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原　　報

STABILITY OF ALKYL ALCOHOLS AS DECOMPOSITION PRODUCTS OF ALKYL NITRITES IN HUMAN WHOLE BLOOD

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亜硝酸エステル分解産物としてのアルキルアルコール類の血液中安定性について

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Summary

Some conditions of storage have been tested for stability of *n*- butyl alcohol, isobutyl alcohol and isoamyl alcohol as decomposition products of alkyl nitrites in human whole blood samples. The above three alkyl alcohols (1 μg/ml each) were spiked to whole blood, and kept at room temperature or at 4℃ for various intervals without any addition of preservatives. The alkyl alcohols were measured by the cryogenic oven trapping gas chromatography. At room temperature, the alkyl alcohols gradually decreased for 7 days; but the decreased rates were only 10－25%. At 4℃, the compounds were almost stable for at least 14 days. There were no postmortem production for each alkyl alcohol. Our results support the idea that the decomposition product alkyl alcohols can be used for detection of alkyl nitrite abuse.

Key words; Alkyl alcohols; *n*- Butyl alcohol; Isobutyl alcohol; Isoamyl alcohol; Alkyl nitrites; Stability; Cryogenic oven trapping gas chromatography

Introduction

The abuse of alkyl nitrites is becoming a serious social problem in the world [1]. The most popular alkyl nitrite being abused is isobutyl nitrite, followed by isoamyl nitrite and *n*- butyl nitrite [2]. Since they are usually not controlled by laws, it is very easy to get them at common markets or by internet sales. Alkyl nitrites rapidly decompose to alkyl alcohols and inorganic nitrite by light or chemical hydrolysis in aqueous or biological matrices [3,4]. Therefore, the decomposition products an alkyl alcohol and/or inorganic nitrite should be analyzed especially for human samples. The inorganic nitrite in blood is, however, very unstable interacting with hemoglobin [5].

In our recent report, we have presented a sensitive method for analysis of alkyl alcohols in human samples using cryogenic oven trapping gas chromatography (GC) in an attempt to detect alkyl nitrite abuse [6]. In this brief report, we have tested whether alkyl alcohols are stable in human whole blood, because it is practically very important and such a study has not appeared to our knowledge.

Experimental

Materials

t- Butyl alcohol, isobutyl alcohol, *n*- butyl alcohol and isoamyl alcohol were purchased from Wako Pure Chemical Industries (Osaka). Other common chemicals used were of the highest purity commercially available. An Rtx-BAC2 medium-bore capillary column (30 m x 0.32 mm i.d., 0.25 μm

film thickness) was purchased from Restek (Bellefonte, PA, USA). Whole blood was obtained from a healthy volunteer; it was heparinized to prevent it from coagulation.

Procedure

Aqueous solution of the mixuture of isobutyl alcohol, *n*- butyl alcohol and isoamyl alcohol (100 μg/ml each) was prepared; the solution of *t*- butyl alcohol (100 μg/ml each) was prepared separately on the day of analysis. To a 7.0-ml screw cap vial containing 1 ml of whole blood, was added 10 μl (1 μg each) of the above aqueous solution of alkyl alcohols, sealed with a silicone-septum cap and mixed well by a vortex. The vials were left at room temperature and 4℃ for various intervals. The 10-μl volume (1 μg) of the aqueous solution of *t*- butyl alcohol (internal standard, IS) and 0.5 g of sodium sulfate were added to each blood sample just before analysis. The vial was rapidly sealed with the silicone-septum cap and placed on an alminum block heater. After heating the vial at 55℃ for 15 min, 5 ml of the headspace vapor was drawn with a gas-tight syringe (10-ml volume with a 23 G needle) and injected into the GC port in the splitless mode at oven temperature of 0℃.

GC conditions

GC analyses were carried out on a Shimadzu GC-15A equipped with flame ionization detection and a cryogenic oven temperature device (Shimadzu, Kyoto). The column temperature was 0 to 240℃ (held for 1 min at 0℃, then from 0℃ to 120℃ at 10℃/ min, and then from 120℃ to 240℃ at 20℃/min); the injection and detection temperature was 240℃; and helium flow rate was 3 ml/min. The 5-ml vapor was injected in the splitless mode, and the splitter was opened 1 min after the completion of the injection.

Results

Figure 1 shows percent concentrations of alkyl alcohols in whole blood spiked with 1 μg each of the compounds as a function of storage days at room temperature. All alkyl alcohols equally showed the tendency of their gradual decrease for 7 days; however the decreased rates were only 10－30%. No postmortem production of the compounds was found.

Figure 2 shows the results obtained from the similar experiments conducted at 4℃. The lines shows that the concentrations of alkyl alcohols were not changed significantly for at least 14 days at this temperature.

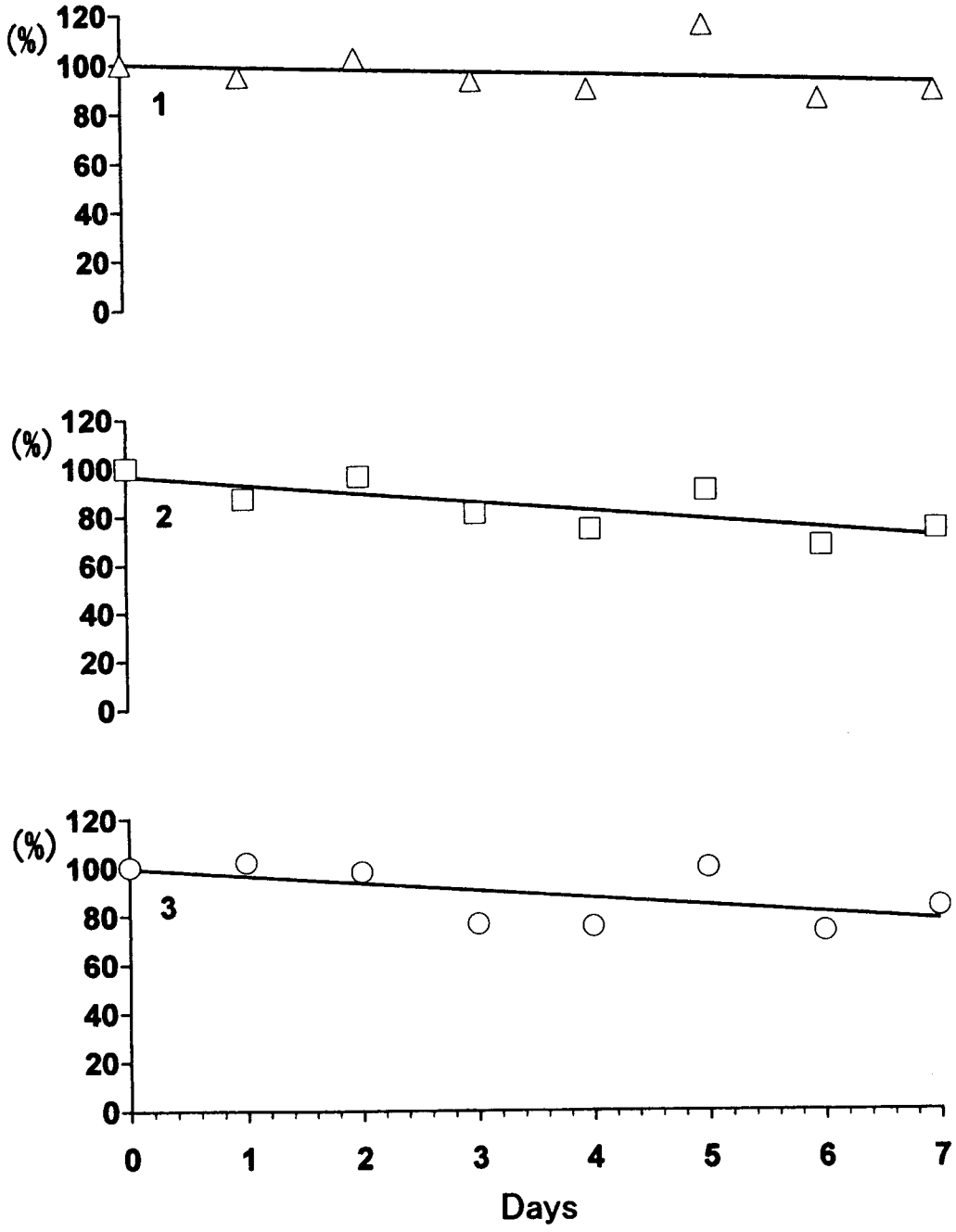

Fig. 1. Stability of alkyl alcohols (1 μg/ml each) in human whole blood at room temperature. The concentration of each alkyl alcohol on day 0 was taken as 100%; all concentrations were expressed as percent values. The straight lines were drawn using the least square method. 1: isobutyl alcohol; 2: *n*- butyl alcohol; 3: isoamyl alcohol.

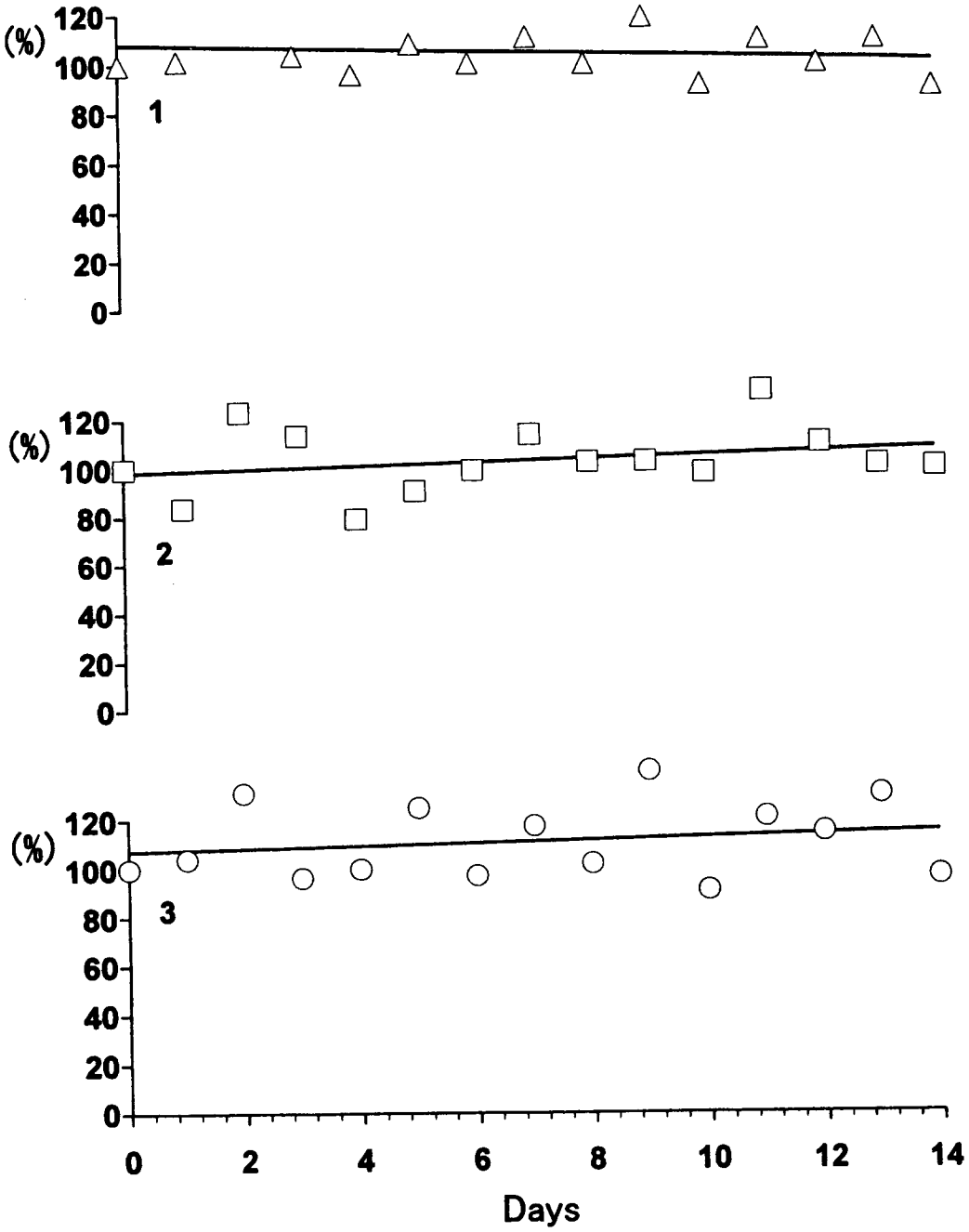

Fig. 2. Stability of alkyl alcohols (1 μg/ml each) in human whole blood at 4℃. The explanation and the numbers (symbols) of the figure is the same as specified in Fig.1.

Discussion

In our previous work [7], we tested stability of ethyl acetate and ethanol in whole blood as a function of storage intervals at room temperature and 4℃; marked postmortem production of ethanol and rapid decomposition of ethyl acetate were found in the absence of any preservative. Such phenomena were not found for the present alkyl alcohol tested (Figs. 1 and 2). They were relatively stable even at room temperature. Our results show that alkyl alcohols can be good indicators for alkyl nitrite abuse.

The other decomposition product from alkyl nitrites is inorganic nitrite, which is easily oxidized to nitrate in blood [5]. Although nitrate was reported stable in whole blood for 1 week at 4℃ [5], the endogeneous concentrations of nitrate were reported to be as high as 0.64－5.7 μg/ml in blood [7]; because of the instability of nitrite and endogenous existence of nitrate, both inorganic compounds cannot be used as indicators of the past presence of an alkyl nitrite in blood.

The present study has shown that inhalation or ingestion of an alkyl nitrite can be proven by detecting an alkyl alcohol from whole blood of a cadaver, which had been left at room temperature for less than 7 days (Fig.1).

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