NOTE

Identification of the Key Aroma Compounds in Dried Roots of *Rubia cordifolia*

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Edited by J. Sekiya, Kyoto Univ., and accepted August 30, 2005 (received for review June 13, 2005)

Abstract: The compositions of the essential oil from *Rubiae Radix* (roots of *Rubia cordifolia*, Rubiaceae) have been investigated by capillary GC and GC/MS. The important odor-active compounds were also detected in the oil using GC-Olfactometry (GC-O) and aroma extract dilution analysis (AEDA). As a result, 43 components, accounting for 91.5 % of the total oil, were detected. The main components in the essential oil were mollugin (19.6%), furomollugin (17.4%), eugenol (12.7%) and (*E*)-anethole (10.6%). AEDA and GC-O showed that geraniol, eugenol and geranyl acetate were the most aroma-compounds.

Key words: *Rubia cordifolia* L., essential oil, gas chromatography-olfactometry, aroma extract dilution analysis, mollugin, furomollugin

1 Introduction

Rubiae Radix ('sensou' in Japanese) is the dried roots of *Rubia cordifolia* L. Rubiaceae. It has been long used as hematopoietic, anastaltic and dyestuff and for the treatment of hematemesis, hematuria and menoxenia in Japan and China. When the crude drug is decocted by hot water, characteristic odor is given off, which is sweet-floral odor and weak pungent-green odor. The genus *Rubia* is a source rich in anthraquinones. For example, many anthraquinones such as 1-hydroxy-2-methylanthraquinone and nor-damnacanthol had been isolated from the roots of *R. cordifolia* (1-5). Naphthohydroquinones such as mollugin and furomollugin had been also isolated from the roots (6-10). However, to the best of our knowledge, there is no report on the volatile components for the Chinese crude drug.

Gas chromatography-olfactometry (GC-O) is the most valuable technique used to identify the key aroma

compounds in foods. There are various methods for the screening odor-active compounds. One of the most frequently used methods is aroma extract dilution analysis (AEDA) (11).

In this paper, the volatile constituents of *R. cordifolia* roots were investigated by GC and GC/MS. The important odor-active compounds were detected in the oil using GC-O and AEDA.

2 Experimental

2.1 Plant

Commercially available air-dried roots (Chinese *Rubiae Radix*) of *R. cordifolia* (Takasago Yakugyou Co. Ltd, Osaka) were used.

2.2 Isolation of the Essential Oil

One hundred grams of dry coarsely powdered plant material were hydrodistilled with a Likens-Nickerson-type apparatus using ethyl ether to yield 0.027 % of yel-

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low oil. The obtained oil was dried over anhydrous sodium sulfate prior to analyses.

2·3 Gas Chromatography-Olfactometry and AEDA

GC was carried out using Agilent Technologies 6890N, fitted with a non-polar capillary column HP-5 (J&W Scientific; 30 m × 0.32 mm, film thickness 0.25 μ m). The oven temperature was programmed from 40 - 240°C at a rate of 4°C/min and held at 240°C for 5 min. The injector temperature was 240°C. The flow rate of the carrier gas (He) was 1.800 mL/min. The Chemstation software acquired two channel signals simultaneously, one channel for the flame ionization detector (FID), and other channel from the olfactometer signal board. A single sniffer, the author, recorded the aroma character manually. The flavor dilution (FD) factor of the odorants in the essential oil was determined by aroma extract dilution analysis (AEDA). The highest sample concentration (1 mg/ml) was assigned a FD-factor of 1. The oil was stepwise diluted (1+1,v/v) by addition of diethyl ether. Aliquots of the dilutions were then analyzed by GC-O. The results are expressed as

FD-factor. An odorant with high FD-factor can be judged as an important contributor to the characteristic flavor.

2·4 Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis was carried out using a Hewlett Packard model 5890 GC with a Hewlett Packard 5972A MS. The capillary column was a DB-5MS (J&W Scientific; 30 m × 0.25 mm, film thickness 0.25 μ m). The oven temperature was programmed from 60 - 240°C at a rate of 2°C/min and held at 240°C for 5 min. The flow rate of the carrier gas (He) was 0.679 mL/min. The detector interface temperature was set at 280°C, with the actual temperature in the MS source reaching approximately 180°C. The ionization voltage was 70 eV.

2·5 Identification of Constituents

The components of the essential oils were identified by direct comparison of their mass spectral pattern and retention index (RI) with those published in the literature (9,12,13).

Table 1 Compounds of Essential Oil from Roots of *Rubia cordifolia*.

No	RI	Compound	peak area (%)	No	RI	Compound	peak area (%)
1	860	furfural	tr	32	1397	methyl eugenol	1.2
2	871	(2E)-hexenal	tr	35	1444	geranyl acetone	0.8
3	948	benzaldehyde	0.1	36	1475	ar-curcumene	0.2
4	1033	benzene acetaldehyde	0.2	37	1479	β -selinene	0.3
5	1090	cis-linalool oxide	tr	38	1492	2-tetradecanone	1.2
6	1147	(2E)-nonenal	tr	39	1503	acenaphthene	1.5
7	1168	borneol	1.6	40	1511	δ -amorphene	0.3
8	1175	menthol	2.7	41	1514	myristicin	1.0
9	1192	α -terpineol	1.9	45	1571	caryophyllene oxide	0.4
10	1195	estragenole	tr	46	1611	dill apiole	0.5
14	1234	pulegone	0.6	47	1618	1- <i>epi</i> -cubenol	0.2
15	1250	<i>p</i> -anisaldehyde + piperitone	1.2	48	1630	hinesol	0.2
16	1253	geraniol	tr	49	1633	cubenol	0.1
18	1268	(E)-cinnamaldehyde	0.8	50	1645	β -eudesmol	0.8
19	1285	(E)-anethole	10.6	51	1657	ar-turmerone	0.6
20	1291	2-undecanone	0.8	52	1760	phenanthrene	0.1
22	1297	carvacrol	0.3	54	1923	methyl palmitate	0.2
23	1305	4-vinyl- <i>o</i> -guaiaicol	0.2	55	2024	4- <i>tert</i> -butyl-2-phenyl phenol	9.9
27	1351	eugenol	12.7	56	2080	furomollugin	17.4
28	1355	γ -nonalactone	0.2	59	2122	linoleic acid	0.2
29	1369	α -yalangene	0.2	62	2237	mollugin	19.6
30	1373	(E)- β -damascenone	0.5				
31	1385	geranyl acetate	tr			total	91.5

RI: Retention Index; tr: trace<0.1

Table 2 Most Odor-active Compounds in Essential Oil from Roots of *Rubia cordifolia*.

No	Aroma quality	RI	FD-factor	Aroma Compound
1	green-fruity, pungent	871	4	(2 <i>E</i>)-hexenal
2	sweet-woody	1090	4	<i>cis</i> -linalool oxide
3	fatty-orrisy	1147	1	(2 <i>E</i>)-nonenal
4	pungent	1153	1	unknown
5	mild and sweet, floral	1253	64	geraniol
6	warm-spicy	1356	32	eugenol
7	sweet, fruity-floral	1385	16	geranyl acetate

3 Results and Discussion

The essential oil from *Rubiae Radix* was analyzed on GC and GC/MS. The percentage composition and modes of identification of the oil components are listed in **Table 1**. In the oil, 43 components, accounting for 91.5% of the total oil, were identified. The main components were mollugin (19.6%), furomollugin (17.4%), eugenol (12.7%), (*E*)-anethole (10.6%), 4-*tert*-butyl-2-phenyl phenol (9.9%), menthol (2.7%), α -terpineol (1.9%). This oil was interesting because it contained large amounts of mollugin and furomollugin. The two naphthohydroquinones were isolated from roots, however there is no report on the identification of two naphthoquinones in any natural essential oil. Because the compounds suppressed the secretion of hepatitis B surface antigen in human hepatoma cells by (10), it is possible that essential oil inhibits chronic and acute hepatitis.

The results of the GC-O and AEDA studies are given in **Table 2**. Geraniol, eugenol, geranyl acetate, (2*E*)-hexenal and *cis*-linalool oxide were the most aroma-compounds in the oil (FD factor > 4). Geraniol, eugenol, geranyl acetate *cis*-linalool oxide and green note such as (2*E*)-hexenal, (2*E*)-nonenal may contribute to sweet-flower note, characteristic odor of the oil.

Acknowledgment

This work was supported by "High-Tech Research Center" project for Private Universities: matching fund subsidy from Ministry of Education, Culture, Sports, Science and Technology, Japan (2004-2008).

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