1,2-cyclohexanediols. In the latter instance, a constant total molar concentration of copper plus hydroxyl groups rather than of copper plus diol groups was maintained. The maximum optical density, therefore, *did not reflect the composition of the copper-diol complex.* Repetition of this work, using reaction mixtures containing various copper-diol proportions, gave results that are in keeping with the formation of a cuprammonium-diol complex in which the copper-diol ratio is 1:1. This is in agreement with the findings of Reeves.[2]

(2) R. E. Reeves and P. Bragg, *J. Org. Chem., in press.*

## Potential Anticancer Agents.[1] LVIII. Analogs of Chlorambucil. VIII. Monofunctional Alkylating Agents Derived from ω-(*p*-Aminophenyl)alkanoic Acids

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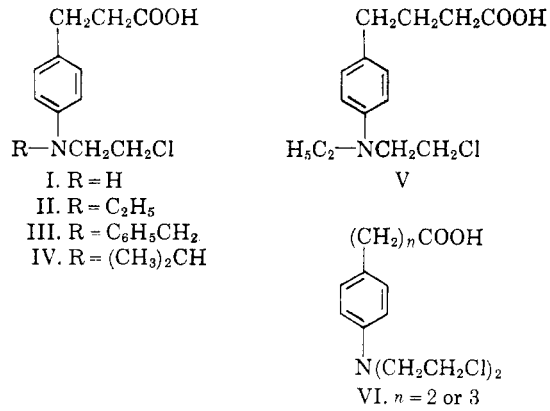
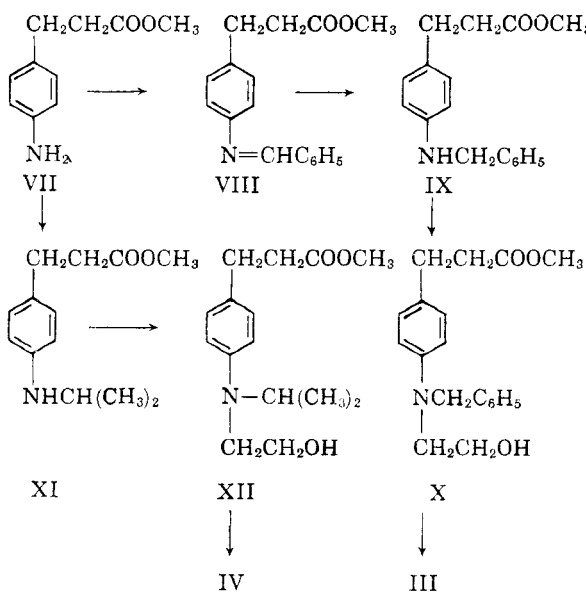
A previous paper[2] described the preparation of the monofunctional alkylating agents (I and II) derived from 3-(*p*-aminophenyl)propionic acid. The "one-armed" mustard (II) was an especially interesting compound for comparison with the difunctional alkylating agent (VI, $n = 2$) as a test of the hypothesis that a monofunctional alkylating group might be as effective as a difunctional agent in effecting irreversible enzyme inhibition.[3] In order to study the effect of the *N*-alkyl group, two other "one-armed" mustards (III and IV) have been prepared, as well as an *N*-ethyl "one-armed" homolog (V) which can be directly compared with chlorambucil[4] (VI, $n = 3$).

Methyl 3-(*p*-aminophenyl)propionate[2] (VII) was used as the starting material for the preparation of both III and IV. The reaction of benzaldehyde with VII[2] gave a 92% yield of the crystalline anil (VIII), which was hydrogenated over Raney nickel to afford the benzylamine (IX) as a low-melting




solid in 89% yield. Hydrogenation at low pressure (20 p.s.i.) was essential; at 50 p.s.i. hydrogenolysis occurred, regenerating the primary amine (VII). The absence of VII in the low-pressure product could be demonstrated by paper chromatography. Treatment of IX with ethylene oxide in aqueous acetic acid gave a good yield of the chromatographically homogeneous sirup (X) which, without purification, was allowed to react with phosphoryl chloride at reflux. The crystalline acid (III) was formed in 82% yield, hydrolysis of the ester group occurring during the decomposition of the chlorination reaction mixture with water.




The conversion of VII to the *N*-isopropyl ester (XI) was accomplished by treatment with 2-bromopropane in the presence of potassium carbonate until paper chromatography of the product showed the absence of VII. Analysis of the crude product suggested that it was contaminated with some dialkylated product although this inference was not obvious from the paper chromatographic data. The crude product, which contained XI, was treated with ethylene oxide in aqueous acetic acid and the hydroxyethylation product was subjected

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center of the 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 not necessarily those of the Cancer Chemotherapy National Service Center. For the preceding paper of this series, see E. J. Reist, R. R. Spencer, M. E. Wain, I. G. Junga, L. Goodman, and B. R. Baker, *J. Org. Chem.*, 26, 2821 (1961).

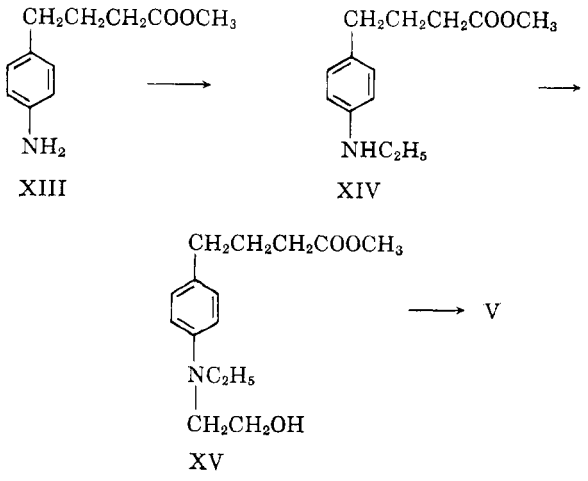
(2) W. A. Skinner, H. F. Gram, and B. R. Baker, *J. Org. Chem.*, **25**, 777 (1960).

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

(4) J. L. Everett, J. J. Roberts, and W. C. J. Ross, *J. Chem. Soc.*, 2386 (1953).

to chromatography on silica gel. Chloroform eluted a material which was probably the dialkylation product but which was not characterized. Methanol eluted the desired 2-hydroxyethylamine (XII), isolated as a chromatographically homogeneous sirup in 46% yield from VII. Reaction with phosphoryl chloride and hydrolysis of the ester during processing of the reaction mixture was carried out as in the preparation of III to yield the crystalline acid IV, which was still contaminated with a trace of ester. The analytically and chromatographically pure acid (IV) was obtained after a further hydrolysis with hydrochloric acid.

The preparation of the "one-armed" mustard (V) of phenylbutyric acid followed the sequence used to prepare the phenylpropionic acid analog (II).[2]




The reductive alkylation of the amino ester (XIII)[4] with acetaldehyde in the presence of Raney nickel afforded 90% of crystalline secondary amine (XIV). Conventional hydroxyethylation of XIV yielded 96% of the hydroxyethylamine, which was a sirup that gave good elemental analyses. Phosphoryl chloride, rather than thionyl chloride as in the preparation of II,[2] was used to convert XV to the acid V, ester hydrolysis taking place during the decomposition of the reaction mixture with water.

*Biological results.*[5] None of the "one-armed" mustards III, IV, or V showed any appreciable inhibiting effect at their maximum tolerated doses on Sarcoma 180, Adenocarcinoma 755, Leukemia L-1210, and Ehrlich Ascites.

## EXPERIMENTAL[6]

*Methyl 3-[(p-benzylideneamino)phenyl]propionate* (VIII). A solution of 1.79 g. (0.010 mole) of methyl 3-(*p*-aminophenyl)propionate[2] (VII), 1.34 g. (0.013 mole) of benzaldehyde, 0.2 g. of anhydrous sodium acetate, and 10 ml. of absolute ethanol was heated at reflux for 2 hr., then cooled to room temperature and diluted with 10 ml. of water. The mixture was extracted with ether (3 × 20 ml.) and the combined extracts were evaporated *in vacuo*, affording 2.45 g. (92%) of crystalline solid, m.p. 63–65°. Recrystallization from aqueous ethanol gave the analytical sample, m.p. 65.5–66.5°; $\lambda_{max(\mu)}^{Nujol}$ 5.74 (ester C=O), 6.13 (C=N and aromatic), 8.52 (ester C—O—C), 12.14 (*p*-disubstituted benzene), 13.19 and 14.35 (monosubstituted benzene). The compound moved as a single spot on chromatography[6] in solvent A on both Whatman No. 1 ($R_f$ 0.88) and acetylated papers ($R_f$ 0.60), and in solvent B on Whatman No. 1 paper. Only in the latter system was there any separation from (VII).

*Anal.* Calcd. for $C_{17}H_{17}NO_2$: C, 76.4; H, 6.41. Found: C, 76.5; H, 6.49.

*Methyl 3-[p-benzylamino)phenyl]propionate* (IX). Commercial Raney nickel catalyst[7] (8.0 g.) was washed free of water with ethanol, then added to a solution of 2.67 g. (0.010 mole) of the anil (VIII) in 50 ml. of methanol. The mixture was shaken under an initial pressure of 20 p.s.i. (gage) of hydrogen at room temperature for 50 min. The catalyst was separated by filtration through a layer of Celite and washed with methanol. The combined filtrate was evaporated *in vacuo*, leaving 2.38 g. (89%) of a yellow oil which solidified on standing at 5°. The white crystals were collected and had m.p. 25–29°; $\lambda_{max(\mu)}^{film}$ 2.97 (NH), 5.74 (ester C=O), 8.3–8.6 (ester C—O—C), 12.2 (*p*-disubstituted benzene), 13.55 and 14.3 (monosubstituted benzene). The compound moved as a single spot in solvent A on both Whatman No. 1 and acetylated papers with $R_f$ 0.93 and 0.35, respectively.

*Anal.* Calcd. for $C_{17}H_{19}NO_2$: C, 75.8; H, 7.11; N, 5.20. Found: C, 76.0; H, 7.23; N, 5.22.

*Methyl 3-[p-(2-propylamino)phenyl]propionate* (XI). A mixture of 25.0 g. (0.14 mole) of the primary amine (VII), 45.0 g. (0.27 mole) of potassium carbonate (anhydrous), and 75 ml. (0.80 mole) of 2-bromopropane was heated at reflux for 64 hr.[8] The mixture was filtered, the separated salts were washed with chloroform, and the combined filtrate was evaporated *in vacuo*, leaving 26 g. of a brown sirup. The infrared spectrum showed the —NH absorption at 2.97 $\mu$ which was characteristic of XI. Paper chromatography in solvent A on acetylated paper showed two spots, a major one at $R_f$ 0.54, presumably that of XI, and a minor one at $R_f$ 0.87, presumably that of the dialkylation product.

*Anal.* Calcd. for 2 [$C_{13}H_{19}NO_2$ (*monoalkylation product*)]: 1 [$C_{16}H_{25}NO_2$ (dialkylation product)]: C, 71.4; H, 9.00; N, 5.95. Found: C, 71.3; H, 8.76; N, 5.92.

*Methyl 4-[(p-ethylamino)phenyl]butyrate* (XIV). Reductive alkylation of 9.30 g. (0.048 mole) of methyl 4-(*p*-aminophenyl)butyrate (XIII)[4] in 150 ml. of absolute ethanol was accomplished with 3.08 g. (0.070 mole) of freshly distilled acetaldehyde, 0.30 g. of anhydrous sodium acetate, and 20 g. of commercial Raney nickel[7] (washed free of water with

(5) These tests were performed at this Institute by Dr. Joseph Greenberg and his staff under contract to the Cancer Chemotherapy National Service Center.

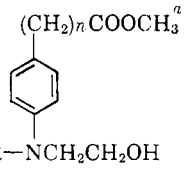
(6) Melting points were taken on a Fisher-Johns apparatus and are uncorrected. Paper chromatography was done by the descending technique on Whatman No. 1 paper or on Schleicher and Schuell No. 2043B acetylated paper as indicated. The solvent systems used were: A, benzene-methanol-water (2/6/1); B, ammonium sulfate–2-propanol-water (2/28/70); C, 1-butanol–acetic acid–water (5/2/3); D, water-saturated 1-butanol; E, 1-butanol saturated with 2*M* aqueous ammonia. The spots were detected by visual examination under ultraviolet light.

(7) Sponge nickel catalyst, Davison Chemical Co., Cincinnati 29, Ohio.

(8) This was the time required to ensure the disappearance of all the primary amine (VII) as detected by paper chromatography. Any unchanged VII might have eventually been converted to bis-mustard (VI, $n$ = 2) which would have confused the biological testing.

TABLE I

HYDROXYETHYL AMINES




| Compound No. | $n$ | R | Yield, % | $R_f$[h] |
|---|---|---|---|---|
| X | 2 | $C_6H_5CH_2$ | 71[b] | 0.47[e] |
| XII | 2 | $(CH_3)_2CH$ | 46[c] | 0.80[f] |
| XV | 3 | $CH_3CH_2$ | 96[d] | 0.83[g] |

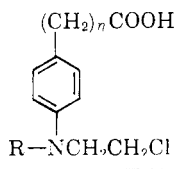
[a] The compounds were sirups. [b] Based on secondary amine (IX). [c] Based on primary amine (VII). [d] Based on secondary amine (XIV). Calcd. for $C_{15}H_{23}NO_2$: C, 67.9; H, 8.74; N, 5.28. Found: C, 68.6; H, 8.63; N, 5.44. [e] Compound IX had $R_f$ 0.25. [f] $R_f$ 0.94 in solvent D on Whatman No. 1 paper. [g] $R_f$ 0.92 in solvent E on Whatman No. 1 paper. [h] $R_f$ in solvent A on acetylated paper.

solved in 10 ml. of chloroform and applied to the top of a column (2.4 × 42 cm.) of 160 g. of silica gel (60–200 mesh). Any dialkylation product was eluted with chloroform until a colorless eluate was obtained. The column was then eluted with methanol until the eluate was colorless. Evaporation of the methanol eluate afforded 8.06 g. (46% based on VII) of a sirup. The pertinent data for the 2-hydroxyethylamines X, XII, and XV are found in Table I.

*The preparations of the 2-chloroethylamines* were carried out with phosphoryl chloride according to the technique described by Skinner *et al.*,[9] except that when the reaction mixture was added to water, the temperature was allowed to rise to 40–50°. The hydrolysis mixture was then stored 3–6 hr. at room temperature to complete hydrolysis of the ester, then the "one-armed" mustard was separated and purified. In the case of IV an additional hydrolysis with 12*M* hydrochloric acid for 1 hr. on the steam bath was required to afford IV, free of ester. Compound V was difficult to obtain as a crystalline solid. It was necessary to extract the crude product with Skellysolve B,[10] decanting from an insoluble tar, in order to obtain pure V. Attempted recrystallization of V from chloroform caused extensive tar formation; the same behavior was noted with the propionic acid analog (I).[2] The data for the 2-chloroethylamines III, IV, and V are found in Table II.

TABLE II

CHLOROETHYLAMINES




| Compound No. | $n$ | R | Yield, % | M.P.[d] | Calcd. C | Calcd. H | Calcd. Cl | Calcd. N | Found C | Found H | Found Cl | Found N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| III | 2 | $C_6H_5CH_2$ | 82[a,e] | 91–92 | 68.0 | 6.34 | 11.2 | 4.11 | 68.4 | 6.41 | 11.5 | 4.41 |
| IV | 2 | $(CH_3)_2CH$ | 31[b,f] | 106–108 | 62.3 | 7.47 | 13.1 | 5.19 | 62.2 | 7.39 | 13.2 | 5.19 |
| V | 3 | $CH_3CH_2$ | 11[c,g] | 61–62 | 62.3 | 7.47 | 13.1 | 5.19 | 62.3 | 7.69 | 13.0 | 5.35 |

[a] Yield of product, m.p. 63–88°. [b] Yield of product, m.p. 95–99°. [c] Yield of analytical sample. [d] Analytical product, all of which were recrystallized from Skellysolve B.[10] [e] $R_f$ 0.41 in solvent A on acetylated paper. [f] $R_f$ 0.80 in solvent D on Whatman No. 1 paper (*cf.* footnote (*f*) in Table I). [g] $R_f$ 0.79 in solvent E on Whatman No. 1 paper (*cf.* footnote (*g*) in Table I).

ethanol), as described for the preparation of methyl 3-[(*p*-ethylamino)phenyl]propionate.[2] The product (9.5 g., 90%) was a sirup which crystallized to a solid on chilling, m.p. 20–22°; $\lambda_{max(a)}^{film}$ 2.97 (NH), 5.74 (ester C=O), 8.2–8.7 (ester C—O—C), 12.15 (*p*-disubstituted benzene). The compound moved as a single spot on paper chromatography in solvent A on both Whatman No. 1 and acetylated papers with $R_f$ 0.60 and 0.97, respectively, whereas the primary amine (XIII) had $R_f$ 0.56 and 0.95, respectively, under these conditions and could be distinguished from XIV.

*Anal.* Calcd. for $C_{13}H_{19}NO_2$: C, 70.6; H, 8.65; N, 6.33. Found: C, 70.3; H, 8.71; N, 6.33.

*Hydroxyethylations* with ethylene oxide were carried out in aqueous acetic acid by the procedure described in Ref. 2. In the case of XI, the crude mixture (12.4 g.) of XI and dialkylated product was allowed to react with ethylene oxide for 65 hr.; hydroxyethylation was incomplete after 24 hr. The crude reaction product, 12.6 of brown sirup, was dis-

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(9) W. A. Skinner, A. P. Martinez, and B. R. Baker, *J. Org. Chem.*, **26**, 152 (1961).

(10) A hydrocarbon fraction, mainly *n*-hexane, b.p. 62–70°.