teristic amino acid absorption peaks appear at 6.3, 7.1 and 7.3 μ. The bands at 6.6, 6.9 and 7.5 μ are absent.[13]

The Low Melting Isomer.—This isomeric modification differs from the high melting isomer in having a hydroxyl band at 2.8 μ and in addition the broad band at 3.1 μ (compare β-phenylserine and β-allophenylserine[13b]). The lactam carbonyl band is at 5.8 μ. The characteristic amino acid peaks are present at 6.3, 6.6, 7.1, 7.3 and 7.5 μ. The band at 6.9 μ is absent.[13b]

DNP Derivatives of the Dioxindole-3-alanines. α-2,4-Dinitrophenylamino-β-dioxindolyl-3-propionic Acid. (A) From the Low Melting Dioxindole-3-alanine.—To a solution of 0.1 g. of dioxindole-3-alanine, m.p. 232°, in 4 ml. of a 4% solution of sodium carbonate, 0.149 g. (100% molar excess) of 1-fluoro-2,4-dinitrobenzene was added, and the mixture stirred vigorously at 40° for 4 hours. The mixture was extracted with ether to remove unreacted fluorodinitrobenzene and the aqueous extract acidified with dilute hydrochloric acid and let stand overnight. A yellow precipitate of 0.15 g., m.p. 160° dec., of the DNP derivative was obtained. Repeated recrystallization from ether–petroleum ether and finally acetone–ether gave yellow needles, m.p. 170° dec.

Anal. Calcd. for $C_{17}H_{14}O_8N_4$: C, 50.74; H, 3.50; N, 13.92. Found: C, 51.21; H, 3.69; N, 13.55.

(B) **From the High Melting Dioxindole-3-alanine.**—A similar preparation from the high melting isomer gave a product which showed no definite melting point, shrinking at about 270° and slowly decomposing as the temperature was raised. The analyses, however, after recrystallization from acetone–ether indicated it to be pure DNP derivative.

Anal. Calcd. for $C_{17}H_{14}O_8N_4$: C, 50.74; H, 3.50; N, 13.92. Found: C, 50.75; H, 3.40; N, 13.64.

Acetyl Derivatives of the Dioxindole-3-alanines. (A) α-Acetamino-β-dioxindolyl-3-propionic Acid Lactone (XXa) (from Low Melting XVIII).—To an ice-cold solution of 0.114 g. of dioxindole-3-alanine, m.p. 232°, in 0.53 ml. of 2 *N* sodium hydroxide (in an atmosphere of nitrogen), 0.46 g. of acetic anhydride was added dropwise with swirling. The reaction mixture at first formed two layers which became a solution on swirling and heating to 35°, whereupon colorless platelets began to separate. Heating was continued at 35–45° for four hours. After cooling in an ice-bath and acidifying with 0.12 ml. of 6 *N* sulfuric acid, 0.1 g. of XXa, m.p. 274° dec., was filtered. The ninhydrin test was negative. Several recrystallizations from acetone gave microcrystals, m.p. 284° dec.

Anal. Calcd. for $C_{13}H_{12}O_4N_2$: C, 59.98; H, 4.64; N, 10.76. Found: C, 59.99; H, 4.71; N, 10.71.

(B) **α-Acetamino-β-dioxindolyl-3-propionic Acid Lactone (XXb) (from the High Melting XVIII).**—A similar preparation to that described in "A" above yielded from dioxindole-3-alanine, m.p. 259°, a lactone amide XXb melting at 226° (no decomposition). The ninhydrin test was negative. It was recrystallized from acetone.

Anal. Calcd. for $C_{13}H_{12}O_4N_2$: C, 59.98; H, 4.64; N, 10.76. Found: C, 59.72; H, 4.62; N, 10.82.

The Dakin–West Reaction with the Isomeric Dioxindole-3-alanines. (A) α-Acetamino-α-aceto-β-dioxindolyl-3-propionic Acid Lactone (XXI). From Low Melting Dioxindole-3-alanine.—A mixture of 0.1 g. of dioxindole-3-alanine, m.p. 232° dec., 0.2 g. of pyridine and 0.35 g. of acetic anhydride was heated on a steam-bath and in a nitrogen atmosphere for five hours. Solution occurred on heating, and after one hour a precipitate formed. The solution was cooled, water carefully added, and the precipitate filtered. Several recrystallizations from acetone gave 0.084 g. of product XXI melting at 265–266° dec.

Anal. Calcd. for $C_{15}H_{14}O_5N_2$: C, 59.59; H, 4.66; N, 9.26. Found: C, 60.16; H, 4.96; N, 9.22.

(B) **XXI from High Melting Dioxindole-3-alanine.**—A similar preparation from dioxindole-3-alanine, m.p. 259°, gave a product identical with that secured at "A" above in melting point and infrared spectra.

Dioxindole-3-alanine (XVIII) from Oxidation of Oxindole-3-alanine (XIII).—To 1.10 g. (0.005 mole) of oxindole-3-alanine (XIII) dissolved in 5 ml. of 1 *N* sodium hydroxide, cooled to 10°, two equivalents of finely ground iodine was added (1.27 g.). The solution was cooled to 0° and 3 ml. of 5 *N* sodium hydroxide was introduced. After warming to room temperature, 4 ml. of 5 *N* sulfuric acid was added, followed by 0.36 g. of iodic acid in 5 ml. of water. The mixture was warmed gently to coagulate the iodine, filtered, and the filtrate concentrated *in vacuo*. The crystals, m.p. 225° dec., which separated were filtered and recrystallized from water in the form of colorless prisms, m.p. 228° dec. This product was identical in all respects to the low melting dioxindole-3-alanine already described. Its infrared curve was identical with that of the low melting isomer and showed none of the characteristically different bands displayed by the high melting diamer.

FRANKLIN PARK, ILLINOIS

[CONTRIBUTION FROM THE WELLCOME RESEARCH LABORATORIES]

Studies on Condensed Pyrimidine Systems. XVII. Some Halogenopurines

BY GERTRUDE B. ELION AND GEORGE H. HITCHINGS

RECEIVED JANUARY 20, 1956

The syntheses of 2,6-dichloropurine and 2,6-dibromopurine from xanthine and of 6-bromopurine from hypoxanthine are reported. The chlorination of xanthine is favored by the addition of half a molecular equivalent of water to the phosphoryl chloride. Treatment of 6-chloropurine and 2,6-dichloropurine with hydriodic acid leads to 6-iodopurine and a monochloro-monoiodopurine, respectively.

The importance of halogenopurines as intermediates for the synthesis of a variety of substituted purines has been amply demonstrated by Fischer's classical syntheses of the naturally occurring purines from 2,6,8-trichloropurine.[1] In addition, the halogenopurines are of interest in themselves as possible antagonists of the purine moieties of the nucleic acids, which have been actively sought by these laboratories for a number of years.[2–4] Consequently, the synthesis of some new 6-monosubstituted and 2,6-disubstituted halogenopurines was undertaken (Reaction Scheme).

The chlorination of methylated xanthines and of uric acid with phosphoryl chloride or with mixtures of phosphoryl chloride and phosphorus pentachloride has been known for a long time,[5–7] but xanthine itself has not been successfully chlorinated. The attempted chlorination of xanthine with phosphoryl chloride and trimethylamine led only to 2,6-bis-(dimethylamino)-purine.[8] The addition of di-

(1) E. Fischer, *Ber.*, **32**, 435 (1899).

(2) G. H. Hitchings, G. B. Elion, E. A. Falco, P. B. Russell, M. B. Sherwood and H. VanderWerff, *J. Biol. Chem.*, **183**, 1 (1950).

(3) G. B. Elion, G. H. Hitchings and H. VanderWerff, *ibid.*, **192**, 505 (1951).

(4) G. H. Hitchings and G. B. Elion, *Ann. N. Y. Acad. Sci.*, **60**, 195 (1954).

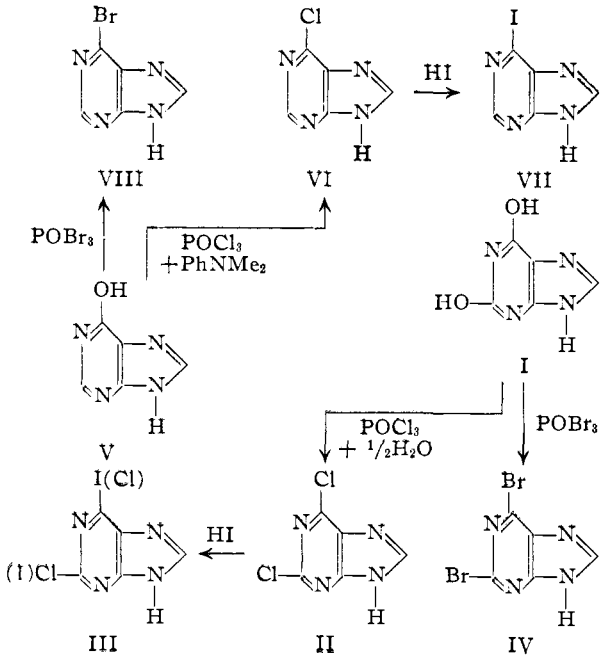
(5) E. Fischer, *Ber.*, **28**, 2480 (1895).

(6) E. Fischer, *ibid.*, **30**, 2220, 2400 (1897).

(7) E. Fischer and F. Ach, *ibid.*, **30**, 2208 (1897).

(8) R. K. Robins and B. E. Christensen, THIS JOURNAL, **74**, 3624 (1952).

REACTION SCHEME



methylaniline to phosphoryl chloride, which has facilitated the chlorination of hydroxypyrimidines,[9] uric acid[10] and hypoxanthine[11] under reflux conditions does not result in the chlorination of xanthine. The finding that the addition of a half of a molecular equivalent of water to phosphoryl chloride favors the formation of oxazolo(5,4-d)pyrimidines from the 4-amino-5-benzamido-6-hydroxypyrimidines[12,13] led to further experimentation with this reagent ("pyrophosphoryl chloride") in the chlorination reactions. It was found to chlorinate xanthine (I) at a temperature around 165° to 2,6-dichloropurine (II). At lower temperatures a considerable amount of xanthine was recovered, at higher temperatures there was formed a brown material which contained organically bound chloride, was insoluble in phosphoryl chloride and appeared to be some polymer of 2,6-dichloropurine.

Since phosphoryl bromide had been used in the preparation of bromopyrimidines[14] and 6-bromo-8-phenylpurines,[13] its utilization for the bromination of hypoxanthine and xanthine was indicated. Both 6-bromopurine (VIII) and 2,6-dibromopurine (IV) have now been prepared. The yields from xanthine were poor and attempts to improve them by increasing the temperature led to the formation of the polymer-like substances noted in the chlorination experiments. The addition of one-half mole of water to the phosphoryl bromide decreased the yield of 2,6-dibromopurine.

The transformation of chloropurines to iodopurines with fuming hydriodic acid and phosphonium iodide has sometimes been accompanied by the reduction of one of the chloro groups. Thus, trichloropurine has been transformed to 2,6-diiodopurine and 2,6-dichloro-7-methylpurine to 2-iodo-7-methylpurine.[15] With cold 47% hydriodic acid, the transformation of 6-chloropurine (VI) to 6-iodopurine (VII) and of 2,6-dichloropurine to a monochloro-monoiodopurine (III) has now been achieved. The fact that 6-iodopurine is considerably more reactive than 6-chloropurine has made possible the synthesis of 6-cyanopurine and related compounds from 6-iodopurine.[16] On the basis of the greater reactivity of the 6-chloro group compared with that of the 2-chloro group, it appears likely that the product from 2,6-dichloropurine is 2-chloro-6-iodopurine. Transformation reactions which should lead to an unequivocal identification are now in progress and will be the subject of another communication.

The ultraviolet absorption spectra of the halogenopurines are given in Table I. As would be anticipated, the heavier the weight of the halogen, the greater is the shift toward the longer wave lengths. The presence of a second halogen in the molecule also produces a bathochromic shift.

Paper chromatography (Table II) indicates a generally greater mobility for the compounds with lower molecular weight in two of the solvents, B and C.

TABLE I

ULTRAVIOLET ABSORPTION SPECTRA

Purines	pH 1 λ_{max} mμ	pH 1 E_m	pH 11 $\lambda_{max.}$, mμ	pH 11 E_m
2,6-Dichloro	250[a]	3930		
	275	8900	280	8500
2,6-Dibromo	247	3900		
	279	8000	283	7750
2(6)-Chloro-6(2)-iodo	252	4800		
	286	10400	289	8800
6-Chloro	264	8950	274	8600
6-Bromo	266	9700	275	8550
6-Iodo	276	10400	280	9300

[a] Inflection.

TABLE II

PAPER CHROMATOGRAPHY

A, *n*-butyl alcohol saturated with water–NH_3 atmosphere[17]; B, 5% ammonium sulfate–5% isopropyl alcohol; C, 5% disodium phosphate–isoamyl alcohol.[18]

Purines	R_f values A	B	C
2,6-Dichloro	0.71	0.56	0.38
2,6-Dibromo	.72	.47	.31
2(6)-Chloro-6(2)-iodo	.71	.46	.31
6-Chloro	.54	.58	.44
6-Bromo	.59	.58	.40
6-Iodo	.59	.52	.36

Experimental

"Pyrophosphoryl Chloride."—To 200 ml. (2.2 moles) of phosphoryl chloride was added dropwise 20 ml. of water. After all the water had been added, the mixture was boiled for 1.5 hours to dispel the hydrogen chloride, cooled and the

(9) J. Baddiley and A. Topham, *J. Chem. Soc.*, 678 (1944).
(10) J. Davoll and B. A. Lowy, THIS JOURNAL, **73**, 2936 (1951).
(11) A. Bendich, P. J. Russell, Jr., and J. J. Fox, *ibid.*, **76**, 6073 (1954).
(12) E. A. Falco, G. B. Elion, E. Burgi and G. H. Hitchings, *ibid.*, **74**, 4897 (1952).
(13) G. B. Elion, E. Burgi and G. H. Hitchings, *ibid.*, **73**, 5235 (1951).
(14) D. R. V. Golding and A. E. Senear, *J. Org. Chem.*, **12**, 293 (1947).
(15) E. Fischer, *Ber.*, **31**, 2550 (1898).
(16) L. B. Mackay and G. H. Hitchings, THIS JOURNAL, **78**, 3511 (1956).
(17) R. Hotchkiss, *J. Biol. Chem.*, **175**, 315 (1948).
(18) C. E. Carter, THIS JOURNAL, **72**, 1466 (1950).

top layer decanted from the thick sirup at the bottom. The top layer was used for the chlorination of xanthine.

2,6-Dichloropurine (II).—A mixture of 8 g. of xanthine and 64 ml. of "pyrophosphoryl chloride" was heated in a sealed glass tube at 165° for 19 hours. After cooling, the brown solution was decanted from the solid residue in the tube and the volatile material was removed under reduced pressure. The sirupy residue was poured onto 200 g. of crushed ice, a small tan precipitate removed and the filtrate extracted six times with 350 ml. portions of ether. The ethereal solution was allowed to stand over anhydrous potassium carbonate for one hour and then over calcium sulfate overnight. On evaporation of the ether, the product (4.3 g., 43%), m.p. 180° dec., was obtained. A small portion was recrystallized from 150 parts of boiling water, and dried at 100°. The m.p. of the purified material was 181° dec.

Anal. Calcd. for $C_5H_2N_4Cl_2$: C, 31.7; H, 1.1; N, 29.6; Cl, 37.6. Found: C, 32.0; H, 1.1; N, 29.8; Cl, 37.7.

2(6)-Chloro-6(2)-iodopurine (III).—To 10 ml. of concentrated hydriodic acid (sp. gr. 1.5) in an ice-bath was added 1 g. (0.0053 mole) of 2,6-dichloropurine. The compound dissolved and a precipitate formed very quickly thereafter. After one hour at 0°, the mixture was filtered. The precipitate was suspended in 20 ml. of cold water and the acidity was adjusted to *p*H 5 with ammonium hydroxide. The pale yellow precipitate was filtered, washed with water and dried at 100° (1 g., 67%), m.p. 203° dec. After two recrystallizations from 15 parts of hot water, the melting point was raised to 208° dec.

Anal. Calcd. for $C_5H_2N_4ClI$: C, 21.3; H, 0.7; N, 20.1. Found: C, 21.3; H, 0.7; N, 20.1.

2,6-Dibromopurine (IV).—A mixture of 10 g. of xanthine and 100 g. of phosphoryl bromide was heated at 135° for 22 hours in a flask equipped with an air condenser and drying tube. The mixture was cooled, shaken with 500 ml. of benzene and filtered through a sintered glass funnel. The insoluble residue was treated with 100 g. of crushed ice and the precipitate collected. The damp filter cake was leached with 300 ml. of acetone and the insoluble residue (7.9 g.) of unreacted xanthine was removed. The acetone filtrate was neutralized with ammonium hydroxide and evaporated to dryness in an air stream to give a product which, after being leached with 50 ml. of cold water to remove inorganic salts, consisted of 1.9 g. of crude 2,6-dibromopurine. It was purified by solution in *ca.* 200 parts of boiling water, filtration and evaporation of the solution to a small volume before chilling.

Anal. Calcd. for $C_5H_2N_4Br_2$: C, 21.6; H, 0.7; N, 20.1. Found: C, 21.4; H, 1.2; N, 19.8.

6-Iodopurine (VII).—To 50 ml. of concentrated hydriodic acid (sp. gr. 1.70[19]) in an ice-bath was added slowly with stirring 5.7 g. (0.04 mole) of 6-chloropurine.[11] The mixture was allowed to stand at 0° for 1.5 hours and then filtered through a sintered glass funnel. The precipitate was suspended in 40 ml. of cold water and adjusted to *p*H 7.5 by the addition of concentrated ammonium hydroxide. After thorough chilling, the precipitate (7.2 g.) was filtered, washed with cold water and dried in a vacuum desiccator. It was purified by solution in 75 ml. of water containing 5 ml. of concentrated ammonium hydroxide and precipitation at *p*H 5 with acetic acid. After drying at 65°, the hemihydrate of 6-iodopurine (5.45 g., 54%), m.p. 167° dec. was obtained. The moisture is lost at 110° with some darkening of the compound.

Anal. Calcd. for $C_5H_3N_4I \cdot {}^1/_2H_2O$: C, 23.5; H, 1.6; I, 49.8; H_2O, 3.5. Found: C, 23.3; H, 1.6; I, 49.9; H_2O, 3.9.

6-Bromopurine (VIII). A mixture of 5 g. of hypoxanthine and 50 g. of phosphoryl bromide was heated at 130° for seven hours in a flask equipped with an air condenser and a drying tube. The mixture was semi-liquid but never clear during this period. After cooling, the reaction mixture was extracted with five 100-ml. portions of benzene to remove excess phosphoryl bromide. The solid residue was treated with 60 g. of crushed ice and filtered, after about ten minutes. The filter cake was leached with 200 ml. of 50% aqueous acetone to dissolve the 6-bromopurine and filtered. The aqueous and acetone filtrates were combined, carefully adjusted to *p*H 7–8 by the addition of ammonium hydroxide, while being kept cold in an ice-bath, and then concentrated to a volume of about 30 ml. under reduced pressure. After chilling, the precipitate of 6-bromopurine was collected, washed with a small amount of ice-water and dried in a vacuum desiccator (4.1 g.). It was recrystallized from 15 ml. of boiling water using Darco. It crystallized as colorless needles (3 g., 40%). At 130° the compound turns dark yellow and decomposes slowly without melting when heated to 200°. It was dried at 105° for analysis.

Anal. Calcd. for $C_5H_3N_4Br$: C, 30.2; H, 1.5; N, 28.1; Br, 40.2. Found: C, 30.6; H, 2.0; N, 28.4; Br, 40.8.

Ultraviolet Absorption Spectra.—The ultraviolet absorption spectra were measured on a Beckman model DU spectrophotometer at a concentration of 10 mg. per l. For *p*H 1, 0.1 *N* hydrochloric acid was used, for *p*H 11, a Sørensen glycine–sodium hydroxide buffer.

Paper Chromatography.—The paper chromatograms were run at 28° on S. and S. #597 paper, in ascending fashion until the front was about 30 cm. from the origin. The ammonium sulfate–isopropyl alcohol system was prepared by adding 5 ml. of isopropyl alcohol to 95 ml. of 5% aqueous ammonium sulfate.

Acknowledgment.—We are indebted to Ralph Kilsheimer for technical assistance and to Samuel Blackman, P. R. W. Baker and Veronica Purdey for the microanalyses.

TUCKAHOE 7, NEW YORK

(19) In subsequent experiments hydriodic acid of sp. gr. = 1.5 was found to be satisfactory.