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Short Communication

Identification of vitamin D_2 by thermospray-interface mass spectrometry

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ABSTRACT

In order to identify vitamin D_2 contained in shiitake mushroom (*Lentinus edodes*), which is taken routinely in Japan, vitamin D_2 was isolated by thin-layer liquid chromatography and high-performance liquid chromatography and identified by thermospray-interface mass spectrometry; this procedure prevents the decomposition of vitamin by heat, which is a common problem in the gas chromatography–mass spectrometry of vitamin D_2.

INTRODUCTION

The identification of vitamin D_2 [1] (hereafter called D_2) and the determination of vitamin D_3 [2] metabolites have usually been conducted by gas chromatography–mass spectrometry (GC–MS). It is well known, however, that the heating process during GC causes ring closure of a steroid ring between the 9- and 10-positions, converting D_2 into pyro D_2 and isopyro D_2 [3] (Fig. 1). In order to identify D_2 contained in shiitake mushroom (*Lentinus edodes*), which is taken routinely in Japan, we isolated D_2 by thin-layer liquid chromatography (TLC) and high-performance liquid chromatography (HPLC) and the isolated substance was identified by thermospray-interface mass spectrometry (TSP-MS). We have subsequently succeeded in obtaining a mass spectrum of D_2 that does not suffer from thermal ring closure.

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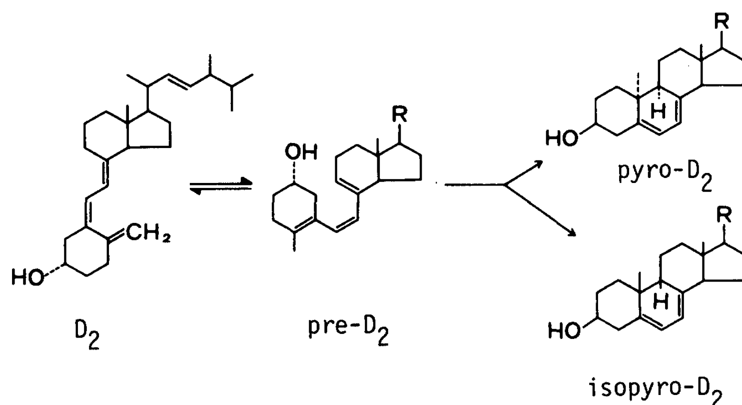

Fig. 1. Thermal isomerization of vitamin D_2.

EXPERIMENTAL

All reagents were of analytical-reagent grade from Wake (Osaka, Japan). Shiitake mushroom (brand name Koshin) produced in Japan from March to May was used as a sample. A 500-g sample was ground with a homogenizer (one fifth at a time), and all of it was placed in a digestion flask and decomposed by heating at 80°C for 30 min after addition of absolute non-aldehyde ethanol (400 ml), pyrogallol (40 g) and 50% potassium hydroxide solution (100 ml). After cooling to room temperature, unsaponifiable matter was extracted with 1000 ml of benzene, followed by washing once each with 500 ml of 1 *M* and 300 ml of 0.5 *M* potassium hydroxide solution and then four times with 100 ml of water [4]. The upper benzene layer was separated by TLC using a Wako-gel B5FM silica gel TLC plate (Wako) with benzene–acetone (95:5) as developing solvent and UV detection (254 nm) so as to obtain a D_2 fraction.

This D_2 fraction was extracted with chloroform and evaporated to dryness below 35°C. To the resulting residue 2 ml of methanol–acetonitrile (1:1) were added and this solution was further purified twice by HPLC on an NSLC Model 100A chromatograph (Nihon Seimitsu Kagaku). In the first step a LiChrosorb RP-18 column (250 × 7.5 mm I.D.) was used with methanol–acetonitrile (1:1) as eluent and in the second step a Nucleosil 100-5 column column (150 × 4.6 mm I.D.) with *n*-hexane containing 0.1% of *n*-amyl alcohol and 0.4% of isopropyl alcohol as eluent.

Finally, the isolated substance was dissolved in 0.5 ml of 0.1 *M* ammonium acetate–methanol (4:6) and directly subjected to identification of D_2 by TSP-MS on a VG Model 12-250 instrument (VG Analytical) without any HPLC column, under the following conditions: solvent flow-rate, 0.4 ml/min; source temperature, 230°C; probe temperature, 250°C; electron energy, 70 eV.

RESULTS AND DISCUSSION

Fig. 2 is a TSP mass spectrum the purified extract of shiitake mushroom. A major peak was detected at m/z 397, $[M+H]^+$, but little fragmentation was observed. Similar results were obtained with standard D_2. Pyro D_2 and isopyro D_2 were also subjected to TSP-MS under the same conditions as those employed for the

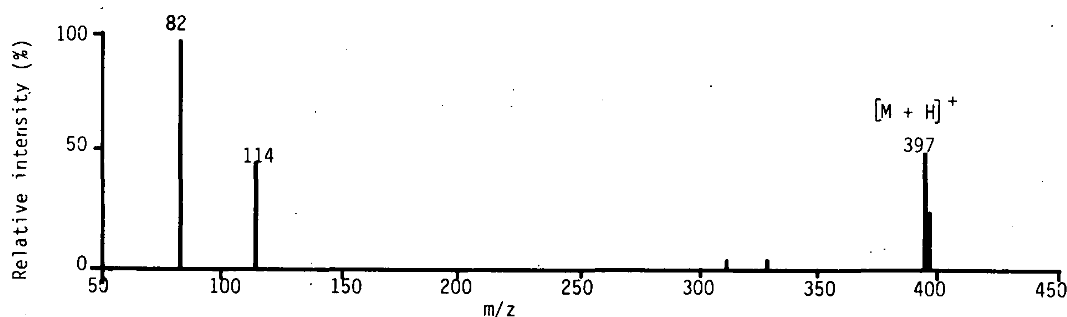

Fig. 2. Mass spectrum of the purified D_2 fraction obtained from shiitake mushroom (concentration 5 $\mu g/\mu l$). Injection volume, 3 μl.

standard D_2. No peak at m/z 397 was obtained with these samples, indicating that D_2 suffering no thermal ring closure can be identified by TSP-MS.

The identification of aldosterone and corticosterone, each having a steroid ring similar to that in vitamin D_3, by LC–MS [5,6] (measurement concentration 1 μg in 100 μl) and the determination of vitamin D_3 metabolites (1,25-dihydroxy-vitamin D_3 and 24,25-dihydroxy-vitamin D_3) by GC–MS [7,8] (determination concentration 0–250 ng/l) have been reported previously. In contrast to dihydroxy-vitamin D_3, the measurement concentration employed in this study was as high as 5 $\mu g/\mu l$, as D_2 is poorly ionized in TSP-MS as there are few functional groups. Previously D_2 has not been identified by TSP-MS.

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