g.) which separated as needles from aqueous solution. The picrate had no sharp m.p. but charred at elevated temperatures.

Anal. Calcd. for $C_{11}H_{14}N_2O \cdot C_6H_3N_3O_7$: C, 48.69; H, 4.09. Found: C, 48.91; H, 4.08.

NEW YORK, N. Y.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF PENNSYLVANIA]

Metabolite Analogs. III. Preparation of Some Benzimidazoles with Substituents on the 4(7)- and 6(5)-Positions[1]

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Benzimidazoles with chloro, nitro and amino groups on the 4(7)- and 6(5)-positions, their hydrochlorides and picrates, have been prepared for testing as potential metabolite (purine, vitamin B_{12}, folic acid) inhibitors.

The interference with purine utilization by benzimidazoles was first reported by Woolley.[2] His findings indicated that 5(6)-aminobenzimidazole is no more active in this respect than the parent compound. The information concerning the inhibitory activity of 4(7)-aminobenzimidazole, prepared by van der Want,[3] is incomplete. Benzimidazoles designed as guanine analogs have been shown to inhibit various guanine-sensitive test systems.[4] Likewise, the inhibition of virus multiplication[5] and of vitamin B_{12} utilization[6] by benzimidazoles have been reported. In view of the antagonistic properties induced in the purine series by modifying the substituents on the 2- and 6-positions, the testing of benzimidazoles containing polar substituents on the equivalent positions, especially those which result in compounds resembling the natural metabolites, appeared to be of interest. In addition to the analogs already reported, a number of derivatives containing amino, nitro, chloro, mercapto, sulfonamido and sulfonic acid groupings on the 4- and 6-positions have been prepared. In most cases, position isomers have been made for use in a study of the effect of grouping and position on the activity. These compounds have been subjected to screening tests, including vitamin B_{12} and purine inhibition. The preparation of the sulfur-containing derivatives as well as the results of the biological studies will be given in later communications.

Picramide served as the starting material for a number of the 4,6-disubstituted benzimidazoles. The complete reduction to 1,2,3,5-tetraaminobenzene, using tin and hydrochloric acid, has been described.[7] Partial reduction with ammonium sulfide results in a mixture of 1,2,3-triamino-5-nitrobenzene and 1,2-diamino-3,5-dinitrobenzene. The procedure of Horner, Schwenk and Junghanns,[8] using methyl acetate as solvent, gives predominately the dinitro compound. Adaptation of the method of Nietzki and Hagenbach (ref. 7) resulted in a more favorable yield of the triamine.

The conversion of 1,2,3,5-tetraaminobenzene to 4,6-diaminobenzimidazole was readily accomplished using Phillips' conditions.[9] Indeed, this appeared to be the method of choice for all of the cyclizations in this series except those containing an acid-labile grouping. This applies also to the preparation of 4-amino- and 4-nitrobenzimidazole previously obtained by treating the corresponding diamine with 85% formic acid.[3]

Replacement of the amino group in 4-amino-6-nitrobenzimidazole *via* the diazonium salt afforded a route to several of the sulfur-containing analogs.[10] The diazonium chloride was readily decomposed by copper to 4-chloro-6-nitrobenzimidazole. For the reduction of the nitro group to give 4-chloro-6-aminobenzimidazole, it was found that stannous chloride and hydrochloric acid gave more satisfactory results than hydrogenation over palladium catalysts. The material from catalytic hydrogenation usually contained impurities which readily underwent decomposition to colored products. These could be removed only with considerable difficulty. This was also true of the reduction of the other nitro compounds in this group.

Preparation of the reversed isomers of the latter two compounds (*i.e.*, 4-nitro-6-chloro- and 4-amino-6-chlorobenzimidazole) was accomplished by using 3-nitro-5-chloro-*o*-phenylenediamine as the starting material. The Phillips reaction was used for closing the ring and stannous chloride for reduction of the nitro group. Both of the chloroaminobenzimidazoles could be converted to 4,6-dichlorobenzimidazole by diazotization followed by decomposition of the diazonium salt with copper or cuprous chloride. This compound has been mentioned by Tamm, Folkers, Shunk and Horsfall[5d] and also by Davies, Mamalis, Petrow and Sturgeon,[11] but the preparative data are very incomplete.

The preparation of 4-nitro-6-aminobenzimidazole for comparison with the 4-amino-6-nitro isomer

(1) This investigation was supported by a research grant (C-2189) from the National Cancer Institute of the National Institutes of Health, Public Health Service.

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

(3) G. M. van der Want, *Rec. trav. chim.*, **67**, 45 (1948).

(4) H. B. Gillespie, M. Engleman and S. Graff, THIS JOURNAL, **76**, 3531 (1954).

(5) (a) R. L. Thompson, *J. Immunol.*, **55**, 345 (1947); (b) G. C. Brown, *ibid.*, **69**, 441 (1952); (c) I. Tamm, K. Folkers, C. H. Shunk, D. Heyl and F. Horsfall, *J. Expt. Med.*, **98**, 245 (1953); (d) I. Tamm, K. Folkers, C. H. Shunk and F. Horsfall, *ibid.*, **99**, 227 (1954).

(6) F. Weygand and A. Wacker, *Z. Naturforsch.*, **5**, 227 (1950).

(7) R. Nietzki and H. Hagenbach, *Ber.*, **30**, 539 (1897).

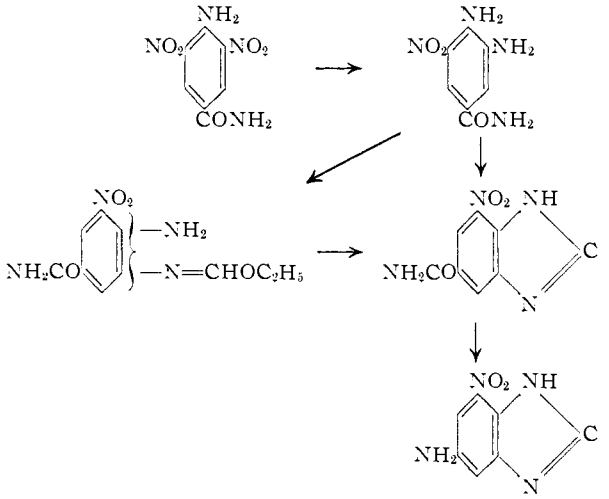
(8) L. Horner, U. Schwenk and E. Junghanns, *Ann.*, **579**, 212 (1953).

(9) M. A. Phillips, *J. Chem. Soc.*, 2393 (1928).

(10) J. R. E. Hoover and A. R. Day, THIS JOURNAL, in press.

(11) M. T. Davies, P. Mamalis, V. Petrow and B. Sturgeon, *J. Pharm. Pharmacol.*, **3**, 420 (1951). These workers merely state that the compound was prepared from the corresponding diamine, listing its melting point as 225–226° and its nitrogen analysis as 14.9%.

utilized the Hoffman reaction[12] in the sequence given below.



Ammonium sulfide reduction gave a good yield of 3-nitro-4,5-diaminobenzamide. The ring could be closed, with the formation of a significant amount of colored material, by refluxing with 98% formic acid. A much smoother conversion utilized ethyl orthoformate which resulted in an easily separated mixture of the desired 4-nitro-6-carboxamidobenzimidazole and the intermediate ethyl isoformanilide. The latter readily was converted to the benzimidazole by heating in the dry state from 150 to 200°.

The nitro-substituted *o*-phenylenediamines listed here crystallized as stable, bright red needles. The corresponding nitroamino- and nitrochlorobenzimidazoles were obtained as stable, light yellow needles (as the free bases), with the exception of 4-nitro-6-aminobenzimidazole which is bright red. The nitroamino derivatives gave stable hydrochlorides while those of the nitrochlorobenzimidazoles lost hydrogen chloride readily to regenerate the free bases on treatment with heat or water. The diamino- and aminochlorobenzimidazoles were isolated as their relatively stable mono- or dihydrochlorides. These compounds, when converted to their free bases, displayed a pronounced tendency to form highly colored oxidation products. Acetylation of 4,6-diaminobenzimidazole resulted in the stable diacetyl derivative which was isolated as its hydrochloride monohydrate. The two nitroaminobenzimidazoles responded differently toward acetic anhydride and sodium acetate. The 4-nitro-6-amino derivative was acetylated at the amino group only while both the amino group and the ring nitrogen atom were acetylated in 4-amino-6-nitrobenzimidazole. The ring acetyl group was readily removed from the latter by treatment with dilute alkali. All of the benzimidazoles formed yellow to orange picrates in aqueous or alcoholic solution.

Experimental[13]

1,2,3,5-Tetraaminobenzene.—The procedure of Nietzki and Hagenbach,[7] using tin and hydrochloric acid, was followed. No yield was reported by these workers. Following their directions, 95% yields of 1,2,3,5-tetraaminobenzene trihydrochloride monohydrate were regularly obtained.

1,2,3-Triamino-5-nitrobenzene.—The experimental data concerning this reduction are very meager. Consequently, the preparation is described here. Picramide (30 g.) was added with stirring to 500 ml. of 2 *N* ammonium hydroxide previously saturated with hydrogen sulfide. The deep red reaction mixture was heated on the steam-bath for 1 hour with the introduction of additional hydrogen sulfide during the first 30 minutes. The precipitate was removed from the cooled reaction mixture, washed with water and evaporated with 100 ml. of 6 *N* hydrochloric acid (steam-bath). The residue was twice extracted with 100-ml. portions of hot water and, after decolorization with Norit A, the aqueous extracts were treated with an equal volume of concentrated hydrochloric acid. The resulting light yellow needles were washed with concentrated hydrochloric acid, absolute alcohol and ether; yield of the trihydrochloride, 15.0 g. (41%), m.p., approximately 270° with decomposition. The free base, obtained by neutralization with ammonium hydroxide, crystallized from alcohol as bright red needles melting at 269–270°.

4,6-Diaminobenzimidazole Dihydrochloride.—Formic acid (3.75 ml., 98–100%) was added to 26.50 g. (0.1 mole) of freshly prepared 1,2,3,5-tetraaminobenzene trihydrochloride monohydrate in 50 ml. of 2 *N* hydrochloric acid and the mixture was heated on the steam-bath for one hour. Two volumes of ethanol and three volumes of ether were added to the cooled reaction mixture. Cooling overnight gave 17.9 g. (81%) of white needles which decomposed at 335°. Recrystallization from 1 *N* hydrochloric acid by adding an excess of absolute alcohol and ether (1:3) resulted in 16.0 g. (73%) of material with the same decomposition point.

Anal. Calcd. for $C_7H_{10}N_4Cl_2$: C, 38.02; H, 4.56; N, 25.34. Found: C, 38.12; H, 4.65; N, 25.27.

Picrate.—The picrate, prepared by passing an aqueous solution of the dihydrochloride through Amberlite (IRA 400) and immediately adding an ethanolic solution of picric acid, was purified by dissolving it repeatedly in methyl cellosolve and precipitating it with water. The final dark orange product became deep red on drying at 100° and decomposed at 231–232°.

Anal. Calcd. for $C_{13}H_{11}N_7O_7$: C, 41.38; H, 2.94; N, 25.99. Found: C, 41.16; H, 3.13; N, 25.96.

4,6-Diacetamidobenzimidazole.—A few drops of concentrated sulfuric acid was added to a mixture of 2.21 g. (0.01 mole) of 4,6-diaminobenzimidazole dihydrochloride in 10 ml. of acetic anhydride. After one hour at room temperature, the mixture was poured into 100 ml. of ice-water and allowed to stand overnight. A 67% yield (1.56 g.) of the white hydrochloride monohydrate, melting at 349–352°, separated from the cooled solution. Recrystallization from water gave 1.31 g. of fine white needles melting at 350–352° with decomposition.

Anal. Calcd. for $C_{11}H_{15}N_4O_3Cl$: C, 46.08; H, 5.27; N, 19.54. Found: C, 47.72; H, 5.39; N, 19.50.

The free base was obtained by neutralizing the hydrochloride with sodium bicarbonate solution or dilute ammonium hydroxide giving white crystals which sintered at 159–161° and melted at 258–260°.

4-Amino-6-nitrobenzimidazole Hydrochloride.—This compound was prepared by a procedure similar to that employed by Bahner, Rutter and Rives.[14] The light yellow monohydrochloride (m.p., 310° dec.) was isolated in a 96% yield by cooling the reaction mixture.

Anal. Calcd. for $C_7H_7N_4O_2Cl$: C, 39.17; H, 3.29; N, 26.11; Cl, 16.52. Found: C, 39.18; H, 3.18; N, 26.21; Cl, 16.42.

Treatment of a solution of the hydrochloride with dilute ammonium hydroxide gave the bright orange free base melting at 247–248°.

Picrate.—The monopicrate, prepared by treating the free base with an alcoholic solution of picric acid, decomposed at 288°.

Anal. Calcd. for $C_{13}H_9N_7O_9$: C, 38.34; H, 2.23; N, 24.08. Found: C, 38.47; H, 2.39; N, 23.81.

(12) E. S. Wallis and J. F. Lane, "Organic Reactions," Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1946, p. 267.

(13) The microanalyses were performed by Dr. Harry W. Galbraith, Knoxville, Tenn.

(14) C. T. Bahner, H. A. Rutter and L. M. Rives, THIS JOURNAL, **74**, 3689 (1952). These workers report the melting point of the free base as 240–241°.

4-Acetamido-6-nitrobenzimidazole.—A suspension of 5.34 g. (0.03 mole) of 4-amino-6-nitrobenzimidazole and 6 g. of sodium acetate in 30 ml. of acetic anhydride was heated on the steam-bath for 10 minutes and, after cooling, was poured into 120 ml. of ice-water. Yellow crystals of 1-acetyl-4(or 7)-acetamido-6(or 5)-nitrobenzimidazole, melting at 287–289°, separated. Purification was accomplished by solution in warm dimethylformamide followed by the addition of an equal volume of water, giving 5.44 g. (69%) of light yellow needles with the same melting point.

Anal. Calcd. for $C_{11}H_{10}N_4O_4$: C, 50.39; H, 3.84; N, 21.37. Found: C, 50.35; H, 4.04; N, 21.49.

This material was insoluble in cold 2 *N* sodium hydroxide. On warming 2.62 g. (0.01 mole) briefly to 50° with 50 ml. of 2 *N* sodium hydroxide, complete solution was effected with the loss of one acetyl group. Neutralization with hydrochloric acid gave yellow needles (1.95 g., 89%) melting at 270–273°. This was readily soluble in cold 2 *N* sodium hydroxide and did not give a positive test for a diazotizable amino group. It was dissolved in dimethylformamide and reprecipitated by adding water; yield 1.55 g., m.p. 275–277°.

4-Chloro-6-nitrobenzimidazole.—A suspension of 21.46 g. (0.1 mole) of 4-amino-6-nitrobenzimidazole in 200 ml. of concentrated hydrochloric acid was cooled to 0° and diazotized by the dropwise addition of 6.90 g. of sodium nitrite in 20 ml. of water. The resulting solution was poured over 2 g. of copper powder and, after the evolution of nitrogen had subsided, the reaction mixture was warmed to 50°. The solution was diluted with two volumes of water and neutralized with ammonium hydroxide. The resulting brown copper salt was dissolved in warm 2 *N* hydrochloric acid and decomposed with hydrogen sulfide. Neutralization of the filtrate with sodium carbonate resulted in 15.9 g. of dark yellow crystals. The dried material was extracted in a Soxhlet apparatus with ethyl acetate giving, after evaporation of the solvent, 11.40 g. (58%) of yellow crystals melting at 196–200°. Recrystallization from 50% alcohol gave 7.12 g. (36%) with a melting point of 222–225°.

Anal. Calcd. for $C_7H_4N_3O_2Cl$: C, 42.55; H, 2.04; N, 21.26. Found: C, 42.53; H, 2.04; N, 21.34.

Hydrochloride and Picrate.—The picrate crystallized from alcoholic solution as yellow plates melting at 214–215°.

Anal. Calcd. for $C_{13}H_7N_6O_9Cl$: C, 36.59; H, 1.65; N, 19.70. Found: C, 36.78; H, 1.74; N, 19.58.

The hydrochloride, obtained by recrystallizing the free base from 2 *N* hydrochloric acid, separated as white needles which lost hydrogen chloride readily on treating it with either heat or water. The material had an indefinite melting point and could not be obtained in an analytically pure condition.

4-Chloro-6-aminobenzimidazole Hydrochloride.—4-Chloro-6-nitrobenzimidazole (1.97 g., 0.01 mole) was added gradually to a stirred solution of 6 g. (5.70 g. is 0.03 mole) of anhydrous stannous chloride in 20 ml. of concentrated hydrochloric acid. The reaction mixture was heated on the steam-bath for 15 minutes and then cooled. The white tin double salt (decomposition point 309°), which separated during the addition of the nitro compound, was removed, dissolved in 75 ml. of alcohol containing several ml. of 6 *N* hydrochloric acid and decomposed with hydrogen sulfide. An equal volume of ether was added to the filtrate giving 1.01 g. (50%) of white needles, m.p., 292–294°, with decomposition. This was purified by dissolving it in 95% alcohol and adding an equal volume of ether, m.p. 294–296° with decomposition. When dried at room temperature, the material analyzed as the hydrochloride monohydrate.

Anal. Calcd. for $C_7H_9N_3OCl_2$: C, 37.85; H, 4.08; N, 18.92. Found: C, 37.62; H, 4.11; N, 18.75.

Picrate.—The picrate crystallized from aqueous solution as yellow needles melting at 235–238°. After several recrystallizations from water, the melting point was 247–249°.

Anal. Calcd. for $C_{13}H_9N_6O_7Cl$: C, 39.36; H, 2.29; N, 21.18. Found: C, 39.47; H, 2.34; N, 21.38.

4-Nitro-6-chlorobenzimidazole.—Freshly reprecipitated 3-nitro-5-chloro-*o*-phenylenediamine (18.76 g., 0.1 mole) was heated for two hours with 5.7 ml. of formic acid in 100 ml. of 4 *N* hydrochloric acid. Neutralization of the reaction mixture with concentrated ammonium hydroxide gave 18.0 g. (91%) of 4-nitro-6-chlorobenzimidazole, melting at 228–230°. Reprecipitation *via* the sodium salt, followed by recrystallization from alcohol, gave yellow needles which melted at 229–230°.

Anal. Calcd. for $C_7H_4N_3O_2Cl$: C, 42.55; H, 2.04; N, 21.26; Cl, 17.95. Found: C, 42.64; H, 2.17; N, 21.28; Cl, 17.79.

Picrate and Hydrochloride.—The monohydrochloride was prepared by recrystallizing the free base from 2 *N* hydrochloric acid and washing the resulting salt with dioxane followed by ether. This compound decomposed with the loss of hydrogen chloride at 247–249° and hydrolyzed to the free base on contact with water.

Anal. Calcd. for $C_7H_5N_3O_2Cl_2$: C, 35.91; H, 2.15; N, 17.96. Found: C, 35.89; H, 2.23; N, 17.83.

The picrate crystallized from alcoholic solution with a melting point of 195–196°.

Anal. Calcd. for $C_{13}H_7N_6O_9Cl$: C, 36.59; H, 1.65; N, 19.70. Found: C, 36.45; H, 1.62; N, 19.74.

4-Amino-6-chlorobenzimidazole Hydrochloride.—The procedure for the reduction of the 4-chloro-6-nitro derivative was applied to 4-nitro-6-chlorobenzimidazole (9.88 g., 0.05 mole), using 29 g. of anhydrous stannous chloride and 50 ml. of concentrated hydrochloric acid. In contrast to 4-chloro-6-aminobenzimidazole, white crystals (9.6 g., 94%) of 4-amino-6-chlorobenzimidazole hydrochloride separated directly from the reaction mixture. The crude product, and a sample purified by several recrystallizations from dilute hydrochloric acid, decomposed at 328–330°. The free base was obtained by neutralization of an aqueous solution of the hydrochloride with sodium hydroxide. It melted at 148–149° and displayed a pronounced tendency to oxidize on standing.

Anal. Calcd. for $C_7H_7N_3Cl_2$: C, 41.20; H, 3.46; N, 20.59; Cl, 34.75. Found: C, 41.40; H, 3.47; N, 20.56; Cl, 34.56.

Picrate.—The picrate was prepared from an aqueous solution of the hydrochloride and was recrystallized from alcohol in which it is slightly soluble. The yellow crystals decomposed between 283 and 290°, depending upon the rate of heating.

Anal. Calcd. for $C_{13}H_9N_6O_7Cl$: C, 39.35; H, 2.29; N, 21.19. Found: C, 39.18; H, 2.34; N, 21.26.

4,6-Dichlorobenzimidazole.—The hydrochloride of 4-amino-6-chlorobenzimidazole (4.08 g., 0.02 mole) was diazotized at 0° with 1.38 g. of sodium nitrite, 40 ml. of concentrated hydrochloric acid and 12 ml. of water. The solution of the diazonium salt was added to 20 ml. of concentrated hydrochloric acid containing 1.98 g. of cuprous chloride. After one hour, the reaction mixture was neutralized with ammonium hydroxide and the resulting pink copper salt of the benzimidazole was decomposed with hydrogen sulfide in dilute hydrochloric acid. The clear filtrate was neutralized with ammonium hydroxide, giving 2.08 g. (56%) of a light gray powder melting at 216–219°. This was dissolved in 2 *N* sodium hydroxide, decolorized with Norit A, made acid with hydrochloric acid, again decolorized and then neutralized with sodium bicarbonate. The resulting white material was dissolved in warm chloroform, a small amount of residue was removed, and the solvent was evaporated, giving 1.35 g., melting at 212–215°. Recrystallization from 50% alcohol gave fine white needles with a melting point of 222–224°. A sample for analysis was dried *in vacuo* at room temperature.

Anal. Calcd. for $C_7H_4N_2Cl_2$: C, 44.95; H, 2.16; N, 14.98. Found: C, 44.75; H, 2.12; N, 15.08.

Picrate.—The picrate was prepared by dissolving equimolar amounts of the benzimidazole and picric acid in a minimal amount of hot water and cooling. The yellow product melted at 217–219° after several recrystallizations from 25% alcohol. It underwent partial dissociation upon heating *in vacuo*. Samples dried at room temperature analyzed as the hemipicrate.

Anal. Calcd. for $C_{20}H_{11}N_7O_7Cl_4$: C, 39.82; H, 1.84; N, 16.26; Cl, 23.51. Found: C, 40.40; H, 1.88; N, 16.22; Cl, 24.20.

3,4-Diamino-5-nitrobenzamide.—A mixture of 22.60 g. (0.1 mole) of 3,5-dinitro-4-aminobenzamide[15] in 300 ml. of

(15) Prepared from *p*-chlorobenzoic acid by the procedures of (a) F. Ullman and N. Wostwesensky, *Ann.*, **366**, 92 (1909); (b) H. Lindemann and W. Wessel, *Ber.*, **58**, 1221 (1925).

2 *N* ammonium hydroxide saturated with hydrogen sulfide was stirred at room temperature for one hour and cooled to 5°. The bright red diamine (18.74 g., 95%), melting at 256–258°, was purified by dissolving it in dimethylformamide, followed by the addition of water to induce crystallization. This resulted in 17.84 g. of deep maroon plates with a melting point of 260–262°.

Anal. Calcd. for $C_7H_8N_4O_3$: C, 42.85; H, 4.11; N, 28.56. Found: C, 42.65; H, 4.06; N, 28.61.

4-Nitro-6-carboxamidobenzimidazole.—Ethyl orthoformate (200 ml.) containing 9.80 g. (0.05 mole) of 3,4-diamino-5-nitrobenzamide was refluxed for two hours. The orange residue, consisting of 4.50 g. of crude 4-nitro-6-carboxamidobenzimidazole (m.p., 300–305°, with decomposition), was removed. This was dissolved in dimethylformamide and precipitated by adding an ether–petroleum ether mixture (2:1) giving 4.10 g. of product with a decomposition point of 318–320°.

The ethyl orthoformate solution was cooled and treated with two volumes of petroleum ether whereupon 5.13 g. of 5-nitro-3(or 4)-amino-4(or 3)-(ethyl isoformamido)-benzamide was obtained. In the vicinity of 165° this compound is converted to the benzimidazole with or without melting, depending upon the rate of heating. It was purified for analysis by repeated recrystallization from ethyl orthoformate.

Anal. Calcd. for $C_{10}H_{12}N_4O_4$: C, 47.62; H, 4.80; N, 22.22. Found: C, 47.77; H, 4.92; N, 22.26.

The intermediate ethyl isoformanilide was converted to the benzimidazole by placing the 5.13 g. of material in an oil-bath at 150° and raising the temperature to 200° over a 30-minute period. The yield of 4-nitro-6-carboxamidobenzimidazole from this treatment was 4.67 g., m.p. 320–325°, giving a total yield of 8.77 g. (85%). A sample, purified for analysis by precipitation from dimethylformamide with a mixture of ether and petroleum ether (2:1), melted at 325°, with decomposition.

Anal. Calcd. for $C_8H_6N_4O_3$: C, 46.59; H, 2.93; N, 27.17. Found: C, 46.86; H, 3.01; N, 27.35.

4-Nitro-6-aminobenzimidazole.—Cold water was added with stirring to 4.12 g. (0.02 mole) of 4-nitro-6-carboxamidobenzimidazole in 40 ml. of 0.5 *N* alkaline sodium hypochloride solution[16] until the solid dissolved (final volume, approximately 200 ml.). After heating on the steam-bath for one hour, the solution was cooled and adjusted to neutrality. This was extracted with ethyl acetate (yellow solution) giving 1.02 g. (29%) of the red free base, m.p. 286–288°, on evaporating the solvent. This was reprecipitated from dilute hydrochloric acid solution with sodium bicarbonate. Further purification was accomplished by adding petroleum ether to an ethyl acetate solution of the material; final m.p., 292–294°.

Anal. Calcd. for $C_7H_6N_4O_2$: C, 47.19; H, 3.40; N, 31.45. Found: C, 47.34; H, 3.36; N, 31.52.

Hydrochloride and Picrate.—The red-orange hydrochloride, prepared by adding absolute alcohol to a solution of the free base in 2 *N* hydrochloric acid, melted at 330–332° with decomposition.

Anal. Calcd. for $C_7H_7N_4O_2Cl$: C, 39.17; H, 3.29; N, 26.11. Found: C, 39.31; H, 3.36; N, 26.25.

The picrate was prepared by dissolving the free base in hot aqueous picric acid solution (saturated at room temperature) and cooling. Recrystallization from water gave bright orange plates which melted at 231–233°.

Anal. Calcd. for $C_{13}H_9N_7O_7$: C, 38.34; H, 2.23; N, 24.08. Found: C, 38.52; H, 2.34; N, 24.23.

4-Nitro-6-acetamidobenzimidazole.—The procedure for acetylating 4-amino-6-nitrobenzimidazole was applied to the 4-nitro-6-amino-isomer (1.8 g., 0.01 mole) giving 2.02 g. (92%) of light yellow product, melting at 330° with decomposition. Recrystallization from water gave light yellow needles decomposing at 341–343°.

Anal. Calcd. for $C_9H_8N_4O_3$: C, 49.09; H, 3.66; N, 25.45. Found: C, 48.84; H, 3.67; N, 25.39.

(16) Refs. 11 and 12.

PHILADELPHIA, PA.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY AND CHEMISTRY, UNIVERSITY OF WISCONSIN]

Xanthomycin A. Degradation Studies[1]

BY K. V. RAO, W. H. PETERSON AND E. E. VAN TAMELEN

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Hydrolysis of xanthomycin ($C_{23}H_{29-31}N_3O_7$) with hydrochloric acid produced ethanolamine, ammonia, methylamine and a large amount of a dark humin-like material. A derivative, methyltetrahydroxanthomycin, suitable for degradation purposes was obtained by simultaneous reduction and methylation of xanthomycin. Oxidation of methyltetrahydroxanthomycin with permanganate produced two crystalline products, xanthomycinic acid I and xanthomycinic acid II. The first contained nitrogen and analyzed for the empirical formula $C_7H_{11}N_3O_{10}$. The second acid was obtained crystalline as the benzylisothiouronium salt and proved to be free of nitrogen. Analysis indicated the empirical formula of this salt was $C_4H_3O_3 \cdot C_8H_{10}N_2S$.

The isolation, purification and properties of the antibiotic xanthomycin A have been described in previous publications.[2,3] The data indicated that xanthomycin A has the molecular formula C_{23}-$H_{29-31}N_3O_7$ and contains a quinonoid nucleus attached to a dibasic nitrogenous moiety.

Xanthomycin A is very sensitive to many of the common reagents, even under mild conditions. It undergoes pronounced decomposition in acidic or basic solutions. Attempted acetylation, benzoylation or methylation at room temperature results in highly colored amorphous products. Almost all the derivatives of xanthomycin A obtained thus far are of very hydrophilic nature and tend to form glassy or amorphous solids. Another difficulty in obtaining useful derivatives of xanthomycin A is the fact that it has two tertiary nitrogen atoms which retain their basic character during many reactions.

Two lines of approach were pursued in the degradation of xanthomycin A. First, drastic acid hydrolysis was employed in the hope of obtaining information regarding the function of the nitrogen atoms in the molecule. Second, reduction of the quinonoid grouping, protection of the resulting hydroxyl groups and oxidation was studied with a view toward determining the nature of the quinone residue.

Degradation of Xanthomycin A.-Hydrolysis of xanthomycin A with 6 *N* hydrochloric acid invariably led to the formation of 35–45% by weight

(1) This investigation was supported in part by research grants from Merck and Co., Inc., and Parke, Davis and Co. Presented in part at the 121st Meeting American Chemical Society, Milwaukee, 1952.

(2) K. V. Rao and W. H. Peterson, THIS JOURNAL, **76**, 1335 (1954).

(3) K. V. Rao and W. H. Peterson, *ibid.*, **76**, 1338 (1954).