Notes

Synthesis of Steroidal Nitrosoureas with Antitumor Activity

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Four steroidal nitrosoureas with structures which may permit specific binding to estrogen receptor were synthesized. Inhibitory activity was observed against the growth of the DMBA-induced transplantable rat mammary tumor 13762.

Among the cancer chemotherapeutic agents, two of the best known groups are the steroid hormones and the alkylating agents. In the last two decades several reports have appeared using estrogenic steroid hormones as carriers of alkylating agents in an attempt to produce antitumor agents with greater specificity of action.[1-5] However, results from this approach have been disappointing. Only two agents, namely, estramustine phosphate and estradiol mustard, have been used in clinical trials.[6-8]

The discovery of estrogen receptor (ER) with specific binding affinity in 50–60% of human breast cancer[9] provided a new approach in this field. More specific antitumor activity may be achieved by taking advantage of the presence of ER which may selectively accumulate certain cytotoxic agents with binding affinity for ER.

It has been established that in order to obtain the highest binding affinity for ER the estrogenic steroid hormone with an estra-1,3,5(10)-triene skeleton must have the 3- and 17β-hydroxyl groups available, presumably to form hydrogen bonds with ER protein at the binding site.[10,11] In estramustine phosphate and estradiol mustard, these hydroxyl groups are used to link the steroid to the alkylating moiety. Binding sites of these two agents suggested that they have very little, if any, binding affinity for ER. It has been suggested that the relatively weak antitumor activity of these agents against breast cancer is due partly to their inability to bind to ER and thereby to accumulate in ER-positive tumor cells.[12,13]

We have been interested in the synthesis of steroidal nitrosoureas with moieties which may permit binding to ER and may demonstrate antitumor activity. This paper reports the synthesis of two groups of steroidal nitrosoureas which are analogues of 3,17β-estradiol. In one group (**5a,b**) the 3- and 17β-hydroxyl functions were preserved and in the other group (**6a,b**) the 17β-hydroxyl function was replaced by a ureidal-NH function so that the hydrogen-bond-forming ability at this position might be retained.

Chemistry. Synthesis of steroidal nitrosoureas was carried out according to Hardegger's one-step approach for the synthesis of streptozotocin and its analogues.[14] Reaction of an amine with an *N*-alkyl-*N*-nitrosocarbamoyl azide gave directly a nitrosourea having the nitroso group in the desired position. The initial step in the synthesis of **5a** and **5b** was conversion of the 3-hydroxyl group of estrone to the corresponding benzyl ether. Reaction of 3-(benzyloxy)estra-1,3,5(10)-trien-17-one (**1**) with dimethylsulfonium methylide yielded the 17β-spirooxirane **2** which was converted into the hydroxy azide derivative **3** by treatment with NaN_3 and H_3BO_3. Hydrogenation reduced the azide group of **3** to amine and at the same time cleaved the benzylic ether to form the amino alchol **4**, which reacted with the alkylnitrosocarbamoyl azide to yield the corresponding nitrosoureas **5a** and **5b**. Similarly, reaction of the 17β-amino-3-hydroxyestra-1,3,5(10)-triene, obtained from reduction of estrone oxime, with the *N*-alkyl-*N*-nitrosocarbamoyl azide furnished **6a** and **6b** (see Scheme I).

Table I. Antitumor Activity of Nitrosourea Derivatives from Estrogenic Steroid Hormone against Rat DMBA-Induced, Transplantable Mammary Tumor 13762

compd	dose, (mg/kg)/-day	% inhibition[a]
5a	2	0
	10	35
	40	85
5b	2	0
	10	10
	40	23
6a	2	0
	10	50
	40	100
6b	2	4
	10	15
	40	15
CCNU	10	80

[a] Tumor growth in groups of six rats each, treated with the dose of compound indicated, was compared to that in 12 control rats treated with sesame oil, and the result is expressed as mean percent inhibition (% inhibition = C – T/C × 100, where T is tumor size in treated rats and C that in control rats).

Biological Data. Antitumor activity of compounds **5a,b** and **6a,b** was evaluated against the 13762 DMBA induced and transplantable rat mammary adenocarcinoma in Fischer/344 rats. The rats were implanted with 2- to 3-mm^3 fragments of tumor. Treatment was initiated 1 day after transplantation and continued for 20 days. Compounds were administered subcutaneously as suspensions in sesame oil. During the first 10 days, groups of six rats each were treated daily with the dose indicated and then with double the dose every 2 days from day 11 through day 19. On day 21, tumor size was measured with a caliper and expressed as the arithmetic mean of two perpendicular diameters. The antitumor activity of the four compounds is presented in Table I. Compounds **5a**, **6a** (at doses of 10 and 40 (mg/kg)/day), and **5b** (at 40 (mg/kg)/day)

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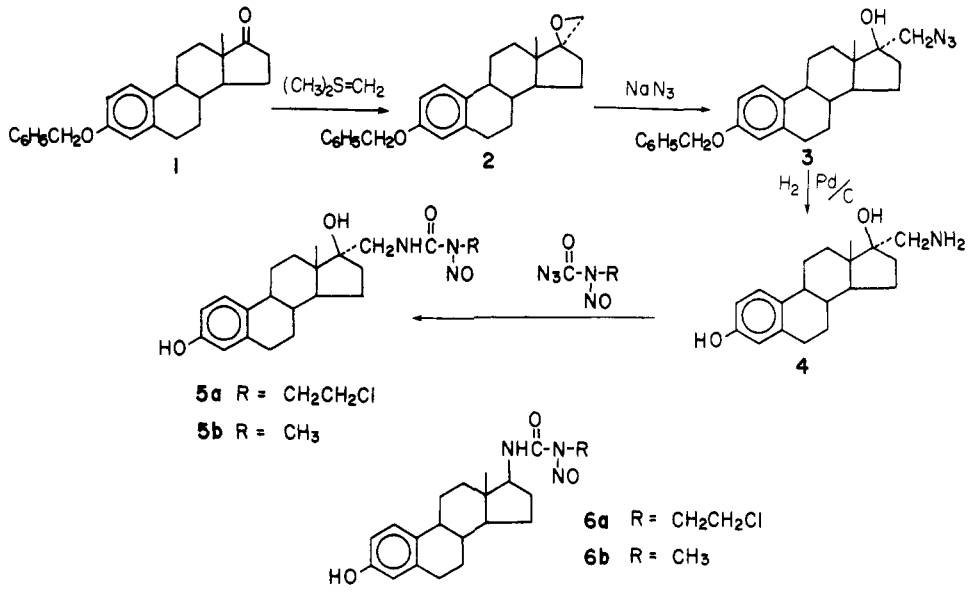
Scheme I



showed definite inhibition of tumor growth; results were statistically significant, as evaluated by the Student's t test. Since N-(2-chloroethyl)-N'-cyclohexyl-N-nitrosourea (CCNU), a nonsteroidal nitrosourea, also showed inhibition of tumor growth, a more detailed pharmacological evaluation will be required to determine whether these steroidal nitrosoureas will demonstrate a higher therapeutic index. Binding studies of these compounds to tumor ER will be undertaken.

Experimental Section

All melting points are uncorrected. IR spectra were recorded on a Perkin-Elmer 701A spectrometer; NMR spectra were obtained with a Varian A-56/60A spectrometer using $CDCl_3$ as solvent unless specified otherwise and tetramethylsilane as internal standard; mass spectra were obtained on a Finnigan 1015, RF-quadruple mass spectrometer. Purity of oily products was established by demonstrating a single spot on TLC. Microanalysis was performed by Dr. C. Daesale, Organic Microanalysis, Montreal.

3-(Benzyloxy)estra-1,3,5(10)-trien-17-one (1). Estrone (12 g, 44 mmol) was refluxed for 44 h with benzyl chloride (120 mL, 1.0 mol) in acetone (600 mL) containing a suspension of pulverized K_2CO_3 (60 g). The mixture was cooled to room temperature, and 200 mL of $CHCl_3$ was added. The precipitated K_2CO_3 was removed by filtration. The solvent and excess benzyl chloride were removed by evaporation in vacuo. Crystallization from $CHCl_3$–MeOH provided the ketone **1** (15 g, 94% yield) as a colorless crystal: mp 127–128 °C, lit.[15] mp 130–131 °C; IR (CH_2Cl_2) 1735 cm^{-1} (C=O); NMR δ 0.92 (s, 3 H, C_{18}-H), 5.08 (s, 2 H, benzylic H), 6.75–7.60 (m, unresolved, 8 H, Ar H).

(17*S*)-Spiro[3-(benzyloxy)estra-1,3,5(10)-trien-17,2′-oxirane] (2). A solution of ketone **1** (15 g, 42 mmol) in THF (100 mL) was added to a solution of freshly prepared dimethylsulfonium methylide (125 mmol) in Me_2SO (100 mL) and THF (100 mL) at 0 °C.[16,17] The mixture was stirred at 0 °C for 10 min and then at room temperature for 1 h. It was then poured into ice water and extracted with ether. The extract was washed (water and brine), dried ($MgSO_4$), and evaporated to dryness. Crystallization from $CHCl_3$–EtOAc furnished **2** as a colorless crystalline product in three crops (15 g, 95% yield): mp 155–157 °C; IR (CH_2Cl_2), 1030 cm^{-1} (oxirane); NMR δ 0.93 (s, 3 H, C_{18}-H), 2.88 (AB quartet, J = 5 Hz, 2 H, oxirane CH_2), 5.08 (s, 2 H, benzylic H), 6.75–7.60 (m, unresolved, 8 H, Ar H); MS m/e 374 ($M^+ - CH_2O$), 91 ($C_6H_5CH_2^+$).

17α-(Azidomethyl)-3-(benzyloxy)-17β-hydroxyestra-1,3,5(10)-triene (3). A mixture of the oxirane **2** (8.8 g, 23 mmol), activated NaN_3 (15 g, 0.23 mol), and pulverized H_3BO_3 (6 g, 97 mmol) in DMF (100 mL) was refluxed under N_2 for 4 h.[18] The mixture was poured into H_2O and extracted with ether. The extract was washed (water and brine), dried ($MgSO_4$), and evaporated to give the azide **3** as a pale-yellow oil (9.2 g, 94% yield), which showed a single spot on TLC (silica; benzene–EtOAc, 9:1; R_f 0.49) and was adequate for further transformation. Crystallization from $CHCl_3$–hexane gave pale-yellow crystals: mp 96–98 °C; IR (CH_2Cl_2) 3600 (OH), 2120 cm^{-1} (N_3); NMR δ 0.93 (s, 3 H, C_{18}-H), 3.44 (AB quartet, J = 12 Hz, 2 H, 17α-CH_2–), 5.02 (s, 2 H, benzylic H), 6.75–7.60 (m, unresolved, 8 H, Ar H).

17α-(Aminomethyl)-3,17β-dihydroxyestra-1,3,5(10)-triene (4). A solution of the azide **3** (5.2 g, 13 mmol) in absolute ethanol (250 mL) was hydrogenated over 600 mg of Pd/C at atmospheric pressure and at room temperature for 39 h. The catalyst was removed by filtration, and the filtrate was evaporated to yield a colorless solid. Crystallization from MeOH–$CHCl_3$ provided a colorless crystalline product (2.6 g, 70%): mp 242–243 °C; IR (Nujol) 3280, 3170 cm^{-1} (NH_2); MS m/e 301 (M^+). Anal. ($C_{19}H_{27}NO_2$) C, H, N.

(2-Chloroethyl)nitrosocarbamoyl Azide. A solution of 2-chloroethyl isocyanate (20 g, 0.19 mol) in dry CCl_4 (200 mL) at 0 °C was bubbled with dry HCl for 3 h. The solvent was evaporated in vacuo to yield (2-chloroethyl)carbamoyl chloride as an oily product (27 g, 99% yield): IR (CH_2Cl_2) 1700 (C=O), 3430 cm^{-1} (NH).

Activated NaN_3 (8.0 g, 0.12 mol) was added to a solution of (2-chloroethyl)carbamoyl chloride (13 g, 89 mmol) in acetone (100 mL) at 0 °C. The mixture was stirred at 0 °C for 3 h and then filtered. The precipitate was washed exhaustively with ether. The filtrate was concentrated in vacuo to a small volume and then combined with the washings. The combined organic solution was washed (ice-cold water and brine), dried ($MgSO_4$), and evaporated to dryness. Crystallization from benzene–hexane gave (2-chloroethyl)carbamoyl azide as a colorless crystalline product (15 g, 82% yield): mp 48–49 °C, lit.[19] mp 49.6–50.2 °C; IR 1710 (C=O), 2150, 2170 (N_3), 3420 cm^{-1} (NH); NMR δ 3.62 (m, unresolved, 4 H, $ClCH_2CH_2N$–), 5.93 (br s, 1 H, NH).

To a solution of (2-chloroethyl)carbamoyl azide (1.2 g, 8.1 mmol) in pyridine, 5 M NOCl (3 mL) in glacial acetic acid was added dropwise over a period of 5 min while the temperature was kept at 0 °C. The mixture was stirred at 0 °C for a further 5 min and then poured into ice water and extracted with ether. The ether layer was washed (ice-cold 2 N HCl two times, 10% $NaHCO_3$, brine successively) and dried ($MgSO_4$). Evaporation of the solvent gave a yellow oil which was applied to a column containing 50 g of silica gel. Elution of the column with ether–hexane (1:3) yielded 0.70 g of (2-chloroethyl)nitrosocarbamoyl azide (49% yield) as a yellow oil, which showed a single spot on TLC (silica; hexane–ether, 6:1; R_f 0.32): IR (CH_2Cl_2) 2180 (N_3), 1720 (C=O), 1530 cm^{-1} (NO); NMR δ 3.52 and 4.17 (A_2B_2 system, 2 t, J = 7 Hz, 4 H, $-N(NO)CH_2CH_2Cl$), lit.[19] δ 3.50 and 4.15.

N-(2-Chloroethyl)-N'-[(3,17β-dihydroxyestra-1,3,5(10)-trien-17α-yl)methyl]-N-nitrosourea (5a). To a solution of the amine **4** (0.70 g, 2.3 mmol) in pyridine (15 mL) at 0 °C, (2-chloroethyl)nitrosocarbamoyl azide (0.42 g, 2.3 mmol) in ether (1.3 mL) was added dropwise with stirring. The mixture was stirred at 0 °C for 3 h and then ice water was added, followed by extraction with ether. The ether extract was washed (ice-cold 2 N HCl, 10% $NaHCO_3$, and brine successively), dried ($MgSO_4$), and evaporated to dryness. Crystallization from ether yielded the nitrosourea **5a** as a pale-yellow crystalline product (0.70 g, 70% yield): mp 116–119 °C dec; IR (CH_2Cl_2) 3560 (OH), 3410 (NH), 1720 (C=O), 1525 (CNH), 1490 cm^{-1} (NO); NMR (acetone-d_6) δ 1.00 (s, 3 H, C_{18}-H), 3.66, 4.28 (A_2B_2 system, 2 t, J = 7 Hz, 4 H, $-N(NO)CH_2CH_2Cl$), 3.70 (m, 2 H, 17α-CH_2N–), 6.60–7.20 (m, 3 H, Ar H); MS m/e 327 (M^+ – $HON{=}NCH_2CH_2Cl$). Anal. ($C_{22}H_{30}ClN_3O_4$) C, H, Cl, N.

N-Methyl-N'-[(3,17β-dihydroxyestra-1,3,5(10)-trien-17α-yl)methyl]-N-nitrosourea (5b). Following the general procedure described for the synthesis of **5a**, reaction of the amine **4** (1.4 g, 4.8 mmol) with methylnitrosocarbamoyl azide[14] (4.8 mmol) gave compound **5b** as a pale-yellow crystalline product after crystallization from CH_3OH–ether–hexane (1.1 g, 60% yield): mp 144–146 °C dec; NMR (acetone-d_6) δ 1.00 (s, 3 H, C_{18}-H), 3.22 (s, 3 H, $-N(NO)CH_3$), 3.40 (s, ~3 H, CH_3 of CH_3OH), 3.65 (m 2 H, 17α-CH_2N), 6.60–7.20 (m, 3 H, Ar H); MS m/e 327 (M^+ – $HON{=}NCH_3$). Anal. ($C_{21}H_{29}N_3O_4{\cdot}CH_3OH$) C, H, N.

N-(2-Chloroethyl)-N'-(3-hydroxyestra-1,3,5(10)-trien-17β-yl)-N-nitrosourea (6a). Following the general procedure described above, reaction of 17β-amino-3-hydroxyestra-1,3,5-(10)-triene[20] (2.5 g, 9.2 mmol) with (2-chloroethyl)nitrosocarbamoyl azide (9.2 mmol), after crystallization from CH_3OH, gave **6a** as a colorless crystal (1.6 g, 43% yield): mp 87–90 °C dec; NMR δ 0.86 (s, 3 H, C-18), 3.66, 4.26 (A_2B_2 system, 2 t, J = 7 Hz, 4 H, $-N(NO)CH_2CH_2Cl$), 4.00 (m, 1 H, C_{17}-H), 6.60–7.35 (m, 3 H, Ar H); MS m/e 405 (M^+), 297 (M^+ – $HON{=}NCH_2CH_2Cl$), 255 (M^+ – side chain). Anal. ($C_{21}H_{28}ClN_3O_3$) C, H, Cl, N.

N-Methyl-N'-(3-hydroxyestra-1,3,5(10)-trien-17β-yl)-N-nitrosourea (6b). Following the general procedure described above, reaction of 17β-amino-3-hydroxyestra-1,3,5(10)-triene (2.4 g, 8.9 mmol) and methylnitrosocarbamoyl azide (8.9 mmol) furnished **6b** (1.7 g, 54% yield): mp 140–142 °C dec; NMR δ 1.00 (s, 3 H, C_{18}-H), 3.24 (s, 3 H, $-N(NO)CH_3$), 4.20 (m, 1 H, C_{17}-H), 6.60–7.35 (m, 3 H, Ar H); MS m/e 357 (M^+), 297 (M^+ – $HON{=}NCH_3$), 255 (M^+ – side chain). Anal. ($C_{20}H_{27}N_3O_3$) C, H, N.

Acknowledgment. We thank Dr. A. E. Bogden, Mason Research Institute, Worcester, Mass., for providing the 13762 tumor, Evelyn Froese for her excellent technical assistance, and Dorothy Faulkner and Pearl Emery for typing the manuscript. This work was supported by a grant from the National Cancer Institute of Canada.

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Phototoxicity of Chlorpromazine

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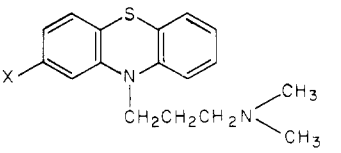
The constitution of chlorpromazine has been studied in the context of its phototoxicity. Electron transfer from the side chain to the aromatic nucleus of the drug contributes to its instability to light. Even without the side chain, however, chlorophenothiazines appear to be very photolabile, so that it is unlikely that nonphototoxic analogues of chlorpromazine can be prepared merely by altering the constitution of the side chain.

Phototoxicity has been noted as a side effect in chlorpromazine therapy for a number of years,[1] but only recently has the photochemical breakdown of the drug been studied. Grant[2] observed that chlorpromazine (**1**) was reduced to promazine (**2**) and also underwent substitution to the hydroxy compound **3** upon illumination in aqueous solution. Formation of **3** was deduced to be a photonucleophilic process, because other nucleophiles such as alcohols and amines could undergo analogous reactions. Reduction and substitution are known photoreactions of other aryl halides.[3]



1, X = Cl
2, X = H
3, X = OH

Photodechlorination of chloro aromatic compounds has been extensively studied in other systems.[3-6] Two reaction

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