- (2) J. Glowinski, I. M. Kopin, and J. Axelrod, J. Neurochem., 12, 25 (1965).
- (3) J. Axelrod, Recent Prog. Horm. Res., 21, 597 (1965).
- (4) A. W. Tank, H. Weiner, and J. A. Thurman, Ann. N.Y. Acad. Sci., 273, 219 (1976).
- (5) V. E. Davis, H. Brown, J. A. Huff, and J. L. Cashaw, J. Lab. Clin. Med., 69, 787 (1967).
- (6) A. Feldstein, H. Hoagland, H. Freeman, and O. Williamson, Life Sci., 6, 53 (1967).
- (7) A. Feldstein, H. Hoagland, K. Wong, and H. Freeman, Q. J. Stud. Alcohol, 25, 218 (1964).
- (8) A. A. Smith and S. Gitlow in "Biochemical Factors in Alcoholism", R. P. Maickel, Ed., Pergamon Press, New York, 1967, p 53.
- (9) R. A. Deitrich and V. G. Erwin, Fed. Proc., Fed. Am. Soc. Exp. Biol., 34, 1962 (1975).
- (10) J. Kim and V. G. Erwin, Biochem. Pharmacol., 24, 2089 (1975).
- J. Kim and V. G. Erwin, Fed. Proc., Fed. Am. Soc. Exp. Biol., 34, Abstract 416 (1975).
- (12) V. E. Davis and M. J. Walsh, Science, 167, 100 (1970).
- (13) V. E. Davis, J. L. Cashaw, and K. D. McMurtrey in "Alcohol

Intoxication and Withdrawal", M. G. Gross, Ed., Plenum Press, New York, 1975, p 65.

- (14) J. Cohen in "Frontiers in Catecholamine Research", E. Usdin and S. H. Snyder, Eds., Pergamon Press, London, 1973, p 1021.
- (15) R. D. Myers and C. L. Melchior, Science, 196, 554 (1977).
- (16) C. L. Melchior and R. D. Myers in "Alcohol and Aldehyde Metabolizing Systems", Vol. 3, R. G. Thurman, Ed., Academic Press, New York, 1977, p 545.
- (17) J. Everse, E. C. Zoll, L. Kahan, and N. O. Kaplan, *Bioorg. Chem.*, 1, 207 (1971).
- (18) M. I. Dolin and K. B. Jacobson, J. Biol. Chem., 239, 3007 (1964).
- (19) B. Tabakoff and V. G. Erwin, J. Biol. Chem., 245, 3263 (1970).
- (20) V. G. Erwin and R. A. Deitrich, Biochem. Pharmacol., 22, 2615 (1974).
- (21) M. M. Ris, R. A. Deitrich, and J.-P. von Wartburg, *Biochem. Pharmacol.*, 24, 1865 (1975).
- (22) M. Javors and V. G. Erwin, Pharmacologist, 20, 184 (1978).
- (23) O. Lindberg and L. Ernster, Methods Biochem. Anal., 3, 1-22 (1956).

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

LaVerne D. Small,\* Harry Rosenberg, Peter U. Nwangwu, Terry L. Holcslaw, and Sidney J. Stohs

College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska 68105. Received March 15, 1979

The synthesis of 6'-hydroxycinchonine [(8R,9S)-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<sub>50</sub> and LD<sub>50</sub> 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  $2-5 \ \mu g/mL$  in plasma and exhibits a moderate duration of action, lasting approximately 4–6 h after a single dose.<sup>1</sup> 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 [(8R,9S)-cinchonan-6',9-diol, also called cupreidine].<sup>2</sup> 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.<sup>3</sup> 10,11-Dihydroquinidine, however, is stable to hot mineral acids and various ethers of the dihydrophenolic derivative have been synthesized.<sup>2</sup>

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.<sup>4</sup>

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  $(5 \,\mu g/mL)$  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.<sup>5</sup> 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

 Table I.
 Onset Time to Aconitine-Induced Initial

 Arrhythmia and Ventricular Tachycardia in Mice

| end point,<br>onset of     | time, s <sup>d</sup>             |                        |  |  |
|----------------------------|----------------------------------|------------------------|--|--|
|                            | control<br>(physiolog<br>saline) | quinidine,<br>30 mg/kg | 6'-hydroxy-<br>cinchonine,<br>30 mg/kg |  |
| arrhythmia                 | $153 \pm 5$                      | $194 \pm 11^{a}$       | $198 \pm 8^{a,b}$                      |  |
| ventricular<br>tachycardia | 194 ± 11                         | $294 \pm 44^{c}$       | $251 \pm 22^{a,b}$                     |  |

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

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

|   | quinidine sulfate   | 6'-hydroxycinchonine<br>dihydrochloride |
|---|---------------------|---|
| $ED_{so}^{a}, mg/kg iy$                     | 22.4 (19.6-25.8)    | 23.5 (20.6-26.8)                        |
| LD <sub>50</sub> , <sup>a</sup><br>mg/kg ip | 202.0 (178.8-228.3) | 304.0 (249.3-370.9)                     |

<sup>a</sup> For both the  $ED_{so}$  and  $LD_{so}$  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  $ED_{so}$  determinations with the drugs being given intravenously. Death within 24 h constituted a positive response for the  $LD_{so}$  determinations, with the drugs being given intraperitoneally. The  $ED_{so}$  and  $LD_{so}$  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.<sup>11</sup>

initial arrhythmia is probably atrial fibrillation.<sup>5</sup> 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.<sup>6</sup> 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,57,8 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.<sup>5</sup> 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<sup>9</sup> and 245  $\pm$  21 mg/kg,<sup>10</sup> 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 highresolution 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<sub>2</sub>Cl<sub>2</sub> 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<sub>3</sub> (23.0 mL, 240 mmol) in 50 mL of dry CH<sub>2</sub>Cl<sub>2</sub> 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% NH4OH and extracted with 1-butanol or a large volume of CHCl<sub>3</sub>. The organic extract was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> 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 P2O5 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  $\delta$  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.

Acknowledgment. This research was supported in part by grants from the University of Nebraska Medical Center and the Nebraska Heart Association.

## **References and Notes**

- G. K. Moe and J. A. Abildskov in "The Pharmacological Basis of Therapeutics", 5th ed, L. S. Goodman and A. Gilman, Eds., Macmillan, New York, 1975, pp 686-694.
- (2) T. A. Henry, "The Plant Alkaloids", 4th ed, Blakiston Co., New York, 1949, pp 430-436.
- (3) W. Solomon in "Chemistry of the Alkaloids", S. W. Pelletier, Ed., Van Nostrand Reinhold, New York, 1970, pp 321-325.
- (4) J. F. W. McOmie, M. L. Watts, and D. E. West, Tetrahedron, 24, 2289 (1968).
- (5) P. U. Nwangwu, T. L., Holcslaw, and S. J. Stohs, Arch Int. Pharmacodyn. Ther., 229, 219 (1977).
- (6) D. E. Drayer, D. T. Lowenthal, K. Restivo, A. Schwartz, C. E. Cook, and M. M. Reidenberg, *Clin. Pharmacol. Ther.*, 24, 31 (1978).
- (7) N. K. Dadkar and B. K. Bhattacharya, Arch. Int. Pharmacodyn. Ther., 212, 297 (1974).
- (8) L. Szekeres and J. Papp, "Experimental Cardiac Ar-

rhythmias and Antiarrhythmic Drugs", Akademiai Kiado, Budapest, 1971.

- (9) B. Calesnick, N. H. Smith, and R. Beutner, J. Pharmacol. Exp. Ther., 102, 138 (1951).
- (10) C. M. Mokler and C. G. Van Arman, J. Pharmacol. Exp. Ther., 136, 114 (1962).
- (11) J. T. Litchfield and F. Wilcoxon, J. Pharmacol Exp. Ther., 96, 99 (1949).

## Hexofuranosyladenine Nucleosides as Substrates and Inhibitors of Calf Intestinal Adenosine Deaminase

Alan J. Grant and Leon M. Lerner\*

Department of Biochemistry, State University of New York, Downstate Medical Center, Brooklyn, New York 11203. Received March 9, 1979

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  $\beta$ -D or  $\alpha$ -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  $V_{\text{max}}$ . Most structural changes resulted in changes in  $V_{\text{max}}$ , whereas  $K_{\text{M}}$  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  $K_{\text{M}}$ . 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.<sup>1-5</sup> The preferred anomeric configuration was  $\beta$ -D or  $\alpha$ -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

Chart I



group, 2,5'-anhydroformycin, was a substrate,<sup>6,7</sup> 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

Table I. Activity of Hexofuranosyladenine Nucleosides with Adenosine Deaminase

|     |   |            | $V_{\rm max}, \mu { m mol}$    |                              |                |
|-----|---|------------|--------------------------------|------------------------------|----------------|
| no. | nucleoside                                    | ref        | $K_{\mathbf{M}},\mu\mathbf{M}$ | $\min^{-1}$ mg <sup>-1</sup> | $K_{i}, \mu M$ |
| 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- $\alpha$ -L-talofuranosyl)adenine | 14, 15, 16 | $21^{a}$                       | $61^a$                       | 32             |
| 4   | 9-(6-deoxy-3-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-a-L-mannofuranosyl)adenine         | 19         | 670                            | 0.326                        | 260            |
| 7   | 9-(α-L-mannofuranosyl)adenine                 | 20         | 280                            | 0.092                        | 120            |
| 8   | 9-( $\beta$ -D-mannofuranosyl)adenine         | 21         | ь                              | ь                            | 670            |
| 9   | 9-(α-D-mannofuranosyl)adenine                 | 22         | Ь                              | ь                            | 240            |
| 10  | 9-(6-deoxy-α-D-mannofuranosyl)adenine         | 23         | Ь                              | ь                            | 240            |
| 11  | 9-(β-L-gulofuranosyl)adenine                  | 24         | ь                              | Ь                            | 120            |
| 12  | 9-(6-deoxy-β-L-gulofuranosyl)adenine          | 12         | Ь                              | Ь                            | 200            |
| 13  | 9-(6-deoxy-β-L-galactofuranosyl)adenine       | 14         | с                              | С                            | 670            |
| 14  | 9-(6-deoxy-α-D-altrofuranosyl)adenine         | 14         | с                              | с                            | 350            |
| 15  | 9-(6-deoxy-β-L-glucofuranosyl)adenine         | 25         | с                              | с                            | 95             |
| 16  | 9-(6-deoxy-α-D-idofuranosyl)adenine           | 12         | с                              | с                            | d              |
| 17  | 9-(a-D-idofuranosyl)adenine                   | 26         | с                              | С                            | d              |
| 18  | 9-(6-deoxy-β-D-gulofuranosyl)adenine          | 12         | с                              | с                            | 240            |

<sup>a</sup> 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  $\mu$ mol min<sup>-1</sup> mg<sup>-1</sup>). This value is almost identical (217  $\mu$ mol min<sup>-1</sup> mg<sup>-1</sup>) with that reported by Evans and Wolfenden.<sup>27</sup> <sup>b</sup> Very weak substrate.  $K_{\rm M}$  and  $V_{\max}$  could not be determined. <sup>c</sup> Not a substrate. <sup>d</sup> Not an inhibitor.