·论著·

蛴螬脂溶性成分的研究

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[摘要] **目的** 研究蛴螬提取物中的脂溶性成分。**方法** 采用乙酸乙酯回流提取蛴螬中的脂溶性成分,对其进行气相色谱-质谱法(GC-MS)分析,并对主要脂溶性成分进行定性分析。**结果** 从蛴螬脂溶性成分中鉴定出 33 个化合物,十五酸、Z-11-十六烯酸、十六酸、十七酸、十八烯酸和十八酸为其主要的脂溶性成分。**结论** 本研究为进一步研究动物药和确认蛴螬的有效成分奠定了基础。

[关键词] 蛴螬;鳃金龟;乙酸乙酯;气相色谱-质谱
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Liposoluble constituent of ethylacetate extract of *Holotrichia diomphalia* larvae

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[**Abstract**] **Objective** To analyze the chemical components of the liposoluble constituent of *Holotrichia diomphalia* larvae. **Methods** Essential liposoluble compounds were extracted from *H. diomphalia* through ethylacetate. GC-MS analysis was used to identify its main components. **Results** 33 peaks were separated and 33 compounds were identified from ethylacetate-extracted *H. diomphalia*. The main compounds were pentadecylic acid, Z-11-Hexadecenoic acid, hexadecanoic acid, heptadecanoic acid, oleic acid and octadecanoic acid. **Conclusion** This work will contribute to the study of medicinal animals and confirmation of active compounds in *H. diomphalia*.

[**Key words**] *Holotrichia diomphalia*; *Melolonthidae*; ethylacetate; GC-MS

1 Introduction

In China, the subfamily *Melolonthidae* is represented in the literature about 500 species[1]. Among them, four species larvae of *Holotrichia diomphalia* Bates, *Holotrichia oblila* Fald, *Holotrichia sauteri* Moser and *Holotrichia parallela* Motschulsky are traditionally used in China to treat arthrolithiasis, tetanus, erysipelas and superficial infection[2-4].

The chemical composition of the medicinal animal has few been studied in detail. We reported previously the composition on the petroleum ether extract of *H. diomphalia* using GC-MS[5], however, liposoluble constituent extracted from *H. diomphalia* has not been studied. Through the investigation of *H. diomphalia*, we discovered that the ethylacetate extract of *H. diomphalia* contained a host of liposoluble constituent. Thus, the extract was identified by GC-MS and we firstly reported the result on GC-MS determination of liposoluble constituent of ethylacetate extract of *H. diomphalia*.

2 Materials and methods

2.1 Materials Medical materials of *H. diomphalia* were purchased from local market and identified by Professor Zhang Hanming, Second Military Medical University, Shanghai, China. All standard compounds were purchased from Sigma (St. Louis, MO, USA). Ultrapure water was produced by a Milli-Q Reagent Water System (Millipore, MA, USA). All experiments were carried out on a Thermo Focus DSQ gas chromatograph and interfaced to a Thermo Focus DSQ mass selective detector.

2.2 Extract methods Dried (50 ℃) and chopped *H. diomphalia* powder was refluxed three times (3 h

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each time) with 75% ethanol. After filtration, the clear supernatant was then concentrated at 60 ℃. Concentrated ethanol extract was partitioned between water and petroleum ether (60-90 ℃). After removing petroleum ether fraction, the aqueous layer was partitioned again with acetic ether. The extract of acetic ether was evaporated and the residue was used for the following experiment.

2.3 GC-MS condition GC-MS analysis was carried out on a Thermo Focus DSQ gas chromatograph fitted with a fused silica HP-5 MS capillary column (30 m×0.25 mm×0.25 μm). The oven temperature was programmed from 50 - 300 ℃ at 15℃/min. Helium was used as a carrier gas at a flow rate of 1.0 ml/min. The gas chromatograph was coupled to a Thermo Focus DSQ mass selective detector. The MS operating parameters were: ionization voltage, 70 eV; ion source temperature, 250 ℃. Identification of components of the petroleum ether extract was based on retention times (Rt) of relative fat acid and computer matching with the NIST98. Library, as well as comparison of the fragmentation patterns of the mass spectra with those reported in the literatures[6,7].

3 Results

In total, as shown in the Table 1, 33 compounds were identified in ethylacetate extract of *H.diomphalia* through GC-MS. Pentadecylic acid, Z-11-Hexadecenoic acid, hexadecanoic acid, heptadecanoic acid, oleic acid and octadecanoic acid were main components of the ethylacetate extract. The retention time (Rt) and relative percentage content (%) of compounds were listed in Table 1.

4 Discussion

In China, *H.diomphalia*, as a traditional medicinal animal, has been used to treat arthrolithiasis and infection, but the chemical composition and active compounds has not been identified and studied. Here, based on the previous research of the composition on the petroleum ether extract of *H.diomphalia*, this time we focused on the liposoluble constituent using GC-MS and 33 compounds were identified. This work will contribute to the study of active compounds in *H. diomphalia* and medicinal animals.

Table 1 Composition of the ethylacetate extract of *H.diomphalia*

Compounds	Rt	%
Glycerin	6.29	0.23
3-hydroxybutyric acid	6.56	0.02
Trans-1,2-Cyclohexanediol	6.85	0.08
Benzoic acid	8.20	0.92
2-piperidone	8.43	0.36
2,3-dihydrothiophene	8.52	0.19
Benzeneacetic acid	8.95	0.20
Hydrocinnamic acid	9.78	0.07
Benzeneacetamide	10.33	0.25
n-dodecanoic acid	11.46	0.05
E-2-hexenyl benzoate	12.71	0.26
Acetosyringone	12.89	0.06
Myristoleic acid	12.92	0.06
Tetradecanoic acid	13.01	0.94
Ethyl myristate	13.17	0.06
Pentadecylic acid	13.48	2.96
Pentadecanoic acid, ethyl ester	13.62	0.10
Cyclo(leucyloprolyl)	13.77	0.66
3,7,11-trimethyl-1-dodecanol	14.04	0.18
Z-11-hexadecenoic acid	14.33	8.17
Hexadecanoic acid	14.56	33.98
Heptadecanoic acid	14.86	1.35
E-9-tetradecenoic acid	14.97	0.22
Z-7-tetradecenoic acid	15.02	0.34
Oleic acid	15.73	44.49
Octadecanoic acid	15.79	1.39
(Z)-octadec-9-enamide	16.90	0.20
Phe-pro-diketopiperazine	17.27	0.10
beta-monopalmitin	17.69	0.19
Heptanoic acid,docosyl ester	18.35	0.17
Monoolein	18.64	1.07
Cholest-5-en-3-ol	21.73	0.57
(3β, 24s)-stigmast-5-en-3-ol	23.88	0.08

Rt: retention time on HP-5 capillary column; %: calculated from GLC data

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表 2　丹参素衍生物对 H9c2 细胞活性的影响

编号	浓度(c_B/μmol·L^{-1})	细胞活力改变率(%)	浓度(c_B/μmol·L^{-1})	细胞活力改变率(%)	浓度(c_B/μmol·L^{-1})	细胞活力改变率(%)
4	5	1.189	50	3.569	100	5.839
5	5	10.703	50	6.318	100	12.440
6	5	7.238	50	0.648	100	8.784
7	5	−2.815	50	0.719	100	1.702
8	5	−0.527	50	−4.234	100	−2.495
9	5	1.262	50	2.528	100	6.639
10	5	0.855	50	−0.765	100	−1.849
11	5	−0.894	50	−1.511	100	−0.984
12	5	10.055	50	1.628	100	−3.466
13	5	7.592	50	7.545	100	4.678
14	5	16.598	50	13.909	100	13.871
15	5	10.366	50	6.115	100	2.316
16	—	—	25	−2.559	100	−13.116
17	5	−0.967	50	1.592	100	5.539
18	5	1.024	50	−2.395	100	−6.279
丹参素钠	5	1.236	50	13.649	100	20.062

性低或不具活性外，其余化合物均表现出或多或少的抗心肌缺氧活性，其中化合物 5、6、12～15 均在浓度为 5 μmol/L 展现出强于丹参素的活性，而且化合物 4、7、9、10、12～16、17、18 随着浓度升高活性增大或减小，均未表现出最适药效浓度时的药理活性大小，需要适当扩大浓度范围做更为全面的测定。此外，硝基的选用可能也有不当，以何种取代基替换，则仍需继续合成大量化合物进行更深一步的研究。

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