Purification of the Isomeric Bases VIa and VIIa and VIc and VIIc.—Ten grams of the redistilled, uncleaved alkylated products obtained in Expt. 5 was fractionally distilled through a Podbielniak-type column (8 × 860 mm.) fitted with a tantalum wire packing and a partial reflux head.[18] Eight fractions were cut, the last two of which proved to be essentially pure O-alkylated product, **1-carbethoxy-2-(β-dimethylaminoethoxy)-cyclohexene (VIa)**, 4.0 g., b.p. 138–139° at 3.2 mm., $n^{20}D$ 1.4849. When a sample of this material was cleaved with 2 *N* hydrochloric acid, IIa was recovered in an amount corresponding to 90% O-alkylation. Purification of the C-alkylated product (VIIa) was accomplished in the same column by distillation of a sample of alkylated product from which the abnormal O-derivative had been removed by cleavage. After a small, low boiling forerun, pure VIIa distilled at 121° at 1.7 mm., $n^{20}D$ 1.4654.

In the same manner, the isomeric bases VIc and VIIc were purified. When 14 g. of the mixed alkylated products obtained in Expt. 9 was fractionated, the higher boiling O-alkylated product, **1-carbethoxy-2-(β-diethylaminoethoxy)-cyclopentene (VIc)** was found in relatively pure form in the end fractions, 4.9 g., b.p. 142–143° at 1 mm., $n^{20}D$ 1.4862. Cleavage of a sample of this material regenerated IIb in an amount corresponding to 80% O-alkylation. The purified C-alkylated product (VIIc) had the following properties: b.p. 132–133° at 1.4 mm., $n^{20}D$ 1.4639.

Absorption Spectra.—The ultraviolet absorption spectra of the purified alkylated products VIa, VIIa, VIc and VIIc were taken in purified cyclohexane with a model DU Beckman spectrophotometer.

LARAMIE, WYOMING

(18) For details of construction see J. Cason and H. Rapoport, "Laboratory Text in Organic Chemistry," Prentice-Hall, Inc., New York, N. Y. 1950, pp. 237–243.

[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY, SCHOOL OF MEDICINE, UNIVERSITY OF PENNSYLVANIA]

Substituted Imidazoles as Precursors of the Purines[1]

BY CHARLES S. MILLER,[2] SAMUEL GURIN AND D. WRIGHT WILSON

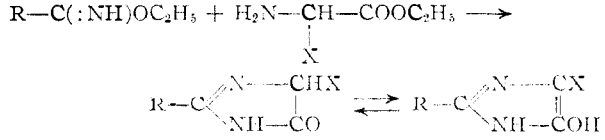
RECEIVED DECEMBER 26, 1951

Syntheses are reported for several new 5(4)-imidazole carboxamide or carbethoxy derivatives containing either hydroxy or amino groups in the 4(5)-position. C^{14}-Labeled 4(5)-hydroxy-5(4)-imidazolecarboxamide is not a biological precursor of the purines. The 4(5)-carbon of C^{14}-labeled 4(5)-amino-5(4)-imidazolecarboxamide is incorporated biologically into carbon 4 of "nucleic acid" guanine.

In an earlier report from this Laboratory,[3] a new and convenient synthesis of C^{14}-labeled 4(5)-amino-5(4)-imidazolecarboxamide was described. It was also demonstrated that this substance is utilized by the rat not only for the biosynthesis of adenine and guanine derived from nucleic acids, but also for nucleotide adenine and urinary allantoin.

As an extension of this work, syntheses have been developed for a number of imidazoles of related structure. One of these 4(5)-hydroxy-5(4)-imidazolecarboxamide was labeled with C^{14}, and its possible role as a precursor of the purines was investigated.

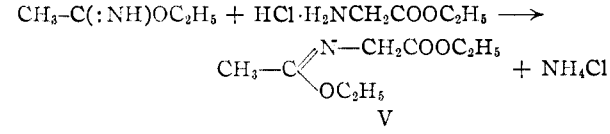
4(5)-Hydroxyimidazoles.—The method reported by Finger[4] for the preparation of 2-methyl-4(5)-hydroxyimidazole (1) was used for the synthesis of (II) and (III) by condensation of the appropriate



I, R = $-CH_3$, X = $-H$
II, R = $-H$, X = $-COOC_2H_5$
III, R = $-CH_3$, X = $-COOC_2H_5$
IV, R = $-H$, X = $-H$

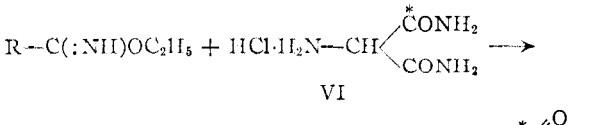
iminoesters and amino acid esters. The preparation of (IV) was not accomplished. Attempts to convert the carbethoxy compounds to their corresponding amides by means of NH_3 were unsuccessful.

Schmidt[5] described the preparation of ethyl acetiminoester-N-ethylacetate (V) by a condensation of ethyl acetaminoester and glycine ethylester hydrochloride. When this reaction was applied

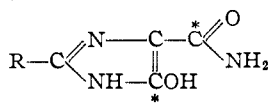


V

to iminoesters and aminomalonamide hydrochloride (VI), a similar condensation resulted which was followed by rapid ring closure to yield the corresponding 4(5)-hydroxy-5(4)-imidazolecarboxamides (VII, VIII).



VI



VII, R = $-H$
VIII, R = $-CH_3$

C^{14}-Labeled 4(5)-hydroxy-5(4)-imidazolecarboxamide (VII) was prepared in 30% yield from carbonyl-labeled aminomalonamide and ethyl formiminoester. (VIII) was similarly prepared (nonlabeled) from ethyl acetaminoester in 70% yield. For these reactions the free iminoesters were pre-

(1) Aided by a grant from the American Cancer Society administered by the Committee on Growth of the National Research Council. One of us (C. S. M.) wishes to express his thanks to Sharp and Dohme, Inc., for financial aid. The C^{14} was received on allocation from the Atomic Energy Commission.

(2) This report as well as an earlier communication[3] are taken from a thesis submitted by Charles S. Miller in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of the University of Pennsylvania.

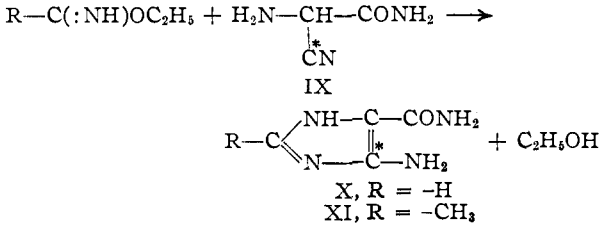
(3) C. S. Miller, S. Gurin and D. W. Wilson, *Science*, **112**, 654 (1950).

(4) H. Finger, *J. prakt. Chem.*, **76**, 93 (1907).

(5) E. Schmidt, *Ber.*, **47**, 2545 (1914).

pared from their respective hydrochlorides by a modification of the procedure of Schmidt.[5]

4(5)-Amino-imidazoles.—Substances of this type were prepared by condensing aminocyanacetamide (IX) with the appropriate iminoester in cold methanol. (X) was obtained in yields of 25–30%.[3] (XI) was similarly prepared in 37% yield from ethyl acetiminoester. This substance was not labeled with C^{14}.



X, R = -H
XI, R = $-CH_3$

Biological.—Although the subcutaneous administration of C^{14}-labeled 4(5)-amino-5(4)-imidazolecarboxamide (X) into rats yielded radioactive purines and allantoin,[3] no activity was found in the isolated uracil, thymine or respiratory CO_2. The radioactive guanine recovered from the nucleic acids was degraded by the method of Wulff[6] as modified by Cavalieri, Tinker and Brown.[7] The resulting tosylglycine was decarboxylated and the CO_2 (representing carbon 4 of guanine) was analyzed for radioactivity. This specimen assayed 630 counts per minute per milligram of carbon (calculated to infinite thickness). The theoretical value based on the radioactivity of the guanine molecule was calculated to be 600 counts per minute per milligram of carbon. It is apparent therefore that essentially all of the activity was located in carbon 4. This suggests (but does not prove) that the imidazole compound may be incorporated into the purines as an intact unit. The fact that no radioactivity was found in the expired CO_2 during the same experiment lends further support to this concept.

In another experiment, 450 mg. of C^{14}-labeled 4(5)-hydroxy-5(4)-imidazolecarboxamide (XI) containing 2930 counts per minute per mg. was administered subcutaneously to rats over a period of 3 days. No radioactivity could be recovered in the respiratory CO_2, the "nucleotide" adenine, the "nucleic acid" purines, or in the allantoin. This substance is apparently not a biological precursor of the purines.

Experimental

Synthesis of C^{14}-Labeled Intermediates.—Labeled ethyl malonate was prepared from radioactive NaCN and sodium chloroacetate by the methods of Weiner[8] and Gattermann and Wieland.[9] Nitrosation of the ethyl malonate[10] followed by reduction of the resulting oximinomalonic ester[11] yielded aminomalonic ester. By means of cold aqueous ammonia, aminomalonamide was obtained.[12] Similar treatment of labeled ethyl cyanoacetate, prepared as previously described,[3] yielded cyanoacetamide.[13]

C^{14}-Labeled oximinocyanoacetamide was prepared by treating a solution of radioactive cyanoacetamide in aqueous acetic acid with a 20% excess of sodium nitrate. After stirring for 6 hours at 10–15° followed by chilling overnight, the reaction mixture was cautiously decomposed with cold concentrated HCl and evacuated with a water-pump to remove oxides of nitrogen. The solution was evaporated *in vacuo* at 50–60° to approximately half-volume, chilled overnight and the precipitate filtered off. The product was extracted into anhydrous alcohol, NaCl removed by filtration, and the solution evaporated *in vacuo* to dryness. A 77% yield of product was obtained melting at 181° with decomposition. A non-labeled specimen, similarly prepared, had a melting point of 182–183° dec. after repeated recrystallizations from water. *Anal.*[14] Calcd. for $C_3H_3O_2N_3$: C, 31.86; H, 2.67; N, 37.16. Found: C, 32.25; H, 2.80; N, 37.16.

The reduction of 4 g. of oximinocyanoacetamide to aminocyanoacetamide (IX) was accomplished with an equal weight of Raney nickel (aged) in a suspension of 16 g. of anhydrous Na_2SO_4 in dry methanol at 3 atmospheres. After the calculated amount of hydrogen had been absorbed, the solid material was removed by filtration and the methanolic solution immediately used for the first step of the synthesis. To identify the product, a similarly prepared solution of non-labeled material was converted by means of acetic anhydride to acetylaminocyanoacetamide. After several recrystallizations from alcohol the product melted at 173.5–174.5°. *Anal.* Calcd. for $C_5H_7O_2N_3$: C, 42.55; H, 5.00; N, 29.78. Found: C, 42.67; H, 5.21; N, 29.69.

4(5)-Hydroxyimidazoles.—Ethyl formiminoester and ethyl acetiminoester were prepared in yields of 50–85% from the corresponding iminoester hydrochlorides by a modification of the procedure of Schmidt.[5] By rigorous drying of the corresponding ethereal solutions with K_2CO_3, the free iminoesters were obtained after removal of the ether by vacuum distillation. Ethyl formiminoester is a straw-colored liquid which freezes to a white solid at about −18° where it is best preserved. Ethyl acetiminoester did not solidify on chilling.

Ethyl 4(5)-hydroxy-5(4)-imidazolecarboxylate (II) was obtained when equimolar quantities of aminomalonic ester and ethyl formiminoester were allowed to react in anhydrous dioxane at 5° for 3 days. The gelatinous precipitate so obtained was washed with cold alcohol and recrystallized from boiling ethanol–benzene (equal parts). Several treatments with charcoal were necessary before obtaining crystals which melted with decomposition at 229–230°. The yield was poor. *Anal.* Calcd. for $C_6H_8O_3N_2$: C, 46.15; H, 5.16; N, 17.94. Found: C, 46.50; H, 5.38; N, 17.94.

Ethyl 2-methyl-4(5)-hydroxy-5(4)-imidazolecarboxylate (III) was similarly prepared in 77% yield from ethyl acetiminoester. After recrystallization from hot anhydrous ethanol the substance charred above 220°. *Anal.* Calcd. for $C_7H_{10}O_3N_2$: C, 49.40; H, 5.92; N, 16.46; OC_2H_5, 26.48. Found: C, 49.39; H, 6.07; N, 16.33; OC_2H_5, 26.63.

C^{14}-Labeled 4(5)-Hydroxy-5(4)-imidazolecarboxamide (VII).—This substance labeled in the 4(5)- and carboxamide positions was prepared from carbonyl-labeled aminomalonamide (in cold water containing an equivalent amount of HCl) by the addition with stirring of a 70% molar excess of ethyl formiminoester at 5°. After standing overnight at low temperature, a white solid was filtered and washed with minimal cold water. The solid could be recrystallized from a small volume of hot freshly boiled water (unboiled water produced a small amount of green pigment). After several such recrystallizations and treatment with charcoal, 1.97 g. of pure material (from 6.79 g. of aminomalonamide) was obtained. The product charred above 250°. *Anal.* Calcd. for $C_4H_5O_2N_3$: C, 37.80; H, 3.47; N, 33.06. Found: N, 32.95. A non-labeled sample similarly prepared contained C, 37.85; H, 3.92; N, 32.87.

Non-labeled 2-methyl-4(5)-hydroxy-5(4)-imidazolecarboxamide (VIII) was similarly prepared from ethyl acetiminoester in 70% yield. After repeated solution in dilute NaOH solution followed by precipitation with HCl the product

(6) C. Wulff, *Z. physiol. Chem.*, **17**, 468 (1893).

(7) L. G. Cavalieri, J. F. Tinker and G. G. Brown, THIS JOURNAL, **71**, 3973 (1949).

(8) N. Weiner, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 376.

(9) L. Gattermann and H. Wieland, "Laboratory Methods of Organic Chemistry," 24th ed., The Macmillan Co., New York, N. Y., 1938, p. 254.

(10) A. Galat, THIS JOURNAL, **69**, 965 (1947).

(11) P. A. Levene and A. Schormuller, *J. Biol. Chem.*, **106**, 595 (1934).

(12) C. Piloty and J. Neresheimer, *Ber.*, **39**, 514 (1909).

(13) B. B. Corson, R. W. Scott and C. E. Vose, ref. 8, p. 173.

(14) We are indebted to K. B. Streeter, E. A. McFadden, J. P. Laux and Joyce L. Pyett of Sharp and Dohme, Inc., for the analyses.

charred above 250°. *Anal.* Calcd. for $C_5H_5O_2N_3$: C, 42.65; H, 4.99; N, 29.78. Found: C, 42.49; H, 5.05; N, 29.61.

2-Methyl-4(5)-amino-5(4)-imidazolecarboxamide (XI).—This substance was obtained by the condensation of aminocyanoacetamide (IX) with ethyl acetiminoester in ice-cold methanol for 24 hours.[3] After removal of the methanol *in vacuo*, the residue was dissolved in water, acidified to congo red with dilute HCl and decolorized with charcoal. The compound was recovered as the picrate which was repeatedly recrystallized from hot water and 50% aqueous acetic acid (yield 37%), m.p. 240° dec. *Anal.* Calcd. for $C_{11}H_{11}O_8N_7$: C, 35.78; H, 3.00; N, 26.56. Found: C, 36.02; H, 3.14; N, 26.57.

Biological.—"Nucleotide" adenine as well as the "nucleic acid" adenine, guanine, uracil and thymine were isolated by the methods previously employed in this Laboratory.[15,3] Urinary allantoin was isolated as described previously.[8]

(15) M. R. Heinrich and D. W. Wilson, *J. Biol. Chem.*, **186**, 447 (1950).

PHILADELPHIA, PENNA.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STATE UNIVERSITY OF IOWA]

Preparation of 4-Alkylpiperidines[1]

BY S. WAWZONEK, M. F. NELSON, JR., AND P. J. THELEN

RECEIVED JULY 19, 1951

4-Alkylpiperidines can be prepared in good yields from 4-alkylpyridines by a sodium and 1-butanol reduction followed by a catalytic reduction in the presence of activated palladium. Sodium and 1-butanol alone give mainly the tetrahydropyridine. Yields are reported for 4-ethyl-, 4-(β-hydroxyethyl)-, 4-*n*-propyl-, 2-methyl-4-ethyl-, 2-*n*-butyl-, 3-methyl-4-ethyl- and 2,6-dimethyl-4-ethylpiperidines.

Piperidines are reported to be formed by the reduction of the corresponding pyridines with tin and hydrochloric acid,[2] or sodium and ethanol.[3] The latter combination, when used with 3-alkyl- and 4-alkylpyridines gives a product in which varying amounts of the tetrahydropyridine are present.[4] In the earlier literature such mixtures were often reported as the piperidines.

In the present work it has been found that sodium and 1-butanol reduction followed by catalytic reduction in the presence of activated palladium will convert 4-alkylpyridines into 4-alkylpiperidines in good yields. A list of the piperidines made in this way together with the yields obtained is given in Table I.

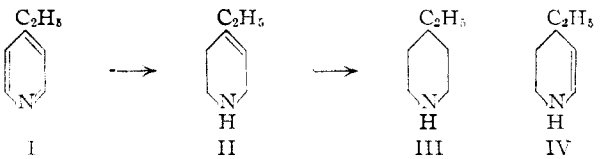
TABLE I

REDUCTION OF PYRIDINES

Pyridine	Yield of tetrahydropyridine	B.p. of tetrahydropyridine °C.	Mm.	Yield of piperidine, %	B.p. of piperidine °C.	Mm.	$n^{23}D$	d^{23}_4
4-Ethyl-	64	158–158.5	749	94.5	151–151.5	753[a]	1.4519	0.862
4-*n*-Propyl-[6]	75	178–179.5	742	95.5	172–172.5	748[b]	1.4465	.864
4-*n*-Butyl-[6]	70.5	196–197	741	94	193–194	745	1.4472	.879
2-Methyl-4-ethyl-	57.5	162–163	747	87	155–156	750[c]	1.4512	.853
3-Methyl-4-ethyl-	87	175–185		85.5	171.5–173	748	1.4530	.901
2,6-Dimethyl-4-ethyl-[10]	72[d]	173–175		71	167–168[e]		1.4433[f]	.831[g]
4-(β-Hydroxyethyl)-	59	140–145	16	85	131–136	17[h]	1.4902	....

[a] Ladenburg using solely a sodium and ethanol reduction on 4-ethylpyridine reports a boiling point of 156–158°. [b] Ahrens[7] lists the boiling point as 178–180°. [c] Schultz[8,9] lists the boiling point as 155–160°. [d] Sodium and ethanol reduction. [e] The literature[17] reports 165–167° (725 mm.). [f] At 30°. [g] At 34°. [h] Meisenheimer[5b] reports a boiling point of 140–141° (13–14 mm.).

Reduction with sodium and butanol was found to give better yields of the tetrahydro compound than reduction with sodium and ethanol. The structure of the product from this reaction was established only in the case of the compound obtained from 4-ethylpyridine; the product was 1,2,5,6-tetrahydro-4-ethylpyridine (II) since it formed a benzenesulfonamide which was stable to alkali. The only other possibility (IV) with the unsaturation in the 2,3-position would be expected to open to an amino-aldehyde under these conditions because of its vinyl amine structure (IV). The unsaturation is probably present in the 3,4-position in all other examples with the exception of the products from 2-methyl-4-ethylpyridine and 3-methyl-4-ethylpyridine. In these two compounds, because of their unsymmetrical structures, the unsaturation is either in the 3,4- or 4,5-positions.



I II III IV

The reduction of the tetrahydro compounds to the piperidines was found to proceed in good yield in the presence of palladium. 4-(β-Hydroxy-

(1) Abstracted in part from the Ph.D. thesis (1949) of M. F. Nelson, Jr., and the Ph.D. thesis (1948) of P. J. Thelen. Presented before the Organic Division of the American Chemical Society, Milwaukee, Wisconsin, March 31, 1952.

(2) W. Koenigs, *Ber.*, **14**, 1852 (1881).
(3) A. Ladenburg, *ibid.*, **17**, 156, 388 (1884).
(4) W. Koenigs, *ibid.*, **40**, 3199 (1907).
(5) A. Ladenburg, *Ann.*, **247**, 72 (1878).
(6) J. F. Arens and J. P. Wibaut, *Rec. trav. chim.*, **61**, 59 (1942).
(7) F. B. Ahrens, *Ber.*, **38**, 155 (1905).
(8) M. Schultz, *ibid.*, **20**, 2720 (1887).
(9) F. Engelmann, *Ann.*, **231**, 378 (1885).
(10) A. Jaeckle, *ibid.*, **246**, 45 (1888).