

Morgan *F* test result in a study would be declared significant at the unilateral probability level of 0.01, if the study used 18 subjects, and if the power of detection of a difference in test and reference product standard deviations were set at 0.80, then the statistically detectable ratio would be (by rough extrapolation of the data in Ref. 9) >2 or 3. In other words, a test product standard deviation that is truly more than double or triple that of the reference product is likely to be detected in a study under these conditions. Any increase in sample size to improve the detection of differences in the standard deviations is believed to be unwarranted since, in view of other sources of inherent variation known in posology but not considered further here and in view of the desirability of minimizing the use of human subjects in bioequivalency studies, the power seems ample.

APPENDIX

It was assumed that the test product values and the reference product values followed a bivariate normal distribution with both means equal to 100, with certain chosen standard deviations and with correlation coefficients (Table I) modified to allow for the intrasubject variability which, among other things, reduces the estimated value of ρ (10).

The assumption that all actual bioequivalency data follow the bivariate normal distribution may be questioned; therefore, it is noted that very large intersubject coefficients of variation for *AUC*, e.g., 150%, are symptomatic of drug products with either a very skewed distribution or some outliers. In such cases, the mean and standard deviation are not sufficient to describe the bivariate nonnormal distribution. However, if differences in only means are tested, the analysis of variance has been found to be a very robust procedure that is practically undisturbed by such things. Remedies after the fact include transformation of the data (e.g., logarithmic) or identification of assignable causes for outliers; remedies *a priori* consist of controlling factors that have been found to cause aberrant results for the particular drug. In cases where the log transformation is used, the 75/75 rule also must be transformed. The performance of that transformed rule would be investigated differently than here, but intuitively the deficiencies of the rule would be substan-

tially the same. The findings in the present study are appropriate for bioavailability parameters that are normally distributed.

The Monte Carlo simulation proceeded according to a FORTRAN program², which used a multivariate normal random deviate generator subroutine, GGNRM³, and a local subroutine, CALC, which applied the 75/75 rule to each of the 1000 studies in turn. The main program then tallied up the number of studies that "passed." One run was made to obtain each cell in Tables I and II. The Pitman-Morgan *F* (9) was calculated for the identical sets of data.

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ACKNOWLEDGMENTS

The author thanks R. Ganesan, S. Heft, R. Kohberger, J. Johnson, A. Weiss, and N. Bauman for helpful comments and Dr. Daniel Weiner for helpful comments and discussion. He also thanks T. Hoffman for the application of Marsaglia's work which provided a different viewpoint.

² Available from the author.

³ International Mathematical and Statistical Libraries, Houston, Tex.

Assignment of Conformation and Configuration to Potassium Permanganate Oxidation Products of Quinidine

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Abstract □ Two epimeric aldehydes [(*R*)- and (*S*)-quinidinals] and the corresponding acids [(*R*)- and (*S*)-norhydroquinidinoic acids] were prepared by the oxidation of quinidine. The π - π interactions of the carbonyl group and the aromatic moiety, as reflected in the NMR spectra, were compared with those of quinidine. NMR spectroscopic analyses made it possible to assign both the stable conformation and their configuration at C-3 to these molecules. The free hydroxyl group at C-9 must be present for the chemical shift values to be concentration dependent.

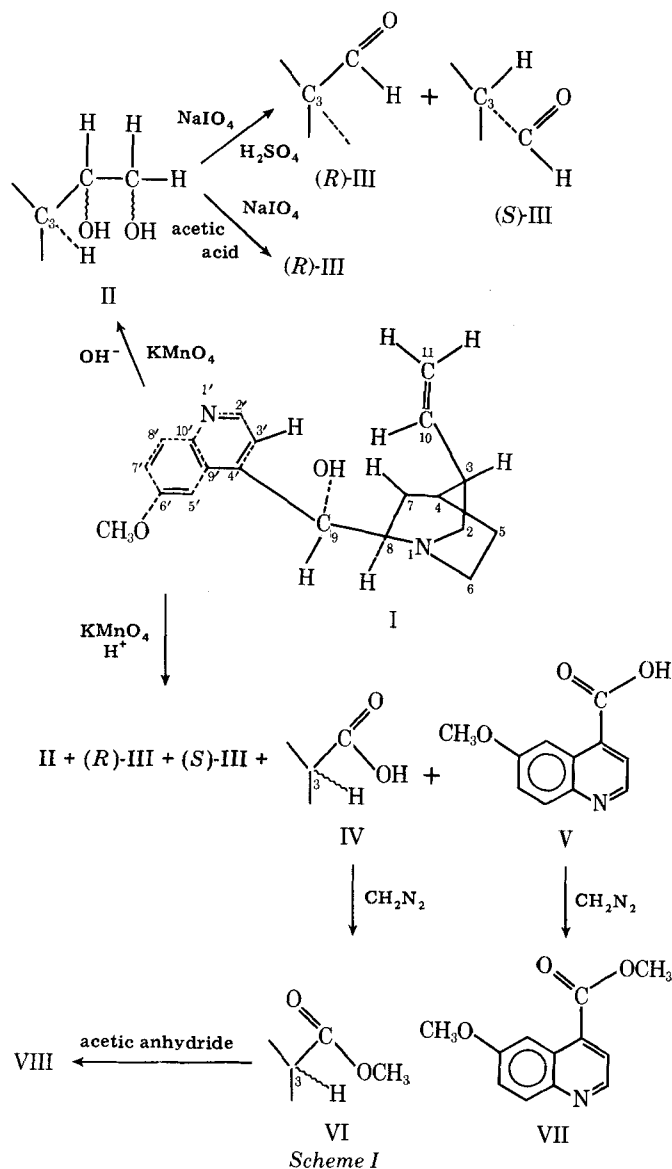
Previous work showed that the NMR spectra of quinidine (I) and hydroquinidine (1) differ significantly. The unique features encountered in the I molecule were attributed to π - π interactions. If changes in these π - π interactions are reflected in the NMR spectra of new compounds compared to the parent substances, it must be determined whether there is any correlation between the NMR data obtained and the biological activity of the compound. The antiarrhythmic activity of I and quinine

These findings provide more information on association in the parent molecules.

Keyphrases □ Quinidine—oxidation products prepared, conformation and configuration assigned, NMR analyses □ Oxidation products—quinidine, conformation and configuration assigned to oxidation products, NMR analyses □ NMR spectroscopy—analyses, quinidine and oxidation products, conformation and configuration assigned

differ greatly because of differences in configuration at C-8 and C-9. Comparison of the NMR data of I and quinine may provide information on the origin of these differences. Intraatomic distances in the molecules play an important role in their respective biological activities. NMR analyses may be valuable in assessing such differences and thus evaluating their possible therapeutic potential.

NMR analysis was used in the present work to measure the extent of π - π interaction by changing the intensity of



the π system in the side chain at C-3. For this purpose, quinidinal¹ (III, a C-10 aldehydic derivative of I) and the corresponding acid norhydroquinidinoic acid (quitenidene, IV) were prepared by potassium permanganate oxidation of the vinylic group of I. Although preparation of III from I by the ozonization route was reported previously (2), the alternative method utilizing potassium permanganate was selected. With the latter method, it was possible to obtain the diol (II), the epimeric aldehydes (*R*)- and (*S*)-III, the corresponding acid (IV), and a fragmentation product (V) in one step (Scheme I). NMR analyses and TLC of the isolated products made it possible to study the characteristics and nature of the new compounds and the details of the experimental conditions.

EXPERIMENTAL

NMR spectra were obtained at 300 MHz using an analytical NMR spectrometer² equipped with an automatic recorder. Tetramethylsilane in deuteriochloroform was the internal standard. IR spectra were mea-

¹ The authors suggest the name norhydroquinidinal (III) instead of quini-dinal.

² Bruker W. H. 300.

Table I—Chemical Shift Values ^a of the Protons of (*R*)-III and (*S*)-III in 1:1 Ratio at Various Concentrations (Milligrams per 0.5 ml) and of Pure (*R*)-III

Proton	Concentration of (R)-III and (S)-III			(R)-III
	8 mg	15 mg	23 mg	
O	9.87 (s)	9.86 (s)	9.85 (s)	9.83 (s)
$\parallel$ C-H	9.78 (s)	9.78 (s)	9.76 (s)	
H-2'	8.66 (t) (<i>J</i> = 4 Hz)	8.63 (t) (<i>J</i> = 4 Hz)	8.58 (t) (<i>J</i> = 4 Hz)	8.58 (d) (<i>J</i> = 4 Hz)
H-8'	7.97 (d) (<i>J</i> = 9 Hz)	7.95 (d) (<i>J</i> = 9 Hz)	7.93 (d) (<i>J</i> = 9 Hz)	7.98 (d) (<i>J</i> = 9 Hz)
H-3'	7.54 (d) (<i>J</i> = 4 Hz)	7.53 (d) (<i>J</i> = 4 Hz)	7.49 (d) (<i>J</i> = 4 Hz)	7.44 (d) (<i>J</i> = 4 Hz)
	7.47 (d) (<i>J</i> = 4 Hz)	7.47 (d) (<i>J</i> = 4 Hz)	7.43 (d) (<i>J</i> = 4 Hz)	
H-7'	— _b	— _b	— _b	— _b
H-5'	7.18 (s) 7.13 (d) (<i>J</i> = 2 Hz)	7.18 (s) 7.13 (d) (<i>J</i> = 2 Hz)	7.15 (d) (<i>J</i> = 2 Hz)	— _b
H-C-9-OH	5.75 (s) 5.62 (d) (<i>J</i> = 4 Hz)	5.72 (s) 5.59 (d) (<i>J</i> = 5 Hz)	5.72 (s) 5.59 (d) (<i>J</i> = 5 Hz)	5.59 (d) (<i>J</i> = 4 Hz)
CH ₃ O	3.91 (s) 3.85 (s)	3.90 (s) 3.84 (s)	3.90 (s) 3.84 (s)	3.92 (s)

^a In parts per million. ^b Increasing overlap with the hydrogen of chloroform with concentration.

sured in potassium bromide disks on a grating IR spectrophotometer³. Mass spectra were recorded on a mass spectrometer⁴ provided with an electric-impact and field-desorption ion source. Pure I was obtained from commercial quinidine sulfate USP⁵. TLC and preparative TLC were carried out on aluminum oxide GF 254⁶ plates.

Oxidation of I by Potassium Permanganate and Dilute Sulfuric Acid—Quinidine (I) (1 g), as the sulfate salt, was dissolved in 20 ml of dilute sulfuric acid (2 N), and two equivalents of potassium permanganate (0.95 g) in aqueous solution was added. The reaction mixture was stirred for 2 hr at room temperature. Sodium bisulfite in a 10% aqueous solution was added dropwise until the precipitated manganese dioxide was dissolved. TLC revealed a mixture of polar and nonpolar products. Separation of these products was accomplished by extraction and chromatography.

Isolation of II and III—The clear solution obtained after the addition of sodium bisulfite was transferred to a separator and made alkaline by the addition of sodium carbonate. The mixture was extracted completely with chloroform containing 10% methanol. After evaporation of the solvents, TLC revealed the presence of hydroquinidine, a small amount of unchanged I, and two new compounds, a diol (II) appearing as two isomers and the aldehyde (III).

The mixture was chromatographed on aluminum oxide plates. The fluorescing material was viewed under UV light and divided into three fractions. The upper strip was scraped and dispersed in 20 ml of 0.1 *N* H₂SO₄. Sodium bicarbonate (240 mg) was added, the mixture was transferred to a separator, and I and hydroquinidine were extracted with chloroform. The middle strip containing III was treated in the same manner but was extracted with chloroform containing 10% methanol. Its melting point was nonspecific; *R*_f 0.20 (ethyl acetate-ethanol, 9:1); IR: 1720 (CO) cm⁻¹; mass spectrum: *m/z* 326. The chemical shifts of III, which consisted of two epimers (*R*)-III and (*S*)-III at various concentrations, are summarized in Table I.

The lower strip, containing a 1:1 mixture of the diastereomeric diols (II), was scraped; the powder obtained was extracted directly with chloroform containing 20% methanol. The two diastereomers were separated by repeated preparative TLC on aluminum oxide plates. The more polar diol, R_f 0.13 (ethanol), yielded crystals from acetone and was sparingly soluble in chloroform. When attempting to determine its melting point, the crystals began to discolor at $\sim 200^\circ$, becoming brown (similar to the caramelization of sugar) and forming a solid lump, which eventually liquified at $225\text{--}227^\circ$. The less polar diol, R_f 0.36 (ethanol), was much more soluble in acetone and chloroform and could not be induced to crystallize. Upon the addition of ether to an acetone solution of the less polar diol, an amorphous material settled out. The melting

³ Perkin-Elmer 457.⁴ Varian Mat CHS DF.

⁵ Sigma Chemical Co., St. Louis, Mo.

⁶ Stahl, Merck.

Table II—Chemical Shift Values ^a of the Protons of VII at Two Different Concentrations (Milligrams per 0.5 ml)

Proton	Concentration of VII	
	11 mg	7 mg
H-3'	8.87 (d) (<i>J</i> = 4 Hz)	8.86 (d) (<i>J</i> = 4 Hz)
H-5'	8.25 (d) (<i>J</i> = 3 Hz)	8.25 (d) (<i>J</i> = 3 Hz)
H-8'	8.08 (d) (<i>J</i> = 9 Hz)	8.08 (d) (<i>J</i> = 9 Hz)
H-2'	7.94 (d) (<i>J</i> = 4 Hz)	7.94 (d) (<i>J</i> = 4 Hz)
H-7'	7.44 (q) (<i>J_m</i> = 3 Hz) (<i>J_o</i> = 10 Hz)	7.44 (q) (<i>J_m</i> = 3 Hz) (<i>J_o</i> = 10 Hz)
CH ₃ O-C-6'	4.04 (s)	4.04 (s)
	3.98 (s)	3.99 (s)

^a In parts per million.

point of this amorphous material was nonspecific; at ~200°, it became brown and finally turned into a viscous liquid.

Isolation of VI and VII—The alkaline solution was made acidic by the addition of acetic acid. The mixture was extracted with chloroform–isopropyl alcohol (3:1). After evaporation of the solvents, the residue was treated with diazomethane, and the methyl esters of IV and V (VI and VII, respectively) were separated by preparative TLC. The mixture was chromatographed on aluminum oxide plates. The fluorescing material was viewed under UV light and divided into two fractions. The upper strip was scraped, and the powder obtained was extracted directly with chloroform containing 10% methanol. It contained the methyl ester of quinic acid (VII), *R_f* 0.80 (ethyl acetate), mp 80–81°; IR: 1715 (CO) cm⁻¹. Table II lists the NMR data.

The lower strip was treated as was the upper strip and was found to contain the methyl ester of norhydroquinidinoic acid (VI), *R_f* 0.14 (ethyl acetate) and *R_f* 0.70 (ethyl acetate–ethanol, 1:1). Recrystallization from methanol gave crystals, mp 140–141°. However, there was a softening of the material at 137°, suggesting that VI consisted of two diastereomers, (*R*)-VI and (*S*)-VI. The IR spectrum showed 1725 (CO) cm⁻¹ with a shoulder at 1740 (CO) cm⁻¹, implying the existence of another diastereomer. The chemical shifts at various concentrations are summarized in Table III.

Methyl Ester of 9-Acetyl Norhydroquinidinoic Acid (VIII)—Pure VI (60 mg) was dissolved in 1 ml of pyridine and 1 ml of acetic anhydride, and this mixture was allowed to stand overnight for ~24 hr at room temperature. The solvents were removed with an air stream, and the solid residue was dissolved in 30 ml of chloroform. The chloroform solution was transferred to a separator, washed with 20 ml of water, and filtered without delay through anhydrous sodium sulfate since the acetylated product was prone to hydrolysis, *R_f* 0.5 (ethyl acetate); IR: 1726, 1736, and 1746 (CO) cm⁻¹. The NMR data are included in Table III.

Oxidation of I by Potassium Permanganate under Basic Conditions—Five grams of I was dissolved in 30 ml of tetrahydrofuran. To this solution was added 40 ml of aqueous 1 *N* KOH followed by two equivalents of potassium permanganate (4.75 g) in aqueous solution. The reaction mixture was stirred magnetically for 7 days at room temperature and then was transferred in its entirety to a separator and extracted with chloroform containing 20% methanol until complete extraction was achieved. Upon removal of the solvents, a substance was readily crystallized. It proved to be a mixture of two diastereoisomers (II), which were identical to those obtained by oxidation of I under acidic conditions.

Oxidation of II by Sodium Periodate—Method A: Dilute Aqueous Strong Acid—One gram of II was dissolved in 20 ml of 1 *N* H₂SO₄. A 10.7% aqueous sodium periodate solution (6.5 ml) was added to the acidic solution of II, and this mixture was stirred at room temperature overnight. The solution was then transferred to a separator, made alkaline with sodium carbonate, and extracted thoroughly with chloroform containing 20% methanol. After evaporation of the solvents, an amorphous material was obtained that would not crystallize. This material was identical in all respects to the amorphous mixture of (*R*)-III and (*S*)-III obtained under acidic conditions.

Method B: Dilute Aqueous Weak Acid—One gram of II was dissolved in 1 ml of acetic acid and diluted with 20 ml of water. The sodium periodate was added, and the reaction mixture was stirred overnight. Once again the solution was made alkaline and extracted as for Method A. After evaporation of the solvents, a crystalline material was obtained and re-

crystallized from acetone, mp 205°, *R_f* 0.20 (ethyl acetate–ethanol, 9:1). NMR analysis revealed only the natural epimer (*R*)-III (Table I).

Epimerization of (*R*)-III—Attempts to purify (*R*)-III by chromatography on aluminum oxide plates brought about complete epimerization, and NMR analysis showed that the final product contained a 1:1 mixture of (*R*)-III and (*S*)-III. On the other hand, NMR analysis of a freshly prepared sample of pure (*R*)-III in deuterated chloroform showed only pure (*R*)-III. After 24 hr, the same sample showed small amounts of the epimeric aldehyde (*S*)-III; after a 7-day standing period, the sample showed equal amounts of (*R*)-III and (*S*)-III.

RESULTS AND DISCUSSION

Assignment of Conformation and Configuration to Oxidation Products of I—The data summarized in Table I show clearly that the chemical shift values of (*R*)-III and (*S*)-III in a mixture depend not only on the concentration of the mixture but also on the relative amounts of these epimers in the mixture. Therefore, to assign the configuration at C-3 in these molecules, NMR analysis was carried out on a mixture containing (*R*)-III and (*S*)-III in a 2:1 ratio.

NMR Analysis of (*R*)-III—The aldehydic proton resonated as a singlet at δ 9.85 ppm. The H-2' proton appeared as a broad singlet at δ 8.54 ppm, H-3' appeared as a doublet at δ 7.45 ppm (*J* = 4 Hz), and H-8' appeared as a doublet at δ 7.90 ppm (*J* = 9 Hz). The H-5' and H-7' protons resonated between δ 7.28 and 7.25 ppm. The H-C-9-OH proton appeared as a doublet at δ 5.60 ppm (*J* = 3.0 Hz). The methoxy protons absorbed at δ 3.87 ppm.

NMR Analysis of (*S*)-III—The aldehydic proton resonated as a singlet at δ 9.76 ppm, at a higher field compared to the crystalline aldehyde. The H-2' proton appeared as a broad singlet at δ 8.54 ppm. The H-3' proton resonated at δ 7.50 ppm (*J* = 4 Hz), at a lower field compared to the crystalline aldehyde; H-8' appeared at δ 7.90 ppm (*J* = 10 Hz), the same as in the crystalline aldehyde. Both H-5' and H-7' appeared separately; the former proton shifted to a higher field at δ 7.11 ppm and the latter proton absorbed at almost the same frequency compared to (*R*)-III. The proton alpha to the hydroxyl group at C-9 (H-C-9-OH) appeared as a slightly broadened singlet at δ 5.71 ppm, at a lower field than (*R*)-III. The peak of the methoxy protons appeared at δ 3.81 ppm, at a slightly higher field than (*R*)-III.

While some protons were less shielded in (*R*)-III compared to those of (*S*)-III, other protons in (*R*)-III experienced more shielding than the same protons of (*S*)-III. In addition, the aldehydic protons in both epimers appeared as singlets while the coupling constants between the aldehydic proton and the proton alpha to the carbonyl group generally ranged between 1 and 3 Hz and in some cases reached a value of 6 Hz (3, 4). This finding indicates that a single rotamer prevails in the aldehydic groups with a dihedral angle of ~90° between the aldehydic proton and the proton alpha to the carbonyl group at C-3.

In a previous work based on NMR analyses (1), we assumed a stable

Table III—Chemical Shift Values ^a of the Protons of VI at Various Concentrations (Milligrams per 0.5 ml) and of VIII

Proton	Concentration of VI			VIII,
	14 mg	36 mg	50 mg	10 and 35 mg
H-2'	8.67 (d) (<i>J</i> = 4 Hz)	8.56 (d) (<i>J</i> = 4 Hz)	8.51 (d) (<i>J</i> = 4 Hz)	8.72 (d) (<i>J</i> = 4 Hz)
H-3'	7.51 (d) (<i>J</i> = 4 Hz)	7.46 (d) (<i>J</i> = 4 Hz)	7.44 (d) (<i>J</i> = 4 Hz)	7.34 (d) (<i>J</i> = 4 Hz)
H-8'	8.00 (d) (<i>J</i> = 9 Hz)	7.94 (d) (<i>J</i> = 9 Hz)	7.87 (d) (<i>J</i> = 9 Hz)	8.01 (d) ^b (<i>J</i> = 9 Hz) 7.96 (d) ^c (<i>J</i> = 9 Hz)
H-C-9-OCH ₃	—	—	—	6.61 (d) (<i>J</i> = 4 Hz)
H-C-9-OH	5.62 (d) (<i>J</i> = 5 Hz)	5.55 (d) (<i>J</i> = 5 Hz)	5.56 (d) (<i>J</i> = 5 Hz)	— 4.00 (s) ^b
CH ₃ O-C-6'	3.92 (s)	3.85 (s)	3.82 (s)	3.88 (s) ^c
	3.75 (s)	3.72 (s)	3.70 (s)	3.77 (s)
	—	—	—	2.14 (s) ^b 2.01 (s) ^c

^a In parts per million. ^b (*R*)-VIII. ^c (*S*)-VIII.

Table IV—Chemical Shift Values ^a of the Protons of Hydroquinidine, VI, I, and (R)-III (~15 mg/0.5 ml)

Compound	H-2'	H-3'	H-8'
Hydroquinidine	8.63 (d) (<i>J</i> = 4 Hz)	7.52 (d) (<i>J</i> = 4 Hz)	7.95 (d) (<i>J</i> = 9 Hz)
VI	8.66 (d) (<i>J</i> = 4 Hz)	7.51 (d) (<i>J</i> = 4 Hz)	8.00 (d) (<i>J</i> = 9 Hz)
I	8.59 (d) (<i>J</i> = 4 Hz)	7.49 (d) (<i>J</i> = 4 Hz)	7.94 (d) (<i>J</i> = 9 Hz)
(R)-III	8.58 (d) (<i>J</i> = 4 Hz)	7.42 (d) (<i>J</i> = 4 Hz)	7.93 (d) (<i>J</i> = 9 Hz)

^a In parts per million.

preferred conformation for I. On the basis of the data presented for the two aldehydes, we concluded that the molecules of each epimer exist as a stable rotamer and the structure of the rotamers depends mainly on steric and π - π interactions in the molecule. As a result of this latter interaction, the coplanar H-C=O group in (R)-III is in close proximity to the nitrogen-containing aromatic ring, and this involves rotation about the C-4'-C-9-C-8 bonds, making the dihedral angle between the protons of C-9 and C-8 <90°. In (S)-III, the steric interaction of the proton at C-3 and the nitrogen-containing aromatic ring causes rotation in the opposite direction, bringing about a change in the dihedral angle between the protons of C-9 and C-8 from <90° to ~90°. At the same time, the H-C=O group is positioned at a greater distance from the nitrogen-containing aromatic ring as compared to (R)-III.

The ups and downs of the chemical shifts of some of the protons could be explained on the basis of the proposed assumption. In (R)-III, the close proximity of the H-C=O group to the nitrogen-containing aromatic ring places H-3' within the positive zone of the carbonyl cone and H-2' near its border. Consequently, H-3' shifts to a higher field, and H-2' appears as a broad singlet. For the same reason, the aldehydic proton in (R)-III is under the deshielding influence of the aromatic ring and absorbs at a lower field compared to the aldehydic proton of (S)-III. In (S)-III the nitrogen atom of the quinuclidine ring is in closer proximity to H-5' than in (R)-III and, consequently, H-5' experiences a shielding effect [due to a long-range shielding by the nitrogen atom (5, 6)].

Whereas the oxidation of I under basic conditions was relatively slow, the oxidation under acidic conditions was much faster and more complex. Its value was in the ability to: (a) prepare, in one reaction, carbonyl-containing compounds other than the aldehydes [(R)-III and (S)-III]; (b) study, with the help of NMR analyses of these additional carbonyl-containing compounds, the intensity of the π - π interaction when the side-chain π system varied from vinyl to aldehydic carbonyl to ester carbonyl; (c) explore, at the same time, their influence on the chemical shifts of some important protons in the molecule; and (d) determine whether they were concentration dependent.

The NMR data of (R)-III and (S)-III made it possible to assign the configuration at C-3. As the carbonyl group departed from the aromatic ring, certain apparent changes took place. The intensity of these changes depended on the mutual π - π interactions between the aromatic ring and the side-chain π systems. In Table IV, three π systems (aldehyde, methoxycarbonyl, and vinyl) are compared; differences in intensity were measured by the chemical shifts experienced by H-2', H-3', and H-8'. For comparative purposes, hydroquinidine was also introduced. Since H-2' is at a greater distance from the side-chain π systems, H-3' is more sensitive to both the intensity of the π systems and the stable conformation of the molecule. As can be seen in Table IV, the shielding of H-3' increased in the following order: (R)-III > VI $\approx$ I. With regard to H-2', the order of shielding was as follows: (R)-III = I > VI.

These differences may be explained on the basis of different orientations of the carbonyl group of the aldehyde and methoxy functions. Since the methoxy group in VI is more bulky than the proton in the aldehydic group, rotation about C-10 takes place and makes the distance between the carbonyl group and H-3' of the aromatic ring greater and thus lowers the shielding effect. Consequently, VI resembles hydroquinidine while (R)-III is closer to I with respect to the shielding effects on H-2' and H-3'. Therefore, it is concluded that the aldehydic and vinylic groups are more appropriate for assignment of the C-3 configuration than the methoxy group, which brings about steric disturbances.

Origin of π - π Interaction—The nitrogen-containing ring is relatively more π deficient due to the electronegative character of the nitrogen atom. This π -deficiency is reflected in the chemical shifts of the protons in the two rings of the quinoline structure. The protons of the nitrogen-containing ring generally are deshielded to a greater extent than the protons of the sister ring. The π - π interactions may be visualized as a

donor-acceptor system. Thus, the π -deficient aromatic ring is the acceptor while the side-chain π system is the donor.

Epimerization at C-3—Oxidation of II under weakly acidic conditions for a short time (as in Method B) yields pure natural (R)-III. Under more acidic conditions (as in Method A), rapid epimerization at C-3 takes place, mainly due to the tertiary nature of the carbon at C-3. In addition, the ease of epimerization suggests that, in the natural configuration (R)-III, the aldehydic derivative III is under steric strain and that this strain is partially relieved in the process of epimerization. An equilibrium is reached between the two epimers. The 1:1 ratio obtained at equilibrium suggests that the energy content of (R)-III and (S)-III is approximately equal. In (R)-III, the energy gained by the π - π interaction is partially offset by the steric interaction developed in the vicinity of C-8-C-9. In (S)-III, the release of steric strain in the vicinity of C-8-C-9 is counteracted by a decrease in the π - π interaction. Complete epimerization was also found when (R)-III was subjected to chromatography or dissolved in chloroform. Therefore, the enhanced sensitivity is due not only to the tertiary character of C-3 but also to the energy content of the molecule.

Concentration Dependence of Chemical Shifts—The concentration dependence of the chemical shifts of I and hydroquinidine were observed previously (1). It was suggested that this concentration dependence is a result of molecular association. In the present study, the same phenomenon was observed in III and IV (Tables I and III). Since the natural epimer (R)-III was only sparingly soluble in deuteriochloroform, a mixture of (R)-III and (S)-III, which was much more soluble, was used. Compound IV was almost insoluble in deuteriochloroform; therefore, the NMR analysis of its methyl ester (VI) was made. Quinic acid (V) was converted to its methyl ester (VII), which was much more soluble. Practically, no concentration dependence was observed in this compound (Table II).

These observations clearly imply that changes in the chemical shifts at different concentrations occur only when the quinuclidine moiety is connected to the quinoline moiety through the carbon atom at C-9 to which a hydroxyl group is attached. Thus, the previous suggestion that molecular association is initiated by hydrogen bonding of the hydroxyl groups has additional support. Aside from its importance in understanding the origin of the association in the molecules, the NMR data of the fragmentation derivative (VII) reveal some interesting features. Theoretically, the carbonyl group of the methoxycarbonyl moiety may be directed toward either H-5' or H-3' if there is no inhibition of resonance in the molecule as a whole. On the other hand, the inhibition of resonance could cause rotation about the C-4'-C-9 bond and bring about a departure of the bulky methoxy group from either H-3' or H-5'. The NMR data of VII unambiguously show that the carbonyl group is positioned near H-5' and that the methoxy group is in the vicinity of H-3'. The model of this molecule reveals that reversing the positions of the carbonyl group and the methoxy is associated with intensified steric strain. On the basis of this assumption, the NMR data may be rationalized. Furthermore, the concentration-independence of its chemical shifts implies that the molecule has one, and only one, stable rotamer.

When the hydroxyl group was replaced by acetoxy in VI, it was found that there was no concentration dependence in the acetyl derivative (VIII) (Table III). This result was in full agreement with previous findings (1) suggesting the absence of molecular association in the acetyl derivative of I. As expected, the presence of the acetoxy group caused a paramagnetic shift of H-2' and a diamagnetic shift of H-3'. Another consequence of the concentration independence of this molecule was clearly shown in its NMR spectrum, namely, the presence of a second epimer (S)-VIII in addition to (R)-VIII. Since the oxidation of I under acidic conditions yielded two epimers, (R)-III and (S)-III in a 1:1 ratio, one might expect that the acids of the same oxidation reaction would also consist of two epimers (R)-IV and (S)-IV in a 1:1 ratio. The NMR data showed that this was not the case; rather, the ratio of the two epimers was ~3:1 in favor of the natural configuration. This observation suggests that I is mainly oxidized directly to an acidic product without the mediation of an aldehyde. The presence of (S)-IV was due to slow oxidation of the epimeric aldehydes (R)-III and (S)-III. The presence of this second epimer was not revealed in the NMR spectrum of VI but only in that of the acetylated product (VIII); this result is a consequence of the lack of molecular association.

REFERENCES

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