The Direct Isolation of Arginine and Lysine Picrates from Gelatin Hydrolysates

By W. ROBSON AND A. S. M. SELIM

*Department of Physiology and Biochemistry, King's College,
Strand, London, W.C. 2*

(Received 1 April 1952)

The original process of Kossel & Kutscher (1900) of isolating arginine and lysine as their respective silver and picric acid salts from the phosphotungstic acid precipitates of protein hydrolysates is costly and tedious. These disadvantages are not entirely overcome by the electrodialysis method of Foster & Schmidt (1926). The direct preparation of arginine by the flavianic acid method (Kossel & Gross, 1924) and of lysine as picrate (Rice, 1939) yields products which require further purification (cf. Cox, 1928; Vickery, 1940; Heathcote, 1949).

In the method now described for the isolation of arginine and lysine, use has been made of the low solubilities of their picrates in water, arginine picrate about 0.5 g./100 ml. at 16° (Abderhalden, 1911) and lysine picrate about 0.54 g./100 ml. at 21° (Lawrow, 1899). The pH of a saturated aqueous solution of the former picrate, determined electrometrically at room temperature, is between 6.5 and 6.8, and of the latter between 5.0 and 5.4. When a quantity of picric acid, equivalent to the arginine present, was added to a concentrated gelatin hydrolysate (pH 8), previously freed from the sulphuric acid used for hydrolysis, no precipitate was formed even when the mixture was kept at 0° for several days. Adjustment of the pH of the mixture, now approximately 5.0, to 6.5–7.0 by the addition of ammonium hydroxide led, on rubbing the sides of the vessel with a glass rod, to the separation of the typical long bright thread-like crystals of arginine picrate. After a few hours the mixture was semi-solid. Collected at the pump and washed with ice-cold water, the compound, on recrystallization from boiling water, yielded a product of excellent melting point and free from histidine, lysine and ammonia. Decomposition of the picrate so obtained was easily effected by hot hydrochloric acid. From the picric acid-free solution arginine monohydrochloride has been obtained in amounts corresponding to 90% of the total amino-acid present in gelatin.

The further addition of picric acid to the solution from which the arginine picrate was removed,

followed by the adjustment of the pH of the mixture to 5, led to the separation of lysine picrate in amounts corresponding to approximately 60% of the theory.

EXPERIMENTAL

Gelatin 'Spa' (600 g.; N, 17.17; moisture, 12.8%) was added to 2.5 l. of 8N-H₂SO₄ and the protein brought completely into solution by heating. When the protein had dissolved the mixture was refluxed for 30 hr. The acid hydrolysate was diluted with twice its own volume of water and neutralized to litmus with a suspension of Ca(OH)₂ in water. The CaSO₄ precipitate, which settled quickly, was removed at the pump, extracted twice with boiling water and the mixture again filtered. The filtrate and washings were combined and any SO₄ ions remaining in solution removed with Ba(OH)₂. Ca ions, still in solution, were removed with oxalic acid; the small precipitate in each case being collected and washed on the filter with boiling water. The hydrolysate freed from oxalate, SO₄, Ca and Ba ions had a pH about 8. It was then concentrated *in vacuo* to 2 l.

Isolation of arginine picrate. An estimation by the colorimetric procedure of Brand & Kassell (1942) showed that the solution contained approximately 44 g. of arginine. Solid picric acid (64 g., 10% in excess of the amount actually required to combine with all the arginine present) having been added, the mixture was heated with stirring until all the reagent was dissolved. The solution was cooled to room temperature and concentrated ammonia added with stirring until the pH was between 6.5 and 7. The solution became slightly turbid due to the precipitation of some ammonium picrate. After re-solution of these crystals, by heating, the mixture was cooled to room temperature under the tap. Long yellow needles soon commenced to form, first at the surface, and spread throughout the solution, until after about 1 hr. the whole solution had turned to a semi-solid mass of crystals. After 12 hr. at 0° the picrate was collected at the pump, sucked dry and then washed thrice with 100 ml. of ice-cold water. The product was then recrystallized from about 500 ml. of boiling water. After chilling the solution for 12 hr., the arginine picrate was again collected, washed first with a little ice-cold water, then with absolute ethanol and finally dried in the oven at 110° for several hours. The mother liquor from the recrystallized product and the washings were added to the original filtrate which was treated as shown below for the isolation of lysine picrate. The yield of recrystallized arginine picrate was 95 g., m.p.

217–218° (Vickery & Leavenworth, 1927). The product gave a negative Pauly reaction for histidine.

Arginine picrate contains 2 mol. of water of crystallization, which are lost at 110°. (Found: water of crystallization, 8.21. Calc. for $C_6H_{14}O_8N_4 \cdot C_6H_3O_7N_3 \cdot 2H_2O$, 8.20%. Found: C, 35.9; H, 4.1; N, 23.4. Calc. for $C_6H_{14}O_8N_4 \cdot C_6H_3O_7N_3$: C, 35.73; H, 4.21; N, 24.29%.)

Preparation of arginine monohydrochloride. The arginine picrate was decomposed by hot HCl. The picric acid was removed from the mixture by extracting three times with hot benzene. The resulting solution was concentrated *in vacuo* to a thick syrup. This was taken up in hot ethanol and concentrated ammonia added drop by drop with stirring until the arginine monohydrochloride started to crystallize. After 12 hr. in the ice box the product was collected and recrystallized by dissolving it in the minimum amount of hot water and precipitating it with ethanol. The recrystallized arginine monohydrochloride melted at 216–217° (Cox, 1928).

Isolation of lysine picrate. The solution remaining after the removal of arginine picrate was acidified with H_2SO_4 and shaken with hot benzene to remove the picric acid. $Ca(OH)_2$ in slight excess of the H_2SO_4 present was added, the $CaSO_4$ removed, washed and the free ammonia removed by partial concentration of the filtrate *in vacuo*. SO_4 and Ca ions were exactly removed in the manner previously described, and the solution was concentrated *in vacuo* to about 500 ml. and allowed to stand overnight in the refrigerator. The mono-amino-acids which had separated were removed and washed several times with ice-cold water; the combined filtrate and washings had a volume of about 800 ml. This was treated with 48 g. of picric acid, heated until all the solid had dissolved and then cooled. The pH of the mixture was adjusted to 5 by the addition of 40% (w/v) NaOH. After

48 hr. in the refrigerator the mixture was filtered, the precipitated picrate collected, washed twice with ice-cold water, and recrystallized from boiling water. The picrate was collected, washed with a little water, then with ethanol and finally dried in the oven at 110°. The dried product weighed 32 g. and when heated decomposed at 261° with the characteristic explosion of pure lysine picrate.

Preparation of lysine monohydrochloride. According to the usual procedure the picrate was decomposed with hot HCl, the picric acid extracted with benzene, and the resulting solution concentrated *in vacuo* to a syrup. This was dissolved in the minimum amount of hot ethanol and pyridine was added gradually until the lysine monohydrochloride commenced to separate. The crude product was recrystallized from aqueous ethanol. The monohydrochloride had a melting point of 258–261° which was not depressed on admixture with an authentic specimen (Rice, 1949).

SUMMARY

1. Procedures for the isolation of arginine picrate and lysine picrate from gelatin hydrolysates are described. These picrates form convenient sources for the preparation of arginine monohydrochloride and lysine monohydrochloride in good yield and purity.

2. The method possesses advantages over the flavianic acid method in that it is economical in time and reagents. Picric acid is cheap, is used only in approximately stoichiometric proportions and, finally, is much easier to recover than flavianic acid.

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