Formation of 3-Methylbutyrylurea from α -Bromoisovalerylurea in Putrefying Biological Sample

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The aim of the present study was to confirm putrefactive formation of 3-methylbutyrylurea (3 M) from α -bromoisovalerylurea (BV). A commercial minced pork sample was spiked with BV and incubated at room temperature (above 25 °C) for 1 month in a closed vessel. After the incubation, BV and 3 M were determined by thin-layer chromatography using guaiac gum reagent combined with treatment of the plate with chlorine gas and potassium iodide reagent. A spot of 3 M was detected and increased with the time of putrefaction, accompanied with a decrease of the BV spot. This finding suggested that 3 M was formed from BV in a putrefying environment.

Keywords— α -bromoisovalerylurea ; 3-methylbutyrylurea ; sample putrefaction ; TLC ; guaiac gum reagent

Introduction

α -Bromoisovalerylurea (BV), a hypnotic, is a drug of abuse, and an overdose may be lethal. BV is rapidly (within 24 h) metabolized to 3-methylbutyrylurea (3 M).¹⁻³⁾ Therefore, the determination of both BV⁴⁾ and 3 M⁵⁾ in body fluids, at least, is important to estimate the amount of BV⁶⁾ ingested, or the period between the ingestion and death. In addition to internal metabolism, BV is metabolized in the gastrointestinal tract by microorganisms as putrefaction proceeds.⁷⁾ The present study was designed to clarify the putrefactive formation of 3 M from BV in a commercial minced pork sample spiked with BV. Both BV and 3 M were detected by thin-layer chromatography (TLC) using guaiac gum reagent combined with treatment of the plate with chlorine gas^{8,9)} and potassium iodide reagent.^{8,9)} It was confirmed that 3 M was formed from BV in a

putrefying environment.

Experimental

Reagents and Apparatus— α -Bromoisovalerylurea (BV), Japan Pharmacopoeia grade, was purchased from Iwaki Pharmaceutical Industries, Ltd., Japan. Guaiac gum, potassium iodide, sodium hypochlorite, and hydrochloric acid were purchased from Wako Pure Chemical Industries, Ltd., Japan. 3-Methylbutyrylurea (3 M) was prepared from BV as described by Sawada.³⁾ Silica gel plates for TLC (No. 5721) were from Merck, Darmstadt.

TLC—An aliquot of supernatant (3 μ l) was applied on a silica gel plate and developed with a solvent system of chloroform-acetone (4 : 1, v/v). The plate was air-dried and exposed for about 5 min to chlorine gas, which was generated by dropping 1 ml of hydrochloric acid onto 1 ml of ca. 8.4 % sodium hypochlorite in a closed glass vessel (25 cm W, 11 cm D, 21 cm H). The plate was taken out and the excess chlorine was removed by heating with a hair-dryer. The chlorine-treated plate was sprayed

Fig. 1. Detection of 3-Methylbutyrylurea (3M) Produced from α -Bromoisovalerylurea (BV) in a Putrefying Pork Sample

A commercial minced pork sample spiked with BV was incubated at room temperature (above 25°C). After incubation for the indicated time(d), aliquots (3 μ l) were subjected to TLC on a silica gel plate with chloroform-acetone, 4 : 1, v/v. The plate was exposed to chlorine gas and sprayed with 1% potassium iodide followed by 2% guaiac gum reagent. The top numbers are the pH values of the medium.

with 1% potassium iodide solution followed by 2% guaiac gum ethanol solution.

Results

Formation of 3M from BV in Putrefying Sample

A mixture of 0.1% BV aqueous solution (100 ml) and commercial minced pork (100 g) was incubated at room temperature (above 25°C) for 1 month in a closed Erlenmeyer flask (500 ml). During the period, 0.5 ml aliquots of the supernatant were taken from the mixture periodically and stored frozen until chromatographic analysis. All of the collected samples were thawed and directly subjected to TLC. As shown in Fig. 1, the spot of 3M gradually increased with time, while the spot of BV decreased. As shown at the top of Fig. 1, the pH of the medium changed from neutral to alkaline *via* acidic during the period. Generation of hydrogen sulfide was recognized on day 2 by passing a 3 ml aliquot of the head space gas of the reaction mixture through a Kitagawa-precision gas detection tube (B type, Komyo Rikagaku Kogyo K.K., Japan). The changes of pH in the medium and the

generation of hydrogen sulfide reflect the stage of putrefaction of the sample.

Discussion

The present study employed a muscle tissue sample (pork) for the observation of putrefactive formation of 3M from BV. In a supplementary experiment, needle-like crystals of 3M (*ca.* 30 mg, 40% recovery, mp 204–205°C) were obtained from an incubation mixture (50 ml) containing rabbit intestinal contents (30 g) spiked with BV (100 mg) after incubation for 1 week at room temperature (above 25°C). The crystals were identified as 3M by infrared spectrometry⁸⁾ and by mass spectrometry: the peak ratio of m/z 145 ($M^+ + 1$) to m/z 102 ($M^+ - \text{CH}_3 - \text{CH} = \text{CH}_2$) was 3 : 100 in the electron impact measurement mode and 100 : 15 in the chemical ionization mode using methane.

Hara reported that the content of BV remaining in the stomach of BV-administered rabbit gradually decreased with the putrefaction period.⁷⁾ In the same report, an unknown ethylether-insoluble product was detected by paper chromatography using a developing sol-

vent of ammonium hydroxide-saturated butanol: R_f values were 0.1—0.2 for the unknown material and 0.8—0.9 for BV. However, its structure has not been identified. In the present study, we confirmed the production of 3M from BV in a putrefying biological sample.

The present study employed guaiac gum

reagent as a substitute for benzidine¹⁻³⁾ for the detection of BV and 3M by TLC. Benzidine is highly sensitive, but is no longer obtainable commercially because of its carcinogenicity. Guaiac gum reagent, traditionally well-known to be harmless, showed detection limit of *ca.* 5 ng and *ca.* 8 ng for 3M and BV, respectively.

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