

ISOLATION OF CHOLINE ASCORBATE FROM *APIUM GRAVEOLENS*

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Apium graveolens L. (Umbelliferae) is a glabrous herb that grows wild and is also cultivated throughout the world as the vegetable, known in English, as celery (1). A survey of the literature indicates that some other choline derivatives had been isolated from natural sources (2). However, this is first report of the isolation of choline ascorbate from *A. graveolens* and from a natural source.

PLANT MATERIAL.—The cultivated plant used in this investigation was collected from the farming areas around Istanbul. The voucher specimen was identified by Dr. P.H. Davis and is deposited in the herbarium of the Department of Botany of Edinburgh University, U.K.

GENERAL EXPERIMENTAL PROCEDURES.—Solvent system used for aluminium oxide tlc was (A) *n*-BuOH-HCOOH-H₂O (60:15:35), and pc with Whatman I was accomplished with solvent systems (B) *n*-BuOH-EtOH-HOAc-H₂O (8:2:1:3) Rf 0.55; and (C) *n*-BuOH-oxalic acid-H₂O (20:1:30) Rf 0.12; Solvent systems for silica gel tlc were (D) *n*-BuOH-HOAc-H₂O (4:1:5) Rf 0.38. Choline ascorbate was detected by spraying with Dragendorff's Reagent while ascorbic acid was detected by spraying with ammonium molybdate reagents (5).

EXTRACTION AND FRACTIONATION.—The leaves of the plant were air dried and milled to a coarse powder. The powdered leaves (1 kg) were subjected to Soxhlet extraction with EtOH (3 liters). The ethanolic extract was evaporated and taken up with distilled H₂O. The aqueous solution of the extract was treated successively in a separatory funnel with petroleum ether (bp 40/60°), CHCl₃, and EtOAc. The aqueous solution remaining after the extraction with these solvents was concentrated to dryness. The residue (5 g) was chromatographed over a 2.5×60 cm column of neutral aluminal (Merck). Elution was started with Me₂CO and continued through a series of solvents containing increasing amounts of EtOH (such as 10%; 25%; 50%; 75%). Twenty-five fractions (25 ml each) from each solvent system were collected and chromatographed on pc and tlc.

IDENTIFICATION OF CHOLINE ASCORBATE.—The fractions (75% EtOH in Me₂CO to pure EtOH) showing a single compound were combined and evaporated to dryness. The substance was obtained pure by preparative tlc. The zone at Rf 0.41 with the solvent system (A) was removed and eluated with EtOH and the solvent was removed in vacuum. Co-chromatography in three solvent systems (A, B, C) showed no modification of the Rf value. The isolated substance is a viscous liquid, very hygroscopic and crystallized by exposure to the air. Uv λ max (EtOH) 266 nm; ir (KBr) 1636, 1470, 953 cm⁻¹; ms: *m/z* M⁺ 297.28 (C₁₁N₂₁NO₇); ¹H nmr (D₂O) 90 Mz δ 3.27 (3×CH₃ s); 3.55 (N-CH₂ q); 4.17 (-CH₂OH m).

By the addition of HCl (pH 1.5) at 45° into the aqueous solution of this substance, the ascorbic acid was identified on the basis of chromatographic data (D) (3). The choline was liberated by addition of alkali and identified by the production of the crystalline ammonium reinekate salt (4).

Isolated choline ascorbate was found to be identical with reference choline ascorbate in its pc and tlc data and ir, ms, uv, ¹H-nmr spectral properties.

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