POTENTIAL ANTICANCER AGENTS
III. URETHANE-TYPE NITROGEN MUSTARDS OF SOME SYNTHETIC ESTROGENS

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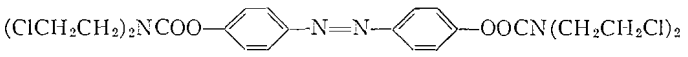
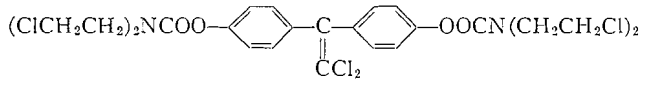
While studying the anticancer activity of the urethane-type nitrogen mustards, we prepared a number of compounds of this type from synthetic estrogens, with the purpose of establishing a relationship between the estrogenic activity of the bis-hydroxyphenyl derivatives and the anticancer action of their nitrogen mustard carbamates. Since compounds I and II that had previously been prepared (1) exhibited 50–60% inhibitions on Walker carcinosarcoma 256 (2), it seemed to be of interest to extend the series of these substances.



I

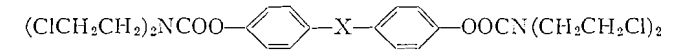


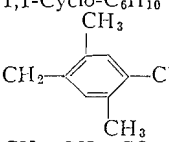
II

The urethane-type nitrogen mustards were obtained by condensation of the bis-hydroxyphenyl derivatives with di-(2-chloroethyl)-carbamyl chloride in pyridine (3) followed, after pyridine removal, by crystallization from ethanol. The nitrogen mustards thus obtained are summarized in Table I.

TABLE I

New urethane-type nitrogen mustards

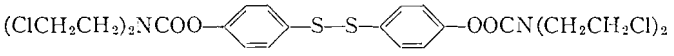


X	Melting point (°C)	Yield (%)	ν_{max}* carbamate group† (cm^{-1})	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
$CH_2—CH_2$	101.5–102	80.5	1712	52.36	5.09	5.09	52.06	5.12	5.08
$C(CH_3)_2$	180–181	36.6	1730	53.19	5.32	4.96	53.00	5.39	4.96
$C(CH_3)(C_2H_5)$	120–120.5	50.4	1725	53.97	5.53	4.84	54.19	5.49	4.66
$C(C_2H_5)_2$	98	52.0	1725	54.73	5.74	4.72	54.52	5.81	4.75
1,1-Cyclo-C_5H_8	105–106	72.4	1712	54.91	5.42	4.74	54.97	5.56	4.80
1,1-Cyclo-C_6H_{10}	89	51.8	1720	55.63	5.62	4.64	55.56	5.70	4.74
	133–134	22.1	1725	58.71	5.50	4.28	58.69	5.49	4.51
CH=CH—CO	89–90	33.5	1724	52.08	4.51	4.84	52.10	4.54	5.01
CH=N	91	38.0	1722	50.27	4.55	7.65	50.27	4.54	7.58
CH=N—N=CH	148	85.0	1712	50.00	4.52	9.72	50.03	4.70	9.70
S—S	46–47	34.7	1725	45.05	4.09	4.77	45.10	4.30	4.76
SO_2‡	114–115	50.0	1715 1740	45.05	4.09	4.77	44.99	4.06	4.73

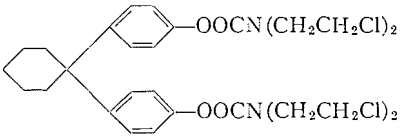
*Infrared spectra were determined on a UR 10 Zeiss-Jena-DDR spectrophotometer, in KBr disks.
†Amide I band.
‡Carbamate groups are in 2,4′ positions.

Some of the carbamates prepared in this way were obtained as gums which could not be purified by crystallization. Column chromatography on alumina was employed, the urethane group not being split as reported by Nogrady for N-di-(2-chloroethyl)-D-1-glucopyranosyl carbamate (4).

Thus, in the case of bis(4-(N,N-di-(2-chloroethyl)-carbamyl)phenyl)disulfide (III), after pyridine removal, the residue was extracted first with benzene and then with benzene – methylene chloride mixtures (from 1:1 to 1:9). The extracts were chromatographed over alumina. The carbamate was recovered from the light-yellow benzene eluate, the other fractions containing small quantities of unidentified dark-colored resinous substances. The chromatography of 1,1-bis(4-(N,N-di-(2-chloroethyl)-carbamyl)phenyl)-cyclohexane (IV) was similarly performed, except that residue extraction, column development, and elution were carried out with benzene and benzene–methanol mixtures (from 99:1 to 90:10). Here also, the carbamate was found in the benzene eluate.

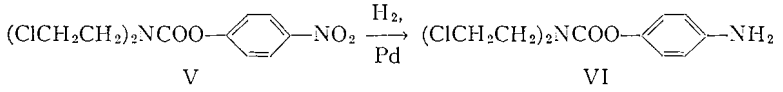


III

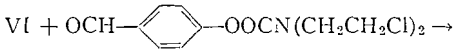


IV

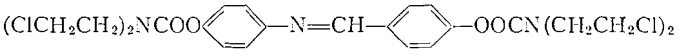
For the same reason (an impossibility to purify), 1,2-bis(4-(N,N-di-(2-chloroethyl)-carbamyl)phenyl)methylenimine (VIII) was synthesized according to the reaction sequence shown in Reaction Scheme 1.



V VI



VII



VIII

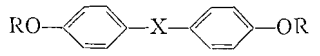
REACTION SCHEME 1.

The carbamates of *p*-hydroxynitrobenzene (V) and *p*-hydroxybenzaldehyde (VII) were prepared by common procedures (3); amine VI was obtained in good yield by the catalytic hydrogenation of the nitro derivative V with palladium chloride on carbon at room temperature.

Biological evaluation of the compounds is in progress. The estrogenic activity of some of the bis-*p*-hydroxyphenyl derivatives used as precursors in the present work and in ref. 1, and the antitumor activity of their bis(N,N-di-(2-chloroethyl)carbamyl) derivatives against Walker carcinosarcoma 256 are shown in Table II. Mention should also be made that preliminary tests showed compounds V and VI (not tabulated) to be inactive against Walker carcinosarcoma 256.

TABLE II

Biological activities*



X	Estrogenic activity† (R = H)	% inhibition against Walker carcinosarcoma 256‡ (R = $OCN(CH_2CH_2Cl)_2$)	References for the estrogenic activity
CH=CH (*trans*)	++	21	5, 6
CH_2—CH_2	++	22	6
CH=CH—CO	+	29	6
CH_2	+	30	7
$C(CH_3)_2$	+	36	7
$CH(CCl_3)$	+	0	8
C ‖ CCl_2	+	52	9
—	+	7, 3	6
N=N	+	59	6
O	+	35	5
S	+	36	6
CH=N	−	T/C > 1§	6
SO_2	−	13	6
2,4′-SO_2	?	30	—
SO	?	25	—
CO	?	0	—
CH=N—N=CH	?	T/C > 1	—

*The table includes derivatives reported in ref. 1.
†Estrogenic activity: ++, 1–100 mg/kg body weight (rat); +, 100 – 1 000 mg/kg body weight; −, over 1 000 mg/kg body weight.
‡Doses of 50 mg/kg body weight (rat) every 2 days, beginning with the 7th day after tumor transplantation up to the 23rd day.
§T = tumor weight (treated animals); C = tumor weight (control animals).

Structural evidence for the new compounds was provided by infrared absorbtion spectra.

EXPERIMENTAL

4-(N,N-Di-(2-chloroethyl)-carbamyl)nitrobenzene (V)

Di-(2-chloroethyl)-carbamyl chloride (7.8 g, 0.038 mole) in 20 ml of pyridine was added to 5 g (0.036 mole) of *p*-nitrophenol. After the solution was allowed to stand for 3 days at room temperature, pyridine hydrochloride separated out and was filtered off. The filtrate was evaporated to dryness *in vacuo* to give a residue, which was triturated with toluene and again evaporated to dryness *in vacuo*. The procedure was repeated 3 times until all the pyridine had been removed; 9.2 g (83%) of V was obtained (after recrystallization from ethanol, m.p. 85–86 °C). Infrared (KBr): ν_{max} carbamate (amide I band) 1 732 cm^{-1}.

Anal. Calcd. for $C_{11}H_{12}Cl_2N_2O_4$: C, 42.99; H, 3.90; N, 9.12. Found: C, 42.97; H, 3.94; N, 9.09.

4-(N,N-Di-(2-chloroethyl)-carbamyl)benzaldehyde (VII) was similarly prepared. The residue after pyridine was removed failed to crystallize and was extracted with benzene; the benzene solution was evaporated to dryness *in vacuo* to give the desired product (viscous yellow oil) of analytical purity. Infrared: ν_{max} carbamate (amide I band) 1 735 cm^{-1}.

Anal. Calcd. for $C_{12}H_{13}Cl_2NO_3$: C, 49.65; H, 4.48; N, 4.82. Found: C, 49.65; H, 4.50; N, 4.78.

4-(N,N-Di-(2-chloroethyl)-carbamyl)aniline (VI)

V (7.9 g, 0.026 mole) was dissolved in 250 ml ethanol and hydrogenated in the presence of 2 g of 5% palladium chloride on carbon catalyst at atmospheric pressure and room temperature, the theoretical amount of hydrogen (1 730 ml) being consumed during 4 h. The catalyst was filtered off and the solvent was removed by distillation under reduced pressure. The residue was dissolved in ether, and dry hydrogen chloride was passed into the solution to give 7.3 g (91%) of the hydrochloride of VI (m.p. 182–183 °C). Infrared (KBr): ν_{max} carbamate (amide band I) 1 730 cm^{-1}.

Anal. Calcd. for $C_{11}H_{15}Cl_3N_2O_2$: C, 42.10; H, 4.78; N, 8.93. Found: C, 42.14; H, 4.80; N, 8.98.

1,2-Bis(4-(N,N-di-(2-chloroethyl)-carbamyl)phenyl)methylenimine (VIII)

Aldehyde VII (3.3 g, 0.011 mole) was added to a solution of 2.9 g (0.011 mole) of amine VI in 110 ml of ethanol. After the solution was allowed to stand for 2 days at room temperature with occasional shaking, a solid was collected and recrystallized from ethanol to give 2.2 g (38%) of pure VIII (see Table I).

SUMMARY

The synthesis of new urethane-type nitrogen mustards from the following bis-hydroxyphenyl derivatives is reported: 1,2-bis(*p*-hydroxyphenyl)ethane; 2,2-bis(*p*-hydroxyphenyl)-propane; 2,2-bis(*p*-hydroxyphenyl)butane; 3,3-bis(*p*-hydroxyphenyl)pentane; 1,1-bis-(*p*-hydroxyphenyl)cyclopentane; 1,1-bis(*p*-hydroxyphenyl)cyclohexane; 2,5-bis(*p*-hydroxybenzyl)-*p*-xylene; *p*,*p*′-dihydroxychalcone; 1,2-bis(*p*-hydroxyphenyl)methylenimine; *p*,*p*′-dihydroxybenzalazine; 1,2-bis(*p*-hydroxyphenyl)disulfide; 2,4′-dihydroxydiphenyl sulfone.

Antitumor activities against Walker carcinosarcoma 256 are given for some of the new compounds.

ACKNOWLEDGMENTS

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