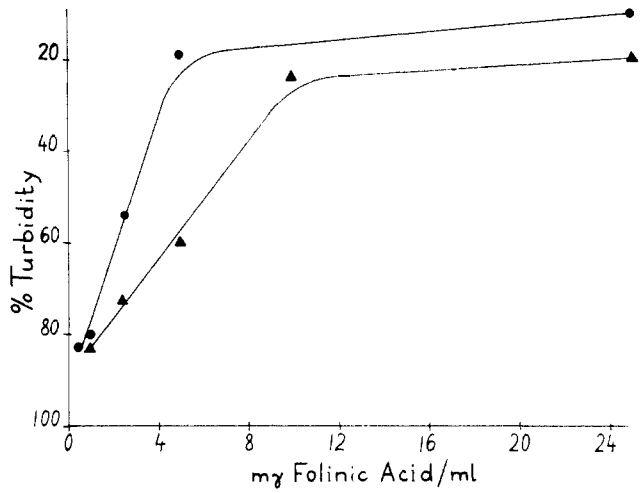


Fig. 1.—Reversal of inhibitory action of pteridinosteroid (III) by folinic acid on *L. leichmannii*: ●—●, III, 10 γ/ml.; ▲—▲, III, 20 γ/ml.

alumina (activity grade III, 15 g.). The product was eluted from the column with benzene (200 ml.) and crystallized as sharp needles from aqueous methanol, m.p. 176–178°; yield, 850 mg. (Ib). It gives one spot by thin layer chromatography (adsorbent, alumina; solvent, ether–alcohol, 9:1).

Anal. Calcd. for $C_{21}H_{31}BrO_3$: C, 61.31; H, 7.54; Br, 19.46. Found: C, 61.13; H, 7.50; Br, 19.30.

17β-Acetoxy-5α-androstano[2,3-*g*]-2′,4′-diaminopteridine (III).—A solution of 1 g. of Ib in methanol (150 ml., 80%, 65°) was kept well stirred while 2,3,4,6-tetraaminopyrimidine bisulfite[11] (II) (3 g.) was added in small portions during the course of 2 hr. The pH of this uniform suspension (6.3) was adjusted to 7.5–8.0 by dropwise addition of 0.5 *N* sodium hydroxide with vigorous stirring. A deep yellow solution resulted, and the reaction was allowed to proceed for 72 hr. at room temperature during which time an ultraviolet absorption peak appeared in the 360–370 mμ region and, at the end of this period, the ratio of absorbancies at 260 *vs.* 370 mμ reached a constant value between 3 and 5.

The solution was neutralized (pH 7) with glacial acetic acid and the dark red solids were filtered (to facilitate concentration of the solution). The filtrate was evaporated to dryness *in vacuo;* the residue was mixed with the dark solids that had been collected by filtration and with some Celite, then extracted in a Soxhlet apparatus with chloroform for 48 hr., the solvent being replaced freshly after every 12 hr. Thin layer chromatography of this extract on alumina with chloroform–ethanol (9:1) gave, on examination under ultraviolet light, an oblong fluorescent spot indicating that probably some deacetylation of the steroid had occurred during the condensation reaction. The combined chloroform extracts were evaporated to dryness and then dried further in a vacuum oven at 110° for 12 hr. (0.8 g.). The residue was dissolved in dry pyridine (10 ml.) and cooled in an ice bath while acetic anhydride (3 ml.) was added with vigorous stirring. The mixture was allowed to stand at room temperature for 6 hr. The solids were collected at the pump, dried (0.6 g.), and dissolved in a minimum volume of chloroform (15 ml.). Examination by thin layer chromatography, using alumina with chloroform–ethanol (9:1), showed only one well-defined spot. The chloroform solution of the pteridinosteroid was adsorbed over a column of Woelm basic alumina (activity grade III, 50 g.). Elution of the column with benzene (200 ml.) gave white solids (probably steroid). Elution with chloroform (500 ml.) gave fractions yielding a yellow residue which had an ultraviolet absorbancy ratio at 260/370 mμ of approximately 3.0. These fractions were combined and dissolved in chloroform (20 ml.) and addition of petroleum ether (b.p. 30–60°) (50 ml.) precipitated yellow solids, m.p. 250–300° dec. (200 mg.).

This was redissolved in benzene (20 ml.) and adsorbed over a column of neutral Woelm alumina (activity grade III, 25 g.). The chloroform–ethanol (9:1, 250 ml.) eluate fraction showed characteristic ultraviolet spectra of the pteridinosteroid. Crystallization from chloroform–petroleum ether (b.p. 30–60°) mixture (2:5 v./v.) gave yellow crystals, which did not melt below 250° (gradual decomposition) (170 mg.). The compound gave a positive Liebermann–Burchard reaction. Sodium fusion test for halogen and Zimmermann color reaction for 3-keto group were negative; $[\alpha]^{28}D$ +57° (*c* 1.0, $CHCl_3$); λ_{max}^{EtOH} 257 mμ (ε 25,600), 373 (9900). Infrared spectrum shows bands for NH_2, C=O, C=N, CH, CH_2, CH_3, and C—O—C. N.m.r. spectrum shows absorption for the angular methyl groups and for the acetyl group.

Anal. Calcd. for $C_{33}H_{34}N_8O_2$: C, 66.65; H, 7.60; N, 18.65. Found: C, 66.50; H, 7.54; N, 18.40.

Microbiological Assay.—The compound was dissolved in alcohol and serial dilutions were made with water. Inhibition and reversal studies on *L. leichmannii* 313 were conducted in the manner described previously with other inhibitors.[9]

Animal Assay.—The compound was assayed for androgenic activity by our standard method.[12] For antiandrogenic activity, we followed the same procedure but gave the rats, in addition to the test compound, 1 γ of testosterone daily, subcutaneously. In the chick assay, the compound was administered locally in addition to 1 γ of testosterone daily on the comb. The final assessment of antiandrogenicity was done by the method of Dorfman[13] where the comb is stimulated by a single intramuscular injection of 0.5 mg. of testosterone enanthate and the antagonist is applied daily to the comb.

(11) M. F. Mallette, E. C. Taylor, and C. K. Cain, *J. Am. Chem. Soc.*, **69**, 1814 (1947).

(12) A. Segaloff and R. B. Gabbard, *Endocrinology*, **71**, 949 (1962).

(13) "Methods in Hormone Research," Vol. II, R. I. Dorfman, Ed., Academic Press, New York, N. Y., 1962, p. 315.

Ethyl N-Benzylleucinate Nitrogen Mustard[1]

HOWARD E. SMITH AND SUSAN L. COOK

Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37203

Received April 13, 1964

In the treatment of some animal tumors, the differences in the activities and toxicities of alkylating agents[2–4] which accompany variations in structure and configuration and the marked effectiveness of some agents, such as sarcolysin (I),[4] incorporating a nitrogen mustard alkylating group attached to an amino acid carrier, are well known. They suggest that the preparation of related amino acid nitrogen mustards may afford active agents which may be of greater use in cancer chemotherapy than those presently available and may also give additional insight into the exact mode of action of alkylating agents.[5]

As a contribution to the achievement of these goals ethyl N-(*p*-[bis(2-chloroethyl)amino]benzylidene)-DL-leucinate (II) was prepared by the condensation of *p*-[bis(2-chloroethyl)amino]benzaldehyde[6] and ethyl DL-leucinate. Reduction of the Schiff base II with hydrogen, using Adams catalyst, produced ethyl N-(*p*-[bis(2-chloroethyl)amino]benzyl)-DL-leucinate (III), isolated as the oxalate salt. Both II and III were

(1) This work was supported by an Institutional Cancer Grant, IN-25-C, Ora Currier Memorial Grant for Cancer Research, from the American Cancer Society to Vanderbilt University.

(2) W. C. J. Ross, "Biological Alkylating Agents," Butterworth and Co. Ltd., London, 1962.

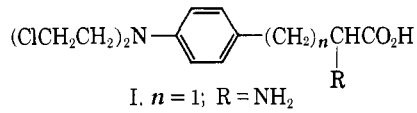
(3) H. E. Skipper and L. H. Schmidt, *Cancer Chemotherapy Rept.*, **17**, 1 (1962).

(4) F. R. White, *ibid.*, **6**, 81 (1960).

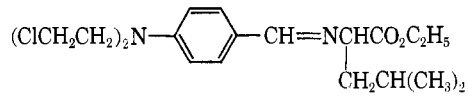
(5) H. F. Gram, C. W. Mosher, and B. R. Baker, *J. Am. Chem. Soc.*, **81**, 3103 (1959).

(6) (a) W. C. J. Ross, G. P. Warwick, and J. J. Roberts, *J. Chem. Soc.*, 3110 (1955); (b) R. H. Wiley and G. Irick, *J. Org. Chem.*, **26**, 593 (1961).

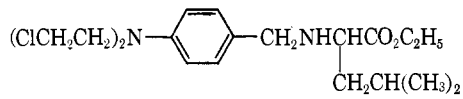
tested[7] for antitumor activities in the KB cell culture[8a] and the Walker 256 carcinosarcoma (subcutaneous)[8b] test systems.[9] Attempts to prepare the carboxylic acid of III by hydrolysis in hydrochloric acid were unsuccessful, but for comparison of activities N-(*p*-[bis(2-chloroethyl)amino]benzyl)aniline (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.



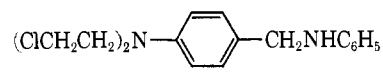
I, $n = 1$; R = NH_2
V, $n = 2$; R = H



II



III



IV

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

MANFRED E. WOLFF AND WINSTON HO

Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California 94122

Received April 23, 1964

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).