Organic Syntheses

A Publication
of Reliable Methods
for the Preparation
of Organic Compounds

Working with Hazardous Chemicals

The procedures in *Organic Syntheses* are intended for use only by persons with proper training in experimental organic chemistry. All hazardous materials should be handled using the standard procedures for work with chemicals described in references such as "Prudent Practices in the Laboratory" (The National Academies Press, Washington, D.C., 2011; the full text can be accessed free of charge at http://www.nap.edu/catalog.php?record_id=12654). All chemical waste should be disposed of in accordance with local regulations. For general guidelines for the management of chemical waste, see Chapter 8 of Prudent Practices.

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These paragraphs were added in September 2014. The statements above do not supersede any specific hazard caution notes and safety instructions included in the procedure.

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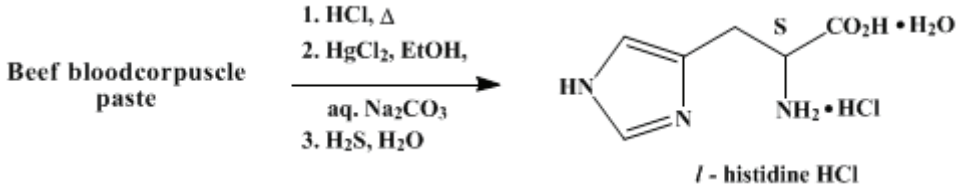
l-HISTIDINE MONOHYDROCHLORIDE



l - histidine HCl

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1. Procedure

In a large round-bottomed flask are placed 1.4 kg. of dried blood corpuscle paste (Note 1) and 4.5 l. of concentrated hydrochloric acid (sp. gr. 1.18). The flask is warmed on a steam bath until the protein has dissolved, and the mixture is boiled gently under reflux for eighteen to twenty hours. After the hydrolysate has been concentrated under reduced pressure to a thick paste, the distillation is continued with the slow addition of a liter of water, thus eliminating most of the excess hydrochloric acid. The residue is taken up in 8 l. of warm water, cooled, and neutralized to *p*H 4.4–4.6 (methyl orange or bromcresol green) by the addition of concentrated sodium hydroxide solution (Note 2). After the mixture has stood overnight, the precipitated pigment is removed by filtering through a large Büchner funnel fitted with two layers of filter paper and a 2-mm. layer of infusorial earth. The filtrate, which is dull red in color, is decolorized by warming and stirring for ten minutes with 60 g. of Norite.

The pale yellow filtrate and washings from the Norite are diluted to 25 l. with tap water, and a solution of 600 g. of mercuric chloride in 2 l. of hot 95 per cent alcohol is added, with stirring. A concentrated solution of sodium carbonate (corresponding to about 350 g. of anhydrous sodium carbonate) is added slowly, with stirring, until the mixture reaches *p*H 7.0–7.5 (phenol red or litmus). After settling for several hours, preferably overnight, the supernatant liquid is siphoned off, and the crock is refilled with water to the original volume (Note 3). The mixture is allowed to settle, the wash liquid siphoned off, and the precipitate washed twice more in the same fashion. After the third washing, the supernatant liquid is siphoned off and the precipitate collected with suction on a large Büchner funnel fitted with two layers of filter paper and a 2-mm. layer of infusorial earth.

The moist histidine-mercury complex is suspended in 5 l. of water and stirred vigorously while a stream of hydrogen sulfide is introduced. When precipitation of mercuric sulfide is complete, the suspension becomes uniformly black and settles sharply on standing. The filtrate and washings from the mercuric sulfide (Note 4) are concentrated under reduced pressure to a volume of about 1 l. and cleared with 5 g. of Norite. The filtrate and washings from the Norite are concentrated further to a volume of about 250 cc. and mixed with three volumes of 95 per cent alcohol. Crystallization is induced by cooling the solution and scratching the walls of the vessel; the histidine monohydrochloride separates in plates. After the material has remained in an ice chest for three or four days, the crystals are separated by suction filtration. The yield of crude histidine monohydrochloride is 85–90 g. The filtrate, on standing for several weeks in an ice chest, usually deposits an additional 4–5 g. of material.

The crude product is dissolved in five times its weight of water, and, after clearing with a little Norite, the solution is diluted with one and one-half volumes of 95 per cent alcohol. The product separates in well-formed, snow-white crystals, and after standing for several days in an ice chest is collected with suction on a Büchner funnel. The yield of purified histidine monohydrochloride is 75–80 g. (Note 5). The compound melts at 251–252°, with decomposition. The amino acid is not racemized by the procedure employed, and it shows the characteristic optical activity, $[\alpha]_D^{26^\circ} = +8.0^\circ$, in the presence of three moles of hydrochloric acid. The recrystallized product is usually analytically pure and shows the correct Van Slyke amino nitrogen content. Occasionally a second recrystallization is necessary to obtain analytically pure material.

2. Notes

1. Commercial "dried blood corpuscle paste" obtained from Armour and Company, Chicago, was used in this preparation. This paste contains about 15 per cent of moisture and ash, and 200 g. contains about the same amount of crude protein as 1 l. of fresh beef blood (170 g. protein per liter).
If fresh blood is used in this preparation, it is convenient to remove much of the water in the following way. Seven liters of beef blood in a 12-l. round-bottomed flask is treated with 50 cc. of glacial acetic acid and heated on a steam bath, with occasional stirring, until a thick, pasty coagulum results. About 4 l. of water is removed by distillation under reduced pressure, using a steam bath, and the residue is hydrolyzed as described above.
2. About 600 cc. of 50 per cent sodium hydroxide is required for neutralization. An excess of alkali should be avoided.
3. If, as occasionally happens, the histidine-mercury complex settles slowly, the supernatant liquid may be siphoned off and filtered. The small amount of material collected on the filter is then returned to the main portion.
4. The mercuric sulfide may be saved and converted to metallic mercury or mercuric chloride by the usual procedures.
5. About 10 g. of crude histidine monohydrochloride may be recovered from the mother liquor by evaporating under reduced pressure to 50–60 cc. and adding one and one-half volumes of 95 per cent alcohol. On recrystallization, 8–9 g. of pure material is obtained.

3. Discussion

The preparation of histidine by the hydrolysis of hemoglobin and precipitation with mercuric chloride in alkaline solution was first carried out by Fränkel.[1], [2], [3] Histidine can also be precipitated as the silver derivative.[4]

References and Notes

1. Fränkel, Monatsh. **24**, 229 (1903).
2. Abderhalden, Fleischmann, and Irion, Fermentforschung **10**, 447 (1928) [C. A. **23**, 2994 (1929)].
3. Gilson, J. Biol. Chem. **124**, 281 (1938).
4. Vickery and Leavenworth, ibid. **78**, 627 (1928).

Appendix
Chemical Abstracts Nomenclature (Collective Index Number); (Registry Number)

metallic mercury

histidine-mercury complex

alcohol (64-17-5)

hydrochloric acid (7647-01-0)

acetic acid (64-19-7)

sodium hydroxide (1310-73-2)

phenol (108-95-2)

hydrogen sulfide (7783-06-4)

sodium carbonate (497-19-8)

Norite (7782-42-5)

mercuric chloride (7487-94-7)

bromcresol

mercuric sulfide

histidine monohydrochloride,
L-Histidine monohydrochloride (1007-42-7)

histidine (71-00-1)

methyl orange (547-58-0)