2-O-(β -L-Arabinopyranosyl)-myo-inositol as a Main Constituent of Tea (*Camellia sinensis*)[†]

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A new main tea constituent, 2-O-(β -L-arabinopyranosyl)-myo-inositol (1, ca. 0.8% of a Japanese green tea) was characterized by ¹³C-NMR spectroscopy. The inositol glycoside (1) has been found not only in all the tea extracts so far examined but also in the homogenate of *Camellia sinensis* var. *sinensis* ("Yabukita cultivar"), suggesting its physiological importance in carbohydrate metabolism in the tea plant.

Extensive chemical analyses have revealed many constituents of tea.¹⁾ Several attempts have been made to use analytical data to determine tea grade, but with little success to date.^{2,3)}

Recent developments in NMR spectroscopy⁴⁾ allow both qualitative and quantitative structural information of multicomponent solutions to be obtained without any pretreatment or purification. We focused our research interests on the application of this attractive analytical method to instantly classify and evaluate made tea. In the course of a complete assignment of signals in the ¹³C-NMR spectra of tea samples, we found a few signals unassignable to any known tea constituents. Being guided by the characteristic signals, we isolated and identified potassium quinate as a major tea component.⁵⁾ Another compound giving previously unidentified signals was tentatively named compound Y.⁶⁾ In this paper we describe the details of the isolation and structural elucidation of this new tea constituent.

Results and Discussion

A comparison of the ¹³C-NMR spectra of a few typical tea samples showed significant differences, suggesting the applicability of this analytical method to a rapid classification and evaluation of tea.⁷⁾ Figure 1 shows the ¹³C-NMR spectra of the extracts of a Japanese green tea ("Sen-cha"), an Oolong tea ("Tie Guan Yin") and an Indian black tea ("Nilgiris"). Most of the signals were easily assigned by directly comparing with those of authentic specimens,⁵⁾ but several signals marked with stars could not be assigned to any known tea component, the size of the signals suggesting that this unknown "compound Y" was a major tea component (Fig. 1). These characteristic signals were observed not only in the spectrum of the green tea but also in the spectra of the Oolong and black tea.

A hot water extract of green tea was extracted with ethyl acetate and then with butanol. The signals in question were found in the spectrum of the aqueous layer. Being guided by

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Fig. 1. ^{13}C -NMR Spectra (22.5 MHz) of Hot Water Extracts of a Few Typical Tea Samples.

these signals, chromatographic purification of the aqueous layer finally gave compound **Y** (**1**, *ca.* 0.8% of the made tea). Crystallization from hot methanol or aqueous ethanol gave beautiful colorless crystals: mp $194\sim 194.5^\circ\text{C}$; $[\alpha]_D^{23} + 146^\circ (c=1.04, \text{H}_2\text{O})$.

In the ^{13}C -NMR spectrum of **1**, eleven oxygenated carbon signals (δ 66~104) including only one anomeric carbon (δ 104.0) were observed. The base peak at m/z 351 ($\text{M} + \text{K}$) in the FD-MS spectrum, as well as analytical data giving the molecular formula, $\text{C}_{11}\text{H}_{20}\text{O}_{10}$ (M.W. 312), suggested that **1** was a cyclitol glycoside of a pentose.

^1H - ^1H -2D NMR spectroscopic analysis of **1** clarified its relative stereostructure. Large vicinal coupling constants ($J=10$ Hz) of five protons (H-1, H-3 to H-6) indicated a sequential *trans*-diaxial relationship for these protons (Fig. 2). The small coupling constants ($J_{1,2}=2.4$ Hz and $J_{2,3}=2.5$ Hz) of H-2 (δ 4.11) implied an equatorial orientation. The configuration of these protons resulted in a *myo*-inositol moiety for the partial structure of **1**.

The stereostructural relationship of the

Fig. 2. Structure of Compound **Y** (**1**).

other five protons was easily established. The larger coupling constant ($J=13$ Hz) indicated the geminal configuration of H-5e and -5a. The *trans* diaxial relationship between H-2' and -3' ($J_{2',3'}=10$ Hz) indicated a pentopyranoside structure. In addition of the small coupling constants ($J_{4',3'}=3.2$ Hz and $J_{4',5'a}=1.2$ Hz) of H-4' (δ 4.02), the pentose moiety proved to be an arabinopyranoside. When compound **Y** (**1**) was subjected to methanolysis, trimethylsilylation and GLC analysis, there were two main peaks, with retention times of 4.9 and 11.0 min. These were identical with the main peaks given by TMS derivatives of the methanolysis products of authentic L-arabinose and

of *myo*-inositol, respectively.

Chromatographic purification of the methanolysis products of authentic L-arabinose yielded an anomeric mixture of methyl L-arabinopyranosides as major products. Their pyranoside structures were confirmed by comparing the ^{13}C -NMR spectrum with those of methyl D-arabinopyranosides reported by Breitmaier *et al.*⁸⁾ HPLC purification of the methanolysis products of **1** in the same way gave an anomeric mixture ($\alpha:\beta=4:6$) of methyl arabinopyranosides, which was confirmed by the ^{13}C -NMR spectrum. The specific rotation, $[\alpha]_D^{23} + 186^\circ (c=0.34, \text{H}_2\text{O})$, of the anomeric mixture indicated the L absolute configuration of the arabinose, based on the $[\alpha]_D$ values of -17.4° and -240.6° for methyl α - and methyl β -D-arabinopyranosides, respectively.⁸⁾

Permethylation of **1** by Hakomori's method⁹⁾ was followed by methanolysis with 4% methanolic HCl and acylation to yield a *p*-Brenzoate (**2**): m/z 434 ($\text{M}^+ + 2$), 432 (M^+). In the ^1H -NMR spectrum of **2** was observed H-1 (δ 5.93, t, $J_{1,2}=J_{2,3}=2.7$ Hz) as well as five methoxymethyls (δ 3.45, 6H, s; 3.61 6H, s; 3.69, 3H, s), an aromatic AB quartet (δ 7.56, 7.86, 2H each, d, $J=9$ Hz) and five unresolved methine protons, confirming the structure of **1** (Fig. 2).

The IR spectrum of **1**, however, showed a fairly strong absorption band at 1660 cm^{-1} , which was rather difficult to explain with proposed structure **1**. An X-ray crystallographic analysis of **1** gave the relative stereostructure shown in Fig. 3, confirming the proposed structure **1**. The X-ray analysis also showed the presence of a water molecule in the crystal, and the unusually strong IR absorption may have been due to the water of crystallization. Thus, the absolute structure of compound **1** was unambiguously elucidated as 2-*O*-(β -L-arabinopyranosyl)-*myo*-inositol.⁶⁾ It is noteworthy that **1** is the first example having a rather unusual axial-axial glycosidic linkage among the inositol glycosides so far isolated.¹¹⁻¹⁴⁾

The characteristic signals ascribed to **1** have

Fig. 3. Perspective View of the Molecule of **1**.

been observed in the spectra of all kinds of tea extracts so far examined. The signals in question were also clearly observed in the spectrum of a homogenate of steamed fresh buds of the tea plant (Fig. 4). The accumulation of so much **1** in tea flush strongly suggests an important physiological role in the tea plant.

Recently, Hakamada has reported the presence of a considerable amount of an unknown compound tentatively named *S'*, which was detected as one of the major metabolites in a tea plant following the uptake of ^{14}C -labeled carbon dioxide.¹⁰⁾ This unknown major metabolite is possibly **1** and we are working to confirm this.

Relatively few inositol glycosides have been isolated and identified so far: glucinol,¹¹⁾ 1- α -galactinol,¹¹⁾ ononitol and pinitol galactosides,^{11,12)} *etc.*^{11,13)} Galactinol is well known to play an important role in galactose transfer, and in the biosynthesis of raffinose, stachyose and their homologues.¹⁴⁾ This report is, to our knowledge, the second example of inositol pentose glycoside,¹¹⁾ and of course the first one of the inositol glycoside as one of the major tea constituents.

It is noteworthy that the new inositol glycoside content is higher in high-quality tea. Its

Fig. 4. ^{13}C -NMR Spectrum (22.5 MHz) of the Homogenate of Fresh Tea Buds.

content is relatively high in a Chinese tea ("Puarl-tea," classified as a microbial fermentation tea), suggesting that the new glycoside is hard for the microorganisms involved in its manufacture to metabolize (K. Sakata, Q. Liu *et al.*, unpublished data). Compound **1** is essentially tasteless and its contribution to the taste of tea remains to be clarified.

Our new application of ^{13}C -NMR spectroscopy to a chemical study on tea and the tea plant suggests the applicability of this method for such uses as the quality examination of liquid-type foods such as vinegar,¹⁵⁾ rapid metabolic profiling of plant tissue, *etc.* We are now examining the hot water extracts of various kinds of plant fresh flush by ^{13}C -NMR spectroscopy and will publish some of our novel findings soon.¹⁶⁾

Experimental

^{13}C FT-NMR spectral measurement. ^{13}C -NMR spectra of the extracts and some authentic specimens were measured on a JEOL FX-90Q FT-NMR spectrometer at 50°C with an external standard method, using a sealed tube containing 3-(trimethylsilyl)-propionic acid- d_4 sodium salt (TSP- d_4) in D_2O (22.5 MHz; 10 mm cell; spectral width, 5000 Hz; 45°C pulse; pulse repetition time, 1 sec).

Sample preparation for ^{13}C -NMR measurement.

(1) For made tea: A tea sample (6 g) was kept in 90 ml (for Japanese green tea or Black tea) or 45 ml (for Chinese tea) of boiling distilled water for 10 min, and then immediately filtered through Toyo No. 2 filter paper. The filtrate (3 ml) was subjected to ^{13}C -NMR analysis after bubbling N_2 gas for a while.

(2) For fresh tea buds and flush: Steamed fresh buds and young leaves of *C. sinensis* var. *sinensis* ("Yabukita"

cultivar) were obtained on the 21st of May, 1986. Each steamed material was cut finely and extracted with hot distilled water for 10 min. The filtered extract (3 ml) was immediately subjected to the ^{13}C -NMR measurement.

Isolation of compound Y (1). The tea sample (first crop, a high grade of "Sen-cha," 50 g) was extracted twice with 500 ml of boiling water for 20 min. The combined extracts were then subjected to centrifugation at 15,000 rpm for 10 min. The resulting supernatant was concentrated and extracted with ethylacetate and then with butanol, the signals in question being observed in the spectrum of the resulting aqueous layer. A half of the aqueous solution was concentrated and fractionated by carbon (H_2O) column chromatography. The fractions showing the signals were combined and purified by Toyopearl HW-40C (H_2O) column chromatography to yield about 200 mg (ca. 0.8% of the made tea) of **1**, mp 194 ~ 194.5°C (from hot methanol), $[\alpha]_D^{25} + 146^\circ$ ($c = 1.04$, H_2O). Anal. Found: C, 41.96; H, 6.44%. Calcd. for $\text{C}_{11}\text{H}_{20}\text{O}_{10}$: C, 42.31; H, 6.46%; $R_f = 0.20$ (RRf to quinic acid = 0.74), silica gel GF₂₅₄ (Merck), EtOAc-MeOH-AcOH- $\text{H}_2\text{O} = 12:3:3:2$. ^{13}C -NMR (D_2O , TSP- d_4 , 100 MHz) δ : 66.23 (t), 71.66 (d), 71.74 (d), 71.85 (d), 73.27 (d), 74.71 (d), 75.50 (d), 75.76 (d), 77.31 (d), 84.23 (d), 104.0 (d, $J = 170$ Hz). ^1H -NMR (D_2O , TSP- d_4 , 400 MHz) δ : 3.29 (1H, t, 9.2), 3.59 (1H, dd, 10, 2.5), 3.62 (1H, dd, 10, 2.4), 3.64 (1H, t, 9.5), 3.66 (1H, dd, 13, 2.4), 3.72 (1H, t, 9.5), 3.89 (1H, dd, 10, 3.4), 3.93 (1, dd, 10, 3.1), 4.02 (1H, broad s, $W_{1/2} = 6.8$), 4.11 (1H, t, 2.5), 4.17 (1H, dd, 13, 1.2), 5.19 (1H, d, 3.4). FD-MS m/z (relative intensity) 351 (M + K, 100), 335 (M + Na, 2), 269 (10), 156 (4), 122 (5), 104 (5). IR ν_{max} (KBr) cm^{-1} 3500 ~ 3100, 1660, 1164, 1100, 1090, 1060, 1040, 1010.

GLC analysis of TMS derivatives of the methanolysis products of 1. Compound **Y** (**1**, 7.8 mg) was subjected to methanolysis with 1% methanolic HCl (5.5 ml) in a sealed tube at 80°C for 2 hr. Authentic L-arabinose was treated in the same manner as this. A small portion of each reaction mixture was evaporated to dryness and reacted with a freshly prepared TMS reagent [bistrimethylsilyl acetamide (BSA): trimethylchlorosilane (TMCS): pyridine = 40:6:

100] at 80°C for 30 min. Authentic *myo*-inositol was also reacted with the reagent under the same conditions. These TMS derivatives were analyzed by GLC [1% SE-30 on Uniport HP, 3 mm i.d. $\times$ 2 m; 100–280°C, 10°C/min; N₂, 21 ml/min]. Two main peaks (t_R = 4.9 and 11.0 min) shown by the reaction mixture obtained from **1** were identical with those from L-arabinose and *myo*-inositol.

Determination of the glycosidic linkage of 1. Compound **Y** (5 mg) was permethylated by Hakomori's method.⁹⁾ A chloroform extract of the reaction mixture was concentrated and chromatographed on Sephadex LH-20 (CHCl₃–MeOH = 2:1) to yield 2.3 mg of permethyl compound **Y**: ¹H-NMR (CDCl₃, 90 MHz) δ 3.4–3.6 (8 $\times$ –CH₃), 5.41 (1H, d). The permethyl compound **Y** (2.3 mg) in 4% methanolic HCl (2 ml) was refluxed for 30 min. The reaction mixture was neutralized with Ag₂CO₃ and filtered. The filtrate was evaporated to dryness and subjected to conventional *p*-bromobenzylation to yield a *p*-bromobenzoate (**2**): MS m/z 434 ($M^+ + 2$), 432 (M^+); ¹H-NMR (CDCl₃, 90 MHz) δ 3.04–3.44 (5H), 3.45 (6H, s), 3.61 (6H, s), 3.69 (3H, s), 5.93 (1H, H-2, t, J = 2.7 Hz), 7.56 (2H, d, J = 9 Hz), 7.86 (2H, d, J = 9 Hz). The signal (δ 5.93) with a small coupling constant (J_{ac} = 2.7 Hz) was assigned to H-2 attached to a *p*-bromobenzyloxy group.

Determination of the absolute stereostructure of 1. The reaction mixture of **1** (16 mg in 11 ml of 1% methanolic HCl; in a sealed tube at 80°C 2.5 hr) was neutralized with Ag₂CO₃ and filtered. The filtrate was concentrated and purified by Toyopearl HW 40SF (H₂O) column chromatography to yield an anomeric mixture of methyl arabinosides: 3.4 mg, $[\alpha]_D^{23} + 186^\circ$ (c = 0.34, H₂O); ¹³C-NMR (D₂O, 22.5 MHz) δ 107.0, 102.9, 75.4, 73.7, 71.8, 71.2, 71.2, 69.0, 65.5, 60.0, 58.2. A comparison of the ¹³C-NMR spectral data with those of methyl D-arabinopyranosides reported by Breitmaier⁸⁾ unambiguously showed that it was an anomeric mixture (α : β = 4:6) of methyl L-arabinopyranosides, together with comparison of the optical rotational data with those of the values of -17.4° and -240.6° for methyl α - and β -D-arabinopyranosides, respectively.⁸⁾

Single crystal X-ray analysis of 1. Crystals were grown as described in the text and belonged to space group P2₁ with a = 10.130(4), b = 9.946(4), c = 6.640(2) and β = 90.90(3)°. The composition of the asymmetric units was C₁₁H₂₀O₁₀·H₂O. All unique diffraction data were collected using a four-circle diffractometer. CuK α radiation (1.54178 Å), and 1° ω -scans. Of the 959 unique reflections, 956 (99.7%) were judged as observed ($|F_o| \geq 3\sigma(F_o)$). The structure was solved by direct methods and refined by block-diagonal least-squares refinement. The final R-factor with anisotropic nonhydrogen atoms and isotropic hydrogens was 0.056. Full crystal data have been deposited with and can be ordered from the Cambridge

Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, U.K. Please give a complete literature citation when ordering.

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