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

Potential Anticancer Agents.[1] XXXIII. Analogs of Chlorambucil. III. Monofunctional Alkylating Agents Derived from 3-(*p*-Acetylphenyl)propionic Acid

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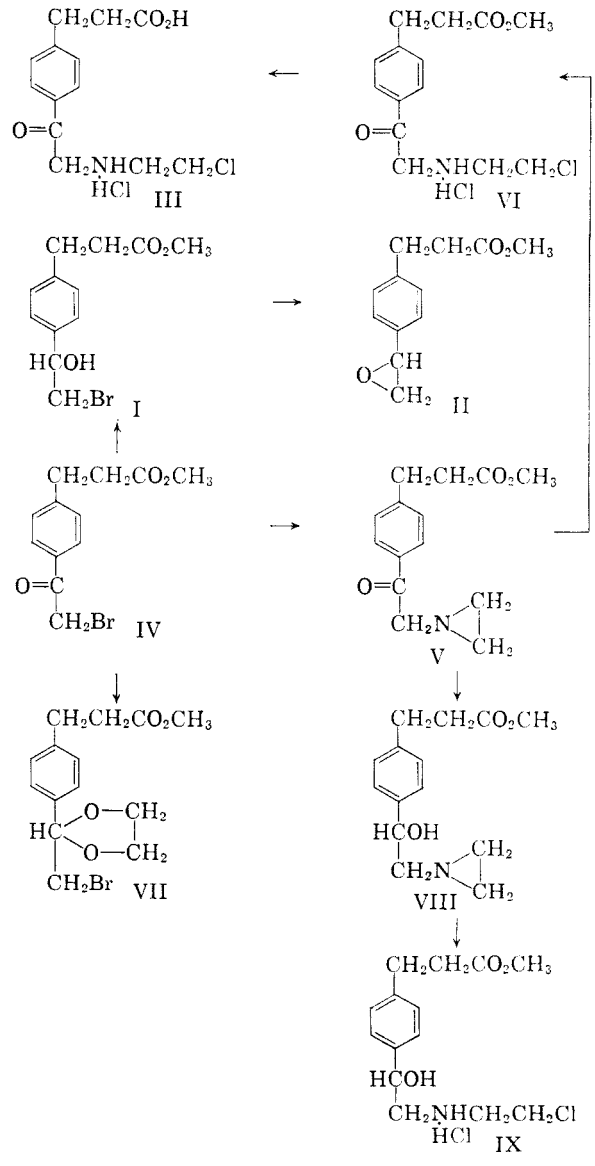
The following monofunctional alkylating agents were synthesized from 3-(*p*-acetylphenyl)propionic acid: 3-{*p*-[*N*-(2-chloroethyl)glycyl]phenyl}propionic acid hydrochloride (III), methyl 3-{*p*-[2-(1-aziridinyl)-1-hydroxyethyl]phenyl}propionate (VIII), and methyl 3-{*p*-[2-(2-chloroethylamino)-1-hydroxyethyl]phenyl}propionate hydrochloride (IX). These compounds can be considered as analogs of chlorambucil, 4-{*p*-[bis(2-chloroethyl)amino]phenyl}butyric acid, and in addition, compound VIII can be considered as an analog of tetramin, α-vinyl-1-aziridinethanol.

In 1953, Everett, Roberts and Ross,[2] reported that a series of *p*-[bis(2-chloroethyl)amino]phenylcarboxylic acids inhibited the growth of the transplanted Walker rat Sarcoma 256, the most active compound being the 4-butyric acid derivative (chlorambucil). The synthesis of derivatives of chlorambucil and norchlorambucil (substituted 3-phenylpropionic acids) has been undertake in these Laboratories.[3a]

Whereas most active anticancer agents of the alkylating type contain bis-alkylating groups, a few anticancer agents can be considered as monofunctional alkylating agents, namely, azaserine[4] (*o*-diazoacetyl-L-serine), DON[5] (6-diazo-5-oxo-L-norleucine), and tetramin[6] (α-vinyl-1-aziridineethanol). With the proper carrier group (metabolite), monofunctional alkylating agents could function as irreversible enzyme inhibitors.[3b] This paper reports the preparation of three monofunctional alkylating agents synthesized from 3-(*p*-acetylphenyl)propionic acid,[3a] namely, 3-[*p*-[*N*-(2-chloroethyl)glycyl]phenyl]propionic acid hydrochloride (III), methyl 3-{*p*-[2-(1-aziridinyl)-1-hydroxyethyl]phenyl}propionate (VIII), and methyl 3-{*p*-[2-(2-chloroethylamino)-1-hydroxyethyl]phenyl}propionate hydrochloride (IX).

The reaction between methyl 3-(*p*-bromoacetylphenyl)propionate (IV)[3a] and ethylenimine was carried out in benzene at 0–5° using triethylamine



(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center of the National Cancer Institute, 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, *cf.* W. A. Skinner, H. F. Gram, and B. R. Baker, *J. Org. Chem.*, 25, 000 (1960).

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

(3)(a) 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); (b) H. F. Gram, C. W. Mosher, and B. R. Baker, Paper XVII of this series, *J. Am. Chem. Soc.*, 81, 3103 (1959).

(4) C. C. Stock, D. A. Clarke, H. C. Reilly, S. M. Buckley, and C. P. Rhoads, *Nature*, 173, 71 (1954).

(5) D. A. Clarke, H. C. Reilly, and C. C. Stock, Abstract of Paper, 129th Meeting, American Chemical Society, Dallas, Texas, April 1956, p. 12-M; H. A. DeWald and A. M. Moore, *J. Am. Chem. Soc.*, 80, 3941 (1958).

(6) H. Oettel, *Angew. Chem.*, 71, 222 (1959); H. Frohberg and H. Oettel, *Arzneimittel-Forsch.*, 2, 189 (1959).

as an acid acceptor. This reaction yielded methyl 3-{*p*-[(1-aziridinyl)acetyl]phenyl}propionate (V) as a semisolid which proved to be too unstable to purify for analysis. However, when V was treated with anhydrous hydrogen chloride in absolute ethanol and chilled in ice, methyl 3-{*p*-[*N*-(2-chloroethyl)glycyl]phenyl}propionate hydrochloride (VI) separated in 52% yield as a crystalline solid, which melted at 180–189° dec. after recrystallization from absolute ethanol.

Hydrolysis of the ester group of VI was accomplished by treatment with hot concentrated hydrochloric acid for two hours. Upon chilling of the acid solution, 3-{*p*-[*N*-(2-chloroethyl)glycyl]phenyl}propionic acid hydrochloride (III) separated in 89% yield as a white solid, m.p. 195–208° dec.

Earlier attempts in these laboratories to treat ethylenimine with methyl 3-(*p*-bromoacetylphenyl)-propionate (IV) in ethanol, using potassium carbonate as the acid acceptor, failed to give the desired product (V) in sufficient yields. That polymeric products were produced even at 0–5°, was shown by treatment of the crude ethylenimino compound with anhydrous hydrogen chloride gas in chloroform, as the resultant product did not move when chromatographed on paper using solvent system A or B.[7]

Tetramin (α-vinyl-1-aziridinethanol) is an anticancer agent active in various experimental systems, whereas the related α-ethyl-1-aziridinethanol is inactive.[6] As methyl 3-(*p*-bromoacetylphenyl)-propionate (IV) was available, it seemed desirable to convert it to VIII, which can be considered an analog of tetramin in which the double bond has been replaced by an aromatic ring. This tetramin analog (VIII) would also be considered an analog of chlorambucil containing a monofunctional alkylating group.

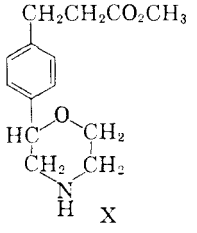
The reduction of methyl 3-(*p*-bromoacetylphenyl)propionate (IV) to methyl 3-[*p*-(2-bromo-1-hydroxyethyl)phenyl]propionate (I) was accomplished in 66% yield by heating with sodium borohydride in aqueous methanol for one hour. The product was a yellow oil that showed OH (2.85μ), C—OH (9.70 μ), C=O ester (5.78 μ), and the absence of C=O ketone (5.90μ) in the infrared spectrum. Without further purification, this oil was treated at 0° in ether with powdered potassium hydroxide to yield quantitatively a yellow sirup which showed the presence of epoxy bands at 7.59 and 11.38μ and no hydroxyl bands in the infrared absorption spectrum. Vacuum distillation of this sirup furnished methyl 3-[*p*-(epoxyethyl)-phenyl]propionate (II) in 58% yield, as a colorless sirup which crystallized upon standing.

Earlier attempts in these laboratories to dehydrobrominate I with ethanolic potassium hydroxide yielded mainly the corresponding glycol or hydroxyether rather than the desired epoxide.

The reaction between methyl 3-[*p*-(epoxyethyl)-phenyl]propionate (II) and ethylenimene failed to yield the desired tetramin analog (VIII). Starting material (II) was recovered when the epoxide and ethylenimine were refluxed in ether for eighteen hours or when a benzene solution of II and ethylenimine was refluxed for eighteen hours. The epoxide (II) did, however, react with anhydrous hydrogen chloride in ethanol to give a compound that showed OH (2.95μ) and C—OH (9.60-μ) in the infrared absorption spectrum and that gave a positive alcohol silver nitrate test for halogen.

Attempts to prepare VIII by a direct reaction between ethylenimine and methyl 3-[*p*-(2-bromo-1-hydroxyethyl)phenyl]propionate (I) by refluxing in benzene in the presence of triethylamine as an acid acceptor failed, starting material and ethylenimine polymer being the only isolable compounds. Without the activation by the keto group, as in the conversion of IV to V, the bromine does not apparently react with the highly heat sensitive ethylenimine.

A successful synthesis of VIII was accomplished by the selective reduction of methyl 3-{*p*-[(1-aziridinyl)acetylphenyl}propionate (V) with sodium borohydride in aqueous methanol. Methyl 3-{*p*-[2-(1-aziridinyl)-1-hydroxyethyl]phenyl}propionate (VIII) was obtained as an analytically pure solid, m.p. 109–112°, in 40% yield by this method. As the OH band in VIII at 2.9μ in the infrared absorption spectrum was very weak, it was necessary to prove that the compound obtained by the sodium borohydride reduction still contained the ethylenimino and hydroxy groupings rather than being the isomeric morpholine derivative (X). Treatment of VIII with hydrogen chloride in benzene successfully opened the ethylenimine ring to form the chloroethylamino derivative (IX) in 85% yield, m.p. 139–140°. The ring of the isomeric morpholine derivative (X) could not be expected to open to IX under the conditions used.



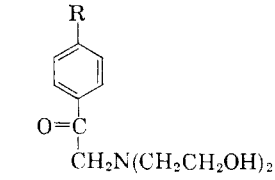
X

The successful reduction of V with sodium borohydride to VIII in which the keto group was selectively reduced without destroying the ethyl-

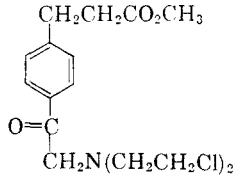
(7) Paper chromatograms were run by the descending technique on Whatman No. 1 paper with the spots being detected by their ultraviolet absorption. In this series, once the ketone has been reduced, the compounds no longer absorb strongly enough in the ultraviolet to be detected on paper chromatograms. The solvent systems used were: Solvent A, *n*-butanol saturated with water, or solvent B, *n*-butanol-acetic acid-water (5:2:3).

enimino group introduces a new route to tetramin analogs via bromoketones rather than from epoxides. This route should prove useful when the epoxides either are too unreactive towards ethylenimine or polymerize too easily.

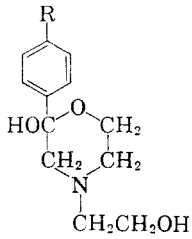
Attempts were made to convert methyl 3-(*p*-bromoacetylphenyl)propionate (IV) to the bis-mustard, methyl 3-{*p*-[*N*,*N*-bis(2-chloroethyl)glycyl]phenyl}propionate (XII) via treatment of IV with diethanolamine followed by chlorination.



XI. R = $CH_2CH_2CO_2CH_3$
XIV. R = H



XII



XIII. R = $CH_2CH_2CO_2CH_3$
XV. R = H

Diethanolamine reacted completely with IV at 90° in one hour, but instead of producing methyl 3-{*p*-[*N*,*N*-bis(2-hydroxyethyl)glycyl]phenyl}propionate (XI), the hemiketal (XIII) was apparently formed. The infrared absorption spectrum of the oily product showed the presence of hydroxyl bands (3.42, 9.50 μ) and an ester band (5.77 μ) but no band for ketone carbonyl (5.90 μ). No ultraviolet-absorbing material could be detected by paper chromatography of this product in solvent system A or B.[7]

Brighton and Reid[8] reported that the reaction of diethanolamine with 2-chloroacetophenone yielded 2-[bis(2-hydroxyethyl)amino]acetophenone (XIV), m.p. 44°. Only analytical data were presented as proof of the structure. This reaction was repeated in this laboratory and the product, m.p. 70–80°, showed no carbonyl absorption near 5.90μ but hydroxyl bands at 3.00 and 9.50μ in the infrared absorption spectrum. This spectrum indicates that the reaction yielded the isomeric hemiketal (XV). Mikhailov and Makarova[9] reported a melting point of 77–78° for this compound and assigned it structure XV. They also converted XV to the ethyl and methyl ketals.

Attempts to effect reaction between diethanolamine and the ethylene ketal VII failed because of the unreactivity of the bromine in that molecule. No reaction with the amine other than amide formation occurred, even when the ketal was heated at 100° for five hours with diethanolamine. The addition of potassium iodide did not increase the reactivity of the compound sufficiently for reaction to occur.

(8) K. W. Brighton and E. Reid, *J. Am. Chem. Soc.*, **65**, 479 (1943).

(9) B. M. Mikhailov and A. N. Makarova, *J. Gen. Chem. (USSR)*, **28**, 149 (1958).

EXPERIMENTAL

Methyl 3-{p-[N-(2-chloroethyl)glycyl]phenyl}propionate hydrochloride (VI). To a solution of 7.13 g. (0.025 mole) of methyl 3-(*p*-bromoacetylphenyl)propionate[3a] in 50 ml. of benzene containing 2.55 g. (0.025 mole) of triethylamine held at 0–5° was added with stirring 2.15 g. (0.055 mole) of ethylenimine. Stirring was continued for 2 hr. and the precipitated triethylamine hydrobromide was removed by filtration. Upon concentration of the filtrate *in vacuo* at room temperature, 6.35 g. of a dark yellow sirup (V) was obtained. This sirup was taken up in benzene and chilled in ice and anhydrous hydrogen chloride gas was bubbled into the solution, causing it to darken. Ether was added to turbidity and the brown solid that separated after chilling was collected on a filter and recrystallized from absolute ethanol to yield 3.25 g. (52%) of white platelets, m.p. 174–188° dec. An analytical sample was obtained by two more recrystallizations from absolute ethanol, m.p. 180–189° dec.; $\lambda_{max(\mu)}^{Nujol}$ 3.65, 3.75 (NH, NH_2^+), 5.72 (ester C=O), 5.90 (ketone C=O), 8.58 (ester C—O—C), 12.00 (*p*-disubstituted phenyl), 13.85 (C—Cl).

Anal. Calcd. for $C_{14}H_{18}ClNO_3 \cdot HCl$: C, 52.5; H, 5.98; Cl, 22.1. Found: C, 52.2; H, 6.27; Cl, 22.2.

3-{p-[N-(2-chloroethyl)glycyl]phenyl}propionic acid hydrochloride (III). A solution of 0.60 g. (2.4 mmoles) of methyl 3-{*p*-[*N*-(2-chloroethyl)glycyl]phenyl}propionate hydrochloride (VI) in 6 ml. of concd. hydrochloric acid was refluxed for 2 hr. and then chilled. The precipitate that formed was collected on a filter and washed with ether; yield, 0.50 g. (89%), m.p. 195–208° dec. A portion, recrystallized twice from 95% ethanol, melted at 198–208° dec.; $\lambda_{max(\mu)}^{Nujol}$ 5.81 (acid C=O), 5.91 (ketone C=O), 6.49 (NH_2^+), 11.67, 12.00 (*p*-disubstituted phenyl), 13.40, 13.70 (C—Cl).

Anal. Calcd. for $C_{13}H_{16}ClNO_3 \cdot HCl$: C, 51.0; H, 5.55; Cl, 23.2; N, 4.57. Found: C, 51.0; H, 5.71; Cl, 22.9; N, 4.52.

Methyl 3-[p-(epoxyethyl)phenyl]propionate (II). To a solution of 2.20 g. (8 mmoles) of methyl 3-(*p*-bromoacetylphenyl)propionate (IV)[3a] in 20 ml. of methanol was added dropwise over a period of about 10 min. 1.2 ml. (5 mmoles) of 15% aqueous sodium borohydride. The solution was refluxed for 1 hr. and then poured into 20 ml. of water. The oil that separated was collected by two extractions with ether. Dried with anhydrous magnesium sulfate, the combined ether extracts were evaporated *in vacuo* to a yellow sirup (I); yield 1.45 g. (66%); $\lambda_{max(\mu)}^{film}$ 2.85 (OH), 5.78, 8.35 (ester), 9.70 (COH), 11.90 (*p*-disubstituted phenyl), and no ketone absorption near 5.90. Because of its weak ultraviolet absorption, it could not be detected on paper chromatograms.

A solution of 3.6 g. (12.5 mmoles) of crude I in 5 ml. of ether was stirred for 1 hr. with 1 g. (16 mmoles) of freshly powdered (under nitrogen) potassium hydroxide. The filtered solution was evaporated to dryness *in vacuo*. The residual oil (2.6 g.) was distilled to give 1.58 g. (58%) of product, b.p. 94–108° (1 mm.), that crystallized on standing and was of sufficient purity for further transformations. Recrystallization from methanol-water gave white crystals, m.p. 33–34°; $\lambda_{max(\mu)}^{film}$ 5.72 (ester C=O), 7.59, 11.38 (epoxy), 8.31, 8.53 (ester C—O—C), and no OH absorption near 2.90.

Anal. Calcd. for $C_{12}H_{14}O_3$: C, 69.9; H, 6.84. Found: C, 70.0; H, 7.03.

Methyl 3-{p-[2-(1-aziridinyl)-1-hydroxyethyl]phenyl}propionate (VIII). To a solution of 1.0 g. (4 mmoles) of V, pre-

pared as reported in the preparation of VI, in 10 ml. of methanol was added in small portions 0.15 g. (3.8 mmoles) of sodium borohydride. When effervescence had ceased, the solution was allowed to stand at room temperature for 2 hr. and then concentrated *in vacuo* to a light brown solid. The material was dissolved in benzene and petroleum ether (b.p. 30–60°) added until a dark brown oil formed. The colorless solution was decanted from the oil and more petroleum ether added until it became turbid. Upon being chilled, the mixture deposited a sandy precipitate that was collected on a filter and washed with petroleum ether; yield 0.40 g. (40%), m.p. 108.5–110.5°. This solid, after two recrystallizations from benzene-petroleum ether, melted at 109–112°; $\lambda_{max(\mu)}^{KBr}$ 3.20 (OH), 5.72 (ester C=O), 8.52, 9.98 (ester C—O—C), 11.92 (*p*-disubstituted phenyl), and no ketone C=O near 5.90.

Anal. Calcd. for $C_{14}H_{19}NO_3$: C, 67.4; H, 7.68; N, 5.62. Found: C, 67.3; H, 7.66; N, 5.69.

Methyl 3-{p-[2-(2-chloroethylamino)-1-hydroxyethyl]-phenyl}propionate hydrochloride (IX). Anhydrous hydrogen chloride gas was passed through a solution of 1.0 g. (4 mmoles) of methyl 3-[*p*-(2-aziridinyl-1-hydroxyethyl)-phenyl]propionate (VIII) in 5 ml. of benzene. A brown gum separated upon adding ether to turbidity and chilling. The solution was decanted from the gum and more ether added. After trituration with ether several times and placing in a refrigerator for 1 hr., the gum solidified, yield 1.1 g. (85%) m.p. 133–140°. Recrystallization from 7 ml. of absolute ethanol by adding ether until turbid and chilling yielded 1.0 g., m.p. 139–140°. An analytical sample, m.p. 140–141°, was prepared by a further recrystallization; $\lambda_{max(\mu)}^{KBr}$ 3.00 (OH), 3.60, 4.00 (NH_2^+), 8.61 (ester C—O—C), 9.59 (C—OH), 12.04 (*p*-disubstituted phenyl).

Anal. Calcd. for $C_{14}H_{20}ClNO_3 \cdot HCl$: C, 52.2; H, 6.52; Cl, 22.0; N, 4.35. Found: C, 52.3; H, 6.52; Cl, 21.8; N, 4.53.

Attempts to acid-hydrolyze IX to the corresponding acid were unpromising.

Acknowledgments. The authors wish to thank Dr. Peter Lim for interpretation of the infrared absorption spectra and his staff for the paper chromatography and spectrophotometry. The authors are also indebted to Mr. O. P. Crews, Jr., and his staff staff for large-scale preparation of certain intermediates.

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[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

Reaction of Some Heterocyclic *vic*-Dicarboxamides with Alkaline Hypobromite

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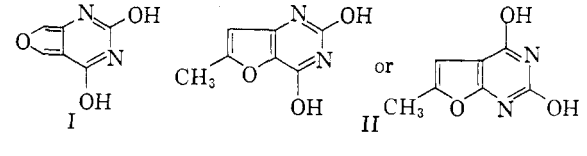
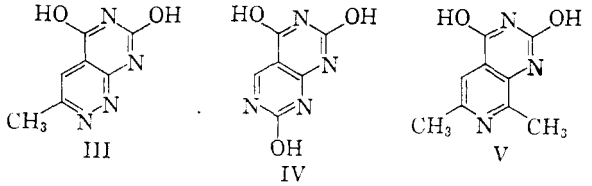
The reaction of alkaline hypobromite with some heterocyclic 1,2-dicarboxamides has led to the preparation of several bicyclic compounds containing the pyrimidine ring fused to furan, pyridazine, and pyrimidine.

This paper concerns the preparation of the new bicyclic pyrimidine derivatives I to V.[1] These were made and tested as possible chemotherapeutic agents against viruses and cancer because of their structural resemblance to certain of the biologically important purines and pteridines.

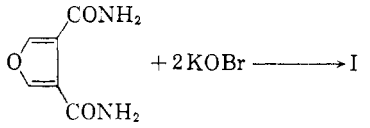


I II



III IV V

The compounds were obtained from appropriate 1,2-dicarboxamides by reaction with alkaline hypobromite under the conditions described by Baxter and Spring.[1] This Hofmann reaction on 1,2-dicarboxamides appears to be a rather general method for the synthesis of the 2,4-dihydroxypyrimidine ring system.[1]



The yields of compounds III, IV, and V were quite satisfactory (70 to 80%), but the yield of compound II was only 20 to 25% and the yield of I was 5 to 8%. One experiment was carried out in which 3,4-thiophenedicarboxamide was allowed to react with hypobromite. A crude product was obtained, but it could not be purified.

From each of the reactions leading to II, III, IV, and V, two isomeric products would appear to be possible. In each case, however, only one compound was isolated. The assignment of structure V is based on analogy with compound VI, which is the exclusive product from the reaction of 3,4-pyridinedicarboxamide with hypobromite.[2] The isomeric 2,3-pyridinedicarboxamide reacts with hypobromite to give exclusively compound VII.[3] By analogy with this latter reaction structures III and

(1) R. A. Baxter and F. S. Spring, *J. Chem. Soc.*, 229 (1945). This reference gives the earlier literature on these reactions.

(2) S. Gabrial and J. Cohnan, *Ber.*, **35**, 2831 (1902).

(3) A. C. McLean and F. S. Spring, *J. Chem. Soc.*, 2582 (1949).