The Identification of α -Ethyl Glucoside and Sugar-alcohols in *Sake*

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α -Ethyl glucoside was found in *sake*, Japanese rice wine, as much as 1~2 mg/ml together with small amounts of erythritol, arabinitol and mannitol.

Quantitative analysis of those components was performed by gas chromatography for various kinds of *sake* and synthetic *sake* obtained commercially.

During the course of analytical investigation on sugars in human urine, we found a unknown compound, which is neither reducing sugar nor sugar-alcohol, in urine of a man who had taken *sake*, Japanese rice wine.

The same compound was detected in *sake* after the examination by gas chromatography. In the present paper, identification and determination of the compound together with sugar-alcohols are described.

MATERIALS AND METHODS

1. Apparatus

Gas chromatograph. A Shimadzu model GC-1C gas chromatograph equipped with a hydrogen flame ionization detector was used. Gas chromatography was carried out under the conditions described in Fig. 3.

GC-MS. A Hitachi model K-53 gas chromatograph was combined with a Hitachi model RMU-6 mass spectrometer by a Watson-Biemann "molecular separator". The ion source was at 200°C, and ionizing potential and current were 40 eV and 80 μ A, respectively.

2. Preparation of samples

Ethyl glucoside. To 2 g of glucose, 100 ml of abs. ethanol and 0.5 ml of sulfuric acid were added and refluxed for 4 hr. The solution was diluted to 300 ml with dist. water and BaCO₃ was added to neutralize the solution. Then, the excess BaCO₃ was

removed by filtration and the filtrate was treated as shown in Fig. 1 and a mixture of α - and β -ethyl glucoside was obtained as syrup.

β -Ethyl glucoside. β -Ethyl glucuronide, which was synthesized according to the usual method,¹⁾ was methylated with CH₃N₂ and reduced with NaBH₄.

3. Determination of α -ethyl glucoside and sugar-alcohols in *sake*

0.5 ml of *sake* was neutralized and evaporated to dryness under reduced pressure below 40°C. The residue was dissolved in 3 ml of dist. water and passed through Amberlite CG-120 (H⁺) column (0.8 $\times$ 15 cm) and Amberlite CG-4B (AcO⁻) column (0.8 $\times$ 15 cm), then the effluent was evaporated to dryness. The residue was dissolved in 3 ml of dist. water and passed through Dowex 1 (OH⁻) column (1 $\times$ 6 cm) and next QAE-Sephadex (borate form) column (0.8 $\times$ 8 cm). The effluent was evaporated to dryness, and to the residue containing α -ethyl glucoside, 500 μ g of mannitol was added as internal standard. The sample was submitted to gas chromatography as trifluoroacetyl (TFA) derivatives according to our method.²⁾

On the other hand, sugar-alcohols in *sake* adsorbed on QAE-Sephadex column were eluted with conc. HCl-MeOH (1:24), and to the solution 100 μ g of galactitol was added. The solution was evaporated to dryness. The residue was analyzed by gas chromatography in a similar manner described above.

1) B. Helferich and A. Berger, *Ber.*, **90**, 2492 (1957).

2) M. Matsui, M. Okada, T. Imanari and Z. Tamura, *Chem. Pharm. Bull.*, **16**, 1383 (1968); T. Imanari, Y. Arakawa and Z. Tamura, *ibid.*, **17**, 1967 (1969).

RESULTS

I. Detection of α -ethyl glucoside and sugar-alcohols in sake

Five ml of sake was treated as shown in Fig. 1 and a syrupy unknown substance was obtained in the glucoside fraction. The compound was not reduced with NaBH_4 , but gave glucose after hydrolysis with 2.5 N trifluoro-

FIG. 1. Isolation of α -Ethyl Glucoside and Sugar-alcohols.

acetic acid at 100°C for 4 hr.

Aliquots of the compound was submitted to gas chromatography as TFA derivatives according to our method for sugar-alcohols.²¹ The gas chromatogram showed one peak and the compound corresponding to the peak was analyzed by GC-MS (Fig. 2). The peak of highest molecular weight was at m/e 592 and this corresponded to TFA derivative of ethyl glucoside. Authentic ethyl glucoside was submitted to GC-MS, and the mass spectrum of it agreed closely with Fig. 2. In order to decide α - or β -configuration of the compound in sake, retention times of α - and β -ethyl glucosides synthetically obtained were measured as TFA, trimethylsilyl (TMS) and acetyl (Ac) derivatives on the different gas chromatographic conditions. Ethyl glucoside in sake corresponded to α -anomer exactly in gas chromatographic properties. From these results, the unknown compound was identified as α -ethyl glucoside. Furthermore, sugar-

FIG. 2. Mass Spectrum of the TFA Derivative of α -Ethyl Glucoside from Sake.

TABLE I. RETENTION TIMES (min) OF ETHYL GLUCOSIDE

Derivatives and column conditions	TFA		TMS		Ac
	2%XF-1105 140°C	2%XF-1105 180°C	2%QF-1 140°C	2%OV-1 180°C	2%XF-1105 190°C
Compound					
α -Ethyl glucoside	6.20	4.06	3.20	6.80	8.60
β -Ethyl glucoside	10.05	5.16	4.35	—	9.17
α -Glucose	6.35	4.34	3.40	7.30	—
β -Glucose	11.10	6.51	5.45	10.85	—

FIG. 3. Gas Chromatogram of Sugar-alcohols from *Sake*.

Peak: 1. Erythritol, 2. Arabinitol, 3. Mannitol.
Condition: 2% XF-1105 (on Gas-Chrom P), glass tube, 1.8 m $\times$ 4 mm *i.d.*
temperature; column 140°C, HFID 200°C.
carrier gas; N₂, 70 ml/min.
sens. 10³ range 0.8 V.

and the results are shown in Table II. Sorbitol was often detected in synthetic *sake*.

DISCUSSION

Sugar components in *sake* have been investigated by many workers³⁻⁵⁾ using paper and column chromatographies, and glucose, isomaltose, panose and other oligosaccharides have been found. Aso³⁾ and Fukinbara⁴⁾ have also found two kinds of nonreducing sugars composed of glucose but the structure of these sugars has been obscure. In order to see whether one of these corresponds to α -ethyl glucoside, paper chromatography was performed using almost similar conditions described in their papers. Although the *R_f* values of the unknown sugars were shown near cellobiose, in their report, α -ethyl glucoside gave larger *R_f* value (Table III). Then, it was clarified that any of their unknown substance was different from α -ethyl glucoside.

In gas chromatograms of ethyl glucoside

TABLE II. CONCENTRATIONS OF α -ETHYL GLUCOSIDE AND SUGAR-ALCOHOLS IN *Sake*, μ g/ml

	<i>Sake</i>					Synthetic <i>Sake</i>		
	A	B	C	D	E ^{a)}	F	G	H
α -Ethyl glucoside	1150	1460	1230	1750	1770	722	1890 ^{b)}	544
Erythritol	116	169	92	202	69	63	61	38
Arabinitol	110	102	61	155	15	35	33	32
Mannitol	61	29	35	40	15	14	17	16
Sorbitol	—	—	—	—	—	83	38	—

^{a)} This sample is addition-free sake which was kindly gifted from Dr. Kenichi Otsuka in Research Institute of Brewing. The other samples were obtained commercially.

^{b)} In this sample, β -ethyl glucoside was also found as much as 100 μ g/ml.

alcohols were examined by gas chromatography using sugar-alcohol fraction of Fig. 1, and erythritol, arabinitol and mannitol were detected as shown in Fig. 3.

II. Determination of α -ethyl glucoside and sugar-alcohols in *sake* and synthetic *sake*

α -Ethyl glucoside and sugar-alcohols in samples were analyzed by gas chromatography according to the procedure described above,

fraction of *sake*, a single peak was observed usually, but in those of synthetic *sake*, un-

3) K. Aso, K. Shibasaki and F. Yamanouchi, *Hakko Kogaku Zasshi*, **30**, 311 (1952); *idem, ibid.*, **31**, 211 (1953).

4) T. Fukinbara and K. Muramatsu, *Nippon Nogeikagaku Kaishi*, **26**, 538 (1952); *idem, ibid.*, **28**, 529 (1953).

5) F. Hemmi and N. Tukiashi, *Nippon Nogeikagaku Kaishi*, **8**, 1319 (1932); *idem, ibid.*, **9**, 179 (1933).

TABLE III. *R_f* VALUES OF SUGARS

Compound	<i>R_f</i> Value
Glucose	0.38
Maltose	0.27
Cellobiose	0.25
Isomaltose	0.15
α -Ethyl glucoside	0.60

Descending paper chromatography

Paper: Toyo Roshi No. 51A

Developing solvent: *n*-BuOH, Pyridine, H₂O (6:4:3)

Dyeing reagent: benzidine-sodium metaperiodate

known peak was often observed in front of the peak corresponding to α -ethyl glucoside. Moreover, β -ethyl glucoside was detected in the synthetic *sake* G in Table II, which tasted bitter. Crude glucose, sucrose, amino acids and many other things are usually added to *sake* and synthetic *sake*, which are commercially obtained, to improve flavors of them. Therefore, the specially brewed *sake* (sample E in Table II) was analyzed, and the results indicated that *sake* contains α -ethyl glucoside naturally.

From some commercial samples of beer, β -ethyl glucoside (about 10 μ g/ml) together

FIG. 4. Gas Chromatograms of Ethyl Glucoside Fraction of *Sake* (A) and Synthetic *Sake* (B).

Condition: the same as Fig. 3.

with a similar amount of α -ethyl glucoside and very small amounts of erythritol, arabinitol and mannitol were found. It is very interesting that *sake* contains a large amount of α -ethyl glucoside but not β -anomer.

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