SYNTHESIS, STRUCTURE, AND ANTITUMOR ACTIVITY OF SOME p-SUBSTITUTED N,N-DI(2-CHLOROETHYL)ANILINES

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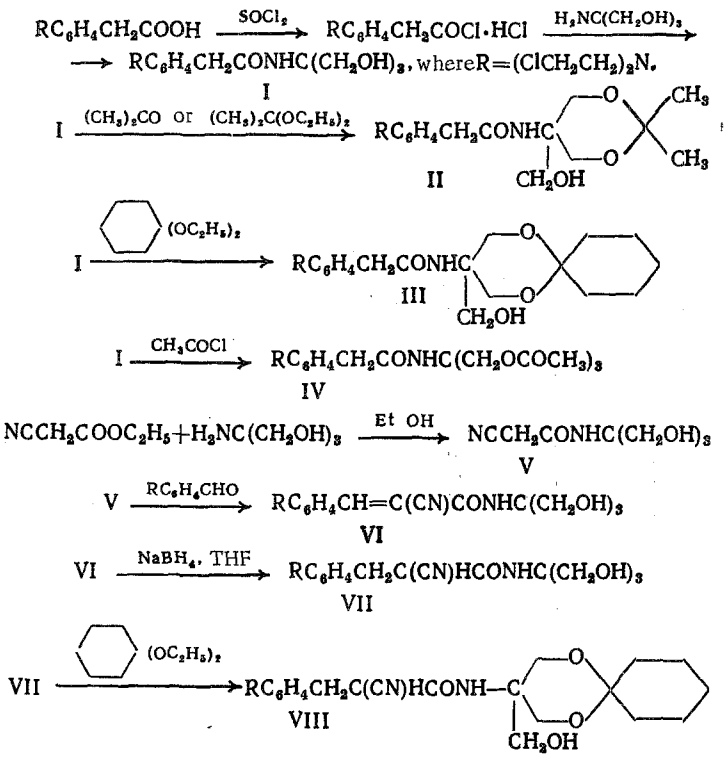
Continuing our search for antitumor agents among some nitrogen mustard derivatives with varying lipophylic:hydrophylic properties, some containing the cytotoxic 1,3-dioxane ring [1], we have synthesized some p-substituted N,N-di(2-chloroethyl)anilines and studied their structure and action on experimental tumors in animals. The synthesis of the substituted amides 4-di(2-chloroethyl) aminophenyl acetic acid (I-IV) and the amides 3-[4-di(2-chloroethyl)aminophenyl]-2-cyano-2-propenoic (VI) and propionic (VII and VIII) acid was carried out as follows:



The composition of the p-substituted N,N-dichloroanilines (I-IV) was confirmed by elemental and functional analysis, the purity by TLC, and the structure by UV and IR spectroscopy. The UV spectra of compounds (I-IV) were characterized by the presence of two absorption bands of different intensities of 261-262 and 300-303 nm. Suspensions of these substances in mineral oil absorbed in the IR at 3260-3390 cm^{-1} (OH and NH), 1030-1230 cm^{-1} (C–OH and C–O–C), 1530 cm^{-1} (aromatic ring), 1645-1675 cm^{-1} (amide-I), 1560, and 1620-1630 cm^{-1} (amide II), and 1750 cm^{-1} (ester C=O group) in compound (IV).

The substituted N,N-di(2-chloroethyl)aniline (VI) was obtained by the condensation of the hydrophylic amide (V) with 4-di(2-chloroethyl)aminobenzaldehyde in anhydrous dioxane in the presence of piperidine as catalyst. Running this reaction in ethanol in the presence of

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TABLE 1. Physicochemical Properties of the p-Substituted N,N-di(2-chloroethyl)anilines

Compound	Yield, %	mp, °C (solvent)	Found, %				Empirical formula	Calculated, %				UV spectrum (EtOH, λ, nm, ε)
			C	H	Cl	N		C	H	Cl	N	
I	50,0	143—4 (dichloroethane or ethyl acetate)	51,10	6,29	18,73	7,35	$C_{16}H_2Cl_2N_2O_4$	50,62	6,38	18,75	7,38	261,5: $2.20 \cdot 10^4$; 300 (shoulder), $2,57 \cdot 10^3$
II	74,5	106—7 (benzene–hexane)	54,97	6,54	17,13	6,68	$C_{19}H_{26}Cl_2N_2O_4$	54,42	6,73	16,91	6,67	261, $2,96 \cdot 10^4$; 303 (shoulder), $8,25 \cdot 10^3$
III	70,0	134—5 (absolute ethanol –hexane)	57,95	7,22	15,70	6,03	$C_{22}H_{32}Cl_2N_2O_4$	57,50	7,02	15,41	6,09	261,5; $2,29 \cdot 10^4$; 302 (shoulder), $2,48 \cdot 10^3$
IV	78,0	122—3 benzene	52,47	5,63	14,13	5,53	$C_{22}H_{30}Cl_2N_2O_7$	52,28	5,98	14,06	5,54	262; $2,38 \cdot 10^4$; 303 (shoulder), $8,25 \cdot 10^3$
VI	70,3	179—0 ethanol	52,29	5,65	17,00	10,73	$C_{18}H_{23}Cl_2N_3O_4$	52,09	5,57	17,08	10,11	245—248; $0,88 \cdot 10^4$ 254; $0,9 \cdot 10^4$; 318; $3,69 \cdot 10^3$; 305—310; $2,66 \cdot 10^3$; 390; $3,04 \cdot 10^4$
VII	63,7	141—2 ethanol	52,14	6,19	16,00	9,14	$C_{18}H_{25}Cl_2N_3O_4$	51,69	5,97	16,90	10,00	262; $2,31 \cdot 10^4$; 302, $1,83 \cdot 10^3$
VIII	52,0	123—4 (benzene–hexane)	57,68	7,12	14,51	9,20	$C_{24}H_{33}Cl_2N_3O_4$	57,83	6,68	14,23	8,43	262; $2,60 \cdot 10^4$; 302; $4,07 \cdot 10^3$

sodium ethoxide gave a mixture of the amide VI and ethyl 3-[4-di(2-chloroethyl)aminophenyl]-2-cyano-2-propionate, which was prepared for identification purposes by reverse synthesis [2].

The UV-spectrum of compound VI in alcohol contains five absorption bands (Table 1). Characteristic of the IR spectrum is the presence of absorption bands at 2250 cm^{-1} (nitrile group), 1530, and 700-800 cm^{-1} (p-substituted phenyl), 1645, 1640 and 1615 cm^{-1} (amide-I and amide-II), 3200-3400 cm^{-1} (stretching) and 1030-1050 cm^{-1} (deformation) vibrations of the hydroxyl groups.

Compound VI was expected to exist as a mixture of cis-trans isomers. However, the calculated value [3] δ_{calc} (—CH=) 8.16 ppm for the trans-isomer ($\overset{H}{}\!\!-C=C\overset{CN}{}$) agrees with that found experimentally in the NMR spectrum* δ_{exp} (—CH=) 8.19 ppm, demonstrating that only the trans form of compound VI is present.

With increasing basicity of the amine nitrogen, the mobility of the halogen atom, and hence also the antitumor effect of the yperite increase [4]. Reduction of the C=C double bond in the aminotriol VI gives a compound in which the electron density on the nitrogen atom of the di(2-chloroethyl)amino group is increased.

Such a compound — the (2-hydroxymethyl-1,3-dioxy-2-propyl)amide of 3-[4-di(2-chloroethyl)aminophenyl] -2-cyanopropionic acid (VII) — was obtained by the reduction of the unsaturated analog VI using sodium borohydride in tetrahydrofuran (THF). The reaction of the amide with cyclohexanone diethyl acetal in benzene in the presence of p-toluenesulfonic acid gave 1,3-dioxanylamide (VIII).

The UV spectra of the amides VII and VIII in the absence of V had two absorption bands characteristic of the "saturated" aromatic nitrogen mustards I-IV. The IR spectra of compounds VII-VIII had absorption bands at 2250 cm^{-1} (nitrile), 1000-1290 cm^{-1} (C—O and C—O—C groups), 3200-3500 cm^{-1} (OH and NH), 1650-1680, and 1580-1620 cm^{-1} (amide-I and amide-II), and 1530 cm^{-1} (p-substituted benzene ring).

Some physicochemical characteristics of the p-substituted N,N-di(2-chloroethyl)anilines are given in Table 1.

The antitumor activity of the compounds synthesized was studied using SHR and $CC_{57}W$ mice and noninbred white rats with transplanted tumors. The preparations were injected intraperitoneally as suspensions in physiological solutions containing 1 drop of Tween-80. The drugs were injected into the animals with solid tumors 3 days after transplantation and tests were begun 5 days after the 10th injection. For mice with Erlich's ascites, the drug was injected 8 times, beginning 1 day after transplantation, and the mice were sacrificed 1 day after the final injection. The results of these tests indicated that the compounds showed a marked antitumor activity and a fairly wide spectrum of activity (Table 2).

EXPERIMENTAL (CHEMICAL)

A VR-10 (GDR) instrument was used to record IR spectra of samples in mineral oil. The UV spectra of alcoholic and aqueous solutions were measured on SF-8 and SF-16 spectrophotometers. NMR spectra were taken in DMSO-D_6 on a Perkin-Elmer R-12 (Sweden) instrument; 60 MHz; internal standard hexamethylenedisiloxane: δ, ppm. ^{13}C NMR spectra were obtained on a Bruker Spectrospin apparatus, 22.63 MHz, DMSO-D_6, relative to DMSO, chemical shift of DMSO, relative to tetramethylsilane 40.48, δ, ppm.

(2-Hydroxymethyl-1,3-dihydroxy-2-propyl)amide of 4-[di(2-chloroethyl) amino]phenylacetic Acid (I). To a solution of 0.138 mole of tris-(hydroxymethyl)aminomethane in 47 ml of water and 23 ml of acetone at a temperature of from —10 to —5°C and with vigorous stirring a solution was added over 30 minutes of 0.046 moles of the hydrochloride of p-di(2-chloroethyl) aminophenylacetyl chloride (obtained by the action of thionyl chloride on p-di(2-chloroethyl) aminophenylacetic acid) in 85 ml of anhydrous acetone. After stirring for 15 min at —10°, 10 ml of ice water was added, and the precipitated material was filtered off and washed with 50 ml of water and then with a mixture of 50 ml of hexane and 5 ml of absolute ethyl alcohol. It was dried in a vacuum desiccator over potassium hydroxide and recrystallized from dichloroethane or ethyl acetate (with charcoal) to give 8.7 g (50%) of I, mp 143-144°. Found; eq_{Cl}

*^{1}H and ^{13}C NMR spectra of compounds V-VIII are given in the "Experimental" section.

TABLE 2. Antitumor Activity of Compounds I-IV, and VI-VIII

Compound	Ehrlich's ascites		Sarcoma 37		Sarcoma 45		Sarcoma 180		PRK*		Walker carcinosarcoma	
	dose mg/kg	% retardation	dose mg/kg	% retardation	dose mg/kg	% retardation	dose mg/kg	% retardation	dose mg/kg	% retardation	dose mg/kg	% retardation
I	10	98	10	40	10	30	10	57	10	38	5	99,5
II	30	100	30	36	30	22	30	54	30	23	20	99,2
III	—	—	160	87	—	—	—	—	—	—	—	—
IV	20	100	20	42	—	—	—	—	—	—	10	96,0
VI	300	100	200	47	200	75	—	—	200	31	—	—
VII	60	100	75	66	100	84	—	—	25	33	—	—
VIII	210	100	250	73	300	91	210	48	210	43	—	—

*Dense-celled skin cancer of $CC_{57}W$ mice (syngeneic tumor).

Footnote. For all values for the percentage inhibition of tumors, $P \leq 0.05$.

189.7 (Folgard). Calculated, eq_{Cl} 189.6. The substance was soluble in THF, moderately soluble in chloroform, alcohol, and slightly soluble in water.

(5-Hydroxylmethyl-2,2-dimethyl-1,3-dioxan-5-yl)amide of 4-[Di(2-chloroethyl)amino]phenylacetic Acid (II). Method A. A mixture of 0.01 mole of I and 0.012 mole of acetone diethylacetal in 30 ml of anhydrous benzene containing a few crystals of p-toluenesulfonic acid or KU-2 in the H^+ form was refluxed until all the solid had dissolved. Activated charcoal was added, and the mixture was heated to boiling and filtered through a 0.5-cm layer of aluminum oxide. The filtrate was diluted with 1.5 times its volume of hexane, and when cool it was filtered to give 3.11 g (74.5%) of colorless crystals with mp 106-107°. Found, eq_{Cl} 213.1. Calculated, eq_{Cl} 209.7. The substance was soluble in chloroform, THF, moderately soluble in alcohol, and insoluble in hexane and water.

Method B. A mixture of 0.005 mole of I and 100 ml of p-toluenesulfonic acid in 15 ml of anhydrous acetone was maintained at 20-25° until the solid had completely dissolved; the solution was made alkaline to pH 8.0 with alcoholic potassium hydroxide, filtered, and evaporated to dryness in vacuum. The residue was recrystallized from a mixture of benzene and hexane to give 0.9 g (50%) of II; the physical constants of this compound agreed with those of the compound obtained by method A.

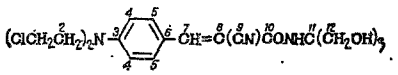
(5-Hydroxymethyl-2,2-pentamethylene-1,3-dioxan-5-yl)amide of 4-[Di-(2-chloroethyl)amino]-phenylacetic Acid (III). A mixture of 0.005 mole of I and 0.0065 mole of cyclohexanone diethylacetal containing a few crystals of p-toluenesulfonic acid or KU-2 in the H^+-form was refluxed until the solid had dissolved. The catalyst was separated, the benzene evaporated in vacuum, and the residue recrystallized from a mixture of anhydrous ethanol and hexane to give 1.58 g (70%) of colorless crystals of III, mp 134-135°C. Found, eq_{Cl} 230.0. Calculated, eq_{Cl} 229.7. The product was soluble in THF, chloroform, moderately soluble in alcohols, ether, carbon tetrachloride, and insoluble in water and cyclohexane.

(2-Acetoxymethyl-1,3-diacetoxy-2-propyl)amide of 4-[Di(2-chloroethyl)amino]phenylacetic Acid (IV). A mixture of 0.001 mole of I and 5 ml of acetyl chloride was refluxed for 2 h and then evaporated in vacuum to dryness. Traces of acetyl chloride were removed by evaporation to dryness in vacuum successively with 3 ml and 5 ml of anhydrous benzene. The residue was recrystallized from benzene to give 0.39 g (78%) IV in the form of colorless crystals with mp 122-123°. Found, eq_{Cl} 251.0. Calculated, eq_{Cl} 252.7. The substance is soluble in THF, chloroform, less soluble in alcohol, and insoluble in hexane and water.

(2-Hydroxymethyl-1,3-dihydroxy-2-propyl)amide of Cyanoacetic Acid (V). A mixture of 12 g of freshly prepared cyanoacetic ester and 12.1 g of tris-(hydroxymethyl)aminomethane in 80 ml of absolute ethanol, protected from atmospheric moisture, was refluxed for 24 h. After cooling for 24 h in the refrigerator the crystals were filtered off, washed with absolute alcohol, ether, and dried to give 13.2 g of V. Recrystallization from absolute ethyl alcohol gave 10.45 g (56%) of colorless crystals of compound V, mp 132-133°. NMR spectrum (37°, DMSO-D_6) δ, ppm: 3.43 (CH_2CN); 3.57, 3.62, 3.66 (CH_2OH); 4.54 (OH), 7.48 (NH) — disappeared on addition of D_2O. Found, %: C 44.89; H 6.35; N 14.97. $C_7H_{12}N_2O_4$. Calculated, %: C 44.73; H 6.41; N 14.86.

(2-Hydroxymethyl-1,3-dihydroxy-2-propyl)amide of 3-[4-Di(2-chloroethyl)aminophenyl]-2-cyano-2-propenoic Acid (VI). A mixture of 4.92 g of di(2-chloroethyl)aminobenzaldehyde, 3.76 g of V, and 0.5 ml of piperidine was refluxed in 50 ml of anhydrous dioxane, and protected from atmospheric moisture until completely dissolved (but not less than 6 h). The moist product was separated either by distillation of dioxane in vacuum, or by filtration of the material when crystallized from the solution on long standing (several days). Two recrystallizations from ethanol gave 5.60 g (67.5%), mp 179-180°. R_f 0.33 (Silufol VV-254, benzene-ethanol, 9:1).* NMR spectra (DMSO-D_6), δ, ppm: 3.74, 3.83, 3.90 (CH_2OH and CH_2CH_2Cl), 4.72 (OH), 7.27 (NH), d 7.06 and d 8.06, I = 9.6 Hz (o-protons in C_6H_4), 8.19 (C=CH).

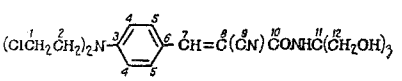
^{13}C NMR spectrum (DMSO-D_6), δ, ppm:



161.6 (C^{10}), 150.7 ($2C^5$), 143.2 ($2C^4$), 120.7 (C^3), 118.5 (C^9), 112.6 (C^6, C^7), 99.2 (C^8), 63.0 (C^{11}), 61.2 ($3C^{12}$), 52.5 ($2C^1$), 41.7 ($2C^2$). The product was slightly soluble in dichloroethane, chloroform, hexane, ether, water, and moderately soluble in alcohols.

(2-Hydroxymethyl-1,3-dihydroxy-2-propyl)amide of 3[4-Di(2-chloroethyl)aminophenyl]-2-cyanopropanoic acid (VII). To a suspension of 3.10 g of VI in 26 ml of anhydrous THF 0.34 g of sodium borohydride, was added portionwise with vigorous stirring at 20-27°, and stirring was continued for a further 2 h at room temperature. On the following day the solution was filtered, and the filtrate was poured into 80 ml of cooled water to which had been added 0.7 ml of concentrated hydrochloric acid. After cooling for 2 h, the white precipitate was filtered off, washed with water to neutral reaction, and dried in a vacuum desiccator over potassium hydroxide to give 2.69 g of VII. Recrystallization from 30 ml of ethanol containing charcoal gave 1.99 g (63.7%) of VII, mp 141-142°. Found eq_{Cl} 212.0. Calculated, eq_{Cl} 209.2. R_f 0.12.

NMR spectrum (DMSO-D_6), δ, ppm: 3.76, 3.86, 3.92 (CH_2CH_2Cl, CH_2CH), 4.80 (OH), d 6.97, 7.12 and d 7.45, 7.60, I = 9 Hz (C_6H_4 o-protons), 7.81 (NH). ^{13}C NMR (DMSO-D_6), δ, ppm:



165.8 (C^{10}), 146 (C^3), 130.6 ($2C^5$), 125.5 (C^6), 119.1 (C^9), 112.8 ($2C^4$), 63.4 (C^{11}), 61.2 ($3C^{12}$), 53.1 ($2C^1$), 42.1 ($2C^2$, C^7).

(5-Hydroxymethyl-2,2-pentamethylene-1,3-dioxan-5-yl)amide of 3-[4-Di(2-chloroethyl)aminophenyl]-2-cyanopropanoic Acid (VIII). A mixture of 1.25 g of VII, 0.57 g of cyclohexanone diethylacetal, and 100 mg of toluenesulfonic acid in 15 ml of anhydrous benzene was refluxed until the solid had dissolved (but not less than 1.5 h). The reaction mixture was made alkaline to pH 8.0 with alcoholic potassium hydroxide and filtered through paper pulp and charcoal; the filtrate was washed with water to neutral reaction and, after drying over sodium sulfate, evaporated to dryness. The residue was recrystallized from a mixture of benzene and hexane, and then from isopropyl alcohol to give 0.74 g (52%) of colorless crystals of VII, mp 123-124°. Found, eq_{Cl} 249.5. Calculated, eq_{Cl} 249.2. R_f 0.28.

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*This system was used further.