hydrolysis of the *N*-chloro compound.

The second-order rate constants for transfer of chlorine from I, III, and IV to morpholine to yield *N*-chloromorpholine are described in Table V. The value for IV was reported earlier; those for I and III were calculated from the pseudo-first-order rate constants for formation of *N*-chloromorpholine in solutions containing I or III (10^{-4} *M*) and morpholine (5×10^{2}–2×10^{-1} *M*). Measurements of UV absorbance at the conclusion of the reactions indicated that stoichiometric amounts of *N*-chloromorpholine were formed. Values of a pH-dependent second-order rate constant, k_{obs}, were calculated from the slope of linear plots of pseudo-first-order rate constants against the concentration of morpholine plus morpholine cation in the solution. A pH-independent rate constant, k, was obtained when k_{obs} values were multiplied by the factor $(1 + [H^+]/K_{a,\text{morpholine}})$. Hence, k values are believed to be the second-order rate constants for a reaction between the *N*-chloro compound and morpholine neutral molecules. Values of both k_{obs} and k are described in Table V.

It can be seen that while I, III, and IV have similar chlorine potentials, the rates at which they transferred positive chlorine to morpholine differed considerably. Compound IV was nearly 300 times more reactive than III and 60 times more reactive than I. This difference in reactivity may explain why IV was less bactericidal than I and III in solutions containing horse serum. These solutions were kept for 30 min before being inoculated with bacteria, and a higher fraction of molecules of IV may have transferred their positive chlorine to protein molecules or other receptors than was the case with I and III.

The results in Table II clearly indicate that III is much more stable with respect to loss of positive chlorine than I or II in aqueous solution. Further confirmation of this fact comes from a comparison of the half-lives, $t_{1/2}$, for the positive chlorine content of solutions of I and III (Table VI). The positive chlorine content was determined by iodometric titration. Hence, III was considerably more stable than I at pH values of 4.6, 7.0, and 9.3. The products of these reactions were not determined, but the relative stabilities of the reactants are consistent with an earlier observation (3) that *N*-chloro compounds that do not contain hydrogen atoms adjacent to the nitrogen–chlorine bond are more stable with respect to loss of positive halogen than are compounds that do contain these α-hydrogen atoms.

The results of this study led to the following three conclusions that should be generally applicable in the design of *N*-chloro compounds:

1. Compounds with chlorine potentials between 7 and 8.5 are ef-

Table VI—Hydrolytic Stabilities of I and III at 40°

Compound	Solvent	Half-Life, hr
I	0.1 *M* Sodium acetate, pH 4.6	1872
	0.1 *M* Sodium dihydrogen phosphate, pH 7.0	42.3
	0.1 *M* Sodium borate, pH 9.3	1.3
III	0.1 *M* Sodium acetate, pH 4.6	9600
	0.1 *M* Sodium dihydrogen phosphate, pH 7.0	3792
	0.1 *M* Sodium borate, pH 9.3	140

fective bactericidal agents against various bacteria.

2. The rate at which chlorine is transferred from an *N*-chloro compound to a nitrogen-containing receptor should be considered along with the chlorine potential of the molecules in estimating the likely bactericidal activity of the compound in solutions containing proteins and other organic material.

3. *N*-Chloro compounds that do not contain hydrogen atoms adjacent to the nitrogen–chlorine bond are likely to be more stable than those that do with respect to loss of bactericidal activity in aqueous solution.

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* Present address: Department of Chemistry, Gunma University, Kiryu, Gunma, Japan.

‡ Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66045

ˣ To whom inquiries should be directed.

Effect of Storage at Specified Temperature and Humidity on Properties of Three Directly Compressible Tablet Formulations

S. T. HORHOTA *, J. BURGIO *, L. LONSKI *, and C. T. RHODES ˣ

Abstract □ Direct compression tablets containing sodium starch glycolate, an alginate derivative, or povidone as a disintegrant, magnesium stearate as a lubricant, amaranth as a tracer, and dibasic calcium phosphate dihydrate as the matrix were stored for 30 days at 23° and 75% relative humidity (R.H.), 45° and 75% R.H., and 65° and 40% R.H. Samples were evaluated after 0, 10, 20, and 30 days for size, hardness, and dissolution characteristics. Although no significant changes in the dimensions or hardness of the three tablet formulations, prepared at three different compaction pressures, were observed, the dissolution efficiency of the systems showed significant changes, some systems dissolving more rapidly and some more slowly after storage. In some cases, the changes were so substantial as to indicate the possibility of significant changes of the bioavailability of drugs formulated in such systems. The relevance of this work to the problem of evaluating aging effects on the physical properties of tablets is discussed.

Keyphrases □ Tablets, direct compression—size, hardness, and dissolution, effect of storage at various temperatures and humidity □ Size, tablet—effect of storage at various temperatures and humidity □ Hardness, tablet—effect of storage at various temperatures and humidity □ Dissolution, tablet—effect of storage at various temperatures and humidity □ Dosage forms—direct compression tablets, size, hardness, and dissolution, effect of storage at various temperatures and humidity

Methods for evaluating the chemical stability of drug substances and pharmaceutical products are well established, and the industry now makes considerable use of storage under temperature stress conditions to predict chemical shelflife. Recently, the problem of biological availability has received considerable attention, both scientific and political, and there is particular concern about factors that may modify the dissolution of drugs from compressed tablets (1, 2).

The problem of tablet aging with accompanying changes in dissolution time has received little attention, although it has been the cause of some recalls, and there now is increasing concern within the industry regarding this problem. Some formulators are using accelerated storage samples to screen for possible aging effects. Furthermore, some workers are placing considerable reliance on tablet hardness as a general indicator of tablet aging, the implicit assumption being that invariance in hardness contraindicates changes in dissolution. Unlike simple chemical decomposition, no well-established theory relates the effects of storage under stress conditions to shelflife.

The present paper reports a study of the effect of storage of compressed tablets, under three sets of stress conditions, on tablet hardness, size, and dissolution properties. The purpose of this investigation was to determine if any simple relationship exists between changes in dissolution properties and storage under