Short Communication

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The Identification of Pungent Components in Hairy Roots and Regenerated Plants of Horseradish (*Armoracia rusticana*)

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Introduction

Horseradish (*Armoracia rusticana*) belongs to the same family (Cruciferae) as wasabi (*Wasabia japonica* Matsum.), and horseradish root tubers contain many of the pungent components found in wasabi[1]. The flavor of horseradish is a little different from that of wasabi because of differences in composition[1]. There are few cases when horseradish root tubers are grated immediately before consumption as is typically done with wasabi; however, the roots are generally used commercially as a raw material for powdered wasabi. Horseradish root tubers are also well-known as the source for commerical peroxidase, which is widely used as a reagent for clinical diagnosis and microanalytical immunoassay.

The production of peroxidase from horseradish hairy roots has been reported by several workers[2-4]; however, there have been no studies to determine if horseradish hairy roots produce pungent components. In this paper, we describe the gas chromatography-mass spectrometry (GC-MS) detection of the pungent components from horseradish hairy roots and plants regenerated from hairy roots.

Materials and Methods

Materials and chemicals Horseradish root tubers were purchased from a department store in Tokyo. Authentic *sec*-butyl isothiocyanate (**1**) and benzyl isothiocyanate (**4**) were obtained from SAN-EI Chemical Industries, Ltd. (Osaka) and Kobayashi Perfumery Co., Ltd. (Tokyo), respectively, *via* Japan Perfumery and Flavouring Association. Authentic allyl isothiocyanate (**2**) and β-phenylethyl isothiocyanate (**5**) were purchased from Tokyo Kasai Kogyo Co. (Tokyo).

Hairy roots Hairy roots of horseradish (*Armoracia rusticana*) were induced and cultivated according to the method previously described[4]. The strain number of *Agrobacterium rhizogenes* was 15834. Every 4 weeks the hairy roots were transferred onto fresh hormone-free Murashige and Skoog's medium (hereafter referred to as MS medium[5]).

Opines (agropine and mannopine) of hairy roots were extracted and analyzed by high voltage

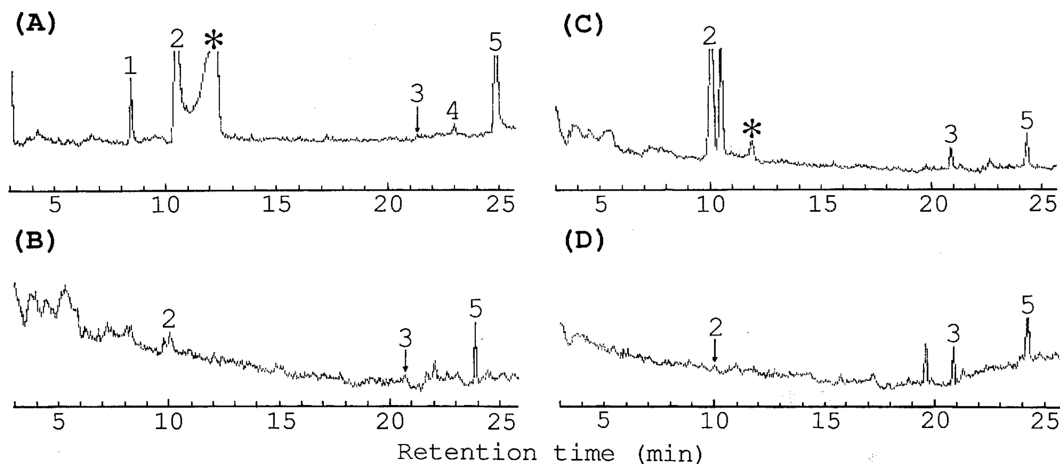

Fig. 1 Total ion chromatograms of hexane extracts of commercial horseradish root tubers (**A**), hairy roots (**B**), aerial part of regenerated plant (**C**) and roots of regenerated plant (**D**). Asterisk indicates the tailing of peak 2.

paper electrophoresis and the presence of T-DNA in genomic DNA was confirmed by Southern blot analysis (data not shown).

Plant regeneration A few weeks after transferring the axenic hairy roots from total darkness to ordinary room illumination, adventitious buds appeared. Those buds with rudimentary roots were excised and maintained on hormone-free MS medium. After the regenerated plants matured, they were transferred every 4 weeks onto fresh hormone-free MS medium. The regenerated roots obtained were thinner than the normal roots.

GC-MS analysis Hairy roots and the aerial parts and the roots of regenerated plants were cut into pieces and separately extracted overnight with hexane. For comparison purposes a sample of commercial horseradish root tuber was subjected to identical treatment. The extracts were analyzed by GC-MS. The GC-MS analyses were carried out by a GCMS-QP2000 Gas Chromatograph/Quadrupole Type Mass Spectrometer System (Shimadzu Co.) equipped with a capillary column of HiCap-CBP20 (25 m×0. 2 mm i. d., Shimadzu Co.). The temperature was programmed from 60°C to 210°C at the rate of 7. 5°C per min. The ion source was operated at 70 eV. The retention times of the authentic samples are as follows; **1**: 8. 5 min, **2**: 10. 5 min, **4**: 23. 1 min, **5**: 24. 9 min.

Results and Discussion

The gas chromatograpic results monitored by total ions are given in **Fig. 1**. The peaks corresponding to the pungent components were identified by direct comparison of the GC retention times and mass patterns with those of the authentic samples (**1**, **2**, **4**, **5**). The mass pattern for peak 3 was compared with those in the literature[6-8].

Peak 1 (**Fig. 1**(**A**)) had the molecular ion peak at m/z 115, and fragments at m/z 86, 72, 57, 56 and 41 (base peak), corresponding to CH_3CHNCS^+, CH_2NCS^+, $C_4H_9^+$, $C_4H_8^+$ and $C_3H_5^+$, respectively. The fragment at m/z 86 showed the larger intensity than that at m/z 72, which is characteristic of *sec*-butyl isothiocyanate. The agreement of the mass pattern as well as the identity of the GC retention time with that of the authentic sample positively identified peak 1 as *sec*-butyl isothiocyanate (**1**). Peak 2 (**Fig. 1** (**A, B, C, D**)) had the characteristic strong fragments at m/z 99 (M^+), 72 and 41 (base peak). Since this mass pattern and the retention time of GC agreed well with those of the authentic sample, peak 2 was established to be allyl isothiocyanate (**2**). Peak 3 (**Fig. 1** (**A,**

B, C, D)) had the molecular ion peak at *m/z* 147, and there were the fragment ions at *m/z* 101, 72 and 61 ($C_4H_7NS^+$, CH_2NCS^+ and $CH_3SCH_2^+$). Since these data were in accord with those in references[6-8], peak 3 was tentatively identified as 3-methylthiopropyl isothiocyanate (**3**). Peak 4 (**Fig. 1** (**A**)) had the molecular ion peak at *m/z* 149 and a characteristic fragment at *m/z* 91 (base peak, $C_6H_5CH_2^+$). Peak 5 (**Fig. 1** (**A, B, C, D**)) had the molecular ion peak at *m/z* 163, and the fragments at *m/z* 105 and 91 ($C_6H_5CH_2CH_2^+$ and base peak, $C_6H_5CH_2^+$). These results and the retention times of GC agreed with those of the authentic samples; therefore, peaks 4 and 5 were identified as benzyl isothiocyanate (**4**) and β-phenylethyl isothiocyanate (**5**), respectively. The peaks eluting with the same retention time as compound **4** in the total ion chromatograms of hairy roots and the aerial parts of regenerated plants (**Fig. 1** (**B, C**)) showed different mass patterns from that of compound **4**. Identifications of the components except for compounds **1-5** are still under study.

It has been reported[1] that compound **2** is the main pungent component in both of horseradish and wasabi root tubers. In addition, a significant amount of compound **5** (about 20% of the amount of compound **2**) was detected in horseradish root tubers. In our studies, compound **2** seemed to be the principal pungent component in the commercial horseradish root tuber and the aerial part of regenerated plants, while compound **5** seemed to be the dominant component in both the hairy roots and the roots of regenerated plants. As for the minor components, compound **3** was detected in all of the samples; however, compounds **1** and **4** were detected only in the commercial horseradish root tubers.

It is known that the pungent components of horseradish are generated from their glucosinolates by myrosinase when horseradish root tubers are grated[8]. Therefore, the myrosinase activity should always be considered when quantitating pungent components. The precise quantitative analyses of compounds **2** and **5** are now being performed with SIM (selected ion monitoring). The control of the myrosinase activity is done by adding the enzyme to the samples. The results will be the subject of a future report.

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《和文要約》

西洋ワサビ（*Armoracia rusticana*）の毛状根および再分化植物体中からの辛味成分の同定

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西洋ワサビ（*Armoracia rusticana*）の毛状根，及び毛状根から得られた再分化植物体より，辛味成分（**1**-**5**）をGC-MSで検出，同定した．主辛味成分は，再分化植物体の地上部では，市販西洋ワサビ根同様，allyl isothiocyanate（**2**）であり，毛状根及び再分化植物体の根では，β-phenylethyl isothiocyanate（**5**）であった．