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

Potential Anticancer Agents. XLVIII.[1] Analogs of Chlorambucil. VI.[2] Ring Isomers

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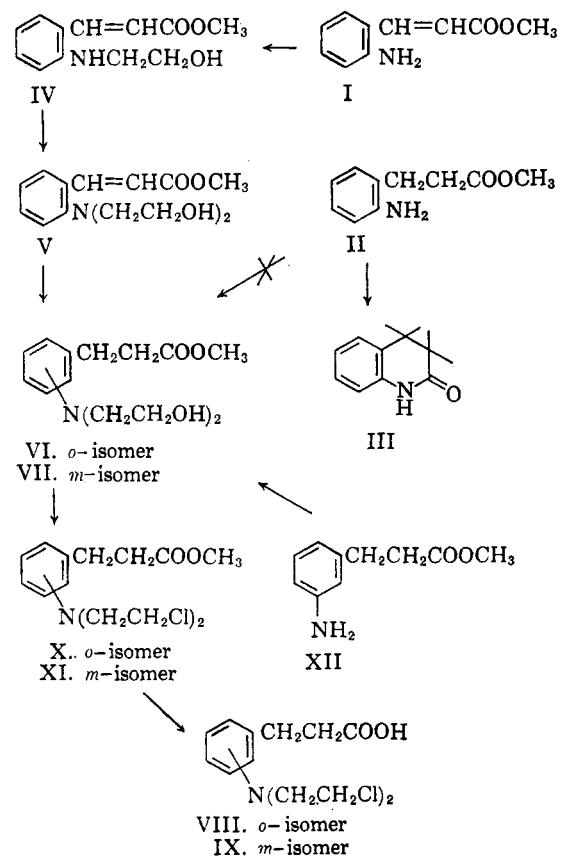
The syntheses of the *ortho*-isomer of Chlorambucil (XV) as well as the *ortho*- and *meta*-isomer (VIII and IX) of its lower homolog, Norchlorambucil, are described. The usual aryl nitrogen mustard synthesis had to be modified for *o*-Norchlorambucil (VIII), due to the ease of cyclization of methyl *o*-aminohydrocinnamate (II) to the lactam (III); the synthesis of VIII was accomplished *via* the key intermediate, methyl *o*-[bis(2-hydroxyethyl)amino]cinnamate (V).

One of the most clinically useful alkylating agents has been Chlorambucil, 4-{*p*-[bis(2-chloroethyl)-amino]phenyl}butyric acid.[3] As part of the continuing search for analogs of Chlorambucil that may have a different tumor spectrum or may be more efficacious in man, this paper describes the synthesis of *o*-Chlorambucil (XV) and the *o*- and *m*-isomers of Norchlorambucil (VIII and IX), particularly since the *m*-isomer of phenylalanine mustard appeared to have a better chemotherapeutic index[4] than the corresponding *p*-isomer against Sarcoma 180 and S-91 Melanoma in mice.

The usual nitrogen mustard synthesis for Chlorambucil and Norchlorambucil[5] had to be modified for *o*-Norchlorambucil (VIII), due to the ease of cyclization of methyl *o*-aminohydrocinnamate (II) to the lactam (III).[6] Reduction of methyl *o*-nitrocinnamate even in the presence of hydrogen chloride did not stabilize the amine (II) as its hydrochloride, the lactam (III) still being formed.

Since cinnamic acids in general and *o*-aminocinnamic acid in particular[7]—providing there are no other α or β substituents—have a *trans*-configuration of carboxyl and phenyl groups, it was reasonable to expect that methyl *o*-aminocinnamate (I)[8] would be sufficiently stable against cyclization that it could be used for further reaction on the amine function. Hydroxyethylation of I with ethylene oxide in aqueous acetic acid was sluggish due to the substituent *ortho* to the amine. The usual conditions,[2,5] room temperature for twenty-four hours, gave a mixture of two products (R_f 0.53 and 0.75) but no starting material (R_f 0.92) as shown by paper chromatography on acetylated paper.[9] Although monohydroxyethylated intermediates such as IV are rarely observed in hydroxyethylation of arylamines, it has been observed in the case of methyl anthranilate[5]; thus one of the two products observed on paper chromatography



(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. For the preceding paper in this series, *cf.* A. P. Martinez, W. A. Skinner, W. W. Lee, L. Goodman, and B. R. Baker, *J. Org. Chem.*, **26**, 860 (1961).

(2) For paper V on analogs of Chlorambucil, see W. A. Skinner, A. P. Martinez, and B. R. Baker, *J. Org. Chem.*, **26**, 152 (1961), Paper XLVI of this series.

(3) For pertinent background references, see W. A. Skinner, A. P. Martinez, H. F. Gram, L. Goodman, and B. R. Baker, *J. Org. Chem.*, **26**, 148 (1961), Paper XLIII of this series.

(4) M. O. Greene, B. R. Baker, and J. Greenberg, *Cancer Research*, **20**, 1160 (1960); the *p*-isomer of phenylalanine mustard is 3-{*p*-[bis(2-chloroethyl)amino]phenyl}-DL-alanine.

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

(6) F. Mayer, H. Philips, F. W. Ruppert, and A. T. Schmitt, *Ber.*, **61**, 1966 (1928).

(7) P. Pfeiffer and G. Haefelin, *Ber.*, **55**, 1769 (1922); S. Gabriel, *Ber.*, **15**, 2294 (1882).

(8) F. Mayer, *Ber.*, **44**, 2298 (1911).

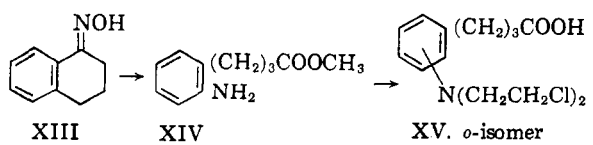
could be expected to be the intermediate IV. That this was indeed the case was shown by chromatography of the reaction mixture on silica gel. Methyl *o*-(2-hydroxyethylamino)cinnamate (IV), m.p. 77.5–78°, was eluted with 1:1 chloroform-ether; the desired product (V) could then be eluted with 1:1 ethanol-ether. Paper chromatography with system A[9] was most useful for rapid determination of column efficiency and separation.

A time and material ratio study of the reaction between methyl *o*-aminocinnamate (I) and ethylene oxide was then made, following the reactions by paper chromatography of aliquots in system A.[9] It was finally established that by doubling the usual conditions of time and amount of ethylene oxide, that is, a second addition of ethylene oxide after twenty-six hours and a total reaction time of forty-eight hours, all of I and IV had been converted to the desired methyl *o*-[bis(2-hydroxyethyl)-amino]cinnamate (V). The crystalline hydrochloride of V was then readily isolated analytically pure in 49% yield without column chromatography purification.

Hydrogenation of the side-chain double bond of V hydrochloride proceeded smoothly in the presence of a palladium-charcoal catalyst, VI being isolated as the crystalline hydrochloride in 81% yield. Treatment of VI hydrochloride with boiling phosphorus oxychloride yielded the ester (X) which was not isolated, but was directly hydrolyzed to the acid (VIII) by allowing the aqueous solution of decomposed phosphorus oxychloride containing X to stand at room temperature for twenty hours. *o*-Norchlorambucil (VIII) was obtained as an analytically pure crystalline solid, m.p. 63–63.5°, that was uniform (R_f 0.48) when chromatographed on paper in system A.[9]

The synthesis of *m*-Norchlorambucil (IX) follows the standard sequence from methyl *m*-aminohydrocinnamate (XII) *via* VII and XI. Again the ester (XI) was not isolated, but was allowed to hydrolyze to the acid (IX) which melted at 88–88.5° and had R_f 0.71 in system A.[9]

With the experience gained in the synthesis of *o*-Norchlorambucil (VIII), the synthesis of *o*-Chlorambucil (XV) gave no serious difficulties. The key intermediate, methyl 4-(*o*-aminophenyl)-butyrate (XIV), was prepared by Beckman rearrangement of α-tetralone oxime (XIII), according to the method of Schroeter, *et al.*[10] The final product (XV) was a low melting (30–30.5°) solid that was chromatographically homogeneous (R_f 0.34) in System A.[9]



XIII XIV XV. *o*-isomer XVI. *m*-isomer

To complete this series, the synthesis of *m*-Chlorambucil (XVI) was investigated. Even after an extensive study of a variety of methods, the conversion of 3-(*m*-aminobenzoyl)propionic acid[11] to the key intermediate, 4-(*m*-aminophenyl)butyric acid, was not sufficiently satisfactory to warrant completion of the synthesis of *m*-Chlorambucil (XVI) unless unusually good anti-tumor activity was observed with VII, IX, or XV. Since this has so far not been the case, the synthesis of XVI has been abandoned.

EXPERIMENTAL[12]

Methyl o-aminocinnamate (I). To a solution of 2.90 g. (0.014 mole) of methyl *o*-nitrocinnamate in 60 ml. of methanol were added 7 ml. of water and 1.3 g. (0.024 mole) of ammonium chloride. The system was stirred and 9.0 g. (0.14 g.-atom) of zinc dust was added over a period of 15 min. When the initial reaction had subsided, the mixture was refluxed for 2 hr., filtered hot through Celite and diluted with 100 ml. of water. Crystals separated on cooling in an ice bath; yield 1.91 g. (77%), m.p. 62–63°[13]; $\lambda_{max(\mu)}^{Nujol}$ 2.90 (NH); 5.86 (ester C=O); 8.50, 8.60 (ester C—O—C); 13.1, 13.3 (*o*-disubstituted benzene). The compound traveled as a single major spot (R_f 0.78) with a minor fluorescent spot (R_f 0.33) in System B.[9]

Methyl o-[bis(2-hydroxyethyl)amino]cinnamate (V) *hydrochloride.* To a solution of 1.00 g. (5.3 mmoles) of methyl *o*-aminocinnamate (I) in 10 ml. of glacial acetic acid and 10 ml. of water was added 2.30 ml. of ethylene oxide (0.047 mole). The system was stoppered and allowed to remain at room temperature for 26 hr.; then an additional 2.30 ml. of ethylene oxide was added and the system allowed to remain at room temperature for an additional 22 hr. The reaction mixture was neutralized with solid sodium hydrogen carbonate and extracted with ethyl acetate (3 × 10 ml.). The combined ethyl acetate extracts were washed twice with 15 ml. portions of water, then dried over anhydrous magnesium sulfate and evaporated to a sirup *in vacuo*. Toluene (25 ml.) was added and the solution again concentrated *in vacuo* to remove acetic acid. The remaining oil (1.04 g.) was dissolved in 15 ml. of benzene and the solution refluxed while hydrogen chloride was passed into the solution. A white, crystalline precipitate soon formed; yield 0.84 g. (49%), m.p. 137–154° dec.; $\lambda_{max(\mu)}^{Nujol}$ 3.10, 3.20 (OH); 5.80 (ester C=O); 6.10, 6.31 (C=C, aryl); 8.33, 8.47 (ester C—O—C); 9.32 (C—OH); 12.9 (*o*-disubstituted benzene). The compound traveled as a single spot (R_f 0.75) in System A.

Anal. Calcd. for $C_{14}H_{19}NO_4.HCl$: C, 55.7; H, 6.64; Cl, 11.8. Found: C, 56.0; H, 6.70; Cl, 11.6.

Methyl o-(2-hydroxyethylamino)cinnamate (IV). Treatment of 4.2 g. (0.023 mole) of methyl *o*-aminocinnamate (I) with ethylene oxide in dilute acid for 24 hr. at room temperature under the usual conditions,[2,5] as described for the prepara-

(9) Paper chromatograms were run by the descending technique. System A was benzene-methanol-water (2:6:1) on Schleicher and Schuell No. 2495 acetylated paper. System B was water-saturated butanol and System C was butanol–acetic acid–water (5:2:3), both on Whatman No. 1 paper. The spots were detected by visual examination under ultraviolet light.

(10) G. Schroeter, A. Gluschke, S. Gotzsky, J. Huang, G. Irmisch, E. Laves, O. Schrader, and G. Stier, *Ber.*, **63**, 1308 (1930).

(11) E. L. Martin, *J. Am. Chem. Soc.*, **58**, 1438 (1936).

(12) Melting points were determined on a Fisher-Johns block and are uncorrected.

(13) Mayer[8] has recorded a melting point of 65° for this compound, prepared in an unspecified manner.

tion of VII, gave 5.3 g. of an oil that showed two spots (R_f 0.58, 0.76) when chromatographed in System A[9]; no I (R_f 0.92) was present. This crude product was dissolved in 15 ml. of benzene and chromatographed on a column (40 × 2.5 cm.) of silica gel (86 g.). Upon elution of the column with a 1:1 mixture of chloroform–ethyl acetate, a yellow band moved down the column; evaporation gave 0.3 g. of a yellow solid, which had no OH in the infrared and was not further identified. The remainder of the material was removed from the column by elution with ethanol. The residue left on evaporation was chromatographed on a similar silica gel column. Elution with a 1:1 mixture of chloroform–ether yielded 2.3 g. of a crystalline solid after removal of the solvent *in vacuo*. Recrystallization from chloroform–petroleum ether (b.p. 30–60°) gave 2.0 g. (40%) of IV m.p. 77.5–78°; $\lambda_{max(\mu)}^{Nujol}$ 2.94 (OH, NH); 5.81 (ester C=O); 9.45 (C—OH); 13.5 (*o*-disubstituted benzene). The compound traveled as a single spot (R_f 0.58) in System A.

Anal. Calcd. for $C_{12}H_{15}NO_3$: C, 65.1; H, 6.83; N, 6.33. Found: C, 65.3; H, 7.06; N, 5.92.

Upon elution of the silica gel column with a 1:1 mixture of ethanol-ether, 2.48 g. (42%) of V was obtained as a viscous oil after evaporation. This material traveled as a single spot (R_f 0.75) in System A.[9] The elementary analysis of this material indicated that it was not quite analytically pure. The paper chromatographic behavior and infrared absorption spectrum indicated the material to be methyl *o*-[bis(2-hydroxyethyl)amino]cinnamate (V).

Methyl o-[bis(2-hydroxyethyl)amino]hydrocinnamate (VI) *hydrochloride.* A solution of 1.02 g. (3.4 mmoles) of methyl *o*-[bis(2-hydroxyethyl)amino]cinnamate (V) hydrochloride in 40 ml. of methanol was added to 0.1 g. of 5% palladium-on-charcoal wetted with 5 ml. of 2-methoxyethanol. The system was shaken with hydrogen at 50 p.s.i.g. for 1 hr. at room temperature at which time hydrogen uptake had ceased. The catalyst was removed by filtration and the filtrate evaporated *in vacuo* at 50° to yield 0.97 g. of a green oil. This oil was dissolved in methanol and treated with Norit at 50°, filtered, and the filtrate concentrated *in vacuo*. The residual oil was triturated with ethyl acetate at 50° and cooled to yield 0.84 g. (81%) of crystals, m.p. 90–97° dec.; $\lambda_{max(\mu)}^{Nujol}$ 3.17 (OH); 3.55, 3.70 (R_3NH^+); 13.0 (*o*-disubstituted benzene). The compound traveled as a single spot (R_f 0.79 in System B and R_f 0.33 in System A).[9]

Anal. Calcd. for $C_{14}H_{21}NO_4 \cdot HCl$: C, 55.5; H, 7.30; Cl, 11.7; N, 4.61. Found: C, 55.7; H, 7.70; Cl, 11.8; N, 5.20.

o-[Bis(2-chloroethyl)amino]hydrocinnamic acid (*o-Norchlorambucil*) (VIII). This compound was prepared in 48% yield from methyl *o*-[bis(2-hydroxyethyl)amino]hydrocinnamate (VI) as described later for the preparation of IX. An analytical sample was prepared by recrystallization from petroleum ether and had m.p. 63–63.5°; $\lambda_{max(\mu)}^{Nujol}$ 3.80–4.00 (acidic OH); 5.83 (carboxyl C=O); 6.26, 6.33, 6.68 (aryl); 12.9 (*o*-disubstituted benzene). The compound traveled as a single spot (R_f 0.48) in System A.[9]

Anal. Calcd. for $C_{13}H_{17}Cl_2NO_2$: C, 53.8; H, 5.85; Cl, 24.5; N, 4.82. Found: C, 54.1; H, 6.16; Cl, 23.8; N, 5.08.

m-Aminohydrocinnamic acid hydrochloride. A mixture of 25 g. (0.13 mole) of *m*-nitrocinnamic acid, 100 ml. of 2-methoxyethanol and 2.5 g. of 5% palladium-charcoal was shaken with hydrogen at 54 p.s.i.g. for 50 min. at which time hydrogen uptake had ceased. The reaction mixture was filtered and the filtrate concentrated *in vacuo*. The crude product was dissolved in 125 ml. of chloroform, treated with Norit, diluted with chloroform to 500 ml. and then hydrogen chloride passed through the solution at 0° causing a white, crystalline hydrochloride to separate; yield 18.5 g. (71%), m.p. 162–165°, that was suitable for the next step. An analytical sample was obtained by recrystallization from concentrated hydrochloric acid and had m.p. 180–181°[14]; $\lambda_{max(\mu)}^{Nujol}$ 5.20 (NH_3^+); 5.80 (carboxyl C=O); 6.25, 6.70 (aryl, NH_3^+); 12.5 (*m*-disubstituted benzene). The compound traveled as a single spot (R_f 0.68) in System C.[9]

Anal. Calcd. for $C_9H_{11}NO_2 \cdot HCl$: Cl, 17.6; N, 6.96. Found: Cl, 17.5; N, 6.97.

Methyl m-aminohydrocinnamate (XII) *hydrochloride.* A solution of 4.60 g. (0.023 mole) of *m*-aminohydrocinnamic acid hydrochloride in 50 ml. of methanol saturated with hydrogen chloride at 3° was refluxed for 2.5 hr., then evaporated to dryness *in vacuo*. This crude product was partitioned between 100 ml. of a saturated solution of sodium hydrogen carbonate and 100 ml. of chloroform. The separated chloroform layer was concentrated to about 50 ml. *in vacuo* and filtered. The filtrate was cooled to 0°, saturated with hydrogen chloride, then ether added until crystallization was complete; yield 2.45 g. (50%), m.p. 107–108.5°; $\lambda_{max(\mu)}^{KBr}$ 6.27, 6.37 (NH_3^+); 5.90 (ester C=O); 8.37, 8.64 (ester C—O—C). The compound traveled as a single spot in System A (R_f 0.70) System B (R_f 0.34), and System C (R_f 0.66).[9]

Anal. Calcd. for $C_{10}H_{14}ClNO_2$: C, 55.7; H, 6.50; Cl, 16.5; N, 6.50. Found: C, 55.7; H, 6.65; Cl, 16.5; N, 6.50.

Methyl m-[bis(2-hydroxyethyl)amino]hydrocinnamate (VII). A solution of 20.3 g. (0.090 mole) of methyl *m*-aminohydrocinnamate (XII) hydrochloride in 150 ml. of water was neutralized with 7.93 g. (0.090 mole) of sodium hydrogen carbonate, then 150 ml. of glacial acetic acid was added. The solution was cooled to 3° and 37.5 ml. (0.76 mole) of ethylene oxide was added. The flask was stoppered and the reaction mixture allowed to remain at room temperature for 24 hr. The volume of the reaction mixture was tripled by the addition of water. The solution was neutralized with solid sodium hydrogen carbonate, then extracted with ether (3 × 150 ml.). The combined ether extracts were washed with water (3 × 100 ml.), dried over anhydrous magnesium sulfate, then evaporated *in vacuo* at 25° to yield 9.8 g. (39%) of a viscous oil; $\lambda_{max(\mu)}^{film}$ 2.95 (OH); 5.75 (ester C=O); 8.48 (ester C—O—C), 9.60 (C—OH); 12.9 (*m*-disubstituted benzene). The compound traveled as a single spot with R_f 0.82 in System B and with R_f 0.57 in System A.[9]

Anal. Calcd. for $C_{14}H_{21}NO_4$: C, 62.9; H, 7.92; N, 5.24. Found: C, 63.0; H, 8.16; N, 5.52.

m-[Bis(2-chloroethyl)amino]hydrocinnamic acid (*m-Norchlorambucil*) (IX). To 12.1 g. (0.054 mole) of methyl *m*-[bis(2-hydroxyethyl)amino]hydrocinnamate (VII) was added 90 ml. of freshly distilled phosphorus oxychloride. The mixture was refluxed for 0.5 hr., then cooled and poured onto 600 g. of crushed ice keeping the temperature below 20°. After standing at room temperature for 20 hr., the solution was neutralized with solid potassium acetate and extracted with benzene (3 × 250 ml.). The combined extracts were dried over anhydrous magnesium sulfate and evaporated to dryness *in vacuo*. Toluene was added and the solution again evaporated *in vacuo* to remove acetic acid, leaving 11.7 g. of brown crystals. The crystals were extracted with petroleum ether (3 × 100 ml.) at 55–60° and the combined extracts cooled at 0° for 2 days to allow crystallization to occur; yield, 8.12 g. (61%), m.p. 88–88.5°; $\lambda_{max(\mu)}^{Nujol}$ 3.50–4.00 (acidic OH of carboxyl); 5.82 (carboxyl C=O); 6.22, 6.30, 6.67 (aryl). The compound traveled as a single spot (R_f 0.71) in System A.[9]

Anal. Calcd. for $C_{13}H_{17}Cl_2NO_2$: C, 53.8; H, 5.85; Cl, 24.5; N, 4.82. Found: C, 53.7; H, 6.04; Cl, 24.2; N, 4.78.

Methyl 4-{o-[bis(2-hydroxyethyl)amino]phenyl}butyrate hydrochloride. Methyl 4-(*o*-aminophenyl)butyrate (XIV) *p*-tolylsulfonate was prepared by the method of Schroeter, *et al.*[10] *via* α-tetralone oxime (68% yield) and *O*-(*p*-tolylsulfonyl)-α-tetralone oxime (53% yield, m.p. 99.5–100°), in 90% yield, m.p. 142–143°, R_f 0.03 in System B; the free base had R_f 0.88 in System A.[9] Hydroxyethylation of XIV *p*-toluenesulfonate was carried out as described for the preparation of V except that one equivalent of sodium bicar-

(14) This compound has been previously prepared by the less convenient tin and hydrochloric acid reduction of *m*-nitrocinnamic acid, but no melting point was recorded, *cf.* S. Gabriel and H. Steundmann, *Ber.*, 15, 842 (1882).

bonate was also added to neutralize the *p*-tolylsulfonic acid; yield, 46%, m.p. 124.5–126°; $\lambda_{max(\mu)}^{Nujol}$ 3.13–3.18 (OH); 5.72 (ester C=O); 8.32, 8.50, 8.60 (ester C—O—C); 9.34, 9.48 (C—OH); 13.2 (*o*-disubstituted benzene). The compound traveled as a single spot (R_f 0.73) in System A.[9]

Anal. Calcd. for $C_{15}H_{23}NO_4 \cdot HCl$: C, 56.7; H, 7.57; Cl, 11.2. Found: C, 56.6; H, 7.70; Cl, 11.3.

4-{o-[Bis(2-chloroethyl)amino]phenyl}butyric acid (XV). Chlorination of methyl 4-{*o*-[bis(2-hydroxyethyl)amino]-phenyl}butyrate hydrochloride was accomplished using phosphorus oxychloride in the same manner as in the preparation of IX; yield 64% of tan, light-sensitive crystals, m.p. 30–30.5°; $\lambda_{max(\mu)}^{Nujol}$ 3.72 (acidic OH); 5.81 (carboxyl C=O); 6.24, 6.68 (aryl); 13.3 (*o*-disubstituted benzene). The compound traveled as a single spot (R_f 0.34) in System A.[9]

Anal. Calcd. for $C_{14}H_{19}Cl_2NO_2$: C, 55.3; H, 6.25; Cl, 23.3. Found: C, 55.5; H, 6.50; Cl, 22.9.

When methyl 4-{*o*-[bis(2-hydroxyethyl)amino]phenyl}-butyrate hydrochloride was chlorinated with thionyl chloride in refluxing chloroform for 30 min., a 52% yield of an oil was obtained that had the infrared absorption spectrum expected for methyl 4-{*o*-[bis(2-chloroethyl)amino]phenyl}-butyrate. This crude material was refluxed in concentrated hydrochloric acid for 30 min. to hydrolyze the ester, yielding, after crystallization from petroleum ether, 18% of XV, m.p. 29.5–30.5°.

4-(m-Aminophenyl)butyric acid hydrochloride. Reduction of the ketone group of 3-(*m*-aminobenzoyl)propionic acid[11] by the Huang-Minlon modified Wolff-Kishner reduction gave the desired product in 0–13% yields as white crystals, m.p. 155–157°; $\lambda_{max(\mu)}^{Nujol}$ 5.82 (carboxyl C=O), absence of ketone at 5.92. The compound traveled as a single spot (R_f 0.69) in System A.[9]

Anal. Calcd. for $C_{10}H_{11}NO_2 \cdot HCl$: C, 55.7; H, 6.54; Cl, 16.4; N, 6.50. Found: C, 55.7; H, 6.69; Cl, 16.4; N, 6.37.

Other methods, such as hydrogenation of 3-(*m*-nitrobenzoyl)propionic acid as its sodium salt at 90–100° in the presence of Raney Nickel or as its hydrochloride in the presence of palladium-charcoal, Clemmenson reduction, or hydrogenolysis of the ethylenethioketal, were no better.

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

Potential Anticancer Agents.[1] LI. Synthesis of 2-Amino-9-(5′-deoxy-β-D-ribofuranosyl)-9-H-purine-6-thiol

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The synthesis of 2-amino-9-(5′-deoxy-β-D-ribofuranosyl)-9-H-purine-6-thiol (III) from 2′,3′-*O*-isopropylideneguanosine (IV) in seven steps is described. The intermediate 2′,3′-*O*-isopropylidene-5′-*O*-(*p*-tolylsulfonyl)guanosine (V) showed much less tendency toward cyclonucleoside formation than did the corresponding adenosine derivative. Thus, displacement of the tosylate of V by mercaptide gave 61% of recrystallized 5′-*S*-ethyl-2′,3′-*O*-isopropylidene-5′-thioguanosine (IX). Deacetonation followed by desulfurization yielded 5′-deoxyguanosine (XI), which was acetylated, then thiated and deacetylated to give the title compound (III).

Thioguanine[2], an analog of guanine, is a potent inhibitor of certain animal tumors[3] and of human leukemia[4]; in addition it is synergistic with azaserine.[5,6] Thioguanine is rapidly converted to its ribonucleotide and partially incorporated into the nucleic acid of thioguanine-sensitive neoplasms.[7] More recently, thioguanosine has been synthesized and evaluated as an antitumor agent.[8]

As part of the continuing study in this institute on the mechanism of action of thioguanine, LePage has given a preliminary report on the metabolism and antitumor effects[9] of 9-methylthioguanine,[10] where conversion to a nucleotide does not take place. The enzymic interconversion of purines at the free base level or at the nucleotides level is well established[11]; in contrast, little is known about interconversion of purines at the nucleoside level. As metabolism of thioguanine at the nucleo-

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(9) Private communication from Dr. G. A. LePage to the Cancer Chemotherapy National Service Center.

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