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STEREOSPECIFIC EPIMERIZATION, OXIDATION AND TOXINE REARRANGEMENT IN CINCHONA ALKALOIDS CATALYZED BY ACETIC ACID

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Abstract - Glacial acetic acid catalyzed a novel stereospecific epimerization of chinchona alkaloids at C - 9. In the presence of water,acetic acid also catalyzed the known toxine rearrangement and oxidation to the corresponding 9- keto derivatives. Addition of acetic anhydride to acetic acid diminished oxidation and epimerization at C-9, and the main products were the results of hydramine fission. Only propionic acid but not other acids, effected similar but not identical transformations. Addition of small quantities of H_2O_2 or exclusion of oxygen produced quantitative oxidation and rearrangement products, respectively. The catalysis by aqueous solutions of acetic acid, involves C-9-OH in the formation of a three-membered ring intermediate. On the other hand,with anhydrous acetic acid, the acetoxyl at C-9 participates in construction of a five-membered ring intermediate. In both cases the reaction appears to be intramolecular. Support for the proposed mechanisms was provided by the isolation of a quarternary salt derived from quinidine, the structure of which was also characterized by X-ray diffraction analysis. Aqueous acetic acid catalyzed the rearrangement of this salt to its corresponding toxine only, and neither oxidation nor epimerization could be observed under conditions employed for the natural alkaloids.

Previous studies with compounds such as ephedrine have established the importance of the conformation of the molecule in determining its pharmacologic activity[1]. Preparation of the epimer of natural quinidine is expected to serve similar purposes in attempts to establish the role of conformation in the pharmacologic potency of chinchona alkaloids.

Reactions causing epimerization at C-8 and C-9 of cinchona alkaloids were reported in the past[2-9]. However, exclusive epimerization at C-9 has never been successful. The first step in epimerization involved conversion of the hydroxyl group in cinchona alkaloids to halogen or tosyl derivatives[9]. However, in the ensuing displacement reactions substantial rearrangements occurred instead of, or in addition to, the desired inversion.

In another set of reactions on the 9-keto derivatives stereospecific reduction with $NaBH_4$ gave a mixture of alcohols in which that with the C-9-epi configuration predominated[6]. However, this approach affected also the configuration at C-8 and therefore separation of all possible isomers was difficult and time consuming[7].

We wish to report here a simple procedure using acetic acid as catalyst,for the epimerization of chinchona alakaloids at C-9 only, in order to compare their properties with the natural C-9,S epimer. This objective could not have been achieved by earlier methods.

Acetic acid can also be used to induce :(a) oxidation of the alkaloids to the corresponding 9-keto derivatives and/or (b) rearrangement of the alkaloids to the corresponding toxine derivatives in very high yields.

Our idea stemmed from earlier observations, in which natural cinchona alkaloids were converted

to toxine derivatives during prolonged boiling in aqueous acetic acid[9,10]. However, the ability of acetic acid to catalyze also oxidation and epimerization, as shown in this study, raised several problems:

1) Does the mechanism of these reactions require a common intermediate?

2) Do these rearrangements occur with acetic acid only?

We wish to stress that all the compounds in this paper, with the exception of the quaternary salt (IV) and its toxine derivative (XIII), have already been prepared and characterized in the past[5,6]. Our contribution lies in presenting a novel and simple procedure for preparation from the natural chinchona alkaloids of pure epi compounds, for oxidations to C-9-keto compounds, and /or formation of toxine products in high yields.

EXPERIMENTAL

Methods and Materials.

^{1}H-NMR spectra were obtained at 300 MHz using an analytical NMR spectrometer (Bruker, W.H.300) equipped with an automatic recorder. Tetramethylsilane in deuterochloroform was added as internal standard.

Mass spectra were recorded on a mass spectrometer (Varian Mat CHS DF) provided with an electric impact and field desorption ion source.

I.R. spectra were measured in KBr discs or in chloroform solutions on a grating IR spectrophotometer (Perkin Elmer model 457).

Colorless crystals of the quaternary salt of quinidine (IV) were subjected to X - ray diffraction analysis as outlined below.

Thin layer chromatography (TLC) was carried out on Al_2O_3 plates (GF_{254} according to Stahl,Merck,W.Germany).

Quinidine and quinine sulfate (USP) were purchased from Sigma Chemicals Co. St.Louis,Mo. All reagents used were of analytical grade.

Preparations.

Purification of commercial quinidine (I) and quinine (III) was done by TLC alumina plates developed with ethyl acetate.The pure products were catalytically hydrogenated with Pd/C, according to Yanuka et al[11].

Conversion of quinidine (I) to epiquinidine (V) and quinotoxine (X) (cf. Scheme 4):

(a) Pure quinidine (0.5 g) was dissolved in a solution of acetic acid (3 mL) and acetic anhydride (3 mL) and heated at 70 - 80°C for 48 h. The solvents were evaporated under reduced pressure and the residue was chromatographed on TLC alumina plates by developing with ethyl acetate once or twice. Two fluoresceing strips were viewed under UV light and divided into 2 portions. The bands were scraped off, collected, wetted with methanol into a slurry and extracted with chloroform. The extract was washed in a separating funnel with water (10 mL), the chloroform layer was filtered through anhydrous Na_2SO_4 and evaporated.

The material of higher polarity (lower R_f value) was homogeneous on TLC although NMR data in Table 1 indicates the presence of two geometric isomers of VIII in almost equal amounts. Their total yield was greater than 90%. Mild hydrolysis quantitatively converted VIII to IX. VIII was dissolved in methanol (20 mL) and 2mL of aqueous KOH (1N)were added. The mixture was left at room temp. for 3 h ,extracted with chloroform, the chloroform solution was washed with water and then filtered through anhydrous Na_2SO_4 and evaporated to dryness. Drastic alkaline hydrolysis of either VIII or of IX (refluxing in ethanolic KOH (1N) for 48 h) yielded quinotoxine (X) which was shown to be identical to that reported by Doering et al[4].

The ^{1}H-NMR data for compounds IX and X are given in Table 1.

The less polar compound (with a higher R_f value) was inhomogeneous on TLC. Mild alkaline hydrolysis at room temp. followed by chromatography on TLC alumina plates (developed in ethyl acetate)yielded quinidine (I) and epiquinidine (V) in yields of 5 - 10%. Only ^{1}H-NMR data of epiquinidine (V) are given in Table 1 since other physical data were identical to reported values[8].

(b) Treatment of quinidine (I),hydroquinidine (II) and quinine (III) with aqueous solutions of acetic acid for short periods.

(1) Anaerobic conditions. Quinidine (1.5 g) was dissolved in a mixture of acetic acid (8 mL) and water (4 mL). The solution was heated under N_2 at 70 - 80°C overnight. The solvents were removed under reduced pressure, the residue was extracted with chloroform , washed first with a solution of 1% aqueous Na_2CO_3, then with water, evaporated and the residue purified by TLC (developed with ethyl acetate). The bands having the high R_f values (less polar) yielded the epi derivative (V).The strips with the lower R_f value (more polar) yielded the toxine derivative (X). Hydroquinidine (II) and quinine (III) subjected to the same treatment also yielded toxine and epi derivatives.

The toxine derivatives from I and of III are identical.

The properties of the epimers at C - 9 of all compounds were identical to those described by Uskokovic and coworkers[5,6], and therefore only their ^{1}H-NMR data are summarized in Table 1.

(2) Aerobic conditions (in presence of H_2O_2).

In the presence of H_2O_2 (0.1 mL of 30%) added to the reaction mixtures described above (b 1), the products obtained were the epimers of the corresponding alkaloids (I,II and III) as well as their 9 - keto derivatives. These 9 - keto compounds were identical to the products obtained either by the Oppenaur oxidation[8] or by its benzophenone modification[4,7]. Compounds I or III each yielded the same mixture of 9-keto derivatives.(i.e., XI and XII).

The NMR data of these derivatives are given in Table 1.

The mass spectra of XI and XII were: M^+ m/e 322, m/e 187 (base peak), m/e 136 (base peak).

(3) Aerobic conditions (in presence of O_2).
When heated under atmospheric oxygen, each of the three alkaloids yielded all three products,i.e., epimer, toxine and 9-keto derivatives, in equal amounts.

(c) Treatment of quinidine,hydroquinidine and quinine with Aqueous solutions of Acetic Acid for long periods.

(1) Anaerobic conditions. Heating the alkaloids under conditions described in section (b1) above for periods up to 96 h yielded only toxine derivatives.

(2) Aerobic conditions (in presence of H_2O_2). Under these conditions only 9-keto derivatives were produced almost quantitatively.

(3) Aerobic conditions (in presence of O_2). With atmospheric oxygen both toxine and 9-keto derivatives were formed.

(d) Reactions of quinidine with glacial acetic acid.
Heating quinidine (I) with glacial acetic acid, at 70 - 80°C caused a gradual and slow conversion to epi quinidine (V). After 7 days, based on NMR analysis,a mixture of nearly equal amounts of (I) and (V) was obtained. The mixture was separated by TLC using ethyl acetate as developing solvent. Very small quantities of (X) and 9-keto derivatives were also detected.

(e) Reaction of quinidine with aqueous solutions of propionic acid. Heating quinidine in an aqueous solution of propionic acid under atmospheric conditions at 70 - 80°C for 7 days yielded the same mixture of products which were formed with aqueous acetic acid Addition of H_2O_2 had no effect.

(f) Reaction of quinidine,hydroquinidine and quinine with aqueous solutions of weak,medium and strong acids.
Heating the alkaloids at 70 -80°C in aqueous solutions of $HClO_4$,HCl,H_3PO_4 or HCOOH at different concentrations for periods up to 7 days either in the absence or presence of H_2O_2 did not affect these compounds at all. In all cases only the starting material was isolated unaltered.

(g) Reaction of quinidine with ethyl bromide.
Quinidine (I,3g) was dissolved in ethanol (30 mL), ethyl bromide (3 mL) was added and the mixture heated under reflux for 48 h.
After cooling to room temperature, the crystals were washed twice with ethanol and recrystallized from methanol. The product (IV) had a m.p.= 237°C. Detailed analysis of the compound is given below:
I.R.($CHCl_3$): 3300-3100 cm^{-1} (OH bonded), 1620 (aromatic), 1000 and 920 (vinyl). The N.M.R. data of compound IV are given in Table 1.
U.V. max (in $CHCl_3$): 241nm ($\varepsilon = 2.7 \times 10^4$),280nm ($\varepsilon = 4.4 \times 10^3$),334nm ($\varepsilon = 5.8 \times 10^3$);(in H_2O): 230nm ($\varepsilon = 3.8 \times 10^4$),284nm ($\varepsilon = 1.5 \times 10^3$),332nm ($\varepsilon = 4.6 \times 10^3$).
M.S.: M^+ m/e 353, m/e 324 ,m/e 189 ,m/e 165 .
The molecular structure obtained by X - ray crystal analysis is shown in Figure 1. The pertinent data were measured on a PW 1100/20 Philips four circle computer-controlled diffractometer. MOK_α ($\lambda = 0.71069$ A) radiation with a graphite crystal monochromator in the incident beam was used. The unit cell dimensions were obtained by a least squares fit of 22 centered reflections in the range of $8^\circ < \theta < 11^\circ$.Intensity data were collected using the $\omega - 2\theta$ technique to a maximum 2θ of 45°. The scan width, $\Delta\omega$, for each reflection was 1° with a scan time of 20 seconds. Background measurements were made for other 20 seconds at both limits of each scan. Three standard reflections were monitored every 60 minutes. No systematic variations in intensities were found. Intensities were corrected for Lorenz and polarization effects. All non-hydrogen atoms were found by using the results of a Multan direct method analysis[12]. After several cycles of refinements[13] the positions of the hydrogen atoms were calculated, and added with a constant isotropic temp. factor of 0.5 A^2 to the refinement process. Refinement proceeded to convergence by minimizing the function $\Sigma w (|F_o|-|F_c|)^2$, where the weight, w , are σ_F^{-2}. A final difference fourier synthesis map showed several peaks less than 0.5 e A^{-3} scattered about the unit cell without a significant feature.
Positional parameters and estimated standard deviations for the atoms in IV are given in Tables 2 and 3.

(h) Rearrangement of IV to XII.
The quaternary salt IV (0.5 g) was dissolved in a mixture of acetic acid (8 mL) and water (4 mL). The mixture was heated without or with H_2O_2 at 70 - 80°C and aliquots were removed and analyzed by TLC and NMR daily for 7 days. Only a small fraction of IV was converted to XII even after 7 days. The product was less polar than IV. Purification on TLC yielded compound XII which was identified by NMR as a toxine derivative. Its NMR data are given in Table 1. Unfortunately, this compound could not be induced to crystaleize.
M.S.: M^+; m/e 187, m/e 323, m/e 352.
The different products which are obtained under various conditions are summarized in Table 4.

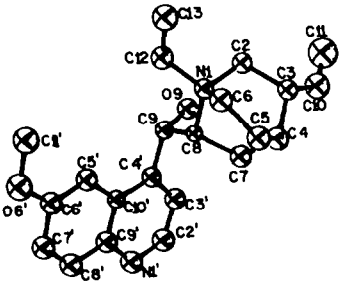


Figure 1. X ray diffraction of the quarternary salt of quinidine (IV)

TABLE 1: CHEMICAL SHIFT VALUES (IN P.P.M.) OF COMPOUNDS DISSOLVED IN $CDCl_3$

PROTON	COMPOUND IV	V	VI	VII	VIIIA+B*	IX	X	XI	XII	XIII	XIV	XV	XVI
H-2'	8.76(d) J=4.4	8.74(d) J=4.5	8.71(d) J=4.4	8.68(d) J=4.4	8.74 (d) J=4.0	8.86(d) J=4.5	8.85(d) J=4.5	8.85(d) J=5.1	8.84(d) J=4.8	8.84(d) J=4.4	8.87(d) J=5.0	8.84(d) J=4.4	8.82(d) J=4.0
H-3'	7.76(d) J=4.4	7.47(d) J=4.5	7.39(d) J=4.4	7.40(d) J=4.9	7.37 (d) J=4.0	7.58(d) J=4.5	7.53(d) J=4.5	7.75(d) J=4.8	7.64(d) J=4.3	7.56(d) J=4.5	7.61(d) J=5.0	7.72(d) J=4.4	7.63(d) J=4.4
H-5'	7.16(d) J=2.2	7.57(d) J=2.7	7.64(d) J=2.6	7.54(d) J=2.7	7.46 (d) J=2.0	7.80(d) J=2.7	7.81(d) J=2.8	7.65(d) J=2.9	7.74(d) J=3.2	7.79(d) J=2.7	7.84(d) J=3.0	7.73(d) J=3.1	7.64(d) J=2.6
H-7'	7.37(q) J_o^m=2.2 J_o^m=9.3	7.36(q) J_o^m=2.7 J_o^m=9.2	7.36(q) J_o^m=2.7 J_o^m=9.2	7.31(q) J_o^m=2.7 J_o^m=9.3	7.38 (q) J_o^m=2.0 J_o^m=9.0	7.40(q) J_o^m=2.7 J_o^m=9.0	7.41(q) J_o^m=2.8 J_o^m=9.2	7.40(q) J_o^m=2.7 J_o^m=9.1	7.40(q) J_o^m=2.7 J_o^m=9.1	8.40(q) J_o^m=2.9 J_o^m=9.3	7.42(q) J_o^m=3.0 J_o^m=9.0	7.38(q) J_o^m=3.1 J_o^m=9.3	7.38(q) J_o^m=3.1 J_o^m=9.3
H-8'	8.04(d) J=9.0	8.02(d) J=9.2	8.02(d) J=9.1	7.96(d) J=9.3	8.02 (d) J=9.0	8.03(d) J=9.0	8.04(d) J=9.2	8.04(d) J=9.2	8.03(d) J=9.3	8.03(d) J=9.2	8.05(d) J=9.0	8.02(d) J=9.8	8.02(d) J=9.8
H-C-9	6.37-bs	5.12(d) J=10.2	5.05(d) J=9.9	5.00(d) J=9.7									
H-8					5.70 (t) J=6.00			4.19(m)	4.19(m)			4.15(m)	4.15(m)
CH=	5.94(m)	5.92(m)	5.63(m)				6.10(m)	6.00(m)	5.64(m)	6.12(m)			
CH_2=	5.28(m)	5.15(m)	5.00(m)				5.10(m)	5.10(m)	5.00(m)	5.11(m)			
CH_3O	3.95(s)	3.94(s)	3.94(s)	3.87(s)	3.93(s)$_A$ 3.93(s)$_B$	3.91(s)	3.94(s)	3.94(s)	3.93(s)	3.93(s)	3.95(s)	3.93(s)	3.92(s)
** XO-C-9					2.19(s)$_A$ 2.18(s)$_B$								
** X-N-1					2.11 (s)	2.06(s)							
CH_2-N^+	4.28(q)												
*** MeC-N^+	1.61(t)												

d : doublet; q : quartet; m : multiplet; s : singlet; bs : broad singlet.
* VIII A+B : denotes two geometric isomers.
** X : $CH_2C{=}O$.
*** Me : CH_3.

TABLE 2. POSITIONAL PARAMETERS AND ESTIMATED STANDARD DEVIATIONS FOR:

ATOM	X	Y	Z	ATOM	X	Y	Z
BR	-.16194(8)	-.15901(7)	-.0705(2)	N(1)'	-.3442(6)	-.4515(4)	-.343(1)
N(1)	.0383(5)	-.3656(4)	-.009(1)	C(2)'	-.2773(7)	-.4533(5)	-.465(2)
C(2)	.1152(6)	-.3684(5)	-.150(2)	C(3)'	-.1970(6)	-.4214(5)	-.436(2)
C(3)	.1445(7)	-.4402(5)	-.172(2)	C(4)'	-.1840(6)	-.3844(5)	-.269(2)
C(4)	.0693(7)	-.4848(5)	-.112(2)	C(5)'	-.2508(7)	-.3420(6)	.031(2)
C(5)	.0617(7)	-.4790(5)	.107(2)	C(6)'	-.3220(7)	-.3385(6)	.151(2)
C(6)	.0631(7)	-.4067(5)	.166(2)	O(6)'	-.3261(5)	-.3028(4)	.317(1)
C(7)	-.0151(6)	-.4604(5)	-.199(2)	C(7)'	-.4004(7)	-.3744(5)	.111(2)
C(8)	-.0436(6)	-.3959(4)	-.101(1)	C(8)'	-.4041(7)	-.4097(5)	-.049(2)
C(9)	-.0961(6)	-.3508(5)	-.233(1)	C(9)'	-.3334(9)	-.4139(5)	-.178(2)
O(9)	-.0493(4)	-.3390(3)	-.408(1)	C(10)'	-.2537(6)	-.3803(5)	-.137(2)
C(10)	.1785(7)	-.4544(6)	-.371(2)	C(11)'	-.2468(7)	-.2681(6)	.370(2)
C(11)	.2251(8)	-.4199(6)	-.472(2)	O14	-.0689(5)	-.2125(4)	-.437(2)
C(12)	.0240(6)	-.2973(5)	.056(2)	C14	-.0483(8)	-.1627(6)	-.564(2)
C(13)	.0919(7)	-.2712(6)	.195(2)				

Estimated standard deviations in the least significant digits are shown in parentheses.

TABLE 3. POSITIONAL PARAMETERS AND ESTIMATED STANDARD DEVIATIONS FOR:

ATOM	X	Y	Z	ATOM	X	Y	Z
H(21)	.1689(6)	-.3395(5)	-.096(2)	H(122)	-.0391(6)	-.2944(5)	.126(2)
H(22)	.0945(6)	-.3496(5)	-.288(2)	H(131)	.0731(7)	-.2207(6)	.199(2)
H(3)	.2005(7)	-.4496(5)	-.081(2)	H(132)	.0887(7)	-.2911(6)	.338(2)
H(4)	.0808(7)	-.5345(5)	-.153(2)	H(133)	.1580(7)	-.2754(6)	.141(2)
H(51)	.1163(7)	-.5039(5)	.173(2)	H(2)'	-.2846(7)	-.4808(5)	-.596(2)
H(52)	.0010(7)	-.5009(5)	.154(2)	H(3)'	-.1460(6)	-.4243(5)	-.063(2)
H(61)	.1281(7)	-.3937(5)	.214(2)	H(5)'	-.1925(7)	-.3143(6)	.063(2)
H(62)	.0167(7)	-.3990(5)	.281(2)	H(7)'	-.4558(7)	-.3719(5)	.208(2)
H(71)	-.0664(6)	-.4961(5)	-.183(2)	H(8)'	-.4639(7)	-.4358(5)	-.080(2)
H(72)	-.0040(6)	-.4514(5)	-.349(2)	H(111)'	-.2660(7)	-.2497(6)	.509(2)
H(8)	-.0901(6)	-.4056(4)	.012(1)	H(112)'	-.1857(7)	-.2942(6)	.383(2)
H(9)	-.1062(6)	-.3032(5)	-.173(1)	H(113)'	-.2385(7)	-.2281(6)	.271(2)
H(10)	.1595(7)	-.5004(6)	-.432(2)	H(141)	-.0893(8)	-.1205(6)	-.545(2)
H(111)	.2198(80	-.4154(6)	-.626(2)	H(142)	.0199(8)	-.1485(6)	-.558(2)
H(112)	.2744(8)	-.3909(6)	-.401(2)	H(143)	-.0620(8)	-.1843(6)	-.702(2)
H(121)	.0239(6)	-.2667(5)	-.070(2)				

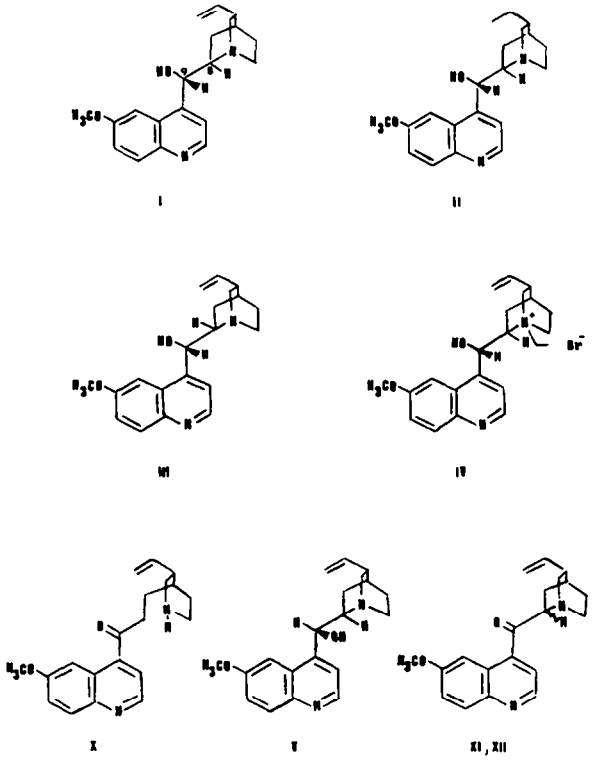
Estimated standard deviations in the least significant digits are shown in parentheses.

RESULTS AND DISCUSSION

1.Conditions for acetic acid catalysis.

The cinchona alkaloids examined in the present study were: quinidine (I), hydroquinidine (II), quinine (III), the 1-N-ethyl quaternary salt (IV), epi quinidine (V), epi quinine (VI) and epi hydroquinidine (VII). The corresponding toxines and the C-9-keto derivatives were also prepared.(cf. Scheme 1).

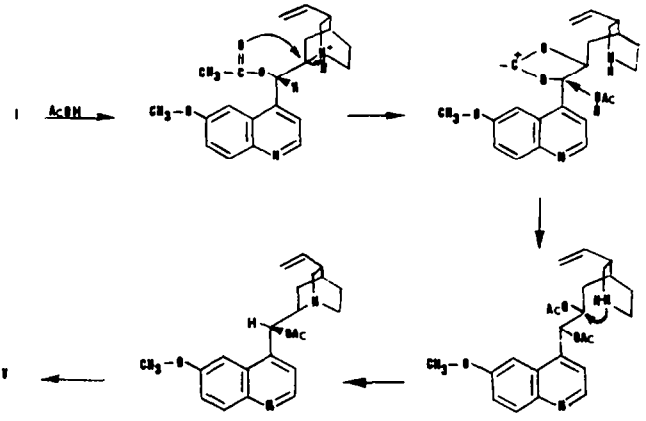
Theoretically, epimerization of quinidine or other chinchona alkaloids required preparation of the corresponding C-9-keto derivatives which seem to be the most adequate starting materials. As a



Scheme 1

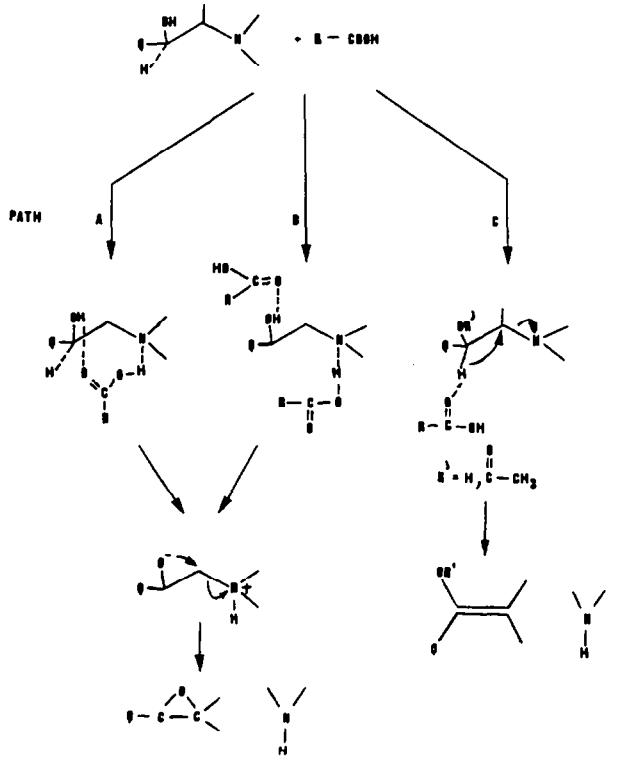
Theoretically, epimerization of quinidine or other chinchona alkaloids required preparation of the corresponding C-9-keto derivatives which seem to be the most adequate starting materials. As a consequence the modified Oppenauer oxidation was considered to be one of the most efficient procedures[5]. However, the conditions employed caused unavoidable epimerization also at C-8. This created serious drawbacks in the subsequent reduction of the 9-keto group.

To avoid such difficulties, alternative routes for epimerization were examined. We expected that 9-acetyl quinidine[14], which is formed by warming quinidine (I) with acetic acid, could serve as a key **precursor** for epimerization Such rearrangements would occur by migration of the acetoxy group to C-8, formerly occupied by the quinuclidine nitrogen, with a concomitant inversion at C-9 (see Scheme 2).



Scheme 2

Acetic acid could also function as an acid-base catalyst causing ring opening and ring closure by one of three possible paths.(Scheme 3).



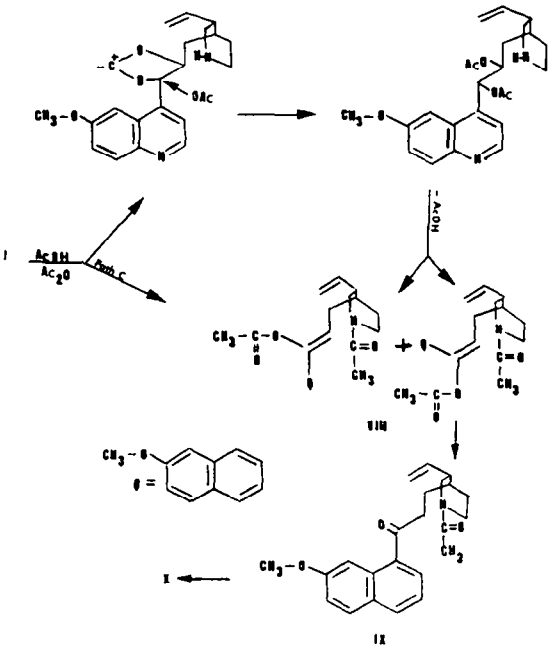
Scheme 3

In paths A and B, epoxide formation can serve as an intermediate for epimerization. In path C, enol (or enol acetate) can be obtained and converted to the corresponding toxine derivatives[15,16].

Evidence given in this study shows that path A is the most rapid among the three paths.

2.Aqueous,anhydrous and glacial acetic acid.

Anhydrous acetic acid (a mixture of glacial acetic acid and acetic anhydride) catalyzed conversion of quinidine (I) primarily to 1-N-acetyl quinotoxine, enol acetate (VIII, Scheme 4).



Scheme 4

Chromatography on Al_2O_3 plates separated compound (VIII) from small quantities of less polar products. ^{1}H-NMR analysis indicated the presence of the two expected geometrical isomers of VIII in equal amounts. Hydrolysis of the less polar compounds followed by NMR analysis showed the sole presence of quinidine and of epiquinidine. Epiquinine was not found.

On the other hand it was expected that aqueous acetic acid would slow or prevent acetylation at the quinuclidine nitrogen, enabling formation of epi quinidine (V) in much higher yields. These predictions were verified .However, additional two compounds, identified as quinotoxine (X) and a 9-keto derivative (XI) were also produced. Their quantity increased with time at the expense of epiquinidine. These results show for the first time that aqueous acetic acid is capable of inducing epimerization and oxidation at C-9, and not only rearrangment into toxine derivatives as the sole reported product[7,8] (see experimental section). Moreover, the relative amounts of each product could be controlled merely by changing the reaction conditions.

Direct conversion of I to XI required an oxidant. Molecular oxygen seemed to be the most reasonable candidate under these condition. Indeed, when the reaction was conducted under nitrogen, only quinotoxine (X) and epi-quinidine (V) were obtained and their separation was easy; compound XI was completely absent. However, with catalytic amounts of H_2O_2, quinidine (I) yielded only oxidation products, i.e., a mixture of quinidinone (XI) and quininone (XII).

Glacial acetic acid (without addition of acetic anhydride) catalysed a slow conversion of cinchona alkaloids to epi-derivatives as major products. After 24 h, only a small quantity of epi-compounds was isolated. After a fortnight almost equal amounts of starting materials and epi-compounds were obtained. In addition, very small quantities of toxines and 9-keto derivatives were also formed.

Other aqueous acids were tested. These included strong aqueous acids, e.g., HCl,$HClO_4$ and relatively weak acids, e.g., H_3PO_4 and HCOOH at different ratios. None of these acids proved to be effective. In all cases the cinchona alkaloids were isolated unchanged. However, propionic acid was found to catalyze conversions of quinidine to (V),(X),(XI) and (XII),similar to acetic acid,in the presence or absence of H_2O_2 but at much slower rates.

3.Unique features of acetic acid - catalyzed reactions.

Evidently, rearrangement of cinchona alkaloids to their corresponding toxine derivatives, catalyzed by acetic acid,is not the only reaction occuring. Two outstanding features characterize the additional reactions:

The first is related to the nature of the products: Partial oxidation of the carbinol at C-9 takes place with atmospheric oxygen **only** when water is present in acetic acid. However, nearly quantitative oxidation occured with H_2O_2. Thus, only aqueous acetic acid can catalyze oxidation of the carbinol to C-9-keto products.

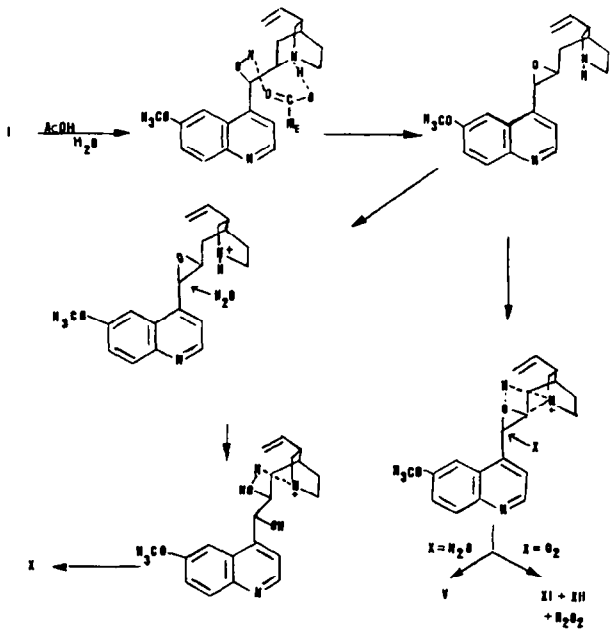
In contrast, oxidation was not observed under N_2 or when acetic anhydride was added to acetic acid. Consequently, when glacial acetic acid yielded small quantities of oxidation products, they must have originated from the possible presence of traces of atmospheric moisture.

The second feature concerns the high degree of stereospecificity observed in the epimerization at C-9. Quinidine (I) and quinine (II) each yielded only the corresponding epimer at C-9, whereas the configuration at C-8 was retained. This property was common to all reactions catalyzed by acetic acid and made the separation of epimers an easy and quantitative process.

4.The catalytic mechanism of acetic acid.

A. Aqueous acetic acid.

Acetic acid functions both as proton donor and proton acceptor. Therefore, it is capable of activating the nucleophilic character of the hydroxyl at C-9 by proton abstraction and at the same time weakens the C - N bond by protonating the quinuclidinic nitrogen (cf. Scheme 5).



Scheme 5

The product is an epoxide which is most probably, formed by an intramolecular process. During the catalyzed epoxide formation, the configuration at C-8 is inverted from 8,R to 8,S by a SN_2 process. The formation of an epoxide intermediate is proposed to be common to all transformations catalyzed by aqueous acetic acid. Atmospheric O_2 or H_2O_2 converted the epoxide to hydroperoxide in already reported pathways[17-19]. This pathway seems to be the faster one. The hydroperoxide is then

internally rearranged to the corresponding C-9 keto derivative with elimination of H_2O_2 . During this process, the configuration at C-8 is re-inverted, resuming its original configuration i.e.,8,R. However, due to keto enol tautomerism, the final products contain both 8,R and 8,S 9-keto derivatives in equal amounts[4-6]. Limiting the amounts of O_2 reduced oxidation, allowing the other two reactions to compete. Under nitrogen, only epimerization at C-9 and toxine formation occurred.(Schemes 3,5).

These reactions occur only if following requirements are met:

(a).The piperidinic nitrogen is completely protonated (Scheme 5).

(b).The protonated nitrogen forms hydrogen bonding with the epoxide,causing a streching of the C-O-C bond.

(c).C - 9 which is part of the epoxide is benzylic, rendering it very reactive to a nucleophilic attack.[20].

Inspite the reactivity of the C-O bond at C-9 neither weak nor strong acids affected direct attack by H_2O or by any other nucleophile present in solution (cf. experimental section). This observation rules out epimerization at C-9 only unless the C-O bond is further activated,e.g., by an epoxide formation in which both inversions at C-8 and C-9 occured simultaneously (cf. Scheme 5). The above crucial requirements provide the basis for the reactions which yielded the above products. O_2 or H_2O_2 reacted only with the reactive benzylic C-9-H to form the hydroperoxide C-O-O-H. Epimerization at C-9 takes place by a simultaneous attack of H_2O at C-9,and of quinuclidine nitrogen at C-8. This unusual combination is made also possible if the above same three requirements are operative, leading to formation of epimers at C-9. If only the piperidinic nitrogen reacts with the epoxide without the participation of H_2O, the starting material ,i.e., quinidine is regenerated. The final product is a result of an inversion of configuration at C-9 and retention of the original configuration at C-8. This explains why quinidine yields only epiquinidine.

Toxine formation involves only an attack by one molecule of H_2O without the requirement of hydrogen bonding with the NH^+,converting the epoxide to C-8, C-9 diol. The diol thus obtained is converted to a toxine derivative by dehydration and tautomerism of the enol to the keto form (cf. Scheme 5).

The exclusive hydrogen bonding between the piperidinic nitrogen and the C-9-OH, explains the formation of the regular toxine in which the carbonyl is at C-9 and not at C-8.

We may conclude that epimerization and oxidation at C-9 take place if the epoxide is activated by the hydrogen bonding and by the presence of the benzylic C-H bond. Toxine formation needs only the presence of benzylic carbon. Therefore, extended anaerobic conditions yielded toxine exclusively in an irreversible reaction. Thus epi compounds are formed preferentially during short periods of reaction. Therefore, optimal quantities of epi products will be obtained only under controlled conditions which include also the time factor. Thus extended reaction periods with acetic acid reduced the amounts of natural and epi-alkaloids, in favor of toxines under anaerobic conditions or to 9-keto compounds upon the addition of H_2O_2.

B.Anhydrous acetic acid.

Under anhydrous conditions the carbinol at C-9 becomes acetylated, preventing an epoxide formation. Nevertheless, epimerization occured and proved to be highly stereospecific, suggesting an alternative mechanism in which the acetyl group participates in an intramolecular rearrangement[21]. The benzylic character of C-9 is reflected by the reactivity of both C-O and C-H bonds. It is unkikely that under anhydrous conditions acetic acid acts as an effective nucleophile. Consequently, epimerization at C-9 is not plausible, otherwise oxidation at C-H would predominate similar to that observed with aqueous acetic acid. The proposed mechanism is shown in Scheme 2. A positive charge on the intermediary five membered ring makes even acetic acid an effective nucleophile driving the competition between inversion at C-9-O and oxidation at C-9-H in favor of inversion.

Considering the proposed mechanism, the following explains why epi formation is incomplete under anhydrous conditions. The starting materials and the epi-compounds are both acetylated at C-9 and undergo similar transformations, i.e., cleavage, closure of ring and then inversion of configuration at C-8.

The individual steps are illustrated for quinidine :

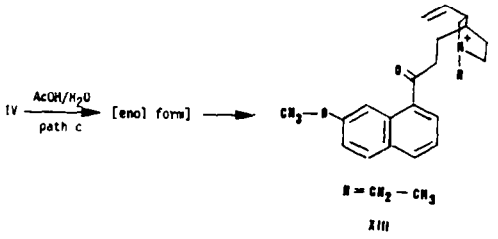
Quinidine ⟶ Acetyl Quinidine ⟷ Acetyl Epiquinidine ⟷ Epiquinidine

When acetic anhydride is present,the quinuclidine nitrogen may also be acetylated preventing ring closure. Conversion of quinidine (I) to toxine enol acetate (Scheme 4, compound VIII, two isomers) may be explained by one of the two possibilities:(A) following path C in Scheme 3, or (B) formation of a five membered ring as shown in Scheme 2. Since under these conditions acylation of the quinuclidine nitrogen is highly favorable the rate of closure to form the original quinuclidine ring is slowed down and elimination of a molecule of acetic acid ,leading to two geometric isomers (VIII A+B),prevailed. This also explains why the yields of epi compounds were much lower than under aqueous conditions.

C. Supporting evidence for mechanisms of catalysis.

1. The first item of supportive evidence for the role of aqueous acetic acid in the catalytic transformations of cinchona alkaloids came from experiments done with other aqueous acids. Strong and medium acids (e.g., $HClO_4$, HCl, H_3PO_4 and HCOOH) failed to catalyze any of the reactions discussed above.

2. Another observation to support the proposed intramolecular mechanism was provided by the quaternary salt of quinidine (IV). Surprisingly, aqueous acetic acid failed to induce oxidation and epimerization under conditions applied for quinidine and other cinchona alkaloids. The only product obtained was the corresponding toxine (XIII) (Scheme 6).



Scheme 6

In contrast to quinidine, its quarternary salt prevented intramolecular catalysis by acetic acid, because the quinuclidine nitrogen was blocked. It may thus be concluded that acetic acid in aqueous solutions serves as an effective catalyst only when it functions as an acid and a base in intramolecular processes. This did not occur with the quaternary salt of quinidine. Consequently, rearrangement of this salt to toxine occurred by a different mechanism which was previously reported. In this process, acetate served only as a base, abstracting a proton from H-C-9 and forming a carbanion which leads to the expected toxine[15]. Evidently these results show that when the reaction cannot follow path A they proceed along path C rather than path B.

3. The intriguing observation provided by the reaction of quinidine (I) with hot propionic acid in presence and absence of H_2O_2 substantiates the proposed mechanism. The reactions were much slower compared to those with hot acetic acid. More surprising was the observation that addition of H_2O_2 to propionic acid did not change the composition of products. All three products,i.e., epi - quinidine (V),quinotoxine (X) and 9-keto derivatives (XI,XII),were formed whether H_2O_2 was present or absent.These reults can be explained as follows: Propionic acid is bulkier compared to acetic acid, and therefore the intramolecular mechanism suggested for acetic acid is not necessarily the only mechanism operating in this case.Rapid formation of oxidation and epimerization products can be rationalized on the basis of intramolecular mechanism.(Scheme 3, Path A). Evidently, these reactions became slower if Path B is operative. Steric strains significantly reduced the probability of the rapid mechanism permitting an alternative one,in which propionic acid acts only as a base with respect to the C-9-H bond (Scheme 3, Path C). This

mechanism is in accord with that forwarded for the reaction of the quaternary salt (IV). Thus,the production of substantial amounts of quinotoxine (X) by the catalysis of aqueous propionic acid containing H_2O_2, supports a simultaneous operation of all the proposed mechanisms (Paths A, B and C).These results also explain: (a) the relative slowness by which aqueous propionic acid catalyzed the rections, and (b) the insensitivity of this reaction to H_2O_2. Namely, the rate of formation of the intermediate epoxide is much slower compared to the decomposition of H_2O_2. This epoxide may be formed by either path A or Path B (Scheme 3).This lag in time permits the escape of some of the generated oxygen.

4. Another mechanism has been proposed for the anaerobic oxidation of quinidine catalyzed by strong bases. This mechanism involves a process of disproportionation and requires that the oxidation product will be accompanied by equal amounts of a reduced product.

During the oxidation of quinidine catalyzed by acetic acid, only XI and XII (the oxidation product) were identified. The absence of any reduced product provided indirect support for the different mechanisms proposed in the present study.

TABLE 4. CONDITIONS FOR CONVERSION OF CHINCHONA ALKALOIDS

	CONDITIONS	TYPE	OF	PRODUCTS
(a)	ACETIC ACID + ACETIC ANHYDRIDE	TOXINE	EPI	-
(b1)	AQUEOUS ACETIC ACID;(N_2); SHORT[1]	TOXINE	EPI	-
(b2)	AQUEOUS ACETIC ACID;(H_2O_2);SHORT	-	EPI	9 - KETO
(b3)	AQUEOUS ACETIC ACID;(O_2); SHORT	TOXINE	EPI	9 - KETO
(c1)	AQUEOUS ACETIC ACID;(N_2); LONG[2]	TOXINE	-	-
(c2)	AQUEOUS ACETIC ACID;(H_2O_2);LONG	-	-	9 - KETO
(c3)	AQUEOUS ACETIC ACID;(O_2); LONG	TOXINE	-	9 - KETO
(d)	AQUEOUS ACETIC ACID; LONG	-	EPI	-

NOTES: 1. SHORT TERM heating at 70 - 80°C for periods up to 48 h.
2. LONG TERM heating for 48 h or longer.

5. Conclusions.

A. The formation of three-membered and five-membered rings, which are proposed as intermediates during the aqueous and glacial acetic acid catalysis, respectively, may account for the stereospecificity of the reactions described in this study.

B. It is the three membered epoxide and hydrogen bonding with the protonated piperidinic nitrogen which allows the activation of the C-H bond at C-9. This activation enables oxidation and epimerization at C-9.

C. The rearrangement and oxidation due to catalysis by acetic acid have proved to be equally applicable to all chinchona alakloids in the present study.

D. Aqueous acetic acid catalyzed anaerobic conversion of chinchona alkaloids to toxines in very high yields.

E. Toxine enol acetate derivatives were major products under totally anhydrous conditions in presence of acetic anhydride. Oxidation products were totally excluded.

F. Propionic acid exhibits only partially the catalytic properties of acetic acid.

G. The intramolecular nature of the catalysis, proposed for acetic acid, may bear close resemblence to reactions catalyzed by enezymes.

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