3, 0.4 ml of pyridine, and 20 ml of CH_2Cl_2. The mixture was stirred for 2.5 hr and evaporated to a solid, which was recrystallized from EtOH to give the desired product.

Compound **20** in Table II was prepared similarly.

Acknowledgment.—The microanalyses were performed by Mr. L. M. Brancone and his associates. The spectral data were obtained from Mr. W. Fulmor and his associates.

Pteridines. XI.[1,2] Pteridines Related to the Diuretic, 2,4-Diamino-6,7-dimethylpteridine

JOSEPH WEINSTOCK, ROBERTA Y. DUNOFF, JOYCE E. CAREVIC, JUNE G. WILLIAMS, AND ANTHONY J. VILLANI

Research and Development Division, Smith Kline and French Laboratories, Philadelphia, Pennsylvania 19101

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Significant diuretic activity of a pteridine was first observed in our laboratory with 2,4-diamino-6,7-dimethylpteridine. In this note we will describe some work carried out in order to explore the structure–activity relationships of compounds related to this lead.

The Isay reaction,[3] which is the condensation of a 4,5-diaminopyrimidine with a 1,2-dicarbonyl compound to form a pteridine, was used to prepare the compounds in Table I. When unsymmetrical dicarbonyl compounds are used it is often possible to direct the course of the synthesis by altering the pH of the reaction medium. The 5-amino group usually is the more reactive of the amino groups and it will react with the most reactive carbonyl group. However, at low pH it also protonates first, and thus allows the less basic 4-amino group to react with the most reactive carbonyl group.[4] Reaction of 4,5,6-triamino-2-phenylpyrimidine and pyruvaldehyde in an acetic acid–potassium acetate buffer gave 4-amino-7-methyl-2-phenylpteridine.[5] Attempts to obtain the 6-methyl isomer by the use of mineral acid failed to alter the course of the reaction. However, the 6-methyl isomer was obtained by allowing pyruvaldehyde to react with 2 moles of hydrazine before addition of the pyrimidine.[6]

In order to extend the Isay reaction to the preparation of a 6,7,8-trialkylpteridine, 2,5-diamino-4,6-bismethylaminopyrimidine was treated with 2,3-butanedione. This gave 2-amino-4-methylamino-6,7,8-trimethylpteridinium chloride (I), the cation of which can be represented by several resonance forms.

TABLE I

PTERIDINES PREPARED BY THE ISAY REACTION

Pteridine	Dicarbonyl reactant	Reaction conditions: Solvent	Time, hr	Temp, °C	Yield, %	Recrystn solvent	Mp, °C	*Rf* (system)	Formula[a]
4-Amino-2-phenyl	Glyoxal bisulfite	H_2O	1	100	45	EtOH	239	0.89 (4)	$C_{12}H_9N_5$
4-Amino-6,7-dimethyl-2-phenyl	Biacetyl	H_2O, pH 5.5	0.5	80	52	DMF–H_2O	308–310	0.71 (5)	$C_{14}H_{13}N_5$
4-Amino-2,6,7-triphenyl	Benzil	EtOH	2	80	70	DMF–H_2O	250–251		$C_{24}H_{17}N_5$
4-Amino-2,6,7-trimethyl	Biacetyl	H_2O, pH 5	1	60	52	H_2O	249–250	0.83 (6)	$C_9H_{11}N_5$[b]
4-Amino-2-phenylcyclopenta[*g*]	1,2-Cyclopentanedione	EtOH	1	80		*c*	295 dec	0.82 (3)	$C_{15}H_{13}N_5$
4-Amino-2-anilino-6,7-dimethyl	Biacetyl	EtOH–H_2O, pH 5.5	0.75	25	54	MeOH	258–258.5	0.89 (4)	$C_{14}H_{14}N_6$
2-Amino-4,6,7-trimethyl	Biacetyl	H_2O, pH 5.5	0.33	100	56	EtOH	>300	*a*	$C_9H_{11}N_5$
2-Amino-4-carbamyl-6,7-dimethyl	Biacetyl	EtOH–H_2O, pH 5.5	3	25		*c*	>330		$C_9H_{10}N_6O$[d]
2,4-Diamino-6(7)-ethyl-7(6)-methyl[d]	2,3-Pentanedione	EtOH–H_2O	0.2	100	67	Dil HCl	>300	0.63 (1)	$C_9H_{12}N_6 \cdot HCl \cdot 0.5H_2O$
			1	25					
2,4-Diamino-cyclopenta[*g*][b]	1,2-Cyclopentanedione	EtOH–H_2O	0.7	100	35	H_2O, pH 2.5	>250		$C_9H_{10}N_6 \cdot HCl \cdot H_2O$
4-Amino-7-hydroxy-6-methyl-2-phenyl	Methyl pyruvate	EtOH	1	100	47	DMF–H_2O	282–284	0.64 (2)	$C_{13}H_{11}N_5O$

[a] $\lambda_{max}^{1\%\ NaOH}$ 364 mμ (log ε 4.08), 364 (sh) (4.00). [b] Hydrochloride hydrate. [c] Dissolve in dilute HCl, precipitate with NH_4OH. [d] Hydrochloride hemihydrate. [e] All compounds were analyzed for C, H, N except as noted. [f] C, H, analysis only. [g] N: calcd, 37.02; found, 37.69.

(1) Previous paper in this series: H. Graboyes, G. E. Jaffe, I. J. Pachter, J. P. Rosenbloom, A. J. Villani, J. W. Wilson, and J. Weinstock, *J. Med. Chem.*, **11**, 568 (1968).

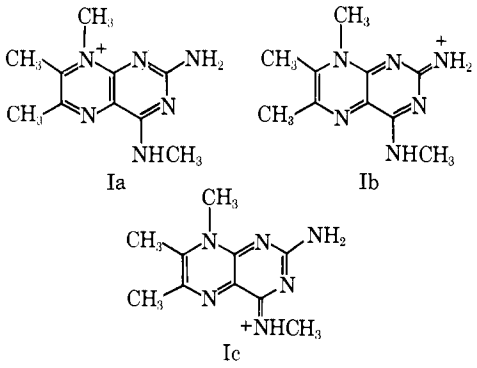
(2) A portion of this work was reported at the 3rd International Pteridine Symposium, Stuttgart, Germany, 1962. See J. Weinstock and V. D. Wiebelhaus in "Pteridine Chemistry," W. Pfleiderer and E. C. Taylor, Ed., Pergamon Press, Oxford, 1964, p 37.

(3) See A. Albert, *Quart. Rev.* (London), **6**, 197 (1952), and W. Pfleiderer, *Angew. Chem. Intern. Ed. Engl.*, **3**, 114 (1964), for brief reviews of this reaction.

(4) (a) G. B. Elion, G. H. Hitchings, and P. B. Russell, *J. Am. Chem. Soc.*, **72**, 78 (1950); (b) W. Pfleiderer and R. Lohrmann, *Ber.*, **94**, 2708 (1961).

(5) I. J. Pachter, P. E. Nemeth, and A. J. Villani, *J. Org. Chem.*, **28**, 1197 (1963).

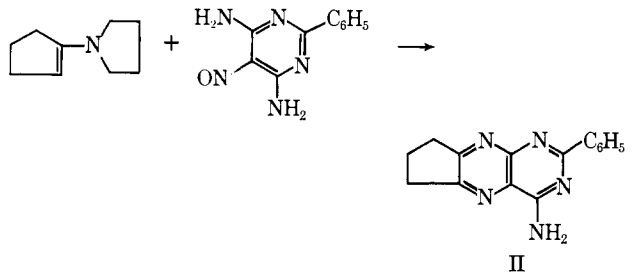
(6) H. S. Forrest and J. Walker, *J. Chem. Soc.*, 2077 (1949).



Ia Ib

Ic

In an attempt to find another way of preparing 6,7-dialkylpteridines, we investigated the ability of an enamine to condense with a 4-amino-5-nitrosopyrimidine. Warming 1-pyrrolidinocyclopentene with 4,6-diamino-5-nitroso-2-phenylpyrimidine in methanol gave II whose structure was established by its synthesis *via* the Isay reaction. The first step of this reaction



II

probably involves the addition of the electron-rich β-carbon of the enamine to the nitroso group of the pyrimidine.

In order to obtain 2,4-diaminopteridine-6-carboxylic acid for conversion to the corresponding amide, we reinvestigated the oxidation of the corresponding 6-methylpteridine.[7a] Oxidation of 2,4-diamino-6-methylpteridine and 4-amino-6-methyl-2-phenylpteridine with permanganate at pH 8 by the procedure of Dion and Loo[7b] gave the corresponding 6-pteridinecarboxylic acids (Table II). However, the corresponding 7-methyl isomers under these conditions did not undergo oxidation; in 1 *N* NaOH oxidation did occur, but the 4-amino groups were hydrolyzed to hydroxy groups under these conditions. The hydrolysis is not surprising in view of the reported[7c] hydrolysis of 2,4-diamino-7-pteridinecarboxylic acid to the corresponding 4-hydroxy analog under these conditions. The more difficult oxidation of the 7-methyl group is somewhat surprising in view of the reported[8] greater instability of the 7 isomer of folic acid in comparison to folic acid. 2,4-Diaminopteridine-6-carboxylic acid was converted to the methyl ester by refluxing methanolic HCl. Some higher homologs were similarly prepared when the methyl ester displayed diuretic activity. Treatment of the methyl ester with ammonia in ethylene glycol gave 2,4-diaminopteridine-6-carboxamide.

(7) (a) C. K. Cain, M. F. Mallette, and E. C. Taylor, *J. Am. Chem. Soc.*, **70**, 3026 (1948); (b) R. L. Dion and T. L. Loo, *J. Org. Chem.*, **26**, 1857 (1961); (c) D. J. Brown and B. T. England, *J. Chem. Soc.*, 1530 (1965).

(8) C. W. Waller, M. J. Fahrenbach, J. H. Booth, R. B. Angier, B. L. Hutchings, J. H. Mowat, J. F. Poletto, and J. Semb, *J. Am. Chem. Soc.*, **74**, 5405 (1952).

Pharmacology.—The diuretic structure–activity relationships of some of the compounds described in this paper are reported in an accompanying paper.[9]

Experimental Section[10]

Melting points are uncorrected and were determined in open capillary tubes. Uv spectra were determined on a Cary Model 14 spectrometer and nmr spectra on a Varian A-60 spectrometer. Paper chromatography was done by the circular system on 3 MM Whatman paper. The systems used were (1) *t*-AmOH–H_2O–HCOOH (5:5:1); (2) *n*-BuOH–6 *N* NH_4OH (1:1); (3) *n*-BuOH–HCOOH–H_2O (5:1:5); (4) *n*-BuOH–H_2O–AcOH (4:5:1); (5) mineral oil pretreated paper, EtOH–H_2O (3:1); (6) C_6H_6–$CHCl_3$–80% EtOH (1:1:2); (7) AcOH–H_2O–*i*-AmOH–*t*-AmOH (1:5:3:3); (8) pretreat paper with castor oil–mineral oil (1:1), EtOH–H_2O (2:1).

4-Amino-6-methyl-2-phenylpteridine.—A solution of 30% aqueous pyruvaldehyde (26.4 ml, 0.11 mole) and 85% hydrazine hydrate (12 g, 0.25 mole) in EtOH (150 ml) was allowed to stand at 25° for 30 min. Then 4,5,6-triamino-2-phenylpyrimidine (20.1 g, 0.10 mole) and glacial AcOH (15 ml) were added and the mixture was refluxed for 5 min, diluted with 50 ml of H_2O, and heated 10 min on a steam bath. Chilling and filtration gave a solid which on recrystallization from DMF–H_2O yielded 11.3 g (48%) of product: mp 240–241°; R_f 0.74 (system 8); $\lambda_{max}^{1\ N\ NaOH}$ 270 mμ (log ε 4.42), 346 (3.90); $\lambda_{max}^{4.5\%\ HCOOH}$ 273 mμ (log ε 4.30), 292 (sh) (4.09), 324 (sh) (4.08), 337 (4.11), 348 (sh) (4.03); nmr peaks (CF_3COOH) at δ 2.82 (s) (6-CH_3), 7.42 and 7.90 (m) (2-C_6H_5), 8.31 (br s) (NH^+), and 8.59 (s) (7-H). *Anal.* ($C_{13}H_{11}N_5$) C, H.

2-Imino-4-methylamino-6,7,8-trimethyl-2,8-dihydropteridine Hydrochloride.—A solution of 2,5-diamino-4,6-bismethylaminopyrimidine hydrochloride[11] (2.04 g, 0.01 mole) and biacetyl (1.0 g, 0.012 mole) in EtOH (100 ml) was refluxed for 1.5 hr and evaporated to dryness under vacuum, and the residue was recrystallized from BuOH to give 0.8 g (29%) of crystals; mp 228–230°; R_f 0.55 (system 3); $\lambda_{max}^{0.1\ N\ NaOH}$ 372, 310, and 230 mμ (log ε 3.93, 4.24, 4.42); $\lambda_{max}^{0.1\ N\ HCl}$ 410, 329 (sh), 275, and 236 mμ (log ε 4.17, 4.08, 4.15, 4.03); nmr peaks (CF_3COOH) at δ 2.72 (6-CH_3), 2.87 (7-CH_3), 3.38 (4-CH_3NH), and 4.12 (8-CH_3), all singlets.[12] *Anal.* ($C_{10}H_{14}N_6 \cdot HCl \cdot H_2O$) C, H, N.

2,4-Diaminopteridine-6-carboxylic Acid.—A suspension of 2,4-diamino-6-methylpteridine (5.5 g, 0.031 mole) in 500 ml of 0.05 *N* NaOH was treated with dilute HCl to bring the pH to 8. $KMnO_4$ (9.8 g) was added and the reaction mixture was stirred on a steam bath for 4 hr. An additional 9.0 g of $KMnO_4$ was added and the mixture was stirred and heated for 2 hr. The excess MnO_4^- was destroyed by addition of Na_2SO_3 and the MnO_2 was removed by filtration. The filter cake was extracted twice with 125 ml of 0.05 *N* NaOH. The combined extracts and mother liquor were brought to pH 2 with dilute HCl and the resulting solid was collected, dissolved in 700 ml of 0.1 *N* NaOH, and reprecipitated at pH 2 with dilute HCl. This gave 3.0 g (43%) of product, mp >300°.

Methyl 2,4-Diaminopteridine-6-carboxylate.—Dry HCl was passed into a stirred suspension of 2,4-diaminopteridine-6-carboxylic acid (15.6 g, 0.076 mole) in MeOH (400 ml) for 1.5 hr causing a mild reflux. The reaction mixture was stirred an additional 1.5 hr at room temperature and the precipitate which formed was collected, dissolved in H_2O, and reprecipitated by addition of concentrated NH_4OH to pH 8. This gave 7.3 g (44%) of product, mp >300°.

2,4-Diaminopteridine-6-carboxamide.—Anhydrous NH_3 was passed for 45 min into a stirred suspension of methyl 2,4-diaminopteridine-6-carboxylate (5.5 g, 0.025 mole) in ethylene glycol (550 ml) which was heated on a steam bath. Chilling and

(9) J. Weinstock, J. W. Wilson, V. D. Wiebelhaus, A. R. Maass, F. T. Brennan, and G. Sosnowski, *J. Med. Chem.*, **11**, 573 (1968), paper XII of this series.

(10) We wish to thank Mrs. Doris Rolston and Miss Margaret Carroll and their staff for microanalytical data, Dr. Walter Thompson and Mr. Richard J. Warren for spectral data, and Mr. Irving Eisdorfer, Mr. Alex Post, and Mr. E. L. Haines for chromatographic data. Where analyses are indicated only by symbols of elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

(11) G. B. Elion and G. H. Hitchings, *J. Am. Chem. Soc.*, **75**, 4311 (1953).

(12) For comparison, 2,4-diamino-6,7-dimethylpteridine has nmr peaks (CF_3COOH) at δ 2.67 and 2.71, both singlets, from the 6- and 7-CH_3.

TABLE II
PTERIDINECARBOXYLIC ACIDS AND DERIVATIVES

Pteridine	Method of prepn	Yield, %	Recrystn solvent	Mp, °C	R_f (system)	Formula[c]	Ultraviolet spectral data: Solvent[d]	Ultraviolet spectral data: λ_{max}, mμ (log ε)
2,4-Diamino- 6-carboxylic acid	*a*	43	*b*	>300	0.21 (7)	$C_7H_6N_6O_2 \cdot 1.33H_2O$	1	257 (4.23), 336 (4.05)
							2	266 (4.35), 370 (3.95)
4-Amino-2-phenyl- 6-carboxylic acid	*a*	8	DMF–H_2O	284–286	0.75 (4)	$C_{13}H_9N_3O_2$	3	278 (4.28), 352 (sh)
							2	278 (4.40), 355 (4.00)
4-Hydroxy-2-phenyl- 7-carboxylic acid	*a*	51	DMF–H_2O		0.58 (4)	$C_{13}H_8N_4O_3$	1	286 (4.19)
							4	271 (4.42), 353 (3.95)
Methyl 2,4-diamino- 6-carboxylate	*c*	44	*f*	>300	0.44 (3)	$C_8H_8N_6O_2$	1	239 (sh), 258 (4.21), 333 (3.98)
							2	218 (4.51), 267 (4.25), 372 (3.86)
Ethyl 2,4-diamino- 6-carboxylate·0.5H_2O	*c*	60	DMF–H_2O	298–301	0.70 (4)	$C_9H_{10}N_6O_2 \cdot 0.5H_2O$	1	242 (sh), 257 (4.30), 337 (4.07)
							4	267 (4.34), 371 (3.94)
Isopropyl 2,4-diamino- 6-carboxylate·0.5H_2O	*c*	40	DMF–H_2O	293–294	0.82 (4)	$C_{10}H_{12}N_6O_2 \cdot 0.5H_2O$	1	254 (4.31), 337 (4.08)
							4	267 (4.34), 371 (3.95)
2,4-Diamino- 6-carboxamide·0.75H_2O	*g*	60	*g*	>300	0.44 (4)	$C_7H_7N_7O \cdot 0.75H_2O$	1	256 (4.34), 338 (4.08)
							4	219 (4.30), 271 (4.32), 377 (3.97)

[a] Oxidation of corresponding methylpteridine. See preparation of 2,4-diaminopteridine-6-carboxylic acid for procedure. [b] Dissolve in 0.1 *N* NaOH and precipitate by adjusting to pH 2 with dilute HCl. [c] All compounds were analyzed for C, H, and N. [d] (1) 0.1 *N* HCl, (2) 0.1 *N* NaOH, (3) 4.5% HCOOH, (4) pH 8. [e] HCl-catalyzed esterification. See preparation of methyl 2,4-diaminopteridine-6-carboxylate for procedure. [f] Dissolve in water as HCl salt, precipitate with NH_4OH at pH 8. [g] See Experimental Section for procedure.

filtering gave a solid which was dissolved in 50% AcOH and reprecipitated at pH 8 with concentrated NH_4OH. This gave 3 g (60%) of product, mp >300°.

4-Amino-2-phenylcyclopenta[*g*]pteridine.—A solution of 4,6-diamino-5-nitroso-2-phenylpyrimidine (5.1 g, 0.024 mole) and 1-pyrrolidino-1-cyclopentene (3.42 g, 0.025 mole) in EtOH (100 ml) was refluxed for 1 hr. Chilling and filtration gave 1.0 g of crystals, mp 290°. This was dissolved in dilute HCl and treated with Darco, and the solution made basic with dilute NaOH to give 0.9 g (16%) of crystals, mp 295° dec, R_f 0.86 (system 3). The ir spectrum of this product was identical with that of the product obtained *via* the Isay reaction.

Antitumor and Antimicrobial Screening of Crosemperine, the Otonecine Ester Occurring in *Crotalaria semperflorens* Vent. II[1]

RAMESHWAR K. SHARMA[2]

School of Pharmacy, Department of Pharmaceutical Chemistry, The University of Mississippi, University, Mississippi 38677

AND PETER HEBBORN

School of Pharmacy, Department of Biochemical Pharmacology, State University of New York at Buffalo, Buffalo, New York 14214

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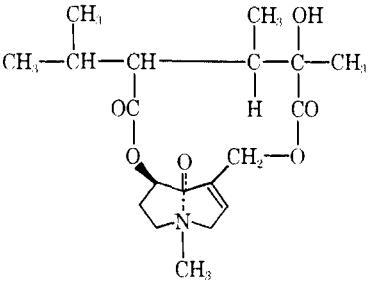
Crosemperine, an otonecine ester, has been recently isolated from *Crotalaria semperflorens*[3] and was shown to possess structure I. Due to reports of the mutagenic[4] and antitumor properties[5,6] of pyrrolizidine esters, *Crotalaria* alkaloids have assumed a significant importance. Since these alkaloids are macrocyclic diesters, such properties could be predicted hypothetically by the enzymatic splitting of the ester of the alkaloid. However, so far, no otonecine ester of this genus has been screened for its antitumor and antimicrobial activity.



I

Biological Results and Discussion.—Crosemperine was evaluated in the Ehrlich ascites tumor test system, 1×10^6 cells being injected intraperitoneally into male, random-bred Swiss mice (20–22 g); the compound was injected intraperitoneally 24 hr later as a single dose, or daily for 5 days. The packed-cell volume of ascites cells was determined on the tenth day.

The data in Table I indicate that the compound was not effective as a tumor-inhibitory agent on acute administration, at a toxic dose level (200 mg/kg). Subacute administration produced tumor inhibition which was of borderline significance also at a toxic dose level (25 mg/kg qd 1–5).

Although this compound has minimal antitumor activity, the LD_{50} value when given for 5 days is 32 mg/kg. This alkaloid is thus considerably more toxic than the related monocrotaline isolated by Kupchan,

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(2) To whom inquiries should be addressed at the Worcester Foundation for Experimental Biology, Shrewsbury, Mass. 10545.

(3) C. K. Atal, C. C. J. Culvenor, R. S. Sawhney, and L. W. Smith, *Australian J. Chem.*, **20**, 805 (1967).

(4) A. M. Clark, *Nature*, **183**, 731 (1959).

(5) S. M. Kupchan, R. W. Doskoch, and P. W. Vanevenhoven, *J. Pharm. Sci.*, **53**, 343 (1964).

(6) S. M. Kupchan and M. I. Suffness, *ibid.*, **56**, 541 (1967).