Authentic 5-nitro-2-furfural phenylhydrazone melts at 190–192°. $\lambda_{max}^{60\%\ EtOH}$ 467 mμ (log ε 4.31).[6,7]

EATON LABORATORIES DIVISION
NORWICH PHARMACAL CO.
NORWICH, N. Y.

(6) Unpublished results obtained in these laboratories.
(7) J. A. Buzard, M. Paul, and V. Ells, *J. Assoc. Offic. Agr. Chemists*, **39**, 512 (1956).

## A Convenient Synthesis of 4(5)-Amino-5(4)-imidazolecarboxamide Hydrochloride[1]

JOHN A. MONTGOMERY, KATHLEEN HEWSON, ROBERT F. STRUCK, AND Y. FULMER SHEALY

*Received August 27, 1958*

4(5)-Amino-5(4)-imidazolecarboxamide was first isolated by Stetten and Fox[2] from a culture of *E. coli* inhibited by sulfanilamide. It has since been shown to be the precursor of purines in the *de novo* synthesis of nucleic acids (in these biosynthetic processes it occurs as the ribotide).[3] It has also been shown that exogenous 4(5)-amino-5(4)-imidazolecarboxamide greatly increases the incorporation of guanine into the nucleic acids of certain tumors in mice and significantly increases its incorporation in normal body tissues.[4] It appears to be an *in vitro* inhibitor of guanase.[5,6]

None of the several synthetic methods which have been developed by various investigators[7] is convenient for the preparation of large quantities of this biologically important compound. An examination of these routes to 4(5)-amino-5(4)-imidazolecarboxamide led to the conclusion that the procedure of Shaw and Woolley[7a] could be most readily adapted to a large scale preparation of the compound. The conversion of ethyl cyanoacetate in three steps to phenylazomalonamamidine hydrochloride is quite good and can be carried out on large amounts of material. The reductive formylation of phenylazomalonamamidine hydrochloride can also be performed on a large scale, but the isolation of the resulting formylaminomalonamamidine and dry fusion of this material to give 4(5)-amino-5(4)-imidazolecarboxamide is not adaptable to large quantities. In addition, the fusion yields a green specimen of the imidazole which is difficult to free from its pigmented impurities.

We have found that phenylazomalonamamidine hydrochloride can be reduced in formic acid solution either catalytically or with zinc dust and, after removal of the palladium-on-charcoal catalyst or the excess zinc dust, cyclized to 4(5)-formylamino-5(4)-imidazolecarboxamide by simply refluxing the now colorless solution.[8] The removal of the excess formic acid gave a white solid which was triturated with ethanol to remove the by-product formanilide and, if the zinc reduction was used, the 4-formylamino-5-imidazolecarboxamide was then recrystallized from water to rid it of zinc salts. When the catalytic reduction was employed simple trituration gave material which was about 98% pure.

The 4(5)-formylamino-5(4)-imidazolecarboxamide was easily converted to 4(5)-amino-5(4)-imidazolecarboxamide hydrochloride by refluxing it in dilute hydrochloride acid. The white material obtained was usually sufficiently pure to use without further purification. Up to 100 g. of both compounds has been prepared in one reaction sequence using the zinc reduction, but better yields were obtained from the runs using catalytic reduction, the average yield of 4(5)-amino-5(4)-imidazolecarboxamide hydrochloride being 70%.

Pure 4(5)-amino-5(4)-imidazolecarboxamide was readily prepared from a water solution of its hydrochloride by treatment with Dowex-1.

### EXPERIMENTAL

*4(5)-Formylamino-5(4)-imidazolecarboxamide and 4(5)-amino-5(4)-imidazolecarboxamide hydrochloride. Method A (zinc reduction).* Phenylazomalonamamidine hydrochloride (25.0 g.) was added in portions to a stirred suspension of zinc dust (50 g.) in 98% formic acid (225 ml.) at 25°. The excess zinc dust was removed by filtration; the filtrate was refluxed for 8 hr. and then taken to dryness *in vacuo*. The solid residue was dissolved in hot water (700 ml.) and the resulting solution allowed to stand overnight in a refrigerator. The 4(5)-formylamino-5(4)-imidazolecarboxamide which crystallized from the solution was removed by filtration and dried *in vacuo* over phosphorus pentoxide: yield, 4.45 g. (28%); $\lambda_{max}^{pH\ 7}$ 269 mμ (ε 12,900) [lit.,[9] $\lambda_{max}^{pH\ 6}$ 268 mμ (ε 10,900)].

*Anal.* Calcd. for $C_5H_6O_2N_6$: C, 38.97; H, 3.94; N, 36.37. Found: C, 39.26; H, 4.20; N, 36.29.

The filtrate from the isolation of the 4(5)-formylamino-5(4)-imidazolecarboxamide was saturated with hydrogen sulfide. The precipitated zinc sulfide was removed by filtration and the excess hydrogen sulfide by concentration of the solution *in vacuo*. The solution was then acidified with dilute hydrochloric acid, refluxed for 15 min., and evapo-

(1) This work was supported by funds from National Institutes of Health, Contract No. SA 43-ph-876 and from the Union Carbide Chemicals Co.
(2) M. R. Stetten and C. L. Fox, Jr., *J. Biol. Chem.*, **161**, 333 (1945).
(3) G. R. Greenberg, *Federation Proc.*, **13**, 745 (1954); M. P. Schulman and J. M. Buchanan, *J. Biol. Chem.*, **196**, 513 (1952); B. Levenberg and J. M. Buchanan, *J. Am. Chem. Soc.*, **78**, 504 (1956).
(4) L. L. Bennett, Jr., and H. E. Skipper, *Cancer Research*, **17**, 370 (1957).
(5) P. E. Carló and G. H. Mandel, *Cancer Research*, **14**, 459 (1954).
(6) A. Roush and E. R. Norris, *Arch. Biochem.*, **29**, 124 (1950).
(7a) E. Shaw and D. W. Woolley, *J. Biol. Chem.*, **181**, 89 (1949). (b) A. Windaus and W. Langenbeck, *Ber.*, **56**, 683 (1923). (c) A. H. Cook, I. Heilbron, and E. Smith, *J. Chem. Soc.*, 1440 (1949). (d) C. S. Miller, S. Gurin, and D. W. Wilson, *Science*, **112**, 654 (1950).
(8) The ultraviolet spectrum of the solution before reflux showed that it contained an appreciable quantity of 4(5)-formylamino-5(4)-imidazolecarboxamide indicating that some cyclization had occurred during the reduction procedure.
(9) E. Shaw, *J. Biol. Chem.*, **185**, 439 (1950).

rated to dryness *in vacuo*. The residue was recrystallized from water-alcohol-ether to give 4.55 g. (27%) of pure 4(5)-amino-5(4)-imidazolecarboxamide hydrochloride: m.p., 254° (lit.,[7a] 255–256°); $\lambda_{max}^{pH\ 7}$ 277 mμ (ε12,300).

*4(5)-Amino-5(4)-imidazolecarboxamide hydrochloride. Method B (catalytic reduction).* To 3 g. of 30% palladium-on-charcoal catalyst[10] wetted with 6 ml. of water and 10 ml. of methyl cellosolve in a 500-ml. pressure bottle was added phenylazomalonamamidine hydrochloride (30.0 g.) suspended in 270 ml. of 98% formic acid.[11] Reduction in a Parr shaker required 3–4 hr. The catalyst was removed by filtration and the formic acid by evaporation *in vacuo*. The resulting white solid was triturated with absolute ethanol (50 ml.) to remove the by-product formanilide and traces of hypoxanthine. The residue, practically pure 4(5)-formylamino-5(4)-imidazolecarboxamide, was suspended in 1*N* hydrochloric acid (150 ml.), and the mixture was refluxed for 15 min.

This solution was evaporated to dryness *in vacuo* and the residual white solid dried *in vacuo* over phosphorus pentoxide: yield, 14.4 g. (71%); m.p., 256°; $\lambda_{max}^{pH\ 13}$ 277 mμ (ε 12,500). Three other runs gave an average yield of 70%.

An aqueous solution of 4(5)-amino-5(4)-imidazolecarboxamide hydrochloride (500 mg.) was converted to the free base by treatment with Dowex 1(carbonate form); yield, 218 mg. (56%); m.p. 170–171° (lit.[12] 169.8–171.4°).

KETTERING-MEYER LABORATORY[13]
SOUTHERN RESEARCH INSTITUTE
BIRMINGHAM, ALA.

(10) Baker and Co., Inc.

(11) When 98% formic acid was added to the dry catalyst, a violent reaction ensued. It was necessary with some batches of phenylazomalonamamidine to pretreat the solution with a portion of catalyst which was then replaced with fresh catalyst before reduction. In all cases this pretreatment increased the rate of reduction.

(12) W. Shive, W. W. Ackermann, M. Gordon, M. E. Getzendaner, and R. E. Eakin, *J. Am. Chem. Soc.*, **69**, 725 (1947).

(13) Affiliated with the Sloan-Kettering Institute.

## Preparation of Hydroperoxide by the Autoxidation of 4-Vinylcyclohexene

WILLIAM F. BRILL

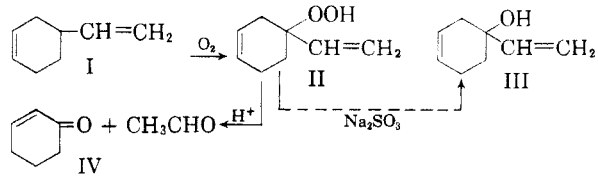
*Received September 2, 1958*

In 1911, Lebedew and Skawronskaja[1] prepared 4-vinylcyclohexene (I) by dimerizing butadiene and observed that it readily took up air. This observation is in agreement with our present knowledge concerning the autoxidation of olefins which indicates that the ease with which olefins react with molecular oxygen, forming hydroperoxide initially, depends upon the presence of reactive allylic hydrogen.[2] 4-Vinylcyclohexene has three positions alpha to a double bond where oxidation may be expected. The rate of its uncatalyzed oxidation and the isolation of hydroperoxide and alcohol fractions from the oxidation product is reported in this paper.

(1) C. B. Lebedew and H. A. Skawronskaja, *Zhur. Russ. Fiz-Khim. Obshchestva*, **43**, 1126 (1911).

(2) A. V. Tobolsky and R. B. Mesrobian, *Organic Peroxides*, Interscience Publishers Ltd., London, 1954, pp. 4–7.

The identification of II in the hydroperoxide isolated indicates the high reactivity of ring hydrogen activated by a vinyl group. Structure II




was demonstrated by acid decomposition of the hydroperoxide to 2-cyclohexenone (IV) and acetaldehyde. If the hydroperoxy function were located elsewhere on the ring, fission of the vinyl group would not be anticipated. The formation of 2-cyclohexenone is expected since it has been reported[3] that the rearrangement of 3-cyclohexenone, the first predicted product, to the conjugated ketone occurs readily under acid conditions. No evidence for the existence of other hydroperoxides was obtained.

Oxidations conducted at 90° give rise to the alcohol III which distills before the hydroperoxide and has an infrared spectra indistinguishable from it. The relative yields of alcohol and hydroperoxide obtained at different reaction times and temperatures indicate that the alcohol arises by thermal decomposition of the hydroperoxide. The same alcohol, as demonstrated by its spectra, may be prepared by reduction of the hydroperoxide with sodium sulfite, a reagent which is known to selectively reduce the hydroperoxide group to the hydroxyl group.[4] Hydrogenation of III required two moles of hydrogen but the products formed were not identified. The lack of any distinguishing differences between the spectra of II and III confirms the statement of Philpotts and Thain,[5] who studied the spectra of a series of alcohols and their corresponding hydroperoxides, that at higher molecular weights it is difficult to distinguish between an alcohol and its hydroperoxide.

The rate at which 4-vinylcyclohexene reacts with oxygen was determined at 70° and 90°. The oxidation begins immediately and the rate increases until it becomes constant and maximum at 20% reacted. The maximum rate is 1.6 times faster than that of cyclohexene under the same conditions. The rapidity of this oxidation can best be seen by comparing it to the frequently studied oxidation of cumene which has a comparable rate only when catalyzed by cobalt naphthenate. The formation of hydroperoxide was followed, as is commonly done, by iodometric analysis of the reaction solution. Only an approximate picture of the relationship between hydroperoxide formation and oxygen consumption was obtained in this way

(3) A. J. Birch, *J. Chem. Soc.*, 593–597 (1946).

(4) E. H. Farmer and A. Sundralingham, *J. Chem. Soc.*, 121 (1942).

(5) A. R. Philpotts and W. Thain, *Analytical Chem.*, **24**, 638 (1952).