

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.

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Pteridines. XI.^{1,2} Pteridines Related to the Diuretic, 2,4-Diamino-6,7-dimethylpteridine

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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-bis-methylaminopyrimidine 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.

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

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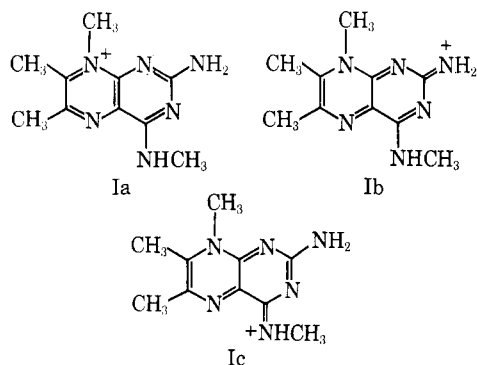
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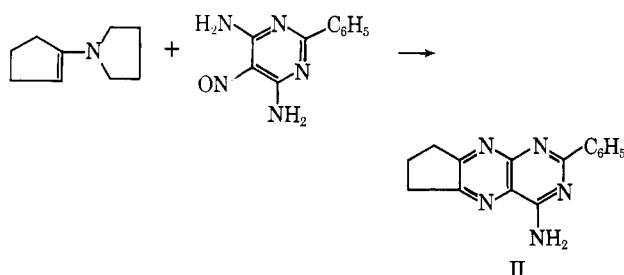
TABLE I
PTERIDINES PREPARED BY THE ISAY REACTION

Pteridine	Dicarbonyl reactant	Reaction conditions Solvent	Time, hr	Temp, °C	Yield, %	Recrystn solvent	Mp, °C	R _f (system)	Formula ^c
4-Amino-2-phenyl	Glyoxal bisulfite	H ₂ O	1	100	45	EtOH	239	0.89 (4)	C ₁₂ H ₉ N ₅
4-Amino-6,7-dimethyl-2-phenyl	Biacetyl	H ₂ O, pH 5.5	0.5	80	52	DMF-H ₂ O	308-310	0.71 (5)	C ₁₄ H ₁₃ N ₅
4-Amino-2,6,7-triphenyl	Benzil	EtOH	2	80	70	DMF-H ₂ O	250-251		C ₂₃ H ₁₇ N ₅
4-Amino-2,6,7-trimethyl	Biacetyl	H ₂ O, pH 5	1	60	52	H ₂ O	249-250	0.83 (6)	C ₈ H ₁₀ N ₆ ^a
4-Amino-2-phenylcyclopenta[g]	1,2-Cyclopentanedione	EtOH	1	80		^c	295 dec	0.82 (3)	C ₁₃ H ₁₃ N ₅
4-Amino-2-anilino-6,7-dimethyl	Biacetyl	EtOH-H ₂ O, 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 ₂ O, pH 5.5	0.33	100	56	EtOH	>300	^a	C ₉ H ₁₁ N ₅
2-Amino-4-carbamyl-6,7-dimethyl	Biacetyl	EtOH-H ₂ O, pH 5.5	3	25		^c	>330		C ₉ H ₁₀ N ₆ O ^f
2,4-Diamino-6(7)-ethyl-7(6)-methyl ^d	2,3-Pentanedione	EtOH-H ₂ O	0.2	100	67	Dil HCl	>300	0.63 (1)	C ₁₃ H ₁₂ N ₆ ·HCl·0.5H ₂ O
2,4-Diamino-cyclopenta[g] ^b	1,2-Cyclopentanedione	EtOH-H ₂ O	0.7	100	35	H ₂ O, pH 2.5	>250		C ₉ H ₁₀ N ₆ ·HCl·H ₂ O
4-Amino-7-hydroxy-6-methyl-2-phenyl	Methyl pyruvate	EtOH	1	100	47	DMF-H ₂ O	282-284	0.64 (2)	C ₁₃ H ₁₁ N ₅ O

^a $\lambda_{\text{max}}^{\text{NaOH}}$ 364 m μ (log ϵ 3.87); 260 (sh); $\lambda_{\text{max}}^{\text{HCl}}$ 352 m μ (log ϵ 4.08), 364 (sh) (4.00). ^b Hydrochloride hydrate. ^c Dissolve in dilute HCl, precipitate with NH₄OH. ^d Hydrochloride hemihydrate. ^e All compounds were analyzed for C, H, N except as noted. ^f C, H, N analysis only. ^g N: calcd, 37.02; found, 37.69.



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



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 re-investigated 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 reported⁸ 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.

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

Experimental Section¹⁰

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-H₂O-HCOOH (5:5:1); (2) *n*-BuOH-*N* NH₄OH (1:1); (3) *n*-BuOH-HCOOH-H₂O (5:1:5); (4) *n*-BuOH-H₂O-AcOH (4:5:1); (5) mineral oil pretreated paper, EtOH-H₂O (3:1); (6) C₆H₆-CHCl₃-80% EtOH (1:1:2); (7) AcOH-H₂O-*i*-AmOH-*t*-AmOH (1:5:3:3); (8) pretreat paper with castor oil-mineral oil (1:1), EtOH-H₂O (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 H₂O, and heated 10 min on a steam bath. Chilling and filtration gave a solid which on recrystallization from DMF-H₂O yielded 11.3 g (48%) of product: mp 240–241°; *R*_f 0.74 (system 8); $\lambda_{\text{max}}^{1\text{N NaOH}}$ 270 m μ (log ϵ 4.42), 346 (3.90); $\lambda_{\text{max}}^{4.5\% \text{HCOOH}}$ 273 m μ (log ϵ 4.30), 292 (sh) (4.09), 324 (sh) (4.08), 337 (4.11), 348 (sh) (4.03); nmr peaks (CF₃COOH) at δ 2.82 (s) (6-CH₃), 7.42 and 7.90 (m) (2-C₆H₅), 8.31 (br s) (NH⁺), and 8.59 (s) (7-H). *Anal.* (C₁₃H₁₁N₅) C, H.

2-Imino-4-methylamino-6,7,8-trimethyl-2,8-dihydropteridine Hydrochloride.—A solution of 2,5-diamino-4,6-bismethylamino-pyrimidine hydrochloride¹¹ (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); $\lambda_{\text{max}}^{0.1\text{N NaOH}}$ 372, 310, and 230 m μ (log ϵ 3.93, 4.24, 4.42); $\lambda_{\text{max}}^{0.1\text{N HCl}}$ 410, 329 (sh), 275, and 236 m μ (log ϵ 4.17, 4.08, 4.15, 4.03); nmr peaks (CF₃COOH) at δ 2.72 (6-CH₃), 2.87 (7-CH₃), 3.38 (4-CH₃NH), and 4.12 (8-CH₃), all singlets.¹² *Anal.* (C₁₀H₁₄N₆·HCl·H₂O) 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. KMnO₄ (9.8 g) was added and the reaction mixture was stirred on a steam bath for 4 hr. An additional 9.0 g of KMnO₄ was added and the mixture was stirred and heated for 2 hr. The excess MnO₄[−] was destroyed by addition of Na₂SO₃ and the MnO₂ 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 H₂O, and reprecipitated by addition of concentrated NH₄OH to pH 8. This gave 7.3 g (44%) of product, mp >300°.

2,4-Diaminopteridine-6-carboxamide.—Anhydrous NH₃ 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

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(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 $\pm 0.4\%$ of the theoretical values.

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(12) For comparison, 2,4-diamino-6,7-dimethylpteridine has nmr peaks (CF₃COOH) at δ 2.67 and 2.71, both singlets, from the 6- and 7-CH₃.

TABLE II
 PTERIDINECARBOXYLIC ACIDS AND DERIVATIVES

Pteridine	Method of prepn	Yield, %	Recrystn solvent	Mp, °C ^a	<i>n</i> _D ^b (system)	Formula ^c	Ultraviolet spectral data	
							Solvent ^d	λ_{\max} , m μ (log ϵ)
2,4-Diamino-6-carboxylic acid	<i>a</i>	43	<i>b</i>	>300	0.21 (7)	C ₇ H ₆ N ₆ O ₂ · 1.33H ₂ O	1	257 (4.23), 336 (4.05)
							2	266 (4.35), 370 (3.95)
4-Amino-2-phenyl-6-carboxylic acid	<i>a</i>	8	DMF-H ₂ O	284-286	0.75 (4)	C ₁₃ H ₉ N ₃ O ₂	3	278 (4.28), 352 (sh)
4-Hydroxy-2-phenyl-7-carboxylic acid	<i>a</i>	51	DMF-H ₂ O		0.58 (4)	C ₁₃ H ₉ N ₃ O ₃	2	278 (4.40), 355 (4.00)
Methyl 2,4-diamino-6-carboxylate	<i>c</i>	44	<i>f</i>	>300	0.44 (3)	C ₈ H ₈ N ₆ O ₂	1	286 (4.19)
							4	271 (4.42), 353 (3.95)
							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 ₂ O	<i>c</i>	60	DMF-H ₂ O	298-301	0.70 (4)	C ₉ H ₁₀ N ₆ O ₂ · 0.5H ₂ O	1	242 (sh), 257 (4.30), 337 (4.07)
							4	267 (4.34), 371 (3.94)
Isopropyl 2,4-diamino-6-carboxylate · 0.5H ₂ O	<i>c</i>	40	DMF-H ₂ O	293-294	0.82 (4)	C ₁₀ H ₁₂ N ₆ O ₂ · 0.5H ₂ O	1	254 (4.31), 337 (4.08)
							4	267 (4.34), 371 (3.95)
2,4-Diamino-6-carboxamide · 0.75H ₂ O	<i>g</i>	60	<i>g</i>	>300	0.44 (4)	C ₇ H ₇ N ₇ O · 0.75H ₂ O	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 NH₄OH 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 NH₄OH. 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. II¹

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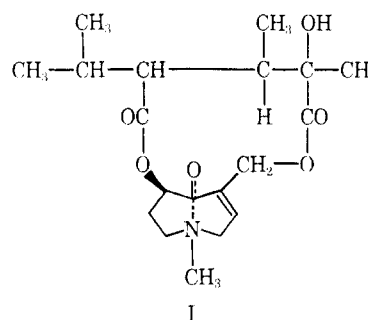
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Crosemperine, an otonecine ester, has been recently isolated from *Crotalaria semperflorens*³ and was shown to possess structure I. Due to reports of the mutagenic⁴ and antitumor properties^{5,6} of pyrrolizidine esters,

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.



Biological Results and Discussion.—Crosemperine was evaluated in the Ehrlich ascites tumor test system, 1 × 10⁶ 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 LD₅₀ 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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