molecules and the bulk material. We have obtained preliminary evidence that denaturation by alkali or acid treatments for brief intervals deforms the DNA sufficiently for the formation of the lead complex. We have not yet investigated the precipitability of the products of enzymatic hydrolysis by DNase. Other cations may substitute for lead ion in the test. The effects of other cations or anions are under investigation.

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

Synthesis of Bis-benzimidazoles

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A number of new bis-benzimidazoles have been prepared. Substituents have been placed on both benzene rings of the benzimidazole nuclei. The chain linking the two benzimidazole units has been varied from simple alkane chains to substituted alkane chains or aryl chains (benzene ring). It was found that polyphosphoric acid is a useful medium for preparing bis-benzimidazoles. In difficult cases the use of a little concentrated sulfuric acid as a catalyst was beneficial.

The benzimidazole nucleus has been of considerable interest since it was noted that benzimidazole inhibits the growth of certain yeasts and bacteria.[2] The discovery of 5,6-dimethylbenzimidazole as a unit in vitamin B_{12} has increased this interest. A number of alkyl benzimidazoles have been tested and found to have some anti-vitamin B_{12} activity and some have been reported to have anti-viral activity also.[3] That structural modifications can produce marked effects on physiological activity has been shown by the test data on the substituted benzimidazoles and naphthoquinone imidazoles which have been synthesized in this Laboratory.[4] Some of these compounds exerted strong inhibitory action not only against purine and B_{12} requiring microörganisms but against folic acid-requiring organisms as well.

Structural modifications of the benzimidazole nucleus can be carried out in several ways. In the present investigation methods were developed to synthesize new bis-benzimidazoles where the two benzimidazole nuclei are united through their 2-positions either directly or through one or more atoms. Such systems can be modified not only by changing the nature and the number of the connecting atoms but by changing the nature of the substituents in the benzimidazole nuclei as well.

A review of the literature revealed the fact that a number of bis-benzimidazoles have been reported but apparently none of them have been screened for physiological activity. In the present study, greatest emphasis was placed on the preparation of substituted bis-benzimidazolylalkanes although a few other types were also prepared.

Phillips' method for the preparation of simple benzimidazoles is well known.[5] The same procedure can be used to prepare bis-benzimidazoles by refluxing two moles of diamine with one mole of a dibasic acid in 4 *N* hydrochloric acid. The method was modified by Shriner and Upson by using a much longer reflux period.[6] In the present work a further modification was made. It was found that the bis-benzimidazoles could be conveniently isolated as their dihydrochlorides by cooling the reaction solution after the refluxing period. The isolation of the dihydrochloride was preferable to neutralization of the whole solution because in general the dihydrochloride of the diamine is more soluble than the dihydrochloride of the bis compound. Thus a separation of most of the excess diamine can be accomplished in this step. The dihydrochloride was then treated with hot bicarbonate solution or aqueous ammonia to remove any benzimidazolylalkylcarboxylic acid which would normally be present.

This modification of Phillips' method gave fair yields in most cases. It failed, however, when used to condense 3-nitro-5-chloro-*o*-phenylenediamine with succinic, adipic and suberic acids. The polyphosphoric acid method, recently reported by Hein, Alheim and Leavitt for the preparation of simple benzimidazoles, was then tried.[7]

The polyphosphoric acid method not only worked for 3-nitro-5-chloro-*o*-phenylenediamine with succinic, adipic and suberic acids but was found to be convenient and fairly general giving about the same yields as the modified Phillips method. The polyphosphoric acid method failed in one case, with 5-nitro-*o*-phenylenediamine.

In the preparation of the bis-benzimidazolylethanes, although 3,4-diaminotoluene and 4-chloro-*o*-phenylenediamine were condensed with succinic acid to give fair yields of the bis compounds, it was found preferable to use succinic anhydride instead of the free acid for the other substituted diamines.

As the alkane chain between the two benzimidazole rings increased in length, the preparation of bis-benzimidazoles became more difficult. The use of polyphosphoric acid was the best method for the condensation of *o*-phenylenediamines with the longer chain dibasic acids. Substituted bis-benzimidazolyloctanes were not easy to obtain even by this method until it was noted that the addition of a small amount of concentrated sulfuric acid facilitated the reaction. Although this modification was not tried with all of the other dibasic acids, it would probably be helpful in those cases also.

(1) To whom all inquiries should be addressed.

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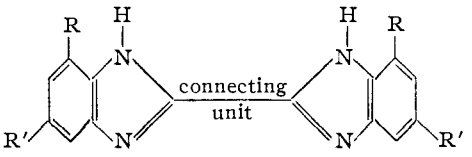
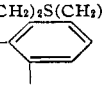
TABLE I

BIS BENZIMIDAZOLES



Substituents R	R'	Connecting unit	Yield, %[d]	M.p., °C.	Formula	Analyses, % Calcd. C	H	N	Cl	Found C	H	N	Cl
H	CH_3	CH_2CH_2	30	265–268	$C_{18}H_{18}N_4$[a,c]	74.45	6.25	19.30	...	74.60	6.38	19.44	...
H	NO_2	CH_2CH_2	31	290–293	$C_{16}H_{12}N_6O_4$[b,c]	54.55	3.44	23.58	...	54.59	3.53	23.77	...
H	Cl	CH_2CH_2	36	270–273	$C_{16}H_{12}N_4Cl_2$[a,c]	58.03	3.65	16.92	21.42	57.88	3.51	16.78	21.22
H	C_2H_5O	CH_2CH_2	42	225–228	$C_{20}H_{22}N_4O_2$[b]	68.54	6.33	15.96	...	68.79	6.37	15.69	...
NH_2	Cl	CH_2CH_2	35	256–258	$C_{16}H_{14}N_6Cl_2$[b]	53.19	3.90	23.26	19.62	53.23	4.04	23.39	19.70
H	CH_3	$(CH_2)_4$	45	235–236	$C_{20}H_{22}N_4$[b]	75.44	6.96	17.60	...	75.62	7.11	17.45	...
H	NO_2	$(CH_2)_4$	35	267–269	$C_{18}H_{18}N_6O_4$[b]	56.84	4.34	22.05	...	56.79	4.32	22.05	...
H	Cl	$(CH_2)_4$	29	276–278	$C_{18}H_{16}N_4Cl_2$[b]	60.17	4.49	15.59	...	59.91	4.65	15.38	...
H	NH_2	$(CH_2)_4$	38		$C_{18}H_{20}N_6$[b]	67.50	6.29	26.23	...	67.41	6.16	26.00	...
H	$NH_2 \cdot 2HCl$	$(CH_2)_4$	65	292–296	$C_{18}H_{22}N_6Cl_2$	46.36	5.19	18.02	30.42	46.32	5.11	18.00	30.52
NO_2	Cl	$(CH_2)_4$	25	288–290[f]	$C_{18}H_{14}N_6O_4Cl_2$[a]	48.13	3.41	18.71	15.78	48.33	3.55	18.54	15.54
NH_2	Cl	$(CH_2)_4$	25	247–250[f]	$C_{18}H_{18}N_6Cl_2$[b]	55.55	4.66	21.59	18.22	55.35	4.87	21.47	18.21
H	CH_3	$(CH_2)_6$	28	221–223	$C_{22}H_{26}N_4$[a]	76.25	7.57	16.18	...	76.07	7.76	16.36	...
H	NO_2	$(CH_2)_6$	30	243–245	$C_{20}H_{20}N_6O_4$[b]	58.81	4.94	20.57	...	58.59	4.91	20.64	...
H	Cl	$(CH_2)_6$	33	204–207	$C_{20}H_{20}N_4Cl_2$[b]	62.03	5.21	14.47	18.31	61.87	5.27	14.57	18.19
NO_2	Cl	$(CH_2)_8$	10	282[f]	$C_{20}H_{18}N_6O_4Cl_2$[a]	50.32	3.80	17.61	14.85	50.12	3.71	17.57	14.72
H	CH_3	$(CH_2)_8$	40	226–228	$C_{24}H_{30}N_4$[a]	76.97	8.07	14.96	...	76.96	8.21	14.82	...
H	Cl	$(CH_2)_8$	40	208–210	$C_{24}H_{24}N_4Cl_2$[a]	63.61	5.82	13.49	...	63.54	5.87	13.52	...
H	H	CHOHCHOH	44	245[f]	$C_{16}H_{14}N_4O_2$[b]	65.31	4.80	19.04	...	65.35	5.08	19.06	...
H	CH_3	CHOHCHOH	42	218–221	$C_{18}H_{18}N_4O_2$[b]	67.06	5.63	17.38	...	66.87	5.49	17.21	...
H	C_2H_5O	CHOHCHOH	35	222–225	$C_{20}H_{22}N_4O_4$[b]	62.80	5.80	14.65	...	62.97	6.02	14.56	...
H	Cl	CHOHCHOH	39	218[f]	$C_{16}H_{12}N_4O_2Cl_2$[b]	52.91	3.33	15.43	19.53	52.71	3.58	15.59	19.62
H	NO_2	CHOHCHOH	30	250–255[f]	$C_{16}H_{12}N_6O_8$[b]	49.99	3.15	21.87	...	49.89	3.08	21.84	...
H	H	$(CH_2)_2S(CH_2)_2$	33	200	$C_{18}H_{18}N_4S$[b]	67.07	5.63	17.38	9.94[g]	66.99	5.50	17.33	10.06[g]
H	Cl		20	365[f]	$C_{20}H_{12}N_4Cl_2$[a]	63.34	3.19	14.77	18.70	63.36	3.22	14.80	18.63

[a] Prepared by method A. [b] Prepared by method B. [c] Listed but not fully described in Swiss Patent. [d] Yields of pure products. [e] Decomposes indefinitely 175–225°. [f] Decomposes. [g] Sulfur, %.

A modified Phillips procedure was also used for the preparation of 1,2-bis-benzimidazolyl-1,2-ethanediols and 2,2′-(thiodiethylene)-bis-benzimidazoles. The polyphosphoric acid method was not tried in these cases.

For the preparation of 2,2′-*o*-phenylenebis-(6-chlorobenzimidazole) only the polyphosphoric acid method was successful. This is in agreement with the observation of others that Phillips' method is not a successful one for the condensation of *o*-phenylenediamines with aromatic acids.

Crippa, Perronato and Sacchetti reported the formation of bis-(2,2′-benzimidazolyl)-thiourea from 2-aminobenzimidazole when the latter was heated with carbon disulfide in dry ethanol.[8] The reaction was repeated, in the present study, but the thiourea derivative was not obtained. The only product isolated proved to be ethyl N-benzimidazolyl thiocarbamate.

Experimental

All of the melting points reported were taken in an apparatus similar to the one described by Wagner and Meyer.[9] The values are uncorrected.

Preparation of Bis-benzimidazolyl Ethane. Polyphosphoric Acid Method. Method A. 1,2-Bis-(6-methyl-2-benzimidazolyl)-ethane.—3,4-Diaminotoluene (6.10 g., 0.05 mole) was mixed with 2.98 g. (0.025 mole) of succinic acid and the mixture poured into 30 ml. of polyphosphoric acid which had been preheated to 100°. The mixture was stirred and heated at 150° for three hours. The reaction mixture was then cooled, diluted with water and allowed to stand overnight. The precipitate was removed by filtration, extracted with hot, dilute ammonium hydroxide and dried. The product was recrystallized from ethanol–water.

The Modified Phillips Method. Method B. 1,2-Bis-(6-nitro-2-benzimidazolyl)-ethane.—4-Nitro-*o*-phenylenediamine (7.65 g., 0.05 mole) and 3.05 g. (0.025 mole) of succinic acid were refluxed for ten hours in 50 ml. of 4 *N* hydrochloric acid. Succinic anhydride may be used in place of succinic acid. The reaction mixture was cooled and the crystalline dihydrochloride which separated was removed by filtration. It was extracted with hot dilute ammonium hydroxide and washed with water. The product was recrystallized from ethylene glycol with the aid of decolorizing carbon. In all other cases where method B was used, the product was recrystallized from ethanol–water with the aid of decolorizing carbon, unless otherwise noted.

1,2-Bis-(6-ethoxy-2-benzimidazolyl)-ethane.—In this case the bis derivative was made from 3-amino-4-nitrophenetole without isolating the intermediate diamine. 3-Amino-4-nitrophenetole (9.1 g., 0.05 mole) was dissolved in 50 ml. of 4 *N* hydrochloric acid, 1 g. of 5% palladium-on-alumina was added and hydrogenation carried out in a Parr apparatus. The catalyst was removed and 2.5 g. (0.025 mole) of succinic anhydride and 2 ml. of concentrated sulfuric acid were added. Method B was followed from this point.

1,2-Bis-(7-amino-5-chloro-2-benzimidazolyl)-ethane.—This bis derivative was prepared from 3-nitro-5-chloro-*o*-phenylenediamine by the procedure described for 1,2-bis-(6-ethoxy-2-benzimidazolyl)-ethane. In this case the crude dihydrochloride was treated with hot sodium bicarbonate solution in place of aqueous ammonia. It was washed with water and dried. Attempts to recrystallize the product from ethylene glycol gave a gum which changed to a crystalline solid when treated with anhydrous acetone.

(7-Nitro-5-chloro-2-benzimidazolyl)-3-propanoic Acid.—Attempts to prepare 1,2-bis-(7-nitro-5-chloro-2-benzimidazolyl)-ethane from 3-nitro-5-chloro-*o*-phenylenediamine were unsuccessful with both methods A and B. The only prod-

(8) G. B. Crippa, G. Perronato and G. Sacchetti, *Gazz. chim. ital.*, **65**, 38 (1935).

(9) E. C. Wagner and J. F. Meyer, *Ind. Eng. Chem., Anal. Ed.*, **10**, 584 (1938).

uct that could be isolated was the benzimidazolylpropionic acid. Since this is a new compound its preparation is included. Method A was used, but in this case the polyphosphoric acid was preheated to 150° and after adding the diamine the mixture was kept at this temperature for five hours. On cooling the reaction mixture and diluting with water a tar formed from which no identifiable product could be obtained. The filtrate from the tar was neutralized and the yellow product which formed was removed and washed with water. It was recrystallized from ethanol–water with the aid of decolorizing carbon, yield 45%, m.p. 240–242°.

Anal. Calcd. for $C_{10}H_8N_3O_4Cl$: C, 44.52; H, 2.99; N, 15.58; Cl, 13.15. Found: C, 44.68; H, 3.12; N, 15.62; Cl, 13.26.

Preparation of Bis-benzimidazolylbutanes. 1,4-Bis-(2-benzimidazolyl)-butane.—This compound was made earlier by Shriner and Upson from *o*-phenylenediamine and adipic acid. It was made in this work, by both methods A and B to get samples for the screening program. The yields were around 40% by both methods.

1,4-Bis-(6-amino-2-benzimidazolyl)-butane.—1,4-Bis-(6-nitro-2-benzimidazolyl)-butane (3.8 g., 0.01 mole) was added to 20 ml. of 4 *N* hydrochloric acid and 0.5 g. of 5% palladium-on-alumina added. When the hydrogenation was complete, 50 ml. of 4 *N* hydrochloric acid was added and the solution warmed. The catalyst was removed and the dihydrochloride separated from the filtrate on cooling. It was recrystallized from 4 *N* hydrochloric acid. Method B was followed from this point.

1,4-Bis-(7-nitro-5-chloro-2-benzimidazolyl)-butane.—This compound was prepared by method A. The temperature was kept at 130° for five hours. The crude product was extracted with hot 10% sodium bicarbonate solution, dried and recrystallized from ethylene glycol.

1,4-Bis-(7-amino-5-chloro-2-benzimidazolyl)-butane.—This bis-benzimidazole was prepared from 3-nitro-5-chloro-*o*-phenylenediamine by reducing it in 4 *N* hydrochloric acid over palladium-on-alumina. The filtrate from the catalyst was refluxed with adipic acid for ten hours as in the preparation of 1,2-bis-(7-amino-5-chloro-2-benzimidazolyl)ethane.

Preparation of Bis-benzimidazolylhexanes.—Suberic acid was the starting organic acid for this series.

Bis-benzimidazolyloctanes were prepared from sebacic acid and the appropriate diamines.

1,8-Bis-(6-chloro-2-benzimidazolyl)-octane.—In this case, 1 ml. of concentrated sulfuric acid was added to the polyphosphoric acid and the reaction mixture was stirred for five hours at 150°.

Preparation of Bis-benzimidazolyl-1,2-ethanediols.—The starting materials were tartaric acid and *o*-phenylenediamines.

1,2-Bis-(6-ethoxy-2-benzimidazolyl)-1,2-ethanediol was prepared from 3-amino-4-nitrophenetole and *d*-tartaric acid by the procedure described for making 1,2-bis-(6-ethoxy-2-benzimidazolyl)-ethane.

1,2-Bis-(6-nitro-2-benzimidazolyl)-1,2-ethanediol was recrystallized from ethylene glycol–water with the aid of decolorizing carbon.

Preparation of 2,2'-(Thiodiethylene)-bis-benzimidazole.—This compound was prepared from *o*-phenylenediamine and 3,3'-dithiopropionic acid.[10]

2,2'-*o*-Phenylenebis-(6-chlorobenzimidazole) was prepared from 4-chloro-*o*-phenylenediamine and phthalic anhydride. The crude product was washed with hot 95% ethanol. The residue (43% yield) was recrystallized from N-dimethylformamide.

Preparation of Ethyl N-Benzimidazolylthiocarbamate.—2-Aminobenzimidazole (13.3 g.) was mixed with 40 ml. of carbon disulfide and 30 ml. of absolute ethanol. The reaction mixture was refluxed on a steam-bath for 50 hours and the product was isolated and purified as described by Crippa, *et al.*[8] The analytical data indicated that the compound was a thiocarbamate rather than the bis derivative reported in reference 8. The yield was 40%, m.p. 202°.

Anal. Calcd. for $C_{10}H_{11}N_3SO$: C, 54.27; H, 5.01; N, 19.00; S, 14.50. Found: C, 54.54; H, 5.15; N, 19.09; S, 14.64.

(10) The 3,3'-dithiopropionic acid was obtained from the American Cyanamid Co.

PHILADELPHIA, PENNA.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTH TEXAS STATE COLLEGE]

Benzo[d]pyrido[a]benzimidazole-4,9-quinone

BY PRICE TRUITT, JAMES ERWIN COOPER, III,[1] AND FRANK M. WOOD, JR.[2]

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The reaction of 2-aminopyridine and 2-acetamido-3-chloro-1,4-naphthoquinone gave the title compound. The same substance was obtained from the reaction of 2-aminopyridine and 2,3-dichloro-1,4-naphthoquinone.

2-Acetamido-3-chloro-1,4-naphthoquinone reacts readily with primary amines to yield 2-acetamido-3-alkyl(aryl)amino-1,4-naphthoquinones.[3,4]

Since Calandra and Adams[5] had reported that the reaction of 2-aminopyridine and 2,3-dichloro-1,4-naphthoquinone yielded a product described as 2-(2-pyridylamino)-3-chloro-1,4-naphthoquinone, it seemed reasonable that 2-aminopyridine should react with 2-acetamido-3-chloro-1,4-naphthoquinone to give 2-acetamido-3-(2-pyridylamino)-1,4-naphthoquinone. When 2-acetamido-3-chloro-1,4-naphthoquinone (I) and 2-aminopyridine (II) were heated together in refluxing butanol, an orange product (III), m.p. 306°, was obtained. It did not contain chlorine. Again, when the 2-acetamido-3-chloro-1,4-naphthoquinone was replaced in the reaction with 2-chloroacetamido-3-chloro-1,4-naphthoquinone, an orange product (III) was obtained, m.p. 306°. It did not contain chlorine. A mixed melting point determination proved that the two substances melting at 306° were identical.

The reaction of 2-aminopyridine (II) and 2,3-dichloro-1,4-naphthoquinone (IV) in refluxing ethanol did indeed give 2-chloro-3-(2-pyridylamino)-1,4-naphthoquinone (V), m.p. 275–276°, as Calandra and Adams indicated.[5]

A suspension of V in glacial acetic acid gave III when heated and cooled.

A solution of I and II were refluxed for 12 hr. in methanol and VI was obtained as orange crystals, m.p. 215°, along with unidentified red platelets. VI was identified as 2-chloro-3-hydroxy-1,4-naphthoquinone.[6] These workers reported VI to melt at 215+°. VI reacted with aniline to yield VII, m.p. 185°. 2-Anilino-3-hydroxy-1,4-naphthoquinone is reported to melt at 183°.[6]

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(2) Research Fellow of Parke, Davis & Co., 1950–1953.

(3) K. Fries and K. Billig, *Ber.*, **58**, 1128 (1925).

(4) J. R. E. Hoover and A. R. Day, THIS JOURNAL, **76**, 1148 (1954).

(5) J. C. Calandra and E. C. Adams, *ibid.*, **72**, 4804 (1950).

(6) T. Zincke and C. Gerland, *Ber.*, **20**, 3222 (1887).