Potential Anticancer Agents.[1] XXV. Monofunctional Alkylating Agents Derived from 2-Methylbenzimidazole

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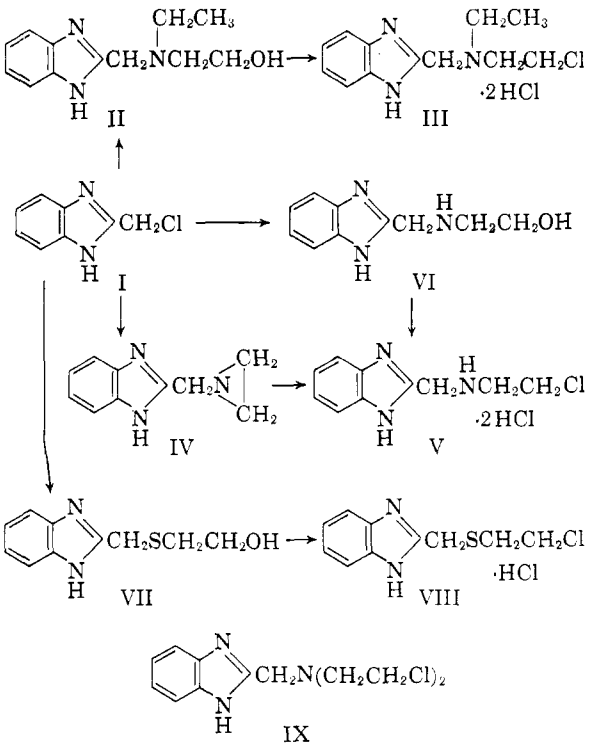
In a recent hypothesis[2] it was suggested that some nitrogen mustard derivatives derived from substrates might operate as irreversible inhibitors by fitting the enzyme site for the substrate, then combining irreversibly with the enzyme by alkylation. If such were the case, then only one alkylating group, rather than the usual two alkylating groups of the bis-nitrogen mustards, would be necessary for irreversible inhibition. One way of evaluating this hypothesis is to synthesize and test monofunctional alkylating analogs of nitrogen mustards derived from carriers that are substrates or substrate inhibitors. Since benzimidazole can act as an antagonist of adenine in some systems,[3-5] and since 2 - [bis(2 - chloroethyl)aminomethyl]benzimidazole (IX) is a mustard with antitumor activity[6] against *Ehrlich ascites carcinoma*, *Adenocarcinoma* 755, *Adenocarcinoma* EO-771, and *Sarcoma* 180, the synthesis and testing of four "one-armed" mustards related to IX were undertaken.

2-(Chloromethyl)benzimidazole (I)[7] reacted with 2-ethylaminoethanol in boiling ethanol to yield crystalline 2-[*N*-ethyl-*N*-(2-hydroxyethyl)aminomethyl]benzimidazole (II) in 52% yield. Treatment of II with thionyl chloride in boiling chloroform yielded the "one-armed" mustard as its dihydrochloride (III) in 26% yield.

Ethylenimine reacted readily with 2-(chloromethyl)-benzimidazole (I) in ethanol at room temperature using potassium carbonate as an acid acceptor. The substituted ethylenimine (IV) was obtained in 71% yield as an unstable oil. Higher temperatures for the reaction were unsuccessful, since the product (IV) was noticeably unstable above 50°. When a solution of IV in chloroform was treated with anhydrous hydrogen chloride, a solid precipitated in 80% yield which proved to be 2-[(2-chloroethyl)-aminomethyl]benzimidazole dihydrochloride (V). That the ethylenimine group of IV had opened to a 2-chloroethylamine (V) was unequivocally proven by the presence of three chlorines in the product, since of the possible products only V could accommodate three chlorines per mole.



A second route to V appeared to be less promising. 2 - [(2 - Hydroxyethyl)aminomethyl]benzimidazole (VI), prepared in 72% yield from 2-aminoethanol and I, readily reacted with thionyl chloride. However, chlorine analyses of the various crystalline preparations inconsistently indicated between two and three chlorine atoms per molecule.

A hemi-sulfur mustard derivative (VIII) of 2-methylbenzimidazole was also synthesized from 2-(chloromethyl)benzimidazole (I). Reaction of I with an excess of potassium 2-hydroxyethylmercaptide in absolute ethanol at room temperature gave a 45% yield of 2-[(2-hydroxyethyl)thiomethyl]benzimidazole (VII) as an oil, characterized as its crystalline picrate. If higher temperatures and lower ratios of the mercaptide were employed, then a bis-benzimidazole derivative of 2-mercaptoethanol was obtained. Replacement of the hydroxyl group of VII with chlorine proceeded smoothly with thionyl chloride in boiling chloroform, the crystalline hydrochloride (VIII) being obtained in 81% yield.

Biological results. The four "one-armed" mustards were evaluated[8] against *Sarcoma* 180, *Carcinoma* 755, *Leukemia* L-1210 and *Ehrlich ascites*. With *Leukemia* L-1210, only V (9 mg./kg.) showed a positive response, giving borderline activity. Only VIII showed activity against *Carcinoma*

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, Contract No. SA-43-ph-1892. For the preceding paper in this series, *cf.* J. DeGraw, L. Goodman, R. Koehler, and B. R. Baker, *J. Org. Chem.*, **24**, 1629 (1959).

(2) H. F. Gram, Carol W. Mosher, and B. R. Baker, Paper XVII of this series, *J. Am. Chem. Soc.*, **81**, 3103 (1959).

(3) L. S. Goodman, A. Gilman, and N. Hart, *Federation Proc.*, **2**, 80 (1943).

(4) D. W. Woolley, *J. Biol. Chem.*, **152**, 225 (1944).

(5) D. W. Woolley, Harvey Lectures, Series XLI, 189 (1945-46).

(6) E. Hirschberg, A. Gellhorn, and W. S. Gump, *Cancer Research*, **17**, 904 (1957).

(7) A. Bloom and A. R. Day, *J. Org. Chem.*, **4**, 14 (1939).

(8) We wish to thank Dr. Joseph Greenberg and staff of this Institute for the test data, performed under contract with the Cancer Chemotherapy National Service Center.

755, being borderline[9] at 60 mg./kg. All four compounds were inactive against *Sarcoma* 180. However, both III (18 mg./kg.) and VIII (60 mg./kg.) gave a 47% extension of life of mice bearing *Ehrlich ascites;* V (18 mg./kg.) gave a less significant 28% extension.

The "two-armed" mustard, 2-[bis(2-chloroethyl)-aminomethyl]benzimidazole (IX) has been reported[6] to give, at 8 mg./kg., a 94% life extension of mice bearing *Ehrlich ascites*, near borderline activity[9] on *Carcinoma* 755 and no activity with *Leukemia* L-1210 or *Sarcoma* 180.[9]

Comparison of the data obtained with the four "one-armed" mustards to that of the "two-armed" mustard (IX) does not allow an unequivocal conclusion on whether the "two-armed" mustard (IX) can or cannot act as an irreversible enzyme inhibitor. Nevertheless, the sought-for increased effectiveness of "one-armed" mustards in the benzimidazol series was not found, except perhaps for *Leukemia* L-1210.

EXPERIMENTAL

2-[N-Ethyl-N-(2-hydroxyethyl)aminomethyl]benzimidazol (II).[10] To a suspension of 75 g. (0.45 mol.) of 2-(chloromethyl)benzimidazole (I)[7] in 175 ml. of absolute ethanol, stirred in an ice bath, was added 112 ml. (1.15 mol.) of 2-ethylaminoethanol. The resulting mixture was heterogeneous, but upon slight heating became homogeneous. The solution was refluxed for 16 hr., cooled, then poured into 500 ml. of 4*N* aqueous sodium hydroxide solution. This solution was evaporated *in vacuo* and the residue partitioned between 200 ml. of distilled water and 500 ml. of chloroform. The aqueous phase was extracted with chloroform (2 × 100 ml.). After being dried with anhydrous magnesium sulfate, the combined chloroform solutions were evaporated *in vacuo*, yielding 98.7 g. of a yellow, tacky solid. The solid was dissolved in methanol, clarified with Norit, and crystallized by the addition of benzene; yield 51.4 g. (52%), m.p. 149–150.0°; $\lambda_{max(\mu)}^{KBr}$ 3.52, (NH), 9.55 (C—OH), 13.40 (*o*-disubstituted benzene); $\lambda_{max(\mu)}^{ethanol}$ 276 (ϵ7600), 282 (ϵ8200). The compound moved as a single spot (R_f 0.73) on paper[11] as detected by its ultraviolet absorption spectrum.

Anal. Calcd. for $C_{12}H_{17}N_3O$: C, 65.7; H, 7.81; N, 19.2. Found: C, 65.9; H, 8.05; N, 19.0.

2-[N-Ethyl-N-(2-chloroethyl)aminomethyl]benzimidazole dihydrochloride (III). A solution of 21.9 g. (0.10 mol.) of 2-[*N*-ethyl-*N*-(2-hydroxyethyl)aminomethyl]benzimidazole (II) in 200 ml. of chloroform was added with shaking to a solution of 100 ml. (1.4 mol.) of thionyl chloride in 150 ml. of chloroform. The system was refluxed for 4 hr. and allowed to remain overnight at room temperature. The solution was evaporated *in vacuo* (bath 40–50°). The solid residue was dissolved in methanol, clarified with Norit, and concentrated *in vacuo* until an oil began to separate. Benzene (200 ml.) was added and the solution again concentrated *in vacuo* until crystals appeared; yield 8.02 g. (26%), m.p. >160° (dec.); $\lambda_{max(\mu)}^{KBr}$ 3.60–3.75 (NH), 4.15 (NH^+), 6.20 (aryl), absence of C—OH, 9.55.

Anal. Calcd. for $C_{12}H_{16}ClN_3 \cdot 2HCl$: C, 46.4; H, 5.84; Cl, 34.2. Found: C, 46.0; H, 5.47; Cl, 34.0.

2-(Ethyleniminomethyl)benzimidazole (IV). To a solution of 5.01 g. (0.030 mol.) of 2-(chloromethyl)benzimidazole (I) in 200 ml. of absolute methanol were added in rapid succession 3.11 g. (0.020 mol.) of finely powdered anhydrous potassium carbonate and 7.80 ml. (0.15 mol.) of ethylenimine. The reaction mixture was stirred vigorously for 6 hr. at room temperature, filtered, and the filtrate evaporated *in vacuo* (bath 25°) to give 8.63 g. of a semisolid residue. The residue was partitioned between 50 ml. of water and chloroform. The chloroform layer was evaporated to dryness *in vacuo* (bath 25°) to yield 5.10 g. (71%) of a pale amber liquid; $\lambda_{max(\mu)}^{liquid}$ 6.15 (aryl), 6.25 (C=N), 6.95, 7.85 (benzimidazole ring), 13.40 (*o*-disubstituted benzene). All attempts to crystallize this liquid have failed.

This compound was characterized by treatment with hydrogen chloride to yield V.

2-[(2-Chloroethyl)aminomethyl]benzimidazole dihydrochloride (V). A solution of 2.0 g. (0.012 mol.) of the oily imine derivative IV in 50 ml. of chloroform was cooled in an ice bath and gaseous hydrogen chloride was bubbled through the solution over a period of 1 hr. The white solid which separated was collected on a filter; yield 2.61 g. (80%) of product, m.p. 228–231° (dec.). Recrystallization of this compound from methanol saturated with hydrogen chloride by the addition of benzene gave a product, m.p. 231–238° (dec.); $\lambda_{max(\mu)}^{Nujol}$ 3.75, 3.92 (NH^+), 6.20 (C=N), 13.35 (*o*-disubstituted benzene), absence of C—OH, 9.55.

Anal. Calcd. for $C_{10}H_{12}ClN_3 \cdot 2HCl$: C, 42.5; H, 5.00; Cl, 37.6. Found: C, 42.7; H, 5.00; Cl, 37.1, 36.5, 35.9. The percentage of chloride decreased upon standing, as shown by the three consecutive analyses.

2-[(2-Hydroxyethyl)aminomethyl]benzimidazole (VI). A solution of 14.4 g. (0.086 mol.) of I and 8.0 g. (0.13 mol.) of 2-aminoethanol in 90 ml. of absolute ethanol was refluxed for 4 hr. The solvent was removed *in vacuo* (bath 50°), yielding a yellow semisolid, which was dissolved in 20 ml. of hot methanol and added to 400 ml. of water and 100 g. of ice; yield 11.8 g. (72%) of white solid, m.p. 101–115°. Recrystallization from aqueous ethanol gave an analytical sample, m.p. 117°; $\lambda_{max(\mu)}^{KBr}$ 3.15, 3.55, 6.50 (NH), 6.20 (C=N), 6.70–7.05 (benzimidazole structure), 9.70 (C—OH), 13.40 (*o*-disubstituted benzene). The compound traveled on paper[11] as a primary spot at R_f 0.85 with a secondary trace spot at R_f 0.82.

Anal. Calcd. for $C_{10}H_{13}N_3O$: C, 62.8; H, 6.85; N, 21.9. Found: C, 62.7; H, 6.42; N, 21.3.

Attempts to convert VI to V with thionyl chloride in boiling chloroform, as described for the preparation of III, gave an unidentified crystalline hydrochloride, m.p. 216–217°, that did not give proper combustion values for V.

Anal. Found: C, 51.6, 51.8; H, 5.02, 4.86; Cl, 24.7; N, 16.1.

2-[(2-Hydroxyethyl)thiomethyl]benzimidazole (VII). To a solution of 350 ml. of absolute ethanol and 14 g. (0.25 mol.) of potassium hydroxide was added with stirring 19.5 g. (0.25 mol.) of 2-mercaptoethanol. When solution was complete, 8.32 g. (0.05 mol.) of finely powdered 2-(chloromethyl)-benzimidazole (I) was added over a period of 15 min. After the addition was complete, the system was stirred for another 3.5 hr. at room temperature.

The reaction mixture was cooled in an ice bath and the potassium chloride (3.54 g., 95%) which had separated was removed by filtration. The filtrate was evaporated *in vacuo* (bath 60°) to a volume of 50 ml., poured into 600 ml. of water, and the resultant solution acidified to *p*H 3 with dilute hydrochloric acid. The acidified solution was extracted 3 times with 100 ml. of ether. The aqueous layer was brought to *p*H 7–8 and extracted 4 times with chloroform. The combined chloroform extracts were evaporated to yield 4.66 g.

(9) Borderline activity is defined as a ratio of tumor weights in treated animals to tumor weights in control animals (T/C) of 0.38–0.54. Above 0.54 is considered inactive; *cf. Cancer Chemotherapy Reports,* No. 1, p. 60 (1959), published by the Cancer Chemotherapy National Service Center, Bethesda, Md.

(10) This compound was first prepared by Dr. S. Fuqua of these laboratories.

(11) Paper chromatograms were run by the descending technique on Whatman No. 1 paper with butanol–acetic acid–water (5/2/3).

(45%) of a viscous oil. This material traveled as a single spot on Whatman No. 1 paper, R_f 0.80 in *n*-butanol saturated with water. In a larger scale preparation, VII crystallized, m.p. 122–124°.

A crystalline picrate of this oil was prepared by adding a saturated solution of picric acid in ethanol to a 10% solution of the oil in ethanol, m.p. 187.5–188.5°; $\lambda_{max(\mu)}^{Nujol}$ 2.82 (OH), 3.48 (NH^+), 6.18 (aryl), 6.51 (NO_2), 9.40 (C—OH).

Anal. Calcd. for $C_{10}H_{12}N_2OS \cdot C_6H_3N_3O_7$: C, 43.9; H, 3.45; N, 16.0; S, 7.33. Found: C, 44.1; H, 3.71; N, 16.1; S, 7.11.

2-[(2-Chloroethyl)thiomethyl]benzimidazole hydrochloride (VIII). To a solution of 4.66 g. (0.023 mol.) of VII in 60 ml. of chloroform was added dropwise with stirring a solution of 22.8 ml. (0.32 mol.) of thionyl chloride in 35 ml. of chloroform. After the addition was complete, the reaction mixture was refluxed for 4 hr., then allowed to stand overnight at room temperature. The system was filtered to yield 4.76 g. (81%) of product, m.p. 177–179°.

An analytical sample was prepared by recrystallization of the crude product from methanol saturated with hydrogen chloride at 40° by the addition of hot benzene, m.p. 180–182°; $\lambda_{max(\mu)}^{Nujol}$ 6.10, 6.35 (aryl, C=N), 13.35 (*o*-disubstituted benzene), no C—OH near 9.40 nor OH near 2.8.

Anal. Calcd. for $C_{10}H_{11}ClN_2S \cdot HCl$: C, 45.6; H, 4.59; Cl, 26.9, S, 12.2. Found: C, 45.7; H, 4.70; Cl, 27.3; S, 11.7.

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Piper Methysticum Forst. II. The Synthesis of *dl*-Methysticin and *dl*-Dihydromethysticin

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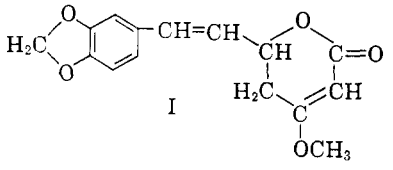
In a previous paper from these laboratories[1] the results of a chemical and pharmacological investigation of *Piper methysticum* Forst were reported. On the basis of ability to antagonize strychnine convulsions and potentiate barbiturate sleep time in mice, it was found that methysticin I and



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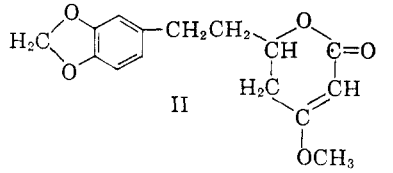

II

dihydromethysticin II possessed a greater degree of activity than the other constituents, kawain, dihydrokawain, yangonin, and desmethoxyyangonin,[2] isolated from this plant. The significant physiological activity evidenced by methysticin and dihydromethysticin on the central nervous system made it of interest to obtain sufficient quantities of these α-pyrone derivatives for further pharmacological studies. Because of the inherent difficulties attendant in securing these compounds from their natural source, a means for obtaining them synthetically was desirable.

The synthesis of kawain[3] and yangonin[4] have been recorded by previous investigators, but the synthesis of methysticin, the first of this class of compounds to be isolated from this plant[5] and its dihydro derivative have not been reported, although their structures have been known since 1929.[6]

Our approach to the synthesis of *dl*-methysticin was by the Reformatsky condensation of 3,4-methylenedioxycinnamaldehyde and methyl γ-bromo-β-methoxycrotonate using tetrahydrofuran as the reaction medium. The condensation proceeded smoothly and *dl*-methysticin was readily obtained by direct crystallization of the product. A comparison of the infrared and ultraviolet spectra of this compound with those of natural methysticin showed them to be indistinguishable. Further evidence for confirming their structural identity was obtained by removing the center of asymmetry at C_6 in the α-pyrone ring of methysticin, by basic hydrolysis, thereby forming methysticic acid which proved to be identical with the acid obtained in the same manner from *dl*-methysticin.

Catalytic reduction of *dl*-methysticin afforded *dl*-dihydromethysticin which exhibited the same infrared and ultraviolet spectra as those of the naturally occurring material.

EXPERIMENTAL[7]

6-(3',4'-Methylenedioxystyryl)-4-methoxy-5,6-dihydro-2-H-pyran-2-one. 3,4-Methylenedioxycinnamaldehyde (58.6 g.;

(1) M. W. Klohs, F. Keller, R. E. Williams, I. M. Toekes and G. E. Cronheim, *Journal of Medicinal and Pharmaceutical Chemistry*, 1, 95 (1959).

(2) This substance had been referred to as compound A in our earlier paper, pending final identification. Compound A has now been compared with a synthetic sample of desmethoxyyangonin [J. Cieślak, *Roczniki Chemii*, 32, 837 (1958) and references therein kindly supplied by Dr. Jerzy Cieślak and they have been found to be identical. This represents the first recorded occurrence of desmethoxyyangonin in *P. methysticum*. Since the completion of this work a publication has appeared citing the presence of this compound in *Aniba firmula* Mez. [Otto Richard Gottlieb and Walter B Mors, *J. Org. Chem.*, 24, 17–18 (1959)].

(3) D. Kosterman, *Nature*, 166, 787 (1950); D. Kosterman, *Rec. Trav. Chim.* 70, 79 (1951); E. M. P. Fowler and H. B. Henbest, *J. Chem. Soc.*, 3642 (1950).

(4) W. Borsche and C. K. Bodenstein, *Ber.* 62, 2515 (1929).

(5) Gobley and O'Rorke, *J. de Pharmacie et Chimi*, 598 (1860), M. Cuzent, *Compt. rend.* 205 (1861).

(6) W. Borsche and W. Peitzsch, *Ber.*, 62, 360 (1929).

(7) All microanalyses by H. V. Tashinian, Microchemical Specialties Company, Berkeley 3, California.