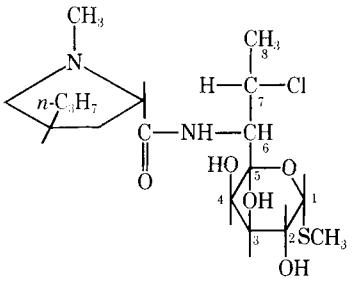
Aqueous Stability of Clindamycin

T. O. OESTERLING

Abstract □ The kinetics and mechanism of degradation of the antibiotic clindamycin were studied in buffered aqueous solution in the pH range 0.4–12. Clindamycin showed maximum stability at pH 3–5; however, high temperature studies indicated that not more than 10% degradation will occur in the pH range 1–6.5 after two years at 25°. In the pH range 0.4–4 the major degradative pathway was hydrolysis of the thioglycoside linkage to form *1*-dethiomethyl-*1*-hydroxy clindamycin and methyl mercaptan. The major degradative pathway in the pH range 5–10 was scission of the 7-(S)-Cl of clindamycin to form the 7-(R)-OH analog, lincomycin. Evidence was obtained that supports the hypothesis that this conversion to lincomycin proceeds through an oxazolonium intermediate and that the extent of conversion depends on the degree of protonation of the amine function of clindamycin. The activation energy for clindamycin degradation in 0.1 *M* HCl, where thioglycoside hydrolysis is predominant, is 38.0 ± 1.2 kcal./mole, and the activation energy in 0.2 *M* citrate buffer adjusted to pH 5, where conversion to lincomycin is predominant, is 29.1 ± 0.6 kcal./mole.

Keyphrases □ Clindamycin—stability, aqueous solution □ Stability—clindamycin aqueous solutions □ Degradation, clindamycin—kinetics, mechanism, product identity □ pH effect—clindamycin stability □ Temperature effect—clindamycin stability □ GLC—analysis □ Mass spectroscopy—identity

The synthesis and biological properties of the antibiotic clindamycin (I) were first reported by Magerlein *et al.* (1, 2). Clindamycin possesses marked antiplasmodial activity (3) and is highly effective in the treatment of infections caused by Gram-positive organisms (2).



I

The purpose of this study was to investigate the degradation of clindamycin in aqueous solution in order to supply basic information necessary for the successful formulation of the drug in liquid dosage forms. The effects of pH and temperature on the rate of clindamycin degradation were studied and the major products of degradation in the pH range 0.4–12 were identified. From these data, the conditions of maximum stability of clindamycin in aqueous solution were established and the stability of clindamycin in pharmaceutical formulations could be predicted.

EXPERIMENTAL

Materials—Clindamycin, lincomycin, lincomycin tetraacetate, 7-epilincomycin, and 7-epiclindamycin (The Upjohn Co.) were used and all other chemicals were reagent grade.

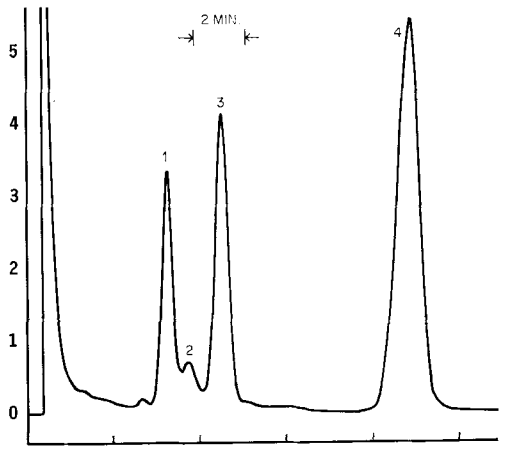


Figure 1—*Typical gas-liquid chromatogram of acetylated sample from low pH (<4) reaction mixtures. Key: Peak 1, 1-dethiomethyl-1-hydroxy clindamycin tetraacetate; Peak 2, suspected anomer of Peak 1; Peak 3, clindamycin triacetate; Peak 4, cholesteryl acetate.*

Kinetic Studies—*pH-Rate Studies*—Reaction mixtures containing clindamycin hydrochloride and buffers were prepared to cover the pH range 0.40–12 using the buffers shown in Table I. Individual buffer systems were prepared from citric acid, tartaric acid, acetic acid, succinic acid, disodium phosphate, boric acid, and sodium carbonate, and adjusted to the desired pH at 70° by the addition of hydrochloric acid or sodium hydroxide.

The concentration of clindamycin hydrochloride was 1% (0.02 *M*) in the pH range 1–6, 0.2% at pH 7, and 0.1% at pH values greater than 7. Reaction mixtures at higher pH contained less clindamycin due to the relatively low solubility of the undissociated species (about 2 mg./ml.). After preparation, volumes of each reaction mixture equivalent to 12 mg. of clindamycin hydrochloride were filled into ampuls, sealed, and placed into a 70° constant-temperature bath. At appropriate times two samples were withdrawn, the pH of one was measured, and an aliquot of the second equivalent to 10 mg. of clindamycin hydrochloride was freeze-dried. The amount of intact clindamycin present in the freeze-dried samples was determined by the gas-liquid chromatographic assay described below.

Temperature-Rate Studies—The effect of temperature on the rate of clindamycin degradation was studied in 0.1 *M* hydrochloric acid and in 0.2 *M* citrate buffer adjusted to pH 5.

Ampuls containing 10 mg./ml. of clindamycin hydrochloride in 0.1 *M* hydrochloric acid were placed into constant-temperature baths set at 47, 53, 70, 79, and 93°. At appropriate times 1.0-ml. samples were withdrawn, freeze-dried, and the amount of intact clindamycin present in the freeze-dried samples was determined by the gas-liquid chromatographic assay described below.

Ampuls containing 10 mg./ml. of clindamycin hydrochloride in the citrate buffer were placed into 47, 53, 60, 70, 80, and 90° constant-temperature baths. At appropriate times two ampuls were withdrawn, the pH of one was measured, and 1.0 ml. of the other was freeze-dried. The pH did not vary by more than 0.2 pH units from the initial pH during the reaction in all of the buffered reaction mixtures including the pH-rate studies described above. The amount of intact clindamycin present in the freeze-dried samples was determined by the gas-liquid chromatographic assay described below.

Gas-Liquid Chromatographic Assay—One milliliter of deionized water was added to the freeze-dried sample, the pH was adjusted to 11 with concentrated ammonium hydroxide, and the solution was extracted with two 1-ml. portions of ethyl acetate. The ethyl acetate extracts were combined and the solvent was removed with a stream

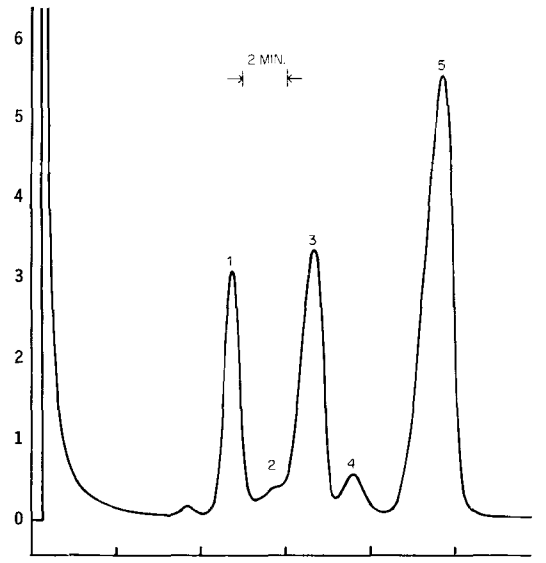


Figure 2—*Typical gas-liquid chromatogram of acetylated sample from high pH (5–8) reaction mixture. Key: Peak 1, clindamycin triacetate; Peak 2, suspected 7-epilincomycin tetraacetate or 7-epiclindamycin triacetate; Peak 3, lincomycin tetraacetate; Peak 4, unknown; Peak 5, cholesteryl acetate.*

of nitrogen. To the residue 1.0 ml. of a mixture of pyridine (dried over KOH)–acetic anhydride (2:1) containing 10 mg./ml. cholesteryl acetate as internal standard was added. After acetylating for 1 hr. at 100°, the solution was cooled to room temperature and 1 μl. was injected into an F & M model 402 gas chromatograph adjusted to the following conditions. Samples were partitioned between helium carrier gas flowing at 40 ml./min. and 3% OV-1 on diatomaceous earth (Gas Chrom Q) 60–80 mesh in a U-shaped glass column 120 cm. long × 3 mm. i.d. Columns were preconditioned by heating at 300° for at least 6 hr. with low helium flow and then for 1 hr. under no flow conditions. The column temperature was 220°, flash heater temperature 230°, and flame ionization detector 240°. Air and hydrogen flow rates were adjusted to give maximum response.

The concentration of clindamycin per sample was obtained by comparing clindamycin–internal standard peak height ratios to the peak height ratios of a standard curve prepared from known amounts of clindamycin. Standard curves were obtained daily.

Determination of pKa of Clindamycin at 70°—Ten milliliters of a 2.0 mg./ml. solution of clindamycin hydrochloride were titrated with 0.05 *N* NaOH in a jacketed vessel at 70° with a Radiometer TTT1C titrator and SBR2C titragraph. The average pKa of six replicate titrations was 6.90 ± 0.10.

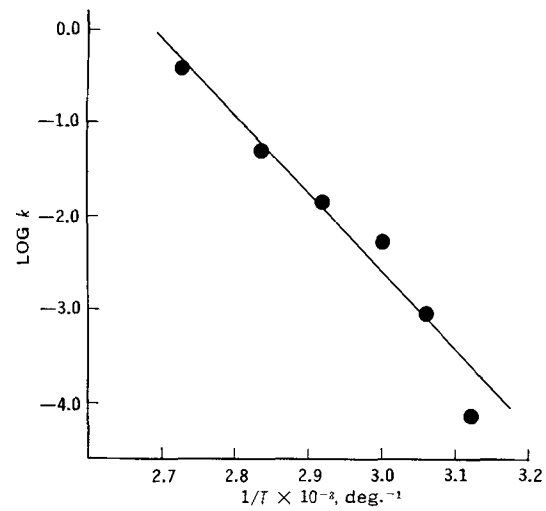


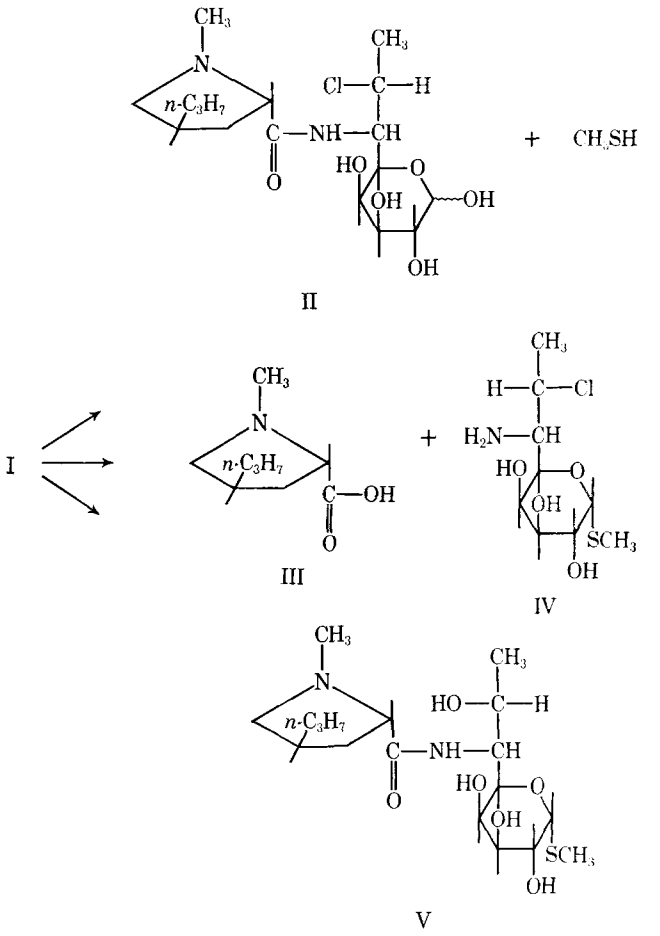
Figure 3—*Arrhenius plot for degradation of clindamycin in 0.1 M HCl.*

Identification of Degradation Products—Degradation products were identified by mass spectral studies and by comparison of gas-liquid chromatographic retention times and thin layer chromatographic R_f values with authentic samples when available. Mass spectra of acetylated clindamycin and degradation products were obtained by processing acetylated reaction mixture samples through an LKB 9000 gas chromatograph-mass spectrometer and recording mass spectra of peaks of interest as they exited from the gas chromatograph column. Thin layer chromatographic studies were carried out by spotting about 50 μl. of clindamycin reaction mixtures on Silica Gel G and developing with chloroform–methanol 90:10. Spots were visualized with iodine vapor.

RESULTS AND DISCUSSION

Identification of Products—Gas-liquid chromatographic data indicated that the mechanism of clindamycin degradation is pH-dependent with a change in mechanism occurring in the vicinity of pH 4–5. For example, chromatograms similar to Fig. 1 were obtained from reaction mixtures whose pH was less than 4, whereas samples from reaction mixtures in the pH range 5–8 yielded chromatograms similar to the one shown in Fig. 2. The major product of degradation in the pH range 0.4–4, represented by Peak 1 in Fig. 1, had a shorter retention time than clindamycin while the major degradation product at higher pH, represented by Peak 3 of Fig. 2, had a longer retention time than clindamycin. Chromatograms from reaction mixtures at pH greater than 8 which were buffered with borate, carbonate, or NaOH showed only disappearance of clindamycin and no other peaks were observed.

The identity of the major degradation product of clindamycin at pH less than 4 was established by mass spectral studies and with a knowledge of the reactivity of lincomycin (V), the 7(R)-OH analog of clindamycin, in acid. Prescott (5) and Herr and Slomp (6) have



Scheme I

Table I—Apparent First-Order Rate Constants of Degradation of Clindamycin

pH	Buffer	Temperature, °C.	k, sec.$^{-1}$ × 10^{-6}
0.44[a]	0.5 *M* HCl	70	19.0
0.54[a]	0.4 *M* HCl	70	19.3
0.66[a]	0.3 *M* HCl	70	14.3
0.83[a]	0.2 *M* HCl	70	8.25
1.10[a]	0.1 *M* HCl	70	4.50
1.10[a]	0.1 *M* HCl	70	4.66
1.10[a]	0.1 *M* HCl	47	0.0199
1.10[a]	0.1 *M* HCl	53	0.252
1.10[a]	0.1 *M* HCl	59	1.43
1.11[a]	0.1 *M* HCl	79	13.0
1.11[a]	0.1 *M* HCl	93	66.5
1.94	0.2 *M* Citrate	70	0.831
1.95	0.2 *M* Citrate	70	0.805
2.90	0.2 *M* Citrate	70	0.295
2.95	0.2 *M* Citrate	70	0.318
3.92	0.2 *M* Citrate	70	0.243
4.00	0.2 *M* Citrate	70	0.249
4.07	0.2 *M* Citrate	70	0.242
4.00	0.2 *M* Citrate	47	0.0124
4.00	0.2 *M* Citrate	53	0.0279
4.00	0.2 *M* Citrate	59	0.0831
4.00	0.2 *M* Citrate	80	0.969
4.00	0.2 *M* Citrate	92	3.62
4.80	0.2 *M* Tartrate	70	0.276
4.85	0.2 *M* Acetate	70	0.278
5.00	0.2 *M* Succinate	70	0.280
5.00	0.2 *M* Citrate	70	0.276
5.95	0.2 *M* Citrate	70	0.665
6.00	0.2 *M* Phosphate	70	0.620
6.00	0.2 *M* Citrate	70	0.946
6.90	0.2 *M* Citrate	70	2.07
6.95	0.2 *M* Citrate	70	2.89
7.25	0.2 *M* Phosphate	70	4.00
7.85	0.2 *M* Phosphate	70	5.91
8.20	0.2 *M* Phosphate	70	4.08
8.30	0.2 *M* Phosphate	70	7.11
8.50	0.2 *M* Borate	70	15.2
9.30	0.2 *M* Borate	70	15.5
9.50	0.2 *M* Borate	70	16.7
9.70	0.2 *M* Borate	70	17.7
9.75	0.2 *M* Borate	70	14.9
10.00	0.2 *M* Borate	70	29.9
11.00	0.2 *M* Carbonate	70	149
11.66[b]	0.1 *M* NaOH	70	204

[a] Calculated from pH = − Log f(HCl): where (HCl) is the experimental molarity and f is the mean activity coefficient for HCl at 70° extrapolated from the literature (4). [b] Calculated from pH = pKw − pOH: where pKw = 12.77 at 70° (4), pOH = − Log f(NaOH), (NaOH) is the experimental molarity, and f is the mean activity coefficient for NaOH extrapolated from the literature (4).

reported that lincomycin (V) degrades in acid *via* thioglycoside hydrolysis to form 1-dethiomethyl-1-hydroxylincomycin and methyl mercaptan. Clindamycin might be expected to react in acid in a manner similar to lincomycin and mass spectral data indicate that the molecular weight of the compound represented by Peak 1 of Fig. 1 is 562. This molecular weight corresponds to acetylated 1-dethiomethyl-1-hydroxyclindamycin which indicates that II in Scheme I is the major product of clindamycin degradation at pH less than 4.

II can be detected gas chromatographically as the predominant reaction product for about three clindamycin half-lives after which it begins to disappear. The decrease in magnitude of the gas-liquid chromatographic peaks of II and clindamycin at extended reaction times is accompanied by the appearance of several small peaks of shorter retention time. This further degradation may result from breakdown of the sugar moiety or amide hydrolysis (6).

Peak 2 of Fig. 1 represents another product of clindamycin degradation at pH less than 4 which is present in the early stages of the reaction. As the reaction proceeds, Peak 2 behaves similarly to Peak 1 and decreases in magnitude after about three clindamycin half-lives. Since thioglycoside hydrolysis proceeds through a carbonium ion intermediate (7), the products of clindamycin would be methyl mercaptan and an anomeric mixture of II. Mass spectral studies indicated that Peak 1 represents one of the anomers and

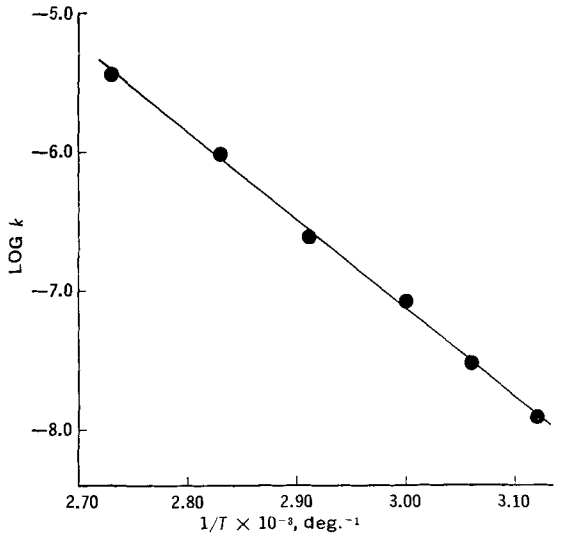


Figure 4—*Arrhenius plot of clindamycin degradation at pH 4 in 0.2* M *citrate buffer.*

Peak 2 may represent the other. The identification of Peak 2 was not attempted.

Lincomycin (V) was identified as the major degradation product of clindamycin at pH greater than 5. Mass spectral studies of Peak 3 of Fig. 2 showed that the molecular weight of this compound was 574, the molecular weight of acetylated lincomycin. Further, the gas-liquid chromatographic retention time of Peak 3 of Fig. 2 is identical to authentic lincomycin tetraacetate. Lincomycin, as well as clindamycin, is unstable in the pH range 5–8 as both Peaks 1 and 3 of Fig. 2 disappear at long reaction times. One possible route of degradation of lincomycin and clindamycin at extended reaction times is amide hydrolysis.

It is obvious from Fig. 2 that lincomycin is not the sole product of clindamycin degradation at pH 5–8. At least two other reaction products are represented by Peaks 2 and 4 of Fig. 2. Both Peaks 2 and 4 represent minor products and neither was ever present in

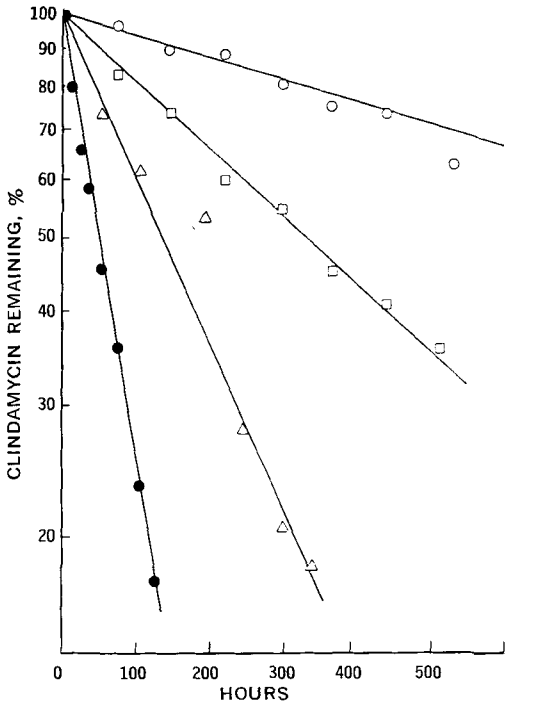


Figure 5—*Apparent first-order disappearance of clindamycin in 0.1* M *HCl at various temperatures. Key:* ○ *47°,* □ *53°.* △ *59°,* ● *69°.*

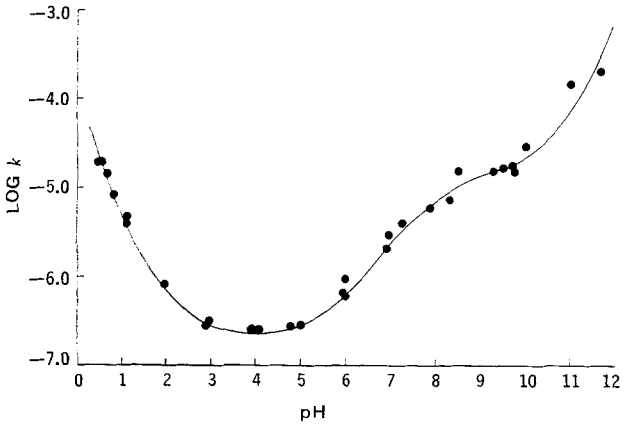


Figure 6—*pH-Rate profile of clindamycin degradation at 70°.*

amounts greater than that shown in Fig. 2. Peak 2 may represent acetylated 7-epilincomycin or acetylated 7-epiclindamycin since its retention time was identical to authentic samples of each. Magerlein has reported the formation of a trace of 7-epilincomycin after refluxing an aqueous solution of clindamycin at pH 7.8 (8). Peak 4 of Fig. 2 was not identified.

Although lincomycin (V) and Peaks 2 and 4 of Fig. 2 could not be detected above pH 8 by GLC, TLC of these reaction mixtures indicated that some of these degradation products were present but were not being extracted for gas chromatographic assay. Thin layer chromatograms of the reaction mixtures above pH 8 showed clindamycin and lincomycin by comparison with authentic samples and three other spots which were not identified. Gas chromatograms of reaction mixtures below pH 8 showed that the major degradation products were extracted by ethyl acetate. The sum of the moles of acetylated IV (Scheme I) and clindamycin between pH 5 and 8 and of acetylated II and clindamycin below pH 5 was greater than 90% of the initial clindamycin concentration in the early stages of the reaction.

Temperature-Rate Studies—Arrhenius plots of clindamycin degradation in 0.1 *M* HCl and at pH 4 are shown in Figs. 3 and 4. The activation energy for clindamycin degradation in 0.1 *M* HCl was calculated to be 38.0 ± 1.2 kcal./mole. The principal degradative pathway in 0.1 *M* HCl is hydrolysis of the *1*-thio-α-D-galactopyranoside moiety of clindamycin at Position 1 to form *1*-dethio-*1*-hydroxy-clindamycin (II) and methyl mercaptan (Scheme I). The activation energy for clindamycin degradation in 0.1 *M* HCl falls in the 30–38 kcal./mole range reported by other investigators for acid degradation of compounds of similar structure such as alkyl *1*-thio-β-D-glucopyranosides (9), alkyl β-D-xylopyranosides (10), and alkyl α- and β-D-glucopyranosides (11).

The activation energy for clindamycin degradation at pH 5, the pH of maximum stability, was calculated to be 29.1 ± 0.6 kcal./mole. Using the rate constants in Table I and the appropriate activation energy, the prediction can be made that clindamycin in pharmaceutical formulations adjusted to pH 1–6.5 will not degrade by more than 10% after 2 years at 25°.

pH-Rate Studies—Clindamycin disappeared from reaction mixtures by an apparent first-order process under all of the conditions studied. Some typical log clindamycin concentration-time curves are shown in Fig. 5.

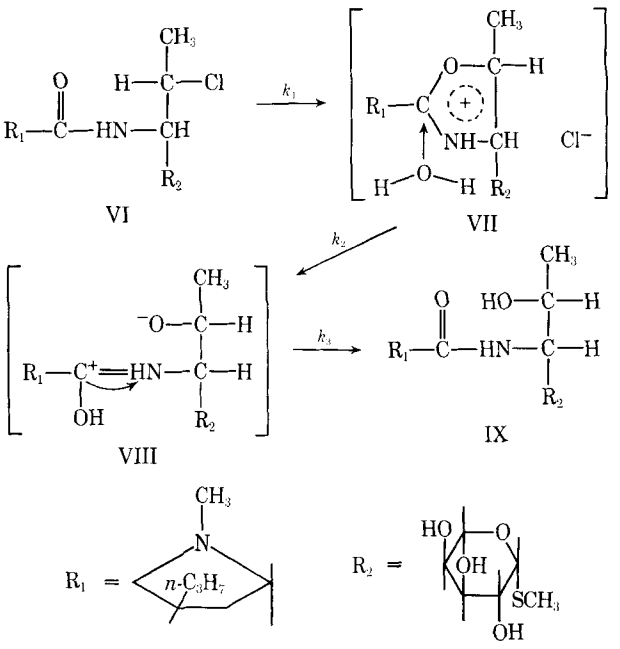
The effect of pH on the rate of clindamycin degradation in the pH range 0.40–12 at 70° is shown in Fig. 6.

Different buffer species did not appear to significantly influence the rate of degradation since a continuous curve was obtained for the pH profile and since the rate constant did not vary with different buffers at the same pH (Table I).

The pH profile of clindamycin (Fig. 6) shows that the rate of degradation increases with decreasing pH in the pH range 0.4–4. This is expected since both major degradative routes in this pH region, thioglycoside and amide hydrolysis, are susceptible to hydrogen ion catalysis. Being of similar structure, lincomycin (V) should also degrade by these two routes in acid media and the rate constant should agree with that of clindamycin when reacted under similar conditions. Forist, *et al.* (12) studied the degradation of V in 0.1 *N* HCl at 70° and the rate constant of 4.85×10^{-6} sec.$^{-1}$ calculated from their data agrees quite favorably with the clindamycin values in Table I.

In the pH range 5–12 the degradative rate increases with increasing pH with an inflection point in the pH 9–10 region. GLC and TLC of reaction mixtures in this pH region show that at least four products are formed by clindamycin degradation. The times of appearance of the various products and their relative concentrations indicate parallel reactions. Assuming there are parallel reactions occurring, the shape of the pH-rate profile in Fig. 6 in the pH 4–12 region might be interpreted as follows. In the pH 4–9 region all degradative rates are increasing with increasing pH with one of the reactions predominating. Above pH 9 the rate constant of the reaction which was predominating at pH 5–9 becomes constant but the rates of the other reactions continue to increase with increasing pH.

Gas-liquid and thin layer chromatographic data of this study show that the predominant degradative reaction at pH 5–9 is conversion to lincomycin (V). Results of studies by Magerlein (8, 13) show how the rate of lincomycin conversion could be predominant in this pH range and then become constant above pH 9. Magerlein has postulated that conversion to lincomycin occurs through the oxazolonium ion intermediate (VII) shown in Scheme II and that the extent of conversion is highly dependent on the participating ability of the substituent on the amide carbonyl (13). For example, solvolysis is anchimerically assisted by the neighboring amide carbonyl if the carbonyl substituent is methyl, whereas when R_1 is a strong electron withdrawing group such as trifluoromethyl, participation does not occur. Magerlein also reports that the 7(S)-Cl position of clindamycin is resistant to direct S_N2 nucleophilic displacement (8).



Scheme II

In the present study no conversion to lincomycin was observed at pH values less than 5, whereas lincomycin was detected by GLC or TLC in all reaction mixtures buffered to pH values greater than 5. Assuming that the protonated form of the *N*-methyl-4-propylpyrrolidine portion of clindamycin (R_1) is analogous to trifluoromethyl as a nonparticipating substituent and $k_1 \ll k_2$, no conversion to lincomycin would be expected in the pH range where R_1 is fully protonated, *i.e.*, at pH values less than 5. On the other hand, it is possible that lincomycin can be detected in the pH 5 reaction mixtures because the small amount of nonprotonated R_1 has facilitated conversion to lincomycin and the increase in rate of clindamycin degradation is partly due to this reaction. As the pH increases the rate of conversion to lincomycin increases due to the presence of more nonprotonated R_1. The leveling trend in the pH profile at pH 9, two pH units above the pKa of 6.90, might be due to a constant rate of lincomycin conversion since at pH 9 and above all of R_1 exists in the nonprotonated form. The further increase in overall rate above pH 10 might then be due to an increase in the rate of other reactions such as amide hydrolysis.

The relationship between pH and the mechanism of clindamycin degradation could be summed as follows. Below pH 4 clindamycin degrades *via* thioglycoside and amide hydrolysis with thioglycoside hydrolysis predominant in the pH range 0.4–4. Above pH 5 clindamycin degrades by conversion to lincomycin and by other reactions such as amide hydrolysis. The extent of lincomycin conversion is dependent on the degree of protonation of the *N*-methyl-4-propylpyrrolidine moiety. At pH less than 5 where the amine is fully protonated no conversion to lincomycin occurs. Then this process can be detected in the vicinity of pH 5 and its rate increases as pH increases to pH 9 and then becomes constant since the amine function is completely nonprotonated. The overall rate of clindamycin degradation continues to increase with increasing pH above pH 9, however, due to the hydroxide ion dependency of the other degradative routes.

REFERENCES

(1) R. D. Birkenmeyer, Abstracts of Papers, Fifth Interscience Conference on Antimicrobial Agents and Chemotherapy and IV International Congress of Chemotherapy, Washington, D. C., October 17–21, 1965.

(2) B. J. Magerlein, R. D. Birkenmeyer, and F. Kagan, *Antimicrobial Agents Chemotherapy*, **1966**, 727.

(3) C. Lewis, *J. Parasitol.*, **54**, 169(1968).

(4) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolyte Solutions," Reinhold, New York, N. Y., 1958.

(5) G. C. Prescott, *J. Pharm. Sci.*, **55**, 423(1966).

(6) R. R. Herr and G. Slomp, *J. Am. Chem. Soc.*, **89**, 2444(1967).

(7) J. N. Be Miller, in "Advances in Carbohydrate Chemistry," vol. 22, M. L. Wolfrom and R. S. Tipson, Eds., Academic, New York, N. Y., 1967, p. 25 ff.

(8) B. J. Magerlein, "Chemical Modifications of Lincomycin," Medicinal Chemistry Symposium, Quebec, Canada, June 23–26, 1968.

(9) M. D. Saunders and T. E. Timell, *Carbohydrate Res.*, **6**, 121(1968).

(10) C. K. De Bruyne and F. Van Wijnendaele, *ibid.*, **6**, 367 (1968).

(11) T. E. Timell, *Can. J. Chem.*, **42**, 1456(1964).

(12) A. A. Forist, L. W. Brown, and M. E. Royer, *J. Pharm. Sci.*, **54**, 476(1965).

(13) B. J. Magerlein and F. Kagan, *J. Med. Chem.* (in press).

ACKNOWLEDGMENTS AND ADDRESSES

Received June 4, 1969 from the *Research Laboratories, The Upjohn Company, Kalamazoo, MI 49001*

Accepted for publication August 28, 1969.

Presented to the Basic Pharmaceutics Section of the APhA Academy of Pharmaceutical Sciences, Montreal meeting, May 1969.

The author wishes to thank Dr. P. B. Bowman for obtaining and aiding in the interpretation of mass spectra, and Mr. G. R. Munting for excellent technical assistance in this work.

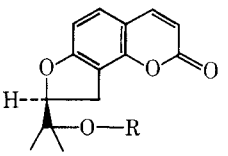
Coumarins XI: A Total Synthesis of (±)-Columbianetin

M. SHIPCHANDLER*, T. O. SOINE†, and P. K. GUPTA‡

Abstract □ (±)-Columbianetin [(±)-I] has been synthesized by a ten-step sequence starting with 2,6-dihydroxybenzoic acid which was converted to the methyl ester, benzylated, and reduced to the benzyl alcohol which was oxidized to the aldehyde and monodebenzylated to provide 2-hydroxy-6-benzyloxybenzaldehyde (VI). Treatment of VI with methyl bromoacetate converted it to methyl 3-benzyloxy-2-formylphenoxyacetate which was cyclized to methyl 4-benzyloxybenzofuran-2-carboxylate, the latter being converted to 2-(α-hydroxyisopropyl)-4-benzyloxybenzofuran (XIV) by the action of CH_3MgI. Reduction and debenzylation of XIV to the corresponding dihydrobenzofuran followed by acid-catalyzed condensation with ethyl propiolate provided (±)-I.

Keyphrases □ (±)-Columbianetin—total synthesis □ TLC—separation identity □ Mass spectroscopy—identity □ UV spectrophotometry—identity □ IR spectrophotometry—identity □ NMR spectroscopy—identity

The isolation of two new coumarins from the umbellifer, *Lomatium columbianum* Math. and Const., was reported in 1964 (1). One of these coumarins, a glycoside assigned the name columbianin, has since been shown to occur in *L. dissectum* var. *multifidum* (Nutt.) Math. and Const. (2) and *L. nuttallii* (A. Gray) Macbr. (3). Acid hydrolysis of columbianin yielded D-glucose and a tertiary coumarinic aglycone, columbianetin (I) and led to the postulation of II as the structure for the glycoside. More recent studies (4) have revised the structure to III, *i.e.*, the β-D-gentiobioside of I (III). The other coumarin, columbianadin, was assigned Structure IV, *i.e.*, the angelate ester of I, and has been found in *Peucedanum palustre* (5) as well as in *Zosimia absinthifolia* (Vent.) Link (6, 7). The absolute configuration of I has been shown to be 8(S) (8).

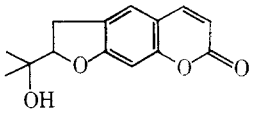


I, R = H

II, R = D-Glucosyl

III, R = β-D-Gentiobiosyl

IV, R = Angeloyl



V

The recent total synthesis of marmesin (V) and its optical antipode, nodakenetin, by Nakajima *et al.* (9) and confirmed by Harada *et al.* (10) in a study of the absolute configuration suggested that a similar synthesis could be applied to the preparation of I by utilizing 2-hydroxy-6-benzyloxybenzaldehyde (VI) as starting material in place of the isomeric 2-hydroxy-4-benzyloxybenzaldehyde employed by these workers. The preparation of VI from 2,6-dihydroxybenzaldehyde (*i.e.*, γ-resorcylaldehyde) was the obvious route but a survey of the literature pertaining to the preparation of the latter (11–15) indicated that all of the published methods were characterized by poor overall yields as well as lengthy synthetic sequences. Thus, a synthesis of