

Voltammetry of the Pteridine Derivative, Triamterene¹⁾

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Triamterene, 6-phenyl-2,4,7-triaminopteridine (1), a diuretic, gave two steps of two-electron reduction waves on Tast polarography (TP). The first step was ascribed to the reversible reduction of 1 into unreducible 5,8-dihydrotriamterene (2) by cyclic voltammetry (CV). Tautomerization of 2 to reducible 7,8-dihydrotriamterene (3) was rapid first-order reaction catalyzed by acid and base. The second step was attributed to the irreversible reduction of 3 into 5,6,7,8-tetrahydrotriamterene (4), which was oxidized to 2,4-diamino-6-phenylpteridine at a more positive potential than those of 2 and 3 into 1 on CV. The oxidation of 3 to 1 was observed at a more positive potential than that of 2 to 1 on CV. The anodic wave of 1 on TP in alkaline solution was ascribed to formation of the mercury complex of 1.

Key words voltammetry; triamterene; pteridine

One of the present authors (Y.A.)²⁾ studied direct current polarography (DCP) of many pteridine derivatives related to folic acid and revealed by NMR and UV that the 2-electron reduction product of 2-amino-4-hydroxy-6-methylpteridine (1a) was 2-amino-4-hydroxy-6-methyl-7,8-dihydropteridine (3a). Kwee and Lund³⁾ investigated electrochemistry of some pteridines including 1a. The reaction path was assumed to be reduction of 1a to 2-amino-4-hydroxy-6-methyl-5,8-dihydropteridine (2a), which tautomerized to 3a, and further reduction of 3a to 2-amino-4-hydroxy-6-methyl-5,6,7,8-tetrahydropteridine (4a), which was oxidized to 2-amino-4-hydroxy-6-methyl-6,7-dihydropteridine (5a). We were interested in triamterene,⁴⁾ 6-phenyl-2,4,7-triaminopteridine (1b), a diuretic, as an object of voltammetry since the structure differed from the naturally occurring pteridines. It was briefly noted⁵⁾ without discussion that 1b showed 2 steps of two-electron reduction waves at pH 2 on DCP.

In this paper the electrode reaction of 1b was elucidated by means of DCP, Tast polarography (TP), cyclic voltammetry (CV), microcoulometry, controlled potential electrolysis (CPE) and spectroscopy (UV, NMR, MS). Furthermore, kinetics of the tautomerization of 5,8-dihydrotriamterene (2b) into 7,8-dihydrotriamterene (3b), the oxidation of 5,6,7,8-tetrahydrotriamterene (4b) to a

new product, 2,4-diamino-6-phenylpteridine (1c), and the reversible reduction of 1c into 2,4-diamino-6-phenyl-5,8-dihydropteridine (2c) were revealed by means of CV.

Experimental

Materials Sample (1b) was provided by Sumitomo Chemical Industries, Ltd. Reagents were of special reagent grade from commercial sources. A stock solution of 0.25 mM 1b containing 1 mM HNO₃ was diluted with 0.1 M acetate or citrate or borate buffers to prepare a test solution of 0.01–0.1 mM 1b.

Polarography DCP and TP were measured on a Yanaco voltammetric analyzer (P-1000). Characteristics of the dropping mercury electrode (DME) were flow rate (*m*) = 0.905 mg/s, drop time (*t*) = 7.44 s (natural), *t*' = 2.00 s (mechanically controlled), and mercury head (*h*) = 50 cm, *k_c* = *m*^{2/3}*t*^{1/6} = 1.32 mg^{2/3}s^{-1/2}. A saturated calomel electrode (SCE) was used as a reference electrode. Polarograms were measured in a thermostat at 25.0 ± 0.1 °C unless otherwise cited after bubbling nitrogen through the solution. Average diffusion current (*i_d*) in DCP was close to 6/7 times maximum current (*i_m*) in TP.

CV CV was performed on an electrochemical analyzer (BAS 100B/W) made in U.S.A. Flow rate (*m*) of a static mercury drop electrode (SMDE) was 15.86 mg s⁻¹ and the drop size was controlled by a solenoid valve in a range of 10–500 ms, usually 100 ms. An Ag/AgCl electrode in 3 M NaCl was used as a reference electrode (*E* = -0.033 V vs. SCE). A Pt wire electrode (*l* = 5 cm, *d* = 0.5 mm) was used as an auxiliary electrode. The potential of SMDE was held at the initial value for a quiet time of 2 s without recording, and then was scanned to run. The peak current (*i_p*) and the peak potential (*E_p*) were read out on a cathode ray tube, and the CV curve was recorded on a printer.

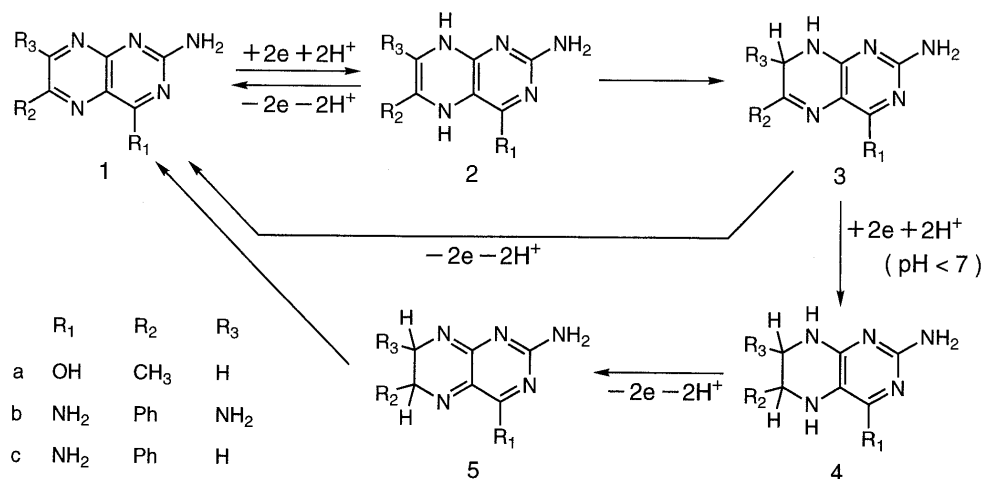


Chart 1

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Microcoulometry The first reduction wave. A solution containing 2 μ mol (0.5 mM) **1b** in dioxane (1.6 ml) and acetate buffer (2.4 ml) at pH 6.1 was electrolyzed on a mercury cathode (1 cm²) in a separate cell at -1.2 V against a SCE (30 cm²) under bubbling nitrogen. The current decreased to almost zero after electrolysis for 90 min. The consumed electricity, 0.39 C, measured by the current-time curve was found to coincide with the theoretical value, assuming two-electron reduction of the pteridine (**1b**) to the dihydropteridine (**3b**). The initial solution of **1b** showed 2 steps of reduction waves, whereas the electrolyzed solution of **3b** gave only the second reduction wave on DCP. The final solution of **3b** was diluted 5 times with 5% formic acid. UV $\lambda_{\max}$ nm (log ϵ): 348 (4.0). Since this UV was similar to those of **1b** and 2,4,6-triamino-5-benzylidenaminopyrimidine ($\lambda_{\max}$ 358 nm)⁴⁾, the reduction product was assigned not to 5,8-dihydropteridine (**2b**) but to conjugated 7,8-dihydropteridine (**3b**).

The second reduction wave. The same initial solution of **1b** (2 μ mol/4 ml) was electrolyzed at -1.4 V vs. SCE. The consumed electricity (0.8 C) corresponded to four-electron reduction of **1b**. The reduction product gave no reduction wave but an oxidation wave ($E_{1/2}$ -0.15 V) at pH 9. UV in 5% formic acid; $\lambda_{\max}$ nm (log ϵ): 294 (4.0), which was similar to UV of pyrimidines rather than pteridines. The product was assumed to be **4b**.

Controlled Potential Electrolysis A solution of **1b** (152 mg, 0.6 mmol) in dioxane (240 ml) and acetate buffer (360 ml, pH 6.1) was electrolyzed on a mercury cathode (30 cm²) at -1.4 V vs. SCE in a separate cell. The resulting solution was immediately electrolyzed on a platinum anode (20 cm²) at 0 V vs. SCE. The final solution was freeze-dried, washed with cold water and crystallized from isopropanol to obtain a yellow product, 2,4-diamino-6-phenylpteridine (**1c**); yield, 77%, mp 266°C (dec.). HREIMS m/z : 238.0971 (Calcd for C₁₂H₁₀N₆: 238.0967). UV $\lambda_{\max}$ nm (log ϵ): 365 (4.0). ¹H-NMR (Me₂SO-*d*₆) δ : 6.67 (2H, NH₂, doublet coalesced over 15°C), 7.75–7.67 (3H, m, Ph), 7.78 (2H, NH₂, doublet coalesced over 40°C), 8.41 (2H, m, Ph), 9.46 (1H, CH). ¹³C-NMR (Me₂SO-*d*₆) δ : 121.4–128.7 (Ph), 135.7, 143.4 (C-4a, 6), 147.4 (CH-7), 154.8, 162.7, 162.9 (C-4, 8a, 2).

UV Spectra **1b** (0.05 mM) in 40% (v/v) dioxane with acetate buffer at pH 5.0, $\lambda_{\max}$ nm (log ϵ): 362 (4.33). Mixture of **1b** (0.05 mM) and Hg(OAc)₂ (0.8 mM) in the same solvent, $\lambda_{\max}$ nm (log ϵ): 401 (4.24). **1b** (0.05 mM) in 0.01 M HNO₃, $\lambda_{\max}$ nm (log ϵ): 356 (4.32). Mixture of **1b** (0.05 mM) and HgNO₃ (0.5 mM) in 0.01 M HNO₃, $\lambda_{\max}$ nm (log ϵ): 369 (4.11).

Results and Discussion

DC and Tast Polarography The pteridine (**1b**) gave 2 steps of reduction waves with equal height at pH 1–13. The wave heights of 0.1 mM **1b** were independent of pH. Those at pH 6.88 were proportional to the concentrations of **1b** in the range of 0.01–0.1 mM. Temperature coefficients of the wave heights were 1.6%/deg. at 15–35°C. The DC wave heights were proportional to square root of the mercury head (*h*) of DME (natural dropping) in a range of *h* = 40–80 cm. It was determined from these facts that the reduction waves were diffusion currents (*i*_d). The diffusion current constant ($k_D = i_d C^{-1} m^{-2/3} t^{1/6}$) for each step at 25°C was estimated to be 3.2 ($\mu A \cdot mm^{-1} mg^{-2/3} s^{1/2}$), which was close to that of the two-electron reduction wave of **1a**.^{2,3)} The slope of linear relationship between potential (*E*) and current (log[(*i*_d − *i*)/*i*]) for the first reduction wave was close to the theoretical value (0.03 V) for the reversible two-electron reduction wave. It was also confirmed by microcoulometry that the two-electron reduction of **1b** at the potential of the first wave resulted in the formation of **3b**. Then the first reduction wave of **1b** was assumed to be due to reversible two-electron reduction of **1b** to unstable **2b**, which was tautomerized to **3b**.

The second reduction wave of **1b** was ascribed to two-electron reduction of **3b** into **4b** in analogy with **1a**.^{2,3)}

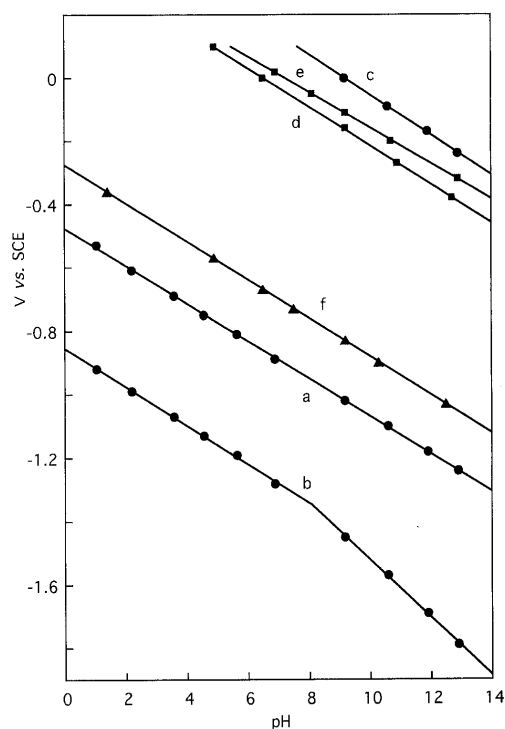


Fig. 1. pH Dependences of Half Wave Potential ($E_{1/2}$) on DCP, Anodic Peak Potential (E_{pa}), and Average Cathodic and Anodic Potentials (E_{av}) on CV

a) **1b** $\rightleftharpoons$ **2b**, $E_{1/2} = -0.48 - 0.059\text{pH}$ ($R = 0.9999$). b) **3b** $\rightarrow$ **4b**, $E_{1/2} = -0.86 - 0.061\text{pH}$ ($R = 0.9993$) ($\text{pH} < 7$), $E_{1/2} = -0.66 - 0.091\text{pH}$ ($R = 0.9994$) ($\text{pH} > 9$). c) Formation of Hg complex of **1b**, $E_{1/2} = 0.59 - 0.064\text{pH}$ ($R = 0.9997$). d) **3b** $\rightarrow$ **1b**, $E_{pa} = 0.40 - 0.061\text{pH}$ ($R = 0.9999$). e) **4b** $\rightarrow$ **5b**, $E_{pa} = 0.41 - 0.057\text{pH}$ ($R = 0.9999$). f) **1c** $\rightleftharpoons$ **2c**, $E_{av} = -0.28 - 0.060\text{pH}$ ($R = 0.9999$). *R*: correlation coefficient.

The slope of the second wave (0.05 V) was larger than the theoretical value (0.03 V) for two-electron reduction wave, so that the transfer coefficient (α) was estimated to be 0.6.

Linear relationships were observed between half-wave potential ($E_{1/2}$) and pH. Their slopes, $\Delta E_{1/2}/\Delta \text{pH}$, for the first wave at pH 1–13, the second wave at pH 1–7, and that at pH 9–13 were -0.059 , -0.061 and -0.091 V, respectively. The numbers of protons concerned with the reduction waves of **1b** were thus assumed to be 2, 2 and 3, respectively.

An anodic wave was observed at 0.0 V (pH 9.2)– -0.24 V (pH 13). This wave was assigned to the formation of a complex of **1b** with mercury ion formed by anodic dissolution of DME because UV of **1b** shifted to a longer wavelength in the presence of mercury (I or II) ion.

CV The important parameters of CV⁶⁾ are cathodic peak potential (E_{pc}), anodic peak potential (E_{pa}), their difference ($\Delta E_p = E_{pa} - E_{pc}$), cathodic peak current (i_{pc}) and anodic peak current (i_{pa}). The number (*n*) of electrons transferred in the electrode reduction for a reversible couple can be determined from $\Delta E_p = 0.059/n$. The formal reduction potential (E'_0) for a reversible couple is average cathodic and anodic peak potentials: $E'_0 = E_{av} = (E_{pc} + E_{pa})/2$. The values of i_{pc} and i_{pa} should be identical ($i_{pc} = i_{pa}$) for a simple reversible couple. However, electrochemical irreversibility and influence of chemical reaction cause increase of ΔE_p and difference between i_{pc} and i_{pa} .

CV of **1b** is interesting, as it varies with the scan rate

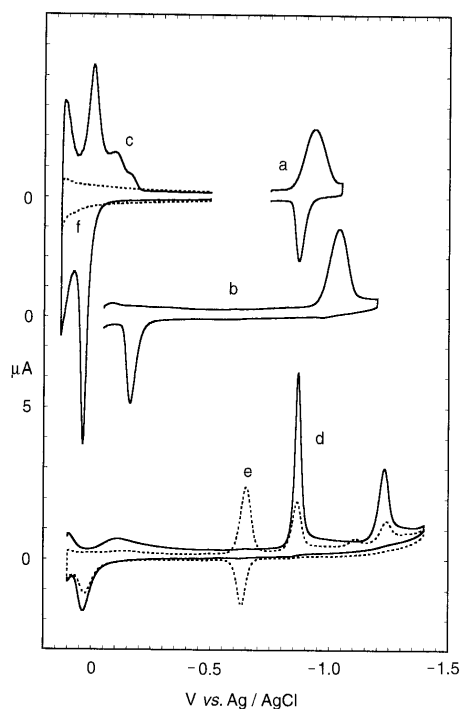


Fig. 2. Cyclic Voltammograms of Triamterene (**1b**)

a) At 0°C, pH 7.4, scan rate (v): 0.5V/s, scan period (t_s): 0.6 s. b, c) At 25°C, pH 9.2, v : 0.7V/s. d, e) At 25°C, pH 6.4, v : 0.2V/s. d) Initial scan. e) Fifth repeated scan. a—e) [**1b**]: 0.025 mM. f) Blank.

(v), the potential range and the repeated times of scan. Reversible redox peaks with equal peak current ($i_{pc} = i_{pa}$) and ΔE_p : 0.03 V at the potential (−0.9 V) of the first reduction wave on DCP were observed only in the neutral solution (pH 7.4) at 0°C on a rapid scan rate (0.5 Vs^{−1}) (Fig. 2a). The cathodic peak current (i_{pc}) was proportional to $v^{1/2}$. These peaks were assigned to the redox system involving **1b** and unstable **2b**. However, the anodic peak disappeared in either acid or basic solutions at a higher temperature (25°C). At the same time, the second anodic peak was observed at a more positive potential ($\Delta E_p = 0.86$ V) than the first of **1b** to **2b** (Fig. 2b). These results were explained by the tautomerization of unstable **2b** into stable **3b**, which gave the second anodic peak due to the oxidation of **3b** to **1b**.

The second cathodic peak of **1b** corresponds to the second reduction wave of **3b** to **4b** on DCP (Fig. 2d). This peak current observed in the local scan range (−1.22—−1.32 V at pH 7) was smaller than that in the wide range (−0.7—−1.4 V) including the first cathodic peak. The second cathodic peak was assumed to be due to the irreversible two-electron reduction of **3b** to **4b** after the rapid tautomerization of **2b** to **3b**. The third anodic peak of **1b** appeared at a more positive potential ($\Delta E_p = 1.3$ V) than that of the second cathodic peak in the wide scan range (+0.1—−1.4 V at pH 6.4) (Fig. 2d). The third anodic peak of **1b** was assumed to be due to the irreversible oxidation of **4b** to 6,7-dihydrotriamterene (**5b**). The two cathodic peaks and the third anodic peak of **1b** on the first scan decreased and new redox peaks with equal peak current ($i_{pc} = i_{pa}$) and ΔE_p : 0.03 V appeared on the following scans at a more positive potential (ca. 0.21 V) than the first cathodic peak of **1b** (Fig. 2e). The new peaks

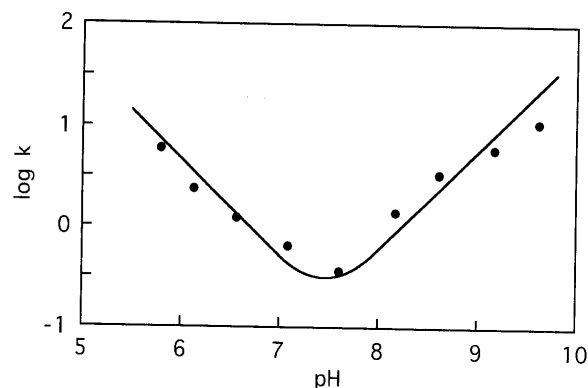


Fig. 3. pH Profile of Rate Constant (k : s^{−1}) for Tautomerization of Dihydrotriamterene (**2b** into **3b**) at 0°C

At pH 5.8—7.1, $\log k = \log k_H - \text{pH}$, $\log k_H = 6.66 \pm 0.17$.^{a)} At pH 8.2—9.6, $\log k = \log k_{OH} - \text{pOH}$, $\log k_{OH} = 6.68 \pm 0.26$.^{a)} a) Mean $\pm$ S.D.

coincided with CV of the product (**1c**) obtained by CPE of **1b**. This redox system was assumed to consist of the pteridine (**1c**) and stable 2,4-diamino-6-phenyl-5,8-dihydropteridine (**2c**). The peak heights of **1b** and **1c** were nearly equal in a steady state after 4—5 scans.

The reaction process of formation of **1c** from **1b** was assumed to be the reduction of **1b** to **4b** and the successive oxidation of **4b** to **5b**, and then elimination of ammonia from the unstable intermediate (**5b**).

The fourth anodic peak of **1b** in alkaline solution was observed on a reverse scan from −0.5 V to +0.13 V (Fig. 2c). This peak corresponds to the anodic wave due to the formation of mercury (I, II) complex with **1b** on DCP. The corresponding cathodic peak was assumed to be due to the reduction of mercury complex to mercury.

Tautomerization of Dihydrotriamterene The tautomerization of **2b** into **3b** was studied kinetically by CV. Many cyclic voltammograms of **1b** (Fig. 2a) were recorded in various scan rates and scan ranges. The first anodic peak current (i_{pa}) corresponds to the residual concentration (C_t) of **2b** and the first cathodic peak current (i_{pc}) corresponds to the initial concentration (C_0) of **2b** as $i_{pc} = i_{pa}$ at $t_s = 0$. A linear relationship was obtained between logarithm of the first anodic peak height (i_{pa}) of **2b** and the time (t_s), which was the scan period between the first redox peaks on CV.

$$kt_s = 2.3 \log(C_0/C_t) = 2.3 \log(i_{pc}/i_{pa}) \quad (1)$$

The rate constant (k) of the first-order reaction was calculated from the slope of the line. The pH profile of the rate constant (k) at 0°C in the buffer solutions of a constant concentration at pH 5.8—9.6 is shown in Fig. 3 and Eq. 2, where k_H and k_{OH} are catalytic constants for H⁺ and OH[−] ions, respectively and $\text{p}K_w = 14.95$ at 0°C.

$$k = k_H[\text{H}^+] + k_{OH}[\text{OH}^-] = 4.5 \times 10^6[\text{H}^+] + 4.8 \times 10^6[\text{OH}^-] \quad (2)$$

Therefore, this reaction is considered to be an acid-base catalyzed reaction. The reaction mechanism is postulated to be subtraction of 5-NH proton by OH[−] ion and addition of proton on 7-C (Chart 1).

References and Notes

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