様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

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研究課題名（和文）Investigation of a novel analysis method for the determination of new biomarkers for alcoholic beverage consumption.

研究課題名（英文）Investigation of a novel analysis method for the determination of new biomarkers for alcoholic beverage consumption.

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研究成果の概要（和文）：アルコール飲料の摂取を特定できる新たなバイオマーカーを探索した。いくつかのアルコール飲料を分析し、飲酒者の尿を調べた結果、ホルデニン（ビール）、エチルグルコシド（ビール、ワイン、日本酒）、リンゴ酸エステル（ワイン）、シリンジン酸（ワイン、ウイスキー）などが候補として検出された。エチルグルコシドは日本酒飲酒者に特異的に高濃度で検出されたが、ウイスキーにも含まれていないことから、体内で生成される可能性が示唆された。さらに実験により、エチルグルコシドは体外でエタノールと糖類から生成されることが確認された。今後はリンゴ酸エステルに注目し、ワイン摂取の特異的なバイオマーカーとしての可能性を検証していく。

研究成果の学術的意義や社会的意義
本研究は、飲酒運転撲滅やアルコール依存症の診断・治療に貢献する新たな一歩を踏み出しました。従来の指標よりも長く体内に残る「エチルグルコシド」を飲酒運転の新指標として発見し、飲酒から時間が経過しても飲酒の有無を正確に判断できるようになりました。
さらに、この研究は、ワイン摂取量の客観的な指標開発にもつながり、個人の飲酒習慣把握やアルコール問題のリスク特定に役立つ可能性があります。ワイン中のリンゴ酸誘導体の存在も確認され、ワインの風味や品質への影響解明が期待されます。
本研究は、飲酒運転事故削減、アルコール依存症対策、ワイン品質評価向上など、社会に多大な貢献をもたらす

研究成果の概要（英文）：In this research, we searched for new biomarkers that can identify alcoholic beverage intake. After analyzing several alcoholic beverages and examining the urine of drinkers, we detected hordenine from beer drinkers, ethyl glucoside from beer, wine, and sake drinkers, a malic acid ester derivative from wine drinkers, and syringic acid from wine and whisky drinkers. Ethyl glucoside was detected at high concentrations specifically in sake drinkers. However, while not being present in whisky itself, it was also found in the urine of whisky drinkers, suggesting that it may be produced within the body. Further experiments confirmed that ethyl glucoside is produced in vitro from ethanol and sugars such as maltose. In the future, we will focus on the malic acid ester derivative by determining its exact chemical structure and by confirming its presence in various red and white wine varieties. We will also attempt to verify its potential as a specific biomarker for wine intake.

研究分野：法医学

キーワード：alcohol biomarker　ethyl glucoside　malic acid derivative　alcoholic beverages　GC-MS　forensic toxicology　forensic autopsy　forensic medicine

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

The purpose of toxicology tests in forensic autopsies is to detect all chemical substances that might directly or indirectly contribute to the cause of death or pathological conditions of the decedent. Our department has developed wide-ranging analytical methods using GC-MS, GC-MS/MS and LC-MS/MS to detect pharmaceutical and illicit drugs, poisons, or endogenous compounds from the decedent's blood, urine, or tissue samples collected at autopsy. Part of our established routine sample preparation method involves using an ion-exchange solid-phase extraction cartridge to simplify the screening process. This method has revealed compounds that were not detected by previous methods, such as 3-hydroxybutyric acid, a marker of diabetic ketoacidosis; ethyl α-D-glucoside, a metabolite of rice malt; and phenylacetylglutamine, a conjugate of phenylacetic acid and glutamine that can be found in human urine. We are currently conducting research to elucidate the forensic significance of these newly detectable compounds. Ethyl α-D-glucoside is a well-known component of Japanese sake and some wines. Therefore, in a previous research project we decided to investigate whether ethyl α-D-glucoside could be used as a marker to prove drinking.

It has been reported that ethyl α-D-glucoside is contained in relatively large amounts in sake and is excreted in urine. In experiments on rats, it was absorbed from the intestines and excreted in the urine. Our analysis of autopsy samples revealed that ethyl α-D-glucoside was clearly detected in the urine, although the blood concentration of ethanol was extremely low. Even in autopsy cases, ethyl α-D-glucoside absorbed from the intestines was thought to be excreted in the urine for a long time after drinking alcohol.

It is socially important to prove that a suspect who committed a hit-and-run while driving drunk was drinking at the time of the accident. We came up with this research idea because we believed that if we could detect chemical substances contained in alcoholic beverages over a long period of time in the suspect's urine, where only a small amount of ethanol was detected, it would be possible to prove that the suspect was under the influence of alcohol at the time of the accident. Car accidents are not the only reason to focus on these compounds. There may be other incidents such as sexual assaults and on-the-job accidents that may be better investigated if the specific alcoholic beverages that were consumed was known.

２．研究の目的

If a suspect in a drunk driving accident commits a hit-and-run, and is taken into custody several hours after the accident, it will not be possible to prove that they were intoxicated unless ethanol is detected. Our goal is to scientifically prove that the suspect was drinking alcohol at the time of the accident by searching for a drinking marker to replace ethanol and analyzing the marker from a sample (blood or urine) collected from the suspect. The purpose was to search for new drinking markers to replace ethanol. We analyzed alcohol components, such as ethyl α-D-glucoside, which were retained in and absorbed from the human gut, and excreted in the urine after drinking, to find out whether they could serve as new drinking markers.

The analysis of these compounds would not be limited to cases of drunk driving, but also other cases where knowing the circumstances surrounding an incident is of utmost importance. Other examples include sexual assault and workplace accidents. Also, the application of these tests could be extended to samples from deceased individuals. The purpose of forensic autopsies is to determine the cause and manner of death. Knowing the specific alcoholic beverage or beverages that a person was consuming shortly before death could be of utmost importance.

３．研究の方法

Alcoholic beverages including various brands and styles of beer, Japanese sake, white and red wine, and whisky were purchased through a vendor. A sample preparation was developed using two types of polymer mix ion-exchange solid-phase extraction columns. These were used to remove interfering compounds while still allowing the desired components (alcoholic beverage congeners) to pass through. After the initial analyses, several possible candidates were isolated. Next, a drinking study was performed where volunteers consumed specific amounts of an alcoholic beverage after a period of abstinence and their urine was collected at specific intervals (i.e., before drinking, 1 hour, 2 hours, 3 hours, and so on). The above sample preparation method with slight alterations was performed on the collected urine. Data analysis was completed and the alcoholic beverage components were targeted. Among the components of alcoholic beverages detected, the compounds that are frequently detected were isolated and examined.

Among the components detected, many were hydrophilic and polar compounds, which were difficult to measure with high precision using commonly used sample preparation and measurement conditions for GC-MS. Thus, a method was developed to solve this problem and led to a rapid and efficient extraction method.

4．研究成果

Ethyl α-D-glucoside (EG) was detected in high concentrations in the urine of drinkers of Japanese sake. There was also EG detected in various concentrations in beer and some kinds of wine, particularly red wine. EG exists as α and β isomers, and they were detected in different concentrations depending on the type of beverage (Fig. 1).

α	β	酒類
＋＋	－	日本酒
＋	＋＋	赤ワイン
＋＋	＋＋	ビール

Figure 1. α and β isomers of ethyl glucoside detected in alcoholic beverages.

Through various experiments with reacting various sugars with ethanol and gut enzymes, it was determined that EG is generated in vivo in the gut in humans. This is important because it shows that the presence of EG is not just because EG exists in the beverage itself. Whisky, for example, does not contain any EG in the beverage itself, yet after the drinking experiments, EG was detected in the urine of whisky drinkers. EG is still a possible candidate as an alcohol beverage biomarker, however, because it relies on the presence of the ethanol in the alcoholic beverage for the EG to be produced in the human gut. Another advantage that EG has with regard to being a possible replacement for ethanol as a drinking marker is its tendency to stay in the drinker's system for longer periods of time. Ethanol is quickly metabolized and eliminated in a relatively short period of time. EG, however, tends to stay in the urine long after consumption of the alcoholic beverage (Fig. 2).

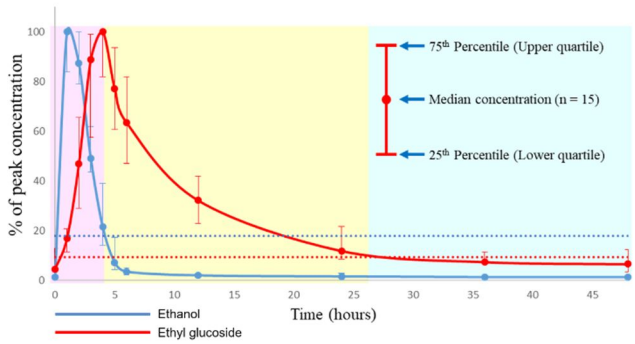

Figure 2. The concentrations of ethanol and ethyl glucoside in urine over time.

Another promising candidate that was detected in some alcoholic beverages and in the urine of drinkers was a compound that appears to be a derivative of malic acid. Malic acid is often used as a congener in alcoholic beverages to add acidity and for flavor enhancement, and it is also a well-known constituent in grapes that are often used for wine making. Thus, it is not unusual to find malic acid in alcoholic beverages. In our experiments, we detected two prominent peaks that corresponded to the GC-MS fragmentation ions *m/z* 89 and *m/z* 71. These ions are associated with malic acid, but since there were two separated peaks it was thought

that they one of them could be a derivative of malic acid that might be produced during the production of the wine. Also detected was a third peak with a prominent ion of *m/z* 117 that is likely an ethyl ester derivative of malic acid, 4-ethoxy-3-hydroxy-4-oxobutanoic acid (Fig. 3).

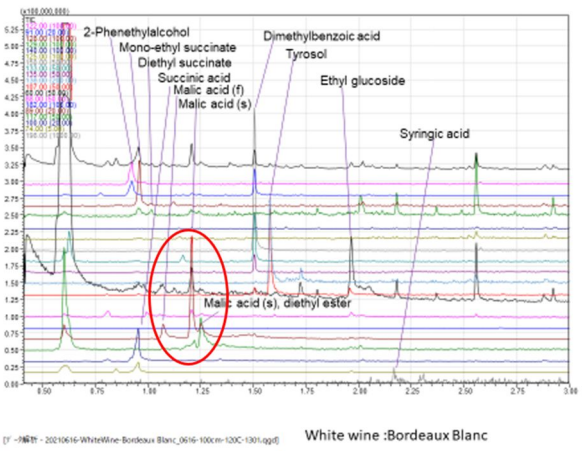

Figure 3. The presence of malic acid derivatives in a sample of white wine.

This ethyl ester derivative is where further work is underway to investigate whether this can be a possible biomarker for the consumption of wine. The first steps would be to determine if the compound is readily detectable from human samples (blood and urine) from drinkers of wine. Next would be to determine whether this compound exists in drinkers of other alcoholic beverages or in the samples of teetotalers. Finally, a robust and efficient method for its detection in human blood and urine, possibly using derivatization with TMS or other reagents will be examined.

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３．雑誌名 Journal of Chromatography B	６．最初と最後の頁 123074～123074
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.jchromb.2021.123074	査読の有無 有
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〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関