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Polarographic Study of Pteridines

By MILTON LAPIDUS and MARVIN E. ROSENTHALE

The electronegativity of the half-wave potentials of a series of pteridine congeners was found to be related to the substituent groups. The 2,4,7-triaminopteridines, 7-substituted 4-amino-2-aryl-6-pteridinecarboxamides, and 4,7-diamino-2-aryl-6-pteridinecarboxamides were characterized, in that order, by decreasingly lower electronegative half-wave potentials.

A NUMBER of attempts have been made to correlate pharmacological activity with the oxidation-reduction potentials of a homologous series of compounds (1). Relationships have seldom been demonstrated; however, there is a report that acridines with reduction potentials (E°_{h}) more negative than -0.400 v. have greater antiseptic activity (2) than those with less negative potentials. That study showed the active acridines to have reduction potentials so electronegative that no physiological system could reduce (inactivate) them. Evidently, with the acridines, the maintenance of the oxidized configuration is necessary for biological activity. Another study showed that the degree to which members of two homologous series of acridine antimalarials inhibit the diamine–oxidase enzyme system parallels the values of the reduction potential of the compounds (3). However, no evidence was found that inhibition of diamine–oxidase is necessary to antimalarial action (4).

The possible importance of the oxidation-reduction potential prompted the authors to determine it for a large number of pteridines which have been under pharmacological review. Some of these pteridines are useful diuretic agents (5–8).

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METHOD

Material.—The pteridines studied were synthesized by Osdene *et al.* (9).

Polarographic Analysis.—A Leeds and Northrup recording polarograph equipped with a dropping mercury electrode was used for determining reduction potentials. The H-type electrolysis cell consisted of a saturated calomel half-cell connected to the test solution through an agar bridge and a fritted-glass diaphragm. Under a potential of -0.50 v. the dropping mercury electrode delivered 2.43 mg. of Hg/sec. with a drop time of 4.31 sec. All measurements were made at 25°. Unless otherwise noted, the solutions for polarographic analysis contained 5×10^{-4} *M* pteridine, 0.1 *M* phosphate buffer (pH 2.0), and 0.005% gelatin. The calibrating solution, which contained zinc chloride (1×10^{-3} *M*) in place of the pteridine, gave an $E^{1}_{1/2}$

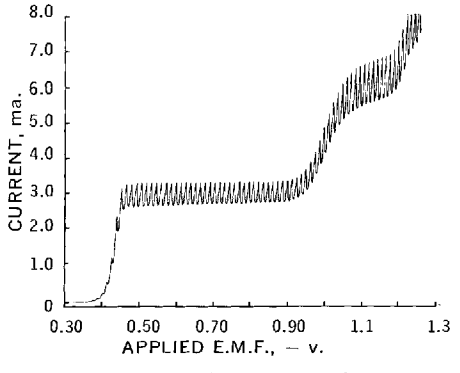
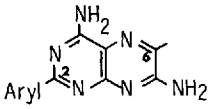
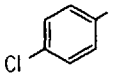
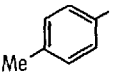
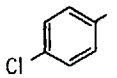
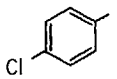
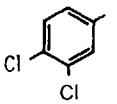


Fig. 1.—Polarographic reduction of Wy-4437 (pH 2.0).

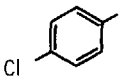
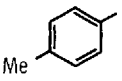
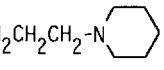
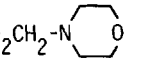
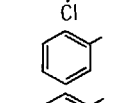
TABLE I.—HALF-WAVE REDUCTION POTENTIALS OF 4,7-DIAMINO-2-ARYL-6-PTERIDINECARBOXAMIDES (CLASS I)



Wy-No.	2	6	$E^1_{1/2}$	$E^2_{1/2}$
1512		-CONHMe	0.430	0.980
1841		-CONHCH$_2$CH$_2$CH$_2$NEt$_2$	0.430	1.010
1843		-CONHCH$_2$CH$_2$NEt$_2$	0.420	0.980
3580		-CONHCH$_2$CH$_2$NMe$_2$	0.430	1.000
3583		-CONH- 	0.405	0.965
3588		-CONEtCH$_2$CH$_2$NMe$_2$	0.445	1.000
3597		-CONMeCH$_2$CH$_2$NMe$_2$	0.440	1.020
3598		-CONHCH$_2$CH$_2$CH$_2$NBu$_2$	0.410	0.985
3599		-CON  NCH$_2$CH$_2$OH	0.440	1.015
3605		-CONHCH$_2$CH$_2$N(CHMe$_2$)$_2$	0.420	0.990
3654		-CONHCH$_2$CH$_2$N 	0.420	0.990
3665		-CONHCH$_2$CH$_2$CH$_2$NEt$_2$	0.410	0.985
3700		-CONHCH$_2$CH$_2$NEt$_2$	0.415	0.960
3873		-CONHCH$_2$CH$_2$CH$_2$NMe$_2$	0.420	0.990
4029		-CONHCH$_2$CH$_2$NEt$_2$	0.415	0.985
4119		-CONHCH$_2$CH$_2$N 	0.395	0.967
4153		-CONHCH$_2$CH$_2$OEt	0.415	0.990
4156		-CONHCH$_2$CH$_2$OMe	0.425	0.985
4196		-CONHCH$_2$CH$_2$NEt$_2$	0.390	0.987

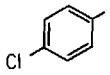
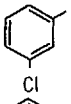
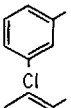
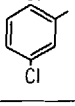
(*Continued on next page.*)

TABLE I.—(*Continued*)

Wy-No.	2	6	$E^1_{1/2}$	$E^2_{1/2}$
4276		-CONHCH$_2$CH$_2$NH$_2$	0.425	0.987
4277		-CONHCH$_2$CH(OEt)$_2$	0.430	1.020
4278		-CONHCH$_2$CH$_2$N(CH$_2$CH$_2$OH)$_2$	0.440	1.000
4376		-CONHCH$_2$CH(OEt)$_2$	0.455	0.950
4377		-CONHCH$_2$CH(OEt)$_2$	0.430	0.995
4436		-CONHCH$_2$CH$_2$CH$_2$-N 	0.440	1.025
4437		-CONHCH$_2$(CH$_2$)$_2$CH$_2$NMe$_2$	0.430	1.015
5121		-CONHCH$_2$(CH$_2$)$_2$CH$_2$NEt$_2$	0.440	1.010
5250		-CONHCH$_2$(CH$_2$)$_4$CH$_2$NMe$_2$	0.400	0.967
5330		-CONHCH$_2$(CH$_2$)$_5$CH$_2$NEt$_2$	0.405	0.995
5331		-CONHCH$_2$(CH$_2$)$_3$CH$_2$NMe$_2$	0.440	1.005
5365		-CONHCH$_2$(CH$_2$)$_3$CH$_2$NEt$_2$	0.410	0.990
5588		-CONHCH$_2$CH$_2$NEt$_2$	0.410	0.980
5589		-CONHCH$_2$CH$_2$-N 	0.425	1.000
6119		-CONHCH$_2$CH$_2$CH$_2$OEt	0.415	0.990
6520		-CONHCH$_2$CH$_2$NEt$_2$	0.410	0.995
7037		-CONHCH$_2$CH$_2$NEt$_2$	0.410	0.980
7038		-CONHCH$_2$(CH$_2$)$_2$CH$_2$NMe$_2$	0.427	1.035
7184		-CONHEt	0.425	1.000
7307		-CONHCH$_2$(CH$_2$)$_2$CH$_2$NMe$_2$	0.430	1.020

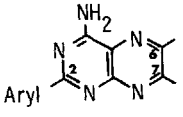
(*Continued on next page.*)

TABLE I.—(*Continued*)

Wy-No.	2	6	$E^1_{1/2}$	$E^2_{1/2}$
7322[a]		-CONHCH$_2$CH$_2$NMe$_2$	0.410	0.975
7323		-CONHCH$_2$CH$_2$NMe$_2$	0.405	0.980
7324[a]		-CONHCH$_2$CH$_2$CH$_2$NMe$_2$	0.400	0.975
7329		-CONHCH$_2$(CH$_2$)$_4$CH$_2$NMe$_2$	0.420	1.020
7330		-CONHCH$_2$(CH$_2$)$_3$CH$_2$NMe$_2$	0.420	1.015

[a] Less than 5×10^{-3} *M*.

TABLE II.—HALF-WAVE REDUCTION POTENTIALS OF 7-SUBSTITUTED 4-AMINO-2-ARYL-6-PTERIDINECARBOXAMIDES (CLASS II)



Wy-No.	2	6	7	$E^1_{1/2}$	$E^2_{1/2}$
4739		-CONHCH$_2$CH$_2$N 	-NHCH$_2$CH$_2$N 	0.480	1.070
4760		-CONHCH$_2$CH$_2$OEt	-NHCH$_2$CH$_2$OEt	0.480	1.050
5120		-CONHCH$_2$CH$_2$NEt$_2$	-NHCH$_2$CH$_2$NEt$_2$	0.490	1.100
5256		-CONHCH$_2$CH$_2$OMe	-NHCH$_2$CH$_2$OMe	0.500	1.070
5587		-CONHCH$_2$CH$_2$SEt	-NHCH$_2$CH$_2$SEt	0.480	1.050
5614		-CONHCH$_2$CH$_2$OMe	-NHCH$_2$CH$_2$OMe	0.515	1.085
5829		-CONHCH$_2$CH$_2$OEt	-NHCH$_2$CH$_2$OEt	0.485	1.055
6218		-CONHCH$_2$CH$_2$CH$_2$OEt	-NHCH$_2$CH$_2$CH$_2$OEt	0.510	1.100
7209		-CONHEt	-NHEt	0.470	1.050
7635		-CONHMe	-NHMe	0.565	1.080
7714		-CONHPr	-NHPr	0.500	1.020
7715		-CONHBu	-NHBu	0.500	...

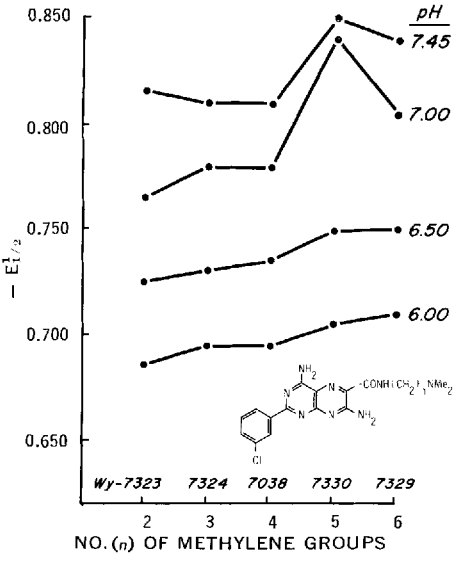


Fig. 2.—Half-wave potential profile for a series of 4,7-diamino-2-(*m*-chlorophenyl)-6-pteridine-carboxamides with varied length of methylene chain in 6-carboxamide substituent.

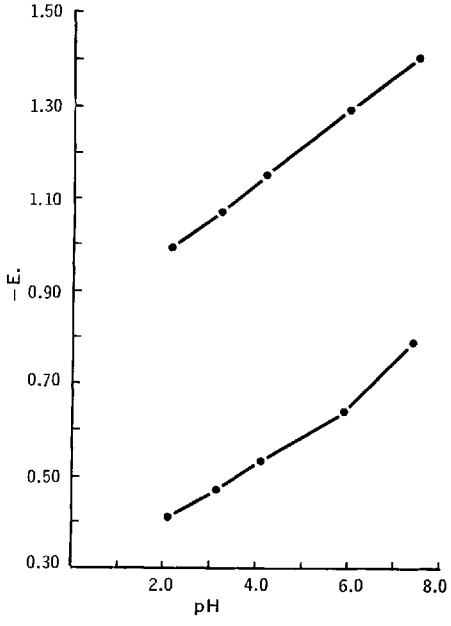


Fig. 3.—Variation in half-wave potential of Wy-4437 with change in pH. Key: top curve, $E^2_{1/2}$; bottom curve, $E^1_{1/2}$.

of −1.075, a value in agreement with the literature (10). Replicate samples varied ±0.015 v.

RESULTS

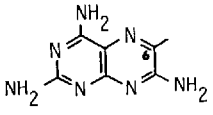
The pteridines gave well-formed 2-step polarographic waves (Fig. 1). The half-wave potentials, designated $E^1_{1/2}$ and $E^2_{1/2}$, were determined by expolation from the current (ma.) *versus* applied voltage (v.) record (11). Characteristic half-wave potentials, $E^1_{1/2}$, were found for each of the 3 general classes of pteridines at pH 2.0.

Class I.—*4,7-Diamino-2-aryl-6-pteridinecarboxamides.*—In this group the compounds had half-wave potentials of −0.390 to −0.460 (Table I).

Class II.—*7-Substituted 4-Amino-2-aryl-6-pteridine Carboxamides.*—Substitution in the 7 position resulted in an increase in the electronegativity of the half-wave potential ($E^1_{1/2}$ from −0.470 to −0.565, Table II).

Included in class II pteridines was a polarographic study investigating the influence of the N—N intra-

TABLE III.—HALF-WAVE REDUCTION POTENTIALS OF 2,4,7-TRIAMINO-6-ARYLPTERIDINES AND 2,4,7-TRIAMINO-6-PTERIDINECARBOXAMIDES (CLASS III)



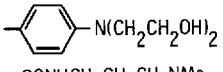
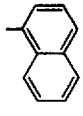
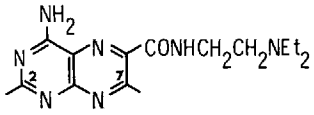
Wy-No.	6	$E^1_{1/2}$	$E^2_{1/2}$
1643		0.690	
1839	-CONHCH$_2$CH$_2$CH$_2$NMe$_2$	0.600	1.060
1840	-CONHCH$_2$CH$_2$NEt$_2$	0.570	1.020
3519		0.625	1.075
4151	-CONHCH$_2$CH$_2$CH$_2$OCHMe$_2$	0.595	1.105
4927		0.655	1.110

TABLE IV.—REDUCTION POTENTIAL OF REPRESENTATIVE COMPOUNDS OF THREE GENERAL CLASSES OF SUBSTITUTED PTERIDINES



Wy-No.	2	7	$E^{1}_{1/2}$
1843 (Class I)		$-NH_2$	0.420
5120 (Class II)		$-NHCH_2CH_2NEt_2$	0.490
1840 (Class III)	$-NH_2$	$-NH_2$	0.570

nuclear distance in the carboxamide structures in the 6 position of a homologous series of pteridines (Fig. 2). The half-wave reduction potential of this series was found to be a function of the N—N intranuclear distance as well as the pH. A methylene bridge of 5 carbons gave the maximal electronegative half-wave potential at pH 7.0 and 7.5.

Class III.—*2,4,7-Triamino-6-arylpteridines and 2,4,7-Triamino-6-pteridinecarboxamides.*—The electronegative potentials found in this general group were from −0.570 to −0.690 (Table III). The reduction potential of the 6-arylpteridines was more electronegative than the 6-pteridinecarboxamides.

Within each of the three general classes, the reduction potential of the pteridines increased in electronegativity with the increase in pH from 2.0 to 7.4. A typical half-wave potential *versus* pH curve is shown in Fig. 3.

DISCUSSION

Polarographic and spectrographic evidence has established that reduction of the pyrazine ring in folic acid and related pteridine structures occurs at the 5–6 and 7–8 double bonds. The reduction occurs in two steps and has been shown to be pH-dependent and to involve 2 electrons per step (12, 13). The authors compared the reduction record of 4,7-diamino-*N*-(4-dimethylaminobutyl)-2-phenyl-6-pteridinecarboxamide (class I, Wy-4437) with that of folic acid at the same molar concentration and pH and found the same 2-step reduction and the same limiting current. The authors consider this to be evidence for a 2-electron-per-step reduction mechanism for both structures (14).

Substitution on the pteridine nucleus had an over-all effect on the reductive process occurring at the 5–6 and 7–8 double bonds. The gain of electrons by the pteridine ring at the dropping mercury electrode and the simultaneous introduction of H+ occurred at voltages which were characteristic for each of the substituted pteridines. One can assume that the more difficult it is for a pteridine to gain electrons and be reduced, the more electronegative the applied voltage has to be. This effect, the ease or difficulty of reduction, involves not only the pteridine nucleus but the substituents as well.

The comparative electronegativity of the half-wave potential of the 3 general classes of pteridines studied was: class III > class II > class I. The substitution of a 2-phenyl group (Wy-1843) for the 2-amino group (Wy-1840) resulted in a decrease in electronegativity of the half-wave potential (Table IV). This decrease may be due to a withdrawal of electrons from the site of reduction by the phenyl group. The result of this resonance effect is to permit reduction to occur at a lower potential. The replacement of a primary amino group (Wy-1843) by a secondary amino group (Wy-5120) at position 7 resulted in an increase in electronegativity of the half-wave potential, indicating that the 2-diethylaminoethyl structure on the 7-amino group must act as an electron donor.

A polarographic study of the effect of pH on a homologous series of 4,7-diamino-2-*m*-chlorophenyl-6-pteridinecarboxamides (Fig. 2) revealed that an increase in the N—N intranuclear distance within the carboxamide tended to raise the electronegative half-wave potential. At acid pH's the protonation of amino and heterocycle nitrogen limited the resonance and inductive effects, but this leveling effect was minimized as the pH was increased. At neutrality the maximal reduction potential was recorded for a methylene chain length of 5 carbons.

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