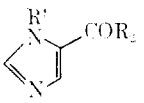
TABLE I



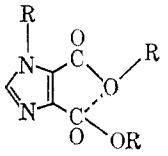
Compd	R_1	R_2	Oral dose, mmole/kg	Act. prior to reserpine (2 mg/kg)	Mean ptotic score	Hexobarb-induced sleep time, min[a]	Effect of compd on reserpine-induced hypothermia, body temp, °C ± SE: 0	1	6	24
Control[b]				Normal	3.66	8.2 ± 0.4	36.2 ± 0.4	29.9 ± 0.5	29.2 ± 0.4	29.2 ± 1.1
III	H	$NHNH_2$	0.275	Increased	2.00	13.5 ± 1.6	35.9 ± 0.1	32.3 ± 0.7	30.9 ± 1.3	32.5 ± 0.7
IV	H	$NHNHCH_3$	0.275	Increased	1.16	14.6 ± 1.4	36.0 ± 0.1	34.8 ± 0.6	33.1 ± 0.8	34.1 ± 1.1
V	CH_3	$NHNH_2$	0.275	Increased	1.50	13.9 ± 1.4	36.6 ± 0.2	34.9 ± 0.1	32.0 ± 0.3	32.2 ± 0.8
VI	CH_3	$NHNHCH_3$	0.275	Increased	2.16	17.0 ± 1.8	36.3 ± 0.1	33.9 ± 0.5	32.9 ± 0.5	31.2 ± 0.9
IX	H	$NHNHC_6H_5$	0.275	Increased	2.33	22.3 ± 2.0	36.2 ± 0.2	33.2 ± 0.4	30.4 ± 1.2	31.9 ± 1.4
X	H	$NHN{=}C(CH_3)_2$	0.275	Increased	2.16	14.2 ± 1.6	36.3 ± 0.2	34.2 ± 0.2	32.3 ± 0.2	32.8 ± 0.6
XI	H	$NHN{=}CHC_6H_5$	0.275	Increased	1.83	16.3 ± 0.7	36.1 ± 0.1	33.3 ± 0.3	32.0 ± 0.6	33.2 ± 0.5
XII	CH_3	$NHN{=}C(CH_3)_2$	0.275	Increased	1.83	13.0 ± 1.0	36.0 ± 0.2	32.4 ± 0.4	31.5 ± 0.7	33.3 ± 0.4
XIII	CH_3	$NHN{=}CHC_6H_5$	0.275	Increased	2.00	12.1 ± 0.3	36.5 ± 0.1	32.3 ± 0.3	32.3 ± 0.7	32.5 ± 0.9
XIV	H	$NH{-}NHCH(CH_3)_2$	0.275	Increased	2.00	14.5 ± 0.7	36.0 ± 0.2	32.8 ± 0.8	33.4 ± 0.5	33.3 ± 1.1
XV	H	$NHNHCH_2C_6H_5$	0.138	Increased	0.16	54.2 ± 2.6	36.3 ± 0.1	35.8 ± 0.4	35.6 ± 0.4	36.2 ± 0.2
XVI	CH_3	$NHNHCH(CH_3)_2$	0.275	Increased	0.66	12.6 ± 0.7	36.7 ± 0.2	34.2 ± 0.3	33.9 ± 0.5	34.0 ± 0.9
XVII[c]	CH_3	$NHNHCH_2C_6H_5$	0.275	Increased	1.83	16.1 ± 2.5	36.1 ± 0.1	32.5 ± 0.3	31.0 ± 0.4	31.7 ± 0.6
Isocarboxazid			0.138	Increased	0.50	10.5 ± 0.5	35.8 ± 0.2	31.7 ± 0.7	34.2 ± 0.7	35.4 ± 0.2

[a] Hexobarbital was given intraperitoneally (55 mg/kg) 2 hr after incubation of the test compound. [b] Control implies reserpine in ptosis and hypothermia test, and hexobarbital in sleeping time prolongation test. [c] The base form rather than the HCl salt was administered.

of the compounds, listed in Table I, for MAO inhibitory activity was carried out as previously reported.[2] The tests consisted of the reversal of reserpine-induced ptosis and hypothermia and hexobarbital sleeping-time prolongation.

Results and Discussion

The result of the present and previous investigation[4] indicates that the reactivity of dimethyl imidazole-4,5-dicarboxylate and its 1-methyl derivative with hydrazine was greater than their monoester analogs I or II. This higher rate of reaction of diesters can be attributed to the existence of a quasi-bicyclic structure (XVIII), in which the elimination of OR group is facilitated.



XVIII

The variations among the C=O stretching in the ir spectra of the esters, however, was not substantial to render support for the presence or absence of XVIII.

As compared with I, the reactivity of II with hydrazines, due to steric and/or electronic influence of the CH_3 group, is retarded.

As illustrated in Table I, the MAO inhibitory activity of the imidazole monohydrazides is in general somewhat higher than their dihydrazide analogs.[2,4] Of particular interest is the significant activity manifested by XV (see Table I). This activity at the present can not be related to any particular moiety in the molecular structure of these imidazolecarbohydrazides. The absence of comparable activity in XVII, the ring-methyl-substituted analog of XV, is an indication of the necessity for further investigation in the series.

Acknowledgment.—The authors wish to thank Mr. Donald Haygood for his efficient assistance in this investigation.

Anthelmintic Quaternary Salts. IV. Aminopentadienylideneammonium Salts

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The synthesis and anthelmintic activity of a group of aminopentadienylideneammonium salts is described. Compounds in which the N atoms were part of a pyrrolidine or piperidine ring were effective against common gastrointestinal nematodes of sheep and had prophylactic activity in protecting swine from infections of *Ascaris suum*.

Schiff bases of glutaconaldehyde have been widely used as chemical intermediates, particularly in the synthesis of azulenes, and have been the subject of extensive spectroscopic investigations. Their effects on biological systems do not appear to have been investigated previously, and it was considered of interest to prepare compounds of this class (Table I) for evaluation as anthelmintics because of their similarity to other cyanine dyes with known anthelmintic potency.

Chemistry.—The general method of preparation of

TABLE I
AMINOPENTADIENYLIDENEAMMONIUM SALTS
[R$^+$=CHC(R')=CHCH=CHR] X$^-$

No.	R	R'	X	Mp, °C	Yield, %	Formula	Analyses
1	Azetidino[a]	H	ClO_4	184–185	83	$C_{11}H_{17}ClN_2O_4$	C, H, N
2a	Pyrrolidino[b]	H	ClO_4	186–187	80	$C_{13}H_{21}ClN_2O_4$	
2b			Pamoate	210–212	83	$C_{49}H_{56}N_4O_6 \cdot 4H_2O$	N; C, H[f]
3a	Pyrrolidino	CH_3	ClO_4	177–179		$C_{14}H_{23}ClN_2O_4$	C, H, N
3b		CH_3	Pamoate	150–152	80	$C_{51}H_{60}N_4O_6 \cdot 2H_2O$	C, H, N, O
4	2,5-Dimethylpyrrolidino	H	Cl	159–160	46	$C_{17}H_{29}ClN_2 \cdot 1.5H_2O$	C, H, N
5a	3-Pyrrolino	H	ClO_4	180–181		$C_{13}H_{17}ClN_2O_4$	C, H, Cl, N
5b		H	Pamoate	225–226 dec	20	$C_{49}H_{48}N_4O_6 \cdot 2H_2O$	C, H, N
6a	Piperidino[c]	H	Cl	102–103	60	$C_{15}H_{25}ClN_2 \cdot H_2O$	C, H, Cl, N
6b		H	Pamoate	176–178	49	$C_{53}H_{64}N_4O_6 \cdot 2H_2O$	H, N, O; C[g]
7a	Piperidino	CH_3	Cl	185 dec	59	$C_{16}H_{27}ClN_2 \cdot H_2O$	C, H; N[h]
7b		CH_3	Pamoate	238–239		$C_{55}H_{68}N_4O_6 \cdot 4H_2O$	C, H, N
8a	4-Methylpiperidino	H	Cl	106–107	79	$C_{17}H_{29}ClN_2 \cdot 1.5H_2O$	C, H, O; N[i]
8b		H	Pamoate	155–158 dec		$C_{57}H_{72}N_4O_6 \cdot H_2O$	C, H, N, O
9	3-Methylpiperidino	CH_3	Cl	139–140	30	$C_{18}H_{31}ClN_2 \cdot H_2O$	C, Cl, N, O; H[j]
10	4-Phenylpiperidino	H	Cl	239–240 dec	89	$C_{27}H_{33}ClN_2$	C, H, Cl, N
11	4-Phenylpiperidino	CH_3	Cl	163–164	39	$C_{28}H_{35}ClN_2 \cdot H_2O$	C, H, Cl, N
12	4-Hydroxypiperidino	H	Cl	85–90	30	$C_{15}H_{25}ClN_2O_2 \cdot 2H_2O$	H, N; C[k]
13	Hexamethyleneimino	H	Pamoate	206–207	48	$C_{57}H_{72}N_4O_6.2H_2O$	C, H, N
14	Hexamethyleneimino	CH_3	Cl	94–98 dec		$C_{18}H_{31}ClN_2 \cdot 1.5H_2O$	H, N; C[l]
15	Heptamethyleneimino	H	Cl	153–155 dec	70	$C_{19}H_{33}ClN_2 \cdot H_2O$	C, H, N
16	Octamethyleneimino	H	Cl	224–225	62	$C_{21}H_{37}ClN_2$	C, H, N
17	Morpholino	H	Cl	125–128	58	$C_{13}H_{21}ClN_2O_2 \cdot 2H_2O$	H, Cl; C[m]
18	1,2,3,4-Tetrahydroquinolino	H	Cl	188–189	72	$C_{23}H_{25}ClN_2 \cdot H_2O$	C, H, Cl, N, O
19	1,2,3,4-Tetrahydro-6-methoxyquinolino	H	Cl	202	62	$C_{25}H_{29}ClN_2O_2 \cdot H_2O$	C, H, N
20	1,2,3,4-Tetrahydroisoquinolino	H	Cl	95–100	72	$C_{23}H_{25}ClN_2 \cdot H_2O$	C, H, N
21	4-Phenylpiperazino	H	Cl	204–205	59	$C_{25}H_{31}ClN_4 \cdot 1.5H_2O$	C, H; Cl[n]
22	Dimethylamino[d]	H	ClO_4				
23	Di(2-hydroxyethyl)amino	H	ClO_4	134–136	47	$C_{15}H_{25}ClN_2O_4$	C, H, Cl, N, O
24	Di-*n*-propylamino	H	ClO_4	105–106	80	$C_{17}H_{33}ClN_2O_4$	C, H, N, O
25	Di-*n*-butylamino	H	ClO_4	206–207	29	$C_{21}H_{41}ClN_2O_4$	C, H, N, O
26	Diisobutylamino	H	ClO_4	91–92	35	$C_{21}H_{41}ClN_2O_4$	C, H, Cl, N, O
27	Dibenzylamino	H	Cl	179–181	29	$C_{33}H_{33}ClN_2 \cdot 1.5H_2O$	C, H; N[o]
28	N-Methylanilino[e]						

[a] See ref 2. [b] S. Dähne and J. Ranft, *Z. Physik. Chem.*, **224,** 65 (1963), reported mp 187° for the perchlorate salt; for photoisomerism see F. Baumgartner, E. Günther, and G. Scheibe, *Z. Elektrochem.*, **60,** 570 (1956). [c] The perchlorate salt was described by Dähne and Ranft. [d] W. König and W. Regner, *Ber.*, **63B,** 2823 (1930). [e] W. König, *J. Prakt. Chem.*, [2] **69,** 134 (1904). [f] C: calcd, 67.88; found, 67.19. H: calcd, 7.21; found, 6.59. [g] C: calcd, 71.59; found, 72.12. [h] N: calcd, 9.31; found, 9.78. [i] N: calcd, 8.65; found, 9.23. [j] H: calcd, 10.11; found, 9.40. [k] C: calcd, 53.48; found, 53.93. [l] C: calcd, 63.97; found, 64.48. [m] C: calcd, 50.56; found, 50.01. [n] Cl: calcd, 7.88; found, 7.44. [o] N: calcd, 5.93; found 5.51.

the desired products was the condensation of secondary amines with 1-(2,4-dinitrophenyl)pyridinium chloride in alcoholic solvents. Most of the amines reacted smoothly at room temperature, but some of the preparations required brief periods of reflux. The progress of the reaction could be observed by the separation of 2,4-dinitroaniline. The dibenzylamino analog was obtained by the condensation of dibenzylamine with glutaconaldehyde dianil. The chloride salts of the products were frequently too hygroscopic for convenient isolation, and the products were isolated as perchlorate and pamoate salts.

It was found that, if desired, the products could be prepared by direct reaction of the amine with glutaconaldehyde. Thus, the condensation of pyrrolidine with glutaconaldehyde gave 1-pyrrolidyl(2,4-pentadienylidene)-1-pyrrolidinium perchlorate in 38% yield. However, this direct process offers no practical advantage over the standard method.

Analogous compounds with Me in the 2 position of the pentadienyl chain were prepared by treating secondary amines with 1-(2,4-dinitrophenyl)-3-methylpyridinium chloride. To determine the effect of chain length on the anthelmintic activity, a number of tri-

TABLE II
ANTHELMINTIC ACTIVITY OF AMINOPENTADIENYLIDENEAMMONIUM SALTS

	Acute toxicity in mice[c]		*N. dubius* in mice[d]		Gastrointestinal nematodes in sheep[e]		*A. suum* in mice[g]		*A. suum* in swine[h]		
	LD$_{50}$, mg/kg			No.		Eggs/g		Lung		Liver	Lung
Compd	Ip	Oral	Dose, mg/kg	of worms, % redn	Dose, mg/kg	of feces, % redn	Dose, mg/kg	lesions, % redn	Dose, % in feed	lesions, % redn	larvae, % redn
1					50	25					
2a	10	100	10	45	200	65	10	90	0.01	88	93
			25	100					0.03	83	98
2b	50	200	50	100	13	54	10	90	0.01	0	93
					25	78			0.02	65	80
					100	88					
					200	100					
3a	10	200	10	95	50	79					
			25	100	100	96					
3b	50	500	150	100	200	72	5	20	0.015	60	99
							10	70			
							25	95			
4	10	50	15	0	200	97					
5a	10	100	10	75							
			15	100							
			25	100							
5b	50	300	25	100	90	87	5	30			
							25	70			
6a	10	50	15	0	50	67	5	60	0.01	75	96
					100	94	10	78	0.02	85	99
6b	>1000	>1000	100	60	200	62	5	70	0.01	69	98
			250	100			10	85	0.015	80	85
									0.02	90	99
									0.03	96	99
7a	10	100			100	0	10	30			
7b	10	500	150	0	200	44	5	30	0.015	0	40
							10	70			
8a	10	50	5	0	200	65	5	20	0.01	88	93
							10	60	0.03	83	98
8b	200	750	100	0	150	72			0.015	78	94
9	10	50	15	0	100	0	10	10			
10	100	750	750	0	100	0	10	30			
11	100	750	250	0	100	0	10	30			
12	500	>1000	750	0	100	58	10	40			
13	300	>1000	100	0	100	72	10	10	0.015	70	96
14	10	100	30	0	100	83					

methine and monomethine cyanines, with substituents similar to those of the active pentamethine analogs, were prepared by standard methods.

Biological Results (Table II).—The compounds were tested first against infections of *Nematospiroides dubius* and *Ascaris suum* in mice, and most of the members of the series were subsequently evaluated for effectiveness against *A. suum* in swine and a group of six gastrointestinal nematodes in sheep. In contrast to other cyanine dyes, this series showed relatively little species specificity, the active compounds tending to be generally effective against all the parasites tested.

The most active of the heterocyclic derivatives were those in which R was pyrrolidino or 3-pyrrolino (**2–5**). The piperidino derivatives (**6–12**) were generally less active, and further ring expansion (**13–16**) caused drastic reduction in activity. The azetidino derivative was also much less active than the pyrrolidino compounds.

Alkyl substitution on the pyrrolidine ring, as in the 2,5-dimethyl derivative **4**, did not affect the activity, but substitution on the piperidine ring had variable effects. The 4-Me and 4-OH derivatives of piperidine retained the activity of the unsubstituted compound with most of the organisms tested, but the 3-Me and 4-Ph analogs were much less active.

Other structural variations which resulted in reduction of activity were the replacement of piperidino by morpholino, tetrahydroquinolino, tetrahydroisoquinolino, and 4-phenylpiperazino.

The introduction of Me in the 2 position of the pentadiene chain had no effect on the activity when R was pyrrolidino, but tended to reduce the activity when the R group was larger.

Of those compounds (**22–28**) which were derived from noncyclic secondary amines, only the di-*n*-propylamino analog **24** showed significant activity; it was of the same order of potency as the pyrrolidino compound in its action against gastrointestinal nematodes in sheep. König's salt (**28**) showed marginal activity against *N. dubius* and *A. suum* in mice.

Compounds in which the number of methine groups in the conjugated chain was reduced from five to three or one (**29–31**) were totally inactive.

Requirements for anthelmintic activity in this group of cyanine dyes include a pentamethine chain between two N atoms which are part of a five- or six-membered ring, or which are substituted with two N-propyl groups. The potency of the active compounds was not enhanced by substitution on either the polymethine chain or the rings.

TABLE II (*Continued*)

Compd	Acute toxicity in mice[a] LD50, mg/kg ip	Acute toxicity in mice[a] LD50, mg/kg Oral	*N. dubius* in mice[b] Dose, mg/kg	*N. dubius* in mice[b] No. of worms, % redn	Gastrointestinal nematodes in sheep[c] Dose, mg/kg	Gastrointestinal nematodes in sheep[c] Eggs/g of feces, % redn	*A. suum* in mice[d] Dose, mg/kg	*A. suum* in mice[d] Lung lesions, % redn	*A. suum* in swine[e] Dose, % in feed	*A. suum* in swine[e] Liver lesions, % redn	*A. suum* in swine[e] Lung larvae, % redn
15	10	50	25	0	100	0	25	40			
16	10	100	25	0	30	32					
17	750	>1000	750	0	200	0					
18	200	1500	500	0	50	37	10	5			
19	50	200	50	0	100	0	10	10			
20	50	500	150	0			10	0			
21	500	>1000	500	0			10	30			
22	50	100	15	0	50	0	10	30			
23	>1000	>1000	500	0	100	0	25	20			
24	10	>1000	15	0	25	50	5	10	0.015	0	0
					50	91	10	40			
25	10	50	15	55			5	20			
26	50	200	15	20			5	40			
27	200	500	400	0			10	30			
28	50	500	150	20	200	0	10	30			

[a] The LD_{50} values recorded in the table are estimates based on acute toxicity studies in which three mice were used per dose level with an average of eight dose levels for each mode of administration. [b] Each of three mice which had been infected with 50 *N. dubius* larvae several weeks earlier was administered a dose of 15–50 mg/kg orally. A similar dose was administered on the following day. On the seventh day the mice were sacrificed and a count was made of the number of worms remaining in the intestine. The unmedicated mice had 35–40 worms; the table records the percentage reduction caused by the action of the test compound. [c] The compounds were tested on sheep which had been experimentally infected with six species of gastrointestinal nematodes: *Haemonchus contortus, Cooperia curticei, Trichostrongylus colubriformis, Trichostrongylus axei, Ostertagia circumcincta,* and *Nematodirus spathiger.* The compounds were administered in two equal doses on consecutive days, and the number of eggs per gram of feces was determined during a 7-day period. The table lists the percentage reduction in the egg count at the end of this period. No particular species specificity in anthelmintic action was observed with this series of compounds. [d] A dose of 10 mg/kg was administered orally to each of three mice, followed by the administration of an infection of 100,000 embryonated *A. suum* eggs. A second dose of 10 mg/kg was administered 4 hr later. After 8 days the mice were sacrificed and the extent of lung lesions was determined by gross examination of the lungs for the number and size of hemorrhagic areas due to the migration of the *Ascaris* larvae. The table lists the percentage reduction in lung lesions of the treated animals as compared with the unmedicated controls. [e] The test compounds were administered at a level of 0.01% in feed for a period of 10 days to two pigs in concrete-floored pens. An infection of 100,000 embryonated *A. suum* eggs was administered 3 days after the start of the inclusion of the test compound in the feed. The animals were sacrificed after 10 days. The percentage reduction in liver lesions due to migrating *Ascaris* larvae in treated animals as compared with controls was determined by counting the small white scars ("milk spots") found on the surface of the liver. The number of lesions was 500 or greater in unmedicated controls (57 animals); beyond 500 the lesions tended to coalesce and could not be counted separately. The procedure used to determine the number of larvae in the lungs of the pigs was based on the method described for mice by D. K. Hass (Ph.D. Thesis, University of Wisconsin, 1962). In 57 control animals, the number of larvae found after sacrifice varied from a low of 10,000 to a high of 49,000, for an average value of 25,000.

Experimental Section[1]

1-(5-Azetidino-2,4-pentadienylidene)azetidinium Perchlorate (1).[2]—A solution of azetidine[3] (7.0 g, 0.122 mole) in MeOH (52 ml) was added to 1-(2,4-dinitrophenyl)pyridinium chloride (17.3 g, 0.061 mole) in MeOH (75 ml) at room temperature. The temperature rose spontaneously to 50°, and the suspension was allowed to stand overnight. The precipitated 2,4-dinitroaniline was filtered, and the filtrate was evaporated to dryness and extracted with H_2O (450 ml), giving a further precipitate of 2,4-dinitroaniline (total 10.8 g, theory 11.2 g). $HClO_4$ was added to the aqueous extract at 0° to give the product, mp 184–185° (from EtOH), yield 13.9 g (83%).

The other compounds (excluding **27**) listed in Table I were prepared in the same way, except that in a few cases, brief periods of reflux were required before the theoretical quantity of 2,4-dinitroaniline was formed. Pamoate salts were prepared by adding sodium pamoate to the aqueous solutions of the chloride salts.

[5-(Dibenzylamino)-2,4-pentadienylidene]dibenzylammonium Chloride (27).—A solution of dibenzylamine (8.0 g, 0.04 mole) and glutaconaldehyde dianil hydrochloride (5.7 g, 0.02 mole) in EtOH (20 ml) was refluxed for 15 min and allowed to stand overnight. The solution was evaporated to dryness and the residue was crystallized from MeOH–EtOAc–Et_2O to give the product in a hydrated form, yield 3.0 g (29%).

1-(Piperidinomethylene)piperidinium Perchlorate (29).—A mixture of piperidine (4.26 g, 0.05 mole), piperidine perchlorate (9.28 g, 0.05 mole), and triethyl orthoformate (7.41 g, 0.05 mole) in EtOH (10 ml) was refluxed for 4 hr and allowed to stand overnight. The product separated on addition of ether giving 11.2 g (80%, mp 210–217°). The analytical sample melted at 233–234° after recrystallization (EtOH). *Anal.* ($C_{11}H_{21}ClN_2O_4$) C, H, Cl, N.

3-Methyl-1-[3-(3-methylpiperidino)allylidene]piperidinium Chloride (30).—The general method of Malhotra and Whiting[4] was employed for the allylidene derivatives. A solution of propiolaldehyde (5.5 g, 0.1 mole) in MeOH (15 ml) was added dropwise to a solution of 3-methylpiperidine (10.9 g, 0.11 mole) in MeOH (25 ml) at 0° and the solution was allowed to stand at 0° for 18 hr. Fractionation yielded 3-(3-methylpiperidino)-propenal, bp 125–130° (0.1 mm), yield 10.5 g (68.5%).

A solution of this compound (6.1 g, 0.04 mole) and 3-methylpiperidine perchlorate (8.76 g, 0.044 mole) in EtOH (35 ml) was refluxed for 2 hr. It was passed through a column of Amberlite IRA-400 in the chloride form prepared in MeOH (200 ml) and the eluate was evaporated. Crystallization of the residue from AcMe–Et_2O yielded 4.0 g (34%) of product, mp 104–105°. *Anal.* ($C_{15}H_{27}ClN_2 \cdot 2H_2O$) C, H, N.

1-(3-Piperidinoallylidene)piperidinium Perchlorate (31).—A solution of propiolaldehyde (5.5 g, 0.1 mole) in MeOH (15 ml) was added dropwise to piperidine (9.5 g, 0.11 mole) in MeOH

(1) Melting points were taken on a Thomas–Hoover melting point apparatus and are corrected.

(2) F. Dörr, J. Kotschy, and H. Kausen, *Ber. Bunsenges. Phys. Chem.*, **69**, 11 (1965). The uv spectrum of the perchlorate salt is given, but the compound is not otherwise described.

(3) D. H. Wadsworth, *J. Org. Chem.*, **32**, 1184 (1967).

(4) S. S. Malhotra and M. C. Whiting, *J. Chem. Soc.*, 3812 (1960).

(25 ml) at 0°, and the solution was allowed to stand overnight. Fractionation of the solution gave 3-piperidinopropenal, bp 115–120° (0.3 mm), yield 4.17 g (30%). This product was refluxed with piperidine perchlorate (6.12 g, 0.033 mole) in 18 ml of EtOH for 15 min. The product separated on cooling, yield 7.5 g (82%), mp 129–130°. *Anal.* ($C_{13}H_{23}ClN_2O_4$) C, H, Cl, N, O.

1-(5-Pyrrolidino-2,4-pentadienylidene)pyrrolidinium Perchlorate (2a).—Pyrrolidine (14.2 g, 0.2 mole) was added to a solution of the sodium enolate of glutaconaldehyde[5] (15.6 g, 0.1 mole) in 300 ml of MeOH. The reaction mixture was refluxed for 15 min and cooled. Acidification with $HClO_4$ yielded the product, mp 184–186°, yield 11.5 g (38%). The product was identical with that prepared by reaction of pyrrolidine with 1-(2,4-dinitrophenyl)pyridinium chloride.

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The Effects of Some Steroidal Alkylating Agents on Experimental Animal Mammary Tumor and Leukemia Systems[1]

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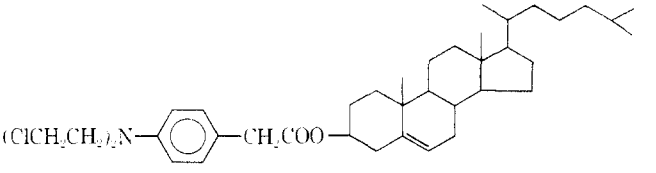
A series of steroid esters of *p*-[N,N-bis(2-chloroethyl)amino]phenylacetic acid (BCAPAA), steroidal sulfides of *p*-(N,N-bis-2-chloroethylamino)thiophenol, and a variety of steroidal ethylenimine derivatives were synthesized and tested for antitumor activity in a number of experimental tumor systems. Activity was found only in those instances in which the steroid and potential oncolytic agent were connected by ester or heterocyclic ether linkages. The steroidal BCAPAA esters were of particular interest showing excellent inhibition of a DMBA-induced and transplantable mammary adenocarcinoma, and marked increase in survival when tested on a variety of rat leukemias. Factors such as route of administration, vehicle, and the nature of the steroid had definite effects and are discussed in detail in the body of the paper. The steroidal BCAPAA esters were judged to be less toxic than some of the well-known nitrogen mustards in general use.

Steroidal alkylating agents have received sporadic attention as potential antitumor agents for some 15 years. The workers in this field were hopeful that useful advantage might be made of the lipophilic nature of the steroid molecule in the transport of various nitrogen and sulfur mustards or that hormonal steroids such as androgens and estrogens could deliver the alkylating agent to a specific target tissue. In his classical treatise on biological alkylating agents, Ross, reviewing this field up to 1960,[3] concluded that the few steroidal nitrogen mustards prepared at this date had no outstanding antitumor properties. In 1961, Burstein and Ringold made an androgen nitrogen mustard which was nontoxic at doses up to 500 mg/kg.[4] Antitumor activity was not reported. In 1962, Rao and Price[5] made a variety of steroidal nitrogen mustards, a few of which were reported active in inhibition of the Gardner ascites tumor. In 1966, Schneider, Hamsher, and Beyler[6] reported that a few steroidal ethylenimine derivatives displayed modest activity in an unspecified C_3H mouse tumor. The reports cited in most cases involved compounds in which the $(ClCH_2CH_2)_2N$ moiety or ethylenimine grouping was linked to the steroid by an N–C bond. Moreover, all the references cited were notable for the paucity of biological data. In contrast to these unpromising results were the reports of a Russian group,[7] Degteva, Larionov, and coworkers.[8] These workers described the preparation of 3β-hydroxy-5-cholestene *p*-[N,N-bis(chloroethyl)amino]phenylacetate which they called phenesterin (**1**) and have made detailed studies of the activity of phenesterin against a variety of solid tumor systems including Sarcoma 45, Walker carcinosarcoma, and alveolar liver carcinoma RS-1,[8a,b] and recently in



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brain tumors.[8c] At levels of 100–200 mg/kg in rats and mice, and subcutaneous administration in olive oil vehicle, these workers reported remarkable inhibition of the above-mentioned tumor systems; however, the compound was inactive against Ehrlich and Sarcoma 180 mice tumors. In addition phenesterin was found to be relatively nontoxic, with an LD_{50} for rats (single injection) of 2.0 g/kg.[8a] Degteva, Larionov, and coworkers claimed that the action of the cholesterol ester was different from that of the parent free acid[8b] and have stressed the importance of the ester function.

With these latter encouraging results in hand, we decided to reinvestigate steroidal nitrogen mustards and steroidal ethylenimine derivatives with the following objectives: (a) to prepare a number of steroid analogs of phenesterin varying the steroid and the location of the BCAPAA ester on the steroid selection; (b) to prepare a number of steroidal ethylenimines in which the active alkylating function would be located on an ester or reac-

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