[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, OREGON STATE COLLEGE]

The Preparation and Properties of Certain 2- and 4-Chloro Substituted Pteridines[1]

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Procedures for the preparation of 2-chloro-, 4-chloro- and 2,4-dichloro-6,7-dimethylpteridines based on the cyclization of the 4,5-diaminochloropyrimidines are described. The chloro substituent in 4-chloro-6,7-dimethylpteridine is very reactive as judged by replacement reactions. On the other hand, the 2-chloro substituent appears to be rather unreactive. When 2,4-dichloro-6,7-dimethylpteridine is subjected to replacement reactions there are frequently sufficient differences in activity as to yield a 2-chloro derivative. The behavior of the three chloropteridines upon dehalogenation with palladized charcoal are different; the 4-chloro yields a pteridinol; the 2-chloro yields 2-chlorodihydro-6,7-dimethylpteridine; while the 2,4-dichloro-6,7-dimethylpteridine gives a resinous product.

The activity of phosphorus oxychloride as a chlorinating agent for nitrogen heterocycles containing a pyrimidine nucleus varies rather widely. While simple pyrimidones and pyrimidinediones are readily chlorinated,[2] the ease of chlorination may be markedly influenced by the other substituents on the ring. In recent years it has been observed that the addition of dimethylaniline[3] or diethylaniline[4] to the reaction mixture has made it possible to chlorinate certain pyrimidine compounds which otherwise, in some instances, would have failed to yield a characterizable halogen derivative.

When the pyrimidine ring is part of a fused ring system, there are striking contrasts in the ease with which they may be chlorinated with the halides of phosphorus. For example, the chlorination of quinazolones and quinazolinediones with phosphorus oxychloride, phosphorus pentachloride and mixtures of these reagents are quite straight forward.[5] Furthermore, the chloro derivatives are quite reactive making these compounds very useful intermediates for synthetic purposes. In 4-chloroquinazoline one has an example of one of the most reactive types of halo derivatives.[6]

On the other hand, the purinones and the purinediones are quite resistant to chlorination with phosphorus oxychloride. Only a few chloropurines are known and their preparation has been somewhat difficult.[7] Furthermore, attempts to prepare chloropurines by the cyclization of chloro-4,5-diaminopyrimidines have as yet not been successful.[7,8] The chloropurines like the chloroquinazolines, however, are fairly reactive.

The literature contains much less information in regard to the chlorination of the pteridinols and pteridinediols than of the aforementioned nitrogen heterocycles. The simple 2-pteridinol, 4-pteridinol and 2,4-pteridinediol are reported to have been destroyed in chlorinating operations.[9] It appears that pteridinols and pteridinediols containing an unsubstituted pyrazine ring will not undergo chlorination. The presence of certain substituents on the pyrazine ring, notably hydroxyl and phenyl, however, have stabilized the pteridine ring so that it will submit to chlorination in the 2- and 4-positions.[10]

Inasmuch as the simple chloropteridines may serve as useful intermediates for the preparation of other pteridine derivatives, this Laboratory undertook a study of their preparation by the cyclization of 4,5-diaminochloropyrimidines. A condensation of 2,4-dichloro-5,6-diaminopyrimidine[11] with glyoxal has been reported.

Although 4,5-diamino-6-chloropyrimidine will cyclize to a purine in anhydrous formamide, it loses the 6-chloro substituent in the process, yielding hypoxanthine.[8] To determine the behavior of the chloro substituted 4,5-diaminopyrimidines in cyclizations yielding pteridines, a number of these diamines were cyclized with biacetyl. 4,5-Diamino-2-chloropyrimidine, 5,6-diamino-4-chloropyrimidine and 5,6-diamino-2,4-dichloropyrimidine readily cyclized to the corresponding chloro-substituted pteridines. Since 2-pteridinol, 4-pteridinol and 2,4-pteridinediol yield resinous products when treated with phosphorus oxychloride, direct cyclization of the corresponding 4,5-diaminochloropyrimidines affords the only method of preparing these interesting intermediates.

In order to determine the reactivity of the chloro-substituents, a number of typical replacement experiments were carried out. In this manner both the 2-mercapto-6,7-dimethylpteridine and 6,7-dimethylpteridinol-2 were synthesized. Experiments with sodium methoxide, however, failed to yield a characterizable product. Furthermore, the chloro substituent was sufficiently unreactive that it would not yield an amino derivative when treated with alcoholic ammonia at reflux temperatures.

4-Chloro-6,7-dimethylpteridine was much more reactive than the 2-chloro isomers. It was readily thionated to the 4-mercapto derivative, hydrolyzed to 6,7-dimethylpteridinol-4, and aminated to yield the 4-amino derivative. Furthermore, the reaction with sodium methoxide proceeded readily in contrast to the behavior of the 2-chloro isomer.

(1) The work described in this paper was made possible by a grant-in-aid from the Research Council of Oregon State College. Published with the approval of the Monograph Publications Committee, Oregon State College, as Research Paper No. 279, School of Science, Department of Chemistry.

(2) G. E. Hilbert and T. B. Johnson, THIS JOURNAL, **52**, 1155 (1930).

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(5) M. T. Bogert and C. E. May, THIS JOURNAL, **31**, 509 (1909).

(6) A. J. Tomisek and B. E. Christensen, *ibid.*, **67**, 2112 (1945).

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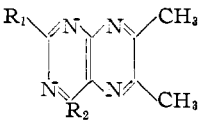
(9) A. Albert, *Quart. Revs.*, **6**, 197 (1952).

(10) C. K. Cain, E. C. Taylor, Jr., and L. V. Daniel, THIS JOURNAL, **71**, 892 (1949); B. Roth, J. M. Smith, Jr., and M. E. Hultquist, *ibid.*, **73**, 2864 (1951); C. Schöpf and R. Reichert, *Ann.*, **548**, 82 (1941); H. Wieland and R. Liebig, *ibid.*, **507**, 226 (1933); H. Wieland, H. Metzger, C. Schöpf and M. Bülow, *ibid.*, **555**, 146 (1944).

(11) G. E. W. Wolstenholme and M. P. Cameron, "Chemistry and Biology of Pteridines," J. and A. Churchill Ltd., London, 1954, p. 117.

TABLE I

ULTRAVIOLET ABSORPTION SPECTRA,



R_1	R_2	*p*H	λmax.			Log ε			λmin.		Log	
Cl	H	6	315			3.99			263		3.14	
SH	H	6	272	323		4.13	4.02		237	301	3.46	3.82
OH	H	6	242	322		3.88	3.90		280		2.95	
H	NH_2	6	244	328		4.23	3.83		287		3.31	
H	OCH_3	6	260	306		3.53	3.95		248	270	3.40	3.42
H	OH	6	229	268	312	3.96	3.66	3.85	252	280	3.43	3.58
H	SH	..[a]	264			4.15			233	316	3.77	3.12
SH	SH	6	271	301		4.10	4.15		230	284	3.88	3.99
OH	OH	6	243	330		4.08	3.76		286		3.39	
OCH_3	OCH_3	6	230	326		4.28	3.96		275		3.04	
Cl	NH_2	6	246	330		4.26	3.88		225	290	3.98	3.35
OH	NH_2	6	245	232		3.85	3.98		273		2.85	
CH_3	OH	6	316	267	229	3.94	3.68	3.78	287	253	3.53	3.47

[a] 0.02 *N* NaOH.

Replacement experiments with 2,4-dichloro-6,7-dimethylpteridine yielded the corresponding-diol-2,4, 2,4-dimethoxy and 2,4-dimercapto analogs in straight forward reactions. Amination experiments showed one of the halogens to be much more reactive than the other, inasmuch as it yielded 4-amino-2-chloro-6,7-dimethylpteridine upon treatment with ethanolic ammonia at room temperature.

The structure of the aminated product was established when it was found to be identical to the cyclization product of 2-chloro-4,5,6-triaminopyrimidine and biacetyl as judged by spectral data. The 2-chloro substituent in this instance reacted with difficulty with sodium methoxide yielding 4-amino-2-methoxy-6,7-dimethylpteridine in low yield. (Repeated attempts at methoxylation were unsuccessful.) The unreactive nature of the 2-chloro substituent was again demonstrated with hydrolysis and thionation experiments which were unsuccessful. This behavior is extremely interesting in view of the reverse order of reactivity reported by Cain and Schenker[12] in the case of 2,4,6,7-tetrachloropteridine (6,7 > 2 > 4) as judged by amination reactions.

The course of the hydrogenation of chloropyrimidines with a palladized charcoal catalyst is markedly influenced by the *p*H of the medium.[13] Under basic conditions the reaction results in a dehalogenation, while under acidic conditions the dehalogenation is accompanied by nuclear reduction yielding tetrahydropyrimidines as the product.

Similar experiments with 2-chloro-6,7-dimethylpteridine run in an acid medium gave 2-chloro-dihydro-6,7-dimethylpteridine. Reductions under basic conditions behaved in the same manner yielding a dihydro derivative, the chloro substituent remaining intact on the ring.

The 2,4-dichloro-6,7-dimethylpteridine responded differently to catalytic hydrogenation. This compound yielded only polymeric products when run under either acid or basic conditions.

The reduction of 4-chloro-6,7-dimethylpteridine using palladized charcoal under both acid and basic conditions yielded 6,7-dimethylpteridinol-4. Apparently the 4-chloro substituent is so reactive that it undergoes hydrolysis rather than hydrogenolysis under these conditions.

(12) C. K. Cain and C. Schenker, Abstract of Papers, 117th Meeting A.C.S., March-April, 1950, p. 41I.

(13) V. Smith and B. E. Christensen, *J. Org. Chem.*, **20**, 829 (1955).

Experimental

2-Chloro-6,7-dimethylpteridine.—2-Chloro-4,5-diaminopyrimidine[14] (2 g.) was suspended in a mixture of 50 ml. of benzene and 10 ml. of methanol. This suspension was brought to a boil, 3 g. of biacetyl was added and the mixture refluxed for one hour. The solvent was removed *in vacuo* and the crude chloropteridine was extracted with two 30-ml. portions of benzene. The combined extracts were made turbid with petroleum ether and then set aside overnight in a refrigerator. The product was removed by filtration; yield 2.2 g. (64%) of light yellow-orange crystals, m.p. 135–137°.

Anal. Calcd. for $C_8H_7ClN_4$: C, 49.36; H, 3.63. Found: C, 49.3; H, 3.70.

4-Chloro-6,7-dimethylpteridine.—6-Chloro-4,5-diaminopyrimidine[8] (5.5 g.) was suspended in a mixture of 75 ml. of benzene and 25 ml. of methanol, and 8 g. of biacetyl was then added. The mixture was refluxed until solution was effected (about 30 minutes) and then the solvent was removed *in vacuo*, leaving the crude product. This was recrystallized from benzene to yield 5.4 g. (73%) of fine yellow crystals, m.p. 149–151° dec.

Anal. Calcd. for $C_8H_7ClN_4$: C, 49.36; H, 3.63. Found: C, 49.5; H, 3.68.

2,4-Dichloro-6,7-dimethylpteridine.—2,6-Dichloro-4,5-diaminopyrimidine[15] (2 g.) was suspended in 50 ml. of benzene; to this suspension was added 20 ml. of a methanol solution containing 4 g. of biacetyl. The mixture was refluxed for two hours and then taken to dryness *in vacuo*. The residue was extracted with benzene and the extract taken to dryness *in vacuo*. The residue (an oil) was dissolved in acetone and set aside in a refrigerator for one to three days to crystallize. The thin yellow-orange platelets were removed and the mother liquor concentrated to yield a second crop of crystals; total yield 1.2 g. (53%), m.p. 146–148°.

Anal. Calcd. for $C_8H_6Cl_2N_4$: C, 41.95; H, 2.64. Found: C, 42.1; H, 2.68.

2-Thio-6,7-dimethylpteridine.—2-Thio-4,5-diaminopyrimidine[16] (1.0 g.) was suspended in 50 ml. of 1 *N* sulfuric

(14) A. Albert, G. Cheeseman and D. J. Brown, *J. Chem. Soc.*, 474 (1951).

(15) P. Bitterli and H. Erlenmeyer, *Helv. Chim. Acta*, **34**, 835 (1951).

(16) G. B. Elion and G. H. Hitchings, THIS JOURNAL, **69**, 2553 (1947).

acid containing 0.2 g. of sodium sulfite. To this mixture was added 1.5 g. of biacetyl and the charge was then refluxed for 45 minutes, filtered while hot and set aside in a refrigerator overnight. The product, a tan powder, yield 1.0 g. (68%), was removed by filtration and washed with cold water. Recrystallization from water gave a product which decomposed without melting at 220°.

Anal. Calcd. for $C_8H_8N_4S$: C, 49.98; H, 4.20. Found: C, 49.9; H, 4.16.

2-Chloro-6,7-dimethylpteridine (1 g.) was added to 50 ml. of water containing 2 g. of sodium hydrosulfide. The mixture was refluxed for two hours, filtered and cooled. Upon acidification with acetic acid a precipitate formed; yield 0.8 g. (80%) of a tan powder. Recrystallization from water gave m.p. 220° dec. The ultraviolet spectrum was identical with that of the product obtained on direct cyclization.

6,7-Dimethylpteridinol-2.—2-Chloro-6,7-dimethylpteridine (2.5 g.) was refluxed with 50 ml. of water for three hours. The *p*H during this period was maintained at about 6 with sodium bicarbonate. Thereupon the suspension was made slightly basic, treated with norite, filtered, then acidified with acetic acid and set aside overnight in a refrigerator; yield 0.8 g. (39%) of a tan powder which darkened without melting at 240°. Recrystallization from water yields a tan powder which darkens without melting at 270°. The water of hydration was not lost at 110° *in vacuo*.

Anal. Calcd. for $C_8H_8N_4O \cdot H_2O$: C, 49.48; H, 5.19. Found: C, 49.8; H, 5.00.

4-Amino-6,7-dimethylpteridine.—4-Chloro-6,7-dimethylpteridine (0.5 g.) was suspended in 10 ml. of absolute alcohol and ammonia was bubbled through the mixture for a period of 15 minutes. The product, an orange precipitate, was removed, washed with cold water and recrystallized again from water; yield 0.35 g. (77%) of crystals which decomposed at 295°.

Anal. Calcd. for $C_8H_9N_5$: C, 54.84; H, 5.18. Found: C, 54.6; H, 5.00.

6,7-Dimethyl-4-methoxypteridine.—To a suspension made from 1 g. of 4-chloro-6,7-dimethylpteridine and 20 ml. of methanol was added slowly with stirring, over a period of one hour, 30 ml. of a sodium methoxide solution prepared from 0.25 g. of sodium.

The dark solution was stirred for two additional hours, saturated with carbon dioxide and then evaporated to dryness *in vacuo*. The residue was extracted with 50 ml. of benzene which in turn was evaporated to dryness *in vacuo*. This residue was dissolved in ethanol, treated with norite and filtered. Upon addition of ether, the product, a white powder, precipitated; yield 0.29 g. (30%); m.p. 128–129°.

Anal. Calcd. for $C_9H_{10}N_4O$: C, 56.83; H, 5.30. Found: C, 56.6; H, 5.18.

6,7-Dimethylpteridinol-4.—4-Chloro-6,7-dimethylpteridine (0.3 g.) in 40 ml. of water was refluxed until solution was effected. During this period the *p*H was maintained at 6 with sodium bicarbonate. The resulting solution was filtered and set aside to cool. The product, a pink powder, was removed by filtration, treated with norite and recrystallized from water; yield 0.13 g. (48%) of a white powder; m.p. > 300°.

The ultraviolet spectrum was identical with that reported by Albert, *et al.*[17]

6,7-Dimethyl-4-mercaptopteridine.—To a suspension made from 0.5 g. of 4-chloro-6,7-dimethylpteridine was gradually added 40 ml. of an aqueous solution containing 1.5 g. of sodium hydrosulfide; the resultant mixture was heated for two hours on a steam-bath. The dark solution was filtered, cooled and acidified with acetic acid. The product, a flocculent yellow precipitate, melted above 300°. The compound was dissolved in 1 *N* sodium hydroxide treated with norite, filtered and reprecipitated with acetic acid, yield 0.32 g. (65%).

Anal. Calcd. for $C_8H_8N_4S$: C, 49.98; H, 4.20. Found: C, 50.1; H, 4.34.

6,7-Dimethyl-2,4-dimercaptopteridine. A.—2,4-Dichloro-6,7-dimethylpteridine (0.5 g.) was suspended in 40 ml. of water containing 1.5 g. of sodium hydrosulfide. The mixture was refluxed for two hours, filtered, cooled and then acidified with acetic acid. After standing overnight, 0.32 g. (82%) of an orange-brown product was obtained. Recrystallization from absolute alcohol gave orange needles; m.p. 250–254° dec.

Anal. Calcd. for $C_8H_8N_4S_2$: C, 42.84; H, 3.60. Found: C, 42.6; H, 3.46.

B.—4,5-Diamino-2,6-dimercaptopyrimidine[18] (2 g.) was suspended in 75 ml. of 1 *N* sulfuric acid containing 3 g. of biacetyl. This suspension was refluxed for three hours, then cooled overnight. A brown powder (1.8 g.) was obtained on filtration which on two recrystallizations from absolute alcohol gave 1.2 g. (47%) yield of orange needles; m.p. 250–254° dec. The ultraviolet spectrum was identical with that obtained from the product in A.

Anal. Found: C, 42.0; H, 3.64.

6,7-Dimethylpteridinediol-2,4.—2,4-Dichloro-6,7-dimethylpteridine (0.5 g.) was suspended in 40 ml. of an aqueous solution containing 1 g. of sodium bicarbonate. After refluxing for three hours the resultant solution was filtered and set aside overnight to cool. The product was removed by filtration; yield 0.32 g. (76%) of a white powder. Recrystallization from water gave product m.p. above 300°.

This compound gave an ultraviolet spectrum which was identical with that of the 6,7-dimethylpteridinediol-2,4, prepared by Weijlard, Tishler and Erickson.[19] The product was chlorinated in an attempt to prepare 2,4-dichloro-6,7-dimethylpteridine. However, only a dark red residue was obtained which confirmed the findings of previous workers.[20]

Anal. Calcd. for $C_8H_8N_4O_2$: C, 50.00; H, 4.20. Found: C, 49.9; H, 4.30.

2,4-Dimethoxy-6,7-dimethylpteridine.—2,4-Dichloro-6,7-dimethylpteridine (0.5 g.) was added to 30 ml. of methanol in which 0.5 g. of metallic sodium had been previously dissolved. The mixture was stirred for 30 minutes at room temperature and then refluxed for an additional 90 minutes. The solution was filtered, saturated with carbon dioxide and then diluted with 20 ml. of water. Upon cooling, 0.34 g. (71%) of a white powder was obtained which upon recrystallization from diethyl ether gave m.p. 184–186°.

Anal. Calcd. for $C_{10}H_{12}N_4O_2$: C, 54.48; H, 5.49. Found: C, 54.5; H, 5.62.

4-Amino-2-chloro-6,7-dimethylpteridine. A.—2,4-Dichloro-6,7-dimethylpteridine (1 g.) was added to 50 ml. of ethanol containing slightly more than 2 moles of ammonia per mole of pteridine. After stirring the mixture for one hour, the orange-red precipitate was removed by filtration and washed with water. Recrystallization from butanol yielded 0.52 g. (55%) of a crystalline yellow-orange product, m.p. > 300°.

Anal. Calcd. for $C_8H_8ClN_5$: C, 45.83; H, 3.85 Found: C, 45.8; H, 3.79.

B.—2-Chloro-4,5,6-triaminopyrimidine[4] (0.25 g.) was added to a mixture composed of 20 ml. of benzene, 10 ml. of methanol and 0.5 g. of biacetyl, which was then refluxed for two hours. Upon cooling, the product, an orange crystalline material, was removed by filtration and recrystallized from butanol to give 0.14 g. (42%) of yellow needles.

Ultraviolet spectrum was identical with that of the pteridine prepared from 2,4-dichloro-6,7-dimethylpteridine.

Anal. Found: C, 45.9; H, 3.98.

4-Amino-6,7-dimethylpteridinol-2.—A mixture consisting of 2-hydroxy-4,5,6-triaminopyrimidine[21] sulfate (2 g.), 50 ml. of water and 1.5 ml. of biacetyl was refluxed for three hours, filtered and the filtrate set aside in a refrigerator overnight. The product, 1 g. (64%) of a white powder, was removed by filtration and recrystallized from water; m.p. > 300°.

Anal. Calcd. for $C_8H_9N_5O$: C, 50.25; H, 4.75. Found: C, 50.1; H, 4.70.

2-Chloro-dihydro-6,7-dimethylpteridine.—2-Chloro-6,7-dimethylpteridine (1 g.) was dissolved in 100 ml. of ether. To this solution was added 200 mg. of 10% palladized charcoal, 5 ml. of 1 *N* sodium hydroxide and the mixture hydro-

(17) A. Albert, D. J. Brown and H. C. S. Wood, *J. Chem. Soc.*, 474 (1951).

(18) K. L. Dille and B. E. Christensen, THIS JOURNAL, **76**, 5087 (1954).

(19) J. Weijlard, M. Tishler and A. E. Erickson, *ibid.*, **67**, 802 (1945).

(20) E. C. Taylor, Jr., and C. K. Cain, *ibid.*, **73**, 4384 (1951).

(21) A. Bendich, J. F. Tinker and G. B. Brown, *ibid.*, **70**, 3109 (1948).

genated over a period of four hours at 45 p.s.i. The solution was then filtered to remove both the catalyst and the insoluble product. This mixture was extracted in a Soxhlet extractor with the original ether. On separation of the ether, 0.6 g. (60%) of a yellow compound was obtained. Benzene extraction removed the color (oxidized product) and left a white residue; m.p. 209–211°.

Anal. Calcd. for $C_8H_{10}ClN_4$: C, 48.8; H, 4.58. Found: C, 48.6; H, 4.72.

2-Methyl-6,7-dimethylpteridinol-4.—2-Methyl-4,5-diamino-6-hydroxypyrimidine[22] (3 g.) was suspended in 150 ml. of an aqueous mixture containing 0.5 g. of sodium sulfite and 2.5 g. of biacetyl. The suspension was refluxed (15 minutes) to effect solution which in turn was then evaporated to dryness. The residue was extracted with two 30-ml. portions of boiling absolute alcohol and the combined extracts were concentrated to one-half the volume and set in the refrigerator overnight. With further concentration the total yield was 2.5 g. (62%) of a white powder; m.p. 256–259°. Recrystallization from absolute alcohol gave m.p. 261–262°.

Anal. Calcd. for $C_9H_{10}N_4O$: C, 56.83; H, 5.30. Found: C, 56.6; H, 5.31.

CORVALLIS, OREGON

(22) A. Maggiolo, A. P. Phillips and G. H. Hitchings, THIS JOURNAL, **73**, 106 (1951).

[CONTRIBUTION FROM THE LANKENAU HOSPITAL RESEARCH INSTITUTE AND THE INSTITUTE FOR CANCER RESEARCH]

The Biosynthesis of Isoleucine[1]

BY MURRAY STRASSMAN,[2] ALICE J. THOMAS, LILLIAN A. LOCKE AND SIDNEY WEINHOUSE

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In previous publications evidence from isotope tracer studies in *Torulopsis utilis* was reported indicating that pyruvic acid may be a direct precursor of the valine carbon chain. A mechanism for the synthesis of valine in this organism was proposed, involving a condensation of acetaldehyde and pyruvic acid to yield acetolactic acid, followed by an intramolecular migration of a methyl group to yield the branched carbon chain of valine. In the present paper data are presented which indicate that the synthesis of the structurally similar isoleucine molecule probably proceeds by an analogous series of reactions, involving a condensation of acetaldehyde with α-ketobutyric acid instead of pyruvic acid, to yield a homolog of acetolactic acid, α-aceto-α-hydroxybutyric acid. Migration of the ethyl group in this intermediate is presumed to produce the carbon skeleton of isoleucine.

In previous isotopic tracer studies of valine synthesis in the yeast, *Torulopsis utilis*,[3,4] evidence was obtained which indicated that this branched chain amino acid is produced *via* a ketol condensation of acetaldehyde and pyruvic acid to yield acetolactic acid, followed by an intramolecular migration of the α-methyl group. Once a reaction of this type was conceived, it seemed probable that the biosynthesis of the homologous, branched-chain amino acid, isoleucine, might proceed by a similar pathway. As shown in Fig. 1, the condensation of acetaldehyde with the homolog of pyruvic acid, α-ketobutyric acid, would yield α-keto-α-hydroxybutyric acid. A shift of the ethyl group from carbon 2 to carbon 3, the carbonyl carbon of the acetaldehyde moiety, would produce the carbon skeleton of isoleucine. In a preliminary note,[5] evidence was presented in support of this mechanism. In the present communication, detailed experimental procedures are described, and more extensive data are reported which further confirm the mechanism of isoleucine biosynthesis shown in Fig. 1.

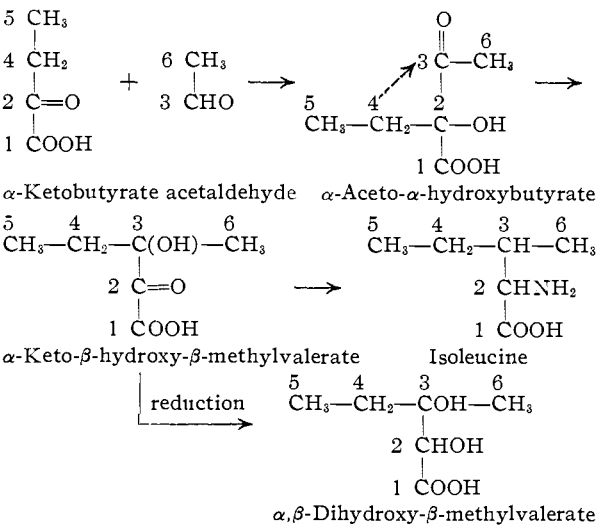

Fig. 1.—Postulated mechanism of isoleucine biosynthesis from acetaldehyde and α-ketobutyric acid. Numbering of carbon atoms is arranged to give isoleucine with the numbering shown in Table II.

Experimental

Complete details concerning cultivation of the organism, radioactivity assays, the isolation of amino acids and other experimental procedures are given in previous publications concerned with lysine[6] and valine[4] biosynthesis.

Isolation of Isoleucine.—Using the procedure previously described[4] a mixture of isoleucine and leucine, containing traces of methionine, was obtained by chromatography on Dowex 50. The two acids were separated from one another in pure form by rechromatographing the mixture on a starch column, 45 × 3.8 cm., following the procedure of Aqvist.[7] This was accomplished by dissolving the mixture in 25–35 ml. of butanol saturated with water, placing the solution on the starch column, and eluting under 14 mm. pressure with butanol saturated with water at such a rate that one 14-ml. fraction was collected every hour. Usually, the isoleucine was completely contained in frac-

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(2) Post-doctoral Fellow of the National Institutes of Health, Department of Health, Education and Welfare.

(3) M. Strassman, A. J. Thomas and S. Weinhouse, THIS JOURNAL, **75**, 5135 (1953).

(4) M. Strassman, A. J. Thomas and S. Weinhouse, *ibid.*, **77**, 1261 (1955).

(5) M. Strassman, A. J. Thomas, L. A. Locke and S. Weinhouse, *ibid.*, **76**, 4241 (1954).

(6) M. Strassman and S. Weinhouse, *ibid.*, **75**, 1680 (1953).

(7) S. E. G. Aqvist, *Acta Chem. Scand.*, **5**, 1031 (1951).