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Potassium Dihydrogen D_sL_s-Isocitrate

Abstract. The monopotassium salt is to be preferred to the lactone as the final product of the synthesis of isocitric acid by the method of Fittig and Miller.

In 1889, Fittig and Miller (*1*) described the synthesis of isocitric acid from sodium succinate and chloral by condensation in the presence of acetic anhydride. The resulting trichloromethylparaconic acid was hydrolyzed with barium hydroxide, and the isocitric acid produced was liberated from the barium salt, dehydrated, and isolated as its lactone. Fittig and Miller's procedure was studied in detail in 1946 by Pucher and Vickery (*2*), and several modifications were found advantageous. A yield of lactone free from alloisocitric lactone of approximately 60 percent was obtained in moderately large-scale operations. Further slight modifications of procedure have since been described by Deutsch and Phillips (*3*) and by Kato and Dickman (*4*), but without improvement in yield or convenience.

The excellent properties of monopotassium L_s-isocitrate obtained from the leaves of plants of the family Crassulaceae (*5*) suggested that the troublesome lactonization step could be avoided if the monopotassium salt were isolated as the final product of the synthesis. It has been found that the yield of trichloromethylparaconic acid can be increased from about 73 percent (*2*) to about 80 percent, and that the reaction goes more smoothly if a liberal excess over 1 molar proportion of chloral is used during the condensation. If hydrolysis of the acid is carried out with an excess of barium hydroxide, and the insoluble barium isocitrate is filtered from the hot solution, most by-products and impurities remain in the filtrate. The monopotassium salt is then easily isolated as described by Vickery and Wilson (*5*).

Trichloromethylparaconic acid is prepared as follows. Twenty grams of anhydrous sodium succinate, 16.0 ml of chloral (33 percent excess over 1 equivalent) and 12.6 ml of acetic anhydride (1 equivalent) are heated in an oil bath for 1 hour at 140°C under a reflux condenser with mechanical stirring. The reaction mixture turns black and becomes viscous, but remains fluid. The tar produced is dissolved in 200 ml of hot water, boiled with 15 g of decolorizing carbon, and the solution is filtered and concentrated *in vacuo* to about 120 ml when separation of sodium salts makes further concentration difficult. The solution is heated to dissolve the salts and 30 ml of concentrated HCl are added. The dark red oil which separates is induced to crystallize by chilling the solution and stirring it with a rod. The mixture is chilled overnight, and the crystals are filtered, pressed down hard in the funnel, and washed with a little ice water. After thorough drying in a vacuum desiccator, the yield is 22 to 24 g (72 to 78 percent). A little more of the acid can be recovered, usually as a few drops of oil, by extraction from the mother liquor with ether. The ether extract is washed with water before being concentrated.

Monopotassium D_sL_s-isocitrate is then prepared as follows. The crude crystalline trichloromethylparaconic acid, together with the material extracted by ether from its mother liquor, is added slowly to a hot solution of 120 g (20 percent excess over 6 equivalents) of barium hydroxide octahydrate in 150 ml of water, and the thick suspension of barium isocitrate is boiled under reflux with mechanical stirring for an hour in an oil bath. The boiling hot solution is filtered on Whatman No. 3 filter paper covered with a thick layer of Celite, and the precipitate is washed with boiling water. The barium salt is then suspended in cold water and decomposed with a slight excess of sulfuric acid, and the monopotassium salt is isolated by crystallization at *p*H 3.50 as described by Vickery and Wilson (*5*, *6*). The yield of this salt is considerably improved if 25 percent of alcohol is added to the concentrated aqueous solution, but the addition of too much alcohol may lead to contamination of the product with alloisocitrate: yield, 14 to 16 g (49 to 56 percent). Most preparations contain between 49 and 50 percent of potassium L_s-isocitrate as determined by the isocitric dehydrogenase method of Ochoa (*7*). Once recrystallized from hot water after addition of 25 percent of alcohol, the salt is essentially pure. The solution from which the monopotassium salt is isolated contains isocitric acid and alloisocitric acid in the ratio of approximately 10:1 as determined by chromatographic analysis on Dowex 1 by the method of Palmer (*8*) with formic acid as eluent.

Monopotassium D_sL_s-isocitrate crystallizes from water in tiny needles which collect in nodular masses and adhere strongly to glass unless the solution is stirred during crystallization. When crystallized slowly from alcohol-containing mother liquors, it forms fascicles of small flattened rhombic needles often aggregated into masses. The salt is soluble in its own weight of water at boiling temperature. The solubility at 0°C of a three-times recrystallized specimen is close to 8 g in 100 ml of water and 1.5 g in 100 ml of 25-percent alcohol. It is thus appreciably more soluble than monopotassium L_s-isocitrate. It decomposes at 175° to 176°C with evolution of gas (*9*).

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Nucleic Acids in Some Deuterated Green Algae

Abstract. In order to determine whether the replacement of hydrogen by deuterium in living organisms is accompanied by changes in amounts and distribution of the cellular components, a preliminary cytochemical investigation has been made on deuterated *Chlorella vulgaris* and *Scenedesmus obliquus*. Cytoplasmic ribonucleic acid is more widely distributed and occurs in higher quantities in deuterated than in nondeuterated algae. Nuclei of deuterated cells are more irregular in shape, and mitotic figures appear with greater frequency in the deuterated organisms.

As part of a study of the effects of deuterium on biological systems, we have made a cytochemical study of some deuterated green algae. The cultivation of *Chlorella vulgaris* and *Scenedesmus obliquus* in 99.6-percent D_2O under conditions that lead to essentially fully deuterated organisms has been previously described (*1*). The organisms used in the studies described here were grown in D_2O for protracted peri-