SHORT REPORTS

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.)

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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-hexafluoroisopropanol (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 2×4 ml of diethyl ether. The combined extracts were dried over anhydrous Na_2SO_4 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. 1 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* $350=[M-CH_3]^+$; *m/e* $334=[M-CH_3O]^+$; *m/e* $306=[M-(CH_3\text{–}SiH\text{–}CH_3)]^+$; *m/e* $293=[M-TMS+H]^+$; *m/e* $276=[M-TMSO]^+$; *m/e* $203=[M-(TMS+TMSO)]^+$.

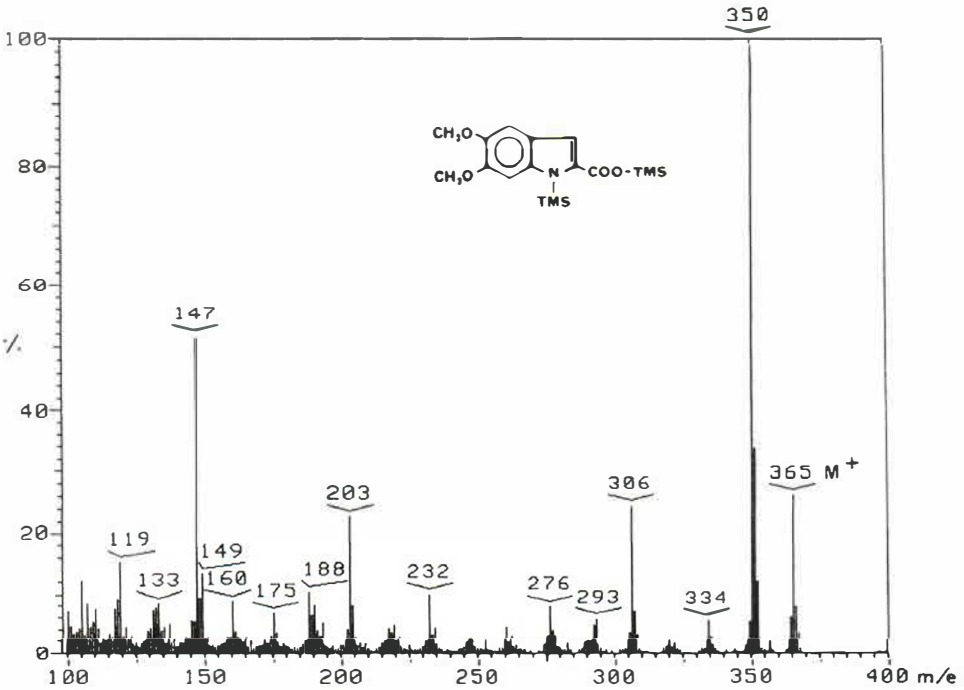

Fig. 1. Mass spectrum of the TMS derivative of synthetic 5,6DMI2C.

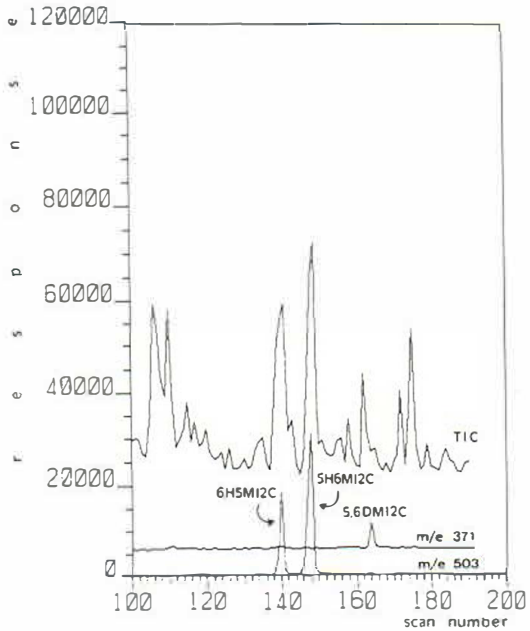

Fig. 2. Part of the total ion current chromatogram with selected mass plots of the HFIP-PFP derivatives of 6H5MI2C and 5H6MI2C (*m/e* 503) and 5,6DMI2C (*m/e* 371) extracted from melanotic urine.

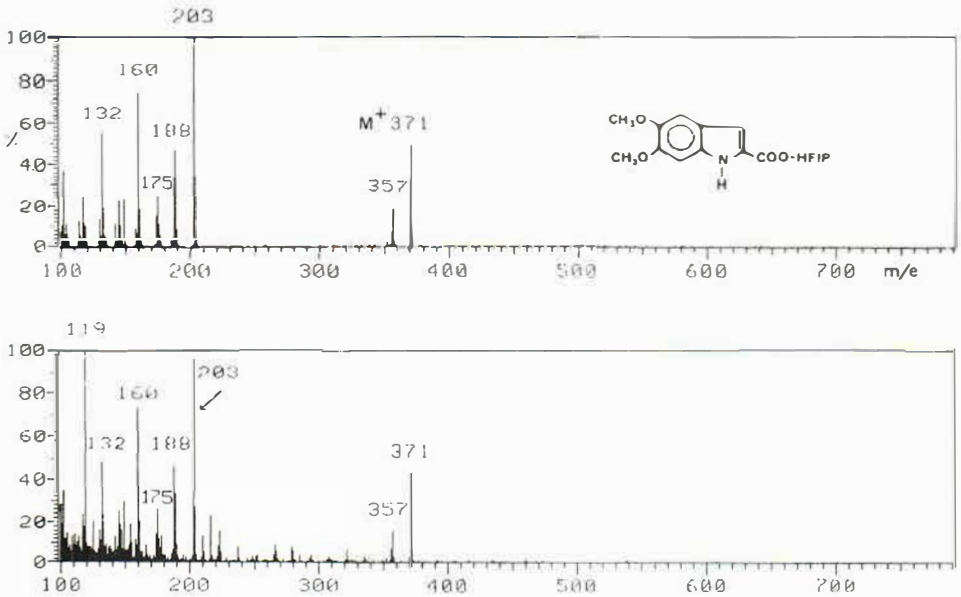

Fig. 3. Mass spectra of the HFIP derivative of synthetic 5.6DMI2C (*upper*) and 5.6DMI2C extracted from melanotic urine (*below*).

For the identification of 5,6DMI2C in melanotic urine, HFIP-PFP derivatives of the synthesized 5,6DMI2C and urinary extracts were prepared and analysed by GC-MS. The mass spectrum of the HFIP derivative of synthetically prepared 5,6DMI2C is shown in Fig. 3 (upper) and can be explained as follows: *m/e* 371=$[M]^+$; *m/e* 203=$[M-HFIP]^+$; *m/e* 188=$[M-(HFIP+CH_3)]^+$ *m/e* 175=$[M-COO-HFIP]^+$; *m/e* 160=$[M-(COO-HFIP+CH_3)]^+$. 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 5,6DMI2C (*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 5,6DMI2C (Fig. 3).

The presence of 5,6DMI2C in melanotic urine is an interesting phenomenon. Analogous to the isomeric 5H6MI2C and 6H5MI2C, one can presume that the formation of 5,6DMI2C 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

1. Agrup, G., Hansson, C., Kennedy, B.-M., Persson, K., Rorsman, H., Rosengren, A.-M. & Rosengren, E.: Analysis of cysteinyldopa isomers and some of their O-methyl derivatives in the urine of melanoma patients by means of gas chromatography – mass spectrometry. Acta Dermatovener (Stockholm) *56:* 491, 1976.
2. Axelrod, J.: The metabolism, storage, and release of catecholamines. *In* Recent Progress in Hormone Research, vol. 21, p. 597. Academic Press, New York, 1965.
3. Axelrod, J. & Lerner, A. B.: O-methylation in the conversion of tyrosine to melanin. Biochim Biophys Acta *71:* 650, 1963.
4. Duchoň, J. & Matouš, B.: Identification of two new metabolites in melanoma urine: 5-hydroxy-6-methoxyindole-2-carboxylic and 5-methoxy-6-hydroxyindole-2-carboxylic acids. Clin Chim Acta *16:* 397, 1967.
5. Pavel, S.,Matouš, B., Duchoň, J. & Bubnová, E.: 5-hydroxy-6-methoxy-2-indolylcarboxylic and 6-hydroxy-5-methoxy-2-indolylcarboxylic acids in the urine of hamsters with melanotic melanoma. Neoplasma *25:* 343, 1978.
6. Pavel, S. & Muskiet, F. A. J.: Synthesis of deuterium labelled eumelanin (precursor) metabolites. J Lab Comp Radiopharm *20*: 101, 1983.
7. Pavel, S. & Muskiet, F. A. J.: Eumelanin (precursor) metabolites as markers for pigmented malignant melanoma. A preliminary report. Cancer Detect Prevent 1982 (in press).
8. Pavel, S., Muskiet, F. A. J., Buděšínská, A. & Duchoň, J.: A qualitative gas chromatographic analysis of substituted 5,6-dihydroxyindoles from the urine of patients with melanoma. Tumori *67:* 325, 1981.
9. Pavel, S., Muskiet, F. A. J., De Ley, L., The, T. H. & Van der Slik, W.: Identification of three indolic compounds in a pigmented melanoma cell culture supernatant by gas chromatography – mass spectrometry. J Canc Res Clin Oncol 1983 (in press).
10. Pavel, S., Muskiet, F. A. J., Nagel, G. T., Schwippelová, Z. & Duchoň, J.: Identification of two Thormählen-positive compounds from melanotic urine by gas chromatography – mass spectrometry. J Chromatogr (Biomed Appl) *222:* 329, 1981.