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# Euscaphic acid, a new hypoglycemic natural product from Folium Eriobotryae

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Folium Eriobotryae has been used as a medicinal plant for a long time, and it is known to have many physiological actions such as anti-inflammatory, anti-tussive, expectorant and anti-diabetic. We have reported that the 70% ethanol extract of Folium Eriobotryae exerted a significant hypoglycemic effect to alloxan-diabetic mice. In this study, we isolated euscaphic acid, a natural product from Folium Eriobotryae, and investigated its hypoglycemic effect in normoglycemic and alloxan-diabetic mice. All effects had been compared with those of gliclazide. The plasma glucose levels were significantly lowered in normoglycemic mice treated with euscaphic acid compared to mice treated with 0.5% CMC-Na solution only. Moreover, the dosage of 50 mg/kg exerted a significant ($P < 0.05$) hypoglycemic effect in alloxan-diabetic mice after orally administration. The research proved that euscaphic acid is one of the active hypoglycemic constituents in Folium Eriobotryae, but the details of the mechanism need to be investigated further.

## 1. Introduction

*Eriobotrya japonica* (Thunb.) Lindl. (Rosaceae) is native to China and Japan that is well known as a kind of delicious fruit tree. Folium Eriobotryae, the dried leaves of *E. japonica*, is a famous Traditional Chinese Medicine to treat various skin diseases and diabetes mellitus. Triterpenoid constituents have been isolated from Folium Eriobotryae, and several of them were reported to have anti-tumor, antiviral, and anti-inflammatory properties (Shimizu et al. 1986, 1996; Liang et al. 1990; Tommasi et al. 1992; Nozato et al. 1994). During our investigation of traditional Chinese herbs, we discovered that Folium Eriobotryae was used to treat diabetes in the rural area of Suzhou, Jiangsu province in east China. Subsequent work was carried out to evaluate the anti-hyperglycemic effect of different extracts of Folium Eriobotryae and their acute toxicity. The result was significant to prove that Folium Eriobotryae is promising as a potential source of agents for diabetes treatment (Li et al. 2007). In this study we keep on to isolate the chemical constituents of Folium Eriobotryae, and assess the hypoglycemic effects of these constituents with animal tests. Euscaphic acid isolated from leaves of Folium Eriobotryae performed remarkable anti-hyperglycemic effects on blood glucose of normal and alloxan-diabetic mice.

## 2. Investigations and results

### 2.1. Characterization of euscaphic acid

The compound was a colorless powder from MeOH–$H_2O$ whose melt-point was 278–279 °C, and the Liebermann-Burchard reaction was positive. It showed a $[M + H]^+$ ion peak at m/z 489, corresponding to $C_{30}H_{48}O_5$. The $^1$H NMR (pyridine-$d_5$, 300 MHz) spectrum of this compound exhibited signals of an olefinic proton at δ 5.57 (1 H, tlike, J = 4.0 Hz, H-12), six singlets (each 3 H) and a doublet at δ 1.10 (3 H, d, J = 6.5 Hz, H-30), which were characteristic of the ursene skeleton. The spectrum also showed a singlet at δ 3.02 (1 H, s, H-18) and two oxygen-bearing methine protons at δ 3.73 (1 H, d, J = 2.3 Hz, H-3β) and δ 4.26 (1 H, ddd, H-2β). The $^{13}$C NMR (pyridine-$d_5$, 100 MHz) spectrum of compound substantiated the presence of a pair of olefinic carbons [δ 128.2 (C-12), 138.5 (C-13)], a carboxylic acid group [δ 180.1 (C-28)] and three hydroxylated carbons [δ 65.8 (C-2), 72.7 (C-19), 78.6 (C-3)] on the ursine structure. The identity of compound as euscaphic acid was confirmed by comparison of spectral data (Ju et al. 2003).

### 2.2. NMR-spectrum data of euscaphic acid

$^1$H NMR (pyridine-$d_5$, 300 MHz) δ: 0.89 (3 H, s, H-24), 0.97 (3 H, s, H-25), 1.09 (3 H, s, H-26), 1.10 (3 H, d, J = 6.5 Hz, H-30), 1.25 (3 H, s, H-23), 1.40 (3 H, s, H-29), 1.62 (3 H, s, H-27), 2.31 (1 H, dt, H-15β), 3.02 (1 H, s, H-18), 3.10 (1 H, dt, H-16α), 3.73 (1 H, d, J = 2.3 Hz, H-3β), 4.26 (1 H, ddd, H-2), 5.57 (1 H, tlike, H-12). $^{13}$C NMR (pyridine-$d_5$, 100 MHz) δ: 41.4 (C-1), 65.8 (C-2), 78.6 (C-3), 38.2 (C-4), 47.9 (C-5), 18.1 (C-6), 32.7 (C-7), 39.5 (C-8), 46.8 (C-9), 38.1 (C-10), 23.5 (C-11), 128.2 (C-12), 138.5 (C-13), 41.7 (C-14), 28.2 (C-15), 25.5 (C-16), 47.3 (C-17), 53.2 (C-18), 72.7 (C-19), 41.3 (C-20), 26.1 (C-21), 37.5 (C-22), 28.7 (C-23), 21.9 (C-24),

**Table 1: Effect of euscaphic acid on plasma glucose of normal mice**

| Group | Dose (mg/kg) | Num. of mice | Plasma glucose level (m mol/L) | |
|---|---|---|---|---|
| | | | 2 h | 4 h |
| Normal | – | 10 | 7.74 ± 1.48 | 6.84 ± 0.91 |
| Euscaphic acid | 15 | 10 | 6.06 ± 1.66* | 6.41 ± 1.54 |
| | 50 | 10 | 5.32 ± 1.57** | 5.97 ± 0.87* |
| Gliclazide | 50 | 10 | 3.69 ± 0.79** | 4.17 ± 1.04** |
| G-A-DGP | 2500 | 10 | 7.63 ± 1.58 | 6.91 ± 1.77 |

Values are mean ± SEM; * $P < 0.05$; ** $P < 0.01$ vs. normal, Student's t-test

**Table 2: Effect of euscaphic acid on plasma glucose of alloxan-diabetic mice**

| Group | Dose (mg/kg) | Num. of mice | Plasma glucose level (m mol/L) | |
|---|---|---|---|---|
| | | | 2 h | 4 h |
| Normal | – | 10 | 6.91 ± 1.25 | 6.55 ± 1.68 |
| Model | – | 10 | 21.96 ± 5.68## | 21.56 ± 6.55## |
| Euscaphic acid | 15 | 10 | 17.86 ± 5.60 | 18.38 ± 5.89 |
| | 50 | 10 | 16.81 ± 4.67* | 14.37 ± 6.41* |
| Gliclazide | 50 | 10 | 14.66 ± 5.26** | 15.59 ± 5.75* |
| G-A-DGP | 2500 | 10 | 17.54 ± 6.98 | 18.05 ± 6.62 |

Values are mean ± SEM; ## $P < 0.01$ vs. normal, * $P < 0.05$; ** $P < 0.01$ vs. model, Student's t-test

16.2 (C-25), 16.8 (C-26), 24.5 (C-27), 180.1 (C-28), 27.1 (C-29), 16.2 (C-30).

### *2.3. Effect of euscaphic acid on plasma glucose in normal mice*

The glycemia of control and drug-treated animals after oral administration is shown in Table 1. Administration of 15 mg/kg of euscaphic acid produced a significant decrease in blood glucose after 2 hours but the effect weakened in 4 hours. When 50 mg/kg of euscaphic acid were administered orally the decrease in blood glucose was significant throughout the entire 4 hours of the test compared with normal.

Gliclazide (50 mg/kg) induced a significant decrease in glucose level throughout the experiment. But 2500 mg/kg 'Ginseng-Astragalus Decrease Glucose Pellets' were not significantly effective ($P < 0.1$) in normal mice.

### *2.4. Effect of euscaphic acid on plasma glucose in alloxan-diabetic mice*

The glycemia of control and drug-treated animals after oral administration is shown in Table 2. We can see that the dosage of 15 mg/kg of euscaphic acid did not cause any significant decrease in blood glucose of alloxan-diabetic mice. However, oral treatment with euscaphic acid 50 mg/kg significantly reduced the glucose level of alloxan-diabetic mice throughout the test.

Similar results were obtained with gliclazide at the 50 mg/kg dose. But 2500 mg/kg Ginseng-Astragalus Decrease Glucose Pellets' effect was still not significant ($P < 0.1$) in alloxan-diabetic mice.

## 3. Discussion

Although oral anti-hyperglycemic agents and insulin are often successful in diabetes treatment, they have prominent side effects and fail to significantly alter the course of diabetic complications. Effective control of the blood glucose level is a key step in preventing or reversing diabetic complications and improving the quality of life in both type 1 and type 2 diabetic patients (Abraira et al. 1995; Ohkubo et al. 1995; Diabetes Control and Complications Trial Research Group 1993; De Fronzo 1999). The present study shows that euscaphic acid from leaves of *Eriobotrya japonica* produces a significant hypoglycemic effect when administered orally to normoglycemic and alloxan-diabetic mice. The dosage of 50 mg/kg is more effective than that of 15 mg/kg and the dosage of 50 mg/kg of euscaphic acid is even more effective on plasma glucose of alloxan-diabetic mice than that of gliclazide 4 hours after administration.

Euscaphic acid both exerted a significant anti-hyperglycemic effect to normal and alloxan-diabetic mice. The alloxan-diabetic mice model shows typical characteristics similar to type 1 diabetes mellitus in which the dosage of alloxan used could damage most of the pancreatic β-cells, so it is possible that euscaphic acid has a mechanism of action similar to that of agents to treat type 1 diabetes mellitus. However, the present tests are only initial research work, further investigations like mechanism research will be carried out.

## 4. Experimental

### *4.1. Collection of materials*

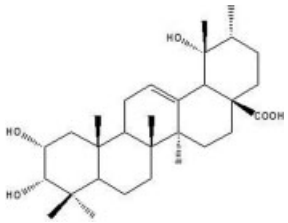
Folium Eriobotryae was collected in September–October in the south region of Jiangsu Province, China, and dried in the air at 25–30 °C, then ground into powder. The plants were identified by Professor Guo Rong-lin in the Institute of Botany, Jiangsu Province and Chinese Academy of Sciences. A voucher specimen (No. 328636) has been deposited at herbarium of the Institute of Botany, Jiangsu Province and the Chinese Academy of Sciences.

### *4.2. Chemicals and instruments*

Alloxan monohydrate was supplied by Sigma Company, USA. Gliclazide (Diamicron) was provided by Servier Company, France. Ginseng-Astragalus Decrease Glucose Pellets were bought from Shandong Lunan Houpu Pharmaceutical Ltd. Company. Silical gel (200–300 mesh) (Qingdao) and RP-18 (40–63 μm) (Merck) were used for column chromatography. NMR spectra were recorded on a Bruker 400 with TMS as internal standard. Autospec-Ultima ETOF was used for Mass spectra record. Melting points were measured by Büchi B-540.

### 4.3. Isolation of euscaphic acid

Powder of dried leaves of *E. japonica* (5 kg) was extracted with 75% ethanol solution three times. The combined ethanol extracts were concentrated under vacuum in a Rotavapor to proper volume to obtain a total extract. The concentrated extract was dispersed in water then extracted with petroleum ether, ethylacetate (EtOAc) and *n*-butanol. The part of EtOAc-extracted weighting 70 g was evaporated and subjected to column chromatography on silica gel, using a solvent gradient system from $CHCl_3$ to MeOH with gradually increasing polarity. The eluted portions were monitored by TLC and similar fractions were combined into six fractions. The fraction eluted by the mixture of $CHCl_3 : CH_3OH$ (100 : 2) was further subjected to silica gel column with a petroleum ether : EtOAc gradient system. Then the fraction (100 : 50) was subjected to column chromatography on RP-18 and eluted by a $H_2O : CH_3OH$ gradient to yield 42 mg of pure euscaphic acid (60 : 40).




### 4.4. Animals

Kunming albino mice (18–22 g) of either sex from the animal center of China Pharmaceutical University were used. The animals received a standard pellet diet and water *ad libitum*, and were maintained under standard environmental conditions (20–25 °C, 12 h of light/dark cycle).

### 4.5. Activity in normoglycemic mice

The mice were fasted for 5 h prior to the experiment but were given water. Then they were divided into 5 groups of 10 mice each in random that received the products orally. Group 1 served as the control and received 0.5% CMC-Na solution in water only. Group 2 and 3 were treated with 15 and 50 mg/kg (body weight) of euscaphic acid respectively. Group 4 received gliclazide 50 mg/kg (body weight). Group 5 received Ginseng-Astragalus Decrease Glucose Pellets (G-A-DGP) 2500 mg/kg (body weight).

### 4.6. Activity in hyperglycemic mice

The mice were fasted for 15 h prior to the experiment but were given water. Then they were injected intraperitoneally with alloxan (200 mg/kg), after 72 h the blood glucose level were measured. Only mice showing high blood glucose levels (>11.12 m mol/L) were considered diabetic and used in the experiments.

The diabetic mice were divided in 5 groups of 10 mice each in random that received the products orally. Group 1 served as the control and received 0.5% CMC-Na solution in water only. Group 2 and 3 were treated respectively with 15 and 50 mg/kg (body weight) of euscaphic acid. Group 4 received gliclazide 50 mg/kg (body weight). Group 5 received Ginseng-Astragalus Decrease Glucose Pellets (G-A-DGP) 2500 mg/kg (body weight).

### 4.6. Preparation and administration of drugs

Euscaphic acid and gliclazide were prepared as a fine suspension in 0.5% CMC-Na solution in water. Drugs were administered orally through an esophageal catheter.

### 4.7. Collection of blood

The blood sample was collected from the vein behind the eye socket 2 h and 4 h after oral administration.

### 4.8. Determination of blood glucose

Glycemia was determined in all the groups of animals. Plasma glucose was measured by the glucose-oxidase method (Glucocard™ Aventis Pharma).

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