Reduction of VIII. A warm solution of 13 g. (0.05 mole) of VIII in 200 ml. of absolute ethanol containing 0.5 g. of Adams' catalyst was hydrogenated on a Parr shaker. The initial hydrogen pressure was 53 p.s.i. The solution absorbed 9.5 lb. of hydrogen in 7 hr. There was no additional absorption in the next 15 hr. The catalyst was filtered and the solvent was removed from the filtrate under reduced pressure. The addition of dry ether to the residue precipitated 12.0 g. of white solid, m.p. 350–352° dec.

Anal. C, 45.06; H, 6.01; N, 7.72; S, 18.29. The solid was soluble in water and when the aqueous solution was made basic an ammoniacal odor was detected. No amine, however, was extracted with ether. An aqueous solution was made strongly alkaline with potassium hydroxide and the solution distilled up to 100°. The distillate was saturated with potassium hydroxide and extracted with ether. To the ether solution, after drying with magnesium sulfate, was added a saturated ether solution of picric acid. Allylamine picrate, m.p. 135–140°, precipitated immediately. A mixed melting point with an authentic sample of allylamine picrate showed no depression.

From the ether solution there was obtained 0.33 g. of ethylene glycol di-*p*-toluenesulfonate, m.p. 120–121° (reported[9] m.p. 125–126°). A sample of ethylene glycol di-*p*-toluenesulfonate was prepared by the reaction of *p*-toluenesulfonyl chloride with ethylene glycol in pyridine solution. The melting point was 123–125°. A mixture of the two solids melted at 123–125°.

ANN ARBOR, MICH.

(9) C. L. Butler, W. L. Nelson, A. G. Renfrew, and L. H. Cretcher, *J. Am. Chem. Soc.*, 57, 575 (1935).

[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL SCIENCES, STANFORD RESEARCH INSTITUTE]

Potential Anticancer Agents.[1] XLIII. Analogs of Chlorambucil. IV.[2] Synthesis of Isochlorambucil and Related Benzylic Type Alkylating Agents

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p-[Bis(2-chloroethyl)aminomethyl]hydrocinnamic acid (XIII) (isochlorambucil), an isomer of chlorambucil containing the more chemically reactive benzylic type alkylating group, has been synthesized for evaluation as an anticancer agent. Several related monofunctional alkylating agents have also been synthesized for test evaluation, namely *p*-(2-chloroethylthiomethyl)-hydrocinnamic acid (II), *p*-[(2-chloroethyl)ethylaminomethyl]hydrocinnamic acid (IVb), methyl *p*-(1-aziridinylmethyl) hydrocinnamate (VIII), and *p*-[(2-chloroethyl)aminomethyl]hydrocinnamic acid (XIVb).

Chlorambucil,[3] 4-*p*-[bis(2-chloroethyl)amino] phenylbutyric acid, is one of the most useful alkylating agents in the clinic.[4] Although chlorambucil is highly effective against the Walker rat Sarcoma 256, it shows little activity against Sarcoma 180, Adenocarcinoma 755, or Leukemia L-1210 in the mouse. As part of the continuing search for analogs of chlorambucil[2,5,6] that may have a different tumor spectrum[4,7] or may be more efficacious in man, this paper describes a series of chlorambucil analogs wherein the alkylating function is separated from the benzene ring by a methylene group such as in Compound XIII. Since aliphatic mustards are chemically more reactive than the corresponding aryl mustards, a change in tumor spectrum or efficiency or both might be anticipated. In addition, some of the monofunctional alkylating agents of this more reactive benzylic type (such as II, IVb or XIVb) described in this paper might be irreversible enzyme inhibitors.[8,9]

Chloromethylation of hydrocinnamic acid with aqueous formaldehyde and hydrochloric acid by the method of Bogdanov[10] afforded *p*-(chloromethyl)-hydrocinnamic acid (VI) in 50% yield. Milder conditions of chloromethylation, namely chloromethyl methyl ether and stannic chloride, were without effect on hydrocinnamic acid since the latter was recovered unchanged. Fisher esterification of VI with methanolic hydrogen chloride

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(2) For paper III on Chlorambucil analogs see W. A. Skinner, H. F. Gram, and B. R. Baker, *J. Org. Chem.*, 25, 953 (1960), paper XXXIII of this series.

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

(4) R. W. Rundles, J. Grizzle, W. N. Bell, C. C. Corley, W. B. Frommeyer, B. G. Greenberg, C. M. Huguley, G. W. James III, R. Jones, Jr., W. E. Larsen, V. Loeb, L. A. Leone, J. G. Palmer, W. H. Riser, Jr., and S. J. Wilson, *Am. J. Med.*, 27, 424 (1959).

(5) W. A. Skinner, H. F. Gram, C. W. Mosher, and B. R. Baker, paper XX of this series, *J. Am. Chem. Soc.*, 81, 4639 (1959).

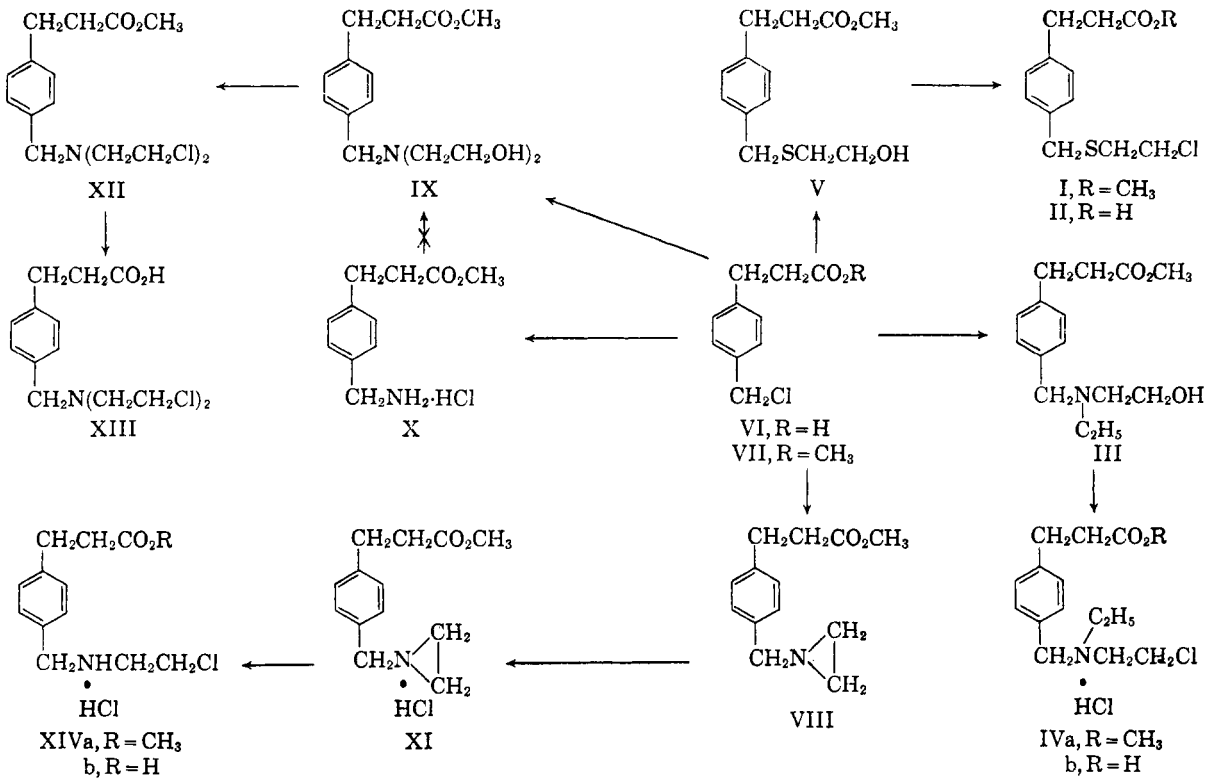
(6) W. A. Skinner, H. F. Gram, and B. R. Baker, paper XXXII of this series, *J. Org. Chem.*, 25, 777 (1960).

(7) J. Scholler, E. Tholen, and L. H. Schmidt, *Proc. Am. Assoc. Cancer Research*, 3, 60 (1959); L. F. Larionov, *Akad. Med. Nauk. Vestnik*, 14, No. 6, 25 (1959).

(8) H. F. Gram, C. W. Mosher, and B. R. Baker, paper XVIII of this series, *J. Am. Chem. Soc.*, 81, 3103 (1959).

(9) B. R. Baker, *Cancer Chemotherapy Reports*, No. 4, p. 1 (1959), published by the Cancer Chemotherapy National Service Center, National Cancer Institute.

(10) M. N. Bogdanov, *J. Gen. Chem. U.S.S.R.*, 28, 1621 (1958), has recorded a yield of 27%.



afforded the crystalline methyl ester (VII) in 93% yield.

The benzylic chloride of methyl *p*-(chloromethyl) hydrocinnamate (VII) was readily replaced when refluxed with sodium 2-mercaptoethanol in ethanol for fifteen minutes. The resultant oil (V) was readily purified by distillation in 60% yield and was uniform (R_f 0.72) on paper chromatography[11] with solvent A when detected both by its ultraviolet absorption and by its color with iodoplatinate spray. Treatment of methyl *p*-(2-hydroxyethylthiomethyl)hydrocinnamate (V) with thionyl chloride in boiling dichloromethane afforded I as a nearly analytically pure oil in 74% yield, which was uniform (R_f 0.63) on paper chromatography[11] in solvent A. Hydrolysis of the ester group of I proceeded smoothly at 50° with 1:2 12*N*-hydrochloric acid-glacial acetic acid. After a fifteen-minute hydrolysis period, crystalline 3-(*p*-chloroethylthiomethyl)hydrocinnamic acid (II) was isolated in 72% yield. A higher temperature (b.p.) and longer reaction time (two hours) for the hydrolysis led to an unidentified, high-melting solid which contained practically no chlorine and was not further investigated.

The chlorine of methyl *p*-(chloromethyl)hydrocinnamate (VII) was also readily replaced by 2-ethylaminoethanol in boiling ethanol to form methyl *p*-[ethyl-(2-hydroxyethyl)aminomethyl]hydrocinnamate (III). Although the latter could be distilled, analysis indicated that it was slightly contaminated with VII; that it had no amine impurity was shown by its homogeneity on paper in solvent B[11] when detected by iodoplatinate spray. Nevertheless, when VII was treated with thionyl chloride in boiling chloroform for fifteen minutes, it was smoothly converted to crystalline, nearly pure methyl *p*-[(2-chloroethyl)ethylaminomethyl]-hydrocinnamate hydrochloride (IVa) in 92% yield, which was readily obtained analytically pure. Hydrolysis of the ester group of XIVa proceeded readily with hot concentrated hydrochloric acid to give an 84% yield of crystalline *p* - [ethyl - (2 - choroethyl)aminoethyl] - hydrocinnamic acid hydrochloride (IVb).

Methyl *p*-(chloromethyl)hydrocinnamate (VII) could also be converted with ethyleneimine to the aziridine, VIII. Considerable difficulty was encountered in finding proper conditions for this reaction since no suitable solvent for paper chromatography of crude VIII could be found and crude VIII was not considered distillable in the presence of unchanged VII. Eventually it was found that a seventy-hour reaction at room temperature between VII and ethyleneimine in methanol in the presence of potassium carbonate as an acid acceptor gave a 69% yield of VIII as a nearly analytically pure oil. The latter could be converted to a crystalline aziridine hydrochloride (XI) in methanol. When

(11) Paper chromatograms were run by the descending technique on Schleicher and Schuell acetylated paper No. 2495 in benzene-methanol-water (2:6:1) (solvent A) or on Whatman No. 1 paper with 1-butanol-acetic acid-water (5:3:2) (solvent B). The compounds were detected by their ultraviolet absorption or by the use of iodoplatinate spray.[12]

(12) L. R. Goldbaum and L. Kazyak, *Anal. Chem.*, 28, 1289 (1956).

VIII was treated with hydrogen chloride in ether, a quantitative yield of pure crystalline methyl *p*-[(2-chloroethyl)aminomethyl)hydrocinnamate hydrochloride (XIVa) was obtained. This conversion of VIII to crystalline XIVa could also be done with impure VIII and was the best method for determining how much VIII was present in a given crude product resulting from interaction of VII and ethyleneimine during the search for proper reaction conditions. Hydrolysis of the ester linkage of XIVa with hot concentrated hydrochloric acid gave a 95% yield of crystalline *p*-[(2-chloroethyl) aminomethyl]hydrocinnamic acid hydrochloride (XIVb).

It is surprising that a crystalline hydrochloride (XI) of methyl *p*-(1-aziridinylmethyl)hydrocinnamate (VIII) could be prepared in view of the ease with which the aziridine group is attacked by hydrogen chloride.[2,13] However, under the conditions employed, the yield of recovered hydrochloride (XI) is only 34% and can be recovered only by the fortuitous circumstance that XI is less soluble and higher melting than the ring opened product (XIVa). Nevertheless, the actual preparation of a crystalline aziridine hydrochloride, fortuitous or not, is of more than theoretical interest.

Methyl *p*-(chloromethyl)hydrocinnamate (VII) was allowed to react with excess 2,2′-iminodiethanol in boiling chloroform for six hours, then the amine was removed by thorough washing with water. The crude oil was isolated as the pure crystalline hydrochloride (IX) in 70% yield. Treatment of IX with thionyl chloride in boiling dichloromethane smoothly afforded crystalline methyl *p*-[bis(2-chloroethyl)aminomethyl]hydrocinnamate hydrochloride (XII) in quantitative yield. Removal of the ester group of XII with hot concentrated hydrochloric acid resulted in an 81% yield of *p*-[bis(2-chloroethyl)aminomethyl]hydrocinnamic acid hydrochloric (XIII) (isochlorambucil), which crystallized directly from the hydrolysis solution.

An alternate synthesis of XIII was also investigated. When methyl *p*-(chloromethyl)hydrocinnamate (VII) was treated with hexamethylenetetramine in boiling 95% methanol, an intermediate adduct was obtained which was treated with methanolic hydrogen chloride[14] to give crystalline methyl *p* - (aminomethyl)hydrocinnamate hydrochloride (X). However, this compound did not react with ethylene oxide in dilute acetic acid containing sodium acetate to give IX, but was recovered unchanged.

EXPERIMENTAL[15]

Methyl p-(chloromethyl)hydrocinnamate (VII). *p*-(Chloromethyl)hydrocinnamic acid (VI) was prepared in 50% yield by the chloromethylation of hydrocinnamic acid.[10] A solution of 6.6 g. (0.030 mole) of VI in 75 ml. of methanol saturated with hydrogen chloride at about 25° was refluxed for 1 hr., then evaporated *in vacuo* to a sirup. A solution of this sirup in 30 ml. of chloroform was washed successively with 30 ml. of water, 30 ml. of 5% sodium bicarbonate, and 30 ml. of water. The dried organic solution was evaporated to dryness *in vacuo* leaving 6.6 g. of a sirup that crystallized on cooling. Recrystallization from petroleum ether (b.p. 62–70°) gave 4.8 g. (67%) of product, m.p. 35–42°. For analysis a sample was distilled at 145° (4 mm.), then recrystallized from petroleum ether (b.p. 62–70°) to give white crystals, m.p. 48–49°: $\lambda_{max\,\mu}^{Nujol}$ 5.75 (ester C=O); 6.15, 6.57 (aryl): 8.35 (ester C—O—C); 11.80 (*p*-disubstituted benzene). No suitable reagent could be found for detection of this compound on paper chromatograms.

Anal. Calcd. for $C_{11}H_{13}ClO_2$; C, 62.1; H, 6.11; Cl, 16.7: Found: C, 62.3; H, 6.44; Cl, 16.7.

On a larger scale using pure VI the yield was 93% (120 g.) and the m.p. was 47–48° without recrystallization.

Methyl p-(hydroxyethylthiomethyl)hydrocinnamate (V). To a solution of 8.0 g. (0.10 mole) of 2-mercaptoethanol and 4.0 g. (0.10 mole) of sodium hydroxide in 300 ml. of absolute ethanol was added 21.3 g. (0.10 mole) of methyl *p*-(chloromethyl)hydrocinnamate (VII). After being refluxed for 15 min., during which time sodium chloride separated, the mixture was spin evaporated *in vacuo* to a sirup. The sirup was partitioned between 50 ml. of chloroform and 50 ml. of water. The separated chloroform layer was washed with two 50-ml. portions of water, then dried with magnesium sulfate and evaporated to dryness *in vacuo*. The resultant sirup, after a forerun with b.p. 180–218° (8 mm.), distilled at 220–222° (7 mm.); yield 15.3 g. (60%) of a colorless oil; $\lambda_{max\,\mu}^{Nujol}$ 2.90 (OH); 5.72 (ester C=O); 8.32, 8.60 (ester C—O—C); 9.55 (C—OH): 11.80 (*p*-disubstituted benzene). The compound traveled as a single spot (R_f 0.72) in solvent A[11] when detected by its ultraviolet absorption and by its color (gray white) with iodoplatinate spray.

Anal. Calcd. for $C_{13}H_{18}O_3S$: C, 61.4; H, 7.10; S, 12.6. Found: C, 61.2; H, 7.27; S, 12.2.

Methyl p-(2-chloroethylthiomethyl)hydrocinnamate (I). To a solution of 2.54 g. (10 mmoles) of methyl *p*-(2-hydroxyethylthiomethyl)hydrocinnamate (V) in 5 ml. of dichloromethane cooled in an ice bath was added 10 ml. of a cold 50% solution of thionyl chloride in dichloromethane. After being refluxed for 90 min., the solution was evaporated to dryness *in vacuo*. A solution of the residual oil in 50 ml. of dichloromethane was washed with water, then dried with anhydrous sodium sulfate and evaporated to dryness *in vacuo*; yield, 2.0 g. (74%) of a light yellow oil with $\lambda_{max\,\mu}^{Nujol}$ 5.72 (ester C=O); 8.30, 8.60 (ester C—O—C); 12.10 (*p*-disubstituted benzene); no COH near 3.0 or 9.5. The oil traveled as a single spot (R_f 0.63) in solvent A[11] when detected by its ultraviolet absorption or by iodoplatinate spray, but was not quite analytically pure.

Anal. Calcd. for $C_{13}H_{17}ClO_2S$: C, 57.2; H, 6.23; Cl, 13.0; S, 11.7. Found: C, 57.6; H, 6.46; Cl, 12.9; S, 11.2.

p-(2-Chloroethylthiomethyl)hydrocinnamic acid (II). A solution of 0.56 g. (1.8 mmoles) of methyl *p*-(chloroethylthiomethyl)hydrocinnamate (I) in 7.5 ml. of 12*N* hydrochloric acid and 15 ml. of glacial acetic acid was heated at 50° for 15 min., then diluted with three volumes of water, and cooled to 0°. The product was collected and washed well with water; yield 0.35 g. (72%), m.p. 75–78°. Recrystallization from ether–petroleum ether (b.p. 30–60°) gave white crystals, m.p. 78–79°; $\lambda_{max\,\mu}^{Nujol}$ 3.60–4.10 (acidic OH); 5.85 (carboxyl C=O); 7.05, 8.35, 10.6 (COOH); 12.00 (*p*-disubstituted phenyl); 13.9 (C—Cl).

(13) W. A. Skinner, M. G. M. Schelstraete, and B. R. Baker, *J. Org. Chem.*, 24, 1827 (1959).

(14) A. Galat and G. Elion, *J. Am. Chem. Soc.*, 61, 3585 (1939).

(15) Melting points were determined on a Fischer-Johns block and are uncorrected.

Anal. Calcd. for $C_{12}H_{15}ClO_2S$: C, 55.6: H, 5.80: Cl, 13.7: S, 12.4. Found; C, 55.9: H, 6.00: Cl, 13.8: S, 12.0.

Methyl p-[ethyl-(2-hydroxyethyl)aminomethyl]hydrocinnamate (III). A solution of 25.5 g. (0.12 mole) of methyl p-(chloromethyl)hydrocinnamate and 22 g. of 2-ethylaminoethanol in 55 ml. of chloroform was refluxed for 2 hr. The cooled reaction mixture was washed three times with water, then dried with magnesium sulfate, and evaporated *in vacuo* to a sirup (30.6 g.). Distillation gave 22.4 g. (70%) of product as a colorless oil, b.p. 140–180° (3 mm.): $\lambda_{max\ \mu}^{Nujol}$ 2.91 (OH); 5.75 (ester C=O); 8.55 (ester C—O—C); 9.58 (C—OH); 11.80 (*p*-disubstituted benzene). The oil traveled as a single spot (R_f 0.76) in solvent B[11] when detected with iodoplatinate spray (gray color). Since the starting material is not detectable by this spray, the paper chromatographic results, the analytical data, and the results of the next experiment are in agreement with about 5% contamination with starting material (VII).

Anal. Calcd. for $C_{15}H_{23}NO_3$: C, 67.9: H, 8.74; N, 5.28. Found: C, 67.4; H, 8.79; N, 4.80.

Methyl p-[(2-chloroethyl)ethylaminomethyl]hydrocinnamate hydrochloride (IVa). To a solution of 0.54 g. (2.0 mmoles) of methyl p-[ethyl-(2-hydroxyethyl)aminomethyl]hydrocinnamate (III) in 5 ml. of chloroform was added 0.22 ml. (3 mmoles) of thionyl chloride. The solution was refluxed for 15 min., then evaporated to a sirup *in vacuo*. The evaporation was repeated three times with fresh 5-ml. portions of chloroform. The residue crystallized after the last evaporation. Recrystallization from absolute ethanol-ether gave 0.59 g. (92%) of product, m.p. 131–133°. A second recrystallization afforded white crystals m.p. 132–133°; $\lambda_{max\ \mu}^{Nujol}$ 3.95 (R_3NH^+); 5.71 (ester C=O); 8.58 (ester C—O—C); 12.2 (*p*-disubstituted benzene); 13.4 (C—Cl); no COH near 3.0 or 9.5. This compound traveled as a single spot (R_f 0.82) in solvent B[11] when detected by iodoplatinate spray (gray color).

Anal. Calcd. for $C_{15}H_{22}ClNO_2.HCl$: C, 56.2; H, 7.20; Cl, 22.2; N, 4.37. Found: C, 56.4: H, 7.39; Cl, 22.3; N, 4.38.

p-[(Chloroethyl)ethylaminomethyl]hydrocinnamic acid hydrochloride (IVb). A solution of 0.50 g. (1.56 mmoles) of methyl p-[(2-chloroethyl)ethylaminomethyl]hydrocinnamate hydrochloride (IVa) in 15 ml. of 12*N* hydrochloric acid was refluxed for 45 min., then evaporated to residue *in vacuo*. Benzene (50 ml.) was added to the residue and benzene (20 ml.) distilled until no more water was removed. Evaporation to residue *in vacuo* left white crystals which were triturated with reagent ether; yield, 0.40 g. (84%), m.p. 141–143°; $\lambda_{max\ \mu}^{Nujol}$ 5.80 (carboxyl C=O); 8.45, 10.55 (COOH); 12.0 (*p*-disubstituted benzene); no ester bands at 5.71 or 8.58. Neither suitable solvents for recrystallization nor suitable detection agents for paper chromatography could be found. The infrared spectrum clearly showed that the ester group had hydrolyzed and no ester (R_f 0.82) could be detected when the product was chromatographed on paper with solvent B[11] and sprayed with iodoplatinate.

Anal. Calcd. for $C_{14}H_{20}ClNO_2.HCl$: C, 54.9; H, 6.87; Cl, 23.2; N, 4.58. Found: C, 54.7; H, 6.90; Cl, 23.2; N, 4.70.

Methyl p-(1-aziridinylmethyl)hydrocinnamate (VIII). To a mixture of 10.8 g. (0.050 mole) of methyl p-(chloromethyl)-hydrocinnamate (VII), 200 ml. of reagent methanol and 10 g. of anhydrous potassium carbonate was added 27 ml. of ethyleneimine. The mixture was stirred for 70 hr. at room temperature. After the addition of 3 g. of Celite,[16] the mixture was filtered and the filtrate evaporated to dryness *in vacuo*. The residue was dissolved in 200 ml. of ether; the solution, clarified by filtration, was evaporated to a sirup *in vacuo;* yield, 7.6 g. (69%) of a colorless nearly pure oil with $\lambda_{max\ \mu}^{film}$ 3.37, 3.40 (CH); 5.72 (ester C=O); 12.10 (*p*-disubstituted benzene).

Anal. Calcd. for $C_{13}H_{17}NO_2$: C, 71.2; H, 7.82; N, 6.39. Found: C, 70.7; H, 7.74; N, 5.99.

(16) Johns-Manville Co. diatomaceous earth.

No suitable solvent system for paper chromatography could be found for this compound even though it could be detected on paper by bromocresol green.

The hydrochloride (XI) of VIII was prepared by saturating a solution of 1.5 g. of VIII in methanol with hydrogen chloride without cooling. The solution was evaporated *in vacuo* and the residue triturated thoroughly with ether. The residue (1.8 g.) was dissolved in 20 ml. of methanol and kept at 3° for 2 weeks, during which time crystals of XI separated; yield, 0.60 g. (34%), m.p. 220–225°; $\lambda_{max\ \mu}^{Nujol}$ 4.25, (R_3NH^+); 5.76 (ester C=O), 11.70 (*p*-disubstituted benzene).

Anal. Calcd. for $C_{13}H_{17}NO_2.HCl$: C, 61.1; H, 7.05; Cl, 13.9; N, 5.48. Found: C, 60.8; H, 7.25; Cl, 13.8; N, 5.66.

Methyl p-[(2-chloroethyl)aminomethyl]hydrocinnamate hydrochloride (XIVa). Through a solution of 2.0 g. (9.1 mmoles) of methyl p-(1-aziridinylmethyl)hydrocinnamate (VIII) in 25 ml. of ether under a reflux condenser was passed hydrogen chloride until the solution was saturated. Evaporation to residue *in vacuo* (bath 30°) gave 2.6 g. (98%) of white crystals, m.p. 197–198°. Recrystallization from hot ethyl acetate containing the minimum amount of methanol to cause solution gave white crystals, m.p. 197–198°; $\lambda_{max\ \mu}^{Nujol}$ 3.65, 3.80, 4.15 (NH_2^+); 5.75 (ester C=O); 8.32, 8.51 (ester C—O—C); 12.1 (*p*-disubstituted benzene); 13.8 (C—Cl).

Anal. Calcd. for $C_{13}H_{17}ClNO_2.HCl$: C, 53.6; H, 6.23; Cl, 24.4; N, 4.79. Found: C, 53.2; H, 6.86; Cl, 24.6; N, 4.97.

p-[(2-Chloroethyl)aminomethyl]hydrocinnamic acid hydrochloride (XIVb). A solution of 1.65 g. (5.64 mmoles) of methyl p-[(2-chloroethyl)aminomethyl]hydrocinnamate (XIVa) in 10 ml. of 12*N* hydrochloric acid was heated on a steam bath for 30 min., then evaporated to dryness *in vacuo;* yield, 1.50 g. (95%) of white crystals, m.p. 173–176°. Trituration with boiling ethyl acetate raised the m.p. to 178–179°; $\lambda_{max\ \mu}^{Nujol}$ 3.20–3.90, 4.10 ($R_2NH_2^+$, acidic OH); 5.79 (carboxyl C=O); 6.29 ($R_2NH_2^+$); 6.15, 6.55 (aryl); 12.00 (*p*-disubstituted benzene); 13.2, 13.8 (C—Cl).

Anal. Calcd. for $C_{12}H_{15}ClNO_2.HCl$: C, 52.0; H, 5.77; Cl, 25.6; N, 5.05. Found: C, 51.9; H, 5.91; Cl, 25.3; N, 5.14.

Methyl p-[bis(2-hydroxyethyl)aminomethyl]hydrocinnamate hydrochloride (IX). To a solution of 2.1 g. (10 mmoles) of methyl p-(chloromethyl)hydrocinnamate (VII) in 25 ml. of chloroform was added 4.2 g. (40 mmoles) of 2,2′-iminodiethanol. After being refluxed for 6 hr., the solution was cooled and washed thoroughly with water (4 × 25 ml.). The organic solution, dried with magnesium sulfate, was chloroform was added 4.2 g. (40 mmoles) of 2,2′-iminodiethanol. After being refluxed for 6 hr., the solution was cooled and washed thoroughly with water (4 × 25 ml.). The organic solution, dried with magnesium sulfate, was evaporated to dryness *in vacuo*. A solution of the residue in 30 ml. of benzene was saturated with hydrogen chloride, then evaporated *in vacuo*. Crystallization from dichloromethane ether gave 2.2 g. (70%) of white crystals, m.p. 82–85°; $\lambda_{max\ \mu}^{Nujol}$ 3.02 (OH): 3.65, 3.75 (NH^+): 5.72 (ester C=O); 8.32, 8.49 (ester C—O—C); 9.32 (C—OH): 12.10 (*p*-disubstituted benzene).

Anal. Calcd. for $C_{15}H_{23}NO_4.HCl$; C, 56.7: H, 7.56; Cl, 10.7; N, 4.42. Found: C, 56.9; H, 7.71; Cl, 10.4; N, 4.11.

Methyl p-[bis(2-chloroethyl)aminomethyl]hydrocinnamate hydrochloride (XII). To a solution of 0.40 g. (1.3 mmoles) of methyl p-[bis(2-hydroxyethyl)aminomethyl]hydrocinnamate hydrochloride (IX) in 5 ml. of dichloromethane was added 3 ml. of thionyl chloride. After being refluxed for 90 min., the solution was evaporated to residue *in vacuo*. The evaporation was repeated with four fresh 10-ml. portions of chloroform, then the residue crystallized on cooling; yield, 0.45 g. (100%) of white crystals, m.p. 105–110°. Recrystallization from dichloromethane-petroleum ether (b.p. 30–60°) raised the m.p. to 113–114°; $\lambda_{max\ \mu}^{Nujol}$ 4.08 (NH^+): 5.72 (ester C=O); 8.32, 8.45, 8.60 (ester C—O—C); 12.2 (*p*-disubstituted benzene); 13.4 (C—Cl).

Anal. Calcd. for $C_{15}H_{21}Cl_2NO_2$.HCl: C, 50.7; H, 6.20; Cl, 30.2; N, 3.95. Found: C, 50.7; H, 6.12; Cl, 30.7; N, 4.02.

p-[Bis(2-chloroethyl)aminomethyl]hydrocinnamic acid hydrochloride (XII). A solution of 0.90 g. (2.5 mmoles) of methyl *p*-[bis(2-chloroethyl)aminomethyl]cinnamate hydrochloride (XII) in 10 ml. of 12*N* hydrochloric acid was refluxed for 30 min., then concentrated to about one-half volume *in vacuo* and cooled in an ice bath. The product was collected on a glass filter; yield, 0.70 g. (81%), m.p. 171–175°. A sample was recrystallized by solution in hot water, then addition of five volumes of 12*N* hydrochloric acid to give white crystals, m.p. 176–177°; $\lambda_{max\ \mu}^{Nujol}$ 3.75, 3.87 (NH^+ and acidic OH); 5.80 (carboxyl C=O); 12.0 (*p*-disubstituted benzene); 13.4 (C—Cl).

Anal. Calcd. for $C_{14}H_{19}Cl_2N_2O_2$.HCl: C, 49.3; H, 5.87; Cl, 31.3; N, 4.12. Found: C, 49.2; H, 5.95; Cl, 31.1; N, 4.15.

Methyl p-(aminomethyl)hydrocinnamate hydrochloride (X). To a stirred solution of 7.0 g. (0.050 mole) of hexamethylenetetramine[14] in 100 ml. of 95% methanol was added 8.5 g. (0.050 mole) of potassium iodide followed by 10.6 g. (0.050 mole) of methyl *p*-(chloromethyl)hydrocinnamate (VII). The reaction mixture was refluxed with stirring for 40 min., then filtered hot to remove potassium chloride. The filtrate was cooled to 0° and deposited 20.0 g. (115%) of the hexamine complex that was contaminated with some salts.

A suspension of 20 g. of the hexamine complex in 150 ml. of methanol was saturated with hydrogen chloride,[14] refluxed for 30 min., then evaporated to dryness *in vacuo*. The residue was dissolved in hot dichloromethane and filtered from some inorganic material. Evaporation of the combined filtrate and washings to dryness *in vacuo* afforded 8.8 g. (77%) of an amorphous solid, m.p. 150–155°, that gave a paper chromatogram and infrared absorption spectrum identical with the analytical sample. Crystallization of a sample from methanol ether afforded white crystals, m.p. 208–217°; $\lambda_{max\ \mu}^{Nujol}$ 3.50, 4.20, 4.85 (NH^+); 5.72 (ester C=O); 8.50 (ester C—O—C); 12.1 (*p*-disubstituted benzene). The compound traveled as a single spot (R_f 0.76) in solvent B[11] when detected by iodoplatinate spray (gray color).

This compound (X), when allowed to react in the usual manner[8] with ethylene oxide in dilute acetic acid containing an equivalent of sodium acetate, was not converted to IX as expected, but was recovered unchanged. It appears that hydroxyethylation in dilute acetic acid fails to take place because of the stronger protonation of aliphatic amines, such as X, compared to the arylamines usually employed under these conditions.[8]

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL SCIENCES, STANFORD RESEARCH INSTITUTE]

Potential Anticancer Agents.[1] XLVI. Analogs of Chlorambucil. V.[2] Alkylating Agents Derived from ω-Phenoxyalkanoic Acids

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Several analogs of the chlorambucil isostere 3-*p*-{[bis(2-chloroethyl)amino]phenoxy}propionic acid (I, $n = 2$) have been synthesized for evaluation as potential anticancer agents and as potential irreversible inhibitors of lactic dehydrogenase.

A series of ω-{*p*-[bis(2-chloroethyl)amino]phenoxy}alkanoic acids (I) have been synthesized[3] and evaluated as anticancer agents against Walker rat Sarcoma 256. All four of these acids showed inhibitory action. The maximum effect was shown by the propionic acid derivative (I, $n = 2$), which was considered[3] to be an isostere of chlorambucil, 4-{*p*-[bis(2-chloroethyl)amino]phenyl}butyric acid.[4] As *m*-phenylalanine mustard[5] appears to be more effective against some tumors[6] than *p*-phenylalanine mustard,[7,8] the synthesis of the *o*- and *m*-isomers of I ($n = 1,2$) was deemed advisable in order to determine whether or not these changes would cause a change in tumor spectrum.[9,10] These

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