hexanenitrile[1] with Adam's catalyst in the presence of acetic anhydride gave the desired compound in 58% yield as a red, viscous liquid, bp 173° (0.5 mm), n^{24}D 1.4679. *Anal.* ($C_8H_{16}N_2O_4$) C, H, N. Nef hydrolysis of this 4-nitro compound gave the previously unreported 4-ketohexanenitrile, bp 75–80° (0.5 mm), n^{20}D 1.4338. *Anal.* (C_6H_9NO) C, H, N.

(4) G. D. Buckley, T. J. Elliott, F. G. Hunt, and A. Lowe, *J. Org. Chem.*, **8**, 10 (1943).

Cysteine Derivatives of Keto Steroids

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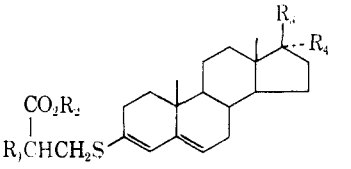
The condensation of L-cysteine derivatives with Δ^4-3-keto steroids in the presence of pyridinium chloride[1] has been found to give the corresponding diene thioethers.[2] When compared with testosterone in mice, **3** was devoid of androgenic, myotropic, and antiestrogenic activity.[3]



1, R_1 = $C_6H_5CH_2CONH$; R_2 = CH_3; R_3 = C_8H_{17}; R_4 = H
2, R_1 = $Cl^-H_3N^+$; R_2 = C_2H_5; R_3 = OH; R_4 = H
3, R_1 = $Cl^-H_3N^+$; R_2 = C_2H_5; R_3 = OH; R_4 = CH_3

Experimental Section[4]

Methyl S-(3,5-Cholestadien-3-yl)-N-phenylacetyl-L-cysteinate (1).—A solution of 500 mg (1.3 mmoles) of cholestenone in 25 ml of C_6H_6 was distilled until 5 ml had collected. A solution composed of 880 mg (5.2 mmoles) of methyl N-phenylacetyl-L-cysteinate,[5] 48 mg of pyridinium chloride, 6 ml of EtOH, and 4 ml of C_6H_6 was added. The solution was refluxed 3 hr, cooled, diluted with 30 ml of ether, and washed with two 25-ml portions of 1 *N* NaOH. After one H_2O wash the ethereal solution was dried (Na_2SO_4) and evaporated leaving 740 mg of semisolid. Precipitation from acetone–petroleum ether (bp 30–60°) gave 264 mg of **1**, mp 119–125°. Further work-up of the mother liquor gave another 100 mg of **1**, mp 100–119°, and 167 mg of recovered cholestenone. The analytical sample (*i*-Pr_2O) had mp 158–159°. *Anal.* ($C_{39}H_{57}NO_3S$) H, N, S.

Ethyl S-(17β-Hydroxy-3,5-androstadien-3-yl)-L-cysteinate Hydrochloride (2).—A similar condensation between testosterone and ethyl L-cysteinate hydrochloride gave **2** as an amorphous solid (acetone), mp 176–179°. *Anal.* ($C_{27}H_{42}ClNO_3S$) C, H, Cl, N, S.

Ethyl S-(17β-Hydroxy-17α-methyl-3,5-androstadien-3-yl)-L-cysteinate Hydrochloride (3).—Similarly, 17α-methyltestosterone and ethyl L-cysteinate hydrochloride gave **3**, needles (Me_2CO–C_6H_6), mp 171–172°. *Anal.* ($C_{25}H_{40}ClNO_3S$) C, H, Cl, N, S.

Acknowledgment.—This work was supported in part by U. S. Public Health Service Research Grant AM-04531.

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(2) Δ^4-3-Keto steroids are reported *not* to react with cysteine or ethyl cysteinate: S. Lieberman, *Experientia*, **2**, 411 (1945).

(3) We are indebted to Dr. R. Kraay, Eli Lilly and Co., for these assays.

(4) Ir spectra were obtained on an Infracord, uv spectra on a Beckman DU. Microanalyses are by Galbraith Laboratories, Knoxville, Tenn.

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17β-(4′-{*p*-[Bis(β-chloroethyl)amino]phenyl}-butanoyloxy)-4-androsten-3-one

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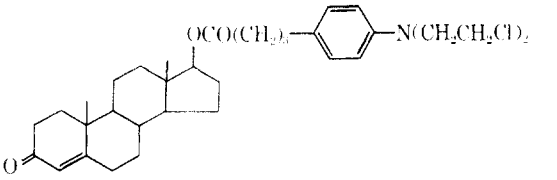
The synthesis and antitumor evaluation of steroidal nitrogen mustards[1] prompted us to synthesize the chlorambucil ester of testosterone by treating chlorambucil chloride with the potassium salt of testosterone in refluxing benzene.



Experimental Section[2]

17β-(4′-{*p*-[Bis(β-chloroethyl)amino]phenyl}butanoyloxy)-4-androsten-3-one.[3]—Testosterone, 0.34 g (1.1 mmoles), was dissolved in dry C_6H_6, excess K was added, and the mixture was then refluxed overnight. After filtration of the unreacted K, the C_6H_6 solution of the potassium salt was added to residual chlorambucil chloride, which was prepared from 0.35 g (1.1 mmoles) of chlorambucil and 2 ml of $POCl_3$ in refluxing C_6H_6 with subsequent solvent removal. The esterification reaction mixture was refluxed 5 hr, then left at room temperature overnight. The solvent was evaporated to give 512 mg of crude product, which was chromatographed on 10 g of Al_2O_3. C_6H_6 eluted 60 mg of acid chloride, 43 mg of the ester (analytical sample), and 34 mg of impure ester, while C_6H_6–Et_2O (4:1) eluted 154 mg of additional ester. Rechromatography of the last two fractions (188 mg) plus 82 mg of similar product from another preparation on 8 g of Al_2O_3 gave 211 mg of ester eluted by C_6H_6–Et_2O (4:1). *Anal.* ($C_{33}H_{45}Cl_2NO_3$) C, H, N.

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(2) Ir spectra were obtained on an Infracord, uv spectra on a Beckman DU. Microanalyses are by Galbraith Laboratories, Knoxville, Tenn.

(3) This compound was submitted to the Cancer Chemotherapy National Service Center, Public Health Service, for an evaluation of its antitumor activity against acute lymphocytic leukemia.

2,2′-Hydrazobis(5-nitropyrimidines)

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2-Amino-5-nitropyrimidine and its derivatives possess pronounced trichomonacidal activity.[1] We have shown that symmetrical 2,2′-hydrazobis(5-nitrothiazoles) also show a very strong antiprotozoal activity.[2] The combination of these two features

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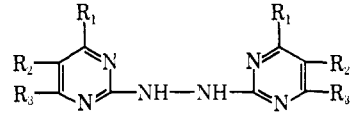
TABLE I
2,2'-HYDRAZOBISPYRIMIDINES



Compd	R_1	R_2	R_3	Method	Mp, °C[c]	Color	Recrystn solvent	Yield, %	Formula[d]
1	H	NO_2	H	B[a]	254–255	Brown	PrOH	35	$C_8H_6N_8O_4$
2	NH_2	NO_2	H	A	>320	Reddish brown	...	57	$C_8H_8N_{10}O_4$
3	$(CH_3)_2N$	NO_2	CH_3	A	224	Yellow	2-PrOH	43	$C_{14}H_{20}N_{10}O_4$
4	$(C_4H_9)_2N$	NO_2	CH_3	A	169–171	Yellow	2-PrOH	30	$C_{26}H_{44}N_{10}O_4$
5	CH_3O	H	H	B[b]	119–120	White	Cyclohexane	47	$C_{10}H_{12}N_6O_2$[e]
6	CH_3O	NO_2	H	C	197	Red	AcOEt	20	$C_{10}H_{10}N_8O_6$

[a] The product was purified by dissolving in DMSO and precipitating with water. [b] The filtered precipitate was dissolved (H_2O) and the free base was precipitated with NH_4OH. [c] All the nitro compounds melted with decomposition. [d] All compounds were analyzed for N. [e] Analyzed for C, H, N.

has led us to prepare a series of 2,2'-hydrazobis(5-nitropyrimidines) as potential antiprotozoal agents. Most of the compounds were obtained by the condensation of the corresponding 2-chloropyrimidines with hydrazine in alcoholic solution (Table I).

Experimental Section[3]

2-Chloro-5-nitropyrimidine,[4] 4-amino-2-chloro-5-nitropyrimidine,[5] 2-chloro-4-dimethylamino-6-methyl-5-nitropyrimidine,[6] and 2-chloro-4-methoxypyrimidine[7] were prepared by procedures described in the literature.

2-Chloro-4-dibutylamino-6-methyl-5-nitropyrimidine.—A solution of 9.7 g (75 mmoles) of dibutylamine and 4.3 ml (75 mmoles) of AcOH in 20 ml of H_2O was added to a solution of 5.2 g (25 mmoles) of 2,4-dichloro-6-methyl-5-nitropyrimidine[8] in 20 ml of dioxane. The mixture was stirred for 2 days and then extracted several times (C_6H_6). The residue, obtained after evaporation of the organic solvent was chromatographed on acid-washed alumina. The fraction eluted with petroleum ether (bp 40–60°) yielded 5.1 g (68%), bp 158° (0.8 mm). *Anal.* ($C_{13}H_{21}ClN_4O_2$) C, H, Cl, N.

2,2'-Hydrazobispyrimidines (Table I). Method A.—A mixture of the corresponding 2-chloropyrimidine (9 mmoles), hydrazine hydrate (4.5 mmoles), and Et_3N (10 mmoles) in *t*-BuOH (35 ml) was refluxed with stirring for 10 hr. The precipitate was filtered, washed (MeOH), dissolved in concentrated HCl, and precipitated with H_2O.

Method B.—One mole of the corresponding 2-chloropyrimidine and 0.5 mole of hydrazine hydrate in absolute EtOH were refluxed with stirring for 10 hr.

Method C.—To a solution of 25 mg of **5** in 2 ml of concentrated H_2SO_4 was added at 0° a solution of 0.12 ml of fuming HNO_3 in 0.6 ml of concentrated H_2SO_4. The mixture was stirred at 0° for 1 hr and poured into ice.

(3) Melting points were taken in capillary tubes and are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. All the hydrazopyrimidines were also identified by their molecular weights, determined by mass spectroscopy.

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Synthesis of 5,7-Dioxo-3-methyl-5,6,7,8-tetrahydropyrimido[5,4-*e*]-*as*-triazine[1]

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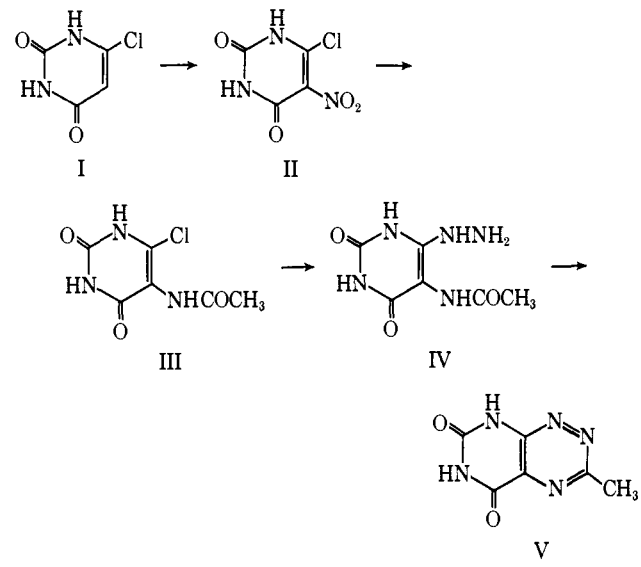
In connection with our investigation of compounds related to a series of pyrimido[5,4-*e*]-*as*-triazine antibiotics [toxoflavin (xanthothricin), fervenulin (planomycin)[2-5]], one of the parent N-unsubstituted derivatives, 5,7-dioxo-3-methyl-5,6,7,8-tetrahydropyrimido[5,4-*e*]-*as*-triazine (V), was synthesized (Scheme I). Compound V is the 7-aza analog of 6-methyllumazine.[6-9]

SCHEME I



As expected, the uv absorption spectra (pH 1 and 11) of V resembled more closely those of 1-demethyltoxoflavin[5] rather than those of toxoflavin[2,4] or fervenulin.[3]

Experimental Section

4-Chloro-5-nitrouracil (II).—The reported procedure gave low yields.[10] The following is a modified procedure. 4-Chlorouracil[10] (11.5 g, 0.08 mole) was added in small portions to 36 ml of concentrated H_2SO_4 at 15° with stirring. To the solution at 0–5° was added, dropwise, 12 ml of fuming HNO_3 (90%). After addition, the mixture was stirred for 30 min at 10°. The resulting yellow solution was poured, with vigorous stirring, into 60 g of

(1) This investigation was supported by the Cancer Chemotherapy National Service Center, National Cancer Institute, of the National Institutes of Health, U. S. Public Health Service, Contract No. PH-43-65-94.

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(3) G. D. Daves, Jr., R. K. Robins, and C. C. Cheng, *J. Org. Chem.*, **26**, 5256 (1961).

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