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Partial Synthesis of 6′-Hydroxycinchonine and Its Antiarrhythmic Activity in Mice

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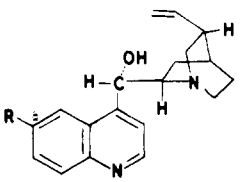
The synthesis of 6′-hydroxycinchonine [(8*R*,9*S*)-cinchonan-6′,9-diol] was achieved by demethylating quinidine with boron tribromide in dichloromethane at −75 °C. The antiarrhythmic activities of 6′-hydroxycinchonine and quinidine were compared following the infusion of aconitine into the tail veins of mice to induce arrhythmias. Comparative ED_{50} and LD_{50} studies for quinidine and 6′-hydroxycinchonine revealed equivalent antiarrhythmic potencies for the two drugs but a smaller acute toxicity for 6′-hydroxycinchonine.

Since its initial use, quinidine has remained one of the



quinidine (6'-methoxycinchonine), R = CH_3O-
cupreidine (6'-hydroxycinchonine), R = HO-

most important and effective drugs for maintaining normal heart rhythm. Patient response to treatment with the drug, however, is often observed to be quite erratic. Side effects frequently require cessation of therapy. Quinidine has a narrow therapeutic range of about 2–5 μg/mL in plasma and exhibits a moderate duration of action, lasting approximately 4–6 h after a single dose.[1] These are undesirable attributes, since the drug is usually administered on a chronic basis for its prophylactic property.

The other major cinchona alkaloids, quinine, cinchonidine and cinchonine, also possess antiarrhythmic activity, although they are somewhat less potent than quinidine. Neither cinchonidine nor cinchonine possesses a 6′-methoxy group. It therefore appears that the 6′-methoxy group of quinidine is not essential for antiarrhythmic activity. Cleavage of the methyl group of the 6′-methoxy grouping of quinidine would form 6′-hydroxycinchonine [(8*R*,9*S*)-cinchonan-6′,9-diol, also called cupreidine].[2] Such cleavage will provide a phenolic group through which a large variety of chemical structures could be attached to yield quinidine analogues having different distribution patterns, tissue affinities, rates of elimination and duration of action. Previous attempts to prepare cupreidine from quinidine failed because the traditional methods for cleaving ethers with hot, concentrated mineral acids produced rearrangements of the quinuclidine and C-3 vinyl groups.[3] 10,11-Dihydroquinidine, however, is stable to hot mineral acids and various ethers of the dihydrophenolic derivative have been synthesized.[2]

Cleavage of the methyl group from the 6′ position of quinidine has been achieved for the first time, by reacting quinidine with boron tribromide in dichloromethane at −75 °C, using a procedure similar to that reported by McOmie, Watts, and West for the demethylation of aryl methyl ethers.[4]

Pharmacological Results and Discussion. The antiarrhythmic activity of 6′-hydroxycinchonine dihydrochloride was compared with that of quinidine sulfate and a control group pretreated with the saline vehicle. Groups of ten mice were pretreated by intravenous injection of either of the test drugs or the saline vehicle. After a period of 3 min, aconitine (5 μg/mL) was infused into the tail vein at a constant rate (0.25 mL/min) to induce arrhythmias, and the electrocardiogram (lead II) pattern was monitored as previously described.[5] The times to onset of aconitine-induced initial arrhythmia and onset of ventricular tachycardia were determined and compared in mice pretreated with quinidine sulfate, 6′-hydroxycinchonine hydrochloride, or the saline vehicle. The time to onset of initial arrhythmia is the time following the initiation of the aconitine infusion to the first discernible sign of persistant (>5 s) deviation from normal sinus rhythm as observed on the ECG recording. This induced

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Table I. Onset Time to Aconitine-Induced Initial Arrhythmia and Ventricular Tachycardia in Mice

end point, onset of	time, s[d] control (physiolog saline)	quinidine, 30 mg/kg	6'-hydroxycinchonine, 30 mg/kg
arrhythmia	153 ± 5	194 ± 11[a]	198 ± 8[a,b]
ventricular tachycardia	194 ± 11	294 ± 44[c]	251 ± 22[a,b]

[a] $p < 0.01$ with respect to saline-pretreatment group. [b] No significant difference between quinidine and 6'-hydroxycinchonine. [c] $p < 0.05$ with respect to saline-pretreatment group. [d] Each value represents the mean ± SE of ten animals.

Table II. ED_{50} and LD_{50} Values for Quinidine and 6'-Hydroxycinchonine in Mice

	quinidine sulfate	6'-hydroxycinchonine dihydrochloride
ED_{50},[a] mg/kg iv	22.4 (19.6-25.8)	23.5 (20.6-26.8)
LD_{50},[a] mg/kg ip	202.0 (178.8-228.3)	304.0 (249.3-370.9)

[a] For both the ED_{50} and LD_{50} determinations, groups of ten animals were given five equally spaced logarithmic doses of either quinidine or 6'-hydroxycinchonine. The time to onset of aconitine-induced arrhythmias were recorded as the end point for each animal for the ED_{50} determinations with the drugs being given intravenously. Death within 24 h constituted a positive response for the LD_{50} determinations, with the drugs being given intraperitoneally. The ED_{50} and LD_{50} values were obtained from the respective probit of response vs. dose plots. The values in parentheses are the 95% confidence intervals, as determined by the method of Litchfield and Wilcoxon.[11]

initial arrhythmia is probably atrial fibrillation.[5] As shown in Table I, no significant differences were observed in the ability of quinidine and 6'-hydroxycinchonine to prolong the onset of the two end points. Table II lists the results of comparative ED_{50} and LD_{50} studies for quinidine sulfate and 6'-hydroxycinchonine dihydrochloride in mice. The data reveal that quinidine and 6'-hydroxycinchonine have equivalent antiarrhythmic potencies, but 6'-hydroxycinchonine has a smaller acute toxicity.

Recently, 6'-hydroxycinchonine, described as *O*-demethylquinidine, was identified as a urinary metabolite in man by Drayer et al.[6] They stated that upon intraperitoneal (ip) injection *O*-demethylquinidine possessed 0.6 the potency of quinidine against chloroform-induced arrhythmia in mice. Aconitine was employed to produce arrhythmia and ventricular tachycardia in our investigations. The induction of arrhythmia by aconitine in a control group is known to occur reliably and predictably,[5,7,8] and it produced arrhythmia and ventricular tachycardia in 100% of the mice in our studies. The specificity of this screening technique for other antiarrhythmic agents has been demonstrated.[5] The ip LD_{50} value for quinidine sulfate in mice of 202 (179–228) mg/kg, as reported in this study, compares very favorably with the previously reported values of 190 mg/kg[9] and 245 ± 21 mg/kg,[10] determined under similar conditions.

The side effects of quinidine may severely limit its use. Thus, the lower acute toxicity and the retention of significant antiarrhythmic activity of 6'-hydroxycinchonine warrants continued investigation. The lessened toxicity of 6'-hydroxycinchonine may be due to more rapid elimination than quinidine. The preparation and screening of various derivatives of this compound to maximize these desirable therapeutic advantages are indicated.

Experimental Section

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. The reaction was monitored routinely on TLC with E. Merck silica gel F-254 coated plastic sheets, which were developed with C_6H_6–CH_3OH–NH_4OH (40:28:1). The compounds were visualized in a "Chromato-Vue" cabinet with both long- and short-wavelength UV (R_f for quinidine 0.61; for cupreidine 0.47). The elemental analysis was performed by Galbraith Laboratories Inc., Knoxville, Tenn. The high-resolution mass spectra were determined by means of a AEI MS-902 mass spectrograph operated at a resolving power of 10000 using a scan speed of 8 s/decade with electron-impact ionization. The mass spectrograph output was processed by a AEI DS-30 data system modified to specifications of VG data systems 2000. The samples were introduced into the mass spectrograph by a direct-probe system. NMR spectra were determined in a Varian EM-360 spectrometer. Quinidine was obtained as the sulfate salt from Sigma Chemical Co., and boron tribromide was obtained from Alfa Division of Ventron Corp.

6'-Hydroxycinchonine. A solution of 19.6 g (60 mmol) anhydrous quinidine in 2 L of dry CH_2Cl_2 contained in a 3-L Morton-type flask was placed in an insulated dry ice–ethanol bath and cooled to −75 °C. The contents of the flask were stirred and continuously flushed with high-purity nitrogen. A solution of BBr_3 (23.0 mL, 240 mmol) in 50 mL of dry CH_2Cl_2 was slowly added to the cooled quinidine solution. The reaction mixture was then allowed to slowly return to room temperature and subsequently refluxed at 40 °C for 1 h and then cooled to 5 °C. While stirring and maintaining the temperature of the mixture at or below 5°C, a solution of 10% NaOH (450 mL) was added. The aqueous, alkaline solution was separated from the organic phase, washed with an equal volume of fresh CH_2Cl_2, and acidified with 50 mL of 37% HCl. The acidic solution was, in turn, basified to pH 9.5 with 58% NH_4OH and extracted with 1-butanol or a large volume of $CHCl_3$. The organic extract was dried over anhydrous Na_2SO_4 and evaporated to dryness to yield 6'-hydroxycinchonine (17.5 g, 95%), mp 167–171 °C dec. A sample of 6'-hydroxycinchonine was dried for 24 h at 100 °C and 0.1 mmHg pressure in an Abderhalden drying apparatus containing P_2O_5 desiccant. Elemental analysis following an additional 2-h drying period indicated the following: Anal. ($C_{19}H_{22}N_2O_2 \cdot 1.25\ H_2O$) C, H, N. Drying at a higher temperature resulted in darkening of the compound. TLC as described above showed only a single spot indicating chromatographic purity of the sample. The NMR spectra of quinidine and 6'-hydroxycinchonine are similar in all respects with the exception that the three protons at δ 4.07 in the quinidine spectrum are totally absent in the spectrum of 6'-hydroxycinchonine. High-resolution mass spectrometry showed a molecular-ion peak at m/e 310.1684 (calcd 310.1681) and confirmed the formula $C_{19}H_{22}N_2O_2$. A strong peak at m/e 136.1122 (calcd 136.1126), C_9H_4N, corresponded to the quinuclidine ion with the C-3 vinyl group.

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Hexofuranosyladenine Nucleosides as Substrates and Inhibitors of Calf Intestinal Adenosine Deaminase

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A series of hexofuranosyladenine nucleosides have been tested as substrates and inhibitors of adenosine deaminase from calf intestinal mucosa. The nucleosides differed from each other in configuration at the various carbon atoms of the hexose and had either a methyl group or hydroxymethyl group at the terminal position. It has been confirmed that the best substrates have the β-D or α-L configuration at the anomeric position and an hydroxyl group on the same side of the furanose ring as adenine. However, these properties are not minimal and other nucleosides will act as substrates even if they do not have the preferred configurations or groups available. The effect of having two hydroxyl groups in the same region of the molecule and in the preferred configurations was to greatly reduce V_{max}. Most structural changes resulted in changes in V_{max}, whereas K_M values remained fairly close. Only a change in configuration of the hydroxyl group at C-5′ caused a dramatic change in affinity, as reflected in the K_M. All nucleosides exhibited competitive inhibitory kinetics. In the latter studies also, a change of configuration at C-5′ greatly affected binding.

Adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) is a ubiquitous enzyme in mammalian tissues that catalyzes the deamination of adenosine to form inosine and ammonia. A large number of structural analogues of adenosine have been studied in order to ascertain the requirements for substrate activity or inhibitory activity. It has been a rather difficult task to clarify the main structural features that will make a substrate, particularly when the sugar moiety is considered. The minimal structural requirements for adenosine deaminase activity have been summarized by several investigators.[1-5] The preferred anomeric configuration was β-D or α-L (*R* configuration for C–N bond) and there had to be an hydroxyl group either at C-5′ or C-3′ in the "up" configuration, as shown in Chart I. However, a nucleoside lacking a 5′-OH group, 2,5′-anhydroformycin, was a substrate,[6,7] and in a recent paper we reported that a series of unsaturated adenine nucleosides were weak substrates for adenosine deaminase even though most of them lacked at least one

Chart I

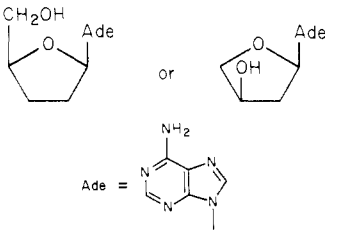

Table I. Activity of Hexofuranosyladenine Nucleosides with Adenosine Deaminase

no.	nucleoside	ref	K_M, μM	V_{max}, μmol min^{-1} mg^{-1}	K_i, μM
1	9-(6-deoxy-α-L-idofuranosyl)adenine	12	320	25	240
2	9-(α-L-idofuranosyl)adenine	13	260	0.178	180
3	9-(6-deoxy-α-L-talofuranosyl)adenine	14, 15, 16	21[a]	61[a]	32
4	9-(6-deoxy-β-D-allofuranosyl)adenine	16, 17	78[a]	0.792[a]	
5	9-(6-deoxy-β-D-galactofuranosyl)adenine	18	230	0.105	230
6	9-(6-deoxy-α-L-mannofuranosyl)adenine	19	670	0.326	260
7	9-(α-L-mannofuranosyl)adenine	20	280	0.092	120
8	9-(β-D-mannofuranosyl)adenine	21	*b*	*b*	670
9	9-(α-D-mannofuranosyl)adenine	22	*b*	*b*	240
10	9-(6-deoxy-α-D-mannofuranosyl)adenine	23	*b*	*b*	240
11	9-(β-L-gulofuranosyl)adenine	24	*b*	*b*	120
12	9-(6-deoxy-β-L-gulofuranosyl)adenine	12	*b*	*b*	200
13	9-(6-deoxy-β-L-galactofuranosyl)adenine	14	*c*	*c*	670
14	9-(6-deoxy-α-D-altrofuranosyl)adenine	14	*c*	*c*	350
15	9-(6-deoxy-β-L-glucofuranosyl)adenine	25	*c*	*c*	95
16	9-(6-deoxy-α-D-idofuranosyl)adenine	12	*c*	*c*	*d*
17	9-(α-D-idofuranosyl)adenine	26	*c*	*c*	*d*
18	9-(6-deoxy-β-D-gulofuranosyl)adenine	12	*c*	*c*	240

[a] Values obtained from ref 2. The values of V_{max} for 3 and 4 were calculated from information supplied in ref 2 using our value of V_{max} for adenosine (220 μmol min^{-1} mg^{-1}). This value is almost identical (217 μmol min^{-1} mg^{-1}) with that reported by Evans and Wolfenden.[27] [b] Very weak substrate. K_M and V_{max} could not be determined. [c] Not a substrate. [d] Not an inhibitor.

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