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Research Article

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The Identification and Quantitation of Thymol and its 4 Isomers in Essential Oil of *Citrus Kinokuni* Tanaka Peel

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ABSTRACT

Thymol and its four isomers were identified and quantified in essential oil of Citrus kinokuni Tanaka peel. Three of them were identified as thymol, carvacrol, and 3-methyl-4-isopropylpheno, respectively. The other two components had the similar structure with the identified three components, and their probably structure were elucidated. The quantitation result demonstrated that the thymol and carvacrol had high content in the five ismomers.

Keywords: Thymol; Isomers; Essential oil; *Citrus reticulata* blanco; *Citrus kinokuni* tanaka; Peel

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INTRODUCTION

Thymol is an important component in the essential oil (EO) from peel of *Citrus reticulata* blanco. Besides thymol, there are always coexisted with carvacrol and 3-methyl-4-isopropylphenol in the peel EO of *C. reticulata*. To correct identification and quantitation of these components maybe have important meaning to clarify the different peel from *C. reticulata*. *C. reticulata* have many varieties, and *C. kinokuni* Tanaka (*Ck*) is one which is a native variety, usually planted in Nanfeng city or nearby, Jiangxi province.

EXPERIMENTAL SECTION

Materials

The peels of *Ck* were collected every month from June 15, 2012 to November 15, 2012 from the same plant grown in Nancheng town, Fuzhou city and Jiangxi province of China. As a result, six batches of peel were collected.

Chemical Standards and Reagents

Hexane (HPLC grade) was purchased from Adamas Reagent Company. Thymol was purchased from Shanghai Titan Chem Company, Ltd., China. Carvacrol was purchased from Tokyo Chemical Industry, and 3-methyl-4-isopropylphenol was purchased from Adamas Reagent Company. EtoAc was purchased from Shanghai Titan Scientific Co., Ltd., China. N-Alkane standard solution of C_{10}–C_{25} purchased from Dr. Ehrenstorfer GmbH, Germany, was used to determine the linear retention index (LRI).

Essential Oil Extraction and Separation

The peels of *Ck* divided into about 0.4 cm × 0.4 cm sections weighed between 30.0-40.0 g were swollen with 10 times volume (v/w) 250-450 mL of pure water in a 500 or 1000 mL round-bottomed flask, then soaked for 0.5 h at 40°C. The EO was extracted by Clevenger-type apparatus for 3-4 h. The EO was prepared according to the procedure described in Chinese pharmacopoeia [1]. The EO was stored in separate screw-capped vials in a refrigerator at 4°C until needed. The yields ranged from 1.17% to 3.00%. To study the components in EO more

thoroughly, the EO from peel collected in July 15, September 15, and November 15 in 2012 were separated by column chromatography, the eluents were *n*-Hexane: EtoAc with different volume ratio in sequence, which is 97:3, 9:1, 8:2, and 0:1, the MeOH was used as eluent in the last. Every eluent was used as two column volumes, and received for two times; every time was one column volume. The oils were diluted as V_{oil}:V_{hexane} equal to 1:10 for detection by gas chromatography-mass spectrometer (GC-MS), except for the oil of sample Ck7, which were diluted as V_{oil}:V_{hexane} equal to 1:20. Every separation part of EO was diluted about V_{oil}:V_{hexane} equal to 1:50.

GC-MS Analysis

An Agilent 7890B gas chromatography (USA) matched with an Agilent 5977A mass spectrometer was used for GC–MS analysis. An Agilent fused silica capillary column – DB-5 (30 m × 0.25 mm i.d., 0.25 μm film thickness) was used. The oven temperature was programmed from 60°C (3-min hold) to 270°C at 3°C min^{-1}, and then held for 2 min. The carrier gas was helium at a constant flow of 1 mL min^{-1}. The injector and ion-source were maintained at 280°C and 200°C, respectively. Transfer line was set to 280°C. The splitting ratio was 20:1. Solvent delay was 3 min. Electron impact mass spectra were taken at 70 eV. Scan at 3.9 scans s^{-1} from m/z 30 to 400 amu. The injection volume was 1 μL.

RESULTS AND DISCUSSION

Thymol and its 4 isomers were identified in the peel EO of *Ck*, and they are mainly identified in the eluent as n-Hexane: EtoAc as 9:1, usually they are identified in the second column volume of 9:1. Their MS spectra were highly similar between each other, which can be seen in Table 1. The three components such as thymol, carvacrol, 3-methyl-4-isopropylphenol were identified by comparison with the corresponding standards. Notes: *Ck*(number 1)-(number 2)-(number 3), number 1 denotes the collected month, number 2 denotes the volume ratio of eluent as *n*-Hexane: EtoAc, for example, 9 denotes the volume ratio of *n*-Hexane to EtoAc is 9:1, number 3 denotes the collection sample number of the eluent. From Table 1, it can be seen that the two unknown compounds were similarly with the other 3 identified components by their mass spectra. But to exam the mass spectrum in detailed, it can be seen that there has some great difference in some parts [2]. For example, as for the ion peaks such as 117 and 115, their abundance of unknow-1 and thymol, respectively was greatly higher than that of the other 3 components, especially for 115. The structure of thymol, carvacrol, and 3-methyl-4-isopropylphenol can be seen in Figure 1.

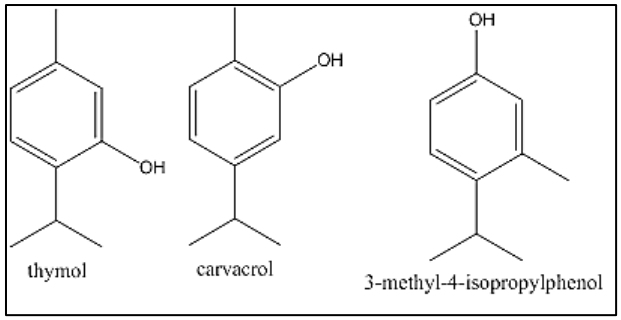

Figure 1: The structure of thymol, carvacrol and 3-methyl-4-isopropylphenol

As for 115, the abundance of thymol is greatly higher than that of carvacrol and 3-methyl-isopropylphenol, which should be related with the hydroxyl and isopropyl are in the ortho-position (o-position), the dissociation pathway was listed below (Figure 2). As a result, the unknown-1 with intensity value as 15 of ion peak 115 was close to that of thymol, which demonstrated that the hydroxyl and isopropyl in unknown-1 should be o-position. Vice versa, the hydroxyl and isopropyl in unknown-2 should not be o-position. As a result, unknown-1 should be the structure from the three candidates, and unknown-2 should be the structure from the four candidates, which can be seen in Figure 3.

Table 1: The abundance of ion peaks in thymol and its 4 isomers (maximum value is 100)

Characteristic ion peaks	The abundance of ion peaks in the components (maximum value is 100)							
	Unknown-1	Thymol		Unknown-2	Carvacrol		3-Methyl-4-isopropylphenol	
	*Ck*9-9-2	*Ck*9-9-2	Standard	*Ck*9-9-2	*Ck*9-9-2	Standard	*Ck*-6	Standard
151	3.6	3.4	3.5	3.2	3.8	3.9	2.4	3.1
150	35	31	33	30	35	36	28	29
149	0.8	0.4	0.5	0.9	1.2	1.3	0	0.3
148	0.3	0.2	0.2	0.5	0.2	0.2	0.1	0.1
136	10	10	10	10	9.9	9.7	10	9.7
135	100	100	100	100	100	100	100	100
134	1.5	1.7	1.8	2.2	1.7	1.7	2.2	1.4
133	3.3	1.8	1.9	3.4	3.1	3.2	1.5	2.7
132	0.3	0.2	0.2	0.2	0.2	0.2	0.4	0.3
131	0.5	0.5	0.5	0.5	0.5	0.5	0.8	1
128	0.6	0.6	0.6	0.1	0.2	0.2	0	0.2
121	2.8	2.6	2.8	2.8	3	3.2	3.2	2.7
120	0.9	0.8	0.9	1.8	1.7	1.7	1.6	2
119	0.8	0.8	0.9	1	0.9	1	1.5	1.1
118	0.8	0.8	0.9	0.9	0.7	0.7	0.5	0.7
117	6.2	7.1	7.7	4.3	4.9	5.2	4.3	4.3
116	3.8	3.7	4	1.6	2	2	1.4	1.6
115	15	15	16	7.3	8.6	8.9	6.8	7.3
109	0.9	0.8	0.8	1	1	1	1.4	1.1
108	1.2	1.1	1.2	1.4	1.7	1.8	1.1	1.1
107	7.8	6.3	7	7.3	9	9.3	8.2	7.2
106	0.7	0.6	0.7	0.8	0.9	1	1.1	0.9
105	4.9	4.6	5	4.7	5.4	5.4	6.2	5.3
103	1.8	1.6	1.8	1.5	1.9	2	1.4	1.8
102	1	1.1	1.1	0.5	0.6	0.6	0.4	0.6
92	1.8	1.7	1.8	2	1.8	1.9	2.9	1.8
91	16	16	17	15	16	16	16	16
89	1.2	1.3	1.4	0.8	1.1	1.1	0.7	0.9
79	3.9	3.7	4	4.5	5.1	5.3	6.5	4.8
78	2	1.9	2.1	1.9	2.1	2.2	1.7	1.7
77	6.7	6.1	6.5	7	7.7	7.8	8.1	7
74	0.5	0.7	0.8	0.7	0.8	0.8	0	0.5
67	1.2	1.1	1.1	1.5	1.2	1.2	0	0
66	1.6	1.4	1.4	1.5	1.7	1.7	2.9	2.8
65	3.4	3.1	3.3	2.7	2.7	2.7	3.5	3.1
63	1.8	1.7	1.8	1.6	1.6	1.5	1.7	1.6
55	0.9	0.8	0.8	1.2	1.1	1.1	0	1.4
53	2.1	1.7	1.8	1.8	2	2	1.6	1.8
52	1.4	0.9	1	0.8	1.1	1.1	0	0.9
51	3.2	2.5	2.6	2.9	2.8	2.9	2.6	2.6
41	1.5	1.4	1.5	2	2	2	0	1.8
39	2.7	2.7	2.8	2.7	2.6	2.6	0	2.9

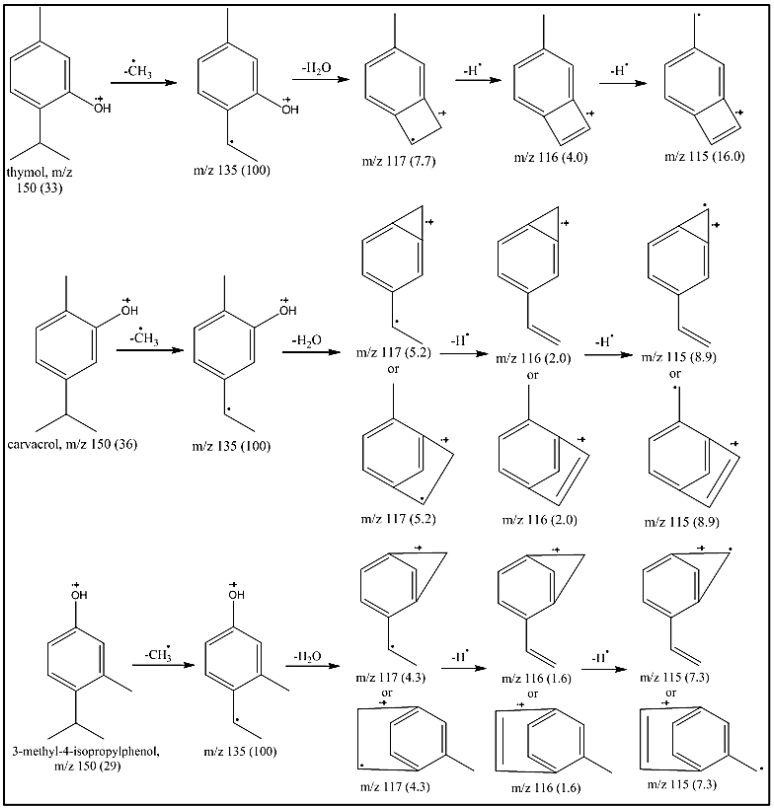

Figure 2: The dissociation pathway of ion peak 115 and 117 in the mass spectra of thymol, carvacrol, and 3-methyl-4-isopropylphenol. The value in the bracket is the intensity of the ion peak

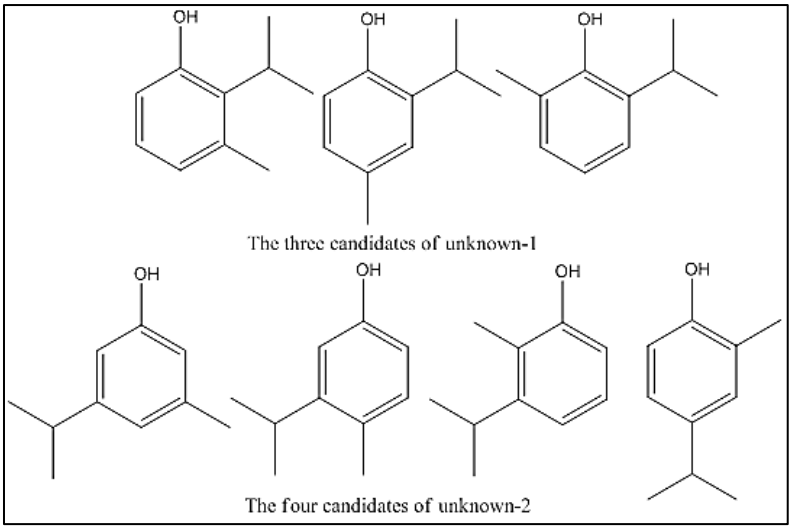

Figure 3: The probably structure of unknown-1 and unknown-2

The LRI and quantitation result of thymol and its four isomers in EO of *Ck* peel can be seen in Table 2.

Table 2: The quantitation result of thymol and its four isomers in EO of Ck peel

Components	LRI[a]	LRI[b]	Peak area percentage (%)					
			*Ck*6	*Ck*7	*Ck*8	*Ck*9	*Ck*10	*Ck*11
Unknown-1	1281		0.11	0.07	0.25	0.25	0.21	0.17
Thymol	1287	1266	4.13	1.42	4.56	3.55	3.68	3.13
Unknown-2	1292		0.08	0.04	0.12	0.16	0.14	0.11
Carvacrol	1297	1278	0.27	0.11	0.52	0.69	0.53	0.41
3-methyl-4 isopropylphenol	1327	1332	0.01	0	0.01	tr	0.01	tr

Note: LRI[a] denotes the value of LRI was gotten in the experiment, LRI[b] denotes the value of LRI was gotten from NIST (National Institute of Standards and Technology) 08 database. Tr (trace) denotes the value is less than 0.005%. The number after *Ck* denotes the collected month

CONCLUSION

Thymol is an important component in the EO of *C. reticulata* peel. Besides it, the carvacrol is another important component in EO of *C. reticulata* peel. The 3-methyl-4-isopropylphenol is a trace component in EO of *Ck* peel. The probably structure of the other 2 isomers was proposed in this paper, and their quantitation value was increased with the delay of collected time as a whole, their value was less than that of carvacrol but larger than that of 3-methyl-4-isopropylphenol.

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REFERENCES

[1] Pharmacopoeia Committee of the People's Republic of China. Pharmacopoeia of the People's Republic of China, Vol. I, People's Medical Publishing House, Beijing, **2010**, 176, 182, appendix 63.
[2] PZ Cong, SY Li. The mass spectra of natural products, publishing house of Chinese medicine and pharmacy, Beijing, China, **2003**.