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Abstract \square The lone-pair ionization energies for N, O, and Cl heteroatoms in four derivatives of piperidine were measured from UV photoelectron spectra. The various lone-pair interactions in each piperidine were investigated. The hydrolytic stability and the antimicrobial activity for the N-chloropiperidines also were determined. The results suggest a correlation between the stability of the nitrogen lone pair, as determined from photoelectron spectroscopy, with the antibacterial activity of the corresponding N-chloramine.

Keyphrases \square Piperidines, *N*-chlorinated—photoelectron spectra, hydrolytic stability, and antimicrobial activity, correlations \square *N*-Chloramines—photoelectron spectra, hydrolytic stability, and antimicrobial activity, correlations \square UV spectrophotometry—determination of photoelectron spectra of four *N*-chlorinated piperidines, correlation of nitrogen lone-pair stability and antimicrobial activity \square Antimicrobial activity—correlated to hydrolytic stability of *N*-chlorinated piperidines, photoelectron spectra

Photoelectron spectroscopy provides a versatile method for determining ionization potentials and, thus, orbital energies for molecules. The energies of the molecular orbitals that describe localized or semilocalized electrons ("lone pairs")¹ depend on the chemical environment of the electronic group in question. Therefore, the corresponding ionization potentials exhibit characteristic "chemical shifts" in the photoelectron spectrum. The nature and magnitude of such shifts can thus provide information concerning the interactions that take place between the localized group and its environment. These interactions may be mediated "through space" (direct) or may be hyperconjugative in nature, occurring through intermediate sigma bonds (indirect) (1, 2).

Recently, the magnitude and mode of the interactions between a carbonyl group and one or more carbon-carbon double bonds were investigated (3). These investigators concluded that through-space interactions have no significant effect upon the ionization potentials corresponding to orbitals primarily confined to the carbon-carbon or carbon-oxygen double bonds. This observation suggests that any $\pi CC \rightarrow \pi CO$ interactions in the compounds investigated must occur through a hyperconjugative through-bond mechanism.

In this study, the photoelectron spectra of several piperidines and their N-chlorinated derivatives were investigated to define the extent of the interaction, if any, between the nitrogen lone pair and a carbonyl

group or an N-chloro function. It is well known that N-chlorinated organic molecules exhibit antibacterial properties. The antibacterial activity of these Nchloramines is affected by a number of factors including concentration, solubility, molecular size, dipole moment, water-lipid partitioning coefficient, and solution pH. It was the hypothesis that the most important factor contributing to the antibacterial activity of these compounds would be the "chlorine potential" (4, 5) of the N-chloramine. The N-chlorine bond in an N-chloramine is covalent. However, the N-chlorine bond has properties different from those of the covalent carbon-chlorine bond. For many purposes, it is convenient to regard chlorine when bonded to a nitrogen atom as "positive." Therefore, the N-chloramine can be considered as a source of "positive" chlorine (Scheme I):

$$\begin{array}{rcl} R_2 \ddot{N} & \longrightarrow & R_2 N \vdots^- & + & Cl^+ \\ & & Scheme \ I \end{array}$$

or in an aqueous solution (Scheme II):

 R_2N — $Cl + H_2O \xrightarrow{Kcp} R_2NH + HOCl$ Scheme II

The reaction in aqueous solution has lead to the development of the chlorine-potential concept, pKcp (Eq. 1b) (4, 5):

$$Kcp = \frac{[R_2NH] [HOCl]}{[R_2NCl] [H_2O]}$$
(Eq. 1*a*)

$$pKcp = -\log Kcp \qquad (Eq. 1b)$$

The chlorine potential has been defined in terms of the equilibrium constant for the hydrolysis of the Nchloramine to hypochlorous acid. When using this approach, the chlorinating ability of N-chloramines can be expressed quantitatively. The existence of a qualitative relationship between N-chloramine's antibacterial activity and its chlorinating ability as defined by its chlorine potential has also been considered².

Although the qualitative relationship between the acidity of an amine (pKa) and its stability relative to the corresponding N-chloramine (pKcp) has been demonstrated (4, 5), no general relationship could be elucidated that would correlate N-chloramines with substantially different chlorine potentials, e.g., N-chloramines and N-chloramides. It is the hypothesis that the orbital energies (ionization potentials) of the

¹ In a rigorous description of ionization phenomena, one should use molecular orbital terminology and ionize nonbonding electrons from nonbonding orbitals rather than equivalent orbital terminology which considers "lone pairs." However, in a qualitative sense (particularly for use in reaction mechanisms), it is sometimes helpful to consider nonbonding orbitals as localized and to call them "lone pairs."

² J. Kaminski and N. Bodor, unpublished results.



Figure 1—Photoelectron spectra of some piperidine derivatives excited by the He I resonance line (21.22 ev). Key: top left, I; top right, II; bottom left, III; and bottom right, IV.

nitrogen lone pair in N-chloramines may give a better and perhaps a unitary basis for comparing the chlorine potentials of structurally different N-chloramines than does comparison of the basicities of the parent amines.

In the present study, the effect of chlorine directly bonded to nitrogen in N-chloramines on the ionization potentials for the nitrogen lone pair was examined. The extension of this study to include novel Nchloramines, for which antibacterial activity has been determined, to establish a possible quantitative relationship between the ionization potentials of the nitrogen lone pair in N-chloramines and the stability of the N-chlorine bond toward hydrolytic displacement (chlorine potential) and/or antibacterial activity is intended.

RESULTS AND DISCUSSION

The relevant regions of the photoelectron spectra for the four piperidines considered are shown in Fig. 1. The ionization potentials derived from the photoelectron spectra are listed in Table I. The assignments of the various bands in the spectra to N, O, or Cl lone pair ionization were based on comparisons with previously assigned spectra for other molecules having the same functional groups (6-9).

As the data in Table I indicate, relative to the first ionization potential for piperidine, 8.31 ev (6), the inductive effect of the four α -methyl groups in 2,2,6,6-tetramethylpiperidine (I) apparently decreases the nitrogen lone-pair ionization potential by 0.92 ev. The magnitude of this difference is in good agreement with that (1 ev) observed (10) between the first ionization potentials of hydrazine and 1,1,2,2-tetramethylhydrazine, although the marginal increase of the difference in the latter case is presumably due to the methyl groups being directly bonded to the nitrogen atom.

Comparison of the ionization potentials for the nitrogen lone pair in 2,2,6,6-tetramethyl-4-piperidone (II) relative to 2,2,6,6-

Table I—Ionization Potentials of Some Piperidines and Their N-Chlorinated Derivatives

Com-	N Lone Pair		O Lone Pair		<i>7</i> -	Cl Lone Pair		
pound	aª	b٥	a	b	Onset	a	b	
I	7.39	8.04	8 75	9.02	9.63	_		
III IV	7.64 8.01	8.31 8.68	9.09	9.27	9.86 10.60	10.86 11.10	$\begin{array}{c} 11.14\\ 11.43\end{array}$	

^a Adiabatic ionization potentials (ev); represents band onsets in Fig. 1. Vertical ionization potentials (ev); represents band maxima in Fig. 1.

 Table II--Ionization Potential Differences between Some

 Piperidines and Their N-Chlorinated Derivatives

ΔIP (ev)	Effect
II - I = 0.35IV - III = 0.37III - I = 0.25IV - II = 3.27	4-Carbonyl 4-Carbonyl N-Chlorine N-Chlorine

tetramethylpiperidine (I) suggests that the 4-carbonyl function in II exhibits a significant stabilizing-delocalizing effect on the nitrogen lone pair: IP (II) – IP (I) = 0.35 ev. A comparison of the activation energies for nitrogen inversion (11) between 1,2,2,6,6-pentamethylpiperidine, $E_{\rm act} = 10.8$ kcal/mole, and 1,2,2,6,6-pentamethyl-4-piperidone, $E_{\rm act} = 10.5$ kcal/mole, also demonstrates the significant stabilizing-delocalizing effect of the 4-carbonyl group on the nitrogen lone pair.

The delocalizing effect is further evidenced by comparison of the difference between the vertical and adiabatic ionization potentials observed in I $(I_v - I_a = 0.65 \text{ ev})$ and II $(I_v - I_a = 0.78 \text{ ev})$. (For completely localized lone-pair orbitals, the adiabatic and vertical ionization potentials should be the same.) The observed ionization potentials are in qualitative agreement and consistent with the trend observed in the basicities of the corresponding amines, pKa (I) = 11.18 (12) and 11.07 (13), and pKa (II) = 7.90 (14). However, the difference in the observed pKa's, $\Delta pKa = 3.17$ or 3.28, is more pronounced than that observed in the nitrogen lone-pair ionization potentials, $\Delta IP = 0.35$ or 0.48 ev. This must reflect the differences existing between the solvation properties of I and II. Undoubtedly, the differences in the solvation energies for these amines is an extremely important factor which affects the pKa value. As a consequence, the solvation phenomenon may substantially alter the electronic environment of the solvated nitrogen lone pair relative to that existing in the gaseous state. In support of this view, I and piperidine have essentially the same pKa of 11.12 (15) and 10.98 (12), respectively. However, the difference between the nitrogen lone-pair ionization potentials for the two amines is 0.92 ev.

Comparison of the ionization potential difference between I and II or 1-chloro-2,2,6,6-tetramethylpiperidine (III) and 1-chloro-2,2,6,6-tetramethyl-4-piperidone (IV) relative to the differences in



Table III—Hydrolytic Stability of 1-Chloro-2,2,6,6tetramethylpiperidine (III) and 1-Chloro-2,2,6,6tetramethyl-4-piperidone (IV) at 40°

Com- pound	Solvent	pН	Half- Life, min	$\begin{array}{c} \text{Rate} \\ \text{Constant} \\ \times 10^{-3} \\ \text{min}^{-1} \end{array}$	Correla- tion Co- efficient ^a
III		4.6	168	4.12	0.993
	0.1 M NaH PO	7.0	221	3.13	0.990
	$\begin{array}{c} \text{Nall}_2 \text{I} \text{ O}_4 \\ \text{O.1 } M \\ \text{Na}_2 \text{B}_4 \text{O}_7 \end{array}$	9.3	226	3.07	0.994
IV	0.1 <i>M</i> №0COCH	4.6	559	1.24	0.990
	0.1 M	7.0	203	3.42	0.997
	$\begin{array}{c} \text{Nall}_2 \text{FO}_4 \\ \text{O.1 } M \\ \text{Na}_2 \text{B}_4 \text{O}_7 \end{array}$	9.3	16	42.6	0.999

^a Correlation coefficients were determined from a linear regression analysis of the experimental data.

the ionization potentials between I and III or II and IV demonstrates that the 4-carbonyl group has a greater effect in stabilizing the nitrogen lone pair than does the N-chlorine group (Table II). In addition, it appears that the effects of the 4-carbonyl group and the N-chlorine function on the ionization potential of the nitrogen lone pair are nearly additive. Comparison between the oxygen lone-pair ionization potentials of IV and II indicates that the presence of the N-chlorine group stabilizes the oxygen lone-pair orbital by about 0.3 ev. Also, comparison between the chlorine lone-pair ionization potentials of IV and III indicates that the presence of the 4-carbonyl group stabilizes the chlorine lone-pair orbital by about 0.3 ev. Furthermore, it can be seen that the 4-carbonyl group in II and IV significantly increases the $\sigma\text{-onsets}$ (0.92 and 0.74 ev, respectively), whereas the presence of the N-chloro function in III and IV has less effect on the onset of σ -ionization (0.23 and 0.05 ev, respectively).

The hydrolytic stability and the antibacterial activity of III and IV were investigated (Tables III and IV). For each N-chloramine, the rate of change in the concentration of positive chlorine in the solution was determined using the iodometric method of analysis. The data were interpreted as pseudo-first-order kinetic processes and the stability of the N-chloramines III and IV under the experimental conditions was characterized using the half-life as the reaction constant. Based on the ionization potential of the nitrogen lone pair determined from the photoelectron spectra, one would predict that the N-chloramine having the more stabilized nitrogen lone pair (higher IP) would also be more susceptible to base-catalyzed and less susceptible to proton-catalyzed hydrolytic cleavage of the N-Cl bond. Comparison of the stability data described in Table III for III and IV supports this hypothesis. For example, IV, which has a more stabilized nitrogen lone pair, IP = 8.01 ev, relative to III, IP = 7.64 ev, also exhibits a significant instability in base, $t_{1/2} = 16$ min, relative to III, $t_{1/2} = 226$ min, under the same experimental conditions (pH 9.3).

The antibacterial activity determined for III and IV is also consistent with the hydrolytic stability of the N-chloramines. As anticipated, the N-chloramine IV, which possesses a higher chlorine potential relative to III, also exhibits a higher antibacterial activity. Unfortunately, due to the limitations in the time scale for sampling in the antibacterial experiment, this observation is only evident in a comparison of the antibacterial activity times required to sterilize only the more resistant microorganisms Staphylococcus aureus (ATCC 6538) and Pseudomonas aeruginosa (ATCC 9027). Hopefully, it will be possible to correlate the stability of the nitrogen lone pair, as determined from photoelectron spectroscopy, with antibacterial activity.

Although no quantitative correlations can be established based on this limited investigation, it is encouraging to observe at least the existence of a qualitative relationship among the electronic, chemical, and microbiological properties of these compounds. This method of analysis will be extended to other N-chloramine derivatives to establish a quantitative correlation between the physical and microbiological properties investigated.

 Table IV—Antibacterial Activity of 1-Chloro-2,2,6,6-tetramethylpiperidine (III) and

 1-Chloro-2,2,6,6-tetramethyl-4-piperidone (IV)

		Concentration Data		Antibacterial Activity Time, min				
Compound	Solvent	Compound	Positive Chlorine	$\frac{\text{ATCC}}{12228^a}$	ATCC 10536 ^b	ATCC 10031 c	ATCC 9027 ^d	ATCC 6538 ^e
III	0.1 M NaOCOCH ₃ pH 4.6	$0.91 \times 10^{-3} M$	30 ppm	0.5	0.5	0.5	2	2
IV	0.1 M NaOCOCH ₃ pH 4.6	$0.97 \times 10^{-3} M$	30 ppm	0.5	0.5	0.5	0.5	0.5

^a Staphylococcus epidermidis .^b Escherichia coli. ^c Klebsiella pneumoniae. ^d Pseudomonas aeruginosa. ^c Staphylococcus aureus.

EXPERIMENTAL

Synthesis—2,2,6,6-Tetramethylpiperidine (I)—A commercial sample³ of 2,2,6,6-tetramethylpiperidine (I) was used after distillation from barium oxide, bp $150-151^{\circ}$.

2,2,6,6-Tetramethyl-4-piperidone (II)—2,2,6,6-Tetramethyl-4piperidone (II) was obtained by basification of a commercial sample of 2,2,6,6-tetramethyl-4-piperidone hydrochloride³. Compound II was used after vacuum sublimation at room temperature (0.1 mm), mp 59-60°.

1-Chloro-2,2,6,6-tetramethylpiperidine (III)—To a solution of 7.05 g (0.05 mole) of I in 20 ml of water was added dropwise, with stirring, 63 ml (0.05 mole) of 0.8 M sodium hypochlorite solution. During the addition, the reaction solution was maintained between pH 4 and 6 using 1 N HCl. The N-chloramine separated from the reaction mixture as an oil and was extracted with ethyl acetate. The aqueous layer was separated and extracted with ethyl acetate. The extracts were combined and dried over anhydrous sodium sulfate. Following filtration, the ethyl acetate was removed at room temperature under reduced pressure to afford a pale-yellow oil. Vacuum distillation of this material gave 7.4 g (0.042 mole), 84%, of III, bp 28-34° (0.2 mm); UV (H₂O): λ_{max} 273 nm, $\epsilon = 152 M^{-1}$ cm⁻¹; PMR (CDCl₃): δ 1.38 (s, 12H) and 1.77 (s, 6H) ppm.

Anal.—Calc. for C₉H₁₈ClN: C, 61.52; H, 10.32; Cl, 20.2; N, 7.97. Found: C, 61.18; H, 9.95; Cl, 20.2; N, 8.11.

1-Chloro-2,2,6,6-tetramethyl-4-piperidone (IV)—To a solution of 4.80 g (0.025 mole) of II-HCl in 10 ml of water was added dropwise, with stirring, 31 ml (0.025 mole) of a 0.8 M sodium hypochlorite solution. During the addition, the reaction solution was maintained between pH 4 and 6 using 1 N HCl. The N-chloramine separated from the reaction mixture as an oil and was extracted with ethyl acetate. The aqueous layer was separated and extracted with ethyl acetate. The aqueous layer was separated and extracted with ethyl acetate. The extracts were combined and dried over anhydrous sodium sulfate. Following filtration, the ethyl acetate was removed at room temperature under reduced pressure to afford a pale-yellow oil. Vacuum distillation of this material gave 3.63 g (0.019 mole), 75%, of IV, bp 44-48° (0.25 mm); UV (H₂O): λ_{max} 270 nm, $\epsilon = 299 M^{-1} \text{ cm}^{-1}$; PMR (CDCl₃): δ 2.73 (s, 4H) and 1.43 (s, 12H) ppm.

Anal.—Calc. for C₉H₁₆ClNO: C, 56.98; H, 8.50; Cl, 18.7; N, 7.39. Found: C, 56.59; H, 8.78; Cl, 18.5; N, 7.29.

Photoelectron Spectra—The spectra were recorded on a photoelectron spectrometer⁴ which incorporates a 10-cm radius cylindrical electrostatic field-deflection analyzer. The apparatus was described previously (16). The He I resonance line at 584 Å (21.22 ev) served as the ionizing source. The samples studied had low vapor pressures and had to be heated to produce sufficient vapor for obtaining good electron fluxes. The temperature of the collision chamber was monitored by a thermocouple placed ~1 cm above the chamber slit. It was possible to maintain the temperature within $\pm 1^{\circ}$ for extended periods. The spectra were recorded at temperatures that gave the best balance between electron flux and sample stability. The spectra were calibrated by use of the Ar²P_{3/2} (15.759 ev) and ²P_{1/2} (15.937 ev) lines with the Kr²P_{3/2} (13.999 ev) and ²P_{1/2} (14.665 ev) lines as internal standards.

Hydrolytic Stability of III and IV—For each determination of the reaction constant for III at a given pH, a stock solution of $\sim 0.80 \times 10^{-3} M$ III was prepared by dissolving 28.00 mg (0.16 mmole) of III in the appropriate buffer solution to a final volume of 200 ml. Approximately 15-ml aliquots of the stock solution were dispensed into ampuls and sealed. The ampuls were equilibrated for 0.5 hr in a constant-temperature bath operating at 40°. Periodically, samples were removed from the bath, and the concentration of positive chlorine in the sample was determined iodometrically using $10^{-3} N$ sodium thiosulfate.

Analysis as a first-order kinetic process gave the half-lives listed in Table II.

For each determination of the reaction constant for IV at a given pH, a stock solution of 1.25×10^{-3} M IV was prepared by dissolving 236.25 mg (1.25 mmoles) of IV in the appropriate buffer solution to a final volume of 100 ml. Approximately 5-ml aliquots of the stock solution were dispensed into ampuls and sealed. The ampuls were equilibrated for 0.5 hr in a constant-temperature bath operating at 40°. Periodically, samples were removed from the bath, and the concentration of positive chlorine in the sample was determined iodometrically using 10^{-3} N sodium thiosulfate.

Analysis as a first-order kinetic process gave the half-lives listed in Table II.

Antibacterial Activity of III and IV—The procedure used to determine the antibacterial activity of III and IV was based primarily on a modification of the serial dilution method of analysis. Instead of determining the minimum inhibitory concentration (MIC) parameters for the N-chloramine, only the bactericidal endpoint for a given concentration of the N-chloramine was determined. Therefore, the screening procedure only determines the time required for complete sterilization of the microorganism when exposed to a given concentration of the N-chloramine.

For each N-chloramine, a stock solution of known concentration was prepared using the appropriate buffer solution. To 5 ml of the N-chloramine solution is added 0.2 ml of an overnight broth culture containing the particular microorganism being investigated. At time intervals of 0.5, 1, 2, 3, 4, 5, ..., min, a loop of this suspension is subcultured into 5 ml of sterile nutrient broth. All samples are then incubated at 37° for 7 days, with daily observation for evidence of bacterial growth. The time intervals reported are for that sample in which no bacterial growth was observed after the incubation period.

In addition, several controls were also conducted.

Control 1—This control is designed basically to check the overnight broth culture.

To 5 ml of sterile 0.9% NaCl solution is added 0.2 ml of an overnight broth culture containing the particular microorganism being investigated. A loop of this suspension is subcultured into 5 ml of sterile nutrient broth and incubated at 37° for 7 days.

Control 2—This control is designed to ensure that the dilution factor of the nutrient broth is beyond any bacteriostatic activity of the N-chloramine solution tested.

To 5 ml of sterile nutrient broth is added a loop of the N-chloramine solution being evaluated and the solution is mixed immediately. To this solution is then added a loop of overnight broth culture which has been diluted 25 times with 0.9% NaCl solution. Incubate at 37° for 7 days.

Control 3—This control is used to ensure that the bacterial growth observed is due to the organism being tested rather than to contamination from a foreign organism.

At the same time intervals used for subculturing the test solution into nutrient broth during the screening procedure, a loop of the test solution is also subcultured onto sterile nutrient agar plates. This technique is useful for observing the morphology of the surface colony characteristic for each organism.

Control 4—This control was used initially to ensure that the pH of the solution and the concentration of the buffer species did not inhibit the bacterial growth during the time intervals used in the screening procedure.

³ Aldrich Chemical Co.

⁴ Perkin-Elmer model PS-16.

The entire screening procedure was conducted for each buffered solution rather than the N-chloramine solution in the procedure.

SUMMARY

The lone-pair ionization energies for the N, O, and Cl heteroatoms in four derivatives of piperidine were measured from UV photoelectron spectra. The interaction between the various lone pairs in each piperidine was studied. The hydrolytic stability and antimicrobial activity of the N-chloro piperidines also were determined. The current results suggest that it may be possible to correlate the stability of the nitrogen lone pair, as determined from photoelectron spectroscopy, with the antibacterial activity of the corresponding N-chloramines.

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Cardiotonic Steroids I: Importance of 14β -Hydroxy Group in Digitoxigenin

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Abstract \Box Analogs of digitoxigenin and its 3-acetate, in which the stereochemistry and nature of the substituent at the 14-position are varied, were synthesized and assayed for inhibition of myocardial Na⁺,K⁺-adenosine triphosphatase. Among the 3-acetates, the 14 β -H and 14 α -H analogs were less active than 3-acetoxydigitoxigenin by a factor of 1000, with the 14 β -chloro analog slightly more active than the others. The corresponding 14 β ,15 β -epoxide was 0.03 as active as 3-acetoxydigitoxigenin, but the isomeric 14 α , 15 α -epoxide was virtually inactive. In the 3-OH series, digitoxigenin was significantly more active than any analog. The 14 β -H analog was 10-fold more active than the 14 α -H analog. The 14 β -H sults suggest the desirability of *cis*-C,D-ring fusion and a highly selective function for the 14 β -OH group of digitoxigenin, possibly

It has been accepted generally that the 14β -OH group of cardiotonic steroids and their glycosides is essential to their inotropic activity (1). Derivative structures such as the corresponding 14(15)-enes and epoxides are considered much less active in assays based upon lethality in cats (1), and 14-epidigitoxigenin showed no activity in a Na⁺,K⁺-adenosine triphosphatase assay (2). These observations tend to confirm the need for a *cis*-C,D-ring fusion as well as a hydroxy group at the 14-position. However, recent reports (3, 4) on uzarigenin derivatives showed that

based upon its ability to receive a hydrogen in hydrogen bonding. Syntheses of the 14 β -H, 14 α -H, and 14 β -Cl analogs were accomplished by converting the known 15-ketones to ethylene thioketals and desulfurizing with Raney nickel. Attempts to prepare a 14 β -NH₂ analog were unsuccessful.

Keyphrases \Box Digitoxigenin, 14 β -H, 14 α -H, 14 β -Cl, and acetate analogs—synthesis and inhibition of Na⁺, K⁺-adenosine triphosphatase \Box Cardiotonic steroids—synthesis of digitoxigenin analogs (14 β -H, 14 α -H, 14 β -Cl, and acetates), inhibition of Na⁺, K⁺-adenosine triphosphatase \Box Structure-activity relationships—digitoxigenin analogs (14 substituents) and inhibition of Na⁺, K⁺-adenosine triphosphatase

the 14β -H analog (I) was one-third as active as uzarigenin itself (II) in the isolated frog heart, indicating that the 14β -hydroxy group is not indispensible for positive inotropic activity. This report prompted a more extensive investigation of the requirements in stereochemistry and the nature of substituents at the 14-position of cardenolides for inotropic activity.

The digitoxigenin nucleus was chosen for investigation since it is more active than the uzariginen nucleus due to its *cis*-A,B-ring fusion. This factor appeared important for structure-activity relation-