

## Anodic Voltammetry of Model Compounds for Anti Cancer Drugs

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**Summary:** The number of electrons involved in the electrooxidation of vindoline, catharanthine and some other model compounds has been determined by large amplitude pulse voltammetry, LAPV, at carbon paste in wet acetonitrile, and in marginally acidic aqueous phosphate buffer.

### Introduction

Vindoline is an *Aspidosperma* alkaloid as well as an aromatic amine. It is frequently used as a nucleophile of choice for attack on an appropriately activated catharanthine molecule [1] to obtain precursors to, and oncolytic agents vinblastine [2,3] and vincristine. One way of activating catharanthine, in principle at least, can be visualised as through electron abstraction from the indole-nitrogen [4-10] of catharanthine, i.e. its electrolytic oxidation [11-13]. It was considered worthwhile to investigate the potential ranges characteristic of electron-removal from the monomeric indole alkaloids vindoline and catharanthine which are building blocks for vinblastine. Specifically, an attempt was made to find out (a) whether the potentials for the oxidations of these alkaloids overlap, and (b) how many electrons are involved in the electrolytic oxidation of these compounds under different solution conditions?

In an earlier paper, large amplitude pulse voltammetry, LAPV, was used to determine the apparent number of electrons transferred during electrooxidation of a few selected anilines [4]. This investigation reports the apparent number of electrons involved in the electrochemical oxidation of vindoline, catharanthine and some selected tetrahydrocarbazoles at carbon electrodes in aqueous buffers, and in neutral wet acetonitrile, as determined by LAPV.

In the technique of LAPV [15], a stationary solid electrode is held at a resting potential,  $E_r$ , at which negligible electrolysis takes place. After a fixed waiting period, usually a few seconds, the potential is changed abruptly to a value  $E$  for a period about 50 ms in duration. This potential pulse

is ended by a return to  $E_r$ . The current is sampled at a time near the end of the pulse. The whole cycle is repeated, except that the potential step is made a few millivolts more extreme with each additional cycle. The output is a plot of sampled current versus potential step  $E$ , and it takes the form of a sigmoid-shaped curve usually with current plateau corresponding to limiting value of current.

Cyclic voltammetric behaviour of these molecules was also examined briefly.

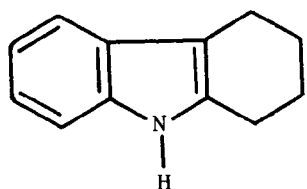
### Experimental

Various substituted tetrahydrocarbazoles, catharanthine, HCl, vindoline, and N,N-dimethyl-m-anisidine were gifts from Professor James M. Bobbitt, University of Connecticut, USA. 1,2,3,4-Tetrahydrocarbazole, obtained from Aldrich Chemical Co. and vinblastine sulfate obtained from Sigma Chemical Co. were used as received. Acetonitrile was ACS reagent grade or spectrograde. All other chemicals were ACS reagent grade. Carbon paste was prepared according to the method described by Adams [16]. Princeton Applied Research Corporation's Model 174 A Polarographic Analyzer and Model 170 Electrochemistry System were used for voltammetry, employing procedures detailed elsewhere [14].

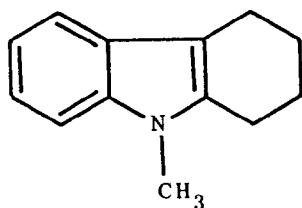
### Results and Discussion

Voltammetric characteristics relating to the electrooxidation of various tetrahydrocarbazoles (Fig. 1), vindoline, N,N-dimethyl-m-anisidine (DMMA) (Fig. 2), and catharanthine (Fig. 3) are

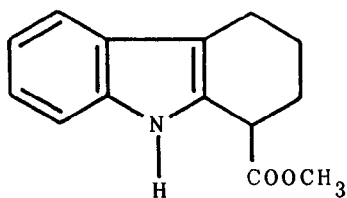
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1,2,3,4-tetrahydrocarbazole

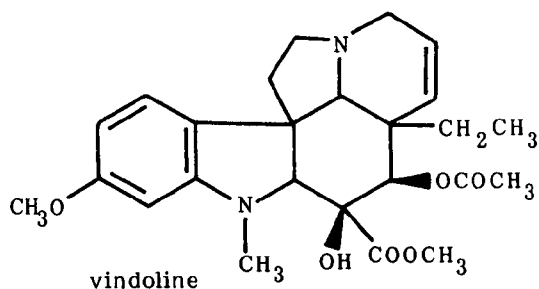


N-methyl-1,2,3,4-tetrahydrocarbazole

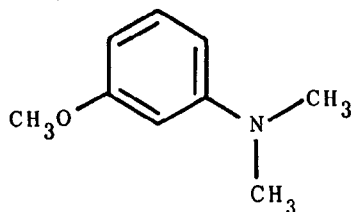


1-Carbomethoxy-1,2,3,4-tetrahydrocarbazole

Fig. 1:



vindoline



N,N-dimethyl-m-anisidine

Fig. 2:

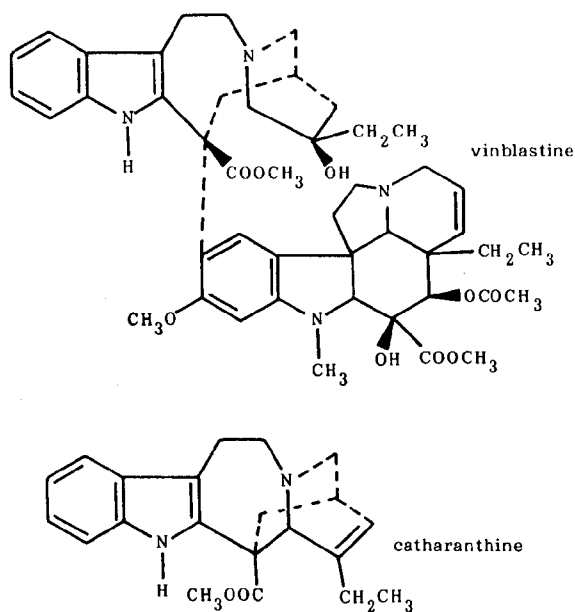


Fig. 3:

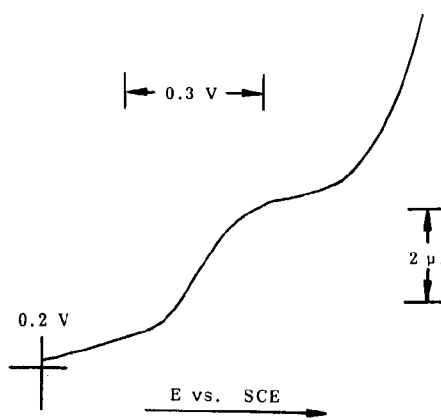


Fig. 4: Large amplitude pulse voltammogram of 0.089 mM catharanthine at carbon paste. Scan rate  $2 \text{ mVs}^{-1}$ . 0.1 M acetate buffer pH 5.6 in 1:1 ethanol-water.

summarized in Tables 1-3. In LAPV, the limiting current,  $i_l$ , and thus,  $i_l C^{-1}$ , is proportional to the apparent number of electrons transferred,  $n(\text{app.})$ , regardless of the rate of electron transfer [18]. To obtain  $n(\text{app.})$ ,  $i_l C^{-1}$  values for the first oxidation waves of these compounds were compared to  $i_l C^{-1}$  values for compounds of known  $n$ , i.e. 1,2,3,4-tetrahydrocarbazole [8,11a,17] in neutral wet acetonitrile and in ethanolic acetate buffer, and *p*-chloroaniline [14] in aqueous phosphate buffer.

**Table 1: Large Amplitude Pulse Voltammetry: Carbazoles, Indole Alkaloids and Related Compounds in CH<sub>3</sub>CN/H<sub>2</sub>O, 9:1.**

Compound	Acidity <sup>a</sup>	Large Amplitude Pulse Voltammetry <sup>b</sup>		
		E <sub>1/2</sub>	E <sub>1/2</sub>	n <sup>c</sup>
tetrahydrocarbazole	A	-	-	-
	N	0.54	0.69	1
	B	0.51	0.68	1
N-methyltetrahydrocarbazole	A	-	-	-
	N	0.50	0.67	1
	B	-	-	-
1-carbomethoxytetrahydrocarbazole	A	-	-	-
	N	0.68	-	2
	B	0.57	-	1
catharanthine	A	0.80	-	2
	N	0.81	-	2
	B	0.34	-	1
vindoline	A	0.66	-	2
	N	0.50	0.66	1
	B	0.55	-	2
N,N-dimethyl-m-anisidine	A	0.55	-	2
	N	0.46	0.58	1
	B	0.46	-	2

<sup>a</sup>All data were obtained using a carbon paste electrode in 0.2 M LiClO<sub>4</sub>. A = 0.04 M HClO<sub>4</sub>, B = 0.002 M tetrabutylammonium hydroxide, N = no acid or base added.

<sup>b</sup>Potentials in volts measured against Ag/Ag<sup>+</sup> (0.01 M). Concentrations of all of the electroactive species were 1.0 mM.

<sup>c</sup>Estimated by comparison of the limiting currents in large amplitude pulse voltammetry with that of tetrahydrocarbazole.

Vindoline was oxidized in a one-electron process in neutral acetonitrile, but gave two-electron oxidations in the presence of dilute acid or base (Table 1). In aqueous pH 6.34 buffer, vindoline again exhibited an n(app.) close to two, but n(app.) was significantly smaller in the ethanolic acetate buffer (Table 3). N,N-dimethyl-m-anisidine was investigated because of its structural similarity to vindoline (Fig. 2). In oxidation by LAPV, the anisidine behaved similarly to vindoline (Table 1), giving a two-electron wave in acidic and alkaline solutions, and an n(app.) close to one in neutral acetonitrile as evaluated from the height of the first

**Table 2: Summary of Cyclic Voltammetric Results for Carbazoles, Indole Alkaloids and Related Compounds in CH<sub>3</sub>CN/H<sub>2</sub>O, 9:1**

Compound	Acidity <sup>a</sup>	Cyclic Voltammetry <sup>b</sup>			
		Anodic		Cathodic	
		E <sub>p1</sub>	E <sub>p2</sub>	E <sub>p3</sub>	E <sub>p4</sub>
tetrahydrocarbazole	A	0.57	0.71	-	-
	N	0.58	0.69	-	-
	B	-	-	-	-
N-methyltetrahydrocarbazole	A	-	-	-	-
	N	0.54	0.70	-	-
	B	-	-	-	-
1-carbomethoxytetrahydrocarbazole	A	0.72	0.84	-	-
	N	0.74	0.86	-	-
	B	0.67	-	-	-
catharanthine	A	0.83	-	-	-
	N	0.83	-	-	-
	B	0.41	-	-	-
vindoline	A	0.64	c	-	-
	N	0.53	0.70	0.39	0.59
	B	0.55	0.85	-	-
N,N-dimethyl-m-anisidine	A	0.58	-	0.30	0.39
	N	0.46	0.59	0.29	0.39
	B	0.51	-	0.22	-

<sup>a</sup>All data were obtained using a carbon paste electrode in 0.2 M LiClO<sub>4</sub>. A = 0.04 M HClO<sub>4</sub>, B = 0.002 M tetrabutylammonium hydroxide, N = no acid or base added.

<sup>b</sup>Potentials in volts measured against Ag/Ag<sup>+</sup> (0.01 M). Concentrations of all of the electroactive species were 1.0 mM. The sweep rate for CV was 100 mVs<sup>-1</sup>.

<sup>c</sup>Broad peak.

wave; the height of the second wave in neutral wet acetonitrile being much smaller than that of first wave. However, in the aqueous phosphate buffer, the analogy was not followed, and DMMA was oxidized in a one-electron wave [14].

Catharanthine was oxidized in a two-electron wave by LAPV in neutral and acidified acetonitrile (Table 1). Addition of base to the acetonitrile medium resulted in a decrease in n(app.) to about one, and a shift in potential of -470 mV. In the al-

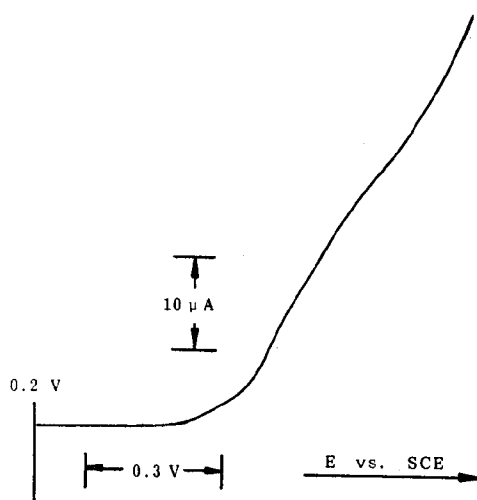


Fig. 5: Large amplitude pulse voltammogram of 0.25 mM vinblastine at carbon paste. Scan rate  $2 \text{ mVs}^{-1}$ . 0.1 M acetate buffer pH 5.6 in 1:1 ethanol-water.

Table 3: Large Amplitude Pulse Voltammetry: Oxidation Waves of Indole Alkaloids Related to Vinblastine

Compound	medium	$E_{1/2}$ / V vs. SCE	$i_1 C^{-1}/$ $\mu\text{Am}$ $\text{M}^{-1}$	$n(\text{app})$
vindoline	a	0.71	207	1.6
vindoline	b	0.75	59	1.3
catharanthine		0.54	45	1.0
vinblastine		0.79	91	2.0

(a) CP electrode,  $A = 0.12 \text{ cm}^2$ ; conc. 0.20 mM. Used p-chloroaniline as standard in determining  $n(\text{app})$ .  $\text{H}_2\text{O}$ , 0.2 M phos. buffer pH 6.34

(b) CP electrode,  $A = 0.08 \text{ cm}^2$ ;  $i_1$  in conc. range: 0.1-1.0 mM,  $E_{1/2}$ 's at 0.8-0.9 mM, Used THC = 1,2,3,4-tetrahydrocarbazole, as standard in determining  $n(\text{app})$ .  $\text{CH}_3\text{CH}_2\text{OH}/\text{H}_2\text{O}$  (1:1), 0.1 M acetate buffer pH 5.6

coholic acetate buffer, catharanthine was also oxidized (Fig. 4) in a one-electron transfer (Table 3).

A value of about 2 was obtained for  $n(\text{app})$  for vinblastine in ethanolic acetate buffer (Table 3) and the shape of the wave in LAPV suggested two severely overlapped waves (Fig. 5).

Cyclic voltammetric behaviour of various tetrahydrocarbazoles, catharanthine, vindoline [19a], and N,N-dimethyl-m-anisidine [19b] in neutral acetonitrile is characterized by irreversible oxidation waves, the characteristics of which are summarized in Table 2.

### Conclusion

The results demonstrate that the simple alkaloids investigated in this study do undergo facile electrooxidations of various types under different solution conditions. Cyclic voltammetric results (Table 2) show that the oxidation potential of catharanthine is markedly reduced in basic solution (from 0.83 V to 0.41 V), whereas that of vindoline is only slightly reduced (0.64 V to 0.55 V). A similar trend is revealed by pulse voltammetric oxidation of these molecules (Table 1). Thus it should be possible to oxidize catharanthine [20] without touching vindoline in basic solution (at 0.45 V for example). This has implications in terms of the possible formation of a coupled product between catharanthine and vindoline if one uses a high concentration of vindoline and a low concentration of catharanthine.

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