Antineoplastic Agents. V. The Aromatic System of Podophyllotoxin (Part B)[1-3]

GEORGE R. PETTIT, MICHAEL F. BAUMANN, AND K. N. RANGAMMAL

Department of Chemistry, University of Maine, Orono, Maine

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Several N-bis(2-chloroethyl)amides have been reduced to their corresponding nitrogen mustard derivatives employing a lithium aluminum hydride-aluminum chloride reagent. The utility of this new approach to nitrogen mustards was illustrated by synthesis of N-bis(2-chloroethyl)-3,4-methylenedioxybenzylamine (X) hydrochloride, N-bis(2-chloroethyl)-3,4,5-trimethoxybenzylamine (XI) hydrochloride and N-bis(2-chloroethyl)-3-(3,4-methylenedioxyphenyl)-3-(3,4,5-trimethoxyphenyl)propylamine (VII) hydrochloride.

A previous investigation was concerned with synthesis of several tertiary amines (*cf.* I) containing the aromatic portion of podophyllotoxin.[1] As part of this approach to potential cancerocidal agents, it was considered important to prepare N-bis(2-chloroethyl)-3-(3,4-methylenedioxyphenyl)-3-(3,4,5-trimethoxyphenyl)-propylamine (VII) since the aromatic system of podophyllotoxin might provide an effective carrier group for the alkylating substituent.[4,5]

The synthetic sequence (III → VII) selected as a feasible route to nitrogen mustard derivative VII first necessitated a review of available procedures leading to 3,4-methylenedioxy-3′,4′,5′-trimethoxybenzophenone (III). The benzophenone derivative III, one of the

(1) Part A, G. R. Pettit and D. S. Alkalay, *J. Org. Chem.*, **25**, 1363 (1960).

(2) Part VII, G. R. Pettit and J. A. Settepani, *J. Med. Pharm. Chem*, **5**, 296 (1962).

(3) This investigation was aided by Grants No. T-79, T-79A, and T-79B from the American Cancer Society.

(4) For a summary of current developments in the nitrogen mustard approach to cancer chemotherapeutic agents, see: G. A. Usbeck, J. W. Jones, and R. K. Robins, *J. Am. Chem. Soc.*, **83**, 1113 (1961); A. Benitez, L. O. Ross, L. Goodman, and B. R. Baker, *ibid.*, **82**, 4585 (1960); W. A. Skinner, K. A. Hyde, H. F. Gram, and B. R. Baker, *J. Org. Chem.*, **25**, 1756 (1960); R. C. Elderfield and R. N. Prasad, *ibid.*, **25**, 1583 (1960); E. J. Reist, R. R. Spencer, and B. R. Baker, *J. Am. Chem. Soc.*, **82**, 2025 (1960); and H. F. Gram, C. W. Mosher, and B. R. Baker, *ibid.*, **81**, 3103 (1959). *Cf.* also: H. H. Lin and C. C. Price, *J. Org. Chem.*, **26**, 266 (1961); H. H. Lin and C. C. Price, *ibid.*, **26**, 264 (1961); W. A. Skinner, A. P. Martinez, and B. R. Baker, *ibid.*, **26**, 152 (1961); A. P. Martinez, W. A. Skinner, W. E. Lee, L. Goodman, and B. R. Baker, *J. Am. Chem. Soc.*, **82**, 6050 (1960); F. Kagan, R. D. Birkenmeyer, and R. E. Strube, *ibid.*, **81**, 3026 (1959); D. A. Lyttle and H. G. Petering, *J. Nat. Cancer Inst.*, **23**, 153 (1959); and R. B. Ross, *J. Chem. Educ.*, **36**, 368 (1959).

(5) A recent compilation of nitrogen mustard derivatives has been prepared by R. B. Ross and P. E. Swartzentruber, *Literature Survey of Nitrogen Mustards*, Cancer Chemotherapy, National Service Center, National Institutes of Health, Bethesda, Md., 1959.

important degradation products of podophyllic acid, was first characterized and subsequently synthesized in very poor yield by Späth from methylenedioxybenzene and 3,4,5-trimethoxybenzoyl chloride.[6] More recently this substance (III) was prepared *via* 1-(3,4,5-trimethoxyphenyl)-6,7-methylenedioxy-3,4-dihydroisoquinoline[7] and employed as starting material for the total synthesis of picropodophyllin.[8,9] In order to develop a convenient route to ketone III, an effort was made to utilize the readily available 3,4-methylenedioxyphenyllithium[10,11] as starting material. Reasonable yields (62–69%) of 3,4-methylenedioxy-3′,4′,5′-trimethoxybenzophenone (III) were eventually obtained when N-(3.4,5-trimethoxybenzoyl)morpholine (II) was allowed to react with an equivalent amount of 3,4-methylenedioxyphenyllithium in tetrahydrofuran solution, cooled to −75°.[12]

The aromatic ketone III was next treated with ethyl bromoacetate in the presence of zinc dust. Following the Reformatsky reaction, tertiary alcohol IV was isolated and dehydrated in acetic anhydride solution containing potassium hydrogen sulfate. Palladium–charcoal catalyzed hydrogenation of the resulting olefin (V) led to the saturated ester VIa.

At this point, it seemed advisable to explore first the remaining route (VI → VII) with appropriate model compounds. Both piperonylic acid (VIIIa) and 3,4,5-trimethoxybenzoic acid (IXa) were individually employed for this purpose. Conversion of acids VIIIa and IXa to nitrogen mustards X and XI, respectively, was also considered a necessary part of the cancer chemotherapy study. In each case, transformation to the acid chloride followed by reaction with two equivalents of N-bis(2-chloroethyl)amine in benzene solution led to good yields of the corresponding amides (VIIIb and IXb). The

(6) E. Späth, F. Wessely, and E. Nadler, *Ber.*, **66**, 125 (1933).

(7) W. J. Gensler and C. M. Samour, *J. Am. Chem. Soc.*, **73**, 5555 (1951).

(8) W. J. Gensler and S. Y. Wang, *ibid.*, **76**, 5890 (1954).

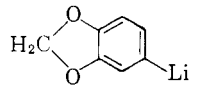
(9) W. J. Gensler, C. M. Samour, S. Y. Wang, and F. Johnson, *ibid.*, **82**, 1714 (1960). This interesting study also included a careful reinvestigation of the Friedel-Crafts procedure described by Späth[6] for the preparation of ketone III. Yields of 80% are now possible using the improved methods of Gensler *et al.* See also, D. C. Ayres and R. C. Denney, *J. Chem. Soc.*, 4506 (1961).

(10) W. J. Gensler and J. E. Stouffer, *J. Org. Chem.*, **23**, 908 (1958).

(11) Although it was found possible to prepare 3,4-methylenedioxyphenylmagnesium bromide employing an entrainment technique [*cf.* D. E. Pearson, D. Cowan, and J. D. Beckler, *J. Org. Chem.*, **24**, 504 (1959)] conversion of 3,4-methylenedioxybromobenzene to this reagent was less satisfactory. Evidence in support of the Grignard intermediate was obtained by carbonation, which gave rise to a 36% yield of piperonylic acid. Previous attempts to prepare 3,4-methylenedioxyphenylmagnesium bromide gave unsatisfactory results (ref. 10, and literature cited therein).

(12) A similar procedure has been used for preparation of several aliphatic ketones: P. T. Izzo and S. R. Safir, *J. Org. Chem.*, **24**, 701 (1959).

amides readily rearranged in the presence of water to the esters represented by structures VIIIc and IXc.[13] The ester formulations were supported by elemental analyses and the appearance of new absorption in the infrared spectrum of each product in the vicinity of 1710



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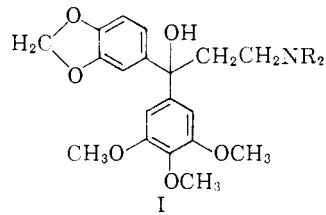

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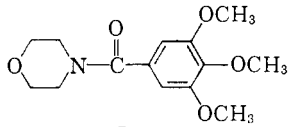

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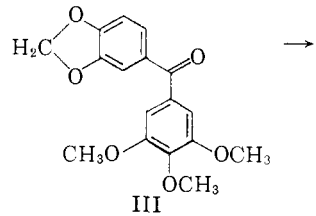
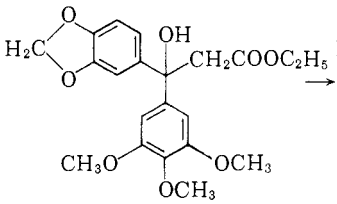


III



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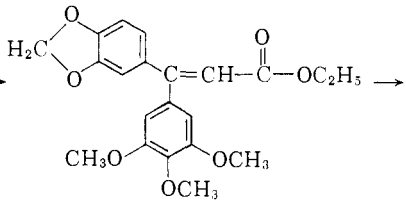
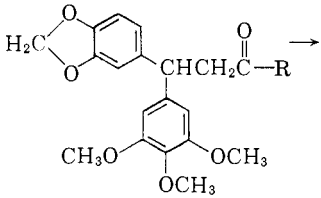
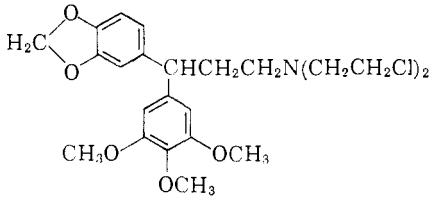


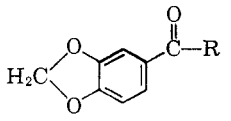
V



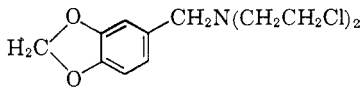
VIa, R = OC_2H_5
b, R = OH
c, R = Cl
d, R = $N(CH_2CH_2Cl)_2$



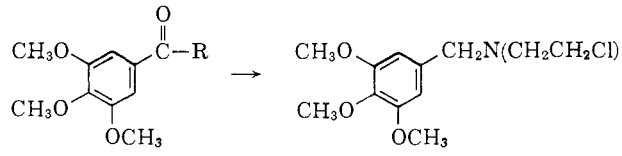
VII



VIIIa, R = OH
b, R = $N(CH_2CH_2Cl)_2$
c, R = $OCH_2CH_2NHCH_2CH_2Cl \cdot HCl$



X



IXa, R = OH
b, R = $N(CH_2CH_2Cl)_2$
c, R = $OCH_2CH_2NHCH_2CH_2Cl \cdot HCl$

XI

cm.$^{-1}$. Absorption assignable to an amide carbonyl group was no longer observed. The lithium aluminum hydride–aluminum chloride reagent[14] was selected for amide → amine reduction in order to prevent hydrogenolysis of the carbon–chlorine bonds. By this means, amides VIIIb and IXb were easily reduced to their respective nitrogen mustard derivatives X and XI.

After saponifying ethyl propionate derivative VIa, the resulting acid (VIb) was converted to the required N-bis(2-chloroethyl)amine VII by adopting the general procedure found suitable with model acids VIII and IX.

A significant increase (5–12 days) in the survival-time of rats with Dunning leukemia[15] was observed following treatment with 2-(2-chloroethylamino)ethyl 3,4,5-trimethoxybenzoate hydrochloride (IXc), N-bis(2-chloroethyl)-3,4,5-trimethoxybenzamide (IXb), or N-bis(2-chloroethyl)-3,4,5-trimethoxybenzylamine (XI) hydrochloride. In the same test system, animals treated subcutaneously with N-bis(2-chloroethyl)-3,4-methylenedioxybenzylamine (X) hydrochloride (5 mg./kg. in oil) were found to survive the entire observation period and were considered "cures."[15] Biological evaluations of these substances and bis(2-chloroethyl)amine derivatives VId, VIIp, VIIIb and VIIIc are being performed by the Cancer Chemotherapy National Service Center.

(13) Analogous rearrangements have been observed: *cf.* E. R. H. Jones and W. Wilson, *J. Chem. Soc.*, 547 (1949); Y. Sakurai and M. Izumi, *Chem. Pharm. Bull.* (*Tokyo*), **1**, 298 (1953); I. Aiko and K. Saruwatari, *Yakugaku Zasshi*, **75**, 418 (1955); and the interesting study reported by W. C. J. Ross and J. G. Wilson, *J. Chem. Soc.*, 3616 (1959).

(14) R. F. Nystrom, *J. Am. Chem. Soc.*, **81**, 610 (1959). After this part of the present study was completed, reduction of several N-bis(2-chloroethyl)amides using lithium aluminum hydride was described by Y. Kuwada, *Chem. Pharm. Bull.* (*Tokyo*), **8**, 77 (1960).

(15) The biological experiments were performed using tumor fragments implanted subcutaneously in inbred albino Fischer F-344 rats. Treatment was begun 24 hr. after implantation and was continued on a schedule of once daily for 12 days. At present, a three-day extension of median life span for treated animals is considered significant and rats surviving the entire observation period (*ca.* 30 days) without development of tumors are termed "cures". We wish to thank Dr. Joseph Leiter, Cancer Chemotherapy National Service Center, National Institutes of Health, for providing this information.

Experimental[16]

Conversion of 3,4-Methylenedioxybromobenzene to Piperonylic Acid (VIIIa).—To a mixture of magnesium (1.4 g.) and 3,4-methylenedioxybromobenzene (5 g.),[10] under nitrogen, was added ethylene bromide (4.8 g.) in 50 ml. of dry ether. The addition of ethylene bromide was carried out over a 3-hr. period with continuous stirring. Before pouring the reaction mixture over 10 g. of Dry Ice, it was heated an additional 4 hr. at reflux. Following evaporation of excess carbon dioxide, the resulting mixture was acidified with dil. hydrochloric acid. The precipitated acid was collected, reprecipitated from 5% sodium hydroxide solution with dil. hydrochloric acid and recrystallized from ethanol; yield, 1.5 g. (36%), m.p. 229–230°. The product (VIIIa) was identical (mixture melting point and infrared comparison) with an authentic specimen (m.p. 226–227°, lit.[17] m.p. 228–229°) prepared in 93% yield by silver oxide oxidation of piperonal.

N-(3,4,5-Trimethoxybenzoyl)-morpholine (II).—Morpholine (9.9 g.) was slowly added to a solution composed of benzene (200 ml.) and 3,4,5-trimethoxybenzoyl chloride (13 g.).[18] The reaction began immediately and was accompanied by evolution of heat and precipitation of morpholine hydrochloride (6.9 g., 98%). After a 3-hr. period, the benzene solution was separated from the solid hydrochloride and concentrated to a crystalline residue which recrystallized from methanol as colorless crystals (14.1 g., 89%); m.p. 120–121°, $\nu_{max}^{CHCl_3}$ 1625 cm.$^{-1}$.

Anal. Calcd. for $C_{14}H_{19}NO_5$: C, 59.14; H, 6.85. Found: C, 59.60; H, 6.76.

3,4-Methylenedioxy-3′,4′,5′-trimethoxybenzophenone (III).—Commercial[19] 1.6 *N* *n*-butyllithium (13.9 ml., 1:2 pentane–heptane as solvent) was added over a 4-min. period to a solution (cooled to −75°) prepared from 3,4-methylenedioxybromobenzene (4.4 g.) and dry tetrahydrofuran (50 ml.). Following a 3-min. reaction period, a solution of N-(3,4,5-trimethoxybenzoyl)morpholine (II, 6.2 g.) in 200 ml. of dry tetrahydrofuran was added all at once. The reaction was carried out under a nitrogen atmosphere and stirred for 1 hr. with continued cooling and for an additional 3 hr. without the cold-bath. Approximately 20 hr. later, the solvent was removed *in vacuo* at 35–40° and the residue diluted with ammonium chloride solution. The solid product was collected and recrystallized from methanol to afford 4.8 g. (69%) of ketone melting at 124–125°, $\nu_{max}^{CHCl_3}$ 1655 cm.$^{-1}$. This procedure consistently gave 62–69% yields of ketone III after one recrystallization.

Anal. Calcd. for $C_{17}H_{16}O_6$: C, 64.55; H, 5.10. Found: C, 64.51; H, 5.22.

The ketone was found (mixture melting point and infrared spectral comparison) to be identical with an authentic sample (m.p. 124–125.5°)[20] of 3,4-methylenedioxy-3′,4′,5′-trimethoxybenzophenone (III).

Preparation of *ca.* 100 g. quantities of this ketone (III) was conveniently ac-

(16) Tetrahydrofuran was purified by initial drying over sodium and redistillation from lithium aluminum hydride. Melting points were determined in a silicone oil bath using open Kimble glass capillary tubes and are uncorrected. Microanalyses were provided by Dr. A. Bernhardt, Mülheim (Ruhr), Germany, and Drs. Weiler and Strauss, Oxford, England, and the infrared spectra were recorded by Dr. R. A. Hill, Department of Chemistry, University of Maine. We also wish to acknowledge assistance from Philip E. Douville during an early phase of the experimental work.

(17) G. Ciamician and P. Silber, *Ber.*, **23**, 1159 (1890).

(18) K. H. Slotta and H. Heller, *ibid.*, **63**, 3029 (1930).

(19) Foote Mineral Company.

(20) We are grateful to Dr. W. J. Gensler for providing a sample of this substance.

complished using the modified Friedel–Crafts procedure of Gensler and coworkers.[9,21]

Ethyl β-Hydroxy-β-(3,4-methylenedioxyphenyl)-β-(3,4,5-trimethoxyphenyl)-propionate (IV).—In a typical experiment, a solution of ethyl bromoacetate (72 g.) and 3,4-methylenedioxy-3′,4′,5′-trimethoxybenzophenone (45.6 g.) in dry benzene (340 ml.) was added over a 1-hr. period to a stirred suspension of zinc dust (28.4 g.)[22] in refluxing benzene (340 ml.). After an additional 2 hr. at reflux, the mixture was cooled and diluted with ammonium chloride solution. The benzene portion was separated, dried over magnesium sulfate and concentrated to a viscous reddish brown residue (65 g.). Recrystallization from methanol (Norit A) led to 45.3 g. of colorless crystals, m.p. 80–82°. A second crop weighed 5.8 g. and melted at 80–83°. Two recrystallizations from the same solvent gave 45.5 g. (78%), m.p. 83–84°, of pure alcohol (IV), $\nu_{max}^{CHCl_3}$ 3450 and 1710 cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{24}O_8$: C, 62.37; H, 5.98. Found: C, 62.35; H, 5.92.

On several occasions, partial dehydration of the Reformatsky product was observed. In these experiments, the mixture of alcohol IV and olefin V was either separated by fractional recrystallization from methanol or, more conveniently, dehydrated as described in the following experiment.

Ethyl β-(3,4-methylenedioxyphenyl)-β-(3,4,5-trimethoxyphenyl)-acrylate (V).—A mixture of tertiary alcohol IV (30 g.), potassium hydrogen sulfate (40 g.) and 250 ml. of acetic anhydride was heated at steam bath temperature for 1.5 hr. The residue obtained, following partial removal of solvent, was diluted with water and the resulting colorless precipitate (28 g., 98%) collected, m.p. 112–113°. A pure sample recrystallized from methanol as colorless needles melting at 119.5–120.5°, ν_{max}^{KBr} 1718, 1619, 1580, 1123 and 1038 cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{22}O_7$: C, 65.27; H, 5.74. Found: C, 64.87; H, 5.71.

Ethyl β-(3,4-Methylenedioxyphenyl)-β-(3,4,5-trimethoxyphenyl)-propionate (VIa).—A suspension of 10% palladium–charcoal catalyst (1.3 g.) in an ethanol (350 ml.) solution of olefin V (5.0 g.) was hydrogenated at room temperature (employing a slightly positive hydrogen pressure) over a 10-min. period. Filtration, followed by evaporation of solvent and recrystallization of the residue from methanol, yielded 4.6 g. (92%) of product (VIa) melting at 62–67°. Five additional recrystallizations from the same solvent gave a pure sample as colorless crystals; m.p. 67–68°, $\nu_{max}^{CHCl_3}$ 1720, 1590, 1130 and 1040 cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{24}O_7$: C, 64.93; H, 6.23. Found: C, 64.96; H, 6.13.

β-(3,4-Methylenedioxyphenyl)-β-(3,4,5-trimethoxyphenyl)-propionic acid (VIb).—The combined ester (VIa, 30 g.) from a number of hydrogenation experiments was heated for 1.5 hr. in a refluxing solution composed of sodium hydroxide (30 g.) and 3:1 ethanol–water (600 ml.). Concentration of the solvent, dilution with water and neutralization with 5% hydrochloric acid afforded an almost quantitative yield (27 g.) of acidic product (VIb). Four recrystallizations from

(21) Palladium–charcoal catalyzed decarbonylation of piperonal provided an efficient source of 3,4-methylenedioxybenzene: F. Dallacker and R. Binsack, *Monatsh. Chem.*, **92**, 492 (1961). During purification of 3,4-methylenedioxybenzene, by distillation through a 120 cm. vacuum jacketed column packed with glass helices, boiling points of 42°/4 mm. and 53°(7 mm.) were recorded. Messrs. M. R. Chamberland and R. E. Hagman provided the boiling point data.

(22) Commercial zinc dust was treated with 2% hydrochloric acid and then washed successively with water, alcohol and ether. Before using the zinc, a final drying period at 100° *in vacuo* was employed. Several milligrams of iodine was added with the first portion of ethyl bromoacetate.

methanol gave pure colorless crystals; m.p. 154–156°, ν_{max}^{KBr} 3400 (broad), 1705, 1590, 1130, and 1040 cm.$^{-1}$.

Anal. Calcd. for $C_{19}H_{20}O_7$: C, 63.33; H, 5.59. Found: C, 62.83; H, 5.70.

N-Bis(2-chloroethyl)-β-(3,4-methylenedioxyphenyl)-β-(3,4,5-trimethoxyphenyl)-propionamide (VId).—Oxalyl chloride (15 ml.) was added dropwise to cold (ice-bath) β-(3,4-methylenedioxyphenyl)-β-(3,4,5-trimethoxyphenyl)-propionic acid (VIb, 5.0 g.) under nitrogen. The mixture was allowed to stand (*ca.* 45 min.) at ice-bath temperature until nearly all the solid phase dissolved. The resulting orange solution was filtered and diluted with dry petroleum ether. Cooling was continued 2 hr. before slowly concentrating (*in vacuo* at 0–5°) the reaction mixture. The crystalline *acid chloride* (VIc, 3.8 g.), m.p. 89–91°, which separated was collected and dried (1 hr.) in a desiccator. A variety of other techniques (*e.g.*, higher temperatures) were employed in early attempts to obtain this acid chloride and all were unsatisfactory.

A benzene (100 ml.) solution of the acid chloride (3.5 g.) was added (40 min.) with stirring to a cool (ice-bath) solution of N-bis(2-chloroethyl)amine (prepared from 10 g. of the hydrochloride derivative)[23] in dry benzene. Stirring and cooling were continued for 2 hr. before collecting precipitated N-bis(2-chloroethyl)amine hydrochloride. Partial removal of solvent (*in vacuo*) and addition of petroleum ether followed by treatment with Norit-A and cooling gave 4.0 g. (87%) of *amide VId* melting at 96–99°. Three recrystallizations from the same solvent mixture led to a pure specimen as colorless crystals; m.p. 103–105°, ν_{max}^{KBr} 1640, 1580, 1130, and 1045 cm.$^{-1}$.

Anal. Calcd. for $C_{23}H_{27}Cl_2NO_6$: C, 57.03; H, 5.62; N, 2.89. Found: C, 57.26; H, 5.66; N, 2.74.

3-(3,4-Methylenedioxyphenyl)-3-(3,4,5-trimethoxyphenyl)-N-bis(2-chloroethyl)-propylamine (VII) hydrochloride.—A sample of acid chloride VIc prepared from 1.5 g. of the corresponding acid (VIb) was converted to N-bis(2-chloroethyl)amide VId by reaction with the free base from 1.7 g. of N-bis(2-chloroethyl)amine hydrochloride.[23] Preparation of the amide (VId) was carried out in benzene solution as described in the preceding experiment. After removal of N-bis(2-chloroethyl)amine hydrochloride (0.59 g.) and solvent from the reaction mixture, the residue was dissolved in dry tetrahydrofuran (30 ml.) and allowed to react with a mixture of aluminum chloride (0.38 g.) and lithium aluminum hydride (0.23 g.) in dry tetrahydrofuran (30 ml.). The reduction and subsequent isolation of crude amine (VII) hydrochloride was performed as illustrated for the synthesis of N-bis(2-chloroethyl)-3,4-methylenedioxybenzylamine (X) hydrochloride. The crude hydrochloride weighed 0.92 g. (44%) and melted at 178–183° with sintering at 172°. Seven recrystallizations from methanol yielded a pure sample as colorless crystals; m.p. 178–180°, ν_{max}^{KBr} 3400, 2300, 1585, 1130, 1040, 1000, and 940 cm.$^{-1}$.

Anal. Calcd. for $C_{23}H_{30}Cl_3NO_5$: C, 54.50; H, 5.97; Cl, 20.98; N, 2.76. Found: C, 54.42; H, 5.73; Cl, 20.95; N, 3.21.

N-Bis(2-chloroethyl)-3,4-methylenedioxybenzylamine (X) hydrochloride.—A mixture of piperonylic acid (13.7 g.) and oxalyl chloride was heated at reflux for 0.5 hr. Removal of solvent (*in vacuo*) followed by addition of dry benzene and reconcentration led to an oily residue which crystallized from benzene–petroleum

(23) K. Ward, *J. Am. Chem. Soc.*, **57**, 914 (1935). After drying over sodium sulfate, additional water was removed from a benzene solution of the amine by azeotropic distillation during *ca.* 20 min.

ether (60–90°). The acid chloride weighed 14 g. (93%) and melted at 78–79°.[24]

The free amine prepared from 25 g. of bis(2-chloroethyl)amine hydrochloride[23] in 100 ml. of dry benzene was treated over a 1-hr. period with a solution of piperonyl chloride (11 g., 0.06 mole) in 200 ml. of dry benzene. After adding the acid chloride, the mixture was heated at reflux for 2 hr. The bis(2-chloroethyl)amine hydrochloride which separated during the reaction weighed 10.3 g. (96%). A portion (10 g., 0.034 mole) of the crude amide (VIIIb) was isolated (by first removing the precipitated amine hydrochloride and then concentrating the filtrate to dryness) and dissolved in dry tetrahydrofuran (75 ml.). The resulting solution was slowly added over a 45-min. period to a cold (ice-bath) mixture of lithium aluminum hydride (1.3 g.) and aluminum chloride (4.7 g.) in 150 ml. of dry tetrahydrofuran. Stirring, cooling and a nitrogen atmosphere were maintained 1 additional hr. before treating the reaction mixture successively with acetone, moist ether and finally water. The solvent was removed *in vacuo* at 35–40° and the residue diluted with 150 ml. of 40% aqueous sodium hydroxide solution. The dry (magnesium sulfate) combined benzene extract of the reaction mixture was treated with ether–hydrogen chloride; the amine (X) hydrochloride which precipitated weighed 7.2 g. (81%), m.p. 153–154°. Five recrystallizations from methanol–acetone afforded an analytical sample as colorless crystals; m.p. 160–161°,[25a] ν_{max}^{KBr} 2700 and 2500 (broad band over the 2700–2400 region) cm.$^{-1}$.

Anal. Calcd. for $C_{12}H_{16}Cl_3NO_2$: C, 46.10; H, 5.18; Cl, 34.02; N, 4.48. Found: C, 45.95; H, 5.32; Cl, 34.27; N, 4.50.

2-(2-Chloroethylamino)ethyl 3,4-methylenedioxybenzoate hydrochloride (VIIIc).—Concentrating a 4:1 ethanol–water (20 ml.) solution of the crude amide (VIIIb, 5.0 g.), obtained as described in the preceding experiment, followed by crystallization of the oily residue from acetone gave 4.8 g. (90%) of product (VIIIc) melting at 158–160°.[25a] Five crystallizations from acetone–methanol led to a pure specimen of the ester in the form of colorless crystals; m.p. 167–168°,[25a] ν_{max}^{KBr} 2850–2450 (broad) and 1715 cm.$^{-1}$.

Anal. Calcd. for $C_{12}H_{15}Cl_2NO_4$: C, 46.75; H, 4.91; N, 4.54. Found: C, 47.13; H, 4.99; N, 4.63.

N-Bis(2-chloroethyl)-3,4,5-trimethoxybenzamide (IXb).—The free amine (in 100 ml. of dry benzene) from bis(2-chloroethyl)amine hydrochloride (16 g.)[23] was allowed to react with 3,4,5-trimethoxybenzoyl chloride (10 g.)[18] in dry benzene (100 ml.) as described for preparation of 3,4-methylenedioxybenzamide VIIIb. After collecting precipitated bis(2-chloroethyl)amine hydrochloride (7 g.), the reaction mixture was concentrated (*in vacuo* at 45–60°) to an oily residue. Following an overnight period (room temperature) in dry benzene (10 ml.)–petroleum ether (20 ml.), the amide was collected; yield 13 g., m.p. 74–78°. Three recrystallizations from dry benzene–petroleum ether (60–90°) afforded pure colorless crystals; m.p. 85–86°, $\nu_{max}^{CHCl_3}$ 1630 cm.$^{-1}$.

Anal. Calcd. for $C_{14}H_{19}Cl_2NO_4$: C, 50.01; H, 5.70; Cl, 21.09; N, 4.17. Found: C, 49.85; H, 5.85; Cl, 21.25; N, 4.01.

2-(2-Chloroethylamino)ethyl 3,4,5-trimethoxybenzoate hydrochloride (IXc).—A solution of N-bis(2-chloroethyl)-3,4,5-trimethoxybenzamide (IXb, 2.5 g.) in 10 ml. of 4:1 ethanol-water was concentrated (*in vacuo*) to dryness and the residue

(24) Piperonyl chloride (m.p. 80°) was originally prepared employing thionyl chloride: G. Barger, *J. Chem. Soc.*, **93**, 563 (1908).

(25) Sample heating was initiated when the bath temperature reached (a) 150°, (b) 125°, (c) 165°, and (d) 170°.

crystallized from acetone. The resulting ester (IXc) weighed 2.0 g. (75%) and melted at 138–140°.[24b] Five additional recrystallizations from acetone gave an analytical sample; colorless crystals, m.p. 141–142°,[25b] $\nu_{max}^{CHCl_3}$ 2900–2450 (broad) and 1710 cm.$^{-1}$

Anal. Calcd. for $C_{14}H_{21}Cl_2NO_5$: C, 47.43; H, 5.98; Cl, 20.03; N, 3.95. Found: C, 47.41; H, 6.17; Cl, 19.60; N, 3.98.

N-Bis(2-chloroethyl)-3,4,5-trimethoxybenzylamine (XI) Hydrochloride.—Lithium aluminum hydride (1.3 g.)–aluminum chloride (4.7 g.) reduction of N-bis(2-chloroethyl)-3,4,5-trimethoxybenzamide (IXb, 11 g.) was carried out as illustrated for reduction of N-bis(2-chloroethyl)-3,4-methylenedioxybenzamide (VIIIb). However, in this example, chloroform was substituted for benzene in the isolation procedure. The crude amine (XI) hydrochloride weighed 6.4 g. (54%) and melted at 172–175°.[25c] Five recrystallizations from methanol–acetone yielded a pure sample as colorless crystals; m.p. 181–182°,[24d] ν_{max}^{KBr} 2700, 2650 and 2450 (broad band over the 2900–2450 region) cm.$^{-1}$.

Anal. Calcd. for $C_{14}H_{22}Cl_3NO_3$: C, 46.88; H, 6.18; Cl, 29.65; N, 3.90. Found: C, 46.86; H, 6.16; Cl, 29.32; N, 3.77.

The Syntheses and Pharmacological Activities of Amide, Sulfamide, and Urea Derivatives of 4,6-Diaminopyrimidines

JOHN J. TRAVERSO, E. BROWN ROBBINS, AND CALVERT W. WHITEHEAD

The Lilly Research Laboratories, Indianapolis, Indiana

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Primary and secondary amino groups of 4,6-diaminopyrimidines were converted to amide or amide-like derivatives. Their diuretic activities were determined in dogs. The amides were generally less active than the parent amines with the exception of N-(4-amino-6-pyrimidyl)acetamide. The latter compound was further studied for pathological and blood pressure effects because of its greatly increased potency.

A series of 6-alkylamino-4-amino-pyrimidines and 4-amino-6-arylaminopyrimidines was shown[1] previously to have diuretic activity. In the present work the diaminopyrimidines were converted to amide, sulfonamide and urea derivatives and their biological activities compared to those of the parent amines. The appropriate derivatives were prepared by the usual reactions with acid anhydrides, acid chlorides and isocyanates. When both primary and secondary amine groups were present the primary amine reacted with the first equiva-

(1) C. W. Whitehead and J. J. Traverso, *J. Am. Chem. Soc.*, **80**, 2185 (1958).