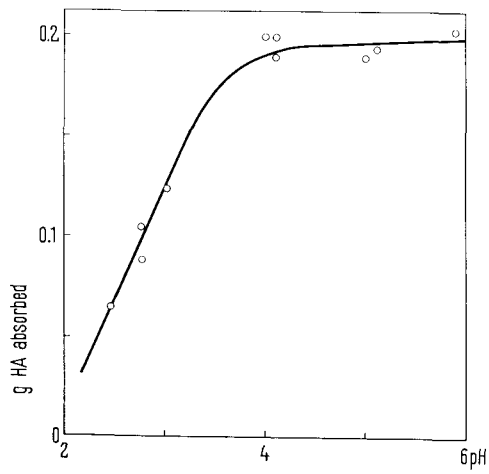
Isolation of Homogentisic Acid from Urine of Alcaptonuric Cases by Means of an Anion-Exchanger

Synthetic preparation of homogentisic acid (2,5-dihydroxyphenylacetic acid, HA), suitable for production on a preparative scale, was not described until the year 1949 by ABBOTT[1]. The total yield of his multistep synthesis does not exceed 40%.

Approximately 5 g of HA a day is excreted in the urine of alcaptonuric patients. Isolation procedures, using either direct precipitation of sparingly soluble HA lead salt[2] or HA ether extraction[3], do not yield reliable or reproducible results. Therefore a new ion-exchange method was developed, useful for HA preparation in even larger quantities with good yield from the urine of alcaptonuric patients.

HA preparation from alcaptonuric urine. Weakly basic polycondensate Anex MFD in acetate cycle (produced by the Research Institute for Synthetic Raisins, Pardubice, Czechoslovakia) has been used in all operations. It takes up only the free dissociated acids present in urine. The results of experiments with alcaptonuric urine acidified to various degrees show that a pH of 4 to 5 is most advantageous. Under these conditions HA is still dissociated enough (its pK = 4.4), but is not yet subject to spontaneous oxidation by atmospheric oxygen.

Urine of alcaptonuric patients was preserved by strong acidification at the time of its collection. The pH was adjusted at 4 to 5 before HA isolation. The urine was placed at a flow velocity of 100 ml/h on a column (3.6 · 25 cm) filled with ground Anex MFD (particle size 0.2–0.6 mm, acetate cycle). The capacity of this column amounted to 20 g HA. The HA absorption is quantitative up to 60% of saturation. If a quantity corresponding to the column capacity is placed on the column, up to 90% HA is still bound.



pH influence upon HA absorption. 250 ml urine, containing 570 mg HA, adjusted to different pH, was passed through the columns (0.8 · 10 cm) filled with Anex MFD (particles 0.125–0.20 mm, acetate cycle, flow velocity 0.25 ml/min) and the quantity of bound HA was determined.

Elution in the routine work was carried out by 2500 ml 5*M* acetic acid. This amount is sufficient for taking up approximately 75% of the bound HA while the column is regenerated for further use at the same time.

HA content in the urine, as in the eluates, was determined by iodometric titration[4].

An example of the treatment of the eluate. The eluate (2550 ml) containing 14.5 g HA was concentrated by distillation under reduced pressure. The dark oily distillation residue (150 ml) was diluted with water (300 ml) and neutralized by the addition of solid sodium bicarbonate to pH 5. After heating, a 20% molar excess of lead acetate was added and the mixture was cooled. The precipitate was sucked off, recrystallized from water and dried at 110 °C. The yield of pure lead homogentisate $(C_8H_7O_4)_2Pb$ was 18.0 g (76%).

HA was set free by means of a calculated amount of 7*N* sulphuric acid. The traces of lead and the pigmented impurities were removed from the slightly brownish solution by passing it through the column filled with Dowex 50 · 4 (H cycle, column 1 · 15 cm). The effluent was concentrated in inert atmosphere under reduced pressure up to crystallization. 6.3 g of quite pure HA was formed. (The purity was checked by spectrophotometry[5] and by paper chromatography.) A further 2.9 g HA were obtained from the mother liquor. The total yield of HA amounted to 9.2 g (63.5%), calculated for its content in the effluate[6].

Zusammenfassung. Die Homogentisinsäure liess sich aus alkaptonurischem Harn selektiv am schwach basischen Anionenaustauscher Anex adsorbieren. Nach der Elution mit Hilfe von 5*M* Essigsäure betrug die Ausbeute 60%. Die Methode ist auch im präparativen Ausmass gut durchführbar.

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