# Potential Anticancer Agents. II. Urethan-Type Nitrogen Mustards of Some Natural Sex Hormones

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In order to obtain drugs active against hormone-dependent forms of cancer, urethan-type nitrogen mustards of androsterone, epiandrosterone, estradiol, and ethynylestradiol have been synthesized. The structure of these derivatives has been confirmed by means of infrared and ultraviolet spectra. The antitumor activity of the new compounds has been studied.

As early as 1952, the steroid nucleus was tested as a specific carrier of the nitrogen mustard group for hormone-dependent tumors, but the compounds thus prepared displayed only moderate carcinostatic activities.[1] Attempts were also made to obtain latent antitumor compounds by attaching the N,N-bis(2-chloroethyl)-carbamoyl moiety to the steroid nucleus of cholesterol,[2a,b] estrone,[2c] testosterone,[2d] and hydrocortisone.[2e] Since estrone 3-[N,N-bis(2-chloroethyl)]carbamate was found to exhibit a 30% inhibition against mammary adenocarcinoma 755,[2a] and testosterone 17$\beta$-[N,N-bis-(2-chloroethyl)]carbamate the same order of inhibition toward Walker 256 carcinosarcoma and 75% inhibition of mammary adenocarcinoma MI,[3] similar derivatives of androsterone, epiandrosterone, estradiol, and 17$\alpha$-ethynylestradiol have now been synthesized.

3-[N,N-Bis(2-chloroethyl)]carbamoyl derivatives of androsterone (I) and epiandrosterone (II) were synthesized according to the method of Ciorănescu, *et al.*,[2d] for testosterone. The compounds crystallized with extreme difficulty. Coupling of the mustard moiety to the alcoholic or phenolic hydroxyl group of estradiol required a more selective procedure. Thus, treatment with phosgene in an inert solvent yielded the 17$\beta$-chlorocarbonate,[4] which in the presence of N,N-bis(2-chloroethyl)amine was converted to estradiol 17$\beta$-[N,N-bis(2-chloroethyl)]carbamate (III), isolated from the reaction mixture by column chromatography on alumina. In support of the location of the carbamate group at the 17 position, III was synthesized according to Scheme I. The infrared spectra of the compounds prepared by the two routes were identical.

17$\beta$-Estradiol 3-[N,N-bis(2-chloroethyl)]carbamate (V) was synthesized in three ways, in order to prove its structure: (a) treatment of estradiol with N,N-bis(2-chloroethyl)carbamoyl chloride in pyridine at room temperature,[3] (b) by heating the sodium salt of estradiol in an inert solvent with the same reagent, and (c) hydrogenation of estrone 3-[N,N-bis(2-chloroethyl)]carbamate with Raney nickel catalyst. The three products were found to be identical by comparison of their melting points, infrared spectra, and specific rotation, $[\alpha]^{20}D$ +50° (*c* 1, dioxane).

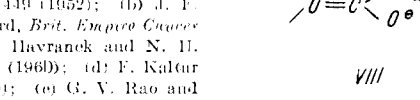
17$\alpha$-Ethynylestradiol 3-[N,N-bis(2-chloroethyl)]carbamate was prepared according to Nogrady.[2e] Unlike the case of estradiol, phosgenation of 17$\alpha$-ethynylestradiol afforded the 3-chloroformyl derivative, which was converted to VI after treatment with N,N-bis(2-chloroethyl)amine (see Scheme II).

Attempts to prepare the 17-carbamoyl derivative of 17$\alpha$-ethynylestradiol failed. Hydrogenation of VI yielded 17$\alpha$-ethylestradiol 3-[N,N-bis(2-chloroethyl)]-carbamate (VII). Data on compounds I–VII are presented in Table I.

As shown in the present and in previous papers[2c] as well as in those of other investigators,[5] the phenolic hydroxyl groups are easily converted to N,N-bis(2-chloroethyl)carbamoyl derivatives, on treatment with N,N-bis(2-chloroethyl)carbamoyl chloride, in pyridine solution at room temperature. Alcoholic hydroxyl groups do not appear to react under such conditions, but they are sensitive to direct phosgenation at room temperature, in an inert solvent, while phenolic hydroxyl groups require the presence of an HCl acceptor.

Infrared and ultraviolet spectra afforded evidence for the location of N,N-bis(2-chloroethyl)carbamoyl group in compounds I–VII. According to literature data[6] the tertiary carbamates should show the amide I band (C=O link) at 1687 ± 4 cm$^{-1}$. The carbonyl absorption of I–III was found to be within that range (see Table II). Compound IV exhibits a spectral shift which is rather difficult to explain, although the synthetic method, analytical data, and ultraviolet spectrum confirm the structure assigned to it.

The band in 3-[N,N-bis(2-chloroethyl)]carbamoyl derivatives is shifted toward higher frequencies, probably due to the electron-attracting effect of the phenyl group and to the larger contribution of a structure of type VII.[7] This explanation is supported by the fact that the shift toward higher frequencies in type IX



VIII IX

compounds depends upon R, namely, R = $NH_2 \cdot HCl$, $\nu_{C=O}$ 1730 cm$^{-1}$; R = $NO_2$, $\nu_{C=O}$ 1732 cm$^{-1}$; and R =

(1) (a) G. G. Hazen, University of Michigan; University Microfilms No. 3761, Ann-Arbor, Mich.; *Dissertation Abstr.*, **12**, 449 (1952); (b) J. F. Danielli, L. Hamilton, M. S. May, and P. J. Barnard, *Brit. Empire Cancer Campaign Ann. Rept.*, **34**, 398 (1956); (c) R. E. Havranek and N. H. Hoereubos, *J. Am. Pharm. Assoc., Sci. Ed.*, **49**, 328 (1960); (d) F. Kaltar and R. Birkenmayer, U. S. Patent 2,905,675 (1959); (e) G. V. Rao and C. C. Price, *J. Org. Chem.*, **27**, 205 (1962).

(2) (a) L. N. Owen, M. H. Beno, and A. M. Creighton, *Brit. Empire Cancer Campaign Ann. Rept.*, **32**, 417 (1954); (b) M. H. Benn, *J. Chem. Soc.*, 2365 (1961); (c) T. Nogrady, M. K. Vagi, and V. W. Adamkievics, *Can. J. Chem.*, **40**, 2426 (1962); (d) E. Ciorănescu and H. Răileanu, *Acad. Rep. Populare Române, Studii Cercetări Chim.*, **10**, 295 (1962); (e) D. Răileanu and E. Ciorănescu, *ibid.*, **11**, 433 (1963).

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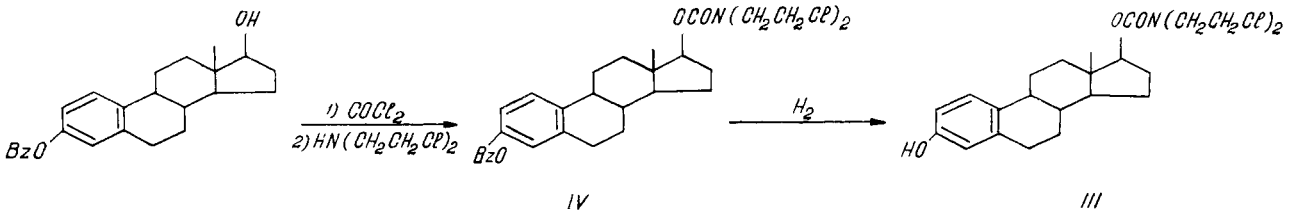
(5) (a) H. E. Skipper, C. E. Bryan, W. H. Riser, Jr., M. Welty, and A. Stelzenmuller, *Natl. Cancer Inst.*, **9**, 77 (1948); (b) C. G. Skinner, T. J. McCord, J. M. Ravel, and W. Shive, *J. Am. Chem. Soc.*, **78**, 2412 (1956).

(6) S. Pinchas and D. Ben Ishai, *ibid.*, **79**, 4099 (1957).

(7) C. M. Lee and W. D. Kumler, *ibid.*, **83**, 4593 (1961).

SCHEME I




SCHEME II

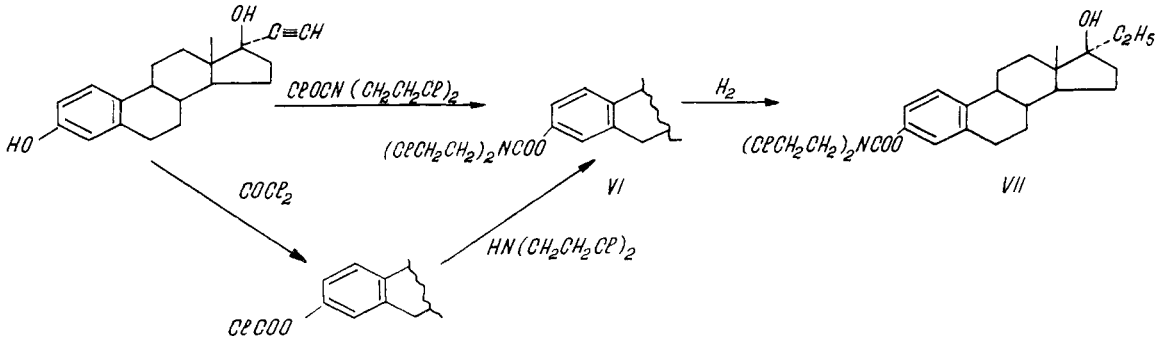



TABLE I

URETHAN-TYPE NITROGEN MUSTARDS OF SOME STEROID SEX HORMONES

| Compd | Mp, °C | Formula | Yield, % | Calcd, % | | | | Found, % | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | C | H | N | Cl | C | H | N | Cl |
| I | 110 | $C_{24}H_{37}Cl_2NO_3$ | 63.3 | 62.88 | 8.08 | 3.06 | 15.50 | 62.50 | 8.20 | 3.04 | 15.59 |
| II | 106–107 | $C_{24}H_{37}Cl_2NO_3$ | 63.2 | 62.88 | 8.08 | 3.06 | 15.50 | 62.80 | 8.08 | 2.98 | 15.51 |
| III | 130–132 | $C_{23}H_{31}Cl_2NO_3$ | 88.3[a] | 62.72 | 7.04 | 3.18 | 16.13 | 62.91 | 7.15 | 3.14 | 16.09 |
| IV | 83–84 | $C_{30}H_{37}Cl_2NO_3$ | 85.3 | 67.92 | 6.98 | 2.64 | 13.39 | 67.85 | 7.05 | 2.56 | 13.20 |
| V | 104–105 | $C_{23}H_{31}Cl_2NO_3$ | 72.7 | 62.72 | 7.04 | 3.18 | 16.13 | 62.64 | 6.95 | 3.18 | 16.30 |
| VI | 142 | $C_{25}H_{31}Cl_2NO_3$ | 73.4 | 64.65 | 6.68 | 3.01 | 15.30 | 64.85 | 6.77 | 3.02 | 15.27 |
| VII | 115–117 | $C_{25}H_{35}Cl_2NO_3$ | 79.6 | 64.10 | 7.47 | 2.98 | 15.16 | 63.98 | 7.43 | 2.97 | 15.39 |

[a] From IV.

TABLE II

SPECTROSCOPIC DATA ON COMPOUNDS I–VII

| Compd | Ultraviolet spectra,[a] mμ | Infrared spectra,[b] cm⁻¹ Amide I band | Infrared spectra,[b] cm⁻¹ OH stretching band |
|---|---|---|---|
| I | ... | 1692 (s) | ... |
| II | ... | 1694 (s) | ... |
| III[c] | 282.3, 288 | 1685 (s) | 3415 (m) |
| IV | 278.5, 286.8 | 1702 (s) | ... |
| V | 270.7, 276.5 | 1713 (s) | 3500 (m) |
| VI | 268.7, 275.5 | 1716 (s) | 3450 (m) |
| VII | 269.1, 285.8 | 1717 (s) | 3512 (m) |

[a] Ultraviolet spectra were determined in alcoholic solution with a CF4 Optic Milano spectrophotometer. [b] Infrared spectra were determined on a U.R.10 Zeiss Jena DDR spectrophotometer in KBr disks. [c] $\lambda_{max}$ 280 mμ for estradiol.

CHO, $\nu_{C=O}$ 1735 $cm^{-1}$. The position of OH group stretching bands in the range of 3520–3400 $cm^{-1}$ also supports the structures attributed by us to the derivatives I–VII.

As shown in Table II, the ultraviolet spectra also permit the differentiation between 3- and 17-substituted derivatives of estrogens, as the N,N-bis(2-chloroethyl)-carbamoyl group in the 3 position causes a constant shift of about 10 mμ toward shorter wavelengths.

**Biological Activity.**—The antitumor activity of androsterone derivatives I and II is shown in Table III. The activity of the estrogen derivatives was tested on rats bearing Walker 256 carcinosarcoma (doses of 50 mg/kg, 0.167–0.1 $LD_{50}$) every 2 days, beginning with the 7th day after tumor transplanation up to the 23rd day. Under these conditions VI caused 60% and V 20% inhibition.

From Zimmel's[3] and our[8] observations on urethan-type nitrogen mustards of certain synthetic estrogens, it follows that derivatives with antitumor activity may retain hormonal properties.

## Experimental Section

**Epiandrosterone 3β-Chloroformate.**—To a solution of 2 g (0.007 mole) of epiandrosterone (mp 174–177°) in 30 ml of dioxane, 4 g (0.04 mole) of phosgene in 15 ml of benzene was added at room temperature. After standing overnight the solution was concentrated to give an oily residue which crystallized after addition of 15 ml of petroleum ether; 2.3 g (95.0%) of the chloroformate, mp 112°, was obtained.

Androsterone 3α-chloroformate (from androsterone, mp 183°), mp 126–127° (yield 95.5%), was prepared similarly.

**Epiandrosterone 3β-[N,N-Bis(2-chloroethyl)]carbamate (II).**—N,N-Bis(2-chloroethyl)amine from 3 g (0.016 mole) of the hydrochloride in 30 ml of benzene was added to 2 g (0.0056 mole) of epiandrosterone chloroformate in 25 ml of benzene at room temperature with stirring. White crystals of nitrogen mustard hydrochloride appeared on standing overnight. Filtration, removal of the solvent under reduced pressure, and trituration with petroleum ether (bp 60–90°) gave 1.7 g of II (63.2%), mp 105–106°. Recrystallization from methanol raised the melting point to 106–107°.

Compounds I and III (from 3-benzylestradiol 17β-chloroformate) and VI were similarly prepared. During the synthesis of 17β-estradiol 3-[N,N-bis(2-chloroethyl)]carbamate (III), the chloroformate was not isolated. The final product was obtained

(8) I. Niculescu-Duvăz, C. Neacsu, V. Dobre, and V. Feyns, *Neoplasma*, in press.

Table III
Antitumor Activity of Urethan-Type Nitrogen Mustard Derivatives from Androgenic Hormones I and II

| No. | Tumor | Dose, mg/day I | Dose, mg/day II | Period of treatment, days I | Period of treatment, days II | Inhibition I T/C | Inhibition I % | Inhibition II T/C | Inhibition II % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Walker 256 carcinosarcoma | 8[a] | 10[a] | 17[b] | 17[b] | 19.9 ± 2.63/39.2 ± 2.29 | 49 | 15.8 ± 3.67/25.3 ± 2.05 | 38 |
| 2 | Erlich carcinoma | 0.2 | 0.5 | 16[c] | 16[c] | 3.1 ± 0.48/5.5 ± 1.34 | 43 | 3.0 ± 0.40/5.5 ± 1.34 | 45 |
| 3 | Mammary adenocarcinoma | 0.2[a] | 0.5[a] | 17[d] | 17[d] | 2.0 ± 0.77/3.7 ± 0.52 | 45 | 2.2 ± 0.44/3.7 ± 0.52 | 40 |
| 4 | Sarcoma 180 | 0.2 | 0.5 | 8[e] | 8[e] | 5.3 ± 0.74/11.3 ± 1.21 | 53 | 3.4 ± 0.82/11.3 ± 1.21 | 69 |
| 5 | Carcinoma O-Ya | ... | 5 | ... | 8[f] | ... | ... | 16.4 ± 2.08/46.6 ± 3.20 | 64 |

[a] Given every 2 days. [b] Treatment begins 7 days after tumor transplantation in rats. [c] Treatment begins 8 days after tumor transplantation in mice. [d] Treatment begins 3 days after tumor transplantation in mice. [e] Treatment begins 24 hr after tumor transplantation in mice. [f] Treatment begins 24 hr after tumor transplantation in rats.

as a gum, purified by chromatography on alumina column. After placing the material on a column in benzene solution, the column was eluted with benzene, benzene–absolute ethanol mixtures (99:1, 98:2, 95:5, 50:50), and absolute ethanol. Compound III was isolated from the 99:1 benzene–absolute ethanol fraction and recrystallized from toluene.

**17α-Ethynylestradiol 3-Chloroformate.**—To 2 g (0.007 mole) of 17α-ethynylestradiol and 3 ml of triethylamine in 20 ml of dioxane, 4 g (0.016 mole) of phosgene in 25 ml of anhydrous benzene at 0° was added with stirring. After standing overnight at room temperature, the triethylamine hydrochloride was removed by filtration and the solution was concentrated under vacuum. The resulting oil, triturated with petroleum ether, gave 2.2 g (85.3%) of the chloroformate, mp 138–139°.

3-Benzylestradiol 17β-chloroformate (IV), mp 104–106° (89.4%), was prepared similarly but without an HCl acceptor ($Et_3N$).

**17α-Ethynylestradiol 3-[N,N-Bis(2-chloroethyl)]carbamate (VI).**—N,N-Bis(2-chloroethyl)carbamoyl chloride (2.4 g, 0.012 mole) was added to a solution of 3 g (0.01 mole) of 17α-ethynylestradiol in 30 ml of pyridine. After 3 days of standing at room temperature, the reaction mixture was poured onto ice–water containing a small quantity of methanol. VI (73.4%, 3.45 g) crystallized on standing. A recrystallization from methanol gave an analytical sample, mp 141–142°.

**17β-Estradiol 3-[N,N-Bis(2-chloroethyl)]carbamate (V).**—Estradiol (1.5 g, 0.0055 mole) was added to 10 ml of anhydrous ethanol in which 0.13 g (0.0056 g-atom) of Na had been dissolved. After removal of the solvent under vacuum the solid was suspended in 20 ml of toluene and 1.2 g (0.006 mole) of nitrogen mustard chloroformate was added with stirring. The mixture was refluxed for 4 hr. After filtration from NaCl and removal of the solvent under reduced pressure, the residue was recrystallized from benzene–petroleum ether, giving 1.6 g (72.7%) of V, mp 104–105°.

**17β-Estradiol 17β-[N,N-Bis(2-chloroethyl)]carbamate (III).** A solution of 3 g (0.0056 mole) of IV in 20 ml of absolute ethanol was hydrogenated in the presence of 2 g of 5% Pt–C. The theoretical amount of $H_2$ was absorbed after 2 hr. After filtration from the catalyst and removal of the solvent, 2.2 g (88.3%) of crystalline III was obtained; mp 128–130°, after recrystallization from toluene, mp 130–132°.

**17α-Ethylestradiol 3-[N,N-Bis(2-chloroethyl)]carbamate (VII).**—Compound VI (2 g, 0.0044 mole) and 1 g of 5% Pt–C in 20 ml of EtOH were shaken under hydrogen at atmospheric pressure. The theoretical amount of $H_2$ was absorbed after 1.5 hr. The catalyst was removed by filtration, and the solvent was distilled under vacuum giving 1.6 g (79.6%) of VII, mp 115–117° after recrystallization from petroleum ether.

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# Histamine Releasers. III. Dibasic Acid Amides of 4-Phenyl-4-aminomethylpiperidines

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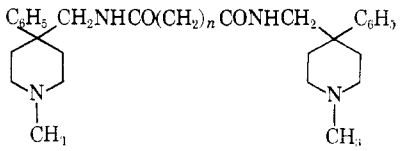
A series of 1-alkyl-4-phenyl-4-aminomethylpiperidine amides of various dibasic acids were found to have histamine-releasing activity in dogs. The most potent compound was 4,4′-dimethyl-N,N′-4-phenyl-4-piperidylmethylterephthalamide (XIII). An exploration of the structure–activity relationship in this area is described.

In 1955, Chiavarelli, *et al.*,[1] in quest of compounds with curare-like activity, discovered a new series of hypotensive agents (later known to be histamine releasers), which were amides derived from 1-methyl-4-phenyl-4-aminomethylpiperidine and aliphatic dibasic acids of formula I. They found that a peak in activity occurred when $n$ equaled 8. It was also reported that



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quaternization of the piperidine nitrogen or reduction of the carbonyl groups caused a loss of activity. With these data we began an exploration of the structure–

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