Identification of 5,6-dimethoxyindolyl-2-carboxylic Acid in Melanotic Urine

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5,6-dimethoxyindolyl-2-carboxylic acid was identified in urine of patients with malignant melanoma by means of gas chromatography - mass spectrometry. Its presence supports the concept of the existence of an efficient methylating activity in melanoma tissue. Key words: Indoles; Malignant melanoma; Melanotic urine; Gas chromatography - mass spectrometry. (Received November 29, 1982.)

In the organism, methylation plays an important role not only in many anabolic, but also in catabolic biochemical processes. With respect to its catabolic role, the inactivating effect of catechol-O-methyltransferase (COMT, EC 2.1.1.6) on catecholamines is well known (2). The presence of COMT activity in melanoma tissue (3) and the existence of O-methylated derivatives of 5,6-dihydroxyindole and 5,6-dihydroxyindolyl-2-carboxylic acid in melanotic urine (4, 5, 8, 10) and in melanoma cell culture supernatants (9) provides evidence that COMT is also responsible for the detoxification of reactive indolic intermediates generated in the course of eumelanin synthesis. The existence of O-methylated derivatives of the cysteinyldopa isomers in melanotic urine has also been reported (1).

In this paper we describe the gas chromatographic - mass spectrometric (GC-MS) identification of a per-O-methylated compound, 5,6-dimethoxyindolyl-2-carboxylic acid (5,6DMI2C), in the urine of melanoma patients.

MATERIAL AND METHODS
Pentafluoropropionic anhydride (PFPA) and 1,1,1,3,3,3-hexafluoropropanol (HFIP) were purchased from Pierce Chemical Company. 5,6-dimethoxyindolyl-2-carboxylic acid was obtained as a co-product in the synthesis of 5-hydroxy-6-methoxyindolyl-2-carboxylic and 6-hydroxy-5-methoxyindolyl-2-carboxylic acids (5H6MI2C and 6H5MI2C, respectively) as previously described (6). All other chemicals were from Merck.

2.5-ml aliquots of melanotic urine from patients with widespread melanotic metastases were acidified to pH 1 with concentrated HCl, saturated with NaCl and extracted with 2x4 ml of diethyl ether. The combined extracts were dried over anhydrous Na2SO4 and evaporated to dryness under a stream of nitrogen at 40°C. Derivatization of urine extracts was performed as previously described (10, 7). GC-MS analysis was performed using a Varian 3700 gas chromatograph coupled to a Varian MAT 44 S mass spectrometer with a Finnigan MAT SS 200 data system as described elsewhere (7).

RESULTS AND DISCUSSION
Fig. I shows a mass spectrum of the TMS derivative of synthetically prepared 5,6DMI2C. The characteristic fragment ions can be interpreted as follows: m/e 365=|M|; m/e 350=|M-CH3|; m/e 334=|M-CH2O|; m/e 306=|M-(CH3-SiH-CH3)|; m/e 293=|M-TMS+H|; m/e 276=|M-TMSO|; m/e 203=|M-(TMS+TMSO)|.
Fig. 1. Mass spectrum of the TMS derivative of synthetic 5,6DM12C.

Fig. 2. Part of the total ion current chromatogram with selected mass plots of the HFIP-PFP derivatives of 6H5M12C and 5H6M12C (m/e 503) and 5,6DM12C (m/e 371) extracted from melanotic urine.
For the identification of 5,6DM12C in melanotic urine, HFIP-PFP derivatives of the synthesized 5,6DM12C and urinary extracts were prepared and analysed by GC-MS. The mass spectrum of the HFIP derivative of synthetically prepared 5,6DM12C is shown in Fig. 3 (upper) and can be explained as follows: m/e 371=[M]+; m/e 203=[M-HFIP]+; m/e 188=[M-(HFIP+CH3)]+; m/e 175=[M-COO-HFIP]+; m/e 160=[M-(COO-HFIP+CH3)]+. The fragment ion m/e 357 belongs to a contaminating compound.

The derivatized urinary extracts were investigated by means of repetitive scanning GC-MS analysis. Fig. 2 shows part of the total ion current chromatogram with selected mass plots of the molecular ions of HFIP-PFP derivatized 6H5MI2C and 5H6MI2C (m/e 503) and 5,6DM12C (m/e 371). In addition to the known presence of 6H5MI2C and 5H6MI2C, the trace at m/e 371 showed a peak at the expected retention time, and the mass spectrum at the top of this peak (scan number 164) was found to be nearly identical with that of the synthetically prepared 5,6DM12C (Fig. 3).

The presence of 5,6DM12C in melanotic urine is an interesting phenomenon. Analogous to the isomeric 5H6MI2C and 6H5MI2C, one can presume that the formation of 5,6DM12C results from a highly efficient methylating activity of COMT, which detoxifies reactive o-dihydroxyindolic compounds and thereby protects the organism’s own cell against self-destruction. The inhibition of O-methylation could therefore lead to an intracellular accumulation of reactive eumelanin precursors with subsequent destruction of the pigment cell. It is suggested that the mechanism of action of several known depigmenting agents should be re-examined from this point of view.

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REFERENCES


Cutaneous Sarcoidosis: An Immunofluorescence Study

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Biopsy specimens from granulomatous skin lesions of 14 patients with active sarcoidosis were examined by immunofluorescence microscopy. In seven lesions, deposits of IgM, IgA or complement C3 were demonstrated in the dermal vessel walls and/or at the dermal–epidermal junction. Similar deposits were found in three of twelve biopsy specimens from clinically normal skin from a buttock of the same patients. The results support the hypothesis that deposition of circulating immune complexes in the vessel walls may be of importance for the development of granulomatous lesions in sarcoidosis. Key words: Sarcoidosis; Immunofluorescence; Vessel walls. (Received December 7, 1982.)

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In 1972 Salo & Hannuksela demonstrated, by means of immunofluorescence microscopy, deposits of IgM and complement C3 in vessel walls in Kveim reactions of 20 patients with sarcoidosis (1). Skin lesions from 5 of the patients were also examined and in 2 of them granular deposits were found at the dermal–epidermal junction (DEJ). These results were confirmed by Quismorio et al. who demonstrated deposits of IgM in the vessel walls in the