II - PS 13 Methoxime-Trimethylsilyl Derivative of β-Ketoadipic Acid for GC/MS

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INTRODUCTION β-Ketoadipic acid is a key intermediate in the aromatic metabolism (Fig. 1). In the previous experiments, we have observed the formation of β-ketoadipic acid in the microbial degradation of phenol [1] and protocatechuic acid [2] using trimethylsilyl (TMS) derivatives by gas chromatography/mass spectrometry (GC/MS). When trimethylsilylated, β-ketoadipic acid was transformed into enol derivatives containing three TMS moieties.

The present paper reports formation of methoxime-trimethylsilyl (MO-TMS) derivative of β-ketoadipic acid to prevent enol ether formation for GC/MS.

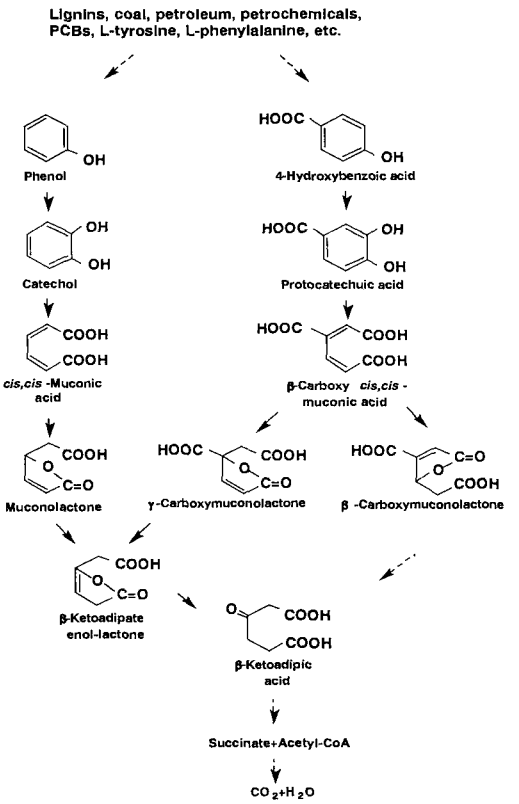

Fig. 1 β-Ketoadipate pathway

MATERIALS AND METHODS MO-TMS derivative of β-ketoadipic acid was prepared according to the method described by Thenot and Horning [3]. β-Ketoadipic acid was transformed into MO derivative prior to silylation to prevent enolization. Reactions were carried out in a glass bial with a Tefron cap liner. β-Ketoadipic acid (Sigma Chemical Co.) was added to 1 ml of a 2% solution of methoxyamine hydrochloride in pyridine (GL Sciences Co.) at a concentration of 0.1 mg/ml. After reaction at 60°C for 15 min, pyridine was evaporated with a nitrogen stream. The silyl donor N,O-bis(trimethylsilyl)acetamide (0.5 ml) was added and the solution was heated at 70°C for 30 min. The solution was used in GC/MS studies.

Mass spectra were obtained with a mass spectrometer (JMS-AX 505W; JOEL Ltd.) operated at the electron impact (EI) of 70 eV. The mass spectrometer was connected with a gas chromatograph (5890;

Hewlett Packard). Samples were analyzed on a fused silica capillary column (DB-5; 30 m by 0.25-mm in diameter; J & W). The temperature was programmed to rise from 50°C (for 1 min) to 140°C at the rate of 25°C/min, and then from 140°C to 250°C at the rate of 5°C/min.

RESULTS AND DISCUSSION Fig. 2 shows a total ion chromatogram (TIC) and mass chromatogram of ions at m/z 333 and 318, corresponding to a molecular ion (M^+) and M-CH_3 ion of MO-TMS derivative of β-ketoadipic acid, respectively. TIC revealed that β-ketoadipic acid was converted successfully to MO-TMS derivative and enol ether formation was not observed. Two peaks, nos. 1 and 2 in Fig. 2 suggested the presence of two stereoisomers of *syn* and *anti* type [4]. The *syn*-isomer may be the small peak no. 1.

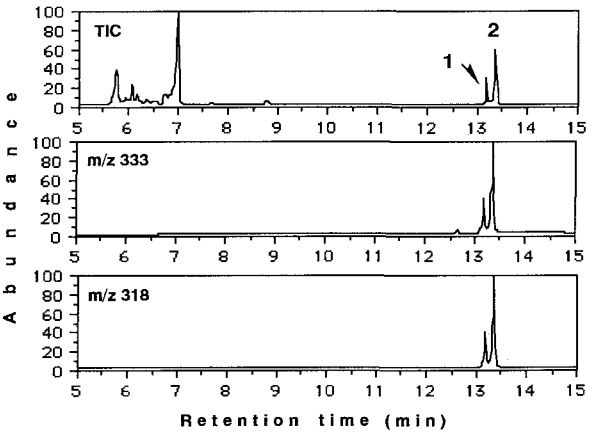

Fig. 2 Total ion chromatogram (TIC) of MO-TMS derivative of β-ketoadipic acid and mass chromatogram of ions at m/z 333 and 318.

Fig. 3 shows EI mass spectrum of MO-TMS derivative of β-ketoadipic acid. Mass spectra of two isomers of MO-TMS derivative of β-ketoadipic acid resembled each other in their fragmentation pattern; they had a molecular ion at m/z 333, and other characteristic ions at m/z 318 (M-CH_3), 302 (M-OCH_3), 243 (M-TMSOH) and 216 (M-COOTMS).

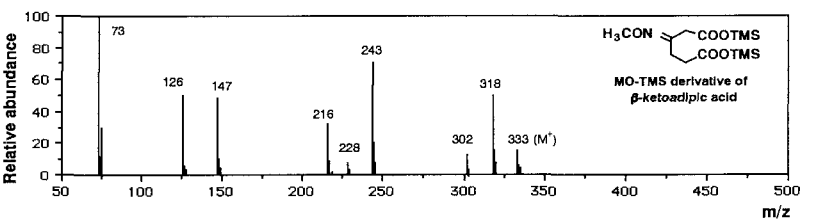

Fig. 3 Electron impact mass spectrum of MO-TMS derivative of β-ketoadipic acid.

The procedure presented here did not work on some compounds such as muconolactone, lacking a reactive ketone group.

This work was supported by a grant from the Ministry of Education, Science and Culture of Japan (No. 03454031).

REFERENCES

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