N-MUSTARD DERIVATIVES OF ESTROGENS

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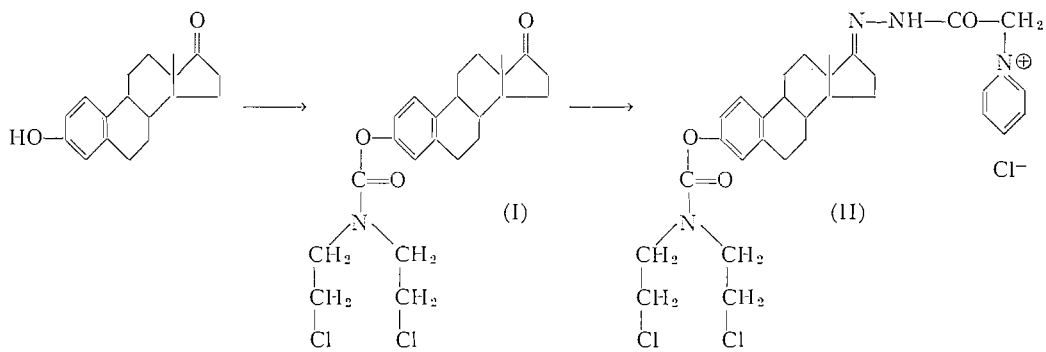
ABSTRACT

N-Mustard urethanes of estrone and stilbestrol, as well as an N-mustard-phosphoramidate of stilbestrol, were synthesized. Only the estrone derivative proved to be slightly active against an adenocarcinoma.

Efforts to influence the transport characteristics of nitrogen mustards resulted in the synthesis of a large number of compounds (1), several among them bearing steroid carriers (2–6). Most of the steroids used were, however, physiologically inactive in themselves, e.g. cholestene, ergostatriene, and stigmastadiene. 3β-[Bis-(β-chloroethyl)-aminoethyl]-Δ^5-cholestene (4) was reported to be inactive; 3-cholesteryl-N-[4′-N-bis-(β-chloroethyl)-aminophenyl]-carbamate (5) and 3-cholesteryl-[4′-N-bis-(β-chloroethyl)-aminophenyl]-sulphonate (6) were found to be nontoxic, but possessed a slight activity only. Steroid hormones were used as carriers by Burstein and Ringold (7) as well as by Rao and Price (8).

We prepared a few N-mustard derivatives of estrone and diethylstilbestrol, hoping to be able to influence estrogen-dependent tumors more selectively.

3-Esteryl-bis-(β-chloroethyl)-carbamate (I) was obtained from estrone and chloroformyl-bis-(β-chloroethyl) amine:



Due to its water insolubility, it was difficult to administer compound I to experimental animals. Therefore a water-soluble but amorphous hydrazone (II) with Girard's P reagent was also prepared.

The same urethane-type mustard (IV) (cf. ref. 9) was also synthesized from diethylstilbestrol, in the usual way.

Stilbestrol-phosphate is reported (10) to have a beneficial effect on prostate tumors, because the high phosphatase activity of prostatic tissue liberates stilbestrol selectively from the ester. It seemed, therefore, interesting to see a N-mustard-phosphate bound to stilbestrol. It was expected that a double selectivity should result: first, the selective

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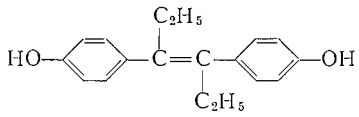

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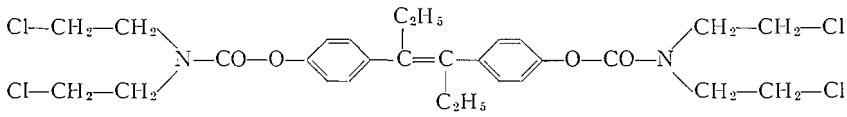
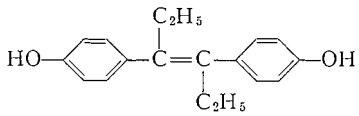


(IV)

accumulation of the compound in prostatic or mammary tissue, due to the carrier, and secondly, the preferential splitting of the phosphate bond at the sites of high phosphatase activity. Moreover, the "hidden" N-mustard group should be nontoxic at sites of low phosphatase activity, thus decreasing the general toxicity.

To test this hypothesis, the diethylstilbesteryl-phosphoramidic chloride (V) was prepared, according to the method of Friedman and Seligman (11). From this, we wished to prepare either the free phosphoric acid or an amide. After lengthy experimentation, it



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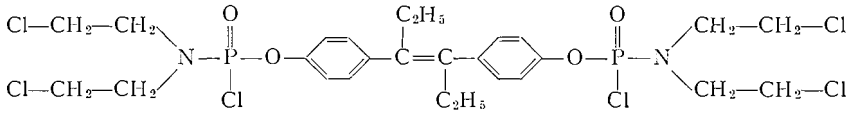

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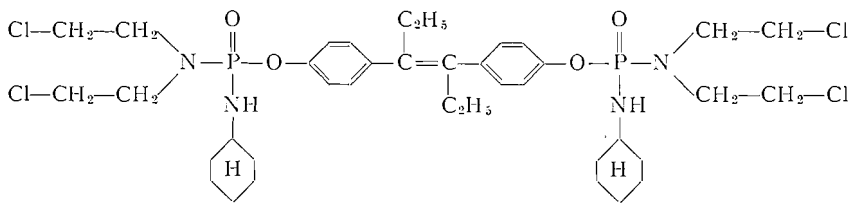

(VI)

was found that only the cyclohexyl amide (VI) could be obtained as a crystalline solid. The free acid and amides with aniline, piperidine, or morpholine were oily.

Pharmacological Results

Screened on mammary adenocarcinoma BN/10232, the estrone urethane (I) showed a 30% inhibition at a dosage level of 5 mg per day per mouse for 14 days, with a 15% mortality. Interestingly, the analogous stilbestrol derivative (IV) proved inactive at the

same dosage level, with a 5% mortality. Tested under similar circumstances, the stilbestrol-phosphoramidate (VI) was inactive too.

EXPERIMENTAL*

3-Esteryl-bis-(β-chloroethyl)-carbamate (I)

Ten grams of estrone (USP grade) and 7.8 g chloroformyl-bis-(β-chloroethyl)amine in 37 ml pyridine solution stood for 4 days at room temperature. When the solution was poured into ice water a solid crystallized out. The precipitate was filtered off, washed with water and methanol, and recrystallized from 250 ml isopropanol, yielding 10.02 g (52.4%) of colorless crystals, m.p. 143–145°. $[\alpha]_D^{20}+109.5°$ (*c* 2, chloroform). Anal. Calc. for $C_{23}H_{29}Cl_2NO_3$: C, 63.01; H, 6.67; N, 3.20. Found: C, 63.2; H, 6.9; N, 3.3.

Hydrazone with Girard's P Reagent (II)

Compound I (0.44 g) and Girard's P reagent (0.19 g) were refluxed in a mixture of 10 ml ethanol and 1 ml acetic acid, for 1 hour. On evaporation, the solution left a colorless water-soluble syrup which solidified to an amorphous precipitate when it was triturated with diisopropyl ether. In a few months it turned brown. Anal. Calc. for $C_{36}H_{37}Cl_3N_4O_3$: Cl, 17.5. Found: Cl, 17.1.

Diethylstilbesteryl bis-[di-(β-chloroethyl)]-carbamate (IV)

The solution of 3.14 g diethylstilbestrol (III) and 4.28 g (3.12 ml) of chloroformyl-bis-(β-chloroethyl)-amine in 10 ml dry pyridine stood at room temperature for 4 days. The solution was decanted from the precipitated pyridine hydrochloride, evaporated under reduced pressure, and the semisolid mass freed from the last traces of pyridine by azeotropic distillation with toluene. Recrystallized from methanol, it yielded 2.94 g (48.8%) colorless crystals, m.p. 139–140°. Anal. Calc. for $C_{28}H_{34}Cl_4N_2O_4$: C, 55.65; H, 5.67; N, 4.67. Found: C, 55.5; H, 5.9; N, 4.7.

Diethylstilbesteryl bis-[di-(β-chloroethyl)-phosphoramidic chloride] (V)

Diethylstilbestrol (1.57 g), bis-(β-chloroethyl)-phosphoramidic dichloride (2.60 g), and triethylamine (1.4 ml) were refluxed and stirred in 25 ml dry benzene for 1½ hours. After the reaction mixture was chilled, the separated triethylamine hydrochloride was filtered off (95.6% of the theoretical amount), and the benzene solution evaporated. The resulting colorless syrup (3.07 g, 86.1%) was used for further experiments. Anal. Calc. for $C_{26}H_{34}Cl_6N_2O_4P_2$: N, 3.93. Found: N, 3.78.

Diethylstilbesteryl bis-[N-di-(β-chloroethyl)-N'-cyclohexyl-phosphorodiamidate] (VI)

The phosphoramidic chloride (V) prepared from 7.6 g diethylstilbestrol was refluxed and stirred with 11.0 ml cyclohexylamine in 150 ml dry toluene for 4 hours, until neutral. The separated cyclohexylamine hydrochloride was filtered off from the hot reaction mixture. On cooling, the solution deposited 9.85 g of colorless crystals, which was recrystallized from 135 ml isopropanol, yielding 7.87 g (38.6%) of compound VI, melting at 206–208°. Anal. Calc. for $C_{38}H_{58}Cl_4N_4O_4P_2$: C, 54.16; H, 6.94; Cl, 16.38; N, 6.65. Found: C, 54.3; H, 7.0; Cl, 16.2; N, 6.7.

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**All melting points are uncorrected. Microanalyses by Dr. C. Daesslé, Microanalytical Laboratory, Montreal.*

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