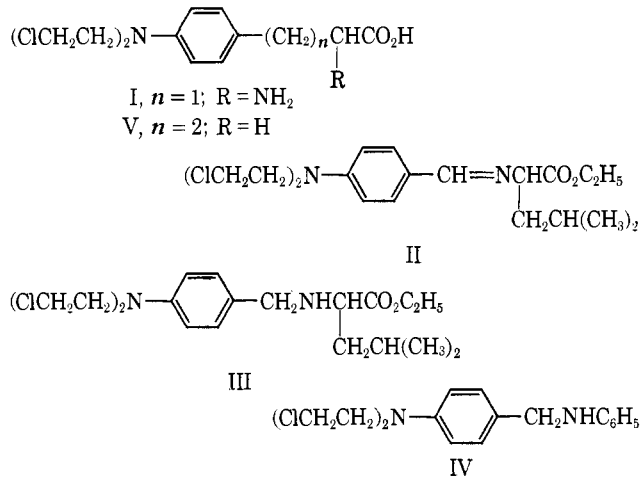
tested[7] for antitumor activities in the KB cell culture[8a] and the Walker 256 carcinosarcoma (subcutaneous)[8b] test systems.[9] Attempts to prepare the carboxylic acid of III by hydrolysis in hydrochloric acid were unsuccessful, but for comparison of activities N-(*p*-[bis(2-chloroethyl)amino]benzyl)aniline (IV), previously isolated as the hydrochloride salt,[6a] was also prepared by reduction of the corresponding Schiff base,[6a] and was also evaluated in the same test systems.



Test Results.—The three nitrogen mustards, II, III, and IV, are nontoxic and inactive in the KB cell culture tests, but all have small but definite activities in the Walker 256 system. The latter results are summarized in Table I and are compared with similar data for sarcolysin (I) and chlorambucil (V), two compounds with high activities in this test system.

TABLE I

ANTITUMOR ACTIVITIES IN THE WALKER 256 CARCINOSARCOMA (SUBCUTANEOUS) TEST SYSTEM

Compound	LD_{10},[a] mg./kg./day	T/C 0.01,[a] mg./kg./day	T.I.[a]
I[b]	6.5	0.45	14
II	>100	181	*ca.* 1
III[c]	>50	19	*ca.* 3
IV	>100	181	*ca.* 1
V[b]	15.5	1.6	10

[a] LD_{10}, T/C 0.01, and T.I. are, respectively, maximum tolerated dose, minimum effective dose, and therapeutic index as defined in ref. 3, pp. 7, 9, and 11. [b] Data from ref. 3, p. 63. [c] As the oxalate salt.

The comparatively small minimum effective dose (T/C 0.01) for III suggests that the preparation of similar substances with the benzyl nitrogen mustard group similarly attached to other amino acid ester or amino acid carriers may afford alkylating agents of substantially higher antitumor activities.

Experimental[10]

Ethyl N-(*p*-[Bis(2-chloroethyl)amino]benzylidene)-DL-leucinate (II).—To a solution of 5.50 g. (0.0224 mole) of *p*-[bis(2-chloroethyl)amino]benzaldehyde,[6b] m.p. 87–88°, in 85 ml. of absolute ethanol was added 8.51 g. (0.0536 mole) of ethyl DL-leucinate, b.p. 85–89° (19 mm.). The mixture was allowed to stand overnight at room temperature. On partial evaporation of the solvent and chilling, there was obtained in three crops 5.09 g. of II (58%) as a white crystalline solid, m.p. 47–50°. Three recrystallizations of this material from absolute ethanol, afforded an analytical sample, m.p. 46–47°.

Anal. Calcd. for $C_{19}H_{28}Cl_2N_2O_2$: C, 58.91: H, 7.29; Cl, 18.31. Found: C, 59.47; H, 7.31; Cl, 18.71.

Ethyl N-(*p*-[Bis(2-chloroethyl)amino]benzyl)-DL-leucinate (III), Oxalate Salt.—In 50 ml. of absolute ethanol 0.249 g. of Adams catalyst was reduced with hydrogen. To the catalyst was then added 5.00 g. (0.0129 mole) of ethyl N-(*p*-[bis(2-chloroethyl)amino]benzylidene)-DL-leucinate in 55 ml. of absolute ethanol, and reduction with hydrogen continued. A 1 mole equiv. of hydrogen was taken up in 15 min. with no additional consumption of hydrogen during another hour. After removal of the catalyst and complete removal of the solvent 5.64 g. of ethyl N-(*p*-[bis(2-chloroethyl)amino]benzyl)-DL-leucinate was obtained as an oil which resisted all attempts at crystallization. To 0.289 g. (0.743 mmole) of this oil in 1 ml. of absolute ethanol was added 0.067 g. (0.53 mmole) of oxalic acid in 1 ml. of absolute ethanol. After warming the mixture briefly and then chilling, 0.169 g. of the oxalate salt of III (65%) was obtained as a white crystalline solid, m.p. 149–150°. Three recrystallizations of this material from absolute ethanol afforded an analytical sample, m.p. 147–148°.

Anal. Calcd. for $C_{19}H_{30}Cl_2N_2O_2 \cdot C_2H_2O_4$: C, 52.61; H, 6.73; Cl, 14.79. Found: C, 52.71; H, 6.78; Cl, 14.76.

N-(*p*-[Bis(2-chloroethyl)amino]benzyl)aniline (IV).—Using the procedure outlined for III, 5.35 g. (0.0167 mole) of N-(*p*-[bis-(2-chloroethyl)amino]benzylidene)aniline,[6] m.p. 64–65°, was reduced in 160 ml. of absolute ethanol using 0.102 g. of Adams catalyst. After removal of the catalyst, evaporation of much of the solvent, and chilling, 4.76 g. of IV (96%) was obtained as a white crystalline solid, m.p. 50–51°. Three recrystallizations of this material from absolute ethanol afforded an analytical sample, m.p. 50–51°.

Anal. Calcd. for $C_{17}H_{20}Cl_2N_2$: C, 63.16; H, 6.24; Cl, 21.94. Found: C, 62.66; H, 6.55; Cl, 21.89.

(7) Testing provided by the Cancer Chemotherapy National Service Center.

(8) (a) *Cancer Chemotherapy Rept.*, **25**, 22 (1962); (b) *ibid.*, **25**, 11 (1962).

(9) Two compounds similar to II and III, ethyl N-(*p*-[bis(2-chloroethyl)-amino]benzylidene)-L-tyrosinate and ethyl N-(*p*-[bis(2-chloroethyl)amino]-benzyl)-L-tyrosinate are listed by R. P. Bratzel, R. B. Ross, T. H. Goodridge, W. T. Huntress, M. T. Flather, and D. E. Johnson in *Cancer Chemotherapy Rept.*, **26**, 247, 248 (1963), respectively. Neither the preparation nor the antitumor activity of either of these compounds appears to have been published.

(10) All melting points were taken in capillary tubes and are corrected. Microanalyses were done by Galbraith Laboratories, Inc., Knoxville, Tenn. All evaporations of solvent were done at reduced pressure under an atmosphere of nitrogen.

C-19 Functional Steroids. VII.[1] Desoxycorticosterone-19-nitrile Acetate

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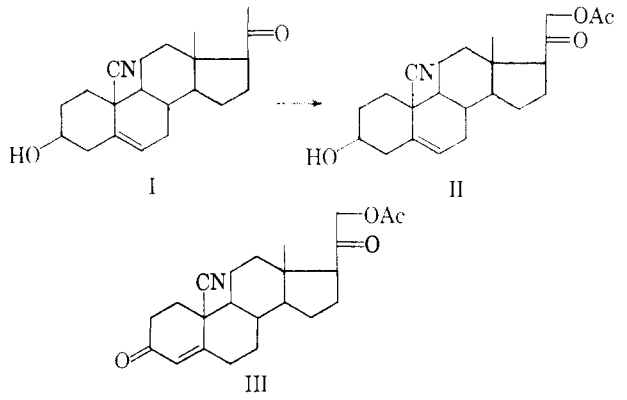
In the preceding paper of this series[2] evidence was obtained which suggested that testosterone-19-nitrile has weak antimyotrophic action at the peripheral level. Since desoxycorticosterone acetate (DOCA) antagonists would be of theoretical as well as practical interest, the preparation and testing of DOCA-19-nitrile (III) was undertaken.

Treatment of I, previously prepared in this laboratory,[2,3] with iodine and calcium oxide in tetrahydrofuran

(1) From the Ph.D. thesis of W. Ho, University of California, 1965. This investigation was supported by a PHS research grant (AM-05016) from the National Institute of Arthritis and Metabolic Diseases, United States Public Health Service.

(2) M. E. Wolff and T. Jen, *J. Med. Chem.*, **6**, 726 (1963).

(3) T. Jen and M. E. Wolff, *ibid.*, **5**, 876 (1962).



solution in the presence of azobisisobutyronitrile[4,5] gave the corresponding 21-iodo steroid. It was found that the use of redistilled tetrahydrofuran was essential for the success of the reaction, probably owing to the presence of chain-breaking stabilizers in the commercial product. The resulting crude iodo ketone was allowed to react with triethylammonium acetate in acetone solution to give the acetate II in 30% over-all yield. Oxidation of II by the Oppenauer method was unsuccessful, but the ketone III was obtained by oxidation with chromic acid in acetone followed by isomerization of the intermediate Δ^5-ketone on alumina.[5]

For the biological assay,[6] the test materials, in sesame oil as vehicle, were administered by subcutaneous injection in divided doses at 0 and +3 hr. The test animals were groups of seven male rats adrenalectomized approximately 48 hr. prior to the initial injection. The urethras were ligated at the end of 4 hr. and urine was collected from the fourth through the sixth hour. Urinary sodium and potassium determinations were made by means of flame photometry after suitable dilutions of the urine. The "t" test was used to evaluate the significance of the data at the 95% level of confidence.

The results of the testing are displayed in Table I. It can be seen that III is inactive in the sodium retention test and has only about 5% of the activity of DOCA as a potassium excretor. The concomitant administration of 20 parts of III and 1 part of DOCA produces an effect which is not significantly different from the effect of DOCA alone. It is concluded that III is not a DOCA antagonist.

TABLE I
MINERAL BALANCE TESTS

Compound (total dose/rat, γ)	Av. final body wt., g./rat	Sodium excreted, mg., mean ± S.D.	Potassium excreted, mg., mean ± S.D.
Control	146	2.03 ± 0.79	2.45 ± 0.60
DOCA (6)	145	0.94 ± 0.63	3.47 ± 1.18
III (120)	149	1.45 ± 0.93	3.84 ± 0.87
DOCA (6) and III (120)	150	0.81 ± 0.49	3.75 ± 0.59

	"t"	Inference[a]	"t"	Inference[a]
Control *vs.* DOCA	2.86	S	2.40	S
Control *vs.* III	1.26	NS	3.38	S
Control *vs.* III + DOCA	3.45	S	3.81	S
DOCA *vs.* III + DOCA	0.04	NS	0.50	NS

[a] S = significant, NS = not significant.

(4) H. J. Ringold and G. Stork, *J. Am. Chem. Soc.*, **80**, 250 (1958).

(5) E. S. Rothman, T. Perlstein, and M. E. Wall, *J. Org. Chem.*, **25**, 1966 (1960).

(6) The biological assays were performed at The Endocrine Laboratories, Madison, Wis.

Experimental[7]

3β,21-Dihydroxy-20-oxo-pregn-5-ene-19-nitrile 21-Acetate (II).—A solution of 1.00 g. (0.002 mole) of I[2,3] in 8 ml. of redistilled tetrahydrofuran and 8 ml. of methanol was treated with 1.7 g. of powdered calcium oxide and 57 mg. of recrystallized azobisisobutyronitrile. To the stirred mixture, immersed in a 25° water bath, there was added 1.2 g. (0.005 mole) of iodine dissolved in a mixture of 5 ml. of tetrahydrofuran and 3 ml. of methanol. The iodine solution was added dropwise but fast enough to slightly exceed the rapid decolorization rate. The mixture was stirred for 2–4 hr. until a pale yellowish color was observed. It was diluted with ether and filtered. The filter cake was washed with ether and was discarded. The ether filtrate was washed free of excess iodine with 15% aqueous sodium iodide solution, dried over sodium sulfate, and evaporated under reduced pressure. Without purification, the 21-iodo residue was dissolved in 30 ml. of acetone, and treated with a mixture of 5 ml. of acetic acid and 8 ml. of triethylamine. The resulting solution was heated under reflux for 1 hr., cooled, and diluted with water. The crude product was filtered and recrystallized from aqueous acetone to afford 0.30 g. (30%) of product, m.p. 197–199°. Further recrystallization gave an analytical sample, m.p. 200–201°, $[\alpha]^{25}D$ −89° (c 1%, $CHCl_3$).

Anal. Calcd. for $C_{23}H_{31}NO_4$: C, 71.66; H, 8.11. Found: C, 71.43; H, 7.96.

3,20-Dioxo-21-hydroxypregn-4-ene-19-nitrile 21-Acetate (III).—A solution of 0.10 g. (0.0002 mole) of II in 20 ml. of acetone was treated at 10–15° with 0.2 ml. of 8 *N* chromic acid reagent for 10 min. Excess oxidant was destroyed with 2-propanol, and the solvent was partially evaporated under reduced pressure. After dilution with water, the product was filtered and dried. The crude product was chromatographed on neutral alumina to effect isomerization of the double bond and gave 0.025 g. (25%) of product, m.p. 125–129°. Recrystallization from aqueous acetone gave an analytical sample, m.p. 129–130°, λ_{max}^{EtOH} 232 mμ (ε 16,500), $[\alpha]^{25}D$ +220 (c 1%, $CHCl_3$).

Anal. Calcd. for $C_{23}H_{29}NO_5$: C, 72.03; H, 7.62. Found: C, 72.01; H, 7.46.

(7) Melting points were determined with a Thomas–Hoover apparatus and are corrected. Ultraviolet spectra were obtained with a Cary Model 11 instrument. Microanalyses were performed by the Microanalytical Department, University of California, Berkeley, Calif. Optical rotations were obtained in a 0.5-dm. cube with a Rudolph photoelectric polarimeter.

Anticholinesterase Activity of Phenylalkyltrimethylammonium Compounds[1]

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Thomas and Marlow[2] have reported on the anti-acetylcholinesterase (red cell) activities of a series of phenylalkyltrimethylammonium compounds. They noted, as the homologous series was ascended, that their inhibitory effect decreased to the fourth member, then increased. The pattern of results obtained differs fundamentally from a "normal" series such as *n*-alkyltrimethylammonium. They interpreted their results in

(1) This investigation was supported in part by Public Health Grant HE-06072-03.

(2) J. Thomas and W. Marlow, *J. Med. Chem.*, **6**, 107 (1963).