4-[N,N-Bis(2-chloroethyl)amino]-2-methylbenzylidenehydrazide of Ferrocenecarboxylic Acid (VI).—A hot solution of 1.17 g. (0.0045 mole) of 4-[N,N-bis-(2-chloroethyl)amino]-2-methylbenzaldehyde in 10 ml. of absolute ethanol was added to 1 g. (0.0041 mole) of ferrocenecarboxhydrazide in 10 ml. of absolute ethanol and the mixture was refluxed for 30 min. and filtered hot. Cold filtration yielded 1.41 g. (71%), of VI, m.p. 195–198°. Recrystallization from absolute ethanol gave material, m.p. 196–197°.

Anal. Calcd. for $C_{23}H_{25}Cl_2FeN_3$: C, 56.81; H, 5.18; N, 8.64; Cl, 14.58. Found: C, 56.80; H, 5.25; N, 8.68; Cl, 14.62.

Acknowledgment.—We wish to acknowledge the technical assistance of Mr. J. Graff and several helpful discussions with Dr. Leo Rane regarding the screening results.

Potential Anticancer Agents.[1] LXXX. Alkylating Agents Related to Phenylalanine Mustard.[2] VI. Enantiomeric *meta*-Phenylalanine Mustards

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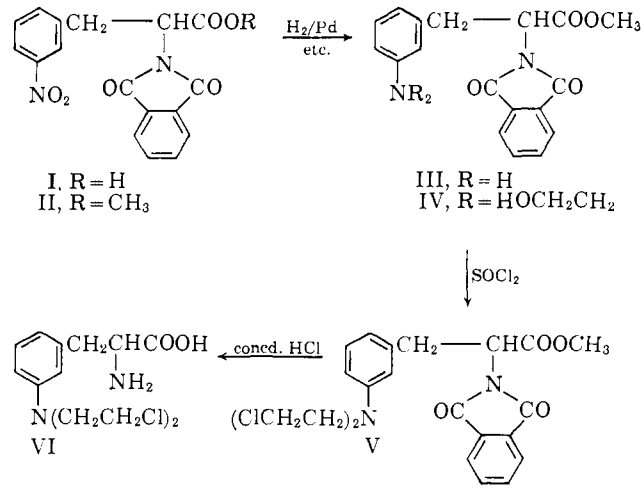
Received July 26, 1962

Preparation of the enantiomorphs of *m*-phenylalanine mustard (D- and L-VI) was prompted by the finding[3] that in the treatment of some transplanted mouse tumors the racemate DL-VI[4] was markedly superior to the *para*-mustard (L or DL); the L-form of the *para*-mustard, in turn, was several times more active than the D-*para*-mustard in certain tests.[5] If this optical selectivity should hold for the *meta*-mustards, then the L-isomer (L-VI) might be one of the most efficacious anticancer drugs of this highly interesting series.

Synthesis of D-VI and L-VI began with the chemical resolution of a precursor, N-phthalyl-*m*-nitrophenylalanine (DL-I), in the sequence[4] to the racemic mustard DL-VI, and was completed by that sequence. Optically pure brucine salts of both D-I and L-I were obtained from one treatment of DL-I with the alkaloid and were converted with methanolic hydrogen chloride directly to the methyl esters, D- and L-II. Intermediate regeneration with aqueous acid to the enantiomorphic acids D- and L-I was inconvenient and less efficient; attempted regeneration in mild base caused partial loss of the phthalyl group. Success in the conversion of IV to V was dependent on use of a carefully controlled excess (13–14%) of thionyl chloride in order to avoid tar formation. Because D-IV and L-IV (unlike racemic IV) could not be crystallized, the degree of purity from



ethylene oxide polymers was best estimated from the optical rotation of a given sample of sirup, and the amount to be used relative to thionyl chloride was verified on a small scale for each batch of D- or L-IV. Optimum yields of D- and L-V were 57–58%. Replacement of the hydroxyl groups in D-IV and L-IV by chlorine could also be accomplished with methanesulfonyl chloride in hot pyridine,[6] in lower yield of D-V and L-V but with no tendency toward darkening and tarring.

A crucial modification in the removal of blocking groups (V to VI) was use of concentrated hydrochloric acid at 95° rather than at reflux[4] (110–130°), otherwise yields of D-VI and L-VI varied from 10–0%. Under optimum conditions, over-all yields of D-VI and L-VI from the nitro esters D-II and L-II were 35% and 27%, respectively. Absence of racemization during the sequence could be expected from the work of Bergel[7] and of Luck[8] and was supported by observation of equal but opposite optical rotations for each enantiomeric pair; use of 12 *M* rather than 6 *M* hydrochloric acid in the final step apparently did not affect the optical center. The absolute configurations were inferred by comparison of optical rotations for the enantiomeric mustards VI and precursors (I, II, and III-hydrochlorides) with the rotations of the analogous *para*-isomers,[7] where starting materials of known absolute configuration were used; the rotations of D- and L-II also agreed very closely with those of N-phthalyl-D- and L-phenylalanine.[9]

Biological Results.—Preliminary studies[10] have been made in which the D- and L-*meta* mustards (D- and L-VI) were compared with the racemic form (DL-VI) and with L-*p*-phenylalanine mustard against four tumors in rats. The results are shown in Table I. The quantity ED_{90} is the dose which causes a 90% reduction in tumor weight, except with Dunning Leukemia where it is the dose causing 90% of the rats to be cured. These preliminary data certainly do not suggest any significant difference in antitumor activity or therapeutic index between the D- and L-forms of *m*-phenylalanine mustard.

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. SA-43-ph-1892. The opinions expressed in this paper are those of the authors and are not necessarily those of the Cancer Chemotherapy National Service Center.

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TABLE I

CARCINOSTATIC ACTIVITY OF SOME PHENYLALANINE MUSTARDS

Tumor	Compound	Daily dose: μ mole/Kg. body wt. LD_{50}	Daily dose: μ mole/Kg. body wt. ED_{90}	Therapeutic index LD_{10}/ED_{50}
Walker Ca-256, subcutaneous	D-VI	10.0	0.8	12
	L-VI	6.0	0.7	9
	DL-VI	10.0	0.7	14
	L-*para*	6.0	0.7	9
Walker Ca-256, pulmonary	D-VI	10.0	1.1	9
	L-VI	6.0	1.1	5
	DL-VI	10.0	1.1	9
	L-*para*	6.0	1.1	5
Yoshida Hepatoma, ascitic	D-VI	10.0	0.90	11
	L-VI	6.0	0.55	11
	DL-VI	10.0	0.38	27
	L-*para*	6.0	0.55	11
Dunning Leukemia	D-VI	6.5	0.8	8
	L-VI	6.5	0.8	8
	DL-VI	6.5	0.8	8
	L-*para*	3.5	1.5	8

Experimental[11]

Chemical Resolution. (1) 3-(*m*-Nitrophenyl)-N-phthalyl-L-alanine (L-I) Brucine Salt.—Hot solutions of 18.8 g. (0.0550 mole) of the racemic acid I in 95% ethanol (420 ml.) and of 23.8 g. (0.0600 mole) of brucine in 95% ethanol (185 ml.) were combined and allowed to cool slowly to room temperature. After 20 hr., the yellow salt that separated was collected on a filter, washed with two 50-ml. portions of ethanol (the filtrates were saved for (2) below), and recrystallized from 1200 ml. of boiling 95% ethanol. The product weighed 20.3 g. (99% yield), m.p. 158–161° dec., $[\alpha]^{25}D$ −107.1 ± 2.5° (50% water-dioxane), and included a second crop (0.9 g.) obtained by concentrating the mother liquor (from the recrystallization) to 400 ml. Easy solubility in methanol was characteristic.

Anal. Calcd. for $C_{39}H_{38}N_4O_{16}$: C, 65.4; H, 5.21; N, 7.62. Found: C, 65.7; H, 5.24; N, 7.47.

(2) 3-(*m*-Nitrophenyl)-N-phthalyl-D-alanine (D-I) Brucine Salt.—The combined filtrate after removal of the yellow salt above was concentrated *in vacuo*. The semisolid residue was dissolved in 200 ml. of hot methanol and the solution allowed to cool slowly to room temperature. After 20 hr., 18.9 g. (92%) of the white salt was collected, m.p. 142–145°. Recrystallization from boiling methanol (200 ml.) afforded 16.8 g. (82%), m.p. 142–146°, $[\alpha]^{25}D$ +83.9 ± 2.0° (50% water-dioxane). This salt was characteristically a white powder, contained methanol of crystallization, and was easily soluble in 95% ethanol.

Anal. Calcd. for $C_{40}H_{38}N_4O_{16}\cdot CH_3OH$: C, 64.2; H, 5.52; N, 7.37. Found: C, 64.0; H, 5.69; N, 7.07.

3-(*m*-Nitrophenyl)-N-phthalyl-L-alanine (L-I).—The yellow brucine salt of L-I (1.5 g., 2.0 mmoles) was suspended in 20 ml. of 4 *M* hydrochloric acid. The resultant gum, when broken up and stirred intermittently, gradually formed a white solid (0.68 g., 100%), m.p. 159–164°. Recrystallization from a chloroform–Skellysolve C[12] mixture (1:1) afforded 0.50 g. (73%), m.p. 162–165°, $[\alpha]^{28}D$ −212.6 ± 3.2° (absolute ethanol).

Anal. Calcd. for $C_{17}H_{12}N_2O_6$: C, 60.0; H, 3.56; N, 8.23. Found: C, 59.7; H, 3.73; N, 8.06.

3-(*m*-Nitrophenyl)-N-phthalyl-D-alanine (D-I) was obtained in 66% yield, m.p. 160–163°, $[\alpha]^{25}D$ +209.7 ± 3.0° (absolute ethanol). A sample recrystallized for analysis melted at 163–165°, $[\alpha]^{15}D$ +212.4 ± 3.0°

Anal. Found: C, 59.6; H, 3.86; N, 8.45.

3-(*m*-Nitrophenyl)-N-phthalyl-D-alanine Methyl Ester (D-II).—A solution of 23.1 g. (31.0 mmoles) of the white brucine salt of D-I in 300 ml. of saturated anhydrous methanolic hydrogen chloride was refluxed for 90 min., concentrated to 150 ml., and chilled to 5–10°. The product crystallized and the total yield, including a second crop formed on further concentration, was 8.9 g. (81%), m.p. 109–113°, $[\alpha]^{28}D$ +218.5 ± 2.0° (methanol). The analytical sample from methanol (20 ml./g.) had m.p. 111–112°, $[\alpha]^{25}D$ +219.3 ± 2.0°, R_f 0.10.

Anal. Calcd. for $C_{18}H_{14}N_2O_6$: C, 61.0; H, 3.98; N, 7.90. Found: C, 61.1; H, 3.86; N, 7.80.

3-(*m*-Nitrophenyl)-N-phthalyl-L-alanine methyl ester (L-II) was similarly obtained in 80% yield, m.p. 109–110°, $[\alpha]^{25}D$ −218.7 ± 2.0° (methanol, same in dioxane), R_f 0.10.

Anal. Found: C, 60.8; H, 3.94; N, 7.89.

3-(*m*-Aminophenyl)-N-phthalyl-L-alanine Methyl Ester (L-III).—A methanol solution of L-II was hydrogenated as described[4] for the preparation of DL-III. The filtered solution, on concentration to one-fourth the volume and chilling, afforded crystals,[13] m.p. 135–137° (99% yield), $[\alpha]^{25}D$ −231.0 ± 2.0° (methanol). The analytical sample from methanol had m.p. 134–136°, $[\alpha]^{25}D$ −230.0 ± 2.0°, R_f 0.17.

Anal. Calcd. for $C_{18}H_{16}N_2O_4$: C, 66.7; H, 4.97; N, 8.63. Found: C, 66.9; H, 5.19; N, 8.44.

The amine from initial, small runs was a sirup and was characterized by conversion in methanol solution (0.5 g./10 ml.) to the *hydrochloride* with anhydrous hydrogen chloride. The salt, initially an oil, was obtained as a crystalline solid by addition of ether (40 ml.) with scratching and chilling; it was twice recrystallized from methanol–ether (1:3), m.p. 177–192° dec., $[\alpha]^{26}D$ −183 ± 2.0° (25% methanol in water).

Anal. Calcd. for $C_{18}H_{17}ClN_2O_4$: C, 59.9; H, 4.74; Cl, 9.82; N, 7.76. Found: C, 60.0; H, 4.68; Cl, 9.82; N, 7.79.

3-(*m*-Aminophenyl)-N-phthalyl-D-alanine methyl ester (D-III) was similarly obtained in 90% yield, m.p. 132–135°, $[\alpha]^{25}D$ +234.0 ± 2.0° (methanol). The analytical sample melted[13] at 133–135°, $[\alpha]^{28}D$ +230.0 ± 2.0°, R_f 0.17.

Anal. Found: C, 66.9; H, 5.11; N, 8.56.

The *hydrochloride* melted at 177–191° dec., $[\alpha]^{26}D$ +182.7° ± 2.0 (25% methanol in water).

Anal. Found: C, 59.7; H, 4.99; Cl, 10.07; N, 8.04.

3-{*m*-[Bis(2-hydroxyethyl)amino]phenyl}-N-phthalyl-L-alanine Methyl Ester (L-IV).—The amine L-III was treated by the procedure for DL-IV,[4] but with only two-thirds the amount of ethylene oxide. The neutralized reaction mixture was extracted three times with methylene chloride, and the combined extracts then were washed with water (four times) until the washings were colorless. Concentration of the dried organic solution formed a sirup (93%, $[\alpha]^{25}D$ −195° in methanol), which was treated twice with boiling ether, separated by decantation of the ether, and dried *in vacuo*. The product, $[\alpha]^{25}D$ −205.0 ± 2.0° (methanol), 87% yield, could not be crystallized, but was chromatographically homogeneous, R_f 0.41. Reduced intensity of infrared bands at 2.98 μ (OH) and 9.63 μ (C-OH) in the purified product suggested that the crude material had contained polymers of ethylene oxide, which would not be detected on a chromatogram under ultraviolet light.

3-{*m*-[Bis(2-hydroxyethyl)amino]phenyl}-N-phthalyl-D-alanine methyl ester (D-IV) was obtained in the same manner and yield, $[\alpha]^{25}D$ +203.0 ± 3.0° (methanol), R_f 0.41.

3-{*m*-[Bis(2-chloroethyl)amino]phenyl}-N-phthalyl-D-alanine Methyl Ester (D-V).—(1) By use of the procedure[4] for the racemate DL-V, 24.3 g. (0.0540 mole) of D-IV was treated with 14.4 g. (0.121 mole) of thionyl chloride in chloroform. In crystallization of the product from hot 95% ethanol (700 ml.), an initial chilling caused separation of a black gum. The supernatant was then decanted and chilled with intermittent scratching. When crystallization was well established, further chilling at −5° for 20 hr. afforded 12.95 g. of light tan product, m.p. 71–74°, $[\alpha]^{25}D$ +177.0 ± 3.0° (methanol), which was suitable for conversion to L-VI. The mother liquor when concentrated to 200 ml. afforded a second crop (2.50 g., total yield 58%), $[\alpha]D$ +174.0 ± 2.0°. After one recrystallization from ethanol, an analytical sample melted at 77–78° $[\alpha]^{25}D$ +178.0 ± 1.0° (methanol), R_f 0.06.

Anal. Calcd. for $C_{22}H_{22}Cl_2N_2O_4$: C, 58.8; H, 4.93; Cl, 15.8; N, 6.23. Found: C, 58.9; H, 5.06; Cl, 15.6; N, 6.40.

(2) Treatment of D-IV in pyridine with methanesulfonyl chloride,[6] at 10° for 10 min. and then at 100° for 2 hr., afforded D-V as a gum, which was crystallized from ethanol in 37% yield

(11) Melting points were observed on a Fisher-Johns apparatus and are uncorrected. Optical rotations were taken in 1% solutions at 1 dcm. path length. Paper chromatograms were run by the descending technique on Schleicher and Schuell No. 2496 acetylated paper in benzene–methanol–water (2:6:1), except as noted with D- and L-VI. Spots were detected visually under ultraviolet light.

(12) A hydrocarbon fraction, b.p. 88–109°.

(13) The racemate DL-III was reported[4] as a sirup, but has subsequently been crystallized, m.p. 85–88°.

and melted at 80–82° after recrystallization, $[\alpha]^{24}D$ +177.0 ± 2.0° (methanol).

3-{*m*-[Bis(2-chloroethyl)amino]phenyl}-N-phthalyl-L-alanine methyl ester (L-V) was prepared with thionyl chloride, as for D-V, in 57% yield, m.p. 70–72°, m.p. after recrystallization 77–79° and $[\alpha]^{23}D$ −177.0 ± 2.0° (methanol), R_f 0.06.

3-{*m*-[Bis(2-chloroethyl)amino]phenyl}-L-alanine (L-VI).—The hydrolysis described[4] for DL-VI was carried out at 90–95° for 4 hr. After filtration from red-stained phthalic acid, the solution was diluted with several vols. of water. The chloroform extracts were washed only twice with water and then not dried before concentration, because in these steps the product tended to separate as a gel. Finally, trituration with refluxing 95% ethanol for no more than several min. was preferred for crystallizing the product, which was washed with acetone while on the filter. The yield was 55%, m.p. 181–183°, $[\alpha]^{26}D$ +26.5 ± 0.5° (1 *M* hydrochloric acid), $[\alpha]^{25}D$ −21.7 ± 1.0° (methanol), R_f 0.34 on Whatman No. 1 paper in water-saturated 1-butanol (ninhydrin-positive).

Anal. Calcd. for $C_{13}H_{18}Cl_2N_2O_2$: C, 51.2; H, 5.92; Cl, 23.2; N, 9.18. Found: C, 51.2; H, 5.84; Cl, 23.1; N, 9.10.

Purity of other samples, of $[\alpha]D$ sometimes as low as +20° in 1 *M* hydrochloric acid, could be improved to $[\alpha]D$ +26° by a second treatment with refluxing 95% ethanol. Small samples for rotation could be recrystallized with low[8] recovery from hot 95% ethanol (0.3 g. in 40 ml.) containing charcoal, by concentrating the solution to one-half the volume and chilling at 0° for 24 hr.

3-{*m*-[Bis(2-chloroethyl)amino]phenyl}-D-alanine (D-VI) was similarly prepared after 4.5 hr. heating in 65–75% yields, m.p. 180–182°, $[\alpha]^{27}D$ −26.5 ± 0.5° (1 *M* hydrochloric acid), R_f 0.34 as for L-VI.

Acknowledgment.—The authors are indebted to Dr. Peter Lim for infrared interpretations, to his staff for paper chromatography and optical rotation measurements, and to Mr. O. P. Crews and staff for preparation of intermediates.

Pyrimidines. III. Some 6-Substituted Di- and Trichloropyrimidines

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Received August 28, 1962

In a previous paper,[2] it was reported that the three monomethyltrichloropyrimidines were prepared and submitted to Cancer Chemotherapy National Service Center, National Institutes of Health, for anticancer screening. It appeared that 6-methyl-2,4,5-trichloropyrimidine was active[3] against Ehrlich ascites tumor in the hands of one screener, but that this activity was not reproducible in other laboratories. Consequently, it was decided to prepare several 6-substituted trichloropyrimidines to determine whether related compounds would show any measure of activity against this tumor.

The 6-substituted pyrimidines were prepared by condensing the appropriate β-ketoester with thiourea in the presence of sodium ethoxide to 6-substituted-2-thiouracils, according to the general procedure of Anderson, *et al.*[4] The conversion of the thiouracils to uracils was accomplished by hydrolysis with chloroacetic and hydrochloric acids. The uracils were chlorinated in the 5-position with sulfuryl chloride by the method of Barrett, Goodman, and Dittmer.[5] The dichloro- and trichloropyrimidines were prepared from the uracils and 5-chlorouracils, respectively, by means of phosphorus oxychloride. This synthetic sequence is summarized in Scheme I.

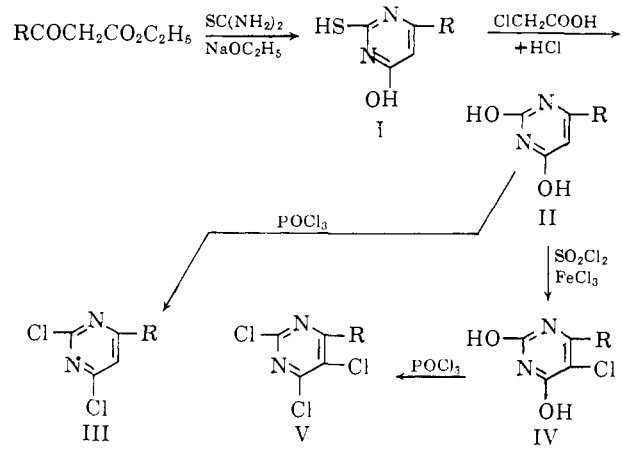
Of the 28 compounds prepared in this study, the ethyl, propyl, isopropyl and phenyl substituted thiouracils and uracils had been reported previously.[4,6,7]

Table I summarizes the pertinent data on the 6-substituted pyrimidines, and Table II contains a summary of the ultraviolet spectral data obtained on the di- and trichloropyrimidines.

These compounds have been submitted for anticancer screening, and the results on the three tumor system (Sarcoma-180, Carcinoma-755, and Leukemia-1210) are listed in Table III. None of the compounds showed reproducible activity. The Ehrlich ascites tumor is no longer included in the screening system.[3]

SCHEME I

R for a = H, b = C_2H_5, c = C_3H_7, d = *i*-C_3H_7, e = *n*-$C_{17}H_{37}$, f = C_6H_5



Experimental[8]

The methods of synthesis of the analogous compounds are similar, and the particular derivatives described in detail are for illustrative purposes.

6-*n*-Heptadecyl-2-thiouracil (Ie).—To a solution of sodium ethoxide, prepared from 6.6 g. (0.287 g.-atom) of sodium dissolved in 300 ml. of ethanol, was added 15.1 g. (0.198 mole) of thiourea and 50.0 g. (0.142 mole) of ethyl stearoylacetate. The mixture was heated on the steam bath for 6 hr. with agitation and allowed to stand overnight. The alcohol was removed in a flash evaporator and the residue was dissolved in water, decolorized with charcoal, and acidified with hydrochloric acid. The product was removed by filtration, washed with water and dried at 70° overnight. The yield was 48.5 g. (93%), m.p. 120–135°. An analytical sample was prepared by recrystallizing several times from methanol, m.p. 147.5–149°.

Anal. Calcd. for $C_{21}H_{38}N_2OS$: N, 7.64; S, 8.73. Found: N, 7.66; S, 8.96.

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