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Voltammetry of the Pteridine Derivative, Triamterene[1)]

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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 Lund[3)] 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 noted[5)] without discussion that **1b** showed **2** steps of two-electron reduction waves at pH 2 on DCP.

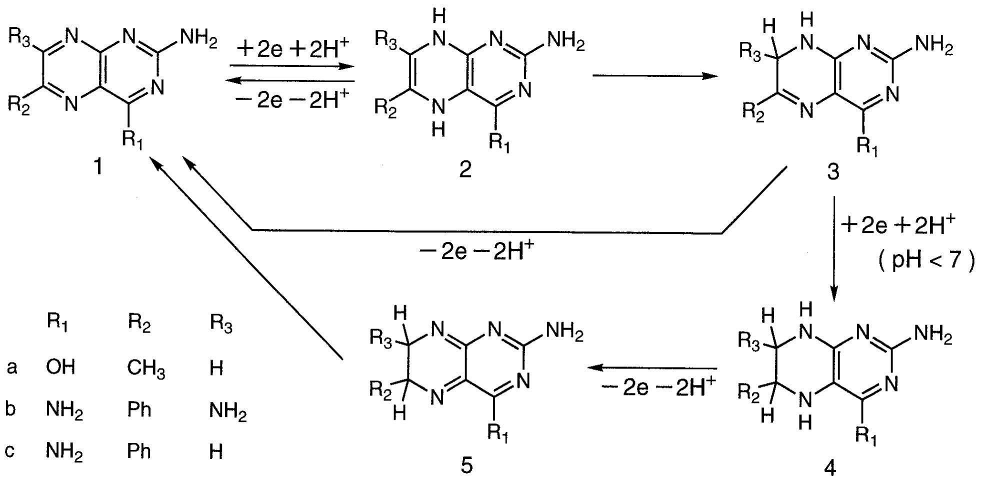
In this paper the electrode reaction of **1b** was elucidated by means of DCP, Tast polarography (TP), cyclic voltammetry (CV), microcoulometry, controlled potential electrolysis (CPE) and spectroscopy (UV, NMR, MS). Furthermore, kinetics of the tautomerization of 5,8-dihydrotriamterene (**2b**) into 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 HNO_3 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, $k_c = m^{2/3} t^{1/6} = 1.32\,\mathrm{mg}^{2/3}\mathrm{s}^{-1/2}$. 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.



	R1	R2	R3
a	OH	CH_3	H
b	NH_2	Ph	NH_2
c	NH_2	Ph	H

Chart 1

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Microcoulometry The first reduction wave. A solution containing 2 μmol (0.5 mM) **1b** in dioxane (1.6 ml) and acetate buffer (2.4 ml) at pH 6.1 was electolyzed on a mercury cathode (1 cm^2) in a separate cell at -1.2 V against a SCE (30 cm^2) 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 λ_{max} nm (log ε): 348 (4.0). Since this UV was similar to those of **1b** and 2,4,6-triamino-5-benzylidenaminopyrimidine (λ_{max} 358 nm)[4], the reduction product was assigned not to 5,8-dihydrotriamterene (**2b**) but to conjugated 7,8-dihydrotriamterene (**3b**).

The second reduction wave. The same initial solution of **1b** (2 μmol/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; λ_{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 cm^2) at -1.4 V *vs.* SCE in a separate cell. The resulting solution was immediately electrolyzed on a platinum anode (20 cm^2) 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 $C_{12}H_{10}N_6$: 238.0967). UV λ_{max} nm (log ε): 365 (4.0). ^{1}H-NMR (Me$_2$SO-d_6) δ: 6.67 (2H, NH$_2$, doublet coalesced over 15 °C), 7.75—7.67 (3H, m, Ph), 7.78 (2H, NH$_2$, doublet coalesced over 40 °C), 8.41 (2H, m, Ph), 9.46 (1H, CH). ^{13}C-NMR (Me$_2$SO-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, λ_{max} nm (log ε): 362 (4.33). Mixture of **1b** (0.05 mM) and Hg(OAc)$_2$ (0.8 mM) in the same solvent, λ_{max} nm (log ε): 401 (4.24). **1b** (0.05 mM) in 0.01 M HNO$_3$, λ_{max} nm (log ε): 356 (4.32). Mixture of **1b** (0.05 mM) and HgNO$_3$ (0.5 mM) in 0.01 M HNO$_3$, λ_{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 ($k_D=i_dC^{-1}m^{-2/3}t^{1/6}$) for each step at 25 °C was estimated to be 3.2 (μA·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] 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.

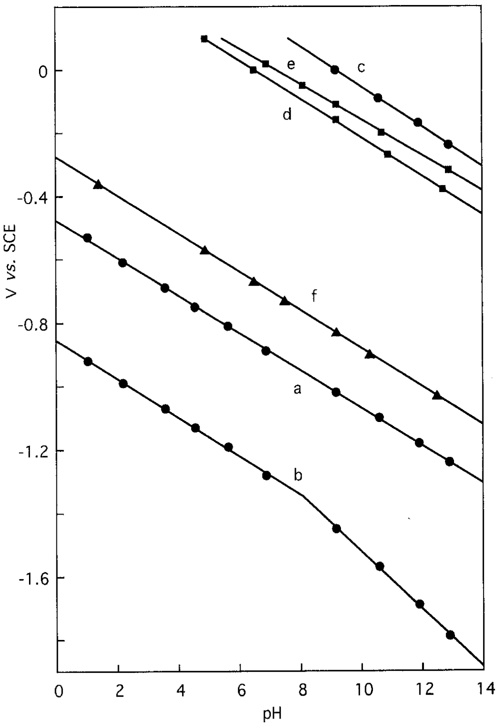

Fig. 1. PH Dependences of Half Wave Potential ($E_{1/2}$) on DCP, Anodic Peak Potential (E_{pa}), and Average Cathodic and Anodic Potentials (E_{av}) on CV

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

Linear relationships were observed between half-wave potential ($E_{1/2}$) and pH. Their slopes, $\Delta E_{1/2}/\Delta$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 CV[6] 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

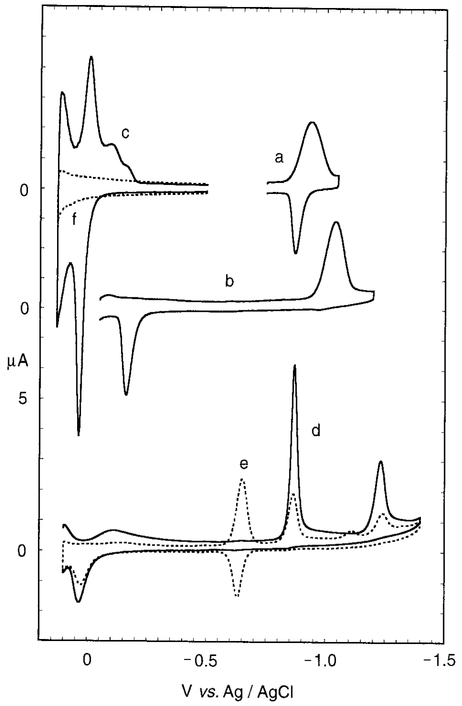

Fig. 2. Cyclic Voltammograms of Triamterene (**1b**)

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 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.

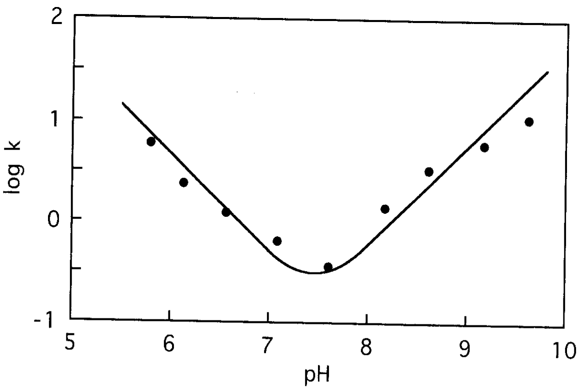

Fig. 3. PH Profile of Rate Constant (k: s^{-1}) for Tautomerization of Dihydrotriamterene (**2b** into **3b**) at 0 °C

At pH 5.8—7.1, $\log k=\log k_H-\text{pH}$, $\log k_H=6.66\pm0.17$.[a)] At pH 8.2—9.6, $\log k=\log k_{OH}-\text{pOH}$, $\log k_{OH}=6.68\pm0.26$.[a)] *a)* Mean ± S.D.

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 ($t_s s$), which was the scan period between the first redox peaks on CV.

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

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 $pK_w=14.95$ at 0 °C.

$$k=k_H[H^+]+k_{OH}[OH^-]=4.5\times10^6[H^+]+4.8\times10^6[OH^-] \qquad (2)$$

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

1) This work was presented at the 104th Annual Meeting of the

Pharmaceutical Society of Japan, Sendai, 1984.

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