another 5 hours under reflux, the solution was slightly acidic. A saturated sodium carbonate solution was added until the solution was slightly alkaline after which it was heated under reflux an additional hour. The solution was cooled and water was added slowly to precipitate the oxime which amounted to 22.9 g. (29%), m.p. 149–155°. Recrystallization from isopropyl alcohol or acetone–water resulted in material melting unsharply from 149–163°.

Anal. Calcd. for $C_{17}H_{14}N_2O_2S$: C, 65.85; H, 4.55. Found: C, 65.72; H, 4.43.

2-Ethyl-3-amino-2,3-dihydro-1H-pyrido[3,2,1-kl]phenothiazine Hydrochloride (IIIc).—We reduced 13.8 g. (0.0445 mole) of IVa with 6.35 g. (0.167 mole) of lithium aluminum hydride in 2 l. of absolute ether by the Soxhlet procedure. The mixture was heated for 21 hours, and then the excess of hydride was decomposed with water. The slurry was filtered and washed thoroughly with dry ether. Dry hydrogen chloride was passed into the solution and the amine hydrochloride was removed by filtration to yield 4.0 g. (28%) of product, of m.p. 249–253°. Recrystallization from ethanol–ether did not raise the melting point.

Anal. Calcd. for $C_{17}H_{19}ClN_2S$: C, 64.15; H, 5.69. Found: C, 63.97; H, 5.83.

TUCKAHOE 7, N. Y.

[CONTRIBUTION FROM ABBOTT LABORATORIES]

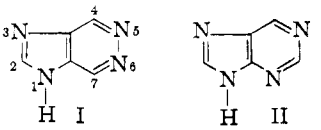
The Preparation of Several 4-Substituted Imidazo[4,5-d]pyridazines as Possible Purine Antimetabolites

BY JOHN A. CARBON

RECEIVED JUNE 23, 1958

A number of 4-substituted imidazo[4,5-d]pyridazines have been prepared for testing as antitumor agents. 1-Benzylimidazo[4,5-d]pyridazin-4(5H),7(6H)-dione (IV, R = $CH_2C_6H_5$) was found to react smoothly with phosphorus oxychloride to give 1-benzyl-4,7-dichloroimidazo[4,5-d]pyridazine (V, R = $CH_2C_6H_5$). Treatment of the latter compound with a variety of nucleophilic reagents gave a series of 1-benzyl-4-substituted-7-chloroimidazo[4,5-d]pyridazines, which were reduced readily with sodium in liquid ammonia to the 4-substituted imidazo[4,5-d]pyridazines. A similar reaction sequence has been applied to 1-methylimidazo[4,5-d]pyridazin-4(5H),7(6H)-dione (IV, R = CH_3) to give 4-amino-1-methylimidazo[4,5-d]pyridazine (XIX) and 7-amino-1-methylimidazo[4,5-d]pyridazine (XX).

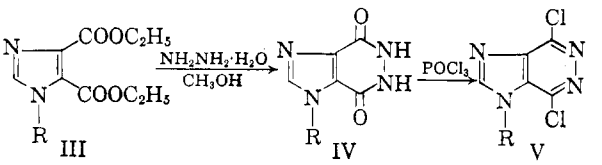
The chemistry of compounds which are structurally related to the naturally occurring purines has received a great deal of attention recently, largely because of the search for more effective antitumor and antileukemic agents. Due to the marked similarity of the imidazo[4,5-d]pyridazine (I) and purine (II) ring systems, we have prepared a series of compounds containing the system I as potential antagonists of purine metabolism.



H I H II

Aside from the preparation of imidazo[4,5-d]-pyridazin-4(5H),7(6H)-dione (IV, R = H) and a few of its 1-substituted derivatives (IV, R = CH_3 and C_6H_5),[1] the chemistry of these compounds has received little attention.[2] We have found that the cyclic hydrazides (IV) of 1-substituted imidazole-4,5-dicarboxylic acids are convenient intermediates for the synthesis of a large variety of imidazo-[4,5-d]pyridazines.

Preliminary attempts to convert imidazo[4,5-d]-pyridazin-4(5H),7(6H)-dione (IV, R = H) to 4,7-dichloroimidazo[4,5-d]pyridazine (V, R = H) by treatment with phosphorus oxychloride or phosphorus pentachloride were without success, IV (R = H) being completely inert to these reagents. The use of a phosphorus oxychloride–N,N-dimethylaniline mixture, a procedure which has been successful in reactions of this type,[3] was also unsatisfactory.[4] However, when 1-phenylimidazo[4,5-d]pyridazin-4(5H), 7(6H)-dione (IV, R = C_6H_5) was treated with refluxing phosphorus oxychloride, a smooth conversion to 4,7-dichloro-1-phenylimidazo[4,5-d]pyridazine (V, R = C_6H_5) was realized in high yield. This remarkably facile replacement of both oxygen functions by chlorine could be extended to produce 4,7-dichloro-1-methylimidazo[4,5-d]pyridazine (V, R = CH_3) and 1-benzyl-4,7-dichloroimidazo[4,5-d]pyridazine (V, R = $CH_2C_6H_5$) from the corresponding imidazo[4,5-d]pyridazin-4(5H),7(6H)-diones.



As might be predicted from the excellent work of Druey, *et al.*,[5] on the behavior of 3,6-dichloropyridazine toward nucleophilic reagents, one of the chlorine atoms in compounds of type V could be replaced with a variety of groups with ease, while the replacement of both chlorine atoms was accomplished only with difficulty. Most of these replacement reactions were carried out on 1-benzyl-4,7-dichloroimidazo[4,5-d]pyridazine (V, R = $CH_2C_6H_5$) since the benzyl grouping can be cleaved easily from an imidazole nitrogen by reduction with sodium in liquid ammonia.[6]

(1) R. G. Jones, THIS JOURNAL, **78**, 159 (1956).

(2) A recent paper (R. N. Castle and W. S. Seese, Abstracts of Papers, 133rd Meeting, American Chemical Society, San Francisco, Calif., April 13–18, 1958, p. 28M) has reported the synthesis of some 4,7-disubstituted imidazo[4,5-d]pyridazines.

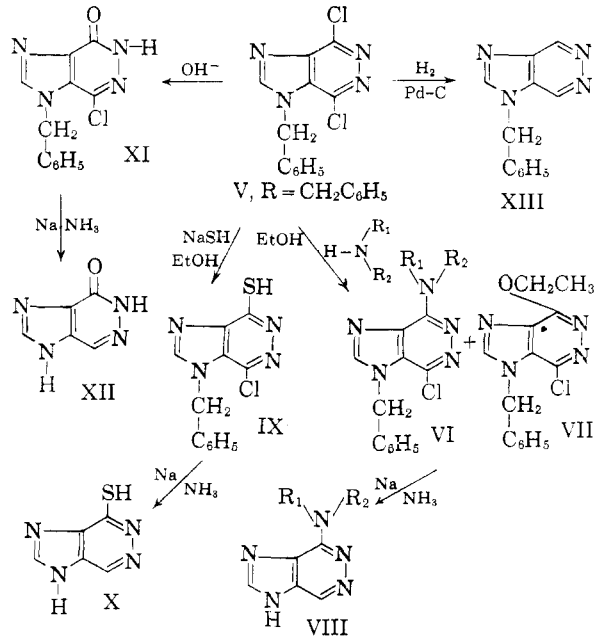
(3) (a) J. Baddiley and A. Topham, *J. Chem. Soc.*, 678 (1944); (b) P. Bitterli and H. Erlenmeyer, *Helv. Chim. Acta*, **34**, 835 (1951); (c) J. R. Marshall and J. Walker, *J. Chem. Soc.*, 1004 (1951); (d) N. Whittaker, *ibid.*, 1565 (1951); 1646 (1953); (e) J. Davoll and B. A. Lowy, THIS JOURNAL, **73**, 2936 (1951); (f) A. Bendich, P. J. Russell, Jr., and J. J. Fox, *ibid.*, **76**, 6073 (1954).

(4) Castle and Seese (ref. 2) have been able to isolate a small quantity of 4,7-dichloroimidazo[4,5-d]pyridazine (V, R = H) from the reaction of imidazo[4,5-d]pyridazin-4(5H),7(6H)-dione (IV, R = H) with phosphorus oxychloride–N,N-diethylaniline.

(5) J. Druey, K. Meier and K. Eichenberger, *Helv. Chim. Acta*, **37**, 121, 510, 837 (1954).

(6) (a) V. duVigneaud and O. K. Behrens, *J. Biol. Chem.*, **117**, 27 (1937); (b) R. G. Jones, THIS JOURNAL, **71**, 383 (1949).

The treatment of V (R = $CH_2C_6H_5$) in ethanol with ammonia or a variety of amines at 100-110° in a sealed autoclave resulted in the formation of 4-amino- or substituted amino-1-benzyl-7-chloroimidazo[4,5-d]pyridazines (VI) (Table I). Although the replacement of a single halogen atom of V (R = $CH_2C_6H_5$) is theoretically capable of leading to two different positional isomers, we were unable to isolate any of the corresponding 7-amino- or substituted amino-1-benzyl-4-chloroimidazo[4,5-d]pyridazines from these reactions. Structure VI has been assigned to the products purely on the basis of steric considerations.[7] Compound VII, 1-benzyl-7-chloro-4-ethoxyimidazo[4,5-d]pyridazine, appeared as a minor by-product in most of these displacement reactions.

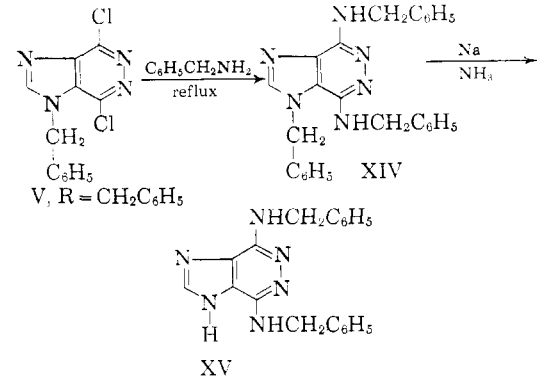


Reduction of the 4-amino- or 4-alkylamino-1-benzyl-7-chloroimidazo[4,5-d]pyridazines (VI, R_1 = H or alkyl, R_2 = H) with sodium in liquid ammonia resulted in the cleavage of the 1-benzyl and 7-chloro groupings to form the corresponding 4-amino- or 4-alkylaminoimidazo[4,5-d]pyridazines (VIII, R_1 = H or alkyl, R_2 = H) (Table II). In contrast to the good yields obtained when the substituent in the 4-position was amino or alkylamino, the similar reduction of the 4-dimethylamino and 4-diethylamino compounds gave only intractable red tars. A successful synthesis of 4-(di-*n*-propylamino)-imidazo[4,5-d]pyridazine (VIII, R_1 = R_2 = *n*-C_3H_7) could be achieved by this method; however, the yield was small (20%).

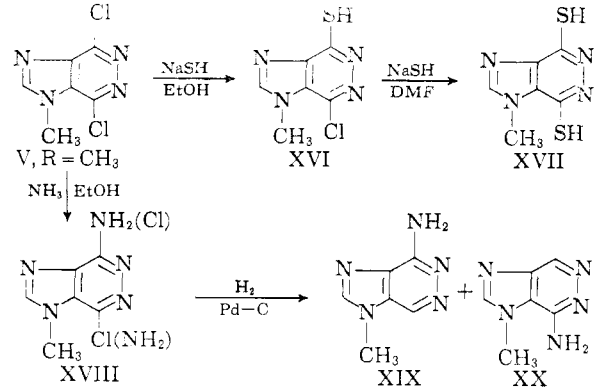
4-Mercaptoimidazo[4,5-d]pyridazine (X), an interesting isomer of the antileukemic agent 6-mercaptopurine,[8] was synthesized by treatment of V (R = $CH_2C_6H_5$) with sodium hydrosulfide in ethanol to form 1-benzyl-7-chloro-4-mercaptoimidazo[4,5-d]pyridazine (IX), followed by reduction with sodium in liquid ammonia.

Similarly, treatment of V (R = $CH_2C_6H_5$) with boiling 10% sodium hydroxide resulted in a good yield of 1-benzyl-7-chloroimidazo[4,5-d]pyridazin-4(5H)-one (XI), which gave imidazo[4,5-d]pyridazin-4(5H)-one (XII) upon reduction with sodium in liquid ammonia. The latter compound is isomeric with the naturally occurring purine, hypoxanthine.

Although the 4,7-dichloro compound V (R = $CH_2C_6H_5$) gave only intractable tars upon reduction with sodium in liquid ammonia, catalytic reduction over a palladium-on-charcoal catalyst resulted in a smooth conversion to 1-benzylimidazo[4,5-d]pyridazine (XIII). This result is not surprising, considering the known reluctance of the benzyl group attached to imidazole nitrogen to undergo cleavage by catalytic hydrogenation.[6]



Several unsuccessful attempts were made to prepare 1-benzyl-4,7-bis-(diethylamino)-imidazo[4,5-d]pyridazine by treatment of V (R = $CH_2C_6H_5$) with ethanolic diethylamine at elevated temperatures in a sealed autoclave. However, both chlorine atoms of V (R = $CH_2C_6H_5$) easily were replaced in refluxing benzylamine to give an excellent yield of 1-benzyl-4,7-bis-(benzylamino)-imidazo[4,5-d]pyridazine (XIV). When XIV was treated with an excess of sodium in liquid ammonia, only one of the three benzyl groups was removed to give 4,7-bis-(benzylamino)-imidazo[4,5-d]pyridazine (XV). It readily was apparent that the benzyl group had been cleaved from the 1-position because of the solubility of XV in aqueous alkali.



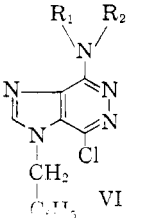
The behavior of 4,7-dichloro-1-methylimidazo[4,5-d]pyridazine (V, R = CH_3) toward nucleo-

(7) Rigorous proofs of the structures of type VI were not undertaken since these compounds served only as intermediates for the synthesis of the more interesting 4-substituted aminoimidazo[4,5-d]pyridazines (VIII), in which the 1-benzyl grouping has been removed.

(8) G. B. Elion, E. Burgi and G. H. Hitchings, THIS JOURNAL, **74**, 411 (1952).

TABLE I

4-SUBSTITUTED AMINO-1-BENZYL-7-CHLOROIMIDAZO[4,5-d]PYRIDAZINES



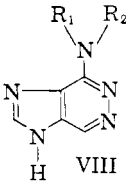
VI

R_1	R_2	Yield, %	M.p., °C.	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
H	H	52	271–273 d.[a]	55.49	3.88	26.97	55.45	4.15	26.84[b]
CH_3	H	58	185–187[c]	57.04	4.42	25.59	57.13	4.60	25.56
C_2H_5	H	42	215–217[c]	58.43	4.90	24.34	58.62	5.09	24.40
n-C_3H_7	H	53	169–171[c]	59.70	5.34	23.21	59.43	5.55	23.12
CH_3	CH_3	44	181–183[d]	58.43	4.90	24.34	58.68	4.92	24.29
C_2H_5	C_2H_5	61	139–140[d]	60.85	5.74	22.18	60.64	5.85	22.08
n-C_3H_7	n-C_3H_7	57	160–161[d]	62.87	6.45	20.37	62.89	6.51	20.27
$HOCH_2CH_2$	$HOCH_2CH_2$	71	158–159[e]	55.25	5.21	20.14	55.49	5.27	20.37[f]

[a] Recrystallized from N,N-dimethylformamide. [b] Calcd.: Cl, 19.31. Found: Cl, 19.15. [c] Recrystallized from 50% ethanol. [d] Recrystallized from ethanol. [e] Recrystallized from 40% ethanol. [f] Calcd.: Cl, 10.20. Found: Cl, 10.37.

TABLE II

4-SUBSTITUTED AMINOIMIDAZO[4,5-d]PYRIDAZINES



VIII

R_1	R_2	Yield, %	M.p., °C.	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
H	H	74	262–263 d.[a,b]	44.44	3.73	51.83	44.20	3.77	51.59
CH_3	H	64	298–300 d.[c]	48.31	4.73	46.96	48.33	4.80	46.95
C_2H_5	H	61	280–281 d.[d]	51.52	5.56	42.92	51.67	5.69	42.90
n-C_3H_7	H	69	215–216[e]	54.23	6.26	39.51	54.09	6.49	39.51
n-C_3H_7	n-C_3H_7	20	146.5–147[e]	60.24	7.82	31.94	60.01	8.12	31.48

[a] Recrystallized from water. [b] The monohydrochloride was obtained as colorless needles from aqueous HCl; m.p. 334–337° dec. Calcd. for $C_5H_6ClN_5$: C, 35.00; H, 3.52; Cl, 20.66; N, 40.82. Found: C, 35.03; H, 3.68; Cl, 20.82; N, 40.73. [c] Purified by dissolving in aqueous HCl, decolorizing with Norit, and reprecipitating by neutralization with aqueous NaOH. [d] Recrystallized from N,N-dimethylformamide–water. [e] Recrystallized from 30% ethanol.

philic reagents was found to be quite similar to the 1-benzyl compound. For example, when V (R = CH_3) was refluxed for extended time periods with a large excess of sodium hydrosulfide in ethanol, the only product was a monomercapto derivative, tentatively designated as XVI. This material could be converted to 4,7-dimercapto-1-methylimidazo[4,5-d]pyridazine (XVII) by refluxing with a solution of sodium hydrosulfide in N,N-dimethylformamide for three hours. Although Druey, *et al.*,[5] were able to prepare 3,6-dihydrazinopyridazine by merely refluxing 3,6-dimercaptopyridazine with ethanolic hydrazine, the mercapto groups of XVII could not be replaced, even when refluxed with hydrazine hydrate in methyl Cellosolve for 24 hours.

The reaction of 4,7-dichloro-1-methylimidazo-[4,5-d]pyridazine (V, R = CH_3) with ethanolic ammonia in a sealed autoclave at 150° gave a 53% yield of 4-amino-7-chloro-1-methylimidazo[4,5-d]-pyridazine (XVIII). Removal of the halogen atom in XVIII by catalytic hydrogenation over palladium-on-charcoal resulted in a moderate yield of 4-amino-1-methylimidazo[4-5-d]pyridazine (XIX) plus an extremely small quantity of an isomeric compound, for which we have assigned structure XX. This isomer presumably arose from a small quantity of 7-amino-4-chloro-1-methylimidazo[4,5-d]pyridazine present as an impurity in XVIII. These isomers were assigned structures XIX and XX on the basis of a comparison of their ultraviolet absorption spectra with those of 9-methyl- and 7-methyladenine[9] (Table III).

TABLE III

COMPARISON OF ULTRAVIOLET ABSORPTION MAXIMA AND MELTING POINTS

	M.p., °C.	λmax, mμ 0.05 *N* HCl	λmax, mμ 0.05 *N* NaOH
4-Amino-1-methylimidazo[4,5-d]-pyridazine (XIX)	295–296	258	255
9-Methyladenine	308–310[a]	260[b]	260[b]
7-Amino-1-methylimidazo[4,5-d]-pyridazine (XX)	318–319	263	261
7-Methyladenine	351 dec.[c]	269[b]	269[b]

[a] E. Fischer, *Ber.*, **30**, 2226 (1897). [b] See ref. 9. [c] E. Fischer, *Ber.*, **31**, 104 (1898).

(9) J. M. Gulland and E. R. Holiday, *J. Chem. Soc.*, **139**, 765 (1936).

The compounds reported in this paper are being screened for antitumor activity at the Sloan-Kettering Institute for Cancer Research. The results of these tests will be reported elsewhere.

Acknowledgment.—The author would like to thank Messrs. M. L. Freifelder and G. R. Stone for carrying out the catalytic hydrogenations and for the preparation of many of the compounds listed in Table I, Mr. E. F. Shelberg and his staff for the microanalyses, and Dr. D. J. Campbell and Mr. F. Chadde for the ultraviolet absorption spectra.

Experimental[10]

Diethyl 1-benzyl-2-mercapto-4,5-imidazoledicarboxylate was prepared using the general method worked out by Jones[11] for the synthesis of other diethyl 1-alkyl-2-mercapto-4,5-imidazoledicarboxylates. From 237.5 g. (1.07 moles) of N-benzyl-N-formylglycine ethyl ester[12] was obtained 240 g. (71%) of product as colorless needles from dilute ethanol, m.p. 131.0–131.5°.

Anal. Calcd. for $C_{16}H_{18}N_2O_4S$: C, 57.48; H, 5.42; N, 8.38. Found: C, 57.61; H, 5.40; N, 8.40.

Diethyl 1-Benzyl-4,5-imidazoledicarboxylate (III, R = $CH_2C_6H_5$).—To a stirred solution of 140 ml. of nitric acid (sp. gr. 1.42) in 400 ml. of water containing about 1 g. of sodium nitrite was added 247 g. (0.74 mole) of diethyl 1-benzyl-2-mercapto-4,5-imidazoledicarboxylate in small portions over a period of 1–1.5 hr. The reaction mixture was kept at 25–35° during this addition by occasional cooling in an ice-bath. After stirring for an additional hour at room temperature, the solution was rendered weakly basic by the addition of solid sodium carbonate and the product extracted into ethyl acetate. The extracts were dried over anhydrous magnesium sulfate and the ethyl acetate removed *in vacuo* to leave 179.5 g. (80%) of a pale yellow oil which slowly crystallized on standing. A small sample was obtained as colorless prisms from chloroform–Skelly B, m.p. 54–55°.

Anal. Calcd. for $C_{16}H_{18}N_2O_4$: C, 63.57; H, 6.01; N, 9.27. Found: C, 63.77; H, 6.24; N, 9.31.

1-Benzylimidazo[4,5-*d*]pyridazin-4(5H),7(6H)-dione (IV, R = $CH_2C_6H_5$).—To a solution of 189 g. (0.63 mole) of diethyl 1-benzyl-4,5-imidazoledicarboxylate in 500 ml. of methanol was added 90 g. (1.80 moles) of hydrazine hydrate. The mixture was refluxed with stirring for one hour, cooled, and the white hydrazonium salt of the product filtered with suction and washed with methanol. This material was suspended in 1 l. of water, heated to 85°, and then rendered acidic to congo red by the cautious addition of concd. HCl. After stirring for 15–20 minutes at 85–90°, the mixture was cooled and the white product filtered with suction and washed with water, ethanol, and finally ether. After drying *in vacuo* at 50° the product weighed 133 g. (88%), m.p. 318–220° dec.

Anal. Calcd. for $C_{12}H_{10}N_4O_2$: C, 59.50; H, 4.16; N, 23.13. Found: C, 59.27; H, 4.43; N, 23.12.

1-Benzyl-4,7-dichloroimidazo[4,5-*d*]pyridazine (V, R = $CH_2C_6H_5$).—Fifty grams (0.21 mole) of 1-benzylimidazo-[4,5-*d*]pyridazin-4(5H),7(6H)-dione (IV, R = $CH_2C_6H_5$) was refluxed with 500 ml. of phosphorus oxychloride for five hours. After removal of the excess phosphorus oxychloride by vacuum distillation, the residual yellow sirup was poured into 1500 g. of crushed ice. The mixture was allowed to stand with frequent stirring until the gummy product had completely solidified (4–5 hours), the lumps broken up, and the product filtered with suction and washed with water. An additional quantity of slightly darker material was obtained by neutralizing the combined filtrate with sodium carbonate. Recrystallization from ethanol gave 40 g. (68%) of pale yellow needles, m.p. 160–161°. An analytical sample, prepared by recrystallization from ethanol, melted at 161.0–161.5°.

Anal. Calcd. for $C_{12}H_8Cl_2N_4$: C, 51.63; H, 2.89; Cl, 25.40; N, 20.08. Found: C, 51.73; H, 2.82; Cl, 25.42; N, 20.25.

4,7-Dichloro-1-methylimidazo[4,5-*d*]pyridazine (V, R = CH_3) was prepared in a similar manner from 32.0 g. of 1-methylimidazo-[4,5-*d*]pyridazin-4(5H),7(6H)-dione (IV, R = CH_3)[13] (0.193 mole) and 400 ml. of phosphorus oxychloride. The product was obtained as 24.1 g. (61.5%) of pale yellow needles from N,N-dimethylformamide–water, m.p. 236–237.5°.

Anal. Calcd. for $C_6H_4Cl_2N_4$: C, 35.50; H, 1.99; Cl, 34.91; N, 27.60. Found: C, 35.60; H, 2.18; Cl, 34.90; N, 27.60.

4,7-Dichloro-1-phenylimidazo[4,5-*d*]pyridazine (V, R = C_6H_5).—A similar treatment of 5.0 g. (0.021 mole) of 1-phenylimidazo[4,5-*d*]pyridazin-4(5H),7(6H)-dione (IV, R = C_6H_5)[1] with 60 ml. of phosphorus oxychloride gave 4.2 g. (76%) of product, obtained as colorless needles from ethanol, m.p. 231.0–231.5°.

Anal. Calcd. for $C_{11}H_6Cl_2N_4$: C, 49.83; H, 2.28; Cl, 26.75; N, 21.14. Found: C, 50.08; H, 2.32; Cl, 26.76; N, 20.93.

4-Substituted Amino-1-benzyl-7-chloroimidazo[4,5-*d*]-pyridazines (VI) (Table I).—1-Benzyl-4,7-dichloroimidazo-[4,5-*d*]pyridazine (V, R = $CH_2C_6H_5$) (27.9 g., 0.10 mole) was mixed with 250 ml. of ethanol containing 0.50 mole of ammonia or the desired amine, and heated at 100–110° in a 1-l. stainless steel autoclave for 12 hours. After cooling to 4°, the products were removed by suction filtration, washed with water, and recrystallized from the appropriate solvent (Table I).

Evaporation of the ethanolic mother liquors from these reactions to dryness *in vacuo* usually left a dark-colored tarry material which slowly solidified when stirred with 5% sodium hydroxide solution. Recrystallization of this solid from ethanol with Norit gave **1-benzyl-7-chloro-4-ethoxyimidazo-[4,5-*d*]pyridazine (VII)** as colorless fine needles, m.p. 190–192°.

Anal. Calcd. for $C_{14}H_{13}ClN_4O$: C, 58.24; H, 4.54; Cl, 12.28; N, 19.41; O, 5.55. Found: C, 58.07; H, 4.53; Cl, 12.80; N, 19.77; O, 5.62.

4-Substituted Aminoimidazo[4,5-*d*]pyridazines (VIII) (Table II).—The appropriate 4-substituted amino-1-benzyl-7-chloroimidazo[4,5-*d*]pyridazine (VI) (0.10 mole) was suppended in 600 ml. of liquid ammonia in a round-bottomed flask fitted with an efficient stirrer and a drying tube. The stirrer was started, and small pieces of metallic sodium were added over a 1-hour period until a permanent deep blue color was obtained. This usually required approximately 0.40–0.42 g. atom of sodium. The mixture was neutralized by the careful addition of 0.45 mole of ammonium chloride, and then allowed to evaporate to dryness. The residual solid was washed well with dry ether to remove toluene and bibenzyl and air-dried. This material was dissolved in aqueous HCl, decolorized with Norit, and reprecipitated by careful neutralization with 10% sodium hydroxide. Additional purification was achieved by recrystallization from an appropriate solvent (see Table II).

When reduction of the 1-benzyl-7-chloro-4-dialkylaminoimidazo[4,5-*d*]pyridazines was attempted by this method, evaporation of the ammonia left a bright red tar which could be separated from the inorganic salts by extraction with warm acetone, filtering the salts with suction, and evaporation of the filtrate to dryness *in vacuo*. In the preparation of 4-(di-*n*-propylamino)-imidazo[4,5-*d*]pyridazine, the product could be isolated from the tarry by-products by extracting with three portions of hot 10% sodium hydroxide (150 ml. each), decolorizing with Norit, and neutralizing to *p*H 5–6 with concd. hydrochloric acid. The resulting precipitate was filtered with suction, and recrystallized from 30% ethanol to constant melting point (see Table II).

4-(Dimethylamino)- and 4-(diethylamino)-imidazo[4,5-*d*]pyridazine could not be prepared by this method.

1-Benzyl-7-chloro-4-mercaptoimidazo[4,5-*d*]pyridazine (IX).—In a 3-necked flask fitted with a mechanical stirrer,

(10) All melting points are uncorrected and were taken on a Fisher–Johns melting point apparatus.

(11) R. G. Jones, THIS JOURNAL, **74**, 1085 (1932).

(12) (a) R. G. Jones, *ibid.*, **71**, 644 (1949); (b) A. H. Cook and I. M. Heilbron, "Chemistry of Penicillin," H. T. Clarke, *et al.*, Princeton Univ. Press, Princeton, N. J., 1949, p. 921.

(13) This compound was synthesized as directed by Jones (ref. 1), with the exception that diethyl 1-methyl-2-mercapto-4,5-imidazoledicarboxylate was used as an intermediate instead of the corresponding dimethyl ester. The diethyl ester was obtained as colorless needles from dilute ethanol, m.p. 124.0–124.5°. Calcd. for $C_{10}H_{14}N_2O_4S$: C, 46.50; H, 5.46; N, 10.85. Found: C, 46.47; H, 5.72; N, 10.93.

reflux condenser and gas entry tube, was placed 100 ml. of absolute ethanol and 0.83 g. (0.036 mole) of metallic sodium. After all of the sodium had reacted, the solution was saturated with dry hydrogen sulfide. Five grams (0.018 mole) of 1-benzyl-4,7-dichloroimidazo[4,5-*d*]pyridazine (V, R = $CH_2C_6H_5$) was added, and the mixture was stirred under reflux for one hour. The sodium chloride was removed by suction filtration, washed with a little absolute ethanol, and the combined filtrates evaporated to dryness *in vacuo*. The solid yellow residue was dissolved in 100 ml. of warm water, acidified to congo red with concd. hydrochloric acid, and the pale yellow solid filtered with suction and washed with water. This material was purified by dissolving in hot dilute potassium hydroxide, filtering, and then precipitated by the addition of glacial acetic acid to obtain 4.7 g. (94%) of a pale yellow powder, m.p. 207–209° with dec.

Anal. Calcd. for $C_{12}H_9ClN_4S$: C, 52.08; H, 3.28; Cl, 12.82; N, 20.25, Found: C, 52.69; H, 3.42; Cl, 12.93; N, 20.64.

4-Mercaptoimidazo[4,5-*d*]pyridazine (X).—In a 3-necked flask fitted with a mechanical stirrer and drying tube was placed 500 ml. of liquid ammonia and 14.1 g. (0.051 mole) of 1-benzyl-7-chloro-4-mercaptoimidazo[4,5-*d*]pyridazine (IX). The stirrer was started, and 6.44 g. (0.28 mole) of metallic sodium cut into small pieces was added over a 1-hour period. The deep blue reaction mixture was allowed to stir for an additional half-hour, and then treated with 16 g. (0.3 mole) of ammonium chloride, added during 15 minutes. The yellow solution was allowed to evaporate to dryness, the residual brown solid taken up in 250 ml. of boiling water, decolorized with Norit, and the clear orange filtrate neutralized with concd. HCl. After cooling overnight, the precipitated product was filtered with suction and washed with water to obtain 2.5 g. (32.5%) of pale yellow needles, which decompose slowly above 315°. An analytical sample was obtained as colorless tiny needles from water, dec. above 315°.

Anal. Calcd. for $C_5H_4N_4S$: C, 39.46; H, 2.65; N, 36.81; S, 21.07. Found: C, 39.20; H, 3.08; N, 36.57; S, 21.85.

1-Benzyl-7-chloroimidazo[4,5-*d*]pyridazin-4(5H)-one (XI).—Five grams (0.018 mole) of 1-benzyl-4,7-dichloroimidazo[4,5-*d*]pyridazine, (V, R = $CH_2C_6H_5$) was suspended in 50 ml. of 10% aqueous sodium hydroxide, and refluxed for 75 minutes. The clear yellow solution was decolorized with Norit, acidified with glacial acetic acid, and the white precipitate filtered with suction and washed with water. Recrystallization from ethanol afforded 3.8 g. (81%) of long colorless needles, m.p. 232–233°. A second recrystallization from ethanol did not raise the melting point.

Anal. Calcd. for $C_{12}H_9ClN_4O$: C, 55.28; H, 3.48; Cl, 13.60; N, 21.49; O, 6.15. Found: C, 55.41; H, 3.63; Cl, 13.92; N, 21.29; O, 6.18.

Imidazo[4,5-*d*]pyridazin-4(5H)-one(XII).—Twenty grams (0.077 mole) of 1-benzyl-7-chloroimidazo[4,5-*d*]pyridazin-4-(5H)-one (XI) was mixed with 600 ml. of liquid ammonia, and 9.2 g. (0.40 mole) of small pieces of sodium metal were added over a 1-hour period. The reaction mixture was stirred vigorously throughout this addition. After stirring for an additional half-hour, the solution was carefully neutralized with 21.4 g. (0.40 mole) of ammonium chloride. The solution was allowed to evaporate to dryness and the residual brown solid washed well with ether and air-dried. This material was dissolved in hot dilute ammonium hydroxide, decolorized with Norit, and precipitated by the addition of hydrochloric acid. The white product was filtered, washed with water, and dried *in vacuo* at 100° to yield 5.9 g. (57%), m.p. >300°. An analytical sample was obtained as colorless needles by precipitation from hot dilute sodium hydroxide with acetic acid, m.p. >300°.

Anal. Calcd. for $C_5H_4N_4O$: C, 44.12; H, 2.96; N, 41.18. Found: C, 44.09; H, 3.08; N, 41.14.

1-Benzylimidazo[4,5-*d*]pyridazine (XIII).—1-Benzyl-4,7-dichloroimidazo[4,5-*d*]pyridazine (V, R = $CH_2C_6H_5$) (7.4 g., 0.026 mole) was suspended in 125 ml. of ethanol containing 17.6 ml. of 3 *N* sodium hydroxide solution, and hydrogenated in the presence of 1.5 g. palladium-on-charcoal (5%) under 30 p.s.i. at 60°. After hydrogen uptake was complete, the mixture was filtered and the filtrate evaporated to dryness *in vacuo*. The solid residue was washed well with water, and recrystallized from 25% ethanol to obtain 3.35 g. (60.5%) of colorless prisms, m.p. 206–207°.

Anal. Calcd. for $C_{12}H_{10}N_4$: C, 68.60; H, 4.79; N, 26.67. Found: C, 68.50; H, 4.94; N, 26.53.

1-Benzyl-4,7-bis-(benzylamino)-imidazo[4,5-*d*]pyridazine (XIV).—1-Benzyl-4,7-dichloroimidazo[4,5-*d*]pyridazine (V, R = $CH_2C_6H_5$) (14.0 g., 0.05 mole) was refluxed with 75 ml. of benzylamine for five hours. Upon cooling to room temperature, an almost colorless precipitate of leaflets separated. Enough water was added to complete the precipitation and the product was filtered and washed with water. The crude product (19.0 g.) was recrystallized from N,N-dimethylformamide–water to obtain 16.4 g. (78%) of colorless prisms, m.p. 202–203°.

Anal. Calcd. for $C_{26}H_{24}N_6$: C, 74.26; H, 5.76; N, 19.98. Found: C, 74.27; H, 5.88; N, 19.94.

4,7-Bis-(benzylamino)-imidazo[4,5-*d*]pyridazine Hydrochloride (XV).—Six grams (0.014 mole) of 1-benzyl-4,7-bis-(benzylamino)-imidazo[4,5-*d*]pyridazine (XIV) was mixed with 200 ml. of liquid ammonia, and, with vigorous mechanical stirring, small pieces of metallic sodium added until a permanent deep blue color was formed. This required about 0.7 g. (0.03 g. atom) of sodium. The thick mixture was treated with 1.6 g. (0.03 mole) of ammonium chloride and then allowed to evaporate to dryness, The residual yellow solid was washed with ether and air-dried. The resulting cream-colored powder was treated with 40 ml. of warm water to dissolve the inorganic salts, and the gummy free base converted to the insoluble, crystalline hydrochloride by the addition of a little concd. hydrochloric acid. This material was filtered with suction, washed with water and recrystallized from 1-propanol to obtain 2.6 g. (51%) of colorless needles, m.p. 201–203°.

Anal. Calcd. for $C_{19}H_{19}ClN_6$: C, 62.21; H, 5.22; Cl, 9.66; N, 22.91. Found: C, 62.04; H, 5.49; Cl, 9.51; N, 22.88.

The free base was a viscous gummy material which could not be obtained crystalline. This substance was insoluble in water, but was readily soluble in 5% sodium hydroxide solution.

7-Chloro-4-mercapto-1-methylimidazo[4,5-*d*]pyridazine (XVI).—To a solution prepared by dissolving 1.86 g. (0.081 mole) of sodium in 100 ml. of absolute ethanol and then saturating with dry hydrogen sulfide, was added 8.16 g. (0.0402 mole) of 4,7-dichloro-1-methylimidazo[4,5-*d*]pyridazine (V, R = CH_3). The resulting mixture was refluxed with stirring for 5 hours, and finally evaporated to dryness *in vacuo*. The yellow residue was treated with 60 ml. of water, acidified to Congo red with concd. hydrochloric acid, and the yellow solid isolated by suction filtration and washed with water. Recrystallization from N,N-dimethylformamide–water gave 6.05 g. (75%) fo pale yellow needles, dec. above 300°.

Anal. Calcd. for $C_6H_5ClN_4S$: C, 35.90; H, 2.51; Cl, 17.69; N, 27.92; S, 15.98. Found: C, 35.99; H, 2.65; Cl, 17.55; N, 27.82, S, 16.30.

An attempt to convert this compound to 4,7-dimercapto-1-methylimidazo[4,5-*d*]pyridazine (XVII) by refluxing for 24 hours with a large excess of sodium hydrosulfide in ethanol gave only an 83% recovery of the starting material (XVI).

4,7-Dimercapto-1-methylimidazo[4,5-*d*]pyridazine (XVII).—A solution of 2 g. of sodium in 100 ml. of absolute ethanol was evaporated to dryness *in vacuo*, and the resulting white residue of sodium ethoxide taken up in 100 ml. of N,N-dimethylformamide. The colorless solution thus obtained was saturated with dry hydrogen sulfide to form a deep blue solution. Five grams (0.025 mole) of 7-chloro-4-mercapto-1-methylimidazo[4,5-*d*]pyridazine (XVI) was added, and the solution was refluxed with stirring for three hours. The reaction mixture was concentrated to about 30 ml. *in vacuo*, and the deep blue solution poured into 90 ml. of water causing immediate decolorization to form a clear, pale yellow solution. The addition of concd. hydrochloric acid to *p*H 2 caused the separation of the product as a cream-colored finely divided solid which was filtered with suction and washed with water. This material was purified by dissolving in dilute sodium carbonate solution, filtering, and acidifying with concd. hydrochloric acid to obtain 4.3 g. (87%) of a pale yellow powder, m.p. 249–251° dec.

Anal. Calcd. for $C_6H_6N_4S_2$: C, 36.34; H, 3.05; N, 28.26; S, 32.35. Found: C, 36.50; H, 3.28; N, 28.51; S, 32.58.

4-Amino-7-chloro-1-methylimidazo[4,5-*d*]pyridazine (XVIII).—4,7-Dichloro-1-methylimidazo[4,5-*d*]pyridazine (V, R = CH_3) (24.1 g., 0.119 mole) was treated with a solution of 20 ml. of liquid ammonia in 200 ml. of ethanol in a stainless steel autoclave at 150° for five hours. After cooling, the product was isolated by suction filtration and washed with water to remove ammonium chloride. This material was dissolved in 150 ml. of hot dilute hydrochloric acid, decolorized with Norit, and precipitated by the addition of 20% aqueous potassium hydroxide. The product was thus obtained as 11.7 g. (53%) of almost colorless microcrystals, m.p. 273–275° dec.

Anal. Calcd. for $C_6H_6ClN_5$: C, 39.25; H, 3.29; Cl, 19.31; N, 38.15. Found: C, 39.30; H, 3.30; Cl, 19.15; N, 38.32.

4-Amino-1-methylimidazo[4,5-*d*]pyridazine (XIX) and 7-Amino-1-methyl-imidazo[4,5-*d*]pyridazine (XX).—Sixteen grams (0.087 mole) of 4-amino-7-chloro-1-methylimidazo-[4,5-*d*]pyridazine (XVIII) was hydrogenated at 30 p.s.i. (55–60°) over 2.0 g. of palladium-on-charcoal catalyst (5%) in 200 ml. of glacial acetic acid containing 7.14 g. of sodium acetate. After hydrogen uptake was complete (19 hours), the mixture was filtered and evaporated to dryness *in vacuo*. The residue was taken up in 150 ml. of water, neutralized with sodium bicarbonate, and evaporated to about 75 ml. *in vacuo*. After standing for two days at 4°, the precipitate was filtered with suction and washed with a little cold water. Recrystallization of this material from water with Norit gave 0.5 g. (3.9%) of **7-amino-1-methylimidazo[4,5-*d*]pyridazine (XX)** as colorless needles, m.p. 318–319° dec.

Anal. Calcd. for $C_6H_7N_5$: C, 48.31; H, 4.73; N, 46.96. Found: C, 47.73; H, 4.67; N, 46.83.

The original aqueous mother liquor plus washings from above was evaporated to dryness *in vacuo* to leave 30.4 g. of product mixed with the inorganic salts. This solid was ground to a fine powder, extracted with 1000 ml. of boiling absolute ethanol for 15 minutes on the steam-bath and filtered while hot. After standing at 4° overnight, the precipitate of colorless needles was filtered with suction and recrystallized from N,N-dimethylformamide to obtain **4-amino-1-methylimidazo[4,5-*d*]-pyridazine (XIX)** as colorless long needles (5.7 g., 44%), m.p. 295–296° dec.

Anal. Calcd. for $C_6H_7N_5$: C, 48.31; H, 4.73; N, 46.96. Found: C, 48.04; H, 4.74; N, 46.76.

The solubility characteristics of these two compounds (XIX and XX) were markedly different; *e.g.*, XIX was quite soluble in hot N,N-dimethylformamide or in cold water, while XX was practically insoluble in boiling N,N-dimethylformamide and only slightly soluble in cold water.

A comparison of the ultraviolet absorption spectra of XIX and XX with those of 9-methyl- and 7-methyladenine will be found in Table III.

NORTH CHICAGO, ILL.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF WAYNE STATE UNIVERSITY AND THE RESEARCH LABORATORIES OF PARKE, DAVIS AND CO.]

Elaiomycin.[1] An Aliphatic α,β-Unsaturated Azoxy Compound

BY CALVIN L. STEVENS, BERNARD T. GILLIS,[2,3] JAMES C. FRENCH[2] AND THEODORE H. HASKELL

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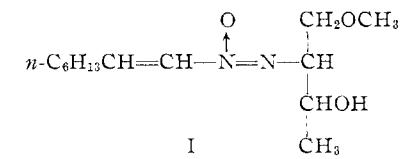
The antibiotic Elaiomycin was shown to have the structure represented by formula I, containing the chemically unique aliphatic α,β-unsaturated azoxy group.

The antibiotic Elaiomycin was isolated from submerged culture filtrates of *Streptomyces hepaticus*.[4] The biological action and chemical structure of the antibiotic are unique. Elaiomycin, which was isolated as a distillable oil, has marked activity only against certain virulent and avirulent mammalian strains of tubercle bacilli[5] and is shown by this investigation to have the chemical structure I.



I

In a previous publication[6] I has been characterized as a stable, optically active oil, $C_{13}H_{26}N_2O_3$, which contained no ionizable groups in the *p*H range 2–10. The presence of one alkoxyl and two terminal methyl groups was indicated by analysis and compound I gave a positive iodoform test.

The hydrogen content of the molecular formula allowed only two double bonds and the ultraviolet absorption spectrum, with λ_{max} 237.5, ϵ 11,000, indicated the double bonds to be in conjugation.

The infrared spectrum[6] of I was relatively simple and had a weak absorption band at 6.03 μ, which was best interpreted as a carbon–carbon double bond. Any of the common functional groups which contain the carbon–oxygen or carbon–nitrogen double bond were excluded.[7] In addition, the infrared spectrum had a strong band at 2.92 μ which indicated an OH or possibly an NH group. The acetate derivative of I was prepared in 70% yield. This derivative II was shown to be a monoacetate, the infrared spectrum of which had no absorption in the 3 μ region. The absorption bands at 5.75 and 8.05 μ indicated an O-acetate and not an N-acetate and this evidence clearly showed Elaiomycin to have only one acylatable hydrogen incorporated in an hydroxyl group.

Elaiomycin absorbed two molar equivalents of hydrogen in alcohol solution in the presence of platinum catalyst to give a deoxydihydro product III.

(1) A preliminary communication appeared in THIS JOURNAL, **78**, 3229 (1956).

(2) Parke, Davis and Co. Fellow.

(3) Abstracted in part from the Doctoral thesis of Bernard T. Gillis, Wayne State University, April, 1956.

(4) L. E. Anderson, P. R. Burkholder, J. Ehrlich and H. S. Sun, *Antibiotics & Chemotherapy*, **6**, 100 (1956).

(5) The biologic studies of Elaiomycin have been reported by J. Ehrlich, L. E. Anderson, G. L. Coffey, W. H. Feldman, M. W. Fisher, A. B. Hillegas, A. G. Karlson, M. P. Kaudsen, J. K. Weston, A. S. Youmans and G. P. Youmans, *ibid.*, **4**, 338 (1954).

(6) The isolation and chemical characterization of Elaiomycin has been reported by T. H. Haskell, A Ryder and Q. R. Bartz, *ibid.*, **4**, 141 (1954). In this article the ultraviolet and the infrared spectra are reproduced and a discussion is presented of the homogeneity and purity of the product.

(7) An oxime of an aliphatic aldehyde or ketone was a possible exception. Heptaldehyde oxime had an absorption band, the intensity and position of which was similar to the 6.03 μ band of I.