hydroxamic acids with no antibacterial properties. It is significant to note, however, that all the compounds which do show activity (compounds *2, 3, 5, 7, 16, 17, 18, 19*) possess this grouping; in some cases, it is apparent that the activity is solely due to the presence of the hydroxamic group. For example, compound *16* shows some activity; the related compound *15* does not. Compound *18* was the most active of the compounds tested, whereas the closely related compound *10* had virtually no activity. The results also show that the nature of the substituents at positions 3 and 4 on the quinoline nucleus influences the antibacterial properties. From the limited number of examples examined, it would seem that an alkyl group is preferred at position 3, and the length and stereochemistry of this side chain contributes to the activity. The most active 3-substituted compounds (compounds *2, 3, 5, 7, 18, 19*) have a methyl, ethyl, isopropyl, or isobutyl group at this position. The only other 3-substituted quinoline hydroxamic acid which shows some activity has an ethoxycarbonyl group (compound *15*). Compounds possessing the greatest antibacterial activity (compounds *18* and *19*) are unsubstituted at position 4. An investigation is being conducted to discover if such a feature is desirable.

It can be concluded that factors in addition to the presence of a hydroxamic acid group are necessary for antibacterial activity; the degree of substitution and the nature of the substituents also are contributing factors.

Through Dr. J. C. MacDonald, National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Saskatchewan, samples of two naturally occurring cyclic hydroxamic acids, aspergillic acid (VII) and pulcherriminic acid (VIII), became available. Their *in vitro* antibacterial activities, determined by our general method of testing, are listed in Table IV. These results indicate that our test is more rigorous than that applied by previous authors (17–19). The activities of compounds *18* and *19* (Table III) compare favorably with those of aspergillic acid and pulcherriminic acid.

REFERENCES

(1) Coutts, R. T., Hooper, M., and Wibberley, D. G., *J. Chem. Soc.*, **1961**, 5058.
(2) Coutts, R. T., and Wibberley, D. G., *ibid.*, **1963**, 4610.
(3) Coutts, R. T., *Can. Pharm. J., Sci. Sect.*, **97** (No. 6), 32(1964).
(4) Newbold, G. T., and Spring, F. S., *J. Chem. Soc.*, **1948**, 1864.
(5) Cunningham, K. G., *et al.*, *ibid.*, **1949**, 2091.
(6) Coutts, R. T., Noble, D., and Wibberley, D. G., *J. Pharm. Pharmacol.*, **16**, 773(1964).
(7) Ornstein, G., *Chem. Ber.*, **40**, 1088(1907).
(8) Mulert, B., *ibid.*, **39**, 1901(1906).
(9) Wislicenus, W., and Elvert, H., *ibid.*, **42**, 1144(1909).
(10) Braun, J., Petzold, A., and Seeman, J., *ibid.*, **55**, 3779(1922).
(11) Kaslow, C. E., and Buchner, B., *J. Org. Chem.*, **23**, 271(1958).
(12) Ohta, A., and Ochiai, E., *Chem. Pharm. Bull. Tokyo*, **10**, 1260(1962).
(13) Gabriel, S., and Gerhard, W., *Chem. Ber.*, **54**, 1067 (1921).
(14) Ohta, T., and Mori, Y., *J. Pharm. Soc. Japan*, **75**, 1162(1955).
(15) Baumgarten, P., and Kargel, W., *Chem. Ber.*, **60**, 832(1927).
(16) Coutts, R. T., and Wibberley, D. G., *J. Chem. Soc.*, **1962**, 2518.
(17) MacDonald, J. C., Micetich, R. G., and Haskins, R. H., *Can. J. Microbiol.*, **6**, 27(1960).
(18) Lott, W. A., and Shaw, E., *J. Am. Chem. Soc.*, **71**, 70(1949).
(19) Shaw, E., *et al.*, *ibid.*, **72**, 4362(1950).

2-Halogenoethylamines as Potential Folic Acid Antagonists I. Synthesis and Biological Activity of Ethyl-*N*-[1-(2-amino-4-hydroxy-6-methyl-5-pyrimidyl)-3-(2-chloro)-propyl]-*p*-aminobenzoate

By A. M. TRIGGLE and D. J. TRIGGLE

The synthesis of the title compound (VII) is described. The key intermediate in this synthesis was prepared from 1-chloro-2,3-epoxypropane and ethyl-*p*-aminobenzoate. Preliminary biological data, including toxicity, and antitumor and folic reductase inhibitory actions, are presented.

THE USE OF nitrogen mustards as antitumor agents has met with varying degrees of success (1, 2). One of the principal disadvantages to the use of these agents in chemotherapy is their generally high toxicity to the host, which results from their undoubted ability to act, in many cases, as rather nonspecific alkylating agents. Nevertheless, the search for selectively and specifically acting (3) alkylating agents is of importance because it offers the hope that appropriately designed compounds will be able to inactivate irreversibly and specifically enzyme and other macromolecular systems of special importance to the tumor cell.

Previous studies have raised the possibility that certain 2-halogenoethylamines, *i.e.*, 4-*N,N*-(di-2-bromoethyl)aminobenzene sulfonamide (I), may produce tumor inhibition through inactivation of one or more stages of the folic acid pathway. As part of a general program intended to investigate the potential of 5-substituted pyrimidines as antitumor agents, the authors are engaged in the synthesis of 2-halogenoethylamines bearing an appropriately substituted pyrimidine nucleus (II). The synthesis and preliminary biological data for one of these compounds, ethyl-*N*-[1-(2-amino-4-hydroxy-6-methyl-5-pyrimidyl)-3-(2-chloro)propyl]-*p*-aminobenzoate (II, R_1 = OH, R_2 = Me, X = Cl, R_3 = H, R_4 = CO_2Et) are reported here. The synthetic route is outlined in Scheme I.

The key intermediate, ethyl-*N*-(2-hydroxy-3-chloropropyl)-*p*-aminobenzoate (III), was con-

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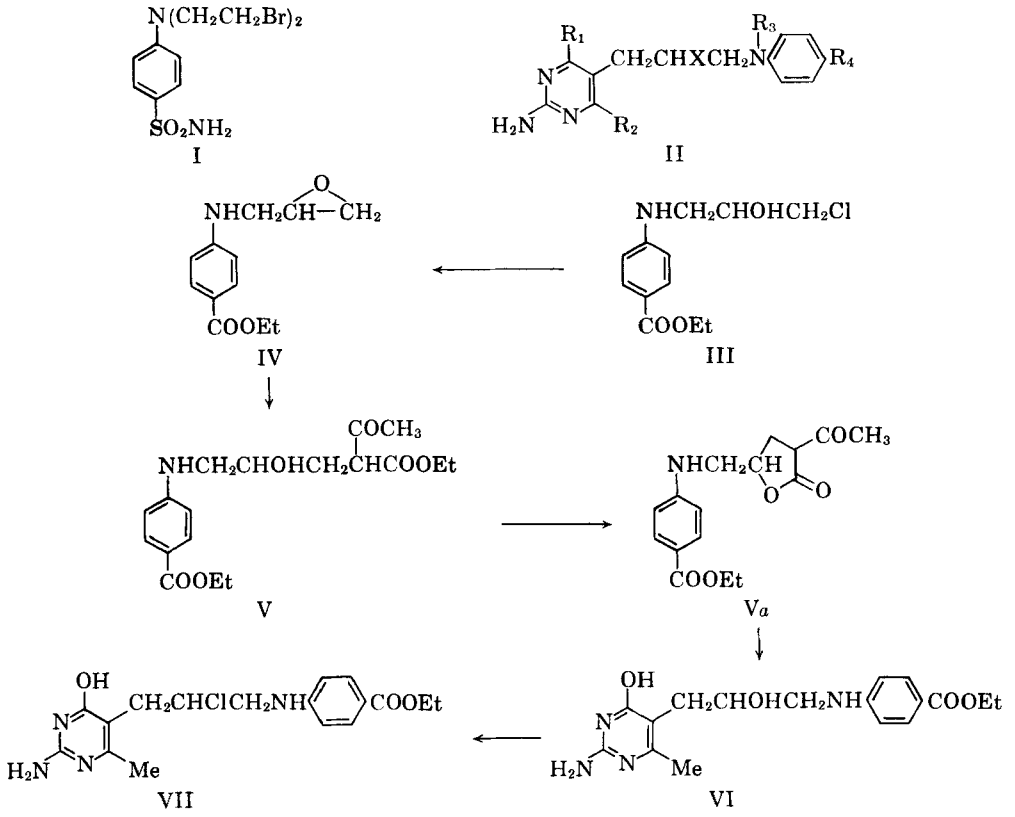

Scheme I

veniently synthesized from 1-chloro-2,3-epoxypropane and ethyl-*p*-aminobenzoate.[1]

The reaction between 1-chloro-2,3-epoxypropane and aromatic amines has been little investigated, although McKelvey and co-workers (4) have described the product from aniline as an unstable oil which polymerizes on standing. The reaction between 1-chloro-2,3-epoxypropane and ethyl-*p*-aminobenzoate proceeds wel. in ethanolic solution at room temperature, with the product slowly precipitating in a practically pure condition.

The reaction between III and ethyl acetoacetate was investigated under a variety of experimental conditions. There is little doubt but that the condensation proceeds through the intermediate epoxide (IV), and the best yields of the final pyrimidine were realized when the chlorohydrin was converted *in situ* to the epoxide prior to the condensation with ethyl acetoacetate. The keto-ester (V), which probably exists as the γ-lactone (V*a*), was not isolated in the normal reaction sequence but was reacted directly with guanidine. This reaction was found to occur most readily when the reaction mixture was stirred at room temperature for 24 hr. before the mixture was refluxed; presumably ethanolysis of the keto-ester is an important side reaction competing with pyrimidine formation (*c.f. Reference 5*).

The chlorination of the pyrimidine (VI) was accomplished by treating it as a suspension in chloroform with thionyl chloride; it proved mpossible, however, to obtain this compound in better than 90% purity. Similar difficulties have been reported in the preparation of 2,4-dihydroxy-5-(2,2′-d chlorodiethyl)amino-pyrimidine (6).

[1] The authors have now isolated this type of product from a number of primary and secondary aromatic amines, *i.e.*, ethyl-*N*-(2-hydroxyethyl)-*p*-aminobenzoate and *N*-(2-hydroxyethyl) aniline. The preparation and properties of the corresponding pyrimidines will be discussed in a later paper.

EXPERIMENTAL

Melting points were recorded on a Kofler hot stage and are uncorrected. Infrared and ultraviolet spectra were recorded with Perkin-Elmer spectrophotometers, models 137B and 202, respectively.

Ethyl - N - (2 - hydroxy - 3 - chloropropyl) - p-aminobenzoate (III).—1-Chloro-2,3-epoxypropane (92.5 Gm., 1.0 mole) was added to a solution of ethyl *p*-aminobenzoate (165 Gm., 1.0 mole) in 1500 ml. of ethanol. Two drops of concentrated hydrochloric acid were added and the mixture set aside at room temperature for 2–3 weeks. White crystals slowly formed and these were filtered to give 130 Gm. of material, m.p. 102–104°. One recrystallization from benzene gave pure III, m.p. 103.5° (120 Gm., 46%).

Anal.[2]—Calcd. for $C_{12}H_{16}ClNO_3$: C, 55.91; H, 6.26; N, 5.44. Found: C, 55.69; H, 6.33; N, 5.51.

Ethyl - N - (4 - carbethoxy - hexan - 2 - ol - 5-one)-p-aminobenzoate (V).—The chlorohydrin III (55.8 Gm., 0.22 mole) in ethanol (200 ml.) was added to an ice cold stirred solution of sodium (5.1 Gm., 0.22 mole) in ethanol (200 ml.). The mixture was stirred for 60 min., then added to a solution of ethyl acetoacetate (28.2 Gm., 0.215 mole) in 200 ml. of ethanolic sodium ethoxide (from 5.1 Gm. of sodium) stirred at 0°. Stirring was continued at room temperature for 24 hr.; the resultant keto-ester was not purified for the

[2] Analyses are by Galbraith Laboratories, Knoxville, Tenn., and Dr. A. E. Bernhardt, West Germany.

subsequent reaction. However, in a separate experiment the keto-ester was isolated by rapid acidification of the reaction mixture to pH 4.5, readjustment to pH 7.0, and extraction of the oil into ether and formation of the hydrochloride of V. Basification of V·HCl, ether extraction, and removal of the ether at 20° gave a viscous uncrystallizable oil. The analysis and I.R. spectra (bands at 1770 cm.$^{-1}$, 1724 cm.$^{-1}$, and 1650 cm.$^{-1}$) suggest that this material contains at least some of the γ-lactone (V*a*).

Anal.—Calcd. for $C_{18}H_{25}NO_6$ (V): C, 61.54; H, 7.17. Calcd. for $C_{16}H_{19}NO_5$ (V*a*): C, 62.89; H, 6.28. Found: C, 61.92; H, 6.77.

Ethyl - N - [1 - (2 - amino - 4 - hydroxy - 6-methyl-5-pyrimidyl)-3-(2-hydroxy)propyl]-p-aminobenzoate (VI).—The ethanolic solution of the keto-ester (V) was added to a solution of guanidine (from 21.2 Gm. of guanidine hydrochloride and 5.1 Gm. of sodium) in ethanol (200 ml.). The mixture was stirred at room temperature for 24 hr., then refluxed for a further 24 hr. The solvent was removed by spin evaporation and the residue dissolved in 10% hydrochloric acid, extracted with ether (2 × 100 ml.), and the aqueous phase basified with 5% ammonium hydroxide to give a semisolid material, which was washed well with water. Trituration with ethanol gave 14.6 Gm. of a pale yellow solid, m.p. 160–163°. Recrystallization (MeOH aqueous) gave pure material, m.p. 162–164° (13.4 Gm., 16.7% over-all from III). $\lambda_{max.}^{pH\ 1}$ 229 (ε, 20,040), 268 (ε, 7,920); $\lambda_{max.}^{pH\ 10}$ 307 mμ (ε, 21,640).

Anal.—Calcd. for $C_{17}H_{22}N_4O_4\ H_2O$: C, 56.1; H, 6.68; N, 15.38. Found: C, 56.23, H, 6.70, N, 15.57.

Prolonged drying at 100° gave a material, m.p. 195–197°, which was free from water of crystallization.

Anal.—Calcd. for $C_{17}H_{22}N_4O_4$: C, 59.2; H, 6.36. Found: C, 59.45; H, 6.36.

N - [1 - (2 - Amino - 4 - hydroxy - 6 - methyl - 5-pyrimidyl) - 3 - (2 - hydroxy)propyl] - p - aminobenzoic Acid.—This carboxylic acid was obtained by acid hydrolysis of the ester (VI), m.p. 176–179° (water–ethanol). $\lambda_{max.}^{pH\ 1}$ 228 (ε, 22,860), 267 (ε, 9,600); $\lambda_{max.}^{pH\ 10}$ 286 mμ (ε, 23,340).

Anal.—Calcd. for $C_{15}H_{18}N_4O_4\ 2H_2O$: C, 51.30; H, 6.23; N, 15.81. Found: C, 51.62; H, 6.10; N, 15.94.

Ethyl - N - [1 - (2 - amino - 4 - hydroxy - 6-methyl - 5 - pyrimidyl) - 3 - (2 - chloro) - propyl] - p-aminobenzoate Hydrochloride (VII).—Two grams of VI (anhydrous) was added to a rapidly stirred solution of thionyl chloride (10% excess in dry $CHCl_3$) at −5°. After 30 min., the solution was brought to room temperature for 30 min. and finally warmed at 30–40° for 10 min. The solution was spin evaporated and the residue dissolved in the minimum of ethanol and poured into an excess of ether. The precipitate was filtered and washed with anhydrous ether to give 2.2 Gm. of VII, m.p. 166–168°. $\lambda_{max.}^{pH1}$ 231 (ε, 15,560), 273 (ε, 9,000), 308 (ε, 11,050); $\lambda_{max.}^{pH\ 10}$ 230 (ε, 12,800), 308 mμ (ε, 24,400).

Anal.—Calcd. for $C_{17}H_{23}Cl_2N_4O_3$: C, 50.8; H, 5.48; Cl, 17.7; N, 13.95. Calcd. for $C_{17}H_{22}Cl_2N_4O_3\ 2H_2O$: C, 46.7; H, 5.98; Cl, 16.21; N, 12.8. Found: C, 47.31; H, 5.88; Cl, 15.99; N, 12.61.

RESULTS AND DISCUSSION

Preliminary toxicity and antitumor data have been obtained for compounds VI and VII. In the toxicity determinations, the compound, suspended in 10% gum acacia, was administered by the intraperitoneal route to groups of three to six animals per dose. Deaths within a 21-day period were recorded and LD_{50} values estimated from percentage mortality/log dose plots.

TABLE I.—TOXICITY AND ANTITUMOR DATA

Compd.	Animal	Toxicity	LD_{50}, mg./Kg./Day
VI	Mouse[a]	>1000	1 day
VI	Rat[b]	> 500	5 day
VII	Mouse	200	1 day
VII	Rat	120	5 day

Compd.	Tumor[c]	Dose, mg./Kg./Day	Growth Inhibition, %
VI	Murphy-Sturm lymphosarcoma	500 (5 days)	0
VII	Murphy-Sturm lymphosarcoma	100 (5 days)	75

[a] Male Holtzman rats. [b] Male Swiss mice. [c] Implanted in male Holtzman rats.

Antitumor activities of these compounds were assessed against the Murphy-Sturm lymphosarcoma. The tumor was implanted into male Holtzman rats, and on the fifth day the compound was administered intraperitoneally for 5 successive days. Control animals received the vehicle only. On the twelfth day, the tumor volumes were calculated from caliper measurements (7) and the mean tumor volumes of the treated rats compared with the mean tumor volumes of control rats. The toxicity and antitumor data are recorded in Table I.

These results show that the potentially alkylating pyrimidine (VII) is significantly more toxic than the nonalkylating analog (VI). This difference is extended to the antitumor activities, since VII produced significant growth inhibition of the Murphy-Sturm lymphosarcoma, although no complete regressions were observed. It is interesting that *N*-(2-chloroethyl)-*p*-aminobenzoic acid is virtually nontoxic and exerts no antitumor action (8). It is apparent that both the pyrimidyl and 2-chloroethyl moieties of VII have contributed to the antitumor action but whether this is due to any selectivity or specificity (3) of action remains to be determined. The determination of folic acid reductase inhibition by VI and VII was carried out according to the method of Werkheiser (9). The *I/S* ratio for 50% inhibition by VI is 400, while for VII, it is less than 400. (This is an approximate figure because VII is only slightly soluble in aqueous media at the pH of the enzyme incubation.) The low activity of VII as a folic reductase inhibitor suggests that this activity may not be related to its antitumor activity. Further investigations concerning this point are currently in progress and will be reported with the complete biological data for these compounds.

REFERENCES

(1) Ross, W. C. J., "Biological Alkylating Agents," Butterworth Press, London, 1962.
(2) Schmidt, L. H., *Ann. N. Y. Acad. Sci.*, **68**, 661(1958).
(3) Triggle, D. J., *J. Theoret. Biol.*, **7**, 241(1964).
(4) McKelvey, J. B., Webre, B. G., and Benerito, R. R., *J. Org. Chem.*, **25**, 1424(1960).
(5) Baker, B. R., Santi, D. V., and Shapiro, H. S., THIS JOURNAL, **53**, 1317(1964).
(6) Lyttle, D. A., and Petering, H. G., *J. Am. Chem. Soc.*, **80**, 6459(1958).
(7) Hebborn, P., and Danielli, J. F., *Biochem. Pharmacol.*, **1**, 19(1958).
(8) Bardos, T. J., *et al.*, *J. Med. Chem.*, in press.
(9) Werkheiser, W. C., *J. Biol. Chem.*, **236**, 888(1961).