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]	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]

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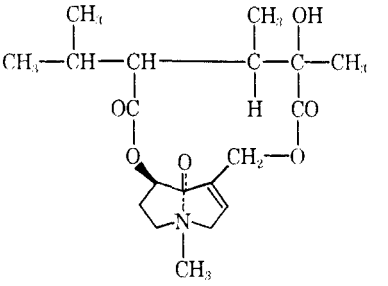
Crosemperine, an otonecine ester, has been recently isolated from *Crotalaria semperflorens*[3] and was shown to possess structure I. Due to reports of the mutagenic[4] and antitumor properties[5,6] of pyrrolizidine esters, *Crotalaria* alkaloids have assumed a significant importance. Since these alkaloids 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,

(1) Paper I: N. S. Bhacca and R. K. Sharma, *Tetrahedron*, in press.

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

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

TABLE I
THE EFFECT OF CROSEMPERINE ON EHRLICH ASCITES TUMOR-BEARING MICE

Dose, mg/kg	No. of daily doses	Survivors	Mean PCV[a] (Test/Control)	T/C
400	1	0/4	...	...
200	1	4/6	2.45 ± 0.4/3.4 ± 0.6	0.72
100	1	6/6	2.46 ± 0.5/3.4 ± 0.6	0.72
50	5	0/5	...	...
25	5	4/5	1.0 ± 0.3/2.2 ± 0.4	0.45

[a] Packed cell volume in milliliters ± standard deviation.

et al.[5] The latter compound was not lethal at 200 mg/kg administered daily for 11 days. If biological activity depends upon opening of the cyclic diester ring structure, these data indicate that crosemperine may be more susceptible to enzymic attack than monocrotaline. Alternatively, the methylated nitrogen of the present compound may alter its distribution properties, resulting in increased toxic effects to the host but reduced toxic effects to the tumor.

Crosemperine was tested up to 1600 μg/ml concentration against *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Saccharomyces cerevisiae, Candida albicans,* and *Aspergillus niger,* using a serial dilution method[7] and was found inactive.

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Cytostatic Bis(haloacyl) Derivatives of Piperazine and 2-Methylpiperazine

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Disubstituted haloacylpiperazines were of interest to us as intermediates which can be used to obtain amine derivatives.[1] Carbon and co-workers[2a] discovered antitumor activity for N,N′-bis(3-bromopropionyl)-piperazine, introduced recently under the name pipobroman,[2b] and N,N′-bis(3-chloropropionyl)piperazine was obtained some years ago by one of us.[1a] We decided to synthesize a series of derivatives[3] and to examine their cytostatic activity. Also the mechanism of action of these compounds has not yet been elucidated.[4] In order to explain the relation between chemical structure and cytostatic activity of the compounds, we varied both the acyl substituent, introducing alkyl groups and a halogen atom in positions 2 or 3, and the amine part, replacing piperazine by 2-methylpiperazine.

(1) (a) S. Groszkowski, *Roczniki Chem.*, **34**, 707 (1960); **38**, 229 (1964); (b) S. Groszkowski and J. Sienkiewicz, *Ann. Pharm. Franc.*, in press.

(2) (a) R. J. Stein, J. A. Carbon, J. Langdon, and R. K. Richards, *J. Lab. Clin. Med.*, **56**, 949 (1960); (b) Vercyte®.

(3) (a) S. Groszkowski, J. Sienkiewicz, and L. Najman, *Farmacia* (Bucharest), **15**, 263 (1967); (b) S. Groszkowski, L. Korzycka, and A. Wesolowski, *Roczniki Chem.*, in press.

(4) (a) J. Louis, R. J. Rohn, and R. W. Monto, *Proc. Am. Assoc. Cancer Res.*, **3**, 246 (1961); (b) T. J. McNair, E. A. Wibin, E. T. Hoppe, J. L. Schmidt, and F. A. de Peyster, *J. Surg. Res.*, **3**, 130 (1963); (c) C. E. Nasjleti, J. M. Walden, and H. H. Spencer, *Cancer. Res.*, **25**, 275 (1965).

The compounds were obtained by condensation of the appropriate haloacyl chlorides or bromides with piperazine hexahydrate or 2-methylpiperazine in chloroform in which almost all derivatives of haloacylpiperazines are readily soluble. The acid chlorides give higher yields than bromides. The piperazine derivatives were obtained in good yield as solids which can be easily purified by recrystallization.[1,3] The new derivatives of 2-methylpiperazine described in this paper, excluding XVI and XIX obtained in the solid state, are (as crude products) oils or resins, crystallizing slowly and decomposing during distillation under reduced pressure. Among them we obtained XII and XVI–XIX in a pure crystalline form. We could not obtain in a pure state the products of condensation of 2-chloropropionyl, 2-bromopropionyl, 3-chloropropionyl, and 3-chlorobutylryl chlorides with 2-methylpiperazine. Therefore, these compounds were not submitted to biological investigations.

The derivatives of 2-methylpiperazine and the derivatives of piperazine previously described[1a,3] were examined for cytostatic activity by the test of Constantinescu and co-workers.[5] The method is based upon the determination of the smallest amount of compound which, dissolved in a 1.0 m*M* caffein solution (0.0212 wt %), will, after 24 hr at 25 ± 1°, induce the characteristic alteration of all the mitotic figures of cariokinesis in the wheat radicular meristemas introduced into the solution. The alterations are identical with those produced by the same agent in healthy and cancerous animal tissues with an intense proliferative activity. These "chromatoclazic"[6] changes consist mainly of mitosis protraction, chromosome fragmentation, the formation of ana- and telophasic bridges, the appearance of micronuclei, and the grouping of chromatin in clusters of various sizes. Under the same conditions, but without the addition of caffein, only a slight inhibition of mitosis with no "chromatoclazic" effects can be observed. On the other hand, the caffeine solution alone, depending on concentration, induces a weak "statmodieretic"[5,7] activity, which consists of the formation of polynuclear cells. It should also be emphasized that cytostatic substances, which show a mechanism of action different from alkylation, do not induce any of the previously described changes in the cariokinesis pictures. They produce, at most, an inhibition of mitosis.

Among 16 substances examined, pipobroman (III) showed (Table I) highest alkylating activity, followed by XII and II. Substances I, X, XIX, and XX did not show cytostatic activity. The remainder increased the statmodieretic activity of caffein and had weak antimitotic or alkylating properties.

On the basis of experimental data, it can be concluded that the bromine derivatives show a higher activity than chloro analogs. Position 3 is much more favorable than 2. The introduction of a methyl radical or branched acyl group into the ring reduces the alkylating activity. The absence of the halogen atom or too small or too large acyl radicals abolish activity.

(5) D. G. Constantinescu, M. Constantinescu, M. Retezeanu, R. Oteleanu, and V. Stoenescu., *Compt. Rend.*, **253**, 176, 1061 (1961); **254**, 1665 (1962).

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