# IDENTIFICATION AND QUANTITATIVE DETERMINATION OF o-, m- AND p-HYDROXYMANDELIC ACID IN HUMAN URINE

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Endogenous p-octopamine (p-hydroxyphenylethanolamine) and its naturally occurring N-methyl derivative are metabolized to p-hydroxymandelic acid (PHMA), which is excreted in human urine in amounts of 1-4 mg $day^{-1}$ (Kakimoto & Armstrong 1962; Gjessing & Armstrong 1963). Similarly exogenous m-octopamine affords m-hydroxymandelic acid (MHMA)(Hengstmann et al 1975). Interest in the biological role of 'octopamine' (Axelrod & Saavedra 1977 and references therein) and the natural occurrence of o- and m-octopamine in mammalian tissue (Williams & Couch 1978) prompted the investigation of human urine for OHMA and MHMA, which have not hitherto been identified in biological tissue or fluid.

Bis(trifluoracetoxy)methyl esters (TFA-ME) of the acids were simply prepared under mild conditions by treatment of the corresponding hydroxymandelic acid with methanolic hydrochloric acid followed by N-methyl-bis(trifluoroacetamide) in pyridine. The three isomeric TFA-ME derivatives were completely resolved from one another by the gas chromatographic procedure employed. The organic acids were extracted by a standard procedure from a volume of urine equivalent to 10 mg creatinine. The acids in the crude solid extract thus obtained were converted to their TFA-ME derivatives and OHMA, MHMA and PHMA were unequivocally identified by gas-chromatography-mass spectrometry-selected ion monitoring (gc-ms-sim). The retention times and ratios of the intensities of the peaks at m/e 315 and m/e 374 obtained from the derivatized authentic samples of OHMA, MHMA and PHMA were identical, within experimental error, to those values obtained from unknowns in the derivatized urine extract.

The isomeric hydroxymandelic acids were deuterated by reacting the non-deuterated acid with a mixture of $D_2O$, $CD_3CO_2D$ and DCl at 190° (Karoum et al 1975). Aliquots of the reaction mixture were examined at intervals by gc-ms-sim of the TFA-ME derivative in order to determine the composition of the deuterated mixture. A calibration curve was prepared for each acid by measuring the ratios of the ion current at m/e 315 to that at m/e 317 for various known quantities of OHMA/100 ng $^2$D-OHMA and for various known quantities of PHMA/20μg $^2$D-PHMA: also at m/e 315 and 318 for various known quantities of MHMA/1μg $^3$D-MHMA. When these values were plotted against the theoretical ratios a straight line was obtained in each case.

A known quantity of each deuterated hydroxymandelic acid was added to the standard volume of urine, which was processed and derivatized as before. The ratio of the ion current at m/e 315 to that at m/e 317 for OHMA and PHMA and at m/e 315 to that at m/e 318 for MHMA showed that the original urine contained 7.5 (± 1.0) OHMA. 12.6 (± 0.9) MHMA, 3030 (± 110) PHMA ng $mg^{-1}$ creatinine. Similar results were obtained from nine other normal adults. Acid hydrolysis of the urine or ingestion of a diet of known composition did not affect these results indicating that the metabolites are excreted as the free acids and probably arise by metabolism of the corresponding endogenous hydroxyphenylethanolamine.

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