trituration with ice water yielded 3.4 g of **2-phenyl-4-chloro-6,8-diaminopyrimido[5,4-*d*]pyrimidine.** This (2.9 g) was treated with liquid NH_3 (25 ml) in a bomb at 150° for 6 hr. The solid residue was washed (H_2O, EtOH). It was recrystallized from DMF–MeOH yielding 0.8 g (31%) of crystals: mp 354–356° dec; R_f 0.69 (system 1); $\lambda_{max}^{4.5\%\ HCOOH}$ 248 mμ (log ε 4.44), 294 (4.27), 336 (sh) (3.85), 354 (sh) (3.72). *Anal.* ($C_{12}H_{11}N_7 \cdot 0.5H_2O$) C, H, N.

2-Phenyl-4-piperidino-6,8-diaminopyrimido[5,4-*d*]pyrimidine.—2-Phenyl-4-chloro-6,8-diaminopyrimido[5,4-*d*]pyrimidine (1.0 g) and piperidine (60 ml) were refluxed 16 hr. The mixture was filtered and the filtrate was concentrated to dryness *in vacuo*. The residue was dissolved in hot aqueous AcOH, decolorized, and filtered, and then the solution was made basic with concentrated NH_4OH. The pale yellow solid was collected (0.4 g) and recrystallized from MeOH–H_2O to give a solid, mp 215°, R_f 0.55 (system 2). *Anal.* ($C_{17}H_{19}N_7$) H; C: calcd, 63.53; found, 64.00.

Methyl 5-Amino-2,6-dihydroxypyrimidine-4-carboxylate.—Methyl 2,6-dihydroxy-5-nitropyrimidine-4-carboxylate (5.0 g, 0.027 mole) was added to a solution of 12.1 g (0.07 mole) of sodium dithionate at 10° and then stirred at room temperature for 15 min. A solid precipitated which was washed with hot MeOH (2.85 g, 66%) and recrystallized twice from DMF to give pale yellow crystals, mp 254–255° dec. *Anal.* ($C_6H_7N_3O_4$) C, H, N.

2,6-Dihydroxy-5-nitropyrimidine-4-carboxamide.—A mixture of methyl 2,6-dihydroxy-5-nitropyrimidine-4-carboxylate (35 g, 0.16 mole), MeOH (720 ml), and liquid NH_3 (72 ml) was heated at 100° for 6 hr in an autoclave. On cooling a solid was obtained which was recrystallized from 540 ml of H_2O to give 21 g (60%) of a yellow solid, mp 269–270°, which on analysis proved to be the ammonium salt of the desired product. *Anal.* ($C_5H_7N_5O_5$) C, H, N.

A portion of the above was treated with aqueous acid and recrystallized from MeOH to give crystals, mp 285° dec. *Anal.* ($C_5H_4N_4O_3$) C, H, N.

2,5,6-Triaminopyrimidine-4-carboxamide.—A suspension of 5.3 g (0.027 mole) of 2,6-diamino-5-nitropyrimidine-6-carboxamide and 300 mg of PtO_2 in 200 ml of glacial AcOH was shaken under 2.8 kg/cm² of H_2 for 2.5 hr. The catalyst was removed by filtration and the solvent was evaporated under vacuum. The residue was washed with MeOH to give 3.8 g (83%) of product which was dissolved in dilute NH_4OH and treated with charcoal, and the solution was filtered. Chilling gave 1.2 g (26%) of white needles, mp 285° dec. A sample was boiled with MeOH to give yellow needles, mp 286–288° *Anal.* ($C_5H_8N_6O$) C, H, N.

2,6-Diamino-5-nitropyrimidine-4-carboxamide.—A suspension of 18 g (0.071 mole) of methyl 2,6-dichloro-5-nitropyrimidine-4-carboxylate in 720 ml of 10% MeOH–NH_3 was heated in a steel bomb at 100° for 2 hr. The product was dissolved in excess MeOH, filtered, and taken to dryness under vacuum. The residue was washed (H_2O) and recrystallized (MeOH) to give 3.2 g (22%) of prisms, mp >285°. *Anal.* ($C_5H_6N_6O_2$) C, H, N.

4,6-Diamino-2-phenylpyrimidine.—A mixture of 3 g (0.013 mole) of 4,6-diamino-2-phenylpyrimidine-5-carboxamide and 9.9 g (0.13 mole) of thiourea was heated at 180° for 5 hr and cooled. Addition of 90 ml of 5% NaOH and warming caused the separation of a yellow-green solid, mp 180–188°. This was dissolved in hot H_2O and acidified with HCl to give a yellow solid, mp 258–260°, which is the hydrochloride of the product. This was dissolved (H_2O), treated with charcoal, and made basic with NH_4OH to give white crystals, mp 193–195°. Repeating the acid–base cycle gave 0.45 g of crystals, mp 194.5–196°, whose ir spectra were identical with that of an authentic sample; lit.[18] mp 195–196°. *Anal.* ($C_{10}H_{10}N_4$) C, H, N.

(18) G. A. Howard, B. Lythgoe, and A. R. Todd, *J. Chem. Soc.*, 476 (1944).

Pteridines. XII.[1] Structure–Activity Relationships of Some Pteridine Diuretics

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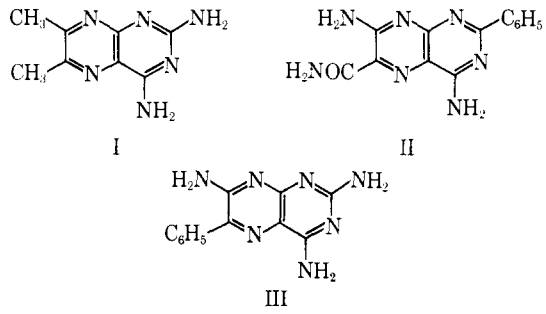
The diuretic activity of pteridines related to 2,4,7-triamino-6-phenylpteridine (triamterene), 2,4-diamino-6,7-dimethylpteridine (I), and 4,7-diamino-2-phenylpteridine-6-carboxamide (II) was studied in the saline-loaded and sodium-deficient rat. A limited number of related pyrimidopyrimidines were similarly studied. Some of the compounds related to triamterene and I not only cause Na^+ excretion but also conserve K^+. All of the 2-phenylpteridines we have studied which are active natriuretic agents also cause K^+ excretion. In the triamterene series, replacement of any of the amino groups by either a large amine or a nonbasic group other than hydrogen leads to reduction of diuretic activity. Replacement of the phenyl by a small, nonbasic group gives active diuretic agents, but an aromatic (or heteroaromatic) group seems desirable for highest activity. Some variation in the substitution pattern on the pteridine ring is permissible as demonstrated by the activity of the triamterene isomers. The 7-phenyl isomer is outstanding as a blocker of K^+ excretion.

The presently known diuretic pteridines[3] may be grouped into three principle classes based on structural features and electrolyte excretion pattern. In order of their discovery these are (1) the 2,4-diamino-6,7-dialkylpteridines, (2) the 2-aryl-4,7-diaminopteridine-6-carboxamides, and (3) the 2,4,7-triamino-6-arylpteridines. In this paper we will discuss the structure–activity relationships within each of these classes and compare these classes to each other. In addition we will discuss the diuretic activity of some related pyrimidopyrimidines. The prototype for the first class is 2,4-diamino-6,7-dimethylpteridine (I), for the second class, 4,7-diamino-2-phenylpteridine-6-carboxamide (II), and for the third class, 2,4,7-triamino-6-phenylpteridine (triamterene, III).

(1) Previous papers in this series: (a) I. J. Pachter and P. E. Nemeth, *J. Org. Chem.*, **28**, 1187 (1963); (b) I. J. Pachter, *ibid.*, **28**, 1191 (1963); (c) I. J. Pachter, P. E. Nemeth, and A. J. Villani, *ibid.*, **28**, 1197 (1963); (d) I. J. Pachter and P. E. Nemeth, *ibid.*, **28**, 1203 (1963); (e) J. Weinstock, R. Y. Dunoff, and J. G. Williams, *J. Med. Chem.*, **11**, 542 (1968); (f) J. Weinstock, R. Y. Dunoff, B. Sutton, B. Trost, J. Kirkpatrick, F. Farina, and A. S. Straub, *ibid.*, **11**, 549 (1968); (g) J. Weinstock, I. J. Pachter, P. E. Nemeth, and G. Jaffe, *ibid.*, **11**, 557 (1968); (h) J. Weinstock, H. Graboyes, G. Jaffe, I. J. Pachter, K. Snader, C. B. Karash, and R. Y. Dunoff, *ibid.*, **11**, 560 (1968); (i) J. Weinstock and R. Y. Dunoff, *ibid.*, **11**, 565 (1968); (j) H. Graboyes, G. E. Jaffe, I. J. Pachter, J. P. Rosenbloom, A. J. Villani, J. W. Wilson, and J. Weinstock, *ibid.*, **11**, 568 (1968); (k) J. Weinstock, R. Y. Dunoff, J. Carevic, J. G. Williams, and A. J. Villani, *ibid.*, **11**, 618 (1968).

(2) (a) Medicinal Chemistry Section. (b) Biochemistry Section.

(3) For a brief discussion of the discovery of useful diuretic activity in the pteridines see J. Weinstock and V. D. Wiebelhaus in "Pteridine Chemistry," W. Pfleiderer and E. C. Taylor, Ed., Pergamon Press, Oxford, 1964, p 37. For discussion of other biological properties of pteridines and related compounds see (a) elsewhere in the above reference; (b) "Chemistry and Biology of Pteridines," G. E. W. Wolstenholme and M. P. Cameron, Ed., Little, Brown and Co., Boston, Mass., 1954; (c) S. Kaufman, *Ann. Rev. Biochem.*, **36**, 171 (1967); (d) G. H. Hitchings and J. J. Burchall, *Advan. Enzymol.*, **27**, 417 (1965).



I II III

Biological Methods.—Three types of diuretic assays were used to characterize the activity of the pteridines. The orally dosed saline-loaded rat was employed as the first screen.[4] Base line data obtained in this protocol with standard diuretic agents indicated that urine volume was a reasonably reliable indicator of natriuresis. In general only those compounds reasonably active in this test were studied in more detail. The orally dosed sodium-deficient rat assay[4] measured the natriuretic potency of the compounds in animals in a state of avid sodium retention due, at least in part, to high levels of endogenous mineralocorticoids. Finally, renal clearance experiments in saline-loaded, 9-α-fluorohydrocortisone treated dogs[5] (referred to hereafter as the saline-9αFHC dog) provided more detailed data on the effects of the compounds on Na^+, K^+, Cl^-, and water excretion, urinary pH, and glomerular filtration rate. Since these three tests measure diuresis in different systems, correlation between these tests was not always good, although in general the compounds highly active in the rat were also active in the dog, while the compounds with poor activity in the saline-loaded rat were also poorly active in the sodium-deficient rat and the dog renal clearances.

In Tables I–XIV the saline-loaded rat data were rated as follows. The urine volume was expressed as a per cent of the original saline load, and the standard control (60% of the saline load) subtracted from this gave the effect due to drug. Since 22% over control is the point of statistical significance (95% confidence limits), responses less than 22% were rated as 0. Responses of 22–45% over control were rated as 1, 46–69% over control as 2, and >69% over control as 3. The greatest response at any dose (usually either 15 or 30 mg/kg) was used for the rating. Although 22% over controls is the point of statistical significance, this cut-off is of limited meaning because the truly interesting compounds usually were those rated 2 or 3. Also, some compounds which were inactive at the usual doses used were quite active at higher doses.

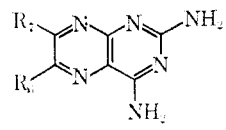
The sodium-deficient rat test was run by giving each rat 10 mg of Na^+ subcutaneously immediately before dosing with drug. The urine was collected for 6 hr and analyzed for Na^+ and K^+. The response was recorded as milligrams of Na^+ excreted per rat. In the tables the maximum response at any dose is reported as follows: <3 mg as 0, 3–6 mg as 1, 6–9 mg as 2, and >9 mg as 3.

(4) See V. D. Wiebelhaus, J. Weinstock, A. R. Maass, F. T. Brennan, G. Sosnowski, and T. Larsen, *J. Pharmacol. Exptl. Therap.*, **149**, 397 (1965), for details of the rat tests as carried out on the pteridines.

(5) See V. D. Wiebelhaus, F. T. Brennan, G. Sosnowski, A. R. Maass, J. Weinstock, and A. D. Bender, *Arch. Intern. Pharmacodyn.*, **169**, 429 (1967), for details of this procedure as carried out on pteridine diuretics.

TABLE I

2,4-DIAMINOPTERIDINES



R_6	R_7	Diuretic act., rat[a] Saline-loaded	Sodium-deficient
H	H[b]	3	0
CH_3	H[c]	3	1
H	CH_3[c]	2	1
CH_3	CH_3[b] (I)	3	3
C_2H_5	C_2H_5[d]	1	0
n-C_3H_7	n-C_3H_7[d]	0	...
n-C_5H_{11}	n-C_5H_{11}[d]	0	...
CH_3	n-C_5H_{11}	1	...
n-C_5H_{11}	CH_3	2	0
CH_3	C_6H_5[e]	0	...
C_6H_5	H	1	...
C_6H_5	C_6H_5[b]	1	0
$C_6H_5CH_2$	$C_6H_5CH_2$[d]	0	...
	$-(CH_2)_3-$	3	1
	$-(CH_2)_4-$[e]	1	...
COOH	H	0	...
$COOCH_3$	H	3	0
$COOC_2H_5$	H	1	...
$COOCH(CH_3)_2$	H	3	...
$CONH_2$	H	0	...
CH_3	OH[f]	0	...
C_6H_5	OH[g]	3	0

[a] Rating scheme for saline-loaded rat assay: maximum response at any dose in volume % of volume compared to volume of 0.9% saline load, less that of untreated controls, <22% = 0, 22–45% = 1, 46–69% = 2, and >69% = 3. Rating scheme for sodium-deficient rat assay: maximum response at any dose in mg of sodium excreted in the urine/rat, <3 mg = 0, 3–6 mg = 2, and >9 mg = 3. References are given only to those compounds whose preparation is not described in the previous papers of this series listed in reference 1. [b] M. F. Mallette, E. C. Taylor, and C. K. Cain, *J. Am. Chem. Soc.*, **69**, 1814 (1947). [c] D. R. Seeger, D. B. Cosulich, J. M. Smith, and M. E. Holtquist, *J. Am. Chem. Soc.*, **71**, 1753 (1949). [d] N. R. Campbell, J. H. Dinsmuir, and M. E. H. Fitzgerald, *J. Chem. Soc.*, 2743 (1950). [e] M. D. Potter and T. Henshall, *ibid.*, 2000 (1956). [f] G. M. Timmis, U. S. Patent 2,858,889 (Jan 8, 1952). [g] A. G. Renfrew, P. C. Piatt, and L. H. Cretcher, *J. Org. Chem.*, **17**, 469 (1952).

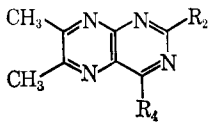
Structure–Activity Relationships.—The diuretic properties of analogs of I are shown in Tables I and II. Although a number of these 2,4-diaminopteridines are active in the saline-loaded rat, only the 6,7-dimethyl compound shows good activity in the sodium-deficient rat. Changes in the 2,4-diamino portion of the molecule cause a more drastic decrease of diuretic activity. 1,3,6,7-Tetramethyllumazine is the only compound other than I in Table II which is highly active in the saline-loaded rat; however, it is not active in the sodium-deficient rat.

The diuretic activity of II is shown in Table III. It is active in both the saline-loaded rat and the sodium-deficient rat. In addition, it is an active natriuretic agent in the saline-9αFHC dog. However, in contrast to I and triamterene, II causes substantial K^+ loss in both the sodium-deficient rat and the saline-9αHFC dog.

In Table III is shown the effect of varying the substituent in the 2 position of II. Substituents in the phenyl other than methyl markedly reduced activity, and

TABLE II

6,7-DIMETHYLPTERIDINES

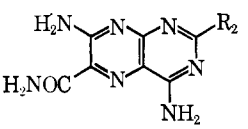


R_2	R_4	Diuretic act.[a] Saline-loaded rat
H	NH_2[b]	0
CH_3	NH_2	0
C_6H_5	NH_2	0
NH_2	NH_2[c] (I)	3
$N(CH_3)_2$	NH_2[d]	1
NHC_6H_5	NH_2	0
SCH_3	NH_2[e]	0
OH	NH_2[f]	1
NH_2	H[g]	1
NH_2	CH_3	0
NH_2	OH[h]	0 (0)
NH_2	$CONH_2$	0
H	OH[i]	0
OH	OH[h]	0 (0)
=O [1,3-$(CH_3)_2$]	=O[j]	3 (0)

[a] Numbers in parentheses refer to sodium-deficient rat data. See note *a*, Table I, for rating scale. [b] J. W. Daly and B. E. Christensen, *J. Am. Chem. Soc.*, **78**, 225 (1956). [c] Note *b*, Table I. [d] B. Roth, J. M. Smith, and M. E. Hultquist, *J. Am. Chem. Soc.*, **73**, 2864 (1951). [e] E. C. Taylor and C. K. Cain, *ibid.*, **74**, 1644 (1952). [f] D. J. Brown and N. W. Jacobsen, *J. Chem. Soc.*, 4413 (1961). [g] Gift from Professor A. Albert. [h] C. K. Cain, M. F. Mallette, and E. C. Taylor, *J. Am. Chem. Soc.*, **68**, 1996 (1946). [i] A. Albert, D. J. Brown, and G. Cheeseman, *J. Chem. Soc.*, 474 (1951). [j] F. F. Blicke and H. C. Godt, *J. Am. Chem. Soc.*, **76**, 2798 (1954).

TABLE III

4,7-DIAMINO-6-PTERIDINECARBOXAMIDES



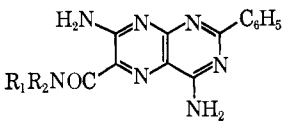
R_2	Diuretic act.[a] Saline-loaded rat
C_6H_5 (II)	3 (3)
p-$CH_3C_6H_5$	3 (3)
m-$CH_3C_6H_5$	3 (3)
o-$CH_3C_6H_5$	1 (1)
p-ClC_6H_5	0
m-ClC_6H_5	1
o-ClC_6H_5	1
p-$CH_3OC_6H_5$	0
4-Cl-3-$H_2NSO_2C_6H_5$	1
3-C_5H_4N	0
H	0
CH_3	0
$CH_2C_6H_5$	0
NH_2	0
NHC_6H_5	0
$N(CH_3)_2$	3(1)

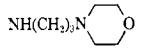
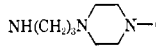
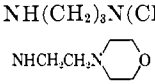
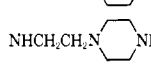
[a] Numbers in parentheses refer to sodium-deficient rat data. See footnote *a*, Table I, for rating scale.

replacement of the phenyl by other groups also decreases activity. The good activity of the 2-dimethylamino compound in the saline-loaded rat was not shown in the sodium-deficient rat. Variations of II in which the 4-amino group was replaced by 4-methylamino, 4-dimethylamino, or 4-anilino were inactive in the

TABLE IV

4,7-DIAMINO-2-PHENYLPTERIDINE-6-CARBOXAMIDES

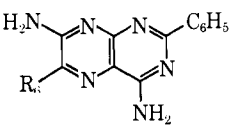


NR_1R_2	Diuretic act., rat[a] Saline-loaded	Sodium-deficient
NH_2 (II)	3	3
$NHCH_3$	2	3
$N(CH_3)_2$	1	1
NHC_2H_5	2	1
$NHCH(CH_3)_2$	1	0
NHC_6H_5	0	...
$NHCH_2CH_2OH$	0	...
$NHCH_2CH_2NH_2$	0	1
$NHCH_2CH(CH_3)NH_2$	0	...
$NHCH_2C(CH_3)_2NH_2$	0	...
$NHCH_2CH_2N(CH_3)_2$	1	3
$NHCH_2CH_2N(C_2H_5)_2$	2	3
	2	0
	0	...
$NH(CH_2)_3N(CH_3)_2$	0	...
	1	...
	1	...
$NHCH_2CHOHCH_2N(C_2H_5)_2$	1	0

[a] See footnote *a*, Table I, for rating scale.

TABLE V

4,7-DIAMINO-2-PHENYLPTERIDINE-6-CARBOXYLIC ACID DERIVATIVES

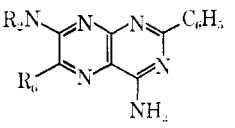


R_6	Diuretic act., rat[a] Saline-loaded	Sodium-deficient
COOH	0	...
$COOCH_3$	1	...
$CONHNH_2$[b]	1	0
$CONHNHCH_3$[b] / $CON(CH_3)NH_2$ mixture	1	1
$CONHN(CH_3)_2$[b]	2	0
$CONHNHCH(CH_3)_2$[d]	1	0
$CONHNHCH(CH_3)CH_2C_6H_5$[d]	1	...
$CONHNHCH(CH_3)CH_2CH(CH_3)_2$[d]	1	...
$CONHNHCH_2C_6H_5$[d]	0	...
$CONHN{=}C(CH_3)_2$[d]	1	1
$CONHN{=}C(CH_3)CH_2CH(CH_3)_2$	1	...
$CONHN{=}CHC_6H_5$[d]	1	...
$CON(CH_3)N{=}CHC_6H_5$[d]	2	1
CN[b]	2	...
$C(=NH)NHNH_2$[b]	2	1
$C(=NH)NHCH_3$[c]	2	0
$C(=NH)NHOH$[e]	1	2

[a] See footnote *a*, Table I, for rating scale. [b] E. C. Taylor and J. Weinstock, U. S. Patent 2,963,480 (Dec 6, 1960). [c] E. C. Taylor, U. S. Patent 2,963,479 (Dec 6, 1960). [d] J. Weinstock, U. S. Patent 3,111,520 (Nov 19, 1963). [e] E. C. Taylor, U. S. Patent 3,012,034 (Dec 5, 1961).

TABLE VI

4,7-DIAMINO-2-PHENYLPTERIDINES

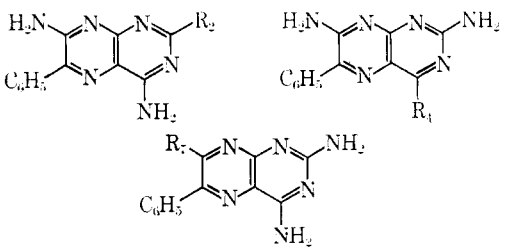


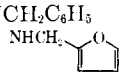
R_6	Diuretic act.[a] Saline-loaded rat
H	1
CH_3	2 (1)
CH_2CH_3	0
	0
$CH_2C_6H_5$	0
C_6H_5	1
COC_6H_5	0
$C(CH_3)=NNHC_6H_5$	0
$CHOHCH_3$	1

[a] Number in parentheses refers to sodium-deficient rat data. See footnote *a*, Table I, for rating scale.

TABLE VII

DIAMINO-6-PHENYLPTERIDINES

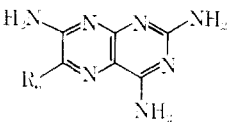


R	Diuretic act., saline-loaded rat[a] R_2	R_4	R_7
NH_2 (III)	3 (3)	3 (3)	3 (3)
$NHCH_3$	2 (2)	1 (1)	2 (3)
$N(CH_3)_2$		1 (2)	1
$NHCH(CH_3)_2$	3 (2)	2 (3)	
$NH(CH_2)_5CH_3$	0		0
$NHCH_2C_6H_5$	0		0
			1
NHC_6H_5	0	0	
	0	1	1
	0		
	0	0	
$NHCH_2CH_2OH$			0
$NHCH_2CH_2OCH_3$			1
$NH(CH_2)_2N(C_2H_5)_2$		1	
$NHNH_2$			1
OH	0	0 (0)	0
OCH_3		0	0
OC_4H_9		1	
$O(CH_2)_2N(CH_3)_2$		0	
SH			0
SCH_3	0	0	
Cl			0
H	3 (2)		1 (1)
CH_2CN	0		
CH_2CONH_2	0		

[a] Numbers in parentheses refer to sodium-deficient rat data. See footnote *a*, Table I, for rating scale.

TABLE VIII

2,4,7-TRIAMINO-6-ARYLPTERIDINES

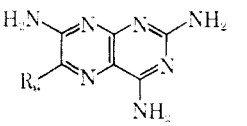


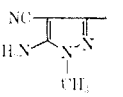
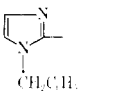
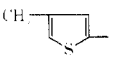
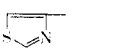
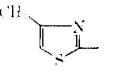
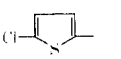
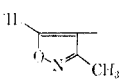
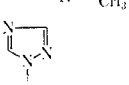
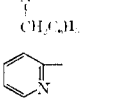
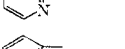
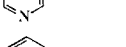
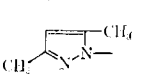
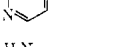
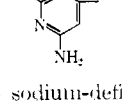
R_6	Diuretic act.[a] Saline-loaded rat	R_6	Diuretic act.[a] Saline-loaded rat
C_6H_5 (III)	3 (3)	4-$CH_3OC_6H_4$	1
2-$CH_3C_6H_4$	2 (1)	$C_6H_5CH_2OC_6H_4$	0
3-$CH_3C_6H_4$	1 (1)	4-HOC_6H_4	0
4-$CH_3C_6H_4$	2 (1)	4-$CH_3CONHC_6H_4$	0
2-FC_6H_4	2	4-$H_2NC_6H_4$	0
4-FC_6H_4	1	4-$O_2NC_6H_4$	0
2-BrC_6H_4	1 (3)	4-$C_6H_5C_6H_4$	0
2-$CH_3OC_6H_4$	3	2-$C_6H_5C_6H_4$	0
		β-$C_{10}H_7$	0

[a] Numbers in parentheses refer to sodium-deficient rat data. See footnote *a*, Table I, for rating scale.

TABLE IX

2,4,7-TRIAMINO-6-HETEROARYLPTERIDINES

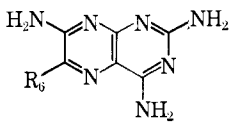


R_6	Diuretic act.,[a] Saline-loaded rat	R_6	Diuretic act.[a] Saline-loaded rat
	0 (1)		0
	1 (2)		
	2 (2)		0
	1 (1)		0
	1 (2)		0
	0		0
	0		0
	1 (2)		0
	2 (3)		0
	0		0
	2 (0)		0
			0

[a] Numbers in parentheses refer to sodium-deficient rat data. See footnote *a*, Table I, for rating scale.

TABLE X

2,4,7-TRIAMINO-6-SUBSTITUTED PTERIDINES

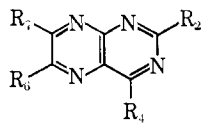


R_6	Diuretic act.[a] Saline-loaded rat
H	2 (3)
CH_3	3 (2)
C_2H_5	3 (2)
$CH(CH_3)_2$	1
$(CH_2)_2CH_3$[b]	2 (2)
$(CH_2)_3CH_3$[c]	2 (2)
	1
$CH_2C_6H_5$	2 (2)
$CH_2CH_2C_6H_5$	1
COC_6H_5	1
COOH	0
$CONH_2$	0 (0)
$CONH(CH_2)_3N(CH_3)_2$	0

[a] Numbers in parentheses refer to sodium-deficient rat data. See footnote *a*, Table I, for rating scale. [b] Prepared by Mrs. R. Y. Dunoff by method of ref 1b. *Anal.* ($C_9H_{13}N_7$) C, H, N.[d] [c] Prepared by Mrs. R. Y. Dunoff by the method of ref 1b. *Anal.* ($C_{10}H_{15}N_7 \cdot {}^1/_8H_2O$) C, H, N.[d] [d] Analytical results were within ±0.4% of the calculated values for the elements indicated by symbols.

TABLE XI

SIMPLE AMINOPTERIDINES



R_2	R_4	R_6	R_7	Diuretic act.[a] Saline-loaded rat
NH_2	H	H	H[b]	3 (2)
$NHCH_3$	H	H	H[c]	2
H	NH_4	H	H[b]	2
H	$N(CH_3)_2$	H	H[b]	1
NH_2	NH_2	H	H[d]	3 (0)
H	NH_2	NH_2	H[e]	2
H	NH_2	H	NH_2[f]	3
NH_2	NH_2	H	NH_2[f]	2 (3)
H	NH_2	NH_2	NH_2[c]	0
NH_2	NH_2	NH_2	NH_2[g]	1 (2)
$N(CH_3)_2$	$N(CH_3)_2$	$N(CH_3)_2$	$N(CH_3)_2$[h]	1

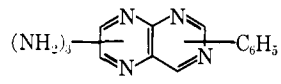
[a] Numbers in parentheses refer to sodium-deficient rat data. See footnote *a*, Table I, for rating scheme. [b] Footnote *i*, Table II. [c] A. Albert, D. J. Brown, and G. Cheeseman, *J. Chem. Soc.*, 4219 (1952). [d] Footnote *b*, Table I. [e] A. Albert, J. H. Lister, and C. Pedersen, *J. Chem. Soc.*, 4621 (1956). [f] T. S. Osdene and G. M. Timmis, *ibid.*, 2036 (1955). [g] E. C. Taylor and W. R. Sherman, *J. Am. Chem. Soc.*, **81**, 2464 (1959). [h] Gift of Professor E. C. Taylor.

saline-loaded rat. Table IV shows the effects of substituents on the carboxamide group of II. Bulky alkyl groups decrease activity, but, as also found by Osdene, *et al.*,[6] certain aminoalkyl groups lead to retention of activity in the rat and dog tests. However, like II, the active natriuretic compounds in the

(6) T. S. Osdene, A. A. Santilli, L. E. McCardle, and M. E. Rosenthale, *J. Med. Chem.*, **10**, 165 (1967).

TABLE XII

TRIAMTERENE PTERIDINE ISOMERS

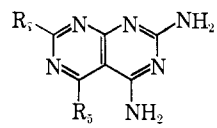


Isomer	Diuretic act., rat[a] Saline-loaded	Sodium-deficient
6-C_6H_5 (III)	3	3
7-C_6H_5	1	3
2-C_6H_5	2	2
4-C_6H_5	1	0

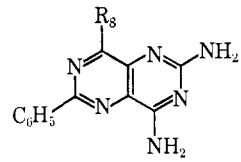
[a] See footnote *a*, Table I, for rating scheme.

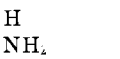
TABLE XIII

PYRIMIDOPYRIMIDINES



R_5	R_7	Diuretic act.[a] Saline-loaded rat
C_6H_5	NH_2	3 (3)
H	NH_2[b]	0
CH_3	NH_2	0
C_2H_5	NH_2	2
NH_2	C_6H_5	0
C_6H_5	H	3
C_6H_5	C_6H_5	0
H	C_6H_5[b]	3
H	CH_3	0
HO	C_6H_5	0



R_3	Diuretic act.[a] Saline-loaded rat
NH_2	0
	0

[a] Number in parentheses refers to sodium-deficient rat. See footnote *a*, Table I, for rating scale. [b] E. C. Taylor, R. J. Knopf, R. F. Meyer, A. Holmes, and M. L. Hoefle, *J. Am. Chem. Soc.*, **82**, 5711 (1960).

TABLE XIV

COMPARISON OF DIURETIC AGENTS IN VARIOUS DIURETIC ASSAYS

Test	Agent: Triamterene	II	I	Chlorothiazide	Spironolactone
Saline-loaded rat[a]	3	3	3	2	0
Sodium-deficient rat[a]	3	3	3	1	1[d]
Glucose-infused dog[b]					
Na^+ (control/drug)[c]	13/13	9/6	6/69[e]	3/57	
K^+ (control/drug)[c]	33/3	7/3	4/16[e]	7/37	
Saline-9αFHC dog[b]					
Na^+ (control/drug)[c]	59/475	10/481	9/221	48/475	7/13
K^+ (control/drug)[c]	21/27	16/70	20/18	15/106	8/9

[a] See footnote *a*, Table I, for rating scale. [b] 25 mg/kg *po*. [c] Average μequiv/min in urine. [d] 10 mg/kg sc. [e] 25 mg/kg iv.

aminoalkylcarboxamides are also active as kaliuretics. Two carbons between the nitrogens appear to be optimal. The higher activity of the dimethylamino and diethylamino compounds in comparison to the primary amino and morpholino compounds is note-

worthy. The dimethylaminoethyl compound is unusual in that relatively speaking it is more active in the sodium-deficient rat than in the saline-loaded rat. Because of the extensive interest in the morpholinoethyl compound,[7] it is interesting to compare its activity to that of the closely related diethylaminoethyl analog in the sodium-deficient rat. The former caused the excretion of 2.92 mg of Na^+ and 10.46 mg of K^+ while the latter caused the excretion of 10.26 mg of Na^+ and 7.91 mg of K^+. Clearly, the diethylaminoethyl compound is not only more strongly natriuretic but also exhibits a higher Na^+/K^+ ratio in the presence of a high level of mineralocorticoids. However, both compounds are kaliuretic.

Table V shows the diuretic activity of some other carboxylic acid derivatives of II. A number of these, such as the hydrazide, the isopropylidene derivative of the hydrazide, and some of the β-alkyl hydrazides have much more activity in the dog than would be predicted from the rat data. At higher doses some of these compounds are natriuretic even in the glucose-infused dog. This renal clearance protocol is not suitable for demonstrating activity for most of the pteridine diuretics we have studied. In Table VI are a series of 6-alkyl and 6-aryl analogs of II. The diuretic activity of these compounds is modest.

Consideration of the structures of I and II suggested that muliple amine groups and an aromatic ring on a pteridine were consistent with good diuretic activity. This led us to prepare a sample of 2,4,7-triamino-6-phenylpteridine (III, triamterene) for diuretic testing. This compound had been originally prepared for antibacterial testing,[8] but had shown no interesting activity. In the diuretic assays it was an outstanding compound. It is very potent in the saline-loaded rat, and in the sodium-deficient rat not only causes a marked excretion of sodium, but simultaneously decreases K^+ excretion. This effect was also seen in dogs and man.[9] In combination with thiazides such as hydrochlorothiazide, it not only blocks the K^+ loss due to the thiazide but also potentiates the Na^+ excretion.[9]

In one approach to studying the structure–activity relationships of compounds related to triamterene, we prepared compounds which differed from the prototype by only one structural feature. In Table VII are listed those compounds in which one of the primary amino groups has been replaced by another group. Replacement of any of the amines by a lower alkylamine led to compounds which retain triamterene-like diuretic activity. More extensive changes generally led to substantially less active compounds. In Table VIII is shown the diuretic activity of compounds in which the phenyl of triamterene has been replaced by a substituted phenyl. Here again, only small changes are permissible if even modest diuretic activity is to be retained. The phenyl and *p*-tolyl compounds are illustrative. The *p*-tolyl compound is only approximately one-half as active as triamterene. In general, *ortho* isomers seem to be more active than the other isomers. The *p*-hydroxy analog[10] of triamterene, which has been reported to be one of its metabolites,[10] is essentially inactive. In an attempt to increase the activity of triamterene by reducing its rate of metabolism, the 4-deuterio analog was prepared. In several diuretic assays its activity was very similar to that of triamterene.

In Table IX is shown the diuretic activity of compounds in which the phenyl group of triamterene has been replaced by a heterocyclic nucleus. In this series the size of the group again appears to be important. High activity is seen only in the case of small, nonbasic groups. The low activity of compounds containing basic centers in this position such as thiazole and pyridine may be rationalized by assuming that the basic centers are highly solvated, and are in effect large substituents. In Table X is listed the diuretic activity of some triamterene analogs in which the 6-phenyl has been replaced by nonaromatic residues. The 6-alkyl analogs are active diuretics and good activity is seen even in the 6-*n*-butyl analog. However, size is important because the isopropyl and cyclohexenyl analogs have only modest activity. The benzyl analog is surprisingly active; in fact, in the sodium-deficient rat it is more active than the isomeric *p*-tolyl analog. This and the activity of the 6-alkyl analogs suggest that the phenyl group in triamterene does not enhance diuretic activity because of electronic interactions with the pteridine ring. Nmr data suggested that in trifluoroacetic acid solution the phenyl and pteridine rings are not co-planar.[1f] Possibly this is also true at the site of biological activity.

The good activity shown by 2,4,7-triaminopteridine and by 2,4-diaminopteridine (Table I) suggested that other simple amino pteridines be studied for diuretic activity. These data are shown in Table XI. This is a surprisingly active series of diuretics. However, some members of this series are also exceedingly toxic. 2-Aminopteridine, for example, after a single oral dose of 5 mg/kg to a dog caused a reduction in glomerular filtration rate from 45 ml/min to 3.2 ml/min. One month later the filtration rate had recovered to only 50% of its normal value.

Since triamterene is a 6-phenylpteridine and II is a 2-phenylpteridine, and both are active diuretic agents, it seemed important to study the isomers of triamterene. The diuretic activity of these compounds is shown in Table XII. The 7-phenyl isomer is interesting because it is one of the most potent K^+ blockers found in the pteridines even though it is only a weak natriuretic agent. In the sodium-deficient rat at 15 and 30 mg/kg it caused the excretion of 9.98 and 12.67 mg of Na^+ with Na^+/K^+ ratios of 12.21 and 20.39. In the saline-9αFHC dog at 25 mg/kg orally in four clearance periods it increased the sodium excretion by an average of 105 μequiv/min while the Na^+/K^+ ratios for these periods were 52.5, 175, 205, and 240. In all of these periods the urinary concentration of K^+ (1.46, 0.34, 0.37, and 0.65 mequiv/l.) was well below that of the average plasma concentration of K^+ (3.67 mequiv/l.). This appears to be the first example of net K^+ reabsorption demonstrated by the clearance technique.

The 2-phenyl isomer of triamterene is very similar in its biological properties of II. It was both natriuretic and kaliuretic in the saline-9αFHC dog. The

(7) M. E. Rosenthale and C. G. Van Arman, *J. Pharmacol. Exptl. Therap.*, **142**, 111 (1963).
(8) R. G. W. Spickett and G. M. Timmis, *J. Chem. Soc.*, 2887 (1954).
(9) A. P. Crosley, Jr., L. M. Ronquillo, W. H. Stickland, and F. Alexander, *Ann. Internal Med.*, **56**, 241 (1962).
(10) K. Lehmann, *Arzneimittel-Forsch.*, **15**, 812 (1965).

4-phenyl isomer had only weak activity in the saline-loaded rat and was not significantly active in the sodium-deficient rat.

Another type of isomerism possible is that involving the pteridine ring system itself. Previously[11] diuretic activity had been reported for some 2,5-diamino- and 2,4,7-triaminopyrimido[4,5-*d*]pyrimidines. In Table XIII is shown the diuretic activity for some pyrimidopyrimidines more closely related to triamterene. Some of these compounds show interesting activity in the saline-loaded rat and the sodium-deficient rat. 2,4,7-Triamino-6-phenylpyrimido[4,5-*d*]pyrimidine was investigated in some detail. Like II it does not block K^+ excretion in the sodium-deficient rat or the saline-9αFHC dog. Some of the considerable natriuretic activity in the dog may be due to the elevation of glomerular filtration rate characteristic of this compound.

As part of our investigation of diuretic activity of pteridines we investigated many compounds not directly related to the three prototypes mentioned above. Most of these were inactive. Among the compounds inactive in the saline-loaded rat were biopterin, folic acid, pterin, xanthopterin, 6-methylisoxanthopterin, leucopterin, aminopterin, and riboflavine.

The pteridines that have been discussed as interesting diuretic agents fall into three general classes: the 2,4-diamino-6,7-dialkylpteridine type related to I, the 4,7-diamino-2-phenylpteridine-6-carboxamide type related to II, and the triamterene type. Among the analogs of I the greatest activity is found in 2,4-diaminopteridines substituted on 6 and 7 with either hydrogen or a lower alkyl group. Of the analogs of II, greatest activity is found in 2-phenyl-4,7-diaminopteridines substituted on position 6 with either a carboxamide or a basic carboxamide-type function such as a hydrazide, an amidrazone, or an aminoalkylcarboxamide. Among the triamterene analogs greatest saluretic activity is found in 2,4,7-triaminopteridines substituted on position 6 with a small aryl, heteroaryl, or alkyl group. Alkylation of the amino groups with lower alkyl groups also gives highly active compounds. Some variation in the relative positions of the groups is permissible as demonstrated by the activity of the isomers.

It is possible to rationalize these data by assuming that the pteridines bind to some active site at two points. The more important site involves a basic center of the drug which in triamterene may be N-1 or N-8 or both. Groups which decrease the base strength of the pteridine nucleus appear to destroy good activity. The other site apparently involves the phenyl of triamterene. This binding may be hydrophobic in nature. There appear to be severe size limitations at this site as evidenced by the sensitivity of the activity to even methyl substitution. Since compounds such as 2,4,7-triaminopteridine are active, the phenyl apparently acts to reinforce activity rather than to be an absolute requirement. The phenyl may either aid to orient the molecule properly on the site and/or increase the degree of binding to the site. Too large a group either at 6 or elsewhere may interfere with the proper fit of the drug to the site.

In order to place the pteridines properly among the diuretics, in Table XIV is shown a comparison of the three pteridine prototypes in several diuretic assays with chlorothiazide and spironolactone (IV), a typical peripheral aldosterone antagonist.[12] The pteridines are highly active in both the saline-loaded and sodium-deficient rat assays in contrast to IV and chlorothiazide. In the glucose-infused dog only chlorothiazide is consistently active. The thiazides are much more active intravenously than orally, but in general, even intravenously, the pteridines are not active in the glucose dog. In the saline-9αFHC dog the pteridines and chlorothiazide cause Na^+ excretion; however, only triamterene accomplishes this with little change in K^+ excretion.

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