tionality is ascribable to a hydroxyl group based on the absorption band at 3250 cm^{-1} in the IR spectrum. The ^{13}C-NMR signals at 108.71 (t), 121.73 (d), 148.55 (s), and 150.13 (s) ppm suggest that the molecule has two double bonds and, therefore, it appears to be bicyclic. Furthermore, the ^1H-NMR spectrum shows the presence of a tertiary methyl group at 0.89 (s, 3), a secondary methyl group at 0.89 (d, 3, J = 6 Hz), and isopropenyl groups at 1.71 (m, 3) and 4.68 ppm (m, 2). From the above data the structure of Ia appears to be similar to that of nootkatone (II), which has been isolated from *Chamaecyparis nootkatensis* (Lamb.)sprach and *Citrus paradisi* Macfad. (4–6). The main difference appears to be the presence of a hydroxyl group in the newly isolated material. The proton attached to the hydroxyl-bearing carbon is observed as a multiplet at 4.06 ppm in the ^1H-NMR spectrum, suggesting the hydroxyl group was β-oriented. To verify the proposed structure, II was reduced with $LiAlH_4$ in ether to yield two products (Scheme I). As expected, the minor one was identical with Ia on the basis of its melting point, IR spectrum, and optical rotation. Thus, Ia is characterized as (2*R*,4*R*,5*S*,7*R*)-eremophil-1(10),11-dien-2-ol.

It has been reported that in vascular smooth muscle, contractile response to potassium chloride is caused for the most part by increasing membrane permeability to Ca^{2+}, while the response to norepinephrine is caused mainly by releasing an intracellular pool of Ca^{2+} (7). Calcium antagonists such as verapamil and gallopamil have been shown to selectively inhibit the contractile activity produced by potassium chloride without having any effect on that induced by norepinephrine (8). In the present experiment, purified Ia (3×10^{-5} M) markedly inhibited the potassium chloride (40 mM)-induced contraction of the aorta, but did not have any effect on that produced by norepinephrine (10^{-6} M). The 50% inhibitory doses of Ia and verapamil for the potassium chloride (40 mM)-induced contraction were $\sim 10^{-5}$ and $\sim 10^{-7}$ M, respectively, indicating that the potency of Ia was approximately 100 times less than that of verapamil[5]. Furthermore, Ia (3×10^{-5} M) profoundly inhibited the increase in the $^{45}Ca^{2+}$ uptake of the aorta induced by potassium chloride[5]. These observations suggest that Ia specifically blocks the Ca^{2+}-influx into the muscle cell of the aorta.

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Reverse-Phase Liquid Chromatography and Pharmacokinetic Study of Two Hydroxylated Analogues of Quinidine in Dogs

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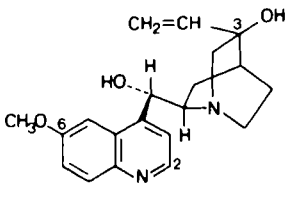
Received August 12, 1982, from the **Department of Analytical Chemistry, Faculty of Pharmaceutical and Biological Sciences, CAEN University, 14032 CAEN Cedex,* ‡*Department of Clinical Biochemistry,* and the §*Department of Pharmacology, Centre Hospitalier, 78000 Versailles, France.* Accepted for publication April 20, 1983.

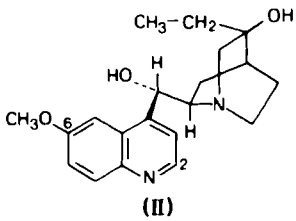
Abstract □ Two hydroxylated analogues of quinidine with antiarrhythmic properties, 3*S*-hydroxyquinidine and 3*R*-hydroxydihydroquinidine were assayed by reverse-phase high-performance liquid chromatography. The analytical technique uses plasma protein precipitation and direct injection on a C_{18} column, with an isocratic mobile phase and spectrofluorometric detection. 3*R*-Hydroxyquinidine is employed as the internal standard. Linearity is verified up to 5 mg/L for the two drugs; concentrations between 0.5 and 2.5 mg/L were measured with a *CV* of 0.5–2.07% for a given day and a sensitivity limit of 50 μg/L. Plasma concentration–time profiles and pharmacokinetic parameters in three dogs are presented after intravenous or oral administration. A significant difference is observed in terminal half-life, terminal rate constant, and total clearance of the two polar analogues of quinidine.

Keyphrases □ Quinidine analogues—hydroxylated, HPLC, pharmacokinetics in the dog □ Pharmacokinetics—hydroxylated analogues of quinidine in the dog, HPLC

Some of the metabolites of quinidine, especially 3*S*-hydroxyquinidine (I), 2′-oxoquinidine, and *O*-demethylquinidine, possess antiarrhythmic activity in mice and rabbits (1) and possibly in humans (2). Other analogues of quinidine, derived from *O*-demethylquinidine (3–6) or 7′-trifluoromethyldihydrocinchonidine (7), have recently been tested. Preliminary studies of I and 3*R*-hydroxydihydroquinidine (II) (8) indicate pharmacological activity of these two compounds when tested against chloroform-induced ventricular fibrillation and aconitine-induced arrhythmia in mice and rats or against coronary ligature in dogs.



(I)



(II)

This report describes a rapid, specific, accurate, and sensitive high-performance liquid chromatographic (HPLC) method for the determination of I and II in plasma. The procedure involves a modification of a recently published reverse-phase HPLC method for quinidine (9) using 3*R*-hydroxyquinidine as internal standard, with an isocratic mobile phase of acetonitrile–acetic acid–water and sensitive spectrofluorometric detection. Preliminary pharmacokinetic parameters of these two hydroxylated analogues of quinidine in the dog are reported.

EXPERIMENTAL

Reagents and Standards—All solvents used were analytical reagent grade[1]: acetonitrile for spectrometry, methanol, and 100% acetic acid. A phosphate buffer (0.05 M, pH 7.4) was prepared in distilled water. Pure standards of

[1] E. Merck, Darmstadt, West Germany.

0022-3549/84/0600-0844$01.00/0

Table I—Variations in Replicate Standards from Spiked Plasma [a]

Compound	Within-Run Concentrations, mg/L				Day-to-Day Concentrations, mg/L			
	0.5	1	2	2.5	0.5	1	2	2.5
I								
SD	0.14	0.12	—	0.12	0.36	0.7	—	2.3
CV (%)	1.5	0.6	—	0.5	4.1	4	—	5.2
II								
SD	0.05	0.09	0.20	0.18	0.07	0.15	0.21	0.34
CV (%)	2.07	1.66	1.89	1.33	2.51	2.71	1.86	2.46

[a] $n = 6$.

Table II—Pharmacokinetic Parameters for I and II after Bolus Injection in Dogs (10 mg/kg iv) [a]

Parameter	I	II	Significance Level
α (h^{-1})	2.25 ± 0.52	2.93 ± 1.27	NS
$T_{1/2\alpha}$ (h)	0.285 ± 0.155	0.27 ± 0.13	NS
β (h^{-1})	0.0875 ± 0.0075	0.1593 ± 0.0096	$p < 0.001$
$t_{1/2\beta}$ (h)	7.95 ± 0.68	4.36 ± 0.27	$p < 0.01$
AUC_∞ (mg/L·h)	55.82 ± 2.36	30.05 ± 5.08	$p < 0.01$
CL_T (L/h·kg)	0.189 ± 0.002	0.325 ± 0.037	$p < 0.01$
V_c (L/kg)	1.04 ± 0.48	0.95 ± 0.18	NS
Vd_β (L/kg)	2.11 ± 0.28	1.96 ± 0.26	NS

[a] Mean ± *SD*; $n = 3$. NS = not significant.

quinidine, dihydroquinidine, I, the internal standard, II, and quinidine *N*-oxide (as bases) were used as supplied[2]. Quinine was used in the sulfate form. Purity of these compounds was verified by HPLC as described here or by TLC on precoated silica gel plates (20 × 20 cm × 0.25 mm)[3], developed in methanol–acetone (4:1) and visualized under UV light (366 nm).

Stock solutions were prepared in methanol at a concentration of 1 g/L (stable for 2 months at 4°C), and working solutions at concentrations of 0.5–5 mg/L were prepared by diluting the stock solutions in acetonitrile–phosphate buffer 0.05 M (1:1). The stock solution of 3*R*-hydroxyquinidine was used to prepare a standard solution at 2.5 mg/L in pure acetonitrile.

Apparatus and Operating Conditions—The analysis was performed using a high-performance liquid chromatograph[4] equipped with an injection system[4] with a 10-μL loop, a stainless steel column[5], 30 cm × 3.9 mm i.d. (particle size 10 μm)[5], a spectrofluorometer[6] (λ_{ex} = 340 nm, λ_{em} = cut-off filter 418 nm) set at 0.5 μAUFS sensitivity with a time constant of 6 s. The mobile phase was a deaerated mixture of acetonitrile–acetic acid–water (8:4:88) with a flow rate of 1.8 mL/min (2200 psi).

Experimental Protocol in Dogs—Fasting mongrel dogs, 10–20 kg, received 10 mg/kg of I or II orally (capsules) or as an intravenous bolus dose (parenteral solutions in acidified saline, pH 4). Sixteen blood samples were collected from the saphenous vein in heparinized tubes and centrifuged at 1200×*g*; the plasma was stored at −18°C until assayed.

After intravenous administration of I or II, plasma concentrations declined biexponentially; pharmacokinetic parameters corresponding to a two-compartment open model were determined with a nonlinear least-squares Graphakin program, according to:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} \quad \text{(Eq. 1)}$$

where C_p is the plasma concentration at time t, A and B are ordinate intercept constants, and α and β are first-order rate constants. For oral administration of II, a one-compartment open model was used with the following:

$$C_p = X(e^{-k_e t} - e^{-k_a t}) \quad \text{(Eq. 2)}$$

where X is a hybrid intercept term, and k_a and k_e are respective apparent first-order absorption and elimination rate constants. Area under the plasma concentration curve from zero to infinity (AUC_∞), total plasma drug clearance (CL_T), central compartment volume (V_c), apparent volume of distribution (Vd) were calculated by the following equations:

$$AUC_\infty = \int_0^{C_{24\,h}} C_p dt + \frac{C_{24\,h}}{\beta} \quad \text{(Eq. 3)}$$

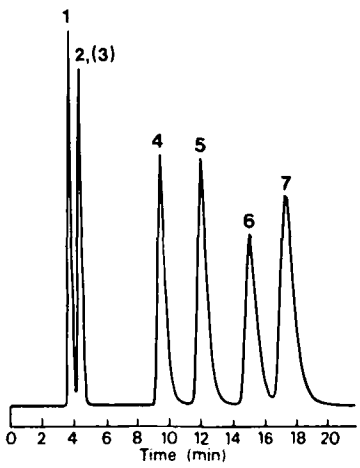


Figure 1—*Chromatogram of pure standards. Key: (1) 3R-hydroxyquinidine (retention time 3.8 min); [2, (3)] I (4.4 min); (4) quinidine N-oxide (9.5 min); (5) quinidine (12.2 min); (6) quinine (15.1 min); (7) dihydroquinidine (17.3 min).*

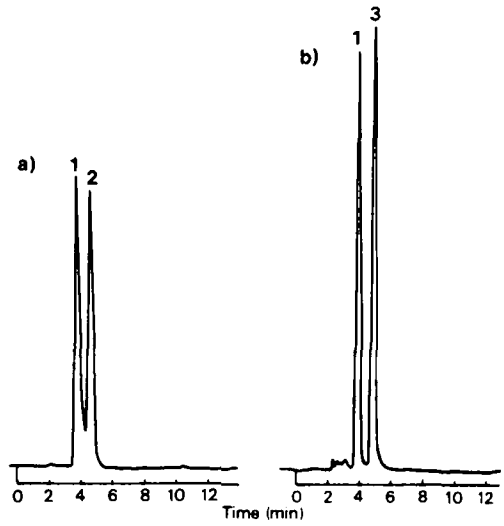


Figure 2—*Chromatogram of a 0.1-mL dog plasma sample after a dose of 10 mg/kg. Key: (1) 3R-hydroxyquinidine, internal standard, 2.5 mg/L; (2) I, (2.78 mg/L, 4.4 min); (3) II (2.95 mg/L, 4.8 min).*

[2] Nativelle Laboratories SA, Paris, France.
[3] Precoated TLC plates ref 5715; E. Merck, Darmstadt, West Germany.
[4] Model 5000 Varian; Varian Instrument, Palo Alto, Calif.
[5] Bondapak C_{18}; Waters Associates, Milford, Mass.
[6] Model FS 970; Schoeffel Instrument Corp., Westwood, N.J.

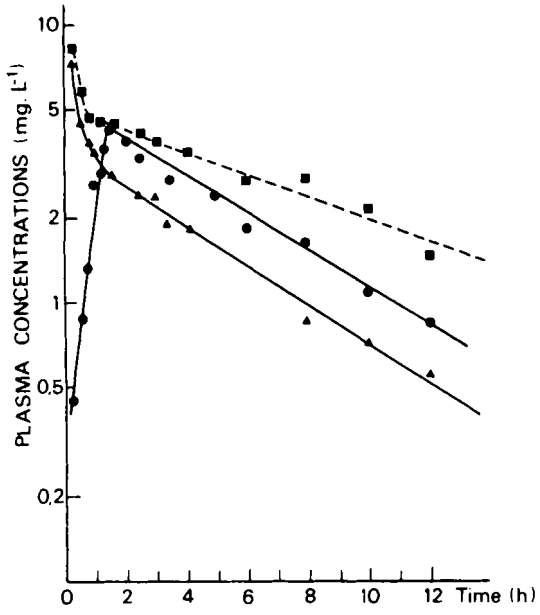


Figure 3—*Plasma concentration in a dog following oral administration of II (●) and intravenous administration of I (■), and II (▲) (10 mg/kg).*

$$CL_T = \frac{\text{Dose}}{AUC_\infty} \quad \text{(Eq. 4)}$$

$$V_c = \frac{\text{Dose}}{A + B} \quad \text{(Eq. 5)}$$

$$Vd_\beta = \frac{CL_T}{\beta} \quad \text{(Eq. 6)}$$

The Student's t test was used to evaluate statistical significance of observed differences between pharmacokinetic parameters of I and II.

Procedure—To precipitate proteins, 100 μL of plasma or diluted plasma was added to 100 μL of a 2.5-mg/L solution of the internal standard in acetonitrile. After closing with laboratory film[7], the mixture was shaken gently on a vortex mixer, centrifuged at 1200×g for 10 min, and 10 μL of the resultant clear supernatant was injected directly into the column. Peak height ratios were measured and reported on standard curves established with loaded plasma sample concentrations.

RESULTS AND DISCUSSION

Figures 1 and 2 show typical chromatograms of pure standards with the retention times observed in these conditions (Fig. 1) and dog plasma samples (Fig. 2). Because of their excessively long retention times, quinidine, quinine, and dihydroquinidine were not used as the internal standard. Quinidine *N*-oxide, a new metabolite described by Guentert *et al.* (10), has the same retention times as an unidentified metabolite in a previous assay for quinidine (9); because of its limited availability, it was not used. 3*R*-Hydroxyquinidine seems to be the most convenient internal standard, because of its same fluorescence characteristics with I and II. As shown in Fig. 2a and b, it is possible to resolve the internal standard from that of I (resolution factor = 1) or II (resolution factor = 1.66); incomplete resolution between I and II (resolution factor = 0.66) is never observed in the determination of plasma levels of either of these two drugs (no simultaneous administration).

The reproducibility assay gave small coefficients of variation (Table I) and recovery was 100 ± 3% when the concentrations of plasma loaded with different compounds were compared with standards solutions in a mixture (1:1) of acetonitrile and 0.05 M phosphate buffer. Linearity of the standard curves was verified by measuring the peak height ratio for concentrations up to 5 mg/L for I and II. An external standardization, by measuring peak height, may be used (no internal standard added to plasma) to detect the possible presence of a peak with retention time identical to that of the internal standard and corresponding to an eventual polar metabolite of I or II. The same results (linearity and plasma levels) are obtained in this procedure. When setting the detector at 0.2 μAUFS, the limit sensitivity, corresponding to a 2:1 signal-noise ratio, is 50 μg/L for the two compounds.

[7] Parafilm; American Com. Company, Dixie Marathon, Greenwich.

Table III—Pharmacokinetic Parameters for II after Oral Administration (10 mg/kg) in Dogs [a]

Parameter	II
k_a (h^{-1})	1.73 ± 1.18
$t_{1/2a}$ (h)	0.56 ± 0.38
k_e (h^{-1})	0.134 ± 0.0073
$t_{1/2e}$ (h)	5.15 ± 0.24
Vd (L/kg)	2.238 ± 0.176
CL_T (L/h·kg)	0.302 ± 0.036
AUC (mg/L·h)	33.42 ± 4.06
C_{max} (mg/L)	4.53 ± 0.34
t_{max} (h)	1.83 ± 0.61

[a] n = 3.

As shown in Fig. 3 for a representative animal, the plasma concentration-times curves for I and II decline biexponentially after intravenous administration (10 mg/kg). Corresponding pharmacokinetic parameters (Table II) indicate a significant difference between the two tested drugs for β, $t_{1/2\,\beta}$, AUC_∞, and CL_T, but not for V_c and Vd_β. The terminal half-life value for I (7.95 ± 0.68 h) is in good agreement with the observed values reported by Jaillon and Kates (11) in dogs (7.27 ± 2.79 h) after oral administration of quinidine, but II is eliminated more rapidly ($t_{1/2\,\beta}$ = 4.36 ± 0.27 h, CL_T = 0.325 ± 0.037 L/h·kg). Similar values for α indicate that I and II are initially distributed in the same order in the body.

For oral administration of II (10 mg/kg), the peak plasma level (4.53 ± 0.34 mg/L) was obtained 1.83 ± 0.61 h after the dose. A higher value for elimination half-life (5.15 ± 0.24 h) was observed than for the intravenous route, but AUC_∞ and CL_T were of the same magnitude, as shown in Table III.

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