TABLE I

STEROIDAL γ-LACTONES

Compd	% yield	Crystn solvent	Mp, °C[a]	λ_{max},[b] mμ	ε	$[\alpha]D$,[c] deg	Formula	Calcd, % C	Calcd, % H	Found,[d] % C	Found,[d] % H
I	69	Benzene–hexane	235–238	249	12,300	+145[g]	$C_{25}H_{34}O_4 \cdot 0.5H_2O$	73.68	8.66	73.94	8.61
		Acetone–benzene	232–234				$C_{25}H_{34}O_4 \cdot 0.5C_3H_6O$	74.50	8.72	74.61	8.46
II	49	Benzene–hexane	217–219	221	15,000	−38[h]	$C_{23}H_{30}O_3$	77.92	8.53	77.46	8.49
III	100	Methanol–water	248–255[e]	220	10,600	−133	$C_{23}H_{32}O_3$	77.49	9.05	77.48	9.21
IV	66	Ether	252–253	219	8,650	−129	$C_{25}H_{34}O_4 \cdot 0.5H_2O$	73.68	8.66	73.93	8.44
V	40	$CHCl_3$–ether	260–263	223	13,800	+102	$C_{23}H_{32}O_3 \cdot H_2O$	75.34	8.60	75.60	8.34
				248[f]							

[a] Capillary tube, corrected. [b] In methanol. [c] At 24–26° and 1% in $CHCl_3$ except as indicated. [d] Microanalyses are by Mr. C. E. E. Childs (Ann Arbor) and by Mr. F. H. Oliver (Hounslow). [e] Lit.[2] mp 257–259°. [f] Inflection. [g] 0.72% in acetone. [h] 0.75% in $CHCl_3$.

TABLE II

INFRARED[a] AND NMR DATA[b]

	Infrared, cm⁻¹						Nmr, δ							
Compd	OH	Lactone	Ketone	Δ^{20}	Δ^4	Ester	Cyclopropyl	18-Me	19-Me	21-Me	C-4	C-6	C-22	C-3
I	3440	1776	1653		1608		0.38	1.11	1.13	1.62	5.85			
II		1744	1665	1632	1618			0.95	1.19	2.15	5.44		5.87	
III	3530, 3480	1743		1634				0.94	1.02	2.15		5.38	5.87	3.50
IV		1750		1632		1730, 1249		0.96	1.04	2.15		5.43	5.88	4.54
V		1745	1658	1635	1604		0.46	1.00	1.26	2.17	5.66		5.87	

[a] Infrared determinations were made by Mr. E. Schoeb (Ann Arbor) using a Beckman Model IR-9. KBr disks were used except for II which was run in $CHCl_3$ solution. [b] Nmr spectra were obtained by Mr. R. B. Scott (Ann Arbor) using a Varian A-60. Except for I, which was run in pyridine, solutions in $CDCl_3$ were used.

TABLE III

PROPORTIONS OF REACTANTS

Product	Me_3SOI, mmoles	NaH, mmoles	DMSO, ml	Steroid, mmoles
I	5	5.3	90	4.75
II		5.6	100	5.6
III		5.3	100	5.3
IV	4.5	4.5	150	4.3[a]
V		5	55	2.4[b]

[a] 3β,17α-Diacetoxypregn-5-en-20-one, mp 174–177°, from pyridine–acetic anhydride acetylation of 17α-acetoxypregnenolone. [b] G. D. Searle & Co., South African Patent 65/4327 (Feb 14, 1966).

ones yielded the corresponding $\Delta^{20(22)}$-lactones:[2] 17α-hydroxy-3-oxo-23-norchola-4,20(22)-dienic acid γ-lactone (II), 3β,17α-dihydroxy-23-norchola-5,20(22)-dienic acid γ-lactone (III), 3β-acetoxy-17α-hydroxy-23-norchola-5,20(22)-dienic acid γ-lactone (IV), and 17α-hydroxy-3-oxo-6-spirocyclopropyl-23-norchola-4,20-(22)-dienic acid γ-lactone (V), when run with sodium hydride in dimethyl sulfoxide. Our work has not permitted the assignment of configuration to the C-20 position.

Compounds I and II were tested for biological activity. Compound II failed to prevent litters being born when fed to mice at 10 mg/kg/day. Neither compound antagonized 1 μg of aldosterone in the salt-loaded rat at dose levels of 30–50 mg/kg. Neither compound showed any progestational effect in the McPhail assay in rabbits at a dose level of 20 times progesterone by subcutaneous and 100 times norethindrone by oral administration.

(2) H. G. Lehmann, *Angew. Chem.*, **77**, 808 (1965), has since reported that the reaction leads to the unsaturated lactone (III) in the presence of equimolar amounts of NaH while catalytic amounts of NaOH for short reaction times allow isolation of the hydroxylactone. Also N. H. Dyson, J. A. Edwards, and J. H. Fried, *Tetrahedron Letters*, 1841 (1966), have reported the conversion of 17α-acetoxypregna-4,6-diene-3,20-dione to the corresponding unsaturated lactone by NaH in DMSO.

Experimental Section

General Procedure.—Sodium hydride was added to dimethyl sulfoxide (DMSO) under nitrogen and stirred at room temperature for about 1 hr. A solution of trimethylsulfoxonium iodide in DMSO was added and stirred for 15–30 min. The steroid was suspended in DMSO and added in one portion. The mixture was then stirred overnight and worked up by pouring into ice–water, separating, and crystallizing. When trimethylsulfoxonium iodide was not used, the remainder of the procedure was unchanged. In two of the preparations, II and V, the reaction mixture was acidified with 3 *N* HCl after being quenched in ice–water. The reactant proportions are given in Table III.

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The Synthesis of Some Aryl Nitrogen Mustard Derivatives of Estrogens[1]

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The synthesis of several new steroidal compounds has been accomplished from the corresponding intermediates 4-aminoestrone 3-methyl ether (I) and 2-aminoestrone 3-methyl ether (II). These mustards were prepared because the literature describes no previous attempts to study aryl nitrogen mustards of steroids and also because of their potential as anticancer agents.

(1) This investigation was supported by the fund from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service, Bethesda, Md. (Grant Ca-06492-03).

(2) The data published here are taken from a thesis submitted by Charles R. Walk in partial fulfillment for the degree of Master of Science.

(3) To whom inquiries should be addressed.

TABLE I

PHYSICAL DATA AND ANALYSES OF SOME ESTRATRIENES

Compd[a]	Mp, °C	Yield, %	Prepn procedure	Formula	Calcd (found), % C	H	N	Cl	P	C	H	N	Cl	P	[α]D, deg (dioxane)[b]	λmax, mμ (ε × 10⁻³)
III	143–146	47	A	$C_{22}H_{31}NO_4$	71.20	8.58	3.61			71.40	8.29	3.49			+82 (24.0)	279–287 (1.95)
IV	Oil	55	C	$C_{22}H_{31}Cl_2NO_2$	65.00	7.36	3.30	16.71		65.11	7.65	3.45	16.6		+74 (24.0)	279–287 (2.21)
V	161–165	75	B	$C_{22}H_{33}NO_4$	70.92	9.06	3.60			70.50	8.73	3.81			+24 (23.0)	222 (8.45), 279–287 (2.80)
VI	Oil	40	C	$C_{22}H_{32}Cl_4NO_4P$	50.84	5.94	2.58	26.1	5.7	50.14	6.28	2.68	21.7	5.3		280–288 (1.57)
VII	118–121	65	A	$C_{24}H_{35}NO_4$	71.20	8.58	3.61			71.18	8.83	3.72			+120 (23.0)	247–258 (3.74), 290 (3.24)
VIII	Oil	50	C	$C_{24}H_{35}Cl_2NO_2$	65.00	7.36	3.30	16.71		65.63	7.76	3.36	16.45		+110 (21.0)	255 (4.56), 288 (3.44)
IX	151–154	85	B	$C_{22}H_{33}NO_4$	70.92	9.06	3.60			70.97	9.34	3.26			+77 (24.0)	244–255 (3.50), 291 (3.12)

[a] All crystalline compounds were recrystallized from ether. [b] Optical rotations were determined as a 1% solution in $CHCl_3$. [c] Ultraviolet spectra were determined in 95% ethanol using a Perkin-Elmer Model 202 spectrophotometer.

I, R = H; R¹, R² = O
III, R = CH_2CH_2OH; R¹, R² = O
IV, R = CH_2CH_2Cl; R¹, R² = O
V, R = CH_2CH_2OH; R¹ = OH; R² = H
VI, R = CH_2CH_2Cl; R¹ = PO_2Cl_2; R² = H

II, R = H; R¹, R² = O
VII, R = CH_2CH_2OH; R¹, R² = O
VIII, R = CH_2CH_2Cl; R¹, R² = O
IX, R = CH_2CH_2OH; R¹ = OH; R² = H

The assignment of structure throughout this work is dependent upon the structures of the two starting materials, *i.e.*, 2-nitro- and 4-nitroestrone. The position of the nitro group in each case was confirmed absolutely by means of its nmr spectrum.[4] Reduction of the nitro compounds[5,6] gave the corresponding 2- and 4-aminoestrones.

The conversions of 2- and 4-aminoestrones to the corresponding bis-2- and -4-hydroxyethylamino compounds (III and VII), according to the procedure of DeGraw and Goodman,[7] proceeded smoothly and in high yield. Treatment of III and VII, respectively, with $POCl_3$ gave IV and VIII. Reduction of III and VII with sodium borohydride gave the corresponding 17β-hydroxy compounds. Treatment of V with $POCl_3$ gave the mustard at the 4 position with a phosphate ester grouping at the 17 position. The ultraviolet spectrum of this compound was identical with that of the 4-nitrogen mustard with a 17-keto group (IV) and remained unaltered when the solution was acidified. This indicated that an aromatic mustard was present. The nmr and infrared spectra agreed with those of previous aromatic mustards. The analysis indicated that one phosphorus and four chlorines were present in the molecule; therefore, the phosphate ester indicated in structure VI was proposed.

Biological Activities.—The mustards, diols, and triols were all found to be inactive at 10^4 μg/ml in the cell culture and cell line KB. These compounds were found to be nontoxic in the Fischer 344 rats at 15 mg/kg for 5 days.[8]

Experimental Section

Melting points were taken in a Thomas–Hoover apparatus and are corrected. The nmr spectra were measured with a Varian A-60 spectrometer at 60 Mc and 26 ± 0.3°. The samples were prepared as dilute solutions in $CDCl_3$. The analytical data were determined by Alfred Bernhardt Mikroanalytisches Laboratorium, Mülheim, Germany.

Syntheses of compounds in Table I were carried out by three general procedures each of which is illustrated by a detailed procedure.

Procedure A. 4-[Bis(2-hydroxyethyl)amino]-3-methoxy-1,3,5-estratrien-17-one (III).—A mixture of 4-amino-3-methoxy-1,3,5-estratrien-17-one (II) (1.0 g), *p*-toluenesulfonic acid (100 mg), absolute ethanol (30 ml), and ethylene oxide (10 ml) was sealed in a stainless steel Parr peroxide bomb and heated on a steam bath for 18 hr. The solvent was removed under reduced

(4) H. H. Lin and T. C. Chou, submitted for publication.
(5) S. Kraychy, *J. Am. Chem. Soc.*, **81**, 1702 (1959).
(6) A. J. Tomson and J. P. Horwitz, *J. Org. Chem.*, **24**, 2056 (1959).
(7) J. DeGraw and L. Goodman, *J. Med. Chem.*, **7**, 213 (1964).
(8) These compounds were tested by the Cancer Chemotherapy National Service Center, National Institutes of Health, U. S. Public Health Service, Bethesda, Md. 20014.

pressure. The residue was treated with 10% aqueous $NaHCO_3$ and then extracted with ether. The organic layer was washed with 10% HCl and the aqueous layer was then made basic with 10% NaOH. The oil that separated was again extracted with ether. The organic layer was washed with water and dried (Na_2SO_4). Upon removal of the solvent a white solid was obtained, which was recrystallized from ether to yield 600 mg of III.

Procedure B. 4-[Bis(2-hydroxyethyl)amino]-3-methoxy-1,3-5-estratrien-17β-ol (V).—A solution of 4-[bis(2-hydroxyethyl)-amino]-3-methoxy-1,3,5-estratrien-17-one (III) (1.4 g) in methanol (50 ml) was treated with excess $NaBH_4$ (2.0 g), stirred for 1 hr at room temperature, and poured into aqueous $NaHCO_3$. The solution was extracted with ether and the organic layer was washed and dried (Na_2SO_4). The solvent was removed and the solid residue was recrystallized from methanol to yield 700 mg of V.

Procedure C. 4-[Bis(2-chloroethyl)amino]-3-methoxy-1,3,5-estratrien-17-one (IV).—A mixture of III (500 mg) and $POCl_3$ (10 ml) was heated on a steam bath for 2 hr. The excess $POCl_3$ was removed under reduced pressure and the remaining oil was dissolved in ether–benzene. The solution was washed with dilute HCl and then water. The organic layer was dried (Na_2SO_4), and the solvent was removed. The residue was dissolved in benzene (5 ml) and absorbed on a column of silica gel G (50 g). After removal of an oily side product by eluting with benzene, IV was eluted with ether–benzene (1:1) as an oil (300 mg), which would not crystallize. This reaction failed with $SOCl_2$.

Treatment of the triol (IX) with $POCl_3$ gave a compound that could not be identified. Acid shifted the ultraviolet absorption from 290 to 282–288 mμ. No hydroxyl absorption appeared in the infrared spectrum. The nmr spectrum showed the aromatic protons as singlets τ 2.3 and 3.17. The 3-methoxy and 13-methyl protons appeared where they did in the starting material. No other protons could be identified. *Anal.* Found: C, 43.23; H, 6.66; N, 2.28; Cl, 17.60; P, 7.6. It is conceivable that this material could be a dimer or polymer.

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Some Alkylating Derivatives of Nicotinic Acid. Synthesis and Antineoplastic Activities

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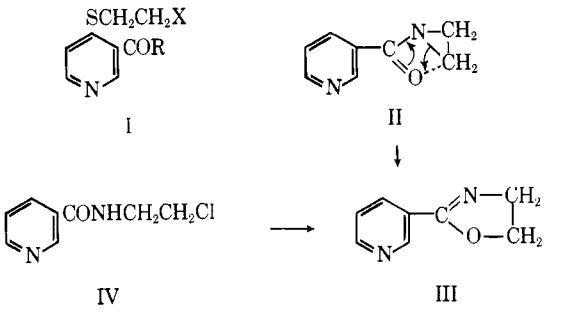
4-Substituted nicotinamides might act as stereospecific inhibitors of the glycolytic process by which many cancer cells derive an appreciable proportion of their energy requirements.[1,2] More effective and potentially irreversible antagonists may be produced by incorporating a chemically reactive grouping into the 4 substituent. The preparation of such derivatives containing alkylating groups is now described.

The preparation of 4-(2-bromoethylthio)nicotinic acid (I, R = OH; X = Br), isolated as its hydrobromide, has already been described.[2] The corresponding amide (I, R = NH_2; X = Br) has now been prepared by the action of ammonia on the mixed anhydride formed from the acid (I, R = OH; X = Br) and isobutyl chloroformate.

The acid (I, R = OH; X = Br) was readily obtained by the action of concentrated hydrobromic acid on the hydroxy acid (I, R = X = OH) but the hydroxy acid was recovered unchanged after prolonged heating with concentrated HCl. When heated with thionyl chloride the hydroxy acid gave an unstable product, presumably the hydrochloride of the acid chloride (I, R = X = Cl), which afforded 4-(2-chloroethylthio)nicotinic acid (I, R = OH; X = Cl), methyl 4-(2-chloroethylthio)nicotinate (I, R = OMe; X = Cl), and 4-(2-chloroethylthio)nicotinamide (I, R = NH_2; X = Cl) on treatment with HCl, methanol, and methanolic ammonia, respectively.



Many difunctional alkylating agents have greater carcinostatic activity than the corresponding monofunctional analogs.[3] The acid (I, R = H; X = Cl) could be converted into a novel type of difunctional alkylating agent, having two different alkylating groups, by the preparation of its ethylenimide (I, R = N=$(CH_2)_2$; X = Cl).

As a model for this synthesis the preparation of N-nicotinoylethylenimine (II) was examined. The action of nicotinoyl chloride on ethylenimine has been reported to give II[4] but the product has not been adequately characterized. When this preparation was repeated, only 2-(3-pyridyl)-2-oxazoline[4] (III), formed by internal alkylation of the initial product, was obtained. Ethylenimides can dimerize to give piperazines but the preparation of N,N′-dinicotinoylpiperazine by an unambiguous synthesis showed that this had not occurred.

Nicotinoylethylenimine (II) was prepared by condensing nicotinic acid with ethylenimine in the presence of dicyclohexylcarbodiimide, and N-4-(2-chloroethylthio)nicotinoylethylenimine (I, R = N=$(CH_2)_2$; X = Cl) was similarly obtained from the acid (I, R = OH; X = Cl).

Biological Data.—The results of screening tests against the Walker 256 tumor[5] and the lymphoid leukemia L1210[6] are given in Tables I and II. Only moderate activity (*ca.* 50% inhibition) at tolerated doses against the Walker tumor was shown by the hydrobromide of the acid (I, R = OH; X = Br) and nicotinoylethylenimine (II). Significant activity against the L1210 leukemia was shown only by the chloro acid (I, R = OH; X = Cl) at the maximum tolerated dose.

(1) W. C. J. Ross, *Ann. Rept. Brit. Empire Cancer Campn.*, **42**, 81 (1964).
(2) W. C. J. Ross, *J. Chem. Soc., Sect. C*, 1816 (1966).
(3) W. C. J. Ross, "Biological Alkylating Agents," Butterworth and Co. (Publishers) Ltd., London, 1962.
(4) G. I. Braz and V. A. Skorodumov, *Zh. Obshch. Khim.*, **26**, 770 (1956); *Chem. Abstr.*, **50**, 14711 (1956).
(5) The protocol for this carcinostatic assay is given by T. A. Connors, R. C. V. Mitchley, V. M. Rosenaner, and W. C. J. Ross, *Biochem. Pharm.*, **13**, 395 (1964).
(6) The protocol for this assay is esentially that given in *Cancer Chemotherapy Rept.*, **1**, 42 (1959); the C_{57}/DBA2 hybrid strain of mouse was used as host.