t, J = 7.3Hz, H-4), 3.69(1H, d, J = 11.5Hz, H-1′a), 3.74(1H, d, J = 11.5 Hz, H-1′b), 3.90(1H, d, J = 9.8 Hz, H-3′), 3.78(1H, dd, J = 9.8, 3.5 Hz, H-4′), 3.82-3.84(1H, m, H-5′), 3.65(1H, dd, J = 12.3, 2.0 Hz, H-6′a), 3.75(1H, dd, J = 12.3, 1.5 Hz, H-6′b)。^{13}C-NMR谱数据见表1。将化合物Ⅻ的^{13}C-NMR谱数据与文献[8]报道的正丁基O-β-D-吡喃果糖苷的数据相比较,两者基本一致。因此鉴定化合物Ⅻ为正丁基O-β-D-吡喃果糖苷。此外根据HMQC谱和DQF-COSY谱准确地归属了β-D-吡喃果糖的碳、氢信号,纠正了文献报道的β-D-吡喃果糖部分1′,3′,4′,5′,6位碳信号归属的错误。

表1 化合物Ⅹ～Ⅻ的碳谱数据

Table 1 ^{13}C-NMR data of compounds Ⅹ-Ⅻ

碳位	Ⅹ	Ⅺ	Ⅻ
苷元			
1	62.0	62.2	61.7
2	33.4	33.4	33.3
3	20.4	20.2	20.5
4	14.2	14.3	14.3
果糖			
1′	61.7	62.1	63.6
2′	108.8	105.2	101.6
3′	83.3	78.6	70.8
4′	78.6	77.4	71.6
5′	84.0	83.4	71.1
6′	62.7	65.0	65.2

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Chemical composition of essential oil in cultured Cordyceps sinensis

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冬虫夏草挥发油成分分析

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1 Introduction

Cordyceps sinensis (Berk.) Sacc. is a parasitic fungus on the larvae of *Leidoptera*. The fruiting bodies of *C. sinensis* have been used as food and tonic herbal medicine for a variety of dieases for centuries, named Dong-Chong-Xia-Cao in mandarin. *C. sinensis* extract has been used for the treatment of hyperglycemia, hyperlipidemia, respiratory and liver diseases, renal dysfunction, renal failure[1], and its antioxidant properties have also been widely used. The extract of *C. sinensis* has been shown to increase the level of high-energy phosphates in mouse liver[2] and steroidogenesis[3], superoxide dismutase activity in red blood cell (RBC), and to decrease plasma malonedialdehyde and oxygen free radicals in older patients. Recent

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reports have shown *C. sinensis* extract can be beneficial to autoimmune diseases and has antitumor properties[4~6]. However, little is known about active ingredients of *C. sinensis* extract or its mechanism of action on anything of the uses described above. Some bioactive constituents from *Cordyceps* species have been reproted including cordyce-pin[7,8], ophicordin and one kind of adenosine derivatives[9] was found, but there is no much attention devoted to essential oil of *C. sinensis*.

Herein the composition of the essential oil from the mycelia of cultured *C. sinensis* has been described, the analytic results by means of GC-MS are reported in the followings.

2 Materials and methods

2.1 Materials: *C. sinensis* (CCTCC AF99009) strain was obtained from China Center for Type Culture Collection (CCTCC), Wuhan University, Wuhan, China. Carrier gases for GC-MS and all chemicals were routinely used of the highest purity grade.

2.2 Cultured of *C. sinensis*: CCTCC AF99009 was first cultured on potato dextrose agar plates for four days at 28 ℃. Then the starter cultures were transferred to nutrient broth containing (g/L): peptone 10, glucose 30, VB1 0.05, KH_2PO_4 1, $MgSO_4$ 0.2, at pH 6.0, on a rotary shaker at 120 r/min for four days at 28 ℃. The mycelium of *C. sinensis* was harvested and then stored at -80 ℃.

2.3 Extraction methods: The mycelium of *C. sinensis* was collected and fragmentated by Sonic Dismembrator in ice water. Purified water, 250 mL was added and subjected to hydrodistillation for four hours to extract the essential oils through distillation apparatus. And ether was the extractive solvent. The essential oil decanted from distillation water was treated with anhydrous sodium sulphate to make it moisture-free, refined, and measured. Oil yield was estimated and the the composition of oil was analyzed by GC-MS. Employing this method, 0.35% of total oil yield was recovered from the hydrosol.

The hydro-distillation of the aerial parts gave transparent yellowish oil with an especially thick odor.

2.4 Analytic conditions of GC-MS: GC-MS (Thermo Finnigon Trace GC + Trace MS^{plus}, capillary DB-1MS system fitted with a 30 m × 0.25 mm). Temperature programme was 20 ℃/min from 60 ℃ to 150 ℃, then 3 ℃/min from 160 ℃ to 170 ℃ and 15 ℃/min from 170 ℃ to 220 ℃, last 8 ℃/min from 220 ℃ to 260 ℃, and kept 6 min at 260 ℃. Helium was used as carrier gas at a flow of 1 mL/min. Mass spectra were recorded over 50- 450 amu with ionization energy 70 eV and ion source temperature 220 ℃. The NIST '98 Libraries database was used.

3 Results and discussion

3.1 Results: Straw yellow essential oil with special odor was extraced from *C. sinensis* by steam distilling instrument, 0.35% of total oil yield (dry weight) was recovered from the hydrosol by this method.

The chemical compositions of the essential oil of *C. sinensis* were analyzed by GC-MS. The result showed that 72 peaks were separated and 41 of them were identified. The main contents of the essential oil were 9, 12-octadecenoic acid, *n*-hexadecanoic acid, oleic acid, 17à-methyltestosterone, and toluene. It was reported for the first time that the essential oil yields were estimated and the essential oil composition of *C. sinensis* analyzed by GC-MS. From the results, the author can get the conclusion that the content of essential oil of *C. sinensis* included plenty of aroma compounds, octadecenoic acid, hexadecanoic acid, and their grease ramification which had special aroma. The essential oil of *C. sinensis* also contained lots of unsaturated fatty acid, sterol, and dipIterpene compound, which worked as the high medicine value of *C. sinensis*.

In herbalism, *C. sinensis* has been used for renal dysfunction and renal failure, that researchers considered related to the sterol sex hormone in *C. sinensis*. In this research, the author found the essential oil of *C. sinensis* contained aromatic compound, diplterpene, and sterol compound, which maybe the intermediate metabolite or ultima metabolite of the sterol metabolizing of *C. sinensis*. Recent researches also showed the sterol matter of *C. sinensis* had anticancer activity[6].

In the test, the verticiol and some structure analog in *C. sinensis* were first found. Verticiol was a substance strongly resembling with caladium alkali in structure which was a medication for aspiratory disease. The author also found the content and compose of the essential oil of *C. sinensis* related with cultured time and condition. Further work is under going in the Laboratory for this.

Table 1 Chemical components of essential oil in C. sinensis

No.	Components	Relative content/%	No.	Components	Relative content/%
1	oxetane, 3-(1-methylethyl)	1.00	23	1*H*-cyclopropa (a) naphthalene, 1a2, 3, 3a,	
2	1.4-dioxane-2, 5-dione	0.08		4, 5, 6, 7b-octahydro-1, 1, 3a-7-tetramethyl	1.94
3	toluene	5.84	24	5-azulenemethanol, 1, 2, 3, 4, 5, 6, 7, 8-octa-	2.89
4	*n*-propyl acetate	2.46		hydro-a, a, 3, 8-tetramethyl (-)-aristolene	1.63
5	furfural	0.21	25	2-naphthalenemethanol, decahydro-à à 4a-	
6	fthylbenzene	0.57		trimethyl-8-methylene-[2r-(2à 4aà 8aâ)]	1.90
7	benzene, 1, 3-dimethyl	0.48	26	selina-6-en-4-ol	0.50
8	*n*-benzyloxycarbonyl-*L*-tyrosine	0.26	27	2-naphthalenemethanel, 1, 2, 3, 4, 4a, 5, 6,	
9	benzene, propyl	0.19		8a-octahydro-a, a, 4a, 8-tetramethyl	0.32
10	phenyletnyl alochol	1.94	28	4-nonylphenol	0.08
11	benzene, 1, 2, 4-triethyl	0.26	29	heptadecane	0.46
12	benzene, 1, 3-bis (1-methylethyl)	0.75	30	nonadecane	0.37
13	tetradecane	0.13	31	2-pentadecanone, 6, 10, 14-trimethyl	0.12
14	cholesterol	0.52	32	verticiol	0.12
15	eudesma-4(14), 11-diene	0.69	33	heneicosane	0.51
16	butylated hydroxytoluene	2.45	34	2-11-pentadecenal	0.43
17	9-octadecenoic acid (*Z*)-methylester	1.81	35	*n*-hexadecanoic acid	16.27
18	2*H*-benzocyclohepten-2-one, 3, 4, 4a, 5,		36	hexadecane	0.59
	6, 7, 8, 9-octahydro-4a-methyl, (*S*)	0.48	37	androst-7-ene, (5a)	2.21
19	eugenol	3.08	38	9, 12-octadecenoic acid	18.47
20	testosterone propionate	3.19	39	oleic acid	9.05
21	testosterone	0.52	40	9, 12-octadecadienoic acid, ethyl ester	0.93
22	17a-methyltestosterone	5.16	41	undecane	2.21

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